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Nutrient Changes During the Fermentation of African Oil Bean (*Pentaclethra macrophylla* Benth) Seeds

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Abstract: The prepared seedslices of African oil bean (*Pentaclethra macrophylla* Benth) were subjected to a 72-hour traditional fermentation to produce 'ugba' a popular food condiment consumed in the rain forest areas of West tropical Africa. The fermentation brought about slight increases in crude protein and ash contents, and a slight decrease in the oil content of the seeds. Amino nitrogen increased steadily from 1.23 $mgNg^{-1}$ DM prior to fermentation to 13.68 mg Ng^{-1} DM after 72-hours, showing a strong indication of appreciable protein hydrolysis. Gas chromatographic analysis of the seed oil showed the principal fatty acid, linoleic acid, increasing from 60.68 to 67.57% of total fatty acids while oleic acid decreased from 26.95 to 22.59%. Palmitic acid and other saturated fatty acids in the seed oil were also slightly affected by the fermentation; while palmitic increased steadily, others decreased markedly. Total titratable acidity increased gradually as fermentation progressed. A further analysis using thin-layer chromatography revealed the accumulation of formic, acetic, lactic and butyric acids in the fermenting seedslices. At the end of 72 hours, 'ugba' contained 0.41 mgg^{-1} butyric acid, 0.35mgg^{-1} lactic acid, 0.18 mgg^{-1} acetic acid and 0.20 mgg^{-1} formic acid, on dry weight basis.

Key words: Oil bean seeds, fermentation, chemical composition, fatty and organic acids

Introduction

The African oil bean tree (*Pentaclethra macrophylla* Benth) is a large leguminous woody plant that belongs to the sub-family Mimosoidae (Keay, 1989). It is frequently cultivated in forest areas, with about eight (8) flat glossy brown edible seeds per pod. The plant grows both as wild and cultivated types. The raw seed is a potential source of edible protein and calories, containing the twenty (20) essential amino acids and essential fatty acids that makes up more than 80% of fatty acids in the oil (Enujiugha and Agbede, 2000; Ikediobi, 1981).

The fermented seed product, ugba, is traditionally prepared by boiling the oil bean seeds overnight for easy removal of the seed coats, slicing of the cotyledons, cooking until the sliced cotyledons become soft with reduced bitterness, washing in five or more changes of water, and fermenting the sliced cotyledons for a period of 3 days (Enujiugha, 2000). The fermented ugba can then be consumed as a snack or used as a condiment in soup mixes and local porridges. Previous research works point to fermentation as bringing about nutritionally-better product than the raw seeds (Achinewhu, 1986; Enujiugha and Olagundoye, 2001) and the enzyme systems, especially α-amylases aid hydrolysis of the seed macromolecules (Enujiugha *et al*., 2002).

However, the nature of the fermentation with respect to the role played by the microbial enzymes in hydrolyzing the seed native nutrients is not known. It is therefore appropriate to examine the changes in chemical composition and the accumulation of some fermentation by-products as a way of monitoring the progress of fermentation. The present study sought to investigate this neglected area.

Materials and Methods

Collection and preparation of materials: African oil bean seeds were collected from Nri in Eastern Nigeria and transported to the laboratory in airtight polyethylene containers. After dehulling, slicing and cooking, the seedslices were fermented at 35 °C for 72 h using a previously described method (Enujiugha, 2000). Briefly, the seeds were parboiled for 30 min at 10 psig using a pressure vessel and the hard seed coats were removed manually. The cotyledons were sliced to about 5.0 cm long x 1.0 cm broad, steamed at 10 psig for 6 h, and soaked in 5% brine at 40 °C for 2 h. After draining, the subsequent fermentation was done using 3% seed culture (traditionally fermented seed product).

Composite samples were collected from different points within the fermenting container at 24-hourly intervals and pooled as individual samples for analysis. Cooked unfermented seedslices served as control. The samples were further ground to pass through a 40-mesh sieve (aperture size 0.42mm) and kept at -20 °C in sealed cellophane containers until required for analysis.

