Irradiation Inactivation of Some Antinutritional Factors in Plant Seeds

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Effects of γ-irradiation (1.0−10 kGy) on trypsin, chymotrypsin, and α-amylase inhibitors of soybean and *Moringa peregrina* seeds on tannin of sorghum, gossypol of cottonseed, and in vitro digestibility of soybean were investigated. A dose of 10.0 kGy caused decreases in trypsin (by 34.9%) and chymotrypsin (by 71.4%) inhibitor activities in soybean defatted flour, whereas its in vitro digestibility increased from 79.8 to 84.2%. The α-amylase inhibitor activity of Al-Yassar (*M. peregrina*) was decreased by 43.6 and 47.8% upon treatment of 7.0 and 10.0 kGy, respectively. Doses of 10.0 and 7.0 kGy significantly reduced the tannin content in Shahlla sorghum but not in Hemaira sorghum. Total and free gossypol contents were slightly reduced by irradiation.

Keywords: *Irradiation; proteinase inhibitors; tannin; gossypol; Moringa peregrina; soybean; sorghum; cottonseed*

INTRODUCTION

Plant seeds are economical sources of protein and other nutrients and can play significant roles in human nutrition. Therefore, they are particularly needed in developing countries, where the average protein intake is less than desirable. Weber et al. (1977) suggested several novel plant protein sources that could be used as food to provide the needed protein. However, the nutritive quality or digestibility of plant proteins is affected by the presence of antinutritional factors such as proteinase inhibitors, especially trypsin and chymotrypsin inhibitors (Griffiths, 1979; Fernandez et al., 1982; Xavier-Filho et al., 1989; Al-Kahtani, 1995; Abu-Tarboush and Ahmed, 1996), α-amylase inhibitors (Singh et al., 1982; Frels and Rupnow, 1985; Cinco et al., 1985; Al-Kahtani, 1995; Grant et al., 1995), phenolic compounds (Griffiths, 1981; Narasinga Rao and Prabhavathi, 1982; Deshpande et al., 1982; Helsper et al., 1993; Al-Kahtani, 1995), and phytate (Thompson and Erdman, 1982; Storey et al., 1983).

Protease inhibitors may inhibit growth, reduce digestibility, and cause pancreatic hypertrophy (Liener and Kakade, 1980). The physiological role of α-amylase inhibitors in plants is still uncertain. There is some evidence to suggest that they may act as a protein reserve in seeds (Sharma and Pattabiraman, 1980). Phytate, a common constituent of plant tissues, has been shown to have an inhibitory action against a proteolytic enzyme (Kratzer, 1965; Kanaya et al., 1976; Knuckles et al., 1985). Tannins are known to impair utilization of proteins in human and animal diets by binding with protein (Butler et al., 1984; Reddy et al., 1985). Growth retardation has been observed in animals fed diets containing tannins (Joslyn and Glick, 1969). Gossypol, a polyphenolic compound, is a constituent of cottonseeds (Murti and Achaya, 1975) and is toxic to monogastric animals (Berardi and Goldblatt, 1980).

Several conventional processing methods, such as germination (Sathe et al., 1983; Nnanna and Philips, 1988; Chang et al., 1989; Vidal-Valverde et al., 1994), soaking, fermentation (Romo-Parada et al., 1985; Hassan and El-Tinay, 1995), and cooking (Dhurandhar and Chang, 1990; Barampama and Simard, 1994; Vidal-Valverde et al., 1994) have been used to inactivate these undesirable components from plant seeds. The abovementioned treatments generally reduce raffinose oligosaccharides and antinutrional factors, but the effect varied with plant cultivars and treatments. In many instances, usage of only one method may not effect the desired removal of antinutritional factors and combination of two or more methods is required. Moreover, destruction of some nutrients and loss of some water-soluble nutrients may occur with heat and soaking treatments.

Irradiation has been suggested to remove or reduce antinutritional factors (Ghazy, 1990; Ghazy et al., 1992; Joseph and Dikshit, 1993). The use of irradiation technology is promising since its effect on nutrients is minimal if suitable doses are applied. However, research in this area is scarce, particularly in newly discovered plants with potential use in human nutrition.

