# Winged Bean (*Psophocarpus tetragonolobus*) Tannin Level, Phytate Content, and Hemagglutinating Activity

Sixteen varieties of winged bean (*Psophocarpus tetragonolobus*) were assayed for tannin level, phytate content, and hemagglutinating activity. Tannin level ranged from 0.3 to 7.5 mg of D-catechin/g of bean, phytate content ranged from 6.10 to 7.52 mg of phosphorus/g of bean, and the hemagglutinating activities of the surveyed beans ranged from 40 to 320 hemagglutinating units/mg of bean. It is found that none of the winged bean varieties surveyed contains amounts of tannin or phytate that may be nutritionally harmful. The winged bean hemagglutinating activity is stable to dry heat treatment (100 °C, 2 h) but could be destroyed completely by 5-min autoclave treatment at 120 °C, 1.05 kg/cm$^2$.

The winged bean (*Psophocarpus tetragonolobus*) is a tropical legume that is grown almost exclusively in Southeast Asia and Papua New Guinea. The seed of this legume has an exceptionally high protein content, and as such this legume has been suggested as a potential food source for the tropics (National Academy of Sciences, 1981).

Phytate and tannins are common constituents of plant tissue (National Academy of Sciences, 1973). Tannins, the phenolic compounds, are nonspecific inhibitors of enzymes and may reduce protein quality by directly complexing with food proteins. On the other hand, phytate, a *myo*-inositol hexaphosphate, has been held responsible for the commonly observed interference that many plant protein sources have on the availability of dietary minerals.

Hemagglutinins (lectins) are commonly present in seeds of legumes and many other species. They have been the subject of intensive investigation because of their possible adverse nutritional qualities, mitogenic properties, and affinity for specific blood cell antigens (Jaffe, 1980).

This paper reports a survey of the tannin level, phytate content, and hemagglutinating activity of 16 varieties of winged beans and the results of our investigation on the thermal stability of the hemagglutinating activity.

## MATERIALS AND METHODS

**Materials.** Sixteen varieties of winged beans were grown locally at the experimental farm of Agricultural University of Malaysia, Serdang, Malaysia. Soybeans were obtained from local commercial outlets. Winged bean meals were prepared by grounding the mature beans manually with a pestle and mortar, and the fine powder was stored below 0 °C in glass container before use. All chemicals are of analytical grade.

**Tannin Analysis.** The method of Burns (1971) as modified by Maxson and Rooney (1972) was used for tannin determination: 1 g of winged bean meal was extracted with 10 mL of 1% HCl in methanol for 24 h at room temperature, with mechanical shaking. After centrifugation at 10000*g* for 5 min, 1 mL of the supernatant was mixed with 5 mL of vanillin–HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% vanillin in methanol), and the absorbance was read at 500 nm after 20 min. A stock D-catechin solution was used as the standard. Values of tannin were read in mg of D-catechin/g of bean.

**Phytate Determination.** The method of Wheeler and Ferrel (1971) was used for phytate determination. To prepare the phytate extract, 500 mg of winged bean was extracted with 15 mL of 3% trichloroacetic acid for 30 min with mechanical shaking.

**Hemagglutinating Activity Assay.** A published microtiter method (Pull et al., 1978) was used for hemagglutinating activity assay. Winged bean extract was prepared by extracting 100 mg of the finely ground winged bean meal with 5 mL of 0.005 N sodium hydroxide for 4 h, with mechanical shaking. Each extract was diluted in 2-fold increments to a final dilution of 1:640 in phosphate-buffered saline. Portions of each dilution (25 μL) were transferred to wells in a microtiter plate (Abott Laboratories), and 25 μL of a 3% suspension of trypsinized type B human erythrocytes was added to each well. The titers were recorded after 3 h at room temperature.

Trypsinized type B human erythrocytes were prepared by treatment of a 3% suspension (v/v) of cells in phosphate-buffered saline for 1 h with Sigma type IX porcine pancreatic trypsin (0.1 mg mL$^{-1}$).

One hemagglutinating unit (HU) is defined as the least amount of hemagglutinin that will produce positive evidence of agglutination of 25 μL of a 3% suspension of washed, trypsinized type B human erythrocytes after 3-h incubation at room temperature.

**Determination of the Thermal Stability of Winged Bean Hemagglutinating Activity.** The thermal stability of the winged bean hemagglutinating activity was determined by assaying the residual hemagglutinating activity in the heat-treated winged bean meals of varieties 207, 188(d), and 201(a). The heat-treated winged bean meals were prepared as follows.

*Preparation of the Dry Heat Treated Winged Bean Meals.* Dry heat treated winged bean meals were prepared by heating 100 mg of the finely ground winged bean meals in an oven maintained at 100 ± 1 °C for 2 h.

*Preparation of the Autoclaved Winged Bean Meals.* The autoclaved winged bean meals were prepared by autoclaving 100 mg of the finely ground bean meals in a test tube at a thickness not exceeding 2 mm at 120 °C, 1.05 kg/cm$^2$, for 5, 10, 15, and 20 min after the desired temperature had been reached. The autoclave oven was preheated before use to minimize the time taken to reach the desired temperature (approximately 7 min).

For comparison, the effects of heat treatments on hemagglutinating activity in soybean were also examined by using the methods described above.

