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Tocols and Food Quality

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Research Article

Discrimination of Geographical Origin of Unroasted Kernels Argan Oil (*Argania spinosa* (L.) Skeels) Using Tocopherols and Chemometrics

Sara Elgadi,[1,2] **Ahmed Ouhammou**,[1] **Hamza Zine**,[1] **Nadia Maata**,[3] **Abderrahmane Aitlhaj**,[4] **Hassan El Allali**,[5] **and Abderraouf El Antari**[2]

[1]*Laboratory of Microbial Biotechnology, Agrosciences and Environment, Regional Herbarium 'MARK', Faculty of Sciences-Semlalia, Cadi Ayyad University, BP. 2390. 40 000, Marrakech, Morocco*
[2]*Laboratory of Agro. Food Technology and Quality, Regional Center for Agronomic Research of Marrakech, National Institute of Agronomic Research (INRA), Marrakech, Morocco*
[3]*Official Laboratory for Chemical Analysis and Research (LOARC), Casablanca, Morocco*
[4]*National Agency for the Development of the Oasis and Argan Zones (ANDZOA), Agadir 80000, Morocco*
[5]*The Interprofessional Federation of the Argan Sector, Agadir 80000, Morocco*

Correspondence should be addressed to Sara Elgadi; sara.elgadi@ced.uca.ma

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Valorisation of Argan oil requires the precise identification of different provenances markers. The concentration of tocopherol is regarded as one of the essential parameters that certifies the quality and purity of Argan oil. In this study, 39 Argan samples from six different geographical origins (Safi, Essaouira, Agadir, Taroudant, Tiznit, and Sidi Ifni) from the central west of Morocco were collected and extracted using cold pressing. The total tocopherol amount was found to range from 783.23 to 1,271.68 mg/kg. Generally, γ-tocopherol has the highest concentration in Argan oil. It should also be noted that the geographical origin was found to have a strong effect on the amounts of all tocopherol homologues studied. Principal component analysis of tocopherol concentrations highlighted a significant difference between the different provenances. The content of tocopherol has also been found to be strongly influenced by the distance from the coast and altitude, whereas no significant effect was found regarding other ecological parameters. The prediction ability of the LDA models was 87.2%. The highest correct classification was revealed in coastal provenances (100%), and the lowest values were from the continental ones (71.4%). These results provide the basis for determining the geographical origins of Argan oil production with well-defined characteristics to increase the product's value and the income of local populations. In addition, this study provides a very promising basis for developing Argan varieties with a high content of tocopherol homologues, as well as contributing to the traceability and protection of Argan oil's geographical indication.

1. Introduction

Argan (*Argania spinosa* (L.) Skeels) is an endemic plant that represents the only species of genus *Argania* and the family of Sapotaceae in North Africa. Currently, the Moroccan Argan forest spans the fertile Souss Valley, the Anti-Atlas mountain range, and the coastal regions between Safi and Agadir [1]. This area exhibits high plant diversity and endemism [2]. The Argan forest was recognised in 1998 as a UNESCO biosphere reserve (Man and the Biosphere Reserve) [3], and it significantly contributes to the economic and social development in Morocco and sometimes even represents the only source of income for the local population [4]. Argan kernels provide a precious oil that is rich in antioxidant compounds, such as saponins and tocopherols [5], as well as fatty acids [6] and sterols [7].

Vitamin E is a vitamin of eight isomers: four tocopherols and four tocotrienols [8]. Tocopherols are the isomers with the greatest biological activity [9]. For example, α-tocopherol (5,7,8-trimethyltocol) is effective against ischemic liver

cell damage thanks to its free radical scavenging properties [10]. Moreover, γ-tocopherol (7,8-dimethyltocol) has been found to have an effect on various types of tumours, even more powerful than α-tocopherol, β-tocopherol (5,8-dimethyltocol), and δ-tocopherol (8-methyltocol) [11]. Tocopherols and tocotrienols are present in fruits and plant seeds [12]. Therefore, fixed oils are a major source of tocopherols [13]. Several studies have compared tocopherol levels in Argan oil and other oils such as olive oil and prickly pear seed oil [14], The results showed that Argan oil has very high concentrations of tocopherols, especially the γ-tocopherol homologue [15]. In general, the presence of tocols prevents lipid oxidation and, hence, maintains the quality and shelf life of oils [16].

Argan oil plays a potential role in the prevention of several diseases [17], including cancer [18], and has lipid-lowering and anti-oxidant properties [19]. These pharmacological properties can be attributed to its high content of tocopherols, especially γ-tocopherol [8]. Argan oil is richer in linoleic acid than olive oil (5.4%–13.2% compared to 32.3%–34.1%, respectively) [6]. The total tocopherol content is considered a purity criterion by the Moroccan 08.5.090 standard for Argan oil, with an established quantity that should fluctuate between 600 and 900 mg/kg of oil [20]. Gharby et al. [21] reported that the tocopherol content ranges between 675 and 871 mg/kg of oil, but a higher range (687.4–1,068 mg/kg of oil) has been observed by Aithammou et al. [22]. This variability in tocopherol concentration can be attributed to many factors, such as the climate [23], variety [24], extraction method [25], storage conditions [26], fruits form [27], and fruits maturity [28].

The genotype has an important impact on oil yield and composition [29]. In the case of apricots (*Prunus armeniaca* L.), the genetic factor influences the composition of tocopherol homologues [29]. Furthermore, the variability of tocopherol composition in various seed oils recovered from the by-products of the apple industry has been attributed to cultivars [30]. Using SRAP and REMAP markers, strong genetic differentiation has been found within Argan populations [31]. *A. spinosa* forests have been considered as "climax" [32]. This state of equilibrium has been reached by spontaneous vegetation under the action of the natural environment, excluding direct or indirect human action [33].

The consumption of cold-pressed oils has increased in recent years [34]. The global Argan oil market is expected to grow at a revenue-based compound annual growth rate of 10.8% between 2020 and 2027 [35]. Such development requires more control to protect the consumer and the producer from fraud. In fact, several marketing and promotional strategies aimed to relate food products to their geographical origin. European Union legislation, for example, allows the reservation of geographical designations for food products, such as Protected Designations of Origin (PDO) and protected geographical indications (PGI) [36]. The combination of chemical composition and chemometric tools such as linear discriminant analysis (LDA) [37] and partial least squares discriminant analysis (PLS-DA) [23] has been used for determining authenticity and quality control of Argan oil. However, there is a lack of studies focussing on the relationship between the tocopherol concentration in Argan oil and geographical origin [22].

The aim of this study was to determine the tocopherols concentration from six Moroccan provinces: Safi, Essaouira, Agadir Ida Outanane, Taroudant, Tiznit, and Sidi Ifni, associated with the chemometric technique LDA, to classify Argan oil according to its geographical origin. This may constitute the basis for geographical origin certification that helps protect the consumer and the producer from fraud and increase the value of Argan oil in the world market. In addition, it can provide a database for updating the Moroccan standard on Argan oil.

2. Materials and Methods

2.1. Plant Material. Argan plants naturally reproduce by seeding, which is the very reason for their great diversity. However, this study focussed on the potential for industrial production representative of a specific environment, very close to that intended for industrial production by local women's cooperatives. To this end, a maximum of trees contributed to the constitution of our batches of samples. Therefore, Argan fruits from natural populations were collected at full maturity from adult trees from six different geographical origins in the central west of Morocco: Safi (for the first time), Essaouira, Agadir, Taroudant, Tiznit, and Sidi Ifni (Figure 1).

This area is characterized by a semiarid to arid climate [1]. Temperature and rainfall data were collected from different weather stations for the period from 1989 to 2019 (Table 1). A total of 39 samples were collected between August and November 2018. After sun drying for two weeks, 20 kg of fruit for each studied point was depulped and crushed manually between two stones, yielding between 800 and 1,500 g of kernels for each sample. The kernels were then tightly closed by vacuum to eliminate oxidation until the extraction process. The moisture within the Argan kernels was measured using the international standard ISO 665 to be in the range from 3% to 5%.

2.2. Oil Extraction. To allow the extrapolation of the results to potential production at the level of cooperatives and local industries, the extraction method used was identical to that used to produce Argan oil in Morocco. For this purpose, unroasted kernels were cold-pressed using an oil press (Komet CA59 G; IBG Monforts Oekotec GmbH Co. KG, Mönchengladbach, Germany). The screw speed was maintained at 30 rpm, and the temperature of the heated press was fixed at 50°C. The temperature of the obtained oil was 20°C. Then, once the oil was decanted, it was preserved in 250 mL dark glass bottles in a refrigerator (at 4°C) filled with nitrogen to avoid oxidation. The oil yield varied between 48.43% and 50.67%.

2.3. Physicochemical Quality Parameters. Free acidity (expressed as percentage oleic acid), spectrophotometric UV indices K_{232} and K_{270}, peroxide value given as

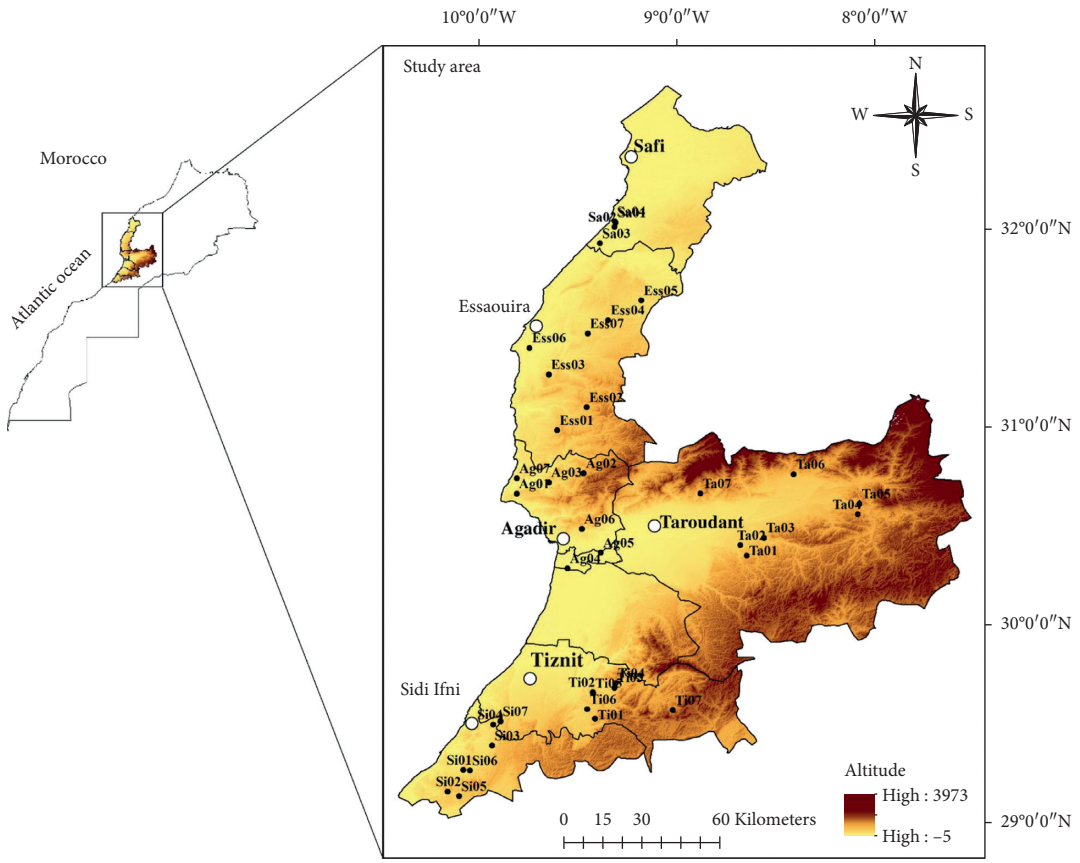

Figure 1: Altitude of sampling provenances and localisation of Argan samples.

Table 1: Geographical parameters of the six sampled provenances of argan trees.

Provenance	Code	Sample size	Latitude	Longitude	Altitude (masl)	Temperature min-max (°C)	Rainfall (mm/year)	Distance from coast (km.a.c.f)
Safi	SA	4	32°02′N	9°18′W	112	13.15–29.60	320.36	5.57
Essaouira	ES	7	30°59′N	9°36′W	138	13.50–26.40	292.17	19.77
Agadir	AG	7	30°22′N	9°23′W	103	12.92–24.55	264.40	22.27
Taroudant	TA	7	30°26′N	8°33′W	592	11.42–31.20	210.37	106.86
Tiznit	TI	7	29°41′N	9°19′W	497	12.70–26.31	205.47	62.54
Sidi Ifni	SI	7	29°23′N	9°56′W	349	13.44–28.70	155.92	21.95

Masl: meters above sea level; km.a.c.f: km as the crow flies.

milliequivalents of active oxygen per kilogram of oil (meq O_2/kg), and oil content (%) were determined according to ISO 660 (2009) [38], ISO 3656 (2002) [39], ISO 3960 (2007) [40], and ISO 659 (2009) [41], respectively.

2.4. Tocopherols Composition. According to ISO 9936 [42], 1 g of Argan oil was dissolved in 25 mL of isooctane/isopropanol (99:1, v/v). Tocopherols were determined using a Shimadzu LC-10 high-performance liquid chromatography system. The sample was first injected into a LiChrospher Si 60 column ($L = 250$ mm, $\Phi = 4.6$ mm id, and $\varnothing = 5\,\mu$m film thickness), and then tocopherols were detected using an RF-10AXL HPLC Fluorescence Detector (Shimadzu, Columbia, MD, USA) at an excitation wavelength of 290 nm and an emission of 330 nm. The eluent used was a 99:1 isooctane/isopropanol (v/v) mixture, and the flow rate was set at 1.2 mL/min. The tocopherol standards α-, β-, γ-, and δ-tocopherols (Sigma-Aldrich, Madrid, Spain) and Argan oil samples were quantified simultaneously. The different compounds of tocopherol were identified by comparing the retention times with authentic standards and confirmed by extrapolating the peak area of the individual tocopherol to the pre-established specific tocopherol calibration curve.

2.5. Statistical Analyses. All statistical analyses were performed using IBM SPSS Statistics version 21 (IBM Corp., Armonk, NY, USA) and R software version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). One-way analysis of variance (ANOVA) was performed followed by Tukey's post hoc test to determine the statistically significant differences between the means of tocopherol concentrations from different provenances

($p < 0.05$). Pearson's correlation heatmap was also assessed to determine the relationship between the geographical parameters and quantity of tocopherols. Furthermore, principal component analysis (PCA) was performed to study whether the means of those regions are significantly different. In addition, linear discriminant analysis (LDA) was applied for creating predictive models that maximize the discrimination of the predefined regions. The difference between means was normalized by a measure of the within-class variability. The statistical significance of each discriminant function was evaluated by Wilk's lambda.

