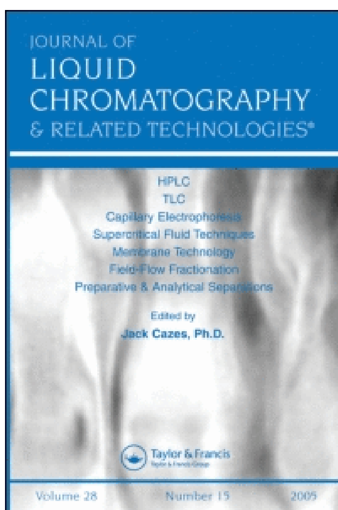
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DEVELOPMENT OF AN HPLC/DIODE-ARRAY/ FLUORIMETRIC DETECTOR METHOD FOR MONITORING TOCOPHEROLS AND TOCOTRIENOLS IN EDIBLE OILS

P. Gama[a]; S. Casal[a]; B. Oliveira[a]; M. A. Ferreira[a]
[a] Universidade do Porto, Porto, Portugal

Online publication date: 15 November 2000

To cite this Article Gama, P. , Casal, S. , Oliveira, B. and Ferreira, M. A.(2000) 'DEVELOPMENT OF AN HPLC/DIODE-ARRAY/ FLUORIMETRIC DETECTOR METHOD FOR MONITORING TOCOPHEROLS AND TOCOTRIENOLS IN EDIBLE OILS', Journal of Liquid Chromatography & Related Technologies, 23: 19, 3011 — 3022
To link to this Article: DOI: 10.1081/JLC-100101839
URL: http://dx.doi.org/10.1081/JLC-100101839

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. LIQ. CHROM. & REL. TECHNOL., 23(19), 3011–3022 (2000)

DEVELOPMENT OF AN HPLC/DIODE-ARRAY/ FLUORIMETRIC DETECTOR METHOD FOR MONITORING TOCOPHEROLS AND TOCOTRIENOLS IN EDIBLE OILS

P. Gama, S. Casal, B. Oliveira,* M.A. Ferreira

CEQUP / Serviço de Bromatologia
Faculdade de Farmácia
Universidade do Porto
R. Aníbal Cunha
4050-047 Porto, Portugal

Downloaded At: 09:41 24 January 2011

ABSTRACT

This paper describes an HPLC procedure for the determination of tocopherols and tocotrienols in crude and edible vegetable oils. The sample preparation only involves a dissolution of the oil in *n*-hexane and filtration. The chromatographic separation was achieved using a normal phase column LiChrosorb SI 60 (5 μm; 25.0 x 0.4 cm). Isocratic elution was carried out using *n*-hexane : 2-propanol (99.7:0.3). The effluent was monitored by using diode-array and fluorimetric detectors.

The determinations were performed in the following linear ranges: 0.5-7.5 μg/mL for α-tocopherol and β-tocotrienol; 0.5-10 μg/mL for β-tocopherol; and 0.5-15 μg/mL for α–tocotrienol, γ-tocopherol, γ-tocotrienol, and δ-tocopherol.

Extensive quality assurance of the proposed method was performed by the standard addition method in both crude and edible oil. For edible oil, the precision obtained (n=10) was better than 1.8, 2.0, and 1.4 CV% for α, β, and γ-tocopherol, respectively; for crude oil it was better than 1.9, 2.4, and 2.4% for α, β, and γ-tocopherol, respectively.

Copyright © 2000 by Marcel Dekker, Inc. www.dekker.com

Furthermore, for edible oil the mean recovery values were between 96-116%, 58-69%, and 111-115% for α, β, and γ-tocopherol, respectively; for crude oil recovery values were between 85-95%, 63-66%, and 99-103% for the same tocopherols, respectively.

The proposed method appears to be an adequate method for quality control and helpful for authenticity verification by the official control in oil industry.

INTRODUCTION

Tocopherols and tocotrienols are a soluble lipid group of compounds recognized as a generic term for vitamin E, being well represented in vegetables, fruits, seeds, nuts, and oils.[1]

Downloaded At: 09:41 24 January 2011

Chemically, vitamin E is a 6-chromanol ring structure with different number of methyl groups at positions 5, 7, and 8 and a saturated or unsaturated 16-carbon isoprenoid side chain. There are four tocopherols (α, β, γ, and δ) and four tocotrienols (α, β, γ, and δ).

