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## Current technologies in the extraction, enrichment and analytical detection of tocopherols and tocotrienols: A review

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### ABSTRACT

During the past few years the scientific and medical community has been confronted with a continual interest in vitamin E with the interest prompted by new discoveries. Tocopherols and tocotrienols, commonly known as vitamin E, are extremely invaluable compounds and have various nutritional functionalities and benefits to human health. Great deals of research projects have been launched in order to develop effective methods for the extraction of vitamin E. By and large, three distinct extractive methods are usually employed: supercritical fluid extraction (SFE), molecular distillation, and adsorption methods. These methods are sensitive to different experimental conditions, such as pressure, temperature, and flow rate with noticeable effects on the efficiency of the extraction and enrichment of vitamin E. This review has covered the most commonly adapted extraction methods and has probed into the extraction yields under variable operational parameters.

### KEYWORDS

Vitamin E; tocopherols; tocotrienols; extraction methods

### Introduction

Vitamins as biologically active compounds are vitally needed in relatively small amounts to sustain life and to promise good health. Vitamin E and other dietary-essential antioxidants have been put to the forefront of the medical and nutrition sciences. This is due to the significant link between oxidative stress in its various forms and the control of many chronic diseases. Of the many such dietary components, vitamin E has commanded most interest because of its availability, strong marketing potential, overall health impact, and central role in preventing oxidation at the cellular level. Vitamin E (Tocopherols and Tocotrienols), is a fat-soluble natural antioxidant. Vitamin E plays important roles in the protection of unsaturated fatty acids lipid-containing food products against oxidative deterioration and free radical-mediated degenerative diseases such as cardiovascular diseases and cancers (Rimm et al., 1993; Ames et al., 1995; Kushi et al., 1996; Nesaretnam, 2000).

Vitamin E was identified as a fat-soluble nutritional factor during the studies on rats by Evans and Bishop (Evans and Bishop, 1922). It was initially called factor X and was later named vitamin E by Bishop. Given the term E was on the ground that its discovery was closely followed by the discovery of vitamin D. Naturally occurring vitamin E has eight members ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -T and the corresponding  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -T3), divided into two groups with four compounds each (Figure 1). It fundamentally comprises of a 6-chromanol ring and a side chain. Compounds with a saturated side chain are

classified as tocopherols, while compounds with a geranylgeranyle side chain with three double bonds are categorized as tocotrienols (Hew, 1989; Sen et al., 2006, 2007). Vitamin E as a natural antioxidant has been receiving great attention due to its benefits in decreasing the risk of coronary heart diseases and cancer (Brigelius-Flohe et al., 2002). Recent studies have indicated that natural vitamin E has more beneficial effects compared to synthetically-produced vitamin E on account of the absence of non-active stereoisomers (Brigelius-Flohe and Traber, 1999) in synthetic products.

Vitamin E is found in oils and fats for the most part. The natural forms of vitamin E are produced from plant and vegetable oils and are the most invaluable while concentrated supply of vitamin E. Vitamin E is found in varied quantities in different plant oil. Moreover, changes in oxidative of vitamin E may occur throughout the harvesting, processing, marketing, and utilization stages. The lower oxidative events of vitamin E can result in large losses in its biological activity. Table 1 shows the composition of tocopherol and tocotrienol in some selected fats and oil. The interest in vitamin E products in pharmaceutical, food, and cosmetic industries has rapidly increased within the last twenty years. Oils and their concentrates are one the natural source of tocopherols and tocotrienols. Vegetable oils are mixtures of minor and major lipid components. These naturally occurring concentrates range from 20% to 90% by weight of tocopherols. These concentrations are essential in foods and supplements. The in-oil tocopheryl ester is mainly used for the fortification of food due to its oxidation stability.

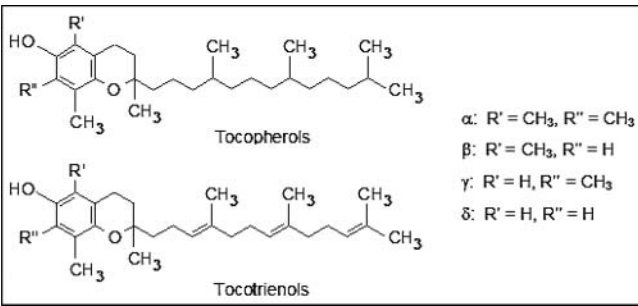


Figure 1. Structures of tocopherols and tocotrienols (Evans and Bishop, 1922).

