

Contribution of Grape Skins and Yeast Choice on the Aroma Profiles of Wines Produced from Pinot Noir and Synthetic Grape Musts

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Table S1. Formulation of synthetic grape must (SGM) simulating Pinot noir grape juice.

component	chemical/solution	concentration
sugar and major components	D-glucose	115 g L ⁻¹ *
	D-fructose	115 g L ⁻¹ *
	DL-malic acid	3 g L ⁻¹
	citric acid	0.2 g L ⁻¹
	K ₂ HPO ₄	1.14 g L ⁻¹
	MgSO ₄ ·7H ₂ O	1.23 g L ⁻¹
	CaCl ₂ ·2H ₂ O	0.44 g L ⁻¹
minerals	potassium tartrate	5 g L ⁻¹
	MnCl ₂ ·4H ₂ O	198.2 µg L ⁻¹
	ZnSO ₄ ·7H ₂ O	287.5 µg L ⁻¹
	CuSO ₄ ·5H ₂ O	25.3 µg L ⁻¹
	H ₃ BO ₃	5.7 µg L ⁻¹
	CoCl ₂ ·6H ₂ O	23.8 µg L ⁻¹
	NaMoO ₄ ·2H ₂ O	24.2 µg L ⁻¹
	KIO ₃	10.8 µg L ⁻¹
	FeSO ₄ ·7H ₂ O	70.1 µg L ⁻¹
	pyridoxine-HCl	2 mg L ⁻¹
vitamins	nicotinic acid	2 mg L ⁻¹
	calcium pantothenate	1 mg L ⁻¹
	thiamine-HCl	0.5 mg L ⁻¹
	p-aminobenzoic acid	1 mg L ⁻¹
	riboflavin	1.2 mg L ⁻¹
	biotin	0.125 mg L ⁻¹
	myo-inositol	100 mg L ⁻¹
amino acids and (NH ₄) ₂ HPO ₄	folic acid	0.2 mg L ⁻¹
	L-alanine	146.27 mg L ⁻¹ **
	L-arginine-HCl	513.52 mg L ⁻¹ **
	L-aspartic acid	47.91 mg L ⁻¹ **
	L-asparagine	6.03 mg L ⁻¹ **
	L-cysteine	5.19 mg L ⁻¹ **
	L-glutamic acid	118.24 mg L ⁻¹ **
	L-glutamine	91.78 mg L ⁻¹ **
	L-glycine	5.93 mg L ⁻¹ **
	L-histidine	19.74 mg L ⁻¹ **
	L-isoleucine	14.42 mg L ⁻¹ **
	L-leucine	21.21 mg L ⁻¹ **
	L-lysine-HCl	5.98 mg L ⁻¹ **
	L-methionine	5.28 mg L ⁻¹ **
	L-phenylalanine	15.79 mg L ⁻¹ **
	L-proline	300 mg L ⁻¹
	L-serine	56.49 mg L ⁻¹ **
	L-threonine	106.64 mg L ⁻¹ **
	L-tryptophan	3.5 mg L ⁻¹ **
	L-tyrosine	7.53 mg L ⁻¹ **

	L-valine	20.63 mg L ⁻¹ **
	(NH ₄) ₂ HPO ₄	185.23 mg L ⁻¹ **
ergosterol	ergosterol	15 mg L ⁻¹
Tween 80	Tween 80	0.5 mL L ⁻¹
glutathione	glutathione	50 mg L ⁻¹

*adjusted so that the total sugars in the SGM was equivalent to the Pinot noir must used in comparable experiments.

**nitrogen components adjusted based on their average concentrations in five Marlborough 2018 Pinot noir juices, with a total YAN of 300 mg L⁻¹.

