A New Method of Terpene Alcohols and Their Stereoisomers in Beers and Its Applications for Improving Beer Hop Aroma

Wei Jiang, Deliang Wang

China National Research Institute of Food & Fermentation Industries

Jiangweigyig@163.com

Background

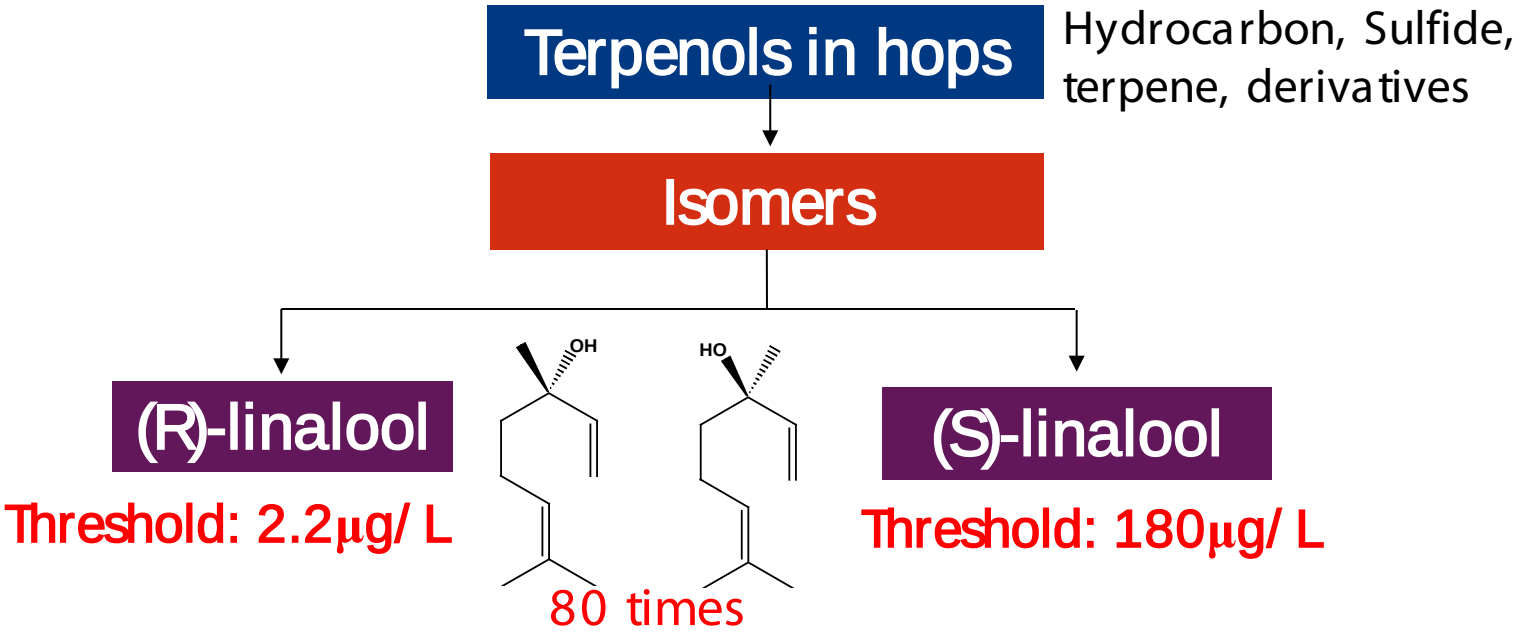
"Hop flavor was considered as the soul of beer."

Three Questions

1. Why the hop flavor is not obvious in beers even after adding lots of hops?
2. Why the hop flavor from different brewing process is significantly different in intensity?
3. How to choose aroma hops?

Question 1

Why the hop flavor is not obvious in beer even after adding lots of hops?



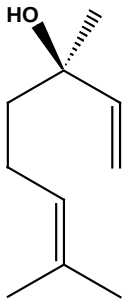
Influence on hop flavor strength in beers:

-Concentration

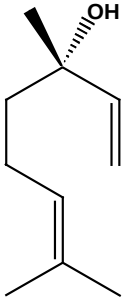
-Isomer ratios?????

