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Nutritional quality evaluation of electron beam-irradiated lotus (*Nelumbo nucifera*) seeds

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Abstract

Nutritional and antinutritional qualities, and functional properties of raw and electron beam-irradiated (doses: 0, 2.5, 5, 7.5, 10, 15 and 30 kGy) lotus seeds were studied. Seeds were rich in protein, amino acids, unsaturated fatty acids and minerals, without heavy metal contamination. Irradiation of seeds revealed a decrease in crude protein and fibre, which was not significant at any of the doses. However, significant increase of ash (10 kGy onwards) and carbohydrates (at 30 kGy) were recorded after irradiation. Seed flours showed a significant dose-dependent decrease in water absorption capacity, while oil absorption capacity significantly increased from 10 kGy onwards ($p < 0.05$). Also, significant increase in protein solubility (5 kGy onwards) and foaming capacity (7.5 kGy onwards) with improvement in the least gelation capacity (5 kGy onwards) of seed flour ($p < 0.05$) was recorded after irradiation. Electron beam irradiation of seeds resulted in significant dose-dependent elevation of total phenolics and tannins, while phytic acid was eliminated at 5 kGy. Seeds of lotus can serve as food and minimise protein-energy malnutrition of economically weaker sections of the population in developing countries. The seed flours also possess great potential for development of new food products and formulations. As a physical method of preservation, electron beam irradiation was effective in the retention of the nutritional qualities of lotus seeds and is recommended for commercial exploitation.

Keywords: Lotus seeds; Electron beam irradiation; Nutritional features; Antinutrients; Functional properties

1. Introduction

Human malnutrition is high in populations dependent mainly on starch-based diets (Siddhuraju, Osoniyi, Makkar, & Becker, 2002). Due to an inadequate supply of proteins of animal origin, nutritionalists, researchers and government organisations worldwide are searching for reliable, cheap and high quality proteins of plant origin (Adebowale & Lawal, 2004). Providing inexpensive plant-based protein supplements has led to the examination of underutilised dicotyledonous seeds for human and livestock consumption. Reports are available on the successful utilisation of underutilised dicotyledonous seeds as food (Arinathan, Mohan, & De Britto, 2003; Glew et al., 1997; Sridhar & Bhat, 2007; Vadivel & Janardhanan, 2004).

Lotus is a nutraceutically valued edible plant that is widely grown and common in Australia, China, India, and Japan (Anonymous, 1966). Generally, the whole plant is considered as a coolant and most of the parts are used for the treatment of diarrhoea and haemostasis (Yu & Hu, 1997). Seeds are reported to be rich in protein and adequate in the amounts of essential minerals (Ibrahim & El-Eraqy, 1996). These seeds are regarded as a popular health food and an alkaloid (liensinine) extracted from lotus serves as an effective drug to treat arrhythmia (Ling, Xie, & Yang, 2005). The seeds are also used in indigenous (e.g., Ayurveda, Chinese) and folk medicines to treat tissue inflammation, cancer, diuretics and skin diseases (e.g., leprosy) (Chopra, Nayar, & Chopra, 1956; Liu et al., 2004).

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In India, lotus plant reportedly grows in almost all lakes and ponds, both at high (1400 m in the Himalayas, North India) (Polunin & Stainton, 1984) and low altitudes (Kanyakumari, Southern India). Seed production in India may vary from 200 to 250 kg/Ha. Lotus seeds are quite uncommon in the Indian market, except in areas where they are sometimes sold as a raw snack. In local markets, the shelled and dried seed fetches a price of US $1.35/kg (Goel, Sharma, & Sharga, 2001), but in the international market it is approximately US $200/1000 seeds. As the utilisation of these seeds is minimal, the rate may vary from year to year.

Stringent laws imposed by the European Union and other countries on the quality and safety of imported food and agricultural commodities have resulted in better physical methods of food preservation. For example, ban on the use of chemical fumigants (e.g., methyl bromide, ethylene dioxide) in developed countries (2005), as well as in developing countries (2015) (Anonymous, 1995) have opened up the possibilities of employing food irradiation as a reliable physical method of preservation. Electron beam (EB) irradiation has been proved to be successful in decontamination, disinfestation and improvement of overall qualities of food and agricultural commodities (Bhat & Sridhar, 2007; Blank, Shamsuzzaman, & Sohal, 1992; Ic, Kottapalli, Maxim, & Pillai, 2007; Kottapalli, Wolf-Hall, Schwarz, Schwarz, & Gillespie, 2003; Palekar et al., 2004). Radiation processing has also been shown to reduce or inactivate some of the antinutritional factors in wild leguminous seeds, thereby enhancing their edibility (Bhat, Sridhar, & Yokotani, 2007; Siddhuraju, Makkar, & Becker, 2002).

Information pertaining to nutritional, antinutritional and safety characteristics of underutilised seeds will be a basic necessity, prior to consumption and commercialisation. The major aim of the present study was to ascertain the impact of electron beam irradiation on the nutritional and antinutritional components, and functional properties of lotus seeds. The result of the present study intends to provide data for the successful utilisation of lotus seeds for human/livestock consumption and for the commercialisation of EB irradiation technology for value addition.

2. Materials and methods

2.1. Seed samples

Mature and dry seeds of lotus (*Nelumbo nucifera* cv. Gaertn.) were purchased from a leading ayurvedic drug dealer (Dhanvantri Ayurveda Pvt. Ltd., Mysore, Karnataka, India). The hard seed coat was removed with the help of a sharp and clean stainless steel knife and the edible cotyledon portion obtained was powdered (particle size 0.5 mm) for analysis.

