

Analysis of Vegetable Oils by High-Performance Liquid Chromatography

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Abstract—A reversed-phase high-performance liquid chromatographic procedure is proposed to monitor the fatty-acid and triglyceride composition of sunflower-seed oil. The procedure uses a column packed with Diasfer-110-C₁₈ (6 μm), a 2 : 8 mixture of acetonitrile and acetone as an eluent, and refractometric detection. Analyses that use simple normalization and normalization with correction factors that take into account the difference between calculated refraction coefficients of different triglycerides are compared. It is shown that the proposed procedure can be used for the quantitative determination of total triglycerides in various vegetable oils as well.

The method of monitoring based on the transesterification of fats by methanol with the further determination of methyl esters by gas-liquid chromatography [1] can (and should) be replaced now by the direct determination of triglycerides by high-performance liquid chromatography [2]. The identification of triglyceride and fatty-acid composition can be easily performed using available oils, for which the triglyceride composition can be predicted by the increment model [3].

In this paper, the main focus is on determining the composition of sunflower-seed oil. For this important source of vegetable oil, the problems of breeding varieties with a high concentration of oleic components [4] and a high concentration of stearic acid [5] are well known. We studied more than 50 samples of sunflower-seed oil (*Helianthus annuus* L.) from known producers and homemade ones available in the market of Belgorodskaya oblast, as well as the planting stock of some important varieties.

EXPERIMENTAL

We used a Gilson liquid chromatograph with an R-401 Waters Millipore refractometric detector. A 20-μL sample was injected with a Rheodyne 7125 injector. The chromatograms were recorded using a Shimadzu C-R3A electronic integrator. The column of 250 × 4.6 mm was packed with Diasfer-110-C₁₈ (6 μm, average performance with respect to triglycerides $N \approx 10000$ theoretical plates) of Russian make. The eluent flow rate was 1 mL/min. The solvents of reagent grade were used without purification to prepare eluents containing 10–25 vol % of acetonitrile in acetone.

We used the following notations for the fatty acids: Ln for α-linolenic (*cis,cis,cis*-9,12,15-octadecatrienoic) acid, Le for linoleic (*cis,cis*-9,12-octadecadi-

enoic) acid, Ol for oleic (*cis*-9-octadecenoic) acid, Pa for palmitic (hexadecanoic) acid, and St for stearic (octadecanoic) acid. Triglycerides were denoted (without specifying positions) by symbols, for example, Le₂Ol is for dilinoleate-oleate. The elution order for triglycerides as a function of the composition was estimated using retention increments and taking into account the decrease in hydrophobicity in the series Le < Ol < Pa < St. The increments for identification of triglycerides by their relative retention were calculated by the formula

$$\Delta(i \rightarrow j) = \log(\tau_{abj} - \tau_0) - \log(\tau_{abi} - \tau_0),$$

where τ_0 is the dead time and τ_{abi} is the retention time for the corresponding triglyceride.

Oil was extracted by acetone for half an hour after crushing the seeds in a mortar with quartz sand.

For quantitative determination, 15–30-mg/mL acetone solutions of oil were prepared.

RESULTS AND DISCUSSION

Use of vacuum-degassed eluent ensures chromatograms with low zero-line drift. In this case, the retention times of triglycerides vary only slightly, by 0.1–0.2 min for 3.5 h of continuous operation. Difficulties in determining the retention times are associated mainly with the peak asymmetry caused by the relatively high concentration of oil in the solutions used: the retention times can decrease by up to 0.2–0.3 min. This is the reason why the increments should be estimated at the lowest possible concentration of oil.

The authors at Belgorod State University performed a study of triglyceride composition of vegetable oil from the seeds of more than 100 various plants. On the basis of this study, we recommend the following data