Vitamin E is an "antioxidant" nutritional agent and is correlated with an antioxidant action against destructive effects of oxygen reactive particles either in foods or in human organism. In some chronic diseases, namely, coronary artery disease, some immunologic diseases, eye disease (age-related cataract), low-density lipoproteins oxidation, and anti-tumor activity, vitamin E has a preventive action and it is also a part of the primary intracellular defense system of the human organism.[2,3,4]

It is well known that vegetable oils and foodstuffs containing high lipid level are the most significant dietary sources of this vitamin; and that the different analogues of tocopherols and tocotrienols do not have a similar antioxidant activity.[1]

Thus, the individual determination of the isomers is more important than the evaluation of the global content of vitamin E. Although the profile of the individual isomers of each matrix may be useful for discrimination of the raw materials, it may also allow the monitoring of the quality of the oil processing.

Several chromatographic methods are found in the literature for the single quantification of vitamin E[5-12] or in association with other chemical classes of compounds.[13-17] The attempts to independently analyze tocopherols and tocotrienols have usually involved reversed-phase HPLC with different detection systems.[6,7,11,14,17]

However, in some of those methods a complete peak separation was not achieved, namely for β- and γ-tocopherols.[5,17] According to other authors, because all isomers have the same chromatographic behavior, the validation of the methodologies are only made for one analogue.[8, 10, 13]

The aim of this paper is to develop a multiparametric methodology to quantify in vegetable oils the individual vitamin E analogues. Despite the similarity of the chemical structure of these seven compounds under analysis the validation of the technique herein proposed includes the individual study of each one.

In order to evaluate the performance of the technique its application in both crude oils and after refining has been carried out.

Downloaded At: 09:41 24 January 2011

EXPERIMENTAL

Apparatus

The chromatographic analyses were carried out in a high performance liquid chromatograph equipped with a PU-980 pump, an AS-950 auto-sampler, a MD-910 multiwavelength diode array detector, a FP-920 fluorimetric detector, all from JASCO, Japan, and a Borwin PDA Controller Software (JMBS, France).

Reagents and Standards

Tocopherols (α, β, γ and δ) and tocotrienols (α, β, and γ) were purchased from Merck (biochemist grade). Also *n*-hexane and 2-propanol (LiChrosolv - gradient grade) were obtained from Merck (Darmstadt, Germany).

Oil Samples

Three different premix vegetable oils (canola, sunflower, and a blend of sunflower and corn oil at 70:30 ratio) were supplied by a local oil industry. Each of the oils was analysed both at crude and at edible stage. Until analyses were performed all the samples were maintained in the dark, at refrigerated temperature.

Sample Preparation

Oils were removed of moisture by filtering through anhydrous sodium sulphate. A 0.1g portion was blended with 10 mL of *n*-hexane and homogenised by stirring.

The sample preparation was conducted in the dark and tubes containing the oil samples were always wrapped in aluminum foil. The mixture was filtered by membrane (Schleicher & Shuell 0.2 μm; ϕ 13 mm, pure polyamide, Dassel, Germany) and analysed by HPLC under the following conditions.

HPLC Analysis

The chromatographic separation of the compounds was achieved with a normal-phase LiChrosorb SI 60 (5 μm; 25 x 0.4cm) column from Merck (Darmstadt, Germany). The effluent used was a mixture of *n*-hexane and 2-propanol (99.7: 0.3). Elution was performed at a solvent flow rate of 1.7 mL/min.

The effluent was monitored with a diode array detector and with a fluorimetric detector, at 290nm and 330nm as excitation and emission wavelengths respectively, connected in series.

The compounds under study were identified by chromatographic comparisons with authentic standards, by coelution and by their UV spectra. Quantification by fluorimetric detection was based on the external standard method.

