

TABLE III  
Iodine Value and Fatty Acid Composition of Rice Bran Lipids and Commercial Oils as Reported by Various Investigators

Sample origin	Iodine value	Fatty acid <sup>a</sup>							
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Philippines (Table I) <sup>b</sup> .....	90.5	0.4	18.3	0.2	1.4	41.0	37.0	1.1	0.6
United States (crude oil) <sup>c</sup> .....	103.4	0.4	17.9	0.2	1.3	41.2	37.7	1.1	0.1
United States (edible oil) <sup>c</sup> .....	105.3	0.5	18.3	0.4	1.5	45.0	33.5	0.5	0.3
Japan (15) <sup>d</sup> .....	.....	0.55 <sup>e</sup>	20.99	.....	1.63	38.14	37.79	0.90	.....
Philippines (5) <sup>d</sup> .....	99.3	0.2	18.7	.....	1.9	49.3	29.9	.....	0.7
Philippines (6) <sup>d</sup> .....	99.5	0.1	18.3	.....	2.8	49.0	29.8	.....	0.5
Various sources (7).....	99-108	0.4-1.0	13-18	.....	1-3	40-50	29-42	0-1	.....

<sup>a</sup> Wt % of total acids.

<sup>b</sup> Mean of 4 varieties. Trace 12:0 present.

<sup>c</sup> Mean of 3 determinations. Trace 12:0 present.

<sup>d</sup> Recalculated to exclude unsaponifiable matter.

<sup>e</sup> Including 12:0.

fatty acid composition of the brown rice lipids of the four Asian rice samples may be calculated as 0.4% myristic, 20.4% palmitic, 0.2% palmitoleic, 1.6% stearic, 41.3% oleic, 34.5% linoleic, 1.0% linolenic, 0.6% arachidic, and trace lauric. This compares well with data based on U.S. whole rice lipids: 13% palmitic, 2% stearic, 4% other saturated acids, 42% oleic, 38% linoleic, and 1% other unsaturated acids calculated as percentage of total acids (8).

#### ACKNOWLEDGMENTS

Crude rice bran oil obtained through R. R. Mickus, Director of Research, Rice Growers Association of California, Sacramento; edible oil, from Harriet Nawry, Comet Rice Mills, Houston, Texas. Moisture and lipids contents by Remedios Santiago.

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[Received September 3, 1963—Accepted November 18, 1963]

## Effect of Oxygen and Other Factors in Selenium Catalyzed Isomerization of Unsaturated Fatty Acid Esters<sup>1,2</sup>

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#### Abstract

In the *trans*-isomerization of ethyl linoleate using 1% selenium as catalyst under nitrogen, 200C was found to be the optimal temperature. Conjugation and polymerization were concurrent with the formation of the *trans* esters. In the isomerization of ethyl oleate with selenium, the double bond did not migrate to any appreciable extent.

Isomerization studies were performed on olive, safflower and linseed oils and ethyl esters of oleic, linoleic and linolenic acids. Time to reach maximal *trans* isomer content was longest with linolenate esters; glycerides reacted more rapidly than ethyl esters, and with lesser polymerization.

Oxygen was found to be an important participant in the *trans*-isomerization reaction. Its exclusion resulted in sharply diminished reaction rates. Benzoyl peroxide and hydrogen peroxide accelerated while an antioxidant (BHT) retarded the reaction.

#### Introduction

HEATING OF AN unsaturated fatty acid with selenium is known to produce changes in configuration; at equilibrium the product has definite proportions of *cis* and *trans* isomers. The rates of isomerization under nitrogen have been determined by various workers; in many of these studies, older methods of estimation of *trans* isomers were used and often led to conflicting results. The present experiments were performed to determine the optimal conditions for the preparation of *trans* fatty acids.

