Protein and Trypsin Inhibitor in Immature Pods of Winged Bean, Psophocarpus tetragonolobus (L.)DC.

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Protein and Trypsin Inhibitor in Immature Pods of Winged Bean, *Psophocarpus tetragonolobus* (L.) DC.

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Introduction

Winged bean, *Psophocarpus tetragonolobus* (L.) DC., is a tropical legume which has attracted much attention as a protein food source in recent years. Mature seeds of the plant have the protein and fat contents equivalent to those of soybean[18]. Flowers, leaves, tubers and young pods are also high in the protein contents and have been used as food in tropical areas[5,18]. Many studies were reported on nutrients and antinutritional factors such as trypsin inhibitor[1-3,11,12,15] of the mature seeds, but rarely on those of other parts.

Several times after 1970, Ishihata, one of the present authors, made field studies on tropical crops in Indonesia and the Philippines, and collected many strains of winged bean[8]. He has cultivated them experimentally at Ibusuki Experimental Botanic Garden, Kagoshima University. He found many strains of winged bean could grow and bear fruit not only in a plastic house but also in the field. He indicates a possibility in cultivating the winged bean in the southern part of Kyushu area as a vegetable crop to utilize their young pods. We made analytical works on these young pods, harvested at Ibusuki Experimental Botanic Garden, with a purpose of evaluating the nutritive value.

The present paper describes the protein and trypsin inhibitor contents and the fractionation and amino acid composition of proteins.

Materials and Methods

1. Plant materials

For the purposes of investigating the individual characteristics and of harvesting seeds, the winged bean plant was made grown with single-stem training in a pot with 30 cm diameter in a plastic house. It gave 20 to 30 pods per stem. The plant cultivated in the field with hedge-row training produced 150 to 300 pods. Table 1 shows the histories and characteristics of some strains of winged bean used in this work. The colors of young pods and immature seeds are green or dark green in most strains and purple in some strains. Wide variations are seen in the shape and length of pods.

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Table 1. Histories and characteristics of green shells and seeds of winged bean

Strain number*1	Introduce		Green shell		Immature seed			Mature seed*3
	Place	Year	Color	Length (cm)	Color	Weight*2 (g)	Size*2 (mm)	Color
KaS- 5	Indonesia	1974	green	15–18	light green	0.43	7×6×4	light brown
KaS- 6	Indonesia	1974	green	14–17	light green	0.23	8×7×5	light brown
KaS- 7	Indonesia	1974	green	22–27	light green	0.29	12×11×9	reddish brown
KaS- 9	Indonesia	1974	purple	15–20	purple	0.27	10×8×4	black
KaS–16	Indonesia	1974	dark green	11–13*6	light green	0.16	10×11×7	bright brown
KaS–29	*4	1979	green	13–16	light green	0.22	6×5×4	reddish brown
KaS–32	*5	1980	green	17–21	light green	0.52	10×9×8	light brown
KaS–33	*5	1980	green	12–16	light purple	0.26	6×4×3	dark purple
KaS–34	*5	1980	green	22–24	light green	0.21	11×10×8	light brown
KaS–36	*5	1980	dark green	14–16	light green	1.02	14×12×10	light brown
KaS–37	Phili-ppines	1981	green	11–17	light green	0.09	8×6×5	light brown
KaS–38	Phili-ppines	1981	green	18–22*7	light green	0.53	11×9×6	light brown

*1 The strain numbers of winged bean were given at Ibusuki Experimental Botanic Garden, Kagoshima University[7].

*2 Average of 10–15 samples.

*3 Colors of other mature seeds are as follows: KaS–8, purplish brown; KaS–11, –30 and –39, reddish brown; KaS–40, bright brown.

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*6 Pods are flat.

*7 Without notches on the circumference of pods.

No flower buds were developed by these plants in the long-day season from May to August. The suitable season for harvesting the pods edible in raw state is the period from October to December.

The young pods collected for the analyses were divided into two portions, namely green shells (pericarp) and seeds. Some portions of green shells and immature seeds were freeze-dried separately and ground into fine powder. Samples of fresh green shells and immature seeds and/or the powdered ones were applied for the determination of the protein and trypsin inhibitor contents and for the fractionation of proteins.

