THE INHIBITION OF DIGESTIVE ENZYMES BY POLYPHENOLS OF CHICKPEA (*CICER ARIETINUM* L.) AND PIGEONPEA [*CAJANUS CAJAN* (L.) MILLSP.]

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ABSTRACT

The role of seed polyphenols of chickpea and pigeonpea in enzyme inhibitory activities of trypsin, chymotrypsin and α-amylase was examined by *in vitro* methods. Polyphenols were extracted from whole seed samples by refluxing with acidified methanol. Chickpea polyphenols inhibited trypsin more than chymotrypsin whereas pigeonpea polyphenols did not show such a distinction. on the basis of the average percent enzyme inhibition in the various cultivars studied, pigeonpea polyphenols were found to be more effective than those of chickpea. The addition of polyvinylpyrrolidone (PVP) remarkably reduced the enzyme inhibitory property of the polyphenols. The polyphenolic compounds of cultivars with dark testa color showed more inhibitory activity than those with light testa color in both chickpea and pigeonpea. Since carbohydrates and proteins are the principal seed constituents of these pulse crops, the observed results have nutritional implications in terms of utilization of these constituents.

INTRODUCTION

Chickpea and pigeonpea are the major pulse crops in India. They occupy an important place in human nutrition providing a rich source of dietary proteins. Considering the importance of food legumes in human nutrition, in recent years increasing emphasis has been placed on their nutritional quality characteristics as well as on their acceptable cooking and organoleptic qualities. Most of the food legumes are known to accumulate antimetabolic and toxic constituents during the course of seed development. Several toxic factors in grain legumes have been reported (1).

Polyphenols (popularly known as tannins) have been the subject of several biochemical and nutritional investigations on cereals and grain legumes (2). Condensed tannins have been reported to occur in some grain seeds that are important as human food or animal feed (3,4). Price et al analysed 10 cultivars each of cowpeas, chickpeas, pigeon-

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peas and mung beans for condensed tannin content and reported tannin content concentrations ranging from 0 to 0.7% for cowpeas, from 0 to 0.2% for pigeonpeas and essentially no tannin in chickpeas and mungbeans (5). Elias et al demonstrated the effects of seed coat color on the protein quality of beans and suggested the possible role of heat resistant tannins and other polyphenols as trypsin inhibitors (6). Trypsin inhibitor activity in heat-processed winged bean was also attributed to the presence of tannin (7) and in field beans it has been shown that the digestive enzyme inhibition caused by extracts of the seed coat of colored varieties is due to the presence of polyphenols (8). In another study, on chickpeas, it was reported that the levels of polyphenolic compounds are significantly and negatively correlated with in vitro protein digestibility and the levels of protease inhibitors (9). This paper describes the inhibition of digestive enzyme activities by polyphenols of chickpea and pigeonpea seeds.

MATERIALS AND METHODS

Seed samples of chickpea and pigeonpea cultivars were obtained from the ICRISAT Pulse Improvement Program. Chickpea cultivars were grown at Hissar, India (29°N) during the postrainy season of 1977-78 and pigeonpea cultivars were grown at ICRISAT Center, Patancheru, near Hyderabad (17°N) during the 1977-78 rainy season. Seed materials were ground to a fine powder in a Udy cyclone mill using a 0.4 mm screen and were defatted in a Soxhlet apparatus using n-hexane. Trypsin and chymotrypsin were purchased from Worthington Biochemical Corporatin, New Jersey, USA. α-amylase (hog pancreas) was purchased from Sigma Chemical Co., USA. All other chemicals and solvents used were of analytical grade.

Extraction and estimation of polyphenols

The polyphenolic compounds were extracted from 500 mg defatted meal by refluxing (boiling) for 2 h with 50 ml of methanol containing 1% HCl. After filtering, the extract was concentrated in a rotary flash evaporator and brought to a known volume with distilled water for the enzyme inhibition study. In order to study the extraction differences of different solvents, the polyphenolic compounds were extracted by refluxing using acetone, methanol, water and methanol-HCl. Methanol-HCl extraction was also carried out at room temperature (25°C) for comparison. The effect of duration of refluxing on extraction of phenolic compounds was also studied by refluxing samples for 1, 2, 3 and 4 h. The extracts were concentrated in a similar way and brought to a known volume with methanol-HCl. The total amounts of total phenolic compounds in the extracts thus obtained were estimated as tannine acid equivalents according to the Folin-Denis procedure (10).

