INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 4, p. 181-187, 2019

RESEARCH PAPER OPEN ACCESS

Extraction and quantification of tocopherols from edible oils using high performance liquid chromatography

Abdul Majid[1*], Farah Naz[1], Abdul Rehman Phull[1], Sanaullah Abbasi[1], Aamir Hassan Khaskheli[1], Muzaffar Husain Sirohi[2], Israr Ahmed[3], Waqar Ahmed[4], Ghulam Fareed Narejo[1]

[1]*Department of Biochemistry, Shah Abdul Latif university, Khairpur, Pakistan*

[2]*Department of Botany, Shah Abdul Latif university, Khairpur, Pakistan*

[3]*Department of Mathemetics, Shah Abdul Latif university, Khairpur, Pakistan*

[4]*School of Mathematics and Physics, University of Lincoln, United Kingdom*

Key words: Adulteration, HPLC, Extraction, Olive oil, Tocopherols, Vegetable oil analysis.

http://dx.doi.org/10.12692/ijb/14.4.181-187 Article published on April 15, 2019

Abstract

Adulteration of Extra Virgin Olive Oil with olive oil and sunflower oil remained major issue in edible oil industry. Herein, rapid, sensitive and precise method for the determination of all type of tocopherols in different vegetable oils includinhg sunflower oil, olive oil and extra virgin olive oil is reported. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) with UV detector was used for tocopherol determination. A simple, quick and sensitive method to estimate the antioxidant quantity is proposed. In this method oils were diluted in methanol and injected directly into column (no saponification procedure). Methanol and water (98:2) mixture was used as mobile phase. Three tocopherols (α, γ and δ) were detected at 292 nm wavelength with UV detector. Method had good limit of detection (LOD) (7ng/g) and reproducibility (C.V% 0.9, 0.8 and 0.4 for α, γ and δ tocopherols, respectively). Result showed the best source for α-tocopherol was sun flower oil (146.65±1.7mg/kg). Oil richest in δ-tocopherol was olive oil and δ-tocopherol was absent in extra virgin olive oil. The current study suggest the new parameter (ratio of α/γ) as first screening indicator of authenticity of purity of extra virgin olive oil for differentiation of various cultivators of same generic source.

* **Corresponding Author:** Abdul Majid ✉ amajid.ansari@salu.edu.pk

Introduction

Vegetable oils are consumed worldwide as food and for different types of industrial purposes. A wide range of vegetable oils are available, however world utilization is dominated by sunflower oil and olive oil with 17.63 and 2.98 million tons, respectively (Statista, 2019). Vegetable oils are different in chemical composition of Fatty acids and antioxidant proportion empowering the performance in food and industrial applications (Bakre *et al.*, 2015). Traditionally, *Olea Europaea* crop used in soap formation, pharmaceutical products, cosmetics and in some places used as fuel for burning lights. Its oil is sold as olive oil (OO) and EVOO. EVOO is more expensive and more popular in European countries. While sunflower oil is synthesised from sunflower (*Helianthus annus)* seeds, contain good portion of fatty acids, lecithin, tocopherols, tocotrienols and carotenoids (Angeloni *et al.*, 2017).

The values of these components in each oil depends on agronomic, environmental and genetic factors (Bajoub *et al.*, 2018). Market of vegetable oils is expending, authenticity has a great importance in both commercial and medical fields (Nazzaro *et al.*, 2018).

Tocopherols along tocotrienols are group of hydrophobic antioxidants containing chromanol ring and tocopherol phytyl side chain. There are four different type of tocopherols (α, ß, γ and δ, these are differ from each other according to number and methyl position present in their side chain. Their structural phenomenon is responsible for their metabolic outcome and biochemical activities, all tocopherols have good absorption rate from gastrointestinal tract. α-Tocopherol (α-T) has highest plasma and tissue level compared to others. α-T resecreted into lipoprotein through α-TTP protein (Reboul, 2018). All tocopherols possess antioxidant activity and are essential in food to prevent the oxidation in body (Phull *et al.*, 2018). α-T has lowest anti-oxidant activity compared to γ-T. Sunflower oil has lowest anti-oxidant property than EVOO due to concentration of α-tocopherol is high in sunflower oil and EVOO has higher concentration of γ-tocopherol (Mohammad *et al.*, 2018). Vegetable oils contain higher amount of total tocopherols compared to animal fat even after refining and hydrogenation of vegetable oils (Barrera-Arellano *et al.*, 2019). Tocopherols play a vital role to decrease several chronic diseases for example, atherosclerosis and cancer. They inhibit the oxidation of poly unsaturated fatty acids (PUFA) in lipoprotein of plasma (Nagham Abdlateef Rasheed and Muhammad, 2018). Oxidation of PUFA can initiate the heart disease. Beside this, tocopherols work as anti-air pollutant for ozone and nitrogen oxide.

