the glucose moiety. The ratio of attachment on glucose and fructose moieties is of the order of 4 to 1. The tosylation method for determining whether the lauroyl group was attached exclusively to a primary alcohol of sucrose proved inadequate. With sucrose monolaurate, recoveries of 61.5 and 81.0% were obtained, assuming two free primary groups. These low recoveries are not uncommon (4). These results suggest, but do not establish that one primary alcohol is bound by the lauroyl radical.

Theoretical values for periodate consumption and formic acid production for various positions of the lauroyl radical on sucrose are given in Table I. The experimental value of 2.9 millimoles of periodate consumed per millimole of ester provides strong evidence to the effect that the lauroyl radical is attached almost entirely to a primary alcohol. Some uncertainty is introduced as to the proportion of bound primary alcohol groups by the fact that only 0.68 molar equivalents of formic acid were produced.

Conclusion

A product corresponding to sucrose monolaurate was prepared by the alcoholysis of methyl laurate with sucrose. This product appears to be a mixture, with the principal component having the lauroyl radical at the 6-position of the glucose portion.

Acknowledgment

The investigation described herein was supported by the Sugar Research Foundation Inc., New York, N. Y.

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[Received April 9, 1956]

Determination of Tocopherol in Autoxidizing Methyl Esters of Fatty Acids[1,2]

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MUCH WORK has been done on the estimation of tocopherol in fats and oils (7). The chief problem has been interference by oxidizing and reducing materials, but, in addition, the fats themselves may affect the values obtained in colorimetric (4, 6) and spectrophotometric (2) procedures. This paper deals with modifications of these procedures developed for use in a study of the stability of tocopherol in autoxidizing methyl esters of fatty acids. Removal of interfering substances was attempted by treatment with sulfuric acid (8), or by saponification. Oxidation of tocopherol to the quinone form (2) was studied as a means of increasing the sensitivity of tocopherol estimation.

Experimental

Treatment with Sulfuric Acid. Pure methyl stearate, oleate, linoleate, or linolenate (obtained from the Hormel Foundation) did not affect the bipyridine colorimetric reaction (8) for *d-a* tocopherol,[3] but oxidized methyl esters reduced the tocopherol values obtained in recovery experiments. Treatment of petroleum ether solutions of tocopherol and oxidized methyl esters with 80% sulfuric acid (80 parts by weight of concentrated acid, sp. gr. 1.84, to 20 parts of water) allowed the colorimetric reaction to develop fully.

Pure methyl esters did not interfere with the characteristic ultraviolet absorption peak of tocopherol at 2980 Å, but oxidized methyl esters obscured the peak, even after treatment with sulfuric acid. A more complete separation of tocopherol from oxidized fat appeared necessary to make spectrophotometry applicable. Treatment with 90 or 95% sulfuric acid, to which tocopherol alone was stable, removed most of the fat but resulted in large losses of tocopherol (Table I), presumably by adsorption on the charred fat (1). Subsequent dilution of the acid phase gave increased recoveries of tocopherol, but appreciable fat was reintroduced into the ether phase (Table I).

It was concluded that sulfuric acid treatment was a useful preliminary to colorimetric determination of tocopherol but could not be used to effect a complete separation of tocopherol from oxidized methyl esters.

Saponification. Saponification in the presence of protective agents was tried at room temperature since earlier applications of this procedure at higher temperatures have not been uniformly successful (4, 11, 12, 13).

At room temperature, potassium hydroxide in water or aqueous alcohol did not completely saponify methyl esters in petroleum ether solution and

TABLE I

Effect of Sulfuric Acid Treatments on Recovery of Tocopherol

Treatment[a]	Tocopherol recovery, % of original amount added[b]	Residual fat, % of original amount
A. 0.01% tocopherol in petroleum ether.		
None	100	—
80% sulfuric acid	100	—
90% sulfuric acid	98.8	—
95% sulfuric acid	97.1	—
Conc. sulfuric acid	23.2	—
B. 0.01% tocopherol and 4% pure methyl oleate in petroleum ether.		
None	100	—
80% sulfuric acid	99.3	99.8
C. 0.01% tocopherol added to 4% oxidizing[c] methyl oleate-tocopherol in petroleum ether.		
None	64.4	—
80% sulfuric acid	98.9	91.8
90% sulfuric acid	86.7	25.9
95% sulfuric acid	31.5	7.7
95% sulfuric acid diluted, then 80% sulfuric acid	97.0	50.9

[a] The acid treatment in each test was followed by washing with 1% potassium hydroxide saturated with sodium sulfate.
[b] Determined colorimetrically by the bipyridine procedure.
[c] Peroxide value about 40 ml. of 0.002 *N* thiosulfate per g. Similar results were obtained with other fatty acid esters.

