occurred on the branches injected by ammonium sulfate and calcium phosphate. This was associated with a total increase in oil and protein content of the fruit. The average weight of olives was increased from 3.35 g. to 3.95 g., the protein content from 8.68% to 13.12%. The increase in yield of olive oil per acre was 27 kilos with nitrogen, 7.5 kilos with phosphorus and 8 kilos with potassium. We think that the injection method could be very useful for experimental purposes in many kinds of fertilization experiments.

Summary

Experiments were conducted in the Research Experiment Station for Agricultural Technology, Athens, Greece, to study the effect of climatic conditions, state of maturity, infection by olive fly, and the effect of various fertilizers upon the quality of olive oil.

Olive oil produced in localities where the temperature is low during the maturity period has more unsaturated fatty acids than oil produced in localities with higher temperatures. With the advance of maturity the proportion of unsaturated fatty acids increased. Infection by olive fly had no appreciable effect upon the relative proportion of saturated fatty acids.

For studying of the effect of fertilizers the injection method was used. Nitrogen fertilizers injected into the branches gave considerable increase in the volume of fruit, its protein content, and the total amount of oil produced per acre.

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The Determination of Tocopherol Content During the Commercial Processing of Soybean Oil*

H. W. RAWLINGS, NOEL H. KUHRT, and J. G. BAXTER

THE increasing study that is being given to the part that tocopherols (vitamin E) play in nutrition emphasizes the need for more information concerning the amount of the tocopherols in primary sources such as vegetable oils. This paper describes a procedure useful for assaying the tocopherol content of soybean oil and presents some data on the effect of commercial processing operations on the tocopherol potency.

The recent discovery of δ-tocopherol in soybean oil has made the accepted methods unapplicable (11). A thorough background of this field is found in other publications (1, 2, 12). A reasonably simple method for soybean oil has been developed by us by modifying the method of Rawlings (10) with these recent findings.

Directions for Modified Emmerie-Engel Procedure for Soybean Oil

A 1-ml. aliquot of sample containing 50 to 300 μg. of tocopherol in redistilled (over KOH and $KMnO_4$) absolute ethanol is added to an opaque 2-oz. glass stoppered bottle. One ml. each of 0.1% $FeCl_3 \cdot 6H_2O$ and 0.25% α,α'-dipyridyl solutions in ethanol are added, in order, followed by 22 ml. of purified ethanol. The color density is read in an Evelyn colorimeter (1.9-cm. cell) at 10 minutes from addition of $FeCl_3$ reagent using a 520 mμ. filter. The apparent tocopherol concentration is determined from a curve prepared from pure α-tocopherol (or pure hydroquinone calculated to read in molecular equivalents of α-tocopherol) and multiplied by 0.91 to obtain the correct assay of mixed tocopherols. (For colorimeters or spectrophotometers using a 1-cm. cell, use a 2-ml. aliquot of sample and 1 ml. each of 0.2% $FeCl_3 \cdot 6H_2O$ and 0.5% α,α'-dipyridyl reagents.)

The important modification of the method is the use of the factor 0.91 to correct the result obtained for the enhanced color due to δ-tocopherol. It was previously found (12) that the tocopherols in the soybean oils examined were a mixture of α-, γ-, and δ-tocopherols and that the ratio was approximately 10:60:30:, respectively. A milligram of such a mixture is found to reduce approximately 10% more ferric chloride than a milligram of α-tocopherol; therefore the correction factor of 0.91 is used to obtain the tocopherol content of Soybean Oil.

Previous work (1, 2) suggests that there are materials in soybean oil which will interfere with the assay method. Parker and McFarlane (7) have published a procedure for removing the carotenoids and phenols with 85% sulfuric acid. Samples so treated have been assayed with and without the addition of a known amount of a concentrate of mixed tocopherols. Corrections were made for inhibition by the graphical method of Kaunitz and Beaver (5). Some results are shown in Table 1, comparing the corrected assays with the original assay, and it is evident that the correction necessary for substances giving spurious color with the Emmerie-Engel reagent is largely compensated for by the repression of color due to the Kaunitz-Beaver effect. As a result, the corrected assays for the potency of crude and refined oils lay within ± 10% of the values obtained by direct assay.

