Facile Enrichment of Tocopherols by Automated Flash Chromatography

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Abstract

Tocopherols are fat soluble vitamins with antioxidant activity found in most vegetable oils. Analysis of tocopherols presents a challenge due to their relatively low concentration and chromatographic similarity to the oil matrix. A single-step method using an automated Flash chromatography system is described to remove the tocopherols directly from the oil matrix that allows easy analysis by HPLC other analytical methods.

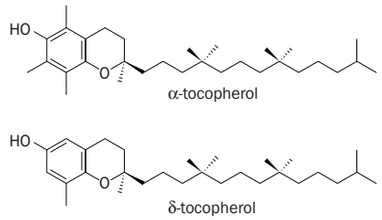
Background

Tocopherols are present in low quantities in plant oils used as biodiesel feedstock. A common method for removing these compounds from the oil is saponification of the glycerol fatty acid ester followed by extraction into diethyl ether[1]. The saponification is time consuming and can incur high disposal costs.

Experimental

All experiments were run with a Teledyne Isco Combi*Flash* Rf 4x system (68-5230-006, 68-5237-046), Lincoln, NE. Vegetable oils used included soybean oil (Wesson, Con Agra foods, Omaha, NE), corn oil and peanut oil (Associated Wholesale Grocers, Kansas City, KS). A mixture was prepared containing 15 g of oil dissolved in methylene chloride (ACS grade, Burdick & Jackson) and mixed with 60 g celite 545 (Acros). Additional methylene chloride was added to make a slurry and the mixture was then evaporated under vacuum to make a free-flowing powder. Loading the sample onto celite allows for easier handling and easier automation on the Combi*Flash* Rf system. Chromatography solvents were ACS grade from Burdick & Jackson.

A weighed portion (sample size 15 g, 3 g oil) of the powder was loaded into a solid load cartridge (Teledyne Isco, part number 69-3873-240) and run on a cyano or diol column as described in the following sections.



Common tocopherols found in vegetable oils

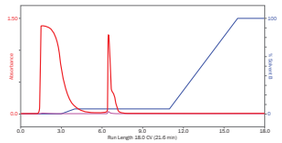
References

1. Adidi, S.L. *J. of Chrom. A.*, **2000**, *881*, 197.
2. Oomah, B.D.; Kenaschuck, E.O.; Mazza, M. *J. Agric. Food Chem.* **1997**, *45*, 2076

Diol Column

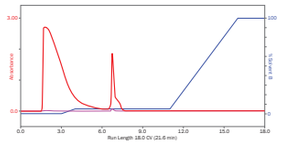
A 50 g Redi*Sep* Rf diol column (Teledyne Isco part number 69-2203-373) was washed with isopropanol followed by hexane. The column was run at 40 mL/min. The column was run with 100% hexane for 3 column volumes after injection followed by a step to 5% isopropanol in hexane for 8 column volumes. This was followed by a gradient to 100% isopropanol to clean the column. The fractions were collected and dried under vacuum.

Fractions were evaluated by TLC, HPLC, and UV-vis spectroscopy.

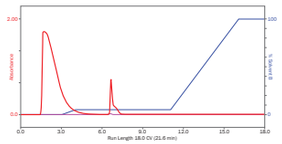


Purification of tocopherols from corn oil

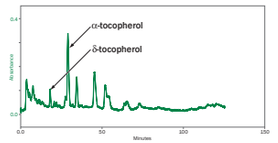
TLC of corn oil (from left to right): α-tocopherol, enriched tocopherol mixture, and δ-tocopherol



Purification of tocopherols from soy oil

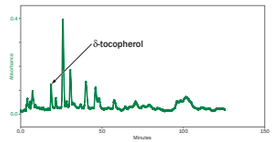


Purification of tocopherols from peanut oil



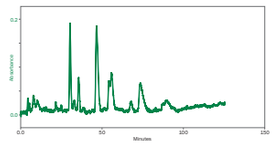
Analytical HPLC of enriched tocopherols purified on RediSep diol column from corn oil

Tocopherols identified by comparison with reference compounds. Yield 0.1457 g (4.9% from 2.992 g oil).



Analytical HPLC of enriched tocopherols purified on RediSep diol column from corn oil

δ-tocopherol identified by comparison with reference compounds. Yield 0.1597 g (5.3% from 3.00 g oil).



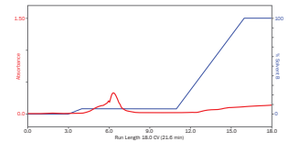
Analytical HPLC of enriched tocopherols from peanut oil

Reference tocopherols not visible in this HPLC. Yield not measured

Cyano Column

A 50 g Redi*Sep* Rf cyano column (Teledyne Isco part number 69-2203-363) was washed with acetone followed by hexane. The column was run at 40 mL/min. The column was run with 100% hexane for 3 column volumes after injection followed by a step to 5% acetone in hexane for 8 column volumes. This was followed by a gradient to 100% acetone to clean the column. The fractions were collected and dried under vacuum.

Fractions were evaluated by TLC, HPLC, and UV-vis spectroscopy.



Tocopherol reference compounds on a cyano column.

Compound elution time would not allow clean enrichment from the oil matrix.

Results and Discussion

The diol column system purified the tocopherol compounds better than the cyano column system. The compounds in the diol column were separated better from the oil than from the cyano column. The oils would elute first in both the cyano and diol column system followed by the desired compounds. The columns were immediately able to be reused for another sample. These columns consist of a short carbon chain bonded to silica gel with a cyano or diol functional group at the free end. The functional groups allow use as a normal or reverse phase column while the carbon chain allowed some control over retention.

Silica gel gave poor resolution of the tocopherols from the oils — there was little control over the retention of the tocopherols. Only a small amount of a polar solvent would release the desired compounds. Reverse phase C18 also performed poorly. The tocopherols would elute, leaving the oils behind. The oils were difficult to remove from the column after completion of the experiment. The oils would elute slowly causing varying baselines during subsequent runs if the column were not properly cleaned during the run.

Yields were about 5% by weight oil. Flaxseed oil, for example, contains 0.046% pure tocopherols[2] suggesting further purification is required to provide pure compounds. The method provides an oil of sufficient purity for analytical purposes

Conclusion

Flash chromatography using cyano and diol columns presents an easy method to enrich tocopherol compounds for further analysis. There is no need for heating the compounds for saponification. The Flash chromatography system also reduces the number of steps required to prepare the sample for analysis. The columns are reusable so cost is limited.

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