The fastest moving (on TLC) component is a waxy solid melting near room temperature. It contains no ester function, is hydrocarbon in nature and presumably is a product of olefin self-condensation.

The second of the three by-products is also a waxy solid, but this material contains an ester group, and, judging from NMR and IR spectra, it is an α-branched ester. Saponification of the ester with 10% alcoholic potassium hydroxide gave a carboxylic acid having a neutralization equivalent of 671. Theoretical neutralization equivalent for an acid formed from one mole lauric acid plus two moles 1-hexadecene is 648.

The slowest of the three byproducts is a liquid. Its IR spectrum indicates the presence of a carboxylic ester function and its NMR spectrum indicates a hydrogen in the 2-position. Saponification of the ester results in an acid having a neutralization equivalent of 683. Thus it appears that the slowest by-product is a slightly more polar isomer of the second by-product and that both resulted from the condensation of one mole of methyl laurate with two moles of 1-hexadecene.

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Cyclopropenoid Fatty Acid Content and Fatty Acid Composition of Crude Oils from Twenty-Five Varieties of Cottonseed

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Abstract

The cyclopropenoid acid content of oils extracted from 22 commercial varieties and 3 botanical species of cottonseed have been determined. The malvalic acid content determined by HBr titration varied from a low of 0.56% to a high of 1.17%. Iodine values of the oils ranged from 96.8 to 111.6. No definite correlation could be established between iodine value and malvalic acid content. Equations for regression lines for the major acids have been calculated from plots of fatty acid composition vs. iodine value. The high degree or correlation suggests that for commercial oils the fatty acid composition can be estimated from the iodine value. Oils of the 3 experimental types of different species showed wide variations in fatty acid composition and represented many of the maximum and minimum values reported.

Introduction

THERE HAVE BEEN NUMEROUS reports dealing with the fatty acid composition of cottonseed oil. Stansbury and Hoffpauir (1) made a systematic study of the relationship between iodine value and fatty acid composition of oils from a number of native varieties and types. Cattaneo et al. (2) reported on the physical-chemical characteristics and fatty acid composition of a number of Argentine cottonseed oils. Harwalkar, Achaya and Saletore (3,4) reported on the fatty acid composition of a number of Indian cottonseed oils. The various methods of analysis employed in these studies are now known to be unreliable. With the advent of modern gas-liquid chromatographic techniques (GLC) more accurate and complete analyses have been reported (5–8). However, these analyses in general were limited to but a few random cottonseed oils. The only reported GLC analyses of a series of cottonseed oils was in connection with the detection of cottonseed oil as an adulterant in olive oil, and no attempt was made to correct for detector response differences. In none of the analyses was there mention of the cyclopropenoid constituents present in cottonseed oil, namely, malvalic or sterculic acid. The recently developed stepwise HBr titration method (9) now makes it possible to determine cyclopropenoid fatty acid moieties to the nearest 0.01%. These factors and the availability of ideally suitable sample material have prompted us to investigate the fatty acid composition of cottonseed oils extracted from a number of cottonseed varieties and species.

Apparatus and Methods

Gas-liquid chromatograms of the cottonseed methyl esters were obtained with the Aerograph Autoprep

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TABLE I

Fatty Acid Composition and Iodine Value of Oils from Commercial Varieties of Cottonseed

