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Research Article

EFFECT OF PROCESSING METHODS ON THE CHEMICAL COMPOSITION AND FUNCTIONAL PROPERTIES OF PIGEON PEA SEED

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ABSTRACT

Objective: The broad objective of the study was to determine the effects of toasting, boiling, germination and fermentation on the chemical composition, antinutrient contents and functional properties of pigeon pea flour.

Methods: Flour samples were prepared from raw, toasted, boiled, germinated and fermented pigeon pea seeds. The flour samples were analyzed for chemical composition, antinutrient contents and functional properties.

Results: Only toasting significantly ($P<0.05$) decreased the moisture content and concentrated the protein, ash Mg, Ca, Fe and Zn contents of the pigeon pea seed. Boiling, germination and fermentation did not significantly ($P>0.05$) affect the proximate composition of the seeds. All the treatments reduced the tannins and trypsin inhibitors in the seeds with boiling and germination exerting greater effect. All the treatments reduced the bulk density and foaming capacity of the pigeon flour. Germination improved the foam stability of the pigeon pea flour by 5%. Toasting, boiling and germination increased the water absorption capacity of pigeon pea flour . While fermentation and toasting increased the emulsion activity of pigeon pea flour, only boiling and germination increased the emulsion stability of the flour. Only germination slightly increased the least gelation concentration from 2% (w/v) for the raw pigeon pea flour to 4% (w/v).

Conclusion: Toasting, boiling, germination and fermentation had effects on pigeon seeds which varied with nutrients, functional properties and treatments. Only toasting increased the concentrations of Mg, Ca, Fe and Zn in the pigeon pea seed but all the treatments reduced the tannins and trypsin inhibitor contents. The variously treated pigeon pea flours possessed functional properties that lend them for various food applications**.**

Keywords: Pigeon pea, Boiling, Toasting, Germination, Fermentation.

INTRODUCTION

Pigeon pea (Cajanus cajan) ranks fifth in importance among edible legumes in the world[1] Although, India remains the major producer of pigeon pea, the interest in pigeon pea in other parts of the world including Nigeria is as a result of its nutritional, medicinal, economical and agronomic usefulness [2]. Based on these attributes, pigeon pea is widely consumed in Africa, India and the Caribbean. Presently, different varieties of pigeon pea are grown in Nigeria. Reports showed that pigeon pea contained 17.9-24.3% protein, 58.7% carbohydrate, 1.2-8.10% crude fiber and 0.6-3.8% fat [2] . Pigeon pea is also a good source of calcium, phosphorus, magnesium, iron, sulphur etc [2,3]. However, pigeon pea like other legumes is deficient in methionine but high in lysine [3].In spite of its high nutritional qualities, pigeon is not popular in the Western and Northern states of Nigeria. It has no industrial use as at now. Like most tropical legumes, pigeon peas, especially the raw seeds contain antinutritonal substances such as trypsin inhibitors and tannins which affect their utilization [4]. They also contain flatulence causing oligosaccharides such as starchyose, raffinose and verbacose [2]. Similarly, the characteristic problem of hard-to-cook phenomenon also hinders the extensive use of pigeon peas as food. The dried seeds are hard and by the traditional processing methods, it takes 24 hours to prepare a meal of pigeon pea [2,3].