3. Results and Discussion

3.1. Physicochemical Quality Parameters. The oil content ranged between 50.94% in Agadir and 55.67% in Taroudant. The results obtained for the oil content are in agreement with the range obtained by Ait Aabd et al. [43] (51.83–57.50%). The ANOVA followed by Tukey's post hoc test confirmed significant differences between Argan provenances. According to the Moroccan Normalization guidelines SNIMA 08.5.090 [20], Argan oils extracted belong to the extra virgin Argan oil category (Table 2). The lowest acidity value was found in Agadir (0.15%). However, the highest value was detected in Safi (0.26%). The spectrophotometric UV indices K_{232} and K_{270} ranged between 0.94 and 1.05 and 0.14 and 0.17, respectively. Furthermore, the highest peroxide value was noticed in Sidi Ifni (2.13 meq O_2/kg oil) and lowest in Taroudant (1.46 meq O_2/kg oil). The overall quality parameters confirmed the high quality of Argan oil samples.

3.2. Tocopherols Composition. As shown in Figure 2, γ-tocopherol was found to be the predominant tocopherol in Argan oil, followed by α-tocopherol and δ-tocopherol (γ-tocopherol > α-tocopherol ≈ δ-tocopherol). γ-tocopherol represents 90% of the total tocopherols [44]. Compared to the literature, similar results were obtained for mechanically pressed unroasted Argan kernels from old trees [22]. The results showed that β-tocopherol is present in Argan oil but in very low concentrations. Generally, the results obtained in this study are similar to those of Taribak et al. [45]. It should be noted, however, that β-tocopherol has not been detected in any of the studied oils, which might be attributed to the extraction method [46]. The amounts of tocopherols established by the Moroccan 08.5.090 standard [20] for extra virgin Argan oil are 18–75 mg/kg for α-tocopherol, 640–810 mg/kg for γ-tocopherol, 54–110 mg/kg for δ-tocopherol, and 600–900 mg/kg for total tocopherol. All values were within the established limits, except for γ-tocopherol in the samples from Safi, Essaouira, Agadir, and Sidi Ifni, which exceeded 810 mg/kg. These provinces presented the coastal Argan samples. Abbasi et al. [47] reported that the amount of tocopherols is strongly affected by abiotic stress, especially γ-tocopherol that has an important role in protecting polyunsaturated fatty acids from oxidation and consequently increasing the seeds' longevity [48]. The results obtained prove that the current standard does not reflect the real performance in terms of the concentration of tocopherols in Argan oil. Considering the geographical origins, ANOVA revealed a high variability for the three tocopherol homologues. The highest total tocopherol content was found in Safi (1,271.68 mg/kg), followed by Agadir (1,167.93 mg/kg) and Sidi Ifni (1,106.87 mg/kg). These values were confirmed by the results obtained by Aithammou et al. [22] for the same extraction method and age of trees. The lowest value was obtained for the samples from Taroudant (783.23 mg/kg), although this value remains very important. Kharbach et al. [23] reported that the samples of Taroudant (865 mg/kg of oil) have the lowest total tocopherol content, which is in line with the results obtained for the same region. The value of γ-tocopherol was found to range from 1,120.75 mg/kg of oil in Safi to 657.10 mg/kg of oil in Taroudant. This range agrees with other results in the literature: 700.30–1,068 mg/kg of oil [22], 664–802 mg/kg [23], 531–756 mg/kg oil [6], and 545.9–701.1 mg/kg oil [49]. The value of α-tocopherol was found to range from 84.59 mg/kg in Taroudant to 48.72 mg/kg in Safi, with both regions exhibiting the lowest and highest altitude (Table 1). The range of variability for δ-tocopherol was found to be between 102.21 mg/kg of oil in Safi and 41.53 mg/kg of oil in Taroudant. These detected values are consistent with the interval found by Aithammou et al. [22] (36.42–132 mg/kg of oil) and Kharbach et al. [23] (58.55–104.36 mg/kg of oil). According to El Kharrassi et al. [14] compared to olive oil, Argan oil has the highest concentration of total and γ-tocopherols, whereas the highest concentration of α-tocopherol was observed in olive oil. In addition, Gharby et al. [50] mentioned that Argan oil has the highest concentration of α-and δ-tocopherols compared to cactus pear seed oil. As reported by Górnaś et al. [29], biotic factors (genotype) also affect the content of tocopherols in fruit kernel oils, such as apple cultivars (*Malus domestica* Borkh.), plums (*Prunus domestica* L.), and apricots (*Prunus armeniaca* L.). However, Dolde, Vlahakis, and Hazebrock [51] reported that the composition of tocopherols in oil seeds, such as sunflower and soybean, is highly dependent on environmental conditions rather than on genetic factors.

3.3. Relation between Geographical Parameters and Tocopherols. Pearson's correlation analysis (Figure 3) revealed that the longitude, minimum temperature, and rainfall have no effect on the tocopherol content. However, distance from the coast was found to exhibit a strong positive correlation with α-tocopherol and a strong negative correlation with γ-tocopherol, δ-tocopherol, and total tocopherols (correlation is significant at the 0.01 level). A significant correlation was also found between altitude and tocopherol homologues. Although latitude was found to have a significant negative correlation with γ-tocopherol and total tocopherol, a significant positive correlation was observed with α-tocopherol. The maximum temperature was also found to have a significant correlation with α-tocopherol. The geographical origin can affect the process of producing effective substances, especially in climatic conditions [52]. To date, few studies have been conducted to investigate the interaction between climatic parameters and tocopherol content in Argan oil. However, levels of

Table 2: Mean values and standard deviations of the oil content and quality parameters of Argan oil from six provenances.

Provenance	Safi	Essaouira	Agadir	Taroudant	Tiznit	Sidi Ifni	SNIMA 08.5.090
Oil content (% dry matter)	54.94 ± 1.6*b*	53.43 ± 0.68*ab*	50.94 ± 1.12*a*	55.67 ± 0.41*b*	51.57 ± 0.80*a*	55.22 ± 0.36*b*	—
Free fatty acid (%)	0.26 ± 0.02*c*	0.24 ± 0.03*b*	0.15 ± 0.01*a*	0.20 ± 0.01*ab*	0.24 ± 0.02*b*	0.20 ± 0.01*b*	≤0.8
K_{232}	1.05 ± 0.07*a*	0.94 ± 0.09*a*	1.14 ± 0.05*a*	1.03 ± 0.07*a*	0.98 ± 0.04*a*	0.99 ± 0.07*a*	≤2.52
K_{270}	0.18 ± 0.03*a*	0.15 ± 0.02*a*	0.17 ± 0.03*a*	0.17 ± 0.01*a*	0.15 ± 0.02*a*	0.14 ± 0.02*a*	≤0.35
Peroxyde value (meq O2/kg oil)	1.68 ± 0.06*ab*	1.57 ± 0.13*a*	1.97 ± 0.06*cd*	1.46 ± 0.04*a*	1.83 ± 0.08*bc*	2.13 ± 0.07*d*	≤15

Values are expressed as mean ± SD. Different letters in the same line designate significant differences ($p < 0.05$). K_{232} and K_{270}: ultra violet specific extinction at 232 and 270 nm, respectively.

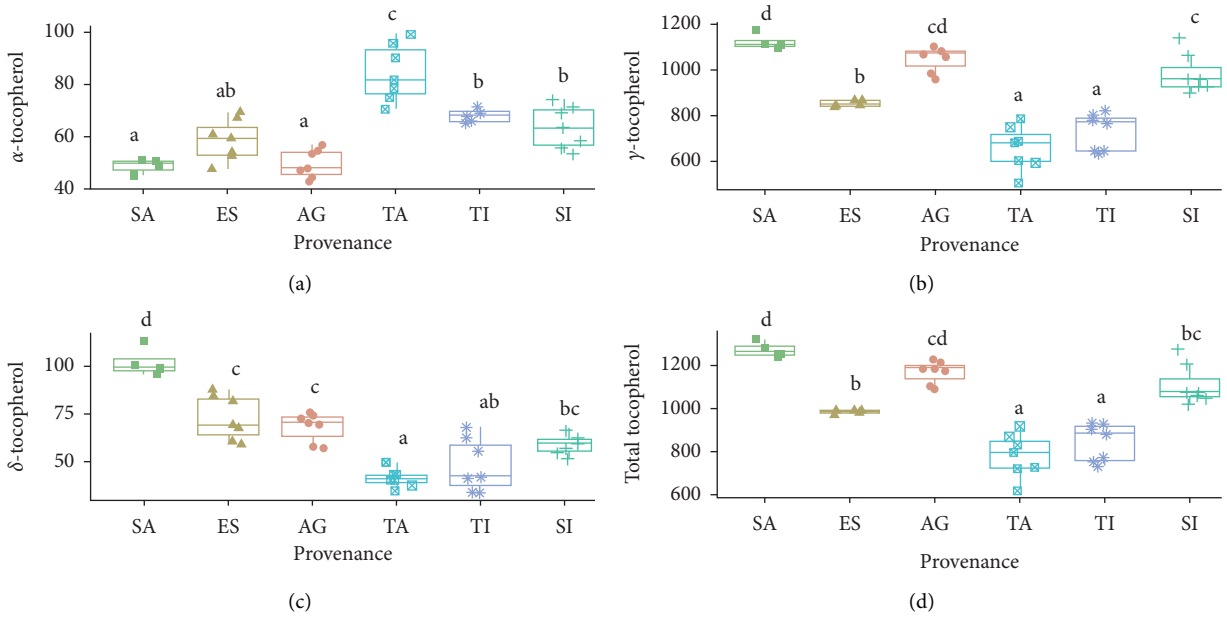

Figure 2: Boxplot of: (a) α-tocopherol, (b) γ-tocopherol, (c) δ-tocopherol, and (d) total to copherolin argan oil samples collected in different provinces (Safi: SA, Essaouira: ES, Agadir: AG, Taroudant: TA, Tiznit: TI, and Sidi Ifni: SI). Significant differences ($p < 0.05$) were expressed by different letters.

γ-tocopherol increased with average temperature and total sunshine and decreased with total rainfall in flaxseed oil [53]. The total tocopherol of olive oil is also strongly affected by altitude [54]. Other Argan oil compounds such as fatty acids were influenced by altitude, latitude, and longitude [37, 43, 55].

3.4. Principal Compound Analysis (PCA). Figure 4 shows a score PCA plot for the 39 Argan oil samples obtained from six provenances according to their tocopherol composition. The first two principal components were found to be very significant, explaining 93% of the total inertia. Although PC1 (Dim1) presented 81.8% of the total inertia, PC2 (Dim2) presented 11.2%. PC1 allowed classifying the coastal sampled locations on the left side of PCA. However, the most continental sampled provinces were plotted on the right side. On the one hand, the coastal locations were characterized by high concentrations of γ-tocopherol, δ-tocopherol, and total tocopherols. On the other hand, α-tocopherol was the most remarkable tocopherol homologue in continental Argan oil samples. Hence, it can be concluded that γ-tocopherol could be used as a good marker of coastal Argan oils. In addition, α-tocopherol can be used as a marker of the continental provinces. The geographical origin has a high impact on tocopherols concentration, showing a distinction between the different studied provenances. This easy, rapid, and precise technique can be used by laboratories to protect this precious oil from fraud such as adulteration by other cheaper oils. Furthermore, it can be combined with other analyses such as fatty acids and phytosterol to enhance the protected geographical indication (PGI).

3.5. Linear Discriminant Analysis (LDA). LDA is a supervised method contrarily to PCA. It was performed to create discriminant models for the classification of Argan oil according to their geographical origin. Figure 5 shows the LDA scatter plot for Argan oils from six provinces. The most continental provinces were plotted on the left of function 1, whereas the coastal origins are plotted on the right. A distinct separation between the provinces was relevant, with some overlap, notably between Tiznit and Taroudant, which can be explained by the geographical parameter similarities.

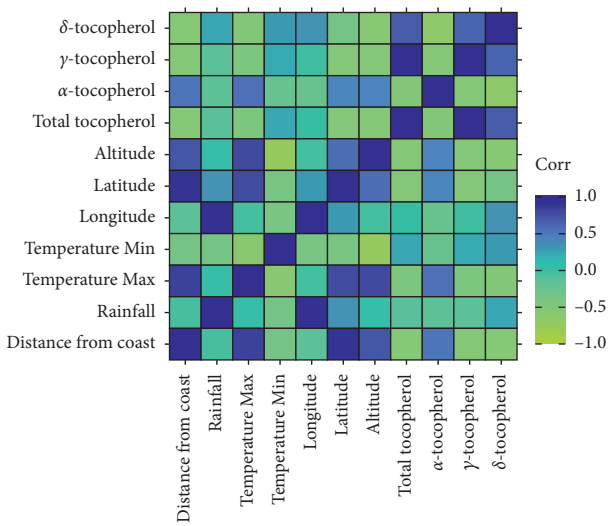

Figure 3: Heatmap of Pearson's correlation coefficient between tocopherol homologues and geographical parameters.

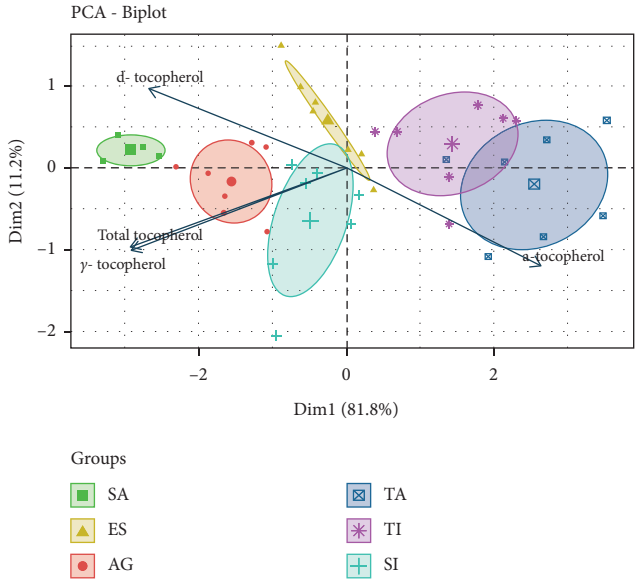

Figure 4: Principal component analysis (PCA) on the tocopherols amount of 39 samples from six different provenances (SA: Safi, ES: Essaouira, AG: Agadir, SI: Sidi Ifni, TA: Taroudant, and TI: Tiznit).

