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Investigations of Antinutritional Factors of the Winged Bean *(Psophocarpus tetragonolobus)*

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ABSTRACT

*Twelve varieties of winged bean (*Psophocarpus tetragonolobus*) were analyzed for tannin, phytic acid, trypsin inhibitor, chymotrypsin inhibitor, α-amylase inhibitor and lectin. Tannin contents ranged from 1·35–6·75 mg/g of bean. Phytic acid contents ranged from 7·77–12·03 mg/g of bean and phytate-phosphorus represented a considerable percentage of the total phosphorus (44·3–54·8%). Chymotrypsin inhibitory activities were about twofold higher than trypsin inhibitory activities. α-amylase inhibitory activities could not be detected in any of the winged bean varieties used in this study. Hemagglutinating activities were observed with respect to all types of trypsinized human erythrocytes (A, B and O). However these activities disappeared completely after heating in a boiling water bath for 10 min.*

INTRODUCTION

Winged bean (*Psophocarpus tetragonolobus*) has attracted much attention as a protein food source in recent years. All parts of this plant are edible and

high in protein and oil contents. Like other legumes, however, winged bean contains several constituents which have adverse effects on enzyme activity, digestibility, nutrition and health.

Tannins and phytic acid are common constituents of plant tissue. Tannins may reduce protein quality by directly complexing with food proteins and may interfere with iron absorption (Radhakrishnan & Sivaprasad, 1980). Phytic acid and its derivatives may decrease the availability of essential dietary minerals (Maga, 1982). The inhibitors of digestive enzymes are generally present in seeds of legumes. de Luman & Salamat (1980) reported that winged bean had the highest trypsin inhibitory activity among several beans studied by them. Jaffé & Korte (1976) reported the presence of α-amylase inhibitor in winged beans. Lectins are carbohyrate-binding, cell-agglutinating proteins widely distributed in legumes and many other species. The occurrence and some properties of lectins in raw winged beans were reported (Pueppke, 1979).

We reported the contents of nutritional constituents in the seed and other parts of winged beans (Ibuki *et al.*, 1983). The purpose of this study is to obtain additional information on the quantitative composition of the antinutritional factors in the seeds of winged beans. Tannin and phytic acid contents, inhibitory activities against trypsin, chymotrypsin and α-amylase, and hemagglutinating activity were analyzed. In this paper, the results from twelve different varieties and the effect of heat treatment on the hemagglutinating activity are reported.

MATERIALS AND METHODS

Materials

Mature winged bean seeds of four Papua New Guinea, four Indonesia and four Okinawa (Japan) varieties were used. The Okinawa varieties were experimentally cultivated in the Okinawa prefecture of Japan and supplied by the Okinawa Branch of the Tropical Agriculture Research Center. The seeds were ground into fine powder and defatted with *n*-hexane. The defatted powder was used as a sample for analyses except for the measurement of tannin and phytic acid contents.

Analytical methods

Tannin content was determined by a vanillin-HCl method of Burns (1971). Phytic acid was isolated by the method of Ogawa *et al.* (1979). Phytate-phosphorus and total phosphorus contents were determined by Allen's

method (1940) after digestion of the sample with 2 N HCl and perchloric acid, respectively. Phytic acid content was estimated by multiplying the amount of phytate-phosphorus by 3·55 based on the empirical formula $C_6P_6O_{24}H_{18}$. The trypsin and chymotrypsin inhibitor assays were carried out using N-benzoyl-DL-arginine-*p*-nitroanilide as substrate for bovine trypsin and benzoyl-L-tyrosine-*p*-nitroanilide for bovine α-chymotrypsin according to the method described by Ibuki *et al.* (1983).

The inhibitory activity against porcine pancreatic α-amylase was measured by the iodine staining method of Kotaru *et al.* (1985). Two g of defatted powder was extracted with 10 ml of distilled water for 2 h and centrifuged at 6000 × g for 30 min. The supernatant was adjusted to pH 4 and heated at 70°C for 15 min to inactivate any amylase present. The solution was clarified by centrifugation and neutralized, and then used for α-amylase inhibitor assay.

