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Influence of gamma irradiation on the nutritional and functional properties of pigeon pea (*Cajanus cajan*) flour

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Effects of gamma irradiation on pigeon pea flour at various doses (0, 5, 10, 15 and 20 kGy) on the proximate composition and functional properties were investigated. Gamma irradiation resulted in a slight increase of crude protein at all doses and the crude lipid. Crude fibre and ash showed no apparent effects of irradiation between the non-irradiated sample and the irradiated. A significant increase of *in vitro* protein digestibility was observed in pigeon pea flour irradiated at 20 kGy. Phytic acid, a major anti-nutritional factor, was reduced significantly on irradiation, indicating the positive effect of gamma irradiation on pigeon pea flour. Significant enhancement in the water absorption and swelling capacities, bulk density of the flour was also recorded after irradiation. Results of the present investigation showed that application of gamma irradiation does not have major effect on the overall nutritional composition and functional properties of the flour and can be used as an effective method of preservation of pigeon pea flour and their products.

Key words: Pigeon pea flour, gamma irradiation, functional properties, anti-nutritional factors.

INTRODUCTION

Legumes play a major role in overcoming protein-energy malnutrition in developing countries, where scarcity of animal proteins prevails. Edible legumes fulfil the basic nutritional requirements as they are rich in proteins and other nutritional components such as essential minerals, unsaturated fatty acids and vitamins. However, the extent of production of legumes has failed to keep pace with the demands of ever-increasing populations (Ali and Kumar, 2000). The scarcity of fertile land and an over dependence on cereal-based food products have also aggravated the protein deficiency problems in humans (Sadik, 1991). To meet the ever-increasing protein demand, the exploitation of nonconventional seeds has become inevitable (Bhat et al., 2009). Such explorations may assuage the problems of food security, agricultural development, self-dependence and enhancement of the economy of developing countries. The little known pigeon pea seed (*Cajanus cajan*) and its flour might significantly contribute to world food production due to its wide distribution and adaptability to adverse environmental conditions. Nutritionally, pigeon pea seeds have been reported to be rich in proteins and the important amino acids such as methionine, lysine and tryptophan. The protein content is comparable with those in other legumes like cowpea and groundnut which have been used in complementing maize. It is rich in mineral quality and fibre content (Nene et al., 1990; El-Tabey, 1992). Pigeon pea grows well in Nigeria but is easily attacked by weevils; hard-to-cook phenomenon and the presence of anti-nutrients have limited its utilization. Irradiation pro-

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cessing of plant product 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 micro flora (Diehl, 1995). Radiation processing has proved to be an effective means of decontamination and disinfestations of food and agricultural products (Anonymous, 1991; Loaharanu, 1994). Irradiation treatment itself or in combination with other processing methods has been shown to reduce or eliminate some of the anti-nutrients in cereals and legumes (Farag, 1989; Sattar et al., 1990).

Ionizing radiations have also been proved effective in improving the overall nutritional attributes, including some desired changes in functional properties of seed flours (Rahma and Mostafa, 1988; Dario and Salgado, 1994; Dogbevi et al., 2000). A joint expert committee convened by the FAO/IAEA/WHO stated that irradiation of any food commodity up to 10 kGy presents no toxicological hazard (Anonymous, 1991). Subsequently, a joint FAO/IAEA/WHO study group reviewed the toxicological, nutritional and radiation-induced chemical and physical aspects of irradiated foods above 10 kGy and concluded that application of ionizing radiation at 10 kGy or higher doses will be safe and nutritionally adequate (WHO, 1999). To fill the existing gap in the knowledge on the effects of γ-rays (low and high dose) on the nutritional potential of pigeon pea seeds, the current study was aimed to investigate the nutritional, anti-nutritional compositions and the functional properties of raw and irradiated pigeon pea flour. The results of this study will be useful to popularize pigeon pea flour for the successful exploitation of its nutritional value in the production of safe and inexpensive food products.

MATERIALS AND METHODS

Pigeon pea was purchased at Odori market in Iwo, Osun State and gamma irradiated at Energy Centre in Abuja at different doses (5, 10, 15 and 20 kGy). The samples were exposed to gamma rays generated by a cobalt-60 source (Gammacell 220, MDS Nordion, Ottawa, Canada) following the procedures described by Helinski et al. (2008) with a dose rate of ca. 2 Gy/min at 25°C and normal relative humidity. Double side irradiation (exposure to both sides) was performed for uniform dose delivery. A dosimetry system was used to measure the dose received by the batch based on the Gafchromic HD-810 film (International Specialty Products, NJ, USA; FAO/IAEA/USDA, 2003). Three dosimeters were included with each batch of pigeon pea seeds and read after irradiation with a radiachromics reader (Far West Technology Inc., CA, USA). All experiments were repeated 3 times and 3 replicates of each sample type were irradiated. The irradiated samples were milled with hammer mill and various analyses were carried out on it.

