Valorization of acorn from oak (*Quercus pyrenaica*) through chemical and nutritional evaluation

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List of abbreviations

AAS: Atomic Absorption Spectroscopy

AOAC: Association of Official Analytical Chemists

ABTS+: 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation

DAD: Diode Array Detection

DPPH: 2-diphenyl-1-picrylhydrazy

dw: dry weight

ESI: Electrospray Ionization

EtOH: Ethanol

FAME: Fatty acid methyl ester

GC-FID: Gas Chromatography- Flame Ionization Detector

HCL: Hydrogen Chloride

HPLC: High-Performance Liquid Chromatography

MS: Mass Spectrometry

MUFA: Monounsaturated Fatty Acid

N: Nitrogen

NaOH: Sodium Hydroxide

OA: Oleic Acid

PMB: Polymer-Modified Binder

PUFA: Polyunsaturated Fatty Acid

PTFE: PolyTetraFluoroEthylene

Q*: Quercus*

SFA : Saturated Fatty Acid

TFAs: Total Fatty Acids

UV-Vis: Ultraviolet-Visible

W: Watt

WCED: World Commission on Environment and Development

Abstract

The present global food system must adapt to the world's expected population expansion. This adaptation will most likely entail an increase in the consumption of edible wild foods, which are high in micronutrients and bioactive substances, as well as a cost-effective and long-term strategy of increasing caloric food security. Despite its potential importance to the rural economy, *Quercus* fruits (acorns) were traditionally utilized mostly for animal food, mostly in the diets of various species of wildlife, insects, birds, and mammals.

Acorns are considered nutritionally valuable, being a good source of carbohydrates, proteins, and fat. In particular, the oil extracted from oak fruit has characteristics similar to those of olive oil, with a composition rich in bioactive compounds and, therefore, with greater potential for industrial application. Due to its nutritional characteristics, the acorn can be ground into flour and used as a functional ingredient to be incorporated in some processed foods, such as bread. Today it is possibleto find some commercial products based on acorns, however, none of them explore the use of *Quercus pyrenaica* acorn. In this context, this work aims to study the chemical composition and nutritional value of *Quercus pyrenaica* acorn, to enhance this product within the food industry. For this, different physicochemical parameters will be evaluated such as water content, ash, proteins, sugars, minerals, phenolic compounds, and characterization of the lipid fraction, which includes fatty acids (saturated and unsaturated).

The results revealed that the fruit and the seed of *Q.pyrenaica* ,*Q.suber* and *Q.ilex* were very rich in Total carbohydrates and energy .Although, the values of lipids and proteins, are around 6 - 7 % and 3 - 4.5% respectively. For ash, the cupule showed the highest value with an average of 2.8%, followed by the seed, the fruit and the shell with closer values around 1.5 to1.8%. As expected, the water content was also higher for the fruit and seed (around 50 to 60%), when compared to the cupule and pericarp (20 to 28%). In addition to that, all the samples were very rich with minerals such as K, Mg and Mn and fatty acids especially palmitic acid which was very abundant in *Q.pyrenaica*. Concerning the phenolic compounds, the fruits present a rich composition in phenolic acids such as gallic acid, followed by hydrolysable tannins, where the earlier stages of development presented a richer composition comparing to the mature stage.

Keywords: *Quercus pyrenaica*, acorn, physicochemical parameters, nutritional value,food application

Resumo

O atual sistema alimentar global tem de se adaptar à expansão esperada da população mundial. Essa adaptação provavelmente irá levar a um aumento no consumo de alimentos silvestres comestíveis, ricos em micronutrientes e substâncias bioativas, bem como uma estratégia económica, a longo prazo para aumentar a segurança alimentar de alimentos calóricos. Apesar de sua potencial importância para a economia rural, os frutos de *Quercus* (bolotas) eram tradicionalmente utilizados maioritariamente para a alimentação animal, maioritariamente na dieta de de várias espécies de animais selvagens, como insetos, aves e mamíferos.

As bolotas são consideradas nutricionalmente valiosas, sendo uma boa fonte de carboidratos, proteínas e gorduras. Em particular, o óleo extraído do fruto do carvalho apresenta características semelhantes às do azeite, com uma composição rica em compostos bioativos e, portanto, com maior potencial de aplicação industrial. Devido às suas características nutricionais, a bolota pode ser moída em farinha e utilizada como ingrediente funcional a ser incorporado em alguns alimentos industrializados, como o pão. Hoje é possível encontrar alguns produtos comerciais à base de bolotas, no entanto, nenhum deles explora o uso da bolota de *Quercus pyrenaica*.

Neste contexto, este trabalho tem como objetivo estudar a composição química e o valor nutricional da bolota *Quercus pyrenaica*, de forma a potenciar este produto dentro da indústria alimentar. Para isso, serão avaliados diferentes parâmetros físico-químicos como teor de água, cinzas, proteínas, minerais, compostos fenólicos, e caracterização da fração lipídica, que inclui ácidos gordos (saturados e insaturados).

Os resultados revelaram que os frutos e a semente de *Q.pyrenaica* , *Q.suber* e *Q.ilex* eram muito ricos em hidratos de carbono e energia totais. As valores dos lípidos e proteínas sejam cerca de 6 - 7 % e 3 - 4,5%, respectivamente. Para as cinzas, o cálice apresentou o valor mais elevado, com uma média de 2,8%, seguido da semente, do fruto e da casca com valores mais próximos em torno de 1,5 a 1,8%. Como se esperava, o teor de água também foi maior para os frutos e sementes (cerca de 50 a 60%), quando comparado com o cupule e o pericarp (20 a 28%). Além disso, todas as amostras eram muito ricas com minerais como K, Mg e Mn e ácidos gordos, especialmente ácido palmítico, que era muito abundante em *Q.pyrenaica*. No que diz respeito aos compostos fenólicos, os frutos apresentam uma composição rica em ácidos fenólicos, como o ácido gálico, seguido de taninos hidrolisados, onde as fases anteriores do desenvolvimento apresentaram uma

composição mais rica em comparação com a fase madura.

Palavras-chave : *Quercus pyrenaica*, bolota, parâmetros físico-químicos, valor nutricional, aplicação alimentar

INTRODUCTION

1. Introduction

Quercus spp. (Fagaceae) are a diverse genus of evergreen and deciduous trees native to temperate and tropical climates. Around 450 species of *Quercus* exist around the world, each with its own flowering and fruiting dynamics as well as maturity index. These species produce an acorn-like fruit that is widely recognized (Terjerina et al., 2011; Sànchez-Burgos et al., 2013).

Acorns are characterized by a single-seeded nut with an achlorophyllous embryo morphologically; they are universally regarded as nutrient-dense products, justifying their usage as secondary human diets (mostly supplies of carbohydrates, proteins, and fat) or food additives, for thousands of years wherever oak trees can be found (Vinha et al., 2016).

Acorn consumption can be divided into three categories based on their possible applications in human nutrition: acorns as nuts (they resemble chestnuts), flour (due to high starch content), and cooking oil (which presents high similarity with olive oil). Combining acorn and wheat flours, for example, was found to have beneficial rheological effects, as the addition of acorn flour enhanced bread volume and improved crumb properties (Korus et al., 2015).

The comprehensive analysis of acorns and components may boost their worth for future applications in the food and pharmaceutical industries, given their nutraceutical, phytochemical, and bioactive potentials (Vinha et al., 2016).

2. *Quercus* spp: different types in Portugal

Forests and woods in the Mediterranean basin have a wide range of architecture, appearance, and woody plant composition because of varying ecological conditions and land-use histories. Oak trees are the most common and important forest species, particularly in the meso- and supra-Mediterranean life zones (Figure1) (Plieninger et al.,2010).

In the north of Portugal, pure woodlands of *Quercus robur, Q. pyrenaica* and mixed woodlands are the most abundant (Marques et al., 2005).

The evergreen cork oak (*Quercus suber L.*) is primarily found in the western Mediterranean basin (Table1). The greatest forest, totaling around 700.000 ha, are found in Portugal and account for 21% of the country's forest area and 30% of the world's cork producing area (Plieninger et al.,2010). However, hybridization of *Q. suber* with other *Quercus* species, specifically the *Quercus aggr. ilex L*. complex, which includes *Q. ilex*, is reported as a natural occurrence and could be regarded as one factor contributing to increased genetic diversity in cork oak (Coelho et al., 2006).

Table 1. Different types of *Quercus* from different sources (Taib et al., 2020).

Source	Acorn species
Turkey	*Q. pontica*
	Q.robur
	Q.hatwissiana
	Q.frainetto
	Q.petraea
	Q.vulcanica
	Q.ithaburensis
	Q.brantii
	Q.libani
	Q.trojana
Spain	*Q.faginea*
	Q.suber
	Q.pyrenaica
	Q. coccifera
	Q. ilex
Portugal	*Q. faginea*
	Q.ilex
	Q.nigra
	Q.suber
Algeria	*Q.ilex*
	Q.suber
Jordan	*Q.aelgilops*
	Q.infectoria
	Q.calliprinos
Latvia	*Q.rubra*
	Q.robur
Serbia	*Q.robur*
	Q.cerris

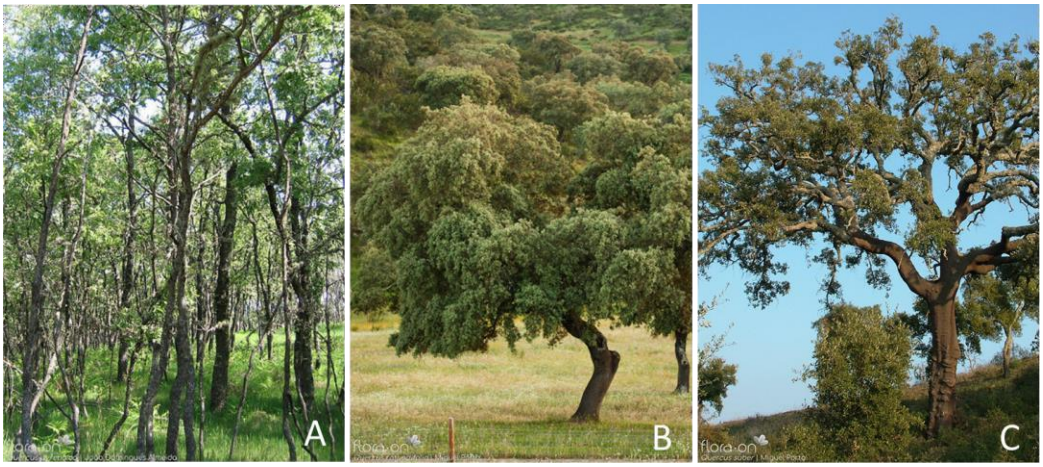


Figure 1. Different types of *Quercus*, A: *Quercus pyrenaica*; B: *Quercus rotundifolia*;C: *Quercus suber*[1][2]

2.1. Acorn morphology

An acorn is a 1-seeded nut, known by the absence of an endosperm and the presence of an achlorophyllous embryo (Figure 2). It is composed of a cupule (the cup), pericarp (the fruit) and the seed coat (or shell for protection) (Figure3) (Vinha et al., 2016).

In general, phylogenetic, and ecological factors are responsible for differences among acorns of *Quercus* species. There is great variability in seed size within and between oak species and it has been proved that acorn size is positively correlated with the length of its development period and with rainfall, (Table 2) (Alegria et al., 2020). However, the size of a fully developed acorn always depends on its growth conditions. Therefore, an acorn size is also positively connected with seedling survival rate under stress conditions (Vinha et al., 2016).

[1] https://flora-on.pt/#/1*Quercus+suber*
[2] https://flora-on.pt/#/1*quercus+pyrenaica*, https://flora-on.pt/#/1*Quercus+rotundifolia*

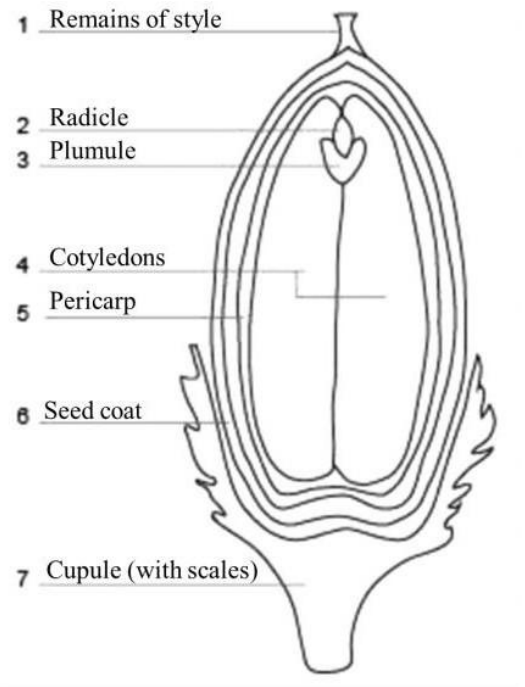


Figure 2. Acorn sketch highlighting the main morphological characters (Vinha et al., 2016)

Figure 3. *Quercus ilex*; *B: Quercus rotundifolia ; C: Quercus suber ; D: Quercus pyrenaica*.[3]

[3] https://flora-on.pt/#/1*quercus+pyrenaica*, https://floron.pt/#/1*Quercus+rotundifolia* https://flora-on.pt/#/1*Quercus+suber*

Table 2. Morphological traits of acorns from two *Quercus* species: *Q.rotundifolia* (n= 15) and *Q.suber* (n=13) (Algeria et al.,2020).

