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Differentiation of Refined and Virgin Edible Oils by Means of the *trans*- and *cis*-Phytol Isomer Distribution

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ABSTRACT: The differentiation of nonrefined (e.g., cold-pressed) and refined edible oils is an important task in food control because of the higher commercial value of the former. Here, we explored the suitability of the relative abundance of *cis*-phytol as a marker for authentication of nonrefined edible oils. Phytol, the tetramethyl-branched, monoenoic alcohol, is found widespread in nature as a part of chlorophyll. In chlorophyll, only *trans*-phytol is found. In this study, we present a method for the analysis of the phytol isomers, considering that traces of *cis*-phytol (contributing 0.1% to the phytol content) can be determined next to *trans*-phytol. For this purpose, phytol was gathered with the unsaponifiable matter from the oil, trimethylsilylated, and analyzed by gas chromatography coupled to mass spectrometry. With this method, 27 samples of edible oils (16 refined and 11 nonrefined edible oils) were analyzed for the abundance of *cis*-phytol relative to *trans*-phytol. In the nonrefined oils (e.g., olive oil, rapeseed oil, maize oil, and sunflower oil), *cis*-phytol contributed 0.1% ($n = 3$) or less ($n = 8$) to the phytol content. In contrast, the refined olive oils ($n = 4$) contained a share of 1.3−3% *cis*-phytol; the refined rapeseed oil ($n = 3$) contained a share of 0.7−1.0% *cis*-phytol; and the refined sunflower oil ($n = 4$) contained a share of 0.3−0.9% *cis*-phytol. Only one refined pomegranate kernel did not contain *cis*-phytol. The phytol concentration was not suited to distinguish nonrefined from refined oils. In contrast, our data suggest that the virtual absence of *cis*-phytol can be used as a marker for nonrefined (e.g., cold-pressed) edible oils.

KEYWORDS: *Edible vegetable oils, cis-phytol, trans-phytol, GC/MS*

INTRODUCTION

Edible plant oils are available in different qualities. Hence, quality and authenticity control is an important issue to avoid consumer fraud. For instance, adulteration of edible vegetable oils with used frying oil has been studied by Fourier transform infrared spectroscopy.[1] Methods to distinguish different edible plant oils were based on differences in the wax components.[2] Further tasks of food control exist with regard to examinations of the quality of given oils. Refined edible oils are cheaper, and they are commonly regarded of lower quality compared to virgin oils. A favorable plant oil in Germany is olive oil. Currently, the preference of consumers for native high-quality oils is on the rise.[3] Retail labels of extra-virgin olive oil and virgin olive oil represent the highest quality, and they must not contain any refined oil. These supreme qualities are exclusively gained by cold pressing.[4] Cold-pressed edible oils are sold at a higher prize, and for this reason, it is important to control the authenticity of the oils with regard to fraud detection caused by the addition of refined oils. Current markers for olive pomace and lampante olive oils are the phytosterols uvaol, erythrodiol, and stigmastadiene.[4] One drawback is, however, that this method is not suited for most other edible oils. However, other types of edible oils (e.g., rapeseed oil and sunflower oil) are increasingly marketed in cold-pressed quality. The use of different methods, in dependence of the type of oil, complicates the analysis. Hence, the availability of a universal marker to distinguish different types of cold-pressed oils from refined analogues would be advantageous. The perfect marker should be present in all plant oils, and it should be changed during the technical refining process. One step forward was provided by Luterotti et al., who determined the distribution of *trans*/*cis* isomers of *β*-carotene by means of thermal lens spectrometry.[5] Unfortunately, this analytical tool is not widely distributed in food control.

In a recent study on the unsaponifiable matter of plant oils, we also detected 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol).[6] Phytol is of general occurrence in nature, especially because it occurs constantly as a component of chlorophyll.[7] Chlorophyll-bound phytol is exclusively *trans*-configurated. Similar to geranylgeraniol, the diterpene phytol plays an important role in the chloroplasts of higher plants because both serve as intermediates in the biosynthesis of chlorophyll, phylloquinone, and tocopherol.[8] The presence of phytol in human food is mainly restricted to spinach, beans, raw vegetables, and asparagus.[9] The reported levels of free phytol in these products were 0.7−2 mg/kg of undried food (i.e., the low micromoles per kilogram range).[9] Higher amounts (12.5−14.7 mg/kg) were only reported in dried tea leaves and beef-stock cubes.[10]