Enzyme assays

Trypsin, chymotrypsin and amylase activities were assayed according to the procedures previously described (9,11). For salivary amylase human saliva was collected and diluted about fivefold in 0.02 M calcium phosphate buffer, pH 6.8. After standing overnight at 5 °C the mixture was centrifuged at 10,000 x g for 15 min and the supernatant used for the assay. Chickpea and pigeonpea seed extracts containing phenolic compounds were added to the reaction mixture. Percent enzyme inhibition was determined by comparing the reduction in activity resulting from the addition of extract with that produced in the absence of any inhibitor. Experiments were conducted to study the effect of polyvinylpyrrolidone (PVP), the tannin complexing agent, on the enzyme inhibition. Seed extract containing polyphenolic compounds were treated with PVP (10% w/v) for 30 min at room temperature (25 °C). PVP treated extracts were used for enzyme inhibition as described above.

RESULTS AND DISCUSSION

Extraction of seed polyphenols

The results of the effect of different solvents on the extraction of total phenolic compounds are presented in Table I. Boiling acidified methanol (methanol-HCl) extraction gave the highest recoveries of these compounds from both chickpea and pigeonpea.

Table I. Effect of different solvents on the extraction of seed polyphenolic compounds of chickpea and pigoenpea

Solvent[a]	Chickpea (G-130)	Pigoenpea (C-11)
	Polyphenols (mg/g sample)	
Acetone	0.62 ± 0.05[c]	0.87 ± 0.04
Methanol	0.28 ± 0.01	0.43 ± 0.02
Water	2.34 ± 0.08	3.06 ± 0.06
Methanol-HCl[b]	3.60 ± 0.07	5.14 ± 0.05
Methanol-HCl	6.18 ± 0.07	14.23 ± 0.07

a, Extraction by refluxing for 2 hr; b, Extraction at 25 °C for 16 hr; c, standard error of estimation based on six determinations.

Earlier workers have reported that methanol-HCl extraction at room temperature produced values twice as high as the methanol extraction in sorghum (12). Considerably higher values were obtained when water rather than methanol was used as a solvent. However, this might have been the result of extraction of some proteins which also give a Folin-Denis positive reaction (10). Extraction of polyphenols with methanol-HCl was significantly higher by refluxing compared with their extraction at room temperature in the same solvent (methanol-HCl). This higher extraction of these compounds as a result of heat treatment in acidic conditions can be attributed to the polymeric nature of flavanoids which generate anthocyanidins as degradation product when they are heated in acid solution (13).

The effects of different durations of extraction using methanol-HCl by refluxing are shown in Table II. The extraction of polyphenols increased up to 2 h and thereafter no noticeable differences were observed. In order to study the enzyme inhibitory properties of the total phenolic compounds of chickpea and pigeonpea it was imperative to extract as many polyphenols as possible. Extraction of polyphenols using methanol-HCl by refluxing for 2 h was found to be satisfactory and was therefore used in the present study.

Table II. Effect of different durations of extraction on polyphenolic compounds of chickpea and pigeonpea[a]

Extraction time (h)	Chickpea (G-130)	Pigeonpea (C-11)
	Polyphenols (mg/g)	
1	4.8 ± 0.05	12.2 ± 0.06
2	6.1 ± 0.04	14.9 ± 0.04
3	5.8 ± 0.06	15.0 ± 0.07
4	6.0 ± 0.05	14.8 ± 0.07

a, Results are averages of six determinations based on the extraction by refluxing in methanol-HCl.

