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Research article

Evaluation of seed protein concentration and storage protein profiles in vegetable soybeans with different seed coat colors during seed development

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Abstract

Importance of the work: Vegetable soybean (*Glycine max* (L.) Merr.) is a good source of protein; however, there are not many of its traits related to nutritional value available in the market.

Objective: To develop a new nutritional value of protein in vegetable soybean, using the evaluation of protein concentration in various unique traits such as seed coat.

Materials & Methods: Twelve vegetable soybeans varieties with different seed coat colors, between stages R5 and R6, were evaluated for their seed protein concentration and seed storage protein profiles. The seed coats of the 12 varieties were classified into 3 groups: yellow, green and brown. The seed protein concentration (SPC) was analyzed at 15 d after flowering (DAF), 25 DAF, 35 DAF and 45 DAF and the seed storage protein profiles were analyzed at 35 DAF and 45 DAF. The soybean plants were grown in randomized complete block design plots with three replications and average mean comparisons were calculated using Tukey's honest significant difference.

Results: The variety Koucha, usually harvested around 30–35 DAF, had the highest SPC. For each variety, the 11S/7S subunit ratio was calculated to determine the seed protein profile as a reflection of the seed nutritional value. The varieties with the highest ratios according to seed coat color were: yellow (Chiang Mai 84-2), green (No.9) and brown (Shounai 2 gou).

Main finding: Overall, the SPC values and 11S/7S ratios were not affected by seed coat color, but rather the stage of seed development and also contributed to by genetic background and physiological development.

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Introduction

Vegetable soybeans (*Glycine max* (L.) Merr.) are high in protein and are a reliable source of carbohydrate and fat; they are commonly consumed when the pods have reached 80–90% of development in their reproductive phase (Konovsky et al., 1994). Soybean as a vegetable can be harvested 60–70 d after planting (DAP) and it is widely considered as a valuable cash crop for farmers and as a food source with high nutritional value for consumers (Chan, 1991; Zhang et al., 2017). Soybeans can be consumed either by boiling or steaming their fresh pods and are suitable for health-conscious and vegetarian consumers as they are a plant-based protein source (Rizzo and Baroni, 2018). Vegetable soybeans have high nutritional value; 100 grams of fresh soybeans contain 68% water, 11% protein, 8% carbohydrates, 5% fat, 5% fiber, 2% sugar, and 1% ash and yield 121 kcal of energy (United States Department of Agriculture Agricultural Research Service, 2016). However, in Thailand, only a few varieties are utilized for domestic production and consumption, with the varieties not differing in term of nutritional content. Therefore, increasing the number of varieties is needed to provide consumers with more options, particularly the introduction of distinctive traits, such as fragrance and different color seed coats.

Protein accumulation in soybean seeds is stored as protein body, transported into the Golgi apparatus, and stored in the vacuole within the cells (Lawrence, 1999). The main storage protein are globulins, which consist of 2S, 7S, 11S and 15S. The most common groups are glycinin (11S) and β-conglycinin (7S; Derbyshire et al., 1976; Singh et al., 2015). The 11S protein has a molecular weight in the range 37–44 kDa acidic polypeptide and 17–22 kDa basic polypeptide subunits (Kitamura et al., 1976). The 7S protein contains subunits with a molecular kDa weight of 72 in α, 68 in α’ and 52 in β (Thanh and Shibasaki, 1977). The sulfur-containing amino acids of methionine and cysteine, which are relatively low in plant protein, can be found in 11S protein (glycinin), in a considerably higher amount than in 7S proteins (β-conglycinin; Ogawa et al., 1989; Nakasathien et al., 2000; Kim et al., 2008). Based on these nutritional facts and in support of strategies to promote soybeans as beneficial source of protein, the current study compared the protein concentration and visualized the protein subunits in a wide variety of fresh soybean seeds. The data from this study can be used to evaluate the properties and composition of the seeds to improve the specified qualitative characteristics of soybeans and to develop varieties that enhance benefits to farmer producers and consumers.

Materials and Methods

Planting and plant sample preparation

The 12 varieties included commercial and newly introduced varieties of vegetable soybean: Chiang Mai 84-2 from the Chiang Mai Field Crops Research Center, Thailand; No. 9 from Chiangmai Frozen Foods Public Co., Ltd. Thailand; and Kinpou dadacha, Koucha, Shounai 1 gou, Shounai 2 gou, Shounai 3 gou, Shounai 4 gou and Shounai 5 gou from Miyazaki University, Japan.

