Oil Fractionation as a Preliminary Step in the Characterization of Vegetable Oils by High-Resolution ^{13}C NMR Spectroscopy

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ABSTRACT: One hundred nine oil samples were separated chromatographically to obtain oil fractions with a decreased TAG content but with enhanced levels of the minor components that define oil genuineness and quality. The oils, which included virgin olive oils from different cultivars and regions of Europe and north Africa and refined olive, "lampante" olive, refined olive pomace, hazelnut, rapeseed, high-oleic sunflower, corn, grapeseed, soybean, and sunflower oils, were fractionated on a silica gel column with hexane/diethyl ether as the mobile phase eluent. The method was highly reproducible, and the fraction obtained contained about 15% unmodified TAG and 85% polar compounds, which included polymeric TAG, oxidized TAG, DAG, MAG, and FFA, in addition to other minor polar components of the oils. The presence of these compounds, in an enriched fraction, should provide information about the thermal, oxidative, and hydrolytic alterations of the oils, as well as many compounds of interest in determining oil genuineness. The results indicate that these fractions can provide more information than the original oils for NMR or other spectroscopic studies used in the determination of oil quality.

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KEY WORDS: Column chromatography, oil analysis, oil fractionation, oil quality, polar lipids, TAG, vegetable oils, virgin olive oils.

The analysis of vegetable oils is dominated by classic determinations, such as acidity, PV, UV absorption, and so on, as well as by the use of chromatographic procedures, including TLC, GC, and HPLC (1–3). These techniques are primarily used for quantitative measurement of known compounds. The availability of this information has led to international regulations based on established analytical criteria to define oil genuineness and quality.

One drawback to these procedures is that there are too many different assays to be applied to routine quality control. In addition, many of these methods require the isolation and analysis of minor compounds from the unsaponifiable matter by means of procedures that are laborious and time-consuming. Therefore, many studies have been carried out to apply new analytical techniques that, with very little or no manipulation of the sample, can give results similar or superior to those obtained by the classical procedures. In this context, instrumental spectroscopic techniques have emerged as potential tools in recent years, although they usually also require the application of multivariate calibration or pattern-recognition techniques to aid interpretation of the data obtained using these methods (4).

One spectroscopic technique with a high potential in this field is high-resolution NMR spectroscopy. Both ^{1}H and ^{13}C NMR have already been applied to the determination of MAG and DAG, to the analysis of the positional distribution of FA in TAG, and to the characterization of virgin olive oils (5–11). However, since oils are composed of more than 95% TAG, obtaining information about other components usually requires extended acquisition time and minimal overlap of their NMR signals with those of TAG.

In an attempt to overcome these limitations, the present investigation was undertaken to isolate an oil fraction with a decreased proportion of TAG. Selective removal of TAG from oils is not easy, although isolation of unsaponifiable matter by saponification of the oil and organic extraction has been applied for many years (12). In fact, this fraction has been shown to be useful for the identification and classification of olive oils (13). However, the use of unsaponifiables has several limitations: It is a time-consuming process, and the structural information on TAG and other saponifiable components is lost.

TAG may be removed chromatographically. This technique is already employed in the isolation of polar compounds from vegetable oils (14). However, this is a nonselective method that eliminates all nonpolar components from the oils, including hydrocarbons, waxes, TAG, and tocopherols. On the other hand, polar compounds include sterols, triterpenic acids, and FFA, as well as the polar compounds produced in the modification of TAG. These last compounds may include polymers, oxidized monomers, and DAG, which are representative, respectively, of the thermal, oxidative, and hydrolytic alterations of the oils (15). The only problem with this method for its application to NMR studies is that when high-quality oils are studied, the structural information on the TAG is almost absent in the fraction. To avoid this, the present study describes a modification of the standard procedure that does not suppress all the TAG. This fraction may be useful not only in characterizing oils but also for determining their quality, because many of the compounds that define oil quality are contained in it. In a subsequent study (16), this fraction was employed for the characterization of vegetable oils by high-resolution ^{13}C NMR.