An average value of the protein content or amino acid composition was obtained on the mixed sample of powdered green shells and on that of immature seeds from the four strains, KaS–21, –27, –28 and –36*1 (harvested in 1980), in a ratio of 1: 0.8: 1: 1. As a representative strain, KaS–7 harvested in 1981, was also analyzed.

2. Reagents

Cellulose tube (27/32) was purchased from Visking Co., Diaflo membrane, PM-10, from Amicon Co., crystalline trypsin from pancreas, type III, from Sigma Chem. Co. and other reagents from Nakarai Co.

3. Fractionation and determination of proteins

Proteins of green shells and immature seeds were fractionated by the method of Maes[13], as modified by Kanamori *et al*[10]. A powdered sample was packed in a column and was extracted successively with 2% sodium chloride, 30% *iso*-propanol, 4% lactic acid and 0.5% potassium hydroxide. The effluents were thus separated into four fractions. The sodium chloride fraction was then dialyzed against distilled water in cellulose tube at 4°C and separated into water-soluble and -insoluble fractions. The five fractions were separately desalted and concentrated by ultrafiltration with Diaflo membrane and lyophilized. Crude protein was determined by multiplying % N by 6.25, % N being estimated by a semi-micro Kjeldahl method.

4. Amino acid analysis of proteins

A sample was hydrolyzed with 6 N hydrochloric acid at 110°C for 24 hours in an evacuated and sealed tube. The hydrolyzate was repeatedly evaporated *in vacuo*. The residue was dissolved in a citrate buffer, pH 2.2, and analyzed on a Yanagimoto amino acid analyzer, SLC-5N, in the usual way. Tryptophan*2 was determined by the *p*-dimethylaminobenzaldehyde method[17].

5. Assay of trypsin inhibitor activity

Trypsin inhibitor activity was assayed for shells and seeds of immature fresh pods of twelve strains, harvested in 1981, and for mature dry seeds of ten strains, harvested in 1980.

A sample (about 1 g of seeds or about 2 g of green shells on a fresh weight basis) was homogenized in a chilled mortar with 15 ml of an ice-cold 0.1 M sodium chloride solution. The suspension was stirred for 1 hour with a magnetic stirrer at 4°C and then centrifuged at 12,000 rpm for 15 minutes. The precipitate was extracted two more times with the same solvent, and the extracts were centrifuged under the same conditions. The supernatant solutions were combined and made up to 50 ml with the same solvent. An appropriate aliquot was assayed for the inhibitor activity according to the method of Kanamori *et al.*[9], with trypsin activity of a sample corrected. One inhibitor unit was defined as the amount of inhibitor required for complete inhibition of 1 mg of crystalline trypsin.

Protein was determined by the modified Lowry method[6].

Results and Discussion

1. Proteins in green shells and immature seeds

The protein contents of green shells and immature seeds are shown in Table 2. They are considerably high and comparable to that of podded pea, *Pisum sativum* L. cv. Kinusaya[14].

The elution profile of green shell proteins from a column by the method of Maes-Kanamori was similar to that obtained on immature seed proteins (Table 3). Most proteins were extracted with 2% sodium chloride and 0.5% potassium hydroxide, and minute amounts of proteins with 30% *iso*-propanol and 4% lactic acid. Green shells and immature seeds have a similar protein

*1 The strain numbers of winged bean were given at Ibusuki Experimental Botanic Garden, Kagoshima University[7].

*2 The analysis was performed by Ms. K. Tajiri, to whom the authors are indebted.

Table 2. Moisture and protein contents of green shells and immature seeds of winged bean

Sample	Moisture (%)	Protein (%) in	
		Fresh matter	Dry matter
Green shells			
Mixture*	89.6	2.5	24.7
KaS–7	89.8	2.0	19.8
Immature seeds			
Mixture*	83.5	6.1	37.0
KaS–7	83.4	5.8	34.7

* Mixture of powdered samples of four strains, KaS–21, –27, –28 and –36.

