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RESEARCH ARTICLE

Seed nutritional quality comparison of vegetable soybean genotypes at fresh pod and mature stage

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ABSTRACT

Vegetable soybean is famous for its better eating quality and taste, which is usually harvested at fresh pod stage. However, no report is available on nutritional values between vegetable soybean seed at fresh pod stage and mature stage. To better understand the seed nutritional quality differences between fresh pod stage and mature stage in vegetable soybean, five vegetable soybean genotypes were examined. The results found that seeds from fresh edible stage had higher total free amino acid, and higher K, Na, Mn and Zn concentrations. The concentrations of soluble sugar, crude oil as well as unsaturated fatty acid were also higher at fresh pod stage. While total isoflavone, Mg and Fe concentrations were generally higher at full maturity stage. No differences in protein concentration were found between the two stages. Significant genotypic differences were found among nutritional parameters. The genotype Line 61 had the highest total soy isoflavone of 4593 μg g^{-1}, whereas the genotype "Heidou" had the lowest total soy isoflavone of 1700 μg g^{-1} at mature stage. Correlation analysis indicated that total free amino acid was significantly positively correlated with soluble sugar, crude oil and total isoflavone. Therefore, the nutritional values at fresh pod stage and mature stage differed from the perspective of nutritional compositions. The findings reported here add new knowledge to vegetable soybean function and is a useful starting point for future breeding program and cultivation towards improving the nutritional compositions of soybean species.

Keywords: Vegetable soybean; Isoflavone; Fatty acid; Free amino acid

INTRODUCTION

Based on the different end uses, soybean can be classified into food bean and oil bean. In order to add value to the soybean crop, soybean breeders are developing specialty soybeans with desired quality attributes for specific applications. The biochemical composition of soybean seed directly affects the health of human beings and livestock (Cober and Voldeng, 2000; Ebert et al., 2017). Soybean seed is different from other legume grains by their higher protein content (above 40% of the dry mass), and it has been treated as an important source of high-quality vegetable protein (Wolf, 1969). Because of the presence of many beneficial secondary metabolites (Messina et al., 1991), soybean is also regarded as a functional food.

Soy isoflavones are becoming of increasing interest as nutritional agents. They have pharmacological activities of antioxidant, cancer-preventive, and anticarcinogenic activities (Adlercreutz, 2002). Isoflavones can be divided into four groups, including aglycons, glucosides, malonylglucosides and acetylglucosides (Kim et al., 2005). The synthesis of isoflavones is executed via the phenylpropanoid pathway through the concerted activities of chalcone synthase (CHS) and isoflavone synthase (IFS) (Dixon, 1995; Wang et al., 2016). Because they are extracted from plants, and have a similar structure with estrogen, soy isoflavones are also called phytoestrogens.

Amino acids composition is vital to protein quality in grain seed. Plants protein mixtures can serve as a complete and balanced source of amino acids to meet human

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physiological needs (Young & Pellett, 1994). Free amino acids also play roles on food taste (Kato et al., 1989). Soybean free amino acids are widely present in soybean tissue cells. Free amino acids have some sweetness, bitterness, sourness, saltiness and umami, and are important taste substances in foods (Kato, 1989).

The major fat of soybean is mainly composed of glycerol and fatty acids, wherein the fatty acid is the main component of the total fat. Soybean seed oil is generally made up of five major fatty acids, palmitic, stearic, oleic, linoleic and α-linolenic acids (Brown et al., 1962). Oleic acid rich foods may have beneficial health effects in human bodies by decreasing low-density lipoprotein (LDL) levels in blood, suppressing tumorigenesis, reducing blood pressure (Lopez-Huertas, 2010; Zengin et al., 2015) and relieving inflammatory diseases. Additionally, high oleic soybean varieties developed for sprout production could also increase valuable health benefits to sprouts (Dhakal, 2014). Traditional soybean is used to squeeze oil or made as soy foods more frequently. Furthermore, with the increasing demand for healthy life living, high oleic acid and low linolenic acid is desirable (Wilson, 2004; Mostafa et al., 2013).

Mineral or trace elements play important roles not only on plant, but human and animal life participating in various metabolic processes such as enzyme catalysis, in the synthesis of proteins and cell growth (Swaine, 2000). However, if the concentration of trace elements exceeds the required level, they may have negative effects on living systems (Zengin, 2005).

With larger seed, higher soluble sugar content, lower oil percentage and higher protein content, vegetable soybean is increasingly popular. Edible quality of vegetable soybean was higher at fresh pod stage with higher soluble sugar concentration and better waxy (Tsou & Hong, 1991, Zhang et al., 2017). In order to add value to the soybean crop, soybean breeders are developing specialty soybeans with desired quality attributes for specific applications (Zhang et al., 2017). The seed size of matured vegetable soybean was generally larger than commercial soybeans, which could also be used as a special soybean types for soy food production. However, few studies assessed the nutritional value of vegetable soybean between fresh pod stage and mature stage. Previous studies only focused on the nutrition composition of fresh vegetable soybean seeds (Primomo et al., 2002), and no comprehensive research has been carried out to assess if fresh seeds are more nutritious. In this study, a field experiment was conducted and five vegetable soybean genotypes were used to evaluate the nutritional compositions of fresh and matured seeds including protein, soluble sugar, oil, fatty acid, free amino acid, isoflavone and mineral nutrients. We intended to add new knowledge to vegetable soybean function and provide potential starting point for future breeding program and cultivation towards improving the nutritional compositions of soybean species.