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EXPERIMENTAL PROCEDURES

Materials. One-hundred nine oil samples were analyzed in this study. These included 45 virgin olive oils from different cultivars and regions of Europe and north Africa (specifically, Spain, Italy, Greece, and Tunisia), 10 (1:1) mixtures of virgin olive oils using Tunisian or Grecian oils and Spanish oils, 7 "lampante" olive oils, 6 refined olive oils, 7 refined olive pomace oils, 3 hazelnut oils, 4 rapeseed oils, 5 high-oleic sunflower oils, 5 corn oils, 4 grapeseed oils, 5 soybean oils, and 8 sunflower oils. Most of the samples were obtained from our Institute's experimental oil mill (Instituto de la Grasa, Sevilla, Spain), the Institute's Department of Analysis, the Institute's pilot plant, and Koipe S.A. (Andujar, Jaén, Spain). In addition, some of the refined oils were prepared and refined in our laboratory using a laboratory-scale apparatus described previously by Dobarganes *et al.* (17). This procedure included degumming with phosphoric acid, neutralization with sodium hydroxide, bleaching with bleaching earth (Trisyl) for 10 min at 90ºC, and deodorization under vacuum (1 mm) at 250ºC for 3 h.

Oil fractionation. Triplicate samples of the oils were fractionated by column chromatography using 19 g of silica gel 60 (particle size 0.063–0.200 mm) as absorbent, which was obtained from Merck (Darmstadt, Germany) and used without any previous treatment. The column was prepared with an elution solvent of a mixture of hexane and diethyl ether (87:13). The oil (6 g) was dissolved in 10 mL of the same solvent and introduced onto the column. Most nonpolar compounds, including a significant portion of the TAG, were eluted with 100 mL of the elution solvent and were discarded. The oil fraction, containing polar compounds as well as a small amount of TAG and other nonpolar compounds, was then eluted with 100 mL of acetone.

Oil fraction analysis. The oil fractions obtained by column chromatography were analyzed for their content of polar and nonpolar compounds as well as the composition of their TAG, DAG, and MAG. These data were compared with the initial composition of the oils. Oil fractions were also analyzed for their sterol, fatty alcohol, and phenol content.

Polar compounds in oil fractions were determined by solid-phase extraction and size-exclusion chromatography according to Márquez-Ruiz *et al.* (15). TAG were determined by GC (18), FA composition was determined by capillary GC of FAME (19), and MAG and DAG were also determined by capillary GC after derivatization with bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane in pyridine (20). The presence and composition of sterols and fatty alcohols were determined by TLC and GLC (21,22), and phenol content was determined with Folin and Ciocalteau's phenol reagent after solid-phase extraction chromatography (23).

Statistical analysis. All results are expressed as mean values ± SD. Statistical comparisons between two groups were made using Student's *t*-test. With several groups, ANOVA was used. When significant *F* values were obtained, group differences were evaluated by the Student-Newman-Keuls test (24). All statistical procedures were carried out using the *Primer of Biostatistics: The Program* (25). Significance level was $P < 0.05$ unless otherwise indicated.

RESULTS

Isolation of the fractions. When oils were fractionated chromatographically according to the method described above, the weight of the fractions obtained was related to the quantity of polar compounds present in each sample. Table 1 describes the weights of the fractions obtained for the 109 oils analyzed. Although the weights obtained differed significantly from some samples to others, the reproducibility of the method was excellent, and the use of triplicate samples for each oil always produced very close results. Thus, the mean of the CV obtained for the 109 samples analyzed was 2.03%.

