Key Aroma Compounds in ‘Centennial’, ‘Citra’ and ‘Nelson Sauvin’ Hop Identified by Aroma Extract Dilution Analysis

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A Word About Hop

- 84% vegetative matter
- 16% Lupulin glands:
  - soft resins ~13%
  - hard resins ~2%
  - essential oils ~0.5-3%
- Depending on variety, growing conditions/harvest time

Hop Oil Composition

- Hydrocarbons and epoxides
  - Monoterpenes
  - Sesquiterpenes
  - Epoxides
- Terpene alcohols and esters
- Aldehydes, Ketones, fatty Acids, esters
  - Simple, well studied
- C_{13}-norisoprenoids
- Benzene derivatives
- Sulfur-containing compounds
  - Very complicated

Which compounds are important?

Objectives

- Identify the most important aroma-active compounds in three different hops
  - Centennial
  - Citra
  - Nelson Sauvin

Gentle Volatile Isolation from Hops

- 30 g of dried hop cones were blended under liquid nitrogen.
- Volatiles were extracted with dichloromethane (3X)
- Solvent Assisted Flavor Evaporation (SAFE) was applied to the hop extract under high vacuum
- Distillates were concentrated to 1 mL with a Kuderna-Danish Concentrator

Aroma Identification Is Extremely Challenging

- Many compounds are co-eluted, what you can identify may not be what you smell
- Many compounds have smell but no MS signal
- Using multiple tools for confirmation
  - Mass spectra and retention index match with pure standards in lab
  - 2D GC-MS with heart-cut technique
  - Pre-fractionation and preparative GC
  - Personal experiences with aroma compounds

Normal Phase Chromatography Fractionation

Aroma Extract

Silica Gel

Eluted with Pentane

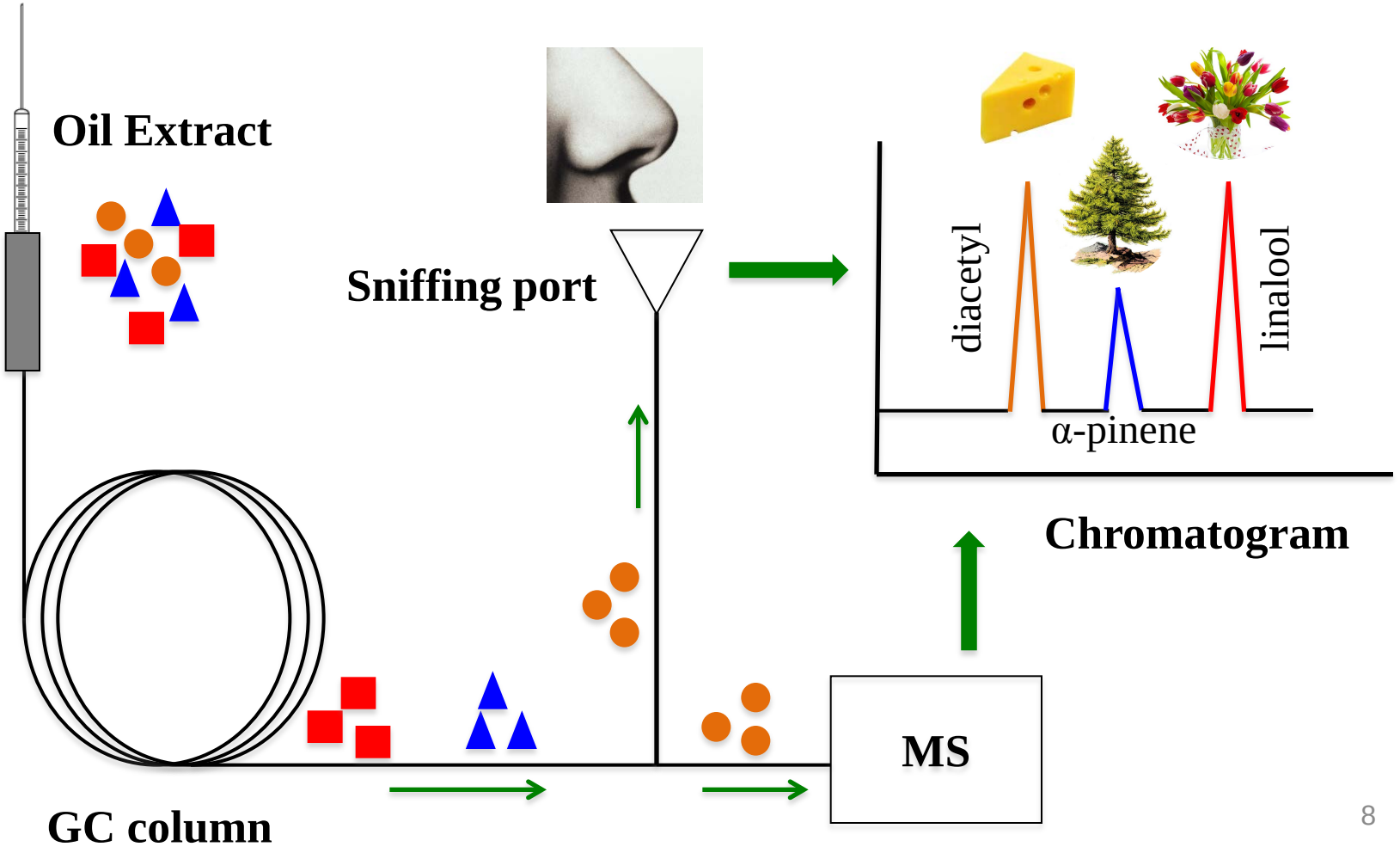
Eluted with CH_2Cl_2

Non-polar fraction

Polar fraction

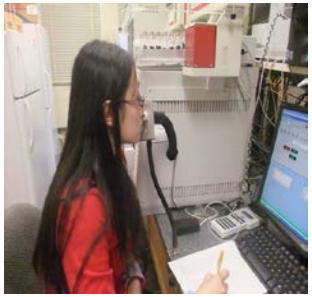
GC-MS/Olfactometry

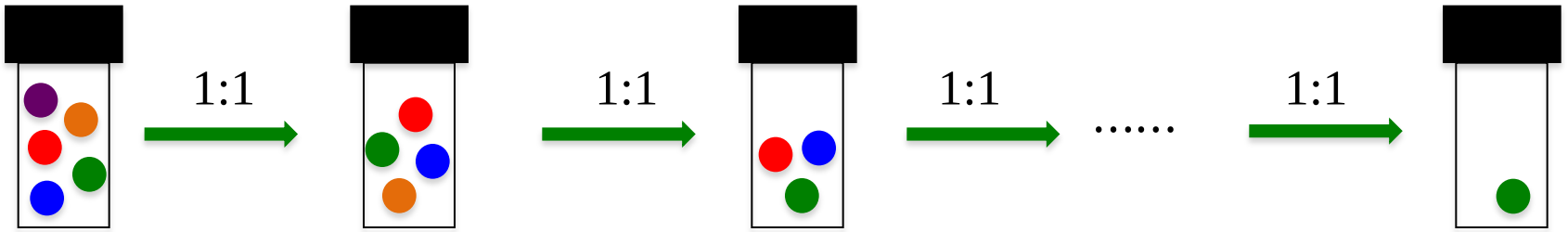
Gas **C**hromatography-**M**ass **S**pectrometry/**O**lfactometry



