



Impact of leaching on the nutritional composition of acorn species

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ABSTRACT

Historically, acorns have been used as a food source; however, nowadays, they are neglected despite their nutritional value. One of the barriers is their high tannin content, which leads to bitterness, reduced nutrient absorption, and may prevent digestion and liver toxicity. This study explored the impact of leaching on the nutritional and physicochemical properties of three endemic Portuguese species (*Quercus pyrenaica*, *Quercus rotundifolia*, and *Quercus robur*). The findings revealed that leaching significantly affected flour's pH, acidity, water activity, and macronutrient profile, which overall leads to reductions in these parameters. Moreover, although rich in insoluble fiber, with potential gut health benefits, substantial fiber losses occurred. Fatty acid analysis showed high oleic, linoleic, and palmitic acid levels, with favourable atherogenic and thrombogenic indices, indicating high fat quality. Leaching also significantly decreased soluble sugars and reduced mineral content by approximately 90%. Overall, these findings emphasise the acorn's potential as a sustainable and nutritious food source. Although leaching reduced soluble sugars, minerals, and part of the fiber and lipid fractions, key macronutrients and a favourable fatty acid profile were preserved, supporting the use of acorn flours in functional food applications.

1. Introduction

Food shortages and waste management have become major global challenges, highlighting the need for innovative and sustainable solutions. In response, the upcycling of agrifood industry by-products and wild foods has emerged as a novel strategy to support sustainability and the circular economy. This approach contributes to United Nations Sustainable Development Goals, namely, number 3: Good health and well-being ensuring people live healthy lives, which can reduce child mortality and increase life expectancy, and number 12: Responsible consumption and production, using resources efficiently and responsibly to minimize environmental impact, reduce waste, and promote resource recovery. The *Quercus* genus comprises a diverse group of evergreen and deciduous trees and shrubs native to temperate and tropical climates, encompassing approximately 600 species. These species produce acorns, a widely available fruit that has been crucial in the diets of various cultures for thousands of years, especially in areas such as Asia, Central and Eastern North America, Sardinia, Spain, Turkey, and Poland (Szablowska and Tańska, 2021; Tantray *et al.*, 2018).

In Portugal, the annual acorn production is estimated at around

401,585 tons (Castro *et al.*, 2022). Furthermore, the *Quercus* genus covers about 34% of Portugal's forest area, spanning 1107,600 ha, which significantly contributes to acorn availability (INE, 2017). However, despite this abundant supply, nearly 20% of the total acorn yield is primarily utilised as animal feed, with less than 1% designated for human consumption, while the remaining portion is left unused in the fields (Agrotec, 2024; LandraTech, 2023). This limited use for human consumption is largely attributed to the high tannin content of most acorn species, which imparts bitterness and reduces digestibility. Additionally, a lack of consumer familiarity, insufficient industrial processing methods, and the absence of established acorn-based food chains have prevented its overall incorporation into modern diets. This scenario is particularly paradoxical for Portugal, a major importer of food products, as well as for other southern countries where *Quercus* species are abundant. These trees represent a crucial resource for sustainability due to their resilience and adaptability to emerging climate challenges, while also reducing pressure on ecosystems. Thus, it is of paramount importance to bring solutions that make it possible to reclaim acorns as a nutritionally valuable resource.

The introduction of acorns in human diets through the development

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of novel functional food products has been proposed as a strategy to add value to this resource. It perfectly aligns with contemporary consumption trends due to its gluten-free nature, high nutritional value, and sustainable character. As a natural ingredient, acorns can enrich traditional foods by incorporating bioactive compounds, particularly phenolic compounds (e.g., ellagic acid, gallic acid derivatives and flavonoids). The presence of these bioactive compounds provides interesting health benefits, primarily attributed to their strong antioxidant properties. They also have a rich mineral profile, including potassium, phosphorus, iron, zinc, and calcium, as well as a considerable amount fiber and vitamin E content (Szabłowska and Tańska, 2024; Vinha et al., 2016). In addition, acorns are carbohydrate-rich, particularly in starch, while also containing significant amounts of lipids, particularly monounsaturated fatty acids (MUFA) (Szabłowska and Tańska, 2024). Therefore, acorn-based food products may contribute to the prevention of cardiovascular and inflammatory diseases, diabetes, gastrointestinal disorders, and microbial infections, among other health conditions (Nogueira et al., 2022; Vinha, Barreira, et al., 2016). Given these advantages, acorns represent a valuable ingredient for enhancing traditional foods and developing high-value-added functional food products. Moreover, this fruit has been incorporated into a variety of food items, including cookies, bread, yogurts, teas, coffee substitutes, hot chocolate-like beverages, and liqueurs (Castro et al., 2022).

Despite their nutritional potential, one of the main challenges limiting generalized acorn consumption is the high tannin content of most species. Tannins are a class of polyphenols that contribute to bitterness and can form complexes with proteins, polysaccharides, and metal ions, reducing digestibility and nutrient absorption. Consequently, tannins are defined as anti-nutritional compounds (Łuczaj et al., 2014; Szabłowska and Tańska, 2021). However, ethnohistoric sources have shown evidence of acorn consumption among Indigenous populations, which, at the time, applied different techniques to remove these anti-nutrients, identifiable by the acorns' bitterness (Hanson et al., 2019). Nowadays, a viable solution that has been applied to address this issue involves processing acorns by debittering through leaching or soaking, which effectively reduces excessive tannin levels. Therefore, this study aims to address a significant research gap by investigating the impact of water leaching on the physicochemical properties and nutritional composition of acorn flours from native *Quercus* species highly represented in Portuguese flora (*Quercus pyrenaica*, *Quercus rotundifolia*, and *Quercus robur*). While acorns hold great potential for human consumption and agro-industrial applications, the challenge lies in effectively reducing their tannin content without compromising their nutritional value. Hence, a deeper understanding of tannin leaching through traditional, scalable, and green extraction methods, while preserving relevant nutritional and nutraceutical features, is essential for obtaining edible acorns. This, in turn, optimizes their use in human consumption and agro-industrial applications, ultimately promoting a more sustainable and efficient utilization of this valuable natural resource.

2. Materials and methods

2.1. Chemicals

Standards of ascorbic acid and α -tocopherol (HPLC \geq 95 %, synth., cryst.); hydrochloric acid (HCl), sodium hydroxide (NaOH), sulphuric acid (H₂SO₄) reagents, and the solvents (n-hexane and methanol) used were purchased from Sigma-Aldrich (St. Louis, Mo, USA).

