

## COMPARISON BETWEEN THE AMINO ACID, FATTY ACID, MINERAL AND NUTRITIONAL QUALITY OF RAW, GERMINATED AND FERMENTED AFRICAN LOCUST BEAN (*PARKIA BIGLOBOSA*) FLOUR

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### ABSTRACT

**Background.** The most popular form of utilization of African locust bean (ALB) is in its traditional fermentation food condiment (*iru/dawadawa*), which adds protein to a protein-poor diet and also as Medicine. In view of the nutritive values of ALB, the present study therefore aimed at investigating the effect of germination and fermentation on the nutritional quality of ALB flour.

**Material and methods.** The ALB was obtained from a local market in Akure, Nigeria. The seeds were divided into three portions, and treated as raw African locust bean (RALB), germinated African locust bean (GALB) and fermented African locust bean (FALB) respectively. Each of the samples was milled, sieved and analysed for chemical, functional properties and nutritional qualities using standard methods.

**Results.** Some most important results of the chemical analysis were as follows: protein content range between  $33.64 \pm 0.41 - 41.49 \pm 1.89$  g/100 g, while the energy value was between  $442.79 \pm 2.32 - 457.20 \pm 2.15$  kcal. The P/Ca and Na/K ratio of the RALB were higher than other flour samples respectively. Total essential amino acid was between 29.960-27.514 mg/100 g. Protein efficiency ratio (PER) was between 1.78-1.87; essential amino acid index 31.43-34.75%; while biological values were 22.56-26.18%. The dominant fatty acid (FA) composition of the samples was linoleic with 33.687%, 31.578% and 28.7% for RALB, GALB and FALB respectively; while the least was lauric acid. The polyunsaturated/saturated FA ratio ranges between 0.589-0.718. The antinutrient concentration of fermented flour sample was significantly reduced than other food samples.

**Conclusion.** The present study investigated the effect of germination and fermentation on the nutritional quality of ALB flour. The finding showed that fermentation technique significantly reduced antinutrient concentration and also improved the nutrient composition, particularly amino acid profile of ALB flour.

**Key words:** African locust bean, amino acids, fatty acids, nutritional quality, germination and germination

## INTRODUCTION

Legumes offer a singular advantage of providing plant proteins with reduced cost of production, less difficulty of processing and provide higher energy value than those supplied by animal protein [Balogun and Fetuga 1986]. High cost of animal protein has directed the interest towards several leguminous seed proteins as potential sources of vegetable protein for human food and livestock feed. Among the plant species, grain legumes are considered as the major source of dietary proteins. They are consumed worldwide, especially in developing and underdeveloped countries where consumption of animal protein may be limited as a result of economic, social, cultural or religious factors.

Among the leguminous plants used by man particularly in some African countries, is the African locust bean tree (*Parkia biglobosa*). The tree is a perennial deciduous *leguminous* tree with pods ranging from pink brown to dark brown, when matured. The pods are reported to contain up to 30 seeds embedded in a yellow pericarp. The seeds having a mean weight of 0.26 g/seed have a hard testa with large cotyledons forming about 70% of their weight. *P. biglobosa* is well known for its high commercial values as food and medicinal agent. The most popular form of consumption of African locust beans is in its traditional fermentation tasty food condiment called *dawadawa* which is used as a flavour intensifier for soups and stews and also adds protein to a protein-poor diet [Campbell-Platt 1980, Ikenebomeh and Kok 1984, Odunfa 1986, Dike and Odunfa 2003]. Nutritionally, African locust bean is such a leguminous plant with an outstanding protein quality and its protein and amino acid composition has been reported by several researchers [Cook et al. 2000, Lockeett et al. 2000, Alabi et al. 2003, Elemo et al. 2011].

To improve the nutritional quality and organoleptic acceptability of leguminous seeds, processing techniques have been reported by several investigators to enhance the nutritional quality and also to reduce or destroy the antinutrients present in them [Esenwah and Ikenebomeh 2008]. Some of the commonly used processing techniques include soaking in water, boiling at high temperatures in water, alkaline or acidic solutions, sprouting, autoclaving, roasting, dehulling,

microwave treatment, steam blanching and fermentation [Esenwah and Ikenebomeh 2008, Słupski 2011 a, b]. The present study, therefore, aims at investigating the nutrient composition of processed African locust bean subjected into germination and fermentation processing methods.

## MATERIAL AND METHODS

### Processing of raw, germinated and fermented African locust beans

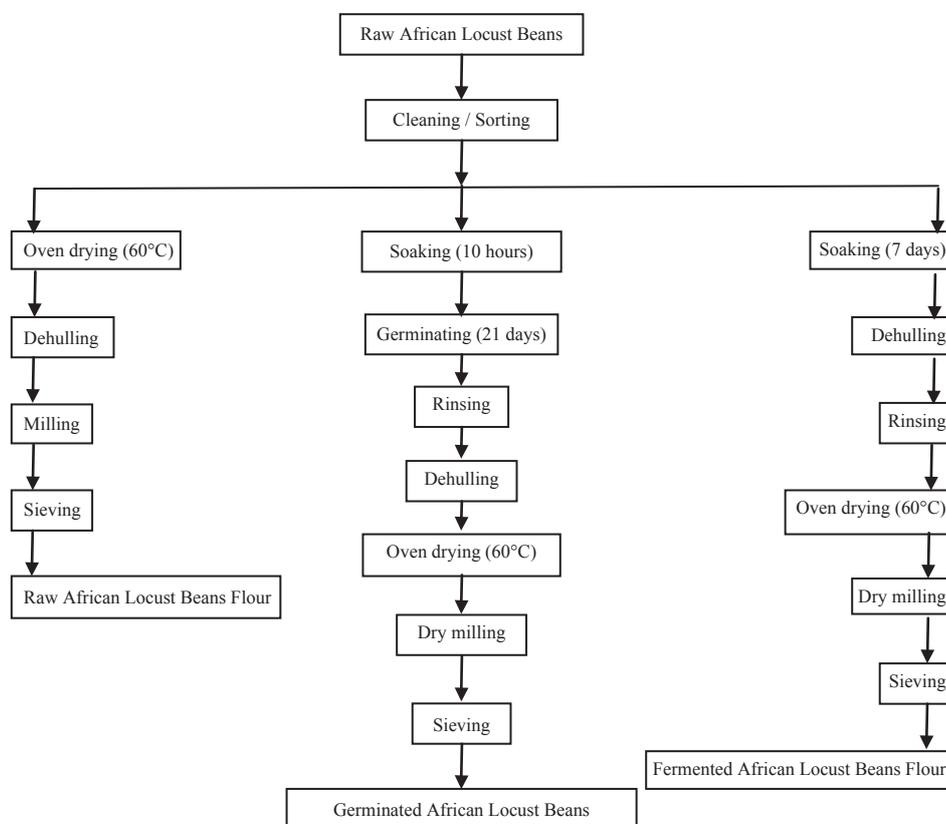
**Raw.** The African locust beans were obtained from a local market (Erekensan), Akure, Nigeria. The seeds were sorted, dehulled, oven dried, milled in attrition mill and sieved through 0.4 mm wire mesh. The raw African locust bean flour was packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses. The flour was prepared according to the flow chart in Figure 1.

