

Screening Methodologies to Extract Polyphenols from Olive Oil Pomace

Martins, V., Ribeiro, T. B., Pintado, M., Morais, R.M.C.S., Morais, A.M.M.B.*

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal; *abmorais@ucp.pt.

Introduction

Olea europaea L. is a plant, which fruits are the source of the olive oil. Each year two million ton of olive oil is produced, causing 30 million m³ of olive mill waste [1], which consist of olive oil pomace and olive wastewater. Under a circular economy approach, it becomes interesting to use the bioactive compounds present in these olive oil by-products [2]. The olive oil pomace may contain polyphenols (hydroxytyrosol, tyrosol, oleuropein, catechol, caffeic acid, verbascoside), compounds with antioxidant and antimicrobial properties, which can have applications in the food industry [3].

Extraction techniques such as maceration in water or in hydroalcoholic solutions [3,4] are used to obtain the bioactive compounds; and other techniques such as ultra-turrax, ultrasounds³ or enzymes⁴ may be used to increase the yield of extraction. This work aims the selection of the best technique to obtain the highest quantity of the principal polyphenols present in the olive oil pomace.

Objectives

The main objective of this study was to select the best methodologies to extract polyphenols from olive oil pomace, in order to incorporate these bioactive compounds in coating formulations for food applications.

Materials and Methods

Extraction of the bioactive compounds from olive oil pomace (from harvest 2021, supplied by Casa Alta – Sociedade Transformadora de Bagaços, Ferreira do Alentejo - Beja):

- hydroalcoholic maceration
- ultra-turrax
- ultrasound
- enzymes (cellulase and viscoenzyme)

To carry out the extraction, the best solvent/solute ratio was selected, as well as the temperature, combination of solvents and techniques, based on literature.



Figure 1. Olive oil pomace sample.

Results

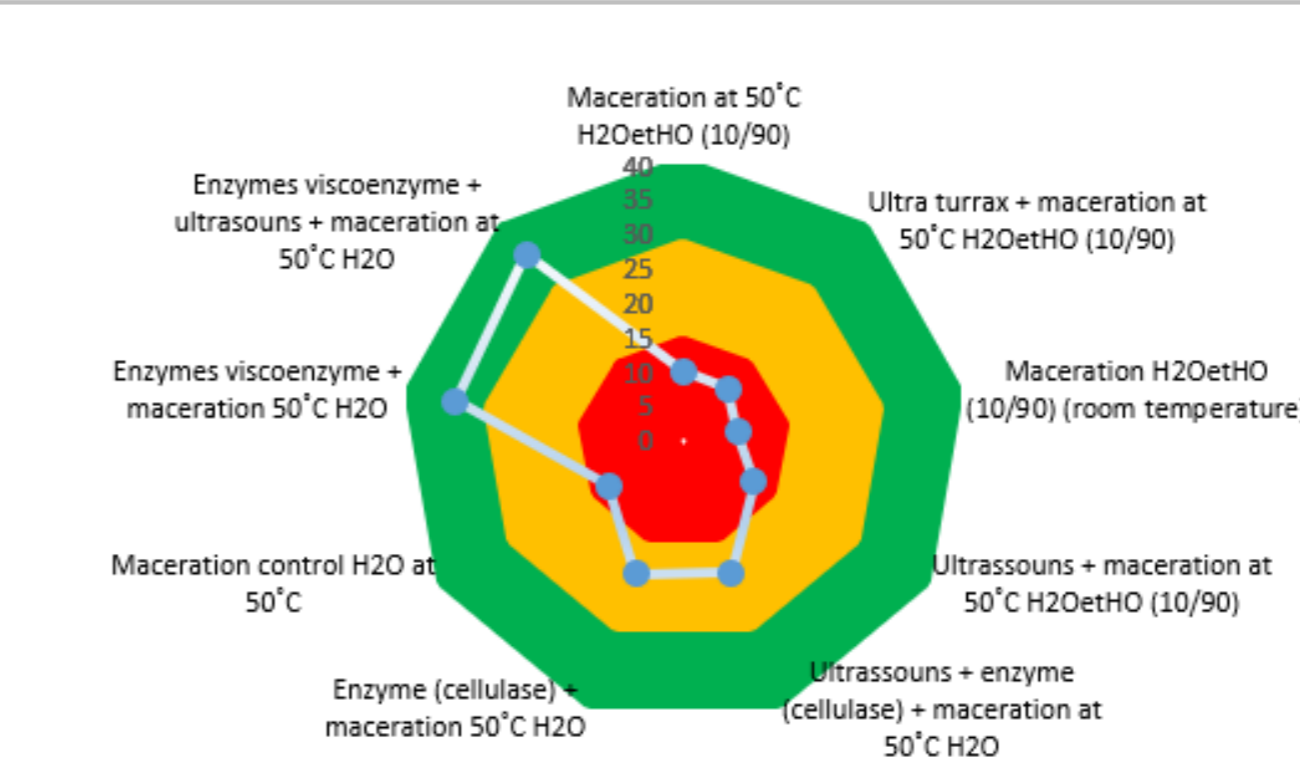


Figure 3. Yield of extraction (mg/g) using different solvents and techniques (different colours mean significant statistical differences, green =a; yellow=b; red=c).

