Comprehensive REVIEWS in Food Science and Food Safety

A New Age for *Quercus* spp. Fruits: Review on Nutritional and Phytochemical Composition and Related Biological Activities of Acorns

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Abstract: The current global food system must adapt to the expected growth of world population (about 9 billion individuals by 2050). This adaptation will probably include an increased consumption of edible wild foods, due to their richness in micronutrients and bioactive compounds, besides providing a cost-effective and sustainable way of improving caloric food security. A striking example of such natural matrices is the *Quercus* genus, which has the additional advantage of being widespread throughout the Northern Hemisphere. In a traditional sense, *Quercus* fruits (acorns) were mainly used in animal feeding, despite their potentially important role on the rural economy. But this preconception is changing. In fact, their nutritional value, high contents in phytochemical compounds, biological activity (such as antioxidant, anticarcinogenic, and cardioprotective properties) and use in the treatment of specific diseases (such as atherosclerosis, diabetes, or Alzheimer's disease) have raised the interest in integrating acorns into the human diet. Accordingly, this comprehensive overview was designed to provide an evidence-based review of the literature, with the objective to achieve useful conclusions regarding the nutritional properties, methodologies of extraction, identification, and characterization of a wide variety of bioactive compounds and scientifically validated bioactivities in *Quercus* species worldwide. The industrial by-products from acorn oil extraction or flour production are also included. Data regarding the analytical techniques, individual compounds, and their bioactivities, are organized in tables. The reported data are discussed and directions for further investigations are suggested, highlighting the use of acorns in food, nutraceutical, and pharmaceutical applications.

Keywords: acorns, biological activity, nutritional composition, phytochemicals, *Quercus* spp., sustainability

Introduction

Quercus spp. (family Fagaceae) represent an important group of evergreen or deciduous trees from temperate and tropical climatic areas. The *Quercus* genus is comprised of around 450 species worldwide, which often differ in their flowering and fruiting dynamics and by maturation index (Tejerina and others 2011; Sánchez-Burgos and others 2013). These species produce a widely known fruit, commonly identified as acorn. Morphologically, an acorn is a 1-seeded nut, characterized by the absence of an endosperm and the presence of an achlorophyllous embryo (Figure 1). However, the acorns produced by different *Quercus* species present significant differences resulting from phylogenetic as well as ecological factors. Actually, there has been a number of attempts to correlate characteristics such as shape, size, and moisture content with ecological factors like climate and vegetation type, since the size of a fully developed acorn usually depends on its growth conditions (Pritchard and others 2004). An acorn size is also positively correlated with seedling survival rate under stress conditions (Aizen and Woodcock 1996).

Besides their association with physiological factors, acorns have also been studied for their nutritional profile and phytochemical contents, revealing great variability among species and sometimes even within the same species. The reported differences are often modulated by soil and climatic conditions, besides being influenced by the oak tree regeneration processes (Koenig and Knops 2002; Liebhold and others 2004). Even so, acorns are invariably considered as nutritionally rich products, justifying their use as secondary human foods (which are essentially the sources of carbohydrates, proteins and fat) or food ingredients for thousands of years wherever oak trees were found. In some specific cases, acorns were included in the human diet, specifically as flour (generally for bread production), or as a coffee substitute beverage (after a roasting process) (Rakić and others 2006, 2007). However, and despite their botanical availability, acorns are currently far from being as widely used as other common nuts (Rakić and others

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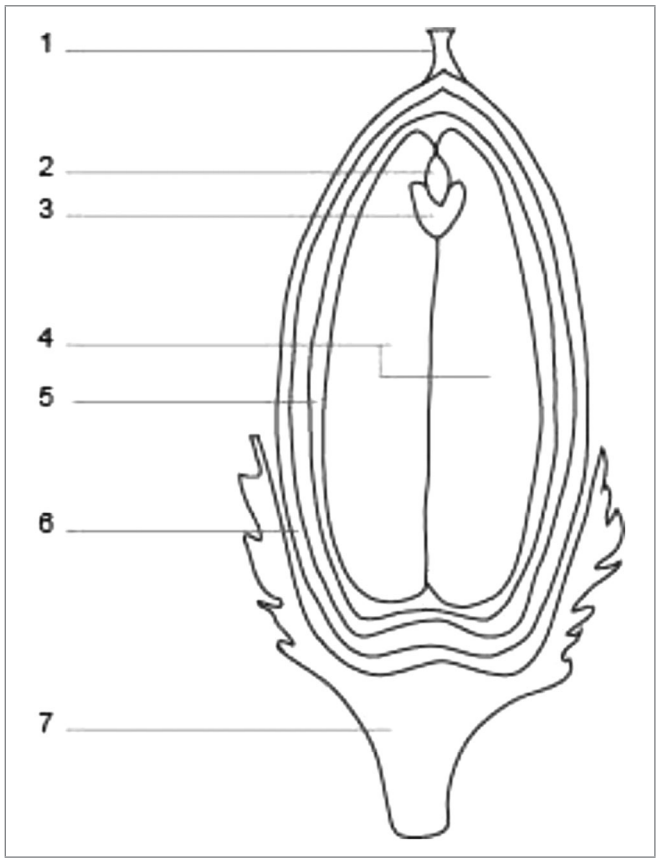


Figure 1–Acorn sketch highlighting the main morphological characters. 1 - remains of style; 2 - radicle; 3 - plumule; 4 - cotyledons; 5 - pericarp (fruit wall); 6 - seed coat; 7- cupule (with scales).

2007). Actually, before the last decade, acorns were mainly used for hog feeding, due to their high contents of carbohydrates, proteins, and lipids, or as raw material for acorn oil production. Even in a more traditional sense, acorns were mostly viewed as fodder or as a raw material against starvation (León-Camacho and others 2004; Lopes and Bernardo-Gil 2005).

Nevertheless, recent scientific reports have validated several advantageous characteristics of acorns, and it seems evident that we are on the brink of drastically increasing the use of this natural resource for various applications. From the point of view of their potential applications in human nutrition, acorn consumption falls into 3 categories: acorns as nuts (they resemble chestnuts), as flour (due to high starch contents), or as cooking oil (which presents high similarity with olive oil). In all of these cases, acorn processing usually includes peeling, roasting, or boiling, which might generate by-products (especially those resulting from acorn oil extraction or flour production) with additional potential applications (Deforce and others 2009).

Some attempts to include acorns in food products have already been made. Mixing acorn and wheat flours, for instance, proved to have advantageous rheological effects, since the incorporation of limited amounts of acorn flour increased bread volume and improved crumb characteristics (Korus and others 2015). However, the main interest in acorns derives from their plentiful phytochemical profile. These fruits have been described as containing high contents of phenolic compounds (Bettinger and others 1997; Bainbridge 2001; Rakić and others 2007), with more than 60 individual phenolics already identified (Saffarzadeh and others 1999; Cadahía and others 2001; Cantos and others 2003; Ferreira-Dias and others 2003; Andrenšek and others 2004; Rakić and others, 2006; Marquart and others 2007; Vanhessche and others 2007; Brossa 2009; Rocha-Guzmán and others 2009; Tejerina and others 2011; Kim and others 2012; Popović and others 2013), including ellagic acid and gallic acid derivatives (such as galloyl and hexahydroxydiphenoyl esters of glucose and tergallic *O*- or *C*-glucosides) and several flavonoids (Table 1). As reported, the presence of these types of compounds provide health benefits, which are mainly correlated to their high antioxidant activity, besides having important functions in decreasing the risk of cardiovascular and inflammatory illnesses, diabetes, cancer, microbial infection, human immunodeficiency virus (HIV) infection and other diseases (Jiang and Dusting 2003; Halliwell and others 2005; Lee and others 2005; Ullah and Khan 2008).

Acorns are also considered as good sources of fibers, proteins, and vitamins (mostly A and E) (Saffarzadeh and others 1999; Gea-Izquierdo and others 2006; Rosenberg 2008), mineral elements (Rakić and others 2006), and unsaturated fatty acids (Gea-Izquierdo and others 2006; Tejerina and others 2011). All these compounds, in addition to other classes such as sterols or aliphatic alcohols, are dealt with detail in the next sections, with particular focus on the phenolic compounds.

From all the above, it becomes obvious that acorns must be considered as functional foods or as alternative sources of several highly-valued food ingredients (Rakić and others 2006). Without doubt, acorns and their components are standing out for their growing relevance in the food industry, stimulating the search for new and/or undervalued components, with promising health benefits and potential disease-prevention properties. Acorn valorization perfectly fits into this future trend, as it additionally improves the sustainability of the agro-food chain by attaining new potential applications (Correia and others 2009). This new orientation will require additional research, particularly to establish novel business approaches. Likewise, there is a strong need for further studies to develop new health-promoting and competitive market products, such as improved acorn oils, acorn chips and crackers, acorn bread and muffins, or pickled acorns (Bainbridge 2001; Rashid and others 2014; Korus and others 2015).

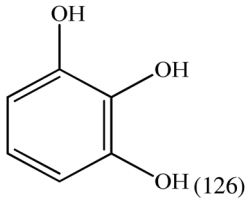
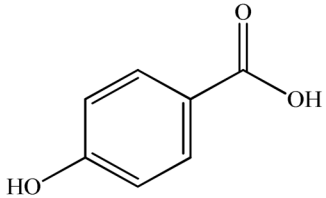
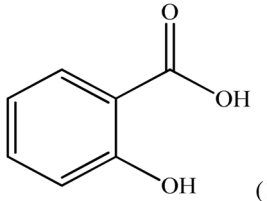
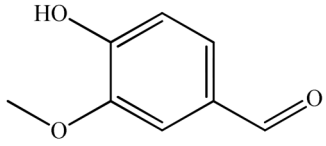
However, the functionality of acorns in food systems is not properly known. Each species presents specific characteristics that may influence functional performance of a given food product. Moreover, the palatability and the variability in potential health benefits are correlated with their phytochemical composition, factors that mandatorily justify future studies. Accordingly, our overview was intended to comprehensively process the scientific published information on different acorn species and their by-products (peel and cupule) to characterize their full nutritional potential and to identify phytochemical compounds with biological properties. Considering their nutraceutical, phytochemical, and bioactive potentials, the complete characterization of acorns and components may increase their value for further applications in the food and pharmaceutical industries.

Data regarding the analytical techniques, number, chemical structures (particularly for the phenolic compounds), and most relevant bioactivities are usually shown as tables.

Sustainability and Food Security

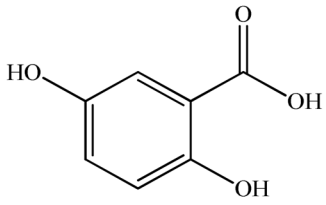
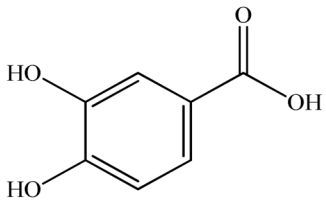
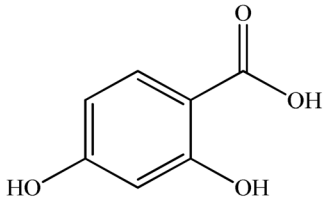
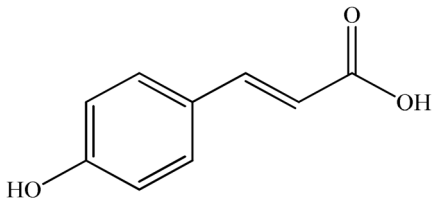
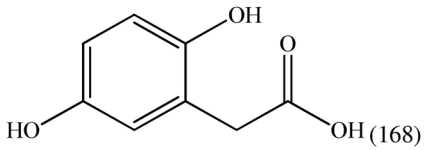
Sustainability is a multidimensional concept that emerged from the World Commission on Environment and Development (WCED 1987). With the growing demands of a global population expected to reach about 9 billion by 2050, it is necessary to know

Table 1—Individual compounds previously reported in acorns or related botanical parts from different *Quercus* species. In some cases, the represented structure corresponds to one of the possible isomers. The superscript numbers indicate the species and respective reference where each compound was reported.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Pyrogallol	 (126)	HPLC-DAD	*Q. acuta*, *Q. glauca*, *Q. myrsinaefolia*, *Q. phillyraeoides*, *Q. salicina*	Kim and others 2012
p-Hydroxybenzoic acid	 (138)	HPLC-DAD[1] HPLC-UV/Vis[2]	*Q. acuta*[1], *Q. glauca*[1], *Q. phillyraeoides*[1], *Q. resinosa*[2], *Q. salicina*[1]	[1]Kim and others 2012 [2]Rocha-Guzmán and others 2009
Salicylic acid	 (138)	HPLC-DAD[1] HPLC-UV/Vis[2]	*Q. acuta*[1], *Q. glauca*[1], *Q. myrsinaefolia*[1], *Q. phillyraeoides*[1], *Q. resinosa*[2], *Q. salicina*[1]	[1]Kim and others 2012 [2]Rocha-Guzmán and others 2009
Vanillin	 (152)	HPLC-DAD[1,3] HPLC-UV/Vis[2]	*Q. acuta*[1], *Q. alba*[3], *Q. faginea*[3], *Q. glauca*[1], *Q. myrsinaefolia*[1], *Q. petraea*[3], *Q. pyrenaica*[3], *Q. resinosa*[2], *Q. robur*[3], *Q. salicina*[1]	[1]Kim and others 2012 [2]Rocha-Guzmán and others 2009 [3]Cadahía and others 2001

(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Gentisic acid	 (154)	HPLC-DAD	*Q. acuta*, *Q. glauca*, *Q. phillyraeoides*, *Q. salicina*	Kim and others 2012
Protocatechuic acid	 (154)	HPLC-DAD[1] HPLC-UV/Vis[2]	*Q. acuta*[1], *Q. glauca*[1], *Q. myrsinaefolia*[1], *Q. phillyraeoides*[1], *Q. resinosa*[2], *Q. salicina*[1]	[1] Kim and others 2012 [2] Rocha-Guzmán and others 2009
β-Resorcylic acid	 (154)	HPLC-DAD	*Q. acuta*, *Q. glauca*, *Q. myrsinaefolia*, *Q. phillyraeoides*, *Q. salicina*	Kim and others 2012
p-Coumaric acid (*meta* and *ortho* isomers were also detected in some species)	 (164)	HPLC-DAD[1] HPLC-UV/Vis[2]	*Q. acuta*[1], *Q. glauca*[1], *Q. myrsinaefolia*[1], *Q. phillyraeoides*[1], *Q. resinosa*[2], *Q. salicina*[1]	[1] Kim and others 2012 [2] Rocha-Guzmán and others 2009
Homogentisic acid	 (168)	HPLC-DAD	*Q. acuta*, *Q. myrsinaefolia*, *Q. phillyraeoides*, *Q. salicina*	Kim and others 2012

(*Continued*)

Table 1–Continued.

