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Analysis of Tocopherol Using Chromatographic and Electrochemical Techniques

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Abstract

Vitamin E is made of tocochromanols which consists of two distinct compounds known as tocopherols and tocotrienols which are produced by plants and serve as an antioxidant that scavenge free radicals. Tocopherol is a vital fat-soluble vitamin which exist in 8 different isoforms namely tocopherol and tocotrienol. These isoforms are widely found present in vegetables, vegetable oil, nuts, grains, seeds, cyanobacteria and supplements. Bioactive compounds are extracted using solvent extraction, ultrasonic assisted extraction, maceration, pressurized liquid extraction and supercritical fluid extraction. Previously, scientist relies on HPLC techniques to determine vitamin E isomers, but these techniques have some challenges such as high cost, longer steps and less sensitivity. Scientists now employ electrochemical method such as differential pulse voltammetry, cyclic voltammetry, Square wave anodic stripping voltammetry and chrono-amperometry to determine antioxidant activity of sample due to affordability, high sensitivity, simplicity, less detection limit and diversification of electrochemical techniques and procedures.

Keywords: Tocopherol; Tocotrienols; DPV; CV; HPLC

Introduction

Vitamin E (Tocopherols)

Vitamin E is general name assigned to a group of fat-soluble organic compounds which was discovered by Evans and Bishop in 1992 [1]. Vitamin E as one of the bioactive vitamins in human nutrition which is made of tocochromanols and consists of two distinct compounds known as tocopherols and tocotrienols which are produced by plants and serve as an antioxidant that scavenge free radicals. It is regarded as the most abundant Lipid soluble antioxidant present in cellular membrane tissue and plasma of higher mammals [2,3]. These distinct chemical compounds contain a hydrophobic side chain and a chromanol ring [4]. Tocopherol is a vital fat-soluble vitamin which exists in 8 different isoforms namely α-, β-, δ-, γ- tocopherol and α-, β-, δ-, γ- tocotrienol. α-Tocopherol is regarded as the most common and biological active form of vitamin E [5,6]. These isoforms are widely found present in vegetables, vegetable oil, nuts (such as almonds), grains (such as corn oil), seeds (such as sunflower), cyanobacteria and supplements [7,8]. Scientist have evaluated it health properties and it has shown to poses antihypertensive, hypolipidemic, anti-inflammatory, antiatherogenic, and nephroprotective activities [9].

Sources of Vitamin E

Oils obtained from vegetable are the main sources of vitamin E, other sources include green leafy vegetable, whole grains and nut also contain desirable amount, fat and oil, meat fish, poultry and eggs as shown in Table 1. Vitamin E can be in the form of chemically stable forms such as α-tocopherol acetate which is produced as supplement of different nutritional and pharmacological benefits [5,10]. Tocopherols and tocotrienols were found to help in prolonging shelf life of food [9]. Different plants contain different proportions of tocochromanols with green leafy vegetable tissues accumulate more of α-tocopherol than total tocopherol. Seeds contain higher amount of total tocopherol (i.e., ten times higher) with γ-tocopherol contributing to a larger percent as shown in Table 2. Plant that contains a higher amount of α-tocopherol is wheat germ, rice bran, sunflower, grape seed and hazel nut. Plants that contain higher amount of γ-tocopherol are soya bean oil, corn, peanut and canola. Some of the plants that are rich in σ-tocopherol are raspberries, sunflower and soya bean [9,11].

Molecular structure and chemistry of Vitamin E

The pattern of methylation (they differ from each other as a result of position of methyl group) of chromanol ring determines the occurrence or form of these compounds into α-, β-, δ-, γ- tocopherol and α-, β-, δ-, γ- tocotrienol [6]. One of the challenges of vitamin E its hydrophobic nature which makes it difficult to absorb, transport and deliver to tissues in the body. After absorption of naturally derived vitamin E, the compound is solubilized in the intestinal lumen by mixing with micelles with amphipathic lipid and bile salt and later passes into the intestinal epithelial cell [12]. While the absorption of synthetic derived vitamin E (such as α-tocopherol acetate) undergoes the process of hydrolysis before micelles solubilisation and uptake by enterocyte [5]. Tocopherols are found to co-exist with other fatty acid in food. Most of γ- tocopherol co-exists with PUFA (poly unsaturated fatty acid) while α-tocopherol co-exist with MUFA (monounsaturated fatty acid) [9].

Source	Examples
Vegetable oils	Sunflower, safflower
Whole grains	Wheat germ, corn
Legumes	Soybean oils
Nuts	Hazelnuts/filberts, peanuts, and almonds
Green leafy vegetables	Broccoli and spinach
Seeds	Olive, Sunflower
Fruit	Avocado, squash, pumpkin, avocado
Fortified Food	Cereals, fruit juices, margarine
Other sources	Meat, poultry and fish
Synthetic	α-tocopherol acetate
Fortified foods are food that contain added vitamins and mostly stated on the package or container label.	

Table 1: Sources of Vitamin E.

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Class	Crop	Tocopherol content		
		α-Tocopherol	**ϒ-Tocopherol**	**σ-Tocopherol**
Seed	Dried pumpkin and squash	2.18	35.10	0.44
	Flaxseed	3.31	19.95	0.35
Nut	Almonds	25.63	0.64	0.07
	Dried pine nut	9.33	11.15	-
	Raw peanuts	8.33	-	-
	Raw pistachio nut	2.86	20.41	0.80
	Dried black walnut	2.08	28.78	1.51
	Pecans	1.40	24.44	0.47
Veg	Dehydrated carrot	5.45	-	-
	Raw green dandelion	3.44	-	-
	Raw green turnip	2.86	-	-
	Raw coriander leaves	2.50	-	-
	Raw taro	2.38	-	-
	Raw green chicory	2.26	-	-
	Raw collards	-	-	-
Species	Chilli powder	38.14	3.41	-
	Paprika	29.10	3.54	0.25
	Dried oregano	18.23	24.42	0.92
	Dried basil	10.70	0.77	-
	Dried parsley	8.96	1.53	-
	Turmeric powder	4.43	0.72	-
	Sweet, green, pepper	4.00	-	-
	Freeze-dried cumin seed	3.33	-	-
	Hot chilli pepper	3.14	-	-
	Sun dried ground mustard seed	5.07	19.82	0.81
	Ground cinnamon	3.32	10.44	0.26
	Poppy seed	1.77	8.82	0.23
Oil	Wheat Germ	149.40	-	-
	Hazelnut	47.20	-	-
	Sunflower	41.08	-	-
	Almond	39.20	-	-
	Rice Bran	32.30	-	-
	Grapeseed	28.80	-	-
	Peanut	15.95	15.95	1.37
	Corn and Canola	14.82	35.37	1.28
	Olive Oil	14.35	0.83	-
	Soya bean	8.18	64.26	21.30
Fruits	Raw avocado (Florida)	2.66	-	-
	Raw mamey sapote	2.11	-	-
	Raw avocado (California)	1.97	-	-
	Raw green kiwifruit	1.46	-	-
	Raw cranberries	1.32	-	-
Others	Dried seaweed spirulina	5.00	-	-