Bertram (1), in 1938, converted oleic acid to elaidic acid by heating with 0.5% selenium at 150C for 28 hr; results showed that the reaction was trimolecular. Kass and co-workers (2,3) have studied isomerization of methyl linoleate and mixed methyl esters of linseed oil. During the preparation of linolelaidate and linolenelaidate with 1% selenium under nitrogen, they found that 6 and 17 hr, respectively, were needed to attain maximum *trans* ester formation. Hilditch and Jasperson (4) from their studies concluded that oleate and linoleate are similar in the amount of time required for complete isomerization. In more recent years Fitzpatrick and Orchin (5) have studied oleic acid isomerization with selenium. They reported that

<sup>1</sup> Journal Paper No. 2046, Purdue University Agricultural Experiment Station.

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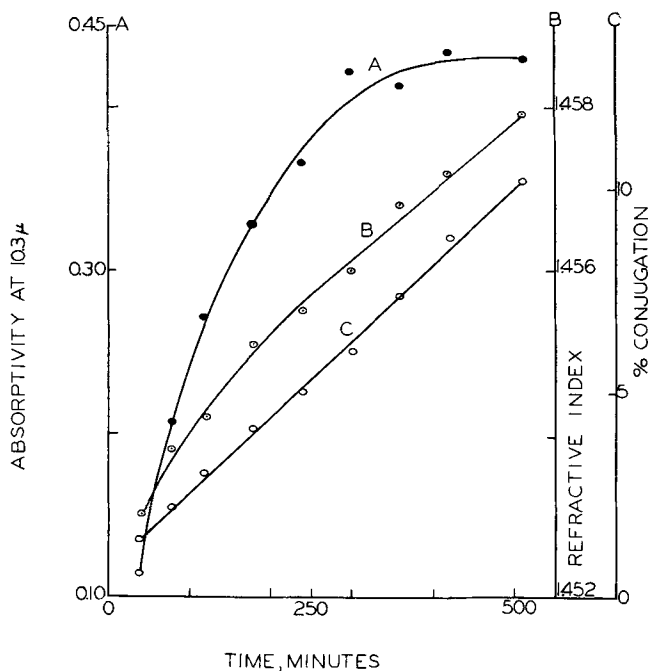


Fig. 1. Effect of 1% selenium treatment of ethyl linoleate at 200C on (A) absorptivity at 10.3  $\mu$ , (B) refractive index at 40C and, (C) % conjugated ester.

in the early stages of heating, selenium complexed with the double bond and on prolonged heating could not be recovered. They suggested that hydroselenides like hydroperoxides may be formed and be responsible for the isomerization.

#### Materials and Methods

Olive, safflower and linseed oils were obtained from reliable commercial sources and their purity was confirmed by gas-liquid chromatographic analysis. Ethyl oleate of 86.5% purity was prepared by two successive crystallizations of the urea adduct from the olive oil esters. Starting from safflower oil, ethyl linoleate was prepared by ethanolysis and urea fractionation, with

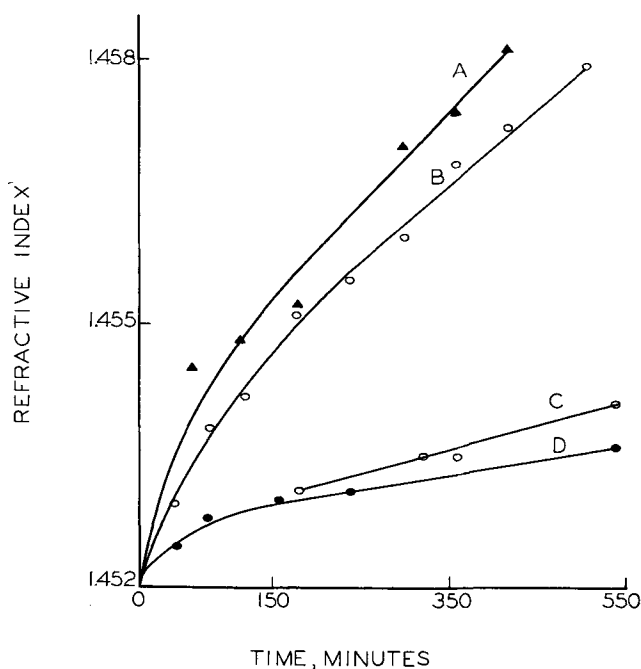


Fig. 2. Effect of temperature on selenium catalyzed isomerization of ethyl linoleate. (A) 210C, (B) 200C, (C) 190C, (D) 180C.

