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ARTICLE

Influence of γ-Radiation on the Nutritional and Functional Qualities of Lotus Seed Flour

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In the present study, we investigated the physicochemical and functional properties of lotus seed flour exposed to low and high doses of γ-radiation (0−30 kGy; the dose recommended for quarantine and hygienic purposes). The results indicated raw seed flour to be rich in nutrients with minimal quantities of antinutritional factors. Irradiation resulted in a dose-dependent increase in some of the proximal constituents. The raw and γ-irradiated seeds meet the Food and Agricultural Organization−World Health Organization recommended pattern of essential amino acids. Some of the antinutritional factors (phytic acid, total phenolics, and tannins) were lowered with γ-irradiation, while the seed flours were devoid of lectins, L-3,4-dihydroxyphenylalanine, and polonium-210. The functional properties of the seed flour were significantly improved with γ-radiation. γ-radiation selectively preserved or improved the desired nutritional and functional traits of lotus seeds, thus ensuring a safe production of appropriate nutraceutically valued products.

KEYWORDS: γ-Irradiation; lotus seed flour; nutrients; antinutrients; protein digestibility; functional properties

INTRODUCTION

The paucity of protein-rich food and protein food supplements poses problems of malnutrition in children and lactating women in developing countries, and it has become a prime concern to food scientists, nutritionists, and local governments (*1*, *2*). The scarcity of fertile land and an overdependence on cereal-based food products have also aggravated the protein deficiency problems in humans (*3*). To meet the ever-increasing protein demand, the exploitation of nonconventional seeds has become inevitable (*4*−*7*). Such explorations may assuage the problems of food security, agricultural development, self-dependence, and enhancement of the economy of developing countries. The little known lotus seed and its flour might significantly contribute to world food production due to its wide distribution and adaptability to adverse environmental conditions. Nutritionally, lotus seeds have been reported to be rich in proteins and consist of adequate amounts of essential minerals (*8*). Lotus seeds are in high demand in Indian Ayurvedic medicinal preparations and are also widely used in folk medicines to treat tissue inflammation and cancer, act as a diuretic (*9*), and treat skin diseases (leprosy), and they are used as an antidote against snake poison (*10*). The seeds also possess hepatoprotective, free radical scavenging (*11*), and antifertility (*12*) properties.

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Radiation processing of plant produce has become an important physical preservation method to overcome the international quarantine barriers and to increase the safety and shelf life of the product, mainly by the elimination of spoilage microflora (*13*, *14*). Irradiation, besides being successful in decontamination and disinfestation, is also known to improve the quality of fresh plant produce (e.g., legumes, seeds, and spices) requiring long-term preservation (*15*, *16*). Radiation processing is also efficient in decreasing or eliminating some of the antinutritional factors in seeds (*17*−*19*). Of late, with the increased database and scientific evidence, health conscious consumers are willing to use irradiated foods for safety concerns (*20*).

To fill the existing gap in the knowledge on the effects of γ-rays (low and high dose) on the nutritional potential of lotus seeds, the current study was aimed to investigate in detail the nutritional and antinutritional compositions and the functional properties of raw and irradiated seeds. The results of this study will be useful to popularize lotus seed flour for the successful exploitation of its nutritional value in the production of safe and inexpensive food products.

MATERIALS AND METHODS

Materials. Freshly harvested and dried lotus seeds (*Nelumbo nucifera* Gaertn.) from a single lot (25 kg) were procured from a local traditional supplier from North India. Seeds free from any apparent physical damage or insect infestation were chosen for the evaluation. The characteristic features (shape, color, length, width, and weight) of randomly selected seeds were also recorded.

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Irradiation. The whole seeds (with the seed coat) (~50 g) packed in polyethylene bags were exposed to various doses of γ-radiation (doses: 0, 2.5, 5.0, 7.5, 10, 15, and 30 kGy) at room temperature (25 ± 1 °C) from a ^{60}Co (cobalt^{60}) source (GC-5000, ISOMED, Bhabha Atomic Research Centre, Trombay, Mumbai, India). Fricke dosimetry was employed to measure the absorbed dose in the irradiated samples (*21*). All of the seed samples were stored in a deep freezer (−20 °C) until further analysis.

Flour Preparation and Nutritional Analysis. The edible portions of the seeds (cotyledon) were separated physically with the help of a sharp stainless knife and were further ground into a fine powder (< 0.5 mm) using a stainless steel blender (Philips India Ltd., India). The green-colored embryos in the seeds were removed before the seed flour was prepared. These embryos are bitter in taste and are usually removed before preparing any food product, probably due to the presence of antinutrients. However, some of the alkaloids in the embryo are also reportedly known to possess certain medicinal properties [e.g., antispasmodic, antidiarrheatic, and anti-human immunodeficiency virus (HIV)] (*7*).

The moisture of the seed flours was gravimetrically determined [drying in a hot air oven, 105 ± 1 °C, until a constant weight was attained (16 ± 1 h)]. The total nitrogen and crude protein were determined by the micro-Kjeldahl method (*22*). The crude lipid, crude fiber, and ash were estimated gravimetrically by employing standard Association of Official Analytical Chemists (AOAC) methods (*23*). The nitrogen-free extractives (NFE) were determined based on Müller and Tobin (*24*) and Siddhuraju et al. (*25*), while the calorific value was calculated according to Ekanayake et al. (*26*).

The minerals in the seed flour were estimated by atomic absorption spectrophotometry (GBC 904AA; Germany) (*22*), while the total phosphorus was determined by sepectrophotometry (Spectronic 21D, Miltonroy, NY) (*27*). The amino acid in the seed flour was estimated using a gas chromatography−combustion−isotope ratio mass spectrometer (GC-C-IRMS/MS) (*28*, *29*) with derivatization of esterification with trifluoroacetylation (*30*). The essential amino acid (EAA) score was determined by considering the Food and Agricultural Organization−World Health Organization (FAO-WHO) (*31*) recommended pattern. The in vitro protein digestibility (IVPD) was evaluated according to Akeson and Stahmann (*32*), and the protein digestibility corrected amino acid score (PDCAAS) of the EAA requirement for adults (*31*) was also estimated.

The fatty acid methyl esters (FAMEs) of the seed flours were measured as per the method of Garces and Mancha (*33*) using gas liquid chromatography (Sigma Instruments, Baroda, India). The polyunsaturated and saturated fatty acid ratio was calculated as follows:

$$\text{P/S ratio} = (\text{sum of polyunsaturated fatty acids}) \div (\text{sum of saturated fatty acids})$$

Antinutritional Analysis. The estimation of the total phenolics in the seed flour was based on Rosset et al. (*34*), while the tannins were evaluated according to the vanillin−hydrogen chloride (HCl) method (*35*). The phytic acid content was determined by the procedures of Deshpande et al. (*36*) and Sathe et al. (*37*). The analysis of the soluble phosphorus was based on Bartlett (*38*).

The phytohemagglutinating activity (lectins) was carried out using a trypsin-treated human erythrocyte suspension (A, B, and O) (*39*). The method described by Fujii et al. (*40*) was employed to determine L-DOPA (L-3,4-dihydroxyphenylalanine). The electrochemical deposition method described by Iyengar et al. (*41*) was employed for the measurement of ^{210}Po in the samples.

