Supplementary Tables and Figures for

Interactions Between Fermentation Temperature and Yeast Strain: Impacts on Polyfunctional Thiol Release and Beer Aroma

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Supplementary Table S1. Analyzed compound names and abbreviations.

Category	Name	Abbreviation	Vendor	Purity
Free Thiols	3-sulfanylhexan-1-ol	3SH	Merck	98 %
	3-sulfanylhexan-1-ol acetate	3SHA	Merck	98 %
	4-methyl-4-sulfanylpentan-2-one	4MSP	Merck	98 %
			Synthesized in the laboratory according to:	
	3-sulfanyl-4-methylpentan-1-ol	3S4MP	Takoi et al., 2009	98 %
	deuterated 3-sulfanylhexan-1-ol	3SH-d_2	Kotseridis et al., 2000	>85 % *
	deuterated 3-sulfanylhexan-1-ol acetate	3SHA-d_5		
	deuterated 4-methyl-4-sulfanylpentan-2-one	4MSP-d_{10}		
Thiol Precursors			Synthesized in the laboratory according to:	
	cysteine-3SH	C3SH	Dagan et al., 2006	>77 % *
	deuterated cysteine-3SH	C3SH-d_2		
	cysteinylglycine-3SH	CG3SH	Bonnaffoux et al., 2017; Fedrizzi et al., 2012	>87 % *
	deuterated cysteinylglycine-3SH	CG3SH-d_2		
	γ-glutamylcysteine-3SH	GC3SH		
	deuterated γ-glutamylcysteine-3SH	GC3SH-d_2		
	gluthation-3SH	G3SH	Roland, Schneider, Guernevé, et al., 2010	
	deuterated gluthation-3SH	G3SH-d_2		
	cysteine-4MSP	C4MSP	Roland, Schneider, Razungles, et al., 2010	>77 % *
	deuterated cysteine-4MSP	C4MSP-d_{10}		
	gluthation-4MSP	G4MSP	Hebditch et al., 2007	>87 % *
	deuterated gluthation-4MSP	G4MSP-d_{10}		
Fermentation Compounds	2-phenylethanol	2PHEN	Merck	99 %
	hexyl acetate	HEAC	Merck	99 %
	isoamyl acetate	IAAC	Merck	99 %
	2-phenylethyl acetate	2PHENAC	Merck	99 %
	ethyl decanoate	ETDEC	Merck	99 %
	ethyl hexanoate	ETHEX	Merck	98 %
	ethyl octanoate	ETOCT	Merck	98 %
	ethyl butanoate	ETBU	Merck	99 %
	ethyl 2-hydroxypropanoate	2HPE	Merck	99 %
	ethyl 3-hydroxybutanoate	3HBE	Merck	99 %
	ethyl 2-methylbutanoate	2MBE	Merck	99 %
	ethyl 2-methylpropanoate	2MPE	Merck	99 %
	ethyl 2-hydroxyisocaproate	2HICE	Merck	96 %
C13-norisoprenoids	1,1,6-trimethyl-1,2-dihydronaphtalene	TDN	Synthesized in the laboratory according to: Dobrydnev et al., 2020	90 %

	α-ionone	AION	Merck	96 %
	β-damascenone	BDAM	Merck	99 %
	β-ionone	BION	Merck	97 %
Terpenes	linalool	LIN	Merck	98 %
	nerol	NER	Merck	98 %
	geraniol	GER	Merck	98 %
	citronellol	CIT	Merck	96 %
	alpha terpineol	ATER	Merck	90 %
	cis-rose oxide	C-ROOX	Merck	99 %
	trans-rose oxide	T-ROOX	Merck	99 %
Sulfur compounds	dimethyl sulfide	DMS	Merck	98 %

* The purity of the natural and deuterated synthetic compounds was assessed by ^{1}H NMR according to Ferreira-Lima et al., 2016. Since purity may vary between batches, only the lowest purity ever obtained is reported. The exact purity of each batch used was accounted for in calibration and quantitation.

Supplementary Table S2. Lexicon and sensory training standards for descriptive analysis.

