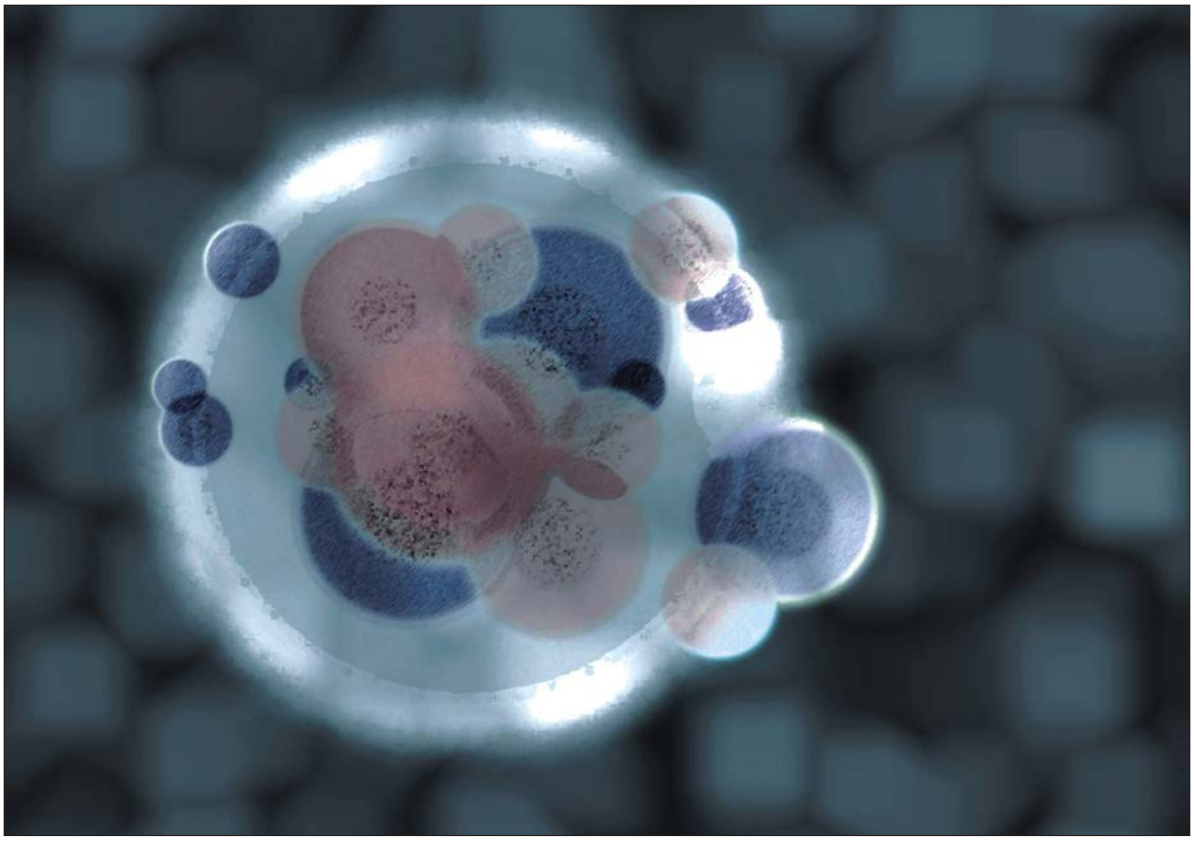

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Anti-nutritional Factors, Essential and Non-essential Fatty Acids Composition of Ugba (*Pentaclethra macrophylla*) Seeds at Different Stages of Processing and Fermentation

