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The Effect of Fermentation on Carbohydrate and Fatty Acid Composition of African Oil Bean Seed (*Pentaclethra macrophylla*)

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ABSTRACT

The effect of fermentation of African oil bean seed (Pentaclethra macrophylla) *on chemical composition was studied. The samples were analysed for carbohydrates and fatty acids using gas–liquid chromatography. There was an increase in the pH, titratable acidity, soluble nitrogen and soluble solids during a 4-day fermentation. Trimethylsilyl (TMS) derivatives of carbohydrates extracted from the samples showed good chromatographic separations giving clear and defined peaks for each sugar. Sucrose and the flatus-forming oligosaccharides, stachyose, verbascose and raffinose were the predominant carbohydrates, with lower contents of monosaccharides in the unfermented sample. Fructose, galactose, glucose and some unidentified monosaccharides were predominant in the fermented sample. Fermentation considerably reduced or eliminated the sucrose and the flatus-forming oligosaccharides. Fermentation did not have much effect on the fatty acid composition except for a very slight reduction in the total saturated fatty acids and an increase in the total unsaturated fatty acids.*

INTRODUCTION

Fermented foods have made significant contributions to the diet of the average Nigerian. Some, such as *gari*, have been used as staple foods and

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others, such as *ogiri*, as condiments. Apart from *gari* and *ogi* (Akinrele, 1964, 1970; Banigo & Muller, 1972), very little is known about the chemical changes during their fermentation. As part of an investigation of the biochemical and nutritional changes of indigenous fermented plant foods, the present study is aimed at investigating the effect of fermentation on the carbohydrate and fatty acid composition of African oil bean seed (*Pentaclethra macrophylla*). Carbohydrates and fats are two important nutrients that supply energy to man and animals. The African oil bean seed is widely consumed in the rural areas of the Eastern States of Nigeria after fermentation. It is consumed alone, mixed with other food ingredients, or as a condiment in soups or salads. Its protein and essential amino acid compositions (Achinewhu, 1982) make it a good source of nutrients.

MATERIALS AND METHODS

Materials

The oil bean seeds belong to the family leguminosae and are obtained from cultivated or wild trees. The seeds are oval but flat in shape, dark in colour with tough seed coats. The mature seeds were harvested fresh from the trees. The standard sugars and chemical used for the gas chromatography of sugars were purchased from British Drug Houses (BDH) and trimethyl silylated sugars (TMS) were purchased from the Sigma Chemical Company Ltd.

Methods

Preparation and fermentation of samples

The seeds were boiled with the seed coats for 7 h (or pressure cooked for 2 h) and the coats were removed with a sharp knife. The seeds were sliced into very small pieces, washed with cold water, drained and sodium chloride was added to the sample (1·0 g per kilogram of sample). The sample was inoculated with a previously oven-dried (vacuum oven at 60 °C for 24 h), fermented sample (3 g per kilogram of sample). The dried sample was made into a slurry with 5 ml of distilled water and thoroughly mixed. The sample was wrapped in aluminium foil and incubated in an oven for 4 days at 31 °C. Subsamples were removed from the sample each day for analysis.

Measurement of N, solids, pH and acidity
Total and soluble nitrogen, total and soluble solids and titratable acidity were measured according to the method of the AOAC (1980). The pH was measured with a Jenway digital pH meter by mixing 1 g of sample with 10 ml of distilled water.

Analysis of carbohydrates (sugars)
The extraction of sugar, the preparation of the stock solutions and the hexamethyldisilazane derivatisation of sugars were the same as described by Li & Schuhmann (1980) with minor modifications.

Preparation of stock solutions
The pyridine reagent was prepared by dissolving, in a 50 ml quantity of pyridine, 25 mg per millilitre of hydroxylamine hydrochloride and 2 mg per millilitre of β-phenyl-D-glucopyranoside as the internal standard and stored at −5 °C until required for use. Standard sugars were prepared with 20 mg of each sugar in 10 ml of water and 1 ml was derivatised, along with the sample, after drying.

