J Food Sci Technol (September 2017) 54(10):3050–3057
DOI 10.1007/s13197-017-2740-3

ORIGINAL ARTICLE

Acorn (*Quercus* spp.) as a novel source of oleic acid and tocopherols for livestock and humans: discrimination of selected species from Mediterranean forest

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Revised: 31 May 2017 / Accepted: 8 June 2017 / Published online: 15 June 2017

Abstract The aim of the present work was to characterization and compare acorns from selected *Quercus* spp. from the Mediterranean forest in Spain, namely, Portuguese oak (QF, *Quercus faginea* Lam.), Cork oak (QS, *Quercus suber* L.), Pyrenean oak (QP, *Quercus pyrenaica* Wild), Kermes oak (QC, *Quercus coccifera* L.), Holm oak (QB, *Quercus ilex* L. subsp. ballota [Desf.]). All physicochemical attributes varied significantly between species. Fat contents ranged from 1.30 to 4.70 g 100 g^{-1} fresh matter. The most abundant fatty acids were oleic (62.44, 56.25, 57.46, 48.02, 65.83%), followed by linoleic (16.42, 20.73, 21.30, 25.38, 14.17%) and palmitic (11.69, 14.27, 12.17, 16.22, 12.28) acids in QF, QS, QP, QC and QB species, respectively. The tocopherol content was high in the range of 31.83–45.25 mg kg^{-1}, and γ-tocopherol constituted 67–78% of total tocopherols. Only an effect of the location on γ-tocopherol content in QB was observed. The present results show the potential of different species of acorn to be used as agricultural and food resources and that geographical location plays a secondary role.

Keywords Fatty acid profile · Physicochemical properties · *Quercus* spp. · Tocopherols

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Introduction

Oak acorns, one of the species of *Quercus* genus, are of vital importance for both humans and animals (Al-Rousan et al. 2013). Usage of acorns in nutrition has a long history. In Europe, oak acorns were especially used in the Mediterranean region, mainly in Italy and Spain (Rakić et al. 2006). In Western Iberia, holm oak occupies large growing extensions either in pure stands or mixed stands, commonly with *Quercus suber* L., *Quercus faginea* Lam. and *Quercus pyrenaica* Willd. Oak acorns have been extensively under exploitation as a fodder for livestock animals (Tejerina et al. 2011). In fact, they are one of the most profitable products in the "dehesa": a woodland ecosystem typical of south-western continental Europe that evolved from the Mediterranean forest. During the oak fruiting period, Iberian pigs are raised extensively and fed on acorns. The quality of the meat products from Iberian pigs highly depend on the feeding background with the products from pigs fed exclusively on acorns and grass at the Mediterranean forest being the most pricey and appreciated commercial category. Most of the benefits of the acorns being used as feeds for Iberian pigs derive from their fatty acid composition and tocopherol content (Ventanas et al. 2005). Currently, acorns are receiving increasing attention for their potential as sources of essential nutrients for humans given their recognized concentration of essential fatty acids and tocopherols.

Acorns are rich in monounsaturated fatty acids, mostly oleic acid and also essential fatty acids such as linoleic (ω-6) and linolenic (ω-3) fatty acids. As monogastric animals, pigs deposit this unsaturated fatty acid in muscle lipid depots contributing positively appreciated quality traits in processed muscle foods such as juiciness, oiliness and intense flavor (Ventanas et al. 2007). The relationship

