Industrial Crops and Products 94 (2016) 143–151

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Chemical and antioxidant profiles of acorn tissues from *Quercus* spp.: Potential as new industrial raw materials

A.F. Vinha[a,b], A.S.G. Costa[a], João C.M. Barreira[a,c,*], R. Pacheco[a], M. Beatriz P.P. Oliveira[a]

[a] REQUIMTE/LAQV, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, no. 228, 4050-313 Porto, Portugal
[b] FP-ENAS (UFP Energy, Environment and Health Research Unit), CEBIMED (Biomedical Research Center), University Fernando Pessoa, Praça 9 de Abril, 349, 4249-004 Porto, Portugal
[c] Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

ARTICLE INFO

Article history:
Received 4 May 2016
Received in revised form 12 August 2016
Accepted 13 August 2016
Available online 25 August 2016

Keywords:
Quercus spp.
Acorn tissues
Nutritional parameters
Phytochemicals
Antioxidant activity
Industrial applications

ABSTRACT

The bioactivity of different organs and tissues of *Quercus* spp. (Fagaceae), an important group of evergreen or deciduous trees from temperate and tropical climatic areas, represents a good starting point for possible industrial applications. Nevertheless, *Quercus* fruits, generally known as acorns, are currently undervalued and underexploited. Accordingly, the proximate composition, fatty acids and tocopherols profiles, chlorophyll, lycopene and β-carotene contents, as well as the antioxidant activity, were studied in different *Quercus* species to boost new applications in food, cosmetic and pharmaceutical industries. In general, significant differences were found among the nutritional parameters, fatty acids and tocopherols contents and bioactivity indicators, either considering phenotypic (studied species) or botanical (acorn tissues) factors. The acorn tissues and the *Quercus* species that optimize the production of each nutrient and bioactive compound, as well as that allowing the highest antioxidant activity were thoroughly identified. The obtained information provides an increased knowledge to define potential industrial applications for acorn tissues, potentially offering economic advantages to this underutilized natural resource.

1. Introduction

The consumption of natural resources has increased substantially, mainly due to the recent consumers' trends. This current concept is mentioned in recent studies, which report several bioactivities on different fruit tissues (Barreira et al., 2008; Vázquez et al., 2008; Baiano, 2014; Costa et al., 2014; Brizi et al., 2016). Furthermore, most natural products have low toxicity and comply with sustainability principles (Islam et al., 2013).

Currently, the potential as a food resource of *Quercus* spp. is being underestimated, comparatively to other plants, *e.g.*, chestnut (*Castanea sativa* Mill.) (Barreira et al., 2009a; Vasconcelos et al., 2010; Barreira et al., 2012), walnut (*Juglans regia* L.) (Amaral et al., 2005; Liao et al., 2016) and hazelnut (*Corylus avellana* L.) (Amaral et al., 2006; Li and Parry, 2011). Acorns are abundant in the Portuguese territory, occupying 1 107 600 ha, an area much higher than that dedicated to chestnut (~41 100 ha) and almond (~36 530 ha). Even so, the inclusion of acorns in human nutrition is still scarce (Cantos et al., 2003; Tejerina et al., 2011; INE, 2014).

Acorns were previously reported as having high contents in starch (48–50%) and low levels of proteins and fat content (*circa* 2% in both cases) (Deforce et al., 2009). Despite not being as nutritionally rich as other common nuts, acorns represent good alternatives to other high-starch content products, such as chestnuts or potatoes. Furthermore, acorn oil also presents relevant features to be considered for industrial purposes, containing mainly oleic and linoleic acids with an average value of 53–65% and 24–50%, respectively (Özcan, 2007), which, together with the oxidative stability, represent similar characteristics to those presented by olive oil. Actually, tocopherols and phenolic compounds, such as phenolic acids, flavonoids and tannins (which have been reported as strong natural antioxidants), are considered as being the primary bioactive compounds in acorn fruits (Cantos et al., 2003; Lopes and Bernardo-Gil, 2005; Rakić et al., 2006, 2007; Tejerina et al., 2011). These components have been associated with biological functions, such as anti-tumoral, anti-allergic, anti-platelet, anti-ischemic and anti-inflammatory activities (Ostertag et al., 2011; Heleno et al., 2015), inclusively when evaluated under epidemiologic studies (Pandey and Rizvi, 2009).

* Corresponding author at: REQUIMTE/LAQV, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, no. 228, 4050-313 Porto, Portugal.
E-mail address: jbarreira@ipb.pt (J.C.M. Barreira).

http://dx.doi.org/10.1016/j.indcrop.2016.08.027
0926-6690/

Some studies reporting the chemical characterization of nut shells have already been published, such as those presented by Shahidi et al. (2007) and Contini et al. (2008), who investigated the bioactive compounds in European hazelnuts and found that extracts of hazelnut shells exhibited higher antioxidant activity than its kernel. Different extracts of chestnut shells were also evaluated regarding the antioxidant activity, which was shown to be correlated with the high tannin contents (Barreira et al., 2008; Vázquez et al., 2008). Nevertheless, and as far as it could be concluded, there are no studies conducted on acorn pericarps alone.

Taking into account the potential of bioactive compounds in acorn components, this study aimed to quantify and compare different chemical parameters and bioactivity indicators in four *Quercus* species: *Q. faginea* (Portuguese oak), *Q. ilex* (evergreen oak), *Q. nigra* (red oak) and *Q. suber* (cork oak), evaluating whole fruits, kernels and pericarps (byproducts of industrial processing). Besides acorns *per se*, the byproducts potentially generated might represent sources of specific bioactive phytochemicals with different industrial applications.

2. Materials and methods

2.1. Standards and reagents

For the macronutrient analysis all analytical grade reagents were purchased from Panreac (Barcelona, Spain) and Merck (Darmstadt, Germany). Tocopherols (α, β, γ and δ) and tocotrienols (α, β, γ and δ) were purchased from Calbiochem (La Jolla, California, USA) and tocol (2-methyl-2-(4,8,12-trimethyl-tridecyl)chroman-6-ol) was obtained from Matreya Inc. (Pennsylvania, USA). The mixture of methyl esters of fatty acids (FAME) standards Supelco37 were obtained from Supelco (Bellefonte, PA, USA). 1,1-diphenyl-2-picrylhydrazyl (DPPH•) free radical, Folin-Ciocalteu's reagent, gallic acid, glycerol, TPTZ (2,4,6-tripyridyl-s-triazine) solution, petroleum ether, potassium hydroxide, anhydrous sodium sulfate, ferrous sulfate heptahydrate were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol reagent grade, sodium acetate, sodium carbonate decahydrate, sodium nitrite, aluminum chloride, acetone, *n*-hexane, methanol and sodium hydroxide were purchased from Merck (Darmstadt, Germany). HPLC-grade *n*-hexane and 1,4-dioxane were from Fluka (Madrid, Spain). HPLC grade *n*-hexane was from Merck (Darmstad, Germany). Purified water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Samples and samples preparation

For the present work, four different acorn species (*Q. suber*, *Q. faginea*, *Q. nigra* and *Q. ilex*) were selected due to the elevated representativeness among Mediterranean forest. Samples were collected in Trás-os-Montes region (Latitude: 41.538°, Longitude: −6.911°). Whole fruits were haphazardly collected from ten trees per species in September, 2014. Freshly collected fruits (approximately 5 kg for each species) were cleaned and used to prepare three distinct sample groups for each component: whole fruits, kernels and pericarps (manually peeled off). All samples were frozen, lyophilized (48 h, −78 °C, 0.015 mbar) (Telstar Cryodos-80, Terrassa, Barcelona), reduced to powder in a mill (Grindomix GM 200, Retsch, Haan, Germany), homogenized and stored in plastic tubes at 4 °C until further analysis.