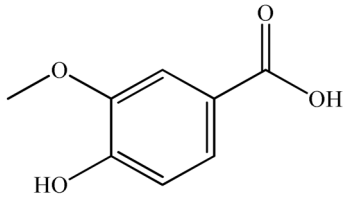
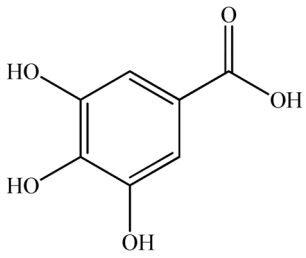
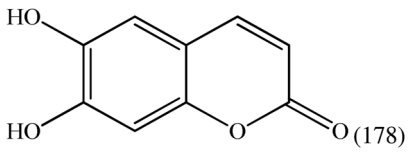
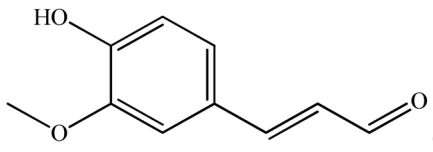
Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Vanillic acid	 (168)	HPLC-DAD[1,3] HPLC-UV/Vis[2]	*Q. acuta*[1], *Q. alba*[3], *Q. faginea*[3], *Q. glauca*[1], *Q. myrsinaefolia*[1], *Q. petraea*[3], *Q. phillyraeoides*[1], *Q. pyrenaica*[3], *Q. resinosa*[2], *Q. robur*[3], *Q. salicina*[1]	[1]Kim and others 2012; [3]Cadahía and others 2001
Gallic acid	 (170)	HPLC-DAD[1,4] HPLC-UV/Vis[2] HPLC-DAD-ESI-MS/MS[3]	*Q. acuta*[1], *Q. alba*[3], *Q. faginea*[3], *Q. glauca*[1], *Q. ilex*[4], *Q. myrsinaefolia*[1], *Q. petraea*[3], *Q. phillyraeoides*[1], *Q. pyrenaica*[3], *Q. robur*[3], *Q. resinosa*[2], *Q. rotundifolia*[4], *Q. salicina*[1], *Q. suber*[4]	[1]Kim and others 2012 [2]Rocha-Guzmán and others 2009 [3]Cadahía and others 2001 [4]Cantos and others 2003
Aesculetin	 (178)	HPLC-DAD	*Q. alba,* *Q. faginea,* *Q. petraea,* *Q. pyrenaica,* *Q. robur*	Cadahía and others 2001
Coniferylic aldehyde	 (178)	HPLC-DAD	*Q. alba, Q. faginea,* *Q. petraea,* *Q. pyrenaica,* *Q. robur*	Cadahía and others 2001

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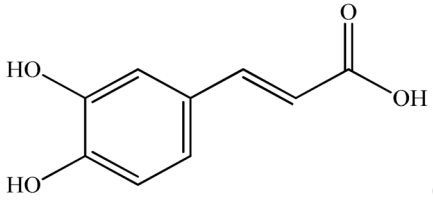
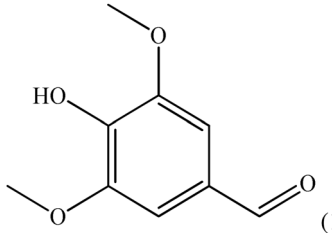
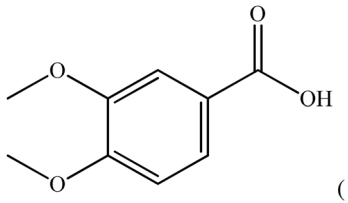
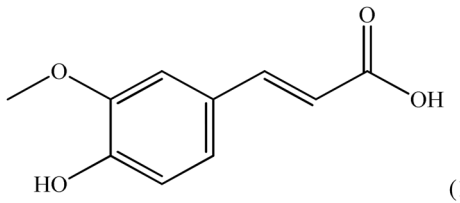
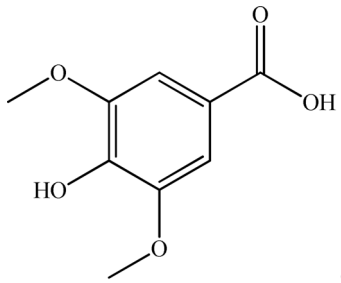
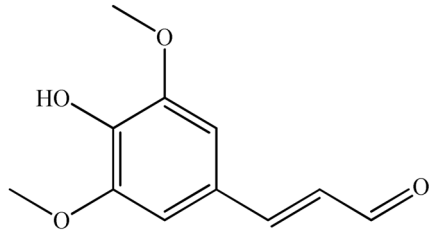
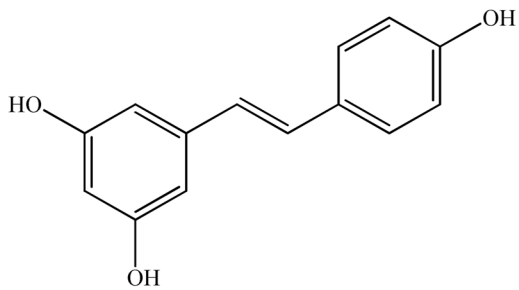
Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Caffeic acid	 (180)	HPLC-DAD[(1)] HPLC-UV/Vis[(2)]	*Q. acuta*[(1)], *Q. myrsinaefolia*[(1)], *Q. phillyraeoides*[(1)], *Q. resinosa*[(2)], *Q. salicina*[(1)]	[(1)] Kim and others 2012 [(2)] Rocha-Guzmán and others 2009
Syringic aldehyde	 (182)	HPLC-DAD	*Q. alba, Q. faginea,* *Q. petraea,* *Q. pyrenaica,* *Q. robur*	Cadahía and others 2001
Veratric acid	 (182)	HPLC-DAD	*Q. acuta, Q. glauca,* *Q. myrsinaefolia,* *Q. phillyraeoides,* *Q. salicina*	Kim and others 2012
Ferulic acid	 (194)	HPLC-DAD	*Q. acuta*[(1)], *Q. faginea*[(3)], *Q. glauca*[(1)], *Q. myrsinaefolia*[(1)], *Q. petraea*[(3)], *Q. phillyraeoides*[(1)], *Q. pyrenaica*[(3)], *Q. robur*[(3)], *Q. salicina*[(1)]	[(1)] Kim and others 2012; [(3)] Cadahía and others 2001

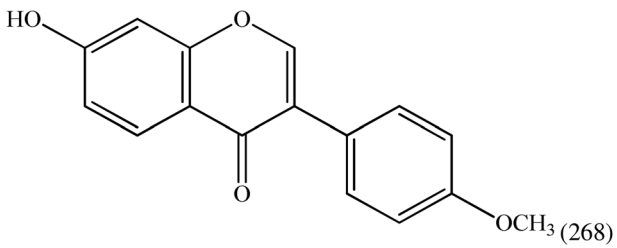
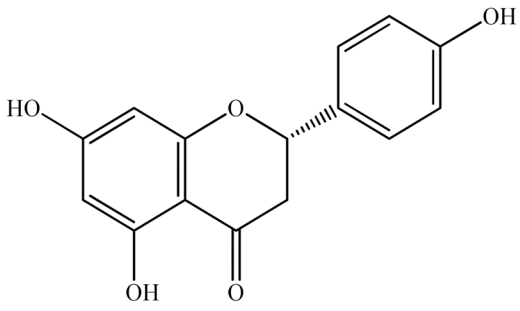
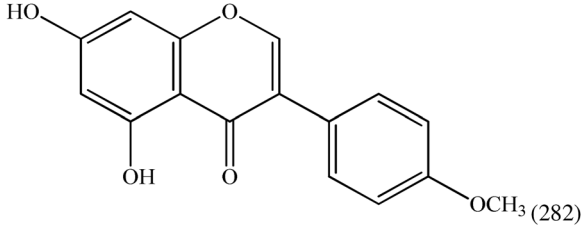
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Syringic acid	 (198)	HPLC-DAD[1,3] HPLC-UV/Vis[2]	*Q. acuta*[1], *Q. alba*[3], *Q. faginea*[3], *Q. glauca*[1], *Q. myrsinaefolia*[1], *Q. petraea*[3], *Q. phillyraeoides*[1], *Q. pyrenaica*[3], *Q. resinosa*[2], *Q. robur*[3], *Q. salicina*[1]	[1]Kim and others 2012 [2]Rocha-Guzmán and others 2009 [3]Cadahía and others 2001
Sinapic aldehyde	 (208)	HPLC-DAD	*Q. alba, Q. faginea,* *Q. petraea,* *Q. pyrenaica,* *Q. robur,*	Cadahía and others 2001
Resveratrol	 (228)	HPLC-DAD	*Q. acuta, Q. glauca,* *Q. myrsinaefolia,* *Q. phillyraeoides,* *Q. salicina*	Kim and others 2012

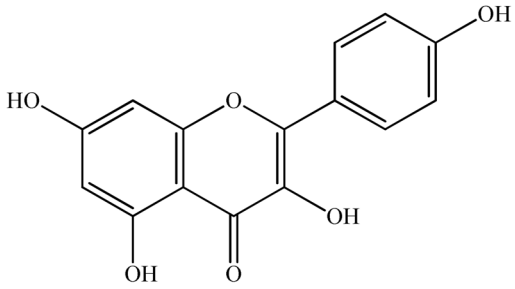
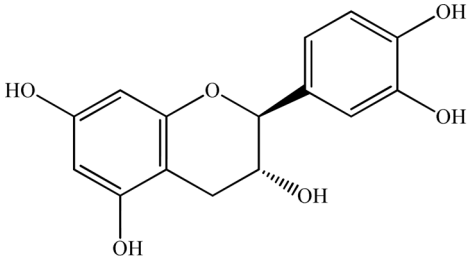
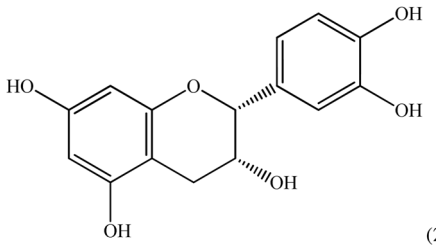
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Formononetin	 (268)	HPLC-DAD	*Q. acuta, Q. glauca, Q. myrsinaefolia, Q. phillyraeoides, Q. salicina*	Kim and others 2012
Naringenin	 (272)	HPLC-DAD	*Q. acuta, Q. glauca, Q. myrsinaefolia, Q. phillyraeoides, Q. salicina*	Kim and others 2012
Biochanin A	 (282)	HPLC-DAD	*Q. acuta, Q. glauca, Q. myrsinaefolia, Q. phillyraeoides, Q. salicina*	Kim and others 2012