MATERIALS AND METHODS

Field conditions and management

Field experiments were conducted in 2014 at the Agronomy Farm of Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin (45°732 N, 126°612 E, and altitude 128 m). Five vegetable soybean genotypes with similar maturity were selected (Table 1). Daily maximum and minimum temperature from May to September covering the whole growth stages of vegetable soybean at Harbin in 2014 is showed in Fig. 1. Genotypes were planted in a randomized complete block design with three replications. Each plot consisted of five rows with 0.65 m spacing and 5.0 m length and the rate of planted seeds were 280,000 ha^{-1}. The soil was a typical Mollisol (Black soil) with 29.0 g kg^{-1} organic matter content, 2.3 g kg^{-1} total N content, 148 mg kg^{-1} available N, 48.1 mg kg^{-1} available P and 74.5 mg kg^{-1} available K. A uniform fertilizer application of 150 kg ha^{-1} diammonium phosphate, 20 kg ha^{-1} urea and 120 kg ha^{-1} potassium sulphate at seeding was applied. Appropriate pesticides were used to control weeds, diseases, and insects. All genotypes were sown on 1 May 2014 and harvested at fresh edible stage (the whole plant is usually pulled when a majority of the pods are well filled but before the pods turn yellow) and full maturity stage (Takeda & Sakuoka, 1997) which was about R6 and R8 stage according to identification of Fehr & Caviness (1977). Harvested samples were dried at 105°C in a forced-air oven for 0.5h immediately and then dried to a constant weight at 65°C.

Analysis for protein, free amino acids, fatty acids, isoflavnoes

The crude protein of vegetable soybean was determined using the method of combustion nitrogen analysis by Elementar-Vario (Elementar Analysensysteme GmbH E-III, Germany) (Li, 2012). Crude protein concentration = 6.25× total nitrogen concentration (Saldivar et al., 2011). Oil concentration was determined using the Soxhlet extractor method (Li, 2012). The free amino acid was determined by reverse-phase high-performance liquid chromatography (RP-HPLC), and fatty acid analyses were performed by gas chromatography (GC) equipped with a flame ionization detector. The protocol was described by Qin et al. (2014). Isoflavone concentration was determined using the HPLC analyses by Hoeck et al. (2000). Preparation of soybean seeds for HPLC analysis was described by Kim et al. (2007).

Table 1: Characteristics of selected five vegetable soybean genotypes

	Heidou	Zhong 09	Line 6	Line 61	Zhong 05
	sub-indeterminate	sub-indeterminate	sub-indeterminate	sub-indeterminate	sub-indeterminate
Seed coat color	Black	Yellow	Yellow	Green	Yellow
Fresh edible stage					
Harvest time (days after flowering)	49	49	47	47	49
100-seed weight (g)	46.3	58.4	49.8	53.3	54.2
Full maturity stage					
Moisture (%)	72.2	70.5	71.6	71.1	70.2
Harvest time (days after flowering)	73	74	72	73	74
100-seed weight (g)	30.9	35.6	31.5	31.3	33.8
Moisture (%)	8.5	9.4	8.6	8.3	9.2

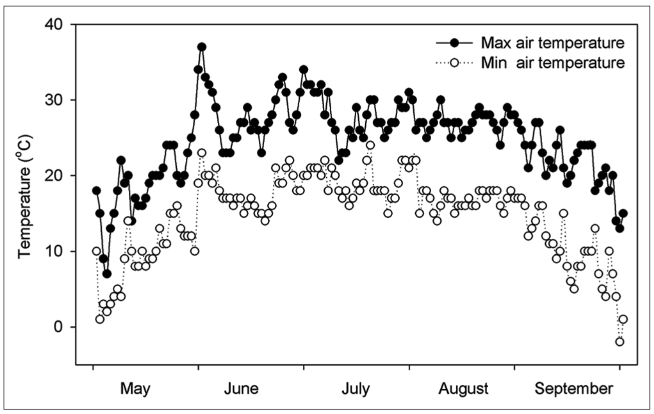

Fig 1. Daily maximum and minimum temperature of May, June, July, August, and September covering the whole growth stages of vegetable soybean at Harbin in 2014.

Soluble sugar and sucrose concentration

For sugar extraction, 0.5 g of dried seed was extracted by 4 mL 80% ethanol with a 10 mL centrifuge tube in 80°C water bath for 40 minutes and was homogenized on a vortex for 10 minutes. Then the mixture was centrifuged at 4500×g for 3 minutes and supernatant was removed to a 50 mL volumetric flask with 80% ethanol to dilute to the 50 mL volume. The supernatant was used to test total soluble sugar concentration and sucrose concentration. The total soluble sugar concentration test was described by Wang in 2002. The concentration of sucrose was analyzed by resorcinol hydrochloric acid method (Li, 2012) where 1 mL of 1% resorcinol and 3 mL of 30% HCl was added in the tube to be incubated at 80°C for 10 minutes and then measured at 480 nm.

