Antioxygenic Properties of Molecularly Distilled Fractions of Peanut Oil[1]

A. E. BAILEY, G. D. OLIVER, W. S. SINGLETON, and G. S. FISHER
Southern Regional Research Laboratory, New Orleans, Louisiana
Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration,
U. S. Department of Agriculture

It has been demonstrated by various investigators (3, 7, 12) that molecular distillation of a vegetable oil results in the recovery of the bulk of the antioxidants of the oil in the first distilled fractions.

The isolation of the tocopherols (1, 2) and the subsequent identification of these substances (9, 10) with the "inhibitols" or antioxidant concentrates of Mattill and his coworkers have provided some insight into the nature of the distillable antioxidants. Tocopherols have been found to be present in vegetable oils from a wide variety of sources. While it is recognized that there are other materials possessing antioxygenic properties in fats and oils, there can be little doubt that the stability of vegetable oils in general is in a large measure due to their presence. Since the molecular weights of the α-, β-, and γ-forms of tocopherol are 404, 390, and 390, respectively, whereas those of most glycerides are above 800, the tocopherols may be expected to be considerably more volatile than their parent oils. Actually, molecular distillation is now being used on a commercial scale for the production of tocopherol (vitamin E) concentrates (6). In the laboratory, Quackenbush, et al (11) have reported an almost quantitative recovery of synthetic α-tocopherol added to a vegetable oil base.

A circumstance which has been puzzling in connection with vegetable oil antioxidants is the failure of concentrates of these materials to stabilize the oils from which they are derived. In explanation of this peculiarity, Olcott (8) postulated the existence in the oil of an unknown substance capable of interfering with the action of the antioxidants. Swift, Rose and Jamieson (13) investigated the effect of different concentrations of pure α-tocopherol in various substrates, and found the effectiveness of this antioxidant to diminish as its concentration was increased. They implied that an explanation of the behavior of the concentrates in natural oils was to be found in the decreased efficiency of the tocopherols at the higher levels of concentration. A similar view was adopted by Golumbic (5), who found that the stability of lard actually decreased with the addition of synthetic α-tocopherol above an optimum level.

Molecular distillation offers a particularly effective means of investigating the effect of antioxidants at varying levels of concentration, since both antioxidant concentrates and antioxidant free substrates may be prepared from the same oil. The concentrates and substrates may be recombined in any desired proportions, and the various recombined mixtures may be compared with each other, as well as with the original oil. The present investigation had the following objectives: (a) To determine the extent to which the antioxidants of peanut oil can be separated by molecular distillation; (b) to determine the stability of the oil at varying levels of antioxidant concentration, and (c) to obtain preliminary information as to the identity of the antioxidants. The oils investigated included both refined peanut oil, and the same oil which had been hydrogenated to the degree to which hydrogenation is commonly carried in the manufacture of commercial edible fat products.

Preparation of the Samples

The oils employed in the investigation were derived from a lot of crude peanut oil obtained by solvent extraction of a mixture of Virginia and Runner varieties in pilot plant equipment. The oil was alkali refined and bleached with Fuller's earth in the conventional manner. It was then divided into two portions, one of which was hydrogenated to an iodine value of approximately 68. Both the hydrogenated and unhydrogenated portions were subjected to steam deodorization at 450° F. before being charged to the molecular still. The characteristics of the two lots of oil corresponded closely, therefore, to those of commercially prepared cooking oil, and all-hydrogenated shortening or margarine oil, respectively.

Approximately 2600 gms. of each oil were molecularly distilled in an all-glass cyclic still of the falling film type. Fractions were first removed at each of the following successively increasing temperatures: 100°, 120°, 140°, 160°, 180°, 200°, and 215° C. At each of these temperatures distillation was continued for approximately 7 hours; the size of the fractions was, of course, variable. The total amount of material removed by distillation within this range of temperature comprised less than 4 percent of the starting material in the case of both oils.