2.2. Raw materials

All acorns were collected at full physiological maturity, after natural abscission and spontaneous fall to the ground. Each sampling location represents a distinct bioclimatic setting within the Iberian Peninsula, covering Atlantic, supra-Mediterranean, and thermo-Mediterranean

contexts. *Quercus pyrenaica* (pyrenean oak) acorns were collected in October 2022 in a *Q. pyrenaica* grove in the village of Ferro (Fundão, Castelo Branco district, Central Portugal). This area corresponds to a supra-Mediterranean climatic belt, marked by cold winters, warm dry summers, and moderate precipitation (900–1100 mm). During the harvest period, environmental conditions were characteristic of mid-autumn, with cooler temperatures (12–18 °C) and variable rainfall. *Quercus rotundifolia* (holm oak) acorns were collected in early January 2023 from isolated trees within a mixed holm-oak–cork-oak agro-silvo-pastoral system in Torre de Miguel Sesmero (Badajoz province, Extremadura, Spain). This region falls within the thermo-Mediterranean climatic zone, defined by hot dry summers, mild winters, and low annual precipitation (450–550 mm). Harvest occurred under typical early-winter Mediterranean conditions, with cool temperatures (10–14 °C) and predominantly dry weather. After collection, all acorns were dried within one week under controlled conditions (45–50 °C, forced-air circulation) in a laboratory drying chamber, ensuring reproducibility and minimising environmental variability in post-harvest moisture loss. *Quercus robur* (pedunculate oak) acorns were collected in mid-September 2023 in Travanca, Amarante (Porto district, Northwestern Portugal), within an adult *Q. robur* woodland. This region belongs to the Atlantic climatic zone, characterised by mild temperatures, high annual precipitation (often >1200 mm), and the absence of pronounced summer drought. At harvest, weather conditions were typical of early autumn, with moderate temperatures (18–22 °C) and occasional rainfall. After harvesting, the acorns' kernels were dried, peeled, and selected using the processes currently employed by Landratech (Azambuja, Portugal), who provided the samples. All samples were stored in hermetically sealed packaging, protected from light and humidity, at –20 °C, until further use.

2.2.1. Taxonomic identification

The taxonomic identification of the *Quercus* species included in this study was performed by the author Pedro Babo (LandraTech, Largo do Esteiro 6, 2050–261 Azambuja, Portugal), who holds an academic background in Molecular Biology and Genetics (Faculty of Sciences, University of Lisbon) and has extensive field experience in the ecological and morphological characterisation of Portuguese and Iberian *Quercus* woodlands. Species determination followed morphological diagnostic criteria described in standard Iberian oak floras and taxonomic keys, ensuring accurate and reproducible identification.

2.3. Acorns Kernel processing

The samples were carefully cleaned and coarsely ground. The kernels from *Q. pyrenaica*, *Q. rotundifolia*, and *Q. robur* were then divided into two groups. One group of each species was directly milled using a grinder (Coffee Grinder TAURUS Aromatic II, Barcelona, Spain). The second group underwent a tannin removal process via leaching. Therefore, the samples were weighed and immersed in ultrapure water (upH₂O) at a 1:6 (w/v) ratio, followed by continuous agitation for 24 h at RT. They were then centrifuged (4000 rpm, 15 min, at 4 °C), the supernatant was discarded, and the pellet was reconstituted with an equivalent volume of upH₂O. The process was repeated three times for each sample. The final set of processed flours obtained for subsequent

Table 1
Acorn samples' species, description, and respective ID.

Sample Species	Sample Description	Flour ID
<i>Q. pyrenaica</i>	Kernel	QP
	Leached Kernel	QPL
<i>Q. rotundifolia</i>	Kernel	QT
	Leached Kernel	QTL
<i>Q. robur</i>	Kernel	QR
	Leached Kernel	QRL

analysis is detailed in Table 1.

2.4. Acorn flours physicochemical characterization

2.4.1. Total acidity and pH assessments

The samples' pH was assessed following the method described by Pourabdollah et al. (Pourabdollah et al., 2020). Briefly, a 10% (w/v) aqueous extract was made from each flour by mixing 10 g of each sample with 100 mL of dH₂O and homogenizing the solution. After, the extracts were filtered using filter paper, the pH value of the supernatant was determined using a potentiometer (SenION™, VWR, West Chester, PA, USA).

The flour's acidity was analyzed following the Association of Official Analytical Chemists (AOAC) 942.15 method. Therefore, 10 g of each sample was mixed with 100 mL 65% EtOH (v/v), followed by agitation for 10 min. The solution was then centrifuged (800 rpm, 10 min, at 4 °C), and its supernatant was filtered using filter paper. Then, using a potentiometer, 25 mL of the filtered supernatant was titrated with 0.1 M NaOH until reaching a pH 8.5. The results were calculated using Eq. 1 (1) and reported in percentage of H₂SO₄ equivalents (eq) (dry weight, DW). Triplicates were made for each sample.

$$\text{Total Acidity (\% H}_2\text{SO}_4 \text{ eq, DW)} = \frac{v \times \text{NaOH (M)} \times \text{H}_2\text{SO}_4(\text{g/mol})}{2 * 1000 * w} \times 100 \quad (1)$$

As v – the added volume of 0.1 M NaOH; NaOH (M) = 0.1; H₂SO₄ (g/mol) = 98.08; w – sample initial weight (g).

2.4.2. Water activity assessment

Each flour's water activity (a_w) was determined using a LabMaster-aw thermoconstanter (Novasina, Lachen, Switzerland), at 25 °C.

2.5. Acorn flours nutritional characterization

2.5.1. Moisture and ash content

The moisture and ash contents were determined for each sample, according to the AOAC 930.15 and AOAC 942.05, respectively. To assess the samples' moisture, 3 g of each sample was oven-dried at 105 °C, until a constant weight was reached. The ashes content was determined by incinerating 3 g of each sample in a muffle furnace at 550 °C for 5 h to remove carbon. The results were expressed as a percentage of moisture (Eq. 2 (2)), and ash contents (Eq. 3 (3)), in each sample. All measurements were performed in triplicate.

$$\text{Moisture (\%)} = \frac{\text{Sample Initial Weight} - \text{Sample Final Weight (g)}}{\text{Sample Initial Weight (g)}} \times 100 \quad (2)$$

$$\text{Ash (\%, DW)} = \frac{\text{Ash Weight (g)}}{\text{Sample Initial Weight (g)}} \times 100 \quad (3)$$

2.5.2. Protein content

The total protein content within each sample was determined using the Kjeldahl method (AOAC, 1997). The results were calculated using the conversion factor (6.25) (Mallek-Ayadi, Bahloul and Kechaou, 2018). The data were expressed as a percentage of DW (Eq. 4 (4)). All measurements were performed in triplicate.

$$\text{Protein Content (\%, DW)} = \left[f \times (V_2 - V_1) \times \frac{100}{m} \right] \times 6.25 \quad (4)$$

As f = 0.0014 (HCL 0.1 M); V₁ = 0.1 N HCL volume (mL) used for the blank titration; V₂ = 0.1 N HCL volume (mL) used for each sample titration; m – mass (g) of each sample.

2.5.3. Total lipid content and fatty acid composition

For the total lipid extraction, 1 g of each acorn sample was processed using the Folch procedure (Folch et al., 1957), substituting chloroform for dichloromethane as previously described by Ozório et al. (OZÓRIO et al., 2012). The analysis was performed in duplicate. The lipid residue obtained was dissolved in n-hexane, and the fatty acid profile of acorns was determined according to Machado et al. (Machado et al., 2022). Briefly, 2.26 mL of methanol was added, followed by 240 µL of sodium methoxide (5.4 M). The samples were then vortexed and incubated at 80 °C for 10 min. Following this, samples were cooled in ice, and 1.25 mL of dimethylformamide and 1.25 mL of sulfuric acid (3 M) in methanol were added. The samples were then vortexed and incubated at 60 °C for 30 min. After cooling, samples were vortexed and centrifuged (1250 g; 5 min). The upper layer containing fatty acids was collected for further analysis. C13:0 was used as an internal standard.

2.5.4. Gas chromatography-flame ionization detection (GC-FID)

Fatty acids were analyzed in a gas chromatograph Agilent 8860 (Agilent, Santa Clara, CA, USA), equipped with a flame ionization de-

tector and a BPX70 capillary column (60 m × 0.25 mm × 0.25 µm; SGE Europe Ltd., Milton Keynes, UK) with analysis conditions being as follows: injector (split 25:1; injection volume 1 µL), injector, and detector temperatures were 250 °C and 275 °C, respectively; hydrogen was used as a carrier gas at a flow rate of 1 mL/min. The initial oven temperature was 60 °C and was then increased to a final temperature of 225 °C. Sample identification was performed using Supelco 37 standard mix (Sigma-Aldrich, St. Louis, Missouri, USA) and each sample was analyzed in duplicate.