Therefore, it was the objective of this study to evaluate the efficiency of irradiation in the inactivation of the most common antinutritional factors, trypsin, chymotrypsin (in soybean), and α-amylase inhibitors (in *Moringa peregrina*), tannins of sorghum, and gossypol of cottonseeds.

MATERIALS AND METHODS

Materials. Seeds of *M. peregrina* (Al-Yassar or Al-Ban) were obtained from the Al-Ola region, northwestern Saudi Arabia. Soybeans (cv. Jupiter) and cottonseeds were obtained from the Agricultural Experimental Station, College of Agriculture, King Saud University, Riyadh, Saudi Arabia. Sor-

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ghum seeds (*Sorghum bicolor*) [two cultivars, Hemaira (reddish color) and Shahlla (slightly brown color)] were obtained from the Gizzan region, southwestern Saudi Arabia. *M. peregrina* kernels were cleaned, hand-cracked, dehulled, and pulverized with a Waring commercial blender (Sanyo Electric Co. Ltd., Japan); the other seeds were milled to pass through a 0.355 mm sieve, using an ultracentrifugal mill (Resh type 2MI, F., Kurt Retsch GmbH and Co., Germany).

Preparation of Defatted Flour from Seeds. The procedure of Abu-Tarboush and Ahmed (1996) was used to prepare defatted flour from soybean and *M. peregrina* flours.

Irradiation Treatments. Samples were irradiated with different doses of 1.0, 3.0, 5.0, 7.0, and 10.0 kGy with a ^{60}Co source at a dose rate of 11.29 kGy/h. Irradiation was carried out using a Gamma cell 220 (Nordion Inc., Kanata, Canada) at King Abdulaziz City for Science and Technology. The absorbed dose was determined using a Fricke dosimeter (ASTM, 1992). The uncertainty of absorbed dose was 3.5% at 95% confidence limit.

Trypsin Inhibitor Activity Assay of Soybean. Trypsin inhibitor activity determination of nonirradiated and irradiated soybean defatted flour was conducted according to the method of Kakade et al. (1969) using N^{α}-benzoyl-DL-arginine *p*-nitroanilide (Sigma Chemical Co., St. Louis, MO) at a concentration of 30 mg/100 mL as a synthetic substrate. One gram of the defatted flour was extracted with 15 mL of citrate buffer (pH 4.6), stirred for 2 h at room temperature (25 °C), and then centrifuged at 4500 rpm for 20 min. The reaction mixture consisted of 0.1 mL of the extract, 0.9 mL of distilled water, 7 mL of the synthetic substrate, and 1 mL of trypsin type III (Sigma). Trypsin type III from bovine pancreas at concentration of 4 mg/100 mL of 0.001 M HCl was used in the reaction mixture. The absorbance was recorded at 410 nm, and the inhibitor activity was calculated using a control sample of trypsin.

α-Chymotrypsin Inhibitor Activity Assay of Soybean. The method of Kakade et al. (1970) was employed for determining chymotrypsin inhibitor activity using bovine pancreas, type II chymotrypsin (Sigma), and 1% casein (BDH Chemicals, Poole, U.K.) as substrate. One chymotrypsin unit (CU) was arbitrarily defined as an increase of 0.01 absorbance unit at 275 nm in 10 min for 10 mL of reaction mixture under the conditions described in this method, and the chymotrypsin inhibitor activity was defined as the number of chymotrypsin units inhibited (CUI).

In Vitro Protein Digestibility of Soybean. The procedure of Hsu et al. (1977) as modified by Satterlee et al. (1979) was used. The drop of pH of casein (control) and the samples after 20 min of hydrolysis by proteolytic enzymes was measured using an Orion Research 501 digital ion analyzer (USA). The enzymes used were trypsin type IX from porcine pancreas, chymotrypsin type II from bovine pancreas, peptidase type III from porcine intestine, and protease type VI from *Streptomyces griseus*. All enzymes were purchased from Sigma.

