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# Inhibitors of Trypsin and Chymotrypsin in Winged Bean (*Psophocarpus tetragonolobus*) Tubers

Trypsin and α-chymotrypsin inhibitors were present in winged bean (*Psophocarpus tetragonolobus* var. Chimbu) tubers. Compared with those of mature seeds, the levels of inhibitors were approximately the same. Tuber inhibitors could be easily inactivated by wet heat with more than 90% loss of activity after a 2-min heating of a water suspension of freeze-dried tubers in boiling water. The two types of inhibitors exhibited similar inactivation patterns. Affinity chromatography demonstrated that the two proteinase inhibitors are distinct and different and do not have overlapping activities. The presence of these inhibitors point to the necessity of processing winged bean tubers before consumption to eliminate these toxic factors.

A survey of underexploited tropical plants by a panel of the National Academy of Sciences (1975) brought attention to the winged bean (*Psophocarpus tetragonolobus*) as a promising source of protein and oil for both animals and humans in the humid tropics. It is interesting to note that the potential of this crop as a possible substitute for soybean was pointed out as early as 1929 by Agcaoili (1929), who published probably the first chemical composition of the mature seed.

The plant is grown and eaten in many parts of Southeast Asia and Melanesia (Burkill, 1906; Purseglove, 1968) and other parts of the tropics. The tender, green pods are the most popular part of the plant and used as a green vegetable, either raw or cooked, in most of these places. All parts of the plant are claimed to be edible and nutritious and efforts are being made to provide information on their chemical composition (Onuma Okezie and Martin, 1980). The leaves and the flowers are popular in many areas where they are eaten either raw or cooked and added to salads and soups (Claydon, 1978).

The tubers of winged bean are consumed as human food but this appears to be limited to Burma and New Guinea highlands (Claydon, 1978). It is possible that the limited use of the tuber in certain areas is due to the fact that people are not aware of the tubers because of the low yield of certain varieties and the adverse effects of tubers when eaten raw (Claydon, 1978). The tubers are reported to be eaten raw or cooked (Burkill, 1906). Chemical composition data reported so far show that the tubers have relatively much higher protein than other edible plant roots. To our knowledge, there have been no studies on antinutritional factors in the tubers. The presence of such compounds in the tubers can be very important due to its consumption in the raw state as pointed out. We are reporting here the presence of trypsin and chymotrypsin inhibitors in winged bean and their heat inactivation patterns.

## MATERIALS AND METHODS

**Seeds and Tubers.** Winged bean seeds (var. Chimbu), were supplied to us by Louis Lazaroff of the International Council on Development of Underutilized Crops, Orinda, CA. The seeds were planted in April 1979 in the University greenhouse and flowered in November, and the dried pods and tubers were harvested in the first week of Jan 1980. Periodic partial pruning by removing young shoots, flowers, and pods is claimed to increase tuber yield (Khan et al., 1977), but since the main objective was to multiply seeds, this was not done in these plants. The average yield of tuber was ~60 g/plant. The seeds used in the experiments for comparison were obtained from the same plants.

**Extraction and Determination of Trypsin and Chymotrypsin Inhibitors.** The extraction and determination of trypsin inhibitor from the seed were done as reported previously (de Lumen and Salamat, 1980). Typically, 1 g of ground sample was extracted with 20 mL of ice-cold distilled water by homogenizing for 2 min with a Potter-Elvehjem homogenizer run with an electric drill. The extract was centrifuged at 4 °C for 30 min at 13600*g* and the supernatant was used for the assay after appropriate dilution.

The whole tubers, after washing and drying, were homogenized in a blender for ~2 min without the addition of additional water and freeze-dried. This material was extracted the same way as the seeds.

Trypsin activity was measured by its hydrolysis of *p*-toluenesulfonylarginine methyl ester (TAME) at 25 °C, pH 8.1, and measuring the absorbance of *p*-toluenesulfonylarginine at 247 nm with a Cary 14 recording spectrophotometer. One unit of trypsin activity is defined as 1 μmol of product released per min. One unit of trypsin activity inhibited is defined as 1 inhibitor unit (TIU).

Chymotrypsin activity was determined by hydrolysis of benzoyl-L-tyrosine ethyl ester (BTEE) as described in the Worthington Biochemical Corp. (1977) manual. The increase in absorbance at 256 nm was recorded as described above. One unit of chymotrypsin activity is equivalent to 1 μmol of substrate hydrolyzed per min at pH 7.8 and 25 °C. One unit of enzyme activity inhibited is defined as 1 chymotrypsin inhibitor unit (CIU).

Trypsin (2× crystallized) was obtained from Sigma Chemical Co. (St. Louis, MO) while chymotrypsin was obtained from Worthington Biochemical Corp. (Freehold, NJ).

Crude fat and crude protein were carried out according to AOAC (1975) procedures. Acid detergent fiber was done according to Van Soest (1963).

