Fast Quality Screening of Vegetable Oils by HPLC–Thermal Lens Spectrometric Detection

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ABSTRACT: Isocratic reversed-phase HPLC with thermal lens spectrometric (TLS) detection enabled identification of linseed, olive, sesame, and wheat germ vegetable oils to control the authenticity of the oils based on characteristic carotenoid/carotene profiles. Four characteristic regions of carotenoids (i.e., lutein, xanthophyll, carotene, and lycopene) have been identified in each type of oil. The concentrations of total β-carotene (BC) and α-carotene (AC), together with *trans*- to *cis*-isomers of β-carotene (TBC/CBC) and BC/AC ratios were shown to be reliable and useful indices for fast screening of oils for nutritional quality. The oil TBC/CBC ratio and the BC concentration (in μg/mL) should meet the following numerical criteria: linseed (≥2:1, ≥1.7), olive (≥3:1, ≥0.4), sesame (≥1:1, ≥0.1), and wheat germ oil (≥1:1, ≥1.7). Based on the above criteria, unsatisfactory olive oils differed significantly from the consumable ones. Likewise, the concentration of AC in consumable wheat germ and sesame oil should not be lower than 0.6 and 0.02 μg/mL, respectively. The AC level in safflower oil should not be higher than 0.04 μg/mL. The BC/AC ratios exceeding 3:1, 6:1, and 8:1 should be used as an additional quality requirement for consumable wheat germ, sesame, and safflower oil, respectively.

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KEY WORDS: Carotenes, HPLC–thermal lens spectrometric detection (TLS), identification, nutritional quality, shelf life, vegetable oils.

Profiling of carotenoids in vegetable oils is of great nutritional and epidemiological interest. The separation, identification, and quantitation of *trans*/*cis*-isomers of β-carotene (TBC, CBC) in edible oils is important to measure antioxidant activity and for quality control. The *cis*-isomer(s) are either naturally present or formed during the processing of oils. In fruits and vegetables all-*trans*-β-carotene has been shown to undergo *cis*-isomerization (1), with the 9-*cis*- and 13-*cis*-stereoisomers being the predominant isomerization products.

Various studies on the distribution of pigments in olives and olive oils have shown that the characteristics of olive oil depend on genetic, agronomic, environmental, and processing factors (2–7). The content of pigments differs greatly among single-variety virgin olive oils. Arbequina variety olives have chlorophyllides, esterified xanthophylls, α-carotene (AC), ξ-carotene, and phytofluene that are exclusive to this variety (2).

The carotenoid (17–19%) and total chlorophyll (81–83%) fractions dominate in fresh olives, whereas lutein (10–11%) and β-carotene (4–5%) are the most abundant among the carotenoids (3). In virgin olive oils the concentration of total xanthophylls (including neoxanthin, mutatoxanthin, antheraxanthin, luteoxanthin, and violaxanthin) varies between 0.3 and 1.6 μg/g, whereas those of pheophytins, lutein, and β-carotene range from 3.3 to 51.4, 0.2 to 9.3, and 0.3 to 7.7 μg/g, respectively (3–7). However, the chlorophyll and carotenoid contents gradually decrease during olive fruit ripening (2). Separation, identification, and quantification of carotenoids and chlorophylls in olive fruit and in olive oils based on normal-phase HPLC (5,6), reversed-phase ion-pair HPLC (2,4), and TLC (3) have been developed and reported. Among other vegetable oils, palm oil is known as one of the richest sources of carotenes, with overall concentrations of α- and β-carotene of 544 μg/mL (8).

Since the natural ratios of pigments in oils can be correlated with parameters such as cultivar, fruit/seed quality, degree of ripeness, storage conditions and losses, and so on, they are useful as markers for assessing the use of added pigment (to enrich the color) or blended vegetable oils. Consequently, this research was carried out to investigate the potential of an isocratic nonaqueous, reversed-phase HPLC procedure using ultrasensitive thermal lens spectrometric (TLS) detection to study the nutritional quality and authenticity of vegetable oils. This report is a continuation of our recent studies (9) on the separation and assay of *trans*- and *cis*-β-carotene isomers in vegetable oils. The use of HPLC–TLS as an analytical means for ultrasensitive and reliable analysis of *trans*-β-carotene in complex oily matrices has already been reported (10).