α-Amylase Inhibitor Activity Assay of Al-Yassar. Amylase inhibitor of Al-Yassar or Al-Ban (*M. peregrina*) assay was conducted according to the procedure of Deshpande et al. (1982). One gram samples were extracted by stirring for 12 h in 10 mL of distilled water at 4 °C. The extract was centrifuged at 5000*g* for 10 min. The clear supernatant (0.25 mL) was incubated with 0.25 mL of α-amylase from porcine pancreas (type I-A, 2× crystallized, Sigma) for 15 min at 37 °C. Starch was added to this mixture and incubated for a further 3 min at 37 °C. One unit of enzyme activity was defined as that which liberated from soluble starch 1 mg of maltose/min at 37 °C and pH 7.0 under the specified conditions.

Tannin Content of the Sorghum Seeds. Tannin content of the sorghum seeds was determined using the modified vanillin−HCl method of Price et al. (1978). One gram of finely ground grain was extracted with 10 mL of 1% HCl in methanol for 24 h at room temperature (25 °C) and then centrifuged at 5000 rpm for 20 min. One milliliter of supernatant was mixed with 5 mL of vanillin−HCl reagent (prepared by mixing equal volumes of 8% concentrated HCl in methanol and 2% vanillin in methanol), and the absorbance at 500 nm was determined after 20 min (LKB Biochrom Ultrospec II 4040 UV−visible). Vanillin and catechin were obtained from Sigma. Different catechin concentrations (0.2−1.0 mg/mL) were used as standards (prepared daily).

Table 1. Effect of Irradiation on Trypsin and Chymotrypsin Inhibitor Activities and in Vitro Digestibility of Soybean Defatted Flour

dose (kGy)	trypsin inhibitor[a] (units/mg)	destruction (%)	chymotrypsin inhibitor[a] (units/mg)	destruction (%)	in vitro digestibility[a] (%)
0.0	76.8 ± 0.17^{a}		9.8 ± 0.15^{a}		79.8 ± 0.47^{d}
1.0	73.3 ± 0.28^{b}	4.6	8.6 ± 0.15^{b}	12.2	81.2 ± 0.41^{c}
3.0	68.2 ± 0.32^{c}	11.2	7.0 ± 0.15^{c}	28.6	81.8 ± 0.35^{bc}
5.0	65.8 ± 0.18^{d}	14.3	4.2 ± 0.18^{d}	57.1	82.3 ± 0.60^{bc}
7.0	56.0 ± 0.07^{e}	27.1	4.1 ± 0.17^{d}	58.2	83.1 ± 0.26^{ab}
10.0	50.0 ± 0.12^{f}	34.9	2.8 ± 0.10^{e}	71.4	84.2 ± 0.27^{a}

[a] Mean ± SE ($n = 5$). Means in column with unlike superscripts differ significantly ($P \leq 0.05$).

Gossypol Content of the Cottonseeds. Free gossypol was determined according to the AOCS method (American Oil Chemists' Society, 1974). Total gossypol was determined by the same method after acid hydrolysis of the bound gossypol. For acid hydrolysis, 25 mL of 0.1% oxalic acid was added to 1 g of the samples in a 100 mL flask. The mixture was heated for 13 h at 75 °C in a water bath, then 25 mL of aqueous acetone (70%) and 5 mL of barium acetate (0.5%) were added, and the mixture was left for 10 min and then filtered. The filtrate was then diluted to 100 mL with aqueous acetone.

Data Analysis. All of the experiments were repeated five times on different days. The results presented are means of five replicates.

RESULTS AND DISCUSSION

Effect of Irradiation on Trypsin and Chymotrypsin Inhibitors and in Vitro Digestibility of Soybean Defatted Flour. Table 1 shows trypsin and α-chymotrypsin inhibitor activities and in vitro digestibility of the nonirradiated and irradiated soybean defatted flours. The inhibitor activities for both trypsin and chymotrypsin were lower for irradiated soybean defatted flour than for nonirradiated samples. The decrease by irradiation was proportional to the dose, and the highest irradiation dose of 10 kGy inactivated 34.9 and 71.4% of trypsin and chymotrypsin inhibitor activities, respectively. This indicates the need for higher irradiation doses, particularly for inactivation of trypsin. The decline in proteinase inhibitor activities was accompanied by an increase in the in vitro digestibility of soybean defatted flour (Table 1). However, the use of 10 kGy did not increase the in vitro digestibility of soybean defatted flour significantly compared to the use of 7.0 kGy. The improvement of in vitro digestibility of safflower oilcake as a result of the decline in proteinase inhibitor activities by irradiation was reported by Joseph and Dikshit (1993).