They decrease the lung damages and play role in maintaining the nervous system (Ueda *et al.*, 2009). Deficiency of α-tocopherol leads to impaired vision and blindness in younger ages (Nian and Lo, 2018). Toxicity of tocopherols compared to other fat soluble vitamins is very low. In animals studies no any significant teratogenic, carcinogenic or mutagenic effect was seen in case of higher doses (Dimery *et al.*, 1997).

Tocopherols are viscous, yellow, heat sensitive and basic without any oxidizing influence. All animals including human beings cannot synthesize tocopherols. 60% to 70% of total tocopherols production per year consumed as supplement to decrease/prevent the deficiency related diseases or to increase the value to stabilize the tissues. In recent years, various methods have been published for the measurement of the tocopherol content in vegetable oils.

These techniques include Raman Spectroscopy (Chen *et al.*, 2011). FTIR and NMR (Lu *et al.*, 2018; Man *et al.*, 2005). As abundant consumption of vegetable oils already discussed, the authenticity of oils is also an emerging problem for consumers. A best way of authentication of vegetable oils can be done by determining amount of active chemical substances. Among all these techniques, few techniques are based on measurement of tocopherols in edible oils for their authenticity (Bajoub *et al.*, 2018). In this study, we

amplified a quick and direct method for the detection and quantification of tocopherols (α, γ and δ) in normal olive oil, EVOO and sunflower oil by HPLC-UV.

The aim of this study was to design and authenticate a method for rapid and reliable measurement of tocopherols in vegetable oils and suggest the tocopherol ratio for the authentication of edible oils.

Materials and methods

Reagents and standards

Methanol of analytical reagent grade (purity 99.99%) was purchased from Fisher Chemicals (USA). Ultrapure water was taken from (Purite Naptune, Thames Oxone, UK). All standard compounds (α-T, γ-T and δ-T) were purchased from Sigma Aldrich (UK) and all parameters were checked, α-T has 96% purity on HPLC, γ-T has 99.1% purity and δ-T has 96% purity with yellow colour.

Stock and Standard Solution Preparation

Separate stock solution of each tocopherol (α-T, γ-T and δ-T, respectively) was prepared in methanol. Briefly, 2.5 mg of α-tocopherol in 5 ml of methanol and 1.6 mg of γ-tocopherol, same way δ-tocopherol. Combine stock standard solution was prepared by transferring of 3ml of each tocopherol in 25ml volumetric flask. Concentration of each tocopherol in combine stock standard solution was 60 ppm, 38 ppm and 24 ppm for α-T, γ-T and δ-T.

It was stored at -20 ºC (protected from light). Five combined working standard solutions were prepared with concentration before the sample analysis, on same day. Calibration graphs were generated with linearity of r^2 =0.9887 for α-T, r^2 = 0.9869 for γ-Tand r^2 = 0.9867 for δ-T. Samples of olive oil, extra virgin olive oil and sunflower oil were purchased from market.

Extraction of Tocopherols

Tocopherols were extracted from oils into methanol using 1:3 of oil to methanol (1g of oil in 3ml of methanol) in 5 ml centrifuge tube. The samples were homogenized by vortaxing for 30 seconds and then these tubes were centrifuged at 3000 rpm for five minutes. Samples were then filtered using 0.45 µm pore size syringe to remove any oil or contamination. Whole preparation work was carried in light sensitive room due to heat sensitivity of analytes.

Instrumentation

The Perkin Elmer 200 Series equipment was used in entire analysis with Micro Series-200 binary pump. Detection system was UV Perkin Elmer 200 Series. Data were analysed using Total-Chrome software. A reverse phase column ACE_5 C_{10} Column (250 X 4.6mm) operating at 10°C was used for separation. Analysis was carried out with mobile phase consisting of 98:2 of methanol to ultrapure water.

The flow rate was 1ml/min and injection volume was 5µL and run time was 20 minutes. The effluent was monitored with UV at 292 nm.

Statistical analysis

All the experiments were performed in triplicate, and results are expressed as mean ± standard deviation.

Results and discussion

Method validation

Initially, the run time was kept 10 minutes with flow rate of 1 ml/min but no any detector response was seen so time was kept at 20 minutes with same flow rate (1ml/min) (Fig. 1. a-d).

The increase in flow rate of mobile phase decreases the retention time peaks were overlapping. The Intraday variability is also seen with difference in retention time however peak area were similar, it might be due to column.