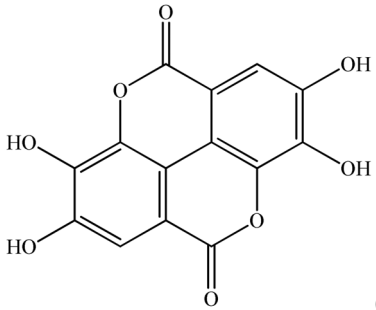
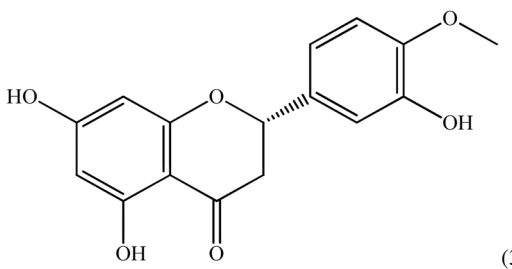
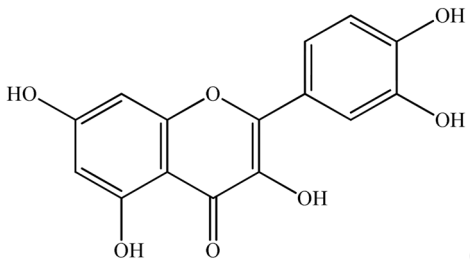
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Kaempferol	 (286)	HPLC-DAD	*Q. acuta, Q. glauca, Q. myrsinaefolia, Q. phillyraeoides, Q. salicina*	Kim and others 2012
Catechin	 (290)	HPLC-DAD[1] HPLC-UV/Vis[2]	*Q. acuta*[1], *Q. glauca*[1], *Q. myrsinaefolia*[1], *Q. phillyraeoides*[1], *Q. resinosa*[2], *Q. salicina*[1]	[1]Kim and others 2012 [2]Rocha-Guzmán and others 2009
Epicatechin	 (290)	HPLC-UV/Vis	*Q. resinosa*	Rocha-Guzmán and others 2009

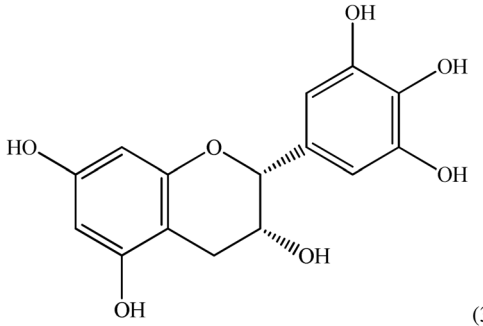
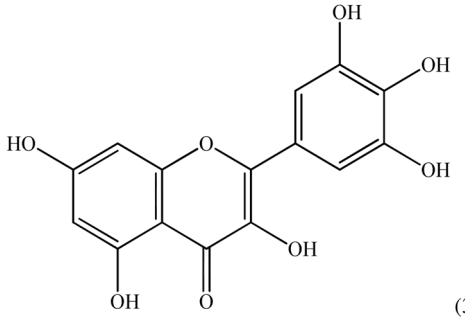
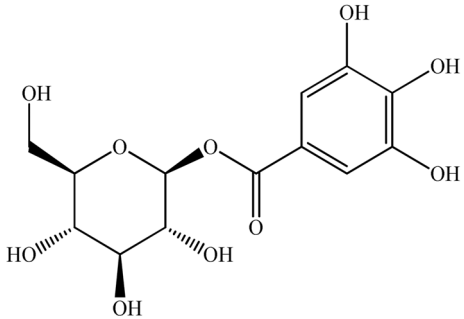
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Ellagic acid	 (302)	HPLC-DAD[3] HPLC-DAD-ESI-MS/MS[4]	*Q. faginea*[3], *Q. ilex*[4], *Q. petraea*[3], *Q. pyrenaica*[3], *Q. robur*[3], *Q. rotundifolia*[4], *Q. suber*[4]	[3] Cadahía and others 2001; [4] Cantos and others 2003
Hesperetin	 (302)	HPLC-DAD	*Q. acuta, Q. glauca,* *Q. myrsinaefolia,* *Q. phillyraeoides,* *Q. salicina*	Kim and others 2012
Quercetin	 (302)	HPLC-DAD	*Q. glauca,* *Q. myrsinaefolia,* *Q. phillyraeoides,* *Q. salicina*	Kim and others 2012

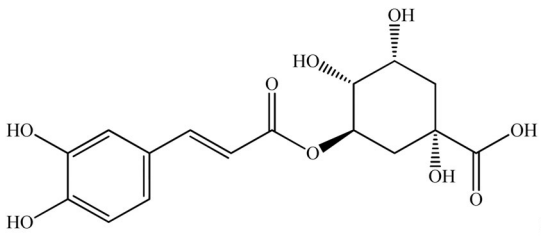
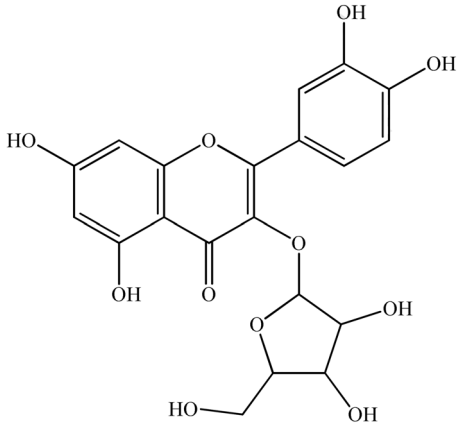
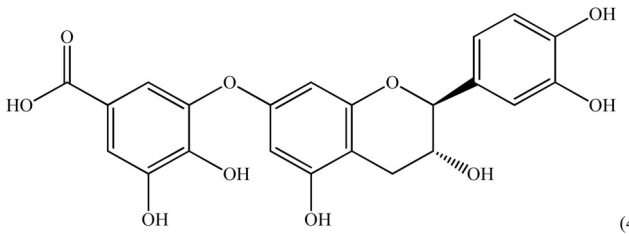
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Epigallocatechin	 (306)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Myricetin	 (318)	HPLC-DAD	*Q. acuta, Q. glauca, Q. myrsinaefolia, Q. phillyraeoides, Q. salicina*	Kim and others 2012
Galloyl glucoside	 (332)	HPLC-DAD-ESI-MS/MS	*Q. ilex*[4, 5], *Q. rotundifolia*[4], *Q. rubra*[5], *Q. suber*[4]	[4]Cantos and others 2003; [5]Brossa and others 2009

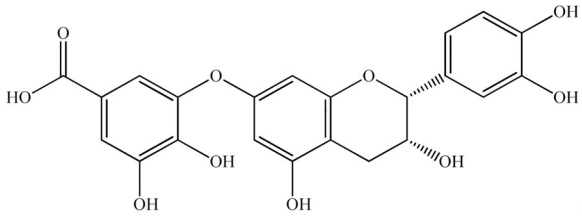
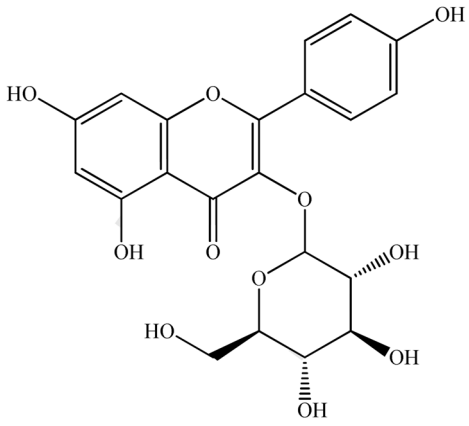
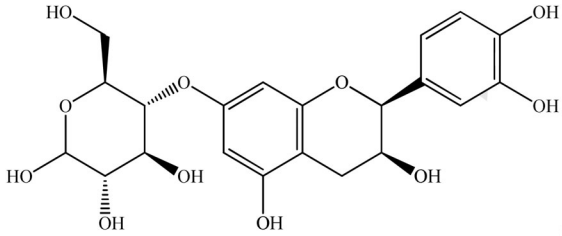
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Chlorogenic acid	 (354)	HPLC-DAD[1] HPLC-UV/Vis[2]	*Q. acuta*[1], *Q. myrsinaefolia*[1], *Q. phillyraeoides*[1], *Q. resinosa*[2], *Q. salicina*[1]	[1] Kim and others 2012 [2] Rocha-Guzmán and others 2009
Quercetin pentoside	 (434)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Catechin gallate	 (442)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009

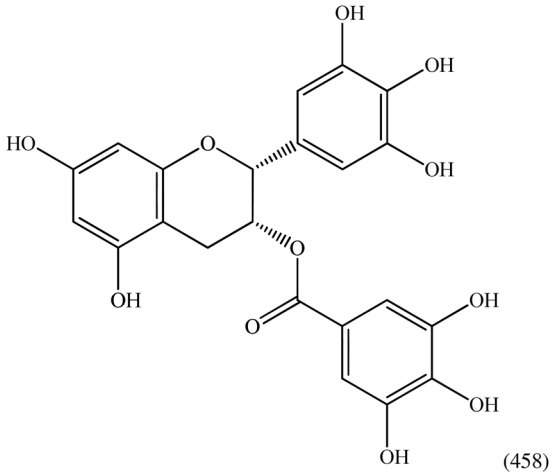
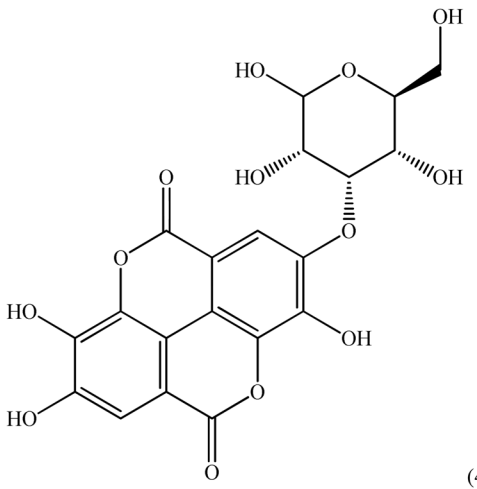
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Epicatechin gallate	 (442)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Kaempferol hexoside	 (448)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Epicatechin hexoside	 (452)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009

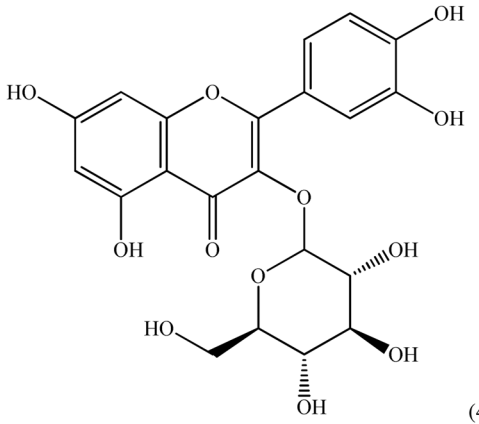
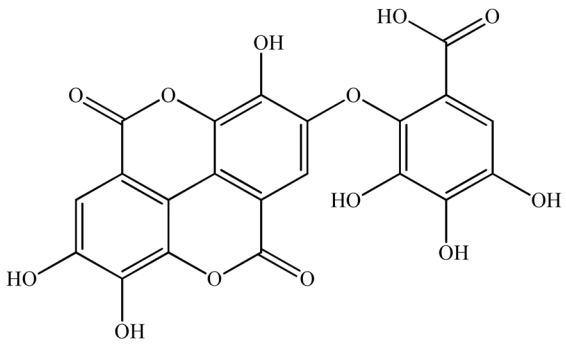
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Epigallocatechin gallate	 (458)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Ellagic acid glucoside	 (464)	HPLC-DAD-ESI-MS/MS	*Q. acutissima*[7], *Q. macrocarpa*[6], *Q. muhlenbergii*[6], *Q. virginiana*[7]	[6] Marquart and others 2007; [7] Vanhessche and others 2007

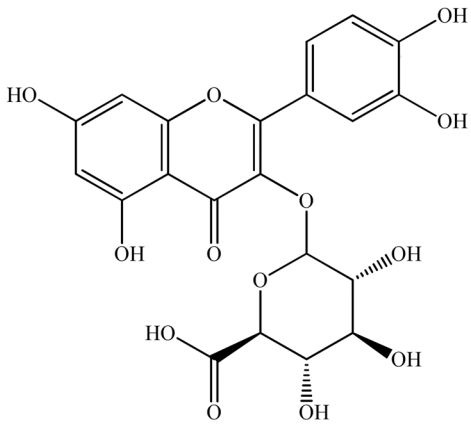
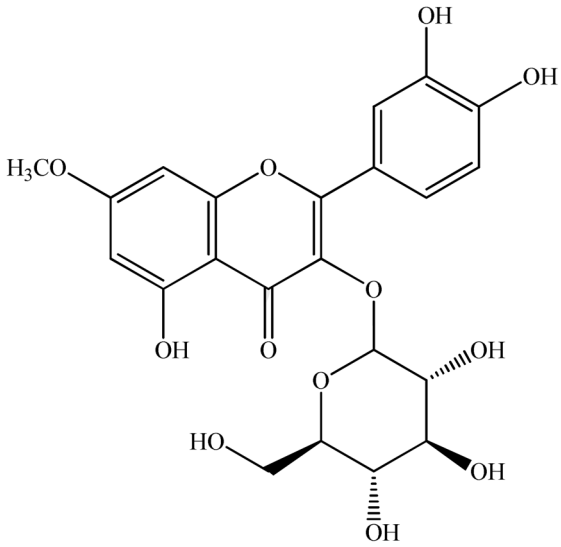
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Quercetin-3-*O*-glucoside (among other quercetin hexosides)	 (464)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Valoneic acid dilactone	 (470)	HPLC-DAD-ESI-MS/MS	*Q. ilex* *Q. rotundifolia* *Q. suber*	Cantos and others 2003

