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Fatty Acid Composition in the Acorn Oil of *Quercus rubra* L. Cultivated in NW Turkey

Tamer ÖZCAN*

Department of Biology, Division of Botany, Faculty of Science, Istanbul University, 34460 Istanbul, Turkey

* Corresponding Author
e-mail: tameroz@istanbul.edu.tr

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Abstract

Total oil and fatty acid composition in the acorns of *Quercus rubra* L. was analysed in this study. Total percentage of acorn oil found at the level of 7,41% (dry wt.%). The major fatty acids were oleic (48,25%), linoleic (35,61 %), and palmitic acids (11,22%) respectively. Lower levels in stearic (1,49%) and α-linolenic acids (0,74%) were quantified. The other fatty acids were also detected in minor concentrations. Total percentage of the mono-unsaturated fatty acids was 49,19%. Poly-unsaturated (36,38%) and saturated fatty acids (14,05%) showed relatively lower concentrations. Total unsaturated fatty acids observed at very high level (85,57%). The ratio of unsaturated fatty acids to saturated ones was also relatively high (6:1). Characteristic fatty acid pattern in this species may be useful in taxonomy of *Quercus* as additional chemometric data. The acorn oil contained valuable concentration of linoleic acid as an essential fatty acid with respect to dietary reference intakes of FAO and may be evaluated as alternative co-product for industrial purposes.

Key words: *Quercus rubra*, acorn oil, fatty acid, taxonomy, nutrition

INTRODUCTION

There are more than 500 species of *Quercus* occurring in North, Central and South America, Europe, Asia and northern Africa. They occur in temperate and subtropical ecosystem and in the tropics at high elevations. Most oaks are trees, but many are shrubs, and some are little more than sprawling ground covers. The oaks can be divided into two main groups; red oak and white oak groups. There are approximately 200 species of red oaks (*Quercus,* section *Lobatae*) [1, 2] restricted to the New World. Jensen [1] included 35 species of red oaks in "Flora of North America," north of Mexico. The northern red oak (*Quercus rubra*) is a powerful and large deciduous tree that normally develops a short trunk and round crown. It sometimes is a dominant species in the forest, but usually grows in association with other trees of the mixed forest at low to moderate elevations in northeast and middle America. Northern red oak is tolerant of urban air pollution and widely planted as a street tree in the American Northeast and Midwest. It is an important source of wood products. The acorns of this species is also valuable food in wild life and human nutrition. Oak species are characterized by unusually high levels of morphological variability which often pose serious taxonomic problems. This variation may be attributed to high intrinsic levels of genetic variation, phenotypic plasticity and gene flow potential among species [4]. A few molecular genetic studies have been conducted on their diversity and phylogeny of red oaks. Guttman and Weigt [5] found most red oak taxa to be similar in mean number of alleles per locus, percent polymorphic loci and mean heterozygosity. It was also reported low levels of genetic differentiation among populations in *Quercus rubra* [6] and between species in Wisconsin [7]. In phylogenetic relationships within subgenus *Quercus*, individual gene trees based on chloroplast DNA (cpDNA) restriction sites, nucleotide sequences of the internal transcribed spacers (ITS) and nuclear ribosomal DNA repeats were reported to be complementary in supporting clades that generally correspond to previously recognized taxonomic groups [8]. On the other hand, fatty acid profiles in the seed oils have great importance for chemotaxonomic differentiation in the plant kingdom [9]. Significantly different concentrations, critical values, total percentages and the relative ratios of saturated and unsaturated fatty acids were suggested to be valuable tools to segregate *Quercus* at the infrageneric level in accord with established phylogenic associations [10]. Acorn steroids and acorn fatty acids as biochemical markers provided strong evidence to support field observations of hybridization between *Q. wislizeni* and *Q. agrifolia* and additionally indicated significant differentiation between Sierra Nevadan and coastal populations of the former species [11]. Acorns were a staple food for many people until after AD 1900 in Europe, Asia, North Africa, the Middle-East, and North America and occured in the early town sites in the Zagros Mountains and at Catal Huyuk (6000 BC) [3]. The acorn is low in protein and rich in fat and starch [12] and a traditional product in the Spanishs Mediterranean diet used in ice creams and other desserts and liqueurs [13]. Valuable quantities of essential amino acids compared to daily requirements of FAO (Food and Agriculture Organization) were also reported in Turkish *Quercus* acorns [14]. Although great diversity of *Quercus*, a limited number of taxon (ca.40) were examined for quantitative compositions of acorn oils as recorded in SOFA (Seed Oil Fatty Acids), and stressed the utility as potential alternative food reserves for crude vegetable oil.

