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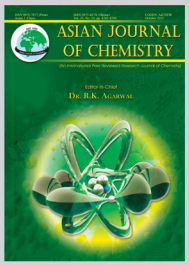

Determination of Vitamin E in Canola Seeds by High Performance Liquid Chromatography

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Tocopherols (vitamin E) are natural phenolic antioxidants present in vegetable oils and are responsible for many healthful properties of these foods. They are effective radical scavengers and defend the body against free radical attack by protecting polyunsaturated fatty acids. Vitamin E plays an important role at the intracellular level since its deficiency increases membrane fragility and promotes the damage of membranes by oxygen-reactive species, ozone or other free radicals. The tocopherols belong to a group of structurally related compounds called tocols. Foods such as nuts, seeds, some grains and vegetable oils are good sources of natural tocopherol antioxidants. The various tocopherols may exist in a free or a esterified form. In seed oils, they are mainly present in the free state and the level of antioxidant materials is of great importance in the stability of vegetable oil products. It is known that vitamin E activity decreases while the antioxidant activity increases in the order α-, β-, γ- and δ-tocopherols. Canola is one of the most important oil seeds growing in many parts of the world. It is very important to grow canola with high oil levels for agronomical benefits. There is a growing interest for the use of oil seeds for nutritional, industrial and pharmaceutical usages. The world production of canola oil is higher than soybean and sunflower. In this study an effective high performance liquid chromatography method for measuring α-tocopherol in canola gathered from different regions.

Key Words: Vitamin E, Canola, Antioxidants, α-Tocopherol.

INTRODUCTION

Synthetic antioxidants are being questioned while natural antioxidants such as tocopherol, polyphenols and carotenoid pigments are having a greater relevance in the protection against lipid oxidation. α and γ-tocopherols are the most abundant natural antioxidants in seed oils. Tocopherols act as antioxidant by donating a hydrogen atom to peroxyl radicals of unsaturated lipid molecules, forming a hydroperoxide and a tocopheroxyl radical, which reacts with other peroxyl or tocopheroxyl radicals forming more stable adducts[1].

Adequate vitamin E supplementation of diets for the newborn can be problematical. In infants, plasma vitamin E concentrations are low at birth[2] and in preterm infants, these low values have been associated with hemolytic anemia, bilirubinemia and neurological complications. In infant diets, it is generally accepted that the ratio of vitamin E to polyunsaturated fatty acids (E: PUFA) not be permitted to fall below 0.6. The types of fatty acid esterified in triglycerides and phospholipids are extremely variable. Genetic and environmental influences can account for a small part of this variability but most is due to nutritional factors, particularly the relative importance of hepatic lipogenesis *versus* the amount and type of dietary fat consumed. Vitamin E is well known for being a lipophilic antioxidant of cell membranes and lipoproteins[3] was used as amodifier (or coating agent) on the blood surface of cellulosic hollow-fiber dialyzer membranes. The ultimate goal of using this vitamin was to increase the biocompatibility and to prevent the oxidative stress that is related to leukocyte activation and other inflammation-related effects that may result from the interaction between blood constituents and cellulosic fibers[4]. The requirement increases linearly with the amount of polyunsaturated fatty acids in the diet. Vitamin E nutrition is compromised by the oxidative rancidity of dietary fats prior to their consumption through two synergistic mechanisms: the vitamin E in the diet is depleted as the fat goes rancid and oxidation products, which result from peroxidation of polyunsaturated fatty acids, deplete α-tocopherol in the tissues of the bird[5,6]. Consequently, rancid dietary fats increase the vitamin E requirement several-fold over that predicted by their polyunsaturated fatty acid content.

Canola is one of the top five oilseed crops cultivated worldwide and is second only to wheat in value and area cultivated in Canada[7]. The global demand for canola is primarily for its edible oil. However, once the oil is removed from the seed, the meal, rich in bioactive components, is left behind. The

defatted substrate is high in protein (35-36 g/100 g) and crude fibre content (*ca.* 12 g/100 g) with a relatively high amount of minerals and vitamins[8]. On the other hand, recent research is rediscovering the potential of these minor components as natural antioxidants. While chlorophyll is very important for photosynthesis, its presence in canola seeds imparts an undesirable green colour to the oil. In addition, chlorophyll promotes photooxidation as well as inhibits the catalysts used for hydrogenation[9]. Canola oil has been found to inhibit cardiac arrythmias in rats compared to olive oil[10]. A recent study suggested that substitution of canola oil for other dietary fats can significantly decrease serum total and LDL cholesterol in humans[11]. Studies on platelet aggregation favour canola over soybean in terms of linoleic acid/linolenic acid ratio in the dietary oil[12].

