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Effects of processing on the nutrient and anti- nutrient contents of African locust bean (*Parkia biglobosa*) flour.

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Abstract
The effects of boiling and fermentation on the nutrient and anti-nutrient contents of African locust bean flours were studied. The seeds were sorted, cleaned and processed into raw, boiled and fermented African locust bean flours. The flours obtained were evaluated for proximate, mineral and anti-nutrient contents using standard analytical methods. The proximate composition of the samples showed that the flours contained moisture (9.45-10.06%), crude protein (21.24-22.62%), fat (10.31-11.32%), ash (3.00-4.19%), crude fibre (3.25-3.84%), carbohydrate (45.80-52.21%) and energy (378.59-388.83 kJ/100 g), respectively. The mineral composition of the samples showed that the flours had a range of calcium, (175.48-181.27 mg/100 g), magnesium, (140.32-146.34 mg/100g), phosphorus, (169.50-173.38 mg/100 g), sodium, (20.23-24.89 mg/100 g), potassium, (54.28-58.35 mg/100 g) and manganese, (0.13-0.73 mg/100 g), respectively. The anti-nutrient contents of the flours revealed that the tannin, alkaloid, trypsin inhibitor, saponin and oxalate levels of the samples were significantly ($p<0.05$) reduced by boiling than the fermentation treatment. The proximate, mineral and anti-nutrient contents of the flours evaluated revealed that the processed African locust bean flours have the potentials to be used as nutritional supplements in the preparation of a number of food products and condiments than the raw sample.

Keywords: African locust bean flour, boiling, fermentation, proximate composition, mineral content, anti-nutrient content

Introduction

Legumes belong to the family *leguminosae*. They are next important food crops after cereals. They are sources of low-cost dietary vegetable protein and minerals when compared to animal products such as meat, egg, fish and poultry. Indigenous legumes therefore are an important source of affordable alternative protein to poor resource people in many tropical countries especially in Africa and Asia where they are predominantly consumed (Achi, 2005) [1]. Legumes are rich in protein and their chemical composition varies depending on variety, soil and climatic conditions and region. Legumes generally are good supplements to cereals which are limiting in essential amino acid, lysine. *Parkia biglobosa* which is also known as the African locust bean or Ugba is a leguminous crop that is found in a wide range of environments in Africa. African locust bean seeds are used to produce some important food products such as food condiments like iru, dawadawa and okpeye which are primarily used in the preparation of soups, stews and porridges etc. The chemical composition and nutritional significance of the African locust bean have been reported by Omafuvbe *et al.* (2000) [18]. The seeds of *Parkia biglobosa* are relatively rich in fat, protein, sugar (soluble), carbohydrate and ascorbic acid. The cotyledon is very nutritious but it is low in fibre and ash contents when compared to the testa. Also, some simple reducing sugars including lactose are also present in the testa of African locust bean. Alabi *et al.* (2005) [6] reported that the oil derived from the cotyledons of the seeds have relatively high saponification value, low iodine and very low acid values, which make *Parkia biglobosa* seed oil to be very useful in soap making.

African locust bean like every other legume contains a number of anti-nutrients which include phytic acid, protease inhibitors, amylase inhibitors, flatulence factors lectins, saponins and tannins which interfere with digestive processes and prevent efficient utilization of their proteins. These anti-nutrients can be reduced or inactivated by the use of simple processing techniques such as roasting, steam blanching, soaking in water, dehulling, boiling at high temperatures in water, alkaline and acidic solutions, fermentation, germination autoclaving and microwave treatment (Esenwah and Ikenebomeh, 2008) [11]. The seeds can also be processed into cakes and preserved for use in the preparation of some indigenous drinks that are more than adequate to meet the FAO/WHO recommended daily allowance of protein of 0.5 g/kg body weight for an average healthy individual and 0.88 g/kg body weight for children aged 1-10 years (Akoma, 1988) [4]. The fermented African locust bean product is used as a flavouring agent. It is also used as a good source of body building protein. Besides its nutritional value, African locust bean seeds also have some medicinal and health benefits. Indigenous traditional medicine practitioners in Africa use different parts of the African locust bean tree for medicinal purposes. *Parkia biglobosa* is one of the most utilized plants in Togo for the treatment of hypertension (Traore *et al*., 2013) [24]. African locust bean seeds contain linoleic acid as the major fatty acid in addition to other fatty acids such as palmitic, stearic, oleic and linolenic acids which are present in appreciable amounts. Therefore, African locust bean seeds are good sources of essential fatty acids like that of omega-3 and omega-6 polyunsaturated fatty acids (PUFA's) that help in the development of neurons in the body (Okaka *et al*., 2006) [16]. The objective of this study, therefore, was to investigate the effects of boiling and fermentation on the proximate, mineral and anti-nutrient contents of *Parkia biglobosa* flours.

