Quick-cooking beans (*Phaseolus vulgaris* L.): II. Phytates, oligosaccharides, and antienzymes‡

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Abstract. Effects of the quick-cooking processes on phytate and oligosaccharide levels, and on trypsin and chymotrypsin inhibitors, were investigated in three bean varieties (*Phaseolus vulgaris* L.: Great Northern, red kidney, and pinto). Beans soaked in distilled water had lower levels of phytate-P than those soaked in a mixed salt solution. Leaching losses of oligosaccharides were nearly the same in different soaking treatments for all the beans except kidney beans. Residual trypsin inhibitor activities (TIA) in cooked quick-cooking beans were about 10% compared with about 20% for chymotrypsin inhibitor activities (CTIA) in the same bean products. γ-Irradiation was more effective in reducing TIA than CTIA and paralleled destruction by moist heat.

Introduction

A worldwide stable supply of nutritionally balanced food is of fundamental importance. It is estimated that over 460 million people in the world today are undernourished and/or malnourished. Proteins, in particular, are of primary concern in this context. Legumes serve as the main source of dietary protein in many parts of the world. They complement cereals in terms of amino acid balance. On an average, grain legumes contain 20%–30% protein on a dry weight basis, and provide about 350 calories of food energy per 100 g. Legumes also serve as an important source of minerals such as calcium, magnesium, iron, zinc, and potassium. Legume proteins are also much cheaper than proteins from animal sources.

Although trypsin inhibitors comprise only 2.5% of the total bean proteins, they contribute 30%–40% of the total cystine content [5]. The nutritional significance of these inhibitors after inactivation is often overlooked. About 40% of the improvement in utilization of processed beans is attributed to inactivation of these inhibitors [6].

Flatulence, due to ingestion of beans, is a factor of social significance and physical discomfort. The raffinose family of oligosaccharides, including stachyose and verbascose, have been reported as the primary cause of bean flatus. These oligosaccharides bypass digestion and absorption due to the lack of the enzyme α-galactosidase in humans. Consequently, the bacteria in the intestinal tract metabolize them to yield carbon dioxide, hydrogen, and methane [10]. The presence of phytic acid (myoinositol hexaphosphate) in

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beans has been studied [9]. Phytic acid may interfere with the mineral utilization when it comprises more than 1% of the diet. Therefore, mineral bioavailability from foods containing phytic acid is of concern.

Cooking quality of beans depends on several factors, such as growing conditions, chemical composition, e.g., mineral content, phytic acid level, and storage and handling. In addition, prolonged cooking may reduce the nutritive quality of the products. Conventional methods for preparing dry beans for food are both time and energy consuming. Reduction in cooking time, through the introduction of quick-cooking beans, can effect significant energy savings in the fuel-deficient, developing areas of the world.

The present study was undertaken to investigate the effects of the quick-cooking process on phytates, oligosaccharides, and proteolytic enzyme inhibitors.

Material and Methods

Lot sizes of 25 lbs of beans (*Phaseolus vulgaris* L.), namely, Great Northern, kidney, and pinto, were obtained from Bean Growers' Warehouse, Filer, Idaho, and stored at 4°C until the experiments were conducted.

Production of quick-cooking beans

Quick-cooking bean products were prepared using procedures described by Rockland and Metzler [13] and Rockland et al. [12]. The beans were blanched in boiling distilled water for 5 min to facilitate hydration and to loosen the seed coat.

Blanched beans were soaked for 0–24 h in different soak solutions. The soak solutions were 2.5% sodium chloride, 1% sodium tripolyphosphate, 1.5% sodium bicarbonate, and 0.5% sodium carbonate (all percentages wt/vol), and a combination of the above salt solutions, hereafter abbreviated as MSS (mixed salt solution), at temperature 22°, 37°, and 45°C at pH values 7.0 and 9.0. Food acidulants (citric, malic, and tartaric), each at concentrations of 0.1%, 0.5%, and 1.0% (wt/vol), were employed. Throughout, the bean-soak solution ratio was 1:3 (wt/vol). The soaked beans were rinsed with distilled water and portions were employed for cooking [in distilled water at 100°C; bean:water::1:5 (wt/vol)], direct freeze dehydration, and dehydration in conventional tray driers.

Soaked beans were dehydrated in conventional tray driers to a final moisture content of 10%–12%. An air velocity of 14 m/min and a temperature not greater than 55°C were maintained during drying. The soaked, dehydrated beans were subjected to γ-irradiation (^{137}Cs). Beans were irradiated at room temperature (22°C), employing doses of 0, 100, 250, and 500 krads at the rate of 12 krads/h.

Physicochemical analyses

Moisture content of the samples was estimated by the AOAC method [1].