Previous gas chromatography with mass spectrometry (GC/MS) analyses of the trimethylsilylated unsaponifiable matter of rapeseed oil indicated that phytol contributed ∼2% to this fraction.[6] A closer inspection by means of high-speed countercurrent chromatographic (HSCCC) fractionation indicated the presence of low amounts of *cis*-phytol.[6] The *cis* isomer eluted slightly prior to *trans*-phytol from the GC column and contributed with ∼6% of *trans*-phytol to the total phytol content. The parallel analysis of a native extra-virgin olive oil did not show the *cis*-phytol peak. For this reason, we

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assumed that the *cis*-phytol was formed as an artifact during the refining process of the crude plant oils. Owing to the known fact that double bonds can be isomerized, it appeared plausible that this reaction could take place during the refining of crude oils.[11] Consequently, the presence of *cis*-phytol was thought to be linked with the refining of edible oils.

The aim of our study was to analyze a range of further samples of nonrefined, mainly cold-pressed oils (n = 11) as well as refined (n = 16) edible plant oils (including olive oils, sunflower oils, and rapeseed oils) for the ratio of *cis*- and *trans*-phytol. For this purpose, the unsaponifiable matter was separated from the fatty acids and silylated and the *cis*/*trans* isomers of the trimethylsilylated phytol were analyzed by GC/MS operated in the selected ion monitoring mode (GC/MS–SIM).

MATERIALS AND METHODS

Samples, Standards, and Chemicals. Edible plant oils were obtained from Heess (Stuttgart, Germany) or bought retail in different shops in Stuttgart (Germany). Synthetic phytol (≥95% purity) was from Merck (Darmstadt, Germany). The ratio of *cis*-phytol (CAS number 5492-30-8) to *trans*-phytol (CAS number 150-86-7) in this standard was ∼1:2. The silylation reagent *N*,*O*-*bis*(trimethylsilyl)-trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS), 99:1, was from Supelco (Bellefonte, PA). *n*-Hexane [high-performance liquid chromatography (HPLC) gradient grade] was from Th. Geyer (Renningen, Germany). KOH was from Carl Roth (Karlsruhe, Germany). Ethanol was from BASF (Ludwigshafen, Germany). Ethanolic KOH was prepared from 50% aqueous KOH and ethanol in the ratio of 1:9 (v/v).

Sample Preparation. About ∼50 mg of plant oil and 2 mL of ethanolic KOH was heated for 1 h at 80 °C. The vial was shaken from time to time. *n*-Hexane (1 mL) and demineralized water (2 mL) were added to the resulting homogeneous solution. An aliquot of the *n*-hexane phase (100 μL) was transferred to a 1.5 mL vial, and the solvent was evaporated. Then, 50 μL of BSTFA/TMCS (99:1) was added, and the solution was heated for 20 min to 70 °C. After the trimethylsilylation, 450 μL of *n*-hexane was added and analyzed by GC/MS.

Gas Chromatography Coupled to Electron Ionization Mass Spectrometry (GC/EI–MS). Analyses were carried out with a 5890 series II plus/5972 GC/MS system (Hewlett-Packard/Agilent, Waldbronn, Germany) equipped with a 7673 autosampler. Separations were performed with a 30 m × 0.25 mm inner diameter fused-silica capillary column coated with 0.25 μm DB-5MS (Hewlett-Packard/Agilent, Waldbronn, Germany).[12] The GC oven programming started at 55 °C (1 min hold time), and then the temperature was increased at 20 °C/min to 255 °C (no hold time), at 1.5 °C/min to 283 °C (no hold time), and finally, at 15 °C/min to 300 °C (5 min hold time).[12] GC/EI–MS full-scan chromatograms (m/z 50–550) were recorded from 7.0 to 35.8 min for peak identification. In selected ion monitoring (SIM) mode, we recorded, from 7.0 to 10.85 min, m/z 143 (base peak) and m/z 353 ($[M - 15]^+$) of the trimethylsilyl ether of phytol, along with m/z 75, 129, 213, and 215 (specific fragment ions of other compounds of the unsaponifiable matter of plant oils[6]). Measurements were performed using a trimethylsilylated phytol standard of 10 ng/μL (1 μL injected). To be able to determine *cis*-phytol in the 0.1% range of oil samples, the area of the trimethylsilylated peak of *trans*-phytol (m/z 143) was requested to account to at least 10% of the area of the reference standard. The limit of determination was about 1 pg of *cis*-phytol.