Materials and methods

Mature African locust bean (*Parkia biglobosa*) seeds used for the study were purchased from Ogige Market, Nsukka, Enugu State, Nigeria. The seeds were sorted, cleaned and divided into four equal portions of 500g each. Three portions were subjected to different processing treatments (boiling and fermentation for 48 and 72 h, respectively), while the fourth batch was processed raw.

Preparation of Raw African Locust Bean Flour

The raw African locust bean flour was prepared according to the method of Madubuike *et al*. (2003) [14]. During preparation, five hundred grammes (500 g) of African locust bean seeds which were free from dirt and extraneous materials were cleaned with 2litres of potable water and dehulled manually by cracking the seeds with stone to remove the hulls. The dehulled seeds were spread on the trays and dried in a hot air oven (Model DHG 9101 ISA) at 60 ºC for 6 h with occasional stirring of the seeds at intervals of 30 min to ensure uniform drying. The dried seeds were milled in a hammer mill and sieved through a 500 micron mesh sieve. The flour produced was packaged in an air tight plastic container, labeled and stored in a freezer until needed for further use.

3.2.2 Preparation of Boiled African Locust Bean Flour

The boiled African locust bean flour was prepared according to the method of Esenwah and Ikenebomeh (2008) [11]. During preparation, five hundred grammes (500g) of African locust bean seeds which were free from dirt and extraneous materials were thoroughly cleaned with 2litres of potable water. The cleaned seeds were boiled with 3litres of potable water at 100 ºC on a hot plate for 8 h with occasional addition of water to avoid the drying up of water. After that, the boiled seeds were drained, rinsed and dehulled manually by pressing out of the seeds. The dehulled seeds were rinsed, spread on the trays and dried in a hot air oven (Model DHG 9101 ISA) at 60 ºC for 10 h with occasional stirring of the seeds at intervals of 30 min to ensure uniform drying. The dried seeds were milled in a hammer mill and sieved through a 500 micron mesh sieve. The flour produced was packaged in an air tight plastic container, labeled and stored in a freezer until needed for further use.

3.2.3 Preparation of Fermented African Locust Bean Flour

The fermented African locust bean flour was prepared according to the method of Esenwah and Ikenebomeh (2008) [11]. During preparation, five hundred grammes (500g) of locust bean seeds which were free from dirt and extraneous materials were boiled with 3litres of potable water at 100 ºC on a hot plate for 8 h. The boiled seeds were drained, rinsed and dehulled manually by pressing out of the seeds. The dehulled seeds were drained, rinsed and wet milled in a hammer mill with 2litres of potable water into fine slurry. The slurry obtained was stirred manually with a wooden stirrer and sieved with a muslin cloth into a clean plastic bowl. The sieved slurry produced was divided into two equal parts and then transferred into separate clean bags and allowed to ferment individually in 1.5litres of water with the aid of naturally occurring microbial flora at room temperature (29 ± 2^0C) for 48 and 72 h, respectively. After that, excess water was decanted and the fermented slurry was separately dewatered manually. The cake obtained in each case was spread on the trays and dried in a hot air oven (Model DHG 9101 ISA) at 60 ºC for 12 h with occasional stirring of the cake at intervals of 30 min to ensure uniform drying. The dried fermented cakes were milled separately in a hammer mill and sieved through a 500 micron mesh sieve. The flours produced were packaged individually in air tight plastic containers, labeled and stored in a freezer until needed for further use.

Chemical Analysis

The moisture, protein, fat, crude fibre and ash contents of the samples were determined in triplicate according to standard analytical methods (AOAC, 2006) [7]. The carbohydrate content of the samples was calculated by subtracting the percentage differences of moisture, crude protein, fat and ash from 100 percent (Onwuka, 2005) [21]. Energy was calculated by multiplying the values of protein, fat and carbohydrate by the Atwater factors of 4, 9 and 4, respectively (Tarek, 2002). The calcium content of the samples was determined according to the method of AOAC (2006) [7]. The magnesium, phosphorus and manganese contents of the samples were determined using the atomic absorption spectrophotometer (Perkin-Elmer, Model 1033, Norwalk, CT, USA) according to the methods of Onwuka (2005) [21]. The sodium and potassium contents of the samples were determined according to the flame photometric methods of AOAC (2006) [7].