EXPERIMENTAL PROCEDURES

Solvents and mobile phase. Chloroform, THF, and methanol (MeOH) were HPLC grade and obtained from Labscan (Dublin, Ireland). THF (5%) in MeOH (vol/vol) served as a mobile phase, whereas the mixture THF/MeOH/$CHCl_3$ (5:45:50, vol/vol) served as a diluent for sample oils and standards.

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Standards. Lycopene standard (Sigma, St. Louis, MO) stock solution in hexane was kindly donated by the Department of Human Nutrition, Wageningen University and Research Centre (The Netherlands), whereas Betatene® 20% Soy and Lyc-O-Mato™ 6% were donated by Cognis Deutschland GmbH, Nutrition and Health (Düsseldorf, Germany) and LycoRed Natural Products Industries Ltd. (Beer-Sheva, Israel), respectively. Betatene 20% Soy, a commercial product obtained from extracts of the alga *Dunaliella salina,* is a mixture of β-carotene (*trans*- to *cis*-isomers *ca.* 65:35), α-carotene, cryptoxanthin, zeaxanthin, and lutein in soybean oil. Lyc-O-Mato 6% is a natural tomato oleoresin containing 6% pure tomato lycopene crystals that are suspended in natural tomato lipids and also containing β-carotene, phytoene, and phytofluene.

Betatene 20% Soy and Lyc-O-Mato 6% were kept at 4°C. Lycopene, Betatene 20% Soy, and Lyc-O-Mato 6% working solutions in diluent, as previously described, were protected from light by wrapping them in aluminum foil and storing at –20°C prior to use.

The above-mentioned products were used for identification and quantitation of α-carotene and *trans*- and *cis*-isomers of β-carotene.

Samples. Several linseed oils, wheat germ oils, olive oils, safflower oils, and sesame oils from different producers and/or production lots were purchased from the local market and analyzed. Each oil was assigned a code number; olive oils 3–11, 13, and 14 were labeled as extra virgin oils. The linseed oils designated 1 and 2; olive oils 1–3, 9, 11, and 12; safflower oils 3 and 4; and sesame oil 4 had expired according to the manufacturer's label or had been stored in our laboratory longer than 2 yr.

Oils were stored in amber glass bottles at 4°C. Prior to HPLC analysis, oils were simply diluted using the described diluent. The dilution factor for sesame and safflower oils was 20, whereas the linseed, olive, and wheat germ oils were diluted 100-fold.

Equipment. The instrument used in this study was constructed in the laboratory (11). The dual beam TLS spectrometer consisted of an intensity-modulated continuous-wave Arion laser (60 mW at 476 nm) used as a pump beam, and the He–Ne laser (20 mW at 632.8 nm) acted as a probe beam. TLS signals were retrieved by a lock-in amplifier with a time constant set at 1 s.

The HPLC system consisted of a polymeric C_{18} stationary phase (Vydac 218TP54 column, length 250 mm, i.d. 4.6 mm, particle size 5 µm, pore diameter 30 nm; Separations Group, Hesperia, CA), an LC 250 isocratic pump (PerkinElmer, Norwalk, CT), an injection valve with a 10-µL loop (Rheodyne, Rohnert Park, CA), and a Milton Roy SM 400 analytical flow cell (length 1 cm, diameter 1 mm; Wokingham, Berkshire, United Kingdom).

Analytical procedures. Diluted oil samples were chromatographed isocratically using a mobile phase flow rate of 1.0 mL/min (3.9–4.5 MPa) 5% THF in MeOH at room temperature. The detection wavelength was 476 nm. The carotene peaks were identified vs. the external standards described above. α-Carotene and *trans*- and *cis*-β-carotenes in the oil samples were quantified vs. Betatene 20% Soy peaks by direct comparison of peak heights. The nonparametric Mann–Whitney test was used for statistical analysis of the data.

