agronomy

MDPI

Supplementary Material

Flavonoids and limonoids profiles variation in leaves from mandarin cultivars and its relationship with alternate bearing

Cecilia Rodríguez Ceraolo [1], Valeria Vázquez [1], Ignacio Migues [1,2], María Verónica Cesio [1], Fernando Rivas [3] and Horacio Heinzen [1,*]

[1] Grupo de Análisis Metabolómicos (GAMe), Laboratorio de Farmacognosia y Productos Naturales, Departamento de Química Orgánica, Facultad de Química, Universidad de la República, Montevideo, Uruguay.
[2] Graduate Program in Chemistry, Facultad de Química, Universidad de la República, Montevideo, Uruguay.
[3] Programa Nacional de Investigación en Produccción Citrícola, Instituto Nacional de Investigación Agropecuaria (INIA), Salto, Uruguay.
* Correspondence: heinzen@fq.edu.uy

Abstract: Alternate bearing in citrus trees has been extensively studied as a key feature for citrus growers. Although the genetic and the biochemical process occurring during alternate bearing has been studied extensively, there is a lack of information pointing out the presence of metabolic indicators during "on" and "off" years. In citrus, leaves play a central role in the metabolic pathway triggering the flowering induction process. To investigate the changes during this transition, a liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis of the leaf profiles of 20 compounds (17 polyphenols, two limonoids, and one furanocoumarin), in bearing and non-bearing branches arising from four different mandarin genotypes, was performed. The same metabolites were found in all the genotypes at both stages: both limonoids and 11 polyphenols. Using these compounds, the chemotaxonomic differentiation between cultivars was assessed. The levels of flavanones and limonoids showed differences in both bearing stages and the transition from vegetative to flowering could be shown by the activation of the polyphenol biosynthetic pathway, from precursors like naringenin to metabolic end-points such as narirutin and polymethoxyflavones. Narirutin levels showed significant differences between both stages, hinting it as a possible marker of the physiological status of the branch.

Keywords: Polyphenols; limonoids; secondary metabolism; bearing branches; non-bearing branches

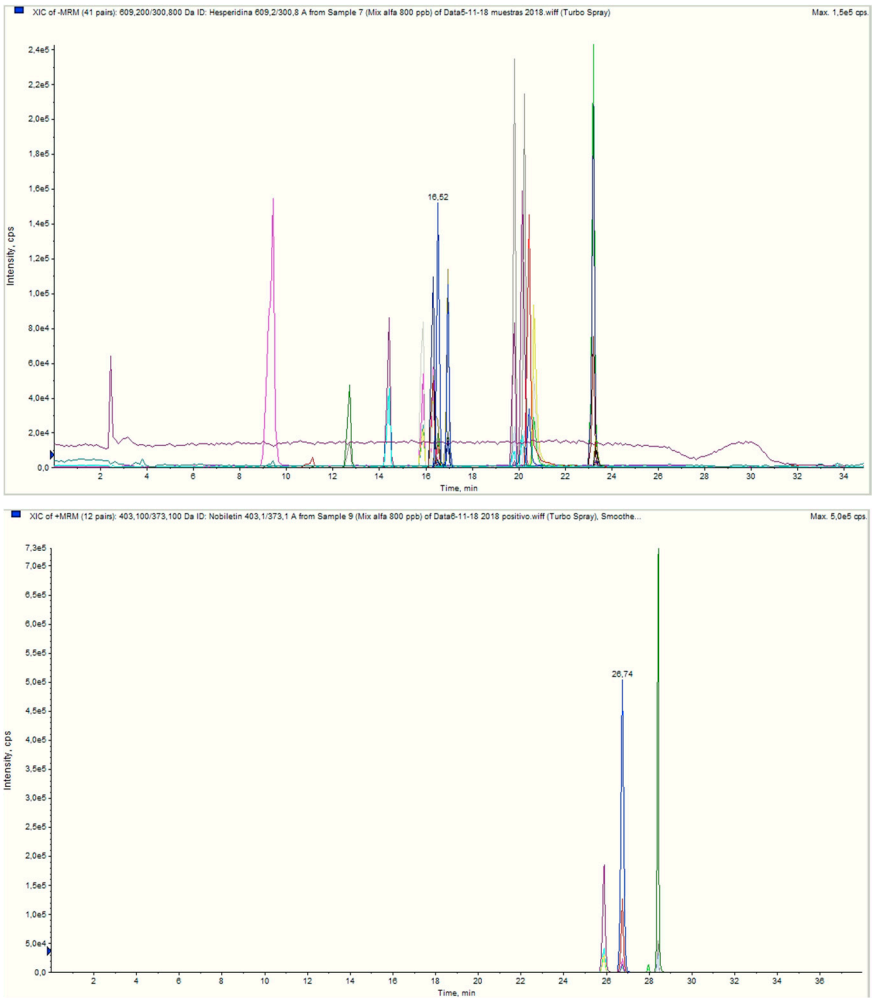
Supplementary material

All standards were selected based on previous studies of flavonoid content in mandarins.
Individual standard solutions of the target compounds were prepared and stored at -20 ° C. Each standard was weighed to approximately 20 mg and dissolved in 10.00 mL of HPLC grade methanol, to obtain a stock solution of 2000 mg/L . Subsequently, a mix (20 mg/L) was prepared by means of the adequate dilution of the stock solutions in acetonitrile, and it was used for the construction of the calibration curve. 10, 50, 100, 200, 400, 600, 800, 1000, 1500 μg/L were the concentrations selected for the curve. In Supplementary Table S1

the evaluated standards are displayed and in Supplementary Figure S1 shows the chromatograms of the mix of standards in negative mode (top) and positive mode (bottom).