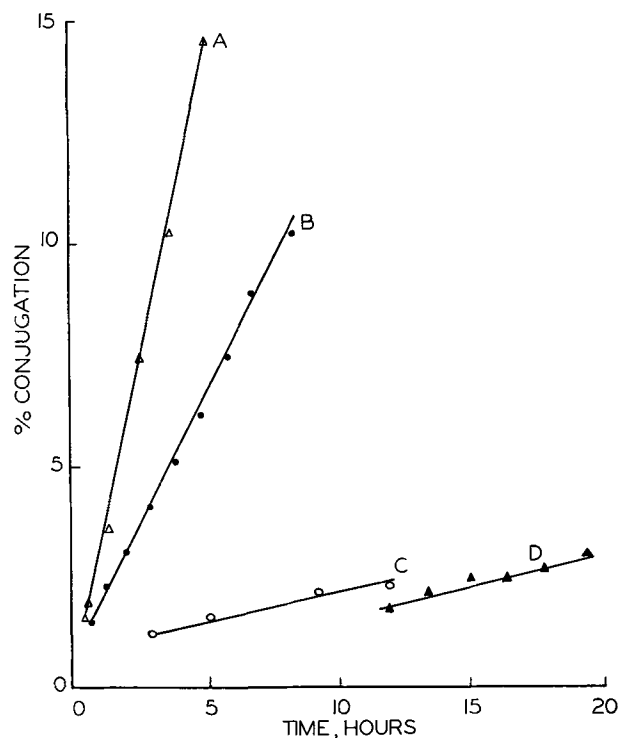


Fig. 3. Linoleate conjugation (from absorbance at 233  $m\mu$ ) at four temperatures. (A) 210C, (B) 200C, (C) 190C, (D) 180C.

a purity of 97.2%, the remainder being oleate as analyzed by gas chromatography (6). Ethyl linolenate with a purity of 93.3% (6.7 linoleate) was prepared by the mercuric acetate adduct procedure of White and Quackenbush (7). Selenium was certified reagent grade (Fisher Scientific Co., Cat. No. S-137). Nitrogen, commercial, of purity 99.7% (0.3% oxygen) unless otherwise specified, was used to blanket the reaction.

For isomerization, 30 g of the ester with 1% selenium in a three-necked flask was heated with controlled agitation in an oil bath, the temperature of which was maintained within  $\pm 1$ C. Nitrogen, dried by passing through a sulfuric acid scrubber, was used to flush the flask initially and a steady stream was allowed to flow through the flask at a rate of 30 ml/min. Nitrogen in the flask was maintained at a slight positive pressure to prevent diffusion of air into the system. Samples were taken at definite intervals and filtered before determining refractive index (Abbe, 40C). The amount of conjugation was determined spectrophotometrically at 233  $m\mu$  (Beckman D U) after diluting to the proper concentration with purified methanol, or with purified isooctane in the case of glycerides. Absorptivity at 10.3  $\mu$  was calculated from measurements with carbon disulfide solutions containing 10–15 g of sample per liter.