Chemical analysis: The proximate chemical composition of the samples was determined using the standard procedures of AOAC (1990). The crude protein content was calculated by multiplying the total nitrogen

Table 1: Fermentation effects on the proximate chemical composition of African oil bean seeds (Mean ± SD)

Fermentation period (hr)	Component determined (% dry wt.)				
	Crude protein	Crude fibre	Ether extract	Ash	NFE
0	34.3 ± 0.4	5.6 ± 0.1	41.4 ± 0.5	2.1 ± 0.1	16.6 ± 0.4
24	34.8 ± 0.2	5.6 ± 0.1	39.9 ± 0.1	2.4 ± 0.2	17.3 ± 0.1
48	35.1 ± 0.4	5.5 ± 0.1	38.5 ± 0.3	2.7 ± 0.1	18.2 ± 0.3
72	35.4 ± 0.1	5.6 ± 0.1	36.3 ± 0.1	2.9 ± 0.1	19.8 ± 0.1

Table 2: Changes in pH, titratable acidity and amino nitrogen during the oil bean seed fermentation (Mean ± SD)

Fermentation period (h)	pH	Titratable acidity (% lactic acid)	Amino nitrogen ($mgNg^{-1}$ dry wt.)
0	6.4 ± 0.1	0.5 ± 0.2	1.23 ± 0.10
24	6.3 ± 0.1	0.8 ± 0.1	3.07 ± 0.07
48	6.1 ± 0.1	1.0 ± 0.1	7.89 ± 0.20
72	5.8 ± 0.1	1.2 ± 0.1	13.68 ± 0.05

by the factor 6.25. The carbohydrate content was estimated by difference and expressed as the nitrogen free extracts (NFE). The pH of each sample homogenate was determined with a Pye Unicam pH meter (Model PW9409), which was previously standardized with buffers pH 4 and pH 7. Total titratable acidity was determined by potentiometric titration against 40% NaOH solution (Pearson, 1976). Amino nitrogen of each fat-free sample concentrate was determined by 0.2N barium hydroxide titration (AOAC, 1990).

Determination of organic acids: The thin-layer chromatographic analysis of organic acids in each fat-free sample extract was determined using the method of Granshirt *et al.* (1965) as earlier modified (Enujiugha, 1990), with pyridine-petroleum ether (1:2) as the developing solvent. The organic acids were eluted on silica gel G alongside acid standards, which were converted into their ammonium salts, and subsequently extracted with benzene.

Analysis of fatty acids: Fatty acid methyl esters were obtained quantitatively from the seed oil by direct transesterification with methanolic sodium hydroxide at room temperature, and subsequent methylation with 14% BF_3 - methanol (Metcalfe *et al.*, 1966). The component fatty acids were determined with a Pye 300 gas chromatograph, using a hydrogen flame ionization detector operated isothermally at 200 °C with a sensitivity of $1x10^{-9}$ A.

Results

The pattern of changes in the proximate chemical composition of the African oil bean seeds during processing into ugba is shown in Table 1. Crude protein increased slightly from about 34.3% in the unfermented seeds to about 35.4% in the produced ugba. The same trend was observed with the carbohydrate content. The ether extract decreased slightly throughout the fermentation period, while ash content increased from 2.1% in the cooked unfermented seeds to 2.9% in the fermented product. Crude fibre content was not affected by the fermentation.

The changes in hydrogen ion concentration (pH) and total titratable acidity during the fermentation of African oil bean seeds are shown in Table 2. While pH decreased from near basic to slightly acidic range, the titratable acidity steadily increased to a significant level in the fermented product. Table 2 also shows the effects of fermentation on the amino nitrogen content of the African oil bean seeds. Amino nitrogen as an index of fermentation efficiency is used to monitor the progress of fermentation of proteinaceous materials. The amino nitrogen increased from 1.23 $mgNg^{-1}$ in cooked unfermented seeds to 13.68 $mgNg^{-1}$ in the fermented product.

Table 3 shows the results of thin-layer chromatographic analysis of organic acids during the 72-h fermentation. Four major organic acids namely formic, acetic, lactic and butyric acids were observed in all the samples except for the cooked unfermented seeds that showed no presence of organic acids. All the four organic acids showed significant increases as fermentation progressed, and had the highest values after 72 hours. Acetic acid had the lowest value of 0.18 mgg^{-1} at the end of fermentation, while the highest value (0.41 mgg^{-1}) was obtained for butyric acid.

The effects of fermentation on the fatty acids profile of the African oil bean seeds is shown in Table 4. The fatty acids present in the samples include capric C_{10} , lauric C_{12} , myristic C_{14} , palmitic C_{16} , stearic C_{18} , oleic $C_{18:1}$, and linoleic $C_{18:2}$. Among the saturated fatty acids present in the seed oil, palmitic acid increased steadily throughout the fermentation period while the others decreased drastically during the first 48 hours only to accumulate slightly after 72 hours. Linoleic acid which was found to be the major fatty acid in the samples increased throughout the fermentation period. On the

Table 3: Profile of organic acids during the fermentation of the oil bean seed slices (Mean ± SD)