Descriptor	Training standard	Brand
Fruity	Fruit cocktail cup (peaches, pear, pineapple, cherry, in fruit juice)	Essential Everyday®
Citrus	Grapefruit peel	n/a
Apple/Pomme	Red + green apple	n/a
Stone fruit	Frozen sliced peaches	Essential Everyday®
Melon	Honeydew	Fresh Garden Highway™
Tropical	Canned tropical mix + canned jackfruit [b]	Dole® (tropical mix), Aroy-D (jackfruit)
White wine	Sauvignon blanc	Whitehaven, Marlborough New Zealand
Grassy/Herbaceous	Fresh parsley + fresh thyme	n/a
Overripe fruit	Ripened banana peels	n/a
Coconut	Coconut milk	n/a
Floral/Perfume	Lavender oil	Majestic Pure™
Sweet aromatic	Marshmallow	Kraft
Malty	10% dried malt extract in water	Briess Malt & Ingredients Co.
Herbal tea	Black tea	Lipton®
Earthy	(none) [a]	n/a
Diacetyl/Buttery	Diacetyl working solution (0.1% in water)	Sigma-Aldrich®
DMS/Vegetal	Canned green beans + canned corn	Essential Everyday®
Sulfur/Egg	(none) [a]	n/a

[a] Training standards were not provided for Earthy and Sulfur/Egg; descriptors were verbally discussed during training.
[b] Two standards were presented separately for Tropical: canned tropical mixed fruit and canned jackfruit.

Supplementary Table S3. Beer chemistry analysis.

Yeast strain	Fermentation temperature	pH	BU (abs@275*50)	Iso α-acid (ppm)	ABV%	RDF%
BRY-97™	15°C	4.54	36	28.1	5.14	65.77
	22°C	4.45	35.1	27.9	5.01	66.27
	30°C	4.27	29.1	24.1	4.88	64.44
Diamond™	15°C	4.28	28.8	24.3	4.93	63.49
	22°C	4.27	31.9	26	5.00	64.29
	30°C	4.19	30.1	21.7	4.83	63.27
London™	15°C	4.41	34.3	26	4.07	53.34
	22°C	4.44	30.7	21.8	4.11	54.15
	30°C	4.41	31.2	21.7	4.10	53.44
Nottingham™ (Rep1)	15°C	4.31	33.6	25.6	4.92	65.13
	22°C	4.23	33.6	25.5	4.99	66.35
	30°C	4.25	30.5	22.4	4.79	74.04
Nottingham™ (Rep2)	15°C	4.35	32.7	28.7	4.90	65.36
	22°C	4.26	30.6	25.5	4.87	67.71
	30°C	4.21	26.9	20	4.93	64.54
Verdant IPA™	15°C	4.45	31.5	25.8	5.02	64.37
	22°C	4.39	36.1	27.2	4.89	66.05
	30°C	4.37	35.9	24.7	4.88	63.83

Supplementary Table S4. Frequencies of CATA sensory analysis descriptors. [a]

Sample		Fruity	Malty	Tropical	Sweet aromatic	Diacetyl/ Buttery	White wine	Apple/ Pomme	DMS/ Vegetal	Citrus	Floral/ Perfume	Overripe fruit	Sulfur/ Egg	Grassy/ Herbaceous	Stone fruit
BRY-97™	15°C	15	10	5	8	9	4	3	4	3	4	3	6	3	2
	22°C	18	12	6	5	3	5	7	4	6	3	3	1	2	7
	30°C	15	11	8	5	3	8	5	5	2	9	3	1	4	4
Diamond™	15°C	16	10	4	8	6	7	5	7	6	3	5	5	4	3
	22°C	12	7	3	4	1	8	7	6	2	3	4	8	3	3
	30°C	13	6	7	7	2	7	9	9	6	5	7	10	3	2
London™	15°C	16	6	1	10	12	2	5	3	2	5	4	1	5	3
	22°C	14	8	4	5	5	5	3	5	4	3	4	6	2	2
	30°C	14	8	5	4	7	5	3	4	7	2	4	2	6	2
Nottingham™ Rep1	15°C	16	9	3	6	4	2	6	9	4	6	3	2	3	2
	22°C	18	13	6	6	4	6	5	4	7	4	4	3	5	2
	30°C	15	13	5	3	6	8	6	5	7	4	5	3	4	2
Nottingham™ Rep2	15°C	17	5	10	4	7	8	2	3	4	5	5	1	3	3
	22°C	17	8	3	2	5	4	3	4	6	3	7	4	4	2
	30°C	17	10	8	7	6	3	4	4	6	8	4	4	3	2
Verdant IPA™	15°C	10	8	8	8	6	5	3	6	4	4	7	3	2	1
	22°C	15	14	9	3	3	3	7	6	6	7	1	2	3	4
	30°C	14	8	9	6	8	6	7	1	5	6	4	2	1	4
	SUM	272	166	104	101	97	96	90	89	87	84	77	64	60	50
	% of Max	100%	61%	38%	37%	36%	35%	33%	33%	32%	31%	28%	24%	22%	18%

[a] Frequency usage of CATA descriptive sensory analysis of twenty-five trained panelists (16 M, 9 F) are shown with descriptors sorted by percent of usage compared to the maximum used descriptor, *Fruity*. Panelists underwent one group training sessions before two separate days of ortho- and retro-nasal evaluations of eighteen beers. Descriptors *Herbal tea, Earthy, Melon, Coconut* not included in table or in subsequent analysis due to lower frequency of usage compared to the maximum frequency - i.e. 17%, 13%, 12%, 10% respectively.