#### Results and Discussion

During isomerization of ethyl linoleate with 1% selenium under nitrogen, refractive index, conjugated diene, and *trans*-absorptivity were found to increase with time (Fig. 1). To reach peak absorptivity, heating for six hours at 200C was necessary and conjugated diene and a refractive index rose to 7.4% and from 1.4521–1.4574, respectively. Heating for longer periods resulted in an apparent loss of *trans* bonds, but the amount of conjugation and hence polymerization increased with further heating. The

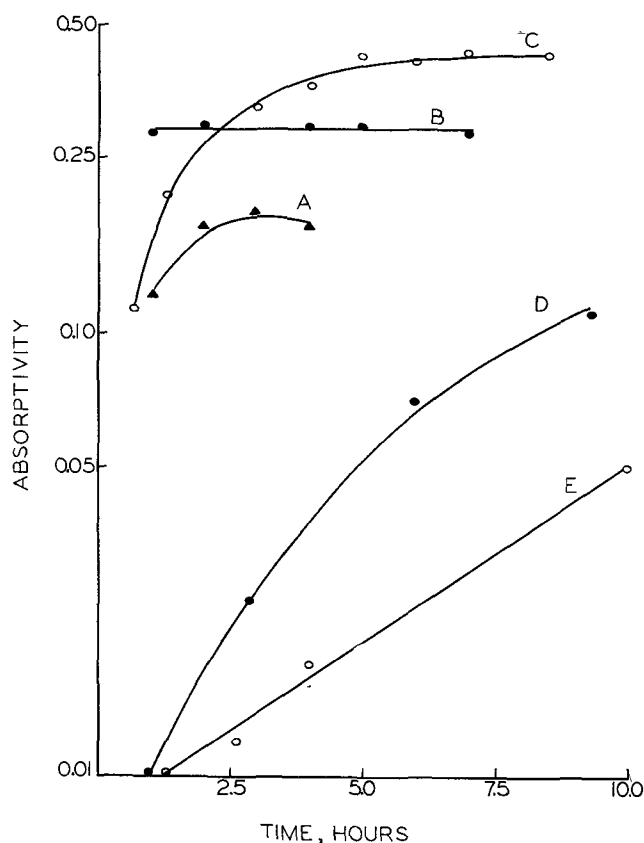


Fig. 4. *Trans* bond formation (absorptivity at  $10.3\mu$ ) of linoleate with 1% selenium. (A) 235C, (B) 210C, (C) 200C, (D) 190C, (E) 180C.

maximum absorptivity reached was lower than the  $\frac{2}{3}$  value for *trans* bond content, but the monomer, freed from polymers by high vacuum distillation, had nearly two thirds of the absorptivity value of the pure linolealaidate.

The effect of temperature, during the isomerization of ethyl linoleate with 1% selenium under nitrogen, on refractive index, percent conjugated diene and absorptivity at  $10.3\mu$  is shown in Figures 2,3, and 4, respectively. In general, with increasing temperatures all three reaction rates increased and 200C appears to be optimal for the preparation of *trans* isomers. At 180 and 190C, although polymerization and conjugated ester formation were negligible in comparison with higher temperatures, *trans*-isomerization was also quite slow; absorptivities were 0.28 and 0.23 after 24 hr as compared to 0.43 at 200C in 4 hr. For temperatures higher than 200C, the equilibrium was reached faster, but the competitive predominancy of unwanted side reactions of conjugation and polymerization gave an apparent lowering of *trans* bond content in the equilibrium mixtures.

A comparison of three ethyl esters and two natural oils revealed some differences in response to selenium catalysis (Table I). There did not appear to be any appreciable difference in isomerization rate of mono- and di-unsaturated acid esters in accord with an earlier observation (4), but the isomerization rate of trienoic esters was much slower. The absorptivities at equilibrium of oleate and linoleate esters also were nearer to the theoretical value for two-thirds of the unsaturated bonds than that of linolenate. This discrepancy arose from differences in the amount of polymerization taking place, as the distilled mono-meric esters had absorptivities nearing the  $\frac{2}{3}$  values. The glycerides reached equilibrium status earlier than

TABLE I  
*Trans*-Isomerization of Various Esters with 1% Selenium under Commercial Nitrogen

Ester or fat	Time for equilibrium hr	Absorptivity at $10.3\mu$	% of theory*
Et. oleate (86.1%)	6.0	0.26	68.5
Et. linoleate (97.2%)	6.0	0.42	60.9
Same after distn.	.....	0.49	70.0
Et. linolenate (93.3%)	28.0	0.47	48.1
Safflower oil.	2.5	0.42	.....
Linseed oil.	22.5	0.47	.....

