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The effect of fermentation on the dietary quality of lipids from African locust bean (*Parkia biglobosa*) seeds

Emmanuel Ilesanmi Adeyeye
Department of Chemistry, Ekiti State University, PMB 5363, Ado-Ekiti, Nigeria.

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ABSTRACT
The levels of crude fat, fatty acids, phospholipids and phytosterols were determined in the unfermented and fermented samples of African locust bean seeds using gas chromatography techniques. Results showed crude fat varied from 16.9-19.5 g/100 g; SFA varied from 45.6 down to 42.1 % of total fatty acids, total unsaturated fatty acids varied from 54.4-57.9 %, PUFA range was 31.8 down to 31.0 % and PUFA/SFA ranged from 0.698-0.735. Both samples had high levels of *n-6* fatty acids but low in *n-3* fatty acids. In the phospholipids, lecithin was highest in the two samples with values ranging from 95.5-107 mg/100 g (46.1-51.4 %). The phytosterol values in the unfermented seeds ranged from 0.00-138 mg/100 g and in fermented seeds the range was 0.00-144 mg/100 g with sitosterol predominating in both samples. Whilst 100 g unfermented seeds would provide 13.5 g fatty acids, 100 g fermented seeds would provide 15.6 g fatty acids. The correlation coefficient was not significantly different in the fatty acids and phospholipids but significantly different in the phytosterols at $r_{=0.05}$.

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Introduction

Dawadawa is the Hausa name for fermented African locust bean (*Parkia biglobosa*). It is the most important food condiment in the entire savannah region of West and Central Africa. Some of the countries where *dawadawa* is important include the northern areas of Nigeria, Ghana, Togo, Benin, Chad, Sierra Leone, Upper Volta, Gambia, Cameroon, Ivory Coast, Guinea, Mali, Senegal and semi-desert country of Niger [1]. Fermented foods constitute some significant part of the diet of many West Africans.

Dawadawa is also known as *iru* inYorubaland of Nigeria, *ogiri-igala* in Iboland, *kpalugu* among the Kusasis and Dagombas of northern Ghana, *kinda* in Sierra Leone, *netetou* or *soumbara* in Gambia. *Damadawa* has undoubtedly been produced in West Africa for centuries, but as with many other traditional arts, there are no written records of its origin. The history of *dawadawa* can be traced back several centuries to when locust bean trees were traditionally first planted around communities. A Scottish surgeon, Mungo Park encountered locust bean trees when he explored the Niger Basin from 1795 to 1799[2]. In 1804 to 1806 he described this tree in his “Travels in the Interior Districts of Africa”[2]. In fact the locust bean tree was subsequently named after him by Robert Brown in 1826[3].

The taxonomy of locust bean trees has been in a state of flux until recently. Because of this, various reports in the literature have referred to the same tree as *Parkia filicoidea, P. bicolor* and *P. clappertoniana*. The currently accepted nomenclature is *P. biglobosa* with *P. clappertoniana* as the accepted synonym[3]. The related species *P. filicoidea* is indigenous to the forest of East and Central Africa. The *P. bicolor* (*Igbaodo yor.*) is found in the forest regions of West Africa; the fruits of it are not eaten by humans, but they serve as food for forest animals. The locust bean tree is planted mainly because of the value of its fruit. These fruit provide a constant source of valuable protein in the dry season (from January to March). The locust bean tree is also used for medicinal purposes and as a source of mouthwash to relieve toothaches[4]. The bean husks (seed coats) are used with indigo dye to improve the luster of fabrics, while the tree bark yields a red tannin for dying leather[4].

Despite its wide use as a food, reliable figures for the production of *dawadawa* are difficult to obtain. Cobley and Steel[5] estimated that about 200, 000 tons of beans are gathered each year in northern Nigeria alone. In addition, large quantities are produced in the savannah regions of Oyo and Kwara States in south-western Nigeria. Some of the beans produced in northern Nigeria are sold to the Yoruba in south-western Nigeria where there is a shortage of locust beans because half of the area is rain forest. Each locust bean tree yields about 25 to 52 kg of pods from which 6 to13 kg of beans may be obtained[6,7]. About 250, 000 tons of locust beans are produced, from which 170,000 tons of *dawadawa* is made[8].

Dawadawa is a strong smelling product and is normally used as a soup or stew flavouring. Low-income families use *dawadawa* as a low-cost meat substitute and they generously add it to soups or stews and sorghum or millet-based dumplings and porridges. The Ekitis of the Ekiti State of Nigeria advertised this food “here is *dawadawa*, the premium meat of the stew”. Stews and soups are essential features of diets of West Africans. The use of stews or soup flavours is widespread in West Africa. Consumer surveys in Nigeria show that flavouring materials constitute an essential ingredient in the preparation of local soups or stews[9].

The quantity of *dawadawa* consumed varies with the country and within the country. The average per capita per day consumption of *dawadawa* in Togo and Ghana is 4 and 2 g, respectively[10, 11]. The Yoruba of southwestern Nigeria consume

Tele:
E-mail addresses: eiadeyeye@yahoo.com
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10 g per day per person[12]. Overall, consumption estimated for parts of Nigeria range from 1 to 17 g per person per day [12, 13, 14].

Busson[6], Oyenuga[15], Campbell –Platt[16], Odunfa[17, 18] carried out some nutritional studies on the fermented African locust bean seeds whilst Ladipo and Adegboye [19], Ogunbunmi and Bassir[20], Eka[21], Aderibigbe and Odunfa [22] also examined some aspects of the fermented seeds. Adeyeye *et al.*[23] studied the proximate, mineral analyses of raw and cooked (fermented and unfermented) and the functional properties of the fermented seeds. Adeyeye[24] had also reported the amino acids composition of fermented African locust bean seeds. The present study mainly focused on the determination of the lipid profiles of cooked (unfermented and fermented) African locust bean seeds.

Materials and methods

Sampling

Samples of cooked unfermented and fermented African locust bean seed (ALBS) were purchased from the local market in Iworoko Ekiti, Ekiti State of Nigeria. All impurities were removed by sorting. The fermented seeds were washed thoroughly with distilled water and all the seeds were oven-dried (55 °C) and later preserved in a deep freezer (-4°C) pending analysis after homogenization in a Kenwood Major blender.