Extraction of vitamin E from natural sources has received rising attention by reason of its function as a highly active antioxidant. In recent years pronounced considerations for the detection and investigation of new alternative techniques for the extraction and enrichment of vitamin E has been taken into account. The extraction of tocopherols and tocotrienols is set out via a distinct number of physical and chemical techniques.

Separation techniques including supercritical fluid extraction (SFE), molecular distillation, and adsorption have been commonly employed for the extraction and production of vitamin E. The SFE is one of the widely implemented techniques for the extraction and isolation of vitamin E. This method has been applied to extract and concentrate tocopherols from different sources such as palm oil (Birtigh et al., 1995) and rice bran or soybean (King et al., 1996) with an enrichment of up to four-fold. Recent studies have employed molecular distillation which deals with the purification of tocochromanols from crude palm oil (CPO) and soy oil deodorizer distillate (SODD) (Gast et al., 2005) with a an enrichments of up to 94%. Other products and residues that have also been studied include wheat germ (Molsa and Ossa, 2000; Ge et al., 2002) and olive tree leaves (De Lucas et al., 2002).

This review paper probes into the technical feasibility of various techniques for the extraction and enrichment of vitamin E from some oily seeds. The main aim of this paper is to provide a detailed summary of the most recent methods and approaches in the extraction and enrichment of vitamin E.

## Separation methods

Potential vitamin E utilization was much to encourage the development of separation technology of this kind of valuable vitamin. There are several types of approaches for the separation of vitamin E such as separation using SFE, molecular distillation and adsorption. In order to improve the level of concentration, different techniques have been investigated.

By direct carbon dioxide extraction, a 10% (w/w) contents was reported (Shishikura et al., 1988). However, when coupled to techniques of esterification or adsorption, it has led to an enhanced yield of up to 64% (w/w) (Shishikura et al., 1988; Lee et al., 1991). When high purity is required, preparative supercritical fluid chromatography (SFC) is often considered, resulting in concentrations of up to 95% (Sayto and Yamamuchi, 1990). The industrial application is highly dependent on the cost of the coupled-technique operations and further purification processes, being of special interest the search of a direct-extraction application which results in enhanced vitamin E concentration. In the following section, the most common separation processes will be discussed.

## Supercritical fluid

A supercritical fluid (SF) is any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist. SFs can be best described by referring to a phase diagram. Figure 2 shows the phase diagram (King et al. 1996) of CO<sub>2</sub>. The critical temperature ( $T_c$ ) is the highest temperature at which a gas can be converted into a liquid by an increase in pressure and the critical pressure ( $P_c$ ) is the highest pressure at which a liquid can be converted to gas by an elevation in its temperature. When the temperature and the pressure of any substance are above its  $T_c$  and  $P_c$ , it is considered to be a SF. Conventional solvents have fixed solvating strength. However, SFs have “tuneable” solvating power. By adjusting the physical parameters such as temperature and pressure, one is able to vary the solvating strengths of SF fluids. The physico-chemical property values (Table 2) of SFs are between those of a gas and a liquid. The higher diffusivity of the SFs compared to conventional solvents makes them ideal for the extraction of solid or powdered matrices. This property also leads to shorter

Table 1. Tocopherol and Tocotrienol content of selected fats and oil (Eitenmiller and Lee, 2004).

Source	Tocopherols (T1) g/ppm				Tocotrienols (T3) g/ppm				T3 Total	T1& T3 Total
	alpha	beta	gamma	delta	alpha	beta	gamma	delta		
Palm Oil	152	—	—	—	205	—	439	94	738	890
Rice bran	324	18	53	—	116	—	349	—	465	860
Wheat Germ	1179	398	493	118	24	165	—	—	189	2377
Barley	350	50	50	—	670	120	120	—	910	1360
Oat	180	20	50	50	180	30	—	—	210	510
Coconut oil	5	—	—	6	5	1	19	—	25	36
Palm Kernel	13	—	—	—	21	—	—	—	21	34
Soybean Oil	101	—	593	264	—	—	—	—	—	958
Safflower Oil	387	—	387	—	—	—	—	—	—	774
Peanut Oil	130	—	216	21	—	—	—	—	—	367
Cocoa Butter	11	—	170	17	2	—	—	—	2	200
Olive Oil	51	—	—	—	—	—	—	—	—	51

T1 : Tocopherol; T3 : Tocotrienol.