\* Based on absorptivities of the crystalline esters prepared in this laboratory.

the corresponding ethyl esters; perhaps they were less prone to side reactions, since we observed relatively lower rates of increase of refractive index and of conjugated ester formation.

When samples of ethyl oleate were isomerized at 200C with 1% selenium under nitrogen and under air, and the fragments resulting from subsequent periodate-permanganate oxidation were esterified with diazomethane and analyzed by gas chromatography (10 ft *DEGS* column with 66.5 ml/min helium flow rate and programmed between 65 and 200C at the rate of 4C/min), the results did not indicate any appreciable extent of double bond migration.

In an attempt to reduce the extent of polymerization, the dissolved air in the ester prior to reaction was replaced with nitrogen by subjecting to high vacuum thrice and introducing nitrogen each time. In another trial, the oxygen present in the nitrogen (Ca 0.3%) was eliminated by reaction in a glass tube packed with fine copper wire, at 300C and the sample treated as above. These procedures reduced polymer and conjugated ester formation rates but also reduced the rate of *trans* ester formation (Fig. 5). The time to reach apparent equilibrium was doubled by the oxygen reduction, and the relative amounts of polymer and *trans* esters formed remained essentially constant. These experiments were repeated at other temperatures and also were performed on safflower oil; the *trans*-isomerization rate was slowed always by removal of oxygen from the system.

Formation of *trans* ester was dependent on the amount of oxygen present in the system and this is

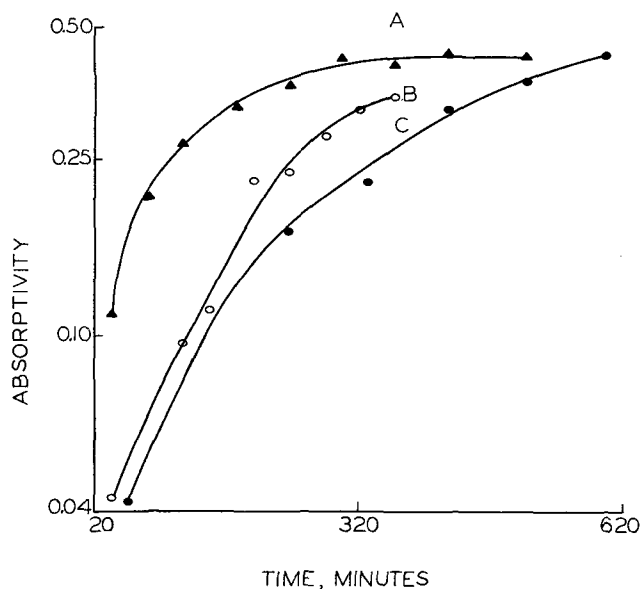


Fig. 5. Effect of oxygen removal on *trans*-isomerization of linoleate. (A) commercial nitrogen (0.3%  $O_2$ ) used to "blanket" the reaction, (B) dissolved air in catalyst and ester replaced by commercial nitrogen before heating, and (C) same as B, using oxygen-free nitrogen (hot-copper treatment).

