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Chemical Changes in Nutritional and Mineral Composition of Fermented Pigeon Pea (*Cajanus Cajan* L.) Seeds.

Nwaemeke David Iweunor[1*]; Ani Abosede Oluwakemi[2]; Adelere Folake Irewunmi[3]; Ogunkoya Adepero[3]; and Abdulsalam Mariam Temitope[4].

Department of Science Laboratory Technology, Ogun State Institute of Technology, Igbesa, Ogun State, Nigeria.

Email: dnwaemeke@gmail.com[1], edunkemy@mail.com[2]; adelerefolake@gmail.com[3]; ogunkoya.adepero@gmail.com[4]; and mariamabdulsalam04@gmail.com[5]

ABSTRACT

The pigeon pea (*Cajanus cajan* L.) is one of the major grain legume crops of the tropical world. Changes in the proximate composition, nutritive and non-nutritive components, and gross energy were studied in seeds of pigeon pea (*Cajanus cajan*) that were fermented for 0, 1, 2, 3, 4 and 5 days. Raw and fermented seeds were analysed for moisture, crude protein, fat, crude fibre, nitrogen free extractives, ash content, copper, iron, zinc, manganese, and average digestible energy. Raw and fermented results shows that moisture (9.63-20.07%), crude protein (17.46-22.38%), fat (2.72-7.84%), crude fibre (8.21-9.16%), nitrogen free extractives (57.44-61.73%), ash (5.10-8.10%), copper (0.018-0.020 mg/g), iron (0.063-0.185 mg/g), zinc (0.035-0.045 mg/g), manganese (0.015-0.019 mg/g), and average digestible energy (369.60-376.74 Kcal/100g). It was concluded that proximate composition and digestible energy value of pigeon pea seeds were significantly affected by fermentation. Fermentation of the seeds up to 3 days improved the total ash, fat, gross energy, digestible energy and the iron contents of the seeds. On the hand, 2 days of fermentation remarkably improved the average digestible energy of the seed.

Key words: Fermentation, Nutritive and Non-nutritive, Pigeon pea and proximate composition.

INTRODUCTION

Pigeon pea is a tropical and subtropical species particularly suited for rain fed agriculture in semi-arid areas due to its deep taproot, heat tolerance and fast growing habit (Mallikarjuna *et al*., 2011). It is the sixth most important grain legume crop grown in the semi-arid tropics of Asia, Africa and the Caribbean under a wide variety of cropping systems (Mula and Saxena, 2010). Pigeon pea is a legume reported to contain 20-22% protein, 1.2 % fat, 65% carbohydrate and 3.8% ash (FAO 1982). Its demand in India is significant because it can provide high quality protein in diet, especially to the vegetarian population (Bhattacharjee *et al*., 2013). It is a fast growing, hardy, widely adaptable and drought resistant (Bekele-Tessema, 2007). It is very heat-tolerant and grows better in places where temperatures range from 20° to 40°C and which are deprived of frost (FAO, 2016). Being environmental friendly by fixing nitrogen, flexibility for mixed cropping or inter crop, due to this it has significant position in dry land farming systems especially adopted by small and marginal farmers in many parts of world (Pandit *et al*., 2015). In addition to being efficient in fixing nitrogen in field conditions, pigeon pea rhizobia also present other biotechnological applications, such as biopolymer production and enzymatic activity (Fernandes *et al*., 2012; Junior *et al*., 2011). Its deep taproot is able to extract nutrients from the low layers lower layers of soil, and bring them to upper layers where they can benefit to other crops (Valenzuela, 2011). Thanks to drought resistance as it can be considered of utmost importance for food security in regions where rain failures are prone to occur (Crop Trust, 2014).