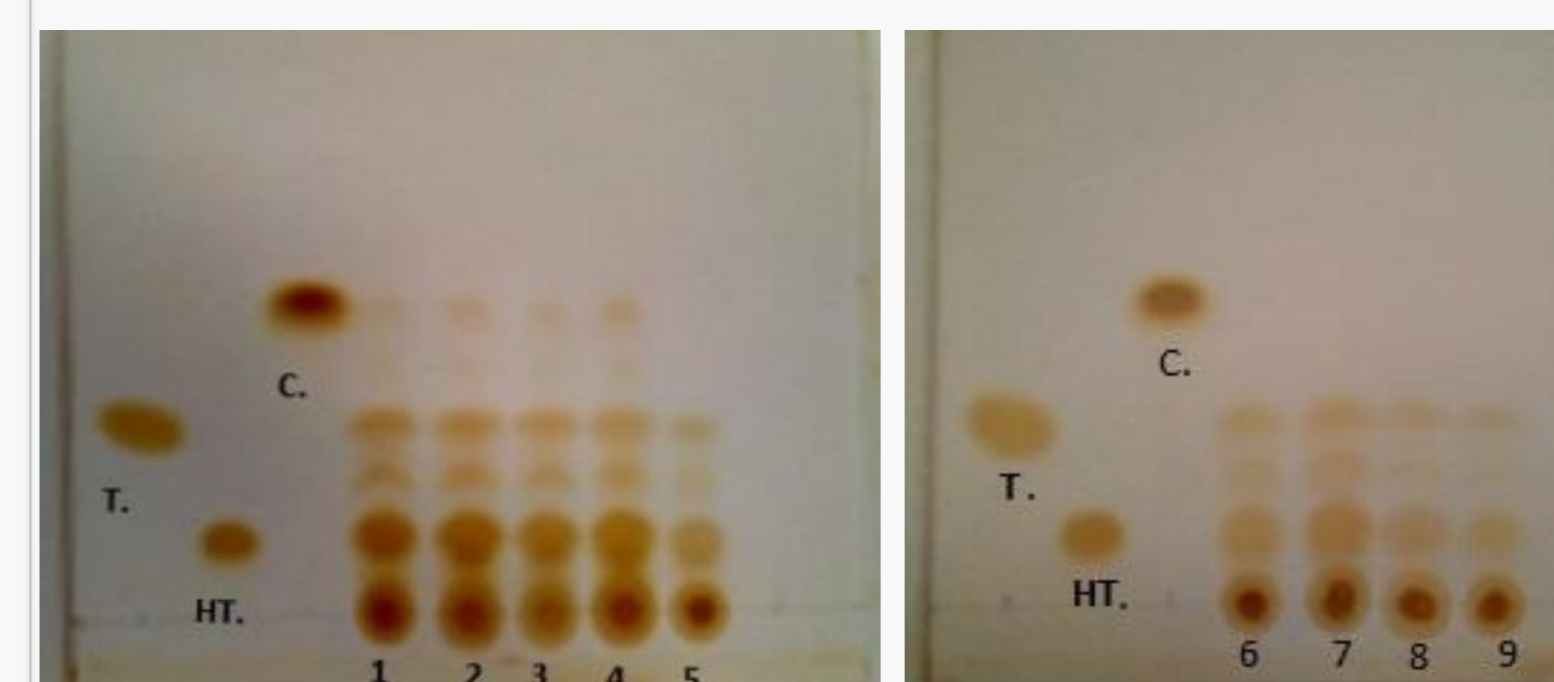


Figure 4. Thin layer chromatography (TLC) results. T – Tyrosol; HT – Hydroxytyrosol; C – Catechol; (standards) 1, 2, 3, 4, 5, 6, 7, 8, 9 – samples; Eluent used - Toluene:Acetone (9:1)

Table 1 – Retention factor (RF) values. (different letters mean significant statistical differences)

Standards	1	2	3	4	5	6	7	8	9
Hydroxytyrosol (0,13 a)	0,12a	0,12a	0,12a	0,12a	0,12a	0,12a	0,12a	0,12a	0,12a
Tyrosol (0,30 b)	0,31b	0,31b	0,31b	0,31b	0,29b	0,41b	0,29b	0,29b	0,29b
Catechol (0,51 c)	0,51c	0,51c	0,51c	0,51c	0	0	0	0	0