Downloaded At: 09:41 24 January 2011

RESULTS AND DISCUSSION

Analytical Curve and Detection Limit

A linear relationship between the concentration of the compounds under study and the fluorimetric detector response was obtained under the assayed conditions. Linearity was observed in the concentration ranges: 0.5-7.5 μg/mL for α–tocopherol and β-tocotrienol; 0.5-10 μg/mL for β-tocopherol; and 0.5-15 μg/mL for δ-tocopherol, γ-tocopherol, γ-tocotrienol, and α-tocotrienol, respectively. The correlation coefficient for all standard curves invariably exceeded 0.99, except for the α-tocopherol, which was 0.98.

The calibration curves were obtained by triplicate determinations of each calibration standard with the peak area values (arbitrary units) plotted as average values. The relative average deviations of triplicates were less than 2% in all cases.

The average regression equations found were: $y = 1917.09x$, $y = 1148,14x$, $y = 1600.91x$, $y=110.81x$, $y = 812.97x$, $y = 959.54x$, $y = 2208.23x$ for α-tocopherol, α-tocotrienol, β-tocopherol, β-tocotrienol, γ-tocopherol, γ-tocotrienol, and δ-tocopherol, respectively.

The detection limit values were estimated as the concentration corresponding to three time the standard deviation of the baseline noise and were 0.1 ng/mL for α-tocopherol, α-tocotrienol, β-tocopherol and δ-tocopherol; 0.2 ng/mL for γ-tocopherol, and γ-tocotrienol; and 1.5 ng/mL for β-tocotrienol.

The quantification limit values, determined by sequential dilutions of a real edible oil sample, were somehow higher. The obtained values were 2 ng/mL for α-tocopherol, β-tocopherol, and δ-tocopherol; 3 ng/mL for α-tocotrienol; 4 ng/mL for γ-tocotrienol; 5 ng/mL for γ-tocopherol; 36 ng/mL for β-tocotrienol.

Validation of the Method

The chromatograms of tocopherols and tocotrienols standards obtained with fluorimetric and DAD detector are depicted in Figure 1. Peaks were separated around the following retention times: RT 5.9 m for α-tocopherol; RT 6.8 m for α-tocotrienol; RT 12.0 m for β-tocopherol; RT 13.6 m for γ-tocopherol; RT 14.0 m for β-tocotrienol; RT 16.0 for γ-tocotrienol, and RT 25.6 m for δ-tocopherol.

Figures 2 and 3 show the tocopherol profiles of a refined blend of sunflower and corn oil (70:30) and refined canola oil.

The fluorimetric and photodiode array detectors connected in series after NP-HPLC allows us to unambiguously identify the tocopherols and tocotrienols under study. By comparison of A and B in Figures 2 and 3, the presence of other peaks besides tocopherols and tocotrienols in the PDA chromatogram is obvious, while the fluorimetric chromatograms are clearer.

Although the quantification was achieved by measuring the peak area obtained with the fluorimetric detector, the PDA data were extremely important allowing a rapid identification of the compounds (Figure 4), especially when there was a retention time drifting due to small variations in the 2-propanol concentration.

Results from the quantification applied to the samples under study are shown in Table 1.

The precision of the analytical method was evaluated by measuring the peak chromatographic areas of the compounds 10 times on the same sample. In the edible sunflower and corn oil blend samples the coefficients of variation were 1.8%, 2.1%, and 1.4% for α, β and γ-tocopherols, respectively. For the corresponding crude samples, the coefficients of variation were respectively 1.9%, 2.4%, and 2.4% for the three tocopherols therein present.