Variety	Year grown	Location	Iodine value (Wijs)	Iodine value (GLC)	Fatty acid composition, %						
					Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Malvalic[a]
Lankart 57	1962	Waco, Tex.	96.8	97.1	1.3	28.0	0.9	3.0	21.2	45.0	0.58
Del Cerro	1962	Las Cruces, N.M.	100.0	101.9	1.1	26.9	0.7	2.9	18.8	49.1	0.64
Lockett 88A	1962	Vernon, Tex.	101.2	102.2	1.2	26.2	0.7	2.9	19.5	48.8	0.66
Deltapine 15	1962	Scott, Miss.	102.0	101.5	1.1	27.1	1.0	3.0	18.1	49.0	0.72
Pima-S2 [b]	1962	Tempe, Ariz.	102.0	103.8	0.8	25.1	0.9	3.1	20.1	49.4	0.58
Auburn 56	1962	Thorsby, Ala.	102.6	103.1	1.1	26.5	0.7	2.7	18.5	50.0	0.70
Acala 44WR	1961	Tempe, Ariz.	102.9	102.8	1.0	27.4	1.1	2.7	17.0	50.3	0.64
Dixie King	1962	Indianola, Miss.	105.5	105.8	1.1	26.6	0.7	2.3	15.9	52.8	0.72
Blightmaster	1962	Locketville, Tex.	105.5	107.2	0.8	23.8	0.6	3.0	19.0	52.0	0.81
Paymaster 54B	1961	Plainview, Tex.	106.8	108.2	0.8	23.9	0.7	3.2	17.2	53.4	0.76
Rex	1962	Marianna, Ark.	107.0	107.6	1.1	25.6	0.7	2.4	15.4	54.0	0.85
Deltapine Smooth Leaf	1962	Scott, Miss.	107.0	105.8	0.9	26.3	0.6	2.7	16.0	52.6	0.93
Empire	1962	Haralson, Ga.	107.1	110.0	1.1	24.3	0.5	2.6	15.0	55.7	0.80
Acala 4-42	1961	Shafter, Calif.	107.4	106.6	1.1	24.8	1.1	3.3	16.2	52.8	0.73
Stoneville 62	1961	Chickasha, Okla.	107.6	109.0	0.9	23.7	0.8	2.9	17.1	53.9	0.74
Coker 100A	1962	Hartsville, S.C.	107.9	109.8	1.0	24.4	0.6	2.6	15.0	55.4	0.84
Fox 4	1962	Scott, Miss.	108.4	109.4	1.0	24.2	0.7	2.9	15.4	55.1	0.70
Paymaster 101A	1961	Plainview, Tex.	109.4	111.4	0.9	21.8	0.5	3.0	18.2	54.9	0.82
Acala 1517D	1962	University Park, N.M.	109.8	110.4	1.1	23.4	0.6	2.5	16.6	55.1	0.68
Stoneville 3202	1962	Stoneville, Miss.	110.5	112.0	0.8	24.2	0.6	2.3	13.9	57.2	0.98
Delfos 9169	1962	Stoneville, Miss.	110.8	111.9	1.0	23.4	0.5	2.7	14.7	57.0	0.66
Stoneville 213	1962	Stoneville, Miss.	111.6	112.1	0.8	21.4	0.9	2.9	17.9	55.2	0.76
Maximum			111.6	112.1	1.3	28.0	1.1	3.3	21.2	57.2	0.98
Minimum			96.8	97.1	0.8	21.4	0.5	2.3	13.9	45.0	0.58

[a] Determined by HBr titration.
[b] The present *G. barbadense* (extra-long staple).

A 700 gas chromatograph equipped with a thermal conductivity cell using a ¼ in. x 7 ft aluminum column with 20% diethylene glycol succinate (DEGS) on an 80–100 mesh support of Gas-Chrom Z. The instrument was operated at 195C with a flow rate of about 70 ml/min. Quantitative estimations of the various components were based on the areas beneath the chromatographic peaks determined by triangulation. The response of the thermal conductivity detector was calibrated by means of a GLC standard mixture of highly purified methyl esters obtained from the Hormel Institute.

The cyclopropenoid fatty acid contents of the oils were determined by the stepwise HBr titration method (9) and calculated as methyl malvalate. The Wijs iodine values were determined on the oils by the AOCS Official Method, Cd 1-25 (10). The methyl malvalate concentration was subtracted from the methyl linoleate concentration determined by GLC since on a DEGS column the methyl malvalate peak is masked by the methyl linoleate peak. The concentration of arachidic and other acids in trace amounts were not determined.