Extraction of sugar
Fat was removed from the 5 g of freeze-dried sample with *n*-hexane and the sample was extracted with 20 ml of 80% methanol for 24 h with constant shaking (600 cycles/min) at room temperature. The sample was centrifuged (2000 rpm for 6 min) and the extract concentrated to 1 ml with a vacuum evaporator at 30 °C, then dried under nitrogen in a water bath at 50 °C. The last traces of water were removed in a vacuum desiccator.

Derivatisation
The dried extracts were heated with 1 ml of pyridine reagent for 30 min, the tubes were cooled, 0·5 ml of hexamethyldisilazane and four drops of trifluoroacetic acid were added and vigorously mixed for 30 s. The mixture was ready to be injected into the gas–liquid chromatograph after 30 min; 1 μl of the sample and standard was injected into the gas–liquid chromatograph.

Gas chromatography
Sugars were analysed using a Pye-104 gas–liquid chromatograph equipped with a flame ionisation detector. The glass column (2·1 m × 6·4 mm inside diameter) was packed with 3% w/w 0V-101 on 80/100 mesh Chromosorb

W and preconditioned for 48 h. The injector and detector temperature were each maintained at 300 °C and the oven temperature was programmed at 5 °C/min from 110 °C to 300 °C with a 30 min hold at 300 °C. The flow rates were: nitrogen carrier gas, 30 ml/min; H_2, 30 ml/min and air, 300 ml/min. Gas chromatographic peaks were identified by comparison of retention times with those of standard sugars. The area of the peak for each sugar was recorded and the sugar contents were quantified (AOAC, 1980) and expressed as milligrams of sugar per gram of sample on a dry weight basis.

Analysis of fatty acids

Fat was extracted from 2 g of the sample with hexane under reflux for 6 h. Component fatty acids were determined after methylation of the fat (AOAC, 1980) with a Pye Unicam 104 GCV gas–liquid chromatograph with a flame ionisation detector. The column (2·7 m × 6·4 mm inside diameter) was packed with GP 10 % SP2 300 on 80/100 Supelcopot. Flow rates were: N_2, 30 ml/min; hydrogen, 33 ml/min and air, 330 ml/min. Detector and injector temperatures were 240 °C and 215 °C, respectively. The column/oven temperature was 215 °C.

RESULTS AND DISCUSSION

pH, titratable acidity, soluble solids, total and soluble nitrogen

Table 1 shows the pH, titratable acidity, soluble solids, total and soluble nitrogen of the fermented oil bean seed. There was an increase in pH and a corresponding increase in titratable acidity. The increase in pH despite an increase in titratable acidity might be due to increased proteolytic activities by the fermenting enzymes. This is evidenced by a rapid increase in soluble nitrogen from 0·3 g to 2·4 g per 100 g of sample. The total nitrogen was not much affected by fermentation. Steinkraus *et al.* (1960) observed a pH increase during a 72-h fermentation of soya bean substrate to produce *tempeh*. They noticed liberation of ammonia in the later stages of fermentation. Similarly, Murata *et al.* (1967) observed a progressive increase in pH during *tempeh* fermentation despite the liberation of acids. They attributed it to active proteolysis and deamination of amino acids. Soluble solids increased from 10·3 % to 29·4 % at the end of the fermentation.