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Abstract: The concentrations of the Anti-nutritional Factors (ANF) and fatty acids of ugba (*Pentaclethra macrophylla*) eaten as a delicacy in different parts of Nigeria and some other African countries, were studied at eight different stages of processing and fermentation. The ANF assayed included cyanide, phytate, tannin and oxalate, while the fatty acids identified included five saturated fatty acids and four unsaturated fatty acids at each of the eight stages. The unsaturated fatty acids however occurred in higher amounts. The raw seeds of ugba were found to contain the highest concentrations of all the ANF determined, ($p<0.01$), except oxalate with the highest concentration of 937.5±0.113 mg/100 g ($p<0.05$) in the boiled seed. Progressive reductions were observed at the different stages of processing and fermentation for all the ANF. For example, cyanide concentrations reduced from 8.3±0.03 mg/100 g, through 7.5±0.03, 7.5±0.03, 4.1±0.00, 3.9±0.02, 3.3±0.01, 2.3±0.03 to 2.2±0.01 mg/100 g, while phytate reduced from 63.5±0.05 to 34.4±0.03 mg/100 g, tannin from 6.80±0.02 to 1.40±0.00 mg/100 g and oxalate from 812.50±1.13 to 187.50±0.197 mg/100 g. The overall percentage reductions obtained in the concentrations between the raw and fully processed and fermented stages were 73.49% for cyanide, 45.98% for phytate, 79.41% for tannin and 76.92% for oxalate. The most dominant fatty acid identified was the unsaturated and essential fatty acid Cis-9, Cis-12, Octadecadienoic acid (linoleic acid). Its concentrations in the samples ranged from 44.7 to 46.3%. The non-essential fatty acid Tetracosanoic acid (lignoceric acid) usually found in trace amounts in most seed oils was found to be the highest occurring saturated fatty acid in the samples being 10% of the total fatty acids concentration. The fatty acid concentrations did not change appreciably with processing and fermentation.

Key words: Ugba, *Pentaclethra macrophylla*, Anti-nutritional Factors (ANF), fatty acids, linoleic acid, lignoceric acid

INTRODUCTION

Fermented *Pentaclethra macrophylla* or oil bean seeds have become a very important delicacy in the life of Nigerians. Its use as food among the south eastern populace of Nigeria and as a delicacy across the different tribes in Nigeria has increasingly been shown and consequently, the ready-to-eat dish is called "African salad". Edward[1] reported that this snack has gained a lot of popularity among different tribes in Nigeria as a result of greater integration and changing food habits.

The oil bean tree has been classified among the plants of tropical West Africa according to Hutchinson and Dalziel[2]. It is a perennial plant that belongs to the family Leguminosae. The local names include "Congo acacia" in Congo, "Duala kombola" in Cameroun and "ugba", "ukpaka" or "ukpakala" in Nigeria.

The oil bean seeds are inedible when raw and bitter until the final stage of fermentation. However, when processed and allowed to ferment fully, they are eaten and relished. They are served to esteemed guests during very important cultural ceremonies. The fermentation improves flavour and taste and has been described as an economic processing method used in homes to improve nutritional quality[3-5].

According to Siggel and Faucett[6], fermentation is the oldest method of processing legumes. Despite this, other workers[7,8] observed that not many fermented foods are produced and consumed in Nigeria and only few studies have been carried out on them. For instance, Onwuliri *et al.*[9] and Onwuliri and Ekpenyong[10] reported the chemical composition of *Pito* and *burukutu*, two Nigerian traditional brands of alcoholic beverages brewed from sorghum and millet, while another worker studied the

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fermentation of cassava[11]. Akingbala *et al.*[12] also studied the laboratory fermentation of "ogi", while Eka[3] highlighted the effect of fermentation on the nutrients status of locust beans. Similarly, others determined the effect of fermentation on the thiamin, riboflavin and niacin contents of melon seeds (*Citrullus vulgaris*) and African oil bean seed (*Pentaclethra macrophylla*)[13]. Also in 1986, Achinewhu[5] reported on the carbohydrate and fatty acid composition of the raw and fully fermented seeds of *Pentaclethra macrophylla*, while the raw seeds were analyzed by some others[14] for fatty acids and amino acids. So far, there has been a paucity of report on the ANF composition of ugba and on the changes in the fatty acid composition of *Pentaclethra macrophylla* during the different stages of fermentation and processing. Furthermore, it has been ascertained that factors like climate, soil, species, nutrition, strain and agricultural methods vary and affect the composition of seeds[15-17]. In addition, earlier studies had been on the raw and the final products. Therefore, this study has followed the ANF components and the fatty acid composition as the "ugba" underwent fermentation from the inedible raw seeds to the edible and palatable boiled and fermented seeds.

MATERIALS AND METHODS

Collection and preparation of samples: The dried *Pentaclethra macrophylla* seeds were obtained from the dried pods fresh from the tree at Amuzi, Ahiara Mbaise Nigeria. The seeds were identified at the Department of Botany, University of Jos, Nigeria and analyzed after one week.