Table 3. Relative amounts of proteins fractionated from green shells and immature seeds of winged bean

Fraction	Mixture*		KaS–7	
	Shells	Seeds	Shells	Seeds
2% NaCl (%)	54.83	52.71	54.65	52.02
Water-soluble (%)	6.95	15.55	3.61	14.15
Water-insoluble (%)	6.21	17.67	4.47	8.59
3% *iso*-PrOH (%)	2.09	4.48	0.32	0.92
4% Lactic acid (%)	3.18	4.51	2.73	4.56
0.5% KOH (%)	18.14	28.53	7.56	9.97
Total (%)	78.24	90.24	65.27	67.47

* Mixture of powdered samples of four strains, KaS–21, –27, –28, and –36.

composition. The main proteins are albumins, globulins and glutelins, as are usual with leguminous seed proteins. Much crude protein of the sodium chloride fraction was lost at the step of dialysis to separate the water-soluble and -insoluble fractions. It happened in both the cases of green shells and immature seeds. No reasonable explanation would be found except that such lower recovery was in part due to the occurrence of non-protein nitrogen in the sodium chloride fraction.

Tables 4 and 5 show the amino acid composition of the fractionated proteins. The proteins of green shells and immature seeds have somewhat similar amino acid pattern: the contents of acidic amino acids and lysine are high and those of sulfur-containing amino acids are low. These values are in fairly good agreement with the reported ones for young pods[5]. Egg scores estimated for some proteins are shown in Tables 4 and 5, the limiting amino acids being the sulfur-containing amino acids in all the cases.

The results described above show that young pods contain proteins of good quality comparable to those of podded pea, as compared with other vegetables.

2. Trypsin inhibitor content

The trypsin inhibitor activity was analyzed for green shells and immature seeds, using fresh samples. The results are expressed as unit/g of fresh matter and unit/g of protein in Tables 6 and 7, in which they are arranged in ascending order of the protein content and the inhibitor activity. Trypsin inhibitor activities of green shells and immature seeds are different to a great extent among different strains. In the case of immature seeds, the strain having a higher protein content tends to

Table 4. Amino acid composition of proteins fractionated from green shells of winged bean (weight percent of each amino acid in total ones)

Amino acid	Whole shells		Water-soluble		Water-insoluble		*iso*-PrOH		Lactic acid		KOH	
	Mix*	KaS-7	Mix*	KaS-7	Mix*	KaS-7	Mix*	KaS-7	Mix*	KaS-7	Mix*	KaS-7
Lys	8.3	9.1	5.0	7.2	6.2	8.3	6.4	5.1	8.0	7.6	6.1	5.9
His	3.6	2.8	2.2	2.2	2.4	3.2	1.9	1.0	3.1	2.5	2.1	1.2
Arg	7.7	5.0	4.1	6.1	6.3	8.4	4.5	6.5	7.0	8.1	5.9	7.2
Asp	13.2	33.0	11.0	12.0	13.7	10.8	11.2	16.3	10.6	10.4	10.1	13.0
Thr	8.0	4.2	6.4	7.6	5.4	5.9	6.2	5.8	5.0	5.3	5.2	6.2
Ser	5.7	4.3	7.0	6.1	6.5	5.5	7.6	7.4	6.2	6.1	6.0	6.7
Glu	10.1	8.6	12.0	10.6	10.2	11.6	11.3	13.0	11.9	10.7	12.5	6.7
Pro	6.2	3.5	7.7	5.9	7.1	4.5	7.1	5.9	6.4	7.0	7.1	6.2
Gly	4.5	3.4	7.5	5.4	5.0	5.4	6.2	5.9	5.9	6.3	5.2	6.2
Ala	5.6	3.8	7.1	5.1	6.4	7.1	6.4	4.9	6.1	6.1	7.3	6.8
Cys	0.1	0.7	2.1	1.6	trace	0.6	trace	trace	trace	1.4	trace	0.1
Val	5.5	5.0	6.9	6.4	5.2	4.4	6.2	6.2	5.9	5.1	5.7	6.9
Met	0.8	0.9	0.6	0.6	1.6	1.5	1.2	1.5	1.5	1.3	1.9	1.4
Ile	4.5	4.1	2.6	4.3	4.1	4.4	4.7	4.4	4.7	5.3	4.5	5.8
Leu	7.5	5.9	5.3	7.9	8.4	7.9	9.1	8.3	8.3	8.1	9.7	10.5
Tyr	4.1	2.2	4.2	4.9	5.3	4.1	4.6	2.9	4.5	4.0	4.2	2.1
Phe	4.6	3.5	8.3	6.1	6.2	4.5	5.4	4.9	4.9	4.7	6.5	6.1
Trp	—	—	—	—	—	1.9	—	—	—	—	—	1.0
Egg score						45						31

