Acorn (*Quercus* spp.) Fruit Lipids: Saponifiable and Unsaponifiable Fractions: A Detailed Study

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ABSTRACT: The composition of the oils extracted from the acorn fruit of three species of Mediterranean oaks, *Quercus ilex* L., *Q. suber* L., and *Q. faginea* L., was characterized. Both major and minor components, including FA, TG, sterols, methyl sterols, triterpenic and aliphatic alcohols, tocopherols, and hydrocarbons, were identified by standard methods and MS. High-resolution GLC and HPLC were used for quantification. The FA profile, together with the equivalent carbon numbers and TG carbon numbers, was compared with data for other edible vegetable oils. Oil yield, expressed as wet weight, was 5% (w/w). Sterol content was remarkable for the three species (8,563–11,420 mg/kg), with β-sitosterol being the most abundant (80%). Oils were also high in tocopherol, with a wide variation between species (165–456 mg/kg) but with γ-tocopherol predominating in all three oils (90% of the total tocopherol content). Also, high terpenic alcohol contents were found (1527–2984 mg/kg), with dammaradienol and β-amyrin being the most abundant (33–60% of the total alcohol content). Bioactive properties and industrial applications of this underutilized native product are also discussed.

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KEY WORDS: Acorn oil, fatty acid, hydrocarbons, *Quercus* spp., sterols, triglycerides.

The genus *Quercus* includes more than 300 species growing in temperate ecosystems. Three species, *Q. ilex* L., *Q. suber* L., and *Q. faginea* L., are very characteristic of the Mediterranean basin, extending from Portugal to Turkey and from France to the north of Africa (1). The human impact on the evergreen oak species is low, except for *Q. suber*, which has been traditionally used to produce high-quality cork; the other species are used to produce firewood and charcoal. In central-southern Spain, they are also considered as a fruit tree for the production of acorns to feed pigs. The Iberian pig consumes the *Quercus* fruit, a traditional local feed resource, occasionally with pasture during the final fattening period. The acorn is low in protein and rich in fat and starch (2), and favors fat deposition while constraining protein accretion (3). The resulting meat products have a high sensory quality and are rich in oleic acid, making them healthier. Among them, a highly appreciated ham stands out as a traditional product in the Spanish Mediterranean diet (4). Hence, the production of the Iberian pig is very strongly connected with the survival of *Quercus* trees. The feasibility of producing commercial feeds from acorns was explored previously, but oxidation phenomena occurred owing to their high fat content (5). The sweet acorn is also considered an edible fruit in some Mediterranean countries (6), including Spain, where it is used in ice creams and other desserts and liqueurs.

Few studies have been done to characterize the oil composition of the acorn fruit (2,4), although a detailed study concerning the phenolic composition has recently been published (7). A better knowledge of the lipid composition of this fruit can be useful in assessing the authenticity of hams claimed as Iberian, in relation to the feed history of the animals, as well as in exploring the feasibility of new applications of this fruit as a raw material for the synthesis of chemical or pharmaceutical products on the basis of the qualitative and quantitative composition of the unsaponifiable and saponifiable matter. The objective of this work was to carry out a detailed and comprehensive study of acorn oil composition. TG and FA were studied, together with waxes, sterols, 4,4-dimethylsterols, terpenic alcohols, tocopherols, and hydrocarbons. The oil composition was compared with that of other edible vegetable oils. The nutritional value as well as the potential uses of this fruit as a source of phytochemicals for the pharmaceutical and food industry was also considered.

EXPERIMENTAL PROCEDURES

Samples. A 1–2 kg sample of acorn fruits was collected directly from two or three individual trees for each of the species included in the study, i.e., *Q. ilex* L., *Q. suber* L., and *Q. faginea* L. The fruits were harvested on privately owned land in Sevilla (Spain) in 1999. The oils were extracted from the whole fruit (including hulls) following IUPAC method 112 (8). The saponifiable and unsaponifiable fractions of the extracted oils were analyzed in duplicate, and the results are presented as mean values.

