Determination of Tocopherols in Vegetable Oils

A.P. CARPENTER, JR., Procter and Gamble Co., Winton Hill Technical Center, 6071 Center Hill Road, Cincinnati, OH 45224

ABSTRACT

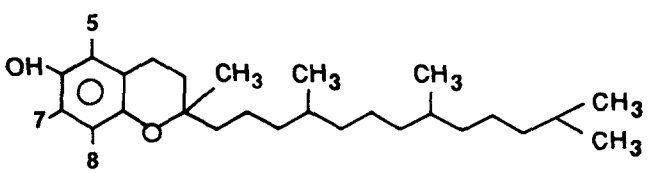
A rapid (ten minute) and selective method for measuring individual tocopherols found in vegetable oils has been developed using High Performance Liquid Chromatography (HPLC) with ultraviolet absorbance detection. The samples are analyzed directly following dissolution in the mobile phase. α- and γ- tocopherols are quantitated based upon their peak areas relative to standard calibration curves. The measurement of β- and δ-tocopherols in the samples is also based upon the calibration data for the α- and γ-tocopherol standards since the individual β- and δ- standards were unavailable. The data obtained are compared with the total tocopherol content as found by a standard colorimetric procedure. The results indicate the HPLC method to be more reliable in the measurement of samples with high α-tocopherol levels. Soybean, safflower, sunflower, cottonseed, corn, peanut and olive oils have been examined using this method.

INTRODUCTION

Studies on the oxidation of natural oils and fats indicate that the qualitative and quantitative determination of the materials which participate directly in the antioxidation process can provide reasonable predictions of stability (1). The greater stability of vegetable oils vs. animal fats under oxidative conditions is known to be due to the higher levels of natural antioxidants in the oils. An important and commonly occurring class of natural antioxidants in vegetable oils is the tocopherols (2).

There are four known naturally occurring tocopherols; these are termed α-, β-, γ- and δ-tocopherol (3). The structures of these compounds are given in Figure 1. There are also four closely related structures termed tocotrienols which occur at much lower levels in most oils. These structures differ only in having three double bonds in the isoprenoid side chain (4).

Several methods for the analysis of tocopherols have been published. These methods can be broadly termed as chemical, electrochemical or chromatographic techniques. The chemical methods generally measure total tocopherol through the chemical oxidation of the phenolic groups (5). Of the methods currently used on a routine basis, the method of Emmerie and Engel (6) is the most widely employed. This method uses ferric chloride solution for oxidation of the tocopherol followed by measurement of the ferrous ion produced. Difficulties encountered with this procedure are the varying rates of reactivity of the different tocopherols and the inhibition of color formation of bipyridine and ferrous ion in the presence of glyceride oils (7). In addition to these difficulties, this type of chemical method can only measure total tocopherol content and yields no information concerning the levels of individual tocopherols. Because the oxidative stabilities of the various tocopherols are different (8), it is important to measure the levels of the α-, β-, γ- and δ- forms in order to truly understand their effect on the stability of the oil.



5,7,8 Trimethyl = α- tocopherol
5,8 Dimethyl = β - tocopherol
7,8 Dimethyl = γ - tocopherol
8 Methyl = δ - tocopherol

FIG. 1. Tocopherol structures.

Polarographic techniques have been successfully applied in measuring individual tocopherols (9,10) in selective cases; however, interference due to additives and lack of resolution of β- and γ-tocopherols (7) can be a problem in the application of this methodology to a wide variety of vegetable oils.

The most successful chromatographic techniques used in the past for quantitation of individual tocopherols have been thin layer chromatography (TLC) (11,12), gas liquid chromatography (TLC) (13) and column liquid chromatography (14,15). Of these techniques, TLC and GC methods require long analysis times with extensive sample workup and/or derivatization for tocopherol measurement. These techniques also allow the possibility of oxidative degradation of the tocopherols during sample handling (5).

Due to these shortcomings of the earlier used methods, High Performance Liquid Chromatography (HPLC) was investigated for use in the quantitation of tocopherols in a variety of vegetable oils. Although the general applicability of liquid chromatography to tocopherol measurement has been shown (10,14-16), very little data have been presented on the tocopherol levels in a variety of oils.

In this work we describe the use of HPLC in the rapid analysis of tocopherols in vegetable oils. The technique is very rapid, requiring less than ten minutes for determination of α-, β-, γ- and δ-tocopherols. Studies on several oil samples indicate that the method is both accurate and precise. This method is also highly sensitive and is capable of measuring less than 10 ppm of individual tocopherols under the experimental conditions which we have used.