*Mixture of powdered green shells of four strains, KaS-21, -27, -28 and -36.

show higher inhibitor activity. The inhibitor content, unit/g of fresh matter, is positively correlated with the protein content in immature seeds ($r=0.96$, $p<0.01$), but not in green shells.

The inhibitor content of immature seeds is likely to be higher than that of green shells in the strains having the high inhibitor content of immature seeds (KaS-5, -32, -34, -36 and -38). This tendency is opposite in the strains having the low inhibitor content of immature seeds (KaS-6, -7, -9 and -16).

The strain KaS-36 is a highly exceptional one introduced from Research Institute of Tropical Agriculture, College of Agriculture, University of the Ryukyu and cultivated at Ibusuki Experimental Botanic Garden. Its seeds are larger (Table 1) and extremely higher in the protein content (Table 7) than those of other strains, and much trypsin inhibitor is found in immature seeds, though it is otherwise in green shells.

A highly positive correlation between the protein and trypsin inhibitor contents of immature seeds suggests that proteins and trypsin inhibitors of the seeds increase with fluctuations during maturation of the seeds. As we estimated the degree of maturation of pods by the naked eye in this work, we can not discuss further on the differences of the inhibitor activity between green shells and immature seeds and among different strains. Another experiment is considered to be necessary, in which the various stages of maturation of pods are followed by definite criteria.

Table 8 shows the protein content and trypsin inhibitor activity of mature seeds. In the table, the results are arranged in ascending order of the protein content and the inhibitor activity. The protein and inhibitor contents of mature seeds are much higher than those of immature seeds, as

Table 5. Amino acid composition of proteins fractionated from immature seeds of winged bean (weight percent of each amino acid in total ones)

Amino acid	Whole seeds		Water-soluble		Water-insoluble		*iso*-PrOH		Lactic acid		KOH	
	Mix*	KaS-7	Mix*	KaS-7	Mix*	KaS-7	Mix*	KaS-7	Mix*	KaS-7	Mix*	KaS-7
Lys	5.5	8.3	4.5	8.0	8.5	7.7	4.9	3.9	9.7	9.0	6.6	5.6
His	3.4	5.1	2.7	3.3	2.2	3.3	1.6	1.2	3.0	2.5	1.3	2.6
Arg	4.6	10.3	5.8	7.9	6.8	8.0	5.0	5.8	7.5	8.3	5.2	7.5
Asp	28.2	10.7	11.7	12.7	10.0	11.4	12.0	11.7	11.2	11.7	13.1	11.4
Thr	4.4	10.1	4.4	4.3	4.3	5.0	5.3	5.7	3.9	4.7	4.8	4.7
Ser	5.1	4.6	6.0	7.2	4.6	5.8	7.3	6.1	5.5	5.7	5.2	6.2
Glu	8.0	11.4	15.3	10.4	14.3	11.7	12.4	12.1	15.8	14.0	12.9	15.1
Pro	5.1	5.5	7.8	5.8	6.8	6.2	8.6	8.5	7.5	6.6	8.1	6.6
Gly	4.1	4.1	4.8	4.3	4.8	4.5	5.9	6.8	4.5	4.5	5.0	4.9
Ala	8.1	4.4	5.0	5.4	7.1	4.1	5.3	4.3	4.4	4.6	4.8	5.1
Cys	0.1	0.6	0.2	1.7	0.8	0.7	1.0	7.1	trace	1.5	1.1	0.1
Val	5.4	4.8	5.6	5.8	5.5	5.5	6.7	4.3	4.7	4.4	6.3	5.4
Met	0.3	0.4	1.4	0.7	1.5	0.6	1.1	1.4	0.8	0.9	1.2	1.0
Ile	4.2	4.3	5.2	4.4	4.7	5.0	5.1	5.5	4.5	4.8	4.7	4.9
Leu	6.4	7.4	9.2	6.7	8.3	8.3	7.7	5.9	8.4	7.6	8.3	8.9
Tyr	3.1	3.2	4.2	4.5	4.3	4.8	3.9	4.1	3.8	4.6	5.3	4.3
Phe	4.0	4.8	6.2	5.1	5.5	5.6	6.2	5.6	4.8	4.6	6.1	5.0
Trp	—	—	—	1.8	—	1.8	—	—	—	—	—	0.7
Egg score				52		27						25

