Varietal dependency of hop-derived water-soluble flavor precursors in beer

Daniel Vollmer, Yanping Qian, Gina Shellhammer, and **Thomas Shellhammer**
Oregon State University

The Science of Beer

Hops composition

Principle Components	Concentration (%w/w)
Cellulose + lignin	40.0 - 50.0
Protein	15.0
Alpha acids	2.0 - 17.0
Beta acids	2.0 - 10.0
Water	8.0 - 12.0
Minerals	8.0
Polyphenols and tannins	3.0 - 6.0
Lipids and fatty acids	1.0 - 5.0
Hop oil	0.5 - 3.0
Monosaccharides	2.0
Pectin	2.0

European Brewery Convention *Hops and Hop Products, Manual of Good Practice*; Getranke - Fachverlag Hans Carl: Nurnberg, Germany, 1997.

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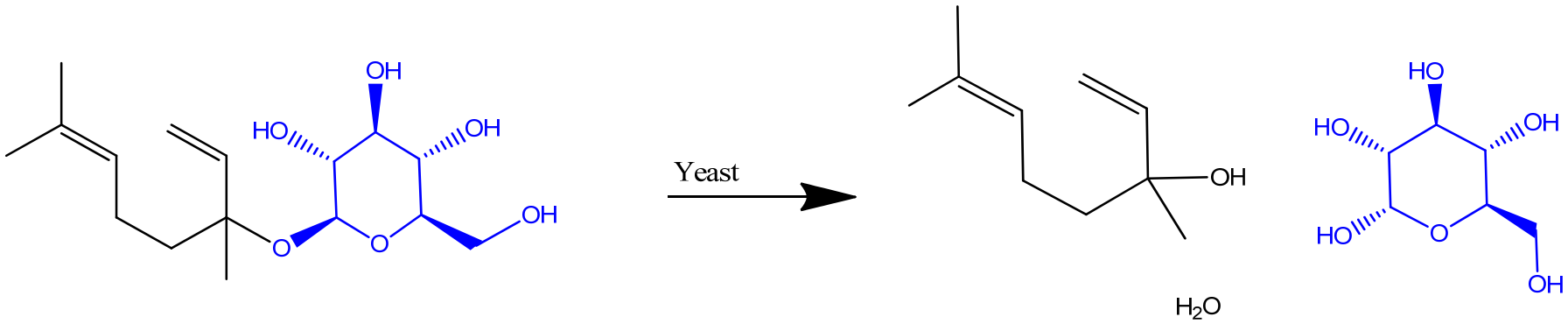
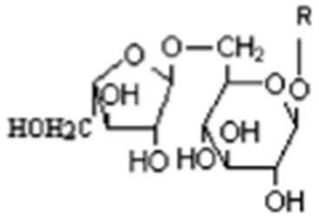
Water-soluble flavor precursors

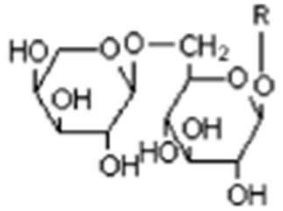
Glycosides of volatile compounds present in plants
Hydrolysis to release aroma compounds
enzymatic hydrolysis – yeast, bacteria, fungi
Acid hydrolysis



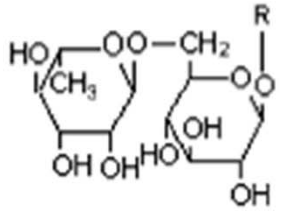
Sugar moieties



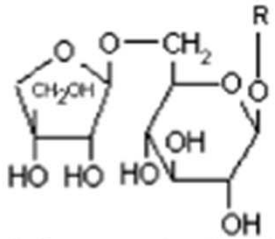
α-L-arabinofuranosyl-β-D-glucoside



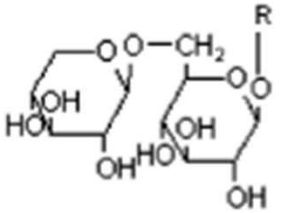
α-L-arabinopyranosyl-β-D-glucoside
(Vicianoside)



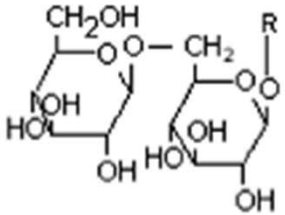
α-L-rhamnopyranosyl-β-D-glucoside
(Rutinoside)



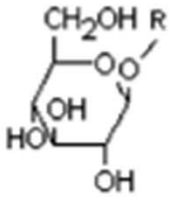
β-D-apiofuranosyl-β-D-glucoside



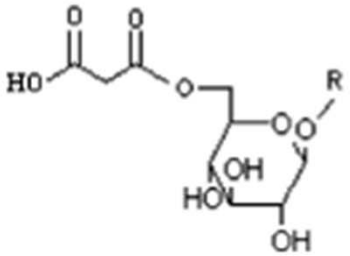
β-D-xylopyranosyl-β-D-glucoside
(Primeveroside)



β-D-glucopyranosyl-β-D-glucoside
(Gentibioside)