Hemagglutinating activity was measured by a serial two-fold dilution method on microtiter plate using a 4% suspension of trypsinized human erythrocytes. Two g of defatted powder was extracted with 20 ml of phosphate-buffered saline (PBS; 10 mM phosphate buffer containing 0·15 M NaCl, pH 7·0) at 4°C overnight and centrifuged at 6000 × g for 30 min. The supernatant was recentrifuged at 100 000 × g for 30 min and then used as the test solution for the hemagglutinating activity assay. The agglutination was determined after incubation at 37°C for 1 h. The effect of heat treatment on hemagglutinating activity was also investigated. The test solution for hemagglutinating activity assay was heated in a boiling water bath for 10 min. Immediately after cooling in ice, it was used in the agglutinating test.

Protein concentration was determined by the Lowry-Folin method (Lowry *et al.*, 1951) with bovine serum albumin as a standard.

All analyses were carried out on triplicate samples.

RESULTS AND DISCUSSION

The tannin contents of the seeds of four Okinawa (Japan), four Papua New Guinea and four Indonesia varieties of winged bean are shown in Table 1. A fivefold variation in tannin content (1·35–6·75 mg/g of bean) was observed, while Tan *et al.* (1983) reported a 25-fold variation (0·3–7·5 mg/g of bean). It is unclear what level of tannin would be noticeably harmful. However, it might be expected that any antinutritive influence of the tannin would be of little significance, because winged beans are high in protein. Although Tan *et al.* (1983) did not indicate the seed coat color of the varieties tested, we attempted to evaluate seed coat color and tannin content but observed no

TABLE 1
Tannin, Phytic Acid and Total Phosphorus of the Winged Bean

Variety	*Source*	*Seed coat color*	*Tannin*	*Phytic acid*	*Phytate-phosphorus*	*Total phosphorus*	*Phytate-phosphorus as % total phosphorus*
			(mg/g of bean)				
001	Okinawa (Japan)	Brown	1·93[a]	9·27[b]	2·61	5·38	48·5
002	Okinawa (Japan)	Brown	3·28	10·76	3·03	5·85	51·8
003	Okinawa (Japan)	Brown	1·95	10·37	2·92	6·09	47·9
004	Okinawa (Japan)	Brown	2·18	9·16	2·58	5·51	46·8
1014	Papua New Guinea	Brown	1·35	12·03	3·39	6·22	54·5
2826	Papua New Guinea	Brown	1·63	10·26	2·89	5·34	54·1
2891	Papua New Guinea	Brown	1·88	9·15	2·58	5·34	48·3
3154	Papua New Guinea	Brown	1·38	10·93	3·08	5·62	54·8
1	Indonesia	Brown	1·68	8·23	2·32	4·49	51·6
2	Indeonsia	Black	1·40	9·00	2·54	4·68	54·3
3	Indonesia	Brown	1·40	7·81	2·20	4·33	50·8
4	Indeonsia	Pale brown	6·75	7·77	2·19	4·94	44·3

[a] D-Catechin equivalents.
[b] Phytate-phosphorus × 3·55.

correlation between these two factors. In fact, variety 2 with a black seed coat had a lower tannin level than variety 4 with a pale brown seed coat.

The phytic acid and total phosphorus contents are also shown in Table 1. The phytic acid contents ranged from 7·77–12·03 mg/g of bean. Except for the values obtained from Indonesia varieties, these results are comparable with that of soy bean seeds and higher than many other legumes (Elkowicz & Sosulski, 1982; Kotaru *et al.*, 1986). The phytate-phosphorus contributed a substantial portion of the total phosphorus (44·3–54·8%). The phytic acid contents of Indonesia varieties were slightly lower than those of others but no significant difference was observed with the ratio of phytate-phosphorus to total phosphorus. de Rham and Jost (1979) reported that phytate in soy bean can bind to its calcium-magnesium and/or to proteins. However, the behavior of phytate as an antinutritional factor in winged beans has not been studied in detail.

