Development of High-Performance Liquid Chromatography Criteria for Determination of Grades of Commercial Olive Oils. Part I. The Normal Ranges for the Triacylglycerols

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Criteria for authentic olive oils were developed from isocratic high-performance liquid chromatography analyses of 99 olive oils from the major Mediterranean producers in the 1983–1986 crop years. Authentic olive oils include extra virgin, virgin and pure or refined oils, but exclude all reesterified and adulterated oils. The extra virgin through pure grades will have a combined area for the LOO ($C_{18:2}C_{18:1}C_{18:1}$), LOP ($C_{18:2}C_{18:1}C_{16:0}$), OOO ($C_{18:1}C_{18:1}C_{18:1}$), POO ($C_{16:0}C_{18:1}C_{18:1}$), POP ($C_{16:0}C_{18:1}C_{18:1}$), and SOO ($C_{18:0}C_{18:1}C_{18:1}$) peaks between 82.0 and 92.6% of the total area (L, linoleic; O, oleic; P, palmitic; S, stearic). Authentic oils will have ratios of LOO/LOP and OOO/POO that coincide with a line defined by OOO/POO = 0.7844(LOO/LOP) + 0.0968; correlation coefficient is 0.885. Authentic oils will not have a trilinolein (LLL) peak over 0.5% in area. Neither triolein (OOO) nor any other single peak suffices to characterize an olive oil sample as one of the authentic grades.

KEY WORDS: Adulteration, HPLC, olive oil, reesterified, refined, refractive index, triacylglycerols.

The United States Customs Service is charged with correctly classifying for tariff purposes all merchandise entering this country and enforcing at the borders laws normally administered by other agencies. In the case of entering olive oil shipments, the Customs Service is confronted with correctly determining a range of olive oil grades, and mixtures thereof, and mixtures of olive oils with other oils, which may involve adulteration or mislabelling of the articles.

The identification and classification of olive oils, which must be within the framework of the Harmonized Tariff Schedule of the United States (1), is complicated due to the nature of olive oil. Under ideal conditions, a super premium edible oil is obtained from olives, which is called "extra virgin." This grade is considered superior to the common edible oils and is many times the cost of ordinary high quality oils, *e.g.* "premium" corn oil or peanut oil. The high price commanded by the highest grades of olive oil constitutes one driving force behind the attendant commercial fraud. In addition, olives themselves are not an inexpensive fruit and must be harvested in timely fashion and with some care to avoid bruising the fruit. Consequently, growers and processors are understandably loathe to allow the least morsel of oil to escape the battery of pressing and extraction processes that have evolved to capture all of the oil. These procedures, in turn, generate a range of grades of olive oils, and our task is to distinguish among them.

We performed high-performance liquid chromatography (HPLC) separations on a total of 99 olive oils from the principal exporters to the United States. This sample set covers importations from Spain, Italy, France, Greece, Turkey and Tunisia for the years 1983–1986. Examination of these data has allowed us to abstract general HPLC characteristics of various grades of olive oil and to set limits for some of those grades based only on the HPLC separation of the triacylglycerols (TAGs). The International Olive Oil Council (IOOC) proposed a set of designations for various grades of olive oils in 1986 (2), which we review here so the correlation to our HPLC work can be understood.

Virgin olive oil is obtained from the olive fruits by purely mechanical means (*e.g.*, pressing) under conditions (especially near room temperature) such that there is no alteration in the oil, and no treatments other than washing, decantation, centrifugation and filtration are permitted. An "extra virgin" oil has absolutely perfect flavor and odor with acidity (as oleic acid) of less than 1 g/100 g. A "fine virgin" oil may have acidity as high as 2.0 g/100 g, but the flavor and odor must still be perfect. A "virgin" (or "semi-fine virgin") may have acidity as high as 3.3 g/100 g, but the flavor and odor must still be considered good. If the processing and/or the fruits were not of the best quality, a virgin oil of inferior quality is obtained, the "lampante" oil, which has an acidity level too high for human consumption without additional treatment. Lampante oil is not to be blended or used as such for human consumption without refining. Refining (usually with a caustic solution) will reduce the acidity to 0.5% and may involve deodorization and decolorizing to achieve palatability. Any of the virgin grades of olive oil that have been refined without altering the original glyceride structure are called "refined oils." The unqualified term "olive oil" is used for any of the blends of the virgin grades and refined virgin oil (*i.e.*, from the "lampante" oil).