EXPERIMENTAL

Vitamin E standard were purchased from Sigma. Hexane and ethanol were purchased from Merck . HPLC analyses were carried out using a HPLC (VARIAN ProStar, Varian, Middelburg, The Netherlands) equipped with a (VARIAN ProStar 363) fluorescence detector and an autosampler (VARIAN Autosampler MODEL 410), The column was packed with reverse-phase Inertsil 5 Si (150 mm × 4.6 mm, 5 µm) (VARIAN, Cat No. 29231) Chromatographic data were collected and analysed with Agilent 1000-HPLC standards were obtained from Calbiochem (Darmstadt, Germany) with a purity of ≥ 95 % for each tocopherol. Chromatographic separation was carried out using continuous isocratic elution with HPLC grade hexane (eluant A) and 0.2 % ethanol (eluant B). The HPLC gradient profile was 99.8 % hexane and 0.2 % ethanol and the flow rate was 1 mL/min throughout the entire separation. The injection volume was 50 µL and detection was monitored with a fluorescence detector at 300 and 330 nm for excitation and emission, respectively.

Sample preparation for HPLC analysis: The canola seeds were crushed in a mill and 1 g samples were taken for each canola seeds. These samples were soaked in 50 mL of EtOH overnight and homogenized in an Ultra-Turrax apparatus by gradually increasing the number of cycles per unit time. The extracts were transferred to centrifuge tubes, centrifuged for 10 min (at 5000 rpm) and subsequently filtered through a blue-band Whatman filter paper into 100-mL flasks. The extracts could be analyzed for their antioxidant capacities on the next day if preserved by storing in stoppered flasks in a freezer at -20 ºC. Plant extracts were filtered through a 0.45 µm microfilter (Whatman, UK) before HPLC analysis.

RESULTS AND DISCUSSION

This work reports the antioxidant assay of α-tocopherol in ethanol solvent media using HPLC. This work brings several contributions to food analytical chemistry (specifically to antioxidant assays). HPLC analysis provided information on α-tocopherol contents. α-Tocopherol contents could be determined for 11 canola seeds. Table-1 shows α-tocopherol content of canola seeds ranged from 0.0007-0.0033 mg/mL. The highest α-tocopherol contents of seeds were obtained from the Tekirdag and Tekirdag Karaevli and were 0.0033 and 0.0027 mg/mL, respectively. Fig. 1 shows the chromatogram

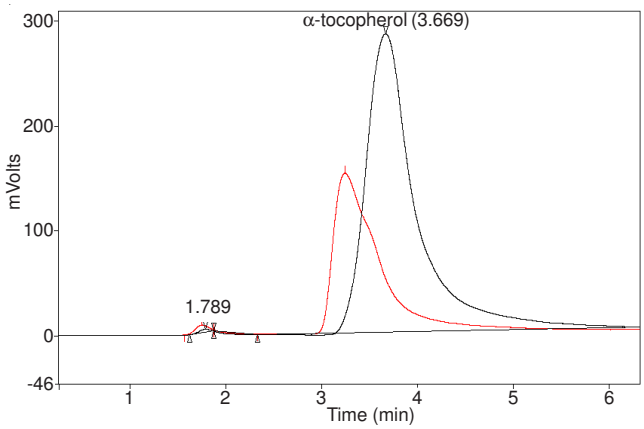

Fig. 1. Chromatogram of standards for 0.002 and 0.004 mg/mL α-tocopherol

TABLE-1
α-TOCOPHEROL CONTENT OF CANOLA SEEDS

Tekirdag Merkez	0.0033 ± 0.01
Tekirdag Karaevli	0.0027 ± 0.01
Çorlu	0.0013 ± 0.01
Muratli	0.0015 ± 0.01
Hayrabolu Merkez	0.0016 ± 0.01
Çorlu Esetçi	0.0007 ± 0.01
Silivri	0.0013 ± 0.01
Hayrabolu Canhidir	0.0009 ± 0.01
Hayrabolu Çikirikçi	0.0014 ± 0.01
Muratli Ballihoca	0.0009 ± 0.01
Malkara Gözsüz	0.0022 ± 0.01

of standards for 0.002 and 0.004 mg/mL α-tocopherol Fig. 2 shows external standard analysis for α-tocopherol. The standards ranged from 0.000025-0.004 mg/mL α-tocopherol. Relative standard deviation (RSD) was 6.802 % and R^2 = 0.998 for standards from the calibration line graphic of α-tocopherol standards in the ethanol. α-tocopherol contents in the standards were found using the equation:

$$X \text{ (mg/mL α-tocopherol)} = 2.4378 \times 10^{-9} \times \text{peak area}$$

