A Simplified Gas Liquid Chromatographic Determination for Vitamin E in Vegetable Oils

KENNETH T. HARTMAN, Frito-Lay Research Laboratory, Irving, TX 75061

ABSTRACT AND SUMMARY

A simple and rapid procedure has been developed for the isolation, concentration, esterification, and gas liquid chromatographic (GLC) quantitation for the Vitamin E content of vegetable oils. Vitamin E is determined by saponification of the oil, ether extraction of the saponified mixture, drying and evaporation of the extract, followed by closed tube esterification and quantitation of the butyrate ester using a gas chromatograph equipped with a hydrogen flame ionization detector. This technique eliminates the conventional thin layer chromatographic isolation of Vitamin E normally used prior to direct or trimethylsilyl (TMS) derivative GLC quantitations. Oils fortified with Vitamin E in the 5 to 40 milligrams per 100 grams range showed recoveries of 93.4 to 98.6%.

INTRODUCTION

The feasibility of tocopherol analysis by gas liquid chromatography (GLC) was suggested by Nicolaides (1) in 1960. Wilson et al. (2) separated tocopherol by GLC using three stationary phases. Sheppard et al. (3) in reviewing gas chromatography of fat soluble vitamins found that GLC analysis appears to be well suited for the determination of tocopherols in a variety of biological materials and pharmaceutical preparations.

The present work was undertaken to develop a specific assay suitable for routine analysis of alpha tocopherol in vegetable oils which would eliminate isolation steps involving column or thin layer chromatographic techniques. This criterion is not met for most food substances using direct GLC analysis of tocopherols or with the trimethylsilyl (TMS), acetate, or propionate esters. Nelson et al. (4) found that the butyrate derivatives would prevent interference from extraneous materials during GLC analysis of soya sludge for tocopherols and sterols. A modification of this procedure has been found to work well for the determination of alpha tocopherol in vegetable oils. This technique has also been used for the analysis of vegetable oils extracted from snack food products using the procedure for Vitamin E, section 43.067 (d) in the "Official Methods of The Association of Official Analytical Chemists" (5).

EXPERIMENTAL PROCEDURES

Reagents and Apparatus

Alpha tocopherol standard: Eastman grade.

Absolute alcohol: Reagent grade absolute ethyl alcohol (SDA, 3-A Absolute) peroxide-free.

Pyridine: Regis Chemical Company, Morton Grove, IL.

Ethyl ether: Reagent grade, peroxide-free.

Potassium hydroxide: Potassium hydroxide, 80 g, was dissolved in 100 ml of distilled water.

Cholesteryl isovalerate and butyric anhydride: Prepared as directed by Nelson et al. (4).

Pyridine-butyric anhydride solution: A solution consisting of 2 parts pyridine and 1 part butyric anhydride was prepared fresh for each day's analysis.

Cholesteryl isovalerate solution: About 50 mg cholesteryl isovalerate was accurately (±0.1 mg) weighed into a 50 ml volumetric flask, dissolved, and diluted to the mark with ethyl ether. This solution was stored in an air-tight bottle having a teflon-lined lid.

Benzene-alcohol solution: Prepared by mixing 5 parts reagent grade benzene with 1 part absolute alcohol.

Vegetable oils: Refined vegetable oils suitable for food processing.

Acetylation tubes: No. 201040, 5 ml capacity, Regis Chemical Company.

Oil bath-130 C: Oil bath was constructed from a glass dish 2 in. in height by 6 in. in diameter heated with a Corning Model PC-351 hot plate stirrer combination.

Gas chromatograph column packing: Gas Chrom Q, 100 to 120 mesh (Applied Science Lab., State College, PA) was coated with SE-30 (Applied Science Lab.) at 1, 3, and 5% levels as directed by Nelson et al. (4).

Gas chromatogaphic columns: Glass columns 6 ft by 5 mm ID were prepared using a pressure packer (Press-Pak 4006, Alltech Associates Inc., Arlington Heights, IL) with 30 psi. nitrogen and gentle tapping. Column packing was secured using silanized glass wool (Applied Science Lab.).

Gas chromatography: Analyses were performed on a Hewlett Packard Model 5750 flame ionization gas chromatograph. The operating conditions of the chromatograph were: column temperature 240 C, injection port temperature 280 C, detector temperature 265 C, hydrogen flow rate 45 cc/min, air flow rate 450 cc/min, and helium flow rate 60 cc/min.