Method of preparation of dawadawa

The processing of locust beans into *dawadawa* is carried out exclusively by women as a specialized trade and commercial activity. A flow diagram for *dawadawa* preparation is presented in Figure 1[8]. A variety of bacteria grows in the fermenting beans during fermentation and produces a mucilaginous substance that covers and links the individual bean cotyledons. The fermented beans have a strongly proteolytic and slightly ammonia-like smell. During the 3-day fermentation, bean cotyledons change in colour from brown to dark brown and become softer. Two kinds of *dawadawa* are produced by the Yoruba of south-western Nigeria. The normal type, *Woro* (*Yor.*), has distinctly discernible cotyledons and a moisture content of 56 %. The softer variety, *Pete* (*Yor.*), has 65 % moisture and contains partly mashed bean cotyledons. The softer variety is prepared by boiling bean cotyledons with potash rock salt. The *Woro* variety was used in this research work. About 70 kg of fresh *dawadawa* is obtained from 100 kg locust bean seeds. It should be noted in Figure 1 that addition of salt is a post fermentation treatment of *dawadawa*.

Extraction of lipid

0.25 g of each sample was weighed into the extraction thimble. 200 ml of petroleum ether (40-60 °C boiling range) was measured and then added to the dried 250 ml capacity flask. The covered porous thimble with the sample was placed in the condenser of the Soxhlet extractor arrangement that has been assembled[25]. The sample was extracted for 5 h. The extraction flask was removed from the heating mantle arrangement when it was almost free of petroleum ether. The extraction flask with oil was oven dried at 55 °C for the period of 1 h. The flask containing the dried oil was cooled in the desicator and the weight of the cooled flask with the dried oil was taken.

Preparation of methyl esters and analysis

50 mg of the extracted fat was saponified for 5 min at 95 °C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralized by 0.7 M HCl. 3 ml of 14 % boron triflouride in methanol was added [25]. The mixture was heated for 5 min at 90 °C to achieve complete methylation process. The fatty acid methyl esters were thrice extracted from the mixture with redistilled *n*-hexane. The content was concentrated to 1 ml for analysis and 1 µl was injected into the injection port of the GC. The fatty acid methyl esters were analysed using an HP 5890 powered with HP gas chromatograph [HP 5890 powered with HP ChemStation rev AO9.01(1206) software (GMI, Inc, Minnesota, USA)] fitted with a flame ionization detector. Nitrogen was used as the carrier gas with a flow rate of 20-60 ml/min. The oven program was: initial temperature at 60 °C, first ramping at 10 °C/min for 20 min, maintained for 4 min, second ramping at 15 °C/min for 4 min and maintained for 10 min. The injection temperature was 250 °C whilst the detector temperature was 320 °C. A capillary column (30 m x 0.25 mm) packed with a polar compound (HP INNOWAX) with a diameter (0.25 µm) was used to separate the esters. Split injection type was used having a split ratio of 20:1. The peaks were identified by comparison with standard fatty acid methyl esters.

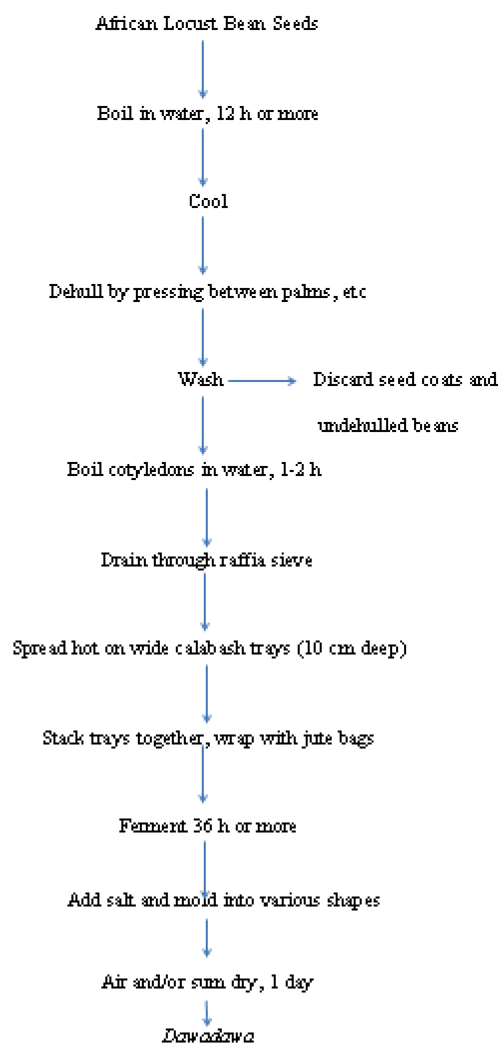

Figure 1. Flow sheet for preparation of *dawadawa*

Sterols analyses

The phytosterol analysis was as described by AOAC[25]. The aliquots of the extracted fat were added to the screw-capped test tubes. The sample was saponified at 95 °C for 30 min, using 3 ml 10 % KOH in ethanol, to which 0.20 ml of benzene had been added to ensure miscibility. Deionised water (3 ml) was added and 2 ml of hexane was used in extracting the non-saponifiable materials. Three extractions, each with 2 ml of hexane, were carried out for 1 h, 30 min and 30 min respectively, to achieve complete extraction of the sterols. The hexane was concentrated to 1 ml in the vial for gas chromatographic analysis and 1 µl was

injected into the injection port of GC. The peaks were identified by comparison with standard sterols. The sterols were analysed using similar conditions as for fatty acid methyl ester analysis.

Phospholipids analyses

Modified method of Raheja *et al* [26] was employed in the analysis of phospholipids. 0.01 g of the extracted fat was added to the test tubes. To ensure complete dryness of the oil for phospholipids analyses, the solvent was completely removed by passing stream of nitrogen gas on the oil. 0.40 ml of chloroform was added to tube followed by the addition of 0.10 ml of the chromogenic solution. The tube was heated at 100 ^{o}C in water bath for about I min 20 sec. The content was allowed to cool to the laboratory temperature and 5 ml of hexane was added and the tube shaken gently several times. The solvent and the aqueous layers were allowed to be separated. The hexane layer was recovered and concentrated to 1.0 ml for analysis. The phospholipids were analysed using an HP 5890 powered with HP gas chromatograph [HP 5890 powered with HP ChemStation rev AO9.01 (1206) software (GMI, Inc, Minnesota, USA)] fitted with a pulse flame photometric detector. Nitrogen was used as the carrier gas with a flow rate of 20-60 ml/min. The oven program was: initial temperature at 50 ^{o}C, first ramping at 10 ^{o}C/min for 20 min, maintained for 4 min, second ramping at 15 ^{o}C/min for 4 min and maintained for 5 min. The injection temperature was 250 ^{o}C whilst the detector temperature was 320 ^{o}C. A capillary column (30 m x 0.25 mm) packed with a polar compound (HP 5) with a diameter (0.25 µm) was used to separate the esters. Split injection type was used having a split ratio of 20:1.