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TABLE 1

Comparison of Direct Assay of Soybean Oils With That by the Parker-McFarlane, and Kaunitz and Beaver Correction Methods

Sample	Direct Assay mg./g.	% Correction P.-McF. Method	% Correction K.-B. Method	Corrected Assay	% Overall Correction
Crude Soybean Oil					
1	1.70	−10.0	+ 9.0	1.67	−1.8
2	1.62	− 9.9	+14.0	1.67	+3.1
Refined Soybean Oil					
1	1.78	−11.2	+ 6.0	1.67	−6.2
2	1.67	−10.2	+ 3.0	1.55	−7.2
3	1.58	− 6.3	+ 4.5	1.55	−1.9
4	1.66	− 7.2	+ 9.0	1.68	+1.2

A second procedure, using the cyclic molecular still, was developed for testing the accuracy of the direct assay method on soybean oil. The procedure is closely related to the distillation method of Dam and coworkers (3) and by Quaife and Harris (9) for the elimination of interfering substances prior to performing the Emmerie-Engel assay. The distillation is performed in a cyclic molecular still of the type described by Hickman (5). The charge of oil is 200 to 250 grams, and by distillation at a temperature of 160 to 210°C. the tocopherols are concentrated from 10 to 20 times in a fraction amounting to about 5 to 10% of the original oil volume. The amount of tocopherol remaining in the residue (undistilled oil) is difficult to determine accurately since the potency is very low, but it is estimated to be 5 to 10% of the original. From an assay of the distillate, using the respective weights of it and the original oil, a value for the potency of the latter may be calculated. Confidence can be placed in this value since very little carotenoid or phenol bodies are present in the distillate, and the potency is sufficiently high (about 2%) so that the Kaunitz-Beaver effect is small. A typical distillation result is given in Table 2 where degummed soybean oil was assayed by this method.

TABLE 2

Test of Accuracy by Molecular Distillation

Sample	Direct Assay mg./g.	% Weight in Distillate	% Tocopherols Recovered in		
			Distillate	Residue	Total
Water-refined crude soybean oil	1.86	8.4	94.8	10.2	105

While the data presented above show that the tocopherol assay method for soybean oils may be in error by a few per cent, it is evident that it will give information that is useful for most purposes. Many samples of crude and processed soybean oils have been tested in our laboratories and the results are summarized in the following sections.

Oils from Different Varieties of Beans. Seed beans of seven different types were extracted and the tocopherol assays of the oils are shown in Table 3.

It is evident that there are no great differences in tocopherol content.

Method of Extracting Oil from Beans. The average potencies of a number of expeller and extracted soybean oils are shown in Table 4. The tocopherol contents are about the same.

The Effect of Soda Refining. The alkali refining of soybean oil by both centrifugal and batch process in the plant and by the A.O.C.S. method in the laboratory have been studied to compare the effect of this operation on the tocopherols. Since tocopherol is unstable in the presence of air and alkali, it is not surprising to learn that centrifugal refining gives the lowest loss. The yield data of a few typical oils are given in Table 5 to illustrate this. It seems that commercial centrifugal refining destroys at most only a few per cent of the tocopherol.

TABLE 3

Tocopherol Content of Soybean Oil from Different Varieties of Beans by Extraction with Petroleum Ether (Skelly F).
(Beans Dried 4 hr. at 130°C. before extraction)

Kind of Bean	% Fat Extraction	Mg./g. Tocopherols in Oil
Lincoln	15.3	2.36
Richland	15.9	2.03
Illini	15.3	2.25
Dunfield	14.6	2.03
Ogden	13.4	1.69
Armyedo	17.7	2.80
Delsta	16.7	2.17
Average	15.9	2.15

Effect of Bleaching. Laboratory bleaching of refined soybean oil using 6% of the A.O.C.S clay usually shows a drop of about 6% tocopherol, but less active clays give even smaller losses. Similar experiments on soybean oil distillates have also shown practically no loss on bleaching, and it can be safely predicted that commercial bleaching operations should show only small losses of tocopherol.

TABLE 4

Tocopherol Content of Crude Soybean Oils by Extraction, Expression, and After Degumming

Process	No. Samples	Average Tocopherol Content, mg./g.
1. Extraction	6	1.72
2. Expression	6	1.80
3. Extracted and degummed	13	1.76

Effect of Hydrogenation. Our experience confirms that repeatedly reported in the literature (6a), viz., that tocopherols are not destroyed by hydrogenation even at temperatures of 150-200°C. In fact, an analytical method (8) is based on the quantitative recovery of tocopherols in such a process. We conclude that commercial hydrogenation probably destroys little or none of the tocopherols in soybean oil.

Table 5

Tocopherol Losses During Soda Refining

Method of Refining	Potency		Loss
	Crude	Refined	
1. Centrifugal—Plant	1.87	1.85	1.1
	1.98	1.90	4.0
	1.93	1.89	2.1
	1.93	1.92	0.5
	2.11	1.99	5.7
Average	.....	.....	2.7
2. Batch Process—Plant	1.91	1.67	12.6
	1.88	1.68	10.6
	1.86	1.61	13.4
Average	.....	.....	12.2
3. Batch Process—Laboratory	1.89	1.48	21.7
	1.78	1.56	12.4
	1.95	1.61	17.4
	1.91	1.62	15.1
Average	.....	.....	16.7

Steam Deodorization. This process removes some of the tocopherols which can be recognized in the

deodorizer distillates from edible oil processing plants. Various deodorizers give different quantities of tocopherol in the distillates. In one controlled experiment on a mixture of refined and hydrogenated soybean and cottonseed oils, a 5% loss of tocopherols was observed. We, therefore, estimate that the loss during commercial deodorization is only a few per cent.