Supplementary Table S5. Free thiols and thiol precursors in beers, wort, and hops.[a]

	Free Thiols (ng/L)				Thiol Precursors (µg/L)					
Sample	3SH	3S4MP	3SHA	4MSP	C3SH	CG3SH	GC3SH	G3SH	C4MSP	G4MSP
BRY (15°C)	26	18	nd	5	29	15	15	169	nd	nd
DIA (15°C)	81	23	nd	11	44	4	17	71	nd	nd
LON (15°C)	24	11	nd	7	51	4	16	129	nd	nd
NOT.R1 (15°C)	35	16	nd	7	28	5	22	156	nd	nd
NOT.R2 (15°C)	27	12	nd	7	28	4	22	149	nd	nd
VER (15°C)	38	17	nd	6	30	13	23	142	nd	nd
BRY (22°C)	39	20	nd	6	27	10	14	164	nd	nd
DIA (22°C)	124	24	nd	12	51	3	17	18	nd	nd
LON (22°C)	47	22	nd	9	57	4	25	79	nd	nd
NOT.R1 (22°C)	65	29	nd	11	13	2	32	117	nd	nd
NOT.R2 (22°C)	59	30	nd	10	14	4	27	115	nd	nd
VER (22°C)	58	20	nd	8	25	8	35	105	nd	nd
BRY (30°C)	55	23	nd	6	24	6	14	159	nd	nd
DIA (30°C)	162	20	nd	10	49	6	19	13	nd	nd
LON (30°C)	86	20	nd	10	39	3	27	46	nd	nd
NOT.R1 (30°C)	76	25	nd	11	12	3	61	70	nd	nd
NOT.R2 (30°C)	72	15	nd	8	13	4	70	72	nd	nd
VER (30°C)	69	20	nd	8	20	12	81	96	nd	nd
Wort no hops R1	nd	nd	nd	nd	2	88	5	31	nd	nd
Wort no hops R2	nd	nd	nd	nd	2	90	7	31	nd	nd
Wort no hops R3	nd	nd	nd	nd	2	88	6	30	nd	nd
Wort hopped R1	nd	nd	nd	4	7	116	15	186	nd	nd
Wort hopped R2	nd	nd	nd	4	6	114	14	176	nd	nd
Wort hopped R3	nd	nd	nd	3	6	111	13	176	nd	nd
	Free Thiols (ng/g)				Thiol Precursors (µg/g)					
Sample	3SH	3S4MP	3SHA	4MSP	C3SH	CG3SH	GC3SH	G3SH	C4MSP	G4MSP
Hops	1.83	3.09	nd	2.03	0.75	2.32	2.16	34	0.01	nd

[a] nd indicates not detected. Precursors C4MSP and G4MSP were analyzed but not detected in beer and wort (LOD/LOQ = 0.1/0.3 ng/L). In the Cascade T90 hop pellets, C4MSP was detected (10 µg/kg), but not G4MSP. Three wort samples (Rep 1, 2, 3) taken from three different brew days (spanning early, mid, and late brew days during experimental brewing trials) were randomly selected for analysis.

Supplementary Table S6. Fermentation compounds in beers. [a]

