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Amino Acid Profile and Physicochemical Properties of African Locust Bean (*Parkia biglobosa*) Seeds as affected by Combined Irradiation and Cooking

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Abstract— *This study examined the impact of cooking, Co-60 gamma irradiation (5 kGy and 10 kGy) and combined irradiation (10 kGy) and cooking treatments on the amino acid profile and physicochemical properties of African locust bean (Parkia biglobosa) seeds, as well as the quality of the seed oil. The treated and untreated (control) seeds were mechanically dehulled and milled into flour using an attrition mill, and subsequently analysed for chemical composition, amino acid profile, functional properties and quality of the seed oil. The results showed a significant increase in the fat content ($p \leq 0.05$) after cooking and post-irradiation cooking treatments. The cooked seed samples recorded a 3.84% increase in total mineral content although there was a loss in the total percentage minerals after combined irradiation and cooking which could be linked to leaching of soluble minerals into cooking water. The 10 kGy irradiation dose improved the total essential amino acids present in the seed possibly due to the lyses of adherrent microflora. Percentage cysteine was highest in the 5 kGy irradiated seed sample with about 4.8% increase compared to control. There was a 92.6% loss in foaming capacity of the seed flour as a result of processing; however, 5 kGy irradiated sample recorded only about 26% loss in the foaming capacity. The anti-nutrients were significantly reduced by cooking, irradiation, post-irradiation cooking up to about 60%, 40%, 26.7% and 60% for cooked, 5 kGy irradiated, 10 kGy irradiated and post-irradiated cooked samples, respectively. Tannin content was not significantly affected ($p<0.05$) by the irradiation treatments; it was however reduced significantly by post-irradiation cooking. The 5 kGy and 10 kGy doses did not significantly affect iodine value of the oil sample. There was however an increase in iodine value as a result of cooking and post-irradiation cooking with values as high as 163.53 g I_2 and 182.77 g I_2 per 100g, respectively. Overall, it could be concluded that medium dose gamma irradiation did not negatively affect the quality of the African locust bean seed, which is desirable for elongation of storage life of the seed.*

Keywords— ***Gamma irradiation, Cooking, Locust bean seed, Physicochemical properties, Oil quality.***

I. INTRODUCTION

The quest for greater value-addition, improved aesthetics and elongation of shelf-life of unconventional and wild uncultivated forest seeds with high nutrient potentials has led to wider exploration and upgrading of traditional foods which hitherto were consumed at the basic subsistence level (Enujiugha, 2000; Oguntimehin *et al.*, 2023). Different techniques are employed to preserve such seeds, one of which is food irradiation that involves exposure of food to

a controlled source of ionizing radiation with a view to reducing microbial load, extending product shelf life, and disinfesting agricultural produce (Enujiugha, *et al.*, 2012; 2023a; Oyinloye et al., 2023). Food irradiation is a more reliable and safer technique of preserving food and improving the nutritional value (Diehl, 2002; Al-Kaisey *et al.*, 2002). Three types of radiation are applicable for use in food preservation: gamma rays, X-rays and rays from high energy electron beams (James, 2006). Gamma irradiation, also known as cold pasteurization, is a technique of food preservation that has been proven to prevent insect infestation in food products during storage and also deter food contamination by microorganisms (Farkas, 1990). Combining low-dose gamma irradiation with cooking in food processing and preservation is a hurdle strategy that allows effective processing while minimizing the severity of treatment (Enujiugha et al., 2012). To maximize the effectiveness of combined treatment, mild irradiation between 1 and 10 kGy is proposed as best (Campbell-Platt and Grandson, 1990). The use of cooking and γ-irradiation as forms of preservation are both proven technologies and the effectiveness of both could be maximized if they are combined in a hurdle effect (Olotu et al., 2014a,b).

The main drive in the application of hurdle technology, and in this case, combination of low dose gamma irradiation with cooking, has been the prevalent food safety concerns and low shelf life of our indigenous foods. Fagbemi *et al.* (2023) observed the high microbial density in selected indigenous foods available for direct human consumption in a typical urban neighbourhood in sub-Saharan Africa, which is a public health concern when the huge numbers of consuming populations are considered. It is widely believed that irradiation of agricultural produce at low doses, before storage and processing, could reduce the normally-high incidence of microbial infestation and curtail the occurrence of disease-causing pathogenic micro-organisms. Combination of this preservation technique with other treatments like cooking in a hurdle effect would ensure effectiveness without adversely affecting the innate nutrients.