Summary. A method for the analysis of the total tocopherols in soybean oil has been presented, and judged by distillation and other procedures is estimated to be accurate to within 10%. A discussion is made of the tocopherol losses in various steps of soybean oil refining.

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Delta-Tocopherol as an Antioxidant in Lard[1,2]

JANICE GRIEWAHN and B. F. DAUBERT

THE antioxidative properties of α-, β-, and γ-tocopherols, isolated and named by Evans and co-workers (1), have been known for some time, having first been demonstrated by Olcott and Emerson (2) in 1937 and later verified by Hove and Hove (3). γ-Tocopherol was shown to be a more effective antioxidant in lard than β-tocopherol, which in turn was more effective than α-tocopherol. "The tocopherols function most effectively at lower levels of concentration and with decreasing efficiency at higher levels," as indicated by Swift, Rose, and Jamieson (4), and also by Golumbic (5). Stern, Robeson, Weisler, and Baxter (6) recently announced the isolation and properties of another tocopherol, which they named δ-tocopherol. They demonstrated that this newly-isolated tocopherol is more effective as an antioxidant for vitamin A acetate at 39° and 55°, and for β-carotene at 39°, in olive oil, than α-, β-, or γ-tocopherol. The purpose of this paper is to compare the relative antioxygenic effects of the four tocopherols in concentrations of 0.02 and 0.1% using lard as a substrate.

Experimental[3]

Oxidation was accelerated by means of a modified Swift Stability Test (7, 8). Compressed air, washed with a solution of potassium dichromate, was dried through a system of concentrated sulfuric acid, sodium hydroxide pellets, and anhydrous calcium chloride. Molten lard with added tocopherol was poured into a three-necked flask and maintained at 100°C. by means of a thermoregulated oil bath. The air was bubbled through the lard at a rate such that the lard was saturated with air. Samples were removed by means of a pipette at intervals in accordance with the rate of oxidation. Peroxides were determined by a modified Wheeler method (9).

[1] The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food & Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

[2] Contribution No. 653 from the Department of Chemistry, University of Pittsburgh.

[3] The δ-tocopherol was supplied through the courtesy of James G. Baxter of Distillation Products, Inc., Rochester, N. Y.

Results and Discussion

The tocopherol effects, at 0.02% concentration, on the oxidation of lard at 100°C. by the modified Swift Stability Test are shown in Fig. 1. The endpoint of

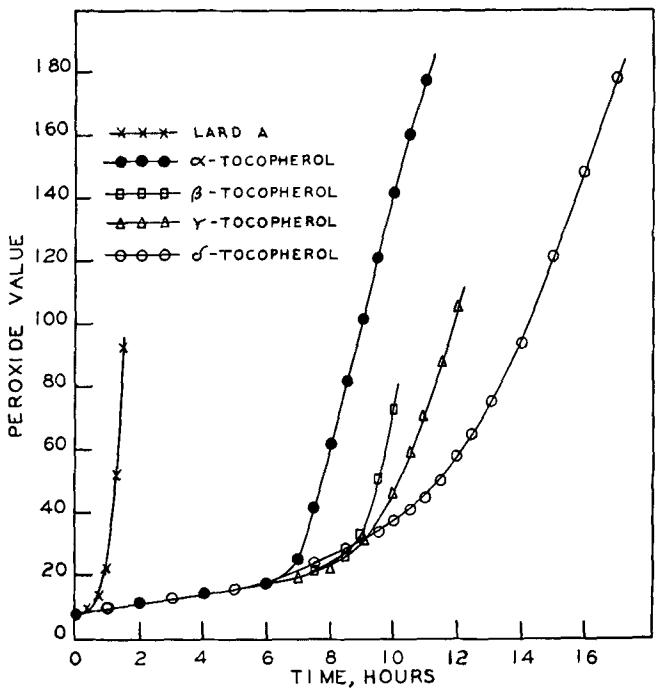

FIG. 1. The tocopherol effect on the oxidation of lard at 100°C. at a concentration of 0.02% in lard.

the induction period was chosen arbitrarily as a peroxide value equivalent to 40 milliequivalents per kilogram of sample. This value is somewhat higher than the value of 30 milliequivalents used by Riemenschneider, *et al.* (10). The induction periods in hours, therefore, for the tocopherols were approximately as follows: α-tocopherol, 7.5; β-tocopherol, 9; γ-tocopherol, 9.5; and δ-tocopherol, 11. The ratios of the relative activities of α-, β-, γ- and δ-tocopherols as