

Effect of Gamma Irradiation on Amino Acids profile, Minerals and Some Vitamins

Content in Pigeon Pea (*Cajanus Cajan*) Flour

Abstract

This study was aimed at checking the affect of gamma irradiation on amino acids profiles, minerals and some vitamins content of pigeon pea (*Cajanus cajan*) flour. The pigeon pea was gamma irradiated at difference gamma doses of 5, 10, 15, & 20 kGy, after which the effect of all these doses were examined on amino acids profile, minerals and some vitamins present in flour. The result showed a significant reduction of amino acids present in the flour at $p < 0.05$, but the gamma irradiated flour still meet the Food and Agricultural Organization-World Health Organization recommended pattern and amount of essential amino acids. The effect of gamma irradiation was not significant on the mineral content of the samples with Sodium content ranging from 5.95mg/100g to 7.09mg/100g and Potassium ranging from 40.83mg/100g to 40.15mg/100g. The effect of vitamin was not significant since the flour was only rich in vitamin A, C and vitamin B3. Vitamin A content of the samples ranged from 458.95 to 463.83mg/100g, Vitamin C ranged from 18.69 to 24.00mg/100g and Vitamin B3 ranged from 2.86 to 3.20mg/100g. In conclusion, gamma irradiation has significant effect on the amino acids profile, but no significant effect on minerals and vitamins content of the flour.

Keywords Amino acids, Irradiation, pigeon pea, flour, vitamin, minerals

Introduction

Legumes are an economically valuable source of protein in the developing countries, wherein animal proteins are scarce. Being rich in proteins, carbohydrates, calorific value, fibre, minerals and vitamins, legumes constitute staple food in many countries [1]. Exploitation of underexplored wild legumes is an important approach to combat the protein-energy malnutrition in developing countries. Pigeon pea (*Cajanus cajan*) is a locally available, affordable and under-utilized grain legume of the tropics and sub-tropics. Pigeon pea varieties have protein content in the range of 23 - 26% [2]. The protein content is comparable with those in other legumes like cowpea and groundnut which have been used in complementing maize [3]. It is rich in mineral quality and fibre content. In agricultural science and food technology, research has shed light upon new potential application for radiation. For example, high ionizing dose of radiation have been shown to prevent growth of microbial infestation in seed [4]. There are also many reports supporting the use of gamma irradiation as a fungicidal agent [5]. However, the capability of growing and sometimes the developmental process of the seedling in plant have been seriously disrupted by radiation [6, 4]. There are insufficient reports about possible effects of gamma irradiation on nutritional composition of the underutilized leguminous seeds made into flour associated with seed sterilization. Seeds of different plants that are consumed as food showed nutrient variations which are dependent on the constituents of such seed protein. Macronutrients in irradiated foods experienced little changes. And as a result the normal value of proteins, lipids and carbohydrates of such foods are maintained [7, 8]. Vitamins have varying sensitivity to radiation treatments and are the only components of foods that need to be considered from a dietary impact standpoint. Like thermal treatments, radiation processing of foods can cause some loss of vitamins. Vitamins have been found to

be affected by irradiation because vitamins presented different sensitivities regarding the treatment with ionizing energy [9]. Amino acids are very important to human health and out of the twenty standard amino acids available; eight are called essential amino acids because they cannot be synthesized by human body from other compounds at the level required for growth, so they must be obtained from food [10]. The amino acids group (-NH₂) is the most radiosensitive group of the amino acids [11, 4]. Extensive research as shown that at 10 kGy dose of irradiation, macronutrients like protein, lipids and carbohydrate content of food are relatively stable. However, the report of Lee [12] that gamma irradiation affects proteins by causing conformation changes, oxidation of amino acids, formation of protein free radicals and rupturing of covalent bonds has been established [13]. Also, chemical changes caused by gamma irradiation on protein include fragmentation, cross-linking aggregation and oxidation caused by oxygen radicals that are generated in water radiolysis [14, 15]. This effect could be related to the structure of each amino acid, simple amino acids increased upon irradiation, such as glycine, which undergo reductive deamination and de-carboxylation [16]. In addition, aliphatic amino acids with increasing chain length provide additional C-H bonds for interaction with OH radicals which reduces the extent of oxidative deamination [16]. Wang & Vonsonntage [17] reported that sulphur containing as well as aromatic amino acids are, in general, the most sensitive to irradiation, while simple amino acids could be formed by destruction of other amino acids. However, there is a scarcity of information relating to the effects of processing with ionizing energy on most underutilized legumes. Therefore, the present work was undertaken to explore the effects of irradiation on amino acids, minerals and some vitamins contents of raw and processed pigeon pea flour.