Irradiation of pure crystalline soybean trypsin inhibitor by a dose of 100 kGy caused no change in the activity (Hafez et al., 1985); however, 98.67% inactivation of the trypsin inhibitor was achieved in the aqueous solution. The same authors found no change in chymotrypsin inhibitor activity after soaking soybeans (15.35−30.47% moisture), while trypsin inhibitor activity decreased under the same conditions. Joseph and Dikshit (1993) studied the effect of irradiation on trypsin and chymotrypsin inhibitor activities of safflower oilcake. They found that trypsin inhibitor activity was inactivated at a dose of 42 Gy, whereas the chymotrypsin inhibitor remained active, even at high dose of 10 kGy. The low

Table 2. Effect of Irradiation on α-Amylase Inhibitor of *M. peregrina* Defatted Flour

dose (kGy)	α-amylase inhibitor[a] (units/g)	destruction (%)
0.0	272.0 ± 6.9[a]	
1.0	261.3 ± 7.1[ab]	3.9
3.0	242.7 ± 3.5[b]	10.8
5.0	213.3 ± 11.6[c]	21.6
7.0	153.3 ± 9.9[d]	43.6
10.0	142.0 ± 6.6[d]	47.8

[a] Mean ± SE ($n = 5$). Means in column with unlike superscripts differ significantly ($P \leq 0.05$).

dose used to inactivate trypsin inhibitor activity in their study (Joseph and Dikshit, 1993) contradicted our finding, and this is due to the heat treatment of the safflower oilcake, which may have contributed to the destruction of trypsin inhibitor activity. Moreover, chymotrypsin inhibitor activity (Joseph and Dikshit, 1993) is more resistant to irradiation in safflower oilcake than in soybean flour, possibly due to structural variation in the inhibitor itself or matrix effects.

El-Morsi et al. (1992) found that irradiation of field bean (*Vicia faba* L.) accompanied by germination caused inactivation of 5.6 and 10.4% of total trypsin inhibitor activity at dose levels of 1 and 2 kGy, respectively. Loss of 45.1% of trypsin inhibitor activity was achieved by the use of 5 kGy in the same study. Ghazy (1990) reported a loss of 16% of activity of kidney bean (*Phaseolus vulgaris* L.) trypsin inhibitor by germination for 96 h and the use of a 5 kGy dose.

Effect of Irradiation on α-Amylase Inhibitor. Table 2 shows the effect of irradiation on α-amylase inhibitor activity of Al-Yassar (*M. peregrina*) defatted flour. Destruction of >40% of the α-amylase was achieved by the use of 7.0 kGy or more. Although 10.0 kGy caused more destruction of α-amylase compared to 7.0 kGy, the difference in destruction was not significant ($P \leq 0.05$). Studies on α-amylase inhibitor of Al-Yassar are rare, and the only study found in the literature was that of Al-Kahtani (1995), who reported an amylase inhibitor activity of 246 units/g for Al-Yassar. Heat treatment at 70 °C for 120 min caused a loss of 50% of the original activity of Al-Yassar α-amylase inhibitor activity. The author also stated that the bacterial α-amylase inhibitor of *M. peregrina* is more heat resistant than that of soybean, but the pancreas α-amylase inhibitor in *M. peregrina* was less heat resistant than that of soybean.

It seems from this study that irradiation treatment of *M. peregrina* defatted flour with 7.0 kGy is as effective as the heat treatment at 70 °C for 120 min by Al-Kahtani (1995). Therefore, irradiation could be useful for reduction of α-amylase inhibitor activity in *M. peregrina*.

Effect of Irradiation on Tannin. The tannin contents of the two varieties of the sorghum grain are shown in Table 3. The tannin content correlated with seed-coat color, and this agrees with the finding of Bate-Smith and Rasper (1969). The Hemaira variety (red color) contained more tannin (0.68% catechin equiv) than the Shahlla variety (0.35% catechin equiv). Irradiation with 7.0 and 10.0 kGy contributed significantly ($P \leq 0.05$) to the reduction of tannin content in Shahlla (only from 0.35 to 0.26 mg of catechin equiv/100 g). The tannin content of Hemaira was not significantly affected by irradiation. However, complete destruction of tannin was not achieved by a dose of 10.0 kGy. The effect of fermentation on the tannin content of sorghum grain is more efficient than irradiation. Romo-Parada et al. (1985) and Hassan and El-Tinay (1995) observed 92 and 62−63% reductions in tannin, respectively. Results of our study indicated 2.9−25.7% reduction of tannin of sorghum as a result of irradiation treatment with a dose of 10.0 kGy.