Effort to improve the health and nutritional status of growing children has focused on the production of nutritious, low cost complementary and breakfast foods from combination of cereals and legume. Cereals are generally low in protein and are limited in some essential amino acids such as lysine and tryptophan (Adekunle and Abiodun 2018); legume, on the other hand, is a major source of nutrients such as protein and minerals. The grains most commonly used in processing of breakfast foods are corn, wheat, oats, rice and barley and the least commonly consumed are acha (fonio), finger millet etc. Fonio (Digitaria exilis), an underutilized cereal, is one of the most nutritious cereals. It is one of the oldest cereals characterized with a pleasant small seeds (Jideani 2012). It covers about 300,000 hectares and serves as staple food for about 4 million people in some parts of Africa particularly northern part of Nigeria (Kwon-Ndung and Ochigbo 2001). It is traditionally used in production of unfermented porridge, gwette, pudding, etc. and consumed as first meal of the day.

Pigeon pea (*Cajanus cajan* L.) is extensively utilized in the form of a pulse and is considered an inexpensive source of proteins. Besides, it is a vital source of nutraceutical and bioactive components (Rizvi *et al.*, 2022). The bioactive components of pigeon pea were examined for their role in increasing the anti-carcinogenic and antioxidant effects, as well as these, have

been reported to play a crucial role in modulating the gut microbiota (Talari and Shakappa, 2018). It is a great source of B-complex vitamins, carbohydrates, and minerals. Pigeon pea when supplemented with other cereals provides a well-balanced diet with all essential amino acids and is equivalent to other protein-rich sources such as soybean and whey (Akporhonor *et al.,* 2006). Due to the existence of various flavonoids and polyphenolic compounds in pigeon pea, it has several nutraceutical characteristics in addition to its high nutritional value. Several studies have shown that consuming pigeon pea reduces the risk of various lifestyle diseases such as diabetes, obesity, cancer, and cardiovascular disorders (Singh and Basu, 2012). Pigeon pea is a dense source of nutrients, but some anti-nutrients such as phytic acid, tannins, and trypsin inhibitors bind with its nutritional elements making them unavailable to our body. Phytic acid binds with dietary minerals, such as iron, calcium, zinc, etc., tannins bind with proteins preventing their absorption, and trypsin inhibitors bind with the enzyme trypsin, thereby reducing its biological activity.

Fermenting is a conventional method used for hydrating the grains in the water (Embaby, 2010) and proved useful for the reduction as well as the elimination of the anti-nutrients existing in the food grains (Singh *et al.,* 2017). It has been reported from various studies that fermenting of food grains for 12–18 h is the best effective processing treatment to decrease the level of anti-nutrients such as trypsin inhibitors, phytic acid, etc. which are wholly or partially soluble in water (Embaby, 2010 and Kajihausa *et al.,* 2014). The present research is aimed to determine the proximate and mineral components of fermented pigeon pea (*Cajanus cajan* L.) seed.

MATERIALS AND METHODS

Sample Collection and Fermentation

The bulk of healthy seeds of pigeon pea were purchased from an open market at Lusada, in Ogun State, Southwest Region of Nigeria. The seeds were sorted manually to remove stones, damaged and immature seeds from the lot. The clean, healthy and matured seeds were used for the study.

The bulk of matured, healthy and clean seeds were boiled in distilled demineralized water (1/10 v/v) for 1 hour to soften the seed coats and then dehulled manually using hand pressure. Sodium chloride (NaCl) was added to cotyledons at the rate of 1g/kg before grinding to a paste.

The paste was divided into six equal portions. The first portion was reserved as control (unfermented seeds), while the remaining five portions were allowed to ferment for 1, 2, 3, 4, and 5 days, respectively. Each of the five portions to be fermented, the paste was wrapped in

50g/pack in flamed-blanched plantain leaves, to provide warm humid environment for natural fermentations to take place (Braber *et al.,* 1989).

Sample Preparation for Analysis

At the end of designated fermentation times, all the fermented pastes and the unfermented (control) seed paste were dried in the hot air oven at 65^0C for 24 hours, cooled and then milled into flour. The milled flour were screened through a standard sieve (40mm mesh) and then kept in airtight containers for subsequent chemical analyses.