Enzyme inhibition by different concentrations of polyphenols

Using the assay conditions described, the inhibition of trypsin, chymotrypsin and amylase enzyme (human saliva and hog pancreas) by different concentrations of polyphenols in chickpea (cv. G-130) and pigeonpea (cv. C-11) were studied. Percent enzyme inhibition of trypsin and chymotrypsin

increased with increasing concentration of polyphenols up to 200 ug/ml of the reaction mixture and thereafter remained constant (Figure I).

In the case of amylase, enzyme inhibition increased up to a concentration of 250 ug/ml of reaction mixture, but additional amounts of polyphenols had no noticeable effect (Figure 2). The use of PVP-treated extracts to a large extent (Table III) prevented the inhibition of enzyme activity, although a complete release of enzyme inhibition was not achieved even in the presence of a higher concentration of PVP in the extract. This indicates the possible presence of compounds other than polyphenols, that inhibit enzyme activity, but are not inactivated by PVP. The temperature at which polyphenols were extracated had a considerable effect on enzyme inhibition. When polyphenolic compounds extracted by refluxing were used, enzyme inibition was greater than when room temperature extracted polyphenols were used (Table III). This implies qualitative differences in the polyphenolic compounds extracted by different procedures.

Enzyme inhibition by polyphenols of cultivars differing in testa color

The results of an experiment conducted to study the effect on enzyme inhibition of polyphenols from cultivars differing in testa colour are presented in Table IV. The polyphenolic compounds from white and dark colored testa chickpeas and pigeonpeas revealed striking differences in their enzyme inhibitory properties. Enzyme inhibition was highest in cultivars with dark testa and lowest in white testa cultivars.

On the basis of average percent enzyme inhibition by the several cultivars studied, the extract of chickpea showed a higher activity towards trypsin than towards chymotrypsin, whereas the pigeonpea extract did not show such a difference (Table IV). This was also observed when different concentrations of phenolic compounds were used (Figure 1). Both chickpea and pigeonpea polyphenols showed higher inhibitory activity towards salivary amylase than towards pancreatic amylase and differences were larger for chickpea. Generally, polyphenols of pigeonpea were found to be more effective than those of chickpea.

CONCLUSION

Earlier studies on several food legumes reported the inhibition of some digestive enzymes by tannins, the heat resistant factors (6,7,8). Tannin concentrations ranged from 0-0.2% in pigeonpea and there was essentially no tannin in chickpea (5). However, in this study these two important grain legumes were shown to contain a considerable amount of

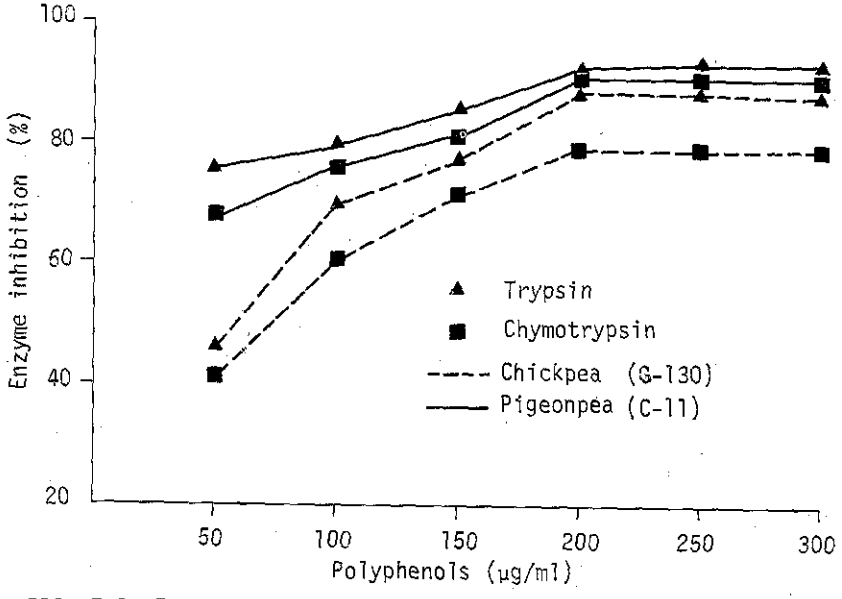

FIGURE 1: EFFECT OF DIFFERENT CONCENTRATIONS OF POLYPHENOLS ON TRYPSIN AND CHYMOTRYPSIN INHIBITION IN CHICKPEA AND PIGEONPEA.

