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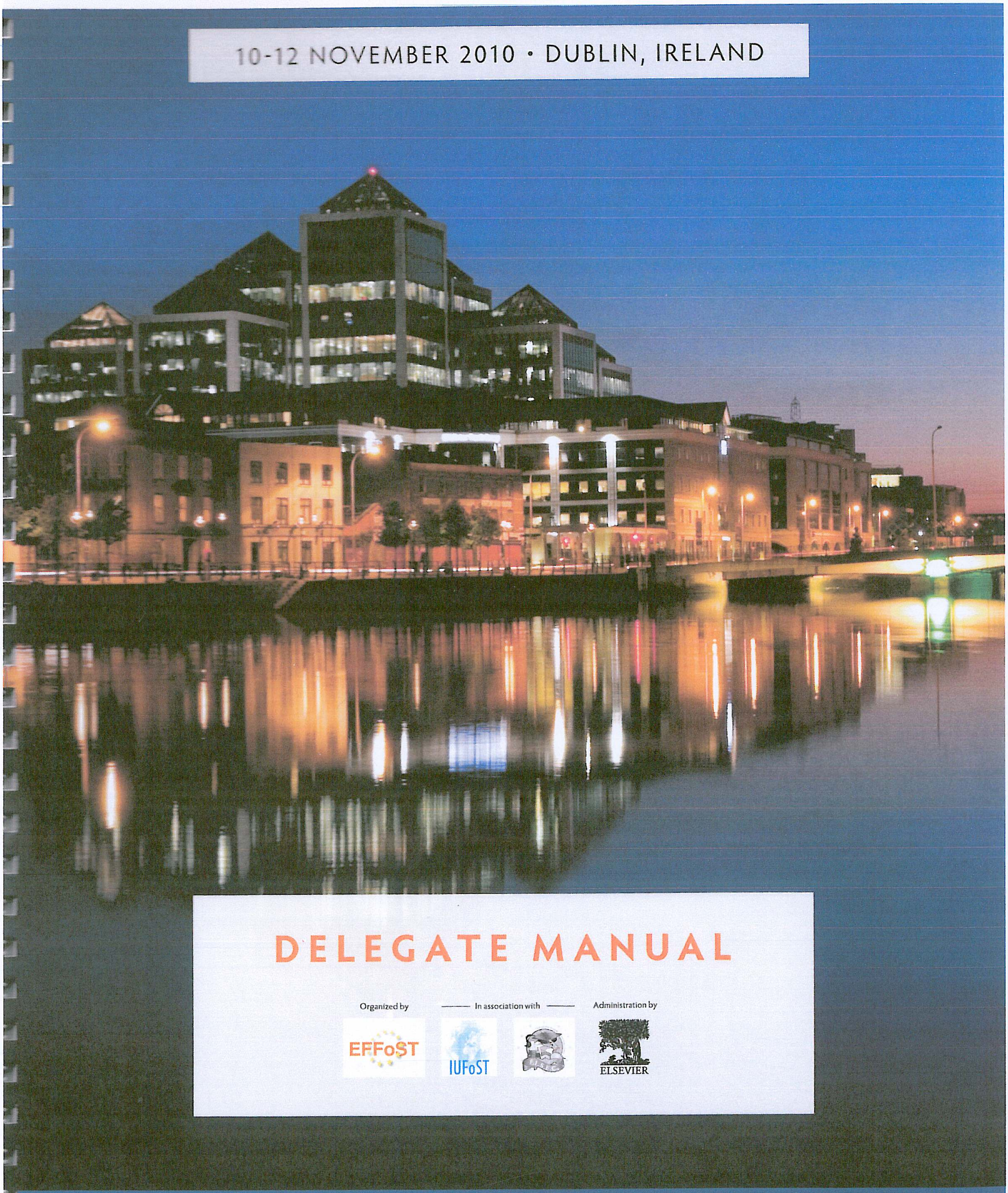

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New biophenols in olive mill wastewaters

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Introduction

Olive mill wastewaters (OMW) are a potential source of biophenols with a wide range of biological activities.

This effluent produced in the olive oil industry is currently exploited for industrial extraction of hydroxytyrosol [1]. Still, the phenolic composition of OMW is extremely complex, and many compounds are yet unidentified.

In this context, the identification of unknown phenolic compounds can encourage the search of new bioactive compounds in OMW and contribute to further valorize this waste.