Functional Properties. The water and oil absorption capacities were estimated according to Beuchat (*42*) by vortexing (for 30 s) the seed flour (1 g) in distilled water (10 mL) or oil (Sundrop, Superlite Refined Sunflower Oil, Agrotech Foods Ltd., Secunderabad, India) (10 mL) in a centrifuge tube. The solution was allowed to stand at room temperature (28 ± 2 °C, 30 min) and centrifuged (5000*g*, 30 min), and the volume of the supernatant was measured in a measuring cylinder (10 mL capacity).

The protein solubility of the seed flour was determined by the method outlined by Were et al. (*43*). In brief, the seed flour (125 mg) was blended with distilled water (25 mL), and the solution was mixed by a magnetic stirrer (1 h, 20 °C) and centrifuged (12000*g*, 20 min, 4 °C). The supernatant was filtered through glass wool, and the nitrogen was estimated by the micro-Kjeldahl method (*22*). The solubility of the protein was determined (nitrogen solubility × 6.25).

The gelation property was assessed according to Coffman and Garcia (*44*). The seed flour suspension (10 mL) in distilled water (pH 7.0) was transferred to test tubes and heated in a boiling water bath (1 h) and cooled to room temperature. The samples in the tubes were further cooled in a refrigerator (4 °C, 2 h), and the least gelation concentration (LGC) was ascertained based on the stability of the gel without slipping or falling down when the tubes were inverted.

The emulsion activity and stability were determined according to the methods explained by Neto et al. (*45*). The seed flour was dispersed in distilled water (10 mg/mL), and the suspension (5 mL) was homogenized in a centrifuge tube (1 min) with oil (5 mL) to develop the emulsion and centrifuged (1100*g*, 5 min), and the height of the emulsified layer and the total height of the contents in the tube were measured to calculate the emulsion activity. The emulsion stability was determined by heating the emulsion in the tube (80 °C, 30 min) before centrifugation.

The foam properties of the seed flour were assessed based on the methods by Coffman and Garcia (*44*). The seed flour was dispersed in distilled water (2 g/100 mL) and whipped vigorously in a kitchen blender (2 min at speed 1) (Philips HL1643, Philips India Ltd., Kolkata, India). The volumes were recorded before and after whipping to calculate the percentage increase in volume. The foam stability was determined based on the volume of stable foam after incubation at room temperature (8 h, 28 ± 2 °C) and expressed as the percentage of the initial foam volume.

Statistical Analysis. Most of the analysis was performed in replicates of five, unless mentioned otherwise. Analysis of variance (ANOVA), followed by the least significant difference test (Duncan's LSD test), was used to compare means at the 5% significance level (SPSS 15.0 software SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Proximate Features. On visual observation, the lotus seeds were ovoid, black with a grayish tinge in color, and possessed a hard seed coat. The cotyledon portion was white with the presence of a green-colored embryo. The average weight per seed was 780 mg (±0.40), and the cotyledon portion weighed about two-thirds of the whole seed (cotyledon weight, 0.55 g ± 0.08, vs coat weight, 0.28 ± 0.03 g). The average length and breadth were 1.47 ± 0.07 and 1.05 ± 0.04 cm, respectively.

Table 1 depicts the details on the proximal composition of the raw and γ-irradiated seed flour. In general, the proximal composition of the raw lotus seeds was comparable to the seeds of some therapeutically valued seeds of India (*46*). The initial moisture of the raw seeds (8.42%) significantly decreased on irradiation ($P < 0.05$). A decrease in the moisture on irradiation can be attributed to the time and dose of radiation delivered, as reported earlier by Warchalewski et al. (*47*). The amount of crude protein significantly increased on irradiation up to a dose of 15 kGy ($P < 0.05$) (in control, 30.38 g/100 g, and at 15 kGy, 33.88 g/100 g). The elevation of the crude protein on irradiation might be attributed to higher extractability due to the dissociation of complex protein molecules into simpler forms. However, the percent moisture content in individual samples might have also contributed to the observed increase in crude protein concentration, as there is every possibility that decreased moisture can be correlated with a corresponding enhancement of the relative amount of major food components in a sample. Interestingly, at the highest dose of 30 kGy, a decrease in the crude protein was recorded. This decrease can be attributed to greater degradation of protein with consequent release of polypeptides. The crude protein of the raw lotus seed flour was relatively higher than many of the edible legumes (e.g., *Phaseolus aureus*, *Cajanmus cajan*, *Cicer srietinum*, and *Vigna unguicilata*) (19.4−25.3%) and wild legumes (e.g., *Atylosia scarbaeoides*, *Erythrina indica*, *Neonotonia wightii*, *Rhynchosia filipes*, *Tamarindus indica*, and *Vigna trilobata*) (14−24.8%) (*48*−*51*).

Table 1. Proximate Composition of the Lotus Seeds Flour Treated with γ-Radiation (on Dry Weight Basis)[a]

	irradiation dose (kGy)						
proximate composition	0	2.5	5	7.5	10	15	30
moisture (%)	8.42 ± 0.39 b	7.56 ± 0.46 a	7.42 ± 0.45 a	7.43 ± 0.17 a	7.43 ± 0.43 a	7.42 ± 0.37 a	7.38 ± 0.33 a
crude protein (g/100 g)	30. 38 ± 0.02 a	31.18 ± 0.01 b	32.36 ± 0.05 b	32.68 ± 0.56 b	32.75 ± 0.67 b	33.88 ± 1.02 c	31.06 ± 1.00 a
crude lipid (g/100 g)	3.70 ± 0.15 d	3.49 ± 0.17 c	3.47 ± 0.26 c	3.47 ± 0.06 c	2.76 ± 0.07 b	2.59 ± 0.36 a	2.50 ± 0.44 a
crude fiber (g/100 g)	4.87 ± 0.11 d	4.76 ± 0.05 d	3.57 ± 0.01 c	3.42 ± 0.74 b	3.26 ± 0.24 b	3.27 ± 1.25 b	2.31 ± 0.30 a
ash (g/100 g)	4.03 ± 0.12 a	4.12 ± 0.02 a	4.15 ± 0.04 a	4.11 ± 3.01 a	4.16 ± 0.51 a	4.18 ± 0.16 a	4.36 ± 0.13 b
nitrogen free extracts (g/100 g)	57.02 ± 0.19 a	57.45 ± 0.53 a	57.85 ± 2.49 a	58.35 ± 0.07 a	58.97 ± 0.69 b	59.08 ± 1.22 b	59.77 ± 1.01 b
gross energy (kJ/100 g)	1599. 07 ± 3.35 a	1636.99 ± 2.12 b	1637.71 ± 7.47 b	1620.0 ± 6.68 b	1622.21 ± 14.69 b	1621.03 ± 25.03 b	1626.18 ± 6.18 b

[a] Values are means ± standard deviations of five determinations (n = 5, mean ± SD). Values followed by different letters in a row are significantly different from each other ($P < 0.05$).