Table 2: Composition of σ, γ and σ in plants.

Free radicals

Free radicals are extremely reactive and sometimes can cause series of several chemical reactions that disrupts millions of cells close by in order to replace their missing electron. Everyday cells are damaged and repaired as a normal part of aging [13]. Free radicals are substances naturally created by the body when we breathe and digest food, but more are formed when individual smoke, expose to pollution or UV light. The presence of high mount of free radicals can cause damage to healthy cells and as a result can lead to increase in the risk of heart diseases, hypertension, cancer, neurodegenerative disorders, type II diabetes, Parkinson, acute respiratory diseases, Alzheimer and other diseases [14,15].

Antioxidants

Antioxidants are substance that prevent, protect and repair cells from damage due to formation of free radicals. They help and regulate or neutralise excess free radical's toxicity that induce cellular apoptosis thereby preventing the body from becoming prone to diseases [15]. They act as defence agents by decreasing the formation of free radicals, scavenging for active radicals and to terminate chain reactions. Human biochemical processes produce antioxidants, but more are needed from food (such as vegetables, whole grains, fruits, nuts beans etc.) and supplements. In 1990, the use of antioxidants supplements became widespread and generated millions of dollars and since then

the markets have been growing and it was estimated that it will reach 3.1 billion dollars in 2020 [16].

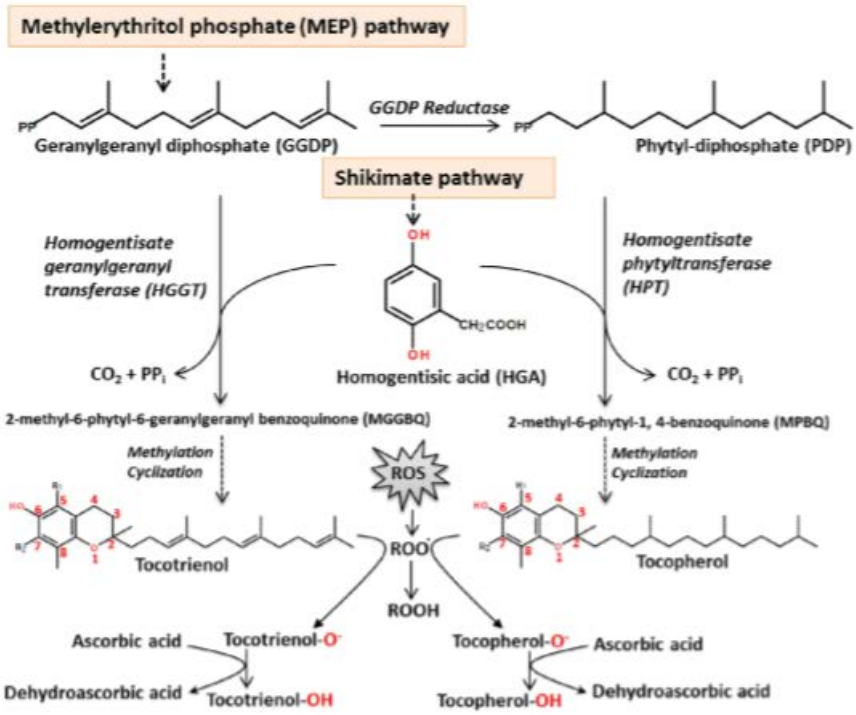
Vitamin E as an antioxidant

The concentrations of vitamin E in membranes are very low but still they serve as antioxidants which act against major lipid-soluble chain. Vitamin E is regarded as one of the essential antioxidants obtained from diets [6]. The antioxidants activity of vitamin E is to supress or inhibit the oxidation of lipid by terminating ROS (radical oxygen species) chain reaction which form as a result of radicals, in both cellular and sub-cellular membrane tocopherol inhibit the peroxidation of Polyunsaturated fatty acid (PUFA) (Figure 1) [3,17]. Tocopherol also serves as peroxyl radical scavenger that stops formation of l cholesterol and low-density lipoprotein (LDL). Both cholesterol, LDL and lipid peroxidation (LP) contribute to risk of serious diseases such as cancer and cardiovascular disorders [2,8]. Food or diets with constituents of Redox modulators can reduce risk of so many chronic diseases such as asthma, diabetes, ocular diseases, neurodegenerative diseases and several viral related infections [10].

α-Tocopherol: α-tocopherol is the most effective (i.e., potent) antioxidant which break fat-soluble chain in human tissues, it poses anti-peroxidative activity [18]. The active side for scavenging radicals is in the chromanol ring 6 hydroxyl groups. Among the 8 isomers, RRR α-tocopherol is the most biologically active of all tocopherol compounds with *in vivo* bioactivity due to it bondage with special transport protein known as α-tocopherol transfer protein (α-TTP) which protect it from degradation unlike other 7 isomers that are easily degradable as shown in Figure 1 [6]. RRR α-tocopherol is the most effective tocopherol isoform which serves a vital function in prevention of free radicals in humans, even though the other isoforms are absorbed by human, the rate of their degradation and retention time within the body varies. Another advantage of RRR α-tocopherol over other isoforms it is the only isoform that is not discriminated by the liver and thus unlike the rest that are easily metabolised and excreted by the body as xenobiotics, RRR α-tocopherol accumulate in the cellular membrane tissues [6].