In grouping the oils by origin or treatment (10 groups, as indicated in Table 1), the mean weight of the fraction obtained for each group did not differ significantly from the mean weight of the other groups, suggesting that the weight of the fraction obtained was independent of the type of oil assayed. In fact, it seemed to depend only on the state of the oil. Thus, old or badly conserved oils with a high content of polar compounds produced a greater quantity of oil fraction than expected. This happened, for example, with sunflower samples no. 102–105, which were produced 2–5 yr ago and conserved in a freezer during this time. The fractions obtained from these samples were 9.81 ± 1.88% of the oil, which was significantly higher ($P = 0.001$) than the 3.91 ± 0.96% of the oil obtained from the recently prepared sunflower samples (no. 106–109).

Composition of the fractions. Analogous to the weights of the fractions, their compositions differed significantly from some samples to others. Table 2 shows the mean compositions of the fractions obtained for the 10 groups of oils analyzed. Fractions were composed of approximately 15% unmodified TAG and 85% polar compounds. There were no significant differences ($P < 0.05$) in this general composition among the different groups of oils assayed.

In contrast, significant differences were observed between the different polar compounds determined by HPLC. As expected, virgin olive oils, which had not been refined or thermally treated, had the lowest quantity of polymeric TAG (PTAG). The polar fraction in these oils was mostly composed of DAG. This fraction was also the main fraction in the olive, olive pomace, high-oleic sunflower, corn, and grapeseed oils assayed. In contrast, the hazelnut, rapeseed, soybean, and sunflower oils assayed had a higher content of oxidized TAG (OTAG).

The differences observed in the polar component groups of the different oils were much more related to the state of the individual oils analyzed than to group differences. For example, highly unsaturated oils, which are more easily oxidized, did not necessarily have the highest content of oxidized TAG. Table 3 shows the mean values obtained for the FA composition of the different groups of oils assayed. No correlation

TABLE 1
Percentage of the Oil Isolated from the 109 Oils Analyzed[a]

