J Chem Biol (2015) 8:45–59
DOI 10.1007/s12154-014-0127-8

REVIEW

A review of characterization of tocotrienols from plant oils and foods

Haseeb Ahsan · Amjid Ahad · Waseem A. Siddiqui

Received: 23 August 2014 / Accepted: 21 December 2014 / Published online: 20 January 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract Tocotrienols, members of the vitamin E family, are natural compounds found in a number of vegetable oils, wheat germ, barley and certain types of nuts and grains. Vegetable oils provide the best sources of these vitamin E forms, particularly palm oil and rice bran oil contain higher amounts of tocotrienols. Other sources of tocotrienols include grape fruit seed oil, oats, hazelnuts, maize, olive oil, buckthorn berry, rye, flax seed oil, poppy seed oil and sunflower oil. Tocotrienols are of four types, viz. alpha (α), beta (β), gamma (γ) and delta (δ). Unlike tocopherols, tocotrienols are unsaturated and possess an isoprenoid side chain. A number of researchers have developed methods for the extraction, analysis, identification and quantification of different types of vitamin E compounds. This article constitutes an in-depth review of the chemistry and extraction of the unsaturated vitamin E derivatives, tocotrienols, from various sources using different methods. This review article lists the different techniques that are used in the characterization and purification of tocotrienols such as soxhlet and solid–liquid extractions, saponification method, chromatography (thin layer, column chromatography, gas chromatography, supercritical fluid, high performance), capillary electrochromatography and mass spectrometry. Some of the methods described were able to identify one form or type while others could analyse all the analogues of tocotrienol molecules. Hence, this article will be helpful in understanding the various methods used in the characterization of this lesser known vitamin E variant.

Keywords Tocotrienols · Extraction · Saponification · Chromatography · Electrochromatography · Mass spectrometry

H. Ahsan
Department of Biochemistry, Faculty of Dentistry, Jamia Millia Islamia, New Delhi 110025, India

A. Ahad · W. A. Siddiqui (✉)
Lipid Metabolism Laboratory, Department of Biochemistry, Faculty of Science, Jamia Hamdard (Hamdard University), New Delhi 110062, India
e-mail: wasiddiqui01@gmail.com

W. A. Siddiqui
e-mail: was.sid@gmail.com

Introduction

In the beginning of twentieth century, it was clearly understood that the diets containing purified carbohydrate, protein, fat and minerals were not adequate to maintain the growth and health of experimental rats, which the natural foods such as milk could do. Hopkins [1] coined the term accessory food factors to the unknown and essential nutrients present in the natural foods. Funk [2, 3] isolated an active principle (an amine) from rice polishing and, later in yeast, which could cure beri-beri in pigeons. He coined the term "vitamine" (Greek, vita-life) to the accessory factors with a belief that all of them were amines. The term, vitamin, is however continued without the suffix final letter "e". Vitamins are classified by their biological and chemical activity, not their structure. Since, the early 1920s, it was found that rats fed on cow's milk could not produce offspring. The active principle from wheat germ that could rectify this deficiency in both male and female rats was named as vitamin E.

Evans and associates [4] isolated the compounds of vitamin E family and named them tocopherols (Greek: Tocos—child birth; pheros—to bear; ol—alcohol). While alpha-tocopherol was the first vitamin E analogue to be recognized, eight chemically distinct analogues are now known, consisting of alpha (α), beta (β), gamma (γ) and delta (δ)-tocopherols (T) and α, β, γ and δ-tocotrienols (T3), all of them are referred to as

vitamin E. The tocopherols are saturated forms of vitamin E, whereas the tocotrienols are unsaturated and possess an isoprenoid side chain.

In 1943, Joffe and Harris demonstrated varying potencies of the eight forms of vitamins [5]. Each form of the vitamin has slightly different biological activity. In general, the L-isomers of tocotrienols lack almost all vitamin activity and the naturally occurring forms are the D-forms. The name "tocotrienol", to denote a tocopherol with a true isoprenoid side chain, was first suggested by Bunyan et al. [6] and tocotrienols were described in nature when isolated from the latex of the rubber plant, *Hevea brasiliensis* [7]. Tocotrienols attracted no real attention until the 1980s and 1990s when their cholesterol-lowering potential [8] and anticancer effects were described [9, 10].

Tocochromanols: tocopherols and tocotrienols

Vitamin E is not a single compound but is at least eight "vitamers", named tocochromanols and can be either "tocopherols" or "tocotrienols". Vitamin E is exclusively synthesized by photosynthetic eukaryotes and other photosynthetic organisms such as cyanobacteria. In order to prevent lipid oxidation, the plants mainly accumulate tocochromanols in oily seeds and fruits or in young tissues undergoing active cell divisions [11]. Vitamin E is an interesting group of compounds, able to exert many and different biological activities in plant, animal and human cells, but the physiological and/or pharmacological role in the cell is not fully described. Vitamin E deficiency is rare in humans, although it may develop in premature infants and in persons with a chronic malabsorption of fats, as well as mild anemia, ataxia and pigmentary changes in the retina. Hence, the vitamin E compounds have to be better evaluated and characterized for understanding their properties [11]. There is a great interest in the natural forms of tocochromanols because they are considered promising compounds able to maintain a healthy cardiovascular system and blood cholesterol levels. Some evidence suggests that the potency of the antioxidant effects may differ between natural or synthetic source of tocochromanols [11].

Vitamin E is a collective term for fat soluble 6-hydroxychroman compounds that have biological activity. Tocol (2-methyl-2-(4,8,12-trimethyltridecyle)-chroman-6-ol) is generally referred to both the parent compounds of tocopherols and tocotrienols. The tocopherols are characterized by the 6-chromanol ring structure methylated at varying degrees at the 5, 7 and 8 positions. At position 2, there is a C16 saturated side chain. The tocotrienols are unsaturated at the 3, 7 and 11 positions of side chain. The specific tocotrienols differ by number and positions of methyl groups on 6-chromanol rings: α-tocotrienol is 5,7,8-trimethyl; β-tocotrienol is 5,8-dimethyl; γ-tocotrienol is 7,8-dimethyl and δ-tocotrienol is 8-monomethyl (Fig. 1). The tocotrienols have the same methyl structure at the ring and methyl notation, but differ from the analogous tocopherols by the presence of three double bonds in the hydrophobic side chain (Table 1). The unsaturation of the tails gives tocotrienols only a single stereoisomeric carbon (and thus two possible isomers per structural formula, one of which occurs naturally). Of the stereoisomers which retain activity, increasing methylation particularly full methylation to the α-form increases vitamin activity. Tocotrienols arising from 2-methyl-2-(4,8,12-trimethyltrideca-3,7,11-trienyl) chroman-6-ol (non-methylated ring structure) have only one chiral centre at position 2. Consequently, only 2R and 2S stereoisomers are possible (3, 7 of phytyl side chains permit four *cis*/*trans* geometric isomers). But only 2R, 3-*trans*, 7-*trans* isomer exists in nature.

Hence, the four tocopherols (α-T, β-T, δ-T and γ-T) and four tocotrienols (α-T3, β-T3, δ-T3 and γ-T3) are collectively called vitamin E. All this eight forms contain a chromanol ring and a hydrophobic side chain, a phytyl in the case of tocopherols and an isoprenyl with three double bonds in tocotrienols. Chemically, T3 and T are closely related: both contain a polar chromanol ring linked to an isoprenoid-derived 16-carbon hydrocarbon chain. The number and position of methyl substituents in the chromanol ring give rise to α-, β-, γ- and δ-isomers. The only difference in the structures of T3 and T is in their isoprenoid chain: while T has a saturated isoprenoid chain, that of T3 contains three isolated double bonds [12]. Therefore, the term vitamin E is commonly used for α-tocopherol (d-α-tocopherol, RRR-α-tocopherol) since it has the highest bioavailability [13]. It is the said to be the gold standard against which all the other similar tocochromanols must be compared. However, it is only one out of eight natural forms of vitamin E. Tocotrienols are similar to tocopherols except that they have an isoprenoid tail with three unsaturations instead of a saturated phytyl tail (Fig. 1).

Tocopherols are the major vitamin E components present in most vegetable oils, while tocotrienols are present especially in palm oil [14]. As the essential vitamin, vitamin E cannot be produced by the human body and needs to be obtained from food. Palm fruit is one of the best sources for tocopherols and tocotrienols besides rice grain and annatto seed. Palm fruit is the richest source of tocotrienols among all vegetable oils. Tocotrienols make up almost 70 % of vitamin E in palm oil, with the remaining 30 % being tocopherols [15]. The most important T3 isomer involved in degradation in T3-rich foods is α-T3 despite the fact that it has the highest absolute antioxidant activity by virtue of its hydrogen-donating power toward free radicals. However, in fats and oils, α-T3 shows the fastest degradation

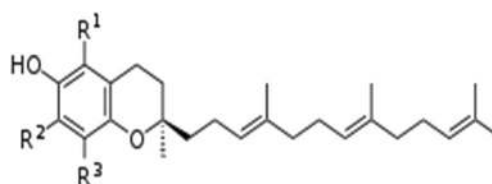

Fig. 1 Chemical structure of tocotrienols

Table 1 Structures of various homologs of tocotrienols

Type	R1	R2	R3	Structure
alpha(α)-Tocotrienol	Me	Me	Me	
beta(β)-Tocotrienol	Me	H	Me	
gamma(γ)-Tocotrienol	H	Me	Me	
delta(δ)-Tocotrienol	H	H	Me	

and the lowest relative antioxidant activity to protect against lipid peroxidation in relation to the other T3 isomers. This phenomenon may be due to the fact that at high temperature, high concentration and in the presence of oxygen, α-tocochromanols can easily act as a prooxidant by undergoing side reactions, leading to the loss of antioxidant efficiency [16].

All tocochromanols are potent antioxidants with lipoperoxyl radical-scavenging activities. Most of the research on vitamin E has primarily focused on αT [17] because αT is the predominant form of vitamin E in tissues, and low intake of this form results in vitamin E deficiency-associated ataxia [18]. However, many human and animal studies on αT supplementation have yielded disappointing results regarding its protective role in prevention or treatment of chronic diseases including cardiovascular diseases and cancer [19, 20]. On the other hand, recent mechanistic studies combined with preclinical animal models have indicated that other forms of vitamin E appear to have different and superior biological properties that may be useful for prevention and therapy against chronic diseases. Furthermore, emerging evidence suggests that some long-chain vitamin E metabolites have even stronger anti-inflammatory effects than their vitamin precursors. These metabolites may be novel anti-inflammatory agents and may contribute to beneficial effects of vitamin E forms in vivo.