Table 2. Mineral Composition of Lotus Seed Flours Treated with γ-Radiation (on Dry Weight Basis) (n = 5, Mean ± SD)[a]

	irradiation dose (kGy)						
mineral (mg/100 g)	0	2.5	5	7.5	10	15	30
sodium	7.03 ± 0.02 a	7.01 ± 0.02 a	6.66 ± 0.02 a	6.61 ± 0.12 a	6.52 ± 0.01 a	6.52 ± 0.01 a	6.52 ± 0.22 a
potassium	23.77 ± 0.24 a	23.14 ± 0.38 a	23.45 ± 0.24 a	23.52 ± 0.23 a	23.95 ± 0.79 a	23.90 ± 0.21 a	23.92 ± 0.10 a
calcium	318 ± 12.02 c	262.01 ± 12.60 b	263.00 ± 9.25 b	263.80 ± 5.12 b	263.01 ± 5.60 b	189.01 ± 0.02 a	189.0 ± 5.82 a
phosphorus	5.29 ± 1.00 a	5.23 ± 5.8 a	5.30 ± 2.34 a	5.24 ± 0.65 a	5.22 ± 3.23 a	5.05 ± 0.01 a	5.03 ± 0.29 a
magnesium	43.90 ± 0.01 a	43.10 ± 0.02 a	42.10 ± 0.2 a	42.10 ± 2.01 a	42.12 ± 0.04 a	42.08 ± 0.02 a	41.00 ± 0.3 a
iron	15.00 ± 0.02 a	15.01 ± 2.35 a	15.00 ± 3.01 a	14.98 ± 0.01 a	14.80 ± 0.02 a	14.90 ± 1.0 a	14.94 ± 0.02 a
copper	2.25 ± 0.05 b	1.63 ± 0.23 a	1.63 ± 0.24 a	1.60 ± 0.19 a	1.60 ± 0.20 a	1.63 ± 0.23 a	1.47 ± 0.06 a
zinc	8.77 ± 1.57 b	8.72 ± 0.01 b	8.27 ± 3.56 b	8.17 ± 0.11 b	8.17 ± 4.61 b	7.60 ± 1.31 b	6.28 ± 0.08 a
manganese	15.32 ± 0.10 c	14.80 ± 3.0 b	14.75 ± 0.15 b	14.70 ± 0.26 b	14.71 ± 0.01 b	13.70 ± 0.1 a	13.74 ± 0.08 a
selenium	1.10 ± 0.01 a	1.10 ± 0.22 a	1.12 ± 0.02 a	1.04 ± 0.01 a	1.04 ± 0.01 a	1.03 ± 0.02 a	1.03 ± 0.01 a

[a] Values are means ± standard deviations of five determinations (n = 5, mean ± SD). Values followed by different letters in a row are significantly different from each other ($P < 0.05$).

The quantity of lipid in the raw seeds showed a significant dose-dependent decrease on irradiation (control 3.70 g/100 g vs 2.50 g/100 g at 30 kGy) ($P < 0.05$). This decrease might be attributed to the fact that γ-rays are an ionizing source of radiation and are capable of breaking the ordered structure of large lipid molecules, resulting in the degradation of lipids as well as conversion to other forms, thus contributing to an overall decrease in the recorded concentration. The crude fiber in the seed flour was in adequate quantity (control, 4.87 g/100 g); however, a significant decrease was recorded at 5 kGy and above doses ($P < 0.05$) (30 kGy, 2.31 g/100). In a normal diet, crude fiber is known to enhance digestibility and promote health benefits (e.g., it decreases cholesterol and reduces the risk of large bowel cancers) (*52*, *53*). However, a low crude fiber in food is also nutritionally appreciated, because it traps less proteins as well as carbohydrates (*54*). The decreased fiber in irradiated seed flour can be attributed to the depolymerization and delignification of seeds as reported earlier by Campbell et al. (*55*). The NFE in the raw lotus seed flour was 57.02 g/100 g, which is comparable or higher than many of the edible and wild legumes (e.g., *N. wightii*, 52.1%; *Mucuna monosperma*, 52.2%; *Cicer arietinum*, 57.1%; *Cajanus cajan*, 57.2%; and *Vigna unguiculata*, 56.9%) (*48*, *56*−*58*). Irradiation resulted in a significant increase in the NFE at a dose of 10 kGy and above (30 kGy, 59.77 g/100 g) ($P < 0.05$). The increase in the NFE due to the irradiation might be attributed to the breakdown of complex sugars (polysaccharides) into simple extractable forms. The increase might be advantageous, as the NFEs in the legume seeds are known to reduce plasma cholesterol (*59*, *60*). The calorific value in the seeds also showed a significant increase at all of the doses of irradiation (control, 1599 vs 1626 kJ/100 g at 30 kGy).

Mineral Composition. Calcium (318 mg/100 g), potassium (23.77 mg/100 g), magnesium (43.90 mg/100 g), and iron (16 mg/100 g) were the major minerals detected in the lotus seed flour (**Table 2**). As an antioxidant (*61*), selenium is known to inhibit the growth of cancer cells (mainly liver tumor) (*62*) and was present in adequate quantities in both the raw (1.10 mg/100 g) and the irradiated seed flour (30 kGy, 1.03 mg/100 g). In the present study, no significant decrease occurred in the mineral concentrations of lotus seed flour upon irradiation ($P > 0.05$). However, calcium, copper, zinc, and manganese did show some significant decrease ($P < 0.05$). Generally, minerals do not degrade on irradiation, but a change in their oxidation state might occur due to their varied extractability in a particular solvent. Apart from this, lotus seed being a biological sample, variations in mineral concentrations might naturally be present between each individual seed, which might give rise to varied results on irradiation. Another possible reason for the observed decrease in some of the minerals might be due to the presence of certain antinutrients at higher concentrations (like oligosaccharides, oxalates, protease inhibitors, saponins, and others) that could have increased on irradiation and possibly be capable of chelating the mineral cations, forming insoluble complexes leading to reduced bioavailability of trace minerals. However, the actual mechanism for decrease in some of the minerals is still obscure, which needs to be further investigated. However, this decrease in some of the minerals should not be an impediment for successful utilization of radiation technology, when considering the overall beneficial aspects related to safety.

Amino Acids. The application of γ-radiation did not have much of a positive effect on the amino acids of the lotus seed flour and showed a significant reduction at all of the delivered doses ($P < 0.05$). In **Table 3**, the amino acid composition of the raw and γ-irradiated lotus seed flour has been compared with the FAO-WHO (*31*) reference patterns and soybeans (*63*). The acidic amino acids, glutamic acid and aspartic acid, were the major amino acids present in the seed flour. Except for valine, isoleucine, and histidine, other EAAs were not comparable with the FAO-WHO (*31*) reference pattern. The sulfur-amino acids cysteine and methionine (1.9 vs 2.5%) and tyrosine and phenylalanine (5.84 vs 6.3%) in the raw seeds fulfilled

Table 3. Amino Acid Composition of the Lotus Seed Flours Treated with γ-Radiation (mg/100 mg Crude Protein; $n = 3$)[a]

	irradiation dose (kGy)								
amino acid	0	2.5	5	7.5	10	15	30	FAO-WHO pattern[b]	soybeans[c]
glutamic acid	16.29	12.97	13.10	13.10	13.13	13.10	13.01		16.9[d]
aspartic acid	7.93	6.29	6.34	6.34	6.37	6.32	6.33		11.3[e]
serine	4.64	3.85	3.85	3.85	3.84	3.90	3.90		5.67
threonine	2.61	2.10	2.11	2.12	2.13	2.13	2.13	3.4	3.76
proline	2.41	1.98	1.97	1.95	1.95	2.02	2.08		4.86
alanine	3.30	2.75	2.77	2.78	2.79	2.76	2.75		4.23
glycine	3.62	2.91	2.94	2.94	2.95	2.94	2.94		4.01
valine	3.55	2.90	2.89	2.89	2.87	2.94	2.98	3.5	4.59
cysteine	0.82	0.77	0.75	0.74	0.72	0.74	0.75		1.70
methionine	1.08	0.76	0.78	0.79	0.80	0.82	0.83	2.5[f]	1.22
isoleucine	2.89	2.36	2.32	2.31	2.27	2.36	2.41	2.8	4.62
leucine	4.90	3.98	3.98	3.98	3.97	4.01	4.03	6.6	7.72
tyrosine	2.67	2.19	2.18	2.18	2.17	2.17	2.14		1.24
phenylalanine	3.17	2.57	2.57	2.56	2.56	2.61	2.64	6.3[g]	4.84
tryptophan	ND	ND	ND	ND	ND	ND	ND	1.1	3.39
lysine	4.24	3.50	3.50	3.52	3.51	3.53	3.54	5.8	6.08
histidine	1.93	1.41	1.38	1.35	1.34	1.46	1.57	1.9	2.50
arginine	6.97	5.68	5.71	5.71	5.72	5.80	5.92		7.13

