Effects of Presoaking on Faba Bean Enzyme Inhibitors and Polyphenols after Cooking

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The effects of presoaking prior to cooking on tannins as well as on trypsin, chymotrypsin, and α-amylase inhibitory activities in the hulls of eight different varieties of *Vicia faba* were investigated. Tannin contents of bean hulls before and after soaking for 18 h and then cooking for 1 h at 121 °C ranged from 1.2 to 4.4 and 0.39 to 0.93 mg of catechin equiv/g of hull, respectively. Cooking after soaking lowered the tannin content of seed hulls by 64.76–78.88%. Presoaking prior to cooking also decreased trypsin, chymotrypsin, and α-amylase inhibitory activities in the seed hulls. Tannins were not detected in white-seeded varieties. Presoaking prior to cooking significantly improved the in vitro digestibility of (whole seed) faba bean proteins.

Faba beans (*Vicia faba*) are the fourth most important pulse crop in the world after dry beans, dry peas, and chickpeas. Faba beans are an important source of dietary protein, particularly in Egypt, Northern Sudan, the highland areas of Ethiopia, and certain rural communities in Maghreb. In Egypt there are many types of faba beans: Giza 1, Giza 2, Giza 3, Giza 4, Rebaeya 1, Rebaeya 2, Rebaeya 4, and Rebaeya 40. The common varieties consumed in Egypt are Giza 1, Giza 2, and Rebaeya 40.

Legumes (including faba beans) require long cooking times, produce methionine deficiency in legume proteins, and show the presence of several heat-stable and heat-labile factors that interfere with digestion, causing gastrointestinal distress and flatulence (Rockland and Nishi, 1979). Tannins are responsible for protein undigestibility in animals and humans, probably either by making protein partially unavailable or by inhibiting digestive enzymes. They are associated with an increase of fecal nitrogen. The increase in nutritional value of beans subjected to heat treatment has been generally attributed to the destruction of protease inhibitors and lectins (Liener, 1979a). The importance, mode of action, nutritional significance and nutritional toxicity, effects of processing, and related aspects of trypsin, chymotrypsin, and α-amylase inhibitors of legumes have been discussed in several reviews (Liener, 1975, 1979a,b; Marshall, 1975).

The seed coats of legumes are sometimes removed prior to their processing and consumption. Kon et al. (1973) studied the effects of seed coat removal on cooking times of dry beans. They observed a reduction of 42, 53, and 70% in cooking times of peeled, unsoaked California small white, Sanilac, and Pinto beans, compared to the corresponding controls. These authors, however, did not report data on the antinutrients of dehulled beans. Progressive dehulling causes an accumulative loss of protein constituents (up to 45%) (Bakshy et al., 1978) and a decline in the content of lysine (Axtell et al., 1975), histidine, and arginine (Bakshy et al., 1978). In this investigation, presoaking prior to cooking and not dehulling was done to minimize certain antinutritional factors (trypsin, chymotrypsin, and α-amylase inhibitory activities) and tannins, which are predominantly located in the pericarp and/or testa of eight cultivars of faba bean commonly grown in Egypt. The effects of presoaking prior to cooking on in vitro protein digestibility were also investigated.

MATERIALS AND METHODS

The tested varieties of Faba bean according to genetic lines were as follows: Giza 2, Giza 3, Rebaeya 40, and Family 402. These varieties were consumed in Egypt, while yellow spot, white flour, double white, and triple white are new varieties. All varieties were provided by the Legume Department, Institute of Field Crops, Agricultural Research Centre, Giza, Egypt.

Preparation of Samples for Analysis. The bean varieties were manually dehulled with a sharp knife. Dehulled seeds and seed coats (hull) were weighed separately, and their percentages were calculated. All hull samples were individually ground to a 60-mesh flour in a UDY Cyclone Mill (Tecator, Inc., Boulder, CO).

Preparation of Cooked Samples after Soaking. Beans were soaked in tap water (1:5 (w/v) bean to water ratio) for 18 h at room temperature and then heated in the same soaking water for 60 min at 1 kg F/cm² (121 °C). Seed hulls were removed, dried in an air-drying oven at 50 °C for 24 h, and then ground in a UDY Cyclone Mill as described above. The tannin content as well enzyme inhibitiors were assayed in the hulls before and after soaking/cooking.