FA. FAME were analyzed by high-resolution GLC (HRGC). FAME were extracted with heptane after a cold methylation with 2 N KOH in methanol (IUPAC method 2301) (8). HRGC was performed with an HP-5890 Series II chromatograph (Palo Alto, CA) using a fused-silica capillary SP-2380 column (60 m × 0.25 mm, 0.2 μm film thickness; Supelco, Bellefonte, PA). The oven temperature was held at 160°C for 13 min and was then raised to 190°C at a rate of 1.5°C/min and held isothermally for 20 min. The injector and detector temperatures were

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held at 225 and 250°C, respectively. Hydrogen (19 psi inlet pressure) was used as carrier gas, and the makeup gas was nitrogen.

The determination of FA in the β-position of the TG was carried out following the methodology just described with a previous enzymatic hydrolysis with lipase (IUPAC method 2210) (8).

TG. These compounds were quantified by HPLC, following IUPAC method 2324 (8). The HPLC system was composed of an HP-1050 low-pressure quaternary pump, a Rheodyne valve (Rohnert Park, CA) with a 20-μL loop, a thermostated column compartment, and an HP-1074 refractive index detector. A LicroSpher 100 RP-18 column (250 × 4 mm, 4 μm; Merck, Darmstadt, Germany) was used with a 1:1 acetonitrile/acetone mobile phase at isocratic elution flow of 1.15 mL/min. The temperatures of the oven and detector were 40°C.

Waxes. Waxes were quantified by HRGC (9) after oil fractionation by means of silica gel column chromatography (Silica gel 60, particle size 0.063–0.2 mm; Merck). An HP-5890 series II chromatograph equipped with a cold on-column injector with an oven track system and an FID were used. The column was a TRB-5 (15 m × 0.32 mm i.d., 0.1 μm film thickness; Tracer, Barcelona, Spain). The initial temperature was 70°C and was raised to 180°C, by 45°C/min, then raised at a rate of 5°C/min to 310°C and held isothermally for 7 min. Hydrogen was the carrier gas, and nitrogen was the makeup gas. The detector temperature was 350°C, and the internal standard was lauryl arachidate (Sigma, St. Louis, MO).

Squalene. This hydrocarbon was isolated with the waxes by a modification of the standard procedure for the analysis of waxes (10). A low-polarity solvent (98.5 hexane/1.5 ethyl ether) was used to separate squalene from esters of aliphatic alcohols and sterol esters on a silica gel column (11). Squalane (Sigma), with a response factor of 0.963, was the internal standard.

Unsaponifiable matter. The method used to determine the unsaponifiable matter involved saponification, isolation, and purification. After saponification, the unsaponifiable matter was isolated by an extraction method in which two different solvents were used: diethyl ether for the isolation of sterols and alcohols (aliphatic and terpenic), and petroleum ether for the isolation of hydrocarbons (11). Sterols and alcohols were purified by TLC (12), and hydrocarbons were purified by column chromatography (13). All these series of compounds were quantified by HRGC, whereas tocopherols were analyzed by HPLC. The quantification by GC was carried out with an HP-5890 series II chromatograph equipped with a split–splitless injector and an FID. An HP-5 fused-silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness; HP 19091J-413) was used. Hydrogen (7 psi inlet pressure) was the carrier gas, and nitrogen was the makeup gas. The oven temperature program was varied from one series to another, as described below.

Alcohols (aliphatic and terpenic). The oven temperature was raised from the initial temperature of 220 to 280°C at a rate of 3°C/min and held isothermally for 23.7 min. The injector temperature was held at 280°C, and the detector was held at 300°C. The internal standard was arachidic alcohol (Sigma).

Sterols. The oven temperature was held isothermally at 265°C for 30 min. The injector temperature was 280°C, and the detector temperature was held at 300°C. The internal standard was α-cholestane (Fluka, Buchs, Switzerland).

Hydrocarbons. The oven temperature was held isothermally at the initial temperature of 110°C for 4 min and then raised to 310°C at a rate of 4.0°C/min and held isothermally for 11 min. The injector temperature was 280°C, whereas the detector temperature was held at 320°C. The internal standard was *n*-eicosane (Fluka).