between acorn intake, tocopherol content in muscle and meat quality has been profusely documented (Cava et al. 1999; Ventanas et al. 2005, 2007). The direct consumption of acorns by humans may also provide health benefits owing to the well-known impact of oleic acid and tocopherols on physiological processes (Sales-Campos et al. 2013). Tocopherols, as natural antioxidants, are present in remarkable amounts in acorn fruits (Rakić et al. 2006). Dietary tocopherols are accumulated in the cell membranes protecting muscle and other tissues against oxidative stress (Soladoye et al. 2015; Ventanas et al. 2005). Other fruits and grains with similar chemical composition such as corn, sesame and walnuts have been already proposed to be included in a balanced diet to provide health benefits to humans (Lemcke-Norojärvi et al. 2001; Rabrenovic et al. 2011). Especially acorns are unique resources of γ-tocopherol isomers and hence, this fruit may be regarded as a relevant natural provider of such micronutrient with proven antioxidant activity. Unfortunately, both the fatty acid composition and tocopherol content in acorns is highly variable depending on the seasonal variations, species and geographical locations. While some of these factors have been already studied, some others such as the species and the location in the Mediterranean forest require further clarification.

The aims of this study were (1) to characterize five different species of the *Quercus* genus from different locations at the Mediterranean forest in southwestern Spain and to evaluate their nutritional suitability for livestock animals and humans on the basis of the fat content and fatty acid composition, and tocopherol content and (2) to examine the effects of two different locations at the Mediterranean forest in southwestern Spain on the characteristics of Holm oaks (*Quercus ilex* L. subsp. *ballota* [Desf.]).

Materials and methods

Chemicals

All chemicals and reagents used in the present work were of ACS analytical grade and purchased from Panreac (Panreac Quimica, S.A., Barcelona, Spain), Merck (Merck, Darmstadt, Germany), Extrasynthese (Genay, France), and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany).

Fruits

Five acorn varieties; Holm oak (QB, *Quercus ilex* L. subsp. *ballota* [Desf.]), Cork oak (QS, *Q. suber* L.), Pyrenean oak (QP, *Q. pyrenaica* Wild), Portuguese oak (QF, *Q. faginea* Lam.) and kermes oak (QC, *Q. coccifera* L.) were collected from different regions of the Caceres region (Spain) during the October and December of 2015. The choice of geographical areas and specimens was made taking into account the most representative areas of each species. In the specific case of holm oak acorns, the samples were collected from two areas: (1) Mediterranean forests in the southwest of Extremadura region, as the most representative area of Iberian pig production in Southwestern Spain, and (2) north of Extremadura where forests are poorly used for livestock production and display greater ecological and edaphoclimatic diversity (Pulido and Díaz 2016). After collecting the samples (200–300 g per analyzed geographical area and species) they were immediately transferred to the laboratory, cleaned and sorted to eliminate damaged fruits. They were immediately measured for their length, width, and weight, and then frozen at −80 °C, until the remaining chemical analysis and extractions were carried out.

Weight and size of acorns

For the weight and size determinations of acorns, five different samples were used for each variety and location. The weight in grams was obtained individually then average was calculated. In order to calculate the dimensions, two measures were taken. Length was measured as the long axis from proximal end to distal end. Diameter was measured at the largest part of the fruit. Both measurements were performed with a Vernier and expressed in centimeters.

Moisture and fat content of acorns

The moisture content of selected fruits was analyzed according to Ensminger (2008) methods. The method of Gossert et al. (2011) was used for determining fat content in the acorn fruits. Extracted fat was dissolved in hexane and kept at −80 °C for further analysis.

Fatty acid profile of acorns

Fatty acid methyl esters (FAMEs) were prepared by acidic esterification in the presence of sulfuric acid following the method described by Sandler and Karo (1992). FAMEs were analyzed by gas chromatography using a Hewlett-Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethyleneglycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA) (60 m × 0.32 mm i.d. × 0.25 μm film thickness). Gas chromatograph oven program temperature was as follows: initial temperature of 190 °C, 2 °C/min to 235 °C; 15 min at this temperature and thereafter 6 °C/min to 250 °C, and then kept for an additional 20 min. Injector and detector temperatures were

250 °C. Carrier gas was helium at a flow rate of 0.8 mL/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO). Tridecanoic acid was used as internal standard. Results were expressed as grams per 100 g of detected FAMEs.

