

Glycosidically-Bound Volatile Phenols Linked to Smoke-Taint: Stability During Fermentation with Different Yeasts and in Finished Wine

Brandon Whitmore^{1,•}, Stephanie McCann¹, Matthew Noestheden^{2,▲}, Eric G Dennis², Sarah M Lyons^{1,♦}, Daniel M Durall¹, and Wesley F Zandberg^{2,*}

¹ The University of British Columbia, Department of Biology, 1177 Research Road, Kelowna, BC, Canada

² The University of British Columbia, Department of Chemistry, 3247 University Way, Kelowna, BC, Canada
Current addresses:

▲ SCIEX, 71 Four Valley Dr., Concord, ON, Canada, L4K 4V8

• University of Calgary, Cumming School of Medicine, 3330 Hospital Dr. NW, Calgary, AB, Canada, T2N 4N1

♦ Supra Research and Development, 2293 Leckie Road, Kelowna, BC, Canada, V1X 6Y5

* Correspondence: wesley.zandberg@ubc.ca ; Tel.: +1-250-807-9821

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Figure S1. Spontaneous hydrolysis of model VP-glycosides in Cabernet Franc wine and model wine matrices. All air/argon samples ($n = 3$) were aliquoted (2 mL) into 4 mL borosilicate glass vials with polyvinyl-faced pulp lined caps and analyzed at the start of the experiment ($t = 0$ weeks), after three months ($t = 12$ weeks). * includes three separate 200 ng / mL fortifications as follows: 1) all β -D-glucopyranosides; 2) guaiacyl-primeveroside; 3) guaiacyl-gentiobioside and syringyl-gentiobioside.

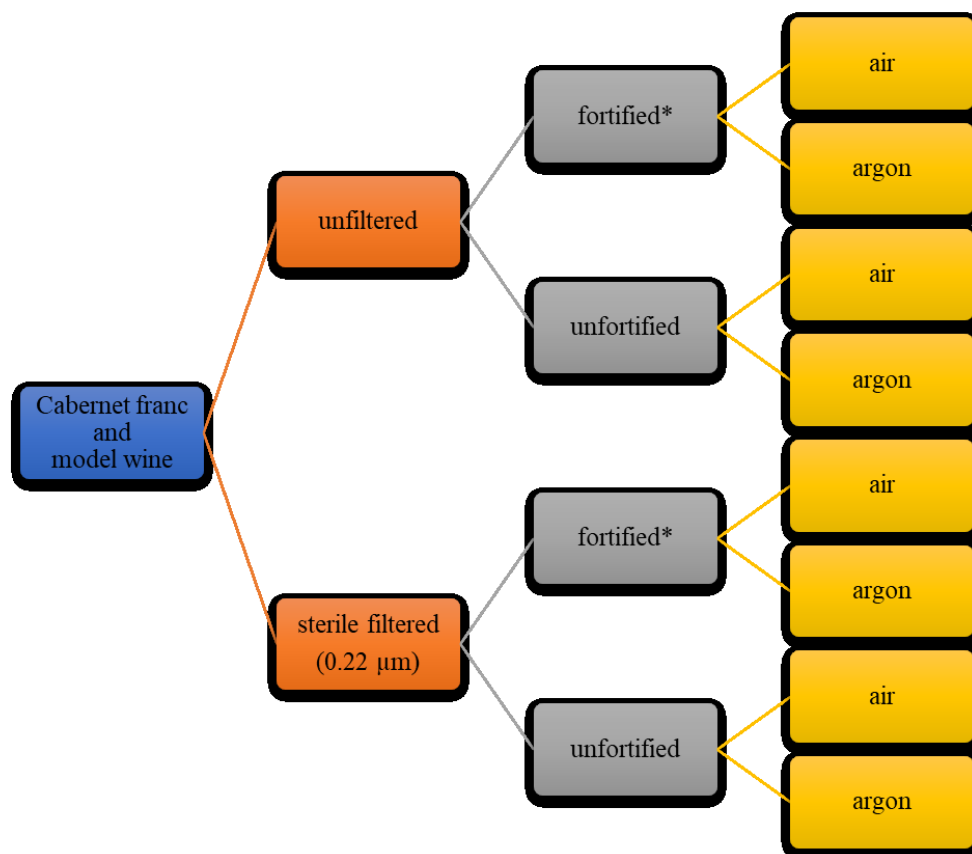


Table S1. Model wine composition. This mixture was used to validate the analysis of VPs in wine by GC-MS/MS, as it was free from incurred residues.