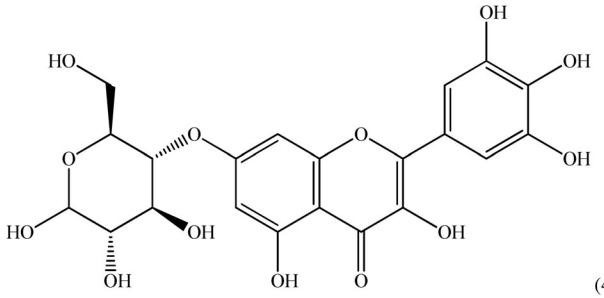
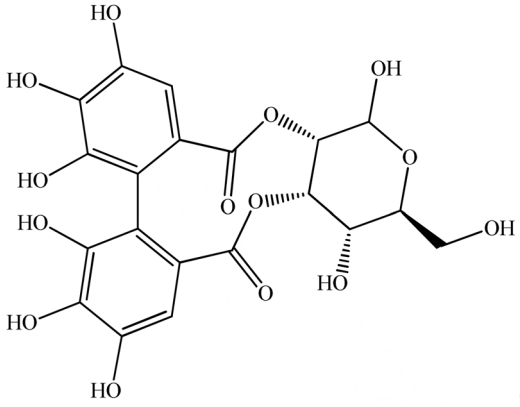
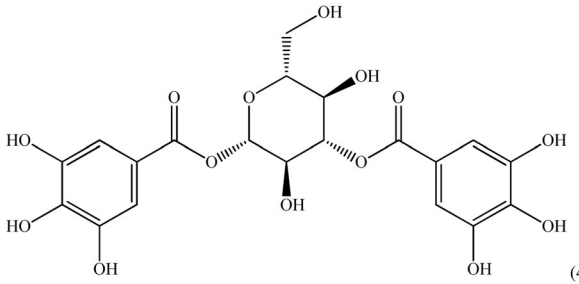
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Quercetin glucuronide	 (478)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Rhamnetin hexoside	 (478)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009

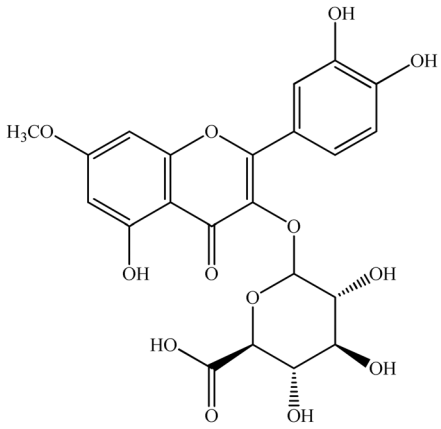
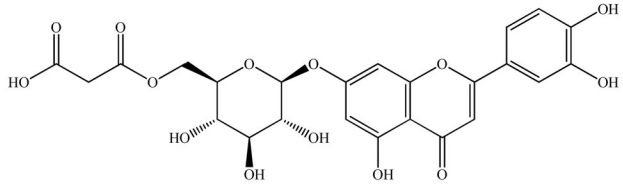
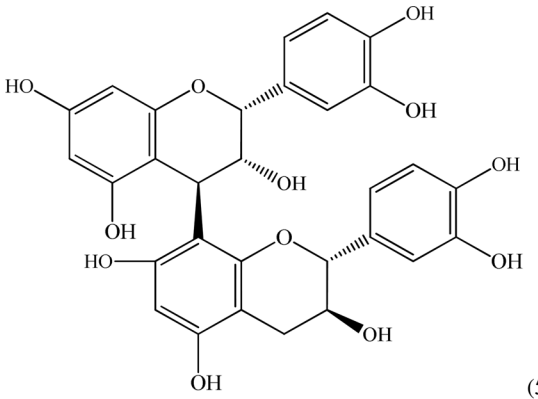
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Myricetin hexoside	 (480)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Hexahydroxydiphenoyl-glucoside	 (482)	HPLC-DAD-ESI-MS/MS	*Q. macrocarpa*, *Q. muhlenbergii*	Marquart and others 2007
Digalloyl glucoside	 (484)	HPLC-DAD-ESI-MS/MS	*Q. acutissima*[7], *Q. ilex*[4, 5], *Q. macrocarpa*[6], *Q. palustris*[6], *Q. rotundifolia*[4], *Q. rubra*[4], *Q. suber*[4]	[4]Cantos and others 2003; [5]Brossa and others 2009; [6]Marquart and others 2007; [7]Vanhessche and others 2007

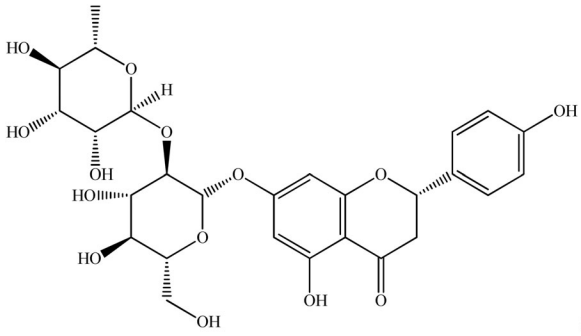
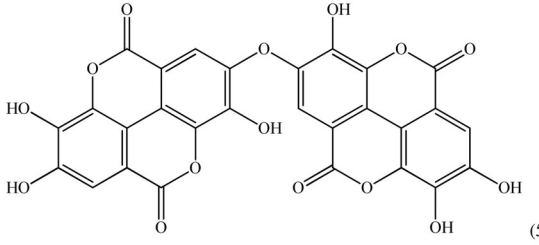
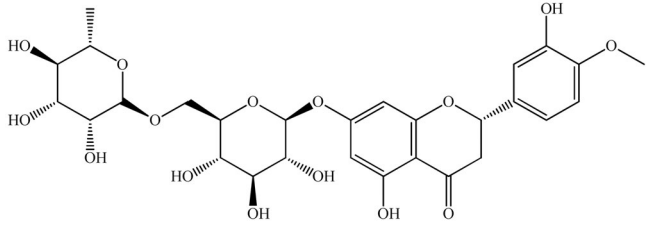
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Rhamnetin glucuronide	 (492)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Luteolin-malonyl-hexoside	 (534)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Procyanidin B1	 (578)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009

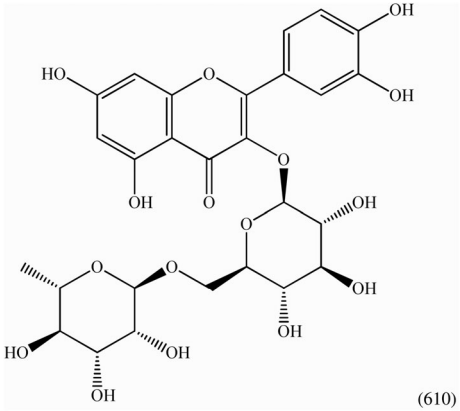
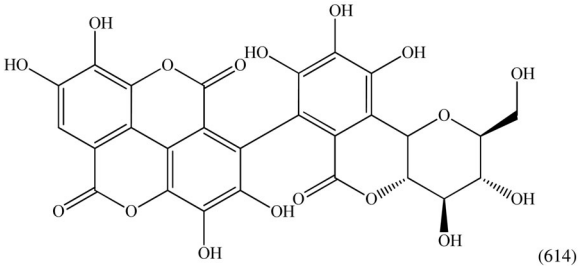
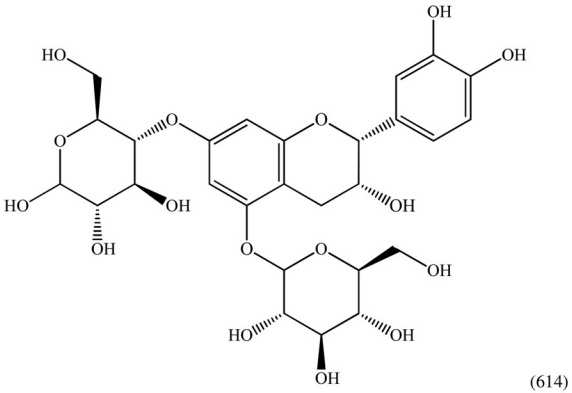
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Naringin	 (580)	HPLC-DAD	*Q. acuta*, *Q. myrsinaefolia*, *Q. phillyraeoides*, *Q. salicina*	Kim and others 2012
Ellagic acid dimer	 (586)	HPLC-DAD-ESI-MS/MS	*Q. ilex* *Q. rotundifolia* *Q. suber*	Cantos and others 2003
Hesperidin	 (610)	HPLC-DAD	*Q. acuta*, *Q. glauca*, *Q. myrsinaefolia*, *Q. phillyraeoides*, *Q. salicina*	Kim and others 2012

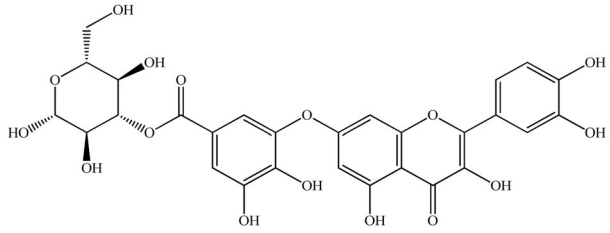
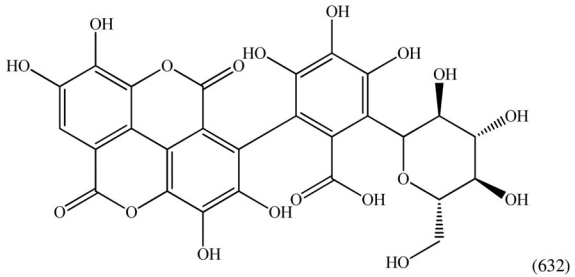
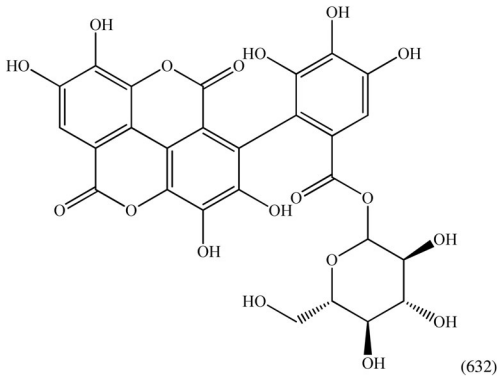
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Rutin	 (610)	HPLC-DAD	*Q. acuta, Q. glauca, Q. myrsinaefolia, Q. phillyraeoides, Q. salicina*	Kim and others 2012
Dehydrated tergallagic *C*-glucoside	 (614)	HPLC-DAD-ESI-MS/MS	*Q. ilex* *Q. rotundifolia* *Q. suber*	Cantos and others 2003
(Epi)catechin-dihexoside	 (614)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009

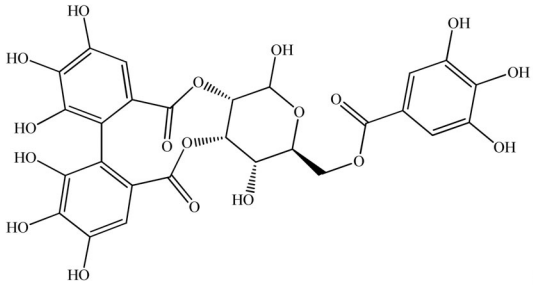
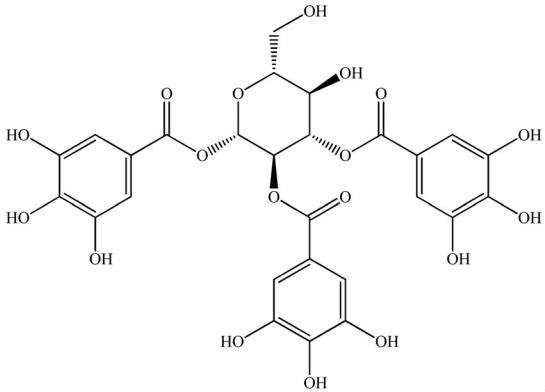
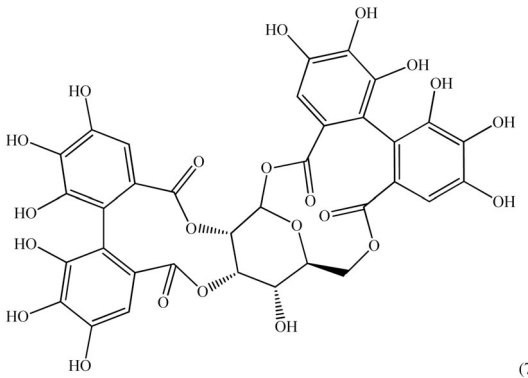
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Quercetin-(galloyl)-hexoside	 (616)	HPLC-DAD-ESI-MS/MS	*Q. ilex*	Brossa and others 2009
Tergallagic *C*-glucoside	 (632)	HPLC-DAD-ESI-MS/MS	*Q. ilex* *Q. rotundifolia* *Q. suber*	Cantos and others 2003
Tergallagic *O*-glucoside	 (632)	HPLC-DAD-ESI-MS/MS	*Q. ilex* *Q. rotundifolia* *Q. suber*	Cantos and others 2003