Tocopherol quantification in acorns

Determination of tocopherols acorns was performed according to the methodology described by Rodriguez-Carpena et al. (2012). For the determination of δ-, γ- and α-tocopherol, the hexane used for dissolving lipids was evaporated and extract subsequently re-dissolved in isopropanol (1:10, v/v) prior to analysis by a Shimadzu "Prominence" HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a quaternary solvent delivery system (LC-20AD), DGU-20AS on-line degasser, SIL-20A auto-sampler, RF-10A XL fluorescence detector, and CBM-20A system controller. Separation was made on a Reversed-phase C18 column (150 mm length × 4.6 mm i.d., 5 μm particle diameter) manufactured by Phenomenex (USA) with the mobile phase being isocratic methanol:water (97:3 v/v) at a flow rate of 1.5 mL/min, and peaks were registered at 285 and 335 nm as excitation and emission wavelength, respectively. The mobile phases were filtered by a Millipore vacuum filtration system equipped with a 0.45 μm pore size filter. The samples (2 μL each) were injected by the aid of the auto-sampler. The system control and data acquisition were performed by Shimadzu "LC solution" software (Shimadzu Corporation, Kyoto, Japan). For quantification purposes, standard curves were prepared using standards of δ-, γ- and α- tocopherol supplied by Sigma-Aldrich (Steinheim, Germany).

Statistical analysis

Statistical analysis of the data was performed using the statistical software SPSS 22.0 for Windows. A One Way Variance Analysis was performed to evaluate the effects of the species on the parameters analyzed. When significant differences were found ($p < 0.05$) Tukey's test was performed to analyze the differences between the means. The study of the differences associated with the two locations of holm oaks was carried out using the statistical test of Student's T test.

Results and discussion

Physicochemical properties of different acorn species

Physicochemical properties of acorn samples from different *Quercus* species are shown in Table 1. These results display that great variability among acorns from different *Quercus* species, in regards to the size and composition of acorns. In agreement with the differences in weight, QB (4.14–1.87 cm) and QP (3.87–2.06 cm) had higher lengths and widths compared to QF (3.06–1.40 cm) and QC (3.10–1.47 cm). Although the number of studies reporting the dimensions of different oak varieties is scarce, data from literature shows variability about the dimensions of oak varieties (Sork et al. 1993) especially in holm oak species (Cantos et al. 2003; Ferreira-Dias et al. 2007; Rakić et al. 2006).

QB and QP were the heaviest acorn samples among acorn varieties under study. The values for holm oak acorn weight found in the literature range from 1.2 to more than 9.5 g, and is likely to be explained by tree individual characteristics (genetics), climate, soil properties, and stand structure (Afzal-Rafii et al. 1992; Cantos et al. 2003). Table 2 shows the results of physicochemical properties of holm oak acorns from two different locations in Spanish "dehesa". In our results, there were no significant differences in the physicochemical properties between the fruits from the north area and southwest of the Spanish Mediterranean forest.

Acorns displayed variable fat content (4–12%) (Lopez-Bote 1998). While a high fat content seems to be a common feature in holm oak fruits, the two morphotypes exhibited differences: *Q. ilex* ssp. *ballota* had a higher fat content (7.3–11.3%) than *Q. ilex* ssp. *ilex* (3.4–4.12%) (Afzal-Rafii et al. 1992). In our results, QB had the highest fat content (4.7 g/100 g fresh matter) followed by QF (4.3 g/100 g fresh matter) while QC (1.3 g/100 g fresh matter) had the lowest fat content among our samples. QB results agree with the those reported by Cantos et al. (2003), Ferreira-Dias et al. (2007) and Rodriguez-Estévez et al. (2008).

Some previous studies showed that fat content of cork oaks (QS) acorns range from 5.2% (Ferreira-Dias et al. 2007) to 7.3% (Fernández et al. 2004), Pyrenean oak (QP) from 3.8% (Ferreira-Dias et al. 2007) to 4.4% (Cañellas et al. 2003) to and Portuguese oak (QF) from 4,12% (expressed as wet matter) and 7.5% (expressed as dry matter) (Cañellas et al. 2003; León-Camacho et al. 2004) and these data are in general higher than the present results.