RESULTS AND DISCUSSION

GC/MS Determination of Phytol in Plant Oils. GC/MS analysis of the trimethylsilylated *cis*-/*trans*-phytol reference standard (see the Materials and Methods) eluted the two isomers in the order *cis*-phytol < *trans*-phytol (Figures 1 and 2a). The mass spectra of the trimethylsilylated phytol isomers (panels b and c of Figure 2) were virtually identical. They featured only traces of both the molecular ion (m/z 368) and

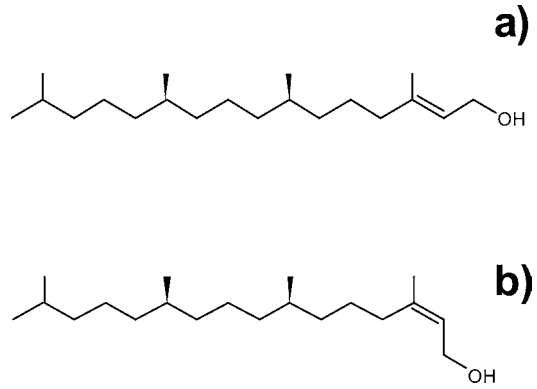

Figure 1. Chemical structures of (a) *trans*-phytol and (b) *cis*-phytol.

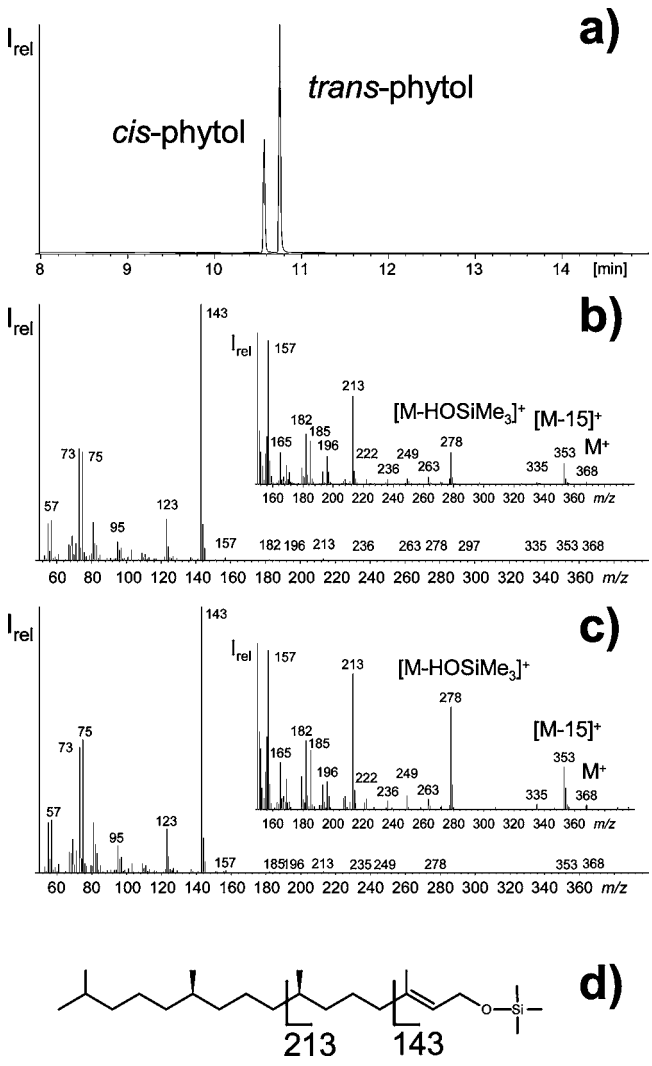

Figure 2. GC/EI–MS full-scan analysis of trimethylsilylated *cis*- and *trans*-phytol. (a) GC/EI–MS full-scan chromatogram of the trimethylsilylated commercial reference standard consisting of *cis*- and *trans*-phytol in the ratio of ∼1:2. Mass spectra of (b) trimethylsilylated *trans*-phytol and (c) *cis*-phytol. (d) Two characteristic fragment ions formed as exemplified with trimethylsilylated *trans*-phytol.