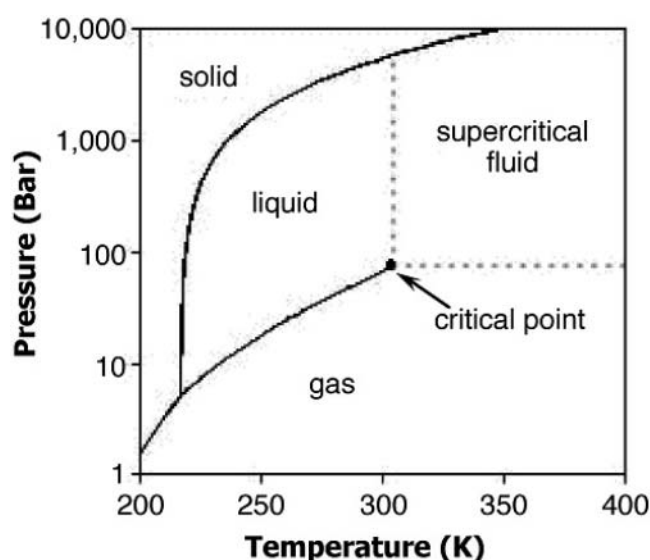


Figure 2. Phase Diagram of CO<sub>2</sub> (Leitner, 2000).

extraction times. SFs can be easily removed after extraction by reducing the pressure, thereby eliminating the need for solvent evaporation.

SFE using carbon dioxide (SC-CO<sub>2</sub>) has been recognised as a potential alternative to conventional solvents due to its relative nontoxicity and non-flammability, as well as its low critical temperature and pressure. SC-CO<sub>2</sub> is a particularly suitable separation method for isolation of the valuable components from plant materials. A natural plant extract, free from chemical alterations brought about by heat and water, and without solvent residues and other artefacts can be obtained by properly applying this method. Carbon dioxide is non-toxic, non-explosive, readily available and easily removed from the extracted products. For extraction of oil seeds a variety of solvents, including alcohols, acetone, and hexane can be used. However, these organic solvents leave adsorbed residues behind and the application of elevated temperatures during desolventization processes can potentially lead to chemical transformation of the oleoresins. The solvent residues must be reduced to very small concentrations, generally down to 25–30 ppm or even less (Reverchon et al., 1994). Variation of pressure changes properties of supercritical fluids continuously from more gas-like behavior to more liquid-like behavior. This behavior may lead to new technologies in processing natural materials (extraction and purification), in processes related to the environment (destruction of waste with supercritical water), and in reaction engineering (hydrogenation with mixtures of hydrogen and supercritical carbon dioxide). Supercritical fluids produce practically solvent-free products and avoid deteriorating reactions. In addition, process temperatures are low to very moderate. Solvent handling is favorable because it can be easily

and effectively removed. Besides, it is abundantly available and cheap and contributes to a huge part of the environment (water, carbon dioxide). Situations where supercritical fluids may be beneficial root from the effects that supercritical fluids have on pure compounds and mixtures. Supercritical fluids change compound properties, phase equilibria, chemical equilibria, and related rate processes (Reverchon et al., 1994). SC-CO<sub>2</sub> extraction has been applied to the extraction of oil enriched in tocopherols and tocotrienols, from several vegetal matrices (Table 3). The detail of each part was individually given in the following.

### Seed

Grape seeds (a residue of the wine industry) were used to obtain oil, enriched in  $\alpha$ -tocopherol, by using supercritical CO<sub>2</sub> (Bravi et al., 2007). The effect of some operational conditions (pressure, temperature, and solvent flow rate) and matrix conditions (size and moisture content) were investigated to find the optimum working values. It was shown that  $\alpha$ -tocopherol concentration in the oil extracted with SC-CO<sub>2</sub> increases with the extraction temperature. The largest extracted amount of  $\alpha$ -tocopherol was reported to be  $265 \pm 17$  ppm at 80°C and pressure of 25 MPa where grape seed were milled to below 0.425 mm. Moreover, they reported that SC-CO<sub>2</sub> extraction can be used to obtain the oil enriched in  $\alpha$ -tocopherol. The  $\alpha$ -tocopherol concentration in the oil and the total oil yield extracted by means of SC-CO<sub>2</sub> under the optimal conditions were found to be higher and lower than the values obtained with *n*-hexane, respectively.