Sample		Fermentation Compounds (µg/L)												
		2PHEN	HEAC	IAAC	2PHENAC	ETDEC	ETHEX	ETOCT	ETBU	2HPE	3HBE	2MBE	2MPE	2HICE
BRY-97™	15°C	31839	nd	332	101	152	80	140	144	nd	44	nd	27	nd
	22°C	43066	nd	432	139	156	69	137	85	nd	52	nd	21	nd
	30°C	40724	nd	398	138	107	79	118	78	nd	46	nd	30	nd
Diamond™	15°C	24319	nd	832	208	163	197	180	88	nd	55	nd	14	nd
	22°C	26265	nd	872	239	130	111	146	79	nd	45	nd	16	nd
	30°C	17129	nd	706	160	201	103	117	73	nd	50	nd	29	nd
London™	15°C	15500	nd	423	114	121	162	170	78	nd	57	nd	10	nd
	22°C	42180	nd	706	284	146	109	156	90	nd	49	nd	13	nd
	30°C	45920	nd	660	289	191	90	118	62	nd	48	nd	17	nd
Nottingham™ Rep1	15°C	40108	nd	819	267	55	107	157	122	nd	46	nd	12	nd
	22°C	55229	nd	1195	555	331	85	148	112	nd	51	nd	15	nd
	30°C	49994	nd	830	362	167	88	117	259	nd	57	nd	21	nd
Nottingham™ Rep2	15°C	42196	nd	1028	364	125	95	149	122	nd	42	nd	15	nd
	22°C	55490	nd	1149	510	61	108	134	131	nd	48	nd	18	nd
	30°C	54146	nd	1087	513	138	78	137	101	nd	51	nd	26	nd
Verdant IPA™	15°C	9826	nd	572	53	139	130	188	196	nd	52	nd	22	nd
	22°C	41464	nd	740	208	149	111	139	104	nd	51	nd	24	nd
	30°C	37213	nd	462	112	162	108	145	263	nd	55	nd	26	nd

[a] nd indicates not detected.

Supplementary Table S7. C13-norisoprenoids, terpenes, dimethyl sulfide concentrations in beers.[a]

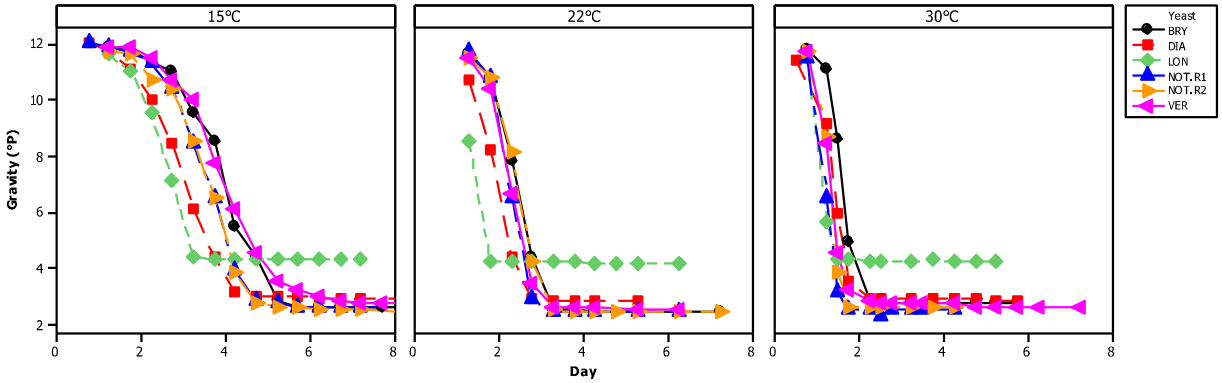
		C13-norisoprenoids (µg/L)				Terpenes (µg/L)							Dimethyl Sulfide (µg/L)
Sample		TDN	AION	BDAM	BION	LIN	NER	GER	CIT	ATER	C-ROOX	T-ROOX	DMS
BRY-97™	15°C	0.06	nd	0.75	0.03	152.9	17.9	29.5	86.2	9.9	nd	nd	88
	22°C	0.06	nd	0.91	0.03	147.9	11.7	21.5	61.8	10.5	nd	nd	91
	30°C	0.05	nd	1.13	0.04	143.6	17.3	32.2	53.3	11.9	nd	nd	57
Diamond™	15°C	0.06	nd	0.99	0.03	156.2	14.2	25.4	64.1	10.6	nd	nd	71
	22°C	0.05	nd	1.11	0.02	152.0	9.1	17.0	71.7	10.5	nd	nd	69
	30°C	0.06	nd	1.47	0.03	151.2	16.6	26.7	77.8	11.5	nd	nd	55
London™	15°C	0.06	nd	0.79	0.02	148.4	8.0	15.5	37.3	9.8	nd	nd	114
	22°C	0.05	nd	0.78	0.03	152.1	7.0	12.9	47.8	10.6	nd	nd	84
	30°C	0.05	nd	0.96	0.04	150.5	14.7	26.9	61.2	11.4	nd	nd	38
Nottingham™ Rep1	15°C	0.06	nd	0.91	0.03	150.9	13.2	23.9	49.8	10.4	nd	nd	126
	22°C	0.06	nd	1.19	0.03	146.4	8.6	17.2	46.7	9.8	nd	nd	78
	30°C	0.06	nd	1.46	0.05	141.5	11.2	20.6	51.1	10.1	nd	nd	43
Nottingham™ Rep2	15°C	0.06	nd	0.93	0.03	152.2	12.9	19.4	41.1	9.7	nd	nd	145
	22°C	0.06	nd	1.12	0.02	152.7	11.0	20.3	53.2	11.2	nd	nd	68
	30°C	0.06	nd	1.48	0.04	146.9	13.3	23.2	52.4	11.0	nd	nd	35
Verdant IPA™	15°C	0.06	nd	0.88	0.04	155.3	15.2	27.2	62.8	10.0	nd	nd	97
	22°C	0.06	nd	0.93	0.02	145.7	10.7	19.4	62.1	9.3	nd	nd	52
	30°C	0.05	nd	1.17	0.04	152.3	18.4	29.8	72.8	11.1	nd	nd	57