Trypsin inhibitory activity (TIA) and chymotrypsin inhibitory activity (CIA) are shown in Table 2. In all winged bean specimens, CIA are about twofold higher than TIA. This observation may be accounted for by the presence of a trypsin inhibitor, which also inhibits chymotrypsin, in winged bean seed (Kortt, 1979). On the other hand, chymotrypsin inhibitor in winged bean seed has no TIA (Kortt, 1980). Recently, Shibata *et al.* (1986) reported that CIA was 2·9-fold higher than TIA in the seed of winged beans harvested in Okinawa. It is known that tannins are nonspecific inhibitors of

TABLE 2
Trypsin and Chymotrypsin Inhibitory Activities of the Winged Bean

Variety	*Trypsin inhibitory activity*	*Chymotrypsin inhibitory activity*
	(units/mg protein)[a]	
001	0·12	0·24
002	0·10	0·22
003	0·14	0·28
004	0·09	0·21
1014	0·12	0·22
2826	0·11	0·20
2891	0·12	0·23
3154	0·14	0·26
1	0·11	0·20
2	0·12	0·21
3	0·13	0·23
4	0·14	0·28

[a] One unit; the amount of inhibitor required for complete inhibition of 1 mg of enzyme.

TABLE 3
Hemagglutinating Activity of the Winged Bean (titer/mg protein)

Variety	*Type of erythrocytes*		
	A	*B*	*O*
001	200	100	100
002	110	110	110
003	94	94	94
004	94	94	94
1014	97	97	97
2826	240	120	120
2891	490	490	490
3154	130	130	130
1	210	210	210
2	220	220	220
3	220	220	110
4	480	480	240

enzymes. However, de Luman and Salamat (1980) reported that TIA, caused by the tannin, occupied only 1% of the total in winged beans. In our study, no correlation was found between tannin contents and TIA.

Jaffé and Korte (1976) examined α-amylase inhibitory activity (AIA) in winged beans by the dinitrosalicylic acid method measuring reducing sugars liberated from starch used as a substrate during digestion with α-amylase, and found AIA in the seed of winged beans harvested in Papua New Guinea. In the present study, the iodine-staining method was adopted in the measurement of AIA by considering the result of our preliminary experiment that crude extract from winged bean seed contained significant amounts of reducing sugars which may interfere with the measurement of AIA by the dinitrosalicylic acid method. Consequently, AIA could not be detected in any of the winged bean varieties used in this study.

Table 3 shows hemagglutinating activity (HA) in winged bean seeds. Winged bean lectins agglutinated all types of trypsinized human erythrocytes (A, B and O). In this study, the agglutination specificity for human erythrocyte types was classified into three patterns. Varieties 001 and 2826 showed that agglutination specificity for human erythrocytes decreased in the order, blood group $A > B = O$; varieties 3 and 4, $A = B > O$; others, $A = B = O$. Turner and Liner (1975) reported that soybean lectins have no apparent deleterious effects on animals while Higuchi *et al.* (1983) showed that winged bean lectins are toxic to rats. In the present study, a fivefold variation in HA was observed. Such variations may be of importance in selection efforts to obtain varieties with low HA

considering the toxicity of winged bean lectins. The effect of heating on HA was also investigated to inactivate winged bean lectin. After heating the test solution in a boiling water bath for 10 min, HA assay was carried out using type A erythrocyte. The boiling resulted in the disappearance of HA from all winged bean varieties used in this study. Tan *et al.* (1983) reported that lectins in winged bean seed could be inactivated easily by 5 min of autoclave treatment. These results suggest that winged bean seed may be acceptable for a food source if cooked. It should be pointed out, however, that animal feeding experiments are needed for conclusion as to the effect of heat-treatment of winged bean seed.

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