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Vitamin E and Beta Carotene Composition in Four Different Vegetable Oils

[1]Eqbal Dauqan, [1]Halimah Abdullah Sani, [2]Aminah Abdullah,
[3]Halimah Muhamad and [3]Ab. Gapor Md. Top
[1]School of Biosciences and Biotechnology,
[2]School of Chemical Sciences and Food Technology,
Faculty of Science and Technology, University Kebangsaan Malaysia,
43600 Bangi Selangor, Malaysia
[3]Malaysian Palm Oil Board, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

Abstract: Problem statement: Some vegetable oils contains natural antioxidants such as beta carotene and vitamin E namely tocopherol and tocotrienol. Different vegetable oils contained different amount of vitamin E and β-carotene. **Approach:** Study was carried out to investigate the natural antioxidants (vitamin E and beta carotene) composition in four different vegetable oils [Red Palm Olein (RPO), palm plein (PO), Corn Oil (CO) and Coconut Oil (COC)]. **Results:** The results showed that RPO contained the highest amount of vitamin E and β-carotene compared to the other three types of vegetable oils studied. **Conclusion:** The RPO can be considered as a good source of natural antioxidant (tocopherol, tocotrienol and β-carotene).

Key words: Vegetable oils, Corn Oil (CO), Red Palm Olein (RPO), beta carotene, high performan liquid chromatography (HPLC), Reactive Oxygen Species (ROS), antioxidant nutrients, tocopherol, tocotrienol

INTRODUCTION

Antioxidant nutrients such as ascorbic acid, α-tocopherol and the carotenoids are present in plant-derived foods. Antioxidants also counterbalance the production of Reactive Oxygen Species (ROS) which may cause oxidative damage to cells and leading to enhance the risk (Jennifer *et al*., 2009). The main biological function of vitamin E active compounds is part of the antioxidants which protects the polyunsaturated fatty acids of cell membranes from free-radical damage and takes part in the oxidative stress (Ubaldi *et al*., 2005; Korchazhkina *et al*., 2006). Chemistry of vitamin E is relatively complex and term includes two big groups of molecules: (tocopherols and tocotrienols). Tocopherols family includes four substances: α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol (Ubaldi *et al*., 2005).

Tocopherols and tocotrienols are fat-soluble vitamin E isomers and the major antioxidants of vegetable oils (Kalyana *et al*., 2003). Tocotrienols family (very similar to tocopherols) includes four different substances. Chemical structure in tocopherols is represented by a saturated carbonic chain bind to a cyclocromarole with methyl groups. In tocotrienols, the carbonic chains are unsaturated. α-Tocopherol has three methyl groups and is the most active form. β-Tocopherol and γ-tocopherol have two methyl groups. δ-Tocopherol has only one methyl group and is the most potent, but the less effective (Ubaldi *et al*., 2005). In general, tocopherols and tocotrienols are considered to have beneficial health effects. The main sources of vitamin E active compounds in the human diet are vegetable fats and oils and products derived from them while tocopherols are generally present in nuts and common vegetable oils. Tocotrienols are mainly concentrated in palm oil (Adam *et al*., 2007). Tocopherols differ from tocotrienols in that there are three double bonds in the side chain of the tocotrienols. There is a possibility that vitamin E isomers with only one or two double bonds in the side chain are present in palm oil (Ng *et al*., 2004). Tocopherol and tocotrienol have different biological activities towards free radicals and may play significant role as signaling molecule in cellular activities (Sue-Mian *et al*., 2010). Tocopherols and tocotrienols are fat-soluble vitamin E isomers and the major antioxidants of vegetable oils. Tocopherols can interrupt lipid oxidation by inhibiting

Corresponding Author: Eqbal Dauqan, School of Biosciences and Biotechnology, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia Tel: 0060172728034

hydroperoxide formation in the chain-propagation step, or the decomposition process by inhibiting aldehyde formation. Beside its free radical scavenging activity, α-tocopherol is highly reactive towards singlet oxygen and protects the oil against photosensitized oxidation (Kalyana *et al*., 2003).

A large number of research papers published have reported the separation and quantification of the eight vitamers from various sources (Choo *et al*., 2005). The vitamin E are relatively non-polar and thus are chromatographed well using either normal phase or reversed phase chromatography using different detectors. The method of choice is the HPLC and the most commonly used detector for vitamin E analysis is the fluorescence detector with normal phase column (Puah *et al*., 2007). β-Carotene is generally regarded as the most commercially important and widely used carotenoid. It is used as a food coloring agent, an antioxidant and safe pro-vitamin A source. Beta-carotene has anti-oxidant activity and it is an efficient quencher of singlet oxygen. It has been shown to act as an immune modulator, quench singlet oxygen and reduce peroxyl radicals at low partial oxygen pressure (Patrick, 2000). Spectrophotometry is still a common technique for the analysis of β-carotene in commercial product forms (Schierle *et al*., 2004). Therefore the main aim of this study was to determine the antioxidants (vitamin E and β-carotene) in four different vegetable oils (red palm olein, palm oil, corn oil and coconut oil) using High Performance Liqid Chromatography (HPLC) and spectrophotometer.

