Studies on Desi and Kabuli Chickpea (Cicer arietinum L.) Cultivars. The Levels of Amylase Inhibitors, Levels of Oligosaccharides and In Vitro Starch Digestibility

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ABSTRACT

Amylase inhibitor activity (AIA) of chickpea extracts was investigated using pancreatic and salivary amylases. The extracts showed higher inhibitor activity towards pancreatic amylase than salivary amylase. Mean values indicated slightly higher inhibitory activity in desi than kabuli cultivars, though clear-cut differences were not observed among the cultivars. While in vitro starch digestibility of meal samples indicated no large differences among desi and kabuli types of chickpea, the mean values of digestibility of isolated starches of kabuli types was higher than those of desi types. The mean values of stachyose were higher in desi cultivars. When desi and kabuli types were considered together, stachyose and raffinose contents were not found significantly related to the concentrations of total soluble sugars while stachyose showed a significant correlation with raffinose.

INTRODUCTION

ALTHOUGH NUTRITIONAL significance of α-amylase inhibitors of cereal grains has been studied (Granum and Eskeland, 1981), amylase inhibitors of grain legumes have not received much attention. The growth inhibiting properties of raw beans have been reported to be due to the presence of heat labile factor which inhibited the in vitro activity of pancreatic amylase (Jaffe and Vega, 1968). A large variation in the inhibitor activity of pancreatic amylase among the several species of food legumes has been reported (Jaffe et al., 1973).

The food legumes are also regarded as notorious inducers of flatulence when they are consumed in large quantity. It has been reported that the two oligosaccharides, raffinose and stachyose, are the causative factors for flatulence and uncomfortable feeling often experienced upon ingestion of soybean products (Steggerda and Rackis, 1967). In particular, the hydrogen component of intestinal gas is formed by the fermentation of low molecular weight galactosido-oligosaccharides raffinose and stachyose which are not digested by human digestive enzymes (Hellendoorn, 1969). Udyashanker Rao and Belavady (1978) reported the presence of considerable amount of stachyose and raffinose in whole-chickpea seeds. Earlier studies revealed marked differences in the in vitro digestibility of carbohydrates of different legumes (Srinivasa Rao, 1969).

Previous studies of some of the antinutritional factors in desi and kabuli types of chickpea showed a considerable variation in the levels of protease inhibitors, in vitro protein digestibility and polyphenolic compounds (Singh and Jambunathan, 1981). In this paper, the levels of amylase inhibitors and oligosaccharides-stachyose and raffinose and the results of in vitro starch digestibility of some desi and kabuli cultivars are reported.

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MATERIALS & METHODS

Materials

Seed samples of eight desi and seven kabuli cultivars grown at Hissar, India (29°N), during the post-rainy season of 1977-78 were decorticated and ground in a Udy cyclone mill to pass a 0.4 mm screen as described earlier (Singh and Jambunathan, 1981).

Methods of Analysis

Soluble sugars and starch content. Sugars were extracted using 80% ethanol in a Soxhlet apparatus and estimated by the phenol-sulphuric acid method (Dubois et al., 1956). Starch content in the dry residue was determined by enzymatic hydrolysis (Singh et al., 1980).

Amylase inhibitor activity. The inhibitor activity of pancreatic amylase (obtained from Sigma Chem. Co., USA) was carried out according to the method of Jaffe et al. (1973). The salivary amylase inhibitor activity was determined according to the procedure of Granum (1978). Human saliva was collected and diluted about five-fold in 0.02M phosphate buffer, pH 6.9. After standing overnight at 5°C, the mixture was centrifuged at 10,000 × *g* for 15 min. Amylase inhibitor was extracted by shaking a finely ground and defatted chickpea sample with 0.02M phosphate buffer, pH 6.9 (1:10, w/v) for 2 hr at room temperature. The suspension was then centrifuged at 10,000 × *g* for 15 min at room temperature. The supernatant was then heated for 10 min at 70°C, centrifuged again at 10,000 × *g* for 15 min at room temperature, and the supernatant so obtained was tested for amylase inhibitor activity.

Estimation of stachyose and raffinose. The oligosaccharides were extracted with 80% ethanol for 6 hr in a Soxhlet apparatus. The separation of these oligosaccharides was accomplished on a Whatman No. 1 chromatographic paper by descending chromatography using the solvents butanol-pyridine-water (5:1:4, v/v). The chromatogram was run for about 72 hr. The paper was removed, dried with hot air and marginal strips were cut off and sprayed with a solution of ammonical silver nitrate (Leslie, 1968). Th strips were heated in an oven at 110°C until the dark spots indicating the position of the sugars appeared. With the aid of lines rules on the central unsprayed portion of the chromatogram and using the developed spots on the marginal strips as indicators, sections of paper corresponding to raffinose and stachyose positions were cut from the central portion. The sugars from the strips were eluted with water and their concentrations estimated colorimetrically by the phenol-sulphuric acid method as mentioned earlier.