γ-Tocopherol: γ-Tocopherol is another abundant vitamin E which is found in large amount of human diet (the most popular and most consume vitamin E in American diet) and mostly found present in vegetable oils [18]. Unlike α-tocopherol that act against ROS, γ-tocopherol act as a scavenger against Reactive Nitrogen Species (RNS) due to undistributed 5 positions on the tocopherol chromanol ring. γ-Tocopherol is less effective as α-tocopherol and can easily be metabolised by cytochrome p450 enzyme. Upon intake, only 10% is retain by cellular membrane tissue [6] (Figure 1).

Tocotrienols: Tocotrienols are less abundant and prevalent isoforms of vitamin E and are found in low quantity and are less consumed as human diets. They are found present in barley, coconuts, oats, chilli spices, bran, paprika and palm oil. Unlike tocopherol, tocotrienols are easily and rapidly metabolised in the body and are found in low amount in the cellular membrane [6,9] (Figure 2).



Isomer	R1	R2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H

Figure 1: Chemical structures, biosynthesis and antioxidants activity of tocopherols and tocotrienols [28].

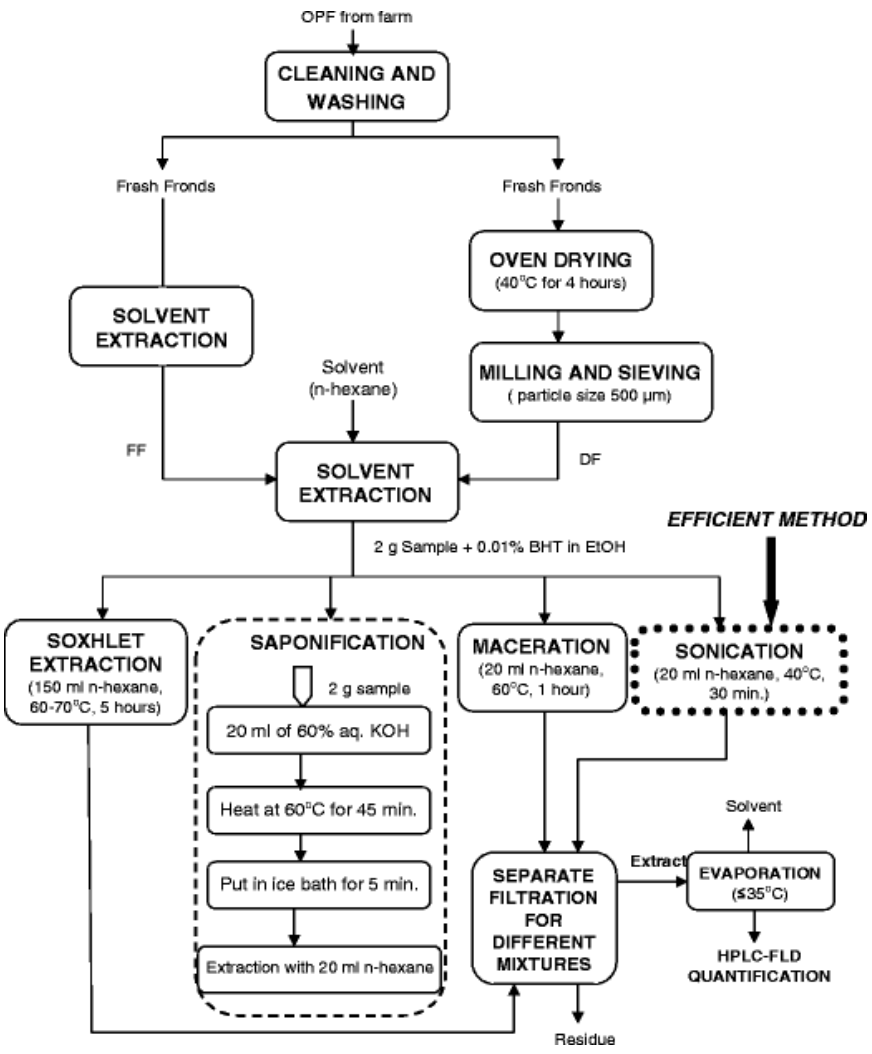


Figure 2: Extraction method for palm oil [36].

Redox reaction of Vitamin E

In both biochemical and chemical analysis, it was reported that after oxidation of vitamin E before it undergoes decomposition, it can be reduced (Figure 1). This reduced form can be generated due to present of ascorbic acid also known as vitamin C and glutathione which are basic water-soluble antioxidant present in the cytosol, depending on their concentration and enzymes that can sustain them in their reduced form [17].

Function of vitamin E in medicine

Function of vitamin E in regulation of cancer: Vitamin E has shown to poses anti-cancer activity due to its various metabolic functions such as stimulation of wild type *p53* suppressor gene, activation of heat shock proteins, mutant *p53* protein downregulation and its anti-angiogenic effect [1]. One of the causes of initiation and promotion of tumour is associated with reactive oxygen species. Vitamin E acts as an antioxidant and result in anti-carcinogenic activity which slows or prevents the growth of cancer cells by destroying free radicals or neutralising them [17]. Researches have shown that α, δ and γ tocopherol all possesses anti-cancer properties. α tocopherol has shown to inhibit the production of collagenase and PKC which promote growth of cancer cells. The use γ tocopherol has shown a significant result (i.e., more effective than α tocopherol) where it is used to stop the proliferation of human prostate cancer cells. δ tocopherol was also stated to stop the growth of mouse mammary cancer cells.

γ tocopherol uses different mechanisms to inhibit cancer cells from proliferating. One of the mechanisms is to trap free radicals mostly reactive nitrogen specie (RNS) molecules which induce mutations in strands of DNAs and transformation of malignant in the cells. The second mechanism of preventing cancer cells from proliferating is by downregulations of cyclins (control molecules). Other mechanism employed by γ tocopherol is by inducing apoptosis through triggering different apoptosis pathways, suppression of tumours from developing new vessels and there by blocking transportation of nutrients [1]. Apart from tocopherols, tocotrienols were also reported to poses both apoptotic and anti- proliferative activities on human healthy and cancerous cells. Tocotrienols inhibit cancer cells by either inducing apoptosis by mitochondrial mediated pathway or by suppressing Cyclin D which will lead to termination of cell cycle. The synergism of γ and δ tocopherol has shown a significant result in inducing apoptosis in prostate cancer cells [1].