Apart from one unpublished study recorded in SOFA, no paper was reported with respect to fatty acid concentrations of

the acorns in this species. A cultivated specimen of *Quercus rubra* as an exotic species for actual flora of Turkey was analysed in order to observe ecological variations of fatty acid quantities of the acorn oil compared to the values of native population in North America and the utility of these parameters in taxonomy of this species in addition to nutritional product potential.

MATERIALS AND METHODS

Acorn specimens of *Quercus rubra* were picked at random in the ripened stage from various branches of the cultivated tree in Demirköy placed in Istranca mountain of Europe-in-Turkey. Collected specimens kept in cool were transported to the laboratory in polypropylene bags and packed in glass vessels in the deep-freezer (-18 °C) until the analysis carried out. Air dried mature acorn samples 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 (W1) with 25–50 ml ether into a weighted extraction pot (W2). 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 dessicator and weighed (W3). The following equation was used to calculate percentages of the oil: Oil % = [(W3-W2)/W1] x 100. The oil was transferred into glass sealed amber dark bottles, capped and stored at –18 °C until analyzed. The methyl esters of 33 fatty acids (FAMEs) were prepared from the acorn oil and determined by gas chromatography (GC) according to the method described by Slover and Lanza [15], and Alasalvar et al. [16]. FAMEs prepared using BF_3 (20%) in methanol were extracted with *n*-hexane and analyzed by GC. Sample (1 µl) was injected into a Supelcowax 10 column (60 m × 0.25-mm i.d., 0.25-µm film thickness; Supelco, Bellefonte, PA) coated with poly- (ethylene glycol). The column was connected to a Hewlett–Packard 5890 Series II (Little Falls, Willmington, DE) GC equipped with a flame-ionization detector. The injector and detector temperatures were 200 and 250 °C, respectively. Helium as the carrier gas was used at a flow rate of 1.5 ml/min. The oven temperature was programmed as follows: 180 °C for 2 min, increased to 200 °C at 2 °C/min, held at 200 °C for a further 10 min, and then increased to 215 °C at 2 °C/min and kept there for 10 min. Identification and quantification of fatty acid methyl esters were accomplished by comparing the retention times of the peaks with authentic standards. All chemical reagents and standards were obtained from Sigma–Aldrich– Fluka Co. Ltd. Each value of the experimental results is the average from dublicate determinations.

RESULTS

High level of total oil (7,41%) was examined in the acorns. The compositions and percentages of the fatty acids were documented in the figure and table (Fig 1 and Table 1). The major fatty acids were oleic (48,25%), linoleic (35,61 %), and palmitic acids (11,22%) respectively. Stearic (1,49%) and α-linolenic acids (0,74%) exhibited the lower levels. The other fatty acids were also quantified below 1%.

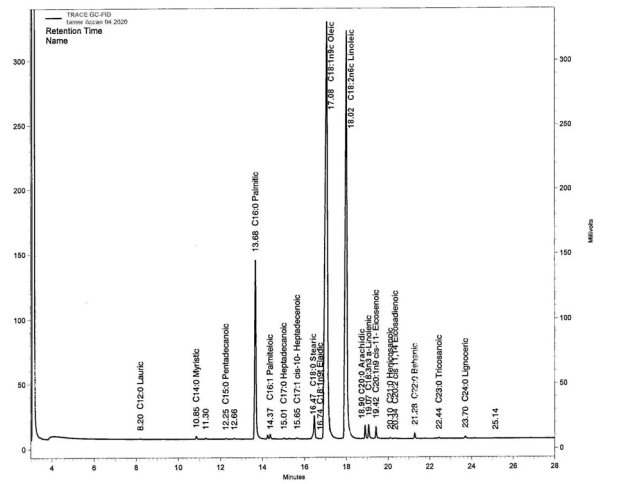

Figure 1. GC spectrum of the fatty acids in the acorn oil of *Quercus rubra* L.

Highest total percentages were observed in the mono-unsaturated fatty acids (49,19%). Poly-unsaturated (36,38%) and saturated fatty acids (14,05%) showed relatively lower concentrations. Unsaturated fatty acids in total was also found at very high level in the acorn oil (85,57%). Characteristically proportions between unsaturated and saturated fatty acids were observed. The proportions of mono- (3,50), poly- (2,59) and total unsaturated fatty acids (6,09) to saturated fatty acids in total were determined at considerably high level.