Isolation of starch and in vitro digestiblity. In vitro digestibility of meal starch and of the isolated starch was determined using pancreatic amylase. Starch was isolated according to the procedure of Garwood et al. (1976). A suitable amount of defatted meal (50 mg) or the isolated starch (25 mg) was dispersed in 1.0 ml of 0.2M phosphate buffer, pH 6.9. Pancreatic amylase (20 mg) was dissolved in 50 ml of the same buffer and 0.5 ml was added to the sample suspension and incubated at 37°C for 2 hr. After the incubation period, 2 ml of 3–5 dinitrosalicyclic acid reagent was quickly added and the mixture was heated for 5 min in a boiling water bath. After cooling, the solution was made to 25 ml with distilled water, and filtered prior to measurement of the absorbance at 550 nm. A blank was run simultaneously by incubating the sample first and 3–5 dinitrosalicyclic acid was added before the addition of the enzyme solution. Maltose was used as the standard and the values were expressed as mg of maltose released per gram of sample.

Table 1—Amylase inhibitor activity (AIA) and in vitro starch digestibility of eight desi and seven kabuli cultivars of chickpea

Cultivar	Starch (%)	Amylase inhibitor activity[e]		*In vitro* starch digestibility		
		Salivary amylase	Pancreatic amylase	a	b	c
Desi						
Range	48.4–53.4	3.7–8.4	7.8–10.5	39.8–50.5	85.4–99.5	108.3–123.0
Mean ± SE	50.8 ± 1.6	5.9 ± 0.2	9.0 ± 0.3	45.2 ± 2.0	89.7 ± 4.6	114.7 ± 5.4
Kabuli						
Range	49.6–54.8	3.1–7.3	5.6–10.0	40.5–51.7	86.6–100.2	120.4–148.5
Mean ± SE[d]	51.5 ± 1.5	4.3 ± 0.2	7.4 ± 0.3	47.1 ± 2.3	91.5 ± 5.1	135.0 ± 5.7

[a] mg maltose released/g meal
[b] mg maltose released/g meal starch
[c] mg maltose released/g isolated starch
[d] Standard error of estimation
[e] Units inhibited/g meal

Table 2—Correlations between starch content and in vitro starch digestibility of 15 cultivars of chickpea

	Correlation coefficients			
	In vitro starch digestibility (IVSD)			Starch content (%)
	a	b	c	
Amylase inhibitor activity[d]	−0.587*	−0.304	0.235	−0.151
IVSD: a	–	0.642**	−0.016	0.203
b	–	–	0.435	0.154
c	–	–	–	0.182

[a] mg maltose released/g meal
[b] mg maltose released/g meal starch
[c] mag maltose released/g isolated starch
[d] pancreatic anylase
*Significant at 5% level
**Significant at 1% level

RESULTS & DISCUSSION

Pancreatic and salivary amylase inhibitor activities

The amylase inhibitor activity of chickpea cultivars, when examined using pancreatic amylase (enzyme units inhibited/g meal), ranged between 7.8 and 10.5 in desi and 5.6 and 10.0 in kabuli cultivars (Table 1) indicating considerable variations among these cultivars. A similar variation but of lower magnitude was observed with salivary amylase. A comparison under similar assay conditions indicated that the amylase inhibitor activity was more towards pancreatic amylase than salivary amylase and this was found to be the case in both desi and kabuli cultivars. Jaffe et al. (1973) have reported that the partially purified kidney bean inhibited the salivary amylase more than the pancreatic amylase. This shows that amylase inhibitors from different legume seeds may behave differently towards the enzyme.

Pancreatic amylase inhibitor is present in most of the legumes, but the highest inhibitor activity has been reported in kidney bean (Jaffe et al., 1973). The inhibitor activity in chickpea cultivars appeared to be considerably lower than in other important food legumes. However, in well-cooked *Phaseolus vulgaris*, the inhibitor was reported to be completely inactivated at 100°C (Hernandex and Jaffe, 1968). We also observed that amylase inhibitors of a few chickpea cultivars became completely inactive when extracts were boiled for 10 min. But the findings reported here suggest that in case of unheated chickpea meal, some inhibition of starch digestion by amylase inhibitors may be expected.