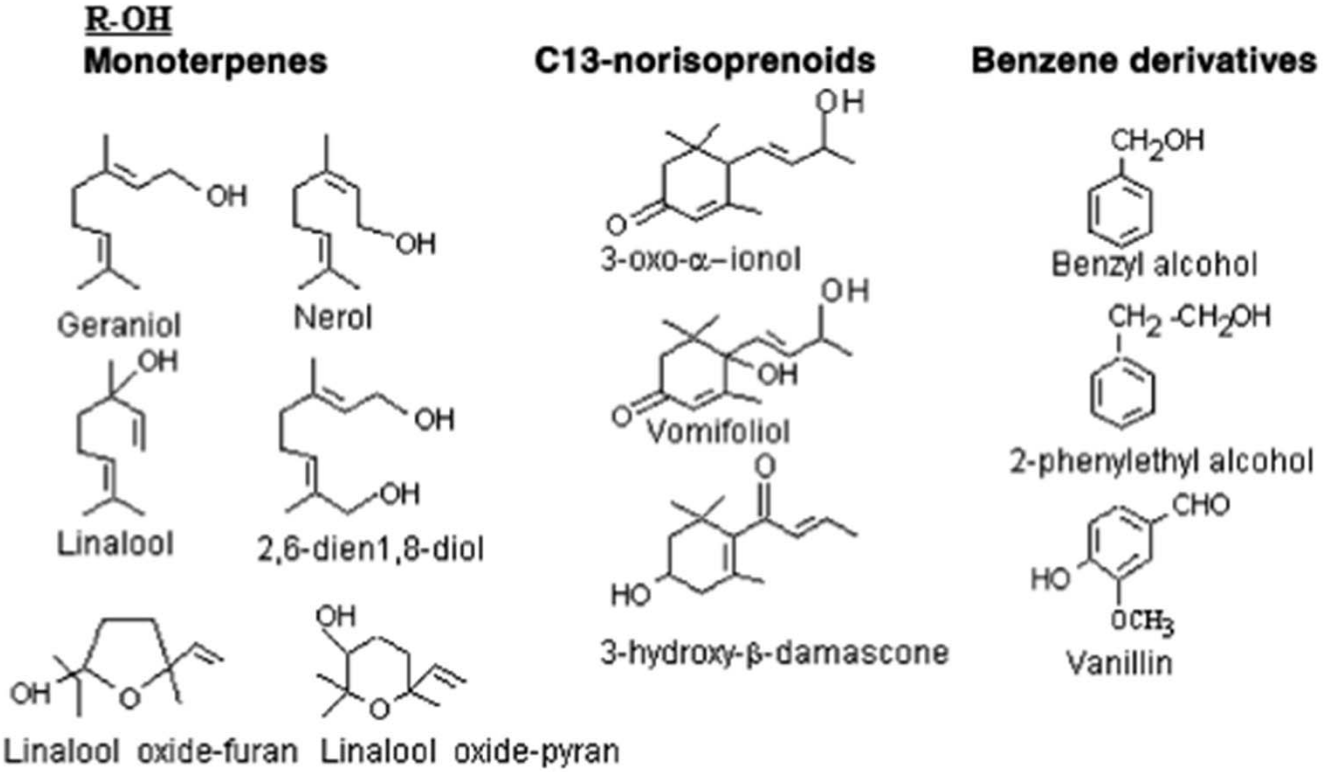
β-D-glucoside



Malonyl-β-D-glucoside

Sarry and Günata, 2004, Plant and microbial glycoside hydrolases: volatile release from glycosidic aroma precursors. Food Chemistry

Aglycones



Sarry and Günata, 2004, Plant and microbial glycoside hydrolases: volatile release from glycosidic aroma precursors. Food Chemistry

Glycosides in wine making

	Monoterpenes		Norisoprenoids		Benzene derivatives[a]	
	Control	Treated	Control	Treated	Control	Treated
Muscat of Frontignan[(1)]	4384	7718	9	542	101	392
Riesling[(1)]	2418	3119	nd	407	107	693
Gewurztraminer[(1)]	129	248	nr	nr	nr	nr
Emir[(1)]	14	25	48	74	809	1105
Trajadura[(1)]	100	233	nr	nr	nr	nr
Muscat[(2)]	805	1407	nr	nr	nr	nr
Muscat of Lunel[(2)]	3916	4646	nr	nr	nr	nr
Sauvignon[(2)]	2018	2178	nr	nr	46	120
Chardonnay[(2),b]	2310	2500	nr	nr	36	97

Sarry and Günata, 2004, Plant and microbial glycoside hydrolases: volatile release from glycosidic aroma precursors. Food Chemistry

Hop derived glycosides

found in Cascade and Mt. Hood

3-methyl butanol glucose (fruity, banana, ethereal)
Benzyl alcohol glucose
2-phenyl ethanol glucose (floral, rose)
1-octanol glucose (waxy, green, citrus, orange)
Vanillin glucose (vanilla)
Linalool glucose (citrus, orange, floral)
α-terpineol glucose (pine, lilac, citrus, woody, floral)

Murakami et al, 2006, Use of hop glycosides extracted from hop plant parts to flavor malt beverages. US Patent 7,001,638 B2

Project Phases

Phase 1: Selection & Supercritical CO_2 Extraction of 18 varieties of hop pellets

Phase 2: Treatment of aqueous extracts with enzyme liberating bound aroma compounds & analysis of extracts with SBSE GC-MS

Phase 3: Descriptive analysis with trained sensory panel comparing treated and untreated extracts

Phase 1: Genetic Groupings

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Cascade	Galena	Cluster	Horizon	Nugget	Challenger	Mt. Hood	Fuggle	Spalter	Perle
Pride of Ringwood	Chinook	Tsingdao Flower		Taurus	Northern Brewer	Liberty	EKG	Saazer	Hersbrucker
Target	Columbus			Magnum	Aurora	HHA	Spalter Select	Tettnanger	Emerald
Simcoe	Brewers Gold			Newport	Northdown	Vangaurd	Willamette		Orion
	Bullion			Millenium		Citra	Golding		
	Glacier			Tsingdao Flower		Crystal	Centennial		
	Apollo					Nelson Sauvin			
	Bramling Cross								
	Bravo								
	Sorachi Ace								
	Summit								
	Super Galena								

