Evaluation of Olive Oils Through the Fatty Alcohols, the Sterols and Their Esters by Coupled LC-GC

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The analysis of the fatty alcohols, the wax esters, the free and the esterified sterols, as well as that of minor components provides a wealth of information about the quality of an oil or fat, its pretreatment and admixture with other oils. Some results obtained by an easy, nearly fully-automated method are shown for olive oils. More specific information is obtained than by the previous saponification methods.

KEY WORDS: Coupled LC-GC, minor components of olive oil, olive oil, sterol fractions, wax esters.

As do most edible oils and fats, olive oil contains some 0.5–1% of nonglyceridic components. These "minor components", such as sterols, fatty alcohols and their esters, are important for the characterization of an oil. They vary more widely between different oils than the fatty acid composition, and their concentrations in the oil are a rich source of information about the oil, providing information on the ripeness of the fruit, the presence of spoiled fruits, the extraction technique, the acidity of the oil (1–3) and the final treatment (4–7).

Despite the wealth of information available, the analysis of the minor components has been carried out in very limited numbers. This is due to the tedious and very time-consuming sample preparation method—some 2–3 samples are analyzed per day per person. The method applied here, involving automated LC-GC, allowed us to analyze some 30–35 samples per day, enabling us to compile a large amount of data.

Distinction between free and esterified components. The minor components were studied mainly in the unsaponifiable fraction. This allows a detailed separation and quantitation of the minor components, but does not provide information about their original structure, i.e., whether or not the component was esterified in the oil—usually these components are present in both forms (8,9). Important information is lost by saponification, as the composition of the free and esterified minor components is not identical, and, e.g., different extraction procedures or refining methods have different effects on free and esterified compounds. For instance, filtration through adsorbents preferentially removes free sterols, while the use of charcoal primarily causes a loss of esters, and in low grade olive oils the concentration of free stigmasterol tends to increase, while the stigmasterol ester content is not increased (10). The analysis in the saponified sample, measuring the sum of the free and esterified components, yields a less significant result.

Only a few papers deal with the analysis of the free and the esterified sterols (11–13), and none of these deal with olive oil. They were mostly used for determining the influence of the refining process on the oil, as reviewed by Kochhar (14). Such information was obtained by removing the triglycerides and fractionating the minor components via chromatography on silica (15). However, the procedure was lengthy and did not find wide application. The analysis of the wax esters provided important information for distinguishing pressed and solvent-extracted olive oil (15). This method was later converted to LC-GC (16).

On-line coupled LC-GC. A method involving on-line coupled LC-GC was developed that promises to resolve several problems at once (17). It allows the determination of free and esterified compounds in the same analysis and eliminates practically all manual work, allowing one person to run large numbers of samples in a short time. An internal standard and some pivalic acid anhydride are added to the oil in order to esterify the free alcohols. LC separates the free monoalcohols (as esters of pivalic acid) and the fatty acid esters of these alcohols from the triglyceride matrix (and other interfering material). This fraction is transferred on-line to GC. Transferring a slightly later LC fraction to GC, erythrodiol and uvaol, two triterpenedialcohols, can be analyzed from the same esterified samples (18).

Coupled LC-GC is a recently developed technique (19) that exploits the high separation efficiency and sophisticated technology of HPLC for sample preparation. Applying specially developed eluent evaporation techniques, LC fractions of more than 1 mL volume can be introduced directly into the GC. Resulting methods are rapid, highly specific, can be fully-automated and produce results of excellent accuracy (20).

Different types of olive oils. This paper reports results obtained by the LC-GC method for different types of olive oils of certified origin. Absolute and relative contents of different components are correlated with the type of olive oil and its treatment. "Extra virgin" oils represent top quality olive oils obtained by pressing the fruit without heating and without any further refinement except of filtration. "Lampante" oils are cold-pressed olive oils with more than 1% of free acids and/or an unpleasant flavor, and are sold only after some refining. Solvent-extracted oils ("sansa") are of far lower value. They are produced from the residues after pressing and require intensive refining. Its production is smaller than that of the pressed oil.

The analysis of the minor components was applied successfully to more than one hundred olive oils from the Swiss market, checking for additions of other oils and for the purity of cold pressed ("extra virgin") olive oils. Two oils contained some 15–20% of other oils, while another pair of oils sold as "extra virgin" contained lampante or solvent-extracted oils. In all four cases, alternative evidence, such as UV spectra, fatty acid composition and isomerized fatty acids would not have been sufficient to take legal actions.

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EXPERIMENTAL PROCEDURES

Analyses were carried out using the fully-automated LC-GC system from Carlo Erba (Milano, Italy). The method corresponded to that described previously (17). Fifty μL pivalic anhydride and 10 μL of an internal standard solution, containing 1% cholesterol and 500 ppm betulinol in pyridine, were added to 100 mg oil in a 10-mL screw cap flask. Acylation occurred on a heating plate at 175°C or in an oil bath at 140°C for 15 min. The flask was then filled with *n*-hexane. The sterols, fatty alcohols and their esters were analyzed in the first LC-GC run ("sterol fraction"), while a second LC-GC run of the same sample solution was required for the determination of uvaol and erythrodiol ("erythrodiol fraction").

