Supplemental Data for:

Englezos V, Di Gianvito P, Peyer L, Giacosa S, Río Segade S, Edwards N, Rolle L, Rantsiou K and Cocolin L. Bioprotective effect of *Pichia kluyveri* and *Lactiplantibacillus plantarum* in winemaking conditions. Am J Enol Vitic 73:294-307.
doi: 10.5344/ajev.2022.22008.

Supplemental Table 1 Chemical parameters of wines produced by the different inoculation protocols in 2020.

Metabolites[a]	Control1	Control2	AFPK	AFLP	Sig[b]
Residual sugars (g/L)	<2.0	<2.0	<2.0	<2.0	-
Acetic acid (g/L)	0.19 ± 0.01 c[c]	0.24 ± 0.01 d	0.11 ± 0.02 b	0.07 ± 0.01 a	***
Glycerol (g/L)	11.52 ± 0.03 b	10.77 ± 0.27 a	12.07 ± 0.02 c	11.72 ± 0.17 b	***
Ethanol (% v/v)	13.55 ± 0.02 a	13.86 ± 0.01 b	13.84 ± 0.01 b	13.83 ± 0.03 b	***
L-Malic acid (g/L)	3.02 ± 0.09 b	3.05 ± 0.12 b	2.97 ± 0.43 b	2.10 ± 0.11 a	***
D-Lactic acid (g/L)	0.29 ± 0.04 b	0.39 ± 0.02 b	0.25 ± 0.06 b	0.13 ± 0.04 a	***
L-Lactic acid (g/L)	0.05 ± 0.04 a	0.09 ± 0.02 a	0.11 ± 0.06 a	1.05 ± 0.04 b	***
Glycerol yield[d]	0.049 ± 0.001 b	0.046 ± 0.001 a	0.051 ± 0.001 c	0.05 ± 0.001 b	***
Ethanol yield[e]	0.057 ± 0.002 a	0.059 ± 0.002 b	0.059 ± 0.001 b	0.059 ± 0.001 b	***
pH	3.47 ± 0.01 e	3.36 ± 0.02 a	3.41 ± 0.02 b	3.46 ± 0.02 b	***
Total acidity (g/L as tartaric acid)	7.16 ± 0.17 a	8.15 ± 0.15 b	7.36 ± 0.09 a	7.02 ± 0.02 a	***
Total anthocyanins (mg/L)[f]	59.0 ± 5.0	56.6 ± 1.8	57.8 ± 2.0	59.8 ± 1.1	NS
Total flavonoids (mg/L)[g]	643 ± 45	719 ± 16	652 ± 24	659 ± 12	NS
Non-anthocyanin flavonoids (mg/L)	557 ± 38 a	637 ± 14 b	567 ± 21 a	571 ± 11 a	*
L*	65.95 ± 0.10 b	64.91 ± 0.65 a	64.32 ± 0.50 a	64.81 ± 0.29 a	***
a*	41.54 ± 0.32 a	44.31 ± 0.24 c	43.60 ± 0.13 b	44.29 ± 0.44 c	***
b*	20.87 ± 0.12 a	22.70 ± 0.02 b	21.13 ± 0.16 a	21.15 ± 0.11 a	***
Color intensity (a.u., 10 mm OP[h])	1.57 ± 0.01 a	1.66 ± 0.03 b	1.66 ± 0.03 b	1.64 ± 0.02 b	***
Color hue	0.80 ± 0.02 b	0.77 ± 0.01 b	0.77 ± 0.02 b	0.75 ± 0.01 a	***
Butanol	10.61 ± 2.5	blq[i]	blq	blq	NS
Hexanol#[j]	1725.25 ± 64.87 bc	1578.21 ± 67.87 bc	1212.53 ± 50.96 a	1827.41 ± 16.26 c	***
Isoamyl alcohol#	250712.18 ± 6638.55 c	6529.74 ± 3086.02 a	75029.04 ± 21162.87 b	74376.95 ± 6119.94 b	***
Isobutanol	721.44 ± 96.06 c	297.92 ± 36.92 ab	326.35 ± 83.45 ab	368.28 ± 16.22 ab	***
Octanol#	4.51 ± 0.32 c	3.7 ± 0.4 b	2.42 ± 0.13 a	3 ± 0.11 a	***
3-ethoxypropanol	14.65 ± 1.23 ab	blq	10.79 ± 2.28 a	17.69 ± 0.69 c	***
1-propanol	60.95 ± 1.3 a	64.49 ± 12.12 a	90.69 ± 3.22 b	145.55 ± 27.99 c	***