component	composition
ethanol	12 % (v/v)
tartaric acid	5.0 g/L
malic acid	3.5 g/L
acetic acid	0.60 g/L
glucose	2.0 g/L
fructose	2.0 g/L
NaCl	0.20 g/L
KH ₂ PO ₄	2.0 g/L
MgSO ₄	0.40 g/L

Table S2. Model VP-glycosides used to fortify ferments with different strains of *S. cerevisiae*.

compound	RT (min)	formula	exact mass (Da)
<i>d</i> ₃ -guaiacyl-β-D-glucopyranoside	7.4	C ₁₃ H ₁₅ D ₃ O ₇	289.1241
guaiacyl-β-D-glucopyranoside	7.5	C ₁₃ H ₁₈ O ₇	286.1053
guaiacyl-β-D-gentiobioside	7.6	C ₁₉ H ₂₈ O ₁₂	448.1581
<i>p</i> -cresyl-β-D-glucopyranoside	8.8	C ₁₃ H ₁₈ O ₆	270.1103
syringyl-β-D-gentiobioside	9.1	C ₂₀ H ₃₀ O ₁₃	478.1686
guaiacyl-β-D-primerveroside	9.7	C ₁₈ H ₂₆ O ₁₁	418.1475
syringyl-β-D-glucopyranoside	10.1	C ₁₄ H ₂₀ O ₈	316.1158
4-methylguaiacyl-β-D-glucopyranoside	11.4	C ₁₄ H ₂₀ O ₇	300.1209
4-ethylphenyl-β-D-glucopyranoside	12.3	C ₁₃ H ₁₈ O ₆	270.1103
eugenyl-β-D-glucopyranoside	13.1	C ₁₆ H ₂₂ O ₇	326.1366

Table S3. Recovery of free VPs from commercial Cabernet franc wine (pH 3.4) fortified with VP-glycosides (200 ng/g) after 12 weeks of spontaneous hydrolysis.

