DEVELOPMENT OF A COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY METHOD FOR THE CHARACTERIZATION OF GRAPE SEED PROCYANIDINS BASED ON THE COUPLING OF HYDROPHILIC INTERACTION AND REVERSED PHASE SEPARATIONS (HILICxRP).

L. Montero, E. Ibáñez, M. Prodanov, A. Cifuentes, M. Herrero
Departament of Bioactivity and Food Analysis, Institute of Food Science Research, CIAL (CSIC-UAM), Nicolás Cabrera 9, Campus Cantoblanco, 28049 Madrid, Spain

Procyanidins are a group of proantocyanidins, also called condensed tannins, widely distributed in the vegetable kingdom. These compounds consist of polymers of flavan-3-ols, in particular, catechin, its isomer epicatechin and epicatechin-3-O-gallate linked by C4-C8 or C4-C6 bonds (B type procyanidins) or by two inter-flavonoids bonds, a C-C bond and an ether C4-O-C7” (A type procyanidins). Grapes (Vitis vinifera) are one of the richest sources of procyanidins, which are mainly found in the seeds.

These compounds have shown some interesting functional and bioactive properties such as antioxidant, antibacterial, antiinflammatory or anticancer activities. For this reason, there is a growing interest on the extraction and characterization of procyanidins. Nevertheless, these compounds constitute a rather complex and huge group of natural compounds, formed by different isomers with diverse degree of polymerization (DP, up to 37 units) and degree of galolilation. Consequently, conventional analytical techniques are not capable to provide the needed separation and identification power. In this regard, multidimensional chromatography offers enhanced separation abilities thanks to the coupling of different independent separations. Comprehensive two-dimensional liquid chromatography (LCxLC) provides much greater resolving power compared to mono-dimensional LC. In a comprehensive LCxLC system all fractions from the first column are continuously sampled and transferred by means of a switching valve to the second dimension column for further separation.

In this work, a novel LCxLC method has been developed for the characterization of grape seeds procyanidins. This new method was based on the challenging coupling between a first dimension separation based on hydrophilic interaction chromatography (HILIC) and a second dimension separation performed under reversed phase conditions (RPLC). A diol stationary phase microbore column was used in the first dimension whereas two different options (C18 solid core and C18 monolithic columns) were studied in the second dimension. The system was coupled to a diode array detector and to a mass spectrometer in series. The use of both detectors under optimum conditions allowed the separation and characterization of more than 50 different procyanidins (up to octamers) on grape seeds without the need of performing any sample pretreatment.

Consequently, this work contributes not only to the development of a new analytical procedure to analyze procyanidins, but also to increase the knowledge on the chemical composition of this interesting complex sample.