Quantifying Glycosidically-Bound Sensory Precursors in Smoke-Exposed *Vitis vinifera* Berries

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Abstract

Volatile phenolic compounds (*e.g.*, guaiacol) and their sugar-bound glycosides are known to negatively influence the sensory attributes of wine made from smoke-exposed *Vitis vinifera* (*V. vinifera L*) berries and have been correlated with unpleasant 'smoky', 'ashy', 'burnt meat' and 'Band-Aid' aromas (*i.e.,* smoke-taint). Understanding this phenomenon is paramount, given that much of the North American wine grape crop is produced near forest fire-prone regions. To date, most volatile phenolic glycosides have been putatively assigned in the absence of direct empirical evidence. While these glycosides are not sensory-active, they are metabolised during fermentation to release volatile phenolic compounds that have distinct sensory descriptors. Therefore, phenolic glycosides constitute a 'sensory potential' that can negatively impact the flavour and aroma of wine following fermentation. It follows then, that the lack of definitive glycoside characterization is problematic since it: a) hinders efforts to develop remedial and preventative strategies; b) precludes absolute quantitative assessment of the total pool of volatile phenolic compounds; and c) confounds any correlation between volatile phenolics and their potential impact on wines made from smoke-exposed berries. We have developed an accurate analytical workflow capable of quantifying phenolic glycosides in *V. vinifera* berries. The systematic approach employed enabled the identification of several issues with existing methods that limit their quantitative accuracy. These deficiencies were critical since they impacted the generation of meaningful risk-assessment strategies based on total volatile phenol load when using smoke-exposed berries. The adaptability of our proposed strategy will enable its use on other glycosylated metabolites integral to wine quality (*e.g.,* terpenoids or norisoprenoids).

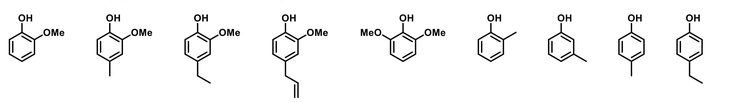
Background



Good ⟵ ⟶ Bad

- Early prediction of crop issues is key to mitigating risk
- ***Can we correlate the chemical composition of smoke-exposed V. vinifera berries to the development of perceptible smoke-taint in wine, without the need for small-batch fermentation?***

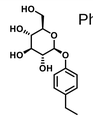
4-Methylguaiacol **Eugenol** ***o*-Cresol** ***p*-Cresol**



Guaiacol **4-Ethylguaiacol** **Syringol** ***m*-Cresol** **4-Ethylphenol**




- Volatile phenolic compounds (VPs) often possess negative organoleptic attributes in smoke-tainted wine
- VPs are only 50-80%[a] predictive of perceptible smoke-taint

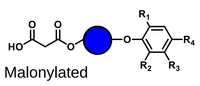


Phenolic Glycoside (odorless)

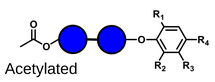
Glucose, Xylose, Apiose, Rhamnose, Arabinose, Galacturonic Acid, Glucuronic Acid

Sensory Potential

4-Ethylphenol → Band-Aid (aroma active)



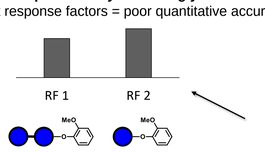
Malonylated



Acetylated

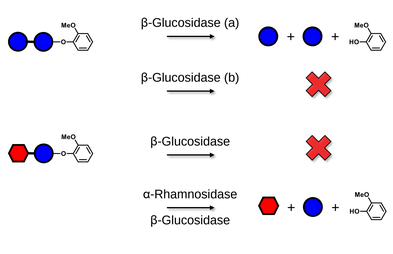
- 'Sensory potential' will determine the total VP load available for release during fermentation
- The glycosidically-bound forms are often present at 2-8x higher concentrations [b] than the free VPs
- The number of potential glycosides makes their direct detection analytically challenging

Mass-spectrometry of intact glycosides
(different response factors = poor quantitative accuracy)



RF 1 RF 2

Enzymatic hydrolysis
(too many enzyme combinations, modified sugars may not hydrolyze)

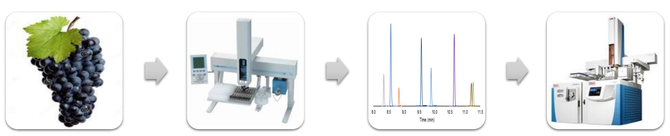


Acid-catalyzed hydrolysis

- The number of glycosidic combinations and the presence of modified sugars makes the use of enzymes or direct analysis via mass-spectrometry challenging, especially if quantitative accuracy is paramount
- Acid-catalyzed hydrolysis is a non-specific hydrolytic approach that should capture all of the potential glycosides and convert them to free VPs, where accurate quantitation is possible