Fermentation period (h)	Organic acid [mgg^{-1} sample][a]			
	Formic [R_f 0.53]	Acetic [R_f 0.59]	Lactic [Rf 0.64]	Butyric [Rf 0.75]
0	-	-	-	-
24	0.04 ± 0.02	0.07 ± 0.01	0.20 ± 0.01	0.15 ± 0.03
48	0.15 ± 0.01	0.10 ± 0.01	0.28 ± 0.04	0.36 ± 0.02
72	0.20 ± 0.02	0.18 ± 0.02	0.35 ± 0.01	0.41 ± 0.01

[a]Developing solvent = pyridine-petroleum ether (1:2)

Table 4: Changes in fatty acids profile during oil bean seed fermentation (Mean ± SD)

Fatty acid (% by wt.)	Fermentation period (hours)			
	0	24	48	72
Capric	0.43 ± 0.05	-	-	0.05 ± 0.01
Lauric	1.44 ± 0.01	0.06 ± 0.01	0.16 ± 0.01	0.57 ± 0.01
Myristic	1.16 ± 0.01	0.15 ± 0.01	-	0.23 ± 0.01
Palmitic	6.65 ± 0.25	6.91 ± 0.05	6.99 ± 0.02	7.02 ± 0.01
Stearic	2.69 ± 0.07	1.66 ± 0.02	1.22 ± 0.02	1.97 ± 0.10
Oleic	26.95 ± 1.20	24.47 ± 1.00	22.67 ± 0.04	22.59 ± 0.03
Linoleic	60.68 ± 0.09	66.73 ± 0.50	66.95 ± 0.15	67.57 ± 0.50

other hand oleic acid, the second unsaturated fatty acid present decreased steadily in the samples.

Discussion

The results have shown that African oil bean seeds in the cooked and fermented forms have enough nutrients to satisfy protein requirements of populations in the developing countries that rely much on starchy staples. The slight increases in crude protein values observed during fermentation (Table 1) could be due to the action of extracellular enzymes produced by the fermenting microorganisms. It has been established (Fogarty and Griffin, 1973) that *Bacillus* species implicated in oil bean seed fermentation are important producers of proteases. These extracellular proteases easily hydrolyze complex plant proteins to amino acids and short chain peptides, thereby causing an increase in total nitrogen content. Crude fibre content not being affected by the fermentation could probably be due to the inability of the microbial agents to synthesize cellulases and hemicellulases for the hydrolysis of complex polysaccharides in the seeds. The decreases in oil content of the seeds could be attributed to glycerol, one of the hydrolytic products of lipids, not being fat-soluble (Landers and Rathmann, 1981).

The increase of ash content by about 35% in the fermented product could be attributed to the increased metabolic activities of the fermenting microorganisms. Some of the biosynthetic mechanisms, especially those involving *Bacillus* species, are capable of synthesizing divalent metals (Moat, 1979). Carbohydrates in the form of nitrogen-free extracts increased slightly but steadily throughout the fermentation period, with a difference of more than 15% between the raw seeds and the fermented product. This increase could be attributed to the hydrolysis of complex oligosaccharides in the seed (Achinewhu, 1986).

The observed increase in the level of amino nitrogen (Table 2) implies protein hydrolysis to amino acids and lower molecular weight peptides. The results obtained in this study agree with the observation of Yong and Wood (1977) that there was a gradual increase in amino nitrogen levels during the fermentation of soybeans to produce "koji", with the highest level been at 72 hours incubation. Table 2 also reveals the production of acids during fermentation. The metabolism of the carbohydrates in the raw seeds would have resulted in the accumulation of organic seeds (Table 3). In almost all fermentation involving *Bacillus* species, there is production of the primary organic acids, especially butyric acid (Moat, 1979). Previous works point to *Bacillus* species, especially *Bacillus subtilis* as the main starters for African oil bean seed fermentation (Isu and Njoku, 1997; Mbajunwa *et al.*, 1998).

All studies on the raw African oil bean seed oil have shown that linoleic acid is the major fatty acid followed by oleic acid (Ikediobi, 1981; Enujiugha, 1990; Achinewhu, 1982). The observed increases in the percentage linoleic acid could probably be due to increased hydrolysis of the glycerides. Linoleic acid, alongside α-linolenic acid and docosahexaenoic acid (DHA), is essential to the well being, growth and development of children, especially breast-fed infants during the first six months (Innis, 1991). The African oil bean seed which is high in linoleic acid could therefore be usefully incorporated into infant formulae and weaning foods in developing countries where infant mortality rates are currently high due to protein-energy malnutrition (PEM).

Conclusion: The fermentation of African oil bean (*Pentaclethra macrophylla* Benth) seeds resulted in higher nutrient availability and digestibility as can be deduced from the higher amino nitrogen and organic and fatty acids contents observed in the fermenting seed slices. There was also an increased unsaturatrion of the seed oil with fermentation. The results tend to confirm *Bacillus* species as the dominant species in the wild fermentation.

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