Quality assurance

Standard chromatographs were prepared for phytosterols, phospholipids and fatty acid methyl esters which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient was determined for each fatty acid parameter, same for phytosterol and phospholipids. Correlation coefficient should be > 0.95 for the result to be acceptable. It was performed with the Hewlett-Packard Chemistry (HPCHEM) software (GMI, Inc 6511 Binker Lake Blvd Ramsey, Minnesota, 55303 USA).

Conversion of percentages of total fatty acids to fatty acids per 100 g of food

At the data source and reference database levels, values for individual fatty acids are usually expressed as percentages of total fatty acids. At the user database levels, values per 100 g of food are required. A conversion factor derived from the proportion of the total lipid present as fatty acids is required[27] for converting percentages of total fatty acids to fatty acids per 100 g of food. Total lipid level was multiplied by a conversion factor of 0.80 to convert it to total fatty acids[27]. For fatty acids expressed in g per 100 g total fatty acids, precision is best limited to the 0.1 g/100 g level, with trace being set at < 0.06 g/100 g of fatty acids[28].

Statistical analysis

Statistical analysis[29] was carried out to determine mean, standard deviation, coefficient of variation in per cent. Also calculated were linear correlation coefficient (r_{xy}), coefficient of determination (r_{xy}^2), linear regression coefficient (R_{xy}) and coefficient of alienation (C_A) in per cent and index of forecasting efficiency (IFE) in per cent[30]. The r_{xy} was subjected to the table (critical) value at $r_{=0.05}$ to see if significant differences existed in the values of fatty acids, phytosterols and phospholipids between the unfermented and fermented samples of African locust bean seeds.

All determinations were in duplicate and all chemicals used were from the British Drugs House (BDH).

Results and discussion

Crude fat

Results in Table I depicted the crude fat, total fatty acids and total energy from the unfermented and fermented African locust bean seeds. The fermented sample had correspondingly higher values than unfermented sample in all the three parameters. The coefficient of variation per cent ranged between 10.1-10.2 in all the three parameters. The value of 19.5 g/100 g crude fat in the fermented value is close to the value of 20.0 g/100 g reported by Oyenuga[15]. Although *dawadawa* is used as flavouring, it contributes to the calorie and protein intake. Simmons[31] reported that the average daily per capita Cal intake of *dawadawa* is 32.6 Cal out of the 2300 total Cal intake in Zaria province. Campbell-Platt[32] reported that on a moisture-free basis, *dawadawa* contains 31.2 g/100 g fat compared to unfermented locust beans, which have 15 g/100 g. In Table II, the differences in the values of crude fat, total fatty acids and total energy between the two samples are shown. The difference was (g/100 g): crude fat, 2.61 (15.5 %); total fatty acids, 2.09 (15.5 %); and total energy, 77.3 kJ/100 g (15.5 %) meaning that for each parameter, the fermented sample was 15.5 % more concentrated than the corresponding unfermented value.

Table I. Crude fat, total fatty acid levels and total energy due to fatty acid levels of African locust bean seeds

Parameter	Unfermented seeds	Fermented seeds	Mean	SD	CV %
Crude fat (g/100 g)	16.9	19.5	18.2	1.84	10.1
Total fatty acids (g/100 g)[a]	13.5	15.6	14.6	1.48	10.2
Total energy (kJ/100 g)[b]	499	576	538	54.4	10.1

SD = standard deviation; CV % = coefficient of variation per cent; [a]Crude fat x 0.80; [b]Total fatty acid x 37 kJ.

Table II. Differences in crude fat, total fatty acid levels and total energy due to fatty acid levels between unfermented and fermented samples of African locust bean seeds

Parameter	Unfermented-fermentedseeds	Percentage difference
Crude fat	-2.61	-15.5
Total fatty acids	-2.09	-15.5
Total energy	-77.3	-15.5

Saturated (SFA) and monounsaturated fatty acid (MUFA)

The total SFA in the unfermented sample was 45.6 % of the total fatty acids whilst the fermented SFA value was 42.1 %; this meant that SFA in unfermented was 3.45 g/100 g better or 7. 56 % (Table III) than in the fermented sample. The most concentrated SFA in both samples was stearic acid (C18:0) with values of 17.2 % (unfermented) and 23.1 % (fermented) and C18:0 was closely followed by palmitic acid (C16:0) with values of 16.2 (unfermented) and 11.2 % (fermented). Oyenuga[15] reported the following SFA values from seed fat of African locust bean (per cent of weight): SFA, 46.0; palmitic acid, 31.0; stearic acid, 7.70; arachidic acid, 4.20; behenic acid, 3.10. Both arachidic acid (C20:0) and behenic acid (C22:0) from literature values are close to the present results with respective values in the fermented sample as 3.08 % and 3.59 %. Out of the reported

nine parameters under the SFA, seven of them or 77.8 % were better concentrated in the unfermented sample than the fermented sample whereas they were similar [either not detected (Nd) or 0.00 %] in six other parameters. Under the SFA, the following acids were not detected: C2:0, C3:0, C6:0 (fermented sample), C8:0 and C12:0 whereas C5:0 recorded 0.00 % in both samples. The differences of the levels of the parameters and the percentage differences in the two samples are also shown in Table III.

Among the short-chain fatty acids in the samples is the C4:0. It constituted just 0.381 % (unfermented) and 0.009 % (fermented). It is mostly found in butterfat from cows. This fatty acid has antimicrobial properties - that is; it protects us from viruses, yeasts and pathogenic bacteria in the gut. They do not need to be acted on by the bile salts but are directly absorbed for quick energy. For this reason, they are less likely to cause weight gain than olive oil or commercial vegetable oils[33]. Short-chain fatty acids also contribute to the health of the immune system[34]. Medium-chain fatty acids have 8-12 carbon atoms and are common in butter fat and the tropical oils. In the present samples C10:0 was present in minor quantities (0.081 % down to 0.001 %) in the samples. Like the short-chain fatty acids, C10:0 has antimicrobial properties; are absorbed directly for quick energy; and contribute to the health of the immune system. Long-chain fatty acids have from 14 to 18 carbon atoms and can either be saturated, monounsaturated or polyunsaturated. Myristic acid (C14:0) is a ubiquitous component of lipids in most living organisms, but usually at levels of 1-2 % only. In the present samples C14:0 ranged from 0.136 % down to 0.002 %. This fatty acid is found very specifically in certain proteolipids, where it is linked via an amide bond to an N-terminal glycine residue, and is essential to the function of the protein components. Palmitic acid (16:0) is usually considered the most abundant SFA in nature, and it is found in appreciable amounts in the lipids in most animal tissues, and it is present in amounts that vary from 10-40 % in seed oils. Although C16:0 fell within this group (10-40 %) with values of 16.2 % down to 11.2 %; the present results are at variance with this earlier observation of usually being the most abundant SFA. Stearic acid (18:0) is the second most abundant SFA in nature, and again it is found in the lipids of most living organisms. In these samples (18:0) occupied the highest position (17.2 %-23.1 %) in the SFA group. In lipids of some commercial importance, it occurs in the highest concentrations in ruminant fats (milk fat and tallow) or in vegetable oils such as cocoa butter, and industrially hydrogenated fats. It can comprise 80 % of the total fatty acids in gangliosides.