Table S2. Ions and retention times (RTs) used to identify aroma compounds and internal standards.

	ions	RTs (mins)
acetate esters		
isobutyl acetate	43, 56 , 73	9.8
isoamyl acetate	70, 55, 87	16.7
ethyl phenylacetate	91, 164	47.9
β -phenylethyl acetate	104, 43 , 91	49.2
alcohols, aldehydes		
isobutanol	43, 41 , 74	9.3
1-butanol	56 , 31, 41	10.9
isoamyl alcohol	55, 42, 70	14.2
phenylethyl alcohol	91 , 92, 122	51.0
methionol	106 , 61, 58	41.1
benzyl alcohol	79, 43 , 91	49.2
benzaldehyde	77, 106 , 105	31.1
fatty acid ethyl esters		
ethyl isobutyrate	43, 71 , 116, 88	8.8
ethyl butanoate	71, 88 , 101	11.0
ethyl 2-methyl butanoate	57, 102 , 85	14.5
ethyl isovalerate	88 , 85, 115	13.65
ethyl hexanoate	88 , 99, 101	25.1
ethyl octanoate	88, 101 , 127	38.2
ethyl decanoate	88 , 101, 155	51.2
fatty acids		
isobutyric acid	43 , 73, 88	39.2
isovaleric acid	60 , 87	33.6
hexanoic acid	60 , 73, 87	48.5
octanoic acid	60 , 73, 101	62.0
decanoic acid	60 , 73, 129	69.0
cinnamates		
ethyl (di)hydrocinnamate	104 , 91, 107	53.7

ethyl cinnamate (<i>trans</i>)	131, 103, 176	63.88
C₁₃-norisoprenoids		
β-damascenone	69, 121, 190	53.4
α-ionone	121, 136, 192	55.8
β-ionone	177, 178, 192	59.6
terpenes		
<i>cis/trans</i> -rose-oxide	139, 69, 83	31.93/33.07
linalool	71, 93, 121	36.278
α-terpineol	59, 93, 121	43.823
β-citronellol	69, 41, 82, 123	47.395
nerol (<i>cis</i> -geraniol)	69, 41, 93, 121	48.505
geraniol (<i>trans</i> -geraniol)	69, 41, 93, 123	51.493
C₆-compounds		
hexanal	44, 56, 57, 72	11.5
<i>trans</i> -2-hexenal	41, 83, 69	16.5
hexanol	56, 43, 69	22.6
<i>trans</i> -3-hexen-1-ol	41, 67, 82	23.597
<i>cis</i> -3-hexen-1-ol	67, 41, 82	25.337
Internal standards		
ethyl butyrate-4,4,4-d ₃	74, 89	10.9
hexanal-d ₁₂	46, 64, 80	12.3
3-methylbutyl acetate-d ₃	46, 90	15.35
ethyl hexanoate-d ₁₁	91, 110	23.3
<i>n</i> -hexyl-2,2,3,3,4,4,5,5,6,6,6-d ₁₁ alcohol	64, 62, 76	23.4
<i>n</i> -hexyl acetate-d ₃	46, 56, 84	26.4
DL-3-octanol	59, 83, 101	27.8
(±)-linalool-d ₃	74, 96, 124	36.187
ethyl octanoate-d ₁₅	91, 105	37.5
4-decanol	73, 97, 115	41.0
α-terpineol-d ₃	62, 139, 124	43.7
hexanoic-d ₁₁ acid	63, 77, 93	47.8
2-phenylethyl acetate-d ₃	46, 91	49.0
3,4-dimethylphenol	107, 122	65.07

Table S3. Species identities of five MSPC isolates (from 96-well positions A3, B3, C3, G2 and H2) selected for Sanger sequencing to represent the three different cohorts identified for MSPC from restriction fragment length polymorphisms (RFLPs) of the ITS1-5.8S rDNA-ITS2 region. BLAST hits of each sequencing were used to identify the closest match for these isolates.

