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DETERMINATION OF NATURAL VITAMIN E FROM ITALIAN HAZELNUT LEAVES

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A new potential source of natural vitamin E from thirteen samples of Corylus avellana *L. leaves was screened: the major Italian cultivar – Tonda romana (collected from Latium and Sardinia localities); ten local genotypes from Sardinia – Moro seme, Suconcale, Moro, Sarda grossa, Sarda grossa seme, Sarda schiacciata, Coccoredda, Sarda lunga, Sarda piccola and Sarda tardiva; wild genotype – Selvatico from Latium. The determination was performed after optimizing the high-efficiency pressurized liquid extraction (PLE) conditions of α-tocopherol from Italian hazelnut tree green leaves. Moro from Sardinia showed the highest content of α-tocopherol (237.4±0.3 μg/g d.w). Leaves of this genotype may be considered as a potential new source for natural α-tocopherol.*

Key words: antioxidant, α-tocopherol, hazelnut, nutrceutical.

α-Tocopherol (vitamin E) occurs naturally in hazelnut tree leaves. Hazelnut plants can tolerate some environmental stress because of their antioxidative system which includes antioxidant such as a-tocopherol and biophenolic compounds [1]. These can interact with free radicals preventing the initiation of potentially lethal process, such as lipid peroxidation and subsequent membrane disorganization [2]. The tocopherol molecule, which is composed of a phytyl chain and a chromanone ring, may be incorporated into biological membranes, thus contributing to their physical stability [3]. The pharmacological action of α-tocopherol is not only as a natural antioxidant but also as a preventive against cancer [4]. Moreover, vitamin E is also used in cosmetology [5]. The nutraceutical demand for natural α-tocopherol continues to increase because it is presumed to be more bioactive than synthetic α-tocopherol. The chloroplast of plants contain significant amounts of α-tocopherol, which is the most biologically active isomer. So far, high-proficiency extraction of leaf α-tocopherol in the extract has not been reported. Therefore, the objective of this work was the optimization of high - efficiency pressurized liquid extraction (PLE) conditions of α-tocopherol from green leaves and the development of a high-performance liquid chromatography (HPLC) method for accurate value measurement. Our main goal was to identify new potential natural sources of antioxidants; thus, a preliminary screening was performed considering different Italian hazelnut tree leaves. This method will allow us to predict which of the Italian hazelnut local genotypes could represent an interesting source of natural α-tocopherol for nutraceutical, pharmaceutical, and cosmetic applications.

Tocopherols are easily oxidized and oxidation losses can be incremented by heat, light, alkaline pH, and by the presence of free radicals [6]. This is especially critical in samples in which vitamin E can be oxidized during the extraction process and/or in the extract until its final analysis. To separate the α-tocopherol fraction, the lyophilized hazelnut leaf powder was mixed with Hydromatrix in the extraction cell and extracted with dehydrated hexane containing 0.01% of butylated hydroxytoluene in an accelerated solvent extraction. Recently, Lagouri and Boskou [7] found that tocopherols in oregano species were largely responsible for the protective effect of hexane extracts in stored cottonseed oil. Lyophilization gave the opportunity to avoid denaturation of α-tocopherol caused by heating, and maintaining samples frozen throughout drying led to completeness of rehydration. Pure nitrogen was used as a carrier gas because it allowed us to avoid denaturation of α-tocopherol during extraction in an ASE and better resolution of chromatographic peaks. Vitamin E is not chemically bound to proteins, lipids, or carbohydrates [8] and using harsh reagents and conditions to free it up (e.g., strong saponification) can destroy the vitamin. But, PLE results on α-tocopherol extraction efficiency showed that 60°C was a suitable temperature for better extraction in an ASE [1] at higher α-tocopherol contents without oxidation.

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TABLE 1. α-Tocopherol Content from Italian Hazelnut Tree Leaves

Major Italian hazelnut cultivar from different agro-climatic areas of Italy	(μg/g d.w)*
Tonda romana (Latinum)	149.3±0.4
Tonda romana (Sardinia)	147.6±0.7
Sardinia local genotypes	
Moro	237.4±0.3
Sarda grossa seme	225.0±0.3
Sarda grossa	187.5±0.5
Moro seme	132.9±0.9
Sarda schiacciata	89.7±0.5
Coccoredda	77.2±0.3
Sarda lunga	58.3±0.8
Suconcale	54.6±0.3
Sarda piccola	49.5±0.4
Sarda tardiva	34.6±0.4
Italian wild genotype	
Selvatico (Latium)	150.5±0.6

*Mean±standard error.

This method was evaluated through repetitive analysis of a standard and hazelnut leaf extracts. PLE can be used to isolate naturally occurring tocopherols from plant sources with the advantages inherent in this technique such as small amount of solvent, speed, and routine analysis [9]. These results show that the α-tocopherol extraction and quantification can be considered accurate.