Anti-Nutrient Analysis

The tannin, alkaloid, trypsin inhibitor and phytic acid contents of the samples were determined in triplicate according to the methods of Onwuka (2005) [21]. The oxalate and saponin contents were determined according to the solvent extraction gravimetric methods of AOAC (2006) [7].

Statistical Analysis

The results were expressed as mean ± standard deviation and the test for statistical significance was carried out using one-way analysis of variance (ANOVA). The Statistical Package for Social Sciences (SPSS, Version 20) software was used to determine the significant differences. Significant means were separated using Turkey’s Least Significance Difference (LSD) test and differences were considered significant at $p<0.05$.

Results and Discussion

Proximate Composition

The proximate composition of raw and processed African locust bean flours are shown in Table 1. The moisture content of the samples was significantly ($p<0.05$) higher in boiled flour than in the fermented samples. The increase could be due to the imbibition of large quantity of water by the seeds during processing as a result of boiling. Omafuvbe *et al.* (2004) reported that the high moisture content of legume and other flours affect their storage stability due to increased microbial action. The observation, however, is in agreement with the report of Madubuike *et al*. (2003) [14] for boiled *Afzelia africana* flour. The crude protein content of the flours was significantly (p<0.05) higher in the flour fermented for 72 h compared to the sample fermented for 48 h and that processed by boiling. The increase in crude protein content could be attributed to decrease in the concentration of carbohydrate which serves as an energy source for the fermentative microorganisms (Ogbadu and Okagbue, 2004) [15]. The fat content of the samples which ranged from 10.31 to 11.32% was significantly (p<0.05) increased by fermentation than the boiling treatment. The increase could be due to decrease in carbohydrate content. However, the result is in agreement with the report of Omafuvbe *et al* (2000) [20] for fermented soybean flour. Fat is important in the diets because it promotes the absorption of fat soluble vitamins and is in itself a high energy yielding nutrient (Okaka *et al*., 2006) [16]. The ash content of the flours varied from 3.00 to 4.19%. The values obtained in this study were lower than the ash content (4.16-4.48%) of boiled and fermented soybean flours. The low ash content of African locut bean flours is an indication that they are not good sources of minerals (Enwere, 1998) [10]. The crude fibre content of flours ranged from 3.25 to 3.84% with the flour fermented for 72 h having the least value followed by the sample fermented for 48 h and that processed by boiling. The reduction in the crude fibre contents of the fermented samples could be attributed to the degradation of fibre components by microbes during fermentation by converting them to volatile fatty acids for their nutrition (Akpet *et al*., 2012) [5]. Fibre has been credited for the promotion of increased excretion of bile salts, sterols and fat which have been implicated in the etiology of certain ailments in humans (Okaka *et al*., 2006) [16]. The carbohydrate content of the samples was significantly (p<0.05) higher in boiled flour than in the samples fermented for 48 and 72 h, respectively. The reduction in carbohydrate content could be due to the utilization of some of the sugars by fermentative microorganisms for growth and metabolic activities during fermentation (Omafuvbe *et al*., 2003) [19]. The energy content of the flours ranged from 378.61 to 388.83 kg/100g. The energy content was significantly (p<0.05) increased by fermentation than the boiling treatment. The differences in energy values of the samples could be attributed to variation in their protein, fat and carbohydrate contents (Esenwah and Ikenebomeh, 2008) [11]. Generally fermentation had a greater effect in increasing the nutrient contents of African locust bean flours than the boiling treatment.