**Fermentation.** The African locust beans were sorted and soaked in hot water and left for 7 days, dehulled, cooked for 1 hour and fermented for 1 day. The fermented seeds were oven dried in hot air oven at 60°C for 20 hours, milled with attrition mill (locally fabricated grinding machine), sieved and packed in plastic container sealed with aluminum foil and stored at room temperature prior to analyses. The flour was prepared according to the flow chart in Figure 1.

**Germination.** The African locust beans were sorted and soaked for 24 hours. The seeds were spread on jute bag and kept wet by frequent spraying of water at every morning and evening for 7 days to sprout. The germinated seeds were washed, oven dried at 60°C for 20 hours, milled and sieved through 0.4 mm wire mesh and stored at room temperature in a well sealed plastic container prior to analyses. The flour was prepared according to the flow chart in Figure 1.

### Proximate analyses

Proximate analysis was carried out on the raw, germinated and fermented African locust bean flour. The moisture content was determined using AOAC [2005], protein was determined by micro-Kjeldahl using the Tecator Digestion System and Kjeltac Auto 1030 Analyzer (Tecator AB, Sweden). Fat was determined by ether extraction using the Soxtec System HT method (Tecator Soxtec System HT 1043 Extraction Unit,



**Fig. 1.** Production of germinated and fermented popcorn flour

Tecator AB, Sweden). Ash was determined by AOAC [2005] method. The carbohydrate content was determined by difference. Addition of all the percentages of moisture, fat crude protein, and ash, crude fibre was subtracted from 100%. This gave the amount of nitrogen free extract otherwise known as carbohydrate.

$$\% \text{ carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ fat} + \% \text{ ash} + \% \text{ crude fibre} + \% \text{ crude protein})$$

The sample calorific value was estimated (kcal/g) by multiplying the percentages of crude protein, crude lipid and carbohydrate with the recommended factors (2.44, 8.37 and 3.57 respectively) as proposed by Martin and Coolidge [1978].

### Mineral analyses

The method described by Association of Official Analytical Chemists [AOAC 2005] was used for mineral analysis. The samples were ashed at 550°C.

The ash was boiled with 10 ml of 20% hydrochloric acid in a beaker and then filtered into a 100 ml standard flask. This was made up to the mark with deionized water. The minerals were determined from the resulting solution. Sodium (Na) and Potassium (K) were determined using the standard flame emission photometer. NaCl and KCl were used as the standards [AOAC... 2005]. Phosphorus was determined colorimetrically using the spectronic 20 (Gallenkamp, UK) Kirk and Sawyer [1991] with  $\text{KH}_2\text{PO}_4$  as the standard. Calcium (Ca), Magnesium (Mg) and Iron (Fe) were determined using Atomic Absorption Spectrophotometer (AAS Model SP9). All values were expressed in mg/100 g.

### Amino acid determination

Amino acid composition of samples was measured on hydrolysates using amino acid analyser (Sykam-S7130) based on high performance liquid

chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stein [1963]. Each of the defatted samples was weighed (200 mg) into glass ampoule, 5 ml of 6M HCl added and hydrolyzed in an oven preset at  $105 \pm 5^\circ\text{C}$  for 22 h. Oxygen was expelled in the ampoule by passing nitrogen gas into it. Amino acid analysis was done by ion-exchange chromatography [Spackman et al. 1958] using a Technicon Sequential Multisample Amino Acid Analyzer (Technicon Instruments Corporation, New York, USA). The period of analysis was 76 min, with a gas flow rate of 0.50 ml/min at  $60^\circ\text{C}$ , and the reproducibility was  $\pm 3\%$ . The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein.

### Nutritional quality determinations

Nutritional qualities were determined on the basis of the amino acid profiles. The Essential Amino Acid Index (EAAI) was calculated using the method of Labuda et al. [1982] according to the equation below:

$$\text{EAAI} = \sqrt{\frac{[\text{Lys} \times \text{Threo} \times \text{Val} \times \text{Meth} \times \text{Isoleu} \times \text{leu} \times \text{Phynylal} \times \text{Histi} \times \text{Trypt}]_a}{[\text{Lys} \times \text{Threo} \times \text{Val} \times \text{Meth} \times \text{Isoleu} \times \text{leu} \times \text{Phynylal} \times \text{Histi} \times \text{Trypt}]_b}}$$

where:  $[\text{lysine} \times \text{threonine} \dots]_a$  in test sample and  $[\text{lysine} \times \text{threonine} \dots]_b$  content of the same amino acids in standard protein [%; egg or casein] respectively.

Nutritional indices of the food samples were calculated using the formula below:

Biological value was calculated according to Oser [1959] cited by Mune-Mune et al. [2011] using the following equation:

$$\text{BV} = 1.09 \times \text{essential amino acid index (EAAI)} - 11.7$$

The Protein Efficiency Ratio (PER) was estimated according to the regression equations developed by Alsmeyer et al. [1974] cited by Mune-Mune et al. [2011] as given below:

$$\text{PER} = -0.468 + 0.454 (\text{LEU}) - 0.105 (\text{TYR})$$

### Fatty acids determination

Fatty acid compositions of the samples were analysed using gas-liquid chromatography (with omega-wax capillary column Supelco, USA). The lipid classes

were separated by thin layer chromatography on silica gel G 60 (Merck, Darmstadt), using n-hexane/ethyl-ether/acetic acid (73/25/2/v/v/v) as developing solvent. The fatty acids of phospholipids and triglycerides were transformed with sodium methylate into methylesters.

