Studies on Desi and Kabuli Chickpea (*Cicer arietinum* L.) Cultivars: Levels of Protease Inhibitors, Levels of Polyphenolic Compounds and in Vitro Protein Digestibility

U. SINGH and R. JAMBUNATHAN

ABSTRACT

The levels of trypsin inhibitor activity were higher in both kabuli and desi seeds of chickpea than their chymotrypsin inhibitor activity. Mean values for the trypsin and chymotrypsin inhibitor units in dhal and seed samples of desi were higher as compared with kabuli cultivars. The presence of seed coat reduced the protein extraction. Mean values of polyphenolic compounds in seed samples of desi were more than twice that of kabuli and these differences disappeared in dhal samples indicating the distribution of these compounds mainly in the seed coat. The in vitro protein digestibility studies showed larger differences between desi seed and dhal samples when compared with kabuli seed and dhal samples. Polyphenolic compounds exhibited a highly significant and negative correlation ($r = 0.872^{**}$) with in vitro digestibility of protein and a significant positive correlation with trypsin ($r = 0.612^{*}$) and chymotrypsin ($r = 0.507^{*}$) inhibitor activities.

INTRODUCTION

IN FOOD LEGUMES, the presence of some well defined antinutritional factors has been well recognised (Liener, 1962). Of the various antinutritional factors, trypsin and chymotrypsin inhibitors have been the subject of several investigations (Laskowski and Sealock 1971; Roy and Bhat, 1974; Tur-Sinai et al., 1972; Bhatty, 1979, Kakade et al., 1972). Recently, biochemical studies on the trypsin and chymotrypsin inhibitors in chickpea seed have been carried out (Smirnoff et al., 1976; Belew and Eaker, 1976). Sumathi and Pattabiraman (1976) examined the levels of these inhibitors in chickpea seeds and have compared them with other crops.

Polyphenolic compounds (loosely termed as tannins) have been reported to interfere with the biological value of grains. Price et al. (1980) have studied the levels of tannins in chickpea. *Phaseolus vulgaris* with different testa colors have been analyzed for the polyphenolic compounds and the effect of these compounds on nutritional quality has been studied (Bressani and Elias 1979; Elias et al., 1979). Griffiths (1979) reported the inhibition of digestive enzymes by the extracts of the seed coat of coloured varieties of field bean (*Vicia faba*).

The major proportion of chickpea grown in the world is consumed after removal of the testa (seed coat). Within the cultivated species of chickpea there are two main groups of practical importance. One group having a light brown testa (although wide variation exists) color is called desi and the other group with a salmon white color is called kabuli. Studies on the chemical composition of these two groups revealed significant differences in their seed coat percentages, crude fiber contents, and in mineral and trace element compositions (Jambunathan and Singh, 1979; Jambunathan and Singh, 1981). Seed protein fractions, electrophoretic patterns and amino acid composition of desi and kabuli types did not show any major differences (Singh et al. 1981). We have examined the levels of trypsin and chymotrypsin inhibitors, levels of polyphenolic compounds and in vitro protein digestibility of these two groups of chickpea cultivars and the results are reported.

Authors Singh and Jambunathan are affiliated with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), ICRISAT Patancheru P.O., 502 324, A.P., India.

MATERIALS & METHODS

Materials

Seed samples of 8 desi (USA-613; 850-3/27; Pant G-114; T-3; Annegiri; BG-203; CPS-1; and P-5462) and 7 kabuli (K-4; C-104; Rabat; L-550; GL-629; Giaza; and No 501) chickpea cultivars grown at Hissar, India (29°N) during the post rainy season of 1977-78 were obtained by pooling seeds from single plots and were received from our chickpea breeding section. For decortication, seed samples were soaked in an excess of water at 5°C overnight and the testa was removed manually. The decorticated material (dhal) was dried overnight at 70°C. Dhal and seed samples were ground in a Udy cyclone mill to pass through a 60-mesh sieve and were defatted in a Soxhlet apparatus using hexane. Trypsin (E.C. 3.4 21.4, 199 u/mg) and chymotrypsin (E.C. 3.4.21.1, 61 μ/mg) were purchased from Worthington Biochemical Corporation (New Jersey, USA). Pepsin, pancreatin and casein were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents used were of AR grade.

