Optimizing hop aroma in beer dry hopped with Cascade utilizing glycosidic enzymes

Young Scientist Symposium – Chico, CA 2016

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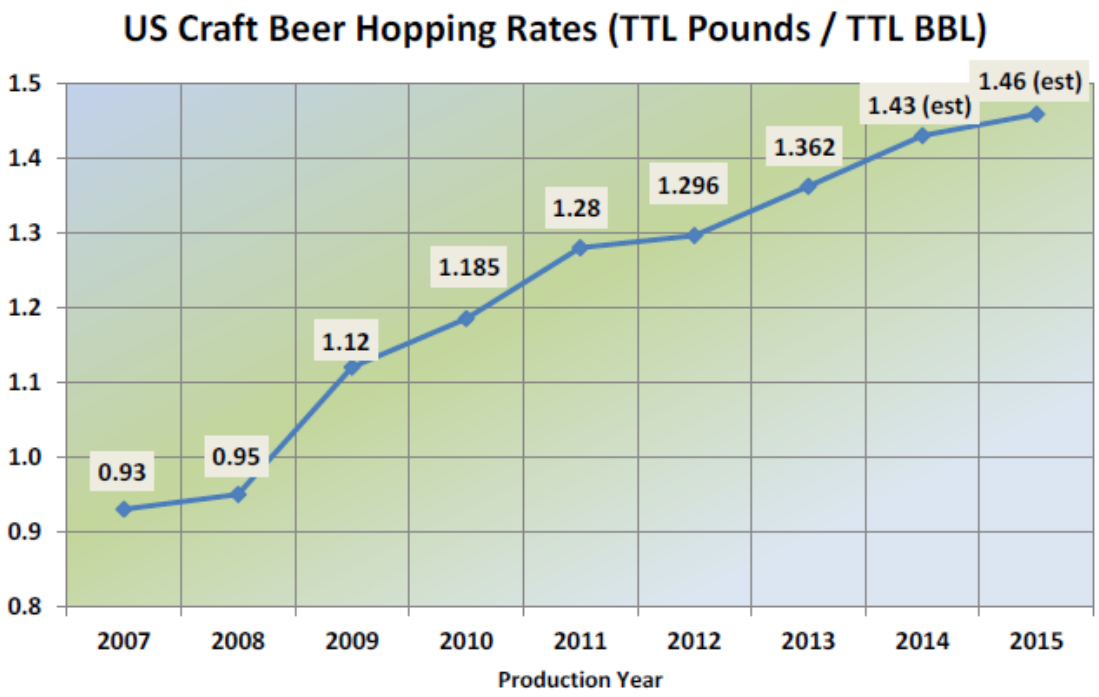
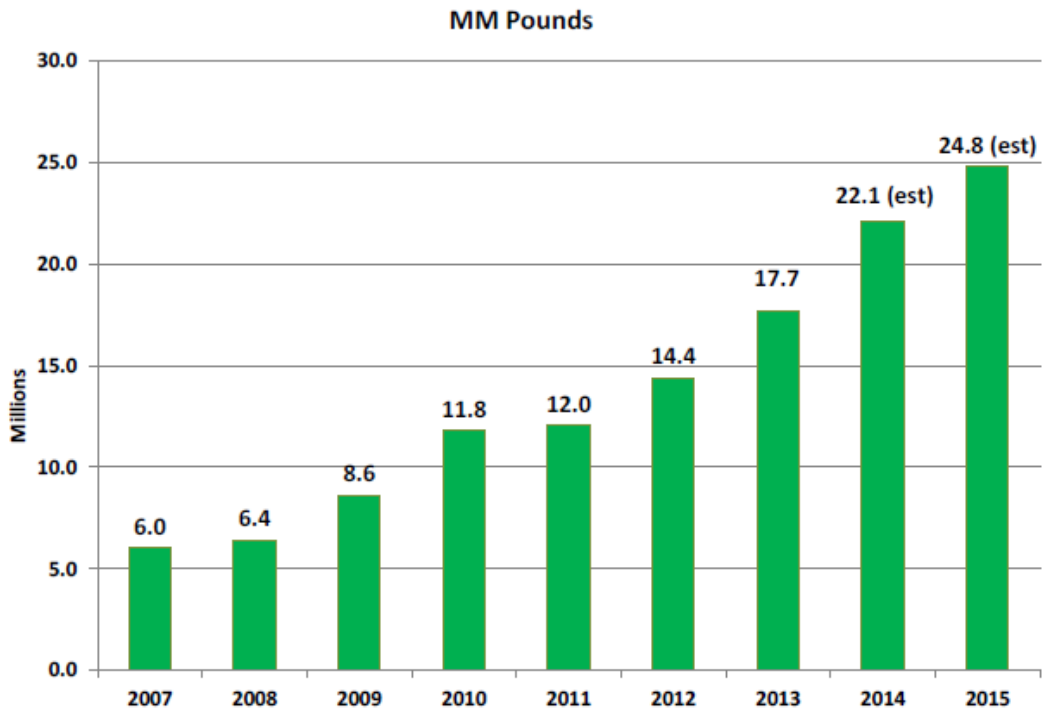
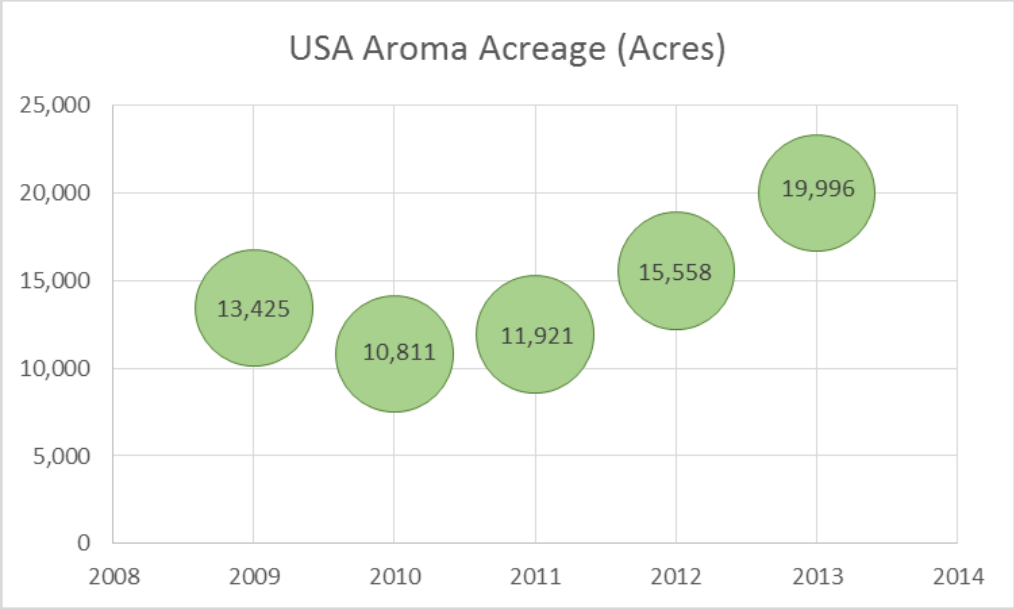
US craft beer hopping rates and usage

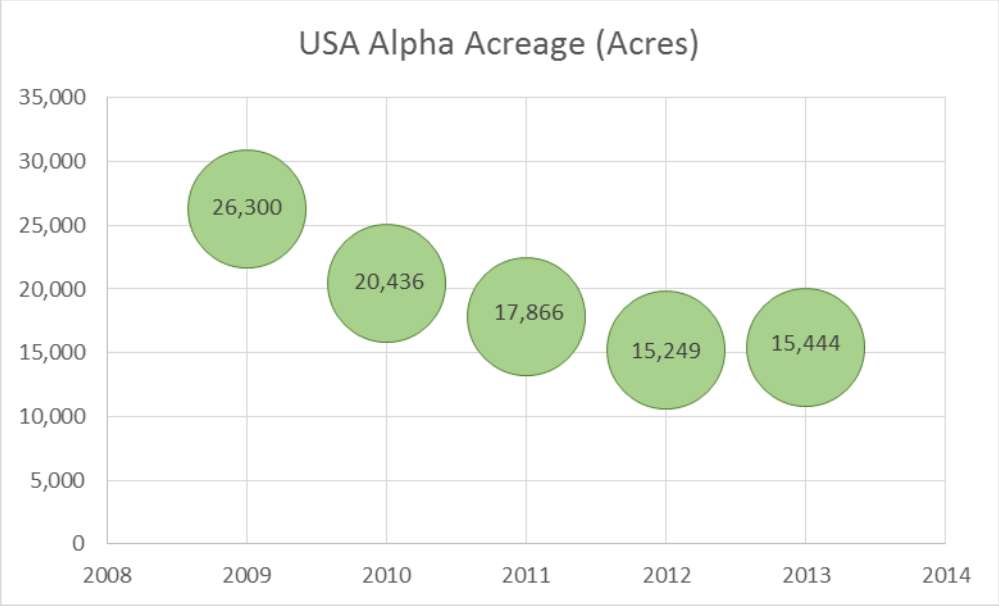




*Source: Brewers Association

US trending towards aroma varieties





From 2012 – 2013…

- USA Aroma increased **28.53%**
- USA Alpha increased 1.28%
- US aroma/alpha acres now number 7/3 (Brewers Association)

*Source: IHGC Economic Commission annual reports

Cascade – a popular choice

Top Hops | 2007

1. Cascade (Aroma)
2. Centennial (Dual)
3. Willamette (Aroma)
4. Chinook (Dual)
5. Amarillo (Aroma)
6. EKG (East Kent Golding) (Dual)
7. Saaz (Aroma)
8. CTZ | Columbus, Tomahawk, and Zeus (Bittering)
9. U.S. Golding (Aroma)
10. Styrian Golding (Aroma)

Top Hops | 2015

1. Cascade (Aroma)
2. Centennial (Dual)
3. Chinook (Dual)
4. Simcoe® (Dual)
5. Citra® (Aroma)
6. Hallertau Mittelfruh (Aroma)
7. Amarillo (Aroma)
8. Crystal (Aroma)
9. Magnum (Bittering)
10. CTZ | Columbus, Tomahawk, and Zeus (Bittering)

