Acknowledgment

The authors gratefully acknowledge the cooperation of Buckeye Cotton Oil Company, Jackson, Miss., and Memphis, Tenn.; Dothan Oil Mill Company, Dothan, Ala.; Kershaw Oil Mill, Kershaw, S. C.; Lever Bros. Company, Edgewater, N. J., Hammond, Ind., and Los Angeles, Calif.; Lookout Oil and Refining Company, a division of Armour and Company, Chattanooga, Tenn.; Mrs. Tucker's Products, Sherman, Tex.; Opelousas Oil Refinery, Opelousas, La.; Procter and Gamble Manufacturing Company, Macon, Ga., and Portsmouth, Va.; Ranchers Cotton Oil, Fresno, Calif.; Southern Cotton Oil Company, Gretna, La.; South Texas Cotton Oil Company, Houston, Tex.; and Wilson and Company Inc., Oklahoma City, Okla., in supplying acidulated soapstocks of known processing histories.

The authors are also indebted to T. H. Hopper for direction and guidance, to R. M. H. Kullman, Julian F. Jurgens, James A. Harris, and Voyce P. Whitley for many of the chemical analyses reported in this paper, and to Elsie F. DuPré and Dorothy C. Heinzelman for spectrophotometric measurements.

REFERENCES

1. American Oil Chemists' Society, "Official and Tentative Methods," 2nd ed., rev. to 1955, Chicago, 1946–1955.
2. Bailey, A. E., "Cottonseed and Cottonseed Products," Interscience, New York, 1948, p. 380.
3. Bailey, A. E., ed., "Industrial Oil and Fat Products," 2nd ed., Interscience, New York, 1951, pp. 646–649.
4. Dechary, J. M., Kupperman, R. P., Thurber, F. H., and O'Connor, R. T., J. Am. Oil Chemists' Soc., *31*, 420–424 (1954).
4a. Earle, F. R., and Milner, R. T., Oil and Soap, *17*, 106–108 (1940).
5. Eaves, P. H., Spadaro, J. J., D'Aquin, E. L., Crovetto, A. J., Cirino, V. O., and Stansbury, M. F., J. Am. Oil Chemists' Soc., *33*, 639–645 (1956).
6. Jancik, V., Pokorny, J., and Mares, E., Prumysl Potravin, *7*, 213–215 (1956); C. A. *50*, 10430.
7. Kaufmann, H. P., Fette u. Seifen, *48*, 53–59 (1941).
8. Keith, F. W. Jr., Blachly, F. E., and Sadler, F. S., J. Am. Oil Chemists' Soc., *31*, 298–302 (1954).
9. Keith, F. W. Jr., Bell, V. G., and Smith, F. H., J. Am. Oil Chemists' Soc., *32*, 517–519 (1955).
10. Lambou, M. G., and Dollear, F. G., Oil and Soap, *22*, 226–232 (1945).
11. Linteris, L., and Handschumaker, E., J. Am. Oil Chemists' Soc., *27*, 260–264 (1950).
12. National Cottonseed Products Association, "Trading Rules, 1956–57," Memphis, Tenn., 228 pp.
13. Pack, F. C., and Goldblatt, L. A., J. Am. Oil Chemists' Soc., *32*, 551–553 (1955).
14. Pons, W. A. Jr., Murray, M. D., LeBlanc, M. F. H. Jr., and Castillon, Leah E., J. Am. Oil Chemists' Soc., *30*, 128–132 (1953).
15. Pons, W. A. Jr., Thurber, F. H., and Hoffpauir, C. L., J. Am. Oil Chemists' Soc., *32*, 98–103 (1955).
16. Pons, W. A. Jr., Mitcham, D., O'Connor, R. T., and Stansbury, M. F., J. Am. Oil Chemists' Soc., *33*, 324–330 (1956).
17. Pons, W. A. Jr., Stansbury, M. F., and Hoffpauir, C. L., J. Assoc. Offic. Agr. Chemists, *36*, 492–504 (1953).
18. Royce, H. D., and Lindsey, F. A. Jr., Ind. and Eng. Chem., *25*, 1047–1050 (1933).
19. Stansbury, M. F., and Hoffpauir, C. L., J. Am. Oil Chemists' Soc., *29*, 53–55 (1952).
20. Wurster, O. H., Govan, W. J. Jr., and Stockmann, G. J., in "Cottonseed and Cottonseed Products," A. E. Bailey, ed., Interscience, New York, 1948, pp. 814–816.