Proximate composition

The level of moisture, crude protein, crude fat, ash, crude fiber and carbohydrates of the irradiated (5, 10, 15, and 20 kGy) and non-irradiated pigeon pea flours were determined by the use of manual methods as outlined by AOAC (2000).

Determination of *in vitro* protein digestibility (IVPD)

IVPD was carried out according to the method described by Monjula and John (1991) with a minor modification. A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1 mg pepsin in 15 ml of 0.1 N HCl at 37°C for 2 h. The reaction was stopped by the addition of 15 ml 10% trichloroacetic acid (TCA). The mixture was then filtered quantitatively, through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-Kjeldahl method (AOAC, 2000). Digestibility was estimated by using the following equation:

$$\text{IVPD } \% = \frac{\text{N in Supernatant} - \text{Enzyme N}}{\text{Nin sample}} \times 100$$

Anti-nutrition determination

Determination of tannin content

The tannin content of the irradiated and non-irradiated samples was determined using the International Standardisation Organisation method (ISO, 1988). Tannic acid was used as a standard and the results were calculated as percentage tannic acid equivalents.

Determination of phytic acid content

The method reported by Reddy et al. (1982) was used for phytic acid and phytate-phosphorus determinations. Phytic acid was extracted from each 3 g flour sample with 3,5 trichloroacetic acid by shaking at room temperature followed by high-speed centrifugation (30, 000 xg for 5 min). The phytic acid in the supernatant was precipitated as ferric phytate, and iron in the sample was estimated. Phytate-phosphorus was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio according to method 970.39 (AOAC, 2000). The phytic acid was estimated by multiplying the amount of phytate-phosphorus by the factor 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$

Determination of trypsin inhibitor

Trypsin inhibitor was determined using the method described by Arntfield et al. (1985). The samples were extracted by weighing 1.000 g of the sample and dissolving it in 50 ml of 0.5 M NaCl solution. The mixture was stirred for 30 min at room temperature and then centrifuged. The supernatant was filtered through Whatman No. 41 filter paper. The filtrate (extract) was used for the determination. Trypsin solution (20 ml) was added to 10 ml of the substrate in a test tube or beaker. A blank (10 ml) of distilled water was prepared. The content of the test tube was allowed to stand for at least 5 min after which its absorbance was measured at a wavelength of 410 nm. Trypsin activity was expressed as number of trypsin unit inhibited (TUI) per unit weight (g) of the sample analysed. Thus,

$$\text{TUI/mg} = \frac{\text{absorbance} \times 0.01\text{F}}{\text{Absorbance of standard}}$$

$$\text{TUI/mg} = \frac{b-a \times F}{0.01}$$

Where b = absorbance of sample, a = absorbance of blank, F = experimental factor.

$F = 1/w \times V_f / Va \times D$

Where w = weight of sample, V_f = total volume of extract, Va =

Table 1. Proximate composition of irradiated pigeon pea flour prior to storage (dry weight basis).

Proximate composition (g/100 g)	Irradiation dose (kGy)				
	0	5	10	15	20
Moisture	7.99 ± 0.120^{d}	7.95±0.220^{cd}	7.54±0.130^{c}	6.84±0.160^{b}	6.22±0.120^{a}
Protein	19.37±0.590^{a}	20.49±0.500^{ab}	21.01±1.010^{bc}	21.96±0.460^{cd}	22.99±0.650^{d}
Crude fat	1.88±0.010^{a}	2.04±0.010^{b}	1.95±0.010^{b}	3.90±0.010^{e}	3.87±0.010^{d}
Crude fibre	5.59±0.153^{e}	3.52±0.012^{a}	3.70±0.010^{b}	3.90±0.044^{d}	3.77±0.010^{c}
Ash	3.62±0.006^{b}	3.47±0.012^{a}	3.59±0.015^{d}	3.46±0.010^{a}	3.55±0.015^{c}
Carbohydrate	61.55±0.015^{b}	62.53±0.010^{d}	62.21±0.010^{c}	59.94±0.015^{a}	59.60±0.025^{a}

Values are means and standard deviation of triplicate of three samples (n = 9); Means followed by the same letter within the same row are not significantly different (p>0.05).

volume of extract used, D = dilution factor (if any).