The variability of tocopherol standard solution in terms of RSD was 1.94% and below 0.04% in peak area for all analytes (N=6). All analytes were separated at base line but base line signal to noise ratio was very high, LOD of instrument was 10ng/ml. Repeatability of this investigating work was determined by C.V % data is given in (Table-1).

Table 1. Method validation parameters.

Analytes	Slope	Standard linearity range	R^2	Retention time in min	Retention time in min (after 24 hours)	LOD ng/g	Repeatability CV%
α-T	2.8±0.02	12-60	0.988	15.43±0.8	17.5±0.3	10	0.9
γ-T	1.1±0.4	7.68-38.4	0.986	13.40±1.0	14.86±0.85	6	0.8
δ-Tl	1.1±0.3	4.8-24	0.986	11.43±1.6	12.78±0.2	6	0.2

UV chromatogram of α, γ and δ tocopherols mixture shows three peaks at 12.46, 14.88 and 17.44 minutes. Identification of each compound was achieved by comparing the retention time of authentic standard's spectra of each α-T, γ-T and δ-T. The retention time of authentic standard solution was taken from separate stock solution.

Table 1. Tocopherols quantification in different vegetable oils.

Samples	α-T	γ-T	δ-T	Total Tocopherols	α-T/ γ-T
Olive oil	62.2± 2.7	9.9±0.5	1.9±0.14	73.61 ±1.38	6.2
Extravirgin olive oil	39.1±1.5	10.9±0.4	00	50.3 ±0.77	3.5
Sunflower oil	146.6±17.8	9.6±0.5	1.3±0.07	157.6 ±10.11	15.2

Tocopherol analysis

Tocopherols content was calculated using slope regression formula. The results are summarized in (Table 2). α-T and γ-T seems to be verified as major tocopherols in all types of oils. Sunflower oil has higher concentration of α-T compared to olive oil and extra virgin olive oil (157.6, 73.6 and 50.3mg/kg, respectively) with RSD of γ-T appeared to be highest 3% more in EVOO than sunflower oil and olive oil respectively. While δ-T could not be found in EVOO, however in other samples, it was lower than α-T and γ-T. α- Tocopherol from olive oil is similar to data base entries (FSA, 2002). Our results for γ-T were lesser and for δ-T these were significantly higher as in published work in (Alghamdi *et al.*, 2018).