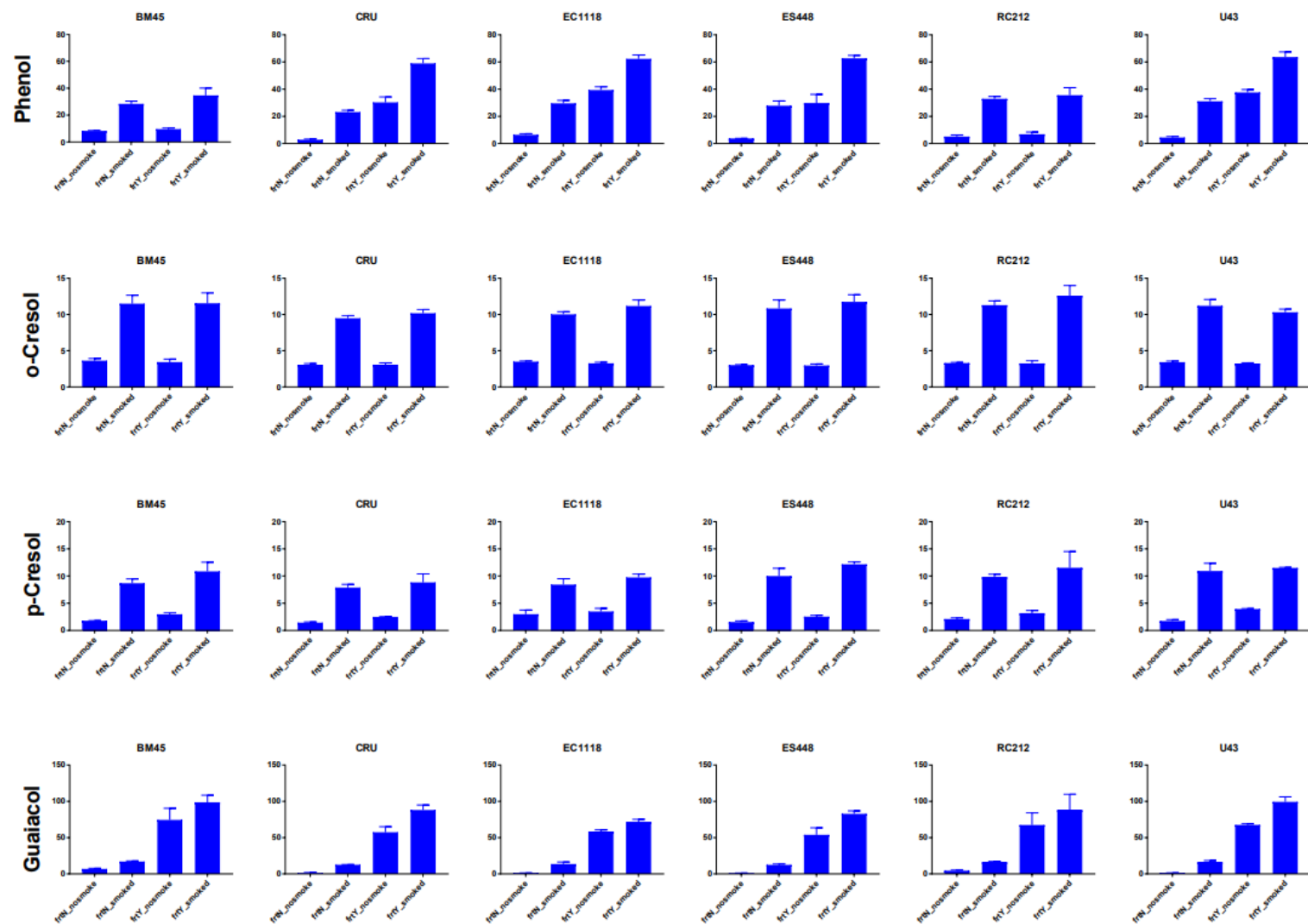
VP-glycoside	treatment		concentration (ng/ g) ^(1,2,3)							
	atmosphere	filtered	4-MG ⁽⁴⁾	Gua	Phe	<i>p</i> -Cre	<i>o</i> -Cre	4-EP	Syr	Eug
none	air	N	1.1 ± 0.05	2.0 ± 0.20	-	-	-	-	1.6 ± 0.31	5.5 ± 0.26
			1.9 ± 0.25	4.1 ± 0.87	2.1	1.6 ± 0.29	2.1 ± 0.10	1.3 ± 0.16	-	10.0 ± 1.23
	air	Y	1.4	2.6 ± 0.18	3.2 ± 0.78	1.2 ± 0.10	2.6 ± 0.20	1.1 ± 0.01	-	7.2 ± 0.19
			1.6 ± 0.06	5.9 ± 0.32	1.7 ± 0.42	1.4 ± 0.12	1.8 ± 0.39	1.7 ± 0.14	6.3 ± 0.70	8.0 ± 0.15
	argon	N	1.2	5.8 ± 1.66	-	1.8 ± 0.05	-	-	65.9 ± 19.53	6.2 ± 0.25
			1.2 ± 0.03	9.4 ± 0.43	2.6 ± 0.22	2.17 ± 0.07	-	3.1 ± 0.22	159 ± 20	7.8 ± 0.58
4-MG-Glc Gua-Glc Phe-Glc <i>p</i> -Cre-Glc <i>o</i> -Cre-Glc 4-EP-Glc Syr-Glc Eug-Glc	air	N	1.8 ± 0.85	6.0 ± 0.52	-	5.0 ± 0.28	1.7 ± 0.01	15.8 ± 0.85	2.7 ± 0.37	15.1 ± 0.54
			1.7 ± 0.04	6.8 ± 0.00	-	28.7 ± 0.09	1.2 ± 0.11	33.0 ± 0.43	6.7 ± 0.05	16.0 ± 0.53
	air	Y	1.5 ± 0.03	5.5 ± 0.43	-	4.3 ± 0.14	-	13.3 ± 0.08	2.4 ± 0.51	12.6 ± 0.30
			2.5 ± 0.12	11.3 ± 0.69	2.9 ± 0.60	55.7 ± 5.79	1.0 ± 0.00	56.5 ± 4.38	14.7 ± 1.53	24.0 ± 1.13
	argon	N	1.3 ± 0.06	11.1 ± 0.31	3.3 ± 0.19	5.0 ± 0.23	-	16.7 ± 0.52	129.1 ± 3.84	11.2 ± 0.85