Morphological Trait	***Quercus rotundifolia* (QR)**	***Quercus Suber* (QS)**
Weight (g)	5.64(±1.21)	6.18(±2.45)
Length (mm)	37.57(±3.24)	33.80(±5.88)
Diameter (mm)	14.75(±1.59)	15.49(±2.50)
Volume (cm^3)	4.34(±1.06)	4.51(±2.05)

Values are mean± SD. Values between brackets correspond to the minimum and maximum value. Between species, no significant differences were found (Kruskal-Wallis test).

2.2. Acorn development

Additional criteria were employed to establish developmental classes because the size of the acorns varied amongst trees in different places on the same collecting day. The presence of a visible endosperm, numerous embryos or a dominating embryo within the growing seed, the cupule covering the acorn, and the pericarp color were among them. It should be noted that most of the acorn mass at maturity is made up of seed tissues,primarily cotyledons (Miguel et al., 2015). Although, the fruits are often defined as seed- bearing structures formed from a mature ovary, many structures that might be defined as fruit are in fact composed of different tissue types (Seymour et al., 2013). At complete maturity cork oak acorns contain a large and fleshy embryo with high water content. The natural shedding of cork oak acorns coincides with complete maturity and acorns left on the ground after shedding will either germinate or lose their viability because of desiccation (Merouani et al., 2003).

3. Acorn as an alternative and sustainable food

Sustainability and food security is a concept that appeared from the World Commission on Environment and Development (WCED 1987). Due to the growing demands of a global population expected to reach about 9 billion by 2050, it is necessary to find other food alternative to compensate the high food demand. These facts led to the creation of the notion of "food security," which refers to a condition in which the entire world's population has access to safe and nutritious food to meet their eating choices and dietary needs (Starin, 2014).

Thus, forest foods, including herbs and natural products collected from trees, can help to improve food security providing affordable and highly nutritious food stuffs, because of 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 (Taib et al., 2020).

Acorns and their by-products (particularly those emerging from oil extraction and flour production) have an immense potential to be involved in such applications. These fruits can be defined as an alternative functional food, specifically considering their high nutritional value and richness in bioactive phytochemicals with biological action. Previous studies showed that acorns contribute greatly to improve the quality of the adipose, hepatic, and muscle tissues of Iberian pigs with a lower rate of saturated fatty acids (Starin, 2014).

Also, acorns can be seen as an alternative food because they are acknowledged for their high importance to the rural economy as components of animal feeding and consequently by their nutritional value and high phytochemical contents which raised the interest to be used in human diet. Additionally, due to the nutritionally rich composition, they are used as secondary human foods or food ingredients, as flours, or as a coffee substitute beverage (Castro et al.,2021). These nutritional indicators show the acorn great potential as high-value nutraceuticals for dietary supplements or as functional foods and for developing new marketable alternatives to their commercialization and valorization (Starin, 2014).

Finally, acorns should be considered as functional foods or as alternative sources of several highly valued food ingredients. Their valorization perfectly fits into this future trend, as it improves the sustainability of the agro-food chain and include new food application. For this reason, they could be a promising ingredient for the food industry with high potential for commercial use (Vinha et al., 2016).

3.1. Nutritional and chemical composition

Vinha et al. (2016) analyzed acorns from 8 different species, *Q. suber, Q. faginea, Q. pyrenaica, Q. ilex, Q. robur, Q. canariensis, Q. coccifera* and *Q. lusitanica*. The results showed that the acorn kernel presents the higher water content than the whole fruit and the pericarp, despite the species, although the acorn parts from *Q. suber* had the highest water contents than those from *Q. ilex*, which showed the lowest contents considering the composition in dried mass (Vinha et al., 2016). Carbohydrates were also the most abundant component, with the highest concentrations in the pericarps and the lowest concentrations in the kernels and entire fruits. Nevertheless, the pericarps are supposed to have high percentages of lignin; thereby, the carbohydrates percentage and they might have been overestimated. Therefore, if we compare species, all of them have presented similar values only for *Q.nigra*, which had the highest carbohydrates contents (Deforce et al., 2009).

The quantities of mineral elements in acorns are also noteworthy (Makhlouf et al.,2018). For instance, considerable amounts of Fe, Cu, Zn, and Mn, besides Ca, Mg, P, and K in lower levels, are described in *Q.robur* acorn samples (Vinha et al., 2016).

The major fatty acids reported in acorns were oleic, palmitic, and linoleic acids, among other lipophilic substances (Gea-Izquierdo et al., 2006; Terjerina et al., 2011; Vinha et al.,2016).

Acorn has also been linked to vitamin E (mostly α- and γ-tocopherol). In general,γ-tocopherol is the most abundant vitamin, reaching levels 4.6- to 8.7-fold greater than α-tocopherol (Terjerina et al.,2011, Rabhi-F et al., 2016, Vinha et al.,2016).

Acorns are also a good source of pro vitamin A, as just a few acorns are needed to meet the necessary daily vitamin A needs. Detected amounts of sterols (of which β-sitosterol was the most prevalent, accounting for over 90% of sterols) and aliphatic alcohols are also important (especially tetracosanol) (Bainbridge et al., 2001, Vinha et al., 2016).

3.1.1. Fatty acids profiles

According to several research, the oil content of white *Quercus* species does not surpass 12%, depending on the specie (Cantos et al., 2003; Özcan et al., 2007; Rabhi-F et al., 2016). However, previous reports found that black and red acorn species have greater oil content, around30% (Vinha et al., 2016).

The percentages of saturated, monounsaturated, and polyunsaturated fatty acids in acorn oils derived from several *Quercus* species exhibited a wide range of variance. As previously stated, acorns are a natural supply of neutral oleic acid, containing a significant level of linoleic acid (Estevez-M et al., 2004). In addition to the benefits of unsaturated fatty acids already discussed, changes in their percentages may be used as a chemical fingerprint to distinguish *Quercus* species (Vinha et al., 2016). However, the percentages of monounsaturated, polyunsaturated, and saturated fatty acids in the lipid profiles of oils varied according to the from different acorn species (Taib et al., 2020). Also, variations in the fatty acid content were observed according to the genetic characteristics, plant species, abiotic factors (such as maturation degree), climate, and geographic origin (Górnaś et al.,2019).

The most abundant fatty acids are oleic, linoleic, and palmitic acid (oleic acid accounts for 63% of total fatty acids), followed by palmitic and linoleic acids at comparable percentages (12–20%), and according to previous findings, the major monounsaturated fatty acid (MUFA) contained in acorn oil is oleic acid (OA) **(**Maguire et al.,2004). Although, linoleic acid (37.2 percent–32.6 percent) and c-linoleic acid (1.8 percent–3.7 percent) are abundant in acorn oils (Petrovic et al., 2004). Stearic acid (1%–4%), eicosanoid acid (0.37 percent), arachidic acid (0.38 percent) (Taib et al., 2020).

Similarly, the amounts of aliphatic alcohols in acorns were reported. The most prevalent chemical in this class was tetracosanol, although considerable amounts of docosanol, hexacosanol, and octacosanol were also identified (Vinha et al., 2016).

3.2. Phytochemical profile

The renewed interest in *Quercus* acorns as a source of bioactive compounds and a secondary food for humans increased the interest in their phytochemical characterization to identify the compounds of interest and correlate their chemical composition with morphometric parameters and biological activities (Galvan et al.,2011, Vinha et al., 2016; López-Hidalgo et al.,2018).

Different compounds were already identified in acorns from different *Quercus* species, including phenolic compounds, chlorophylls, carotenoids, sterols, among others. Several compounds were already identified in different acorn species, Table 3 (Vinha et al., 2016).

Taib et al., (2020) identified twenty phenolic compounds in acorn oil, where the predominant compounds were hydrolysable tannin derivatives, gallotannin or ellagitannin, in the form of hexahydroxydiphenoyl esters of glucose and galloyl glucose esters (trigalloyl glucose and pentagalloylglucose) (Taib et al., 2020). Concerning the total phenolic content (expressed in gallic acid equivalent), the values ranged between 195.6 and 322.06 mg GAE/kg oil while flavonoid contents ranged between 122.99 and 131.6 mg CE/kg of oil (Makhlouf et al., 2018).

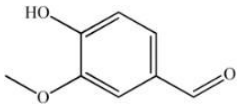
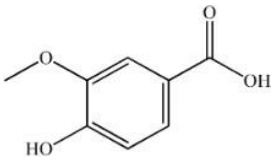
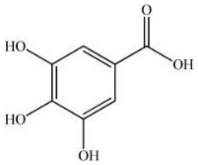
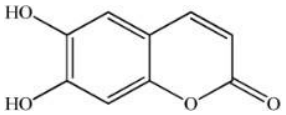
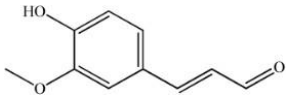
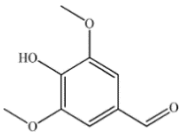
In general, phenolic compounds are responsible for physiological, biological, and biochemical functions, mainly because of their strong antioxidant activity, also due to their properties as membrane stabilizers (Kodad et al., 2014 Żyżelewicz et al.,2014; Vinha et al., 2016). Furthermore, these compounds are important in the human diet containing an adequate level of antioxidants. Despite the phylogenetic variability, phenolic acids (particularly gallic and ellagic acids and their derivative compounds), flavonoids (particularly flavan-3-ols), and tannins are somehow omnipresent in all *Quercus* species,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* (Cadahia et al., 2001 ; Cantos et al., 2003 ; Ferreira-Dias et al.,2003 ; Andrenšek et al., 2004 ; Rakic et al.,2006 ; Marquart et al., 2007; Vanhessche et al. ,2007 ; Brossa et al., 2009 ; Terjerina et al., 2011 ; Jong et al.,2012 ; Popović et al., 2013, Vinha et al., 2016).

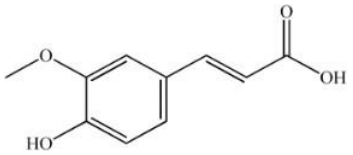
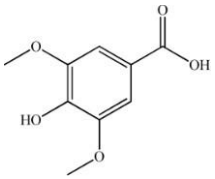
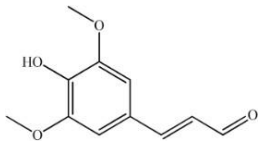
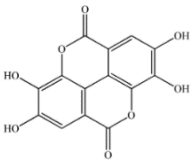
Another obvious conclusion from Vinha et al., (2016) is the high number of tannin

compounds, that provide acrid aroma and astringent taste. These compounds are produced as part of the defense mechanism against parasites specifically for their anticarcinogenic and antimutagenic properties. Like other polyphenols, tannins possess antioxidant and antimicrobial activities, justified by their ability to inhibit hydrolytic enzymes (proteases and carbohydrolases), bind cell envelope transport proteins, and inactivate microbial adhesions (Sung-SH et al.,2012, Vinha et al., 2016).

Also, the acorns showed the presence of chlorophylls, mostly on the pericarps, which had the maximum chlorophyll content, being the concentration very similar within different *Quercus* species. The lycopene content, on the other hand, varied significantly between species, with *Q. suber* having the lowest quantity and *Q. faginea* having the highest quantity The entire fruits had the greatest lycopene concentration among the acorn tissues. The results were different for β-carotene, which was found in roughly fivefold higher concentrations in the pericarps than in the remaining acorn components (Vinha et al.,2016).

Table 3. Individual compounds previously reported in acorns or related botanical parts from *Quercus pyrenaica* (Cadahia et al., 2001; Vinha et al., 2016).

Compound	**Chemical structure (m/z)**	**Detection methodology**
Vanillin		HPLC-DAD
Vanillic acid		HPLC-DAD
Gallic acid		HPLC-DAD-ESI-MS/MS
Aesculetin		
Coniferylic aldehyde		
Syringic aldehyde		

Ferulic acid		HPLC-DAD
Syringic acid		
Sinapic aldehyde		
Ellagic acid		

3.3. Bioactive properties

Acorns from different species present significant differences in their chemical composition due to their high variability. However, some of their biological activity indicators, such as antioxidant activity which presents some similarity independently of the species. However, having many benefits regarding the abundance of acorn and the easiness of the consumption could define their potential industrial applications (Rakic et al., 2006; Siró et al.,2008; Vinha et al., 2016).

3.3.1. Antioxidant capacity

In the literature, the antioxidant activity was measured, mainly by two tests, the first using the DPPH•+ radical and the second using the ABTS•+ radical. The antioxidant activity is mostly attributed to the presence of phenolic compounds, where the most abundant compounds were hydrolysable tannins derivatives, showing their potential as antioxidant in this food matrix (Makhlouf et al., 2018).

However, Sánchez-Burgos et al., (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• radicals, as well as antimicrobial and anti-topoisomerase activities (Sánchez-Burgos et al.,2013). On the one hand, 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-sulfonicacid) (ABTS) assays (Vinha et al., 2016). On the other hand, the methanolic leaf extracts exhibited the strongest inhibitory activity against acetylcholinesterase, butyrylcholinesterase, α-amylase, and α-glucosidase (Custódio et al.,2015; Vinha et al.,2016).