Compounds

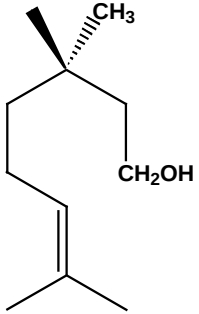
Structure for 12 kinds of terpenols, isomers and esters



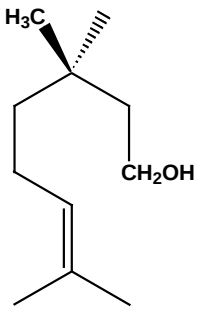
(R)- (-)-linalool



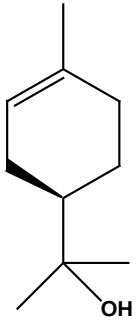
(S)- (+)-linalool



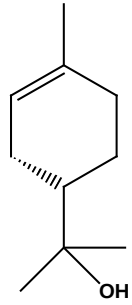
S-(-) -β- citronellol



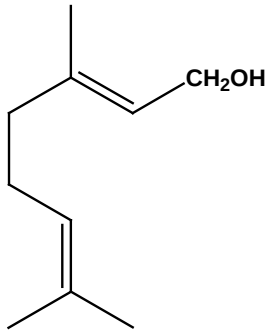
R-(+)-β-citronellol



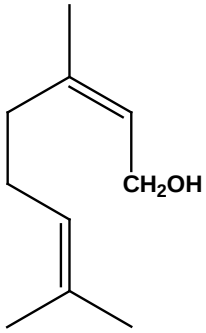
(-)-α-terpineol



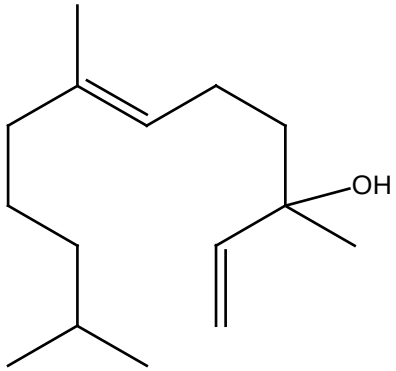
(+)-α-terpineol



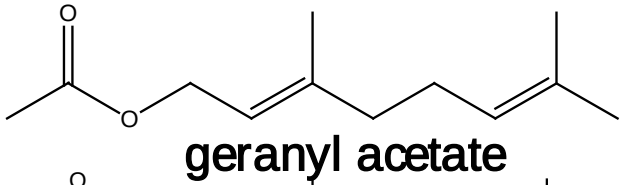
geraniol



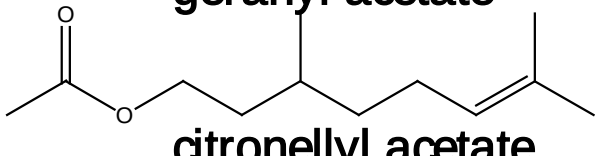
nerol



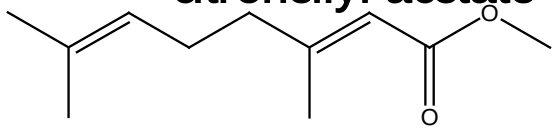
nerolidol



geranyl acetate



citronellyl acetate



methyl geranate

Find out the thresholds for all the compounds

Outline

1. Sensory Threshold Test
2. Establishment of Determination Method
3. Transformation Mechanism
4. Changes During Brewing

Threshold Tests

ASTM 679 Method

- Team: at least 8 members.
- Five grades concentration for each compound.

Threshold of R-(-)-linalool

(unit: μg/ L)

Panelist	1.1	2.2	4.4	8.8	17.6	Individual thresholds
A	0	0	0	+	+	6.2
B	0	0	+	+	+	3.1
C	0	+	+	+	+	1.6
D	+	0	+	+	+	3.1
E	0	+	+	+	+	1.6
F	0	0	+	+	+	3.1
G	0	+	+	+	+	1.6
H	0	0	+	+	+	3.1
Group thresholds						2.6

Note: The concentration is doubled at each step.