Various methods are used to improve the food value of pigeon pea by improving its processing, storage, preservation and utilization. Such methods include germination, fermentation, toasting, boiling, irradiation[4,5] etc. These processing methods may affect the physicochemical properties of the seeds and hence, their potential food applications. Moderate heat treatment improves the digestibility of plant proteins without developing toxic derivatives and inactivates several enzymes such as proteases, lipases, lipoxygenases, amylases and other oxidative and hydrolytic enzymes in foods [6,7,8]. Roasting causes physical, chemical, structural and sensorial changes in foods [9]. Roasting could promote the development of flavor, desired color and increase palatability of foods[9]. One of the main desired outcomes of roasting process is the increase in antioxidant activity due to the formation of Maillard reaction products[9].Boiling destroyed protease inhibitors and cyanogens in pigeon peas [5]. Toasting and boiling have been reported to enhance taste and flavor of foods [5.9]. Fermentation gives the food longer keeping quality, develops flavor and decreases anti-nutritional factors in foods [10,11]. Germination increased the water absorption capacity of seeds and protein solubility of winged bean [12]. Germination was reported to increase the capacity of millet flour to bind oil but decreased its water absorption capacity [13]. However, these methods in some cases, rather than improving the nutritive value of foods, adversely reduced them [10,11,13].Therefore, the objective of this study was to determine the effects of toasting, boiling, germination and fermentation on the chemical composition, antinutrient contents and functional properties of pigeon pea flour.

METHODS

The pigeon pea seeds were purchased from Oja-Oba market in Ijare town, Ondo State. The seeds were cleaned of extraneous materials and stored in jute bags prior to use. Wheat flour was purchased from a local market in Idah Township, Kogi State, Nigeia. The wheat hour was screened through a 60 mesh sieve (0.1mm) (British standard) and stored in high density polyethylene (HDPE) bags (0.77mm thick) before use.

Preparation of raw pigeon pea flour

The pigeon pea seeds were hydrated in cold water (30 ± 2^oC) for 60min, dehulled manually, oven dried (60^oC, 3h) and milled in attrition mill. The powder was screened through a 60 mesh sieve (0.1 mm), packed in HDPF bags and kept on a laboratory bench (30 ± 2^oC) until used.

Preparation of toasted pigeon pea flour

The cleaned pigeon pea seeds were toasted on trays (120°C, 30min) in an air convection oven with intermittent mixing. The toasted seeds were dehulled manually and the kernels were milled in attrition mill and screened though a 60 mesh sieve (0.1 mm).

Preparation of boiled pigeon pea flour

The pigeon pea seeds were boiled in hot water (100°C) for 60 min, cooled, dehulled manually, oven dried (60°C, 3h), milled in attrition mill and sieved through a 60 mesh sieve (0.1mm).

Preparation of germinated pigeon pea flour

The pigeon pea seeds were surface sterilized with 1.5% sodium hypochorite solution followed by soaking in 70% ethanol for 20min. The seeds were rinsed thoroughly with tap water and soaked for 6h in tap water. The hydrated seeds were spread evenly on layers of wet jute bags in large petrish dishes (in 3 replicates) and germinated for 5 days in the dark. The jute bags were moistened at regular intervals. The ungerminated seeds were discarded. The sprouted seeds were rinsed with tap water and then oven dried (60°C, 3h). The kernels were milled in attrition mill and screened through a 60 mesh sieve.

Preparation of fermented pigeon pea flour

A portion of the raw pigeon pea flour (RPPF) was mixed with water at 3.2 (water: flour) ratio in a covered plastic bowl as described by Ariahu et al.[14]. The paste was fermented for 5 days, oven dried (60°C, 3h), milled in attrition and screened through a 60 mish sieve (0.1 mm). All the flour samples were stored in high density polyethylene (HDPE) (0.77mm thick) bag prior to use.

Evaluation of Functional Properties

Water and oil absorption capacities were determined as described by Sosulski et al. [15]. The foaming capacity and foam stability were determined by the method of Narayana and Narasinga Rao [16]. Bulk density was determined as outlined by Okaka and Potter [17]. The method of Onimawo and Akubor [18] was used to determine the least gelation concentration. Emulsion activity and emission stability were measured as described by Onimawo and Akubor [18].

Chemical evaluation

Moisture content was determined by hot air oven drying at 105%C to constant weigh (19). Ash, protein (N x6.25), crude fiber and fat (solvent extraction) were determined by the AOAC [19] methods. Carbohydrate was calculated by difference as 100-(% Protein + % Fat + % Crude fiber + %Ash + % Moisture). The caloric value of samples was calculated by multiplying the % protein, fat and carbohydrate contents by Atwater factors of 4, 9 and 4, respectively (19).Tannins and trypsin inhibitors contents were determined as described byAOAC [19].