Tocotrienols

The tocotrienols (T3) are members of the vitamin E family, have excellent antioxidant properties and are able to prevent the autocatalytic lipid peroxidation process [21]. While T have been intensively investigated, in recent years, interest in T3 has increased due to their special health benefits. Unlike T, T3 are reported to inhibit cholesterol biosynthesis and to have neuroprotective properties [22, 23]. Moreover, α-T3 has outstanding antioxidant activity in liver microsomes, 40–60 times greater than that of α-tocopherol (α-T) [24], and shows special anti-carcinogenic properties [16].

Tocotrienols possess neuroprotective, antioxidant, anticancer and cholesterol-lowering properties that often differ from the properties of tocopherols [25]. Micromolar amounts of tocotrienol suppress the activity of HMG-CoA reductase, the

hepatic enzyme responsible for the synthesis of cholesterol [26, 27]. Tocotrienols are thought to have more potent antioxidant properties than α-tocopherol [28, 29]. The unsaturated side chain of tocotrienol allows for more efficient penetration into tissues that have saturated fatty layers such as the brain and liver [30]. Experimental research examining the antioxidant, free radical scavenging, effects of tocopherol and tocotrienols have found that tocotrienols appear superior due to their better distribution in the lipid layers of the cell membrane [30]. One major conclusion often used to undermine tocotrienol research is the relative inferiority of the bioavailability of orally taken tocotrienols as compared to that of α-tocopherol. The hepatic α-tocopherol transfer protein (α-TTP), together with the tocopherol-associated proteins (TAP), is responsible for the endogenous accumulation of natural α-tocopherol. Although these systems have a much lower affinity to transport tocotrienols, it has been evident that orally supplemented tocotrienol results in plasma tocotrienol concentration in the range of 1 μM [31]. Despite such promising potential of tocotrienol, the experimental analysis accounts for about 1 % of all vitamin E research. The unique vitamin action of α-tocopherol, combined with its prevalence in the human body and the similar efficiency of tocopherols as chain-breaking antioxidants, led biologists to almost completely discount the "minor" vitamin E molecules as topics for basic and clinical research. However, recent findings have enforced a serious reconsideration of this traditional perception [32, 33].

This review article takes a closer look at the methods for the purification and characterization of the lesser known forms of vitamin E, i.e. tocotrienols from different plant sources (Tables 2 and 3). Tocotrienols are found in certain cereals and vegetables such as palm oil, rice bran oil, coconut oil, barley germ, wheat germ and annatto [34, 35]. Palm oil and rice bran oil contain particularly higher amounts of tocotrienols (940 and 465 mg/kg, respectively) [36]. Other sources of tocotrienols include grape fruit seed oil, oats, hazelnuts, maize, olive oil, Buckthorn berry, rye, flax seed oil, poppy seed oil and sunflower oil (Fig. 2) [37].

Tocotrienols: methods of extraction, identification and characterization

Tocotrienols occur in photosynthetic plants in varying amounts, and the vegetable oils such as sunflower, corn, safflower and cottonseed provide a useful source for these vitamin E forms. A number of researchers have developed methods and techniques for extraction, analysis, identification and quantification of tocotrienols from various sources such as solvent extraction, supercritical fluid extraction (SFE), column chromatography, thin layer chromatography, normal and reversed high-performance liquid chromatography (HPLC), etc. The different methods that have been employed in the purification and characterization of tocotrienols have been summarized in the following in Fig. 3. Several different methods have been used for the extraction of vitamin E such as solvent extraction (direct, soxhlet, pressure-liquid and supercritical-fluid), enzymatic method, chemical method (saponification, esterification), adsorption, molecular distillation, microwave-assisted extraction and membrane technology [15].

The efficiency, economy and sufficient degree of purity and quantity are the common primary objectives of any purification procedure. It applies equally well to preparation of sample for characterization and to large-scale production. It is, therefore, necessary to set objectives for purity, amount, retention of biological activity and economy for any process. Although HPLC methods have been proven to be suitable for the separation, determination and isolation of αT oxidation products, only a few methods allow the simultaneous separation of polar and nonpolar oxidation products, although both the normal phase and the reversed phase are used for separation as the stationary phase. The detection of αT oxidation products is generally achieved by diode array detection

Table 3 Tocotrienol content of various palm-derived oil materials (adapted from Maarasyid et al. [15])

Raw material	α-Tocotrienol (%)	γ-Tocotrienol (%)	δ-Tocotrienol (%)
Crude palm oil	20–25	36–45	7–10
Palm pressed fiber oil	13–20	18–23	8–10
Refined bleaching deodorized palm oil	23–29	36–50	6–10
Palm fatty acid distillate	23–24	36–38	13–15
Palm olein	26–28	25–40	6–10
Palm phytonutrient concentrate	17–21	55–60	6–7

The remaining percentage is tocopherols

Table 2 Presence of different tocotrienols in various vegetable oils (adapted from Liu et al. 2008) [38].

	α-Tocotrienol (mg/L)	γ-Tocotrienol (mg/L)	δ-Tocotrienol (mg/L)	Total tocotrienols (mg/L)
Palm	205	439	94	738
Rice bran	236	349	–	585
Wheat germ	26	–	–	26
Coconut	5	1	19	25
Cocoa	2	0	0	2

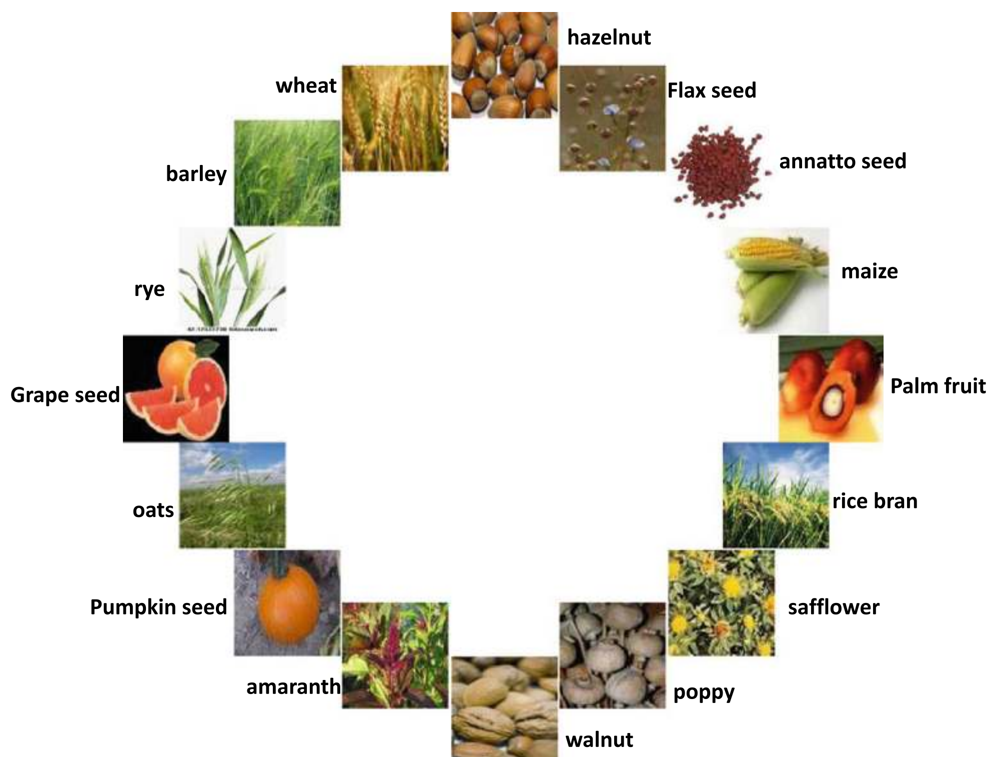

Fig. 2 Natural sources of tocotrienols (reprinted from [37], with kind permission from Springer Science and Business Media)

(DAD) and mass spectroscopy (MS) because of the additional spectroscopic information provided by these methods, and by fluorescence (F) and electrochemical detection. However, no method has yet been developed for the simultaneous determination of αT3 oxidation products with other tocochromanol isomers [16]. Some of the most common methods mentioned have been discussed in detail in this review article.

The determination of tocochromanols in vegetable oils includes the classical physico-chemical techniques to the chromatographic-, spectroscopic- and molecular-based methodologies [14], among others such as liquid-liquid extraction without saponification or solvent extraction after saponification before a chromatographic analysis by HPLC or gas chromatography (GC) [39]. HPLC using both normal (NP) and reversed phases (RP) is the most common methodology used for the analysis of tocochromanols. When comparing RP to NP columns for separation, the latter show the main advantage over the former by completely separating all isomers [39, 40]. HPLC used in the analysis of these compounds includes UV, fluorescence, ELSD, electrochemical and amperometric detection. Fluorescence detection is described as more sensitive and selective than UV. ELSD has been successfully applied in the analysis of different compounds and, consequently, has been increasingly used in many analytical laboratories [14, 40].

Soxhlet extraction

Soxhlet extraction is a process in which the ground plant parts are packed in a Soxhlet apparatus and then extracted with the solvent of choice. In an experiment, tocopherols and tocotrienols were simultaneously determined from hazelnuts. The chopped hazelnuts were extracted on a Soxhlet apparatus (Buchi, Switzerland) with light petroleum ether (b.p. 40–60 °C) during 1.5 h, and the solvent that remained was removed under a stream of nitrogen. An accurately weighted sample of the obtained oil was diluted in hexane, filtered through a 0.22-μm disposable LC filter disc and then directly injected in the HPLC system [41]. In another method, a sample of chopped hazelnuts (~300 mg) was accurately weighed in glass screw cap tubes (Supelco, Bellefonte, PA, USA) and homogenized with 2 mL of ethanol by mixing. Subsequently, 4 mL of hexane was added and again vortex mixed for 1 min. After that, 2 mL of saturated NaCl aqueous solution was added and the mixture was homogenized (1 min), centrifuged (2 min, 5,000×*g*), and the clear upper layer was carefully transferred to another glass screw cap tube. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, at room temperature, on a Reacti-Therm module (Pierce, Rockford, IL, USA), transferred to microcentrifuge tubes with 1.5 mL of hexane and, finally, dehydrated with anhydrous sodium sulfate. The extract was centrifuged (10,000×*g*, 20 s), transferred into a dark injection vial and analysed by HPLC [41]. Hence, the above methods can be used for the characterization of tocotrienols from hazelnuts normal phase liquid extraction methods.

