TOCOPHEROL CONTENTS OF PAKISTANI SEED OILS STUDIED BY NORMAL PHASE HPLC

FAZLI MANAN*

SUMMARY: A high performance liquid chromatographic (HPLC) method was developed for the quantitative determination of tocopherol vitamers (α, β, T and σ) in different seed oils. After saponification the oil samples and extracts in n-hexane, tocopherol vitamers were quantitatively analyzed by HPLC with fluorimetric detector. Assay of tocopherol vitamers added to margarine resulted in ≥ 97% recovery, showing high precision of the method. The tocopherol vitamers were eluted isocratically by an eluent containing 95% hexane, 5% diethyl ether, at a pump flow rate of 1.5 ml/min. A silica column (Si-60) with a temperature of 45°C was used. The method developed in this investigation enables better separation of the tocopherol vitamers and their quantitative analysis in seed oils.

Key Words : Tocopherol, Pakistani seed oils, normal phase HPLC, liquid chromatography (HP), vitamin E.

INTRODUCTION

Vitamin E, a strong anti-sterility factor belongs to the family of tocopherols found in oils and fats. Due to the presence of methyl (-CH_3) group in its ring, it is stable to heat, alkali or acid. However, in the presence of oxidizing agents or UV light, this vitamin undergoes degradation and isomerization and yields four major vitamers (i.e., α, β, T, and σ-tocopherols). Three of these vitamers lower biological activities i.e., 30, 15, and 5% for β , T, and σ-tocopherols, respectively, while a-tocopherol has 100% biological activity (1).

For the determination of tocopherol vitamers in foods and food products, various methods have been documented, such as AOAC, colorimetric, spectrophotometric, thin layer chromatography (TLC), and fluorimetric methods are mostly used (2-5). These methods have been found time consuming, require considerable skill and experience, and their precision is low. Recently several authors (6-13) have recommended high performance liquid chromatography (HPLC) as most proficient, sensitive and speedy method for analysis of tocopherols in oils and foods.

Normal phase HPLC has been found capable of separating isocratically all the vitamers of tocopherol in seed oils (14), which involves dissolving the oils in hexane for injection on to a silica column. Muralidharan and Husain (15) reported that both reverse and normal phase HPLC is used for tocopherol analysis, the latter is however more suitable for separating different tocopherol vitamers. The present study was, therefore, initiated to evaluate the quality of different brands of seed oils for their tocopherol contents by an HPLC assay with a silica (Lichrosorb Si-60) column and n-hexane based solvent.

*From Department of Agricultural Chemistry and Nutrition, NWFP Agricultural University Peshawar, Pakistan.

MATERIALS AND METHODS

Different brands of seed oil samples (corn, sunflower, soybean, olive and sesame) were collected from the local market in Peshawar, North West Frontier Province, Pakistan. Standard α - (alpha) and β - (beta) tocopherols were obtained from Sigma Chemical Co, T - (gamma) and σ - (delta) tocopherol from Rathburn Chemical Co, while diethyl ether and n-hexane were procured from BDH Chemical Co. All other reagents used were of AnalaR grade.

Liquid chromatography

High performance liquid chromatography (HPLC) (Perkin Elmer, USA, Isocratic LC pump 250, with 20 μL loop injector, Model 7125 Rheodyne value), equipped with a spectrophotometer fluorescence detector (Perkin Elmer, USA, LC-3) was employed. Elution profiles were displayed on a PE Nelson computer integrator (Perkin Elmer, USA) attached with a Oki-data Microcline 320 printer.

HPLC column

The column used was Lichrosorb Si-60 (E. Merck AG Darmstadt, F. R. Germany), length 250 nm and internal diameter 4 mm. The column was fitted in a Perkin Elmer oven LC 101.

HPLC conditions

The following HPLC conditions were used.

Stationary phase : Lichrosorb Si-60
Eluent composition : Hexane : Diethyl ether (95:5)% v/v
Column temperature : 45°C
Loop Capacity : 20 μL
Flow rate : 1.5 μL/min
Detection : by fluorimetry at 290/325 nm

Recovery of tocopherols

The percent recovery of different tocopherols (α, β, T, and s-tocopherols) added to margarine was determined to obtain the level of tocopherols for analysis and ascertain the precision of the method. This also shows any interference by other substances if any, present in the sample.

A weighed quantity (2 g) of margarine was taken in a clean and dried round bottom flask covered with aluminum foil and 0.01 g of α, β, T, and s-tocopherols were also added. Nitrogen gas was flushed into the flask and stoppered. Percentage recoveries of different tocopherols added to margarine were calculated on five different days.