isolate	cohort	name	bit score	description	E value	grade
A3	2	MH041896	981.691	<i>Saccharomyces bayanus</i> strain SF5-310-4I2 26S ribosomal RNA gene, partial sequence	0	100%
A3	2	MH041895	981.691	<i>Saccharomyces uvarum</i> strain SF5-310-4II8 26S ribosomal RNA gene, partial sequence	0	100%
A3	2	LT594198	981.691	<i>Saccharomyces cerevisiae</i> x <i>Saccharomyces eubayanus</i> x <i>Saccharomyces uvarum</i> genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, strain NCAIM00677	0	100%
B3	1	MK722474	887.512	<i>Saccharomyces cerevisiae</i> strain FBKL2.8022 large subunit ribosomal RNA gene, partial sequence	0	100%
B3	1	MK722467	887.512	<i>Saccharomyces cerevisiae</i> strain FBKL2.8012 large subunit ribosomal RNA gene, partial sequence	0	100%
B3	1	MK722464	887.512	<i>Saccharomyces cerevisiae</i> strain FBKL2.8009 large subunit ribosomal RNA gene, partial sequence	0	100%
C3	2	MH041896	972.458	<i>Saccharomyces bayanus</i> strain SF5-310-4I2 26S ribosomal RNA gene, partial sequence	0	100%
C3	2	MH041895	972.458	<i>Saccharomyces uvarum</i> strain SF5-310-4II8 26S ribosomal RNA gene, partial sequence	0	100%
C3	2	LT009475	972.458	<i>Saccharomyces uvarum</i> partial 26S rRNA gene, strain VA12	0	100%
G2	3	MK581178	922.598	<i>Hanseniaspora uvarum</i> isolate FBKL2.9h702 large subunit ribosomal RNA gene, partial sequence	0	100%
G2	3	MK581177	922.598	<i>Hanseniaspora uvarum</i> isolate FBKL2.9696 large subunit ribosomal RNA gene, partial sequence	0	100%
G2	3	MK581174	922.598	<i>Hanseniaspora uvarum</i> isolate FBKL2.9633 large subunit ribosomal RNA gene, partial sequence	0	100%
H2	2	MH595098	900.439	<i>Saccharomyces uvarum</i> strain UCDFST:48-40 26S ribosomal RNA gene, partial sequence	0	100%
H2	2	MH041896	900.439	<i>Saccharomyces bayanus</i> strain SF5-310-4I2 26S ribosomal RNA gene, partial sequence	0	100%
H2	2	MH041895	900.439	<i>Saccharomyces uvarum</i> strain SF5-310-4II8 26S ribosomal RNA gene, partial sequence	0	100%

Table S4. Microsatellite genotypes of the 19 *S. cerevisiae* isolates from the MSPC community. NP: no peak. EC1118: *S. cerevisiae* EC1118.

sample name/index	A1	A5	B9	B12	C5	C10	D3	E2	E6	E9	F5	F8	F11	G3	G6	G7	G10*	H3	H7	H ₂ O	EC1118
allele size																					
YGL139W - 1	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	125	122	122	NP	114
YGL139W - 2	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	122	125	122	122	NP	122
YLL049W - 1	278	278	278	278	278	278	278	278	278	278	278	278	278	278	278	278	272	278	278	NP	278
YLL049W - 2	278	278	278	278	278	278	278	278	278	278	278	278	278	278	278	278	272	278	278	NP	291
YBR240C - 1	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	NP	358
YBR240C - 2	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	358	NP	358
YPL009C - 1	446	446	446	446	446	446	446	446	446	446	446	446	446	446	446	446	450	446	446	NP	423
YPL009C - 2	446	446	446	446	446	446	446	446	446	446	446	446	446	446	446	446	450	446	446	NP	434
MAT α - 1	457	457	457	457	457	457	457	457	457	457	457	457	457	457	457	457	461	457	457	NP	457
YFR028C - 1	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	NP	122
YFR028C - 2	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	156	NP	132
YOL109W - 1	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	NP	311
YOL109W - 2	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	260	NP	311
MAT α - 1	481	481	481	481	481	481	480	481	481	481	481	481	480	480	481	481	480	481	481	NP	481
YGL014W - 1	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	NP	139
YGL014W - 2	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	146	NP	146
YML091C - 1	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	NP	270
YML091C - 2	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	290	NP	318
YDR160W - 1	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	NP	389
YDR160W - 2	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	393	NP	484

*data acquired from a second run under the same protocol.