### Anti-nutritional composition of the samples

**Determination of trypsin inhibitor activity (TIA).** Trypsin inhibitor activity of sample was determined by the method of Kakade et al. [1974]. The digest contained 1.0 g of the sample, 40  $\mu\text{g}$  of trypsin and 2 mg of benzoyl-DL-arginine-P-nitroanilide (BAPA) in Tris buffer. The absorbance of sample was read at 410 nm.

**Determination of tannin content.** The method of estimation of tannin content in extract by Joslyn [1970] was used for the determination of tannin content in samples. Finely ground sample (0.5 g) was defatted with 5% ethyl ether for 15 min. The tannin in the defatted sample was then extracted with methanol and the absorbance at 760 nm was measured.

**Determination of phytic acid.** An indirect colorimetric method of Wheeler and Ferrel [1971] was used for phytate determination. This method depends on an iron to phosphorus ratio of 4:6. Five grams of the test sample were extracted with 3% tri-chloro acetic acid. The phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding sodium hydroxide. The precipitate was dissolved in hot 3.2 N HNO and the colour read immediately at 480 nm. The standard solution was prepared from  $\text{Fe}(\text{NO}_3)_3$  and the iron content was extrapolated from a  $\text{Fe}(\text{NO}_3)_3$  standard curve. The phytate concentration was calculated from the iron results assuming a 4:6 iron:phosphorus molecular ratio.

**Determination of oxalate content.** Oxalate was determined by AOAC [2005] method. 1 g of the sample was weighed into 100 ml conical flask. 75 ml of 3 M  $\text{H}_2\text{SO}_4$  was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and then filtered using Whatman No. 1 filter paper. The sample filtrate (extract; 25 ml) was collected and titrated against hot ( $80\text{-}90^\circ\text{C}$ ) 0.1 N  $\text{KMnO}_4$  solution to the point when a faint pink colour appeared that persisted for at least 30 s. The concentration of oxalate in each sample was obtained from the calculation: 1 ml 0.1 permanganate = 0.006303 g oxalate.

## Functional properties

**Water absorption capacity.** Water and oil absorption capacities of the flour samples were determined by Beuchat [1977] methods. Each of the formulated sample was weighed (20 g) and hydrated with 100 ml of distilled water at 25°C for one hour with manual stirring at 10 minutes intervals. Excess water was drained with a Whatman number 2 filter paper with slight suction. The water absorption index was calculated as follows:

$$\text{WAC} = \frac{\text{weight gain upon hydration} \times 100}{\text{dry weight}}$$

**Bulk density.** A 50 g flour sample was put into a 100 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density ( $\text{g} \cdot \text{cm}^{-3}$ ) was calculated as weight of flour (g) divided by flour volume ( $\text{cm}^3$ ) [Okaka and Potter 1979].

**Swelling capacity.** This was determined with the following method. One gram of the flour sample was mixed with 10 ml distilled water in a centrifuge tube and heated at 80°C for 30 min. This was continually shaken during the heating period. After heating, the suspension was centrifuged at  $1000 \times g$  for 15 min. The supernatant was decanted and the weight of the paste taken. The swelling power was calculated as: swelling power = weight of the paste / weight of dry flour.

## Statistical analysis

The data were analysed using SPSS version 15.0. The mean and standard error of means (SEM) of the triplicate analyses of the samples were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means, while the means were separated using the new Duncan multiple range test.

## RESULTS

### Macronutrient and mineral composition of African locust bean flour

The proximate compositions of raw, germinated and fermented African locust bean flour were presented in Table 1. The protein content of ALB samples range between  $33.64 \pm 0.41$  g/100 g for raw

**Table 1.** Mean ( $\pm$ SEM) of macronutrient composition (g/100 g dry weight matter) of raw, germinated and fermented African locust beans flour

Nutrient/ Sample	Raw African locust bean flour	Germinated African locust bean flour	Fermented African locust bean flour
Protein	$33.64^b \pm 0.41$	$41.49^a \pm 1.84$	$35.36^b \pm 0.23$
Fat	$18.21^a \pm 0.38$	$18.64^a \pm 0.83$	$18.63^a \pm 0.19$
Ash	$3.99^a \pm 0.49$	$4.34^a \pm 0.38$	$2.34^b \pm 0.21$
Fiber	$8.08^a \pm 0.36$	$7.28^{ab} \pm 0.35$	$6.65^b \pm 0.50$
Carbohydrate	$36.08^a \pm 0.24$	$28.24^b \pm 1.92$	$37.01^a \pm 0.38$
Energy, kcal	$442.79^b \pm 2.32$	$446.71^{ab} \pm 7.06$	$457.20^a \pm 2.15$

Mean values with the same superscript in a row are not significantly different ( $P > 0.05$ ).

African locust bean flour (ALB) and  $41.49 \pm 1.89$  g/100 g for germinated African locust bean flour. The germinated sample was significantly higher in protein content when compared with the fermented and raw sample respectively ( $p < 0.05$ ). The fat content of both germinated ( $18.64 \pm 0.83$  g/100 g) and fermented ( $18.63 \pm 0.19$  g/100 g) African locust bean flour was higher than the raw sample ( $18.21 \pm 0.38$ ), but there was no significant difference between these values ( $p > 0.05$ ). The ash content of the germinated ALB ( $4.34 \pm 0.38$  g/100 g) was significantly higher than that of fermented ALB ( $2.34 \pm 0.21$  g/100 g) ( $p < 0.05$ ); but insignificantly different when compared with the raw ALB ( $3.99 \pm 0.49$  g/100 g) sample ( $p > 0.05$ ). The fiber contents of both germinated ( $7.28 \pm 0.35$  g/100 g) and fermented ( $6.65 \pm 0.50$  g/100 g) ALB flour were lower when compared with the raw ALB flour ( $8.08 \pm 0.36$  g/100 g). The energy value of fermented ALB sample ( $457.20 \pm 2.15$  kcal) was higher than germinated ( $446.71 \pm 7.06$  kcal) and raw ( $442.79 \pm 2.32$  kcal) ALB respectively.