The dried seeds were cleaned and de-shelled before processing, fermentation and analysis as described below. At different stages, samples were collected and dried to constant weight, ground to powder and stored in screw-top containers at room temperature, from where portions were taken for analyses.

The stages were as follows:

Stage 1: Raw seeds without shell (A)
Stage 2: Parboiled seeds without shell (B)
Stage 3: Parboiled, sliced and re-boiled ©
Stage 4: Parboiled, sliced, re-boiled and soaked for 12 h (D)
Stage 5: Washed, drained, wrapped in fresh leaves and covered for fermentation (E)
Stage 6: 12 h of fermentation (F)
Stage 7: 24 h of fermentation (G)
Stage 8: 48 h of fermentation (H)

Anti-nutritional Factors (ANF) estimation: Two grams of powdered samples were used for the analysis of the different anti-nutritional factors. The procedures of Oke[18], Trease and Evans[19] and, Onwuliri and Obu[20] were employed in the determination of the total cyanide content. Tannin was assayed by using the methods of AOAC[21] and Onwuliri and Obu[20]. Phytate was estimated by the procedure of Davies and Reid[22], whereas total oxalate was analyzed according to the methods of Munro and Bassir[23], Onwuliri and Obu[20].

Lipid and fatty acid analysis: The powdered samples were extracted in 20 g portions in a soxhlet extractor for 8 h with the Folch mixture and the total lipid was determined by gravimetry[20,21,24]. Fatty acid methyl esters were prepared from the lipids with methanol in the presence of boron triflouride in benzene solution. The fatty acid methyl esters were analyzed by gas chromatography according to earlier methods[24,25].

RESULTS AND DISCUSSION

The concentrations of some Anti-nutritional Factors (ANF) in the seeds of ugba undergoing processing and fermentation are presented in Table 1. The ANF assayed which included cyanide, phytate, tannins and oxalate were found to be in the highest concentrations in the raw seeds, except for oxalate that had the highest value of 937.5±0.113 mg/100 g in the boiled seed. The results indicated that the range of values for cyanide samples were from 8.3±0.03 to 2.2±0.01 mg/100 g. The initial values of 8.3±0.03 mg/100 g were above the tolerable level of 3 mg/100 g dry weight sample for humans, as asserted by Ikediobi *et al.*[26]. The concentration of 2.2±0.01 mg/100 g in the final fermented ugba fell below the tolerable limits. These values are all below the 42.0 mg/100 g DM reported for raw lima beans[27]. The reduced values of the hydrocyanic acid with processing and fermentation can

Table 1: Anti-nutritional contents of ugba

	Concentration of ANF (mg/100 g)			
Samples	CN	Phytate	Tannin	Oxalate
A	8.3±0.03	63.5±0.05	6.80±0.02	812.50±1.13
B	7.5±0.03	53.4±0.03	4.08±0.00	937.50±0.113
C	7.5±0.03	48.9±0.03	3.83±0.03	562.50±0.197
D	4.1±0.03	48.7±0.01	2.05±0.02	502.50±0.02
E	3.9±0.02	48.6±0.01	2.03±0.01	486.50±0.01
F	3.3±0.01	48.4±0.02	1.98±0.01	312.50±0.463
G	2.3±0.03	44.1±0.02	1.75±0.01	250.00±0.114
H	2.2±0.01	34.4±0.03	1.40±0.00	187.50±0.197
% Reduction from raw to fully fermented	73.49%	45.98%	79.41%	76.92%

Table 2: Fatty acid composition of ugba seeds at different stages of processing and fermentation

Fas	A	B	C	D	E	F	G	H
C12	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
C14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C14-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C15-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C16	3.5	3.0	3.0	3.0	3.0	2.9	2.9	2.9
C16-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C17-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C18	4.5	3.7	3.9	3.9	3.8	3.7	3.7	3.7
C18-1	26.3	26.2	26.1	26.1	26.0	25.8	25.7	25.8
C18-2	44.7	45.1	45.7	45.6	45.5	46.3	45.6	46.1
C18-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C20	4.2	4.0	4.0	3.9	3.9	3.9	4.1	3.9
C20-1	0.8	0.8	0.7	0.7	0.7	0.7	0.8	0.8
C20-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C22	4.2	4.4	4.0	4.2	4.2	4.3	4.5	4.3
C22-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C22-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C22-6	1.7	2.0	2.0	2.1	2.1	2.0	2.0	2.1
C24	10.1	10.8	10.3	10.5	10.5	10.4	10.7	10.4
C24-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Fas-Fatty acids

be explained by the report of Okolie and Ugochukwu[28] that food processing like soaking in water and cooking, drastically reduce the cyanide levels in food.

