

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

Ana Nogueira, Cristina Soares ^{*}, Clara Grosso and Maria João Ramalhosa

REQUIMTE/LAQV, Instituto Superior de Engenharia do Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4249-015 Porto, Portugal; 1150654@isep.ipp.pt (A.N.); claragrosso@graq.isep.ipp.pt (C.G.); mjr@isep.ipp.pt (M.J.R.)

^{*} Correspondence: cmdss@isep.ipp.pt

[†] Presented at the 3rd International Electronic Conference on Foods: Food, Microbiome, and Health—A Celebration of the 10th Anniversary of Foods' Impact on Our Wellbeing; Available online: <https://foods2022.sciforum.net>.

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 10.07 ± 0.24 , 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 $29.7 \mu\text{g/mL}$; however, no inhibition of the α -amylase was registered.

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

Citation: Nogueira, A.; Soares, C.; Grosso, C.; Ramalhosa, M.J. Acorns as a Functional Food for Cardiovascular Disease Prevention: Chemical Characterization and Bioactivity. *Biol. Life Sci. Forum* **2022**, *2*, x. <https://doi.org/10.3390/xxxxx>
Published: 1 October 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Since the turn of the century, cardiovascular diseases continue to be the leading cause of mortality worldwide and are currently regarded as one of the most important public health problems. These pathologies affect the cardiovascular system and may be associated with several risk factors that can cause diseases such as diabetes, hypertension, and hypercholesterolemia, among others [1].

Sustainability and 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 typical of 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 a 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 contents in phytochemical compounds, and biological activity (such as antioxidant, anticarcinogenic, and cardioprotective properties) have raised the interest in integrating this nut into the human diet as a potential functional food alternative, bringing potential beneficial effects for health and for the treatment of diseases [3].

Acorns were already reported as having high contents in carbohydrates (75–84%), mainly starch (51–57%), fiber (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.

Overall, this study aimed to characterize acorns by analyzing their chemical composition and bioactivity against α -amylase and α -glucosidase activities.

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, 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) 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 on 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 a buffer.

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 enzyme the absorbance was measured at 405 nm, after incubation at 37 °C for 10 min, and 540 nm for the α -amylase enzyme. The IC₅₀ values were calculated using Graph Pad Prism Software Version 8. All the assays were carried out in triplicate.

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.

Table 1. Acorn chemical composition.

Parameters	Content (%) ¹
Moisture	10.07 ± 0.24
Ash	1.94 ± 0.03
Total lipids	3.94 ± 0.34
Total protein	3.72 ± 0.07

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

The moisture and ash content are one of the most frequently determined parameters to analyze the proximate composition of a matrix. The values obtained were $10.07 \pm 0.24\%$ for moisture and $1.94 \pm 0.03\%$ 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 ($3.94 \pm 0.34\%$) and low protein content ($3.72 \pm 0.07\%$). 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.

3.2. Bioactivity

Regarding acorn's bioactivities, the acorn extract analyzed showed to be a good inhibitor for the α -glucosidase as illustrated in Figure 1. The concentration of acorn extract that inhibits 50% of the enzyme was $29.7 \mu\text{g/mL}$, which turns out to be a better value than the positive control (acarbose).

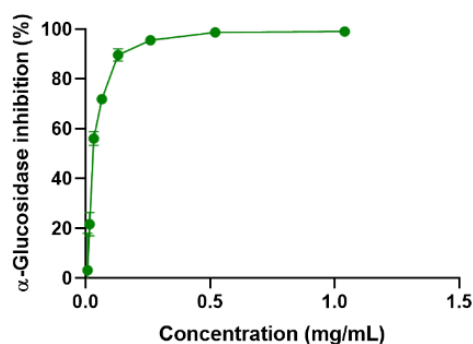


Figure 1. Dose-response curve of acorn extract in α -glucosidase inhibition assay.