Materials and Method

Pigeon pea seeds were purchased at Odori market in Iwo, Osun State Nigeria. The gamma irradiation process was carried out 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 following the procedure by Helinski [18] 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 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, [19]). 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 with 3 replicates of each sample. The irradiated samples were milled with hammer mill and packaged inside low density polythene bags. All of the flour samples were stored in a deep freezer (-20° C) until further analysis.

Mineral analysis

Minerals (sodium, potassium, calcium, magnesium, iron, copper, zinc, manganese and selenium) of the seed flour were determined employing the AOAC [20] method on digestion of flour with a mixture of concentrated nitric acid, sulphuric acid and perchloric acid (10:0:5:2, v/v) using an atomic absorption spectrophotometer (GBC 904AA, Germany). The ascorbic acid method was used to determine the total phosphorous as orthophosphate after acid digestion and neutralization using phenolphthalein indicator and APHA, [21] combined reagent. The absorbance was read at 880nm (Spectronic 21 D, Miltonroy, NY, USA) and KH₂PO₄ (Merck, India Limited, Mumbai, India) served as the standard.

85 Amino acid analysis

86 Amino acids of the pigeon pea flour samples were assessed by the method outlined by Hofmann [22, 23].
87 A known quantity of pigeon pea flour was hydrolysed with 6 N HCl (15 ml) for 4 h at 145° C. After
88 cooling, HCl was removed in a rotoevaporator combined with a diaphragm vacuum pump (MC2C;
89 Vacuubrand GmbH, Wertheim, Germany). Internal standard, trans -4-(aminomethyl) -
90 cyclohexanecarboxylic acid was added to each sample. The discovery step consisted of esterification with
91 trifluoroacetylation [24]. Samples of standard amino acids were weighed out in reaction vials and dried
92 using CH₂Cl₂ under a gentle stream of helium and slow heating in an oil bath (40-60 ° C) to remove the
93 traces of water. A 12 ml freshly prepared acidified isopropanol (acetyl chloride, 3 ml+2-propanol, 12 ml)
94 was added and the mixture was heated at 100° C for 1 h. After cooling, the reagent was eliminated with a
95 gentle stream of helium at 60° C, followed by combined evaporation with three successive aliquots of
96 CH₂Cl₂ to remove propanol and water. The dry residue was trifluoroacetylated with 200 ml
97 trifluoroacetic anhydride overnight at room temperature. An aliquot of this solution was used without
98 further treatment for gas chromatography-combustion-isotope ratio mass spectrometry. Hewlett-Packard
99 58590 II gas chromatograph, connected through a split with a combustion interface to the IRMS system
100 were used to carry out the GC-C-IRMS/MS measurements for the isotopic determination of nitrogen and
101 through a transfer line with a mass spectrometer ((GCQ; Finnigan MAT) for qualitative analysis and
102 quantification of the amino acids. The capillary column of GC was a 50 m x 0.32 mm i.d. x 0.5 µm BPX5
103 (SGE), operating with a carrier gas flow of 1.5 ml/min with the following temperatures and pressure:
104 initial 50° C (1 min), increased to 100° C at 10° C/min (10 min), increased to 175° C at 3° C/min (10 min),
105 increased to 250° C/min (10 min); head pressure, 13 psi (90 kPa). The essential amino acid (EAA) score
106 was determined employing the formula:

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$$\text{EAA score} = [\text{EAA (mg) in 100 mg test protein}] / [\text{EAA (mg) in 100 mg FAO/}$$

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$$\text{WHO [25] reference pattern}] \times 100$$

110 Protein digestibility

111 The Akesson and Stahmann [26] method was employed to determine the in vitro protein digestibility
112 (IVPD) of pigeon pea flours (100 mg). The defatted flours were incubated (37°C, 3 h) with pepsin (3,165
113 units/mg protein; Sigma-Aldrich, St. Louis, MO, USA) (1.5 mg/2.5 ml of 0.1N HCl) followed by
114 inactivation (0.25 ml of 1 N NaOH). Incubation was continued (24 h, 37° C) with trypsin (16,100
115 units/mg protein; Sigma-Aldrich, St. Louis, MO, USA) and a-chymotrypsin (76 units/mg protein; Sigma-
116 Aldrich, St. Louis, MO, USA) (2 mg each/2.5 ml potassium phosphate buffer, pH 8.0, 0.1 M) followed
117 by inactivation (0.7 ml of 100% Trichloroacetic acid (TCA)). The inactivation of the enzyme was
118 maintained at zero-time control before addition of substrate. The supernatant of inactivation reaction
119 mixture that were centrifuged were collected. The residue was washed (2 ml, 10% TCA) and centrifuged.
120 The combined supernatant was washed twice with 10 ml diethyl ether, and the ether layer was removed
121 by aspiration. The aqueous layer was heated on a boiling water bath (15 min) to remove traces of ether
122 and the solution was made up to 25 ml with distilled water. The nitrogen content (in 5 ml aliquots) was
123 determined to calculate protein in the digest:

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$$\text{In vitro digestibility (\%)} = (\text{protein in digest}) / (\text{protein in defatted flour}) \times 100$$

126 The protein digestibility corrected amino acid score (PDCAAS) of EAA was calculated based on EAA
127 requirements for adults [25]

PDCAAS (%) = [(amino acid content in food protein) x true digestibility] / (amino acid content of reference pattern)

Vitamins Analysis

Ascorbic acid was extracted and estimated according to the method described by Sadasivam and Manickam [27]. 3 g of each sample was mixed with 25 ml. To 10 ml of the filtrate, bromine water was added drop by drop until it turned orange-yellow to remove the enolic hydrogen atom. The excess bromine was removed by blowing in the air. Filtrate was made up to 25 ml with 4 % oxalic acid and used for ascorbic acid estimation. Two ml of the extract was made up to 3 ml with distilled H₂O in a test tube. One ml of 2% 2, 4-dinitrophenyl hydrazine reagent and a few drops of thiourea were added. The contents of the test tube were mixed thoroughly. After 3 hours incubation at 37° C, 7 ml of 80% H₂SO₄ was added to dissolve the osazone crystals and the absorbance was measured at 540 nm against a reagent blank. The ascorbic acid content present in the sample was calculated by referring to a standard graph and expressed as milligrams per 100 grams of powdered samples.

For the extraction of thiamin, riboflavin and niacin, method of AOAC was followed [20]. Content present was calculated by comparison with standard curve developed using standards for vitamins, such as thiamine hydrochloride, riboflavin, and nicotinic acid, purchased from sigma St, Louis, USA.

Vitamin A in form of β -Carotene was determined using the method of AOAC [20], which involved extraction of the samples with acetone and hexane. Concentrations were determined by comparison with a standard curve developed using β -Carotene from sigma St, Louis, USA.

Statistical analysis

Each determination was carried out on three separate samples, analysed in triplicate, and the mean taken. Data obtained from the mineral analysis, amino acids profiles and vitamins analysis were assessed using analysis of variance (ANOVA) and the means were separated by lowest standard deviation (LSD) test (SPSS version 16) [28]. Significant level was accepted at 5%.

