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ORIGINAL PAPER

Characterization of Turkish *Quercus* L. Taxa Based on Fatty Acid Compositions of the Acorns

Tamer Özcan

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Abstract Total oil content and the composition of fatty acids were analyzed in the acorns of 16 *Quercus* taxa from Turkey. The range of total fat varied between 0.7 and 7.4%. Oleic (10.2–54.4%), linoleic (24.2–49.1%), palmitic (13.4–30.4%), alpha linolenic (1.5–8.6%) and stearic acid (1.5–4.5%) were major fatty acids for all taxa. Significantly differences at section level were found ($p < 0.05$) for palmitic, stearic and oleic acid concentration. Saturated (17.0–38.6%), mono unsaturated (11.0–55.5%) and unsaturated fatty acids (57.4–81.6%) in total oil were also significantly different between section *Quercus*, *Cerris* and *Ilex* ($p < 0.05$). In addition, sectional differences were significant ($p < 0.02$) for the relative concentrations of saturated fatty acids compared to mono, poly and total unsaturated fatty acids. Considerable variation of individual fatty acid levels were observed in related species and varieties. The species from section *Ilex* Loudon exhibited the highest levels of saturated fatty acid while the lowest levels were found in *Q. brantii*, *Q. libani* and *Q. trojana* from section *Cerris* Loudon. These species also had the highest levels of unsaturated fatty acids. Whereas the lowest values were detected in the species of section *Ilex*. Both varieties of *Q. cerris* showed significant differences ($p < 0.05$) from the other species in section *Cerris* for all parameters, except for stearic acid and exhibited little variations among their individual populations. Different concentrations of fatty acids may be useful biochemical markers for the characterization of *Quercus* at the infrageneric level. Interesting ratios of linoleic:α-linolenic acid especially in *Q. robur* ssp. *robur*, *Q. hartwissiana*, *Q. vulcanica*, *Q. ithaburensis* ssp. *macrolepis* and *Q. libani* also were detected with respect to dietary reference for fatty acid intake.

Keywords *Quercus* · Taxonomy · Acorn · Fatty acid composition · Total oil

T. Özcan (✉)
Faculty of Science, Department of Biology,
Division of Botany, Istanbul University,
34460 Istanbul, Turkey
e-mail: tameroz@istanbul.edu.tr

Introduction

Quercus is one of the most important woody genera with large number of species including trees and shrubs in the Northern Hemisphere. Major distribution centers in the world are North America, Europe and Eastern Asia. Eastern Asia has the highest diversity with about 250 species. Oaks such as Turkish oak, sessile oak, pubescent oak and English oak have been an important source of fuel, fodder, and building materials throughout history. Tannins, dyes and valuable nutrient are important coproducts from acorns. These trees are tolerant to different environmental and climatic conditions and also contribute to erosion control. Eighteen of these species are native to Turkey. Related subspecies, varieties and natural hybrids cover about 6.5 million ha in Thrace and Anatolia [1, 2]. However, the classification of individual trees to a given species is quite challenging. Widespread hybrid combinations are known to occur only in the wild within the members of the same sub-genus. For example, no hybrids are reported between *Lepidebalanus* (white oaks) and *Erythrobalanus* (black and red oaks) [3]. Still, intermediate types, intraspecific variation and environmental influences may have obscured detection of hybridization.

Extreme variability occurs especially in the populations of fairly broad geographic distributions. Several problems

of nomenclature and typification particularly in the wide-ranging groups are still unresolved [4] and modern monographic treatment is needed to improve phylogenic analysis. Some molecular genetic studies have been conducted on the diversity and phylogeny of *Quercus*. Phylogenetic relationships within *Quercus* subgenus *Quercus* using chloroplast DNA (cpDNA) restriction sites, nucleotide sequences of the internal transcribed spacers (ITS) and nuclear ribosomal DNA repeats were analyzed and individual gene trees were reported to be congruent and often complementary in supporting clades that generally correspond to previously recognized taxonomic groups [5]. Good resolution among most species groups and distribution patterns were obtained in the phylogeny of Asian *Quercus* species based on the non-coding region sequences of chloroplast genome and ITS sequences of nuclear rDNA. Yet, ITS and chloroplast DNA phylogeny were largely congruent with regard to the infrageneric classification system, thus, the species relationships among very closely related species were unresolved [6]. However, chloroplast DNA variation at the intraspecific level were detected in *Quercus* and the geographical distribution of cpDNA variants was reported to be largely dependent on species migration [7, 8].