Low income earners in developing countries like Nigeria can hardly afford animal products, such as milk, meat, fish and eggs, which are rich sources of protein. In these areas, staple diets consist mainly of cereal grains or starchy root and tuber crops thus leading to various health problems associated with protein and vitamin/mineral deficiencies (Enujiugha, 2020). This has led to various researches being directed towards the utilization of plant protein sources in the form of nuts, oilseeds and legumes to tackle the prevailing protein-energy malnutrition ravaging many parts of less-developed nations (Enujiugha and Ayodele-Oni, 2003; Enujiugha et al., 2023b; Talabi et al., 2023). In the exploration of plant protein and vitamin substitutes, the African locust bean (*Parkia biglobosa*) has become very popular especially in the fermented 'Dawadawa' and 'iru' forms, which are commonly-derived products from the seeds (Enujiugha, 2009; Gernah *et al.*, 2007). Dawadawa or iru (fermented *Parkia biglobosa* seeds) is used for seasoning of traditional soups in all parts of Nigeria and indeed the West Coast of Africa (Enujiugha et al., 2006). The sticky, sour solid contains sufficient amounts of protein, vitamins, energy (Oyedokun et al., 2016; 2020) and has appreciable shelf life even without refrigeration. Combined irradiation and cooking treatments could further extend the shelf life but have also been shown to effect various changes in the physical, chemical and functional properties of foods (Olotu et al., 2014a). This study examined the effect of combined low-dose gamma irradiation and post-irradiation cooking on the functional and physicochemical properties of African locust bean seed and its oil quality characteristics, in comparison to single irradiation and cooking treatments. This was with a view to highlighting the potentials of such hurdle treatments in the preservation of the nutrient potentials of the seeds while at the same time achieving the twin functions of disinfestation and processing.

II. MATERIALS AND METHODS

Material Procurement and Preparation

Wholesome African locust bean seeds (*Parkia biglobosa*) were obtained from a local market (*Oja Oba*), in Akure, Ondo State of Nigeria. Upon reception, the seeds were visually inspected and defective ones were discarded. The seeds were then kept in air tight polyethylene containers in a dry and cool environment until ready for use. All the chemicals and reagents used in the study were of analytical grade. The raw grains were treated at room temperature with gamma rays at doses of 5 and 10 kGy using a multipurpose gamma irradiator with a cobalt 60 source (compact-type commercial radiator) at the Shedan Science and Technology Complex (SHESTCO), Abuja Nigeria (Oyinloye et al., 2023). Raw untreated locust bean seeds served as control in the experiment. The 5 kGy sample was milled immediately while the 10 kGy irradiated sample was divided into two parts; a part was milled while the other part and half part of the raw (uncooked and un-irradiated) samples were subjected to hydrothermal treatment in the proportion of 1:3 (seed to water ratio) for 6 h at 100 ^{o}C. The samples were placed in aluminum trays and dried with forced circulation of air at 50-55 ^{o}C until constant weight (approximately 24 h).

Determination of Proximate chemical composition

Quantitative composition was determined on each of the flour samples using the following analytical methods: Moisture content using the air oven method at 105 ^{o}C until constant weight was achieved (AOAC, 2012); crude protein using the micro-Kjeldahl apparatus (AOAC, 2012); crude fat extracted overnight in a Soxhlet extractor with n-hexane and quantified gravimetrically; crude ash via exhaustive combustion in a Muffle furnace at 550 ^{o}C for 8 h (AOAC, 2012); crude fibre estimated after digesting known weights of fat-free samples in refluxing 1.25% sulphuric acid and 1.25% sodium hydroxide; and carbohydrate determined by difference method (subtracting the percent crude protein, crude fibre, crude fat, and ash from 100% dry matter). All analyses were carried out in triplicates. The gross energy was calculated based on the formula reported by Enujiugha and Ayodele-Oni (2003).

Mineral analysis

Analysis of sodium and potassium contents of the samples was carried out using flame photometer, while phosphorus was determined colourimetrically by the phosphovanado-molybdate (yellow) method (AOAC, 2012). The other elemental concentrations were determined after wet digestion of sample ash with a mixture of nitric and perchloric acids (1:1 v/v), using atomic absorption Spectrophotometer (AAS, Buck Model 20A, Buck Scientific, East Norwalk, CT06855, USA). All the determinations were carried out in triplicates.

Determination of Amino Acids Profile

The amino acids profile in the sample was determined using the method of Olotu et al. (2014b), with slight modifications. The samples were dried to constant weight, exhaustively defatted, acid-hydrolyzed (or alkaline hydrolyzed, in the case of tryptophan), evaporated in a rotatory evaporator and loaded into the technicon sequential multi sample amino acid analyzer (TSM). Briefly, a known weight of sample powder was weighed into extraction thimble and any remaining fat was extracted with chloroform/methanol (2:1) using Soxhlet extraction apparatus as described by AOAC (2012); the extraction lasted for 15 hours. A known weight of the defatted sample was then weighed into glass ampoule. Exactly 7 ml of 6N HCL (or 6N KOH) was added and oxygen was expelled by passing nitrogen into the ampoule in order to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cysteine. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 ± 5 ^{o}C for 22 hours. The ampoule was allowed to cool before broken open at the tip arid the content was filtered to remove the remains. The filtrate was evaporated to dryness at 40 ^{o}C under vacuum in a rotator evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer. The amount of hydrolysate loaded into the TSM analyser was between 5 to 10 microliters. This was dispensed into the cartridge of the analyzer. The TSM analyser is designed to separate and analyze free acidic, neutral and basic amino acid of the hydrolysate. The period of an analysis lasted for 76 minutes. All determinations were carried out in triplicates.