After the initial pressings, the olive fruits are often extracted with organic solvents, typically hexane, to afford "crude olive pomace oil." This grade is generally in need of refining to achieve palatability. Crude olive pomace oil after refining is reduced to 0.5% acidity and referred to as "refined olive pomace oil." Refined olive pomace oil may be blended with virgin grades to yield "olive pomace oil." Such oil may have a maximum acidity of 1.5%. Oils that are extensively hydrolyzed may be reesterified and then refined to produce an oil that will not have the original glyceridic structure. This reesterified oil may not be sold for human consumption in at least some of the European Community countries (3). Fortunately, this plethora of possible grades is seldom encountered in United States commerce. The principal items of commerce are the virgin grades, refined, pomace oil and blends among them. The purpose of the present work is to identify all these grades collectively as acceptable olive oils and to exclude from the acceptable category all olive oils that are adulterated and/or reesterified.

Additional insight into the descending series of grades is provided in a much earlier description by Gracian (4). The successively more rigorous pressings of the olive fruits, with addition of warm water, and often the passage

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of time before solvent extraction result in: (i) a declining gradient in which the trace components responsible for the flavors are largely removed in the first pressings (the virgin grades); (ii) bitter or "off" flavors are released from the crushed olive pits; and (iii) lypolytic enzymes have time to hydrolyze the TAGs. (Oxidation reactions also become more important with the passage of time but are not treated in this paper.) Consideration of these factors led us to focus on an HPLC method to develop a screening procedure for the various olive oil samples. The HPLC separations achieved conveniently quantitate both the diacylglycerols (DAGs) and TAGs in one run. The information about the DAGs and TAGs is necessarily lost if one proceeds with the conventional fatty acid methyl ester gas chromatograpy analysis.

EXPERIMENTAL PROCEDURES

For samples numbered 1-46. HPLC separations were performed on a 40-cm length (25-cm and 15-cm columns, closely coupled) of 5-micron C18-coated particles (Supelcosil LC-18, Supelco Inc., Supelco Park, Bellefonte, PA). Elution was at 1.5 mL/min with acetone/acetonitrile (63.6:36.4, vol/vol) at room temperature. The detector was a Waters 401 differential refractometer (Waters Chromatography Division, Millipore Corp., Milford, MA), usually at 8× attenuation. Samples were prepared to about 9% concentration (wt/vol) in the mobile phase, with sufficient chloroform added to achieve completely clear solutions. The sample solutions were filtered through 0.45-micron filters (AcroDisc CR, Gelman Sciences Inc., Ann Arbor, MI). Twenty-microliter injections were done manually through a Waters U6K injector. Injections were performed six times for statistical calculations. Integrations were performed with an M-1 Computing Integrator (Perkin-Elmer, Norwalk, CT), with the minimum area set to 5,000 counts. Samples typically gave over 5,000,000 counts for total area.

For samples numbered 47-105. The following changes were made: HPLC separations were performed on two closely coupled 15-cm columns, of 3-micron C18-coated particles (Supelcosil LC-18, Supelco Inc.). The same eluant was used at 1.5 or 2.0 mL/min. The detector was a Waters 410 differential refractometer. Samples were injected automatically *via* a WISP 710B (Waters/Millipore Corp.) in a range of 10–15 microliters. Integrations were performed on a Hewlett-Packard 3390A Integrator (Palo Alto, CA). Samples were now filtered through 0.2-micron filters before injection (AcroDisc CR, Gelman Sciences Inc.).

Each sample was run at least six times on the same day. Ten minutes after the last peak of each chromatogram had appeared, the next injection was performed. Peaks were never observed to appear in successive chromatograms due to previous injections. The columns were washed for 30 min at the end of the day with pure acetone.

