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Effort to improve the quantitative determination of oxidation and hydrolysis compound classes in edible vegetable oils

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Abstract

This paper proposes an analytical method to evaluate the classes of products of polymerization, oxidation and hydrolysis as well as the polar compounds present in refined edible oils in a more reliable fashion. The polar compounds of a marketed refined peanut oil were analyzed by preparative gel permeation chromatography and the classes of substances corresponding to single chromatogram peaks were collected by means of a fraction collector, purified and used as standards for high-performance size-exclusion chromatographic analysis. The linearity of detector response, the precision and accuracy of the method for each class of compounds and for polar compounds were assessed. Another aim was to verify whether this method may be applied to other refined peanut oils and to edible vegetable oils in general, even of different botanical origin, using the standards that had already been prepared for that particular peanut oil. The results obtained showed that this was possible and the analytical method developed can be extended to the most common edible vegetable oils.

Keywords: Food analysis; Fats; Vegetable oils; Triglycerides; Diglycerides

1. Introduction

Evaluation of the classes of products of oxidation, polymerization and hydrolysis has provided greater insight into the assessment of the state of degradation of oils and fats used for cooking, e.g., for frying [1,2] and has made it possible to better establish the extent of oxidation and hydrolysis of edible vegetable oils, especially the recently refined ones [3,4]. This approach has currently been also used in studies on oxidation [5].

A two-phase analytical procedure is used with the first phase consisting of the separation of polar compounds (PCs) from the oil as described by the IUPAC method [6] with silica gel column chromatography. The PCs, which are substances having a polarity greater than that of unaltered triglycerides, mainly comprise triglyceride oligopolymers, oxidized triglycerides, partial glycerides, sterols, triterpene diols and fatty acids [7]. The quantitative determination of these classes of compounds is carried out during the second phase when the PCs are analyzed by high-performance size-exclusion chromatography (HPSEC). One to three columns connected in series are packed with highly cross-linked porous styrene–divinylbenzene copolymer with a $d_p < 10$ μm and pore diameters ranging from

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500 Å to 100 Å [8–11]. Analyses were carried out isocratically and toluene, tetrahydrofuran or dichloromethane were used as elution solvents at flow-rates ranging from 0.6 to 1.0 ml/min. A differential refractometer was generally used as a detector though more recently some authors have used an evaporative light scattering detector [11].

HPSEC analysis has been used for PCs since the late 1980s [12]. Satisfactory separations may generally be obtained between the various classes of compounds but their quantitative determination has been difficult to achieve since each chromatogram peak may correspond to a complex group of substances. This has induced some researchers to divide the mass of the PCs, after it had been obtained gravimetrically [13] or by means of an internal standard [14], amongst the various peaks on the bases of their respective areas on the chromatogram and assuming that they had identical response factors. Other authors have relied on standards corresponding to single substances or to their appropriate mixtures [10,15–18]. In any case, evaluations are not easy because the standards utilized may yield roughly approximate results since each of them must serve to quantitate a class of compounds. The same holds true when the areas alone are used since, for instance, classes of different compounds of the same mass may produce different responses from the detector and therefore determine different areas for the corresponding chromatogram peaks.

The first aim of this paper was to define a method for a more reliable HPSEC analysis of the various classes of polar compounds. To this purpose, the PCs of the refined peanut oil were analyzed by preparative gel permeation chromatography (GPC) at a low pressure. The classes of compounds corresponding to the single peaks on the chromatogram were collected by means of a fraction collector, weighed and then used as standards in the HPSEC method. In this way the standards used are the classes of compounds themselves and not single standard substances thereby enhancing the reliability of the results obtained.

The second aim was to assess whether this method may be applied to other refined peanut oils and to edible vegetable oils in general, even of different botanical origin, using the standards that had been prepared for that particular peanut oil. Our working hypothesis was founded on the assumption that the classes of compounds constituting the PCs of different oils are similar.

