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ORIGINAL RESEARCH PAPER

NUTRITIONAL CONSTITUENTS OF SOYBEAN GROWN IN NORTHEAST REGION OF CHINA

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Nutritional constituents of soybeans (*Glycine max* L. Merr.) grown in the Northeast Region of China were evaluated through measuring amino acid composition, fatty acid profile and mineral content. Results showed that there were eighteen detectable amino acids in soybean samples. The fatty acid profile indicated linoleic acid, oleic acid, palmitic acid and linolenic acid as the most abundant fatty acid followed by stearic acid, lauric acid, arachidonic acid, behenic acid and gondoic acid. Mineral content analyses indicated that the most abundant mineral in soybeans was potassium, followed by calcium, iron, magnesium, sodium, zinc and manganese. The soybean cultivar “JinYou” contained higher ($P<0.05$) values of essential amino acids, essential fatty acids, calcium and iron contents compared to “HeFeng”, “NongKe” and other cultivars previously reported.

Keywords: soybean, amino acids, fatty acids, minerals

Introduction

Soybean (*Glycine max* L. Merr.) is a very old legume consumed worldwide, but mainly in Asian countries (Liu *et al.*, 2008). In the Orient, soybean seed has been used as human food for more than 6000 years (Agarwal *et al.*, 2013). In China it is consumed as cooked beans, soy sauce, soy milk or tofu (Yu *et al.*, 2013). Nowadays, as one of the exotic healthy and fashionable food, the importance of soybean for human consumption on Chinese markets is increasing.

Soybean is an inexpensive source of dietary proteins, fats and carbohydrates. Other important chemical components of soybean are isoflavones and lignans, well-known for their beneficial effects on human health due to their antioxidant activity (Shao *et al.*, 2009). Essential amino acids, essential fatty acids and minerals are necessary nutrients for health maintenance. The human body cannot synthesize these compounds therefore they must be obtained through the diet (Koyuncu *et al.*, 2014). Soybean is rich in essential amino acids, essential fatty acids and minerals.

Thus, the nutritional value of soybean is very important for the Chinese population because various processed soy-based foods are consumed on a daily basis supplying thus required nutrients.

Factors like genotype and environmental condition are known to influence the nutritional constituents of soybeans (Jurgonski *et al*., 1997). In literature, there were several reports about the chemical composition of different soybean cultivars grown under different ecological conditions in the United States, Brazil, India and Argentina (Esteves *et al*., 2010; Kaushik *et al*., 2010; Bøhn *et al*., 2014; Adie *et al*., 2015). A recent study reported data about crude protein, total lipids, and ash contents of soybean grown in China (Yu *et al*., 2013). However, there is no further detailed information on amino acid, fatty acid and individual mineral element of soybean cultivated in China. Therefore, the specific objective of this study was to evaluate amino acid composition, fatty acid profile and mineral content of three soybean cultivars ("HeFeng", "JinYou" and "NongKe") grown in the Northeast Region of China. In addition, the contents in essential amino acids, essential fatty acids and minerals (calcium, iron and zinc) of the studied cultivars were investigated and discussed.

Materials and methods

Materials

Three soybean cultivars ("HeFeng", "JinYou" and "NongKe") were harvested from the Northeast Region of China in 2014, grinded for 2 min (FSD-100A, Xinen Instrument Co. Ltd., Taizhou, China), and sifted through a 0.15 mm sieve. The resulted soy flour was stored in dark bags at -20 °C. All standards used in the experiments were purchased from Sigma–Aldrich Chemical Co. (St. Louis, Mo, USA). All other reagents were from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China) and were of analytical grade.

Determination of amino acid composition

Amino acid composition analysis was performed using the method previously described by Tu *et al*. (2009) with a minor modification. Briefly, the sample (100 μg) was mixed with 0.4 mL of 6 M HCl heated at 120 °C for 24 h in a vacuum sealed tube. The hydrolysate was evaporated to dryness in a vacuum and was dissolved in 800 μL of glycine-HCl buffer (pH 2.2). Further, for amino acid composition analysis, an amino acid analyzer (L-8800, Hitachi Ltd., Tokyo, Japan) was used. Standard amino acids were used for identification. The chromatograms were recorded and the area of individual peaks was calculated. The amino acids were identified by comparing their retention times with standards and the area under the peaks was calculated in order to determine amino acids content present in the sample. The results were expressed as g/100 g of soybean sample.

