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Full Length Research Paper

Bioactive ingredients in adzuki bean sprouts

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Adzuki bean sprouts are consumed as food and herb medicine in Chinese folk. In this study, the main bioactive ingredients of adzuki bean sprouts were evaluated. As seeding days progressed, the free amino acids in the sprouts were rapidly increased while mineral elements and unsaturated fatty acids were reduced. After seeding for 3 days, the highest content of γ-aminobutyric acid could be investigated, and the bioactive value of the sprout seeding for 3 days was superior to that of the sprout seeding for 7 days.

Key words: Adzuki bean, sprout, nutrient, γ-aminobutyric acid, inductively coupled plasma atomic emission spectroscopy (ICP-AES).

INTRODUCTION

The adzuki bean has been cultivated and used for thousands years in East Asian countries such as China, Japan, and Korea, where it is a popular ingredient for desserts, snacks, and confectionery items. In West countries, it has been little more than a curiosity until recently. The adzuki bean contains about 50% starch and 20 to 25% protein (Yousif and Deeth, 2003; Sato et al., 2005). In Chinese folk, the adzuki bean can be consumed as the sprouts, named as “Yuweiya” (seeding for about 3 days) and “Yuweimiao” (seeding for about 7 days), for its true leaves like the fish tails. The adzuki bean sprouts have a soft and slightly crispy texture and an attractive fragrance. But to the best of our knowledge, there were no reports about the bioactive ingredient evaluation of the adzuki bean sprouts, which was just the purpose of this study.

MATERIALS AND METHODS

Materials and chemicals

The adzuki beans (cv. Hongxiaodou 1) for this study were provided by Shanxi Academy of Agricultural Sciences (Taiyuan, China). Fatty acid methyl esters used as standards, amino acids and γ-amino butyric acid were purchased from Sigma Chemicals Co. (USA). Other chemicals were of analytical grade.

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Preparation of adzuki bean sprouts

The preparation process of adzuki bean sprouts was shown in Figure 1. The soaked adzuki beans were put in an artificial climate incubator (PQX-330B-22H, Ningbo Scientific Co., Ltd, China) at the temperature and humidity of 25°C and 80%, respectively, without sunlight and sprayed with water at intervals of 8 h everyday. The water used to grow was de-ionized water. General characteristics of the sprouts were measured according to days after seeding (DAS). Randomly collected sprouts were immediately frozen in -40°C refrigerator. After freeze-dried (Alpha 1-4, Christ, Germany), the sprouts were finely grounded to be used for preparing the samples of following analysis.

Analysis of fatty acid composition

The procedure of free fatty acids was as follows: 3 g of freeze-dried sprouts was heated with a reagent containing methanol, heptane, benzene, 2, 2-dimethoxypropane, H_2SO_4 (37:36:20:5:2, v/v). The simultaneous digestion and lipid transmethylation took place in a single phase at 80°C. After, they were cooled at the room temperature, the upper phases containing the fatty acid methyl esters (FAMEs) were prepared for the capillary GC analysis. The GC analysis was performed on a HP 6890 system (HP Co., USA) equipped with a FID by using a HP-Innowax capillary (cross-linked polyethylene glycol) column (0.25 μm, 30 m). The initial temperature of 150°C was raised to the final temperature of 280°C at a rate of 4°C/min. Carrier gas was nitrogen at a flow rate of 10 mL/ min. During the analysis, the temperatures of inlet and detector were maintained at 250 and 300°C, respectively. The fatty acids were identified with reference to the retention times of standard fatty acid methyl ester performed at the same conditions.