2. Experimental

2.1. Chemicals

Silica gel 60 (0.063–0.200 mm) for column chromatography and pre-coated silica gel 60 thin-layer chromatography (TLC) plates of 0.25 mm thickness were purchased from Merck (Darmstadt, Germany). CH_2Cl_2 was HPLC grade and light petroleum (b.p. 40–60°C) and diethyl ether were of analytical grade.

2.2. Samples

The following refined oils were purchased in supermarkets of the area of Bari (Southern Italy): peanut, soybean, sunflower, corn, as well as one sample of olive oil (a mix of refined olive oil and virgin olive oil) and one of extra virgin olive oil.

2.3. Polar compound separation

PCs were separated from each oil as described by the IUPAC method [6]. The efficacy of separation was checked by TLC as recommended by the same method. One refined peanut oil was considered for standard preparation. PC separation from this oil was repeated 10 times to collect a sufficient amount of PCs.

2.4. Preparative gel permeation chromatography

The PCs of the refined peanut oil were analyzed by preparative GPC at a low pressure using a glass column, 1.9 m×1 cm I.D., packed with a spherical porous styrene–divinylbenzene copolymer with 2% crosslinkage Bio-Beads SX-2, 200–400 mesh (Bio-Rad Labs., Richmond, CA, USA). The elution solvent was CH_2Cl_2 for high-performance liquid chromatography (HPLC) kept at a constant flow-rate of 1 ml/min by a peristaltic pump (LKB, Bromma, Sweden). The detector was a differential refractometer (Milton Roy, Riviera Beach, FL, USA), with $128 \cdot 10^{-6}$ refractive index units full scale (RIUFS),

connected to an integrator. The analysis was performed at room temperature. Up to 200 mg of PCs could be put into the column without jeopardizing the type of separation obtained.

2.5. Preparation of standards

The substances corresponding to the peaks eluted in the preparative GPC were collected separately by means of a fraction collector. The fractions corresponding to a single peak were pooled together, the solvent made to evaporate at reduced pressure and the GPC repeated for purification, which was obtained by collecting only the substances that eluted in the main peak. The pureness of each standard was subsequently evaluated for HPSEC.

2.6. High-performance size-exclusion chromatography

The HPSEC of the PCs was carried out utilizing the following chromatographic system: a series 10 pump (Perkin-Elmer, Norwalk, CT, USA), a 7125 S sample injector (Rheodyne), 50 μl injector loop, and a series of three PL-gel columns (Perkin-Elmer, Beaconsfield, UK), 30 cm×0.75 cm I.D. The columns were packed with highly cross-linked styrene–divinylbenzene copolymer with a d_p=5 μm and pore diameters of 500 Å, 500 Å and 100 Å, respectively. A PL-gel guard column, 5 cm×7.5 mm I.D. was used. The detector was a deflection type differential refractometer (RID 6 A, Shimadzu, Japan) connected to an integrator. The detection limit was 35 ng whereas the lowest detection limit for this method was 0.01% for each component with respect to the oil. Under our experimental condition the RIUFS was $8 \cdot 10^{-6}$. The elution solvent used was CH_2Cl_2 for HPLC at flow-rate of 1.0 ml/min. The analyses were performed at room temperature. The procedure for identifying the peak on each chromatogram were carried out as described elsewhere [9].

2.7. Linearity, precision and accuracy

The linearity of the detector responses for the standards we prepared was assessed by means of a linear regression analysis regarding the amounts of each standard (measured in μg) introduced in the loop of the chromatographic system and the area of the corresponding peak on the chromatogram. Accuracy and precision were evaluated by adding known escalating amounts of each standard and of PCs to a solution of a known PC concentration whose analysis was replicated three times. HPSEC analysis was then performed with the usual procedure and replicated thrice. Precision was expressed as relative standard deviation (RSD) and accuracy as found amount (%).

2.8. Statistical analysis

The data from different methods were compared by means of the two-tailed *t*-test independent samples.