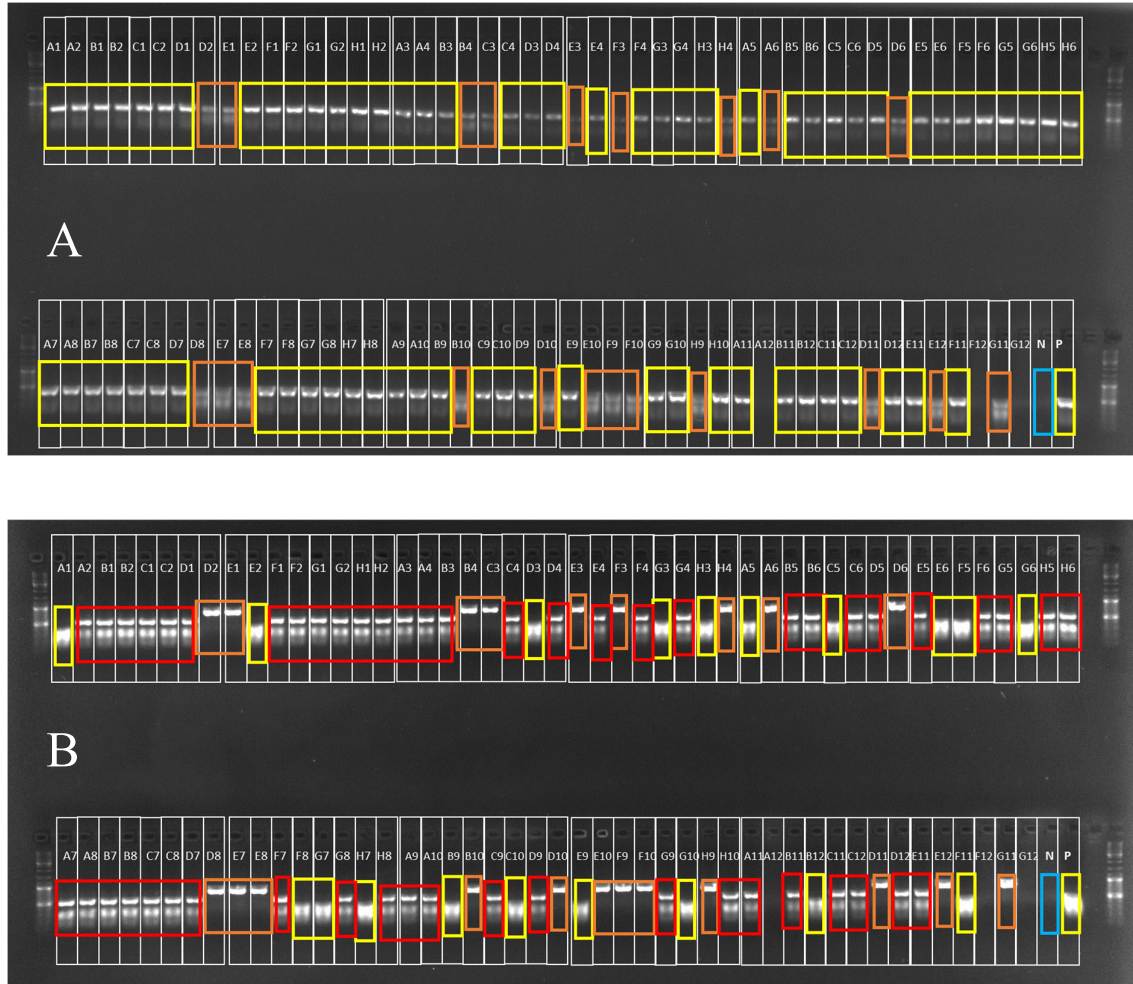


Figure S1. Cohort determination of the MSPC single vineyard community using the amplification of the ITS1-5.8S rDNA-ITS2 region, followed by RFLP analysis using *HinfI* (A) and *HaeIII* (B). The 1 kb+ ladder is shown to the left and right of all lanes. N = negative control (no DNA) surrounded by a blue box. P = *S. cerevisiae* positive control. Yellow boxes around *HinfI* and *HaeIII* digests represent cohort 1, *Saccharomyces cerevisiae*. Yellow *HinfI* and red *HaeIII* boxes represent cohort 2, *Saccharomyces uvarum*. Orange *HinfI* and *HaeIII* boxes represent cohort 3, *Hanseniaspora uvarum*.

