

Differences in the volatile profile of apple cider fermented with *Schizosaccharomyces pombe* and *Schizosaccharomyces japonicus*

Nicola Ferremi Leali¹, Elisa Salvetti^{1,2}, Giovanni Luzzini¹, Andrea Salini¹, Salvatore Fusco¹, Maurizio Ugliano¹, Sandra Torriani^{1*}, Renato L. Binati¹

¹ Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona (VR), Italy

² Verona University Culture Collection – Dept. of Biotechnology (VUCC-DBT), University of Verona, Strada Le Grazie 15, 37134 Verona (VR), Italy

* Correspondence: sandra.torriani@univr.it; Tel.: +39-045-8027921

Supplementary Table S1. Chemical composition of apple cider fermented with *Schizosaccharomyces pombe* SzpT and Szp, *Schizosaccharomyces japonicus* SzjT and Szj, and *Saccharomyces cerevisiae* Sc at half fermentation. Data are the averages of three independent fermentations. Different letters for the same data series indicate a significant difference in Tukey's HSD test ($p<0.05$).

N.D.=not detected

Compound (g/L)	Strain				
	SzpT	Szp	SzjT	Szj	Sc
Fructose	41.68 ± 2.47 ^a	43.64 ± 0.50 ^a	34.63 ± 2.91 ^a	37.82 ± 7.19 ^a	40.24 ± 3.68 ^a
Glucose	5.95 ± 2.71 ^b	9.47 ± 0.89 ^{ab}	8.40 ± 1.00 ^{ab}	12.47 ± 2.76 ^a	5.44 ± 0.96 ^b
Sucrose	N.D.	N.D.	N.D.	1.59 ± 0.36 ^a	1.42 ± 0.09 ^a
Citrate	N.D.	N.D.	N.D.	N.D.	N.D.
Malate	3.25 ± 0.04 ^a	1.64 ± 0.01 ^b	0.93 ± 0.01 ^d	0.17 ± 0.00 ^c	3.25 ± 0.04 ^a
Glycerol	2.77 ± 0.51 ^c	3.35 ± 0.42 ^c	6.44 ± 0.66 ^a	5.04 ± 0.11 ^b	2.79 ± 0.31 ^c
Sorbitol	N.D.	N.D.	N.D.	N.D.	N.D.
Ethanol (v/v, %)	3.65 ± 0.60 ^b	4.39 ± 0.51 ^{ab}	4.49 ± 0.32 ^{ab}	3.80 ± 0.15 ^b	5.37 ± 0.56 ^a
pH	3.56 ± 0.04 ^c	3.58 ± 0.05 ^c	3.58 ± 0.03 ^c	4.74 ± 0.10 ^a	3.74 ± 0.02 ^b
Ratio Fructose/ Glucose	7.34 ± 1.39 ^a	4.63 ± 0.39 ^b	4.13 ± 0.18 ^b	3.04 ± 0.09 ^b	7.49 ± 0.85 ^a

Supplementary Table S2. Volatile organic compounds (µg/L) in apple cider fermented with *Schizosaccharomyces pombe* SzpT and Szp, *Schizosaccharomyces japonicus* SzjT and Szj, and *Saccharomyces cerevisiae* Sc.