Sterol fraction. Ten μL of the acylated sample solution were automatically injected into a 100 × 2 mm i.d. LC column packed with silica gel Spherisorb S-5-W, used with *n*-hexane/0.5% *tert.*-butylmethylether (MTBE) at 500 μL/min. A 750 μL fraction was transferred through the loop-type interface into a 15 m × 0.32 mm i.d. glass capillary column coated with PS-255 (a methylsilicone) of 0.15 μm film thickness. The GC column was equipped with a 2 m × 0.32 mm i.d. uncoated pre-column consisting of phenyldimethylsilylated fused silica and a 3 m × 0.32 mm i.d. retaining pre-column taken from the separation column. An early vapor exit (21) was installed between the retaining pre-column and the separation column, using a press-fit T (Y) piece and a 50 cm × 0.32 mm i.d. raw fused silica capillary. The exit was automatically opened upon starting transfer and closed (by switching to a 1 m × 0.1 mm i.d. resistance capillary) 30 seconds after evaporation was completed. Transfer occurred at 120°C and 200 kPa inlet pressure. During analysis, the carrier gas was flow-regulated at 3 mL/min. After completion of the transfer (7 min after starting transfer), column temperature was increased at 30°C/min to 230°C, then by 7°/min to 350°C (12 min). The LC column was back-flushed with 1 mL of MTBE shortly after transferring the fraction of interest.

Erythrodiol and uvaol. The analysis of the triterpenedialcohols followed the method previously described (18). Twenty μL of the above sample solution was injected into the same silica gel column, using *n*-hexane/2% MTBE as mobile phase at 300 μL/min. A 625 μL fraction was transferred into the same GC system, using the same conditions as described above. Oven temperature was programmed at 30°/min to 250°C, then at 7°/min to 340°C.

Quantitation. Quantitative determinations within the sterol fraction were based on the internal standard (cholesterol) representing a 1000 ppm concentration in the oil. All concentrations are given in ppm.

Free sterols and fatty alcohols were determined with a response factor of 1. For the fatty alcohols, the peaks of the alcohols C_{22}, C_{24}, and C_{26} were summed up. Wax esters were quantitated using a response factor of 1.22, accounting for the increased response of the internal standard due to acylation. Peaks of the esters 40–46 were added. Resulting wax ester concentrations are substantially lower than those found by the conventional method, as the esters 36 and 38 were not included. The main components of these esters were missing in the LC-GC chromatograms, either because the components were outside the transferred window or (more probably) because these esters degraded. They probably represent esters of diterpene alcohols, phytol and geranylgeraniol, and therefore do not belong to the wax esters in the true sense. With the esters 40–46, stable wax esters were analyzed, and did not interfere with other components. Furthermore, it is this range of esters that varies the most strongly.

Sitosteryl-C_{18} esters, of which sitosteryloleate is the predominant component, was quantitated by calibration with a 1:1 mixture of cholesterol and cholesterylstearate, acylated as the samples. The resulting correction factor considers the fact that the internal standard molecule is enlarged by derivatization, but also that a noticeable proportion of the sterol esters are degraded in the capillary column.

The total sterol ester content was estimated by multiplying the sitosteryl-C_{18} ester concentration with 1.15, assuming that 10% of the esters are C_{16} esters and that 5% of the sterols are campesterol and stigmasterol. To determine the total concentration of the sterols (comparable with the results obtained through saponification), the sterol ester concentration was multiplied by the proportion of the molecular weight of the sterols in the esters, i.e., by 0.60.

Free erythrodiol and uvaol were quantitated using betulinol as internal standard (50 ppb in the oil) and a response factor of 1. Concentrations relative to the total sterol plus triterpenedialcohol content (the quantitation prescribed by official methods) were calculated using total sterol contents determined as outlined above.

Comparison with results of conventional method. Several oils were analyzed by the conventional method involving saponification, clean-up by thin-layer chromatography, silylation and GC, in order to compare the total sterol contents obtained with those derived from LC-GC (Table 1). As the LC-GC method distinguishes between free and esterified sterols, the total content was calculated, summing the free sterols and the proportion of the sterols in the sterol esters. The results show deviations up to 12%, although deviations appear to be random and not the results of a systematic difference.

RESULTS AND DISCUSSION

Average contents of trace components. Table 2 lists average concentrations and concentration ratios of the components analyzed for different types of olive oils and provides some indication about which parameter may serve for distinguishing different oils. Although not further discussed in this paper, it should be noted that an admixture of oils other than olive oil is usually detectable by an increase of the stigmasterol and campesterol concentration, and possibly by the presence of brassicasterol.

Differences between lampante and extra virgin oils are relatively small, as both are produced by pressure. Raw lampante oils contain more free stigmasterol than an extra virgin, also reflected by a lower ratio of campesterol/stigmasterol. The content of free sitosterol does not differ significantly (in fact it is surprisingly constant for almost all olive oils), but that of sitosteryl esters is significantly higher in lampante oils. As concentrations of sterylesters are nevertheless low as compared to those of free sitosterol, this increase is hardly detected by the total concentration of sitosterol as determined by saponification, which presupposes separate analysis of

TABLE 1

Comparison of Results Obtained by the Conventional Analysis Via Saponification and by the Proposed LC-GC Method[a]

Oil	Sterols total nonsaponifiable	Sterols free LC-GC	Sterol esters LC-GC	Sterols in Esters × 0.6	Sterols total LC-GC	Difference (%)
1	1263	812	900	547	1359	8
2	1012	589	558	339	928	−8
3	1241	713	937	569	1282	3
4	876	642	536	325	967	10
6	1017	637	928	381	1018	0
5	1292	896	906	550	1446	12
7	1085	682	603	366	1048	−3
8	988	640	562	341	981	−1

[a]Concentrations in ppm.