TABLE 1

The pH, Titratable Acidity, Soluble Solids, Total and Soluble Nitrogen of the Fermented Oil Bean Seed ± SE (Mean of Four Determinations)

	Days				
	0	*1*	*2*	*3*	*4*
pH	5·1 ± 0·02	5·3 ± 0·02	6·3 ± 0·05	6·8 ± 0·05	7·1 ± 0·01
Acidity (% lactic acid)	0·06	0·12	0·45 ± 0·001	0·82 ± 0·003	0·98 ± 0·001
Soluble solids (%)	10·3 ± 0·08	10·9 ± 0·05	17·5 ± 0·1	26·9 ± 0·3	29·4 ± 0·2
Total N (grams per 100 g of sample)	4·5 ± 0·03	4·4 ± 0·01	4·5 ± 0·02	4·8 ± 0·01	5·1 ± 0·02
Soluble N (grams per 100 g of sample)	0·3 ± 0·005	0·5 ± 0·001	1·6 ± 0·006	2·1 ± 0·004	2·4 ± 0·002

TABLE 2
Carbohydrate Composition (mg/g Sample Dry Weight) of the Extracts from the Unfermented Oil Bean Seed ± SE (Mean of Five Determinations)

Peak No.	*Sugars*	*Concentration (mg/g dry weight)*	*Per cent total carbohydrate*
1	Unknown	0·66 ± 0·004	1·04
2	Fructose	0·22 ± 0·001	0·34
3	Galactose	0·34 ± 0·001	0·53
4	Glucose	0·34 ± 0·002	0·53
5	Glucose + Galactose	0·54 ± 0·001	0·85
Total monosaccharides		1·76 ± 0·009	2·78
7	Sucrose	33·4 ± 1·8	52·8
8	Maltose	0·22 ± 0·003	0·34
9	Unknown	0·05	0·08
10	Raffinose	2·1 ± 0·08	3·32
11	Stachyose	20·9 ± 1·3	33·1
12	Verbascose	4·41 ± 0·2	6·97
Total di/oligosaccharides		61·1 ± 3·4	96·7
Total carbohydrates of the extracts		63·2 ± 3·4	

Chromatography of carbohydrates

Figures 1 and 2 show the chromatographic separation of the sugar standard and the extracts from the unfermented and fermented oil bean seed, respectively. There were defined peaks of various sugars in the test samples. Chromatography had been successfully used to analyse sugars in food products (Sweeley *et al.* , 1963; Li & Schuhmann, 1980; Fleming, 1981; Chompreeda & Fields, 1984).

Tables 2 and 3 show the carbohydrate composition of the seeds. The unfermented sample contained mostly sucrose (52·8 %) followed by stachyose (33·1 %), verbascose (6·9 %) and raffinose (3·3 %). These sugars were decreased by fermentation which caused a corresponding increase in total monosaccharides from 2·7 % to 88·5 % of the total carbohydrate. Fructose (24·5 %) and galactose (15·5 %) were the predominant sugars in the fermented sample. There was an increase in glucose but the concentration was lower than that of galactose and fructose. The increase in fructose and galactose was probably due to the breakdown of