Tocopherols. These compounds were quantified by an HPLC system composed of an HP-1050 low-pressure quaternary pump, a Rheodyne valve with a 20-μL loop, a thermostated column compartment, and an RF-235 fluorescence detector. A LicroSpher Si-60 column (250 × 4 mm, 5 μm thickness) (Merck) was used with a 99:1 *n*-hexanol/isopropanol mobile phase at an isocratic elution flow of 1 mL/min. Quantification was carried out from a calibration based on standards (IUPAC method 2411) (8).

HRGC–MS. An MAT 95S MS system was used in combination with an HP-5890 Series II gas chromatograph equipped with a DB5-MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA). The oven temperature program has already been described for each series. For all the analyses, helium was the carrier gas, at a flow rate of 1 mL/min. Mass spectra in the CI mode (MS–CI) were recorded at 70 eV, and the ion source temperature was 250°C.

Mathematical analysis. Quantification of the chemical compounds was carried out with HP ChemStation software, version 5.01. Statistica release 5.5 (Statsoft, Tulsa, OK) was used for hierarchical cluster analysis based on the Euclidean distance and the Ward algorithm. This method was selected as a linkage rule because it uses ANOVA to evaluate the distances between clusters and is considered very efficient.

RESULTS AND DISCUSSION

The crude fat content of the acorns ranged from 6.92% for *Q. ilex* L. to 4.12% for *Q. faginea* L. (based on wet weight), with a mean value of 5.1% (w/w), consistent with previous findings that reported a fat content of 7–10% (2,6). Although the acorn cannot be considered an oil-bearing seed, the oil content was in the range of other vegetable materials that are used because of their health components or their industrial or pharmaceutical applications, such as wheat germ (8–14% fat content) (14) or amaranth (6.34%) (15).

FA. As one can observe in Table 1, the most abundant FA found in acorn oil were oleic (60%) and linoleic (27%), which together constituted more than 80% of the total FA. Also, a high amount of palmitic acid was found (about 14%), whereas stearic and linolenic concentrations were low (about 2%). For a meaningful comparison of the FA profile of acorn oil with those of other vegetable oils, a multivariate exploratory technique was needed. Cluster analysis, which is designed specifically to identify patterns in multivariate data sets, was applied to the major FA (palmitic, stearic, oleic, linoleic, and linolenic acids) of several edible vegetable oils (16,17), including those

TABLE 1
FA Profiles (as the percentage of total FAME identified) of the Three Species of the Genus *Quercus* Analyzed, Including FA in the β-Position

	Q. ilex L.		*Q. suber* L.		*Q. faginea* L.	
FA	%	β-position	%	β-position	%	β-position
14:0	0.10	0.00	0.1	0.17	0.12	0.00
16:0	13.57	0.79	14.36	0.60	13.74	0.63
16:1	0.43	0.46	0.5	0.45	0.62	0.35
17:0	0.13	0.00	0.1	0.00	0.12	0.00
17:1	0.05	0.07	0.0	0.00	0.11	0.00
18:0	2.33	0.18	1.2	0.00	2.70	0.39
18:1n-9	62.88	66.07	57.15	58.95	60.29	70.15
18:1n-7	0.77	0.20	0.8		1.24	
18:2	17.35	30.55	21.95	36.71	18.42	27.17
20:0	0.4		0.2		0.55	
18:3	1.03	1.40	2.6	3.12	1.70	1.30
20:1	0.66		0.6		0.40	

under study. As shown in Figure 1, the FA profile of the three species of *Quercus* analyzed was very similar to those of other edible vegetable oils such as peanut, olive, hazelnut, avocado, tea seed, and kapok seed. Note that in this classification, the higher the level of aggregation, the less similar the members in the respective classes are.

The profile of FA in the β-position confirmed the similarity between acorn oil and these vegetable species, although the percentage of oleic acid in the β-position was of the same order as in olive oil and hazelnut oil (between 59 and 70%) (17). The unsaturated/saturated ratio (ratio of the sum of unsaturated FA to the sum of saturated FA) was relatively high, at 5:1, and the relatively high content of linoleic acid makes acorn oil especially prone to oxidation. However, this profile may have nutritional implications and beneficial effects in the prevention of coronary heart disease.