Table 3. Effects of Irradiation on Tannin Content of Two Varieties of Sorghum Whole Flour

dose (kGy)	tannin content[a,b] (mg of catechin equiv/100 g)	
	Hemaira	Shahlla
0.0	0.680 ± 0.006[ab]	0.35 ± 0.006[a]
1.0	0.683 ± 0.003[a]	0.33 ± 0.003[b]
3.0	0.670 ± 0.006[ab] (1.0)	0.34 ± 0.007[ab]
5.0	0.677 ± 0.012[ab]	0.34 ± 0.003[ab]
7.0	0.673 ± 0.003[ab] (1.0)	0.31 ± 0.006[c] (11.4)
10.0	0.660 ± 0.006[b] (2.9)	0.26 ± 0.003[d] (25.7)

[a] Mean ± SE ($n = 5$). Means in column with unlike superscripts differ significantly ($P \leq 0.05$). [b] Number in parentheses is percent destruction.

Table 4. Effect of Irradiation on the Gossypol Content of Cottonseed

dose (kGy)	gossypol content (%)			
	total	destruction (%)	free	destruction (%)
0.0	0.93 ± 0.007[a]		0.76 ± 0.003[a]	
1.0	0.90 ± 0.006[b]	3.2	0.72 ± 0.004[b]	5.3
3.0	0.83 ± 0.001[c]	10.8	0.71 ± 0.002[c]	6.6
5.0	0.80 ± 0.007[d]	14.0	0.70 ± 0.001[d]	7.9
10.0	0.80 ± 0.005[d]	14.0	0.70 ± 0.00[d]	7.9

[a] Mean ± SE ($n = 5$). Means in column with unlike superscripts differ significantly ($P \leq 0.05$).

Agrawal and Chitnis (1995) studied the effect of five different treatments on the tannin content of sorghum grain. The treatments included soaking the seeds in 0.2 N HCl for 48 h, soaking in 0.05 N sodium bicarbonate for 48 h, and soaking in 2% boric acid for 48 h, followed by resoaking in water for 16 h and steeping in 0.005 N sodium hydroxide for 48 h. Destruction of 43−100% of tannin in sorghum varieties was achieved by these treatments, and responses to each treatment depended on the varieties of sorghum.

Effect of Irradiation on Gossypol of the Cottonseed. The effect of irradiation on free and total gossypol contents of cottonseed is given in Table 4. Irradiation reduced both total and free gossypol by 14.0 and 7.9%, respectively, but was not effective in reducing the gossypol content of cottonseed to the permissible level of 0.06%. Extraction with different solvents achieved reduction in gossypol content to varying extent. Rahma and Narasinga Rao (1984) reported 0.069% free gossypol after extraction of cottonseed with 1:1 mixture of 85% 2-propanol and hexane. Cherry and Gray (1981) obtained cottonseed flours with free gossypol content of 0.011−0.24% by using methylene chloride with other suitable solvents.

Gossypol is a constituent of cottonseeds (Murti and Achaya, 1975) and is toxic to monogastric animals (Berardi and Goldblatt, 1980). Cottonseed meal is being utilized for human consumption in Latin America and, to a limited extent, in other parts of the world (Noyes, 1969). According to the Protein Advisory Group of the United Nations, free gossypol content of edible-grade cottonseed flour should not exceed 0.06%. Therefore, several solvent extraction methods have been used to reduce the gossypol content of cottonseed meal (Damaty

and Hudson, 1975; Canella and Sodini, 1977; Cherry and Gray, 1981; Rahma and Narasinga Rao, 1984). Although solvent extraction reduces the gossypol content, the flavor of the meal was objectionable (Alyevand et al., 1967). Therefore, an attempt was made in this study to use irradiation to reduce the gossypol content. Such an approach requires fewer steps and does not affect the flavor of the meal compared to solvent extraction methods. However, a high dose (>10.0 kGy) is needed to reduce the gossypol content.