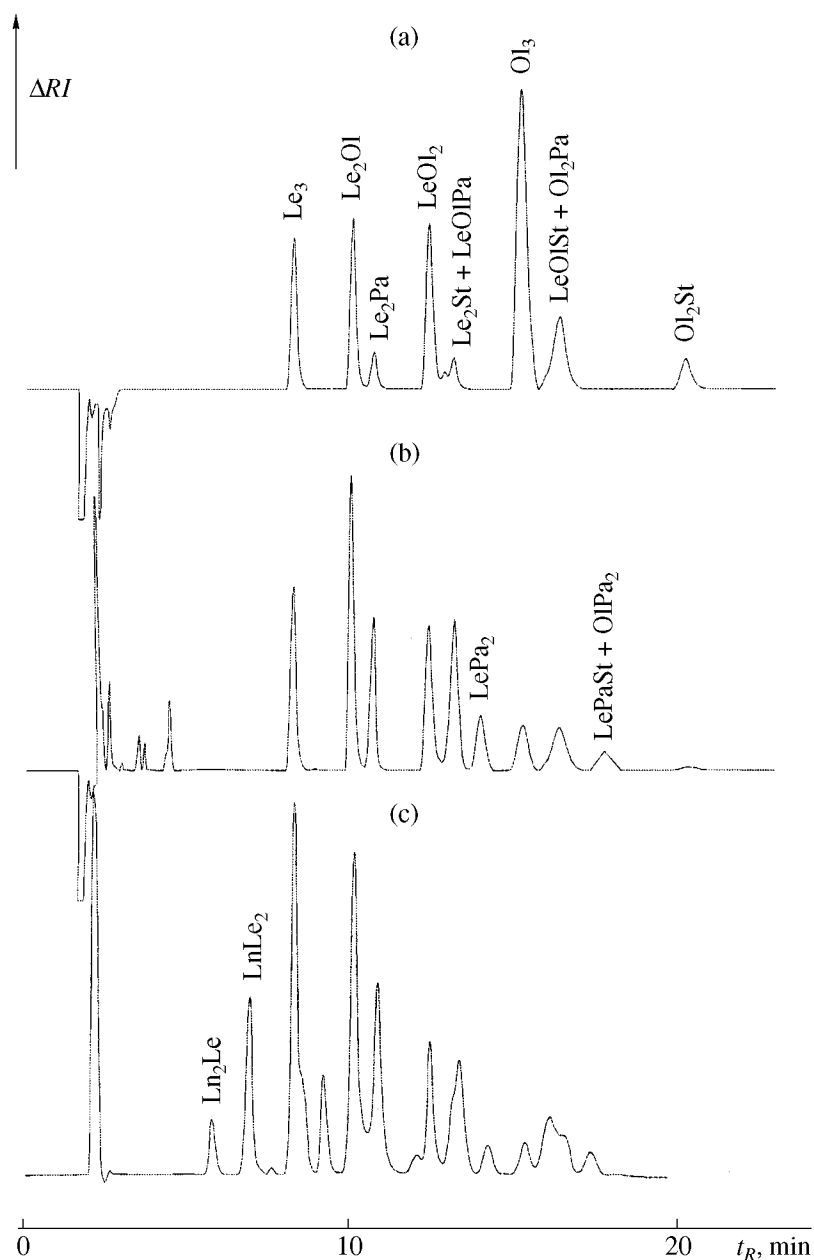


Fig. 1. Chromatograms of (a) peach pit oil, (b) fodder beet-seed oil, and (c) soybean oil eluted by a 2 : 8 acetonitrile–acetone mixture.

for calculating the increments: peach pit or beet-seed oil for $\text{Le} \rightarrow \text{Ol}$ (three equal transitions in the triglyceride series $\text{Le}_3 \rightarrow \text{Le}_2\text{Ol} \rightarrow \text{LeOl}_2 \rightarrow \text{Ol}_3$, Figs. 1a and 1b); soybean oil for $\text{Ln} \rightarrow \text{Le}$ (two equal transitions in the series $\text{Ln}_2\text{Le} \rightarrow \text{LnLe}_2 \rightarrow \text{Le}_3$, Fig. 1c); beet-seed oil for $\text{Ol} \rightarrow \text{Pa}$ (five equal transitions in the triglyceride series $\text{Le}_2\text{Ol} \rightarrow \text{Le}_2\text{Pa}$, $\text{LeOl}_2 \rightarrow \text{LeOlPa} \rightarrow \text{LePa}_2$, and $\text{LeOl}_2 \rightarrow \text{LeOlPa} \rightarrow \text{LePa}_2$, Fig. 1b); and peach pit oil for $\text{Pa} \rightarrow \text{St}$ ($\text{Ol}_3 \rightarrow \text{Ol}_2\text{St}$ transition, Fig. 1a).

Ordinary sunflower seed oil is semi-drying: the predominant acid in its triglycerides is linoleic, the oleic

acid content is lower, and the content of palmitic and stearic acids is even lower. The main peak corresponds to trilinoleate Le_3 ; next is dilinoleate-oleate Le_2Ol , and right after it, the palmitate derivative Le_2Pa is eluted. After LeOl_2 , a poorly resolved pair, Le_2St and LeOlPa , appears. Under studied HPLC conditions, the resolution R_s for this pair cannot be better than 0.5. This is the first obstacle in calculating the acid composition from the triglyceride composition. However, the proportion between Le_2St and LeOlPa can be roughly estimated from the shape of the overall peak: fronting is observed when Le_2St prevails; tailing is observed otherwise.