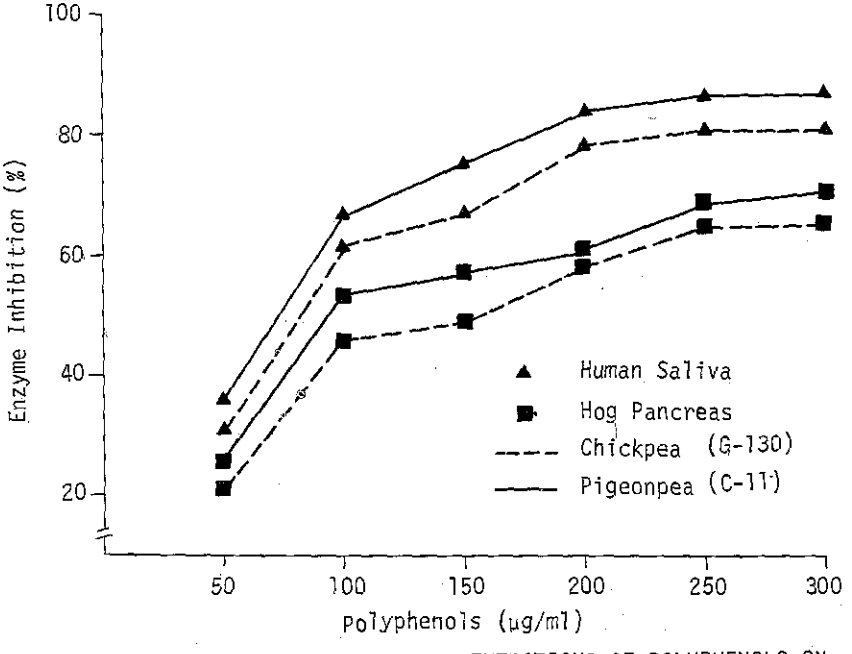

FIGURE 2: EFFECT OF DIFFERENT CONCENTRATIONS OF POLYPHENOLS ON AMYLASE INHIBITION IN CHICKPEA AND PIGEONPEA.

Table IV. Varietal differences in the enzyme inhibitory property of polyphenols of chickpea and pigeonpea

Cultivar	Testa colour	Polyphenols (mg/g sample)	Enzyme Inhibition (%)[a]			
			Trypsin	Chymo-trypsin	Human saliva	Hog pancreas
Chickpea						
Rabat	White	1.9	33.6	26.3	29.8	17.5
L-550	Salmon white	2.3	34.5	25.7	31.5	20.8
Pant G-114	Light brown	5.3	86.4	72.5	73.4	56.9
G-130	Brown	5.8	88.7	79.0	80.3	64.5
USA-613	Brown	6.1	81.6	70.9	78.6	61.0
Mean	-	4.8	65.0	54.9	58.7	44.1
SE ±	-	0.1	1.8	1.6	1.7	1.5
Pigeonpea						
Hy-3c	White	3.7	37.9	36.0	34.5	21.8
NP(WR)-15	White	6.0	40.5	38.6	32.7	19.7
C-11	Light brown	14.2	91.5	90.3	86.0	80.9
BDN-1	Brown	15.2	90.3	91.6	79.4	69.3
No-148	Brown	14.9	88.0	85.9	75.8	68.5
Mean	-	10.8	69.7	68.5	61.7	52.0
SE ±	-	0.2	2.1	1.7	1.4	1.3

a, Based on assay carried out using 200 ug polyphenols for trypsin and chymotrypsin and 250 ug polyphenols for amylase inhibitions.