### Leaves

De Lucas et al. (2002) used supercritical CO<sub>2</sub> for the extraction tocopherol from olive tree leaves (a residue obtained during the harvest of olives). They studied about the effect of pressure, particle size, solvent flow and temperature on the extraction rates of tocopherol from olive tree leaves. In all analyses,  $\alpha$ -tocopherol was the major isomer (>90%) in comparison to the other isomers. The effect of pressure was investigated at three levels of 25, 35, and 45 MPa. It was observed that the maximum amount of tocopherol could be obtained at 25 MPa and a further increase of pressure up to 35 and 45 MPa adversely affected tocopherol recovery. Moreover, the optimal extraction conditions were reported to be achieved with a particle size of 1.5 mm, a solvent flow rate of 1 SL/min and an operational temperature of 313 K. A comparison was also drawn between the obtained results and those reported by the extraction of olive tree leaves with soxhlet. They stated that the hexane extract showed no tocopherol content. It was observed that these conditions would lead to a decent amount of valuable extracts of 74.5% (w/w) (flow rate for 2 h) and 97.1% (w/w) (flow rate for 1 h) tocopherol concentration, respectively.

Table 2. Physico-chemical properties of Gases, SF's, and Liquids (Taylor, 1996).

	Density (g/mL)	Dynamic viscosity (g/cm-sec)	Diffusion coefficient (cm <sup>2</sup> /sec)
Gas (ambient)	$6 \times 10^{-4}$ – $2 \times 10^{-3}$	$1 \times 10^{-4}$ – $3 \times 10^{-3}$	$10^{-1}$ – $4 \times 10^{-1}$
Supercritical fluid ( $T_c$ , $P_c$ )	$2 \times 10^{-1}$ – $5 \times 10^{-1}$	$1 \times 10^{-4}$ – $3 \times 10^{-4}$	$7 \times 10^{-4}$
Liquid (ambient)	$6 \times 10^{-1}$ – $16 \times 10^{-1}$	$2 \times 10^{-3}$ – $3 \times 10^{-2}$	$2 \times 10^{-6}$ – $2 \times 10^{-5}$

**Table 3.** Extraction and enrichment of tocopherols and tocotrienols by using SC-CO<sub>2</sub>.

Material	Supercritical/SC Technique	References
Grape seeds	SC-CO <sub>2</sub>	Bravi et al. (2007);
Almond seeds	SC-CO <sub>2</sub>	Leo et al. (2005);
Olive tree leaves	SC-CO <sub>2</sub>	de Lucas et al. (2002)
Palm leaves	SC-CO <sub>2</sub>	Birtigh et al. (1995);
Olive husk	SC-CO <sub>2</sub>	Gracia et al. (2011)
Wheat germ	SC-CO <sub>2</sub>	Saito et al. (1988)
Palm Oil	SC-CO <sub>2</sub>	Choo et al. (2004)
Rice bran Oil	SC-CO <sub>2</sub>	Sarmiento et al. (2006)
Soybean Oil	SC-CO <sub>2</sub>	Jokić et al. (2012)
Soybean Oil	SC-CO <sub>2</sub>	Fang et al. (2007)

The supercritical fluid method was used to obtain high concentrations of tocopherols from the leaves of palm oil trees (Birtigh et al., 1995). They reported that the maximum amount of extracted tocopherol could be reached under an operational pressure of 30 MPa. However, the final concentration would decrease at higher extraction pressures. The maximum amount of extracted tocopherols from the residues and palm leaves has been reported to be 0.113 kg/kg and is uncovered to be not high enough to allow an economically industrial extraction.

### Husk

Vitamin E (tocopherols and tocotrienols) has also been extracted from olive husk (Gracia et al., 2011). The influence of pressure (ranging from 25 to 35 MPa) and temperature (ranging from 313 to 333 K) on oil yield and the quality of the extract were evaluated. It was understood that under the optimum pressure of 35 MPa and the temperature of 333 K, adapting supercritical CO<sub>2</sub> technology is a viable method for recovering concentrated vitamin E with a concentration of two to four times better than that of the classical hexane.