Add to that, the significant antioxidant activity of *Quercus* fruit is due to the inductive effect of the natural antioxidants present in the fruit such as phenolic compounds and flavonoids which reduce and discolor free radicals (DPPH•, ABTS•+) because of their ability to yield hydrogen (Turkmen et al., 2007; Servili et al., 2009).

3.4. Food applications

Acorns from oak are commonly used as a feed source for animals especially pigs. Findings indicated that acorn might be replaced barely without any impact on growth performance (Cantos et al., 2003). However, studies have showed that acorns and grass are natural sources of antioxidants and fatty acids in the ‘‘montanera” feeding of Iberian pig,which are important from the point of view of consumer health (Terjerina et al. 2011).

Acorns are a new source of valuable chemical components that could be considered as functional food with antioxidant properties (Taib et al.,2020). Acorn extracts were found to be effective antioxidants in the prevention of lipid and protein oxidation. Also, previous studies showed that acorn extracts can be used to improve the sensory qualities of chicken meat (color, juiciness, astringency, and general conveying improvingproduct color and odor acceptance (Özünlü et al., 2018, Taib et al.,2020).

Nevertheless, acorns are currently employed in the human diet as flour (usually for the creation of bread and biscuits) or as a coffee substitute beverage. Acorn flour has recently been shown to improve bread specific volume and crumb texture while also increasing the amount of phenolic chemicals (Skendi et al., 2018). Furthermore, it was shown than supplementing bread with acorn flour not only strengthened dough structure

but alsoenriched bread with proteins, minerals, and nutritional fiber, making acorn flour an intriguing ingredient for gluten-free bread development (Taib et al., 2020).

According to Sekeroglu et al., (2017), coffee made from acorns can be regarded a good source of macronutrients (P, Ca, K, Mg, and S) and micronutrients (Fe, Cu, Mn, and Zn) without dangerous heavy metals.

As Taib et al., (2021) reported acorns are underutilized and represent a good alternative sourceof starch. Starch is a mixture of two biopolymers: amylose and amylopectin, the two polysaccharides are homoglycans with only two types of chain linkages, α-(1–4) bonds of the main chain and α-(1–6) bonds in the branching point. However, amylose content of the acorn starches is reported to vary in the range of approx. 20–39%. In addition, since this polysaccharide is present as resistant starch in a high percentage, it can be very usefulas a prebiotic growth promoter, constituting a good alternative to other current prebiotic agents such as fructo-oligosaccharides, inulin, isomalto-oligosaccharides, polydextrose, and lactulose (Siró et al., 2008; Vinha et al., 2016; Taib et al.,2021).

4. Objectives

This work aims to study the chemical composition and nutritional value of *Quercus pyrenaica* acorn, in order to enhance this product within the food industry. For this, different physicochemical parameters will be evaluated such as water content, ash, content, proteins, minerals, phenolic compounds, and characterization of the lipid fraction, which includes fatty acids (saturated and unsaturated). Also, to study other species (*Q.ilex* and *Q.suber*) and the effects of the stages of development and different parts of the acorn (fruit, shell, seed and cupule).

MATERIALS AND METHODS

5. Materials

5.1. Chemicals and reagents

Ethanol methanol, sodium phosphate (Na_3HPO_4), potassium phosphate (KH_3PO_4), potassium ferrocyanide ($C_6FeK_4N_6 \bullet 3H_2O$), trichloroacetic acid ($C_2HCl_3O_2$), acetonitrile (CH_3N), formic acid (CH_2O_2), sulfuric acid (H_2SO_4), diethyl ether $(C_2H_5)_2O$, sodium hydroxide (NaOH), hydrochloric acid (HCl), petroleum ether, and gallic acid were purchased from Fisher Scientific (Pittsburgh, PA). Folin-Ciocalteu's reagent, Kjeldahl catalyst tablets, and acetic acid glacial were purchased from Panreac Applichem (Barcelona, Spain). Water was treated in a Milli-Q water purification system (TGI pure system, Houston, TX, USA).

5.2. Samples collection and preparation

Samples of *Quercus pyrenaica* and *Quercus ilex* were collected from the Montesinho Natural Park, Bragança (Portugal), from different places and in different acorn development stages (earlier stage, middle stage, mature stage). The different samples, collection times and places are described in Figure 4 and Table 4. Also, samples from *Q. ilex* and *Q. suber* in the last development stage (mature fruit) were collected in Herdade do Freixo do Meio, Évora, Figure 4 and Table 4.

The acorns were manually separated into cupule, shell, seed and fruit (all part of the acorn). The samples were freeze-dried using a lyophilized (FreeZone 4.5 model 7750031, Labconco, USA) and grind to a smooth flour then stored at -20 °C for further analysis.

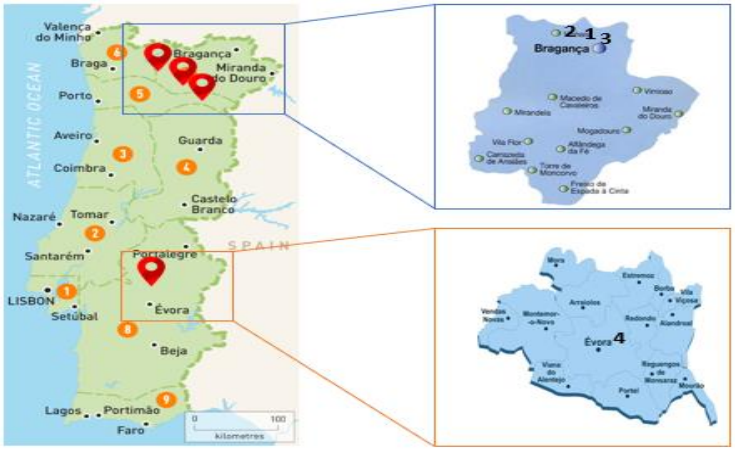


Figure 4. Identification of the localization of the collection of the samples

1: Donai, Bragança, 2: Monteishno Natural Park of Bragança, 3: Devesa,Bragança, 4 : Herdade do Freixo do Meio ,Evora

Table 4.. Identification of the species, time, development stage and collection location of the samples.

Sample code	Specie	Time of collection	Development stage	Place of collection
DB1*pyrenaica*	*Q.pyrenaica*	18.08.2021	Earlier	Donai, Bragança
DB2*pyrenaica*		10.09.2021	Middle	
DB3*pyrenaica*		22.10.2021	Mature	
DPB*pyrenaica*	*Q.pyrenaica*	22.10.2021	Mature	Devesa,Bragança
DB1*ilex*	*Q.ilex*	18.08.2021	Earlier	
DB2*ilex*		10.09.2021	Middle	Donai,Bragança
DB3*ilex*		22.10.2021	Mature	
HFM*ilex*		22.10.2021	Mature	Herdade do Freixo do Meio ,Évora
HFM*suber*	*Q.suber*	22.10.2021	Mature	Herdade do Freixo do Meio ,Évora

6. Methods

6.1. Nutritional analysis

6.1.1. Water content

The water content was determined in 2 g of sample using the PMB Moisture Analyzer (Kingston, Milton Keynes, U.K.) (Figure 5) following the AOAC 925.45. The analysis was performed in triplicates for each part of the acorn (cupule, shell, fruit, and seed).

Figure 5.PMB Moisture Analyzer (Kingston, Milton Keynes, U.K.)

6.1.2. Ash content

The ash content was estimated through the incineration of the samples (1 g) in a muffle (Optic Ivymen System) at 550 ± 5 °C for 5 hours according to the AOAC 923.05. The ash content were the results of the substitution of the initial weight of the crucibles containing 1g of sample before the incineration and the final weight of the crucibles after the incineration and the cool down to room temperature.The analysis was performed in triplicate for each part of the acorn.

6.1.3. Protein content

The protein content estimation was performed through Kjeldahl nitrogen determination using a copper catalyst in 0.25 g of each sample, in Kjeldahl steam distillation unit (Pro-Nitro A, Selecta, Spain) according to the AOAC 920.87 Method. For the conversion of nitrogen levels to protein, the factor 6.25 was used. The analysis was performed in triplicates for each part of the acorn (shell, fruit, seed, and cupule).

6.1.4. Total Lipid

The total lipid was determined through the AOAC 989.05, 1 g of sample was treated with petroleum ether in a Soxhlet apparatus for 4 hours (Figure 6). The petroleum ether extract was evaporated under reduced pressure to dryness, the residues were

weighed, then the lipid content was expressed as a mass percentage. The analysis was performed in triplicates for each part of the acorn.

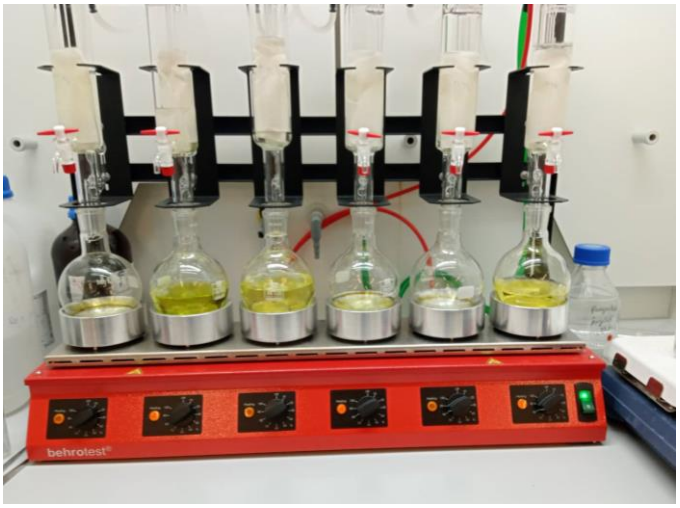


Figure 6. Total lipid extraction using Soxhlet apparatus

6.1.5. Fiber content

The total dietary fiber content was performed using a fiber assay kit (Megazyme K-TDFR-200A, Wicklow, Ireland) following the AOAC 991.43 Method. Briefly, 0.125 g of dried sample was subjected to 6.25 mL of phosphate buffer, and then 6.25 µL of α-amylase solution was added and kept at 100 °C for 15 min. After this process, the samples were allowed to cool to room temperature and the pH was adjusted to 7.5 mL with 1.25 mL of 0.275 N NaOH solution. Then 12.5 µL of protease solution was added and left for 30 min in incubation at 60 °C. The samples were allowed to cool again and then the pH was adjusted 4.5 with 1.25 mL 0.325 N HCl solution. Then, 25 µL of amyloglucosidase solution was added and incubated at 60 °C for 30 min. After incubation, the samples were allowed to cool. The sample was then precipitated with 95% EtOH (12.5 mL) and preheated to 60 °C. The analysis was performed in quadruplicates for each part of the acorn (shell, fruit, seed, and cupule).

After filtering the samples, 1 g of celite and 10 mL of 95% EtOH were added sequentially. Following this procedure, the samples were washed successively with three 5 mL portions of 78% EtOH, two 2.5 mL portions of 95% EtOH and two 2.5 mL portions of acetone. The sample was dried overnight in a 105 °C air oven. The crucible and celite weights were then removed to determine the weight of the samples. Then, the residue

from the sample was analyzed by AOAC 920.87 method using N x 6.25 as the conversion factor. The second residual sample was burned at 525 °C for 5 h and cooled in a desiccator. Crucible and celite weights were removed to determine ash.

6.1.6. Total carbohydrates and energy

Total carbohydrate content **(Equation 1)** and energy values **(Equation 2)** were determined from the difference between 100 grams of the correspondent flour on a dry basis and the partial sum of the protein, ash, and lipid contents.

The samples were calculated using the following equations (Li et al.,2015):

:

$$Total\ carbohydrates = 100 - (g\ ash + g\ proteins + g\ lipids)$$

Equation 1 – determination of carbohydrates

$$Energetic\ value\ (Kcal) = 4 \times (g\ protein + g\ carbohydrate) + 9 \times (g\ lipids)$$

Equation 2– determination of total energy

6.1.7. Mineral content analysis

Mineral elements were analyzed by atomic absorption spectroscopy (AAS) using a Perkin Elmer PinAAcle 900T Spectrometer (Waltham, MA, USA). Potassium, sodium, calcium, magnesium, zinc, and iron were analyzed by flame ionization AAS, while atomic absorption spectrophotometry in a graphite chamber were applied for manganese, copper, cadmium and lead.

The sample preparation was carried out through microwave-assisted extraction, using a MARS 5 Digestion Microwave System (CEM Corporation, Matthews, NC, USA). Approximately 1 g of the sample was weighed into a PTFE digestion tube followed by the addition of 10 mL of concentrated nitric acid. The digestion was performed setting the ramp temperature program: 15 min until 200 ºC with a power of 1200 W, following by additional 15 min at the same temperature and power conditions. After cooling down, the resulting solutions were diluted up to 50 mL with deionized water and analyzed by AAS, with prior treatment for specific elements. For the determination of K and Na, the sample was diluted in a cesium chloride solution (1 $g.L^{-1}$); for Ca and Mg, the sample was diluted

in a lanthanum chloride solution (1 $g.L^{-1}$); for Mn and Cu, a magnesium nitrate solution was used as a matrix modifier; and Fe and Zn were directly analyzed. The determination of elements was achieved by comparing the absorbance responses to pure analytical solutions. The analysis was performed in triplicate.