[a] ND, not detectable. [b] FAO-WHO pattern (*31*). [c] Bau et al. (*63*). [d] Glutamic acid + glutamine. [e] Aspartic acid + asparagine. [f] Methionine + cysteine. [g] Tyrosine + phenylalanine.

Table 4. EAA Score, IVPD ($N = 5$, Mean ± SD), and PDCAAS[a] of the Flours of Lotus Seeds Treated with γ-Radiation[b]

	irradiation dose (kGy)						
	0	2.5	5	7.5	10	15	30
				EAA score			
threonine	76.76	61.76	62.06	62.35	62.65	62.64	62.65
valine	101.43	82.86	82.57	82.57	82.00	84.00	85.14
cysteine + methionine	76.00	61.20	61.2	61.20	60.80	62.40	63.20
isoleucine	103.21	84.29	82.86	82.50	81.07	84.29	86.07
leucine	74.24	60.30	60.30	60.30	60.15	60.76	61.07
tyrosine + phenylalanine	92.70	75.56	75.40	75.24	75.80	75.87	76.51
lysine	73.10	60.34	60.34	60.69	60.52	60.86	61.03
histidine	101.58	74.21	72.63	71.10	70.53	76.84	82.63
IVPD (%)	37.90 ± 5.10 d	27.40 ± 1.3 c	20.17 ± 0.03 b	20.99 ± 1.92 b	20.23 ± 4.28 b	15.78 ± 0.24 a	15.41 ± 0.42 a
				PDCAAS (%)			
threonine	29.09	17.20	12.52	13.09	13.30	9.89	9.65
valine	38.44	23.07	16.65	17.33	17.41	13.46	13.12
cysteine + methionine	28.80	17.04	12.34	12.85	12.91	9.85	9.73
isoleucine	39.12	23.47	16.71	17.32	17.21	13.30	13.26
leucine	28.13	16.79	12.16	12.66	12.77	9.59	9.41
tyrosine + phenylalanine	35.13	21.03	15.21	15.79	15.94	11.97	11.79
lysine	27.71	16.80	12.17	12.74	12.85	9.60	9.41
histidine	38.50	20.66	14.65	14.91	14.97	12.13	12.73

[a] Calculated based on the FAO-WHO pattern (*31*). [b] Values across the row (for IVPD) with different letters are significantly different from each other ($P < 0.05$).

76 and 93% of the FAO-WHO (*30*) pattern, respectively. Except for tryptophan, the rest of the EAA in the raw and γ-irradiated seeds is on par with the Food and Agricultural Organization−World Health Organization−United Nations University (FAO-WHO-UNU) (*64*) pattern. The tyrosine in the raw as well as the irradiated seeds was higher than soybeans (2.14−2.67 vs 1.24%). The irradiation might have possibly changed the oxidative state, affecting the results of amino acids. According to Baudoin and Maquet (*65*), ecological conditions are known to markedly influence the total nitrogen of seeds, which in turn affect the relative proportions of the EAA (e.g., lysine, methionine, and cysteine).

IVPD. In our study, the IVPD of the seed flour showed a significant, dose-dependent decrease ($P < 0.05$) (control, 37.90%, vs 30 kGy, 15.41%) (**Table 4**). Such a decrease in the protein digestibility in the irradiated seed flour can be attributed to the increased high molecular weight polyphenolic compounds, which are capable of binding to the digestive enzymes and can act directly on the digestion of dietary proteins. Saunders et al. (*66*) have hypothesized that "IVPD" could be comparable to "in vivo protein digestibility" in rat models. However, the observed decrease in the IVPD should not be considered a hindrance when considering the overall beneficial aspects (for example, elevation of crude protein at certain doses, increase in linoleic acid concentration, water and oil absorption capacities, emulsion capacities, and decrease in some of the antinutrients) of radiation treatments of lotus seed flour. Further studies are warranted wherein studies can be pursued to evaluate the effects of radiation treatments on the in vivo protein digestibility to provide more clear information.

Table 5. Fatty Acid Composition of the Lotus Seed Flours after γ-Radiation (mg/g Lipid) (n = 3, Mean)[a]

	irradiation dose (kGy)						
fatty acid	0	2.5	5	7.5	10	15	30
saturated fatty acids							
myristic acid (C 14:0)	0.07	0.05	0.041	0.08	0.08	0.10	0.1
pentadecanoic acid (C15:0)	0.01	0.01	0.01	0.02	0.03	0.03	0.06
palmitic acid (C16:0)	4.24	4.28	4.36	4.35	4.36	4.37	4.47
heneicosanoic acid (C21:0)	0.09	0.08	0.08	0.07	–	–	–
behenic acid (C22:0)	0.60	0.001	0.001	0.001	0.0003	0.0003	–
polyunsaturated fatty acids							
myristoleic acid (C14:1)	0.02	0.02	0.02	0.02	0.003	0.004	0.01
elaidic acid (C18:1)	2.14	1.20	0.20	0.14	–	–	–
oleic acid (C18:1)	7.02	6. 40	6.00	1.20	–	–	–
linoleic acid (C18:2)	–	–	2.97	6.12	6.88	7.63	14.53
linolelaidic acid (C18:2)	5.84	5.80	5.77	4.00	2.21	1.82	1.44
linolenic acid (C18:3)	1.42	1.40	1.37	1.22	–	–	–
sum of saturated fatty acids	5.01	4.42	4.49	4.52	4.47	4.50	4.63
sum of polyunsaturated fatty acids	16.44	14.82	16.33	12.7	9.09	9.45	15.98
P/S ratio	3.28	3.35	3.63	2.81	2.03	2.10	3.45

[a] –, not detectable; SDs for all of the values were <±0.001.

The EAA score and the PDCAAS were also significantly decreased with irradiation ($P < 0.05$), indicating the limitation of available amino acids on digestion with enzymes. Even though γ-irradiation decreased the EAA of the lotus seed flour, the irradiated seed flour still fulfilled the FAO-WHO (*30*) EAA requirement pattern.