EXPERIMENTAL PROCEDURES

HPLC Separation and Quantitation

The chromatographic separation was performed on a 4 mm x 30 cm μ-Porasil column (Waters Assoc.) with a mobile phase of 1.5% iso-propyl alcohol (IPA) in hexane (HPLC grade, Burdick & Jackson, Millipore filtered .45 μm) at a flow of 1.8 to 2.0 ml/min. A Waters Model 6000 LC pump was used. Sample injections were made with a 100 μl Valco loop injection valve. A Perkin-Elmer LC-55 spectrophotometer set at 295 nm, the absorption maxima for tocopherols, was the detector. A Hewlett-Packard 3380A integrator was used for the determination of the standard calibration curves and for the calculation of the amounts of the tocopherols in the oil samples.

Standards

Reagent grade α- and δ-tocopherol standards were used as received from Eastman Organic Chemicals. Rochester, NY Commercial sources of analytical grade β- and δ-tocopherols could not be located; however, a mixed tocopherol standard containing α-, β-, γ- and δ-tocopherols

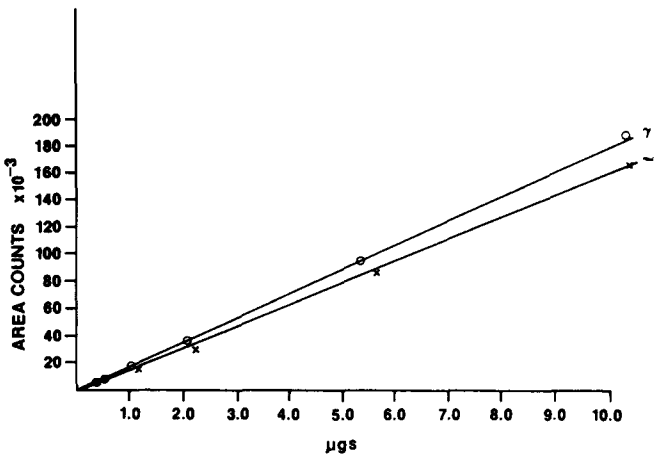


FIG. 2. Calibration curves α and γ tocopherols.

(Polyscience Corp., Rochester, NY) was obtained in order to determine retention times of each of these components. The α- and γ-tocopherol standards were made up in 1.5% IPA in hexane at concentrations ranging from 0.1 to 4.0 µg/ml. Calibration curves prepared from area counts and µgs injected were made (Fig. 2). The response factors of β- and δ-tocopherols were taken as the average of the relative responses of α- and β-tocopherols due to their similar absorption coefficients (17). This practice is believed to contribute negligibly to any error in the analytical method.

Samples

The samples studied were commercially processed oils available in most grocery stores; they were examined without any special sample preparation. Five grams of sample was weighed into a 100 ml actinic glass volumetric flask. The sample was brought to volume with 1.5% IPA in hexane before injection of 100 µl onto the HPLC column. The samples and standards were kept in the actinic glassware in a refrigerator for no more than one week in order to avoid possible tocopherol degradation, solvent evaporation or other adverse effects.

Recovery Studies

A standard addition technique was employed in order to determine if the oil matrix significantly affected tocopherol quantitation. A soybean oil solution was spiked with known amounts of α- and γ-tocopherols. The amounts of α- and γ-tocopherols were determined after spiking in the same manner as in the unspiked soybean oil sample. The percent recovery of tocopherol was calculated as:

$$\% \text{ Recovery} = [C/(A+B)] \times 100$$

where C = µg tocopherol found in spiked sample

B = µg tocopherol added to sample solution

A = µg tocopherol found in original sample solution

Column Regeneration

At the end of each day, methanol was pumped through the HPLC column for ca. 30 min. This removed the more polar oil constituents which are adsorbed on the column during the analyses. After this washing step, ca. 30 min. of pumping the mobile phase (1.5% IPA in hexane) through the column was found adequate for re-equilibration before injection of additional samples.

RESULTS AND DISCUSSION

Several different oils were examined for levels of α-, β-, γ- and δ-tocopherols by direct injection of the oil dissolved in the mobile phase. A detection wavelength of 295 nm was found to be selective for the measurement of tocopherols in