Results and Discussion

Mineral Composition

The calcium content of un-irradiated pigeon pea flour was (119 mg/100 g), potassium (38.80 mg/100 g), Zinc (28.57mg/100 g) and manganese (14.33 mg/100 g) were the major minerals in the pigeon pea flour (Table 3.1). The study showed no decrease occurred in the mineral concentrations of pigeon pea flour upon irradiation ($P < 0.05$). The moderate increase observed occurred from dose 5 kGy to that of 20 kGy except that of Zinc where the lowest value was recorded at dose 15 kGy. This result was directly opposite to the findings of Bhat [29]. They recorded a decrease in mineral content of irradiated lotus seed flour at dose 0-30 kGy. 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 [29]. Apart from this, pigeon pea 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. The mineral constituent of legumes is dependent on the soil edaphic features as well as the efficacy of mineral uptake [30]. The mineral composition of Pigeon pea can be considered to be present in adequate quantities when compared with the Recommended Dietary Allowances, which infers that any diet containing two-thirds of the Recommended Dietary Allowances is deemed as a potential source of minerals.

Amino Acids

The application of gamma irradiation did not have positive effect on the amino acids of the pigeon pea flour, it showed a significant reduction at all of the delivered doses ($P < 0.05$). In Table 3.2, the amino acids composition of the raw and gamma irradiated pigeon pea flour has been compared with the [25] reference patterns and soybeans [31]. The acidic amino acids, glutamic acid and aspartic acid, were the major amino acids present in the flour. The sulfur-amino acids cysteine and methionine gave a value of 2.38% compared with the FAO/WHO pattern of 2.5% and tyrosine and phenylalanine gave 8.78% against 6.3% of FAO/WHO. Except for tryptophan, the rest of the essential amino acids in the raw and gamma irradiated samples are in line with the Food and Agricultural Organization-World Health Organization-United Nations University (FAO-WHO-UNU) [32] pattern. The tyrosine in the raw flour was higher than soybeans (2.63 - 1.24 %), but lower in the irradiated sample compared with soybeans (1.00, 1.15 and 1.24%). The changes in the concentration of amino acids induced by irradiation may probably be due to free radicals that might be formed in association with splitting of the peptide bonds, deamination and decarboxylation reactions of amino acids followed by chains of chemical reactions forming other new radicals [33]. This finding is not in line with the report of Abdel-Ghaffar [34], who reported an increase in some amino acids content of soy flour. According to Baudoin and Maquet [35], ecological conditions are known to markedly influence the total nitrogen of seeds, which in turn affect the relative proportions of the essential amino acid. The essential amino acid 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 gamma irradiation decreased the essential amino acid of the pigeon pea flour, the irradiated seed flour still fulfilled the FAO-WHO [24] essential amino acid requirement pattern.

In vitro protein digestibility

Table 3.3 presents the EAA, IVPD and PDCAAS of raw and irradiated pigeon pea flour. Cystine and methionine were the limiting amino acids in raw flour, which further decreased on irradiation ($P < 0.05$). The IVPD of raw flour (60.67%) was significantly affected by irradiation doses. There was a dose increase in IVPD through all the irradiated samples (64 to 75%). The increase can be attributed to the degradation of proteins into fractions susceptible to enzymes or partial destruction of trypsin inhibitors. Similarly, decrease in some of the major anti-nutrients (e.g. phenols, protease inhibitors, phytates, oxalates, lectins, goitrogens) might have contributed to increase IVPD. Abu-Tarboush [36] correlated the elevated IVPD of soybean defatted flour with decreased anti-nutrients such as trypsin and chymotrypsin. The PDCAAS also revealed a significant dose-dependent decrease on irradiation ($P < 0.05$), indicating the limitation of available amino acids on digestion with enzymes.

