DISCUSSION

The route selected for the preparation of methyl 9,12-epoxyoctadeca-9,11-dienoate is presented in Scheme I.

Pure ricinoleic acid was readily prepared from castor oil according to Gunstone's partition procedure (5). The methyl ester derivative was oxidized according to the two-phase method recommended by Brown et al. (7). The resulting product was subsequently refluxed with mercury (II) acetate in glacial acetic acid, and the crude product was loaded on top of a *dry* silica gel column and developed with a mixture of diethyl ether/petroleum ether (1:9, v/v). This chromatographic technique was rapid, efficient and economical. A portion of the developed column of silicic acid was sectioned off and the support extracted with diethyl ether to furnish (~57%, based on methyl ricinoleate) pure C_{18} furanoid ester. The entire operation required less than 8 hr of actual (manual) laboratory time. The time for refluxing of reaction mixtures and evaporation of solvents was not included in this count.

ACKNOWLEDGMENTS

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⁂Heterogeneity within Commercial Contract Analysis Samples of Shea-Nut Kernels

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ABSTRACT

Shea-nuts are a commercial oilseed crop with relatively large mean kernel weight. Results are presented to demonstrate that the individual kernels making up single contractual samples are heterogeneous in their individual analyses for oil, free fatty acids in oil and moisture contents. These results are discussed and an effective technique giving reproducible results is described.

INTRODUCTION

Shea-nut kernels are the oil-bearing seeds of the deciduous Shea-nut or Shea butter tree, *Butyrospermum paradoxum* (Gaertn. f.) Hepper subsp. *parkii* (G. Don) Hepper. The major source of this crop is the Savannahs of West Africa (estimated annual production 0.5 million tons), where the trees occur naturally but may be protected and cultivated (1). The oil extracted from imported kernels (45-60%) is important in the U.K. as a cocoa butter substitute in chocolate manufacture. The extreme heterogeneity in the oil and free fatty acid contents of individual kernels within analysis samples representing single parcels from single sources has been noted in this laboratory (2). The long periods to first fruition (12-15 years) and maturation (30 years), as well as passive cultivation of this crop are prohibitive regarding selective breeding, but this study is relevant to the commercial analysis of this crop, because the kernel weight for the Shea-nut (up to 8 g) is relatively much greater than for most other commercially available oilseeds.

METHODS

Samples of West African Shea-nuts were made available subsequent to contractual analyses having been completed in this laboratory. Reduction of contract samples, removal of impurities, and determination of oil, free fatty acids in oil, and moisture and volatile matter were based on ISO methods (3-7). Mineral matter was determined by ashing at 550 C (8).

Analysis samples of Shea-nuts of at least 1 kg, taken from contract samples after determination of admixture, and Shea-nut shell were each milled in specially modified mechanical mills, without expression of oil, to a meal not exceeding 4-mm particle size. Individual Shea-nut kernels, or parts of kernels, selected at random from samples, were grated using a Moulinex metal hand grater, and analyzed in accordance with the applicable methods. Milled or grated materials were subjected to analysis immediately after preparation. Oil determinations were made by continuous extraction with light petroleum (bp 40-60 C). The regime for extraction of portions of milled whole sample, done in quadruplicate, was: extracted 2 hr, hand-ground after drying, extracted 1 hr, micropulverized in prolabo mill after drying, extracted 2 hr, and solvent removed. Individual kernels or parts of kernels were extracted for 2 hr, exhaustively hand-ground, and extracted for a further 2 hr before removal of the solvent. All grinding operations were aided by the additon of portions of 40-60 mesh, neutral pH silver sand.

The preparation of methyl esters and gas liquid chromatographic (GLC) analysis for determination of fatty acid composition followed IUPAC recommendations (9), and results were calculated by hand-integration of peaks identified by comparison with known standards.