Table 1. Total oil percentages, fatty acid compositions and some of their ratios in the acorns of *Quercus rubra*

species	*Q.rubra* (Subgen. *Erythrobalanus* sect.*lobatae*)
C6:0 Caproic acid	0
C8:0 Caprylic acid	0
C10:0 Capric acid	0
C11:0 Undecanoic acid	0
C12:0 Lauric acid	0,020
C14:0 Myristic acid	0,150
C15:0 Pentadecanoic acid	0,037
C16:0 Palmitic acid	**11,227**
C16:1 Palmitoleic acid	0,247
C17:0 Heptadecanoic acid	0,050
C17:1cis-10-Heptadecanoic acid	0,065
C18:0 Stearic acid	**1,497**
C18:1n9t Elaidic acid	0,021
C18:1n9c Oleic acid	48,258
C18:2n6t Linolelaidic acid	0
C18:2n6c Linoleic acid	**35,616**
C18:3n6 Gamma linolenic acid	0
C20:0 Arachidic acid	0,600
C18:3n3 Alpha linolenic acid	**0,742**
C20:1n9 cis-11-Eicosenoic acid	0,599
C21:0 Henicosanoic acid	0,052
C20:2 cis-11,14-Eicosadienoic acid	0,027
C20:3n3 cis-11,14,17-Eicosatrienoic acid	0

C22:0 Behenic acid	0,251
C22:2 cis 13,16 Docosadienoic	0
C20:4n6 Arachidonic acid	0
C22:1n9 Erucic acid	0
C23:0 Tricosanoic acid	0,043
C22:2cis-4,7,10,13,16,19-Docosahexaenoic acid	0
C20:5n3cis-5,8,11,14,17-Eicosapentaenoic acid	0
C24:0 Lignoceric acid	0,125
C24:1n9 Nervonic acid	0
C22:6n3 cis-4,7,10,13,16,19-Docosahexaenoic acid	0
Undetermined %	0,373
Saturated %	14,052
Mono unsaturated %	49,190
Poli unsaturated %	36,385
Total unsaturated	85,575
Mono unsaturated /Saturated	3,500
Poli unsaturated /Saturated	2,589
Total unsaturated /Saturated	6,089
Mono unsaturated / Poli unsaturated	1,351
Linoleic acid / α-linolenic acid	48,000
Total oil amount %	**7,41**
Linoleic acid (AIs for life stage groups) g/day	**4,4-17**
α-linolenic acid (AIs for life stage groups) g/day	**0,5-1,6**

Each value of fatty acid concentrations is the average of dublicate determinations

Higher concentration of mono-unsaturated fatty acids than poly-unsaturated fatty acids was detected at the ratio of 1,35%. Calculated some proportions of fatty acids reflect more stable pattern than individual fatty acid concentrations. Linoleic and α-linolenic acid as essential fatty acids exhibited valuable concentrations with respect to adequate intakes (AIs) of fatty acids according to dietary refence intakes (DRIs) for different life stage groups [17]. Higher proportion of linoleic acid to α-linolenic acid (48,0) than the ratios of adequate intakes (AIs) reported in DRIs was found. Investigated major fatty acids, total ratios of saturated and unsaturated fatty acids and their some relative proportions represent characteristic pattern as additional chemometric data in the species concept of *Quercus rubra*.

