Quantifying Vitamin E in vegetable oils with reversed-phase high performance liquid chromatography

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Abstract

Vitamin E is an important antioxidant in the human diet and comes in two primary structures: tocopherols and tocotrienols. Reversed phase high performance liquid chromatography was used to separate and quantify α- and δ-tocopherols in canola and soybean oil. Using the novel methanol extraction and a methanol/water mobile phase method of Gimeno et.al, the tocopherols were resolved with complete baseline separation. Canola oil was determined to contain 19 ± 2.3 ppm α-tocopherol and 22.9 ± 0.65 ppm δ-tocopherol. Soybean oil was determined to contain 16 ± 2.3 ppm α-tocopherol and 147 ± 1.8 ppm δ-tocopherol.

Introduction

Vitamin E is a fat-soluble molecule that is essential in the human diet. Once absorbed by the blood stream, vitamin E works to prevent oxidative reactions of unsaturated fatty acids and other biomolecules. Protection against oxidation reactions is important to human health because oxidants can form free-radical molecules and become mutagenic to DNA.\(^1\)

Vitamin E deficiency is caused by mutations of tocopherol transfer protein, and a low dietary intake of the vitamin.\(^2\) Symptoms of low vitamin E levels include ataxia, anemia caused by the breakdown of red blood cells and peripheral neuropathy. Vitamin E deficiency studies conducted on rats also caused changes in retina color.\(^1\)

Tocopherol, Fig. 1, and tocotrienol, Fig. 2, are two forms that vitamin E is found. Each of these forms can be further subdivided into α, β, δ, and γ forms and all have differing antioxidant potency.\(^3\) γ-Tocopherol is the primary form of vitamin E found in a normal diet in the United States, but this form is ultimately converted to α-tocopherol once inside the body.\(^4\)

α and δ tocopherol are found in large quantities in vegetable oils. Canola oil contains 0.021% wt or 192 ppm α-tocopherol and 0.394 ppm δ-tocopherol. Soybean oil contains 0.0075% wt or 69 ppm α-tocopherol and 0.0266% wt or 245 ppm δ-tocopherol.\(^3\) These values have been obtained primarily using high performance liquid chromatography (HPLC) in both reverse and normal phase. Normal phase HPLC has the benefit of being able
to separate all derivatives of tocopherol whereas reversed phase can only separate α, δ, and a third peak containing γ+β.\textsuperscript{5} HPLC has not been shown to be effective at separating tocotrienols.

In this experiment, we quantified both α and δ tocopherols in Crisco® brand canola and soybean oils following the method of Gimeno et.al. using reversed phase HPLC.

**Experimental**

**Standards Preparation**

A stock solution of δ-tocopherol (F.W. 402.65 g/mole) was prepared by placing 60.3 mg of δ-tocopherol in a 25-mL volumetric flask and diluting to the mark with ethanol (5.99 \times 10^{-3} \text{ M}). Five standard solutions, concentrations of 5.00, 10.00, 15.00, 20.00, and 25.00 ppm, were prepared by adding 20.7 µL, 41.5 µL, 62.2 µL, 82.9 µL, and 103.6 µL, respectively, of stock solution to 10 mL volumetric flasks and diluting to the mark with ethanol. A stock solution of α-tocopherol (F.W. 430.71 g/mole) was prepared by placing 100 mg of α-tocopherol in a 50 mL volumetric flask and diluting to the mark with ethanol (4.64 \times 10^{-3} \text{ M}). Five standard solutions with concentrations of 43.07, 86.14, 129.21, 172.28, and 215.36 ppm were made by adding 215.4 µL, 430.8 µL, 646.1 µL, 861 µL, and 1076.9 µL, respectively, to 10-mL volumetric flasks and diluting to the mark with ethanol. The ten standard solutions were then pipetted into ten individually labeled vials (δ1-δ5 and α1-α5).

**Sample Preparation**

The samples analyzed were Crisco® brand canola oil and soybean oil. Four trials of each oil were prepared for a total of eight trials. Tocopherol was extracted into methanol from the oil using a 1:3 ratio of oil to methanol in a 2-mL microcentrifuge tube. The sample was then vortexed and centrifuged at 3000 rpm for five minutes. A 0.45-µm pore size syringe filter was used to remove the remaining oil and the samples were placed into individually labeled vials C1-C4 and S1-S4.

**Instrumentation**

A Varian ProStar HPLC system was used to carry out the separations. This system consists of a piston-based pump, an autosampler, a reversed-phase chromatographic column (Kinetex 50 × 4.6 mm), and a variable-wavelength UV-vis spectrophotometric detector. The column was housed in a column heater at 35°C, and the mobile phase was a 95:5 ratio of methanol to ultra-pure water (double-deionized water that was vacuum filtered). Analysis was carried out with a mobile phase flow rate of 1 mL/min with detection at 292 nm. Each run had an injection volume of 10 µL and a run time of five minutes.
Data Analysis

Chromatograms for the eight samples and ten standards were collected. Calibration plots were constructed in which area was plotted versus concentration.