Trace elements measurement

Mineral nutrients (P, K, S, Ca and Mg) and trace elements (Fe, Na and Zn) were extracted by wet oxidation with concentrated HNO_3 under pressure in a microwave digester. Analysis of mineral nutrients and Fe in the extracts thus obtained was performed by ICP-OES (inductively coupled plasma–optical emission spectrometry). Analysis of Zn was performed by ICP-MS (inductively coupled plasma-mass spectrometry) (Madejón et al., 2003).

RESULTS

Comparison of soluble sugar and sucrose concentration

Soluble sugar concentration is an important factor assessing the eating quality of vegetable soybean which is usually eaten at fresh stage with green pods. The present study revealed that soluble sugar concentration was significantly higher at full maturity stage than that of the fresh edible stage over the five genotypes (Fig. 2a). The highest soluble sugar concentration of 120 mg g^{-1} was found in genotype Zhong 09, while at fresh edible stage, the highest soluble sugar concentration of 90 mg g^{-1} was found in Zhong 05. Over the five vegetable soybean genotypes, soluble sugar concentration increased 31.7%~94.4% from fresh edible stage to full maturity stage.

Similar to soluble sugar concentration of the five vegetable soybean genotypes, the sucrose concentration also had an increasing trend from fresh edible stage to full maturity stage. Fig. 2b showed that the highest sucrose concentration of 99 mg g^{-1} was found in Zhong 05 at full maturity stage. At the same stage, the lowest sucrose concentration of 76 mg g^{-1} was found in Heidou. Comparing the sucrose concentration of vegetable soybean from fresh edible stage to full maturity stage, sucrose concentration increased from 55.6% to 70.5%.

At fresh edible stage in genotypes Heidou, Zhong 09, Line 6, Line 61 and Zhong 05, sucrose concentration accounted for 85.1%, 79.1%, 91.1%, 73.4% and 64.8% of the total soluble sugar respectively. As for full maturity stage, sucrose concentration accounted for 75.9%, 73.4%, 73.3%, 84.2% and 83.9% respectively.

Comparison of protein and free amino acid concentration

The accumulation of protein concentration over the five genotypes at fresh edible stage and full maturity stages is shown in Fig. 2c. Protein concentrations were around 34.6~43.9% at fresh edible stage and 38.4~42.5% at full maturity stage. The highest protein concentration was 43.9% at fresh edible stage in Heidou. Seed protein accumulation of the five genotypes differed between fresh edible stage and full maturity stage. For genotypes Heidou and Line 6, protein concentration was higher at fresh edible stage, and protein concentration of Line 6 was significantly higher in particular. But for genotypes Zhong 09, Line 61 and Zhong 05, protein concentrations were higher at full maturity stage and significant differences were found between Line 61 and Zhong 05.

Table 2 compared the 16 free amino acid concentrations of five soybean genotypes at fresh edible stage and full maturity

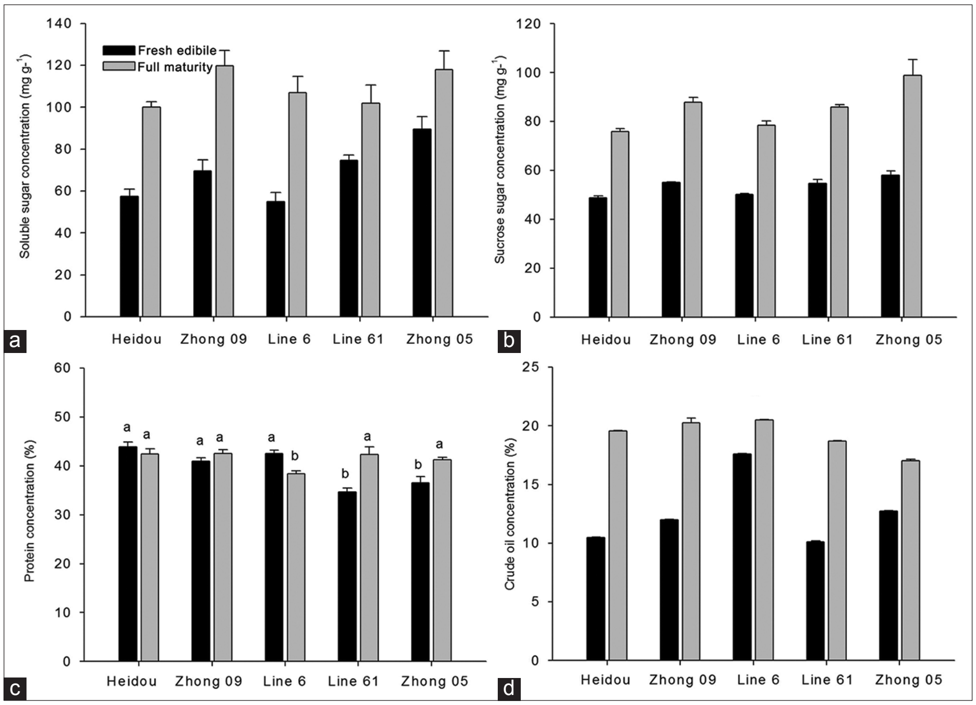

Fig 2. Comparison of soluble sugar (a), sucrose (b), protein (c) and crude oil (d) concentrations of five vegetable soybean genotypes between fresh edible and full maturity stage.