The tested vegetable soybeans were classified into three groups, identified by their distinctive seed coat colors: 1) yellow seed coat (AGS292 and Chiang Mai 84-2), 2) green seed coat (No. 9 and No. 75); and 3) brown seed coat (Chamame, Kinpou dadacha, Koucha, Shounai 1 gou, Shounai 2 gou, Shounai 3 gou, Shounai 4 gou and Shounai 5 gou).

The experiment was conducted under greenhouse conditions from March to May 2018. Two seeds of each variety were sown in pots (25 cm diameter), filled with commercial potting soil containing, loamy soil, coconut peat, and rice husk ash, and then later thinned to one plant per pot. The plants were given foundation fertilizer at the time of planting (8-24-24 for nitrogen, phosphorous and potassium, respectively, at the rate of 93.75 kg/ha, fertilizer formula 13-13-21 at 15 DAP at the rate of 156.25 kg/ha and formula 46-0-0 at 35 DAP at the rate of 125 kg/ha. Each pot was watered by hand twice daily 0700 hours and 1700 hours. Water was applied into the pot until soil surface was wet and the residual water drained. Pod samples were collected at 15 d after flowering (DAF), 25 DAF, 35 DAF and 45 DAF. Flowering day was determined when the plants reached 50% of full bloom. Harvesting day was indicated by the day that the plants reached their full seed pod stage (not less than 95% or R6 stage). At the R6 stage, the seed size was nearly maximized and the accumulation of dry weight and nutrients was about the decline (R6.5; Herman, 1985).

Weather data were obtained from the Faculty of Environment, Kasetsart University, Bangkok, Thailand. Over the course of this study, the cumulative rainfall and average daily temperature were 407 mL and 29.04°C, respectively. The maximum and minimum temperatures were 32.82°C and 25.98°C, respectively.

Protein extraction

Fresh seed samples stored at -80°C were crushed using a chilled mortar. The protein was extracted with a buffer of 0.03 M Tris-HCl pH 8.0 and 0.05 M NaN_3 in 1:10 proportions (weight:volume), then the supernatant was separated using a centrifuge for 30 min calibrated at 12,000 revolutions per minute (rpm) and 4°C. Then, the supernatant was used for the analysis of seed protein concentration and seed storage protein profile.

Seed protein concentration and seed storage protein profile analysis

Seed protein concentration was analyzed according to Bradford assay (Robertson et al., 1997). Protein concentration analysis used a spectrophotometer at wavelength 595 nm and the values were compared with bovine serum albumin standard solution. The seed storage protein profiles were analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein supernatant was added with Tris-HCl pH 6.8, 10% SDS, 20% glycerol, 2% β-mercaptoethanol and bromphenol blue in 1:1 (volume:volume) proportions, put in boiling water for 10 min (Nakasathien et al., 2000; Ruangpanit, 2005) and then centrifuged for 60 s at 12,000 rpm. The protein subunits were separated by Gel Documentation NuPAGE™ 4-12% Bis-Tris Gel, thickness 1.0 mm × 12 wells, separated by 100 V. Then, the gel was stained with Coomassie staining solution (0.25% Coomassie Brilliant Blue R-250, 50% methanol and 10% acetic acid). After 2 hr, the stained gel was removed using a destaining solution (50% methanol and 10% acetic acid), with a volume of 200 mL per gel, applied for 6 hr. The gel documentation Omega Lum G and the GeneTools (-File version: 4.02.03) software were used to analyze band intensity and for calculation of the 11S/7S ratio.

Experimental design

The pot experiment was conducted in an open-roofed greenhouse using a randomized complete block design with three replications. Data were subjected to analysis of variance. Mean comparison used Tukey's honest significant difference. The Statistical Tool for Agricultural Research (STAR) 2.0.1 was used to analyze statistical results. The tests were considered significant at $p < 0.05$.

Results and Discussion

Flowering and harvest days

The average number of days to reach 50% of full bloom (flowering days) of the 12 varieties of fresh soybeans could be classified into two groups: early flowering (less than 30 DAP), consisting of Chiang Mai 84-2, No. 9, Chamame, Kinpon dadacha, Shounai 2 gou and Shounai 3 gou; and late flowering: (30 or more DAP), consisting of AGS292, No. 75, Koucha, Shounai 1 gou, Shounai 4 gou and Shounai 5 gou.