Determination of functional properties

The determination of water and oil absorption capacities followed a modification of the method of Prinyawiwatkul *et al.* (1999). Each flour sample (5 g) was thoroughly mixed, without pH adjustment with 25 ml of deionized water or oil in 50 ml centrifuge tubes. Suspensions were stirred intermittently over a 30 min period at room temperature (28±2 ^{o}C) and then centrifuged at 12,000 x g for 30 min at 25 ^{o}C. The volume of decanted supernatant was centrifuged and the water and oil absorption capacities were calculated. Triplicate samples were analyzed for each flour sample category.

Least gelation concentration was carried out as described by Enujiugha and Akanbi (2005). Triplicate suspension of 1-20% seed flour sample (dry w/v at 1% increment) were prepared in 10 ml of deionized water and mixed thoroughly without pH adjustment. The slurries were heated in 125 x 20 mm screw-capped test tubes in a water bath with in-built magnetic stirrer (Julabo Model SW22, Julabo Labortechnik GMBH, Seelbatch, Germany) at 95 ± 2 ^{o}C. After 1 h of heating, tubes were immediately cooled in tap water for 30 s and then in ice water for 5mins to accelerate gel formation. All tubes were then held at 4 ^{o}C for 3 h. The least gelation concentration (%) was determined as the concentration above which the sample remained in the bottom of the inverted tube.

The foaming properties of the samples were determined using the method of Coffmann and Garcia (1977), with slight modifications. Two grams (2 g) of the sample was weighed into 60 ml distilled water in a 100 ml cylinder. Solid material was dispersed with spatula and the suspension was whipped for 5 min using ultra-Turax T25 mixer at high speed. Volumes before and after whipping were noted and volume increase due to whipping was calculated. The volume of foam in the standing cylinder was also recorded for foam stability at 1, 5, 10, 20, 30, 60, 90, 120 and 180 min after whipping. The results were expressed in percentages (g/g basis).

Emulsifying properties were determined with a slight modification of the method described by Ige *et al.*, (1984). A known quantity (1.8 g) of sample was dispersed in 25 ml distilled water and 25ml vegetable oil (pure groundnut oil)

was added. The 50 ml mixture was emulsified at high speed using ultra-Turax T25 mixer for 1 min. emulsion was filled into centrifuge tubes and centrifuged for 5 min at 1,300 x 6 rpm. Percentage emulsion was then expressed as:

$$\% \text{ Emulsion} = 100\ x/y$$

Where x = height of emulsified layer

y = height of whole solution in centrifuge tube.

The results were expressed in percentages (g/g basis).

Determination of anti-nutritional factors

The modified method of Reddy *et al.,* (1982) was used for phytic acid and phytate-phospphorus determinations. Phytic acid was extracted from each 3 g flour sample with 3% trichloroacetic acid by shaking at room temperature followed by high-speed centrifugation (30,000 x g for 5 min), the phytic acid in the supernatant was precipitated as ferric phytate and iron in the sample was estimated. Phytate-phosphorus (phytate-P) was calculated from the iron results assuming a 4:6 iron: phosphorous molecular ratio (AOAC, 2012). The phytic acid was estimated by multiplying the amount of phytate-phosphorous by the factor 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$ (Enujiugha and Olagundoye, 2001).

Tannin contents were determined by the modified vanillin-HCl method (Price *et al.*, 1978). A 2 g sample was extracted with 50 ml 99.9% methanol for 20 min at room temperature with constant agitation. After centrifugation for 10 min at 653 x g, 5 ml of vanillin-HCl (2% vanillin, 1% HCl) reagent was added to 1 ml aliquots and the colour developed after 20 min at room temperature was read at 500 nm. Correction for interference by natural pigments in the sample was achieved by subjecting the extract to the conditions of the reaction but without vanillin reagent. A standard curve was prepared using catechin (Sigma Chemical, St. Louis, MO) after correcting for blank, and tannin concentration was expressed in mg/g.