Fatty Acids. Among the saturated fatty acids, palmitic acid was high (4.24 mg/g) in raw seed flour, which was elevated significantly at 30 kGy (4.47 mg/g) ($P < 0.05$). Behenic acid, one of the antinutritional fatty acids that are known to enhance cholesterol levels (*67*), decreased significantly upon irradiation (0.60 vs 0.001−0.0003 mg/g). Raw seed flour possessed high amounts of polyunsaturated fatty acids (oleic acid, elaidic acid, linolenic acid, and linolelaidic acid) than saturated fatty acids (**Table 5**). According to Omode et al. (*68*), a higher proportion of unsaturated fatty acids in the diet lowers the risk of cardiovascular diseases. Interestingly, linoleic acid, which was absent in the raw seed flour, could be detected after radiation doses of 5 kGy and above. In a biological sample such as lotus seeds, most of the active ingredients like that of lipids are embedded in the seed matrix in a complex form. Irradiation treatments at high dose (like that of 30 kGy) can disrupt this complex form to facilitate their release from the seed matrix, thus enabling the increase in the total amount of recovered individual fatty acids. The increase in linoleic acid might be a result of conversion from highly unstable oleic acid due to the presence of double bonds (bonds that could be easily oxidized when irradiated in air) as well as due to molecular rearrangements. Also, γ-irradiation at higher dose might be effective in contributing to higher extractability in the solvent used. Even though, in general, γ-radiation decreased most of the unsaturated fatty acids, a higher P/S ratio was recorded even at a 30 kGy dose as compared to the raw seeds (3.28 vs 3.45). This was mainly due to the dose-dependent increase of the essential fatty acid, linoleic acid, which was not present in the raw seeds.

Antinutritional Features. The total amount of phenolics significantly decreased from 10 kGy and up (control, 3.38%; 30 kGy, 1.32%) ($P < 0.05$). The tannins had a significant dose-dependent decrease (raw, 3.30%; 30 kGy, 1.11%) ($P < 0.05$) (**Figure 1**). The trend was similar in the phenolics and tannins of

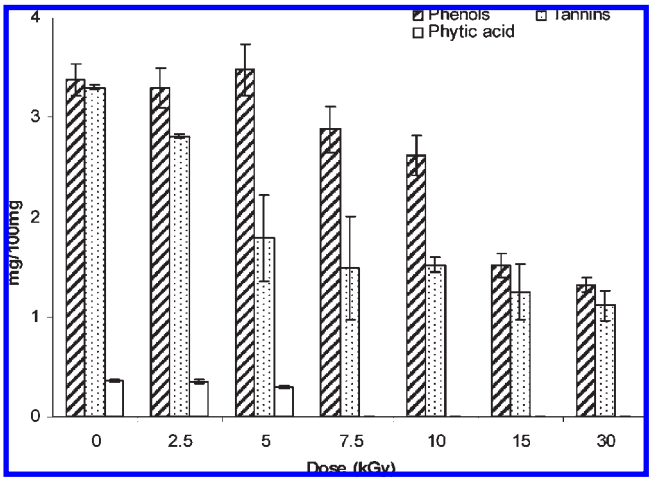

Figure 1. Changes in the antinutrients in lotus seed flour after γ-irradiation; the error bar indicates standard deviation.

Brazilian beans (*Phaseolus vulgaris* L. var. *carioca*; *V. unguiculata* L. Walp var. *macaçar*) (*69*). A decrease in the phenols and tannins might be advantageous, as they possess antinutritional properties. However, phenolics and tannins are also known to serve as free radical scavengers and are reported to play a major role in combating cardio-cerebrovascular diseases and cancer (*70*, *71*). Hence, a retention or elimination of the phenolics and tannins in lotus seed flour depends on the requirements of specific foods or pharmaceutical preparations.

The amount of phytic acid was low in the raw seeds (0.36 mg/100 mg) and was significantly decreased by up to 5 kGy (0.29%) ($P < 0.05$); its presence was undetectable at doses above 7.5 kGy. A similar trend in phytic acid has been reported with the irradiation (20 kGy) of *P. vulgaris* seeds (*72*). A decrease in phytic acid has been assigned to the production of free radicals during irradiation, thus leading to breaking of a chemical bond of the phytic acid (*73*) (**Figure 1**). Similar to phenolics, phytic acid is also known to affect the nutritional value of the edible seeds, mainly through limiting the bioavailability of minerals and essential trace elements (*74*). However, phytic acid possesses antioxidant, anticarcinogenic, and hypoglycemic properties (*75*, *76*), and thus, its retention or elimination depends on the nature of the nutraceutical product to be developed from the lotus seed flour.

The lotus seed flour was devoid of hemagglutinins (lectins), which are known to interfere with the brush border functions, especially absorption (*77*). The seed flour was also devoid of a major antinutrient, L-DOPA. The presence of some of the natural radionuclides in vegetation or edible plant produce (e.g., beans, legumes, rice, and fresh vegetables) has been proven to be harmful due to biomagnification (*78*). A natural radionuclide, polonium-210 (as hazardous as plutonium and 5-fold more toxic than ^{226}Ra) (*79*) was below the detection limit in all of the seed flours analyzed, indicating it to be much safer.

Functional Properties. The water absorption capacity (WAC) of the lotus seed was significantly elevated at doses of 10 kGy and above (control, 2.50 mL/g; 30 kGy, 3.31 mL/g) ($P < 0.05$) (**Figure 2**). Similarly, the oil absorption capacity (OAC) of the flours also increased significantly with irradiation (control, 1.45 mL/g; 30 kGy, 2.60 mL/g) ($P < 0.05$). The dissociation and denaturation of the proteins might have increased the WAC and OAC in the irradiated lotus seed flour, as attributed by Rahma and Mostafa in peanut flour (*80*). The high WAC is a desirable trait in many food preparations (e.g., sausages, custards, and dough), as they imbibe and become thick and viscous without dissolving the proteins. The high OAC is also another desired trait