## RESULTS AND DISCUSSION

**Tannin Level of Winged Beans.** The tannin content of the surveyed winged beans is reported in Table I. The data show a high degree of variability in the tannin content among the winged bean specimens. The upper and lower limits of the data presented in the table reveal a 25-fold variation in tannin content (0.3–7.5 mg/g of bean). It is interesting to note that Price et al. (1980) failed to detect tannin in four varieties of winged beans (TPT-1, TPT-2, TPT-6, and Chimbu) examined.

The minimum amount of dietary tannin needed to elicit a negative growth response has not been established. It is still unclear what level of tannin would be noticeably harmful (National Academy of Sciences, 1973). Among the samples surveyed, variety 048(a) has the highest level

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Table I. Winged Bean Tannin Level, Phytate Content, and Hemagglutinating Activity[a]

| variety | native source | tannin level,[b] mg of D-catechin/g of bean | phytate content,[b] mg of phosphorus/g of bean | hemagglutinating act. HU/mg of bean |
|---|---|---|---|---|
| 207 | Malaysia | 4.2 ± 0.1 | 7.08 ± 0.32 | 320 |
| 046(b)/ba | New Guinea | 3.1 ± 0.1 | 6.88 ± 0.19 | 320 |
| 051(b) | New Guinea | 5.3 ± 0.2 | 6.76 ± 0.19 | 320 |
| 079(c)/b(a) | New Guinea | 5.7 ± 0.2 | 7.02 ± 0.18 | 80 |
| 201(a) | New Guinea | 4.1 ± 0.1 | 7.00 ± 0.16 | 80 |
| 195(b) | New Guinea | 4.6 ± 0.2 | 7.50 ± 0.34 | 320 |
| 039(a)a | New Guinea | 5.6 ± 0.2 | 7.48 ± 0.30 | 320 |
| 034(b) | New Guinea | 3.6 ± 0.1 | 7.13 ± 0.19 | 320 |
| 050(a) | New Guinea | 3.8 ± 0.2 | 7.43 ± 0.22 | 320 |
| 044(a) | New Guinea | 2.6 ± 0.1 | 6.93 ± 0.20 | 320 |
| 042(a) | New Guinea | 3.9 ± 0.2 | 6.10 ± 0.12 | 320 |
| 048(a) | New Guinea | 7.5 ± 0.2 | 7.27 ± 0.27 | 320 |
| 184(a)(b) | Thailand | 1.6 ± 0.2 | 7.48 ± 0.21 | 40 |
| 157(d) | Thailand | 0.3 ± 0.03 | 7.50 ± 0.26 | 160 |
| 188(d) | Thailand | 7.1 ± 0.2 | 6.97 ± 0.18 | 160 |
| 181(b) | Thailand | 1.5 ± 0.1 | 7.52 ± 0.25 | 320 |
| soybean | | 0 | 6.82 ± 0.34 | 320 |

[a] As-is moisture basis. [b] Mean ± standard deviation, $n = 3$.

of tannin (7.5 mg of D-catechin/g of bean). This, however, is still much lower than that found in a few representative high-tannin sorghum grains [28.3–43.2 mg of D-catechin/g of sample (Maxson and Rooney, 1972)]. Because winged beans are high in protein, it might be expected that the tannin would be of even less significance than, for example, the tannin in sorghum grains. On the basis of our limited surveyed of 16 varieties of winged beans, however, none seems to have sufficiently high levels of tannin to be of concern in nutrition. It is interesting to note, however, that de Lumen et al. (1982) showed that inclusion of the hulls (which contain more than 50% of total tannin) may adversely affect the nutritional quality of winged bean meal in chicken.

**Phytate Content of Winged Beans.** The phytate content of the surveyed winged beans is also reported in Table I. The data show only minor varietal variations in the phytate content: 6.10–7.5 mg of phytate phosphorus/g of bean. The phytate content of winged beans examined here is comparable to that found in soybean, and as such it might be expected that the phytate would not be noticeably harmful to the nutritional value of winged beans.

**Winged Bean Hemagglutinating Activity.** The hemagglutinating activities of the surveyed winged beans are reported in Table I. These data show a high degree of variability in hemagglutinating activities among the 16 winged bean varieties examined. The upper (320 HU/mg of bean) and lower (40 HU/mg of bean) limits of the data presented in Table I reveal about an 8-fold variation in hemagglutinating activity. The level of hemagglutinating activity in the soybean sample examined is comparable to that found in winged beans.

**Thermal Stability of Winged Bean Hemagglutinating Activity.** Dry heat at 100 °C for 2 h inactivated less than 5% of the hemagglutinating activities in the winged beans and the soybean sample examined. Autoclave treatment, on the other hand, is much more effective in destroying the hemagglutinating activities. In fact, 5 min of autoclave treatment at 120 °C, 1.05 kg/cm$^2$, of winged bean meals generally destroyed the hemagglutinating activities of the beans. The same treatment also destroyed 100% of the hemagglutinating activities of soybean.

**Nutritional Importance of the Winged Bean Tannin, Phytate, and Hemagglutinins.** The above studies suggested that the levels of tannin and phytate in winged beans might not be sufficiently high to be of concern in nutrition. Also, the hemagglutinins could be detoxified easily by 5-min autoclave treatment. It should be pointed out, however, that animal feeding experiments are needed for the final answer as to the nutritional significance of the findings.

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**Registry No.** Phytate, 83-86-3; D-catechin, 154-23-4.

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