On the other hand, fruits of oaks are a stable and generative organ, and phenotypic differences in acorn composition are an important diagnostic element [9]. Chemical content of seeds is determined ultimately by genetic factors and varies widely among species and their varieties and cultivars [10]. Fatty acid profiles of the seed oils have great taxonomic value in the plant kingdom [11]. Biochemical systematics has been frequently used as a tool in studies of some plant groups at different taxonomic levels [12–16]. Chemosystematic differentiation based on differences in acorn fatty acid composition between Italian and Spanish populations of *Q. ilex* and *Q. rotundifolia* yielded partial separations of the individual populations [17]. Such separation using acorn fatty acids for native and hybrid populations of *Q. agrifolia* and *Q. wislizenii* was also achieved [18]. French Mediterranean evergreen oak populations were reported to be intermediate and heterogeneous for the fatty acid profiles between Spanish and Italian oak, suggesting a zone of hybridization [19]. In addition to genetic factors, some environmental conditions may also influence variations of this parameter in *Quercus*. Acorns of *Q. brantii* from different climatic conditions in the Zagross mountains produced different contents of crude fat, although fatty acid values were not significantly different [20]. A few studies have examined the chemical contents of Turkish *Quercus* acorns from a taxonomical point of view [21, 22]. This investigation examined oaks that were very polymorphic, taxonomically problematic and important woody groups in the ''Flora of Turkey''. Total oil contents and compositional fatty acid levels in the mature acorns of 16 *Quercus* taxa were associated with taxonomical and diagnostic parameters at the infrageneric levels. These data have provided biochemical markers that help establish phylogenic associations of this genus, and also reveal potentially account as an alternative source for dietary nutrition.

Materials and Methods

Mature acorn specimens were collected at random from ten individual trees of native populations of *Q. pontica* C. Koch (pontine oak), *Q. robur* L. subsp. *robur* (pedunculate oak) *Q. hartwissiana* Steven (strandzha oak), *Q. frainetto* Ten. (Hungarian oak), *Q. petraea* (Mattuschka) Liebl. subsp. *petraea* (sessile oak), *Q. vulcanica* (Boiss. & Heldr. ex) Kotschy (kasnak oak), *Q. pubescens* Willd. (pubescent oak), both variety of Turkish oaks; *Q. cerris* L. var. *cerris* and *Q. cerris* L. var. *austriaca* (Willd.) Loudon, *Q. ithaburensis* Decne. subsp. *macrolepis* (Kotschy) Hedge & Yalt. (tabor oak), *Q. brantii* Lindl. (Brant's oak), *Q. libani* Olivier (Lebanon oak), *Q. trojana* P.B. Webb (Macedonian oak), *Q. ilex* L. (holm oak), *Q. aucheri* Jaub. & Spach (boz-pirnal oak), *Q. coccifera* L. (kermes oak). In addition, acorn samples of two varieties of *Q. cerris* were collected from three individual populations in the geographically different Marmara and Eagean regions. Three values of total oil and fatty acid compositions were presented for each variety of this species to show potential environmental influence on these traits. Each species was verified by the classification criteria in ''Flora of Turkey'' [4] and by comparison with identified specimens from the ISTF (Herbarium of Faculty of Science of Istanbul University) and the ISTO herbarium (Herbarium of Faculty of Forestry of Istanbul University). Voucher specimens were deposited with the Division of Botany, Istanbul University.

Sample Preparation and Analysis

Air dried mature acorn samples of each *Quercus* taxon were dehulled, and the kernels (dicotyledons) were ground into meal and homogenized with pestle and mortar. Total oil content was detected with ''Tecator Soxtec System HT''. Powdered material (3 g) from each sample was added to oil in cartridge (*W*1) with 25–50 mL ether into a weighted extraction pot (*W*2). Extraction was carried out for 15 min with rinsing for 30–45 min. The extracted seed meals were air dried to remove traces of solvent and oven dried at 100 °C. The pots were cooled in a desiccator and weighed (*W*3). The following equation was used to calculate percentages of the oil;

$$\text{Oil } \% = ((W3 - W2)/W1) \times 100$$

The oil was transferred into glass sealed amber dark bottles, capped and stored at –18 °C until analyzed. The IUPAC standard method for the preparation of the fatty acid methyl esters were used [23]. Approximately 0.150 g. of powdered material from each sample was added to 5 mL 0.5 N NaOH in 50% aqueous methanol with the glass. The solution was incubated at 100 °C for 15 min in a water bath for saponification, then boiled for 5 min with 5 mL BF_3 and to the addition of 2–5 mL *n*-heptane. This mixture (25 mL) was washed with saturated NaCl. An aliquot (1–2 mL) of the heptane phase was dehydrated with a few crystals of anhydrous Na_2SO_4. The methyl esters of 33 fatty acids were quantified by thermoquest trace GC equipped with a SP-2330 fused silica capillary column (30 m, 0.25 mm, ID-0.20 μm). The oven temperature was held at 120 °C for 2 min and increased at a rate of 5 °C/min and held at 220 °C for 8 min. Injector and detector temperatures were 240 and 250 °C, respectively. Hydrogen was used as carrier gas at a flow rate of 35 mL/min. Split flow, split ratio and sample injection was 75 mL/min, 1/150 and 0.5 μL. Identification and quantification of fatty acid methyl esters was accomplished by comparing the retention times of the peaks with authentic standards (Sigma).