Downloaded At: 09:41 24 January 2011

Downloaded At: 09:41 24 January 2011

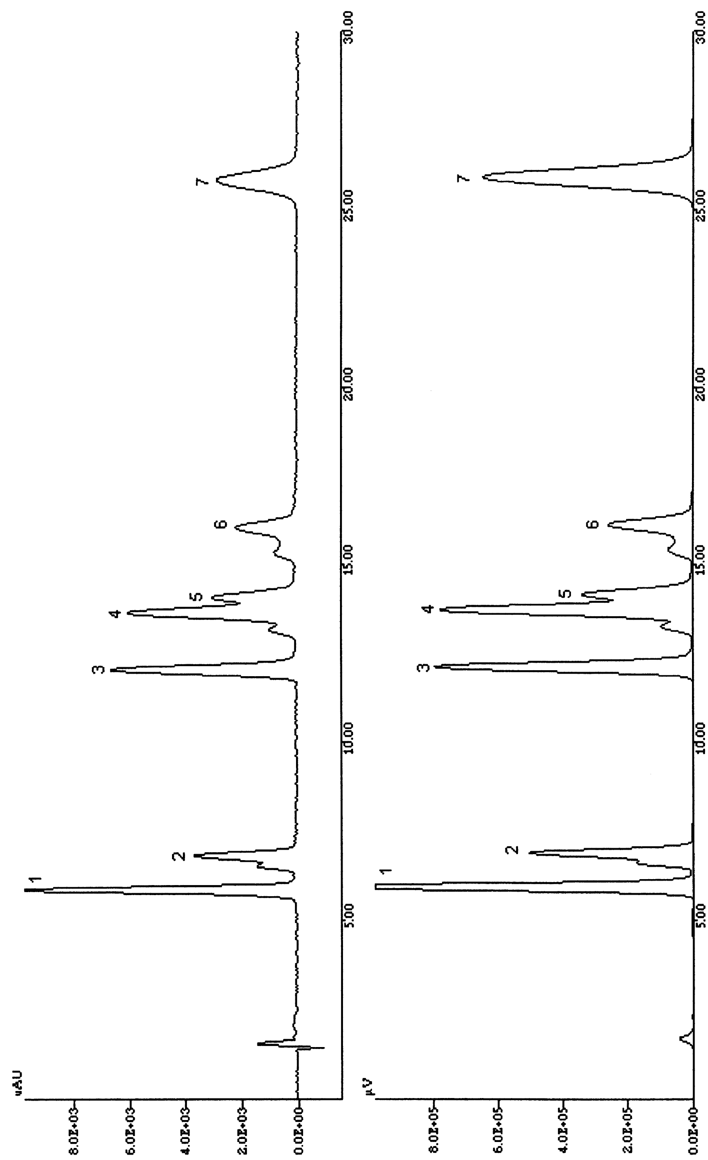


Figure 1. HPLC profile of a standard solution of tocopherols and tocotrienols. Fluorimetric detection (A) was performed at 290nm and 330nm as excitation and emission wavelengths and PDA (B) was recorded at 299 nm. (1) α-tocopherol, (2) α-tocotrienol, (3) β-tocopherol, (4) γ-tocopherol, (5) β-tocotrienol, (6) γ-tocotrienol and (7) δ-tocopherol.