* Mixture of powdered immature seeds of four strains, KaS-21, -27, -28 and -36.

Table 6. Protein and trypsin inhibitor contents of green shells of winged bean

Protein (mg/green shell*1, g)	Inhibitor, specific activity	
	(unit/green shell*1, g)	(unit/protein, g)
4.5 (KaS-34)*2	0.06 (KaS-37)*2	10 (KaS-37)*2
5.2 (KaS-29)	0.14 (KaS-33)	21 (KaS-33)
5.4 (KaS- 7)	0.19 (KaS-34)	22 (KaS-36)
5.7 (KaS-16)	0.26 (KaS- 6)	41 (KaS- 6)
6.1 (KaS-37)	0.30 (KaS- 5)	43 (KaS-34)
6.1 (KaS-38)	0.34 (KaS-36)	45 (KaS- 5)
6.5 (KaS- 6)	0.38 (KaS-16)	51 (KaS-32)
6.6 (KaS-33)	0.38 (KaS- 9)	54 (KaS- 9)
6.8 (KaS- 5)	0.42 (KaS-32)	67 (KaS-16)
7.1 (KaS- 9)	0.43 (KaS- 7)	80 (KaS- 7)
8.3 (KaS-32)	0.45 (KaS-29)	86 (KaS-29)
15.3 (KaS-36)	0.82 (KaS-38)	134 (KaS-38)

*1 Fresh weight.

*2 The figures in parentheses represent the strain numbers of winged bean.

clarified by comparison between Tables 7 and 8. Except for two strains, KaS–11 and –16, the strain having the higher protein content tends to be higher in the inhibitor content. The inhibitor content is positively correlated with the protein content ($r=0.86$, $p<0.01$). There is no significant difference in the inhibitor activity, unit/g of protein, among ten strains excepting the two strains, KaS–11 and –16. It is likely that trypsin inhibitor activities, unit/g of protein, increase to a similar level, at maturity of the seeds, in most of the strains.

The inhibitor contents of immature and mature seeds of an identical strain are compared in Table 9 made up from Tables 7 and 8. The inhibitors apparently increase with the increasing content of protein during maturation of the seeds in all the strains. The strain KaS–36 is an exceptional one which shows the high inhibitor activity at the immature stage. The detailed changes in the inhibitor activity are now being studied in another experiment in which seeds of the same plant

Table 7. Protein and trypsin inhibitor contents of immature seeds of winged bean

Protein (mg/immature seed*1, g)	Inhibitor, specific activity	
	(unit/immature seed*1, g)	(unit/protein, g)
10.6 (KaS– 7)*2	0.12 (KaS– 6)*2	9 (KaS– 6)*2
11.2 (KaS–29)	0.15 (KaS–37)	9 (KaS–37)
12.4 (KaS–33)	0.19 (KaS–16)	10 (KaS– 9)
13.2 (KaS– 6)	0.19 (KaS– 9)	12 (KaS–16)
14.3 (KaS–34)	0.24 (KaS– 7)	23 (KaS– 7)
15.9 (KaS–16)	0.46 (KaS–33)	37 (KaS–33)
16.5 (KaS–37)	0.51 (KaS–29)	41 (KaS– 5)
18.8 (KaS– 9)	0.84 (KaS–34)	46 (KaS–29)
23.9 (KaS– 5)	0.97 (KaS– 5)	66 (KaS–34)
36.8 (KaS–32)	4.70 (KaS–32)	128 (KaS–32)
38.0 (KaS–38)	7.07 (KaS–38)	186 (KaS–38)
65.8 (KaS–36)	19.06 (KaS–36)	290 (KaS–36)

*1 Fresh weight.