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LITERATURE CITED

Abu-Tarboush, H. M.; Ahmed, S. B. Studies on Karkade (*Hibiscus sabdariffa*): protease inhibitors, phytate, *in vitro* protein digestibility and gossypol content. *Food Chem*. **1996**, *56*, 15−19.

Agrawal, P.; Chitnis, A. U. Effect of treatments on phytate phosphorus, iron bioavailability, tannins and *in vitro* protein digestibility of grain sorghum. *J. Food Sci. Technol*. **1995**, *32*, 453−458.

Al-Kahtani, H. A. Some antinutritional factors in *Moringa peregrina* (Al-Yassar or Al-ban) and soybean products. *J. Food Sci*. **1995**, *60*, 395−398.

Alyevand, F.; Coleman, G.; Haisman, D. R. Catty odours in food: the reaction between mesityloxide and sulfur compounds in food stuffs. *Chem. Ind. (London)* **1967**, *37*, 1563.

American Oil Chemists' Society. *Official and Tentative Methods*, 3rd ed.; Chicago, IL, 1974.

ASTM. Standard practice for using the fricke reference standard dosimetry system. ASTM Standard E1026; American Society for Testing and Materials: Philadelphia, PA, 1992.

Barampama, Z.; Simard, R. E. Oligosacchardies, antinutritional factors and protein digestibility of dry beans as affected by processing. *J. Food Sci*. **1994**, *59*, 833−838.

Bate-Smith, E. C.; Rasper, V. Tannins of grain sorghum: Luteoforol (leucoluteolinidin), 3,4,4,5,7-Pentahydroxyflavan. *J. Food Sci*. **1969**, *34*, 203−209.

Berardi, L. C.; Goldblatt, L. A. Gossypol. In *Toxic Constituents of Plant Foodstuffs*; Liener, I. E., Ed.; Academic Press: New York, 1980; pp 184−237.

Butler, L. G.; Riedle, D. L.; Lebryk, D. G.; Blytt, H. J. Interaction of proteins with sorghum tannin: mechanism, specificity and significance. *J. Am. Oil Chem. Soc*. **1984**, *61*, 916−920.

Canella, M.; Sodini, G. Extraction of gossypol and oligosaccharides from oil seed meals. *J. Food Sci*. **1977**, *42*, 1218−1219.

Chang, K. C.; Chang, D. C.; Phatak, L. Effect of germination on oligosacchardies and nonstarch polysaccharides in navy and pinto beans. *J. Food Sci*. **1989**, *54*, 1615−1619.

Cherry, J. P.; Gray, M. S. Methylene chloride extraction of gossypol from cottonseed products. *J. Food Sci*. **1981**, *46*, 1726−1733.

Cinco, F.; Frels, J. M.; Holt, D. L.; Rupnow, J. H. Determination of the number and heat stability of α-amylase inhibitors in white and red kidney bean (*Phaseolus vulgaris*). *J. Food Sci*. **1985**, *50*, 533−534.

Damaty, S.; Hudson, B. J. F. Preparation of low gossypol cottonseed flour. *J. Sci. Food Agric*. **1975**, *26*, 109−115.

Deshpande, S. S.; Sathe, S. K.; Salunkhe, D. K.; Cornforth, D. P. Effects of dehulling on phytic acid, polyphenols, and enzyme inhibitors of dry beans (*Phaseolus vulgaris* L.). *J. Food Sci*. **1982**, *47*, 1846−1850.

Dhurandhar, N. V.; Chang, K. C. Effect of cooking on fimness, trypsin inhibitors, lectins and cystine/cysteine content of navy and red kidney beans (*Phaseolus vulgaris*). *J. Food Sci*. **1990**, *55*, 470−474.

El-Morsi, E. A.; Abdel-Salam, H. S.; Ismail, H. A.; Aboul-Fetouh, S. A.; Fawzay, A. M. Effect of gamma-Irradiation on trypsin inhibitor and subunit structure of proteins in field bean (*Vicia faba* L.) seeds during germination. *Minia J. Agric. Res. Dev*. **1992**, *14*, 849−871.