2.5.5. Nutritional quality indices

The nutritional quality of the fatty acids present in the acorn samples was evaluated by taking into consideration the following indices: atherogenicity index (AI), thrombogenicity index (TI), and hypocholesterolemic/hypercholesterolemic ratio (HH), calculated according to the respective equations indicated below (5) – (7). In addition, the polyunsaturated/saturated fatty Acid (PUFA/SFA) ratio was also calculated (Chen and Liu, 2020; Ratusz et al., 2018).

$$\text{AI} = \frac{\text{C12 : 0} + (4 * \text{C14 : 0}) + \text{C16 : 0}}{\sum \text{MUFA} + \sum \omega 3 + \sum \omega 6} \quad (5)$$

$$\text{TI} = \frac{\text{C14 : 0} + \text{C16 : 0} + \text{C18 : 0}}{(0.5 * \sum \text{MUFA}) + (0.5 * n6\text{PUFA}) + (3 * n3\text{PUFA}) + \left(\frac{\omega 3}{\omega 6}\right)} \quad (6)$$

$$\text{HH} = \frac{\text{C18 : 1} + \text{C18 : 2} + \text{C18 : 3} + \text{C18 : 4} + \text{C20 : 4}}{\text{C14 : 0} + \text{C16 : 0}} \quad (7)$$

2.5.6. Total Carbohydrate Content

The flour's total carbohydrate content was determined by calculating the difference between the initial sample weight (DW) and the sum of the protein, ash, and lipid contents. The results were expressed as a percentage of DW.

2.5.7. Dietary fiber content

The sample's soluble, insoluble, and total dietary fiber content was

analyzed according to the Megazyme total dietary fiber assay kit procedure (K-TDFR-200A, Megazyme, Bray, Ireland), based on the AOAC 991.43 and AACC method 32–07.01. Briefly, this methodology involves the removal of starch and protein from the samples, followed by the determination of total dietary fiber by the sum of insoluble and soluble fiber content. This analysis was conducted in duplicate.

2.5.8. Total sugar content

To assess the total sugar content, an extract was made for each sample by weighing 150 mg of flour and subjecting it to triple extraction with 5 mL of 80 % (v/v) EtOH. Each extraction was performed by boiling the samples in capped glass tubes submerged in an 80 °C water bath for 20 min. Following each extraction, the samples were centrifuged at 2500 rpm for 5 min to separate the supernatant. The supernatants from all three extractions were combined and reserved for subsequent sugar analysis. The total sugar of each flour was then determined according to the phenol-sulphuric acid method (Dubois et al., 1951; Gu et al., 2024), in triplicate. Results were expressed as mg of glucose equivalents (GE)/100 g of sample DW.

2.5.9. Total starch content

To assess the sample's total starch content was analyzed according to the Megazyme total starch assay kit procedure (K-TSTA-100A, Megazyme, Bray, Ireland), based on the AOAC 996.11 and AACC 76–13.01 methods. This analysis was conducted in duplicate.

2.5.10. Mineral content analysis by ICP-MS

For each sample, about 500 mg of flour was digested with 10 mL of HNO₃ 67 % - 69 % (v/v), into a vessel, using a microwave digester (Berghof, Germany) (900–1800 W, 800 psi, 200 °C, 15 min ramp, 15 min hold) (CEM, 2019). The digested samples were completely cooled at room temperature (RT) and collected into 50 mL Falcon tubes, which were then filled to 20 mL with ultrapure water. The mineral profile of each sample was assessed by injecting 10 µL of digested sample into an ICP-MS equipment (Optima 7000 DV ICP-OES, PerkinElmer, USA). Nitrogen, air, and argon were used as carrier gases. External calibration curves of zinc (Zn; 213.857 nm), phosphorus (P; 214.914 nm), manganese (Mn; 257.610 nm), iron (Fe; 259.939 nm), magnesium (Mg; 279.077 nm), calcium (Ca; 317.933 nm), copper (Cu; 324.752 nm), selenium (Se; 196.026 nm), sodium (Na; 589.592 nm) and potassium (K; 769.896 nm) were prepared with HNO₃ 5 % (v/v) from a standard mix from Inorganic Ventures, USA (UCP-3-mL; REF: P2-MEB687579). Results were expressed as mg/100 g of sample DW. Each sample was extracted in duplicate, and injections were performed in duplicate. The limit of detection (LOD) and limit of quantification (LOQ) were determined by considering the slope of the calibration curve and the residual standard deviation of the regression line.

2.5.11. Energy value

The energy content was calculated based on the following conversion factors: 4 kcal/g for carbohydrate and protein, and 9 kcal/g for fat content (EU, 2011).

2.5.12. Vitamin E quantification by HPLC-DAD-FLD

To assess the samples' vitamin E content, an extraction was carried out for each flour, following the method described by Vieira et al. (Vieira et al., 2022). Therefore, 100 mg of sample were mixed with 100 mg of sodium sulphate anhydrous, 6 mg of ascorbic acid (internal standard), and 1.5 mL n-hexane, and then vortexed for 30 s. The mixture was further homogenized in a roller mixer (Roller 10 Digital, IKA, Staufen, Deutschland) for 20 min. Subsequently, samples were centrifuged (3 min, 14,500 g) and 1 mL of the resulting supernatant was evaporated using a speed vacuum apparatus (Martin Christ Gefrier-trocknungsanlagen GmbH, Osterode am Harz, Germany) (40 °C, 1000 rpm, 45 min). The obtained pellet was reconstituted in methanol, vortexed (10 s), and filtered (22 µm filter), into an amber vial. An

integrated system with data transmitter analyses (Thermo Fisher Scientific Inc., MA, USA), a pump (Vanquish, ThermoFisher Scientific Inc., MA, USA), an autosampler (Vanquish, Thermo Fisher Scientific Inc., MA, USA), a DAD (Vanquish Detector, Thermo Fisher Scientific Inc., MA, USA), and a FLD (Vanquish Detector, ThermoFisher Scientific Inc., MA, USA) was used to carry out the chromatographic analyses. Data were analyzed using Software Empower 3, and the chromatographic separation was achieved with a reversed-phase C18 column (ZORBAX Eclipse XDB-C18, 80 Å; 4.6 × 250 mm; 5 µm; Agilent, Santa Clara, CA, USA) eluted with a total run time of 40 min, and a mobile phase of 95 % methanol (v/v). The flow rate was set at 1 mL/min, maintaining the temperature at 22 °C, and the injection volume at 20 µL. The compounds were identified by chromatographic comparisons against an α-tocopherol standard (Sigma-Aldrich, St. Louis, Missouri, USA). The quantification was made using the fluorescence signal response 295/325 nm, and the results were expressed as mg/100 g of sample DW.

2.6. Statistical analysis

All experiments were conducted in duplicate or triplicate, and values were expressed as mean ± standard deviation (SD). The GraphPad Prism 9 (GraphPad Prism 9 for Windows, GraphPad Software Inc., San Diego, CA, USA) was used to perform the statistical analysis, using the one-way or two-way ANOVA followed by Tukey's test, considering $p < 0.05$ significant. Data processing was also carried out using Microsoft Excel 2021. For statistical calculations, results below the LOD or LOQ were assumed as LOD/√2 or LOQ/√2, a commonly used procedure for imputing these results (Hornung and Reed, 1990).