Phytates. The modified method of Wheeler and Ferrel [15] was employed; 1–2 g meal (60 mesh) was extracted with 50 ml of 3% (wt/vol) trichloroacetic acid (TCA) solution for 1.5 h with mechanical shaking. The slurry obtained was centrifuged at 5000*g* for 15 min; 10 ml of supernatant were used for each assay. Phytate in the supernatant was precipitated as ferric phytate. The conversion of ferric phytate to ferric hydroxide was carried out by Makower's procedure [8]. The precipitate (ferric hydroxide) was dissolved in 0.5 ml of 0.5 *N* hydrochloric acid in a boiling water bath for 15 min. The solutions were then transferred quantitatively and made to volume with 0.1 *N* hydrochloric acid. The Fe^{+++} ion concentration was determined by the AOAC method [1].

Oligosaccharides. The oligosaccharides from bean samples were extracted according to the method of Hymowitz et al. [4], separated by the paper chromatographic method of Shallenberger and Moores [14], and quantitatively determined by the phenol-sulfuric acid method [3]. The sources of standard oligosaccharides were: verbascose (a gift from Dr. E. Cristofaro, Nestle's Products Technical Assistance, Ltd., Switzerland), stachyose tetrahydrate (a gift from Dr. R.S. Shallenberger, NY Agric Exp Stn, Geneva, NY), raffinose pentahydrate (Mann Research Labs, Inc., New York, NY), and sucrose (ICN Nutritional Biochemicals, Cleveland, Ohio).

Trypsin and chymotrypsin inhibitors. One gram of bean meal (60 mesh) was blended with 15 ml of 0.05 *N* hydrochloric acid for 2 min and extracted with mechanical shaking for 1 h. The slurry was then centrifuged at 5000*g* for 15 min; 3 ml of 30% TCA were added to the supernatant, and the supernatant was recentrifuged. The supernatant was then neutralized with sodium hydroxide, the pH adjusted to 8.0, and made to a volume of 250 ml. Trypsin and chymotrypsin activities were determined as outlined in the Worthington Enzyme Manual [2]. The corresponding inhibitors were assayed as follows. An equal volume of the inhibitor-containing solution and the respective enzyme was incubated at room temperature (22°C) for 2–3 min. The residual enzyme activity was determined as before.

Results and Discussion

Phytates

Phytate-P analyses of raw dry, water-soaked, and salt-soaked beans and their corresponding cooked products are shown in Table 1. Conventionally soaked and cooked beans contained considerably less phytate than dry raw, salt-soaked, or salt-soaked cooked beans. Water-soaked cooked beans contained

Table 1. Effects of soaking and cooking on phytate[a] concentrations in Great Northern, kidney, and pinto beans

Treatment		Great Northern	Kidney	Pinto
Control (dry beans)		(4.6)	(5.8)	(5.5)
Soaking[b]		30.4	48.3	47.3
Soaking[c]		91.3	77.6	81.8
Soaking and cooking (15 min)		86.9	68.7	74.5
Conventional cooking (90 min)		23.9	43.2	38.2
LSD	0.05	0.59	0.58	0.38
	0.01	0.84	0.83	0.54

[a]Mean of triplicate determinations, expressed as phytate-phosphorus in mg/g beans on moisture-free basis. Numbers in parentheses are absolute values for control samples. The remaining values are expressed as percentages of the controls.
[b]Soaking in distilled water, 18 h, room temperature (22°C).
[c]Soaking in MSS, 18 h, room temperature (22°C).

considerably less phytate than dry raw, salt-soaked, or salt-soaked cooked beans. Cooked water-soaked beans contained only slightly less phytate than the raw, water-soaked samples. The salt-soaked and salt-soaked cooked beans contained one and one-half to three times as much phytate as the samples soaked and/or cooked in distilled water. Analogously, the salt-soaked cooked beans contained only slightly less phytate than the corresponding raw beans, retaining 70%–80% of the phytate found in the raw dry beans. It would appear that the diffusibility of phytate during rehydration is retarded when the beans are soaked in MSS. It is possible that divalent cations, i.e., calcium and magnesium, released from protein-carbohydrate complexes during rehydration in the salt solution, react with soluble phosphate phytate to form insoluble or nondiffusible calcium and/or magnesium phytate compounds.

Oligosaccharides

Oligosaccharide content in the processed beans and its loss in processing are presented in Table 2 for Great Northern, kidney, and pinto beans. Leaching losses in different soaking solutions, including distilled water, were practically the same for the oligosaccharides in all the beans studied, with the exception of raffinose in kidney beans. Conventional processing and cooking facilitated greater removal of oligosaccharides than those processed in MSS. This may be due to the longer duration of cooking in the case of the former over the latter. Raffinose leached preferentially compared with sucrose, verbascose, and stachyose. Traces of verbascose were detected in raw kidney beans, but not in soaked and cooked beans. Stachyose content was highest in all the samples followed by sucrose and raffinose. Quick-cooking cooked Great Northern, kidney, and pinto beans lost 43%, 45.8%, and 64.4% in raffinose + stachyose, respectively, compared with raw beans.

