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

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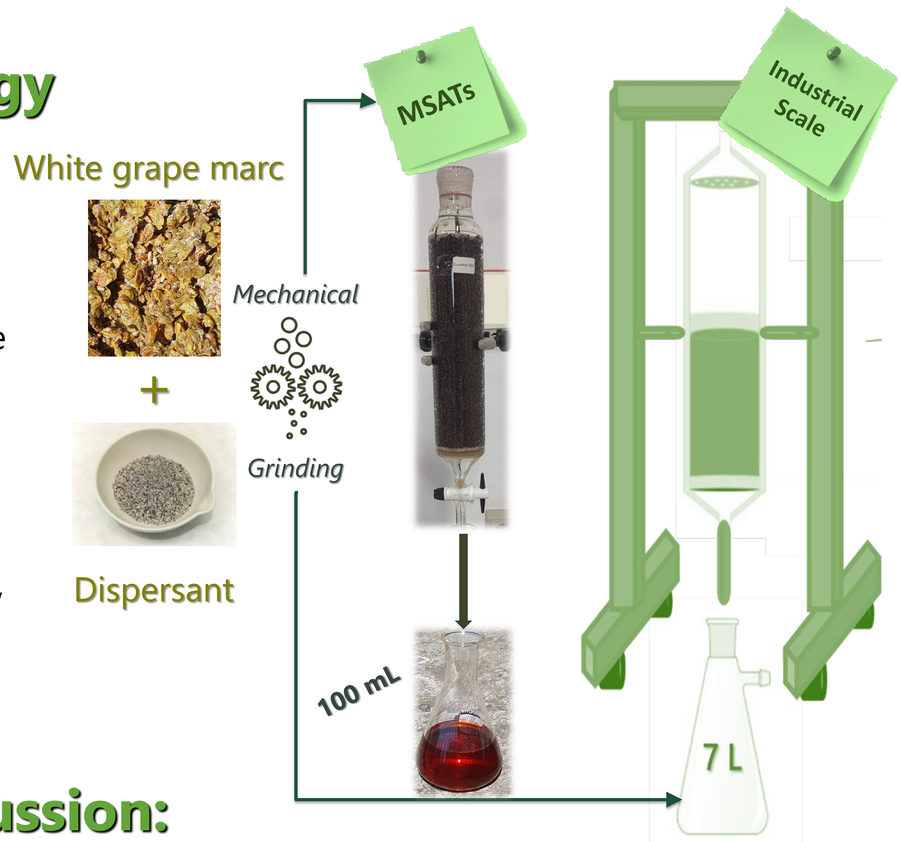
Introduction & Methodology

Vitis vinifera var. Albariño

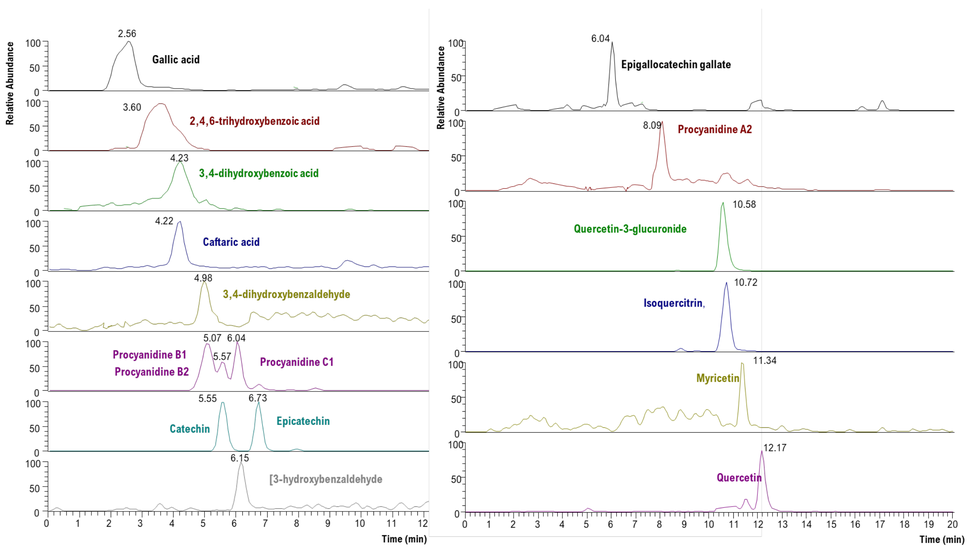
Grapevines (*Vitis vinifera*), like many other plant species, naturally produce a wide range of secondary metabolites that act as a chemical line of defense against microbial pathogens, animal herbivores, solar radiation (screens out DNA damaging UV-B light), high temperatures, and so on. This phytochemical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