3. Results and discussion

Fig. 1 shows the GPC chromatogram for the PCs of the peanut oil examined. An effective separation was obtained between the main PC classes, namely the triglyceride oligopolymers (PTGs), oxidized triglycerides (ox-TGs) and diglycerides (DGs); the sterols and triterpene diols eluted in peak 6 [7] while peak 5 was small, had the same retention time as monoglycerides and may be ascribed also to other substances having a similar molecular mass since the content of monoglycerides is negligible in refined vegetable oils.

Fig. 2A shows the HPSEC chromatogram for the PCs of the same oil. Comparison of the two figures evinces that in the GPC chromatogram the oligopolymers eluted as a single peak while in the HPSEC chromatogram the triglyceride trimers were separated from the dimers. Very small amounts of fatty acids are present in refined oils so they were determine by means of acid–base titration as described in the official analytical methods [19].

The substances eluted in each peak of the GPC chromatogram were collected, purified and used as standards in calibrating the HPSEC method. Fig. 2B, C, D, E and F depict the HPSEC chromatograms of the different classes of compounds collected and purified by GPC. The area corresponding to the main peak for each standard ranged from 98.9% to 99.7%

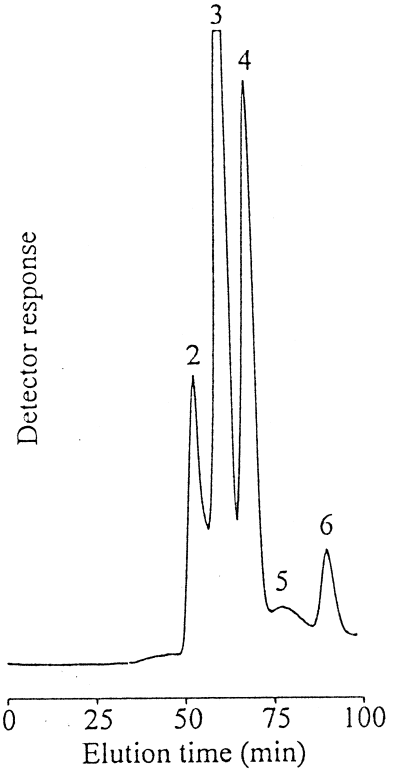

Fig. 1. Preparative GPC of the polar compounds in the refined peanut oil considered. Glass column 1.9 m×1 cm I.D. packed with Bio-Beads SX-2, 200–400 mesh. Differential refractometer as detector. CH_2Cl_2 as elution solvent at a flow-rate of 1 ml/min; injection volume 1 ml; injection solvent CH_2Cl_2. (2) Triglyceride oligopolymers 10.0 mg; (3) oxidized triglycerides 35.9 mg; (4) diglycerides 21.1 mg; (5) monoglycerides and non-identified substances 0.4 mg; (6) free sterols, triterpene diols and non-identified substances 2.1 mg.

thus demonstrating the high degree of purity obtained in the preparation.

Assessment of whether the responses of the detector were linear with the standards we prepared and fell within the range normally found in refined vegetable oils was carried out as follows. The amount collected for each standard corresponding to a given class of compounds was used to prepare a stock solution in CH_2Cl_2 and solutions containing different concentrations after successive dilutions. These solutions were analyzed by HPSEC following the analytical methods we developed. The calibration curves were obtained by plotting the amounts of standards (μg) that had been put into the HPSEC