Statistical analysis

The data were analyzed by analysis of variance using statistical package for social sciences (SPSS) soft ware version 18 [20] in completely randomized design. The least significant difference (Lsd) test was used to separate significantly different means [20]. Means were accepted at $P < 0.05$.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of pigeon pea seed as affected by the processing treatments is presented in Table 1. Only toasting significantly ($P<0.05$) decreased the moisture content of the seed. However, all the treatments except boiling slightly increased the protein content of pigeon pea. Toasting may have concentrated the proteins in the pigeon pea seed. On the other hand, soluble proteins of the pigeon pea seed may have leached into the boiling water during the boiling process. Synthesis of new protein was reported to occur during fermentation [21]. Activities of proteolytic bacteria during fermentation were reported to improve the digestibility and availability of proteins due to the breakdown of protein-tannin and protein-phytate complexes [21]. High proteolytic activity in germinating millet grains was reported by Hamad and Fields'[6,13]. Germination may have increased the amino acids content of the pigeon pea seed storage protein in this study. Hamad and Field [13] reported significant improvement in the protein content of sorghum and other grains during malting.

Table 1: Effect of treatments on the proximate composition of pigeon pea seed flour

Composition(%)	Raw	Toasted	Boiled	Germinated	Fermented
Moisture	10.5^{a} ±0.8	9.0^{b} ±0.7	11.0^{a} ±0.5	10.9^{a} ±0.4	10.7^{a} ±0.4
Crude protein	23.0^{b} ± 0.2	24.9^{a} ±0.3	22^{c} ±0.1	24.4^{b} ±0.2	24.8b ± 0.1
Ash	2^{a} ±0.3	3.8^{b} ±0.5	1.5^{a} ±0.3	1.7^{a} ±0.8	1.8^{b} ±0.5
Crude fiber					
Carbohydrate	57.7^{a} ±0.5	56.3^{a} ±0.2	58.7^{a} ±0.7	56.9^{a} ±0.4	57.5^{a} ±0.2

Values are means ± SD of 3 replications. Mans within a within a row with the same superscript were not significantly different ($P>0.05$).Fermentation and malting not only improved the nutrient density and availability but also improved flavor, palatability and shelf life of foods [21a]. Alpha amylase rich foods which are produced from malted cereals reduced viscosity, increased starch digestibility and nutrient densities of gruels [12,13,21a]. All the treatments slightly decreased the fat content but increased the crude fiber content of the pigeon pea seeds. The fat in the seeds was probably utilized for the energy need of the microorganisms during fermentation and germination. Decrease in fat content due to sprouting could be due to the activities of lipases which were activated during sprouting[12,13] . Leaching may be responsible for the decrease in fat content of the boiled seeds. Only toasting increased the ash of the pigeon pea flour due to concentration effect. Only toasting and germination increased the crude fiber content of the pigeon pea seed flour. The increase in the crude fiber content of the pigeon seed on germination may be attributed to the synthesis of more of the cell wall material to support the roots and rootlets [13].On the other hand, toasting concentrated the crude fiber content of the seed by loss of moisture. The carbohydrate contents of the germinated and fermented seed flours were slightly lower than those of the other flours. Similar decrease in carbohydrate content occurred during germination of millet[12,13].The decrease in carbohydrate content could be due to alpha amylase activity which broke down complex carbohydrates into simpler and more absorbable sugars which are utilized by the growing seedling during the early stage of germination. Carbohydrate was probably utilized as energy source and carbon skeleton for synthesis of other compounds during the fermentation of the pigeon seeds.