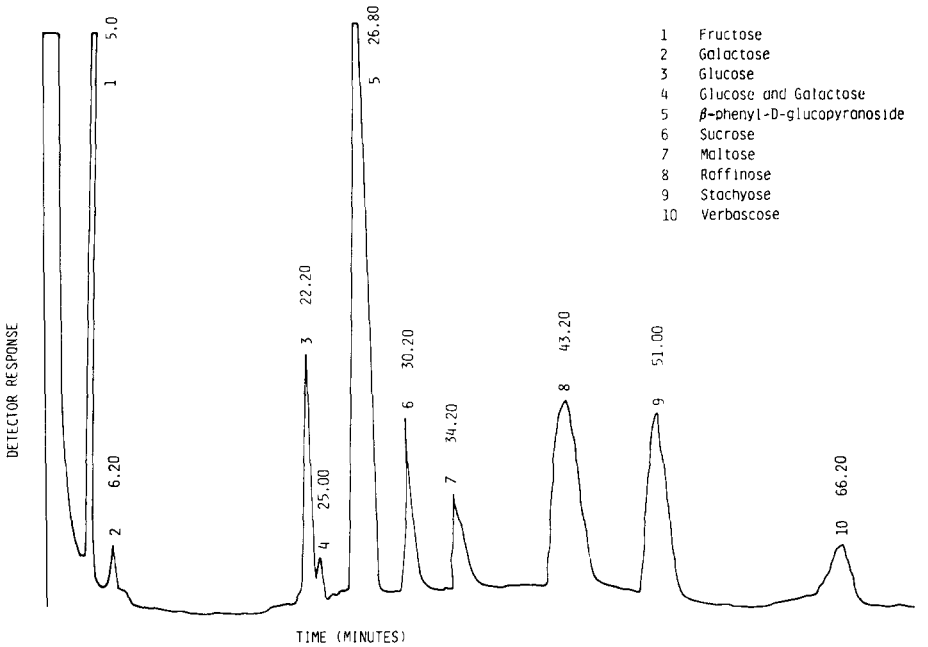

Fig. 1. Chromatography of trimethylated (TMS) derivatives of sugar standards.

raffinose, stachyose and verbascose by the fermenting enzymes. Raffinose (*O*-α-D-galactopyranosyl-(1,6)-*O*-α-D-glucopyranosyl-(1,2)-β-D-fructofuranoside), stachyose (6-*O*-α-D-galactopyranosylraffinose) and verbascose (6-*O*-α-D-galactopyranosylstachyose) are tri-, tetra- and pentasaccharides, respectively. The source of fructose could also be sucrose. There is no doubt that the fermenting organisms would have derived metabolic energy from the breakdown of the oligosaccharides. More sugars were probably broken down than were needed for metabolic processes. Glucose concentration was lower than fructose and galactose, probably because the organisms would have preferentially utilised glucose than other sugars. It is known that glucose enters the fermentation pathway directly (Vedamuthu, 1982), whereas galactose goes through a series of reactions first. Fermentation considerably reduced the concentration of raffinose and stachyose and eliminated verbascose. These oligosaccharides are known to produce flatulence in persons consuming legumes. The α-galactosidase enzyme necessary to cleave the galactose unit is not present in the human digestive system. Fermenting microflora do possess this enzyme (Fleming, 1980). These oligosaccharides have been shown to cause elevated hydrogen excretion