2.2. Electron beam irradiation

Seed samples, packed in polyethylene bags (6 × 6 cm) were exposed to electron beam radiation at the Microtron Centre, Mangalore University (Microtron accelerator, designed by Centre for Advanced Technology, India) to various doses (2.5, 5.0, 7.5, 10, 15 and 30 kGy) at room temperature (25 ± 1 °C). The conditions of the Microtron accelerator were: dose rate, 2 kGy min^{-1}; beam energy, 8.3 MeV (variable) and beam current, 30 mA (time duration required for irradiating seeds up to 30 kGy was 15 min). Double side irradiation (exposure to both sides) was performed for uniform dose delivery. The absorbed dose was measured employing Fricke dosimetry (Fricke & Hart, 1966). Similarly packed seed samples without irradiation served as a control.

2.3. Nutritional properties

2.3.1. Proximal analysis

Moisture content of the seeds (cotyledon portion) was determined by oven drying to a constant mass at 105 °C (16 ± 1 h). The crude protein (Humphries, 1986), crude lipid, crude fibre and ash contents (AOAC, 1990) were determined as per the standard procedures.

The method of Müller and Tobin (1980) was used to calculate total crude carbohydrates:

$$\text{Total crude carbohydrates (\%)} = 100 - [\text{crude protein (\%)} + \text{crude lipid (\%)} + \text{crude fibre (\%)} + \text{ash (\%)}]$$

The gross energy was calculated based on the formula used by Ekanayake, Jansz, and Nair (1999):

$$\text{Gross energy (kJ 100 g}^{-1}\text{ DM)} = (\text{protein} \times 16.7) + (\text{lipid} \times 37.7) + (\text{carbohydrates} \times 16.7).$$

2.3.2. Mineral analysis

Minerals content (sodium, potassium, calcium, magnesium, iron, copper, zinc, manganese and selenium) of the seed flour was determined employing AOAC (1990) method. Flour was digested with a mixture of concentrated nitric acid, sulfuric acid and perchloric acid (10:0.5:2, v/v and analysed using an atomic absorption spectrophotometer (GBC 904AA; Germany). The total phosphorus was determined as orthophosphate by the ascorbic acid method after acid digestion and neutralisation using phenolphthalein indicator and combined reagent (APHA, 1995). The absorbance was read at 880 nm (Spectronic 21 D, Miltonroy, NewYork, USA) and KH_2PO_4 (Merck, Mumbai, India) served as a standard.

2.3.3. Amino acids

The amino acid composition of lotus seed samples was determined by the method outlined by Hofmann, Jung, Bender, Gehre, & Schüürmann (1997) and Hofmann, Gehre, & Jung (2003). A known quantity of seed flour

was hydrolysed with 6 N HCl (15 ml) for 4 h at 145 °C. After cooling, HCl was eliminated in a rotary evaporator (Büchi Laboratoriumstechnik AG RE121; Switzerland), combined with a diaphragm vacuum pump (MC2C; Vacuubrand GmbH, Germany). Internal standard, *trans*-4-(aminomethyl)-cyclohexanecarboxylic acid (Aldrich, purity, 97%) was added to each sample. The derivatisation step consisted of esterification by trifluoroacetylation (Brand, Tegtmeyer, & Hilkert, 1994).

Samples of standard amino acids were weighed out in reaction vials and dried using CH_2Cl_2 under a gentle stream of helium and slow heating in an oil bath (40–60 °C) to remove water traces. A 15 ml aliquot of freshly prepared acidified isopropanol (acetyl chloride, 3 ml + 2-propanol, 12 ml) was added and the mixture was heated at 100 °C for 1 h. After cooling, the reagent was eliminated with a gentle stream of helium at 60 °C, followed by combined evaporation with three successive aliquots of CH_2Cl_2 to remove propanol and water. The dry residue was trifluoroacetylated with 200 µl trifluoroacetic anhydride overnight at room temperature. An aliquot of this solution was used without further treatment for gas chromatography–combustion-isotope ratio mass spectrometry (GC–C-IRMS/MS).

The GC–C-IRMS/MS measurements were carried out with a Hewlett-Packard 5890 Series II gas chromatograph, connected *via* a split with a combustion interface to the IRMS system (MAT 252, Finnigan MAT; Germany) for the isotopic determination of nitrogen, and, *via* a transfer line, with a mass spectrometer (GCQ, Finnigan MAT; Germany) for qualitative analysis and quantification of the amino acids. The capillary column for GC was 50 m × 0.32 mm i.d. × 0.5 µm BPX5 (SGE), operating with a carrier gas flow of 1.5 ml min^{-1} at the following temperature and pressure: initial 50 °C (1 min), increased to 100 °C at 10 °C min^{-1} (10 min), increased to 175 °C at 3 °C min^{-1} (10 min), increased to 250 °C min^{-1} (10 min); head pressure, 13 psi (90 kPa).

The Essential Amino Acid (EAA) score was determined employing the formula:

$$\text{EAA score} = 100 \times [\text{mg of EAA in 100 mg test protein}] \div [\text{mg of EAA in 100 mg FAO/WHO(1991)reference pattern}]$$

2.3.4. In vitro protein digestibility

In vitro protein digestibility (IVPD) of seed flours were determined using the standard methods described by Akeson & Stahmann (1964). Defatted flours were incubated (37 °C, 3 h) with pepsin (Sigma, 3165 units mg^{-1} protein) (1.5 mg in 2.5 ml 0.1 N HCl) followed by inactivation (0.25 ml 1 N NaOH). Incubation was continued (24 h, 37 °C) with trypsin (Sigma, 16,100 units mg^{-1} protein) and α-chymotrypsin (Sigma, 76 units mg^{-1} protein) (2 mg each in 2.5 ml potassium phosphate buffer, pH 8.0, 0.1 M) followed by inactivation (0.7 ml of 100% TCA). Zero-time control was maintained by inactivating the enzyme before addition of substrate. The inactivated reaction mixtures were centrifuged and supernatant was collected. The residue was washed (2 ml, 10% TCA) and centrifuged. The combined supernatant was washed with 10 ml diethyl ether twice and the ether layer was removed by aspiration. The aqueous layer was heated on a boiling water bath (15 min) to remove traces of ether and the solution was made up to 25 ml with distilled water. Nitrogen content (in 5 ml aliquots) was determined to calculate protein in the digest:

$$\textit{in vitro}\ \text{digestibility}(\%) = 100 \times (\text{Protein in digest}) \div (\text{Protein in defatted flour})$$