The comparison and interpretation of our results was complex due to the lack of references in literature regarding physicochemical properties of different oak varieties (excluding holm oaks). This study might have been the first to analyze and display the physicochemical properties of Kermes oak (QC) fruits.

Fatty acid compositions of different acorn species

Fatty acids profiles of acorn samples from different *Quercus* species are shown in Table 3. As shown, the most abundant fatty acids were oleic (62.44, 56.25, 57.46, 48.02,

Table 1 Physicochemical properties of acorn samples from different *Quercus* species

	QF	QS	QP	QC	QB
Moisture[B]	39.67 ± 1.37	44.30 ± 4.02	40.96 ± 2.29	42.30 ± 0.79	38.38 ± 4.76
Fat[B]	3.76 ± 0.30^{ab}	2.06 ± 0.53^{c}	2.47 ± 0.32^{bc}	1.30 ± 0.18^{c}	4.70 ± 1.30^{a}
Weight[C]	4.30 ± 0.75^{b}	7.69 ± 2.72^{ab}	10.92 ± 1.32^{a}	4.10 ± 0.74^{b}	8.77 ± 2.82^{a}
Length[D]	3.06 ± 0.40^{b}	3.65 ± 0.35^{ab}	3.87 ± 0.47^{a}	3.10 ± 0.13^{b}	4.14 ± 0.17^{a}
Width[D]	1.40 ± 0.11^{c}	1.77 ± 0.26^{abc}	2.06 ± 0.12^{a}	1.47 ± 0.15^{bc}	1.87 ± 0.32^{ab}

The mean ± standard deviation

[B] Results expressed as g/100 g fresh matter

[C] Results expressed as g

[D] Results expressed as cm

[a–c] Different superscripts in the same row indicate significant differences $p < 0.05$

QF, Portuguese oak (*Q. faginea* Lam.); QS, Cork oak (*Q. suber* L.); QP, Pyrenean oak (*Q. pyrenaica* Wild); QC, Kermes oak (*Q. coccifera* L.); QB, Holm oak (*Quercus ilex* L. subsp. *ballota* [Desf.])

Table 2 Physicochemical properties content of holm oak acorns (*Quercus ilex* L.) from two different locations in the Extremadura region

	North	South	P[a]
Moisture[b]	38.17 ± 3.45	38.38 ± 4.76	0.938
Fat[b]	3.98 ± 1.15	4.70 ± 1.30	0.382
Weight[c]	6.74 ± 1.55	8.77 ± 2.82	0.195
Length[d]	3.88 ± 0.64	4.14 ± 0.17	0.396
Width[d]	1.68 ± 0.10	1.87 ± 0.32	0.236

The mean ± standard deviation

[a] Values are significantly different ($p < 0.05$)

[b] Results expressed as g/100 g fresh matter

[c] Results expressed as g

[d] Results expressed as cm

65.83%), followed by linoleic (16.42, 20.73, 21.30, 25.38, 14.17%) and palmitic (11.69, 14.27, 12.17, 16.22, 12.28) acids in QF, QS, QP, QC and QB species, respectively.

The present results show significant differences between the species under study. Researches mostly have been focused on fatty acid profile of Holm Oak acorn species. For QB, the oleic, linoleic and palmitic acid percentages were in accordance with earlier findings (Cantos et al. 2003; Ferreira-Dias et al. 2007; León-Camacho et al. 2004; Rodriguez-Estévez et al. 2008; Tejerina et al. 2011). It reflects a very high content of oleic acid, >63% of total fatty acids, followed by palmitic and linoleic acids at similar concentrations (12–20%).

