

Utilization of the Dicarbonyl Compounds 3- Deoxyglucosone and 3-Deoxymaltosone during Beer Fermentation by *Saccharomyces* Yeasts

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- Supplementary Material -

Table S1. Yeast strains used in current studies

species	Strain number	origin
<i>Saccharomyces pastorianus</i>	SafLager W34/70	dry beer yeast (bottom fermented), Fermentis, Marcq-en-Barceul, France
<i>Saccharomyces cerevisiae</i>	BY4741	laboratory strain, Euroscarf, Frankfurt, Germany
	DSM 1848	beer yeast (bottom fermented), freeze-dried pellet, Leibniz-Institut DSMZ, Braunschweig, Germany
	DSM 70449	beer yeast (top fermented), freeze-dried pellet, Leibniz-Institut DSMZ, Braunschweig, Germany
	Kitzinger Reinhefe Steinberg (KRS)	dry wine yeast, Paul Arauner GmbH & Co. KG, Kitzingen, Germany
	S402-OA*	yeast from natural habitat, substrate: sap flux oak origin: Württemberg, Germany sampling date: 28.12.2014
	SafAle BE-256	dry beer yeast (top fermented), Fermentis, Marcq-en-Barceul, France
	SafAle T-58	dry beer yeast (top fermented), Fermentis, Marcq-en-Barceul, France
	SafAle WB-06	dry beer yeast (top fermented), Fermentis, Marcq-en-Barceul, France
<i>Saccharomyces uvarum</i>	S648-OA*	yeast from natural habitat,

	S91-OA*	substrate: sap flux cherry tree origin: Rheinhessen, Germany sampling date: 17.03.2016 yeast from natural habitat, substrate: rotten apple origin: Württemberg, Germany sampling date: 08.02.2014
<i>Saccharomyces paradoxus</i>	S445-OA*	yeast from natural habitat, substrate: forest soil (fir) origin: Ruwertal, Germany sampling date: 22.01.2015
	S466-OA*	yeast from natural habitat, substrate: sap flux cherry tree origin: Rheinhessen, Germany sampling date: 22.01.2015
	S96-OA*	yeast from natural habitat, substrate: mummified grape origin: Württemberg, Germany sampling date: 08.02.2014

*from an earlier investigation of Brysch-Herzberg et al. [24]

Table S2. Cell counts/mL of yeast suspensions at an optical density of 5 and determination of yeast growth during incubation of yeast strains with 3-DG in Pilsner wort

strain number	cell count/mL ^[a] OD5	yeast growth 3-DG incubation ^[b] [%]
SafLager W34/70	$5.7 \times 10^7 \pm 2.6 \times 10^6$	116.8 ± 6.4
DSM 1848	$6.5 \times 10^7 \pm 1.2 \times 10^6$	116.8 ± 3.4
DSM 70449	$6.8 \times 10^7 \pm 1.4 \times 10^6$	125.0 ± 5.6
SafAle T-58	$5.1 \times 10^7 \pm 9.2 \times 10^6$	95.6 ± 6.4
SafAle WB-06	$6.4 \times 10^7 \pm 1.5 \times 10^6$	96.4 ± 3.7
SafAle BE-256	$5.1 \times 10^7 \pm 1.4 \times 10^6$	108.1 ± 7.4
Steinberg	$4.9 \times 10^7 \pm 1.1 \times 10^6$	85.9 ± 8.7
S648-OA	$7.3 \times 10^7 \pm 1.9 \times 10^6$	88.2 ± 6.6
S91-OA	$6.3 \times 10^7 \pm 1.7 \times 10^6$	93.8 ± 1.5
S96-OA	$6.0 \times 10^7 \pm 1.6 \times 10^6$	81.5 ± 3.5
S466-OA	$4.7 \times 10^7 \pm 1.2 \times 10^6$	98.4 ± 13.2
S445-OA	$6.1 \times 10^7 \pm 1.2 \times 10^6$	87.2 ± 6.5
S402-OA	$7.1 \times 10^7 \pm 1.6 \times 10^6$	89.8 ± 6.7
BY4741	$5.9 \times 10^7 \pm 2.0 \times 10^6$	80.9 ± 4.5

[a] Data in mean value \pm SD (n = 6). [b] Percentage yeast growth is calculated as OD600 of the incubated mixture after 24 h incubation divided by the OD600 of the included yeast blank without addition of 3-DG, data in mean value \pm SD (n = 6).