RESULTS AND DISCUSSION

We show examples of typical high-quality oils in Figures 1 and 2, with the TAGs labelled in abbreviated form. The fatty acid abbreviations are: palmitic acid, P; oleic, O; stearic, S; linoleic, L; linolenic, Ln; palmitoleic, Po. The TAGs are abbreviated, *e.g.* LOP, without prejudice to the existence of other possible positional isomers. The 99 olive

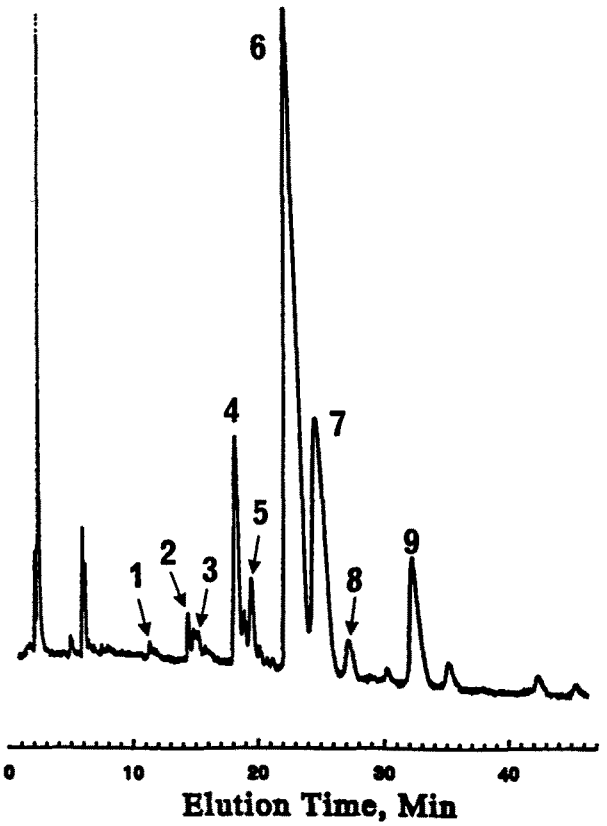

FIG. 1. Sample #56. Triacylglycerols are: 1) LLL, 2) LLO, 3) LLP, 4) LOO, 5) LOP, 6) OOO, 7) POO, 8) POP, 9) SOO. Abbreviations: P, palmitic; O, oleic; S, stearic; L, linoleic; Ln, linolenic; Po, palmitoleic.

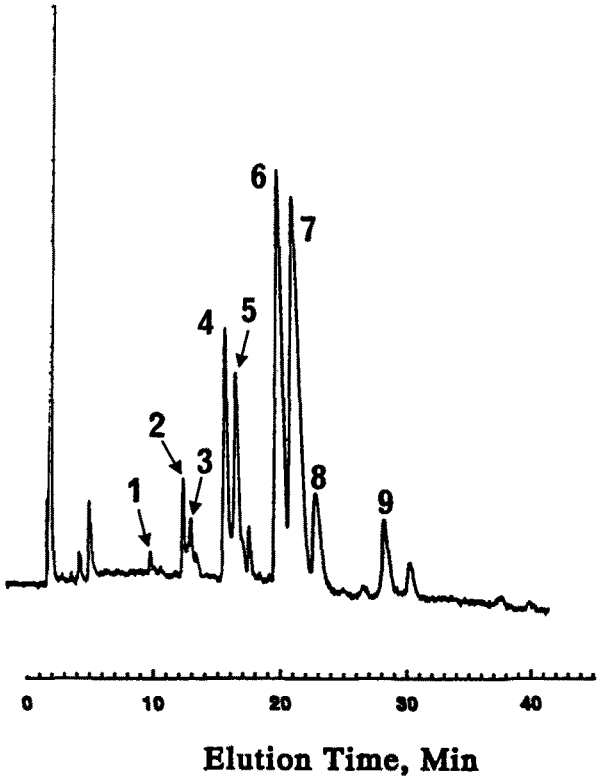

FIG. 2. Sample #52. Triacylglycerols as in Figure 1.

oil samples that we examined were reduced to a set of 67 by removing all those that were (i) not olive oils at all; (ii) adulterated with other vegetable oils; or (iii) reesterified. This set of 67 samples combines the extra virgin through "pure" grades into a single group, which is shown in Table 1. Here the samples are shown in ascending order of their Six Peak Sums (6PeakS) which is the sum of the area percentages of LOO, LOP, OOO, POO, POP and SOO. Early in this work, we sought to find simple criteria based on the TAG peak areas and/or ratios, which would allow us to set ranges for identifying the various grades of olive oils and to distinguish them from other oils. The first criterion to emerge was the sum of the largest TAGs. We considered the sum of LOO, LOP, OOO and POO (4PeakS); and also the sum of LOO, LOP, OOO, POO and POP (5PeakS); as well as the 6PeakS. We settled upon the 6PeakS as representing the best compromise of a small number of peaks sufficient to generate a reliably large area, without subjecting the defining criteria obtained to distortion by inadvertent inclusion of samples which had low (*e.g.*, less than 5–10%) levels of adulteration with polyunsaturated oils. The ratios of LOO/LOP and OOO/POO are also given in Table 1, and they are plotted as a scatter diagram in Figure 3.