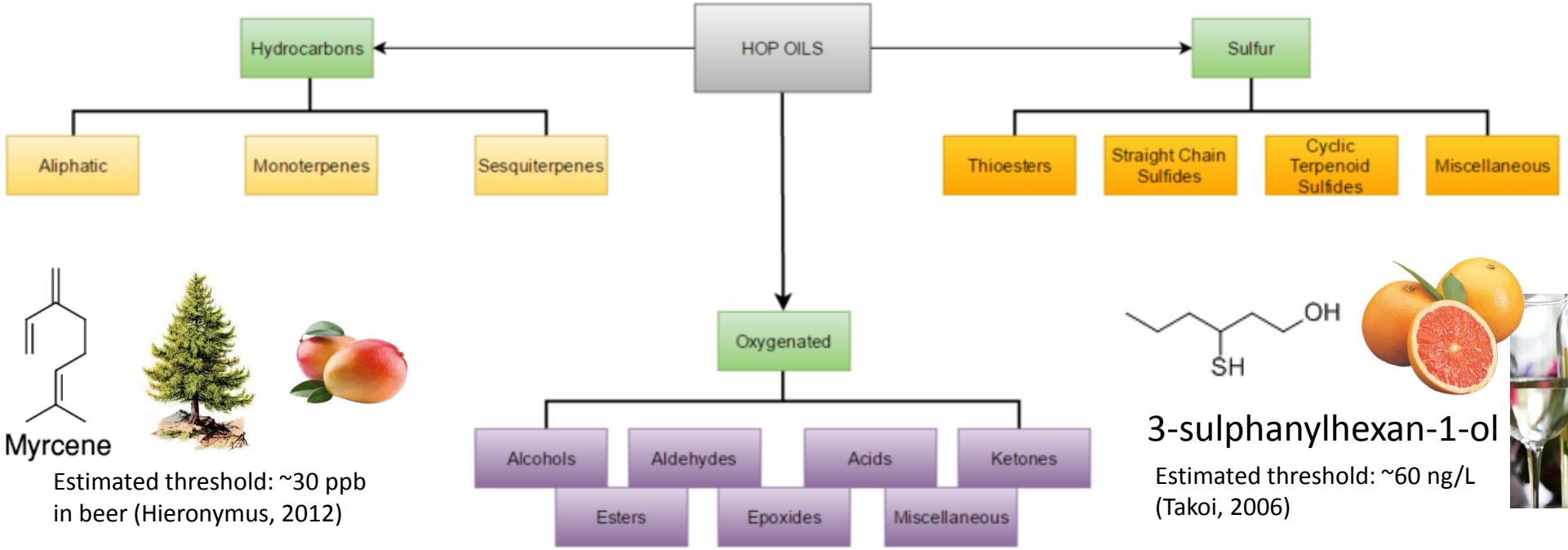
Source: Brewers Association 2015 Hop Usage Survey/

Acres Harvested by AOHGA Growers*		
Hop Variety	2012 Acreage	2013 Acreage
Ahtanum™	2	2
Cascade	60	61
Centennial	35	36
Chinook	20	20
Citra®	37	38
Fuggle	3	7
Golding	3	4
Hallertau	6	6
Liberty	1	1
Magnum	4	6
Nugget	< 1	5
Palisade®	8	10
Perle	0	2
Simcoe®	5	19
Sterling	15	21
Summit™	2	2
Willamette	0	< 1

Exploring hop aroma – brewing industry

- Sustainability
  - How can we make the best use of our natural resources?
- New product development
  - How can we create unique and desirable hop flavor for consumers?
- Process efficiency
  - How can we use the tools that we have to optimize and better control hop utilization in brewing process?

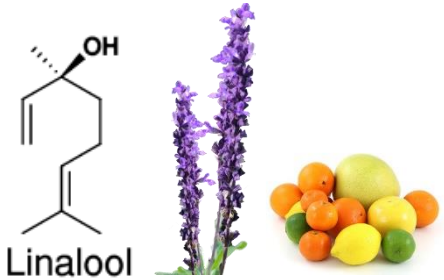
Hop oil fractions



Estimated threshold: ~30 ppb in beer (Hieronymus, 2012)

3-sulphanylhexan-1-ol

Estimated threshold: ~60 ng/L (Takoi, 2006)



Estimated threshold: ~10 ppb in beer (Almaguer, 2014)

Glycosides – potential flavor precursors in hops

- Yeast, enzyme or acid treatment
- Aliphatic alcohols, terpene alcohols, phenols, norcarotenoids
- O, S, and N-linked glycosidic bonds
- 41 polyfunctional thiols recently found in hops (Gros, 2013)

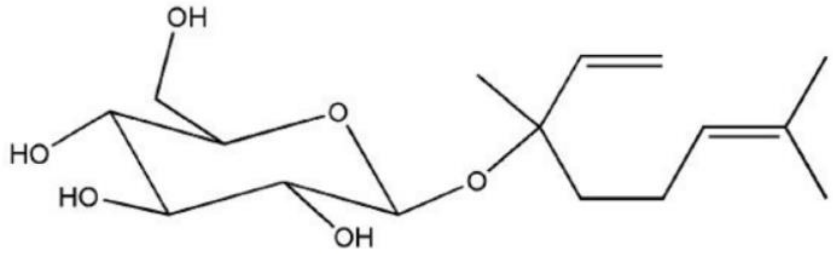
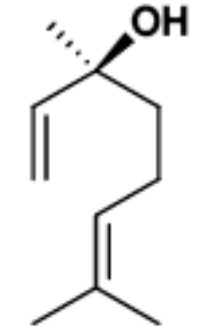


Figure 1. Glycoside from beta-D-glucose and linalool.

Enzymatic hydrolysis



Linalool

Kollmannsberger, H., Biendl, M., and Nitz, S. (2006). Occurrence of glycosidically bound flavor compounds in hops, hop products and beer. Monatsschr. Brauwissenschaft 59(May/June):83-89.

Glycosides in hopped pilsner beer

Enzymatic hydrolysis ß-Glucosidase, pH 5, 24 h, 40 °C	Addition of enzyme	Without enzyme
3(Z)-Hexenol	9	0
1-Octen-3-ol	484	0
1,5-Octadien-3-ol	39	0
Linalool	9	0
α -Terpineol	17	0
8-Hydroxy-linalool I	6	0
8-Hydroxy-linalool II	32	0
Benzylalcohol	82	15
3-Hydroxy-7,8-dihydro-β-ionol	10	0

Linalool is a key hop aroma compound with strong contribution to “hoppy flavor”

Kollmannsberger, H., Biendl, M., and Nitz, S. (2006). Occurrence of glycosidically bound flavor compounds in hops, hop products and beer. Monatsschr. Brauwissenschaft 59(May/June):83-89.

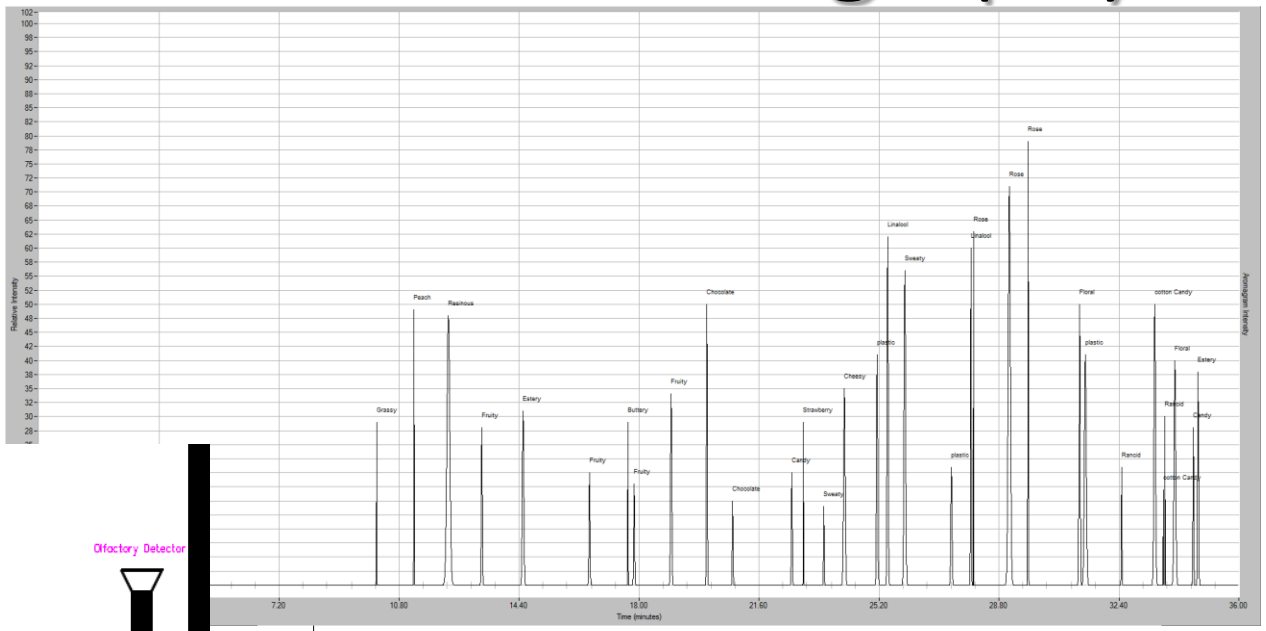
Targeted analysis using SPME GC-MS

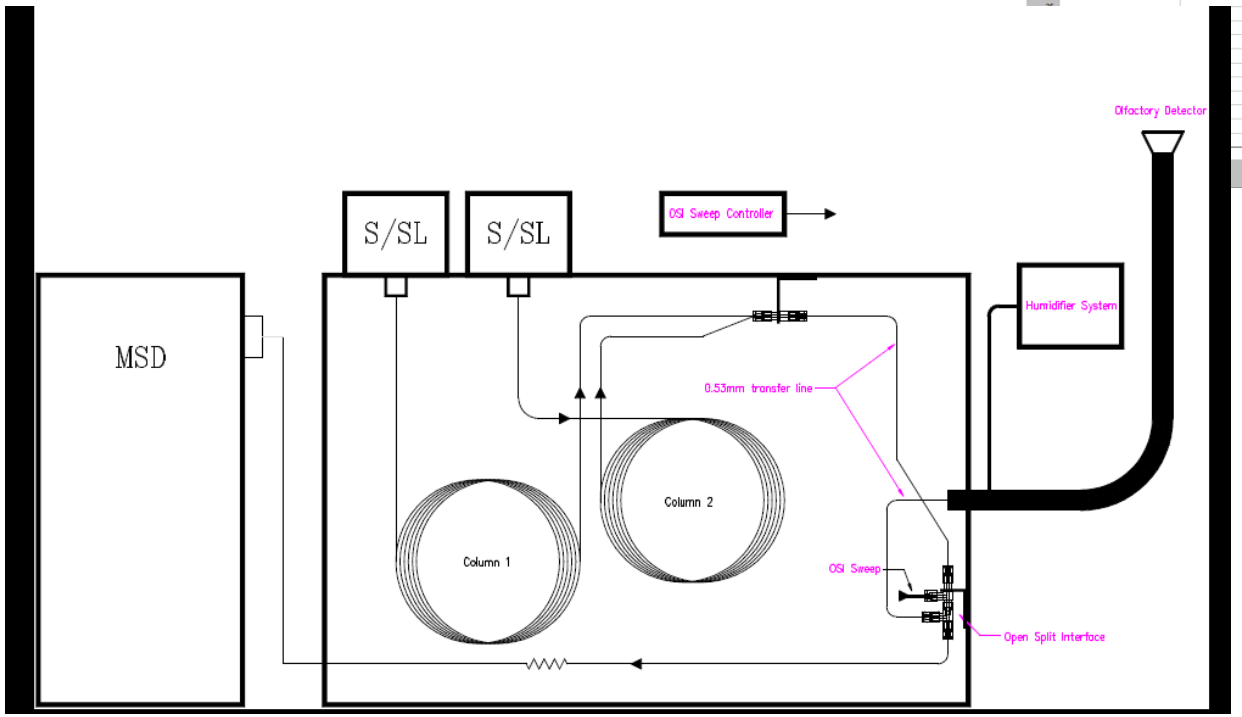
- DVB/CAR/PDMS SPME fiber
- Gerstel MPS auto sampler
- Agilent DB-5MS
- Agilent 7890A GC and 5975C MSD
- Compounds targeted via SIM:

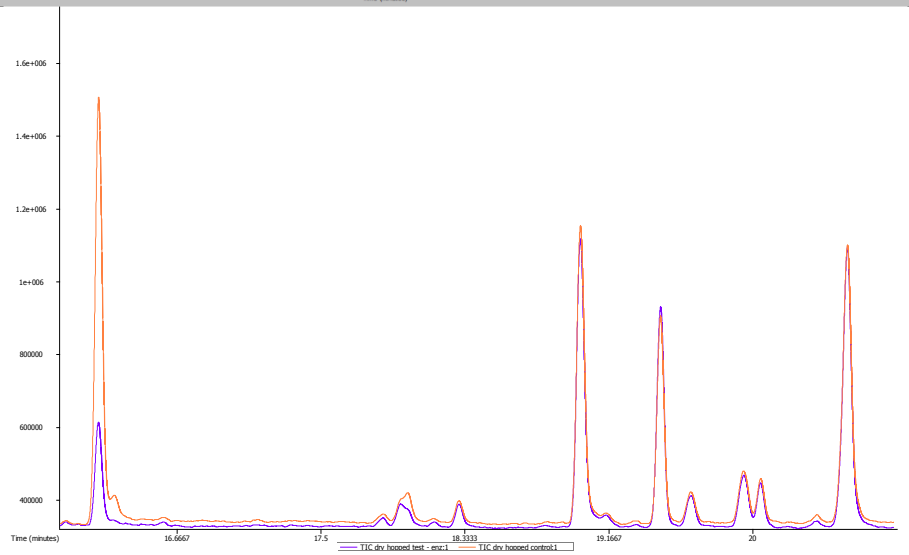
	Myrcene	Limonene	Linalool	2-Undecanone	Geranyl Acetate	Caryophyllene	Humulene
Relative RT	1	1.09	1.22	1.56	1.60	1.79	1.85
Standard	Myrcene 90% technical grade, Acros	(R)-(+)-Limonene, Fluka	(-)-Linalool, Fluka	2-Undecanone, SAFC	Geranyl Acetate, Fluka	(-)-trans-Caryophyllene, Sigma Aldrich	α-Humulene, Sigma Aldrich
Calibration (ppb)	125 – 1000	125 – 1000	12.5 – 100	12.5 – 100	12.5 – 100	12.5 – 100	12.5 – 100

Non-targeted analysis: Gas Chromatography Olfactory & TOF

- GCO – link to sensory
- Broad view of aroma actives







Experimental design: phase 1

Enzyme treatment	Description	Active ingredients
Rapidase Hoptimase (HOP)	Aroma precursor extraction in hops	Polygalacturonase (pectinase) & β-glucosidase
Rapidase Expression Aroma (WG)	Aroma precursor extraction in white grape	Polygalacturonase and N-arabinofuranosidase
Rapidase Extra Fruit (RG)	Aroma precursor extraction in red grape	Polygalacturonase

9 conditions x 5 repetitions = 45 dry hop reactions

3 enzymes

Factor	A	B
Row #	Enzyme	Hopping Rate
1	HOP	100
2	HOP	200
3	HOP	300
4	WG	100
5	WG	200
6	WG	300
7	RG	100
8	RG	200
9	RG	300

3 hopping rates

Determine interactions between hop dosing rate and enzyme to optimize aroma compounds

Can we access exogenous enzyme to enhance hop aroma and better utilization of dry hop load?

Experimental design flow: phase 1

Component Name	Type	Value	Units
Ea	N	2.82	Deg Plato
ABV	N	6.26	% ABV
ABW	N	4.89	% ABW
RDF	N	66.69	RDF
SG 20/20	N	1.011025	SG
pH	N	4.55	pH
Color	N	37.92	EBC
Er	N	5.05	Er
Calories	N	192.07	Calories
BCOG	N	14.41	Deg Plato

