DEVELOPMENT OF AN ADVANCE METHOD FOR THE DETERMINATION OF TOCOPHEROLS IN EDIBLE OILS

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Abstract. We have developed a method for the determination of tocopherols in different edible oils by RP-HPLC, using a FLD detector, a Poroshell column as a stationary phase, and a mixture of acetonitrile, methanol and water (5:4.5:0.5) as mobile phase (flow rate 0.75 ml/min). The structure of the compounds was identical, so it could not be separated by reverse phase liquid chromatography. The obtained peaks show an important overlapping and we have used chemometric tools to get a calibration model, which assumes a linear behavior, i.e., the total signal is the sum of individual signals of components. Recovery percentages were between 90.3% and 95.7%. The method has been successfully applied to edible oils and dietary supplements. No pretreatment of the samples was needed.

Keywords: tocopherols, HPLC, sunflower oil, pumpkin oil,

INTRODUCTION

Cold pressed oils are a growing market due to changes in the diet of consumers and their knowledge of food quality and safety. The demand for these oils is constant throughout the year, but the production is seasonal, which makes them no longer widely available in animate periods. The high price at which these oils are sold, offers a significant opportunity to earn, especially if the products sold are counterfeited by various methods (Racolta et al., 2002). There are two main ways in which oils are adulterated: 1. By declaring the oil to be cold pressed, when in fact a high temperature or even solvent technique was used to extract the oil; 2. By declaring the oil to be pure, not a mixture, but in its composition they have different percentages of one or more types of oil (EU, 2014).

The adulteration of oils has a direct effect on the health of the consumers, but also on the producers of non-counterfeit oils, due to the unfair competition created by the counterfeit products and the decrease of the consumers confidence in the authenticity of the products (Thi Kieu et al., 2015).

Vitamin assays in foods are carried out for several purposes, among them, to provide data for food composition and for quality assurance. Eight vitamers of vitamin E occur in nature: four tocopherols and four tocotrienols. Tocopherols are methyl-substituted derivatives. Tocopherols and tocotrienols are designated as a-, b-, c- and d according to the number and position of the methyl groups in the chromanol ring. The role of vitamin E in the body can be explained in general as a lipid antioxidant in stabilizing subcellular membranes, but it is worth noting that every vitamer varies widely in biological activity (Zhang et al., 2020). It is well known that vitamin E is present in a wide range of foods naturally, including green leafy vegetables and fatty foods such as vegetable oils, nuts, seeds, and egg yolk.

Tocopherols and tocotrienols represent a class of benzopyranols (or methyl tocols) that are synthesized in plants and other photosynthetic organisms, where they have many important functions. One form, α-tocopherol, is recognized as having biological activity at humans. A-tocopherol deficiency is called ataxia and is characterized by very low concentrations of α-tocopherol in plasma (Webera et al., 2020). Although the pathogenetic mechanism is not yet known, the disease is manifested by muscle weakness, coordination difficulties, tingling and numbness, impaired vision and problems with the immune system (Bi et al., 2019). The evolution of the disease can be prevented only by administering this form of tocopherol. Tocopherols are essential compounds, but the body cannot synthesize. Thus, they must be supplied through the diet in small amounts. Vegetable oils are a major dietary source of tocopherols for humans (Bakre et al., 2015). They have a high nutritional value, and also help to prevent oxidation of lipids, which would result in the formation of undesirable compounds producing oil deterioration (Elisia et al., 2013). Depending on the source of the oils, the ratio between the main types of tocopherols (α, β, γ, δ) differs, thus being able to establish a tocopherol profile specific to the type of oil (Diaz et al., 2007).

Different methods related to the analytical determination of tocopherols in edible oils have been described in literature (Robledo et al., 2013), most of which require a preliminary stage of extraction combined with separation techniques, being the most used the HPLC chromatography (Li et al., 2017).

