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Changes of Total Tocopherol and Tocopherol Species During Sunflower Oil Processing

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Abstract The changes of total and individual tocopherols were investigated during different sunflower oil processing stages by reverse-phase high-performance liquid chromatography. The results revealed that the levels of total and individual tocopherol content were decreased during the neutralization, bleaching, and deodorization processes. The overall loss of total tocopherols during these stages was found to be 37.9%, although the general reduction trend of delta (δ), gamma (γ), and alpha (α) tocopherols is very similar during neutralization (35.3%), bleaching (38.3%), and deodorization (37.8%). However, in contrast to the neutralizing and deodorizing stages, the bleaching process caused relatively less reduction for individual tocopherol contents. Deodorizer distillates were also analyzed and were found to be rich with tocopherols content (29,348.24 μg/ml). The results of the study indicated that most parts of the tocopherols are wasted during processing. Therefore, the proper concentration of nutritionists, industrialists, and manufacturers is needed for the necessary improvements in processing technology to avoid the major loss of tocopherols and to increase the shelf life, as well as the nutritive value of processed oil.

Keywords Sunflower oil · Tocopherols · Refining stages · Reverse-phase HPLC

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Introduction

Vegetable oils extracted from seeds are in the crude state and are inedible, except for olive oil. In modern society, the consumers do not like the use of crude oils directly without proper processing due to the unacceptable color and odor. Generally, crude oils contain many unwanted matter, such as free fatty acids, color pigments, metals, gums, waxes, phosphatides, and odoriferous materials, which must be removed to yield a stable product with a bland or pleasant taste. Therefore, efficient industrial processing involves removing these unpleasant impurities with the least possible effect on the desired components and the least possible loss of neutral oil [1].

The processing involves a series of purifying steps, which may be chemical (caustic refining) or physical (bleaching, deodorization) [2]. The principal difference between the two procedures is the removal of free fatty acids, which could be either performed chemically (caustic/alkali neutralization) or physically (steam distillation). Other steps involved in both types of refining are common. Therefore, chemical refining includes neutralization, bleaching, and, finally, deodorization as separate processes, while physical refining includes only bleaching and deodorization processes [3]. This means that, in physical refining, the neutralization is also carried out at the deodorization stage. Tocopherols (α, γ, and δ) are potent natural antioxidants that prevent the rancidity of oils during storage and, thus, increase the shelf life of edible oils [4]. Additionally, tocopherols have an important role in the prevention of many types of diseases (such as Parkinson's disease, ataxia with vitamin E deficiency, and various cancers, etc.). Also, they enhance the body's immune system and reduce cellular aging [5]. Among the tocopherols, α-tocopherol exhibits the

maximum vitamin E activity (in vivo), but its in vitro activity is relatively low [6]. On the other hand, γ-tocopherol is a more effective free radical scavenger than α-tocopherol in vitro [7]. These astonishing properties have made the tocopherols an incredibly essential nutrient and which must be present in edible oil in a significant amount. But, unfortunately, tocopherols are decreased during each refining process and are markedly reduced during the deodorization stage of industrial processing [8]. However, these are recovered in the deodorizer distillate as by-products. The deodorizer distillate is a mixture of the valuable components obtained during the deodorization stage and is considered to be the most concentrated source of tocopherols [9]. The assessment of tocopherol level in deodorizer distillate is of prime importance due to its possible cosmetic and pharmaceutical applications [10]. There are around 150 edible oil processing plants installed in Pakistan, producing 2.71 million tons per annum of edible oil, with approximately 25,000 tons per annum of deodorizer distillate [11]. The consequences of refining processes on the changes of total and individual tocopherol contents in various types of oil has rarely been investigated. In the literature, only limited data are available on the losses of valuable tocopherols during the different processing stages of palm oil [12], sunflower oil [2], rice bran oil [13], corn, soybean, and rapeseed oil [8]. Although, Tasan and Demirci [2] have reported on the effects of individual processing stages on the tocopherols content of sunflower oil, the data on the content of tocopherol in deodorizer distillate is missing in their study. Also, in Pakistan, no such study has been carried out. Furthermore, each industry has its own design and parameters for each step of processing. Therefore, the objective of the present study was to investigate the loss of valuable tocopherols during different refining stages of sunflower oil processing, to compare the results with the reported values, and to check the recovery of tocopherols in deodorizer distillate.