Function of Vitamin E in reducing cardiovascular risk: One of the complications leading to cardiovascular disease is inflammation as a result of oxidation of low density lipoproteins [1]. Vitamin E serves a significant function in reducing risk of cardiovascular diseases by different mechanisms such as preventing the formation of free radical through detection of exited oxygen species [19]. It also serves a significant role in red blood cell formation, widening of blood vessels and blood clotting. Development of atherosclerosis relates to excessive aggregation of blood platelet. Some researches studies have reported that the use of vitamin E helps decrease excessive platelet aggregation in both patients with high level of blood lipids and healthy adults [17]. A study has revealed the use of γ-tocopherol to increase the activity of nitric oxide NO_2 synthase to produce a relaxing vessel NO_2 which significantly improves cardiovascular activity. Different research has shown that consuming 100 mg of vitamin E supplement by humans can reduce the risk of arterial clotting, formation of cholesterol and platelet aggregation. Another study has shown that tocotrienols inhibit biosynthesis of cholesterol by suppressing 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase which signals the liver to produce less cholesterol. Other research has shown the negative implication of vitamin E as it can increase the risk of haemorrhagic stroke among participant. The use of vitamin E to cure and prevent coronary heart disease and related cardiovascular diseases still need extensive research [1].

Function of vitamin E in reducing risk of cataracts: Cataract is the most common disease that causes loss of vison in elderly people which occurs as a result of accumulation of damage proteins due to the action of free radicals. Different researches have reported a correlation between the use of vitamin E and the risk of cataracts. A comparative study has shown participant who consumes vitamin E have superior lens clarity than participant who do not engaged in consuming vitamin E [1].

Other functions of tocopherols: Tocopherols play a significant function in gene regulation and expression, it contributes to anti-inflammatory activity, angiogenesis and tumour suppression, it enhances immune system to fight against bacteria and viruses and helps inhibit cell proliferation, it poses anti-atherogenic and anti-proliferative activity [2,15]. Research has shown the use of vitamin E to decreased symptoms in patient with arthritis [17].

Vitamin E deficiency

Both animals and human with low level (below required amount) of vitamin E have higher risk of neuromuscular and neurological disorders. A study has shown that deficiency of vitamin E (i.e., <12 μmol/l) are at more risk of miscarriage can lead to pregnant women given birth to premature babies or children that will end up with anaemia, neurological, neuromuscular diseases and other embryogenic related disorders. Researches have shown that increasing the dosage of vitamin E is very useful for the management of the deficiency (Table 3) [6-21].

Separation techniques

In the last five decades several techniques have evolved for the separation and analysis of tocopherols present in food, some of these techniques include Reverse Phase High Performance Chromatography (RP-HPLC), Normal Phase High Performance Chromatography (NP-HPLC), Gas Chromatography (GC), Capillary Liquid Chromatography (CLC), Thin Layer Chromatography (TLC), Capillary Electrochromatography (CEC), Modification of chromatography techniques with nanomaterials such as Nano Liquid Chromatography (NLC), other approaches include Supercritical Fluid Chromatography (SFC), Fourier Transform Infrared Spectroscopy (FT-IR) and Synchronous Fluorescence Spectroscopy (SFC) [9,22].

Method of extraction

Scientist have developed wide range of techniques for extraction of tocopherols and other phytochemical compounds such as carotenoids, polyphenols, flavours, caffeine etc. below methods have both advantage and disadvantage (Table 4), the factors that determine extraction techniques include phytochemical and physical properties of plants, availability of instruments and resources. Some of the techniques includes (Figure 3):

1. Maceration (MAC),

Individuals /group	Age (years/months)	Vitamin E Requirement
Infants	0-6 months	4 mg/day
	7-12 months	5 mg/day
Children	1-3 years	6 mg/day
	4-8 years	7 mg/day
	9-13 years	11 mg/day
Adolescent and Adults	14 and above	15 mg/day
Pregnant Teens and Women	-	15 mg/day
Breastfeeding teens and women	-	19 mg/day

Table 3: Vitamin E recommended intakes for individuals [1-18].

Extraction Method	Plant/solvents	References	Advantages	Disadvantages
Maceration (MAC)	Rice bran/ Ethanol and Ethanol-water	Vasilescu I, et al. [44]	A simple technique that does not involve sophisticated equipment	Time consuming High amounts of solvents are needed. After extraction the waste must be manage properly
Ultrasonic Assisted Extraction (UAE)	Palm oil/ ethanol, -hexane acetone and η	Ofori-Boateng and Lee [27]	Cheap, easy, effective and fast (CEEF) technique, require small volume of solvent and little quantity of sample, efficient solvent penetration is achieved using ultrasound.	Lack of reproducibility, the technique result in generation of large amount of heat and sonication vessel must undergo cooling process. Using ultrasound energy above 20kHz can have effect on the bioactive component as a result of free radical formation
Solvent Extraction (SE)	*Brassica rapa*/ ethanol, methanol and chilled acetone	Annunziata et al. [30]	Simple and easy technique Addition of saponification help remove interference and impurities when carrying out chromatographic method	Time consuming It can only extract hydrophobic (i.e. lipid soluble compound).
Supercritical Fluid Extraction (SCFE)	Rice bran/ Carbon dioxide	Imsanguan et al. [32]	Suitable for extraction of thermal bioactive compounds	It is only operated under atmospheric pressure Not suitable for industrial scale extraction
Extraction using Matrix Solid Phase Dispersion (MSPD	Barely grains/ Methanol	Tsochatzis and Tzimou-Tsitouridou [33]	it requires low volume of solvents and small quantity of sample	Time consuming

Table 4: Advantage and disadvantage of extraction methods.