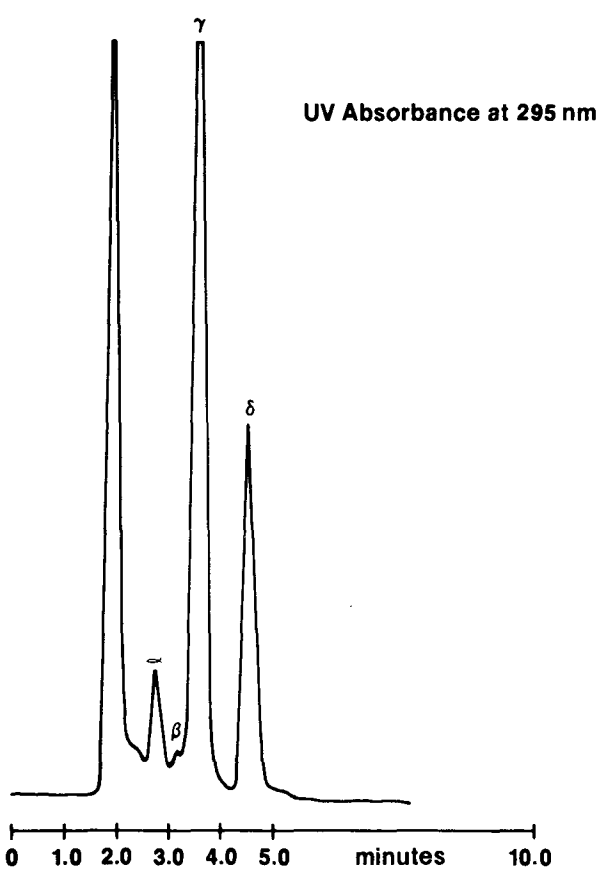


FIG. 3. HPLC tocopherol profile of a soybean oil.

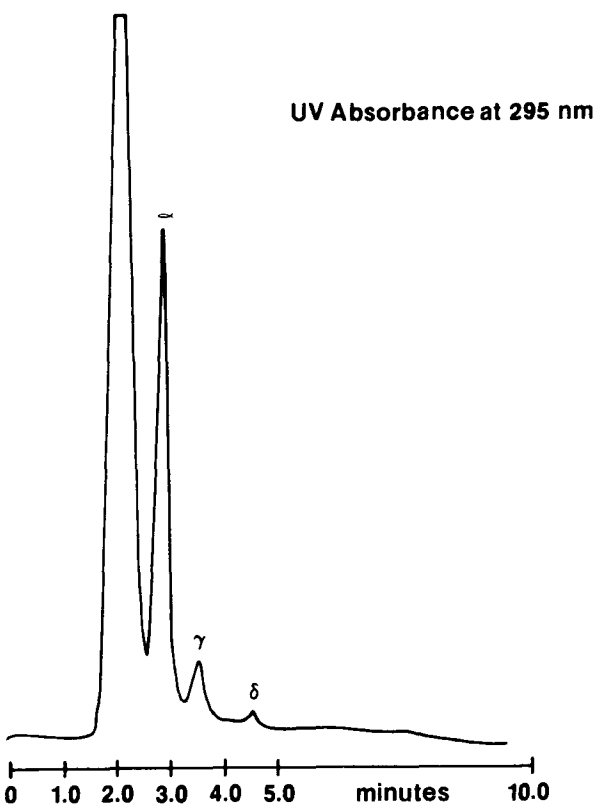


FIG. 4. HPLC tocopherol profile of a sunflower oil.

TABLE I

Tocopherol in Oils by HPLC[a]

Sample	α (μg/mg)	β (μg/mg)	(γ (μg/mg)	δ (μg/mg)	(μg/mg) Total HPLC	(μg/mg) Total colorimetry
Soybean oil A	.070 ± .003	Trace	.924 ± .014	.370 ± .006	1.36	1.3
Soybean oil B	.039 ± .002	Trace	.600 ± .005	.235 ± .002	.87	.8
Corn oil A	.123 ± .011	ND[b]	.468 ± .008	.021 ± .001	.61	.6
Corn oil B	.284 ± .012	ND	.758 ± .004	⩽ .010	1.04	.3
Sunflower oil A	.570 ± .003	ND	.039 ± .001	⩽ .010	.61	.2
Sunflower oil B	.904 ± .031	ND	.049 ± .005	⩽ .010	.95	.2
Safflower oil A	.367 ± .003	ND	.044 ± .001	⩽ .010	.41	.2
Safflower oil B	.477 ± .014	ND	Trace	⩽ .010	.48	.2
Olive oil	.070 ± .004	ND	.020 ± .001	⩽ .010	.09	.2
Peanut oil	.304 ± .004	ND	.192 ± .005	.031 (2)	.53	.2
Cottonseed oil	.573 ± .004	.040 ± .001	.317 ± .002	⩽ .010	.89	.5

[a]Average of four determinations unless marked otherwise.
[b]ND = None detected.

TABLE II

Tocopherol Recovery Study

Sample	α	γ
Soybean A 50 mg/ml	.315 μg	4.158 μg
Standard solution added	1.00 μg	1.06 μg
Spiked oil solution	[a]1.318 μg	[a]5.193 μg
100 μl injections	% Recovery = 100.2	% Recovery = 99.5

[a]Avg. of 6 determinations.

the oils studied. The majority of the oil components (triglycerides absorb at wavelengths less than 225 nm, so no interference from the major components is observed at the detection wavelength used in this method.