Saito et al. (1990) devised a method for the extraction and fractionation of substances from complex mixtures such as natural products by using supercritical carbon dioxide both as an extraction medium and as a mobile phase in preparative chromatography. They have demonstrated the enrichment of tocopherols from wheat germ. The method was evolved based on coupled SFE-preparative SFC. It was reported that tocopherols were enriched from 0.05 to about 5%, i.e., by a factor of 100, in a single run of coupled SFE-preparative SFC. However, even after repeated chromatography of the  $\alpha$ -tocopherol fraction, the final concentration was only about 20% owing to the low initial concentration (0.05%) of the compounds in the complex matrix of wheat germ.

### Oil

Birtigh et al. (1995) extracted tocotrienols from CPO by SFC by utilizing a silica gel column. They used programmed extraction elution method (PEEM) for the fractionation of CPO and managed to attain four fractions. Fraction 1 included substances that eluted from 0 to 66 minutes while using pure carbon dioxide at 18 MPa pressure as the eluent. Fraction 2 included substances that eluted during the next 20 minutes with a mixture of carbon dioxide and ethanol (5.0/0.2 mL/min). Fraction 3 included solutes that eluted in the following 54 minutes by increasing ethanol flow rate to 0.5 mL/min. Finally, fraction 4

included substances that eluted in the final 10 minutes under the same elution conditions as were used for fraction 3. They reported that tocopherols and tocotrienols were successfully isolated in fraction 2.

Rice bran has been another precursor for the extraction of tocopherol and tocotrienol from its oil by using supercritical CO<sub>2</sub> method (Sarmiento et al., 2006). These researchers probed into a range of different experimental conditions to identify the optimum temperature and pressure for oil fractionation, after the extraction process with supercritical CO<sub>2</sub>. They conducted their experiments in two distinct paths (without separators and with separators). The operational conditions, i.e., temperature and pressure were evaluated in order to obtain the highest oil yield and the highest level of tocols in the oil. In the first series of experiments (without separators), the operational pressures for extractions were set to 150, 200, and 250 bar with a temperature range of 25, 40, 50, and 60°C and an average solvent flow rate of 0.0756 kg h<sup>-1</sup>. Moreover, two different procedures were followed for each set of operational conditions. The first one was favored in order to pinpoint the yield of a total extraction process of 8 h. The second procedure was to obtain the extraction curve, with solute collected at 1 h intervals, during an extraction process of 8 h. They reported that at a pressure of 150 bar for all studied and temperatures at 25, 50, and 60°C for all studied pressures, the process yield increased with decreasing process temperature. Also, it was observed that at 40°C an increase in pressure from 150 to 250 bar led to an increase in the percentage of oil yield from 20% to 80%. They reported the rate of tocols extraction increased with temperature for the range evaluated. Moreover, they observed that larger quantities of tocotrienols than of tocols were always found under any extraction condition. The highest amount of tocols (939.12 mg/g) was reported achievable at 200 bar and 40°C. In the second arrangement, two vessels in series were used to obtain the tocopherol- and tocotrienol-enriched fractions with extraction conditions of 200 bar/40°C and 200 bar/25°C. The separation conditions were set as 2, 25, and 40°C and 100 and 150 bar for vessel 1. In vessel 2, the operational conditions were maintained constant at 2°C and 25 bar. They reported the fractions of oil obtained in the two separators had different characteristics and larger quantities of isomers of tocotrienol than of tocopherol isomers were found in all of the experiments.

Jokić et al. (2012) inspected the concentration of tocopherol isomers in different fractions of soybean oil taken at different extraction process conditions, i.e., at different pressure, temperature and CO<sub>2</sub> mass flow. They reported that the total content of tocopherols in collected fractions was statistically different and was in the range between 287 and 1410 mg/kg, depending on the extraction conditions. The effects of pressure, temperature, and flow rate were studied. It was confirmed that an increase in pressure would lead to a decrease in tocopherol concentration at a constant temperature. Similarly, an increase in temperature would decrease tocopherol content. The content of total tocopherols was obtained during the 40°C extraction temperature decreases from 1410 mg/kg (first fraction) to 445 mg/kg (final fraction). Similarly, at a temperature of 60°C, the content of total tocopherols decreased from 668 to 32 mg/kg. The effect of solvent mass flow rate on the tocopherol concentration in obtained oil fractions were reported to have a significant effect



on tocopherol content. When a smaller solvent flow rate was set up, the highest contents of individual and total tocopherols were obtained.