RESULTS AND DISCUSSION

Our recent paper on an HPLC–TLS study of vegetable oils (multifold diluted, directly injected, and analyzed), reports on an efficient separation of TBC and CBC that was achieved within a few minutes using 10% THF in MeOH as the mobile phase (9). With the exception of a modified mobile phase (5% THF in MeOH), the same procedure was followed in the current investigation to achieve the separation of carotenoid pigments [particularly that of *trans*-β-carotene from *cis*-isomer(s) and of lycopene isomers] in various vegetable oils and in several food supplements (Betatene 20% Soy and Lyc-O-Mato 6%) (Figs. 1–6). AC, TBC, and CBC could be identified according to their chromatographic retention: The elution of AC (8.2 min) was followed by that of TBC (8.9 min) and CBC (9.5–10.2 min, possibly also at 11.0 min). However, without the efficient separation of all of the *cis*-isomers of β-carotene, one can only speculate about their identities and relative amounts. The interference of other oil pigments such as chlorophylls, pheophytins, and pheophorbides was avoided due to analytical detection wavelength and differences in retention times (3,10).

In Figure 1 (traces 1 and 2) are the chromatograms obtained from the diluted Betatene 20% Soy. Likewise, traces 3 and 4 refer to a lycopene standard and to diluted Lyc-O-Mato 6%. Note that the *trans*-β-carotene and *cis*-β-isomers in Figure 1 are resolved efficiently; however, the separation of the *cis* isomers was inadequate. On the other hand, Lane *et al.*

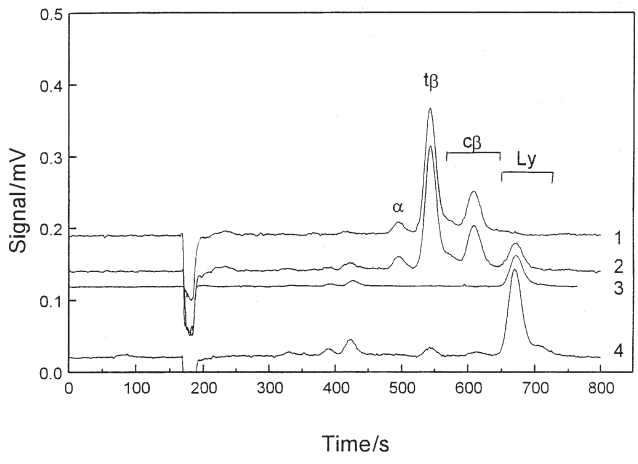

FIG. 1. Chromatograms of (1), Betatene® 20% Soy (dil. 2.4×10^6×; Cognis Deutschland GmbH Nutrition and Health, Düsseldorf, Germany); (2), Betatene® 20% Soy (dil. 2.4×10^6×) plus lycopene standard; (3), lycopene standard; (4), Lyc-O-Mato™ 6% (dil. 6.6×10^5×; LycoRed Natural Products Industries Ltd., Beer-Sheva, Israel). Abbreviations: α, α-carotene; cβ, *cis*-β-carotene; Ly, lycopene; tβ, *trans*-β-carotene.

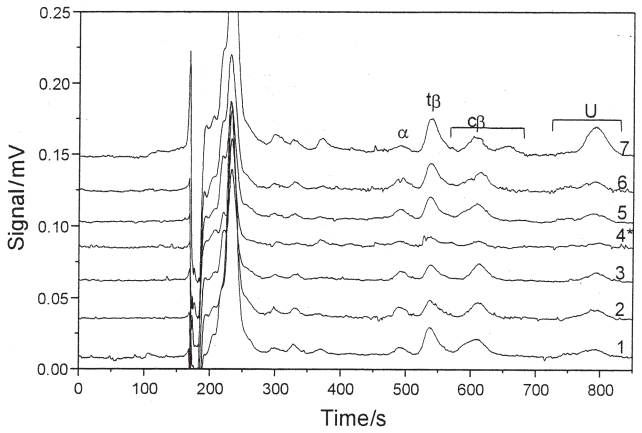

FIG. 2. Chromatograms of sesame oils (dil. 20×). U, unidentified; for other abbreviations see Figure 1. Inadequate oil is marked by an asterisk.