[1] N. R. C. No. 4053.
[2] Presented in part at meeting of American Oil Chemists' Society, Minneapolis, Minn., Oct. 11–13, 1954.
[3] Similar results were obtained throughout with *dl-a* tocopherol.

caused considerable destruction of tocopherol. Better results were obtained by the addition of pyrogallol and by the use of potassium hydroxide or sodium methylate in absolute ethanol to form a single phase with the petroleum ether. A closed tube containing this mixture was shaken to dissolve the alkali and to bring about absorption of oxygen by pyrogallate. Although 0.025 g. of pyrogallol per tube was adequate, 0.1 g. was used to give a wide margin of protection. Pyrogallol gave better protection to tocopherol than did stannous chloride, *p*-hydroxyacetanilide, *α*-naphthol, or butylated hydroxyanisole under the conditions studied. The extent of saponification of methyl esters was the same with all concentrations of pyrogallol tested.

Extraction of the saponification mixture with petroleum ether yielded a tocopherol solution containing less than 1% of the original fat. The ultraviolet spectrum of the solution was essentially the same as that of pure tocopherol, and indicated satisfactory recovery of the tocopherol originally present.[4]

Increasing the Sensitivity of the Tocopherol Determination. The sensitivity of the spectrophotometric determination can be increased by oxidizing tocopherol to quinones, which show a greater absorption in the ultraviolet than the parent substance (2). Conversion to tocopheryl-*p*-quinone appeared to be more suitable than conversion to the *o*-quinone since the former has a greater absorption at low concentrations. Attempts were made therefore to develop a simple procedure for converting tocopherol quantitatively to the *p*-quinone. For purposes of comparison, the *p*-quinone was prepared by gold chloride oxidation (5), and the *o*-quinone by concentrated nitric acid oxidation (10).

Petroleum ether solutions of tocopherol were shaken with a series of reagents. Hydroxylamine salts or 2 *N* nitric acid, under suitable conditions, eliminated the tocopherol absorption and gave the *p*-quinone double peak at 2,600–2,700 Å (5) without formation of *o*-quinone. The use of 2 *N* nitric acid was preferred as the reaction was more rapid. The quinone obtained by treatment with 2 *N* nitric acid gave the same ultraviolet (5) and infrared (9) spectra as did authentic *p*-quinone.

[4] The room temperature saponification procedure was also found applicable to determination of tocopherol in tocopherol acetate and succinate.

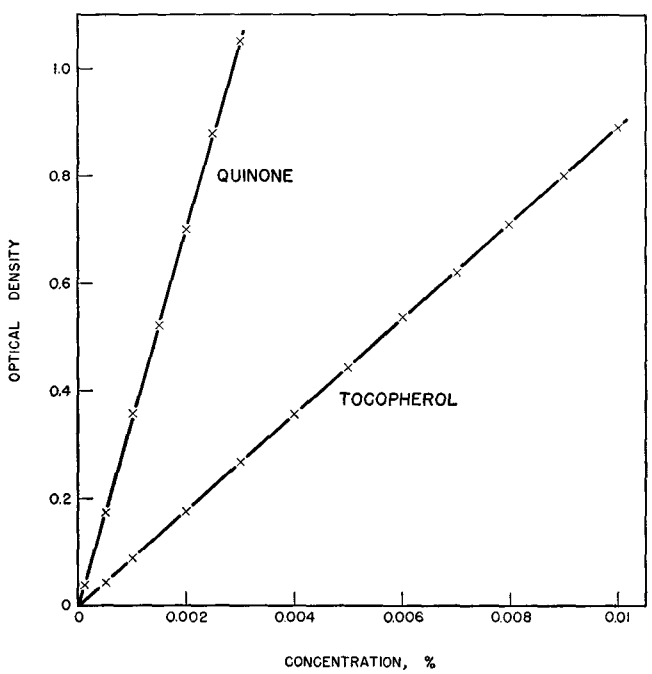

FIG. 1. Absorbancies of *d*-*α* tocopherol at 2,980 Å and *α* *p*-quinone at 2,600–2,700 Å. Determined in petroleum ether and corrected for petroleum ether blank.

TABLE II

Recovery of Added Tocopherol and Agreement of Duplicate Determinations [a] for Tocopherol Added to Autoxidizing Methyl Oleate-Tocopherol Mixtures

Total tocopherol concentration, g./100 ml. of petroleum ether	Range of tocopherol recovery, %	Max. difference between duplicates, % of the mean
A. Colorimetric method, after sulfuric acid treatment, 1 to 5 ml. aliquot.		
0.0001–0.0005	85–103	15.8
0.0005–0.01	96–101	3.1
B. Spectrophotometric method, after saponification treatment, direct reading of tocopherol, 1 to 5 cm. cell.		
0.0001–0.0005	78–105	22.2
0.0005–0.01	95–101	4.2
C. Spectrophotometric method, after saponification treatment and conversion of tocopherol to *p*-quinone, 1 to 5 cm. cell.		
0.0001–0.0005	93–102	6.6

[a] Eight duplicate tests in each tocopherol concentration range; oxidized methyl oleate present in each solution at 4% concentration. Similar results were obtained with other fatty acid esters.

