Metabolomic profiling of smoke-exposed red wine by LC-HRMS/MS

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Introduction

- US wine industry is facing more smoke exposure issues due to the increasing incidence of wildfires.
- **Exacerbated by climate change,** wildfires increase in both number and size in recent years, intensifying smoke exposure issues in wine.

- During a wildfire, smoke constituents, such as volatile phenols, can permeate the grape cuticle and bind to sugars molecules, creating volatile phenol glycosides.
- These glycosides can be hydrolyzed during the winemaking process, releasing the aglycone volatile moieties such as guaiacol or cresols in wine.

- Smoke taint is an off-aroma described as smoky, dirty, burnt, medicinal, and ashy character in the wine[1].

- Although GC-MS techniques offer a powerful tool to analyze the volatiles, they cannot shed light on the chemical nature of intact phenolic glycosides or other smoke precursor compounds. LC-HRMS/MS systems can complement the GC-MS analysis.

Materials & Methods

LC-HRMS/MS and the latest generation of bioinformatics tools to discriminate smoke-related compounds present in wines from 2020 wildfire and control wines (N=14 for control wine; N=26 smoke exposed wine).

- ☐ Untargeted high-performance liquid chromatography (HPLC) combined with high resolution accurate LC–MS/MS was conducted using a Shimadzu Nexera UHPLC system connected to an AB SCIEX TripleTOF® 5600 mass spectrometer equipped with a Turbo V ionisation source.
- ☐ Chromatographic separation was achieved using an Inertsil Phenyl-3 column (4.6 mm × 150 mm, 100 Å, 5 µm; GL Sciences).
- ☐ Gradient elution was performed using solvent A, water containing 0.1% v/v formic acid, and solvent B, methanol containing 0.1% v/v formic acid.
- ☐ Mass spectrometer settings: spray voltage 4500 V; source temperature 550°C and a period cycle time of 950 ms was used. Full scan with ion accumulation of 150 ms, followed by a dynamic MS/MS; collision energy 35 V with collision energy spread (CES) of 15 V ramped through each MS/MS scan using a range of m/z 100–1200.
- ☐ For tentative annotations of volatile phenol glycosides, raw data processing was performed using Progenesis QI™ software (NonLinear Dynamics) and entailed peak picking, alignment and searching of multiple databases to assist in compound annotations.
- ☐ Putative annotations are based on exact mass, isotopic pattern and MS/MS spectral data[2].