2.3. Proximate analysis

Macronutrients (moisture, ash, fat, protein and carbohydrates) were analyzed following the Association of Official Analytical Chemists methods (AOAC, 2012).

The moisture content was instrumentally determined using an infrared moisture analyzer (SMO 01, Scaltec Instruments, Heiligenstadt, Germany). The ash content was determined by incineration at 550 ± 15 °C approximately during 5 h, until the sample was converted in a whitish ash. The crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus. The protein content ($N \times 6.25$) was determined using the Kjeldahl procedure. Total carbohydrates were calculated as the difference between 100 g and the sum of the contents obtained for ash, crude fat and protein. Energy value was calculated according to the general Atwater factors (Atwater and Benedict, 1902): Energy (kcal) = 4 × (g protein) + 3.75 × (g carbohydrate) + 9 × (g fat). The results are expressed as g per 100 g of dried mass.

2.4. Fatty acids composition

Fatty acids methyl esters (FAME) were prepared, in triplicate, according to ISO (129662:2011).

The analysis was carried out with a Shimadzu GC-2010 *Plus* gas chromatograph equipped with a split-splitless Shimadzu AOC-20i injector and a FID detector (Shimadzu, Tokyo, Japan). A CP-Sil 88 silica capillary column for FAME (50 m × 0.25 mm i.d, 0.20 μm film thickness; Varian, Middelburg, Netherlands) was used. Helium was used as gas carrier (40 mL/min) and separation was achieved with the following temperature program: 5 min at 120 °C, increase of 3 °C/min from 120 °C to 220 °C, maintaining 220 °C for 10 min. The temperature of the injector and detector was 250 °C and 270 °C, respectively; a split ratio of 1:25 was used and the injection volume was 1 μL. FAME were identified by comparing the relative retention times with a standard mixture (FAME 37, Supelco, Bellefonte, PA, USA) and analyzed using the Shimadzu software GC Solution (v. 2.30, Shimadzu GC Solution, Shimadzu, Tokyo, Japan) based on the relative peak areas. The results were expressed in relative percentage of each fatty acid.

2.5. Vitamin E

For identification and quantification of individual compounds standard solutions of α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol were prepared in *n*-hexane (25, 18.75, 12.5, 6.25, 2.5 and 1.25 mg/mL). Each of these solutions contained 20 μL of tocol (internal standard, 1 mg/mL).

The lipid fraction for tocopherols quantification was obtained by Soxhlet extraction with petroleum ether (2.5 h). Analysis was carried out in an HPLC integrated system equipped with an AS-2057 automated injector, a PU-2089 pump, a MD-2018 multiwavelength diode array detector (DAD) and a FP-2020 fluorescence detector (Jasco, MD, USA), programmed for excitation at 290 and emission at 330 nm (Rodrigues et al., 2015). The chromatographic separation was achieved on a normal phase Supelcosil™ LC-SI (3 μm; 75 mm × 3.0 mm; Supelco, Bellefonte, PA, USA). Chromatographic data were analyzed using JASCO-Chrom NAV Chromatography Software (Jasco, Japan). The compounds were identified based on the UV/vis spectra and respective retention time patterns. Quantification was based on the fluorescence signal, using the internal standard method. Final results were expressed in mg/100 g of fat.

2.6. Chlorophylls and carotenoids quantification

The chlorophylls (*a* and *b*), β-carotene and lycopene were determined according to the methodology proposed by Nagata and Yamashita (1992), slightly modified by Vinha et al. (2014). Briefly, ~0.5 g acorn samples were extracted with 10 mL of acetone/hexane (4:6, v/v) and centrifuged at 5000 rpm, during 30 min. Then, the absorbance of the supernatants was measured at 453,

505, 645, and 663 nm (BioTek Synergy HT microplate reader, GEN5, Winooski, Vermont, USA). The contents in chlorophylls and carotenoids were calculated according to the following equations: Chlorophyll *a* (mg/100 mL) = $0.999A_{663} - 0.0989A_{645}$; Chlorophyll *b* (mg/100 mL) = $-0.328A_{663} + 1.77A_{645}$; β-carotene (mg/100 mL) = $0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$; Lycopene (mg/100 mL) = $-0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$, and further expressed in mg/100 g of dried ample.

2.7. Antioxidant activity

2.7.1. Preparation of hydro-alcoholic extracts

The hydro-alcoholic extracts were obtained using 0.5 g of each powdered sample with 100 mL of ethanol:water (1:1, v/v) at 40 °C for 60 min (Mirac, Thermolyne, USA), according to Costa et al. (2014). Afterwards, extracts were filtered through Whatman no. 1 filter paper, and kept at −25 °C until being used for quantification of total phenolics and antioxidant activity assays.

2.7.2. Total phenolics quantification

The amount of total phenolics was determined according to Alves et al. (2010). Briefly, 500 μL of each hydro-alcoholic diluted (1:10 v/v) extract were mixed with 2.5 mL of the Folin-Ciocalteau reagent (1:10) and 2.5 mL of a sodium carbonate solution (7.5% m/v). The mixture was first incubated at 45 °C, during 15 min, followed by 30 min incubation at room temperature (~25 °C). Absorbance readings were performed at 765 nm using a microplate reading Synergy HT (Bio Tek Instruments, Synergy HT GENS5, EUA). Total phenolic contents were calculated from a calibration curve prepared with gallic acid (0–150 mg/L; $r = 0.9986$) and results were expressed as mg of gallic acid equivalents (GAE)/g of dried mass.

2.7.3. DPPH radical-scavenging activity

The anti-radical ability of the extracts was evaluated according to Brand-Williams et al. (1995) with minor modifications. The reaction mixture was prepared directly on a 96 well plate, consisting of a solution of different sample concentrations (20 μL) and an ethanolic solution (180 μL) containing DPPH radicals (6×10^{-5} mol/L) in each well. The reduction of the DPPH radical was determined by measuring the absorption at 525 nm, during 40 min until stabilization of the reaction. The radical scavenging activity (RSA) was calculated as a percentage of DPPH inhibition using the equation: % $RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution at initial time.