TG. The so-called "critical pairs" (18) have been defined as those structures that have the same equivalent carbon number (ECN = CN – 2*n*, where CN is the actual carbon number and *n* is the number of double bonds per molecule). The isolation of TG critical pairs can be applied to all oils containing TG of long-chain FA. Thus, high values of ECN44, ECN46, ECN48, and ECN50 characterize olive oil, whereas all oils rich in linoleic acid (corn, sunflower, and soybean) are characterized by a high value of ECN42; a high value of ECN48 characterizes canola oil (19). For the acorn oils, the value for ECN46 was highest (58%), followed by ECN48 (22%) and ECN44 (12%) (Table 2). The quantification of TG also allowed us to

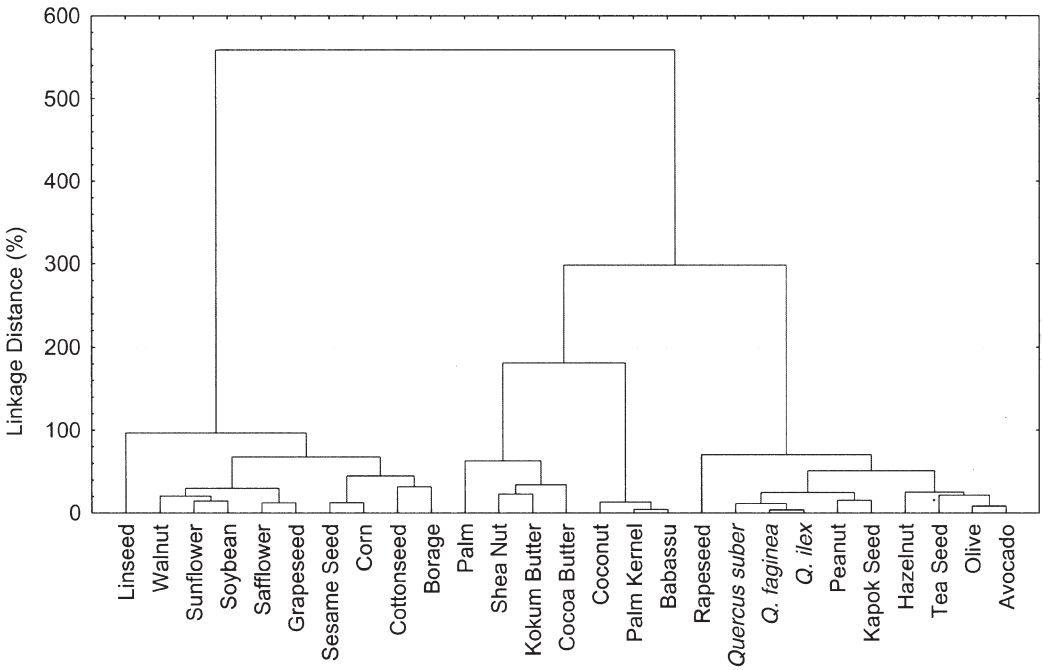

FIG. 1. Cluster analysis (Ward's method) of the major FA in a selection of edible vegetable oils.

TABLE 2
Percentage (%) of TG in the Three Species of *Quercus* Analyzed as Classified by Equivalent Carbon Number (ECN)

ECN	TG[a]	*Q. ilex* L.	*Q. suber.* L.	*Q. faginea* L.
40	LLLn	0.28	1.48	0.95
42	LLL	0.95	1.98	1.68
	LnLO	0.69	1.70	1.14
	LnPL	0.40	1.07	0.78
44	OLL + OLnO	5.93	7.09	5.03
	PLnO	3.59	6.03	5.08
46	OLO	0.55	1.17	0.72
	PLO + SLL	15.00	15.69	13.83
	PPL	10.01	11.58	10.14
48	POO + SOL	1.39	1.88	1.52
	OOO	33.23	27.83	31.26
	POO	20.95	18.42	18.10
	POP + PSL	2.85	2.69	3.17
50	SOO	3.30	1.37	5.10
	SOS	0.90	0.00	1.50

[a]L, linoleic acid; Ln, linolenic acid; O, oleic acid; P, palmitic acid; S, stearic acid.

calculate their carbon numbers. The values of major carbon numbers in the *Quercus* oils (C_{52}–C_{54}) are within the range of cottonseed oils and similar to those of groundnut oil, maize oil, or soybean oil and are quite different from those of the other vegetable oils (Table 3) (20).