3. Results and discussion

3.1. Physicochemical analysis

The physicochemical properties of the acorn flours (Table 2), namely water activity (a_w), pH, and total acidity (TA), were assessed to understand the impact of leaching on these parameters, which are highly relevant for the nutritional quality and processing potential of these fruits.

As shown in Table 2, the water activity is relatively low for all samples ($a_w < 0.680$). Moreover, a_w was significantly reduced ($p < 0.05$) after leaching: QP/QPL (0.507 ± 0.001 and 0.213 ± 0.001 , respectively), QT/QTL (0.672 ± 0.001 and 0.350 ± 0.001 , respectively), and QR/QRL (0.552 ± 0.000 and 0.541 ± 0.001 , respectively). This is particularly relevant since water activity influences microbial growth, enzymatic activity, and overall shelf life. Leaching may reduce water activity in acorns by removing soluble components such as sugars, salts, and other hydrophilic substances, which attract and retain water. As these compounds are drawn out during the leaching process, the osmotic pressure is reduced, lowering the water activity (Wang and Feng, 2023).

While pH directly influences acidity perception, TA provides

Table 2

Chemical characterization of the different acorn flours. Results are presented as mean ± standard deviation.

Flour ID	Water Activity (a_w)	pH	Acidity (% w/w, H ₂ SO ₄ eq)
QP	0.507 ± 0.001^a	5.88 ± 0.00^a	0.105 ± 0.001^a
QPL	0.213 ± 0.001^b	5.32 ± 0.00^b	0.029 ± 0.003^b
QT	0.672 ± 0.001^c	5.78 ± 0.00^c	0.049 ± 0.003^c
QTL	0.350 ± 0.001^d	5.68 ± 0.00^d	0.013 ± 0.002^d
QR	0.552 ± 0.000^e	5.01 ± 0.00^e	0.089 ± 0.002^e
QRL	0.541 ± 0.001^f	5.02 ± 0.00^e	0.023 ± 0.001^f

Different lowercase letters in the same column indicate statistically significant differences ($p < 0.05$) between samples, as determined by one-way ANOVA, followed by Tukey's test for multiple comparisons. Data are presented as mean ± SD.

additional information on the buffering capacity of the flour, that is, its ability to resist changes in pH. Thus, both pH and TA play an important role in food preservation, texture, and sensory perception (Ognean, 2015). Regarding the pH results, all samples were slightly acidic, which is in accordance with the values reported in the literature (Purabdollah et al., 2020). These pH values correlate with these species' preferred edaphoclimatic growing conditions, namely acidic soils (M. Castro and Fernandez-Nuñez, 2014; Ikaunieca et al., 2013). Moreover, the leaching process significantly reduced ($p < 0.05$) the pH of *Q. pyrenaica* (QP: 5.88; QPL:5.32) and *Q. rotundifolia* flours (QT: 5.78; QTL: 5.68), as well as the TA for all samples (QP: 0.105 ± 0.001 ; QPL: 0.029 ± 0.003 ; QT: 0.049 ± 0.003 ; QTL: 0.013 ± 0.002 ; QR: 0.089 ± 0.002 ; QRL: 0.023 ± 0.001 % (w/w) H_2SO_4 equivalents). In addition, *Q. pyrenaica* showed the greatest reduction in both pH and TA after leaching, suggesting stronger compositional shifts during aqueous extraction compared with the other species. Thus, it is important to consider that the pH and TA changes have practical implications, particularly for formulation and fermentation processes, which may be positively or negatively influenced depending on the desired technological characteristics of the final product (Martins et al., 2022).

3.2. Centesimal composition and energy value

The nutritional profile of acorn flours was analyzed (Table 3) to assess their potential benefits for human consumption and to understand the impact of leaching on their properties. The findings revealed that QP exhibited higher ash and protein contents (2.82 ± 0.01 and 8.60 ± 0.07 % DW, respectively) compared to the other species. In contrast, QT showed a higher lipid content (11.27 ± 0.04 % DW), while QR had the highest content of carbohydrates (76.30 ± 0.05 % DW). In addition, the moisture content of all samples ($5.84 - 11.77$ %) was found to be within acceptable limits, ensuring safe storage conditions (Tizemmour et al., 2024). The nutritional profile of these species aligns with previous studies conducted by Castro et al. (Castro et al., 2022), which obtained similar values for QP (approximately 2.2 % DW ashes; 6.5 % DW protein; 5.2 % DW total lipids; 85.8 % DW total carbohydrates; 378 kcal/100 g DW) and QR (approximately 2.2 % DW ashes; 7.9 % DW protein; 4.1 % DW total lipids; 86.2 % DW total carbohydrates; 381 kcal/100 g DW), as well as with another study by Tizemmour et al. (Tizemmour et al., 2024) which reported identical values for QT (approximately 3.0 % DW ashes; 7.7 % DW protein; 13.95 % DW total lipids; 65.00 % DW total carbohydrates; 416 kcal/100 g DW).

It was also concluded that the leaching process significantly affected the flour's ash content (QPL: 0.35 ± 0.00 % DW; QTL: 0.35 ± 0.00 % DW; QRL: 0.35 ± 0.00 % DW), potentially due to the removal of soluble minerals, as well as the total lipid content (QPL: 5.28 ± 0.13 % DW; QTL: 4.94 ± 0.79 % DW; QRL: 6.08 ± 0.78 % DW). This reduction in lipid content may be explained by partial dispersion of lipids into the leaching water, especially when cell walls become more permeable during prolonged hydration. Additionally, the repeated washing steps may facilitate the removal of lipid-phenolic complexes or emulsified lipid fractions (Kummerdsiri et al., 2024; Monsoor and Proctor, 2002). As a result, the energy value of QTL and QRL decreased compared to the

non-leached samples, primarily due to lipid reduction (Steele and Bogdziewicz, 2024). In contrast, QPL did not exhibit statistically significant changes in energy value compared to QP. It is also important to note that the nutritional profile of acorn flours may vary depending on environmental conditions such as year-to-year climatic variability and harvest season, as well as species, maturation stage, and post-harvest storage conditions (Castro et al., 2022). 3.3. Fatty Acids Content and Nutritional Quality Indices