[Received April 29, 1957]

Determination of Tocopherol in Oxidized Fats[1]

E. N. FRANKEL, C. D. EVANS, and J. C. COWAN, Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois

SEVERAL MODIFICATIONS of the Emmerie-Engel method (2) for the determination of tocopherol in fats have been developed to remove substances which interfere with the ferrous-bipyridine color reaction (1, 2, 7, 8). Fats were reported to interfere with this color reaction (4, 6). Kaunitz and Beaver (6) introduced a proportionality factor to correct for the color-depressing effect observed in the presence of increasing concentrations of fat. However Gupta and Basu (3) found that this correction factor did not apply to oxidized groundnut oil. They showed that this oil when free of peroxides did not inhibit the color development in the Emmerie-Engel procedure. Therefore this interference of fats with the color reaction may be caused by peroxides in the fats. Lips (7) used the sulfuric-acid treatment of Parker and McFarlane (9) and saponification at room temperature to remove interfering substances in autoxidized methyl esters of fatty acids.

This paper describes a simple method for tocopherol determination in oxidized fats where peroxides are removed by thermal destruction since the presence of peroxides gives erroneously low values. The method is currently being used in a study of the fate of tocopherols in oxidizing fats.

Experimental

The method of Stern and Baxter (11) was used for tocopherol, except 10 min. were allowed for color development instead of 2.5 min. Preliminary observations showed that soybean oil immediately after deodorization at 210°C. did not interfere with the Emmerie-Engel color reaction for tocopherol. When a series of determinations was carried out with different concentrations of freshly deodorized oil (20 to 140 mg. per 10-ml. solution), the color developed was proportional to the sample weight. However, when allowed to oxidize, the oils interfered with the determination of tocopherol (Figure 1). These results are in agreement wih those of Gupta and Basu (3) in showing the interference of fat peroxides with the Emmerie-Engel color reaction for tocopherol.

A study was made of the effect of deodorization on the tocopherol and peroxide contents of soybean oil and lard. The fats were heated at 210°C. under reduced pressure (less than 1 mm. Hg.) in 50-ml., round-bottom flasks immersed in a thermostatically

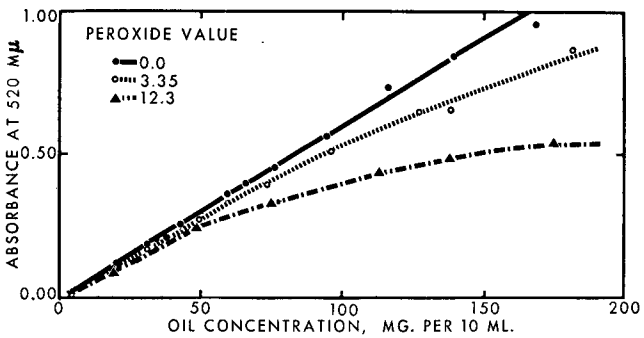

FIG. 1. Effect of peroxides in soybean oil on the ferrous-bipyridine color reaction for tocopherol.

[1] Presented at annual meeting of American Oil Chemists' Society, New Orleans, La., April 28–May 1, 1957.

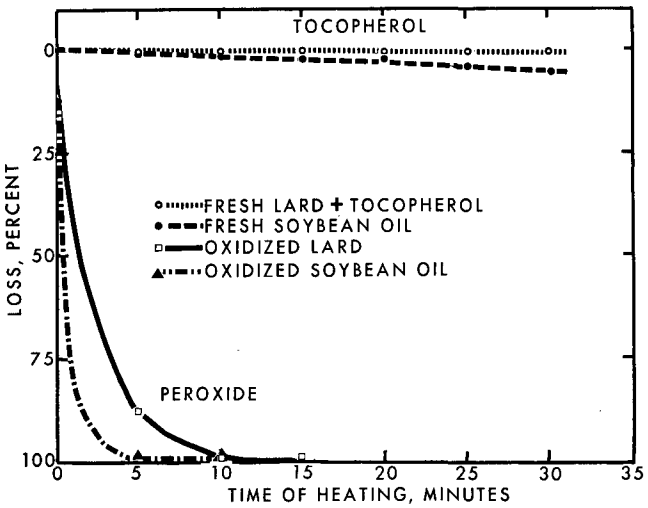

FIG. 2. Effect of heating at 210°C. *in vacuo* on the tocopherol content of fresh fats (initial tocopherol concentration: 1,500 microgram per gram) and the peroxide value of oxidized fats (initial peroxide value: 22).