Table 1. Triglyceride composition (molar fraction of triglycerides, mol. %) of sunflower-seed oil ($n = 3$, $P = 0.95$)

Sample	Met hod	Le ₃	Le ₂ Ol	Le ₂ Pa	LeOl ₂	Le ₂ St + LeOlPa	Ol ₃	LeOlSt + Ol ₂ Pa
Trademarks of sunflower-seed oil:								
Zlato, Razdol'e, and Oleina	A	29.8 ± 0.4	29.8 ± 0.4	9.9 ± 0.5	12.1 ± 0.4	12.8 ± 0.4	2.0 ± 0.1	3.3 ± 0.2
	B	28.7 ± 0.4	29.5 ± 0.4	10.0 ± 0.5	12.4 ± 0.4	13.3 ± 0.5	2.2 ± 0.1	3.3 ± 0.2
Homemade	A	32.2 ± 0.4	29.2 ± 0.4	10.9 ± 0.5	9.8 ± 0.4	13.1 ± 0.4	1.6 ± 0.1	0.9 ± 0.2
	B	31.0 ± 0.4	29.0 ± 0.4	11.1 ± 0.5	10.0 ± 0.4	13.4 ± 0.5	1.8 ± 0.1	1.0 ± 0.2
Sloboda	B	31.8 ± 0.4	29.8 ± 0.4	11.1 ± 0.5	9.4 ± 0.4	12.1 ± 0.5	2.6 ± 0.1	1.4 ± 0.2
Sunflower varieties:								
Veidelevskii super elite	A	32.7 ± 0.4	29.2 ± 0.4	10.4 ± 0.5	10.6 ± 0.4	11.5 ± 0.4	1.3 ± 0.1	3.3 ± 0.2
	B	31.5 ± 0.4	29.0 ± 0.4	10.6 ± 0.5	10.8 ± 0.4	12.0 ± 0.5	1.4 ± 0.1	0.9 ± 0.2
Konditerskii super elite	B	31.5 ± 0.4	29.0 ± 0.4	10.6 ± 0.5	10.8 ± 0.4	12.0 ± 0.5	1.4 ± 0.1	0.9 ± 0.2
Prokhorovskii elite	B	28.5 ± 0.4	32.3 ± 0.4	9.4 ± 0.5	10.5 ± 0.4	10.8 ± 0.5	2.1 ± 0.1	3.7 ± 0.2
Pochin	B	20.5 ± 0.4	28.2 ± 0.4	6.3 ± 0.5	15.9 ± 0.4	14.8 ± 0.5	5.5 ± 0.1	5.0 ± 0.2
Krasotka	B	26.7 ± 0.4	31.4 ± 0.4	9.2 ± 0.5	11.9 ± 0.4	11.9 ± 0.5	2.5 ± 0.1	4.5 ± 0.2
Krasnoe solnyshko (ornamental)	B	22.4 ± 0.4	31.7 ± 0.4	8.8 ± 0.5	14.7 ± 0.4	12.4 ± 0.5	4.1 ± 0.1	5.4 ± 0.2
Sesame oil:								
India, a food additive	B	11.2 ± 0.2	21.2 ± 0.4	7.3 ± 0.5	19.5 ± 0.4	14.6 ± 0.5	9.9 ± 0.2	11.4 ± 0.2

Note: A is simple normalization method, B is normalization with correction factors.

Table 2. Fatty-acid composition (molar fraction of fatty acids, mol. %) of sunflower-seed oil ($n = 3$, $P = 0.95$)

Sample	Method	Le	Ol	Pa	St
Trademarks of sunflower-seed oil:					
Zlato, Razdol'e, and Oleina	A	67.4 ± 2.1	23.5 ± 2.1	6.1 ± 2.1	2.9 ± 2.1
	B	66.7 ± 2.1	23.8 ± 2.1	6.1 ± 2.1	3.1 ± 2.1
Homemade	A	70.5 ± 1.8	20.1 ± 1.8	5.3 ± 1.8	3.8 ± 1.8
	B	69.6 ± 1.8	20.5 ± 1.8	5.5 ± 1.8	4.0 ± 1.8
Sloboda	B	69.3 ± 2.3	21.4 ± 2.3	6.2 ± 2.3	2.8 ± 2.3
Sunflower varieties:					
Veidelevskii super elite	A	69.6 ± 2.1	21.3 ± 2.1	8.2 ± 2.1	2.8 ± 2.1
	B	69.1 ± 2.1	21.4 ± 2.1	6.3 ± 2.1	3.1 ± 2.1
Konditerskii super elite	B	71.1 ± 1.5	22.3 ± 1.5	4.4 ± 1.5	2.1 ± 1.5
Prokhorovskii elite	B	66.3 ± 1.9	24.3 ± 1.9	6.0 ± 1.9	2.9 ± 1.9
Pochin	B	59.2 ± 1.9	30.2 ± 1.9	6.2 ± 1.9	4.4 ± 1.9
Krasotka	B	65.4 ± 1.9	25.2 ± 1.9	5.3 ± 1.9	3.7 ± 1.9
Krasnoe solnyshko (ornamental)	B	61.7 ± 1.8	29.0 ± 1.8	5.9 ± 1.8	3.2 ± 1.8
Sesame oil:					
India, a food additive	B	46.5 ± 2.2	39.8 ± 2.2	8.9 ± 2.2	4.5 ± 2.2