Mineral composition

The Mg, Ca, Fe, and Zn contents of raw pigeon pea seed were 146, 110, 5 and 3 Mg/100g, respectively (Table 2). All the treatments except toasting decreased the levels of these minerals in the seed. Toasting may have concentrated the minerals by loss of moisture. The seedlings may have used some of the minerals during the sprouting process.Obizoba(22) had earlier documented similar report for sprouted pigeon pea flour.The decrease in the mineral contents due to boiling was probably caused by leaching of the minerals into the boiling water(23).

Table 2: Effect of treatments on the mineral composition of pigeon pea seeds flour

	Raw	Toasted	Boiled	Germination	Fermentation
Mg	$146^{b} \pm 0.3$	$148^{a} \pm 0.3$	$140^{d} \pm 0.6$	$132^{e} \pm 0.5$	$142^{c} \pm 0.2$
Ca	$110^{b} \pm 0.1$	$112^{a} \pm 0.1$	$104^{c} \pm 0.5$	$92^{d} \pm 0.4$	$104^{c} \pm 0.6$
Fe	$5.0^{a} \pm 0.5$	$5.5^{a} \pm 0.4$	$4.0^{b} \pm 0.2$	$3.0^{c} \pm 0.3$	$5.0^{a} \pm 0.1$
Zn	$3.0^{b} \pm 0.9$	$4.0^{a} \pm 0.5$	$2.5^{b} \pm 0.3$	$2.0^{c} \pm 0.3$	$2.8^{b} \pm 0.4$

Values are Means $\pm$ SD of 3 replications. Means without a row with the same superscript were not significantly different (P>0.05).

Antinutrient contents

The effect of the processing treatments on the antinutrient contents of pigeon pea flour is presented in Table 3. The raw pigeon pea seed contained 6.5mg/100g tannins and 4.5 Tiu/mg trypsin inhibitors. The treatments had varied effects on these antinutrients in the pigeon pea seed. All the treatments reduced the level of tannins in the seed but germination exerted greater effect. Reduction of tannins by germination and fermentation may be due to activation of polyphenol oxidase which is responsible for the degradation of polyphenol[4,5]. Tannins are water soluble complexes and may be destroyed by heat and some may have leached into the boiling water [4]. The effects of tannins on the availability of nutrients are widely reported[,5]. Tannins decrease the digestibility and palatability of proteins and carbohydrates by forming insoluble complexes with them[4]. They also reduce bioavailability of minerals [23]. Dry heating (toasting) was less effective in reducing typsin inhibitors than the other treatments. Boiling and germination exerted greater effect in reducing trypsin inhibitors. This was followed by fermentation.The reduction in the trypsin and chymotrypsin inhibitor activities in some leafy vegetables was strongly influenced by the presence of water, the amount of heat applied and the heating time [23] . Moist heating was more effective in reducing trypsin and chymostypsin activities in some grains[4,23]. The boiling process used in this study caused thermal breakdown of these compounds and the subsequent leaching of the soluble components into the boiling water .Complete elimination of trypsin inhibitor activity was reported on fermentation of sorghum[24]. An inhibitor is an organic substance capable of reducing enzyme activity by either binding to the enzyme or rendering it unavailable to substrate [24], interfering with the biosynthesis of the enzyme or affecting a hormone which in turn changes the level of enzyme activity [24]. Trypsin and chymotrypsin inhibitors are natural organic compounds which interact with proteolytic enzymes particularly trypsin and chymotrypsin rendering them unavailable for protein digestion. The inhibitors, therefore reduce protein bioavailability and contribute to the poor nutritional quality of human diets [24]. Trypsin and chymotrypsin inhibitors are widely distributed in plants especially beans, tubers and leaves [24]. However, the conditions employed in this study reduced these inhibitors in pigeon pea seeds.