2.7.4. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to Benzie and Strain (1999) with minor modifications. This method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous form (Fe^{2+}-TPTZ). An aliquot (90 μL) of each extract (with appropriate dilution, if necessary) was added to 270 μL of distilled water and 2.7 mL of the FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ solution and 1 part of 20 mM $FeCl_3 \cdot 6H_2O$ solution). The reaction mixture was then incubated at 37 °C. The increase in the absorbance at 595 nm was measured after 30 min. Solutions of known Fe(II) concentrations ($FeSO_4 \cdot 7H_2O$) were used for calibration. A calibration curve was prepared with ferrous sulfate (linearity range: 0–840 mg/L, $r = 0.9976$), and the results were expressed as ferrous sulfate equivalents/g of dried plant material.

2.8. Statistical analysis

All statistical tests were performed at a 5% significance level using IBM SPSS Statistics for Windows, version 22.0. (IBM Corp., Armonk, NY, USA).

Data were expressed as mean ± standard deviation, maintaining the significant numbers allowed by the magnitude of the standard deviation. An analysis of variance (ANOVA) with type III sums of squares was performed using the general linear model (GLM) procedure. The dependent variables were analyzed using 2-way ANOVA with the factors "acorn tissues" (AT) and "*Quercus* species" (QS). When a statistically significant interaction among factors was detected, these were evaluated simultaneously by the estimated marginal means plots for all levels of each factor. On the other hand, if no statistical significant interaction was found, the means were compared using Tukey's multiple comparison test, after a previous assessment of the equality of variances through the Levene's test.

Principal components analysis (PCA) was applied as a pattern recognition unsupervised classification method. The number of dimensions to keep for data analysis was assessed by the respective eigenvalues (which should be greater than one), by the Cronbach's alpha parameter (that must be positive) and also by the total percentage of variance (that should be as high as possible) explained by the number of components selected. The number of plotted dimensions was chosen to allow meaningful interpretations.

3. Results and discussion

In view of the potential use of acorns in industrial applications, it should be borne in mind that it is often difficult to ensure that the raw materials are obtained from the same *Quercus* species. Accordingly, it is mandatory to evaluate each considered acorn tissue independently of its phylogenetic origin, *i.e.*, understand its potential regardless of the species in analysis. Likewise, it might also be useful to find which *Quercus* species has the highest suitability for a determined purpose, independently of the acorn part to be used. Accordingly, the average values presented for each species were calculated considering all the acorn parts; similarly, the average values for each acorn tissue comprise the results obtained for all species. Hence, the standard deviations result from samples prepared from different fruit parts or species and should not be regarded as a measure of accuracy of the applied methodologies. The interaction among factors (AT × QS) was also evaluated. As indicated in the **Materials and methods** section, when a significant interaction was found ($p < 0.050$), the results obtained from the multiple comparisons could be indicated. Therefore, the influence of each factor was assessed by interpreting the estimated marginal means (EMM) plots, since the interaction among factors was always significant.

3.1. Macronutrients analysis

In fresh mass (fm) basis (Table 1), the acorn kernel presented higher water content (46 ± 9 g/100 g fm) than the whole fruit (40 ± 6 g/100 g fm) and the pericarp (27 ± 5 g/100 g fm), independently of the species. Likewise, the acorn parts from *Q. suber* had the highest water contents (46 ± 8 g/100 g fm), whilst those from *Q. ilex* showed the lowest contents (30 ± 3 g/100 g fm). Considering the composition in dried mass (dm) basis, carbohydrates were the major component, reaching maximum values in the pericarps (92 ± 3 g/100 g dm) and indistinguishable values in the kernels and whole fruits (87 ± 3 g/100 g dm). Nevertheless, it should be reminded that pericarps are expected to have high percentages of lignin; thereby, the carbohydrates percentage might have been overestimated. In terms of species comparison, all the species pre-

Table 1
Macronutrients profile and energy value of *Quercus* spp. components. Results are presented as mean ± standard deviation.

		Water (g/100 g fm)	Fat (g/100 g dm)	Protein (g/100 g dm)	Ash (g/100 g dm)	Carbohydrates (g/100 g dm)	Energy (kcal/100 g dm)
AT	Kernel	46 ± 9	6 ± 3	6 ± 2	1.7 ± 0.5	87 ± 3	399 ± 21
	Whole fruit	40 ± 6	5 ± 3	7 ± 2	1.9 ± 0.5	87 ± 3	393 ± 18
	Pericarp	27 ± 5	0.8 ± 0.2	5 ± 2	1.5 ± 0.5	92 ± 3	375 ± 2
	p-value[1] (n = 36)	<0.001	<0.001	0.010	0.003	<0.001	<0.001
QS	*Q. faginea*	35 ± 10	2 ± 1	6 ± 1	2.0 ± 0.2	89 ± 1	381 ± 7
	Q. ilex	30 ± 3	8 ± 4	4 ± 1	1.2 ± 0.2	87 ± 6	412 ± 26
	Q. nigra	39 ± 12	2 ± 1	5 ± 1	1.2 ± 0.3	92 ± 2	381 ± 3
	Q. suber	46 ± 8	3 ± 1	9 ± 1	2.5 ± 0.4	86 ± 2	382 ± 6
	p-value[2] (n = 27)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
AT × QS	*p*-value[3] (n = 108)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

fm: fresh mass; dm: dry mass; AT: acorn tissues; QS: *Quercus* species. [1]$p < 0.05$ indicates that the mean value of the evaluated parameter of at least one acorn part differs from the remaining. [2]$p < 0.05$ indicates that the mean value of the evaluated parameter of at least one *Quercus* species differs from the remaining. [3]$p < 0.05$ indicates that the interaction among factors is significant (in this case, the multiple comparison tests could not be performed).

sented similar values (86–89 g/100 g dm), except for *Q. nigra*, which had the highest carbohydrates contents (92 ± 2 g/100 g dm). Protein was the second component in all cases except *Q. ilex*, in which the second most abundant component was fat (8 ± 4 g/100 g dm). On the other hand, proteins reached maximal contents in *Q. suber* (9 ± 1 g/100 g dm), while the acorn parts had lower differences (5–7 g/100 g dm) among each other. In general, the obtained values were higher than the reported in acorns obtained from *Q. robur* (pedunculate oak) and *Q. petraea* (Irish oak) (Deforce et al., 2009) and similar to the ones reported in Spanish acorns produced by *Q. rotundifolia* (holm oak) (4.8 g/100 g dm) and *Q. suber* (6.3 g/100 g dm) (Aguilera et al., 2002). Besides the fat content highlighted for *Q. ilex*, the remaining species had similar values (2–3 g/100 g dm), while the pericarps showed minimal contents (concerning the comparison among acorn tissues). In contrast, the kernels, whole fruits and pericarps presented similar values in mineralized material (1.5–1.9 g/100 g dm), which, in turn, presented significant differences among *Quercus* species (1.2 ± 0.2 g/100 g dm in *Q. ilex* and 1.2 ± 0.3 in *Q. nigra*, 2.0 ± 0.2 g/100 g dm in *Q. faginea* and 2.5 ± 0.4 g/100 g dm in *Q. suber*). Finally, the kernels had the highest energetic value (399 ± 21 kcal/100 g dm), followed by the whole fruits (393 ± 18 kcal/100 g dm) and the pericarps (375 ± 2 kcal/100 g dm). *Q. ilex*, perhaps in result of its higher fat content, presented the highest energy value (412 ± 26 kcal/100 g dm), while the remaining species presented nearly the same value (381–382 kcal/100 g dm).