Note: A is simple normalization method, B is normalization with correction factors.

Note that, at high concentration of palmitic acid, the LePa₂ peak eluted after LeOlPa can be noticeable. With a mobile phase containing more than 10 vol % of acetonitrile, trioleate Ol₃ is separated easily, but the poorly resolved groups LeOlSt + Ol₂Pa and LePaSt + OlPa₂ are eluted next. These can be partially resolved by using

elutents with more than 20 vol % of acetonitrile. At high concentrations of oleic acid, Ol₂St peak is noticeable. Other combinations, as a rule, are rather minor.

The presence of poorly resolved pairs is not a drawback in the selected mobile and stationary phases. Unlike [6], in which only Le₃, Le₂Ol, LeOl₂, Ol₃, and

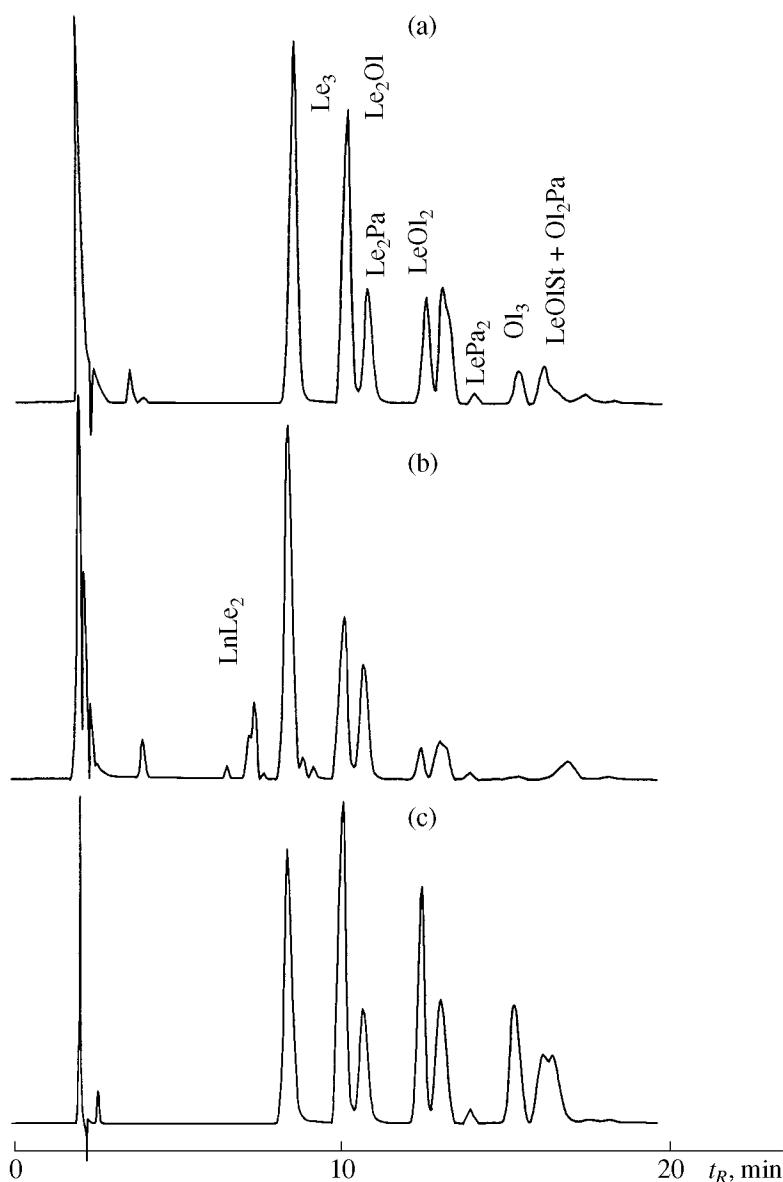


Fig. 2. Chromatograms of (a) oil of the Konditerskii sunflower variety, (b) dandelion-seed oil, and (c) sesame oil eluted by a 2 : 8 acetonitrile–acetone mixture.