controlled oil-bath; agitation was provided by a fine stream of nitrogen. After heating, the oils were immediately cooled to room temperature, and the vacuum was broken with nitrogen. The results represented in Figure 2 show that heating soybean oil and lard at 210°C. for 10 and 15 min., respectively, is sufficient to remove all peroxides from the oxidized fats. This heat treatment caused a loss of 1–2% of the tocopherol in fresh fats. It is interesting to note that the loss of both tocopherol and peroxides by heat is more rapid in soybean oil than in lard. The more rapid destruction of peroxides in oxidized soybean oil would be in agreement with the finding of Privett and Quackenbush (10) that tocopherol caused an increase in the rate of thermal destruction of fat-peroxides.

To determine the recovery of tocopherol in oxidized fats, fresh lard containing a known concentration of added α-tocopherol was mixed in different proportions with oxidized lard (peroxide value, 23.8) containing no added tocopherol. The tocopherol was determined in these mixtures after heating at 210°C. for 10 min. *in vacuo*. The linear relation obtained

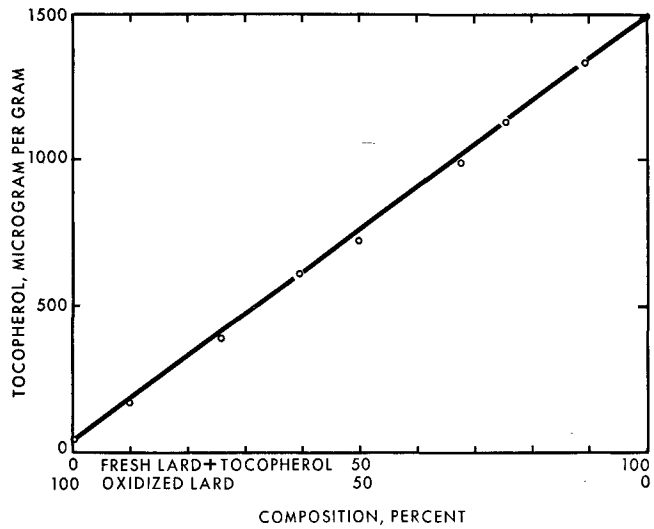

FIG. 3. Tocopherol content of mixtures of fresh lard + 1,500 microgram per gram α-tocopherol with oxidized lard (peroxide value, 23.8) after heating at 210°C. for 10 min. *in vacuo*.

between tocopherol and the extent of dilution (Figure 3) demonstrates that the heat treatment does not affect the tocopherol content in oxidized fat.

In another experiment the heat treatment (210°C. for 10 min.) was applied to a series of soybean oil samples containing different concentrations of *d*-α-tocopherol. These samples were prepared by first removing the natural tocopherols from the oil by shaking with carbon black in pentane and then addment with carbon black was carried out as follows. A solution of 500 g. of oil in 1 liter of redistilled pening different amounts of *d*-α-tocopherol. The treattane was shaken mechanically with 100 g. each of carbon black (Darco G-60[2]) and 100 g. of Celite in a 3-l. bottle for 30 min. The oil suspension was filtered through a thin layer of Celite, and the adsorbent was washed thoroughly with pentane. The volume of the oil solution was reduced to 1.5 liters with a rotating evaporator. This treatment with carbon black-Celite was repeated three or four times to remove the tocopherols completely from the oil. The results (Table I) show that no loss of tocopherol resulted from this heat treatment.