Table 2: Comparison of 16 free amino acids concentration of five soybean genotypes between fresh edible (FE) stage and full maturity (FM) stage

Free Amino Acids	Heidou ($mg\ g^{-1}$)		Zhong 09 ($mg\ g^{-1}$)		Line 6 ($mg\ g^{-1}$)		Line 61 ($mg\ g^{-1}$)		Zhong 05 ($mg\ g^{-1}$)		LSD0.05	Genotype	Stage	Genotype× Stage
	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM				
Aspartic Acid	14.5	5.3*	5.7	7.3*	6.5	5.9*	6.3	6.1*	15.3	8.2*	0.21	<0.001	<0.001	<0.001
Threonine	4.5	3.1*	1.6	2.6*	3.1	2.4*	2.4	3.0*	3.7	2.8*	0.23	<0.001	0.009	<0.001
Serine	17.0	8.3*	10.1	2.9*	19.2	1.2*	13.0	0.9*	21.8	7.8*	0.26	<0.001	<0.001	<0.001
Glutamic Acid	15.1	4.2*	20.3	6.6*	17.5	3.6*	12.9	3.4*	25.3	7.5*	0.31	<0.001	<0.001	<0.001
Proline	1.3	0.1*	0.5	0.1*	0.9	0.1*	0.9	0.1*	0.5	0.1*	0.19	<0.001	<0.001	<0.001
Glycine	2.7	0.5*	1.7	0.7*	3.0	0.3*	1.1	0.3*	1.6	1.2*	0.27	<0.001	<0.001	<0.001
Alanine	19.7	1.8*	14.1	1.3*	27.1	0.6*	6.5	0.6*	15.4	4.6*	0.24	<0.001	<0.001	<0.001
Valine	2.5	0.4*	2.1	0.6*	2.2	0.4*	1.9	0.4*	2.8	0.5*	0.26	<0.001	<0.001	<0.001
Methionine	0.2	0.1*	0.1	0.1^{ns}	0.2	0.1^{ns}	0.1	0.1^{ns}	0.2	0.2^{ns}	0.19	0.255	0.845	0.743
Isoleucine	0.8	0.3*	0.9	0.2*	0.9	0.2*	0.8	0.2*	1.3	0.4*	0.24	0.082	0.750	<0.001
Leucine	2.2	0.3*	1.2	0.1*	1.4	0.2*	1.5	0.1*	2.3	0.1*	0.20	<0.001	<0.001	<0.001
Tyrosine	1.5	0.1*	1.5	0.1*	1.1	0.2*	1.2	0.2*	1.3	0.2*	0.20	0.023	<0.001	<0.001
Phenylalanine	9.0	0.2*	0.6	0.3*	0.4	0.5^{ns}	5.3	0.2*	10.0	0.3*	0.26	<0.001	<0.001	<0.001
Histidine	1.1	2.6^{ns}	1.6	1.3^{ns}	3.3	0.4*	7.0	0.2*	7.6	2.9*	0.19	<0.001	<0.001	<0.001
Lysine	2.4	0.5*	1.7	0.5*	2.3	6.2*	1.6	0.3*	0.5	0.5ns	0.25	<0.001	<0.001	<0.001
Arginine	34.7	29.1*	10.2	15.3*	20.7	22.2*	11.8	8.1*	8.6	35.5*	0.27	<0.001	<0.001	<0.001
Total	129.2	56.9*	74.0	40.0*	109.9	44.4*	74.3	24.3*	118.2	72.7*	1.00	<0.001	<0.001	<0.001

* and ns indicate significant and non-significant difference (t-test) between fresh edible stage and full maturity, respectively, for individual genotypes. LSD (Least Significant Difference) values correspond to the genotype × stage interaction (two-way ANOVA).

stages. Amongst the five soybean genotypes, free amino acids generally decreased from fresh edible stage to full maturity stage. For aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine and histidine, all the five genotypes generally had higher concentrations at fresh edible stage. The major contributors to the free amino acid pool at fresh edible stage were aspartic acid, serine, glutamic acid, alanine and phenylalanine, accounting for 58.3%, 68.6%, 64.3%, 59.2% and 74.3% in Heidou, Zhong 09, Line 6, Line 61 and Zhong 05 respectively. In contrast, arginine increased from fresh edible stage to full maturity in Zhong 09, Line 6 and Zhong 05 but decreased in Heidou and Line 61. As for methionine, the changes were irrelevant over five genotypes at two stages but its concentration was the lowest at fresh edible stage compared with other amino acids for all genotypes.

In genotype Heidou, the highest free amino acid was arginine accounting for 26.9% of the total free amino acid at fresh edible stage. While, in genotypes Zhong 09 and Zhong 05, glutamic acid was higher at fresh edible stage accounting for 27.4% and 21.4% of the total free amino acid respectively. In Line 6, alanine accounted for 24.7% of the total free amino acid at fresh edible stage. In Line 61, the major free amino acid was serine accounting for 17.5% of the total free amino acid at fresh edible stage. Meanwhile, at full maturity stage, arginine was the major free amino acid for all genotypes accounting for 33.3% to 51.1%.

