Nature of Lipids in African Locust Beans (*Parkia filicoidea* Welw.) and Changes Occurring during Processing and Storage

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Total lipids were extracted from raw and processed locust beans (*Parkia filicoidea* Welw.) with chloroform–methanol and analyzed for component fatty acids by gas chromatography. A portion of the lipid from raw seeds was resolved on a silicic acid column into neutral and polar classes and their corresponding fatty acid composition analyzed. Total lipid contents for raw beans, cooked dehulled beans (CDB), and fermented beans (FB) were 16.56, 18.87, and 19.51%, respectively. Neutral lipids constituted 62.80% of the total lipid of raw seeds and 37.20% for polar lipids. Components of the neutral lipids included triglycerides (66.70%), free sterols (15.43%), sterol esters (10.86%), free fatty acids (FFA) (6.40%), diglycerides (0.31%), and monoglycerides (0.30%). The lipids had high levels of unsaturation (62.11% for raw seed lipids), but the level of unsaturation decreased after fermentation, indicating lipid oxidation had occurred during fermentation. FFA increased during fermentation, and TBA values increased during 10 days of room-temperature storage.

INTRODUCTION

The African locust bean (*Parkia filicoidea* Welw.), which is extremely hard and inedible in the raw state, is commonly processed and fermented into a tasty product known by several names in West Africa. This product, called "Iru" in the Yoruba-speaking areas of Nigeria, is used as a condiment in numerous dishes. However, the smell of the fermented product as it is sold in the market is commonly undesirable, and this makes the product unpopular among some people.

Researchers have studied African locust bean seeds in terms of their composition, nutritive value, and processing (Platt, 1964; FAO, 1970; Fetuga et al., 1974; Oke and Umoh, 1975; Ikenebomeh and Kok, 1984), but little has been published concerning the flavor of the processed seeds. The understanding of both desirable and undesirable flavor of the fermented seeds is important in developing sound technology for effective processing and storage of this product.

Of the three main classes of foodstuffs, proteins, lipids, and carbohydrates, lipids are probably the most important source of derived flavors. Forss (1969) discussed the role of lipids in flavors and showed that lipids are important precursors of volatile flavors. To fully understand the contribution of lipids to the flavor of fermented locust beans, it is important to understand the nature of the lipids present in the seeds and it is equally important to understand the changes that occur in the lipids during processing and storage. Girgis and Turner (1972) reported the fatty acid composition of the oil of locust beans but did not study the lipid changes occurring during processing and storage. Thus, this paper reports the lipid classes and their corresponding fatty acid composition as well as the lipid-related changes during processing and storage.

EXPERIMENTAL SECTION

Materials and Processing. Dried locust bean seeds were collected from a local market in Ilesha, Oyo State of Nigeria. The procedure outlined by Ikenebomeh and Kok (1984) was used for the processing and fermentation of the locust beans. Tap water was added to the dried seeds to give a bean to water ratio of 1:5 (w/w) in a flask and gently boiled for 8 h on a hot plate with replacement of evaporated water every 2 h. After heating and cooling, the softened testa were removed by rubbing the seeds between the palm and washing the seeds with water. The beans free of testa are referred to as cooked dehulled beans (CDB). The CDB was further cooked for 30 min to soften the seeds.

To obtain the fermented product, 25 g of CDB was weighed into a 100-mm-diameter filter paper lined glass Petri plate and covered with filter paper and the lid. This fermentation unit was placed in an incubator at 37 °C for 72 h. The resulting product is referred to as fermented beans (FB).

Lipid Extraction and Classification. Dried raw and CDB seeds were finely ground in a Udy Cyclone sample mill, and total lipids were determined by a 12-h chloroform–methanol (2:1) extraction using a Goldfisch extractor. Tocopherol acetate in chloroform was used as antioxidant. Following the method of Ronser et al. (1967), a known amount of total lipid of the raw seeds was resolved on a 100–200-mesh silicic acid column using chloroform to pull out neutral lipids and acetone followed by methanol for the polar lipids. Each fraction was isolated in a rotary flash evaporator and weighed. The total recovery for all fractions added together was about 78%. A portion of the neutral lipids was further fractionated on a silica gel G thin-layer chromatographic (TLC) plate using hexane–ether–acetic acid (80:20:1) as developing solvent. The spots were detected with 50% sulfuric acid and identified by comparison with standards. The relative percentage of each spot was measured on a Transidyne Model 2955 scanning densitometer.