The heat-treatment procedure was compared with other methods used to remove interfering peroxides (and/or substances) prior to the determination of tocopherol in oxidized fats. These methods include treatment with 80% sulfuric acid and a hot and a cold saponification. The sulfuric acid treatment and the cold saponification were carried out by the method of Lips (7). The hot saponification was carried out as follows. Ten grams of oil were saponified with 20 ml. of 3.5 *N* alcoholic KOH in the presence of 5 ml. of 5% alcoholic pyrogallol by refluxing for 10 min. After saponification 40 ml. of distilled water were added to the saponification mixture, and the solution was immediately cooled to room temperature. The unsaponifiable matter was extracted in the dark with peroxide-free ethyl ether according to the procedure described by Zscheile *et al.* (13). The results given in Table II show that a greater recovery of tocopherol is obtained in the fats with the heat treatment than with the acid or saponification treatments. The cold saponification resulted in considerable loss of tocopherol in soybean oil while the hot saponification method yielded a recovery equivalent to that obtained with the sulfuric-acid treatment. The interference of peroxides with the Emmerie-Engel color reaction is evident from the low values obtained with the control oxidized-fats.

The possibility that tocopherol may be regenerated in the oxidized oils by the heat treatment at 210°C. *in vacuo* was investigated. Two lots of fresh soybean oil were oxidized at 60°C. with a stream of oxygen for four days to a peroxide value of 60 and 72, re-

[2] Since the Department of Agriculture does not recommend the products of one company over those of another, these names are furnished for information only.

TABLE I
Effect of Heat Treatment on Tocopherol Contents of Soybean Oil

Oil samples[a]	Tocopherol	
	Before heat treatment[b]	After heat treatment[b]
	μg./g.	μg./g.
1	662	661
2	1,052	1,050
3	1,220	1,250
4	1,330	1,310
5	1,770	1,710

[a] Treated with carbon black + various concentrations of *d*-α-tocopherol.
[b] 210°C., *in vacuo*, 10 min.

TABLE II

Effect of Different Peroxide-Removal Treatments on Tocopherol Contents of Fats

Fats	Tocopherol				
	Control, not treated	Heat treatment[a]	Sulfuric acid	Saponification	
				Cold	Hot
	μg./g.	μg./g.	μg./g.	μg./g.	μg./g.
Fresh soybean oil A	1,530	1,460	1,260	841	1,264
Oxidized soybean oil A	414	1,210	1,140	633	1,115
Fresh soybean oil B	1,615	1,592	1,352	719	1,375
Oxidized soybean oil B	887	1,294	1,164	—	—
Fresh lard plus *d*-α-tocopherol	500	483	442	—	—
Fresh lard plus *d*-α-tocopherol	1,540	1,513	1,497	1,302	1,324
Oxidized lard plus *d*-α-tocopherol	308	379	373	—	—

[a] 210°C., *in vacuo*, 10–15 min.

spectively. The tocopherol content of the fresh oil was compared with that of the oxidized oils before and after heating at 210°C. for 10 min. under vacuum. Tocopherol was determined in the oils directly and in their unsaponifiable matter, which was obtained by the hot saponification method. The data (Table III) show that tocopherol is not regenerated in the oxidized oils by the heat treatment since this treatment had essentially no effect on the tocopherol concentration in their unsaponifiable matter. It is interesting to note the relatively small loss of tocopherol incurred in the oxidized oils which have been taken far beyond the induction period. The loss of tocopherol observed in different oxidized soybean oils at the end of the induction period was less than 10%.

Peroxides were also effectively removed from oxidized oils by adsorption chromatography on silicic acid. The procedure used was to pass 100 ml. of a 10% solution of soybean oil in benzene through a column of silicic acid (1.9 x 10 cm., 100 mesh) under nitrogen pressure to obtain a rate of approximately 3 ml. per minute. The column was then washed with 100 ml. of benzene. This procedure did not affect the tocopherol content of fresh soybean oil, and the tocopherol values in oxidized oils corresponded to those obtained with the heat treatment. In a representative experiment the concentration of tocopherol of an oxidized soybean oil sample (peroxide value = 22.1) was 1,412 micrograms per gram after passage through a silicic-acid column and 1,426 micrograms per gram after heating *in vacuo* at 210°C. for 10 min. The agreement between the two methods is additional evidence that tocopherol is not regenerated in oxidized oils by the heat treatment.