TABLE 2

Average Concentrations (ppm) of the Minor Components in Various Types of Olive Oils

Oil	Alcoh	Campe free	Stigma free	c/st free	Sito free	Sito-C18	Sito total	% Sito free	Waxes	Eryth	e + u (%)
Extra virgin 1987/88	73	36	23	3.2	995	256	1164	86	63	9	1.0
Extra virgin 1988/89	61	29	10	3.2	834	182	954	88	16	16	2.0
Lampante raw	84	33	21	1.8	945	877	1524	63	292	10	0.6
Lampante refined	44	25	12	2.2	692	544	1051	66	180	8	0.8
Extraction oil raw	725	51	34	1.6	1234	2702	3018	44	3294	283	13.5
Extraction oil refined	75	25	19	1.5	659	2624	2390	27	3277	116	5.6

Abbreviations: Alcoh, sum of the fatty alcohols C_{22-26} (see Fig. 1); campe, campesterol; stigma, stigmasterol; c/st, ratio campesterol/stigmasterol; sito, sitosterol; sito-C18, sitosteryl-C_{18}-esters; waxes, sum of wax esters C_{40-46}; eryth, erythrodiol; e + u (%), percent erythrodiol + uvaol relative to the sum of the total sterols and triterpenedialcohols.

free and esterified sterols. The percentage of free sitosterol is a sensitive indicator of the oil quality. Wax ester concentrations in lampante oils are much higher also. On the other hand, the concentration of free erythrodiol is rather lower in the lampante oils and cannot be used for distinguishing lampante and extra virgin oils.

After refining, lampante oils contain free campesterol and stigmasterol at concentrations not very different from those of extra virgin oils. However, as both components are removed at similar proportions, the campesterol/stigmasterol ratio remains low. Concentrations of free sitosterol are substantially lower, too. As sterol esters are removed at the same time, the percentage of free sitosterol remains similar. However, the losses of wax esters are small. Thus, high wax ester concentrations remain an indication for lampante oils.

Differences between pressed and solvent-extracted oils are spectacular. Raw extraction oils contain most of the minor components in high concentrations (with the exception of free sitosterol). They contain about ten times more fatty alcohols, sterylesters, wax esters and erythrodiol. Only about 40% of the sitosterol is free. However, solvent-extracted oils require strong treatment for removing acids, the nearly black color and the unpleasant odor and taste. After such treatment, the concentrations of the minor components are strongly reduced, and the concentrations of the fatty alcohols, campesterol, and stigmasterol are back at the level of an extra virgin oil. The concentration of free sitosterol, however, is considerably lower than in the extra virgin oil. Concentrations of sitosteryl and wax esters are not significantly reduced and still allow the detection of small admixtures of solvent-extracted oils to extra virgin oils. Concentrations of the free triterpene dialcohols (erythrodiol and uvaol) are lower, but still far above those of higher quality olive oils.

Figure 1 shows chromatograms for typical extra virgin, lampante and solvent-extracted olive oils. Differences are noted upon first sight, primarily considering the wax esters and the steryl esters. Running the analysis of the extraction oil at the same sensitivity as the two others, the chromatogram was completely overloaded. The chromatogram shown was obtained after diluting the sample by a factor of five.

Extra virgin oils—1988/89. Table 3 shows results for extra virgin olive oils of the 1988/89 season from Apulia (Italy) and Greece. Most acidities were far below the legal limit for extra virgin oils (1%), indicating the use of high quality olives. Quality of an extra virgin oil may be reduced either due to expression of olives of low quality (mostly overripe fruits) or by forced extraction. Oil 8 is a typical example for an oil produced of olives of poorer quality, since acidity of the oil was near the legal limit. As observed for other oils of increased acidity (e.g., lampante oils or extra virgin oils 31, 41, 50), this went along with increased stigmasterol and wax ester concentrations, and, as a consequence of this, with a campesterol/stigmasterol ratio as low as for typical lampante oils.