- Among winemaking by-products, **White grape marc** stands out as an unquestionable source of such bioactive compounds.
- 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).
- This use requires **an in-depth and comprehensive chemical-analytical characterization**, so that these extracts can be registered as additives in compliance with the relevant regulations.
- The 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.

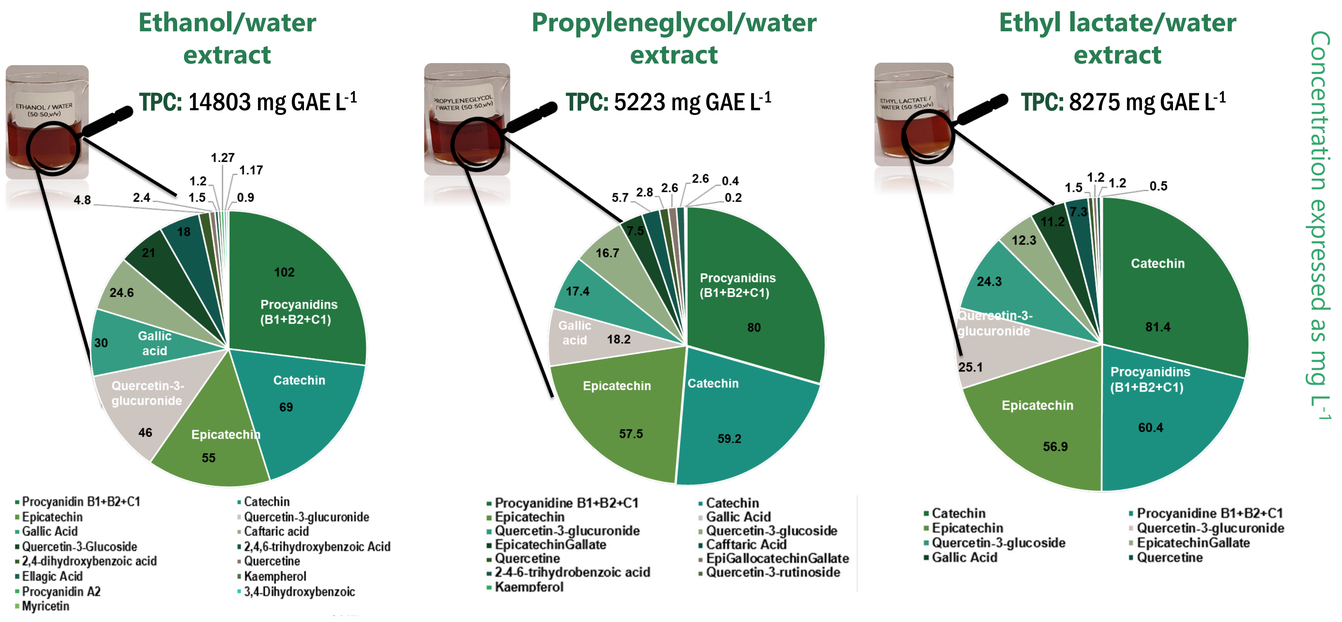
Results & Discussion:

Target analysis: Quantification of bioactive compounds



✓ 60 target polyphenols were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. 18 of them were detected in the analyzed extracts

✓ The ∑ of individual bioactive compounds was similar in all extracts, between 270-378 mg L^{-1}



✓ As expected, the profile for each plant phenolics group was slightly different depending on the solvent employed to obtain the extracts

✓ Highlights the presence of ***flavan-3-ols*** (***catechin*** and ***epicatechin***) as well as their ***oligomeric derivatives*** (***procyanidines B1, B2 and C1***)

Non-Target analysis: High Resolution Mass Spectrometry (HRMS)

The untarget analysis was carried out in two phases:

—by ***SmartFormula*** an average of **35 compounds** were detected for **each solvent**, of which **15 were common to all extracts (see Table 1)** and the others were discriminating compounds

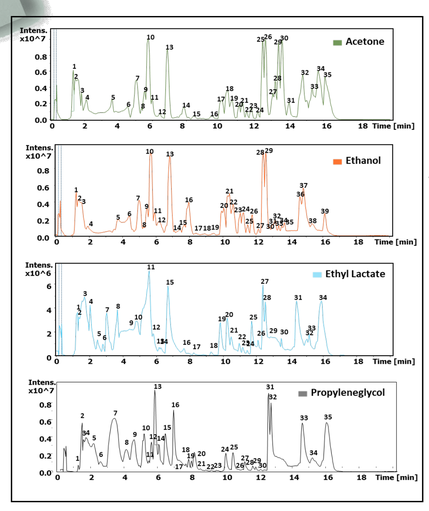
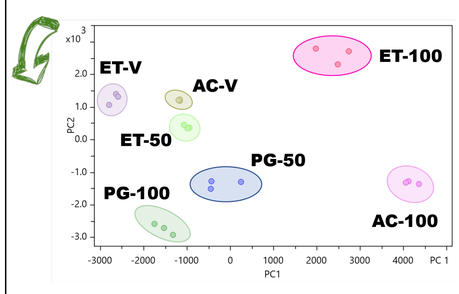


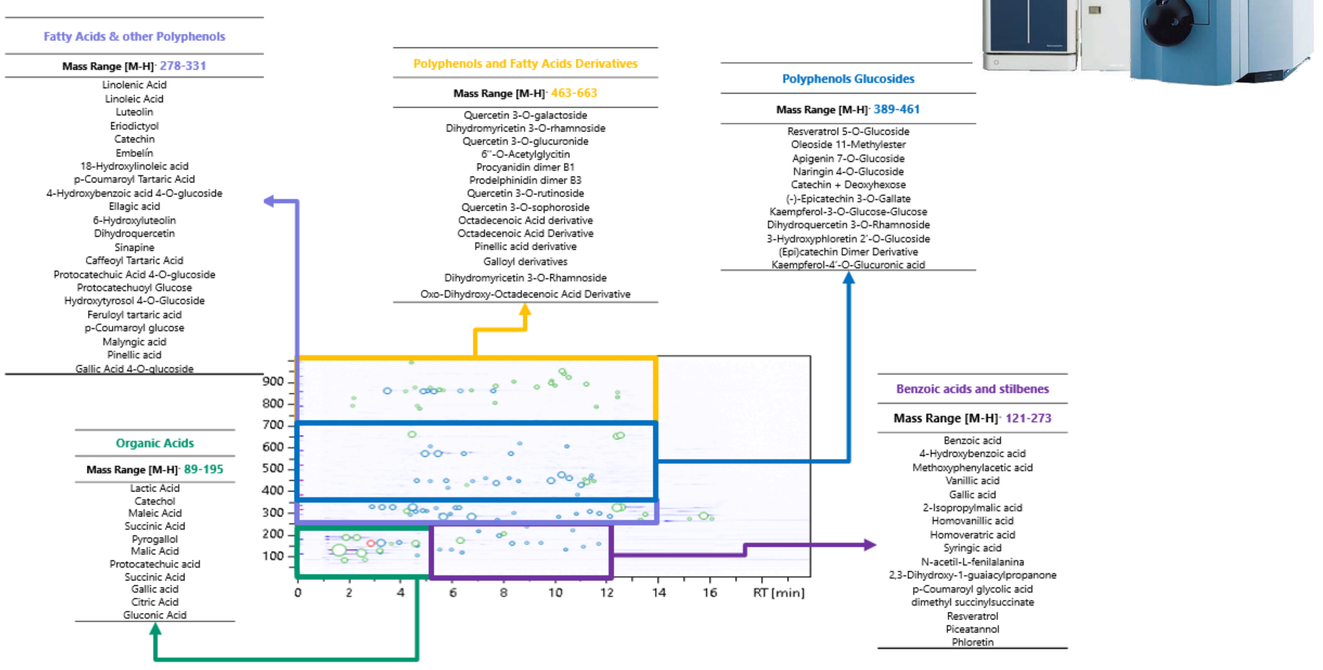
Table 1: common analytes for all hydro-organic extracting mixtures