49 proposed varieties

- 10 genetically distinct groups – 49 varieties
- Hops selected based on information provided by John Henning & Shaun Townsend (Oregon State University)

Phase 1: Selections from Groups

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Cascade	Galena	**Cluster**	**Horizon**	**Nugget**	Challenger	Mt. Hood	Fuggle	**Spalter**	**Perle**
Pride of Ringwood	Chinook	Tsingdao Flower		Taurus	**Northern Brewer**	Liberty	EKG	Saazer	**Hersbrucker**
Target	**Columbus**			**Magnum**	**Aurora**	**HHA**	Spalter Select	**Tettnanger**	Emerald
Simcoe	Brewers Gold			Newport	Northdown	Vangaurd	**Willamette**		Orion
	Bullion			Millenium		Citra	Golding		
	Glacier			Tsingdao Flower		Crystal	**Centennial**		
	Apollo					**Nelson Sauvin**			
	Bramling Cross								
	Bravo								
	Sorachi Ace								
	Summit								
	Super Galena								

18 varieties selected

- HRC Member Survey – ***Spring 2013***

Hop acids reduction by SC-CO_2 extraction

	UV-Vis			HPLC			
Variety	Alpha %	Beta %	HSI	Alpha %	Beta %	Co-Hum %	Co-Lup %
Simcoe (2011)	0.2	0.4	1.52	1.2	0.7	2	30
Cascade	0.3	0.4	0.50	0.2	0.0	32	0
Summit	0.8	0.3	0.90	0.1	0.0	29	0
Columbus (2011)	0.6	0.6	0.96	0.4	0.0	28	0
Cluster	0.8	0.4	0.37	0.4	0.2	36	62
Horizon	0.1	0.1	1.46	0.0	0.0	12	0
Magnum	1.2	0.4	0.67	1.0	0.2	28	50
Nugget	0.6	0.4	0.69	0.5	0.1	34	0
Northern Brewer	0.2	0.1	0.96	0.1	0.0	35	44
Styrian Aurora	0.1	0.2	1.46	0.1	0.0	25	0
Hallertauer mittlefrüh (HHA)	0.2	0.2	1.29	0.1	0.0	35	31
Nelson Sauvin	0.0	0.3	2.72	0.1	0.0	49	40
Willamette	0.3	0.3	0.58	0.2	0.1	33	61
Centennial	-0.4	0.3	2.46	0.2	0.0	0	0
German Spalt	0.3	0.2	0.86	0.1	0.0	34	32
Tettnang	0.3	0.1	0.58	0.1	0.0	28	0
Hersbrucker	0.2	0.1	0.92	0.0	0.0	38	21

The SFE resulted low residual hop acid content with only two varieties having more than 1% residual hop acids

Spent Hop Processing

Spent hops were boiled for 3 hours in 3 L (aq.) buffer

Brewing Salt	Concentration
Citric Acid	1.00 g/L
$CaSO_4$	0.31 g/L
$MgCl_2$	1.41 g/L
NaCl	0.11 g/L
$(NH_4)\ SO_4$	1.79 g/L

Dosage: **50 grams/liter**

- Spent extracts were coarse filtered & cooled
- Exogenous enzyme added after cooling overnight
- Stored at -25° C until instrumental & sensory analysis

Enzymatic Hydrolysis

Previous experiments revealed that enzymatic hydrolysis yielded comparable results to yeast and acid treatments

Selected Enzyme: Rapidase AR2000 - DSM Food Specialties

Pectolytic enzyme with specific glycosidase activity used in white wine processing to enhance aroma

Dosage: 1000 ppm

Incubation: 48 hours at 30°C

Instrumental Analysis of Volatile Compounds

GC-MS with stir bar sorptive extraction (SBSE)

- 20°C for 3 hours, stirred at 1000 RPM using a PDMS coated stir bar.

External standard curves in ethanol and dichloromethane

- concentrations of 1, 10, 25, 50, 100, 250, and 500 ppb

Glycoside Internal Standard (IS)

(β- d-octyl glucopyranoside) at 100 ppb to measure extent of hydrolysis.

Targeted Aglycones:

- Linalool
- Terpinen-4-ol
- Citronellol
- Nerol
- Beta-damascenone
- Geraniol
- Eugenol

Sensory Protocol

14 trained panelists participated in **12** sessions

Sensory Protocol:

- Descriptive Analysis
- 0-7 scale (none – very high)
- Blocked by replication & varietal combination
  - 3 blocks (contain both control & enzyme)
  - 1 block = 36 samples
- Control v. Enzyme Aroma Profile Evaluation
- Shared Samples (4 people per sample set)

Descriptive Attributes

- Overall Intensity
- Citrus
- Dark Fruit
- Floral
- Earthy/Mushroom
- Vegetal/Hay
- Meaty/Broth
- Iced Tea
- Spicy
- Burnt Rubber