2-phenylethanol#	3653.08 ± 8986.43 a	96266.39 ± 11810.16 d	39566.73 ± 6789.78 b	53018.91 ± 756.81 c	***
2-ethylhexanol	27.67 ± 8.49 b	14.81 ± 2.48 a	14.98 ± 4.67 a	23.77 ± 2.02 ab	**
(E)-3-hexenol#	25.11 ± 4.18 a	32.98 ± 8.44 b	blq	blq	***
Diethyl succinate#	52.22 ± 3.83 b	110.07 ± 11.37 c	57.75 ± 5.65 b	33.51 ± 2.13a	***
Ethyl acetate#	174446.57 ± 22014.86 c	29269.78 ± 11037.62 a	63089.78 ± 5282.99 b	54177.89 ± 2612.33 b	***
Ethyl butanoate#	160.54 ± 6.22 ab	194.31 ± 25.55 b	163.24 ± 28.44 ab	191.85 ± 3.44 b	***
Ethyl decanoate#	47.27 ± 13.3 ab	110.31 ± 23.36 c	63.75 ± 13.45 ab	43.49 ± 3.18 a	***
Ethyl dodecanoate#	24.62 ± 2.82 a	46.59 ± 2.04 c	37.93 ± 10.37 b	32.15 ± 2.86 ab	***
Ethyl heptanoate#	50.52 ± 12.29 c	21.12 ± 1.54 b	18.27 ± 0.2 ab	11.56 ± 0.85 ab	***
Ethyl hexanoate#	158.08 ± 8.66 abc	239.94 ± 18.63 d	179.96 ± 13.35 c	141.49 ± 0.75 a	***
Ethyl lactate#	blq	blq	blq	4163.19 ± 68.58 a	***
Ethyl nonanoate#	0.82 ± 0.09 b	0.75 ± 0.15 b	0.58 ± 0.1 bb	0.29 ± 0.01 a	***
Ethyl octanoate#	130.07 ± 11.9 a	239.74 ± 26.82 b	151.29 ± 19.54 a	125.54 ± 8.76 a	***
(E/Z) - ethyl 2-hexenoate	21.32 ± 3.06 bc	18.76 ± 1.52 b	23.32 ± 1.34 c	12.99 ± 0.8 a	***
(E/Z) - ethyl-4-hexenoate	blq	blq	blq	blq	***
Ethyl 9-decenoate	25.78 ± 2.08 b	26.14 ± 2.41 b	17.68 ± 1.67 a	18.63 ± 1.69 a	***
Hexyl acetate#	14.2 ± 0.85 a	10.51 ± 0.65 a	54.49 ± 7.65 b	12.34 ± 0.3 a	***
Isoamyl acetate#	873.44 ± 139.89 a	1256.76 ± 95.26 a	2341.26 ± 531.9 b	978.38 ± 18.84 a	***
Methyl octanoate#	blq	0.13 ± 0.01 a	blq	blq	***
2-phenylethyl acetate#	106.46 ± 18.22 a	242.77 ± 40.5 ab	916.08 ± 176.67 d	462.3 ± 206.09 c	***
3-methylbutyl octanoate	15.26 ± 1.62 a	63.95 ± 17.67 b	14.26 ± 3.92 a	blq	***
3-methylbutyl propanoate	19.21 ± 3.11 a	19 ± 1.65 a	19.04 ± 1.49 a	18.5 ± 0.9 a	***
Ho-trienol	7.98 ± 1.65 b	blq	5.16 ± 0.2 a	blq	***
(+/-)-β-citronellol	16.15 ± 0.64 a	15.67 ± 3.48 a	20.71 ± 1.79 b	20.88 ± 0.55 b	***
Linalool#	3.51 ± 0.11 b	2.91 ± 0.26 a	8.5 ± 0.22 c	6.9 ± 0.25 c	***
Hexanoic acid	29.4 ± 2.01 a	59.53 ± 6.6 c	41.67 ± 3.71 b	29.99 ± 2.15 a	***
Methionol#	2557.42 ± 189.83 b	6922.59 ± 611.4 c	1980.88 ± 92.23 a	2398.37 ± 126.05 ab	***
trans-b-damascenone	21.51 ± 2.08 ab	24.65 ± 4.94 b	21.2 ± 3.98 ab	17.41 ± 0.46 a	ns
(Z)-b-nerolidol	16.39 ± 3.43	15.9 ± 2.05	13.3 ± 3.24	14.15 ± 0.94	ns
(Z)-dihydrofarnesol	8.42 ± 1.72 a	17.72 ± 2.96 c	10.33 ± 2.11 b	8.45 ± 0.88 ab	***
2,3-dihydrobenzofuran	90.73 ± 20.51	93.43 ± 11.69	89.76 ± 12.84	78.29 ± 3.44	ns