Fatty acid profile analysis

Lipids were obtained from soybean sample by Soxhlet extraction using petroleum ether as solvent under reflux for 6 h, according to the method described by Herchi *et al*. (2014). The lipid fractions were initially subjected to esterification of fatty acids, which were converted into fatty acid methyl esters using the method described by Hartman and Lago (1973). The analysis of fatty acid methyl esters was performed using a gas chromatograph (6890N, Agilent Technologies, Santa Clara, USA) equipped with flame ionization detector with a fused silica Supelcowax 10 capillary column (30 m × 0.25 mm i.d., 0.20 µm film thickness). Split injection mode (1:50) was used, and helium was used as the carrier gas at a constant flow of 1.2 mL/min. The gradient temperature program started from 60°C, maintained for 2 min, and raised, at 6 °C/min rate, up to 220°C, then maintained for 20 min. The injector and the detector were set at 220 and 250°C, respectively. Identification of the fatty acid methyl esters was carried out by comparison of their retention times with that of the standards, and the quantities were calculated from the area obtained by the recorded integrator. The results were expressed as g/100 g of soybean sample.

Mineral content determination

Mineral content was evaluated according to the method detailed by Tu *et al*. (2009) with slight modifications. In short, the sample (500 mg) was weighed into a beaker, digested in 7 mL of HNO_3-H_2O_2 (5:2) for 10 min, and the mixture heated to near dryness. After cooling, the residue was treated with 0.1 M HNO_3 and brought to 50 mL with bidistilled water. Minerals were determined with an inductively coupled plasma-optical emission spectrometry (Teledyne Leeman Labs Ltd., Prodigy, Hudson, NH, USA). Calibration curves of standard elements (sodium, potassium, calcium, zinc, magnesium, iron and manganese) were performed and their contents in samples were calculated with regression equation (Excel 2007, Microsoft Corporation, Redmond, USA). The results were expressed as mg/100 g of soybean sample.

Statistical analysis

The experimental results were expressed as means ± standard deviation (SD) of triplicate. Differences among means were evaluated using Fisher's *F*-test at a significance level of P<0.05.

Results and discussion

Amino acid composition

Proteins and amino acids are essential for human health. Amino acids are required for the growth, development, regeneration and reconstruction of the body and are responsible for the production of antibodies, blood cells, hormones, and enzymes (Sousa *et al*., 2014). Amino acids composition of three soybean cultivars grown in the Northeast Region of China was analyzed and results are given in Table 1. Obtained data revealed only eighteen identified amino acids in soybean samples, which agreed with result reported by Esteves *et al*. (2010). Results also showed that the main amino acids of soybean samples were glutamic acid, histidine, aspartic acid, tryptophan, leucine, arginine and lysine (above 4 g/100 g), followed by valine, isoleucine, proline, serine, tyrosine, alanine, glycine and threonine (1-4 g/100 g). The contents of phenylalanine, cysteine and methionine (below 1 g/100

g) were the lowest, as compared to other amino acids. These results were similar to those previously reported by Bøhn *et al*. (2014).

Table 1. Amino acid composition of different soybean cultivars

Amino acid	Cultivar		
	HeFeng (g/100 g)	JinYou (g/100 g)	NongKe (g/100 g)
Aspartic acid	4.58±0.08^{b}	4.80±0.11^{a}	4.77±0.10^{a}
Glutamic acid	7.34±0.12^{a}	7.07±0.13^{b}	7.09±0.09^{b}
Serine	2.05±0.06^{c}	2.19±0.04^{b}	2.29±0.03^{a}
Arginine	3.13±0.07^{b}	3.41±0.07^{a}	3.02±0.06^{b}
Glycine	1.77±0.04^{b}	1.85±0.02^{a}	1.86±0.02^{a}
Threonine	1.62±0.06^{c}	1.93±0.06^{a}	1.76±0.04^{b}
Proline	2.06±0.04^{c}	2.53±0.06^{a}	2.41±0.04^{b}
Alanine	1.82±0.03^{a}	1.88±0.04^{a}	1.75±0.04^{b}
Valine	2.17±0.04^{c}	2.30±0.04^{b}	2.39±0.02^{a}
Methionine	0.19±0.01^{c}	0.50±0.01^{a}	0.32±0.01^{b}
Cysteine	0.21±0.01^{b}	0.37±0.01^{a}	0.38±0.01^{a}
Isoleucine	2.10±0.04^{b}	2.15±0.05^{b}	2.23±0.05^{a}
Leucine	3.27±0.07^{a}	3.32±0.04^{a}	3.36±0.05^{a}
Tryptophan	3.31±0.10^{a}	3.40±0.09^{a}	3.21±0.07^{b}
Phenylalanine	0.89±0.01^{c}	1.13±0.02^{a}	1.04±0.02^{b}
Histidine	4.65±0.11^{b}	5.43±0.10^{a}	4.75±0.08^{b}
Lysine	3.09±0.08^{c}	3.68±0.09^{a}	3.28±0.05^{b}
Tyrosine	1.86±0.04^{b}	2.19±0.03^{a}	2.22±0.04^{a}