The mineral composition of African locust bean flour is shown in Table 2. The mineral composition of the samples range as follows: raw ALB sample was between the range of  $1.29 \pm 0.02$  mg/100 g of manganese and  $108.50 \pm 0.20$  of phosphorous, for the germinated ALB sample was between  $1.62 \pm 0.04$  mg/100 g of sodium and  $90.85 \pm 0.45$  of phosphorous; while that

**Table 2.** Mean ( $\pm$ SEM) of mineral composition (mg/100 g) of raw, germinated, fermented African locust bean flour

Nutrient/ Sample	Raw African locust bean flour	Germinated African locust bean flour	Fermented African locust bean flour
Phosphorous	108.50 <sup>a</sup> $\pm$ 0.20	90.85 <sup>b</sup> $\pm$ 0.45	75.75 <sup>c</sup> $\pm$ 0.15
Potassium	2.23 <sup>b</sup> $\pm$ 0.05	3.76 <sup>a</sup> $\pm$ 0.15	1.30 <sup>c</sup> $\pm$ 0.15
Sodium	1.43 <sup>b</sup> $\pm$ 0.25	1.62 <sup>a</sup> $\pm$ 0.04	1.32 <sup>c</sup> $\pm$ 0.15
Calcium	1.22 <sup>c</sup> $\pm$ 0.14	1.73 <sup>a</sup> $\pm$ 0.04	1.44 <sup>b</sup> $\pm$ 0.05
Magnesium	4.86 <sup>a</sup> $\pm$ 0.01	4.16 <sup>b</sup> $\pm$ 0.05	3.60 <sup>c</sup> $\pm$ 0.10
Iron	2.20 <sup>c</sup> 0.10	6.20 <sup>a</sup> $\pm$ 0.10	2.63 <sup>b</sup> $\pm$ 0.02
Zinc	2.55 <sup>b</sup> $\pm$ 0.15	3.03 <sup>a</sup> $\pm$ 0.01	1.27 <sup>c</sup> $\pm$ 0.01
P/Ca	0.89 <sup>a</sup> $\pm$ 0.00	0.53 <sup>b</sup> $\pm$ 0.01	0.52 <sup>b</sup> 0.00
Na/K	0.64 <sup>b</sup> $\pm$ 0.00	0.43 <sup>c</sup> $\pm$ 0.00	1.02 <sup>a</sup> $\pm$ 0.00
Copper	4.15 <sup>a</sup> $\pm$ 0.15	2.26 <sup>b</sup> $\pm$ 0.01	1.25 <sup>c</sup> $\pm$ 0.01
Manganese	1.29 <sup>c</sup> $\pm$ 0.02	1.72 <sup>b</sup> $\pm$ 0.03	2.20 <sup>a</sup> $\pm$ 0.10
Nickel	–	–	–
Rubidium	–	–	–
Molybdenum	–	–	–
Cadmium	–	–	–
Bromine	–	–	–
Strontium	–	–	–
Astatine	–	–	–
Lead	–	–	–
Aluminium	–	–	–
Iodine	–	–	–

– not detected.

Mean values with the same superscript in a row are not significantly different ( $P > 0.05$ ).

of fermented ALB sample was between 1.27  $\pm$ 0.01 mg/100 g of zinc and 75.75  $\pm$ 0.15 mg/100 g of phosphorous. The mineral compositions of germinated African locust bean flour were significantly higher in terms of phosphorous, potassium, sodium, calcium, magnesium, iron and zinc when compared with the fermented African locust bean flour ( $p < 0.05$ ); but lower in the minerals like phosphorous, magnesium,

and copper than the raw sample. Heavy metals like nickel, rubidium, molybdenum, cadmium, bromine, strontium, astatine, lead, aluminum and iodine were not detected in the samples. The P/Ca ratios of the sample were significantly higher in raw ALB sample (0.89) than the germinated (0.53) and fermented (0.52) ALB samples respectively ( $p < 0.05$ ). Similarly, the Na/K ratios were highest in raw sample (0.64) compared with the germinated (0.43) and fermented (1.02) sample respectively ( $p < 0.05$ ).

#### Amino acid composition and nutritional quality of African locust bean flour

The amino acid composition and nutritional quality of African locust bean flour are presented in Table 3 and 4 respectively. The total non essential amino acids composition of the raw, fermented and germinated ALB samples ranged between 44.975 mg/100 g of the germinated ALB and 48.865 mg/100 g of fermented ALB sample. The highest concentration of non essential amino acids of the sample was aspartic acid, while the least concentration was serine. For conditionally essential amino acids composition, the concentration ranged between 48.965 mg/100 g of fermented ALB flour and 15.340 mg/100 g of germinated ALB flour. The highest concentration of conditionally essential amino acids for the raw, fermented and germinated flour were proline, glycine and arginine respectively; while that of the least was cysteine for all the food samples. Also, for essential amino acids, the concentration ranged between 29.960 mg/100 g for fermented ALB and 27.514 mg/100 g for raw sample. Lysine was the highest concentration while methionine was the least concentration for the raw, fermented and germinated ALB samples.