Sample Preparation

The oils analyzed were obtained from cottonseed samples representing a wide range of genetic types. Twenty-one were commercial varieties of American Upland cotton *(Gossypium hirsutum)*, most of which were included in Regional Variety Tests (11). Also included were the following: an extra-long staple variety of *G. barbadense*, Pima S-2, and 3 botanical types. The latter include seed of the Asiatic species—*G. arboreum* A2-47; a tropical *G. barbadense*—CB 551, the same species as Pima S-2; and "Palmeri"—a tropical *G. hirsutum* cotton related to American Uplands. Except for the last 3 mentioned, the seeds were obtained from the collections maintained by the USDA Crops Research Division to supply agronomists with small amounts of planting seeds for variety tests.

The kernels were mechanically separated from the hulls and residual lint, and then ground in a Wiley mill. The oil was Soxhlet-extracted with petroleum ether (bp 30–60C) and the solvent removed at reduced pressure using a rotary evaporator and maintaining a temperature of approximately 60C. Methyl esters were prepared from the oil by methanolysis with sodium methoxide, a procedure which does not affect the cyclopropenoid acid content (9).

Results and Discussion

The fatty acid composition and iodine value of the oils extracted from 22 commercial varieties of cottonseed are summarized in Table I. It should be noted that the analyses are representative only of the specific specimens analyzed. It is well known that the fatty acid composition and iodine value of the oil of a given variety vary over a wide range depending upon environmental factors during development of the seed.

The varieties are arranged in order of increasing iodine value. The percentage of malvalic acid in the different varieties varied from 0.58 for Lankart 57 and Pima S-2 to 0.98 for Stoneville 3202. The av-

TABLE II

Fatty Acid Composition and Iodine Value of Oils from Experimental Types of Cottonseed

Type	Year grown	Location	Iodine value (Wijs)	Iodine value (GLC)	Fatty acid composition, %						
					Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Malvalic[a]
G. barbadense (CB-551)	1955–56	Iguala, Mex.	101.6	100.2	1.0	25.6	0.9	3.5	22.1	46.3	0.56
G. arboreum (A2-47)	1957	Stoneville, Miss.	104.0	104.6	0.3	21.2	1.4	3.0	27.2	45.9	1.17
G. hirsutum (Palmeri)	1959–60	Iguala, Mex.	111.4	111.4	0.9	24.2	1.0	2.9	13.1	57.2	0.71

[a] Determined by HBr titration.

erage malvalic acid content was 0.74%. The percentages of the major fatty acids in the 22 varieties varied over a wide range. Lankart 57 was especially noteworthy. It had the largest amounts of palmitic and oleic acids, 28.0 and 21.2%, respectively, and the smallest amount of linoleic acid, 45.0%. Stoneville 3202 had the highest linoleic acid content, 57.2%, and the lowest oleic acid content, 13.9%. Stoneville 213 had the lowest concentration of palmitic acid, 21.4%.

Though the difference between the maximum value for stearic acid, 3.3%, and the minimum value, 2.3%, can be considered significant, there was little variation between the individual varieties. This was true also for palmitoleic acid, which varied from a low of 0.5% to a high of 1.1%, and for myristic acid, which ranged from 0.8 to 1.3%. Lankart 57 had the highest myristic acid content.

Lankart 57, which had the lowest malvalic acid content, also had the lowest iodine value, and Stoneville 3202, which had the highest malvalic acid content, had very nearly the highest iodine value. In general, however, there was no apparent correlation between malvalic acid content and iodine value. The iodine values calculated from the GLC analyses of the methyl esters are in good agreement with the Wijs determinations made on the crude oils.