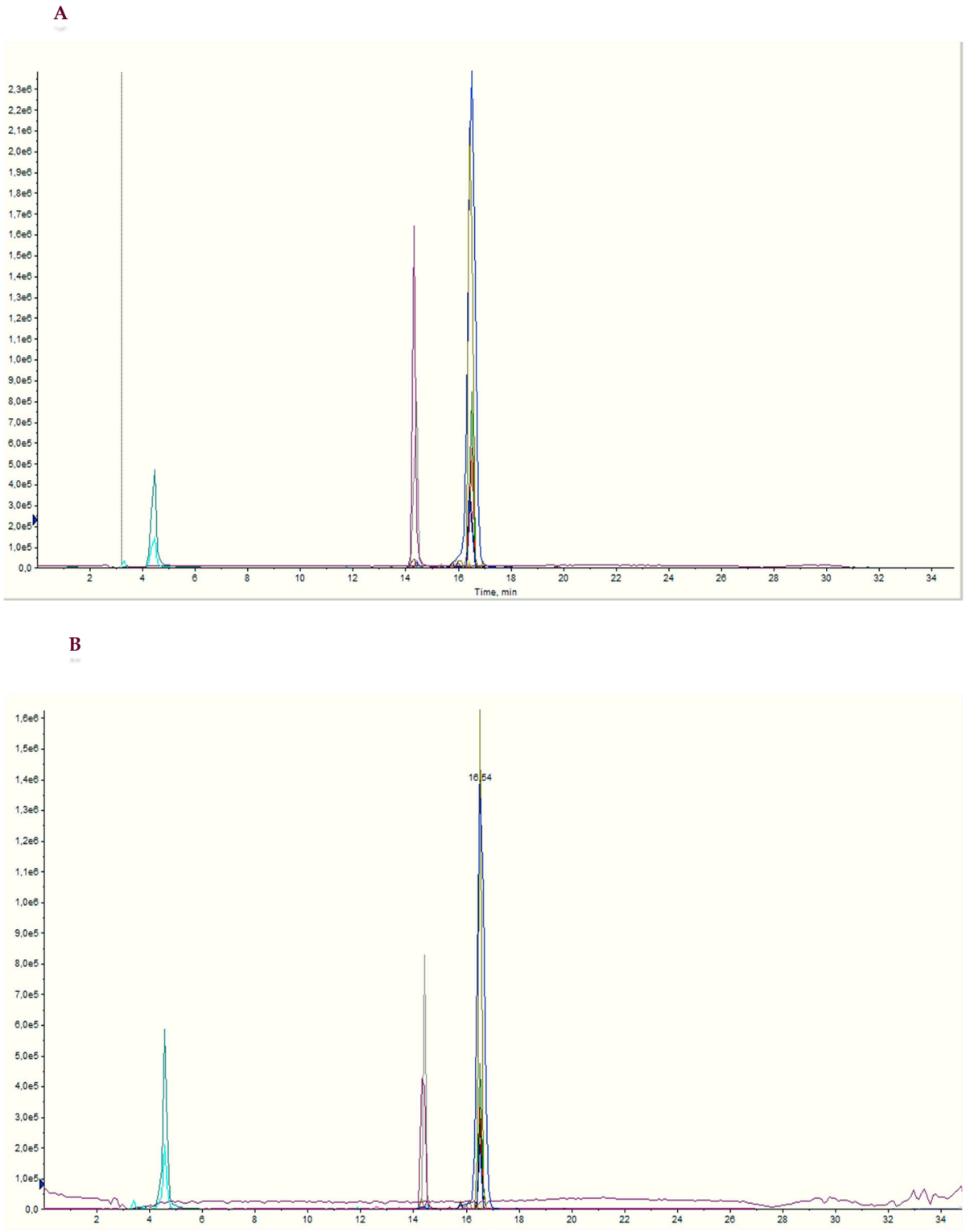
Supplementary Table S1. Target compounds selected for the analysis.

Analytes			
Protocatechuic acid	Naringin	Poncirin	Bergapten
Chlorogenic acid	Quercitrin	Luteolin	Limonin
Caffeic acid	Hesperidin	Quercetin	Nobiletin
Eriocitrin	Neohesperidin	Kaempferol	Nomilin
Narirutin	Didymin	Naringenin	Tangeretin



Supplementary Figure S1. Chromatogram of the mix of standards at 800 g/L injected into the LC-MS/MS in negative (Top) and positive (Bottom) mode.

As stated in the manuscript, the compounds found in "on"and "off" bearing stages was the same. Supplementary figure S2 shows the differences in the chromatograms between "on"and "off" bearing stages in 'Willowleaf' genotype, which was one of the genotypes that presented more differences in the levels of the identified compounds.



Supplementary Figure S2. Chromatograms of 'Willowleaf' genotype a non-bearing ("off") stage (A) and at a bearing ("on") stage (B) in negative acquisition mode.