Chemical Composition of Winged Bean (*Psophocarpus tetragonolobus*) Varieties

Fumio IBUKI,¹ Makoto KOTARU,² Kuang Kung KAN,^{1*}
Tsuneo IKEUCHI,² and Masao KANAMORI¹

¹Department of Agricultural Chemistry, Faculty of
Agriculture, Kyoto Prefectural University,
Kyoto 606, Japan

²Department of Home Economics, Koka Women's
Junior College, Kyoto 615, Japan

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Summary The chemical composition of dry seeds of four varieties, pods, stalks and leaves of winged beans (*Psophocarpus tetragonolobus*) was determined. The seeds had a high range of protein (27.8–36.6%) and fat (14.8–17.9%), which were similar to soybeans. The seeds contained high phosphorus, calcium and magnesium. The leaf was highest in protein content (33.7%) of all the parts studied except for the seeds. The protein and fat content of pods decreased as pods ripened. The calcium content in the leaf was much higher than in the other parts. Protein was extracted sequentially with 2% NaCl, 30% isopropyl alcohol, 4% lactic acid and 0.5% KOH from dry seeds of four varieties of winged beans. The NaCl extract showed the highest range of protein concentration (60.2–77.6%). The NaCl extract was separated into two fractions based on solubility in water. The amino acid composition of the flour from the seeds and of the two fractions from the NaCl extract were determined. Contents of lysine, aspartic acid, glutamic acid and leucine were large, while the sulfur-amino acid content was small. Trypsin and chymotrypsin inhibitory activities of 2% NaCl extract from the seeds were determined, and chymotrypsin inhibitory activity was higher than the trypsin.

Key Words winged bean, chemical composition, protein extraction, amino acid composition, trypsin inhibitory activity, chymotrypsin inhibitory activity

The winged bean (*Psophocarpus tetragonolobus*), a high protein and oil crop, has been cultivated in Papua New Guinea, Southeast Asia and other humid tropics. The plant is unique among leguminous crops because every part of it (seeds, pods, leaves, flowers and roots) is edible and some parts have medicinal properties (1, 2).

¹ 伊吹文男, ² 小垂 真, ³ 甘 耿光, ² 池内常郎, ¹ 金森正雄

* Present address: Chung-Itwa Junior College of Medical Technology, Taiwan.

Okezie and Martin (3) reported on the chemical composition of dry seeds and fresh leaves of winged bean varieties grown in the U. S. and Puerto Rico. They reported the protein content of undehulled seeds to range from 31.56 to 41.35% and the fat content to be in the range of 16.24–19.71%. Garcia and Palmer (4) also reported that whole seeds of winged beans contained a range of 33.7–37.8% protein and 14.0–17.0% fat. The winged bean was noted as a promising legume due to its high protein and fat content. The amino acid composition of the winged bean seed was reported in comparison with that of the soybean (1, 3, 5). Recently, antinutritional factors such as enzyme inhibitory activity and hemagglutinating activity were investigated with the winged bean (6–8). In Japan, the winged bean varieties introduced from Indonesia and some countries were cultivated in Okinawa prefecture since 1975.

The purpose of this study, therefore, was to provide a knowledge based on nutrient composition, especially seed protein, and on trypsin and chymotrypsin inhibitory activity as an antinutritional factor of the winged beans grown particularly in the Okinawa prefecture of Japan.

MATERIALS AND METHODS

Sample preparation. The dry seeds of four varieties (WB 001, 002, 003 and 004), pods, stalks and leaves of winged bean were obtained from the Institute of Tropical Agriculture, Okinawa branch. The dry seeds were ground in a Mini-Crusher, Model 3 (Sibata Chemicals Co., Tokyo). Samples of immature, half-mature and mature pods, mature leaves and stalks were carefully washed and freeze-dried followed by pulverizing.

Chemical analyses. Moisture, fat, ash and protein ($N \times 6.25$) were determined by the standard method (9). Starch was determined by the method described by Osborne and Voogt (10). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined according to the methods of Van Soest (11, 12) as described by Southgate (13). The residues after amylase treatment of samples for the starch determination were used for the NDF analysis. Tannin was determined by the Folin-Denis method (14). Samples for the mineral analysis were prepared by ignition at 500–550°C. The minerals (except phosphorus) were assayed using an atomic absorption spectrophotometer. Phosphorus was determined by the Bartlett method (15). Amino acids were determined according to the method described by Kanamori *et al.* (16). All analyses were performed at least in duplicate.