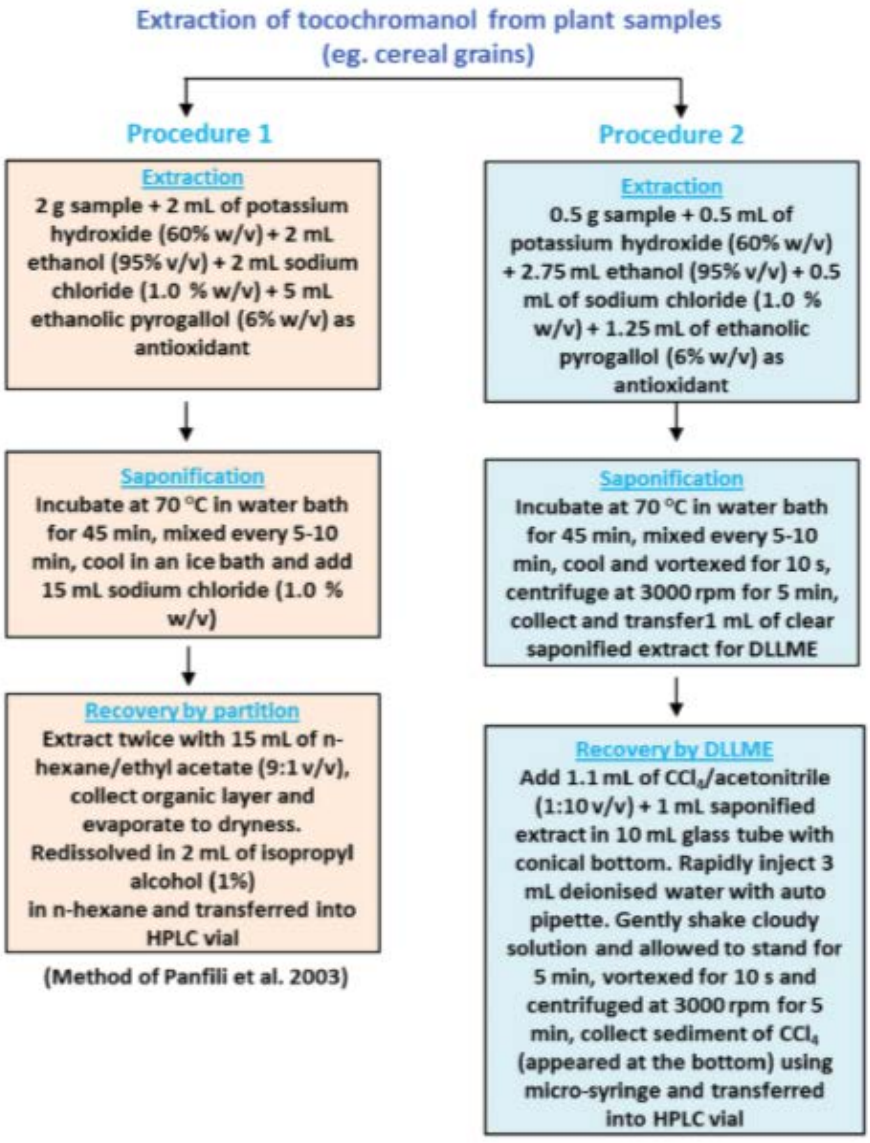


Figure 3: The most common outlines for tocopherol extraction [28].

2. Ultrasonic Assisted Extraction (UAE),
3. Solvent Extraction (SE) (which include direct solvent extraction, saponification and Soxhlet extraction)
4. Supercritical Fluid Extraction (SCFE)
5. Extraction using Matrix Solid Phase Dispersion (MSPD) and
6. Pressurize Liquid Extraction (PLE).

Maceration (MAC): Maceration is an old traditional and a popular method which is utilizes by brewing companies and is used to extract bioactive components such as phenolic compounds, caffeine, flavonoids and tocopherols mostly using 70% ethanol as the main solvents [23,24]. The process involved mixing plant material (i.e., raw material) and solvent and soaking for some days (i.e., 3-10 days) at room temperature. Soaking of substrate with solvent allow the release of phytochemical compounds such as carotenoids, flavonoids and tocopherols [24]. One of the challenges of this method is time consuming as it takes 2-10 days to achieve extraction and result in low extract yield compare to UAE and MAE. This technique has been used to extract phenolic compounds from olive and green tea leaves [23]. Rafiee et al. in 2011 employed maceration and MAE techniques to extract phenolic compounds from olive leaves using solvents such as ethanol and acetone. The result has shown that MAE produced higher extract than maceration [25]. Vongsak et al. 2013 used maceration technique to extract phenolic compounds, antioxidant activity, flavonoids and ferric reducing power (FRP) using dried leaves of *Moringa oleifera* and 70% ethanol [26]. Ghasemzadeh et al. in 2015 utilized MAC and UAE method to extract secondary metabolite such as tocopherols and tocotrienols, flavonoid and total phenolic compounds from rice bran. MAC extract result is compared with UAE and UAE method was found to produce more tocopherol extract than MAC. Gharibzahedi et al. in 2013 evaluated tocopherol and other physiochemical compounds from walnut oil

extracted using MAC, Cold Press (CP) and MBD (Modified Bligh-Dyer). MBD technique has shown to extract tocopherol content compare to MAC and CP (Table 5).

Ultrasonic Assisted Extraction (UAE): UAE is the most utilized extraction method for bioactive components such as polyphenols, gingerols and carotenoids in plants samples such as oil and proteins. To enhanced solvent penetration into plants tissue, ultrasound disrupt the tissue matrix making the most process effective with high efficiency. UAE technique is rapid, and extraction can be achieved at low temperature and short period of time using less amount of solvents and sample. UAE has been used to extract polyphenol contents from apple and black tea, oil from soya bean seed and almonds, red grape, β-carotene from carrot, gingerol from ginger. Ofori-Boateng and Lee in 2014 employed UAE to extract α-tocopherol form *Elaeisis guinensis* (palm oil) using two additional techniques Response Surface Methodology (RSM) coupled with central composite design (CCD) along with UAE (Figure 2) [27]. The same study included a comparative analysis and employed Solvent Extraction (SE), MAC and saponification using three different solvents (ethanol, acetone and η-hexane) for the extraction of α-tocopherol from palm oil and UAE with η-hexane as solvent has shown to be the most effective extraction method compare to other techniques.