Determination of oxalate was by the AOAC (2012) method. One gram (1 g) of finely ground sample was dissolved in 75 ml of 1.5 N H_2SO_4. The solution was carefully stirred intermittently with a magnetic stirrer for about 1hr and filtered using Whatman no. filter paper. A 25 ml sample of the filtrate (extract) was collected and titrated hot (80-90 ^oC) against 0.1 N $KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30 s. The concentration of the oxalate in each sample got from the calculation: 1 ml 0.1 N permanganate = 0.006303 g oxalate. All procedures were carried out in triplicates.

Determination of seed oil characteristics

The seed oils of the samples were extracted using Soxhlet apparatus (Talabi and Enujiugha, 2014) and the rancidity indices (peroxide value, saponification value, iodine value, free fatty acids content and acid value) were determined according to the standard methods of AOAC (2012). The peroxide values were expressed as miliequivalents of peroxide oxygen per kg of sample (mEq/kg) while the free fatty acids were expressed as g oleic acid per 100 g of sample (g/100 g). The acid value was expressed as mg NaOH per g of sample (mg NaOH/g). The saponification value was expressed as mg KOH per g of sample (mg KOH/g). Iodine value was determined by the AOAC (2012) method using Wij's iodine solution.

Statistical analysis

Data collected from the study were subjected to one-way analysis of variance (ANOVA). Differences among means were separated using Duncan's new multiple range test, and significances were accepted at 5% confidence level ($P \leq 0.05$). The statistical software used was SPSS 16.0 for windows.

III. RESULTS AND DISCUSSION

Proximate Composition of *Parkia biglobosa*

Table 1 shows the effects of gamma radiation on the proximate compositions of raw and cooked *Parkia biglobosa* seeds. There was an increase in the fat content of the cooked seeds after irradiation. This may be due to the rupture of oil cells in the seed skin. Badiani *et al.* (2002) reported that most nutrients increased their concentration as a consequence of moisture loss through cooking. The protein content of the cooked seeds was reduced by 2.9% from the raw. This is in line with the report of Attia *et al.* (1994) who observed a reduction in protein contents after cooking chickpea. There was also a slight decrease in the protein content of seed samples upon irradiation. This might be due to damage on sulphur-containing amino acids. The most radiation sensitive amino acids are those that contain sulphur, notably cysteine, methionine and tryptophan. Desulphurization is one of the principal effects of ionizing radiation on amino acids and proteins (Singh *et al.*, 1991). The single and combined irradiation and cooking treatments did not significantly ($p \geq 0.05$) affect the ash, fibre and carbohydrate contents of the locust bean seeds.

Table 1: Proximate composition of raw and treated Parkia biglobosa seeds

Parameters (g/100g)	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
Moisture Content	4.59^{a}±0.13	4.50^{a}±0.24	4.53^{a}±0.09	4.57^{a}±0.10	4.62^{a}±0.12
Ash content	6.18^{a}±0.02	5.97^{a}±0.03	5.89^{a}±0.03	5.75^{a}±0.02	5.76^{a}±0.04
Total fat/oil	33.92^{b}±0.12	34.06^{ab}±0.10	33.55^{b}±0.11	33.87^{b}±0.09	34.04^{a}±0.12
Crude Fibre content	5.57^{a}±0.02	5.24^{a}±0.01	5.82^{a}±0.02	5.60^{a}±0.02	5.71^{a}±0.02
Crude protein	32.82^{a}±0.14	31.88^{b}±0.12	32.73^{a}±0.13	32.48^{a}±0.13	32.22^{ab}±0.12
Carbohydrate content	20.45^{a}±0.32	20.56^{a}±0.24	21.57^{a}±0.29	21.47^{a}±0.26	21.41^{a}±0.42
Gross Energy (KJ 100g^{-1} DM)	2168.393	2212.59	2171.645	2165.8	2250.559

*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different (p≥0.05).

