

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.

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.

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 (Ahmed et al., 2013). There are also many reports supporting the use of gamma irradiation as a fungicidal agent (Aziz et al., 2007).

However, the capability of growing and sometimes the developmental process of the seedling in plant have been seriously disrupted by radiation (Casarett, 1996; Ahmed et al., 2013). 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 (WHO, 1999; Magda and Nelida, 2003).

Vitamins have been found to be affected by irradiation because vitamins presented different sensitivities regarding the treatment with ionizing energy (Ana Paula Dionise et al., 2009). 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 (Elishazali et al., 2010). The amino acids group ($-NH_2$) is the most radiosensitive group of the amino acids (Siddhuraju et al., 2002 and Ahmed et al., 2013). Extensive research has 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 et al. (2005) 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 (Bamidele and Akanbi, 2013). 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 (Piri et al., 2011 and Babiker et al., 2013).

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 (Erkan and Ozden, 2007). 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 (Erkan and Ozden, 2007). Wang & vonsonntage (1991) 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 Methodology

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 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 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 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 (2000) 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, (1995) combined reagent. The absorbance was read at 880nm (Spectronic 21 D, Miltonroy, NY, USA) and KH_2PO_4 (Merck, India Limited, Mumbai, India) served as the standard.

Amino acid analysis

Amino acids of the pigeon pea flour samples were assessed by the method outlined by Hofmann et al. (1997, 2003). A known quantity of pigeon pea flour was hydrolysed with 6 N HCl (15 ml) for 4 h at 145° C. After cooling, HCl was removed in a rotoevaporator combined with a diaphragm vacuum pump (MC2C; Vacuubrand GmbH, Wertheim, Germany). Internal standard, trans -4-(aminomethyl) - cyclohexanecarboxylic acid was added to each sample. The discovery step consisted of esterification with trifluoroacetylation (Brand et al., 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 the traces of water. A 12 ml 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 ml trifluoroacetic anhydride overnight at room temperature. An aliquot of this solution was used without further treatment for gas chromatography-combustion-isotope ratio mass spectrometry. Hewlett-Packard 58590 II gas chromatograph, connected through a split with a combustion interface to the IRMS system were used to carry out the GC-C-IRMS/MS measurements for the isotopic determination of nitrogen and through a transfer line with a mass spectrometer ((GCQ; Finnigan MAT) for qualitative analysis and 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 (SGE), operating with a carrier gas flow of 1.5 ml/min with the following temperatures and pressure: 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), increased to 250° C/min (10 min); head pressure, 13 psi (90 kPa). The essential amino acid (EAA) score was determined employing the formula:

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

$$\text{WHO (1991) reference pattern}] \times 100$$

Protein digestibility

The Akesson and Stahmann (1964) method was employed to determine the in vitro protein digestibility (IVPD) of pigeon pea flours (100 mg). The defatted flours were incubated (37C, 3 h) with pepsin (3,165 units/mg protein; Sigma-Aldrich, St. Louis, MO, USA) (1.5 mg/2.5 ml of

0.1N HCl) followed by inactivation (0.25 ml of 1 N NaOH). Incubation was continued (24 h, 37° C) with trypsin (16,100 units/mg protein; Sigma-Aldrich, St. Louis, MO, USA) and a-chymotrypsin (76 units/mg protein; Sigma-Aldrich, St. Louis, MO, USA) (2 mg each/2.5 ml potassium phosphate buffer, pH 8.0, 0.1 M) followed by inactivation (0.7 ml of 100% Trichloroacetic acid (TCA)). The inactivation of the enzyme was maintained at zero-time control before addition of substrate. The supernatant of inactivation reaction mixture that were centrifuged were collected. The residue was washed (2 ml, 10% TCA) and centrifuged. The combined supernatant was washed twice with 10 ml diethyl ether, 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. The nitrogen content (in 5 ml aliquots) was determined to calculate protein in the digest:

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

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

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

Vitamins Analysis

Ascorbic acid was extracted and estimated according to the method described by Sadasivam and Manickam (1996). 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.

References

- Babiker, EE, Ismat, GA., Khogali, EA and Azhani, OA. Effects of irradiation process followed by traditional treatments on nutritional and anti-nutritional attributes of Sorghum cultivar. International Food Research Journal. 2013; 20(5): 3221-3228
- Magda S.Taipina and Nelida L. del Mastro. Radiation effects on Vitamin A and β-carotene in Liver products. NUKLEONIKA 2003; 45(1): 9-11
- Piri Issa, Medadi Babayan, Abolfazi Tavassoli and Mehdi Javaheri. The use of gamma irradiation in agriculture. African Journal of Microbiology Research. 2011;5(32) 5806-5811
- Bamidele, OP and Akanbi, CT. Influence of gamma irradiation on nutritional and functional properties of Pigeon pea (*Cajanus cajan*) flour. 2013; 7(9) 285-290
- Ahmed Ismat, G, Ahmed Khogali, E, Abdelgagi Azhari, O and Babiker Elfadil, E. The impact of radiation process followed by traditional treatment of nutritional Quality of low tannin sorghum cultivar. Innovative Romanian Food Biotechnology. 2013; 12