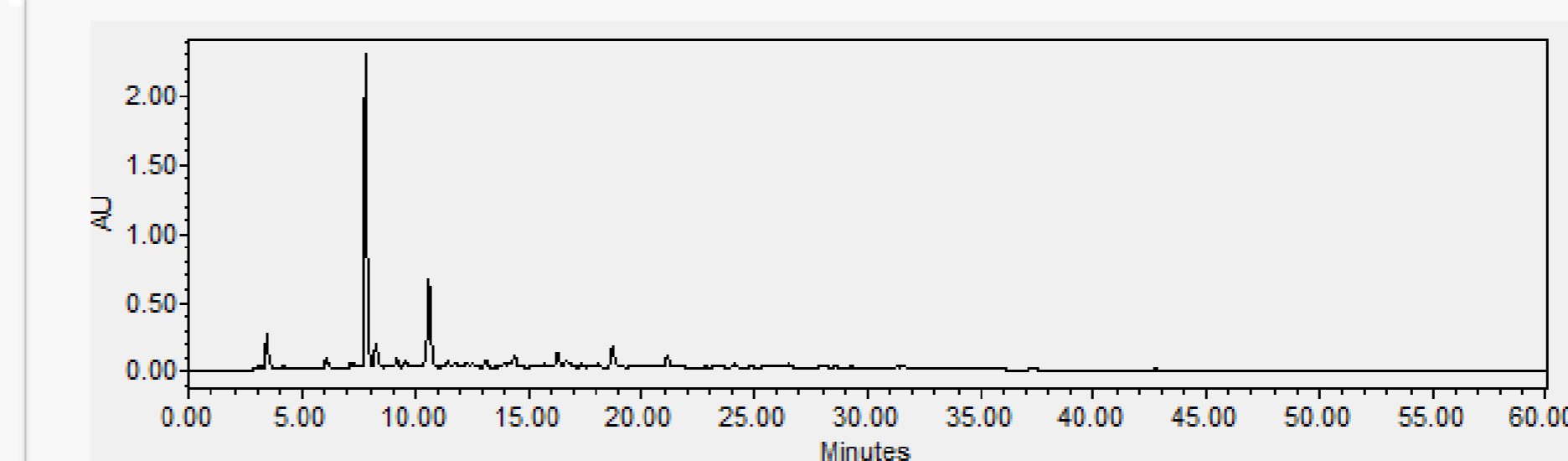


Figure 5. High performance liquid chromatography (HPLC) results: example of chromatogram and graphs with quantity extracted for each compound (different letters means statistical differences)

TLC showed the presence of the phenolic compounds tyrosol and hydroxytyrosol in all analyzed samples, but catechol is present only after hydroalcoholic extraction (samples 1, 2, 3, 4), this compound being absent in all aqueous extractions (samples 5, 6, 7, 8, 9). The values of RF were significantly different between standards, but they were equal between samples of the same standard, in all standards.

HPLC showed the presence of various phenolics: hydroxytyrosol, tyrosol, vanillin, caffeic acid and verbascoside. Most compounds were present in different concentrations, but the extraction techniques used revealed no difference in the content among different compounds. Vanillin was detected only in methodologies 1, 2, 4, 7 and 8. The maximum concentration found was near 0.03 mg/g olive oil pomace using methodology 2. The main difference among methodologies was the phenolic compounds concentrations of the extracts, e.g. the methodology 1 with 110.9 mg of extract had the same content in phenolics than methodology 9 with 404.7 mg of extract, for about the same mass of olive oil pomace.