Table 1 shows the α-tocopherol content (mg/g d.w.) of hazelnut leaf samples. Moro leaves from Sardinia local genotypes showed the highest content of α-tocopherol (237.4±0.3); Sarda grossa seme, Sarda grossa, and Moro seme (225.0±0.3, 187.5±0.5, 132.9±0.9, respectively) and Sarda schiacciata, Coccoredda, Sarda lunga, Suconcale, Sarda piccola, and Sarda tardiva (89.7±0.5, 77.2±0.3, 58.3±0.8, 54.6±0.3, 49.5±0.4, 34.6±0.4, respectively) recorded the lowest.

One of the major Italian varieties, Tonda romana (a traditional cultivar from Central Italy) collected from different places such as Latium (149.3±0.4) and Sardinia (147.6±0.7) showed a minor content when compared to Moro, Sarda grossa seme,and Sarda grossa, but a higher content than Moro seme and other local Sardinia genotypes. No significant differences were found between the same varieties from different localities. Regarding the α-tocopherol content in wild genotype, Selvatico from Latium showed a similar value (150.5±0.6) when compared to Tonda romana, but in Moro, Sarda grossa seme, and Sarda grossa the opposite was observed. With respect to leaf developmental stage and environmental conditions, there was a tendency for varying accumulation of α-tocopherol content. This was also noted by other workers [10, 11]. This was understood as being due to the synthesis of the α-tocopherol in the early stages of development of the leaves and some portion transferred to nuts as maturation progressed. Because of its hydrophobic nature, almost all cellular α-tocopherol is in the membrane fraction of cells. In that location it can readily donate an electron to a fatty acid hydroperoxyl radical, thus breaking the chain reaction associated with lipid peroxidation [12]. However, wide variations in α-tocopherol levels have been attributed to differences in hazelnut cultivars, maturity, growing practices, climates, etc. [13].

In conclusion, PLE shows several advantages when compared to other extraction methods such as higher extraction efficiency, small amount of solvent, time consumption, etc. Our HPLC screening data from hazelnut leaves could allow natural manipulation of ruminant diets to increase uptake of the natural antioxidant α-tocopherol by meat-producing cattles, thereby enhancing the color and oxidative stability of fresh meat [11]. It is well established from vitamin E deficiency studies that adequate amounts of α-tocopherol are necessary to prevent mitochondrial disfunction. Thus, not only is α-tocopherol required for mitochondrial stability, but it may also prevent radical release. Furthermore, the selection of Italian hazelnut varieties and genotypes could represent an interesting tool to find natural sources of α-tocopherol for nutraceutical, pharmaceutical, and cosmetic applications.

EXPERIMENTAL

Sampling and Sample Preparation. Thirteen samples of leaves from *Corylus avellana* L., collected during June 2004 in two different geographical conditions of Italy [major cultivar – Tonda romana from Latium and Sardinia]; ten local genotypes from Sardinia – Moro seme, Suconcale, Moro, Sarda grossa, Sarda grossa seme, Sarda schiacciata, Coccoredda, Sarda lunga, Sarda piccola, and Sarda tardiva; and wild genotype – Selvatico from Latium were analyzed. Three representative samples were collected from each cultivar, immediately frozen in liquid nitrogen, and milled to a fine powder by a Waring blender unit for 20 s. Then fine leaf powder samples were dehydrated on lyophilizer (Edwards Pirani 501) shelves to –60°C in two days. During all the steps that followed, care was taken to protect the samples from light and atmospheric oxygen.

Pressurized Liquid Extraction (PLE). The extraction was performed on ASE 100 (Dionex) using extraction cells of 10 mL, with cellulose filters. The lyophilized leafs powder (1g) was mixed homogeneously with a drying agent (Hydromatrix, VARIAN) in the extraction cell. After placing the cell in the ASE, the selected assay conditions were applied. α-Tocopherol was extracted with dehydrated hexane containing 0.01% of butylated hydroxytoluene (BHT 99%, Sigma-Aldrich, which was added to inhibit the oxidative degradation of α-tocopherol during extraction) in an ASE. The assay cycle on the ASE was temperature 60°, pressure 1500 psi, flush volume 60%, purge time 100 s, number of static cycle 1, and total assay time 15 min. This solution (20 µL) was injected into the HPLC system. The extraction unit was wrapped in aluminum foil.

High-performance Liquid Chromatography (HPLC). The normal-phase HPLC system (VARIAN, Prostar) consisted of a VARIAN Prostar 210 pump equipped with a VARIAN Lichrosorb Si 60-5 column (15 × 4mm, 5µm) and a variable wavelength UV-visible detector (VARIAN, Prostar 325), the wavelength of which was set at 292 nm. The isocratic mobile phase contained 0.5% isopropanol in dehydrated hexane and the flow rate was 1 mL/min. The mobile phase was degassed with helium. Pure α-Tocopherol standard was purchased from Sigma. Stock standard solution was prepared in extraction solvent and stored at –20°C in dark bottles. The α-tocopherol peak was identified by adding the standard to the samples before PLE extraction. The α-tocopherol was quantified using the external standard method. The relationship between the concentration of α-tocopherol and peak height value was established by the calibration curve and expressed in µg/g dry weight of α-tocopherol.

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