400 mL ale base for dry hopping

Stirred @ 25°C

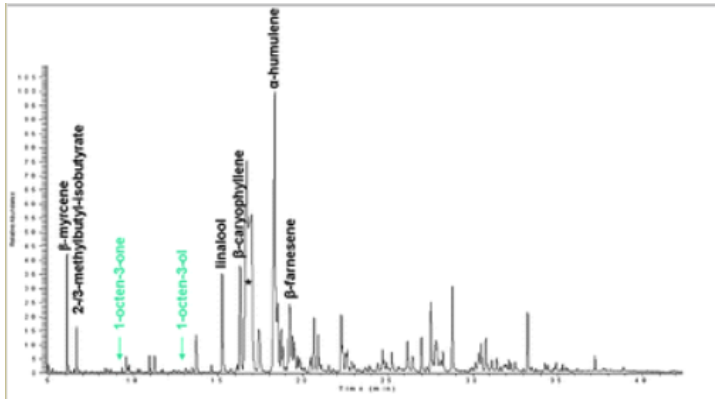
Enzyme @ 1000 ppm

1. Rapidase Expression Aroma (WG)
2. Rapidase Extra Fruit (RG)
3. Rapidase Hoptimase (HOP)

Cascade @ 100/200/300 g/hL

Incubate for 48 hours; centrifuge

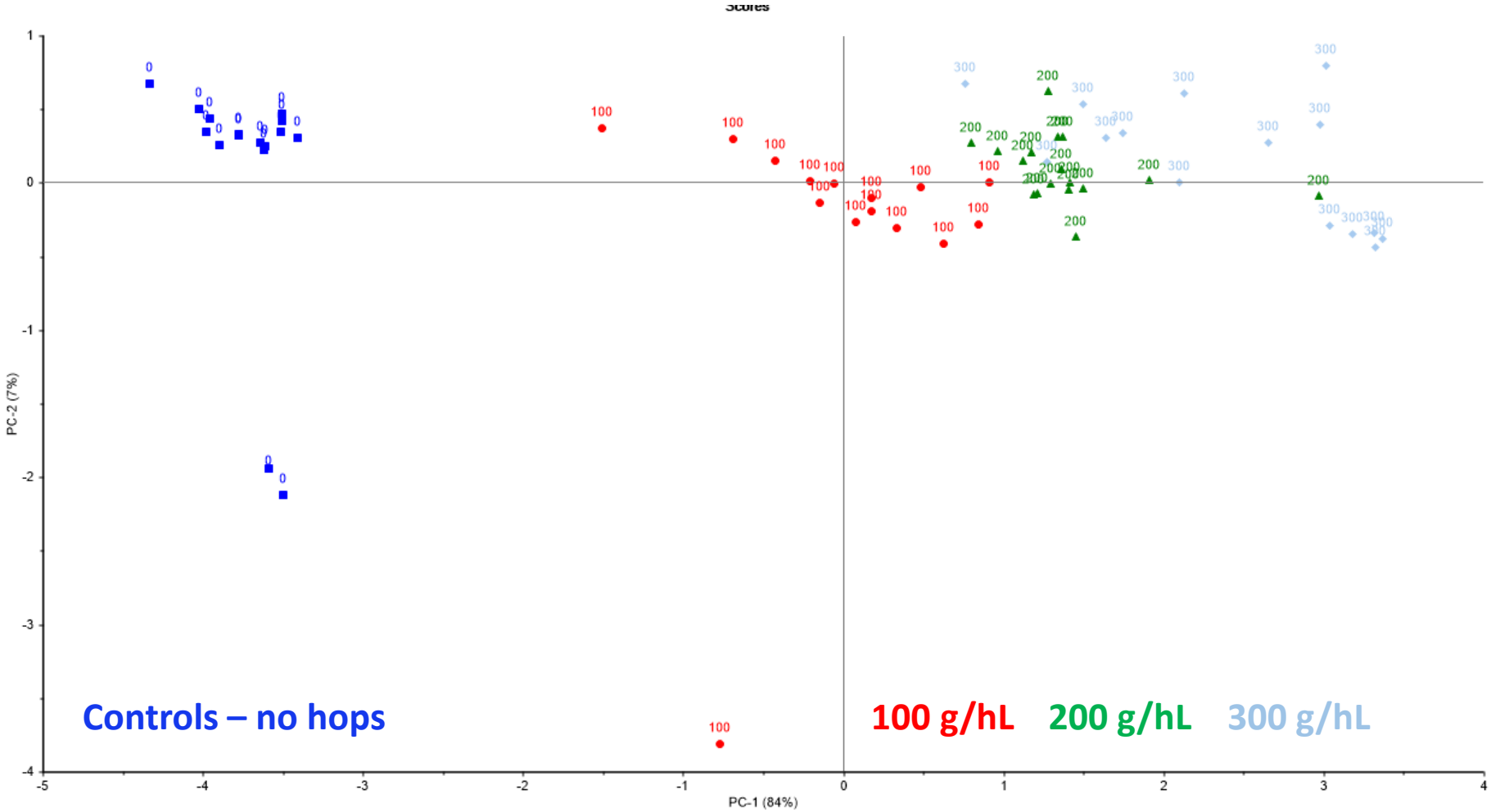
GC-MS

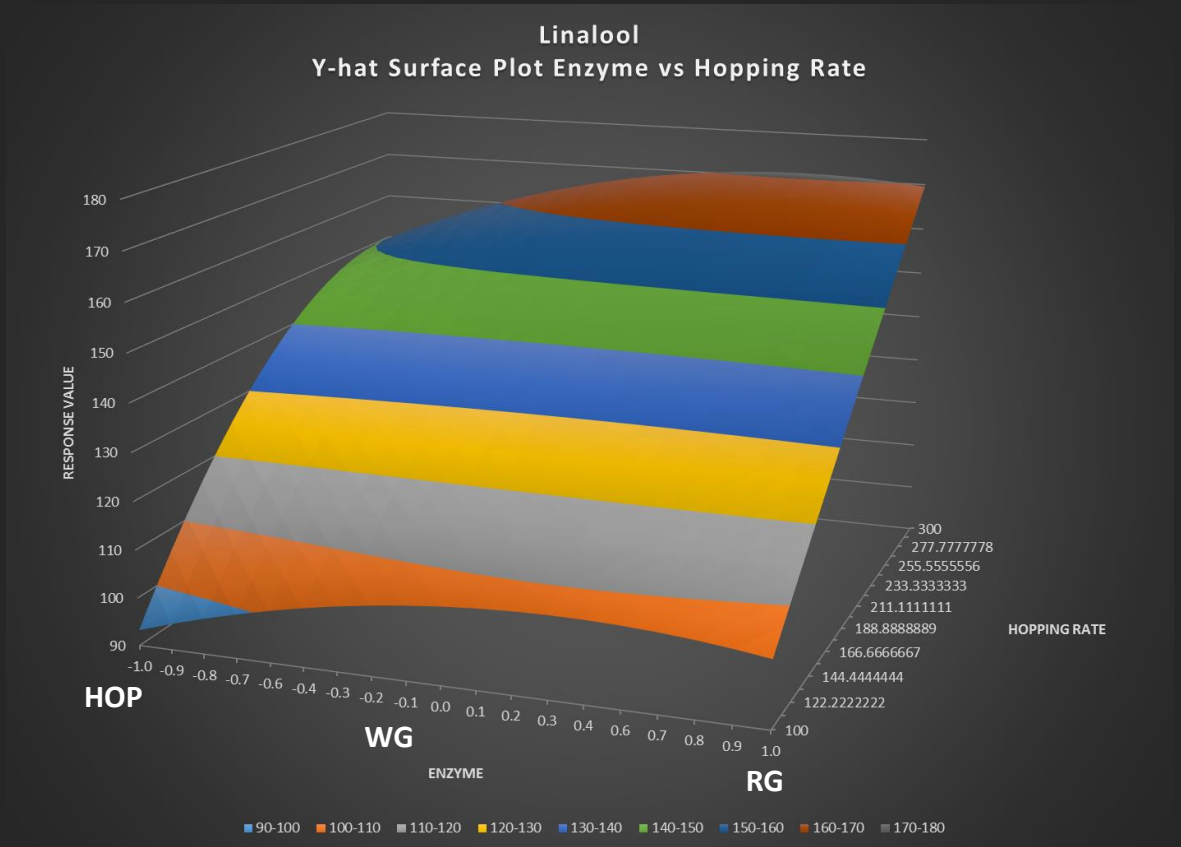


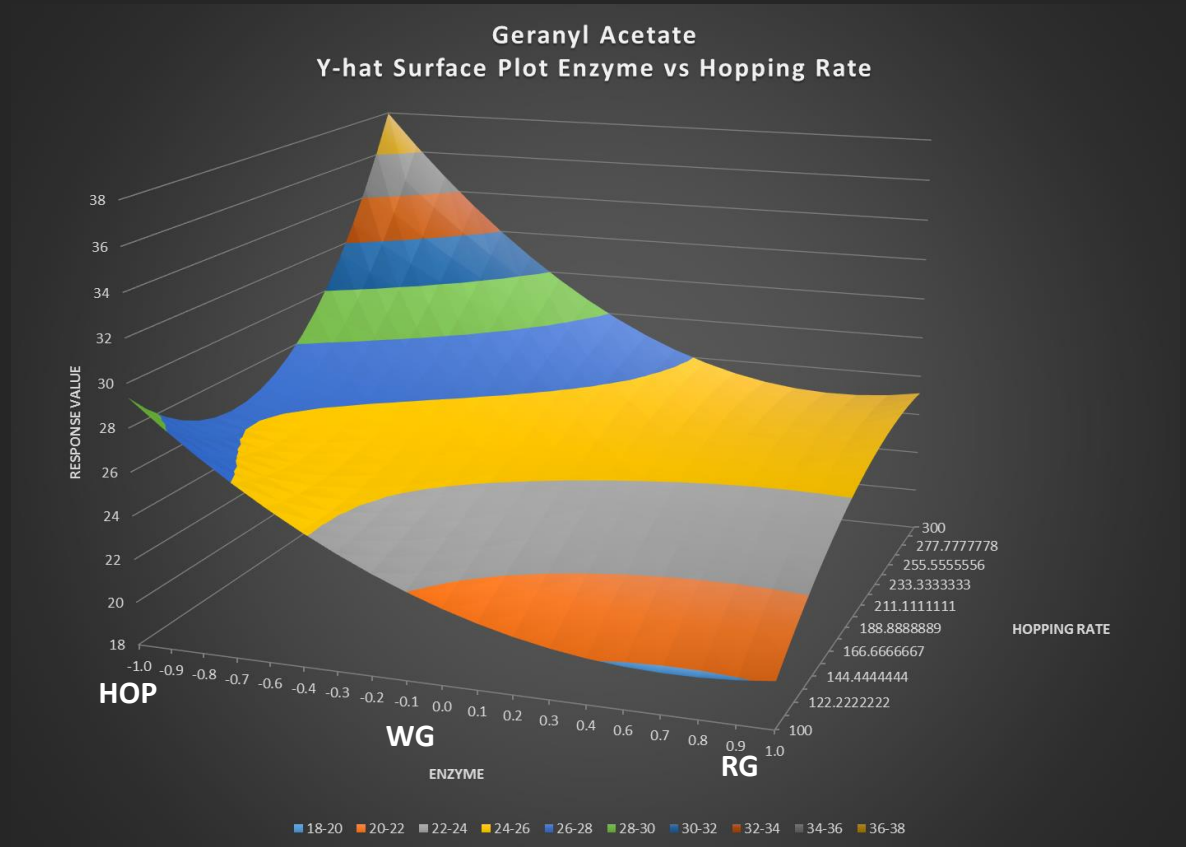
Identification, quantification, and data analysis

Description		Myrcene Resu		Limonene Resu		Linalool Resu		2-Undecanone Resu		Geranyl Acetate Resu
	Acq. Date-Time	Final Conc.	Area	Final Conc.	Area	Final Conc.	Area	Final Conc.	Area	Final Conc.
200HOP	3/18/2016 18:07	313.048	72569	5.30825	29639	155.0371	30100.5	11.52935	48014	24.04855
300HOP	3/28/2016 0:03	251.74965	54658	6.99015	39957.5	199.3749	42396.5	12.5741	54115	25.0469
100WG	3/24/2016 7:01	179.95865	33681	3.48275	18438	99.74455	14767	9.1972	34397	19.4837
200WG	3/30/2016 0:37	209.05085	42182	5.36275	29972.5	129.49545	23017.5	11.09965	45505.5	24.63805
300WG	3/22/2016 5:14	207.1331	41621	6.2144	35198.5	161.12645	31789.5	10.77685	43620.5	24.3641
100RG	3/26/2016 2:30	211.43985	42880	3.75005	20078.5	105.9086	16476.5	9.1386	34055	21.13755
200RG	3/18/2016 18:33	212.62535	43226	5.0219	27882	141.66585	26392.5	10.5666	42393.5	24.054
300RG	3/22/2016 3:02	218.6026	44973	6.0977	34482.5	169.94225	34234.5	10.468	41817.5	25.7685
100HOP	3/24/2016 5:16	193.9888	37781	3.26095	17078	107.3476	16875.5	8.2253	28722.5	18.95625
200HOP	3/22/2016 3:55	220.4448	45511	5.1754	28823.5	137.08795	25123	8.93515	32867	20.45075
300HOP	3/17/2016 5:27	223.9987	46549	4.1773	22700	126.2328	22113	13.6929	60647	47.2829
100WG	3/26/2016 5:07	221.1239	45709	3.61205	19232	103.57885	15830.5	8.8371	32294.5	20.92855
200WG	3/30/2016 1:56	216.69205	44415	5.52105	30944.5	136.28955	24901.5	11.51675	47941	25.3029
300WG	3/30/2016 2:22	244.74515	52611	6.40305	36355.5	174.25505	35430.5	12.19555	51904.5	28.02375
100RG	3/24/2016 6:35	196.82725	38610	3.70415	19797	104.72915	16149.5	8.37805	29614	20.6872
200RG	3/30/2016 1:03	238.2931	50726	5.5525	31137.5	148.8968	28398	11.16675	45897	23.1335
300RG	3/30/2016 0:11	328.87865	77195	6.07525	34344	182.3255	37668.5	11.41605	47352.5	24.5116
100HOP	3/17/2016 5:00	121.39785	16570	1.8362	8336	83.08955	10148	11.51715	47943	37.40615
200HOP	3/28/2016 3:07	340.59895	80619	4.7091	25962.5	145.64515	27496	9.1477	34108	20.8809
300HOP	3/17/2016 4:08	179.54345	33560	3.4971	18526.5	127.3466	22421.5	14.99765	68265.5	45.4567
100WG	3/18/2016 17:41	200.4131	39658	4.00685	21654	114.61765	18892	9.96905	38904	22.2884
200WG	3/24/2016 10:13	209.4381	42295	4.54185	24936.5	137.5205	25243	9.9196	38615	23.2367
300WG	3/22/2016 5:40	207.47005	41720	5.92995	33453	157.6433	30823.5	10.41545	41510.5	23.5799
100RG	3/18/2016 20:44	179.13685	33441	3.4647	18328	104.7573	16157.5	8.8901	32604.5	18.90205
200RG	3/26/2016 3:49	264.72905	58450	4.38895	23998.5	125.53775	21920	9.21685	34512	22.57035
100HOP	3/26/2016 4:41	223.99875	46549	3.3682	17735.5	99.70185	14755.5	8.5689	30728.5	18.81125
200HOP	3/17/2016 5:53	182.52015	34430	3.2932	17275.5	104.2781	16024.5	12.70675	54889	43.96245
300HOP	3/22/2016 3:28	255.2609	55684	5.70955	32101	163.21995	32370	9.53645	36377.5	23.5118

