

# **Supplementary data for**

## **Increase in fruity ester production during spine wine**

### **fermentation by goal-directed amino acid**

### **supplementation**

**Zijian Zhu <sup>1</sup>, Kai Hu <sup>1</sup>, Siyu Chen <sup>1</sup>, Sirui Xiong <sup>1</sup> and Yongsheng Tao <sup>1,2,\*</sup>**

<sup>1</sup> College of Enology, Northwest A&F University, Yangling, Shaanxi 712100, China

<sup>2</sup> Shaanxi Engineering Research Center for Viti-viniculture, Yangling, Shaanxi 712100, China

\*Corresponding author: Yongsheng Tao

Email: [taoyongsheng@nwsuaf.edu.cn](mailto:taoyongsheng@nwsuaf.edu.cn)

Tel: 0086-87092107, Fax: 0086-87091994

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Table S1 Qualitative and quantitative information of chromatographically pure standards in HPLC analysis.

Standards	Purity	SKU-Pack size*	RT (min)	Regression equation	R <sup>2</sup>	Calibrated interval
<b>Column: Welch Ultimate AQ-C<sub>18</sub></b>						(mg/L)
L-malic acid	≥99.0%	5g	4.48	Y = 586136 X - 8809	0.9991	3.13-4000
Acetic acid	≥99.8%	1mL	5.21	Y = 653519 X + 1824	0.9999	3.13-4000
Citric acid	≥99.5%	0.1g	7.81	Y = 688652 X - 5359	0.9998	3.13-4000
Succinic acid	≥99.0%	0.25g	9.17	Y = 296691 X - 6416	0.9991	1.56-2000
<b>Column: Welch Ultimate XB-NH<sub>2</sub></b>						(mg/L)
Glycerol	≥99.5%	1mL	7.84	Y = 315934 X - 2320		
<b>Column: Agilent ZORBAX SB-C<sub>18</sub></b>						(mmol/L)
L-aspartic acid	≥98.0%	B21934-200mg	3.92	Y = 2079746 X + 11424	0.9966	0.001-0.1
L-glutamic acid	≥98.0%	YZ140690-100mg	5.06	Y = 2171807 X + 7568	0.9984	0.003-0.1
L-arginine	≥98.0%	B21920-200mg	10.81	Y = 1666352 X + 5590	0.9976	0.02-0.8
L-proline	≥99.0%	B21914-200mg	12.25	Y = 2202082 X + 20061	0.9999	0.08-5.6
γ-aminobutyric acid	≥99.0%	B21979-100mg	13.16	Y = 2233401 X + 26225	0.9967	0.006-0.1
L-alanine	≥98.0%	B21911-200mg	13.87	Y = 2050799 X + 14230	0.9964	0.006-0.1
L-valine	≥98.0%	SV8010-200mg	21.89	Y = 2519184 X + 6045	0.9968	0.006-0.1
L-methionine	≥98.0%	B21913-200mg	23.08	Y = 938054 X + 13076	0.9971	0.01-0.5
L-isoleucine	≥98.0%	B21937-200mg	27.49	Y = 1493240 X + 12448	0.9957	0.01-0.5
L-leucine	≥99.0%	YZ140687-100mg	28.02	Y = 1356899 X + 35778	0.9962	0.01-0.5
L-tryptophan	≥98.0%	B21930-200mg	29.22	Y = 1452635 X - 1620	0.9986	0.01-0.8
L-phenylalanine	≥98.0%	B21910-200mg	29.76	Y = 1720549 X + 4175	0.9967	0.01-0.5
L-tyrosine	≥99.0%	B21924-200mg	37.31	Y = 811523 X - 5991	0.9978	0.01-0.5

\*SKU-Pack size was given by DrE, Yuanye and Solarbio.

Y: peak area versus; X: concentration.

Table S2 Qualitative and quantitative information of chromatographically pure standards in SPME-GC-MS analysis.