Data detailing the fatty acid profile (mg/100 g) and quality indices for the acorn samples are presented in Table 4. The primary fatty acids identified in the acorn samples were oleic acid (C18:1 n-9c) at 46–69 %, linoleic acid (C18:2 n-6c) at 18–33 %, palmitic acid (C16:0) at 10–16 %, and stearic acid (C18:0) at 1–3 %. This fatty acid composition is consistent with previous studies (Martins et al., 2022; Vinha, Barreira, et al., 2016). In terms of differences among acorn species regarding saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), and poly-unsaturated fatty acids (PUFA), the total SFA content was highest in QT (1433.00 ± 62.64 mg/100 g DW), followed by QR (854.94 ± 59.87 mg/100 g DW) and QP (711.61 ± 2.34 mg/100 g DW). For the total MUFA, the values were 8247.87 ± 418.19 mg/100 g DW for QT, 2493.29 ± 0.76 mg/100 g DW for QP, and 2189.94 ± 150.28 mg/100 g DW for QR. The total PUFA content was 2194.71 ± 51.88 mg/100 g DW for QT, 1654.39 ± 98.10 mg/100 g DW for QR, and 1118.28 ± 8.58 mg/100 g DW for QP. In leached samples, the values for total SFA, MUFA, and PUFA were consistently lower than those in non-leached samples, with significant reductions observed in the QT and QR samples. The reduction in fatty acids after leaching may be linked to the partial removal of lipid fractions associated with phenolic-lipid complexes or located within cells disrupted by prolonged hydration. Species with lower phenolic content may experience less antioxidant protection, increasing susceptibility to oxidative degradation during processing (Maqsood et al., 2014). Therefore, species (QT) with potentially lower phenolic content increased lipid oxidation could be occurring, making the lipids more easily removed through the leaching process. Moreover, small amounts of fatty acids can be lost during leaching due to the formation of natural emulsions, or due to chemical bond disruption (e.g., lipid-protein matrices). The most substantial difference was seen between the QT and QTL samples. The PUFA/SFA ratio, commonly used to evaluate the nutritional value and cardiovascular health effects, ranged from 1.18 to 1.94, with leached samples generally showing lower values, except for QP. These findings fall within the range reported for other acorn species, with ratios of 1.24 for *Q. rotundifolia* and 1.63 for *Q. faginea* (QF) (Taib et al., 2023), all above the recommended value of < 0.45 . Regarding the Quality Indexes, the atherogenicity index (AI), assesses the relationship between primary SFAs and the major unsaturated fatty acids (UFA) categories. SFAs are considered pro-atherogenic, promoting lipid adhesion to the blood vessel walls, whereas UFAs are anti-atherogenic, helping to inhibit platelet aggregation. Lower AI values indicate better nutritional quality (Soares et al., 2021). In this study, AI values ranged from 0.12 for QT to 0.20 for QR. Literature reports indicate values of 0.15 for QT and 0.16 for QF (Taib et al., 2023). The TI reflects the potential for thrombosis by analyzing the relationship between SFA (pro-thrombogenic) and MUFA

Table 3
Centesimal composition of the acorn flours.

Flour ID	Moisture (%)	Ash (% DW)	Protein (% DW)	Total Lipids (% DW)	Total Carbohydrates (% DW)	Energy Value (Kcal/100 g DW)
QP	10.34 ± 0.00^a	2.82 ± 0.01^a	8.60 ± 0.07^a	7.19 ± 0.79^a	71.11 ± 1.02^a	395.15 ± 2.66^a
QPL	5.84 ± 0.00^b	0.35 ± 0.00^b	7.12 ± 0.43^b	5.28 ± 0.13^{bd}	81.41 ± 0.42^b	398.58 ± 0.09^a
QT	11.76 ± 0.00^c	2.00 ± 0.01^c	4.35 ± 0.00^c	11.27 ± 0.04^c	70.33 ± 0.17^a	400.16 ± 0.44^a
QTL	9.29 ± 0.00^d	0.36 ± 0.00^b	4.72 ± 0.13^c	4.94 ± 0.79^{bd}	80.02 ± 1.27^{bc}	383.42 ± 5.34^b
QR	9.58 ± 0.01^c	1.95 ± 0.02^d	6.09 ± 0.22^d	6.08 ± 0.78^{ab}	76.30 ± 0.05^d	384.34 ± 4.48^b
QRL	11.77 ± 0.01^c	0.37 ± 0.01^b	5.91 ± 0.11^d	3.95 ± 0.79^d	77.98 ± 0.74^{cd}	371.20 ± 4.09^c

Different lowercase letters in the same column indicate statistically significant differences ($p < 0.05$) between samples, as determined by one-way ANOVA, followed by Tukey's test for multiple comparisons. Data are presented as mean \pm SD.

Table 4

Fatty acid composition of the different acorn flours.

SFA (mg/100 g DW)	Flour ID					
	QP	QPL	QT	QTL	QR	QRL
C6:0	5.40 ± 0.74 ^a	2.31 ± 0.37 ^b	3.60 ± 0.18 ^b	2.45 ± 0.31 ^b	2.71 ± 0.37 ^b	2.66 ± 0.80 ^b
C8:0	0.92 ± 0.10 ^a	0.42 ± 0.19 ^b	0.90 ± 0.14 ^{ac}	0.58 ± 0.02 ^{bc}	1.03 ± 0.08 ^a	1.93 ± 0.14 ^d
C10:0	1.76 ± 0.06 ^a	6.08 ± 0.44 ^b	17.17 ± 2.47 ^c	2.01 ± 0.02 ^{ab}	2.65 ± 0.55 ^{ab}	3.81 ± 0.02 ^{ab}
C12:0	2.44 ± 0.12 ^a	1.47 ± 0.21 ^b	ND	ND	0.39 ± 0.05 ^c	0.25 ± 0.00 ^c
C14:0	7.18 ± 0.09 ^a	4.77 ± 0.41 ^b	5.81 ± 0.58 ^c	2.60 ± 0.20 ^d	3.48 ± 0.42 ^e	3.12 ± 0.10 ^d
C15:0	2.71 ± 0.24 ^a	3.59 ± 0.27 ^b	4.99 ± 0.47 ^c	0.91 ± 0.13 ^d	3.53 ± 0.32 ^b	2.19 ± 0.34 ^a
C16:0	605.83 ± 4.26 ^a	575.62 ± 44.11 ^a	1226.59 ± 64.47 ^b	296.34 ± 32.27 ^c	748.70 ± 49.40 ^d	509.53 ± 7.06 ^a
C17:0	3.49 ± 0.55 ^a	6.61 ± 0.76 ^b	4.21 ± 0.65 ^a	1.82 ± 0.27 ^c	3.79 ± 0.18 ^a	2.83 ± 0.06 ^{ac}
C18:0	78.05 ± 0.41 ^a	92.11 ± 4.72 ^a	170.94 ± 7.55 ^b	52.54 ± 3.57 ^c	80.90 ± 7.66 ^a	59.49 ± 5.32 ^c
C20:0	2.92 ± 0.75 ^a	3.91 ± 0.76 ^a	ND	2.84 ± 0.20 ^a	5.68 ± 0.40 ^c	6.27 ± 0.61 ^c
C21:0	0.42 ± 0.03 ^a	ND	0.68 ± 0.08 ^b	ND	1.25 ± 0.15 ^c	0.98 ± 0.11 ^d
C24:0	0.92 ± 0.29 ^a	0.94 ± 0.14 ^a	1.03 ± 0.15 ^a	ND	2.60 ± 0.30 ^b	1.94 ± 0.33 ^c
MUFA (mg/100 g DW)						
C16:1 n-7	16.66 ± 0.61 ^a	8.18 ± 0.64 ^b	2.23 ± 0.07 ^c	2.20 ± 0.23 ^c	12.91 ± 1.38 ^d	8.16 ± 0.36 ^b
C17:1 n-7	2.98 ± 0.31 ^{ab}	4.63 ± 0.70 ^c	1.59 ± 0.19 ^b	2.32 ± 0.27 ^{bd}	3.34 ± 0.42 ^a	3.06 ± 0.28 ^{ad}
C18:1 n-9c	2467.60 ± 0.67 ^a	2049.20 ± 121.97 ^a	8237.77 ± 419.70 ^c	1265.54 ± 109.46 ^d	2156.85 ± 146.52 ^a	1467.55 ± 14.63 ^d
C20:1 n-9	7.64 ± 0.09 ^a	13.61 ± 0.97 ^b	10.92 ± 0.16 ^{ab}	4.60 ± 0.76 ^a	16.85 ± 1.96 ^c	12.04 ± 1.09 ^b
PUFA (mg/100 g DW)						
C18:2 n-6c	1069.72 ± 8.90 ^{ab}	1190.09 ± 83.75 ^a	2116.56 ± 54.33 ^c	402.41 ± 34.29 ^d	1553.06 ± 91.58 ^e	1006.16 ± 36.62 ^b
C18:3 n-3	8.51 ± 0.61 ^a	15.41 ± 0.88 ^{ab}	32.53 ± 8.11 ^c	9.39 ± 0.79 ^a	21.09 ± 1.86 ^{bd}	13.97 ± 1.54 ^{ad}
C18:3 n-6	36.97 ± 0.23 ^a	53.77 ± 4.65 ^b	37.81 ± 4.39 ^a	11.96 ± 1.10 ^c	71.62 ± 3.92 ^d	45.01 ± 3.58 ^a
C20:3 n-6	2.05 ± 1.78 ^a	4.71 ± 0.43 ^b	7.82 ± 1.16 ^c	1.13 ± 0.10 ^a	8.62 ± 0.74 ^c	6.45 ± 1.05 ^{bc}
Σ (mg/100 g DW)						
ΣSFA	711.61 ± 2.34 ^a	696.05 ± 52.69 ^a	1433.00 ± 62.64 ^b	359.89 ± 38.63 ^c	854.94 ± 59.87 ^d	591.58 ± 9.26 ^b
ΣMUFA	2493.29 ± 0.76 ^a	2075.62 ± 124.04 ^a	8247.87 ± 418.19 ^b	1274.10 ± 111.11 ^c	2189.94 ± 150.28 ^a	1490.82 ± 15.45 ^c
ΣPUFA	1118.28 ± 8.58 ^{ab}	1263.99 ± 88.74 ^a	2194.71 ± 51.88 ^c	424.60 ± 35.70 ^d	1654.39 ± 98.10 ^e	1071.60 ± 37.69 ^b
Σω3	8.51 ± 0.61 ^a	15.41 ± 0.88 ^{ab}	32.53 ± 8.11 ^c	9.39 ± 0.79 ^a	21.09 ± 1.86 ^{bd}	13.97 ± 1.54 ^{ad}
Σω6	1109.77 ± 9.19 ^{ab}	1248.58 ± 88.28 ^a	2162.18 ± 49.99 ^c	415.21 ± 35.07 ^d	1633.30 ± 96.24 ^e	1057.63 ± 39.15 ^b
Ratios (mg/100 g DW)						
PUFA/SFA	1.57	1.82	1.53	1.18	1.94	1.81
AI	0.18	0.18	0.12	0.18	0.20	0.20
TI	0.15	0.14	0.13	0.19	0.14	0.15
HH	5.78	5.61	8.43	5.61	4.96	4.85

SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids; AI- Atherogenicity index; TI- Thrombogenicity index; HH- Hypocholesterolemic/hypercholesterolemic ratio; ND- Not Detected. Different lowercase letters in the same row indicate statistically significant differences ($p < 0.05$) between samples, as determined by one-way ANOVA, followed by Tukey's multiple comparisons test. Data are presented as mean ± SD.

and PUFA (anti-thrombogenic) (Soares et al., 2021). In this study, thrombogenicity index (TI) values varied between 0.13 for QT and 0.19 for QTL, while literature values for QT and QF were reported as 0.22 and 0.17, respectively (Taib et al., 2023). The AI and TI values were notably low across all analyzed samples, indicating good nutritional quality (Chen and Liu, 2020). Furthermore, when comparing leached and non-leached samples, leaching generally led to slight increases in both AI and TI values, reflecting a relative reduction in unsaturated fatty acids. However, the indices remained low across all samples, indicating that despite these changes, the nutritional quality of the lipid fraction remained favourable. Conversely, higher values of the hypocholesterolemic/hypercholesterolemic ratio (HH) are considered more beneficial, with HH ratios ranging from 4.85 for QRL to 8.43 for QT. These values align with previously reported ranges for other acorn species, such as 6.36 for QT and 6.17 for QF (Taib et al., 2023). The quality indices were comparable to those typically found in sesame and olive oils (Khalili Tilami and Kourimská, 2022), and in linseed (Awasthi et al., 2024). Consequently, acorn flour may be considered a good source of essential fatty acids for human consumption.

3.3. Dietary fiber, total sugar, and total starch content

Fiber content is known to be an important component to evaluate in foods, due to its potential health benefits. Hence, this parameter was assessed for all acorn flours (Fig. 1). Among non-processed flours, QP exhibited the highest total fiber content (31.16 g/100 g DW), while QT and QR presented similar values (22.81 and 22.28 g/100 g DW, respectively). These variations between species can also be attributed to

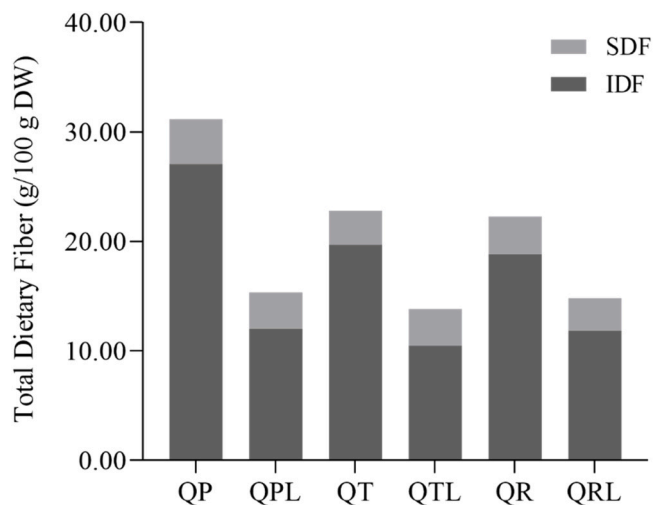


Fig. 1. Flour's total dietary fiber content. IDF – insoluble dietary fiber; SDF- soluble dietary fiber. Data are presented as g/100 g DW.

differences in growing conditions and ecological factors.

Moreover, all flours demonstrated a higher proportion of insoluble fiber (IF) compared to soluble fiber (SF) content (QP: 27.07 and 4.09 g/100 g DW; QT: 19.70 and 3.11 g/100 g DW; QR: 18.84 and 3.44 g/100 g DW, respectively). These results are in accordance with those reported in

the literature for acorn species (19.70–30.07 g/100 g DW) (Inácio et al., 2024). Furthermore, leaching led to the reduction of total fiber content, with losses ranging from 49.29 % to 66.56 %. The most affected sample was QRL, while QPL showed the least fiber loss. In addition, the decrease in total fiber was mainly driven by the loss of IF, likely due to the mechanical processes involved in leaching. Nonetheless, IF remained the predominant fiber fraction in all leached samples (QPL: 12.02 and 3.34 g/100 g DW; QTL: 10.49 and 3.35 g/100 g DW; QRL: 11.85 and 2.98 g/100 g DW, respectively).

Fiber is widely recognized as an important nutritional component, due to its impact on human health, and can contribute to the development of functional food products (Martins et al., 2022). Therefore, acorn fiber offers several health benefits, including the potential to improve gut health due to its prebiotic effects (Ahmadi et al., 2019). Additionally, as a rich source of non-digestible fiber, acorn flours may help manage conditions like diabetes by inducing a hypoglycemic response (Sasani et al., 2023).