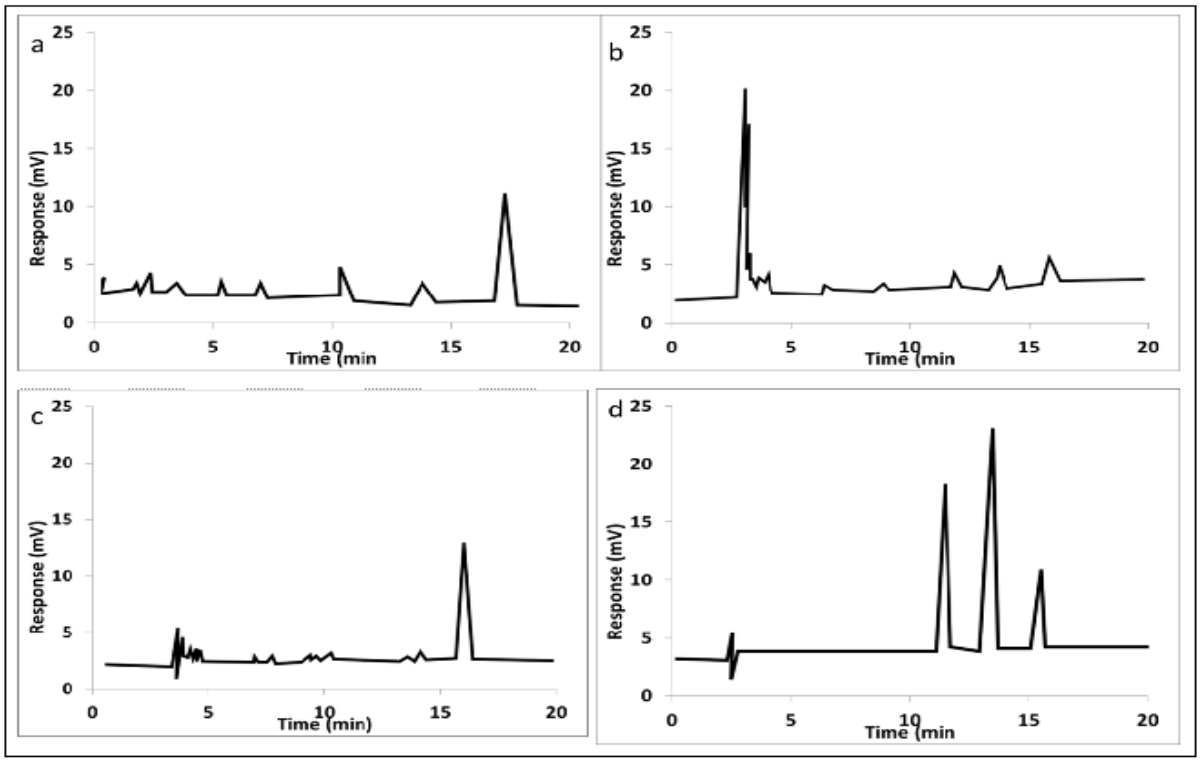

Fig. 1. HPLC chromatograms (a) olive oil, (b) EVOO, (c) Sunflower oil and (d) combined standards of tocopherols.

There are several reason behind this difference, these variations can be seen in with different cultivators such as α-tocopherol content (148 to 265 mg/kg) in vegetable oils has been published with different cultivators (Rodrigues *et al*., 2018). Influence of gene background has also been observed for example, gene *tph1* and *tph2* mutation shown α/γ tocopherol content varied from 40/60 to 60/40% (Havlickova *et al*., 2018) and also agronomic and processing factors have influence in terms of tocopherol content (Miho *et al*., 2018).

α/γ Tocopherol Ratio Assessment for the Authenticity of oils

Olive oil contains δ-T higher in concentration 1.9 mg/kg and sunflower oil contains 1.3 mg/kg. α-T/γ-T ratio for olive oil was 6.2:1, EVOO 3.5:1 and 15.2:1. This can be a good idea to assess the fraudulent addition of other oils into EVOO samples.

It was also found that EVOO contain traces of δ-tocopherol in samples less than six months from harvest (Azadmard-Damirchi *et al*., 2010). For this reason, we can argument that proposed method of α/γ tocopherol ratio in specific oil samples can detect the fraudulent addition of different oil. While δ/ß tocopherol ratio is less sensitive because both have low concentration.

Conclusion

This study presents rapid, sensitive and precise method for the determination of all type of tocopherols in different vegetable oils. In comparison with other analysis methodologies, this method based on good extraction (1:3 dilution of oil in methanol, no saponification) direct injection in C_{18} column and great sensitivity to UV detector for all three tocopherols. It is very straightforward and reduces the undesired sample loses. The tocopherols are light sensitive, proposed method for sample preparation preserve their stability and decrease the quantification error. RP Column has greater stability, good reproducibility for retention time, faster equilibration and shorter analysis time. Furthermore, solvents used in RP-HPLC have less environment effect then solvent used in NP-HPLC. Method described here should be used for routine analysis of vitamin E homologous family in vegetable oils with improved LOD compared to published literature. Moreover, accurate measurement of tocopherols is very essential for food science, for health and wellbeing of society. This method gave a fresh and innovative idea that helps to formulate the α/γ ratio to differentiate the sort of oils and differentiate the various cultivators of same generic source.

References

Alghamdi E, Piletsky S, Piletska E. 2018. Application of the bespoke solid-phase extraction protocol for extraction of physiologically-active compounds from vegetable oils. Talanta **189,** 157-165. https://doi.org/10.1016/j.talanta.2018.06.070

Angeloni P, Echarte MM, Irujo GP, Izquierdo N, Aguirrezábal L. 2017. Fatty acid composition of high oleic sunflower hybrids in a changing environment. Field crops research **202,** 146-157. https://dx.doi.org/10.1016/j.fcr.2016.04.005

Azadmard-Damirchi S, Nemati M, Hesari J, Ansarin M, Fathi-Achachlouei B. 2010. Rapid separating and enrichment of 4, 4′-dimethylsterols of vegetable oils by solid-phase extraction. Journal of the American Oil Chemists' Society **87**, 1155-1159. http://dx.doi.org/10.1007/s11746-010-1595-9

Bajoub A, Bendini A, Fernández-Gutiérrez A, Carrasco-Pancorbo A. 2018. Olive oil authentication: A comparative analysis of regulatory frameworks with especial emphasis on quality and authenticity indices, and recent analytical techniques developed for their assessment. A review. Critical reviews in food science and nutrition **58,** 832-857. http://dx.doi.org/10.1080/10408398.2016.1225666

Bakre SM, Gadmale DK, Toche RB, Gaikwad VB. 2015. Rapid determination of alpha tocopherol in olive oil adulterated with sunflower oil by reversed phase high-performance liquid chromatography. Journal of food science and