In vitro starch digestibility

The starch digestibility was studied using pancreatic amylase. An increase in digestibility was observed with increasing periods of incubation up to 2 hr and thereafter no measurable changes were noticed. Therefore, for comparing the digestibility of cultivars, an incubation period of 2 hr was followed. The results expressed as mg maltose released/g meal and mg maltose released/g meal starch are reported in Table 1. No large variations in the starch digestibility of meal was observed among the cultivars studied and apparently no large differences in the digestibility of meal starches were noticed between desi and kabuli types. However, the mean value for digestibility of isolated starch was slightly higher for kabuli than for desi types (Table 1). On the other hand, digestibility of isolated starch from kabuli types was found to be higher than desi types. Moreover, the digestibility of isolated starch was apparently higher than that of the meal starch. There appeared to be no relationship between the digestibility of meal starch and isolated starch of chickpea (Table 2). Perhaps, some interfering substances are present in meal samples and in higher concentration in desi than in kabuli ones. In order to confirm this hypothesis, determination of in vivo digestibility of starch of these cultivars is required.

A statistically significant negative correlation was obtained between the amylase inhibitor activity and digestibility of meal (Table 2) indicating that the digestibility of starch is adversely affected by the levels of amylase starch is adversely affected by the levels of amylase inhibitor. But there was no significant correlation between amylase inhibitor activity and digestibility of isolated starch. It is known that oligosaccharides such as raffinose, stachyose, and verbascose are present in considerable amount in several grain legumes (Nigam and Giri, 1961). However, due to the nonsignificant differences in these oligosaccharides among the legumes, the observed differences in the α-amylolysis of different legumes could not be explained on the basis of the presence of these oligosaccharides (Srinivasa Rao, 1969). Also, our results revealed no relationship between the in vitro starch digestibility and the stachyose and raffinose contents of chickpea.

Stachyose and raffinose content

Data on the concentrations of total soluble sugars and oligosaccharides in chickpea cultivars are given in Table 3. While the percentages of soluble sugars in these cultivars did not differ considerably, fairly large variations in stachyose and raffinose contents were observed. When the results of desi and kabuli were considered together, it was noticed that on average, stachyose accounted for 26.7% and raffinose accounted for 10.2% of the total soluble sugars.

These results are comparable to those of earlier workers who reported that in chickpea, stachose and raffinose account for 27.3 and 7.7% of total soluble sugars, respec-

Table 3—Soluble sugars, stachyose and raffinose contents in eight desi and seven kabuli cultivars of chickpea

Cultivar	Soluble sugars (%)	Stachyose		Raffinose	
		a	b	a	b
Desi:					
Range	4.15–5.08	1.06–1.85	25.01–42.82	0.36–0.66	8.91–13.65
Mean ± SE	4.47 ± 0.12	1.34 ± 0.03	29.97 ± 1.58	0.50 ± 0.01	11.07 ± 0.50
Kabuli:					
Range	4.68–5.67	0.82–1.38	17.94–28.87	0.36–0.62	7.25–12.95
Mean ± SE[c]	5.06 ± 0.15	1.16 ± 0.04	23.41 ± 1.03	0.46 ± 0.02	9.27 ± 0.48

[a] g/100g sample
[b] g/100g soluble sugars
[c] Standard error of estimation

tively (Lineback and Ke, 1975). Earlier workers (Udashanker Rao and Belavady, 1978) reported that chickpea also contained one more oligosaccharide-verbascose. But in the present study, this oligosaccharide was not determined because the standard verbascose required for determination, was not available commercially.

In order to know if any relationship exists between the oligosaccharides and total soluble sugars, correlation coefficients among these variables were worked out (Table 4). Total soluble sugars were not significantly correlated with either of these two oligosaccharides expressed either as g/100g sample or as g/100g soluble sugars. The present study gave enough indication that the concentration of these oligosaccharides is independent of the levels of total soluble sugars in these cultivars. On the other hand, stachyose and raffinose were positively and significantly correlated with each other when the results were expressed either as g/100g sample or as g/100g soluble sugars.

The ingestion of large quantities of legumes is known to cause flatulence in experimental animals and humans due to the presence of these oligosaccharides. Germinated or cooked chickpea or mungbean did not greatly alter their flatus-inducing capacity as compared to the raw forms (Shurpalaker, 1973). In view of the observed variations in the levels of oligosaccharides among the chickpea cultivars and their implication in human nutrition, attempts should be made to screen and then select cultivars having lower amounts of these oligosaccharides.

Table 4—Correlation coefficients between total soluble sugars, stachyose and raffinose in 15 cultivars of chickpea

Oligosaccharides	Raffinose		Stachyose		Soluble sugars (%)
	a	b	a	b	
Raffinose					
a	–	0.923**	0.765**	0.696**	0.091
b	–	–	0.692**	0.781**	–0.289
Stachyose					
a	–	–	–	0.917**	0.154
b	–	–	–	–	–0.244

[a] g/100g sample
[b] g/100g soluble sugars
**Significant at 1% level

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