Extraction of protein fractions. Continuous extraction of the protein fractions based on their differential solubilities on four various solvents was carried out according to the method of Maes (17). In brief, the ground mixture of seed flour, celite and sea sand was packed into a column with quartz sand and sea sand, and the protein fractions were extracted successively with four different solvents in the following order: 2% NaCl, 30% isopropyl alcohol, 4% lactic acid and 0.5% KOH. The fraction extracted with 2% NaCl was separated according to the different

protein solubilities in water by dialysis against distilled water. The water-soluble (WS) and water-insoluble (WI) fractions separated from 2% NaCl extract were lyophilized, respectively.

Trypsin and chymotrypsin inhibitory activity. 1.5 g of the seed flour were extracted with 20 ml of 2% NaCl at 4°C overnight (with constant magnetic stirring) and centrifuged at $3,500 \times g$ for 60 min. The residue after centrifugation was re-extracted with 20 ml of 2% NaCl at 4°C for 2 h and centrifuged as above. The supernatant was combined and further centrifuged at $100,000 \times g$ for 60 min. The resultant supernatant was used as the sample of protease inhibitory activity assay. Protease inhibitory activity was measured using α -N-benzoyl-DL-arginine-*p*-nitroanilide (BANA) as substrate for trypsin and benzoyl-L-tyrosine-*p*-nitroanilide (BTNA) as substrate for chymotrypsin according to the method described by Kanamori *et al.* (18). Trypsin (2 $\times$ crystallized, from bovine pancreas) and α -chymotrypsin (3 $\times$ crystallized, from bovine pancreas) were purchased from the Sigma Chemical Co. (St. Louis, U.S.A.). BANA and BTNA were from the Protein Research Foundation (Osaka, Japan).

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of dry seeds of the four varieties (WB 001, 002, 003 and 004) are shown in Table 1. The protein content ranged from 27.8% to 36.6% and the fat content ranged from 14.8% to 17.9%. These values are similar to those of soybeans and compare favourably with the other reports (3, 4). The ash content was approximately 4%. The moisture content ranged from 8.0% to 9.7%. The starch content (7.9–10.0%) was much lower than that of the mung bean (42.0%) or chick pea (34.1%) reported by Aman (19). NDF and ADF were analyzed because they

Table 1. Proximate composition (%) of winged bean seeds.

	Winged bean seeds			
	WB 001	WB 002	WB 003	WB 004
Moisture	8.5	8.3	9.7	8.0
Crude protein ^a	27.8	30.8	30.6	36.6
Crude fat	17.9	15.4	14.9	14.8
Ash	3.9	3.6	3.8	4.0
Carbohydrates				
Starch	10.0	9.9	8.5	7.9
NDF	14.6	13.2	14.3	12.3
ADF	13.5	12.4	12.7	11.6
Tannin ^b	0.77	0.87	0.89	0.97

^a N $\times$ 6.25. ^b The values are expressed as tannic acid equivalents.

Table 2. Proximate composition of pods, stalk and leaf of winged bean.

	Parts of winged bean ^a				
	m-pod ^b	hm-pod ^c	im-pod ^d	Stalk	Leaf
Moisture (%)	55.0	85.7	91.2	79.8	76.3
(% of dry matter)					
Crude protein ^e	7.3	11.6	17.5	12.5	33.7
Crude fat	0.7	1.0	1.6	n.d.	5.0
Ash	8.5	7.5	5.3	5.6	8.4
NDF	65.9	52.7	27.7	—	—
ADF	45.9	34.2	16.2	—	—

^a The samples were grown in Okinawa Prefecture. ^b Mature pod. ^c Half-mature pod.

^d Immature pod. ^e N × 6.25.

provide a more reliable estimate of the digestibility than crude fiber. NDF content ranged from 12.3% to 14.6% while the ADF varied from 11.6% to 13.5%. These values are similar to the results reported by Garcia and Palmer (4). The difference between NDF and ADF estimated hemicellulose. Hemicellulose content of winged bean tested in this study seemed to be low. This agrees with other reports (3, 4). Besides fiber and starch, the oligosaccharide content (9.8–13.8%) of winged beans was reported by Garcia and Palmer (20). Tannin content expressed as tannic acid equivalents ranged from 0.77% to 0.97%. The parts other than the seed of winged bean also are useful as a food resource but they have not been studied as much as the seed. The proximate composition of pods, stalks and leaves analyzed in this study is shown in Table 2. Data of pods were obtained from three samples of different extent of ripeness. The young pods are a very popular edible part. Mature stalks and leaves were used. The leaves of winged bean may be useful as protein source due to their high protein content. Okezie and Martin (3) also reported a high protein content of the leaves.