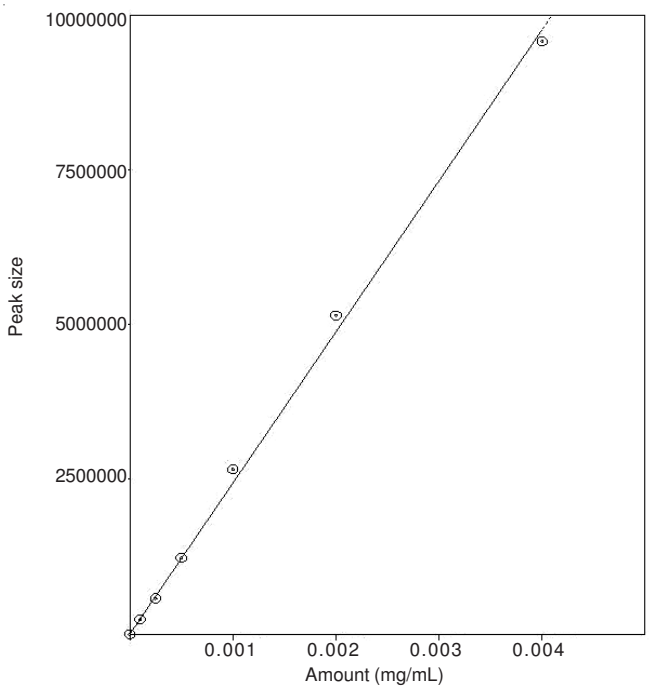

Fig. 2. External standard analysis for α-tocopherol

Fig. 3 shows chromatogram from a canola seed sample collected from Malkara Gözsüz. Relative standard deviation (RSD) was 5.278 % and $R^2 = 0.998$ for samples. α-Tocopherol contents in the samples were found using the equation:

$$X \text{ (mg/mL } \alpha\text{-tocopherol)} = 0.4102 \times 10^{-9} \times \text{peak area}$$

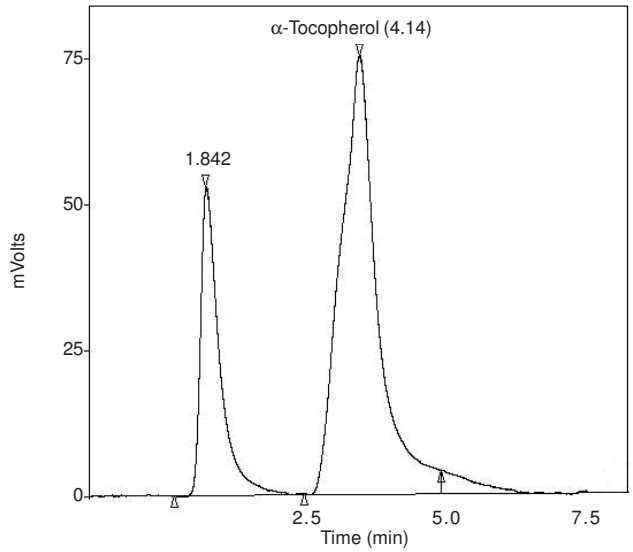

Fig. 3. Chromatogram from a canola seed sample collected from Malkara Gözsüz

Conclusion

A simple, rapid and versatile HPLC assay was presented for determination of vitamin E in canola seeds. It is to be emphasized that widely available laboratory equipment and inexpensive chemicals were used. The results of this work may be useful in recognizing the nutritive antioxidant values of commercially canola seed and comparing their antioxidant capacities for possible health benefits through diet. The decreasing order of α-tocopherol for canola seed with respect to HPLC was: Tekirdag > Tekirdag (Karaevli) > MalkaraGözsüz > Hayrabolu > Muratli > Hayrabolu Çikirikçi > Çorlu ≥ Silivri > Hayrabolu Canhidir ≥ Muratli Ballihoca > Çorlu Esetçi.

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