Protein and quality analyses of accessions from the USDA soybean germplasm collection
for tofu production

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Abstract

Food-grade soybeans with large seed size, uniformity, clear hilum and a high 11S/7S ratio are favoured by the food industry for making tofu. In order to search for soybean lines with desirable characteristics for making foods, twenty-two soybean lines were selected from the USDA-Soybean Germplasm Collection, were grown in Stoneville, MS for biochemical analysis and tofu texture and sensory quality tests. Eight lines were identified, from twenty-two lines harvested in 2014, to be suitable for tofu making, as judged by chemical composition and sensory quality of pressed tofu. In the filled tofu making and texture analysis study, the correlation between A₃ subunit content and filled tofu firmness was significant (N=22. $r = 0.77$, $P < 0.001$). The results indicated that the A₃ subunit could be an indicator for predicting the firmness of tofu. The results provided important food quality information for the selection of soybean genotypes for improving food quality.

Key words: Soybean quality, soybean selection, tofu quality, sensory evaluation, 11S/7S ratio, A₃ subunit.

1. Introduction

Tofu is very popular in East Asian countries and has been gaining acceptance in the United States and Canada. Among all the factors influencing the quality of tofu, the soybean variety (genotype) is one of the most important. Good tofu can only be made by using quality soybeans (Chang, 2015). The yield and quality of tofu have been reported to be affected by soybean varieties, due to the differences in their chemical compositions (Cai, Chang, Shih, Hou, & Ji, 1997; Skurray, Cunich, & Carter, 1980).

It is generally known by soybean breeders and processors that soybeans with high protein and large seeds will result in better tofu quality and create higher tofu yields (Bhardwaj et al., 1999). In the market, soybeans with clear hilum, round and uniform shape are favoured. Besides total protein content, the distribution of storage protein composition also affects the quality of tofu. It has been reported that glycinin (11S) and β -conglycinin (7S) proteins in soybeans have different effects on tofu firmness and smoothness (Saio & Watababe, 1978). Previous studies have demonstrated that the ratio of 11S/7S soybean proteins may influence the textual quality of tofu (Cai & Chang, 1999; Chang, 2015; Kim & Wicker, 2005; Zhang & Chang, 1996) among a wide range of soybeans. However, the positive relationship between 11S/7S ratio and tofu firmness has not been universally agreed by researchers, since opposite findings have been reported.

The science and technology for making purified protein gel is different from that for making tofu from soymilk. In purified protein gel making, a high concentration (6-8%) of soy protein isolate without coagulant is used. In tofu making, much lower protein content (2-3%) in soymilk and a coagulant, such as Ca and Mg salts or glucono- δ -lactone, are needed for coagulation. Gels made from purified soy protein, containing various

concentrations of glycinin's A₃ subunit, show A₃ subunit content is correlated with the firmness of the purified protein gel (Nakamura, Utsumi, Kitamura, Harada, & Mori, 1984; Fukushima, 2000). However, the quantified influence of A₃ subunit on tofu made from soymilk, which is a complex mixture of soy protein, lipids, phytate and other constituents, has not been demonstrated. A study by Poysa, Woodrow and Yu (2006) found that the average effect of the A₃ subunit was to increase tofu firmness without providing a quantified correlation. Further demonstration of the role of A₃ subunit in the complex system of soymilk may provide useful peptide marker for breeders to select genotypes for tofu making in the early generations of the breeding program. Since tofu manufacturers desire to control the texture of tofu products with a range of firmness to meet the needs of the consumers (Cai & Chang, 1999).

In the state of Mississippi, there are no known commercial non-GMO food-grade soybean available for planting. In order to find soybean lines that are suitable for tofu production and that may be suitable for growing in Mississippi, 68 soybean 'Plant Introductions' (referred to as "lines" or "varieties" in this study) were selected from USDA Germplasm Collection. Initial selections were made from thousands of lines using GRIN (Germplasm Resources Information Network) based on the following parameters (Hill, et al, 2001): protein (42% or higher), lodging (2.5 or less), seed weight (18.5-25/100 seeds), seed quality (1-3), mottling (1-2). Then the 11S/7S protein ratio was tested to be greater than 1.45, which is a ratio reported to be good for tofu making (Cai & Chang, 1999).

Health-promoting effects of phenolic substances, including isoflavones and antioxidant capacity, in various soybean varieties and in tofu have previously been

studied (Liu, Chang, & Wiesenborn, 2005; Xu & Chang, 2008). Besides isoflavones, polyphenols, such as flavonoids, condensed tannins and free phenolic acids are important for their antioxidant effects, which are affected by soybean varieties and planting location (Xu & Chang, 2008). Therefore, selecting soybean based on phenolic content and antioxidant capacity can be helpful for human health improvement.

The objectives of this study were (1) to screen and select suitable soybean lines for tofu production; (2) to determine if there was a correlation between storage protein profile (11S/7S ratio or A₃ subunit content) and tofu quality (firmness); and (3) to investigate the phenolic and antioxidant properties of these lines.

2. Material and methods

2.1. Materials

Using the USDA Soybean Germplasm “Plant Introduction” Collection database, sixty-eight soybean lines (varieties) were pre-selected from thousands of entries in 2012 using the parameters given above, and planted in Stoneville, Mississippi for increase in the summer of 2013. Twenty-two lines were then selected from these 68 lines based on seed size, appearance and 11S/7S ratio, and were further increased in the winter 2013-2014 in Puerto Rico, through the help of the Illinois Crop Improvement program. To increase to adequate quantity of seed for tofu quality testing, the selected 22 lines were grown again in the summer 2014 in Stoneville, Mississippi. The seeds were not dried in a dryer and were sufficiently dry at harvest. After harvest the seeds were stored in a cool, humidity controlled seed storage room. These soybeans were coded as MS-01 to MS-22. All the chemicals and reagents for protein analyses were from Sigma Chemical

Co. (St. Louis, Mo., U.S.A.). The chemicals and reagents for phenolic and antioxidant analyses had been described in a previous study (Xu & Chang, 2007).

2.2. Moisture and protein analysis of soybean

Soybeans were ground to pass a 0.5 mm screen using a Retsch ZM 200 ultra-centrifugal mill (Retsch GmbH, Germany). The flour was analyzed for moisture, protein, and other chemical constituents. Moisture was determined using an oven method (AOAC International, 2012; Method 934.01). Crude protein was determined by the Kjeldahl method (AOAC International, 2012; Method 955.04). The nitrogen to protein conversion factor was 6.25.

2.3. One hundred-seed weight and seed appearance score

One hundred-seed weight was measured by randomly selecting 100 seeds from the total samples and repeated 3 times. Seed appearance quality score (0: bad; 10: excellent) was assigned by evaluating seed size, wrinkling, defective seed coat, greenish or diseased seeds.

2.4. Protein extraction analysis

Protein extraction procedures of Cai and Chang (1999) were modified as follows: Soybean flour (0.3 g) was defatted in 10 ml of acetone with an orbital shaker for 1 h. The suspension was centrifuged (2795g for 20 min) by the centrifuge (SorvallTM LegendTM X1, Thermo, Marietta, OH) and the pellet was dried under an exhaustion hood overnight at < 40 °C to dryness. Three milliliters of water were added and the pH was adjusted to 8.5-9 with 1N NaOH. The mixture was shaken at room temperature for 60 min to extract protein. The extract was then centrifuged at 2800×g by the centrifuge (SorvallTM

LegendTM X1, Thermo, Marietta, OH) for 20 min. Protein content of the supernatant was analyzed according to the Bradford's method (Bradford, 1976).