QS and QP results agree with the results previously reported (Cantos et al. 2003; Ferreira-Dias et al. 2007). After literature review, we can state that this research might have been the first to report data on the fatty acids profiles of QF and QC species. Moreover, it should be noted that fatty acid profile of QF is similar to QB. Hence, it might be an interesting alternative for feeding livestock animals destined to the production of quality products. However, this fruit is not abundant in the Mediterranean forest.

As aforementioned, the fatty acid profile of acorns is one of the main factors affecting the quality of the meat and products from the Iberian pig fed extensively on the natural resource (Ventanas et al. 2005, 2007). The high content of C:18:1n-9 in acorns is responsible for the high concentration of this fatty acid in adipose (Ordonez et al. 1996; Regueiro et al. 1994), hepatic (de la Hoz et al. 1993), and muscle (Cava et al. 1997; Martín et al. 1999) tissues and even in dry hams (de la Hoz et al. 1996) from Iberian pigs raised extensively. Several relevant sensory properties in dry-cured hams from Iberian pigs fed on acorns are directly linked to the high levels of oleic acid, including brightness, oiliness, juiciness and pleasant flavor (Ventanas et al. 2005).

In relation to the impact that the intake of these fruits may exert on humans in terms of nutritional value or health benefits, it is worth highlighting that the concentration of oleic acid in this fruit is considerably higher than in other fruits generally considered natural sources of oleic acid such as peanut (38.41%) and walnut (21%) (Maguire et al. 2004) with the concentration of this acid in acorn being only surpassed by olive oil (Al-Rousan et al. 2013). The benefits of this fatty acid on human health is proven and involves a positive influence on lipid metabolism and plasma lipoproteins levels, increasing HDL and decreasing LDL (Lemcke-Norojärvi et al. 2001; Maguire et al. 2004).

Table 4 shows that fatty acids profiles of holm oak acorn samples from different locations at the Mediterranean forest. Our results show that there were no significant differences in the fatty acid profiles of holm oak acorns collected at two different locations. This is also a relevant finding as using the northern forest for extensive pig feeding, much less exploited than the southern counterpart,

Table 3 Fatty acid composition of acorn samples from different *Quercus* species (%)