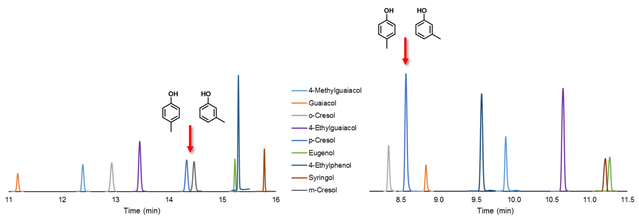
Methods

- Gas chromatography-tandem mass spectrometry (GC-MS/MS) was utilized for separation and quantitation of the free VPs



Preparation → Injection → Separation → Detection

Gas Chromatography



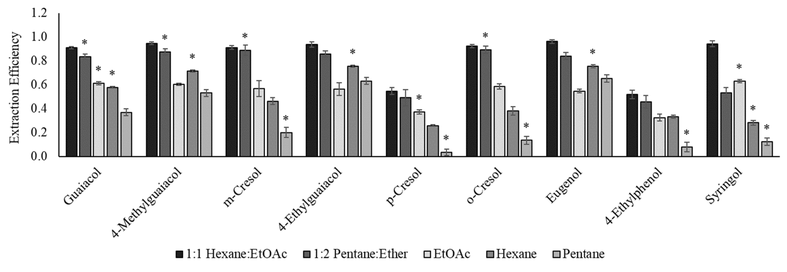
Column	WAX
Length	30 m
Coating	50 µm
ID	0.35 mm
Run Time	12.5 min
Max. Temp	250 °C

Column	DB5
Length	60 m
Coating	50 µm
ID	0.25 mm
Run Time	12.0 min
Max. Temp	300 °C

Sample Preparation

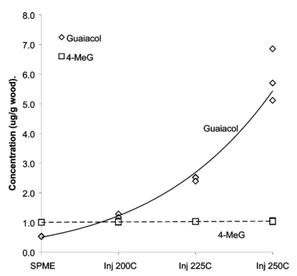
Extraction Solvent	$NaCl_{Sat}$	Matrix	Inj. Temp. (°C)
1:1 Hexane:EtOAc [f]	Y	Berries	240
2:1 Pentane:Ether [c]	-	Wine	200
EtOAc	Y	Berries	220
Hexane [e]	Y	Berries	250
Pentane [d]	-	Wine	200

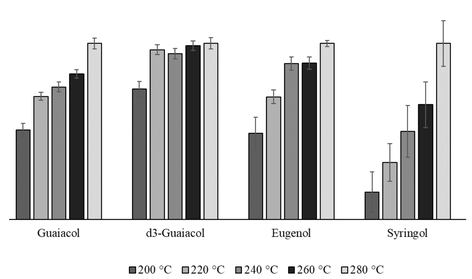
- A variety of liquid-liquid extraction conditions have been published, but not systematically compared
- Hypothesized differences in extraction efficiency would impact quantitation of VPs
- Five extraction conditions were evaluated (left) to identify optimal parameters



- 1:1 hexane:EtOAc with saturated NaCl yielded the highest absolute extraction efficiency for all compounds investigated
- Most compounds were recovered near 100%, expect for *p*-cresol and 4-ethylphenol
- ***Care should be used when comparing quantitative results from methods with different extraction parameters***

Injection Port Temperature

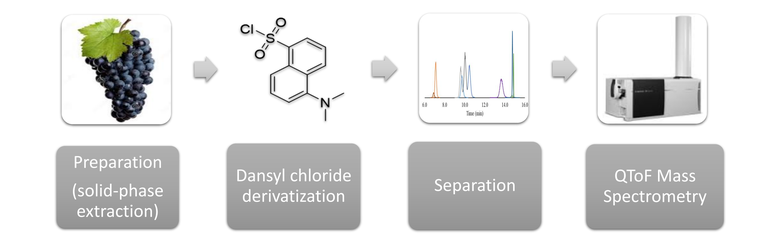


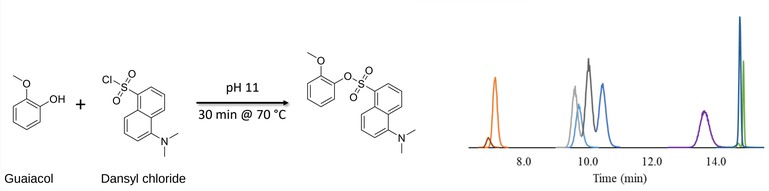


- Pollnitz, *et al* reported increased guaiacol response in wine as a function of injection port temperature [g] (top left)
- Attributed to thermal decomposition of co-extracted lignin
- Berry extracts show similar effect for guaiacol, but not d_3-guaiacol
- Broad panel of volatile phenolics identified similar issues with eugenol and, more prominently, for syringol
- ***Existing methods use injection port temperatures that will yield inaccurately high concentrations for these compounds***
- 220 °C selected as the optimal injection port temperature

Novel Analytical Method

- Despite evaluation, injection port issue raised concerns regarding the quantitative accuracy of our GC-MS/MS method
- Orthogonal method based on ultra-high pressure liquid chromatography-quadrupole time-of-flight mass spectrometry (uHPLC-QToF) was ***developed to cross-validate the quantitative accuracy of the GC-MS/MS method***