Results

LC-HRMS/MS untargeted metabolomics analysis of wine

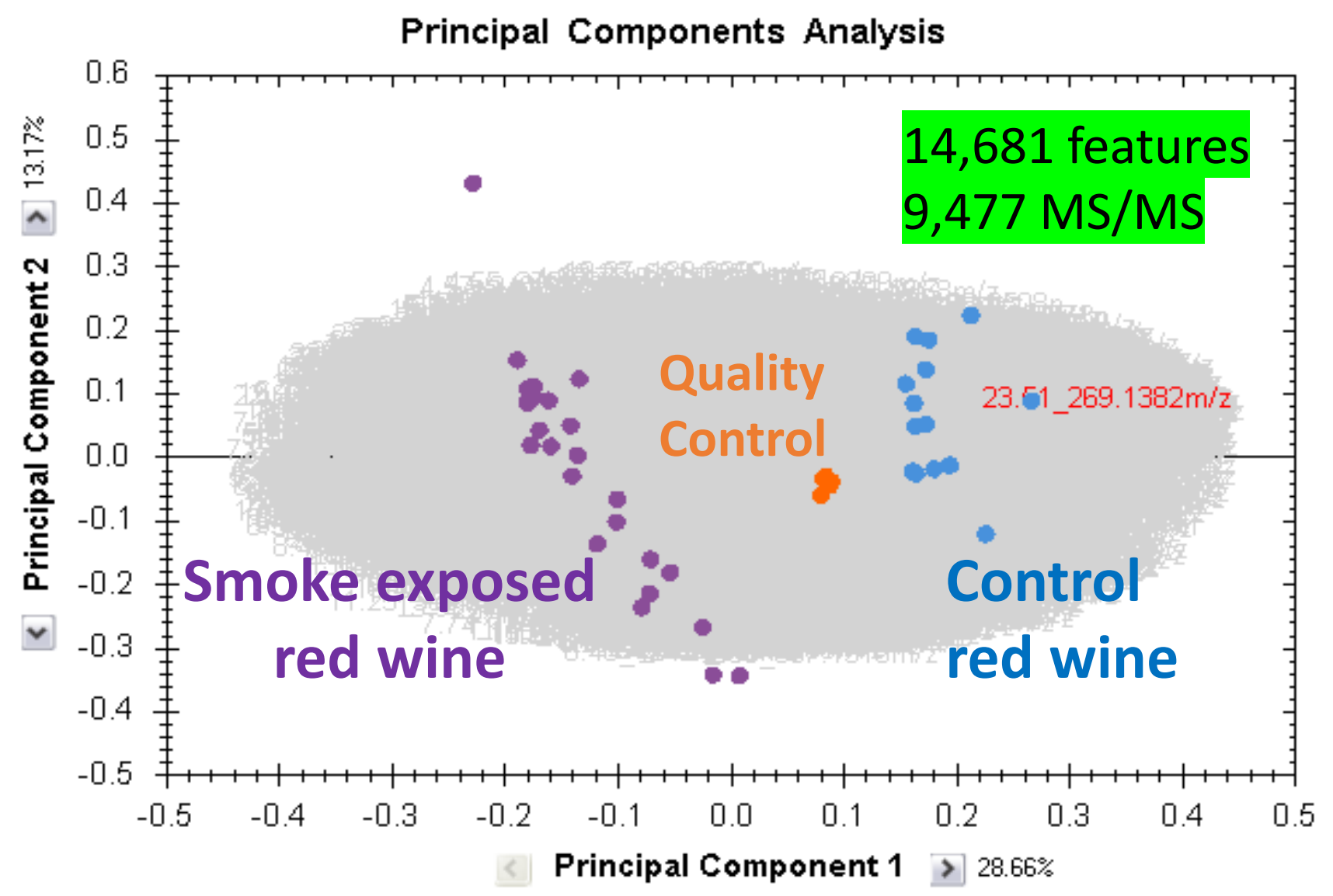

Fig. 1.- **Principal component analysis (PCA).** PCA computed using 4271 ions containing MS/MS data in positive ion mode. All sapless presented a complete clustering according to their class. Aliquots of 20 µL from each wine were pooled to generate a quality control sample (QC) used for evaluating LC–MS/MS platform performance.