Following the removal of the preceding fractions, the temperature was raised to 225° C. and distillation was continued until the total amount of distillate was approximately 10 percent of the original oil (leaving 90 percent undistilled). The temperature of distillation was then increased to 240°, at which temperature all of the remaining oil except a residual 10 percent was distilled. The 80 percent of the oil which was distilled at this temperature was taken off in successive fractions amounting respectively to approximately 10, 10, 20, 20, and 20 percent of the original oil. Altogether, this procedure resulted in a separation of the original oil into 13 distilled fractions and one residual fraction, ranging in size from less than 1 gm. to approximately 520 gms.

The data pertaining to the separation of the fractions, together with certain analytical data, are recorded in Tables 1 and 2.

Examination of the Samples

The various fractions from the two oils, as well as the original oils, were analyzed for their content of tocopherols by the colorimetric method of Furter and

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TABLE 1

Molecular Distillation of Refined, Bleached and Deodorized Peanut Oil, and Analysis of the Distilled Fractions

Fraction number	Temp. of distillation, °C.	Weight of fraction gms.	Portion of total charge in fraction, percent	Portion of total charge, cumulative percent	Chromogenic substances in fraction, percent, (calculated as α-tocopherol)	Chromogenic substances in fraction, gms. (calculated as α-tocopherol)	Iodine value	Thiocyanogen value
Original oil		.....	.....	.....	0.051	1.34	92.8	72.8
1	100	1.20	0.046	0.046	.....	.....	.....	.....
2	120	.49	.019	.065	2.35	.012	.....	.....
3	140	.67	.025	.090	3.81	.026	.....	.....
4	160	2.45	.093	.183	5.99	.147	.....	.....
5	180	5.88	.223	.406	2.73	.161	.....	.....
6	200	32.95	1.25	1.66	.464	.153	.....	.....
7	215	70.22	2.67	4.32	.054	.038	93.5	71.3
8	225	153.2	5.82	10.14	.016	.024	92.0	70.9
9	240	261.3	9.92	20.06	.010	.026	92.5	71.4
10	240	258.4	9.81	29.87	.009	.023	93.0	71.9
11	240	522.3	19.84	49.71	.006	.031	93.8	72.9
12	240	520.2	19.76	69.47	.004	.021	94.8	74.2
13	240	525.3	19.95	89.42	.002	.011	94.5	73.9
Residue	.....	278.8	10.58	100.00	.184	.512	95.1	65.0

TABLE 2

Molecular Distillation of Refined, Bleached, Hydrogenated and Deodorized Peanut Oil, and Analysis of the Distilled Fractions

Fraction number	Temp. of distillation, °C.	Weight of fraction gms.	Portion of total charge in fraction, percent	Portion of total charge, cumulative percent	Chromogenic substances in fraction, percent, (calculated as α-tocopherol)	Chromogenic substances in fraction, gms. (calculated as α-tocopherol)	Iodine value	Thiocyanogen value
Original oil		.....	.....	.....	0.028	0.74	68.0	66.3
1	100	1.02	0.039	0.039	.185	.002	.....	.....
2	120	.55	.021	.060	1.75	.010	.....	.....
3	140	1.70	.065	.125	6.57	.111	.....	.....
4	160	2.55	.097	.222	7.40	.189	.....	.....
5	180	6.35	.242	.464	3.16	.201	.....	.....
6	200	24.85	.947	1.41	.310	.077	.....	.....
7	215	64.78	2.47	3.88	.048	.031	57.0	54.8
8	225	163.5	6.23	10.11	.014	.023	66.3	64.1
9	240	263.9	10.06	20.17	.006	.016	68.2	67.0
10	240	261.8	9.98	30.15	.004	.010	68.7	67.3
11	240	526.2	20.05	50.20	.002	.011	69.4	68.1
12	240	529.6	20.18	70.38	.000	.....	70.4	69.1
13	240	532.3	20.28	90.66	.000	.....	69.7	68.9
Residue	.....	245.1	9.34	100.00	.069	.169	61.6	60.5

Meyer (4) as modified by Quackenbush, Gottlieb and Steenbock (11).