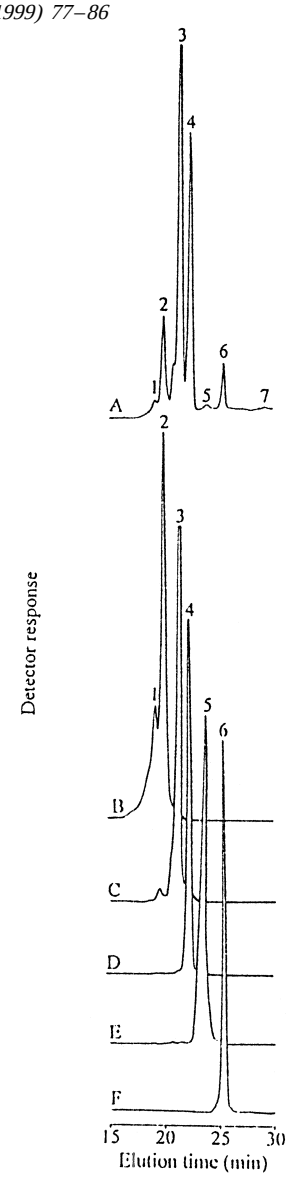

Fig. 2. (A) HPSEC of polar compounds in the refined peanut oil considered. (1) Triglyceride trimers 3.7 μg; (2) triglyceride dimers 17.9 μg; (3) oxidized triglycerides 77.6 μg; (4) diglycerides 45.6 μg; (5) monoglycerides and non-identified substances 0.8 μg; (6) free sterols, triterpene diols and non-identified substances 4.6 μg; (7) free fatty acids (determined by acid–base titration). N.B. The sample was diluted five-times before the injection. The same calibration line was used for triglyceride dimers and trimers. HPSEC of substance classes of polar compounds separated and purified by preparative GPC: (B) triglyceride oligopolymers; (C) oxidized triglycerides; (D) diglycerides; (E) peak 5 (t_R=24.5 min); (F) peak 6 (t_R=25.9 min). Deflection type differential refractometer as detector. Elution solvent CH_2Cl_2 at a flow-rate of 1.0 ml/min; injection volume 50 μl; injection solvent CH_2Cl_2.

system loop against the areas 10^{-3} of the corresponding chromatogram peaks. The linear regression equations were:

- $y=0.0264x+0.7902$ ($n=10$) for PTGs, where the response proved to be linear within the range 1.5–154 μg; the standard errors for regression, intercept and slope were 0.5372, 0.2211 and 0.000099, respectively; the regression coefficient was 0.9999 ($p<0.001$).
- $y=0.0297x+0.1889$ ($n=10$) for ox-TGs, where the range of the linear response assessed was 1–136 μg; the standard errors for regression, intercept and slope were 0.6822, 0.3166 and 0.00015, respectively; the regression coefficient was 0.9999 ($p<0.001$).
- $y=0.0295x+0.7955$ ($n=10$) for DGs, where the range of the linear response assessed was 1–137 μg; the standard errors for regression, intercept and slope were 1.0249, 0.4715 and 0.00021, respectively; the regression coefficient was 0.9998 ($p<0.001$).
- $y=0.0177x+0.5419$ ($n=7$) for peak 5 ($t_R=24.5$, Fig. 2) where the range of the linear response assessed was 1–36 μg; the standard errors for regression, intercept and slope were 0.6188, 0.3208 and 0.00035, respectively; the regression coefficient was 0.9989 ($p<0.001$).
- $y=0.0182x+0.7794$ ($n=7$) for peak 6 ($t_R=25.9$, Fig. 2) where the range of the linear response assessed was 2–50 μg; the standard errors for regression, intercept and slope were 0.5400, 0.2978 and 0.00023, respectively; the regression coefficient was 0.9996 ($p<0.001$).

With the calibration curves thus obtained, the percent mass of the single classes of compounds in the original oil was then directly computed on the basis of the peak areas on the HPSEC chromatogram without needing to know the mass of the PC. In our case, the PCs had been brought to volume in a 5-ml volumetric flask before the HPSEC analysis and the injection loop was 50 μl, so:

$$\text{PTGs (\%)} = \frac{\text{PTG (numerical value of the calibration curve)}}{\text{Ws}} \cdot 10$$

$$\text{ox-TGs (\%)} = \frac{\text{ox-TG (numerical value of the calibration curve)}}{\text{Ws}} \cdot 10$$

$$\text{DGs (\%)} = \frac{\text{DG (numerical value of the calibration curve)}}{\text{Ws}} \cdot 10$$

where Ws indicates the mass of the oil sample collected for the analysis expressed in mg. The same occurred for peaks 5 and 6.