Calibration of Flame Ionization Detector

Calibration was performed using two known mixtures. These solutions were prepared to have the following composition. (a) Mixture A: 0.50 mg, alpha tocopherol and 2.00 mg, cholesteryl isovalerate in 3.0 ml, pyridine-butyric anhydride solution. (b) Mixture B: 3.00 mg, alpha tocopherol and 2.00 mg, cholesteryl isovalerate in 3.0 ml, pyridine-butyric anhydride solution. The above solutions were each contained in a closed acetylation tube, heated, and stirred for 15 min in 130 C oil bath using teflon-coated stirring bars (1/2 in. x 1/8 in.), cooled and chromatographed in duplicate. Response factor (K) was calculated using the procedure of Sawardeker and Sloneker (6).

$$K = \frac{A_s \times W_s}{A_s \times W}$$

where A, peak area of alpha tocopherol butyrate: A_s, area of cholesteryl isovalerate; W, milligrams of alpha tocopherol; W_s, milligrams of cholesteryl isovalerate.

Assay Procedure

Accurately weigh 5.00 g of the well mixed vegetable oil into a 125 ml glass stoppered boiling flask, add 20 ml of absolute alcohol, and 1.5 G of ascorbic acid (Eastman 4640). Attach a water cooled reflux condenser and heat to boiling in a boiling water bath. Raise condenser and add 5 ml of potassium hydroxide solution, replace condenser and reflux 15 min. Stopper boiling flask and cool rapidly under cold running water. Transfer the solution to a 250 ml separatory funnel using 100 ml of distilled water. Add 3/4 teaspoon of sodium chloride and mix until salt dissolves. Wash the boiling flask with 25 ml of ethyl ether. Add ether washing to the separatory funnel, shake for 1 min, and allow layers to

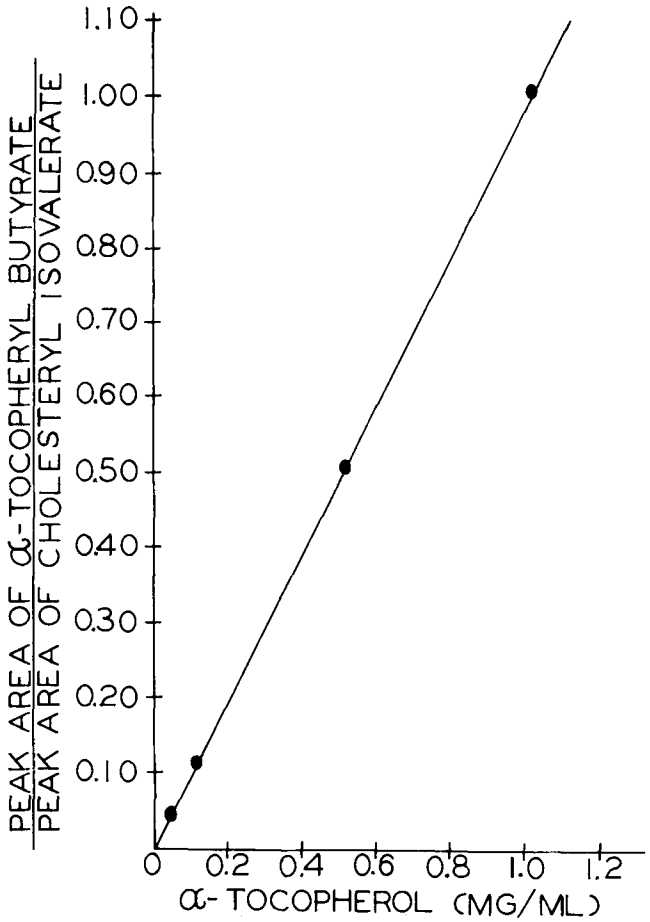

FIG. 1. Linearity plot. Plot of relative response values for alpha tocopheryl butyrate vs. the concentration of alpha tocopherol.

TABLE I

Recovery of α-Tocopherol from Fortified Olive Oil

Number	α-Tocopherol added (mg/100g oil)	α-Tocopherol found (mg/100g oil)	% Recovery
1	5.0	4.67	93.4
2	10.0	9.83	98.3
3	20.0	19.26	96.3
4	30.0	29.16	97.2
5	40.0	38.72	96.8