			1.6 ± 0.04	12.3 ± 0.20	2.4 ± 0.20	38.2 ± 1.84	-	42.3 ± 1.70	186 ± 7.3	17.3 ± 1.13
Gua-Gent	air	N	1.1 ± 0.04	8.2 ± 0.45	1.8 ± 0.34	5.0 ± 0.34	-	15.8 ± 0.59	63.3 ± 5.11	8.6 ± 0.41
			1.7 ± 0.22	14.8 ± 1.22	3.8 ± 0.96	44.6 ± 10.6	1.2 ± 0.09	49.9 ± 9.41	215 ± 5.6	21.4 ± 2.90
	air	Y	1.4 ± 0.05	3.7 ± 0.31	-	1.2	1.5 ± 0.03	1.0 ± 0.02	-	7.3 ± 0.18
			1.1 ± 0.06	3.8 ± 0.38	-	-	1.3 ± 0.07	-	3.3 ± 0.58	6.1 ± 0.30
	air	Y	1.4 ± 0.04	3.6 ± 0.37	-	1.3 ± 0.10	2.1 ± 0.02	1.0 ± 0.01	-	6.9 ± 0.27
			1.3 ± 0.13	6.6 ± 1.23	-	1.4 ± 0.15	1.5 ± 0.38	1.8 ± 0.15	8.8 ± 2.35	6.9 ± 0.74
Gua-Prim	air	N	1.5 ± 0.09	4.4 ± 0.54	-	1.3 ± 0.10	1.6	1.2 ± 0.10	-	7.7 ± 0.42
			1.2 ± 0.06	3.7 ± 0.27	-	-	-	1.1 ± 0.07	4.1 ± 0.42	6.1 ± 0.20
	air	Y	1.1 ± 0.03	2.9 ± 0.19	-	-	-	-	-	5.7 ± 0.15
			1.4 ± 0.14	5.8 ± 0.66	-	1.2 ± 0.15	1.5 ± 0.27	1.4 ± 0.09	7.4 ± 0.56	6.7 ± 0.55
	air	N	1.5 ± 0.07	4.6 ± 0.19	-	1.3	1.6 ± 0.08	1.5 ± 0.16	1.6	8.5 ± 0.37
			1.2 ± 0.04	3.9 ± 0.62	-	1.1 ± 0.01	1.4 ± 0.09	1.5 ± 0.07	4.1 ± 0.80	6.7 ± 0.62
Syr-Gent	air	Y	1.2 ± 0.16	4.9 ± 0.67	1.1	1.3 ± 0.08	1.1	1.6 ± 0.30	1.6 ± 0.40	7.7 ± 0.98
			1.4 ± 0.22	5.5 ± 0.96	1.8	1.3 ± 0.17	-	1.7 ± 0.27	8.0 ± 0.94	7.7 ± 0.78