Values with different letters in same row are significantly different (P<0.05)

Total amino acid contents of soybean cultivars "HeFeng", "JinYou" and "NongKe" were 46.11, 48.73 and 48.13 g/100 g, respectively. It accounted for 46, 49, and 48% of the samples' mass, which indicated that protein was the most prevalent component in soybean. Several authors had reported previously that protein contents of soybean cultivars grown in other regions ranged from 36.40 to 42.40 g/100 g (Redondo-Cuenca *et al*., 2006; Darmawan *et al*., 2010; Adie *et al*., 2015), lower than the amount of protein determined in studied cultivars. Thus, protein provided by "HeFeng", "JinYou" and "NongKe" might be used as an important nutrient source to meet the nutritional requirements of the Chinese population.

Among three soybean cultivars, "JinYou" contained significantly higher (P<0.05) amounts of arginine, threonine, proline, phenylalanine, histidine and lysine. In particular, the content of eleven essential amino acids (threonine, valine, methionine, cysteine, isoleucine, leucine, tryptophan, phenylalanine, tyrosine, histidine and lysine) in “JinYou” (26.40 g/100 g) was approximately 1.13 - and 1.06-times higher compared to “HeFeng” (23.36 g/100 g) and “NongKe” (24.93 g/100 g). In general, essential amino acids of three soybean cultivars exceed the requirements established by WHO/FAO/UNU (2007) (Table 2) except methionine and cysteine with amino acid score (AAS) of 35, 69, and 58, respectively. This result indicated that methionine and cysteine are the main limiting amino acids of soybeans grown in the Northeast Region of China.

Table 2. Amino acid score for essential amino acids from different soybean cultivars

Amino acid	FAO	HeFeng		JinYou		NongKe	
		Content	AAS	Content	AAS	Content	AAS
Isoleucine	31	46	>100	44	>100	46	>100
Leucine	63	71	>100	68	>100	70	>100
Lysine	52	67	>100	76	>100	68	>100
Methionine + Cysteine	26	9	35	18	69	15	58
Phenylalanine + Tyrosine	46	60	>100	68	>100	68	>100
Threonine	27	35	>100	40	>100	37	>100
Tryptophan	8	72	>100	70	>100	67	>100
Valine	42	47	>100	47	>100	50	>100
Histidine	18	100	>100	111	>100	98	>100

Results are expressed in mg amino acid /g protein

FAO: Requirements in essential amino acids (mg/g protein) proposed by the WHO/FAO/UNU (2007)

AAS: Amino acid score

Fatty acid profile

Lipids are one of the body’s energy sources, and they provide fuel for the central nervous system and for other organs of the human body (Sousa *et al*., 2014). Fatty acids profile of studied soybean samples is given in Table 3. The experimental results showed linoleic acid (8.35-8.75 g/100 g), oleic acid (3.64-3.91 g/100 g), palmitic acid (1.82-2.04 g/100 g) and linolenic acid (1.44-1.64 g/100 g) to be the most abundant, followed by stearic acid (0.61-0.68 g/100 g), lauric acid (0.11-0.19 g/100 g), arachidonic acid (0.04-0.05 g/100 g), behenic acid (0.04-0.05 g/100 g) and gondoic acid (0.02-0.03 g/100 g). The current findings on fatty acids composition were in agreement with those reported in previously published studies (Bøhn *et al*., 2014; Sawada *et al*., 2014).

Unsaturated fatty acids (oleic acid, linoleic acid, linolenic acid and arachidonic acid) have an important role in reducing blood cholesterol levels and improving treatment of atherosclerosis (Uchôa-Thomaz *et al.*, 2014). Meanwhile, the essential fatty acids, including linoleic acid and linolenic acid, are indispensable for human health (Özcan and Juhaimi, 2014). In the present study, contents of fatty acids unsaturated and essential of "JinYou" was 14.07 and 10.39 g/100 g, being markedly higher (P<0.05) compared to "HeFeng" and "NongKe" grown in China and "OCEPAR-19" and "UFV-116" grown in Brazil (Esteves *et al.*, 2010).