RESULTS AND DISCUSSION

Contract samples of Shea-nut kernels have been analyzed in this laboratory since about 1930. It has been common practice for two independent laboratories to receive con-

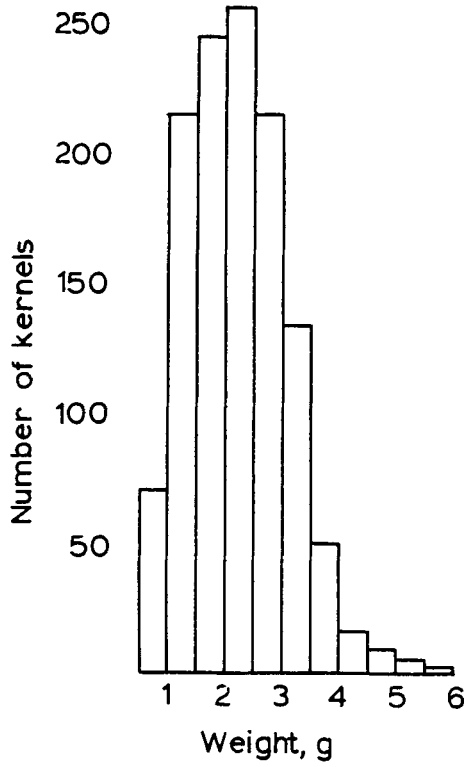

FIG. 1. Distribution of kernel weights of a single contract analysis sample. Number of kernels, 1,210; range, 0.43-5.71; mean, 2.41.

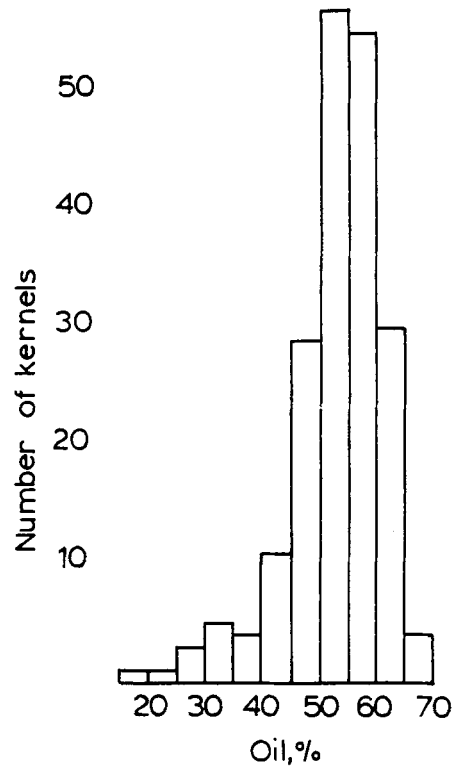

FIG. 2. Distribution of kernel oil contents within kernels taken from a single contract analysis sample. Number of kernels tested, 200; range, 19.7-67.3; mean, 51.1; (calculated standard deviation, 13.5); coefficient of variation, 180.5.

tract samples representing the same parcel and results of two analyses are averaged. All too frequently, however, the two sets of results have been in poor agreement and a third analysis has been required. Preliminary research done in this laboratory in 1965 led us to conclude that the most likely explanation for these disparate results was the application of less than optimal laboratory subsampling techniques to an extremely heterogeneous material. We note that disparate results still occur far too often, as judged from the number of third analyses we do in a season, and effective laboratory subsampling remains the key. A number of Shea-nut samples have been studied in detail recently and we are satisfied that the conclusions we reach here are generally applicable.

The variation among kernels within single parcels arises from the method of making up parcels at source. Single sackfuls, gathered by hand, are collected together at intermediate staging-points, and loads are taken from several staging-points to make up parcels for shipping. Shea-nut kernels within a single analysis sample may be vastly different in their physical appearance. The flesh may be any color from buff to dark brown or black, or from orange or salmon-pink to dark red. Similarly, the oil extracted from individual kernels within the same sample may be solid or liquid, and vary in color from colorless or white to bright yellow, or occasionally, green, presumably due to contamination with chlorophyll-containing material.