Table 2: Amino acid composition of raw and treated P. biglobosa seeds

Parameters	Raw	Cooked	Irradiated(10kGy)	Irradiated+Cooked
Lysine*	3.54^{c}±0.01	3.60^{b}±0.02	3.76^{a}±0.01	3.19^{d}±0.04
Histidine*	2.29^{c}±0.12	2.54^{b}±0.02	2.57^{a}±0.03	2.07^{d}±0.02
Arginine*	10.03^{b}±0.04	10.04^{b}±0.03	10.30^{a}±0.03	9.36^{c}±0.22
Asparagine	10.60^{a}±0.03	10.15^{c}±0.04	10.59^{a}±0.02	10.28^{b}±0.14
Threonine*	3.27^{a}±0.02	3.00^{b}±0.02	3.27^{a}±0.01	3.27^{a}±0.02
Serine	2.91^{b}±0.12	2.55^{c}±0.03	3.05^{a}±0.02	2.54^{c}±0.03
Gluthamine	16.20^{a}±0.04	14.14^{c}±0.21	15.14^{b}±0.30	13.24^{d}±0.02
Proline	3.29^{c}±0.02	4.14^{b}±0.02	4.46^{a}±0.01	2.97^{d}±0.01
Glycine	4.69^{b}±1.02	4.52^{c}±0.13	5.01^{a}±0.13	4.17^{d}±0.20
Alanine	3.94^{b}±0.03	3.90^{c}±0.72	4.05^{a}±0.42	3.59^{d}±1.33
Cysteine*	1.65^{c}±0.22	1.69^{b}±0.66	1.72^{a}±0.92	1.39^{d}±0.12
Valine*	3.83^{c}±0.14	5.08^{a}±0.13	5.00^{b}±0.34	3.60^{d}±0.87
Methionine*	1.41^{a}±0.26	1.30^{c}±0.01	1.38^{b}±0.12	1.20^{d}±0.01
Isoleucine*	3.95^{b}±0.03	3.83^{c}±0.05	4.14^{a}±0.03	3.39^{d}±0.18
Leucine*	6.05^{a}±0.01	5.66^{c}±0.01	5.85^{b}±0.01	5.02^{d}±0.01
Tyrosine*	1.61^{d}±0.00	2.74^{b}±0.02	2.90^{a}±0.04	2.25^{c}±0.02
Phenyalanine*	4.56^{b}±0.02	4.31^{c}±0.18	4.65^{a}±0.14	3.97^{d}±0.06
Tryptophan	2.10^{a}±0.05	1.97^{a}±0.02	2.08^{a}±0.07	1.95^{a}±0.01

*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different (p≥0.05).

Effects of processing on the amino acid profile of *Parkia biglobosa*

Table 2 presents the results of the effect of processing (cooking, irradiation and a combination of both) on the amino acids composition of *Parkia biglobosa* seeds. Amino acids are needed for the synthesis of most body tissues, enzymes, hormones and other metabolic molecules (Olotu *et al.*, 2014b). Glutamate and Aspartate accounted for about 30% of the amino acids in the sample which is similar to amino acid profiles reported previously in some oil seeds (Olotu *et al.*, 2014b; Igwe *et al.*, 2012). Sulphur-containing amino acids (methionine and cysteine) were the least

concentrated with values ranging from 1.20 g to 1.41 g and 1.39 g to 1.72 g, respectively. The reduced concentration of methionine could be attributed to the simple amino acids undergoing reductive deamination and decarboxylation during irradiation (Enujiugha *et al.*, 2023a). Cooking significantly ($p \leq 0.05$) reduced the amino acid contents except for valine which is heat stable. The reductive effects of cooking on protein and amino acid compositions of the seeds may be attributed to Amadori rearrangements that may go beyond the deoxy-ketosyl stage. It may also be due to the formation of D-amino acids which results from high and prolonged heat treatment (Olaofe *et al.*, 1994). This was likely the case because the method used for amino acid analysis will only detect L-amino acids from animal and plant proteins that do not produce racemisation (Adeyeye *et al.*, 2010). Increase in the amino acid concentration of the 10 kGy irradiated sample was observed with arginine, histidine, tyrosine, glycine, lysine, proline, threonine, phenylalanine and serine. The increase of alanine, glutamic acid, valine, methionine, isoleucine and cystine were significant ($p > 0.05$). The changes in the concentration of amino acids induced by irradiation may be due to free radicals of the peptide bonds, deamination and decarboxylation reactions of amino acids followed by chains of chemical reactions forming other new radicals (Bamidele, 2015). This finding is in line with the report of Abdel-Ghaffar (2013), who reported an increase in some amino acids of soy flour.

Effect of processing on the mineral composition of *Parkia biglobosa*

The results of the mineral analysis as shown in Table 3 indicate that the locust bean seeds were richer in iron and potassium after cooking, which could be interpreted as a consequence of the probable inactivation of anti-nutrients. Sodium was lost in the cooked as well as post-irradiated cooked samples. This may be due to its solubility and its ability to leach-off in cooking water. It can however be stated from the findings that mineral bioavailability can be increased with cooking with little impact on the mineral constituent of the seed. Zinc was significantly increased in cooked as well as 10 kGy irradiated samples

These minerals act as stabilizers of the structures of membranes and cellular components. Zinc is an important component of several enzymes and their biochemical functions, especially in the synthesis and degradation of macromolecules such as carbohydrates, proteins, lipids and nucleic acids as well as wound healing (Frossard *et al.*, 2000).