Results

Instrumental Results

	Linalool					Geraniol	
Variety	linalool	(-) terpinene-4-ol	citronellol	nerol	beta-damascenone	geraniol	eugenol
Nelson Sauvin	4.6	1.0	0.3	2.1	0.0	8.7	1.7
Cascade	27.0	0.8	0.3	5.5	0.0	15.3	0.1
Centennial	11.9	4.3	5.3	12.7	0.3	88.7	8.3
Cluster	1.6	0.1	0.0	2.7	-0.1	21.7	2.6
Columbus	82.8	6.6	0.9	16.8	0.2	178.3	5.1
German Spalt	20.4	0.8	1.1	3.6	0.3	18.9	4.0
Hersbrucker	11.1	0.3	0.1	4.5	0.2	36.0	4.2
HHA	15.4	1.1	-0.5	4.7	0.2	39.4	2.6
Horizon	28.1	2.8	0.5	5.2	0.1	21.5	2.8
Magnum	30.2	3.1	2.5	4.1	0.3	17.8	4.5
Northern Brewer	29.5	2.2	-0.1	2.7	-0.1	9.3	0.5
Nugget	45.0	3.3	1.3	3.6	0.2	13.1	2.3
Perle	7.9	-2.2	0.7	4.4	-0.2	46.6	-0.1
Simcoe	27.6	1.4	0.9	8.0	0.2	44.5	2.9
Styrian Aurora	61.4	1.7	0.9	3.0	0.2	22.5	3.9
Summit	39.4	1.4	0.9	5.8	0.1	21.5	-0.1
Tettnang	9.5	0.6	0.0	2.7	0.0	17.8	3.3
Willamette	5.8	0.2	0.1	3.2	0.0	43.8	1.5

Δ

delta = *Treatment - Control*

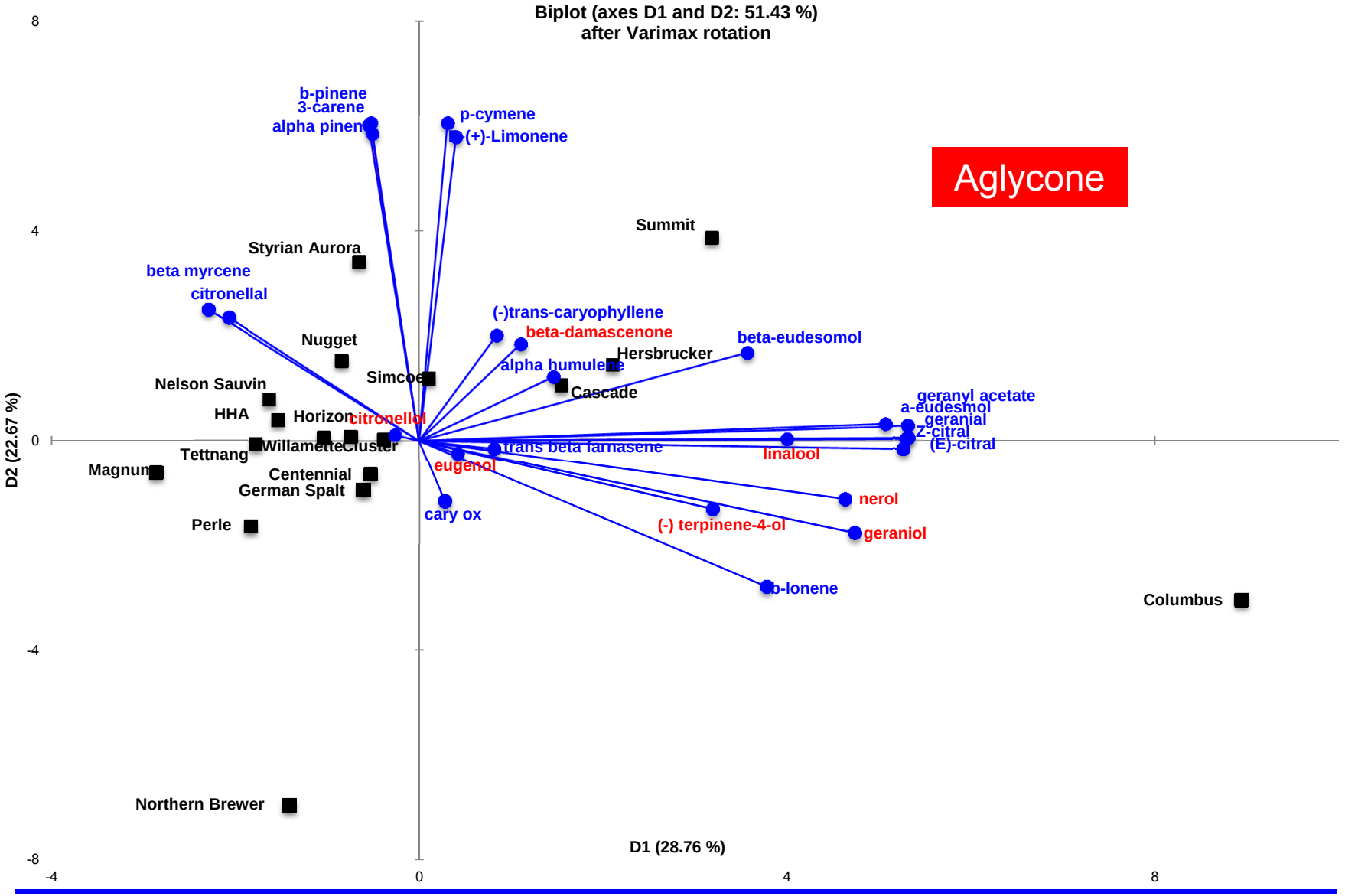
- Averages of duplicate injections using SBSE
- Centennial & Columbus
- Geraniol & linalool driving instrumental changes