The purpose of the study is to develop a method for the determination of tocopherols in different edible oils that can be used for their authentication since every oil has specific tocopherol content depending on the raw material it was made from.

MATERIAL AND METHODS

Reagents and standards

ISO-propanol, methanol and acetonitrile where HPLC Grade from Merk. All standards used for the calibration curve were from Sigma Aldrich: (±)-α-Tocoferol, sintetic, ≥96% (HPLC), 100G, rac-β-Tocoferol, (+)-γ-Tocoferol ≥96% (HPLC), 100MG, δ-Tocopherol. Ultra-pure water was obtained using a water filtration system from Siemens.

Determination of tocopherols

Due to the sensitivity of the compounds to UV, it is essential that the extraction of the samples be done in a short time, and dark colored bottles be used during this process. The extraction stages are dilution 1: 100 with iso-propanol and filtration on a 45 µm cartridge.

Perkin Elmer 200 Series High Performance Liquid Chromatograph (HPLC) with fluorescence detector was used to determine the tocopherols. The mobile phase was a mixture of acetonitrile, methanol and water 5:4.5:0.5 in isocratic mode at a flow rate of 0.75 ml/min. Injection volume was 5 µl. For the chromatographic separation a Poroshell 120, EC-C18, 3.0x150 mm, 2.7 µm column was used and the temperature of the column oven was set at 30 ^{o}C. The fluorescent detector (FLD) was set at 290 nm excitation and 330 emission wave lengths.

RESULTS AND DISCUSSION

Due to the almost identical structure and properties of the compounds β-tocopherol and γ-tocopherol the two could not be separated by reverse phase liquid chromatography (RP-HPLC) and they will be quantified as the sum. Calibration curves were performed at concentrations of 5, 10, 25 and 50 mg/l. (fig 1-3)