3.2. Fatty acids (FA)

The most representative FA are presented in Table 2. In addition to the tabled molecules, C4:0, C8:0, C10:0, C12:0, C14:0, C15:0, C17:0, C20:1, C21:0 and C22:2 were also detected, but in percentages below 0.5%. Oleic acid (C18:1n9c) was the main fatty acid in all cases, varying from 49 ± 9% in the pericarps to 58 ± 7% in the whole fruits, and from 48 ± 2% in *Q. nigra* to 64 ± 7% in *Q. ilex*. Linoleic acid (C18:2n6c) was also present in relevant percentages, showing some statistically significant differences among species (*Q. faginea*: 20 ± 2%, *Q. ilex*: 14 ± 2%; *Q. nigra*: 26 ± 6%; *Q. suber*: 21 ± 3%) and acorn parts (kernels: 22 ± 7%, whole fruits 21 ± 5%, pericarps: 18 ± 2%). Oleic and linoleic acids were previously reported as the predominant fatty acids in *Quercus* species (Cantos et al., 2003; Petrović et al., 2004; Gea-Izquierdo et al., 2006; Tejerina et al., 2011), emphasizing the interest of these natural matrices (especially in the case of *Q. ilex*, considering its fat content), considering the health-promoting effects attributed to unsaturated fatty acids (Lands, 2014). Among the saturated fatty acids (SFA), palmitic acid (C16:0) was quantified in the highest percentages, once again with significant differences among species (*Q. faginea*: 18 ± 4%, *Q. ilex*: 14 ± 1%; *Q. nigra*: 17 ± 1%; *Q. suber*: 14 ± 1%) and acorn tissues (kernels: 15 ± 2%, whole fruits 14 ± 1%, pericarps: 17%). In general, acorns FA profiles were similar to those typically detected in mastic, sunflower, peanut, cotton, olive and avocado oils (Charef et al., 2008).

3.3. Vitamin E composition

Five different isoforms of vitamin E were detected, with α-tocopherol and γ-tocopherol as the predominant compounds (Table 3). The vitamin E profiles varied greatly among *Quercus* species and different botanical parts, as indicated by the high standard deviation presented below. Among the identified vitamers, γ-tocopherol was the only one lacking statistically significant differences ($p = 0.094$), despite the tendency to higher contents shown by *Q. nigra* (8 ± 6 mg/100 g oil). *Q. faginea*, on the other hand, showed the maximum average contents in α-tocopherol (7 ± 7 mg/100 g oil). α-Tocopherol (6 ± 6 mg/100 g oil) was maximized in whole fruits, while γ-tocopherol (11 ± 4 mg/100 g oil) reached the highest levels in the kernels of all species. β-tocopherol, δ-tocopherol and α-tocotrienol were also quantified, but in minor levels (≤0.3 mg/100 g oil). Regarding total vitamin E, the results herein indicate lower quantities than those reported previously in related *Quercus* species (Gea-Izquierdo et al., 2006; Tejerina et al., 2011), where a different extraction technique was followed. Considering that tocopherols are strong lipophilic antioxidants (Barreira et al., 2009b) the low detected quantities might be interpreted as a reliable indicator of the oxidative stability of the lipid fraction of acorns.

3.4. Carotenoids, chlorophylls and antioxidant activity

As a preliminary indicator of the potential bioactivity of the studied acorns, the antioxidant activity was evaluated by measuring the scavenging activity against DPPH radicals and the ferric reducing power (Table 4).

Interestingly, the kernels tended to have the highest activity in both assays, which is a surprising result as the corresponding parts of similar fruits present less activity than the pericarps (Barreira et al., 2008). When comparing the *Quercus* species (using extracts at 1 mg/mL), the results were similar for all cases (presenting approximately 70% of inhibition), except for *Q. ilex*, which tended to show lower scavenging activity (42 ± 6% of inhibition). Concerning the ferric reducing power, *Q. faginea* stood out as the species with the highest power (827 ± 302 μmol FSE/g of dm), whilst *Q. ilex* showed the least strong activity in this assay (638 ± 297 μmol FSE/g of dm). In either case, the measured activities are slightly higher than those described in acorns (Custódio et al., 2015). Besides the antioxidant assays, the contents in chlorophyll a, chlorophyll *b*, lycopene, β-carotene and phenolics were also evaluated. As

Table 2
Fatty acids profiles of *Quercus* spp. components. Results are presented as mean ± standard deviation.

		C16:0 (%)	C16:1 (%)	C18:0 (%)	C18:1n9c (%)	C18:2n6c (%)	C20:0 (%)	C18:3n3 (%)	C22:1n9 (%)	C24:0 (%)	SFA (%)	MUFA (%)	PUFA (%)
AT	Kernel	15 ± 2	0.3 ± 0.2	1.2 ± 0.5	57 ± 8	22 ± 7	0.4 ± 0.1	2 ± 1	0.2 ± 0.1	0.1 ± 0.1	16 ± 2	61 ± 8	23 ± 7
	Whole fruit	14 ± 1	0.8 ± 0.8	1.2 ± 0.5	58 ± 7	21 ± 5	0.4 ± 0.1	3 ± 2	0.2 ± 0.1	0.1 ± 0.1	16 ± 2	62 ± 7	22 ± 5
	Pericarp	17 ± 4	0.4 ± 0.3	3.5 ± 0.3	49 ± 9	18 ± 2	0.4 ± 0.2	7 ± 4	0.7 ± 0.4	2 ± 2	25 ± 3	57 ± 4	19 ± 2
	p-value[1] (n = 36)	<0.001	<0.001	<0.001	<0.001	0.001	0.132	<0.001	<0.001	<0.001	<0.001	0.001	0.003
QS	*Q. faginea*	18 ± 4	0.3 ± 0.1	2 ± 1	51 ± 12	20 ± 2	0.5 ± 0.1	5 ± 5	0.6 ± 0.5	0.4 ± 0.4	22 ± 6	57 ± 5	21 ± 2
	Q. ilex	14 ± 1	0.3 ± 0.2	1 ± 1	64 ± 7	14 ± 2	0.4 ± 0.1	2 ± 1	0.3 ± 0.2	0.3 ± 0.3	16 ± 4	69 ± 6	15 ± 2
	Q. nigra	17 ± 1	0.5 ± 0.2	2 ± 1	48 ± 2	26 ± 6	0.5 ± 0.1	4 ± 2	0.5 ± 0.3	0.1 ± 0.1	20 ± 3	53 ± 3	27 ± 6
	Q. suber	14 ± 1	1.0 ± 1.0	2 ± 1	55 ± 2	21 ± 3	0.1 ± 0.1	4 ± 1	0.1 ± 0.1	2 ± 2	18 ± 3	60 ± 2	21 ± 3
	p-value[2] (n = 27)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
AT × QS	*p*-value[3] (n = 108)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