Solvent Extraction (SE): It is the most common method employed by scientists for tocopherol extraction. Due to hydrophobic nature, it is suitable for extraction of tocopherol from crops such as oily seeds, grains and extraction from biological tissues. Tocopherols coexist with other macromolecules such as proteins, lipids and carbohydrates [9]. In order to easily extract tocopherols from samples (i.e., plants), alkaline hydrolysis is employed during saponification stage. Other techniques are used such as grounding, sonication, homogenization, ultrasound and vortexing to enhance proper extraction from solid food matrix. There are wide range of solvent that are used for extraction procedure, ethanol is the most utilized solvent used for extraction of tocopherol from oil, grain samples and bakery [28]. Hidalgo et al. in 2009 used einkorn wheat to extract tocopherols using four distinct extraction procedures which include methanol, water saturated 1-butanol, hot saponification and room temperature saponification [29]. Their result has shown the use of hot saponification produced higher extraction tocopherol content [9]. In order to prevent protein interference with tocopherols, biological samples such as milk and plasma undergoes deproteinization with ethanol after extraction. Annunziata et al. in 2012 conducted a comparative analysis utilize leaves of *Brassica rapa* with 3 extraction techniques which include ethanol with saponification, methanol and chilled acetone and their result has shown the use of methanol resulted in extraction of high content of tocopherol compare to other method [30]. For analysis of tocochromanols in raw vegetable, sample stabilization is required during homogenization and extraction stages in an inert surrounding under subdues light in order to avoid tocopherol oxidation while freeze drying can be utilized to stabilize fresh vegetable and fruit before homogenization. Normally during extraction stage, antioxidants such as BHT (Butylated hydroxytoluene), ascorbic acid and pyrogallol can be used to stabilized tocopherols. The main reason of saponification when extracting tocopherol from leafy samples is to remove interfering lipid and chlorophyll which interfere with tocopherol in mass spectrophotometric analysis for detection of tocopherol. In fluorescence and UV detection method, these interfering agents do not pose a serious concern [31].

Technique	Sample	Reference
Near Infrared Spectroscopy (NIRS)	Olive oil	Cayuela and Garcia [3]
HPLC	Blood serum	Huang et al. [2]
HPLC-CEAS and HPLC-MS	Tissue homogenate	Lee et al. [7]
HPLC-FLD	Cooked vegetables	Knecht et al. [4]
HPLC-ES and HPLC-MS	Acrocomia aculeata	Schex et al. [40]
UPCC	Moringa oleifera	Qi et al. [8]
LC-MS and LC-HRMS	Blood and plasma	Giusepponi et al. [18]

Table 5: Technique and sample for analysis of tocopherol.

Supercritical Fluid Extraction (SCFE): Supercritical Fluid Extraction is the extraction approach that is used to extract bioactive constituents, lipids, extraction of alcohol from beer and wine and flavours. The common solvent used in SCFE is Carbon dioxide. After extraction the solvent can easily be recovered from the process without inducing serious damage to extracts and substrates. The advantage of using carbon dioxide as a solvent for SCFE is due to it non-toxic, non-corrosive (in the presence of water), non-flammable and less expensive (affordable at a cheap cost) and moderate temperature (i.e., close to environmental temperature) [9]. SCFE has been established as the best suitable extraction method for thermally bioactive compounds but it has so many limitations for industrial scale extraction due to it batch processing mode (stages) and operation under pressure. In the last 4-5 years scientists have developed new method and modifications to enable industrial scale extraction such as addition of more extractor vessels, incorporation of SCFE with LPSE (Low Pressure Solvent Extractor) with proportion of ethanol and water to remove bixin [9]. Imsanguan et al. in 2008 extracted α-tocopherol from rice bran using both SCFE-CO_2 and Solvent Extraction (SE) using hexane and ethanol as solvent [32]. The result has shown that SCFE with carbon dioxide as solvent result in higher extraction efficiency. The result also shown that both hexane and ethanol can be used to extract α-tocopherol under atmospheric pressure. The use of hexane as extraction solvent for SE has shown a better efficiency than ethanol.

Extraction using Matrix Solid Phase Dispersion (MSPD): MSPD technique involves blending of suitable dispersion sorbent with sample and the resulting transfer of the mixture into a column for elution. One of the advantages of this process it is require low quantity of solvents and small period. MSPD has been used to extract and isolate distinct components from drugs, pollutants, bioactive compounds and pesticides from plants tissue and debris [9]. For the extraction of tocopherols, MSPD has been employed by Tsochatzis and Tzimou-Tsitouridou in 2015 to extract tocopherols from barely grains using methanol and alumina as dispersion sorbent [33].

Pressurized Liquid Extraction (PLE): PLE is an extraction process that involves subjecting both samples and extraction solvents to temperature of sample and solvent boiling point and higher pressure to improved mass transfer and solubility. PLE has been used to extract carotenoids, ligans, essential oils, phenolic compounds and other components from crops and herbal plants [34]. The advantage of using PLE is due to it environmentally friendly quality which reduce the effect of toxic organic solvents during extraction. It runs in a short period of time and utilize low amount or volume of solvents. The technique is found to be effective for the isolation of thermolabile components. PLE has also been used to extract tocopherol in cereals sample. Bustamante-Rangel et al. in 2007 utilized PLE to extract tocopherol using methanol as extraction solvent at a low temperature of (i.e., 50 degrees) with 110 bar pressure, the process lasted for 5 minutes [35]. Dos Santos et al 2008 employed PLE to extract α-tocopherol from cereals, vegetables and fruit such as Brazilian grape seed for wine industry [36]. A study by Vinas et al 2014 employed PLE to extracts tocopherol from corn, spinach, cranberry, mango and apple juice and pomegranate at 50 degrees temperature and a pressure of 1600 psi (equivalent to 110.3 bar) with a static time of 5 minutes [37].