Sterols. Tocols and phytosterols are probably the most important and most abundant minor components in the unsaponifiable matter of most vegetable oils. Phytosterols are plant sterols that are structurally similar to cholesterol. Their recovery from oils is of interest because they constitute the starting material for the synthesis of sex hormones and vitamin D (14). The distribution of sterols in acorn oil and other vegetable oils is shown in Table 4. The total sterol content ranged from 8,563 mg/kg in *Q. faginea* to 11,420 mg/kg in *Q. suber.* β-Sitosterol was the major sterol, constituting about 80% (76–89%) of the total sterol fraction. This sterol is widely distributed and is the major sterol in hazelnut, olive, kapok seed, and walnut oils (17). In addition to the pharmacological and industrial interest in phytosterols, several potentially beneficial nutritional effects of these compounds have been reported. The hypolipemic activity of phytosterols and their derivatives in foods has been well established (21). Sitostanol, a 5-α-reduced metabolite of sitosterol that was detected in low concentration in *Q. faginea*, is a potent inhibitor of intestinal cholesterol absorption (22). Clerosterol (found at levels of about 1% in acorn oil) has shown activity against bacteria (21). The levels of the major sterols found, including campesterol, stigmasterol, β-sitosterol, Δ^5-avenasterol, and Δ^7-stigmastenol, were compared among the three acorn oils as well as with the sterol profiles of other vegetable oils (23) by means of cluster analysis (Ward's method). Greater similarities were found between the sterol profiles of *Q. ilex* and hazelnut oil, *Q. suber* and kapok seed oil, and *Q. faginea* and olive oil than among the sterol profiles of the three acorn oils.

Tocopherols (vitamin E isomers). These are well-known natural antioxidants whose presence in seeds is often correlated with a relatively high abundance of unsaturated FA. Three tocopherols were clearly identified and quantified in the oils (Table 5). The major tocopherol found was γ-tocopherol, accounting for about 90% of the total tocopherol content. Important quantitative differences were found among the oils of the three species studied. The total tocopherol content in *Q. faginea* oil was almost triple that in *Q. ilex* oil, whereas that in *Q. suber* oil was almost double that in *Q. ilex* oil. Compared with other vegetable oils with similar FA profiles, the total tocopherol contents of the acorn oils, as well as their isomer distributions, could be considered as being within the range found in other oleic-rich oils, such as peanut and walnut oils, but was quite different from those of olive oil or avocado oil. Tocotrienols were not detected in any of the acorn oils studied.

Alcohols. Both terpenic and aliphatic alcohols were investigated. In the latter, the aliphatic chains had an odd number of carbon atoms, similar to olive oil. In comparison, those in seed oils have an even number. Table 6 shows the total and esterified alcohols found in acorn oils. Little information is available on this series of compounds in vegetable oils, but results have been published for amaranth and olive oils (15).

TABLE 3
TG Carbon Numbers (%) of the Three *Quercus* Oils and of Other Edible Vegetable Oils[a]

Oil	C_{50}	C_{52}	C_{54}	C_{56}	C_{58}
Q. ilex L.	12.9	40.0	45.8	—	—
Q. suber L.	14.3	41.2	42.6	—	—
Q. faginea L.	13.3	37.8	47.4	—	—
Canola	0–2.6	10.5–17.5	68.0–80.0	3.5–6.8	0–5.3
Coconut	0.7–2.1	0.1–2.0	0–1.7	—	—
Cottonseed	12.6–19.9	40.2–45.5	32.2–43.6	0.1–5.4	0–2.2
Groundnut	2.5–5.5	21.2–30.2	48.5–59.6	6.3–10.4	5.3–9.2
Maize	2.6–6.6	22.9–32.1	56.9–69.8	1.1–2.9	0.2–1.7
Olive	2.0–10.0	—	—	—	—
Palm kernel	1.5–5.8	1.5–7.8	1.5–7.9	0–0.3	—
Palm	34.2–44.0	37.5–43.1	8.5–14.5	0–0.7	—
Safflower	0.9–1.6	14.3–20.7	74.5–82.1	0.8–7.5	0–0.7
Soybean	2.1–4.1	25.9–29.5	62.5–68.9	1.5–4.4	0.2–1.1
Sunflower	1.2–1.7	16.4–18.5	75.1–79.7	1.2–3.0	1.1–2.9