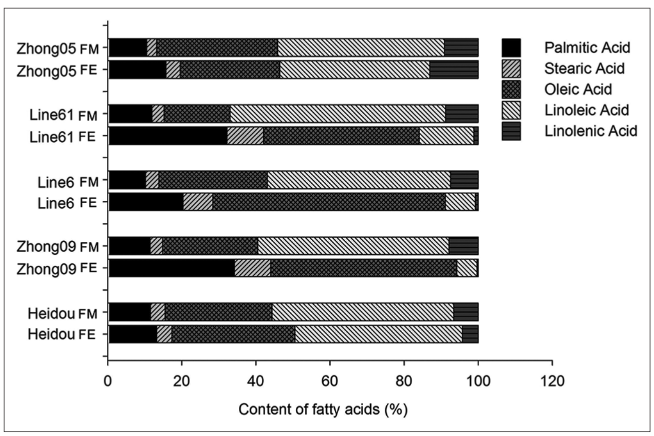

Fig 3. Comparison of fatty acid composition of five vegetable soybean genotypes between fresh edible and full maturity stage.

As showed in Table 2, the total free amino acid concentration was higher at fresh edible stage compared with full maturity stage. Heidou, Line 6 and Zhong 05 had higher total free amino concentration. From fresh edible stage to full maturity, the total free amino acid concentration of Heidou, Zhong 09, Line 6, Line 61, and Zhong 05 dropped 56.0%, 45.9%, 59.6%, 67.3% and 38.5% respectively.

Comparison of crude oil and fatty acids

Fig. 2d revealed changes of crude oil concentration of the five soybean genotypes between fresh pod stage and full maturity stage. The highest crude oil concentration at fresh edible and full maturity stage was both found in Line 6 by 17.6% and 20.5% respectively. The concentration of crude oil was consistently higher at full maturity stage. The increase rates among genotypes were quite different, e.g. from fresh edible stage to full maturity crude oil concentration increased 86.5% in Heidou, but only 16.7% in Line 6.

Variation of fatty acids between fresh edible stage and full maturity stage is showed in Fig. 3. The relative content of saturated fatty acid (palmitic acid + stearic acid) was generally higher at fresh edible stage. In contrast, matured vegetable soybean seed obtained more unsaturated fatty acid (oleic acid + linoleic acid + linolenic acid). Furthermore, the highest saturated fatty acid content was found in Heidou at full maturity stage. Saturated fatty acid in Line 61 and Zhong 09 declined dramatically by 26.9% and 29.2% respectively from fresh edible stage to full maturity stage.

Table 3: Comparison of isoflavones concentration of five vegetable soybean genotypes between fresh edible (FE) stage full maturity (FM) stage

Isoflavones	Daidzin μg g^{-1}		Glycitin μg g^{-1}		Genistin μg g^{-1}		Daidzein μg g^{-1}		Glycitein μg g^{-1}		Genistein μg g^{-1}		Total μg g^{-1}	
	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM
Heidou	70	653*	26	138*	51	821*	43	35*	28	48*	28	5.5*	246	1700*
Zhong 09	480	1353*	215	205*	420	1518*	40	20*	49	26*	51	27*	1255	3150*
Line 6	582	1130*	292	445*	498	1680*	36	25*	99	101ns	41	14*	1548	3395*
Line 61	378	2294*	144	291*	187	1911*	15	27*	49	41*	11	28*	1548	4593*
Zhong 05	326	1444*	99	259*	149	1281*	23	23^{ns}	20	70*	23	28*	641	3104*
LSD 0.05	17.7		6.2		17.2		0.8		2.0		0.8		43.3	
Significance level														
Genotype	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Stage	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Genotype × Stage	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	

* and ns indicate significant and non-significant difference (t-test) between fresh edible stage full maturity stage, respectively, for individual genotypes.
LSD (Least Significant Difference) values correspond to the genotype × stage interaction (two-way ANOVA).

Table 4: Comparison of mineral elements concentration of five vegetable soybean genotypes between fresh edible (FE) and full maturity (FM) stage

	Na mg g^{-1}		Mg mg g^{-1}		P mg g^{-1}		S mg g^{-1}		K mg g^{-1}		Ca mg g^{-1}		Mn µg g^{-1}		Zn µg g^{-1}		Fe µg g^{-1}		Cu µg g^{-1}	
	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM	FE	FM
Heidou	2.67	2.15*	2.6	1.5*	6.4	4.4*	3.9	1.8*	16.4	15.9*	3.6	1.7*	18.1	16.4*	46.5	44.0*	106.2	60.1*	19.7	23.3*
Zhong 09	4.77	3.67*	5.7	7.9*	8.2	8.2ns	4.9	5.1*	18.8	18.0*	3.1	3.6*	18.2	16.0*	29.4	35.4*	64.3	100.0*	20.2	15.6*
Line 6	2.8	3.07*	1.9	2.1*	4.4	6.4*	4.7	2.6*	14.8	16.0*	4.6	2.8*	24.8	12.7*	41.6	26.3*	71.9	103.9*	20.3	18.2*
Line 61	3.33	2.19*	2.9	5.1*	4.9	7.7*	1.3	6.3*	19.3	18.9*	0.2	2.2*	14.8	15.3*	42.6	38.7*	53.7	72.3*	19.4	21.0*
Zhong 05	3.66	2.10*	3.0	3.3*	6.9	4.1*	4.2	3.8*	18.5	14.3*	3.5	3.1*	27.6	14.5*	42.4	29.5*	64.7	83.0*	22.5	17.5*
LSD 0.05	0.04		0.03		0.04		0.03		0.65		0.04		0.05		0.15		1.27		0.34	
Significance level																				
Genotype	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Stage	<0.001		<0.001		1.000		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Genotype ×Stage	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	

* and ns indicate significant and non-significant difference (t-test) between fresh edible stage and full maturity stage, respectively, for individual genotypes.
LSD (Least Significant Difference) values correspond to the genotype×stage interaction (two-way ANOVA).