No.	G	Fraction (% of the oil)	CV (%)	No.	G	Fraction (% of the oil)	CV (%)	No.	G	Fraction (% of the oil)	CV (%)
1	V	3.66 ± 0.02	0.67	38	V	7.61 ± 0.14	1.87	75	P	4.82 ± 0.09	1.87
2	V	2.95 ± 0.09	2.98	39	V	2.75 ± 0.06	2.03	76	H	2.42 ± 0.21	8.54
3	V	5.56 ± 0.07	1.22	40	V	2.45 ± 0.02	0.90	77	H	5.33 ± 0.10	1.85
4	V	4.27 ± 0.20	4.71	41	V	6.88 ± 0.04	0.59	78	H	5.04 ± 0.01	0.11
5	V	6.63 ± 0.17	2.50	42	V	2.93 ± 0.01	0.37	79	R	5.68 ± 0.11	1.86
6	V	2.98 ± 0.03	1.03	43	V	2.29 ± 0.01	0.18	80	R	3.82 ± 0.21	5.44
7	V	2.50 ± 0.05	2.06	44	V	2.80 ± 0.13	4.69	81	R	7.82 ± 0.12	1.56
8	V	2.73 ± 0.29	10.5	45	V	2.98 ± 0.33	11.3	82	R	3.86 ± 0.01	0.32
9	V	3.08 ± 0.09	3.00	46	V	6.49 ± 0.05	0.79	83	X	3.49 ± 0.08	2.19
10	V	2.55 ± 0.06	2.49	47	V	6.51 ± 0.09	1.31	84	X	3.76 ± 0.05	1.21
11	V	2.14 ± 0.04	1.82	48	V	7.09 ± 0.04	0.54	85	X	3.07 ± 0.03	0.91
12	V	4.67 ± 0.22	4.70	49	V	6.49 ± 0.11	1.65	86	X	4.43 ± 0.05	1.23
13	V	2.43 ± 0.05	1.93	50	V	7.29 ± 0.09	1.22	87	X	2.61 ± 0.03	1.07
14	V	2.85 ± 0.02	0.87	51	V	3.29 ± 0.08	2.30	88	C	3.63 ± 0.04	1.19
15	V	2.94 ± 0.01	0.17	52	V	3.76 ± 0.06	1.69	89	C	5.17 ± 0.28	5.39
16	V	3.24 ± 0.02	0.75	53	V	3.18 ± 0.05	1.64	90	C	9.32 ± 0.49	5.23
17	V	3.35 ± 0.04	1.21	54	V	3.17 ± 0.06	1.90	91	C	4.40 ± 0.09	1.95
18	V	4.16 ± 0.11	2.64	55	V	2.92 ± 0.01	0.25	92	C	4.07 ± 0.04	1.09
19	V	2.45 ± 0.05	2.07	56	O	4.96 ± 0.09	1.90	93	U	6.51 ± 0.06	0.97
20	V	2.33 ± 0.04	1.77	57	O	6.32 ± 0.10	1.57	94	U	5.51 ± 0.08	1.46
21	V	3.74 ± 0.04	0.95	58	O	3.58 ± 0.24	6.64	95	U	3.73 ± 0.03	0.82
22	V	3.26 ± 0.02	0.55	59	O	4.24 ± 0.06	1.33	96	U	28.7 ± 0.49	1.70
23	V	4.03 ± 0.05	1.13	60	O	10.5 ± 0.14	1.36	97	S	3.02 ± 0.04	1.22
24	V	2.38 ± 0.02	1.03	61	O	14.1 ± 0.12	0.82	98	S	4.04 ± 0.02	0.62
25	V	2.73 ± 0.05	1.76	62	O	16.0 ± 0.06	0.36	99	S	4.81 ± 0.08	1.76
26	V	2.42 ± 0.10	3.93	63	O	12.7 ± 0.13	1.05	100	S	4.22 ± 0.08	1.81
27	V	10.2 ± 0.06	0.61	64	O	3.55 ± 0.06	1.56	101	S	6.11 ± 0.26	4.33
28	V	2.37 ± 0.09	3.66	65	O	4.99 ± 0.16	3.22	102	F	7.25 ± 0.02	0.28
29	V	2.38 ± 0.02	0.89	66	O	4.25 ± 0.01	0.34	103	F	10.5 ± 0.09	0.90
30	V	2.68 ± 0.16	6.03	67	O	9.58 ± 0.06	0.65	104	F	9.79 ± 0.06	0.60
31	V	2.47 ± 0.06	2.58	68	O	3.60 ± 0.06	1.55	105	F	11.7 ± 0.13	1.09
32	V	3.58 ± 0.06	1.61	69	P	9.17 ± 0.20	2.18	106	F	4.86 ± 0.19	3.85
33	V	4.61 ± 0.16	3.54	70	P	7.58 ± 0.07	0.90	107	F	2.85 ± 0.02	0.54
34	V	2.19 ± 0.03	1.58	71	P	6.28 ± 0.03	0.53	108	F	4.58 ± 0.03	0.70
35	V	2.03 ± 0.03	1.69	72	P	4.39 ± 0.06	1.32	109	F	3.35 ± 0.04	1.20
36	V	3.73 ± 0.08	2.07	73	P	6.44 ± 0.08	1.28				
37	V	3.29 ± 0.23	6.92	74	P	5.02 ± 0.06	1.24				

[a]No., sample number; G, group; V, virgin olive oil; O, olive oil (lampante, pure or refined); P, olive pomace oil; H, hazelnut oil; R, rapeseed oil; X, high-oleic sunflower oil; C, corn oil; U, grapeseed oil; S, soybean oil; F, sunflower oil. Values are presented as mean ± SD.