PCA results –SPME GC-MS of 7 hop aroma compounds

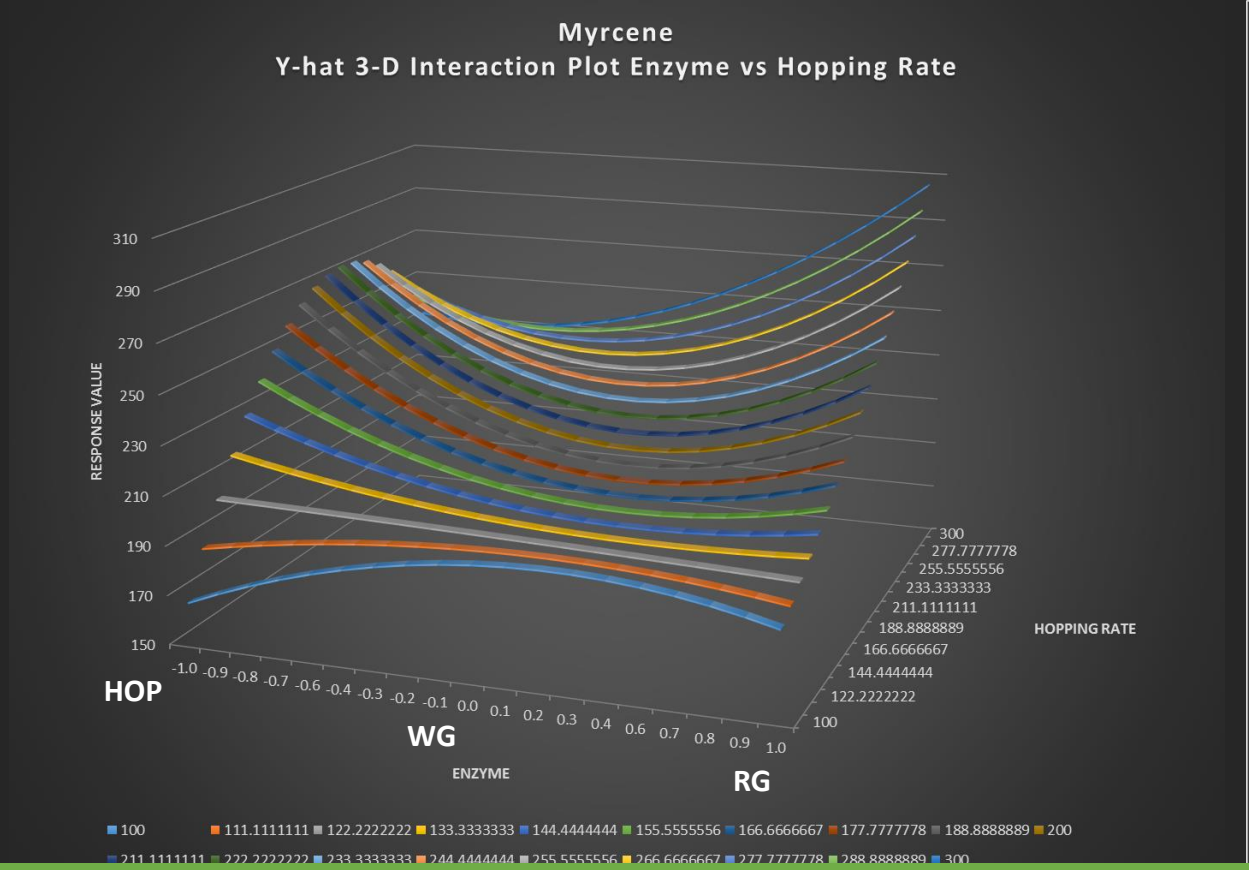


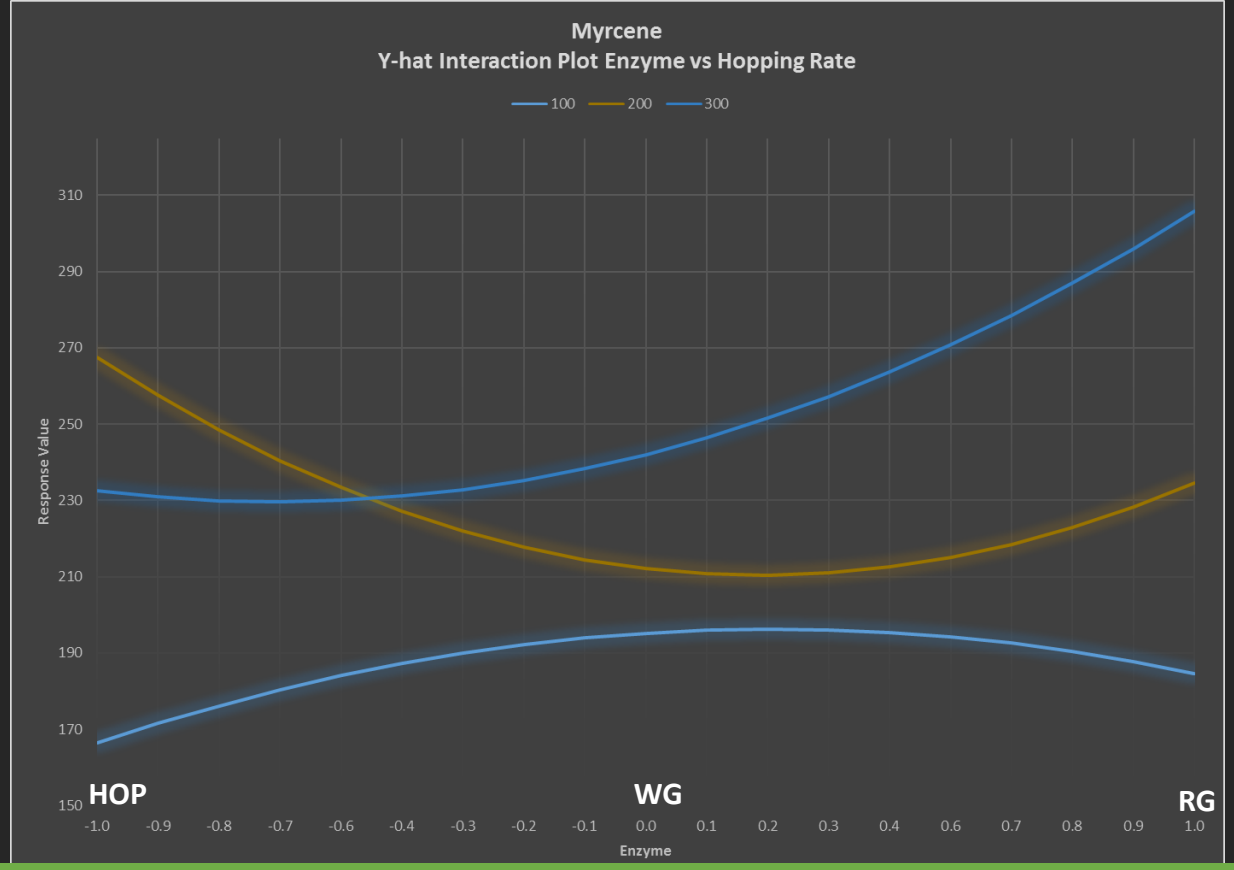




Enzyme treatment	Description	Active ingredients	Observed effects
Rapidase Hoptimase (HOP)	Aroma precursor extraction in hops	Polygalacturonase (pectinase) & β-glucosidase	• Increase in linalool with hopping rate, but appears to drop off between 200-300 g/hL • Increase in geranyl acetate with hopping rate, though increase is more pronounced at 300 g/hL
Rapidase Expression Aroma (WG)	Aroma precursor extraction in white grape	Polygalacturonase and N-arabinofuranosidase	• Increase in linalool with hopping rate • Little effect seen on geranyl acetate with hopping rate
Rapidase Extra Fruit (RG)	Aroma precursor extraction in red grape	Polygalacturonase	• Steep increase in linalool with hopping rate – enzyme may not be saturated • Little effect seen on geranyl acetate with hopping rate

Hoptimase β-glucosidase enzyme activity may enhance geranyl acetate concentration at 300 g/hL hopping rate

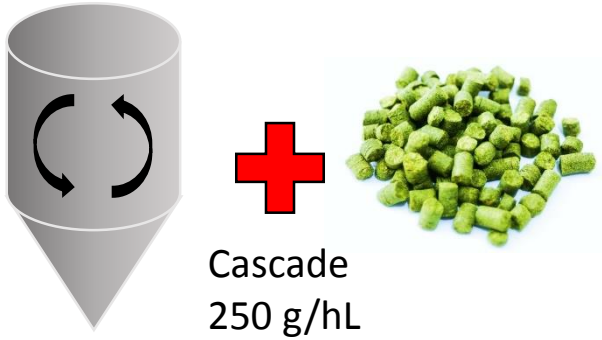




Enzyme treatment	Description	Active ingredients	Observed effects
Rapidase Hoptimase (HOP)	Aroma precursor extraction in hops	Polygalacturonase (pectinase) & β-glucosidase	• Increase in myrcene with hopping rate, but appears to decrease once reaching 300 g/hL – biotransformation?
Rapidase Expression Aroma (WG)	Aroma precursor extraction in white grape	Polygalacturonase and N-arabinofuranosidase	• Little observed effect –larger increase in concentration between 200-300 g/hL than 100-200 g/hL
Rapidase Extra Fruit (RG)	Aroma precursor extraction in red grape	Polygalacturonase	• Steep increase in myrcene with hopping rate – enzyme may not be saturated

Hoptimase β-glucosidase activity may cause myrcene concentration to decrease at higher hopping rates

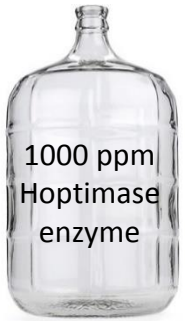
Experimental design flow: phase 2



Cascade
250 g/hL

65 hours
@ 15°C

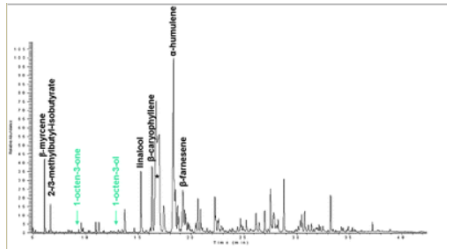
Control



Test

GC-MS

Data



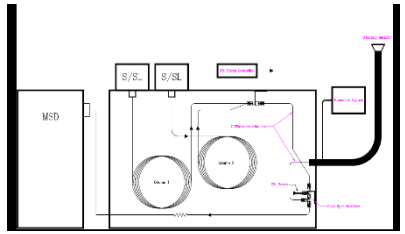
Sensory

- Are they different?
- Scaling attributes
- Descriptive analysis

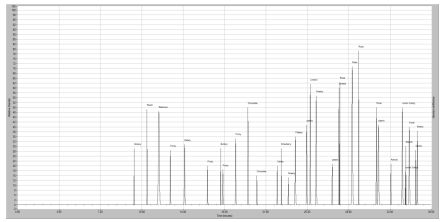
Data

Brewfile	Session	Quantit	Size	Alcohol	Apricot	Caramel	Catty	Garlic	Geranio	Grapefr	Grassy	Green	Linalool	Myrcene	Acetaldehyde
control	4/6/2016	0	1	4	3	3	1	3	2	1	1	3	3	6	0
control	4/6/2016	5	0.5	3	3	1	0.5	2	0.5	6	6	2	0.5	4	0
control	4/6/2016	9	0	4	5.5	3	5	4	0	5	3	3	0	6	0
control	4/6/2016	2	0	3	2	1.5	1	0	2.1	2.6	1.5	1	3	6	0
control	4/6/2016	0	0.1	3	3.6	1.4	3	4	3	4.9	3	2	1.3	3	0
control	4/6/2016	7	2.6	3.5	4.4	2.7	1.4	1.5	1.5	3.8	3	2.5	3.8	3.9	0
control	4/6/2016	0.9	0.2	4.4	2	0.6	3	3.5	2	3	3	5	1	3	0
control	4/6/2016	8	1	3	3	4	1	2	0.5	3	2	2	0	1	0
control	4/6/2016	4.6	1	4.1	3.5	4.1	0	0	2.1	4	2.2	1.6	3.6	5.7	0
control	4/6/2016	6.8	0.2	2	3.6	3.5	2.7	2.6	6.9	3.4	0.6	1.8	2.1	2.9	0
control	4/6/2016	5.5	2	1.5	3	3.3	0.9	2	3	2.5	2	2.1	2.9	3.4	0
control	4/6/2016	6	1	2	1	3	1	2.1	1	1	0	2	1	1	1
control	4/6/2016	6	0.5	3.5	4	5.5	0	1	3	3	4	2	1	5.5	2
test	4/6/2016	4	0	7	3	4	1	5	2	4	1	2	1	6	0
test	4/6/2016	5	0.5	1	4	7	1	4	2	3	5	2	1	3	0
test	4/6/2016	9	0	4	6	3	4	3	0	6	4	3	0	6	0
test	4/6/2016	1.5	0	2.2	2.5	1.5	0	0.5	2	2.5	3.5	3.6	2.4	1.5	0
test	4/6/2016	0	0.1	2	3	5	2.1	4	5	4	3	6	8	5	0
test	4/6/2016	7	2.9	3.5	4.5	2.5	1.2	1.5	1.5	4.4	3.8	1.8	2.5	4	0
test	4/6/2016	0.1	0	3.5	3	3	5	3	4	6	5	3	3	3	0
test	4/6/2016	8	1	1	2	4	1	1	6.5	4	2	1	0	4	0
test	4/6/2016	4.5	1	5.1	3.2	2	0	0	2.5	4	1.8	1.6	4.2	4.8	0
test	4/6/2016	7.3	0.1	4.2	6.5	6.2	1.8	0.3	4.4	0.7	0.4	0.8	3	1.1	0
test	4/6/2016	5	3	2.1	0.7	2.8	0.8	2.3	2	1.9	3.6	3.3	1.7	2.2	0
test	4/6/2016	0	3	2	3	4	0	2	0	0	0	3	3	1.9	0
test	4/6/2016	6	0.5	2	4	5.5	1	0	3	3	3	1	1	3	2.5
	0.802062	1	0.04313	0.606764	0.400485	0.833361	0.911294	0.291884	0.971472	0.560053	0.736967	0.420451	0.633196	0.88296	