Table III also contains values for the monounsaturated fatty acids. The total MUFA levels ranged from 22.6 %-26.9 % with the unfermented sample predominating. Whereas the unfermented monoenes in *cis*-forms were more concentrated in the unfermented sample, the monoenes in the *trans* forms were more concentrated in the fermented samples. The most predominant *cis*-MUFA in both samples was petroselinic acid C18:*1 cis-6*) with values of 6.15 % down to 4.35 %, this is closely followed by oleic acid (C18:*1 cis-9*) with values of 4.89 % down to 4.17 %. In *trans*-MUFA, elaidic acid (C18:*1trans-9*) was highest in unfermented sample (4.79 %) whereas *trans*-petroselinic acid (C18:*1 trans-6*) was highest in the fermented sample (7.05 %). The ratio of *cis*-MUFA: *trans*-MUFA in the unfermented sample was 1.01:1, similar ratio in fermented was 0.464:1. The report of Oyenuga[15] showed that *dawadawa* has 8.80 % oleic acid and 2.70 % palmitoleic acid. The present report showed that C16: 1 *cis-9* ranged from 0.049 % down to 0.001 %.

Oleic acid [9c-18:1or 18:1(*n-9*)] is by far the most abundant monoenic fatty acid in plant and animal tissues, both in structural lipids and in fat depots. Olive oil contains up to 78 % of oleic acid, and it is believed to have especially valuable nutritional properties as part of the Mediterranean diet. It has a number of important biological properties, both in the free and esterified form. Oleic acid is the biosynthetic precursor of a family of fatty acids with the (*n-9*) terminal structure and with chain-lengths of 20-24 or more. Petroselinic acid (6*c* – 18:1) occurs up to a level of 50 % or more in seed oils of the Umbelliferae family, including carrot, parseley and coriander.

Tissues of ruminant animals, such as cows, sheep and goats, can contain a number of different 18:1 isomers like: C18:*1 trans-9* (5.0 %) and C18:1 *tran-9* (85 %), C18:1 *trans-11* (47 %) and C18:1 *cis-11* (47 %)[35] with the *cis*-isomers, 9-and 11-18:1 slightly predominate as might be expected. 11*t*-18:1 makes up 50 % of *trans*-monoenes in ruminant animal tissues (which can comprise 10-15 % of the total fatty acids). In the present report C18:1 *trans-11* had a range of 3.28 % -5.54 % of the total fatty acids and 29.0 %-30.1 % of the *trans*-monoenes. *cis*-Vaccenic acid [11*c*-18:1 or 18:1 (*n-7*)] is a common monoenoic fatty acid of bacterial lipids, and it is usually present but as a minor component of plant and animal tissues. It is occasionally a more abundant constituent of plants, for example those containing appreciable amounts of its biosynthetic precursor, 9-16:1 (e. g. the fruit of sea buckthorn). Note that vaccenic acid per se is the *trans* isomer.

The relative proportion of MUFA/SFA is an important aspect of phospholipid compositions and changes to this ratio have been claimed to have effects on such disease states as cardiovascular disease, obesity, diabetes, neuropathgological conditions and cancer. For example, they have been shown to have cytoprotective actions in pancreatic β-cells. *cis*-Monoenoic acids have desirable physical properties for membrane lipids in that they are liquid at body temperature, yet are relatively resistant to oxidation. They are now recognised by nutritionists as being beneficial in the human diet.

Current nutritional thinking appears to be that dietary *trans*-monoenoic fatty acids, both from ruminant fats and from industrial hydrogenation processes, should be considered as potentially harmful and in the same light as saturated fatty acids. In Table IV, the five important long-chain and very-long-chain fatty acids were C18:2 *cis-9*, 12, C18:3 *cis-6*, 9, 12, C18:2 *cis-9*, *trans-11*, C18:3 *cis-9*, 12, 15 (all in the group of long-chain fatty acids) and C20:2 *cis-11*, 14 (under very-long-chain fatty acids). The two essential fatty acids are C18:2 *cis 9*, 12 and C18:3 *cis 9*, 12, 15 with respective values of 21.4 % down to 14.3 % and 0.329 % down to 0.032 %. Another important long-chain fatty acid was gamma-linolenic acid (GLA). It formed a minor level of 0.239 % down to 0.024 % in the samples. It is found in evening primrose, borage and black currant oils. The body makes GLA out of omega-6 linoleic acid and uses it in the production of substances called prostaglandins, localized tissue hormones that regulate many processes at the cellular level. Eicosadienoic acid [C20:2 *cis-11*, 14 or 20:2 (*n-6*) all-*cis-11*, 14-eicosadienoic acid] or homo-gamma-linoleic acid is an uncommon naturally occurring PUFA. It is not enriched in any particular tissue, it is rare in all lipid classes. It formed a very low level in the present samples.