Table 3: Effect of treatments on the antrnutrtional factors in pigeon pea seed flour

	Tannins (mg/100g)	**Trypsin inhibitors Tiu/mg**
Raw	6.5 ± 0.9^{a}	4.5 ± 0.6^{b}
Toasted	3.1 ± 0.5^{a}	3.5 ± 0.8^{b}
Boiled	25 ± 0.4^{a}	1.0 ± 0.3^{b}
Germinated	$1.5 \pm 0.1_{a}$	0.08 ± 0.7^{b}
Fermented	2.8 ± 0.6^{a}	1.2 ± 0.1^{b}

Values are means $\pm$ SD of 3 replications. Means within a column with the same superscript were not significantly different (P>0.05).

FUNCTIONAL PROPERTIES

The effects of the processing treatments on the functional properties of pigeon flour are shown in Table 4.

Bulk density and foaming properties

Functional properties are the intrinsic physicochemical characteristics which may affect the behavior of food systems during processing and storage [25].All the treatments reduced the bulk density and foaming capacity of pigeon pea flour but only germination increased the foaming capacity of the flour by 5%. The bulk density of the raw PPF was 0.70g /cm^3 and reduced to 0.56-0 for the treated flours. Bulk density is dependent on factors such as method of measurement, geometry, size, solid density and surface properties of the molecules and could be improved when the particles are small, compactible, properly tapped/vibrated and when suitable packaging material is used[25]. The low bulk density helps to reduce transportation and storage costs. Germination and fermentation are traditionally used for preparing low bulk weaning foods [14]. Thus, the reduction in the bulk density would be an advantage in the use of pigeon pea flour for preparation of supplementary foods. Akubor and Obiegbuna [13] had previously reported that germination lowered foaming capacity of millet but slightly increased its foaming stability. Akubor et al. [25] reported that toasting decreased the foaming capacity of African breadfruit seeds and that the foaming capacity was dependent on the toasting time. Foamability is related to the rate of decrease of the surface tension of air water interface caused by absorption of protein molecules [26]. It is also a function of the type of protein ,pH, processing methods etc [26]. The treatments employed in this study may have increased the surface tension of the protein molecule, thus reduced the foamability of the flours. High foam stability is enhanced by native proteins [10], suggesting that the germinated PPF had higher proportion of native proteins than the other flours . Foams are used to improve texture, consistency and appearance of foods. The low foaming properties of the raw and treated pigeon pea flour suggested that they may not be suitable for food products where high porosity is required. This probably explains why pigeon pea flour is not used in the preparation of *akara* balls in Nigeria. However, the raw pigeon flour and germinated pigeon pea flour may find application in baked and confectionery products.

Table 4: Effect of processing treatments on the functional properties of pigeon seed flour

Functional Properties	Raw	Toasted	Boiled	Germinated	Fermented
Bulk density (g/Cm^3)	0.73^a ±0.1	0.56^a ±0.8	0.67^a ±0.3	0.56^a ±0.7	0.70^a ±0.8
Foaming capacity (%)	20.75^a ±0.1	9.52^d ±0.1	5.88^e ±0.3	17.67^b ±0.3	16.67^c ±0.1
Foam stability (%)	63.64^b ±0.4	2. 0^e±0.7	8.0^d ±0.6	66.6^a ±0.1	12.25^c ±0.4
Water absorption capacity(%)	120^c ±0.8	190^a ±0.6	108^c±0.2	140^b ±0.2	105^d ±0.4
Oil absorption capacity (%)	110^a ±0.3	70^c ±0.3	30^d ±0.5	70^c ±0.6	100b ±0.7
Emulsion stability (%)	35.0^c ±0.5	25.6^d ±0.4	41.1^b ±0.7	42.7^a ±0.9	12.5^e ±0.2
Least gelation Concentration (%, W/V)	2.0^b ±0.1	2.0^b ±0.3	2.0^b ±0.3	4.0^a ±0.3	2.0b ±0.6

Values are means ± SD of 3 replications. Means within a row within a row with the same superscript were not significantly different ($P > 0.05$).