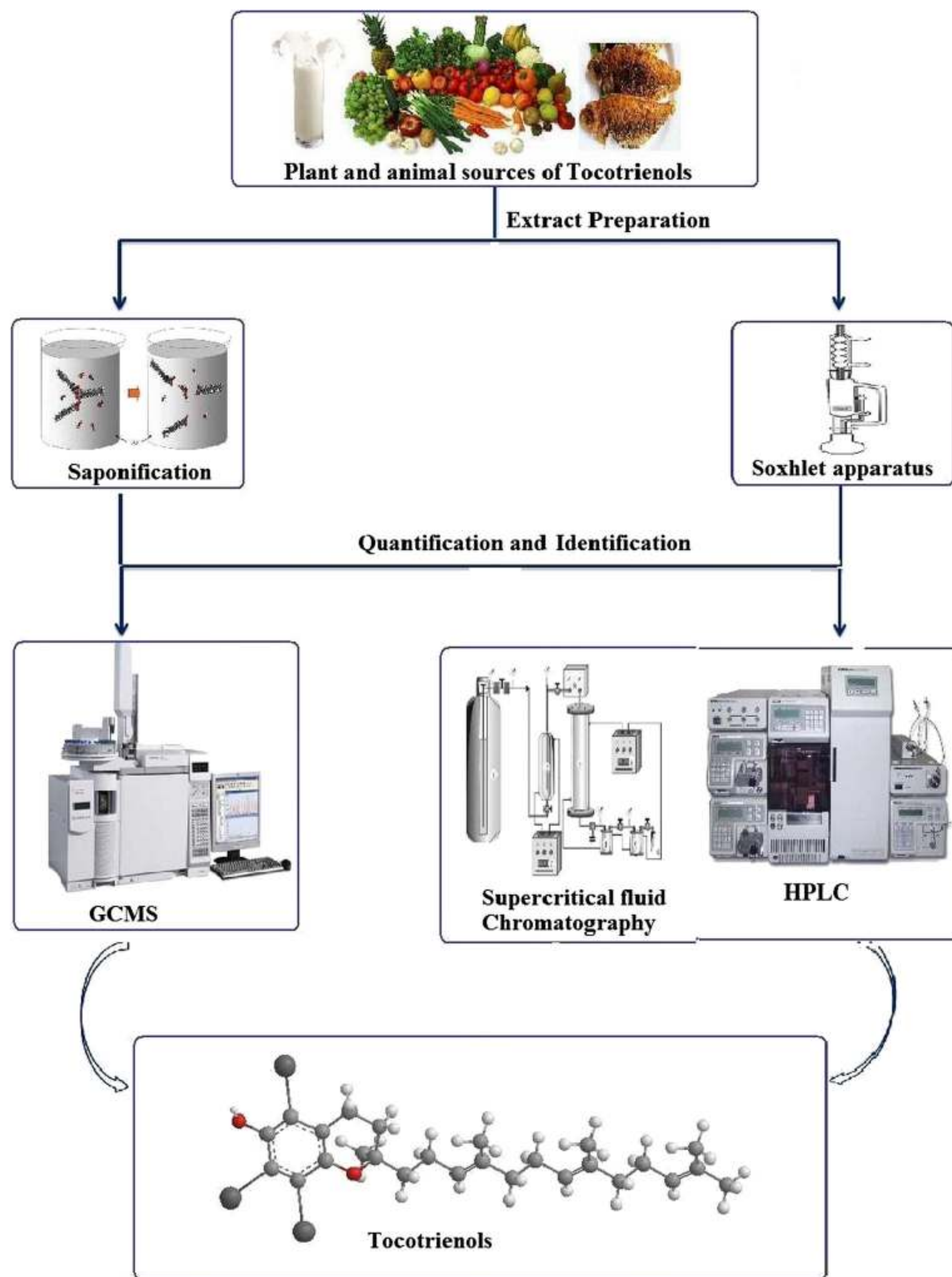

Fig. 3 Different methods for the extraction of tocotrienols

Saponification

Saponification has been regarded as one of the best method for the isolation of tocotrienols from plant as well as animal sources [42]. In one experiment, the method involved an alkaline digestion prior to extraction of the unsaponifiable compounds with hexane. Ethanol (2.5 mL), water (2.5 mL) and 10 M NaOH (0.5 mL) were added to each sample of chopped hazelnuts accurately weighted in a glass screw cap tube and homogenized for 1 min, by vortex mixing. After that, the tubes were flushed with nitrogen and closed. Saponification was performed at 60 °C during 20 min on a Reacti-Therm module. After the addition of water (2.5 mL) and hexane (5.0 mL), it was vortex mixed for 1 min. The tubes were then centrifuged (5 min, 5,000×*g*) and the clear upper layer was carefully transferred to another glass screw cap tube. The sample was re-extracted twice with hexane and processed as described in method II [41].

Chromatography

Chromatography is a separation process based on distribution between two phases, a solid or liquid stationary phase and a liquid or gas mobile phase. The sample is propelled by fluid mobile phase which percolates the stationary phase. Different

chromatographic processes based on characteristic principles may be used for the separation of a wide variety of substances. A given chromatographic process may be run both in low and high pressure systems, although the basic principle remains same.

Thin layer chromatography (TLC) is one of the oldest and widely used chromatographic techniques for the identification, isolation, purification and quantification of assay samples. Tocotrienols from plant origin have been separated efficiently by TLC. Several researchers have developed TLC methods for the isolation, purification and quantification of tocotrienols [42, 43]. In one method, a plant extract was spotted on a silica gel and developed either with hexane-ethylacetate (92.5:7.5) or with chloroform in one dimension and hexane-isopropyl ether (80:20) in another dimension leading to the separation of six compounds; α, β, δ-tocopherols and α, γ, δ-tocotrienols. Visualization of samples is usually carried out under UV light [43]. Liquid–solid column chromatography (CC) has been used by various researchers for the identification and isolation of tocotrienols as it separates the undesirable impurities with high precision as compared to TLC method [44, 45]. Silica gel, Keiselgel and hydrated florisil have been widely used as stationary matrix so as to afford quantitative recovery of tocols.

A number of scientists have developed GC techniques for the accurate measurement of tocols in various oils [46–50]. GC instrument consists of a packed capillary column to afford a high degree of detection, sensitivity and component resolution. A GC column is also attached to a flame ionization detection system (FID) to monitor common effluents or to a mass spectrometer for structural identification and quantification. Some of the reported methods for the analysis of tocopherols and tocotrienols in vegetable oils and related samples are summarized in Table 4. Capillary column possesses some advantages over packed column, as it allows the detection of tocols in nanogram quantities with higher precision, resolution and sensitivity. It also possesses thermal stability [46]. To volatilize the hydroxyl-containing GC detectants, these compounds are more frequently analysed as their ester [47, 48] or trimethylsilane [49, 50] derivatives.

Supercritical fluid chromatography (SFC) employs the use of an inert, low temperature supercritical carbon dioxide as mobile phase eluent which allows it to be advantageous over gas chromatography and HPLC in food industry. SFC allows sample extraction, pre-concentration, chromatographic quantification and preparative fractionation in a single operation. Various tocopherols and tocotrienols have been isolated using SFC [52–57]. Capillary SFC methods for the analysis of tocopherols and other compounds in edible oils and fish oils have been reported [58]. In one method, a successful isolation of tocotrienols from palm oil using SFC was performed [58]. The experiment was performed on JASCO Model SUPER-200 SFC system with a UV–Vis detector equipped with high-pressure flow cells. Columns used consisted of Lichrosorb silica 4.6×250 mm and Macherey-Nagel EC 250/4.6 Nucleosil 100-50 H diol column. SFC conditions included a temperature of 60 °C, pressure of 180 kg/cm^2 and a flow rate at 3.0 mL/min for CO_2 and 0.12 mL/min for ethanol. The supercritical fluid in the study was supercritical CO_2. The column yielded a complete separation of tocol isomers following the sequence: αT, αT_3, βT, βT_3, γT, γT_3 and δT_3.

HPLC (sometimes also referred to as high-pressure liquid chromatography) is a chromatographic technique used to separate a mixture of compounds for various analytical and preparative purposes. HPLC finds enormous number of uses including those in medicine, research and industry. HPLC is distinguished from traditional ("low pressure") liquid chromatography as it operates at significantly higher pressures (50–350 bar), while ordinary liquid chromatography typically relies on the force of gravity and operates at normal atmospheric pressure. Also HPLC columns are made with very fine sorbent particles (2–5 μm in average particle size) that can withstand high pressure generated during the separation. The schematic of an HPLC instrument typically includes a sampler, pumps and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. Some models of mechanical pumps in a HPLC instrument can mix multiple solvents together in

Table 4 Gas chromatographic (GC) analysis of tocotrienols

Sample no.	Source	Column	Detector	Eluted form	Reference
1	Vegetable oils	Packed, 8 ft×4 mm, 6 % SE-52 on chromosorb W AW-DMCS	Flame ionization detector	Tocotrienol propionates	[47]
2	Vegetable oils	Packed, 6 ft×5 mm, 3 % SE-30 on Gaschrom Q	Flame ionization detector	Tocotrienol butyrates	[48]
3	Palm oil	Packed, 3 ft×4 mm, 2 % silicon oil on Chromosorb W AW- DMCS	Flame ionization detector	Native tocotrienols	[51]
4	Fats and oils	Capillary, 150 ft×0.25 mm, Dexsil 400	Flame ionization detector	Tocotrienol (TMS ether)	[50]
5	Oil distillate	Capillary, 90 ft×0.25 mm, 0.25 μm DB-5	Flame ionization detector	Tocol (TMS ether)	[49]
6	Edible oils	Capillary, 30 ft×0.53 mm, HP-1	Flame ionization detector	Tocol acetate	[46]

ratios changing in time, generating a composition gradient in the mobile phase. Though the principle of any chromatographic procedure remains same whether in normal or in high pressure mode, HPLC offers several distinct advantages over the normal pressure chromatography such as high separation capacity, enabling the batch analysis of multiple components, superior quantitative capability and reproducibility, moderate analytical conditions, generally high sensitivity, low sample consumption easy preparative separation and purification of samples. With the advent of HPLC technology, a majority of researchers have shifted their attention towards the use of HPLC methods for the analysis of tocols. It is a very simple technique and can be performed in both normal phase and reverse phase. Both normal and reverse phase HPLC techniques have been extensively used for the analysis of tocols in various sample matrices. Normal phase HPLC possesses some shortcomings such as long equilibration times and employment of hazardous volatile organic solvents. Different silica-based stationary phases have been employed by different researchers for the analysis of tocopherols and tocotrienols such as Lichrosorb Si 60 [59–61], Zorbax Si [10, 35], Ultrasphere Si [62], etc. Table 5 summarizes the various normal phase and reverse phase HPLC methods used by various researchers to separate the tocols from different origins.