Measurements of color intensity were made with a Coleman spectrophotometer, [model 10-S DM, 7.5 m(mu) slit width], at 490 m(mu). A calibration curve of color intensity versus tocopherol content was established from the examination of samples of lard containing known added amounts of pure synthetic α-tocopherol; all of the reported values for contents of tocopherol or other chromogenic substances were calculated upon the basis of this calibration.

The stabilities of all of the larger molecularly distilled fractions were determined by aeration in the Swift apparatus at approximately 99° C. It is to be noted that in the present experiments molecular distillation resulted in no important degree of fractionation of the glycerides according to their degree of unsaturation (cf. iodine and thiocyanogen values of Tables 1 and 2). Also tested for stability were two series of samples of varying antioxidant content. In making up these samples, an antioxidant concentrate was first prepared from each oil by combining the fractions distilling at 140°, 160° and 180° C. The concentrate from each oil was then added in varying amounts to a molecularly distilled fraction of the same oil which was practically devoid of antioxidants. This procedure produced a series of unhydrogenated oil samples assaying from 0.00046 to 0.2368 percent tocopherols by the Furter-Meyer method and a series of hydrogenated samples assaying from 0.00118 to 0.1512 percent tocopherols. The results of the various stability tests are shown graphically in Figures 1, 2, 3, and 4.

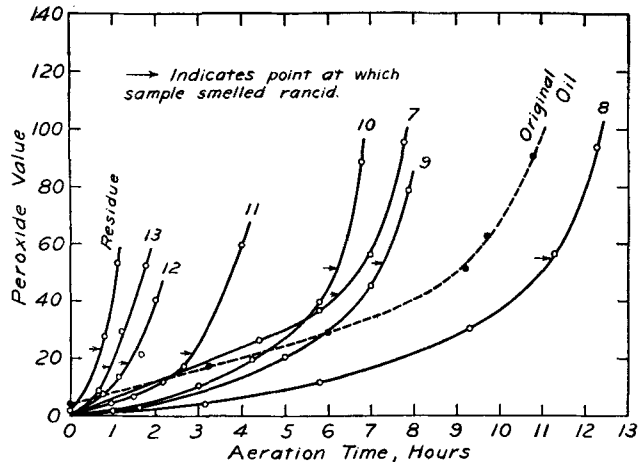

FIG. 1. Stability of the different molecularly distilled fractions from unhydrogenated peanut oil.

Stability tests were also conducted on both hydrogenated and unhydrogenated oils reconstituted from the distilled fractions and the undistilled residue, in the proper proportions (Figure 5). Also, in order to effect a comparison between the hydrogenated and unhydrogenated antioxidant concentrates, portions of the hydrogenated concentrates were added to the unhydrogenated substrate and portions of the unhydrogenated antioxidants were added to the hydrogenated substrate. Results of the stability tests on these mixtures are shown in Figures 6 and 7.

Finally, each of the original oils was tested for stability after the addition of a portion of its own antioxidant concentrates (Figure 8).

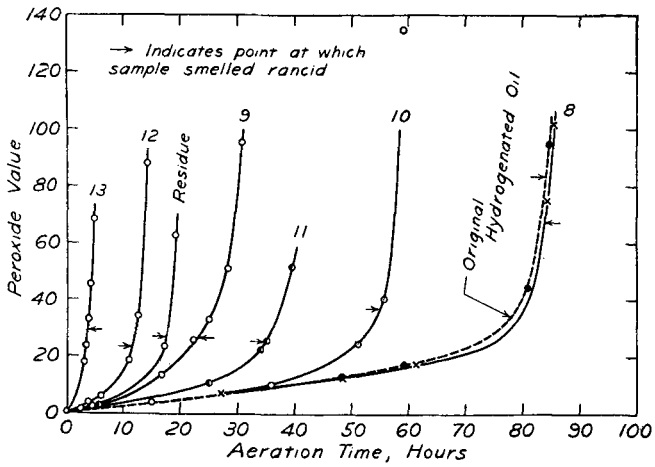

FIG. 2. Stability of the different molecularly distilled fractions from hydrogenated peanut oil.

