

Supplementary material - Brewing potential of the wild yeast species *Saccharomyces paradoxus*

Running head: Brewing potential of *Saccharomyces paradoxus*

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Table S1 Acetaldehyde and aroma characteristics of *S. paradoxus* wild isolates and control strains used to ferment an all-malt 15 °P wort. Fermentations were conducted at 100 ml scale at 20 °C. Values (mg l⁻¹) are mean of two independent replicates. Range shown. Values above the threshold are shown in bold.

	Sp3	Sp5	Sp6	Sp8	Sp12	Sp17	Sp20	Sp23	Lager A15	<i>S. paradoxus</i> C-09850	Ale A62	<i>S. cariocanus</i> C-15951	Threshold [38]
Acetaldehyde	13.2 ± 6.1	13.0 ± 3.0	9.4 ± 0.6	10.8 ± 0.8	19.2 ± 17.2	12.3 ± 5.7	13.3 ± 1.4	10.6 ± 1.1	42.02 ± 0.1	5.6 ± 0.9	1.3 ± 0.6	5.1 ± 0.3	25
Propanol	25.0 ± 2.9	23.1 ± 3.3	22.9 ± 0.7	21.8 ± 3.6	22.3 ± 0.1	23.8 ± 5.3	16.4 ± 1.2	18.9 ± 0.5	42.2 ± 3.0	29.6 ± 3.1	35.2 ± 0.8	32.8 ± 0.1	800
2-Methylbutanol	27.4 ± 0.9	29.5 ± 2.4	31.7 ± 1.6	29.9 ± 0.4	29.7 ± 4.6	26.1 ± 2.2	29.0 ± 2.9	54.7 ± 44.1	46.9 ± 3.4	29.0 ± 0.6	15.0 ± 0.1	47.5 ± 3.0	65
3-Methylbutanol	78.7 ± 5.6	85.5 ± 5.32	88.4 ± 5.4	82.75 ± 1.5	71.9 ± 0.8	70.3 ± 12.5	79.1 ± 6.2	119.3 ± 74.1	116.4 ± 5.0	88.5 ± 1.6	73.6 ± 0.6	148.7 ± 10.7	70
2-Methylpropanol	28.8 ± 3.4	34.3 ± 3.1	39.21 ± 4.9	35.1 ± 2.4	34.1 ± 8.2	27.7 ± 2.8	30.9 ± 4.6	63.3 ± 54.3	67.9 ± 7.6	35.3 ± 2.1	19.9 ± 0.6	82.0 ± 1.4	
2-phenylethanol	21.8 ± 3.2	28.5 ± 3.9	29.7 ± 2.0	29.1 ± 2.2	19.2 ± 1.0	18.2 ± 4.42	21.7 ± 2.9	47.4 ± 42.9	50.1 ± 1.0	27.7 ± 0.9	28.1 ± 0.1	78.4 ± 3.4	125
3-Methylbutylacetate	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.2	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.2	1.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	1.6
2-Phenylethylacetate	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	3.8
Ethylhexanoate	0.0 ± 0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0	0.0 ± 0.01	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.2
Ethyl octanoate	0.0 ± 0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.9
Ethyl decanoate	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.5
Ethyl acetate	13.3 ± 3.4	17.5 ± 0.2	18.6 ± 4.8	16.3 ± 1.3	16.9 ± 3.2	13.3 ± 1.2	11.7 ± 2.1	9.6 ± 5.8	20.5 ± 0.3	20.7 ± 2.7	26.8 ± 3.2	22.3 ± 0.1	33

Table S2 Acetaldehyde and aroma characteristics of *S. paradoxus*, wild isolates and control strains used to ferment an all-malt 15 °P wort at 15 °C and 12 °P at 20 °C. Fermentations were conducted at 2 L-scale and performed at 15 °C. Values (mg l⁻¹) are means of two independent replicates. The range is included. Values with different superscript letters under aroma compound differ significantly (p < 0.05) as determined by one-way ANOVA and Tukey's test.