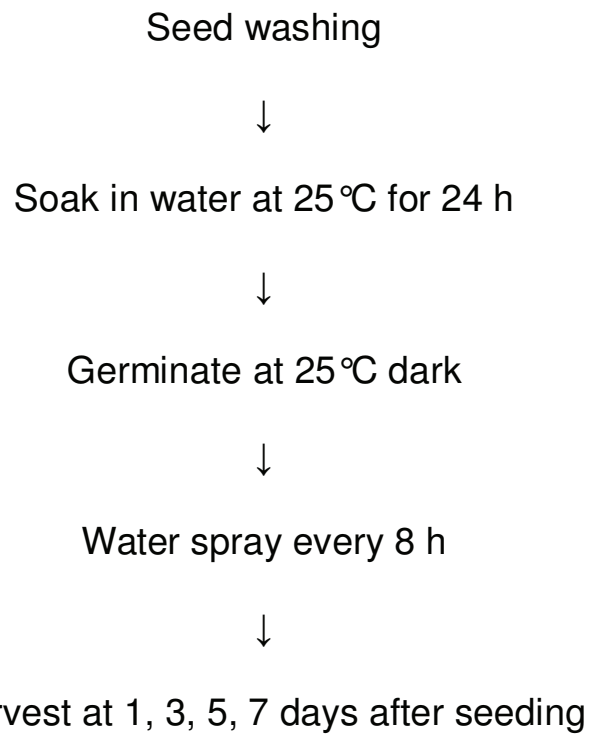

Figure 1. Procedure for the production of adzuki bean sprouts.

Analysis of free amino acids

Free amino acid composition of protein isolate samples was determined with an amino acid analyzer (Waters M510, USA). The different amino acids recovered were presented as g/100 g dry basis. The 0.5 g of the sample was soaked in 10 ml of de-ionized water, and then sonicated for 15 min. The resulting mixture was then centrifuged at 10000 rpm/min for 15 min. Determination was obtained by pre-column derivation of the sample with phenyl isothiocyanate and separating the corresponding derivatives with RP-HPLC on a PICO.TAG NH_2 analytical column at 38°C and UV detection (254 nm).

Analysis of γ-aminobutyric acid

The content of γ-aminobutyric acid was determined by L-2000 HPLC. The 0.3 g of sprouts was diluted with 7 ml of 8% trichloroacetic acid solution, and then sonicated for 40 min. The resulting mixture was centrifuged at 9000 rpm/min for 10 min. Determination was obtained by pre-column derivation of the sample with OPA and separating the corresponding derivatives with RP-HPLC on a Waters C18 column (3.9 ×150 mm, 4 µm,) at 40°C and UV detection (338 nm).

Mineral analysis

The mineral analysis was determined by using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The dried sample (1.5 g) was accurately weighed in a porcelain crucible and subjected to a calcination step in a muffle furnace at 550°C for about 8 h. The residue was solved in dilute nitric acid for mineral analysis. The concentrations of 7 elements (Ca, Cu, Fe, K, Mg, Na, Zn) were measured using ICP-AES (Perkin-Elmer, model Optima 2100 DV, 3 Australia). Measurement conditions were adjusted to a power of 1.kW, coolant gas flow 15 ml/min, auxiliary gas flow of 0.2 ml/min, nebuliser gas flow of 0.8 ml/min, and sample uptake rate of 1.5 ml/min. The standards were prepared in the range from 0.05 to 10 mg/mL. The elements were monitored at the following wavelength: K (766.49 nm), Na (589.59 nm), Ca (317.93 nm), Mg (279.08 nm), Cu (324.75 nm) and Zn (213.86 nm).

Statistical analysis

The data obtained in this study were expressed as the mean of three replicate determinations. Statistical comparisons were made with student's test. P values of < 0.05 were considered to be significant.