6.2. Chemical analysis

6.2.1. Fatty acids analysis

6.2.1.1. Fatty acids extraction

For the fatty acids extraction, the same procedure of fat content was preformed, and the total fatty acids content was expressed as a mass percentage. The analysis was performed in duplicate. The extract was kept at -20 º C for the fatty acids analysis by GC-MS.

6.2.1.2. Determination of the fatty acid composition by GC-FID

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID) (Obodai et al.,2017),based on the following trans-esterification procedure: fatty acids were methylated with 4450 µL of methanol: sulfuric acid: toluene 2:1:1 (v: v) and 550 µL of internal standard (pentanoic acid; 0,5 mg/mL), for at least 12 h in a bath at 50 °C and 160 rpm; then, 3 mL of deionized water was added, to obtain phase separation; the FAMEs were recovered with 3 mL of diethyl ether by shaking in vortex, and the upper phase was passed through a microcolumn of sodium sulfate anhydrous to eliminate the water; the sample was recovered in a vial with Teflon, and before injection, the sample was filtered with 0.2 µm nylon filter.

For the analysis of the fatty acid methyl esters (FAME) profile was used a YOUNG IN Chromass 6500 GC System instrument equipped with a split/splitless injector set at 250 ºC with a split ratio of 1:50, a flame ionization detector (FID) set at 260 ºC and a Zebron-Fame column (30 m × 0.25 mm ID × 0.20 µm df, Phenomenex, Lisbon, Portugal). The following oven temperature program was used: initial temperature of 100 ºC, held for 2 min, increase 10 ºC/min to 140 ºC, followed by a 3 ºC/min ramp to 190 ºC and 30 ºC/min ramp to 260 ºC. The carrier gas (hydrogen) flow rate was 1.2 mL/min, measured at 250

ºC. Fatty acids identification and quantification was performed by comparing the relative retention times of FAME peaks from samples with standards (standard mixture 47885-U, Sigma, St. Louis, USA) and results were recorded and processed using the Software Clarity DataApex 4.0 Software (Prague, Czech Republic) and expressed in relative percentage of each fatty acid.

6.2.2. Phenolic compounds

6.2.2.1 Phenolic compounds extraction

To perform the maceration extraction, 1 g of the previously crushed raw material was placed in a beaker with 30 mL of the mixture of ethanol: water in the proportion 80:20 (v/v). (Obodai, et al.,2017) Then, the sample was kept under magnetic stirring at a room temperature for 1h. Subsequently, the extracts obtained were filtered employing a Whatman No. 4 paper and collected. This extraction procedure was performed in triplicate. Afterwards, the ethanolic fraction was removed from the obtained extract employing a Heidolph rotary evaporator that operates at 40°C. Finally, the aqueous extract was placed in a plastic flask, frozen and lyophilized to obtain the dry extracts, which were stored at -80°C, until needed for analysis (FreeZone 4.5 model 7750031, Labconco, Kansas City, MO, USA) This procedure was performed in triplicates only for the fruit of the following samples DB1*pyrenaica*, DB2*pyrenaica* , DB3*pyrenaica*,DPB*pyrenaica* , DB3*ilex* and HFM*suber* .

6.2.2.2 Phenolic compounds characterization by LC-MS

The phenolic extracts were re-dissolved in ethanol/water (20:80, v/v; 1 mL), and filtered through 0.22-μm disposable LC filter disks. Phenolic compounds were separated and analyzed by high performance liquid chromatography (HPLC) on a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA), according to the method described by Barros et al. (2013) with some modifications. The detection was carried out in a diode array detector (DAD), with chromatograms processed at 280 nm and 370 nm, and in an Orbitrap Exploris 120 mass spectrometer (MS) (ThermoFinnigan, San Jose, CA, USA) equipped with an electrospray ionization (ESI) operating in negative mode. Spectra were acquired in full scan mode and MS2 in the m/z range 100–1500 with Xcalibur® software (ThermoFinnigan, San Jose, CA, USA).

Phenolic compounds were identified by comparing their retention time and their UV-Vis and mass spectra ($([M+H]^-$) and MS/MS fragments) with authentic standards, when available, or by comparison with available data in the literature. Quantification was performed with standard substance calibration curves for ellagic acid (y = 14957 x +14559; R^2 = 0.9985) and gallic acid (y = 45933x-19932; R^2 = 0.9996). When standards were not available, the compounds were expressed by equivalents of the structurally more similar phenolic compound.

RESULTS AND DISCUSSION

7. Results and discussion

7.1. Nutritional analysis

The nutritional parameters such as water content, ash content, proteins, total lipids, total carbohydrates, dietary fiber, minerals, fatty acids, and phenolic compounds were analyzed in different development stages of ***Quercus pyrenaica***. Also, samples of ***Quercus ilex*** in different development stages, as well as ***Quercus ilex*** and ***Quercus suber*** from a different geographical origin were analyzed for comparison of the results.

7.1.1. Water content

The results of water content of the cupules, shells, fruits, and seeds of the different species (*Quercus pyrenaica*, *Quercus ilex* and *Quercus suber*) are presented in Table 5.

Comparing the different development stages of *Quercus pyrenaica*, a decrease can be observed when going from the stage 1 to the stage 3 corresponding to the mature phase. The highest amount was observed for the fruit of DB1*pyrenaica* with (68.5±0.5 %), while the lower value was observed for the cupule in DB3*pyrenaica* (16.30±0.49%).

Generally, for *Q. pyrenaica*, in the mature stage, the fruit and seed presented higher water content, between (29.34-31.70%), when comparing to the cupule and shell (16.30-22.12%).

However, the same decrease was observed when going from the stage 1 to 3 in the mature stage of the sample *Quercus ilex.* The sample coded DB2*ilex* fruit presented the greatest value (72.44±0.62%) and the sample DB3*ilex* cupule had the lowest value (27.47±0.38%).

For HFM*ilex* and HFM*suber* in the mature stage, the fruit and seed presented

higher water content, respectively (34.40-37.84%) and (37.98-40.12%), when comparing to the cupule and shell (17.84-26.00%) and (19.74-27.66%).

These results were confirmed by the literature in which Vinha et al. (2016) found similar values in *Q.ilex* and *Q.suber* respectively (30±3) and (46±8) g/100g of fruit .As well as Castro et al. (2022) who identified the water content in *Q.pyrenaica* (42.0± 0.1) g/100g and *Q.ilex* (45.3± 0.3) g/100g .

Table 5. Water content of the samples according to different development stages, acorn parts and species (% of fresh weight).

	Water content (%)			
	Fruit	**Seed**	**Shell**	**Cupule**
DB1*pyrenaica*	68.50(±0.55)	67.27(±0.35)	64.88(±0.60)	47.08(±0.59)
DB2*pyrenaica*	46.36(±0.64)	47.66(±0.60)	25.76(±0.90)	31.22(±0.57)
DB3*pyrenaica*	29.34(±0.85)	31.70(±0.65)	18.81(±0.74)	16.30(±0.49)
DPB*pyrenaica*	30.72(±0.82)	29.69(±0.69)	22.12(±0.75)	17.27(±0.46)
DB1*ilex*	60.86(±0.73)	59.32(±0.35)	68.35(±0.20)	40.08(±0.78)
DB2*ilex*	72.44(±0.62)	68.55(±0.64)	72.13(±0.38)	48.31(±0.45)
DB3 *ilex*	49.36(±0.63)	50.91(±0.83)	49.21(±0.52)	27.47(±0.38)
HFM*ilex*	34.40(±0.26)	37.84(±0.65)	26.00(±0.84)	17.84(±0.35)
HFM*suber*	37.98(±0.29)	40.12(±0.60)	27.66(±0.44)	19.74(±0.35)

Each value is the mean ± standard deviation

7.1.2. Ash content

The results of ash content of the different acorn parts, and in different species (*Quercus pyrenaica, Quercus ilex* and *Quercus suber*) were analyzed after lyophilization and the results are presented in Table 6.

Concerning the ash content, the higher the value, the lower the organic matter content. The ash content also makes it possible to give an approximate estimate of the total mineral content present in the food matrix, being an important nutritional parameter. The values obtained for the *Quercus pyrenaica* varied between (1.48%) and (3.88%) of dw however for *Q. ilex* the ash content varied between (1.37-3.98%) of dw and *Q.suber* between (1.76-2.53%) of dw.

For *Q.pyrenaica* in the earlier stages of development(DB1*pyernaica* and

DB2*pyrenacia*) the cupule(2.35-3.88%) of dw and the shell (2.70-2.78%) of dw presented higher ash content comparing to seed (1.48-1.93%) of dw and the fruit (1.85-1.57%) of dw.

In the mature stages of development (DB3*pyernaica* and DPB*pyrenacia*) the cupule (2.86-2.75%) of dw and the seed (3.10-1.79%) of dw presented ash content comparing to shell (2.39-.1.52 % of dw) and the fruit (2.63-1.61%) of dw.

According to Silva e t al. (2016) who have characterized the acorn flour of *Q.ilex* and *Q. rotundifolia* and analyzed the ash content and found a very similar values for *Q.ilex* with (1.81± 0.09)g/100 g of dried fruit .

Vinha et al. (2016) and Castro et al. (2022) studied several species of *Quercus* and found the following results (2.2±0.1)g/100g for *Q.pyrenaica* , (2.5±0.4)g/100g for *Q.suber* and (2.1±0.2)g/100g for *Q.ilex.*

Table 6. Ash content of the samples according to different development stages, acorn parts and species, (% of dry weight).

	Ash Content (%)			
	Fruit	**Seed**	**Shell**	**Cupule**
DB1*pyrenaica*	1.85(±0.21)	1.48(±0.09)	2.70(±0.56)	2.35(±0.05)
DB2*pyrenaica*	1.57(±0.23)	1.93(±0.22)	2.78(±1.08)	3.88(±0.34)
DB3*pyrenaica*	2.63(±0.10)	3.10(±0.07)	2.39(±0.05)	2.86(±0.20)
DPB*pyrenaica*	1.61(±0.41)	1.79(±0.14)	1.52(±0.24)	2.75(±1.84)
DB1*ilex*	3.02(±0.31)	2.99(±0.14)	2.20(±0.28)	2.03(±0.21)
DB2*ilex*	3.37(±0.24)	2.90(±0.25)	3.98(±0.34)	3.51(±0.27)
DB3 *ilex*	2.64(±0.12)	2.82(±0.07)	2.33(±0.07)	1.37(±0.29)
HFM*ilex*	2.77(±0.49)	3.43(±0.04)	1.92(±0.66)	2.32(±0.55)
HFM*suber*	2.42(±0.75)	2.08(±0.08)	1.76(±0.34)	2.53(±0.04)

Results are expressed on the dry weight; each value is the mean ± standard deviation.

7.1.3. Protein content

The results of protein content of the cupules, shells, fruits, and seeds of the different species (*Quercus pyrenaica, Quercus ilex* and *Quercus suber*) are presented in Table 7.

Proteins are highly important constituents of foods, as they play an extremely important biological role, exerting functions at the structural, enzymatic, energetic, hormonal and defense. According to several studies *Quercus* is quite rich in protein.

Comparing the samples, the protein content of *Q.pyrenaica* varied between (9.18-2.73 g/100g of dw, however ,*Q.ilex* and *Q.suber* varied between (6.69-2.93) g/100g of dw and (6.31-5.41)g/100g of dw.

In the mature stages of *Quercus pyrenaica* the seed (5.69-6.03)g/100g of dw and the fruit (4.73-5.65)g/100g of dw presented a higher protein content than the shell (3.13-2.73) g/100g of dw and the cupule (3.71-3.22)g/100g of dw. While in the earlier stages, the shell (9.18-6.75)g/100g of dw and the seed (5.60-3.75)g/100g of dw showed better results than the cupule (4.66-3.65)g/100g of dw and the fruit (5.60-3.71)g/100g dw.

Silva et al., 2016 have found that *Quercus ilex* has a low protein content (4–5 %) when comparing the nutritional characterization of the flours with the results reported for acorns from other authors.

These data are similar to the data previously reported for holm oak populations in which Galván et al. (2011) have studied of variability in Holm oak (*Quercus ilex* subsp. ballota [Desf.] Samp.) by analyzing the acorn protein profile through SDS-PAGE and 2-DE coupled to mass spectrometry and the proteins were extracted from acorn flour showing a significant correlation with acorn weight, length and diameter, latitude and altitude date, and average monthly maximum temperature.

Özcan et al. (2006) studied the total protein and amino acid compositions in mature acorns of 20 *Quercus* taxa from Turkey in which *Quercus ilex* was evaluated finding that it has the lowest protein content in the average of 3.35% compared to *Quercus Cerris* (4.22%) and other *Quercus* section (5.11%) but in this study *Quercus pyrenaica* was not included in this evaluation.

Table 7. Protein content of the samples according to different development stages, acorn parts and species, (g/100g of dry weight).

