Occurrence and Stability of Trypsin Inhibitors in Iraqi Local Legumes

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Trypsin inhibitor activity was assayed in local cultivars of broad bean, chickpea, black-eyed pea, lentil, and mung bean. Dry seeds of the tested legumes showed trypsin inhibitor activity. The inhibitor activity decreased in most seeds after soaking in water at room temperature for 24 h. However, black-eyed pea showed increased inhibitor activity after soaking. Heating the soaked legume seeds at 121 °C for 30 min brought about complete inactivation of inhibitor activity. While chickpea trypsin inhibitor retained only 17% of its activity after being heated at 80 °C for 30 min, black-eyed pea inhibitor retained 94 and 58% of its activity after heating for 30 min at 80 and 100 °C, respectively.

Trypsin inhibitors are widespread in nature, being present in many plants and animals. These naturally occurring proteins have the property of combining with and inactivating trypsin. They have been studied extensively in the Leguminosae, Solanaceae, and Gramineae, since the majority of species in these families are considered as important food sources. A great deal of research effort has been focused on the seeds of Leguminosae because they have been considered as excellent sources of protein.

Proteins inhibitory of trypsin have been reported in soybean seeds (Ham and Sandstedt, 1944; Bowman, 1944; Kunitz, 1947b; Birk, (1961), lima bean (Fraenkel-Conrat et al., 1952; Haynes and Feeney, 1967), navy bean (Wagner and Riehm, 1967), and black-eyed pea (Ventura and Filho, 1966). Several of these inhibitors are "double headed", i.e., capable of inhibiting both trypsin and chymotrypsin (Haynes and Feeney, 1967; Richardson, 1977). In addition, they occur in multiple molecular forms separable by ion-exchange chromatography and isoelectric focusing. Since these inhibitors are relatively heat stable, suitable processing treatments are required for their destruction.

Dry legume seeds have long been recognized as important sources of protein in Iraq. *Vicia faba* (broad bean), *Lens esculenta* (lentils), *Cicer arietinum* (chickpea), *Vigna sinensis* (black-eyed pea), and *Phaseolus aureus* (mung bean) are largely consumed by various segments of society. In spite of the importance of local cultivars of these legumes, few attempts have been reported on their content of naturally occurring antinutritional factors. The purpose of this study was to determine the levels of trypsin inhibitors in Iraqi local legumes and the effect of processing treatments on their inhibitory activity.

EXPERIMENTAL SECTION

Materials. Legume seeds were obtained from the Department of Agronomy, Ministry of Agriculture, Baghdad, Iraq.

Bovine trypsin (2× crystallized) and bovine serum albumin were from Fluka AG. All other compounds were of reagent grade, and deionized water was used.

Extraction of Inhibitor. Dry legume seeds were cracked in a mortar and pestle and ground into flour in an electrical coffee grinder. The flour was extracted with deionized water (1:5 w/v) for 2 h at room temperature by using a magnetic stirrer. The extract was centrifuged at 10000*g* for 30 min, the pellet was discarded, and the supernatant was saved. The pH of the supernatant was adjusted to 4.0 with 1 N HCl. At this pH the suspension was heated at 60 °C for 10 min, cooled rapidly in an ice bath, and centrifuged at 10000*g* for 30 min. The supernatant was adjusted to pH 7.0 with 1 N NaOH and the pellet discarded.

Soaked samples were prepared by soaking 50 g of dry seeds in 1 L of deionized water for 24 h at room temperature. The cooking treatment involved heating of soaked seeds in a pressure cooker for 30 min at 121 °C. Seeds from these treatments were drained and ground in a mortar and pestle. The ground material was extracted as described for the dry seeds.

Inhibitor Activity. Trypsin inhibitor activity was determined by using the casein digestion method of Kunitz (1947a) at pH 7.8 and 35.0 °C. The change in absorbance at 280 nm was measured by a Pye Unicam SP 1800 spectrophotometer. One inhibitory unit is defined as the amount of inhibitor that will produce an absorbance decrease of 0.001 in 1 min when carried out under the assay conditions.