*2 The figures in parentheses represent the strain numbers of winged bean.

Table 8. Protein and trypsin inhibitor contents of mature seeds of winged bean

Protein (mg/mature seed*1, g)	Inhibitor, specific activity	
	(unit/mature seed*1, g)	(unit/protein, g)
124.5 (KaS–29)*2	8.19 (KaS–11)*2	47 (KaS–11)*2
134.9 (KaS– 8)	10.74 (KaS–16)	57 (KaS–16)
137.9 (KaS–30)	14.27 (KaS–30)	103 (KaS–30)
157.5 (KaS–33)	18.17 (KaS–29)	144 (KaS–36)
158.9 (KaS–36)	22.95 (KaS–36)	146 (KaS–29)
163.5 (KaS– 7)	23.60 (KaS– 8)	158 (KaS– 7)
173.1 (KaS–40)	25.23 (KaS–33)	160 (KaS–33)
175.2 (KaS–11)	25.80 (KaS– 7)	164 (KaS–40)
186.5 (KaS–39)	28.42 (KaS–40)	172 (KsS–39)
188.0 (KaS–16)	32.05 (KaS–39)	175 (KaS– 8)

*1 Fresh weight.

*2 The figures in parentheses represent the strain numbers of winged bean.

Table 9. Comparison of trypsin inhibitor content of immature seeds with that of mature seeds

Strain number	Inhibitor, specific activity					
	(unit/seed*, g)			(unit/protein, g)		
	Immature	Mature	Mature / Immature	Immature	Mature	Mature / Immature
KaS-16	0.19	10.74	56.5	12	57	4.8
KaS- 7	0.24	25.80	107.5	23	158	6.9
KaS-33	0.46	25.23	54.9	37	160	4.3
KaS-29	0.51	18.17	35.6	46	146	3.2
KaS-36	19.06	22.95	1.2	290	144	0.5

* Fresh weight.

are assayed for the inhibitor activity at the various stages of maturation after flowering and fruiting.

From the results described above, we can conclude as follows. Young pods of winged bean contain proteins of good quality comparable to those of podded pea and soybean, as compared with many other vegetables. A nutritional advantage of young pods over mature seeds is the low activity of trypsin inhibitor. Immature pods can be served as delicious salad or cooked food by boiling only for a short period. Dry hard mature seeds must be soaked in water prior to cooking. Soaking process necessary to make seeds sufficiently tender and to improve their digestibility is time-consuming[4,16]. Modified technique to remove this defect was proposed for tendering seeds[15].

Young pods are harvested in the season when we can not obtain enough other leguminous crops as vegetables. The winged bean is, therefore, promising as a vegetable crop in this country and as a special product of Kagoshima Prefecture, comparable to podded pea.

Summary

The protein contents of green shells and immature seeds of winged bean, *Psophocarpus tetragonolobus* (L.) DC., were considerably high. The proteins mainly consisted of albumins, globulins and glutelins and had a high lysine content, similarly as with many other leguminous proteins. The proteins, like those of podded pea, *Pisum sativum* L. cv. Kinusaya, can be utilized as good quality proteins.

The trypsin inhibitor activities of green shells and immature seeds were different to a great extent among different strains. The inhibitor level in green shells was generally lower than that of immature seeds. The inhibitor contents of both the immature and mature seeds were positively correlated with the protein contents. The trypsin inhibitor of the seeds increased greatly with maturation of the seeds.

Young pods can be served as delicious salad or cooked food by boiling only for a short period. They are harvested in the season when we can not obtain enough other leguminous crops as vegetables. The winged bean is, therefore, promising as a vegetable crop in this country and as a special product of Kagoshima Prefecture, comparable to podded pea.

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