TABLE 2
Composition of the Fractions Obtained[a]

	Composition (% of fraction)				
		Polar compounds			
G	TAG	PTAG	OTAG	DAG	FA
V	15.09 ± 6.83	0.85 ± 0.72^{b}	22.67 ± 8.80^{b}	49.59 ± 7.63^{b}	11.81 ± 3.06^{b}
O	16.43 ± 8.79	5.49 ± 7.52^{c}	19.89 ± 8.92^{b}	$45.87 \pm 9.51^{b,c}$	12.32 ± 7.36^{b}
P	9.80 ± 5.98	11.25 ± 4.91^{c}	15.70 ± 4.92^{b}	59.98 ± 6.29^{d}	$3.28 \pm 2.07^{c,d}$
H	16.85 ± 1.64	$3.94 \pm 1.15^{b,c}$	$47.65 \pm 1.13^{c,d}$	$24.25 \pm 0.11^{e,g}$	$7.31 \pm 0.53^{b,d}$
R	9.82 ± 6.83	11.99 ± 1.00^{c}	$41.27 \pm 15.21^{d,e}$	$28.36 \pm 6.73^{e,f}$	$8.56 \pm 2.65^{b,d}$
X	18.17 ± 8.36	5.83 ± 1.15^{c}	$25.58 \pm 12.76^{b,e}$	$40.09 \pm 8.05^{b,e}$	$10.33 \pm 1.03^{b,e}$
C	13.92 ± 5.49	11.17 ± 2.92^{c}	18.46 ± 1.22^{b}	$46.43 \pm 6.52^{b,c}$	$10.02 \pm 2.92^{b,e}$
U	11.47 ± 8.05	19.19 ± 5.50^{d}	$26.96 \pm 14.77^{b,c,e}$	$37.62 \pm 15.95^{c,e}$	$4.75 \pm 1.52^{c,d,e}$
S	14.38 ± 7.51	8.63 ± 3.21^{c}	$45.86 \pm 11.48^{c,d}$	$22.11 \pm 6.33^{f,g}$	$9.02 \pm 1.96^{b,d}$
F	16.54 ± 6.88	9.04 ± 4.16^{c}	55.95 ± 20.41^{d}	12.80 ± 11.16^{g}	$5.67 \pm 3.12^{c,d,e}$

[a]Values are presented as mean ± SD. Means in the same column with different roman superscript letters (b–g) are significantly different. TAG, triacylglycerols; PTAG, polymeric TAG; OTAG, oxidized TAG; DAG, diacylglycerols; FA, fatty acids and other low molecular weight polar compounds. For other abbreviations see Table 1.

TABLE 3
FA Composition of Assayed Oils[a]

	FA (%)						
G	16:0	16:1	18:0	18:1	18:2	18:3	Others
V	11.64 ± 2.17[b]	0.74 ± 0.32[b,c]	2.83 ± 0.54[b]	75.99 ± 5.72[b]	7.36 ± 4.02[b]	0.64 ± 0.08[b]	0.82 ± 0.18[b]
O	11.12 ± 1.07[b]	0.76 ± 0.13[b,c]	2.81 ± 0.56[b]	76.11 ± 2.78[b]	7.57 ± 2.16[b]	0.70 ± 0.18[b]	0.94 ± 0.16[b]
P	10.52 ± 1.91[b]	0.67 ± 0.34[b,c]	2.83 ± 0.37[b]	74.85 ± 1.31[b]	9.52 ± 1.46[b,d]	0.52 ± 0.21[b,e]	1.09 ± 0.14[b,c]
H	5.43 ± 0.38[c]	0.11 ± 0.08[b]	2.25 ± 0.22[b,c]	81.70 ± 1.39[b]	10.17 ± 1.87[b,d]	0.13 ± 0.00[b,e]	0.22 ± 0.07[d]
R	5.01 ± 0.08[c]	0.13 ± 0.10[b]	1.84 ± 0.21[c]	57.65 ± 2.35[c]	24.33 ± 1.19[c]	7.26 ± 1.88[c]	3.78 ± 1.03[e]
X	4.20 ± 0.14[c]	0.10 ± 0.01[b]	4.05 ± 0.18[d]	75.96 ± 2.04[b]	13.78 ± 1.92[d]	0.07 ± 0.01[b,e]	1.85 ± 0.11[f]
C	10.84 ± 0.39[b]	0.11 ± 0.00[b]	2.00 ± 0.09[c]	28.27 ± 1.21[d]	56.96 ± 0.87[e]	0.76 ± 0.07[b]	1.08 ± 0.06[b,c]
U	6.90 ± 0.53[c]	1.23 ± 2.04[c]	4.32 ± 0.34[d]	19.95 ± 3.07[e]	66.30 ± 5.98[f]	0.22 ± 0.05[b,e]	1.10 ± 0.67[b,c]
S	11.19 ± 0.52[b]	0.08 ± 0.04[b]	3.96 ± 0.25[d]	22.53 ± 1.32[e]	55.35 ± 1.13[e]	5.56 ± 0.81[d]	1.33 ± 0.16[c]
F	6.68 ± 0.32[c]	0.12 ± 0.02[b]	4.32 ± 0.42[d]	28.34 ± 2.29[d]	58.82 ± 2.58[e]	0.07 ± 0.01[e]	1.65 ± 0.23[f]