Methods of analysis

Total nitrogen in each defatted sample was determined by the micro-Kjeldahl procedure (AOAC, 1975) and the crude protein content was calculated by using a factor of 6.25.

Trypsin inhibitor activity

The trypsin inhibitor activity (TIA) was assayed according to Kakade et al. (1969). Trypsin inhibitor was extracted by shaking 200 mg of defatted material with 10 ml of 0.1M phosphate buffer (pH 7.6) at room temperature for 1 hr. The extract was diluted fourfold. The aliquots containing 0.2, 0.4, 0.6, and 0.8 ml were assayed for trypsin inhibitor activity. Protein content of extract was determined according to Lowry et al. (1951) and the percentage of protein extracted was calculated.

Chymotrypsin inhibitor activity

Chymotrypsin inhibitor activity (CIA) was assayed according to Kakade et al (1970). Chymotrypsin inhibitor was extracted as described above except that 0.1M borate buffer (pH 7.6) was used. Protein content of extract was determined according to Lowry et al. (1951) and the percentage of protein extracted was calculated.

In vitro protein digestibility (IVPD)

An amount of sample containing 6.75 ± 0.1 mg N was placed into a 50 ml conical flask and 5 ml of HCl solution (pH 2.0) containing 2 mg of pepsin was added. The flask was incubated in a water bath shaker for 16 hr at 37°C. Then 2 ml of pancreatin solution was added and the contents were further incubated for 24 hr. The pancreatin solution was prepared by dissolving 50 mg of pancreatin in 100 ml of 0.1M borate buffer (pH 6.8) containing 0.025M calcium chloride and the solution was filtered and used. Toluene (2–3 drops) was added during incubation and samples were stirred slowly on a mechanical shaker. After 24 hr, the reaction was stopped by adding 7.0 ml of 10% (w/v) trichloracetic acid (TCA) and the suspension was centrifuged (10,000 x g, 15 min). The residue was washed twice with 5 ml of 5% TCA and the pooled supernatants made upto 25 ml with 5% TCA. An aliquot (5.0 ml) was taken and evaporated to dryness at low temperature (80–90°C) and the nitrogen content was determined by the micro-

Kjeldahl procedure. The digestibility of each sample was calculated as nitrogen in sample supernatant minus nitrogen in enzyme blank supernatant expressed as percentage of nitrogen in the starting material.

Polyphenolic compounds

The polyphenolic compounds were extracted from each defatted sample (500 mg) by refluxing with 50 ml of methanol containing 1% HCl for 4 hr. The extract was concentrated in a rotary flash evaporator and brought to 25 ml with methanol-HCl. The amounts of phenolic compounds were estimated as tannic acid equivalent according to the Folin-Denis procedure (Swain and Hills, 1959).

RESULTS & DISCUSSION

Effect of seed coat on protein solubility

The effect of the seed coat of desi and kabuli varieties on protein extraction is shown in Table 1. The average seed coat in desi seed was 16.0% while in kabuli seed the average was 7.1%. Lower percentage of meal protein was extracted from desi seed samples (53.9) as compared to dhal (64.7) when phosphate buffer was used for extraction. Similar differences in the extractability of proteins were also observed in the case of borate buffer (Table 1). The differences in the extraction of protein from the seed and dhal samples of kabuli cultivars were not large. This could be due to the lower amount of seed coat in kabuli and perhaps to their chemical nature. The observed differences in protein extractions of desi and kabuli seed samples influenced the trypsin and chymotrypsin inhibitor values and this is discussed in the following section.