Capillary electrochromatography (CEC) is a hybrid technique combining aspects of both HPLC and capillary electrophoresis (CE). As in the case of CE, the separation is performed in a capillary column with electro-osmotic flow as the driving force for the mobile phase and solutes transported along the column containing the stationary phase either packed or bound to the capillary wall. The advantages of CEC include the ability to analyse both neutral and changed solutes, low sample requirements and reduced organic solvent consumption. Moreover, it has been reported that CEC provides higher efficiencies compared to HPLC, better resolution, higher peak capacity and shorter analysis time. CEC has proven to be a successful technique in the separation of neutral compounds, pharmaceuticals, food/biological samples and environmental chemicals. It has also been successfully used by various researchers to separate the tocotrienols from their different sources. The technique allows high speed microanalysis with minimal solvent consumption. The instrument includes a CEC apparatus equipped with a diode array detector (290 nm) and a HP Chem Station software for system control. Three commercial CEC capillary columns (25 cm×100 μm I.D.) packed with 3 μm alkyl or aryl bonded silica and one 3 μm silica column obtained from a different source is widely used. These columns include CEC Hyposil C8, CEC-Hypersil C18, CEC-Hypersil phenyl and Unimicre CEC silica, 25 cm×75 μm I.D. The samples are run electrokinetically onto the column at 10 kV for 10 s. Column temperature and separation voltage were maintained at 25 kV and 30 °C, respectively. CEC coupled with calibration data enables speedy sample analysis with adequate component resolution and enhanced detection sensitivity. CEC method can serve as a viable alternative to existing RP-HPLC method and can be used in the routine analysis of tocopherols and tocotrienols in oil samples [42, 102–104].

Mass spectrometry

Spectroscopic methods represent one of the main tools of modern chemistry for the determination of the authenticity of edible oils. Most spectroscopic methods for the detection are based on NMR spectroscopy of ^{1}H and ^{13}C, infrared spectroscopy, Raman spectroscopy, fluorescence spectroscopy and mass spectrometry. The primary advantage of these methods is related to the nondestructive character, simplicity (relative easy sample preparation and adaptation for the use by untrained personnel), rapidness and moderate cost instrumentation [14].

Mass spectrometry has not been widely used for the analysis of vitamin E owing to the difficulty involved in the ionization of non-polar molecules. Atmospheric pressure chemical ionization (APCI) has been used as an ionization technique for LC–MS analysis of tocopherols in biological samples. Unlabeled α-T and deuterated tocopherols have been quantified in human plasma [105], and retinol, α-T and β-carotene in human serum by normal phase liquid chromatography–atmospheric pressure chemical ionization-tandem mass spectrometry (LC–APCI-MS–MS) [106]. LC–APCI-MS–MS has also been proposed to determine α-tocopherol and carotenoids [107] in plant materials. It was reported by Bustamante-Rangel et al. [108] that detection using APCI mode is 100 times more sensitive than that with an electrospray (ESI) mode. ESI is the most frequently used ionization technique for LC–MS. However, the lack of a site for protonation or deprotonation on non-polar substances such as fat-soluble vitamins hinders their ionization. The addition of a metal salt to themobile phase enhances the ionization of tocopherols; this ionization technique is called coordinated ion spray (CIS). Silver is the metal usually added, forming Ag^{+}-tocopherol adducts that facilitate ionization. This approach has been used for the detection and identification of tocopherols and carotenoids in several food products [84], and tocotrienol isomers, α-tocoenol and α-T in a crude palm oil extract rich in vitamin E homologues [87]. Using the same method, α-T, α-tocopheryl succinate and α-tocopheryloxyacetic acid in mouse serum were analysed [109]. Some authors have enhanced the ESI, in positive ion mode, of tocopherols by the addition of an acid to the mobile phase to produce, [M + H]+ species. Thus, α-T and α-tocopherol quinone have been quantified in human plasma by the addition of formic acid to the mobile phase without problems due to the large amounts of this compound in human plasma [110]. Negative ion mode electrospray ionization was used for the determination of unlabelled and deuterium-labelled α-T in blood components [111]. ESI is not usually used in food samples because food matrices are

Table 5 High-performance liquid chromatographic (HPLC) analysis of tocotrienols

Sample no.	Source	Stationary/mobile phase	Detector	Elution order	Reference
	Foods, tissues	LiChrosorb Si 60, 5 μm 250×3.2 mm, hexane/isopropanol (99.8:0.2)	Florescence, $290_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma T_3 \rightarrow \delta T$	[61]
	Palm oils	ZorbaxSil, 5 μm 250×4.6 mm, hexane/tetrahydrofuran/methanol (97.25:2.5:0.25)	Florescence, $298_{Excitation}$ $325_{Emission}$	$\alpha T \rightarrow \alpha T_3 \gamma T_3 \rightarrow \delta T_3$	[10]
	Corn grain	Ultrasphere Si, 5 μm 250×4.6 mm, hexane/isopropanol (98.8:1.2)	Florescence, $205_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \gamma T \rightarrow \gamma T_3 \rightarrow \delta T$	[62]
	Seed oils	Partisil PAC, 5 μm 250×4.6 mm, hexane/tetrahydrofuran (94:6)	Florescence, $210_{Excitation}$ $325_{Emission}$	$\alpha T \rightarrow \alpha T_1 \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma T$ $_3 \rightarrow \delta T \rightarrow \delta T_3$	[63]
	Seed oils	Naphthylethylsilica, 5 μm, 750×0.53 mm, hexane/hexafluoroisopropanol (99.9:0.1)	UV 295 nm	$\alpha T \rightarrow \gamma T \rightarrow T \rightarrow \delta T$	[64]
	Palm oils	ZorbaxSil, 5 μm 250×4.6 mm, hexane/isopropanol (99:1)	UV 295 nm	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[35]
	Seed oils, Cereals	LiChrospher 100 Diol, 5 μm 250×4.0 mm, hexane/t-butylmethyl ether (96:4)	Florescence, $295_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma_3 \rightarrow \delta T \rightarrow \delta T_3$	[65]
	Stillingia oil	Nucleosil 50 Si, 5 μm 250×4.0 mm, hexane/dioxane (95:5)	Florescence, $295_{Excitation}$ $330_{Emission}$	$\alpha T_3 \rightarrow \beta T \rightarrow \gamma T_3 \rightarrow \delta T_3$	[66]
	Foods	LiChrospher 100 Diol, 5 μm 250×4.0 mm, hexane→hexane/t-butylmethyl ether (97:3)→hexane/t-butylmethyl ether (95:5) (gradient elution)	Florescence, $295_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow AC$ -$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \beta T_3 \rightarrow \gamma T$ $_3 \rightarrow \delta T \rightarrow \delta T_3$	[65]
	Rice bran	Supelcosil LC-Si, 5 μm 250×4.6 mm: (i) isooctane–ethyl acetate (97.5:2.5), (ii) isooctane/ethyl acetate/acetic acid/2, 2-dimethoxypropane (98.15:0.9:0.85:0.1)	Florescence, $290_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T$-$\beta T_3 \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[60]
	Margarine	Hypersil Si, 5 μm 100×2.1 mm hexane/isopropanol (99.8:0.2)	Florescence, $290_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \beta T \rightarrow \gamma T \rightarrow \delta T$	[67]
	Vegetable oils	Cyclobond I, 5 μm 250×4.6 mm, cyclohexane/diisopropyl ether (95:5)	Florescence, $298_{Excitation}$ $345_{Emission}$	$\alpha T \rightarrow \zeta T \rightarrow \beta T \rightarrow \gamma T \rightarrow \delta T$	[44]
	Tissues	LiChrosorb Si 60, 5 μm 125×4.6 mm, hexane/dioxane (97:3)	Florescence, $295_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[59]
	Soybean oil, wheat bran	Econosil Si, 10 μm 250×10 mm, hexane/tetrahydrofuran (gradient 0→15 % tetrahydrofuran)	Florescence, $290_{Excitation}$ $330_{Emission}$	(i) $\alpha T \rightarrow \beta T \rightarrow \gamma T \rightarrow \delta T$ ii) $\alpha T_3 \rightarrow \beta T_3 \rightarrow \gamma T_3 \rightarrow \delta T_3$	[60]
	Vegetable oils	LiChrosorb Si 60, 5 μm 250×4.0 mm, hexane/isopropanol (99.7:0.3)	Florescence, $290_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[68]
	Foods	Nucleosil 100-5 NO_2, 5 μm 250×4.0 mm, hexane→hexane/t-butylmethyl ether (98:2)→hexane/t-butylmethyl ether (98:2)→hexane/t-butylmethyl ether (85:15) (gradient elution)	Florescence, $295_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow AC \rightarrow \alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \beta T_3 \rightarrow \gamma T$ $_3 \rightarrow \delta T \rightarrow \delta T_3$	[69]
	Foods	Chromega Diol, 5 μm 250×4.6 mm, hexane/diisopropyl ether (95:5)	FL, $298_{Excitation}$ $345_{Emission}$	$\alpha T \rightarrow \zeta T \rightarrow \beta T \rightarrow \gamma T \rightarrow \delta T$	[44]
	Tissues, diet	Supelcosil LC-Diol, 5 μm 250×4.6 mm, hexane/isopropanol (99:1)	Florescence, $290_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[70]
	Foods	Vydac C_8, 10 μm 250×3.2 mm, methanol/water (95:5) + acetic acid	Florescence, $295_{Excitation}$ $330_{Emission}$	$\delta T \rightarrow (\beta + \gamma)T \rightarrow \alpha T$	[71]
	Feeds	Yanapack ODS-T C_{18}, 5 μm 250×4.0 mm, methanol + 50 mM $NaClO_4$	ED, +0.8 V	$\delta T \rightarrow (\beta + \gamma)T \rightarrow \alpha T$	[72]
	Vegetable oils	Spheri-5 RP-18, 5 μm 100×2.1 mm, methanol/water (95:5)	UV, 300 nm	$\delta T \rightarrow (\beta + \gamma)T \rightarrow \alpha T$	[73]
	Tissues	Ultrasphere ODS, 5 μm 250×4.6 mm, methanol/ethanol (1:9) + 20 mM $LiClO_4$	ED, +0.8 V	$\delta T \rightarrow \gamma T \rightarrow \alpha T$	[74]
	Tissues	Spherisorb ODS II, 3 μm 150×4.6 mm, methanol/water (96:4) + $NaClO_4$	ED, +0.6 V	$\delta T \rightarrow \gamma T \rightarrow \alpha T$	[75]