Chemical Analyses

Unfermented and fermented seed flours were analyzed for proximate composition and mineral composition as described below:

Proximate composition

Moisture, total nitrogen, ether extract, crude fibre and total ash were determined in accordance with the procedures of AOAC (1990). The crude protein content was calculated by multiplying the total nitrogen by 6.25. Nitrogen free extractive (NFE) was estimated by difference. The total ash was fractionated into soluble and acid-insoluble ash as described by Egan *et al.,* (1981). The energy content of the samples was estimated by multiplying the percentages of crude protein, ether extract, and NFE by the factors of 4, 9, and 4 respectively (Osborne and Voogt, 1978).

Determination of moisture content

Procedure:

A clean, dried and cooled silica dish was tare-weighed. 1g of milled test food sample was weighted into the dish, and thereafter, the dish and the weighted sample was placed in the oven. The oven temperature was adjusted to 105^0C, and the drying of the sample was done overnight.

The dried sample was cooled in a desiccator, and then weigh. Loss in weight was noted. Moisture content was calculated using the following formula:

$$\%\ Moisture\ Content = \frac{Loss\ in\ Weight}{Weight\ of\ food\ Sample} \times\ 100$$

Dry matter was calculated as:

% Dry matter = 100 - % Moisture Content

Determination of crude protein content

Procedure:

0.35g mercuric oxide, 3g powdered potassium sulphate and 15 cm^3 concentrated sulphuric acid were added to 0.2g milled food sample in a micro Kjeldahl flask. The mixture was heated on sand bath until the resultant solution became clear i.e. colourless. The clear and colourless digest was allowed to cool before making up to 100 cm^3 mark with distilled water. The distillation unit was assembled and warmed for 5-10 minutes by boiling about 25cm^3 of distilled water in the unit. Using the funnel, 5cm^3 of the dilute digest was introduced into the steamed distillation unit, followed by1cm^3 of 1% of sodium thiosulphate. The funnel was filled with 40% of potassium hydroxide solution. 10cm^3 of 1% boric acid solution containing mixed indicator was transferred into a receiving conical flask. About 10 cm^3 of 40% of potassium hydroxide solution was introduced through the funnel into the distillation flask and ammonium (distillate) was collected in the receiving flask for the next 15-20 minutes after noticing colour change (Wine to Green). The content of the receiving flask was titrated against 0.01M hydrochloric acid (the colour change indicated Green to Pink).

Calculation of the percentage crude protein (%CP) was done using the following formula:

$$\%CP = 0.000014 \times V_T \times \left(\frac{100}{5}\right) \times \left(\frac{100}{W}\right) \times 6.25$$

Where V_T = Titre value, and W = Weight of Sample

Determination of ether extract

Procedure:

1g (W_1) of milled food sample was introduced into a dry soxhlet thimble, and placed in a soxhlet extractor. The extractor is attached to a round bottom flask containing 250 cm^3 petroleum ether. With a heating mantle, the set up was heated to keep the ether boiling gently at a temperature of about 65^0C. Continuous extraction was allowed to go on for 2 hours, after heating was stopped, and the thimble with the residue was removed, dried, and cooled in a desiccator overnight. The cooled residue was weighed (W_2).

The percentage ether extract was calculated as follows:

$$\%\ Ether\ Extract = \left(\frac{(W_1 - W_2}{W_1}\right) \times 100$$

Determination of crude fibre

Procedure:

1g of milled sample was weighed and transferred quantitatively into a 500 cm^3 round bottom flask. 100cm^3 of digestion reagent was added to the food sample while washing down the sides of the flask. The mixture was boiled under reflux for exactly 40 minutes; counting from the time heating commenced. A long 3ft air condenser was used for the refluxing to prevent loss of liquid. The flask was removed from the heater and the content was filtered hot through a sintered glass funnel under suction. The residue was washed six times with hot water, and once with industrial spirit. Thereafter, the residue was transferred into a silica dish, dried in the oven over night at 105^0C, cooled in a desiccator and weighed (W_1). The dried residue was ignited at 600^0C for 3 hours in a furnace, cooled in a desiccator and then weigh (W_2).