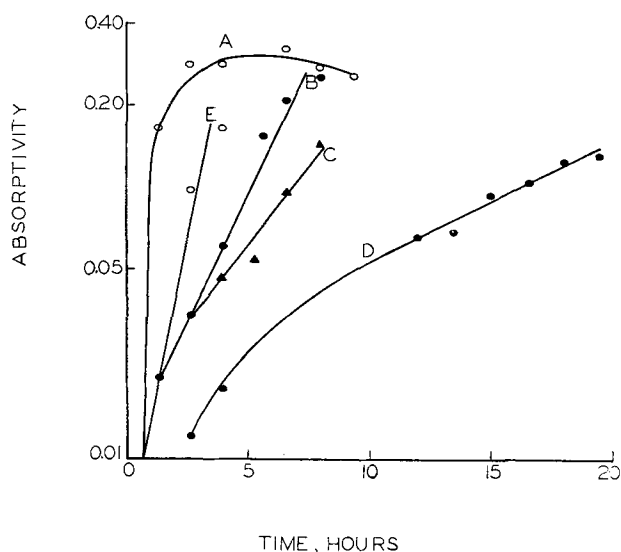


Fig. 6. Effect of oxygen supply on *trans*-isomerization of linoleate at 180C, O<sub>2</sub> in N<sub>2</sub>: (A) 21%, (B) 10%, (C) 5%, (D) 0.3%, (E) 0.5% benzoyl peroxide added to flask contents.

shown by the results of isomerization of ethyl linoleate at 180C (Fig. 6). For 21, 10, and 5% of oxygen in the atmosphere at 400 min the absorptivities were 0.32, 0.21, and 0.09 as compared to 0.06 at 720 min for 0.3% oxygen. Conjugation and polymerization were also diminished. For the above conditions the % conjugated diene was 9.5, 7.9, 6.2, and 1.5 and the refractive index at 40C was 1.4601, 1.4575, 1.4560, and 1.4539, respectively. Addition of benzoyl peroxide (0.5%), initially, increased the rate of *trans* ester formation for isomerization of ethyl linoleate under commercial nitrogen.

The catalytic effect of benzoyl peroxide was diminished by the addition of an antioxidant, 1% BHT (Fig. 7). The rates of polymerization and conjugation also were diminished. For the three tests: a) no additive, b) benzoyl peroxide added, and c) benzoyl peroxide and BHT added, at 80 min the absorptivities at 10.3  $\mu$  were 0.21, 0.27, and 0.09 and the conjugated diene contents were 2.2, 4.8, and 1.2%,

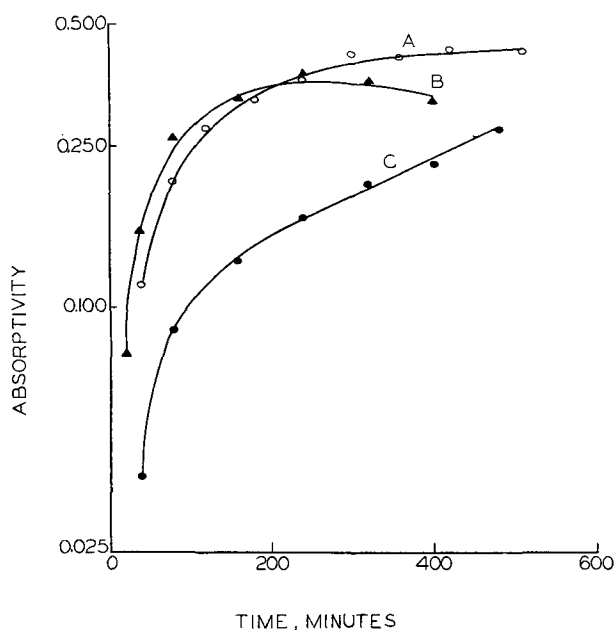


Fig. 7. Antioxidant effect on peroxide-catalyzed *trans*-isomerization of linoleate at 200C. (A) no additives, (B) with 0.5% benzoyl peroxide, (C) with 1% BHT.