MATERIALS AND METHODS

Instruments: High performance liqid chromatography (HPLC) (Hewlett Packard HP1100, germany) was used for the measurement of vitamin E and spectrophotometer (Perkin Elmer, Lambda 35, UV/VIS spectrophotometer) was used for the measurement of β-carotene.

Samples of oil: The evaluated red palm olein (RPO) samples consist of carotenes (576 ppm), vitamine E (>800 ppm) and free fatty acids (0.045%) provided by Carotino SDN BHD company and and palm olein (PO) (Seri Murni), Corn Oil (CO) and Coconut Oil (COC) were obtained commercially.

Determination of concentration of vitamin E by HPLC analyses: The concentration of vitamin E in the vegetable oils (RPO, PO, CO, COC) was determined by HPLC (Hewlett Packard HP1100, FLD). HPLC analysis was performed using YMC column 150×6 mm I.D. The mobile phase used was composed of 0.5% isopropylalkhol/hexane and the flow rate was 1 ml/min. Total runtime for each standard and sample was 40 minutes. The injection volume was 20 µL. Detection was performed using a fluorescence detector at excitation 295 nm and emission 330. All standards were obtained from the Malaysian Palm Oil Board (MPOB). The standard concentration was 40 ppm for each component. Quantification of vitamin E was done by using the following formula:

$$\chi \text{ ppm} = Vs/Ws \times As/ Astd \times VIstd/ VIs \times Cstd$$

Where:

Vs = Volume of sample
Ws = Weight of sample
As = Area of sample
Astd = Area of standard
VIstd = Volume of standard injected
Vis = Volume of sample injected
Cstd = Concentration of standard

Determination of concentration of β-carotene by spectrophotometer: Carotenes content in the samples were analysed by Ultraviolet-Visible (UV-vis) spectrophotometer at 446 nm using MPOB test method. The sample was homogenized and weighed to the nearest ±0.0001 g into a 25 mL volumetric flask. The sample was dissolved with ***n***-hexane and diluted to the mark. The solution was transferred into a 1 cm quartz cuvette and the absorbance was measured at 446 nm against n-hexane. The carotene content of different vegetable oils is defined and calculated as β-carotene in parts per milliom (ppm).

The calculation was as follows:

$$\text{Carotenoids content} = [V \times 383 \times (As-Ab)]/ (100\times W)$$

Where:

V = The volume used for analysis
383 = The extinction coefficient for carotenoids
AS = The absorbance of the sample
Ab = The cuvette error
W = The weight of the sample in g

RESULTS

Composition of the Vitamin E (Tocopherol and Tocotrinols) in Four Different Vegetable Oils (RPO, PO, CO and COC) in Table 1 shows the concentration and the percent composition of the vitamin E (tocopherol and tocotrinols) in all samples analyzed. It was observed that the concentration and the percent composition of vitamin E were higher in RPO and PO compared to CO while the current study did not find of vitamin E in COC which was used in this study.