Table 3: Mineral composition of raw and treated Parkia biglobosa seeds

Parameters (mg/100g)	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
Potassium	$18.21^{a}\pm1.65$	$14.47^{b}\pm0.65$	$15.25^{b}\pm0.15$	$17.30^{a}\pm0.65$	$15.57^{b}\pm0.15$
Sodium	$2.88^{a}\pm0.10$	$2.68^{ab}\pm0.07$	$2.57^{b}\pm0.18$	$2.54^{b}\pm0.10$	$2.18^{c}\pm0.13$
Calcium	$14.68^{c}\pm0.55$	$18.30^{a}\pm1.08$	$18.17^{b}\pm0.21$	$15.86^{c}\pm1.91$	$11.18^{d}\pm0.19$
Magnesium	$4.61^{a}\pm0.82$	$3.49^{b}\pm0.24$	$3.40^{b}\pm0.10$	$3.60^{b}\pm0.14$	$2.85^{b}\pm0.25$
Zinc	$4.19^{c}\pm0.02$	$5.19^{b}\pm0.03$	$4.59^{b}\pm0.75$	$6.34^{a}\pm0.06$	$4.19^{c}\pm0.59$
Iron	$1.46^{b}\pm0.02$	$1.73^{a}\pm0.04$	$1.42^{bc}\pm0.11$	$1.24^{cd}\pm0.20$	$1.09^{d}\pm0.03$
Phosphorus	$3.67^{bc}\pm0.39$	$5.75^{a}\pm0.03$	$4.25^{b}\pm0.06$	$4.00^{b}\pm0.55$	$3.36^{c}\pm0.18$

*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different ($p \geq 0.05$).

Effect of processing on Functional properties

The effects of cooking, irradiation and combined treatment of both on the functional properties of locust bean seeds are presented in Table 4. Water absorption capacity (WAC) is an important functional property of proteins and is a measure of the quality (juiciness, texture, binding of structure, appearance and mouth feel) of flour. There was a significant decrease in the water absorption capacities of the cooked (as a single treatment) sample and the irradiated cooked sample compared to the raw. Cooking led to significant increase in oil absorption capacity (OAC) of the seed flour. Dissociation and denaturation results in increased oil absorption of treated proteins compared to native proteins (Siddharaju *et al.*, 2002). This observation is consistent with the results of Sosulski *et al.* (1976) who reported similar results using sunflower seeds.

Cooking significantly reduced the foaming capacity by about 92.6%. This finding is in agreement with that reported by Yusuf *et al.* (2008) for Bambara groundnut which was attributed to protein denaturation. Irradiation at 10 kGy decreased the foaming capacity of the seed flour significantly by 25.9%, probably owing to extensive

denaturation and protein cross-linking. However, given the foaming capacity values in the present study, African locust bean seed flour has its foaming capacity compromised by all the treatments. The 5 kGy irradiated sample has the highest foam stability while the cooked sample has the lowest foam stability. This agrees with the findings of Lin *et al.* (1974) which stated that native proteins give higher foam stability than denatured protein.

Significant reduction in least gelation concentration (LGC) was observed at 5 kGy gamma irradiation dose ($p < 0.05$). Such a decrease in LGC might be attributed to increased interaction of proteins with water (Adebowale and Lawal, 2004). Improvement in gelation property is beneficial as it allows the utility of seed flour in preparation of food products like custards, ice creams, sausages and other bakery products (Bhat and Sridhar, 2008). The current findings are in agreement with results obtained for *Pentaclethra macrophylla* (Enujiugha *et al.*, 2012) and groundnut (Enujiugha *et al.*, 2023a).

Emulsion capacity denotes the maximum amount of oil that can be emulsified by protein dispersion. The high emulsion capacity in the present study could be as a result of high content of free fatty acids which leads to increased oil absorption (Ihekoronye and Ngoddy, 1985). Irradiation at 5 kGy caused a significant decrease in the emulsion capacity of the seed flour accounting for more than 10% loss of emulsion capacity. Irradiation at 10 kGy had no significant ($p<0.05$) effect on emulsion capacity of the oil seed flour when compared with the non-irradiated (control) samples. The decrease in emulsion capacity has been speculated to have resulted from changes, such as protein aggregation as well as surface hydrophobicity and charge characteristics (Cheftel *et al.*, 1985).

Table 4: Functional properties of raw and processed Parkia biglobosa seeds

Parameters	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
WAC (ml/g)	$380.00^{a}\pm0.21$	$360.00^{ab}\pm0.35$	$360.00^{b}\pm0.08$	$380.00^{a}\pm0.02$	$360.00^{ab}\pm0.03$
LGC (m/v)	$8.00^{a}\pm0.01$	$6.00^{a}\pm0.02$	$2.00^{b}\pm0.03$	$4.00^{b}\pm0.01$	$2.00^{b}\pm0.02$
OAC(ml/g)	$190.00^{ab}\pm0.03$	$210.00^{a}\pm0.02$	$175.00^{b}\pm0.02$	$180.00^{b}\pm0.01$	$200.00^{ab}\pm0.02$
FC (%)	$45.00^{a}\pm0.03$	$3.33^{e}\pm0.12$	$33.33^{b}\pm0.02$	$25.00^{c}\pm0.04$	$6.67^{d}\pm1.23$
EC (%)	$380.00^{a}\pm0.09$	$360.00^{b}\pm0.01$	$340.00^{c}\pm0.31$	$380.00^{a}\pm0.03$	$360.00^{b}\pm0.01$

*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different ($p\geq0.05$).