Downloaded At: 09:41 24 January 2011

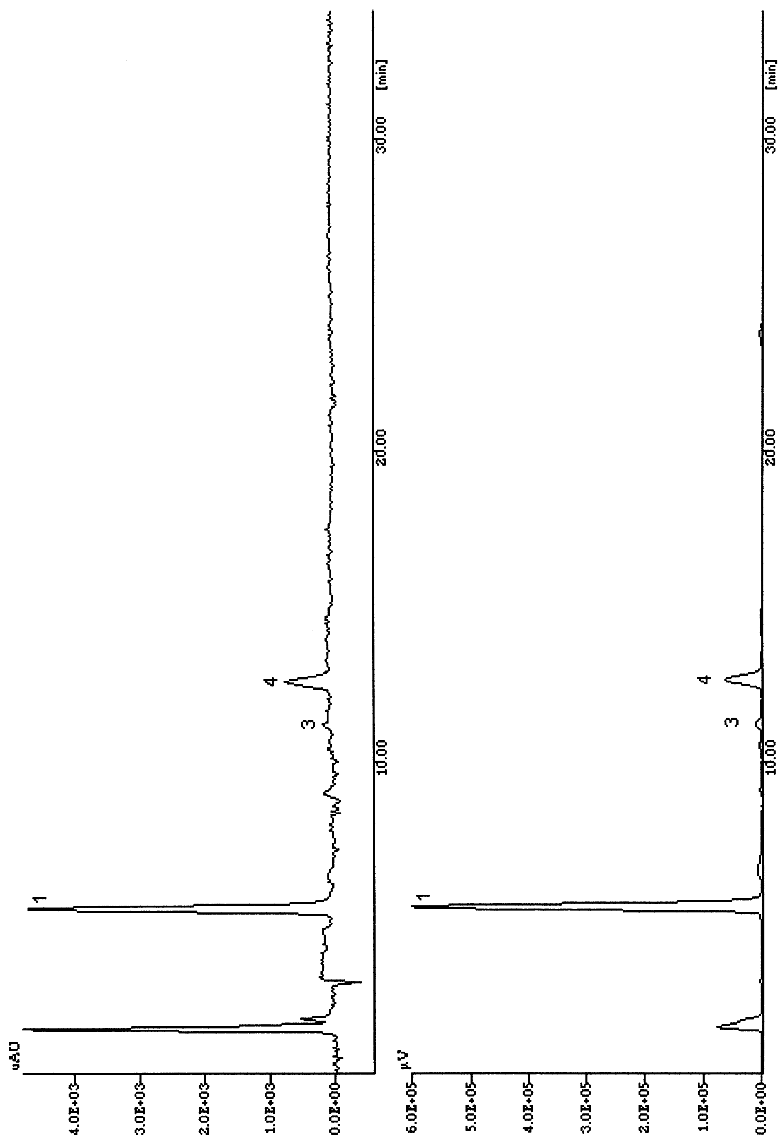


Figure 2. HPLC profile of a edible sunflower and corn oil blend sample (70:30). Fluorimetric detection (A) was performed at 290nm and 330nm as excitation and emission wavelengths and the PDA (B) was recorded at 299 nm. (1) α-tocopherol, (3) β-tocopherol and (4) γ-tocopherol.