Technology **52,** 3093-3098.
http://dx.doi.org/10.1007/s13197-014-1309-7

Barrera-Arellano D, Badan-Ribeiro AP, Serna-Saldivar SO. 2019. Corn Oil: Composition, Processing, and Utilization. In Corn (p 593-613). AACC International Press.
https://doi.org/10.1016/B978-0-12-811971-6.00021-8

Chen H, Angiuli M, Ferrari C, Tombari E, Salvetti G, Bramanti E. 2011. Tocopherol speciation as first screening for the assessment of extra virgin olive oil quality by reversed-phase high-performance liquid chromatography/fluorescence detector. Food chemistry **125,** 1423-1429.
http://dx.doi.org/10.1016/j.foodchem.2010.10.026

Dimery IW, Hong WK, Lee JJ, Guillory-Perez C, Pham F, Fritsche HA and Lippman SM, 1997. Phase I trial of alpha-tocopherol effects on 13-cis-retinoic acid toxicity. Annals of Oncology **8,** 85-89.

Havlickova L, He Z, Wang L, Langer S, Harper AL, Kaur H, Broadley MR, Gegas V, Bancroft I, 2018. Validation of an updated Associative Transcriptomics platform for the polyploid crop species Brassica napus by dissection of the genetic architecture of erucic acid and tocopherol isoform variation in seeds. The Plant Journal **93,** 181-192.
https://doi.org/10.1111/tpj.13767

Lu Y, Li H, Geng Y. 2018. Analysis of the Effects of δ-Tocopherol on RAW264. 7 and K562 Cells Based on 1H NMR Metabonomics. Journal of agricultural and food chemistry **66,** 1039-1046.
https://doi.org/10.1021/acs.jafc.7b04667

Man YC, Ammawath W, Mirghani MES. 2005. Determining α-tocopherol in refined bleached and deodorized palm olein by Fourier transform infrared spectroscopy. Food Chemistry **90**, 323-327.
https://doi.org/10.1016/j.foodchem.2004.05.059

Miho H, Díez CM, Mena-Bravo A, de Medina VS, Moral J, Melliou E, Magiatis P, Rallo L, Barranco D, Priego-Capote F. 2018. Cultivar influence on variability in olive oil phenolic profiles determined through an extensive germplasm survey. Food chemistry **266,** 192-199.
https://doi.org/10.1016/j.foodchem.2018.06.002

Mohammad NA, Anwar F, Mehmood T, Hamid AA, Muhammad K, Saari N. 2018. Phenolic compounds, tocochromanols profile and antioxidant properties of winter melon [Benincasa hispida (Thunb.) Cogn.] seed oils. Journal of Food Measurement and Characterization, 1-9.
https://doi.org/10.1007/s11694-018-0008-x

Nagham Abdlateef Rasheed, Muhammad MF. 2018. The effect of feed additive supplementation in broiler diet on performance and α–tocopherol. International Journal of Biosciences, **13**, 151-160.

Nazzaro F, Fratianni F, Ombra MN, d'Acierno A, Coppola R. 2018. Recovery of biomolecules of high benefit from food waste. Current Opinion in Food Science **22,** 43-54.
https://doi.org/10.1016/j.cofs.2018.01.012

Nian S, Lo AC. 2018. Lutein and the Aging Eye. In Progress in Carotenoid Research. IntechOpen.

Phull AR, Nasir B, ul Haq I, Kim SJ. 2018. Oxidative stress, consequences and ROS mediated cellular signaling in rheumatoid arthritis. Chemico-biological interactions **281**, 121-136.
https://doi.org/10.1016/j.cbi.2017.12.024

Reboul E. 2018. Vitamin E intestinal absorption: Regulation of membrane transport across the enterocyte. IUBMB life.
https://doi.org/10.1002/iub.1955

Rodrigues N, Casal S, Peres AM, Baptista P, Bento A, Martín H, Manzanera MCAS, Pereira JA. 2018. Effect of olive trees density on the quality and composition of olive oil from cv.

Arbequina. Scientia Horticulturae **238,** 222-233. https://doi.org/10.1016/j.scienta.2018.04.059

Statista. 2019. Consumption of vegetable oils worldwide from 2013/14 to 2018/2019, by oil type. In, 2019).

Ueda N, Suzuki Y, Rino Y, Takahashi T, Imada T, Takanashi Y, Kuroiwa Y. 2009. Correlation between neurological dysfunction with vitamin E deficiency and gastrectomy. Journal of the neurological sciences **287,** 216-220. https://doi.org/10.1016/j.jns.2009.07.020