Table 5 (continued)

Sample no.	Source	Stationary/mobile phase	Detector	Elution order	Reference
	Tissues	Ultrasphere ODS, 5 µm 150×4.6 mm, isopropanol/acetonitrile/ water/tetraethyl ammonium hydroxide/acetic acid (60:20:19.4:0.5:0.1)	ED, +0.3 V	$\delta T \rightarrow \gamma T \rightarrow \alpha T$	[76]
	tissues	Polymeric C_{18}, 5 µm 750×0.53 mm, acetonitrile/hexane (91.5:8.5)	UV, 295 nm	$\delta T \rightarrow \beta T \rightarrow \gamma T \rightarrow \alpha T$	[64]
	Palm oil	Zorbax ODS, 5 µm 250×4.6 mm, acetonitrile/methanol/CH_2Cl_2 (60:35:5)	UV, 295 nm	$\delta T_3 \rightarrow \gamma T_3 \rightarrow \alpha T_3 \rightarrow \delta T \rightarrow (\beta+\gamma)T \rightarrow \alpha T$	[35]
	Tissues	YMCPack-A-ODS, 5 µm 150×4.6 mm, isopropanol/water (65:35)	Florescence, $298_{Excitation}$ $325_{Emission}$	$\delta T \rightarrow \gamma T \rightarrow \beta T \rightarrow \alpha T$	[77]
	Tissues	Resolve C_{18}, 5 µm 300×3.9 mm, acetonitrile/CH_2Cl_2/methanol/ octanol (90:15:10:0.1)	UV, 300 nm	$\gamma T \rightarrow \alpha T$	[78]
	Foods	Bakerbond C_{18}, 5 µm 250×4.6 mm, acetonitrile/methanol + ammonium acetate/ethyl acetate (gradient elution)	Florescence, $295_{Excitation}$ $335_{Emission}$	$\epsilon T \rightarrow \delta T \rightarrow \gamma T \rightarrow \alpha T$	[79]
	Tissues	Superspher 100RP-18, 4 µm 250×4 mm, methanol/ethanol (1:9) + 2.5 mM $HClO_4$ + 7.5 mM $NaClO_4$	ED, 0.35 V	$\delta T \rightarrow (\beta+\gamma)T \rightarrow \alpha T$	[80]
	Tissues	Ultrasphere ODS, 5 µm 250×4.6 mm, acetonitrile/tetrahydrofuran/ methanol/1 % ammonium acetate (684:220:68:28)	Florescence, $298_{Excitation}$ $328_{Emission}$	$\delta T_3 \rightarrow (\beta+\gamma)T_3 \rightarrow \alpha T_3 \rightarrow \delta T \rightarrow (\beta+\gamma)T \rightarrow \alpha T$	[59]
	Vegetable oils	Taxsil PFP, 5 µm, methanol/water (92:8)	UV, 290 nm	$\delta T \rightarrow \beta T \rightarrow \gamma T \rightarrow \alpha T$	[81]
	Tissues	C_{18} Vydac201TP54, 5 µm 250×4.6 mm, methanol/acetonitrile (9:1)	PDA, 200 nm→800 nm	$\delta T \rightarrow \alpha T$	[82]
	Vegetable oils	Spherisorb ODS, 5 µm 250×4.5 mm, methanol/water (90:10) + $NaClO_4$	ED, +0.6 V	$(\beta+\gamma)T_3 \rightarrow (\beta+\gamma)T \rightarrow \alpha T$	[68]
	Vegetable oils	Asahipak ODP, 5 µm 250×4.6 mm, acetonitrile/water (85:15)	Florescence, $298_{Excitation}$ $345_{Emission}$	$\delta T \rightarrow \zeta T \rightarrow \beta T \rightarrow \gamma T \rightarrow \alpha T$	[44]
	Vegetable oils	YMCPack-ODS-A, 5 µm 250×4.6 mm, methanol/water (95:5)	Florescence, $298_{Excitation}$ $345_{Emission}$	δT-AC→ζT-AC→βT-AC→γT-AC→αT-AC	[44]
	Tissues	Suplex pKb-100, 5 µm 250×4.6 mm, methanol/t-butylmethyl ether/water (80:20:5)	PDA, 200 nm→800 nm	αT-AC→γT→αT	[83]
	Foods	YMCPack-C30, 3 µm 250×4.6 mm, acetone/water + $AgClO_4$ (90:10)→(100:0) (gradient elution)	UV, 295 nm MS	δT→γT→βT→αT→αT-AC	[84]
	Tissues	Microsorb MV-C_{18}, 3 µm 100×4.6 mm A: methanol/water (3:1) + ammonium acetate B: methanol/CH_2Cl_2 (4:1) A→B→B (gradient elution)	PDA, 200 nm→800 nm	$\gamma T \rightarrow \alpha T$	[85]
	Tissues	Asahipak ODP, 5 µm 250×4.6 mm, acetonitrile–water (70:30)	Florescence, $290_{Excitation}$ $330_{Emission}$	$\delta_1 T_3 \rightarrow \delta_2 T_3 \rightarrow \delta_3 T_3 \rightarrow \delta_4 T_3 \rightarrow \beta_1 T_3 \rightarrow \gamma_1 T_3 \rightarrow \beta_2 T_3 \rightarrow \gamma_2 T_3 \rightarrow \beta_3 T_3 \rightarrow \gamma_3 T_3 \rightarrow \beta_4 T_3 \rightarrow \gamma_4 T_3 \rightarrow \alpha_1 T_3 \rightarrow \alpha_2 T_3 \rightarrow \alpha_3 T_3 \rightarrow \alpha_4 T_3$	[86]
	Palm oil	YMCPack-C30, 3 µm 250×4.6 mm, methanol	UV, 295 NMR. MS	$\delta T_3 \rightarrow \gamma T_3 \rightarrow \beta T_3 \rightarrow \alpha T_3 \rightarrow \alpha T_1 \rightarrow \alpha T$	[87]
	Tissues	Ultrasphere ODS, 5 µm 250×4.6 mm, methanol/water/ethanol + 0.2 % $LiClO_4$ (gradient elution)	ED, 0.5 V	$\gamma T_3 \rightarrow \alpha T_3 \rightarrow \gamma T \rightarrow \alpha T$	[88]
	Tissues	Hypersil ODS, 5 µm 150×4.6 mm, methanol/water (96:4)	Florescence, $296_{Excitation}$ $340_{Emission}$	$\epsilon T \rightarrow \delta T \rightarrow \gamma T \rightarrow \alpha T$	[89]
	Tissues	SuperPacPeP-S $RP_{C2/C18}$, 5 µm 250×4.6 mm, methanol/ethanol/ isopropanol (88:24:10) + 13 mM $LiClO_4$	ED, +0.6 V	$\gamma T_3 \rightarrow \gamma T \rightarrow \alpha T$	[90]
	Vegetable oils	Silica Sep-pak, mu-Bondapak $RP_{C2/C18}$ column 5 µm 250×4.6 mm, methanol/water (95:5)	Florescence, $296_{Excitation}$ $340_{Emission}$	$\delta T_3 \rightarrow \gamma T_3 \rightarrow \beta T_3 \rightarrow \alpha T_3 \rightarrow \alpha T_1 \rightarrow \alpha T$	[91]
	Synthetic α-tocotrienol	Chiralcel OD-H column (250×4.6 mm, 5 mm particle size, adsorbent cellulose derivated with 3,5-dimethyl phenyl carbamate), 0.05 % 2-propanol in isohexane	Fluorescence, $295_{Excitation}$ $330_{Emission}$	RS, Z-Z-αT_3→E/Z-αT_3→E/Z-αT_3→E-E-αT_3→ E-E-αT_3→E/Z-αT_3→E/Z-αT_3→Z-Z-αT_3→f E/Z-αT_3→S, E-E-αT_3→R, E-E-αT_3	[92]

Table 5 (continued)