[a]Values are presented as mean ± SD. Means in the same column with different roman superscript letters (b–f) are significantly different. For abbreviations see Table 1.

was observed between FA composition of assayed oils and composition of polar compounds in the fractions obtained.

Characterization of TAG of the fractions. Because fractions contained only a part of the TAG of the oil, analysis of the TAG composition of the original oils was carried out to learn whether the relative composition of TAG changed during chromatographic fractionation. Table 4 shows the TAG composition of the analyzed oils as determined by GC. As expected, high-oleic oils (groups V, O, P, H, R, and X) were mainly composed of triolein (OOO). On the other hand, oils rich in linoleic acid had high amounts of trilinolein (LLL), 1,2-dilinoleyl-3-oleylglycerol (OLL), and 1,2-dilinoleyl-3-palmitoylglycerol (PLL), among other TAG containing linoleic acid residues.

When oils were fractionated chromatographically, the composition of the TAG remaining in the fractions showed significant differences from the original. Table 5 gives the TAG composition of the fractions obtained. When compared with data obtained for the original oils, two types of behaviors were observed. Most of the fractions obtained from high-oleic oils (groups V, O, P, H, R, and X) had a decreased proportion of POO, POL, and OOL when compared with original oils and an increased proportion of PLL, OLL, and LLL. This was most likely a consequence of the stronger adsorbance on silica gel of TAG containing unsaturated FA than those containing saturated FA of the same chain length, similar to that observed for chromatographic fractionations using silicic acid (26). This effect was not so clearly observed in high-linoleic oils. Nevertheless, very good correlations were observed between TAG compositions in oils and fractions, indicating that the original TAG content may be deduced from the TAG content determined in the fraction. Table 6 shows the correlations obtained for the major TAG. The worst results were obtained for 1,2-dilinoleyl-3-oleylglycerol (OLL) and trilinolein (LLL).

Other components in the fractions. In addition to unmodified TAG, PTAG, OTAG, DAG, MAG (observed only in minor amounts by GC, data not shown), and FFA, the fractions also included other polar components of the oils, which were recovered completely. In particular, the presence of three components was investigated because these may be of interest either for use of the fractions in characterizing the oils or in determining oil stabilities, i.e., sterols and fatty alcohols for the former, and phenols for the latter.

Sterols and fatty alcohols were determined by GLC after fractionation of oil fractions by TLC. The results obtained (data not shown) indicated that these components passed from the oils to the fractions. In addition, the signals corresponding to both types of compounds were clearly observed in the ^{13}C NMR spectra of the fractions (16). Analogous results

TABLE 4
TAG Composition of Oils Assayed[a]