Discussion of the Results

It is apparent that molecular distillation of peanut oils results in the removal of the greater part of their antioxygenic substances, within the temperature interval of 120° to 200° C. Of the total content of distillable substances responding to the Furter-Meyer test, 72 percent appeared in the 120 to 200° fractions in the case of the unhydrogenated oil and 85 percent in the case of the hydrogenated oil. When due allowance is made for the fact that there is some degree of

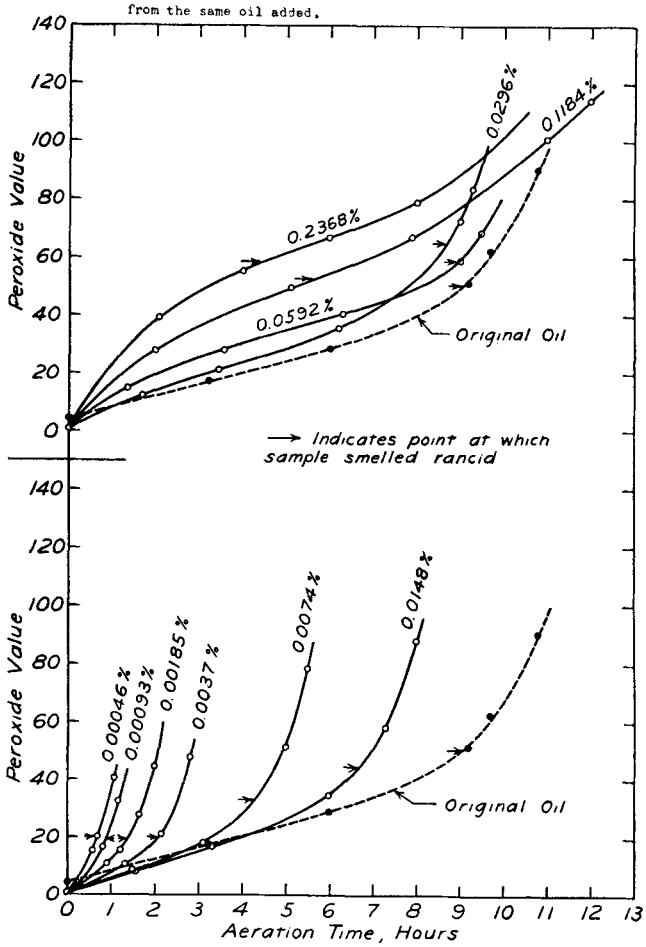

FIG. 3. Stability of a molecularly distilled unhydrogenated peanut oil substrate (Fraction No. 13), with different percentages of antioxidants (calculated as tocopherols) from the same oil added.

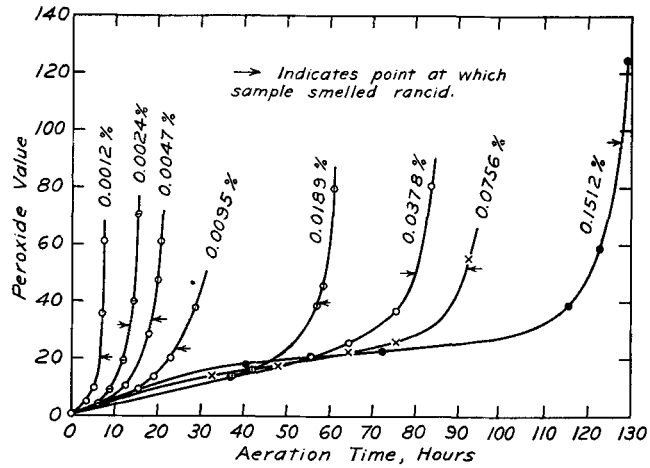

FIG. 4. Stability of a molecularly distilled hydrogenated peanut oil substrate (Fraction No. 13), with different percentages of antioxidants (calculated as tocopherols) from the same oil added.