TABLE II

α-Tocopherol in Vegetable Oils

Oil	α-Tocopherol (mg/100 g oil)
Soybean-A	3.3
Soybean-B	6.7
Cottonseed-A	18.0
Cottonseed-B	34.4
Peanut	13.6
Corn	13.9

separate. Transfer ether layer to a second 250 ml separatory funnel. Repeat ethyl ether extraction five more times. Wash the combined ether extracts with 50 ml portions of distilled water until the wash water is neutral to phenolphthalein. Add 2.00 ml of the cholesteryl isovalerate solution after the water has been drained from the third washing, mix by swirling, and proceed with the fourth water washing. Four to six washings are usually required. Dry the ether extract by shaking with 25 G of anhydrous sodium sulfate. Evaporate the dried extract to 1 to 2 ml using a 100 ml Geotz centrifuge tube, a 45 C water bath and a slow stream of dried nitrogen (nitrogen is dried by passing it through a calcium sulfate drying trap). Transfer the concentrated extract to an acetylation tube using a disposable Pasteur pipet (No. 13-678-5B, Fischer Scientific Company, Pittsburg, PA) and evaporate to about 0.5 ml. Wash the Geotz tube with two, 2 ml portions of anhydrous ethyl ether (ether shaken with anhydrous sodium sulfate), transfer each washing to the acetylation tube, and evaporate the ether using the 45 C water bath and a slow stream of dry nitrogen. Dry the residue by adding 0.5 ml of benzene-alcohol mixture and evaporate in the same manner as previous. Repeat the drying step two additional times. Add a small teflon-coated stirring bar (1/2 in. x 1/8 in.), 3.0 ml of pyridine-butyric anhydride solution, secure top, heat, and stir for 15 min in a 130 C oil bath. Allow the solution to cool to room temperature and inject 3 to 5 microliters of the solution into the gas chromatograph.

Calculations

The milligrams of alpha tocopherol per 100 g vegetable oil was calculated using the following equation.

$$\text{Alpha tocopherol (mg/100 g)} = \frac{W \times A \times 100}{S \times K \times A_S}$$

where A, area of tocopheryl butyrate peak; A_S, area of cholesteryl isovalerate peak; W, milligrams of cholesteryl isovalerate added to the sample; S, original sample weight in grams; and K, response factor for tocopheryl butyrate obtained from detector calibration.

Linearity of Response

Linearity tests were conducted with four alpha tocopherol solutions. These solutions were prepared to have 0.15, 0.30, 1.50, and 3.00 mg alpha tocopherol in 3.0 ml of pyridine-butyric anhydride solution containing 2.00 mg cholesteryl isovalerate. These mixtures were heated and stirred in a 130 C oil bath for 15 min, cooled, and injected into the gas chromatograph. Duplicate tests were completed for each mixture.

Method Accuracy

Olive oil found to contain a low level of alpha tocopherol was fortified with alpha tocopherol at levels of 5, 10, 20, 30, and 40 mg per 100 G. Fortified oil samples were treated as directed under "Assay Procedure."

Corn oil, peanut oil, cottonseed oil, and a mixture of soybean and cottonseed oils, purchased from a grocery store, were analyzed for alpha tocopherol using the proposed method. Triplicate analyses were completed for each of these oils.

Portions of soybean, peanut, corn, and cottonseed oils were each put through a ceric sulfate column as directed in AOAC Official Methods, section 43.074 (5). The treated oils were analyzed for alpha tocopherol by the proposed method.

RESULTS AND DISCUSSION

Three columns were tested for quantitation of alpha tocopherol in vegetable oil. The glass columns were all 6 ft by 4 mm ID packed with SE 30 at levels of 1,3, and 5% on 100 to 120 mesh Gas Chrom Q. The 3% SE 30 coating level was found to be the column packing of choice based on resolution at 230 to 250 C with a carrier gas flow rate of 40 to 60 cc/min.

Esterification solution volumes of 1.0, 2.0, and 3.0 ml were tested. Three ml of pyridine-butyric anhydride solution was found to be optimum for 5.0 g of vegetable oil.

TABLE III

Method Reproducibility Tests

Vegetable oil	Analyses[a]	Average[a]	Standard deviation
Mixture of Cottonseed & Soybean oils	12.2 12.6 12.6	12.5	1.92
Corn oil	11.7 11.4 11.5	11.5	1.29
Peanut oil	15.1 14.8 15.2	15.0	1.73
Cottonseed oil	21.7 21.5 21.8	21.7	1.29

[a]Milligrams α-tocopherol per 100 grams oil.

Peak broadening resulting in poor GLC resolution occurred for corn oil when 1.0 ml of pyridine-butyric anhydride solution was used for esterification. When 2.0 ml of solution were used, only an occasional corn oil sample, having a high level of unsaponifiable material, showed some reduction in the resolution of butyrate esters. GLC resolution problems were eliminated when the solution volume was raised to 3.0 ml.

Alpha tocopherol preparations ranging from 0.15 to 3.00 mg per 3.0 ml of solution were put through the esterification procedure. The quantities of alpha tocopherol were plotted vs. relative response values as shown in Figure 1. This plot shows a linear response for alpha tocopheryl butyrate over the range tested.