Mineral Composition

The mineral composition of raw and processed African locust bean flours are shown in Table 2. The calcium content of the samples which ranged from 175.48 to 181.27 mg/100g was significantly (p<0.05) lower in the boiled flour compared to the samples fermented for 48 and 72 h, respectively. This increase in calcium content of the fermented samples could be due to its insolubility in water which makes it not to be easily leached out into the processing water during fermentation (Oladunmoye, 2007) [17]. Calcium is one of the macro minerals and it is involved in bone formation in conjunction with magnesium, phosphorus and protein (Okaka *et al*., 2006) [16]. The magnesium content of the flours was significantly (p<0.05) increased by fermentation compared to the sample processed by boiling. Magnesium helps in the maintenance of electrical potential of the nerves (Graham and Welch, 1996) [13]. The phosphorus content which ranged from 169.50 to 173.38 mg/100g was significantly (p<0.05) increased by the fermentation than the boiling treatment. Phosphorus helps in the maintenance of the acid-base balance of the body. It is also required in reasonable amounts by young children, pregnant and nursing mothers (Okaka *et al*., 2006) [16]. The sodium content of the samples which varied from 20.23 to 24.89 mg/100g was significantly (p<0.05) reduced by boiling than the fermentation treatment. The decrease could be due to leaching of the mineral into boiling water during processing. Sodium is one of the major extra-cellular ions of the body which helps in the maintenance of osmotic equilibrium and volume of the body fluid (Graham and Welch, 1996) [13]. The potassium content of the flours was significantly (p<0.05) increased by the fermentation compared to the sample processed by boiling. The increase could be due to the microbial synthesis of the mineral element during processing as a result of fermentation (Ajeigbe *et al*. 2012). Potassium is very essential in blood clotting and muscle contraction (Okaka *et al*., 2006) [16]. The manganese content of the samples which ranged from 0.13 to 0.73 mg/100g decreased significantly (p<0.05) by boiling than the fermentation treatment. Manganese is one of the micro minerals that helps in the formation of connective tissues, bones, blood clotting factors and sex hormones. It also plays a significant role in fat and carbohydrate metabolism, calcium absorption and blood sugar regulation in the body (Apet *et al*. 2012).

Generally, fermentation had a greater effect in improving the micro nutrient contents of African locust bean flours than the boiling treatment.

Anti-Nutrient Composition

The anti-nutrient composition of raw and processed African locust bean flours are shown in Table 3. The tannin content of the samples was significantly (p<0.05) reduced by boiling than the fermentation treatment. Tannins have been reported to be one of the metal chelating compounds that precipitate proteins. Tannin interacts with protein and starch to form complexes that are resistance to hydrolysis by enzymes which result in depressed utilization of protein and starch after consumption (Aremu *et al*., 2006) [8]. Tannins at their safe level (below 100 ppm) (Frias *et al*., 2005) [12] have some health benefits. They help to prevent cavities, diarrhoea, tooth decay and even heart diseases. The alkaloid content of the samples which ranged from 0.28 to 1.18% was significantly (p<0.05) lower in the boiled flour compared to the samples processed by fermentation. The values obtained in the study are within the safe level recommended for alkaloid which is in the range of 17-23 mg/g (Vinay *et al*., 2014) [26]. Alkaloids contain nitrogen in them and so are alkaline in nature. They are less soluble in water and they are used for the treatment of heart failure, blood pressure and migraine. The trypsin inhibitor content of the samples was significantly (p<0.05) lower in the boiled flour than in the fermented samples. Trypsin inhibitors are globular proteins found in most legumes which have the capacity to reduce the biological activity of trypsin enzyme (Okaka *et al*., 2006) [16]. The phytate content of the samples which ranged from 0.60 to 0.83% was significantly ($p<0.05$) reduced by fermentation compared to the sample processed by boiling. The values obtained in this study are within the safe level which is in the range of 5-10 mg/g (Schlemmer, 2009) [22]. Phytate related compounds have been reported to have beneficial effect as an antioxidant (Echendu *et al*., 2009) [9]. The oxalate content of raw African locust bean flour which was 0.28% was significantly ($p<0.05$) reduced by boiling than the fermentation treatment. The observation is in accordance with the report of Esenwah and Ikenebomeh (2008) [11] for fermented and boiled African locust bean flours. Oxalates affect calcium and magnesium metabolism. They also react with proteins to form complexes which have an inhibitory effect on peptic digestion (Akande *et al*., 2010). Oxalates also tend to bind to calcium to form calcium oxalates which are responsible for the formation of kidney stone in human subjects. The saponin content of the flours ranged from 0.13% to 0.28% with the boiled sample having the least value. Saponins at the recommended safe dosage (below 22.4 mg/g) reduce the blood lipids and possess antioxidant effect (Vinay *et al*., 2014) [26]. In effect, the result showed that fermentation had a greater effect in reducing the levels of anti-nutrients present naturally in African locust bean seeds than the boiling treatment.