The *G. arboreum* A2-47 also proved to be exceptional. It had a malvalic acid content of 1.17% as compared to the maximum of 0.98% for the commercial varieties (see Table II). Its oleic acid content, 27.2%, was nearly 6 percentage units greater than the highest oleic acid content of all the varieties analyzed and its palmitic acid content was the lowest found. It was the only sample containing more oleic acid than palmitic acid.

In contrast, *G. barbadense* CB 551 represented the lowest malvalic acid content, 0.56%, and the highest percentage of stearic acid, 3.5%, of the 25 varieties analyzed. The commercial *G. barbadense*, Pima S-2, also had an equally low malvalic acid content.

The relationship between the fatty acid compositions and iodine values (I.V.) for the 22 commercial varieties is illustrated in Figure 1 using the method of plotting of Stansbury and Hoffpauir (1). The equations for the regression lines for oleic, linoleic, and palmitic acid calculated by the method of least squares are as follows:

$$\% \text{ palmitic acid} = -0.3880(\text{I.V.}) + 66.04$$
$$\% \text{ oleic acid} = -0.3625(\text{I.V.}) + 55.51$$
$$\% \text{ linoleic acid} = +0.7882(\text{I.V.}) - 30.84$$

In general the changes in oleic and linoleic acid content with iodine value reported by Stansbury and Hoffpauir are corroborated. Their values tended to be 4–5 percentage units low for linoleic acid, however, and about 8 units too high for oleic acid, as would be expected since their analyses were based upon iodine-thiocyanogen values. The linoleic acid contents of the same oils determined spectrophotometrically by O'Connor et al. (12) were also low, but by only 2–3 units.

It would appear from the results that a reasonably accurate estimate of the fatty acid compositions of American commercial cottonseed oils can be made from the iodine value by use of these equations. The calculated average deviations of the individual analyses are ±0.6% for linoleic acid, ±0.8% for palmitic acid, and ±1.0% for oleic acid. This was not true for 2 of the 3 experimental varieties. The compositions of *G. arboreum* A2-47 and *G. barbadense* CB 551 are markedly different from those which would have been expected on the basis of their iodine values (Figure 1). *G. hirsutum* Palmeri showed good agreement with the predicted analyses, but was unique since it had the highest linoleic and lowest oleic acid content of all 25 oils.

From the regression lines obtained for the commercial varieties it is apparent that the increase in iodine value results from an increase in linoleic acid with a concomitant decrease in both oleic and palmitic acids at approximately equal rates. The slight increase in malvalic acid content with iodine value is of questionable significance. There is no significant

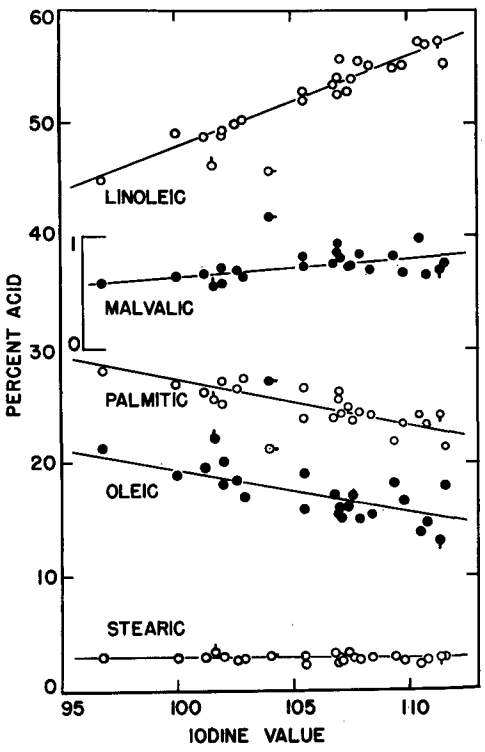

FIG. 1. Fatty acid composition vs. iodine value for oils from 25 varieties of cottonseed including three experimental types: | ○ *G. barbadense* CB 551; ○ *G. hirsutum*, Palmeri; and ○– *G. arboreum* A2-47.

change in stearic acid. Palmitic acid rather than stearic acid is associated with the increase in linoleic acid. This might have some implications in connection with fatty acid biosynthesis in cottonseed.