TABLE II  
Absorptivities of Linseed Oil During Isomerization with Selenium under Commercial Nitrogen at 200C

Time hr	Additive			
	1% BHT	None	1% Benzoyl peroxide	H <sub>2</sub> O <sub>2</sub> (0.2 ml 30%)
0.5.....	0.02	.....	0.12	0.13
1.0.....	0.04	0.05	0.13	0.27
2.0.....	0.05	0.11	0.22	0.32
4.0.....	0.08	0.19	0.24	0.35
6.0.....	0.12	0.23	0.33	0.37
8.0.....	0.16	0.30	0.37	0.38
10.0.....	0.20	0.32	0.38	0.39

respectively. After heating 240 min the refractive indices rose from 1.4521–1.4555, 1.4561, and 1.4539, respectively. The same general observations were made on safflower, linseed and olive oils. Hydrogen peroxide too, when added (0.2 ml/50 g) promoted the rate of isomerization. It was noted that heating with either air or benzoyl peroxide alone in the absence of selenium resulted in negligible amounts of *trans* esters even after prolonged heating, but the other two reactions, i.e. conjugation and polymerization, were taking place.

In an attempt to eliminate completely peroxides as well as dissolved oxygen, safflower oil was preheated with magnetic stirring for 2 hr at 180C under vacuum (0.1 mm). Selenium (1%), which was supported in the vessel, was tipped into the oil after raising the temperature to 200C, and heating with agitation was continued for three more hours. The resultant oil had an absorptivity of only 0.03, as compared to 0.42 when no attempt was made to remove oxygen.

Because of the above observations, progress of the *trans*-isomerization reaction was observed with linseed oil under a series of treatments (Table II). BHT clearly inhibited the reaction, even though no peroxides were added. Hydrogen peroxide accelerated the reaction more than benzoyl peroxide.

Some practical application may be derived from these experiments for those who wish to prepare *trans* from *cis* esters. One should a) choose a glyceride rather than a mono-ester as the starting material, b) maintain the reaction temperature near 200C, c) provide for a source of oxygen, and d) keep the antioxidant content of the reaction mixture at a minimum.

*Trans*-isomerization and its accompanying polymerization and conjugation reactions were shown to be dependent on the oxygen pressures. The dependence of *trans*-isomerization upon oxygen and its inhibition by antioxidant action suggest that peroxidation in some form precedes the reaction. The mere presence of performed peroxide of the benzoyl peroxide type was not sufficient for immediate catalysis of the reaction. Perhaps a more active form of oxygen than that present in the relatively stable peroxides is involved under ordinary conditions. That such active form exists in incipient peroxidation of fatty acid esters had been observed in polarographic studies in our laboratory (8). Perhaps these primary oxidation products act as intermediates for the *trans* ester formation, the oxygen adding in the vicinity of the unsaturated linkage to form an intermediate, which was postulated for auto-oxidation at relatively lower temperature (9,10), and with which the selenium then acts to release the unsaturated bond in its new configuration. Bertram (1) from studies on the reaction kinetics has suggested that this reaction could be trimolecular. Perhaps the reaction proceeds as two successive bimolecular reactions: in one oxygen adds, and in the second it is removed, selenium participating

in either one or both of these reactions. The position of equilibrium is well-known to favor the *trans*-isomer. This fact, and the comparatively slow rate of conversion of *trans* acids to *cis* acids may reflect, at least in part, the slower rate of oxygen attack upon the *trans* configuration.

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[Received January 8, 1963—Accepted September 23, 1963]

## Search for New Industrial Oils. IX.

### *Cuphea*, a Versatile Source of Fatty Acids

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## Abstract

Seed oils from five species of *Cuphea* show three distinct patterns of fatty acid composition. *C. hookeriana* and *C. painteri* oils contain ca. 70% caprylic acid, *C. ignea* and *C. llavea* oils have over 80% capric acid, and *C. carthagenensis* oil contains 57% lauric and 18% capric acids.

## Introduction

THE FIRST REPORT of a high concentration of capric acid in seed oil of herbaceous plants was based on the discovery early in this survey that oil from seed of *Cuphea llavea* var. *miniata* contained over 80% (2) of this fatty acid component. Earlier known sources of capric acid containing comparable concentrations were oils from *Ulmus americana* (4), *Zelkova serrata* (5), and more recently other species of *Ulmus* (10) and *Sassafras albidum* (2).