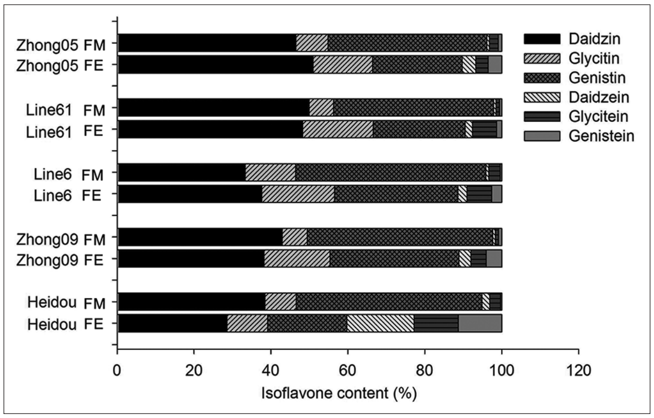

Fig 4. The ratios of each isoflavone group to average total isoflavone of five soybean genotypes. Table S1. Correlation of nutritional compositions of selected five soybean species. *,**, significant level at $p \leq 0.05$ and $p \leq 0.01$; NS, not significant.

All the five genotypes had higher linoleic acid content at full maturity stage. In Heidou, Zhong 09, Line 6 and Line 61, oleic acid decreased by 4.3~33.5% from fresh edible stage to full maturity stage, but increased 5.8% in Zhong 05. Genotype Zhong 05 had highest linolenic acid relative content of 13.1% at fresh edible stage, whereas genotype Zhong 09 got the lowest linolenic acid relative content of 0.3%. As for palmitic acid, all the five genotypes got higher palmitic acid content at fresh edible stage, and palmitic acid contributed a major part to the fatty acid pool at this stage. A decreased trend from fresh edible stage to full maturity stage was found declining from 3.9~9.9% to 3.2~2.7%.

Comparison of soy isoflavones

Six isoflavones in the seeds of five soybean genotypes were quantified. As showed in Table 3, total concentrations of isoflavone in the fully matured seeds were significantly higher than fresh seeds. With seed growth, isoflavones accumulated rapidly. For instance, total isoflavone concentration of Heidou increased 6.9 times from fresh edible stage to full maturity stage. Over the five selected soybean genotypes, the highest total isoflavone concentration of 4593 µg g^{-1} was found in Line 61 at full maturity stage.

Daidzin is a main ingredient of soy isoflavones which contributes a big part of total isoflavone either at fresh edible stage or full maturity stage. The highest daidzin concentration of 2294 µg g^{-1} was found in Line 61 at full maturity stage, while Line 6 got the highest daidzin concentration of 582 µg g^{-1}at fresh edible stage. Daidzin concentration in different genotypes was distinct, though the percentage was close (Fig. 4). The percentage of daidzin in Heidou, Zhong 09 and Line 61 increased from fresh edible stage to full maturity stage, but decreased in Line 6 and Zhong 05. Glycitin concentration generally increased from fresh edible stage to full maturity stage in five soybean genotypes, but percentage all declined (Fig. 3). This means that glycitin has a lower accumulation rate in soybean seeds. Genistin is another big ingredient of isoflavone, which increased in both concentration and percentage from fresh edible stage to full maturity stage. Line 61 also got a higher genistin concentration of 1912 µg g^{-1} at full maturity stage. Concentration changes in daidzein, glycitein and genistein from fresh edible stage to full maturity stage were distinct, but percentage variation declined similarly, which contributed less to the isoflavone pool especially at full maturity stage.

The correlation of protein, crude oil, soluble sugar, total free amino acids and soy isoflavone in the five vegetable soybean genotypes revealed that protein was not significantly correlated with the other four nutritional compositions (Table S1). And total free amino acid was negatively correlated with soluble sugar, crude oil and isoflavone (-0.714*, -0.667*, -0.873**, respectively. *,

$p<0.05$, **, $p<0.01$). Crude oil was positively correlated with soluble sugar and isoflavone (0.673* and 0.733*), while soluble sugar was positively correlated with isoflavone (0.720*).

Mineral elements

As showed in Table 4, potassium concentration was the highest among the selected ten mineral elements in the five genotypes. The potassium concentration in genotype Line 61 reached 19.3 mg g^{-1} at fresh edible stage. The concentration of Cu was lowest above 20 μg g^{-1}. The concentration of K, Na, Zn and Mn was generally higher at fresh edible stage, and the concentration of Mg and Fe was generally higher at full maturity stage. The concentration of P and S was second highest, but no significant trend was found from the five selected genotypes. The concentration of Ca was around 0.2 to 4.6 mg g^{-1}, with the highest Ca concentration of 4.6 mg g^{-1} found in Line 6 and the lowest of 0.2 mg g^{-1} in Line 61 at the fresh edible stage.