Fatty Acid Composition. Methyl esters were prepared from total lipids of raw seeds, CDB, and FB as well as from neutral and polar lipids by transesterification with methanolic base (0.5 *N* sodium methoxide) (Supelco, Inc.). About a 20-mg sample of the lipid was dissolved in a mixture of 1 mL of benzene and 1 mL of methanolic base and the solution heated for 20 min at 80 °C. The mixture was then cooled to room temperature; 3 mL each of water and diethyl ether was added and mixed well. The ether layer was removed, washed with 2 mL of water, and dried over anhydrous sodium sulfate.

The methyl esters were analyzed on a Hewlett-Packard Model 5830A gas chromatograph with a flame ionization detector. A 2 m × 4 mm stainless-steel column, packed with 10% Silar 10C on 100–120-mesh Gas Chrom. Q was used, and the peak areas were analyzed on a Hewlett-

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Table I. Lipid Classes and Fatty Acid Composition of Raw Locust Beans[a]

lipid class	% compn	% fatty acid compn											% unsatd FA	% satd FA	US:S ratio
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	18:0	20:0	22:1	20:4			
total lipids	16.56[b] ± 0.78	0.04 ± 0.01	9.00 ± 0.04	0.25 ± 0.03	14.53 ± 0.10	13.96 ± 0.08	44.90 ± 0.09	0.91 ± 0.04	0.81 ± 0.02	13.51 ± 0.10	0.54 ± 0.0l	1.55 ± 0.09	62.11 ± 0.17	37.89 ± 0.15	1.64
polar lipids	37.20[c] ± 1.23	1.72 ± 0.05	14.48 ± 0.09	1.26 ± 0.02	13.30 ± 0.07	14.13 ± 0.11	39.06 ± 0.26	1.04 ± 0.02		15.01 ± 0.03	...	...	55.49 ± 0.28	44.51 ± 0.13	1.25
neutral lipids	62.80[c] ± 1.11	0.07 ± 0.01	8.20 ± 0.10	0.20 ± 0.00	14.40 ± 0.05	13.19 ± 0.04	43.58 ± 0.21	1.01 ± 0.02	0.95 ± 0.01	15.08 ± 0.02	0.97 ± 0.03	2.35 ± 0.04	61.30 ± 0.22	38.70 ± 0.12	1.58
sterol esters	10.86[d] ± 0.90														
triglycerides	66.70[d] ± 2.31														
free fatty acids	6.40[d] ± 1.01														
free sterols	15.43[d] ± 1.22														
diglycerides	0.31[d] ± 0.08														
monoglycerides	0.30[d] ± 0.06														

[a] Values show the average of three deteriminations. [b] Percentage in whole seeds. [c] Percentage in total lipids. [d] Percentage in neutral lipids.

Table II. Total Lipid and Fatty Acid Composition of Raw, Cooked Dehulled Beans (CDB), and Fermented Beans (FB)[a]

	% compn[b]	% fatty acid compn											% unsatd FA	% satd FA	US:S[b] ratio
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	22:1	20:4			
raw	16.56 ± 0.79[a]	0.04 ± 0.01	9.00 ± 0.04	0.25 ± 0.03	14.53 ± 0.10	13.96 ± 0.08	44.90 ± 0.09	0.91 ± 0.04	0.81 ± 0.02	13.51 ± 0.10	0.54 ± 0.06	1.55 ± 0.09	62.11 ± 0.17	37.89 ± 0.15	1.64[a]
CDB	18.87 ± 0.62[b]	0.06 ± 0.01	8.97 ± 0.09	0.29 ± 0.02	14.64 ± 0.09	13.77 ± 0.11	45.10 ± 0.15	0.79 ± 0.08	0.84 ± 0.06	13.43 ± 0.14	0.54 ± 0.08	1.57 ± 0.03	62.06 ± 0.22	37.94 ± 0.19	1.64[a]
FB	19.51 ± 0.80[b]	0.11 ± 0.03	9.10 ± 0.07	0.25 ± 0.01	14.92 ± 0.11	13.38 ± 0.09	44.98 ± 0.10	0.66 ± 0.04	1.11 ± 0.05	14.40 ± 0.12		1.39 ± 0.07	60.66 ± 0.16	39.34 ± 0.19	1.54[b]

[a] Values show average of three determinations. [b] Values followed by different letters are significantly different at $p \leq 0.05$.

Table III. Changes in TBA Values of Cooked Dehulled Beans (CDB) during Fermentation and Room-Temperature Storage of the Fermented Product (FB)

	days	TBA values[a] [malonaldehyde content, μg/g]
CDB	before ferment	2.60 ± 0.12
FB	0	5.91 ± 0.30
	2	7.32 ± 0.23
	4	8.20 ± 0.28
	6	8.61 ± 0.19
	8	8.70 ± 0.17
	10	8.83 ± 0.28

[a] Values are significantly different at $p \leq 0.05$.