RESULT AND DISCUSSION

Fatty acid composition

The GC analyses of the samples are shown in Table 1. The fatty acid composition of adzuki bean seeds showed that adzuki bean seeds were abundant in unsaturated fatty acids, with only lower amounts present in saturated fatty acids. The composition rates of unsaturated fatty acids, such as oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) were 7.06, 35.62 and 26.92%, respectively. Meanwhile, major saturated fatty acid of buckwheat seeds is palmitic acid (C16:0), which comprised approximately 22.48% of total fatty acids. Accordingly, only small quantities of myristic acid (C14:0), stearic acid (C18:0), arachidic acid (C20:0), and behenic acid (C22:0) were observed. As seeding days progressed, however, all the saturated fatty acids were rapidly increased. For 1, 3, 5 and 7 days after seeding, the content of total saturated fatty acid was 26.49, 32.46, 45.69 and 46.05%. After seeding for 3 days, the sprouts still contained more 67% unsaturated fatty acids. Polyunsaturated fatty acids such as linoleic acid (C18:2), linolenic acid (C18:3), and arachidonic acid (C20:4) are called essential fatty acids (EFA) because of their necessity in the human body. Linolenic acid is a known enhancer for transporting bioactive compounds into the skin, and it is converted to arachidonic acid, which serves as a precursor for powerful hormone-like compounds (Kim et al., 2004). After seeding for 3 days, the content of linoleic acid (C18:2) increased to 39.00%, and the content of linolenic acid (18:3) reduced slightly to 23.47%. But after seeding for 7 days, the content of linoleic acid (18:2) and linolenic acid (18:3) was decreased to 30.05 and 17.83%, respectively.

Free amino acids

In this study, HPLC analysis showed that most of free amino acid contents were increased as seeding days progressed (Table 2). And total free amino acid content in 7 DAS sprouts was almost 12-times higher than that of

Table 1. Fatty acid composition of adzuki bean sprouts according to days after seeding (%).

Fatty acid	Seed	Day after seeding (DAS)			
		1	3	5	7
C14:0	0.35	0.29	0.28	0.49	0.46
C16:0	22.48	19.39	24.61	34.97	33.77
C16:2	0.29	0.34	-	-	0.66
C18:0	3.96	3.75	3.44	4.88	5.11
C18:1	7.06	7.93	5.08	6.02	5.39
C18:2	35.62	39.06	39.00	29.64	30.05
C18:3	26.92	26.14	23.47	17.85	17.83
C20:0	0.94	0.72	0.77	0.94	1.01
C22:0	2.36	2.34	3.36	4.45	5.70
C22:1	-	-	-	0.73	-

Table 2. Free amino acid contents of adzuki bean according to days after seeding (mg/100 g).

Free amino acid	Seed	Day after seeding (DAS)			
		1	3	5	7
Asp	22.40	14.65	41.11	58.06	78.15
Glu	60.01	150.66	183.20	132.48	97.45
Ser	4.82	14.82	59.60	155.30	269.36
Gly	5.40	4.67	3.15	14.43	25.87
His	8.54	28.24	62.82	180.12	261.61
Arg	33.13	46.38	82.66	139.19	201.55
Thr	6.69	41.31	124.47	206.27	288.03
Ala	10.28	43.25	46.45	53.93	94.79
Pro	9.49	16.51	26.98	70.21	102.13
Tyr	25.55	40.60	59.48	129.89	212.93
Val	6.46	13.02	48.35	187.38	350.12
Met	2.24	3.08	8.34	34.02	59.97
Cys	1.63	3.57	0.00	0.00	0.00
Ile	3.99	9.16	28.04	117.70	242.87
Leu	2.01	7.61	22.33	86.85	176.88
Trp	16.83	20.98	25.35	60.84	81.88
Phe	4.90	13.61	41.51	202.60	330.34
Lys	3.44	6.73	14.68	32.43	52.06
Total	227.83	478.84	878.52	1861.71	2925.98

seed. The increment in free amino acid content is favorable as the protein quality of vegetable depends not only on its amino acids but also on the availability of these amino acids (Hamad and Field, 1979). It was considered that the high contents of necessary amino acids such as Lysine threonine, valine, methionine, isoleucine, leucine, tryptophane and phenylalanine, would provide a high bioactive value.

