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Polymeric biophenols in olive mill wastewaters

Cardoso S M[1,2], Falcão S I[2], Peres A M[2,3], Pereira O R[1,4], Domingues M R M[5]

[1]CERNAS - Escola Superior Agrária, Instituto Politécnico de Coimbra, Bencanta, 3040-316 Coimbra, Portugal;
[2]CIMO, [3]LSRE - Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, 5301-855 Bragança, Portugal;
[4]Escola Superior de Saúde, Instituto Politécnico de Bragança, Av. D. Afonso V, 5300-121 Bragança, Portugal;
[5]Centro de Espectrometria de Massa, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

Introduction

Olive mill wastewater (OMW), the effluents generated in the olive oil extraction industry operating in three-phases mode, are phytotoxic mainly due to its high phenolic content [1]. On the other hand, attending to the potential health-benefits of some of their phenolic compounds, OMW are now regarded as a potent source of biophenols for food and pharmaceutical industries. An important portion of the OMW biophenols include the secoiridoids found in olive pulp and their derivatives formed along the olive oil extraction process [2]. Still, due to the complex composition of OMW, many phenolic compounds remain unknown. Their structural identification can encourage the search of new bioactive compounds in OMW and contribute to further valorize this waste.

Methods

Samples: Two olive oil mill wastewaters were collected in continuous three-phases olive oil factories at the north of Portugal, namely Mirandela and Amarante.

Extraction and purification of phenolic compounds: The phenolic compounds were extracted from the OWM by an adaptation to the method described by a previous work [3]. The freeze-dried OMW (2 g) were defatted with 20 mL of *n*-hexane, for 3 times, and the residue was extracted with 20 mL of methanol at pH 2, for 5 times during 20 min each. The resulting residue was extracted with acetone/water (6:4 v/v) and the extracted solutions were filtered, combined, concentrated, frozen at –20ºC and freeze-dried, to give the aqueous acetone extract.

Mass spectrometry analysis: The samples were directly injected into the ESI source by means of a syringe pump, at a flow rate of 8 μL min^{-1}. Studies were performed in the negative mode using a Linear Ion trap LXQ (ThermoFinnigan, San Jose, CA, USA). Typical ESI conditions were the same as previously described [4].

RESULTS

New major polymeric compounds were detected as $[M-H]^-$ ions at *m/z* 909, 1071, 1457, 1075 and 1613

According to their ESI- MSn analysis, these new compounds could be distinguished as:

I- derivatives of a ligstroside glucoside isomer

II- oleuropein oligomeric compounds.

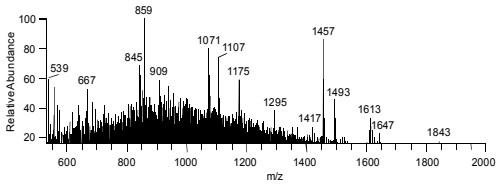
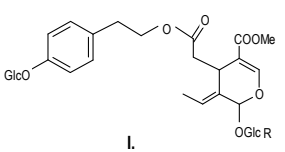


Fig. 1- Representative ESI-MS spectrum of the purified acetone extract extract obtained from olive mill wastewaters



I.

R= elenoic acid MW = **910 Da**
R= oleoside 11-methyl ester MW = **1072 Da**
R= di-oleoside 11-methyl ester MW = **1458 Da**

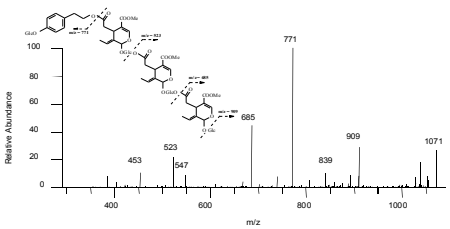
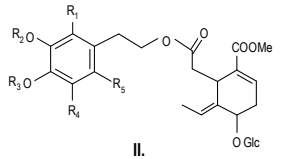


Fig. 2- ESI-MS^2 spectrum of the $[M-H]^-$ ion at *m/z* 1071. The tentative structure of the compound and the interpretation of relevant product ions are enclosed



II.

R_1, R_2, R_3, R_4 or R_5= one oleuropein residue MW = **1076 Da**
R_1, R_2, R_3, R_4 or R_5= two oleuropein residues MW = **1614 Da**

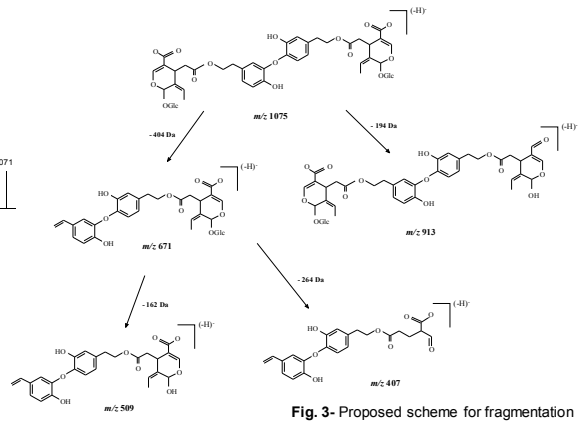


Fig. 3- Proposed scheme for fragmentation of the $[M-H]^-$ ion at *m/z* 1075

CONCLUSIONS

The present study is a valuable contribution for understanding the complex phenolic composition of OMW , as several new compounds were detected:

→ Elenoic acid derivative of a ligstroside isomer glucoside (compound of MW 910 Da).

→ Mono- and di-oleoside 11-methyl ester derivatives of a ligstroside isomer glucoside (compounds of MW 1072 and 1458 Da, respectively) .

→ Oleuropein dimer and trimer (compounds of MW 1076 and 1614 Da, respectively) .

Attending that the scavenging ability of a polyphenolic compound is increased by its degree of polymerization [5], bioactivities related to that capacity are expected at least for some of these compounds.

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