Sample no.	Source	Stationary/mobile phase	Detector	Elution order	Reference
	Vegetable oils	(i) polymethacrylic adsorbent I.D. column 150×4.6 mm, 10 μm (ii) silica-based I.D. column 250×4.6 mm, 5 μm: (i) hexane–EtOH (98:2), (ii) hexane–EtOH (99:1)	UV, 254 nm	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \beta T_3 \gamma T \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[93]
	Tissues	MicrosorbuMV C18, 12 cm, 3 μm, 100 A column, methanol/ethanol (1:3) containing 20 mM lithium perchlorate	LC-4B amperometric detector, 500 mV	$\alpha T_3 \rightarrow \gamma T_3 \rightarrow \alpha T \rightarrow \gamma T$	[94]
	Chicken meat	Lichrosphere Si 100 silica column (5 μm, 250 mm×4.6 mm)	Fluorescence, $295_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[95]
	Hazelnuts	Inertsil 5 SI column (250×3 mm), hexane/1,4-dioxane (95.5:4.5)	Diode array detector (DAD) connected in series with an FP-920 fluorescence detector, $290_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \beta T_3 \rightarrow \gamma T \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[41]
	Olive oils	Inertsil 5 SI normal-phase column (250 mm×3 mm I.D.), 1,4-dioxane/*n*-hexane (3.5:96.5, *v*/*v*)	(i) Diode array detector (DAD) connected in series with an FP-920 fluorescence detector, $290_{Excitation}$ $330_{Emission}$, (ii) UV, 295 nm; (iii) ELSD (evaporator temperature 40 °C; air pressure 3 bar; and photomultiplier sensitivity 4)	$\alpha T \rightarrow \beta T \rightarrow \gamma T \rightarrow \delta T \rightarrow \alpha T_3$	[96]
	Sea Buckthorn	Phenomenex Luna 5 μm silica (2) 100A, 250×4.60 mm column with a Security Guard silica 4 mm L×3.0 mm precolumn, *n*-hexane/ethyl acetate/acetic acid (97.3:1.8:0.9)	Fluorescence detector, $290_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma T$ $_3 \rightarrow \delta T \rightarrow \delta T_3$	[97]
	Simvastatin-tocotrienolrich fraction nanoparticle	RP-C_{18} column (4.6 mm×100 mm), water/methanol (15:85, gradient elution)	UV, 238 nm, 295 nm	$\delta T_3 \rightarrow \gamma T_3 \rightarrow \alpha T_3 \rightarrow \alpha T$	[98]
	Vegetable oils	Nano-C18 silica monolithic column (150 mm×0.1 mm), acetonitrile/ methanol/water (acidified with 0.2 % acetic acid)	UV, 295 nm	$\alpha T \rightarrow \gamma T \rightarrow \delta T$	[99]
	Rice bran	Inertsil CN-3, SIL-100A 5 μM (4.6 mm×250 mm) column hexane/ isopropanol/ethylacetate/acetic acid (97.6:0.8:0.8:0.8)	UV, 295 nm	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[100]
	Palm oils	Silica column, 4.6 mm I.D.×250 mm: (i) hexane/THF/IPA (95:4:1), (ii) heptane/ethyl acetate (95:5)	Photodiode array detector, 280 nm	αT, αT_3, γT, and γT_3, and δT_3	[52]
	Rose hips	Phenomenex Luna 5 μm silica (2) 100A, 250×4.60 mm column with a Security Guard silica 4 mm L×3.0 mm precolumn, *n*-hexane/ethyl acetate/acetic acid (97.3:1.8:0.9)	Fluorescence detector, $290_{Excitation}$ $330_{Emission}$	$\alpha T \rightarrow \alpha T_3 \rightarrow \beta T \rightarrow \gamma T \rightarrow \beta T_3 \rightarrow \gamma T_3 \rightarrow \delta T \rightarrow \delta T_3$	[101]

generally more complex than clinical samples for the quantification of tocopherols [108].

Bustamante-Rangel et al. [108] have developed a rapid and simple method for the determination of tocopherols and tocotrienols in cereals. The analytes were extracted from cereals using pressurized liquid extraction (PLE) and separation was carried out by liquid chromatography, and detection was performed with electrospray ionization mass spectrometry (LC–ESI-MS). MS was chosen as the detection technique because it can provide additional information about analytes and hence allows confirmation of the presence of tocotrienols in the samples analysed. Extraction of analytes from the sample using PLE allowed their direct injection into the chromatographic system, which afforded simplicity, speed and the possibility of automation of the methodology. PLE provides some advantages over other extraction techniques, such as low solvent consumption, the possibility of automation and speed. Moreover, the reduction in sample manipulation leads to a decrease in error propagation. Negative ion mode electrospray ionization was used for the ionization of tocopherols and tocotrienols. The difficulty involved in the ionization of these molecules was avoided by adding a base, specifically the presence of ammonia in the mobile phase was essential to enhancing their ionization. The sensitivity of this LC–ESI-MS method is higher than other LC–APCI-MS. The proposed method was found to be reliable for the determination of tocopherols and tocotrienols in cereals. The repeatability and accuracy of the proposed procedure was verified by analysing the vitamin E content of wheat and rye samples [108].

Therefore, PLE-LC-ESI MS is a technique that uses solvents at high pressure and temperature above their boiling point to extract substances from solid matrix. PLE offers several advantages over other extraction techniques, such as possibility of automation, low solvent volume and reduced extraction times. PLE has been used for the extraction of vitamin E prior to chromatographic determination. Thus, Bustamante-Rangel et al. [108] developed a rapid and simple method including PLE followed by LC-ESI-MS for the determination of tocopherols and tocotrienols in cereals. Extraction of analytes from the sample using PLE allowed their direct injection into the chromatographic system, which afforded simplicity, speed and possibility of automation of the methodology. Separation was carried out by LC and detection was performed with ESI mass spectrometry. Negative ion mode electrospray ionization was used for the ionization of tocopherols and tocotrienols. The difficulty in the ionization of these molecules was avoided by adding a base. Almost all of the tocopherols and tocotrienols were analysed successfully by this method.

Conclusion

Tocotrienols, members of the vitamin E family, are natural compounds found in a number of vegetable oils, wheat germ, barley and certain types of nuts and grains. The unsaturated chain of tocotrienol allows an efficient penetration into tissues that have saturated fatty layers such as the brain and liver. Recent mechanistic studies indicate that other forms of vitamin E, such as γ-T, δ-T and γ-T3, have unique antioxidant and anti-inflammatory properties that are superior to those of α-tocopherol against chronic diseases. Tocotrienols are detectable at appreciable levels in the plasma after supplementations. However, there is inadequate data on the plasma concentrations of tocotrienols that are sufficient to demonstrate significant physiological effect and biodistribution studies show their accumulation in vital organs of the body. Considering the wide range of benefits that tocotrienols possesses against some common human ailments and having a promising potential, the current state of knowledge deserves further investigation into this lesser known forms of vitamin E.

In recent years, the basic research on vitamin E has expanded from primarily focusing on αT and its antioxidant effects to investigation of different tocopherols and tocotrienols, their metabolism and their non-antioxidant activities including anti-inflammatory property [8, 9]. In contrast to αT, accumulating evidence suggests that γT, δT and tocotrienols seem to have unique properties that are superior to αT and relevant to prevention and therapy against chronic diseases even under conditions with adequate αT status [112]. In spite of the promising potential, the experimental analysis of tocotrienols accounts for only a small portion of total vitamin E research.

Thus, the literature shows a very broad spectrum of medicinal properties of these vitamin E variants. α-T3, γ-T and δ-T3 have emerged as vitamin E molecules with functions in health and disease that are clearly distinct from that of α-T. An expanding body of evidence support that members of the vitamin E family are functionally unique. The current state of knowledge deserves further investigation on tocotrienols. Therefore, it becomes imperative that an assessment in order to regulate the quality, authenticity and consistency of delivery of the compound product to the pharmaceutical industry should be carried out. Hence, this review article containing the methods for analysis and characterization of tocotrienols will be helpful in fulfilling the experimental and methodological information on this vitamin E variant.

Acknowledgments The authors would like to acknowledge and thank the reviewers for their critical reading, comments and suggestions for the improvement of manuscript.

Conflict of interest The authors declare that there is no conflict of interest.

