Oligopolymer, Diglyceride and Oxidized Triglyceride Contents as Measures of Olive Oil Quality[1]

Tommaso Gomes*
Istituto di Industrie Agrarie, University of Bari, I-70126 Bari, Italy

The objective of this study was to test the qualities of olive oils of different commercial grades by quantifying oligopolymer compounds by high-performance size-exclusion chromatography (HPSEC). The method required no sample manipulation and was accurate and rapid. The mean level of oligopolymers in refined olive oils was 0.70% and was more than twice as high in refined olive pomace oils. Conversely, edible virgin olive oils had no oligopolymer compounds. HPSEC analyses of polar compounds by silica gel column chromatography also allowed determination of oxidized triglycerides and partial glycerides, which help define levels of oxidative degradation and hydrolysis.

KEY WORDS: HPSEC, oligopolymer compounds, olive oil, oxidized triglycerides, partial glycerides.

Oligopolymer determinations in oils and fats have been the subject of a considerable amount of research due to the importance of oligopolymers in ascertaining the level of oxidative and thermal degradation during heat exposure, especially during food frying (1–3).

High-performance size-exclusion chromatography (HPSEC) is the most widely used method. Generally, two or three columns, connected in series, are packed with styrenedivinylbenzene copolymer (particle diameter < 10 μm and pore diameters between 100 and 1,000 Å). The refractive-index detector is commonly used, although an ultraviolet (UV) detector is preferred by some authors (4). The method is quite simple; the oil is dissolved in an appropriate solvent, then injected into the chromatographic system.

While oligopolymers in thermally oxidized oils are rather easily quantified, sensitivity problems are observed when the effects of refining and autooxidation on the formation of polymer compounds are investigated. Such problems are due to the extremely low oligopolymer contents and to the amount of oil that can be introduced into the chromatographic system without reducing the degree of separation obtainable.

Dobarganes and co-workers (5) used two columns (100 and 500 Å ultrastyragel) connected in series and a 10-μL loop in their work on heated and unheated oils. They concluded that direct oil analysis of oligopolymers in unheated oils was not possible because triglyceride levels were more than 95% of the fat and, therefore, no other components present in the sample could be quantified.

The present study reports the development of a time-saving method by which small amounts of oligopolymer compounds can be determined by direct oil analysis without concentrating them. The study also reports determinations of partial glycerides and oxidized triglycerides by HPSEC of polar compounds (6) for a better evaluation of the hydrolytic and oxidative state. Olive oil samples differing in quality were taken into account.

EXPERIMENTAL PROCEDURES

This paper reports the results obtained by analyzing different types of olive oils of certified origin. "Virgin" olive oil is oil obtained from olives (Oleaceae, *Olea europaea*) by mechanical or other physical means without any further refinement except filtration. "Extra virgin" olive oil is high-quality virgin olive oil with free fatty acids (FFA) <1.0% and faultless taste and aroma. "Lampante" olive oil is a virgin olive oil with more than 3.3% FFA and/or unpleasant flavor and is sold only after refining. "Refined" olive oil is oil obtained by refining "lampante" olive oil. "Refined olive pomace" oil is oil extracted with solvents from the olive residue of mechanical extraction of virgin oil and made edible by intensive refining.

Samples of extra virgin olive oil, refined olive oil and refined olive pomace oil were analyzed for FFA (as percent oleic acid), peroxide values (PV) and conjugated diene contents at 232 nm (K_{232}) (7).

Oligopolymers were determined by HPSEC. The chromatographic system was composed of a Perkin-Elmer pump series 10, a 7125 S sample injector (rheodyne), a 50-μL injector loop and a series of three PL-gel columns (Perkin-Elmer Ltd., Beaconsfield, Great Britain) of 7.5 mm i.d. × 30 cm length. The column packing material was highly crosslinked styrenedivinylbenzene copolymer with particle diameter of 5 μm and pore diameters of 500 Å, 500 Å and 100 Å, respectively. A column inlet filter (2 μm) between the injector and the column prevented blockage of the column inlet frit. Elution was carried out with CH_2Cl_2 for high-performance liquid chromatography (HPLC) at a flow rate of 0.9 mL/min. The detector consisted of a differential refractometer connected to an integrator.

The oil samples, dissolved in CH_2Cl_2 to a concentration of about 50 mg/mL, were filtered through 0.5 μm polytetrafluoroethylene filters (Millipore, Bedford, MA) and injected directly into the chromatographic system.

Polystyrene standards (Supelchem, Milan, Italy) of known molecular weights (4,000 and 2,000 g/mol), and tristearin, distearin, monostearin and stearic acid standards (Sigma, St. Louis, MO) were used for peak identification.