	QF	QS	QP	QC	QB
C14	0.19 ± 0.05^{bc}	0.31 ± 0.06^{a}	0.18 ± 0.02^{c}	0.30 ± 0.09^{ab}	0.18 ± 0.04^{c}
C15	0.15 ± 0.02^{a}	0.15 ± 0.03^{a}	0.15 ± 0.01^{a}	0.16 ± 0.03^{a}	0.08 ± 0.01^{b}
C16	11.69 ± 1.19^{b}	14.27 ± 1.48^{ab}	12.17 ± 0.55^{b}	16.22 ± 0.99^{a}	12.28 ± 1.55^{b}
C16:1 (n-7)	0.36 ± 0.12^{ab}	0.26 ± 0.03^{ab}	0.38 ± 0.02^{a}	0.37 ± 0.15^{a}	0.17 ± 0.04^{b}
C17	0.16 ± 0.01^{a}	0.19 ± 0.01^{a}	0.15 ± 0.01^{a}	0.18 ± 0.01^{a}	0.19 ± 0.03^{a}
C18	3.50 ± 0.66^{a}	2.74 ± 0.54^{a}	3.23 ± 0.66^{a}	3.31 ± 0.44^{a}	4.03 ± 0.86^{a}
C18:1 (n-9)	62.44 ± 2.46^{ab}	56.25 ± 6.84^{bc}	57.46 ± 3.46^{ab}	48.02 ± 4.55^{c}	65.83 ± 3.75^{a}
C18:1 (n-7)	2.49 ± 0.24^{a}	2.23 ± 0.41^{ab}	2.36 ± 1.18^{a}	3.11 ± 0.76^{a}	1.40 ± 0.31^{b}
C18:2 (n-6)	16.42 ± 0.22^{bc}	20.73 ± 4.43^{abc}	21.30 ± 3.54^{ab}	25.38 ± 2.54^{a}	14.17 ± 2.98^{c}
C18:3 (n-3)	0.74 ± 0.21^{b}	1.34 ± 0.42^{a}	0.80 ± 0.10^{b}	1.57 ± 0.25^{a}	0.54 ± 0.18^{b}
C20	0.58 ± 0.18^{a}	0.26 ± 0.06^{b}	0.54 ± 0.15^{a}	0.29 ± 0.03^{b}	0.36 ± 0.04^{ab}
C20:1 (n-9)	0.43 ± 0.12^{a}	0.39 ± 0.08^{a}	0.39 ± 0.07^{a}	0.38 ± 0.04^{a}	0.36 ± 0.04^{a}
C20:2 (n-6)	0.04 ± 0.03^{bc}	0.08 ± 0.02^{a}	0.04 ± 0.04^{bc}	0.06 ± 0.02^{ab}	0.03 ± 0.05^{c}
C20:3 (n-6)	0.11 ± 0.04^{a}	0.09 ± 0.03^{a}	0.11 ± 0.02^{a}	0.09 ± 0.02^{a}	0.07 ± 0.01^{a}
C22	0.34 ± 0.17^{a}	0.35 ± 0.15^{a}	0.41 ± 0.11^{a}	0.28 ± 0.06^{a}	0.18 ± 0.01^{a}
C23	0.12 ± 0.05^{ab}	0.14 ± 0.07^{a}	0.12 ± 0.03^{ab}	0.10 ± 0.02^{a}	0.05 ± 0.06^{b}
C24	0.22 ± 0.10^{a}	0.23 ± 0.12^{a}	0.21 ± 0.04^{a}	0.16 ± 0.04^{a}	0.09 ± 0.01^{a}
Σ SFAs	16.96 ± 0.88^{b}	18.64 ± 1.87^{ab}	17.15 ± 1.44^{b}	21.01 ± 1.35^{a}	17.44 ± 1.01^{b}
Σ MUFAs	65.73 ± 2.36^{a}	59.13 ± 6.50^{ab}	60.59 ± 3.55^{ab}	51.89 ± 4.05^{b}	67.76 ± 3.93^{a}
Σ PUFAs	17.31 ± 2.14^{bc}	22.24 ± 4.74^{ab}	22.26 ± 3.61^{ab}	27.11 ± 2.80^{a}	14.80 ± 3.12^{c}

The mean ± standard deviation

$^{a–c}$ Different superscripts in the same row indicate significant differences $p < 0.05$

% ∑SFAs, total saturated fatty acids; ∑MUFAs, total mono-unsaturated fatty acids; ∑PUFAs, total poly-unsaturated fatty acids, QF, Portuguese oak (*Q. faginea* Lam.); QS, Cork oak (*Q. suber* L.); QP, Pyrenean oak (*Q. pyrenaica* Wild), QC, Kermes oak (*Q. coccifera* L.); QB, Holm oak (*Quercus ilex* L. subsp. *ballota* [Desf.])

may lead to similar dietary effects to the typical southern forest as long as the fatty acid profile is concerned.