Compound	IUPAC Name	OD	Strain					p- value
			SzpT	Szp	SzjT	Szj	Sc	
<i>Esters</i>								
ethyl acetate (mg/L)	ethyl acetate	Ethereal, fruity, sweet	32.58 ± 4.37 ^c	17.61 ± 3.91 ^c	180.96 ± 14.21 ^c	315.44 ± 73.07 ^a	63.29 ± 3.41 ^b	3.05e-06
ethyl butanoate	ethyl butanoate	Fruity sweet apple	125.4 ± 25.61 ^c	104.4 ± 6.17 ^c	182.83 ± 4.51 ^a	127.73 ± 8.33 ^c	225.7 ± 9.72 ^b	3.05e-06
ethyl hexanoate	ethyl hexanoate	Sweet, fruity, pineapple	127.77 ± 24.97 ^c	171.7 ± 4.24 ^c	148.77 ± 9.52 ^c	276.77 ± 20.39 ^b	448.77 ± 22.06 ^a	4.87e-09
ethyl octanoate	ethyl octanoate	Fruity, winey, waxy	74.23 ± 19.81 ^c	137.62 ± 8.63 ^b	19.7 ± 0.26 ^d	37.5 ± 1.57 ^d	526.7 ± 20.57 ^a	2.52e-12
ethyl decanoate	ethyl decanoate	Sweet, waxy, fruity	49.13 ± 25.05 ^c	112.42 ± 6.23 ^b	101.33 ± 20.1 ^b	33.3 ± 2.56 ^c	352 ± 23.06 ^a	6.05e-09
ethyl di-2 hydroxyhexanoate	ethyl 2- hydroxyhexanoate	Sweet, fruity	0.57 ± 0.06 ^a	0.7 ± 0.1 ^a	0.07 ± 0.06 ^b	0.03 ± 0.06 ^b	0.1 ± 0 ^b	2.19e-07
isoamyl acetate	3-methylbutyl acetate	Floral, rose, sweet	183.53 ± 28.67 ^d	145.03 ± 3.28 ^d	2166.77 ± 254.84 ^c	5994.03 ± 135.5 ^a	774.17 ± 28.26 ^b	4.22e-13
2 phenethyl acetate	2-phenylethyl acetate	Sweet, fruity	139.03 ± 1.76 ^c	130.98 ± 4.71 ^c	1174.47 ± 138.86 ^a	658.33 ± 69.38 ^b	196.13 ± 5.41 ^c	1.47e-08
		Σ ESTERS (mg/L)	33.27	18.41	184.75	322.57	65.81	
<i>Alcohols</i>								
isoamyl alcohol	3-methylbutan-1-ol	Roasted, spicy, sweet	42671.97 ± 4821.3 ^{cd}	24884.02 ± 2162.82 ^d	177672.2 ± 10105.07 ^b	247762.43 ± 15190 ^a	71098.37 ± 15597.49 ^c	8.82e-10
1 pentanol	pentan-1-ol	Sweet solvent, fusel	92.4 ± 4.99 ^{ab}	91.49 ± 6.86 ^{ab}	45.57 ± 5.02 ^c	111.87 ± 2.94 ^a	70.63 ± 19.58 ^{bc}	0.000103
1 hexanol	hexan-1-ol	Sweet, resin, ether	2031 ± 14.05 ^a	1884.05 ± 191.28 ^{ab}	926.53 ± 143.59 ^c	1953.57 ± 103.57 ^{ab}	1612.53 ± 117.49 ^b	5.66e-06
benzyl alcohol	phenylmethanol	Floral, rose, phenolic	51.7 ± 19.92 ^b	46.14 ± 19.61 ^b	41.9 ± 3.21 ^b	96.5 ± 3.05 ^a	17.27 ± 6.18 ^b	0.000332
phenylethyl alcohol	2-phenylethanol	Sweet, floral, fresh	2876.27 ± 502.83 ^c	2727.31 ± 143.16 ^c	6195.87 ± 515.27 ^a	4842.67 ± 274.79 ^b	3432.67 ± 220.25 ^c	1.49e-06
		Σ ALCOHOLS	47723.34	2457.01	184882.07	254767.04	76231.47	