DISCUSSION

Red oak grows best in Euro-Siberian region of Turkey presenting suitable conditions for its cultivation. The distinguishing characteristics include an acorn cup that is flat, thin and papery leaves and winter buds on twig tips hairless and not angled. It isn't always possible to identify oaks to species with complete certainty. Many other species of oaks are similar to northern red oak, and several species are known to hybridize with it. But, no hybrids were reported between *Lepidebalanus* (white oaks) and *Erythrobalanus* (black and red oaks) [18]. Compositions and concentrations of fatty acids stored in seeds express the characteristic patterns in biochemical systematic at different plant groups [9]. Fatty acid pattern of the acorn oil of *Quercus rubra* as additional chemotaxonomic data may reflect its genotype, apart from environmental conditions. It was reported that most genetic variation and isoenzyme diversity in red oaks was contained within the population. However, low estimates of genetic diversity were reported between populations of red oaks [19, 20]. On the other hand, the range of the areals for any species may account for the reason of biochemical polymorphism derived from both original genotypes and the ecological factors. Some differences in fatty acid patterns may be the result of disjunct distribution of the plant populations indicating genetical divergency. In general, parallel results with current study were announced in red oak group which contain decreasing levels of major fatty acids; oleic, linoleic and palmitic acids respectively [3,11,21,22]. Obtained results for fatty acid concentrations and their some proportions are generally correspond with the values from section *Cerris* Loudon as red oak group in Flora of Turkey, apart from *Quercus cerris*. Other sections exhibited completely different profiles of the fatty acids [10]. Such similar patterns may indicate the synapomorphy explaining phylogenetic relations and common pathways involved in the biosynthesis and accumulation of the fatty acids. Acorn fatty acids as diagnostic parameters could also significantly separated related species from each other [11]. But, some ecological parameters should be considered. A separation of xeric zone populations from mesic populations based on fatty acid profiles was reported in 13 natural populations of *Austrocedrus chilensis* from Andean Chile and Argentina. These differences were associated with a greater abundance of C20 unsaturated acids in the mesic group [23]. Significant differences between the fatty acid results were also found in different years for each species [24]. In general, fatty acid results in the acorn oil of *Quercus rubra* from two different continent are in good agreement. Our results from cultivated specimen in Turkey are correspond with the findings of Ivanov and Aitzetmüller obtained from native population of *Quercus rubra* in North America (SOFA data, unpublished results, 1998) for oleic (63,80%), linoleic (18,40%), palmitic (11,0%) and stearic acids (1,60%). But, lower level for oleic (48,25%), higher concentration for linoleic (35,61%) and similar values for palmitic (11,22%) and stearic acid (1,50%) were determined in this study. Unsaturated fatty acids exhibited some variations between two studies, while strictly agreement present in saturated fatty acids. More variable quantities in unsaturated fatty acids and consistent levels of saturated fatty acids were also examined for two varieties of *Quercus cerris* collected from three different populations in Turkey. Significantly differences at sectional level of *Quercus* were reported for palmitic, stearic and oleic acid concentrations. In addition, sectional differences were also significant for some relative concentrations of the fatty acids [10]. Saturated fatty acids may be more stable parameters which are relatively little affected from ecological conditions and determined primarily with genetical factors.

The acorns of *Quercus* are rich in total oil. Some varieties contain more than 30 percent oil, equal or greater than the best oil olives [25, 26]. As reported in *Q. rubra*, acorn dominated diets high in metabolizable energy provide optimal fat deposition for winter survival in deers [27]. Considerably high level of total oil in the acorns of *Q. rubra* (7,41%) was quantified in this study.

The percentage of total fat may exhibit some variation with respect to environmental factors. In cold climate conditions, the higher level of seed lipid contents, and decreasing levels of seed oils correlated with dry seasons were reported for different plant groups exhibiting constant profiles of fatty acid quantities in general [28, 29]. Such differences in total oil contents may be partly explained with different geographical, ecological and growing conditions in addition to ripening stages of the seeds. It was reported that temperate variety oils are less saturated due to a natural selection in northern latitudes for oils with a higher energy storage capacity or which remain liquid at a lower temperature [30]. Considering as a biomonitor or marker specimen, our observations on this species provide the information for the range of variation of fatty acid composition in the acorn oils based on geographical and growing conditions in order to evaluate the results for chemotaxonomic utility as stable parameters. In general, parallel findings with the native population of this species growing in two different continent of Holoarctic region may primarily indicate consistent and genotypic characteristic of fatty acid pattern in the acorn oil.

Data reported by Garcia and Buron [32] show that acorn oil should be classified as a non-drying oil, with its density similar to peanut oil, its viscosity similar to corn, sunflower and soybean oils, and with many other physical and chemical parameters similar to olive oil. The unsaturated/saturated ratio in the acorns of *Quercus rubra* was relatively high, at 6:1, and the relatively high content of linoleic acid as essential fatty acid (35,61%) makes acorn oil of this species especially prone to oxidation. However, this profile may have nutritional implications and beneficial effects in the prevention of coronary heart disease. Actually, Portuguese legislation [31] includes the acorn oil in the category of directly alimentary oils, although no industrial oil is produced. FA profile of *Quercus rubra* was very similar to those of other edible vegetable oils such as peanut, olive, hazelnut, avocado, tea seed, and kapok seed. This species can be planted for use as food, in addition to valuable feed for domestic animals, birds and wildlife. In suitable conditions, the yield of acorns per acre compares well with grains and acorn production can be very high, with yields of more than 5,280 kg/ha [3]. High acorn yields can be maintained on hilly lands where annual grain crops cause severe soil erosion. The production of acorn oil could add value to an underutilized agricultural product. Turkey that is in the same latitudes with the native populations of *Quercus rubra* exhibits suitable climatic conditions for the cultivation of this species. High ecological tolerance as observed with high acorn production in the northern district of Europe-in-Turkey may imply its alternative utility in forestry.

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