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Table I. Chemical Composition of Winged Bean Tuber Used in This Study[a]

| | composition, % | |
|---|---|---|
| | wet basis | dry basis |
| moisture | 57.8 | |
| crude fat | 0.7 | 1.6 |
| crude protein (% N × 6.25) | 8.6 | 20.3 |
| acid detergent fiber (ADF) | 1.5 | 3.5 |
| carbohydrate (by difference) | 31.4 | 74.6 |

[a] Values are averages of three replicates.

Table II. Trypsin and α-Chymotrypsin Inhibitors in Winged Bean Tubers and Mature Seeds Taken from the Same Plants[a]

| | tuber | seed |
|---|---|---|
| trypsin inhibitor | 2278 ± 287 | 2086 ± 301 |
| α-chymotrypsin inhibitor | 2104 ± 312 | 2394 ± 289 |

[a] Inhibitor units per gram. Mean ± standard error of mean of four determinations. Tuber values are based on freeze-dried samples (4–6% moisture) while the seed values are based on dried seeds with ~12% moisture.

**Affinity Chromatography.** A 10-fold dilution of 5% tuber–water extract was made. Two milliliters of the diluted extract was applied to an agarose–trypsin column (Sigma Chemical Co.) with a 1.5-mL bed volume which had been washed and equilibrated with 0.05 M Tris-HCL, pH 8.0, buffer containing 0.1 M KCl and 0.02 M $CaCl_2$. The column was then eluted with the same buffer until the 280-nm absorbance was zero. The trypsin inhibitor bound to the column was eluted with 0.2 M KCl, pH 2.0. Eluates were collected (3.0 mL/tube) and assayed for protein (OD 280), trypsin, and chymotrypsin inhibitors.

**Heat Inactivation Tests.** Ten milliliters of a 5% (w/v) water suspension of freeze-dried tuber powder (100 mesh) was heated in a boiling water bath for 0, 20, 40, 60, 120, 240, and 360 s. The maximum temperature attained was noted. The mixture was cooled down to room temperature, homogenized for 2 min in an Elvehjem homogenizer, and centrifuged at 13600*g* for 30 min, and the supernatant assayed for trypsin and chymotrypsin inhibition.

## RESULTS AND DISCUSSION

The results of proximate analysis are shown in Table I. The protein content of 20.3% on a dry weight basis is relatively high compared to that of other plant tubers such as cassava. This value is within the range of values reported by Claydon (1978). The unusually high protein content of this tuber is attributed to its ability to fix nitrogen from the atmosphere. One report on the amino acid composition of the tuber protein indicated that it is superior to that of cassava (Wong, 1975), but the biological quality of the protein as determined by animal feeding experiments has not been studied. It should be pointed out that the AOAC (1975) method does not distinguish between protein and low molecular weight nitrogenous compounds such as amides and the protein may be overestimated. However, from a nutritional standpoint, low molecular weight peptides and free amino acids are as important as proteins. The values for fiber and total carbohydrates are similar to those reported by Claydon (1975), but the total carbohydrates is higher than that reported by Wong (1975).

The trypsin and α-chymotrypsin inhibitor levels in the tubers and mature seeds obtained from the same plants are compared in Table II. To our knowledge, this is the first report on the presence of such inhibitors in winged bean tubers. The levels of both types of inhibitors are similar in the seeds and tubers. The presence of proteinase inhibitors in plant storage organs such as seeds and tubers is well documented. They have been isolated mainly from Leguminosae, Graminae, and Solanaceae but also from other families as well (Dechary, 1970; Laskowski and Sealock, 1971; Leiner and Kakade, 1969; Puztai, 1967; Vogel et al., 1966). Plant proteinase inhibitors are generally small proteins having molecular weights more commonly below 20000. Inhibitors from potatoes have molecular weights below 10000 (Belitz et al., 1971) and often exist as dimers or tetramers. Potato inhibitors had been reported by Ryan et al. (1968) as mostly concentrated in the cortex of the tubers and constituted up to 10% of the soluble proteins of the tubers.

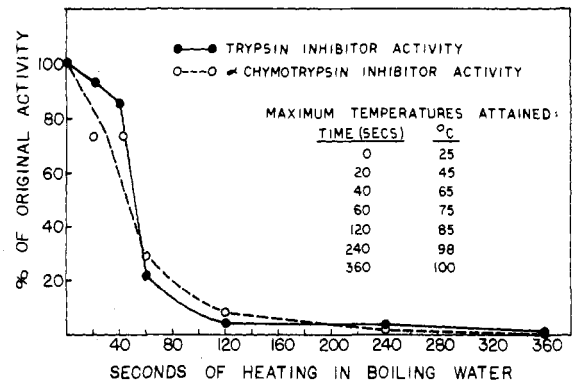


**Figure 1.** Effects of heating on trypsin and α-chymotrypsin inhibitors in winged bean tubers. Ten milliliters of a 5% (w/v) suspension of tuber powder in water was immersed in boiling water for the time periods shown. Due to the short heating times, the actual temperatures attained by the samples were measured as indicated.

Hemagglutinins, trypsin inhibitor, cyanide, and saponins had been reported in ripe seeds, but none of these toxic compounds had been studied in the tubers. It is possible that other toxic compounds may be present in the raw tuber besides these protease inhibitors. Eating raw tubers had been reported to cause nausea, and cooked roots eaten with cocoa produce severe intoxication (Claydon, 1978). The presence of protease inhibitors may account partially for these physiological effects.