Greater than 30 ppb

Between 10 – 30 ppb

Less than 10 ppb

Principle Component Analysis – Instrumental *Differences*



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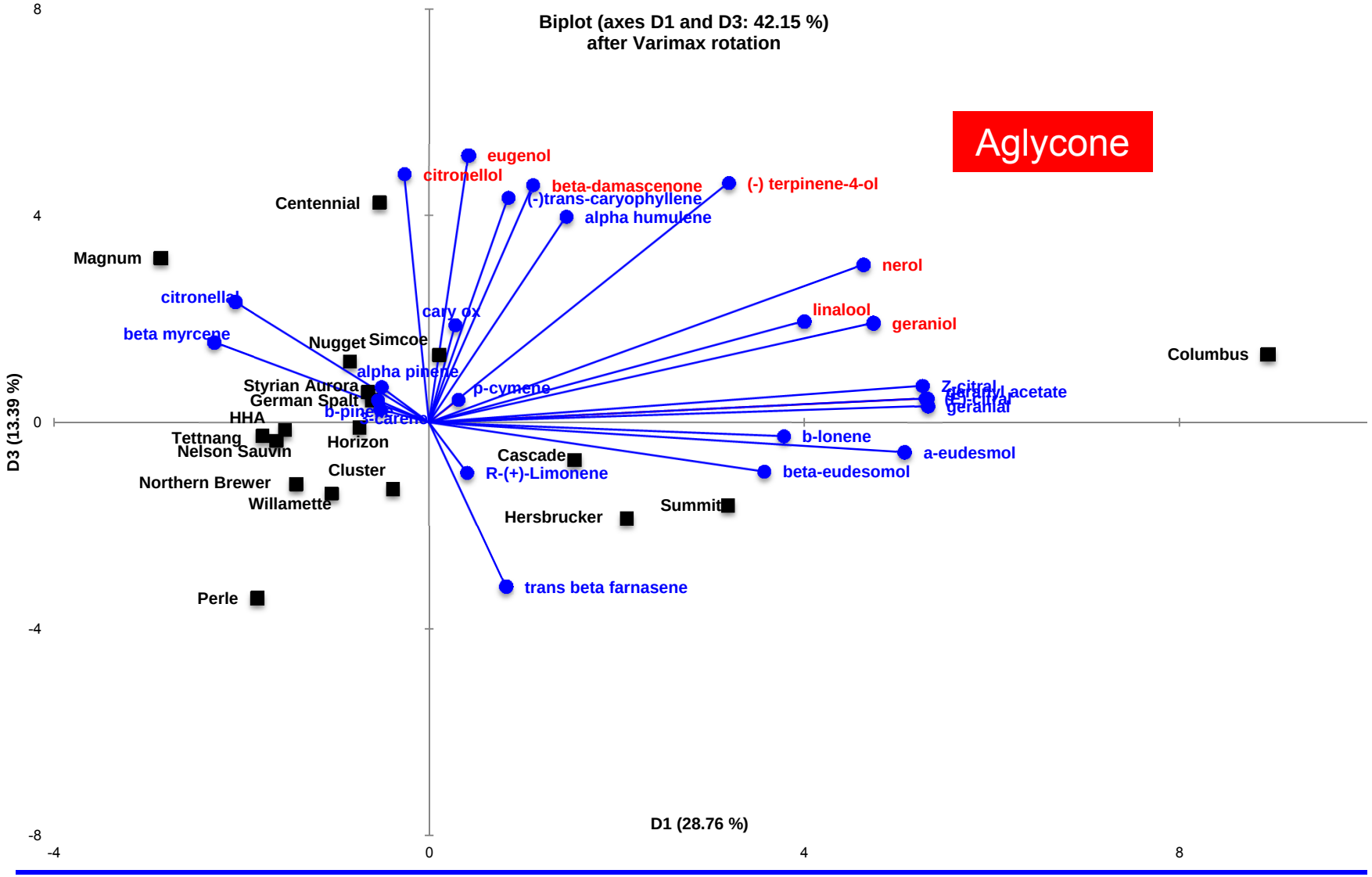
Principle Component Analysis – Instrumental *Differences*