Regarding the total sugar content (Fig. 2), statistically significant ($p < 0.05$) differences were found between non-processed flours. QT showed higher soluble sugar content (40.51 ± 1.13 mg/g DW), followed by QP (27.19 ± 5.86 mg/g DW), and QR (12.03 ± 0.55 mg/g DW). These values are similar to those reported by Yang et al. (Yang et al., 2023), who assessed the soluble sugar in *Q. variabilis* (31.95 ± 8.08 mg/g) and *Q. aliena* (20.77 ± 6.55 mg/g), and mentioned that these contents might vary according to the maturation state of acorns. Also, the results show that the leaching process significantly reduces the soluble sugar content for all flours (QPL: 0.13 ± 0.00 mg/g DW; QTL: 0.28 ± 0.00 mg/g DW; QRL: 0.28 ± 0.00 mg/g DW) since these sugars are removed during washing, which might also contribute to a lower glycemic impact, compared to the non-processed flours (Giuntini et al., 2022). Moreover, there are no statistically significant differences between leached samples.

Starch is a complex carbohydrate that is broken down into glucose during digestion, impacting blood sugar levels (Maningat and Seib, 2010). Different starches have varying glycemic indices, affecting how quickly they raise blood sugar, which is particularly significant for individuals with diabetes or insulin resistance. Additionally, the starch content of each flour can influence its texture, viscosity, and stability in developing new food products (Pereira et al., 2024). Consequently, the total starch content of each acorn flour was measured. As shown in Fig. 3, the total starch content of the non-processed flours is highest in QR (39.28 ± 5.65), followed by QP (30.35 ± 0.50), and then QT (25.23 ± 0.32). These values are within those reported in the literature for acorn species (Castro et al., 2022). Moreover, leaching appears to have a substantial effect on the increase in starch concentration, which is notably evident ($p < 0.05$) in QPL (47.64 ± 1.76) and QTL (68.06 ± 3.27). This may result from the removal of specific compounds during

the leaching process (e.g., fibers, lipids, minerals), leading to an increase in relative starch content. Despite the higher total starch concentration in QRL (48.84 ± 0.51) compared to QR, no statistically significant differences were observed for these samples.

3.4. Mineral composition

The mineral profile of acorn flours (mg/100 g DW) is summarized in Table 5. The total mineral composition in leached samples ranged from 89.09 ± 10.26 mg/100 g DW for QRL to 137.14 ± 3.69 mg/100 g DW for QTL. In contrast, the non-leached samples exhibited total mineral content ranging from 856.11 ± 74.49 mg/100 g DW for QR to 1305.10 ± 17.59 mg/100 g DW for QP. This indicates that the leached samples showed a reduction of approximately 90 % in total mineral content compared to their non-leached counterparts. The total mineral content of the non-leached samples falls within the previously reported range for other acorn species (QT), which is 622 – 1670 mg/100 g DW (Castro et al., 2022; Martins et al., 2022). The primary minerals present in the non-leached samples were K, P, Mg, and Ca, respectively, consistent with earlier findings (Martins et al., 2022). For the leached samples, this profile changes depending on the species. Among the non-leached samples, the highest concentrations were found for K (1020.99 ± 24.66 mg/100 g DW), P (118.08 ± 6.25 mg/100 g DW), and Ca (41.67 ± 3.59 mg/100 g DW) in the QP sample, while the highest Mg content (58.41 ± 1.78 mg/100 g DW) was found in the QT sample. Interestingly, Al levels were higher in the leached samples, which may be attributed to increased handling during laboratory analysis, potentially leading to contamination. Some biological interactions between minerals have been examined, such as the interaction between Na and K, which is noteworthy in the context of hypertension and cardiovascular diseases; a high Na/K ratio has been linked to an increased risk of these conditions. The recommended intake suggests that for a healthy diet, the Na/K ratio should be less than 0.6 (Morrissey et al., 2020). The acorn samples tested showed very low Na levels, below the limit of detection (LOD), resulting in a Na/K ratio of less than 0.01 for all samples, indicating a healthy balance. Moreover, a Ca/Mg ratio of ≤ 2 is associated with potential benefits in preventing metabolic syndrome dysfunctions (Afonso et al., 2022). The Ca/Mg ratio remained below 2.3 for all acorn flour samples analyzed, with a mean of 0.59 ± 0.28 for non-leached samples and 1.65 ± 0.89 for leached samples. Among the species, whether leached or non-leached, QT showed the most promising mineral balance.

3.5. Vitamin E content

Acorns are highly abundant in tocopherols (vitamin E), which can contribute to the functional properties of acorn flours (Taib et al., 2020; Vinha et al., 2016). Therefore, the vitamin E content of each flour was evaluated before and after leaching, to assess the impact of this process on this micronutrient concentration, which might affect each flour's bioactive properties since tocopherols can act as an antioxidant (Rather et al., 2023). According to the results (Fig. 4), the vitamin E content varied significantly ($p < 0.05$) among all samples. Regarding non-leached flours, QR exhibited the highest vitamin E content (20.71 ± 0.23 mg/100 g DW), while QT (17.06 ± 0.36 mg/100 g DW) and QP (15.96 ± 0.28 mg/100 g DW) showed statistically similar values, which all were slightly above the results reported by Vinha et al. (Vinha, Costa, et al., 2016). Furthermore, the results showed that the leaching process led to a decrease in vitamin E content for all species (QR: 11.38 ± 0.37 mg/100 g DW; QT: 4.61 ± 0.10 mg/100 g DW; QP: 14.76 ± 0.30 mg/100 g DW), which corroborates the observed reduction in lipid content, since tocopherols are mainly lipophilic compounds. As presented in the results, the species with a greater reduction in total lipid content also exhibited the highest reduction in vitamin E content after leaching (Rather et al., 2023).

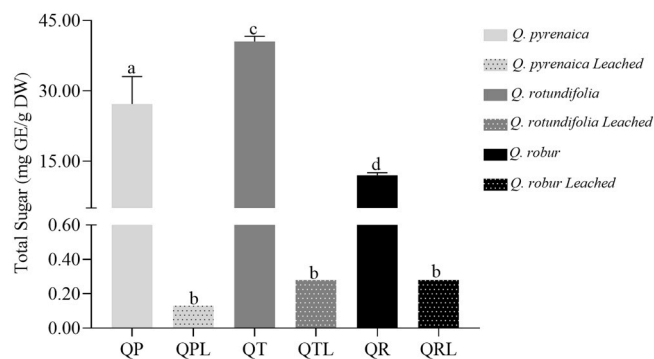


Fig. 2. Flour's total sugar content. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between samples, as determined by one-way ANOVA, followed by Tukey's test for multiple comparisons. Data are presented as mean \pm SD of mg glucose equivalents (GE)/100 g DW.

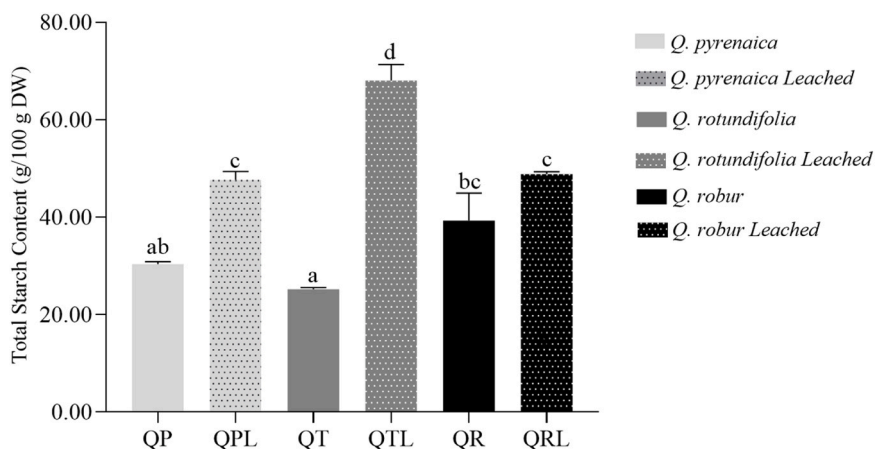


Fig. 3. Flour's total starch content. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between samples, as determined by one-way ANOVA, followed by Tukey's test for multiple comparisons. Data are presented as mean \pm SD of g/ 100 g DW.