The protein digestibility corrected amino acid score (PDCAAS) of EAA was calculated based on EAA requirements for adults (FAO/WHO, 1991):

$$\text{PDCAAS}(\%) = [\text{Amino acid content in food protein}] \times [\text{true digestibility}] \div [\text{Amino acid content of reference pattern}]$$

2.4. Fatty acid methyl esters

The method of Garces & Mancha (1993) was followed to determine fatty acid methyl esters in the seed flour. Samples (50 mg) together with respective fatty acids [American Oil Chemists Society (AOCS); Merck, Germany] as the internal standard were placed in tubes with teflon-lined caps and methylated with a mixture containing methanol, benzene, DMP (2,2-dimethoxypropane) and H_2SO_4 (37:20:5:2) (v/v). The sample was placed in a water bath at 80 °C for 2 h and the mixture was made up to a total volume of 5 ml with heptane. The tubes were cooled and shaken to separate the two phases. The upper layer containing the fatty acid methyl esters (FAMEs) was injected onto a GC (Sigma Instruments, Baroda, India) containing a glass column (Silar, 10%) packed with 5% ethylene glycol succinate on Supelcoport 80/100 at 200 °C. Conditions for the analysis were as follows: carrier gas, N_2; injector temperature, 225 °C; FID detector temperature, 265 °C; oven temperature, 200 °C; flow rate: N_2, 35 ml min^{-1}, H_2, 30 ml min^{-1}, O_2, 75 ml min^{-1}.

The polyunsaturated to saturated fatty acid ratio was calculated as follows:

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

2.5. Antinutritional features

2.5.1. Phenolics

Total phenolics of the seed flours were assayed by adapting the method outlined by Rosset, Bärlocher, & Oertli (1982). A known amount of the seed flour was extracted

twice with methanol (50%, 5 ml) in a water bath (95 °C, 10 min). The pooled extract was made up to 10 ml, the extract (0.5 ml) was mixed with an equal quantity of distilled water and treated with 5 ml Na_2CO_3 (in 0.1 N NaOH). After 10 min, 0.5 ml Folin-Ciocalteu's reagent (diluted 1:1 with distilled water) was added and the colour developed was read at 725 nm. The phenolics determined were expressed as gallic acid equivalents (GAE).

2.5.2. Tannins

The vanillin–HCl method was adapted to determine tannins in the seed flours (Burns, 1971). A known amount of the seed flour (1 g) was extracted with methanol (10 ml, 28 °C, 12 h), vortexed and decanted. This process was repeated and the supernatant was pooled and made up to 25 ml. The extract (1 ml) was treated with reagent mixture (5 ml) (4% vanillin in methanol and 8% concentrated HCl in methanol, 1:1). After 20 min, the colour developed was read at 500 nm (Spectronic 21, Miltonroy, USA) using catechin (50–250 μg) as standard.

2.5.3. Phytic acid

Phytic acid was extracted from the seed flour was extracted and determined by adapting standard procedures (Deshpande, Sathe, Salunkhe, & Cornforth, 1982; Sathe, Deshpande, Reddy, Goll, & Salunkhe, 1983). A known amount of the sample (2 g) was extracted (2 h) with 1.2% HCl (10 ml) containing sodium sulfate (10%), at room temperature (25 ± 1 °C) and centrifuged. The volume was made up to 10 ml with the extracting solvent. Phytic phosphorus was estimated before and after precipitation of phytic acid by $FeCl_3$. Five milliliters of the above extract was taken and 3 ml of $FeCl_3$ solution ($FeCl_3$, 2 g + concentrated HCl, 16.3 ml, diluted to 1 L) was added, stirred, boiled for 75 min in a boiling water bath, cooled and left at room temperature (1 h) prior to centrifugation (2000*g*, 10 min) and filtration (Whatman # 1). The supernatant (made up to 10 ml with distilled water) was used for assay. Analysis of soluble phosphorus was done by the method described by Bartlett (1959), using ammonium molybdate reagent. The absorbance was read at 430 nm after 30 min with KH_2PO_4 as standard.

Phytate phosphorus was determined by the following formula:

$$\text{Phytate phosphorus} = [A \times 28.18] \div [100]$$

(where, A = phytic acid).

2.6. Functional properties

2.6.1. Water and oil absorption capacity

Water and oil absorption capacities (WAC and OAC) were determined according to Beuchat (1977). One gram of the seed flour was vortexed with 10 ml distilled water or oil (Sundrop superlite refined sunflower oil, Agrotech Foods Ltd., Secunderabad, India) for 30 s in centrifuge tube. The solution was allowed to stand at room temperature (28 ± 2 °C) for 30 min, centrifuged (5000*g*, 30 min) and the volume of supernatant was measured in a 10 ml graduated cylinder.