Packard 18850A integrator. Fatty acid methyl esters (FAME) standards were obtained from Supelco Co. (Bellefonte, PA). The column temperature was started at 150 °C for 2 min and then programmed at 5 °C/min up to 230 °C. Nitrogen was used as carrier gas with a flow rate of 20 mL/min.

The percentage of free fatty acids (FFA) contained in the extracted CDB and FB lipids was determined by titration with standard alkali (AOAC, 1980).

Thiobarbituric Acid (TBA) Analysis. The method described by Tarladgis and Watts (1960) was used to follow the rate of lipid oxidation during processing and storage.

Statistics. Data are presented as means plus/minus their standard error; differences between means were analyzed for significance by the Student's t-test.

RESULTS AND DISCUSSION

Nature of Lipids. The total lipid content, lipid classes, and the corresponding fatty acid composition of raw locust bean seeds are presented in Table I. The total lipid content of these leguminous seeds is 16.56%. Girgis and Turner (1972) reported only 14.5% for the total lipid content of locust bean seeds that were collected from another location different from where those used in this study were collected. The variance between Girgis and Turner's value and that obtained in this study may be due to differences in the growing condition and state of maturity at the time of harvest. Both values are relatively high when compared with values for most legumes. Total lipid content of all legumes except peanut, soybean, and winged beans was reported to vary from 1.00 to 7.20% (Pattee et al., 1981). The total lipid content of locust beans is similar to that of winged beans of 16.8% and obviously falls within the few exceptions among the legumes. The neutral lipids are the predominant class in locust bean lipids and constitute 62.8% of the total lipid while the polar lipids comprise 37.2%. The value for the neutral fraction is slightly higher than the general trend among most legumes. The distribution of neutral and polar lipids in most legumes varies from 32 to 51% for the neutral fraction and from 49 to 68% for polar lipids (Pattee et al., 1981).

TLC of the neutral lipid fraction showed six spots. The most abundant component was triglyceride while sterol esters, free sterols, and free fatty acids were present in appreciable amounts. Only slight indications of diglycerides and monoglycerides were shown.

The major fatty acid of locust bean lipid is linoleic acid (44.9%). Palmitic, stearic, oleic, and behenic acids are also present in appreciable amounts. The unsaturated fatty acid content is high (62.11%), thus making the lipid highly susceptible to oxidation. The fatty acid composition of the neutral lipid fraction is similar to that of the total lipid, but the polar lipid fraction lacks some of the long-chain unsaturated fatty acids contained in both the total lipid and the neutral lipid fraction. Longer chained fatty acids exceeding 20 carbons, which were not reported by Girgis and Turner (1972), were detected in this study. Factors mentioned above to explain differences in lipid content may also account for the lack of detection by the earlier workers. Experimental variations may also be a factor. Some of the longer chained fatty acids are highly unsaturated and can be lost easily by autooxidation if not well protected during analysis. Tocopherol acetate was used in this study as an antioxidant, but Girgis and Turner (1972) did not report the use of any antioxidant.

Lipid-Related Changes during Processing. Table II shows the lipid content and fatty acid composition of the raw, CDB, and FB lipids. From this table, it can be seen that the lipid contents of the CDB and FB samples are significantly higher than that of raw beans. This could be explained in terms of the removal of testa in the CDB and FB, which would result in an overall higher percentage for the lipid content. The total lipid contents of the FB and CDB are not significantly different.

The major difference in the fatty acid content is in terms of the saturated to unsaturated ratio. The raw beans and CDB have significantly higher levels of unsaturated fatty acids than the FB samples. This could be due to autoxidation of the lipids during fermentation. This observation was confirmed by the results of the TBA analysis on the CDB and FB, which showed higher TBA values for the fermented product (Table III). TBA analysis on FB during 10 days of room-temperature storage also revealed increasing TBA values. This observation could partially explain why the fermented products purchased in local markets in Nigeria have undesirable off odors. Good packaging and/or addition of antioxidants should improve the off odor situation considerably.

The FFA value of FB was found to be 223 mg/g of lipid, and that of CDB was 71.0 mg/g of lipid. These figures indicate that there was some lipolytic activity during fermentation and the production of FFA could also contribute to the off flavor of the fermented beans. Investigations are in progress to explore and characterize other sources of off odors in fermented locust beans.

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