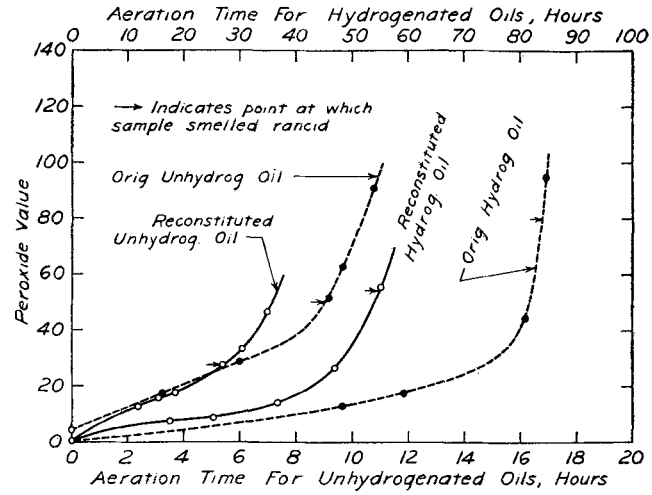

FIG. 5. Stability of oils reconstituted from molecularly distilled fractions, in comparison with the original oils.

hold-up of the various fractions on the condensing surface of the still, the temperature of maximum elimination of these substances may be said to correspond

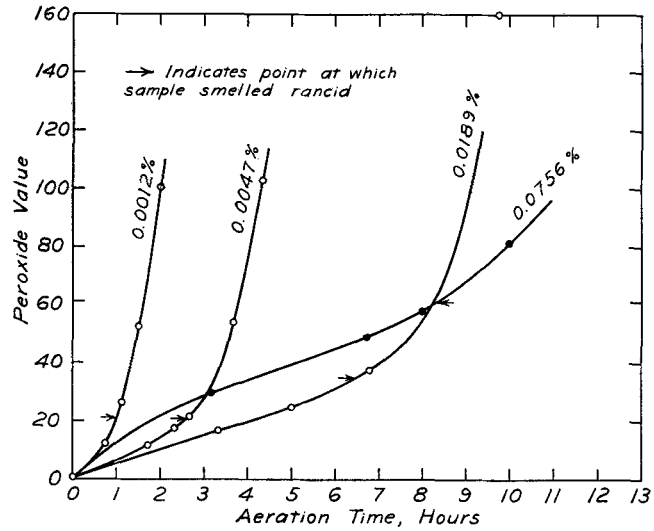

FIG. 6. Stability of a molecularly distilled unhydrogenated peanut oil substrate (Fraction No. 13), with different percentages of antioxidants (calculated as tocopherols) from hydrogenated peanut oil added.

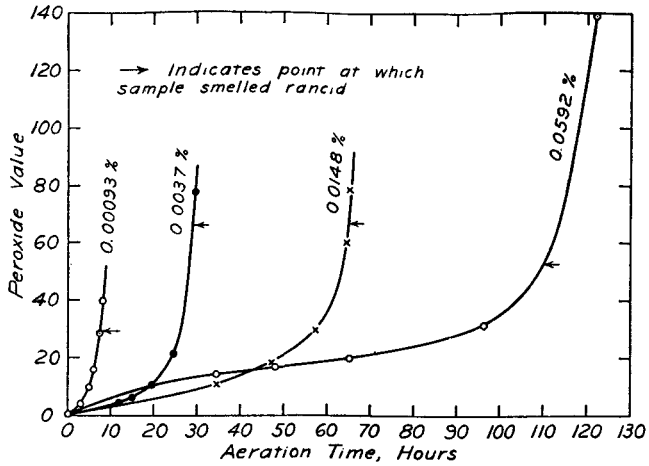

FIG. 7. Stability of molecularly distilled hydrogenated peanut oil substrate (Fraction No. 13), with different percentages of antioxidants (calculated as tocopherols) from unhydrogenated peanut oil added.