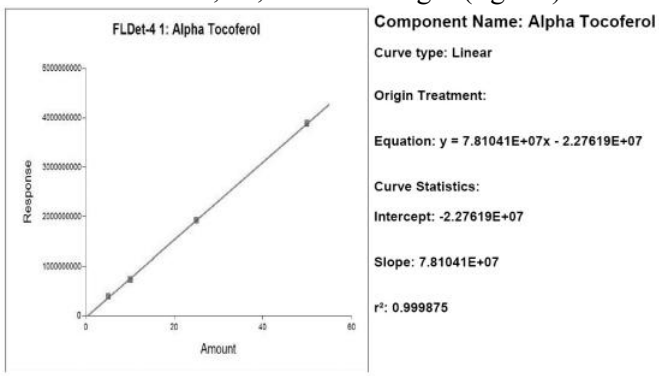

Fig. 1. Calibration curve parameters for α -tocopherol

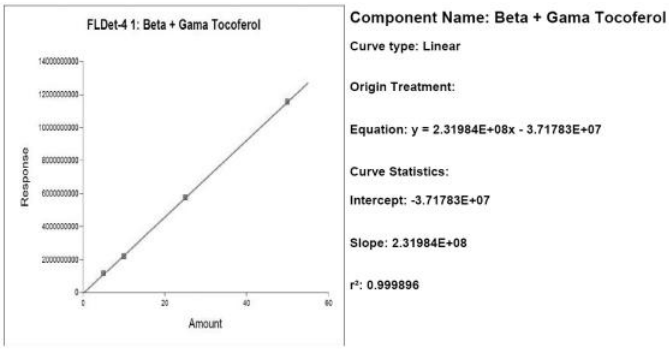

Fig. 2. Calibration curve parameters for β-tocopherol + γ-tocopherol

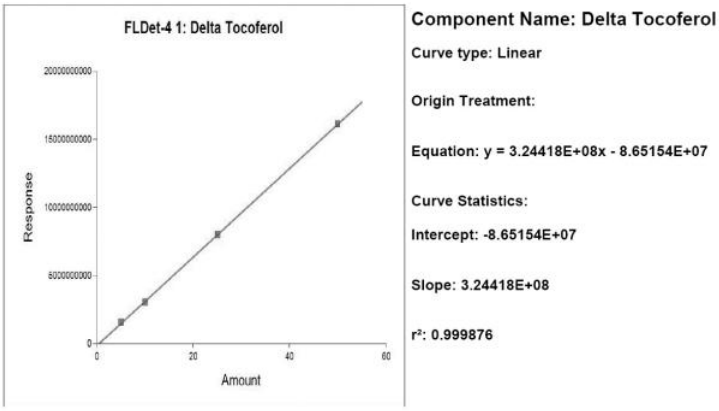

Fig. 3. Calibration curve parameters for δ-Tocopherol

The integration features of the peaks specific to this method are:
• grouping factor: 8
• area threshold: 68,026.25
• background noise threshold: 13,605.25
Recovery of the compounds is between 90.3-95.7%.

Validation of the method

Validation of the method was done using sun cold press sunflower oil and pumpkin seed oil available on the Cluj-Napoca Market. (fig 4 -5, table 1)

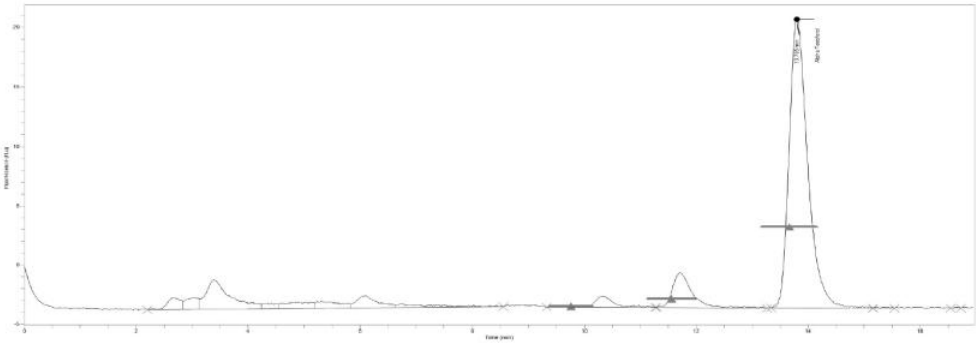

Fig. 4. Tocopherol chromatogram for cold press sunflower oil

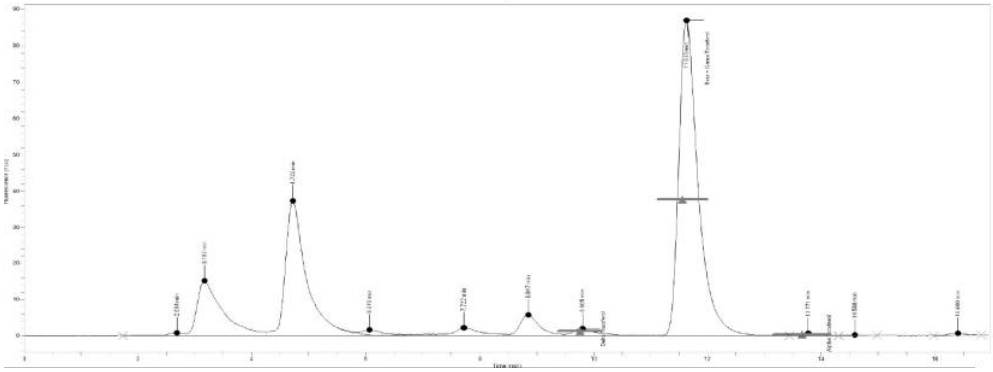

Fig. 5. Tocopherol chromatogram for pumpkin oil

Table 1

Concentration of tocopherol in edible oils

Crt. no.	Oil type	Concentration mg/l			
		α-Tocopherol	β-Tocopherol + γ - Tocopherol	δ-Tocopherol	Total
1	Cold press sunflower oil	749.24	47.12	27.99	824.35
2	Pumpkin seed oil	50.99	902.73	45.33	999.05