The protein extract was diluted to 2 mg/ml with distilled water, and 0.5 ml of diluted extract was mixed with 0.5 ml of SDS sample buffer, containing 10% 2-mercaptoethanol (Cai & Chang, 1999). After boiling for 10 min, 25 µl of the cooled solution, containing equivalent of 25 µg protein, was loaded onto a gradient gel containing 8-16% polyacrylamide. SDS-polyacrylamide gel electrophoresis (SDS-PAGE), based on the procedure of Laemmli (1970), was performed in a BioRad Protean II chamber at 100 V for 1 h, followed by 125 V for 6 h. At the end of electrophoresis, gels were stained with Coomassie Brilliant Blue R-250. For the quantification of glycinin and β-conglycinin, gels were scanned and analyzed by a Molecular Imager (Bio-Rad ChemidocTM XRS+, Hercules, California) equipped with Image LabTM Analysis Software (version 5.2). The determination of glycinin and β-conglycinin and their relative composition were based on the mobility, band intensity and total area of their subunits (Cai & Chang, 1999) using molecular weight markers (Product No. S8445, Sigma, St. Louis, Mo., U.S.A.). The relative amounts of glycinin and β-conglycinin were estimated by using purified glycinin (purified in our own lab) as a standard based on a standard curve of different concentrations of glycinin.

2.5. Phenolic compound extraction

The extraction method was according to the optimal extraction method for soybean by Xu, Yuan, and Chang (2007). The extractions were conducted in duplicates for each individual line.

2.6. Total phenolic content

Total phenolic content (TPC) was determined by a Folin-Ciocalteu assay (Singleton & Rossi, 1965, Xu & Chang, 2007), with gallic acid (GA) as standard. Total phenolic content was expressed as gallic acid equivalents per gram of sample (mg of GAE/g sample) through the calibration curve of gallic acid. Linearity range of the calibration curve was 50 to 1000 $\mu\text{g/ml}$ ($r=0.99$).

2.7. Total flavonoid content

Total flavonoid content was determined using a colorimetric method (Heimler, Vignolini, Dini, & Romani, 2005) with some modifications (Xu & Chang, 2007). The results were calculated and expressed as micrograms of (+)-catechin equivalents per gram of sample (mg of CAE/g sample) using the calibration curve of (+)-catechin. Linearity range of the calibration curve was 10 to 1000 $\mu\text{g/ml}$ ($r=0.99$). The extraction was conducted in duplicate.

2.8. Condensed tannin content

Analysis of condensed tannin content (CTC) was carried out according to the method of Broadhurst and Jones (1978), with slight modifications (Xu & Chang, 2007). In brief, three hundred microliters of concentrated hydrochloric acid were added to 10 μl of properly diluted sample, and then 600 μl of 4% vanillin in methanol was added. The mixture was vortexed and allowed to stand at room temperature for 15 min. Two hundred microliters of the reaction mixture were added into a 96-well plate, and the absorption was measured at 500 nm against the blank (sample was replaced by methanol) by a plate reader (FlexStation 3, Molecular Devices, Inc. CA). The content of condensed tannin was calculated and expressed as mg catechin equivalents (mg of CAE/g sample) using the

calibration curve of (+)-catechin. Linearity range of the calibration curve was 31.25 to 750 $\mu\text{g/ml}$ ($r=0.99$).

2.9. Radical DPPH scavenging activity

DPPH-free radical scavenging capacity of legume extracts was evaluated according to the method of Chen and Ho (1995), with slight modifications (Xu & Chang, 2007). The free radical scavenging activity of soybean extracts was expressed as Trolox equivalent. Every sample was extracted in duplicate, and the results were calculated and expressed as micromoles of Trolox equivalents (TE) per gram of soybean, using the calibration curve of Trolox. Linearity range of the calibration curve was 20 to 1000 μM ($r=0.99$).

2.10. Oxygen radical absorbing capacity assay

The ORAC assay was performed according to Prior et al. (2003) and Xu and Chang (2007) using a Molecular Device plate reader. The final ORAC values were calculated automatically by software based on the net area under curve (AUC). The data were analyzed using Microsoft Excel (Microsoft, Roselle, Ill., U.S.A.). Linearity range of the calibration curve was 12.5 to 50 μM ($r=0.99$).

2.11. Preparation of laboratory pressed tofu and yield determination

Laboratory tofu making method was based on Yuan and Chang (2010), with some modifications. Soybeans (130 g) were soaked in tap water at 20-22 $^{\circ}\text{C}$ for 8 h to obtain ~2.1 times of their original weight. The soaked water was discarded, soaked beans were added to 1300 g (bean: water=1:9 w/w) with fresh tap water and ground for 4 min at a high speed in a blender (model 908-2, Hamilton Beach Co., Washington, NC). The slurry was squeezed through a muslin cloth manually by the same researcher (Mr. Shi) to

maintain consistency. The test of soymilk yield and solid content showed very small variations among different replicates of the same soybean line. A portion of 1100 g soymilk was placed into a small stainless steel pot (18 cm diameter × 11.5 cm height). The small pot was put into a larger pot with boiling water on the stove for 10 min. When soymilk reached 85 °C, the small pot was then placed directly on the stove with medium heat to prevent burning at the bottom. Stirring by hand continuously was needed to keep it boiling slightly without burning for 5 min. When the cooked soymilk was cooled to 85 °C, calcium sulfate (CaSO₄ at 2% of raw bean weight, equivalent to 0.29% of cooked soy milk) suspended in 20 ml of water was added. The mixture was stirred with a Caframo stirrer (model RZR1, Caframo Ltd., Warton, ON, Canada), equipped with a stainless steel paddle (14 cm length *1.5 cm width), at 150 rpm (speed at 4) for 15-20 s. The pot was taken off with cover on and put in an insulated container covered with several layers of muslin cloth to prevent heat loss for 12 min to promote protein coagulation. After 12 min, the bean curd was cut into in each direction to become dices (1 inch square) and stood for another 1 min. The diced curd was poured into a wooden mold (12.5 cm length × 12.5 cm width × 5.5 cm height). The bean curd was pressed sequentially by placing weights of 24 lb for 15 min and 48 lb for another 15 min, which were equivalent to pressures of 69.7 and 139.3 g/cm² of tofu, respectively. After pressing and weighing, the tofu was stored in cold water at 4 °C prior to colour measurement, textural analysis and sensory evaluation. The yield of tofu (or soymilk) was calculated as the weight (g) of fresh tofu (or soymilk) per 100 g soybeans used to make tofu.

2.12. Texture analysis of laboratory pressed tofu

The tofu texture measurement method used was according to Yuan and Chang (2007) with minor modifications. In this study, 75% plunger penetration was used and the plunger speed was set at 100 mm/min to determine the force-time response using the TPA method (Bourne, 2002) with an Instron universal testing machine (Model 1011, Instron Co., Canton, Mass., U.S.A.). Each preparation and Instron analysis was repeated at least 3 times. The textural profile parameters, including firmness, brittleness (fracturability) and springiness were calculated according to the method of Yuan and Chang (2007).

2.13. Sensory evaluation of laboratory-pressed tofu

Sensory evaluation was conducted in the Garrison Sensory Evaluation Laboratory in the Department of Food Science, Nutrition and Health Promotion at the Mississippi State University. A continuous linear-scale (15 cm) descriptive sensory evaluation method was used to determine the sensory properties (Sidel & Stone, 1993; Yuan & Chang, 2007) using a Linear spectrum method. Twelve panellists, consisting of graduate students, staff, and faculty were recruited and trained using known commercial tofu products as references. During the training, each sensory attribute was evaluated and discussed until the panel reached consensus. The commercial soft tofu (Silken tofu, Nasoya, Ayer, MA) reference was assigned at 2 cm for firmness and elasticity, respectively. The commercial extra-firm tofu (Nasoya, Ayer, MA) reference was assigned at 14 cm for firmness and elasticity, respectively. The commercial soft tofu reference was designated at 12 cm for smoothness, while the extra-firm tofu reference was designated at 5 cm for smoothness. These assigned scores for references were agreed upon by the panellists after the discussion. The sensory testing of the tofu products made from

selected soybean lines was conducted in individual booths. A fork, a pencil, a napkin, a cup of spring water and an empty cup were prepared for each panellist. Replicates of tofu samples in random code number were evaluated in different sessions on different days. Four tofu products were evaluated in each session.