Functional properties determination

Water absorption determination

The water absorption was determined by modified centrifuge method of Lin and Zayas (1987). Each sample (2.0 g) was transferred into lagged 50 ml centrifuge tube and weighed (W_1). Exactly 30 ml of hot distilled water (70°C) was added to each sample and at the same time washing down the inside of the centrifuge tubes using a glass stirring rod, the sample and the water was mixed for 30 min. The suspension was allowed to rest for 10 min; the flour adhering to the side of the centrifuge was scrubbed down with glass rod to prevent it from drying. Additional 10 ml of hot distilled water was used to wash the sample adhering to the stirring rod into the sample. The suspension was centrifuged at 1165 g for 25 min at 50°C. The tube was cooled in desiccator and weighed (W_2).

$$\text{Water absorption} = \frac{W_2 - W_1\,(\text{ml})}{\text{Weight of the sample (g)}}$$

Bulk density determination

Bulk density was determined using the gravimetric method as described by Okaka and Potter (1976). The sample (10 g) was weighed into a 25 ml graduated cylinder. The cylinder was gently tapped ten times against the palm of the hand. The bulk density was expressed as the sample per volume occupied by the sample.

Swelling capacity determination

Swelling capacity of the samples were determined by modification of Lin and Zayas (1987) method. Each sample (2 g) was dispersed in 40 ml distilled water. The resultant slurry was heated at a temperature of 70°C for 30 min in a water bath, cooled to room temperature and centrifuged at 598 xg for 30 min. The supernatant liquid was decanted and the centrifuge tube was placed in a hot air oven and dried for 25 min at 50°C. The residue was weighed (W_2). Centrifuge tube containing sample alone was weighed prior to adding distilled water (W_1).

$$\text{Swelling capacity} = \frac{W_2 - W_1\,(\text{ml})}{\text{Weight of the sample (g)}}$$

Statistical analysis

All experiments were carried out in triplicate using three samples at each replicate. Mean and standard deviation were calculated for each treatment. Data obtained from the phyisco-chemical results were subjected to analysis of variance (ANOVA) and the means were separated by lowest standard deviation test (SPSS version 16, 2008). Significant level was accepted at 5%.

RESULTS AND DISCUSSION

Proximate composition of irradiated pigeon pea flour (dry weight basis)

The proximate composition of raw and gamma-irradiated pigeon pea flour is presented in Table 1. The moisture content of pigeon pea flour was not substantially affected by gamma irradiation treatment. Similar findings were observed by Rady et al. (2002) who reported that gamma irradiation has no real effect on moisture content of oil seeds. Crude protein and carbohydrate constitute the major chemical constituents of the seeds. Gamma irradiation resulted in a moderate increase (p>0.05) in crude protein at all the irradiated doses (control, 19.37%; 20 kGy, 22.99%). The protein content is comparable with the values reported by Faris et al. (1987). The carbohydrates constitute over 50% of the pigeon pea flour constituents, probably due to low lipid content. Irradiation did not significantly alter the carbohydrates (p>0.05) and was slightly decreased at all doses except for 5 and 10 kGy. Stability of carbohydrates in pigeon pea flour on irradiation is advantageous as carbohydrates contribute to the calorific value as well as acting as an anti-marasmus in infant nutrition (Vadivel and Janardhanan, 2002). The crude fat on irradiation showed a dose-dependent increase which was significant at doses 15 and 20 kGy and this may be due to higher dose of irradiation. These results are in agreement with previous finding of Abu et al. (2005) who reported that at low dose irradiation (2 kGy); the oil absorption capacity of cow pea flour was not affected. However, an increase in oil absorption capacity was observed at higher doses (10 and 50 KGys); this may

Table 2. Functional properties of irradiated pigeon pea flour prior to storage (dry weight basis).

Functional properties	Irradiation dose (kGy)				
	0	5	10	15	20
Water absorption	1.88±0.020^{a}	1.89±0.150^{a}	1.96±0.020^{b}	2.04±0.010^{c}	2.06±0.010^{c}
Swelling capacity	2.31±0.010^{b}	2.60±0.010^{c}	2.67±0.010^{d}	2.29±0.020^{a}	2.29±0.020^{a}
Bulk density	50.31±0.015^{ab}	50.20±0.010^{a}	50.22±0.015^{ab}	50.34±0.025^{b}	50.27±0.015^{ab}

Values are means and standard deviation of triplicate of three samples (n = 9); Means followed by the same letter within the same rows are not significantly different (p>0.05).

Table 3. Effect of gamma irradiation on anti-nutritional factors and *in-vitro* protein digestibility of pigeon pea flour.

