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Separation and determination of tocopherols in vegetable oils by solid phase extraction on porous polymers SPE cartridges and capillary gas chromatography analysis

Research Article

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Abstract: In this study we present the solid phase extraction selectivity of tocopherols from vegetable oils using four porous polymers (Porapak P, Porapak Q, Porapak QS, Porapak N). The tocopherols elution from SPE cartridges was performed using several hexanes:ethyl acetate mixtures (100:0, 95:5, 90:10, 85:15, v/v). Tocopherols (α, γ and δ-tocopherol) were analyzed by gas chromatography without any derivation steep. The amount of NaOH used for triglyceride removal was optimized. Particularly liquid-liquid and solid phase extraction methods for the extraction of tocopherols from vegetable oils were compared. The results confirmed that porous polymers represent promising SPE alternatives for the extraction of tocopherols from oils.

Keywords: *Tocopherols • Solid phase extraction • Porous polymers • Vegetable oils • Capillary gas chromatography*

1. Introduction

Vitamin E is a generic term that defines a family of organic compounds with similar structure consisting of a chromanol core and an aliphatic chain named phythol. In nature, vitamin E exists in eight different forms: α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol. These tocopherols have been intensively studied due to their medical, biological and physiochemical significance. Usually, the role of vitamin E inside human body is to prevent unwanted oxidative processes and to stop the formation of free radicals [1].

Humans, as well as a considerable number of animal species, do not synthesize their own vitamin E, but they acquire this vitamin from a large variety of nutrients. The absence of vitamin E from nourishment leads to neuropathy, fetal death or musclular diseases and is directly connected to different regulatory processes [2].

Based on their critical significance, it is very important to design techniques able to extract tocopherols efficiently from various sources and analyze them with high accuracy and precision. The most commonly used analytical techniques for tocopherol analyses include spectrophotometry [3,4], voltametry [5,6], thin-layer chromatography, gas chromatography and high performance liquid chromatography [7-25].

Even though the tocopherols are not chemically bonded to the proteins, lipids or carbohydrates, they could be assosciated with other components of food matrices such as membranes, lipoproteins *etc.* [1,2]. There are two major sample preparation approaches for food samples: direct extraction of the tocopherols from

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the food matrix [7,14,15,17] and extraction of tocopherols after saponification of fatty material [11-13,15,17,19,21]. Saponification further assists in releasing the vitamins by degrading the food matrix and removing the bulk of fat [1]. Tocopherols and tocotrienols are relatively unstable in alkaline condition and care must be taken to avoid their destruction [15]. The most frequently used methods for tocopherol extractions involve organic solvent extraction [14-17], solid phase extraction (SPE) [10-12,18-22] and supercritical fluid extraction (SFE) [23-25]. Of all of these methods, solid phase extraction seems to be the most suitable technique for extraction of tocopherols, because it uses a low amount of organic solvent and it is in the same time a very good method for cleaning the samples and concentration of targeted species.

The materials used as supports for SPE of tocopherols are limited. Usually for SPE of tocopherols cartridges with C-8 [18], Cyclohexyl [18], C-18 [19,20], diol [20], silica gel [12,21], XAD-2 [10-11] are used and most recently molecularly imprinted polymers (MIP) [22]. The purpose of this work is to evaluate the extraction efficiency of four porous polymers in solid phase extraction of tocopherols from vegetable oils, subsequently analyzed by capillary gas chromatography.

2. Experimental Procedure

2.1 Sample materials, chemicals and standards

Pumpkin, sunflower, walnut and sesame oils have been obtained directly by pressing crushed seeds. The refined sunflower oils, virgin olive oils and refined olive oils were purchased from the grocery market.

The porous polymers: Porapak P (styrene-divynilbenzene), Porapak Q (divinylbenzene/ethyl-vinylbenzene) Porapak QS (silanized divinylbenzene / ethyl-vinylbenzene) and Porapak N (Divinylbenzene/ vinyl pirolidone) were acquired from Carlo Erba, Italy. The characteristics of these sorbents are presented in Table 1.