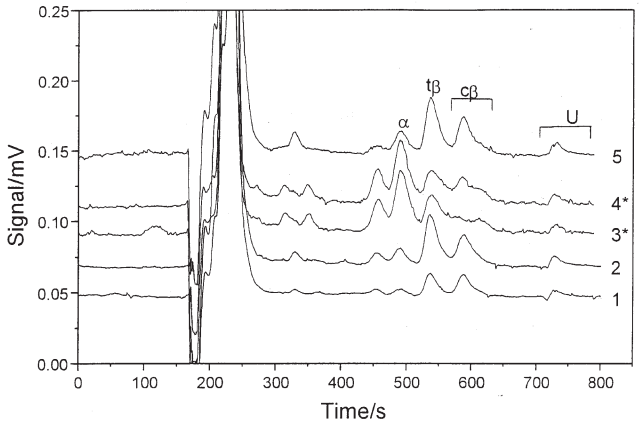

FIG. 4. Chromatograms of safflower oils (dil. 20×). For abbreviations see Figures 1 and 2. Inadequate oil is marked by an asterisk.

(12) identified all-*trans*- and 9-*cis*-, 13-*cis*-, and 15-*cis*-geometric isomers of β-carotene, as well as all-*trans*-α-carotene in Betatene.

Each type of oil shows a characteristic carotenoid profile, a fingerprint that can be used for identification and authentication purposes. The analysis of chromatograms recorded for five types of oils (see Figs. 2–6) indicated four distinct carotenoid regions, i.e., those of lutein (3–5 min), xanthophyll (5–7 min), carotene (7–11 min), and lycopene (11–14 min). Some early-eluting carotene (7.5–7.8 min) was clearly differentiated in wheat germ and safflower oils. The proportion of the early-eluting carotene peak and that of α-carotene was almost constant in consumable wheat germ oils. The peaks following *cis*-β-carotene, namely those in the lycopene region, appear at different retention times and have not been identified; in addition to lycopene isomers, the peaks could possibly be attributed to some carotenol FA esters.

The CBC interval within the carotene region may comprise one to two peaks and/or shoulders arising from various *cis*-β-carotene isomers and/or their mixtures. With the exception of safflower oil, retention times for all other oils were practically the same. The olive and linseed oils examined exhibited similar features in the carotene region based on TBC and CBC peaks, with the excess of TBC above CBC being more pronounced in olive oils (2:1 to 7:1) than in linseed oils (2:1 to 4:1). The latter, however, contained relatively high concentrations of xanthophylls. In sesame, safflower, and wheat germ oils, the TBC/CBC ratio ranged from 1:1 to 2:1.

Whenever more than one *cis*-β-isomer is clearly evident from the chromatogram (as observed in the case of wheat germ, safflower, and/or olive oils), their total concentration should be taken into account when calculating the TBC/CBC ratio.

Screening a number of oils of each kind resulted in the typical data shown in Table 1.

Peaks of AC, TBC, and CBC characterize the carotene region in the chromatograms of sesame oils (Fig. 2): Levels of both TBC and CBC were almost equal (0.1 μg/mL) in all consumable sesame oils.

Wheat germ and safflower oils showed an early-eluting

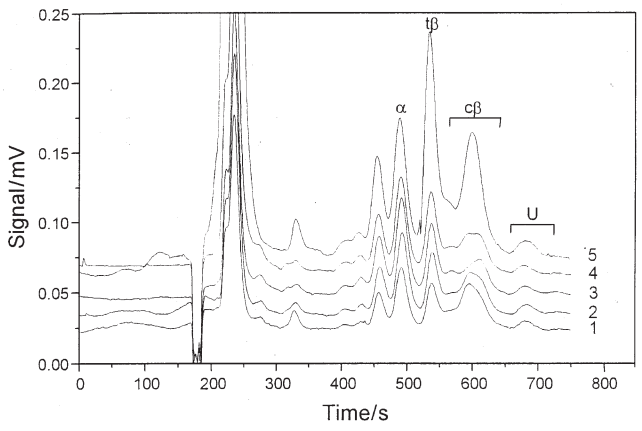

FIG. 3. Chromatograms of wheat germ oils (dil. 100×). For abbreviations see Figures 1 and 2.