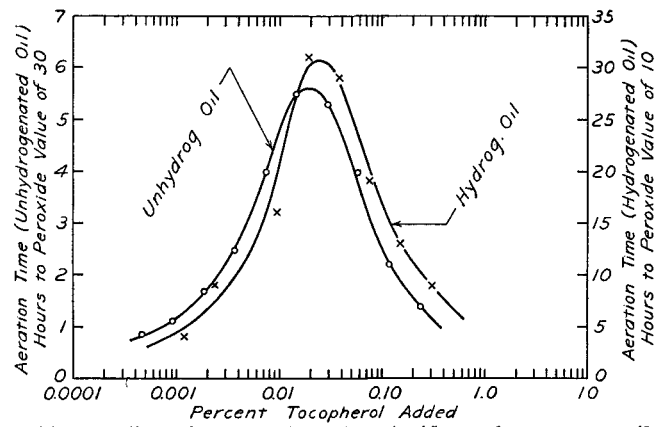

Fig. 8. Effect of the addition of antioxidants from peanut oil (calculated as tocopherols) on the initial rate of peroxide formation in unhydrogenated and hydrogenated peanut oil substrates.

fairly closely to published values for the pure tocopherols (11). Consequently, it appears reasonable to assume that the chromogenic substances present in the antioxidant concentrates consist chiefly of tocopherols.

However, it is also apparent that there is a large fraction of the chromogenic substances which is either devoid of antioxygenic properties or exhibits these properties only slightly. In the unhydrogenated oil, approximately 40 percent of the chromogens did not distill and were concentrated in the residue, which comprised 10 percent of the total oil. In the hydrogenated oil, approximately 20 percent of the chromogens similarly failed to distill. In the case of the unhydrogenated oil, the chromogen residue was virtually devoid of antioxygenic activity. The residue of this oil had a stability lower than that of any of the distilled fractions. Also, its stability test curve had the form characteristic of fats low in antioxidant content. The hydrogenated residue obviously contained antioxidants, as its stability was greater than that of the latter distilled fractions. Its stability, however, was by no means proportionate to its chromogen content.

It is further apparent that the oils contain difficultly distillable antioxidant substances which either do not give the Furter-Meyer reaction or else give this reaction but weakly in proportion to their antioxygenic activity. Fraction No. 8, from the unhydrogenated oil, for example, had a keeping time of 11.2 hours although its content of chromogenic substances, calculated as α-tocopherol, was but 0.016 percent. The results shown in Figure 3 indicate that an oil containing this amount of chromogens from the distilled concentrate should have a keeping time of not more than about 8 hours. As a matter of fact, it was found to be impossible to increase the keeping time of the substrate to 11 hours by the addition of any amount of concentrate. Similarly, fraction No. 8 from the hydrogenated oil had a keeping time of 84 hours, with a chromogen content of but 0.014 percent. Thus, its stability was equivalent to that of the original oil containing 0.028 percent of chromogen, and was slightly superior to that of the substrate to which 0.0378 percent of chromogens had been added.

Hydrogenation of the oil resulted in a reduction of its content of chromogenic substances from 0.051 to 0.028 percent. However, the chromogens which disappeared consisted largely of the nondistillable substances which are devoid of antioxygenic activity in the unhydrogenated oil. The yield of tocopherols at 140° to 200° was in fact higher from the hydrogenated than from the unhydrogenated oil, and the total yield of distillable chromogens was the same in each case, namely 0.68 gms. The stability tests of Figures 3, 4, 6, and 7 show the chromogens from the unhydrogenated and hydrogenated concentrates to be virtually identical in antioxidant properties. There is no difference in the characteristic form of the stability curves in the two cases.

In the case of both oils, molecular distillation either caused some destruction of antioxidants or otherwise injured the stability of the oils, as the oils reconstituted from the separated fractions were less stable than the original oils.