Tannin Content. Tannin contents of bean hulls were determined according to the modified vanillin–hydrochloric acid method of Price et al. (1978) using appropriate sample blanks. Catechin (Sigma Chemical Co., St. Louis, MO) was used as a reference standard and the tannin content expressed in terms of catechin equivalents.

Trypsin and Chymotrypsin Inhibitors. The sources of chemicals for the assays were as follows: Trypsin enzyme, substrate, and *p*-tosyl-L-arginine methyl ester (TAME) were obtained from Sigma. α-Chymotrypsin was from P. L. Biochemicals, Milwaukee, WI. Benzoyl-L-tyrosine ethyl ester (BTEE) was from Aldrich Chemical Co., Milwaukee, WI.

A 1-g flour sample (hull) was blended with 15 mL of 0.05 N HCl for 2 min in a Sorvall Omni-mixer (Ivan Sorvall, Inc., Newtown, CT) and extracted for 12 h at 4 °C. The slurry was then centrifuged at 5000*g* for 15 min. A 3-mL portion of 30% TCA was added to the supernatant and the mixture recentrifuged. The supernatant was then neutralized with sodium hydroxide to pH 8.0 and made up to 25 mL. The enzyme inhibitory activities were determined as described by Decker (1977). The details of the procedure are described by Sath and Salunkhe (1981).

α-Amylase Assay. α-Amylase (Type I-A, from porcine pancreas) was obtained from Sigma, and soluble potato starch was obtained from J. T. Baker Chemical Co., Phillipsburg, NJ. All reagents were preincubated for 15 min at 37 °C in a water bath. To 0.5 mL of 1% starch solution in 0.2 M sodium phosphate buffer (pH 7.0) and 0.25 mL of the buffer was added 0.25 mL of α-amylase enzyme solution (30 μg/mL in 0.2 M sodium phos-

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Table I. Tannin Content of Hulls from Different Faba Beans before and after Cooking Previously Soaked Cultivars

		tannin content,[a] mg catechin equiv/g hull		
cultivar	color	hull of raw beans	hull of cooked beans after soaking	% redn on cooking after soaking
Giza 2	brown	3.56	0.81	77.25
Giza 3	dark brown	3.9	0.88	77.43
Family 402		4.25	0.90	78.88
Rebaeya 8		4.4	0.93	78.86
yellow spot	brown	3.5	0.89	74.57
white flour	bronze	2.1	0.74	64.76
double white	yellowish	1.2	0.39	67.50
triple white	white	ND[b]	ND	ND
				74.17[c]
mean ± SE		3.27 ± 1.18	0.79 ± 0.19	
SD		1.097	0.174	
t-test			5.910	
stat signif			$P > 0.001$	

[a] Means of triplicate determinations on a dry weight basis. [b] ND = not detected. [c] Mean percent decreases on cooking after soaking.

phate buffer (pH 7.0) containing 0.006 M NaCl). At the end of 30 min, the reaction was stopped by addition of 2 mL of dinitrosalicylic acid reagent (Sumner, 1924) and heating in a boiling water bath for 10 min. The test tubes were then cooled under running cold tap water and made to a final volume of 13 mL with distilled water. The absorbance was recorded at 540 nm in a Beckman DB-G spectrophotometer. The liberated reducing sugars were expressed as maltose. One unit of enzyme activity was defined as that which liberates from soluble starch 1 μmol of reducing groups (calculated as maltose)/min at 37 °C and pH 7.0 under the specified conditions (Deshpande et al., 1982).

α-Amylase Inhibitor Assay. α-Amylase inhibitor activity was evaluated according to the method of Deshpande et al. (1982). A 1-g sample was extracted with 10 mL of distilled water for 12 h at 4 °C and centrifuged at 5000*g* for 20 min, and the supernatants were tested for α-amylase inhibitory activity. Extract containing inhibitor (0.25 mL) was incubated with 0.25 mL of enzyme solution for 15 min at 37 °C. To this mixture after precincubation was added 0.5 mL of 1% starch solution. The assay was conducted as described above. One unit of α-amylase activity inhibited was defined as 1 α-amylase inhibitory unit.