The nutritional quality of raw, germinated and fermented African locust bean (Table 4) showed that the percentage of essential amino acids (EAAs) in fermented flour sample (35.69%) was higher than raw (35.24%) and germinated (34.88%) flour sample respectively. The ratios of total essential amino acids to total non-essential amino acids of the flour samples were 0.54, 0.65 and 0.54 for the raw, fermented and germinated respectively. Protein efficiency ratio (PER) of the samples were 1.87 for raw ALB, 1.78 for fermented ALB and 1.80 for germinated ALB. Essential amino acid index (EAAI) of fermented ALB sample (34.75%) was higher than germinated (34.34%)

**Table 3.** Amino acid composition (mg/100 g protein) of raw, germinated and fermented African locust bean flour

Amino acids	Raw African locust bean flour	Fermented African locust bean flour	Germinated African locust bean flour
<b>Non essential amino acids</b>			
Alanine	4.720 <sup>b</sup> ±0.010	5.020 <sup>a</sup> ±0.010	4.315 <sup>c</sup> ±0.015
Aspartic acid	22.820 <sup>b</sup> ±0.010	23.150 <sup>a</sup> ±0.020	21.790 <sup>c</sup> ±0.010
Serine	3.855 <sup>b</sup> ±0.055	4.195 <sup>a</sup> ±0.015	3.540 <sup>c</sup> ±0.010
Glutamic acid	14.825 <sup>b</sup> ±0.015	16.500 <sup>a</sup> ±0.010	15.150 <sup>b</sup> ±0.150
Total	46.220	48.865	44.975
<b>Conditionally essential amino acids</b>			
Proline	4.290 <sup>a</sup> ±0.020	4.285 <sup>a</sup> ±0.015	3.880 <sup>b</sup> ±0.010
Glycine	3.420 <sup>c</sup> ±0.010	5.295 <sup>a</sup> ±0.015	3.420 <sup>b</sup> ±0.010
Arginine	4.150 <sup>b</sup> ±0.010	4.840 <sup>a</sup> ±0.010	3.960 <sup>c</sup> ±0.010
Cysteine	1.700 <sup>a</sup> ±0.020	1.760 <sup>a</sup> ±0.010	1.845 <sup>a</sup> ±0.255
Tyrosine	2.550 <sup>a</sup> ±0.010	2.515 <sup>a</sup> ±0.025	2.235 <sup>b</sup> ±0.015
Total	16.110	18.965	15.340
<b>Essential amino acids</b>			
Lysine	5.335 <sup>b</sup> ±0.015	5.915 <sup>a</sup> ±0.025	5.035 <sup>c</sup> ±0.015
Threonine	2.545 <sup>b</sup> ±0.005	3.165 <sup>a</sup> ±0.015	2.265 <sup>c</sup> ±0.005
Valine	4.175 <sup>a</sup> ±0.015	4.235 <sup>a</sup> ±0.015	3.790 <sup>b</sup> ±0.020
Methionine	0.884 <sup>a</sup> ±0.006	0.900 <sup>a</sup> ±0.010	0.812 <sup>b</sup> ±0.001
Isoleucine	3.315 <sup>b</sup> ±0.015	3.500 <sup>a</sup> ±0.010	3.285 <sup>b</sup> ±0.015
Leucine	5.740 <sup>b</sup> ±0.010	5.805 <sup>a</sup> ±0.015	5.515 <sup>c</sup> ±0.005
Phenylalanine	3.795 <sup>b</sup> ±0.025	4.120 <sup>a</sup> ±0.010	3.695 <sup>c</sup> ±0.015
Histidine	1.725 <sup>b</sup> ±0.025	2.320 <sup>a</sup> ±0.010	1.735 <sup>b</sup> ±0.035
*Tryptophan	ND	ND	ND
Total	27.514	29.960	26.132

Mean values with the same superscript in a row are not significantly different ( $P > 0.05$ ).  
ND – not determined.

and raw (31.43%) sample respectively. For biological values (BV), fermented ALB flour was 26.18% and higher when compared with germinated and raw flour sample respectively. Similarly, nutritional index of

**Table 4.** Calculated nutritional quality of raw, germinated and fermented African locust bean flour

Parameters	Raw African locust bean flour	Fermented African locust bean flour	Germinated African locust bean flour
TAA, mg/100 g	89.84 <sup>b</sup> ±0.19	97.52 <sup>a</sup> ±0.08	86.27 <sup>c</sup> ±0.44
TEAA+His+Arg/TAA, %	39.86	40.65	39.47
TEAA/TAA, %	35.24	35.69	34.88
TNEAA/TAA, %	64.76	64.31	65.12
TSAA (Meth+Cys)	2.58	35.45	60.73
ArEAA (Phe+Tyr)	6.35	4.25	31.66
TEAA/TNEAA	0.54	0.65	0.54
PER	1.87	1.78	1.80
EAAI, %	31.43	34.75	34.34
BV, %	22.56	26.18	25.73
Nutritional index, %	10.57	14.42	12.14

Mean values with the same superscript in a row are not significantly different ( $P > 0.05$ ).

fermented flour (14.42%) was higher when compared with that of germinated flour (12.14%) and raw flour sample (10.57%) respectively.

Figure 2 shows the comparison between the total essential amino acids recommended daily requirements by FAO/WHO [1991] and African locust bean flour samples. Total amino acid composition of fermented flour sample was higher when compared with the raw and germinated flour samples; however, the total amino acids of the raw and processed African locust bean flour were lower than the recommended daily requirements of FAO/WHO [1991].

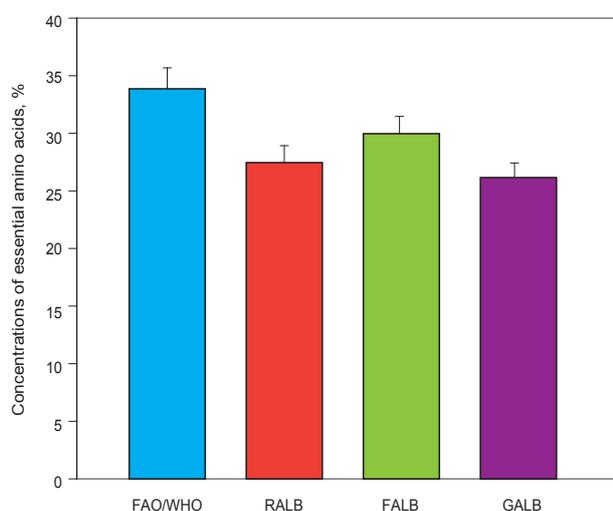
#### Fatty acids composition of raw, germinated and fermented African locust bean

The fatty acid compositions of the raw, germinated and fermented samples are presented in Table 5. The result showed that linoleic was the dominant fatty acid