2.14. Preparation of filled tofu and texture measurement.

A tofu-making device was prepared using a 60 ml plastic syringe (Terumo SS-60L, Terumo, Tokyo). The needle site of the plastic syringe was cut in order to fill in soymilk to form tofu. This device was 110 mm in length and 34 mm in diameter internal. Tofu was prepared according to the procedure described in Toda et al. (2003) with minor modifications. In brief, soybean seeds were washed and soaked with 7 times of deionized water at 20 °C for 15 h. Soaked soybeans were drained and ground at 10,000 rpm with a final bean-to-water ratio of 1:7 (w/w) using an Osterizer Mixer (Oster Co., Milwaukee, WI) for 3 min. The soybean slurry was filtrated by 120 mesh cloth to get soymilk. The soymilk, in a beaker, was put into boiling water until a temperature of 90 °C was reached and the soymilk was further heated for 2 min. After cooling to 4 °C in an ice bath, forty-nine milliliters of cooled soymilk were transferred to a 60 ml syringe. One milliliter of MgCl₂ solution (12.5%) was added and stirred quickly. The syringe was covered with a glass ball as a lid and was incubated in a water bath for 45 min at 85 °C. The tofu prepared using this tofu-making device was placed in a refrigerator (4 °C) for 18-24 h. The tofu in the syringe was pushed out and the upper end was discarded. The tofu was cut into 10-mm thick pieces with a sharp knife. Each piece of tofu specimen, with a 10 mm height and 34 mm diameter, was placed on a measuring plate. A compression test was carried out at a compression rate of 60 mm/min with 95% penetration with an 8 mm

diameter cylindrical plunger using an Instron universal testing machine (Model 3345. Instron Co., Canton, Mass., U.S.A). Breaking stress of a curd was expressed as the mean value of three measurements.

2.15. Statistical analysis

Experiments were performed in duplicate or triplicate with a completely randomized design. Data were analyzed by 2014 SAS (Version 9.4, SAS Inst. Inc., Cary, N.C., USA). Statistical significance of differences between means was determined by the Duncan multiple range test procedure for independent samples at $p < 0.05$. Pearson's correlation coefficient analyses were determined. Significance levels were defined using $P < 0.05$.

3. Results and discussion

3.1. Physical and chemical properties of soybean

Tables 1 and 2 showed the physical (seed size and quality score) and chemical (total protein, moisture) properties of the 22 soybean samples harvested in the Fall of 2014 (Stoneville, Mississippi). One-hundred seeds weight varied from 13.30 g to 25.96 g, and 17 out of 22 soybean samples were above 20 g. Seed size is an important trait of soybean for commercial use, since small beans are not favoured by farmers and the processing industry customers for tofu making. The preference may be that the larger seeded soybeans would have a lower ratio of seed coat component which is not utilized in the tofu making and would result in higher tofu yield (Aziadekey, Schapaugh, & Herald, 2002). In our study, the correlation between seed size and tofu yield is not statistically significant. This result is consistent with an earlier report (Wang & Chang, 1995).

However, in Japan, the seed size requirement for tofu production by the tofu processing industry is at least 20 g per 100 seeds (Brar & Carter Jr, 1993).

The seed quality (based on 10-point scale, 10 is excellent) varied from 4 to 8 according to appearance. Yellow coloured, round, big and uniform soybean sample without defective seedcoat had a high quality score. Eight out of 22 samples were above 7 score, which means a relatively good quality by appearance. Meanwhile, the eight selected bean lines had 100-seed weight over 20 g. Total protein contents of all of soybean lines were above 40%, which indicated that all beans had a high protein content and were preferred by the soy food industry for food making according to protein content.

3.2. Protein subunit distribution of soybean

The 11S/7S ratio varied from 1.31 to 1.92 as shown in Table 2 and Fig. 1. The 11S protein content (% of total soluble protein) varied from 36.50 % to 46.36 %. The 7S protein content (% of total soluble protein) varied from 21.97 % to 32.65 %. The 11S+7S protein content (% of total soluble protein) varied from 61.43 % to 75.53 %. There have been several studies on the correlations between 11S/7S ratio and tofu's firmness. Some studies showed positive correlations between tofu firmness and the 11S/7S ratios among various soybean cultivars (Cai & Chang, 1999; Hou & Chang, 2004; Kim & Wicker, 2005; Zhang & Chang, 1996). However, some researchers did not find significant correlations between 11S/7S ratio with tofu firmness. Murphy, Chen, Hauck, Wilson, and Giese (1997) reported a negative relationship between tofu firmness and the 11S/7S protein ratio of food soybeans. Skurray, Cunich, and Carter (1980) and Taira (1990) found that there was little correlation between the 11S/7S protein ratio and tofu quality. Therefore, the contribution of soybean storage protein composition patterns to tofu

texture is controversial, and may be partly due to different processing methods used for tofu making, and lack of a standard method for analysis of texture. In previous studies, a relatively consistent processing method was used and results showed that the 11S/7S ratio positively correlated with the firmness of tofu, and a ratio of 1.45 or higher was found to produce good tofu texture (Cai & Chang, 1999; Ji, Cai & Chang, 1999). Therefore, in this study, soybean lines with 11S/7S ratio under 1.45 were purposely not selected for tofu making. Consequently, the 11S/7S ratio range was narrow, ranging from 1.46 to 1.92 (Table 1). The narrow 11S/7S range might be responsible for the lack of a significant correlation between 11S/7S ratio and firmness of tofu made from selected soybean lines.

3.3. Phenolic profile of soybean

The phenolic profile and antioxidant activity analysis were conducted on the same 22 lines of soybeans used for protein composition and tofu analysis (results shown in Table 2). The phenolic profile of soybean was composed of three major parts: total phenolic content (TPC), total flavonoid content (TFC) and condensed tannin content (CTC).

For the total phenolic content, the results varied from 1.74 to 2.55 mg GAE/g among 22 soybeans. It has been reported (Xu & Chang, 2008) that the TPC of 28 yellow soybeans varied from 2.07 to 2.90 mg GAE/g. The data in this current study were slightly lower. The soybean with highest TPC (MS-22) was greenish. For food making, this variety was not chosen even though the phenolic profile and antioxidant activity was relatively high since the tofu industry prefers a yellow colour soybean.

The total flavonoid content of 22 soybean lines varied from 0.68 to 1.73 mg CAE/g. Compared with an earlier study (Xu & Chang, 2008) data varying from 0.18 to

0.59 mg CAE/g for 28 soybean varieties, the total flavonoid content in this study was higher than this reported range. The difference may be due to different varieties and planting locations of soybeans between these two studies. The 28 soybeans studied by Xu and Chang (2008) were collected from local farmers or seed companies in the region of North Dakota-Minnesota. However, the 22 soybeans of this study were harvested in Mississippi. The environmental differences might have partly contributed to the discrepancies in the TFC values.

The condensed tannin content of the 22 soybean lines varied from 0.3 to 0.8 mg CAE/g. These values were comparable to that of an earlier study (Xu, Yuan, & Chang, 2007) with the same method, varying from 0.37 to 0.79 mg CAE/g. Of the three correlations among the three phenolic profiles, the only significant correlation existed between CTC and TFC ($r = 0.51$, $P < 0.05$). Similar positive correlation ($r = 0.56$, $P < 0.0001$) was found in a previous study (Xu & Chang, 2008).