Table S3. Relative amounts of extracellular (EZ) and intracellular (IZ) 3-DG and its metabolites 3-DF and 3-DGA following incubation of the yeast strains in the presence of 0.25 mM 3-DG for 24 h at 30 °C in Pilsner wort.^[a]

Strain number	3-DG [%]	3-DF [%]	3-DG [%]	3-DG [%]	3-DF [%]	3-DGA [%]
	EZ	EZ	adsorbed	IZ	IZ	IZ
SafLager W34/70	88.4 ± 4.4	-	-	11.8 ± 0.1	-	0.8 ± 0.6
DSM 1848	58.8 ± 2.3	38.5 ± 5.6	-	9.4 ± 0.1	1.9 ± 0.4	0.5 ± 0.0
DSM 70449	62.0 ± 0.6	27.2 ± 1.8	-	9.8 ± 0.2	4.0 ± 0.2	0.5 ± 0.2
SafAle T-58	55.8 ± 2.5	51.4 ± 6.4	6.9 ± 0.2	12.0 ± 0.1	3.9 ± 0.3	1.0 ± 0.2
SafAle WB-06	74.4 ± 2.0	9.3 ± 6.4	-	11.3 ± 0.2	3.9 ± 0.6	1.3 ± 0.4
SafAle BE-256	70.2 ± 1.6	3.9 ± 1.6	24.8 ± 9.3	9.6 ± 0.0	0.3 ± 0.2	0.4 ± 0.1
Steinberg	36.8 ± 2.3	24.1 ± 1.5	-	12.2 ± 0.0	27.0 ± 1.4	0.8 ± 0.1
S648-OA	79.9 ± 1.0	5.8 ± 2.3	8.8 ± 2.0	12.2 ± 0.4	10.1 ± 0.1	0.7 ± 0.0
S91-OA	86.7 ± 3.5	4.2 ± 2.5	6.6 ± 4.9	10.6 ± 0.2	-	0.3 ± 0.0
S96-OA	83.4 ± 3.8	17.4 ± 4.5	-	9.2 ± 0.1	4.3 ± 2.3	0.5 ± 0.1
S466-OA	86.6 ± 5.0	11.5 ± 6.4	13.5 ± 5.2	8.3 ± 0.0	3.6 ± 1.9	0.2 ± 0.1
S445-OA	88.4 ± 0.9	8.6 ± 2.5	5.9 ± 1.0	4.9 ± 0.5	3.0 ± 1.3	0.3 ± 0.2
S402-OA	68.7 ± 0.6	8.0 ± 2.7	16.6 ± 4.4	12.0 ± 0.0	9.4 ± 0.2	0.7 ± 0.3
BY4741	91.6 ± 1.1	1.9 ± 0.4	0.6 ± 0.2	0.7 ± 0.1	0.3 ± 0.1	0.1 ± 0.0

[a] Data in mean value ± SD (n = 3).

Table S4. Determined percentages of dead cells in Pilsner wort spiked with 0.25 mM 3-DG as well as yeast blanks (YB) after 24 h relative to the total cell count.^[a]