Percentage recovery = 100 (a-b)/c

Where 'a' is the total tocopherols (added and natural), 'b' is the tocopherol contents of the sample and 'c' is the total tocopherols added (pure tocopherols).

Saponification and extraction of tocopherols

A weighed quantity (2-5 g) of margarine samples was taken in a 250 mL Erlenmayer flask covered with aluminum foil. Nitrogen gas was flushed in and a freshly prepared 50 mL 8% ethanolic KOH solution, 5 mL petroleum ether and 10 mL absolute alcohol were added to the flask. A 20 mg BHT (16,17) was added as an antioxidant to prevent the oxidation of tocopherols. Nitrogen gas was again flushed in, the flask was stoppered and swirled gently to mix the reagents. The contents of the flask were saponified under reflux on a boiling water bath for 30 min. The flask was then cooled to room temperature. Diethyl ether (100 mL) was added to the flask with constant shaking. The contents were then transferred to a 500 mL separating funnel. Following separation of the two layers, the lower aqueous layer was discarded. The ether layer was washed with 100 mL distilled water. After drying the mixture in a vacuum rotary evaporator, residue was dissolved in 100 mL n-hexane. The aliquot was then injected on to HPLC for tocopherols analysis. All operations were carried out in subdued light and in the presence of nitrogen gas wherever possible. The same procedure was employed for the analysis of different tocopherol vitamers in different oil samples.

A direct extraction of tocopherols added to margarine was also performed by dissolving the seed oils (2 g) in n-hexane : diethyl ether (90:10% v/v) containing 20 mg/100 ml BHT. The mixture was injected directly on to the normal phase HPLC with silica column with the conditions set-up previously.

Table 1: Composition of different tocopherol vitamers in margarine.*

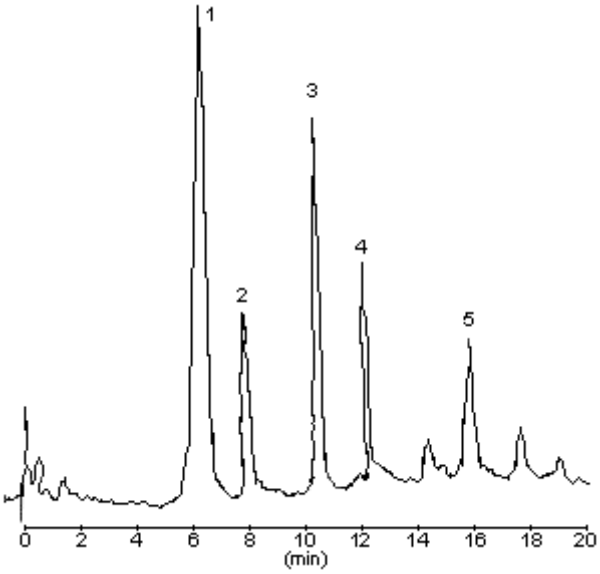
Tocopherol	Mean content gKg^{-1}	Standard deviation
α	0.285	± 1.65
β	0.056	± 1.12
T	0.735	± 1.15
σ	0.081	± 1.25
Total	1.152	± 1.29

* Mean of three determination.

Standard tocopherols solutions

A series of standard tocopherol solutions (0.01g containing 20 mg/100 mL, BHT) were prepared in clean and dried flasks covered with aluminum foil. The standard solutions

Figure 1: HPLC chromatogram of spiked tocopherol vitamers on margarine. Peaks: 1. α-tocopherol, 2. α-tocotrienol (expected), 3. β-tocopherol, 4. T-tocopherol, 5. σ-tocopherol.



were saponified and extracted in the presence of nitrogen gas as described earlier. The standard solution and the extract from the margarine/oil samples were subjected to analysis by the same chromatographic conditions. The concentration of different tocopherol vitamers were calculated by comparing the peak area of extracted tocopherol with the peak area of a standard solution of known concentration.

RESULTS AND DISCUSSION

The tocopherol vitamers of margarines (Table 1) determined in this study are in fair agreement with the value reported by Sivell *et. al.* (18), but are slightly higher than those of McCance and Widdowson (19). The extraction procedure employed indicated no significant difference between percent recovery of tocopherols added to margarine whether the samples were analyzed after saponification or without saponification procedure. McMurray and Blanchflower (9) reported that saponification is essential where tocopherol acetate is added to foods in order to hydrolyze it to free tocopherol. Similar results were achieved by Manz and Philipp (10).