Oils 2 and 6 are examples of oils obtained by forced

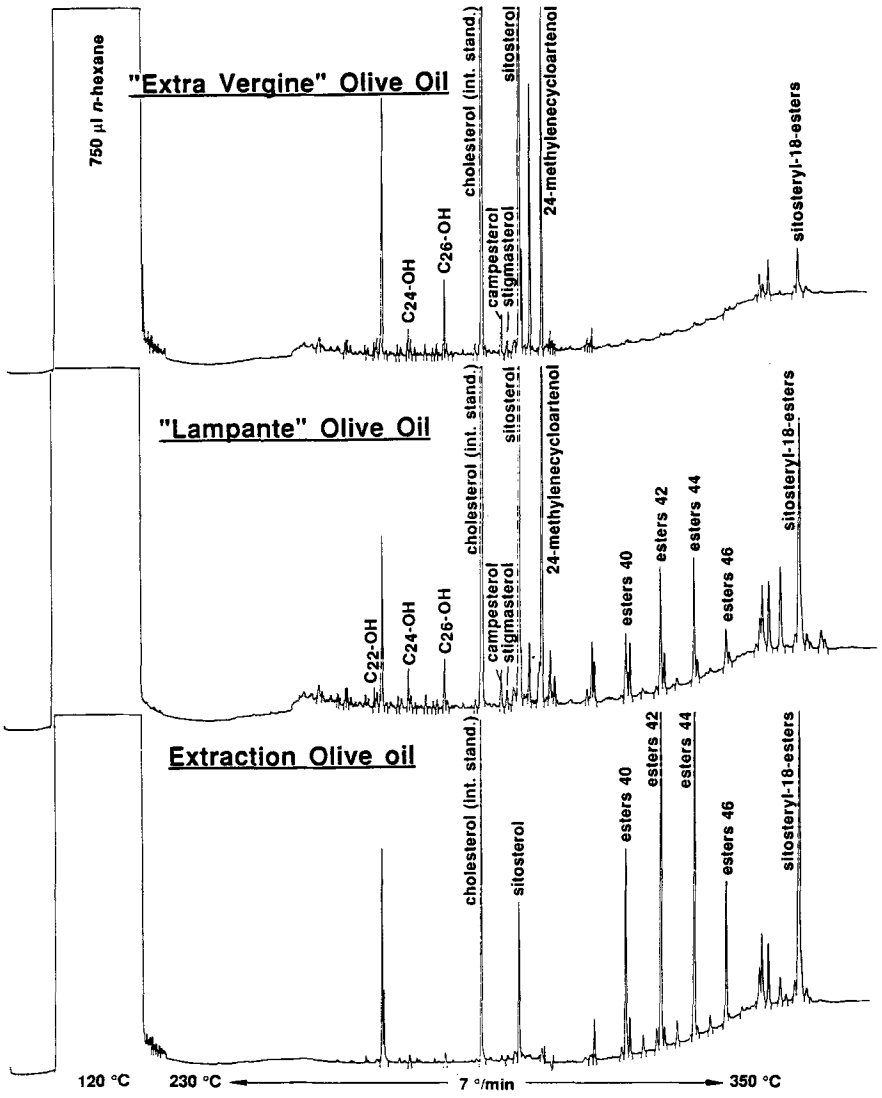

FIG. 1. LC-GC-FID chromatograms for typical olive oils. Nearly complete absence of wax ester (esters 40-esters 46) and very low concentrations of steryl esters indicate a high quality extra virgin oil. The concentration of free stigmasterol is low. C_{24-26}-OH, fatty alcohols. In lampante oils, more wax esters and steryl esters are found. The concentration of stigmasterol increases more than that of campesterol if the oil was prepared from olives of low quality. Run at the same sensitivity, chromatograms of solvent-extracted oils are completely overloaded. The refined extraction oil was diluted 1:5 before running the chromatogram shown. Wax ester and steryl ester concentrations are very high.

extraction. Both were obtained by second pressing, number 6 after regrinding the oil cake left behind by oil 5. Concentrations of nearly all minor components are increased, whereby the increase is strong for components of which only a small proportion is extracted by cold pressing, such as for the esters, fatty alcohols, erythrodiol and, to a lesser degree, for stigmasterol. As a result, the percentage of free sitosterol and the campesterol/stigmasterol ratio decrease. Thus far, oils obtained by forced extraction resemble oils from overripe olives. However, acidity usually remains low.

The Greek oils (numbers 24-27 and 31), as well as the Apulian oil 29, contained high concentrations of fatty alcohols, steryl esters and wax esters. The percentage of free sitosterol is relatively low. Little further information is available about these oils except that the Greek oils contained Freon 11 and the Apulian oil 29 contained tetrachloroethene (the significance of which is unknown).

Concentrations of free cycloartenol might give some indication about the origin of the oil. Italian oils are relatively rich in cycloartenol (most exceeding 100 ppm), while the concentrations in the Greek oils were about 40-70 ppm. In Spanish oils, concentrations were below 15 ppm (Table 4).

Extra virgin oils—1987/88. Table 4 presents data for extra virgin oils which have been stored for one year (the above oils were only a few months old). The analytical data suggest that the oils were generally of somewhat lower quality than the oils from 1988/89, but storage could also be part of the reason for the observed differences.

TABLE 3

Extra Virgin Olive Oils from the 1988/89 Season

Oil	Origin	Type	Acid	K232	K268	Alcoh	Campe free	Stigma free	c/st free	Sito free	Sito-C18	Sito total	% Sito free	Waxes	Cyclo-arte	Eryth	% e + u
1	Apulia	1st press.	0.17	1.289	0.116	17	27	7	3.9	794	121	874	91	⩽5	145	3.7	0.5
2	Apulia	2nd press.	0.18	1.475	0.164	35	33	10	3.3	1047	180	1166	90	⩽5	114	12.2	1.2
3	Apulia	centrif.	0.11	1.793	0.219	25	25	8	3.1	743	110	816	91	⩽5	161	4	0.6
4	Apulia	centrif.	0.21	1.648	0.169	21	26	6	4.3	737	150	836	88	⩽5	137	10.2	1.7
5	Apulia	centrif.	0.18	1.552	0.140	30	30	9	3.3	845	148	943	90	⩽5	149	4.1	0.5
6	Apulia	regrinded	0.33	1.719	0.139	131	48	18	2.7	1304	396	1565	83	39	120	63.2	4.5
7	Apulia	centrif.	0.15	1.509	0.110	33	29	7	4.1	781	142	875	89	⩽5	160	3.7	0.4
8	Apulia	pressed	0.96	1.507	0.157	60	32	21	1.5	951	193	1078	88	35	208	51.4	6.7
9	Apulia	pressed	0.17	1.381	0.130	95	29	8	3.6	846	122	927	91	⩽12	224	38.9	5.7
10	Apulia	centrif.	0.27	1.585	0.136	73	27	10	2.7	774	110	847	91	⩽13	332	12.7	1.9
11	Apulia	pressed	0.22	1.474	0.128	86	27	9	3.0	779	98	844	92	⩽14	242	35.6	5.5
12	Apulia	pressed	0.32	1.645	0.104	42	23	7	3.3	727	117	804	90	⩽10	372	14.2	2.4
13	Apulia	pressed	0.24	1.193	0.093	45	30	8	3.8	853	128	937	91	⩽8	232	12.9	1.9
14	Apulia	pressed	0.2	1.687	0.127	70	30	8	3.8	859	147	956	90	⩽20	236	18.7	2.9
15	Apulia	centrif.	0.24	1.717	0.127	117	32	10	3.2	938	135	1027	91	29	242	41.5	5.7
16	Apulia	pressed	0.17	1.444	0.113	25	28	7	4.0	852	142	946	90	⩽5	184	10.6	1.7
17	Apulia	centrif.	0.18	1.734	0.152	23	20	6	3.3	610	86	667	91	⩽5	354	3.2	0.5
18	Apulia	centrif.	0.18	1.708	0.134	38	26	9	2.9	791	112	865	91	⩽5	220	4	0.5
19	Apulia	centrif.	0.23	0.144	0.113	15	23	6	3.8	640	82	694	92	⩽5	225	⩽1	⩽0.3
20	Apulia	centrif.	0.26	1.510	0.111	29	20	9	2.2	617	79	669	92	⩽5	303	7.6	1.5
21	Apulia	centrif.	0.19	1.515	0.092	18	22	7	3.1	577	59	616	94	⩽5	528	2.9	0.5
22	Apulia	centrif.	0.26	1.678	0.097	73	29	11	2.6	860	150	959	90	⩽8	135	11.9	1.5
23	Apulia	centrif.	0.38	1.714	0.133	21	29	9	3.2	828	137	918	90	⩽5	169	8.9	1.3
24	Greece	centrif.	0.51	2.290	0.208	146	33	9	3.7	906	339	1130	80	34	65	12.5	1.4
25	Greece	centrif.	0.46	3.178	0.219	86	42	24	1.8	1032	447	1327	78	65	70	14.8	1.2
26	Greece	centrif.	0.5	2.540	0.208	142	35	10	3.5	942	395	1203	78	37	42	12.5	1.3
27	Greece	centrif.	0.5	2.440	0.229	142	34	10	3.4	921	360	1159	79	37	51	11.7	1.2
28	Apulia	centrif.	0.6	1.574	0.152	36	29	9	3.2	847	192	974	87	51	188	19	2.7
29	Apulia	centrif.	0.41	1.720	0.141	79	28	10	2.8	919	331	1137	81	24	63	10	0.6
30	Apulia	centrif.	0.3	1.432	0.136	30	23	7	3.3	633	112	707	90	⩽5	302	4.8	0.9
31	Greece	centrif.	0.64			108	35	12	2.9	894	318	1104	81	47	41.0	10.6	1.1
averages			0.31	1.66	0.143	61	29.2	10	3.2	834	182	954	88	16	200	15.7	2.0