Table III. Effect of polyvinylpyrrolidone (PVP) and methods of extraction of polyphenols on enzyme inhibitory activity of polyphenols of chikpea (G-130) and pigeonpea (C-11)[a]

Enzyme	Methanol-HCl[b]				Methanol-HCl[c]	
	Chickpea		Pigeonpea		Chickpea	Pigeonpea
	Control	+PVP	Control	+PVP	Control	Control
	..... Enzyme Inhibition (%)					
Trypsin	88.7	13.4	91.5	14.6	80.7	86.5
Chymotrypsin	79.0	12.3	90.3	11.0	70.6	81.4
Amylase						
. human saliva	80.3	17.8	86.0	18.6	71.5	77.8
. hog pancreas	64.5	12.5	80.9	15.3	60.7	62.3

a, Averages of three replications using 200 ug polyphenols for trypsin and chymotrypsin and 250 ug polyphenols for amylase inhibitions; b, Extraction by refluxing (boiling); c, Extraction at room temperature (25°C).

polyphenolic compounds which may or may not be tannins. Although the nutritional role of such compounds ramains unclear, based on the results of this present study it may be concluded that the polyphenolic compounds of chickpea and pigeonpea adversely effect the activities of digestive enzymes and that this effect will have nutritional implications in terms of nutrient utilization.

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REFERENCES

1. Liener, I.E. Toxic factors in edible legumes and their elimination. Am. J. Nutr. 2, 281-298 (1962).

2. Hulse, J.H. Polyphenols in Cereals and Grain Legumes. Proceedings of a Symposium held during the 36th annual meeting of the Institute of Food Technologists, St. Louis, Missouri, 10-13 June 1979, p1-7.

3. Martin-Tanguy, J., Vuillaume, J. and Kossa, A. Condensed tannins in horse bean seeds: Chemical structure and apparent effects on poultry. J. Sci. Food Agric. 28, 757-765 (1977).

4. Ma Yu and Bliss, F.A. Tannin content and inheritance in common beans. Crop Sci. 18, 201-204 (1978).

5. Price, M.L., Hagerman, A.E. and Butler, L.G. Tannin content of cowpeas, chickpeas, pigeonpeas, and mung beans. J. Agric. Food Chem. 28, 459-461 (1980).

6. Elias, L.G., Fernandez, D.G. and Bressani, R. Possible effects of seed coat polyphenols on the nutritional quality of bean protein. J. Food Sci. 11, 524-527 (1974).

7. Lumen, B.O., de, and Salamat, L.A. Trypsin inhibitor activity in winged bean (Psophocarpus tetragonolobus) and the possible role of tannins. J. Agric. Food Chem. 28, 533-536 (1980).

8. Griffiths, D.W. The role of field bean polyphenolics in digestive enzyme inhibition. In Proc. Sym. on Vicia faba. Feeding value, Processing and Viruses, EEC EAEC Brussels-Luxembourg, p.145-157 (1980).

9. Singh, U. and Jambunathan, R. Studies on desi and kabuli chickpea (Cicer arietinum L.) cultivars: Levels of protease inhibitors, levels of polyphenolic compounds and in vitro protein digestibility. J. Food Sci. 46, 1364-1367 (1981).

10. Swain, T. and Hillis, W.E. The phenolic constituents of Prumus domestica 1. The qualitative analysis of phenolic constituents. J. Sci. Food Agric. 10, 63-68 (1959).

11. Singh, U., Kherdekar, M.S. and Jambunathan, R. Studies on desi and kabuli chickpea (Cicer arietinum L.) cultivars. The levels of amylase inhibitors, levels of oligosaccharides and in vitro starch digestibility. J. Food Sci. 47, 510-512 (1982).

12. Earp. C.F., Abingbala, J.O., Ring, S.H. and Rooney, L.W. Evaluation of several methods to determine tannins in sorghum with varying kernel characteristics. Cereal Chem. 58, 234-238 (1981).

13. Sarkar, S.K., Howarth, R.E. and Goplen, B.P. Condensed tannins in Herbaceous Legumes. Crop Sci. 16, 543-546 (1976).

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