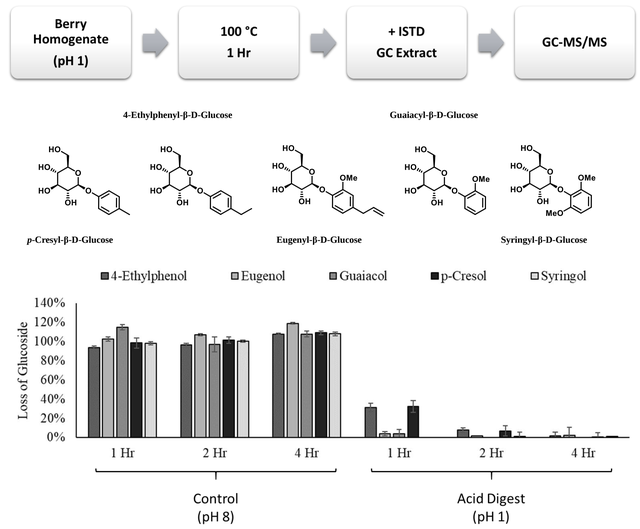


Method Validation

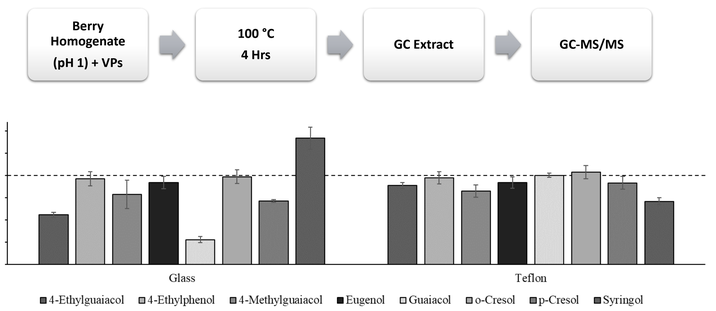
Compound	GC-MS/MS LOQ (ng/g)	GC-MS/MS Accuracy (2/100 ng/g)	GC-MS/MS Repeatability (2/100 ng/g)	uHPLC-QToF LOQ (ng/g)	uHPLC-QToF Accuracy (5/200 ng/g)	uHPLC-QToF Repeatability (5/200 ng/g)	HOR_{RAT}
4-Ethylguaiacol	1.62	97/85	8/9	2.72	105/107	14/1	-
4-Ethylphenol	0.55	95/82	5/5	3.39	90/100	17/2	-
4-Methylguaiacol	0.49	90/75	6/3	1.22	98/111	9/4	-
Eugenol	1.05	113/106	13/3	1.22	112/94	6/5	1.5
Guaiacol	1.67	93/90	8/7	1.06	110/111	9/1	0.6
m-Cresol	-			0.40	89/100	5/4	-
o-Cresol	0.85	86/67	14/2	0.83	90/106	10/6	1.2
p-Cresol	0.77	76/82	6/3	0.94	92/114	10/3	-
Syringol	0.84	126/99	6/4	2.64	90/87	13/3	-

- Both methods are suitably sensitive, precise and accurate for the analysis of smoke-exposed berries
- uHPLC-QToF method would be more sensitive if using a triple quadrupole detector
- ***Cross-validation using Horwitz ratios (HOR_{RAT}) demonstrated the quantitative equivalence of both methods and, by extension, the quantitative accuracy of the developed GC-MS/MS method (with optimized extraction and injection port parameters)***

Acid Hydrolysis



- Model glucosides were synthesized to facilitate development of a method that yields quantitative recovery of free volatile phenolic compounds following acid-catalyzed hydrolysis
- ***Current methods use 1 Hr @ 100 °C, which is insufficient to hydrolyze the model glucosides (4 Hrs is required)***



- Existing methods do not specify reaction vessels for acid digests
- ***Our data show that quantitative recovery of liberated volatile phenols is not possible in glass vials, while switching to Teflon vials lead to near quantitative yields***
- Cause of this effect is unclear, although our data suggest it is a function of non-specific surface interactions under the harsh hydrolytic conditions employed

Summary and Future Works

- Predicting the presence of smoke-taint in wine based on the chemical composition of smoke-exposed berries will require a quantitatively accurate assessment of the total volatile phenol load in the berry
- Existing methods should be carefully evaluated regarding their extraction parameters and, in the case of GC-MS/MS, their injection port temperatures
- Independent and cross-validation of the developed GC-MS/MS method demonstrated quantitative accuracy
- Acid-catalyzed hydrolysis required 4 hours to liberate bound glycosides and needed to be carried out in teflon vials to ensure quantitative recovery of volatile phenolic compounds

- The developed quantitative work-flows will be applied to the analysis of field trials from 2016
- Build model for predicting sensory properties of wine based on VP composition

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