Olive oil fortified with alpha tocopherol at levels of 5, 10, 20, 30, and 40 mg per 100 of oil resulted in recovery values ranging from 93.4 to 98.6%. (Table I). Each result is the average of two determinations.

Results for the determination of alpha tocopherol in refined soybean, cottonseed, peanut, and corn oils are shown in Table II. Each result is the average of duplicate analyses. Soybean-A and soybean-B were oils obtained from two different refining processes. Cottonseed-A and cottonseed-B were also oils obtained from the two refining processes.

Reproducibility tests were conducted with vegetable oils purchased from a grocery store. Table III shows the average alpha tocopherol and standard deviation values obtained for each oil.

Tests were conducted to determine if interfering compounds were eluting from the GLC column at the same time as alpha tocopheryl butyrate. Alpha tocopherol was removed from soybean, peanut, corn, and cottonseed oils by ceric sulfate oxidation. Chromatograms obtained for these oils were found to be void of any peaks having the same elution time as alpha tocopheryl butyrate. Additional tests involved analyzing unoxidized portions of these oils by the proposed method. These preparations were subjected to GLC temperature programming in order to better resolve any compounds that might be eluting with the alpha tocopheryl butyrate. These tests did not reveal the presence of any compounds that would cause interference with the GLC determination of alpha tocopherol by the proposed method.

A typical chromatogram for the butyrate derivatives of the unsaponifiables for soybean oil is shown in Figure 2 and their relative retention times in Table IV. Alpha tocopherol, beta plus gamma tocopherol, delta tocopherol, campesterol, stigmasterol, and beta sitosterol are all detected with this technique, however, the beta and gamma tocopherol isomers are not resolved.

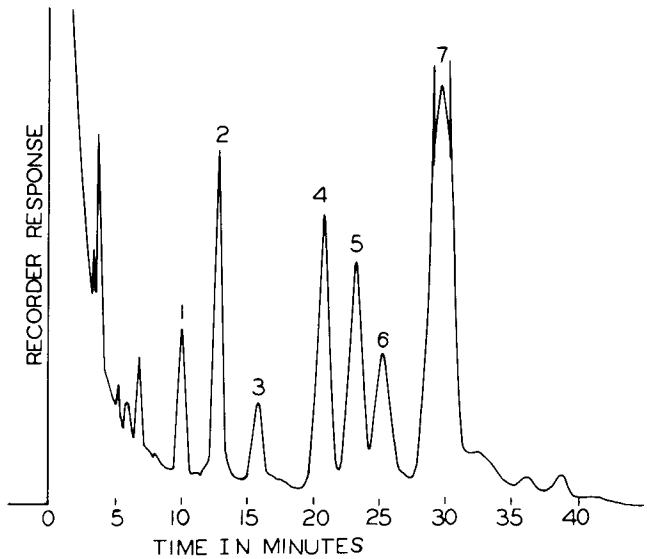

FIG. 2. Chromatogram for the butyrate derivatives of soybean oil unsaponifiables. Identity of peaks: 1. delta tocopheryl butyrate; 2. beta plus gamma tocopheryl butyrate; 3. alpha tocopheryl butyrate; 4. cholesteryl isovalerate; 5. campesteryl butyrate; 6. stigmasteryl butyrate; 7. beta sitosteryl butyrate.

TABLE IV

Relative Retention Times (RRT)

Derivative	RRT
δ-Tocopheryl butyrate	0.48
β + γ-Tocopheryl butyrate	0.62
α-Tocopheryl butyrate	0.76
Cholesteryl isovalerate	1.1.00
Campesteryl butyrate	1.12
Stigmasteryl butyrate	1.21
β-Sitosteryl butyrate	1.43

The lower limit of detection for alpha tocopherol was found to be about 0.5 mg per 100 g vegetable oil. This technique has also been successfully employed for vegetable oils extracted from snack food products.

REFERENCES

1. Nicolaides, N., J. Chromatography 4:496 (1960).
2. Wilson, P.W., E. Kodicek, and V.H. Booth, Biochem. J. 84:524 (1962).
3. Sheppard, A.J., A.R. Prosser, and W.D. Hubbard, JAOCS 49:619 (1972).
4. Nelson, J.P., A.J. Milun, and H.D. Fisher, Ibid. 47:249 (1970).
5. "Official Methods of Analysis," 12th Edition, Association of Official Analytical Chemists, Washington, DC, 1975.
6. Sawardeker, J.S., and J.H. Sloneker, Anal. Chem. 37:945 (1965).

[Received March 14, 1977]