A sample chromatogram of a commercially processed soybean oil is shown in Figure 3. The tocopherols corresponding to the chromatographic peaks shown are indicated. This profile is characteristic of a high γ-tocopherols oil. An example of a high α-tocopherol oil is sunflower oil which is illustrated in Figure 4. The comparison of Figures 3 and 4 suggests that the tocopherol distribution is quite characteristic of the type of oil being examined.

Quantitation of tocopherols in several different commercial oils was done by electronic integration of the chromatographic peaks and comparison to the appropriate calibration curves. The results are presented in Table I. As seen from this Table, the reproducibility of this method is good with relative standard deviations being less than 2% in most cases. Table I also contains the total tocopherol content as found by the colorimetric method of Emmerie and Engel (6) as modified by Stern and Baxter (7). In general, the HPLC method gives higher values for total tocopherols. The trend of the data indicates that the ferric chloride method does not respond well to α-tocopherol, as the colorimetric results on high α-tocopherols oils are extremely low. On the other hand, the HPLC method gives a more selective analysis and is reliable for many types of oils, including high α-tocopherol oils.

Although β-tocopherol was found by Woziwodzki (18) in the LC of the unsaponifiable fraction of many oils, the only commercial oils examined in this study which showed measurable levels of the β isomer were cottonseed (40 ppm) and soybean (trace, ⩽ 10 ppm) oils. The separation of the β and γ isomers, however, is still adequate to allow measurement of low levels of β-tocopherol in the presence of high levels of γ-tocopherol (Fig. 3).

The results of the standard addition experiment on the soybean A oil sample are given in Table II. These data indicate the good accuracy of the HPLC method, as 100% of the added α- and γ-tocpherols was found in the final solution.

CONCLUSIONS

An HPLC method has been developed for the direct measurement of the individual tocopherols in vegetable oils. The technique is highly selective for the measurement of tocopherols relative to the commonly used ferric chloride colorimetric procedure and has the ability to detect less than 10 ppm of the individual tocopherols which is also an improvement relative to colorimetric measurement. This method requires only ten minutes of analysis time and is both accurate and precise. We have successfully applied the method to a variety of oils with no apparent loss in chromatographic efficiency after several analyses. We have, however, found it helpful to flush the column with methanol for thirty minutes each day in order to remove any adsorbed components. By doing this we are assured of retaining the column efficiency for an extended period of time.

The information obtained by this type of analysis will be helpful in assessing the effect of various tocopherols on the oxidative stabilty of vegetable oils and in some cases may be useful in determining unknown mixtures of oils based on the type of tocopherol distribution found.

ACKNOWLEDGMENT

I extend thanks to R.J. Healy for help in completing the necessary analyses. I also thank H.W. Wharton and T.S. Turan for their review of this paper.

REFERENCES

1. "Analysis and Characterization of Oils, Fats and Fat Products," Edited by H.A. Boekenoogen, Interscience Publishers, London, England, 1964, pp. 52-53.
2. "Scientific Status Summary by the IFT Expert Panel," Food Technology, January 1977, pp. 77-80.
3. "Bailey's Industrial Oil and Fat Products," Edited by Daniel

Swern, Interscience Publishers, 1964, pp. 38-41.
4. "Methods of Vitamin Assay," Edited by The Association of Vitamin Chemists, Interscience Publishers, New York, 1966, pp. 363-402.
5. Strohecker, R., and H.M. Henning, "Vitamin Assay – Tested Methods," CRC Press, Cleveland, OH, 1966, pp. 283-308.
6. Emmerie, A., and C. Engel, Recl. Trav. Chim. 57:1351 (1938.
7. Stern, M.H., and J.G. Baxter, Anal. Chem. 19:902 1947.
8. Bauernfeind, J.C., CRC Critical Reviews in Food Science and Nutrition, 8 No. 4:337 (1977).
9. Waltking, A.E., M. Kiernau, and G.W. Bleffort, J. Assoc. Off. Anal. Chemists, 60(4):890 (1977).
10. Podlaha, O., A. Eriksson, and B. Toregard, JAOCS 55:530 1978.
11. Bollinger, H.R., in "Thin Layer Chromatography," Edited by E. Stahl, Academic Press, Inc., New York, 1957, pp. 229-232.
12. Seher, A., Nehrung, 4:466 1960.
13. Hartman, K.T., JAOCS 54:421 (1977).
14. Van Niekerk, P.J., Anal. Biochem. 52:533 1973.
15. Matsuo, M., and Y. Tahara, Chem. Pharm. Bull. 25(12):3381 1977.
16. Vatassery, G.T., V.R. Maynard, and D.F. Hagen, J. Chrom. 161, 299, 1978.
17. "Handbook of Chemistry and Physics," 55th Ed., Edited by R.C. Weast, CRC Press, Cleveland, OH, 1974, p. C512.
18. Woziwodzki, H., AOAC 67th Annual Spring Meeting, April 1976, Paper No. 146.

[Received January 15, 1979]