3.4. Antioxidant activity of soybean

For antioxidant capacity, ORAC value varied from 20.87 to 46.29 $\mu\text{mol TE/g}$ among 22 soybeans (Table 2). Compared to a previous study (Xu & Chang, 2008) with ORAC values varying from 21.2 to 91.3 $\mu\text{mol TE/g}$ in 28 soybeans, the ORAC value was lower and the range was narrower. The sample with the highest ORAC value (MS-22) also possessed the highest TPC, TFC and CTC. The correlation coefficient between TPC and ORAC in this study was 0.60 ($P < 0.01$), which showed a significant positive correlation. The correlation coefficient between TFC and ORAC in this study was not significant ($r = 0.30$, $P > 0.1$). The correlation coefficient between CTC and ORAC in this study was 0.41 ($P < 0.1$), which showed a relatively moderate positive correlation but

was not significant. Among the three major phenolic profiles, the correlation ($r = 0.6$) between TPC and ORAC was the strongest, which indicated that the amount of TPC in these 22 lines of soybeans might be one of the major contributors to the ORAC value. The above correlation patterns supported earlier findings (Xu & Chang, 2008) that TPCs correlated positively with ORAC of yellow soybeans.

DPPH scavenging capacity varied from 1.95 to 5.25 $\mu\text{mol TE/g}$ among the 22 soybean lines (Table 2). There was a lack of significant correlation between ORAC value and DPPH scavenging capacity. The result was very similar to a previous study (Xu & Chang, 2008). Antioxidant activity is reaction-mechanism dependent, and using at least two assay methods with different free-radical scavenging mechanisms is necessary to provide a more reliable antioxidant profile (Xu, Yuan, & Chang, 2007). The correlation coefficient between TPC and DPPH scavenging capacity in this study was not significant ($-0.26, P > 0.1$). No correlation existed between TFC and DPPH scavenging capacity ($-0.08, P > 0.1$), and between CTC and DPPH scavenging capacity ($-0.10, P > 0.1$). These results reconfirmed a study (Xu & Chang, 2008) that the DPPH value was less correlated with all the three phenolic profiles of yellow soybean lines than ORAC values.

3.5. Selection of soybean for soymilk and tofu making

For all soybean lines grown in Mississippi (Tables 1 and 2), the phenolic profile and antioxidant capacity did not correlate well with seed quality (appearance), one hundred seed weight, and 11S/7S ratio. The only correlation significant at 0.05 level was between seed appearance quality and total phenolic content ($r = -0.44$). The reason for the negative correlation was partially due to the greenish coloured seed, which may have a higher TPC with a low seed quality score. For example, MS-02 and MS-22 had high total

phenolic content, but low in seed quality score (green or small size). For making high quality soymilk and tofu, the selection criteria was based on the protein content ($\geq 40\%$), 11S/7S ratio (≥ 1.45) and general seed quality (≥ 7) traits. As a result, eight soybean lines were selected and prepared for soymilk and tofu making. These soybean lines were MS-01, MS-06, MS-07, MS-12, MS-15, MS-16, MS-17 and MS-20.

3.6. Texture properties of pressed tofu and correlation with 11S/7S and A₃ subunit

Among the eight soybean lines, the firmness varied from 3.64 to 8.66 Kg force (Table 3). Compared with the literature in the texture analysis that was conducted utilizing the same measuring conditions as previously reported (Yuan & Chang, 2007), the Mori-nu Firm tofu and Mori-nu Extra Firm tofu (commercial products) ranged from 4.62 to 6.53 Kg force. Firm and extra firm tofu of other brands had higher firmness values. The tofu made in this study was within the range of commercial firm and extra firm tofu. The correlations between tofu firmness and 11S/7S ratio and A₃ subunit content (%) of the eight selected soybean lines were calculated in order to understand the effect of storage protein subunit composition on pressed-tofu firmness. The results from the correlation study (Table 4) showed that a moderate correlation ($r = 0.63$, $P < 0.1$) existed between the content of A₃ subunit and firmness of pressed tofu. This is a new finding, consistent with the relationship between purified protein gel firmness and A₃ subunit as reported by Nakamura, Utsumi, Kitamura, Harada, and Mori (1984), and Fukushima (2000). The reason why P value was not significant at 0.05 may be due to the relatively small sample size ($N = 8$), since the objective of this study was to screen and select soybean lines for tofu making, and the pre-screening process deleted many soybean lines and hence reduced the soybean population size. If the soybean population size increases,

the P value might have been lower. The relationship between 11S/7S ratio and firmness of pressed-tofu was weak and not significant. The result showed that the 11S/7S ratio in the narrow range from 1.46 to 1.92, which was used to select soybean for planting in Stoneville, MS, did not correlate well with the firmness of pressed tofu. The screening process deleted all soybean lines with low 11S/7S ratio (<1.45), and reduced the population size from 22 to 8. The content of A₃ subunit correlated better with firmness of pressed-tofu when compared with 11S/7S ratio in this study, indicating this single peptide was more indicative of the quality of texture as opposed to the overall storage protein composition.

3.7. Firmness of filled tofu and correlation with 11S and 7S profile

Tofu processing method is a factor which affects tofu quality. Filled tofu making method did not require pressing, it was expected that the firmness of filled tofu would better reflect the molecular structures of storage proteins. In order to understand the correlations between filled tofu firmness and 11S/7S and A₃ subunit content better, filled tofu was made from all 22 lines (the 8 lines for pressed tofu were included) harvested in the fall of 2014 in Stoneville, MS. The correlation of 11S content, 7S content, 11S+7S, other 11S subunits (A_x subunit and B_x subunit) content and tofu firmness were also calculated to understand the effect of storage protein distribution in a more comprehensive manner.

The A₃ subunit content of the 22 soybean lines varied from 2.12% to 4.23% (Table 2). The breaking stress (firmness value per unit area) of filled tofu varied from 54.4 g force/cm² to 107 g force/cm² (Table 2, Figure 2). As Table 5 shows, correlations of tofu breaking stress between 11S/7S ratio and A₃ subunit content (% of total soluble

protein) were 0.06 ($P > 0.1$) and 0.77 ($P < 0.001$), respectively. The correlation between A_3 subunit content and filled tofu breaking stress was significant at 0.001 level with $r = 0.77$ after changing tofu making method from pressed tofu to filled tofu method, and increasing the soybean population size to 22. The correlation between total protein content and filled tofu breaking stress was 0.56 ($P < 0.01$). As shown in Table 4, the correlation coefficient between protein content and tofu firmness was 0.56 ($P < 0.01$). The result was in agreement with a recent study of Huang et al. (2014), in which protein content was significantly correlated (r ranged from 0.45 to 0.6, $P < 0.002$) with the texture of tofu made from 48 soybean cultivars. This result further confirmed the importance of high protein content for tofu making. However, A_3 subunit exhibited higher correlation with tofu firmness than protein content. A_3 subunit is the largest constituent of acidic polypeptide of glycinin. This result showed that A_3 subunit has a significant influence on tofu structure that may be pertaining to its molecular feature. This finding supports the results of Poysa, Woodrow, and Yu (2006) who found that A_3 glycinin subunit null lines were difficult to form tofu gels and the average effect of the A_3 glycinin subunit was to increase tofu hardness and firmness. The correlation of 11S/7S ratio and filled tofu breaking stress was not significant at 0.1 level with $r = 0.06$. The reason may be due to the relatively narrower range of 11S/7S ratio 1.31-1.92 in this study as compared with other studies, Cai and Chang (1999) reported a wider range of 11S/7S ratio varied from 1.64 to 2.51 among 13 varieties and that was significantly correlated with firmness of tofu. Since the relationship of tofu and 11S/7S ratio is controversial, more varieties of soybean need to be cultivated and made into tofu to give more convincing conclusion in the future.

In the result of correlation between 11S and/ or 7S content and filled tofu firmness as shown in Table 4, the correlation coefficients between 11S content, 11S+7S content and tofu firmness were significant at 0.05 level with $r = 0.52$ and $r = 0.49$, respectively. However, the correlation between 7S content and tofu firmness was not significant at 0.05 level with $r = 0.32$. This result indicated that 11S, not 7S, was the main contributor to the firmness of tofu. No significant correlation was observed between tofu firmness and other 11S subunits (A_x subunit and B_x subunit) (Table 4). This result further confirms the important role of A_3 subunit in tofu firmness.