Chromatographical analysis of tocopherol

Cayuela and Garcia in 2017 estimated α-tocopherol and total tocopherol contents of olive oil by employing Partial Least Square (PLS) model and discriminating analysis (DA) using Near Infrared Spectroscopy (NIRS) [3]. Tocopherols were extracted from the oil using lag mill based on Abenscer system. Spectrophotometer equipped with 3 detectors at different wavelength (350-1000 nm, 1000-1800 nm and 1800-2500 nm). The result has shown that predicting α-tocopherol with NIRS model displayed a valid estimate of vitamin E in olive oil. Lee et al. in 2018 quantified both tocopherol and tocotrienols and their chain degradation metabolites by employing techniques such as a High liquid performance chromatography (HPLC) for determination of tocopherol and tocotrienols [38]. Serum is used as sample and mix with ethanol for extraction using hexane where tocopherol and tocotrienols are extracted from tissue homogenate. Sample were analysed on HPLC and the result displayed proficient and clear separation of analytes with less interfering peaks. For detecting analytes, HPLC/CEAS is more sensitive compare to MS and fluorescence method. These methods have shown to be effective, sensitive and less expensive in carrying out routine tocopherol and tocotrienols analysis for bot epidemiological analysis and large number of samples in the laboratories. Knetch et al. in 2015 developed and validated HPLC-FLD technique for analysis of tocopherol and tocotrienols extracted from cooked vegetables (green beans tomatoes, carrots, spinach, red and green pepper, broccoli, and celery) [4]. Different stabilization and homogenization processes were employed. Samples were extracted using acetone. Saponification technique was carried out to extract both tocopherol and tocotrienols. HPLC was used to analyse sample extract. The result has shown that some of the sample contain high amount of tocopherol such as carrots and green vegetables contain high amount of α-tocopherol while some has shown no amount of both tocopherol and tocotrienols.

Schex et al. in 2018 carried out an analysis on α-tocopherol and carotenoids extracted from *Acrocomia aculeata* fruit which is grown in Costa Rica [39]. Acetone was used to extract analyte under dimmed light in order to prevent both α-tocopherol and carotenoids from degradation and isomerization. Saponification method is employed to obtain sample for use in HPLC analyses. The results of compounds obtain were assigned by comparing UV/vis absorption, retention time and mass spectra with the ones commercially available. The result has shown that α-tocopherol is present in Acromia aculeate fruit with amount ranging from 468-2943 μg in 100 g fruit mesocarp. Qi et al. in 2016 evaluated all 8 isomers of vitamin E (α-, β-, δ-, γ-Tocopherol and α-, β-, δ-, γ- tocotrienol) from *Moringa oleifera* leaf using Ultra Performance Convergence Chromatography (UPCC) equipped with diode array detector [8]. Analytes were extracted using ethanol with some modifications such as addition of potassium hydroxide solution and the use of ether after funnel separation for sample extraction. For the first time the 8 isomers of vitamin E were successfully determined using UPCC. This method has shown to be fast (3 minutes), highly reproducible separation and sensitive. Giusepponi et al. in 2017 determined tocopherols (α-, γ- Tocopherol) and their metabolites extracted from human serum and plasma using Liquid chromatography coupled with tandem mass spectroscopy [18]. The plasma and serum sample undergo deproteinization before extraction using hexane/methyl butyl ether in the volume ratio 2:1. Reversed phase chromatography is used to separate analytes and further successfully detected by quadrupole mass analyser using electrospray ionization. Other unknown analyte detected in the samples were additionally evaluated using Liquid Chromatography Couple with High Resolution Mass Spectrometry (LC-HRMS).

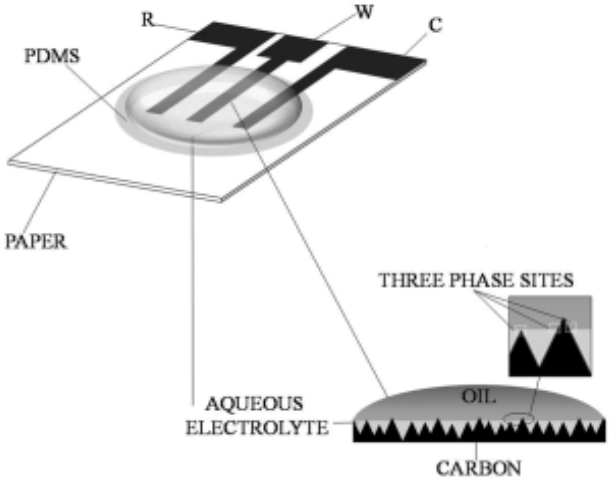


Figure 4: Schematic representation of simple drawn paper-based device: Magnification of 3 phases active sites where the WE, aqueous electrolyte, and oil sample [25].

Electrochemical analysis of tocopherol

Electrochemical techniques: Voltammetric techniques is a popular and widely technique that is used in chemistry for the quantitative determination of pharmaceutical compounds, determination of metals ion in water, determination of wide array of redox potential, determination of eluted analytes in High Performance of Liquid Chromatography (HPLC) and flow injection analysis. Cyclic Voltammetry (CV) is the first voltammetric technique employed to study electrochemical reactions of chemical compounds, biological materials and surface of electrode [40]. The use of electrochemical method such as Differential Pulse Voltammetry (DPV), Cyclic Voltammetry (CV), Square Wave Anodic Stripping Voltammetry (SWASV), Chrono-Amperometry (CA) etc. to determine antioxidant activity of sample is trending in the field of chemistry, food science and engineering, biomedicine due to low cost, high sensitivity, ease of use, less detection limit, rapid analysis time and diversification of electrochemical techniques and procedures [41] (Table 6).

Differential Pulse Voltammetry (DPV) is the most widely employed pulse voltammetric technique which is a powerful method for qualitative and quantitative analysis of specie, where as a result of change in potential, faradic current is obtained which allows the equilibration of background current and lead to increase in signal noise ratio. DPV is regarded as the most effective method for determination of antioxidants. Scientist have employed this technique to analyse thousands of compounds such as vitamin E (tocopherol isomers), vitamin C (ascorbic acid), vitamin A (retinol), free radical scavengers etc. [41].