	Protein content (g/100g)			
	Fruit	**Seed**	**Shell**	**Cupule**
DB1*pyrenaica*	5.60(±0.30)	5.60(±0.60)	9.18(±0.63)	4.66(±0.20)
DB2*pyrenaica*	3.71(±0.11)	3.75(±0.14)	6.75(±0.21)	3.65(±0.19)
DB3*pyrenaica*	4.73(±0.53)	5.69(±0.14)	3.13(±0.47)	3.71(±0.55)
DPB*pyrenaica*	5.65(±0.66)	6.03(±0.52)	2.73(±0.10)	3.22(±0.98)
DB1*ilex*	6.69(±0.26)	5.69(±0.20)	4.07(±0.11)	4.65(±0.55)
DB2*ilex*	5.21(±0.88)	3.70(±0.27)	6.21(±0.86)	3.09(±0.69)
DB3 *ilex*	5.50(±0.17)	4.18(±0.07)	5.51(±0.27)	4.47(±0.44)
HFM*ilex*	4.22(±0.59)	3.51(±0.27)	4.24(±0.50)	2.93(±1.10)
HFM*suber*	5.97(±0.25)	5.51(±0.37)	6.31(±0.96)	5.47(±0.16)

Results are expressed on the dry weight; each value is the mean ± standard deviation

7.1.4. Total lipid

The results of Total lipid of the cupules, shells, fruits, and seeds of the different species (*Quercus pyrenaica, Quercus ilex* and *Quercus suber*) are presented in Table 8.

Lipids are an important macronutrient that forms the nutritional composition of the acorns which are known of having a relevant quantity of lipids (8-14%) (Silva et al., 2016).

Acorrding to our analysis, the lipid content of *Q.pyrenaica* varied between (18.60-1.15%)of dw while *Q.ilex* varied between (9.44-0.53%) of dw and *Q.suber* varied between (6.43-0.45%) of dw .

Moreover, in the mature stages of *Q.pyrenaica* the fruit (4.16-4.30%) of dw and the seed (4.70-5.41%) of dw presented a higher lipid content than the shell (1.34-1.27%) of dw and the cupule(1.30-1.15 %) of dw .

In the earlier stages, the greatest values of lipid content were observed in the shell (18.60% of dw) and the cupule (8.32% of dw). However, the lowest lipid content was detected in the fruit (4.03% of dw and the cupule (5.93% of dw)

Castro et al. (2022) studied the effect of dehulling methods on the nutritional

composition of *Quercus pyrenaica*, *Quercus ilex* and *Quercus robur* who found that the seeds of *Quercus pyrenaica* and *ilex* have around (5.2g/100 g) and (4.7g/100 g) respectively which was in accordance with the results of the present study. Also, Silva et al. (2016) have identified the nutritional characterization of the different flours dry and roasted of *Q. rotundifolia* and *Q. ilex* which were collected at Herdade do Freixo do Meio (Montemor-o-Novo, Portugal) by studying the total lipids of both species finding that the dried flours of *Q. rotundifolia* and *Q. ilex* are respectively (8.44±0.32) and (13.41± 0.36) g/100 g , these values are quite higher than the lipid found in the sample coded HFM*ilex* which is collected from the same region within (3.52±0.63 %).

As well as Vinha et al. (2016) who have studied the chemical and antioxidant profiles of acorn tissues from *Q.faginea, Q.ilex, Q.nigra* and *Q.suber* were collected in Trás-os-Montesregio and have found a very similar results in *Q.suber* and *Q.ilex* .

Table 8.lipid content of the samples according to different development stages, acorn parts and species, (% of dry weight).

	Total lipid (%)			
	Fruit	**Seed**	**Shell**	**Cupule**
DB1*pyrenaica*	4.03(±0.21)	5.93(±0.58)	18.60(±0.59)	8.32(±0.24)
DB2*pyrenaica*	1.82(±0.04)	2.90(±0.32)	1.37(±0.22)	1.31(±0.06)
DB3*pyrenaica*	4.16(±0.15)	4.70(±0.08)	1.34(±0.04)	1.30(±0.13)
DPB*pyrenaica*	4.30(±0.15)	5.41(±0.21)	1.27(±0.14)	1.15(±0.14)
DB1*ilex*	7.03(±1.00)	5.88(±1.86)	9.44(±0.84)	2.68(±1.52)
DB2*ilex*	1.73(±0.24)	3.00(±0.20)	0.75(±0.05)	0.53(±0.06)
DB3*ilex*	5.49(±0.37)	9.13(±0.63)	1.53(±0.23)	1.84(±0.70)
HFM*ilex*	3.52(±0.63)	6.43(±0.26)	0.68(±0.21)	0.77(±0.20)
HFM*suber*	2.84(±0.23)	6.58(±0.26)	1.12(±0.05)	0.45(±0.40)

Results are expressed on the dry weight; each value is the mean ± standard deviation

7.1.5. Total carbohydrates and Energy:

The results of Total carbohydrates and Energy of the cupules, shells, fruits, and seeds of the different species (*Quercus pyrenaica, Quercus ilex* and *Quercus suber*) are presented in Table 9. and Table 10.

Acorns are recognized with their high carbohydrates content (75-85%) (Silva et al.,

2016). The results of proteins, lipids and ash were involved to determine the total carbohydrates of the three different species studies (*Q.pyrenaica, Q.ilex* , and *Q.suber*) and according to the results presented in table 9. *Q.pyrenaica* had a very high carbohydrates content in all stage of development. However, the highest and the lowest values were detected in the shell of the samples DPB*pyrenaica* and DB1*pyrenaica* respectively (94.7±0.2) and (53.6±0.8) g/100g of dw, otherwise the other samples and parts of the species *Q.pyrenaica* were very rich in carbohydrates with average of (86.8 ±0.74)g/100g of dw . Comparing the stage of development, the mature phase showed a higher carbohydrates content than the first and second stages specially for the cupule and shell increasing respectively from (78.7±0.5)g/100g of dw in DB1*pyrenaica* to (93.7±0.7)g/100g of dw in DB3*pyrenaica* and from (53.6±0.8)g/100g of dw in n DB1*pyrenaica* Shell to (91.4±0.6)g/100g of dw in DB3*pyrenaica*. Although, *Q.ilex* has shown a close values in all stage of development and the difference was not significant comparing to *Q.pyrenaica* , also the samples HFM*ilex* and HFM *suber* had similar results in all parts of the acorn.

Table 9.Total carbohydrates content of the samples according to different development stages, acorn parts and species, (g/100g of dry weight).

	Total carbohydrates (g/10)			
	Fruit	**Seed**	**Shell**	**Cupule**
DB1*pyrenaica*	86.3(±0.7)	83.1(±0.6)	53.6(±0.8)	78.7(±0.5)
DB2*pyrenaica*	92.6(±0.2)	90.4(±0.5)	92.8(±4.0)	93.7(±0.2)
DB3*pyrenaica*	86,9(±0.3)	84.9(±0.1)	94.2(±0.4)	93.7(±0.7)
DPB*pyrenaica*	85.7(±0.5)	83.1(±0.9)	94.7(±0.2)	94.5(±1.3)
DB1*ilex*	79,2(±1.8)	82.5(±3.7)	77.1(±1.6)	90.0(±3.6)
DB2*ilex*	92.6(±0.2)	90.3(±0.5)	91.3(±1.4)	95.8(±0.6)
DB3 *ilex*	83.5(±0.8)	77.5(±1.2)	91.4(±0.6)	95.8(±0.6)
HFM*ilex*	90.1(±1.0)	83.3(±0.5)	93.5(±0.6)	96.2(±1.5)
HFM*suber*	91.3(±1.4)	90.3(±0.7)	92.3(±1.3)	93.0(±0.3)

Results are expressed on the dry weight; each value is the mean ± standard deviation.

Meanwhile, having a high carbohydrates content means also having a high energetic value, the calculation results presented in table 10 showed that in general the acorn has a high energy content, and the results seems to be very close for all species and different parts. The highest value was detected in the sample coded DB1*pyrenaica* Shell

with (418.60±0.59) Kcal/100g of dw and the lowest value was detected in the sample HFM *ilex* Cupule (400.46±0.40) Kcal/100g of dw.

Comparing the stage of development, *Q.pyrenaica* had a higher energetic value compared to *Q.ilex* and *Q.suber* . In the first stage of development DB1*pyrenaica* had a greatest values in the cupule shell, fruit and a very close value in the seed. In the second stage, DB2*ilex* showed a lower energetic value than DB2*pyrenacia*. However, in the mature phase, DB3*ilex* had higher values than DB3*pyrenacia*. The seed of the different species studied showed the greatest values compared to other parts due to the high proteins, lipids and carbohydrates contents that contain.

The results obtained are superior to those found by Vinha et al. (2016) and Castro et al. (2022) for *Q.suber* and *Q.pyrenaica* but inferior for *Q.ilex*.

Table 10.Energetic value t of the samples according to different development stages, acorn parts and species, (Kcal/100g of dry weight).

	Energetic value (Kcal/100g)			
	Fruit	**Seed**	**Shell**	**Cupule**
DB1*pyrenaica*	404.03(±0.21)	405.93(±0.58)	418.60(±0.59)	408.32 (±0.24)
DB2*pyrenaica*	401.82(±0.05)	402.91(±0,33)	401.37(±0.22)	401.32(±0.06)
DB3*pyrenaica*	404.17(±0.15)	404.70(±0.08)	401.35(±0.05)	401.31(±0.13)
DPB*pyrenaica*	404.31(±0.15)	405.42(±0.21)	401.28(±0.14)	401.16(±0.14)
DB1*ilex*	407.03(±1.00)	405.89(±1.86)	409.44(±0.84)	402.68(±1.52)
DB2*ilex*	401.73(±0.25)	403.01(±0.20)	400.76 (±0.05)	400.53(±0.07)
DB3 *ilex*	405.50(±0.38)	409.14(±0.63)	401.53(±0.24)	401.85(±0.70)
HFM*ilex*	402.85(±0.23)	406.58(±0.26)	401.12(±0.06)	400.46(±0.40)
HFM*suber*	403.52(±0.63)	406.43(±0.27)	400.68(±0.22)	400.78(±0.21)

Results are expressed on the dry weight,each value is the mean ± standard deviation

7.1.6. Dietary fiber

The results of dietary fiber for the different acorn parts and different species (*Quercus pyrenaica, Quercus ilex* and *Quercus suber*) are presented in Table 11.

The results of fiber content showed that the species *Q.pyrenaica*, *Q.ilex* and *Q.suber*

that have been studied are rich in fiber. According to the analysis, *Q.pyrenaica* showed a fiber content values between (65.36-16.48%) of dw , *Q.ilex* between(68.52-13.94%) of dw and *Q.suber* between (42-20.37%) of dw.

In tha mature stages *Q.pyrenaica* presented the highest value in the sample coded DB3*pyrenaica* shell (41.08% of dw)while the sample coded DPB*pyrenaica* cupule showed the lowest fiber content (20.22% of dw) . Meanwhile, in the earlier stages DB2*pyrenaica* fruit reveled the greatest fiber content (66.36% of dw) and the lowest value was detected in the cupule of the same sample (16.48% of dw).

These results are superior to the results found by Silva et al. (2016) and Castro et al. (2022) who studied *Q.ilex* respectively (10.89 g/100 g of dry fruit) and (31.4 ± 1.0 g/100 g of dry fruit) also *Q.pyrenaica* (36.7 ± 0.9 g/100 g of dry fruit).

Table 11. Dietary fiber content of the samples according to different development stages, acorn parts and species, (% of dry weight).

	Dietary fiber (%)			
	Fruit	**Seed**	**Shell**	**Cupule**
DB1*pyrenaica*	42.96(±0.5)	20.72(±0.3)	50.96(±0.6)	17.20(±0.4)
DB2*pyrenaica*	65.36(±0.6)	36.52(±0.6)	60.80(±0.7)	16.48(±0.5)
DB3*pyrenaica*	30.64(±0.2)	20.70(±0.6)	41.08(±0.7)	22.48(±0.4)
DPB*pyrenaica*	31.52(±0.5)	26.96(±0.5)	26.88(±0.7)	20.22(±0.4)
DB1*ilex*	56.08(±0.7)	27.53(±0.3)	68.52(±0.2)	27.36(±0.7)
DB2*ilex*	52.20(±0.6)	39.44(±0.6)	45.52(±0.3)	26.96(±0.4)
DB3 *ilex*	47.40(±0.6)	13.37(±0.8)	30.48(±0.5)	36.40(±0.3)
HFM*ilex*	22.60(±0.2)	13.94(±0.6)	39.96(±0.8)	51.68(±0.8)
HFM*suber*	20.37(±0.2)	31.32(±0.6)	38.41(±0.4)	42.00(±0.3)

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7.1.7. Minerals

The results of minerals of the cupules, shells, fruits, and seeds of the different

species (*Quercus pyrenaica, Quercus ilex* and *Quercus suber*) are presented in Table 12.

Minerals are so important in human's diet. Having useful minerals and lower heavy metal concentrations, acorn could be consumed in safe and probable with health benefits (Sekeroglu et al.,2017).

According to our analysis heavy elements such as Pb, Cd, Co, and Mo were not detected and only macronutrients (Ca, K and Mg), micronutrients (Fe, Zn, Cu and Mn) and light metals (Na) where present in the samples.

As the results presented in table 12 showed, the samples were quite rich in macronutrients and low in metal and micronutrient except Mn which showed a significant value in all parts of the acorns. All the species (Q.*pyrenaica ,Q ilex and Q suber*) that have been studied presented very close and similar results ,but *Q.pyrenaica* and *Q.suber* had a bit greater values comparing to *Q.ilex.*

On hand in the mature phase, DPB*pyrenaica* seed had the highest value in K (7.02±0.37 mg/g of dw), while the cupule of the same sample was rich Ca (3.78 ±0.3mg/g of dw) .