Chromatograms of the polyunsaturated vegetable oils generally have large peaks for LLL, LLO and LLP but usually smaller LOO and LOP peaks (5–8). Examination of those references shows that HPLC chromatograms for corn, cottonseed, soybean, sunflower and safflower oils have such large peaks. Additionally, HPLC chromatograms of soybean oil have a distinctively large (*ca.* 7%) LnLL peak, and canbra rapeseed oil has an equally large LnLO peak. HPLC chromatograms of peanut oil (9) display a relatively small LLL peak (*ca.* 3.5%) but much larger LLO and LLP peaks (18.2%, 5.9%). We avoided using LLO and LLP as defining peaks; consequently, the database and conclusions drawn from it should be undistorted by the presence of the common adulterants. Samples that have sufficient vegetable oil(s) admixed to elevate the LOO, LOP and OOO peak areas of olive oils are recognizable because their LLL, LLO and LLP peaks are dramatically larger than expected for genuine olive oils. By not using peaks eluting before LOO, we also exclude distortion due to palm kernel or coconut oils. Also, palm kernel and coconut oils have such distinctive HPLC patterns (9) that significant (*e.g.*, 5%) admixture of either should be recognizable. Addition of either palm oil or palm stearine is easy to detect because the POP, and especially the PPP, peaks would be much larger than usual in the chromatogram. By following these guidelines, we are confident that our database of 67 samples is free of olive oils adulterated with any of the vegetable oils important in commerce.

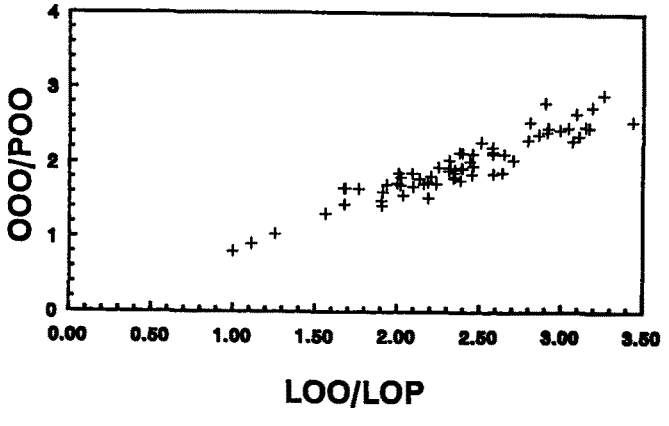

FIG. 3. Plot of Area Ratios OOO/POO *vs.* LOO/LOP for all 67 authentic olive oils. Abbreviations as in Figure 1.

The range for the 6PeakS was *ca.* 82.0–92.6% for the samples we analyzed that were considered authentic. Olive oil samples are unlikely to have 6PeakSs more than *ca.* 1–2% above the presently reported 92.6% because there will be at least some LLO, LLP, LnOO, LnOP and DAGs even in the highest quality oils. Much more important is the lower limit for authentic olive oils. It is possible to find a sample with a 6PeakS well below 80% because it is heavily adulterated with a polyunsaturated oil. For example, a sample was found where *ca.* 30% corn oil lowered the 6PeakS to 74.4%. More frequently, we encountered reesterified olive oils with 6PeakS just below 80%, although most were lower than 76%. The conclusion is that genuine olive oils will not have 6PeakS more than 1–2% below 82% because the remaining peak area would appear as peaks that are diagnostic of adulteration and/or reesterification.

We have developed the additional criterion that samples containing more than 0.5% LLL are presumed to be adulterated with polyunsaturated oil. This matter will be fully discussed in a subsequent paper and is mentioned here as a result to be used in the general guidelines we are presenting. This cutoff is important because we have 16 adulterated samples where the 6PeakS is within the 82.2–92.2% range. Without this exclusion, those 16 oils would be considered authentic, *i.e.*, not adulterated.