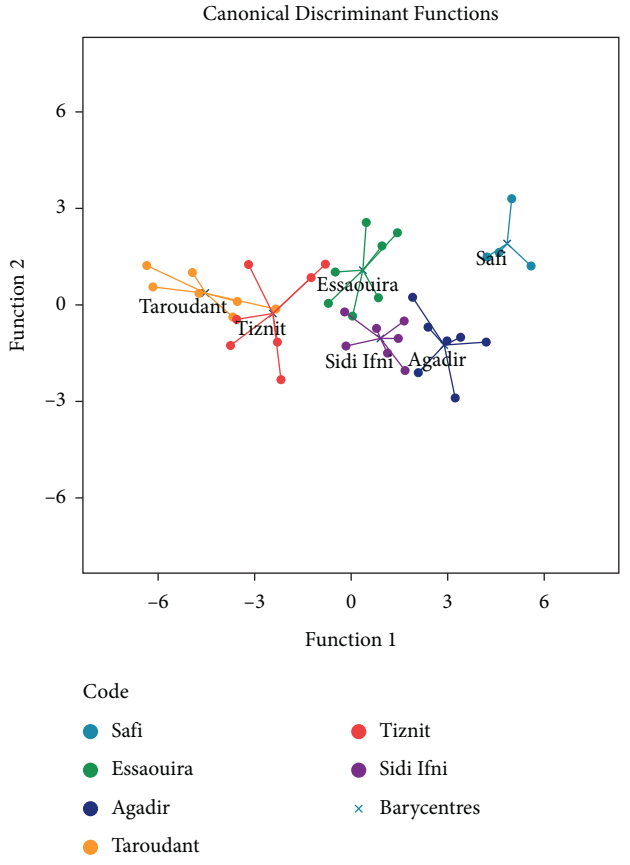

Figure 5: Linear discriminant analysis based on the tocopherols amount of 39 samples from six different provenances.

Table 3 presented three discriminant functions created based on Wilks' lambda values, which explained 100% of the variance (Table 3); 85.7% of the total variance was explained by function 1, 10.7% explained by function 2, and 3.6% explained by function 3. The Wilks' lambda values (Table 3) for the functions 1, 2, and 3 were 0.02, 0.30, and 0.69, respectively, with p-values 0.0001, 0.0001, and 0.007. The LDA showed a good predictive ability, which can reach up to 87.2% for the geographical origin classification.

Discriminant models allowed a good prediction with an accuracy of 87.2% (Table 4). Essaouira, Safi, and Sidi Ifni presented the highest correct classification rate (100%) followed by Agadir (85.71%). Taroudant and Tiznit presented the lowest classification rate (71.42%). According to the results obtained by Elgadi et al. [37] using the LDA models based on fatty acids and isotope combination, the classification rate fluctuate between 85.7% and 100%, which was near to the obtained accuracy. Furthermore, the results obtained by Miklavčič et al. [56] based on fatty acid profile using OPLS-DA showed a similar rate (82%–100%). The high accuracy confirms the performance of tocopherols in the prediction of geographical origin.

Table 3: Discriminant functions elaborated based on the tocopherols composition.

Functions	Wilks' lambda	p value	Variance (%)	Cumulative (%)	Canonical correlation
Function 1 = $-0.70 - 0.07 \times \alpha$-tocopherol + $0.01 \times \gamma$-tocopherol + $0.035 \times \delta$-tocopherol	0.02	0.000	85.7	85.73	0.95
Function 2 = $-4.33 + 0.05 \times \alpha$-tocopherol $- 0.01 \times \gamma$-tocopherol + $0.11 \times \delta$-tocopherol	0.30	0.000	10.7	96.42	0.75
Function 3 = $-14.36 + 0.11 \times \alpha$-tocopherol + $0.01 \times \gamma$-tocopherol + $0.01 \times \delta$-tocopherol	0.69	0.007	3.6	100.00	0.55

Table 4: Performance of the LDA classification models for the geographical origin prediction.

Provenance of origin	Predicted origin					
	Agadir	Essaouira	Safi	Sidi Ifni	Taroudant	Tiznit
Agadir	6 (85.71%)	1 (14.28%)	0.0	0.0	0.0	0.0
Essaouira	0.0	7 (100%)	0.0	0.0	0.0	0.0
Safi	0.0	0.0	4 (100%)	0.0	0.0	0.0
Sidi Ifni	0.0	0.0	0.0	7 (100%)	0.0	0.0
Taroudant	0.0	0.0	0.0	0.0	5 (71.42%)	2 (28.57%)
Tiznit	0.0	2 (28.57%)	0.0	0.0	0.0	5 (71.42%)

4. Conclusion

The results obtained in this study highlight the impact of the geographical origin on the α-tocopherol, γ-tocopherol, and δ-tocopherol content. Pearson's correlation analysis showed that the longitude, minimum temperature, and rainfall have no effect on the content of tocopherols. However, distance from the coast, latitude, and altitude was found to exhibit a strong correlation with the majority of tocopherol homologues. PCA also revealed a distinction between provenances and confirmed the relationship between the geographical origin and tocopherol concentration. In addition, α-tocopherol and γ-tocopherol could present promising markers to protect the geographical origin of Argan oil. The prediction ability of the LDA models was 87.2%. Our study provided interesting results for the variability of tocopherol homologues concentration in six principal production areas. This technique is easy, not expensive, and rapid for laboratories to control the fraud related to geographical origin. These results present a preliminary basis for determining the geographical origins of Argan oil and highlight the real tocopherols potential in Argan oil that varies from 1,271.68 to 844.05 mg/kg of oil. Further future studies more exhaustive are planned to confirm the obtained results. In addition, more studies focussing on biotic factors (genotype/variety/cultivar) are necessary to have a clear overview of tocopherol homologues variation, which is very useful particularly for varietal selection objectives.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

An Efficient Solid-Phase Extraction-Based Liquid Chromatography Method to Simultaneously Determine Diastereomers α-Tocopherol, Other Tocols, and Retinol Isomers in Infant Formula

Baifen Huang, Zengxuan Cai, Jingshun Zhang, and Jiaojiao Xu

Department of Physicochemical & Toxicology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou 310051, China

Correspondence should be addressed to Jiaojiao Xu; jjxu@cdc.zj.cn

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The separation and simultaneous quantitation of diastereomers of DL-α-tocopherol, eight tocol forms, and retinols (trans and cis) have been conducted by reversed-phase liquid chromatography followed by solid-phase extraction. A chiral silica stationary phase modified with polysaccharide derivative on the monodisperse macroporous silica gel (Unichiral OD-5H column, 150 mm × 4.6 mm, 5 μm, NanoMicro Technology Co., Ltd.) was employed for eluting each target compound. Instead of conventional solvent extract, a green and eco-friendly solid-phase extraction column, packing with nonpolar polystyrene divinylbenzene, was optimized in terms of capacity and solvent used in steps. Validation of the method was examined and confirmed to be satisfactory, with excellent linearity regression ($r > 0.9999$), acceptable accuracy (74.66%~112.92%), and precision (0.20%~10.52%) results. Limit of detection ranged from 0.05 mg·kg^{-1} (retinols) to 0.4 mg·kg^{-1} (tocols). The method was checked by infant formula reference material SRM 1849a as well, which illustrated good agreement of mass fraction with certified value and enriched the important isomer data.

1. Introduction

Measurement of vitamins in foods and supplements is important for monitoring and controlling nutrient intakes of various populations, especially for specific groups (like elders and infants). Excess and deficient intakes of fat-soluble vitamins could cause a disorder of protein metabolism [1], immune system, version and regulation of cell growth, and differentiation [2].

Vitamin A belongs to the fat-soluble vitamin group that helps maintain normal reproduction, vision, and immune function. It comes in several forms (like retinol, retinal, retinoic acid, or retinyl ester). Isomers of vitamin A have different activities. All-trans-retinol is defined to the 100% reference activity level, while 13-cis-retinol and 11-cis are 75% and 30% active, respectively, and the other isomers have activities lower than 20% [3]. In general, all-trans and 13-cis retinol are the most common forms found in foods and supplements. However, the determinations of vitamin A always focus on total vitamin A or total retinol only, which could lead to underestimation of vitamin A when cis-isomers are also present.

Vitamin E, another main group of fat-soluble vitamins, plays an important role in animal reproduction, antioxidant, and anticancer activities. Consisting of tocopherols and tocotrienols (α-, β-, γ-, and δ-form), both natural and synthetic forms of vitamin E are used as additives in food and food supplement. For the sake of different presentence of 2, 4', and 8' asymmetric carbon atoms in tocopherol molecule, the natural α-tocopherol (D-α-tocopherol) and synthetic tocopherol (DL-α-tocopherol) result in eight stereoisomers [4, 5]. Natural D-α-tocopherol is the most effective assigned 1.49 IU vitamin E equivalent, whereas DL-α-tocopherol (all-rac) was assigned 1.10 IU vitamin E

equivalent [6]. Besides, β-, γ-, δ-tocopherol and tocotrienol congeners act out significantly different activities. Consequently, the distinction of α-tocopherol forms and vitamin E isomers is important for quality control and analysis.

Liquid chromatography is the method most frequently employed for the analysis of retinol and tocol isomers. Normal-phase liquid chromatography (NP-LC) has successfully been applied to separations of isomers of retinol [7–9], and tocol isomers, which has been reviewed by Ruperez et al. and Fanali et al. [5, 10]. When saponification is not essential, the NP-LC method could conduct direct quantitative of target compounds through sample extraction and elution with hexane. However, considering robustness of the chromatographic columns, reproducibility of chromatographic peak characteristics, and reduction of volatile and hazardous solvents, reverse-phase liquid chromatography (RP-LC) offers greater suitability, especially in the aspect of multiple vitamin isomers separation. Silica-C30, pentafluorophenyl (PFP), and high-density C18 stationary phase with polymeric stationary phase have been employed in RP-LC for the separation of β- and γ-tocols [4, 11, 12], while it has been a bit rarely used for retinol isomers.

To distinguish the natural tocopherol (D-α-tocopherol) in a product, it is only necessary to demonstrate a single peak using the chiral stationary column. To date, several publications have been reported to separate α-tocopherol stereoisomers, based on the three chiral centers in the phytyl tail. With different polymeric bonding modified chiral stationary phases, some scientific researches have been done to separate isomers of DL-α-tocopherol into more than two peaks [4, 13, 14]. Although there is rarely a report showing the differentiation between diastereomers of vitamin E and retinol congeners simultaneously, the differentiation by RP-LC would be usefully considered for the sake of reversed-phase mode advantages and versatilities.

Solvent extraction is a classical method in vitamin A and vitamin E analysis, as in the case of the standardized method in authority [15–17]. Except for tedious steps, those methods are not satisfied with the economy and environmental friend. For their peculiarities, extraction methods, including solid-phase extraction (SPE), supercritical fluid extraction, and pressurized liquid extraction, have been developed to meet the scientific trend of simplification, speediness, wastage reduction, costs, and safety. Among them, SPE is a rapid, effective, and versatile technique and has been employed in various matrices for fat-soluble compounds extraction, such as the concentration of tocols in rice brans [18], and tocols and carotenoids in cereal samples [19]. To the best of our knowledge, there is no study to describe simultaneous extraction of vitamin E congeners and retinols (cis and trans isomers) in infant formula by SPE.

The present study aims to develop and validate an accurate, precious, sensitive, and eco-friendly RP-LC method for the determination of tocols (tocopherols and tocotrienols) and retinol (cis and trans) isomers simultaneously using polysaccharide derivative modified silica stationary phase (Unichiral OD-5H column, 150 mm × 4.6 mm, 5 μm), which was proven to be sufficient for the distinction of the DL-α-tocopherol and D-α-tocopherol. A green sample preparation technique was employed instead of solvent extraction and was applied in infant formula samples successfully.

2. Materials and Methods

2.1. Chemicals and Materials. All-trans-retinol, 9-cis-retinol, and 13-cis-retinol were obtained from Toronto Research Chemicals (Irvine, CA, USA). D-Tocopherols (D-α-, β-, γ-, and δ-tocopherol) and tocotrienols (D-α-, β-, γ-, and δ-tocotrienol) were obtained from Supelco (Bellefonte, PA, USA), as well as DL-α-tocopherol. Stock solutions (1 mg·mL^{-1} of all-trans-retinol, 100 μg·mL^{-1} of cis-retinol, 20 mg·mL^{-1} of DL-α-tocopherol, 5 mg·mL^{-1} of tocopherols and tocotrienols) were prepared in anhydrous alcohol and stored in brown glass bottles at −20°C. Their concentrations were evaluated spectrophotometrically based on their specific absorption coefficients: α-tocopherol = 75.8 at 292 nm, β-tocopherol = 89.4 at 296 nm, γ-tocopherol = 91.4 at 298 nm, δ-tocopherol = 87.3 at 298 nm, α-tocotrienol = 91.0 at 292 nm, β-tocotrienol = 87.5 at 295 nm, γ-tocotrienol = 103.0 at 298 nm, δ-tocotrienol = 83.0 at 292 nm, all-trans-retinol = 1830 at 325 nm, and 13-cis-retinol = 1686 at 328 nm [15, 20]. Take one milliliter of each stock standard solution and makeup to 100 mL with methanol in a 100-mL volumetric flask. Working solutions were prepared by methanol in available dilution times.

HPLC grade of methanol (MeOH) and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from Millipore (Bedford, MA, USA). All other reagents were of analytical grade and were purchased from local suppliers. The packings with polystyrene divinylbenzene (PS-DVB) SPE cartridges in three brands were evaluated, SelectCore PSN from NanoMicro Technology Co., Ltd. (Suzhou, China), Bond Elut Plexa from Agilent Technologies, Inc. (CA, USA), and Welchrom PS/DVB column from Welch Technologies Shanghai Co., Ltd. (Shanghai, China). The infant powder matrix sample was purchased and information was collected from a local supplier.

2.2. Instrumentation. LC system was composed of I-Class Waters Acquity™ UPLC with a fluorescent detector (Large volume flow cell), a photo-diode array detector, and a 20-μL sample loop. The chiral column, 150 mm × 4.6 mm inner diameter, 5 μm particle size, Unichiral OD-5H column (NanoMicro Technology Co., Ltd, China), which was packed with polysaccharide derivative on the surface of spherical silica stationary phase, was conducted at 35°C. The two-component mobile phase (A-water, B-75% ACN/25% MeOH) was delivered at a flow rate of 1.2 mL·min^{-1} as the following time table: 0–23 min, 75% B; 23–32.5 min, 75%~80% B; 32.5–35 min, 80%~100% B; 35–39 min, 100% B, 39–39.5 min, 100%~75% B; total run time was 45 min. Tocopherols and tocotrienols were detected with fluorescence at 294 nm excitation and 328 nm emission, while retinols were detected with a photo-diode array detector at 328 nm.

2.3. Method Validation. The established RP-LC method was validated in an aspect of specificity, linearity, range, limits of detection (LOD), limits of quantification (LOQ), precision, and accuracy. The linearity of each analyte was evaluated by calculating the slope, intercept, and correlation coefficient of each calibration curve. The LODs and LOQs were determined by spiking various low concentration levels and determined as the lowest concentrations that produce chromatographic peaks at a signal-to-noise ratio (S/N) of 3 and 10, respectively. Accuracy and precision of the method were conducted by adding three levels of standard working solution to infant formula sample in six parallel levels, whereas spiking concentrations were based 0.75-, 1.5-, and 3-folds on the content of analytes in infant formula sample (which is mainly calculated based on the content of D-α-tocopherol). The selectivity of the method was analyzed by comparing the chromatograms of analyte-free samples and the spiked ones. Furthermore, the method was validated and applied for an infant/adult nutritional formula SRM 1849a of reference material supplied by the National Institute of Standards and Technology (NIST).

