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From Forest to Table: Optimizing the Nutritional Value of Acorns through Effective Tannin Extraction †

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Abstract: The acorn, a fruit of the *Quercus* genus, is abundant in Portugal and offers a high nutritional content, including essential minerals and vitamins, unsaturated fatty acids, and bioactive compounds like tannins, phenolic acids, and flavonoids. Despite their antioxidant properties, excessive consumption of tannins can cause health problems due to their effect on protein bioavailability. This study evaluated two extraction techniques, water immersion at room temperature for seven days and thermal hydrolysis via boiling for 30 min, and their effects on acorn's tannin and mineral content. The results reveal that both extraction methods are viable options for extracting tannins, although they also reduce some important minerals.

Keywords: acorn; extraction techniques; minerals; tannins

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1. Introduction

Acorns are the fruits of species from the genus *Quercus*, which includes oaks (*Quercus coccifera* L. and *Quercus robur* L.), cork oaks (*Quercus suber* L.), and holm oaks (*Quercus rotundifolia* Lam.). These are abundant in the Portuguese territory and occupy about 36% of the national forest area [1].

In the past, oaks were extremely important to the country, being used for food, building materials, energy and shelter for hunting. However, following the improvement of economic conditions, the consumption of acorn by humans was becoming increasingly scarce. In fact, before the last decade, acorns were mainly used for feeding pigs, due to their high content in carbohydrates, proteins and lipids, or as a raw material for the production of acorn oil [2].

Recently, interest in the research of these fruits as food has increased since they are nutritious, with good content of calcium, iron, magnesium, potassium, phosphorus, vitamins A and E, and unsaturated fatty acids. In addition, they have biologically active compounds, namely tannins, phenolic acids, and flavonoids that are essential to maintain an adequate level of antioxidants, which provide important functions in reducing the risk of cancer and preventing degenerative diseases, among other disorders [3]. However, the consumption of tannins in high amounts can cause some health problems because they affect the bioavailability of proteins and are often considered anti-nutrients [4].

There are several extraction techniques to determine tannins in acorns: water immersion, thermal, chemical, and enzymatic hydrolysis [5]. This research aimed to assess two tannin extraction techniques from acorns of the Quercus species, specifically *Q. robur*, prevalent in Northern Portugal. Their effects on mineral content were also evaluated, aiming to optimize health benefits while minimizing potential risks. While offering insights into dietary applications, the study's scope is limited to these techniques and does not investigate other compounds or extraction methods.

2. Materials and Methods

2.1. Samples

Acorns of the oak-*alvarinho* species (*Q. robur*) were collected in October/November 2021 in the Minho region, Portugal. The taxonomic analysis of the samples was conducted by us according to the morphological characters described in Iberian flora [6] and also based on the distribution of Quercus species in Portugal as described in the maps of Flora-on (https://flora-on.pt/?q=Quercus+robur) [7]. The acorns were first dehydrated in a food dehydrator (Excalibur 9 Tray Dehydrator, Model 4926 T, USA) at 41 °C for about 72 h. Then, the acorn's pericarp and seed were separated, thus obtaining only the embryo.

2.2. Tannin Extraction Techniques

In this work, two extraction techniques were carried out without agitation: (1) water immersion: the acorns were submerged in water at room temperature. The water was changed daily over fourteen days, and (2) thermal hydrolysis: acorns were boiled for 30 min, after which the water was discarded. This boiling process was repeated, with a fresh batch of water introduced for the second boil, ensuring two boiling sessions, each lasting 30 min. During the washing stage, all the waters utilized for immersion and boiling were collected and stored for subsequent analysis of their tannin content. The total tannin content was determined using methylcellulose, ammonium sulphate, and epicatechin, as described by Sarneckis et al. (Figure 1) [8]. Tannin content was expressed as mg Epicatechin Equivalents (EE)/g of sample and as mg EE/L of wash water. All the analyses were done in triplicate.

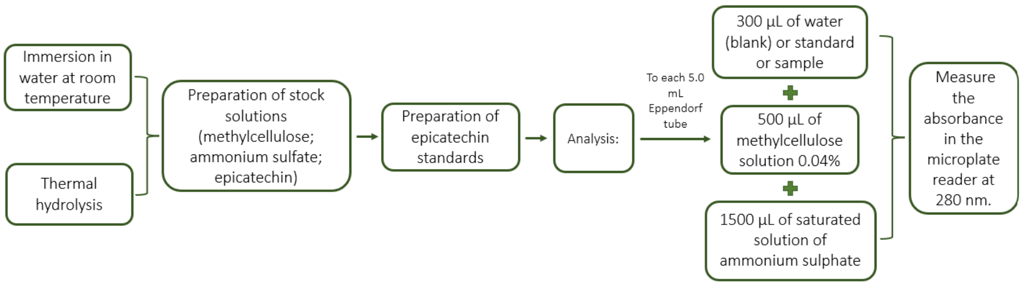

Figure 1. Quantitative determination of tannins content.