Standards	Purity	SKU-Pack size*	RT (min)	Regression equation	R <sup>2</sup>	Calibrated interval (µg/L)
Ethyl acetate	≥99.9% (GC)	58958-5ML	7.98	Y = 31328X + 444	0.9947	600-90200
Isobutyl acetate	≥99.8% (GC)	94823-1ML-F	11.72	Y = 2522 X - 5	0.9995	2-240
Isoamyl acetate	≥99.7% (GC)	79857-1ML	19.16	Y = 409X + 48	0.9998	53-8000
Hexyl acetate	≥99.7% (GC)	25539-1ML	20.96	Y = 126X + 25	0.9982	7-1000
Phenethyl acetate	≥97.0% (GC)	73747-1ML	37.25	Y = 401X + 13	0.9982	4-600
Ethyl butyrate	≥99.5% (GC)	75563-1ML	12.16	Y = 3367X - 45	0.9993	7-1080
Ethyl hexanoate	≥99.5% (GC)	08375-1ML	19.71	Y = 1047X + 206	0.9952	24-3600
Ethyl octanoate	≥99.0% (GC)	44879-1ML	26.99	Y = 958X + 150	0.9901	20-3000
Ethyl decanoate	≥99.0% (GC)	00733-1ML	33.81	Y = 2925X - 11	0.9990	5-800
Isobutyl alcohol	≥99.8% (GC)	82059-1ML-F	14.39	Y = 111711X + 2766	0.9981	600-90000
Isoamyl alcohol	≥98.5% (GC)	77664-1ML	18.47	Y = 21262X - 2751	0.9994	2157-323600
2-phenylethanol	≥99.0% (GC)	77861-250ML	39.42	Y = 355959X + 32	0.9983	1333-200000
Hexanoic acid	≥99.0% (GC)	21529-5ML	37.63	Y = 12213X + 194	0.9952	120-3600
Octanoic acid	≥99.5% (GC)	21639-5ML	43.08	Y = 581X + 128	0.9993	40-6000
Decanoic acid	≥99.5% (GC)	21409-5G	48.48	Y = 512X + 46	0.9977	47-1400

\*SKU-Pack Size was given by Sigma-Aldrich.

Y: concentration (µg/L); X: relative peak area versus the area of the internal standard (2-octanol).

Table S3 Concentrations of yeast assimilable nitrogen in different nitrogen treatments and controls.

	YAN (mg N/L) (Before treating)	Nitrogen nutrient supplements (mg/L)				YAN (mg N/L) (After treating)*
		DAP	Ala	Phe	Ile	
CK-CS	187.0±2.8	-	-	-	-	-
CK-SP	136.2±3.6	-	-	-	-	-
IN	136.7±3.1	236	-	-	-	185.9±1.6
AA-Ala	136.6±2.3	-	318	-	-	185.3±1.9
AA-Phe	137.2±2.3	-	-	589	-	187.9±2.0
AA-Ile	136.0±1.8	-	-	-	468	187.1±3.7
MAA	136.5±1.6	-	163	71	170	187.6±1.5
IN+MAA	136.1±3.0	178	39	17	39	186.7±3.8

\* No significant difference after nitrogen nutrient supplementation.

CK-CS: Cabernet Sauvignon control; CK-SP: spine grape control. IN: DAP supplement; AA-Ala: single alanine supplement; AA-Phe: single phenylalanine supplement; AA-Ile: single isoleucine supplement; MAA: mixed nitrogen supplement with alanine, phenylalanine and isoleucine; IN+MAA: mixed nitrogen supplement with DAP, alanine, phenylalanine, and isoleucine.

Table S4 Evolution of amino acid contents during alcoholic fermentation of spine grape and Cabernet Sauvignon grape (μmol/L)

