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Extraction and Estimation of Alpha-Tocopherol from Commercially Available Vegetable Cooking Oils by HPLC

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Abstract: Vitamin E is an essential micronutrient required by the body for proper functioning found commonly in vegetable oils and plays very useful role in prevention from degenerative diseases. Alpha-tocopherol is an important antioxidant from the family of Vitamin E. The study was conducted for the extraction and estimation of alpha- tocopherol content in commercially available vegetable cooking oils from the local market of Quetta city. Alpha-tocopherol was determined by Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) equipped with UV-Vis detector. Results showed concentration value of different vegetable oils examined in range of 0.67 µg/L - 97.9 µg/L. Highest concentration of alpha-tocopherol was observed in Eva canola oil and least in Kisan sunflower oil. Thus use of vegetable cooking oils enriched in vitamin E must be encouraged as they are nutritionally valuable and proved to be important sources in overcoming vitamin E deficiency in developed countries like Pakistan.

Key words: Vitamin E · Antioxidant · Edible Oils · RP-HPLC

INTRODUCTION

Vitamin E is a fat soluble vitamin that is responsible for antioxidant activity against oxidative damage that causes many degenerative diseases as heart disease, Alzheimer disease and others [1]. Many functions of vitamin E are related to its antioxidant property. It hinders the non-enzymatic oxidation of unsaturated fatty acids by free radicals as super oxides (O_2^-) and hydrogen peroxide and reactive molecular oxygen [2]. In disease like atherosclerosis, diabetes mellitus, cancer and during aging, increased production of peroxidation products occurs. Vitamin E protects the body against these diseases by inhibiting free radicals and peroxides formation. Talwar *et al*., reported that vitamin E used as a Supplement in diet prevent vascular complications in diabetes by enhancing thromboxane A_2 levels and thereby lowering aggregating of platelets and also increase cell mediated immunity in elderly person of over 60 years [3].

A study by Zeynab *et al*. [4] discovered the antioxidant effects of Vitamin E for Hepato-renal protection. Al- Hayani *et al*. [5] reported that Vitamin E has protective and antioxidant effect against Sodium Flouride in brain, so its dietary intake is recommended. Nekoubin *et al*. [6] carried out a study to determine positive effects of dietary vitamin E on the growth performance, survival rate and reproduction. Jamilah *et al*. [7] reported that Vitamin E is good for males who have infertility problems due to oxidative stress. Roshan *et al*. [8] found that short term Vitamin E supplementation increased some indexes of athletic performance although decreased Lipid-peroxidation.

The best and richest natural source of vitamin E is vegetables oil such as sunflower oil, corn oil and Soya bean oil. Highest concentration of α-tocopherol is present in sunflower oil whereas γ-tocopherol is present predominantly in Soya bean and corn oil while cotton seed oil, rice germ oil and palm oil contains varying concentration of α- and γ-tocopherols. The concentration of α-tocopherol was determined by Goossens and Marion

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and was also found higher in canola oil whereas soybean oil contained higher concentration of δ-tocopherol in this study [9]. Similarly a research in Malysia also showed highest vitamin E content in Sesame blended oils and least in palm olein [1]. Sarmidi reported that palm oil contains free fatty acids, sterols and tocopherols etc, so it is a valuable product [10]. Olive oil is very nutritive and healthy, especially against heart diseases due to presence of phytosterol and tocopherols [11].

Intake of vitamin E is directly linked with consumption of poly unsaturated fatty acids. The requirement for this vitamin increases with increase intake of polyunsaturated fatty acids. The daily recommended value for this vitamin is 10 mg or 15 IU [2]. A study by Jafar *et al.,* in University students living in Amman and Zarqa, Jordan showed that about 90% of these students had low intake of Vitamin E, below two third of the RDA [12].

Crude oil contains higher concentrations of tocopherol isoforms (alpha, beta, gamma) than refined oil because this vitamin is lost during refining process. The primary aim of refining process is to obtain high-quality vegetable oil by eliminating all impurities present in crude oil but many important compounds such as vitamin E are lost during refining due to high temperature. The important step carried out during refining is deodorization which removes undesirable odour and flavors in oil by eliminating free fatty acid aldehydes, unsaturated hydrocarbons and ketones but due to high temperature involved in it, thermal degradation of vegetable oil may occur resulting in tocopherols lost [13]. It has been reported by Safinaz *et al*. [14] that during production of vegetable edible oils, a by-product (distillate) is made in refining steps which contains large amount of tocopherols and sterols.