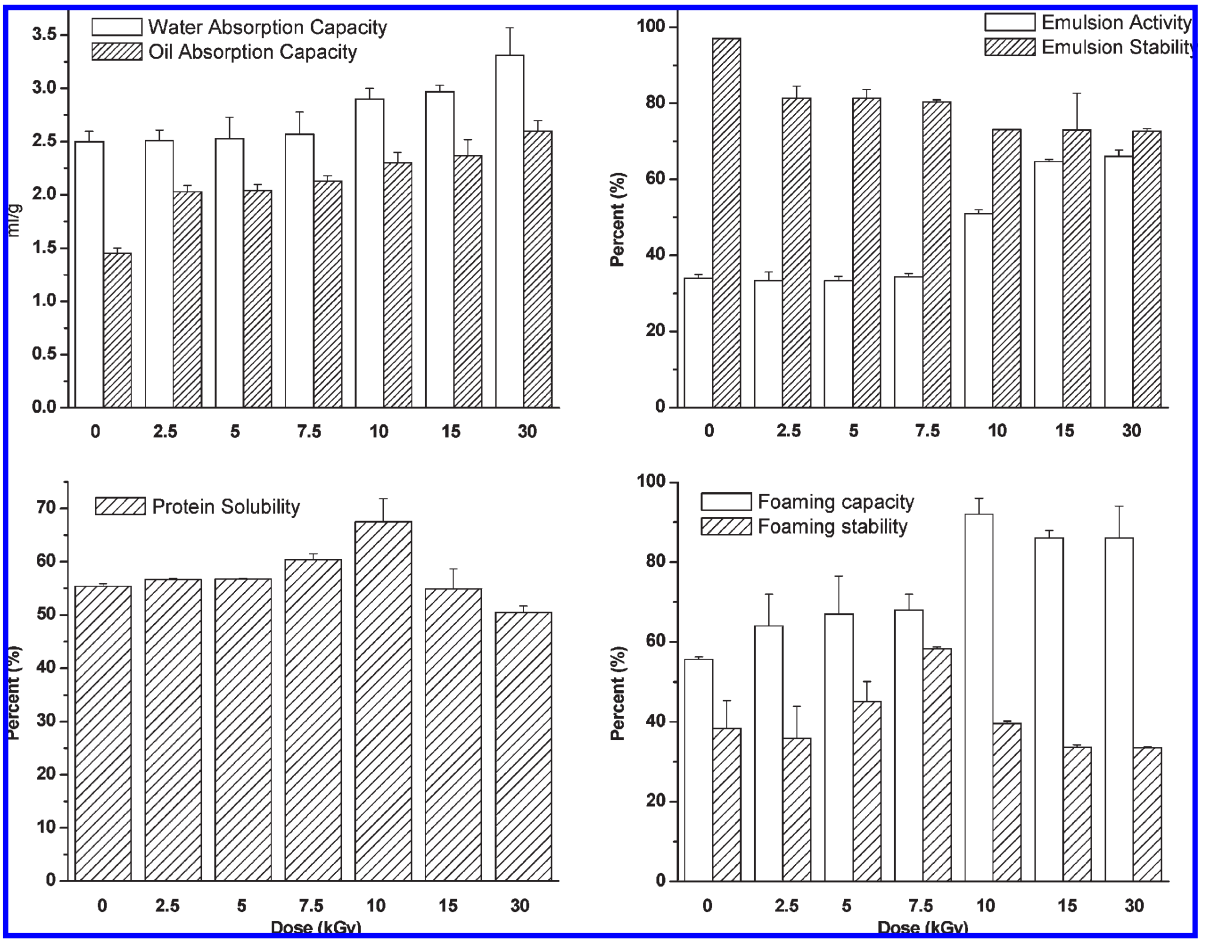

Figure 2. Effects of γ-irradiation on the functional properties of the lotus seed flour; the error bar indicates standard deviation.

in the retention of flavor and also improves the palatability and extends the shelf life of bakery products, meat products, doughnuts, pancakes, baked goods, and soups.

The application of γ-radiation significantly lowered the protein solubility of the lotus seed flour at dose of 30 kGy ($P < 0.05$) (control, 55.38%, and at 30 kGy, 50.50%) (**Figure 2**). However, an increase in the protein solubility was recorded at 7.5 and 10 kGy doses. Such an increase due to irradiation can be attributed to higher protein extraction from the seed matrix. A decrease in the protein solubility with irradiation has been predicted due to the denaturation of proteins from the exposure of hydrophobic groups or protein−protein aggregation. γ-radiation significantly reduced the least gelation capacity (LGC) at doses of 5 kGy and above (from 16% in control up to 4% at 30 kGy). The LGC of the flour at 2.5 kGy (16%) decreased to 8% (nearly 50% reduction) at 5 kGy and got saturated at 7.5 kGy and above doses (4%).

The results on the emulsion activity of the flour with irradiation revealed a dose-dependent increase that was significant at 10 kGy and above (control, 34.0%; 30 kGy, 66.0%) ($P < 0.05$) (**Figure 2**). However, the irradiation decreased the emulsion stability, which was significant at all of the doses (in control, 97.04%, and at 30 kGy, 72.67%) ($P < 0.05$) (**Figure 2**). Good emulsion properties are necessary in the preparation of milklike beverages and meat analogues. The irradiation also resulted in a significant elevation of the foaming capacity at 7.5 kGy and above (control, 55.67%; 30 kGy, 88.07%) ($P < 0.05$), while the foam stability was lowered by higher doses (control; 38.32%; 30 kGy, 33.56%) (**Figure 2**). A good foaming property of flours has been positively correlated to the amount of protein and its solubility (*81*).

In conclusion, our study revealed lotus seed flour to be rich in nutrients with minimal quantity of antinutrients. The raw seeds are a good source of proteins, carbohydrates, minerals, EAAs, and fatty acids, which are comparable to some of the edible legumes and other dicotyledonous seeds. γ-radiation resulted in a dose-dependent increase of the crude proteins and carbohydrates with an elevation in the overall functional properties of the seed flour. Also, a drastic reduction in the antinutritional factors was recorded in the seed flour post γ-radiation treatments. Lotus seed flour has a great potential for exploitation for food and pharmaceutical purposes. As these seeds are an economically valued commodity in national and international markets, the application of a specific dose of ionizing radiation along with other appropriate food processing methods might facilitate the exploitation of the nutritional and pharmaceutical potential for assured product safety.

ABBREVIATIONS USED

ANOVA, analysis of variance; AOAC, Association of Official Analytical Chemists; ^{60}Co, cobalt^{60}; EAA, essential amino acids; FAMEs, fatty acid methyl esters; FAO-WHO, Food and Agricultural Organization−World Health Organization; FAO-WHO-UNU, Food and Agricultural Organization−World Health Organization−United Nations University; GC, γ-chamber; GC-C-IRMS-MS, gas chromatography−combustion−isotope ratio mass spectrometer; HCl, hydrogen chloride; HIV, human immunodeficiency virus; IVPD, in vitro protein digestibility; kGy, kiloGray; L-DOPA, L-3,4-dihydroxyphenylalanine; LGC, least gelation concentration; NFE, nitrogen-free extractives; OAC, oil absorption capacity; PDCAAS, protein digestibility corrected amino acid score; WAC, water absorption capacity.

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LITERATURE CITED

(1) Friedman, M. Nutritional value of proteins from different food sources. A review. *J. Agric. Food Chem.* **1996**, *44*, 6–29.

(2) Conway, G.; Toenniessen, G. Feeding the world in the twenty-first century. *Nature* **1999**, *402*, 55–58.

(3) Sadik, N. Population growth and the food crisis: food, nutrition and agriculture/alimentation. *Food Nutr. Agric.* **1991**, *1*, 3–6.

(4) Bhat, R.; Karim, A. A. Exploring the nutritional potential of wild and underutilized legumes. *Comp. Rev. Food Sci. Food Saf.* **2009**, *8*, 305−331.

(5) Hossain, M. A.; Becker, K. Nutritive value and antinutritional factors in different varieties of *Sesbania* seeds and their morphological fractions. *Food Chem.* **2001**, *73*, 421–431.

(6) Sridhar, K. R.; Bhat, R. Agrobotanical, nutritional and bioactive potential of unconventional legume—*Mucuna*. *Livestock Res. Rural Dev.* **2007**, *19*, 1–28.

(7) Sridhar, K. R.; Bhat, R. Lotus—A potential nutraceutical source. *J. Agric. Technol.* **2007**, *3*, 143–155.

(8) Ibrahim, N.; El-Eraqy, W. Protein content and amino acid composition of *Nelumbo nucifera* seeds and its evaluation as hypoglycaemic agent. *Egypt. J. Pharmacol. Sci.* **1996**, *37*, 635–641.

(9) Liu, C. P.; Tsai, W. J.; Lin, Y. L.; Liao, J. F.; Chen, C. F.; Kuo, Y. C. The extracts from *Nelumbo nucifera* suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells. *Life Sci.* **2004**, *75*, 699–716.

(10) Chopra, R. N.; Nayar, S. L.; Chopra, I. C. *Glossary of Indian Medicinal Plants #22*; Council of Scientific and Industrial Research: New Delhi, 1956.