Vitamins

Table 3.4 summarizes the β -carotene, thiamin, riboflavin, niacin and ascorbic acid content of both raw and irradiated pigeon pea flour (*Cajanus cajan*). A slight decrease in vitamin β -carotene occurred throughout the doses of irradiation and the decrease became significant in ascorbic acid at doses of 15 and 20 kGy. For un- irradiated sample of Vit A and the ascorbic acid, the values were 469.67 and 25.00mg/100g, respectively. From the result, it showed that pigeon flour is richer in β -carotene and ascorbic acid. The remaining vitamins; thiamine, riboflavin and niacin followed the same trend as the first two vitamins. The result followed the report of Tresina [37] who reported a significant decrease in vitamins (niacin and ascorbic acid) which occurred only at high doses of irradiation. The reduction in the vitamins may be due to the ionizing radiation reacts with water in the food, causing the release of electrons and the formation of highly reactive free radicals. The free radicals interact with vitamins in ways that can alter and degrade their structure and/or activity [38].

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Conclusion

The observations about amino acids profile, minerals and some vitamins content of pigeon pea (*Cajanus cajan*) flour in the studied samples tend to suggest that gamma irradiation had a significant effect on amino acids profile of the flour but the amount left still meet the recommendation of FAO/WHO. Gamma irradiation has no significant effect on the minerals and some vitamins tested for in the samples. Therefore, gamma irradiation can be applied to alleviate any problem relating to production of the flour. Moreover, irradiation process when compared to chemicals or heat treatment emerges as an attractive and healthy alternative.

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Table 3.1, Effects of Gamma Irradiation on Mineral content of Pigeon Pea Flour (mg/100g)

Dose kGy	Minerals						
	Na	K	Fe	Cu	Zn	Mn	Ca
0	6.99±0.03 ^c	38.80±0.27 ^a	8.30±0.10 ^b	4.57±0.06 ^b	28.57±0.20 ^b	14.33±0.20 ^a	119.67±0.12 ^a
5	5.95±0.05 ^a	40.13±0.06 ^b	7.07±0.06 ^a	5.17±0.15 ^{bc}	31.73±0.15 ^d	14.10±0.10 ^a	120.90±0.85 ^a
10	6.70±0.20 ^b	40.12±0.02 ^b	8.43±0.20 ^b	4.73±0.12 ^d	30.57±0.08 ^c	18.67±0.58 ^c	121.53±1.16 ^a
15	7.07±0.15 ^c	40.12±0.08 ^b	8.7±.60.58 ^{bc}	5.10±0.10 ^b	27.70±0.10 ^a	15.43±0.15 ^b	121.33±2.40 ^a
20	7.09±0.17 ^c	40.15±0.06 ^b	9.37±0.058 ^d	5.47±0.06 ^d	33.30±0.10 ^e	18.53±0.12 ^c	122.13±2.16 ^a

Values are means and standard deviation of three determinations (n=3)
Means followed by the same letter within the same column are not significantly (p>0.05) different according to LSD test

339 Table 3.2 Amino acid profile of Pigeon pea flour treated with gamma irradiation (mg/100 mg crude
340 protein).

Amino Acids	Dose					FAO/WHO Pattern ^a	Soybean ^b
	Control	5 kGy	10 kGy	15 kGy	20 kGy		
Glutamic acid	9.23	5.06	4.97	4.88	4.82		16.9 ^c
Aspartic acid	11.56	6.59	6.44	6.37	6.34		11.3 ^d
Serine	3.59	2.49	2.47	2.36	2.32		5.67
Threonine	3.12	2.41	2.41	2.32	2.30	3.4	3.76
Proline	3.17	2.22	2.20	2.18	2.16		4.86
Alanine	3.79	2.86	2.81	2.75	2.65		4.23
Glycine	3.07	2.17	2.15	2.06	2.02		4.01
Valine	5.85	3.45	3.42	3.40	3.35	3.5	4.59
Cystine	1.19	0.12	0.10	0.08	0.06	2.5 ^e	1.70
Methionine	1.19	0.08	0.04	0.02	0.01		1.22
Isoleucine	3.47	2.47	2.40	2.35	2.30	2.8	4.62
Leucine	6.78	4.50	4.44	4.32	4.30	6.6	7.72
Tyrosine	2.63	1.15	1.05	1.03	1.00	6.3 ^f	1.24
Phenylalanine	6.15	4.01	3.98	3.88	3.78		4.84
Tryptophan	ND	ND	ND	ND	ND	1.1	3.39
Lysine	7.79	5.72	5.45	5.20	5.15	5.8	6.08
Histidine	3.66	2.12	2.09	2.09	2.07	1.9	2.50
Arginine	5.86	3.89	3.76	3.54	3.43		7.13
Total amino acid	82.10	51.31	50.18	48.83	48.06		
Total EAA	41.83	29.92	29.14	28.23	27.75		