Abbreviations: Cycloarte, cycloartenol; for others see Table 2.

TABLE 4

Extra Virgin Olive Oils from the 1987/88 Season

Oil	Origin	Acid (%)	K232	K268	Alcoh	Campe free	Stigma free	c/st free	Sito free	Sito-C18	Sito total	% Sito free	Waxes	Cycloarte	Eryth	% e + u
41	Sicily	0.88	1.915	0.16	112	39	15	2.6	1098	363	338	82	131	147	16.1	1.5
42	Apulia	0.68	2.045	0.165	107	37	9	4.1	1075	259	1246	86	58	252	14.9	1.7
43	Apulia	0.67	1.85	0.135	107	40	9	4.4	1104	268	1281	86	62	264	15.3	1.7
44	Apulia	0.4	1.605	0.13	42	27	8	3.4	761	121	841	91	⩽10	346	4.8	0.6
45	Apulia	0.31	1.55	0.08	31	27	8	3.4	840	125	923	91	⩽10	321	8.7	1.2
46	Apulia	0.65	1.712	0.16	115	40	10	4.0	1110	284	1297	86	74	181	13.9	1.5
47	Apulia	0.39	1.605	0.136	84	33	13	2.5	1075	364	1315	82	54	69	5.8	0.5
48	Apulia	0.58	2.045	0.165	71	35	13	2.7	1029	325	1244	83	114	86	6.6	0.5
49	Sicily	0.4	1.929	0.137	40	28	8	3.5	693	59	732	95	⩽5	5	1.9	0.3
50	Apulia	0.87	1.9	0.155	80	42	19	2.2	1012	624	1424	71	72	44	7.0	0.6
51	Apulia	0.89	2.22	0.175	79	33	12	2.8	1012	299	1209	84	81	217	9.0	0.9
42	Apulia	0.64	2.045	0.165	127	40	9	4.4	1087	276	1269	86	86	194	23.2	2.6
53	Apulia		2.045	0.165	53	31	12	2.6	912	327	1128	81	65	105	12.9	1.5
54	Spain	1	2.1	0.15	117	41	16	2.6	1145	279	1329	86	145	13	11.8	1.1
55	Spain	1	2.365	0.14	62	41	16	2.6	1126	290	1317	85	112	13	6.2	0.5
56	Spain	0.6	2.35	0.165	187	39	9	4.3	1077	185	1199	90	⩽10	10	2.1	0.2
57	Spain	1	2.04	0.195	56	50	11	4.5	1033	256	1202	86	87	7	23.0	2.3
58	Provence				51	43	14	3.1	1135	269	1313	86	31		7.7	0.6
59	Greece				121	39	10	3.9	744	183	865	86	98		7.9	1.2
60	Spain				77	32	11	2.9	1055	241	1214	87	65		5.4	0.5
61	Sicily				27	28	10	2.8	754	99	819	92	11		3.2	0.4
62	Toscana				90	39	21	1.9	1276	292	1469	87	29		3.8	0.3
63	Sicily				20	26	9	2.9	736	92	797	92	9		3.2	0.4
averages		0.7	1.96	0.15	73	36	12	3.2	995	256	1164	86	63	134	9.3	1.0

Acidity and UV absorptivities were mostly higher than for the oils from 1988/89, and correlate with increased contents of free sitosterol, sitosterylesters, wax esters and erythrodiol. As a consequence, the percentage of free sitosterol also tends to be somewhat lower (82–90% instead of above 90%). The finding that low ester concentrations correlate with low acidity of an oil contradicts the common opinion that acidity increases due to hydrolysis of the esters of minor components—in fact, it increases due to hydrolysis of triglycerides. Stigmasterol concentrations also often exceed those of Table 3, resulting in reduced ratios of free campesterol/stigmasterol. In most cases, low percentages of free sitosterol, as well as high fatty alcohol and wax ester concentrations correlate with high acidity, indicating that the high acidity was due to the use of olives of poor quality rather than due to extended storage. Low percentage of free sitosterol again correlates with low campesterol/stigmasterol ratios (with the exception of oils 60–63). These data also confirm that there is a fair correlation between the concentrations of wax esters and steryl esters (or the percentage of free sitosterol).