The Japanese tofu industries set the requirement for the breaking stress of filled tofu at 90 g force/cm². As shown in Table 2 and Figure 2, five out of 22 soybean lines grown in Stoneville, MS were suitable for filled tofu making. Among the eight lines which were selected for pressed tofu making, two lines (MS-1 and MS-16) were proven to be suitable for making filled tofu by the Japanese industry standard. The reason why only five soybean lines reached the firmness requirement of Japanese industry may be due to a fixed amount of coagulant (0.25% $MgCl_2$) being used for filled tofu making for all soybeans. According to an earlier study (Liu & Chang, 2003), the optimal coagulant concentration varied among different soybean cultivars and could be measured by a rapid titration method. Toda et al. (2003) also found that different soybean varieties may require different amount (0.25~0.4%) of coagulant ($MgCl_2$) for maximum breaking stress of tofu. These researchers found that 0.25% concentration was preferred when $MgCl_2$ was used as coagulant. That's also the reason why 0.25% $MgCl_2$ was chosen for tofu making for the 22 soybean lines. We expect more soybean lines would reach the Japanese

industry requirement if optimal coagulant concentration is measured and applied for tofu making (Yu, Kumari, Zhang, & Chang, 2014).

3.8. Sensory testing of tofu

Sensory evaluation was conducted twice for pressed tofu made from each soybean to test their quality. Among the eight soybean lines, in most cases, there was no significant difference in overall quality (Table 4). Tofu made from MS-15 had a higher overall quality score than that from other soybean lines. MS-12 had a lower overall quality score than the other seven soybean lines. For the firmness, all tofus varied from 8.69 to 12.94 (0-15; 15 means very firm). Compared with commercial extra firm's score of 14, the pressed tofu showed lower firmness but was significantly firmer than the commercial soft tofu (score: 2). The elasticity and firmness scores showed a high correlation ($r = 0.99$, $P < 0.0001$), and varied from 8.63 to 12.78. The elasticity of tofu from the eight soybean lines was also significantly higher than commercial soft tofu and slightly lower than the extra-firm tofu. The smoothness of tofu was a relatively important sensory attribute which is difficult to be tested by instrument. The sensory smoothness of eight tofu products varied from 9.81 to 11.06. The sensory smoothness of commercial soft tofu was 12, while the smoothness of commercial extra firm tofu was 5. Compared with commercial tofu, the pressed tofu from the eight soybeans all showed higher smoothness than commercial extra-firm tofu, and some of the tofu's were as smooth as commercial soft tofu (less than 1 score's different). Off-flavour was also evaluated to determine whether the tofu had objectionable beany odour or other undesirable flavour. The results showed that none of the tofus had an off-flavour. Overall quality of all tofu products made from the 8 lines were high when compared to the commercial products.

4. Conclusions

Eight soybean lines, which were selected from the original 68 lines according to the traits of protein content and composition, seed size and seed appearance quality, were made into tofu products. Results from instrumental texture analysis and sensory evaluation showed they were very suitable for pressed tofu making. Filled tofu making and breaking stress analysis demonstrated a significant correlation of A₃ subunit content and firmness of filled tofu. The results suggested that the soybean A₃ subunit could be used as an effective marker for predicting the firmness of tofu in the soybean breeding program. The phenolic profile and antioxidant activities of yellow soybean were not correlated with the physical quality of seed. This study provided valuable information for the growers and food industries in the selection of soybean lines for food making.

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497 **References**

- 498 AOAC Int. 2012. Official methods of analysis of AOAC International (19th ed).
499 Gaithersburg, MD: Association of Official Analytical Chemists
- 500 Aziadekey, M., Schapaugh, W., & Herald, T. (2002). Genotype by environment
501 interaction for soymilk and tofu quality characteristics. *Journal of food*
502 *quality*, 25(3), 243-259.
- 503 Bhardwaj, H., Bhagsari, A., Joshi, J., Rangappa, M., Sapra, V., & Rao, M. (1999).
504 Yield and quality of soymilk and tofu made from soybean genotypes grown at
505 four locations. *Crop science*, 39(2), 401-405.
- 506 Bourne, M. (2002). Food texture and viscosity: concept and measurement: Academic
507 press.
- 508 Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of
509 microgram quantities of protein utilizing the principle of protein-dye binding.
510 *Analytical biochemistry*, 72(1), 248-254.
- 511 Brar, G. S., & Carter Jr, T. E. (1993). Soybean Glycine max (L.) Merrill. In G. Kalloo
512 and B. O. Bergh (Eds), *Genetic Improvement of Vegetable Crops*, (pp.
513 427-463). New York: Pergamon Press.
- 514 Broadhurst, R. B., & Jones, W. T. (1978). Analysis of condensed tannins using
515 acidified vanillin. *Journal of the Science of Food and Agriculture*, 29(9),
516 788-794.
- 517 Cai, T., & Chang, K.C. (1999). Processing effect on soybean storage proteins and
518 their relationship with tofu quality. *Journal of Agricultural and Food*

519 *Chemistry*, 47(2), 720-727.

520 Cai, T., Chang, K., Shih, M., Hou, H., & Ji, M. (1997). Comparison of bench and
521 production scale methods for making soymilk and tofu from 13 soybean
522 varieties. *Food Research International*, 30(9), 659-668.

523 Chang, S. K. C. (2015). Science and Technology of Tofu Making. In Y. H. Hui and E.
524 O. Evranuz (Eds), *Handbook of Fruits and Vegetable Processing*, 2nd edition
525 (pp. 817-840). Boca Raton, FL: CRC Press.

526 Chen, C. W., & Ho, C. T. (1995). Antioxidant properties of polyphenols extracted
527 from green and black teas. *Journal of food lipids*, 2(1), 35-46.

528 Fukushima, D. (2000). Recent progress in research and technology for processing and
529 utilization of soybeans. In the Organizing Committee for ISPUC. In Proc. 3rd
530 Int. Soybean Processing and Utilization Conference (ISPUC-III). Korin
531 Publishing, Tokyo, (pp. 11-16).

532 Heimler, D., Vignolini, P., Dini, M. G., & Romani, A. (2005). Rapid tests to assess
533 the antioxidant activity of *Phaseolus vulgaris* L. dry beans. *Journal of*
534 *Agriculture and Food Chemistry*, 53(8), 3053-3056.

535 Hou, H., & Chang, K. (2004). Storage conditions affect soybean color, chemical
536 composition and tofu qualities. *Journal of Food Processing and Preservation*,
537 28(6), 473-488.

538 Hill, J.L., Peregrine, E.K., G.L. Sprau, C.R., Nelson, R.L., Kenty, M.M.,
539 Kilen, T.C. & Thomas, D.A. (2001). *Evaluation of the USDA Soybean*
540 *Germplasm Collection: Maturity Groups VI–VIII (FC 03.659–PI 567.235B)*.

541 <http://www.ars.usda.gov/is/np/SoybeanGermplasm/SoyGerm2001.pdf>. p. 6-7

542 Huang, M., Kaletunc, G., St Martin, S., Feller, M., & McHale, L. K. (2014).

543 Correlations of seed traits with tofu texture in 48 soybean cultivars and

544 breeding lines. *Plant Breed*, 133, 67-73.

545 Ji, M., Cai, T., & Chang, K. (1999). Tofu yield and textural properties from three

546 soybean cultivars as affected by ratios of 7S and 11S proteins. *Journal of Food*

547 *Science*, 64(5), 763-767.

548 Kim, Y., & Wicker, L. (2005). Soybean cultivars impact quality and function of

549 soymilk and tofu. *Journal of the Science and Food Agriculture*, 85(15),

550 2514-2518.

551 Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the

552 head of bacteriophage T4. *Nature*, 227(5259), 680-685.

553 Liu, J., Chang, S. K., & Wiesenborn, D. (2005). Antioxidant properties of soybean

554 isoflavone extract and tofu in vitro and in vivo. *Journal of Agricultural and*

555 *Food Chemistry*, 53(6), 2333-2340.