Trypsin inhibitor activity in dhal and seed samples

The trypsin inhibitor activities of dhal and seed samples of desi and kabuli cultivars are shown in Table 2. The results showed that trypsin inhibitor activity was higher both in the dhal and seed of desi when compared with kabuli cultivars. One might expect greater trypsin inhibitory differences between seed samples of desi and kabuli as compared to dhal samples, but this was not observed. This may be due to the observed lower protein extraction in the case of seed samples of desi cultivars. When the results were expressed as trypsin inhibitor units per mg of extracted protein, desi seed samples exhibited higher values (52.6 trypsin units inhibited) when compared with kabuli seed samples (31.9 trypsin units inhibited) and the observed differences for dhal samples of both types were small (Table 2). The inhibition was about 70% higher in desi seed as compared to kabuli seed samples and only 25% higher in desi as compared with kabuli dhal samples. The higher amount of trypsin inhibition in desi seed samples might have occurred due to the extraction of polyphenolic compounds from the seed coat.

Chymotrypsin inhibitor activity of dhal and seed samples

The results of CIA of dhal and seed samples of desi and kabuli cultivars are shown in Table 2. Less variability was observed in the CIA though the mean inhibitor activity was slightly higher for seed and dhal samples of desi as compared with kabuli cultivars. The mean chymotrypsin units inhibited (CUI/mg meal) were 7.7 units for desi dhal and 6.5 units for kabuli dhal while it was 8.1 units for desi seed

Table 1—Effect of seed coat on protein extraction[a]

Cultivar	Seed coat (%)		Phosphate buffer[b]		Borate buffer[b]	
	Range	Mean	Range	Mean	Range	Mean
Desi (n = 8)						
Dhal			59.6–70.2	64.7	59.4–64.9	62.6
Seed	12.8–17.6	16.0	48.0–61.3	53.9	50.4–57.5	54.8
Kabuli (n = 7)						
Dhal			65.4–71.1	67.8	63.9–69.9	66.3
Seed	5.7–8.8	7.1	61.7–68.4	63.5	60.6–68.4	64.0

[a] 0.1M, pH 7.6
[b] % protein extracted; determined according to Lowry et al. (1951); n = number of cultivars.

Table 2—Trypsin and chymotrypsin inhibition in decorticated (dhal) and seed samples of 8 desi and 7 kabuli cultivars

Cultivar	Protein (%)[c]		Trypsin inhibition				Chymotrypsin inhibition			
			Dhal		Seed		Dhal		Seed	
	Dhal	Seed	a	b	a	b	a	b	a	b
Desi:										
Range	23.5–29.3	19.8–23.5	9.3–14.6	26.0–41.2	9.9–15.7	37.4–60.4	7.1–9.0	20.6–26.8	7.6–8.8	29.8–39.2
Mean	26.0	21.7	12.0	36.0	12.7	52.6	7.7	23.8	8.1	34.4
SE±[d]	0.3	0.4	0.3	0.7	0.3	0.8	0.1	0.5	0.1	0.4
Kabuli:										
Range	22.8–27.3	22.5–25.3	6.7–12.3	20.0–39.8	8.1–12.1	25.2–39.2	5.7–9.4	18.2–23.4	6.1–8.0	20.1–26.8
Mean	24.6	24.1	9.4	29.2	10.3	31.9	6.5	21.2	7.3	23.2
SE±[d]	0.3	0.3	0.2	0.5	0.2	0.6	0.1	0.5	0.1	0.5

[a] Inhibitor units/mg meal
[b] Inhibitor units/mg extracted protein
[c] N x 6.25, moisture free basis
[d] Standard error of estimation

Table 3—In vitro protein digestibility and polyphenolic compounds in decorticated (dhal) and seed samples of 8 desi and 7 kabuli cultivars