Variety	Amino acid	Must	1 d	2 d	3 d	5 d	7 d	10 d	12 d
Cabernet Sauvignon	Asp	11.7 ± 2.6	8.5 ± 0.1	4.6 ± 0.4	4.5 ± 0.3	1.6 ± 0.2	0.8 ± 0.1	0.7 ± 0.2	ND
	Glu	14.9 ± 0.2	11.4 ± 1.2	6.6 ± 0.1	3.0 ± 1.2	2.4 ± 0.2	2.0 ± 0.1	1.8 ± 0.2	2.5 ± 0.1
	Arg	273.2 ± 1.5	271.0 ± 3.0	148.5 ± 19.0	87.1 ± 21.5	18.4 ± 1.6	18.0 ± 0.1	17.1 ± 0.3	9.4 ± 0.2
	Pro	10868.6 ± 108.0	11414.0 ± 154.0	11212.7 ± 1432.4	10906.0 ± 5453.0	11725.8 ± 414.1	10248.6 ± 15.9	9391.4 ± 198.3	10349.4 ± 171.8
	GABA	59.9 ± 0.5	44.1 ± 1.6	23.9 ± 2.3	7.3 ± 0.9	ND	ND	ND	ND
	Ala	48.1 ± 4.4	49.8 ± 0.3	51.6 ± 6.7	33.7 ± 0.1	21.6 ± 5.0	5.4 ± 0.1	3.2 ± 0.1	1.5 ± 0.2
	Val	14.3 ± 0.3	8.4 ± 0.1	3.4 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.4 ± 0.3	ND
	Met	19.9 ± 2.9	11.4 ± 2.3	6.6 ± 1.3	4.0 ± 0.7	1.0 ± 0.3	0.2 ± 0.1	ND	ND
	Ile	37.6 ± 2.5	24.0 ± 2.5	9.6 ± 1.4	3.7 ± 0.2	1.4 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	0.9 ± 0.1
	Leu	20.1 ± 3.4	16.5 ± 2.1	8.9 ± 1.7	3.7 ± 1.9	0.2 ± 0.3	ND	ND	ND
	Trp	22.3 ± 2.1	13.6 ± 1.7	3.8 ± 0.41	3.0 ± 0.38	1.5 ± 0.39	1.0 ± 0.27	0.5 ± 0.17	ND
	Phe	15.4 ± 0.9	14.4 ± 0.2	13.3 ± 1.8	7.6 ± 0.6	0.8 ± 0.3	ND	ND	ND
	Tyr	21.1 ± 0.4	20.1 ± 0.1	19.0 ± 0.7	18.7 ± 0.9	12.1 ± 0.1	12.5 ± 0.1	13.1 ± 6.6	13.2 ± 0.1
Spine grape	Asp	28.1 ± 4.0	30.9 ± 0.7	25.5 ± 2.3	24.1 ± 0.5	18.0 ± 1.4	13.5 ± 0.2	9.8 ± 0.4	8.5 ± 0.1
	Glu	63.4 ± 18.0	64.0 ± 0.5	56.9 ± 3.2	37.9 ± 1.9	7.5 ± 0.4	5.5 ± 0.4	4.1 ± 0.7	3.9 ± 0.1
	Arg	814.7 ± 33.8	85.1 ± 0.5	81.1 ± 5.3	67.6 ± 2.2	47.7 ± 3.7	32.9 ± 1.6	21.7 ± 4.3	46.3 ± 28.7
	Pro	1316.4 ± 79.4	1523.0 ± 3.0	1515.4 ± 35.4	1774.0 ± 15.0	1936.5 ± 53.9	1936.0 ± 9.6	2021.6 ± 193.2	3472.2 ± 228.0
	GABA	463.0 ± 3.2	418.5 ± 7.5	406.2 ± 31.7	338.5 ± 17.5	212.0 ± 53.9	128.6 ± 4.0	ND	ND
	Ala	5.1 ± 0.1	3.7 ± 0.3	3.5 ± 0.7	2.5 ± 0.2	1.0 ± 0.1	2.1 ± 0.1	0.5 ± 0.2	1.2 ± 0.1
	Val	31.6 ± 0.6	27.0 ± 2.5	10.7 ± 0.1	9.8 ± 0.1	8.8 ± 0.4	5.2 ± 0.1	3.5 ± 0.6	ND
	Met	15.2 ± 1.9	14.0 ± 1.4	7.1 ± 0.8	5.3 ± 0.5	1.6 ± 0.6	ND	ND	ND
	Ile	7.6 ± 0.5	6.0 ± 0.1	5.5 ± 0.7	4.5 ± 0.1	3.2 ± 0.5	1.6 ± 0.1	0.2 ± 0.1	ND
	Leu	24.6 ± 2.2	18.5 ± 1.1	11.2 ± 0.1	4.6 ± 0.5	0.8 ± 0.2	0.4 ± 0.1	ND	ND

Variety	Amino acid	Must	1 d	2 d	3 d	5 d	7 d	10 d	12 d
	Trp	28.6 ± 3.1	16.9 ± 2.8	5.9 ± 1.0	2.8 ± 0.3	1.1 ± 0.2	ND	ND	ND
	Phe	5.4 ± 1.0	8.5 ± 0.1	7.5 ± 1.1	4.3 ± 2.1	4.0 ± 1.4	3.3 ± 0.1	2.5 ± 0.1	1.4 ± 0.1
	Tyr	16.9 ± 1.2	16.5 ± 0.1	16.0 ± 0.1	17.3 ± 0.5	18.6 ± 1.3	18.5 ± 0.1	15.4 ± 1.9	16.1 ± 0.5

Data are mean values of two independent experiments ± standard deviation.