Table 5
Mineral composition of the acorn flours.

Minerals (mg/100 g DW)	Flour ID					
	QP	QPL	QT	QTL	QR	QRL
P	118.08 \pm 6.25 ^a	15.34 \pm 0.65 ^b	76.01 \pm 2.05 ^c	15.36 \pm 1.11 ^b	71.87 \pm 5.47 ^c	14.70 \pm 2.10 ^b
K	1020.99 \pm 24.66 ^a	58.99 \pm 2.63 ^b	847.56 \pm 17.40 ^c	82.94 \pm 1.45 ^b	713.06 \pm 58.47 ^d	53.48 \pm 4.10 ^b
Na	< LOD	< LOD	< LOD	< LOD	< LOQ	< LOQ
Ca	41.67 \pm 3.59 ^a	15.54 \pm 0.55 ^b	15.42 \pm 0.94 ^b	13.17 \pm 1.51 ^b	28.24 \pm 6.05 ^c	11.93 \pm 2.25 ^b
Mg	53.35 \pm 0.68 ^a	6.80 \pm 1.00 ^b	58.41 \pm 1.78 ^a	20.63 \pm 1.52 ^c	39.06 \pm 5.07 ^d	5.88 \pm 1.65 ^b
Fe	< LOD	4.55 \pm 0.12 ^a	0.17 \pm 0.01 ^b	0.89 \pm 0.04 ^c	0.20 \pm 0.04 ^b	0.28 \pm 0.09 ^b
Zn	0.13 \pm 0.01 ^a	0.18 \pm 0.03 ^a	0.37 \pm 0.01 ^b	0.30 \pm 0.01 ^b	0.17 \pm 0.04 ^a	< LOQ
Cu	0.73 \pm 0.04 ^a	0.69 \pm 0.01 ^a	0.47 \pm 0.01 ^b	0.53 \pm 0.00 ^b	0.70 \pm 0.07 ^a	0.74 \pm 0.04 ^a
Mn	1.94 \pm 0.05 ^a	0.70 \pm 0.01 ^b	3.27 \pm 0.05 ^c	1.92 \pm 0.03 ^a	1.20 \pm 0.12 ^d	0.60 \pm 0.05 ^b
Co	0.01 \pm > ^a	0.03 \pm 0.00 ^b	0.01 \pm > ^a	0.01 \pm > ^a	0.01 \pm > ^a	0.01 \pm > ^a
Mo	ND	ND	ND	ND	ND	ND
Ni	0.05 \pm 0.00 ^a	< LOQ	0.11 \pm 0.00 ^b	0.05 \pm 0.01 ^a	< LOD	< LOD
B	3.51 \pm 0.05 ^a	2.23 \pm 0.05 ^b	1.00 \pm 0.05 ^c	0.89 \pm 0.04 ^c	1.40 \pm 0.14 ^d	1.23 \pm 0.15 ^{cd}
Cd	ND	ND	ND	ND	ND	ND
Pb	0.02 \pm 0.01 ^a	0.03 \pm 0.01 ^a	0.03 \pm 0.01 ^a	< LOQ	0.05 \pm 0.01 ^a	0.03 \pm 0.02 ^a
Al	0.11 \pm 0.02 ^a	0.53 \pm 0.04 ^b	0.20 \pm 0.01 ^{ac}	0.47 \pm 0.20 ^{bc}	0.11 \pm 0.01 ^a	0.14 \pm 0.02 ^{ab}
Total	1305.10 \pm 17.59 ^a	105.58 \pm 3.97 ^b	1003.01 \pm 18.37 ^c	137.14 \pm 3.69 ^b	856.11 \pm 74.49 ^d	89.09 \pm 10.26 ^b
Ratios (mg/100 g DW)						
Na/K	0.01 >	0.01 >	0.01 >	0.01 >	0.01 >	0.01 >
Ca/Mg	0.78	2.29	0.26	0.64	0.72	2.03

DW- dry weight; LOD- limit of detection; LOQ- limit of quantification. ND- not detected. Different lowercase letters in the same row indicate statistically significant differences ($p < 0.05$) between samples, as determined by one-way ANOVA, followed by Tukey's multiple comparisons test. Data are presented as mean \pm SD.

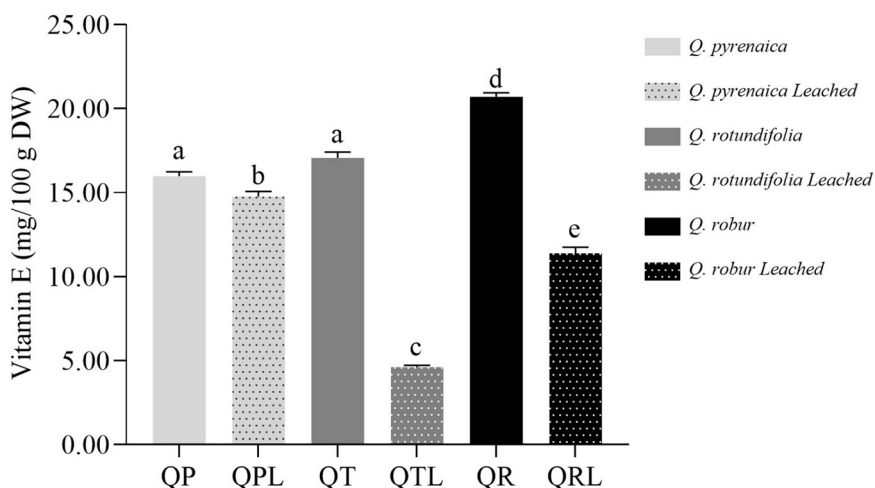


Fig. 4. Flour's vitamin E content. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between samples, as determined by one-way ANOVA, followed by Tukey's test for multiple comparisons. Data are presented as mean \pm SD.

4. Conclusion

This study provides an evaluation of the impact of leaching on the physicochemical properties and nutritional composition of acorn flours from *Q. pyrenaica*, *Q. rotundifolia*, and *Q. robur*. The findings confirm that leaching results in a substantial reduction in fiber and mineral content. Nonetheless, the leached flours retain key macronutrients and favorable fatty acid profiles, supporting their potential as functional food ingredients.

In summary, this work emphasizes the potential of acorns as a sustainable and underutilized food resource with valuable nutritional properties. Therefore, future research should focus on optimizing leaching methods to minimize the loss of important nutrients while developing the applicability of acorn flours in functional food formulations. Additionally, exploring alternative debittering techniques and assessing their effects on the functional and sensory characteristics of acorn flours could further facilitate their integration into mainstream food markets. In conclusion, acorn-based products offer promising avenues for sustainable food innovation and the diversification of gluten-free options, contributing to the advancement of the functional food industry.

CRedit authorship contribution statement

Cristina V. Rodrigues: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Luz Maia:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pedro Babo:** Writing – review & editing, Validation. **Manuela Pintado:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2025.108788](https://doi.org/10.1016/j.jfca.2025.108788).

Data availability

Data will be made available on request.

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