Aroma Extract Dilution Analysis (AEDA)

- The two fractions of hop oils were stepwise diluted with dichloromethane/pentane at 1:1 ratio and analyzed by GC–O.
- Flavor dilution (FD) factor of each odorant was calculated.





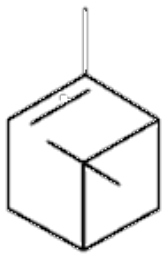
Original sample	1:2 dilute	1:4 dilute		1:512 dilute
FD=1	FD=2	FD=4		FD=512

AEDA-Nonpolar Fraction

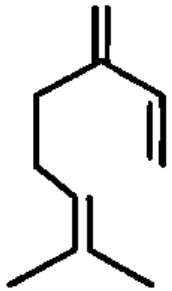
- Nonpolar fraction (hydrocarbons) can be classified into two major groups: monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$).

Nonpolar Fraction					
			FD-Factor		
RI	Aroma Compounds	Descriptor	CE	CI	NS
1032	α-pinene	orange peel, pine	32	32	32
1152	myrcene	celery, balsamic	512	512	512
1177	β-phellandrene	mint, turpentine	8	4	4
1628	β-caryophyllene	woody	4	2	4

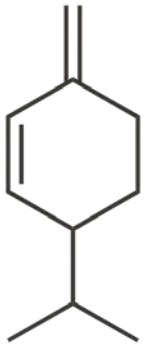
CE: Centennial, CI: Citra, NS: Nelson Sauvin



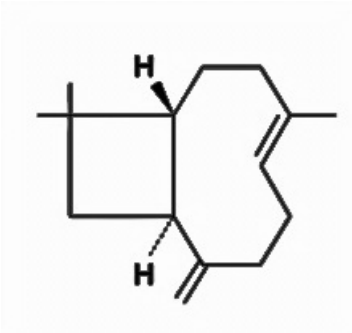
α-pinene



myrcene



β-phellandrene



β-caryophyllene

AEDA-Polar Fraction

- Oxygenated components (alcohols, aldehydes, acids, esters， etc.) and sulfur-containing components (thioesters, miscellaneous sulphur compounds, etc.).

Polar Fraction					
			FD-Factor		
RI	Aroma Compounds	Descriptor	Centennial	Citra	Nelson Sa
987	diacetyl	cheesy, buttery	64	8	16
1130	2-methylbutyl acetate	fruity, nail polish	16	16	8
1173	unknown	pine, turpentine	32	64	64
1461	methional	potato, soy sauce	16	8	4
1550	linalool	floral	64	64	16
1609	methyl (Z)-4-decenoate	milky, green	16	16	4
1659	isovaleric acid	smelly, rancid	128	256	64
1701	(2E,4E)-nona-2,4-dienal	steamed grain, oily	16	64	16
1730	S-methylthiomethyl 2-methylbutanethioate*	garlic, preserved vegetable	16	32	8
1837	geraniol	citrus, lemon	256	128	128
1897	S-methylthiomethyl 4-methylpentanethioate*	garlic, fatty	16	16	16
1973	S-methyl methanethiosulfonate	radish, cabbage	128	64	16
2000	unknown	preserved vegetable	16	64	16
2546	vanillin	vanilla	64	64	64

*Compounds were tentatively identified but have not confirmed by pure standards yet.

