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Acorns as a Functional Food for Cardiovascular Disease Prevention: Chemical Characterization and Bioactivity †

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Abstract: Acorns are one of the most promising natural resources to be considered nationally and internationally due to their nutritional benefits. Thus, their valorization is an essential factor of sustainability nowadays. Therefore, this project aims to characterize acorns by analyzing their chemical composition and bioactivity toward cardiovascular disease-related enzyme inhibition. Proximate analysis was performed where moisture, ash, lipids, and proteins were determined, and the values obtained in mass percentage on a dry basis were 10.1 ± 0.2, 1.94 ± 0.03, 3.94 ± 0.34 and 3.72 ± 0.07, respectively. The concentration of acorn extract that inhibits 50% of the enzyme α-glucosidase is 5.36 ± 0.66 µg/m, showing their great potential as an anti-diabetic agent, one of the risk factors for cardiovascular diseases; however, no inhibition of the α-amylase was registered.

Keywords: acorns; cardiovascular diseases; chemical characterization; functional foods; sustainability

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1. Introduction

Since the turn of the century, cardiovascular diseases (CVD) have continued to be the leading cause of mortality worldwide and are currently regarded as one of the most critical public health problems. These pathologies affect the cardiovascular system and may be associated with several risk factors, such as diabetes, hypertension, and hypercholesterolemia [1].

Sustainability and the circular economy of food production are among the main market trends that have led to the search for innovative solutions, such as using raw materials not typically used for human food. In this sense, the exploitation of undervalued natural resources, particularly regarding low-cost and highly available vegetable matrices in Portugal, such as acorns, is possible.

Acorns are dry fruit from trees of the *Quercus* genus that belong to the *Fagaceae* family. In Portugal, there are more than 300 species, and the most predominant are the common oak (*Quercus robur* L.), Portuguese oak (*Quercus faginea* Lam.), pyrenean oak (*Quercus pyrenaica* Willd.), holm oak (*Quercus ilex* L.) and cork oak (*Quercus suber* L.) [2].

While typically perceived as animal feed, this fruit is still part of the traditional gastronomy of several Mediterranean countries, being consumed in the form of flour to make bread or even various traditional drinks such as coffee or acorn liqueur. However, its nutritional value, high content regarding phytochemical compounds, and biological activity (such as antioxidant, anticarcinogenic, and cardioprotective properties) have raised interest in integrating this nut into the human diet as a potential functional food alternative, bringing potential beneficial effects for health and the treatment of diseases [3].

Acorns were already reported as having a high content of carbohydrates (75–84%), mainly starch (51–57%), fibre (10–18%) and low levels of proteins (4–5%) and fat content

(8–14%), presenting a high nutritional value comparable to the most common cereals [2]. However, due to the high variability of the genus and depending on the species, acorns can differ in chemical composition.

This study aimed to characterize acorns by analyzing their chemical composition and bioactivity against α-amylase and α-glucosidase activities to assess their potential use as a functional food with anti-diabetic properties.

2. Materials and Methods

2.1. Samples

Mature acorn fruits were manually collected from common oak (*Quercus robur* L.), in mid-October/November 2021 in a private forest in the Minho region, Portugal. The acorns were washed and dehydrated at 41 °C for 72 h in a food dehydrator (Excalibur 9 Tray Dehydrator, Model 4926 T, Sacramento, CA, USA). After this process, the seed and pericarp of the fruit were separated.

For the analyses, approximately 100 to 200 g of sample were ground in a mill (ZM 200, Retsch, Haan, Germany), and particles were separated by size with a 2 mm sieve shaker (AS 200 Basic, Retsch, Haan, Germany) until a fine powder was obtained.

2.2. Proximate Analysis

Several nutritional parameters of the acorn were analyzed, such as moisture, ash, total lipids and total proteins, using sample triplicates for each analysis.

The moisture content was estimated through the mass variation after oven drying at 105 °C, and the ash content was determined by the dry incineration method in a muffle furnace at 600 °C for 6 h. The determination of total lipid content was performed by solid-liquid extraction using a Soxhlet extractor (Soxtest, Raypa, Barcelona, Spain) with *n*-hexane, according to Soares et al. [4], and the total protein was assayed through the Kjeldahl method as described in Vieira et al. [5].

2.3. Bioactivity Analysis

The acorn extract for this analysis was prepared from a concentration of 50 mg/mL, where 1 g of acorn powder was dissolved in 20 mL of buffer (potassium phosphate buffer solution 10 mM (K_2HPO_4/KH_2PO_4, pH 7)).

The enzyme inhibition assays were performed in 96-well plates and analyzed in a microplate reader (BioTek Synergy HTX Multimode Reader, Winooski, Vermont, EUA) as reported by Figueiredo-González et al. [6]. For the α-glucosidase, the absorbance was measured at 405 nm after incubation at 37 °C for 10 min [7,8]. To evaluate the inhibitory effect of the samples against the α-amylase (porcine pancreatic enzyme), the absorbance was measured at 540 nm according to Lordan et al. [8] after dissolving the extract in buffer (20 mM sodium phosphate buffer (Na_3PO_4 with 6 mM NaCl, pH 6.9)). The IC_{50} values were calculated using Graph Pad Prism Software Version 8 (JMP Statistical Discovery, Marlow, Buckinghamshire, United Kingdom). All the assays were carried out in triplicate, and acarbose was used as the positive control.

3. Results and Discussion

3.1. Chemical Composition

The values obtained for the proximate analysis in mass percentage on a dry basis are shown in Table 1.