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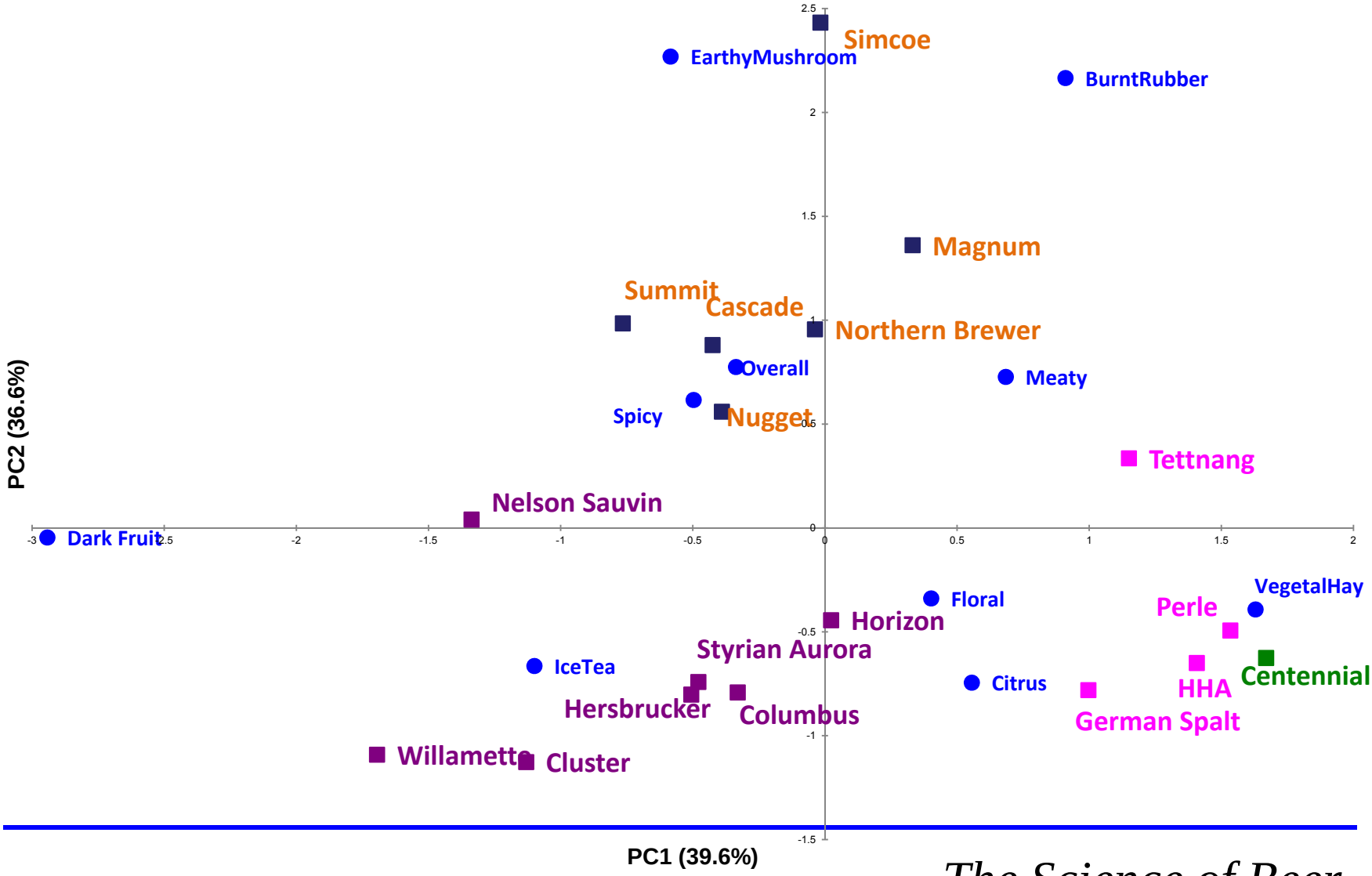
Sensory Results – Individual Attributes

- Analysis of Variance of (ANOVA) mean sensory scores
- Enzyme treatment effect significant for every attribute except: *burnt rubber*
- Interaction significant for every attribute except: *E/M, Iced Tea*

Attribute	Hop Variety	Enzyme Treatment	Variety * Treatment
Overall	8.81*	37.38*	4.34*
Citrus	6.85*	10.03*	5.26*
Dark Fruit	5.54*	1095.63*	6.15*
Floral	4.69*	62.17*	3.55*
Earthy/Mushroom	7.94*	81.08*	2.32
Vegetal/Hay	2.36*	143.62*	3.34*
Meaty/Broth	5.80*	387.55*	3.59*
Iced Tea	3.89*	80.43*	2.46
Spicy	8.18*	41.96*	3.20*
Burnt Rubber	9.67*	3.36	4.61*