Data Evaluation

Statistical analysis of the experimental results at the $p < 0.05$ significance level (SPSS 10.0). Principle component analysis of the ratios of total percentages of the fatty acids was conducted with statistiXL.

Results and Discussion

The range of total oil concentration among all taxa was between 0.7% (w/w) (*Q. vulcanica*) and 7.4% (w/w) (*Q. brantii*). The average total oil concentration in section *Cerris*, *Quercus* and *Ilex* was 3.91, 1.35, 0.85% (w/w), respectively. Minor variations in total oil content were detected between samples of two varieties of *Q. cerris* collected from geographically different populations, with relatively higher values in *Q. cerris* var. *austriaca*. Percentages of the fatty acids in the examined species were documented in Tables 1, 2, 3, 4. The major fatty acids were oleic (10.2–54.4%), linoleic (24.2–49.1%), palmitic (13.6–30.4%), alpha linolenic (1.5–8.6%) and stearic acid (1.5–4.5%). The values for palmitic, stearic and oleic acid at the section level were significantly different ($p < 0,05$). Total percent of saturated fatty acids were between 17.01 and 38.63%. The concentration of mono, poly and unsaturated fatty acids in total oil were between 11.05 and 55.47%, 26.15–54.72% and 57.44–81.62%.

The concentration of total saturated fatty acids among species compared to mono, poly and total unsaturated fatty acids at the section level was significantly different ($p < 0.05$). The highest ratios of mono:poly unsaturated fatty acid were found in *Q. brantii* and *Q. trojana* (section *Cerris*). Whereas, *Q. aucheri* and *Q. coccifera* (section *Ilex*) exhibited lower ratios. The lowest ratios of total saturated:total unsaturated fatty acids were found in *Q. brantii* and *Q. libani* (section *Cerris*), whereas, the species from section *Ilex* exhibited a high value. Similar values in related species and infraspecific hybrids were observed in both varieties of *Q. cerris*. Both varieties of *Q. cerris* could be delineated from the other species in section *Cerris* for almost all parameters examined, except for stearic acid ($p < 0.05$). In addition, significant variation in palmitic acid values was the only difference between both varieties of *Q. cerris* ($p < 0.05$).

In contrast, the highest oleic acid concentrations obtained are in *Q. brantii* and *Q. trojana* from that section. A remarkably high value for this fatty acid also was detected in *Q. vulcanica* from section *Quercus* (Table 1). Considerably lower levels for oleic acid were observed in both varieties of *Q. cerris*, but, the lowest levels are in the species from section *Ilex*. The highest value of linoleic acid was observed in *Q. hartwissiana* (section *Quercus*) (49.1) and the lowest concentrations were obtained in the species of section *Cerris* except for *Q. cerris*, in addition to *Q. vulcanica* from section *Quercus*. Both varieties of *Q. cerris* expressed high levels for this fatty acid (Table 3). The levels of α-linolenic acid as the other poly unsaturated fatty acid are generally higher in section *Ilex* (Table 4), but lower in section *Cerris*. As observed for linoleic acid, α-linolenic acid levels in both varieties of *Q. cerris* were greater than the section averages. Different ratios of linoleic:α-linolenic acids were also compared at the sectional and specific level. Significant differences were found only between the two varieties of *Q. cerris* compared to other species from its section ($p < 0.01$).

Principle component analysis based on five different ratios of fatty acids applied to the whole series of *Quercus* species show that the first two axes of this analysis accounted for 88.7% of the variation in the data. Principle components 1 and 2 explain 65.9 and 22.8% of total variation, respectively. This analysis defines these relatively distinct groups. Section *Ilex* is clearly segregated, however two varieties of *Q. cerris* (Table 3) appear to be aligned with the section *Quercus* gene pool, and *Q. vulcanica* from section *Quercus* appear to be more closely related to section *Cerris*. In addition, *Q. petraea* subsp. *petraea* appear to be aligned with *Q. vulcanica* in section *Cerris* group (Fig. 1).

Table 1 Total oil percentages, fatty acid compositions and some of their ratios for the acorns of examined taxa in section *Quercus*