The percent sum of the single classes of compounds made it possible to determine the percent mass of the PCs in a fully independent fashion.

The accuracy and precision of the method were assessed for the main classes of compounds and for the PCs as indicated in Section 2.7. The results obtained are reported in Tables 1 and 2. The amounts added for the single standards and for the PCs are expressed as μg introduced in the HPSEC system loop. Precision was expressed as RSD while accuracy as found amount (%).

The found amounts (%) ranged: from 95.3 to 99.9

Table 1
Accuracy and precision of triglyceride oligopolymers, oxidized triglycerides and diglycerides determinations

Added (μg) (loop of HPSEC)	Triglyceride oligopolymers		Oxidized triglycerides		Diglycerides	
	Found (%)	RSD (%)	Found (%)	RSD (%)	Found (%)	RSD (%)
5	97.7	3.06	101.8	1.48	105.9	2.30
10	99.9	3.29	105.7	1.15	102.2	0.95
15	96.0	2.19	103.9	2.38	100.4	1.63
20	95.3	1.77	106.9	0.23	100.4	1.44
25	98.5	1.18	101.8	1.79	96.1	1.45
30	96.4	1.18	101.3	1.35	98.2	2.82

RSD = (standard deviation / mean found) · 100, $n=3$.

Table 2
Accuracy and precision of polar compounds determination

Added (μg) (loop of HPSEC)	Found (%)	RSD (%)
10	100.9	0.89
20	101.9	0.53
30	103.9	3.01
40	97.6	0.29
50	100.6	1.94
60	99.2	1.72

RSD = (standard deviation / mean found) · 100, $n = 3$.

for the PTGs with an RSD ranging from 1.18 to 3.29%; from 101.3 to 106.9 for the ox-TGs with an RSD ranging from 0.23 to 2.38%; from 96.1 to 105.9 for the DGs with an RSD ranging from 0.95 to 2.82% and from 97.6 to 103.9 for the PCs with an RSD ranging from 0.29 to 3.01%.

Table 3 reports the results obtained from 10 independent replicate analyses of the PCs. The PCs were determined both gravimetrically and by our method. With our method the PC values closely approximated the PCs determined by means of the IUPAC method. Overall there was a slight difference which amounted to only 0.5%. Furthermore, as the SD and RSD values show, the variability of the results obtained with our method was very limited with a reduction of the RSD amounting to as much as 22.9%. This may be ascribed to the difficulty in eliminating the last traces of solvent during the IUPAC gravimetric analysis of the PCs. The average values obtained for the single classes of compounds were: 1.05% for the PTG with an RSD = 2.80%; 3.86% for the ox-TGs with an RSD = 2.13%; 2.28% for the DGs with an RSD = 1.66%. As could have been expected the RSD values all fell within the ranges that had been found experimentally for the single classes of compounds and for the PCs when accuracy and precision had been assessed.

Finally, the results in Table 3 obtained for the PCs with both the IUPAC method and the proposed method were statistically compared by means of the two-tailed *t*-test independent samples. No statistically significant differences were found to exist between the two methods at a confidence level of 95%.

At this point we decided to check whether the results obtained with our method were comparable to the ones obtained with the other analytical methods currently used. As mentioned in the introduction, one of the methods used consists in subdividing the mass of the PCs amongst the peak areas on the chromato-

Table 3
Determination of polar compounds and of their constituting substance classes of the refined peanut oil considered