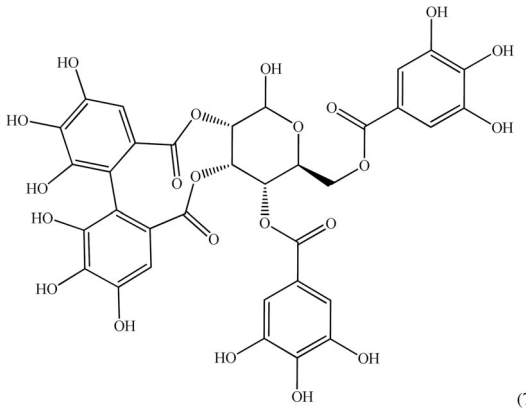
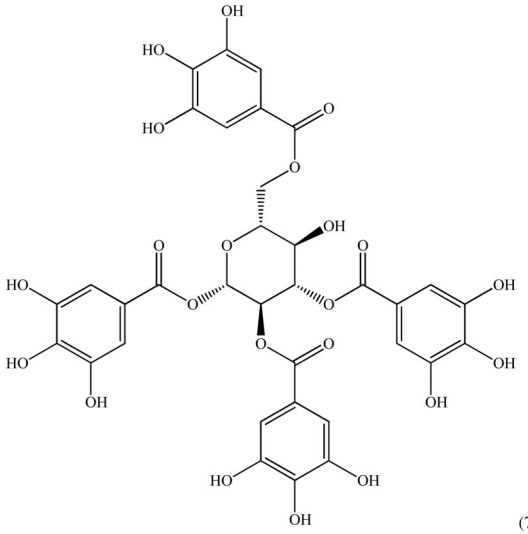
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Galloyl-hexahydroxydiphenoyl-glucoside	 (634)	HPLC-DAD-ESI-MS/MS	*Q. marilandica*[7], *Q. muhlenbergii*[6], *Q. palustris*[6], *Q. rubra*[7]	[6] Marquart and others 2007; [7] Vanhessche and others 2007
Trigalloyl glucoside	 (636)	HPLC-DAD-ESI-MS/MS	*Q. ilex*[4, 5], *Q. rotundifolia*[4], *Q. suber*[4],	[4] Cantos and others 2003; [5] Brossa and others 2009
Dihexahydroxydiphenoyl-glucoside	 (784)	HPLC-DAD-ESI-MS/MS	*Q. acutissima*[7], *Q. macrocarpa*[6], *Q. muhlenbergii*[6]	[6] Marquart and others 2007; [7] Vanhessche and others 2007

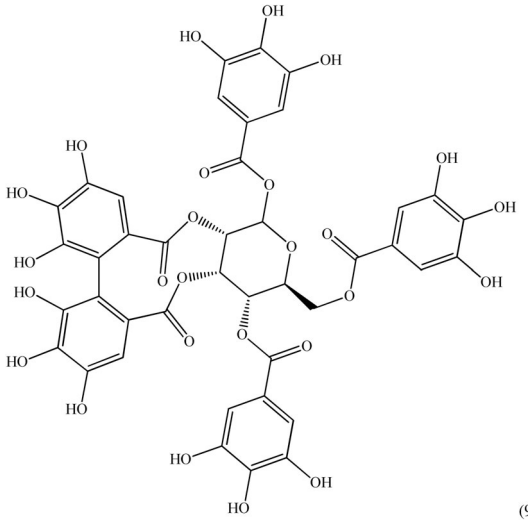
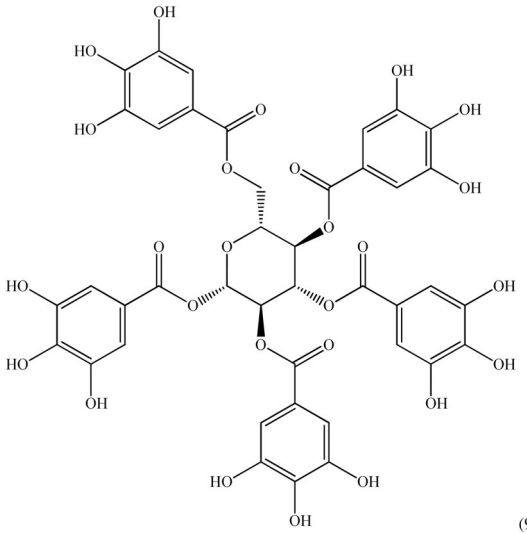
(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Digalloyl-hexahydroxydiphenoyl-glucoside	 (786)	HPLC-DAD-ESI-MS/MS	*Q. marilandica*[7], *Q. ilex*[4], *Q. palustris*[6], *Q. rotundifolia*[4], *Q. rubra*[6], *Q. suber*[4]	[4]Cantos and others 2003 [6]Marquart and others 2007 [7]Vanhessche and others 2007
Tetragalloyl glucoside	 (788)	HPLC-DAD-ESI-MS/MS	*Q. ilex* *Q. rotundifolia* *Q. suber*	Cantos and others 2003

(*Continued*)

Table 1–Continued.

Compound	Compound name and chemical structure (*m/z*)	Detection methodology	Species	Reference
Trigalloyl-hexahydrodiphenoyl-glucoside	 (938)	HPLC-DAD-ESI-MS/MS	*Q. ilex* *Q. rotundifolia* *Q. suber*	Cantos and others 2003
Pentagalloyl glucoside	 (940)	HPLC-DAD-ESI-MS/MS	*Q. ilex* *Q. rotundifolia* *Q. suber*	Cantos and others 2003

about the current global food system and to increase the consumption of edible wild foods, which are definitely underutilized (Spiertz 2010). This trend intends to guarantee that all populations may have access to safe and nutritious food, produced in an environmentally and socioculturally sustainable manner (Sibbel 2007; Vinceti and others 2013; Grunert and others 2014).

Furthermore, the ongoing climate change, the continuing depletion of fundamental resources, and the already emphasized expansion of the world population require the development of new agricultural systems (besides maintaining the current actual capacity) to ultimately assure a consistent food supply (Sibbel 2007).

These facts led to the development of a new concept of "food security" defined by the World Summit on Food Security (WSFS 2009) as a situation when the global population has access to safe and nutritious food, to satisfy food preferences and essential dietary requirements, promoting an active and healthy lifestyle (Oosterveer and others 2014).

Forest foods, including herbs and natural products collected from trees, can contribute to improve food security providing affordable and often highly nutritious foodstuffs (Jamnadass and others 2011; Powell and others 2011). Particularly, because the consumption of foods rich in micronutrients and bioactive compounds seems to be a viable, cost-effective, and sustainable way to improve life quality and diversify diets (Tontisirin and others 2002; Johns and Sthapit 2004; Vinceti and others 2013).

This sustainable approach is also committed to the valorization of food wastes. According to Gustavsson and others (2011), 1.3 billion tons of edible material are wasted every year in the world, which represents one-third of the global food production (European Commission 2015). Nonetheless, many studies have shown that underutilized food, food wastes, and also their by-products may provide bioactive compounds for pharmaceutical, cosmetic, food, and nonfood applications (Barreira and others 2010; Costa and others 2014; Braga and others 2015). Regarding the potentially marketable components present in underutilized food or food wastes and by-products, the aim is to explore high-value components such as proteins and essential amino acids, polysaccharides, fibers, flavor compounds (volatiles such as alcohols, aldehydes, or esters, among others), and phytochemicals as nutritionally and pharmacologically functional ingredients (Baiano 2014).

As examples of this type of approaches, the work of Baiano and others (2014) can be cited. They focused on the single and interactive effects of process variables on the yield and antioxidant concentrations of aqueous extracts of cauliflowers, celery, chicory, and asparagus obtained by conventional and microwave-assisted extraction. In a similar study, Costa and others (2014) have studied the optimization of antioxidant extractions from coffee silverskin. This same matrix was also used to evaluate the effects of its dietary fiber (obtained by alkaline extraction) on the quality and shelf-life of Iranian Barbari bread (Sourki and others 2013). Several other studies are currently in progress, including the evaluation and sustainable application of *Castanea sativa* by-products (Braga and others 2015).

Acorns and by-products (particularly those emerging from oil extraction and flour production) have an immense potential to be included in analogous studies and applications. According to the information described onward, it is intended to define these fruits as alternative functional foods, specifically considering their high nutritional value and richness in bioactive phytochemicals with biological action, contributing to the consumer well-being. It is expected that acorns and related botanical parts might be fully acknowledged as ingredients for novel food and pharmaceutical applications.

Acorns as an Alternative Food

There is an increasing interest in the study of wild food plants to validate their consumption as an alternative to other agricultural fruits or as a new ingredient for the food industry. This current research trend has been gradually pointing out acorns as a valuable resource.

Acorns were typically acknowledged for their high importance to the rural economy as components of animal feeding; however, their nutritional value and high phytochemical contents have raised the interest of many researchers looking for undervalued food to be integrated in the human diet. In addition, an increase in their consumption can have a positive impact at social and economic levels (Rosenberg 2008).

In general, these fruits are described as a new "healthy food," containing about 48% to 50% starch, 2% to 5% proteins, and generally a low fat content, presenting a higher nutritional value than cereals (Aguilera and others 2002; Özcan 2006; Rababah and others 2008; Deforce and others 2009). Their contents in starch and other carbohydrates, as well as fibers, proteins, and vitamins (mostly A and E), make them feasible to be used as an important source of dietary energy (Saffarzadeh and others 1999; Gea-Izquierdo and others 2006; Rosenberg 2008). Acorn starch, in particular, presents high paste consistency, allowing its use as a food ingredient, especially as thickening and stabilizing agents. In fact, interest in new sources of starch has been increased to enforce industrial applications. For this reason, acorns could be a promising ingredient for the food industry with high potential for commercial use (Correia and others 2013).

Nevertheless, their profile of bioactive compounds might be modulated by genetic and physiological aspects (such as the maturation degree), but also by several extrinsic factors, such as soil composition, climate, and geographic origin (Tejerina and others 2011). Therefore, these aspects should be considered when comparing the chemical compositions of acorns. Afazal-Raffi and others (1992), for instance, studied 2 species of *Quercus* (*Q. ilex* and *Q. rotundifolia*) acorns collected in Spain and Italy, verifying that Spanish acorns have higher protein and fat contents. In a similar study, acorns from *Q. rotundifolia* and *Q. suber* collected in the same geographic area (Spain) presented comparable protein contents (Aguilera and others 2002). Likewise, the fat content may vary from percentages as low as 2% up to a maximum value of 30% (Ofcarcik and others, 1971; Özcan 2007; Rababah and others 2008).

Besides the phylogenetic variability, climate and soil composition (including microbiota) are also relevant because chemical compounds and minerals may produce considerable changes in the sensory and nutritional attributes. These data were collected by Cañelas and others (2003), who analyzed the kernel (cotyledons) and seed coats of 4 acorn species (*Q. suber*, *Q. ilex*, *Q. faginea* and *Q. pyrenaica*) collected during different seasons of the year (summer and autumn). The authors found differences in protein and fat contents in different seasons, wherein the highest levels of protein and fat were obtained in spring and in autumn, respectively. Despite these changes, the fiber content presented similar values during all seasons (25% to 35%).

The quantities of mineral elements in acorns are also noteworthy. Rakić and others (2006), for instance, described considerable amounts of Fe, Cu, Zn, and Mn, besides Ca, Mg, P, and K in lower levels, in *Q. robur* acorn samples.

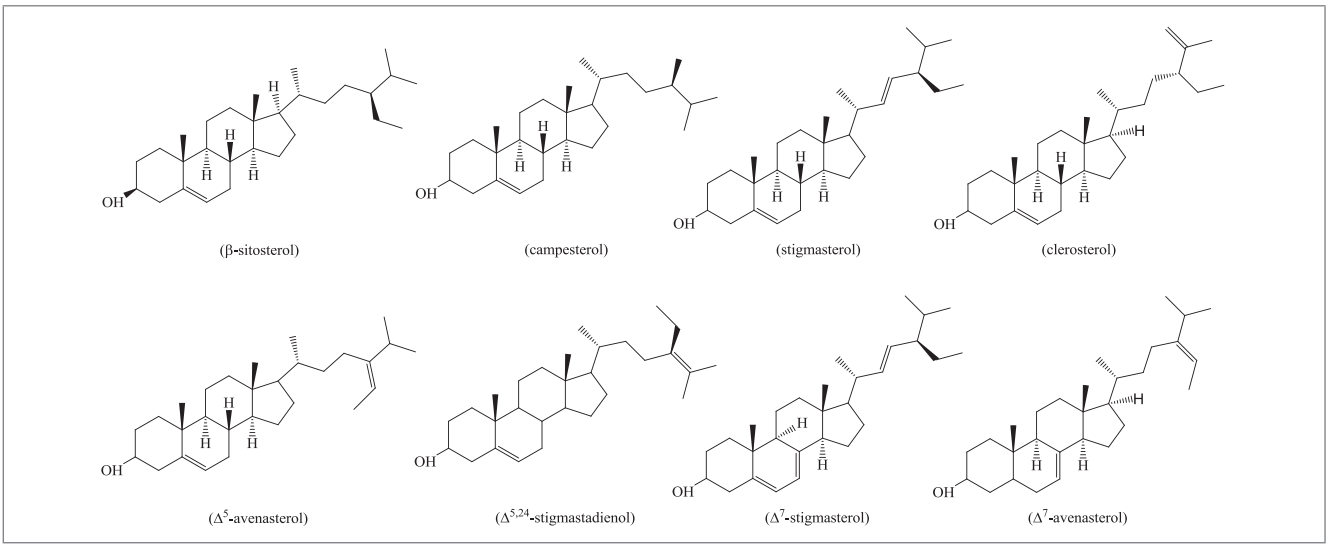


Figure 2–Chemical structures of the main sterols found in acorns.