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Nutritional Evaluation of Inter-Esterified Fats

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Abstract

Studies with inter-esterified fats prepared to maintain a high level of linoleic acid content have been undertaken in several series of experiments with rats. These fats are as digestible as the liquid nonhydrogenated oils and the biological value of the linoleic acid is not impaired by the inter-esterification. Investigations involving growth, reproduction and lactation, longevity, tissue cholesterol levels and histological tissue examination have revealed that these inter-esterified fats are utilized by the animal similarly to cottonseed oil. No tissue pathology or interferences with any of the nutritional indices are observed. When the inter-esterified fats are included in atherogenic diets, the atherosclerotic lesions which develop in the coronary arteries and aorta of the animals are similar to, but less marked than, those found when animals are fed cholesterol-containing diets with butter oil or conventional margarine oil of the all-hydrogenated type. It is concluded that these inter-esterified fats are at least nutritionally equal to other similar edible fats of equivalent essential fatty acid content.

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Introduction

IN RECENT YEARS production of solid fats with high linoleic acid content has been achieved through an inter-esterification process. Nonhydrogenated vegetable seed oils are mixed with small amounts of highly hydrogenated fats and the blend is then inter-esterified to rearrange the fatty acid radicals; this imparts the firmness characteristic of margarine fats to the blend (1). Since the nutritional value of inter-esterified fats had not been investigated to the same extent as had been done for hydrogenated fats, an evaluation of inter-esterified fats in rats was undertaken and has now been completed. These investigations include determination of the digestibility and the essential fatty acid activity of these fats. The more extended studies include measurements of growth, reproduction, longevity and tissue cholesterol levels as criteria for nutritional value and safety. Similar long-term studies have been performed with animals fed (a) an unhydrogenated cottonseed oil, (b) a mixture of the inter-esterified fat with hydrogenated coconut oil, and, in some experiments, (c) butter oil and (d) a conventional margarine oil.

Experimental Procedures

The description of the fats fed is shown in Table I. The fatty acid composition was determined by spectrophotometric (2) and the trans isomers by in-

TABLE I

Description of Fats

Fat	Iodine No. (Wijs)	Fatty acid composition (%)					Digestibility[d]
		Saturated[a]	Oleic[a]	Trans[b]	Linoleic		
					Spec.[a]	Bioassay[c]	
MBO 1[e]	89.6	39.2	13.6	1.8	42.8	44.0	not tested
MBO 4[f]	89.6	39.2	13.6	1.8	42.8	41.5	94.3
MBO 45[g]	76.3	47.3	11.7	1.5	36.4	34.5	95.6
Cottonseed oil	112.0	23.6	20.1	....	51.9	.....	.....
Coconut oil (hydrogenated)	1.0	94.5	1.0		0.0	.....	.....
Butter oil	41.0	55.0	37.2	7 (approx)	3.5	.....	.....
Margarine oil (all hydrogenated)	80.0	19.4	69.5	35.0	9.7	6.9	92.9

[a] By spectrophotometric assay (2); as % of triglycerides.
[b] According to infrared absorption (3).
[c] Bioassay, based upon weight gain of male rats following supplementation of the basic fat-free diet (5).
[d] Based upon the amount of dietary fat absorbed from the digestive tract; correction is made for the metabolic fat found in the total fecal fat.
[e] Inter-esterified blend of 82.5% unhydrogenated and 17.5% completely hydrogenated cottonseed oil (1).
[f] Same as MBO 1 but containing added antioxidants, 0.02% of butylated hydroxytoluene (in solution) and 0.002% of ethylenediaminetetracetic acid (in suspension).
[g] Composed of 85% MBO 4 + 15% saturated coconut oil.