Ol_2St were found in sesame (*Sesamum indicum*) oil; the triglyceride composition of this oil found with the chromatographic system studied is much more varied (Fig. 2c). All the studied sunflower seed oils have a similar composition, namely, they are rich in linoleic acid (Tables 1, 2). The shapes of the chromatograms of

oils with higher oleic acid content or, vice versa, with higher linoleic acid content are shown in Fig. 2 with the examples of sesame and dandelion seed oils, respectively.

For the quantitative determination of the triglyceride and fatty-acid composition of an oil, we used two

Table 3. Parameters of the calibration plot $c = a + bS$

Oil	Method	a	b	RSD, rel %	n
Apricot pit	A	14.31	0.286	2.62	11
Apricot pit + soybean + sunflower seed + olive	A	15.30	0.282	5.63	23
	B	14.85	0.289	3.08	23

Note: A is simple normalization method, B is normalization with correction factors

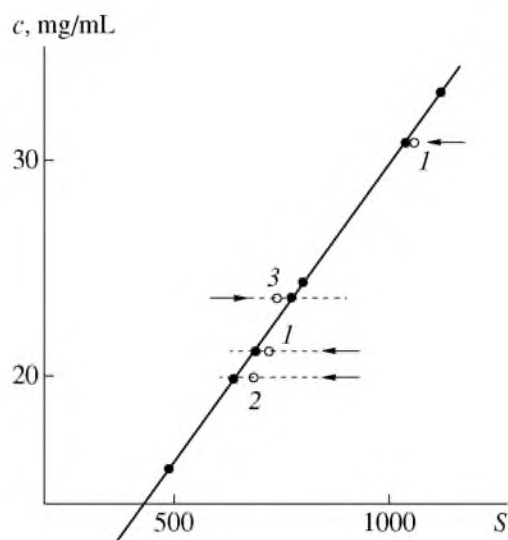


Fig. 3. Calibration plot for the quantification of total triglycerides of (no mark) apricot pit, (1) soybean, (2) sunflower seed, and (3) olive oil with (solid circles) and without (open circles) correction factors.

normalization methods: simple normalization and normalization with correction factors. Both methods are easy to implement in the computer processing of chromatograms. Using simple normalization (Table 1), the molar fractions of triglycerides were calculated from the areas of the corresponding peaks; next, the fatty-acid composition was calculated (Table 2). The instrumental error of the triglyceride composition determined by both methods of internal normalization was less than 1% ($n = 3$, $RSD < 0.2\%$). The estimated quantification error for triglycerides and fatty acids was mainly governed by the fraction of poorly resolved triglycerides. Half the range of the triglyceride content obtained under the assumptions that only Le_2St or only $LeOlPa$ was present was taken as the quantification error. The obtained results as a whole correspond to the known literature data [7, 8].

The proposed procedure is rapid (an analysis takes 10 min) and sample-efficient (one sunflower seed is sufficient). It makes it possible to distinguish high-oleic oils from high-linoleic ones by the peak ratio of the corresponding triglycerides without calculations.

The applicability of the developed procedure to the determination of total triglycerides in different oils was also studied. In this case, we used 15–35-mg/mL solutions of oil and measured the total area of the peaks corresponding to all the detected triglycerides. We used

oleic (olive), oleic–linoleic (apricot pit), linoleic (sunflower-seed), and linolenic–linoleic (soybean) oils. Simultaneous processing of the data obtained shows that the relative response of the soybean oil is somewhat higher than that of the apricot pit and sunflower seed oils, and the response from the olive oil was the lowest (Fig. 3). The possible reason for this is the difference between the refraction coefficients of different triglycerides. Because it is difficult to obtain all necessary individual triglycerides for calibration, we calculated the refraction coefficients using the ACD Labs program package and found the difference between the resulting coefficients and the refraction coefficient of the mobile phase. The ratio between these values and that for trioleate gives the correction factors for other triglycerides for the normalization with correction factors. After the recalculation, the difference between the used oils disappeared (Fig. 3, Table 3). Moreover, the method used made it possible to obtain close values for highly saturated cocoa butter: the deviation from the overall calibration line was less than 2%, although no true solution was obtained from the used cocoa butter in sample preparation.

Because the normalization with correction factors has only a small effect on the estimated triglyceride and fatty-acid composition of oil (Tables 1 and 2), simple normalization can be recommended for studying the composition of vegetable oils.

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