Table III. Saturated and monounsaturated fatty acid composition of the unfermented and fermented African locust bean seeds

Fatty acid	Unfermented Seeds	Fermented seeds	Unfermented -fermented seeds	Percentage difference
Acetic acid (C2:0)	Nd	Nd	-	-
Propionic acid (C3:0)	Nd	Nd	-	-
Butanoic acid (C4:0)	0.381	0.009	+0.371	+97.5
Pentanoic acid (C5:0)	0.000	0.000	-	-
Hexanoic acid (C6:0)	0.262	Nd	-	-
Octanoic acid (C8:0)	Nd	Nd	-	-
Decanioc acid (C10:0)	0.081	0.001	+0.080	+98.2
Lauric acid (C12:0)	Nd	Nd	-	-
Myristic acid (C14:0)	0.136	0.002	+0.134	+98.2
Palmitic acid (C16:0)	16.2	11.2	+5.02	+30.9
Stearic acid (C18:0)	17.2	23.1	-5.94	-34.6
Arachidic acid (C20:0)	2.90	3.08	-0.179	-6.17
Behenic acid (C22:0)	6.49	3.59	+2.90	+44.7
Lignoceric acid (C24:0)	1.88	1.08	+0.800	+42.5
Total SFA	45.6	42.1	+3.45	+7.56
Myristoleic acid (C14:*1cis-9*)	0.189	0.006	+0.183	+96.8
Palmitoleic acid (C16:*1cis-9*)	0.049	0.001	+0.048	+98.2
Petroselinic acid (C18:*1cis-6*)	6.15	4.35	+1.80	+29.2
Oleic acid (C18:*1cis-9*)	4.89	4.17	+0.715	+14.6
Gondoic acid (C20:*1cis-11*)	0.081	0.001	+0.079	+98.2
Erucic acid (C22:*1cis-13*)	0.000	0.000	-	-
Nervonic acid (C24:*1cis-15*)	0.000	0.000	-	-
MUFA (*cis*)	11.4	8.53	+2.82	+24.9
trans-Petroselinic (C18: 1*trans*-6)	3.19	7.05	-3.86	-121
Elaidic acid (C18:1*tran*-6)	4.79	5.79	-1.00	-20.9
Vaccenic acid(C18:1*trans*-11)	3.28	5.54	-2.26	-68.9
MUFA (*trans*)	11.3	18.4	-7.12	-63.2
MUFA (total)	22.6	26.9	-4.30	-19.0
MUFA/SFA	0.496	0.639	-	-

Nd = not detected; - = not determined; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.

Table IV. *n*-6 and *n*-3 fatty acid composition of African locust bean seed samples

Fatty acid	Unfermented seeds	Fermented seeds	Difference	Percentage difference
Linoleic acid (LA) (C18:2*cis*-9, 12)	21.4	14.3	+7.05	+33.0
Gamma-linolenic acid (GLA) (18:3 *cis*-6, 9, 12)	0.239	0.024	+0.215	+90.1
Eicosadienoic acid (C20:2 *cis*-11, 14)	0.081	0.001	+0.079	+98.2
Dihomo-γ-linolenic acid (DGLA) (C20:*3cis*-8, 11, 14)	0.000	0.000	-	-
Arachidonic acid (AA) (C20:4 *cis*-5, 8, 11, 14)	0.000	0.000	-	-
Docosadienoic acid (C22:2 *cis*-13, 16)	0.000	0.000	-	-
n-6 PUFA (*cis*)	21.7	14.3	+7.34	+33.9
Rumenic acid (RA) (C18:2 *cis-9, trans-11*)	9.82	16.6	-6.79	-69.1
n-6 PUFA (total)	31.5	30.9	+0.552	+1.75
Alpha-linolenic acid (ALA) (C18:3 *cis-9*, 12, 15)	0.329	0.032	+0.297	+90.2
Eicosatrienoic acid (ETE)				
(C20:3 *cis-11*, 14, 17)	0.000	0.000	-	-
Timnodonic acid (EPA)				

(C20:5*cis-5*, 8, 11, 14, 17)	0.000	0.000	-	-
Cervonic acid (DHA) (C22:6 *cis-4*, 7, 10, 13, 19)	0.000	0.000	-	-
n-6 + *n*-3 (PUFA)	31.8	31.0	+0.849	+2.67
Totals (SFA+MUFA+PUFA)	100	100	-	-
Totals (MUFA+PUFA)	54.4	57.9	-3.45	-6.33
PUFA/SFA	0.698	0.735	-	-
2*n*-6/3*n*-3	64.8	443	-	-
Ratio	1:1	1:1	-	-

PUFA = polyunsaturated fatty acid.

Table V. Energy contribution (kJ/100 g) of African locust bean seeds by the unfermented and fermented samples from their fatty acids

Parameter	Unfermented seed	Fermented Seeds	Mean	SD	CV %
SFA	227 (45.6 %)	243(42.1 %)	235	11.3	4.81
MUFA (*cis*)	56.7	49.2	53.0	5.30	10.0
MUFA (*trans*)	56.2	106	81.1	35.2	43.2
MUFA (total)	113 (22.6 %)	155 (26.9 %)	134	29.7	22.2
n-6 PUFA (*cis*)	108	82.6	95.3	18.0	18.8
n-6 PUFA (*cis, trans*, RA)	49.0	95.6	72.3	33.0	45.6
n-6 (total)	157(31.4 %)	178(30.9 %)	168	14.8	8.87
n-3	1.64(0.330 %)	0.186(0.032%)	0.913	1.03	113
Total energy	498	576	537	55.2	10.3
PUFA energy	158(31.8 %)	178(31.0 %)	168	14.1	8.42
Total unsaturated fat	271 (54.4 %)	333 (57.9 %)	302	43.8	14.5

Table VI. Statistical analysis of the results from Tables III and IV

Fatty acid	Unfermented Seeds	Fermented seeds	r_{xy}	r_{xy}^2	R_{xy}	C_A	IFE	Remark
SFA	45.6	42.1						
MUFA (total)	22.6	26.9	0.9889	0.98	10.9	14.9	85.1	NS
PUFA	31.8	31.0						

r_{xy} = correlation coefficient; r_{xy}^2 = coefficient of determination; R_{xy} = regression coefficient; C_A = coefficient of alienation; IFE = index of forecasting efficiency; NS = results not significantly different at n-2 and $r_{=0.05}$.