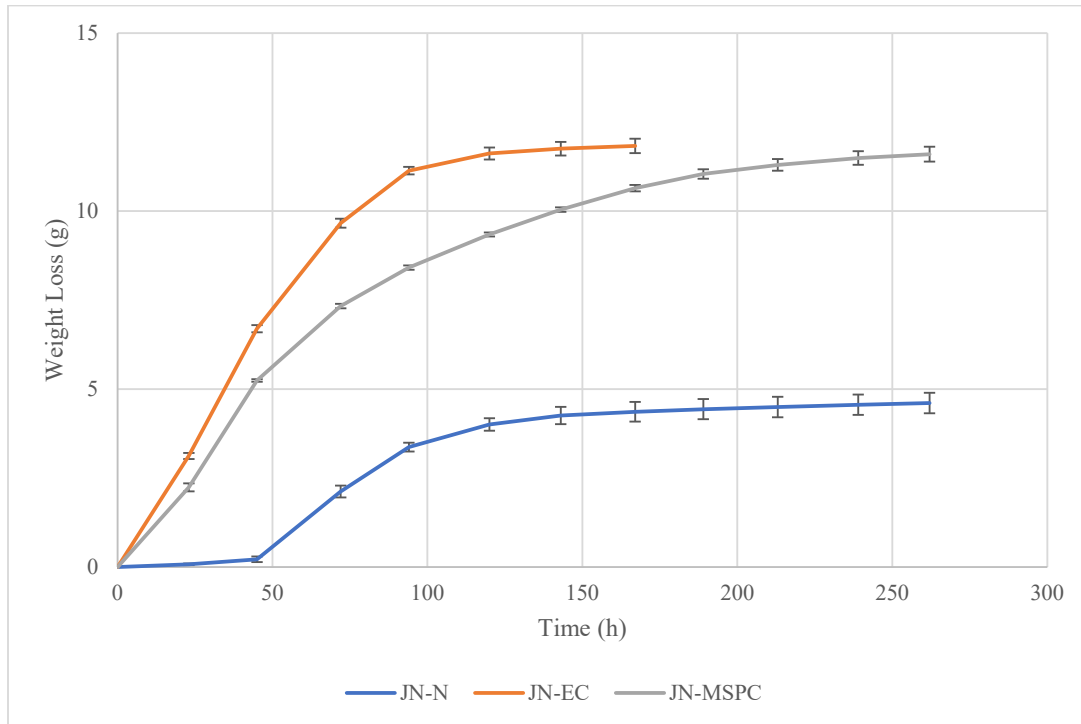


Figure S2. Cumulative weight loss (g) over time (h) of fermentations in Pinot noir juice without grape skins (JN). N = no inoculation, EC = inoculation of *S. cerevisiae* EC1118, MSPC = inoculation of the MSPC yeast community. Error bars represent the 95% confidence intervals, (n = 3).