WAC = Water absorption capacity; OAC = Oil absorption capacity; LGC = Least gelation concentration; FC = Foaming capacity; EC = Emulsion capacity

Effect of processing on anti-nutritional factors

The levels of some anti-nutritional components in the raw and processed seed flour samples are presented in Table 5. Anti-nutritional factors are generally reported to have the effect of lowering digestibility and absorption of important dietary nutrients.

Oxalate can have deleterious effects on human nutrition and health, particularly by decreasing calcium absorption and aiding the formation of kidney stones (Noonan and Savage, 1999). Cooking has been found effective in the reduction of oxalate content in oil seeds (Chai and Liebman, 2005). A decrease in the oxalate content was observed in both cooked samples. The higher percentage of oxalate reduction occurs during boiling and may be due to its solubility in water. Boiling may cause skin rupture and facilitate the leakage of soluble oxalate from seeds into cooking water (Albihn and Savage, 2001). Comparing the different treatments, cooking and post-irradiation cooking are to be preferred to irradiation alone in order to keep oxalate intake as low as possible. Observations were comparable to the reports of Albihn and Savage (2001) and Wanasundera and Ravindran (1992). The reduction in oxalate levels on cooking is to enhances the bioavailability of essential dietary nutrients of the African locust bean seeds

The results obtained indicate that phytate content decreased slightly with treatment. Irradiation alone effectively reduced the phytate level in the oil seed by 4.8% compared to the control. Decrease in the phytate content in the irradiated and cooked sample was as high 24.8%. The reduction in phytate content during cooking may be partly due to the formation of insoluble complexes between phytates and other components, such as phytate-protein and phytate-protein-mineral complexes, or to the inositol hexaphosphate being hydrolyzed (Siddhuraju and Becker, 2001). Cooking has been reported to lower the phytate levels in several plant foodstuffs (Badifu, 2001). Taghinejad-Roudbaneh, (2010) in a study where canola

meal was irradiated using electron beams up to 45 kGy dose observed a total disappearance of phytic acid at 30 kGy and a reduction of up to 89.66% at 15 kGy irradiation dose. Currently there is evidence that dietary phytate at low levels may have beneficial effect as an antioxidant, anticarcinogen and may likely play an important role in the control of hypercholesterolemia and atherosclerosis (Kumar *et al.*, 2010; Phillippy *et al.*, 2004).

The high tannin contents of the seed might have been a major cause of the astringency observed when tasted in its unprocessed form. Tannins usually form insoluble complexes with proteins thereby interfering with their bioavailability (Enujiugha and Agbede, 2000). Reduction of tannins might have resulted in a significant increase in Zinc content at 10kGy irradiation dose.

Rancidity indices of oil from *Parkia biglobosa*

The products of rancidity are known to be hazardous to health since they are associated with aging, membrane damage, heart disease and cancer (Cosgrove *et al.*, 1987). The rancidity indices of oil as affected by processing are presented in Table 6.

Table 5: Anti-nutritional factors in raw and treated Parkia biglobosa seeds

Parameters	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
Oxalate(mg/g)	$0.15^{a}\pm0.02$	$0.06\pm^{d}0.13$	$0.09^{c}\pm1.04$	$0.11^{b}\pm0.18$	$0.06^{d}\pm0.05$
Phytate(mg/g)	$1.25^{b}\pm0.02$	$1.19^{a}\pm0.02$	$1.57^{a}\pm0.02$	$1.11^{b}\pm0.02$	$0.94^{b}\pm0.03$
Tannin(mg/g)	$0.02^{a}\pm0.01$	$0.02^{a}\pm0.01$	$0.02^{a}\pm0.01$	$0.02^{a}\pm0.02$	$0.02^{b}\pm0.01$
Phytate-P	$0.35^{b}\pm0.12$	$0.34^{bc}\pm0.05$	$0.44^{a}\pm0.04$	$0.31^{bc}\pm0.01$	$0.33^{c}\pm0.26$

*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different ($p\geq0.05$).