AT: acorn tissues; QS: *Quercus* species; nd: non detected. [1] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one acorn part differs from the remaining. [2] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one *Quercus* species differs from the remaining. [3] $p < 0.05$ indicates that the interaction among factors is significant (in this case, the multiple comparison tests could not be performed). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 3
Vitamin E composition of *Quercus* spp. components. Results are presented as mean ± standard deviation.

		α-Tocopherol (mg/100 g oil)	β-Tocopherol (mg/100 g oil)	γ-Tocopherol (mg/100 g oil)	δ-Tocopherol (mg/100 g oil)	α-Tocotrienol (mg/100 g oil)	Vitamin E (mg/100 g oil)
AT	Kernel	2 ± 1	0.05 ± 0.02	11 ± 4	0.3 ± 0.1	0.03 ± 0.01	13 ± 5
	Whole fruit	6 ± 6	0.3 ± 0.3	4 ± 2	0.3 ± 0.2	0.05 ± 0.04	11 ± 8
	Pericarp	2 ± 1	0.07 ± 0.06	2 ± 1	0.1 ± 0.1	0.02 ± 0.01	4 ± 2
	p-value[1] (n = 36)	<0.001	<0.001	0.010	0.003	<0.001	<0.001
QS	*Q. faginea*	7 ± 7	0.3 ± 0.3	5 ± 1	0.3 ± 0.2	0.06 ± 0.04	13 ± 9
	Q. ilex	0.7 ± 0.1	0.026 ± 0.004	5 ± 3	0.2 ± 0.1	0.03 ± 0.01	6 ± 3
	Q. nigra	2 ± 1	0.12 ± 0.05	8 ± 6	0.2 ± 0.1	0.01 ± 0.01	10 ± 6
	Q. suber	2 ± 1	0.07 ± 0.03	5 ± 5	0.2 ± 0.1	0.03 ± 0.01	7 ± 4
	p-value[2] (n = 27)	<0.001	<0.001	0.094	0.027	<0.001	0.001
AT × QS	*p*-value[3] (n = 108)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

AT: acorn tissues; QS: *Quercus* species; [1] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one acorn part differs from the remaining. [2] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one *Quercus* species differs from the remaining. [3] $p < 0.05$ indicates that the interaction among factors is significant (in this case, the multiple comparison tests could not be performed).

Table 4
Chlorophylls, carotenoids and antioxidant profiles of *Quercus* spp. components. Results are presented as mean ± standard deviation.

		Chlorophyll *a* (mg/g dm)	Chlorophyll *b* (μg/g dm)	Lycopene (μg/g dm)	β-carotene (μg/g dm)	Phenolics (mg GAE/g dm)	DPPH (% inhibition)	FRAP (μmol FSE/g of dm)
AT	Kernel	1 ± 1	1 ± 1	95 ± 63	448 ± 539	29 ± 7	70 ± 13	841 ± 119
	Whole fruit	2 ± 1	1 ± 1	193 ± 92	475 ± 381	26 ± 6	65 ± 15	742 ± 264
	Pericarp	8 ± 2	4 ± 1	106 ± 158	2457 ± 876	27 ± 9	52 ± 15	580 ± 208
	p-value (n = 36)[1]	<0.001	<0.001	<0.001	<0.001	0.478	<0.001	<0.001
QS	*Q. faginea*	4 ± 3	2 ± 2	183 ± 141	1312 ± 890	31 ± 7	70 ± 10	827 ± 302
	Q. ilex	2 ± 2	1 ± 1	148 ± 74	473 ± 497	18 ± 3	42 ± 6	638 ± 297
	Q. nigra	5 ± 4	3 ± 2	115 ± 127	1421 ± 1290	32 ± 7	74 ± 7	745 ± 91
	Q. suber	3 ± 3	1 ± 1	79 ± 103	1301 ± 1412	28 ± 4	64 ± 16	674 ± 96
	p-value[2] (n = 27)	0.011	0.005	0.008	0.006	<0.001	0.001	0.012
AT × QS	*p*-value[3] (n = 108)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

dm: dry mass; AT: acorn tissues; QS: *Quercus* species; GAE: gallic acid equivalents; FSE: ferrous sulfate equivalents. DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; [1] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one acorn part differs from the remaining. [2] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one *Quercus* species differs from the remaining. [3] $p < 0.05$ indicates that the interaction among factors is significant (in this case, the multiple comparison tests could not be performed).

it would be expectable, the pericarps showed the highest content in chlorophylls, which did not vary significantly among the *Quercus* species. On the other hand, the lycopene content showed significant changes among species, with *Q. suber* giving the least quantity (79 ± 103 μg/g dm), in contrast with *Q. faginea*, which gave the top quantity (183 ± 141 μg/g dm) in this carotenoid. Among the acorn tissues, the whole fruits (193 ± 92 μg/g dm) showed the highest contents in lycopene. The results were different for β-carotene, which was present in the pericarps in contents nearly fivefold higher than in the remaining acorn parts. Among *Quercus* species, *Q. ilex* presented the lowest β-carotene contents (473 ± 497 μg/g dm), whilst the remaining species presented similar values (1301–1421 μg/g dm). Finally, the acorn tissues did not show statistically significant differences ($p = 0.478$) for the phenolic compounds, while *Q. ilex* gave lower contents (18 ± 3 mg GAE/g dm) than the remaining species (28–32 mg GAE/g dm). Considering previous findings (Cantos et al., 2003; Rakić et al., 2006; Tejerina et al., 2011), these phenolic compounds should include phenolic acids (gallic acid and its derivatives and ellagic acid), flavonoids and tannins. The high antioxidant activity of these compounds is a reasonable explanation for the highest correlations (evaluated by the Spearman rho), which were verified in this work among DPPH radicals scavenging inhibition (0.653) and among FRAP (0.596) and phenolic compounds contents.