Tocopherol contents in different acorn species

It is well-known that the occurrence of natural antioxidants such as tocopherols in agricultural products has a positive correlation with the degree of fat unsaturation (León-Camacho et al. 2004). Table 5 shows a comparison of the tocopherol contents in the fat from the five spp. selected in the current study. The results reveal that the total content of tocopherols in the present study ranged from 31.60 to 45.25 mg kg^{-1} fat. The main tocopherol was γ-tocopherol with this isomer having higher antioxidant capacity than the α-isomer (Duthie et al. 1991). The content of γ-tocopherol ranged from 21.16 mg/kg (QS) to 32.19 mg/kg (QB). According to this study, only δ-tocopherol results showed significant differences among the species. QF and QP had lower δ-tocopherol contents compared to other acorn species. As regards to the tocopherol content of holm oak, some previous studies have revealed a notable variability in the content of this compound in relation to the year and season of study (Tejerina et al. 2011). According to these authors, these variations could be attributed to the action of climate, soil properties, physiological conditions (maturation and germination) or health status (drying, putrefaction, pest attack) of acorns in each season. In addition to this, Table 6 shows the results obtained after the analysis and quantification of the tocopherols content of holm oak acorns from two the different locations under study. The results of this study showed significant differences for the total content of tocopherols as well as for the γ-tocopherol content (majority isomer) between the two geographical locations analyzed. Of note, holm oak acorns from the north area of the forest had a considerably lower content of γ-tocopherol (18.47 mg/kg) than acorns from forests from southwest region (32.19 mg/kg). Nevertheless, significant differences were not found in the content of α- and δ-tocopherol between the studied areas. Comparing the results obtained with the available literature we can conclude that the data derived from this study are consistent with other previous studies, though in most cases the previous work is restricted to the analysis of tocopherols in holm oak, cork oak and Portuguese oak (Cantos et al. 2003; León-Camacho et al. 2004; Tejerina et al. 2011) and there are no previous bibliographical references to the content in

Table 4 Fatty acid composition of acorn samples from different *Quercus* species (%)

	North	South	p[a]
C14	0.22 ± 0.08	0.18 ± 0.04	0.260
C15	0.10 ± 0.01	0.08 ± 0.02	0.145
C16	13.56 ± 1.57	12.28 ± 1.55	0.231
C16:1 (n-7)	0.20 ± 0.04	0.17 ± 0.04	0.218
C17	0.18 ± 0.03	0.19 ± 0.03	0.573
C18	3.59 ± 0.63	4.03 ± 0.86	0.382
C18:1 (n-9)	62.25 ± 2.60	65.83 ± 3.75	0.117
C18:1 (n-7)	1.85 ± 0.37	1.40 ± 0.31	0.068
C18:2 (n-6)	16.30 ± 1.80	14.17 ± 2.98	0.209
C18:3 (n-3)	0.58 ± 0.08	0.54 ± 0.18	0.593
C20	0.35 ± 0.05	0.36 ± 0.04	0.781
C20:1 (n-9)	0.37 ± 0.02	0.36 ± 0.04	0.686
C20:2 (n-6)	0.03 ± 0.006	0.03 ± 0.004	0.508
C20:3 (n-6)	0.07 ± 0.01	0.06 ± 0.01	0.656
C22	0.21 ± 0.05	0.18 ± 0.01	0.286
C23	0.05 ± 0.008	0.06 ± 0.006	0.412
C24	0.09 ± 0.01	0.09 ± 0.01	0.691
Σ SFAs	18.35 ± 1.31	17.44 ± 1.01	0.251
Σ MUFAs	64.66 ± 2.83	67.76 ± 3.93	0.191
Σ PUFAs	16.99 ± 1.86	14.80 ± 3.12	0.216

The mean ± standard deviation

[a] Values are significantly different ($p < 0.05$)

% ∑SFAs, total saturated fatty acids; ∑MUFAs, total mono-unsaturated fatty acids; ∑PUFAs, total poly-unsaturated fatty acids)

tocopherols in acorns from different geographical locations. The differences observed in the tocopherol content between the different geographical areas might directly affect the tocopherol content accumulated in the tissues of livestock animals and humans with the consequences that this fact may have in terms of meat quality and health status, respectively (Cava et al. 1999). Hereby, further studies are required to examine the influence of geographical location on the content of tocopherols.

This work represents the first reference to the presence of δ-tocopherol in acorns from assorted *Quercus* species. In the available literature, only a single study providing results on *Q. suber* has been found (León-Camacho et al. 2004) The differences between the present results and those found by León-Camacho et al. (2004) can be attributed to the lower limit of detection of the technique used in this study for the determination of tocopherols.