Taxa	*Q. pontica*	*Q. robur* ssp. *robur*	*Q. hartwissiana*	*Q. frainetto*	*Q. petraea* ssp. *petraea*	*Q. vulcanica*	*Q. pubescens*
C6:0 caproic acid	0	0	0	0	0	0	0
C8:0 caprylic acid	0	0	0	0	0	0	0
C10:0 capric acid	0	0	0	0	0	0	0
C11:0 undecanoic acid	0	0	0	0	0	0	0
C12:0 lauric acid	0.2	0	0.1	0.1	0	0.6	0.3
C14:0 myristic acid	0.5	0.2	0.2	0.4	0.2	0.6	0.7
C15:0 pentadecanoic acid	0.2	0.2	0.2	0.2	0.1	0	0.3
C16:0 palmitic acid	18.2	18.9	17.1	20.9	19.6	13.8	22.8
C16:1 palmitoleic acid	0.8	0.8	0.5	0.6	0.4	0.5	1.8
C17:0 heptadecanoic acid	0.1	0.1	0.1	0.2	0.1	0	0.2
C17:1 *cis*-10-heptadecanoic acid	0.1	0.1	0.1	0.1	0.1	0	0.2
C18:0 stearic acid	2.1	2.0	1.8	3.2	1.8	4.0	3.1
C18:1n-9t elaidic acid	0.1	0.1	0.1	0.0	0.0	0	0.1
C18:1n-9c oleic acid	32.6	22.6	21.4	25.7	41.4	50.2	16.9
C18:2n-6t linolelaidic acid	0	0	0	0	0	0	0.1
C18:2n-6c linoleic acid	35.9	41.9	49.1	38.7	31.1	25.1	38.7
C18:3n-6 gamma linolenic acid	0	0	0	0	0	0	0
C20:0 arachidic acid	0.4	0.7	0.6	0.8	0.5	0.5	0.8
C18:3n-3 alpha linolenic acid	5.5	4.0	5.3	4.4	2.1	2.6	6.1
C20:1n-9 *cis*-11-eicosenoic acid	0	0.8	0	0.1	0.4	0.5	0
C21:0 henicosanoic acid	0.1	0.1	0.2	0.1	0.1	0	0.2
C20:2 *cis*-11,14-eicosadienoic acid	0.1	0.2	0.1	0.1	0.2	0.2	0.1
C20:3n-3 *cis*-11,14,17-eicosatrienoic acid	0	0	0	0	0	0	0
C22:0 behenic acid	0.5	0.7	0.6	0.7	0.4	0.4	0.8
C22:2 *cis*-13,16 docosadienoic	0	0	0	0	0	0	0
C20:4n-6 arachidonic acid	0	0	0	0	0	0	0
C22:1n-9 erucic acid	0	0	0	0	0	0	0
C23:0 tricosanoic acid	0.1	0.1	0.2	0.2	0.1	0	0.2
C22:2 *cis*-4,7,10,13,16, 19-docosahexaenoic acid	0	0	0	0	0	0	0
C20:5n-3 *cis*-5,8,11,14, 17-eicosapentaenoic acid	0.1	0.1	0.1	0.1	0	0	0.1
C24:0 lignoceric acid	0.4	0.4	0.1	0.7	0.2	0.5	0.5
C24:1n-9 nervonic acid	0	0	0	0	0	0	0
C22:6n-3 *cis*-4,7,10,13,16, 19-docosahexaenoic acid	0	0	0	0.2	0	0.1	0
Undetermined (%)	1.2	5.3	1.8	2.0	0.8	0.1	5.6
Total saturated (%)	23.1	23.9	21.3	27.7	23.1	20.4	29.9
Mono unsaturated (%)	33.8	24.4	22.1	26.6	42.4	51.2	19.1
Poly unsaturated (%)	41.7	46.3	54.7	43.6	33.5	28.1	45.2
Total unsaturated	75.6	70.7	76.8	70.2	75.9	79.3	64.4
Total saturated/mono unsaturated	0.68	0.97	0.96	1.00	0.54	0.39	1.50
Total saturated/poly unsaturated	0.55	0.51	0.38	0.63	0.69	0.72	0.66
Total saturated/total unsaturated	0.30	0.33	0.27	0.39	0.30	0.25	0.46
Mono/poly unsaturated	0.80	0.52	0.40	0.61	1.20	1.81	0.42
Linoleic/α-linolenic acid	6.48	10.30	9.20	8.70	14.30	9.53	6.20
Total oil amount (%)	1.59	0.99	1.01	1.17	2.75	0.75	1.16

Each value is the average of duplicate determinations

Table 2 Total oil percentages, fatty acid compositions and some of their ratios for the acorns of examined taxa in section *Cerris* Loudon