3.5. Principal components analysis (PCA)

In the former sections, the differences among the acorn tissues and the *Quercus* species were compared analyzing each parameter individually. In most cases, the detected differences were statistically significant, but the parameters with the highest differences for each acorn part or *Quercus* are yet to be identified. Accordingly, the results were evaluated considering data for all parameters (nutritional, chemical and antioxidant activity) simultaneously, by applying principal components analysis (PCA).

The correlations among the first three PC and each of the 41 included parameters are indicated in Table 5, showing which parameters contributed mostly to define the obtained PCs. The parameters more highly correlated with PC1 were carbohydrates, C12:0, C14:0, C15:0, C17:0, C18:0, C20:1, C18:3n3, C22:1n9, SFA, chlorophyll a, chlorophyll *b* and β-carotene. Analyzing the 3D plot (Fig. 1A), it is obvious that markers corresponding to the pericarps were the only ones scoring positively in PC1, indicating that these components present particularly high levels of all parameters (except C20:1) with the highest correlation with PC1. The higher levels of chlorophyll a, chlorophyll *b* and β-carotene could have been anticipated, considering the color of the pericarps (brownish-green) and the kernels (white). Likewise, the higher abundance of SFA is probably related with the fact that pericarps are more exposed to environmental stressors, thereby presenting fatty acids less prone to oxidation. Concerning the distribution of markers according to PC2 and PC3 it was not possible to obtain a clear separation among kernels and whole fruits, thereby indicating that the parameters more highly correlated with PC2 (moisture, fat, energy, C18:1n9c, C18:2n6c, MUFA, PUFA, phenolics and DPPH scavenging activity) and PC3 (protein and ash) scored similarly for those acorn parts.

On the other hand, the markers corresponding to each *Quercus* species were not clustered individually by PC1 (Fig. 1B), which indicates the similarity among species for all the parameters more highly correlated with PC1. According to PC2, *Q. ilex* was significantly different from the remaining species, mostly due to its higher levels in fat, energy, C18:1n9c and MUFA, and the lower (negative correlations) levels in all the remaining parameters highlighted as having the highest correlations with PC2. *Q. faginea* and *Q. suber* scored positively on PC3, in opposition to *Q. ilex* and *Q. nigra*, prob-

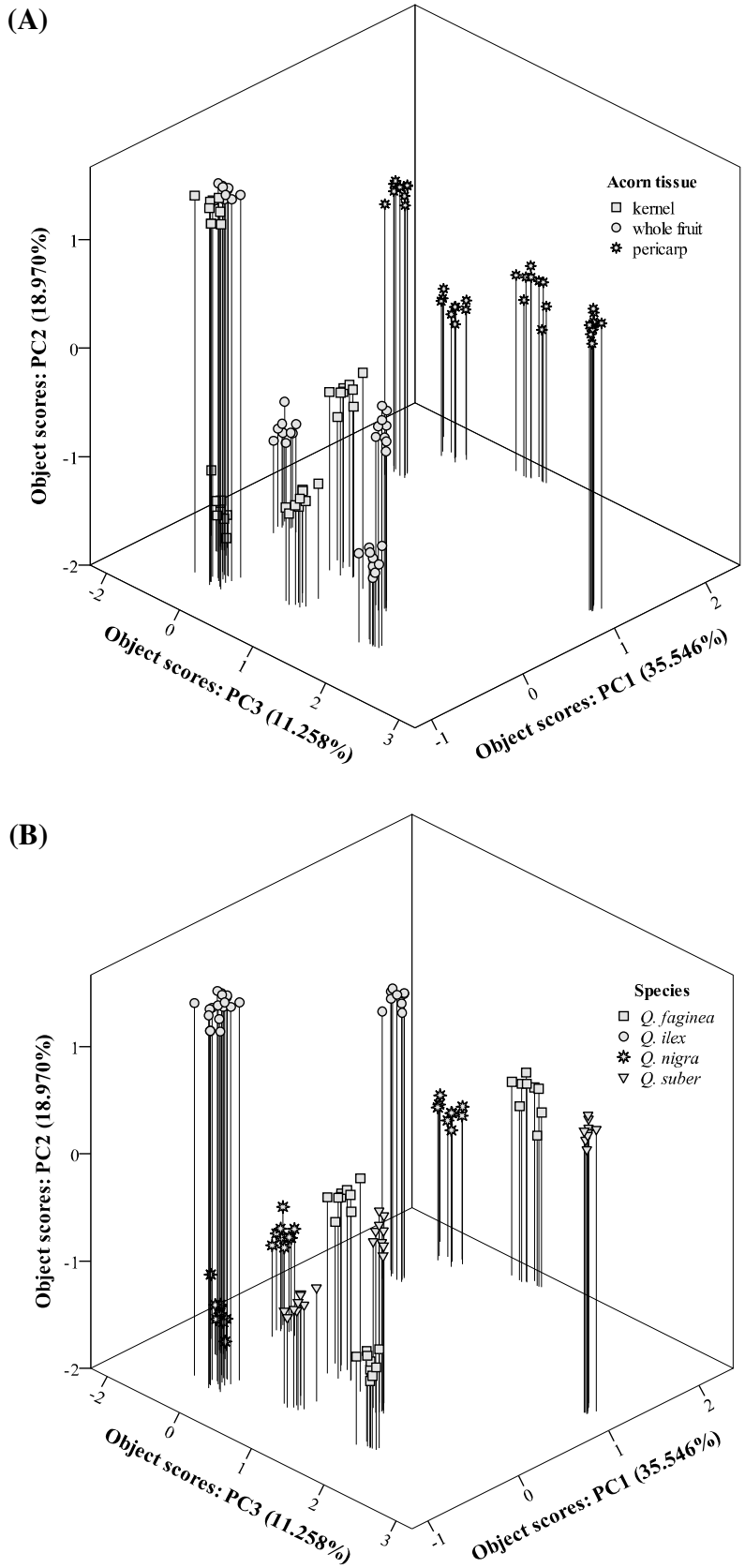

Fig. 1. Tridimensional plots (defined by the firs three defined principal components) of object scores highlighting differences among *Quercus* species (A) and acorn tissues (B). The percentage of variance explained by each principal component is indicated in each axis. Spikes were represented in the vertical axis to allow a better visualization of the tridimensional projection of objects.

Table 5
Correlations among variables (assayed parameters) and the first three defined principal components (PC). The percentage of variance included in each PC is given in the bottom line of the table. Numbers highlighted in bold represent the highest absolute correlations among variables and principal components.