Conclusion

The boiling and fermentation treatments affected the nutritional value of African locust bean flours. The higher protein, calcium, phosphorous, sodium and manganese contents observed in processed African locust bean flours showed significant improvement in their nutrient density. In addition, the levels of tannin, oxalate, phytate, saponin, and alkaloid and trypsin inhibitor were also reduced drastically by boiling and fermentation treatments. Although boiling drastically improved the nutritional value of the product, fermentation resulted in the production of better quality flours that will extend the utilization of the products as nutritional supplements in the preparation of a wide range of food condiments.

Table 1: Proximate composition (%) of African locust bean flours.

Parameter	Raw	Boiled	Fermented for 48 h	Fermented for 72 h
Moisture	9.45^{d}± 0.04	10.06^{a}± 0.03	9.66^{c}± 0.00	9.74^{b}± 0.06
Crude protein	21.26^{c}± 0.06	21.24^{c}± 0.06	21.45^{b}± 0.04	22.62^{a}± 0.01
Fat	10.31^{d}± 0.01	10.71^{c}± 0.01	11.19^{b}± 0.00	11.32^{a}± 0.01
Ash	4.19^{a}± 0.01	3.93^{b}± 0.00	3.37^{c}± 0.03	3.00^{d}± 0.14
Crude fibre	3.84^{a}± 0.04	3.66^{b}± 0.00	3.54^{c}± 0.05	3.25^{d}± 0.04
Carbohydrate	45.80^{d}± 3.54	54.21^{a}± 0.01	49.99^{b}±0.08	49.66^{c}± 0.11
Energy (kJ/100g)	382.61^{c}± 13.	378.59^{d}± 0.07	387.66^{b}± 038 387.66 ^{b}± 0.20	388.83^{a}± 0.33

Values are mean ± standard deviation of triplicate determinations. Values followed by different superscripts in the same row are significantly different ($p<0.05$).

Table 2: Mineral composition (mg/100g) of African locust bean flours.

Parameter	Raw	Boiled	Fermented for 48 h	Fermented for 72 h
Calcium	178.37^{c}± 0.12	175.48^{d}± 0.00	179.61^{b}± 0.01	181.27^{a}± 0.05
Magnesium	143.45^{c}± 0.24	140.32^{d}± 0.00	145.76^{b}± 0.06	146.34^{a}± 0.02
Phosphorus	169.50^{d}± 0.03	170.83^{c}± 0.01	172.57^{b}± 0.05	173.38^{a}± 0.04
Sodium	20.27^{d}± 0.10	23.77^{c}± 0.04	24.71^{b}± 0.13	24.89^{a}± 0.03
Potassium	54.32^{c}± 0.04	56.28^{d}± 0.11	57.71^{b}± 0.13	58.35^{a}± 0.01
Manganese	0.13^{d}± 0.01	0.38^{c}± 0.00	0.47^{b}± 0.02	0.73^{a}± 0.04

Values are mean ± standard deviation of triplicate determinations. Values followed by different superscripts in the same row are significantly different ($p<0.05$).

Table 3: Anti-nutrient composition (%) of African locust bean flours.

Parameter	Raw	Boiled	Fermented for 48 h	Fermented for 72 h
Tannin	0.42^{b}± 0.01	0.15^{c}± 0.00	0.36^{a}± 0.08	0.34^{a}± 0.00
Alkaloid	1.18^{a}± 0.01	0.28^{d}± 0.01	1.12^{b}± 0.00	0.85^{c}± 0.01
Trypsin Inhibitor	15.83^{a}± 0.10	6.32^{d}± 0.03	8.86^{b}± 0.06	6.56^{c}± 0.34
Phytate	0.83^{a}± 0.01	0.54^{c}± 0.00	0.60^{b}± 0.02	0.60^{b}± 0.02
Oxalate	0.28^{a}± 0.00	0.16^{d}± 0.00	0.24^{b}± 0.01	0.18^{c}± 0.01
Saponin	0.28^{a}± 0.00	0.13^{b}± 0.01	0.05^{b}± 0.01	0.19^{c}± 0.01

Values are mean ± standard deviation of triplicate determinations. Values followed by different superscripts in the same row are significantly different ($p<0.05$).

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