The antioxidant family of vitamin E is lost during refining and storage of oils, as proofed by a study that was aimed to determine fatty acids and tocopherols content in eight refined and ten cold pressed commercially available vegetable oils from different manufacturers in Poland showed that highest vitamin E concentration was found in sunflower oil and the amount of tocopherol varied much between oil types and between same types of oils from different manufacturers [15]. Another study on Dabai pulp and kernel oils of *Canarium odontophyllum* also compared commercially available olive and palm oil by chromatographic techniques which showed only traces of Vitamin E in Dabai pulp oil while the palm oil had very high concentration of Vitamin E [16].

This present aim of this study is extraction and determination of α-tocopherol from vegetable oils available in the local market of Quetta in order to compare amount of α-tocopherol present in different vegetable oils available in Quetta.

MATERIAL and METHODS

Standards Preparation: A stock solution of α-tocopherol was prepared by weighing accurately 100 mg α-tocopherol in 50 mL pre-weighted volumetric flask and diluting it with 50mL of ethanol. Five standard solutions were further prepared by making concentrations 43.07, 86.14, 129.21, 172.28 and 215.36 ppm by diluting 215.4 µL, 430.8 µL, 646 µL, 861 µL and 1076.9 µL of α-tocopherol stock solution with 10 mL of ethanol in respective volumetric flask [9].

Sample Preparation: Twelve different brands of vegetable oils were purchased from the local retail stores in Quetta, Pakistan as shown in Table 1. α-tocopherol, an isomer of vitamin E found in vegetable oils was directly extracted into methanol from vegetable oils. α-tocopherol was extracted in 2 mL microcentrifuge tube from different oils into methanol by 1:3 ratio. Samples were vortexed for 5-6 minutes and then centrifuged for 5 minutes at 3000 rpm [9]. The entire samples were covered with aluminum foil and stored in the refrigerator until analysis.

Instrumentation: Shimadzu Reverse phase High Performance Liquid Chromatography (RP-HPLC) equipped with UV-Vis spectrophotometer was used for identification and quantification of α-tocopherol concentration in extracts of vegetable oils. Sonicated methanol was used as mobile phase at a flow rate of 1mL/min. α-tocopherol was detected at 292 nm. Retention time was 5 minutes for each analysis. Individual chromatograms were obtained and peaks were identified by comparing retention times of the oil samples with α-tocopherol standard solutions [15]. Data on peak area,

Table 1: Peak area of HPLC chromatograms of standard solution of Alpha-tocopherol.

S/No.	Concentration (µg/L)	Peak area (m^3)	Retention time (min)
1	43.07	36022	2.760
2	86.14	430418	2.872
3	129.21	700903	2.915
4	172.28	943298	2.988
5	215.36	1174710	3.088

peak height, retention time and concentration of individual oil samples were collected and processed by computer integrator software.

RESULTS

Twelve different brands of vegetable cooking oils were collected (Table 3) and estimated for α-tocopherol concentration by RP-HPLC and quantified by UV-Vis spectrophtometric detector. Table 2 represents different concentrations of standard α-tocopherol determined by HPLC and represented in the calibration curve (Figure 1). The results in Table 3 showed that highest concentration of α-tocopherol was found in Eva cooking oil and least in Kisan sunflower oil. Chromatographic peaks of alpha tocopherol appeared in the samples of oils at 2.00 to 3.2 min and were compared with the peak areas of α-tocopherol standards. The cooking oils were observed to contain α-tocopherol concentration in the decreasing order as follow (Table 3): Eva canola oil 97.9 µg/L, Paris soybean oil 93 µg/L, Maryam cooking oil 85 µg/L, Habib cooking oil 32.36 µg/L, Kainat cooking oil 31 µg/L, Soghat cooking oil 19.94 µg/L, Canola vegetable oil 16 µg/L, Dalda cooking oil 11.45 µg/L, Virgine olive oil 9.68 µg/L, Asal cooking oil 7.6 µg/L, Canola cooking oil 5 µg/L and Kisan Sunflower oil 0.67 µg/L of α-tocopherol.