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[a]Values are expressed as mean ± standard deviation of three independent replicates.
[b]*, ***, and NS indicate significance at $p < 0.05$, <0.001, and not significant, respectively. Sugars concentration before fermentation: 236 g/L (glucose 120 g/L, fructose 122 g/L). Values are expressed as mean ± standard deviation of three independent replicates.
[c]Different letters in the same column indicate significant difference at $p < 0.05$ (Tukey's honest significant difference test).
[d]Glycerol yield: glycerol production/sugar consumption.
[e]Ethanol yield: ethanol production/sugar consumption.
[f]mg/L as malvidin-3-*O*-glucoside chloride.
[g]mg/L as (+)-catechin.
[h]OP, optical path.
[i]blq, below quantitation limit.
[j]Concentrations of compounds identified with a # was calculated by a calibration with standard solutions analyzed under the same conditions as the wine samples. The other volatile compounds were semi-quantified in relation to a 1-heptanol internal standard.

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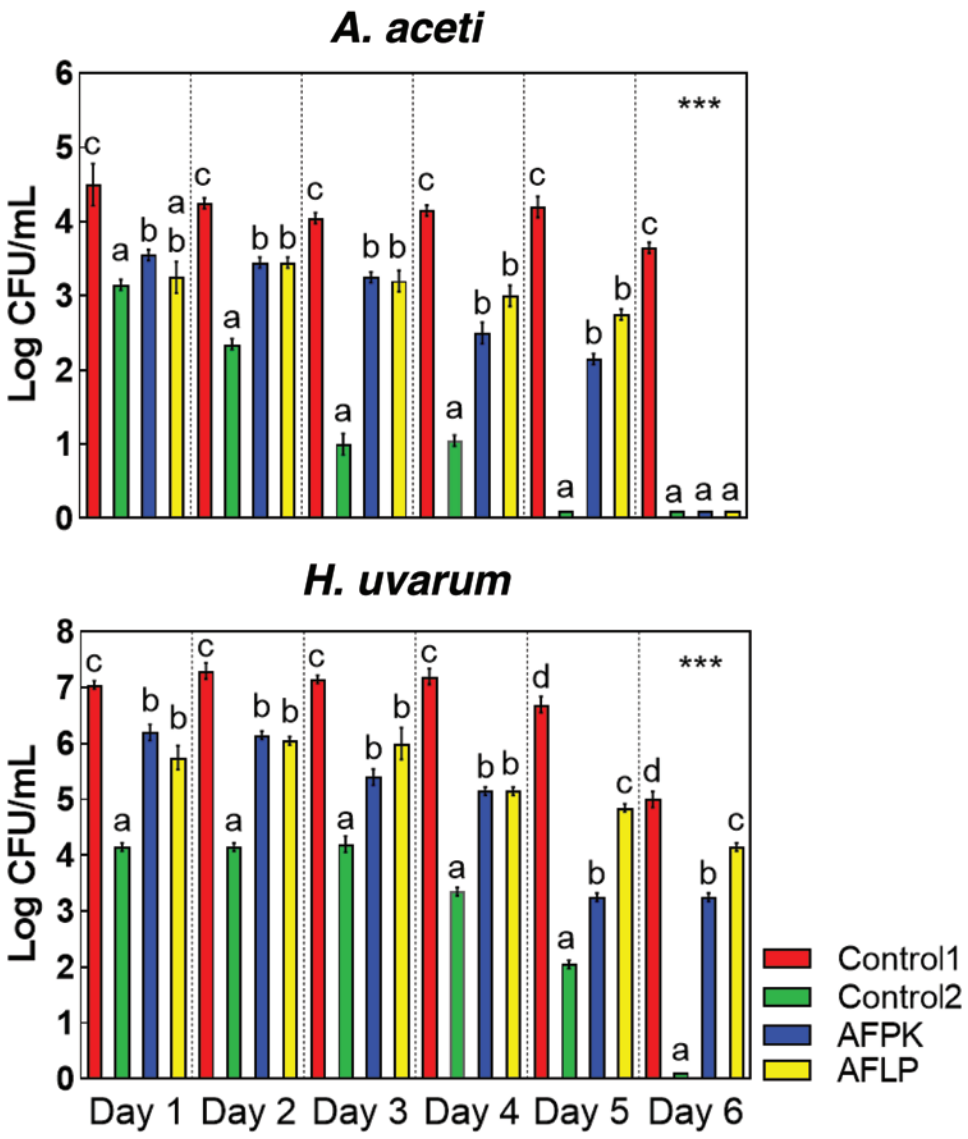

Supplemental Figure 1 Viable counts (log CFU/mL) of *Acetobacter aceti* and *Hanseniaspora uvarum* populations during fermentation (2019 vintage). Data are the means ± standard deviation of three independent experiments. Different letters in each column indicate significant differences according to analysis of variance and Tukey's honest significant difference test ($p < 0.001$).