Materials and Methods

Sunflower Oil Samples

Crude, neutralized, bleached, and deodorized sunflower oils processed from the same batch in addition to deodorizer distillates were obtained from a commercial refinery located in Karachi, Pakistan. Samples were obtained three times over a period of 3 months. Amber-colored glass bottles were purged with nitrogen gas after filling with each sample in order to avoid oxidation and stored at −4 °C until they were analyzed.

Standards and Chemicals

A tocopherol kit consisting of α, γ, and δ tocopherol (purity >95%) was purchased from Sigma–Aldrich and used as the reference standard for the separation, identification, and quantitative analysis of individual tocopherols present in the different refining stages. High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were purchased from Fisher Scientific UK Ltd.

HPLC Analysis

All HPLC analyses of tocopherols (vitamin E) were performed at ambient temperature on a Hitachi high-performance liquid chromatograph (model 6200 Hitachi, Ltd., Tokyo, Japan) equipped with a Hitachi L6200 intelligent pump, a 20-μl injection loop, and a Hitachi F1050 fluorescence detector controlled by HP ChemStations software (Hewlett-Packard, Palo Alto, CA, USA), along with a Kromasil-100 C18 column (25 × 0.46 cm, I.D., 5 μm particle size, Teknokroma, Barcelona, Spain). A mixture of methanol–acetonitrile (1:1 v/v) at a flow-rate of 1.0 ml/min (isocratically) was used as a mobile phase. The fluorescence signal was measured at λ_{295} nm excitation and λ_{325} nm for emission wavelengths, as per the previously reported procedure [14].

Samples of crude, neutralized, bleached, and deodorized sunflower oils were prepared by dissolving 0.04 g of each in a 2 ml mixture of methanol–acetonitrile (30:70 v/v). The mixture of 30:70 v/v was chosen for the best solubility of tocopherol. Each sample was centrifuged for 30 s and the supernatant (10 μl) was injected into the HPLC column. By the same procedure, deodorizer distillate samples were prepared by taking 0.0025 g of distillate. A lesser amount of deodorizer distillate was taken due to the greater content of tocopherols. All samples were prepared carefully in an amber vial and purged with nitrogen to avoid air and light exposure until analysis.

Standards Preparation for Calibration

All of the standards and samples were run in triplicate. The stock solutions of α, γ, and δ-tocopherol were prepared by dissolving 0.001 g of each tocopherol in 10 ml of methanol–acetonitrile (30:70 v/v) in amber vials, giving a final concentration of 100 μg/ml. From the individual stock solution, a series of calibration standards for α-tocopherol (5–50 μg/ml), γ tocopherol (1–50 μg/ml), and δ-tocopherol (0.04–20 μg/ml) were prepared and stored at −4 °C. For the determination of individual tocopherols, the calibration was achieved by plotting the mean peak heights of standards versus their known concentrations, and the slope of the standard curve was calculated using the method of least

squares due to the linear relationship. The regression equations acquired from three different plots were used to calculate the amount of α, γ, and δ-tocopherols present in each sample.

Statistical Analysis

The results of all of the analyzed samples were expressed as mean values with standard deviations. The significant differences between the means of all of the analyzed results were obtained from Tukey's test at a *P*-value <0.05 associated with the one-way analysis of variance (ANOVA) using SPSS 16.0 for Windows.

Results and Discussion

HPLC Analytical Characteristics

After optimization of the HPLC conditions, peaks were observed at 14.37 min for δ tocopherol, 16.12 min for γ tocopherol, and 18.5 min for α tocopherol. Figure 1 shows the chromatogram of a standard mixture of δ, γ, and α tocopherols (10 μg/ml), which illustrates good separation of individual tocopherols within a reasonable time period.

The calibration plots of δ, γ, and α tocopherol standards in the methanol–acetonitrile (30:70 v/v) solvent system are shown in Fig. 2. Linearity of the calibration curve was in the range of concentration 0.04–20 μg/ml for δ-tocopherol, 1–50 μg/ml for γ tocopherol, and (5–50 μg/ml) for α-tocopherol. The coefficient of determination for all of the standard curves exceeded 0.995, as shown in the individual calibration plots of each standard.

In the present study, the extraction and saponification steps were totally avoided, and only a very small amount of sample (0.002–0.04 g) was diluted in 2 ml of the methanol–acetonitrile (30:70 v/v) solvent system. The chromatograms in Fig. 3 illustrate the excellent and reproducible elution profiles of the three isomers (δ, γ and α) of tocopherol from samples obtained at different stages of the sunflower oil refining process (neutralization, bleaching, and deodorization).

