CHARACTERIZATION OF POLYPHENOLIC COMPOSITION OF EXTRACTS FORM WINERY WASTES BY HPLC-UV-MS/MS

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INTRODUCTION

Polyphenols display a wide range of positive health effects, thus contributing to the prevention or treatment of some diseases due to their great antioxidant, anti-inflammatory, antimicrobial and antineoplastic properties. The use of polyphenols is not limited to pharmaceutical purposes, but they are also applied to cosmetics, nutraceuticals, etc.

Currently, there is a growing interest in the recovery of polyphenols from agrifood wastes. In particular, winery wastes are especially rich in phenolic compounds. Recovery consists of several stages, including extraction and purification, but also chemical characterization.

This work aims at characterizing the polyphenols recovered from wastes generated during the winemaking processes using chromatographic techniques.

MATERIALS AND METHODS

SAMPLES

Samples are aqueous extracts, obtained from wine lees, and submitted to ultrafiltration or reversed osmosis purification processes.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Chromatograph	Agilent Series 1200 HPLC equipped with diode array detector
Column	Kinetex C18 (100 mm x 4.6 mm, 2.6 µm)
Mobile phase	Solvent A: 0.1% formic acid in water; Solvent B: acetonitrile
Elution program	Time (min), % B: (0, 3%), (10, 15%); (20, 45%); (22, 90%); (24, 90%); (24.2, 3%); (30, 3%).
Flow rate	0.7 mL min^{-1}
Injection volume	5 µL
UV detection	250, 280, 325 and 370 nm

MASS SPECTROMETRY

Mass spectrometer	4000 Qtrap (AB Sciex)	LTQ Orbitrap Velos (Thermo Fisher Scientific)
Resolution	Low	60,000 FWHM (at m/z 200)
Mode	Enhance MS (m/z 100-650)	Full scan (m/z 100-1500) Data-dependent scan (MS/MS)
Polarity	negative	
Source voltage	-2500V	
Source temperature	700 ºC	350 ºC
Capillary temperature	---	320 ºC
Declustering potential	-80 V	---
S-Lens RF voltage	---	50 V
Gas (N_2)	20 a.u. (CUR) 50 a.u. (GS1) 50 a.u. (GS2)	60 a.u. (HESI-II sheath) 0 a.u. (ion-sweep) 10 a.u. (auxiliary)

RESULTS AND DISCUSSION

The HPLC-UV chromatogram (Figure 1) indicates that wine lees extracts are quite complex. This work focuses on the identification of the most remarkable phenolic compounds.

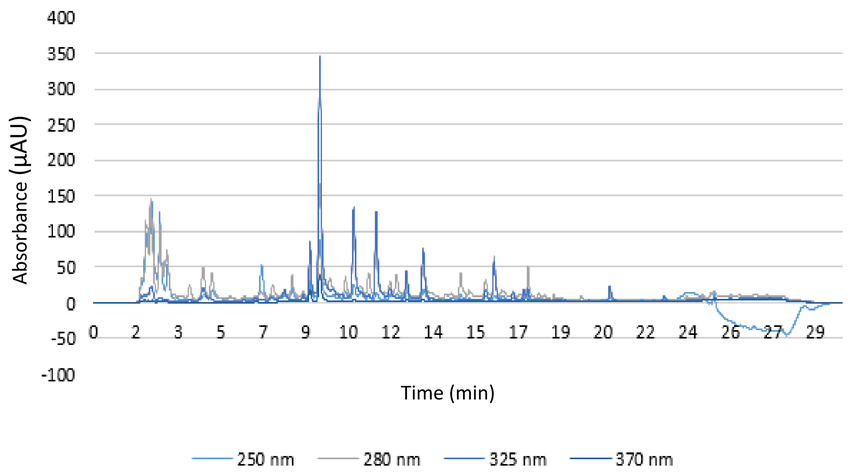

Figure 1. HPLC-UV chromatograms of an aqueous lees extract.

COMPOUND IDENTIFICATION USING MASS SPECTROMETRY

Both LRMS and HRMS were applied to confirm the identity of phenolic compounds in wine lees extracts. Different strategies and acquisition modes were used.

HPLC-LRMS

Full scan mode: The MS spectrum of the suspect peak was compared with that of the standard when available (Figure 2).

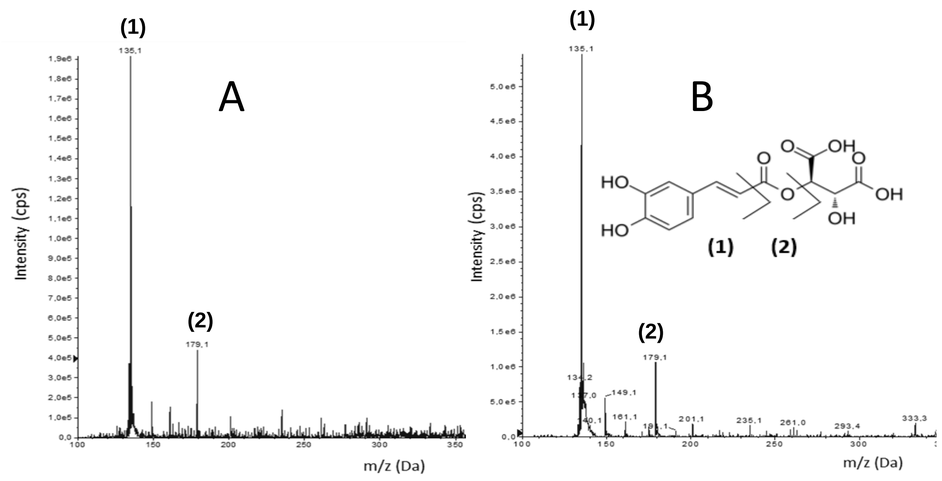

Figure 2. Mass spectrum of the chromatographic peak at 12.3 min: (A) caftaric acid standard solution, (B) wine lees extract.