**Table 5.** Fatty acids composition (mg/100 g protein) of raw, germinated and fermented African locust bean

Fatty acids	Raw	Germinated	Fermented
	African locust bean flour %	African locust bean flour %	African locust bean flour %
Lauric acid (C12:0)	0.0003	0.001	0.001
Myristic acid (C14:0)	0.0003	0.001	0.001
Palmitic acid (C16:0)	17.256	15.479	12.879
Palmitoleic acid (C16:1)	0.014	0.006	0.142
Stearic acid (C18:0)	18.086	18.073	19.721
Oleic acid (C18:1)	18.594	20.833	20.154
Linoleic acid (C18:2)	33.687	31.578	28.700
Linolenic acid (C18:3)	0.0002	0.001	0.403
Arachidic acid (C20:0)	3.815	3.306	5.165
Behenic acid (C22:0)	7.742	8.444	11.678
Lignoceric acid (C24:0)	0.734	2.022	0.008
<b>Saturated fatty acids (SFA)</b>			
Myristic acid	0.0003	0.001	0.001
Palmitic acid	17.256	15.479	12.879
Stearic acid	18.086	18.073	19.721
Arachidic acid	3.815	3.306	5.165
Behenic acid	7.742	8.444	11.678
Total	46.8993	45.3030	49.4440
<b>Poly unsaturated fatty acids (PUFA)</b>			
Linoleic	33.687	31.578	28.7
Linolenic acid	0.0002	0.001	0.403
Arachidonic acids	–	–	–
Docohexanoic acid	–	–	–
Total	33.6872	31.779	29.103
<b>Mono unsaturated fatty acid (MUFA)</b>			
Palmitoleic acid	0.014	0.006	0.142
Oleic acid	18.594	20.833	20.154
Total	18.608	20.839	20.296
P:S	0.718	0.701	0.589

Mean values with the same superscript in a row are not significantly different ( $P > 0.05$ ).



**Fig. 2.** Comparison of total essential amino acid of FAO/WHO reference [1991], raw (RALB), germinated (GALB) and fermented African locust beans (FALB) flour

in the raw ALB (33.687%), germinated ALB (31.578%) and fermented ALB (28.7%) sample, while lauric acid was the least amino acid. For the saturated fatty acids (SFA), stearic acid was the most dominant fatty acids, while myristic acid was the least. The total saturated fatty acid of fermented flour (49.444%) sample was the highest when compared with raw flour sample (46.8993%) and germinated flour samples (45.3030%). The total polyunsaturated fatty acid of the raw ALB sample was the highest fatty acid concentration when compared with other samples, that is, germinated and fermented ALB samples. The linoleic was the dominant fatty acid in the polyunsaturated fatty acid. For the monounsaturated fatty acid, oleic acid was the predominant fatty acid, while arachidonic acids and docohexanoic acids were not detected. The monounsaturated fatty acid concentration of fermented sample was more than germinated and raw sample respectively. The ratio of polyunsaturated/saturated ranged between 0.718 for raw sample and 0.589 for fermented sample.

#### Antinutritional composition of raw, germinated and fermented African locust bean flour

The antinutritional composition of African locust bean samples is presented in Table 6. The oxalate concentration ranged between  $0.013 \pm 0.003$  g/100 g for

**Table 6.** Antinutritional composition (g/100 g) of raw, germinated and fermented African locust bean flour

Antinutritional factors	Raw African locust bean flour	Fermented African locust bean flour	Germinated African locust bean flour
Oxalate	$3.57^a \pm 0.020$	$0.013^c \pm 0.003$	$0.140^b \pm 0.010$
Tannin	$2.100^a \pm 0.020$	$0.012^b \pm 0.002$	$0.014^b \pm 0.001$
Phytate	$3.340^a \pm 0.030$	$0.008^c \pm 0.001$	$0.122^b \pm 0.002$
Trypsin inhibitor	$12.25^a \pm 0.005$	$0.007^b \pm 0.001$	$0.014^b \pm 0.001$

Mean values with the same superscript in a row are not significantly different ( $P > 0.05$ ).

fermented African locust bean and  $3.57 \pm 0.020$  g/100 g for the raw African locust bean flour sample. Tannin concentration was between  $0.012 \pm 0.002$  g/100 g for fermented ALB flour sample and  $2.100 \pm 0.020$  g/100 g for the raw sample. The concentration of the phytate in the samples ranged between  $0.008 \pm 0.001$  for the fermented ALB flour sample and  $3.340 \pm 0.030$  g/100 g raw sample. While trypsin inhibitor concentration ranged between  $0.007 \pm 0.01$  g/100 g for fermented sample and  $1.225 \pm 0.005$  g/100 g for raw sample. Statistically, both the germinated and fermented ALB samples were significantly lower in oxalate, tannin, phytate and trypsin inhibitor when compared with the raw ALB sample ( $p < 0.05$ ).

#### Functional properties of raw, germinated and fermented African locust bean samples

Table 7 shows the functional properties of raw and biological processed African locust bean flour. Swelling capacity (SW) of the samples ranged between  $4.224 \pm 0.005\%$  for germinated ALB flour and  $4.958 \pm 0.020\%$  fermented ALB flour. Bulk density range between  $0.783 \pm 0.001\%$  for germinated sample and  $0.821 \pm 0.012\%$  raw ALB flour; while water absorption capacity was between  $1.964 \pm 0.014\%$  for germinated sample and  $2.061 \pm 0.005\%$  for raw sample. The bulk density and water absorption capacity of raw ALB samples were significantly higher than the processed samples. However, germinated and fermented ALB samples were significantly lower in swelling capacity when compared with the raw sample.

**Table 7.** Functional properties of raw, germinated and fermented African locust bean flour

Functional attributes/ Sample	Raw African locust bean flour	Germinated African locust bean flour	Fermented African locust bean flour
Swelling capacity	4.626 <sup>b</sup> ±0.002	4.224 <sup>c</sup> ±0.005	4.958 <sup>a</sup> ±0.020
Bulk density	0.821 <sup>a</sup> ±0.012	0.783 <sup>b</sup> ±0.001	0.797 <sup>ab</sup> ±0.004
Water absorption capacity	2.061 <sup>a</sup> ±0.005	1.964 <sup>b</sup> ±0.014	2.111 <sup>a</sup> ±0.044

Mean values with the same superscript in a row are not significantly different ( $P > 0.05$ ).