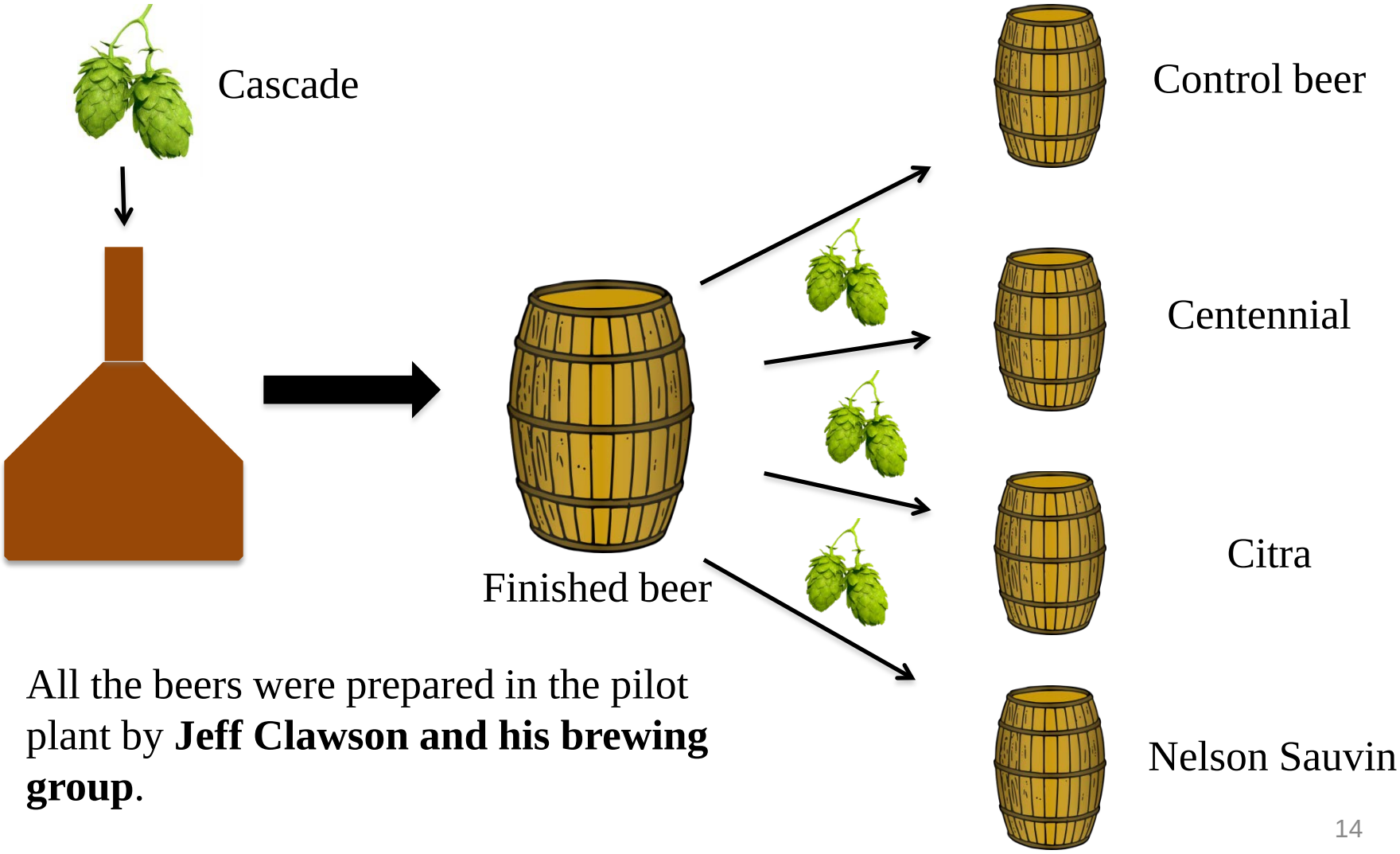
AEDA-Polar Fraction

Polar Fraction					
			FD-Factor		
RI	Aroma Compounds	Descriptor	Centennial	Citra	Nelson Sauvin
1101	hexanal	grassy	4	8	1
1181	isoamyl propionate	fruity	4	2	4
1195	2-methylbutyl isobutyrate	fruity, soda	2	<1	<1
1199	2-methyl-1-butanol	pear, fruity, sweet	8	4	8
1228	unknown	pine tree, almond	8	4	1
1271	methyl heptanoate	fruity, sweet	2	2	<1
1354	S-containing compounds	preserved vegetable	8	2	2
1380	methyl octanoate	citrus, soapy, fatty	8	8	4
1398	nonanal	citrus, floral	8	<1	4
1404	S-methylthiohexanoate	preserved vegetable, cabbage	8	8	2
1409	cis-3-hexenol	green, grassy	8	2	4
1433	acetic acid	vinegar, sour	2	2	2
1493	methyl nonanoate	floral, cooked rice	8	4	8
1547	isobutyric acid	cheese, rancid	8	8	<1
1704	geranial	citrus, soapy	4	<1	<1
1756	nerol	sweet, floral, citrus	8	<1	<1
1845	Unknown	fatty, nutty	8	16	1
1970	caryophyllene oxide	woody, incense	8	16	1
2041	octanoic acid	sweaty, rancid	16	8	2
2133	Unknown	ink, rancid	8	16	<1
2206	nonanoic acid	sweaty	2	4	8
2227	Unknown	rancid, cheese	<1	16	<1

Odor Active Compounds in Three Hops

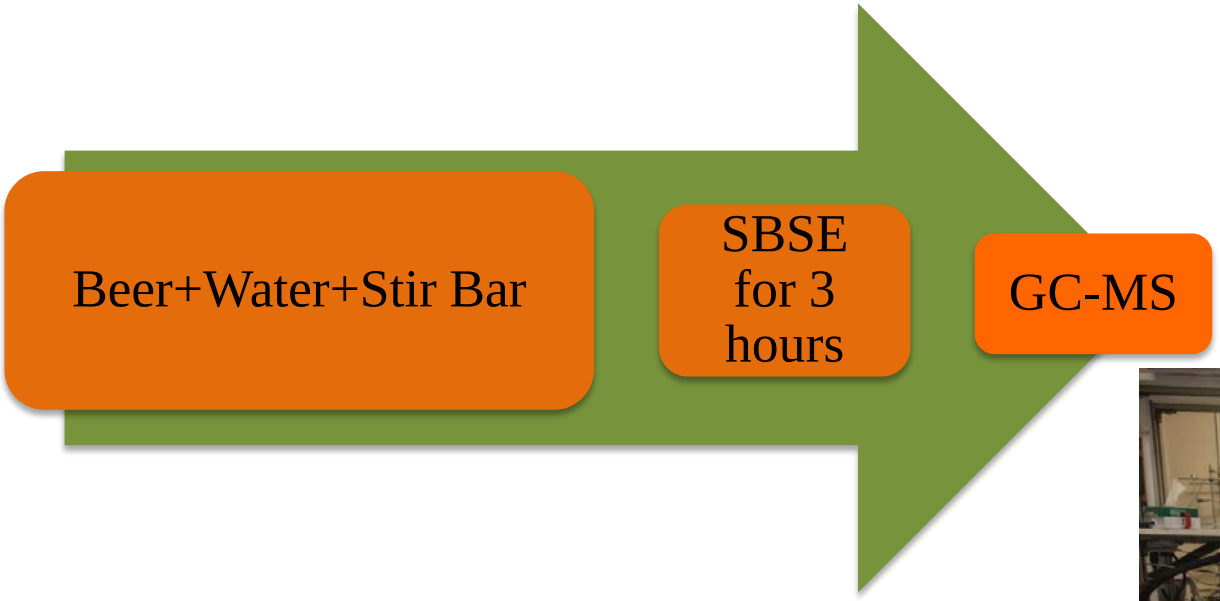
- In the nonpolar fractions, myrcene possessed the highest FD factor while α-pinene also stood out as an odor-active compound.
- In the polar fractions, geraniol and isovaleric acid were proved to have the highest FD values followed by S-containing compound, linalool and vanillin.
- S-methyl methanethiosulfonate was detected in the hops for the first time and showed high FD factor in Centennial and Citra hops, further work is needed to confirm this finding.
- Sulfur-containing compounds were identified to be important contributors to hop aroma profile, many needs to be further confirmed

Dry-Hopped Beer

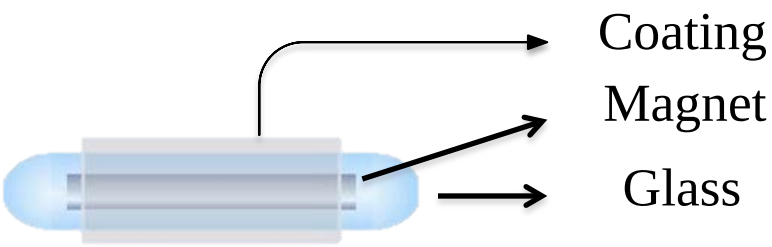


- All the beers were prepared in the pilot plant by **Jeff Clawson and his brewing group**.