strain number	3-DG incubation		
	YB [%]	Samples with 0.25 mM 3-DG [%] ^[b]	Total dead cell number [%] ^[c]
SafLager W34/70	28.0 ± 7.3	29.1 ± 5.8	0.92 ± 5.9
DSM 1848	10.2 ± 1.8	9.1 ± 1.3	-1.1 ± 1.0
DSM 70449	10.6 ± 4.6	16.9 ± 8.0	6.3 ± 4.4
SafAle T-58	17.1 ± 6.9	18.5 ± 6.1	1.0 ± 2.4
SafAle WB-06	13.3 ± 2.1	13.9 ± 4.6	0.5 ± 5.6
SafAle BE-256	11.9 ± 6.8	17.4 ± 5.2	6.0 ± 1.7
Steinberg	8.9 ± 1.6	17.3 ± 2.8	8.4 ± 3.0
S648-OA	14.6 ± 7.4	26.1 ± 6.0	11.7 ± 3.2
S91-OA	17.1 ± 4.6	32.4 ± 7.6	15.3 ± 4.3
S96-OA	11.9 ± 4.0	22.6 ± 3.5	11.2 ± 0.9
S466-OA	14.1 ± 7.3	31.6 ± 7.4	17.7 ± 0.9
S445-OA	10.8 ± 2.0	23.3 ± 4.4	12.6 ± 1.3
S402-OA	11.6 ± 2.4	24.5 ± 2.1	12.8 ± 2.1
BY4741	10.2 ± 6.1	26.2 ± 2.6	16.0 ± 4.0

[a] Data in mean value ± SD (n = 6). [b] Without subtraction of YB. [c] With subtraction of YB.

Table S5. Concentrations in $\mu\text{mol/L}$ of 3-DG, 3-DF and 3-DM during fermentation experiments with selected yeast strains in two different worts for 14 days at RT.^[a]

Fermentation sample	Day	3-DG [μM]	3-DF [μM]	3-DM [μM]
Pilsner wort				
Pilsner wort	0	261.6 ± 2.8	-	27.5 ± 0.2
	1	251.4 ± 1.4	-	26.1 ± 0.1
	2	254.5 ± 0.3	-	25.9 ± 0.2
	3	252.0 ± 1.5	-	25.3 ± 0.1
	5	252.8 ± 1.4	-	23.8 ± 0.0
	7	249.9 ± 2.2	-	23.2 ± 0.1
	14	260.7 ± 1.8	-	23.0 ± 0.1
<i>S. cerevisiae</i> T-58	0	267.8 ± 0.6	-	26.1 ± 0.3
	1	257.9 ± 0.1	-	23.9 ± 0.6
	2	240.2 ± 7.2	11.5 ± 0.2	23.7 ± 0.3
	3	225.9 ± 2.5	28.3 ± 0.0	19.2 ± 0.3
	5	203.2 ± 5.2	67.4 ± 2.8	11.9 ± 0.2
	7	186.7 ± 5.4	73.5 ± 0.9	11.6 ± 0.2
	14	164.2 ± 9.2	77.9 ± 1.3	10.1 ± 0.2
<i>S. pastorianus</i> w34/70	0	258.2 ± 2.0	-	26.9 ± 0.4
	1	257.0 ± 2.1	-	26.6 ± 0.3
	2	245.8 ± 3.0	-	25.1 ± 0.5
	3	239.1 ± 3.6	10.2 ± 0.2	20.6 ± 0.2
	5	225.6 ± 3.9	57.9 ± 1.8	17.7 ± 0.4
	7	212.4 ± 6.7	80.7 ± 1.3	16.5 ± 0.2
	14	194.1 ± 0.3	78.7 ± 0.5	14.8 ± 0.1
<i>S. paradoxus</i> S445-OA	0	253.1 ± 0.4	-	22.3 ± 1.1
	1	253.4 ± 1.6	-	22.2 ± 0.2
	2	254.2 ± 0.4	7.4 ± 0.4	22.1 ± 0.5
	3	248.2 ± 1.4	11.5 ± 0.5	21.5 ± 0.8
	5	239.4 ± 5.2	14.5 ± 0.2	20.4 ± 0.5
	7	231.0 ± 6.0	14.6 ± 0.0	20.2 ± 1.1
	14	214.1 ± 6.4	13.9 ± 0.7	18.7 ± 0.3
<i>S. cerevisiae</i> BY4741	0	246.4 ± 2.1	-	28.2 ± 0.7

