# Mass spectrometry-based metabolomics in grapevine and wine fingerprinting

***By Marta Sousa Silva and Carlos Cordeiro***

In the omics universe, the metabolome are the biochemical products that arise from cellular processes and is the final response of a biological system to environmental or genetic changes. Being highly dynamic and time-dependent, metabolomes are exquisitely complex (Aretz and Meierhofer, 2016). In plants, it is predicted that more than 200,000 metabolites are involved in physiological processes, in controlling plant growth and development and plant responses to environmental changes or biotic stresses (Fiehn, 2002). Besides their importance for plant physiology, there is a wide range of compounds with high nutritional value, organoleptic properties and potential human health benefits, which is particularly relevant in crop plants. Due to its economic importance in the wine industry, grapevine (*Vitis vinifera* L.) is one of the most important fruit plants cultivated worldwide. The characterization of the grapevine's metabolome is essential to understand its physiology, its response to stress conditions, improve resistance to pathogens, increase productivity, quality and assess food safety. Concerning wine, one of the most widely appreciated drinks worldwide, its metabolome results from a complex process, involving grapes, yeast, bacteria, terroir effects and its ageing in wooden barrels. Wine metabolome fingerprint tells the story of its origin, production and quality. Its characterization is of paramount importance for quality control, authenticity and quality improvement. Wine metabolome characterization remains one the greatest challenges of analytical chemistry and biochemistry that challenges all conventional approaches to metabolomics. Indeed, metabolome characterization is often achieved through integrated analytical technologies such as nuclear magnetic resonance spectroscopy (NMR) and a combination of different mass spectrometry (MS)-based methods. Over the last five years, MS-based metabolomics superseded NMR-based metabolomics, because of its higher sensitivity, selectivity, number of compounds detected and identified, as well as the ability to perform very high-throughput assays (Markley et al, 2017; Sousa Silva et al, 2019). Typical MS-based metabolomics experiments are performed using liquid (LC) or gas (GC) chromatography coupled to MS instruments. The choice for one or both technologies depends on the chemical characteristics of the molecules of interest and the sensitivity and speed of the instrument, often resulting in the identification of dozens to hundreds of compounds (Boccard et al, 2010).

**Figure 1.** Grapevine, grapes and wine. Mass spectrum from grapevine leaves obtained by FT-ICR mass spectrometry at Laboratório de FT-ICR e Espectrometria de Massa Estrutural – Ciências, Universidade de Lisboa.

LC-MS and GC-MS technologies have been widely used in grapevine's metabolic profiling, mainly in a targeted approach for the characterization of specific classes of secondary metabolites from grapes, leaves and stems (Anesi et al, 2015; Billet et al, 2018; Monagas et al, 2006; Souquet et al, 2000). These studies pointed out grapevine as a potential source of bioactive ingredients with antioxidant properties, paved the way to grapevine genotype discrimination based on a small picture of their metabolism, and confirmed the terroir effect in the grape's metabolic profile, from the same grapevine cultivar. In wine analysis by LC- or GC-MS, the main target are phenolic compounds, as they contribute to the colour, astringency, bitterness and aroma of wine, sugars and aminoacids (Cuadros-Inostroza et al, 2016; Monagas et al, 2007; Rubert et al, 2014). Despite these promising results, only small subset of these metabolomes was uncovered.

The analysis of extremely complex samples calls for disruptive analytical techniques, able to provide extreme resolution to resolve tens of thousands of individual compounds in very high-throughput assays, without the need for time-consuming chromatographic separation. At present, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) provides the ultra-highest mass resolution available, with the latest instrument developed (Bruker Daltonics) able to exceed 20 million resolution. This technology is bringing grapevine and wine research to a higher level. In our group, we have been developing methods for metabolite extraction from grapevine leaves compatible with direct infusion-FTICR-MS-based metabolomics (Maia et al, 2016), capable to identify thousands of chemical species in a few minutes, to perform a thorough metabolome characterization of grapevine leaves, validating their valorisation as a source of bioactive compounds with high nutritional and nutraceutical value (Maia et al, 2019). Grapes and their corresponding wines are also discriminated based on their chemical fingerprints, and the effect of the terroir in their composition was confirmed to an unprecedent depth (Roullier-Gall et al, 2014a; Roullier-Gall et al, 2014b). And, although it is possible to characterize ten years of wine bottle ageing through its chemical profile (Karbowiak et al, 2019), only FT-ICR-MS uncovered the comprehensive chemical signatures still present in 170-year-old champagne bottles recovered from the Baltic Sea (Jeandet et al, 2015). This work contributed to the knowledge of the winemaking process used in Champagne at mid-19th century, giving an invaluable input for the World's cultural heritage. FT-ICR-MS is thus poised to revolutionise our understanding of wine, from grapevine to glass.

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