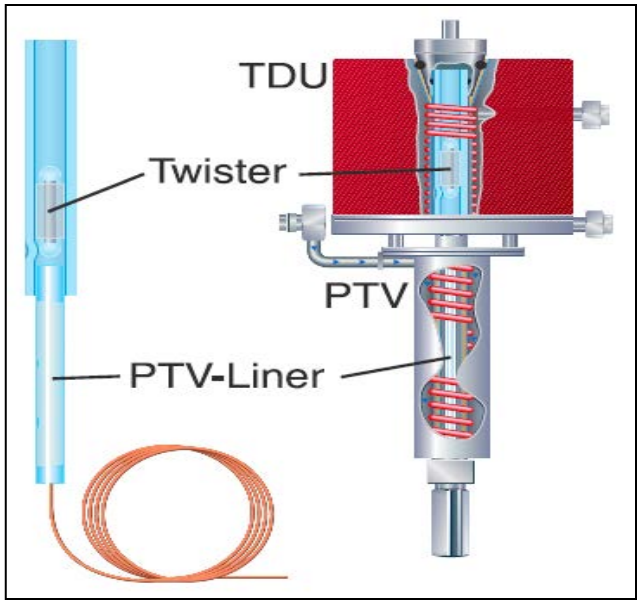
Complete Hop Profiling in Beer by Stir Bar Sorptive Extraction (SBSE)-GC-MS



Stir Bar Sorptive Extraction (SBSE)-GC-MS



PDMS (Polydimethylsiloxane) Stir Bar

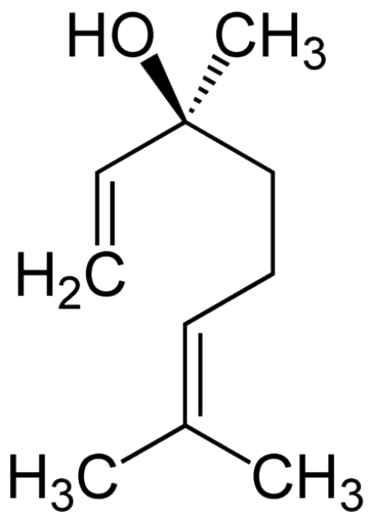


Hop Aroma in Beer

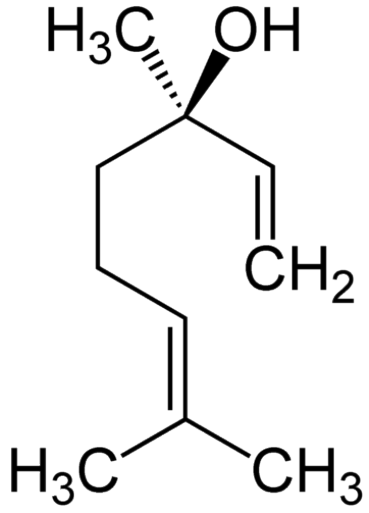
Target compound	Threshold(ppb)	Concentration (ppb)			
		Control	Centennial	Citra	Nelson Sauvin
α-pinene	2.5-62	<1	<1	<1	<1
β-pinene	140	<1	<1	<1	<1
myrcene	13	27.4±1.9	48.2±4.7	131±3.5	110±3.9
limonene	4-229	109±7	56.9±3.1	118±13.8	105±6.0
β-caryophyllene	64-90	<1	<1a	5.63±0.5	<1
α-humulene	120	<1	1.07±0.2	9.38±0.6	8.61±0.3
linalool	4-10	46.8±0.7	78.4±4.9	144±2.7	70.2±0.0
neral	28-120	<1	<1	<1	<1
geranial	n/a	<1	<1	<1	<1
geranyl acetate	9-460	<1	<1	<1	<1
nerol	680-2200	<1	11.71±0.4	7.71±0.2	<1
geraniol	4-75	40.0±1.4	97.0±0.9	86.4±0.2	49.3±1.9
caryophyllene oxide	n/a	9.3±0.5	12.5±1.3	14.7±0.5	8.0±0.1

Linalool Enantiomer

- Linalool occurs naturally as two isomeric forms. These two enantiomers have identical physical properties such as boiling point, melting point and spectroscopic features.