[a]Data on other vegetable oils from Reference 15.

TABLE 4
Concentration (mg/kg) and Distribution (%) of Sterols[a] in *Quercus* spp. Oils and Other Vegetable Oils[b]

Sterol	*Q. ilex* L.	*Q. suber* L.	*Q. faginea* L.	Hazelnut	Peanut	Walnut	Olive oil
I	1.54	1.65	1.16	0–0.7	0–0.2		0–0.1
II	0.07	0.16	0.36	—	0–03.8		0–0.5
III	2.34	9.79	3.40	5–6	12.0–99.8	5	0–0.4
IV	0.11	0.08	0.11				
V	1.98	3.89	11.45	1	5.4–13.2		0–4.0
VI	0.02	0.38	0.24				
VII	1.07	0.96	1.16				
VIII	88.94	81.72	75.92	82–93	47.4–67.7	89.0	75–80
IX	0.00	0.00	0.76				
X	2.94	0.88	4.20	2–8	8.3–18.8	5	4–14
XI	0.74	0.33	0.75				
XII	0.15	0.09	0.40	1–3	0–5.1		0–0.5
XIII	0.10	0.07	0.09	2–3	0–5.5		
Total (mg/kg)	8,747	11,420	8,563	1,200–2,000	901–2,854	1,760	100

[a]I, cholesterol; II, brassicasterol; III, campesterol; IV campestanol; V, stigmasterol; VI, Δ^7-campesterol; VII, clerosterol; VIII, β-sitosterol; IX, sitostanol; X, Δ^5-avenasterol; XI, $\Delta^{5,24}$-stigmastadienol; XII, Δ^7-stigmastenol; XIII, Δ^7-avenasterol.
[b]Data on other vegetable oils from Reference 17.

TABLE 5
Concentration of Tocopherols (mg/kg) in *Quercus* spp. Oils and Other Vegetable Oils

	Tocopherols				
Oil	α-	β-	γ-	δ-	Total
Q. ilex L.	16	ND	159	ND	165
Q. suber L.	20	ND	257	12	289
Q. faginea. L.	29	ND	427	ND	456
Peanut[a]	49–373	0–41	88–390	0–22	175–1300
Olive oil[a]	63–135	—	7–17		70–150
Avocado oil[a]	64–100	—	0–19	—	83–100
Walnut[a]	10–20	—	263–400	40–60	309–455
Hazelnut[a]	200–409	6–17	18–150	1–7	225–583
Soybean[b]	110	10	1280	465	1865
Sunflower[b]	860	25	20	2	907
Rapeseed[b]	215	55	630	15	915

[a]Data from Reference 17.
[b]Data from Reference 16. ND, not detected.

TABLE 6
Concentration (mg/kg) and Distribution (%) of Total and Esterified Aliphatic Alcohols in *Quercus* spp. Oils

	Aliphatic alcohols			Esterified alcohols		
Compound	*Q. ilex* L.	*Q. suber* L.	*Q. faginea* L.	*Q. ilex* L.	*Q. suber* L.	*Q. faginea* L.
Total (mg/kg)	1975	3905	2858	1893	2356	1562
C_{22}	23.78	25.53	20.61	5.92	2.47	11.06
C_{24}	46.97	49.96	47.36	10.19	5.33	16.90
C_{26}	10.16	11.32	13.67	22.73	20.30	11.46
C_{28}	19.09	13.19	18.36	17.35	28.91	16.09

The content of aliphatic alcohols is two to three times higher than that in amaranth oil and six times higher than that in olive oil. Also, whereas the major alcohol in acorn oil is tetracosanol, in amaranth oil it is docosanol, and in olive oil it is hexacosanol.