The soybean varieties were classified by number of days before they achieved harvest growth (harvesting days): 1) harvested before 66 DAP, AGS292, Chamame, Kinpon dadacha, Koucha, Shounai 2 gou and Shounai 3 gou; and 2) harvested more than 66 DAP, Shounai 1 gou, Chiang Mai 84-2 No. 75 No. 9 Shounai 5 gou and Shounai 4 gou.

Variety Shounai 4 gou had the longest period before reaching harvest time of 76 DAP (Table 1). Pa-oblek et al. (2015) reported that AGS292 and Chiang Mai 84-2 were harvested during 59 and 60 DAP (based on research conducted in December 2018 at the Chainat Field Crops Research Center, Thailand) which was within the same range as the current study. Santana et al. (2012) reported that the whole grain harvest (R6) had lower protein content compared to the harvested grains at maturity (R8) and that the longer the period after flowering to maturity, the higher the SPC that could be gained. However, this should be considered concomitantly with the soybean genetics or varieties, as there are differences depending on the type or maturity group of soybean (Robert, 2001).

Seed protein concentration and seed storage protein profile

This experiment determined the seed protein concentration (SPC) at 15 DAF, 25 DAF, 35 DAF and 45 DAF using Bradford's assay, prior to conducting seed protein profile analysis. Thus, discussion regarding the pattern of SPC along embryonic developmental stages and seed storage protein profiles are based on the results from Bradford's assay.

Table 1 Agricultural characteristics of seed coat and flower color, 50% full bloom and harvesting day of 12 vegetable soybeans

Variety	Seed coat color	Flower color	To 50% full bloom (DAP)	Harvesting (DAP)	Harvesting (DAF to 50% full bloom)
AGS292	Yellow	Purple	32.60±1.14^{b}	63.20±1.30^{cd}	30.60±1.67^{g}
Chiang Mai 84-2	Yellow	Purple	27.00±1.22^{d}	67.00±2.65^{bc}	40.00±1.58^{a}
No. 9	Green	White	28.40±1.14^{d}	68.20±1.92^{b}	39.80±1.10^{ab}
No. 75	Green	White	29.60±1.52^{bcd}	66.80±2.17^{bc}	37.20±1.64^{bc}
Chamame	Brown	White	27.20±0.84^{d}	61.60±1.14^{d}	34.40±0.55^{def}
Kinpon dadacha	Brown	White	29.40±2.07^{cd}	61.60±2.07^{d}	32.20±0.84^{fg}
Koucha	Brown	White	31.60±1.34^{bc}	64.20±1.30^{cd}	32.60±0.55^{dfg}
Shounai 1 gou	Brown	White	29.80±0.84^{bcd}	66.20±1.64^{bc}	36.40±1.14^{cd}
Shounai 2 gou	Brown	White	28.60±1.14^{cd}	64.00±2.55^{cd}	35.40±2.30^{cd}
Shounai 3 gou	Brown	White	28.80±1.30^{cd}	63.80±2.05^{cd}	35.00±1.22^{cde}
Shounai 4 gou	Brown	White	36.40±2.30^{a}	76.20±1.92^{a}	39.80±0.84^{ab}
Shounai 5 gou	Brown	Purple	31.60±1.52^{bc}	68.20±1.30^{b}	36.60±1.52^{cd}
F-test	-	-	**	**	**
Mean ± SD	-	-	30.08±2.63	65.92±3.98	35.83±3.10
Coefficient of variation (%)	-	-	4.58	2.71	3.44

DAF = days after flowering; DAP = days after planting

** = highly significant difference at $p < 0.01$; values (mean ± SD) within the same column superscripted with different lowercase letters are highly significantly ($p < 0.01$) different.

From Table 2, the SPC of 12 varieties determined at 15 DAF, 25 DAF, 35 DAF and 45 DAF could be grouped into three patterns. In pattern A, the SPC of four varieties (AGS292, Chamame, Kinpon dadacha and Shounai 2 gou) demonstrated an increasing linear trend up to stage R6 (Fig. 1A). Observed values were in the ranges 20.87–35.96 mg/g fresh weight (FW) at 15 DAF and 74.95–84.97 mg/g FW at 45 DAF. At 45 DAF, there were no significant differences among the varieties within

Table 2 Protein concentration in 12 fresh soybean seeds at different days after flowering (DAF)