A considerable divergence in kernel weight exists, up to 10 times or greater, clearly indicating that the crop is not a cultivated one, in which case a more uniform seed size might have been expected. Samples with a mean kernel weight as high as 4 g are common, and single kernels weighing up to 7.9 g have been noted. An example of the distribution of kernel weights within a single contract analysis sample is shown in Figure 1.

Oil contents of individual kernels taken from the same contract analysis sample are shown in Figure 2. Oil content can be seen to vary considerably between kernels and to follow a normal distribution. Kernel flesh color bore no relationship to either oil content or oil color. The grouping of points in a plot of oil content vs kernel weight indicated

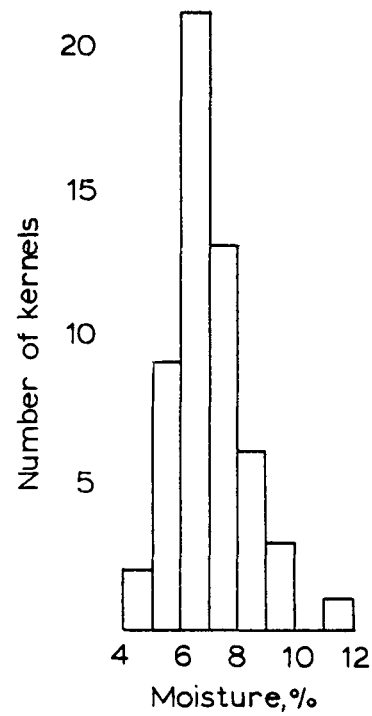

FIG. 3. Distribution of moisture contents within kernels taken from a single contract analysis sample. Number of kernels tested, 55; range, 4.0-11.3; mean, 6.9; (calculated standard deviation, 1.3); coefficient of variation, 1.7.

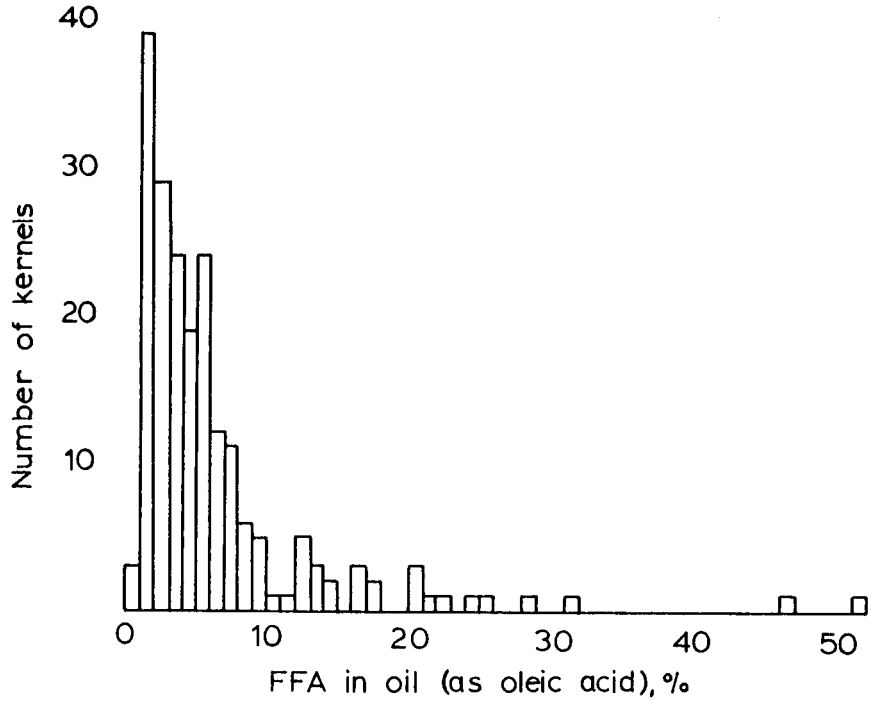

FIG. 4. Distribution of FFA in oil contents in the oils extracted from kernels taken from a single contract analysis sample. Number of kernels tested, 200; range, 0.8-51.9; mean, 6.2; (calculated standard deviation, 6.9); coefficient of variation, 47.6.

the likely presence of a single biotype with wide divergence. No correlation of oil content with kernel weight was apparent.