2.6.2. Protein solubility

The protein solubility was determined by the method outlined by Were, Hettiarachchy, & Kalapathy (1997). One hundred and twenty five milligrams of seed flour were blended with 25 ml distilled water. The solution was mixed using a magnetic stirrer (1 h at 20 °C) and centrifuged (12,000*g* for 20 min at 4 °C). The supernatant was filtered through glass wool and nitrogen was estimated by micro-Kjeldahl's method (Humphries, 1986). The soluble protein (%) profile was determined (nitrogen solubility × 6.25):

$$\text{Protein solubility}(\%) = 100\,(\text{Amount of nitrogen in the supernatant}) \div (\text{Amount of nitrogen in the flour})$$

2.6.3. Gelation properties

The gelation properties were determined by the method outlined by Coffman & Garcia (1977). Ten milliliters of each of a seed flour suspension in distilled water (pH 7) was transferred to test tubes and heated in a boiling water bath for 1 h and cooled. The samples in the tubes were further cooled for 2 h at 4 °C and the least gelation concentration (LGC) was detected when the sample from the inverted test tube did not fall or slip. The effect of radiation treatments on gelation property was carried out and the LGC was determined.

2.6.4. Emulsion properties

Emulsifying activity and emulsion stability were determined in triplicate following the methods of Neto, Narain, Silvia, & Bora (2001). Five milliliters of seed flour dispersion in distilled water (10 mg ml^{-1}) were homogenised (1 min) with 5 ml oil (Sunflower refined oil). The emulsions were centrifuged (1100*g*, 5 min) and the height of the emulsified layer and the total contents in the tube were determined. The emulsifying activity was calculated:

$$\text{Emulsifying activity}(\%) = 100(\text{height of the emulsified layer}) \div (\text{height of the total content})$$

Emulsion stability was determined by heating the emulsion (80 °C, 30 min) before centrifuging (1100*g*, 5 min):

$$\text{Emulsion stability}(\%) = 100(\text{height of the emulsified layer after heating}) \div (\text{height of emulsified layer before heating})$$

2.6.5. Foaming properties

Foaming capacity of the seed flour was determined according to the method outlined by Coffman & Garcia (1977). Two grams of the flour were dispersed in 100 ml of distilled water and whipped vigorously for 2 min in a kitchen blender at speed 1 (Philips HL1643, Philips India

Ltd.). The volumes were recorded before and after whipping and the percentage volume increase was calculated:

$$\text{Volume increase}(\%) = 100(V_2 - V_1) \div (V_1)$$

(where, V_1 = initial volume of solution; V_2 = volume of solution after whipping).

Foam stability was determined as the volume of foam that remained after 8 h at room temperature (28 ± 2 °C) and expressed as the percentage of initial foam volume.

2.7. Statistical analysis

One-way analysis of variance (ANOVA) (using ORIGIN®, version 6.0, Microcal Software Inc., Northampton, MA) was carried out to compare the mean values of control and irradiated seed samples. The data presented are the mean of five independent determinations (±SD), unless mentioned otherwise. The level of significance was determined at $p < 0.05$.

3. Results and discussion

3.1. Nutritional qualities

3.1.1. Proximal features

The moisture content of raw lotus seed (cotyledon portion) was 8.25% and, except for at 2.5 kGy, irradiation caused significant loss of moisture ($p < 0.05$) (Table 1). A low moisture content will be advantageous in maintenance and improvement of shelf life. The crude protein and crude fibre content in raw seed flour were 24.4% and 3.15%, respectively. The crude protein of lotus seeds is higher than parboiled rice (7.7%), wheat, (8.55%), egg (12.6%) (Statens Livsmedelsverk, 1988) and some tribal pulses (12.9–20.2%) (Arinathan et al., 2003). The high protein content in lotus seeds emphasises their value as a vital source of nutrients. EB irradiation decreased the crude protein and crude fibre of seeds, but not significantly at any of the doses delivered. It would be interesting to determine total, soluble and insoluble dietary fibre fractions in raw and EB-irradiated lotus seeds, to gain a better insight into the fibre contents. Crude lipid was significantly reduced on EB irradiation (control, 3.68%; 30 kGy, 2.72%) ($p < 0.05$), while ash significantly increased at 10 kGy onwards (control, 4.03%; 30 kGy, 4.47 %) ($p < 0.05$). The quantity of ash in any seed sample assumes importance, as it determines the nutritionally important minerals (Vadivel & Janardhanan, 2004). Lotus seeds contained a high amount of carbohydrates (64.6%), which might be due to low lipid content. However, EB irradiation increased the crude carbohydrates, which was significant only at 30 kGy (30 kGy, 73.51%) ($p < 0.05$). The increase in carbohydrates might be attributed to radiation-induced breakdown of complex sugars (polysaccharides) into simple extractable forms (e.g., free sugars). The calorific value of seeds was not altered significantly at any dose of radiation (1624 kJ per 100 g) and the values exceeded those of cowpea, green gram, horse gram, moth beans and peas (1318–1394 kJ per 100 g) (Narasinga Rao, Deosthale, & Pant, 1989).

3.1.2. Mineral composition

Adequate amounts of potassium, calcium, magnesium and iron were found in lotus seeds (Table 2). High levels of potassium in diets are beneficial for those suffering from hypertension and those who suffer excessive excretion of potassium through the body fluids (Siddhuraju, Becker, & Makkar, 2001). Iron and selenium act as antioxidants (Talwar, Srivastava, & Mudgil, 1989), and are involved in strengthening the immune system, while magnesium and zinc are known to prevent cardiomyopathy, muscle degeneration, growth retardation, alopecia, dermatitis, immunologic dysfunction, gonadal atrophy, impaired spermatogenesis, congenital malformations and bleeding disorders (Chaturvedi, Shrivastava, & Upreti, 2004). Electron beam irradiation significantly decreased ($p < 0.05$) potassium at 10 kGy onwards, while zinc was reduced at 5 kGy onwards. A similar decline was registered in calcium on irradiation, which was significant at 10 kGy onwards ($p < 0.05$). The other minerals studied were not altered by irradiation. Lotus seeds were devoid of heavy/toxic metals such as copper, cobalt, lead, chromium and nickel. Since wheat flours used in the baking industry are usually deficient in some of the essential minerals (e.g., calcium and iron) (El-Adawy & Khalil, 1994), the fortification of wheat flours with lotus seed flour will definitely improve the dietary requirements.