	TAG (%)							
G	POO	POL	PLL	OOO	OOL	OLL	LLL	Others
V	28.16 ± 1.86[b]	5.08 ± 2.84[b]	1.57 ± 0.76[b]	41.44 ± 6.54[b]	5.59 ± 2.46[b]	2.62 ± 0.69[b]	1.81 ± 0.35[b]	12.61 ± 1.45[b]
O	27.54 ± 0.72[b]	5.75 ± 1.49[b]	1.18 ± 0.38[b]	41.42 ± 3.84[b]	7.07 ± 2.01[b,c]	3.03 ± 0.78[b]	2.01 ± 0.22[b,c]	12.02 ± 0.57[b,c]
P	27.29 ± 3.47[b]	6.14 ± 0.87[b,c]	2.00 ± 0.66[b]	40.41 ± 4.55[b]	7.52 ± 1.84[b,c]	2.64 ± 0.87[b]	1.90 ± 0.21[b,c]	12.09 ± 1.68[b,c]
H	16.02 ± 0.69[c]	2.88 ± 0.23[b,d]	0.72 ± 0.24[b]	56.19 ± 0.89[c]	9.72 ± 1.08[c]	4.37 ± 0.15[c]	3.22 ± 0.37[c,d]	6.88 ± 0.48[d]
R	8.83 ± 0.42[d]	6.73 ± 0.36[b,c]	4.18 ± 0.70[c]	33.68 ± 1.69[d]	21.64 ± 0.51[d]	14.74 ± 1.14[d]	6.02 ± 1.37[e]	4.19 ± 0.56[e]
X	11.79 ± 0.26[e]	2.06 ± 0.19[d]	1.25 ± 0.30[b]	60.00 ± 0.81[c]	5.63 ± 0.64[b,c]	5.05 ± 0.61[c]	3.34 ± 0.93[d]	10.89 ± 0.56[c]
C	7.15 ± 0.41[d]	15.13 ± 0.65[e]	16.24 ± 1.00[d]	5.65 ± 0.52[e]	15.15 ± 0.81[e]	19.51 ± 1.03[e]	13.95 ± 1.06[f]	7.22 ± 0.74[d]
U	2.49 ± 0.28[f]	9.37 ± 0.59[c,f]	16.79 ± 2.05[d]	3.69 ± 1.64[e]	7.93 ± 1.61[b,c]	22.29 ± 1.75[f]	26.04 ± 3.62[g]	11.39 ± 0.69[b,c]
S	4.97 ± 0.30[f]	14.23 ± 0.62[e]	18.26 ± 0.82[e]	4.25 ± 0.54[e]	9.41 ± 0.71[c]	17.70 ± 0.86[g]	17.62 ± 0.57[h]	13.56 ± 0.55[b]
F	4.38 ± 1.08[f]	10.53 ± 1.03[f]	11.24 ± 1.48[f]	7.76 ± 2.32[e]	14.35 ± 1.14[e]	24.56 ± 1.69[h]	16.14 ± 1.53[i]	11.06 ± 1.13[c]

[a]Values are presented as mean ± SD. Means in the same column with different roman superscript letters (b–i) are significantly different. P, palmitic acid; O, oleic acid; L, linoleic acid; for other abbreviations see Table 1.

TABLE 5
TAG Composition of Enriched Polar Fractions[a]