Examination of Table 2 reveals that every one of the six principal TAGs varies at least twofold in its own range but still corresponds to a perfectly acceptable olive oil in our set of 67 samples. Except for OOO, this is also true for the 141 Spanish oils that have been examined by Graciani Constante (7). Given a combined total of 208 samples, we suggest that any sample which exhibits TAG peaks outside two of those ranges be rejected. We have found that reliance upon a single TAG instead of the 6PeakS does not provide a reliable guide to olive oil quality. The poor correlation of OOO to 6PeakS (correlation coefficient = 0.56) warns us that even the (usually) largest single peak will not serve as a substitute for the 6PeakS.

We have found that the ratios of the largest TAGs serves to link seemingly weakly related olive oil samples. Figure 3 shows the plot of OOO/POO *vs.* LOO/LOP for all 67 olive oils. The correlation coefficient is 0.885 for the straight line given by the relation OOO/POO = (0.7844)LOO/LOP + 0.0968. Figures 1 and 2 illustrate the extremes in TAG distribution that we encountered, particularly with respect to OOO and POO. Nevertheless both samples are on the line defined by Figure 3. It is this correlation line that enables us to further classify oil samples. Lack of adherence to this line means that a sample is defective in some manner, *e.g.*, it is not an olive oil, or it is adulterated or reesterified.

We searched for other published data to fit into the scheme presented here. No other suitable data were found. Histograms were found, *e.g.*, of the fatty acid distributions