Polar compounds were prepared from each oil sample by silica-gel column chromatography (CC) as described by IUPAC method (6). The efficacy of column separation was checked by thin-layer chromatography (TLC) for absence of nonpolar triglycerides in the polar fraction. When TLC did not reveal complete and sharp separation, polar compounds were again subjected to CC. After that, inspection by TLC always showed absence of nonpolar triglycerides in polar compounds. Lastly, polar compounds in CH_2Cl_2 were brought to volume in a 5-mL volumetric flask, filtered and analyzed by HPSEC.

[1]Research supported by National Research Council of Italy, Special Project RAISA, Sub-project 4, Paper No. 321.

*Address correspondence at Istituto di Industrie Agrarie, University of Bari, Via Amendola 165, I-70126 Bari, Italy.

RESULTS AND DISCUSSION

Peaks on the chromatograms were identified by polystyrene standards of known molecular weight (MW), as well as tristearin, distearin and monostearin standards. For each standard, the elution volume was measured under the same conditions as used in our analysis. The log of MW as a function of elution volume was plotted, and the line of best fit was drawn by using the least squares method (Fig. 1). From the elution volume of each separate peak in a chromatogram, the corresponding MW could then be obtained.

Figure 2 shows the HPSEC chromatogram of a refined olive oil. Peak 2 appeared to be triglyceride dimers (MW = 1,800). Although present in the sample in small amounts, triglyceride dimers were clearly separated and were readily quantified. Peak 3 was triglycerides and peak 4 was diglycerides. Peak 5, partially separated from the diglyceride peak, and peak 6 are unidentified and are being investigated. Peak 7 had the same retention time as stearic acid and comprised FFA.

Thus, the method quantified not only triglyceride dimers but also fatty acid and diglyceride contents. Diglycerides were also determined by polar compound analysis, where the peak was more sharply separated and more convenient to evaluate (Figs. 2 and 3).

Known amounts of oligopolymers, originating from thermal oxidation of vegetable oils and prepared as previously described (8), were used for quantitative analyses of oligopolymer substances. The line of best fit was drawn by plotting polymer weight as a function of the corresponding chromatogram area. The lowest observable oligopolymer concentration was 0.10% of oil. Reproducibility of the method was investigated by twelve consecutive analyses of a refined soybean oil. The mean was 0.97 mg/100 mg oil; the standard deviation, 0.030 mg/100 mg oil; and the coefficient of variation, 3.09%, showing high degree of precision for the technique.

Figure 3 shows an HPSEC chromatogram of polar compounds derived from the same refined olive oil as the oil sample in Figure 2. The diglyceride peak (no. 4) showed the most widely represented component. Triglycerides were in peak 3. Because these triglycerides were contained in polar compounds, they were oxidized triglycerides and, therefore, represent another important class of oxidative degradation products.

Both oxidized triglycerides and diglycerides were quantified by means of calibration curves obtained from

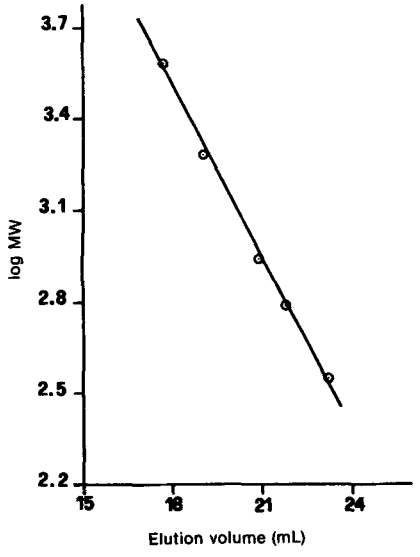

FIG. 1. Standard elution volumes *vs.* log of molecular weights.

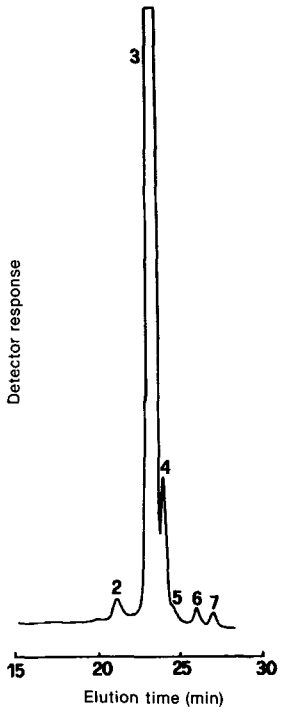

FIG. 2. Direct high-performance size-exclusion chromatography of a refined olive oil: 2) triglyceride dimers (0.73% of the oil), 3) triglycerides, 4) diglycerides, 5) unidentified, 6) unidentified, 7) fatty acids.