The standards α-, γ- and δ-tocopherol of 99.99% purity were obtained from Supelco (USA). Organic solvent (methanol, ethanol, hexane, ethyl acetate and acetone) were obtained in HPLC grade from Merck (Darmstadt, Germany).

2.2 Equipment and gas chromatography

For all GC analysis we used a Hewlett Packard gas chromatograph HP 4890 D series, equipped with a splitless injector, a fused-silica capillary column (30 m × 0.32 mm ID) coated with 1.0 μm thick film of 100% dimethylpolysiloxane (Supelco SPB-1) and a flame ionization detector (FID). Data acquisition and evaluation were achieved using the Clarity chromatographic software. The chromatographic conditions are described in detail as follows: the initial temperature was set at 140°C, followed by a gradient of 7°C min^{-1} to 310°C and held for 5 minutes. The injector and flame ionization detector temperatures were set at 250°C respectively 310°C. Nitrogen of 99.999% purity was used as the carrier gas, at a flow rate of 1.5 mL min^{-1}. Tocopherols were analyzed without any derivation step and the concentrations were determined by the calibration curve method. The calibration curve equations are given in Table 2.

2.3 Sample pretreatment

Generally, the isolation of tocopherols from oil samples involves the alkaline hydrolysis of fatty material, followed by the extraction of the tocopherols from the unsaponifiable material. In this study, for saponification we used the following ingredients: a 400 mg sample of oil was weighted in a screw-capped tube (made by Sovirel, France), 0.2 g of ascorbic acid, 15 mL of absolute ethanol and 1.5 mL of 50% sodium hydroxide solution were added under a stream of nitrogen. The tube was heated to 70ºC for 30 minutes. After cooling the tube, 3 mL of sodium chloride solution (50 g L^{-1}) was added. In this way, the tocopherols are released from the oil matrix in unsaponifiable matter and they can be easily isolated.

2.4 Liquid-Liquid Extraction

For liquid-liquid extraction of tocopherol from vegetable oils, the sample was saponified using the same protocol described above and extracted three times with 15 mL of a mixture of hexane:ethyl acetate (85:15, v/v)

Table 1. The characteristics of porous polymers used in SPE

Type of polymer	Structure	Particle size (mesh)	Specific surface (m^2 g^{-1})	Polarity of stationary phases
Porapak P	Styrene/divinylbenzene	80-100	100-200	Non-polar
Porapak Q	Divinylbenzene/ethylvinylbenzene	80-100	500-600	Weakly-polar
Porapak QS	Silanizated-Divinylbenzene/ethylvinylbenzene	80-100	500-600	Moderately polar
Porapak N	Divinylbenzene/vinylpirolidone	80-100	250-350	Polar

Table 2. The equations of calibration curves used for tocopherols quantification

Compounds	The calibration curve equations	R^2	$F_{(1,16)}$	p	s_r
α -tocopherol	A = -119.68 + 2.89c	0.9968	4926	<0.0000	67.4
γ -tocopherol	A = -59.07 + 3.51c	0.9969	5080	<.0000	84.1
δ -tocopherol	A = -90.39 + 3.10c	0.9988	13778	<0.0000	36.0

[4]. The resulting sample was evaporated to dryness under nitrogen and the residue was dissolved in 2 mL of the same mixture and analyzed by capillary gas chromatography.

2.5 Solid-Phase Extraction

The porous polymer SPE cartridges were prepared in the laboratory and consist of a 4 mL syringe filled with 0.5 g of porous polymer. Right before extraction, the cartridges were conditioned with acetone and methanol.

To evaluate the porous polymers SPE selectivity, 10 µL of a standard solution containing 133 µg α-tocopherol, 83 µg γ-tocopherol, 105 µg δ-tocopherol dissolved in methanol was applied on the cartridge and eluted with 2 mL of a solvent mixture consisting of a non-polar (hexane) and a polar (ethyl acetate) solvent. The resulting samples were evaporated to dryness under nitrogen and the residue was dissolved in 100 µL of methanol and analyzed by capillary gas chromatography.