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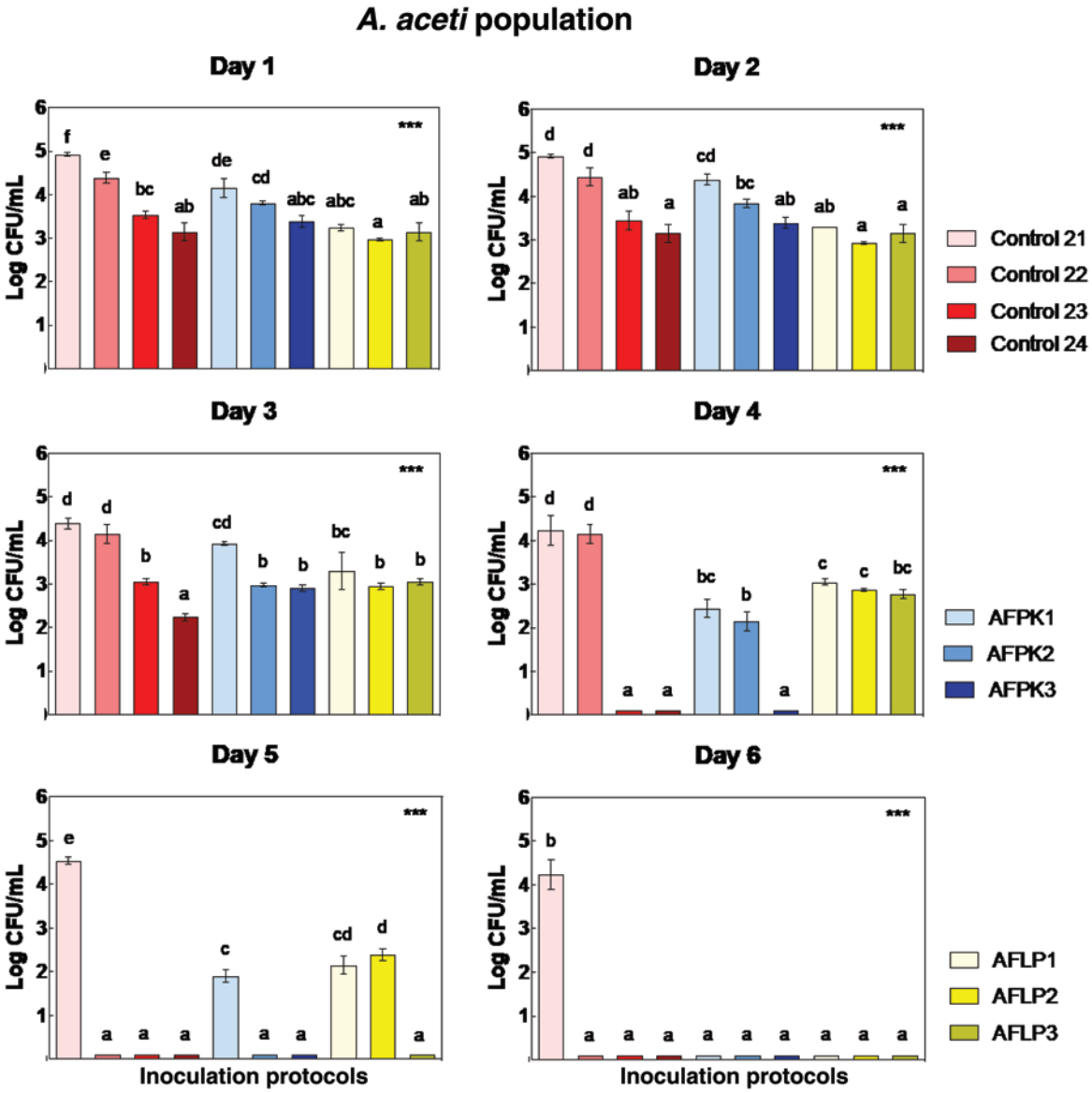

Supplemental Figure 2 Viable counts (log CFU/mL) of *Acetobacter aceti* populations during fermentation (2020 vintage). Inoculation protocols are indicated in the symbol key. Data are the means ± standard deviation of three independent biological experiments. Different letters in each column indicate significant differences according to analysis of variance and Tukey's honest significant difference test ($p < 0.001$).