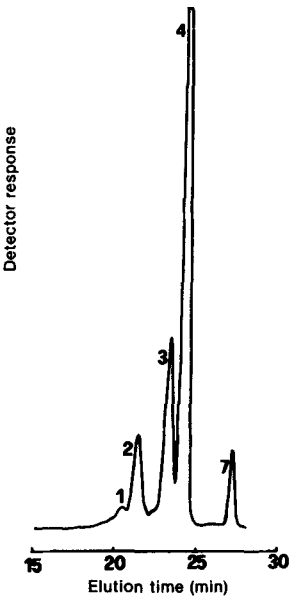

FIG. 3. High-performance size-exclusion chromatography of polar compounds derived from the same refined olive oil as in Figure 2: 1) triglyceride trimers, 2) triglyceride dimers, 3) oxidized triglycerides, 4) diglycerides, 7) fatty acids.

distearin and tristearin standards. The reproducibility of the method was demonstrated by ten consecutive analyses of olive oil polar compounds. For oxidized triglycerides the mean was 0.52 mg/100 mg oil; the standard deviation, 0.014 mg/100 mg oil; and the coefficient of variation, 2.70%. For diglycerides the mean was 2.47 mg/100 mg oil; the standard deviation, 0.030 mg/100 mg oil; and the coefficient of variation, 1.22%. Our method did not detect the presence of monoglycerides, possibly due to the low amounts contained in refined and virgin olive oils (9).

Triglyceride oligopolymers collected with the polar compounds appeared to be concentrated and were determined at lower levels than by direct oil analysis. The lowest observable concentration by this method was 0.02%. However, direct oil analysis was a valid tool because results were obtained rapidly and without manipulating the sample. Also, it allowed rapid screening of samples for the presence of oligopolymer compounds, diglycerides and fatty acids. A careful perusal of Figure 3 indicated that the main dimer peak was preceded by another peak which, according to its elution volume, corresponded to trimer triglycerides. Similarly, between the dimer peak and the oxidized triglyceride peak, there was yet another, rather small peak with a MW of approximately 1,200 and possibly ascribable, as also suggested by Perrin (10), to polymerization reactions between diglycerides.

Thus, triglyceride trimers were in fact found in refined oils in addition to oxidized triglycerides and triglyceride dimers. This means that vegetable oil refining may always involve the appearance, although in extremely small amounts, of oxidized compound classes that are characteristic of frying oils (3).

The chromatogram obtained by directly injecting an extra virgin olive oil is shown in Figure 4. No trace of oligopolymers was observed. The diglyceride content was less than that of refined olive oil. The same amount of oil was injected into the column in direct oil analysis. The chromatogram for polar compounds in the same oil (Fig. 5) did not reveal any presence of oligopolymers. It showed the presence of oxidized triglycerides, which could provide an additional important measure of quality.

Figure 6 shows the chromatogram obtained by direct analysis of a refined olive pomace oil. This clearly showed the relatively high oligopolymer content, which was higher than that of refined olive oil. Still more apparent was the intermediate peak between the dimer and the monomer

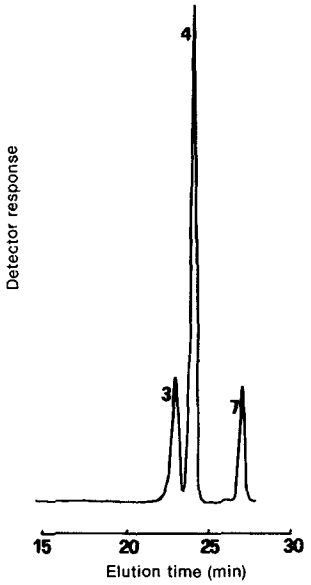

FIG. 5. High-performance size-exclusion chromatography of polar compounds derived from the same extra virgin olive oil as in Figure 4: 3) oxidized triglycerides, 4) diglycerides, 7) fatty acids.

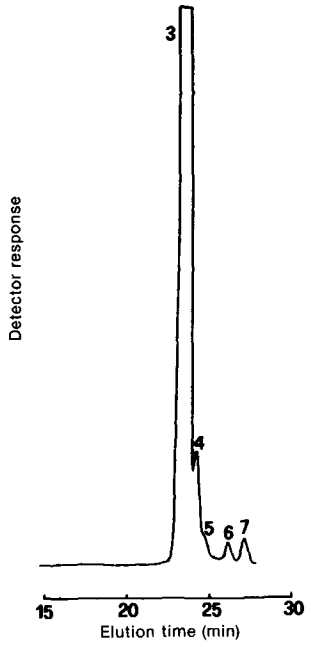

FIG. 4. Direct high-performance size-exclusion chromatography of an extra virgin olive oil: 3) triglycerides, 4) diglycerides, 5) unidentified, 6) unidentified, 7) fatty acids.