In Vitro Digestibility. In vitro protein digestibility of whole beans before and after cooking the previously soaked seeds was determined by the multienzyme technique of Hsu et al. (1977). The details of the procedure were described by Sath et al. (1982). The enzymes peptidase and trypsin were from Sigma, and α-chymotrypsin was from P. L. Biochemicals.

Statistical Analysis. Statistical analysis of the results was carried out as described by Fisher (1950). A *t* was calculated and compared with its tabulated value at the 5% level of significance and with a degree of freedom of $n_1 + n_2 - 2$. The *p* values corresponding to the *t* values were obtained from statistical tables.

RESULTS

Tannin contents in the hulls of different raw and cooked faba bean cultivars ranged from 1.20 to 4.40 and 0.39 ± 0.93 mg of catechin equiv/g of hull, respectively (Table I). Tannins were not detected in the white-seeded variety (triple white). Among the colored seeds, Rebaeya 40 and Family 402 contained more tannin than did the Giza beans. Double white beans had the lowest tannin content of the colored faba beans (Table I). Presoaking prior to cooking removed most of the tannin from the colored hulls. The percent decrease in tannins of all tested samples ranged from 64.76 to 78.88 with an average of 74.17%, and it was statistically significant at $P > 0.001$.

Data on trypsin, chymotrypsin, and α-amylase inhibitor activities of the raw and cooked hulls after soaking are presented in Table II. Soaking and then cooking significantly ($P > 0.001$) decreased only the activities of α-amylase inhibitor in the investigated samples.

Soaked and cooked whole beans had significantly higher in vitro protein digestibility than did the raw seeds (Table III). The in vitro protein digestibilities ranged from 54.2 to 72.0% and from 63.9 to 84.9% for the raw and cooked beans, respectively. Triple white bean proteins were significantly more digestible than those of the other varieties investigated, whereas Rebaeya 40 bean proteins were the least digestible (Table III).

DISCUSSION

The decrease of trypsin, chymotrypsin, and α-amylase inhibitor activities in bean hulls after soaking and cooking of all eight different varieties investigated may be attributed to at least two factors: First, these antinutritional factors may normally be present in the seed coat fractions of the beans. Second, the seed coat contributes a substantial portion of the whole seed weight. Cooking after soaking may lead to a decrease in the concentration of these antinutrients on a unit weight basis. Presoaking prior to cooking caused a decrease in the relative contents of these antinutrients within an average percent as follows: trypsin inhibitor, 11.84%; chymotrypsin inhibitor, 11.54%; α-amylase inhibitor, 45.85% (Table II). This decrease suggests that presoaking prior to cooking may improve the extraction efficiency of the inhibitors from the seed hull. In many cases the contents of inhibitory factors are not considered to be absolute and may vary depending upon the variety and/or cultivar, climatic condition, location, irrigation conditions, type of soil, and year during which they are grown (Bassiri and Nahapetian, 1977; Singh and Sedeh, 1979; Miller et al., 1980a,b). The low enzyme inhibitor content of certain cultivars in the present study may be largely be due to these factors (Table II).

A review of the literature suggests that milling lowers the α-amylase inhibitor activity of rice (de Boland et al., 1975; Reddy and Solunkhe, 1980). Davies and Nightingale (1975) suggested that enzyme inhibition was dependent on the "quality and nature" and not solely on the quantity of the polyphenols present; enzyme inhibition gave the best measure of the inhibitory polyphenols.

The white-seeded cultivar in the present study (triple white) had significantly lower enzyme inhibitor activities than did the others. In general, the colored beans exhibited more enzyme inhibitor activities. The wide variation in the inhibitor activities observed among the faba bean varieties investigated suggests that genetic selection for beans with low inhibitory activities should be effective.

A relationship was observed between enzyme inhibitory activity and bean color in the present study. Dry bean protease inhibitors are not considered to be a practical problem. Cooking or heat treatment of beans generally destroys these heat-labile antinutritional factors (Liener, 1979a).

Tannin contents of the hull of dry faba bean seed cultivars investigated were within the range reported for several legumes (Ma and Bliss, 1978; Price et al., 1980; Davis, 1981).