The methyl sterols and terpenic alcohols are a structurally complex group, consisting of five condensed cyclohexane rings that have 30 carbon atoms. The total amount of terpenic alcohols in the studied samples ranged from 1527 ppm in *Q. ilex* to 2984 ppm in *Q. suber*. Of the 15 compounds present, only 7 were identified by MS (Table 7). The major compounds identified were β-amyrin (15–40%) and dammaradienol (18–23%). Noteworthy amounts of cycloartenol and 24-methylenecycloartenol, gramisterol, citrostadienol, and taraxerol also were detected. These compounds are widely distributed in members of the vegetable kingdom, and some of them have been shown to have bioactive properties. β-Amyrin isolated from *Ixeris sonchifolia* has been found to inhibit the growth of

TABLE 7
Concentration (ppm) and Distribution (%) of Terpenic Alcohols and Methyl Sterols in *Quercus* spp. Oils

Compound	*Q. ilex* L.	*Q. suber* L.	*Q. faginea* L.
Total (mg/kg)	1527	2984	2072
Unidentified	3.36	2.86	3.82
Unidentified	0.00	0.87	0.00
Unidentified	0.00	0.58	0.00
Unidentified	3.61	2.15	0.79
Taraxerol	1.88	5.71	2.95
Dammaradienol	22.87	22.95	17.64
β-Amyrin	24.61	14.96	39.61
Unidentified	3.09	7.22	2.56
Gramisterol	6.31	10.44	4.77
Cycloartenol	10.27	4.75	3.11
Unidentified	7.41	12.30	13.64
Unidentified	1.03	1.82	0.56
Unidentified	2.16	1.08	0.88
24-Methylene-cycloartenol	7.27	9.38	5.60
Citrostadienol	6.13	2.91	4.08

TABLE 8
Concentration (mg/kg) and Distribution (%) of Aliphatic Hydrocarbons in *Quercus* spp. Oils

Compound	*Q. ilex* L.	*Q. suber* L.	*Q. faginea* L.
Total (mg/kg)	1526	1089	1736
$C_{20:1}$	0.00	36.05	0.00
C_{21}	1.68	4.90	3.65
C_{22}	2.29	5.45	4.51
C_{23}	4.27	6.10	7.29
C_{24}	6.26	8.71	10.24
C_{25}	45.17	13.50	18.92
C_{26}	13.89	13.83	14.93
$C_{27:1}$	32.35	18.51	13.02
C_{27}	372.80	69.91	306.58
C_{28}	55.85	37.24	73.43
$C_{29:1}$	31.13	20.80	6.25
C_{29}	876.08	771.01	1220.76
$C_{30:1}$	16.18	13.83	9.55
C_{30}	20.45	12.74	9.37
$C_{31:1}$	14.34	18.51	12.33
C_{31}	33.11	74.05	24.82