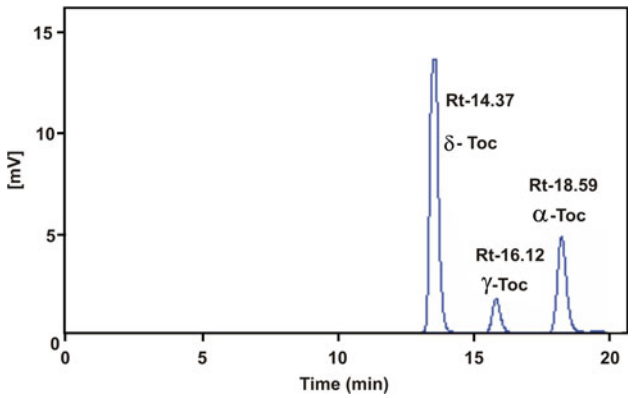

Fig. 1 High-performance liquid chromatography (HPLC) chromatogram of a standard mixture of δ, γ, and α tocopherols

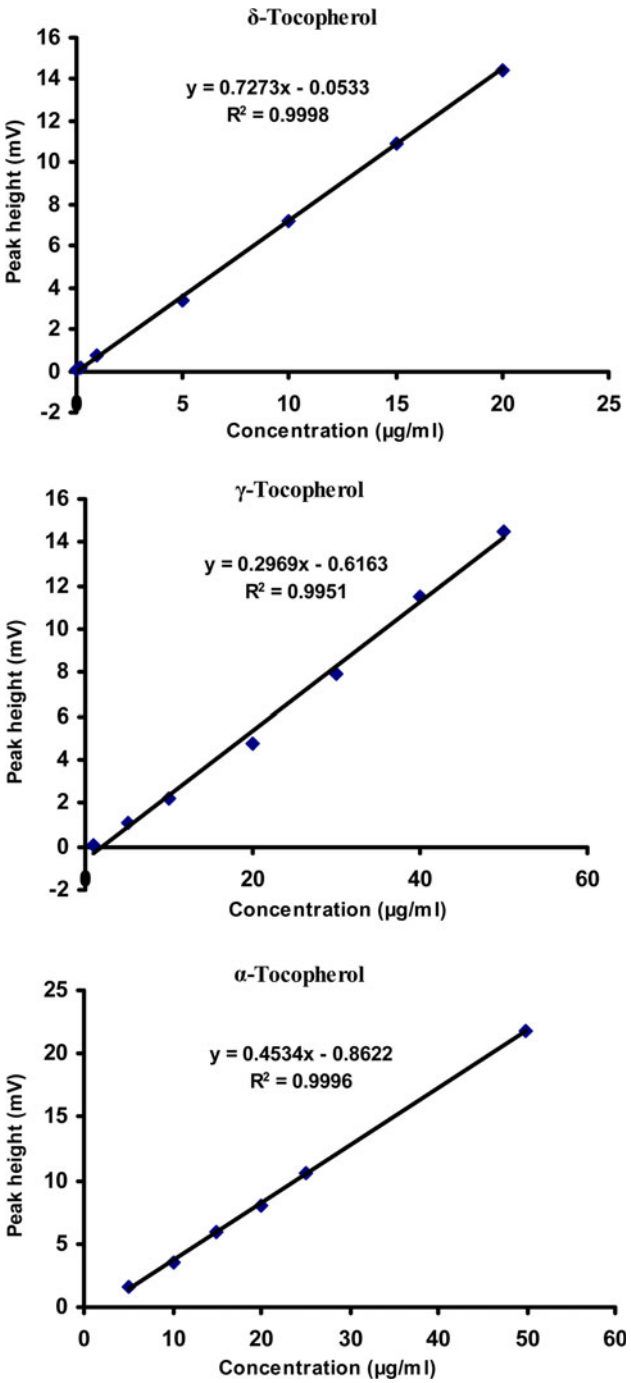

Fig. 2 Calibration plots of δ, γ, and α tocopherol standards

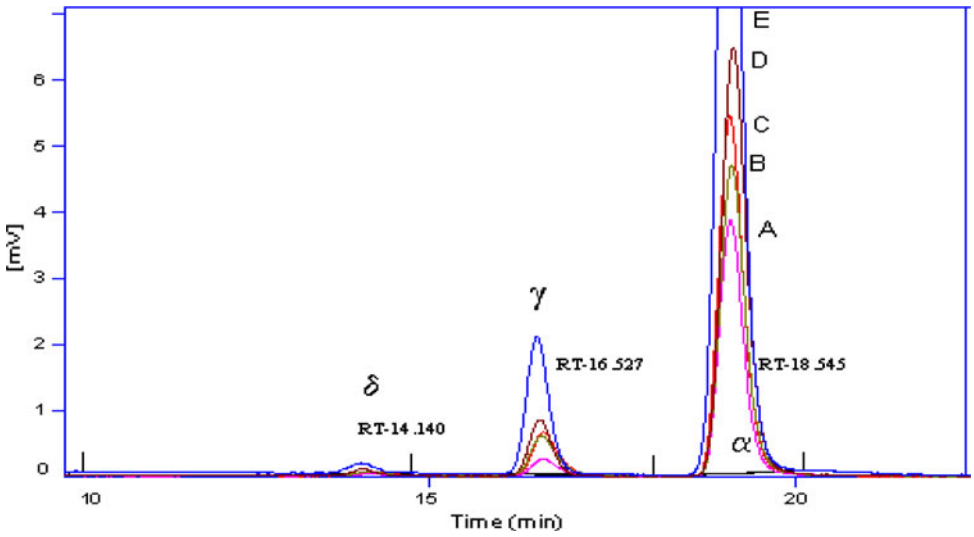

Fig. 3 Typical reverse-phase (RP)-HPLC chromatograms of **a** deodorized, **b** bleached, **c** neutralized, **d** crude, and **e** deodorizer distillate of sunflower oil samples. The optimized conditions were: mobile-phase methanol–acetonitrile (1:1) on a Kromasil-100 C18 column with fluorescence detection

Quantification of Tocopherols in Sunflower Oil

Table 1 shows the level of δ, γ, and α tocopherol (μg/ml) contents in sunflower oil samples taken from the neutralization, bleaching, and deodorization stages compared to the reported values [2]. All values are an average of three determinations with low standard error (<4%). The results achieved from three different refining stages showed that the total tocopherol content was significantly ($P < 0.001$) higher in the crude oil sample (1,095.90 ± 21.60 μg/ml) compared to the literature value (749.4 ± 8.0) [2]. The level continuously decreased in neutralized (917.22 ± 3.87 μg/ml), bleached (851.77 ± 6.20 μg/ml), and deodorized (680.02 ± 16.93 μg/ml) oil. Table 1 illustrates that the total and individual tocopherols were significantly decreased during each stage of the refining process ($P < 0.001$). In the present study, no β-tocopherol was observed in any sample, while in the reported study [2], no δ-tocopherol was detected, which may be due to the different varieties of sunflower oil used and the diverse environmental conditions. The decrease in the total tocopherol level during the caustic neutralization is in agreement with the results obtained by the reported study, according to which alkali treatment affected the tocopherol content of oils [2]. A considerable decline of δ, γ, and α tocopherol content was observed at each refining stage ($P < 0.001$). The processing loss of the total and individual tocopherols during deodorization was also confirmed with their high level found in the deodorizer distillate sample, i.e., 29,348.24 ± 220.55 μg/ml.

The overall losses of tocopherol content in each refining stage are summarized in Table 2. The reduction of the total tocopherol content during the neutralization, bleaching, and deodorization processes was confined to 43.6%, which is significantly higher than that reported (30.2%) by Tasan et al. [2]. Caustic refining resulted in the maximum reduction of α (17.4%), followed by δ (16.9%) and γ tocopherols (12.5%). The decline of tocopherols may be due to the fact that tocopherols are unstable in the presence of longer contact time with air and alkali [2]. In the present

Table 1 α, γ, and δ tocopherol (μg/ml) content in a sunflower oil sample from different refining stages with the results of the analysis of variance (ANOVA)