556 Liu, Z.-S., & Chang, K. (2003). Development of a rapid titration method for

557 predicting optimal coagulant concentration for filled tofu. *Journal of*

558 *Agricultural and Food Chemistry*, 51(18), 5214-5221.

559 Murphy, P. A., Chen, H.-P., Hauck, C. C., Wilson, L. A., & Giese, J. (1997). *Soybean*

560 *protein composition and tofu quality. Food Technology*, 51(3), 86-88.

561 Nakamura, T., Utsumi, S., Kitamura, K., Harada, K., & Mori, T. (1984). Cultivar

562 differences in gelling characteristics of soybean glycinin. *Journal of*

563 *Agricultural and Food Chemistry*, 32(3), 647-651.

564 Poysa, V., Woodrow, L., & Yu, K. (2006). Effect of soy protein subunit composition
565 on tofu quality. *Food Research International*, 39(3), 309-317.

566 Poysa, V., & Woodrow, L. (2002). Stability of soybean seed composition and its
567 effect on soymilk and tofu yield and quality. *Food Research International*,
568 35(4), 337-345.

569 Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L.,
570 Hampsch-Woodill, M., Huang, D., Ou, B., & Jacob, R. (2003). Assays for
571 hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance
572 capacity (ORACFL)) of plasma and other biological and food samples.
573 *Journal of Agriculture and Food Chemistry*, 51(11), 3273-3279.

574 Saio, K., & Watanabe, T. (1978). Differences in functional properties of 7S and 11S
575 soybean proteins. *Journal of Texture Studies*, 9(1-2), 135-157.

576 Sidel, J. L., & Stone, H. (1993). The role of sensory evaluation in the food industry.
577 *Food Quality and Preference*, 4(1), 65-73.

578 Singleton, V., & Rossi, J. A. (1965). Colorimetry of total phenolics with
579 phosphomolybdic-phosphotungstic acid reagents. *American journal of*
580 *Enology and Viticulture*, 16(3), 144-158.

581 Skurray, G., Cunich, J., & Carter, O. (1980). The effect of different varieties of
582 soybean and calcium ion concentration on the quality of tofu. *Food Chemistry*,
583 6(2), 89-95.

584 Taira, H. (1990). Quality of soybeans for processed foods in Japan. *Japan*

585 *Agricultural Research Quarterly*, 24, 224-230.

586 Toda, K., Ono, T., Kitamura, K., Hajika, M., Takahashi, K., & Nakamura, Y. (2003).

587 Seed protein content and consistency of tofu prepared with different

588 magnesium chloride concentrations in six Japanese soybean varieties.

589 *Breeding Science*, 53(3), 217-223

590 Wang, C. C. R., & Chang, S. K. C. (1995). Physicochemical properties and tofu

591 quality of soybean cultivar Proto. *Journal of Agricultural and Food Chemistry*,

592 43(12), 3029-3034.

593 Xu, B., & Chang, S. K. (2008). Characterization of phenolic substances and

594 antioxidant properties of food soybeans grown in the North Dakota–

595 Minnesota region. *Journal of Agricultural and Food Chemistry*, 56(19),

596 9102-9113.

597 Xu, B. J., & Chang, S. K. (2007). A comparative study on phenolic profiles and

598 antioxidant activities of legumes as affected by extraction solvents. *Journal of*

599 *Food Science*, 72(2), S159-166.

600 Xu, B. J., Yuan, S. H., & Chang, S. K. (2007). Comparative analyses of phenolic

601 composition, antioxidant capacity, and color of cool season legumes and other

602 selected food legumes. *Journal of Food Science*, 72(2), S167-177.

603 Yu, H. S., Kumari, S., Zhang, Y., Chang, S. K. (2014) Improved Small-Scale

604 Methods for Evaluating Pressed Tofu and Filled Tofu. Abstract of Annual

605 Meeting of Institute of Food Technologists, New Orleans, LA..

606 Yuan, S., & Chang, S. K. (2007). Texture profile of tofu as affected by instron

607 parameters and sample preparation, and correlations of instron firmness and
608 springiness with sensory scores. *Journal of Food Science*, 72(2), S136-145.

609 Yuan, S., & Chang, S. K. (2010). Standardization of physical parameters for instron
610 texture analysis of tofu products. In *ACS symposium series*, vol. 1059 (pp.
611 231-248); American Chemical Society, Washington, DC.

612 Zhang, G., & Chang, K. (1996). Tofu quality as related to soy-bean and soymilk
613 composition. In Abstract of Annual Meeting of Institute of Food
614 Technologists, New Orleans, LA, (pp. 121).

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616 Figure Legend:

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618 Figure 1. SDS-PAGE protein subunits (polypeptides) of β -conglycinin (7S) and
619 glycinin (11S) in 22 soybean lines harvested in Mississippi Stoneville Fall 2014.
620 Gel A's Lane 1-11 are polypeptides in 11 lines of soybeans (MS-01~MS-11),
621 respectively. Gel B's Lane 1-11 are polypeptides in other 11 lines of soybeans
622 (MS-12~MS-22), respectively. Lane M represents protein molecular mass markers
623 (molecular masses are shown beside the markers). A3, Ax (A1a, A2 and A4), and Bx
624 (B1a, B1b, B2, B3 and B4) are subunits of 11S (A, Acidic; B, basic), and α , α' and β
625 are subunits of 7S protein.

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627 Figure 2. Pearson correlation coefficient analysis between A3 subunit content (% total
628 soluble protein) and firmness of filled tofu (N = 22). The maroon colour spots (N = 8)
629 are the samples selected as suitable for pressed tofu making. The green line represents
630 the firmness requirement for filled tofu in Japan (> 90 g force/cm²). The number
631 labeled beside the spot represent a code number (MS-Number). For example, 1
632 represents MS-01.

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1 Table 1. Seed quality, protein profile and firmness of filled tofu of 22 soybean lines (MS-1~MS-22) (Stoneville, Mississippi 2014)

Code No.	Seed quality score	Total protein (%)	11S/7S ratio	7S content (% of TSP*)	11S content (% of TSP)	11S+7S content (% of TSP)	A3 subunit content (% of TSP)	Firmness of filled tofu (gf/cm ²)
MS-01	8	44.0±0.01	1.75±0.18 <i>b</i>	26.38±4.16 <i>bcdefg</i>	46.14±7.28 <i>a</i>	72.51±11.44 <i>abc</i>	3.94±0.25 <i>ab</i>	107.0±6.41 <i>a</i>
MS-02	5	39.2±0.24	1.53±0.10 <i>gf</i>	24.90±1.55 <i>cdefg</i>	38.13±2.37 <i>bd</i>	63.03±3.92 <i>bc</i>	2.12±0.28 <i>k</i>	54.4±2.37 <i>j</i>
MS-03	7	45.1±0.13	1.66±0.16 <i>bcdef</i>	26.91±3.09 <i>bcdef</i>	44.58±5.11 <i>abc</i>	71.50±8.20 <i>abc</i>	4.42±0.10 <i>a</i>	105.3±5.33 <i>a</i>
MS-04	5.5	43.5±0.20	1.53±0.04 <i>fg</i>	26.94±2.78 <i>bcdef</i>	41.30±4.27 <i>abcd</i>	68.24±7.05 <i>abc</i>	3.21±0.14 <i>cdefg</i>	81.6±1.03 <i>d</i>
MS-05	5	44.3±0.09	1.74±0.05 <i>bc</i>	22.85±3.22 <i>fg</i>	39.65±5.58 <i>abcd</i>	62.50±8.79 <i>bc</i>	3.20±0.52 <i>cdefgh</i>	78.2±1.51 <i>def</i>
MS-06	8	43.0±0.04	1.71±0.14 <i>bcd</i>	22.79±3.27 <i>fg</i>	39.05±5.60 <i>abcd</i>	61.84±6.87 <i>c</i>	2.57±0.23 <i>ijk</i>	60.7±0.65 <i>i</i>
MS-07	7	44.5±0.08	1.79±0.06 <i>ab</i>	25.96±3.74 <i>cdefg</i>	46.56±6.71 <i>a</i>	72.52±7.44 <i>abc</i>	3.79±0.09 <i>bc</i>	83.6±1.81 <i>de</i>
MS-08	5	45.6±0.16	1.48±0.15 <i>g</i>	25.84±5.34 <i>cedfg</i>	38.22±7.90 <i>bcd</i>	64.06±9.22 <i>abc</i>	3.65±0.39 <i>bc</i>	84.3±2.15 <i>gh</i>
MS-09	6.5	42.6±0.19	1.58±0.07 <i>defg</i>	25.04±4.42 <i>cdefg</i>	39.55±6.99 <i>abcd</i>	64.59±9.44 <i>abc</i>	3.43±0.10 <i>bcde</i>	75.8±3.26 <i>efg</i>
MS-10	5.5	42.0±0.23	1.59±0.04 <i>defg</i>	23.76±2.16 <i>efg</i>	37.67±3.43 <i>cd</i>	61.43±4.59 <i>c</i>	3.33±0.12 <i>cdef</i>	74.8±1.53 <i>h</i>
MS-11	5.5	44.1±0.30	1.49±0.06 <i>g</i>	24.43±3.93 <i>defg</i>	36.50±4.84 <i>d</i>	60.93±8.77 <i>c</i>	3.68±0.05 <i>bc</i>	85.4±2.55 <i>gh</i>