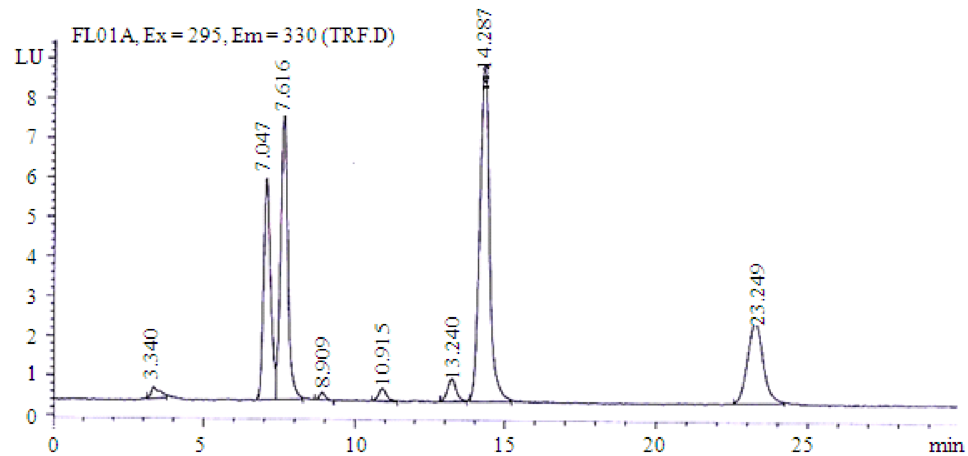

Fig. 1: Typical HPLC chromatogram of RPO

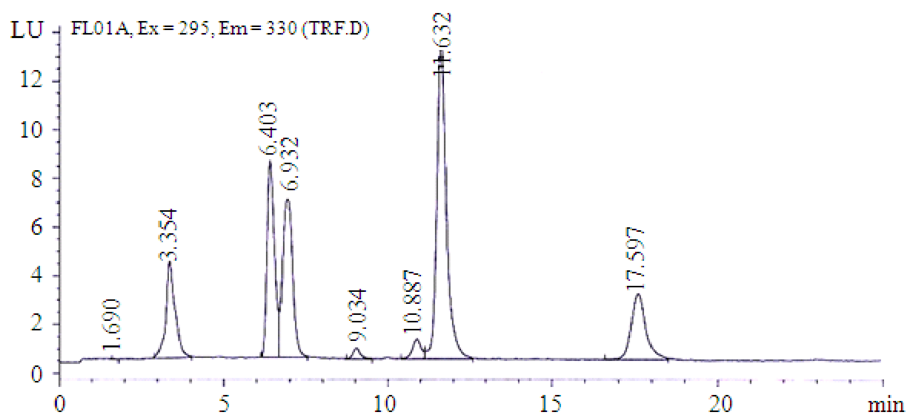

Fig. 2: Typical HPLC chromatogram of PO

Table 1 Concentration of vitamin E in four different vegetable oils

Sample	α –T (ppm)	α -T3 (ppm)	γ -T3 (ppm)	δ -T3 (ppm)	T+T3 (ppm)	% T+T3
RPO	171	294	367	126	953	0.10%
PO	218	289	395	111	1013	0.10%
CO	254	24	39	0	318	0.03%
COC	0	0	0	0	0	0.00%

Abbreviations: α-T, α-tocopherol; α-T3, α-tocotrienol; γ-T3, γ-tocotrienol; δ-T3, δ-tocotrienol; RPO, red palm olein; PO, palm olein; CO, corn oil; COC, coconut oil

A typical HPLC profiles of RPO obtained is shown in Fig. 1. The chromatogram shows a good baseline separation of the vitamn E. It illustrates identifiable peaks which correspond to α-T (7.047 min), α-T3 (7.616 min), γ-T3 (14.267 min) and δ-T3 (23.249 min). The total analysis time was less than 25 mins.

A typical HPLC profiles of PO obtained is shown in Fig. 2 and the identifiable peaks corresponded to α-T (6.403 min), α-T3 (6.932 min), γ-T3 (11.632 min) and δ-T3 (15.597 min). The total analysis time was less than 25 mins.

A typical HPLC profiles of CO obtained is shown in Fig. 3. The chromatogram shows identifiable peaks which correspond to α-T (7. 097 min), α-T3 (7.674 min), γ-T3 (12.986 min) and δ-T3 (20.797 min). The total analysis time was less than 25 mins.

A typical HPLC profiles of COC obtained is shown in Fig. 4. The chromatogram shows identifiable peaks which correspond to α-T (7. 743 min), γ-T3 (9.006 min) and δ-T3 (14.377 min). The total analysis time was less than 25 mins.