2.4. Sample Preparation. According to significantly different uniformity of dry and wet blended powder samples, sample homogenization was conducted as follows: transfer 20 g of dry blended/nonhomogeneous infant formula powder samples, accurately weighed, to a 250-mL bottle. Dissolve in warm water (about 50–60°C) until no obvious granule, cool down, and make up to 100 g with water. Transfer 5 g reconstituted sample to a screw-top centrifuge tube. For wet blended/homogeneous powder samples, transfer 1.0 g to a screw-top 50 mL centrifuge tube. Add 5 mL warm water of approximately 50°C and shake to dissolve [21].

For extraction, samples were submitted to a modified saponification procedure as described in mandate standardized method [17], with 0.2 g ascorbic acid, 6 mL ethanol containing 0.1% butylated hydroxytoluene (BHT), and 3 mL 50% potassium hydroxide for 30 min at 80°C constant temperature oscillation water bath. The tube was placed in an ice bath to cool down. Then onefold saponification solution bulk of the water was added. The test tube was centrifuged and the supernatants were loaded to SPE cartridge. To avoid the destruction of labile vitamins, all saponification work was carried out under subdued light.

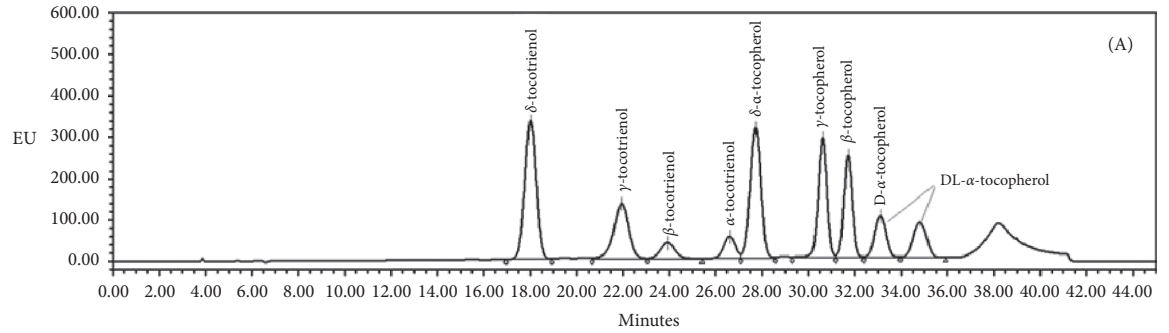
The total of the above supernatants was passed through PS-DVB cartridges, which were conditioned with 3 mL of methanol and 3 mL of water. After washing with 5 mL of 10% aqueous methanol solution repeated twice, retained constituents were eluted with 7.5 mL of ACN/MeOH mixture (75/25, v/v). Making up to 10 mL by water, the lotions were filtered and injected into the HPLC column. All measurements were performed in triplicate. The results of all measurements are expressed as means ± SD.

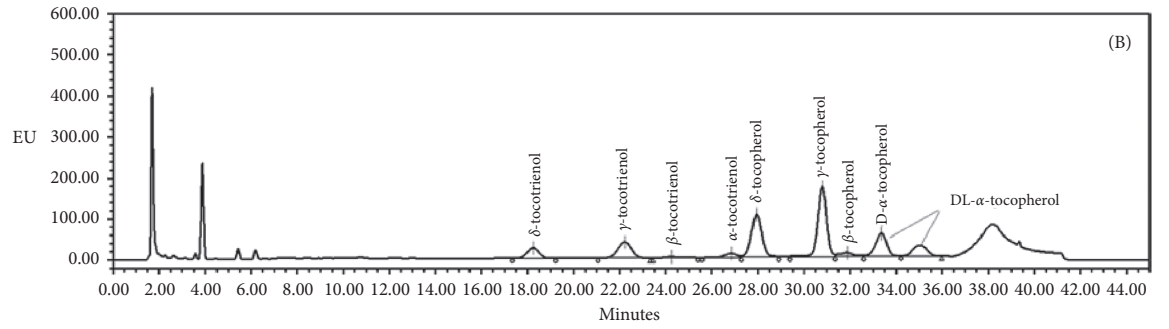
3. Results and Discussion

3.1. Separation of Asymmetric α-Tocopherol and Retinol Isomers. The first and crucial study was carried out here to pick up the analytical column and address the optimization of the mobile phase. Different stationary phase columns were considered, as alkyl-bonded C30 silica, high-density C18 stationary phase with polymeric bonding, and PFP column were reported to separate β and γ tocol isomers. In an aspect of retinols, few pieces of literature were discussed about the RP-LC method for trans and cis isomers separation. In the present study, C30 and PFP stationary phases were proven to be of satisfying performance for the separation of trans and cis retinols, while both of them could not distinguish DL-α-tocopherol and D-α-tocopherol. Normal C18 stationary phase could not be used for the separation of β and γ tocol isomers. According to previous reports, chiral stationary phases were available for asymmetric α-tocopherol. In this study, a chiral silica stationary phase modified with polysaccharide derivative on the monodisperse macroporous silica gel (Unichiral OD-5H column, 150 mm × 4.6 mm, 5 μm) was tested. Methanol and acetonitrile were primarily examined as mobile phases. Starting isocratic elution with methanol, the overlapping peak of δ-tocopherol and γ-tocotrienol was observed, as well as the longer retention times of all analytes. With acetonitrile solvent, the elution was so quick that the complete separation of trans and cis retinols could not be achieved. Different proportions of these two solvents were tested consequently. The best separation was conducted by the gradient elution system started with 75% of ACN/MeOH (75/25, v/v) mixture. Under optimization conditions, those compounds were separated sufficiently by the Unichiral OD-5H column and the whole elution lasted less than 40 min. Figure 1(c) illustrates that there would be two peaks in DL-α-tocopherol standard solution, while it would be only one peak in the D-α-tocopherol standard, which would be employed to distinguish the α-tocopherol form in samples.

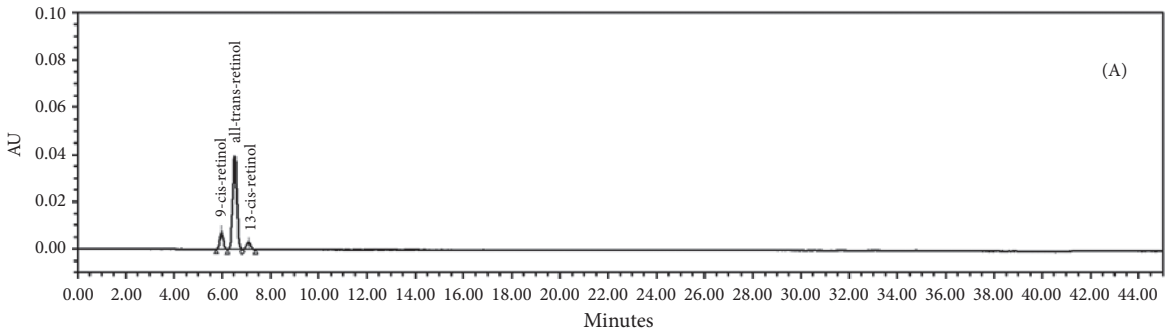
3.2. Optimization of SPE Parameters. For disadvantages of solvent extraction in long extraction time with a lot of toxic solvents consumption and tedious steps, several environmental extraction methods have been developed for the release of tocols depending on the characteristics of the samples. In this study, a green SPE method was established and optimized, including the choice of sorbent, wash, and eluent solvent.

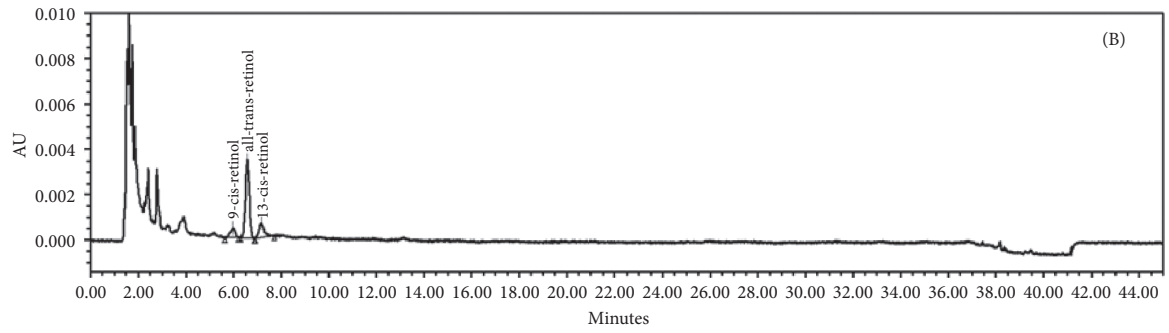
The simultaneous extraction of tocols and retinols was more complicated. A commercial nonpolar polystyrene divinylbenzene (PS-DVB) packing column was taken on researchers' interest based on its advantage of high-throughput assays, alkali resistance, and strong hydrophobicity. Bond Elut Plexa column (500 mg, 6 mL), Bond Elut Plexa column (200 mg, 6 mL), SelectcCore PSN column (200 mg, 6 mL), and Welchrom PS/DVB column (200 mg, 6 mL) were compared and illustrated in Figure 2. The simulated saponification extract solution consists of available tocols and retinols standards mixture, and 40 % potassium hydroxide in ethanol solution. Figure 2 illustrates that different brand packing columns showed different retention capabilities for analytes. There was no significant difference between the capabilities of SelectcCore PSN and that of Welchrom PS/DVB. Bond Elut Plexa columns demonstrated fewer capacities for partial analytes, especially





(a)





(b)

Figure 1: Continued.

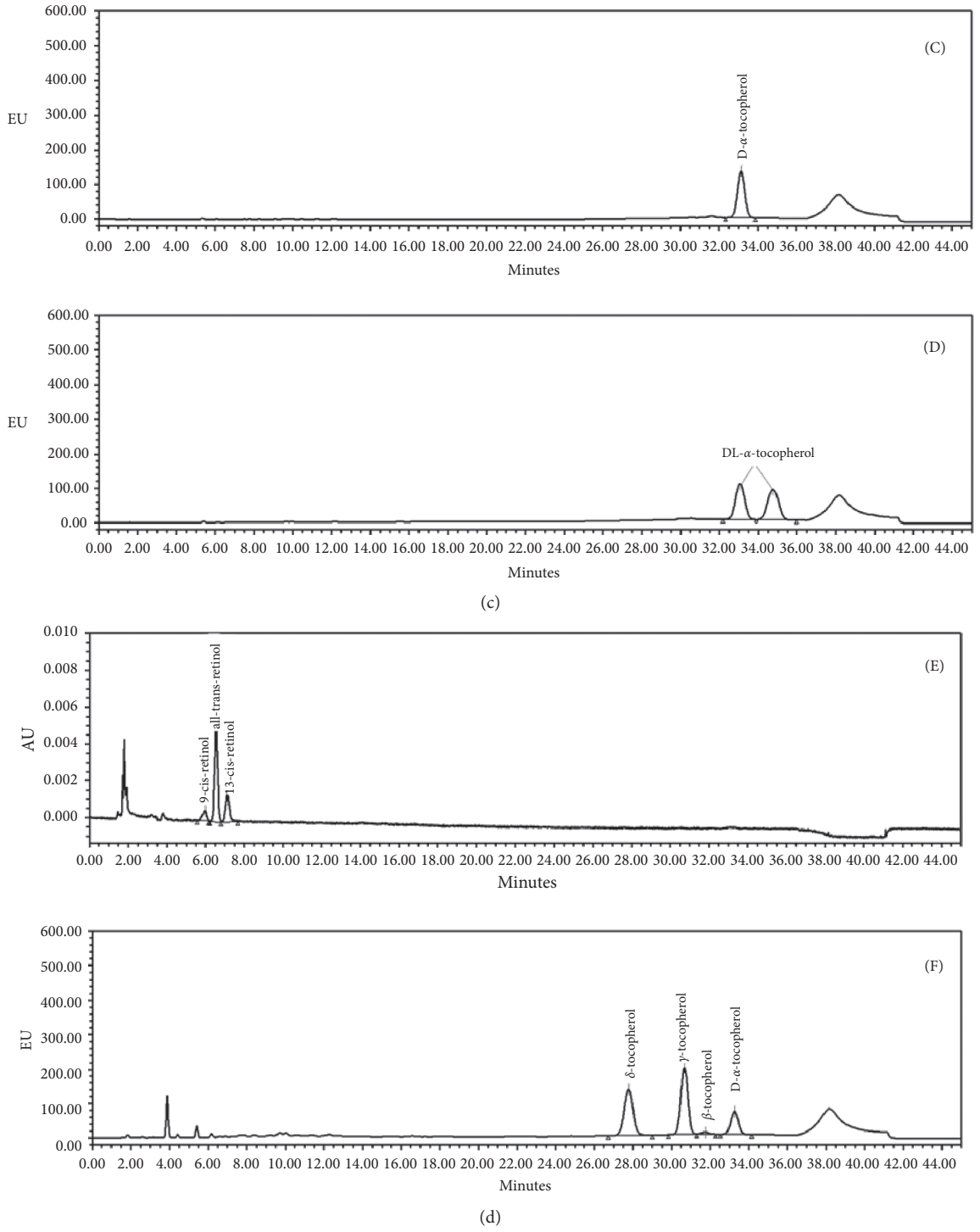


Figure 1: Typical chromatograms of (a) tocopherols and tocotrienols in fluorescent detector and (b) retinols in photo-array detector: (A) presents all analytes in mixture standard solution, (B) presents analytes in matrix extraction, (c) presents peaks of D-α-tocopherol (C) and DL-α-tocopherol (D) in the relative standard solution, (d) presents chromatograms of components in SRM 1849a, (E) retinols in photo-array detector, (F) tocopherols and tocotrienols in fluorescent detector.

200 mg size column. And the speed of extracts passed by Bond Elut Plexa column (500 mg, 6 mL) was much slower during the loading step. Thus, both of SelectcCore PSN (200 mg, 6 mL) and Welchrom PS/DVB (200 mg, 6 mL) could be chosen in the following steps.