DISCUSSION

Vitamin E is required with increase intake of polyunsaturated fatty acids. To meet the Daily Recommended Value of 10 mg/day of Vitamin E [2], we need to include it daily in our diet. In a study by Jafar *et al*. [12] among university students it was found that about 90% of them had low intake of Vitamin E, below two thirds of the RDA.

Table 2: HPLC determined concentration of α-tocopherol in different vegetable cooking oils.

Ser No.	Samples Collected	Samples ID	Retention Time (mins)	Peak Area (m3)	Height of Peak mV	Concentration (µg/L)
1	Kainat cooking oil	S1	2.994	15235	1497	31.0488
2	Paris soybean oil	S2	2.875	303420	45569	93.1764
3	Canola cooking oil	S3	2.866	4161	528	5.0829
4	Kisan sunflower oil	S4	2.737	1276	183	0.6767
5	Canola vegetable oil	S5	3.192	9691	1168	16.1074
6	Soghat cooking oil	S6	3.157	41144	2713	19.9410
7	Asal cooking oil	S7	2.707	23679	1801	7.6385
8	Maryam cooking oil	S8	2.862	206770	31390	85.0289
9	Eva canola oil	S9	3.171	6952060	292412	97.9007
10	Habib cooking oil	S10	3.130	51606	2173	32.3683
11	Dalda cooking oil	S11	3.181	22995	1175	11.4555
12	Virgin olive oil	S12	3.181	26082	2532	9.6887

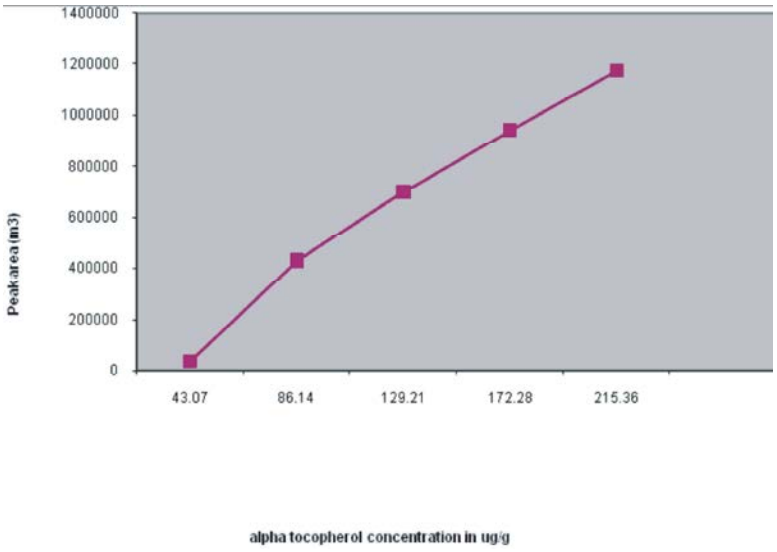

Fig 1: Calibration curve for different standard concentrations of alpha- tocopherol

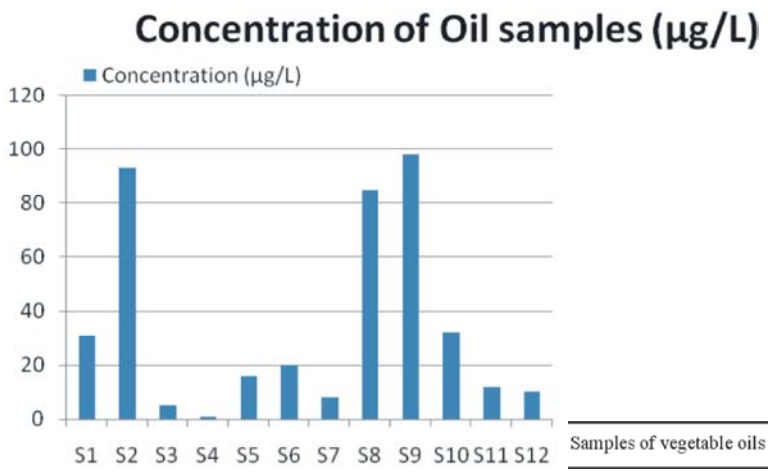

Fig 2: Concentration of 12 samples of vegetables oils collected from market of Quetta

Highest concentration of α-tocopherol was found in Eva canola oil (97.9µg/L) and Paris soybean oil (93µg/L) as represented in Figure 2, which showed that they are rich source of antioxidant vitamin E. It was found previously that the richest source of α-tocpherol were sunflower oil and soybean oil [3]. While Canola oil and Palm oil contained varying amount of α- and γ-tocopherols. The results of conducted study has shown that soybean oil contained α-tocopherol amount which is in accordance with literature values but after analysis concentration of α-tocopherol showed by Kisan sunflower oil did not agreed with literature values. The reason for that expectation might be improper storage or inappropriate refining [13].