From vegetable oils, the tocopherols were extracted from unsaponifiable matter on Porapak Q with a flow rate of 2 mL min^{-1}. After that, the SPE cartridges were washed with distilled water until free of sodium hydroxide. Next, the retained compounds (tocopherols) were eluted with 2 mL of hexane:ethyl acetate mixture (90:10, v/v) and analyzed by capillary gas chromatography.

3. Results and Discussion

3.1 Extraction efficiency of porous polymers

Extraction efficiency of porous polymers was investigated by considering the analytes recovery after SPE procedure. Here, the mixtures consisting of a non-polar (hexane) and a polar (ethyl acetate) solvent can yield satisfactory recovery rates. Several ratios of hexane:ethyl acetate (100:0, 95:5, 90:10 and 85:15 v/v) were tested to improve the overall recovery rates. Overall, we determined that the optimal composition depends largely on the polymer polarity. The influence of the solvent mixture composition on the recovery rates of tocopherols are illustrated in Fig. 1.

As illustrated in Fig. 2, the non-polar and moderately polar polymers (*e.g.* Porapak P, Porapak Q and Porapak QS) can be successfully used in solid phase extraction of tocopherols, while more polar porous polymers (*e.g.* Porapak N) provide inferior results based on a very strong absorption that occurs at their surface. For each polymer and solvent mixture, the experiments were performed three times and the calculated RSD was under 5%. The limits of quantification (LQ) calculated based on calibration curve equations [26] were 6.24 mg Kg^{-1} α-tocopherol, 6.69 mg Kg^{-1} γ-tocopherol and 8.09 mg Kg^{-1} δ-tocopherol.

3.2 Optimization of saponification

The amount of sodium hydroxide used for saponification is crucial for tocopherol stability. Usage of a large quantity of sodium hydroxide leads to analyte loss through certain oxidation processes, even in the presence of an antioxidant (ascorbic acid) and nitrogen. The quantitative and qualitative composition of the tocopherols was determined by performing SPE followed by capillary gas chromatography analysis. The losses of tocopherols during the saponification process have been studied using different amounts of sodium hydroxide and following the same protocol as described above. For this purpose, in a screw-capped tube, 15 mL of absolute ethanol was spiked with a known amount of tocopherols and the resulting sample was subjected to the saponification process using different amounts of 50% sodium hydroxide solution (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mL). After saponification, the tocopherols were extracted on Porapak Q and analyzed by capillary gas chromatography. Table 3 illustrates that losses during the saponification process are directly dependent to the amount of sodium hydroxide and on the type of isomers used.

The most unstable isomer is δ-tocopherol that was 90 % oxidized by an addition of 3 mL 50% sodium hydroxide solution, followed by γ- and α-tocopherol. Taking into account the isomer molecular structure, it seems that the stability of tocopherols isomers during the saponification process increases with the number of methyl groups directly linked to phenolic ring. In conclusion, the amount of 1.5 mL sodium hydroxide solution (50%) does not affect the stability of tocopherols and the recoveries are around 100% for all isomers. In addition, a volume smaller than 1.5 mL of sodium hydroxide solution (50%), is not enough for an efficient alkaline saponification of fatty matrices.