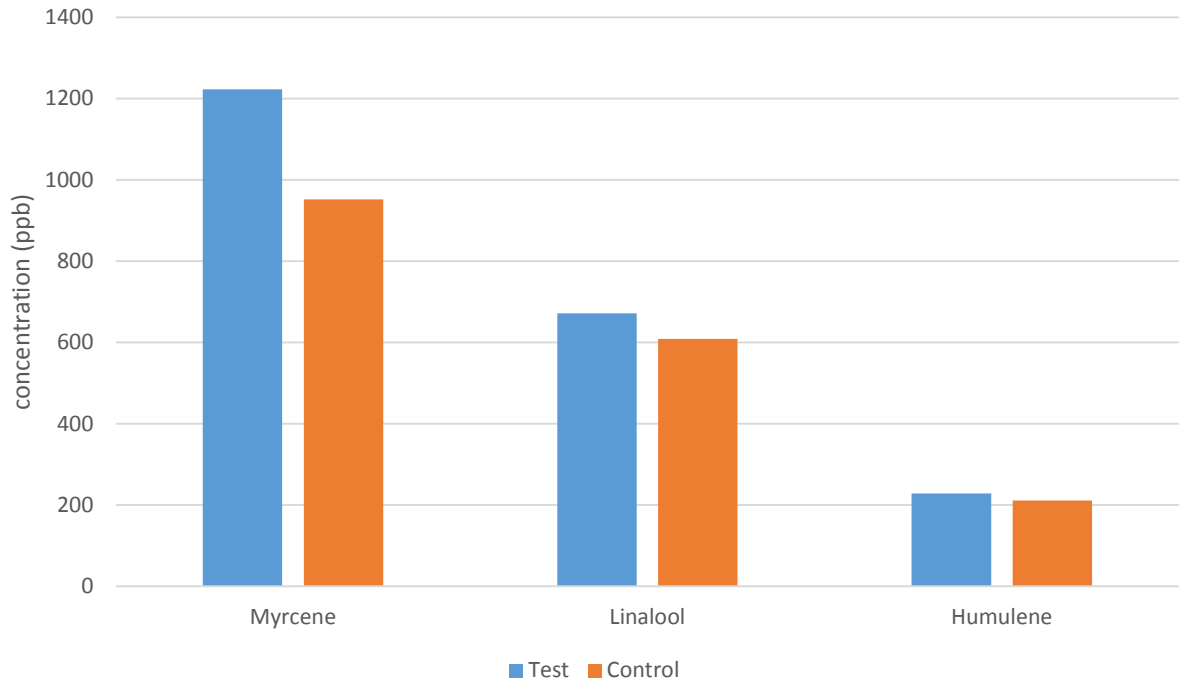
GCO/TOF

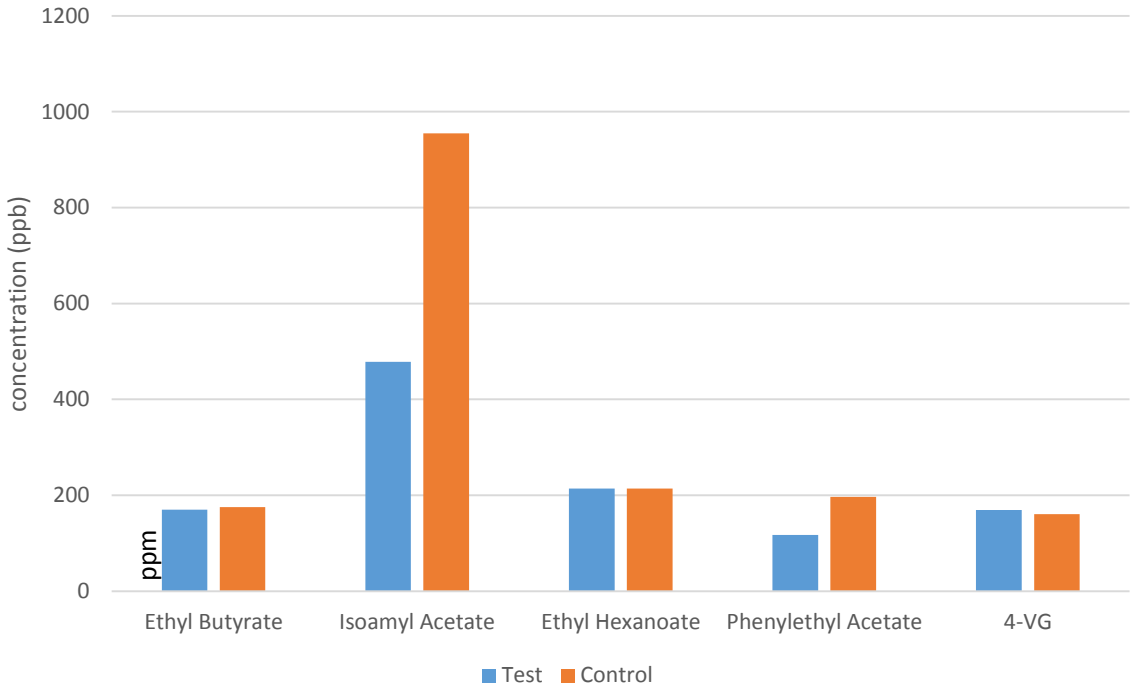


Data



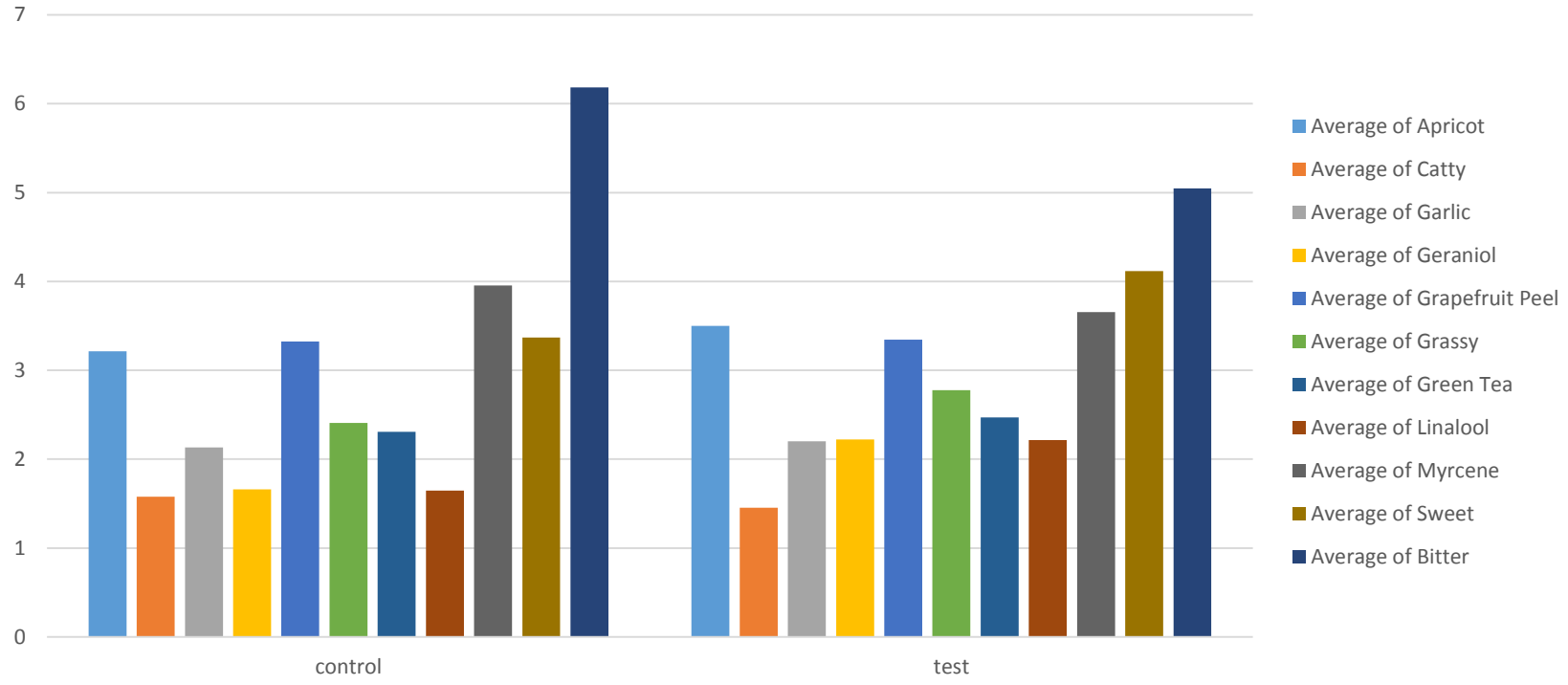
SPME-GCMS – IPA dry hopped with enzyme





- Isoamyl acetate loss in test = esterase side reactions (Daenen, 2012)
- Acetate esters hydrolyze more rapidly than ethyl esters – isoamyl acetate both chemically and enzymatically (Preedy, 2011)

Sensory – IPA dry hopped with enzyme



- **If there is a difference, must be qualitative and outside scope of test**
- Investigate further → descriptive analysis

Sensory – IPA dry hopped with enzyme

Descriptor	Control	Test
Visual	Golden amber color with an orange glow, creamy white form and a heavy sheen	Same as control
Aroma	Mostly grapefruit and pine with some tropical pineapple, orange and caramel backin' it up	Mostly pine with some **citrus, orange and grapefruit**, followed by **tropical pineapple** aroma as well, slight caramel and isoamyl acetate
Taste	Low sweetness into intense lingering bitter	Low to moderate sweetness with an intense bitter linger
Mouthfeel/Body	Medium body with a lingering astringency	Medium body, sl. creamy, finishes astringent

- Descriptive Analysis (n = 7 validated taste panelists)
- Overall most panelists noted that the **citrus, stone fruit and tropical aromas** were more pronounced in the test than the control

Next steps →Non-targeted GCO & TOF

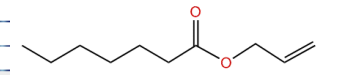
- What are the aromas responsible for **citrus, stonefruit and tropical fruit** detected in sensory?
- GCO & TOF: What is different between test and control?
- Can we quantitate? Is it significant?
- Does this data correlate to sensory?