Table VII. Fatty acid per 100 g sample as food in African locust bean seeds

Parameter	Unfermented seed	Fermented Seeds	Mean	SD	CV %
C4:0	0.051	0.001	0.026	0.035	136
C6:0	0.035	-	-	-	-
C10:0	0.011	0.0002	0.006	0.008	136
C14:0	0.018	0.0004	0.009	0.012	135
C16:0	2.19	1.75	1.97	0.311	15.8
C18:0	2.32	3.60	2.96	0.905	30.6
C20:0	0.391	0.479	0.435	0.062	14.3
C22:0	0.874	0.558	0.716	0.223	31.2
C24:0	0.253	0.168	0.211	0.060	28.6
C14:*1cis-9*	0.025	0.001	0.013	0.017	131
C16:*1cis-9*	0.007	0.0001	0.004	0.005	137
C18:*1cis-6*	0.829	0.678	0.754	0.107	14.2
C18:*1cis-9*	0.659	0.650	0.655	0.006	0.972
C20:*1cis-11*	0.011	0.0002	0.006	0.008	136
C18:*1trans-6*	0.430	1.10	0.765	0.474	61.9
C18:*1trans-9*	0.646	0.902	0.774	0.181	23.4
C18:*1trans-11*	0.442	0.862	0.652	0.297	45.5
C18:2*cis*-9, 12	2.88	2.23	2.56	0.460	18.0
C18:3*cis*-6, 9, 12	0.032	0.004	0.018	0.020	110
C20:2*cis*-11, 14	0.0002	0.0002	0.0002	0.00002	10.2
C18:2*trans-9, cis-11*	1.32	2.59	1.96	0.898	45.9
C18:3 *cis-9*, 12, 15	0.044	0.005	0.025	0.028	113
Total	13.5	15.6	14.6	1.48	10.2
Difference	0.011(0.079%)	0.00 (0.00 %)	-	-	-

Table VIII. Phospholipid levels (mg/100 g) of the unfermented and fermented African locust bean seeds

Phospholipid	Unfermented seeds	Fermented seeds	Difference	Percentage difference
Cephalin (PE)	13.4 (6.48 %)	36.4 (17.5 %)	-22.9	-171
Lecithin (PC)	95.5 (46.1 %)	107(51.4 %)	-11.5	-12.1
(PS)	37.8 (18.2 %)	36.3(17.5 %)	+1.45	+3.85
(LPC)	49.4 (23.9 %)	16.3 (7.82 %)	+33.2	+67.1
(PI)	11.2 (5.38 %)	12.1 (5.83 %)	-0.931	-8.82
Total	207	208	-0.824	-0.397

PE = phosphatidylethanolamine/cephalin; Lecithin = phosphatidylcholine/lecithin; PS = phosphatidylserine; LPC = lysophosphatidylcholine; PI = phosphatidylinositol

Table IX. Statistical analysis of the results from Table VIII

Phospholipid	Unfermented seeds	Fermented Seeds	r_{xy}	r_{xy}^2	R_{xy}	C_A	IFE	Remark
Cephalin	13.4	36.4						
Lecithin	95.5	107						
PS	37.8	36.3	0.8377	0.70	2.89	54.6	45.4	NS
LPC	49.4	16.3						
PI	11.2	12.1						

Table X. Phytosterol levels (mg/100 g) of the unfermented and fermented African locust bean seeds

Phytosterol	Unfermented seeds	Fermented seeds	Difference	Percentage difference
Cholesterol	9.33 (3.77 %)	9.84 (3.86 %)	-0.511	-5.48
Cholestanol	0.000	0.00	-	-
Ergosterol	0.000	0.000	-	-
Campesterol	92.0(37.1 %)	91.6 (35.9 %)	+0.382	+0.415
Stig-masterol	8.47 (3.42 %)	8.63 (3.38 %)	-0.154	-1.82
5-Avenasterol	0.000	0.000	-	-
Sitosterol	138(55.7 %)	144 (56.8 %)	-6.86	-4.97
Total	248	255	-7.15	-2.89

Table XI. Statistical analysis of the results from Table X

Phytosterol	Unfermented seeds	Fermented Seeds	r_{xy}	r_{xy}^2	R_{xy}	C_A	IFE	Remark
Cholesterol	9.33	9.84						
Campesterol	92.0	91.6						
Stig-masterol	8.47	8.63	0.9994	0.999	-0.604	3.51	96.49	*
Sitosterol	138	144						

*= results are significantly different at n-2 and $r_{=0.05}$.

The fatty acid inhibits the binding of [^{3}H] - 1TB$_4$ to pig neutrophil membrane with a Ki of 3µm. The levels of C18: 2 *cis-9*, *trans-11* ranged from 9.82 %-16.6 % as seen in Table IV. The rumenic acid (RA) was more concentrated in the fermented African locust bean seeds than the linoleic acid (LA), as seen in Table IV. In Table III vaccenic acid levels ranged from 3.28 %-5.54 %. Conjugated linoleic acids (CLA) make up a group of polyunsaturated fatty acids found in meat and milk from ruminant animals and exist as a general mixture of conjugated isomers of LA. Of the many isomers identified, the *cis-9*, *trans-11* CLA isomer (also referred to as rumenic acid or RA) accounts for up to 80-90 % of the total CLA in ruminant products[36]. Naturally occurring CLAs originate from two sources: bacterial isomerization and /or biohydrogenation of *trans*-fatty acids in the adipose tissue and mammary glands[37]. Microbial biohydrogenation of LA and aLA by an anaerobic rumen bacterium *Butyrivibrio fibrisolvens* is highly dependent on rumen pH[38]. *De novo* synthesis of CLA from 11*t*-C18:1 has been documented in rodents, dairy cows and humans. Studies suggest a linear increase in CLA synthesis as the TVA content of the diet increases in human subjects[39]. True dietary intake of CLA should therefore consider native 9*c*11*t*-C18:2 (actual CLA) as well as the 11*t*-C18:1 (potential CLA) content of food[40].

Over the past two decades numerous studies have shown significant health benefits attributable to the actions of CLA, as demonstrated by experimental animal models, including actions to reduce carcinogenesis, atherosclerosis, and onset of diabetes[41]. Conjugated LA has also been reported to modulate body composition by reducing the accumulation of adipose tissue in a variety of species including mice, rats, pigs, and now humans[42]. Optimal dietary intake remains to be established for CLA. It has been hypothesized that 95 mg CLA/day is enough to show positive effects in the reduction of breast cancer in women utilizing epidemiological data linking increased milk consumption with reduced breast cancer[43]. Ha *et al*[44] published a much more conservative estimate stating that 3 g/day CLA is required to promote human health benefits. Ritzenthaler *et al*[45] estimated CLA intakes of 620 mg/day for men and 441 mg/day for women are necessary for cancer prevention. Obviously, all these values represent rough estimates and are mainly based on extrapolated animal data. What is clear is that we as a population do not consume enough CLA in our diets to have a significant impact on cancer prevention or suppression. Reports indicate that Americans consume between 300-400 mg/day [45], and the Australians seem to be closer to the optimum concentration at 500-1000 mg/day according to Parodi [46].