Downloaded At: 09:41 24 January 2011

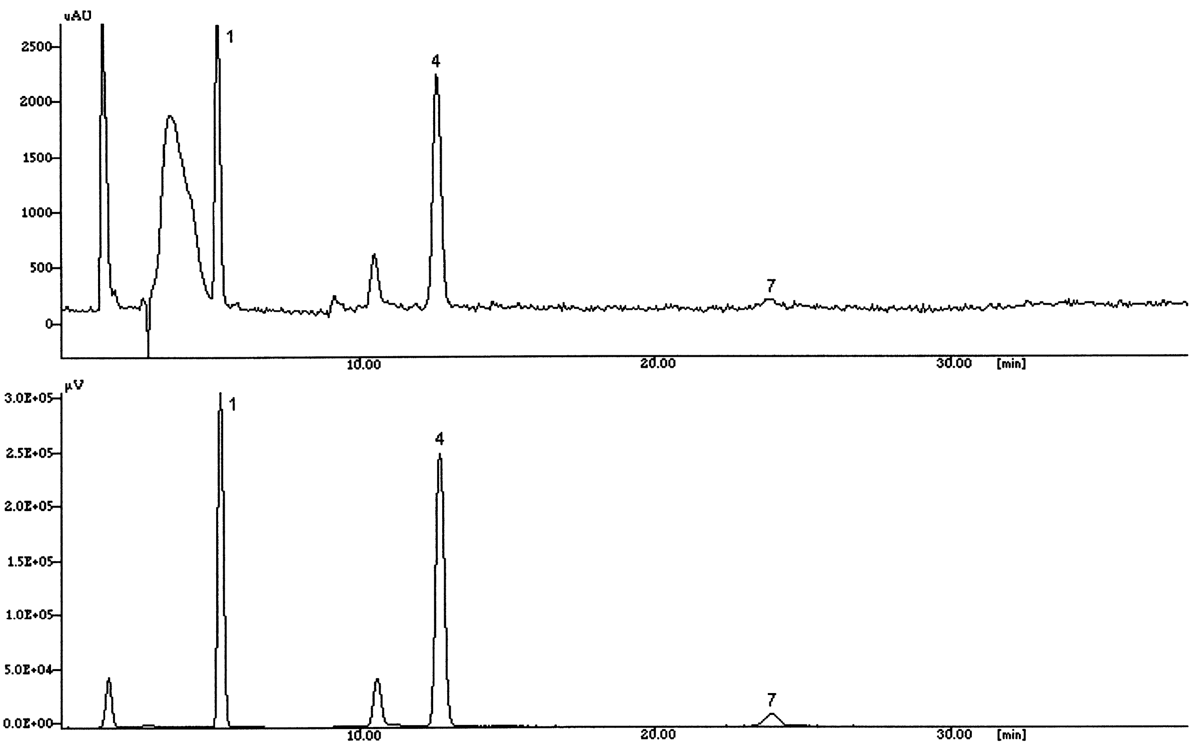


Figure 3. HPLC profile of an edible sample canola oil. Fluorimetric detection (A) was performed at 290nm and 330nm as excitation and emission wavelengths and PDA (B) was recorded at 299 nm. (1) α-tocopherol, (4) γ-tocopherol and (7) δ-tocopherol.

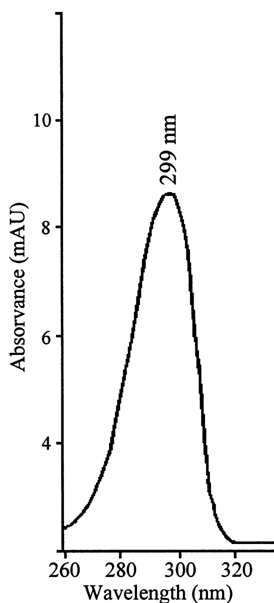


Figure 4. α-Tocopherol spectrum obtained by the PDA detector.

Downloaded At: 09:41 24 January 2011

Table 1

Content of Tocopherols (Toc) in Crude and Edible Oil Samples*

	α-Toc	β-Toc	γ-Toc	δ-Toc
		mg/100g (x ± sd)		
Canola Oil				
Crude	12.5 ± 0.25	n.d.	44.6 ± 0.55	0.4 ± 0.02
Edible	11.1 ± 0.64	n.d.	47.2 ± 0.26	0.5 ± 0.00
Sunflower oil				
Crude	40.3 ± 0.60	1.4 ± 0.02	n.d.	n.d.
Edible	41.3 ± 0.26	1.5 ± 0.05	n.d.	n.d.
Sunflower and Corn Oil Blend (70:30)				
Crude	26.4 ± 0.49	0.4 ± 0.01	15.6 ± 0.28	n.d.
Edible	26.9 ± 0.27	0.5 ± 0.02	11.4 ± 0.48	n.d.

* Values are expressed as mean ± SD of three determinations; n.d. - not detected.

In order to study the recovery of the procedure and the accuracy of the proposed method, two samples of a blend of sunflower and corn oil (70:30), crude and edible oil, were added to known quantities of α, β and γ-tocopherols, which were the tocopherols detected in the samples. The samples were analysed in triplicate before and after the addition of such compounds. The results are listed in Tables 2 and 3.

The mean recovery values for α, β and γ-tocopherols were, respectively, 92 ± 6%, 65 ± 2%, and 100 ± 2% in crude oil and 108 ± 11%, 62 ± 6% and 113 ± 2% in edible oil. The lower recovery values for crude oil compared with the edible oil can be explained by interferences effects from the matrix composition, which could not be identified. The reason for the low recovery of β-tocopherol was also not fully understood.

During the refining process the tocopherols were retained, as apparent from inspection of Table 1. Some publications refer that the processing of vegetable oils can result in losses of tocopherols and tocotrienols.[1, 8] However, the results presented herein show that the oil refining procedures were made under good manufacturing conditions and the tocopherols were not significantly lost.

Downloaded At: 09:41 24 January 2011

Table 2

Recovery of α-Toc, β-Toc, and γ-Toc from a Spiked Crude Sample of Sunflower and Corn Oil Blend

	Present µg/g	Added µg/g	Found µg/g	Standard Deviation	CV %	Recovery %
		41.4	235.4	3.0	1.3	94.6
α-Toc	207.4	96.6	289.4	6.7	2.3	95.3
		193.3	343.3	4.8	1.4	85.0
		5.5	7.2	0.1	1.2	66.2
β-Toc	5.4	11.0	10.4	0.0	0.0	62.9
		16.6	14.4	0.1	0.6	65.4
		2.5	153.5	2.4	1.6	99.0
γ-Toc	152.5	6.4	16.3	2.2	1.3	102.6
		13.0	163.9	3.6	2.2	99.3

* Values are expressed as mean ± SD of three determinations. Toc - tocopherol.