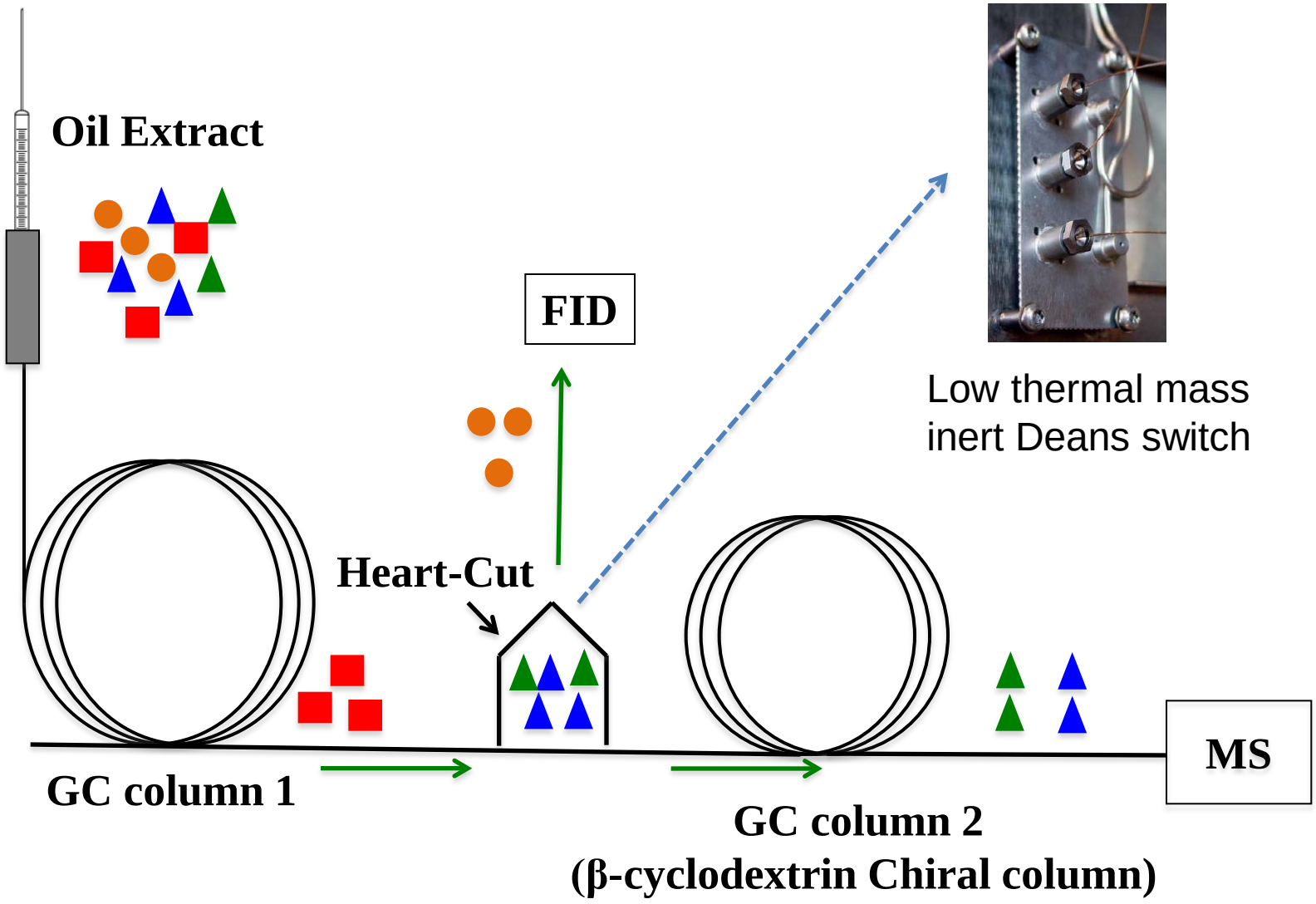


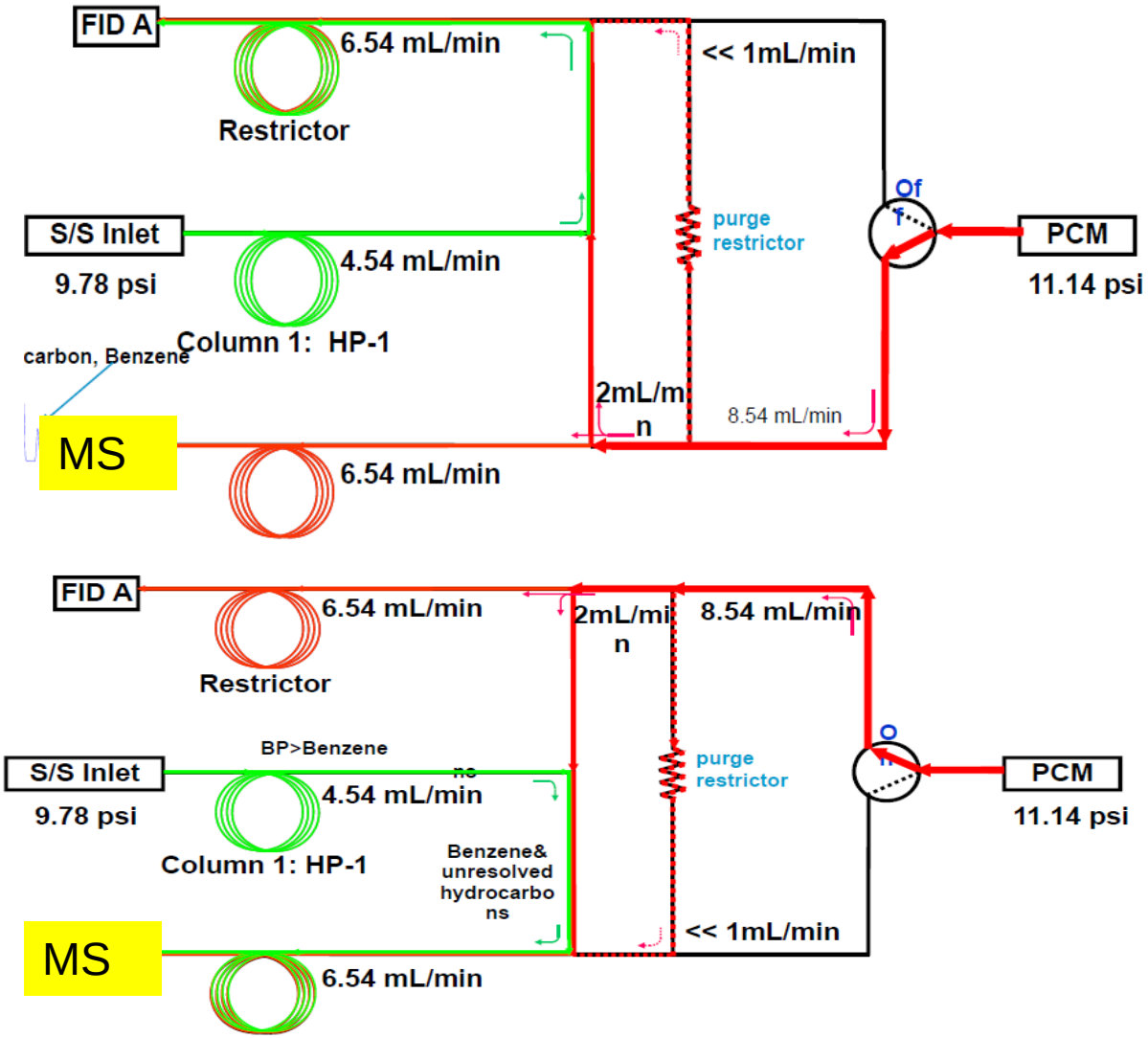
(S)-(+)-linalool
Threshold:**7.4** ppb
Odor: sweet, floral