Protein Determination. Protein concentration in all extracts was determined by the procedure of Lowry et al. (1951) using bovine serum albumin as a standard.

Heat Stability. The heat stability of trypsin inhibitors in dry chickpea and soaked black-eyed pea extracts was studied (selected because they had the highest inhibitor activity among dry and soaked samples, respectively). Extracts at pH 7.0 were incubated at 80 and 100 °C for 5, 10, 15, 20, 25, and 30 min, cooled rapidly in an ice bath, and assayed for remaining inhibitor activity by using the general assay conditions described above.

RESULTS AND DISCUSSION

All dry legume samples showed trypsin inhibitor activity (Table I). The presence of variable amounts of inhibitor activity appears to be in agreement with data reported by various authors (Richardson, 1977; Whitaker and Feeney, 1973). Chickpea and broad bean contained the largest and smallest amounts of inhibitor activity, respectively. Al-Nouri (1979) studied 12 broad bean cultivars and found considerable variation in trypsin inhibitor activity among them. The activity varied from 50 to 70% inhibition of bovine trypsin. Heating of the extracts for 10 min at 60 °C at pH 4.0 was effective in removing the turbid appearance of extracts. Thus the preparations obtained were clear and quite suitable for analysis.

Soaking appeared to reduce the levels of trypsin inhibitor activity in all legumes examined except black-eyed pea (Table I). While the inhibitor activity disappeared in broad beans, an increase of 2-fold was observed in black-eyed peas after soaking. However, cooking for 30 min at 121 °C destroyed the inhibitor(s) in all samples (Table I). The concentration of trypsin inhibitors in many legumes fluctuates during germination, development, and maturation (Richardson, 1977). Although soaking and germination reduce proteinase inhibitor content in most legume

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Table I. Trypsin Inhibitor Activity in Legume Partially Purified Extracts[a]

cultivar	protein in extract, mg/mL	inhibitor act., units/mg of protein[b]
chickpea		
dry	4.8	46.5
soaked	2.1	19.3
soaked and cooked	1.9	0
broad bean		
dry	7.5	4.0
soaked	1.8	0
soaked and cooked	c	
lentil		
dry	5.5	40.0
soaked	3.4	2.9
soaked and cooked	3.5	0
black-eyed pea		
dry	5.9	33.9
soaked	3.2	68.8
soaked and cooked	2.9	0
mung bean		
dry	5.2	28.7
soaked	2.1	9.7
soaked and cooked	2.0	0

[a] Legume seed flour extracted with deionized water as described in the text under Extraction of Inhibitor. [b] One inhibitor unit is that amount of inhibitor that produced an absorbance decrease at 280 nm of 0.001 in 1 min at pH 7.8 and 35.0 °C. [c] Not included since the inhibitor activity disappeared after soaking.

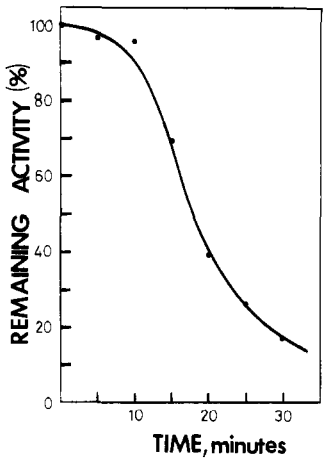

Figure 1. Heat stability of chickpea trypsin inhibitor. The inhibitor was incubated at 80 °C for various time intervals followed by rapid cooling and assay for the remaining activity.

seeds, kidney bean seeds show higher inhibitor content during germination (Richardson, 1977).