*indicates significance $p < 0.0001$

Principle Component Analysis
Impact of enzyme treatment on sensory attributes



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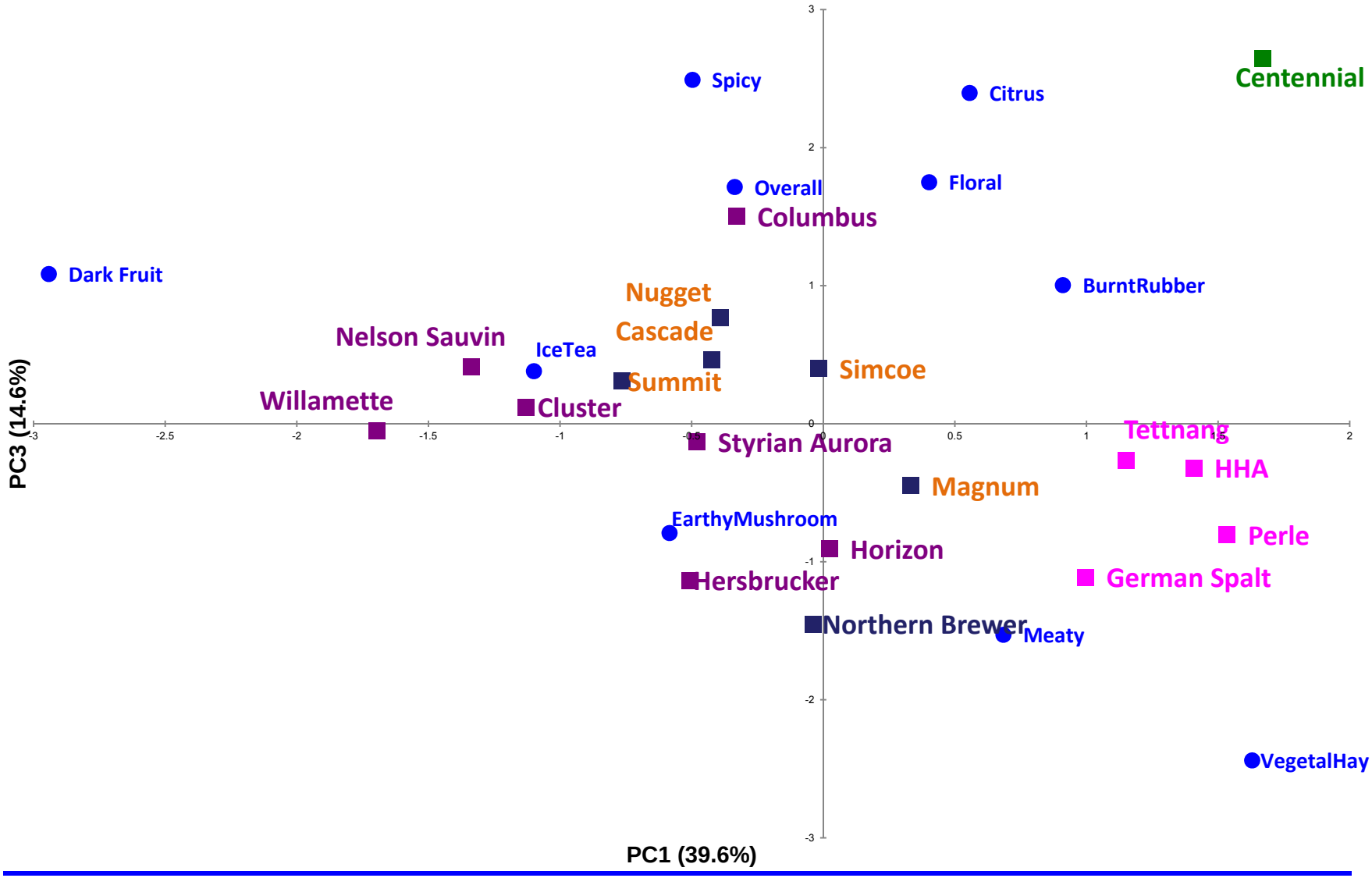
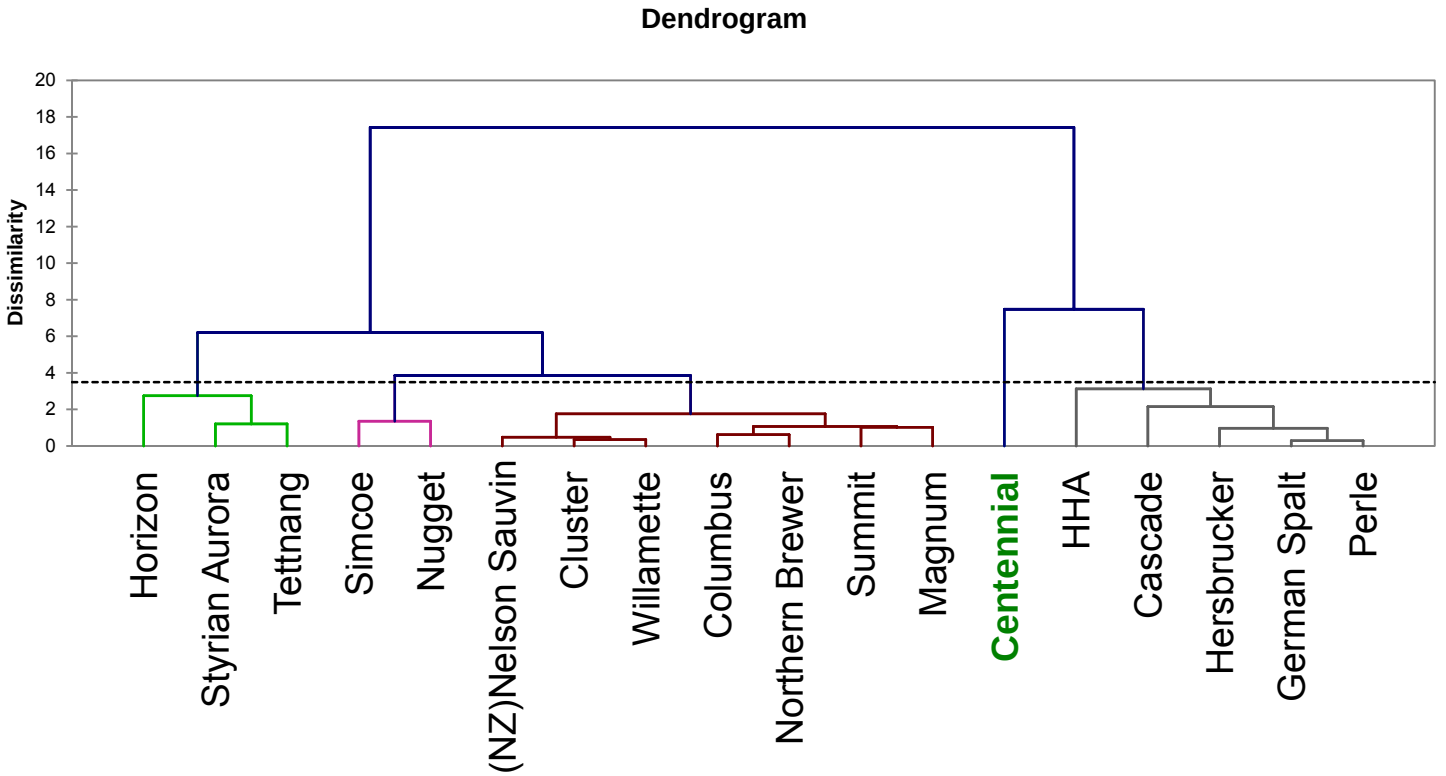
Principle Component Analysis
Impact of enzyme treatment on sensory attributes