In this work, ESI-MS was used to analyse purified methanolic extracts from two Portuguese OMW samples and tandem mass spectrometry (MS/MS) was used to elucidate the structure of phenolic compounds.

Methods

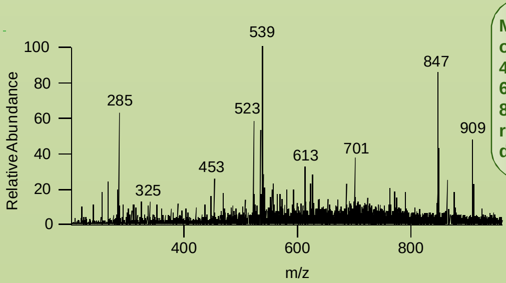
Samples: Two olive oil mill wastewaters were collected in continuous three-phases olive oil factories at the north of Portugal, namely Mirandela and Amarante.

Extraction and purification of phenolic compounds: The freeze-dried OMW (2 g) were defatted 3 times with 20 mL of *n*-hexane, and the residue was extracted 5 times with 20 mL of methanol at pH 2, for 20 min each. The extracted solutions were filtered and freeze-dried, to give the methanolic extract. This extract was purified by solid-phase chromatography on Sep Pack C18, according to the methodology described by Cardoso et al. [2].

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 [3].

RESULTS

I. ESI-MS analysis of the purified methanolic extract



Major $[M-H]^-$ ions were observed at *m/z* 285, 447, 453, 523, 535, 539, 557, 613, 627, 685, 701, 775, 789, 847, 863 and 909, although their relative intensity could differ for the two samples.

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

II. New oleuropein Derivatives

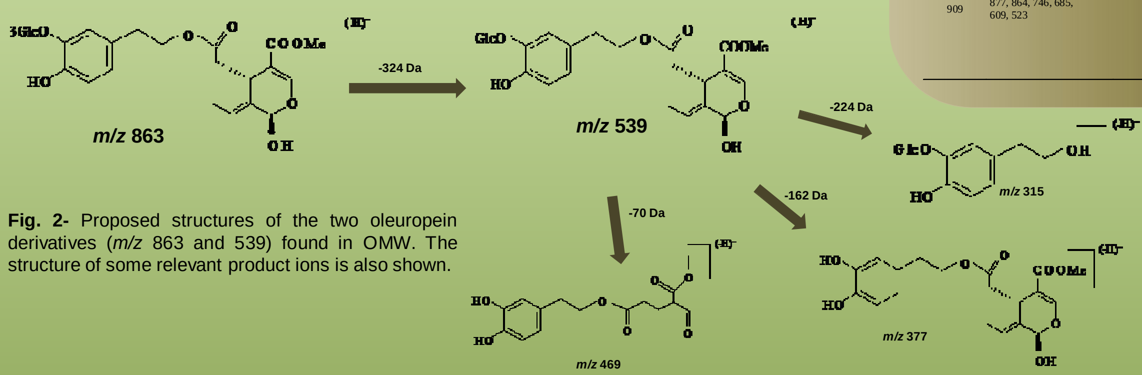
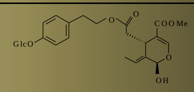
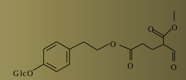
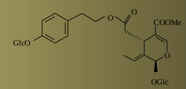
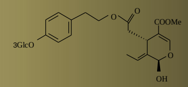
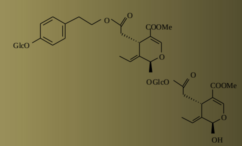


Fig. 2- Proposed structures of the two oleuropein derivatives (*m/z* 863 and 539) found in OMW. The structure of some relevant product ions is also shown.

III. New ligstroside derivatives

Table 1 – Resume of ligstroside derivatives found in OMW.

$[M-H]^-$	Main Fragments ESI⁻MS[a]	Tentative structure
523	453, 421, 361, 299	
453	421, 299, 291	
685	667, 653, 641, 523, 453, 361	
847	815, 777, 745, 685, 665, 623	
909	877, 864, 746, 685, 609, 523	

CONCLUSIONS

-The following compounds were detected for the first time in OMW

- Oleuropein/ligstroside isomers (ions at *m/z* 539 and 523, respectively)
- Diglucoside derivative of the oleuropein/listroside isomer (ions at *m/z* 863 / 847)
- Degradative compound of the ligstroside isomer (ion at m/z 453)
- Monoglucoside derivative of ligstroside isomer (ions at *m/z* 685) and its elenoic acid derivative (ion at *m/z* 909)

References:

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