2.3. Mineral Analysis

To determine and quantify the sample minerals, atomic absorption spectroscopy (ContrAA 700 High-Resolution Continuum Source Flame Atomic Absorption Spectrometer, Analytik Jena, Jena, Germany) [9] and X-ray fluorescence (XRF) (Thermo Fisher Scientific, Billerica, MA, USA) were employed. Sodium and magnesium were analyzed at 589.6 nm and 285.2 nm, respectively, using atomic absorption spectrophotometry. The other minerals were assessed using an XRF spectrometer, with solid samples placed in capsules for automated analysis by the equipment.

2.4. Statistical Analysis

The assessment of data normality involved conducting the Kolmogorov–Smirnov and Shapiro–Wilk tests. Determined values were expressed as mean ± standard deviation, and group comparisons utilized the Mann-Whitney test at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Condensed Taninns

Tannins, polyphenolic compounds in plants, impact nutritional bioavailability and are termed “anti-nutrients” because they hinder nutrient absorption. They bind strongly to proteins, particularly digestive enzymes, forming complexes that reduce protein digestibility [10]. Tannins inhibit enzymes like amylase and lipase, affecting carbohydrate and fat digestion [10]. They also bind to minerals like iron and calcium, reducing their absorption and potentially leading to deficiencies [11]. While tannins can disturb metabolism and irritate the gastrointestinal tract, moderate consumption offers health benefits, including antioxidant properties and potential protection against heart disease and cancer [12]. Balancing these effects depends on intake quantity and diet. Extraction techniques affecting tannin content, especially in foods like acorns, are crucial for maximizing benefits and minimizing problems.

As depicted in Figure 2, initial immersion extraction at room temperature yielded 14,385.7 ± 719.0 mg EE/L. However, tannin content decreased notably in subsequent daily extractions. After fourteen days, only 265.1 ± 58.2 mg EE/L was extracted. In contrast, thermal hydrolysis extracted 22,811 ± 1894.0 mg EE/L after one boil and 15,525 ± 2531.7 mg EE/L after two boils.

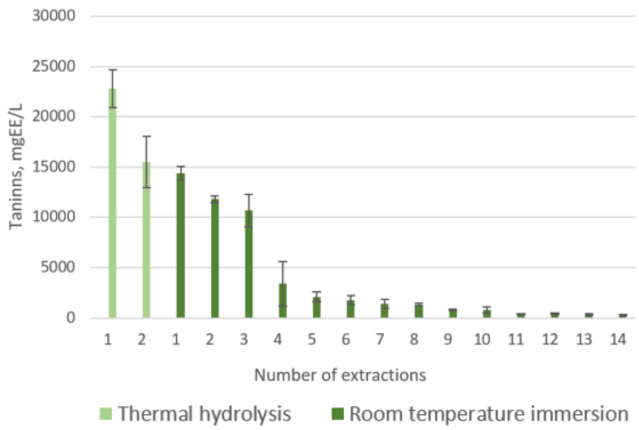

Figure 2. Concentration of tannins in different extractions techniques (n = 3).

Both extraction techniques resulted in no waste; the obtained solutions were transformed into tannin powder for subsequent use. Therefore, the selection of the technique depends on the intended objective.

Post-extraction, the remaining condensed tannins in the acorns were quantified using the method described in Section 2.2. The results are presented in Table 1, indicated in mg EE/g of the sample.

Table 1. Concentration of tannins present in each sample after water immersion and thermal treatment (*n* = 3).

Acorn Samples	Tannins (mg EE/g)
No treatment	
Raw	77.1 ± 2.5 [c]
Thermal hydrolysis	
1 boil	57.7± 12.1 [b]
2 boils	34.8 ± 6.5 [a]
Water immersion	
7 days in water	34.5 ± 1.3 [a]
10 days in water	27.0 ± 1.3 [a]

14 days in water	20.5 ± 1.0 a

Within columns, homogeneous groups are shown by the same letter (Mann-Whitney test) for $p < 0.05$.

Upon examining Table 1, it is evident that the tannin concentrations in acorns subjected to two boils and immersion in water for seven, ten, and fourteen days are notably similar ($p < 0.05$). The decline in acorn tannin content over 14 days results from natural diffusion, cumulative extraction, and equilibrium dynamics. Daily water changes are crucial, promoting continuous extraction and preventing saturation. The increased tannin concentration after one boil in Table 1 arises from initial extraction efficiency and varying boiling dynamics. Prolonged heat and compound interactions might reduce the second boil's effectiveness. As anticipated, untreated acorns exhibit higher tannin content, showing significant statistical differences from other samples ($p < 0.05$). Cooking and processing can reduce tannin levels in certain foods. For instance, after cooking, reductions in rhubarb, broad beans, and pears were 28%, 58%, and 26%, respectively [11]. Łuczaj et al. documented a tannin content range of 2.43–5.17% in Poland's *Q. robur* raw acorns [13], aligning closely with our findings of 7.71%. Factors like region, variety, processing methods, storage duration, agricultural techniques, and cultivar variations significantly influence tannin content [11,13].