DISCUSSION

Soluble sugar is the most important factor assessing the edible quality of vegetable soybean, since sucrose accounted for over 70% of soluble sugar concentration (Li et al. 2012; Song et al., 2013). The accumulation of soluble sugar concentration increased with seed growth, and the highest sugar concentration was found after fresh edible stage (Saldivar et al., 2011, Li et al., 2012). In the present study, we found that sucrose accounted for 64.8~91.0% and 73.3~84.3% of the soluble sugar at fresh edible stage (R6) and full maturity stage (R8) respectively. While the soluble sugar concentration of 100~120 mg g^{-1}at R8 stage was higher than that of 650~90 mg g^{-1} at R6 stage. This shows that soluble sugar accumulates until the end of seed development. Saldivar et al. (2011) also found that soluble sugar concentration at R6 and R8 stage ranged from 40~70 mg g^{-1} and 70~110 mg g^{-1} for seven soybean genotypes respectively. This indicates that (1) seed soluble sugar accumulation does not reach the peak at R6 stage, (2) and vegetable soybean has higher soluble sugar concentration. Therefore, selecting higher soluble sugar genotypes at fresh pod stage such as genotype Zhong 05 should be encouraged.

Protein is the major nutrient component of soybean seed, accounting for above 40% of the dry mass of soybean seed (Wolf, 1969). Jiang et al. (2018) found out that concentration of protein, oil, and soluble sugar of edamame at fresh edible stage was about 420mg g^{-1}, 200~220mg g^{-1}, and 100mg g^{-1}, respectively in 86 edamame breeding lines and cultivars over two years. The present study obtained the similar results. In our investigation, higher protein concentration was found in Heidou and Line 6 at fresh edible stage, and protein concentrations were higher at full maturity stage for genotypes Zhong 09, Line 61 and Zhong 05. However, we did not find greater protein differences between fresh edible and full maturity stage. Hill & Breidenbach (1974) showed a slight change in protein accumulation as soybean seed desiccated. Therefore, harvesting soybean at either of fresh edible or full maturity stage has less effect on protein concentrations. Many evidence indicated that there was an inverse relationship between seed yield and seed protein content (Burton, 1987), and single cross and rapid back cross breeding methods could achieve both high seed yield and high protein content (Cober & Voldeng, 2000).

Dajanta et al. (2011) once demonstrated that fermented soybean (thua nao) had much higher concentrations of free amino acids than had their unfermented samples. Free amino acid of plants is a good source for human body to remedy the lack of synthesis, and also has strong cytotoxic activity against cancer cells (Kim et al., 1999). But fewer studies investigated the differences in free amino acids compositions between fresh pod stage and maturity stage of grain soybean or vegetable soybean. The present study indicated that total free amino acid concentration of vegetable soybean was significantly higher at fresh edible stage, but dropped rapidly to full maturity. This might be due to the fact that protein synthesis at R6 stage requires more free amino acids (Song et al., 2013; Postles et al., 2016). Similar results were also reported in rye grain (Postles et al., 2016). Present study also found that from fresh edible to full maturity, the concentration of aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), valine (Val), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe) and histidine (His) generally decreased while arginine (Arg) and methionine (Met) concentration changed slightly. This indicates that the compositions of the free amino acids varied greatly between two growing stages. The increases of Gly, Glu, Asp and Arg in chungkukjang (Korean traditional soybean paste fermented for a few days) could enhance the sweet and savory taste characteristics (Lee et al., 2010). Based on the different taste characteristics, amino acids can be grouped as monosodium glutamate-like (MSG-like) (Asp + Glu), sweet tasting (Ala + Gly + Ser + Thr), bitter tasting (Arg + His + Ile + Leu + Met + Phe + Trp + Val) and tasteless (Cys + Lys + Pro) (Tseng et al., 2005). The present study found that the concentration of free amino acids was 2.43 times higher for MSG-like group, 5.18 times higher for sweet tasting group in the five genotypes at fresh edible stage than full maturity stage. In contrast, no consistent trend was found for bitter tasting group (Trp was not included). This explains why fresh vegetable soybean seeds are famous for their good taste.

It was suggested that the most desirable phenotype for soybean oil is <7% saturates (palmitic acid + stearic acid), >55% oleic acid, and <3% linolenic acid (Wilson, 2004). With seed maturing, crude oil concentration elevated rapidly (Bachlava et al., 2009). Our investigation found a significantly higher oil concentration of 170~205 mg g^{-1} at full maturity stage than 101~176 mg g^{-1} at fresh edible stage. This indicates that harvesting at different stage will influence the end use of soybean oil. Li et al. (2007) reported that linoleic acid and oleic acid accounted for 80% of the total fatty acids in mature seed, and C16:1 disappeared in the halfway of seed growth. The present study found that unsaturated fatty acid increased and saturated fatty acid concentration dropped down with seed growth. This was particularly evident for the linoeic acid and oleic acid. Kim et al. (2015) reported that oleic acid of high oleic acid genotype *JD11-0070* accounted for about 80% of total fatty acid content, but only 20~30% in commercial soybean cultivars *Taekwang* and *Wooram*. The present results showed that the relative content of oleic acid was higher at fresh edible stage (26~50%) than at full maturity stage (17~33%), fresh pod vegetable soybean could be a good source of oleic acid, because oleic acid has beneficial health effect in humans (Lopez-Huertas et al., 2010).