Taxa	*Q. ithaburensis* ssp. *macrolepis*	*Q. brantii*	*Q. libani*	*Q. trojana*
C6:0 caproic acid	0	0	0	0
C8:0 caprylic acid	0	0	0	0
C10: capric acid	0	0	0	0
C11:0 undecanoic acid	0	0	0	0
C12:0 lauric acid	0	0	0	0
C14:0 myristic acid	0.2	0	0.3	0.2
C15:0 pentadecanoic acid	0.1	0	0.1	0
C16:0 palmitic acid	19.0	13.5	15.7	17.0
C16:1 palmitoleic acid	0.2	0.2	0.4	0.2
C17:0 heptadecanoic acid	0.1	0.1	0.1	0.1
C17:1 *cis*-10-heptadecanoic acid	0	0.1	0.1	0.1
C18:0 stearic acid	2.5	1.9	1.5	1.8
C18:1n-9t elaidic acid	0.1	0	0	0
C18:1n-9c oleic acid	46.8	54.3	49.7	51.4
C18:2n-6t linolelaidic acid	0	0	0	0
C18:2n-6c linoleic acid	26.1	24.2	26.0	24.7
C18:3n-6 gamma linolenic acid	0	0	0	0
C20:0 arachidic acid	0.5	0.4	0.3	0.3
C18:3n-3 alpha linolenic acid	2.4	1.5	2.6	2.0
C20:1n-9 *cis*-11-eicosenoic acid	0.6	0.7	0.5	0.5
C21:0 henicosanoic acid	0.1	0.1	0	0
C20:2 *cis*-11,14-eicosadienoic acid	0	0.1	0.1	0
C20:3n-3 *cis*-11,14,17-eicosatrienoic acid	0	0	0	0
C22:0 behenic acid	0.4	0.3	0.3	0.3
C22:2 *cis*-13,16 docosadienoic	0	0	0	0
C20:4n-6 arachidonic acid	0	0	0	0
C22:1n-9 erucic acid	0	0	0	0
C23:0 tricosanoic acid	0.1	0.1	0.1	0.1
C22:2 *cis*-4,7,10,13,16,19-docosahexaenoic acid	0	0	0	0
C20:5n-3 *cis*-5,8,11,14,17-eicosapentaenoic acid	0	0.3	0.1	0.1
C24:0 lignoceric acid	0.2	0.2	0.1	0.1
C24:1n-9 nervonic acid	0	0	0	0
C22:6n-3 *cis*-4,7,10,13,16,19-docosahexaenoic acid	0	0	0	0
Undetermined	0.3	1.3	1.4	0.5
Total saturated (%)	23.1	17.0	18.6	20.2
Mono unsaturated (%)	47.8	55.4	51.2	52.2
Poly unsaturated (%)	28.6	26.1	29.0	27.0
Total unsaturated	76.5	81.6	80.3	79.2
Total saturated/mono unsaturated	0.48	0.30	0.36	0.38
Total saturated/poly unsaturated	0.80	0.65	0.64	0.74
Total saturated/total unsaturated	0.30	0.20	0.23	0.27
Mono/poly unsaturated	1.60	2.10	1.70	1.90
Linoleic/α-linolenic acid	10.60	16.30	9.80	12.10
Total oil amount (%)	3.58	7.45	2.75	6.90

Each value is the average of duplicate determinations

Besides distinctive morphological features, fatty acid profiles in acorns may reflect the delineation of *Quercus* at the infrageneric level in the absence of ecological factors, climatic and seasonal variations. For example, remarkable variation in linolenic acid contents were experienced in the different genotypes, cultivars and mutant lines in the seed samples of *Linum* species [24]. Fatty acid patterns revealed lower intraspecific variability and higher taxonomic

Table 3 Total oil percentages, fatty acid compositions and some of their ratios for the acorns of two varieties of *Q. cerris* (section *Cerris* Loudon) collected from three different populations