The results obtained in the present study and in the available scientific literature, show that acorns are a remarkable source for tocopherols (Cantos et al. 2003; León-Camacho et al. 2004; Perez-Palacios et al. 2009; Tejerina et al. 2011). Some researchers have demonstrated the role of tocopherols (α- and γ-tocopherol) in modulating the susceptibility to lipid oxidation of different tissues in vivo, in vitro, and in post-mortem muscle (Soladoye et al. 2015). Furthermore, León-Camacho et al. (2004) showed that total tocopherol contents of *Q. faginea* oil, *Q. ilex* oil and *Q. suber* oil 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, albeit lower than in olive or avocado oil. According to our findings, there were no significant differences between *Q. faginea*, *Q. suber*, *Q. pyrenaica* and *Q. coccifera* regarding to their tocopherol contents and their isomer distributions ($p > 0.05$). Given the current theories relating aging, age-related diseases and lifespan with oxidative stress, an appropriate delivery of essential nutrients such as tocopherols and other antioxidant compounds in the diet seems to be a feasible strategy to alleviate oxidative stress

Table 6 Tocopherol contents of holm oak acorns (*Quercus ilex* L.) from two different locations in the Extremadura region (mg/kg)

	North	South	p[a]
δ-Tocopherol	0.93 ± 0.31	1.44 ± 0.68	0.164
γ-Tocopherol	18.47 ± 5.96	32.19 ± 7.83	0.014
α-Tocopherol	12.10 ± 4.38	11.63 ± 6.78	0.899
Σ tocopherol	31.50 ± 8.88	45.25 ± 15.66	0.044

The mean ± standard deviation

[a] Values are significantly different ($p < 0.05$)

Table 5 Tocopherol contents of acorn samples from different *Quercus* species (mg/kg)

	QF	QS	QP	QC	QB
δ-Tocopherol	0.54 ± 0.05[b]	0.94 ± 0.29[ab]	0.60 ± 0.04[b]	1.01 ± 0.26[ab]	1.44 ± 0.68[a]
γ-Tocopherol	24.33 ± 2.03[a]	21.16 ± 4.33[a]	21.44 ± 3.02[a]	26.05 ± 5.97[a]	32.19 ± 7.83[a]
α-Tocopherol	6.96 ± 1.26[a]	9.50 ± 0.56[a]	10.25 ± 4.75[a]	5.48 ± 0.82[a]	11.63 ± 6.78[a]
Σ tocopherol	31.83 ± 2.48[b]	31.60 ± 4.09[b]	32.29 ± 4.05[b]	32.54 ± 6.85[b]	45.25 ± 8.21[a]

The mean ± standard deviation

[a–c] Different superscripts in the same row indicate significant differences $p < 0.05$

QF, Portuguese oak (*Q. faginea* Lam.); QS, Cork oak (*Q. suber* L.); QP, Pyrenean oak (*Q. pyrenaica* Wild); QC, Kermes oak (*Q. coccifera* L.); QB, Holm oak (*Quercus ilex* L. subsp. *ballota* [Desf.])

and its negative health consequences (Estévez and Luna 2016). Acorns and other natural sources of natural antioxidants may be seriously considered to be used for the development of innovative foods with healthy properties.

Conclusion

This study brings new insight into the five species of acorns of the *Quercus* species naturally present in the Mediterranean forests located in Spain. Significant differences were found between species in relation to their physicochemical characteristics, fatty acid profile and tocopherol content. This fact must be taken into account when designing the extensive feeding strategies of the livestock animals. As regards to animal and human nutrition, QB is the species with the highest fat, monounsaturated fatty acids and tocopherols contents, with these parameters being highly appreciated in terms of nutritional value and health benefits. Similarly, according to its fatty acid profile, QF might be considered an interesting alternative for acorn consumption. However, QF is less abundant in the Mediterranean forest compared to QB. The geographical area of origin (Mediterranean forests located in the south vs. the north of the Extremadura region, in Spain) significantly affects the tocopherol content of oak acorns while this effect is negligible compared to the genetic background of the fruit.

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