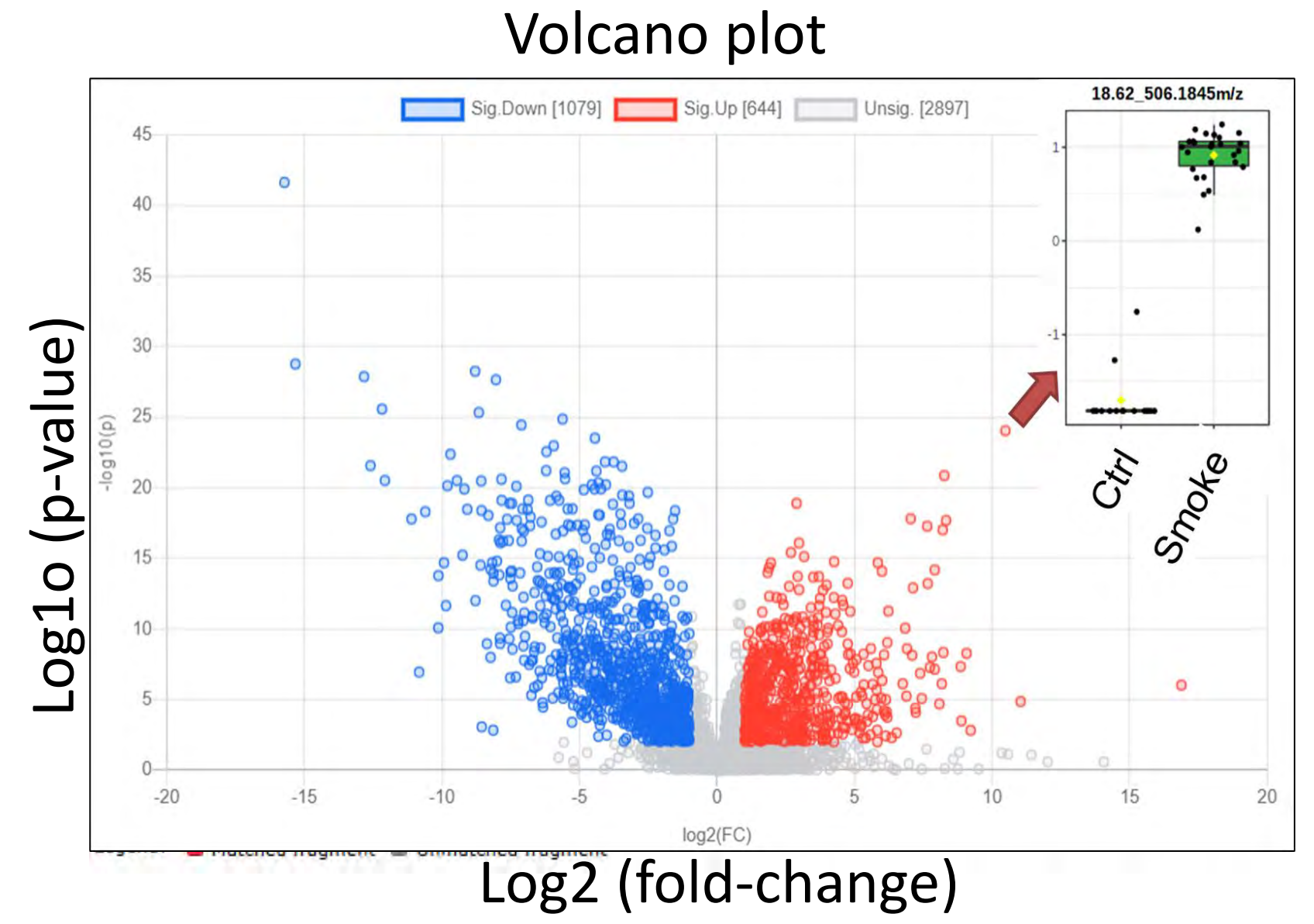

Fig. 2. Volcano plot. Molecular features with MS/MS data and CV<25% on quality control samples (QCs). Six hundred forty-four features were significantly higher in smoke wine after applying false discovery correction (p< .05, fold-change >2).