Moisture contents of individual kernels taken from this contract analysis sample are shown in Figure 3. Moisture appears to be relatively evenly distributed between the kernels, as might be expected.

The results of analysis for free fatty acid (FFA) contents of the oils extracted from individual kernels taken from this same contract analysis sample are shown in Figure 4, and can be seen to follow a distinctive pattern with an obviously modal distribution. A predominance of the kernels tested (86%) had FFA in oil content, calculated as oleic acid, of less than 10%, with relatively fewer kernels having high, and occasionally very high, acidity. Further investigation showed the development of high FFA content to be very localized (Table I), and to be associated particularly with the outer 2 mm of the kernel (Table II). The outer surface of the decorticated kernel must be considered prone to the effects of external factors facilitating lipolysis, e.g., air and excessive moisture (including intentional watering to increase weight, a practice currently being eliminated among the native gatherers), and to microbial lipase attack (10).

While whole, nearly whole, or recognizable parts of decorticated kernels form the major part of contract

TABLE I

Variation in Oil and FFA in Oil Contents of the Halves of Individual Shea-Nut Kernels[a]

Weighted mean oil for kernel (%)	FFA in oil (as oleic acid) (%)	
	Values for two halves	Weighted mean for kernel
50.70	0.43, 1.34	0.89
51.19	1.13, 1.76	1.45
51.14	1.48, 1.59	1.53
62.08	1.68, 1.72	1.70
64.56	3.11, 3.57	3.39
32.46	3.50, 3.50	3.50
59.65	3.32, 3.81	3.52
62.29	3.22, 5.65	4.07
59.21	3.79, 5.31	4.50
31.51	3.03, 6.13	4.58
57.79	2.01, 6.89	5.17
63.39	4.76, 5.77	5.26
57.72	7.00,11.03	5.47
49.53	5.86, 6.19	6.03
55.30	4.89, 9.01	6.87
59.46	6.94, 9.55	8.40

[a]Individual whole Shea-nut kernels were cut in half along their long axes and the two halves analyzed separately in the usual manner.

TABLE II

Comparison of the FFA in Oil Content in the Outer 2 mm and Heart of Individual Shea-Nut Kernels[a]

FFA in oil (as oleic acid) (%)			
Interior	Exterior	Weighted mean for kernel	Ratio exterior/interior
0.26	1.27	0.51	4.9
0.39	1.73	0.79	4.4
0.55	1.72	0.79	3.1
0.42	1.93	0.84	4.6
0.55	2.17	0.94	3.9
0.11	2.55	0.96	23.2
0.50	4.70	1.74	9.4
1.14	6.29	2.53	5.5
2.74	3.15	2.84	1.1
2.65	5.90	3.37	2.2
3.00	6.48	3.69	2.2
3.07	11.06	6.25	3.6
0.63	23.55	8.44	37.4
8.65	16.07	10.12	1.9
12.77	39.45	22.97	3.1
69.51	69.14	69.34	1.0

[a]Kernels had the external 2 mm (approximately) cut away and the inner and outer parts were analyzed separately in the usual manner. The range of weighted mean oil contents for the kernels in this experiment was 45.4-61.7%.