Oil 47 is abnormal, as the analysis of the minor components points towards an oil of reduced quality (percentage of free sitosterol is only 82%). As acidity is nevertheless fairly low, this might be an oil obtained under forced conditions. Oil 50 contained the highest steryl ester concentration observed in an extra virgin olive oil (including some 100 oils from the Swiss market not shown here). This went along with a high stigmasterol concentration, but wax esters and erythrodiol are not particularly high. The cycloartenol concentration is untypically low for an Apulian oil. Oil 62 is from a very small producer and was described as a top quality oil, but the campesterol/stigmasterol ratio corresponds to that of an oil from spoiled olives.

Lampante oils. Table 5 shows characteristics for some lampante oils. Oils listed as "lampante" were untreated. For some of the oils, raw as well as treated oils were available, allowing recognizable effects of oil refining methods on the absolute and relative concentrations of the minor components.

As shown in Table 2, the concentrations of fatty alcohols, free campesterol and free sitosterol in raw lampante oils are similar to those observed for extra virgin oils. However, stigmasterol, wax ester and steryl ester contents are clearly increased, as for extra virgin oils made of olives of poor quality (with which they are related). This results in a reduced campesterol/stigmasterol ratio (typically around 2) and a reduced percentage of free sitosterol (average of ca. 65%).

Oil 70 has an extremely high stigmasterol concentration; the latter even exceeds that of campesterol. As found previously, free stigmasterol is increased for certain types of spoiled olives (10). The oil also contains extremely high concentrations of sitosteryl-C_{18} esters (only 41% free sitosterol) and wax esters, while the free erythrodiol concentration is rather low.

Oils 79–81 are those used by Morchio (22), while oil 71 is a mixture of the same oils with some extra virgin. They are characterized by extremely low UV absorption and were produced to be added to extra virgin oils without being detectable by classic methods. Even with the presently described method, additions of these oils to extra virgin oils would hardly be detected, since all minor components are present at very low concentrations. Morchio assumed that these oils were obtained using high temperatures during deodorization, as confirmed by the noticeable presence of *trans* fatty acids. However, the low concentration of the high boiling esters may also indicate removal of these components by the use of large amounts of bleaching earth at temperatures above those usually applied.

In refined lampante oils, concentrations of the minor components generally are reduced. Losses of minor components are most pronounced during neutralization (easily exceeding 30%). This in agreement with the findings of Morchio *et al.* (1) as well as Leone *et al.* (6). Losses were usually attributed to the removal of the more polar, nonesterified components [at least for seed oils, Johannsson (23)]. However, our results show that losses of esters are at least similar to those of the free alcohols. This was confirmed by the analysis of olein, the oil recovered from filtration, where high concentrations of sterylesters were found.

In contrast to all other minor components, fatty alcohol concentrations increased several-fold during the neutralization step. Apparently, free alcohols were liberated by hydrolysis of some wax esters—in fact, increase in fatty alcohol contents tends to be high where the decrease of the wax ester content is more pronounced. Neglecting removal of free alcohols by physical effects, more than half of the losses of wax esters could be explained by hydrolysis. This effect is most obvious for oils with very low starting fatty alcohol concentrations (e.g., oils 88, 92 and 96).

Solvent-extracted oils. As mentioned above, raw solvent-extracted olive oils strongly differ from pressed oils in almost all respects (Table 6). Wax ester and sitosteryl ester contents are extremely high; the percentage of free sitosterol is correspondingly low. However, oils added to commercial olive oils are strongly refined, which brings the concentrations of fatty alcohols and campesterol back to those of pressed oils. Stigmasterol contents are high, reflected by low campesterol/stigmasterol ratios. Sitosterol is present at abnormally low concentrations, and as the sitosteryl ester concentrations remain high, the percentage of free sitosterol is very low. However, solvent-extracted olive oils are most easily detected by the high wax ester concentrations. Concentrations of free erythrodiol are far above those found for pressed oils. Figure 2 shows the chromatogram of an extra virgin oil containing 4% extraction oil. The high concentrations of wax esters (228 ppm) and sitosteryl-C_{18} esters (556 ppm) are obvious indications that the oil does not correspond to an extra virgin oil. However, at this low concentration of extraction oil, the oil cannot be distinguished from a lampante oil.

The last two oils in Table 6 were refined by molecular distillation [Lanzini *et al.* (24)]. This vacuum distillation from a thin film of oil removes a considerable part of the materials analyzed, leaving behind concentrations approaching those of pressed oils. Thus, corresponding solvent-extraction oils could be admixed to lampante oils without being detected by the minor components. However, the concentrations of the wax esters and the sitosteryl esters remain too high (low percentage of free sitosterol) to remain undetected in extra virgin oils.