Variable	PC1	PC2	PC3	Variable (cont.)	PC1	PC2	PC3
Moisture	−0.646	−0.602	0.235	C22:2	−0.670	−0.542	−0.100
Fat	−0.671	0.631	−0.225	C24:0	0.525	0.292	0.576
Protein	−0.281	-0.293	0.712	SFA	0.950	−0.191	0.067
Ash	−0.319	−0.226	0.677	MUFA	−0.538	0.768	0.049
Carbohydrates	0.731	−0.306	−0.279	PUFA	−0.189	−0.865	−0.130
Energy	−0.645	0.630	−0.291	α-tocopherol	−0.206	−0.474	0.461
C8:0	−0.308	−0.519	−0.267	α-tocotrienol	−0.602	0.028	0.471
C10:0	0.220	-0.489	−0.387	β-tocopherol	−0.129	−0.439	0.318
C12:0	0.819	−0.068	−0.171	γ-tocopherol	−0.540	−0.354	−0.348
C14:0	0.834	0.157	0.351	δ-tocopherol	−0.543	−0.349	0.042
C15:0	0.840	0.207	0.358	Tocopherols	−0.611	−0.542	0.115
C16:0	0.693	−0.392	−0.313	Chlorophyll *a*	0.919	0.127	0.109
C16:1	0.059	−0.202	−0.097	Chlorophyll *b*	0.921	0.161	−0.073
C17:0	0.774	-0.037	−0.314	Lycopene	−0.022	0.179	−0.235
C18:0	0.910	−0.158	0.184	β-carotene	0.843	0.035	0.330
C18:1n9c	−0.648	0.607	0.152	Phenolics	0.058	−0.611	0.211
C18:2n6c	−0.187	−0.862	−0.127	DPPH	−0.243	−0.850	-0.025
C20:0	0.425	−0.226	−0.605	FRAP	−0.459	−0.223	0.171
C18:3n3	0.757	−0.283	0.034	Eigenvalue	15.350	7.967	4.728
C20:1	−0.700	−0.086	−0.577	Proportion of total variance (%)	36.546	18.970	11.258
C21:0	−0.597	−0.116	−0.478				
C22:1n9	0.799	−0.062	−0.398				

DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power.

ably due to higher contents of protein and ash (the parameters more correlated with PC3) in *Q. faginea* and *Q. suber*.

4. Conclusions

Overall, the *Quercus* species gave significantly different results in the analyzed parameters (except for the γ-tocopherol). Likewise, the difference among the studied acorn tissues were also significant in most cases, except for C20:0 and phenolics content. The kernels emerged as the main source of oil, energy, C18:2n6c, γ-tocopherol and total tocopherols, while the whole fruits presented the highest levels of protein, α-tocopherol and lycopene. These two components showed also similar values in C18:1n9c, MUFA and PUFA; the pericarps, on the other hand stood out for the high carbohydrates and β-carotene contents. From the comparison among species, *Q. faginea* gave the highest α-tocopherol, total tocopherols and lycopene contents; *Q. ilex* emerged as a particularly interesting source of oil, energy, C18:1n9c and MUFA; the components from *Q. nigra* stood out for the contents in carbohydrates, C18:2n6, MUFA and β-carotene; finally, *Q. suber* showed the highest protein levels. The best antioxidant effectiveness was obtained in the kernels extracts and in the components of *Q. nigra* (for DPPH scavenging activity) and *Q. faginea* (for FRAP assay). From the industrial application standpoint, these results might be useful for designing novel food and pharmaceutical products. Considering sustainability principles, the results could be useful in providing added-value to byproducts from industrial processing of acorns or the generated agricultural wastes.

Acknowledgements

The authors are grateful for the financial support to REQUIMTE/LAQV (UID/QUI/50006/2013 – POCI/01/0145/FEDER/007265) and CIMO (UID/AGR/00690/2013) from FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020. J.C.M. Barreira thanks FCT, POPH-QREN and FSE for his grant (SFRH/BPD/72802/2010).

References

Aguilera, J.F., Nieto, R., Rivera, M., Garcia, M.A., 2002. Amino acid availability and energy value of acorn in the Iberian pig. Livest. Prod. Sci. 77, 227–239.
Alves, R.C., Costa, A.S.G., Jerez, M., Casal, S., Sineiro, J., Núñez, M.J., Oliveira, M.B.P.P., 2010. Antiradical activity, phenolics profile and hydroxymethylfurfural in expresso coffee: influence of technological factors. J. Agric. Food Chem. 58, 12221–12229.
Amaral, J.S., Alves, M.R., Seabra, R.M., Oliveira, M.B.P.P., 2005. Vitamin E composition of walnuts (*Juglans regia* L.): a 3-year comparative study of different cultivars. J. Agric. Food Chem. 53, 5467–5472.
Amaral, J.S., Casal, S., Alves, M.R., Seabra, R.M., Oliveira, M.B.P.P., 2006. Tocopherol and tocotrienol content of hazelnut cultivars grown in Portugal. J. Agric. Food Chem. 54, 1329–1336.
AOAC, 2012. In Official methods of analysis of AOAC International, ed. W. Horwitz, Gaithersburg, USA, 19th ed.
Atwater, W.O., Benedict, F.G., 1902. Experiments on the Metabolism of Matter and Energy in the Human Body, 1898–1900. US Office of Experiment Stations Bulletin No. 109. Government Printing Office, Washington DC.
Baiano, A., 2014. Recovery of biomolecules from food wastes—a review. Molecules 19, 14821–14842.
Barreira, J.C.M., Ferreira, I.C.F.R., Oliveira, M.B.P.P., Pereira, J.A., 2008. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food Chem. 107, 1106–1113.
Barreira, J.C.M., Casal, S., Ferreira, I.C.F.R., Oliveira, M.B.P.P., Pereira, J.A., 2009a. Nutritional: fatty acid and triacylglycerol profiles of *Castanea sativa* Mill. cultivars: a compositional and chemometric approach. J. Agric. Food Chem. 57, 2836–2842.
Barreira, J.C.M., Alves, R.C., Casal, S., Ferreira, I.C.F.R., Oliveira, M.B.P.P., Pereira, J.A., 2009b. Vitamin E profile as a reliable authenticity discrimination factor between chestnut (*Castanea sativa* Mill.) cultivars. J. Agric. Food Chem. 57, 5524–5528.
Barreira, J.C.M., Casal, S., Ferreira, I.C.F.R., Peres, A.M., Pereira, J.A., Oliveira, M.B.P.P., 2012. Chemical characterization of chestnut cultivars from three consecutive years: chemometrics and contribution for authentication. Food Chem. Toxicol. 50, 2311–2317.
Benzie, I.F.F., Strain, J.J., 1999. Methods in enzymology. In: Lester, P. (Ed.), Methods in Enzymology, vol. 299. Academic Press, pp. 15–27.
Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free-radical method to evaluate antioxidant activity. LWT Food Sci. Technol. 28, 25–30.
Brizi, C., Santulli, C., Micucci, M., Budriesi, R., Chiarini, A., Aldinucci, C., Frosini, M., 2016. Neuroprotective effects of *Castanea sativa* Mill. bark extract in human neuroblastoma cells subjected to oxidative stress. J. Cell. Biochem. 117, 510–520.
Cantos, E., Espín, J.C., López-Bote, C., de La Hoz, L., Ordoñez, J.A., Tomás-Barberán, F.A., 2003. Phenolic compounds and fatty acids from acorns (*Quercus* spp.): the main dietary constituent of free-ranged Iberian pigs. J. Agric. Food Chem. 51, 6248–6255.
Charef, M., Mohamed, Y., Saidi, M., Stocker, P., 2008. Determination of the fatty acid composition of acorn (*Quercus*), *Pistacia lentiscus* seeds growing in Algeria. J. Am. Oil Chem. Soc. 85, 921–924.