TABLE III

Typical Analytical Data for Shea-Nut Kernel, "Dust" and Shell[a]

Material	Sample 1	Sample 2			
	Oil (%)	Oil (%)	FFA in oil (%)	Moisture (%)	Mineral matter (%)
Clean kernels, whole sample	45.7	49.2	9.9	5.7	3.7
"Dust:" 2-2.5 mm	9.6	12.8	47.6	8.9	20.2
1-2	6.0	9.6	51.6	8.4	19.5
0.5-1	4.7	8.0	53.6	7.5	35.1
<0.5	3.8	7.0	61.6	5.2	51.4
Shell	0.6	1.5	18.6	12.0	2.8

[a]Shea-Nut sample material passing a 2.5-mm square hole sieve, designated "dust," was further subdivided by size using 2-mm and 1-mm round hole and 0.5-mm square hole brass sieves. The fractions obtained were analyzed separately in the usual manner at the same time as milled shell and clean-picked, whole kernel materials from the same sample.

TABLE IV

Fatty Acid Composition of Oil Extracted from Shea-Nut Material[a]

Material	Fatty acid (wt %)				
	16:0	18:0	18:1	18:2	18:3
Clean kernel, whole sample (2)	2.8	47.6	46.4	2.7	0.5
"Dust" (4)[b]	5.5	48.2	41.8	3.5	1.0
Shell (2)	8.5	49.0	35.9	5.1	1.5

[a]Minor components (<0.5%) ignored. Results are mean of number of determinations stated in parentheses.

[b]See footnote to Table III.

samples of Shea-nuts in this laboratory, other components making up the remainder of the sample must not be ignored. The greater proportion of the kernels have been decorticated by the native gatherers after harvest and before acceptance by the shippers, normally leading to only a limited amount of shell (similar in appearance to horse-chestnut shell) being included in the parcel. A limited amount of other relatively large foreign or non-oleaginous material may be present, viz., foreign beans, twigs, bark and stones.

A proportion of most samples consists of fragmented kernels and more finely divided material. An arbitrary limit of 2.5-mm particle diameter was taken and material passing a square hole sieve of this size, designated "dust," was separated, subdivided by size, and analyzed in parallel with portions of milled, clean-picked whole kernels and milled shell. The results of these analyses are presented in Table III. It can be seen that the oil content decreases and FFA in oil content increases with decreasing particle size. Moisture is quite evenly distributed but the mineral matter (ash) content increases considerably with decreasing particle size, showing that a large proportion of the fine particulate matter present is made up of nonvegetable dust. Some part of the mineral matter figure found for the clean-picked kernel may be due to the adherence of fine particulate matter to the outer surface of the oilseed kernel (S.J. Kershaw and J.F. Hardwick, unpublished results).

Contractual analysis usually requires dust of less than a certain particle diameter to be treated as admixture and this portion of the sample would be termed the "fines." Table III indicates that the sieve size taken as the limit for determination of the "fines" would be arbitrary, as is the case for most oilseeds. Calculation shows that the presence of shell or fines in a contract sample would have relatively little consequence on the overall oil or FFA in oil analyses unless either or both were present in significant proportion. A substantial shell content would serve to increase the oil content while causing only a minimal decrease in the FFA content, whereas a high proportion of fines would both reduce the oil content and elevate the FFA content.

Table IV shows the results of GLC analysis to determine the fatty acid composition of the different components of Shea-nut contract samples. The fatty acids present are predominantly 18:0 and 18:1 (greater than 90%), accounting for Shea-butter's solid fat form. In "dust" and shell, the C12-C16 fatty acids were more significant than in whole kernels, but in all cases C12 + C14 acids totaled less than 2.5%. Fatty acid compositions of the oils extracted from individual kernels showed a greater degree of uniformity than might be expected from their otherwise disparate analyses. In a comparison of oils from the outer 2 mm and heart of individual kernels, there was a tendency for oils from the outer layer of the kernel to contain a slightly lower proportion of 18:1 but much less 18:2 and 18:3 acids.

The high mean kernel weight for the Shea-nut together with the heterogeneity in individual analysis demonstrates the necessity for effective analytical technique. In our opinion, contract samples of 5 kg might be sufficiently representative of a parcel, whereas samples currently received are usually in the range 2.5-3.5 kg. Occasionally, contract samples of less than 1 kg have been received representing parcels of 500 MT.