TABLE 5

Lampante[a] Olive Oils

Oil	Origin	Type	Alcoh	Campe free	Stigma free	c/st free	Sito free	Sito-C18	Sito total	% Sito free	Waxes	Cycloarte	Eryth	e + u (%)
70	Apulia	lampante	36	33	57	0.6	817	1781	1992	41	849	93	7	0.4
71	Spain	refined	20	21	8	2.6	637	250	802	79	38	18	4	0.6
72	Spain	refined	56	22	10	2.2	639	377	888	72	307	42	11.5	1.4
73	Apulia	lampante	178	40	26	1.5	1148	488	1470	78	278	69	24.5	
74	Spain	lampante	63	50	22	1.8	1095	606	1495	73	368	15	13.1	1.8
75	Spain	refined	34	21	10	2.1	608	376	857	71	132	19	8.8	0.9
76	Greece	lampante	120	27	18	1.5	966	1033	1648	59	220	269	9.3	0.5
77	Greece	lampante	141	26	17	1.6	872	912	1474	59	207	232	10.3	0.3
78	Greece	lampante	138	30	17	1.8	948	818	1488	64	198	296	11.8	0.3
79	Spain	ref. low UV	31	13	4	2.9	382	159	487	78	37	9	3.9	0.4
80	Spain	ref. low UV	25	14	5	2.7	398	197	528	75	46	8	3.2	0.4
81	Spain	ref. low UV	40	20	8	2.6	590	158	694	85	48	16	4.7	0.3
82	Spain	refined	11	17	8	2.2	457	865	1028	44	355	20	4.1	0.5
90		lampante 1	59	34	19	1.8	871	898	1463	59	225	120	10.2	0.3
91		refined 1	59	22	12	1.8	569	767	1075	53	205	104	9.2	0.1
92		lampante 2	62	38	13	2.9	1084	338	1307	83	89	32	6.4	0.5
93		refined 2	57	33	11	3.0	930	316	1139	82	71	26	5.6	0.5
94		lampante 3	52	39	17	2.3	1107	720	1582	70	203	57	11.3	0.8
95		refined 3	48	27	12	2.3	787	478	1102	71	143	36	7.5	0.8
96		lampante 4	56	39	18	2.2	1097	674	1542	71	194	52	11.5	0.8
97		refined 4	69	30	13	2.3	812	437	1100	74	147	43	10.3	1.0
98		lampante 5	15	30	20	1.5	762	989	1415	54	327	72	6.5	0.5
99		neutro 5	77	23	15	1.5	574	624	986	58	162	76	11.4	1.4
100		bleached 5	72	22	15	1.5	549	569	925	59	172	64?	10.2	1.3
101		deodor. 5	66	22	15	1.5	552	612	956	58	155	72	10.2	1.2
102		lampante 6	⩽10	27	13	2.1	673	1029	1352	50	283	37	3.3	0.3
103		neutro 6	29	26	13	2.0	653	671	1096	60	215	41	6.9	0.9
104		bleached 6	28	26	12	2.2	624	648	1052	59	224	50	6.2	0.8
105		deodor. 6	20	25	12	2.1	605	715	1077	56	221	52	5.5	0.6
106		lampante 7	⩽10	31	20	1.6	845	1121	1585	53	360	90	0.6	0.7
107		bl. coal 7	47	24	15	1.6	643	661	1079	60	173	93	12	1.4
108		bl. earth 7	37	23	15	1.5	599	690	1054	57	206	49	7.4	0.9
109		deodor. 7	37	23	14	1.6	603	617	1010	60	184	89	10.9	1.3
averages		raw oil	84	33	2.1	1.8	945	877	1524	63	292	110	10	0.6
		refined oil	44	25	12	2.2	692	544	105	166	180	48	8	0.8

[a]Oils characterized as "lampante" are raw oils.

TABLE 6

Solvent-Extracted Oils

Oil	Origin	Type	Alcoh	Campe free	Stigma free	c/st free	Sito free	Sito-C18	Sito total	% Sito free	Waxes	Cycloarte	Eryth	e + u (%)
111	Italy	raw	122	37	25	1.5	1246	4379	4136	30	4765	32		
112	Italy	raw	1116	46	27	1.7	939	1351	1831	51	2110	66	475	25.1
113	Italy	raw	1648	60	24	2.5	1112	1271	1951	57	3559	37	410	20.6
114	Italy	raw	134	57	40	1.4	1703	2528	3371	51	2100	82	169	6
115	Italy	raw	607	53	60	0.9	1171	3983	3800	31	3938	68	80	2.4
116	Italy	neutr.	177	55	35	1.6	1318	1978	2623	50	2477	48	413	16.5
117	Italy	refined	49	26	27	1.0	770	1760	1932	40	1699	25	137	7.9
118	Italy	refined	164	49	33	1.5	1162	3492	3467	34	5118	36	152	5.1
119	Italy	refined	79	20	14	1.4	529	2785	2367	22	3311	24	122	5.8
120	Italy	refined	56	19	16	1.2	452	2336	1994	23	2778	15	124	7.9
121	Italy	refined	63	22	20	1.1	528	2780	2363	22	3214	17	116	6.4
122	Spain	refined	41	16	6	2.6	511	2589	2220	23	3540	5	45	0.5
123	Italy	mol. dist.	34	20	8	2.5	514	795	1039	49	608	37	7	0.8
124	Italy	mol. dist.	43	19	7	3.0	456	890	1044	44	805	35	7	0.2
averages		raw oils	725	51	35	1.6	1234	2702	3018	44	3294	57	283	13.5
		refined	75	25	19	1.5	659	2624	2390	27	3277	20	116	5.6