The absorption of tocopherol and *p*-quinone in petroleum ether at 2,980 Å, and 2,600–2,700 Å, respectively, obeyed Beer's Law in the concentration ranges tested (Figure 1). The molar extinction coefficient for the *p*-quinone, 16,070, was 4.2 times that for *d*-*α* tocopherol, 3,870, in petroleum ether (1-cm. cells).

Choice of Method. Recovery experiments (Table II) indicated that for the higher concentrations of tocopherol, colorimetry could be satisfactorily applied to sulfuric acid treated solutions, and ultraviolet spectrophotometry to saponified solutions. For very small concentrations of tocopherol, accuracy and precision were improved by the use of the saponification procedure, followed by conversion of tocopherol to the *p*-quinone. The method finally adopted is as follows.

Pipette 10 ml. of a 4% (w/v) solution of the fat in purified petroleum ether (3), b.p. 100°–120°C., into a 30-ml. glass-stoppered centrifuge tube and proceed according to A, B, or C:

A. Add 2 ml. of 80% H_2SO_4, close the tube, and shake it continuously for 8 min. Centrifuge, and remove most of the top layer to a second tube. Add 5 ml. of 1% potassium hydroxide solution saturated with sodium sulfate, shake the tube for 8 min., and centrifuge. Determine tocopherol by the bipyridine colorimetric method (8).

B. Add 5 ml. of 2% alcoholic pyrogallol and 1 g. of solid potassium hydroxide, close the tube at once, and shake it for 2 hr. Add 10 ml. of 5% sodium sulfate solution, shake the tube for 8 min., and centrifuge. Remove most of the ether layer to a 25-ml. "low actinic" volumetric flask. Extract the soap layer twice more with fresh petroleum ether, and make the combined extract to volume. Determine tocopherol in the final extract by ultraviolet absorption at 2,980 Å.

C. If the tocopherol concentration is less than 0.002% add 2 ml. of 2 *N* nitric acid to 10 ml. of petroleum ether extract from B. Shake the mixture for 2 hr. and centrifuge. Read the absorption of the

ether layer at 2,700 Å and check also at 2,980 Å for complete conversion of tocopherol. Compare with standard curve prepared by oxidizing known amounts of tocopherol.

Summary

Autoxidizing methyl esters of fatty acids interfered with the determination of α-tocopherol by ultraviolet spectrophotometry or by the bipyridine colorimetric method. Interference with the colorimetric method was removed by sulfuric acid treatment, but spectrophotometry was applicable only when the tocopherol was completely separated from oxidized fat. This separation could not be obtained by sulfuric acid treatment but was accomplished by room temperature saponification in an alcohol-petroleum ether system protected by pyrogallol. The sensitivity of the spectrophotometric method was increased by oxidizing tocopherol quantitatively to the *p*-quinone with 2 *N* nitric acid.

Acknowledgment

The technical assistance of J. W. Halcro is gratefully acknowledged.

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[Received March 26, 1956]

ABSTRACTS . . . . . R. A. Reiners, Editor

• Oils and Fats

S. S. Chang, Abstractor
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The polymorphism of glycerides—an application of x-ray diffraction. E. S. Lutton(Procter & Gamble Co., Cincinnati, O.). *J. Soc. Cosmetic Chemists* 6, 26–34(1955). Methods used in the study of the modes of crystallization of a number of mono-, di-, and triglycerides are reviewed. (*C. A.* 50, 7481)

Continuous refining of rapeseed oil. A. M. Zharskii and T. E. Romanova(Fat Combine, Kharkov). *Masloboino-Zhirovaya Prom.* 21(8), 12–3(1955). Rapeseed oils of acid number 3.5–4.5 were refined by the continuous process of A. A. Schmidt. The oils were hydrated with steam, held two hours and centrifuged. Refining was with 100% excess lye solution of 130 g. per liter concentration. Tests on 10 oils yielded refined oils containing 0.36–1.25% soap and 0.24–0.38% free fatty acids. The foots contained 9–12% soap and a saponified fatty acid to neutral oil of ratio of 1:0.49 to 1:0.3. The refined oil was efficiently decolorized with 2% active earth when the moisture present was 0.5–1.5%. (*C. A.* 50, 7481)