Thresholds of Terpenols in Beers

Isomer Forms

Compound	Reference Thresholds (μg/ L)		Lab Test Thresholds (μg/ L)	Odour Description
R-(-)-linalool	2.2	Linalool 5;100;27;10	2.60	Flowery, Fruity, Lavender
S-(+)-linalool	180		—	Flowery, Coriandrol,
(-)-α-terpineol	—	Terpineol 460；40；20	—	Lilac
(+)-α-terpineol	—		449	
Geraniol	132; 20; 6; 36		7.10	Waxy sweet rose ordour
R-(+)-β-citronellol	—	Citronellol 8；10	23.78	Rose-like, Citronella
S-(-)-β-citronellol	—		7.00	Geranium oil
Nerolidol	—		21.44	Rose, Apple
Nerol	80 μg/ L (in 5% of alcohol)		70	Sweet rose, citrus
Methyl geranate	—		21.44	Lemon, Rose, Lavender
Geranyl acetate	—		14.14	fruity-rose
Citronellyl acetate	—		16.25	fresh fruity rose lime bergamot lavender

Outline

1. Sensory Threshold Test
2. Establishment of Determination Method
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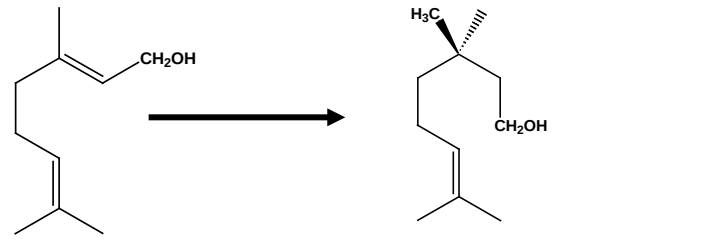
4. Changes During Brewing

Existing Methods for the Determination of Terpenol Isomers

1. Gramatica et al, 1982; King et al, 2000;



geraniol

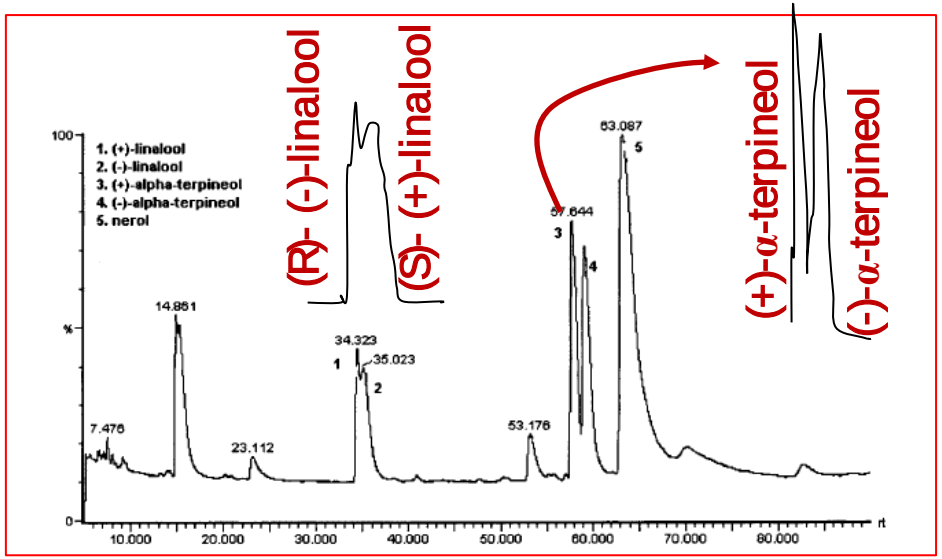
100% R-(+)-β-citronellol

2. Fritsch et al, 2003; Steinhaμs et al, 2003; Preedy, 2009; Takoi, 2010; Kaltner, 2003

R-linalool in hop oil: 92%-95%. After wort boiling, S-linalool: 30%. The total hop flavor decreased.