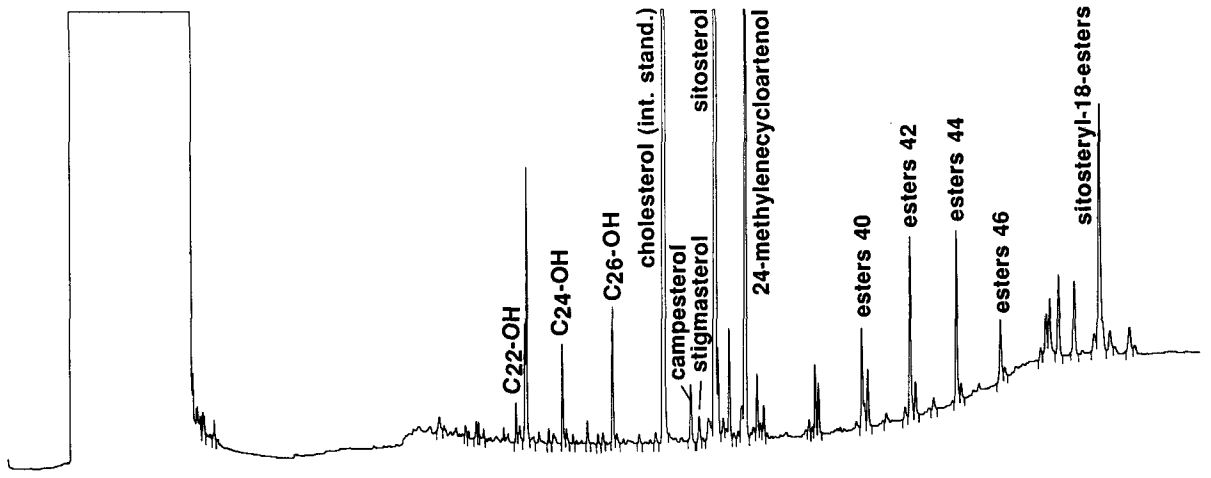

FIG. 2. Extra virgin oil containing 4% extraction oil. Wax ester and steryl ester concentrations in solvent-extracted oils are high enough that small admixtures of extraction oil can be detected. However, with such small admixtures, the oil might be mistaken for a lampante oil.

Proportion of extracted material. Concentrations of free sitosterol in various types of oils (e.g., extra virgin and raw solvent-extracted oil) are similar, presumably because the pressed oil already extracts most of it. As sitosterol is well extracted by the first pressing, its concentrations only weakly increase on forced extraction methods or by better availability from overripe fruits.

Concentrations of fatty alcohols, steryl esters and wax esters are drastically increased when applying solvent extraction, indicating that pressed oils extract less than 10% of these components from the olives. Any measures for increasing the yield of oil should increase the extraction of these components. As most of the material of these components is in the skin of the fruit, softening of the skin as a result of overripening or better availability due to the decay of cell compartmentation in spoiled fruits also results in increased availability to the extraction, agreeing with the finding that forced extraction and use of olives of low quality may result in similar phenomena.

Interpretation of the above results is certainly not exhaustive, as the data reported are the first to be used to analyze minor components without saponification. With additional data from olives of known quality, oils of specific origin or treatment, more information could be derived from such analyses. At the present stage, the above data suggests the interpretation of the determined parameters as follows:

Free campesterol and brassicasterol. Concentrations of free campesterol in pressed olive oils should not exceed 40 ppm. Higher concentrations are an indication for the addition of seed oils. Brassicasterol, eluted just after cholesterol (17), should not be present. A 10 ppm concentration of brassicasterol (producing a peak of the size similar to that of stigmasterol) indicates the presence of ca. 2.5% rape seed oil.

Free stigmasterol. In high quality extra virgin olive oils, concentrations of free stigmasterol are below 10 ppm. Higher concentrations are indicators for low quality olives (overripe or spoiled fruits), forced extraction procedures or the addition of other oils. As absolute sterol concentrations vary, the ratio of free campesterol/stigmasterol appears to be the most significant parameter. It exceeds 3.0 for high quality extra virgin oils.

Sitosteryl esters. Concentrations of sitosteryl-C_{18}-esters in high quality extra virgin olive oils are below 200 ppm, but up to 400 ppm must be considered acceptable. Lampante oils are usually characterized by higher concentrations; up to 1000 ppm appear to be normal. As refined solvent-extracted oils contain approximately 2500 ppm sitosteryl-C_{18}-esters, the addition of 10% extraction oil increases the sitosteryl ester concentration by about 250 ppm.

Percent free sitosterol. The percentage of free sitosterol is a key parameter for assessing the quality of an olive oil. In high quality extra virgin oils of low acidity, the percentage of free sitosterol exceeds 90%. The acceptable minimum is around 80%. Lower relative concentrations indicate the use of low quality olives or forced extraction procedures. This parameter might be useful for setting a limit between extra virgin and lampante oils, particularly for those oils which appear to be extra virgin oils after a gentle neutralization.

Wax esters. Besides the percentage of free sitosterol, the wax ester concentration appears to be the other key parameter for evaluating olive oils. High quality extra virgin olive oils contain less than 50 ppm wax esters, whereby up to ca. 150 ppm were found in others. Higher concentrations suggest a lampante oil or admixture of solvent-extracted oil. Each addition of 10% refined solvent-extracted oil causes the wax ester content to increase by approximately 300 ppm.

Free cycloartenol and erythrodiol. The concentration of free cycloartenol found in Italian olive oils considerably exceeded 100 ppm, while concentrations in Spanish oils only reached 20 ppm (45 ppm for a refined solvent extraction oil). Erythrodiol (often together with uvaol) is commonly used as an indicator for extraction oils. In fact, absolute concentrations of free erythrodiol in pressed oils were usually below 30 ppm (for extra virgin as well as lampante oils), although with surprisingly high deviations. They were between 80 and 475 ppm for the raw solvent-extracted oils. After rafination of the latter, however, concentrations were reduced to ca. 130 ppm.

Usually triterpenedialcohol concentrations (erythrodiol + uvaol) are given as a percentage of the whole sterol fraction. On the basis of our data, this appears to be an

inadequate method because such calculations reduce the observed differences, as high erythrodiol (and uvaol) concentrations usually go along with high sitosteryl ester concentrations. For instance, in the extraction oil 115, the sum of erythrodiol and uvaol represent 2.4% of the total sterol content (calculated from free and esterified sterols). Thus, the oil would not be recognized as an extraction oil (European legislation states a limit of 5%). This is due to the presence of some 4000 ppm sitosteryl-C_{18}-esters, strongly reducing the relative concentration of the triterpenedialcohols. Calculated for the total sitosterol content of an extra virgin oil (1200 ppm), the relative erythrodiol + uvaol concentration would be 8%, and clearly exceed the legal limit of 5%. This shows that determinations in absolute concentrations are clearly preferable.

Triterpenedialcohols must be analyzed in a separate run. It is questionable whether this is worth the effort, since the wax esters are more suitable for detecting admixtures of solvent-extracted oils. In addition, there appear to be various methods which eliminate triterpenedialcohols from the oil (25).

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