<i>Terpenes</i>								
β -myrcene	7-methyl-3-methylideneocta-1,6-diene	Peppery, terpenic, spicy	1.04 ± 0.43 ^b	0.79 ± 0.16 ^b	0.97 ± 0.25 ^b	1.47 ± 0.27 ^b	2.33 ± 0.29 ^a	0.000566
limonene	1-methyl-4-prop-1-en-2-ylcyclohexene	Citrus, orange, fresh	0.35 ± 0.06 ^{ab}	0.33 ± 0.09 ^b	0.32 ± 0.06 ^b	0.51 ± 0.06 ^{ab}	0.63 ± 0.19 ^a	0.0183
linalool	3,7-dimethylocta-1,6-dien-3-ol	Citrus, floral, sweet	4.37 ± 0.53 ^{ab}	4.33 ± 0.61 ^b	2.28 ± 0.4 ^c	1.92 ± 0.21 ^c	5.67 ± 0.58 ^a	1.3e-05
α -terpineol	2-(4-methylcyclohex-3-en-1-yl) propan-2-ol	Woody, terpenic, lemon	0.25 ± 0.03 ^b	0.24 ± 0.06 ^b	0.22 ± 0.02 ^b	0.16 ± 0.03 ^b	0.39 ± 0.06 ^a	0.000759
geraniol	(2E)-3,7-dimethylocta-2,6-dien-1-ol	Sweet, floral, fruity	1.26 ± 0.37 ^b	2.28 ± 0.47 ^{ab}	1.33 ± 0.18 ^b	1.83 ± 0.55 ^{ab}	3.04 ± 0.57 ^a	0.00364
nerolidol 2	(3S,6Z)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol	Mild, floral	0.82 ± 0.42 ^b	0.85 ± 0.06 ^b	0.79 ± 0.1 ^b	0 ± 0 ^b	2.58 ± 0.74 ^a	0.000137
		Σ TERPENES	8.09	8.82	5.91	5.89	14.64	
<i>Fatty acids</i>								
hexanoic acid	hexanoic acid	Sour, fatty, sweaty	1803.7 ± 160.44 ^d	2721.14 ± 147.14 ^c	3102.1 ± 142.53 ^c	8040.03 ± 208.39 ^a	4510.27 ± 99.93 ^b	2.62e-12
octanoic acid	octanoic acid	Fatty, waxy, rancid	10806.03 ± 678.07 ^c	14036.47 ± 776.31 ^b	2040.5 ± 285.69 ^d	2145 ± 201.12 ^d	25047.97 ± 855.22 ^a	3.08e-12
3-methylbutanoic acid	3-methylbutanoic acid	Tropical, acid, sweaty	16.27 ± 0.95 ^b	34.37 ± 2.05 ^b	2362.73 ± 436.96 ^a	300.07 ± 34.11 ^b	304.6 ± 106.66 ^b	2.37e-07
		Σ FATTY ACIDS	12662.00	16791.98	7505.33	10485.1	29862.84	
<i>Sulphur compound</i>								
methionol	3-methylsulfanylpropan-1-ol	Vegetable, sulphurous	731.9 ± 85.81 ^b	562.74 ± 25.93 ^b	3726.5 ± 715.89 ^a	954.5 ± 190.19 ^b	861.87 ± 282.41 ^b	3.39e-06
<i>Benzoid</i>								
methyl salicylate	methyl 2-hydroxybenzoate	Vanilla, spices, herbal	0.21 ± 0.05 ^c	0.21 ± 0.04 ^c	0.28 ± 0.04 ^{bc}	0.80 ± 0.15 ^b	1.52 ± 0.41 ^a	3.53e-05
4-vinyl guaiacol	2-methoxy-4-(prop-1-en-1-yl) phenol	Spicy, clove-like	3.33 ± 0.43 ^a	4.11 ± 1.92 ^a	11.79 ± 5.62 ^a	13.20 ± 2.79 ^a	150.21 ± 7.25 ^b	0.000179

4-vinyl phenol	4-Ethenylphenol	Phenolic,pungent, and slightly sweet	2.97 ± 0.69 ^a	3.82 ± 0.98 ^a	5.85 ± 4.58 ^a	5.05 ± 0.68 ^a	375.88 ± 12.17 ^b	7.01e-15
4-ethyl guaiacol	4-ethyl-2- methoxyphenol	Smoky, spicy, and phenolic	0.01 ± 0.02 ^a	0.03 ± 0.01 ^a	0 ± 0 ^a	0.22 ± 0.03 ^b	0.06 ± 0.01 ^a	1.64e-07
4- ethyl phenol	para-ethylphenol	Strong, phenolic, smoky	0.12 ± 0.09 ¹	0 ± 0 ^a	0.08 ± 0.08 ^a	0 ± 0 ^a	0 ± 0 ^a	0.0581
<i>Norisoprenoids</i>								
β-damascenone	(E)-1-(2,6,6- trimethylcyclohexa- 1,3-dien-1-yl) but-2-en- 1-one	Natural, sweet, fruity	0.72 ± 0.12 ^b	1.04 ± 0.04 ^b	0.82 ± 0.13 ^b	0.66 ± 0.10 ^b	2.49 ± 0.69 ^a	0.000179

Mean ± standard deviation values of three independent replicates are indicated. Different letters in each row indicate significant difference according to HSD Tukey test (p<0.05). OD = Odour Descriptor