The relative values of PUFA in all the samples made them important in diet. The eicosanoids help regulate blood clot formation, blood pressure, blood lipid (including cholesterol) concentration, the immune response, the inflammation response to injury and infection and many other body functions[47]. A deficiency of *n-6* fatty acids in the diet leads to skin lesions. A deficiency of *n-3* fatty acids leads to subtle neurological and visual problems. Deficiencies in PUFA produce growth retardation, reproductive failure, skin abnormalities and kidney and liver disorders. However, people are rarely deficient in those fatty acids[48]. The relative amounts of PUFA and SFA in oils is important in nutrition and health. The ratio of PUFA/SFA (P/S ratio) is therefore important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is the oil. This is because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by SFA and PUFA [49]. The present PUFA/SFA varied between 0.698-0.735 which were averagely normal. The *n-6* and *n-3* fatty acids have critical roles in the membrane structure[50] and as precursors of eicosanoids, which are potent and highly reactive compounds. Since they compete for same enzymes and have different biological roles, the balance between the *n-6* and *n-3* fatty acids in the diet can be of considerable importance[51]. The ratio of *n-6* to *n-3* or specifically LA to aLA in the diet should be between 5:1 and 10:1[51], or 4-10 g of *n-6* fatty acids to 1.0 g of *n-3* fatty acids[52]. As LA is almost always present in foods, it tends to be relatively abundant in animal tissues. The 2n-6/3n-3 (LA/aLA) range was 64.8:1 to 443:1 in the present samples. These fatty acids are the biosynthetic precursors in animal systems of C20 and C22 PUFAs, with 3-6 double bonds, via sequential desaturation and chain-elongation steps[53]. Whilst it would be easy for the human body to synthesise arachidonic acid [AA, 20:4 (*n-6*)] from [18:2 (*n-6*)], it may be difficult to synthesize the *n-3* PUFA series especially eicosapentaenoic acid [20:5 (*n-3*) or EPA] because of the low level of C18:3 (*n-3*) and so the diet must be enhanced in this PUFA.

Pierson *et al* [8] reported that *dawadawa* contains 60-80 % of total unsaturated fatty acids with linoleic acid being the major fatty acid. The present total unsaturated fatty acids ranged from 54.4 %-57.9 % in the samples. Oyenuga [15] reported a value of 42.5 % linoleic acid which is much higher than the present results of 21.4 %-14.3 %.

Although oil constitutes 31 %-40 % of the locust bean, lipase activity is low in fermenting locust beans, and there is a fluctuation in activity throughout the fermentation[8]. Ikenebomeh [54] did not detect lipase in a laboratory fermented *dawadawa*. Of 95 bacterial isolates from 10 *dawadawa* samples, only 7 % were lipolytic, while most of them were proteolytic[8]. Of the six strains of *Bacillus subtitis* characterized in the laboratory of Pierson *et al* [8], only one strain had lypolytic activity. The lipase in the fermenting locust beans is possibly from the *Staphylococcus* sp. and *B. licheniformis*[8]. Campbell-Platt [16] suggested that lipolytic activity occurred in the later stages of locust bean fermentation. This observation was based on the increase in the number of lypolytic microorganisms. Another significant change during the fermentation of locust beans is a decrease in percentage of free fatty acids, i.e., from 0.06 % in the cooked unfermented locust beans to 0.1 % in *dawadawa* [8]. The decrease in free fatty acids is desirable since large amounts of free fatty acids in foods can result in an objectionable taste and cause rancidity[8]. From the present samples, the fermented beans fatty acids were enhanced only in C18:0 and C20:0 in the SFA but fully enhanced in all *trans*- MUFA and *trans*-PUFA which might have been promoted by the lypolytic bacteria.

The energy contribution by each fatty acid group to the overall energy contribution is depicted in Table V. Total energy contribution from the unfermented bean seeds was 498 kJ/100 g. Total energy from SFA ranged from 45.6 % down to 42.1 %; MUFA (total) from 22.6 %-26.9 %, *n-6* fatty acid total was 31.4 % down to 30.9 %, *n-3* fatty acid total contribution was low at 0.330 % down to 0.032 %; PUFA energy was 31.8 % down to 31.0 % and total unsaturated fatty acid energy was 54.4 %-57.9 %. A cursory look at these figures will show them to be equivalent to the fatty acids values of each group/each fatty acid per cent. All the coefficient of variation per cent (CV %) were low except in *n-6* (*cis, trans*, RA (45.6 %) and *n-3* (113 %). For optimum weight loss, reduce your overall fat/oil consumption to a sensible level. A level of 15-20 % of your total calories should come from fat- and the majority of that should be essential fatty acids. To determine how many grams of fats this translates into, you multiply your total daily calories by 15 % (20 % for the high-end of the range) and then divide the result by 9, which is the number of calories in a gram of fat. Here is an example: 2, 500 daily calories x 0.15 = 375. 375/9 = 41.6 or 42 grams of total fat per day- the bulk of which should be essential fatty acids. It is known that 20 % energy from fat is consistent with good health. With 41.6 g of total fat per day, the African locust bean seeds that certain 13.5 g/100 g fatty acids will only be able to just be above one-third of body requirement of energy unless > 100 g – 15.6 g/100 g is consumed as the main source of fatty acids.

The statistical analysis of the results in Tables III and IV as summarised in Table VI showed that all the results were not significantly different among themselves. The correlation coefficient (r_{xy}) was positively high at 0.9889 but not significant because it is lower than the critical value of 0.997 at $\alpha_{=0.05}$. The regression coefficient (R_{xy}) showed that for every 1 unit increase in SFA, MUFA and PUFA in unfermented sample, there was a corresponding increase of 10.9 units in the fermented sample. The coefficient of alienation (C_A (non-relationship) was low at 14.9 % whereas the index of forecasting of relationship (IFE) was high at 85.1 %. The IFE actually represents the reduction in the error of relationship between the two samples which meant 100-85.1 = 14.9 %, showing the relationship is easily predictable. Since IFE was high it means the unfermented sample could carry out all the functions that the fermented fatty acids could exercise.

Fatty acid per 100 g sample as food in African locust bean seeds is shown in Table VII. From Table I total fatty acids in unfermented locust bean seeds was 13.5 g/100 g and 15.6 g/100 g in fermented seeds. Table VII showed that 0.011 g/100 g (0.079 %) was not utilised or accounted for in the unfermented sample but all the 15.6 g/100 g was accounted for in the fermented sample. In the SFA group, C18:0 contributed highest levels of 2.32-3.60 g/100 g as food, in MUFA group C18:*1 cis-6* contributed the highest level of 0.659 down to 0.650 g/100 g and in PUFA group C18:*2cis-9, 12* contributed the highest level of 2.88 down to 2.23 g/100 g.