γ-Aminobutyric acid

γ-Aminobutyric acid (GABA) is an important ubiquitous non-protein amino acid in both prokaryotic and eukaryotic organisms. It is a representative depressive neurotransmitter in the sympathetic nervous system and has been proved to be effective for lowering the blood pressure of experimental animals and humans (Zhang et al., 2006). Besides improving hypertension, GABA could also be used as a dietary supplement and/or nutraceutical to help treat sleeplessness, depression and autonomic disorders, chronic alcohol-related symptoms and to stimulate immune cells (Oh et al., 2003). HPLC analysis of GABA was shown in Table 3 and Figure 2. The GABA content in the seeds was only 21.31 mg/100 g while the

Table 3. γ-Aminobutyric acid content of adzuki bean according to days after seeding (mg/100 g).

	Seed	Day after seeding (DAS)			
		1	3	5	7
GABA	21.31	16.98	63.29	60.74	44.54

Table 4. Mineral content of adzuki bean according to days after seeding.

Mineral content (μg/g)	Day after seeding (DAS)		
	0	3	7
Ca	992.56	914.78	745.70
Cu	24.24	6.91	2.53
Fe	83.95	57.29	40.32
K	12.03	10.56	7.74
Mg	1466.94	1295.83	775.80
Na	55.86	8.57	-
Zn	23.16	12.14	5.22

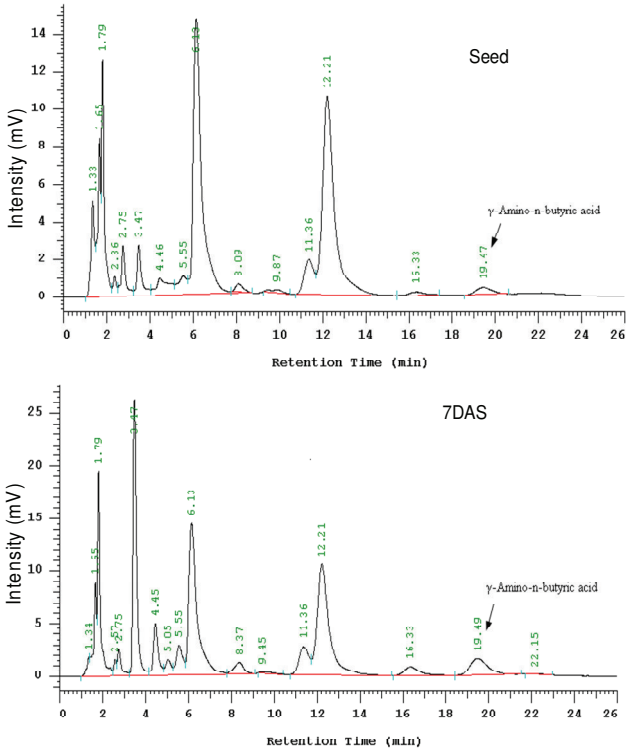

Figure 2. HPLC profiles of adzuki bean seed and sprout (7DAS).

GABA content was 63.29 mg/100g at 3 DAS, almost 3 times higher than that of seeds. But after 5 DAS, the content of GABA decreased.

Mineral content

Inductively coupled plasma atomic emission spectroscopy (ICP-AES), also referred to as inductively coupled plasma optical emission spectrometry (ICP-OES), is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample (Stefánsson et al., 2007). In this study, seven metal elements in seed, 3 DAS, 7 DAS were determined by ICP-AES, including 4 major elements (K, Ca, Mg, Na) and 3 trace elements (Cu, Fe, Zn), as shown in Table 4. Among the measured elements, K was the leading one in citrus peels, followed by Mg and Ca. It was found than all mineral contents decreased as seeding days progressed, which should be attributed to watering. And the mineral loss of 3 DAS was less than that of 7 DAS.

Conclusion

In Chinese folk diet, the adzuki bean can be consumed as the sprouts, named as “Yuweiya” (seeding for about 3 days) and “Yuweimiao” (seeding for about 7 days). According to the result of this study, the total free amino acid content of 3 DAS (Yuweiya) was less than that of 7 DAS (Yuweimiao), but in view of its high contents of polyunsaturated fatty acids, γ-aminobutyric acid, minerals, the bioactive value of 3 DAS was higher that of 7 DAS.

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