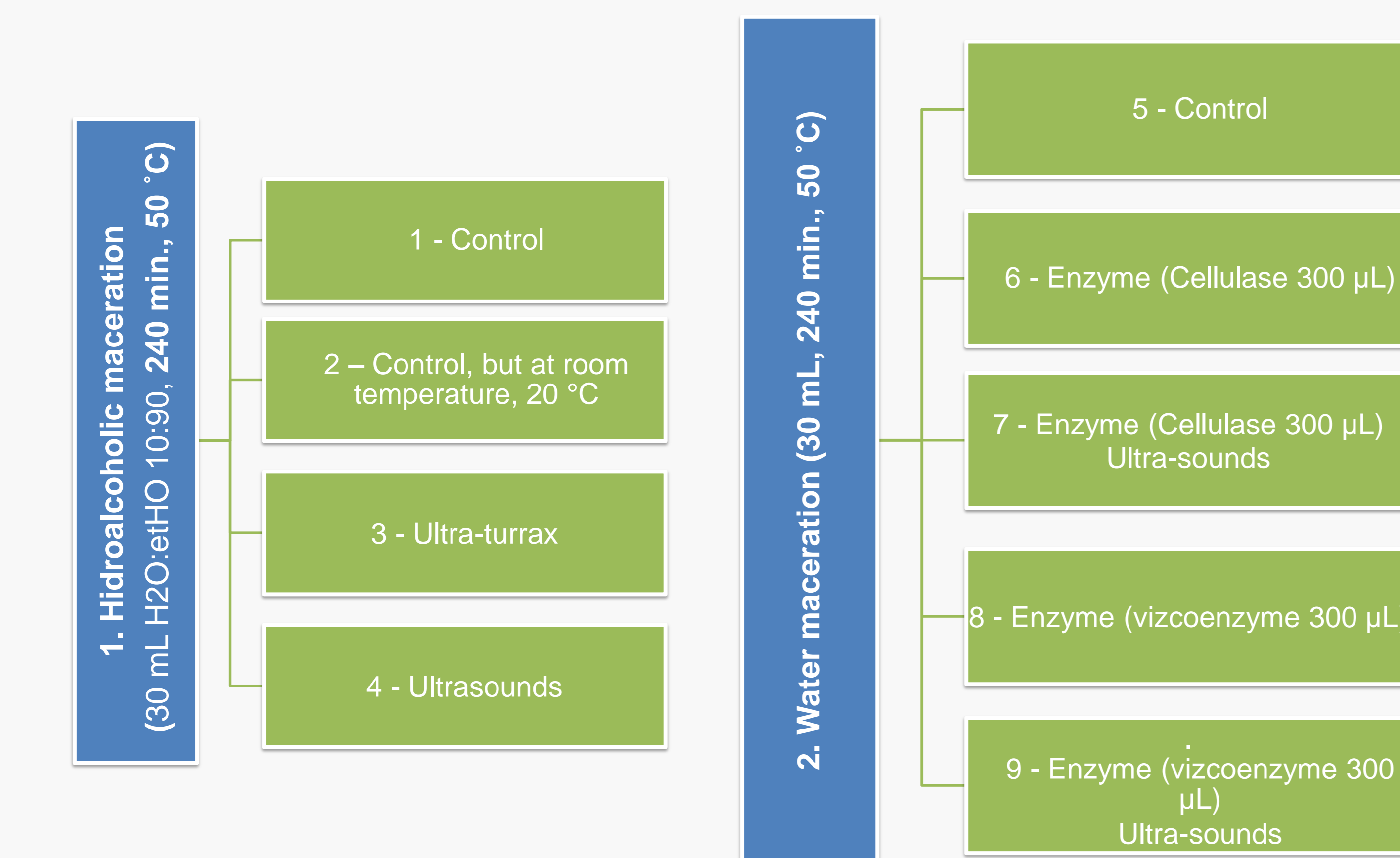


Figure 2. Description of the methodologies of extraction used to obtain polyphenols from olive oil pomace.

Conclusions

Different methodologies to extract phenolic compounds were tested. The extraction with enzymes led to a higher mass of extract. The TLC proved that the extracts contained hydroxytyrosol, tyrosol and catechol, which is agreement with the results of the HPLC. The analysis by HPLC proved the presence of hydroxytyrosol, tyrosol, vanillin, caffeic acid and verbascoside in different concentrations in the extracts, the most relevant being hydroxytyrosol and tyrosol. Hydroalcoholic extracts presented higher phenolic compounds concentrations in olive oil pomace than other aqueous extracts.

Bibliography

- H. Azaizeh, H.N. Abu Tayeh, Y. Gerchman, Valorisation of olive oil industry solid waste and production of ethanol and high value-added biomolecules. In: Bivalourisation of Wastes to Renewable Chemicals and Biofuels (edited by N.K. Rathinam & R. Sani), pp. 27–40, 2020.
- D. Klisovic, A. Novoselic, A.R. Jambrak, K. B. Bubola, The utilization solutions of olive mill by-products in the terms of sustainable olive oil production: a review. *Int. J. Food Sci. Technol.* 56 (2021) 4851-4860.
- P. Xie, L. Huang, C. Zhang, Y. Deng, X. Wang, J. Cheng, Enhanced extraction of hydroxytyrosol, maslinic acid and oleonic acid from olive pomace: Process parameters, kinetics and thermodynamics, and greenness assessment. *Food Chem.* 276 (2019) 662-674.
- G.A. Macedo, A.L. Santana, L.A. Crawford., S.C. Wang, F.F.G. Dias, J.M.L.N. Moura bell. Integrate microwave and enzyme assisted extraction of phenolic compounds from Olive pomace. *LWT – Food Science and Technology.* 138 (2021) 110621.

Acknowledgements

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through Project UID/Multi/50016/2020. The first author thanks FCT for the PhD research grant with reference UI/BD/152825/2022. All authors thank company “Casa Alta - Sociedade Transformadora de Bagaços” for the supply of the olive oil pomace.