Table 2. Effects of soaking and cooking on oligosacchardies in Great Northern, kidney, and pinto beans

Treatment	Sucrose[a]			Raffinose[a]			Stachyose[a]			Verbascose[a]		
	Great Northern	Kidney	Pinto	Great Northern	Kidney	Pinto	Great Northern	Kidney	Pinto	Great Northern	Kidney	Pinto
Control (dry beans)	(2.02)	(1.92)	(2.19)	(0.56)	(0.93)	(0.63)	(2.40)	(2.44)	(2.95)	ND[d]	(0.06)	ND[d]
Soaking[b]	70	83	83	61	26	75	69	59	60	–	–	–
Soaking[c]	68	69	85	46	57	70	71	64	61	–	–	–
Conventional cooking (90 min)	65	55	56	23	11	11	31	38	22	–	–	–
Quick cooking (15 min)	56	52	53	34	19	43	61	52	34	–	–	–
LSD 0.05	0.34	0.52	0.39	0.20	0.29	0.19	0.26	0.32	0.26			
0.01	0.49	0.74	0.56	0.29	0.39	0.28	0.38	0.50	0.38			

[a]Mean values of triplicate determinations. Numbers in parentheses indicate absolute values in g/100 g dry beans for control samples. The remaining values are expressed as percentages of the controls.
[b]Soaking in distilled water, 18 h, room temperature (22°C).
[c]Soaking in MSS, 18 h, room temperature (22°C).
[d]ND, not detectable.

Table 3. Effects of processing on trypsin and chymotrypsin inhibitor activities[a] in Great Northern, kidney, and pinto beans

Treatment	Trypsin inhibitory activity[b] × 10^3			Chymotrypsin inhibitory activity[c] × 10^3		
	Great Northern	Kidney	Pinto	Great Northern	Kidney	Pinto
Control (dry beans)	(349.9)	(395.6)	(344.5)	(198.2)	(199.5)	(260.2)
Soaking[d]	96.7	89.5	86.7	98.9	96.1	84.6
Soaking[d] + dehydration[e]	98.9	82.0	91.4	99.5	93.1	79.9
Soaking[d] + dehydration[e] + cooking (15 min)	12.3	11.4	7.9	19.9	21.1	19.5
Conventional cooking (90 min)						
Soaking[d] + dehydration[e] + irradiation 100 krads	62.7	49.8	18.5	87.5	94.4	72.1
250 krads	60.3	36.3	15.3	88.2	86.4	73.1
500 krads	56.3	28.4	16.7	73.2	83.5	71.5
LSD 0.05	25.9	22.2	36.8	27.0	20.7	29.7
0.01	34.0	30.9	51.2	37.5	28.9	41.2

[a]Numbers in parentheses indicate absolute values of the inhibitor activities for control samples. The remaining values are residual activities expressed as percentages of the control values.

[b]Mean of triplicate determinations. One unit of trypsin inhibitor is that which reduces the activity of trypsin by one unit.

[c]Mean of triplicate determinations. One unit of chymotrypsin inhibitor is that which reduces the activity of chymotrypsin by one unit.

[d]Soaking in MSS, 18 h, room temperature (22°C).

[e]Dehydration to a final moisture content of 10%–12%.

Trypsin and chymotrypsin inhibitors

Table 3 contains results of trypsin- and chymotrypsin-inhibiting activities per gram beans. Quick-cooking, cooked (15 min) Great Northern, kidney, and pinto beans had about 20% residual chymotrypsin-inhibiting activity (CTIA). The corresponding figures for residual trypsin-inhibiting (TIA) activity ranged from 8%–12%. The susceptibility of CTIA and TIA towards heat inactivation varied within and among the bean samples. Rockland [11] reported the complete destruction, during cooking of TIA, in both standard and quick-cooking large lima beans and pink beans. Destruction of TIA and CTIA by moist heat may be attributed to hydration of these inhibitors, which may change their secondary and tertiary structures, increasing their susceptibility to denaturation.

Summary

Effects of quick-cooking processes on antinutritients in Great Northern, kidney, and pinto beans were evaluated. Beans soaked in distilled water had lower phytate-P contents than those soaked in salt solutions. Conventional processing of beans facilitated greater removal of oligosaccharides than the quick-cooking process. Stachyose was the major oligosaccharide found in all three bean varieties.

Residual chymotrypsin-inhibiting activity (CTIA) in quick-cooking cooked Great Northern, kidney, and pinto beans was about 20% compared with a range of 8%–12% for residual TIA activity. γ-Irradiation was more effective in reducing TIA than CTIA, and paralleled destruction of TIA and CTIA by moist heat.

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