Variety	Protein concentration (mg/g fresh weight) 15 DAF	25 DAF	35 DAF	45 DAF
Group 1				
AGS292	35.96±1.47^{abc}	49.01±7.06^{abc}	68.13±11.73^{ab}	84.97±2.04^{a}
Chamame	23.39±3.58^{bcde}	47.50±9.08^{abc}	64.82±17.59^{abc}	83.54±4.25^{a}
Kinpon dadacha	23.19±3.11^{cde}	42.90±4.03^{abc}	64.80±17.70^{abc}	74.95±5.47^{a}
Shounai 2 gou	20.87±1.59^{de}	41.59±9.34^{abc}	64.92± 6.14^{abc}	80.22±2.71^{a}
Group 2				
No. 75	39.19±2.68^{a}	42.41±2.38^{abc}	76.44±5.81^{ab}	77.07±2.17^{a}
Koucha	15.29±7.13^{e}	44.06±8.82^{abc}	84.01±3.44^{a}	84.58±8.63^{a}
Shounai 1 gou	15.77±5.97^{e}	37.42±4.86^{bc}	75.36±6.81^{ab}	78.82±5.27^{a}
Group 3				
Chiang Mai 84-2	36.12±3.51^{abc}	61.41±9.52^{ab}	67.19±5.14^{abc}	85.00±2.57^{a}
No. 9	36.48±6.48^{ab}	64.17±6.36^{a}	67.13±4.11^{abc}	76.25±7.57^{a}
Shounai 3 gou	20.69±0.55^{de}	46.68±7.94^{abc}	57.22±10.37^{abc}	80.04±5.03^{a}
Shounai 4 gou	29.12±6.21^{abcd}	35.96±4.06^{c}	39.98±1.61^{c}	58.47±8.08^{b}
Shounai 5 gou	22.08±3.99^{de}	44.06±16.69^{abc}	55.08±9.71^{bc}	76.90±2.83^{a}
F-test	**	**	**	**
Mean ± SD	26.51±2.16	46.43±3.75	65.43±5.26	78.41±2.37
Coefficient of variation (%)	16.71	17.58	14.10	6.87

** = highly significant difference at $p < 0.01$; values (mean ± SD) within the same column superscripted with different lowercase letters are highly significantly ($p < 0.01$) different.

this pattern in SPC; however the average SPC determined in the three patterns differed: pattern A (80.92 mg/g FW SPC), pattern B (80.16 mg/g FW SPC) and C (75.33 mg/g FW SPC).

The varieties grouped in pattern A, based on a fitted straight line, showed significant differences in SPC at 15 DAF between AGS292 (35.96 mg/g FW) and Shounai 2 gou (20.87 mg/g FW), but there were no significant differences in SPC at 25 DAF (mean 45.25 mg/g FW), 35 DAF (mean 64.85 mg/g FW) and 45 DAF (mean 80.92 mg/g FW).

Three soybean varieties were grouped in pattern B: No.75, Koucha and Shounai 1 gou. As illustrated by the fitted polynomial curve in Fig. 1B, the SPC of these varieties slowly increased during 15–25 DAF and then had sharp incremental changes during 25–35 DAF. After 35 DAF, the SPC increased but at a diminishing rate until it reached its maximum during 35–45 DAF, before declining. However, the actual SPC of the grouped soybean varieties in pattern B during 35–45 DAF different only slightly from the predicted polynomial values (Table 2), indicating that the polynomial curve fitted well to predict the average SPC between 15 DAF and 35 DAF. The SPC values of the varieties in this pattern were in the ranges 15.29–39.19 mg/g FW (15 DAF) and 77.07–84.58 mg/g FW (45 DAF). At 15 DAF, No.75 had the greatest significant difference (156.99% SPC compared to the other two varieties (average SPC 15.53 mg/g FW). There were no significant differences in SPC among the pattern B varieties at 25 DAF, 35 DAF and 45 DAF.