15 °C	Sp3	Sp6	Sp17	<i>S. paradoxus</i> C-09850	Ale A62	Lager A15	Threshold [38]
Acetaldehyde	10.1 ± 0.1 ^{c,d}	13.9 ± 4.4 ^c	10.5 ± 2.3 ^{c,d}	57.9 ± 1.2 ^a	33.8 ± 2.5 ^b	7.9 ± 0.6 ^{c,d}	25
Propanol	11.5 ± 0.6 ^{b,c,d,e}	8.4 ± 2.4 ^{e,f}	8.1 ± 1.1 ^f	9.0 ± 0.76 ^{e,f}	13.6 ± 0.1 ^{a,b,c}	12.7 ± 0.2 ^{b,c,d}	800
2-Methylbutanol	19.2 ± 0.3 ^b	14.1 ± 2.9 ^{c,d}	12.9 ± 1.7 ^{d,e}	17.7 ± 0.5 ^{b,c}	9.2 ± 0.1 ^e	18.8 ± 0.2 ^b	65
3-Methylbutanol	37.8 ± 1.9 ^{b,c,d}	32.8 ± 5.9 ^{c,d,e}	27.6 ± 3.2 ^e	45.8 ± 1.4 ^b	28.9 ± 1.0 ^{d,e}	47.2 ± 0.5 ^b	70
2-Methylpropanol	18.0 ± 0.1 ^{b,c,d}	13.0 ± 2.3 ^{f,g}	12.0 ± 1.6 ^{g,h}	18.9 ± 0.6 ^{b,c}	8.9 ± 0.1 ^h	12.9 ± 0.2 ^{f,g}	
2-phenylethanol	11.8 ± 0.3 ^{c,d}	10.2 ± 0.7 ^{d,e}	8.3 ± 0.5 ^e	8.5 ± 0.8 ^e	5.3 ± 0.2 ^f	13.1 ± 0.2 ^c	125
3-Methylbutylacetate	0.6 ± 0.0 ^{d,e,f}	0.4 ± 0.1 ^{f,g}	0.3 ± 0.1 ^g	0.7 ± 0.1 ^{c,d,e}	0.8 ± 0.1 ^c	2.7 ± 0.1 ^a	1.6
2-Phenylethylacetate	0.1 ± 0.0 ^{d,e,f}	0.1 ± 0.0 ^{e,f}	0.1 ± 0.0 ^{e,f}	0.13 ± 0.0 ^{d,e,f}	0.1 ± 0.0 ^f	0.4 ± 0.0 ^b	3.8
Ethylhexanoate	0.4 ± 0.0 ^a	0.2 ± 0.1 ^c	0.2 ± 0.1 ^{b,c}	0.1 ± 0.0 ^c	0.4 ± 0.0 ^a	0.2 ± 0.0 ^{b,c}	0.23
Ethyl octanoate	0.1 ± 0.0 ^{d,e}	0.1 ± 0.0 ^e	0.1 ± 0.0 ^e	0.5 ± 0.0 ^e	0.2 ± 0.0 ^c	0.1 ± 0.0 ^b	0.9
Ethyl decanoate	0.1 ± 0.0 ^{b,c}	0.1 ± 0.0 ^{b,c}	0.1 ± 0.0 ^{c,d}	0.1 ± 0.0 ^{c,d}	0.0 ± 0.0 ^d	0.1 ± 0.0 ^a	1.5
Ethyl acetate	30.2 ± 0.3 ^{b,c}	16.4 ± 7.7 ^{d,e}	17.0 ± 4.6 ^{d,e}	8.0 ± 0.9 ^e	34.1 ± 3.2 ^{a,b}	43.0 ± 1.5 ^a	33

20 °C	Sp3	Sp6	Sp17	<i>S. paradoxus</i> C-09850	Ale A62	Lager A15	Threshold [38]
Acetaldehyde	9.1 ± 2.1 ^{c,d}	4.7 ± 0.6 ^d	7.1 ± 0.2 ^{c,d}	5.2 ± 0.9 ^d	30.3 ± 1.3 ^b	5.4 ± 0.8 ^d	25
Propanol	9.6 ± 0.5 ^{d,e,f}	10.9 ± 0.0 ^{c,d,e,f}	11.3 ± 0.4 ^{b,c,d,e,f}	14.4 ± 0.6 ^{a,b}	16.3 ± 0.0 ^a	12.9 ± 0.5 ^{a,b,c,d}	800
2-Methylbutanol	16.0 ± 0.9 ^{b,c,d}	18.0 ± 0.6 ^{b,c}	19.1 ± 0.1 ^b	26.3 ± 0.3 ^a	12.9 ± 0.1 ^{d,e}	17.8 ± 0.9 ^{b,c}	65
3-Methylbutanol	38.5 ± 2.1 ^{b,c}	44.8 ± 1.4 ^b	40.1 ± 0.2 ^{b,c}	65.6 ± 0.6 ^a	44.0 ± 0.6 ^b	58.1 ± 3.2 ^a	70
2-Methylpropanol	16.1 ± 1.2 ^{c,d,e,f}	17.5 ± 0.4 ^{b,c,d,e}	19.9 ± 0.1 ^b	27.9 ± 0.3 ^a	14.8 ± 0.1 ^{d,e,f,g}	14.0 ± 0.6 ^{e,f,g}	
2-phenylethanol	13.2 ± 0.9 ^c	15.4 ± 0.5 ^b	11.8 ± 0.1 ^{c,d}	24.7 ± 0.5 ^{c,d}	12.9 ± 0.4 ^c	25.4 ± 0.2 ^a	125
3-Methylbutylacetate	0.5 ± 0.0 ^{e,f,g}	0.5 ± 0.0 ^{e,f,g}	0.4 ± 0.0 ^{f,g}	0.8 ± 0.0 ^{c,d}	0.7 ± 0.0 ^{c,d}	1.9 ± 0.1 ^b	1.6
2-Phenylethylacetate	0.1 ± 0.0 ^{d,e,f}	0.2 ± 0.0 ^{d,e}	0.2 ± 0 ^{d,e}	0.3 ± 0.0 ^c	0.2 ± 0.0 ^d	0.6 ± 0.0 ^a	3.8
Ethylhexanoate	0.2 ± 0.0 ^{b,c}	0.2 ± 0.0 ^c	0.2 ± 0.0 ^c	0.1 ± 0.0 ^c	0.3 ± 0.0 ^{a,b}	0.2 ± 0.0 ^c	0.23