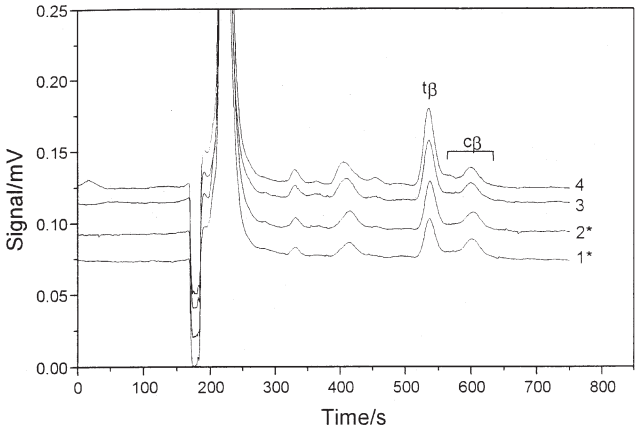

FIG. 5. Chromatograms of linseed oils (dil. 100×). For abbreviations see Figures 1 and 2. Inadequate oil is marked by an asterisk.

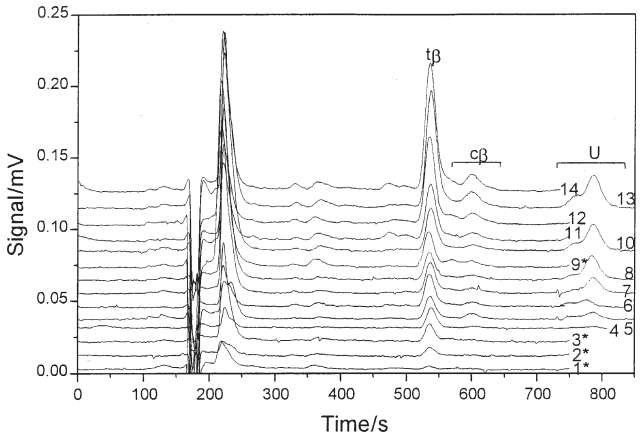

FIG. 6. Chromatograms of olive oils (dil. 100×). For abbreviations see Figures 1 and 2. Inadequate oil is marked by an asterisk.

carotene peak as well as AC, TBC, and CBC peaks in the carotene region (Figs. 3 and 4). The AC peak was quite intense, and several *cis*-β-carotene isomers were evidently present in wheat germ oils; moreover, the intensity of the TBC and CBC peaks varied considerably, even in consumable wheat germ oils (Fig. 3). TBC and CBC peaks were characteristic for the carotene region of olive and linseed oils: A clear dominance of *trans*-β-carotene was observed (Figs. 5 and 6).

The intensity of the CBC peak was almost constant in all linseed oils examined, whereas the TBC concentration varied greatly between inadequate (0.7–0.8 μg/mL) and consumable (1.1–1.3 μg/mL) linseed oils (Fig. 5). The highest variability of TBC and CBC signal intensities as well as of their ratios was observed in olive oils (Fig. 6). In olive and linseed oils the presence of AC was uncertain and/or within the background noise: AC was detected in only two olive oils.

The characteristics of the carotene fingerprint region, the intensity of the peak in the lutein region, the contour of the xanthophyll region, and the position of the peak in the lycopene region (see Figs. 2–6) enable one to differentiate between the various oils. Moreover, the oils are characterized by varying amounts of total β-carotene (BC) and variable relative amounts of TBC and CBC, as well as by the concentration of AC and the ratio of BC to AC.

Inspection of the data obtained from fresh oils and from those that had expired allowed us to assign the tentative numerical criteria for the shelf life of the oils. According to Table 1, the consumable oils should meet at least the lower figures quoted in columns 3, 4, 7, and 8. These values represent the limits that correlate very well with the aging of the product. On the basis of these values, as many as 9 out of 11 expired vegetable oils (see the Samples section) were identified as inadequate for consumption. In Figures 2–6 the inadequate samples are marked by an asterisk. From Table 1 it is evident that in consumable oils the amount of TBC should be higher than that of CBC. A multifold excess of BC over AC was characteristic for consumable wheat germ and safflower oils; for sesame oils absolute concentration values for total BC and AC were characteristic. The positive correlations between concentrations of total BC and AC have been found in wheat germ, sesame, and safflower oils (see Table 1).

In consumable linseed oils, total BC should exceed 1.7 μg/mL and the TBC/CBC ratio should preferably be larger than 2; the inadequate linseed oils (linseed oils 1 and 2) are marked in Figure 5. Inadequate sesame oil (sesame oil 4; Fig. 2) was characterized by a concentration of total BC (<0.1 μg/mL) and AC (<0.02 μg/mL). Neither the ratio of TBC/CBC nor of BC/AC was characteristic of the inadequate sesame oil. For safflower oils neither the BC concentration nor the TBC/CBC ratio was found to be characteristic. Instead, an AC concentration below 0.04 μg/mL and a BC/AC ratio ≥8 were indicative of consumable safflower oils. Inadequate safflower oils exhibited a featureless CBC region as well (safflower oils 3 and 4; Fig. 4).