The soybean varieties grouped in pattern C (Fig. 1C) were late development varieties. A fitted polynomial curve satisfactorily depicted the increase in the mean SPC during 15–45 DAF whereas the averaged SPC values during both 15–25 DAF and 35–45 DAF increased more than during 25–35 DAF. The five soybean varieties in this group were Chiang Mai 84-2, No. 9, Shounai 3 gou, Shounai 4 gou and Shounai 5 gou. These varieties had SPC values in the ranges 20.69–36.48 mg/g FW (15 DAF) and 58.47–85.00 mg/g FW (45 DAF). At 15 DAF, the SPC values of Chiang Mai 84-2, No.9 and Shonai 4 gou were significantly higher than for Shounai 3 gou and Shounai 5 gou. However, at 25 DAF, the SPC for Shoinai 4 gou was significantly lower than for Chiang Mai 84-2 and No.9. At 35 DAF, there were no significant differences in the SPC among the five varieties, with the averaged SPC being 61.66 mg/g FW. At 45 DAF, Shounai 4 gou had a significantly lower SPC (26.49%) than the other varieties, but there were no significant differences among the others that had an average SPC of 79.55%. The lowest SPC was for Shounai 4 gou, at 25 DAF, 35 DAF and 45 DAF.

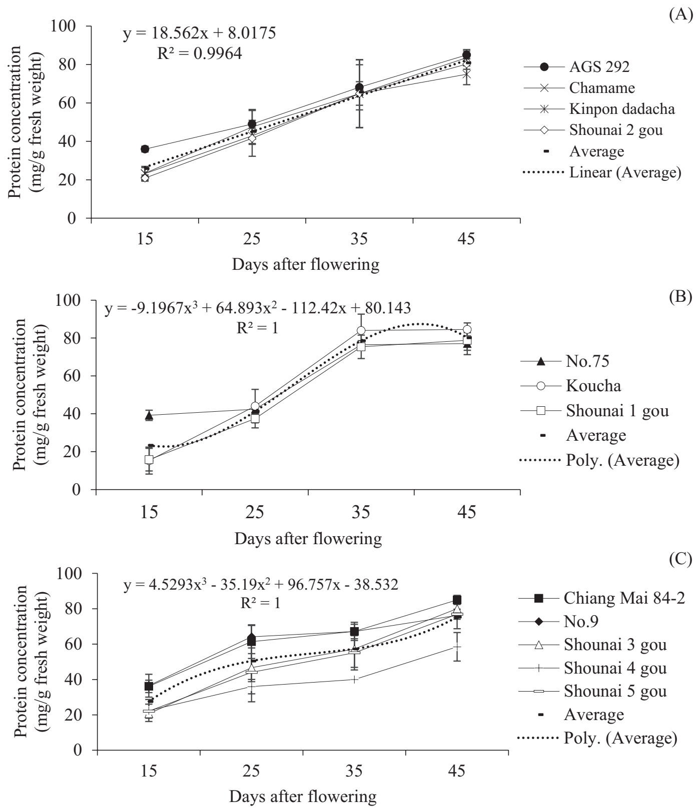

Fig. 1 Protein concentration curves of seeds at different days after flowering: (A) pattern A (AGS292, Chamame, Kinpou dadacha and Shounai 2 gou); (B) pattern B (No.75, Koucha and Shounai 1 gou); (C) pattern C (Chiang Mai 84-2, No.9, Shounai 3 gou, Shounai 4 gou and Shounai 5 gou), where values are mean ± SD and R^2 = coefficient of determination

Generally, vegetable soybean can be harvested around 35 DAF; in this study, Shounai 4 gou had a lower SPC (41.30%) than the average, being lower than for Koucha (having the highest SPC) by 52.41%. Collectively, the protein concentration analysis indicated that there were significant differences in SPC among soybean varieties. These findings were consistent with Herman (2014), who noted that protein biosynthesis and the protein composition of seeds were controlled by genetics and physiological processes. In addition, nitrogen fixation and nitrate assimilation are important physiological processes involved in protein biosynthesis and deposition, particularly during the flowering stage (R2) until the whole kernel (R6) phase (Fabre and Planchon, 2000).

Based on the fitted curve analysis, three curves were obtained (linear pattern A, polynomial pattern B, and polynomial pattern C). The polynomial patterns in B and C indicated that the soybean varieties with these patterns accumulated SPC at an uneven rate during their seed development. It appeared that the carbon-skeleton compound in these varieties may be utilized for the other chemical compound synthesis during seed development (Saldivar et al., 2011) rather being solely for seed storage protein accumulation. For crop production, soybean varieties with pattern B that had an unaltered SPC during 35–45 DAF may be harvested early. Besides the soybean varieties with B and C patterns, soybean varieties having linear pattern A had a certain rate of protein accumulation during the seed-filling stage, which may be beneficial in maintaining vegetable soybean quality.