References

1. Hopkins FG (1912) Feeding experiments illustrating the importance of accessory factors in normal dietaries. J Physiol 44(5–6):425–460
2. Funk C (1912) The etiology of the deficiency diseases. Beri-beri, polyneuritis in birds, epidemic dropsy, scurvy, experimental scurvy in animals, infantile scurvy, ship beri-beri, pellagra. J State Med 20: 341–368
3. Funk C (1975) The Journal of State Medicine. Volume XX: 341-368, 1912. The etiology of the deficiency diseases, beri-beri, polyneuritis in birds, epidemic dropsy, scurvy, experimental scurvy in animals, infantile scurvy, ship beri-beri, pellagra. Nutr Rev 33(6):176–177
4. Evans HM, Emerson OH, Emerson GA (1936) The isolation from wheat germ oil of an alcohol, a-tocopherol, having the properties of vitamin E. J Biol Chem 113(1):319–332
5. Joffe M, Harris P (1943) The biological potency of the natural tocopherols and certain derivatives. J Am Chem Soc 65:925–927
6. Bunyan J, Mc HD, Green J, Marcinkiewicz S (1961) Biological potencies of epsilon and zeta-1-tocopherol and 5-methyltocol. Brit J Nutr 5:253–257
7. Whittle KJ, Dunphy PJ, Pennock JF (1966) The isolation and properties of delta-tocotrienol from *Hevea* latex. Biochem J 100:138–145
8. Qureshi AA, Burger WC, Peterson DM, Elson CE (1986) The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J Biol Chem 261(23):10544–10550
9. Guthrie N, Gapor A, Chambers AF, Carroll KK (1997) Inhibition of proliferation of estrogen receptor-negative MDA-MB-435 and -positive MCF-7 human breast cancer cells by palm oil tocotrienols and tamoxifen, alone and in combination. J Nutr 127(3):544S–548S
10. Kato A, Gapor A, Tanabe K, Yamaoka M, Mamuro H (1981) Esterified & alpha-tocopherol and tocotrienols in palm oils. J Jpn Oil Chem Soc 30:590–591
11. Colombo ML (2010) An update on vitamin E, tocopherol and tocotrienol—perspectives. Molecules 15:2103–2113
12. Kamal Eldin A, Appelqvist LA (1996) The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31(7): 671–701
13. Vagni S, Saccone F, Pinotti L, Baldi A (2011) Vitamin E bioavailability: past and present insights. Food Nutr Sci 2:1088–1096
14. Cunha SC, Amaral JS, Oliveira MB (2011) Authentication of vegetable oils. Current topics on food authentication: ISBN: 978-81-7895-510-0.ed: M. Beatriz PP Oliveira, Isabel Mafra, JS Amaral
15. Maarasyid C, Muhamad II, Supriyantob E (2014) Potential source and extraction of vitamin E from palm-based oils: a review. J Teknologi 69(4):43–50
16. Büsing A, Ternes W (2011) Separation of α-tocotrienol oxidation products and eight tocochromanols by HPLC with DAD and fluorescence detection and identification of unknown peaks by DAD, PBI-EIMS, FTIR, and NMR. Anal Bioanal Chem 401(9): 2843–2854
17. Jiang Q, Christen S, Shigenaga MK, Ames BN (2001) gamma-Tocopherol, the major form of vitamin E in the US diet, deserves more attention. Am J Clin Nutr 74(6):714–722
18. Brigelius-Flohé R, Traber MG (1999) Vitamin E: function and metabolism. FASEB J 13(10):1145–1155
19. Moya-Camarena SY, Jiang Q (2012) The role of vitamin E forms in cancer prevention and therapy—studies in human intervention trials and animal models. In: Sarkar FH (ed) Nutraceuticals and cancer. Springer, New York, pp 323–354
20. Myung SK, Ju W, Cho B, Oh SW, Park SM, Koo BK, Park BJ, Korean Meta-Analysis Study Group (2013) Efficacy of vitamin and antioxidant supplements in prevention of cardiovascular disease: systematic review and meta-analysis of randomised controlled trials. BMJ 346:f10
21. Seppanen CM, Song QH, Csallany AS (2010) The antioxidant functions of tocopherol and tocotrienol homologues in oils, fats, and food systems. J Am Oil Chem Soc 87(5):469–481
22. Khanna S, Parinandi NL, Kotha SR, Roy S, Rink C, Bibus D, Sen CK (2010) Nanomolar vitamin E α-tocotrienol inhibits glutamate induced activation of phospholipase A2 and causes neuroprotection. J Neurochem 112(5):1249–1260
23. Sen CK, Khanna S, Roy S (2007) Tocotrienols in health and disease: the other half of the natural vitamin E family. Mol Asp Med 28(5–6):692–728
24. Serbinova E, Kagan V, Han D, Packer L (1991) Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. Free Radic Biol Med 10(5):263–275
25. Sen CK, Khanna S, Roy S (2006) Tocotrienols: vitamin E beyond tocopherols. Life Sci 78(18):2088–2098
26. Pearce BC, Parker RA, Deason ME, Dischino DD, Gillespie E, Qureshi AA, Volk K, Wright JJ (1994) Inhibitors of cholesterol biosynthesis. 2. Hypocholesterolemic and antioxidant activities of benzopyran and tetrahydronaphthalene analogues of the tocotrienols. J Med Chem 37(4):526–541
27. Pearce BC, Parker RA, Deason ME, Qureshi AA, Wright JJ (1992) Hypocholesterolemic activity of synthetic and natural tocotrienols. J Med Chem 35(20):3595–3606
28. Serbinova E, Kagan V, Han D, Packer L (1991) Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. Free Rad Biol Med 10(5):263–275
29. Serbinova EA, Packer L (1994) Antioxidant properties of alpha-tocopherol and alpha-tocotrienol. Meth Enzymol 234:354–366
30. Suzuki YJ, Tsuchiya M, Wassall SR, Choo YM, Govil G, Kagan VE, Packer L (1993) Structural and dynamic membrane properties of alpha-tocopherol and alpha-tocotrienol: implication to the molecular mechanism of their antioxidant potency. Biochemistry 32(40): 10692–10699
31. O'Byrne D, Grundy S, Packer L, Devaraj S, Baldenius K, Hoppe PP, Kraemer K, Jialal I, Traber MG (2000) Studies of LDL oxidation following alpha-, gamma-, or delta-tocotrienyl acetate supplementation of hypercholesterolemic humans. Free Rad Biol Med 29(9):834–845
32. Hensley K, Benaksas EJ, Bolli R, Comp P, Grammas P, Hamdheydari L, Mou S, Pye QN, Stoddard MF, Wallis G, Williamson KS, West M, Wechter WJ, Floyd RA (2004) New perspectives on vitamin E: gamma-tocopherol and carboxyelthylhydroxychroman metabolites in biology and medicine. Free Rad Biol Med 36(1):1–15
33. Das S, Lekli I, Das M, Szabo G, Varadi J, Juhasz B, Bak I, Nesaretam K, Tosaki A, Powell SR, Das DK (2008) Cardioprotection with palm oil tocotrienols: comparison of different isomers. Am J Physiol Heart Circ Physiol 294:H970–H978
34. Qureshi AA, Mo H, Packer L, Peterson DM (2000) Isolation and identification of novel tocotrienols from rice bran with hypocholesterolemic, antioxidant, and antitumor properties. J Agric Food Chem 48:3130–3140
35. Tan B, Brzuskiewicz L (1989) Separation of tocopherol and tocotrienol isomers using normal- and reverse-phase liquid chromatography. Anal Biochem 180:368–373
36. Aggarwal BB, Sundaram C, Prasad S, Kannappan R (2010) Tocotrienols, the vitamin E of the 21st century: its potential against cancer and other chronic diseases. Biochem Pharmacol 80:1613–1631
37. Kannappan R, Gupta SC, Kim JH, Aggarwal BB (2012) Tocotrienols fight cancer by targeting multiple cell signaling pathways. Genes Nutr 7(1):43–52
38. Liu DH, Shi J, Rodríguez Posada L, Kakuda Y, Xue SJ (2008) Separating Tocotrienols from Palm Oil by Molecular Distillation. Food Reviews International, 24(4):376–391

39. Cert A, Moreda W, Pérez-Camino MC (2000) Chromatographic analysis of minor constituents in vegetable oils. J Chromatogr A 881(1–2):131–148
40. Cunha SC, Amaral JS, Fernandes JO, Oliveira MB (2006) Quantification of tocopherols and tocotrienols in Portuguese olive oils using HPLC with three different detection systems. J Agric Food Chem 54(9):3351–3356
41. Amaral JS, Casal S, Torres D, Seabra RM, Oliveira BP (2005) Simultaneous determination of tocopherols and tocotrienols in hazelnuts by a normal phase liquid chromatographic method. Anal Sci 21:1545–1548
42. Abidi SL (2000) Chromatographic analysis of tocol-derived lipid antioxidants. J Chromatogr A 881:197–216
43. Chow CK, Draper HH, Saari Csallany A (1969) Method for the assay of free and esterified tocopherols. Anal Biochem 32:81–90
44. Abidi SL, Mounts TL (1994) Separations of tocopherols and methylated tocols on cyclodextrin-bonded silica. J Chromatogr A 670: 67–75
45. Kormann AW (1980) Routine assay for determination of alpha-tocopherol in liver. J Lipid Res 21:780–783
46. Ballesteros E, Gallego M, Valcarcel M (1996) Gas chromatographic determination of cholesterol and tocopherols in edible oils and fats with automatic removal of interfering triglycerides. J Chromatogr A 719:221–227
47. Feeter DK (1974) Determination of tocopherols, sterols, and steryl esters in vegetable oil distillates and residues. J Am Oil Chem Soc 51:184–187
48. Hartman K (1977) A simplified gas liquid chromatographic determination for vitamin E in vegetable oils. J Am Oil Chem Soc 54: 421–423
49. Marks C (1988) Determination of free tocopherols in deodorizer distillate by capillary gas chromatography. J Am Oil Chem Soc 65:1936–1939
50. Slover HT, Thompson RH, Merola GV (1983) Determination of tocopherols and sterols by capillary gas chromatography. J Am Oil Chem Soc 60:1524–1528
51. Meijboom PW, Jongenotter GA (1979) A quantitative determination of tocotrienols and tocopherols in palm oil by TLC-GLC. J Am Oil Chem Soc 56:33–35
52. Han NM, May CY (2012) Chromatographic analyses of tocopherols and tocotrienols in palm oil. J Chromatogr Sci 50:283–286
53. Choo YM, Ng MH, Ma AN, Chuah CH, Hashim MA (2005) Application of supercritical fluid chromatography in the quantitative analysis of minor components (carotenes, vitamin E, sterols, and squalene) from palm oil. Lipids 40:429–432
54. King JW, Favati F, Taylor SL (1996) Production of tocopherol concentrates by supercritical fluid extraction and chromatography. Sep Sci Technol 31:1843–1857
55. Manninen P, Laakso P, Kallio H (1995) Method for characterization of triacylglycerols and fat-soluble vitamins in edible oils and fats by supercritical fluid chromatography. J Am Oil Chem Soc 72: 1001–1008
56. Saito M, Yamauchi Y (1990) Isolation of tocopherols from wheat germ oil by recycle semi-preparative supercritical fluid chromatography. J Chromatogr A 505:257–271
57. Yarita T, Nomura A, Abe K, Takeshita Y (1994) Supercritical fluid chromatographic determination of tocopherols on an ODS-silica gel column. J Chromatogr A 679:329–334
58. Han NM, May CY, Ngan MA, Hock CC, Ali Hashim M (2004) Isolation of palm tocols using supercritical fluid chromatography. J Chromatogr Sci 42:536–539
59. Schüep W, Rettenmaier R (1994) Analysis of vitamin E homologs in plasma and tissue: high-performance liquid chromatography. In: Lester, P. (Ed.), Methods Enzymol. Academic Press, pp. 294-302
60. Shin TS, Godber JS (1993) Improved high-performance liquid chromatography of vitamin E vitamers on normal-phase columns. J Am Oil Chem Soc 70:1289–1291
61. Thompson JN, Hatina G (1979) Determination of tocopherols and tocotrienols in foods and tissues by high performance liquid chromatography. J Liq Chromatogr Relat Technol 2:327–344
62. Weber E (1984) High performance liquid chromatography of the tocols in corn grain. J Am Oil Chem Soc 61:1231–1234
63. Rammell CG, Hoogenboom JJL (1985) Separation of tocols by HPLC on an amino-cyano polar phase column. J Liq Chromatogr 8:707–717
64. Wahyuni WT, Jinno K (1988) Separation of tocopherols on various chemically bonded phases in microcolumn liquid chromatography. J Chromatogr A 448:398–403
65. Balz M, Schulte E, Thier HP (1992) Trennung von Tocopherole n und Tocotrienolen durch HPLC. Lipid/Fett 94:209–213
66. Aitzetmüller K, Xin Y, Werner G, Grönheim M (1992) High-performance liquid chromatographic investigations of stillingia oil. J Chromatogr A 603:165–173
67. Micali G, Lanuzza F, Currò P (1993) Analysis of tocopherols in margarine by on-line HPLC–HRGC coupling. J High Res Chromatogr 16:536–538
68. Dionisi F, Prodolliet J, Tagliaferri E (1995) Assessment of olive oil adulteration by reversed-phase high-performance liquid chromatography/amperometric detection of tocopherols and tocotrienols. J Am Oil Chem Soc 72:1505–1511
69. Balz M, Schulte E, Thier HP (1995) Simultaneous determination of retinol esters and tocochromanols in foods using nitro-column HPLC. Lipid/Fett 97:445–448
70. Kramer JG, Blais L, Fouchard R, Melnyk R, Kallury KR (1997) A rapid method for the determination of vitamin E forms in tissues and diet by high-performance liquid chromatography using a normal-phase diol column. Lipids 32:323–330
71. DeVries JW, Egberg DC, Heroff JC (1979) Concurrent analysis of vitamin A and vitamin E by reversed phase high performance liquid chromatography. In: George, C. (Ed.) Liquid chromatographic analysis of food and beverages. Academic Press, pp. 477-497
72. Ueda T, Ichikawa H, Igarashi O (1993) Determination of α-tocopherol stereoisomers in biological specimens using chiral phase high-performance liquid chromatography. J Nutr Sci Vitaminol 39: 207–219
73. Mulholland M (1986) Linking low dispersion liquid chromatography with diode-array detection for the sensitive and selective determination of vitamins A, D and E. Analyst 111:601–604
74. Lang JK, Gohil K, Packer L (1986) Simultaneous determination of tocopherols, ubiquinols, and ubiquinones in blood, plasma, tissue homogenates, and subcellular fractions. Anal Biochem 157: 106–116
75. Pascoe GA, Duda CT, Reed DJ (1987) Determination of α-tocopherol and α-tocopherylquinone in small biological samples by high-performance liquid chromatography with electrochemical detection. J Chromatogr B: Biomed Sci Appl 414:440–448
76. Murphy ME, Kehrer JP (1987) Simultaneous measurement of tocopherols and tocopheryl quinones in tissue fractions using high-performance liquid chromatography with redox-cycling electrochemical detection. J Chromatogr B: Biomed Sci Appl 421:71–82
77. Satomura Y, Kimura M, Itokawa Y (1992) Simultaneous determination of retinol and tocopherols by high-performance liquid chromatography. J Chromatogr A 625:372–376
78. Barua AB, Furr HC, Janick-Buckner D, Olson JA (1993) Simultaneous analysis of individual carotenoids, retinol, retinyl esters, and tocopherols in serum by isocratic non-aqueous reversed-phase HPLC. Food Chem 46:419–424
79. Epler KS, Ziegler RG, Craft NE (1993) Liquid chromatographic method for the determination of carotenoids, retinoids and