Table 3

Recovery of α-Toc, β-Toc, and γ-Toc from a Spiked Edible Sample of Sunflower and Corn Oil Blend

	Present µg/g	Added µg/g	Found µg/g	Standard Deviation	CV %	Recovery %
		40.8	360.4	3.2	0.9	116.0
α-Toc	207.1	95.4	407.5	2.4	0.6	111.5
		190.7	437.6	5.6	1.3	94.9
		5.5	8.3	0.1	1.2	68.7
β-Toc	6.6	10.9	10.2	0.1	1.3	58.3
		16.4	14.0	0.1	0.5	61.1
		2.5	98.9	0.2	0.2	110.7
γ-Toc	86.8	6.3	107.30	0.7	0.6	115.2
		12.9	113.8	3.4	3.0	114.1

* Values are expressed as mean ± SD of three determinations. Toc - tocopherol.

Downloaded At: 09:41 24 January 2011

Nevertheless, in analyzed oils only tocopherols have been detected that doesn't exclude the usefulness of this methodology when applied in other oils having tocotrienols in their compositions.

It is also clear from these results that this parameter can be helpful in the identification and the differentiation of vegetable oils. Furthermore, it may be helpful in the verification of the adulteration of such foods during quality control.

REFERENCES

1. R. R. Eitenmiller, Food Technol., **51(5)**, 78-81 (1997).

2. J. Giese, Food Technol., **49(5)**, 110-122 (1995).

3. H. S. Garewal, **Antioxidants and Disease Prevention**, CRC Press, Boca Raton, New York (1997).

4. P. C. F. S. Gama, M. B. Oliveira, M. A. Ferreira, Rev. Port. de Farmácia, **XLVIII(2)**, 61-70 (1998).

5. D. C. Liebler, J. A. Burr, L. Philips, A. J. L. Ham, Anal. Biochem. **23(6)**, 27-34 (1996).

6. G. W. Chase, Jr, C. C. Akoh, R. R, Eitenmiller, JAOCS, **71(8)**, 877-880 (1994).

7. F. Lavedrine, A. Ravel, A. Poupard, J. Alary, Food Chem., **58(1,2)**, 135-140 (1997).

8. F. Dionisi, J. Prodolliet, E. Tagliaferri, JAOCS, **72(12)**, 1505-1511 (1995).

9. R. A. Ferrari, E. Schulte, W. Esteves, L Bruhl, K. D. Mukherjee, JAOCS, **73(5)**, 587-592 (1996).

10. G. Micali, P. Curro, La Riv. Ital. Delle Sostanze Grasse, **LXI (2)**, 95-98 (1984).

11. E. J. M. Konings, H. H. S. Roomans, P. R. Beljaars, JAOAC, **79(4)**, 902-906 (1996).

12. S. L. Richheimer, M. C. Kent, M. W. Bernart, J. of Chromatogr. A, **677**, 75-80 (1994).

13. F. Yao, G. Dull, R. Eitenmiller, J. Food Sci., **57(5)**, 1194-1197 (1992).

14. Y. F. Lozano, C. D. Mayer, C. Bannon, E. M. Gaydou, JAOCS, **70(6)**, 561-565 (1993).

15. J. I. Rader, C. M. Weaver, L. Patrascu, L. H. Ali, G. Angyal, Food Chem., **58(4)**, 373-379 (1997).

16. G. L. Catignani, J. G. Bieri, Clin. Chem., **29(4)**, 708-712 (1983).

17. G. Panfili, P. Manzi, L. Pizzoferrato, Analyst, **119(6)**, 1161-1165 (1994).

18. E. J. Rogers, S. M. Rice, R. J. Nicolosi, D. R. Carpenter, C. A. McClelland, L. J. Romanczyk, Jr., JAOCS, **70(3)**, 301-307 (1993).

Received July 18, 1999
Accepted December 6, 1999
Author's Revisions April 20, 2000
Manuscript 5127

Downloaded At: 09:41 24 January 2011