3. Andrew King et al., 2000

Level of terpenols: mg/ L.
Terpenol isomer transformation: no details.



Existing Methods for the Determination of Terpenol Isomers

4. Martin Steinhaus et al, 2003 (Helge T et al., 2005)

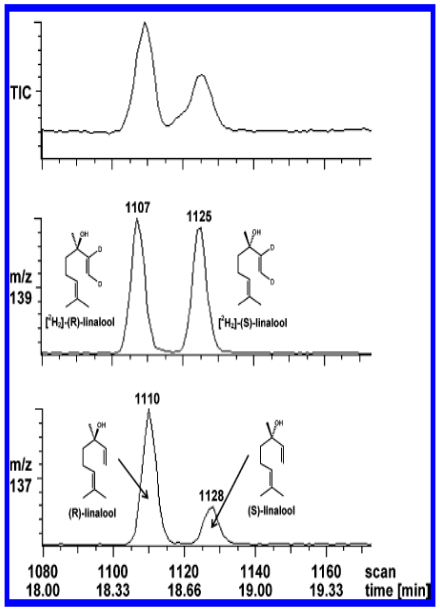


Figure 7. Mass chromatogram obtained by application of the SIDA on beer no. 4 (method I).

(R)- (-)-linalool **(S)- (+)-linalool**

Quantitation by Two-Dimensional HRGC/SIDA Using SPME Isolation (Method I). Beer samples (0.5−10 mL) were placed in headspace vials (20 mL) equipped with a stir bar and topped up to 10 mL with tap water, if necessary. An aliquot (50−100 μL) of an ethanolic solution of [$^2H_2$]linalool (c = 2.38 μg/mL) was added by subsurface pipetting. After short agitation, sodium chloride (4 g) was added and the vials were capped. After 30 min with continuous stirring, the vials were placed into the tray of a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) held at 20 °C. Extraction was performed using 65 μm PDMS/DVB fibers (Supelco, Sigma-Aldrich Chemie). Compounds were desorbed during 1 min into the hot injector (PPKD injector, Thermo Finnigan, Egelsbach, Germany) of a GC (Trace GC, 2000 Series, Thermo Finnigan) and transferred onto the column in the first dimension (DB-FFAP, WCOT Fused Silica, 30 m × 0.32 mm internal diameter, 0.25 μm; J&W Scientific, Agilent Technologies, Waldbronn, Germany) held at 40 °C. After 2 min, the volatiles were desorbed and separated using a temperature gradient of 6 °C/min. At the elution time of linalool/[$^2H_2$]linalool (~13 min), the effluent was quantitatively transferred to a cold trap (SGE, Darmstadt, Germany) using a moving column stream switching system (Thermo Finnigan). After the cooling was turned off, the trapped material was further separated using either a DB-1701 column (WCOT Fused Silica, 30 m × 0.32 mm internal diameter, 0.25 μm; J&W Scientific, Agilent Technologies) or a chiral BGB-176 column (BGB Analytik, Adliswil, Switzerland) installed in the second GC oven (CP 3800, Varian, Darmstadt, Germany). Separa-

Two-dimensional high resolution GC-MS

Two columns used
1st column：DB-FFAP
2nd column：DB-1701 or BGB176

(R/S)-linalool studied only

Improvement Opportunity

How to detect terpenol and isomers QUICKLY, SIMPLY and ECONOMICALLY?

Challenges:

- **Interference of impurity in hops and beers**
- **Trace content at $\mu g \cdot L^{-1}$ Level**
- **Expensive and complicated pretreatment**
- **Lack of studies on the isomers of α-terpineol and β-citronellol**