Ethyl octanoate	$0.2 \pm 0.0^{c,d}$	$0.2 \pm 0.0^{c,d}$	$0.2 \pm 0.0^{c,d}$	$0.1 \pm 0.0^{d,e}$	$0.2 \pm 0.0^{c,d}$	0.6 ± 0.1^a	0.9
Ethyl decanoate	$0.1 \pm 0.0^{a,b}$	$0.1 \pm 0.0^{a,b}$	$0.1 \pm 0.0^{a,b}$	$0.1 \pm 0.0^{c,d}$	0.0 ± 0.0^d	0.1 ± 0.0^a	1.5
Ethyl acetate	$18.8 \pm 1.5^{d,e}$	$18.0 \pm 0.4^{d,e}$	$23.1 \pm 0.2^{b,c,d}$	$22.5 \pm 0.9^{c,d}$	$31.1 \pm 0.7^{b,c}$	$25.1 \pm 0.9^{b,c,d}$	33

Table S3 Aroma characteristics of green beers of 40 L-scale fermentations. Values are in mg l⁻¹. Values above the threshold are shown in bold.

	A15 green beer	Sp3 green beer	Threshold [38]
Acetaldehyde	6.4	3.4	
Propanol	11.5	12.4	800
2-Methylbutanol	18.9	15.6	65
3-Methylbutanol	38.7	31.8	70
2-Methylpropanol	11.7	14.6	
2-phenylethanol	16.7	7.0	125
3-Methylbutylacetate	1.6	0.4	1.6
2-Phenylethylacetate	0.4	0.06	3.8
Ethylhexanoate	0.24	0.35	0.23
Ethyl octanoate	0.8	0.2	0.9
Ethyl decanoate	0.3	0.1	1.5
Ethyl acetate	34.4	23.7	33

Table S4 Aroma characteristics of bottled beers of 40 L-scale fermentations. Values are in mg l⁻¹.

	A15 bottled beer	Sp3 bottled beer	Threshold [38]
Propanol	4.0	7.2	800
2-Methylbutanol	7.8	9.9	65
3-Methylbutanol	16.2	20.3	70
2-Methylpropanol	6.5	6.6	
2-phenylethanol	8.4	5.3	125
3-Methylbutylacetate	0.4	0.2	1.6
2-Phenylethylacetate	0.2	0.0	3.8
Ethylhexanoate	0.1	0.1	0.23
Ethyl octanoate	0.2	0.1	0.9
Ethyl decanoate	0.1	0.1	1.5
Ethyl acetate	11.6	12.9	33

Table S6 Sensory analysis results for A15 and Sp3 beers fermented in pilot-scale (40 L) at 15 °C. The samples were evaluated on a scale from 0, “nothing” to 5, “extreme”.

	A15	Sp3
Alcoholic	0.36	0.36
Solvent-like	0.29	0.40
Estery	0.79	1.72
Fruity	0.97	1.61
Acetaldehyde	0	0.22
Floral	0.22	0.29
Hoppy	0	0.52
Resinous	0.29	0.41
Nutty	0	0.22
Grassy	0	0
Grainy	0	0.07
Malty	0.22	0.22
Worty	0.07	0.32
Caramel	0	0
Burnt	0.15	0
Phenolic	0	2.54
Fatty acid	1.34	0
Diacetyl	0	0
Rancid	0	0
Oily	0	0
Sulphury	0.22	0
Sulphitic	0	0
Sulphidic	0.54	0
Cooked vegetable	0.57	0
Yeasty	0.40	0
Stale	0	0
Catty	0	0
Papery	0.13	0
Leathery	0	0
Mouldy	0	0
Acidity	0.61	0.82
Sweet	0.68	0.68
Salty	0.15	0.32
Bitter	0.65	1.07
Mouthfeel	0.63	0.59
Alkaline	0.15	0.07
Metalic	0.15	0.07
Astringent	0.29	0.22
Powdery	0.07	0.07
Carbonation	0.15	0.72
Warming	0.61	0.65
Fullness	0.88	1.06