Independent replicate analyses	IUPAC method Polar compounds (%)	Proposed method						
		Triglyceride oligopolymers (%)	Oxidized triglycerides (%)	Diglycerides (%)	Peak 5 (%) (t_R = 24.5 min)	Peak 6 (%) (t_R = 25.9 min)	FFAs (%)[a]	Polar compounds (%)[b]
1	7.73	1.08	3.91	2.26	0.04	0.25	0.05	7.59
2	7.25	1.01	3.73	2.23	0.05	0.24	0.05	7.31
3	7.31	1.01	3.94	2.29	0.05	0.23	0.05	7.57
4	7.57	1.07	3.89	2.32	0.04	0.24	0.05	7.61
5	7.69	1.03	3.79	2.25	0.05	0.26	0.05	7.43
6	7.58	1.05	3.73	2.24	0.04	0.25	0.05	7.36
7	7.37	1.08	3.88	2.28	0.04	0.23	0.05	7.56
8	7.42	1.09	3.96	2.35	0.04	0.23	0.05	7.72
9	7.48	1.05	3.90	2.31	0.05	0.22	0.05	7.58
10	7.39	1.03	3.87	2.28	0.04	0.23	0.05	7.50
Mean	7.48	1.05	3.86	2.28	0.04	0.24	–	7.52
SD	0.16	0.03	0.08	0.04	0.005	0.01	–	0.12
RSD (%)[c]	2.14	2.80	2.13	1.66	11.74	5.17	–	1.65

[a] FFAs = Free fatty acids (determined by acid–base titration).
[b] Total sum of the percent amounts of: triglyceride oligopolymers, oxidized triglycerides, diglycerides, peak 5, peak 6, free fatty acids.
[c] RSD was obtained considering all the decimal places of both mean and SD.

Table 4
Polar compounds (IUPAC method) and their constituting substance classes of the refined peanut oil considered utilizing identical response factors

Independent replicate analyses	Polar compounds (%)	Triglyceride oligopolymers (%)	Oxidized triglycerides (%)	Diglycerides (%)	Peak 5 (%) (t_R = 24.5 min)	Peak 6 (%) (t_R = 25.9 min)	FFAs (%)[a]
1	7.73	1.15	3.86	2.24	0.11	0.32	0.05
2	7.25	1.04	3.60	2.16	0.09	0.31	0.05
3	7.31	1.03	3.72	2.14	0.08	0.29	0.05
4	7.57	1.14	3.70	2.23	0.12	0.33	0.05
5	7.69	1.13	3.83	2.27	0.10	0.31	0.05
6	7.58	1.14	3.73	2.24	0.11	0.31	0.05
7	7.37	1.11	3.66	2.15	0.10	0.30	0.05
8	7.42	1.13	3.67	2.19	0.10	0.28	0.05
9	7.48	1.12	3.72	2.20	0.10	0.29	0.05
10	7.39	1.07	3.71	2.18	0.09	0.29	0.05
Mean	7.48	1.11	3.72	2.20	0.10	0.30	–
SD	0.16	0.04	0.08	0.04	0.01	0.02	–
RSD (%)[b]	2.14	3.93	2.06	1.99	11.55	5.17	–

[a] FFAs = Free fatty acids (determined by acid–base titration).
[b] RSD was obtained considering all the decimal places of both mean and SD.

gram assuming that they have identical response factors [13,17]. Other authors [16], instead, have utilized external standards, such as triolein, to assay triglyceride oligopolymers and oxidized triglycerides, and diolein to measure diglycerides. In a subsequent paper, some of these authors [20] found that triolein and diolein had very similar response factors when using a differential refraction index as a detector, thus practically reverting to the method based on the identical response factors. Table 4 contains the PC values for the ten independent determinations reported in Table 3 ascertained following the IUPAC method and their corresponding subdivision based on the peak areas considered using identical response factors. The data differ from the results obtained with the method we propose by approximately 5%, at least for the main classes of substances. The statistical analysis reported in Table 5 shows a clear-cut difference with $p<0.01$ for the oligopolymers and $p<0.001$ for the oxidized triglycerides and the diglycerides. In particular, there proved to be an overestimation of the oligopolymers and an underestimation of the oxidized triglycerides and the diglycerides when identical response factors were relied on. The peaks with $t_R = 24.5$ min and $t_R = 25.9$ min also appear to have been overestimated though their presence in the PCs may be considered quantitatively less relevant. Finally, de Greyt et al. [18] used tristearin, distearin, monostearin and a standard reference material with a certified amount of dimeric and polymeric triglycerides (CHEK, The Netherlands) to identify and quantitate the peaks obtained from HPSEC of PCs. The same authors, however, added that calibration is particularly difficult for dimeric triglycerides because they can be composed of both oxidized and non-oxidized lipids.