For cleaner samples and reducing ion suppression, the washing solution was optimized. Different percentages of organic solution (10 mL of 0%, 2%, 5%, 10% methanol solution, and 40% ethanol, respectively) were compared and the results showed 10% methanol solution was the best with

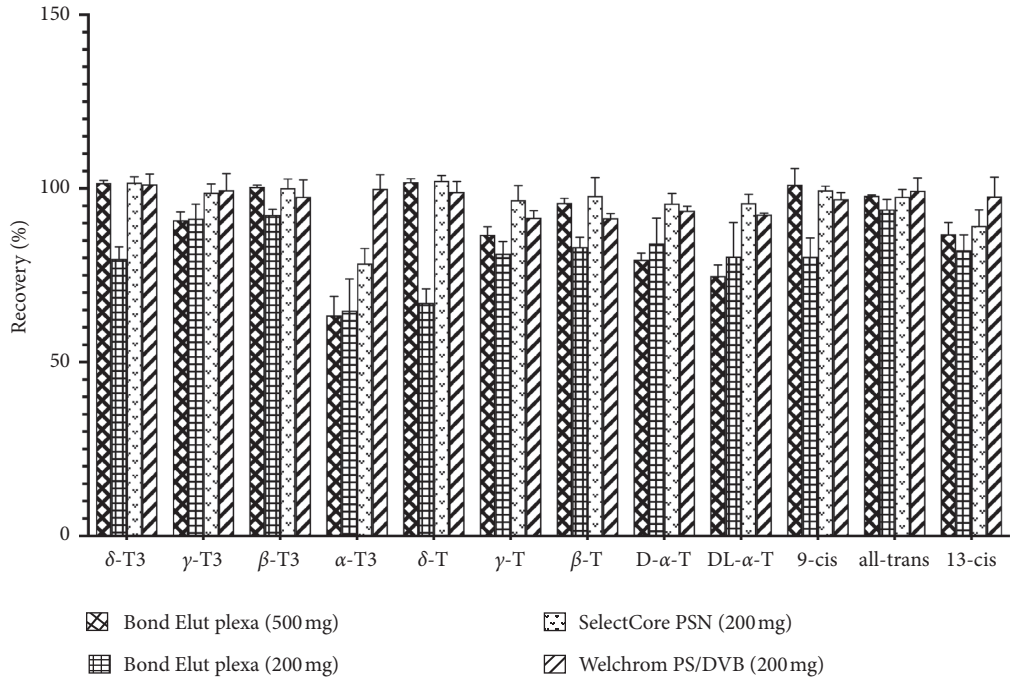


Figure 2: Appearances of different brand SPE columns on retinols, tocopherols, and tocotrienols (T: tocopherol; T3: tocotrienol; 9-cis: 9-cis-retinol; 13-cis: 13-cis-retinol; all-trans: all-trans-retinol).

higher than 80% recoveries of all analytes. Recoveries of δ-tocols were lower than 70% when 40% ethanol solution was used for washing, which explicated a higher organic washing solution was undesirable. In the elution step, 7.5 mL of ACN/MeOH mixture (75/25, v/v) was necessary to provide high recoveries of analytes, which ranged from 90.25% to 103.3% for tocotrienols, 89.54% to 98.36% for tocopherols, and 93.13 to 116.03% for retinols.

Besides being satisfied with the simulated saponification solution, it would be available for real infant formula samples. To confirm the SPE conditions, a mixture of several different brands of infant formula samples was conducted for the sake of enriching tocols and retinols instead of spiking standards. Also, it is more economical and effective to investigate the purification ability and applicability of the SPE method to potential impurities. The results were evaluated by each compound content in the mixture sample. In practice, there was no difference in washing and eluting steps between simulation solution and mixture infant formula sample, except the loading step. When loading sample saponification extract directly, the contents of δ-tocols would be half of that from the loading sample with a onefold volume of water. This is matched with the simulation extract recoveries obtained from 40% ethanol used as a washing solution. The high percentage of ethanol in saponification extract would cause less reservation of analytes. Onefold bulk of the water was added to decrease the percentage of organic solvent.

3.3. Analytical Characteristics of the Method. The typical chromatograms of analytes extracted from the mixture infant formula sample and standards solution are presented in Figures 1(a) and 1(b), as well as DL-α-tocopherol and D-α-tocopherol standard solution (Figure 1(c)). The separation of each retinol and tocol compound exhibited good specificity. No unidentified peaks in the selected samples interfered with the analytes.

The linearity calculation was based on the six increasing concentrations of the standard solution of each isomer. Ranging from 0.01 $\mu g \cdot mL^{-1}$ to 25 $\mu g \cdot mL^{-1}$, all of retinols and tocols compounds showed good linear regressions ($r > 0.999$) as displayed in Table 1, which allowed acquiring reliable and effective data for infant formula samples and relative modified products with low and high contents of vitamin E and retinols. The LODs and LOQs, from 0.05 mg/kg to 0.4 $mg \cdot kg^{-1}$, and from 0.15 $mg \cdot kg^{-1}$ to 1.2 $mg \cdot kg^{-1}$, respectively, were reported here matched with small amounts of analytes in infant formula samples, which referred to the sensitivity of the instrument.

Accuracy and precious constructed for the spiked infant formula sample with approximate standard concentration, prepared as described in Section 2.4, are presented in Table 2. It is noticed that the results performed excellent repeatability and satisfactory precision, with RSD values lower than 11%, and mean recoveries were between 74.66% and 112.92%.

Finally, the reliability of the method was further checked by using the reference material SRM 1849a. The results obtained are listed in Table 3 and illustrated in Figure 1(d). Figure 1(d) displays the variety of components in SRM 1849a, containing cis and trans retinols, and four isomers of tocopherols (α-, β-, γ-, and δ-), compared with NIST official document report. The form of α-tocopherol is D-α-tocopherol mainly, for the sake of one

Table 1: Parameters of the RP-LC method for determination of vitamin E and retinol isomers.

Analyte	RT (min)	Calibration curve	r	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Range (μg mL^{-1})
All-trans-retinol	6.53	$y = 0.0042x - 3.00$	0.9999	0.05	0.15	0.1~5
13-cis-retinol	7.09	$y = 0.0046x - 0.36$	1.0000	0.05	0.15	0.01~0.5
9-cis-retinol	5.97	$y = 0.0041x - 1.43$	1.0000	0.05	0.15	0.01~0.5
DL-α-tocopherol[a]	33.34/35.10	$y = 0.0006x - 74.28$	0.9998	0.4	1.2	2~100
D-α-tocopherol	33.34	$y = 0.0006x - 119.01$	0.9998	0.4	1.2	0.5~25
D-β-tocopherol	31.93	$y = 0.0002x - 10.63$	0.9999	0.4	1.2	0.5~25
D-γ-tocopherol	30.84	$y = 0.0001x + 49.20$	0.9999	0.4	1.2	0.5~25
D-δ-tocopherol	27.99	$y = 0.0001x + 10.72$	0.9999	0.4	1.2	0.5~25
D-α-tocotrienol	26.92	$y = 0.0006x + 108.41$	0.9999	0.4	1.2	0.5~25
D-β-tocotrienol	24.32	$y = 0.0002x + 7.62$	0.9996	0.4	1.2	0.5~25
D-γ-tocotrienol	22.29	$y = 0.0002x + 47.58$	0.9999	0.4	1.2	0.5~25
D-δ-tocotrienol	18.30	$y = 0.0001x - 12.00$	0.9999	0.4	1.2	0.5~25

[a] Two ideal peaks appeared in retention time (RT) of 33.34 min and 35.10 min, within the approximate peak area.

Table 2: Accuracy and precious in spiked infant formula samples ($n = 6$).

Analyte	Blank[a] (mg kg^{-1})	Spiking level 1[b]		Spiking level 2		Spiking level 3	
		Recovery (SD) %	RSD%	Recovery (SD) %	RSD%	Recovery (SD) %	RSD%
All-trans-retinol	2.25 (0.10)	95.31 (3.46)	3.63	82.49 (3.18)	3.85	82.54 (3.21)	3.89
13-cis-retinol	0.74 (0.10)	74.66 (2.72)	3.64	95.12 (9.80)	10.30	98.63 (9.61)	9.74
9-cis-retinol	0.39 (0.10)	112.92 (5.71)	5.05	96.40 (6.27)	6.50	97.23 (4.38)	4.51
DL-α-tocopherol	114.19 (2.02)	103.22 (4.19)	3.9	91.34 (2.59)	2.86	85.70 (1.07)	1.25
D-α-tocopherol	69.85 (2.33)	104.12 (6.20)	5.68	93.35 (2.65)	2.84	87.24 (0.84)	0.97
D-β-tocopherol	1.26 (0.15)	92.22 (1.41)	1.53	89.29 (1.49)	1.67	91.62 (0.59)	0.65
D-γ-tocopherol	23.14 (0.92)	92.70 (9.75)	10.52	90.93 (5.28)	5.81	90.90 (3.57)	3.92
D-δ-tocopherol	10.42 (0.47)	81.15 (6.67)	8.22	84.14 (4.28)	5.08	83.16 (2.24)	2.69
D-α-tocotrienol	2.42 (0.00)	76.38 (4.03)	5.28	90.31 (2.69)	2.97	89.53 (2.70)	3.02
D-β-tocotrienol	ND	109.38 (9.95)	9.10	97.18 (4.21)	4.33	95.35 (2.90)	3.05
D-γ-tocotrienol	1.07 (0.00)	79.81 (0.16)	0.20	82.03 (4.49)	5.47	83.83 (1.90)	2.27
D-δ-tocotrienol	0.78 (0.00)	82.98 (3.38)	4.07	85.15 (1.00)	1.18	85.92 (1.16)	1.35

[a]ND represents mass fraction of the analyte in sample was lower than LOD. [b]Spiking concentrations were based 0.75-, 1.5-, and 3-folds on the content of analytes in infant formula sample, which was mainly calculated based on the content of D-α-tocopherol.

Table 3: Retinol and vitamin E isomer contents in certified reference materials (SRM 1849a).

	Vitamin A (mg kg^{-1})				Vitamin E (mg kg^{-1})[b]			
	All-trans-retinol	13-cis-retinol	9-cis-retinol	Total[a]	D-α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol
Content	5.91 ± 0.13	1.80 ± 0.05	0.81 ± 0.04	7.71 ± 0.14	221 ± 4	5.59 ± 0.36	141 ± 4	76.1 ± 2.1
Certified value	7.68 ± 0.23 mg·kg^{-1} retinol equivalents, total (cis + trans) retinol without any biological activity correction				219 ± 16 mg·kg^{-1} α-tocopherol equivalents, including natural α-tocopherol and added α-tocopheryl acetate			

[a]The total listed here is equal to all-trans-retinol and 13-cis-retinol without any biological activity correction. The total is equal to 8.52 ± 0.18 mg·kg^{-1}. If calculating all cis and trans retinols without any biological activity correction. [b]Mass fractions of tocotrienols were detected lower than LODs in SRM 1849a.

peak, appeared in relative retention time. Table 3 shows the mass fraction of total (cis and trans) retinols was a little bit higher than the certified content, whereas the total of all-trans and 13-cis retinols was in agreement. With respect to vitamin E, the result of D-α-tocopherol was in the range of assigned value, and the contents of γ- and δ-tocopherols were also abundant. Such data confirmed the efficacy of the methodology and the extraction procedure. And what is more, it is necessary to identify each isomer of retinols and tocols compound in infant formula when evaluating biological activity and estimate the equivalent by rule and line.

4. Concluding Remarks

The optimized RP-LC method offers advantages over previous literature, such as simultaneous quantitation of variety analytes, quicker distinction of α-tocopherol form, and estimation of common retinol isomers. The choice chiral chromatographic column was recommended to utilize in routine practice for the relatively low cost and available effective time. For the trend of quicker, simpler, cheaper, rugger, and safer requests in sample preparations, the SPE method takes place of the conventional solvent extraction method. Although the sorbent (PS-DVB) in the packing

column is not a novel material, it is the first time to be employed in vitamin E and retinols concentrated.

Abbreviations

NP-LC: Normal-phase liquid chromatography
RP-LC: Reverse-phase liquid chromatography
PFP: Pentafluorophenyl
SPE: Solid-phase extraction
MeOH: Methanol
ACN: Acetonitrile
LOD: Limits of detection
LOQ: Limits of detection
S/N: Signal-to-noise ratio
BHT: Butylated hydroxytoluene
PS-DVB: Polystyrene divinylbenzene.

Data Availability

The data used to support the study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

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Review Article

Occurrence of Tocols in Foods: An Updated Shot of Current Databases

Alessandra Durazzo,[1] **Amirhossein Nazhand**,[2] **Massimo Lucarini**,[1] **Amélia M. Delgado**,[3] **Maryna De Wit**,[4] **Kar Lin Nyam**,[5] **Antonello Santini**,[6] **and Mohamed Fawzy Ramadan**[7,8]

[1]*CREA-Research Centre for Food and Nutrition, Via Ardeatina 546, Roma 00178, Italy*
[2]*Department of Biotechnology, Sari Agricultural Science and Natural Resource University, Sari 48181 68984, 9th km of Farah Abad Road, Mazandaran, Iran*
[3]*Mediterranean Institute for Agriculture, Environment and Development, Universidade do Algarve, Edf 8, Campus de Gambelas, 8005-139 Faro, Portugal*
[4]*Department of Sustainable Food Systems and Extention, University of the Free State, Bloemfontein, South Africa*
[5]*Department of Food Science with Nutritio, Faculty of Applied Sciences, UCSI University, 56000 Kuala Lumpur, Malaysia*
[6]*Department of Pharmacy, University of Napoli Federico II, Via D. Montesano 49, Napoli 80131, Italy*
[7]*Agricultural Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt*
[8]*Deanship of Scientific Research, Umm Al-Qura University, Makkah, USA*

Correspondence should be addressed to Alessandra Durazzo; alessandra.durazzo@crea.gov.it

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Tocols are present in various foods, mostly in fruits and in plant seeds. Edible oils are the most important natural dietary sources of tocopherols and tocotrienols, collectively known as tocols. Tocopherols and tocotrienols are considered beneficial for their antioxidant effect which impacts on prevention of different health conditions. This perspective is addressed to give an updated picture of the tocol occurrence in foods. Moreover, the current state of the art of tocols in updated databases is explored and commented outlining their importance and future trends.

1. Introduction

Tocols (tocopherols and tocotrienols), as shown in Figure 1, are monophenols obtained from 6-hydroxy-2-methyl-2-phytylchroman, which are applied as food additives in the food and pharmaceutical industries [1]. Some of the chemical characteristics of the tocols include their solubility in polyethylene glycol, propylene glycol, chloroform, acetone, surfactants, oils, and ethanol. They are not water soluble, while they are resistant to heat, and acid-stable, although they are instable when exposed to alkali, light, and oxygen [2].

The chemical structure of tocopherols and tocotrienols is different so that tocopherols (α, β, γ, and δ) contain a chromanol ring and a 16-carbon phytyl side chain in their structure with methylation at three positions of 5, 7, and 8 in the chromanol ring of the α-tocopherols, at two positions of 7 and 8 in the chromanol ring of the γ-tocopherols, and the positions of 8 in the chromanol ring of the δ-tocopherols. Simultaneously, the same substitution of methyl groups can be seen in the tocotrienols on the chromanol ring with unsaturation in the 16-carbon side chain having double bonds at the positions of 3′-, 7′-, and 11′ [3].