[a] nd indicates not detected.

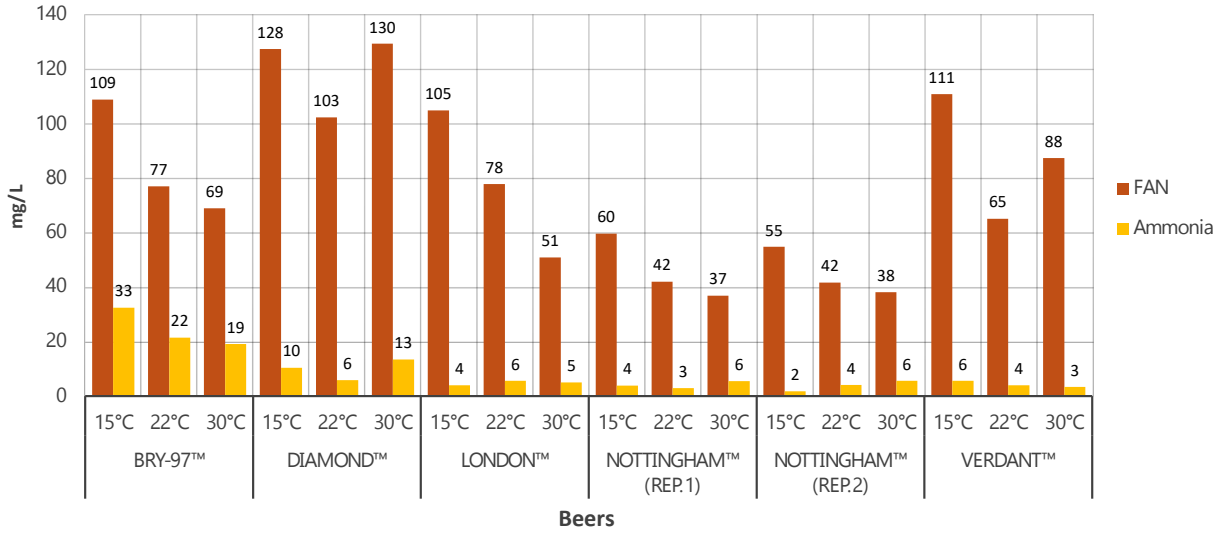
Supplementary Table S8. Analysis of variance F-values for temperature and yeast effects on free thiol expression plus Tukey HSD groupings.[a]

	3SH	4MSP	3S4MP
Temperature Effect	30.56 (p <0.001)	12.25 (p = 0.002)	5.44 (p = 0.025)
Yeast Effect	22.94 (p <0.001)	12.10 (p = 0.001)	0.90 (p = 0.517)
Groupings: 15/22/30°C	C / B / A	B / A / A	B / A / AB
Groupings: BRY / DIA / LON / NOT.R1 / NOT.R2 / VER	B / A / B / B / B / B	C / A / AB / AB / B / BC	A / A / A / A / A / A

[a] Two-way ANOVA of temperature and yeast effects with resulting F-values (and p-values in parenthesis). Tukey HSD means comparisons grouping results are indicated by letters A, B, C; different letters indicate statistically different grouping at an a of 0.05. Mean thiol concentrations per comparison are grouped in A (highest concentration), followed by B, then C (lowest concentration).



Supplementary Figure S1. Fermentation curves of gravity measurements in degrees Plato.



Supplementary Figure S2. Free amino acid (FAN) and ammonia analysis of beers. The starting wort prior to fermentation averaged 237 mg/L FAN and 61 mg/L ammonia.