Table 1
Proximate composition of lotus seeds treated with electron beam irradiation (on dry weight basis) ($n = 5$, mean ± SD)

Component	Irradiation dose (kGy)						
	0	2.5	5	7.5	10	15	30
Moisture (%)	8.25 ± 0.24	7.80 ± 0.16	7.64 ± 0.23	7.50 ± 0.10	7.40 ± 0.20	7.44 ± 0.04	7.45 ± 0.02
Crude protein (g 100 g^{-1})	24.4 ± 5.22	21.3 ± 2.02	20.9 ± 1.05	20.3 ± 2.03	21.2 ± 2.83	21.8 ± 0.96	17.4 ± 3.76
Crude lipid (g 100 g^{-1})	3.68 ± 0.73	4.5 ± 0.17	4.10 ± 0.26	3.8 ± 1.22	2.78 ± 0.08*	2.68 ± 0.28*	2.72 ± 0.07*
Crude fibre (g 100 g^{-1})	3.15 ± 1.74	1.75 ± 0.4	1.28 ± 0.03	2.1 ± 0.15	2.2 ± 0.39	1.92 ± 0.10	1.78 ± 0.06
Ash (g 100 g^{-1})	4.03 ± 0.21	4.63 ± 0.55	4.23 ± 0.21	4.40 ± 0.90	4.83 ± 0.06*	4.45 ± 0.50*	4.57 ± 0.15*
Crude carbohydrates (g 100 g^{-1})	64.6 ± 5.69	66.8 ± 2.91	68.5 ± 0.61	69.4 ± 1.32	69.0 ± 2.67	69.0 ± 0.05	73.5 ± 3.37*
Gross energy (kJ 100 g^{-1})	1624 ± 38.64	1679* ± 11.0	1685* ± 2.46	1641* ± 37.02	1610* ± 8.67	1677* ± 34.40	1624* ± 6.49

* Significantly different from control ($p < 0.05$).

Table 2
Mineral composition of raw and electron beam irradiated lotus seeds (mg 100 g^{-1}) (on dry weight basis) ($n = 5$, mean ± SD)

Mineral	Irradiation dose (kGy)						
	0	2.5	5	7.5	10	15	30
Sodium	7.86 ± 0.01	7.86 ± 0.12	6.90 ± 0.02	6.55 ± 0.03	6.56 ± 0.14	6.54 ± 0.01	6.21 ± 0.12
Potassium	48.5 ± 0.46	47.6 ± 1.1	47.4 ± 0.65	48.1 ± 0.25	46.9 ± 0.89*	44.2 ± 5.22*	44.2 ± 5.34*
Calcium	313 ± 12.30	252 ± 13.70	261 ± 10.00	229 ± 5.00	234 ± 4.52*	188 ± 0.01*	189 ± 5.62*
Phosphorus	6.25 ± 0.02	6.13 ± 5.30	5.98 ± 3.89	5.63 ± 1.78	5.75 ± 3.54	5.75 ± 3.25	5.75 ± 3.58
Magnesium	43.9 ± 1.24	43.090 ± 0.01	41.590 ± 1.15	40.992 ± 1.91	42.992 ± 1.23	41.9 ± 0.01	40.3 ± 0.03
Iron	16.4 ± 4.44	14.3 ± 5.47	15.8 ± 3.2	14.2 ± 2.54	13.8 ± 3.52	10.3 ± 0.95	9.25 ± 1.13
Copper	2.51 ± 0.38	2.14 ± 0.16	2.19 ± 0.09	1.99 ± 0.04	2.04 ± 0.11	1.96 ± 0.12	1.94 ± 0.10
Zinc	7.72 ± 1.78	7.49 ± 3.49	7.84 ± 1.99*	7.71 ± 0.56*	7.58 ± 0.55*	7.14 ± 0.01*	6.7 ± 0.02*
Manganese	16.6 ± 2.23	15.0 ± 0.02	13.7 ± 0.85	14.9 ± 1.08	14.5 ± 0.55	15.1 ± 0.70	15.0 ± 0.57
Selenium	1.04 ± 0.02	1.30 ± 0.01	1.43 ± 0.12	0.39 ± 0.02	0.39 ± 0.52	0.26 ± 0.01	0.264 ± 0.02

* Significantly different from control ($p < 0.05$).

3.1.3. Amino acids and in vitro protein digestibility (IVPD)

The results revealed significant increase and higher concentration of essential amino acids (EAA) (threonine, valine, leucine, tyrosine + phenylalanine, and lysine) after EB irradiation of lotus seeds, compared to the FAO/WHO (1991) reference pattern (Tables 3 and 4). Unlike legumes, lotus seeds possess adequate sulfur amino acids (methionine + cysteine), comparable to the FAO/WHO (1991) and soybean reference patterns (Bau et al., 1994). These results indicate the efficacy of EB irradiation in maintaining the nutritional potential of lotus seeds.

Although the IVPD decreased on irradiation, it was only significant at 30 kGy ($p < 0.05$) (Table 4). The EAA score showed a significant increase ($p < 0.05$) at high doses, while protein digestibility corrected amino acid score (PDCAAS) significantly decreased after EB irradiation ($p < 0.05$).

