

# Metabolic diversity conveyed by the process leading to glutathione accumulation in inactivated dry yeast: A synthetic media study

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## Highlights

- Combined MS analyses for a comprehensive characterization of GSH enriched IDY extractable fractions.
- Hundreds of peptides associated with the glutathione enrichment process.
- 16 identified markers not yet associated with glutathione enrichment.

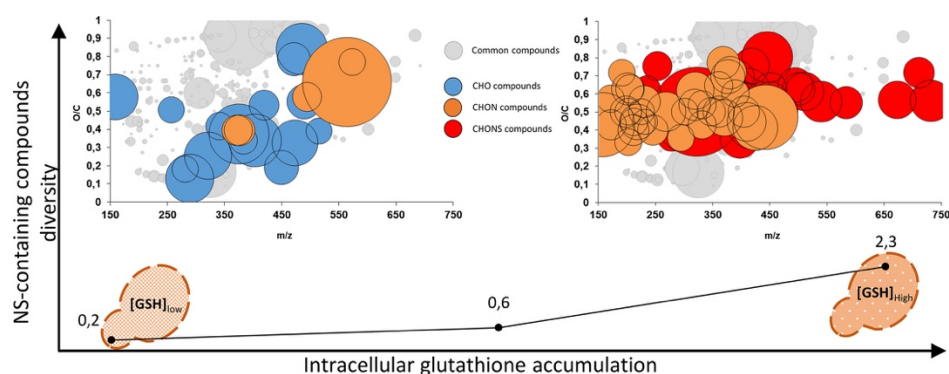
## Abstract:

Glutathione-rich inactivated dry yeasts (GSH-IDY) are purported to accumulate glutathione intracellularly and then released into the must. Glutathione is beneficial for wine quality, but research has highlighted that GSH-IDYs have a synergic antioxidant effect similar to that of molecular GSH. Combination of negative mode ultra-high-resolution Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry ((-)FT-ICR-MS), ultra-high-performance liquid chromatography coupled to a Quadrupole-Time of Flight mass spectrometer (UHPLC-Q-ToF-MS) and HPLC/Diode Detector Array (DAD)-Fluorescence spectroscopy was applied to three inactivated dry yeasts soluble fractions, with increasing intracellular glutathione concentration, in order to explore the chemical diversity released in different synthetic media.

Using the mean of size exclusion chromatography/DAD and fluorescence detection we report that most of the signals detected were below the 5–75 kDa-calibrated region of the chromatogram, indicating that most of the soluble protein fraction is composed of low molecular weight soluble peptides. In light of these results, high-resolution mass spectrometry was used to scan and annotate the low molecular weight compounds from 50 to 1500 Da and showed that GSH level of enrichment in IDYs was correlated to a discriminant chemical diversity of the corresponding soluble fractions. Our results clearly show an impact of the GSH accumulation process not only visible on the glutathione itself, but also on the global diversity of compounds.

Within the 1674 ions detected by (-)FT-ICR-MS, the ratio of annotated elemental formulas containing carbon, hydrogen, oxygen, nitrogen and sulfur (CHONS) to annotated elemental formulas containing carbon, hydrogen, oxygen (CHO) increased from 0.2 to 2.1 with the increasing levels of IDYs GSH content and 36 unique CHONS annotated formulas were unique to the IDY with the highest concentration of GSH. Amongst the 1674 detected ions 193 were annotated as potential peptides (from 2 to 5 residues), 61 ions were annotated as unique amino acid combinations and 46% of which being significantly more intense in GSH-rich IDY. Thus, the process leading to the accumulation of glutathione also involves other metabolic pathways which contribute to an increase in CHONS containing compounds potentially released in wine, notably peptides.

## Graphical abstract



## Keywords

Mass spectrometry; Wine; Oenology; Untargeted analysis

## Chemical compounds studied in this article

Glutathione (PubChem CID: 124886)  
 Leucyl-arginine (PubChem CID: 3800205)  
 Leucyl-lysine (PubChem CID: 14299197)  
 Adenosine (PubChem CID: 60961)  
 Glutamyl-cysteine (PubChem CID: 123938)  
 Pantothenic acid (PubChem CID: 6613)  
 Cysteinyl-glycine (PubChem CID: 439498)  
 Pipecolic acid (PubChem CID: 439227)  
 Homocitric acid (PubChem CID: 5460287)  
 Deoxy-methylthioadenosine (PubChem CID: 439176)

## Abbreviations

IDY - inactivated dry yeast  
 GSH - glutathione  
 MS - mass spectrometry  
 WSF - water soluble fraction  
 MWSF - model wine soluble fraction  
 MSF - methanol soluble fraction

GPC - gel permeation chromatography  
HPLC - high pressure liquid chromatography  
DAD - diode array detector  
LMW - low molecular weight  
UHPLC-Q-ToF-MS - Ultra-High-Performance Liquid chromatography triple quadrupole time of flight mass spectrometry  
RP-LC - reversed phase liquid chromatography  
(-)FT-ICR-MS - Negative mode Fourier-transform ion-cyclotron-resonance mass spectrometry  
S/N - Signal/Noise  
kDa - kilo Dalton  
MAAC - Multiple Amino Acid Combination  
UAAC - Unique Amino Acid Combination  
CHO - Carbon-Hydrogen-Oxygen  
CHON - Carbon-Hydrogen-Oxygen-Nitrogen  
CHONS - Carbon-Hydrogen-Oxygen-Nitrogen-Sulfur

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