The moisture and ash content are the most frequently determined parameters to analyze the proximate composition of a matrix. The values obtained were 10.07 ± 0.24% for moisture and 1.94 ± 0.03% for ash content, both results being within the expected range of values according to what Silva et al. [2] reported (5–22% for moisture and 1–2% for ash).

From a nutritional standpoint, acorns exhibited low levels of lipids (3.94 ± 0.34%) and low protein content (3.72 ± 0.07%). However, the values obtained are slightly lower than those previously reported by Silva et al. [2] (lipids levels of 8%–14% and protein values of 4–5%), but still in conformity.

Table 1. Acorn chemical composition.

Parameters	Content (%) [1]
Moisture	10.1 ± 0.2
Ash	1.94 ± 0.03
Total lipids	3.94 ± 0.34
Total protein	3.72 ± 0.07

[1] The values are presented as a percentage on a dry basis (%).

3.2. Bioactivity

CVD, kidney damage and neuropathy (neurological disorder) are the leading cause of high mortality rates among individuals with diabetes [9]. A therapeutic approach to the treatment of diabetes is to reduce postprandial hyperglycemia by inhibiting digestive enzymes such as α-glucosidase and α-amylase as they are responsible for digesting complex carbohydrates, converting them into easily digestible simple monosaccharides, such as glucose [10]. These enzymes play their role in different parts of the body in mammals. For example, α-glucosidase is an intestinal enzyme bound to the epithelial membrane of the small intestine that catalyzes the final step in the digestive process of carbohydrates into glucose. At the same time, α-amylase is an enzyme that participates in digestion in two ways, one produced in the salivary glands, and another produced in the pancreas, catalyzing the hydrolysis of starch that transforms it into simpler sugar molecules such as glucose and maltose [10,11].

Currently, some synthetic inhibitors of both enzymes, such as acarbose, miglitol and voglibose, are widely used in clinics to control blood glucose levels in patients. However, they can cause negative gastrointestinal symptoms. In this sense, the scientific community has been looking for new natural compounds with anti-diabetic properties to overcome any resistance developed by patients to the drugs currently used. Ideally, the discovery of α-glucosidase and α-amylase inhibitors from natural materials, such as food matrices, will be very useful in developing new anti-diabetic drugs for treating diabetes and its complications [12].

Regarding acorn's bioactivities, the extract analyzed showed it to be a suitable inhibitor for the α-glucosidase, as illustrated in Figure 1. The concentration of acorn extract that inhibits 50% of the enzyme was 5.36 ± 0.66 μg/mL, which turns out to be a much better value than for the acarbose positive control with IC_{50} = 304 ± 37 μg/mL (more than 50 times lower).

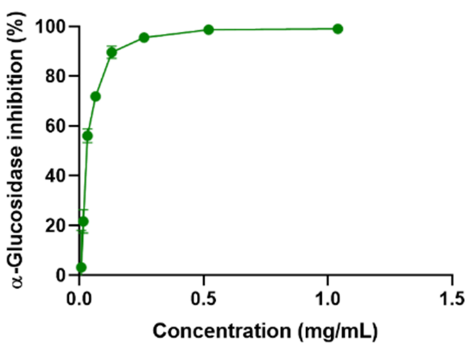

Figure 1. Dose-response curve of acorn extract in an α-glucosidase inhibition assay.

Interestingly, there are not many studies on the inhibitory effect of acorns on this enzyme. However, a study carried out by Güvenalp et al. [13] investigated the inhibitory activity of methanolic extracts of acorn of the species *Quercus robur* L., reporting an IC_{50} value = 7.1 μg/mL, very close to that obtained in this work for this matrix (IC_{50} = 5.36 ± 0.66 μg/mL).

α-Amylase (derived from the porcine pancreas) was another enzyme used to evaluate the potential of the studied sample to suppress postprandial hyperglycemia through its inhibition capacity. An IC_{50} value = 913 µg/ mL was obtained for acarbose (positive control). Acorn showed some inhibitory effect on the activity of this enzyme for the highest concentration tested, 1 mg/mL, with an inhibition percentage of 44.5 ± 8.5%.

In the literature, no information was found on the ability to inhibit α-amylase by any acorn species.

Although acorns did not show much capacity to inhibit the α-amylase, it is essential to carry out additional studies to test different solvents and extraction techniques to verify if they can achieve a more significant inhibition of this enzyme.

4. Conclusions

For its national and international importance, the acorn is one of the most promising natural food resources to consider. This study showed several nutritional benefits and potentially cardiovascular disease prevention properties by inhibiting an enzyme related to diabetes, one of the main risk factors for CVD. Thus, this study showed that acorns have a high potential use as a functional food for the prevention of cardiovascular diseases, while their valorization represents an important sustainability factor nowadays. Furthermore, in the bioactivity study, acorn samples showed their potential as anti-diabetic agents since the extracts presented stronger inhibition of α-glucosidase compared to acarbose (positive control). On the other hand, low inhibitions were recorded for α-amylase.

Following the analyses performed, it was demonstrated that acorns exhibited low levels of lipids and low protein content.

As future work, it is intended to perform further analyses of the chemical composition of the acorn, such as the content of carbohydrates, amino acid and fatty acid profiles, fibres, minerals, and phenolic profile, among others, and study more of their biological potential to assess their use as a functional food with anti-diabetic properties.

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Conflicts of Interest: The authors declare no conflict of interest.

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