Table 5
Difference between the corresponding substance classes present in polar compounds, obtained with the two analytical methods considered

Determination	Two-tailed *t*-test independent samples	Ten independent replicate analysis (mean±SD)	
		Proposed method	Method with usual response factors
Triglyceride oligopolymers	$p<0.01$	1.05±0.03	1.11±0.04
Oxidized triglycerides	$p<0.001$	3.86±0.08	3.72±0.08
Diglycerides	$p<0.001$	2.28±0.04	2.20±0.04

Another aim of the research was to verify whether the analytical method developed for a particular refined peanut oil could be applied to other commercially available refined peanut oils with the same degree of reliabilitìy. In fact it could not be excluded, a priori, that different peanut oils produced at different processing plants contained classes of compounds of oxidation, polymerization and hydrolysis that were not similar in terms of the types of molecules present. In this case it would be necessary to prepare other standards for each oil studied. Four refined peanut oils marketed by different companies were examined for this purpose. Four independent PC determinations following the IUPAC method and four independent HPSEC analyses of the PCs were carried out on each oil sample.

The data obtained are reported in Table 6. The values of the PCs obtained with the method proposed always closely approximated those determined by the IUPAC method with a maximum error of 1.55% when compared to the tabulated means. The error was computed considering the values obtained with the IUPAC method as reference values. Furthermore, the variability of the results obtained with the method proposed was smaller for all the samples as may be demonstrated by the lower RSD values. The PC values reported in Table 6 obtained with both the IUPAC method and the proposed method were statistically compared by means of the two-tailed *t*-test independent samples. No statistically significant differences were found to exist between the two methods at a confidence level of 95%.

The use of the classes of PCs present as standards, the reduced variability of the experimental results and the observation that the PC values measured with the proposed method are statistically indistinguishable from the ones obtained with the IUPAC method demonstrated that the results obtained had the same characteristics as those we had initially found for the reference peanut oil the standards were prepared from. This confirmed the validity of our initial hypothesis that the corresponding classes of compounds constituting the PCs of refined oils are similar and warranted extending the analytical method developed with a particular refined peanut oil to all the other refined peanut oils.

The experimental investigation was thence extended to oils of different botanical origins to assess

Table 6
Determination of polar compounds and of their constituting substance classes of the refined peanut oils

Samples	IUPAC method Polar compounds (%)	Proposed method						
		Triglyceride oligopolymers (%)	Oxidized triglycerides (%)	Diglycerides (%)	Peak 5 (%) (t_R = 24.5 min)	Peak 6 (%) (t_R = 25.9 min)	FFAs (%)[a]	Polar compounds (%)[b]
Peanut oil 1 (n^c = 4)								
Mean	6.07	0.58	3.23	1.87	0.03	0.26	0.14	6.13
RSD (%)	1.90	2.62	1.76	2.47	17.32	2.19	–	1.80
Peanut oil 2 (n^c = 4)								
Mean	6.84	1.05	3.61	1.89	0.03	0.26	0.06	6.90
RSD (%)	2.41	1.98	2.22	1.40	17.32	4.50	–	1.09
Peanut oil 3 (n^c = 4)								
Mean	5.01	0.66	2.47	1.43	0.06	0.34	0.11	5.04
RSD (%)	4.04	0.88	1.24	2.24	21.65	2.94	–	1.10
Peanut oil 4 (n^c = 4)								
Mean	7.75	1.02	3.84	2.38	0.06	0.26	0.06	7.63
RSD (%)	1.96	2.59	1.23	2.31	9.12	2.19	–	1.56