HPLC-HRMS

Data-dependent acquisition mode *(Full scan+MS/MS)*:

a) Standard available at the lab: the MS spectrum of the peak of the extract was compared to that of the standard.
b) Standard not available at the lab: HRMS data bases were used.
i)The chromatogram corresponding to m/z of $[M-H]^-$ was extracted. If a peak was detected, the exact mass was compared with the actual mass of the compound.
ii)The MS/MS spectrum of the peak of the extract was compared with the spectrum of the standard reported in the data base.

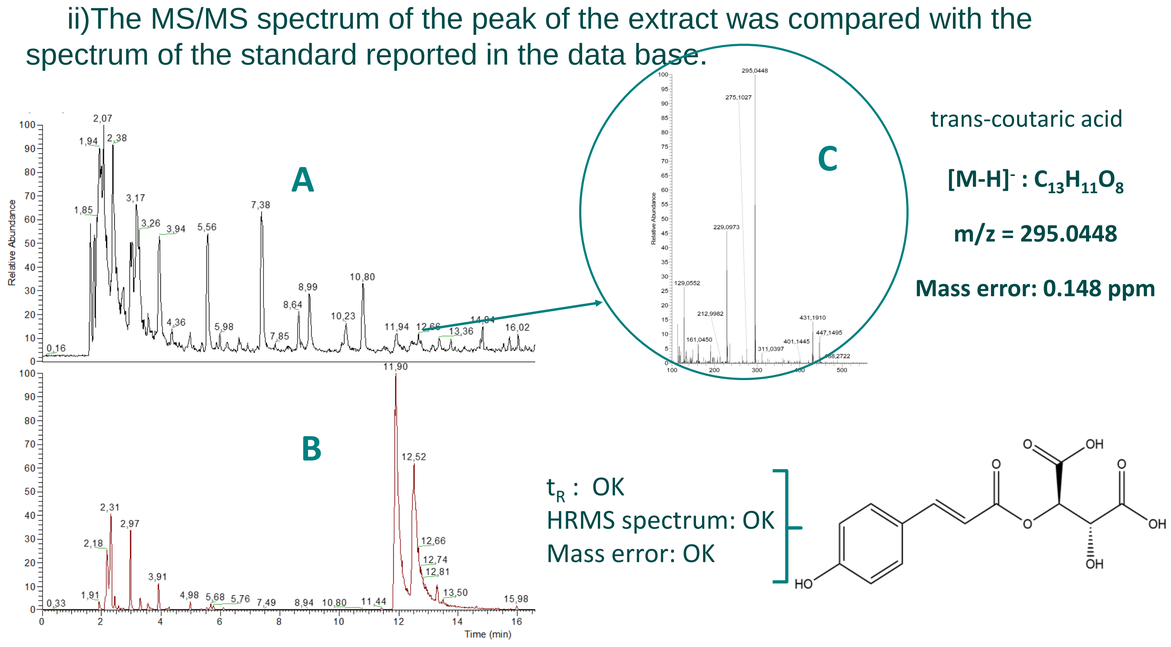

Figure 3. Total ion chromatogram (A), extracted chromatogram (m/z 295-296) (B), and the corresponding mass spectrum of the chromatographic peak at 12.5 min (C) of a lees extract.

POLYPHENOL CONTENT IN LEES EXTRACTS

Multiple reaction monitoring (MRM) mode was used for confirmation and quantification of compounds, previously identified by HRMS, using the corresponding standards

Table 1. Polyphenols identified in lees extract and concentration levels.

Compound	t_R (min)	Concentration (mg L^{-1})
(1) Gallic acid	6.41	13.3
(2) 3,4-hydroxybenzoic acid	10.05	0.5
(3) Caftaric acid	12.32	21.1
(4) Chlorogenic Acid	14.05	5
(5) Catechin	14.11	11.1
(6) cis-coutaric acid	14.17	
(7) 2,5-Dihydroxybenzoic acid	14.33	2.8
(8) trans-coutaric acid	14.92	16.3
(9) Caffeic acid	15.47	20.2
(10) Syringic acid	15.52	0.08
(11) Epicatechin	15.8	11. 7
(12) Ethyl gallate	17.27	10.8
(13) Rutin	17.55	0.02
(14) p-coumaric acid	17.81	2.86
(15) Ferulic acid	18.34	0.5
(16) Astilbin	18.44	1
(17) Resveratrol	19.21	0.51

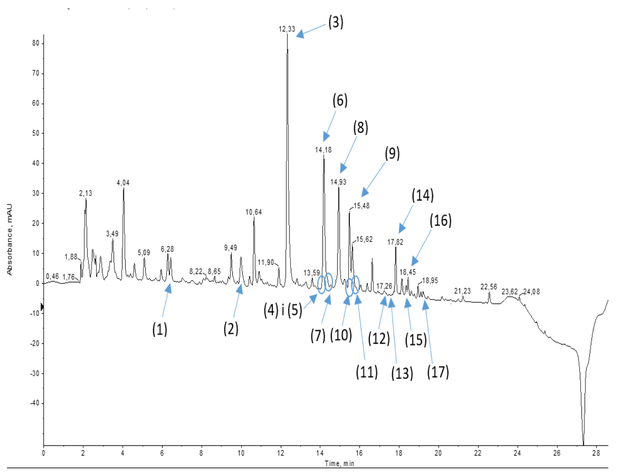

Figure 4. Chromatogram of a lees extract. *See Table 1 for peaks assignation.*

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