The observed progressive reductions of ANF at the different stages is in line with the assertion that the antinutrients levels (phytate, tannin and HCN) were generally reduced due to fermentation and the microflora activity[29]. Onwuliri and Obu[20] also noted that prolonged processing like soaking, cooking, discarding water used and removal of testa reduce cyanide too. In the same vein, it was observed that a 20-34% reduction in the phytate levels occurred in fermented legumes samples[30]. Ihekoronye and Ngoddy[31] asserted that phytate levels can be reduced through soaking, dehydration and cooking. This agrees with the fact that microflora enzymes which break down organic complexes to release antinutrients which leach out into the surrounding medium are produced by fermentation. Soaking, another process employed here has been reported to lead to the slight decrease in phytate[32] and to appreciable reduction of cyanide[33].

The tannin content reduced by 73.49% from 6.80±0.02 to 1.40±0.00 mg/100 g. Boiling reduced tannin content to 4.08±0.00 mg/100 g whereas washing and slicing reduced the concentration to 2.05±0.02. The reduction continued with fermentation and after 48 h, the tannin level fell to 1.40±0.00 mg/100 g. This is low and therefore, agrees with reported observations[20, 34-36]. Tannin in food depresses growth by decreasing protein quality and digestibility. Fortunately, the levels of tannin detected in these samples would not affect the nutritional potential of ugba[36,37].

The oxalate concentrations in the samples ranged from 812.50±1.13 to 187.50±0.197 mg/100 g, a reduction of 76.92%. This is consistent with other observations[38] and lower than the lethal dose of 3 g given by Munro and Bassir[23].

The results of the present study showed that ugba lipids contain both saturated and unsaturated fatty acids of long chain lengths. The chain lengths of 16 to 24 obtained are in line with the chain length of 12 to 24 reported by Ononogbu for commonly occurring fatty acids in seed oils[39].

The saturated fatty acids identified in this study are the non-essential fatty acids hexadecanoic or palmitic (C16) acid ranging from 2.9 to 3.5%, Octadecanoic or stearic (C18) acid from 3.7 to 4.5%, eicosanoic acid or arachidic acid (C20) from 3.9 to 4.2%, docosanoic or behenic acid (C22) 4.0 to 4.5% and tetracosanoic or lignoceric acid (C24) from 10.1 to 10.8%. These results agree with earlier observations that palmitic acid generally forms less than 5% of the total fatty acids in vegetable oils and that stearic, arachidic and behenic acids occur in small amounts in seed oils[33] (Table 2).

Lignoceric (C24) acid was found to be about 10% of the total fatty acids in "Ugba" at the different stages. This is opposed to the assertion that lignoceric acid is present in trace levels in plants oil except in groundnut oils where it is up to 1%. However, there are other exceptions like in *Adenanthera, pavonina* a leguminous seed and in carnauba wax where lignoceric acid levels are about 25 and 30%, respectively[39].

In all, the saturated fatty acids account for 25.2 to 26.5% of the fatty acids in Ugba undergoing

processing and fermentation while the unsaturated fatty acids constitute between 73.5 and 74.7% with cis-9, cis-12-octadeca dienoic acid, (18-2) or linoleic acid being most predominant with values ranging from 44.7 and 46.3%. Linoleic acid is an ω-6 fatty acid predominantly found in sunflower and corn oil[40]. Furthermore, Cis-9, cis-12-octadecadienoic acid is one of the three essential fatty acids and a precursor of Arachidonic acid, another ω-6 essential fatty acid. Both of them are important precursors of prostaglandins, thromboxanes and leukotrienes[41]. Finally, the observation that the concentrations of the fatty acids in Ugba did not change with processing and fermentation are in line with that of Achinewhu[42].

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