Mineral composition

The mineral composition of all the parts studied are shown in Table 3. The seeds contained high phosphorus, calcium and magnesium. Similar results except for iron content were reported (3, 5, 21). The iron content of the seeds was approximately half of the values of the above reports. The parts other than the seed had a similar content of mineral. Quite a high calcium content of the leaf was noted.

Extraction of protein fractions

The extraction profiles of the soluble protein fractions from seed flour of four varieties are shown in Table 4. A high proportion (60.2–77.6%) of 2% NaCl extract was contained in all samples but the protein contents of the extracts of other

Table 3. Mineral composition of different parts of winged beans.

	Mn	Zn	Fe	Ca	Mg	P
Winged bean seeds	(mg per 100 g of whole seed)					
WB 001	8.5	2.8	5.2	297	366	492
WB 002	8.3	3.0	5.9	212	372	536
WB 003	6.7	3.1	4.9	102	338	550
WB 004	6.0	2.6	5.7	342	312	507
Other parts	(mg per 100 g of dry matter)					
Mature pod	2.7	0.6	5.1	156	131	156
Half-mature pod	5.6	1.4	9.1	299	177	226
Immature pod	11.3	1.3	8.9	454	264	265
Stalk	7.0	1.0	3.8	610	290	263
Leaf	16.5	1.6	13.5	1,551	469	311

Table 4. Relative concentrations of the four soluble protein fractions from winged bean seeds.^a

		Winged bean seeds			
		WB 001	WB 002	WB 003	WB 004
2% NaCl sol.	(%)	60.2	71.2	77.6	68.4
WI ^b	(%)	(37)	(45)	(52)	(42)
WS ^c	(%)	(23)	(26)	(24)	(26)
30% isopropyl alcohol sol.	(%)	6.0	4.1	5.0	6.3
4% lactic acid sol.	(%)	6.8	5.2	1.6	4.5
0.5% KOH sol.	(%)	12.6	5.1	10.2	8.2
Total	(%)	85.6	85.6	94.4	87.4

^a All values are given in terms of Kjeldahl nitrogen. ^b WI fraction from 2% NaCl extract.^c WS fraction from 2% NaCl extract.

solvents were low. Total recovery of protein in the extracts was high (85.6–94.4%). Kanamori *et al.* (16) reported that the same extraction method gave a similar high recovery of protein from twelve legumes grown in the tropics. A 2% NaCl extract was separated into two fractions based on its protein solubilities in water. The WS fraction consisted of albumins and the WI fraction of globulins. In all the samples, the amount of the WI fraction was 1.6–2.2 fold greater than that of the WS fraction. Sathe and Salunkhe (6), however, reported that the ratio of albumins to globulins in two winged bean varieties (Chimbu and HF-10) was 4.66 and 1.88, respectively.

Table 5. Amino acid composition of winged bean flour and protein fractions (WI and WS).