3.1.4. Fatty acid composition

Unsaturated fatty acids constituted the major portion (oleic acid, linolelaidic acid, elaidic acid and linolenic acid) of the lotus seed flour (Table 5). All the saturated fatty acids detected showed decline on EB irradiation. Linoleic acid, which was absent in raw seeds, showed a dose-dependent increase (5 kGy, 2.95 mg g^{-1}; 30 kGy, 7.64 mg g^{-1} lipid). One of the major antinutritional fatty acids, behenic acid (Fernando & Bean, 1985) was totally absent at 5 kGy.

Table 3
Amino acid composition of lotus seeds treated with electron beam irradiation (mg 100 mg^{-1} crude protein) (mean of 3 independent determinants)

Amino acid	Irradiation dose (kGy)							FAO/WHO pattern[a]	Soybean[b]
	0	2.5	5	7.5	10	15	30		
Glutamic acid	20.30	19.42	18.80	18.48	18.20	21.00	23.43		16.9[c]
Aspartic acid	9.89	9.47	9.30	9.10	8.97	10.20	11.29		11.3[d]
Serine	5.78	5.58	5.46	5.40	5.33	6.08	6.86		5.67
Threonine	3.26	3.13	3.07	3.05	3.01	3.42	3.80	3.4	3.76
Proline	3.00	2.88	2.87	2.86	2.86	3.20	3.63		4.86
Alanine	4.14	3.94	3.91	3.89	3.86	4.40	4.86		4.23
Glycine	4.51	4.33	4.30	4.26	4.19	4.80	5.31		4.01
Valine	4.43	4.32	4.23	4.21	4.13	4.72	5.30	3.5	4.59
Cystine	1.03	1.02	1.00	1.01	1.10	1.21	1.43	2.5[e]	1.70
Methionine	1.35	1.11	1.22	1.25	1.27	1.40	1.52		1.22
Isoleucine	3.60	3.48	3.39	3.38	3.32	3.79	4.25	2.8	4.62
Leucine	6.09	5.86	5.74	5.71	5.61	6.40	7.11	6.6	7.72
Tyrosine	3.33	3.17	3.12	3.10	3.07	3.53	3.96	6.3[f]	1.24
Phenylalanine	3.94	3.86	3.74	3.73	3.66	4.19	4.71		4.84
Tryptophan	ND	ND	ND	ND	ND	ND	ND	1.1	3.39
Lysine	5.33	5.14	5.08	5.01	5.00	5.70	6.30	5.8	6.08
Histidine	2.76	2.43	2.40	2.34	2.33	2.55	3.07	1.9	2.50
Arginine	8.69	8.30	8.19	8.13	8.07	9.06	10.80		7.13

ND, Not detectable.
[a] FAO/WHO (1991) pattern.
[b] Bau et al. (1994).
[c] Glutamic acid + glutamine.
[d] Aspartic acid + asparagine.
[e] Methionine + cystine.
[f] Tyrosine + phenylalanine.

Table 4
Essential amino acid score, in vitro protein digestibility ($n = 5$, mean ± SD) protein digestibility corrected amino acid score of lotus seeds treated with electron beam irradiation (mean of three independent determinations)

Parameter	Irradiation dose (kGy)						
	0	2.5	5	7.5	10	15	30
Essential amino acid score							
Threonine	95.9	92.1	90.3	89.7	88.5	101	112
Valine	127	123	121	120	118	135	151
Cystine + methionine	95.2	85.2	88.8	90.4	90.0	104	118
Isoleucine	129	124	121	119	135	135	152
Leucine	92.3	88.8	87.0	86.5	85.0	97.0	108
Tyrosine + phenylalanine	115	112	109	108	97.3	123	138
Lysine	91.9	88.6	87.6	86.4	86.2	98.3	109
Histidine	145	128	126	123	123	134	162
In vitro protein digestibility (%)							
Digestibility	43.0 ± 1.02	41.4 ± 4.06	39.8 ± 2.11	39.8 ± 0.94	39.8 ± 3.98	40.5 ± 3.68	24.1 ± 1.74[a]
Protein digestibility corrected amino acid score (%)							
Threonine	41.3	38.1	36.0	35.7	35.2	40.8	26.9
Valine	54.5	51.1	47.9	47.9	47.0	54. 6	36.5
Cystine + methionine	41.0	35.3	35.4	36.0	37.7	42.3	28.4
Isoleucine	55.3	51.5	48.2	48.1	47.2	54.8	36.6
Leucine	39.7	36.8	34.6	34.5	33. 8	39.3	26.0
Tyrosine + phenylalanine	49.6	46. 2	43.4	43.2	42.5	49.6	33.2
Lysine	39.5	36.7	34.9	34.4	34.3	39.8	26.2
Histidine	62. 5	53.0	50.3	49.0	48. 8	54.4	39.0

[a] Calculated according to FAO/WHO (1991) pattern.

3.2. Antinutritional features

The highest impediment to consume any wild or underutilised seeds is the presence of antinutritional factors, particularly those which are heat-stable and difficult to eliminate on processing (Liener, 1994; Nowacki, 1980). These antinutritional factors decrease the digestibility and bioavailability of nutrients in the intestine. Most of the methods used to deactivate the antinutrients (e.g., dry heating, cooking, roasting, germination, fermentation) need not necessarily reduce or completely eliminate antinutrients, instead some methods reduce the nutritive value of plant produce.

3.2.1. Phenolics and tannins

Total phenolics and tannins of EB-irradiated lotus seeds revealed a significant dose-dependent decrease ($p < 0.05$) compared to the control (Table 6). Decrease in total phenolics was significant at all of the irradiation doses (control, 4.00%; 30 kGy, 1.30%), while tannins were reduced significantly at 5 kGy onwards (control, 3.91%; 30 kGy, 2.20%).