The present commercial source of capric, caprylic, and lauric acids is coconut oil imported from the tropics (6). These acids of intermediate chain length are highly important to the chemical industry as raw material for the production of surface-active agents, plasticizers, physiologically active compounds, perfumes, and flavors. In 1962 the United States imported 807 million lb of lauric acid oils, ca. 90% of which came from coconuts (12). A domestic source of capric and lauric acids could be of great strategic importance and, if sufficiently economical, could prove of value to the chemical industry in normal times. Accordingly, special attention was given to the recollection of *C. llavea* and to collection of other species of the genus.

*Cuphea* of the family Lythraceae is a large genus of over 200 species of herbs and shrubs in the tropics and subtropics of the Americas. Varieties of *C. ignea*, cigar flower, and *C. llavea*, cinnabar cuphea, are popular ornamentals in warmer sections of the United States.

## Materials and Methods

Samples were prepared and analyzed as described previously (3). Methyl esters were derived from the oils by methanolysis catalyzed by HCl. Analysis by gas chromatography (GLC) followed previously reported procedures (8).

Evaporation of solvents under a stream of nitrogen on a steam bath gave low yields of recovered ester, and GLC analyses of methyl esters gave low values for acids having less than 10 carbon atoms in chain.

To avoid losses of lower mol wt methyl esters from *C. hookeriana* and *C. painteri*, the following modification of the usual procedure was used. Ethyl ether was used in place of benzene to increase the solubility of oil in the methanol-HCl reagent. After methanolysis, solvent removal was accomplished by fractional distillation through a column packed with glass helices. Removal of the ether was essentially complete with no detectable loss of fatty acids as determined by weighing the esters and examining the GLC curve for evidences of residual solvent. Other means of avoiding such losses have been reported recently (1,7,11).

## Results and Discussion

All five species of *Cuphea* analyzed produce seed oils rich in intermediate chain-length acids, but three distinct patterns of composition are noted (Table I). Three accessions of *C. llavea*, including the previously reported sample (2) and one of *C. ignea*, produce oil with more than 80% capric acid. Oils from seeds of *C. hookeriana* and *C. painteri* contain ca. 70%

TABLE I  
Analytical Data on *Cuphea* Seeds and Derived Oils

	<i>C. carthagenensis</i>	<i>C. hookeriana</i>	<i>C. painteri</i>	<i>C. ignea</i>	<i>C. llavea</i> <sup>a</sup>
Seed analysis					
Weight/1,000, g....	0.7	0.3	0.5	1.0	1.0; 3.3; 0.9
Protein (N × 6.25), % DB.....	16	19	23	18	17; 21; 15
Oil, % DB.....	33	16	36	34	21; 30; 17
Oil analysis					
Iodine value <sup>b</sup> .....	18	18	12	10	18; 12; —
Percent methyl ester in mixed methyl esters (by area on GLC curve)					
6:0.....	....	0.1	0.2	....	....
8:0.....	3	65	73	3	0.8; 0.6; 1
10:0.....	18	24	20	87	83; 85; 86
12:0.....	57	0.1	0.2	0.8	1; 3; 1
14:0.....	8	0.2	0.3	0.2	0.8; 1; 0.6
16:0.....	3	2	2	2	3; 2; 2
18:0.....	0.6	0.5	0.4	....	0.5; 0.3; 0.5
18:1.....	5	2	2	2	5; 4; 2
18:2.....	5	6	2	4	6; 4; 4
18:3.....	....	0.4	....	....	tr; —; —

<sup>1</sup> No. Utiliz. Res. and Dev. Div., ARS, USDA.

<sup>2</sup> ARS, USDA.

<sup>a</sup> One of these samples is the one previously reported (2).

<sup>b</sup> Approximately 0.2 g samples used.