Analysis of tocopherol using electrochemical techniques

Sys et al. in 2017 employed Square Wave Anodic Voltammetry (SWASV) using carbon paste as working electrode and 0.1 M HNO_3 as electrolyte to analyse vitamin E (α-tocopherol) in margarines sample [42,43]. 0.3 g of the sample along with 0.8 g of edible oil undergoes dissolution step using ultrasonic bath. For SWASV analysis, Ag/AgCl with 3.0 M KCl is used as Reference electrode and Platinum (Pt) wire as counter electrode. The peaks obtained show the present of vitamin E isomers in margarines. The electrochemical result that

Electrochemical Technique	Sample	Electrode	Reference
Square wave anodic voltammetry (SWASV)	Margarines	WE: Glassy Carbon electrode (GCE) and RE: Ag/AgCl with saturated KCl CE: Platinum (Pt)	Sys et al. [43,46]
Differential Pulse Voltammetry (DPV) and Cyclic Voltammetry (CV)	Olive oil	WE: Platinum screen printed electrode (Pt-SPE) RE: Silver (Ag) CE: Platinum (Pt)	Vasilescu et al. [44]
Differential Pulse Voltammetry (DPV)	Fish Oil	WE: Glassy Carbon electrode (GCE) and RE: Ag/AgCl with saturated KCl CE: Platinum (Pt)	Lubeckyj et al. [41]
Square Wave Voltammetry	D, L-α tocopherol (synthetic form)	WE: Glassy Carbon electrode (GCE) and RE: Ag/AgCl with saturated KCl CE: Platinum (Pt)	Filik et al. [45]
Adsorptive Stripping Voltammetry	Lipophilic Vitamins	WE: Glassy Carbon electrode (GCE) and RE: Ag/AgCl with saturated KCl CE: Platinum (Pt)	Sys et al. [43,46]
Paper device	Sunflower oil	WE: pencil drawn electrode and RE: Ag/AgCl	Dossi et al. [47]

Table 6: Summary of electrochemical analysis for determination of tocopherol.

have been obtained are compared with HPLC and SWASV results and they displayed efficient result than HPLC. Vasilescu et al. in 2015 determined the anti-radical (antioxidant) properties which include vitamin E isomers (α-, β-, δ-, γ- tocopherol) extracted from olive oil using Differential Pulse Voltammetry (DPV) and Cyclic Voltammetry (CV) techniques [44]. Olive samples were prepared in the volume ratio 1:10 with 2-propanol. For DPV and CV, Platinum Screen Printed Electrode (Pt-SPE) was used as working electrode, silver (Ag) as a reference electrode and Platinum (Pt) as a counter electrode. CV was recorded at scan rate of 50 mv/s. DPV voltammograms were recorded between the potential of +600 to -50 mv. The DPV results have shown different peaks for α-, β-, δ-, γ- tocopherol. When compared with HPLC, it was found that DPV result agrees with the one of HPLC.

Lubeckyj et al. in 2017 applied Differential Pulse Voltammetry (DPV) to determine efficiency of vitamin E (i.e., stripping tocopherol) from fish oil and compared with the result obtain from HPLC [41]. Tocopherols were extracted from fish oil with slight modifications using aluminium oxide and hexane. For DPV analysis glassy carbon is used as working electrode, Platinum (Pt) as counter electrode and Ag/AgCl as Reference electrode. Potentials were scanned from 0 to 1.0 V. the peaks for α-, β-, δ-, γ- tocopherol is obtained, and the results were compared with HPLC result. Due to redox reaction of analyte at different potential DPV is more useful in determining antioxidant than HPLC. Filik et al. in 2016 evaluated α-tocopherol (Vitamin E) and Retinol (Vitamin A) using electrochemical analysis where working electrode is modified on a Poly (2, 2′-(1,4 phenylenedivinylene)-bis-8-hydroxyquinaldine)-multi walled Carbon Nanotube [45]. A Modified Glassy Electrode (GCE) was used as a working electrode Ag/AgCl with a saturated KCl which employed as a Reference electrode and Platinum (Pt) as a counter electrode. A synthetic form of α-tocopherol is used in the form of D, L-α-tocopherol acetate without any purification and dissolves in ethyl alcohol. The electrolyte consists of acetate buffer and sodium acetate salt. The pH of the medium was adjusted using HCl and NaOH. Square wave voltammetry was used for the analysis with potential range of 0.15-0.95. The results were compared between modified and unmodified electrode. Small anodic peak was observed when using unmodified electrode and well-defined wave was observed using modified type. This indicated that using nanoparticles to the modified electrode has increased sensitivity and thus for suitable for tocopherol analysis.

Sys et al. in 2016 employed absorptive stripping voltammetry to detect lipophilic vitamins such as Cholecalciferol, tocopherol, retinol and phylloquinone [46]. 3 electrodes system was used which includes glassy carbon electrode (GCE) as working electrode, Ag/AgCl with 3 Mol/L KCl as a reference electrode and Platinum wire as a Counter electrode. The result has shown the use of adsorptive voltammetry as a candidate for determination of tocopherol and other lipophilic vitamins.

Dossi et al. in 2015 developed simple pencil drawn paper-based devices that can be used for one spot detection of different electroactive species present in oil samples [47]. The papers used are small circular pads that are hydrophilic and possess hydrophobic barriers. The device was designed to contain electrolyte (1 M KCl and 0.1 M HCl as supporting electrolyte), pencil drawn electrode as working electrode and Ag/AgCl as reference electrode. The portion of edible oil (sunflower oil) samples was applied on top of the wet (i.e., moist) cell. This device not only allows detection of α-tocopherol but also polyphenols, ortho-diphenols and monophenols (Figure 4).

Conclusion

Vitamin E is considered as one of the most vitamin produced by both plants and animals. They are made of 8 distinct isomers known as α-, β-, δ-, γ- tocopherol and α-, β-, δ-, γ- tocotrienols which differ as a result of position of methyl group of chromanol ring. Vitamin E has shown to play a significant role in human and animal's metabolism; they are widely used as antioxidants which terminate the chain reaction of free radicals which causes oxidative stress. Vitamin has shown a positive effect in treatment and prevention of cancer diseases, cardiovascular diseases, cataracts, arthritis and inflammations. Scientist employ HPLC for the analysis of vitamin E isomers, but this method is limited to due to its longer steps, less sensitivity and high cost of apparatus and chemicals. The use of electrochemical techniques such as DPV, CV, CA and SWASV to determine the antioxidant activity of vitamin E isomers is highly promising as it is more affordable, simple, rapid, highly sensitive and less detection limit.

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