## DISCUSSION

The present study investigated the influence of germination and fermentation on the nutrient composition of African locust bean flour. From the result, it was observed that the nutrient compositions of germinated and fermented African locust bean flour were dominantly increased when compared with the raw sample. For instance, the protein content of germinated African locust bean flour was significantly higher than that of the fermented and raw sample respectively. The increase in nutrient composition of germinated and fermented African locust bean flour could be attributed to the biochemical activities of the sprouting seeds and also due to the activities of micro-organism that responsible for the fermentation process. Quite a number of scientific researchers have reported that germination and fermentation processing techniques improved the nutritional quality and bioavailability of essential nutrients in food products [Cronk et al. 1977, Ochanda et al. 2010]. The energy value of germinated ALB flour was insignificantly lower than the fermented ALB flour, but higher than that of raw flour sample; this observation could be a result of the activities of the growing seeds that utilized parts of the protein, fat and carbohydrate content of the seeds for their growth and other biochemical activities. Several studies have reported that germination method improved on the nutritional quality of food products [Ohtsubo et al. 2005, Khatoon and Prakash 2006, Kaushik et al.

2010], but also reduces carbohydrate and fiber content of the sample, hence, energy value of the food products [Syed et al. 2011].

The mineral composition of the germinated ALB flour sample was significantly higher than the raw and fermented ALB flour. This observation is similar to other investigators who have reported that germination increases retention of all minerals and B-complex vitamins compared to other processing methods [Gibson et al. 1998, Ariaahu et al. 1999, Egli 2001, Helland et al. 2002, El-Adawy 2002]. For instance, it is documented that germination increased the amount of thiamin, riboflavin, niacin and ascorbic acid in both soybean and mung bean [Abdullah and Baldwin 1984]. Mineral values in germinated legumes/cereals increased with germination except of iron. Iron values decreased in germinated seeds but its availability increased due to an increase in phytase activity during seed germination [Bates et al. 1977, Walker and Kochhar 1982].

Comparatively, the total values of essential, conditional and non-essential amino acid profiles of the germinated African locust bean flour samples were lower when compared with the raw sample, but the fermented sample was higher. The increased amino acids of fermented flour sample could be attributed to the activities of microorganisms that covert some of the nutrients in the food into amino acids for their utilization during the sprouting period. Contrary, the amino acids composition of germinated food sample was lower and this could also be attributed to the fact that some of the amino acids were utilized for growth by the germinating seeds. Also, by comparing the total essential amino acid profile with the FAO/WHO [1991] reference standard (Fig. 1), it was observed that the values of both germinated and fermented samples were lower than the recommended values. This finding shows that the amino acid composition of African locust bean is incomplete; hence, it needs to be complemented with other food materials like cereal to attain complete amino acid profile.

The arginine and histidine content of fermented and germinated African locust bean flour was higher than the FAO/WHO [1991] recommendations for infants (Arginine 2.0 mg, Histidine 1.9 mg). These amino acids are very important for the growing and development of infants, therefore, incorporation of African locust beans into infant complementary foods would

enhance the growth and development of the children particularly in developing countries where animal-based complementary foods are expensive. The percentage ratios of TEAA to the TAA in the samples were 35.24% (raw ALB), 35.69% (fermented ALB) and 34.88% (germinated ALB), which were within the range of the 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults [FAO/WHO/UNU 1985]. The TEAA/TAA percentage contents were strongly comparable to that of eggs (50%) [FAO/WHO 1990]; pigeon pea flour (43.6%) [Oshodi et al. 1993]; beach pea protein isolate (43.6-44.4%) [Chavan and McKenzie 2001]; coconut endosperm (55.3%) [Adeyeye 2004]; *P. biglobosa* (46.8%) [Adeyeye 2006]; *A. occidentale* (51.0%), *C. acuminata* (38.4%) and 47.1% reported for *G. kola* [Adeyeye et al. 2007].

It is well documented that food fermentation process increased the protein content of fermented food products through the activities of the microorganisms [Cronk et al. 1977]. Thus, this process provides a means by which the protein content of high starch substrates can be increased for the benefit of consumers needing higher protein intakes, particularly children, living in the communities where animal proteins are very expensive [Rao 1961, Rajalakshmi and Vanaja 1967, Steinkraus et al. 1967, Cronk et al. 1977].

Nutritionally, the calculated protein efficiency ratios (PER) of fermented and germinated African locust beans flour were higher than cowpea (1.21), millet (1.62), sorghum (0.27) and comparable to pigeon pea (1.82), casein (2.5) [Oyarekua and Eleyinmi 2004]; but similar to the report of Adeyeye [2006] that reported 2.0 for fermented African locust bean. The essential amino acid index (EAAI), biological value (BV) and nutritional index of fermented flour sample were higher than the raw and germinated flour samples. In comparison, the EAAIs of processed ALB samples (34.75% fermented ALB, and 34.34% germinated ALB) were lesser when compared with the values of other food products like defatted soy flour (126%) [Nielsen 2002]. The biological values of fermented ALB (26.18%) and germinated (25.73%) samples were also lower compared to *A. bisporus* (45%) and *P. florida* (39%). However, the nutritional index of the flour samples was comparable to the *A. bisporus* (13.69%) and *P. florida* (12.59%). The essential amino acid index can be useful

as a rapid tool to evaluate food formulations for protein quality [Nielsen 2002]. However, it does not account for differences in protein quality due to various processing methods or certain chemical reactions [Nielsen 2002]. Protein material is said to be of good nutritional quality when its biological values (BV) are high (70-100%) and also when the essential amino acid index (EAAI) is above 90% and to be useful as food when the values are around 80% and are inadequate for food material when below values are 70% [Oser 1959]. From the present study it is observed that the BV and EAAI values were generally low and could be attributed to the fact that African locust bean, a leguminous plant, is deficient in some essential amino acids notably methionine and tryptophan.