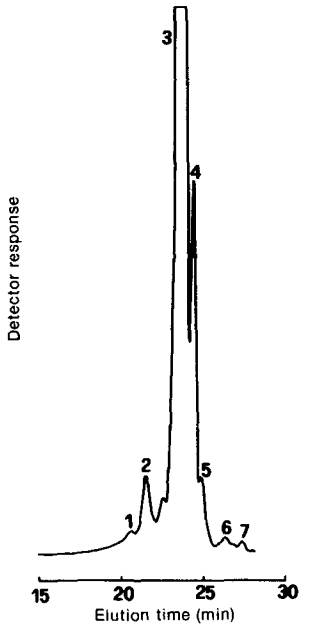

FIG. 6. Direct high-performance size-exclusion chromatography of refined olive pomace oil: 1) triglyceride trimers, 2) triglyceride dimers (1.92% of oil), 3) triglycerides, 4) diglycerides, 5) unidentified, 6) unidentified, 7) fatty acids.

TABLE 1

Chemical Characteristics of Extra Virgin Olive Oils[a]

Samples	FFA (% oleic acid)	Diglycerides (%)	PV (meq/kg)	K_{232}	Polar compounds (%)	Oxidized triglycerides (%)	Triglyceride oligopolymers (%)
1	0.30	1.20	8.6	1.624	2.03	0.39	n.d.
2	0.48	1.83	9.3	1.635	2.89	0.47	n.d.
3	0.83	2.42	5.8	1.509	3.79	0.44	n.d.
4	0.66	2.28	10.2	1.640	3.52	0.49	n.d.
5	0.85	2.10	8.3	1.690	3.73	0.40	n.d.

[a]Abbreviations: FFA, free fatty acid; PV, peroxide value; n.d., not detected.

TABLE 2

Chemical Characteristics of Refined Olive Oils and Olive Pomace Oils[a]

Samples	FFA (% oleic acid)	Diglycerides (%)	PV (meq/kg)	K_{232}	Polar compounds (%)	Oxidized triglycerides (%)	Triglyceride oligopolymers (%)
Refined olive oil	0.14	3.08	2.1	3.467	4.98	0.83	0.75
Refined olive oil	0.35	3.42	0.9	3.155	5.56	0.78	0.81
Refined olive oil	0.30	2.69	2.4	3.083	4.51	0.86	0.46
Refined olive pomace oil	0.18	5.81	1.5	5.920	9.98	1.40	2.52
Refined olive pomace oil	0.39	5.64	2.1	4.911	8.68	1.02	1.51
Refined olive pomace oil	0.09	7.14	2.5	4.189	9.82	1.20	1.30

[a]See Table 1 footnote for abbreviations.

triglyceride peaks, which also contributed to differentiation between the two types of oils. Diglycerides occurred in much higher amounts than in the other oils and were an unmistakable sign of hydrolytic degradation. The above findings were confirmed in greater detail by the chromatogram obtained for polar compounds.

Table 1 gives the data obtained for extra virgin olive oils. Triglyceride oligopolymers were absent in all. Oxidized triglycerides were found in all samples at a mean value of 0.44%, never exceeding 0.49%, and contributed, along with PV and K_{232}, to better establishment of the existing level of oxidation. Percent diglyceride content changed according to the level of oil acidity, and the data obtained with HPSEC of polar compounds were in good agreement with those reported in the literature (11).

Data obtained for refined oils are given in Table 2. Oxidized triglyceride levels were higher than those in extra virgin oils and as high as 1.40% in olive pomace oil. Oxidized triglyceride determination of refined oils provided better indications for the oxidation states than did PV and K_{232}, because the peroxides were destroyed and K_{232} was subject to variations during refining. Diglyceride contents of refined oils also provided useful information about hydrolytic degradation, whereas FFA were of little significance because they are removed in the refining process. Polar compounds provided information not only on oxidative, but on hydrolytic degradation as well because they also comprise partial glycerides and FFA. Thus, a virgin oil with relatively high acidity will show high levels of polar compounds without being extensively oxidized. Especially noteworthy were the data concerning oligopolymers which, as reported by some authors (2,12), derive from oxidative degradation and from thermal and catalytic activity at certain stages during refining. Oligopolymers were absent in extra virgin oils but were present in refined olive and pomace oils. Our data for refined olive oils indicated an average 0.70% and rose to more than twofold levels in refined pomace oils, due to the poorer quality of the raw material and to the more severe refining procedures that were used.

Amounts of polymer compounds, generally ranging between those of refined olive oils and of refined pomace olive oils, are also found in refined seed oils (5,13,14).

Thus, absence of oligopolymers in virgin oils can be used as a quality standard and rules out the possibility of mixing with refined oils. On the other hand, a comprehensive survey of an adequate number of certified refined olive oils could provide information on average contents of oligopolymer compounds. It would then be up to regulatory agencies to set maximum limits for oligopolymer levels, thus differentiating refined olive oils from refined olive pomace oils.

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[Received October 15, 1991; accepted July 23, 1992]