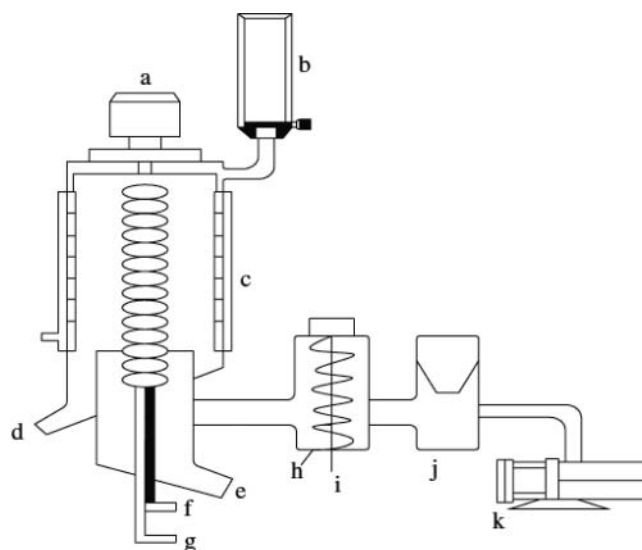
Separation of natural tocopherols from oil deodorizer distillate (DOD), a by-product from the soybean oil refining process, with supercritical carbon dioxide was studied by Fang et al. (2007). They ran the experiments in a fractionation column with semi-continuous counter current process. Operational parameters, i.e., pressure, the ratio of solvent to feed and the temperature of the column were researched. They found out that a higher pressure resulted in more tocopherols being extracted corresponding to a total content of 3.2% at 18 MPa. The produced product contained about three times tocopherols than that extracted at 14 MPa.

The experimental results indicated that supercritical CO<sub>2</sub> effectively extracts fatty acid methyl esters at 16 MPa with a column temperature gradient of 313–348 K. By increasing pressure, natural tocopherols (>50%) can be successfully recovered to a high degree (>80%). The initial pressure, feed location, temperature gradient, and the ratio of CO<sub>2</sub> to methyl esterified DOD were optimized for separating FAMES. For the following tocopherol concentration step, a final pressure of 20 MPa resulted in the greatest average tocopherol content (>50%) and tocopherol recovery (about 80%). According to the results derived from the separation experiments, it is concluded that supercritical CO<sub>2</sub> fractionation is technically feasible for concentrating natural tocopherols from methyl esterified DOD.

In another study (Saito and Yamauchi, 1990), tocopherols were enriched from wheat-germ oil through recycling in a semi preparative SFC using two 250 mm × 10 mm I.D. Columns packed with 5-µm silica gel. Tocopherol was fractionated after recycling twice while α-tocopherol was extracted after two additional subsequent recycles. Peak assignment was performed by comparing the UV spectra of the fractionated substances, which were obtained during the chromatographic run using a photodiode-array UV detector, with those of standard tocopherols. The purities of α- and β-tocopherols were found to be about 85% and 70%, respectively, using capillary gas chromatography.

### Molecular distillation

Molecular distillation has shown promising potentials in the separation, purification, and/or concentration of natural products, usually constituted by complex and thermally sensitive molecules, such as vitamins and polyunsaturated fatty acids due to its capability to minimize losses by thermal decomposition. Furthermore, this process has advantages over other techniques that use solvents as the separating agent, avoiding problems with toxicity. In this process, the distilled liquid continuously passes downward over the heated evaporating cylinder, evaporates partially and the vapors then condense on the cooled condenser placed close to the evaporating cylinder. The advantage of using molecular distillation technique is that the separation processes under vacuum which makes it possible to keep the temperature of the separation to the minimum in order to avoid damage to thermally sensitive materials (Shao et al., 2007). Several studies of tocopherol isolation and enrichment by means of molecular distillation has been carried out



**Figure 3.** Schematic of wiped-film molecular distillation (Jiang et al., 2006) (a) motor drive; (b) feed flask; (c) wiper; (d) residue receiver; (e) distillate receiver; (f) one-stage condenser inlet; (g) one-stage condenser outlet; (h) two-stage condenser inlet; (i) two-stage condenser outlet; (j) cold trap; (k) rotary vane vacuum pump.

with CPO (Setyawan et al., 2011) and rapeseed oil deodorizer distillate (RODD) (Jiang et al., 2006) as the raw material as shown in Figure 3.