A number of pertinent facts are revealed by the tests in which the antioxidant concentrates were added in varying concentrations to distilled nearly antioxidant-free substrates from the two oils (Figures 3 and 4). It is presumed that the chromogens in these concentrates consisted chiefly of tocopherols, hence they are hereafter referred to as such.

The progressively increased addition of these concentrates had the peculiar effect of first diminishing and then increasing the initial rate of peroxide formation in the stability tests. The comparative times required to reach specific low peroxide values in the two oils, as a function of the percentage of tocopherols added to the substrates, are shown graphically in Figure 8. The tocopherol concentration at which the rate of initial peroxide formation is at a minimum is slightly higher for the hydrogenated than for the unhydrogenated oil.

In both the hydrogenated and unhydrogenated substrates, an increase in the concentration of added tocopherols also resulted in a progressive increase in the peroxide level at which organoleptic rancidity was developed. The development of organoleptic rancidity was reasonably sharp and definite in all cases except those of the unhydrogenated substrate containing 0.1184 and 0.2368 percent tocopherols, respectively. At these high concentrations of tocopherol, the first odor of rancidity was fugitive. Although it first appeared at a peroxide value of about 60, it was inclined to alternately appear and disappear until a peroxide value of about 120 was reached.

The addition of tocopherols above a certain level had no stabilizing effect on the unhydrogenated substrate, and in fact, actually resulted in a decrease in its stability. On the other hand, the hydrogenated substrate was increasingly stable with increased concentrations of tocopherol up to the maximum amount used (0.1512 percent). The stabilities of the two substrates, as functions of the amount of tocopherol added, are shown graphically in Figure 9.

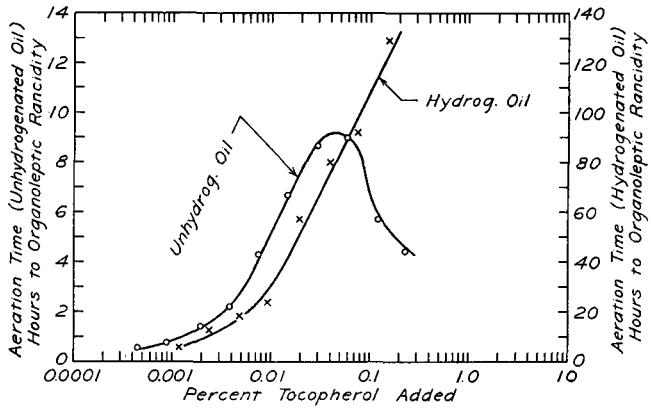

FIG. 9. Effect of the addition of antioxidants from peanut oil (calculated as tocopherols) on the stability of unhydrogenated and hydrogenated peanut oil substrates.

For some reason, which is at present unknown, increments of added tocopherol are increasingly effective up to a concentration of about 0.008 percent. Beyond this point there is a range of concentrations within which the stability of the oil varies linearly with the logarithm of the tocopherol concentration. In the case of the unhydrogenated oil, this linear relationship continues to a concentration of about 0.025 percent, after which the addition of tocopherol is decreasingly effective. A maximum stability is then reached at a concentration of about 0.05 percent, after which the further addition of tocopherol decreases the stability. In the case of the hydrogenated oil, the linear relationship between the stability and the logarithm of the tocopherol concentration persists to the highest level to which the latter was carried (0.1512 percent).

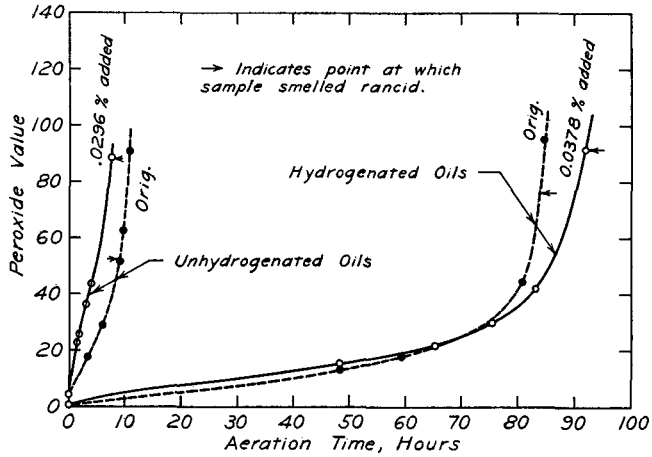

FIG. 10. Effect of the addition of antioxidants from peanut oil (calculated as tocopherols) on the stability of unhydrogenated and hydrogenated peanut oils.