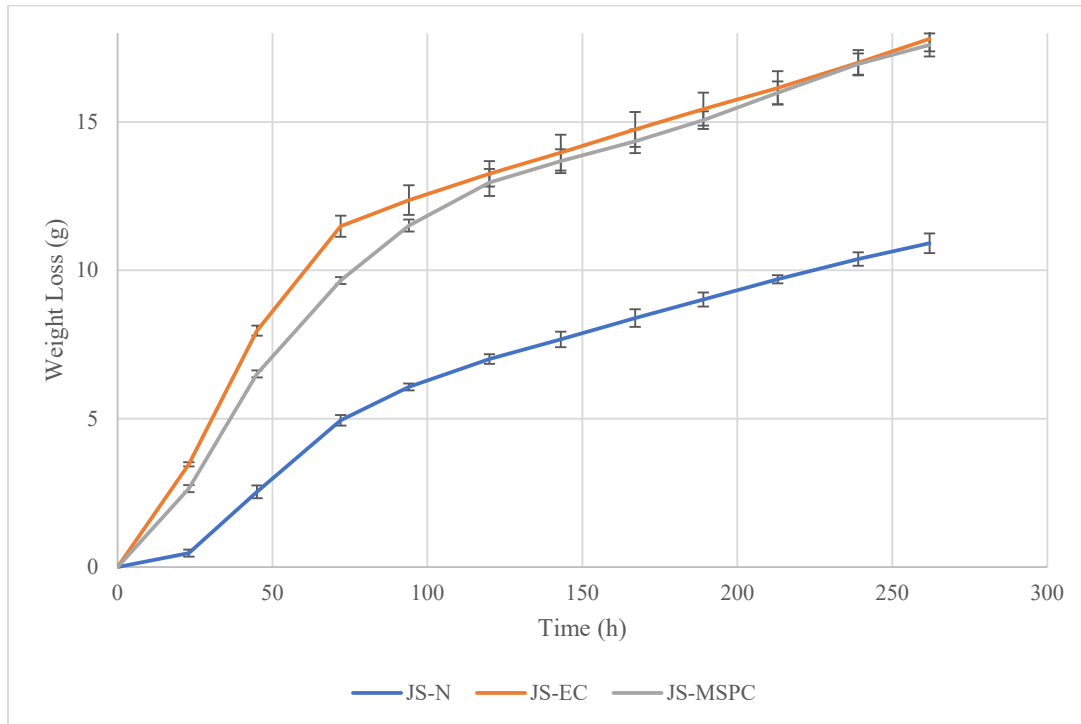


Figure S3. Cumulative weight loss (g) over time (h) of fermentations in Pinot noir juice with grape skins (JS). N = no inoculation, EC = inoculation of *S. cerevisiae* EC1118, MSPC = inoculation of the MSPC yeast community. Error bars represent the 95% confidence intervals, (n = 3).