Notes: (1) Fortified Cabernet franc wines were stored under an atmosphere of air or argon gas. When indicated, samples were filtered (Y) through a 0.22 µm filter prior to storage; untreated samples are denoted with a (N). (2) Data are provided as mean VP concentrations (in ng/g) recovered from each sample ± the standard error of the mean (SEM). For each sample, volatile phenol levels were determined after zero (top, shaded cells) or 12 weeks (bottom, unshaded cells) of storage. (3) Pairs of values in bold represent a statistically significant difference (two-tailed Welch's test of unequal variance, $n = 3/\text{condition}$, $\alpha = 0.05$) between samples analyzed at the two different time points for each condition. (4) The following abbreviations are used: Glc = β -D-glucopyranoside, Gent = gentiobioside, and Prim = primeveroside; 4-EP = 4-ethylphenol, 4-MG = 4-methylguaiacol, Eug = eugenol, Gua = guaiacol, *o*-Cre = *o*-cresol, *p*-Cre = *p*-cresol, Phe = phenol, and Syr = syringol.

Table S4. Change in total soluble solids (°Bx) as a metric of fermentation progress (days).

strain	treatment	control						smoked					
	fortified	yes			no			yes			no		
	replicate	1	2	3	1	2	3	1	2	3	1	2	3
EC1118	0	21.7	22.2	22.1	21.9	21.3	22.2	22.7	23.1	23.2	22.9	23.4	21.7
	1	22.9	22.2	22.0	21.9	21.6	22.4	22.7	21.4	23.1	22.9	23.3	21.9
	2	20.7	18.5	21.4	19.2	18.7	19.4	17.6	16.6	18.9	19.3	20.7	18.9
	3	18.8	16.1	19.3	17.4	16.9	17.0	15.0	14.2	16.9	16.3	17.5	15.9
	4	14.5	13.1	16.1	13.7	13.4	13.7	11.3	10.8	12.3	12.6	14.4	12.2
	5	10.4	9.0	10.9	9.9	6.4	9.8	7.1	7.6	7.6	8.1	8.5	5.8
	6	6.8	5.9	6.5	7.8	5.9	7.1	6.7	6.2	7.4	7.4	8.2	7.1
	7	5.7	6.6	5.6	7.2	6.8	6.5	6.2	7.4	4.9	7.3	6.1	6.5
	8	6.9	7.9	6.0	6.1	5.5	6.9	5.9	6.3	6.2	5.8	6.5	5.9
	9	4.8	5.4	7.0	6.7	6.5	6.6	6.9	6.8	5.0	6.7	4.9	7.1
RC212	0	21.4	21.2	21.5	20.2	20.4	21.7	17.4	18.3	19.4	18.4	18.4	18.0
	1	16.2	15.4	16.6	17.7	16.1	17.0	12.2	16.1	15.3	12.7	14.3	16.0
	2	12.1	13.2	14.3	13.4	14.8	13.7	10.1	10.2	11.0	12.4	13.6	10.6
	3	9.2	11.4	10.0	7.9	6.2	9.9	9.0	8.0	7.1	8.0	7.3	6.7
	4	8.9	8.6	6.6	8.5	7.9	8.6	6.2	6.8	5.5	6.6	5.9	5.8
	5	7.3	6.5	5.4	7.2	6.2	7.2	5.5	5.7	5.3	4.8	5.8	5.5
	6	5.3	5.5	6.7	6.2	5.3	6.0	5.2	5.3	5.6	5.6	5.2	5.2
	7	-	6.0	5.1	5.7	5.2	6.0	4.2	3.3	5.8	5.0	3.9	5.9
BM45	0	20.4	22.0	19.1	20.3	21.4	20.6	19.6	19.2	20.1	19.4	19.7	19.8
	1	17.6	17.3	17.1	17.0	17.2	16.0	17.2	16.0	18.2	16.4	14.9	18.5
	2	10.4	9.2	11.4	11.9	11.4	12.6	15.7	11.3	13.0	12.3	10.8	-
	3	8.5	7.4	7.7	9.3	8.3	8.4	9.6	9.8	8.3	9.9	9.2	9.9
	4	7.3	7.0	5.9	6.3	6.9	5.8	9.4	9.3	9.7	8.4	9.6	9.8
	5	5.8	6.2	6.0	5.9	5.5	6.0	6.8	7.0	6.8	6.3	8.4	6.7
	6	6.3	7.1	6.0	6.0	6.4	5.6	6.4	5.7	5.3	6.2	6.7	6.1
	7	5.4	3.3	5.9	4.5	7.0	5.5	6.2	5.2	6.3	5.3	5.2	5.4
U43	0												
	1												
	2	18.1	18.7	17.4	18.4	18.2	16.9	21.6	23.4	22.7	23.3	21.6	19.4
	3	16.1	20.7	17.7	17.2	15.4	14.9	19.5	18.7	18.5	22.6	17.5	17.5
	4	11.7	14.9	9.3	13.1	13.2	10.6	14.2	14.6	12.9	13.8	13.1	11.3
	5	8.2	6.4	8.9	9.0	9.2	6.8	6.7	8.4	9.0	6.8	9.7	6.6
	6	5.2	6.5	6.0	7.3	7.8	4.9	7.9	8.0	7.9	5.1	6.2	5.8
	7	4.4	6.4	3.7	4.0	5.9	5.9	2.5	6.0	5.7	4.3	6.7	5.4
	8	2.7	7.0	3.6	5.4	6.3	3.7	5.5	6.7	6.8	5.3	6.3	4.3