TABLE III

Effect of Heat Treatment on Tocopherol Contents of Fresh and Oxidized Soybean Oil and Their Unsaponifiable Matter

Oil samples	Tocopherol in the	
	Oil	Unsaponifiable matter
	μg./g.	μg./g.
Fresh oil A, heated[a]	1,530	1,264
Oxidized oil A,[b] heated[a]	1,320	1,043
Oxidized oil A,[b] not heated	314	1,115
Fresh oil B, heated[a]	1,583	1,375
Oxidized oil B,[c] heated[a]	1,495	1,239
Oxidized oil B,[c] not heated	465	1,273

[a] 210°C., *in vacuo*, 10 min.
[b] Peroxide value = 60.
[c] Peroxide value = 72.

Ultraviolet spectrophotometric measurements were made on soybean oil and its unsaponifiable matter in petroleum ether and ethyl ether solutions, respectively. With the unsaponifiable matter prepared by the hot saponification method, the absorption at 298 mμ gave values for tocopherol concentration of the same order of magnitude as the values obtained by the colorimetric method. With the oils, other substances interfered with the absorption of tocopherols. The unsaponifiable matter prepared by the cold saponification method of Lips (7) showed absorption at 258 mμ but not at 298 mμ. Since the absorption at 258 mμ would be due to tocopheryl-*p*-quinone (5), it would appear that the cold saponification method of Lips causes oxidation of tocopherol in soybean oil, thus explaining the low tocopherol values obtained by this method. The unsaponifiable matter obtained by hot saponification from oxidized soybean oil (peroxide value of 72) showed no absorption at 258 mμ before or after the heat treatment (210°C., *in vacuo*, 10 min.). Therefore tocopheryl-*p*-quinone does not appear to form in soybean oil at this level of oxidation. This observation is consistent with the relatively small decrease in tocopherol observed in the oxidized soybean oils.

Absorption measurements in the visible range showed an increase in general absorption between 400 and 500 mμ in oxidized soybean oil (peroxide value of 72), which may be attributed to browning. No absorption maximum was evident at 470 mμ which is characteristic of the red chroman-5,6-quinone. Therefore, unlike cottonseed oil, chroman-5,6-quinone does not appear to form in autoxidized soybean oil (12).

Summary

Heating oxidized fats to 210°C. under reduced pressure for 10 to 15 min. removed peroxides without affecting the tocopherol content. This simple heating method yielded a higher recovery of tocopherol in oxidized fats than other modifications of the Emmerie-Engel method used to remove interfering substances. The data indicate that tocopherol is not regenerated in the oxidized oils by the heat treatment. The loss of tocopherol in soybean oil which had been oxidized beyond the induction period was relatively small.

Acknowledgment

The authors wish to acknowledge the help of E. H. Melvin and Jean Mallan for the spectrophotometric analyses and their interpretation.

REFERENCES

1. Baxter, J. G., Biol. Symposia, *12*, 484–507 (1947).
2. Emmerie, A., and Engel, C., Rec. Trav. Chim., *57*, 1351–1355 (1938); *58*, 283–289 (1939).
3. Gupta, M. L. S., and Basu, U. P., J. Indian Chem. Soc., Ind. and News Ed., *17*, 171–176 (1954).
4. Hove, E. L., and Hove, Z., J. Biol. Chem., *156*, 601–610 (1944).
5. Karrer, P., and Geiger, A., Helv. Chim. Acta, *23*, 455–459 (1940).
6. Kaunitz, M., and Beaver, J. J., J. Biol. Chem., *156*, 653–659 (1944).
7. Lips, H. J., J. Am. Oil Chemists' Soc., *33*, 426–428 (1956).
8. Mattill, H. A., "The Vitamins," vol. 3, pp. 506–509, New York, Academic Press Inc., 1954.
9. Parker, W. E., and McFarlane, W. D., Can. J. Research, *B18*, 405–409 (1940).
10. Privett, O. S., and Quackenbush, F. W., J. Am. Oil Chemists' Soc. *31*, 281–283 (1954).
11. Stern, M. H., and Baxter, J. G., Analyt. Chem., *19*, 902–905 (1947).
12. Swift, C. E., Mann, G. E., and Fisher, G. S., Oil and Soap, *21*, 317–320 (1944).
13. Zscheile, F. P., Nash, M. A., Henry, R. L., and Green, L. F., Ind. Eng. Chem., Anal. Ed., *16*, 83–85 (1944).

[Received May 13, 1957]