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Short communication

Hellenoinos: NMR and LC-MS metabolic profiling of grape skin extracts of six Greek grape varieties

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Introduction

Grape (Vitis Vinifera) is a unique fruit, not only because it constitutes one of the most important fruit crop in the world, but also because it is connected to the history and evolution of humanity (Stavrakakis, 2013). It is a fleshy fruit, which consists of the skin, the flesh and the seeds (Ollat, 2002). The skin is about 15% of total grape fresh weight and the flesh and the seeds 80% and 5%, respectively (Dai et al., 2010). Wine comes mainly from the flesh (Kennedy, 2002). Wine aroma consists of volatile organic compounds, which are non-polar small molecules and it can be divided in three categories: varietal, fermentation and ageing or wine bouquet (Ilc et al., 2016; Liu et al., 2017). It is considered that most of the precursors aroma compounds exist in grape and become aroma compounds of wine after fermentation and ageing (Ilc et al., 2016). In this study, for the first time, NMR and LC-MS based metabolic profiling approaches are applied in 6 red and white Greek varieties (Agiorgitiko, Asyrtiko, Malagouzia, Moschofilero, Roditis, Xinomavro). Additionally, for each variety three different clones have been analysed, except for Malagouzia. 1H-NMR and LC-ESI-QTOF-MS have been employed in combination with Multivariate Statistical Analysis for the interpretation of the results.

Materials and methods

Plant material

Plant material was collected from the vineyards of VBN – Bakasieta form different Greek territorries. The six varieties were Agiorgitiko, Asyrtiko, Malagouzia, Moschofilero, Roditis, Xinomavro.

Sample preparation

A husking protocol was developed in order to use only skins of grape for the analysis. The protocol has the following steps: grapes are on ice, each grape is hulled, it is placed on a tissue and wiped, the skins of hulled grapes stored temporarily in a strainer on ice, then immersed in 100 mL of water and rinsed with 100 mL of water, they are placed on a tissue and wiped again and finally the skins are weighted and stored in freezer. Afterwards, fresh samples are grinded with liquid nitrogen in mortar and eventually follows the lyophilisation of samples for 90 hours. For each sample, 0.4900-0.5100 g of grape skin powder is placed in a 15 mL falcon and 5 mL of acetone HPLC grade is added. The samples get into the ultrasonic bath for 20 minutes and then centrifuged for 15 minutes at 4000 rpm and 20 °C. Finally, supernatant is removed and placed in penicillin vial, where solvents are removed with N2. For NMR experiment, dry extract is dissolved again with acetone : methanol 90:10 at the concentration of 7.5 mg/mL with sonication (1 min.). 1 mL is placed in weighed eppendorf and solvents is evaporated. Then, D6- acetone is added in order to have the concentration of 7.5 mg/mL in each eppendorf. Finally, 650 mL is placed in 5 mm NMR tube. For LC-MS experiment, acetone : methanol 90:10 used to

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dissolve again dry extract at the concentration of 3 mg/mL with sonication (1 min). In eppendorf is diluted the extract at concentration of 1 mg/mL adding methanol:water 50:50. 10 μL of each sample is transferred in each of three QCs eppendorfs. QCs and samples were filtered through PVDF filter (13 mm, 0,45 μm, Philic) and then were transferred in HPLC vials.

NMR experimental parameters

NMR spectra were acquired on a Bruker Avance III 600 MHz spectrometer with 1D NOESY pulse program with water suppression and were processed with TopSpin and AMIX software.

LC-MS experimental parameters

The analysis was conducted in a UHPLC system (Dionex UltiMate 3000 RSLC, Thermo Fisher Scientific, Germany). Also, an Electrospray Ionization connected to an Ultra High Resolution QTOF spectrometer (Maxis Impact, Bruker Daltonics, Bremen, Germany) was used for this analysis. Samples were injected in an Acclaim RSLC 120 C18 (2.1 × 100 mm, 2.2 μm) column from Thermo Fischer Scientific (Dreieich, Germany) with a C18 1.7 μm precolumn VanGuard from Waters (Dublin, Ireland). LC-MS chromatograms were processed with MZmine 2.54.

Multivariate statistical analysis

Multivariate statistical analysis for NMR and LC-MS data was performed with SIMCA 14.1.

Results and discussion

NMR results

PCA and PLS-DA models of NMR based multivariate statistical analysis facilitated the clustering of unripe and harvest maturation stages, while samples of 10-11 Baume stage did not group. Oleanolic and malic acid had an important role in the clustering of grapes in unripe stage, whereas sugars were responsible for the clustering of the other two maturation stages. OPLS-DA models were employed for the discrimination of unripe and harvest but also unripe and stage of 10-11 Baume. Finally, the majority of clones of each grape variety could not form clusters when analysed with PCA and PLS-DA models.

LC-MS results

PCA and PLS-DA models of LC-MS based multivariate statistical analysis facilitated the clustering of all maturation stages (unripe, 10-11 Baume and harvest).

Oleanolic, malic, quinic, tartaric and dehydroascorbic acid were responsible for the grouping of samples in the unripe stage, while sugars were significant for the clustering of samples at the stage of harvest. OPLS-DA S-plots indicated malic acid derivatives and sugars as potential responsible for the clustering of samples of 10-11 Baume when plotted with unripe and harvest stage, respectively.

Conclusion

PCA analysis of both analytical approaches revealed that skin grape extracts of unripe and harvest stage showed tight clustering, while samples of 10-11 Baume were intermediate. For the grouping of samples of unripe stage, oleanolic (NMR, LC-MS), malic (NMR, LC-MS), quinic (LC-MS), tartaric (LC-MS) and dehydroascorbic acid (LC-MS) were responsible. Sugars seemed to play an important role in the grouping of harvest samples with both techniques. When 10-11 Baume and harvest samples were compared, the aliphatic region of NMR spectra was indicated responsible for the grouping of samples, whereas in the comparison with samples of harvest stage, sugars were significant for the separation. Finally, ferulic acid and derivatives of malic and succinic acid were identified with LC-MS.

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