The apparent fact that hydrogenated peanut oil can be improved in stability by the addition of tocopherol or other related natural antioxidants, whereas unhydrogenated oil cannot, was checked by adding an amount of the concentrates corresponding to approximately 0.03 percent tocopherol to each of the original oils (Figure 10). The stability of the unhydrogenated oil was decreased by this addition, whereas that of the hydrogenated oil was increased.

Summary

1. Samples of unhydrogenated and hydrogenated peanut oils, which had been refined, bleached, and deodorized, were separated into two comparable series of fractions by molecular distillation. The various fractions were analyzed for tocopherols (and related chromogens) by the method of Furter and Meyer, and stability tests by the Swift method were made on the larger distilled fractions.

2. Molecular distillation at 140°, 160°, and 180° C. yielded antioxidant concentrates (presumably of tocopherols) from each oil; distillation at 240° yielded fractions almost devoid of antioxygenic substances.

3. In the unhydrogenated and hydrogenated oils, approximately 40 percent and 20 percent, respectively, of the chromogenic substances reacting in the Furter-Meyer tests were undistillable and remained in the residue comprising 10 percent of the original oil.

4. Evidence was found of the presence of distillable antioxidants other than tocopherols, which either do not respond to the Furter-Meyer test or else respond to it weakly, in proportion to their antioxygenic activity.

5. Hydrogenation of the oil had no appreciable effect on the activity of its distillable antioxidants.

6. The progressively increased addition of tocopherol-rich concentrates to fractions almost devoid of antioxidants resulted in first decreasing and then increasing the initial rate of peroxide formation in the stability tests.

7. In the case of the unhydrogenated oil, there was an optimum level of antioxidant concentration above which the addition of these substances had no stabilizing action. However, hydrogenated oil showed an increase in stability with the addition of antioxidants up to the highest level to which the concentration of the latter was carried (approximately 0.15 percent, calculated as α-tocopherol).

Acknowledgment

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BIBLIOGRAPHY

1. Emerson, O. H., Emerson, G. A., Mohammad, Ali, and Evans, H. M., J. Bio. Chem. *122*, 99-107 (1937).

2. Evans, H. M., Emerson, O. H., and Emerson, G. A., J. Biol. Chem. *113*, 319-32 (1936).

3. Fawcett, E. W. M., J. Soc. Chem. Ind. *58*, 43-50T (1939).

4. Furter, M., and Meyer, R. E., Helv. Chim, Acta *22*, 240-50 (1939).

5. Golumbic, C., Oil and Soap *20*, 105-07 (1943).

6. Hickman, K., Ind. Eng. Chem. News Ed. *20*, 1561-62 (1942).

7. Hickman, K., and Baxter, J. G., Brit. Pat. 507,471 (1939).

8. Olcott, H. S., Oil and Soap *18*, 77-80 (1941).

9. Olcott, H. S., and Emerson, O. H., J. Am. Chem. Soc. *59*, 1008-09 (1937).

10. Olcott, H. S., and Mattill, H. A., J. Am. Chem. Soc. *58*, 1627-30 (1936).

11. Quackenbush, F. W., Gottlieb, H. L., and Steenbock, H., Ind. Eng. Chem. *33*, 1276-78 (1941).

12. Riemenschneider, R. W., Swift, C. E., and Sando, C. E., Oil and Soap, *17*, 145-48 (1940).

13. Swift, C. E., Rose, W. G., and Jamieson, G. S., Oil and Soap *19*, 176-80 (1942).