Among the lipophilic compounds (which will be characterized with higher detail in the section dedicated to acorn oil) oleic, palmitic, and linoleic acids were the major fatty acids described in acorns (Gea-Izquierdo and others 2006; Tejerina and others 2011). Besides the potential effects of their direct consumption, the inclusion of acorns in animal feeding might improve the quality of their meat. Cantos and others (2003), for instance, suggested that higher quantities of acorns fed to Iberian pigs contributed greatly to improve the quality of their adipose, hepatic, and muscle tissues, thereby producing a "healthier" type of meat, with a lower rate of saturated fatty acids.

Vitamin E (mainly α- and γ-tocopherol) was also described in acorn (Gea-Izquierdo and others 2006). In general, γ-tocopherol is the most abundant vitamer, reaching levels 4.6- to 8.7-fold higher than those detected for α-tocopherol (Tejerina and others 2011; Rabhi and others 2016). This is a very interesting nutritional point, considering that γ-tocopherol is the most abundant isoform of vitamin E in the diet of some populations, such as it is verified in North America, due to the high consumption of soybean and corn oils (Brigelius-Flohé and Traber 1999).

Acorns are also an excellent source of provitamin A, since it has been reported that a small amount of acorns would guarantee the recommended daily requirements of vitamin A, which might be a great advantage in areas (particularly in some African and Southeast Asian low-income countries) where vitamin A deficiency is a common problem (Bainbridge 2001).

Equally relevant are the detected levels of sterols (among which β-sitosterol was the major compound, representing more than 90% of sterols) and aliphatic alcohols (especially tetracosanol) (Rabhi and others 2016). The values reported for sterols were inclusively higher than those obtained in almond, soybean, olive, pistachio, and pine oils, despite being in the same range as those reported for sesame and corn oils (Abidi 2001; Phillips and other 2005; Nasri and others 2007).

These nutritional indicators demonstrate that acorns have great potential as high-value nutraceuticals for dietary supplements or as functional foods, showing the importance of developing new marketable alternatives to their commercialization and valorization.

Acorn oil

Due to the recent development in agricultural and genetic engineering fields, new wild crops are being used to obtain edible oils. Acorns are considered an edible fruit in several Mediterranean countries providing desirable characteristics to be used in cooking procedures, cosmetics, and in folk medicine for the treatment of burns and injuries (Bainbridge 2001; Rosenberg 2008; Al-Rousan and others 2013). Several studies have reported that the oil content of white species of *Quercus* did not exceed 12% (Cantos and others 2003; Özcan 2007; Rababah and others 2008). However, Ofcarcik and others (1971) reported higher oil contents (about 30%) in black and red acorn species. In addition, acorn oil presents good nutritional quality, and its flavor is similar to that of olive oil (Bainbridge 2001; Özcan 2007; Al-Rousan and others 2013). Other characteristics that make acorn oil comparable to olive oil are the similarity in color, refractive index, UV extinction coefficient, and saponification and iodine values. Charef and others (2008) also reported that the fatty acid composition of acorn oil from *Q. suber* and *Q. ilex* is similar to that of *Pistacia lentiscus* oil and other edible vegetable oils such as those obtained from sunflower, peanut, cotton, olive, and avocado.

Fatty acid composition and their percentage in the β-position were also described (Lopes and Bernardo-Gil, 2005; Al-Rousan and others 2013). Nonetheless, the fatty acid profiles of acorn oils obtained from different *Quercus* species showed a high variation in the percentages of saturated, monounsaturated, and polyunsaturated fatty acids. As described previously, acorns are a natural source of neutral oleic acid (Estévez and others 2004) and contain high amounts of α-linolenic acid (Petrović and others 2004; Karolyi and others 2007), which is important in eicosanoid synthesis, promoting the decrease of blood serum triglycerides and the increase of HDL-cholesterol levels. In addition to the already mentioned advantageous effects of unsaturated fatty acids, the differences in their percentages may be useful as a chemical fingerprint to differentiate *Quercus* species.

The composition of sterols is also important. The *Quercus* genus is generally characterized by elevated percentages of β-sitosterol, besides minor amounts of campesterol, stigmasterol, clerosterol, Δ^5-avenasterol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol, and Δ^7-avenasterol (Figure 2) (Rabhi and others 2016). Phytosterols, either

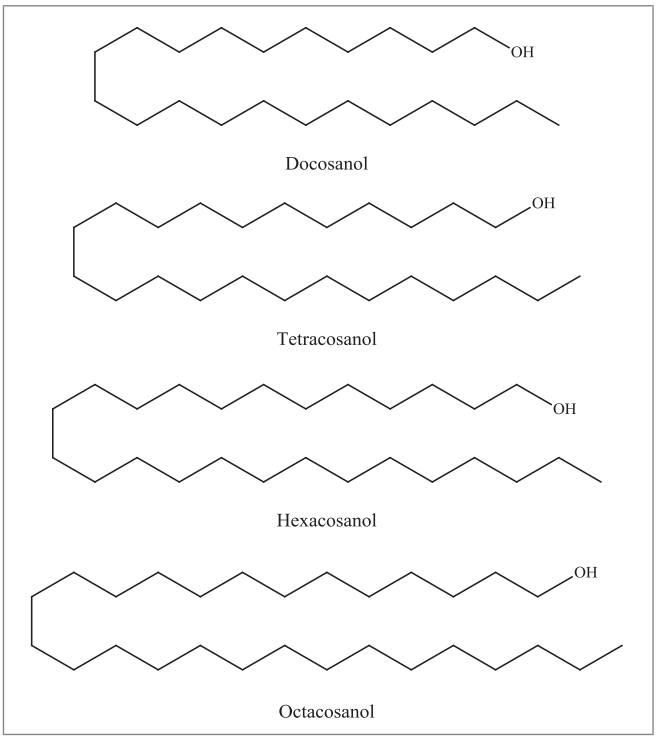


Figure 3–Chemical structures of the main aliphatic alcohols found in acorns.

consumed through the daily diet or as food additive are important to reduce the cholesterol levels in the blood (Ostlund and Lin 2006), besides being biologically active molecules in the prevention of several diseases (Barreira and Ferreira 2015).

Likewise, acorns were reported for their relevant levels of aliphatic alcohols. Tetracosanol was the most abundant compound of this class, but significant percentages of docosanol, hexacosanol, and octacosanol were also found (Figure 3) (Rabhi and others 2016). The presence of aliphatic alcohols in acorns oil might have industrial relevance. In fact, these compounds are used as emulsifiers, emollients, and thickeners in food and personal care products. In addition, aliphatic alcohols were also reported for their antimicrobial and antitumor activities (Volin 2001; Hilmarsson and others 2007).

Furthermore, data on chemical composition of acorn species are being developed in order to find new applications, useful for educational purposes, for compiling local food composition tables, and also to improve the sustainability concept of acorn production and uses (Léon-Camacho and others 2004; Rosenberg 2008; Pinna 2013; Korus and others 2015).

Acorn flour

Nowadays, different plant sources, including seeds, tubers, and fruit pulps are being used as an alternative raw material for flour production, or as an ingredient in food products, such as bread and pastry (Rashid and others 2014; Korus and others 2015).

The grinding of acorns into flour can be done with fresh or dehydrated fruits, but the preservation of acorn flour obtained from fresh fruits might be difficult, because it has a high content of moisture (about 18%) (Correia and others 2009).

In general, the edible use of flours from different sources depends greatly on their physicochemical and functional properties. Starch-based materials have received considerable interest in the past few years due to their biodegradability and low cost. As previously stated, starch is the main component of acorns, usually constituting more than 50% of the kernel (Rababah and others 2008).

According to Kaur and Singh (2007), functionality is any property of a food ingredient, without considering its nutritional value, which conveys an advantageous effect upon its utilization. Viscosity, water-binding ability, emulsification, and foaming capabilities are some of those properties. Also, the functionality of flours is related to chemical components determined by phylogenetic factors, postharvest conditions, and soil and climate conditions (Correia and Beirão-da-Costa 2011). Kinsella (1979) and Prinyawiwatkul and others (1997) emphasized that functional properties of flours are also controlled by the physicochemical characteristics of proteins and starch constituents. Several studies have reported that acorn flour is rich in fiber, besides being a good source of minerals (such as P, K, Ca, and Mg), which are not available in wheat flour (Rakić and others 2006; Jawarmeh and others 2013; Rashid and others 2014).

For the development of new flour products, their physical and chemical properties need to be evaluated. This is particularly important because there are several new potential applications for acorn flour. An example is the significant importance of acorn flour in the production of bakery products for consumers with celiac disease, which requires the use of preselected raw materials free of specific proteins (Korus and others 2015; Witczak and others 2016). The application of maize, rice, several types of millet and sorghum, or pseudocereals like buckwheat, amaranth, and quinoa has already been described (Torbica and others 2010; Matos and Rosell 2013; Pongjaruvat and others 2014). These flours may significantly increase nutritional value of bakery products, particularly by adding protein, essential amino acids, vitamins, macro- and micro-elements and dietary fiber, especially if the formulations are based on starch (Morais and others 2013; O'Shea and others 2014; Matos and Rosell 2015). Moreover, the use of acorn starch may also be considered in many other industrial applications beyond the food industry, like in the cases of paper, plastics, textile, pharmaceutical, and cosmetic industries (Rodrigues and Emeje 2012).

The development of these new products using acorn flour allows designating them as a functional food, since they may be seen as a good source of biologically active compounds (Özcan and Baycu 2005; Rakić and others 2006). However, and despite the fact that acorn flour presents good functional properties and sensory quality, which may be important factors for its acceptability by consumers, the available information on its nutrients and the chemical composition of acorns is far from being exhaustive. Accordingly, more research should be carried out to achieve a comprehensive characterization of this raw material, thereby boosting its potential applications (Correia and Beirão-da-Costa 2011).

Acorn toxicity

The acorns from specific *Quercus* species, particularly when consumed without processing (raw), seem to present some toxicity to chickens, rabbits, horses, and goats (Martinson and others 2007). This toxicity has been attributed to their high amounts of tannins, mostly the hydrolyzable forms, which can be metabolized to pyrogallol and gallic acid; their contents' are significantly higher in immature acorns. Spier and others (1987) observed renal damage and gastroenteritis in cattle after ingestion of different parts of oak trees, including leaves, buds, and acorns. Also, Plumlee and others (1998) and, more recently, Pérez and others (2011)

considered that hydrolyzable tannins and volatile phenols in oak leaves, buds, and acorns were responsible for those clinical signs. It is important to note that the mechanism of toxicity in acorns is not fully understood, but many studies have suggested that tannins and their metabolites may cause negative effects, due to binding to protein (including salivary proteins) and endothelial cells (indirectly complexing microbial proteins and interfering with the gastrointestinal microbial flora) (Smith and others 2015).

The sensitivity to acorn toxicity appears to vary between species and individuals. Herbivores may be more susceptible than other mammalian species. However, acorn exposure has been well described as a cause of pathology in some mammalian species, causing ulceration of the mouth, esophagus, and rumen, with anorexia and progression to hemorrhagic diarrhea. In ruminants, for example, gastroenteritis and nephrotoxicity caused by acorns is well-reported. On the other hand, pigs appear to develop protection when exposed to oak leaves or acorns in the diet, by increasing the production of tannin-binding salivary proteins (Smith and others 2015). Despite the importance of the topic, there is no published study that has reported acorn toxicity in humans.

Acorn as a source of bioactive compounds

As previously stated, acorns from different species present significant differences in their chemical composition, mainly due to the high variability within the *Quercus* genus. Nevertheless, some of their biological activity indicators, such as those regarding antioxidant compounds, present some similarity independently of the species, which might be interpreted as conveying many benefits regarding the easiness of predicting the effects of acorn consumption, as well as in defining their potential industrial applications (Rakić and others 2006; Silva and others 2008).

Extraction methodologies, chemical constituents and biological effects

In the last several decades, studies regarding the extraction of phytochemicals from natural products have attracted special interest. However, it is generally known that when natural compounds with biological properties are combined, several interactions may occur, causing changes in the expected outcomes, thereby producing synergistic, antagonistic, or additive effects. Several modern pharmacological tools have been employed to investigate the phytochemical composition and to validate different assays to evaluate the bioactivity of the of a wide variety of natural products (Lapornik and others 2005; Caridi and others 2007; Naimaa and others 2015).