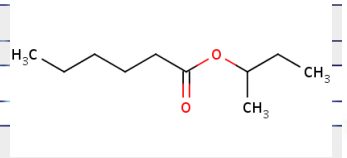
The Good Scents Company Information Listings

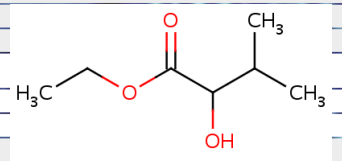
Odor Descriptors for pineapple

Primary (First) - pineapple

FL/FR		allyl cyclohexyl propionate
		odor: sweet pineapple tropical fruity candy waxy
		flavor: Fruity, pineapple, waxy, with green sweet apple nuances
FL/FR		allyl heptanoate
		odor: Sweet, pineapple-like, fruity with a slight waxy ripe fermented note
		flavor: Fruity, pineapple-like with a waxy tropical nuance
FL/FR		allyl hexanoate
		odor: sweet fruity pineapple tropical ethereal rum arrack fatty cognac
		flavor: Sweet, fresh, juicy pineapple and fruity
FL/FR		allyl hexanoate
		odor: sweet fruity pineapple tropical ethereal rum arrack fatty cognac
		flavor: Sweet, fresh, juicy pineapple and fruity
FL		allyl valerate
		odor: pineapple
		ananas comosus extract
		odor: heavy ripe pineapple
FL/FR		butyl hexanoate
		odor: Fruity, pineapple, waxy, green, juicy, apple
		flavor: Fruity, pineapple, green, waxy, tutti-frutti, juicy with a slight fermented fruit note
FL	9-	decen-2-one
		odor: pineapple fruity pear apple green fatty
FL/FR		ethyl heptanoate
		odor: fruity pineapple cognac rum wine
		flavor: Fruity, pineapple, banana and strawberry with a spicy, oily nuance
FL/FR		ethyl hexanoate
		odor: sweet fruity pineapple waxy green banana
		flavor: Sweet, pineapple, fruity, waxy and banana with a green, estry nuance
		ethyl 2-hydroxy-3-methyl butyrate
		odor: pineapple strawberry tea honey







Polyfunctional thiols – hidden players?

- Primarily from fermentation by **microbial metabolism of non-volatile precursors** (Musumecci, 2015)
- Low sensory threshold, low concentrations, challenging to quantitate analytically
- Nelsen Sauvin– fruity volatile thiols (Gros, 2012)
- 3-sulfanyl-4-methylpentan-1-ol (3S4MP)
  - grapefruit, rhubarb aroma
  - May enhance flavor of terpene alcohols linalool & geraniol (Takoi, 2009)

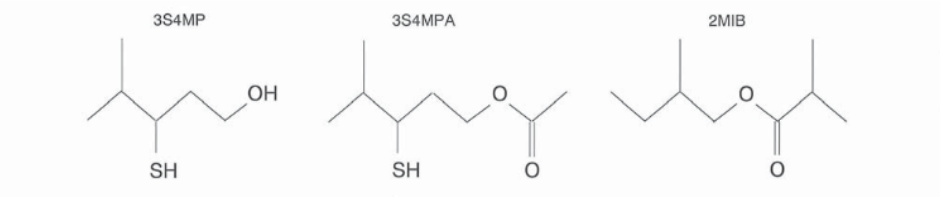
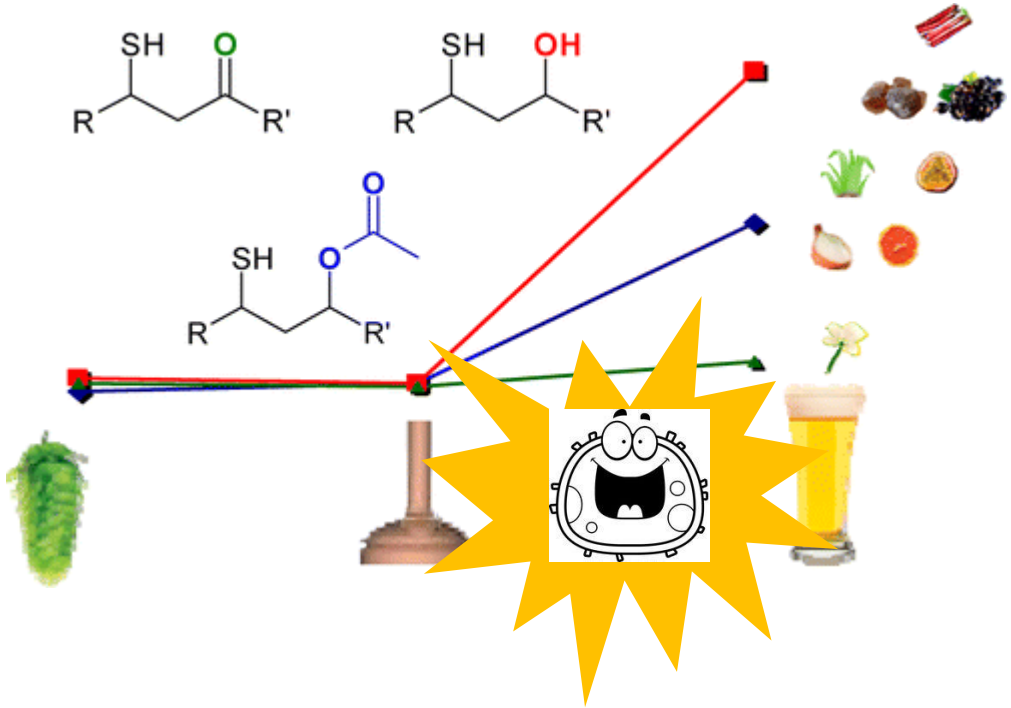


Fig. 1 Specific flavor compounds derived from NS hop



In conclusion…

- Aroma active compounds are elusive – low concentrations may contribute to overall flavor due to synergistic and additive effect (Almaguer, 2014)
- Need sensory to validate analytical data
- Application and effectiveness of exogenous products may depend on process specifications
- **Enzymatic reactions at dry hop warrant further investigation**

Future directions

- Uses in process – dry hop? Enhance late/kettle hop additions?
- Enzyme action during fermentation (on yeast)
- Polyfunctional thiols – flavor enhancement potential
- Fruited and spiced beer
- Shelf life studies – flavor stability
- What glycosides are present in beer without hops?
- New method development to quantify compounds of interest

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Acknowledgements

- New Belgium Brewing Co. & the Quality Assurance Team
- DSM for the use of enzymes
- Our gracious hosts at Sierra Nevada

Thank you for your attention…

Questions?

Extras

Aroma precursors present in hops

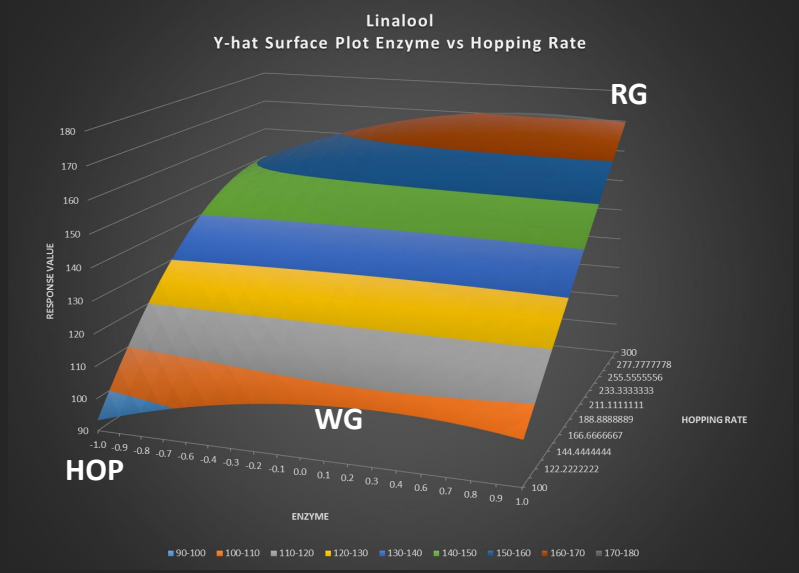
- **Alcohols**: Aliphatic alcohols, terpene alcohols (sa. **Linalool**) (Kollmannsberger, 2006)
- B-damascenone glycosides in beer (Preedy, 2008)
- Increase in phenolic content when used in wine (Stepanova, 2006)
- **Polyfunctional thiols**
  - 41 thiols recently found in hop (Gros, 2012)
  - Varietal specific cysteine-S-conjugate
  - **Cascade** hops have high 3-sulphanylhexan-1-ol (grapefruit-like) potential (Gros, 2013)

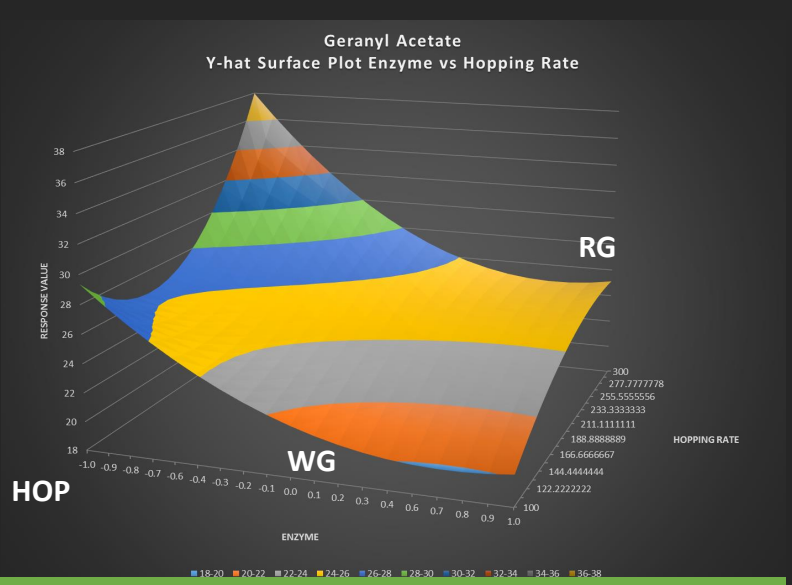
Targeted analysis using SPME GC-MS

- Supelco 2 cm DVB/CAR/PDMS SPME fiber (10 minute extraction at 60°C)
- Gerstel MPS auto sampler (sample incubation 60°C x 5 min, agitation time of 60 sec)
- Agilent DB-5MS UI:2891.71150 60m x 320um x 1um
  - 40°C x 5 minutes
  - Ramp 4°C/min → 190°C
  - Ramp 30°C/min → 235°C x 1 min
- Agilent 7890A GC and 5975C MSD
- Compounds targeted via SIM:
  - **Myrcene**
  - **Linalool**
  - Caryophyllene
  - Humulene
  - Limonene
  - Geranyl acetate
  - 2-undecanone