CONCLUSIONS

Each calibration curve had a slope above 0.9998, which proves that the method developed is accurate and with high repeatability and reproducibility. Because the extraction of the samples has only 2 steps, the recovery for each of the tocopherols is high and very similar. There is a clear difference in tocopherols profile between the types of oils tested. Pumpkin oil has the highest concentration of β-Tocopherol + γ-

Tocopherol 902.73 mg/l and the lowest concentration has sunflower oil 47.12 mg/l. The highest concentration of α-Tocopherol is present in sunflower oil 749.24 mg/l and the lowest in pumpkin oil 50.99 mg/l.

The results obtained prove that method developed can be successfully used for the evaluation of the tocopherol profile of different edible oils.

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REFERENCES

1. Bakre S. M., Gadmale D. K., Toche R. B., and Gaikwad V. B. (2015). Rapid determination of alpha tocopherol in olive oil adulterated with sunflower oil by reversed phase high-performance liquid chromatography. J Food Sci Technol. 52 (5): 3093–3098.

2. Daniela Webera, Bastian Kochlika, Ilja Demuthc, Elisabeth Steinhagen-Thiessenc, Tilman Grunea, Kristina Normanf. (2020). Plasma carotenoids, tocopherols and retinol - Association with age in the Berlin Aging Study II. Redox Biology 32: 101461

3. European Parliament. (2014). Reporte On the food crisis, fraud in the food chain and the control there of, Rapporteur (chair). Esther de Lange, Committee on the Environment, Public Health and Food Safety.

4. Ingrid Elisia, John W. Young, Yvonne V. Yuan, David D. Kitts. (2013). Association between tocopherol isoform composition and lipid oxidation in selected multiple edible oils. Food Research International 52: 508–514

5. Jun Li, Yanlan Bi, Shangde Sun, Dan Peng. (2017). Simultaneous analysis of tert-butylhydroquinone, tert-butylquinone, butylated hydroxytoluene, 2-tert-butyl-4-hydroxyanisole, 3-tert-butyl4-hydroxyanisole, a-tocopherol, c-tocopherol, and d-tocopherol in edible oils by normal-phase high performance liquid chromatography. Food Chemistry 234: 205–211

6. Liming Zhang, Zhanli Liu, Yang Sun, Xiaomin Wang, Ling Li. (2020). Effect of α-tocopherol antioxidant on rheological and physicochemical properties of chitosan/zein edible films. LWT - Food Science and Technology 118: 108799

7. Racolta Emil, Crina Mureşan, Tehnologia uleiului şi margarinei, (2002). Editura AcademicPres Cluj-Napoca. ISBN 973-8266-80-7.

8. Sebastián Noel Robledo, Vanessa Gimena Lourdes Zachett, María Alicia Zon, Héctor Fernández. (2013). Quantitative determination of tocopherols in edible vegetable oils using electrochemical ultra-microsensors combined with chemometric tools. Talanta 116: 964–971

9. T. Galeano Dıaz, M.I. Rodriguez Ca´ceres, B. Roldan Murillo. (2007). Chemometrics for the resolution of co-eluting peaks of b- and c-tocopherols in RP-HPLC: Determination in edible oils and dietary supplements. Food Chemistry 105: 1583–1590

10. Thi Kieu Tiên Do, F. Hadji-Minaglou, S. Antoniotti, X. Fernandez. (2015). Authenticity of essential oils. Trends in Analytical Chemistry 66: 146–157.

11. Xinyan Bi, Yi Ting Loo, Penny Liu Qing Yeo, Christiani Jeyakumar Henry. (2019). Are α-tocopherol levels associated with improved glycaemia?. Journal of Nutrition & Intermediary Metabolism 18: 100110