A deep proteomics perspective into grape berry quality traits during ripening

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Abstract

Discovery-based proteomics studies have an important role in the understanding of the biochemical processes that occur during grape berry ripening. The ripening process is relevant in determining grape berry quality. For a proteome analysis of grape berry ripening, Kambiranda et al., (2018) applied a label-free mass spectrometry based quantitative approach. The authors reported the identification of proteins associated with the production flavour, aroma and ethylene production. Despite the valuable contribution of discovery-based proteomics studies, the picture is still incomplete. Future efforts in gaining proteome coverage would benefit the identification of proteins associated with grape berry quality traits.

Keywords

Grape berry proteomics, secondary metabolism, quality traits, berry ripening, MRM

Grapes (*Vitis vinifera* sp.) are the most important commercial fruit crops on the planet and their production is mainly for winemaking. The onset of ripening triggers a profound biochemical and physiological transformation that continues along the ripening phase of the fruit. Grape berries change from being an acidic, firm fruit with little sugar and desirable aroma to a sweeter, softer and highly-flavoured fruit. The biochemical changes that grape berries undergo during their development have been widely studied [1]. The development of desirable quality traits is controlled not only genetically in the fruit but also by environmental conditions and viticultural practices; these factors define the fruit quality and consequently the wine quality. Thus, the understanding of grape berry ripening is crucial in controlling the quality of the grape berries.

Hypothesis-free discovery-based approaches have been applied since 2004 as a tool to gain insight into the biochemical processes at protein level that occur during the ripening phase. The development of the proteomics technologies including better instrumentation for LC-MS has allowed profiling of hundreds of proteins implicated in primary and secondary metabolism (SM). The study presented in this issue by Kambiranda et al. [2], focuses on the identification of those proteins involved in the synthesis and regulation of the flavour and aroma compounds that accumulate during the ripening of the American hybrid grape cv. Blanc du Bois. Forty-three quantified proteins have been identified to be associated with flavour, aroma and ethylene production. They represent

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7.8 % of the analysed proteome which is a similar identification rate consistent with previous discovery proteomics applied to grape berries. In this study, a pre-fractionation of the sample was performed by 1D SDS-PAGE previously to the label-free quantification of the proteome. The authors reported on proteins involved in the production of monoterpenes, volatile fatty acid derivatives, phenylpropanoids, benzenoid volatiles and methoxypyrazines.

Few proteomic studies have shown protein profiles regarding proteins involved in the production of aroma and flavour [3], either because these proteins were not identified, or it was not in the scope of the investigation. To date there are only two publications that have focused entirely on the study of the profiles of proteins in ripening involved in the production of aroma and flavour volatiles in the grapes of two different varieties [2,4]. The cultivar-specific development of the aroma and flavour makes it necessary to collect proteomic data from different grape cultivars. Additionally, the proteomic technology has been reported to be consistent in distinguishing between cultivar variations. To predict the biochemical pathways involved, which are complex and can vary significantly among cultivars, more data needs to be collected [5].

The main observation from these studies is that only the major proteins acting upstream in the metabolic pathways are identified. For example, Kambiranda et al. [2], reported the identification of proteins involved in the biochemical pathways of terpenoid biosynthesis. While two proteins from the mevalonate pathway destined to the terpenoid backbone formation (isopentenyl diphosphate isomerase and 4-hydroxy-3-methylbut-2-enyl diphosphate) were quantified, the downstream acting enzymes responsible for the synthesis of monoterpenes were barely detected. The same phenomenon is also observed in other biochemical pathways of the SM responsible for the biosynthesis of other important metabolites from a wine quality perspective such as the polyphenols, non-flavonoids hydroxycinnamic acids (CAD) and the flavonoids anthocyanin (ANR, ANS) and proanthocyanidins (CHS, CHI, F3H) [3,6]. The most frequently identified proteins (shown within brackets) belong to the early or mid-pathway and the common central pathway of the phenylpropanoids (PAL). The main reasons are the lack of knowledge about the acting enzymes which is reflected in the public databases and the lack of sufficient proteome coverage which is commonly obtained by the discovery hypothesis-free proteomics experiments.

The top ten proteins which are mostly identified in ripe grape berry proteomic experiments are reported to belong to defence type proteins, such as pathogenesis-related proteins (thaumatins, osmotins, chitinases), non-specific lipid-transfer proteins and other ripening-related proteins (grip22, grip23) [5]. Functional analysis on the quantified data in discovery-based proteomics studies of grape berries shows an overrepresentation of GO categories related to carbohydrate and malate metabolism, photosynthesis, protein degradation, cellular component organization and biogenesis, cell wall remodelling while secondary metabolism (SM) is quite underrepresented.

Thus, the current challenge in the studies of grape berry ripening as well as in the studies of the metabolic pathways leading to the aroma composition in ripe berries is to increase the proteome coverage in order to detect the underneath low abundance proteome. The challenges in proteomics for the analysis of plant SM have been recently reviewed [7]. A common issue in grape berry proteomics shared with the rest of the plant proteomics analyses is the protein preparation and fractionation which is an important step in achieving a comprehensive and deeper coverage of the fruit proteome. An additional factor that hinders the detection of a higher protein dynamic range is

the type of proteomic approach applied. The application of a targeted proteomic approach would allow a gain in sensitivity which contrasts with the classical, but necessary first action proteomic tool, discovery-based proteomics approaches. Targeted proteomics using MRM methodology is a powerful tool that is being applied for a long time in other fields such as small molecule quantitative analysis. Most recently, MRM has been applied in proteomics, but its use is rather scarce in the analysis of SM in plants. The application of the MRM hypothesis-driven approach requires a selection of proteotypic peptides and unique transitions. This means that the development of an MRM method requires protein sequence information and/or spectral libraries. The use of MRM includes not only the validation of the proteomic experiments but also the enormous potential as indicated by Rudnick, P.A. [8] to become an alternative discovery proteomics tool with the targeted approach sensitivity benefit.

Examples of an application of MRM to study protein profiles in fruits can be found in the literature [9,10]. In line with the topic of the commented article, it is worth to mention the study published by Song et al., [10] showing the quantitative changes in proteins that regulate volatile biosynthesis of strawberry fruit during ripening by applying MRM.

Nevertheless, discovery proteomics studies shed some light on the metabolic pathways implicated in the ripening of the fruit and as reported by Kambiranda et al. [2] important quality traits such as the flavour composition of the ripe berries. These types of studies are valuable in contributing and deploying of the information into public data repositories that can be used in future for gene annotation, model predicting pathways and the design of targeted approaches.

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