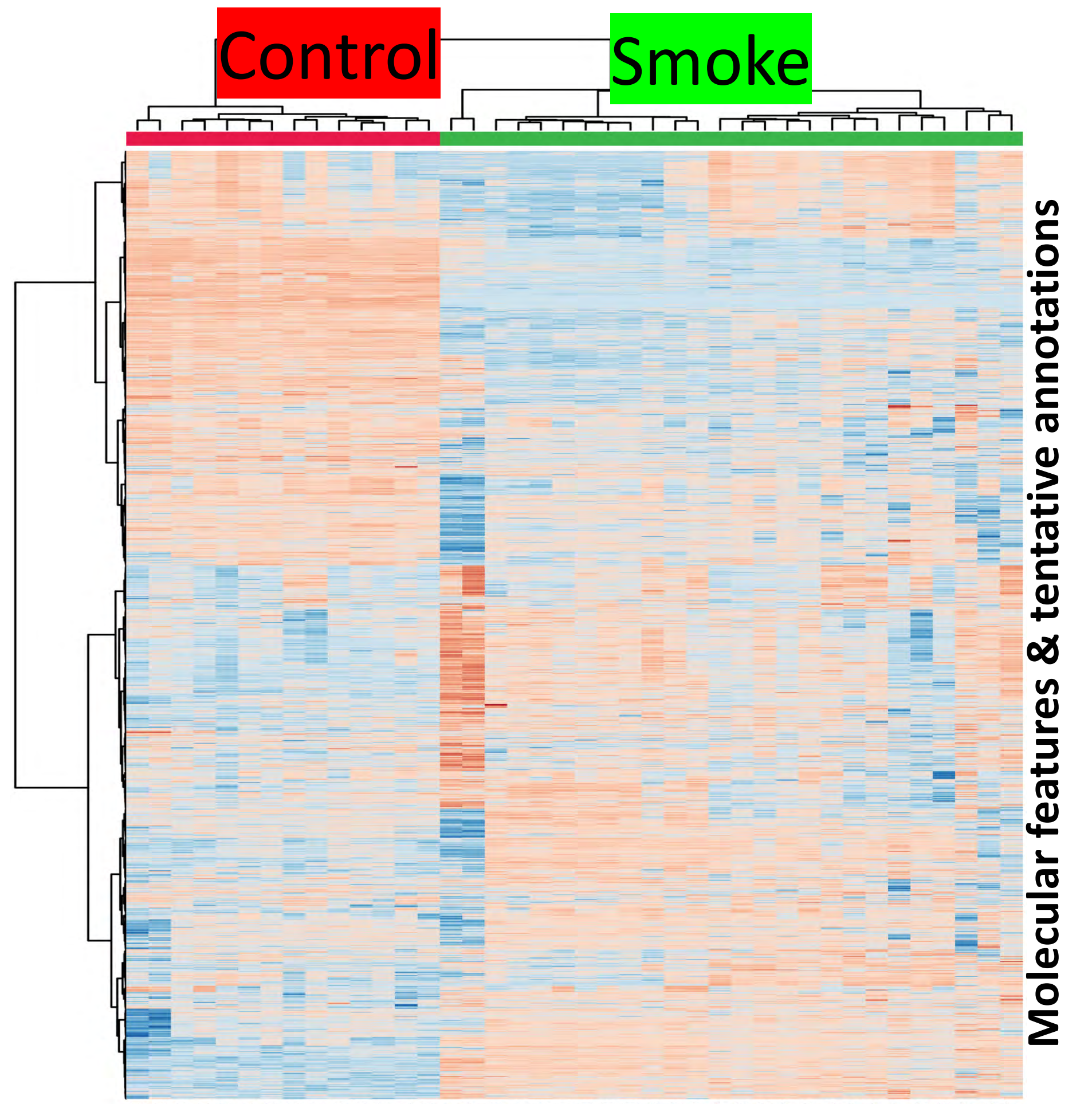

Fig. 3. Heatmap. Tentative annotations and molecular features with MS/MS data and CV<25% on quality control samples (QCs). Mass spectral data were searched against our *in-house* ENZO library, METLIN, Human Metabolome Database (HMDB), and the MassBank of North America (MoNA).