Cultivar	Seed coat (%)	In vitro digestibility (%)[a]		Polyphenols (mg/g meal)	
		Dhal	Seed	Dhal	Seed
Desi:					
Range	12.8–17.6	63.7–76.0	52.4–69.0	1.7–2.4	4.1–6.1
Mean	16.0	71.0	63.1	2.1	4.7
SE±[b]	0.2	1.3	1.4	0.1	0.1
Kabuli:					
Range	5.7–8.8	72.7–79.1	70.2–77.6	1.4–2.1	1.9–2.3
Mean	7.1	75.3	72.7	1.8	2.1
SE±[b]	0.1	1.3	1.3	0.1	0.1

[a] Percent digestible nitrogen
[b] Standard error of estimation

Table 4—Correlation coefficients between protease inhibitors, polyphenols, seed coat percentage and in vitro protein digestibility in seed samples of 15 chickpea cultivars

	Protein (%)	Seed coat (%)	TIA[a]	CIA[a]	Polyphenols (%)
Seed coat (%)	−0.625**	–			
TIA[a]	−0.509*	0.493*	–		
CIA[a]	−0.331	0.457	0.530*	–	
Polyphenols (%)	−0.627**	0.938**	0.612*	0.507*	–
IVPD[b]	0.134	−0.731**	−0.439	−0.339	−0.872**

[a] Units inhibited/mg meal
[b] In vitro protein digestibility
*Significant at 5% level
**Significant at 1% level

and 7.3 units for kabuli seed samples. As observed for trypsin inhibitor, the chymotrypsin units inhibited (CUI/mg protein) were higher in the case of desi cultivars and the mean value was 34.4 units for desi seed and 23.2 units for kabuli seed indicating the possible role of seed coat constituents in these determinations.

It may be mentioned here that most of the protease inhibitors in legumes are inactivated by heat and most of them probably destroyed after cooking. We also studied this aspect and found most of the inhibitors were inactivated when extracts were heated for 60 min. The levels of protease inhibitors could not be assayed in cooked samples because the protein extractability was considerably reduced as the result of cooking (unpublished data).

In vitro protein digestibility and polyphenolic compounds

Results of in vitro digestibility studies and the levels of polyphenolic compounds in chickpea are shown in Table 3. The mean values for protein digestibility of desi seed and dhal were 63.1 and 71.0% respectively and for kabuli seed and dhal were 72.7 and 75.3% respectively. Determination of in vitro protein digestibility of cooked samples was tried and lower values were obtained. This might have happened as a result of the fact that protein extractability was considerably reduced in cooked samples (unpublished data).

The mean value of polyphenolic compounds (mg/g meal) in desi seed (4.7 mg) was more than twice the amount that was present in desi dhal (2.1 mg/g) while a comparison of the mean values between kabuli seed (2.1 mg/g) and dhal (1.8 mg/g) showed no such differences. This observation could again be related to the variability in the seed coat percentages in desi and kabuli cultivars (Table 3). This was confirmed by by analyzing the dhal, whole seed and seed coat samples for polyphenolic compounds which showed that seed coat contributed to about 75% of total polyphenolic compounds of seed in desi cultivars. Similar results were obtained by Bressani and Elias (1979) when they compared the polyphenolic compounds of field bean varieties having white and red seed color.

The interrelationships between the levels of protease inhibitors, polyphenolic compounds, seed coat content and in vitro digestibility of proteins in chickpea were worked out and results are shown in Table 4. Statistical analysis was carried out as described earlier (Snedecor and Cochran, 1967). There was a positive and significant relationship between the seed coat percentage and polyphenols. The seed coat percentage was also negatively and significantly correlated with the in vitro protein digestibility. A highly significant and negative correlation was observed between the in vitro protein digestibility and the concentration of polyphenolic compounds in seed samples. Trypsin and chymotrypsin inhibitor activites were positively correlated with the amount of polyphenols. These results are in agreement with the results of Milic et al. (1972) who studied the activity of purified tannins on the trypsin digestion of casein. Water extracts of field beans with different colored testa have also been reported to inhibit digestive enzymes (Griffiths, 1979).