Two of the most common extraction solvents are acidified methanol and ethanol (Kapasakalidis and others 2006). Nevertheless, several other solvents have been described in literature, regarding their phytochemical characterization and the further evaluation of their biological activity (Table 2). With no doubt, the phenolic compounds stand out as the phytochemicals with the highest number of reported studies on acorns (therefore, this chemical group is particularly highlighted in the next subsection). Likewise, the antioxidant and the antimicrobial activities gather the highest number of dedicated studies. However, there is a considerable number of publications dedicated to other chemical constituents and their bioactivities (Table 2).

Lopes and Bernardo-Gil (2005), for instance, have characterized fatty acids, triacylglycerols, sterols, tocopherols, and phospholipids contents of *Q. rotundifolia* and *Q. suber* oil extracted with *n*-hexane by Soxhlet and by supercritical carbon dioxide extractions. Few differences were observed in the contents of the main fatty acids (oleic, linoleic, and palmitic acids), triacylglycerols, and sterols (mainly β-sitosterol) present in the oils extracted by the different procedures. Furthermore, supercritical fluid extraction from plant material has become a very popular process due to its advantages over traditional extraction techniques, mainly for being a flexible process owing the possibility of continuous modulation of solvent selectivity by inducing changes in its density (achieved through pressure and temperature alterations), but also because it does not use polluting organic solvents (Reverchon and De Marco 2006). However, the supercritical carbon dioxide extraction did not yield the phospholipids fraction in both species, whereas *n*-hexane solvent was able to extract these compounds (Lopes and Bernardo-Gil 2005). Considering this limitation, future supercritical carbon dioxide experiments of polar compounds extraction may benefit from using a polar modifier (cosolvent) together with the supercritical CO_2 to enhance the solubility (Oman and others 2013).

As previously indicated, oleic, palmitic, and linoleic acids are the major fatty acids in acorns (Gea-Izquierdo and others 2006; Tejerina and others 2011). The presence of elevated percentages of oleic and linoleic acids is always noteworthy, considering the potential therapeutic human health benefits of *n*-3 and *n*-6 polyunsaturated fatty acids (PUFAs). Some of their main effects are associated with modulation of the immune system, particularly in reducing the action of inflammatory compounds (Darlington and Stone 2001) and with the decrease of cardiovascular disease risk, besides contributing to decrease the levels of glucose, low-density lipoprotein-cholesterol, fibrinogen, and C-reactive protein (Livingstone and others 2013).

Other important lipophilic compounds detected in acorns are vitamin E and provitamin A. The presence of vitamin E (mostly in the form of γ-tocopherol, as already described) is an advantageous feature, since it has several important biological effects. In recent epidemiological studies, vitamin E has been reported as preventing neurodegenerative disorders associated with PUFA peroxidation and protein oxidation, and other markers, all caused by oxidative stress (Butterfield and others 2002; Morris and others 2005). In fact, vitamin E is a powerful antioxidant and a scavenger of hydroxyl radicals, exerting important anti-inflammatory properties in different tissues (Tahan and others 2011), besides preventing aging pathologies (Mocchegiani and others 2014). Equally important is the mutualistic association among vitamin E and vitamin C, which might be regenerated from intermediate forms to reinstate their antioxidant potential (Carocho and Ferreira 2013). Thus, it is mandatory to characterize vitamin E profiles in new food products or natural products to accurately monitor its dietary uptake by humans (Saini and Keum 2016).

The levels of provitamin A are also remarkable. This vitamin is essential for human growth and development, in addition to its contribution to the proper functioning of the immune system (particularly in the defense against infections) and its vital action in preserving vision acuity. On the other hand, vitamin A deficiency can lead to several disorders such as xerophthalmia, susceptibility to infections, anemia, and night blindness in childhood, as well as low resistance to infections (Grilo and others 2014).

There are some reports indicating clinical applications of some *Quercus* species, including the use of acorn and its bark in the treatment of some chronic dermatological diseases (Kaur and others 2004; Aslani and others 2009, 2013). In addition, *Quercus* species have been reported to have antibacterial (Güllüce and others 2004; Basri and Fan, 2005; Jamil and others 2012), antiviral (Muliawan and others 2006; Karimi and others 2013), as well

Table 2–Extraction solvent, evaluated phytochemicals, and biological activities of *Quercus* spp. reported in the literature. The characteristics belonging exclusively to a single (or a determined group) *Quercus* species are identified by superscript numbers.

Species	Solvent extractor	Phytochemicals	Biological activities	References
Q. brantii	Ethanol[1,2,3] Methanol[2] Butanol[2] Chloroform[2] Hexane[2]	Total phenolics, flavonoids	Antiseptic[1] Antimicrobial activity[1] Antioxidant activity[2] Antiproliferative activity[3] Apoptosis inducing activity[3]	[1]Aslani and others 2013 [2]Karimi and Moradi 2015 [3]Moradi and others 2016
Q. cerris	Methanol[4] Ethanol[5]	Total phenolics[4], tannins[4], flavonoids[4]	Antioxidant activity[4] Antimicrobial activity[5]	[4]Rakić and others 2007 [5]Hobby and others 2012
Q. dilatata	Methanol	Alkaloids, saponins, anthraquinones, couramins, terpenoids, flavonoids, tannins, cardiac glycosides	Antimicrobial activity	Jamil and others 2012
Q. durifolia *Q. eduardii* *Q. sideroxyla*	Water	Total phenolics, flavonoids, tannins	Antioxidant activity Anti-inflammatory activity Antitumoral activity	Moreno-Jimenez and others 2015
Q. grisea *Q. laeta* *Q. obtusata* *Q. resinosa*	Water + hexane fraction	Total phenolics, flavonoids, condensed tannins	Antimicrobial activity Antioxidant activity Antitopoisomerase activity Gastroprotective effect	Sánchez-Burgos and others 2013
Q. ilex	Methanol/water 80:20 (v/v)[8] Ethanol[7] Butanol[7] Water[7,8] Methanol[9]	Phenolic compounds[6,8], tocopherols[6]	Antioxidant activity[6] Antimicrobial activity[7,9] Gastroprotective effect[8]	[6]Cantos and others 2003 [7]Berahou and others 2007 [8]Gharzouli and others 2007 [9]Güllüce and others 2004
Q. infectoria	Ethanol[10] Acetone[11] Water[11]	Total phenolics[11], tocopherols[11], steroids[11]	Anti-inflammatory[10] Antimicrobial activity[11]	[10]Kaur and others 2004 [11]Basri and Fan 2005
Q. lusitanica	Methanol	Non determined	Antiviral activity	Muliawan and others 2006
Q. persica	Ethanol	Total phenolics[12] Flavonoids[12] Tannins[12]	Antimicrobial activity Antiviral activity[12] Antioxidant activity[12] Cytotoxicity[12]	Bajalan and others 2014 Nourafcan and others 2013 [12]Karimi and others
Q. petraea	Ethanol 80:20 (v/v) Water	Total phenolics, tannins, flavonoids	Antioxidant activity	Popović and others 2013
Q. resinosa	Water	Phenolic compounds	Antioxidant activity Genotoxic effect	Rocha-Guzmán and others 2009
Q. robur	Methanol/water 80:20 (v/v)[13] Ethanol 80:20 (v/v)[14] Water[14,15] Methanol[4,16]	Total phenolics[4,15,16], tannins[4,14,15,16], flavonoids[4,14,16], terpenoids[16], alkaloids[16], anthraquinones[16], steroids[16]	Antioxidant activity Antimicrobial activity[13,17]	[13]Andrenšek and others 2004 [14]Popović and others 2013 [15]Rakić and others 2006 [4]Rakić and others 2007 [16]Uddin and Rauf 2012
Q. rotundifolia	Methanol/water 80:20 (v/v)	Phenolic compounds, tannins, tocopherols	Antioxidant activity	Cantos and others 2003 Tejerina and others 2011
Q. suber	Methanol/water 80:20 (v/v)[17] Methanol[18] n-Hexane[18] Water[18]	Phenolic compounds[17], total phenolics[18], tannins[18], flavonoids[18], tocopherols[17]	Antioxidant activity[17,18] Haemolytic activity[18]	[17]Cantos and others 2003 [18]Custódio and others 2015
Quercus spp.[a]	Ethanol[19,20,22] Chloroform[20] Methanol/water 70:30 (v/v)[20] Water[20,21,22] Hexane[22]	Total phenolics[20,21], flavonoids[20, 21], tannins[19,21,22]	Anti-hemorroidal[19] Antioxidant activity[20] Acute toxicity[20] Neuroprotective activity[21] Anti-cholinesterase activity[21] Hepatoprotective activity[22] Antimicrobial activity[22]	[19]Aslani and others 2009 [20]Sung and others 2012 [21]Custódio and others 2013 [22]Toori and others 2013

[a]The authors did not specify the *Quercus* species.

as gastroprotective effects (Gharzouli and others 1999; Sánchez-Burgos and others 2013). More recently, acorns have also been highlighted for their antiproliferative and apoptosis-inducing activities (Moradi and others 2016), and also for their neuroprotective activity (Custódio and others 2013).

In the next section, the antioxidant and antimicrobial activities of acorns and related botanical components will be analyzed in detail.

Phenolic compounds, antioxidant activity, and antimicrobial activity

Phenolic compounds are classified as 1 of the 5 categories of phytochemicals found in food (the other categories are carotenoids, alkaloids, nitrogen-containing compounds, and organosulfur compounds), having a wide range of physiological activities, such as activating, or limiting, the gene expression associated with some diseases or with the production of natural antioxidant enzymes (Yeh and others 2009). In general, phenolic compounds are responsible for physiological, biological, and biochemical functions, mainly because of their strong antioxidant activity, but also due to their properties as membrane stabilizers (Kodad and others 2014; Żyżelewicz and others 2014). Moreover, these compounds are important in the human diet to maintain an adequate level of antioxidants (endogenous and exogenous), and to counteract the production of reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS), as well as their subsequent neutralization (Moo-Huchin and others 2015).

Despite the phylogenetic variability, phenolic acids (particularly gallic and ellagic acids and their derivative compounds), flavonoids (particularly flavan-3-ols), and tannins are somehow ubiquitous in all *Quercus* species (Table 1), as verified in *Q. acuta*, *Q. acutissima*, *Q. alba*, *Q. cerris*, *Q. faginea*, *Q. glauca*, *Q. ilex*, *Q. macrocarpa*, *Q. marilandica*, *Q. muhlenbergii*, *Q. myrsinaefolia*, *Q. palustris*, *Q. petraea*, *Q. phylliraeoides*, *Q. pyrenaica*, *Q. robur*, *Q. rubra*, *Q. rotundifolia*, *Q. salicina*, *Q. suber*, and *Q. virginiana* (Saffarzadeh and others 1999; Cadahía and others 2001; Cantos and others 2003; Ferreira-Dias and others 2003; Andrenšek and others 2004; Rakić and others 2006; Marquart and others 2007; Vanhessche and others 2007; Brossa 2009; Rocha-Guzmán and others 2009; Tejerina and others 2011; Kim and others 2012; Popović and others 2013).

At this stage, it should be noted that the reports presented by Kuliev and others (1997), who isolated more than 20 phenolic compounds (catechins and oligomeric and polymeric proanthocyanidins) from the bark of *Quercus robur*, as also the recent review on phenolic compounds in oak wood samples reported by Zhang and others (2015), were not included in Table 1, because this table comprises exclusively the phenolic compounds found in acorn components and *Quercus* leaves.

As it can be easily concluded from Table 1, the phenolic profiles vary greatly among species. For instance, in a study conducted on the leaves of 5 different *Quercus* species (*Q. acuta*, *Q. glauca*, *Q. myrsinaefolia*, *Q. phylliraeoides*, and *Q. salicina*), the authors reported particularly high levels (especially in *Q. salicina*) of gentisic, chlorogenic acids, as well as of the flavonoids naringin and rutin; but none of these compounds was detected in any other *Quercus* species (Kim and others 2012). On the other hand, Brossa and others (2009) reported flavanols and flavonols as the major constituents in holm oak (*Q. ilex*) leaves, some of them being exclusively found in this species. Likewise, Cantos and others (2003) reported several gallic acid derivatives as being solely found in *Q. ilex*, *Q. rotundifolia*, and *Q. suber*. However, these are merely 3 examples of the high heterogeneity found among *Quercus* species. As it can be seen in Table 1, some phenolic compounds were only detected in a particular species, while others are present in 10 species or more. Likewise, despite the 66 compounds in Table 1, the maximum number of compounds in a single species was 31, in *Q. ilex*.