strain	treatment		control					smoked					
	fortified replicate	yes			no			yes			no		
		1	2	3	1	2	3	1	2	3	1	2	3
ES488	0												
	1												
	2	17.1	17.4	16.3	17.6	17.6	17.7	19.2	18.3	20.4	19.7	19.4	20.6
	3	16.7	17.9	16.5	14.8	16.5	19.2	19.7	17.7	20.4	18.3	19.1	21.2
	4	13.0	15.7	13.9	17.5	18.2	12.3	20.1	17.2	18.0	13.3	18.1	17.5
	5	10.0	13.2	11.3	14.7	11.2	9.7	15.4	9.5	11.4	16.3	13.4	10.8
	6	10.0	13.0	12.4	10.2	11.7	11.2	10.7	7.9	11.9	13.0	13.6	10.4
	7	9.6	9.5	8.5	6.5	6.6	8.9	8.7	7.8	7.0	8.8	7.4	8.0
	8	4.1	4.8	7.8	9.8	8.0	7.0	4.9	6.2	7.2	6.2	6.2	3.8
Cru	0												
	1												
	2	17.1	16.2	15.5	16.1	17.4	16.1	18.2	19.8	19.6	19.4	19.4	18.7
	3	16.6	15.2	13.6	16.0	14.3	14.6	13.7	19.9	17.1	20.7	20.6	18.3
	4	13.3	10.2	6.7	15.6	15.6	7.9	10.8	9.0	9.6	11.4	8.1	15.4
	5	11.6	11.2	8.7	6.9	10.6	10.2	5.5	8.9	6.7	11.8	10.6	10.5
	6	10.4	7.6	4.1	9.1	10.6	7.2	4.4	4.9	6.7	5.8	9.7	5.9
	7	5.4	8.9	7.3	5.3	8.0	7.9	5.1	3.5	3.4	7.8	5.2	6.0
	8	3.1	3.3	8.1	7.9	7.4	4.0	5.8	3.0	7.1	5.9	6.0	4.3