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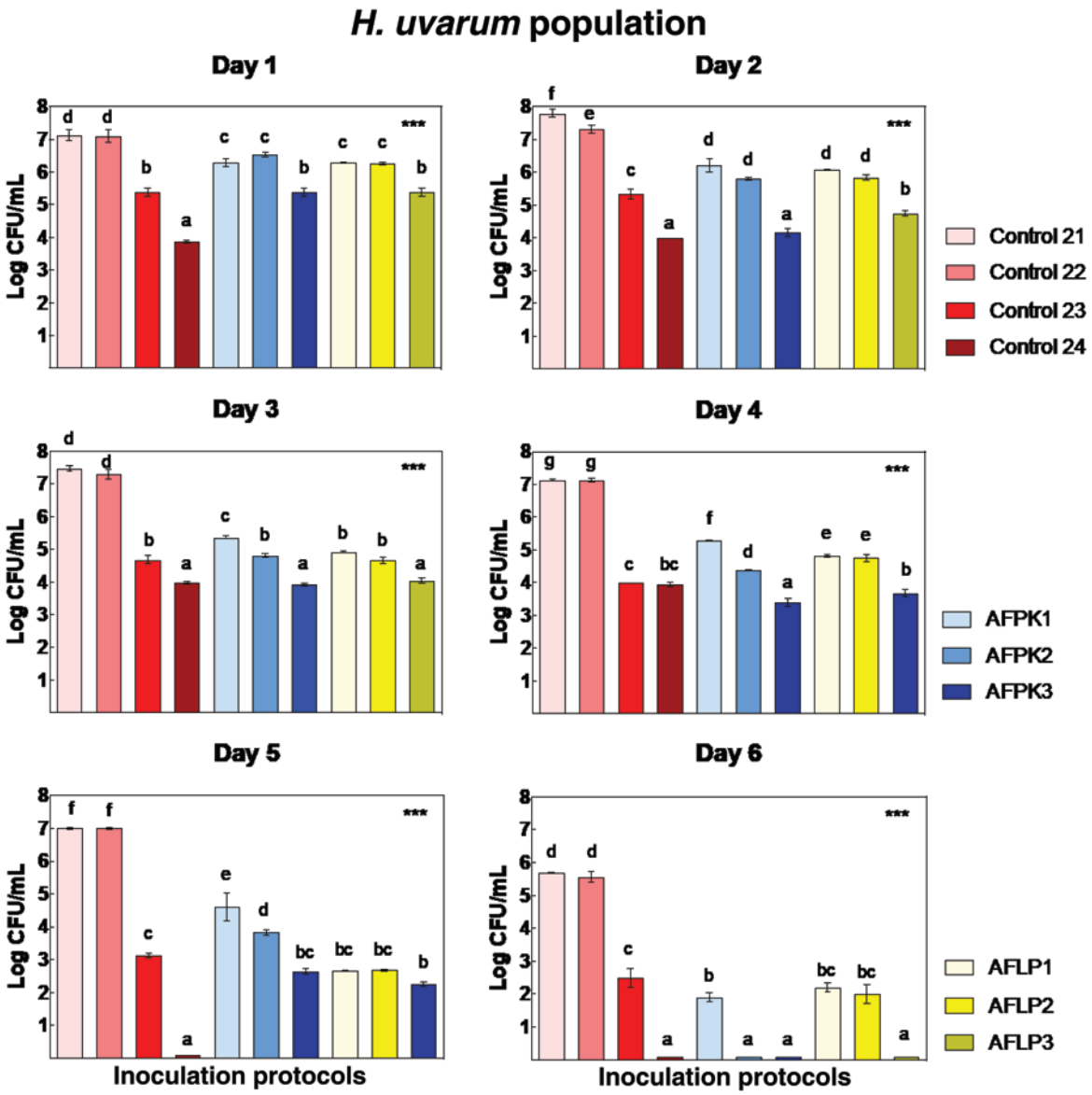

Supplemental Figure 3 Viable counts (log CFU/mL) of *Hanseniaspora uvarum* populations during fermentation (2020 vintage). Inoculation protocols are indicated in the symbol key. Data are the means ± standard deviation of three independent biological experiments. Different letters in each column indicate significant differences according to analysis of variance and Tukey's honest significant difference test ($p < 0.001$).