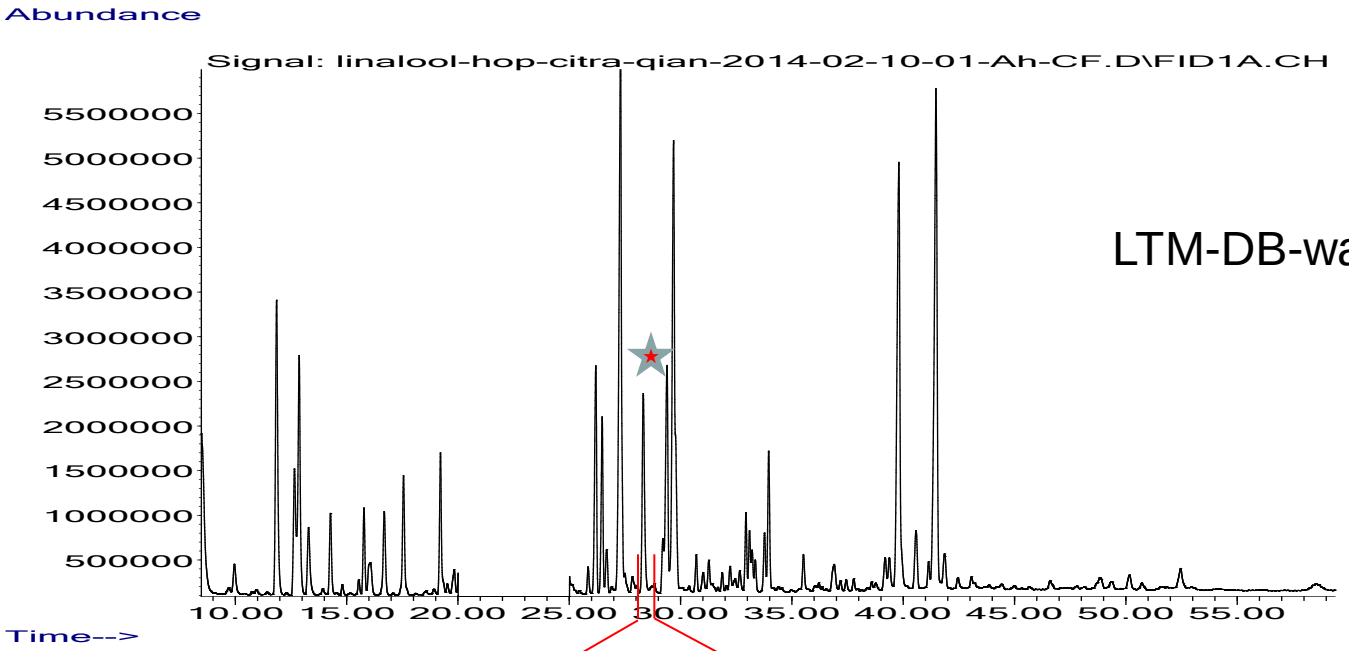


(R)-(-)-linalool
Threshold: **0.8** ppb
Odor: woody, lavender, floral

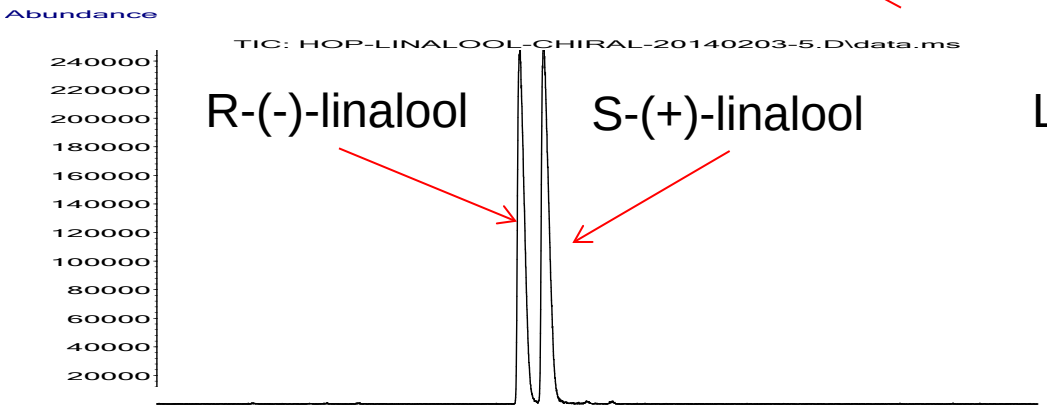
Two-Dimensional GC-MS




FID A
6.54 mL/min
Restrictor
<< 1mL/min
S/S Inlet
9.78 psi
4.54 mL/min
Column 1: HP-1
purge restrictor
Of
PCM
11.14 psi
carbon, Benzene
MS
2mL/m
n
8.54 mL/min
6.54 mL/min
FID A
6.54 mL/min
Restrictor
2mL/mi
n
8.54 mL/min
BP>Benzene
S/S Inlet
9.78 psi
4.54 mL/min
Column 1: HP-1
purge restrictor
O
PCM
11.14 psi
Benzene& unresolved hydrocarbo ns
MS
<< 1mL/min
6.54 mL/min