Another obvious conclusion from Table 1 is the high number of tannin compounds. This is an important issue, because the high contents in tannins provide acrid aroma and astringent taste. These compounds are produced as part of the defense mechanism against parasites and they have been extensively reported as providing benefits to human health, specifically for their anticarcinogenic and antimutagenic properties; however, their antinutritional activity must always be taken into consideration, despite that dose which causes this negative effect is far beyond the level that one person would ingest during normal food intake (Chung and others 1998). In addition, the high contents of tannins might be decreased by submitting acorns to a cooking procedure, therefore also reducing their astringent properties. Furthermore, Rakić and others (2006) studied the influence of thermal treatment on physical and nutritive characteristics of acorn samples, as in their contents of total polyphenols, gallic acid, nitrogen compounds, and macro- and microelements, concluding that all the components were maintained upon treatment. In fact, in some cases, the functional properties of acorns were improved.

Like other polyphenols, tannins possess antioxidant and antimicrobial activities, the latter being mainly justified by their ability to inhibit hydrolytic enzymes (proteases and carbohydrolases), bind cell envelope transport proteins, and inactivate microbial adhesions (Sung and others 2012).

Even so, the majority of reports describing the biological activity of acorns are focused on their strong antioxidant activity, which might be related to other biological functions, such as antimutagenity, anticarcinogenicity, and antiaging effects, and reducing risks or symptoms of cardiovascular diseases, diabetes, microbial infection, and inflammatory diseases (Rakić and others 2006; Silva and others 2008; Karimi and Moradi 2015). The antioxidant activity was mostly attributed to the presence of high amounts of phenolic compounds in the acorn extracts (Cantos and others 2003; Rakić and others 2006, 2007; Tejerina and others 2011).

In one particular study, Sánchez-Burgos and others (2013) established that the aqueous extracts obtained from the leaves of different white *Quercus* species (*Q. grisea*, *Q. laeta*, *Q. obtusata*, and *Q. resinosa*) displayed high radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and $HO^{\bullet}$ radicals, as well as antimicrobial and antitopoisomerase activities.

In addition, Andrenšek and others (2004) considered that the cortex of *Q. robur* could be a natural plant material with antioxidant and antimicrobial activities (Table 2). The antimicrobial activity was described in extracts obtained by step-gradient elution with different solvents in a multifunctional ExtraChrom® instrument. Interestingly, the extracts obtained with the mixtures methanol:ethyl acetate 50:50 (v/v) and methanol:water 75:25 (v/v) were bactericidal for *S. aureus*, but the less polar extracts (ethyl acetate:hexane 75:25, ethyl acetate, and ethyl acetate:methanol 95:5) were especially active against the Gram-negative *Enterobacter aerogenes* and the fungus *Candida albicans*. Likewise, there were significant differences concerning the antioxidant activity, having been verified that the extracts obtained with methanol:ethyl acetate 50:50 (v/v) presented the highest activity. Either way, *Q. robur* extracts seem to be a promising starting material, with high amounts of secondary metabolites able

to confer protection against microbial contamination (Andrenšek and others 2004).

In a similar study, the antioxidant and inhibitory activities of leaves and acorn fruits of *Q. suber* were investigated using 3 different solvents (hexane, methanol, and water). The aqueous extracts showed the highest antioxidant activity, as measured by the DPPH and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. On the other hand, the methanolic leaf extracts exhibited the strongest inhibitory activity against acetylcholinesterase, butyrylcholinesterase, α-amylase, and α-glucosidase. Similarly, the methanolic extracts showed the highest recovery rates of phenolic and flavonoid compounds (Custódio and others 2015).

Contrarily, water proved to be a better extraction solvent for tannins, which might explain the better results obtained with aqueous extracts in a study conducted to evaluate the antioxidant activity of twigs, leaves, and acorns of *Q. robur* and *Q. petraea* (Popović and others 2013). The same authors reported acorns as valuable sources of phytochemicals with potential use in food and pharmaceutical industries.

Besides the influence of using different solvents, the potential effects of thermal treatment on the physical and nutritional characteristics of acorns were also evaluated in *Q. robur*, having been verified that hydrolyzable tannins and gallic acid were present in all samples and nontannin phenolic contents were higher in thermally treated samples, while tannin content showed a marked decrease. Once tannins were degraded during thermal treatment, and according with the increase of nontannin phenolics content (especially gallic acid), thermally treated samples possess higher antioxidant activity than the raw material (Rakić and others 2007).

Recently, Toori and others (2013) evaluated the antioxidant activity and hepatoprotective effects of acorn extracts on carbon tetrachloride-induced liver damage in rats. This study showed that chloroform and methanolic and aqueous extracts of the internal layer of oak fruit (cotyledons) presented high antioxidant activity, as determined by DPPH, ferric antioxidant power (FRAP), and trolox equivalent antioxidant capacity (TEAC) assays. Aqueous extracts at 250 and 500 mg/kg showed the highest antioxidant activity and superior hepatoprotective potential, suggesting that this solvent is a better alternative, with no toxic effects.

Uddin and Rauf (2012) evaluated the content of bioactive compounds in *Q. robur* aerial parts, and different classes of phytochemicals were determined and associated with antimicrobial and antioxidant activities. For antimicrobial activity, n-hexane, ethyl acetate, chloroform, and methanolic fractions of *Q. robur* were evaluated against 5 different bacteria (*B. subtilis*, *E. coli*, *K. pneumoniae*, *S. aureus*, and *S. epidermidis*) showing moderate activities. The antioxidant activity was evaluated by DPPH assay, not exhibiting an outstanding scavenging capacity among whole extracts, as well as in the partioned fractions (particularly the chloroform fraction, which was the least active). This study also suggested further toxicological studies to ensure the potential use of *Q. robur* as antimicrobial agent and natural antioxidant (Uddin and Rauf 2012).

Nourafcan and others (2013) also reported an effective antibacterial activity in *Q. persica* ethanolic extracts against *S. aureus*, *B. subtilis*, *E. coli*, and *K. pneumoniae*.

Finally, the antibacterial activity of *Q. ilex* was evaluated in different extracts against 11 reference strains (Berahou and others 2007) and also in a comprehensive study with methanolic extracts, where 55 bacterial species were screened (Güllüce and others 2004).

Overall, the reported antimicrobial activities are noteworthy, considering the present situation of antimicrobial resistance to several agents, resulting from the indiscriminate use of different antimicrobials. Furthermore, the demand for convenient, ready-to-eat food has increased in the last decades, but convenience food offers a suitable growth environment for toxin-producing bacteria such as *S. aureus* (Balaban and Rasooly 2000). To overcome this urgent problem of the increased resistance of pathogenic microbes, researchers are also considering the traditional knowledge to help in the development of new drugs with high antimicrobial potential. In fact, the use of phytochemicals as natural antimicrobial agents, commonly called "biocides," is gaining popularity, even though the extraction conditions to obtain those compounds may strongly influence their antimicrobial activity.

Accordingly, studies focused on natural plant extracts, as the cases presented above, might be essential to find novel agents to produce human or veterinary medicines. In some countries, acorns and other aerial parts of oak trees have actually been used as condiments and as food preservatives, due to their antimicrobial properties. This traditional practice has been scientifically validated, since the ethanolic extract of acorns presented good results in controlling various bacterial species (Bajalan and others 2014).

Future Perspectives

Acorns and their by-products have diverse beneficial effects, mainly due to the presence of specific functional groups of phytochemicals. In this context, it is somehow surprising that these fruits were almost exclusively used for animal feeding. Nevertheless, there are some current trends toward the incorporation of acorns in different food products, such as the already indicated acorn bread and pastry products, which will be adequate for consumers with celiac disease. Likewise, acorn starch might be used as thickening and stabilizing agent, owing to its high paste consistency. In addition, since this polysaccharide is present as resistant starch in a high percentage, it can be very useful as a prebiotic growth promoter, constituting a good alternative to other current prebiotic agents such as fructo-oligosaccharides, inulin, isomalto-oligosaccharides, polydextrose, and lactulose (Siró and others 2008). Acorn starch might also be used in other industrial applications, such as paper, plastics, textile, pharmaceutical, and cosmetic industries. In addition, the aliphatic alcohols in acorns oil may be industrially applied as emulsifiers, emollients, and thickeners in food and personal care products.

Nevertheless, these marketing tendencies, together with several other possible examples, are far from reaching their maximum magnitude, since the current applications of acorns are still not profitable enough to attract investors, mostly because they are still scarcely diffused among consumers.

But this situation will surely change soon, considering the number of studies designed to valorize acorns as a natural source of bioactive compounds or as base of new foodstuffs.

Moreover, there is the ever-important question of sustainability and waste management handling. In fact, acorns and acorn by-products could also replace several other products, usually more expensive and with more negative impact on the environment, namely as an innovative source of oil and flour, as well as a diverse set of different food products prepared with these 2 key ingredients, such as bread, breakfast cereals, pastry products, yogurt components, among others. The upgrading of the by-products of the forestry industry constitutes, with no doubt, an important challenge within the development of a sustainable economy and environmentally friendly industrial processes, but this obviously

requires more studies in oak tree management in order to increase the exploitation of acorn species and their by-products on an industrial scale, focusing on sustainability and human health benefits.

In general, acorns can (and should) be used as a source of dietary energy, starch and fiber, providing an attractive low-cost food. In addition, acorns contain essential fatty acids and high levels of provitamin A and vitamin E. Therefore, acorn flour can be an interesting substitute of wheat flour in bread production, fulfilling the purposes of innovation and valorization of traditional products. The edible fruit (kernel) and its waste (shell) could also be considered as an added-value ingredient for other purposes, including gluten-free formulations. In this way, it is reasonably expected to expand the current limited examples of the utilization of acorns to produce oil, flour, or specific beverages (only applied by small population groups) (Pinna 2013).

The present review aims at contributing to this global change, highlighting acorns as an added-value resource not only for their nutritional quality, but also for the beneficial health effects due to their bioactive compounds, particularly helping in the prevention of several diseases (Rakić and others 2006; Tejerina and others 2011). Accordingly, there are immense opportunities for the development of new food and pharmaceutical products based on this fruit, providing valued benefits for consumers and a stimulating opportunity for the food and pharmaceutical industries (Correia and others 2013).

Research Gaps

The limited diffusion of acorns in the food industry might be explained by some current consumer trends, but also due to the lack of a number of chemical characterization studies sufficient enough to validate their true potential. There is also the additional constraint of their astringency, which, in some cases, might compromise their acceptability as food. In this specific case, further studies should be performed to conclude the best processing practices to counteract astringency, while maintaining as much as possible the nutritional and phytochemical profiles of acorns. It is true that a wide variety of individual compounds with acknowledged biological activities have already been reported in acorns. Nevertheless, the inclusion of acorns in industrial applications must also be preceded by an evaluation of processing effects, since these procedures frequently interfere in the sensory and nutritional properties of foods, changing their organoleptic quality, bioavailability of nutrients, and shelf-life (De Vasconcelos and others 2010; Cruz and others 2013).

Regarding their most common applications, it seems obvious that acorn flour presents good functional properties, sensory quality, and considerable consumer acceptance, but the available information on nutrients and the chemical composition of acorns is far from satisfactory. Accordingly, more research should be carried out on their chemical composition and bioactivity evaluation to achieve a comprehensive characterization of this raw material, increasing its potential applications. The chemical composition of acorn oil had already been studied to some extent, but extraction effectiveness could be improved.

Likewise, the mechanisms of toxicity in acorns must be further studied in order to be fully understood. Studies have also suggested additional toxicological investigations to ensure the potential use of *Quercus* extracts as antimicrobial agents and natural antioxidants.

Conclusion

In general, research focusing on characterizing plant extracts, identifying their individual compounds, and evaluating the related biological activities might be considered as an essential task to find sustainable sources of novel agents for industrial application, while improving their processing and preservation techniques.

Acorn had some secondary uses as food in the past, but the establishment of acorns as an alternative and competitive food source is emerging. Among the suggested applications, the use of acorn as food is, at this stage, more obvious and convenient, since acorn oil has important properties similar to other usual ingredients as, for example, olive oil. In addition, acorn flour might improve the rheological properties of bakery products, besides providing several components that cannot be found in commonly used flours. And, of course, other new food products might be developed, using acorns as one of their main ingredients.

Nevertheless, the phytochemicals listed in this state-of-the-art review, which are known to provide beneficial effects to human health because of their biological activities, also raise the potential for new applications of acorns. Some specific species such as *Q. acuta*, *Q. glauca*, *Q. ilex*, *Q. myrsinaefolia,* and *Q. salicina*, proved to have particularly high potential as phenolic compounds sources. Besides their irreproachable interest as a natural low-cost edible source, acorns may provide essential constituents for a healthy diet, but to be also included in pharmaceutical and cosmetic formulations.

Taking into account current consumer trends and industrial approaches, the information gathered here will certainly be useful to validate the future application of acorns as a functional food or as a starting material for related industries, improving the food chain sustainability, considering economic or environmental standpoints.

Author Contributions

Ana F. Vinha contributed to the conception of the idea, literature review, and manuscript writing. João C.M. Barreira contributed to the literature review, graphical elements preparation, writing, and overall interpretation of data. Anabela S.G. Costa contributed to the conception of the idea and literature review. M. Beatriz P.P. Oliveira contributed to the framework writing, conception of the idea, and interpretation of data.

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