Looking deeper at winemaking by-products composition: target & untarget analysis

CONTEXT AND AIMS OF THE MANUSCRIPT

This study is framed within the valorization of white grape marc for the design and obtaining of bioactive functional extracts with applications in livestock and aquaculture. The antimicrobial activity of these extracts allows us to seriously consider their use as an alternative or complement to classical antibiotics as another tool in the fight against antimicrobial resistance (AMR). These developments are being carried out in the context of the European H2020 NeoGiANT project that is supporting this study.

The aim of this work is to investigate in depth the composition of white grape marc extracts obtained with different GRAS solvents using a sustainable and patented technology. The use of these extracts as functional ingredients in *enhanced feed, natural therapies for animal production and semen extenders* requires the identification and quantification of target bioactive compounds (target analysis by LC-MS/MS), and the investigation of other analytes present with potential bioactivities by means of high-resolution techniques (UPLC-QToF) that also guarantee the absence of unwanted compounds, such as potential pesticide residues.

The proposed extraction process (applied both on medium and pilot scale) uses non-contaminating materials, takes place under gentle conditions and prevents the obtained eluates from containing suspended solids, enabling ready-to-use liquid extracts rich in bioactive polyphenols to be obtained. We work with a zero-waste philosophy, so that the exhausted biomass generated after extraction, which is otherwise very efficient, is subjected to a vermicomposting process to produce an organic biofertilizer that can be reused in the vineyards that produced the grapes whose by-product we process once winemakers have obtained excellent quality white wines. It is pure circular economy and total absence of second-generation by-products.

If your publisher considers this approach to be of interest, I undertake to adequately develop the content summarized in the abstracts detailed below, to prepare the manuscript under the corresponding editorial guidelines, and to send it on time in the framework of the CHANIA 2023 Special Issue.

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Looking deeper at winemaking by-products composition: target & untarget analysis

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Keywords: grape marc, GRAS solvents, MSAT, QToF, AMR
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Grapevines (*Vitis vinifera*), like many other plant species, naturally produce a wide range of secondary metabolites that act as a chemical line of defence against microbial pathogens, animal herbivores, solar radiation (screens out DNA damaging UV-B light), high temperatures, and so on. This phytochemical arsenal is mainly, but not exclusively, composed of plant phenolics.

Among winemaking by-products, grape marc stands out as an unquestionable source of such bioactive compounds. White grape marc is very rich in all kinds of polyphenols except for anthocyanidins and their derivatives. All types of extraction techniques and virtually all available solvents have been used to obtain them. In our research group, we have developed and patented (Lores *et al*, 2022) a green and scalable technique called MSAT (Medium Scale Ambient Temperature System). The extraction process is characterized by minimum energy requirements in combination with solvents generally recognized as safe (GRAS) and their respective hydro-organic mixtures, and produces multicomponent extracts ready-to-use as liquid ingredients in various innovative formulations (Castillo *et al*, 2022).

The polyphenolic content of the grape marc extracts depends on the phenotypic and agro-environmental conditions, and the extraction technique and solvents used. Existing data suggest that the antimicrobial activity of a multi-component extract containing most of the original polyphenols will be higher than that of a single-component extract. This is due to the foreseeable synergistic activity between the different polyphenols of the plant, as the mechanisms to counteract oxidative stress and those of the antibacterial action are diverse, and the plant synthesizes a whole battery of compounds to attack its potential enemies and/or to react in stressful situations. This makes raw, unfractionated extracts powerful broad-spectrum antibacterials and potent free radical scavengers. So process that not include fractionation or purification stages of specific compounds, seems a very good strategy.

The use of these multi-component extracts as ingredients in formulations with antimicrobial and antioxidant properties, however, requires an in-depth and comprehensive chemical-analytical characterisation, so that these extracts can be registered as additives in compliance with the relevant regulations. For this purpose, not only their polyphenolic composition must be known in detail (target analysis), but also the presence of other compounds of interest as well as the absence of unwanted compounds must be evaluated (untarget analysis). This is the focus of the present study.

A high-resolution technique has been used to address this task, UHPLC-QToF (Ultra High-Performance Liquid Chromatography Quadrupole Time-of-Flight). The hybridization of quadrupole and TOF technology resulted in high-resolution and high mass accuracy analysis of all ions simultaneously, which allows to identify and quantify complex mixtures or unknown compounds. Besides, target analysis has been accomplished by HPLC-QqQ (High-Performance Liquid Chromatography Triple Quadrupole) considering sixty polyphenolic compounds commonly found in white grapes, white wines and related winemaking by-products.