The germinated African locust bean flour was high in polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid composition compared with the fermented African locust bean flour and raw samples. The slight increase might be due to non-conversion of free fatty acids to carbohydrates which may lead to increase in fat composition during germination [Afam-Anene and Onuoha 2006]. It is evident that essential fatty acid like oleic and linoleic acid are good fatty acids and their consumption should be encouraged; hence the consumption of African locust bean would serve as a good source. Quite a number of studies have reported that fat/oil containing high value of polyunsaturated:saturated fatty acid ratio is desirable for human consumption, because of their potential health benefits [Bonvehi and Coll 1993, Cunnane et al. 1993, Zwarts et al. 1999]. With the current emphasis on lowering consumption of saturated fats, minimizing or eliminating trans fat, and increasing polyunsaturated and monounsaturated fats intake the consumption of African locust bean would therefore improve the integrity of cardiovascular system and thereby prevent cardiovascular disease and other nutritional related diseases.

The oxalate, tannin, phytate and trypsin concentrations of germinated and fermented African locust bean flour were lower when compared with the raw sample. However, fermented sample had the lowest concentration of antinutritional factors. This could be attributed to the fact that fermentation is usually accompanied with soaking, hydration and cooking of the raw seeds; and these processes have reduction effects on the level of antinutritional factors in fermented food products.

This finding was similar to the report of other investigators [Siddhuraju and Becker 2001, El-Adawy 2002, Ugwu and Oranye 2006].

The swelling capacity, bulk density and water absorption capacity of germinated ALB flour were lower than the fermented ALB and the raw ALB samples. It has been proved by researchers that processing methods, such as soaking, sprouting, fermentation and cooking improve the nutritional and functional properties of plant seeds [Jirapa et al. 2001, Yagoub and Abdalla 2007]. Functional properties of food materials are very important for the appropriateness of the diet, particularly, for the growing children [Omueti et al. 2009]. The consistency of energy density [energy per unit volume] of the food and the frequency of feeding are also important in determining the extent to which an individual will meet his or her energy and nutrient requirements [Omueti et al. 2009]. The bulk density value is of importance in packaging [Snow 1974]. The lower loose bulk density implies that less quantity of the food samples would be packaged in constant volume thereby ensuring an economical packaging. However, the packed bulk densities would ensure more quantities of the food samples being packaged, but less economical. Nutritionally, loose bulk density promotes easy digestibility of food products, particularly among children with weak digestive system [Osundahunsi and Aworh 2002, Gopaldas and John 1991]. The water absorption capacity is an index of the maximum amount of water that a food product would absorb and retain [Marero et al. 1988, Mosha and Lorri 1987]. With respect to water absorption capacity, Giami and Bekeham [1992] reported that the microbial activities of food products with low water absorption capacity would be reduced. Hence the shelf-life of such a product would be extended. The swelling capacity is an important factor used in determining the amount of water that diets would absorb and the degree of swelling within a given time.

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## **PORÓWNANIE ZAWARTOŚCI AMINOKWASÓW, KWASÓW TŁUSZCZOWYCH, ZWIĄZKÓW MINERALNYCH ORAZ WARTOŚCI ŻYWIENIOWEJ MĄKI OTRZYMANEJ Z SUROWYCH, SKIEŁKOWANYCH ORAZ FERMENTOWANYCH NASION AFRYKAŃSKIEGO DRZEWA NÉRÉ (*PARKIA BIGLOBOSA*)**

### **STRESZCZENIE**

**Wstęp.** Najczęściej nasiona z afrykańskiego drzewa néré (African locust bean, ALB) są wykorzystywane w tradycyjnej afrykańskiej kuchni w formie fermentowanej jako przyprawa do produktów spożywczych zwiększająca zawartość białka w diecie niskobiałkowej oraz jako produkt leczniczy. Celem pracy było zbadanie wpływu kiełkowania i fermentacji na wartość żywieniową mąki otrzymanej z nasion drzewa néré.

**Materiał i metody.** Nasiona z afrykańskiego drzewa néré zakupiono w sieci handlowej miasta Akure (Nigeria). Nasiona podzielono na trzy części, z których pierwszą stanowiły nasiona nieprzetworzone, drugą – nasiona skiełkowane i trzecią – poddane procesowi fermentacji. Każda grupa nasion została zmielona, odsiana i podana analizie pod względem właściwości chemicznych, funkcjonalnych oraz wartości żywieniowej.

**Wyniki.** W wyniku przeprowadzonych analiz prób mąki oznaczono zawartość białka w granicach od 33,64 ±0,41 do 41,49 ±1,89 g/100 g oraz wartość energetyczną na poziomie od 442,79 ±2,32 do 457,20 ±2,15 kcal. Stwierdzono, że stosunek P/Ca oraz Na/K w mące otrzymanej z nasion nieprzetworzonych był wyższy niż w pozostałych próbach. Całkowita zawartość aminokwasów wyniosła od 29,960 do 27,514 mg/100 g. Wydajność wzrostowa białka (PER) zawierała się w granicach 1,78-1,87; indeks aminokwasów egzogennych kształtował się na poziomie od 31,43 do 34,75%, natomiast wartość biologiczna wyniosła od 22,56 do 26,18%. Największym udziałem procentowym spośród kwasów tłuszczowych wyróżniał się kwas linolowy: odpowiednio 33,687%, 31,578% i 28,7% w próbkach mąki RALB, GALB oraz FALB, natomiast najmniejszym – kwas laurynowy. Stosunek kwasów tłuszczowych nienasyconych do nasyconych wynosił od 0,589 do 0,718. Zawartość substancji antyżywnościowych w mące otrzymanej z ziarna fermentowanego była mniejsza niż w pozostałych próbach.

**Wnioski.** W przedstawionej pracy zbadano wpływ kiełkowania oraz procesu fermentacji na wartość odżywczą mąki otrzymanej z nasion afrykańskiego drzewa néré. Na podstawie wyników stwierdzono, że proces fermentacji zmniejsza właściwości antyodżywcze oraz poprawia wartość żywieniową, w szczególności profil aminokwasów badanej mąki.

**Słowa kluczowe:** nasiona afrykańskiego drzewa néré, aminokwasy, kwasy tłuszczowe, kiełkowanie i proces fermentacji

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