The seed storage protein profiles at 35 DAF and 45 DAF were separated using SDS-PAGE and the patterns of the protein subunits of each variety are shown in Fig. 2. The acidic and basic subunits were in the ranges 33–38 kDa and 17–20 kDa, respectively, while the 7S proteins had subunits in the ranges α’ (72–75 kDa), α (66–69 kDa) and β (54–47 kDa, as shown in Fig. 2. According to Ogawa et al. (1989); Nakasathien et al. (2000); and Kim et al. (2008), the calculations of 11S/7S proteins reflect the nutritional value of seed storage proteins. From the current analysis, the mean subunit ratios of the 11S/7S protein were at 35 DAF were in the range 0.65–1.41 which were not significantly different and at 45 DAF were in the range 0.81–1.81. At 45 DAF, Chiang Mai 84-2 had the highest 7S/11S value which differed significantly from Shounai 5 gou (Table 3). The varieties with the highest 11S/7S ratio, classified by seed coat color were: yellow seed coat (Chiang Mai 84-2) ratio1.81; green seed coat (No. 9) ratio 1.62; and brown seed coat (Shounai 2 gou) ratio 1.73.

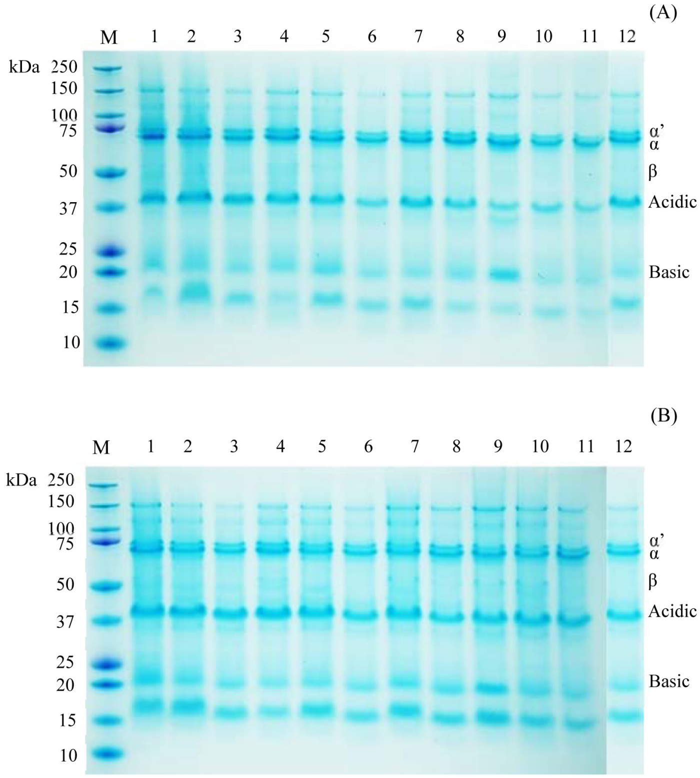

Fig. 2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein subunit separation of 12 fresh soybean seeds at seed stages: (A) 35 days after flowering (DAF); (B) 45 DA, where 1 = AGS292, 2 = CM 84-2, 3 = No. 9, 4 = No.75, 5 = Chamame, 6 = Kinpon dadacha, 7 = Koucha, 8 = Shounai 1 gou, 9 = Shounai 2 gou, 10 = Shounai 3 gou, 11 = Shounai 4 gou and 12 = Shounai 5 gou.

Table 3 Ratio of 11S to 7S protein subunits (11S/7S) of 12 fresh soybeans at 35 days after flowering (DAF) and 45 DAF

Variety	At 35 DAF	Variety	At 45DAF
	11S/7S		11S/7S
Shounai 5 gou	0.65 ± 0.22	Shounai 5 gou	0.81 ± 0.37^{b}
AGS292	0.71 ± 0.36	No. 75	0.96 ± 0.13^{ab}
No. 75	0.76 ± 0.16	Shounai 1 gou	0.98 ± 0.23^{ab}
Shounai 4 gou	0.85 ± 0.63	AGS292	1.09 ± 0.45^{ab}
Shounai 2 gou	0.92 ± 0.17	Shounai 4 gou	1.22 ± 0.54^{ab}
Kinpon dadacha	0.95 ± 0.70	Shounai 3 gou	1.49 ± 0.20^{ab}
Koucha	1.01 ± 0.75	Koucha	1.52 ± 0.45^{ab}
Shounai 3 gou	1.02 ± 0.31	Chamame	1.55 ± 0.69^{ab}
Chamame	1.13 ± 0.46	Kinpon dadacha	1.59 ± 0.43^{ab}
Shounai 1 gou	1.14 ± 0.47	No. 9	1.62 ± 0.57^{ab}
Chaing Mai 84-2	1.17 ± 0.23	Shounai 2 gou	1.73 ± 0.82^{ab}
No. 9	1.41 ± 0.51	Chaing Mai 84-2	1.81 ± 0.82^{a}
F-test	ns	*F*-test	*
Mean ± SD	0.98 ± 0.22	Mean ± SD	1.35 ± 0.32
CV (%)	27.28	CV (%)	17.86