### Oil

Molecular distillation method has been practiced for separation of tocopherol from CPO (Setyawan et al., 2011). They stated that tocopherol separation from CPO biodiesel using molecular distillation under a flow rate of 1.3 L/h to 7 L/h, the distillation temperature of 175–220°C, and a rotational wiper's speed of 200–400 rpm successfully separated fraction tocopherol from palm oil biodiesel rich. Tocopherol as much as 22.9–605 mg of a total of 1302 mg tocopherol contained in any treatment (2 L of biodiesel CPO) was effectively separated. Tocopherol recovery was calculated to be around 35%. It is noticeable that biodiesel did not indicate any quality changes before and after the molecular distillation process.

### Seed

Tocopherol was successfully recovered from rapessed oil deodoriser distillate (RODD), a by-product of vegetable refining edible oil (Jiang et al., 2006). These researchers looked into the effects of various experimental conditions including evaporating temperature, feed flow rate and wiper rolling speed on the measured yield of tocopherol. The tocopherol content did not change much over the studied evaporating temperature range. They highlighted that the fraction collected at 200–230°C at 2.66 Pa was rich in tocopherol (nearly 35%). Additionally, they managed to recover around 50% of the tocopherol originally contained in RODD.

### Adsorption

Palm fatty acid distillate (PFAD) has been reported to be another source for the concentration of vitamin E (tocopherols and tocotrienols) by adsorption chromatography (Chu et al.,

**Table 4.** Results obtained in enrichment of tocopherol and tocotrienol from different natural materials by using different method.

Material	Technique	Optimal Conditions	Tocopherol enriched mg/kg (ppm)	Tocotrienol enriched mg/kg (ppm)	Ref.
Rice Bran	SC-CO <sub>2</sub>	20 Mpa, 313 K	9390	90	Sarmiento et al. (2006)
Palm Leaves	SC-CO <sub>2</sub>	30 MPa, 343 K	1710	—	Birtigh et al. (1995)
Soybean Oil	SC-CO <sub>2</sub>	25 MPa, 313 K	1410	—	Jokic et al. (2012)
Wheat germ	SC-CO <sub>2</sub>	25 MPa	950	—	Saito et al. (1988)
Almond Seeds	SC-CO <sub>2</sub>	42 Mpa, 323 K	600	—	Leo et al. (2005)
Soybean Oil	SC-CO <sub>2</sub>	20 MPa	570	—	Fang et al. (2007)
Rapeseed Oil	M.D	473—503 K	363	—	Jiang et al. (2006)
Crude Palm Oil	M.D	448—493 K	228	—	Setyawan et al. (2011)
Grape Seeds	SC-CO <sub>2</sub>	25 Mpa, 353 K	265	—	Bravi et al. (2007)
Olive Husk	SC-CO <sub>2</sub>	35 Mpa, 333 K	157	—	Gracia et al. (2011)
Olive tree leaves	SC-CO <sub>2</sub>	25 MPa, 313 K	110	—	de Lucas et al. (2002)

SC = supercritical; M.D = Molecular Distillation.

2003). They explored several experimental conditions, such as flow rate, column temperature, loading size, and column bed height in order to optimize the purity and recovery of vitamin E. They applied low temperature (60°C) and mild alkali conditions which minimized the loss of vitamin E during the separation. They mentioned that neutralization, concentrated the vitamin E content of the oil by 5.6 times, while hydrolysis followed by neutralization resulted in 11.8 times concentration compared to the original PFAD. Tocopherols and tocotrienols were then concentrated in adsorption chromatography. They utilized silica normal-phase chromatography with samples being eluted with hexane followed by isopropanol. They realized that the oil recovered from hexane elution was higher compared to that of isopropanol elution of around 97%.