2 Means within a column followed by different letters are significantly different (P < 0.05). *TSP: total soluble protein.

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7 Table 1 (Continued)

Code No.	Seed quality score	Total protein (%)	11S/7S ratio	7S content (% of TSP*)	11S content (% of TSP)	11S+7S content (% of TSP)	A3 subunit content (% of TSP)	Firmness of filled tofu (gf/cm ²)
MS-12	7.5	43.3±0.09	1.51±0.10 <i>g</i>	28.67±0.62 <i>abcd</i>	43.21±0.93 <i>abcd</i>	71.88±1.55 <i>abc</i>	2.81±0.47 <i>fghij</i>	77.8±0.93 <i>def</i>
MS-13	6.5	42.9±0.13	1.31±0.09 <i>h</i>	32.65±3.48 <i>a</i>	42.88±4.56 <i>abcd</i>	75.53±8.04 <i>a</i>	3.48±0.46 <i>bcde</i>	81.1±6.96 <i>fg</i>
MS-14	7.5	42.1±0.52	1.46±0.04 <i>g</i>	31.08±2.11 <i>ab</i>	45.32±3.07 <i>ab</i>	76.40±5.18 <i>a</i>	3.52±0.66 <i>bcd</i>	90.7±2.82 <i>c</i>
MS-15	7	43.2±0.03	1.60±0.09 <i>cdefg</i>	27.06±0.92 <i>bcdef</i>	43.31±1.48 <i>abcd</i>	70.38±2.40 <i>abc</i>	2.42±0.24 <i>jk</i>	56.3±2.37 <i>j</i>
MS-16	7	45.7±0.27	1.56±0.07 <i>efg</i>	27.25±0.17 <i>bcdef</i>	42.44±0.26 <i>abcd</i>	69.69±0.43 <i>abc</i>	2.66±0.25 <i>ij</i>	98.3±1.55 <i>b</i>
MS-17	4	43.5±0.33	1.92±0.13 <i>a</i>	21.97±1.32 <i>g</i>	42.14±2.54 <i>abcd</i>	64.12±3.86 <i>abc</i>	2.99±0.58 <i>efghi</i>	81.6±0.93 <i>d</i>
MS-18	5	42.2±0.23	1.68±0.11 <i>bcde</i>	25.17±1.40 <i>cdefg</i>	42.35±2.35 <i>abcd</i>	67.52±3.74 <i>abc</i>	2.69±0.36 <i>hij</i>	74.2±0.99 <i>fgh</i>
MS-19	5	42.0±0.05	1.54±0.03 <i>fg</i>	29.39±2.68 <i>abc</i>	45.20±4.12 <i>abc</i>	74.59±6.79 <i>ab</i>	4.23±0.03 <i>a</i>	97.5±2.06 <i>b</i>
MS-20	7	43.1±0.05	1.46±0.11 <i>g</i>	27.95±3.79 <i>abcde</i>	40.88±5.54 <i>abcd</i>	68.83±9.32 <i>abc</i>	2.78±0.10 <i>ghij</i>	79.4±0.18 <i>de</i>
MS-21	6.5	43.0±0.01	1.57±0.10 <i>efg</i>	27.81±3.74 <i>abcde</i>	43.59±5.86 <i>abcd</i>	71.40±9.59 <i>abc</i>	3.70±0.06 <i>bc</i>	82.4±2.77 <i>gh</i>
MS-22	4	41.1±0.23	1.48±0.10 <i>g</i>	26.56±0.33 <i>bcdefg</i>	39.42±0.49 <i>abcd</i>	65.98±0.81 <i>abc</i>	3.05±0.09 <i>defghi</i>	71.9±1.55 <i>gh</i>

8 Means within a column followed by different letters are significantly different (P < 0.05).*TSP: total soluble protein.

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15 Table 2. Twenty-two soybean line's 100-seed weight, moisture content, phenolic profile and antioxidant capacity (MS-01~MS-22) (Stoneville,
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17 Mississippi 2014)

Code No.	100-seed Weight(g)	Moisture Content (%)	TPC*, mg CAE/g	TFC*, mg CAE/g	CTC*, mg CAE/g	ORAC*, μmol TE/g	DPPH*, TE/g
MS-01	22.64±0.59 h	10.0	1.74±0.08 i	1.31±0.01 d	0.59±0.01 de	20.87±1.31 n	2.90±0.02 ijk
MS-02	14.30±0.14 m	7.5	2.59±0.11 a	0.76±0.01 m	0.35±0.02 jk	36.07±0.63 $fghi$	1.95±0.02 n
MS-03	22.75±0.64 h	8.2	2.09±0.12 $cdefgh$	1.24±0.02 ef	0.69±0.02 b	31.00±0.90 kl	3.34±0.4 fgh
MS-04	24.29±0.16 efg	8.2	2.15±0.05 $bcde$	1.24±0.01 e	0.57±0.04 ef	36.06±0.84 $fghi$	2.40±0.25 lm
MS-05	20.67±0.70 i	8.2	2.07±0.03 $cdefgh$	1.06±0.01 jk	0.58±0.02 def	29.72±2.67 l	3.34±0.55 fgh
MS-06	25.21±0.57 $bcde$	7.8	1.75±0.17 i	0.84±0.02 l	0.41±0.03 hi	32.81±0.75 jk	3.76±0.14 edf
MS-07	23.61±0.47 gh	8.5	2.01±0.12 fgh	0.68±0.02 n	0.41±0.02 hi	34.16±0.80 ij	3.55±0.23 efg
MS-08	25.74±0.96 abc	9.2	1.99±0.13 gh	0.87±0.01 l	0.66±0.04 bc	35.44±0.62 ghi	3.04±0.17 hij
MS-09	25.33±0.77 bcd	8.5	1.94±0.05 h	1.04±0.02 k	0.30±0.02 k	27.23±2.39 m	4.24±0.19 bc
MS-10	25.73±0.95 abc	7.7	2.06±0.06 $defgh$	1.08±0.01 ij	0.36±0.04 ij	35.05±1.88 hi	3.11±0.01 hij
MS-11	19.54±1.00 j	9.0	2.03±0.14 $efgh$	1.11±0.01 h	0.44±0.02 h	37.00±0.05 $efgh$	3.99±0.12 cd

18 Data are expressed as means± standard deviations (n=3) on dry weight basis. Means within a column followed by different letters are
19 significantly different (P < 0.05). *TPC: total phenolic content; TFC: total flavonoid content; CTC: condensed tannins content; ORAC: oxygen
20 radical absorbance capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; units/g is on a dry basis.