Calculation of the percentage crude fibre was done as follows:

$$\% \text{ Crude fibre} = (W_1 — W_2) \times 100$$

Determination of total ash

Procedure:

1g of milled sample was weighed into a silica dish, which had been previously ignited at 600^0C for 3 hours in muffle furnace, cooled and weighed (W_1). The dish containing the food sample was transferred into the muffle furnace, and allowed to ash at 600^0C for 3 hours. Finally, the dish containing the ash was cooled in a desiccator and weigh (W_2).

Calculation of the percentage total ash was done as follows:

$$\% \text{ Total Ash} = (W_1 — W_2) \times 100$$

Determination of nitrogen free extractives (NFE)

Nitrogen free extractives, was calculated by difference as follows:

% NFE = 100 - (% Moisture + % Crude Protein + % Ether Extract + % Crude Fibre + % Total Ash)

Determination of dry matter content

Dry matter content of the food sample was obtained from the equation:

% Dry Matter = 100 – (% Moisture content)

Mineral composition

Wet oxidation:

Prior to mineral analysis, samples were subjected to wet oxidation as follows:

2.0g of milled sample was weighed into 300 cm^3 Kjeldahl flask that has previously been acid and glass-distilled water washed. 4 cm^3 of perchloric acid (A.R. 60%) and 25 cm^3 of concentrated nitric acid (A.R.) were added. By gently swilling the content of the flask were mixed well. Thereafter, the contents of the flask were heated gently on small burner in the Kjeldahl rack. After 5 minutes dense brown fumes were evolved, then the flask were removed from the burner until the initial vigorous reactions was subsided. The flasks were replaced in the burner and heated slowly for about 2 hours. The completion of digestion was indicated by the appearance of dense white fumes and was discontinued after 5 minutes of the first appearance. At this stage the digest was cleared. Finally, the flask was heated strongly for 1 minute to remove the traces of perchloric and nitric acids and allowed to cool. Later, 50 cm^3 of glass distilled water was added and boiled for half a minute, cooled and was transferred to 100 cm^3 standard volumetric flask and was made up with distilled deionized water when cooled. This solution was reserved for the determination of mineral elements using Atomic Absorption Spectrophotometric method (AOAC, 1980).

Mineral elements analyses by AAS:

The digested elements were transferred into a teflon boat and analyzed on direct reading arc-spark emission spectrograph.

Results of the proximate composition and energy value of raw and fermented pigeon pea seeds are presented in Table 1 below. Results show that there is marked variation in the raw and fermented seeds. The seeds had 9.58 - 20.07% moisture, 79.93 – 90.42% dry matter, 5.10 -8.10% total ash, 17.46 – 22.38% crude protein, 2.72 – 7.84% fat, 8.21 – 9.39% crude fibre, 57.44 – 61.73% nitrogen free extractives and 358.90 – 373.30 Kcal/100g gross energy.

Table 1: Proximate composition and energy value of pigeon pea as affected by fermentation

Fermentation (day)	Moisture (%)	Dry Matter (%)	Total Ash (%)	Crude Protein (%)	Ether Extract (%)	Crude Fibre (%)	Nitrogen Free Extractives (%)	Energy (Kcal/100g)
0	9.81	90.19	5.10bc*	22.38	2.72b	8.21	61.24	358.90
1	10.44	89.52	6.20ab	19.87	4.45b	8.25	61.73	362.45
2	9.58	90.42	6.70ab	18.56	6.64a	8.42	59.67	368.09
3	9.63	90.37	6.90ab	18.56	6.65a	9.39	58.50	368.09
4	20.07	79.93	7.60a	18.30	7.50a	8.80	58.15	373.30
5	19.57	80.43	8.10a	17.46	7.84a	9.16	57.44	370.16
±SEM^{+}	2.103	2.101	0.182	0.710	0.809	0.202	0.616	2.146

*Mean values followed or denoted by different subscripts in a column are significantly different at P (O.05)

$^{+}$SEM, standard error of the mean

Results of the mineral element compositions of raw and fermented pigeon pea seeds are presented in Table 2. The results showed that the seeds contained 0.018 – 0.020 mg/g copper, 0.063 – 0.185 mg/g iron, 0.035 – 0.045 mg/g zinc and 0.015 - 0.019 mg/g manganese. The contents of all the mineral elements except iron were not significantly ($P<0.05$) affected by fermentation.