Water and oil absorption capacities

The raw pigeon pea flour was characterized by relatively high water absorption capacity (120 %).While only boiling and fermentation failed to improve the water absorption of pigeon pea flour, all the treatments decreased the oil absorption capacity with boiling exerting greater effect. The dissociation of the pigeon pea protein into subunits by toasting and germination may have occurred. This may have unmasked the polar residues from the interior of the proteins molecules, which lead to increased water absorption capacity but not oil absorption capacity. The boiled samples had the least water absorption capacity due to the fact the seed had absorbed much water by the length of time they stayed while being boiled. The toasted sample had the highest water absorption capacity due to loss of water during the roasting process. The increase in water absorption capacity due to toasting could also be attributed to the heat dissociation of proteins, gelatinization of carbohydrate in the flour and swelling of crude fiber [18]. According to Onimawo and Akubor [18], more hydrophobic proteins show superior binding of lipids implying that non polar amino acids chains bind the paraffin chains of fats. Based on this suggestion, it could be inferred that raw pigeon pea flour which showed higher oil absorption capacity had more available non polar side chains in its protein molecules than the other treated flours These results suggest that only fermented pigeon pea flour would be unsuitable for bakery products where hydration to improve handling characteristics is required. Oil absorption capacity assesses the ability of flour to absorb fat. Ingredients with high oil absorption capacity play important role in stabilizing food systems with high fat content and can also act as emulsifiers[10]. The ability to absorb water or oil is a very important property of all flours used in food preparation. Water binding capacity is a measure of the strength of starch inter granular bond. Low water binding capacity is attributable to tight association while high water binding capacity is indicative of a loose association of native starch polymers or low lipid content[18]. The ability of foods to absorb oil may help to enhance sensory properties such as flavor retention and mouth feel.

Emulsifying properties

Fermentation (42.50%) and toasting (37.50) significantly ($P < 0.05$) improved the emulsion activity of raw PPF (24%) but only boiling (41.10%) and germination (42.7%) increased the emulsion stability.The emulsion stability of the raw pigeon pea flour was 35 %. Fermentation and toasting may have caused unfolding of the PPF protein chains and thereby exposed the hydrophilic section of the peptide which enhanced emulsion activity [10]. The pigeon pea flours showed high emulsion activity when compared to flours like African oil bean (6%) [14]. The high emulsion activity of pigeon pea flour could be due to its high protein content. Efficiency of emulsification varies with the type of protein, its concentration, pH, ionic strength, viscosity of the system, temperature and method of preparation, the rate of oil addition, sugars and moisture content [13]. The high emulsion property of pigeon pea flours suggests their possible use in sausage and in stabilizing colloidal food systems[14].

Gelation property

The least gelation concentration of pigeon pea flour pigeon pea flour was slightly increased from 2% (w/v) to 4% (w/v) by germination while other treatments did not have any effect. The least gelation concentration reported for other legume flours were 14% for lupin seed [Sathe et. al, 1982]. 6% for defatted sesame seeds [10], 8% for African breadfruit seeds [25] and 14% for fermented African oil bean [10]. The variation in gelling properties of different flours was associated with the relative ratios of different constituents such as protein, carbohydrate and lipids that make up the flours [25].Onimawo and Akubor [18] suggested that interactions between such components may also play significant role in functional properties. The pigeon pea flours would be good gel forming agents and would be useful in food system such as puddings and snacks where thickening and gelling are needed.

CONCLUSION

Toasting, boiling, germination and fermentation which are traditionally employed for the preparatory of pigeon pea seeds to human foods affected the chemical composition and functional properties of the seeds. The effects varied with nutrients, functional properties and treatments. Only toasting increased the concentrations of Mg, Ca, Fe and Zn in the pigeon pea seed. However, all the treatments reduced the tannins and trypsin inhibitor contents of pigeon pea seeds. The various capacity tests showed that the raw, toasted, boiled, germinated and fermented pigeon pea flours possessed functional properties that lend them for various food applications.

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