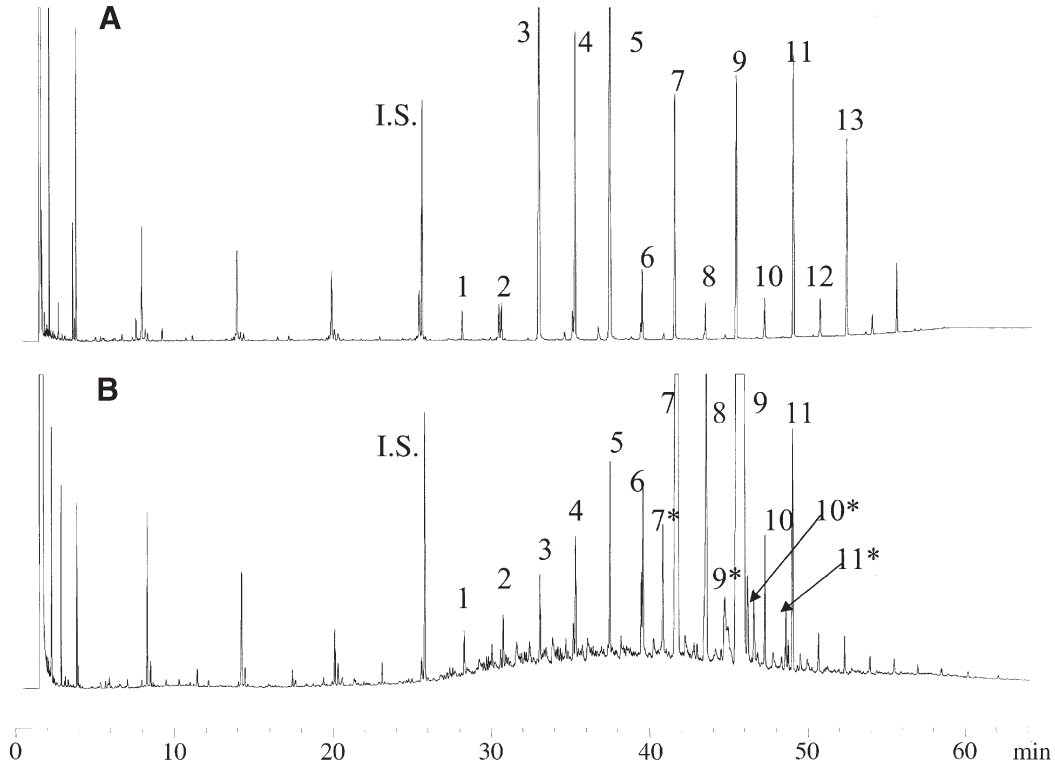

FIG. 2. Chromatograms of the hydrocarbons (*n*-alkanes and *n*-alkenes) of (A) olive oil, and (B) *Quercus faginea* oil. The peaks of the different hydrocarbons identified are cited in the figure. 1, C_{21}; 2, C_{22}; 3, C_{23}; 4, C_{24}; 5, C_{25}; 6, C_{26}; 7, C_{27}; 7*, $C_{27:1}$; 8, C_{28}; 9, C_{29}; 9*, $C_{29:1}$; 10, C_{30}; 10*, $C_{30:1}$; 11, C_{31}; 11*, $C_{31:1}$; 12, $C_{31:2}$; 13, C_{33}.

some human cancer cell lines *in vitro* (24). Dammaradienol has shown antiviral activity against herpes simplex virus types II and I *in vitro* (25). In recent studies, cycloartenol and 24-methylene-cycloartenol showed anti-inflammatory activity against 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation (1 µg/ear) in mice (26).

Hydrocarbons. Most fats contain small quantities (0.1–1.0%) of saturated and unsaturated hydrocarbons, mainly *n*-alkanes from C_{10} to C_{35}, with the odd-numbered being the more abundant (27). Squalene, the most important and widely distributed hydrocarbon, was not detected in any of the *Quercus* species analyzed. Other than squalene, the hydrocarbons in vegetable oils

have been poorly studied. The hydrocarbon profile has been used to assess the authenticity of both vegetable (27) and animal fats (28). As one can observe in Table 8, medium-chain alkanes (C_{29} and C_{27}) predominate in acorn oils, constituting about 80% of the total hydrocarbons detected, whereas other *n*-alkanes accounted for less than 10%. This distribution is quite similar to that reported for other vegetable oils such as sesame, peanut, and safflower and, conversely, is quite different from that of olive oil (Fig. 2) in which other medium-chain *n*-alkanes (C_{23}, C_{24}, and C_{25}) also are significant (29). Unsaturated hydrocarbons ($C_{20:1}$, $C_{27:1}$, $C_{29:1}$, $C_{30:1}$, and $C_{31:1}$) were identified by means of MS.

The potential use of acorn oil appears to be promising, as indicated by a chemical composition that is rich in phytochemicals, especially sterols, tocopherols, and terpenic alcohols, suggesting possible applications in the pharmaceutical industry. The production of acorn oil could add value to an underutilized agricultural product. In terms of its use in feeds, it seems feasible to obtain feeds more stable to oxidation from *Q. faginea,* based on its oil composition, mainly the tocopherol content.

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