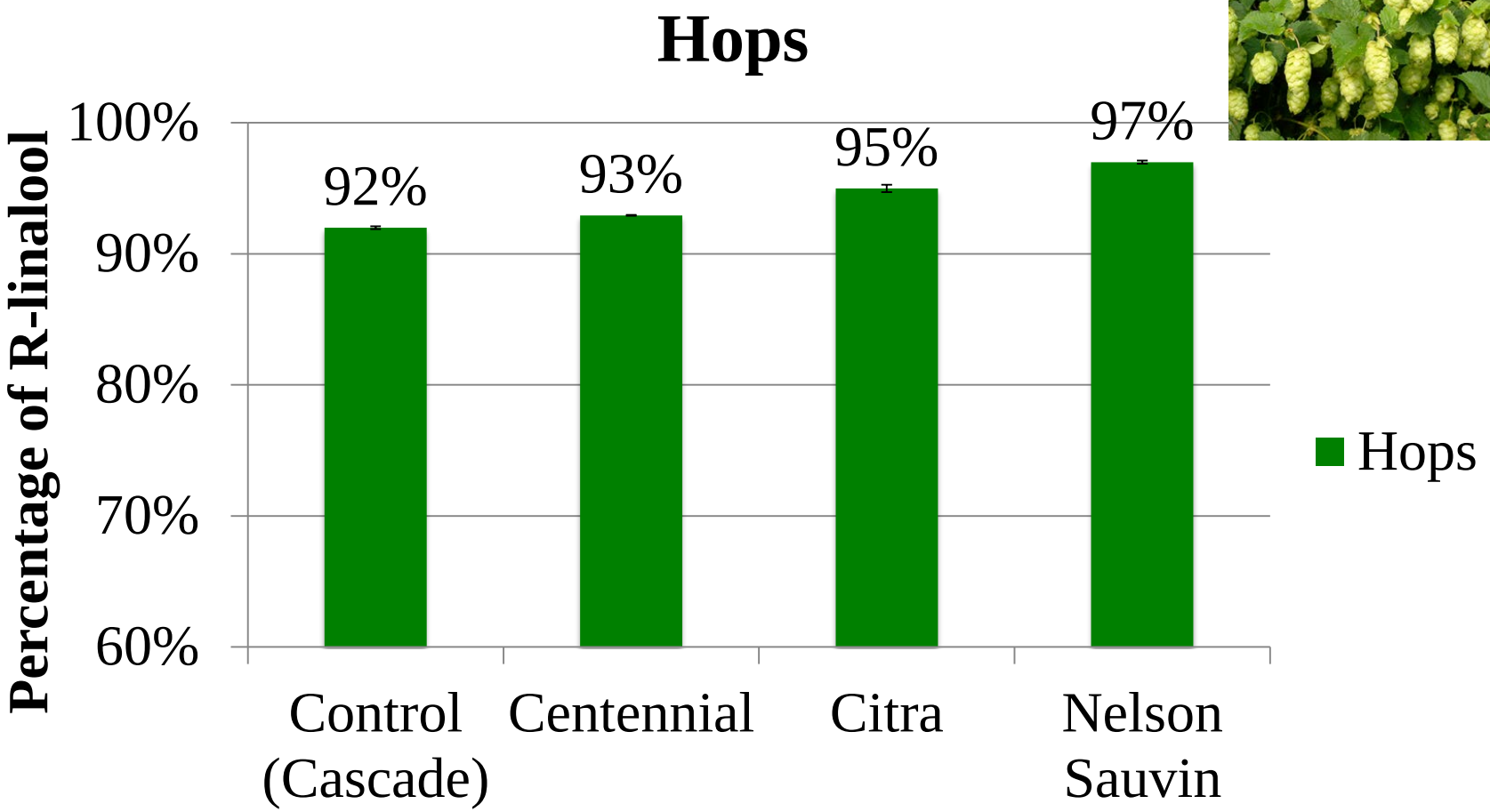


LTM-DB-wax column



LTM-DB-cyclosil B

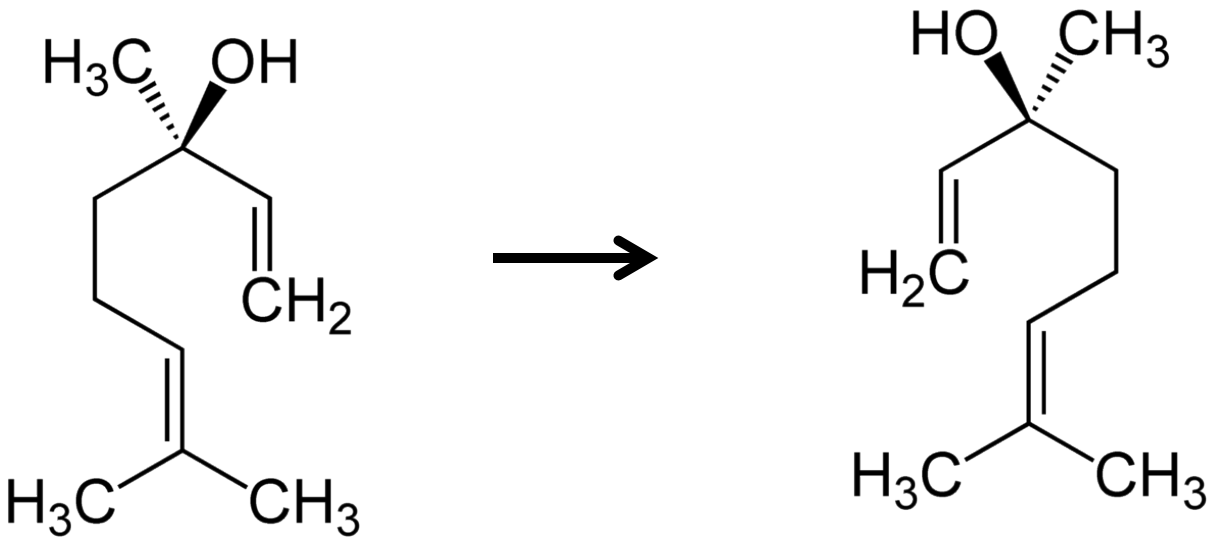
Chiral Distribution of Linalool



Linalool Chiral Distribution in Hops

Hops	% R(-)-linalool	Hops	% R-(-) Linalool
Azacca	93.7± 0.1	Horizon	95.7± 0.1
Chinook	94.2± 0.4	Maridian	93.8± 0.01
Citra	94.1± 0.07	Mt. Hood	94.3± 0.1
Cluster	92.8± 0.4	N. Brewer	92.6± 0.1
Crystal	94.3± 0.2	Topaz	93.3± 0.1
Fuggle	94.2± 0.06	UK-Golding	94.8± 0.2
Galaxy	93.8± 0.2	Ultra	94.1± 0.3
Galena	94.1± 0.1	Warrior	94.2± 0.1
Glacier	94.8± 0.2	Willamette	94.7± 0.06