On the other hand, in the earlier stage DB1*pyrenaica* shell had the greatest value of Mg reaching (4.38±1.05)mg/g of dw and DB2*pyrenaica* cupule had the highest value of Na with (0.97±0.01) mg/g of dw. However, the mature and the earlier stages showed very low values of Zn and Cu.

The species *Q. ilex and Q. suber* had very similar results to *Q pyrenaica,*. These results seem to be in accordance with the previously reported in the literature from Rakic et al. (2006), Sekeroglu et al. (2017) and Castro et al. (2022) in which they evaluated the minerals in different species such as *Q. robur*, *Q. Coccifera L*, *Q. pyrenaica*, and *Q. ilex.*

Rakic et al. (2006) detected considerable amounts of Fe, Cu, Zn, and Mn, and lower content in Ca, Mg, P, and K in *Q. robur* acorn samples. Sekeroglu et al. (2017) have studied the mineral composition of acorn coffees from *Q. Coccifera L* finding that the samples have a higher calcium concentration than P, Mg and S.

Finally, according to Castro et al. (2022) who studied the unraveling the effect of

dehulling methods on the nutritional composition of acorn *Quercus spp* ,and characterized *Q.pyrenaica* and *Q.ilex* detecting very similar values of minerals to our analysis results in Zn (0.6 mg/100g of dw) , Mn (2.4mg/100g of dw) , Fe (1.1 mg/100g of dw) , Mg (32.6 mg/100g of dw) , Ca (26.7 mg/100g of dw) , Cu (0.5 mg/100g of dw) , Na (4.8 mg /100g of dw) and K (557 mg/100g of dw) .

Table 12. Minerals content of the samples according to different development stages, acorn parts and species, (mg/g of dry weight).

	DB1*pyrenaica*				**DB2*pyrenaica***				**DB3*pyrenaica***			
mg /g	Fruit	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**
[K]	6,62± 0,44	5,94± 0,56	5,98± 0,49	4,91(± 0,06)	6,66± 0,15	6,18± 0,43	5,53± 0,19	4,97± 0,14	6,37± 0,49	4,92± 1,09	3,23± 0,32	4,47± 0,52
[Na]	0.43± (0.01)	0.45(± 0.02)	0.41(± 0.01)	0.40(± 0.06)	0.56(± 0.08)	0.56(± 0.06)	0.56(± 0.01)	0.97(± 0.01)	0.57(± 0.01)	0.77(± 0.05)	0.56(± 0,03)	0.54(± 0.03)
[Ca]	2,36± 0,13	1,06± 0,06	3,42± 0,20	3,48± 0,28	1,56± 0,17	1,44± 0,67	2,78± 0,25	3,72± 0,35	1,48± 0,14	2,63± 0,32	2,81± 0,17	3,73± 1,37
[Mg]	3,86± 0,25	3,30± 0,13	4,38± 1,05	4,08± 0,01	3,62± 0,29	3,38± 0,13	3,93± 0,33	3,93± 0,33	3,53± 0,40	3,59± 0,04	3,77± 0,25	3,53± 0,49
[Fe]	0.21(±0.008)	0.26(±0.004)	0.20(±0.002)	0.05(±0.004)	0.15(±0.001)	0.13(±0.006)	0.16(±0.001)	0.58(±0.002)	0.35(±0.008)	0.26(± 0.017)	0.81(±0.005)	1.73(±0.003)
[Mn]	1.67(±0.02)	0.44(±0.02)	3.01(±0.08)	2.88(±0.05)	0.82(±0.07)	0.41(± 0.08)	1.80(±0.05)	3.04(±0.03)	0.60(±0.06)	0.83(±0.04)	1.75(±0.07)	3.56(±0.02)
[Cu]	0.009(±0.0004)	0.007(±0,0008)	0.013(±0,0005)	0.007(±0,0004)	0.006(±0,0003)	0.006(±0,0001)	0.007(±0,0003)	0.005(±0.0002)	0.006(±0.0002)	0.007(±0.0004)	0.015(±0,0006)	0.005(±0.0005)
[Zn]	0.01(±0.003)	0.01(±0.001)	0.02(±0,003)	0.01(±0.003)	0.02(±0,001)	0.01(±0.001)	0.02(±0.005)	0.01(±0.008)	0.01(±0.006)	0.02(±0,001)	0.01(±0,004)	0.02(±0.002)
	DB1*ilex*				**DB2*ilex***				**DB3*ilex***			
mg /g	Fruit	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**
[K]	5,22± 0,43	5,11± 0,20	5,42± 0,52	5,17± 0,75	4,90± 0,21	5,04± 0,60	5,29± 0,76	5,18± 0,62	5,00± 0,71	5,44± 0,52	3,19± 1,65	5,02± 0,22
[Na]	0.04(± 0.03)	0.41(± 0.01)	0.40(± 0.01)	0.41(± 0.02)	0.41(±0.01)	0.39(± 0.07)	0.41(± 0.01)	0.41(± 0.02)	0.41(± 0.02)	0.39(± 0.02)	0.39(± 0.03)	0.40(± 0.01)
[Ca]	2,01± 0,89	2,16± 1,03	2,00± 1,34	2,08± 1,18	2,02± 1,50	1,80± 1,40	2,71± 1,22	2,90± 0,73	1,61± 0,26	1,22± 0,28	1,87± 0,59	3,45± 0,12
[Mg]	3,02± 0,51	3,16± 0,33	2,74± 0,71	2,89± 1,05	3,22± 1,02	3,14± 0,93	3,52± 0,81	3,16± 0,55	3,49± 0,22	4,14± 0,53	3,46± 0,50	3,98± 0,33
[Fe]	0.31(±0.009)	0.23(±0.004)	0.29(±0.002)	0.24(±0.004)	0.33(±0.009)	0.23(±0.003)	0.37(±0.007)	0.30(±0.006)	0.33(±0.008)	0.33(±0.004)	0.30(±0.001)	1.37(±0.003)
[Mn]	1.48(±0.02)	1.73(±0.02)	2.00(±0.09)	1.92(±0.09)	0.87(±0.08)	0.74(±0.01)	3.45(±0.03)	1.62(±0.05)	0.85(±0.04)	0.38(±0.01)	1.52(±0.01)	2.74(±0.02)
[Cu]	0.007(±0.0006)	0.008(±0.0006)	0.008(±0,0005)	0.009(±0.0006)	0.007(±0.0001)	0.007(±0.0001)	0.008(±0.0001)	0.008(±0.0007)	0.008(±0.0002)	0.009(±0.0005)	0.007(±0.0002)	0.009(±0.0002)
[Zn]	0.01(±0.003)	0.02(±0.006)	0.01(±0.001)	0.01(±0.001)	0.01(±0.002)	0.01(±0.002)	0.01(±0.004)	0.01(±0.003)	0.01(±0.001)	0.01(±0.003)	0.01(±0.003)	0.01(±0.003)

	DPB*pyrenaica*				HFM*ilex*				HFM*suber*			
mg /g	Fruit	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**
[K]	7,02± 0,37	4,92± 1,47	6,56± 0,45	5,98± 1,25	6,90± 2,39	8,68± 0,39	6,15± 2,26	3,43± 0,17	7,50± 0,40	7,98± 0,89	5,82± 0,83	8,75± 1,37
[Na]	0.56(±0.03)	0.86(± 0.03)	0.40(± 0.01)	0.41(± 0.07)	0.56(± 0.02)	0.57(± 0.03)	0.52(± 0.06)	0.51(± 0.02)	0.55(±0.07)	0.64(± 0.08)	0.61(± 0.03)	0.94(± 0.03)
[Ca]	1,12± 0,24	2,63± 0,98	1,92± 1,86	3,78± 0,30	1,51± 0,75	1,33± 0,10	1,98± 1,72	3,32± 0,71	1,93± 0,21	1,22± 0,42	1,67± 0,52	1,58± 0,65
[Mg]	3,14± 0,19	3,59± 0,23	3,19± 0,10	3,69± 0,36	3,13± 0,11	3,20± 0,16	3,17± 0,26	3,53± 0,02	3,29± 0,11	3,01± 0,05	4,17± 1,81	3,14± 0,17
[Fe]	0.44(±0.004)	0.26(±0.005)	0.18(±0,004)	1.33(±0.003)	0.50(±0.001)	0.18(±0,002)	1.41(±0.006)	0.69(±0.002)	0.23(±0.002)	0.30(±0.003)	0.35(±0.003)	0.57(±0.001)
[Mn]	0.29(±0.07)	1.07(±0.08)	0.19(±0.01)	2.30(±0.10)	0.62(±0.05)	0.46(±0.04)	2.31(±0.03)	2.93(±0.01)	0.85(±0.06)	0.74(±0.05)	1.39(±0.10)	1.13(±0.03)
[Cu]	0.009(±0,0006)	0.007(±0.0001)	0.008(±0.0001)	0.006(±0.0001)	0.006(±0.0001)	0.005(±0,0001)	0.007(±0.0001)	0.006(±0.0007)	0.005(±0.0001)	0.005(±0.0001)	0.005(±0.0001)	0.0005(±0.0001)
[Zn]	0.05(±0.001)	0.02(±0.004)	0.01(±0.006)	0.01(±0.004)	0.09(±0.001)	0.05(±0.002)	0.08(±0.002)	0.06(±0.001)	0.03(±0.003)	0.02(±0.002)	0.06(±0.001)	0.02(±0.002)

Results are expressed on the dry weight; each value is the mean ± standard deviation

7.2. Chemical analysis

7.2.1. Fatty acid

The results of fatty acid of the cupules, shells, fruits, and seeds of the different species (*Quercus pyrenaica, Quercus ilex* and *Quercus suber*) are presented in Tables 13,14 and 15.

The results of our analysis showed that the three species that have been studied (*Q.pyrenacia, Q.ilex* and *Q.suber*) are very rich with fatty acids , and the most abundant were C16:0(palmitic acid), C18:1n9c (oleic acid) and C18:0(Stearic acid).

The distribution of the fatty acids was very different depending on the stage of development and the specie, In the earlier stage of *Q.pyrenaica* ,the sample coded DB1*pyrenaica* presented a mixture of saturated and unsaturated acid with different proportions and the most abundant are C16:0 (palmitic acid)(59,47±0.01%) and C18:0(Stearic acid) (10,05±0.02%). In the mature phase, DB3*pyreniaca* showed a better percentage of the C16:0 in the fruit than in DB2*pyrenaica* (55,17±0,9%). Regarding the saturated fatty acids (SFA) in the earlier and the mature stages the fruit had the greatest values with (2.71±0.1%) and (101.47±0.32%), respectively. For monounsaturated fatty acid (MUFA) in the earlier and the mature stages the seed showed the highest percentage with (29.49±0.8) and (49.97±0.05), respectively. However, the polyunsaturated fatty acids (PUFA) were only present in the sample DPB*pyrenaica in* the fruit , seed and shell. The total fatty acids (TFAs) in the earlier and the mature stages the fruit had the greatest values with 96.23±0.09 and 159.15±0.17%, respectively.

Akan et al. (2017) has reported the same fatty acids in his study of the acorn ‘species as a novel source of oleic acid and tocopherols for livestock and humans finding the most abundant fatty acid was C18:1n9 (57.45%) which is higher than the percentage found in our samples, also detected the palmitic acid (12.17%) that is inferior to our results.

The table 14 shows the percentage of the fatty acids that were detected in the specie *Q.ilex* in all stage of development in which thirteen fatty acids were quantified (C12:0, C14:0, C15:0, C16:0, C18:0,C18:1n9c, C18:2n6c, C18:3n3, C20:00,C20:1,C22:0, C23:0 and C24:0) . C16:0 was very abundant in the fruit of the earlier stages between (38,36- 51,55%),and with lower concentration in the mature stages C18:1n9c and

C18:2n6c were also present in all stage of development but with higher percentage in the earlier stages .

As regards, in the earlier stage Q.*ilex* presented a greatest value of SFA (68.51±0.02%) and TFAs (112.01±0.01%) respectively in the shell and the fruit .Although , in the mature stage the seed presented the highest MUFA with (52.57±0.03%) and the greatest PUFA percentage in shell (17.45±0.01%).

For the *Q.suber*, the cupule had the greatest values SFA with (48.56 ±0.00%) and the lowest in the fruit with (8.82±0.02%). The seed showed the highest percentage of MUFA with (52.57±0.03%) and the lowest in the cupule (7.34±0.00%), The PUFA was present in a great percentage in the shell (8.44± 0.00 %). Thus, the seed had the highest TFAs value with 85.59±0.15% and the lowest value was present in the shell with (56.91±0.01%).

These results were similar to those found by Castro et al. (2022) who studied unraveling the effect of dehulling methods on the nutritional composition of acorn after detecting a high percentage of palmitic acid in *Q.pyrenaica* and *Q.ilex* no matter was the dehulling method but C18:1n9c and C18:2n6c were not detected .

C16:0 was found very abundant in *Q. pontica Q. robur Q. hartwissiana Q. frainetto Q. petraea Q. vulcanica and Q. pubesce*ns reaching 22.8% also C18:1n9c and C18:2n6c were quantified by Özcan (2007) in his characterization of Turkish *Quercus* species based on their fatty acid compositions.

Akan et al. (2017) confirmed the presence of C18:1n9t and C18:1n9cin Q.*suber* with very similar percentage (56.25%) which was detected in the seed of HFM*ilex* (55,37±0,1%).