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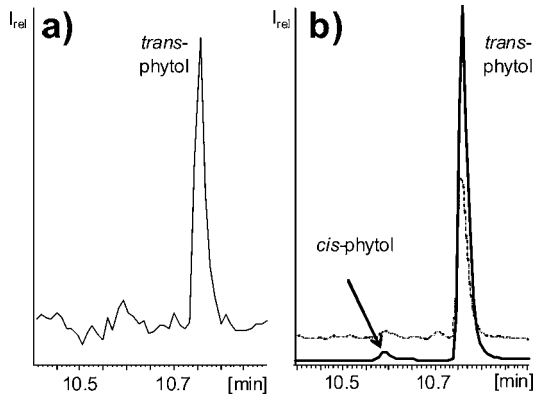

Figure 3. GC/EI−MS chromatograms of the analysis of phytol in a deodorized olive oil in (a) full scan (m/z 50−500) and (b) SIM of m/z 143 (black line) and m/z 75 (gray, dotted line). The retention time of *cis*-phytol is marked. Especially, m/z 143 provided the required selectivity and sensitivity for the determination of low amounts of *cis*-phytol.

the $[M - 15]^+$ fragment ion at m/z 353. The base peak was formed at m/z 143. This ion is produced by cleavage between C-3 and C-4, with the charge remaining on the headgroup (Figure 2d). Next to the base peak, m/z 123 was the most abundant fragment ion in the mass range of >100 Da. The mass spectrum also featured m/z 213 (cleavage between C-7 and C-8; Figure 2d) and an even mass fragment at m/z 278, which is formed from M^+ by the elimination of H−$OSiMe_3$ ($[M - 90]^+$). Similarly, m/z 123 mentioned above is most likely formed by elimination of H−$OSiMe_3$ from m/z 213. The slight deviations in the mass peak abundances between the trimethylsilylated *cis*- and *trans*-phytol (panels b and c of Figure 2) could not be used for differentiation.

On the basis of the mass spectrometric features, we developed a GC/MS−SIM method that facilitated the sensitive and selective determination of the phytol *cis*/*trans* isomers by means of the base peak at m/z 143. In addition, we recorded

Table 1. Relative *cis*-Phytol Content (Percentage of *trans*-Phytol) in a Sample of Olive Oil[a]

	I (%)	II (%)	III (%)	mean (%)	STD (%)[b]	RSTD (%)[b]
olive oil, deodorized, 1	2.26	2.29	2.20	2.25	0.046	2.06
olive oil, deodorized, 2	2.14	2.29	2.23	2.22	0.076	3.43
olive oil, deodorized, 3	2.17	1.88[c]	2.07	2.04	0.147	7.22
olive oil, deodorized, 4	2.29	2.20	2.31	2.27	0.060	2.66
olive oil, deodorized, 5	2.18	2.15	2.30	2.21	0.081	3.67

[a]The same sample was silylated 5 times (1−5) and measured 3 times (I−III). [b]STD standard deviation; RSTD, relative standard deviation. [c]Outlier, not eliminated to present the full array of variability for routine analysis.

m/z 353 ($[M - 15]^+$) of the trimethylsilyl ether of phytol for verification. Figure 3 shows the gain in sensitivity by switching from full scan to SIM mode. Only the SIM mode provided the required sensitivity for the determination of *cis*-phytol. Moreover, m/z 143 provided the required selectivity and sensitivity for the determination of low amounts of *cis*-phytol. Using this GC/MS−SIM method, we were able to determine less than 0.1% *cis*-phytol next to the dominant peak of *trans*-phytol. For the reference standard, three concentrations were prepared and the repeatability of the measurements was 0.30−0.34%. Further tests were carried out with a sample of deodorized olive oil, which contained a lower abundance of *cis*-phytol (Figure 4a and Table 1). The individual values varied less than 10% from the mean, which is considered excellent in view of the low abundance of *cis*-phytol (~2%) relative to *trans*-phytol (Table 1). In comparison to the deodorized sample, a native olive oil measured delivered only a peak for *trans*-phytol (<0.1% relative abundance of *cis*-phytol; Figure 4b). For this reason, the analytical method (e.g., the silylation step) could not be responsible for the presence of *cis*-phytol in the