Results

Tentative identification of volatile phenol glycosides

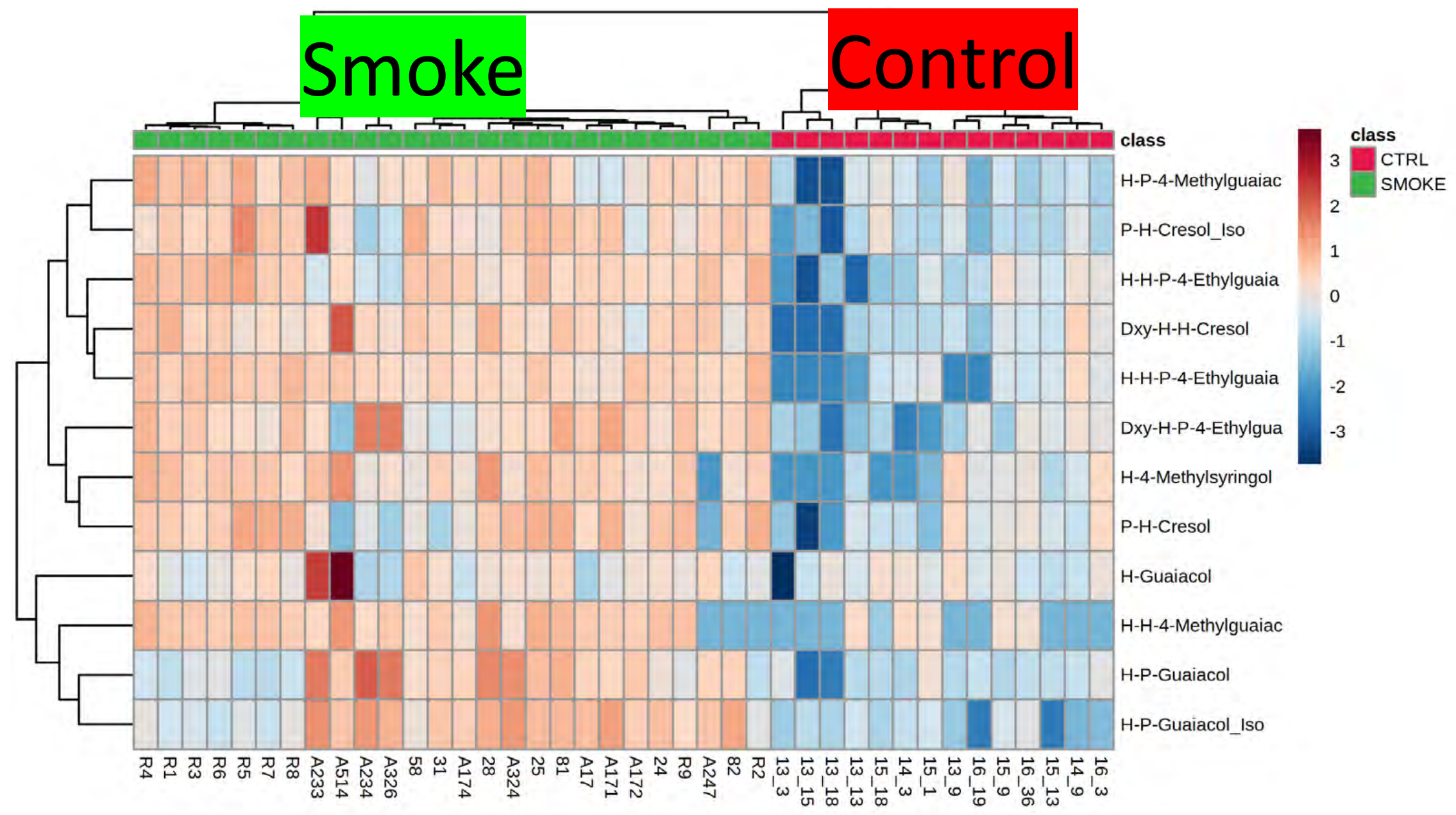

Table 1. t-test. Tentative identification of volatile phenol glycosides. Fold change corresponds to the ratio of smoke wine/control wine.

Compound	FC SMK/CTR	p-value
H-P-4-Methylguaiacol	3.6	1.46E-08
H-H-P-4-Ethylguaiacol	5.5	1.65E-06
Dxy-H-P-4-Ethylguaiacol	2.6	8.53E-06
H-H-P-4-Ethylguaiacol_Iso	22.4	3.47E-05
H-P-Guaiacol_Iso	11.4	1.98E-04
P-H-Cresol	2.8	2.26E-04
Dxy-H-H-Cresol	4.0	2.57E-03
H-P-Guaiacol	4.1	4.56E-03
H-4-Methylsyringol	13.4	4.07E-02

H-Hexose, **P-** Pentose, **Dxy-** Deoxy

Fig. 4. Heatmap. Tentative volatile phenol glycosides annotations. Assignations were based on exact mass, isotopic pattern and MS/MS spectral data. According to the t-test (Table 1). All assigned phenol glycosides were significantly higher in smoked wine.