Table 13. Fatty acid of the different acorn parts from samples DB1*pyrenaica*, DB2*pyrenaica* and DB3*pyrenaica* (%).

	DB1*pyrenaica*				**DB2*pyrenaica***				**DB3*pyrenaica***			
%	**Fruit**	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**
C12:0	0,57(±0,01)	0,37(±0,00)	0,47(±0,04)	0,42(±0,01)	0,12(±0,01)	0,12(±0,01)	0,82(±0,00)	1,48(±0,04)	nd	nd	nd	nd
C13:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C14:0	1,81(±0,00)	1,10(±0,01)	1,46(±0,08)	1,28(±0,01)	0,45(±0,01)	0,32(±0,00)	2,74(±0,00)	5,08(±0,00)	0,16(±0,00)	0,24(±0,01)	0,27(±0,00)	2,76(±0,01)
C14:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C15:0	1,27(±0,01)	0,60(±0,07)	0,94(±0,01)	0,77(±0,02)	0,3(±0,03)	0,22(±0,07)	1,89(±0,70)	2,66(±0,4)	0,25(±0,05)	0,21(±0,01)	0,2(±0,00)	0,25
C15:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C16:0	59,47(±0,01)	33,98(±0,07)	46,72(±0,07)	40,35(±0,01)	21,00(±0,00)	19,93(±0,8)	50,34(±0,4)	53,44(±0,00)	55,17(±0,9)	41,06(±0,6)	51,02(±0,01)	47,03(±0,01)
C16:1	0,13(±0,00)	0,36(±0,05)	0,25(±0,03)	0,31(±0,01)	0,73(±0,01)	0,21(±0,9)	1,77(±0,00)	2,72(±0,00)	0,26(±0,51)	0,28(±0,05)	0,2(±0,01)	0,19(±0,01)
C17:0	1,06(±0,02)	0,49(±0,01)	0,7(±0,01)	0,63(±0,09)	0,29(±0,01)	0,16(±0,00)	8,57(±0,065)	11,23(±0,70)	8,47(±0,01)	9,67(±0,1)	17,67(±0,15)	24,87(±0,07)
C17:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C18:0	10,05(±0,02)	4,32(±0,08)	7,18(±0,08)	5,75(±0,11)	41,99(±0,6)	41,20(±0,6)	4,01(±0,00)	8,39(±0,04)	45,02(±0,01)	44(±0,01)	8,37(±0,01)	6,35(±0,01)
C18:1n9c	7,12(±0,01)	18,88(±0,01)	13,00(±0,8)	15,94(±0,01)	24,39(±0,01)	29,28(±0,00)	2,58(±0,00)	7,52(±0,01)	39,54(±0,11)	41,53(±0,81)	28,00(±0,01)	9,57(±0,00)
C18:2n6c	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C18:3n6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C18:3n3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C20:0	0,81(±0,21)	1,12(±0,4)	nd	nd	1,2(±0,01)	0,98(±0,09)	nd	nd	1,12(±0,01)	1,55(±0,01)	nd	nd
C20:3n3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C20:1	0,81(±0,01)	1,12(±0,00)	nd	nd	0,68(±0,01)	0,66(±0,00)	nd	nd	2,357(±0,01)	2,37(±0,01)	nd	nd
C22:0	4,64(±0,00)	2,40(±0,41)	nd	nd	1,25(±0,01)	1,08(±0,00)	nd	nd	2,57(±0,01)	1,12(±0,00)	nd	nd
C23:0	1,51(±0,1)	0,92(±0,01)	nd	nd	0,40(±0,01)	0,26(±0,74)	nd	nd	1,64(±0,22)	1,55(±0,00)	nd	nd
C24:0	6,98(±0,5)	1,62(±0,01)	nd	nd	0,82(±0,01)	0,55(±0,00)	nd	nd	2,60(±0,05)	2,24(±0,30)	nd	nd
SFA	72,71(±0,1)	40,89(±0,03)	55,83(±0,06)	47,8(±0,03)	64,76(±0,12)	62,55(±0,47)	57,91(±0,4)	68,39(±0,04)	101,47(±0,32)	86,85(±0,23)	59,66(±0,01)	56,14(±0,02)
MUFA	7,25(±0,01)	19,24(±0,04)	13,25(±0,43)	16,25(±0,01)	25,12(±0,01)	29,49(±0,8)	4,35(±0,4)	10,24(±0,01)	39,8(±0,5)	41,81(±0,7)	28,2(±0,01)	9,76(±0,01)
PUFA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
TFAs	96,23(±0,09)	67,28(±0,1)	70,72(±0,4)	65,45(±0,03)	93,62(±0,06)	94,97(±0,4)	72,72(±0,29)	92,52(±0,28)	159,15(±0,17)	145,82(±0,19)	105,73(±0,03)	91,02(±0,06)

Results are expressed on the dry weight; each value is the mean ± standard deviationSFA: Saturated fatty acids; MUF: Monounsaturated fatty acids; PUFA :Polyunsaturated fatty acids ;TFAs: Total Fatty acids. C12:0: Lauric acid, C13:0: Tridecylic acid, C14:0: Myristic acid, C14:1: Myristoleic acid, C15:0: Pentadecylic acid, C16:0: Palmitic acid, C16:1: Palmitoleic acid, C17:0: Margaric acid, C18:0: Stearic acid, C18:1n9c: Oleic acid, C18:2n6c: Linoleic acid, C18:3n6: γ-Linolenic acid, C18:3n3: α-linolenic acid, C20:0: Arachidic acid, C20:3n3: Eicosatrienoic acid, C20:1: Eicosenoic acid, C22:0: Behenic acid, C23:0: Tricosylic acid, C24:0: Lignoceric acid

Table 14. Fatty acid of the different acorn parts of samples DB1*ilex*, DB2*ilex* and DB3*ilex* (%).

	DB1*ilex*				DB2*ilex*				DB3*ilex*			
	Fruit	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**
C12:0	0,67(±0,01)	0,45(±0,50)	1,43(±0,01)	1,26(±0,00)	nd	nd	nd	nd	nd	nd	nd	nd
C13:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C14:0	1,44(±0,00)	1,27(±0,10)	2,14(±0,00)	2,26(±0,00)	1,21(±0,01)	0,47(±0,01)	0,84(±0,01)	0,56(±0,00)	nd	nd	nd	nd
C14:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C15:0	1,06(±0,00)	0,74(±0,80)	2,01(±0,00)	1,34(±0,01)	1,36(±0,00)	0,32(±0,06)	0,84(±0,00)	0,5(±0,06)	nd	nd	nd	nd
C15:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C16:0	38,36(±0,00)	38,15(±0,00)	51,37(±0,04)	46,69(±0,30)	51,55(±0,01)	30,46(±0,00)	41,01(±0,01)	42,55(±0,01)	5,86(±0,00)	0,67(±0,7)	0,41(±0,00)	0,14(±0,00)
C16:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C17:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C17:1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C18:0	6,63(±0,00)	6,62(±0,00)	13,57(±0,00)	1,40(±0,00)	7,39	5,13(±0,01)	6,26(±0,3)	7,21	2,45(±0,00)	2,47(±0,01)	8,96(±0,02)	20,37(±0,00)
C18:1n9c	13,91(±0,01)	14,90(±0,00)	4,14(±0,00)	11,68(±0,2)	31,94	31,94(±0,00)	31,94(±0,00)	30,25(±0,00)	45,31(±0,00)	52,57(±0,03)	18,39(±0,01)	7,34(±0,00)
C18:2n6c	21,78(±0,01)	20,64(±0,00)	10,06(±0,00)	6,20(±0,00)	12,84(±0,00)	10,74(±0,09)	33,98(±0,00)	44,25(±0,00)	25,75(±0,00)	27,50(±0,00)	8,93(±0,00)	18,39(±0,01)
C18:3n6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C18:3n3	6,65(±0,01)	5,61(±0,00)	1,62(±0,00)	10,08(±0,00)	nd	nd	nd	nd	0,49(±0,01)	0,35(±0,00)	8,44(±0,00)	nd
C20:0	3,40(±0,21)	3,66(±0,00)	7,13(±0,00)	2,19(±0,00)	2,13	2,13(±0,02)	2,13(±0,00)	2,12(±0,07)	1,52(±0,08)	1,65(±0,01)	nd	nd
C20:3n3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C20:1	nd(±0,1)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C22:0	3,16(±0,01)	3,30(±0,8)	nd	12,51(±0,07)	1,93(±0,00)	1,19(±0,02)	1,56(±0,00)	1,46(±0,00)	nd	nd	nd	nd
C23:0	0,79(±0,01)	0,98(±0,00)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C24:0	2,16(±0,00)	2,65(±0,02)	nd	Nd	1,66(±0,00)	0,74(±0,02)	1,20(±0,02)	1,10(±0,00)	0,51(±0,02)	0,38(±0,02)	11,78(±0,00)	28,05(±0,00)
SFA	49,26(±0,01)	49,14(±0,2)	68,51(±0,02)	51,61(±0,00)	61,81(±0,01)	36,8(±0,01)	49,31(±0,08)	51,42(±0,01)	8,82(±0,02)	3,52(±0,24)	21,15(±0,02)	48,56(±0,00)
MUFA	13,91(±0,01)	14,9(±0,00)	4,14(±0,00)	11,68(±0,2)	31,94(±0,00)	31,94(±0,00)	31,94(±0,00)	30,25(±0,00)	45,31(±0,00)	52,57(±0,03)	18,39(±0,1)	7,34(±0,00)
PUFA	6,65(±0,01)	5,61(±0,00)	1,62(±0,00)	10,08(±0,00)	nd	nd	nd	nd	0,49(±0,01)	0,35(±0,00)	8,44(±0,00)	nd
TFAs	100,01(±0,04)	98,97(±0,44)	93,47(±0,02)	95,61(±0,14)	112,01(±0,01)	83,12(±0,03)	119,76(±0,08)	130(±0,04)	81,89(±0,03)	85,59(±0,15)	56,91(±0,01)	74,29(±0,01)

Results are expressed on the dry weight; each value is the mean ± standard deviation.SFA : Saturated fatty acids; MUF: Monounsaturated fatty acids; PUFA :Polyunsaturated fatty acids ;TFAs: Total Fatty acids. C12:0: Lauric acid, C13:0: Tridecylic acid, C14:0: Myristic acid, C14:1: Myristoleic acid, C15:0: Pentadecylic acid, C16:0: Palmitic acid, C16:1: Palmitoleic acid, C17:0: Margaric acid, C18:0: Stearic acid, C18:1n9c: Oleic acid, C18:2n6c: Linoleic acid, C18:3n6: γ-Linolenic acid, C18:3n3: α-linolenic acid, C20:0: Arachidic acid, C20:3n3: Eicosatrienoic acid, C20:1: Eicosenoic acid, C22:0: Behenic acid, C23:0: Tricosylic acid, C24:0: Lignoceric acid

Table 15. Fatty acid of the different acorn parts of samples DPB*pyrenaica* ,HFM*ilex* and HFM*suber* (%).

	DPB*pyrenaica*				HFM*ilex*				HFM*suber*			
	Fruit	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**	**Fruit**	**Seed**	**Shell**	**Cupule**
C12:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C13:0	nd	nd	nd	nd	0,41(±0,00)	0,28(±0,01)	nd	nd	0,42(±0,06)	0,24(±0,03)	nd	nd
C14:0	0,16(±0,01)	0,13(±0,01)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C14:1	nd	nd	nd	nd	0,42(±0,01)	0,17(±0,08)	4,81(±0,01)	nd	nd	nd	nd	nd
C15:0	0,13(±0,01)	0,11(±0,01)	16,42(±0,00)	19,31(±0,1)	nd	nd	nd	nd	nd	nd	nd	nd
C15:1	nd	nd	nd	nd	11,95(±0,00)	10,22(±0,03)	30,41(±0,08)	44,55(±0,01)	9,57(±0,07)	8,02(±0,01)	16,21(±0,03)	11,88(±0,08)
C16:0	17,48(±0,1)	17,07(±0,00)	nd	nd	nd	nd	nd	nd	11,02(±0,01)	2(±0,00)	nd	nd
C16:1	1,19(±0,00)	0,26(±0,01)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C17:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C17:1	nd	nd	nd	nd	2,98(±0,00)	13,48(±0,03)	nd	15,13(±0,00)	2,21(±0,09)	3,52(±0,01)	5,94(±0,05)	7,25(±0,01)
C18:0	2,66(±0,00)	2,29(±0,01)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C18:1n9c	47,86(±0,07)	49,71(±0,1)	28,47(±0,01)	35,93(±0,01)	38,89(±0,1)	32,38(±0,01)	8,10(±0,03)	12,68(±0,00)	43,00(±0,01)	55,37(±0,1)	46,28(±0,01)	55,32(±0,00)
C18:2n6c	0,19(±0,07)	0,16(±0,01)	26,94(±0,00)	38,82(±0,01)	nd	nd	nd	nd	28,26(±0,01)	30,64(±0,1)	31,56(±0,00)	36,99(±0,00)
C18:3n6	26,23(±0,01)	26,84(±0,00)	6,66(±0,00)	nd	4,79(±0,00)	2,16(±0,00)	6,65(±0,03)	nd	4,34(±0,01)	3,10(±0,00)	nd	nd
C18:3n3	2,07(±0,01)	1,89(±0,01)	2,12(±0,01)	nd	0,79(±0,01)	0,48(±0,01)	10,80(±0,01)	nd	nd	nd	nd	nd
C20:0	0,55(±0,06)	0,48(±0,6)	nd	nd	1,54(±0,11)	1,71(±0,01)	nd	nd	nd	nd	nd	nd
C20:3n3	nd	nd	nd	nd	1,06(±0,02)	0,44(±0,01)	nd	27,62(±0,00)	1,14(±0,01)	0,61(±0,01)	nd	nd
C20:1	0,45(±0,01)	0,43(±0,01)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C22:0	0,42(±0,41)	0,25(±0,05)	nd	nd	0,67(±0,01)	0,27(±0,0)	nd	nd	nd	nd	nd	nd
C23:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
C24:0	0,36(±0,01)	0,2(±0,05)	nd	nd	1,16(±0,01)	0,51(±0,01)	nd	nd	nd	nd	nd	nd
SFA(%)	20,66(±0,04)	19,69(±0,02)	nd	nd	1,16(±0,01)	0,51(±0,01)	nd	nd	11,02(±0,01)	2(±0,00)	nd	nd
MUFA(%)	49,05(±0,00)	49,97(±0,05)	28,47(±0,01)	35,93(±0,01)	38,89(±0,1)	32,38(±0,01)	8,1(±0,03)	12,68(±0,00)	43(±0,01)	55,37(±0,1)	46,28(±0,01)	55,32(±0,00)
PUFA(%)	28,3(±0,01)	28,73(±0,01)	8,78(±0,01)	nd	5,58(±0,01)	2,64(±0,01)	17,45(±0,01)	nd	4,34(±0,01)	3,1(±0,00)	nd	nd
TFAs	99,33(±0,07)	80,61(±0,07)	117,86(±0,00)	94,06(±0,05)	64,66(±0,03)	62,1(±0,02)	60,77(±0,03)	99,98(±0,01)	99,54(±0,03)	101,26(±0,04)	99,99(±0,03)	111,44(±0,04)

