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Review

Tocopherols and tocotrienols in plants and their products: A review on methods of extraction, chromatographic separation, and detection

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ABSTRACT

Vitamin E consists of four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ-, and δ-tocotrienols), collectively referred to as tocochromanols or tocols. Tocols are well-known for potent antioxidant, anticancer, anti-inflammatory, immuno-stimulatory and nephroprotective properties. For human nutrition, diet is the major source of tocols (vitamin E) in the body. Thus, there is a need to analyze the different forms of tocols in the diet for the recommendations and to monitor the intake in the body accurately. Several methods have been developed for effective extraction, selective chromatographic separation and sensitive detection of tocols in food. Major advancements also have been made in the field of mass spectrometry for high throughput analysis of primary and secondary metabolites in fruits, vegetables, and grains. This review discusses the theoretical aspects and modern developments in methods of extraction, chromatographic separation, and detection of tocols in plants and their products. Additionally, future research challenges in this perspective are also identified.

Contents

1. Introduction

In nature, vitamin E consists of four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ-, and δ-tocotrienols), determined by the numbers and position of methyl groups ($-CH_3$) present

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on the chromanol ring. The tocopherols and tocotrienols are collectively referred to as tocochromanols or tocols. Vitamin E is the major bioactive constituent of human diet and is well-known for its potent antioxidant and anticancer activities. In addition, numerous studies have demonstrated the potential health benefits, since the discovery of vitamin E in 1922, which includes, hypolipidemic (Minhajuddin, Beg, & Iqbal, 2005), antiatherogenic (Kirmizis & Chatzidimitriou, 2009), antihypertensive (Kizhakekuttu & Widlansky, 2010), allergic dermatitis suppressive (Tsuduki, Kuriyama, Nakagawa, & Miyazawa, 2013), nephroprotective (Siddiqui, Ahsan, Khan, & Siddiqui, 2013), neuroprotective (Rashid Khan, Ahsan, Siddiqui, & Siddiqui, 2014) and anti-inflammatory (Mocchegiani et al., 2014) activities. Tocopherols and tocotrienols are also used to increase the shelf life and the stability of foods (Kamal-Eldin & Budilarto, 2015; Seppanen, Song, & Csallany, 2010). The relative antioxidant activity of tocopherols and tocotrienols generally depends on the food system. α-Tocopherols have shown superior antioxidant activity than γ-tocopherols in oils and fats (Seppanen et al., 2010). Vitamin E is also receiving growing attention in cosmetic and clinical dermatology because of its photoprotection and antioxidant properties (Thiele, Hsieh, & Ekanayake-Mudiyanselage, 2005).

Unlike any other antioxidant molecule; evolutionary, genetic and biochemical evidence suggest cell signaling as the prime activity of α-tocopherol in a cellular system (Azzi, 2007). α-Tocopherol plays a significant role in maintaining the integrity of long-chain polyunsaturated fatty acids in the cell membranes. Thus, bioactive lipids such as tocopherol are the important signaling molecules that change their levels differentially in response to the external environment, depending on the extent and type of the stimulations. This phenomenon is the key to cellular events that are governed by α-tocopherol and responded to by cells (Traber & Atkinson, 2007). With this mechanism only, α-tocopherol plays a vital role in plant stress tolerance. It is assumed that increased level of α-tocopherol contributes to stress tolerance while decreased levels favor oxidative damage in plants (Munné-Bosch, 2005). In plants, tocopherol also plays an important role as singlet oxygen scavenger in photosystem II (Kruk, Holländer-Czytko, Oettmeier, & Trebst, 2005).

Several separation techniques have been developed for the analysis of tocopherols in food. These include gas chromatography (GC), normal phase- or reverse phase-high performance liquid chromatography (NP/RP-HPLC), capillary electrochromatography (CEC), nano-liquid chromatography (Nano-LC), capillary liquid chromatography (CLC), supercritical fluid chromatography (SFC), capillary liquid chromatography (CLC) and thin layer chromatography (TLC). Also, Fourier transform-infrared spectroscopy (FT-IR) and synchronous fluorescence spectroscopy (SFS) techniques have been developed and applied for the direct determination of α-tocopherol in various oils.

NP/RP-HPLC with UV–visible, fluorescent and mass spectrometric detection is routinely used for analysis of tocopherols (Lanina, Toledo, Sampels, Kamal-Eldin, & Jastrebova, 2007). With the introduction of new stationary phases, such as long-chain alkyl-bonded C30-silica and solid-core Penta fluorophenyl (PFP) column, separation of different tocopherols isomers in RP–HPLC has become easier. This review discusses the theoretical aspects and modern developments in methods of extraction and analysis of tocopherols and tocotrienols. Additionally, future research challenges in this perspective are also identified.