(11) Sohn, D. H.; Kim, Y. C.; Oh, S. H.; Park, E. J.; Li, X.; Lee, B. H. Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*. *Phytomedicine* **2003**, *10*, 165–169.

(12) Mazumder, U. K.; Gupta, M.; Pramanik, G.; Mukhopadhyay, R. K.; Sarkar, S. Antifertility activity of seed of *Nelumbo nucifera* in mice. *Indian J. Exp. Biol.* **1992**, *30*, 533–534.

(13) Diehl, J. F. *Safety of Irradiated Foods*, 2nd ed.; Marcel Dekker Inc.: New York, 1995; p 189.

(14) Bhat, R.; Sridhar, K. R.; Velmourougane, K. Microbial quality evaluation of velvet beans (*Mucuna pruriens* L. DC.) exposed to ionizing radiation. *Trop. Subtrop. Agroecosys.* **2007**, *7*, 29–40.

(15) Farkas, J. Irradiation as a method for decontamination food. A review. *Int. J. Food Microbiol.* **1998**, *44*, 189–194.

(16) Hayashi, T. Decontamination of dry food ingredients and seeds with soft electrons (low energy electrons). *Food Sci. Technol. Tokyo* **1998**, *4*, 114–120.

(17) Siddhuraju, P.; Osoniyi, O.; Makkar, H. P. S.; Becker, K. Effect of soaking and ionizing radiation on various antinutritional factors of seeds from different species of an unconventional legume, *Sesbania* and a common legume, green gram (*Vigna radiata*). *Food Chem.* **2002**, *79*, 273–281.

(18) Brigide, P.; Canniatti-Brazaca, S. G. Antinutrients and in vitro availability of iron in irradiated common beans (*Phaseouls vulgaris*). *Food Chem.* **2006**, *98*, 85–89.

(19) Bhat, R.; Sridhar, K. R.; Yokotani, K. T. Effect of ionizing radiation on antinutritional features of velvet bean seeds (*Mucuna pruriens*). *Food Chem.* **2007**, *103*, 860–866.

(20) Hunter, C. Changing attitudes to irradiation throughout the food chain. *Radiat. Phys. Chem.* **2000**, *57*, 239–243.

(21) Fricke, H.; Hart, E. J. Chemical dosimetry. In *Radiation Dosimetry*; Attix, F. H., Raesch, W. C., Eds.; Academic Press: New York, 1966; pp 167−239.

(22) Humphries, E. C. Mineral composition and ash analysis. In *Modern Methods of Plant Analysis*; Peach, K., Tracey, M. V., Eds.; Springer-Verlag: Berlin, 1956; Vol. *1*, pp 468−502.

(23) AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990; p 1230.

(24) Müller, H. G.; Tobin, G. *Nutrition and Food Processing*; Croom Helm Ltd.: London, 1980; p 302.

(25) Siddhuraju, P.; Vijayakumari, K.; Janardhanan, K. Nutritional and chemical evaluation of raw seeds of the tribal pulse *Vigna trilobata* (L.) Verdc. *Int. J. Food Sci. Nutr.* **1992**, *43*, 97–103.

(26) Ekanayake, S.; Jansz, E. R.; Nair, B. M. Proximate composition, mineral and amino acid content of mature *Canavalia gladiata* seeds. *Food Chem.* **1999**, *66*, 115–119.

(27) APHA. *Standard Methods for Examination of Water and Waste Water*, 19th ed.; American Public Health Association: Washington, DC, 1995; pp 113−114.

(28) Hofmann, D.; Jung, K.; Bender, J.; Gehre, M.; Schüürmann, G. Using natural isotope variations of nitrogen in plants an early indicator of air pollution stress. *J. Mass Spectrom.* **1997**, *32*, 855–863.

(29) Hofmann, D.; Gehre, M.; Jung, K. Sample preparation techniques for the determination of natural 15N/14N variations in amino acids by gas chromatography combustion-isotope ratio mass spectrometry (GC-C-IRMS). *Isot. Environ. Health Stud.* **2003**, *39*, 233–244.

(30) Brand, W. A.; Tegtmeyer, A. R.; Hilkert, A. Compound-specific isotope analysis: Extending towards 15N/14N and 13C/12C. *Org. Geochem.* **1994**, *21*, 585–594.

(31) FAO-WHO. *Protein Quality Evaluation. Reports of a Joint FAO/WHO Expert Consultation*; Food and Agriculture Organization of the United Nations: Rome, 1991.

(32) Akeson, W. R.; Stahmann, M. A. A pepsin pancreatin digest index of protein quality. *J. Nutr.* **1964**, *83*, 257–261.

(33) Garces, R.; Mancha, M. One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Anal. Biochem.* **1993**, *211*, 139–143.

(34) Rosset, J.; Bärlocher, F.; Oertli, J. J. Decomposition of conifer needles and deciduous leaves in two Black Forest and two Swiss Jura streams. *Int. Rev. Gesamten. Hydrobiol.* **1982**, *67*, 695–711.

(35) Burns, R. R. Methods for estimation of tannins in grain sorghum. *Agron. J.* **1971**, *63*, 511–512.

(36) 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.

(37) 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.

(38) Bartlett, G. R. Phosphorus assay in column chromatography. *J. Biol. Chem.* **1959**, *234*, 466–468.

(39) Hankins, C. N.; Kindinger, J. I.; Shannon, L. M. Legume alphagalactosidases which have hemagglutinin properties. *Plant Physiol.* **1980**, *65*, 618–622.

(40) Fujii, Y.; Shibuya, T.; Yasuda, T. L. 3,4-dihydroxyphenylalanine as an allelochemical from *Mucuna pruriens* (L.) DC. var *utilis*. *Agric. Biol. Chem.* **1991**, *55*, 617–618.

(41) Iyengar, M. A. R.; Ganapathi, S.; Kannan, V.; Rajan, M. P.; Rajaram, S. Procedure manual. India: Workshop on environmental radioactivity, April 16−18, **1990**.

(42) Beuchat, L. R. Functional and electrophoretic characteristics of succinylated peanut flour protein. *J. Agric. Food Chem.* **1977**, *25*, 258–261.

(43) Were, L.; Hettiarachchy, L.; Kalapathy, U. Modified soy proteins with improved foaming and water hydration proteins. *J. Food Sci.* **1997**, *62*, 821–824.

(44) Coffman, C. W.; Garcia, V. V. Functional properties and amino acid content 561 of protein isolate from mung bean flour. *J. Food Technol.* **1977**, *12*, 473–484.

(45) Neto, V. Q.; Narain, N.; Silvia, J. B.; Bora, P. S. Functional properties of raw and heat-processed cashew nut (*Anacardium occidentale* L.) kernel protein isolate. *Nahrung* **2001**, *45*, 258–262.

(46) Indrayan, A. K.; Sharma, S.; Durgapal, D.; Kumar, N.; Kumar, M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Curr. Sci.* **2005**, *89*, 1252–1255.

(47) Warchalewski, J. R.; Gralik, J.; Zawirska-Wojtasia, R.; Zabielski, J.; Kuśnierz, R. The evaluation of wheat grain odour and colour after gamma and microwave irradiation. *Electron. J. Pol. Agric. Univ. Food Sci. Technol.* **1998**, *1*, 1–11.

(48) Nwokolo, E. Nutritional evaluation of pigeon pea. *Plant Foods Hum. Nutr.* **1987**, *37*, 283–290.