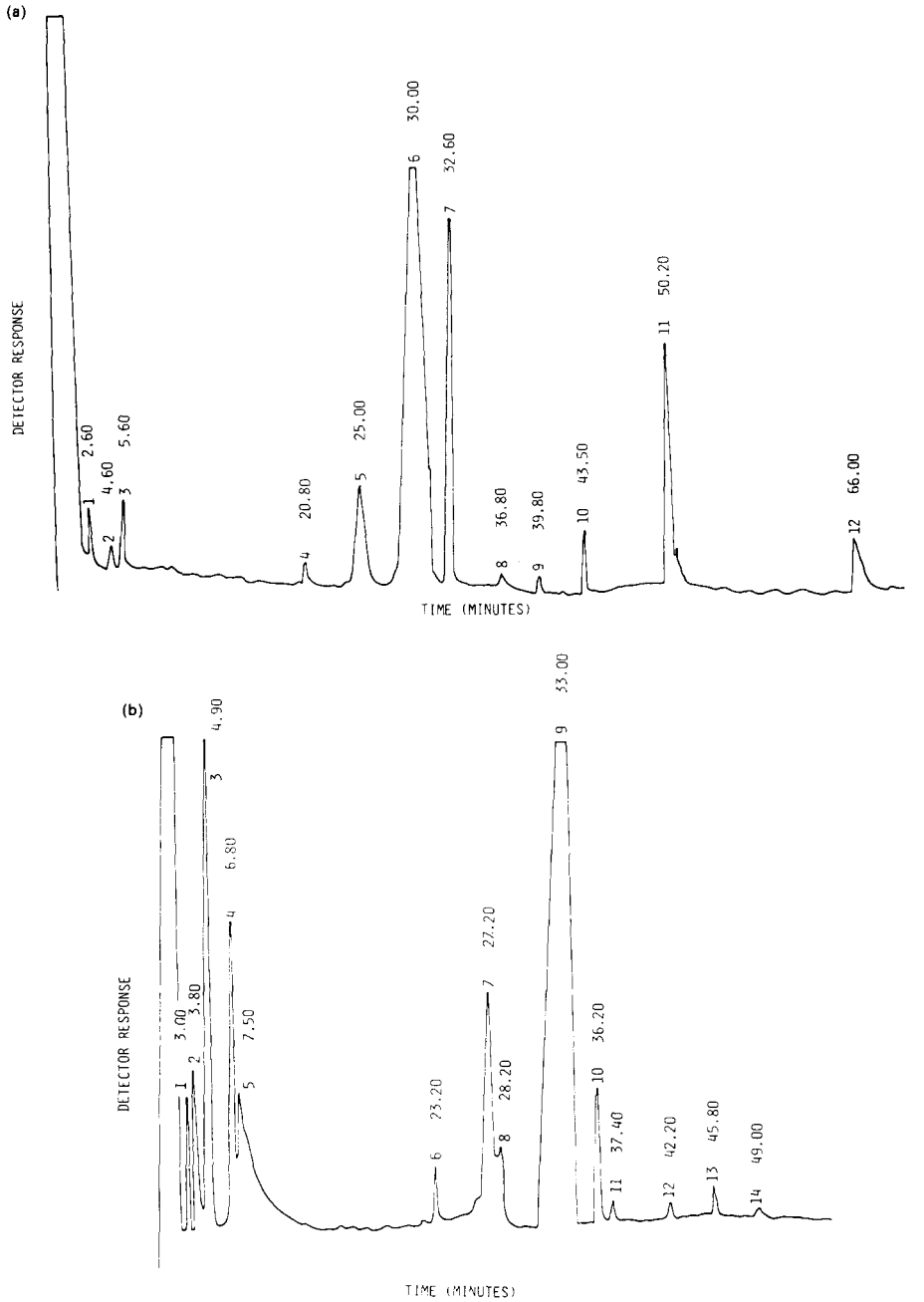

Fig. 2. (a) Chromatogram of trimethylated (TMS) sugar extracts from unfermented oil bean seed. (b) Chromatogram of trimethylated (TMS) sugar extracts from fermented oil bean seed.

TABLE 3
Carbohydrate Composition (mg/g Sample Dry Weight) of the Extracts from the Fermented Oil Bean Seed (Mean of Five Determinations)

Peak No.	*Sugars*	*Concentration (mg/g dry weight)*	*Per cent total carbohydrate*
1	Unknown	0·16 ± 0·001	0·47
2	Unknown	1·52 ± 0·006	4·49
3	Fructose	8·30 ± 0·8	24·5
4	Galactose	5·24 ± 0·08	15·5
5	Unknown	3·44 ± 0·08	10·2
6	Glucose	1·74 ± 0·009	5·14
7	Glucose + Galactose	7·98 ± 0·9	23·6
8	Unknown	1·74 ± 0·02	5·14
Total monosaccharides		30·0 ± 1·9	88·5
10	Sucrose	2·62 ± 0·2	7·74
11	Maltose	0·64 ± 0·003	1·89
12	Raffinose	0·15	0·44
13	Unknown	0·32 ± 0·001	0·94
14	Stachyose	0·16 ± 0·001	0·47
Total di/oligosaccharide		3·89 ± 0·2	11·5
Total carbohydrate of the extracts		33·9 ± 2.1	

in humans (Calloway *et al.*, 1971) and rats (Fleming, 1980; Wagner *et al.*, 1976). The human intestine has bacteria that can metabolise them to CO_2, H_2 and methane.