Both extraction methods are viable options, but each comes with its own set of advantages and disadvantages. An increased number of extractions through thermal hydrolysis is needed to achieve the same total tannin extraction using immersion in water. While the latter method is quicker, it also entails higher energy consumption. However, both extraction methods can be utilized for subsequent research or applications.

3.2. Minerals

The results emphasize the practicality of both extraction methods for tannin extraction, even though they lead to a reduction in essential minerals. For this study, thermal hydrolysis was chosen to assess crucial minerals in acorns. The analysis encompasses untreated acorns, one-time boiled, and two-times boiled samples. The mineral content outcomes are outlined in Table 2 and expressed in mg/g of the sample.

Table 2. Amount of some minerals in each sample (n = 3).

Extractions / Minerals, mg/g	-	1 Boil	2 Boils
Calcium (Ca)	11.3 ± 0.2 b	0.688 ± 0.089 a	0.396 ± 0.086 a
Copper (Cu)	0.0349 ± 0.0044 b	0.0227 ± 0.0037 a	0.0212 ± 0.0036 a
Iron (Fe)	0.351 ± 0.015 c	0.0609 ± 0.0080 b	0.0292 ± 0.0072 a
Magnesium (Mg)	0.299 ± 0.044 a	0.278 ± 0.075 a	0.332 ± 0.068 a
Potassium (K)	23.4 ± 0.3 c	19.4 ± 0.3 b	18.0 ± 0.3 a
Sodium (Na)	0.142 ± 0.019 a	0.287 ± 0.033 b	0.256 ± 0.002 b
Zinc (Zn)	0.0193 ± 0.0025 b	0.00462 ± 0.00189 a	0.00352 ± 0.00179 a

Within lines, homogeneous groups are shown by the same letter (Mann-Whitney test) for $p < 0.05$.

According to Table 2, along with the extractions of tannins, some minerals are reduced, namely calcium, copper, iron, potassium, and zinc. These minerals have an important role in human health. The calcium cation (Ca^{2+}) is essential in signal transduction, regulating target cell activity, and cardiac excitation-contraction [14–16]. Specifically, calcium emerges as the predominant mineral, but extractions show a noTable 96.5% reduction with significant statistical differences ($p < 0.05$). Potassium helps lower blood pressure and reduces the progression of kidney and cardiovascular diseases [17]. Analyzing Table 2, potassium, like calcium, is one of the more abundant minerals in acorns. Significant statistical differences exist between untreated and treated acorns ($p < 0.05$), although with less than a 20% reduction in K content. Finally, transition metals such as iron, zinc, and copper are essential in cellular metabolism, defense against oxidative stress, and enzymatic and antioxidant function, and all of them have significant changes ($p < 0.05$) [18,19]. Even though iron presents a low concentration, it decreases by 91.6%.

On the other hand, the amount of magnesium, which is fundamental in muscle contraction, nerve conduction, and bone strength and acts on transmembrane ion transport pumps, does not show significant changes ($p < 0.05$) during extractions of tannins [20].

Excessive sodium intake raises blood pressure [17]. In this instance, sodium levels are low, and the extraction has no variations in sodium amounts. However, an unexpected increase in sodium content is observed when comparing the untreated acorn to the one boiled. Sodium is known to have organic forms that might not be easily extracted through water immersion [21]. Compared to the raw acorn, the rise in Na content after boiling could be clarified by the breakdown of cell walls and membranes during boiling. This breakdown potentially releases sodium previously held within these structures [22]. Boiling may leach minerals and tannins from acorns into the water, leading to a relative rise in the acorn's sodium concentration. This observed increase might result from multiple factors, considering the acorn's complex composition and interactions during boiling. Determining the precise cause requires analyzing the experimental conditions.

This study explored tannin extraction techniques from acorns, emphasizing the impact of boiling on mineral content. Strengths include a detailed comparison of two extraction methods and a comprehensive mineral impact assessment, supported by statistical analysis. However, limitations include a lack of depth in energy consumption assessment and exploring alternative extraction methods. An unanticipated sodium increase post-boiling suggests unexpected extraction interactions. Moreover, concerns arise about the extraction consistency across various acorn types. Despite its insights, the research highlights potential techniques for further exploration in acorn processing efficiency and nutritional valorization.

4. Conclusions

In conclusion, the choice of extraction method depends on specific needs. Thermal hydrolysis suits those seeking speed, while water immersion is cost-effective for budget-conscious individuals. In this instance, thermal hydrolysis was chosen, reducing critical minerals necessary for human health. Calcium and potassium are the most abundant minerals in acorns, with calcium showing a more pronounced reduction during tannin extraction. Further laboratory tests on extraction water and samples should be conducted for future endeavors.

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