TABLE 1

Area Percentages and Area Ratios for Olive Oil Samples

Sample number	Six Peak Sum (%)	%								
		LLL	LOO	LOP	OOO	POO	POP	SOO	LOO/LOP	OOO/POO
59	82.1	0.3	17.0	13.6	21.7	21.0	5.3	3.4	1.26	1.03
20	82.8	0.5	12.7	6.3	35.8	20.8	2.9	4.3	2.00	1.72
85	84.0	0.4	11.8	7.0	35.3	21.5	3.5	4.9	1.67	1.64
17	84.1	0.3	12.0	5.1	38.7	20.7	3.2	4.4	2.34	1.87
92	84.2	0.4	15.2	13.6	22.0	24.5	5.6	3.2	1.11	0.90
28	84.7	0.4	14.8	8.4	33.7	20.5	3.3	3.9	1.76	1.64
22	85.0	0.4	12.4	5.8	37.0	21.6	3.2	5.0	2.16	1.72
93	85.6	0.3	11.3	5.2	39.2	22.6	3.4	4.0	2.19	1.74
63	86.2	0.3	10.8	5.2	39.9	21.5	3.3	5.5	2.09	1.86
51	86.3	0.4	12.6	6.2	38.3	21.2	3.0	5.0	2.02	1.80
82	86.5	0.3	11.8	5.8	39.3	21.2	3.0	5.5	2.01	1.86
84	86.5	0.4	11.1	5.3	38.8	23.1	3.3	4.9	2.10	1.68
4	86.9	0.3	10.2	4.1	44.4	19.5	2.8	5.9	2.51	2.28
69	86.9	0.4	11.7	4.5	42.5	20.0	2.8	5.4	2.58	2.13
56	87.0	0.4	9.4	9.4	25.3	31.7	6.6	4.6	1.00	0.80
26	87.1	0.3	15.4	9.1	34.5	21.0	3.3	3.8	1.68	1.65
25	87.1	0.3	13.5	6.0	40.1	20.6	2.3	4.5	2.25	1.94
86	87.3	0.3	11.7	6.0	38.9	22.9	3.4	4.5	1.93	1.70
24	87.6	0.5	14.0	5.8	39.8	20.7	2.3	4.8	2.40	1.93
88	87.6	0.4	10.1	5.3	37.2	26.2	3.9	4.9	1.90	1.42
8	87.8	N.I.[a]	10.1	4.4	43.0	21.1	3.2	6.1	2.32	2.04
23	87.9	0.3	14.7	8.8	33.4	23.3	3.9	3.8	1.68	1.43
9	88.1	0.4	11.0	4.5	43.5	20.4	2.8	5.9	2.46	2.12
90	88.2	0.3	9.8	4.3	42.8	22.6	2.8	5.8	2.31	1.89
75	88.5	0.2	12.1	5.1	41.2	21.5	3.1	1.3	2.35	1.91
78	88.5	0.2	12.2	6.0	38.2	24.5	3.3	4.4	2.04	1.56
21	88.6	0.2	14.2	6.1	39.0	21.8	2.7	4.8	2.34	1.79
13	88.7	N.I.[a]	10.8	4.5	44.4	20.8	2.6	5.6	2.38	2.14
99	88.6	0.1	10.5	3.3	48.4	17.6	2.0	6.7	3.19	2.76
19	88.8	0.2	15.8	8.2	35.9	22.4	3.1	3.3	1.91	1.60
66	88.8	0.2	10.3	6.6	34.8	26.7	4.2	6.1	1.56	1.30
18	88.8	0.2	16.7	7.1	37.5	20.7	2.6	4.3	2.35	1.81
70	88.9	0.3	9.7	4.0	43.6	22.5	2.7	6.4	2.39	1.94
58	89.1	0.2	11.7	4.2	46.8	18.3	2.2	5.9	2.80	2.55
50	89.2	0.5	12.9	4.4	47.8	17.0	1.6	5.4	2.90	2.81
72	89.3	0.3	11.7	5.3	41.0	22.6	3.2	5.3	2.20	1.82
16	89.5	0.5	14.2	5.5	42.4	20.0	2.2	5.3	2.58	2.12
71	89.6	0.2	11.3	4.4	45.0	20.3	2.5	6.0	2.58	2.22
68	89.8	0.2	10.2	4.5	41.9	24.4	3.5	5.4	2.24	1.72
61	89.9	0.5	12.7	3.9	49.0	16.8	1.5	6.0	3.26	2.92
3	89.9	0.2	9.6	3.1	49.2	18.4	2.2	7.4	3.09	2.66
15	89.9	0.2	14.4	7.1	38.3	22.6	2.8	4.7	2.02	1.70
95	90.4	0.2	9.2	3.8	43.5	23.6	3.0	7.4	2.45	1.85
62	90.4	0.1	9.9	4.2	42.5	24.0	2.7	7.1	2.38	1.77
12	90.4	N.I.[a]	13.0	6.1	40.8	22.8	2.8	4.9	2.14	1.78
11	90.5	N.I.[a]	9.9	4.1	46.2	21.6	2.4	6.1	2.40	2.14
76	90.5	0.1	11.2	4.3	45.5	21.2	3.0	5.3	2.58	2.15
87	90.6	N.I.[a]	9.8	5.1	40.4	27.2	3.3	4.7	1.90	1.48
10	90.6	0.2	11.2	4.5	43.9	22.3	2.8	5.7	2.46	1.97
102	90.7	0.1	10.7	3.4	47.6	19.1	2.8	7.0	3.14	2.49
94	91.0	N.I.[a]	8.6	3.3	44.9	24.0	2.7	7.6	2.64	1.87
7	91.1	0.3	12.3	4.4	46.3	20.0	1.9	6.1	2.79	2.31
6	91.3	0.2	11.8	5.1	44.0	22.7	2.5	5.3	2.32	1.94
65	91.3	0.3	9.0	3.1	49.3	20.7	2.4	6.9	2.86	2.38
5	91.4	N.I.[a]	10.6	4.3	45.7	22.5	2.4	6.2	2.44	2.03
79	91.4	0.4	10.3	3.4	49.6	20.0	1.9	6.1	3.04	2.48
101	91.8	0.1	7.1	2.4	51.7	21.1	2.0	7.4	2.99	2.45
64	91.8	0.1	8.2	2.6	50.8	21.5	2.2	6.4	3.11	2.36
77	92.1	N.I.[a]	10.5	3.9	47.8	22.6	2.7	4.6	2.65	2.12
97	92.1	0.3	9.7	3.7	45.6	24.6	2.9	5.5	2.58	1.85
96	92.1	0.2	9.3	4.2	42.8	28.0	3.5	4.2	2.19	1.53
98	92.2	0.1	8.0	2.3	52.6	20.5	1.8	7.0	3.44	2.56
1	92.2	0.1	8.8	3.0	50.6	20.9	1.9	7.1	2.91	2.42
103	92.3	0.1	8.2	2.6	51.7	20.8	2.2	6.7	3.17	2.48
80	92.4	0.2	10.7	4.0	47.0	23.0	2.5	5.2	2.70	2.04
52	92.6	0.1	7.3	2.5	52.2	21.1	2.2	7.2	2.92	2.47
100	92.6	0.1	7.8	2.6	51.7	22.5	2.3	5.7	3.07	2.30

[a]Not integrated. Abbreviations as in Figure 1.