	(g per 16 g N)											
	WB 001				WB 002				WB 003			
	Flour	WI	WS	Flour	Flour	WI	WS	Flour	WI	WS	Flour	WS
Aspartic acid	10.7	13.2	11.9	11.6	16.0	10.1	10.9	11.9	10.3	11.7	10.6	13.4
Threonine	4.0	4.1	4.6	2.8	3.0	3.0	4.1	2.9	3.9	4.5	4.2	4.9
Serine	4.8	5.4	5.3	3.7	4.4	4.4	4.8	4.0	4.6	6.6	3.7	5.5
Glutamic acid	13.3	18.9	15.3	16.0	16.0	16.0	12.7	16.5	15.0	12.0	14.0	12.0
Proline	2.9	6.6	6.5	2.3	5.4	5.4	5.7	2.2	6.6	6.0	3.1	5.8
Glycine	4.7	4.4	4.6	4.2	3.1	3.1	3.7	3.9	3.9	3.6	4.4	4.6
Alanine	5.7	6.3	7.6	5.5	5.9	5.9	6.9	5.8	7.3	5.0	5.2	6.1
Valine	5.3	4.3	5.7	5.1	4.0	4.0	5.0	5.2	4.6	5.7	5.0	5.3
Cystine	1.6	0.6	1.0	1.3	0.5	0.5	0.6	0.9	1.0	0.8	1.1	0.7
Methionine	0.7	0.8	1.0	0.8	0.7	0.7	0.9	0.9	1.0	0.7	0.8	0.9
Isoleucine	4.2	4.1	4.6	4.0	3.9	3.9	4.0	4.2	5.2	4.6	4.0	4.5
Leucine	8.7	10.5	8.3	8.3	8.6	8.6	7.0	8.7	11.6	7.5	8.6	7.2
Tyrosine	4.1	4.1	4.5	2.3	5.0	5.0	4.6	2.4	5.3	4.8	4.0	4.7
Phenylalanine	3.9	4.5	4.9	5.5	5.5	5.5	5.3	5.0	4.7	4.3	3.8	4.9
Lysine	7.7	8.7	7.8	7.6	11.8	11.8	8.8	8.0	9.3	8.1	7.5	8.0
Histidine	1.7	2.2	1.9	1.9	3.3	3.3	2.0	2.1	1.4	1.1	1.8	2.1
Arginine	4.5	5.2	4.7	4.8	8.1	8.1	5.0	5.1	5.3	6.0	5.2	4.1
Tryptophan	1.0	0.5	1.1	1.0	0.5	0.5	1.2	1.0	0.7	0.8	0.9	0.6

They fractionated albumins with distilled water as the first solvent of extraction. Therefore, it seemed that small amounts of inorganic salts contained in raw legumes might have affected protein solubility. More investigations on globulins and albumins of winged bean are necessary. After lyophilization, the protein content of WS and WI fractions were determined. The protein content ranged in WS fraction from 74% to 84%, and in WI fraction from 84% to 97%. In all the samples, the content of WI fraction was higher than that of WS.

Amino acid composition

The amino acid profiles of seed flour and the main soluble fractions (WS and WI) are shown in Table 5. The seed flour of four varieties was generally high in lysine, leucine, aspartic acid and glutamic acid but low in the sulfur-amino acids. Similar results were reported elsewhere (1, 3, 5). The protein of winged bean can be utilized in supplementing cereal-based diets. There are, however, little differences from the reports described above with essential amino acids. The reports (1, 5) indicated the threonine content in winged bean seed compare with that in soybean but the other (3) showed the value lower than soybean. In our results, WB 001 and 004 are similar to the former and the others are to the latter. Besides the report (3) alone indicated the high tryptophan content. These may be due to the difference of the variety of winged bean. More investigations on these points are needed. The amino acid profiles of WS and WI fractions are generally similar to that of seed flour except for a high content of proline in two soluble fractions. There were little differences between the WS and WI fractions. Threonine, valine, sulfur-amino acids (except for WB 003) and tryptophan of the WI fraction were lower than that of WS while lysine, glutamic acid and leucine of WI were higher. In WB 003, the sulfur-amino acids of WI was higher than that of the seed flour.

Trypsin and chymotrypsin inhibitory activity

Trypsin and α -chymotrypsin inhibitory activity of seed are shown in Table 6. Though de Lumen and Salamat (22) described that water was better than a NaCl

Table 6. Trypsin and chymotrypsin inhibitory activity in 2% NaCl extracts of winged beans.

Winged bean	Trypsin inhibitory activity (unit) ^a	Chymotrypsin inhibitory activity (unit)
WB 001	44.0	87.9
WB 002	40.8	86.4
WB 003	50.0	102.6
WB 004	46.0	109.6

^a One unit was the amount of inhibitor required for complete inhibition of 1 mg of the enzyme.

solution for extracting trypsin inhibitory activity (TIA) in raw and cooked beans, a 2% NaCl solution was used in extracting both TIA and chymotrypsin inhibitory activity (CIA) since the purpose of this assay was the measurement of TIA and CIA in protein fractions. CIA varied more than TIA. In all of the samples, CIA was higher than TIA. Similar results were reported (7, 23). This observation may be due to the presence of trypsin inhibitor with CIA (24) and chymotrypsin inhibitor without TIA (25) in winged bean seed. Tannins have been reported to be able to inhibit trypsin (6, 22, 26). It seemed that there is no relationship between the tannin content of seed (Table 1) and TIA. But the correlation between tannin content and TIA may be found when seed coats were used as the samples, because colored seed coats such as winged bean contained generally a high concentration of tannins. The detailed data on tannins will be published elsewhere.

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