Fernandez, R.; Elias, L. G.; Braham, J. E.; Bressani, R. Trypsin inhibitor and hemagglutining in beans (*Phaesolus vulgaris*) and their relationship with the content of tannins and associated polyphenols. *J. Agric. Food Chem*. **1982**, *30*, 734−739.

Frels, J. M.; Rupnow, J. H. Purification and partial characterization of two α-amylase inhibitors from black bean (*Phaseolus vulgaris*). *J. Food Biochem*. **1985**, *8*, 281−301.

Ghazy, M. A. Effect of gamma-irradiation on some antinutritional factors in kidney bean (*Phaseolus vulgaris* L.) seeds. *Minia J. Agric. Res. Dev*. **1990**, *12*, 1965−1980.

Ghazy, M. A.; Abdel-Salam, H. S.; Aboul-Fetouh, S. A.; Ismail, H. A.; Fawzy, A. M. Effect of γ-irradiation on soluble sugars and raffinose oligosacchardies in field bean (*Vicia faba* L.) seeds during germination. *Minia J. Agric. Res. Dev*. **1992**, *14*, 873−896.

Grant, G.; Edwards, J.; Pusztai, A. α-Amylase inhibitor levels in seeds generally available in Europe. *J. Sci. Food Agric*. **1995**, *67*, 235−238.

Griffiths, D. W. The inhibition of digestive enzymes by extracts of field bean (*Vicia faba*). *J. Sci. Food Agric*. **1979**, *30*, 458−462.

Griffiths, D. W. The polyphenolic content and enzyme inhibitory activity of testas from bean (*Vicia faba*) and pea (*Pisum* spp.) varieties. *J. Sci. Food Agric*. **1981**, *32*, 797−804.

Hafez, Y. S.; Mohamed, A. I.; Singh, G.; Hewedy, F. M. Effect of γ-irradiation on protein and fatty acids of soybean. *J. Food Sci*. **1985**, *50*, 1271−1274.

Hassan, I. A. G.; El-Tinay, A. H. Effect of fermentation on tannin content and *in-vitro* protein and starch digestibilities of two sorghum cultivars. *Food Chem*. **1995**, *53*, 149−151.

Helsper, J. P. F. G.; Hoogendijk, J. M.; Norel, A. V.; Burger-Meyer, K. Antinutritional factors in faba beans (*Vicia faba* L.) as affected by breeding toward the absence of condensed tannins. *J. Agric. Food Chem*. **1993**, *41*, 1058−1061.

Hsu, H. W.; Vavak, D. L.; Satterlee, L. D.; Miller, G. A. A multi-enzyme technique for estimating protein digestibility. *J. Food Sci*. **1977**, *42*, 1269−1273.

Joseph, A.; Dikshit, M. Effect of irradiation on the proteinase inhibitor activity and digestibility (*in vitro*) of safflower oilcake. *J. Am. Oil Chem. Soc*. **1993**, *70*, 935−937.

Joslyn, M. A.; Glick, Z. Comparative effects of gallotannic acid and related phenolics on the growth of rats. *J. Nutr*. **1969**, *98*, 119−126.

Kakade, M. L.; Simons, N.; Liener, I. E. An evaluation of natural vs synthetic substrates for measuring the antitryptic activity of soybean samples. *Cereal Chem*. **1969**, *49*, 518−526.

Kakade, M. L.; Swenson, D. H.; Liener, I. E. Note on the determination of chymotrypsin and chymotrypsin inhibitor activity using casein. *Anal. Biochem*. **1970**, *33*, 255−258.

Kanaya, K.; Yasumoto, K.; Mitsuda, H. Pepsin inhibition by phytate contained in rice bran. *Eiyo To Shkuryo*. **1976**, *29*, 341−346.

Knuckles, B. E.; Kuzmicky, D. D.; Betschart, A. A. Effect of phytate and partially hydrolyzed phytate on *in vitro* protein digestibility. *J. Food Sci*. **1985**, *50*, 1080−1082.

Kratzer, F. H. Soybean protein-mineral interrelationships. *Fed. Proc. Fed. Am. Soc. Exp. Biol*. **1965**, *24*, 1498−1500.

Liener, I. E.; Kakade, M. L. Protease inhibitors. In *Toxic Constituents of Plant Foodstuffs*; Liener, I. E., Ed.; Academic Press: New York, 1980; pp 7−71.