Fermentation sample	Day	3-DG [μ M]	3-DF [μ M]	3-DM [μ M]
	1	260.6 \pm 0.2	-	28.0 \pm 1.6
	2	252.0 \pm 2.3	-	24.4 \pm 0.5
	3	248.6 \pm 6.1	7.5 \pm 0.4	22.8 \pm 0.2
	5	242.5 \pm 9.9	16.3 \pm 0.9	23.1 \pm 0.1
	7	238.2 \pm 0.4	20.0 \pm 0.4	20.4 \pm 0.2
	14	235.8 \pm 2.5	20.4 \pm 1.0	18.9 \pm 0.0
Dark beer wort				
Dark beer wort	0	581.8 \pm 7.7	-	255.4 \pm 10.5
	1	576.0 \pm 9.9	-	253.6 \pm 1.8
	2	573.4 \pm 6.4	-	228.9 \pm 4.3
	3	574.9 \pm 20.8	-	263.8 \pm 1.5
	5	572.1 \pm 8.5	-	255.9 \pm 2.1
	7	571.7 \pm 0.7	-	265.0 \pm 0.6
	14	582.9 \pm 1.1	-	279.1 \pm 1.1
<i>S. cerevisiae</i> T-58	0	548.7 \pm 5.0	-	231.8 \pm 1.1
	1	560.5 \pm 4.1	-	206.2 \pm 16.4
	2	547.0 \pm 16.0	29.4 \pm 0.6	142.7 \pm 2.9
	3	520.4 \pm 1.0	69.9 \pm 4.5	71.8 \pm 1.6
	5	489.1 \pm 6.1	181.3 \pm 0.4	40.3 \pm 2.6
	7	461.9 \pm 11.6	201.3 \pm 0.9	36.1 \pm 0.7
	14	467.9 \pm 0.0	213.0 \pm 2.0	35.2 \pm 0.1
<i>S. pastorianus</i> w34/70	0	553.4 \pm 10.5	-	227.1 \pm 13.5
	1	531.6 \pm 0.5	-	231.0 \pm 4.4
	2	560.8 \pm 8.9	-	208.0 \pm 3.7
	3	520.0 \pm 0.5	17.9 \pm 0.6	129.9 \pm 1.9
	5	512.3 \pm 0.7	126.6 \pm 3.5	63.9 \pm 0.9
	7	458.6 \pm 5.8	160.3 \pm 4.0	49.4 \pm 1.1
	14	457.8 \pm 3.0	196.6 \pm 9.3	40.7 \pm 3.0
<i>S. paradoxus</i> S445-OA	0	578.4 \pm 10.9	-	232.6 \pm 6.6
	1	557.1 \pm 3.7	-	204.3 \pm 21.0
	2	546.6 \pm 3.5	16.6 \pm 0.8	127.5 \pm 1.7
	3	545.9 \pm 3.8	27.1 \pm 0.0	102.9 \pm 2.4
	5	498.6 \pm 3.5	42.2 \pm 0.9	75.7 \pm 0.3

Fermentation sample	Day	3-DG [μ M]	3-DF [μ M]	3-DM [μ M]
	7	486.5 \pm 19.2	48.5 \pm 0.5	73.3 \pm 1.5
	14	471.9 \pm 10.4	48.3 \pm 0.1	74.9 \pm 2.2
<i>S. cerevisiae</i> BY4741	0	551.5 \pm 5.5	-	247.3 \pm 7.0
	1	534.8 \pm 10.0	-	222.8 \pm 1.8
	2	538.0 \pm 5.6	-	139.3 \pm 1.9
	3	555.6 \pm 21.3	14.7 \pm 0.3	77.5 \pm 1.9
	5	534.2 \pm 3.0	32.7 \pm 0.0	73.8 \pm 0.7
	7	506.5 \pm 6.3	37.5 \pm 0.0	69.7 \pm 2.0
	14	510.7 \pm 0.2	42.6 \pm 2.4	70.2 \pm 5.2

[a] Data are given as mean value \pm SD (n = 2)

Table S6. Intracellular concentrations of 3-DG, its metabolites 3-DF and 3-DGA, and 3-DM following fermentation experiments of selected yeast strains in the presence of 0.25 mM 3-DG / 0.02 mM 3-DM in Pilsner wort and 0.5 mM 3-DG / 0.25 mM 3-DM in Dark beer wort for 14 days at RT.^[a]