Compound	RT [min]	Formula	Ion [m/z]	Mass (Da)	Family
Gluconic Acid	1.3	C6H12O7	195	196	Organic Acid Derivative
Mallic Acid	1.5	C4H6O5	133	134	Organic Acid Derivative
Glucogalin (Derivatives)	4.5	C13H16O10	331	332	Organic Acid Derivative
Procyanidin B#	5.1	C30H26O12	577	579	Procyanidins
Procyanidin B#	5.5	C30H26O12	577	579	Procyanidins
Catechin	5.8	C15H14O6	289	290	Catechin Derivatives
Procyanidin B#	6	C30H26O12	577	579	Procyanidins
Epicatechin	6.9	C15H14O6	289	290	Catechin Derivatives
Procyanidin B1 3-O-gallate	9.5	C37H30O16	729	731	Procyanidins
Flavanomarein	10.1	C21H22O11	449	450	Polyphenol
Quercetin 3-O-glucuronide	10.4	C21H18O13	477	478	Quercetin Derivatives
Engeletin or prunin	11.1	C21H22O10	433	434	Polyphenol
Malignyc acid /Fulgidic acid / Fatty acid FA18:2+3O	12.5	C18H32O5	327	328	Fatty Acids
9,12,13-TriHOME / 5,8,12-TriHOME(9) / FA18:1+3O	12.7	C18H34O5	329	330	Fatty Acids
Leptospermone	14.9	C15H22O4	265	266	Phloroglucinol derivative

—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

—by ***Metaboscape:*** deep search for all analytes detected in each sample. 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**.





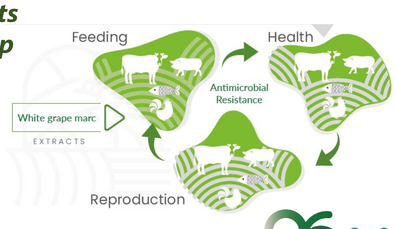
Conclusions

Extracts from grape marc were obtained by a *sustainable procedure* called MSATs and successfully scaled-up

All the *extracts* obtained are *rich in bioactive compounds*, highlighting the presence of polyphenols, mainly flavan-3-ols and their oligomeric derivatives, along with fatty & organic acids

Applications development: H2020 NeoGiANT project

The ***ready-to-use extracts*** are being used to ***develop novel natural products*** for the ***control and prevention*** of the most ***relevant diseases*** in ***animal production and aquaculture***



The final formulations: ***enhanced feed, plant-extract based therapies and semen extenders*** will not only avoid the growth of microorganisms (**antimicrobial**), but also improve the health and welfare of the animals (**antioxidant**), increasing profitability.

NeoGiANT works under the perspective of animal health, reducing environmental impacts & contributing to the circular economy in the wine sector

References

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