2. Chemistry, biosynthesis, biological activities, and RDA of tocols

Tocochromanols or tocols (vitamin E) are the group of four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ- and δ-tocotrienols), determined by the numbers and position of methyl groups ($-CH_3$) present at the 5- and 7-positions on the chromanol ring. All these tocols have a 16-carbon phytyl side chain attached to chromanol ring, in which tocopherols are saturated, and tocotrienols have three double bonds. Tocols are produced at different levels by photosynthetic organisms. However, their chemistry and function are originally studied in animals due to the vitamin E activity in the diet. The outline of biosynthesis, general structure and antioxidant activity of tocols are shown in Fig. 1. The polar head group (chromanol ring) of tocols are derived from homogentisic acid (HGA), biosynthesized from aromatic amino-acid metabolism (shikimate pathway), whereas the hydrocarbon tail is derived from geranylgeranyl diphosphate (GGDP), biosynthesized from methylerythritol phosphate (MEP) pathway. Tocols interact with polyunsaturated acyl groups of membrane lipids and protect from peroxidation by scavenging reactive oxygen species (ROS) (Szarka, Tomasskovics, & Bánhegyi, 2012). During scavenging reaction with ROS, tocopherols are converted to the corresponding quinone (DellaPenna & Pogson, 2006). The presence of other antioxidant such as ascorbic acid (vitamin C) is required to regenerate the tocols (Jiang, 2014) (Fig. 1).

The content and composition of tocols vary immensely among plant tissues, with photosynthetic tissues (green leafy vegetables) accumulating low levels of total tocols and a high proportion of α-tocopherol (Saini, Prashanth, Shetty, & Giridhar, 2014), whereas seeds accumulate 10–20 times higher amount of total tocols, with large proportion of γ-tocopherol (DellaPenna & Pogson, 2006). The summarized data on tocopherol content in selected food crops (in Table 1) shows that α-tocopherol is predominantly found in wheat germ, hazelnut, sunflower, almond, rice bran, grapeseed oil, whereas γ-tocopherol is the major proportion of vitamin E in peanut, corn, canola and soybean oil. Surprisingly, the significant high content of α-tocopherol (53.3 mg/100 g FW) is recorded in black chokeberry (*Aronia melanocarpa*) leaves collected from greenhouse-grown in vitro plants (Sivanesan, Saini, & Kim, 2016). Among the natural sources of tocopherols, wheat germ oil is one of the most abundant, containing 149.40 mg of tocopherol in 100 g wheat germ (Table 1). Soybean and sunflower seeds and raspberries are the rich sources of δ-tocopherol. Tocotrienols are much less prevalent then tocopherols (≈1%), found commonly in paprika and chili spices, oat bran (raw), and coconut oil as listed in USDA, ARS, National nutrient database for standard (http://ndb.nal.usda.gov/ndb/search, Release 28, Accessed on 7th November 2015). Due to the extensive use of soybean and corn oil in food preparation, γ-tocopherol represents ≈60–70% of the total vitamin E, in typical U.S. diet, whereas, α-tocopherol accounts only 20–30% (Jiang, 2014).

In food, tocols are coexisting with fatty acids. Interestingly, the majority of γ-tocopherol is mainly associated with polyunsaturated fatty acids (PUFA), especially with omega-6 fatty acids ($\Omega-6$ or $n-6$) whereas α-tocopherols are associated with monounsaturated fatty acids (MUFA). Thus, collectively γ-tocopherol and $\Omega-6$ fatty acids plays a vital role in disease prevention (Jiang, 2014). The antioxidant, non-antioxidant and radical scavenging functions of tocols are well studied. α-Tocopherol functions as a fat soluble chain-breaking (hydrogen atom donor) antioxidant, intercepting the chain-carrying peroxyl radicals during lipid oxidation (ROO•) in cellular membranes and low-density lipoproteins (LDLs) (Higdon, 2015) (Fig. 1). LDLs play a significant role in cholesterol transportation from the liver to the tissues of the body. The oxidized LDLs are associated with the development of cardiovascular disease, due to lipid deposition in the arterial wall (Trpkovic et al., 2015). The antioxidant function of α-tocopherol is also important in cell-mediated immunity by strengthening the immune synapse between $CD4^+$ T cells and antigen-presenting cells (APC), and enhanced production of interleukin-2 (IL-2), resulting in provoked T cell activation and proliferation (Molano & Meydani, 2012). In contrast with α-tocopherols, γ-tocopherols are capable of trap electrophiles and reactive nitrogen species (RNS) during inflammation (Constantinou, Papas, & Constantinou, 2008). Recent studies have demonstrated that tocotrienols have potent antioxidant and anti-inflammatory properties that are superior to tocopherols in prevention and treatment against major chronic diseases (Jiang, 2014). The unsaturated side chain of tocotrienols allows an efficient penetration into tissues with saturated fatty layers. Tocotrienols possess potent