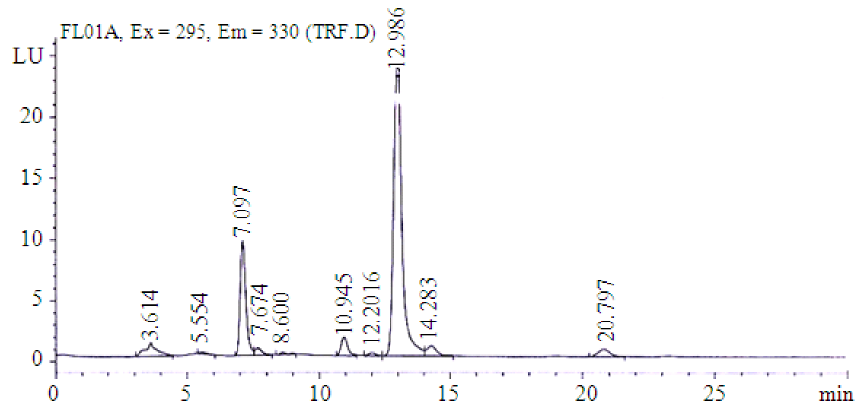

Fig. 3: Typical HPLC chromatogram of CO

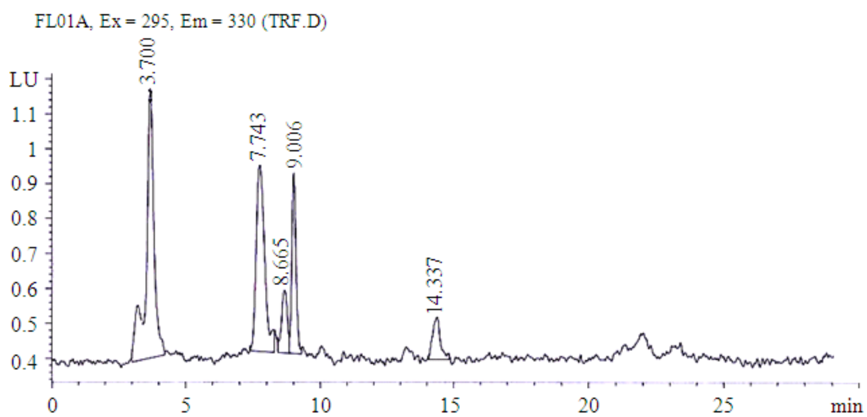

Fig. 4: Typical HPLC chromatogram of COC

Table 2: Concentration β-carotene in four different vegetable oils

Sample code	β-Carotene (ppm)
RPO	542.09
PO	0.00
CO	0.91
COC	0.00

Abbreviations: RPO, red palm olein; PO, palm olein; CO, corn oil; COC, coconut oil

Composition of the Beta Carotene in Four Different Vegetable Oils (RPO, PO, CO and COC): The Table 2 values have been found for the β-carotene content of four fifferent vegetable oils (RPO, PO, CO and COC). Red palm olein has highest (542.09 ppm) beta caroten content and corn oil the lower (0.91 ppm) than RPO. It can be seen from the table that PO and COC did not contain beta carotene.

DISCUSSION

Vitamin E is an important antioxidant that plays an important role in prevention of chronic diseases. The major biological role of vitamin E is to protect unsaturated fatty acids contained in vegetable oils from oxidation by free radicals. On the other hand the RPO and PO has lower levels of PUFA, antioxidants constitute the major substrates that are more easily reactive with oxygen (Adam *et al*., 2007). There is now a growing interest in the nutritional and physiological properties of vitamin E in palm oil, especially those of the tocotrienols. This has recently been reviewed (Kalyana *et al*., 2003). Vitamin E is also an important structural component of biological membranes, contributing to their stability. It is the first line of defense against lipid peroxidation. By its free radical quenching activity, it breaks chain propagation and thus terminates free radical attack at an early stage and the methyl groups of tocopherol interact with the cis double bounds of the fatty acids to form a stable complex in membrane phospholipids. Supplementation with vitamin E in humans decreases the susceptibility of LDL to oxidation *in vivo* (Madhavi *et al*., 2009). Some studies have shown that vitamin E has beneficial effects on plasma lipids (Park and Choi, 2002; Gazis *et al*., 1999; Maria, 2001; Jain and Palmer, 1996; Halliwell, 2002). In Paolisso's study vitamin E administration reduced triglycerides, total cholesterol and LDL (Paolisso *et al*., 1993). In another research 100 IU/day vitamin E in diabetic patients reduced serum triglycerides significantly (Khabaz *et al*., 2009). While Solanki and Bhatt (2010) did not show the effect of vitamin E on lipids. Kalyana and Yusof reported that the red palm oil usually contains about 500 ppm vitamin E.

Carotenoids also play an important potential role in human health by acting as biological antioxidants protecting cells and tissues from the damaging effects of free radicals and singlet oxygen (Zeb and Mehmood, 2004). Van Rooyen *et al*. (2008) suggested that RPO, as natural carotenoid rich oil, may protect the heart against an episode of oxidative stress in the rat and another study found that the treatment with beta carotene and vitamin E can significantly reduce lipid peroxidation in erythrocytes membranes (Mahjoub *et al*., 2007). Kritchevsky *et al*. (2002) suggest that vitamin E and/or carotenoids may be protective against coronary heart disease and lung cancer.

Beta- carotene is the most abundant form of provitamin A in fruits and vegetables (Hathcock, 2004). β-Carotene is currently incorporated in a wide variety of dietary supplements, including multivitamin, vitamin A and antioxidant formulations (Schierle *et al*., 2004). There is limited evidence to suggest that beta-carotene supplementation reduces the risks of chemically induced tumours in animals (Wald *et al*., 1988). The carotenoids, together with vitamin E, ascorbic acid, enzymes and proteins, are members of the biological antioxidant network converting highly reactive radicals and free fatty peroxyl radicals to less active species (.

CONCLUSION

The results conclude that red palm olein contained the highest amount of vitamin E and β-carotene compared to the other three types of vegetable oils studied. The red palm olein can be considered as a good source of natural antioxidant (tocopherol, tocotrienol and β-carotene).

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