Table 5
Fatty acid composition of flours of lotus seeds treated with electron beam irradiation (mg g^{-1} lipid) (mean of three independent determinants)

Fatty acid	Irradiation dose (kGy)						
	0	2.5	5	7.5	10	15	30
Saturated fatty acids							
Myristic acid (C 14: 0)	0.10	0.10	0.04	0.08	0.08	0.09	0.17
Pentadecanoic acid (C15:0)	0.01	0.01	0.01	0.02	0.02	0.03	0.03
Palmitic acid (C16:0)	4.20	2.11	2.32	2.34	2.56	2.57	2.47
Heneicosanoic acid (C21:0)	0.10	0.10	–	–	–	–	–
Behenic acid (C22:0)	0.64	0.63	–	–	–	–	–
Lignoceric acid (C24:0)	–	–	–	0.01	0.01	0.01	0.03
Polyunsaturated fatty acids							
Myristoleic acid (C14:1)	0.01	0.01	0.01	0.03	0.03	0.04	0.01
Elaidic acid (C18:1)	2.16	1.16	0.16	–	–	–	
Oleic acid (C18:1)	7.04	6.32	5.82	1.14	–	–	–
Linoleic acid (C18:2)	–	–	2.95	6.12	7.00	7.63	7.64
Linolelaidic acid (C18:2)	6.00	5.89	4.05	3.14	2.20	1.82	1.43
Linolenic acid (C18:3)	1.43	1.42	–	–	–	–	–
Sum of saturated fatty acids	5.05	2.95	2.37	2.44	2.44	2.70	2.70
Sum of polyunsaturated fatty acids	19.50	17.74	15.36	12.87	11.67	12.19	11.78
P/S ratio[a]	3.86	6.01	6.48	5.27	4.78	4.51	4.36

–, Not detectable.
[a] Ratio of polyunsaturated/saturated fatty acids.

Table 6
Antinutritional features of raw and electron beam irradiated lotus seeds (on dry weight basis) ($n = 5$, mean ± SD)

Antinutrients	Irradiation dose (kGy)						
	0	2.5	5	7.5	10	15	30
Total phenols (mg 100 mg^{-1})	4.00 ± 0.20	3.31 ± 0.01[a]	3.00 ± 0.30[a]	2.90 ± 0.01[a]	2.60 ± 0.02[a]	1.48 ± 0.20[a]	1.30 ± 0.10[a]
Tannins (mg 100 mg^{-1})	3.91 ± 0.57	3.81 ± 0.28	3.68 ± 0.30[a]	3.47 ± 0.08[a]	3.40 ± 0.10[a]	2.23 ± 0.13[a]	2.20 ± 0.88[a]
Phytic acid (mg 100 mg^{-1})	0.37 ± 0.01	0.35 ± 0.04	NP	NP	NP	NP	NP

NP, Not present.
[a] Significantly different from control ($p < 0.05$).

Aw & Swanson (1985) have reported that tannins adversely affect the nutritive value of black beans by decreasing the proteolytic enzymes' digestibility. However, epidemiological studies have shown that polyphenolic compounds possess rich antioxidant properties and are effective in reducing cardio-cerebrovascular diseases and cancer mortality (Hertog, Sweetnam, Fehily, Elwood, & Kromhout, 1997). Hence, retention or elimination of phenolic compounds will entirely rest on the consumer's needs.

3.2.2. Phytic acid

Phytic acid was completely eliminated on EB irradiation at 5 kGy. Similar observations have been made earlier, wherein antinutrients were reduced after exposure to ionising radiation (Bhat et al., 2007). The degradation of phytic acid by radiation can be attributed to cleavage in the structure of phytic acid or to the formation of inositol and inositol phosphates' due to the action of free radicals generated during irradiation (De Boland, Garner, & O'bell, 1975). As a physical process, EB irradiation definitely has an upper hand against conventional methods in reduction of antinutrients while having a low impact on nutritional qualities.

3.3. Functional properties

3.3.1. Water and oil absorption capacities

Significant dose-dependent decline in the WAC was witnessed on EB irradiation (control, 2.90 ml g^{-1}; 30 kGy, 2.23 ml g^{-1}) ($p < 0.05$), while OAC significantly increased

