ASSESSMENT OF THE POLYPHENOLIC COMPOSITION OF ORANGE WASTES FROM AGRI-FOOD INDUSTRIES BY HPLC-UV-MS/MS

Àlex Cáceres[a], Mercè Granados[a], Javier Saurina[a,b], Sònia Sentellas[a,c]

a Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, E08028 Barcelona, Spain.
b Research Institute in Food Nutrition and Food Safety, University of Barcelona, Av. Prat de la Riba 171, Edifici Recerca (Gaudí), E08921 Santa Coloma de Gramenet, Spain.
c Serra Húnter Lecturer, Generalitat de Catalunya, Spain.

INTRODUCTION

The industrial production of orange juices generates large amounts of wastes highly rich in polyphenolic compounds with great antioxidant properties. Hence, in the framework of circular economy, orange residues result in an excellent source of valuable by-products that can be recovered and purified to be further used in pharmaceuticals, nutraceuticals and functional foods. In this context it is crucial to identify the most remarkable phenolic species they contain.

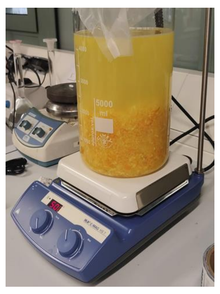
In this work, orange wastes consisting of solid mixtures of skin and pulp residues were treated with water under mechanical shaking to recover the polyphenolic components. The resulting extracts were centrifuged and filtered and were analyzed by high performance liquid chromatography (HPLC) using UV and mass spectrometry (MS) detection.

MATERIALS AND METHODS

EXTRACTION

5 g of orange solid waste (skin and pulp) was extracted with 50 mL of water at 70 ºC for 15 min using mechanical shaking.

The extracts were centrifuged at 1800 g for 15 min, and filtered trough a cellulose membrane (0.22 µm).



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Chromatograph	Agilent Series 1200 HPLC with diode series 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%), (15, 30%); (20, 65%); (22, 90%); (24.5, 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 (@ 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 the complexity of the orange extracts. Although a complete characterization was beyond the scope, it was intended to identify the most remarkable phenolic compounds.

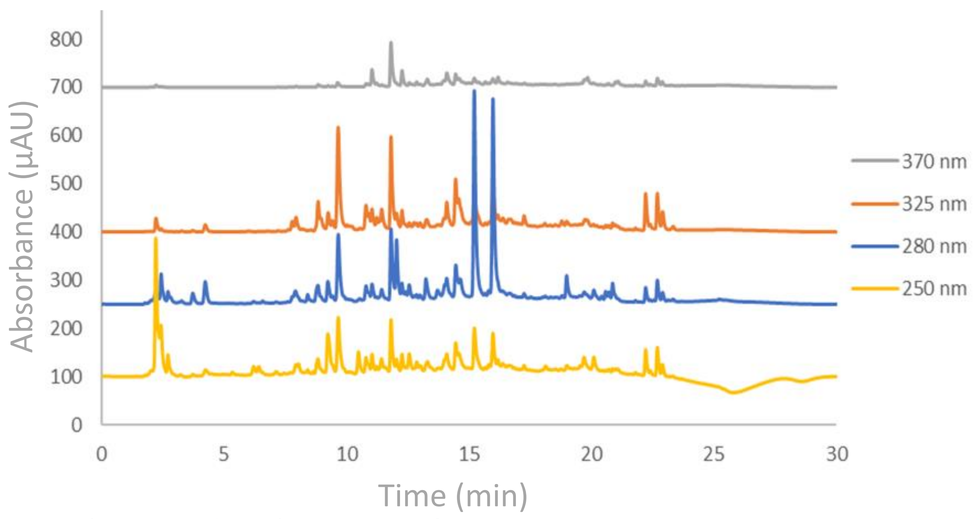


Figure 1. HPLC-UV chromatograms of an aqueous orange waste extract

COMPOUND IDENTIFICATION USING MASS SPECTROMETRY

Both LRMS and HRMS were applied to confirm the identity of phenolic compounds in orange 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).