There are reports on different functional features of tocopherols and tocotrienols, including anticancer [4], antiobesity [5], antidiabetic [6], and cardioprotective [7] effects. Moreover, the functions of tocotrienols and tocopherols are different, and a recent study indicated a more effective activity of the tocotrienols than that of the

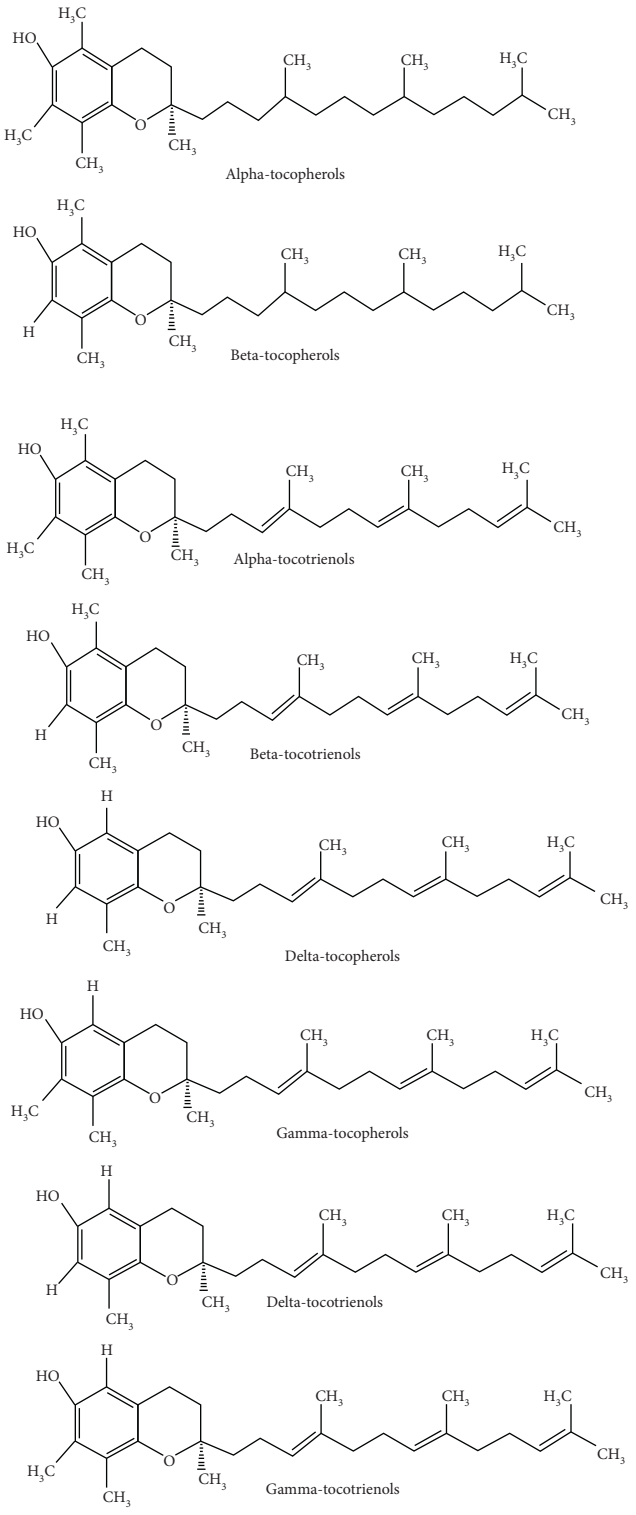

Figure 1: Structure of main tocols.

α-tocopherol in the control of chronic disorders [8]. The results of a review article on noncommunicable diseases revealed the inhibition of hormonal changes, oxidative stress, inflammatory response, and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase following the administration of tocotrienols, wherein the efficiency of tocotrienols was higher than that of tocopherol. The tocotrienols alone had a better influence on the treatment of diseases rather than the combination of tocotrienols and tocopherol [9]. Idriss et al. [10] found an *in vitro* anticancer activity for beta-tocotrienol, which was related to the induction of p53-independent apoptosis and the stop of the cell cycle G1 phase, and a higher anti-tumorigenic potential for beta-tocotrienol when compared with gamma-tocotrienol was also noted. The administration of tocotrienol-rich fraction (200 mg/kg) for about three months showed a positive impact on the myocardial antioxidative system in rats *via* new GSH synthesis [11]. The administration of gamma-tocotrienol reduced adipose tissue macrophages' recruitment and systemic and adipose inflammation in mice after a month, confirming the antiobesity activity [12].

A study aimed to investigate pharmacokinetics' impact of δ-tocotrienol at different high concentrations (750–1000 mg/d), the results of which showed Tmax value of 3–4 hours for all the isomers of tocotrienols and tocopherols, apart from α-tocopherol. According to this finding, it can be concluded that such high concentrations of tocotrienols are safe for human use especially as therapeutic agents in the management of some disorders, e.g., Alzheimer's disease, diabetes, and cancer [13]. Liang et al. produced α-tocopherol succinate modified chitosan (CS-TOS) and then encapsulated it using paclitaxel (PTX) to obtain micelles [14]. They could improve the performance and safety of PTX-loaded CS-TOS micelles via prolonged systemic circulation time and slow down the elimination rate than those of Taxol formulation. Based on the results from an *in vivo* study, the U14 tumor growth was significantly inhibited by PTX-loaded polymeric micelles, mitigating the toxicity of formulation [14].

2. Distribution of Tocols in Foods: Occurrence

Tocols are present in various foods, predominantly in fruits and plant seeds (see Table 1). Bastías-Montes et al. conducted a study to identify the tocols using the HPLC technique and reported the presence of β-sitosterol, tocotrienols, and α, β, γ, and δ-tocopherols from seed oil of Maqui berry (*Aristotelia chilensis*) fruit [15]. In a study, the two methods of direct injection and solid-phase microextraction (SPME) were combined with gas chromatography-mass spectrometry (GC-MS). The results detected α-tocopherol (LOD = 0.001 μg mL^{-1} and LOQ = 0.004 μg mL^{-1}) and α-tocopheryl acetate (LOD = 0.002 μg mL^{-1} and LOQ = 0.006 μg mL^{-1}), as well as the relative standard derivation (RSD) percent on days 4.8 and 8.8 in vegetables such as curly kale, celery, carrot, and onion [16]. The tocopherols were detected in vegetable oils using a novel flow-through column electrolytic cell for supercritical fluid chromatography system, the results of which reported 3.55 RSD percent [17]. Mezni et al. found α- and γ-tocopherols at the concentrations of 119 mg/kg and 23 mg/kg of oil, respectively, using HPLC analysis [18]. In a recent study, the yield of edible oil was 8.6 ± 1.2 g oil/100 g of guava seeds by supercritical CO_2 extraction, and then the γ-tocopherol with a concentration of 82.6 ± 3.7 mg/100 g oil was detected by the GC-MS method [19]. One of the most important products derived from fruit is Hass avocado (*Persea americana* Mill.)

Table 1: Levels of tocols in different foodstuff.

	Compound	Content	Reference
Raw foods			
Macauba fruits	α-Tocopherol	4373 μg/100 g	[29]
Cauliflower	α-Tocopherol and γ-tocopherol	23.47 mg/100 g and 74.55 mg/100 g	[30]
Yellow passion fruit (*Passiflora edulis*)	γ-Tocopherol	0.045 mg/100 g	[31]
Broccoli	Tocopherols	286 μg/g	[21]
Pitaya, jackfruit, durians, mango, and papaya fruits	α-Tocopherol	0.45, 0.20, 0.36, 0.16, and 0.26 mg/100 g DW	[32]
Oat, corn, spelt, buckwheat, wheat, rye, and rice bran	Total tocochromanol	5.5, 16.2, 15.8, 14.7, 12.8, 10.7, and 9.1 mg/100 g DW	[33]
Annatto seeds	γ–Tocotrienol, total tocotrienols	3.7 and 28.9 g/100 g extract	[34]
Barely grain	α-Tocotrienol, γ-tocotrienol, α-tocopherol, and γ-tocopherol	16.26, 4.67, 7.14, 0.55 mg/100 g DW	[35]
Einkorn wheat (*Triticum monococcum* ssp. *monococcum* L.)	α- and β-tocopherol and tocotrienol	12.2 mg/g dm, 4.79 mg/g dm, 12.7 mg/g dm, and 48.2 mg/g dm	[36]
Sesamum angustifolium	Tocopherol	7.34 mg α-TE/100 g	[37]
Hazelnuts	Tocols	41.9 mg/100g	[38]
Barley genotypes (*Hordeum vulgare* L.)	Total tocols	39.9 and 81.6 μg/g	[39]
Irish barley	α-Tocotrienol	46–58 μg/g dw	[40]
Fresh goji berries	α-Tocopherol and β-tocopherol	1.4 and 1.0 mg/100 g	[41]
Oils			
Sea buckthorn berries pulp oil	Total tocols	666–1788 mg/kg	[42]
Cold-pressed Moringa oleifera and Moringa peregrina seed oils	α-Tocopherol	139.61 and 137.89 mg/kg	[43]
Cane berry seed oils	Total tocopherols	75–290 mg/100 g	[44]
Apple, Japanese quince, and sea buckthorn seed oils	α-Tocopherol	58.77, 121.79, and 198.94 mg/100 g	[45]
Rapeseed, sunflower seed, linseed, sesame, and maize oils	α-Tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol	0.6–46.1 mg/100 g	[46]
Cold-pressed pumpkin seed (*Cucurbita pepo* L.) oil	Tocopherol and tocotrienol	94.29–97.79 mg/100 g	[47]
Soybean, corn, olive, and camellia oils	Total tocopherols	39.9 mg/100 g, 36.06 mg/100 g, 29.42 mg/100 g, and 17.72 mg/100 g	[48]
Sunflower, soybean, corn, hazelnut, peanut, and canola oils	Total tocols	488.88–913.51 mg/kg	[49]

oil, which has a great market value and is the richest source of tocopherols. Accordingly, Santana et al. extracted Hass avocado oil from dried fruit using conventional methods and then identified the α-tocopherol with the concentration of 11.6–21.0 mg/100 g using normal phase HPLC with a photon-diode array detector (PDA) [20]. In another study, the analytical method of LC-APCI-MS/MS was used to detect the main compounds in 12 vegetables from the Brassicaceae family, the results of which reported total levels ranging from 1.83 to286 μg/g DW for tocopherols [21]. Niro et al. employed the HPLC method to detect the tocols (tocopherols and tocotrienols) in the cereals [22]. According to the findings, total levels of tocols were 3.80 mg/100g d.w. in millet, 3.09 mg/100g d.w. in sorghum, 5.99 mg/100g d.w. in tef, 0.36 mg/100g d.w. in wild rice, 9.10 mg/100g d.w. in quinoa (white and pigmented), 18.06 mg/100g d.w. in cañihua, 6.31 mg/100g d.w. in amaranth, and 14.43 mg/100g d.w. in chia. Labuschagne et al. used the HPLC method and reported the maximum tocol level of 59.8 mg kg^{-1} in the whole flour of South Africa's wheat [23]. Dąbrowski et al. applied the HPLC-FLD technique to detect tocols such as β/γ-tocopherols in flaxseed oils using *n*-hexane (1%, m/V) and isopropanol (0.7%) solutions [24]. Bertolín et al. [25] implemented a fast, accurate, and simple method to determine carotenoids, tocopherols, retinol, and cholesterol in ovine lyophilised meat, liver, and milk and raw samples using the UHPLC method. Another recent study developed a UHPLC-LTQ-Orbitrap-HRMS-based method to determine the nutrients in rice; as a result, 21 nutrients have been identified and reported in less than 13 min [26]. The range of regression coefficients was between 0.05 and 10 μg/mL for tocopherols, tocotrienols, and β-carotene, between 0.1 and 50 μg/mL for phospholipids, and between 0.001 and 10 μg/mL for γ-oryzanols. Besides, the limit of detection was between 0.2, and 1.9 ng/mL, the limit of quantitation was between 0.7 and 6.3 ng/mL, the relative standard deviations were between 2.3 and 9.6%, and the recoveries were between 80.6 and 109.6% for all the analytes. Moreover, the total ion current fingerprint profile showed significant differences between the brown and white rice samples. The developed method provided a convenient analytical method to identify the nutrients in rice, confirming the effectiveness of this approach for food testing [26].

Knecht et al. [27] developed and validated an HPLC-FLD method for tocochromanol (tocopherols and tocotrienols) analysis equally suitable for raw and cooked vegetables. The recent study of Wu et al. [28], reported the integrated analysis of fatty acid, sterol, and tocopherol components of seed oils obtained from four varieties of industrial and environmental protection crops, i.e., *Amygdalus pedunculata* Pall. (Amygdalus), *Elaeagnus mollis* Diels (Elaeagnus), *Xanthoceras sorbifolium* Bunge (Yellowhorn), and *Paeonia suffruticosa* Andr. (Paeonia); particularly, three tocopherol homologues, α-, γ-, and δ-tocopherols, were present in four varieties of seed oils, and *Elaeagnus* oil contains the highest α-tocopherol (7.48 mg/100 g) and γ-tocopherol (109.58 mg/100 g) content [28].

A shot of the occurrence of tocols in different foodstuffs is given in Table 1, taking into account both more and less rich sources and more and less consumed foods.

The occurrence of tocols in food groups is described here. It is worth mentioning that the reviews [50–54] summarized common and emerging dietary sources of tocols, with particular attention to oils as the major natural dietary sources of tocopherols and tocotrienols, as well as main analytical methods and effects in food and biological systems.

3. Tocols and Databases: The Current State of the Art

Nowadays, the need of the categorization of bioactive compounds is emerging. A bioactive compound can be defined as a "compound that occurs in nature, part of the food chain, that can interact with one or more compounds of the living tissue, by showing an effect on human health" as reported by Biesalski et al. [55]. Databases can be viewed as a system that can generate and collect any data, information, and documentation especially organized for a rapid search and retrieval by using a computer (Encyclopaedia Britannica) [56]. They represent tools developed to simplify the storage, retrieval, modification, and deletion of data, all this in combination with several data-processing operations [57].

The development of specialized databases of components with nutritional and nutraceutical properties [58], at a National and European level, represents a current challenge to explore better the relationship between food, nutrition, health, and environment. Researches on the relationship between diet and health have led to a great interest in all bioactive substances present with the nutrients in food, and data on these and other compounds are increasingly required in the database system. Specialized databases could be useful for planning and evaluating clinical and epidemiological research studies on biologically active food contained compounds. They may represent a crucial tool to evaluate exposure measurement and, indeed, understanding the potential benefits of substances and extracts with nutritional and nutraceutical properties [59–62]. In the formulation of complete and comprehensive harmonized databases, possible limitations, as highlighted by Scalbert et al. [63], could be given both by the diversity of the chemical features of the compounds, the numerous dietary sources, the variability in content from a source to another, and by the different extraction procedures as well as the analytical techniques and methodologies used. Moreover, additional factors that should be considered in some cases are as follows: (i) only a few compounds within a class are investigated in literature studies and (ii) there is a lack of appropriate analytical methods.