In consumable wheat germ oils, total BC was >1.7 μg/mL and AC was >0.6 μg/mL; furthermore, TBC/CBC and BC/AC ratios were higher than 1 and 3, respectively (Fig. 3).

In consumable olive oils, the amount of BC was found to be between 0.4 and 2.6 μg/mL, a range that can be considered typical. Such a broad range for the natural abundance of β-carotene is wider than the values reported by Stancher *et al.* (7) and Psomiadou and Tsimidou (5) (1.0 to 2.5 μg/g) but is in accordance with the reports for virgin olive oils (5), especially those obtained from semiblack olives (6). Inadequate olive oils were identified by a concentration of BC <0.4 μg/mL and/or a TBC/CBC ratio <3:1 (olive oils 1, 2, 3, and

TABLE 1
Qualitative Scheme of Chromatograms of Consumable Oils

Oil	Carotene region fingerprint	TBC/CBC ratio	Concentration (μg/mL)				BC/AC ratio	Correlation BC conc. vs. AC conc. (*r*)
			Total BC	TBC	Total CBC	AC		
Wheat germ	4 principal peaks:	1:1 to 2:1[b]	1.7–6.4[b]	0.8–4.0	0.9–2.5	0.6–1.3[b]	3:1 to 5:1[b]	0.904
Safflower	EEC + AC + TBC + CBC	1:1 to 2:1	0.2–0.4	0.1–0.2	0.1–0.2	0.01–0.04[b]	8:1 to 13:1[b]	0.992
Sesame	3 principal peaks: AC + TBC + CBC	1:1 to 2:1	0.1–0.2[b]	0.1	0.1	0.02–0.03[b]	6:1 to 10:1	0.556
Linseed	2 principal peaks:	2:1 to 4:1[b]	1.7–1.8[b]	1.1–1.3	0.4–0.6	—	—	—
Olive	TBC + CBC	3:1 to 7:1[b]	0.4–2.6[b]	0.4–2.2	0.1–0.4	—	—	—

[a]Concentration in original oil.
[c]The lower numerical values represent the limits for consumable oils. Abbreviations: AC, α-carotene; BC, β-carotene; CBC, *cis*-β-carotene; TBC, *trans*-β-carotene.

9; Fig. 6); such oils may also contain little lutein. The directional Mann–Whitney test confirmed our criteria: It demonstrated that consumable olive oils tend to have higher BC levels ($P < 0.05$) and higher TBC/CBC ratios ($P < 0.025$) than the unsatisfactory ones.

Stancher *et al*. (7) suggested that the analysis of carotenoids in olive oils may represent a simple method to distinguish between various types of oils. However, their investigation was confined only to olive oils and required a time-consuming saponification pretreatment of the samples. Further, Gandul-Rojas *et al*. (13,14) reported that ratios of chlorophylls/carotenoids and lutein/β-carotene could be used as indicators of the treatment process used for an olive oil and to verify its authenticity. Kaufmann (15) and Lee *et al*. (16) used the TAG composition as a basis for detecting oil adulteration. Based on the experimental evidence presented, we suggest that in addition to the values mentioned above, or even instead of them, the following criteria may be used to screen oils for their nutritional quality and shelf life: (i) the characteristic carotenoid profile (carotenoid/carotene fingerprint), (ii) the total β-carotene concentration, (iii) the TBC/CBC ratio, (iv) the α-carotene concentration, and (v) the BC/AC ratio. The latter two values might be proposed as indices for α-carotene-containing oils. These criteria seem reliable and applicable to a variety of vegetable oils.

The superb sensitivity achievable with the TLS detector coupled with HPLC (in comparison with conventional UV-vis or diode array detector) allows one to avoid the sample pretreatment step and enables rapid analysis of highly diluted vegetable oils by simply injecting them on the column. The proposed method is simple and applicable for a routine control, thereby contributing to simplifying the overall complex process of quality evaluation of vegetable oils.

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