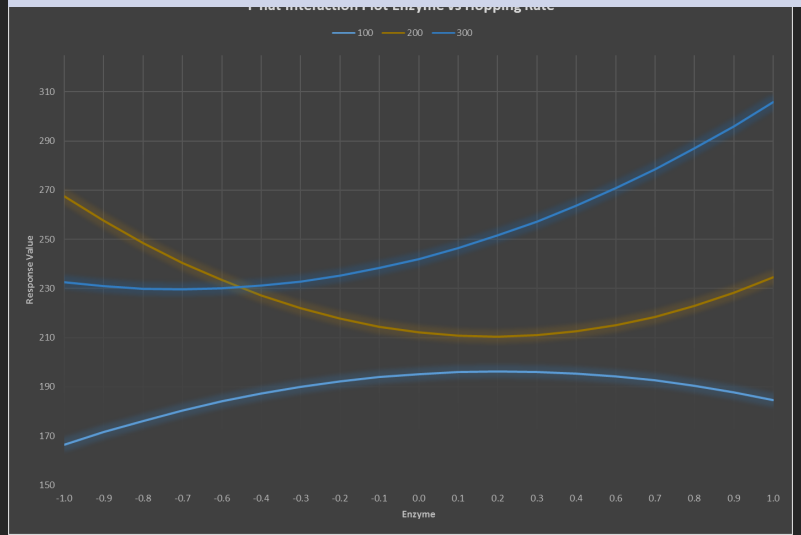
Design of experiment (DOE)

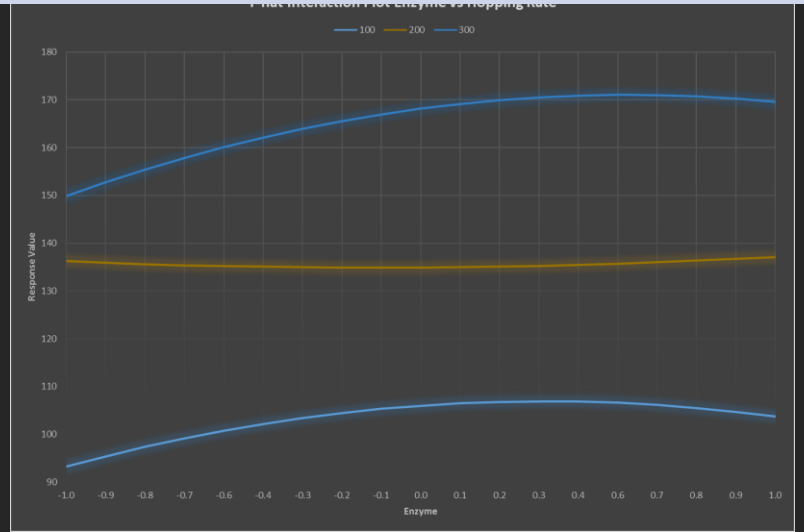
- Two factors (enzyme and hopping rate)
- 7 responses and 5 repetitions
- Quantify changes using 7 hop aroma compounds with SPME GC-MS
  - **Myrcene**, limonene, **linalool**, 2-undecanone, geranyl acetate, caryophyllene, humulene
- GOAL:
  - Determine interactions between hop dosing rate and enzyme to optimize aroma compounds
  - *Can access exogenous enzyme to enhance hop aroma and better utilization of dry hop load?*

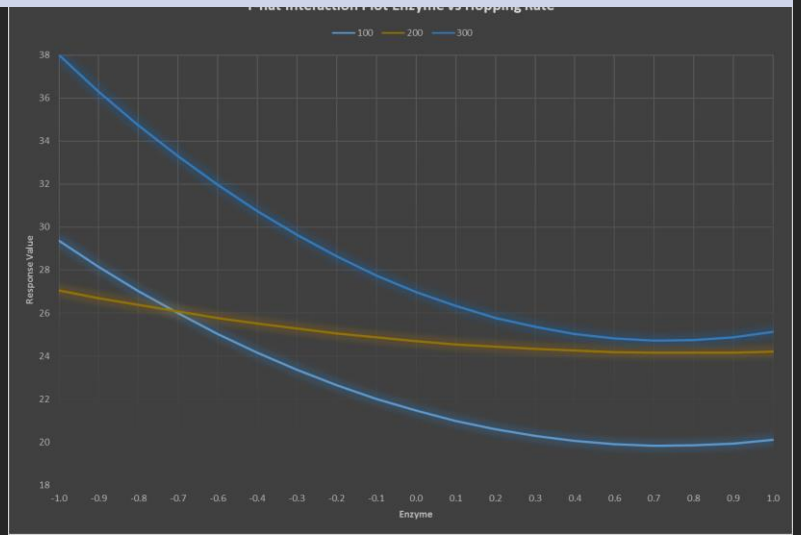




Enzyme treatment	Description	Active ingredients	Observed effects
Rapidase Hoptimase (HOP)	Aroma precursor extraction in hops	Polygalacturonase (pectinase) & beta-glucosidase	High geranyl acetate with high hopping rate, myrcene saturation?
Rapidase Expression Aroma (WG)	Aroma precursor extraction in white grape	Polygalacturonase and N-arabinofuranosidase	Hard to say what effects are (if any)
Rapidase Extra Fruit (RG)	Aroma precursor extraction in red grape	Polygalacturonase	High myrcene with high hopping rate, low geranyl acetate



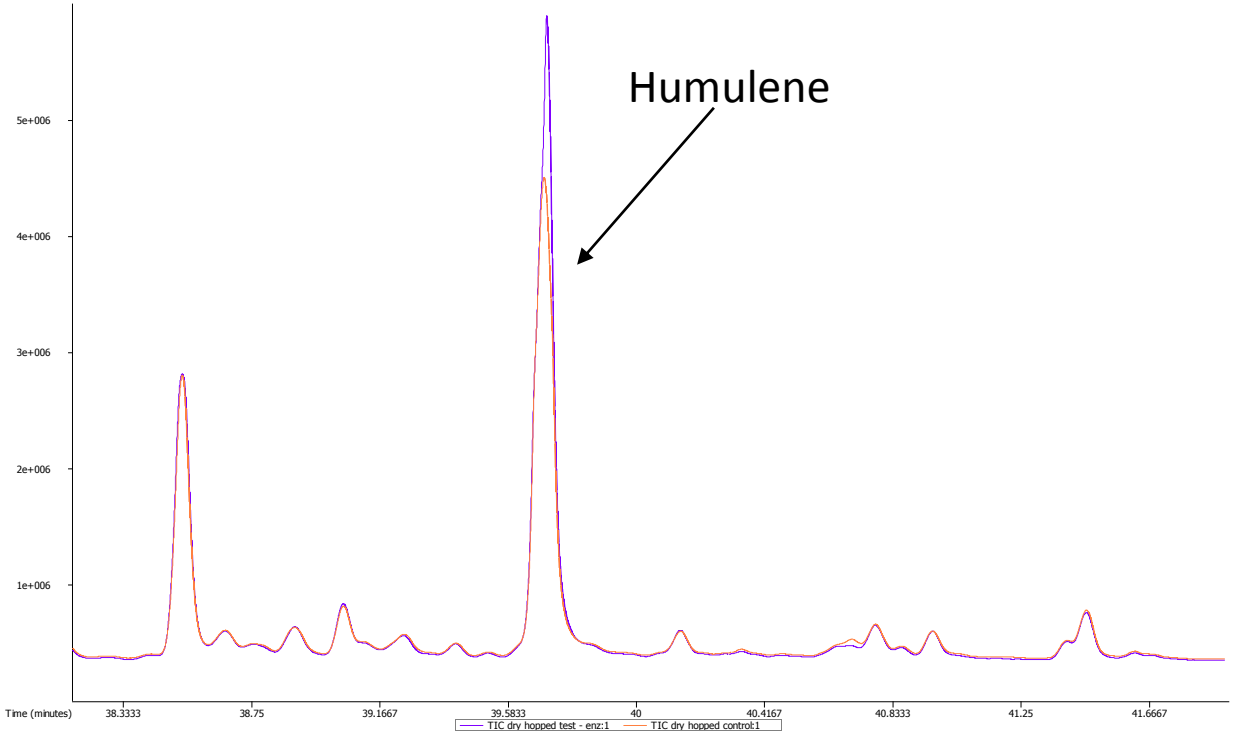


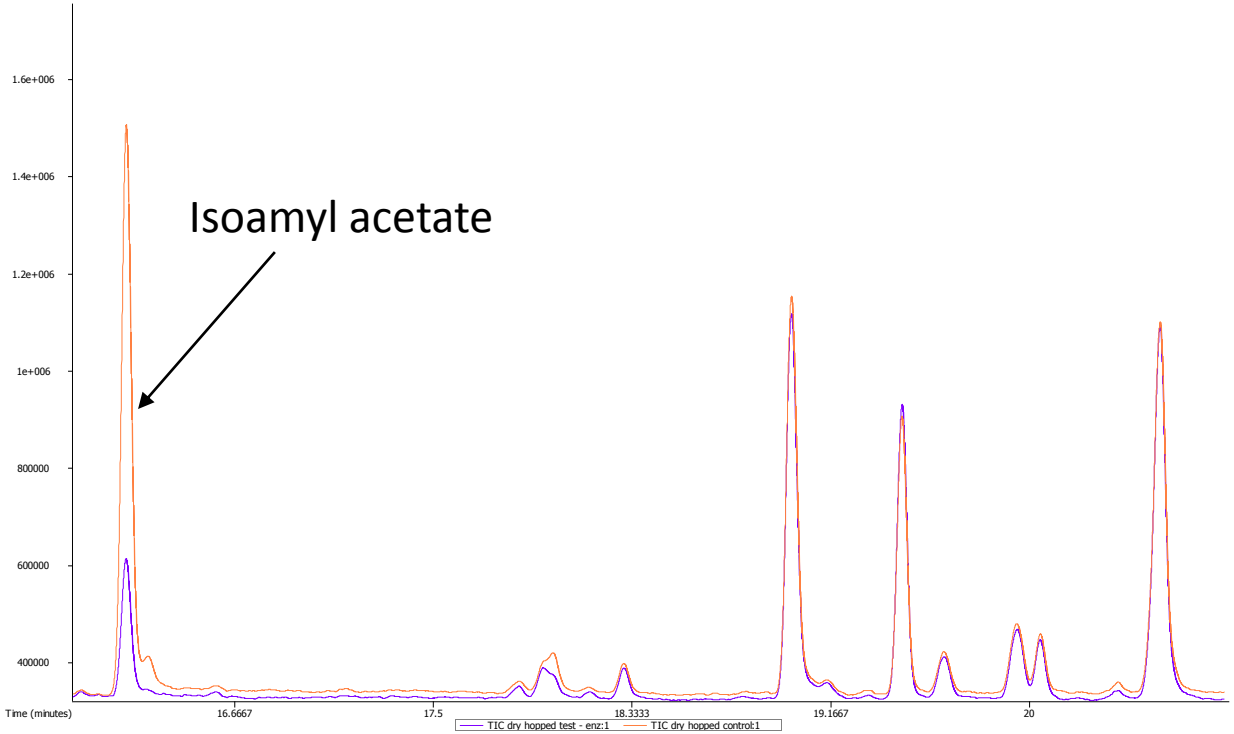


DOE Results

- Alpha .05, but R^2 only 0.79 for linalool
- Challenge in measurement variation (SPME)
- Hopping rate = biggest driver
- Depends on enzyme (saturation and conditions)
- Red grape enzyme could be of interest (polygalacturonase)
- Enzyme effects likely to be unique per brand

TOF – IPA dry hopped with enzyme





- What is different? Can we quantitate? Is it significant?
- Does this data correlate to sensory?