In order to draw a careful comparison among the methods and find the most suitable ones for the extraction and enrichment of vitamin E, the fraction of tocopherol and tocotrienol were investigated (Table 4). As shown, supercritical separation technique is the most concentration-effective method which can enrich tocopherol olive tree leaves, wheat germ, and soybean oil up to 97%, 95%, and 57%. The second best method in terms of final concentration of vitamin E is molecular distillation. Tocopherol-enriched fraction of rapeseed oil and CPO has been about 35% by exercising molecular distillation approach.

## Analysis methods

### Gas chromatography (GC)

In early gas chromatographic procedures for vitamin E analyses packed columns were used; stationary phases including Apiezon L, SE-30 and OV-17 coupled with flame ionization detection (FID). In the majority of methods, tocopherols and tocotrienols were derivatized to their trimethylsilyl ethers to improve thermal stability and volatility. Analysis using GC has some advantages as well as a few downsides. The main gains of using GC are:

1. Free and esterified  $\alpha$ -tocopherol ( $\alpha$ -T) could be quantified in the same product.
2. Sterols and other fat-soluble vitamins could be simultaneously quantified.
3. Assay values are normally higher, indicating less destruction and/or better extraction.

4. Reproducibility of the analysis is generally better.

5. GC analysis is faster in nature.

However, it has some disadvantages in comparison with other methods as follows:

1. GC analysis requires higher extract purity when dealing with low levels of vitamin E.
2. Instrument and column parameters have to be carefully controlled and adjusted.
3. Calibrations of the apparatus and standards have to be constantly checked.
4. Column overloading had to be prevented.

### High-performance liquid chromatography (HPLC)

A large number of publications are dealing with HPLC applications to the analysis of vitamin E in food within the last few decades. Parrish (1980) published a comprehensive review of vitamin E analyses methods that included initial reports on HPLC. He presented HPLC as a method with superior advantages over other methods. At that time, Parrish cited only 19 publications that applied HPLC to vitamin E analysis and discussed advantages of the technique over GC. Since 1980, in a large number of publications HPLC application has been benefitted for the quantitative and qualitative analyses of vitamin E in foods, feeds, tissues and other highly varied biologicals.

Van Niekerk's et al. work was the first published HPLC-oriented paper dealing with food-based analysis of vitamin E (Van Niekerk, 1988). In that study normal-phase chromatography on Corasil II with a mobile phase of isopropanol ether:hexane (5:95) was applied. Van Niekerk's work set important principles for the application of HPLC to vitamin E analysis that eased the work of later vitamin E analysts. These included the followings:

1. Oils could be injected directly into a silica column; therefore, no sample preparation other than dilution of the oil was required.
2. Fluorescence provided an ideal, sensitive, and specific detection mode.
3. Positional isomers,  $\beta$ - and  $\gamma$ -T, could be resolved.
4. Good reproducibility became possible.
5. Recoveries of added tocopherols to oils were high, i.e., approaching 100%.
6. The procedure was fast and easy.

Van Niekerk predicted that HPLC would promisingly find a wide range of applications for the routine assay of vitamin E in foods.

## Conclusion

In this review, current approaches in the extraction of vitamin E are extensively illuminated. Vitamin E, consisting of tocopherols and tocotrienols, has proven to play vital roles in human body's metabolism. In contemporary literature distinct methods have been applied to extract these compounds from a series of various sources. Each of these approaches has observed to inherit advantages as well as downsides and limitations which are resultants of the operational conditions. As we move into the future, decisions on food and nutrition would increasingly be influenced not only by quality but by social and environmental factors which will subsequently need to be considered. Therefore, it is desired to develop and apply an extraction process build upon green technology and eco-friendliness. Among these processes, SFE is considered to be a green technology on the grounds that they require much less solvent than competing techniques. Although CO<sub>2</sub> is considered a greenhouse gas and used in this process; however, it does not contribute to the greenhouse effect because CO<sub>2</sub> is either a by-product of an existing process or is withdrawn from the environment, used in the SFE process and returned to the environment. In addition to this, optimum process design for the chosen technology is crucial in order to develop an industrially feasible recovery of vitamin E translated into high separation time efficiency, production cost's attractiveness and acceptable safety levels of the production process and products. Moreover, molecular distillation and adsorption methods require a large amount of organic solvent, additional process equipment and also have long batch times compared to SFE method. This review concludes that still there is much room for the development of a greener technology which should be further diversified and manifested for sustainable process of vitamin E.

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