tocopherols in human serum and in food. J Chromatogr B: Biomed Sci Appl 619:37–48

80. Sarzanini C, Mentasti E, Vincenti M, Nerva M, Gaido F (1993) Determination of plasma tocopherols by high-performance liquid chromatography with coulometric detection. J Chromatogr B: Biomed Sci Appl 620:268–272
81. Richheimer SL, Kent MC, Bernart MW (1994) Reversed-phase high-performance liquid chromatographic method using a pentafluorophenyl bonded phase for analysis of tocopherols. J Chromatogr A 677:75–80
82. Ben-Amotz A (1995) Simultaneous profiling and identification of carotenoids, retinols, and tocopherols by high performance liquid chromatography equipped with three-dimensional photodiode array detection. J Liq Chromatogr 18:2813–2825
83. Lane JR, Webb LW, Acuff RV (1997) Concurrent liquid chromatographic separation and photodiode array detection of retinol, tocopherols, all-trans-α-carotene, all-trans-β-carotene and the mono-cis isomers of β-carotene in extracts of human plasma. J Chromatogr A 787:111–118
84. Rentel C, Strohschein S, Albert K, Bayer E (1998) Silver-plated vitamins: a method of detecting tocopherols and carotenoids in LC/ESI-MS coupling. Anal Chem 70(20):4394–4400
85. Barua AB, Olson JA (1998) Reversed-phase gradient high-performance liquid chromatographic procedure for simultaneous analysis of very polar to nonpolar retinoids, carotenoids and tocopherols in animal and plant samples. J Chromatogr B: Biomed Sci Appl 707:69–79
86. Abidi SL (1999) Reversed-phase retention characteristics of tocotrienol antioxidants. J Chromatogr A 844:67–75
87. Strohschein S, Rentel C, Lacker T, Bayer E, Albert K (1999) Separation and identification of tocotrienol isomers by HPLC-MS and HPLC-NMR coupling. Anal Chem 71(9):1780–1785
88. Podda M, Weber C, TraberMG, Milbradt R, Packer L (1999) Sensitive high-performance liquid chromatography techniques for simultaneous determination of tocopherols, tocotrienols, ubiquinols, and ubiquinones in biological samples. In: Lester, P. (Ed.), Method Enzymol. Academic Press, pp. 330-341
89. Huo JZ, Nelis HJ, Lavens P, Sorgeloos P, De Leenheer A (1997) Determination of E vitamers in microalgae using high-performance liquid chromatography with fluorescence detection. J Chromatogr A 782:63–68
90. Finckh B, Kontush A, Commentz J, Hübner C, Burdelski M, Kohlschütter A (1999) High-performance liquid chromatography-coulometric electrochemical detection of ubiquinol 10, ubiquinone 10, carotenoids, and tocopherols in neonatal plasma. In: Lester, P. (Ed.) Method Enzymol. Academic Press, pp. 341-348
91. Bonvehi JS, Coll FV, Rius IA (2000) Liquid chromatographic determination of tocopherols and tocotrienols in vegetable oils, formulated preparations, and biscuits. J AOAC Int 83:627–634
92. Drotleff AM, Ternes W (2001) Determination of RS, E/Z-tocotrienols by HPLC. J Chromatogr A 909:215–223
93. Adachi T, Isobe E (2003) Use of synthetic adsorbents in preparative normal-phase liquid chromatography. J Chromatogr A 989:19–29
94. Fuchs J, Weber S, Podda M, Groth N, Herrling T, Packer L, Kaufmann R (2003) HPLC analysis of vitamin E isoforms in human epidermis: correlation with minimal erythema dose and free radical scavenging activity. Free Rad Biol Med 34:330–336
95. Hewavitharana AK, Lanari MC, Becu C (2004) Simultaneous determination of vitamin E homologs in chicken meat by liquid chromatography with fluorescence detection. J Chromatogr A 1025:313–317
96. Cunha SC, Amaral JS, Fernandes JO, Oliveira MB (2006) Quantification of tocopherols and tocotrienols in Portuguese olive oils using HPLC with three different detection systems. J Agric Food Chem 54:3351–3356
97. Andersson SC, Rumpunen K, Johansson E, Olsson ME (2008) Tocopherols and tocotrienols in sea buckthorn (*Hippophae rhamnoides* L.) berries during ripening. J Agric Food Chem 56:6701–6706
98. Ali H, Nazzal S (2009) Development and validation of a reversed-phase HPLC method for the simultaneous analysis of simvastatin and tocotrienols in combined dosage forms. J Pharm Biomed Anal 49:950–956
99. Cerretani L, Lerma-Garcia MJ, Herrero-Martinez JM, Gallina-Toschi T, Simo-Alfonso EF (2010) Determination of tocopherols and tocotrienols in vegetable oils by nanoliquid chromatography with ultraviolet-visible detection using a silica monolithic column. J Agric Food Chem 58:757–761
100. Huang SH, Ng LT (2011) An improved high-performance liquid chromatographic method for simultaneous determination of tocopherols, tocotrienols and gamma-oryzanol in rice. J Chromatogr A 1218:4709–4713
101. Andersson SC, Olsson ME, Gustavsson KE, Johansson E, Rumpunen K (2012) Tocopherols in rose hips (*Rosa* spp.) during ripening. J Sci Food Agric 92:2116–2121
102. Abidi SL, Rennick KA (2001) Capillary electrochromatographic evaluation of vitamin E-active oil constituents: tocopherols and tocotrienols. J Chromatogr A 913:379–386
103. Abidi SL, Thiam S, Warner IM (2002) Elution behavior of unsaponifiable lipids with various capillary electrochromatographic stationary phases. J Chromatogr A 949:195–207
104. Aturki Z, D'Orazio G, Fanali S (2005) Rapid assay of vitamin E in vegetable oils by reversed-phase capillary electrochromatography. Electrophoresis 26:798–803
105. Lauridsen C, Leonard SW, Griffin DA, Liebler DC, McClure TD, Traber MG (2001) Quantitative analysis by liquid chromatography-tandem mass spectrometry of deuterium-labeled and unlabeled vitamin E in biological samples. Anal Biochem 289(1):89–95
106. Andreoli R, Manini P, Poli D, Bergamaschi E, Mutti A, Niessen WM (2004) Development of a simplified method for the simultaneous determination of retinol, alpha-tocopherol, and beta-carotene in serum by liquid chromatography-tandem mass spectrometry with atmospheric pressure chemical ionization. Anal Bioanal Chem 378(4):987–994
107. Kalman A, Mujahid C, Mottier P, Heudi O (2003) Determination of alpha-tocopherol in infant foods by liquid chromatography combined with atmospheric pressure chemical ionisation mass spectrometry. Rapid Commun Mass Spectrom 17(7):723–727
108. Bustamante-Rangel M, Delgado-Zamarreno MM, Sanchez-Perez A, Carabias-Martinez R (2007) Determination of tocopherols and tocotrienols in cereals by pressurized liquid extraction-liquid chromatography-mass spectrometry. Anal Chim Acta 587:216–221
109. Al-Talla ZA, Tolley LT (2005) Analysis of vitamin E derivatives in serum using coordinated ion spray mass spectrometry. Rapid Commun Mass Spectrom 19(16):2337–2342
110. Mottier P, Gremaud E, Guy PA, Turesky RJ (2002) Comparison of gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry methods to quantify alpha-tocopherol and alpha-tocopherolquinone levels in human plasma. Anal Biochem 301(1):128–135
111. Hall WL, Jeanes YM, Pugh J, Lodge JK (2003) Development of a liquid chromatographic time-of-flight mass spectrometric method for the determination of unlabelled and deuterium-labelled alpha-tocopherol in blood components. Rapid Commun Mass Spectrom 17(24):2797–2803
112. Jiang Q (2014) Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic Biol Med 72C:76–90