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25 Table 2 (Continued)

Code No.	100-seed Weight(g)	Moisture Content (%)	TPC*, mg CAE/g	TFC*, mg CAE/g	CTC*, mg CAE/g	ORAC*, μ mol TE/g	DPPH*, TE/g
MS-12	24.20 $\pm$ 0.45efg	7.9	2.17 $\pm$ 0.13bcd	1.20 $\pm$ 0.01g	0.66 $\pm$ 0.04bc	37.26 $\pm$ 0.24defg	3.29 $\pm$ 0.01ghi
MS-13	26.38 $\pm$ 0.08a	8.4	2.04 $\pm$ 0.09efgh	1.21 $\pm$ 0.01fg	0.63 $\pm$ 0.02cd	38.96 $\pm$ 2.19de	3.71 $\pm$ 0.05edf
MS-14	25.63 $\pm$ 0.3abc	7.9	2.20 $\pm$ 0.10bc	1.26 $\pm$ 0.01e	0.59 $\pm$ 0.03de	43.78 $\pm$ 0.68b	2.81 $\pm$ 0.06jkl
MS-15	21.10 $\pm$ 0.54i	9.4	2.13 $\pm$ 0.07bcdef	1.58 $\pm$ 0.02b	0.53 $\pm$ 0.03fg	39.29 $\pm$ 0.65d	2.20 $\pm$ 0.29mn
MS-16	24.52 $\pm$ 0.59defg	8.7	2.25 $\pm$ 0.03b	1.73 $\pm$ 0.01a	0.65 $\pm$ 0.02bc	43.97 $\pm$ 0.63b	4.47 $\pm$ 0.01b
MS-17	15.44 $\pm$ 0.20l	7.9	2.06 $\pm$ 0.04degh	1.03 $\pm$ 0.03k	0.56 $\pm$ 0.07ef	37.70 $\pm$ 1.32def	3.77 $\pm$ 0.20ed
MS-18	17.44 $\pm$ 0.32k	7.6	2.01 $\pm$ 0.05fgh	0.76 $\pm$ 0.02m	0.64 $\pm$ 0.03bc	41.47 $\pm$ 1.24c	2.61 $\pm$ 0.17klm
MS-19	25.96 $\pm$ 1.55ab	10.0	2.13 $\pm$ 0.19bcdef	0.68 $\pm$ 0.04n	0.49 $\pm$ 0.04g	31.58 $\pm$ 1.20kl	5.25 $\pm$ 0.01a
MS-20	24.71 $\pm$ 0.25cdef	7.5	2.18 $\pm$ 0.02bcd	1.46 $\pm$ 0.01c	0.54 $\pm$ 0.02efg	34.88 $\pm$ 1.06hij	4.32 $\pm$ 0.11bc
MS-21	23.83 $\pm$ 0.16fg	7.8	2.10 $\pm$ 0.09cdefg	1.10 $\pm$ 0.02hi	0.63 $\pm$ 0.06cd	36.63 $\pm$ 0.76fgh	4.34 $\pm$ 0.07bc
MS-22	25.66 $\pm$ 0.08abc	11.6	2.55 $\pm$ 0.18a	1.73 $\pm$ 0.03a	0.80 $\pm$ 0.02a	46.29 $\pm$ 1.62a	2.84 $\pm$ 0.18jk

26 Data are expressed as means $\pm$ standard deviations (n=3) on dry weight basis. Means within a column followed by different letters are
 27 significantly different (P < 0.05). *TPC: total phenolic content; TFC: total flavonoid content; CTC: condensed tannins content; ORAC: oxygen
 28 radical absorbance capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; units/g is on a dry basis.

29 **Table 3.** Texture analysis, yield and sensory evaluation of laboratory pressed-tofu made from eight selected soybean lines

Code No.	Texture analysis*			Yield*	Sensory evaluation score**			
	Brittleness (Kg force)	Firmness (Kg force)	Elasticity (%)	(g/ 100 g soybean)	Firmness	Elasticity	Smoothness	Overall quality
MS-01	2.44±0.20ab	8.66±1.35a	12.0±1.09e	253±15b	12.94±1.11a	12.78±1.08a	9.81±0.95c	11.78±1.55bc
MS-06	2.38±0.55ab	5.55±1.73bcde	27.0±2.28cd	274±28ab	10.81±1.83b	11.31±2.02b	10.63±1.04ab	12.53±1.02ab
MS-07	2.58±0.34ab	7.00±1.22abc	30.7±1.89bc	294±19a	9.63±1.74c	9.59±1.88c	11.06±0.83a	11.88±1.77abc
MS-12	3.10±0.34a	7.65±1.99ab	36.0±1.89a	282±25ab	8.69±1.90c	8.63±2.00c	11.00±1.22ab	11.25±1.35c
MS-15	2.12±0.88b	5.27±0.74cde	31.6±4.75abc	272±10ab	11.00±1.46b	11.19±1.41b	11.04±0.68a	12.75±1.20a
MS-16	2.22±0.38b	4.31±0.63ed	33.8±0.77ab	268±18ab	11.09±1.95b	11.28±1.93b	10.65±1.00ab	12.19±1.38abc
MS-17	1.79±0.10b	3.64±0.23e	24.3±2.96d	281±10ab	10.94±1.69b	11.03±1.61b	10.34±1.03bc	12.09±1.40abc
MS-20	3.18±0.80a	5.82±1.73bcd	35.7±5.26a	291±27a	9.19±1.64c	9.59±1.68c	11.06±1.00a	11.81±1.12abc
Soft tofu	-	-	-	-	2	2	12	13
Extra-frim	-	-	-	-	14	14	5	11

30 *Means of three treatment replications. ** All tests were conducted twice on different days for the tofu made from the same line, using the
31 commercial tofu (Soft tofu and Extra-firm tofu shown in the last two rows) as references. Means within a column followed by different letters
32 are significantly different (P < 0.05).

Table 4. Pearson correlation coefficient analysis between the firmness of tofu and 11S and /or 7S protein profile.

Correlation coefficients (r)	Pressed tofu hardness N=8	Filled tofu hardness N=22
Total protein	-	0.56***
11S/7S ratio	-0.14	0.06
A₃ subunit, %	0.63*	0.77****
A_x subunit, %	-	0.146
B_x subunit, %	-	-0.165
11S, %	-	0.52**
7S, %	-	0.32
11S+7S, %	-	0.48**

* Correlation is significant at 0.1 level. **Significant at 0.05 level. ***Significant at 0.01 level. ****Significant at 0.001 level.

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4 Figure 1. SDS-PAGE protein subunits (polypeptides) of β -conglycinin (7S) and
5 glycinin (11S) in 22 soybean lines harvested in Mississippi Stoneville Fall 2014.
6 Gel A's Lane 1-11 are polypeptides in 11 lines of soybeans (MS-01~MS-11)
7 respectively. Gel B's Lane 1-11 are polypeptides in other 11 lines of soybeans
8 (MS-12~MS-22) respectively. Lane M represents protein molecular mass markers
9 (molecular masses are shown beside the markers). A_3 , A_x (A_{1a} , A_2 and A_4), and B_x
10 (B_{1a} , B_{1b} , B_2 , B_3 and B_4) are subunits of 11S (A, Acidic; B, basic), and α , α' and β
11 are subunits of 7S protein.

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15 Figure 2. Pearson correlation coefficient analysis between A3 subunit content (% total
16 soluble protein) and firmness of filled tofu (N = 22). The maroon color spots (N = 8)
17 are the samples we selected as suitable for pressed tofu making. The green line
18 represents the firmness requirement for filled tofu in Japan (> 90 g force/cm²). The
19 Number labeled beside the spot represent a code number (MS-Number). For example,
20 1 represents MS-01.

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