Since chickpea and black-eyed pea had the largest amount of trypsin inhibitor activity among dry and soaked seeds, respectively, they were used to study thermal stability of the inhibitor. Chickpea inhibitor retained 17% of its original activity after being heated at 80 °C for 30 min (Figure 1). However, black-eyed pea inhibitor retained all of its original activity when heated at 80 °C for 5 min and 94% after heating for 30 min at the same temperature (Figure 2). It was observed that 58% of the inhibitor activity was retained after heating at 100 °C for 30 min. Thus the results suggest that black-eyed pea inhibitor is more heat stable than chickpea inhibitor. Heat stability is a common characteristic of proteinase inhibitors regardless of their sources (Feeney and Allison, 1969; Richardson, 1977). The bovine basic pancreatic inhibitor

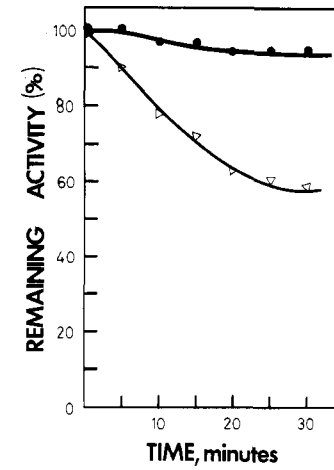

Figure 2. Heat stability of soaked black-eyed pea trypsin inhibitor. The inhibitor was incubated at 80 (○) and 100 °C (△) for various time intervals followed by rapid cooling and assay for the remaining activity.

retained all of its original activity after being heated in 2.5% trichloroacetic acid to 80 °C. It retained 50% of the original activity after 24 h at room temperature and pH 12. While heating at 80 °C for 5 min had no effect on inhibitor activity in chickpea, 50% of the original activity was retained after boiling at 100 °C for 5 min (Belew et al., 1975). Trypsin inhibitors in faba beans retained 20% of their original activity after being heated for 60 min in a boiling water bath (Bhatty, 1975).

It appeared that the inhibitor activity of chickpea and black-eyed pea was not affected by temperature and duration of heat treatment in the same magnitude. This may be due to the differences in inhibitor activity levels, nature of the inhibitors present, or composition of these local legumes. However, none of these parameters was studied extensively yet. Further investigations are required to achieve a better understanding of the biochemical characteristics and physiological role of these inhibitors.

LITERATURE CITED

Al-Nouri, F. F. Ph.D. Thesis, College of Agriculture, University of Baghdad, 1979.
Belew, M.; Porath, J.; Sundberg, L. *Eur. J. Biochem.* **1975**, *60*, 247.
Bhatty, R. S. *Can. J. Bot.* **1975**, *53*, 3075.
Birk, Y. *Biochim. Biophys. Acta* **1961**, *54*, 378.
Bowman, D. E. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 139.
Feeney, R. E.; Allison, R. G. "Evolutionary Biochemistry of Proteins"; Wiley: New York, 1969.
Fraenkel-Conrat, H.; Bean, R. C.; Ducay, E. D.; Olcott, H. S. *Arch. Biochem. Biophys.* **1952**, *37*, 393.
Ham, W. E.; Sandstedt, R. M. *J. Biol. Chem.* **1944**, *154*, 505.
Haynes, R.; Feeney, R. E. *J. Biol. Chem.* **1967**, *242*, 5378.
Kunitz, M. *J. Gen. Physiol.* **1947a**, *30*, 291.
Kunitz, M. *J. Gen. Physiol.* **1947b**, *30*, 311.
Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.
Richardson, M. *Phytochemistry* **1977**, *16*, 159.
Ventura, M. M.; Filho, J. X. *Acad. Bras. Cienc. Anais.* **1966**, *38*, 553.
Wagner, L. P.; Riehm, J. P. *Arch. Biochem. Biophys.* **1967**, *121*, 672.
Whitaker, J. R.; Feeney, R. E. "Toxicants Occurring Naturally in Foods"; National Academy of Sciences: Washington, DC, 1973; Chapter 13.

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