Table S5 Concentrations of wine fruity esters during alcoholic fermentation of Cabernet Sauvignon grape ( $\mu\text{g/L}$ )

	Compounds	Fermentation time							
		Must	1 d	2 d	3 d	5 d	7 d	10 d	12 d
1	Ethyl acetate	1846 $\pm$ 2	1951 $\pm$ 35	3438 $\pm$ 240	8608 $\pm$ 1403	24509 $\pm$ 827	25681 $\pm$ 69	26713 $\pm$ 508	27764 $\pm$ 1252
	AHAs	ND	ND	8 $\pm$ 0	423 $\pm$ 13	1548 $\pm$ 49	1663 $\pm$ 75	2143 $\pm$ 84	1867 $\pm$ 128
1	Isobutyl acetate	ND	ND	ND	59 $\pm$ 12	100 $\pm$ 11	62 $\pm$ 2	37 $\pm$ 9	9 $\pm$ 0
2	Isoamyl acetate	ND	ND	ND	345 $\pm$ 0	1383 $\pm$ 35	1506 $\pm$ 59	1994 $\pm$ 74	1753 $\pm$ 127
3	Hexyl acetate	ND	ND	1 $\pm$ 0	1 $\pm$ 0	2 $\pm$ 0	3 $\pm$ 0	1 $\pm$ 0	1 $\pm$ 0
4	$\beta$ -phenylethyl acetate	ND	ND	7 $\pm$ 0	18 $\pm$ 1	63 $\pm$ 3	92 $\pm$ 14	111 $\pm$ 1	104 $\pm$ 1
	SCFAEEs	ND	ND	ND	28 $\pm$ 6	111 $\pm$ 5	127 $\pm$ 4	138 $\pm$ 5	143 $\pm$ 10
1	Ethyl butyrate	ND	ND	ND	28 $\pm$ 6	111 $\pm$ 5	127 $\pm$ 4	138 $\pm$ 5	143 $\pm$ 10
	MCFAEEs	ND	ND	20 $\pm$ 1	851 $\pm$ 152	2289 $\pm$ 176	1837 $\pm$ 123	1458 $\pm$ 44	1410 $\pm$ 108
1	Ethyl hexanoate	ND	ND	ND	440 $\pm$ 93	1146 $\pm$ 77	841 $\pm$ 15	638 $\pm$ 20	530 $\pm$ 40
2	Ethyl octanoate	ND	ND	11 $\pm$ 0	238 $\pm$ 33	846 $\pm$ 74	587 $\pm$ 68	417 $\pm$ 7	505 $\pm$ 29
3	Ethyl decanoate	ND	ND	9 $\pm$ 1	173 $\pm$ 26	297 $\pm$ 25	409 $\pm$ 40	403 $\pm$ 17	375 $\pm$ 39

Data are mean values of two independent experiments  $\pm$  standard deviation.



Table S6 Concentration of wine fruity esters during alcoholic fermentation of spine grape (µg/L)

	Compounds	Fermentation time							
		Must	1 d	2 d	3 d	5 d	7 d	10 d	12 d
1	Ethyl acetate	2352 ± 0	1861 ± 17	2913 ± 105	3608 ± 7	8644 ± 526	11383 ± 1051	13204 ± 499	16168 ± 117
	AHAs	ND	ND	ND	9 ± 0	192 ± 41	112 ± 7	87 ± 0	84 ± 11
1	Isobutyl acetate	ND	ND	ND	ND	18 ± 0	7 ± 0	ND	1 ± 0
2	Isoamyl acetate	ND	ND	ND	ND	161 ± 41	87 ± 5	66 ± 0	63 ± 11
3	Hexyl acetate	ND	ND	ND	1 ± 0	1 ± 0	1 ± 0	1 ± 0	ND
4	β-phenylethyl acetate	ND	ND	ND	8 ± 0	12 ± 0	17 ± 2	20 ± 0	20 ± 0
	SCFAEEs	ND	ND	ND	ND	59 ± 7	85 ± 4	101 ± 6	127 ± 3
1	Ethyl butyrate	ND	ND	ND	ND	59 ± 7	85 ± 4	101 ± 6	127 ± 3
	MCFAEEs	ND	ND	47 ± 2	308 ± 31	1449 ± 108	1219 ± 44	972 ± 39	945 ± 25
1	Ethyl hexanoate	ND	ND	ND	73 ± 12	707 ± 73	531 ± 11	414 ± 21	429 ± 9
2	Ethyl octanoate	ND	ND	35 ± 1	132 ± 10	485 ± 22	373 ± 18	295 ± 1	282 ± 4
3	Ethyl decanoate	ND	ND	12 ± 1	103 ± 9	257 ± 13	315 ± 15	263 ± 17	234 ± 12