Taxa	*Q. cerris* var. *cerris*			*Q. cerris* var. *austriaca*		
Sample	I	II	III	I	II	III
C6:0 caproic acid	0	0	0	0	0	0
C8:0 caprylic acid	0	0	0	0.14	0	0
C10:0 capric acid	0.1	0.2	0.1	0.1	0.1	0
C11:0 undecanoic acid	0	0	0	0	0	0
C12:0 lauric acid	0.2	0.4	0.2	1.5	0.2	0.3
C14:0 myristic acid	0.7	1.0	0.6	0.8	0.5	0.7
C15:0 pentadecanoic acid	0.2	0.2	0.2	0.1	0.1	0.2
C16:0 palmitic acid	20.0	21.9	21.5	18.6	19.0	17.7
C16:1 palmitoleic acid	0.4	0.4	0.3	0.3	0.4	0.6
C17:0 heptadecanoic acid	0.1	0.2	0.2	0.1	0.1	0.1
C17:1 *cis*-10-heptadecanoic acid	0.1	0.1	0.1	0	0.2	0.2
C18:0 stearic acid	2.4	3.0	2.7	2.1	2.4	2.1
C18:1n-9t elaidic acid	0.3	0.4	0	0.1	0.2	0.1
C18:1n-9c oleic acid	22.6	17.1	26.5	36.0	27.6	25.2
C18:2n-6t linolelaidic acid	0	0	0	0	0.1	0
C18:2n-6c linoleic acid	40.8	38.4	38.2	32.7	38.1	41.8
C18:3n-6 gamma linolenic acid	0	0	0	0	0	0
C20:0 arachidic acid	0.4	0.8	0.5	0.3	0.5	0.6
C18:3n-3 alpha linolenic acid	7.0	8.7	4.7	4.5	6.9	6.7
C20:1n-9 *cis*-11-eicosenoic acid	0.6	0.1	0.3	0.6	0.3	0.3
C21:0 henicosanoic acid	0.1	0.2	0.1	0	0.1	0.1
C20:2 *cis*-11,14-eicosadienoic acid	0.2	0.3	0.1	0.1	0.3	0.2
C20:3n-3 *cis*-11,14,17-eicosatrienoic acid	0.1	0.1	0	0	0.1	0.1
C22:0 behenic acid	0.5	0.8	0.7	0.3	0.4	0.5
C22:2 *cis*-13,16 docosadienoic	0	0	0	0	0	0
C20:4n-6 arachidonic acid	0	0	0	0	0	0
C22:1n-9 erucic acid	0	0.1	0.1	0	0.1	0.1
C23:0 tricosanoic acid	0.2	0.2	0.2	0.1	0.1	0.1
C22:2 *cis*-4,7,10,13,16,19-docosahexaenoic acid	0	0	0	0	0	0
C20:5n-3 *cis*-5,8,11,14,17-eicosapentaenoic acid	0	0	0	0	0	0
C24:0 lignoceric acid	0.1	0.7	0	0.1	0.5	0.1
C24:1n-9 nervonic acid	0	0	0	0	0	0
C22:6n-3 *cis*-4,7,10,13,16,19-docosahexaenoic acid	0	0	0	0	0	0
Undetermined	2.4	4.2	2.4	0.9	1.3	1.6
Total saturated (%)	25.1	29.7	27.1	24.6	24.2	22.7
Mono unsaturated (%)	24.0	18.3	27.3	37.1	28.8	26.5
Poly unsaturated (%)	48.3	47.6	43.0	37.2	45.5	49.0
Total unsaturated	72.4	66.0	70.4	74.4	74.4	75.6
Total saturated/mono unsaturated	1.00	1.60	0.98	0.66	0.83	0.85
Total saturated/poly unsaturated	0.52	0.62	0.63	0.66	0.53	0.46
Total saturated/total unsaturated	0.34	0.45	0.38	0.33	0.32	0.30
Mono/poly unsaturated	0.49	0.38	0.63	0.99	0.63	0.54
Linoleic/α-linolenic acid	5.70	4.40	8.10	7.20	5.50	6.20
Total oil amount (%)	1.08	0.68	1.84	2.28	1.29	1.23

Each value is the average of duplicate determinations

Table 4 Total oil percentages, fatty acid compositions and some of their ratios for the acorns of examined taxa in section *Ilex* Loudon

Species	*Q. ilex*	*Q. aucheri*	*Q. coccifera*
C6:0 caproic acid	0	0	0
C8:0 caprylic acid	0	0	0
C10: capric acid	0	0	0
C11:0 undecanoic acid	0	0	0
C12:0 lauric acid	0.2	0.7	0.9
C14:0 myristic acid	0.6	1.0	1.0
C15:0 pentadecanoic acid	0.3	0.2	0.3
C16:0 palmitic acid	27.7	27.2	30.3
C16:1 palmitoleic acid	0.7	0.5	0.5
C17:0 heptadecanoic acid	0.3	0.2	0
C17:1 *cis*-10-heptadecanoic acid	0.1	0	0
C18:0 stearic acid	4.4	4.2	3.9
C18:1n-9t elaidic acid	0	0	0
C18:1n-9c oleic acid	15.3	10.1	11.8
C18:2n-6t linolelaidic acid	0.1	0	0
C18:2n-6c linoleic acid	31.9	41.1	38.3
C18:3n-6 gamma linolenic acid	0	0	0
C20:0 arachidic acid	1.0	1.3	1.0
C18:3n-3 alpha linolenic acid	8.5	7.5	7.1
C20:1n-9 *cis*-11-eicosanoic acid	0	0.4	0.3
C21:0 henicosanoic acid	0.3	0.2	0
C20:2 *cis*-11,14-eicosadienoic acid	0.1	0	0.3
C20:3n-3 *cis*-11,14,17-eicosatrienoic acid	0	0	0
C22:0 behenic acid	1.1	1.0	0.6
C22:2 *cis*-3,16 docosadienoic	0	0	0
C20:4n-6 arachidonic acid	0	0	0
C22:1n-9 erucic acid	0.1	0	0
C23:0 tricosanoic acid	0.4	0.2	0
C22:2 *cis*-4,7,10,13,16,19-docosahexaenoic acid	0	0	0
C20:5n-3 *cis*-5,8,11,14,17-eicosapentaenoic acid	0.2	0	0.3
C24:0 lignoceric acid	0.1	0.8	0.3
C24:1n-9 nervonic acid	0	0	0
C22:6n-3 *cis*-4,7,10,13,16,19-docosahexaenoic acid	0	0	0
Undetermined	5.9	3.1	2.6
Total saturated (%)	36.6	37.1	38.6
Mono unsaturated (%)	16.4	11.0	12.6
Poly unsaturated (%)	40.9	48.6	46.0
Total unsaturated	57.4	59.7	58.7
Total saturated/mono unsaturated	2.20	3.30	3.00
Total saturated/poly unsaturated	0.89	0.76	0.83
Total saturated/total unsaturated	0.63	0.62	0.65
Mono/poly unsaturated	0.40	0.22	0.27
Linoleic/α-linolenic acid	3.70	5.40	5.30
Total oil amount (%)	0.80	0.96	0.78