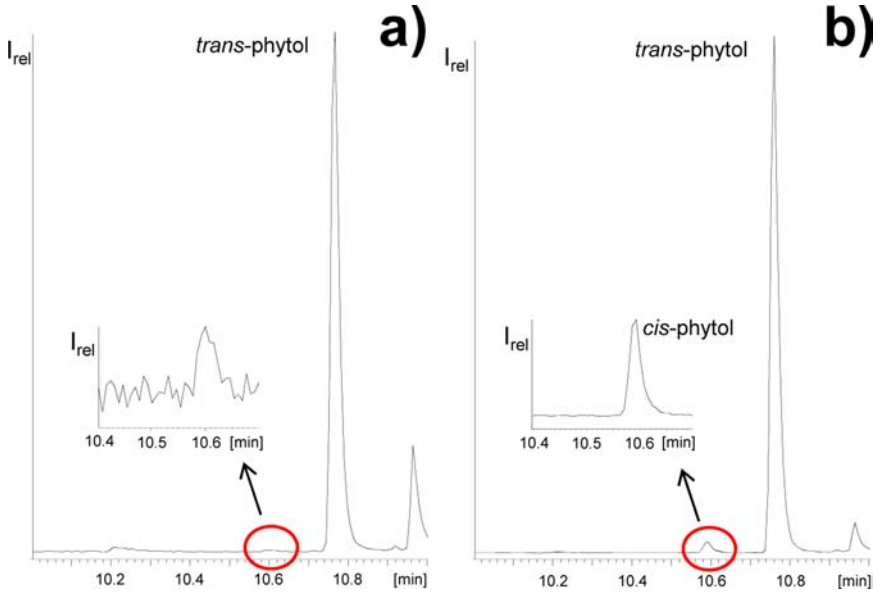

Figure 4. GC/EI−MS−SIM chromatograms (m/z 143) of trimethylsilylated samples of the unsaponifiable matter of (a) native olive oil (no *cis*-phytol detected) and (b) deodorized olive oil (*cis*-phytol detected), with insets of intensity-enlarged excerpts of the retention time range of trimethylsilylated *cis*-phytol.

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Table 2. Percentage Contribution of *cis*-Phytol and *trans*-Phytol to the Phytol Content of Nonrefined and Refined Edible Oils

	refined oils			nonrefined oils		
edible oil	treatment	*cis*-phytol (%)	*trans*-phytol (%)	treatment	*cis*-phytol (%)	*trans*-phytol (%)
olive oil	deodorized	2.3	97.7	native (Crete)	0.1	99.9
olive oil	refined	1.2	98.8	extra virgin	0.0	100.0
olive oil	refined	3.0	97.0			
olive oil	pomace oil	2.7	97.3			
rapeseed oil	refined (1)	0.8	99.2	cold pressed	0.0	100.0
rapeseed oil	refined (2)	1.0	99.0	raw	0.1	99.9
rapeseed oil		0.7	99.3			
sunflower oil	refined HO[a]	0.4	99.6	cold pressed, organic	0.0	100.0
sunflower oil	refined HO[a]	0.3	99.7			
sunflower oil	refined	0.5	99.5			
sunflower oil		0.9	99.1			
soy oil		0.7	99.3			
maize (germ) oil	refined	2.2	97.8	cold pressed	0.0	100.0
sesame oil	refined	6.4	93.6	cold pressed	0.0	100.0
apricot kernel oil	refined	2.3	97.7	cold pressed, organic	0.1	99.9
pomegranate kernel oil	refined	0.0	100.0	cold pressed, organic	0.0	100.0
rosehip kernel oil	refined	1.4	98.6	cold-pressed, organic	0.0	100.0
almond oil	refined	1.5	98.5	cold pressed	0.0	100.0
phytol standard		25.5	74.5			

[a]High oleic (HO).

deodorized sample. Thus, the method proved to be suited to determine the *cis*-phytol in the sub-percentage range of *trans*-phytol in edible oils.

Analysis of Different Oils. A total of 11 native cold-pressed oils were measured. Irrespective of the plant source (olive, maize, rapeseed, sunflower, and others), the relative abundance of *cis*-phytol was generally 0.1% or lower (Table 2). In most occasions (n = 8), *cis*-phytol could not even be detected. In contrast, the relative abundance of *cis*-phytol in refined edible oils was up to 6.4% (Table 2). Four refined olive oils were analyzed, and these showed a relative abundance of *cis*-phytol of 1.2−3.0%, which is more than 1 order of magnitude higher than in the cold-pressed olive oils (Table 2). Three refined rapeseed oils contained 0.7−1.0% *cis*-phytol, which was lower than in the samples of the refined olive oils but still much higher than in the nonrefined, cold-pressed oils (Table 2).