Figure 1 shows an example of the target analysis on an extract obtained with an isovolumetric mixture of ethanol and water. Seventeen out of the sixty target polyphenols analyzed were detected, highlighting the presence of procyanidins (B1, B2, C1), catechin, epicatechin, and miquelianin.

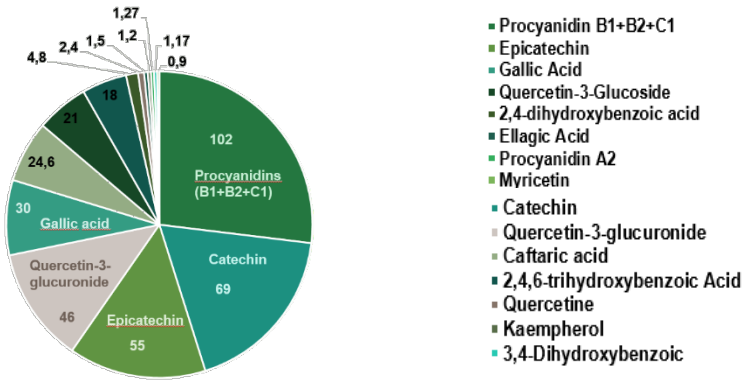


Figure 1. Target polyphenolic composition of an hydroethanolic extract (Concentration of polyphenols in mg/L)

High Resolution Mass Spectrometry (HRMS) was employed for non-target analysis, allowing the detection of a number of additional compounds, not only other polyphenols not considered in the target analysis, but also interesting compounds from other families such as fatty acids or short-chain organic acids. Figure 2 shows, as an example, a chromatogram of the untargeted analysis of an aqueous extract of white grape marc.

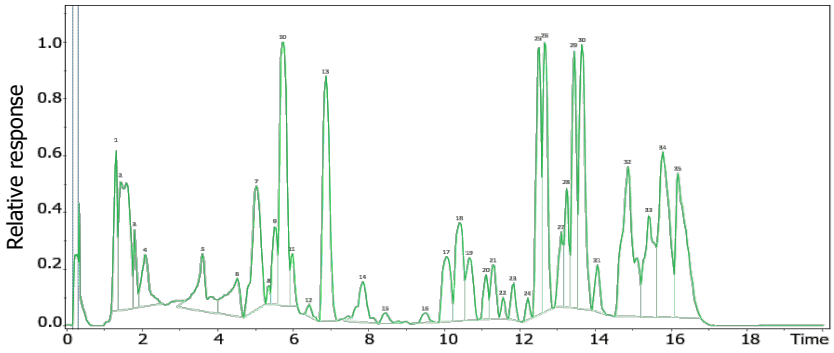
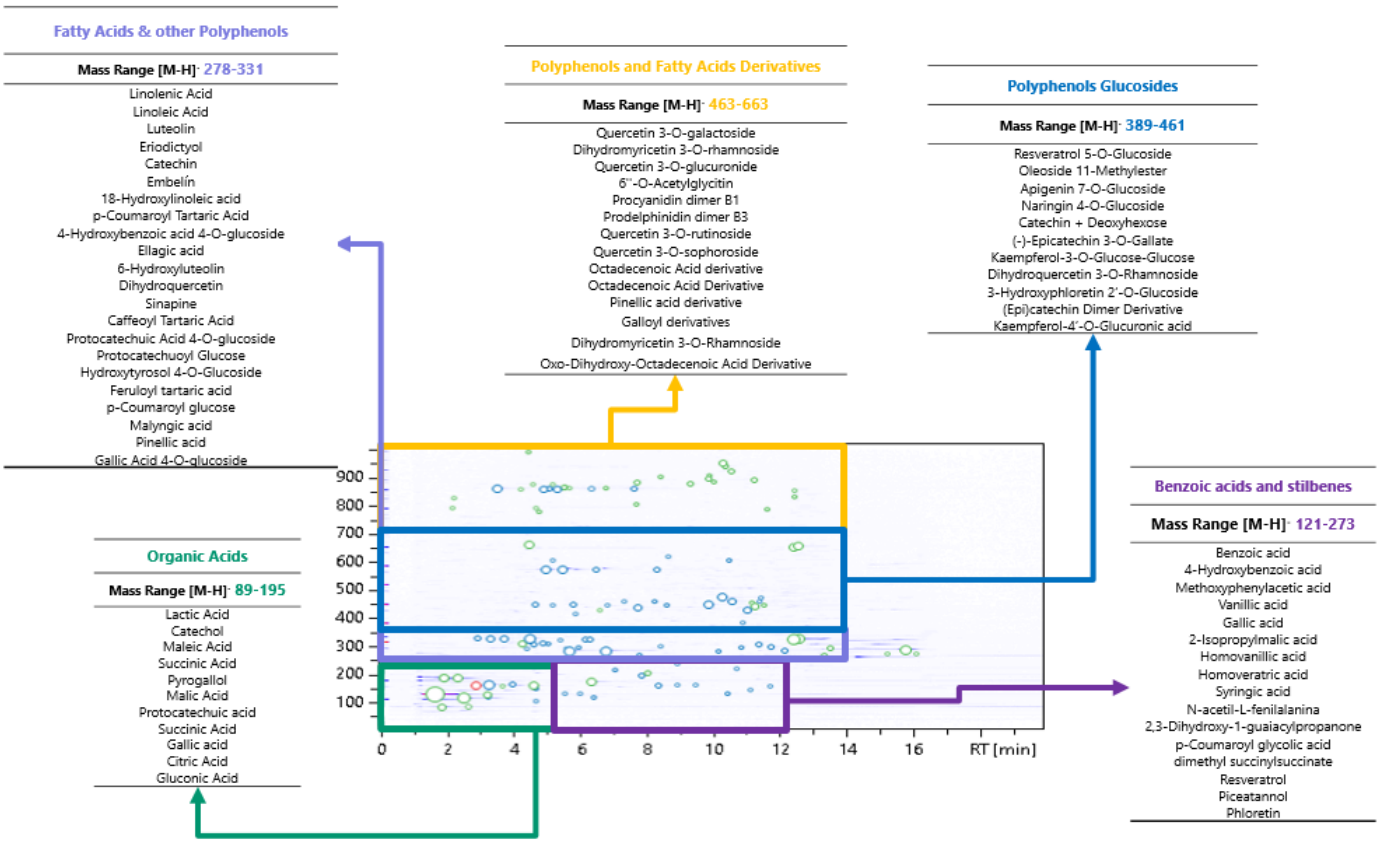