The results showed considerable differences in the levels of trypsin and chymotrypsin inhibitors and phenolic compounds among desi and kabuli cultivars. Presumably the varieites with higher trypsin and chymotrypsin inhibitors and tannins would not be utilized by man as readily as those varieties that are low in these antinutritional factors. But additional studies are needed to investigate the role of phenolic compounds of desi and kabuli types in the bioavailability of other nutrients. As most of the phenolic compounds are located in the seed coat, it appears that breeding for varieties having lower seed coat percentages would be desirable.

REFERENCES

AOAC. 1975. "Official Methods of Analysis," 12th ed, Washington, DC.

Belew, M. and Eaker, D. 1976. The trypsin and chymotrypsin inhibitors in chickpea. Eur. J. Biochem. 62: 499.

Bhatty, R.S. 1979. Trypsin Inhibitors of Fababeans. Partial purification and some properties. Can Inst. Food Sci. Technol. 12: 135.

Bressani, R. and Elias, L.G. 1979. The nutritional role of polyphen-

ols in beans. In Proc. Symp. Polyphenols in cereals and legumes. Institute of Food Technologits, St. Louis, MO.
Elias, L.G., de Fernandes, D.G., and Bressani, R. 1979. Possible affects of seed coat polyphenols on the nutritional quality of bean protein. J. Food Sci. 44: 524.
Griffiths, D.W. 1979. The inhibition of digestive enzymes by extracts of field bean (Vicia faba). J. Sci. Food Agric. 30: 458.
Jambunathan, R. and Singh, U. 1979. Studies on desi and kabuli chickpea (Cicer arietinum) cultivars. 1. Chemical composition, In Proc. International workshop on chickpea improvement, ICRISAT, Hyderabad, India (In press).
Jambunathan, R. and Singh, U. 1981. Studies on desi and kabuli chickpea (Cicer arietinum L.) cultivars—3. Mineral and trace element composition. J. Agric. Food Chem. (In press).
Kakade, M.L., Swenson, D.H., and Liener, I.E. 1969. An evaluation of natural vs. synthetic substrates for measuring the antitrypitic activity of soybean samples. Cereal Chem. 46: 518.
Kakade, M.L., Swenson, D.H., and Liener, I.E. 1970. Note on the determination of chymotrypsin and chymotrypsin inhibitor activity using casein. Anal. Biochem. 33: 255.
Kakade, M.L., Simons, N.R., Liener, I.E., and Lambert, J.W. 1972. Biochemical and nutritional assessment of different varieties of soybeans. J. Agric. Food Chem. 20: 87.
Laskowski, M. Jr. and Sealock, R.W. 1971. In "The Enzymes," Ed. Boyer, P.D., Vol. 3, p. 375. Academic Press, New York.
Liener, I.E. 1962. Toxic factors in edible legumes and their elimination. Am. J.Clin. Nutr. 2: 281.
Lowry, O.H., Rosebrough, N., Farr, A.L., and Randall, R.J. 1951. Protein measurement with Folin-phenol reagent. J. Biol. Chem. 193: 265.
Milic, B.L., Stojanovie, S., and Vucurevic, N. 1972. Lucerne tannins. Isolation of tannins from lucerne, their nature and influence on the digestive enzymes in vitro. J. Sci. Food Agri. 23: 1151.
Price, M.L., Hagerman, A.E., and Butler, L.G. 1980. Tannin contents of cowpeas, chickpeas, pigeonpeas and mungbeans. J. Agri. Food Chem. 28: 459.
Roy, D.N. and Bhat, R.V. 1974. Trypsin inhibitor content in some varieites of soya bean (Glycine max L.) and sunflower seeds (Helianthus annus L.). J. Sci. Food Agric. 25: 765.
Singh, U., Raju, S.M., and Jambunathan, R. 1981. Studies on desi and kabuli chickpea (Cicer arietinum L.) cultivars. 2. Seed protein fractions, electrophoretic patterns and amino acid composition. J. Sci. Food Technol. (In press).
Smirnoff, P., Khalif, S., Birk, Y. and Applebaum, S.W. 1976. A trypsin and chymotrypsin inhibitor from chickpea (Cicer arietinum L.). Biochem. J. 157: 745.
Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods," 6th ed. Oxford and IBH Publishing Co., New Delhi.
Sumathi, S. and Pattabiraman, T.N. 1976. Natural plant enzyme inhibitors, Part II. Protease inhibitor of seeds. Ind. J. Biochem. & Biophys. 13: 52.
Swain, T. and Hills, W.E. 1959. The phenolic constituents of Prumus domestica 1. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63.
Tur-Sinai, A., Birk, Y., Gertler, A., and Rigbi, M. 1972. A basic trypsin and chymotrypsin inhibitor from groundnuts (Arachis hypogaea). Biochem. Biophys. Acta. 263: 666.
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factors are more in line with the factor listed for soybeans in Agricultural Handbook No. 8 (1975) of 5.71, as well as with the factor recommended by AOAC of 6.25 (Anonymous, 1979; 1980). However, the factor based on amino acid composition (Factor Method) should provide the most reliable conversion factor (Heidelbaugh et al., 1975).