As an example, as reported by the NDA Panel of the European Food Safety Authority (EFSA) in 2015 [64], the most of food composition databases in EU countries contain values for vitamin E as α-tocopherol equivalents (α-TEs), and only two countries (Finland and Sweden) considered in the intake assessment by EFSA have vitamin E values in their food composition databases as α-tocopherol values. Food Explorer, an innovative interface for finding food composition data, allows to simultaneously search information from most of the available databases from the European Union (EU) Member States, as well as Canada, United States, New Zealand, and Japan. Searching, for example, "Vitamin E" and selecting all the 39 databases, 398 records can be retrieved [65, 66].

The eBASIS database [67, 68] contains composition data and biological effects of over 300 major European plant-derived foods organized in 24 classes of compounds (i.e., glucosinolates, polyphenols, isoflavones phytosterols, glycoalkaloids, and xanthine alkaloids).

The EuroFIR eBASIS (Bioactive Substances in Food Information Systems) is an Internet-deployed food composition and biological effects resource based on a compilation work of experts that critically evaluated data extracted from peer-reviewed literature as raw data. eBASIS could be considered as the first EU-harmonized food composition database. Concerning tocols, in eBASIS, 4 data points are present for α-tocopherols [69].

Tocols represent essential ingredients in many dietary supplements. Nowadays, a great attention is given to the use of natural substances in different fields such as nutraceutical and cosmeceutical ones [70–72].

Recently, information on the compositions reported on labels of selected dietary supplements has been collected for the development of a Dietary Supplement Label Database according to products' availability on the Italian market, including also items consumed in the last Italian Dietary Survey [73]. Five hundred and fifty-eight products were entered into the aforementioned database, giving a homogeneous picture of the major classes of food supplements consumed in Italy. It is worth underlining that, for each item, a code was assigned following the food classification system FoodEx2 developed by EFSA [74], a tool for the standardization and harmonization of the data among different countries to guarantee interoperability between different databases [75].

In particular, in Italian Dietary Supplement Label Database, tocols are present as ingredients in different categories of products containing vitamin E and, in particular, as main ingredients in vitamin only supplements [A03SL], combination of vitamin and mineral only supplements [A03SN], mixed supplements/formulations [A03TC], or as minor ingredients in formulations containing special fatty acids (e.g., omega-3 and

essential fatty acids) [A03SX], protein and amino acid supplement [A03SY], and micronutrient supplement for sports people [A03SB] [73]. The ingredient vitamin E was indicated using the facet [F04.A0EXL]. The code in square brackets identify the category of products.

Moving towards the scenario of metabolic pathways and the benefits of bioactive compounds in humans, Human Metabolome Database (HMDB) has been considered, in particular, the version 4.0 [76, 77]. It is a freely available electronic database of information regarding small molecule metabolites found in the human body. It well linked chemical data, clinical data, and molecular biology/biochemistry data. In the above database, for instance, the following information is reported including metabocard for α-tocopherol (https://hmdb.ca/metabolites/HMDB0001893) and β-tocopherol (https://hmdb.ca/metabolites/HMDB0006335) reporting information on record information, metabolite identification, chemical taxonomy, ontology, physical properties, chemical spectra, biological properties, normal concentrations, abnormal concentrations, associated disorders, diseases, external links, and references.

4. Conclusion

Alongside the increasing attention towards the standardization and need of food categorization and classification, this perspective paper gives an updated shot of the occurrence of tocols in food and existing databases as useful tool in nutrition-related studies, i.e., dietary intake assessment and exposure studies.

Although the tocols and their different homologous derivates have been consuming as additives in food and pharmaceutical industries and evaluated using advanced analysis methods during the last decade, extracting and analyzing them from complex food matrices is still time-consuming and needs a significant quantity of organic solvents. Therefore, there is need for simple, fast, and green extraction protocols using environmentally friendly solvents. Despite the absence of any evidence of possible adverse effects following the use of tocols, caution should still be exercised in recommending the use of supplement containing them, with special attention to the recommended intake and dosage.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Contribution of Tocols to Food Sensorial Properties, Stability, and Overall Quality

Amélia Delgado,[1] **Said Al-Hamimi**,[2] **Mohamed Fawzy Ramadan**,[3] **Maryna De Wit**,[4] **Alessandra Durazzo**,[5] **Kar Lin Nyam**,[6] **and Manel Issaoui**[7]

[1]*MED-Mediterranean Institute for Agriculture, Environment and Development, University of Algarve, Gambelas Campus, Faro 8005-191, Portugal*
[2]*OQ, SablaX, P.O. Box 261, Muscat 118, Oman*
[3]*Deanship of Scientific Research, Umm Al-Qura University, Makkah, Saudi Arabia*
[4]*Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Nelson Mandela Road 205, P.O. Box 339, Bloemfontein, Free State, South Africa*
[5]*CREA-Research Centre for Food and Nutrition, Rome, Italy*
[6]*UCSI University, Kuala Lumpur, Malaysia*
[7]*Lab-NAFS 'Nutrition-Functional Food & Vascular Health', Faculty of Medicine, University of Monastir, Monastir 5019, Tunisia*

Correspondence should be addressed to Amélia Delgado; amdelgado@ualg.pt

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This paper reviews the contribution of tocopherols and tocotrienols (tocols) to food quality as well as their bioactivity and health-promoting properties, which have attracted researchers and food technologists. Tocols are lipophilic phenolic antioxidants encompassing tocopherols that are characterized by a saturated side chain and tocotrienols with an unsaturated isoprenoid side chain. Tocols are natural constituents of several foods like dairy, vegetable oils, nuts, and grains. Their presence in foods, namely, as food additives, helps prevent lipid oxidation, which negatively affects the sensorial quality of foods, and even the nutritional value and safety. Supplementation of animals' diets with tocopherols has proven its effectiveness in preserving fresh color and flavor of the meat. Although alfa-tocopherol displays much higher vitamin E activity than other tocols, health outcomes have been reported for tocotrienols, thus calling for more studies.

1. Introduction

The interest in the study of tocols has dramatically increased in the last decades, most probably in raising awareness on the health impact of individual food items and diets. Tocols include tocopherols and tocotrienols, whose biosynthetic pathways are exclusive of photosynthetic organisms (plants and cyanobacteria) and are essential nutrients in mammals mostly due to their vitamin E activity and/or antioxidant and other bioactivities [1]. Tocols are lipophilic phenolic antioxidants that protect polyunsaturated fatty acids (PUFA) from lipid peroxidation in food matrices and in the human body, where reactive oxygen species (ROS) may come from environmental exposure or are formed as side products of cell metabolism. Tocols and other antioxidants accept unshared highly energetic electrons from ROS, thus preventing damage to unsaturated fatty acids, whether part of a food or biological membrane.

The primary dietary sources of tocols are lipids, notably butter and vegetable oils (as virgin olive oil). The current review encompasses a rapid overview of tocols' chemistry, their main features, and occurrence in foods, a brief review on sensorial assessment, and other factors that determine food choice. We also describe the contribution of main tocols to color and flavor of foods, as well as their role in sensorial food quality. We aim to reach students and researchers in food quality and nutrition and food technologists, in search of focused information to support their research and innovations efforts.

2. Sensorial Perceptions and Food Choices

Humans associate wider roles and significance to food, mainly surpassing its primary function of supplying essential nutrients. Food is associated with pleasure, social and religious occasions, and, more recently, healthy and sustainable lifestyles. A myriad of diets and processed foods have been arising to the market, advertised as healthy and/or environmentally friendly, often by blaming or amplifying certain features or constituents of foods to align them with consumers' preferences at the time of shopping [2]. International organizations have been raising attention to climate change and environmental issues, such as soil degradation and biodiversity loss, and the burden of obesity and noncommunicable diseases associated with the so-called "western diet" [3, 4].

Combining the constraints related to human health and environmental dimensions while appealing to the senses seems to be a complicated equation in a diet. A very few dietary patterns, notably the Mediterranean Diet (MD), can simultaneously address all these factors, translated in the "one health" approach [5, 6]. The MD can stimulate the senses through a wide range of colors, flavors, aromas, and scents, which are mainly conveyed by the large quantities and variety of plant-based foods, thus valuing agrobiodiversity and addressing other sustainability factors. In the MD, the food components that bring nutritional, health, and environmental benefits are the same ones that convey colors and flavors [7–9].

When appreciating food, all senses are involved, and our preferences are also dictated by memories, beliefs, cultural aspects, and other subjective factors. In respect to sensorial aspects, the taste of foods is detected by receptors on the tongue and interpreted in the brain. Basic tastes are categorized into sweet (e.g., fig), bitter (e.g., coffee), sour (e.g., yogurt), salty (e.g., table olive), and umami (cheese). Aroma detected by olfactory pathways is usually interlinked with taste, playing an essential role in the sensations caused by a food. The physical sensations (color, temperature, texture, and hardness) and chemical sensations (chemical irritation in the mouth and throat) also affect the overall perception of food [10].

The term "flavor," as used by sensory analysis specialists, refers to all these sensations. When a piece of food is introduced into the mouth, it is smelled in the process and sensed through the gut, and a whole cascade of chemical reactions and nerve responses are triggered. When interpreted in the brain, other factors such as perceptions from the surrounding environment to cultural and ethical judgments are all considered. Thus, the feeling of how enjoyable a meal is or how and when we like or dislike a particular food is highly subjective, variable, and quite complex [11, 12].

When examining foods at the molecular level, plant foods stand out for their pigments, like carotenoids, tocols, xanthophylls, chlorophylls, and polyphenols that, besides the color, also act as vitamins, provitamins, or antioxidants; plant foods also contain molecules that convey aroma (e.g., tocols, aldehydes, and aliphatic and triterpenic alcohols) which are often bioactive too, displaying multiple features of interest in foods (color, flavor, and bioactivity) [13].

3. Tocols and Vitamin E Activity

The molecular structure of tocopherols consists of a chromanol ring connected to a long carbon side chain. Variations in the number and position of the methyl groups on the ring result in different forms named α-, β-, γ-, and δ-tocopherol, all provided as a blend in the abovementioned dietary sources, although in different proportions and depending on the species and the considered edible part. Tocopherols are more abundant than tocotrienols, which are only found in some plant species' fruits and seeds [1, 14].

It has been argued that α-, β-, γ-, and δ-tocopherols are all forms of vitamin E with different levels of activity and bioavailability. α-Tocopherol is the preferred form of vitamin E, absorbed and accumulated in humans and other mammals. On the other hand, β-, γ-, and δ-tocopherols are referred to as having little vitamin E activity, but they retain similar antioxidant activity and may convey additional health benefits [14–16].

There has been some debate in relation to vitamin activity of tocols. Some authors claim that the 8 isomeric forms of tocopherols and tocotrienols all have vitamin E activity, though to a different extent [1, 17–19], while the EFSA NDA Panel [20] considers vitamin E as being α-tocopherol only, despite acknowledging that other tocopherol isomers and tocotrienols may have antioxidant activity. On its turn, NIH [21] accepts that vitamin E may exist in 8 different chemical forms but states that α-tocopherol is the only form maintained in plasma and recognized to meet human requirements for vitamin E.

4. Colors and Flavors of Main Tocols

Tocopherols and tocotrienols are naturally transparent and viscous substances with colors ranging from light yellow to reddish-brown [14]. When in the form of powders, tocopherols take a tan or tan-to-reddish color. Besides the beneficial health properties, tocols play a vital role in the stability of color and flavor of foods.

Carotenoids and tocopherols are closely related in their functions and location in plants, as both are lipid-soluble antioxidants found in chloroplasts. In addition to their roles in photosynthesis, carotenoids and tocols are essential components of animal diets, including humans, for their vitamin and antioxidant activities [16]. Synergy and reactions between tocopherols, carotenoids, ascorbic acid, and other components have been reported to affect food quality [16].

For instance, the autoxidation of lipids observed in vegetable oils is initiated by the free radicals, leading to the formation of lipid peroxy radicals and finally lipid hydroperoxides, which are unstable and can trigger further propagation reactions. As such compounds play a crucial role as intermediates of oils' autoxidation reactions, the "peroxide value" is a parameter that gives a measure of the extent of primary oxidation of edible oils and is of capital

importance in their grading and hence in their quality [22]. Propagation reactions triggered by hydroperoxides are known as branching steps or secondary decomposition reactions, and their products are responsible for causing rancid off-flavors. Oxidation of lipids is a major cause of deterioration of the quality of foods affecting flavor, color, texture, and even the nutritional value and safety. Safety is of particular concern when speaking of ultra-processed and frying foods, in which further degradation reactions may occur with the formation of toxic compounds [23, 24].

Tocopherols and tocotrienols are best known for their ability to accept high energy electrons (free radicals) and terminate oxidation chain reactions, thus preventing changes in color and flavor of foods containing natural (e.g., hazelnut) or added tocols (e.g., margarine).

Supplementation of feed with tocopherols (in poultry, cattle, and fish diets) has shown effective results in delaying lipid oxidation and subsequently increasing the shelf life with preserved freshly color and flavor. Researches on feed quality and the relation to food quality started in the 1970s and are still a field of interest. The detailed information on the use of tocols in the feed (as vitamin supplements or as preservatives) was reported.

5. The Role of Tocols in Food Quality

5.1. Tocols as Natural Constituents in Food. As lipophilic molecules, tocols are natural constituents of a range of foods, as dairy (e.g., butter), vegetable oils (as virgin olive oil), nuts (as almonds and hazelnuts), vegetables, and grains (notably wheat germ), which are known sources of vitamin E and other lipophilic vitamins, as the presence of fat is required for active absorption [20, 21]. In addition to the activities referred to above as an antioxidant, vitamin E is involved in immune function, anti-inflammatory processes, inhibition of platelet aggregation, cell signaling, regulation of gene expression, and other metabolic processes [20, 21].

According to EFSA [20], the average α-tocopherol absorption from a usual diet is about 75% and defines adequate intakes for α-tocopherol (based on observed intakes in healthy populations) in 13 mg/day for men, 11 mg/day for women, and 9 mg/day for children of both sexes, aged 3 to <10 years, and 6 mg/day if aged <3 years, considering that no vitamin E deficiencies have been reported in Europe. NIH [21] sets higher levels for the American population, with recommended dietary allowance for α-tocopherol of 15 mg/day for adults irrespective of sex and 11 mg/day for children aged 9 to <13 and decreasing until 6 mg/day for children aged <3 years [25].

The bioavailability of vitamin E is influenced by a range of factors, including fixed ones, like gender, age, and genetic constitution, as well as others that depend on the environment and can be changed, as food habits and lifestyle, impacting dietary guidelines for different population groups.

Vitamin E deficiencies are rare and reported in premature babies of very low birth weight, rare inherited disorders, Crohn's disease, cystic fibrosis, or medical conditions interfering with the ability to secrete bile from the liver into the digestive tract [21]. Thus, vitamin E supplements are justifiable only in some instances as, in general, a balanced diet provides the necessary levels of vitamin E and other tocols for health benefits. It should also be noted that naturally occurring α-tocopherol exists in only one stereoisomeric form, known as RRR α-tocopherol. In contrast, synthetically produced forms contain equal amounts of all stereoisomers and are known as all-racemic α-tocopherol, with about half the potency of the natural form [21, 26].