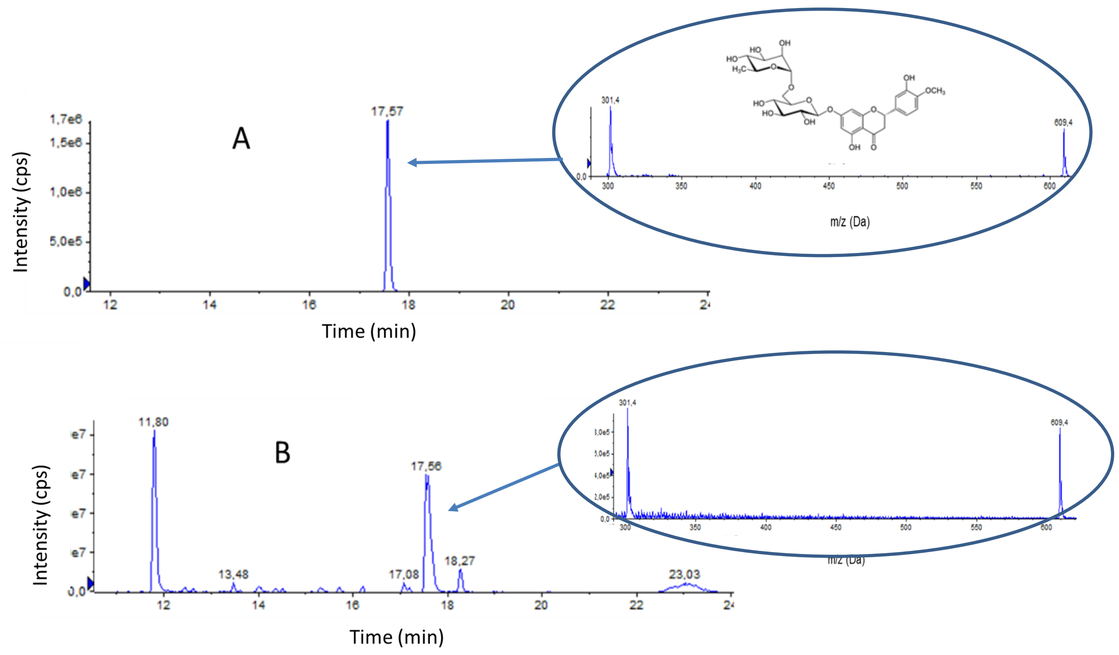


Figure 2. Extracted chromatograms (m/z 609-610) of hesperidin standard solution (A) and orange extract (B), and the corresponding mass spectra of the chromatographic peaks at 17.6 min.

HPLC-HRMS

Data-dependent acquisition mode *(Full scan+MS/MS)*:
MS/MS conditions: CE: 35V; minimum m/z: 50; required MS intensity: $1.0 \cdot 10^6$

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 databases were used.
i)The chromatogram corresponding to m/z of $[M-H]^-$ is extracted. If a peak is detected, exact mass was compared with the actual mass of the compound
ii)The MS/MS spectrum of the peak of the extract is compared with the spectrum of the standard reported in the data base (Figure 3).

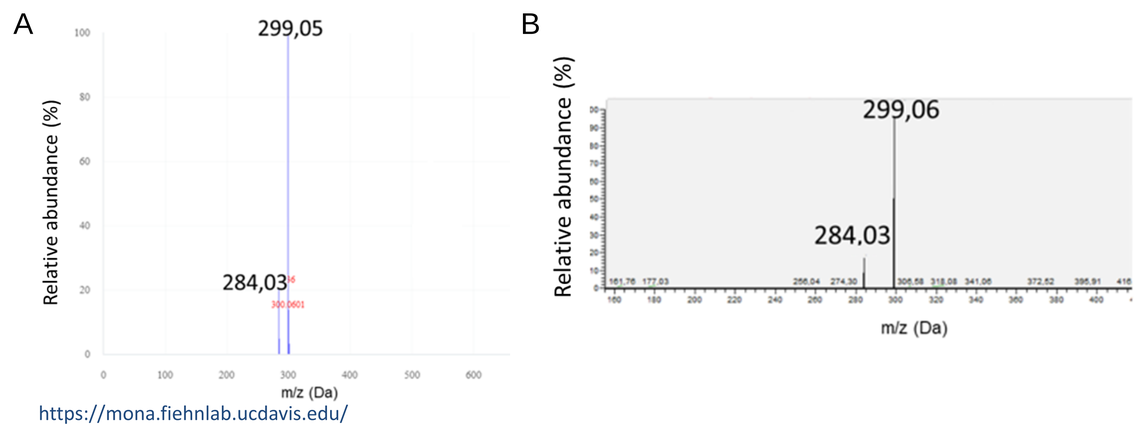


Figure 3. (A): MS/MS spectrum of diosmin by HPLC-ESI-TOF (MoNA database); (b) MS/MS spectrum of extract peak by HPLC-HRMS.

POLYPHENOL CONTENT IN ORANGE WASTE

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 the orange waste extract and concentration levels.

Compound	Concentration (mg L^{-1})
Astilbin	0.024
p-coumaric acid	0.028
Rutin	0.10
Ferulic acid	0.17
Diosmin	0.35
Caffeic acid	0.75
Hesperetin	3.2
Naringenin	14
Hesperidin	34

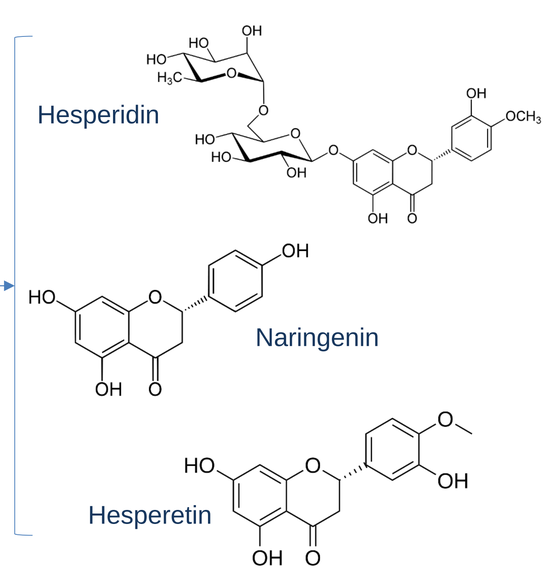


Figure 4. Structure of the most abundant polyphenols

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