Data are mean values of two independent experiments ± standard deviation. AHA: acetates of higher alcohols; SCFAEE: short-chain fatty acid ethyl ester; MCFAEE: medium-chain fatty acid ethyl esters.

Table S7 Pearson's correlation coefficients ( $r$ ) between amino acids and wine fruity esters during alcoholic fermentation.

Compounds	Cabernet Sauvignon			Spine grape		
	AHAs	SCFAEEs	MCFAEEs	AHAs	SCFAEEs	MCFAEEs
Total AAs	-0.886	-0.875	-0.825	-0.612	-0.864	-0.750
Arg	-0.884	-0.885	-0.849	-0.699	-0.875	-0.880
Asp	-0.852	-0.847	-0.754	-0.666	-0.927	-0.752
Glu	-0.799	-0.781	-0.777	-0.837	-0.904	-0.920
GABA	-0.819	-0.820	-0.811	-0.655	-0.969	-0.767
Leu	-0.858	-0.750	-0.818	-0.715	-0.761	-0.816
Met	-0.812	-0.728	-0.788	-0.711	-0.823	-0.860
Trp	-0.728	-0.640	-0.698	-0.605	-0.644	-0.722
Val	-0.710	-0.858	-0.759	-0.574	-0.794	-0.804
Ala	-0.974	-0.961	-0.703	-0.723	-0.805	-0.681
Ile	-0.735	-0.732	-0.729	-0.636	-0.712	-0.572
Phe	-0.975	-0.967	-0.895	-0.569	-0.701	-0.658

AHA: acetates of higher alcohols; SCFAEE: short-chain fatty acid ethyl ester; MCFAEE: medium-chain fatty acid ethyl esters.

Table S8 Physiochemical indices of wine samples.

Indices	CK-CS	CK-SP	IN	AA-Ala	AA-Phe	AA-Ile	MAA	IN+MAA
Residual sugar (g/L)	2.10 ± 0.26a	1.83 ± 0.36cd	1.74 ± 0.24d	1.93 ± 0.38bc	1.98 ± 0.28abc	1.97 ± 0.16abc	2.02 ± 0.14ab	2.03 ± 0.29ab
Alcohol content (%)	11.98 ± 1.08	12.03 ± 0.93	11.95 ± 0.88	12.09 ± 1.16	12.01 ± 1.34	12.06 ± 0.96	12.05 ± 1.16	12.05 ± 0.98
Titrateable acid (g/L as tartaric acid)	7.56 ± 0.52a	6.49 ± 0.95b	6.46 ± 0.83b	6.45 ± 0.78b	6.61 ± 0.98b	6.46 ± 1.23b	6.86 ± 0.86b	6.76 ± 1.33b
Volatile acid (g/L as acetic acid)	0.36 ± 0.07abc	0.36 ± 0.05c	0.38 ± 0.04bc	0.43 ± 0.06a	0.41 ± 0.06ab	0.37 ± 0.05bc	0.39 ± 0.05abc	0.42 ± 0.05a
pH	3.3 ± 0.01f	3.47 ± 0.01bc	3.51 ± 0.02a	3.5 ± 0.01ab	3.47 ± 0.02bc	3.44 ± 0.01	3.41 ± 0.01e	3.43 ± 0.01de

CK-CS: Cabernet Sauvignon control; CK-SP: spine grape control. IN: DAP supplement; AA-Ala: single alanine supplement; AA-Phe: single phenylalanine supplement; AA-Ile: single isoleucine supplement; MAA: mixed nitrogen supplement with alanine, phenylalanine and isoleucine; IN+MAA: mixed nitrogen supplement with DAP, alanine, phenylalanine, and isoleucine.