Each value is the average of duplicate determinations

resolution [25]. Thus, characteristic fatty acid profiles of each taxa may reflect different pathways involved in oil biosynthesis and accumulation. The percentage of palmitic, stearic, oleic, linoleic and α-linolenic acid, total saturated and unsaturated fatty acids and their ratios in acorns appear to be useful parameters in the delimitation of *Quercus* taxa, especially for *Quercus* species [17, 18]. Our observations support that concept. Palmitic, oleic, α-linolenic acid

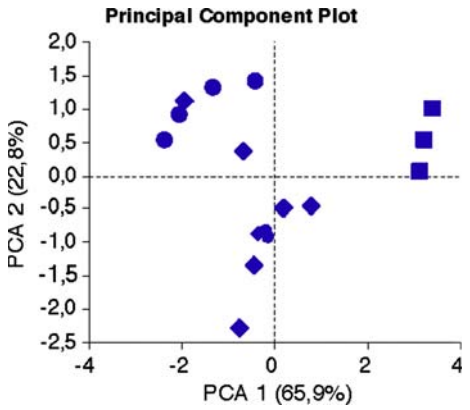

Fig. 1 Principle component analysis of 16 *Quercus* species based on five calculated ratios of the fatty acid percentages (total saturated/mono unsaturated, total saturated/poly unsaturated, total saturated/total unsaturated, mono/poly unsaturated, linoleic/α-linolenic acid). *Filled diamond*s section *Quercus*, *filled circles* section *Cerris*, *filled squares* section *Ilex*

concentrations and total percent of saturated and mono-unsaturated fatty acids are distinctive characteristics between *Q. brantii* and *Q. trojana*. *Quercus brantii* is a polymorphic species that hybridizes with *Q. libani* which is one of the most characteristic oak-trees of east Anatolia and Iraq. But, hybridization between *Q. brantii* and *Q. trojana* has not been reported [4, 9]. Our results for crude fat content are in agreement with the findings on *Q. brantii* from Mediterranean climate of Iran, but, palmitic and oleic acid show relatively lower, and linoleic acid higher levels [20]. In addition, α-linolenic acid and total percent of saturated and poly unsaturated fatty acids have considerably different levels between *Q. libani* and *Q. trojana*. The latter species is reported to vary little in its characters and is closely related to *Q. libani*. The distribution areas of these two oaks touch in the mountains of the central Taurus [4, 9]. However, *Q. libani* is completely different from *Q. brantii* for all examined parameters. This species, having more limited distributional area between *Q. brantii* and *Q. trojana*, may be better classified as a subspecies of *Q. trojana* on the basis of some morphological features [9]. However, specific separation was expressed as being merited in the ''Flora of Turkey''; because of has fewer distinctive variants than most Turkish species of oak and is clearly related to the West Anatolian *Q. trojana* [4].

Although there is considerable similarity among fatty acid profiles between these species, the most distinctive parameters are the ratio of mono:poly unsaturated fatty acids in total and linoleic:α-linolenic acid concentrations. These species are close to *Q. ithaburensis* subsp. *macrolepis* for many morphological features and hybridize frequently with it. They also express considerably different saturated, monounsaturated, total unsaturated fatty acid concentrations and ratios of saturated and unsaturated fatty acids. In addition, hybridizations are frequent among *Q. cerris* from the same section [4]. Palmitic and stearic acid levels in both taxon present similar values. *Quercus cerris* is widespread, variable and moderately mesophyllic species [9], and hybridizes with *Q. pubescens* and *Q. libani* [4]. *Quercus cerris* and *Q. pubescens*, from different sections show some similarity for general fatty acid profiles. Both varieties of *Q. cerris* exhibit highly stable concentrations within variety for palmitic acid, even though samples were collected from three different populations. Significant differences for this trait may then be a useful tool at the variety identification level in this species.