ns = non-significant difference; * = significant difference at $p < 0.05$; values (mean ± SD) within the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Even though statistical analysis revealed no significant differences in the 11S/7S ratios among the soybean varieties at 35 DAF and found only one pair significantly different (Shounai 5 gou and Chiang Mai 84-2) at 45 DAF, the ratios of the 11S/7S protein subunits of 12 soybean varieties could be grouped into low and high 11S/7S ratios to help in the evaluation of their nutritional value. The 11S/7S ratios obtained from the 12 soybean varieties at 35 DAF and 45 DAF could be grouped into: 1) low-moderate (11S/7S ratio < 1); and 2) high values (11S/7S ratio ≥ 1). At 35 DAF, the varieties with low-moderate 11S/7S were: Shounai 5 gou, AGS292, No. 75, Shounai 4 gou, Shounai 2 gou and Kinpon dadacha; the varieties with high 11S/7S ratios were: Koucha, Shounai 3 gou, Chamame, Shounai 1 gou, Chaing Mai 84-2 and No. 9. At 45 DAF, the low-moderate 11S/7S varieties were: Shounai 5 gou, No. 75 and Shounai 1 gou; the varieties with high 11S/7S ratios were: AGS292, Shounai 4 gou, Shounai 3 gou, Koucha, Chamame, Kinpon dadacha, No. 9, Shounai 2 gou and Chaing Mai 84-2. The varieties Shounai 4 gou, Shounai 2 gou and Kinpon dadacha had lower 11S/7S ratios at 35 DAF but could increase the deposition of subunits in the proportion that reflected a higher 11S/7S ratio, similar to the high 11S/7S value group.

The protein subunit deposition at 45 DAF was of particular interest because it is at the harvest stage (R6) suitable for vegetable soybeans. The vegetable soybeans with a high 11S/7S ratio at 45 DAF are likely to have high nutritional value and consequently are good candidates for further development. This was in agreement with the findings by Hill and Breidenbach (1974), who explained that the 11S protein usually contains sulfur-containing amino acids, such as cysteine and methionine at higher levels than in the 7S protein. Thus, the greater the 11S/7S ratio, the more nutritional value that can be obtained.

In conclusion, the Chiang Mai 84-2 variety had the shortest mean time to flowering (27 DAP), while Shounai 4 gou had the longest (36 DAP). The average harvesting time for all 12 varieties was 66 DAP (or 36 DAF). The development of SPC could be represented graphically using three patterns based on fitting linear or polynomial curves, with diminishing rate and increasing rate, respectively. Koucha (brown seed coat) had an early harvesting time (approximately 35 DAF) and was in the group with the highest SPC at that stage. AGS292 (seed coat yellow) and No. 9 (seed coat green) were also in the group with the highest SPC. Pattern B showed an unchanged SPC during 35–45 DAF, indicating that soybean varieties with this pattern may be harvested early. At 35 DAF, there were no significant differences among the varieties in their 11S/7S ratios, which can be used as a nutritional index; however, the 11S/7S ratios at 45 DAF were significantly different. The seed coat colors of the varieties with the greatest 11S/7S ratios were: yellow (Chiang Mai 84-2), green (No.9) and brown (Shounai 2 gou). Overall, the SPC and 11S/7S ratios were not significantly related to seed coat color; however, this might be attributed to the stage of seed development, which was influenced by genetic background and physiological development.

Understanding SPC accumulation, SPC patterns and 11S/7S ratios is helpful in assessing high-protein and high-nutritional soybean cultivars, as well as for selecting cultivars to be used for further breeding. In addition, the data obtained from this study can be used in guidelines for the further improvement of vegetable soybean varieties to improve their nutritional characteristics or to provide more diversity to consumers.

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