Computations of protein content of each of the above soy protein products from their Kjeldahl nitrogen values and a common conversion factor, such as 6.25, would undoubtedly introduce substantially more error into the determination of proteins in food products than by using a specific conversion factor based upon each protein's amino acid composition. Since the food industry commonly utilizes computer technology in the formulation of food products, it should be capable of utilizing the Kjeldahl nitrogen content multiplied by a conversion factor based on the amino acid composition of the protein source materials. It can be estimated that upwards of 20% error in protein determination could be expected by utilizing conversion factors ranging from 5.64–6.84 (Table 2). An additional and most important advantage to using the Factor Method is that the amino acid compositional data would enable the food processor to determine the limiting amino acid and chemical score of the protein as indicators of its nutritional quality.

REFERENCES

Agricultural Handbook No. 8, 1975. "Composition of Foods," U.S. Dept. of Agriculture, Washington, DC.
Anonymous. 1979. J. Assoc. Official Anal. Chem. 62(2): 370.
Anonymous. 1980. The refereee. Association of Official Analytical Chemists. 3(5): 2.
AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.
Finley, J.W., Johnston, P.H., and Friedman, M. 1975. "Protein Nutritional Quality of Foods and Feeds." Marcel Dekker Inc., New York.
Heidelbaugh, N.D., Huber, C.S., Bednarczyk, J.F., Smith, M.C., Rambaut, P.C., and Wheeler, H.O. 1975. Comparison of three methods of calculating protein content of foods. J. Agr. Food Chem. 23: 611.
Moore, S. 1963. On the determination of cystine as cysteic acid. J. Biol. Chem. 238(1): 235.
Rackis, J.J., Anderson, R.L., Sesame, H.A., Smith, A.K., and Van Etten, C.H. 1961. Amino acids in soybean hulls and oil meal fractions. J. Agr. Food Chem. 9: 409.
Smith, A.K. and Circle, S.J. 1971. Chemical composition of the seed. In "Soybeans: Chemistry and Technology." Avi Publishing Co., Inc., Westport, CT.
Spackman, D.H., Stein, W.H., and Moore, S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190.
Torun, B. 1979. Nutritional quality of soybean protein isolates: Studies in children of preschool age. In "Soy Protein and Human Nutrition," Academic Press, New York.
Wolf, W.J. 1971. Purification and properties of the proteins. In "Soybeans: Chemistry and Technology." Avi Publishing Co., Inc., Westport, CT.
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