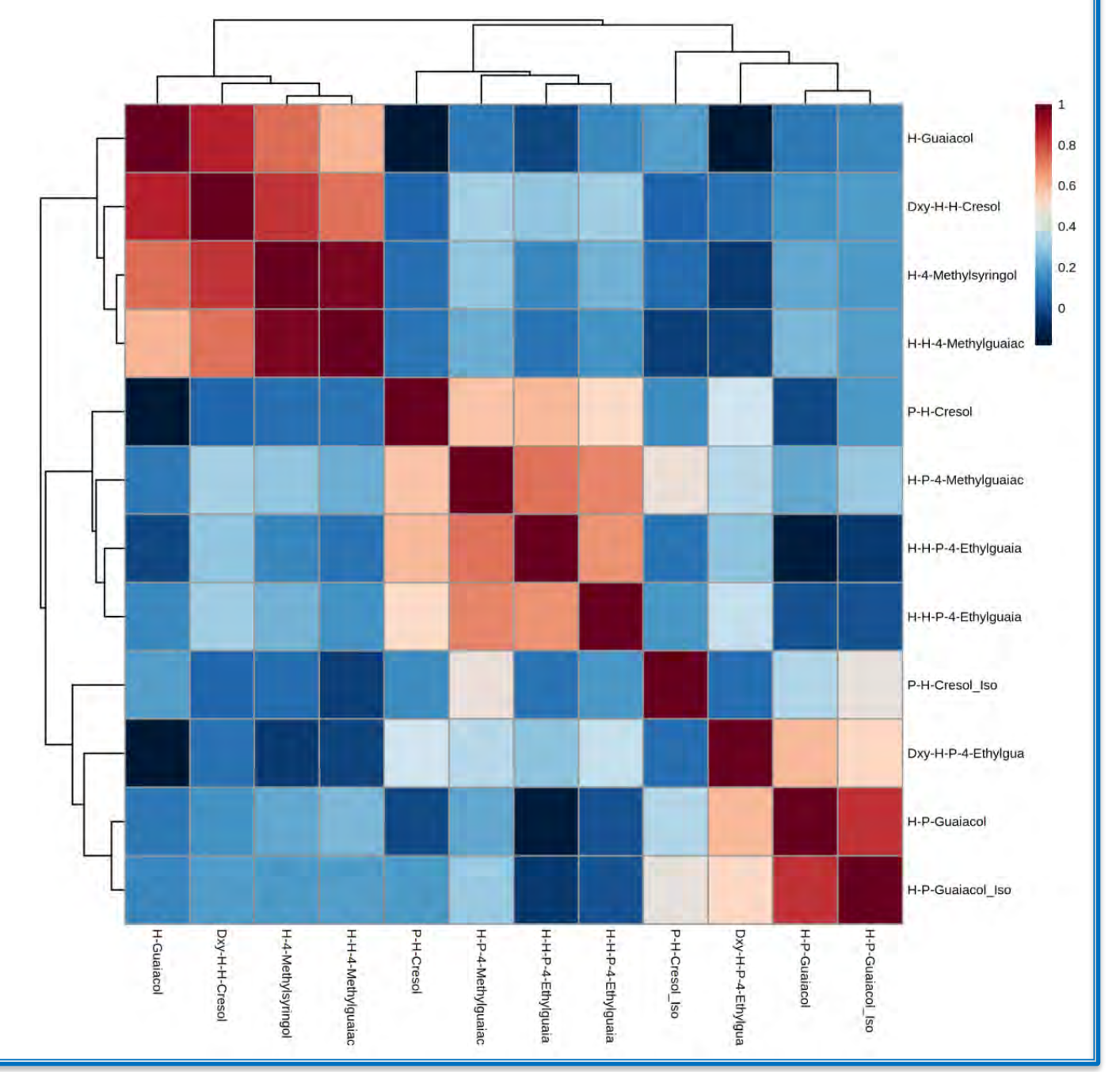

Fig. 5. Correlation analysis. Tentative volatile phenol glycosides annotations. Hexose-guaiacol, deoxy-hexose-hexose cresol, hexose-4-methylsyringol and hexose-hexose-methyguaicol showed a positive correlation among them. Furthermore, pentose-hexose-cresol, hexose-pentose-4-methylguaicol and two isomers of hexose-hexose-pentose-4-ethylguaiacol were positively correlated among them.

Conclusions

1. This study employed a state-of-the-art untargeted metabolomics approach using high-performance liquid chromatography combined with high-resolution accurate mass LC–MS/MS.
2. By using latest generation of bioinformatics tools, we detected hundreds of molecular features (containing structural and elemental composition) only present in smoke-exposed wine in addition to diverse volatile phenol glycosides.
3. Univariate data analysis was further performed to establish differences or similarities between the control and smoke-exposed wines, including twelve volatile phenol glycosides reaching up to 22-fold-change higher in smoke-exposed wine.

Acknowledgements

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References

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