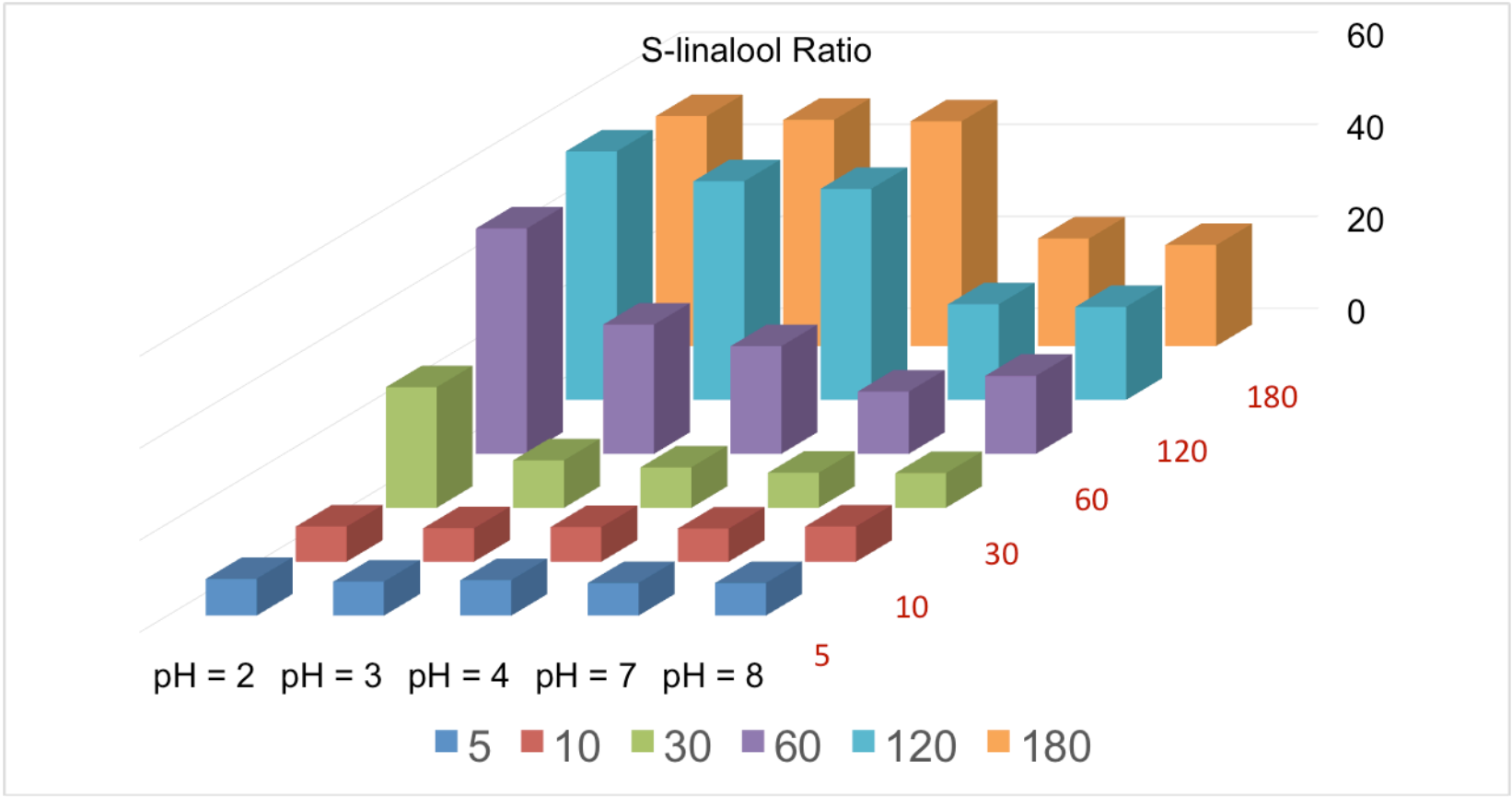
(R)-Linalool Conversion



(R)-(-)-linalool
Threshold: **0.8** ppb
Odor: woody, lavender, floral

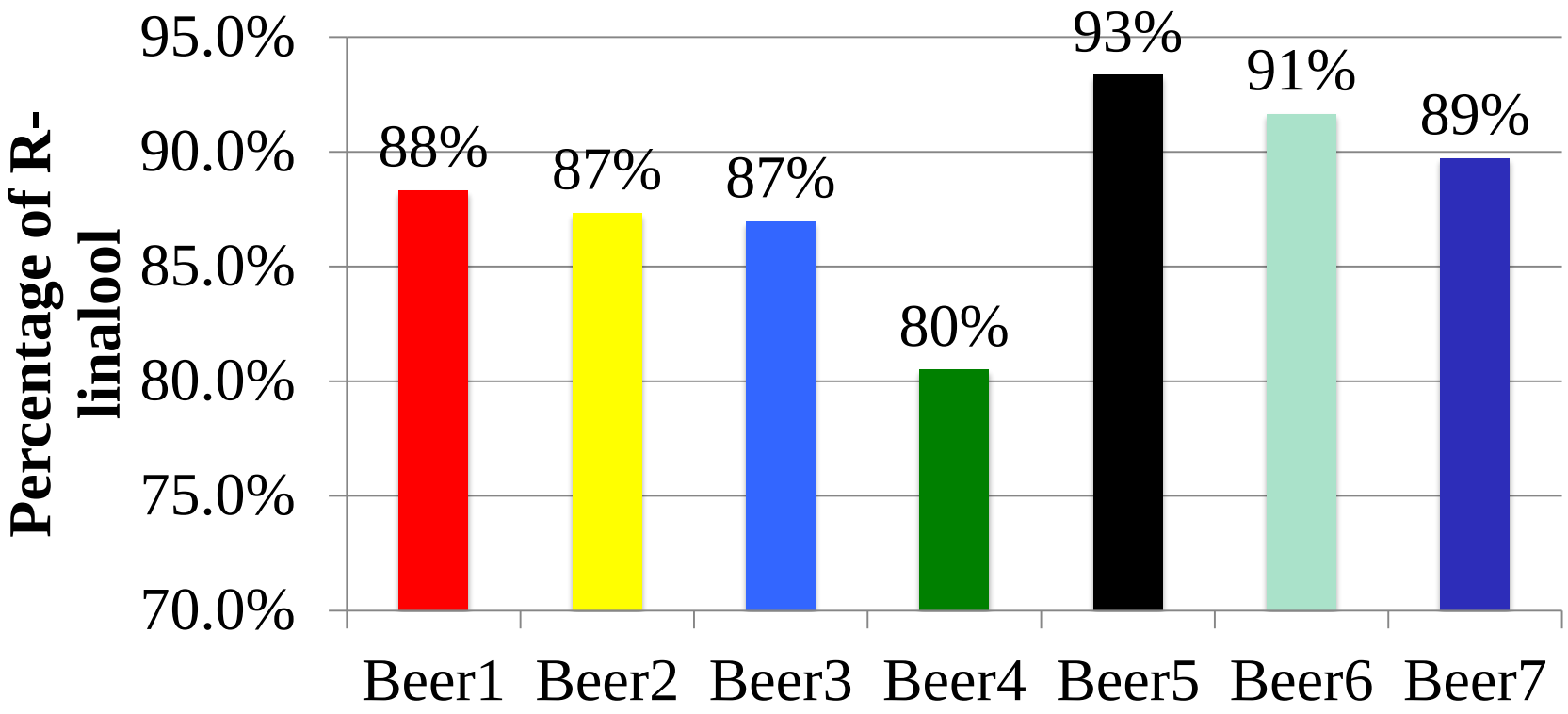
(S)-(+)-linalool
Threshold:**7.4** ppb
Odor: sweet, floral

Conversion to S-linalool at 100ºC



(Ratio x Time [min])

Chiral Distribution of Linalool in Commercial Beer



Conclusion

- Hop aroma chemistry is very complicated and facinating
- Understanding hoppy aroma in beer is even more challenging
- Tremendous of challenges and opportunities