Table 3. Fatty acid profile of different soybean cultivars

Fatty acid	Cultivar		
	HeFeng (g/100 g)	JinYou (g/100 g)	NongKe (g/100 g)
Lauric acid	0.15±0.01^{b}	0.19±0.01^{a}	0.11±0.10^{c}
Palmitic acid	1.82±0.03^{c}	1.96±0.04^{b}	2.04±0.02^{a}
Stearic acid	0.65±0.02^{a}	0.68±0.02^{a}	0.61±0.01^{b}
Oleic acid	3.91±0.06^{a}	3.64±0.04^{c}	3.80±0.03^{b}
Linoleic acid	8.44±0.17^{b}	8.75±0.14^{a}	8.35±0.19^{b}
Linolenic acid	1.44±0.02^{b}	1.64±0.04^{a}	1.59±0.04^{a}
Arachidonic acid	0.05±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}
Gondoic acid	0.02±0.01^{a}	0.03±0.01^{a}	0.02±0.01^{a}
Behenic acid	0.04±0.01^{a}	0.05±0.01^{a}	0.04±0.01^{a}

Values with different letters in same row are significantly different (P<0.05)

Mineral content

Minerals are one of the most important nutritional quality factors in many crops and have many biological activities in the human body. In a similar study, Kaushik *et al*. (2010) found that the most abundant mineral in soybean was potassium, which agrees with the data obtained in this study (Table 4). The level of potassium was higher (P<0.05) than that of sodium, which could realize a mineral balance and favor hypertension control. Moreover, a diet rich in potassium could lower blood pressure and, consequently, the risk of morbidity and mortality due to cardiovascular diseases (Sousa *et al*., 2014). In addition, potassium intake could decrease urinary calcium excretion and, consequently, reduce the risk of developing osteoporosis (Çetin *et al*., 2011).

Calcium, iron and zinc are considered essential for the human body. Calcium is one of the most important compound in human bone. Iron is associated with the production of blood cells and zinc is essential for the immune system (Sousa *et al*., 2014).

As shown in Table 4, the contents of calcium and iron of "JinYou" were significantly higher (P<0.05) than the values of "HeFeng" and "NongKe". The measured zinc content of "JinYou" was similar to those of "HeFeng" and

"NongKe", but approximately 1.4-2.2 times higher than the values reported by Elsheikh *et al*. (2009) and Plaza *et al*. (2003) for soybeans. It is important to note that the intake of the minerals, particularly calcium, iron and zinc, is often low in some mountain areas of China. Thus, the consumption of soybean could increase the amount of minerals in the daily diet in these regions of China.

Table 4. Mineral contents of different soybean cultivars

Mineral element	Cultivar		
	HeFeng (mg/100 g)	JinYou (mg/100 g)	NongKe (mg/100 g)
Sodium	7.49±0.21[a]	5.41±0.17[b]	5.69±0.11[b]
Potassium	4363.12±22.74[c]	5068.32±20.41[b]	5228.19±16.58[a]
Calcium	297.61±7.71[c]	389.32±8.83[a]	367.61±9.15[b]
Zinc	5.57±0.18[a]	5.32±0.11[a]	5.47±0.16[a]
Magnesium	7.69±0.23[a]	3.35±0.08[c]	6.27±0.12[b]
Iron	23.39±0.56[b]	25.35±0.30[a]	21.98±0.21[c]
Manganese	2.51±0.06[c]	2.93±0.08[b]	3.18±0.08[a]

Values with different letters in same row are significantly different (P<0.05)

Conclusions

The soybeans (cultivars "HeFeng", "JinYou" and "NongKe") grown in the Northeast Region of China were evaluated in relation to amino acid composition, fatty acid profile and mineral element content. Results showed that there were eighteen detectable amino acids in soybean samples. The fatty acid profile indicated linoleic acid, oleic acid, palmitic acid and linolenic acid as the most abundant. Analyses of mineral content indicated that the most abundant minerals in soybeans were potassium, followed by calcium, iron, magnesium, sodium, zinc and manganese. The soybean cultivar "JinYou" contained higher (P<0.05) contents of essential amino acids, essential fatty acids, calcium and iron contents compared to cultivars "HeFeng" and "NongKe". Obtained results showed that soybean consumption can be considered to improve diet and dietary deficiencies in certain nutrients.

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