Frequently but not generally, the phytol content was higher in the cold-pressed oils. For instance, the maximum phytol content of nonrefined native olive oils (6−57 mg/100 g of lipids) was slightly lower than that of refined olive oils (11−68 mg/100 g of lipids), while the opposite was found for rapeseed oil (65−92 mg/100 g of lipids in cold-pressed rapeseed oil versus 47−50 mg/100 g of lipids in treated oils). Wide and overlapping ranges in the phytol content were observed for both nonrefined and refined oils. The highest amounts were present in both pomegranate oils (>200 mg/100 g of oil). Accordingly, the phytol amount cannot be used as a marker for nonrefined oils. It appears that the phytol content in the oil is influenced by different varieties of the plant and/or geographic and seasonal (ripeness) factors. The relative high amount of phytol in some treated oils indicated that the removal of chlorophyll in the refining step could be accompanied with the liberation of phytol, which mainly remained in the oil. Irrespective of these uncertainties, the abundance of *cis*-phytol in the oils was found to be a suitable marker for the treated oils.

In general, the contribution of *cis*-phytol to the phytol content in cold-pressed edible oils was 0.1% or less, whereas its contribution to the phytol content of refined oils was at least 0.3% and up to 6.4%. On the basis of this characteristic difference, a differentiation of classic cold-pressed from refined oils (i.e., olive oil, rapeseed oil, and sunflower oil) was possible without exception. Only the sample of the uncommon refined pomegranate kernel oil did not contain the *cis* isomer of phytol. Furthermore, initial experiments in our laboratory indicated that *cis*-phytol was not formed by water steam treatment, which is mandatory for different oils.[13] For this reason, the *cis*-phytol content can also be used to distinguish steam-treated, nonrefined oils from refined oils. Accordingly, the *cis*-phytol determination appears to be difficult to falsify because it would require its elimination from the oil. The addition of pure *trans*-phytol would be another option because it would increase (only) the *trans*-phytol content and, thus, decrease the relative abundance of *cis*-phytol. In principle, this could be achieved by the addition of high amounts of chlorophyll after the refining process. However, this measure would be uneconomic because of the large amounts required to dilute the proportion of *cis*-phytol below 0.1% contribution to the phytol content.

Isomerization of double bonds is a known side reaction of oil-refining processes. It cannot be excluded that there are further markers that also react in the same way, although we could not identify a second suitable compound during our detailed analysis of the unsaponifiable matter of different plant oils.[6] However, one advantage was that phytol can be easily gained with the unsaponifiable matter. After silylation, it was easily determined at low amounts. Thus, phytol can be analyzed by existing standard methods in most laboratories involved in the analysis of plant oils.

Natural Occurrence of *cis*-Phytol. As mentioned above, *trans*-phytol is the natural constituent of chlorophyll. However, phytol concentrations in food are low, typically with pronounced dominance of *trans*-phytol.[9,10,14] Occasionally, the occurrence of traces of *cis*-phytol [frequently labeled (*Z*)-phytol] has been mentioned in wholemeal bread, soy chunks, dried peppers and peas, baked beans, and sardines (0.06−0.5 mg/kg).[10] In rare cases, *cis*-phytol was more abundant than

trans-phytol.[10] Surprisingly, none of the phytol isomers had been reported to occur in plant oils (including olive oil, rapeseed oil, soy oil, and maize oil).[10] In addition to food, *cis*-phytol was reported to occur in some essential oils, such as *Tortula muralis* and different varieties of *Hypericum* sp.[15−17] In these studies phytol was analyzed without derivatization. Using our GC/MS system, the commercial (non-silylated) phytol standard (see above) gave five peaks whose mass spectra were characterized by intense fragmentation and low abundance in the mass range above 120 Da. Moreover, the signal intensity was much lower. Accordingly, phytol should preferably be determined after silylation. The silylation method was also used by June Brown et al.[10] Noteworthy, the toxicity of phytol (usually tested as a mixture of *cis*- and *trans*-phytol) was reported to be low. The acute oral median lethal dose (LD_{50}) of phytol (50% in olive oil) in rats was >10 g/kg of body weight.[18]

In conclusion, on the basis of the different relative *cis*-phytol abundances (<0.2% in cold-pressed versus >0.5% in refined oils), the phytol isomer determination seems to be a suitable marker for edible oil qualities. Especially in combination with other marker substances, its determination could support the authentication of virgin plant oils. It should be noted that details on the oil-refining processes were not available because the samples were bought retail. More future research has to be directed to identify the steps and conditions under which *cis*-phytol is formed during the refining of edible oils. Determination of the thermal degradation product pyropheophytin has been suggested to identify thermally heated virgin olive oil.[13] Related to this process may be the formation of *cis*-phytol.

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Notes

The authors declare no competing financial interest.

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