Determination of the Molecular Transformations and Pathways that Occur During the Winemaking Process

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Overview
Purpose: To determine the molecular transformations and pathways that occur during the winemaking process.
Methods: Samples were analyzed by injection onto an LC-MS and using post column derivatization analysis software.
Results: The results of this study provide a catalogue of the components present in the samples and a statistical analysis reflecting the changes in the component levels observed across the samples. This data set may serve as a first approximation of those changes occurring during the winemaking process, providing insights into the changes that occur during the production of the first wine product. Differentiation of known compounds as well as detection of unknown components is an important area of research for the agricultural industry.

Introduction
Wine is a very complex mixture and a rich source of beneficial and antioxidant compounds. Identification and quantitation of the components is a challenging process. High Pressure Liquid Chromatography coupled to a Thermo Scientific Q Exactive Mass Frontier benchtop Orbitrap mass spectrometer was used for analysis of several wines from the Ballard Winery. Using SIEVE software, an easy-to-use automated solution for evaluating chromatograms, we were able to identify and quantitate several wine components and their variable content across the study samples.

Methods
High Performance Liquid Chromatography
The wine was stored at room temperature until analysis. Chromatography was performed using a gradient elution. The samples were eluted using a flow rate of 300 L/min by linearly increasing solvent B concentration from 0% to final 80% over 15 min. The wavelengths were then scanned with 80% solvent A (3 mm) and re-equilibrated with 100% solvent A, 3% solvent B (2 min). The compounds were eluted using flow rate 300 L/min by linearly increasing solvent B concentration from 0% to final 80% over 15 min. The total run time, including column wash and equilibration, was 20 min.

Mass Spectrometry
Q Exactive benchtop Orbitrap mass spectrometer was operated in positive ion mode at 70,000 resolving power (defined as FWHM @ 200) for full scan analysis (mass range 70 – 1000 amu). The measurements were done in duplicate with an internal standard added to each sample.

Component Clustering
The Q Exactive benchtop Orbitrap LC-MS/MS highlighting the front-end ion optics which enhance sensitivity, the quadrupole mass filter for selectivity, the HCD cell used for Higher Energy Collisional Dissociation (HCD) fragmentation, and the simultaneous detection and relative quantitation of the wine’s components.

PCA Analysis
A principal component analysis (PCA) was performed on the data from the Q Exactive benchtop Orbitrap LC-MS/MS measurements using R.

Conclusions
Despite the complexity of the wine samples, exhibiting numerous individual compounds, our initial analysis showed that many of these compounds can be grouped together to form a few clusters. These clusters represent different aspects of the wine’s composition and can be used to identify unique characteristics of different wines. Further analysis is needed to confirm the identity and significance of these clusters. The results from this study will be further subjected to more in-depth analysis to elucidate the molecular transformations that occur during the winemaking process.

[Figures and tables as described in the text]