In the body, tocols undergo a series of complex metabolic processes comprising intestinal absorption, vascular transport to the liver, and hepatic sorting by intracellular binding proteins, such as the significant α-tocopherol-transfer protein (α-TTP), which preferentially binds α-tocopherol rather than other tocopherols or tocotrienols [20, 27]. According to EFSA panel on dietetic products, nutrition, and allergies (NDA) [20], because α-TTP and ω-hydroxylase (a key enzyme in the liver) have a much higher affinity towards α-tocopherol than other tocopherols, the former one predominantly accumulates in body tissues. In contrast, other tocols are preferentially catabolized in the liver. However, doubt persists on the functions and health outcomes of other tocols, namely, tocotrienols, which have not been so thoroughly studied, and some authors discuss their probable hypocholesterolemic, anticancer, and neuroprotective properties, as well as tocotrienols' potential action against inflammation-associated diseases [17, 19].

In the case of ingested natural vitamin E, it is necessary to take into account the interactions with the food matrix, resulting in enhanced bioactivities when synergies are established with other food constituents, such as vitamin K [27], ascorbic acid, and carotenoids [28].

In this context, the vitamin content of plant foods, and hence nutritional quality, varies widely with a range of factors, including agronomic techniques. Due to the lack of robust data, debate exists on the existence of sufficient differences in sensorial and nutritional quality of organic produce vs. intensive systems [29–31]. When considering the nutritional quality of foods, it is necessary to recognize that competing nutrients can reduce the bioavailability of certain compounds as tocopherols in the food matrix [27]. There is not enough science-based evidence supporting the superior composition on bioactive compounds of organic produce, even though evidence shows that organic foods are lower in toxic compounds bringing proven benefits to human health and the environment. A more in-depth discussion is out of the scope of the present review. For sensorial and nutritional quality, the food matrix seems to have a much higher impact, potentiating synergies between distinct classes of compounds (e.g., tocols and other antioxidants), or reactions with antinutrients that decrease the bioavailability of vitamins and others [32].

The content in vitamin E of primary dietary sources is given in Table 1, which indicates available data on the composition in other tocols. As deduced from Table 1 and corroborated by authorities [20, 21], a balanced diet should provide the necessary amounts of vitamin E and other potentially health-promoting tocols. As shown in Table 1, the primary natural sources of vitamin E to the diet are edible oils (notably olive oil) and certain nuts.

Table 1: Natural dietary sources of vitamin E and average content (mg/100 mg) according to ANSES-Ciqual food composition table.

Food group	Description	Average concentration of α-tocopherol (mg/100 g)
Dairy	Butter (80% fat)	2.11
	Cheddar cheese (cow's milk)	0.78
Vegetable oils	Olive oil (virgin)	22.3
	Palm oil*	15.9
	Sunflower oil*	57.3
Grains	Wheat germ	10.2
Vegetables	Spinach, boiled	3.98
	Basil, fresh	0.8
	Tomato, raw	0.66
Fruits	Mango, pulp, raw	2.05
	Avocado, pulp, raw	2.23
	Kiwi	0.96
Nuts	Almond, with peel	22, 3
	Walnut, fresh	1.6
	Hazelnut	16.3

*Commercial oils that may include additional vitamin E as a food additive. Data retrieved from ANSES-Ciqual food composition table: https://ciqual.anses.fr.

Despite the differences of natural and synthetic compounds (including esterification to prolong its shelf life while protecting its antioxidant properties), the organism absorbs and metabolizes different isomers and esters as efficiently as natural molecules [21].

5.2. Tocols as Food Additives. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) defines food additives as "substances added to food to maintain or improve its safety, freshness, taste, texture, or appearance" [33]. The safety of additives that can be used in foods traded internationally should be evaluated firstly by the joint FAO/WHO Expert Committee on Food Additives [33]. Food additives are thus substances intentionally added to foodstuffs in small quantities generally aiming to improve their sensorial features and/or increase their time-span for consumption, and tocols, most particular tocopherols, are recognized as safe food additives by official food authorities [34–36]. Codex Alimentarius code numbers for d-α-tocopherol, dl-α-tocopherol, and tocopherol concentrated mix (a mixture of several different types of vitamin E) are 307a, 307c, and 307b [33]. The maximum levels of tocopherols in foods have been established by the Codex Alimentarius Commission and are summarized in Table 2.

As mentioned above, and again stressed, main tocols have vitamin E activity, although to different levels, in addition to other potential health benefits. In this respect, JECFA derived an acceptable daily intake (ADI) for vitamin E of 0.15–2 mg/kg body weight (bw)/day for dl-α-tocopherol [34] and Codex Alimentarius recommends the incorporation of tocopherols in some foods, namely, in vegetable oils to prevent rancidity, as the often necessary oil refining process causes a decrease in the concentration of this vitamin, as well as of other antioxidants that could be present in crude oil fractions. According to [38], for named vegetable oils, the authorized concentration of tocopherols (tocopherol, d-α, tocopherol concentrate, mixed and tocopherol, dl-α) is about 300 mg/kg (Table 2). The standard for olive oils and olive-pomace oils [39] recommend an addition level of tocopherols [d-α-tocopherol (INS 307a), mixed tocopherol concentrate (INS 307b), and/or dl-α-tocopherol (INS 307c)] to refined olive oil and other grades, stating that the concentration of α-tocopherol in the final product shall not exceed 200 mg/kg [40].

From the European perspective, the use of food additives is regulated by specific laws in the European Union, supported by the evidence-based and expert opinions of the European Food Safety Authority (EFSA). These legal regulations consider the specificity of the food, in which the additive is incorporated, the maximum permitted quantity, the chemical structure, and the degree of purity [41] (Table 3).

EFSA derived a tolerable upper intake level (UL) for vitamin E of 300 mg/day for adults [34]. The EFSA ANS panel has reevaluated the safety of tocopherols-rich extract of natural origin (E 306), synthetic α-tocopherol (all-rac α-tocopherol; dl-α-tocopherol; E 307), synthetic γ-tocopherol (dl-γ tocopherol; E 308), and synthetic δ-tocopherol (E 309) on food additives, and nutrient sources added to food [34] and claimed that "tocopherols (E 306-E 309) are not of safety concern at the levels used in food".

Tocopherol occupies the category of antioxidants in the list of food additives. It is used in an extensive series of foodstuffs to abolish the oxidation of fatty acids and vitamins [42]. A considerable number of studies have focused on using tocopherols as additives in food [43–45]. Wagner and Elmadfa [45] have tested the effects of tocopherols and their mixtures on the oxidative stability of olive oil and linseed oil under heating. These authors registered an antioxidant activity at all levels of the addition of tocopherols that depended on the concentration level and the mixture of tocopherols. Incorporation of α-tocopherol at up to 0.2% increased the oxidative stability of refined olive oil and decreased the formation of phytosterol oxidation products, as reported by Tabee et al. [44]. A comparative study on the impact of certain antioxidant compounds on the stability and prolongation of the mayonnaise's shelf life was carried

Table 2: Example of general standard for food additives' provisions for tocopherols.

Food category	Max level (mg/kg)
Aromatized alcoholic beverages (e.g., beer, wine, and spirituous cooler-type beverages, low alcoholic refreshers)	5
Batters (e.g., for breading or batters for fish or poultry)	100
Beverages whiteners	200
Breakfast cereals, including rolled oats	200
Butter oil, anhydrous milk fat, ghee	500
Dried fruit	200
Flavored fluid milk drinks	200
Vegetables oils and fats	300
Fish oil and other animal fats	300

Adapted from the update to the 42nd session of [37] http://www.fao.org/gsfaonline/groups/details.html?id=2.

Table 3: Use of tocopherols as food additives in accordance with European legislation.

General data	The additive is authorized to be used in the following category (ies)	Legislation (details on European Regulation/Directive)
Tocopherol-rich extract E 306	(i) Fats and oils essentially free from water (excluding anhydrous milk fat)/ individual restriction/exception: quantum satis, except virgin olive oils and olive oils	(i) No. 1129/2011, applicable as from 01/06/2013
	(ii) Infant formulae as defined by directive 2006/141/EC (13.1.1)	(ii) No. 1129/2011, applicable as from 01/06/2013
	(iii) Follow-on formulae as defined by directive 2006/141/EC (13.1.2)/ Individual restriction/exception: ML = 10 mg/kg	(iii) No. 1129/2011, applicable as from 01/06/2013
	(iv) Processed cereal-based foods and baby foods for infants and young children as defined by directive 2006/125/EC (13.1.3)/individual restriction/exception: ML = 100 mg/kg; only fat-containing cereal-based foods including biscuits and rusks and baby foods	(iv) No. 1129/2011, applicable as from 01/06/2013
	(v) Other foods for young children (13.1.4)/individual restriction/ exception: ML = 100 mg/kg	(v) (EU) No. 1129/2011, applicable as from 01/06/2013
Alpha-tocopherol E 307	(i) Fats and oils essentially free from water (excluding anhydrous milk fat) (2.1)/individual restriction/exception: quantum satis, except virgin oils and olive oils; ML = 200 mg/kg, only refined olive oils, including olive pomace oil	(i) No. 1129/2011, applicable as from 01/06/2013
	(ii) Infant formulae as defined by directive 2006/141/EC (13.1.1)	(ii) No. 1129/2011, applicable as from 01/06/2013
	(iii) Follow-on formulae as defined by directive 2006/141/EC (13.1.2)/ individual restriction/exception : ML : 10 mg/kg	(iii) No. 1129/2011, applicable as from 01/06/2013
	(iv) Processed cereal-based foods and baby foods for infants and young children as defined by directive 2006/125/EC (13.1.3)/individual restriction/exception: ML = 100 mg/kg, only fat-containing cereal-based foods including biscuits and rusks and baby foods	(iv) No. 1129/2011, applicable as from 01/06/2013
	(v) Other foods for young children (13.1.4)/individual restriction/ exception: ML : 100 mg/kg	(v) No. 1129/2011, applicable as from 01/06/2013
Gamma-tocopherol E 308	(i) Fats and oils essentially free from water (excluding anhydrous milk fat) (2.1)/individual restriction/exception : ML : quantum satis, except virgin oils and olive oils	(i) No. 1129/2011, applicable as from 01/06/2013
	(ii) Infant formulae as defined by directive 2006/141/EC (13.1.1)	(ii) No. 1129/2011, applicable as from 01/06/2013
	(iii) Follow-on formulae as defined by directive 2006/141/EC (13.1.2)/ individual restriction/exception: ML = 10 mg/kg	(iii) Legislation: (EU) no. 1129/2011, applicable as from 01/06/2013
	(iv) Processed cereal-based foods and baby foods for infants and young children as defined by directive 2006/125/EC (13.1.3)/Individual restriction/exception: ML = 100 mg/kg, only fat-containing cereal-based foods including biscuits and rusks and baby foods	(iv) No. 1129/2011, applicable as from 01/06/2013
	(v) Other foods for young children (13.1.4)/individual restriction/ exception: ML = 100 mg/kg	(v) No. 1129/2011, applicable as from 01/06/2013

Table 3: Continued.

General data	The additive is authorized to be used in the following category (ies)	Legislation (details on European Regulation/Directive)
Delta-tocopherol E 309	(i) Fats and oils essentially free from water (excluding anhydrous milk fat) (2.1)/individual restriction/exception: ML = quantum satis, except virgin oils and olive oils	(i) No. 1129/2011, applicable as from 01/06/2013
	(ii) Infant formulae as defined by directive 2006/141/EC (13.1.1)	(ii) No. 1129/2011, applicable as from 01/06/2013
	(iii) Follow-on formulae as defined by directive 2006/141/EC (13.1.2)/ individual restriction/exception: ML = 10 mg/kg	(iii) No. 1129/2011, applicable as from 01/06/2013
	(iv) Processed cereal-based foods and baby foods for infants and young children as defined by directive 2006/125/EC (13.1.3)/individual restriction/exception: ML = 100 mg/kg, only fat-containing cereal-based foods including biscuits and rusks and baby foods	(iv) No. 1129/2011, applicable as from 01/06/2013
	(v) Other foods for young children (13.1.4)/individual restriction/ exception: ML = 100 mg/kg	(v) No. 1129/2011, applicable as from 01/06/2013)

out by Alizadeh et al. [43]. This study used tocopherols, rosemary essential oil, and *Ferulago angulata* extract, showing the high potency of tocopherol in maintaining the stability of mayonnaise. 10% of the extract from the tocopherol solution was able to scavenge up to 99% of free radicals from DPPH (2, 2-diphenyl-1-picrylhydrazyl)·. Tocopherol was notable in controlling the primary oxidation steps (after four months of storage), showing a considerable capability to inhibit the formation of some secondary products, such as hexanal and heptanal [43]. The overall acceptability of the mayonnaise supplemented with tocopherol was good in terms of the sensory score, and the molecule seems to be compatible with mayonnaise's flavor. Based on this study, the authors recommended using tocopherols as an alternative to synthetic antioxidants in food [43].

6. Final Remarks

Tocols encompass tocopherols and tocotrienols, collectively known as vitamin E, and are associated with lipids in animal-based (e.g., dairy) and vegetable-based (e.g., oils and nuts) food. Chemical reactions, which lead to the degradation of food constituents under processing and storage conditions, may cause the accumulation of compounds that compromise the sensorial and nutritional quality of foodstuffs. Notably, the oxidative deterioration of fat-rich food can be protected by tocols. Under food processing and storage conditions, tocols offer protection against oxidative deterioration of foodstuffs.

The consumption of natural and organic foods is becoming more and more fashionable and is gaining new markets in spite of ultra-processed foods. However, the vulnerability of certain foodstuffs, such as the oxidation of fats and oils, poses the problem of the addition of additives to avoid color changes, rancidity, and the appearance of undesirable tastes and odors. A scan of scientific research confirms the relevance of tocopherols in maintaining the sensory properties of foods in addition to their role as effective antioxidants. In fact, their physicochemical properties, low volatility, and good solubility in fats and oils give them the necessary resistance to processes using high temperatures. They have been incorporated into many formulations including baked goods, grains, dehydrated potatoes, fried nuts and noodles, meat and eggs, and tuna fillets. On the other side, while the tocopherols have been investigated extensively, little is known about the tocotrienols but some studies suggest that both the molecular and therapeutic targets of the tocotrienols are distinct from those of the tocopherols, and their role in cancer prevention and treatment, as well as in cardiovascular and neurological diseases, awaits further investigation.

Data Availability

The data (from literature review and databases) used to support the current work are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to the present work.

Authors' Contributions

AM Delgado and MFR Hassanien structured the manuscript; AM Delgado and M Issaoui wrote the manuscript with collaboration of S Al-Hamimi and MRF Hassanien; AM. Delgado, M Issaoui, and MFR Hassanien reviewed the manuscript with collaboration of M De Wit, A Durazzo, and KL Nyam.

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