Contini, M., Baccelloni, S., Massantini, R., Anelli, G., 2008. Extraction of natural antioxidants from hazelnuts (*Corylus avellana* L.) shell and skin wastes by long maceration at room temperature. Food Chem. 110, 659–669.
Costa, A.S.G., Alves, R.C., Vinha, A.F., Barreira, S.V.P., Nunes, M.A., Cunha, L.M., Oliveira, M.B.P.P., 2014. Optimization of antioxidants extraction from coffee silverskin, a roasting by-product, having in view a sustainable process. Ind. Crops Prod. 53, 350–357.
Custódio, L., Patarra, J., Alberício, F., Neng, N.R., Nogueira, J.M.F., Romano, A., 2015. Phenolic composition, antioxidant potential and in vitro inhibitory activity of leaves and acorns of *Quercus suber* on key enzymes relevant for hyperglycemia and Alzheimer's disease. Ind. Crops Prod. 64, 45–51.
Deforce, K., Bastiaens, J., Calster, H.V., Vanhoutte, S., 2009. Iron age acorns from Boezing (Belgium): the role of acorn consumption in prehistory. Arch. Korresp. 39, 381–392.
Gea-Izquierdo, G., Cañellas, I., Montero, G., 2006. Acorn production in Spanish holm oak woodlands. Invest. Agric. Sist. Rec. For. 15, 339–354.
Heleno, S.A., Martins, A., Queiroz, M.J.R.P., Ferreira, I.C.F.R., 2015. Bioactivity of phenolic acids: metabolites vs. parent compounds. Food Chem. 173, 501–513.
INE. Estatísticas Agrícolas, 2013. Instituto Nacional de Estatística IP., 2014. Portugal ISBN: 978-989-25-0265-6.
ISO 12966-2, 2011. Animal and vegetable fats and oils—Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids. www.iso.org/iso/catalogue.
Islam, S., Shahid, M., Mohammad, F., 2013. Perspectives for natural product based agents derived from industrial plants in textile applications—a review. J. Clean Prod. 57, 2–18.
Lands, B., 2014. Historical perspectives on the impact of n-3 and n-6 nutrients on health. Prog. Lipid Res. 55, 17–29.
Li, H., Parry, J.W., 2011. Phytochemical compositions, antioxidant properties, and colon cancer antiproliferation effects of Turkish and Oregon hazelnut. Food Nutr. Sci. 2, 1142–1149.
Liao, W., Lai, T., Chen, L., Fu, J., Sreenivasan, S.T., Yu, Z., Ren, J., 2016. Synthesis and characterization of a walnut peptides-zinc complex and its antiproliferative activity against human breast carcinoma cells through the induction of apoptosis. J. Agric. Food Chem. 64, 1509–1519.
Lopes, I., Bernardo-Gil, M., 2005. Characterisation of acorn oils extracted by hexane and by supercritical carbon dioxide. J. Lipid Sci.Technol. 107, 12–19.
Nagata, M., Yamashita, I., 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J. Jpn. Soc. Food Sci. Nutr. 39, 925–928.
Ostertag, L.M., O'Kennedy, N., Horgan, G.W., Kroon, P.A., Duthie, G.G., Roos, B., 2011. In vitro anti-platelet effects of simple plant-derived phenolic compounds are only found at high, non-physiological concentrations. Mol. Nutr. Food Res. 55, 1624–1636.
Özcan, T., 2007. Characterization of Turkish *Quercus* L. taxa based on fatty acid compositions of the acorns. J. Am. Oil Chem. Soc. 84, 653–662.
Pandey, K.B., Rizvi, S.I., 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxid. Med. Cell. Longev. 2, 270–278.
Petrović, S., Šobajić, S., Rakić, S., Tomić, A., Kukić, J., 2004. Investigation of kernel oils of *Quercus robur* and *Quercus cerris*. Chem. Nat. Comp. 40, 420–422.
Rakić, S., Povrenović, D., Tešević, V., Simíc, M., Maletić, R., 2006. Oak acorn, polyphenols and antioxidant activity in functional food. J. Food Eng. 74, 416–423.
Rakić, S., Petrović, S., Kukić, J., Jadranin, M., Tešević, V., Povrenović, D., Šiler-Marinković, S., 2007. Influence of thermal treatment on phenolic compounds and antioxidant properties of oak acorns from Serbia. Food Chem. 104, 830–834.
Rodrigues, F., Santos, J., Pimentel, F.B., Braga, N., Palmeira-de-Oliveira, A., Oliveira, M.B.P.P., 2015. Promising new applications of *Castanea sativa* shell: nutritional composition, antioxidant activity, amino acids and vitamin E profile. Food Funct. 6, 2854–2860.
Shahidi, F., Alasalvar, C., Liyana-Pathirana, C.M., 2007. Antioxidant phytochemicals in hazelnut kernel (*Corylus avellana* L.) and hazelnut byproducts. J. Agric. Food Chem. 55, 1212–1220.
Tejerina, D.S., García-Torres, M., Cabeza de Vaca, F.M., Vázquez, R.C., Cava, R., 2011. Acorns (*Quercus rotundifolia* Lam.) and grass as natural sources of antioxidants and fatty acids in the montanera feeding of Iberian pig: intra- and inter-annual variations. Food Chem. 124, 997–1004.
Vázquez, G., Fontenla, E., Santos, J., Freire, M.S., González-Álvarez, J., Antorrena, G., 2008. Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. Ind. Crops Prod. 28, 279–285.
Vasconcelos, M.C.B.M., Bennett, R.N., Rosa, E.A.S., Ferreira-Cardoso, J.V., 2010. Composition of European chestnut (*Castanea sativa* Mill.) and association with health effects: fresh and processed products. J. Agric. Food Chem. 90, 1578–1589.
Vinha, A.F., Alves, R.C., Barreira, S.V.P., Castro, A., Costa, A.S.G., Oliveira, M.B.P.P., 2014. Effect of peel and seed removal on the nutritional value and antioxidant activity of tomato (*Lycopersicon esculentum* L.) fruits. LWT Food Sci. Technol. 55, 197–202.