Figure 1 shows the heat inactivation pattern of the inhibitors. Preliminary experiments indicated that almost all the activities were eliminated after immersing a water suspension of the freeze-dried powder in boiling water for 2 min. Therefore, shorter times of heating were investigated. The inhibitors exhibited approximately similar patterns of activity loss with roughly 25% of the original activity remaining only after 60 s of heating. The remainder of the activities were destroyed after 6 min of heating. Rapid inactivation of the inhibitors would indicate that they are probably proteins. It is to be noted that due to the short time of heating, all the samples except the longest heated (360 s) attained temperatures below 100 °C. It is evident that the tuber inhibitors can be easily inactivated by heat in contrast to the trypsin inhibitor activity (TIA) of the mature seeds which was relatively more resistant to heat (de Lumen and Salamat, 1980). Heating whole seeds for 2 h at 100 °C inactivated only 65% of TIA while heating at 60 °C did not have any effect after 2 h. However, when the hull was removed, 2 h of heating at 80 °C inactivated ~68% and almost 99% inactivation was achieved at 100 °C after 2 h. Since the heat inactivation tests were carried out on water suspensions of freeze-dried tuber powders, it is possible that the rate of loss of TIA and chymotrypsin inhibitor activity in whole tubers would be less due to a slower heat penetration.

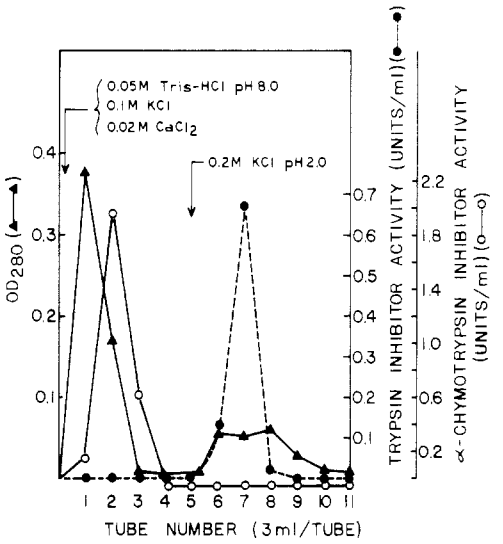


**Figure 2.** Elution patterns of trypsin and α-chymotrypsin inhibitors on an agarose–trypsin column. The column was washed and equilibrated with 0.05 M Tris-HCl, pH 8.0, buffer containing 0.1 M KCl and 0.02 M $CaCl_2$. After 2 mL of winged bean tuber extract was introduced, the column was eluted first with the above solvent and then with 0.2 M KCl, pH 2.0, as indicated.

Heating whole tubers is closer to actual cooking conditions, but this was not done because of insufficient samples. When sufficient whole tubers become available to our laboratory, heat inactivation tests will be carried out.

Trypsin and chymotrypsin inhibitors from plants are known to possess overlapping activities. Since this is the first report on the presence of these protease inhibitors in winged bean tubers, and because of the similarities in their inactivation behavior, experiments to distinguish between the two activities were carried out. Complete separation of activities were achieved when a water extract was injected into an agarose–trypsin column (Figure 2). The majority of the protein and all of the α-chymotrypsin inhibitor activity were eluted with the first solvent. No trypsin inhibitor activity was detectable in these fractions. Subsequent elution with 0.2 M KCl, pH 2.0, yielded a minor protein peak with a broad band and the trypsin inhibitor activity without α-chymotrypsin activity. These results show that the two types of proteinase inhibitors are due to two different and separate compounds. Nearly all plant inhibitors that inhibit animal or microbial proteinases have either trypsin- or chymotrypsin-specific inhibition (Ryan, 1973). These specificities are due to the presence of unique sequences in the active sites (Laskowski and Sealock, 1971). The trypsin-specific inhibitors always have either a lysine-x or arginine-x sequence at the binding sites while the chymotrypsin inhibitors usually have a leucine-x sequence. The presence of trypsin-specific and chymotrypsin-specific inhibitors in winged bean tubers is consistent with this information.

It is reported that winged bean tubers can be eaten raw or cooked like yam bean tubers (Burkill, 1906) in Burma and New Guinea highlands. Baked whole tubers can be bought in Papua New Guinea markets during tuber season while tubers raw or boiled are available in the markets of Burma. Because the tuber is eaten raw in some places, the presence of trypsin and chymotrypsin inhibitors is significant, pointing to the necessity of processing the tuber to destroy these toxic compounds. The presence of other toxic compounds in the tubers remains to be established.

In summary, we are reporting the presence of two distinct inhibitors of trypsin and α-chymotrypsin in winged bean tuber without overlapping biological activities. The levels of these inhibitors in the tubers are approximately the same as those found in the mature seeds. In contrast to the inhibitors in the seeds, the tuber inhibitors could be easily inactivated by wet heat under the conditions used in these experiments. The inactivation patterns are similar in both types of inhibitors.

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