Table 6: Rancidity indices of the raw and treated seed oil extract

Parameters	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
Peroxide value (mEq./kg)	$0.71^{c}\pm0.05$	$0.73^{c}\pm0.10$	$0.73^{c}\pm0.28$	$0.79^{b}\pm0.05$	$0.86^{a}\pm0.06$
Iodine Value (mg/100g)	$140.13^{a}\pm1.80$	$133.53^{b}\pm2.82$	$142.48^{a}\pm0.17$	$144.40^{a}\pm2.05$	$122.77^{b}\pm8.85$
Acid Value (mgNaOH/g)	$1.07^{c}\pm0.03$	$1.11^{ab}\pm0.01$	$1.07^{c}\pm0.01$	$1.09^{bc}\pm0.02$	$1.14^{a}\pm0.02$
Saponification Value (mgKOH/g)	$176.33^{c}\pm0.21$	$184.00^{b}\pm0.10$	$173.33^{b}\pm0.01$	$180.67^{b}\pm0.02$	$186.67^{a}\pm0.09$
Free Fatty Acid (% Oleic acid)	$1.31^{cd}\pm5.51$	$1.62^{ab}\pm2.00$	$1.63^{d}\pm2.08$	$1.69^{c}\pm1.15$	$1.85^{a}\pm1.53$

*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different ($p\geq0.05$).

The peroxide value is an indicator of oxidative rancidity. As oxidation takes place, the double bonds in the unsaturated fatty acids are attacked leading to the formation of peroxides. Fresh oils have been shown to have peroxide values lower than 10 mg/g and oils become rancid with peroxide value ranging from 20 to 40 mg/g (Oyinloye and Enujiugha, 2017). The highest peroxide value was observed in the oil from the seeds with combined treatments of irradiation and cooking. These results are generally in agreement with those of Mexis *et al.* (2009) for gamma irradiated almonds where the peroxide values increased with dosage. Cooking alone accounted for 3.6% increase in peroxide value of the oil while 5kGy irradiation dose resulted in 2.82% increase in the peroxide value. This increase conforms to the work of Farag *et al.* (1992) who reported acceleration of cottonseed oil oxidation during irradiation heating which as indicated by an increase in peroxide value. Increases in peroxide values are expected as irradiation produces large amounts of free radicals that enhances lipid peroxidation (Sajilata and Singhal, 2006).

Iodine value is a measure of the unsaturation of fats and oils. It is based on the ability of an unsaturated carbon to carbon bond to add halogen atoms. Irradiation treatments alone (5 kGy and 10 kGy) did not significantly affect the iodine

value of the resultant oil. However, significant decreases in iodine value were observed for the cooked and post-irradiated cooked samples (133.53 g I_2 per 100 g and 122.77 g I_2 per 100 g respectively). These were in agreement with the reports of Omafuvbe *et al.*, (2004) who observed a decrease in iodine value in African Oil Bean seeds as a result of cooking.

The acid value of oils indicates the total acidity as estimated by the fatty acids in the sample. The acid value obtained from the raw sample (1.07 mgKOH/g) correlates with the report of Omafuvbe *et al.*, (2004) for African Locust bean seed oil extract. Cooking of African locust bean seeds during processing increased acid value significantly (1.11 mgKOH/g for cooked and 1.14 mgKOH/g for irradiated and cooked).

Saponification value (SV) is a measure of the alkali-reactive groups in fats and oils which is useful in predicting the type of glycerides in a sample. Glycerides containing short-chain fatty acids have higher SV than those with longer chain fatty acid. The saponification values of the locust bean seed oils as shown in Table 5 ranged from 173.33 mg KOH/g for 5 kGy irradiated sample to 186.67 mg KOH/g for post-irradiated cooked samples. These results conform with the ranges reported in literature for vegetable oils: olive oil (184-196 mg KOH/g), rapeseed oil (168-181 mg KOH/g), sunflower seed oil (188-194 mg KOH/g) and pumpkin seed oil (174-197 mg KOH/g) (Nichols and Sanderson, 2003). This is an indication that there was formation of short chain fatty acid from the breakage in the longer chain length as a result of radiolysis.

IV. CONCLUSION

Gamma irradiation at doses up to 10 kGy has been shown to potentially reduce anti-nutritional factors in African locust bean seeds. Combined gamma irradiation and post-irradiation cooking however, can be concluded to be viable hurdle technique for the treatment of African locust bean (*Parkia biglobosa*) seed as it effectively enhances the nutritional component of the oil seed by inactivating anti-nutrients and allowing for bioavailability of minerals needed for body development. The findings highlight the great potentials of low dose gamma irradiation in the preservation of neglected and underutilized forest seeds that commonly dot the African agro-ecological landscape.

DATA AVAILABILITY

The collated data presented in this work is available for whatever scientific purpose that is required.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This work is part of a wider research which was approved by the Ethics Committee of the School of Agriculture and Agricultural Technology, Federal University of Technology with assigned number FUTA/SAAT/ETH/2011/14

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