Sample name	α-Tocopherol (μg/ml)	γ-Tocopherol (μg/ml)	δ-Tocopherol (μg/ml)	Total tocopherol (μg/ml)
Crude oil	835.14 ± 21.80^{a}	250.41 ± 3.70^{a}	10.50 ± 0.90^{a}	1,095.90 ± 21.60^{a}
Neutralized oil	689.44 ± 2.60^{b}	219.10 ± 1.10^{b}	8.68 ± 0.31^{ab}	917.22 ± 3.87^{b}
Bleached oil	625.96 ± 8.95^{b}	217.74 ± 2.75^{b}	8.06 ± 0.24^{bc}	851.77 ± 6.20^{b}
Deodorized oil	519.06 ± 16.16^{c}	154.20 ± 1.76^{c}	6.76 ± 0.20^{c}	680.02 ± 16.93^{c}
F statistics at *df* 3	52.279	66.663	14.470	92.105
Significance at 5% level	0.000	0.000	0.001	0.000
Deodorizer distillate	21,847.15 ± 192.70	7,248.14 ± 17.05	252.95 ± 16.29	29,348.24 ± 220.55

The tocopherol values represent the mean of three replicates with standard deviations (SD). Means followed by different superscripts in the same column differ significantly (Tukey's HSD test at $P < 0.05$)

df degrees of freedom

Table 2 Losses (%) of individual and total tocopherol at different stages of refining with the results of ANOVA

Refining stages	α-Tocopherol (%)	γ-Tocopherol (%)	δ-Tocopherol (%)	Total tocopherols loss (%) in each stage
Neutralization (caustic)	17.44[b]	12.51[b]	16.89[ab]	16.30[b]
Bleaching	9.21[b]	0.61[c]	7.13[b]	7.14[c]
Deodorization (steam)	17.08[b]	29.18[a]	16.20[ab]	20.16[b]
Total (%) loss of individual tocopherol	37.84[a]	38.42[a]	35.31[a]	43.60[a]
F statistics at *df* 3	18.499	65.974	7.450	47.414
Significant at 5% level	0.001	0.000	0.001	0.000

Means followed by different superscripts in the same column differ significantly (Tukey's HSD test at $P < 0.05$)

df degrees of freedom

study, bleaching shows much lower impact than neutralization and deodorization on the decrease of δ, γ, and α-tocopherol content, which is very clear from the results shown in Table 2. The deodorization caused the utmost reduction of γ-tocopherols (29.2%). The minimum loss of total γ-tocopherols was observed (0.6%) during the bleaching stage. During bleaching, the total tocopherols concentration was slightly reduced (7.1%), due to their possible adsorption on bleaching clay [15]. It was observed that the deodorization stage caused the greatest overall reduction of total tocopherol content (20.16%) compared to the reported value (11.0%), whereas Tasan et al. quoted that the greatest overall reduction of total tocopherol content (14.7%) was determined in the neutralization process. Therefore, our result (16.30%) is slightly different. However, significant differences in the total tocopherol content was observed at the neutralization and deodorization processes ($P < 0.001$) compared to bleaching.

The loss of tocopherol during deodorization may be due to the thermal degradation at high temperature (>240 °C) by oxidation reaction or by chemical reaction, such as the formation of tocopherol esters [16]. During deodorization, all tocopherols present in the incoming bleached oil will be distributed either in deodorized oil or in deodorizer distillate. The results showed (Table 1) that the maximum amount goes into deodorizer distillate rather than in deodorized oil. Sunflower oil is the richest in α-tocopherol content [17] and from the results, it is clear that the highest amount of α-tocopherols are wasted into the deodorizer distillate. From a nutritional point of view, α-tocopherol exhibits the maximum vitamin E activity and it is the most potent antioxidant in vivo than compared to other isomers [18]. Generally, in the industries, synthetic antioxidants are added to enhance the shelf life of the edible oils. Numerous studies have reported that these synthetic antioxidants are toxic to human health and result in carcinogenesis and liver damage [19]. Comparatively, natural antioxidants (vitamin E) are safer and are considered to be better than the synthetic antioxidants [20]. Therefore, consumers demand natural and healthier products, which leads to increased beneficial positive heath effects [21]. Therefore, it is essential to avoid the reduction of natural antioxidants, i.e., vitamin E, by improving processing practices to enhance the nutritive value and shelf life of the oil.

Conclusion

The present study concluded that each refining stage was found to be responsible for the loss of total and individual tocopherols. The variation of the results when compared to the already reported study indicated that the loss of total and individual tocopherol may depends on the tocopherol distribution of sunflower oil, different operating parameters, and different designs of the processes. Therefore, there is a strong need to improve the processing technology in order to reduce the loss of tocopherols to as little as possible and to obtain better nutritive values as well as longer shelf lives of the finished edible oils.

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