Obtaining easily refinable extracted cottonseed oil. I. V. Gavrilenko and I. E. Bezuglov. *Masloboino-Zhirovaya Prom.* 21(8), 5–9(1955). Method of processing and characteristics of products are reported from operation at some Russian plants. In pressing with a "FP prepress" oil in cake is reduced to 12% on 1–4 grade seed and 15% for 5- and 6-grade seed. The gossypol content was 0.05–0.11% in the cake and 0.11–0.17% in the prepress oil. The cakes were extracted with benzene to yield miscella of 6.22–9.35% concentration. Miscella at one plant was concentrated in 2-stage equipment: in the first stage miscella passed through 4 compartments of increasing temperature of 57–92° where it was concentrated from 9.35 to 81.42% and final concentration was done in a second single step stage at 115°. Another factory uses a 3-stage distillation, the first 2 stages being each 4-compartment units as the first stage of the above. (*C. A.* 50, 7481)

Mineral constituents of peanut oil. K. S. Srinivasa Varadan. *Indian Pharmacist.* 10, 263–4, 271(1955). The minerals found in the ash in terms of their oxides were P_2O_5 55.82, Fe_2O_3 8.76, CaO 6.5, CuO 5.18, MgO 2.85, and SiO_2 1.10%. The other minerals present were thought to be Na_2O and K_2O, but no data are given. Chlorides were found but only traces of sulfates were detected. (*C. A.* 50, 7481)

Synthesis of some some higher fatty acids. E. D. Bergmann and M. Ish-Shalom(Ministry Defense, Tel Aviv). *Bull. Research Council Israel* 5A, 65–6(1955). Pentadecanoic, nonadecanoic, and decosanoic acids were prepared by the method of Fieser and Szmuszkowicz. (*C. A.* 50, 8447)

Chromatographic behavior of fatty acids containing an oxygenated function supplemented in their chains. P. Desnuelle and M. Burnet(Fac. sci., Marseilles). *Bull. soc. chim. France* 1956, 268–74. The best technique for the separation of a group of oxygenated fatty acids is that of partition chromatography in inverse phases with powdered rubber as the support and aqueous solution of acetone as the eluant. 12-Hydroxystearic acid, ricinoleic acid, 12-oxostearic acid, *trans*-9,10-dihydroxystearic acid, and lauric, myristic, and palmitic acids were prepared and tested. (*C. A.* 50, 8398)

Electrical characteristics of animal fats. A. A. Lapshin(Leningrad Technol. Inst. Refrig. Ind.). *Myasnaya Ind. S.S.S.R.* 27(1), 13–5(1956). The dielectric constants and conductivities of animal fat(lard) in relation to temperature and moisture content are graphically presented. The dielectric constants plotted with respect to temperature show a maximum within the crystallization zone; the other relationships are linear. (*C. A.* 50, 9039)

Determination of glyceride structure of fats. M. K. Yakubov (Polytech. Inst., Kharkov). *Masloboino-Zhirovaya Prom.* 21 (1), 14–7(1956). A few changes are recommended for Kartha's and Hilditch's methods of acetone-potassium permanganate oxidation in determination of saturated, mono-, di-, and triunsaturated glycerides. (*C. A.* 50, 9038)

The catalytic air oxidation of vegetable oils at elevated temperatures. J. M. Martínez Moreno and J. M. Huesa López (Inst. grasa y sus derivados, Seville, Spain). *Compt. rend. 27° congr. intern. chim. ind. Brussels 1954*, 3; *Industrie chim. belge* 20, Spec. No., 717–20(1955). Cottonseed oil, olive oil (both crude and refined), and mixtures of oils were air oxidized at 120° with manganese dioxide catalyst. The results indicate that hydroxyl groups are introduced in the initial stages of the reaction followed by the hydroxylation of double bonds and polymerization. (*C. A.* 50, 9038)

Operating of the expeller press "EP." M. Chernov. *Myasnaya Ind. S.S.S.R.* 27(1), 19–20(1956). This expeller press has a shaft containing four stages of different diameters. The diameter is large at the inlet, smallest in the next stage, followed by 2 stages of increasing diameters. Its operation for the pressing of cracklings is described. (*C. A.* 50, 9039)

Cold degreasing of bones. V. Petrovskii. *Myasnaya Ind. S.S.S.R.* 27(1) 9–12(1956). Data for operation of an impulse degreaser are presented. (*C. A.* 50, 9039)

Determination of glyceride composition in vegetable fats. M. Filajdić(Univ. Zagreb). *Kemija u Industriji(Zagreb)* 4, 235–48(1955). Methods of determining the glyceride composition of vegetable fats are reviewed. (*C. A.* 50, 6813)

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