Anti-nutritional factors (mg/100 g)	Irradiation dose (kGy)				
	0	**5**	**10**	**15**	**20**
Phytic acid	94.67±1.522^{e}	83.33±0.575^{d}	50.67±5.125^{c}	41.00±1.725^{b}	25.00±0.000^{a}
Tannin	2.47±0.050^{d}	2.27±0.020^{c}	1.43±0.055^{b}	0.83±0.055^{a}	0.50±0.000^{a}
Trypsin inhibitor	47.67±2.520^{d}	32.33±2.520^{d}	23.67±1.525^{c}	16.33±1.525^{b}	13.00±1.725^{a}
IVPD (%)	60.67±1.160^{a}	64.00±1.730^{b}	69.00±1.000^{c}	72.33±1.530^{d}	75.00±2.000^{e}

Values are means and standard deviation of triplicate of three samples (n = 9); Means followed by the same letter within the same row are not significantly different (p>0.05).

be due to exposure to non-polar protein site.

Crude fibre and ash on irradiation showed no apparent effect except that between non- irradiated sample and this was not in line with the report of Bhat et al. (2008) who reported a decrease in crude fibre and ash content of velvet bean seed. Low crude fibre is nutritionally valued as it traps less proteins and carbohydrates (Balogun and Fetuga, 1986). The dose-dependent decrease in fibre on irradiation has been attributed to depolymerization and delignification of the plant matrix (Bhat et al., 2008).

Functional properties of irradiated pigeon pea flour (dry weight basis)

Table 2 shows the result of functional properties of irradiated pigeon pea flour. Irradiation at 10 kGy and above showed a slight increase in water absorption capacity. Similar result was reported by A/Azim et al. (2009) which reported no apparent effect of gamma irradiation on water absorption capacity of groundnut flour. Seed flours with high water absorption capacity are suitable for production of soups and gravies (Oshodi and Adeladun, 1993). As polar amino acids are responsible for binding of water molecules by proteins, differences in water absorption capacity suggest variations in the polar amino acid composition in pigeon pea flour. Also, irradiation at 5 kGy and above showed a significant increase and a better swelling capacity except that of 15 kGy which showed a slight decrease. There was no significant difference in bulk density. This result was in line with the report of A/Azim et al. (2009) which reported that, the bulk density of ground nut flour was not affected significantly by gamma irradiation.

Functional properties of seed flours assume importance for the development of food products. Acceptability of legume flour depends entirely on the nutritional value as well as functional properties (Pour-El, 1981). Physicochemical changes in proteins influence functional properties such as the water absorption capacity, oil absorption capacity, protein solubility and foaming properties in cowpea products (Krer et al., 2000).

Effect of gamma irradiation on anti-nutritional factors

Table 3 present the result of effect of gamma irradiation on anti-nutritional factors in pigeon pea. Phytate, tannin and trypsin inhibitor in the samples decrease with increase in irradiation dose. Similar trend was reported by El-Niely (2007) that radiation processing significantly ($P \leq 0.05$) reduced the levels of phytic acid and tannins of legumes. Also, Bhat et al. (2009) who irradiated lotus seed at gamma dose range of 0 to 30 kGy. 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 phytic acid (Bhat et al., 2009). Phytic acid in legumes has been reported to lower the nutritional value by limiting the bioavailability of minerals and essential elements (Gustafsson and Sandbreg, 1995). Tannin concentration of raw pigeon pea is low compared to trypsin inhibitor and phytic acid concentration. Tannin is said to be present in the seed coat of legumes and pre-processing operation of the seed can result into reduction in tannin content (Gurumoorthi et al., 2003).

Effect of gamma irradiation on *in-vitro* protein digestibility

The *in-vitro* protein digestibility of pigeon pea flour shows a dose dependent increase (Table 3). The result is similar to that of Rehman and Shah (2001) that the removal of tannin and phytate create a large space within the matrix of flour which increase the susceptibility to enzymatic attack and consequently improve the digestibility of protein after radiation treatment. Bhat et al. (2008) also reported an increase in *in-vitro* protein digestibility of velvet beans seed due to decrease in some of the anti-nutritional factors such as phytates, trypsin and chymotrypsin inhibitors present in the seed; the analysis showed a significant difference ($p<0.05$).

Conclusion

The results of the present investigation showed that pigeon pea flour is a valuable source of nutrition due to high proteins and carbohydrates. The seed flour exhibited good functional properties, which will be of immense value in food industries in production of quality food formulations. Application of gamma irradiation as a method of preservation of pigeon pea flour quality did not show any adverse effect on the nutritional composition. However, it led to improvement of some of the functional properties (for example, water absorption and swelling of the flour) which is advantageous in food manufacturing/production.

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