Fermentation sample	3-DG [μ M]	3-DF [μ M]	3-DGA [μ M]	3-DM [μ M]
Pilsner wort				
<i>S. cerevisiae</i> T-58	5.2 \pm 0.4	2.1 \pm 0.2	1.1 \pm 0.1	-
<i>S. pastorianus</i> w34/70	5.0 \pm 0.1	2.1 \pm 0.1	1.3 \pm 0.2	-
<i>S. paradoxus</i> S445-OA	5.8 \pm 0.2	0.5 \pm 0.0	0.9 \pm 0.1	-
<i>S. cerevisiae</i> BY4741	5.9 \pm 0.9	0.7 \pm 0.1	1.3 \pm 0.1	-
Dark beer wort				
<i>S. cerevisiae</i> T-58	8.3 \pm 1.2	5.1 \pm 0.8	1.3 \pm 0.2	1.0 \pm 0.1
<i>S. pastorianus</i> w34/70	6.9 \pm 0.8	4.2 \pm 0.6	1.0 \pm 0.1	1.0 \pm 0.0
<i>S. paradoxus</i> S445-OA	7.9 \pm 0.8	1.1 \pm 0.1	1.3 \pm 0.1	2.0 \pm 0.3
<i>S. cerevisiae</i> BY4741	7.7 \pm 0.6	0.9 \pm 0.1	0.8 \pm 0.0	0.8 \pm 0.1

[a] Data in mean value \pm SD (n = 3).

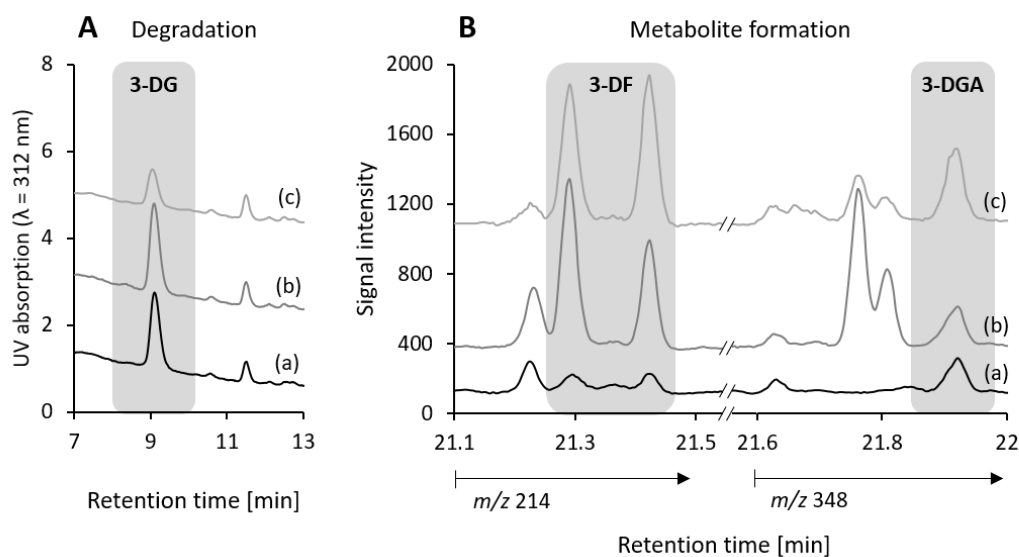


Figure S1. (A) RP-HPLC with UV detection at 312 nm of the supernatants of (a) the 3-DG stability blank (SB) after 24 h of incubation in pilsner wort (black line), (b) the *Saccharomyces cerevisiae* beer yeast T-58 with added 3-DG (0.25 mM) after 0 h (dark grey line) and (c) after 24 h (light grey line) of incubation in pilsner wort. (B) GC-MS (selected ion monitoring at m/z 214 and 348) of (a) a standard mix consisting of 2 μ M of each 3-DF and 3-DGA in pilsner wort (black line), (b) the extracellular supernatants of the *Saccharomyces cerevisiae* beer yeast T-58 after 24 h of incubation with 0.25 mM 3-DG in pilsner wort (dark grey line) and (c) the extracellular supernatants of the *Saccharomyces cerevisiae* beer yeast T-58 after cell disruption of the 24 h incubation samples (light grey line). Samples were prepared for measurement by oximation and silylation.