



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



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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.

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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).

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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 (Darmstadt, 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, Scotec 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) = $4 \times (\text{g protein}) + 3.75 \times (\text{g carbohydrate}) + 9 \times (\text{g fat})$. 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 SupelcosilTM 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,

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