Murti, K. S.; Achaya, K. T. Gossypol. In *Cottonseed, Chemistry and Technology*; Publications and Information Directorate, CSIR: New Delhi, 1975; pp 48−71.

Narasinga Rao, B. S.; Prabhavathi, T. Tannin content of foods commonly consumed in India and its influence on ionisable iron. *J. Sci. Food Agric.* **1982**, *33*, 89−96.

Nnanna, I. A.; Phillips, R. D. Changes in oligosaccharides content, enzyme activities and dry matter during controlled germination of cowpea (*Vigna ungiculata*). *J. Food Sci.* **1988**, *53*, 1782−1786.

Noyes, R. Protein food supplements. In *Food Processing Review No.3*; Noyes Development Corp.: Park Ridge, NJ, 1969; pp 232−245.

Price, M. L.; Scoyoc, V. S.; Shokuryo, L. G. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* **1978**, *26*, 1214−1218.

Rahma, E. H.; Narasinga Rao, M. S. Gossypol removal and functional properties of protein produced by extraction of glanded cottonseed with different solvents. *J. Food Sci.* **1984**, *49*, 1057−1060.

Reddy, N. R.; Pierson, M. D.; Sathe, S. K.; Salunkhe, D. K. Dry bean tannins: a review of nutritional implications. *J. Am. Oil Chem. Soc.* **1985**, *62*, 541−549.

Romo-Parada, M. L.; Simard, R. E.; Larrea-Reynoso, S. S. Influence of germination, nixtamalization and fermentation on the nutritional value of sorghum protein. *Microbiol. Alim. Nutr.* **1985**, *3*, 125−132.

Sathe, S. K.; Deshpande, S. S.; Reddy, N. R.; Goll, D. E.; Salunkhe, D. K. Effects of germination on proteins, raffinose oligosaccharides and antinutritional factors in the Great Northern beans (*Phaseolus vulgaris* L.). *J. Food Sci.* **1983**, *48*, 1796−1800.

Satterlee, L. D.; Marshall, H. F.; Tennyson, J. M. Measuring protein quality. *J. Am. Oil Chem. Soc.* **1979**, *56*, 103−109.

Sharma, K. K.; Pattabiraman, T. N. Natural plant enzyme inhibitors. Purification and properties of an amylase inhibitor from yam (*Dioscorea alata*). *J. Sci. Food Agric.* **1980**, *33*, 255−262.

Singh, U.; Kherdekar, M. S.; Jambunathan, R. J. Studies on Desi and Kabuli chickpeas (*Cicer arietinum* L.) cultivars: The levels of amylase inhibitors, levels of oligosaccharides and *in-vitro* starch digestibility. *J. Food Sci.* **1982**, *47*, 510−512.

Storey, R.; Bower, N.; Lovejoy, C.; Tagget, R. Analysis of selected nutritional and antinutritional factors in Jojoba seed from the United States and Mexico. In *Jojoba and Its Uses, Proceedings of the 5th International Confercene*; Cesnick, A. E., Ed.; University of Arizona: Tucson, AZ, 1983; p 25.

Thompson, D. B.; Erdman, J. W., Jr. Phytic acid determination in soybeans. *J. Food Sci.* **1982**, *47*, 513−517.

Vidal-Valverde, C.; Frias, J.; Estrella, I.; Gorospe, M. J.; Ruiz, R.; Bacon, J. Effect of processing on some antinutritional factors of lentils. *J. Agric. Food Chem.* **1994**, *42*, 2291−2295.

Weber, C. W.; Berry, J. W.; Philip, I. Citrullis, apodanthera, cucurbita and hibiscus seed protein. *Food Technol.* **1977**, *31*, 182−183.

Xavier-Filho, J.; Campos, F. A. P.; Ary, M. B.; Silva, C. P.; Carvalho, M. M.; Macedo, M. L. R.; Lemos, F. J. A.; Grant, G. Poor correlation between the levels of proteinase inhibitors found in seeds of different cultivars of cowpea (*Vigna unguiculata*) and the resistance, susceptibility to predation by *Callosobruchus maculatus*. *J. Agric. Food Chem.* **1989**, *37*, 1139−1143.

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