The detection limits (1.5-0.3 μg/mL) of tocopherol vitamers achieved in the present study was much smaller than that obtained by Brubacher *et. al.* (14) for α-tocopherol analysis. Isocratic elution was used for α, β, T, and σ-tocopherols instead of gradient elution, the aim was to develop a single system to enable separation and measurement. Gradient elution facility although available with the HPLC in our laboratory, but was not availed since this involved a number of different solvents and hence may prove to be a more complicated and time consuming method.

Table 2 shows the percentage recoveries of each tocopherol vitamers on five different days, while Figure 1 shows the chromatogram of the spiked margarine extract. The percentage recoveries of each tocopherols were ≥ 97% indicating that the method gives very high and perfect recovery rates. McMurray and Blanchflower (9) using reverse phase HPLC with fluorimetric detector also reported a recovery of 97.3% for α-tocopherol.

The contents of tocopherol vitamers of different seed oils estimated, are given in Table 3. The results obtained are different from those reported by Niekerk (20) and Carpenter (6).

The extraction procedure and the normal phase

Table 2: Percentage recovery of different tocopherol vitamers added to margarine in five different days.*

Days	α-tocopherol	β-tocopherol	T-tocopherol	σ-tocopherol
After Saponification [1]				
1	97.62 ± 4.99	96.67 ± 5.16	98.66 ± 4.33	99.16 ± 5.66
2	96.70 ± 5.61	98.00 ± 4.99	96.70 ± 5.01	95.17 ± 5.22
3	100.01 ± 6.11	97.61 ± 5.00	97.13 ± 6.11	98.66 ± 4.23
4	97.00 ± 5.67	95.91 ± 4.12	99.11 ± 5.22	96.22 ± 5.12
5	98.22 ± 4.88	98.21 ± 6.01	100.21 ± 5.13	98.33 ± 5.95
Mean	97.98 ± 5.32	97.28 ± 5.06	98.36 ± 5.16	97.73 ± 5.20
Without Saponification [2]				
1	96.13 ± 5.55	98.77 ± 4.99	95.66 ± 6.33	94.33 ± 5.33
2	96.00 ± 4.67	99.88 ± 3.44	100.09 ± 4.66	99.12 ± 4.88
3	99.99 ± 4.98	98.89 ± 6.66	97.98 ± 7.45	98.99 ± 4.44
4	98.99 ± 4.55	97.00 ± 4.35	99.99 ± 4.44	98.97 ± 5.12
5	96.88 ± 3.44	97.00 ± 4.76	98.99 ± 6.00	97.77 ± 4.78
Mean	97.60 ± 4.64	98.31 ± 4.84	98.54 ± 5.77	97.84 ± 4.91

* Mean of three determinations.

1. Margarine samples were saponified and then extracted for HPLC analysis.

2. Margarine samples were directly injected on to HPLC after extraction with hexane: diethyl ether (90:10 v/v).

Table 3: Composition of different tocopherol vitamers in different brands of oils.*

Type of oil	α-tocopherol gKg^{-1}	β-tocopherol gKg^{-1}	T-tocopherol gKg^{-1}	σ-tocopherol gKg^{-1}	Total tocopherol gKg^{-1}
Corn oil	0.344	0.014	0.729	0.036	1.123
Sunflower oil	0.622	0.019	0.008	0.003	0.652
Soyabean oil	0.636	0.031	0.202	0.388	1.257
Olive oil	0.225	0.004	0.011	0.002	0.202
Saesame oil	0.113	0.003	0.400	0.005	0.521

* Mean of three determinations.

HPLC employed in this study gave an excellent separation of different tocopherol vitamers in shortest possible time, which provide a fast and reliable method for these determinations. Many workers have used reverse phase HPLC for the separation of vitamin A, D, K, and E using both isocratic and gradient elution with UV detection (21), whereas others used normal phase HPLC (22-24). Still others used the reverse phase HPLC and UV detector with an eluent composed of methanol : water 98:2 and 90:10% v/v, for determination of tocopherol vitamers in animal feeds and milk, respectively. The method developed in the present investigation, using a simple isocratic elution with 95:5 v/v hexane : diethyl ether conveniently allows a number of extractions and determinations of tocopherols vitamers to be carried out in one day.

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Correspondence:
Fazli Manan
Department of Agricultural Chemistry and Nutrition,
NWFP Agricultural University,
Peshawar, PAKISTAN.