TABLE V

Typical Contract Analysis Results for Shea-Nut Samples[a]

Experiment	A	B	C	D
Oil (%)	45.68 ± 0.31	40.88 ± 0.93	49.48 ± 0.18	48.95 ± 0.85
FFA in oil (as oleic acid) (%)	7.17 ± 0.21	8.78 ± 0.37	10.33 ± 0.77	9.47 ± 0.34
Moisture (%)	8.11 ± 0.15	8.18 ± 0.03		

[a]Determinations done in quadruplicate. Values shown are mean ± standard deviation.

Internationally accepted methods recommend 500 g as being an adequate analysis sample for Shea-nuts after reduction of the contract sample, whereas in this laboratory a subsample of at least 1 kg is milled and the meal used for routine analysis as described in Methods. We find that our procedure gives results with low standard deviations as shown in Table V. Individual nuts may be grated using a hand grater, but this method is not practicable for kilogram quantities of nuts. Equally, the cutting of pieces from each of a sample of nuts is most unreliable, particularly in view of the localization of FFA.

In our experience, we emphasize that the size of contract samples provided is very often too small. It is only possible to obtain reproducible results from such a heterogeneous material if a large subsample is taken from an adequately large sample, and that the subsample be milled to a relatively small particle size meal in order that thorough mixing is possible before subsequent analysis. Analyses done by different laboratories on contract samples of Shea-nuts representing the same parcel must be expected to differ slightly with such a heterogeneous material, but should not show a consistent bias.

Those who trade in Shea-nuts will be familiar with the respective allowances for oil and FFA, and must be aware of the need for satisfactory methods for the analysis of Shea-nuts.

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TLC-FID Assessment of Lipid Oxidation As Applied to Fish Lipids Rich in Triglycerides[1]

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ABSTRACT

Changes in the polar lipid content of fatty fish oils were studied by quantitative thin layer chromatography-flame ionization detection (TLC-FID) during an accelerated oxidation test. There was a direct linear correlation between increase in polar lipids and increase in weight of the oil samples. Comparison of increases in polar lipids with thiobarbituric acid values gave a hyperbolic correlation curve. However, a linear correlation was obtained in a semilogarithmic system. The simple and time-saving TLC-FID method for the analysis of the polar lipid content of oils as compared to a column chromatographic method suggests that this new method could be used in quality control of edible oils.

INTRODUCTION

Oxidative rancidity of fats and oils in foods is a major problem resulting in a decreased quality and nutritive value of the product. This is especially true for food fish which usually contain highly unsaturated lipids. Autoxidation of unsaturated fatty acids leads to the formation of relatively stable hydroperoxides (1) which later may break down to the lower molecular weight carbonyl compounds responsible for the unpleasant off-flavor in rancid fish oils (2).

Oxidation of lipids consisting mainly of triglycerides results in compounds with a higher polarity than their parent triglycerides (3). The significance of the polar lipid content in edible oils has been recognized by the German Society for Fat Research (DGF, Deutsche Gesellschaft fur Fettwissenschaft) which has recommended that the content of polar artifacts should be taken as a basis for the quality assessment of used frying oils (4). This led us to study the changes in the polar lipid content as a possible indicator of lipid oxidation in some fatty fish lipids during an accelerated oxidation test. Direct analysis of the polar lipid content was achieved by quantitative thin layer chromatography-flame ionization detection (TLC-FID). The results are compared with thiobarbituric acid (TBA) values and weight gain.

EXPERIMENTAL PROCEDURES

Preparation of Fish Oils

Lipids of the skin, including the subcutaneous fat layer, and the meat from common food fishes, Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*) and tuna (*Thunus obesus*) were extracted by the method of Bligh and Dyer (5).

Accelerated Oxidation Test

Duplicate samples of ca. 2.0 g of each oil were accurately weighed in glass Petri dishes (id 50 mm). These samples

[1]Presented in part at the ISF/AOCS World Congress, New York City, April 1980.