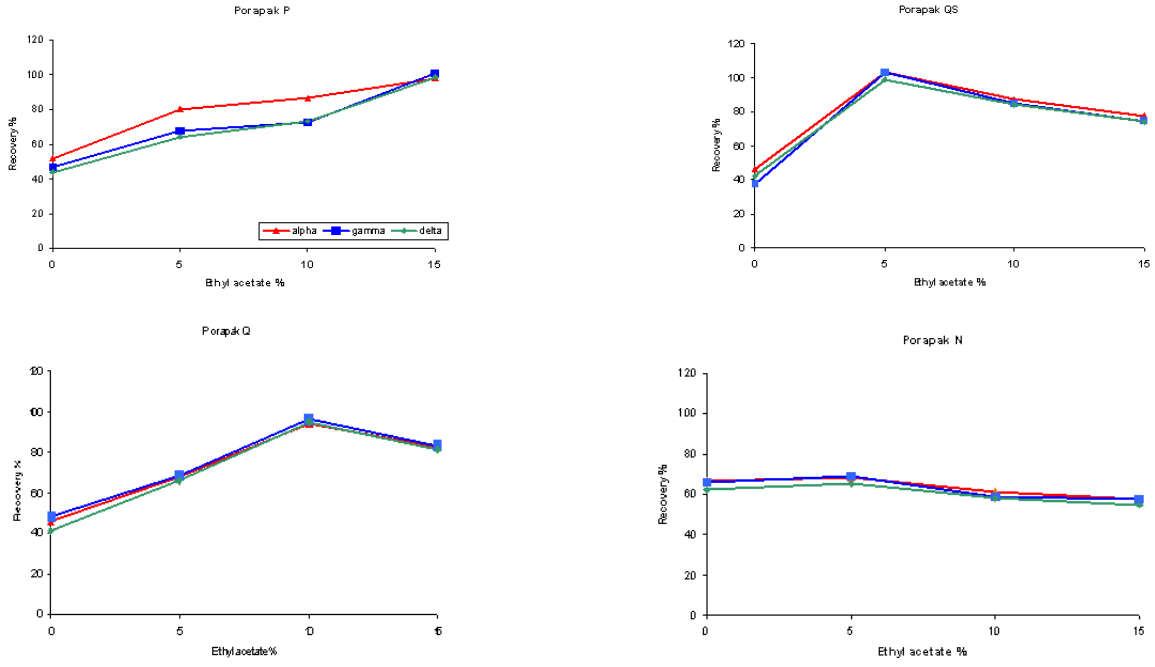

Figure 1. Influence of ethyl acetate addition on tocopherols recovery.

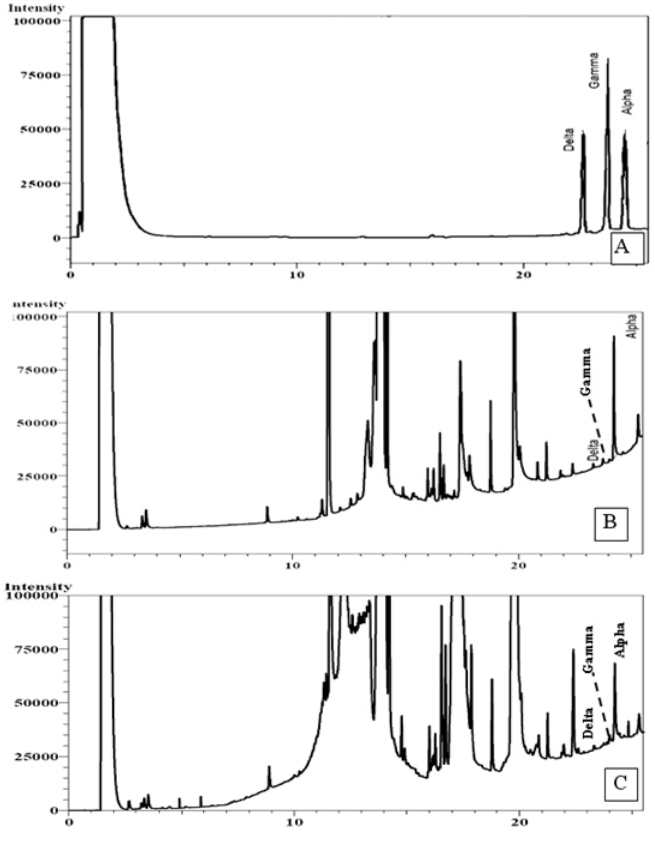

Figure 2. The chromatograms of tocopherols standards (A) and sunflower oil extract obtained by SPE with Porapak Q (B) and liquid-liquid extraction (C).