On the other hand, no inhibition of the α -amylase was registered, therefore, acorn extract proved not to be a good inhibitor of this enzyme.

4. Conclusions

For its national and international importance, acorn is one of the most promising natural food resources to consider. This study showed some of its several nutritional benefits and potential cardiovascular disease prevention properties.

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

In terms of acorns bioactivities, acorns showed their great potential as antidiabetic agent since the extract displayed strong α -glucosidase inhibition. However, no inhibition of the α -amylase was registered.

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.

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, fibers, minerals, phenolic profile, among others, and study more of their biological potential.

Author Contributions: Conceptualization, C.S., C.G. and M.J.R.; methodology, A.N., C.S., C.G., M.J.R.; validation, C.S., C.G. and M.J.R.; formal analysis, C.S., C.G. and M.J.R.; investigation, A.N., C.S. and C.G.; resources, C.S., C.G. and M.J.R.; writing—original draft preparation, A.N.; writing—review and editing, A.N., C.S., C.G. and M.J.R.; supervision, C.S., C.G. and M.J.R.; project administration, C.S., C.G. and M.J.R.; funding acquisition, C.S., C.G. and M.J.R. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are grateful for the financial support from REQUIMTE/LAQV—UIDB/50006/2020, UIDP/50006/2020 and LA/P/0008/2020, financed by FCT/MCTES and the project SYSTEMIC, “An integrated approach to the challenge of sustainable food systems: adaptive and mitigatory strategies to address climate change and malnutrition”. The Knowledge hub on Nutrition and Food Security received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI) in a joint action of JPI HDHL, JPI-OCEANS, and FACCE-JPI launched in 2019 under the ERA-NET ERA-HDHL (n° 696295).

Acknowledgments: Clara Grosso is thankful for her contract (CEECIND/03436/2020) financed by FCT/MCTES—CEEC Individual 2020 Program Contract.

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

References

1. Bays, H.E.; Taub, P.R.; Epstein, E.; Michos, E.D.; Ferraro, R.A.; Bailey, A.L.; Kelli, H.M.; Ferdinand, K.C.; Echols, M.R.; Weintraub, H.; et al. Ten things to know about ten cardiovascular disease risk factors. *Am. J. Prev. Cardiol.* **2021**, *5*, 100149.
2. Silva, S.; Costa, E.M.; Borges, A.; Carvalho, A.P.; Monteiro, M.J.; Pintado, M.M.E. Nutritional characterization of acorn flour (a traditional component of the Mediterranean gastronomic folklore). *J. Food Meas. Charact.* **2016**, *10*, 584–588.
3. Vinha, A.F.; Barreira, J.C.; Costa, A.S.; Oliveira, M.B.P. A new age for *Quercus* spp. fruits: Review on nutritional and phytochemical composition and related biological activities of acorns. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 947–981.
4. Soares, C.; Sousa, S.; Machado, S.; Vieira, E.; Carvalho, A.P.; Ramalhosa, M.J.; Morais, S.; Correia, M.; Oliva-Teles, T.; Domingues, V.F.; et al. Bioactive lipids of seaweeds from the portuguese north coast: Health benefits versus potential contamination. *Foods* **2021**, *10*, 1366.
5. Vieira, E.F.; Soares, C.; Machado, S.; Correia, M.; Ramalhosa, M.J.; Oliva-Teles, M.T.; Carvalho, A.P.; Domingues, V.F.; Antunes, F.; Oliveira, T.A.C.; et al. Seaweeds from the Portuguese coast as a source of proteinaceous material: Total and free amino acid composition profile. *Food Chem.* **2018**, *269*, 264–275.
6. Figueiredo-González, M.; Grosso, C.; Valentão, P.; Andrade, P.B. α -Glucosidase and α -amylase inhibitors from *Myrcia* spp.: A stronger alternative to acarbose? *J. Pharm. Biomed. Anal.* **2016**, *118*, 322–327.