Results are expressed on the dry weight; each value is the mean ± standard deviation

SFA : Saturated fatty acids; MUF: Monounsaturated fatty acids; PUFA :Polyunsaturated fatty acids ;TFAs: Total Fatty acids. C12:0: Lauric acid, C13:0: Tridecylic acid, C14:0: Myristic acid, C14:1: Myristoleic acid, C15:0: Pentadecylic acid, C16:0: Palmitic acid, C16:1: Palmitoleic acid, C17:0: Margaric acid, C18:0: Stearic acid, C18:1n9c: Oleic acid, C18:2n6c: Linoleic acid, C18:3n6: γ-Linolenic acid, C18:3n3: α-linolenic acid, C20:0: Arachidic acid, C20:3n3: Eicosatrienoic acid, C20:1: Eicosenoic acid, C22:0: Behenic acid, C23:0: Tricosylic acid, C24:0: Lignoceric acid

7.2.2. Phenolic compounds

The results of phenolic compounds of fruits of the different species (*Quercus pyrenaica* , *Quercus ilex* and *Quercus suber*) as well as different stages of development are presented in Table 16.

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 et al., 2014; Żyżelewicz et al., 2014), they vary greatly among species (Vinha et al.,2016).

In our work the analysis permitted the identification of 27 compounds as shown in the table 16 indicating that the samples included a wide range of phenolic chemicals.

The major compound was the gallic acid that was detected in all samples and mostly abundant in DB1*pyrenaica*, DB2*pyrenaica* and HFM*suber* with the values of the (36,27±1,44 -28,26±0,73)mg/g and (14,36±0,09)mg/g , respectively. However, a low amount of Methylellagic acid-pentose isomer II, Methylellagic acid-pentose isomer I and Ellagic acid pentoside iosmer III were only detected in DB3*ilex* with less than 1mg/g and weren't found in the other tested samples.

In the term of total phenolic compounds, total hydrolysable tannins and phenolic acids , the mature stage of *Q.pyrenaica* showed the lowest value comparing to the earlier stages in which the samples codes DB2*pyrenaica* had the highest value of total phenolic compounds (267,92±3,72 mg/g), total hydrolysable tannis (126,58±0,99 mg/g) and phenolic acids(159,31±2,77 mg/g)

These results are in accordance with the study of Cantos et al. (2003) in who quantified the phenolic compounds and fatty acids in *Q. rotundifolia,* Q. *ilex,* and Q. *suber* using UV and HPLC-MS/MS Analyses.

According to Mezni et al. (2022) who studied the phenolic profile and in vitro anti-diabetic activity of acorn from four African *Quercus* species (*Q. suber*, *Q. canariensis*, *Q. coccifera* and *Q. ilex*) using HPLC-UV-DA analysis found that the major compound in *Q.ilex* was Chlorogenic acid with is not detected in our sample. .

Table 16. Tentative of identification of the phenolic compounds of the fruit from the species (*Q. pyrenaica* , *Q.ilex and Q.suber*) (mg/g)

Peak	Rt	λmax	[M-H]- m/z	MS^2	Tentative Identification	**DB1*pyrenaica***	**DB2*pyrenaica***	**DB3*pyrenaica***	**DB3*ilex***	**DPB*pyrenaica***	**HFM*sbuber***
1	3,93	270	169	125(100)	Gallic acid	36,27(±1,44)	28,26(±0,73)	7,87(±0,07)	3,95(±0,04)	3,25(±0,12)	14,36(±0,09)
2	4,18	272	483	331(100),313(25),169(30)	Digalloyl glucoside isomer I	Nd	34,45(±1,38)	5,04(±0,05)	4,38(±0,02)	2,99(±0,09)	5,03(±0,10)
3	4,41	280	783	481(100),301(33)	Pedunculagin (bis-HHDP-glucose)	13,76(±0,67)	12,52(±0,06)	nd	0,96(±0,01)	nd	nd
4	4,59	271	785	615(100),463(13),301(46)	Digalloyl-HHDP-hexose	14,98(±0,30)	17,98(±0,04)	1,14(±0,01)	2,22(±0,00)	0,60(±0,01)	4,31(±0,03)
5	4,87	273	935	633(100),301(21)	Galloyl-bis-HHDP-glucose isomer I	8,21(±0,58)	11,75(±0,12)	nd	1,50(±0,00)	nd	nd
6	5,12	273	935	633(100),301(11)	Galloyl-bis-HHDP-glucose isomer II	9,73(±0,63)	12,91(±0,03)	1,03(±0,00)	1,62(±0,00)	0,68(±0,00)	1,52(±0,00)
7	5,37	273	483	331(41),313(25),169(30)	Digalloyl glucoside isomer II	9,3(±0,64)	9,97(±0,07)	nd	1,37(±0,00)	nd	nd
8	5,65	273	787	635(18),617(21),483(54),465(100),447(16),423(63),169(5)	Tetragalloyl-glucose isomer I	9,54(±0,32)	15,17(±0,09)	0,65(±0,00)	0,95(±0,00)	0,71(±0,00)	3,29(±0,10)
9	5,96	274	635	465(100),313(15),169(6)	Trigalloyl glucose isomer I	5,08(±2,29)	15,17(±0,06)	0,39(±0,00)	0,68(±0,00)	nd	nd
10	6,23	353	463	301(100)	Ellagic acid hexoside	1,71(±0,42)	5,75(±0,02)	3,45(±0,00)	0,72(±0,00)	2,71(±0,00)	1,37(±0,02)
11	6,64	270	635	465(100),313(25),169(16)	Trigalloyl glucose isomer II	3,33(±0,14)	6,13(±0,06)	nd	0,93(±0,00)	nd	nd
12	7,25	276	937	637(100),467(2),301(14)	Trigalloyl-HHDP-glucose isomer I	4,70(±0,41)	4,37(±0,03)	nd	1,38(±0,00)	nd	nd
13	7,99	288	1083	781(57),601(16), 301(100)	Punicalagin	7,45(±5,18)	15,09(±0,06)	0,49(±0,00)	1,15(±0,00)	nd	nd
14	8,27	282	935	633(100),301(18)	Galloyl-bis-HHDP-glucose	7,09(±0,9)	7,09(±0,04)	nd	1,11(±0,00)	nd	nd

15	10,51	362	433	301(100)	Ellagic acid pentoside isomer I	22,95(±0,15)	32,89(±0,46)	1,13(±0,00)	3,55(±0,00)	0,91(±0,00)	2,45(±0,00)
16	11,82	278	787	635(8),617(11),483(34),465(100),447(16),423(52),169(15)	Tetragalloyl-glucose isomer II	9,47(±2,06)	8,32(±0,09)	nd	0,39(±0,00)	nd	0,69(±0,02)
17	12,58	356	433	301(100)	Ellagic acid pentoside isomer II	nd	23,94(±0,19)	1,67(±0,00)	2,56(±0,07)	1,91(±0,00)	0,41(±0,00)
18	13,11	367	301	135(100)	Ellagic acid	3,57(±0,15)	nd	11,55(13,50)	0,25(±0,00)	1,21(±0,02)	7,07(±0,23)
19	13,53	281	937	637(100),467(22),301(64)	Trigalloyl-HHDP-glucose isomer II	nd	nd	nd	1,40(±0,00)	nd	nd
20	13,97	281	937	637(100),467(32),301(14)	Trigalloyl-HHDP-glucose isomer III	nd	nd	nd	2,18(±0,00)	nd	nd
21	14,38	281	937	637(100),467(18),301(4)	Trigalloyl-HHDP-glucose isomer IV	nd	nd	nd	1,55(±0,00)	nd	nd
22	14,89	281	937	637(100),467(22),301(44)	Trigalloyl-HHDP-glucose isomer V	nd	nd	nd	0,90(±0,00)	nd	nd
23	15,09	280	939	631(31),469(66),169(100)	Pentagalloyl glucose isomer I	29,62(±2,06)	15,44(±0,12)	nd	2,56(±0,02)	nd	nd
24	16,28	340	433	301(100)	Ellagic acid pentoside iosmer III	nd	nd	nd	0,14(±0,00)	nd	nd
25	16,51	281	939	631(31),469(66),169(100)	Pentagalloyl glucose isomer II	nd	nd	nd	1,00(±0,00)	nd	nd
26	16,75	353	447	301(100)	Methylellagic acid-pentose isomer I	nd	nd	nd	0,10(±0,00)	nd	nd
27	17,32	368	447	301(100)	Methylellagic acid-pentose isomer II	nd	nd	nd	0,14(±0,00)	nd	nd
					Phenolic Acids	99,51(±6,19)	159,31(±2,77)	30,25(±13,65)	22,13(±0,21)	12,79(±0,25)	14,99(±0,27)
					Total hydrolysable Tannins	97,35(±12,97)	126,58(±0,99)	3,70(±0,02)	16,47(±0,06)	2,20(±0,02)	9,88(±0,06)
					Total Phenolic Compounds	196,87(±19,16)	267,92(±3,72)	39,77(±0,28)	39,77(±0,28)	14,99(±0,27)	42,12(±0,63)

CONCLUSIONS AND FUTURE PERSPECTIVES

8. Conclusion

The aim of this work was the valorization of acorn from oak (*Quercus pyrenaica*) through chemical and nutritional evaluation, through this study we were able to identify several nutritional parameters in *Q.pyrenaica* ,*Q.ilex* and *Q.suber* in different stage of development collected from different locations in Portugal mainly the Monteishno Natural Park of Bragança and Herdade do Freixo do Meio,Evora.

The results of the analysis that have been effected on the different parts of the *Quercus* showed that *Q.pyrenaica* , *Q.ilex* and *Q.suber* were very rich in macros and micros nutritional completeness such as proteins , lipids , ash, carbohydrates and energy in which the specie *Q.pyrenaica* had the greatest values also dietary fiber and moisture were in high concentrations in *Q.ilex* . The three species were rich with minerals specially micronutrients (Fe, Zn, Cu and Mn).*Q.pyrenaica* was the richest specie with fatty acids with a great percent of saturated fatty acids , monounsaturated fatty acids and total fatty acids that improves insulin sensitivity and reduces cholesterol levels .

Having a wide range of physiological activities, twenty-seven phenolic compounds were identified. In the earlier stage of development, *Q.pyrenaica* had the greatest tannins concentration and this high contents in tannins provide acrid aroma and astringent taste.

Based on the results that have been discussed before *Q.pyrenaica* , *Q.ilex* and *Q.suber* seems to be a good matrix to explore withn food industry as an alternative and competitive food source is emerging especially *Q.pyrenaica* which is the most abundant and unexplored specie in the north of Portugal .

9. Future perspective

Mainly most of the objectives were achieved. However, some recommendations for future research are given below:

• Since studies on these products are very limited in the literature, more research is needed focusing on the identification of other parameters such as tocopherols, starch and free sugar to better understand the specie.

• Incorporating acorns in different food products as an attractive of low-cost food

• Acorns are known of their high starch content which might be used as stabilizing agent and prebiotic growth promoter.

• Acorns and acorn by-products are eco-friendly products they can be used as an innovative source of oil, flour and beverages and can be easily introduced to the human' diet as bread, breakfast cereals, pastry products or yogurt components.

• Acord interest to introduce acorn in the and pharmaceutical industries and providing more valued benefits for consumers.

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