Other legumes also contain some of these flatus-forming oligosaccharides. Fleming (1981) showed that navy bean, red kidney bean, garbanzo bean and mung bean contained 0·37 to 0·67 % raffinose, 1·67 % to 4·0 % stachyose and 0·5 to 1·7 % verbascose. Odunfa (1983) showed contents of sucrose, raffinose and stachyose of 31·0 mg/g, 12·0 mg/g and 28·0 mg/g dry weight, respectively, in unfermented African locust bean. He observed a reduction in these oligosaccharides after fermentation. Similarly, Chompreeda & Fields (1984) showed that soya bean contained 1·48 g per 100 g of raffinose and 5·03 g per 100 g of stachyose and that fermentation considerably reduced or eliminated the oligosaccharides. Other workers (Vishalakshi *et al.*, 1980), also found sucrose, raffinose,

stachyose and verbascose in three varieties of *Phaseolus vulgaris* and raw kidney bean.

Fatty acid composition

Table 4 shows the fatty acid composition of the fermented and unfermented sample. Fermentation did not have much effect on the fatty acid composition except a slight decrease in the total saturated fatty acids and a slight increase in the total unsaturated fatty acids. The fermenting organisms probably did not utilise much lipid as carbon or energy source as they did carbohydrates. Beuchat & Worthington (1974) have shown that microorganisms do not appear to utilise lipids during the fermentation of peanuts.

Carbohydrates and fats are very important energy-giving nutrients in foodstuffs. Carbohydrate digesting enzymes, probably produced by the

TABLE 4
Fatty Acid Composition of the Oil Bean Seed (Per cent Total Oil) ±SE (Mean of Four Determinations)

	Unfermented	*Fermented*
Saturated		
8:0 Caprilic	—	0·023
10:0 Capric	—	0·05
12:0 Lauric	—	0·02
14:0 Myristic	—	0·03
16:0 Palmitic	5·03 ± 0·3	4·52 ± 0·03
17:0 Margaric	—	—
18:0 Stearic	4·35 ± 0·2	3·39 ± 0·3
22:0 Behenic	3·78 ± 0·3	3·93 ± 0·4
24:0 Lignoceric	10·0 ± 0·6	10·0 ± 0·8
Total saturated	23·2 ± 1·4	22·0 ± 1·8
Unsaturated		
18:1 Oleic	30·3 ± 1·3	31·2 ± 1·8
18:2 Linoleic	42·8 ± 1·1	40·9 ± 1·5
18:3 Linolenic	2·75 ± 0·08	4·51 ± 0·5
20:1 Gadoleic	0·66 ± 0·002	1·1 ± 0·003
Total unsaturated	76·6 ± 2·5	77·8 ± 3·8
Total fatty acids	99·7 ± 3·9	99·7 ± 5·6
Per cent saturated	23·2	22·0
Per cent unsaturated	76·8	78·0

fermenting organisms, hydrolyse the oligosaccharides, and possibly starch, contained in the seed. This might increase available carbohydrates and thereby make the fermented food a high energy food. The increase in soluble nitrogen indicates an increased action of the proteolytic enzymes. Predigestion by enzymes may improve the protein quality of the products. An improved digestibility of the fermented oil bean seed has been shown by Achinewhu (1983*b*) with rats. Fermentation, however, did not have much effect on the total essential amino acid composition (Achinewhu, 1983*a*). Other workers (Zamora & Fields, 1979; Au & Fields, 1981; Chompreeda & Fields, 1984) have shown improvement, by fermentation, of microbiologically available lysine, methionine, tryptophan and relative nutritive value (RNV) in soya bean and sorghum. The oil bean seed with a high protein and essential amino acid composition (Achinewhu, 1982) is a potential source of nutrient from plant. It is easily available, relatively cheap and is locally fermented for immediate family use or for sale in the local market. The fermented seed is being investigated for use as a possible baby food supplement. The reduction in flatus-forming oligosaccharides, the increase in soluble nitrogen, soluble solids and monosaccharides may make it an acceptable ingredient for such supplement. Further biochemical and nutritional studies of the fermented products are in progress.

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