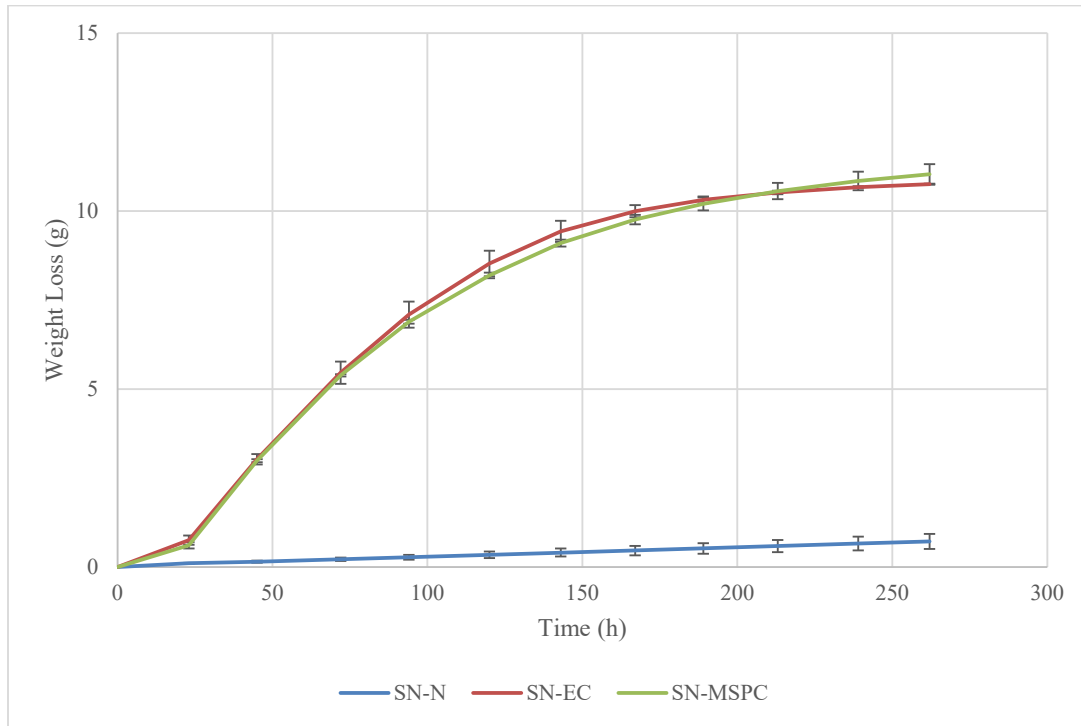


Figure S4. Cumulative weight loss (g) over time (h) of fermentations in synthetic grape medium (SGM) without grape skins (SN). N = no inoculation, EC = inoculation of *S. cerevisiae* EC1118, MSPC = inoculation of the MSPC yeast community. Error bars represent the 95% confidence intervals, (n = 3).

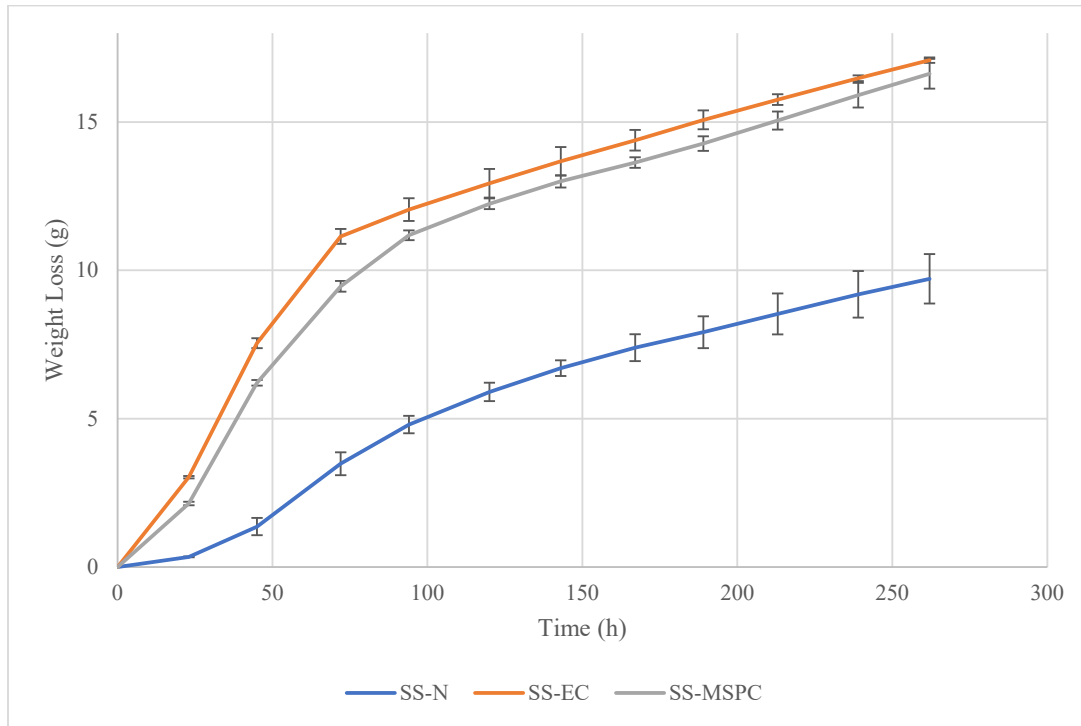


Figure S5. Cumulative weight loss (g) over time (h) of fermentations in synthetic grape medium (SGM) with grape skins (SS). N = no inoculation, EC = inoculation of *S. cerevisiae* EC1118, MSPC = inoculation of the MSPC yeast community. Error bars represent the 95% confidence intervals, (n = 3).