Total saturated, total unsaturated fatty acid concentrations and their ratio also show considerable stability between two varieties of *Q. cerris*. On the contrary, remarkable fluctuations within each variety for individual unsaturated fatty acid were observed according to the populations from different regions. Unsaturated fatty acid levels may be more susceptible to environmental conditions or population characteristics in *Q. cerris*. Significant difference of fatty acid profiles in both varieties of *Q. cerris* apart from general characteristics of its section may account for some of the genetic distance. This species covers the largest distributional areas in Anatolia compared to other members of the section. It was reported that *Q. hartwissiana* could be placed between *Q. robur* and *Q. petraea* in its morphological features and quite often there is no distinction between them [9]. In our observations, *Q. petraea* ssp. *petraea* differs from the others with higher mono-unsaturated and lower poly unsaturated fatty acid contents resulting in the high ratio of mono:poly unsaturated fatty acids. Additionally, the ratio of linoleic:α-linolenic acid shows a considerably higher value in this subspecies. This taxon has differences in fatty acid composition compared to all taxa from its section (section *Quercus*), except for *Q. vulcanica* endemic to Turkey. However, from the latter it differs in higher palmitic and lower stearic acid levels. The ratios of mono:poly unsaturated and linoleic α-linolenic acid are also distinctive parameters. All other traits examined in both taxa show considerable similarity in general. *Quercus vulcanica* is reported to be similar to *Q. petraea* ssp. *pinnatiloba*, but also to *Q. frainetto*. It differs from *Q. petraea* ssp. *pinnatiloba* in the flat scales of the cupule, secondary leaf lobes and intercalary veins. From *Q. frainetto* it differs in the longer petioles and the leaves evenly distributed over the shoots [4]. Similarity in higher stearic acid levels and considerably differences for almost all parameters were observed between *Q. vulcanica* and *Q. frainetto*, a typical species of forest tree growing fast and characterized by a great resistance to drought [9]. A high level of saturated fatty acids were also examined in *Q. frainetto* and *Q. pubescens* apart from other members of

the section. *Quercus pubescens* belongs to the most xeric oak species in west Anatolia growing as xerothermic scrubs in anthropogenic steppe or semi-steppe terrain; rarely in macchie [4, 9]. On the other hand, *Q. coccifera* is thought to be a dominant member of the phryagana and macchie flora of Turkey [26]. This species is related to *Q. ilex* and *Q. aucheri* which are endemic to Turkey, and it exhibited very similar values within its section and the highest saturated fatty acid concentrations among all examined taxa. But, high oleic and low linoleic acid levels were found in *Q. ilex*, a typical component of evergreen forests and maquis. All examined taxa having xerophytic nature mainly contained higher concentrations of saturated fatty acids. But, the information on the effect of environmental variation of concentrations for the fatty acids growing in different regions is needed to evaluate the utility of this characteristic. The lowest oil contents in species from section *Ilex* were associated with partly xerophytic characteristic. Different acorn samples of *Q. brantii* collected from different climatic zones in the Zagrossian region of Iran were reported to show no significant difference in fatty acid composition, but, crude fat contents varied significantly [20]. In addition, the acorn specimen of *Q. cerris* var. *cerris* from a Mediterranean climate resulted the lowest amount of total oil in our study. Similar total oil contents were detected for both geographically close populations of *Q. cerris* var. *austriaca* having similar ecological and climatic conditions. But, some differences for total oil contents were obtained in the acorn samples collected from individual population of *Q. cerris*. On the other hand, the relative percentages of fatty acids may be more stable and determinative in order to understand taxonomic and phylogenetic relations in *Quercus*. Many studies have reported phylogenetic relationships are associated with differences in the fatty profile of the seed oils [27–29]. In this study, similar fatty acid compositions for related species plus their morphology may account for its common ancestral stock. In that regard, each close related taxa examined here shows typical fatty acid composition. It is also possible to delineate taxa for fatty acid profile at the section level. Some ratios between saturated and unsaturated fatty acids have significantly difference implying its reliable characteristic for sectional and specific delineations in *Quercus*. Segregation of the three sections could be accomplished with using all ratios as a marker set. These results also provide an insight to the source potential of Turkish *Quercus* acorns based on fatty acid analysis.

Acorn oils are reported to have good nutritional quality with a flavor comparable to olive oil [30, 31]. Crude fat content of the acorns can be compared generally with the values of some grains [10, 20]. The highest levels of total oil are in *Q. brantii* and *Q. trojana*. Four species examined here from section *Cerris* (except for *Q. cerris*) show similar composition with olive, canola and hazelnut oils. Both varieties of *Q. cerris* correspond with cotton oil composition generally. The oil of *Q. hartwissiana* especially from section *Quercus* has very similar compositional fatty acid characteristics to sunflower oil. In addition, *Q. pubescens* has similarity with maize oil. *Quercus hartwissiana* and *Q. robur ssp. robur* show also common characteristics in fatty acid composition compared with soyabean oil. It is possible to say that sesame oil may be compared in general with *Q. petraea* ssp *petraea* and *Q. vulcanica*. Acorns of *Quercus* may be used as potential alternative food reserves for crude vegetable oil.

Mature acorn samples of examined taxa growing in their natural habitats showed a characteristic fatty acid composition. Our results suggest that these parameters may be valuable tools to segregate taxa at the sectional and specific level. Significantly different concentrations, critical values, total percentages and the relative ratios especially of saturated and unsaturated fatty acids seem to be useful for the characterization of *Quercus* at the infrageneric level in accord with established phylogenic associations. But, expanded evaluation is needed to understand how much of the variation of these parameters in the genotypes is valid for any examined species collected from different regional localities and climatic conditions.

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