Figure 2. Untarget bioactives composition of an aqueous extract showing 35 additional compounds belonging to the following groups: flavan-3-ols and oligomeric derivatives, flavonols, flavanones, fatty acids and derivatives and aliphatic hydroxy acids.

Untarget analysis was carried out in progressive stages. First, by *SmartFormula* an average of 35 compounds were detected for each solvent (as shown in figure 2), of which 15 were common to all extracts and the others were discriminating compounds. Then, by *Metaboscape*: a deep search for all analytes detected in each sample was carried out. Using chemometric tools, the different industrial extracts of Albariño white grape marc were analyzed, and 925 analytes were detected. Initially, by means of PCA, the characteristic analytes of the different extracts were screened, leaving 142 outstanding compounds (intensity and recurrences) allowing the perfect discrimination of extracts obtained with different solvents. Finally, by combining metabolomics tools: SmartFormula, Compound Crawler, Metfrag; together with the systematic comparison of the mass profile of each analyte with the other 924 compounds & the position in the topographical space (classification by compound family) the identification of these bioactives was obtained (see an approach in Figure 3).



Application development: H2020 NeoGiANT project (https://www.neogiant.eu/)

To improve the waste management in the wine sector and, in parallel, to overcome the challenges related to AMR (antimicrobial resistance) derived from the misuse and abuse of antibiotics in farmed animals' production (in feeding, health and reproduction areas) NeoGiANT offers innovative solutions. They are based on the known potent natural antimicrobial and antioxidant activities of the above-described grape marc extracts, due to their arsenal of phytochemicals, in particular their phenolic compounds content. Suitable bioactive molecules from white grape marc are identified, with a validation of their benefits (*in-vitro* and *in-vivo*) to produce final formulations to be used in animal production: *enhanced feed, natural therapies for animal production and semen extenders* that will not only avoid the growth of microorganisms, but also improve the health and welfare of the animals, increasing profitability. NeoGiANT works under the perspective of animal health, reducing environmental impacts and contributing to the circular economy.

References

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Acknowledgements

This study has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 10103676.