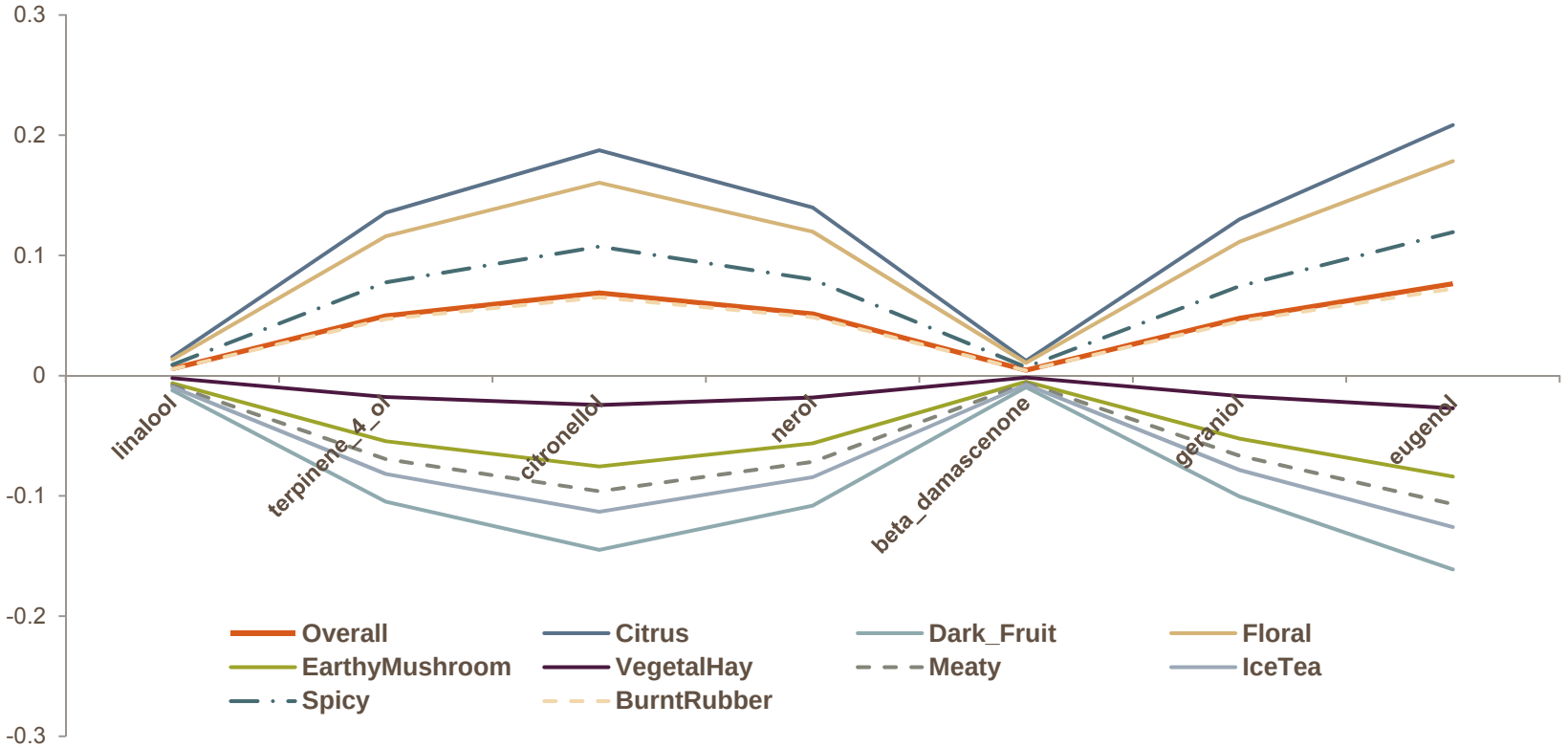
AHC Cluster Analysis – Sensory Attributes *Differences*



Group Centroids

Class	Overall	Citrus	Dark Fruit	Floral	Earth/Mush	VegetalHay	Meaty	IceTea	Spicy	BurntRubber
1 (Perle)	0.429	-0.143	1.452	0.476	0.571	-0.357	-0.952	0.357	-0.214	0.476
2 (Nugget)	0.000	0.143	3.024	0.929	0.691	-1.476	-2.476	1.500	-0.595	0.500
3 (Willamette)	0.310	-0.810	3.000	0.000	0.476	-1.119	-1.857	0.881	-0.690	-0.357
4 (Styrian Aurora)	0.167	-0.286	1.976	0.452	0.214	-1.167	-1.262	0.643	-0.191	-0.429
5 (Centennial)	1.190	1.405	0.847	1.500	-0.119	-0.738	-2.024	0.286	0.286	1.405

Partial Least Squares Regression



- Increases in *citronellol* and *eugenol* were associated with increases in the **Citrus** & **Floral**
- PLS-R model accounts for **54.8%** variability in chemical data and **17.4%** in sensory data

Conclusions

Instrumental:

- Columbus & Centennial map strongly with changes in aglycones
- Linalool, geraniol, nerol, eugenol, citronellol, terpinen-4-ol
- Analytes observed do not represent all possible sources of aroma

Sensory:

- Enzyme treatment had significant effect for all except *Burnt Rubber*
- Effect of enzyme treatment varied by hop variety for nearly all attributes except: *Earthy/Mushroom, Iced Tea*
- 5 distinct sensory groupings – did not match original pedigree
- Centennial experienced largest impact of enzyme hydrolysis (*Overall, Citrus, Floral, Burnt Rubber*)

Final Remarks

- Concentration of spent extracts ***(50 g/L)*** is much greater than typical hopping rates used in practical brewing
- GC-MS in Selective Ion Monitoring (SIM) mode does not look at all possible contributions to aroma
- Assessing the impact of sulfur based aroma in the extracts would require different detection technology
- Glycosidically bound aroma compounds appear to play a role in total hop aroma

Acknowledgements

Hop Research Council

OSU - Jeff Clawson

Yakima Chief

Missy Raver and YC Pilot CO_2 extraction team

Thank you

Questions?