	TAG (%)							
G	POO	POL	PLL	OOO	OOL	OLL	LLL	Others
V	17.96 ± 2.92[b]	4.06 ± 2.74[d]	3.28 ± 1.40[c]	41.91 ± 8.39[d]	6.32 ± 3.70[d]	8.74 ± 4.96[c]	7.81 ± 3.40[c]	9.91 ± 2.57[b]
O	18.83 ± 4.61[b]	3.21 ± 1.22[b]	3.71 ± 1.51[c]	41.20 ± 5.14[d]	6.47 ± 2.47[d]	9.36 ± 4.09[c]	8.35 ± 2.54[c]	8.86 ± 2.85[b]
P	18.70 ± 3.68[b]	2.99 ± 0.66[b]	2.72 ± 1.22[d]	41.65 ± 9.42[d]	7.00 ± 2.17[d]	12.54 ± 6.79[c]	6.63 ± 3.45[c]	7.78 ± 1.92[b]
H	6.56 ± 0.09[b]	2.15 ± 0.81[d]	3.66 ± 0.21[c]	29.60 ± 0.07[b]	2.89 ± 0.77[b]	19.18 ± 0.96[c]	26.47 ± 0.06[c]	9.51 ± 0.49[c]
R	7.02 ± 1.70[d]	4.76 ± 2.62[d]	8.01 ± 2.67[c]	29.28 ± 4.06[d]	19.76 ± 1.29[b]	13.62 ± 3.40[d]	8.56 ± 0.23[c]	9.01 ± 1.99[c]
X	7.39 ± 0.82[b]	0.96 ± 0.15[b]	0.77 ± 0.49[d]	51.44 ± 4.78[b]	3.90 ± 0.77[b]	15.75 ± 3.51[c]	8.59 ± 1.31[c]	11.21 ± 6.30[d]
C	5.29 ± 1.00[b]	14.70 ± 0.57[d]	17.00 ± 1.17[d]	7.90 ± 0.57[c]	14.66 ± 0.62[d]	20.14 ± 1.11[d]	8.31 ± 0.79[b]	12.00 ± 1.64[c]
U	3.55 ± 2.78[d]	9.36 ± 3.06[d]	16.48 ± 3.08[d]	6.90 ± 1.92[c]	11.10 ± 2.52[d]	18.00 ± 4.30[d]	19.72 ± 7.21[d]	14.90 ± 2.33[c]
S	5.93 ± 1.91[d]	9.63 ± 3.80[b]	20.86 ± 5.51[d]	8.37 ± 2.77[c]	11.00 ± 4.47[d]	10.67 ± 5.42[b]	17.10 ± 12.56[d]	16.44 ± 3.44[d]
F	3.44 ± 2.22[d]	9.38 ± 3.94[d]	13.26 ± 2.25[d]	5.65 ± 3.64[d]	13.39 ± 4.61[d]	16.75 ± 4.59[b]	21.63 ± 6.91[c]	16.50 ± 2.06[c]

[a]For abbreviations see Table 1.
[b]Values significantly lower ($P < 0.05$) than corresponding values in the original oil (Table 4).
[c]Values significantly higher ($P < 0.05$) than corresponding values in the original oil (Table 4).
[d]No significant difference ($P < 0.05$) grom corresponding values in the original oil (Table 4).

were also obtained when determining total phenols with Folin and Ciocalteau's phenol reagent, although the low concentration of these compounds negated a clear identification of their signals in the ^{13}C NMR spectra (16).

DISCUSSION

The main problem in obtaining complete information from the NMR spectra of vegetable oils is that the minor components in the oils, usually very important for the full characterization of the oils, have NMR signals that are either too small to be accurately acquired during a normal acquisition time or are overlapped by the very large signals of TAG.

In an attempt to increase the signals of these minor components, we showed that the chromatographic fractionation of the oil produced a fraction that contained many of the components of the oils that define oil genuineness and quality, including unmodified TAG, PTAG, OTAG, DAG, MAG, and FFA, in addition to other minor polar components of the oils (sterols and phenols, for example).

Polar components were recovered completely from the oil. However, TAG composition of the fractions was slightly different from that of the oil because the proportion of TAG rich in unsaturated FA was increased. Nevertheless, the TAG composition of the fraction remained characteristic of the original oil because there was a correlation between the original TAG composition of the oil and the TAG composition of the obtained fraction.

These results suggest that it is possible to remove chromatographically a significant part of the TAG from the oils, leaving in the fractions obtained many of the components that define oil genuineness and quality. These fractions may be more useful than original oils for spectroscopic characterization.

TABLE 6
Correlations Between TAG Content in Oils and Fractions Obtained[a]

TAG	Correlation coefficient	SD of the fit	*P*-value
POO	0.901	2.905	<0.0001
POL	0.883	1.840	<0.0001
PLL	0.916	2.341	<0.0001
OOO	0.877	7.572	<0.0001
OOL	0.722	3.188	<0.0001
OLL	0.459	5.331	<0.0001
LLL	0.583	5.687	<0.0001

[a]For abbreviations see Table 4.

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