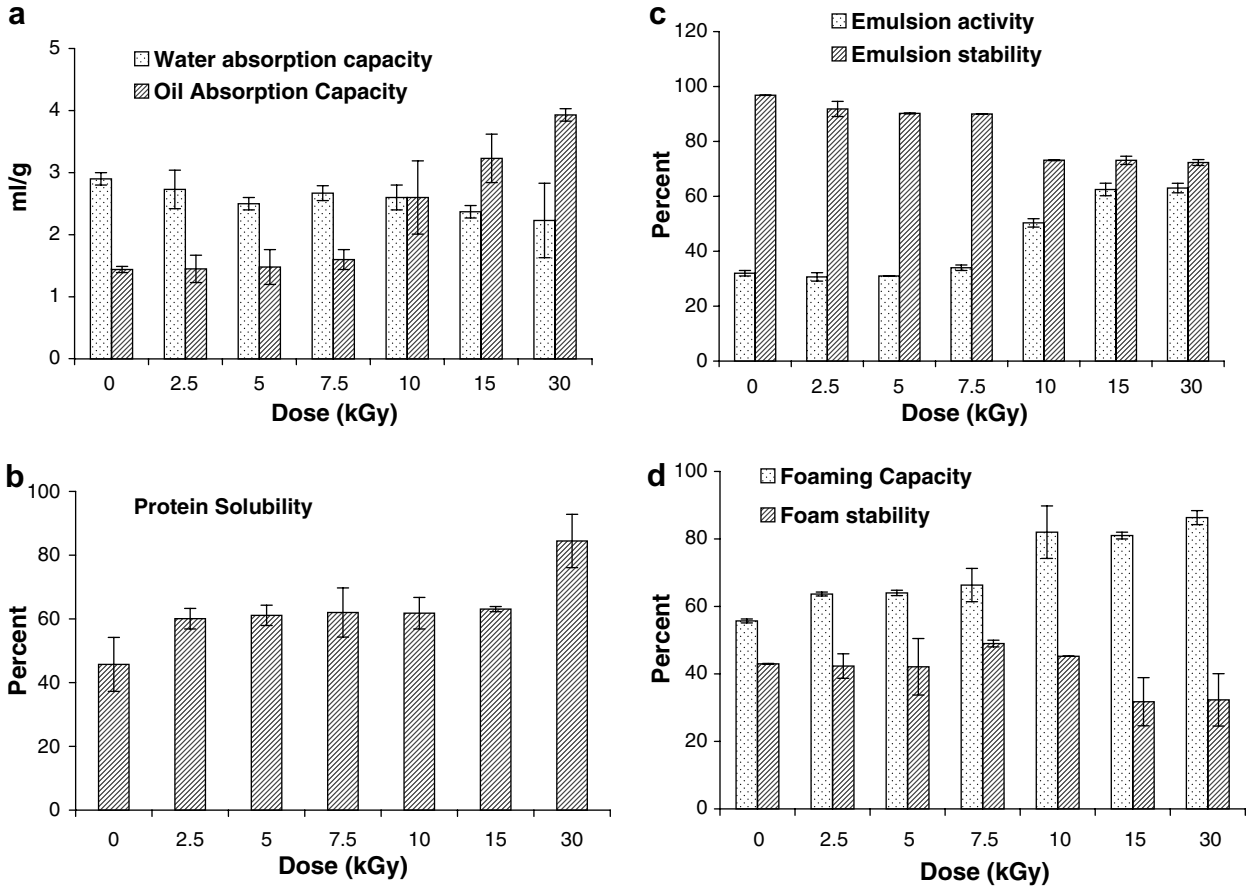

Fig. 1. Functional properties of raw and electron beam irradiated lotus seeds: (a) water and oil absorption capacity; (b) protein solubility; (c) emulsion activity and stability and (d) foaming capacity and foam stability.

from 10 kGy onwards (control, 1.44 ml g^{-1}; 30 kGy, 3.93 ml g^{-1}) ($p < 0.05$) (Fig. 1). Retention of liquid in seed flours is an index indicating the ability of protein to absorb and retain water and/or oil, which in turn influences the texture and mouthfeel of foods particularly in comminuted meats, meat analogues and baked products (Okezie & Bello, 1988). Retention of liquid can also be attributed to dietary fibre in the seed flours. Similarly, OAC influences how the food acts as a flavour retainer and hence affects the palatability of foods (Kinsella, 1976).

3.3.2. Protein solubility

Proteins in seed flours significantly influence the functional properties (oil and water absorption capacity, protein solubility, emulsification and foaming) (Kinsella, 1979; Kerr, Ward, Mc Watters, & Resurreccion, 2000) (Fig. 1). Results on the protein solubility of lotus seeds, as a result of EB irradiation, showed significant increases from 5 kGy onwards (control, 45.7%; 5–30 kGy, 61.1–84.5%) ($p < 0.05$). Such an increase due to irradiation can be attributed to higher protein extraction from the seed matrix.

3.3.3. Gelation capacity

Significant improvement of least gelation concentration (LGC) was seen on EB irradiation dose 5 kGy onwards (control, 16%, 30 kGy, 4%) ($p < 0.05$). Such a decrease in LGC might be attributed to increased interaction of proteins with water. Improvement in gelation property is beneficial and enhances the utility of lotus seed flour in preparation of food products like custards, ice creams, sausages and other bakery products.

3.3.4. Emulsion properties

Emulsion capacity usually denotes the maximum amount of oil that can be emulsified by protein dispersion, while emulsion stability denotes the ability of an emulsion with certain composition to remain unchanged (Enujiugha, Badejo, Iyiola, & Oluwamukomi, 2003). The emulsion activity of lotus seed flour revealed dose-dependent increase in irradiated samples, which was significant at 10 kGy onwards (control, 32.0%; 30 kGy, 63.0%) ($p < 0.05$) (Fig. 1). Comparatively high emulsion capacity values denote the high desirability of lotus flour for preparing comminuted meats. EB irradiation caused a significant decrease in emulsion stability at 5 kGy onwards (control, 96.8%; 30 kGy, 72.3%) ($p < 0.05$). This might be attributed to changes in protein aggregation, as well as surface hydrophobicity, which significantly influence the emulsifying capacity.

3.3.5. Foaming properties

A significant increase in the foaming capacity of lotus seed flour due to EB irradiation was seen at 7.5 kGy onwards (control, 55.7%; 30 kGy, 86.3%) ($p < 0.05$), while the foam stability decreased, but not significantly. The decline in foam stability can be attributed to the denaturation and dissociation of the proteins. Foaming properties of lotus seed flours may be improved by addition of sodium chloride, as it enhances the ionic strength of water and solubilised protein.

4. Conclusions

The present study reveals that lotus seeds constitute a rich source of nutrients, with the presence of minimal amounts of antinutrients, and can be successfully utilised as an important source of protein and carbohydrates for humans and livestock. The high amount of unsaturated fatty acids in lotus seed fat might form a suitable substitute or alternative to highly unsaturated oils. The seed flours exhibited better functional properties than many of the conventional grains or legumes, indicating the potential for addition to food systems, especially bakery products, as nutrient supplements as well as functional agents. A specific dose of electron beam irradiation can be chosen depending on the technical criteria to be achieved (e.g., increase in protein, improvement in gelation capacity, decrease in antinutrients). Electron beam irradiation as a physical method of preservation proved its efficacy in maintaining the overall quality of lotus seeds.

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