Tannins in beans are located in the seed hull (Ma and Bliss, 1978), and thus its removal and or/treatment (presoaking prior to cooking) may be expected to reduce the tannin content of beans. Tannins were detected only in the colored cultivars, and there was a direct relationship between the intensity of color and tannin content in the

Table II. Enzyme Inhibitory Activities of Hulls from Different Faba Beans[a,b] before and after Cooking Previously Soaked Cultivars

	trypsin inhib act. units, g × 10^{-3}			chymotrypsin inhib act. units, g × 10^{-3}			α-amylase inhib act. units, g		
cultivar	A	B	C	A	B	C	A	B	C
Giza 2	34.93	33.41	4.35	44.72	43.55	02.61	114.55	69.03	39.72
Giza 3	36.66	30.29	6.66	47.45	41.99	11.49	115.44	54.60	52.70
Family 402	37.57	35.10	6.50	48.23	43.04	10.78	123.50	72.80	41.05
Rebaeya 8	56.29	47.19	16.16	48.15	41.99	12.79	127.70	74.01	41.71
yellow spot	34.19	31.20	8.74	41.34	37.32	12.44	114.66	74.49	35.03
white flour	33.44	28.08	15.95	37.44	33.8	09.73	110.24	82.29	25.35
double white	31.85	58.85	9.14	35.33	28.21	19.92	109.72	42.90	60.90
triple white	14.15	10.18	28.05	24.58	20.22	17.73	60.45	33.95	43.83
			11.94[c]			10.60[c]			42.53[c]
mean ± SE	34.89 ± 11.40	33.65 ± 14.64		40.91 ± 8.22	36.18 ± 8.34		109.53 ± 20.77	63.01 ± 17.22	
SD	10.666	13.695		7.691	7.797		19.433	16.109	
t-test		0.2013			1.1423			5.213	
stat signif at *P*		<0.8			<0.2			>0.001	

[a] Means of triplicate determinations on a dry weight basis. A–C are, respectively, results from the hull of raw beans, beans that have been soaked then cooked, and the percent decrease of B on A. [b] One unit of inhibitor activity is that which reduces the activity of the corresponding enzyme by 1 unit under the assay conditions. [c] Mean percent decreases on cooking after soaking.

Table III. In Vitro Digestibility of Whole Different Faba Beans[a] before and after Cooking Previously Soaked Cultivars

	% in vitro digestibility		
cultivar	raw beans	cooked beans after soaking	% inc on cooking after soaking
Giza 2	59.45	69.20	16.40
Giza 3	57.95	67.70	12.83
Family 402	57.20	65.33	14.21
Rebaeya 40	54.20	63.96	18.00
yellow spot	60.83	68.40	12.44
white flour	61.70	70.70	14.58
double white	63.70	73.5	15.38
triple white	72.07	84.95	17.87
			15.21[b]
mean ± SE	60.89 ± 5.38	70.47 ± 6.56	
SD	5.032	6.136	
t-test		3.414	
stat signif		$P < 0.001$	
casein digestibility		97.7%	

[a] Means of duplicate determinations. [b] Mean percent increases on cooking after soaking.

present study (Table I). Cooking after soaking slightly but significantly improved the in vitro protein digestibility of the investigated faba bean cultivars. This may be attributed to the removal of the polyphenols in the seed hull and to the removal of enzyme inhibitors (Tables I and II).

The in vitro digestibility of bean proteins observed in the present study was much less than that reported for casein (97.7%, a well-established phenomenon) (Acton et al., 1982). The low in vitro digestibility of bean proteins may be due to their structural characteristics. Major storage proteins of dry beans are known to be resistant to proteolysis (Liener and Thompson, 1980; Chang and Satterlee, 1981). Numerous studies have indicated that the globulin fraction of legumes, which represents the major storage protein and comprises 50–75% of the total protein of the dry seed (Romero et al., 1975), is quite resistant to attack by proteolytic enzymes in vitro (Romero and Ryan, 1978; Seidl et al., 1969; Vaintraub et al., 1976; Liener and Thompson, 1980; Chang and Satterlee, 1981). Our results also indicated the inherent resistance of bean proteins to proteolytic digestion.

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