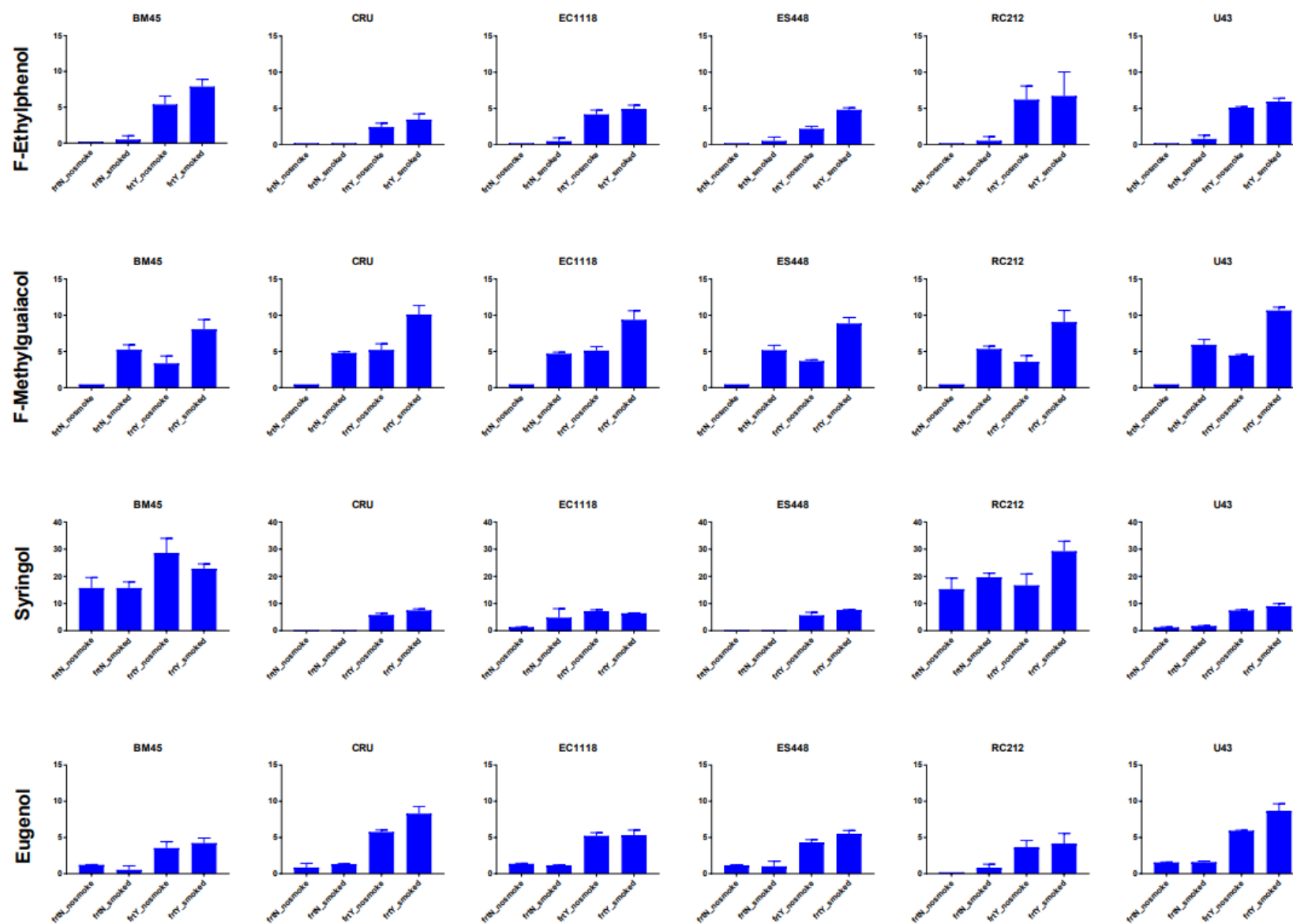


Figure S2. Comparison of the concentration (ng/g) of VPs released by different yeast strains after primary fermentation. Note that not every VP has the same *y*-axis scale. Error bars indicate the standard errors of the mean of $n = 3$ replicate fermentations. frtN and frtY indicate control or VP-glycoside-fortified samples, respectively, while “nosmoke” and “smoke” indicate Pinot Noir grapes were not or were exposed to simulated forest fire smoke while ripening.