Table 3. The influence of NaOH addition on the tocopherols recovery

Compounds	Amount added (μg)	NaOH solution 50% (mL)	Amount found (mean) (μg)	Recovery (%)
δ-tocopherol	210	0.5	212	100.9
γ-tocopherol	166		167.3	100.8
α-tocopherol	266		268	100.7
δ-tocopherol	210	1.0	209	99.5
γ-tocopherol	166		166.3	100.2
α-tocopherol	266		268	100.7
δ-tocopherol	210	1.5	204.2	97.2
γ-tocopherol	166		164.3	98.9
α-tocopherol	266		265	99.6
δ-tocopherol	210	2.0	121.8	58.0
γ-tocopherol	166		116.0	69.9
α-tocopherol	266		224.5	84.4
δ-tocopherol	210	3.0	26.7	12.7
γ-tocopherol	166		54.4	32.8
α-tocopherol	266		158.8	59.7

Table 4. Tocopherols content of several vegetal oils, extracted with Porapak Q SPE cartridges and GC analysis

Type of oil	Tocopherols content (mg kg^{-1})			Total tocopherols (mg kg^{-1})
	α-	γ-	δ -	α+γ+δ
Pumpkin oil	42.5	119.1	361.8	523.4
Olive oil – virgin	530	48.5	825	1403.5
Olive oil – refined	365	45.2	330.6	740.8
Sunflower oil – refined	416.4	10.6	11.4	438.4
Sunflower oil – unrefined	678.4	7.5	26.3	712.2
Walnut oil	28.3	127.6	22.8	178.7
Sesame seed oil	1436.4	172.1	9.3	1617.8

3.3 The comparison of liquid-liquid extraction and SPE on Porapak Q

Liquid-liquid and solid phase extraction methods for the extraction of tocopherols from vegetable oils were compared. For this purpose, two samples of sunflower oil were pretreated in the same way as described above. After saponification of the fatty matrix, one sample was subjected to liquid-liquid extraction using a mixture of hexane:ethyl acetate 85:15 v/v [4] while the other sample was extracted by SPE using Porapak Q as sorbent. The resultant extracts were analyzed by capillary gas chromatography. As can be seen in fig. 2 the solid phase extraction of tocopherols from unsaponifiable matter using Porapak Q proves to be a very good method for cleaning the samples and for the concentration of targeted species compared liquid-liquid extraction.

3.4 Quantitative analysis

The developed method was applied for analysis of tocopherol content in seven different vegetable oils by SPE on Porapak Q after alkaline saponification of fatty matrix and subsequently by capillary gas chromatography analysis. The quantitative and qualitative compositions of the individual tocopherols were determined based on calibration curve method.

As can be seen in Table 4, the results showed that the tocopherol content and the type of structural isomer is related to the origin of the raw material from which it was obtained.

According to our data, sesame seed and olive oils appear to have the highest tocopherol content. Taking into account the tocopherol content as well as the type of isomers, the results obtained in this work are comparable with other results from literature [12,14,16,17,21].

Repeatability of the quantitative results was examined by consecutive injections of identical sunflower oils and

by repeating the entire analysis sequence, including sample preparation. The RSD in this case was under 5%.

4. Conclusions

The separation of minor components from oil matrices by capillary gas chromatography involves an essential step of alkaline saponification necessary to remove the bulk of lipid material. We have determined that the amount of sodium hydroxide used for triglyceride removal is crucial and the tocopherol losses and may be reduced by using an optimum volume of solution with a determined concentration. We also determined that during the saponification process the stability of tocopherol isomers increases with the number of methyl groups directly linked to the phenolic ring.

The solid phase extraction of tocopherols from unsaponifiable matter using Porapak Q SPE cartridge proved to be a very good method for cleaning the samples and for the concentration of targeted species compared to solvent extraction.

Most of the Porapak porous polymers offer good selectivity for tocopherols as well as good chemical stability during the extraction process. Therefore, non-polar and moderately polar polymers such as Porapak P, Porapak Q and Porapak QS can be successfully used in the solid phase extraction of tocopherols. Polar polymers such as Porapak N provided less capable results due to a very strong absorption that occurs at their surface.

The excellent efficiency of porous polymers for tocopherol recovery and the associated excellent repeatability of the analysis yields great promise as an alternative to traditional supports (C-18, C-8, diol, silica gel) for solid phase extraction of tocopherols.

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