Cooking oils are major source of Vitamin E in our daily diet. Commonly crude oils contain higher amounts of vitamin E than the refined oils as this vitamin is lost during refining process especially in the step of "Thermal Degradation" s[13]. It was also reported by Safinaz *et al* that while manufacturing cooking oils, in refining steps a by-product (distillate) contained large amounts of Vitamin E and sterols [14].

Alpha tocopherols are often lost in stored commercial oil due to improper storage [15]. In commercially available cooking oils, manufacturing parameters were applied in the makeup procedure for cooking oils. Storage in high intensity of light is responsible for significant loss of α-tocopherol. The amount of α-tocopherol printed on oil packets might be different from their actual concentration due to excessive exposure to light, storage in sunlight, high temperature and moist atmosphere. Alpha-tocopherols concentration in refined cooking oil was different from that of crude oil because significant loss might occurred during refining especially during deodorization, part of the tocopherol loss was a result of distillation or as a result of thermal degradation [4]. Major factor that caused loss during distillation was pressure and temperature. Thus α-tocopherol value found in refined oil is different from respective crude oil due to inappropriate high temperature involved during refining.

Alpha tocopherol is sensitive to light and alkaline conditions. Long term exposure to light resulted in loss of considerable amount of α-tocopherol from tested oils [15]. So for proper quantification of α-tocopherol in sample oils, condition such as exposure to light and would be minimum and appropriate preserving α-tocopherol extracted oils in dark but would help in the attainment of proper and suitable conditions. Although α-tocopherol is the predominant isomer of vitamin E found to occur in vegetable oils but minutes amount of others isomers are also present sometimes.

In commercially available cooking oils, in this study although it was mentioned that oils had additional amount of vitamin E but these oils were found to be low in tocopherol concentration and that amount was not detected by HPLC. The results of this study showed that the highest amount of α-tocopherol was detected in Eva canola oil (97.9 µg/L) and the lowest concentration was detected in Kisan Sunflower oil (0.67 µg/L). Therefore from nutritional point of view it can be inferred that Eva cooking oil, Paris soybean oil and Maryam cooking oil have sufficient amount of α-tocopherol amount to fulfill the Daily Recommended amount of Vitamin E. Although other cooking oils like Habib cooking oil, Soghat cooking oil, Dalda cooking oil, Virgine olive oil, Asal cooking oil, Canola cooking oil and Kisan sunflower cooking oil are low in α-tocopherol concentration but this significant decrease might be due to refining, temperature,

saponification in alkaline medium and exposure to high intensity light. As Dalda cooking Oil, Virgine olive oil and Canola cooking oil are the most popular cooking oils in Pakistan, they had significant amount of α-tocopherol, but if it is comparatively less that is because they are well refined cooking oils.

CONCLUSION

The study shows that RP-HPLC is a simple and reliable method for determination of tocopherols in various cooking oils usually available in Pakistan. The result of this study showed that from nutritional point of view, Eva cooking oil, Paris soybean oil and Maryam cooking oil have a beneficial effect on health as they contain α-tocopherol amount sufficient enough to fulfill daily requirement. Significant decrease in α-tocopherol level occurs due to refining, temperature, saponification in alkaline medium and exposure to high intensity light.

Recommendation: It is usually recommended to include cooking oil in daily diet which contains sufficient quality of vitamin E. This vitamin plays important role as antioxidant and prevent damage caused by oxidation as well as formation of free radicals which are the main cause of degenerative disease. Commercial cooking oils should be properly stored under condition required for maintenance and preservation of antioxidant levels. It is therefore suggested that best cooking oils that contain good quality of tocopherol are Eva cooking oil and Paris soybean oil available in Pakistan. These can be used in diet to accomplish the sufficient daily requirement of Vitamin E.

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