Isoflavone concentration may be different depending on distinct soybean varieties, tissue types, and growth conditions (Lee et al., 2010). The present study found that the increased concentration of total isoflavone from R6 to R8 stages in soybean seed, which was also demonstrated by Kim et al. (2007). Soy isoflavone content was influenced by genetics, crop years, and growth location, especially crop years (Song, 1998; Wang & Murphy, 1994). For instance, isoflavone contents in three Japanese varieties (*Keburi, Kuro diazu, Raiden*) ranged from 2041 to 2343 μg g^{-1} and from 1261 to 1417 μg g^{-1} for soybeans grown in two years, respectively. In the present study, the total soy isoflavone concentrations differed among genotypes, ranging from 250 to 1548 μg g^{-1} at fresh edible stage, and 1700 to 4593 μg g^{-1} at full matured stage. Moreover, the daidzin, glycitin and genistin accounted for the majority of the total isoflavones over two stages. Therefore, full matured vegetable soybean would provide more isoflavone.

There are many mineral elements in soybean seeds, most of which are essential for human bodies. The elements accumulated in crops are affected by soil types or environmental factors (Wang et al., 2000). Ebert et al. (2017) found that with regard to nutritional value, the vegetable soybean was superior to soybean sprouts in terms of content of protein (14% increase), Zn (45%), Ca (72%) and Fe (151%). The present study found that K concentration was significantly higher than the others especially at fresh edible stage. Na, Mn and Zn also got higher concentrations at fresh edible stage, but the concentration of Fe and Mg were higher at full maturity stage. Regardless of the harvest time, the concentration of trace elements was generally in the same order of magnitude. Therefore, vegetable soybean provides a good source of elements for human beings at both fresh edible stage and full maturity stage.

A negative relationship between protein and oil content is well established (Burton, 1987), however in present study we did not find significant correlation between any composition with protein in the two stages (Table S1). This might be due to the completion of seed protein accumulation after full seed stage (Ghassemigolezani et al., 2010). Hymowitz et al. (1972) reported that total sugar content of soybean seed, as well as sucrose, is significantly positively related to total oil and negatively to total protein. In the present study, free amino acid was significantly negatively correlated with crude oil, soluble sugar and total isoflavone concentration. Furthermore, significantly positive correlation among crude oil, soluble sugar and total isoflavone concentration was also revealed. Therefore, with seed maturing, concentration of total free amino acid was decreased, but crude oil, soluble sugar and total isoflavone were increased. Therefore, in order to obtain the balanced nutrition match, the relationship between total free amino acid and other nutritional compositions should be considered.

In summary, present observations presented distinct compositions and contents of major and minor metabolites between fresh edible and full maturity stage. Seeds harvested at full maturity stage had higher protein, higher unsaturated fatty acid, and higher soy isoflavone and mineral elements, which add nutritional values to soy food processing and production. Genotypes with large seed size, light hilum color, low oligosaccharides and high water absorption, high protein and sucrose concentration are always desirable for soymilk, tofu and bean cans (Saldivar et al. 2011). The present study provides an insight on how to make harvest strategy based on different nutritional value of vegetable soybean types. The findings also suggest that not only green fresh seed but maturated seed of vegetable soybean genotypes could be used as high quality material for soy food production.

CONCLUSIONS

This study was designed to figure out if a significant difference existed in the nutritional value of vegetable soybean genotypes between fresh edible (R6) and full matured (R8) stage. The results indicated that the protein concentration was less changed since R6 stage. The highest protein concentration was found at fresh edible stage in Heidou (43.9%). However, higher free amino acids and mineral elements K, Na, Mn and Zn concentrations were found at R6 stage. Whereas the concentration of protein, crude oil, soluble sugar, unsaturated fatty acid, soy isoflavones and some trace elements (Fe and Mg) was higher at R8. Correlation analysis revealed that the concentration of total free amino acid was significantly higher at R6 stage which was negatively correlated with soluble sugar, crude oil and total soy isoflavone. There were also genotypic differences among the nutritional compositions. The highest total soy isoflavone was 4593 μg g^{-1} in Line 61, and the lowest was 1700 μg g^{-1} in Heidou. The findings reported here will provide an insight for future breeding program and cultivation towards improving the nutritional compositions of soybean species. The time of harvesting could maximize the nutritional value of vegetable soybean.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPLYMENTARY TABLE

Table S1: Correlation of nutritional compositions of selected five soybean species.

	Protein	Crude oil	Soluble sugar	Total free amino acid
Crude oil	0.377NS			
Soluble sugar	-0.019NS	0.673*		
Total free amino	-0.053NS	-0.667*	-0.714*	
Isoflavone	0.141NS	0.733*	0.720*	-0.873**

*,**, significant level at $P \leq 0.05$ and $P \leq 0.01$; NS, not significant.