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342 Notes: Data presented as the mean, n=3. ND, not determined. ^aFAO/WHO (1991). ^bBau et al. (1994).
343 ^cGlutamic acid_glutamine. ^dAspartic acid_asparagine. ^eMethionine_cystine. ^fPhenylalanine_tyrosine.
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345 Table 3.3 Essential amino acid score and protein digestibility corrected amino acid score ^a of gamma-
346 irradiated Pigeon pea flour.

EAA	Dose				
	Control	5 kGy	10 kGy	15 kGy	20 kGy
EAA Score					
Threonine	91.76	70.88	70.88	68.24	67.64
Valine	167.14	98.57	97.71	97.14	95.57
Cystine + Methionine	95.20	8.00	5.60	4.00	2.80
Isoleucine	123.93	88.21	85.71	83.93	82.14
Leucine	102.72	68.18	67.27	65.46	65.15
Tyrosine + Phenylalanine	139.37	81.91	79.84	77.94	75.87
Lysine	134.31	98.62	93.97	89.66	88.79
Histidine	192.63	111.58	110.00	110.00	108.95
IVPD (%)	60.67±1.60 ^a	64.00±1.73 ^b	69.00±1.00 ^c	72.33±1.53 ^d	75.00±2.00 ^e
PDCAAS (%)					
Threonine	45.61	35.23	35.23	33.91	33.62
Valine	83.07	48.99	48.56	48.28	47.57
Cystine + Methionine	47.31	39.80	27.80	19.90	15.90
Isoleucine	61.59	43.84	42.60	41.71	40.83
Leucine	51.06	33.89	33.44	32.53	32.38
Tyrosine + Phenylalanine	69.26	40.71	39.68	38.73	37.71
Lysine	66.75	49.02	46.70	44.56	44.13
Histidine	95.74	55.46	54.67	54.67	54.15

347 ^a Calculated according to FAO/WHO (1991).

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Table.3.4 Vitamins content of Pigeon Pea (*Cajanus cajan*) flour treated with gamma irradiation
(mg 100 g⁻¹)

Components	Dose				
	Control	5 kGy	10 kGy	15 kGy	20 kGy
Carotene (Vit A)	469.67± 2.08 ^b	463.83± 2.02 ^a	462.00± 2.00 ^a	460.21± 2.19 ^a	458.95± 1.69 ^a
Thiamin (Vit B1)	0.30± 0.20 ^b	0.25± 0.50 ^{ab}	0.19±0.03 ^a	0.19± 0.03 ^a	0.18±0.02 ^a
Riboflavin (Vit B2)	0.31± 0.04 ^b	0.27±0.03 ^{ab}	0.22±0.02 ^a	0.21±0.03 ^a	0.21±0.03 ^a
Niacin (Vit B3)	3.20± 0.20 ^b	2.99±0.10 ^{ab}	2.98±0.20 ^{ab}	2.90±0.04 ^a	2.86±0.09 ^a
Ascorbic acid (Vit C)	25.00± 1.00 ^b	24.00±2.00 ^b	22.00±2.00 ^{ab}	20.08±0.88 ^a	18.69±1.52 ^a

Means ± SE (N = 3) means in the column with unlike superscript differs significantly (p<0.05)