Table 2: Mineral element compositions of pigeon pea as affected by fermentation

Fermentation (day)	Copper ($mg.g^{-1}$)	Iron ($mg.g^{-1}$)	Zinc ($mg.g^{-1}$)	Manganese ($mg.g^{-1}$)
0	0.019	0.111b*	0.035	0.015
1	0.019	0.119b	0.040	0.016
2	0.018	0.063c	0.040	0.019
3	0.019	0.109b	0.041	0.018
4	0.020	0.115b	0.045	0.019
5	0.018	0.185a	0.042	0.016
$\pm SEM^{+}$	0.0077	0.0477	0.0165	0.0070

*Mean values followed or denoted by different subscripts in a column are significantly different at P (O.05)

$^{+}$SEM, standard error of the mean

DISCUSSION

Results in Table 1 shows that the proximate composition of unfermented pigeon pea seeds are somewhat in agreement with that reported by Adebowale and Maliki (2011) for dry matter (89.80%), ash (4.61%), protein (21.80%) and ether extracts (2.74%) contents. Lower values compared to those obtained in the present study were reported for nitrogen free extractives (53.40%) and gross energy (325.46 Kcal/100g). On the other hand, values obtained for NFE (61.24%) and gross energy (358.90 Kcal/100g) in the raw seed in the present study are similar to those reported (62.6% NFE and 362 Kcal/100g) by Oloyo (2004a).

Variability in the proximate composition and energy values of the raw and fermented seeds suggested that fermentation remarkably affected the nutrient values where dry matter, protein and NFE decreased as fermentation progressed, ether extract, ash and gross energy increased.

Adebowale and Maliki (2011) had reported that whereas fermentation resulted in reduction of dry matter and NFE in pigeon pea seeds, total ash content increased.

Fermentation is a metabolic process in which an organism converts a carbohydrate, such as starch or a sugar, into an alcohol or an acid. Consequently, the NFE that is essentially carbohydrate is the most preferred substrate on which the microorganism acts upon and thus will diminish as the fermentation process progressed.

The results also revealed that whereas fermentation for up to 3 days increased total ash marginally, fermentation for 2 day was adequate for increasing the ether extract. The decrease in the NFE with fermentation is expected to cause decrease in the gross energy of the seeds. The increase in the energy observed in the fermented seeds might be attributable to the significant increase in the ether extract content. A gram of fat supplies two and a half times the amount of energy a gram of carbohydrate will produce (Osborne and Voogt, 1978).

The results therefore tended to suggest that fermentation of the pigeon pea seeds for a minimum of 2 day was adequate to improve on the gross energy value of the seeds.

Results in Table 2 show that while fermentation significantly ($P>0.05$) affected iron contents of the pigeon pea seeds, copper, zinc and manganese contents were not ($P<0.05$). The highest concentration of iron was found in seeds fermented for 5 days, and the least in those fermented for 3 day.

The raw and unfermented pigeon pea seeds had comparable copper and zinc contents with values reported by Oloyo (2004b). Iron and manganese contents were higher and lower, respectively. The difference in the observed values and earlier reported ones might be due to variability in the source (Kay, 1979).

CONCLUSION

From the foregoing, it may be concluded that proximate composition and digestible energy value of pigeon pea seeds were significantly affected by fermentation. Fermentation of the seeds up to 3 days improved the total ash, fat, gross energy, digestible energy and the iron contents of the seeds. On the other hand, 2 days of fermentation remarkably improved the average digestible energy of the seed.

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