(49) Arinathan, V.; Mohan, V. R.; De Britto, A. J. Chemical composition of certain tribal pulses in South India. *Int. J. Food Sci. Nutr.* **2003**, *54*, 209–217.

(50) Pugalenthi, M.; Vadivel, V.; Gurumoorthi, P.; Janardhanan, K. Comparative nutritional evaluation of little known legumes, *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa*. *Trop. Subtrop. Agroecosyst.* **2004**, *2*, 107–123.

(51) Rehman, Z.; Shah, W. H. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chem.* **2005**, *91*, 327–31.

(52) Anderson, J. W.; Johnstone, B. M.; Cook-Newell, M. E. Meta-analysis of the effects of soy protein intake on serum lipids. *N. Engl. J. Med.* **1995**, *333*, 276–282.

(53) Salvin, J.; Jacobs, D. R.; Marquart, L. Whole grain consumption and chronic disease: Protective mechanisms. *Nutr. Cancer* **1997**, *27*, 14–21.

(54) Balogun, A.; Fetuga, B. L. Chemical composition of some under exploited leguminous crop seeds in Nigeria. *J. Agric. Food Chem.* **1986**, *34*, 189–192.

(55) Cambell, G. L.; Sosulski, F. W.; Classen, H. L.; Ballam, G. M. Nutritive value of irradiated and β-glucanase treated wild oat groats (*Avena fatua* L.) for broiler chickens. *Anim. Feed Sci. Technol.* **1987**, *16*, 243–252.

(56) Nwokolo, E.; Oji, D. I. M. Variation in metabolizable energy content of raw or autoclaved white and brown varieties of three tropical grain legumes. *Anim. Feed Sci. Technol.* **1985**, *13*, 141–146.

(57) Mohan, V. R.; Janardhanan, K. Chemical analysis and nutritional assessment of lesser known pulses of the genus, *Mucuna*. *Food Chem.* **1995**, *52*, 275–280.

(58) Vishwanathan, M. B.; Thangadurai, D.; Ramesh, N. Biochemical evaluation of *Neonotonia wightii* (Wight and Arn.) Lackey (Fabaceae). *Food Chem.* **2001**, *75*, 275–259.

(59) Leeds, A. R. Legumes and gastrointestinal function in relation to diets for diabetics. *J. Plant Foods* **1982**, *4*, 23–27.

(60) Walker, A. F. Physiological effects of legumes in the human diet: A review. *J. Plant Foods* **1982**, *4*, 5–14.

(61) Combs, G. F.; Gray, W. P. Chemo preventive agents: Selenium. *Pharmacol. Ther.* **1998**, *79*, 179–92.

(62) Underwood, E. J. *Trace Elements in Human and Animal Nutrition*, 4th ed.; Academic Press Inc.: New York, 1977; p 640.

(63) Bau, H. M.; Villaume, C.; Lin, C. F.; Evard, F.; Quemener, B.; Nicolas, J. P.; Mejean, L. Effect of solid-state fermentation using *Rhizopus oligosporus* sp. T-3 on elimination of antinutritional substances and modification of biochemical constituents of defatted rapeseed meal. *J. Sci. Food Agric.* **1994**, *65*, 315–22.

(64) FAO-WHO-UNU. *Energy and Protein Requirements*; Technical Report Series #724; WHO: Geneva, 1985.

(65) Baudoin, J. P.; Maquet, A. Improvement of protein and amino acid content in seeds of food legumes—A case study in *Phaseolus*. *Biotechnol. Agron. Soc. Environ.* **1999**, *3*, 220–224.

(66) Saunders, R. M.; Connor, M. A.; Booth, A. N.; Bickoff, E. M.; Kohler, G. O. Measurement of digestibility of alfaalfa concentrates by in vivo and in vitro methods. *J. Nutr.* **1973**, *103*, 530–535.

(67) Fernando, T.; Bean, G. Variation of the antinutritional behenic acid content among the cultivars of winged bean (*Psophocarpus tetragonolobus* (L.) (DC.) seeds. *Food Chem.* **1985**, *185*, 265–269.

(68) Omode, A. A.; Fatoki, O. S.; Olaogun, K. A. Physico-chemical properties of some under-exploited and non-conventional oil seeds. *J. Agric. Food Chem.* **1995**, *43*, 2850–2583.

(69) Villavicencio, A. L. C. H.; Mancini-Filho, J.; Delincée, H.; Greiner, R. Effect of irradiation on antinutrients (total phenolics, tannins and phytate) in Brazilian beans. *Radiat. Phys. Chem.* **2000**, *57*, 289–293.

(70) Alothman, M.; Bhat, R.; Karim, A. A. Effects of radiation processing on phytochemicals and antioxidants in plant produce. *Trends Food Sci. Technol.* **2009**, *20*, 201–212.

(71) Hagerman, A. E.; Riedl, K. M.; Jones, G. A.; Sovik, K. N.; Ritchard, N. T.; Hartfield, P. W.; Riechel, T. L. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem.* **1998**, *46*, 1887–1892.

(72) Pinn, A. B. R. O. *Efeitos das Radiacões Gama Sobre a Disponibilidade do Ferro em Feijões* (*Phaseolus vulgaris*), *Dissertacão* (*Mestrado*); Facuuldade de Cincias Farmacuticas Universidade de São Paulo: Brazil, 1992.

(73) De Boland, A. R.; Garner, G. B.; O'Bell, B. L. Identification and properties of 'phytate' in cereal grains and oil seed products. *J. Agric. Food Chem.* **1975**, *23*, 1186–9.

(74) Ryden, P.; Selvendran, R. R. Phytic acid: Properties and determination. In *Encyclopedia of Food Science, Food Technology and Nutrition*; Macrae, R., Robinson, R. K., Sadler, M. J., Eds.; Academic Press: London, 1993; pp 3582−3587.

(75) Rickard, S. W.; Thompson, L. U. Interactions and biological effects of phytic acid. In *Antinutrients and Phytochemicals in Food*; Shahidi, F., Ed.; ACS Symposium Series #662; American Chemical Society: Washington, DC, 1997; pp 294−312.

(76) Shamsuddin, A. M.; Vucenik, I.; Cole, K. E. IP_6: A novel anticancer agent. *Life Sci.* **1997**, *61*, 43–54.

(77) Jaffe, W. G. Phytohaemagglutinins. In *Toxic Constitutes of Plant Food Stuffs*; Liener, I. E., Ed.; Academic Press: New York, 1980; pp 69−101.

(78) Bhat, R.; Sridhar, K. R.; Rajashekara, K. M.; Narayana, Y. 210-Po bioaccumulation in coastal sand dune wild legumes *Canavalia* spp. of southwest coast of India. *J. Environ. Monit.* **2005**, *7*, 856–860.

(79) McDonald, P.; Fowler, S. W.; Heyraud, M.; Boxter, M. S. Polonium-210 in mussels and its implications for environmental alpha-autoradiography. *J. Environ. Radioact.* **1986**, *3*, 293–303.

(80) Rahma, E. H.; Mostafa, M. M. Functional properties of peanut flour as affected by different heat treatments. *J. Food Sci. Technol.* **1988**, *25*, 11–15.

(81) Aluko, R. E.; Yada, R. Y. Relationship of hydrophobicity and solubility with some functional properties of cowpea (*Vigna unguiculata*) protein isolate. *J. Sci. Food Agric.* **1993**, *62*, 331–335.

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