Phospholipids

Table VIII shows the level of various phospholipids in the samples. Phospholipids are not essential nutrients: they are just another lipid and, as such, contribute 9 kcalories per gram of energy. The total phospholipids ranged between 207 and 208

mg/100 g. Cephalin, lecithin and phosphatidylinositol were all better concentrated in the fermented sample than the unfermented sample whereas phosphatidylserine and lysophosphatidylcholine were in the opposite. Lecithin is usually the most abundant phospholipid in animals and plants, often amounting to almost 50 % of the total, and as such it is the key building block of membrane bilayers. This observation is true for lecithin values in these results with percentage values ranging from 46.1-51.4 %. Phosphatidylcholines (PC) are a class of phospholipids that incorporate choline as a headgroup. They are a major component of biological membranes and can be easily obtained from a variety of readily available sources such as egg yolk or soy beans from which they are mechanically extracted or chemically extracted using hexane. They are also a member of the lecithin group of yellow-brownish fatty substances occurring in animal and plant tissues. Phosphatidylcholines are such a major component of lecithin that in some contexts the terms are sometimes used as synonyms. However, lecithin extract consists of a mixture of phosphatidylcholine and other compounds. While lysophosphatidylcholine (LPC) was the second highest in the unfermented sample [49.4 mg/100 g (23.9 %)], cephalin (PE) and phosphatidylserine (PS) about shared that position in fermented sample with respective values of [36.4 mg/100 g (17.5 %)] and [36.3 mg/100 g (17.5 %)]. Cephalin is found in all living cells, although in human physiology it is found particularly in nervous tissue such as the white matter of brain, nerves, neural tissue and in spinal cord. The US Food and Drug Administartion (USFDA) have stated that consumption of PS may reduce the risk of cognitive dysfunction in the elderly[30]. In addition to the increased caloric burden of a diet rich in fats like phosphatidylcholine, a recent report has linked the microbial catabolites of phosphatidylcholine with increased atherosclerosis through the production of choline, trimethylamine oxide and betaine [55]. The present samples were low in both total fat and phosphatidylcholine. The statistical analysis of result in Table VIII is shown in Table IX. The r_{xy} was high but not significant, the value in fermented sample increased by 2.89 as against one unit increase in the unfermented sample, the C_A value was 54.6 % whereas IFE was 45.4 % making the prediction of relationship difficult.

Phytosterols

Phytosterol results in Table X showed the values to be close to the total values in the phospholipids: in phospholipids, total value range was 207-208 mg/100 g and in phytosterols range was 248-255 mg/100 g. The following phytosterols: cholestanol, ergosterol and 5-avenasterol had 0.00 mg/100 g each. Sitosterol was highest in both samples with 138-144 mg/100 g or 55.7 %-56.8 %, followed by campesterol with values of 92.0 down to 91.6 mg/100 g or 37.1 % down to 35.9 %, cholesterol at 9.33-9.84 mg/100 g or 3.77 %-3.86 % and lastly stig-masterol at 8.47-8.63 mg/100 g or 3.42 %-3.38 %. Out of the four phytosterols with values greater than 0.00 mg/100 g, three of them (75 %) were more concentrated in the fermented than the unfermented sample. In the samples of groundnut (*Arachis hypogaea*) seeds, cholestanol and 5-avenasterol recorded 0.00 mg/100 g in raw, roasted and cooked seeds. Also sitosterol occupied the first position in concentration among all phytosterols in all the samples of raw, roasted and cooked groundnut seeds [56]. β-sitosterol is one of several phytosterols with chemical structures similar to that of cholesterol. It is widely distributed in the plant kingdom and is found in cumin seed, *Nigella sativa*, pecans, corn oils, wheat germ, etc. Alone and in combination with similar phytosterols, β-sitosterol reduces blood levels of cholesterol and is sometimes used in treating hypercholesterolemia. In Europe, β-sitosterol plays a major role in treatment of herbal therapy of being prostatic hypertrophy; it is also used in Europe for the treatment of prostatic carcinoma and breast cancer although the benefits are still being evaluated in the USA[57]. β-sitosterol was also the major sterol in the three seed oils of *Collocynthis citrullus* (CLCT), *Cucurbita moschata* (CCBT) and *Cyperus esculentus* (CYP) [58]. While β-sitosterol occupied the second position in *Plukenetia conophora* (PKCP), it occupied the first position in *Adenopus breviflorus* (ADB) seed oils[59]. Both CLCT, CCBT, CYP [58]; raw, roasted and cooked groundnut seeds[56] also contained some quantities of cholesterol, campesterol and stigmasterol just like we have in the African locust bean seeds. Actually the cholesterol levels in the African locust bean seeds are close to the values in the three different groundnut seeds with a range of 8.93-9.28 mg/100 g.

Table XI depicts the statistical analysis of the results shown in Table X. Very high values were shown by r_{xy}, r_{xy}^2, IFE but low values in R_{xy} and C_A. The R_{xy} of -0.604 showed a reversible value between fermented and unfermented locust seeds whereas high IFE showed that the relationship between the sterol levels between the unfermented and fermented locust bean seeds could easily be predicted. For the first and only time, significant difference was observed between the values of unfermented and fermented locust bean seeds at $\alpha_{=0.05}$.

Quality assurance

The correlation determined for all the standards: fatty acids, phospholipids and phytosterols, all had values ranging as follows: 0.99833-0.99997 (fatty acids), 0.99909-0.99999 (phospholipids) and 0.99920-0.99994 (phytosterols); all the correlation values were greater than 0.95 which is the critical correlation for acceptance of these types of analytical results, thus attesting to the quality assurance of the determinations.

Conclusion

The results of these analyses and other calculations showed that the samples contained unequal distribution of most of the parameters determined. The samples were high in *n-6* fatty acids but low in *n-3* fatty acids. The samples had unsaturated fatty acids as the predominant fatty acids. All the samples were good sources of lecithin and phytosterols. Quality assurances of the determinations were highly satisfactory. Fermentation enhanced the following parameters: crude fat, total fatty acids, total energy, C18:0, C20:0, C18:*1 trans-6*, C18:*1 trans-9*, C18:*1 trans-11*, C18:*2 cis-9*, *trans-11,* lecithin, cephalin, phosphatidylinositol, cholesterol, stigmasterol and sitosterol to various levels. Only the phytosterol levels were significantly different among themselves. The totality of the results showed that 2.91 g/100 g (17.2 %) unfermented seeds crude fat was not accounted for by the total fatty acids, phospholipids and phytosterols whereas the level of 3.43 g/100 g (17.6 %) crude fat was not accounted for in the fermented African locust bean seeds.

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