TABLE 2

Summary of Ranges of Area Percentages of Principal Triacylglycerols of Olive Oils[a]

Triacylglycerol	% Lowest value	Median value	Highest value
LLL[b]	<0.05[c] [1]	0.2 [6]	0.5 [82]
d	—	0.0	—
e	<0.06 [94]	0.1 [99]	0.5 [16]
LLO[b]	0.6 [101]	1.9 [78]	6.1 [28]
d	0.0	0.3	2.0
e	0.6 [101]	1.3 [8]	5.6 [15]
LOO[b]	7.2 [101]	11.1 [84]	17.0 [59]
d	4.2	10.4	16.6
e	7.1	10.2 [4]	16.7 [18]
LOP[b]	2.3 [98]	4.5 [69]	13.6 [92]
d	<0.7 [98]	4.5	8.3 [111]
e	<2.3 [4]	4.1 [4]	7.1 [15]
OOO[b]	21.7 [59]	42.8 [90]	52.6 [98]
d	30.2 [111]	43.1 [111]	56.0
e	37.5 [18]	45.7 [5]	52.6 [98]
POO[b]	16.8 [61]	21.5 [63]	31.7 [56]
d	15.1	23.1	31.1
e	17.6 [99]	20.8 [103]	24.0 [94]
POP[b]	1.5 [61]	2.8 [4]	6.6 [56]
d	0.3	2.9	5.4
e	1.8	2.4 [11]	3.2 [8]
SOO[b]	3.2 [92]	5.4 [69]	7.6 [94]
d	2.3	5.8	9.3
e	4.3 [18]	6.1 [8]	7.6 [94]
6PeakS[b,f]	82.1 [59]	89.1 [58]	92.6 [103]
6PeakS[e,f]	86.9 [4]	90.5 [11]	92.6 [103]

[a] Our sample's number is given in brackets.

[b] Taken from our 99 olive oil samples, from all sources. Abbreviations as in Figure 1.

[c] Eight of our samples failed to give integrated peaks. This is the value of the smallest peak that was integrated, from sample #1 of our work.

[d] See Table III in ref. 7.

[e] These are the results of our 19 Spanish olive oils.

[f] The Six Peak Sums (6PeakS) is the sum of the areas of: LOO, LOP, OOO, POO, POP and SOO.

(10) and of the TAGs (11). Unfortunately, histograms are designed to agglomerate data into meaningful patterns by consolidating data points, but we needed individual points for use here. We were able to reconstruct the ranges reported in Graciani Constante's work (7,11) from the average values and the ranges found by him. Those ranges are incorporated into our Table 2. In Table 2 we compare the full range of Graciani Constante's 141 Spanish olive oils with our multinational set of 67 oils and with the subset of our 19 Spanish oils. It is a curious finding that Graciani Constante encountered olive oils with such low levels of some major TAGs. We observed that he did not report values below 0.10%, which accounts for his LLL value being listed as 0%. Our experience has been that many high-quality olive oils have such low levels of LLL that this peak is integrated only intermittently in some of our runs. We were unfortunate in not encountering samples where the LLO, LOO and LOP peaks are also extraordinarily low. Our upper ranges are quite compatible with his, given that we have a smaller sample set. Most striking is our mutual finding that the levels of OOO and SOO for Spanish oils are high relative to olive oils from other sources, *e.g.*, Italy.

The most useful data in the literature are the TAG areas reported by Castilho *et al.* (12) for their ultraviolet (UV) analysis of 48 Portuguese olive oils. We calculated the LOO/LOP and OOO/POO ratios for the 30 virgin oils (as proclaimed by the producers) and performed a regression analysis. We obtained a correlation coefficient of 0.269 for this set, which compares unfavorably to the 0.885 found in our 67 oils. It appears that the UV extinction coefficients of even closely similar TAGs differ sufficiently to invalidate the underlying approximation that area percentages can be used directly for weight percentages. We are able to utilize this approximation with our refractive index (RI) data because it has been shown to be workable for the classes of fully saturated TAGs and the POO, PPO, OOO group (13,14).

The present work allows identification of a sample as being within the specified ranges for an authentic olive oil. Further subdivision within the virgin grades is perhaps best started with a titration of the free fatty acids (15) to determine whether the sample is within the specifications for its nominal grade. We have observed that the HPLC runs allow integration of the free stearic and oleic acids only intermittently due to interference from the added chloroform. Consequently, we do not recommend using this chromatography to determine free fatty acid levels. In following papers we shall address issues of identifying adulteration and reesterification of olive oils.

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