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СЕКЦІЯ: ХАРЧОВА ХІМІЯ, БІОХІМІЯ, БІОТЕХНОЛОГІЯ ТА ФУНКЦІОНАЛЬНІХАРЧОВІ ПРОДУКТИ

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MODIFICATION OF THE FLUORIMETRIC METHOD OF DETERMINATION OF VITAMIN E (A-TOCOPHEROL)

Vitamin E, as one of the important biologically active vitamins in the human diet, consists of two different compounds known as tocopherols and tocotrienols, which are produced by plants and serve as an antioxidant that scavenges free radicals. It is considered the most common fat-soluble antioxidant present in the tissues of cell membranes and plasma of higher mammals and humans [Huang J., et al, 2019; Ozsoz M et al., 2019].

Various forms of vitamin E and its derivatives can act as modulators of enzymes mainly involved in signal transmission, affect gene expression (for example, redox-regulated). In addition, tocopherols are able to interact with cellular lipids and other molecules, including DNA, protecting them from oxidation or peroxide damage [Galli F., Azzi A., Birringer M., et al., 2017]. The antioxidant activity of vitamin E consists in suppressing or inhibiting lipid oxidation by stopping the chain reaction of ROS (radical oxygen species), which are formed as a result of radicals in both cellular and subcellular membranes. Tocopherol inhibits the peroxidation of polyunsaturated fatty acids (PUFA) [Ozsoz M, et al, 2019]. It is known that the consequences of deficiency states of vitamin E are cellular destruction of erythrocyte membranes; degeneration of nerve cells; atropopathy, weakness of bones and smooth muscles; atrophy of reproductive organs; possible increase in the risk of cancer, atherosclerosis, arthritis and cataracts.

Tocopherol exists in 8 different isoforms namely α-, β-, δ-, γ- tocopherol and α-, β-, δ-, γ- tocotrienol. The α-Tocopherol (α-T) is regarded as the most common and biological active form of vitamin E [Riggotto A., 2007; Raederstorff D., et al, 2015]. These isoforms are widely found present in vegetables, vegetable oil, nuts (such as almonds), grains (such as corn oil), seeds (such as sunflower), cyanobacteria and supplements [Tang Y., et al (2015) ; Qi N., Gong X., Feng C et al., 2016; Ozsoz M, et al., 2019].

Given the fact that the study of the content of tocopherols is extremely important for the food industry, pharmacology continues today to search for sensitive methods of analyzing the forms of tocopherols. A wide range of techniques for extraction of tocopherols and other phytochemical compounds was discribed. The factors that determine extraction techniques include phytochemical and physical properties of plants, availability of instruments and resources [Pinheiro H.M. et al., 2011; Ozsoz M., et al., 2019].

In the last decades several techniques have evolved for the separation and analysis of tocopherols present in food, some of these techniques include Reverse Phase High Performance Chromatography (RP-HPLC), Normal Phase High Performance Chromatography (NP-HPLC) or HPTLC and Gas Chromatography (GC), Capillary Liquid Chromatography (CLC), Thin Layer Chromatography (TLC), Capillary Electrochromatography (CEC), Modification of chromatography techniques with nanomaterials such as Nano Liquid Chromatography (NLC), other approaches include Supercritical Fluid Chromatography (SFC), Fourier Transform Infrared Spectroscopy (FT-IR)

and Synchronous Fluorescence Spectroscopy (SFC) including even the AAS method [Pinheiro-Sant Y.M. et al, 2011; Palombini S.V., et al, 2012; Gornas P. et al, 2014; Prevc T., et al. 2015 ; Saini R,, Keum Y., 2016; Saeed Ah.M., Al-Kadumi A. Sh., Ali N. J. M. 2017;Kulkarni M.B., et al, 2018; Pan Q., Shen M. Yu T., et al., 2020]. When necessary, all tocopherols can be fully separated using normal phase chromatography (Bele C. et al. 2013). Use of electrochemical method such as Differential Pulse Voltammetry (DPV), Cyclic Voltammetry (CV), Square Wave Anodic Stripping Voltammetry (SWASV), Chrono-Amperometry (CA) has also shown good results for the determination of tocopherols [Delgado-Zamarreno M.M. et al, 2004;Hossu A.M., et al., 2011; Ozsoz M., Ibrahim A.U., Coston P.P.; 2019]. Different literary sources were used as the basis for the modification of the method in our case.

Materials and method. The α-T were extracted from different seeds samples according to the method descraibed by R. Gutiérrez-Peña et al. (2018), Ozsoz M., et al (2019) and Irías-Mata A., et al., (2017) with the following minor modification. The 50 mg were weighed into a glass tybe with screw-cap and 1 mL 1% ascorbic acid in water, 2 mL isopropanol and 1 mL saturated potassium hydroxide (40% in water) were added. Samples were saponified at 70 °C in a shaking water bath for 1 hour. For extraction added 5 mL of n-Hexane, mix by vortexing. Centrifugated at 5000 rpm during 4 min (temp 20 ºC) the supernatant was separated for further analysis. The aqueous phase with pelletes was again subjected to extraction 2 times. Content of α‑T in seeds was carried by method fluorimetric detection was performed at exilation wavelength λex = 295 nm and emission waveleng that λem = 325 nm. Slit width for both detector was set at 5 nm. Three replicates of standartes and samples were measured in each group. The limits of detection (LOD) calculated as the concentration corresponding to three times the standard deviation of the baseline noise were not higher than 0,41µg /mL.

Results. The content α-T dominates in quinoa, less content saw in amaranth minimal content determined in kmin seeds. The concentration of α -T in all seeds is not higher than 2,6 mg/100 g. The results obtained are generally in agreement with the literature data which were obtained by chromatographic methods (Palombini S. V., et al, 2012; Ogrodowska D., et al., 2014).

It should be noted that extraction with hexane gave a higher intensity of the tocopherol peak than with heptane. Intense staining in the case of saponification of cumin seeds, obviously, was the reason for the matrix effects and incomplete determination of the concentration of tocopherol in these seeds. We optimized the method by choosing 2-propanol (acetone, ethyl or methyl alcohol and a number of other solvents are used in the literature). In 2-propanol reactivity is independent of tocopherol concentration, reactions are faster and solvent is fully compatible with polypropylene labware.

In conclusion, the UV-detection spectrometric method and modification of the seed tocopherol extraction method presented here are sensitive, selectively accurate, rapid, simple, cheap, and convenient for the routine determination of α-tocopherol in various seeds.

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