[a] FFAs = Free fatty acids (determined by acid–base titration).
[b] Total sum of the percent amounts of: triglyceride oligopolymers, oxidized triglycerides, diglycerides, peak 5, peak 6, free fatty acids.
[c] Number of independent replicate analyses.

whether the analytical method proposed could be employed continuing to use the standards available or whether corresponding standards had to be prepared for each oil studied. Refined soybean, sunflower and corn oils as well as one sample of olive oil and one of extra virgin olive oil were examined for this purpose. These samples were submitted to the same analytical procedures as the peanut oils we had already analyzed. Table 7 contains the relevant results obtained. As with the peanut oils, even in this case, the PC determined by the method proposed closely approximated the values obtained with the IUPAC method with a maximum error of 1.33% when compared to the tabulated means. The variability of the independent replicate analyses also proved to be smaller for all the samples with the method proposed. The greater differences in the RSD values were registered in the extra virgin olive oil sample where the RSD value with the IUPAC method was 6.86% and only 0.75% with the method proposed, namely a reduction of as much as 89%. In our opinion, such a conspicuous difference may be ascribed to low percent PC value found in this oil along with the fact that the last traces of solvent are not eliminated in a uniform fashion in the independent replicate analyses on the PCs with the IUPAC gravimetric method.

Even in this case, statistical comparison by means of two-tailed *t*-test independent samples between the PCs measured with the IUPAC method and the PCs obtained with the proposed method revealed no statistically significant differences at a confidence level of 95%.

The fact that oils of different botanical origins responded to the analytical method proposed in the same way as the refined peanut oils means that this method may also be applied to other edible vegetable oils. It is particularly convenient to use it with the higher quality oils as they contain a very low percent amount of PCs.

Table 7
Determination of polar compounds and of their constituting substance classes of the commercial edible vegetable oils

Samples	IUPAC method Polar compounds (%)	Proposed methods						
		Triglyceride oligopolymers (%)	Oxidized triglycerides (%)	Diglycerides (%)	Peak 5 (%) (t_R = 24.5 min)	Peak 6 (%) (t_R = 25.9 min)	FFAs (%)[a]	Polar compounds (%)[b]
Soybean oil (n^c = 4)								
Mean	5.31	0.84	2.49	1.43	0.06	0.30	0.12	5.24
RSD (%)	2.23	1.38	1.52	2.46	9.12	1.90	–	1.30
Sunflower oil (n^c = 4)								
Mean	5.40	0.68	2.99	1.45	0.05	0.21	0.06	5.42
RSD (%)	2.16	2.52	1.92	1.51	10.53	4.51	–	1.29
Corn oil (n^c = 4)								
Mean	4.67	0.46	1.36	2.33	0.04	0.25	0.28	4.73
RSD (%)	3.98	3.30	2.12	1.51	13.32	4.00	–	1.20
Olive oil (n^c = 4)								
Mean	4.51	0.21	0.65	3.11	0.04	0.31	0.27	4.57
RSD (%)	3.14	4.61	2.32	1.83	16.50	1.89	–	1.46
Extra virgin olive oil (n^c = 4)								
Mean	2.60	nd[d]	0.47	1.63	0.01	0.26	0.21	2.57
RSD (%)	6.86	–	2.70	1.28	0.00	2.25	–	0.75

[a] FFAs = Free fatty acids (determined by acid–base titration).
[b] Total sum of the percent amounts of: triglyceride oligopolymers, oxidized triglycerides, diglycerides, peak 5, peak 6, free fatty acids.
[c] Number of independent replicate analyses.
[d] nd = Not detected.

Based on the results obtained, the method proposed in this paper appears to be precise, accurate and suitable for tracking the evolution of the classes of the products of oxidation, polymerization and hydrolysis in studies regarding the changes technological processes produce in fatty substances, lipid oxidation and the effect of substances having an anti-oxidant or pro-oxidant activity on the shelf-life of oils, fats or foods containing a lipid fraction.

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