Results and Discussion

Peak areas from the chromatograms in Fig. 3 were used to make calibration curves seen in Fig 4. The α tocopherol standards displayed a retention time shift as concentration increased. This could be due to changes in pressure of the column and could potentially be eliminated in future trials with the same concentrations.

Figure 3. α-tocopherol standard (left) and δ-tocopherol standard (right)

Figure 4: Calibration curves for α (left) and δ tocopherol (right) generated in Microsoft Excel.

Error in the calibration curves was calculated using the propagation of uncertainty with a calibration curve method of Harris. As can be seen in Fig.5, the left most peak, appearing at approximately 2.7 min, is associated with δ tocopherol. The right most peak, appearing at approximately 3.85 min, is associated with α tocopherol. The large peak between the α and δ peaks is likely the β+γ forms of tocopherol which cannot be separated by reversed phase HPLC.
Canola oil was observed to contain $19 \pm 2.3$ ppm $\alpha$-tocopherol and $22.9 \pm 0.65$ ppm $\delta$-tocopherol while soybean oil was observed to contain $16 \pm 2.3$ ppm $\alpha$-tocopherol and $147 \pm 1.8$ ppm $\delta$-tocopherol. These values differ by $90.0\%$, $5700\%$, $76.8\%$, and $40.0\%$ respectively from the literature values for tocopherol in vegetable oil. These values were obtained using calibration curves of both $\alpha$ and $\delta$-tocopherols comparing peak area (mAU/min) vs. the concentration of the standard.

One peculiar observation is the oddly low observed concentration of $\alpha$-tocopherol in both oil samples. This could be due to several factors ranging from an incomplete extraction of tocopherol to degradation of tocopherol within the oil. Although we were able to reproduce the chromatograms with high precision, a considerable amount of tocopherol may remain in the oil. Many more separately prepared trials will need to be conducted to eliminate this discrepancy. The observed amount of $\delta$-tocopherol in canola is considerably larger than the literature value. The canola oil that was used in this experiment was not virgin oil and contained an added amount of tocopherol that was observed in the $\delta$ form. A tocopherol concentration literature value for the canola oil used in this experiment could not be found.

With concentrations and absorbencies being rather low, standard additions may be beneficial in determining the concentrations of tocopherols. This would allow quantification of a smaller concentration and minimize matrix effects of the instrument.
Several major adjustments were made to the method of Gimeno et al. The first was the dilution factor of the oil samples. After several trials, we determined that diluting the samples did not increase peak separation as stated in Gilmeno et al. Rather, we prepared our samples by a single extraction of oil to methanol 1:3 v/v. The second adjustment that was made was the temperature of the column. Rather than the 45°C of Gimeno et al., 35°C was used and better peak separation was observed. The final and most important changes were made to the instrument itself. A different column was used as well as a different injection volume. Both of these changes produced identifiable, separated, and symmetric peaks.

A number of factors contributed to the error in the experiment. First of all, the tocopherol that was ordered came as a highly viscous liquid. Despite being approximately 100 mg each (noted by Supelco™), a problem was presented in weighing and transferring the tocopherols accurately. For the α-tocopherol, an exact weight was not determined; it was assumed to be 100 mg. After a failed attempt at mixing a δ-tocopherol stock solution, a more accurate weight was determined by weighing the container before and after transferring. Error could have also arisen due to improper storage of the tocopherols. The packaging suggested storing at −20°C, but they were kept at room temperature. This could possibly explain the large peak near the solvent front and could be a result of degradation. Another source of error may have come from a lack of extractions when preparing the sample solutions. Additional extractions would increase the concentration of tocopherol in the sample and may possibly produce larger peaks.

Conclusions

Reversed phase HPLC, under the proper conditions, is an effective tool to separate and quantify tocopherol species within vegetable oils. The method of Gimeno et al. was a useful starting point for this experiment but was not completely reproducible in our laboratory. Canola oil was determined to contain 19 ± 2.3 ppm α-tocopherol and 22.9 ± 0.65 ppm δ-tocopherol. Soybean oil was determined to contain 16 ± 2.3 ppm α-tocopherol and 147 ± 1.8 ppm δ-tocopherol.

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References

3. Anonymous In vitamin E; Benders’ Dictionary of Nutrition and Food Technology; Woodhead Publishing Ltd: Cambridge, United Kingdom, 2006.