Result

High Cost

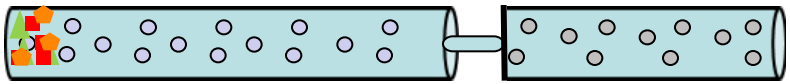
Low Cost

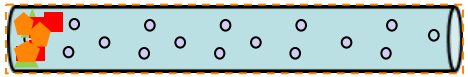
Existing method

- SIDA
- Two-Dimensional HRGC-MS
- Two columns

Our method

- GC-MS with single quadrupole
- Only one column
- Easy to introduce

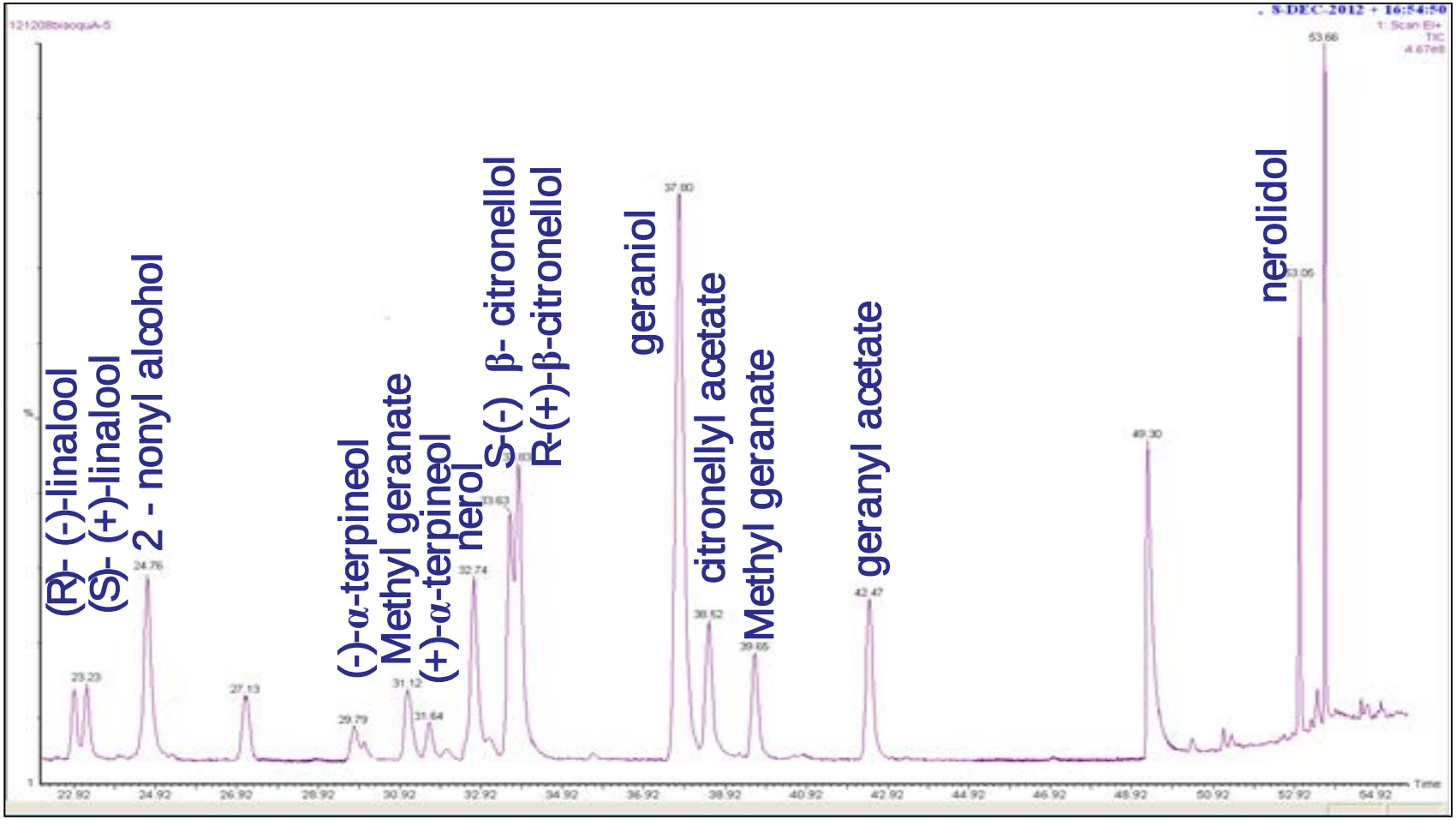




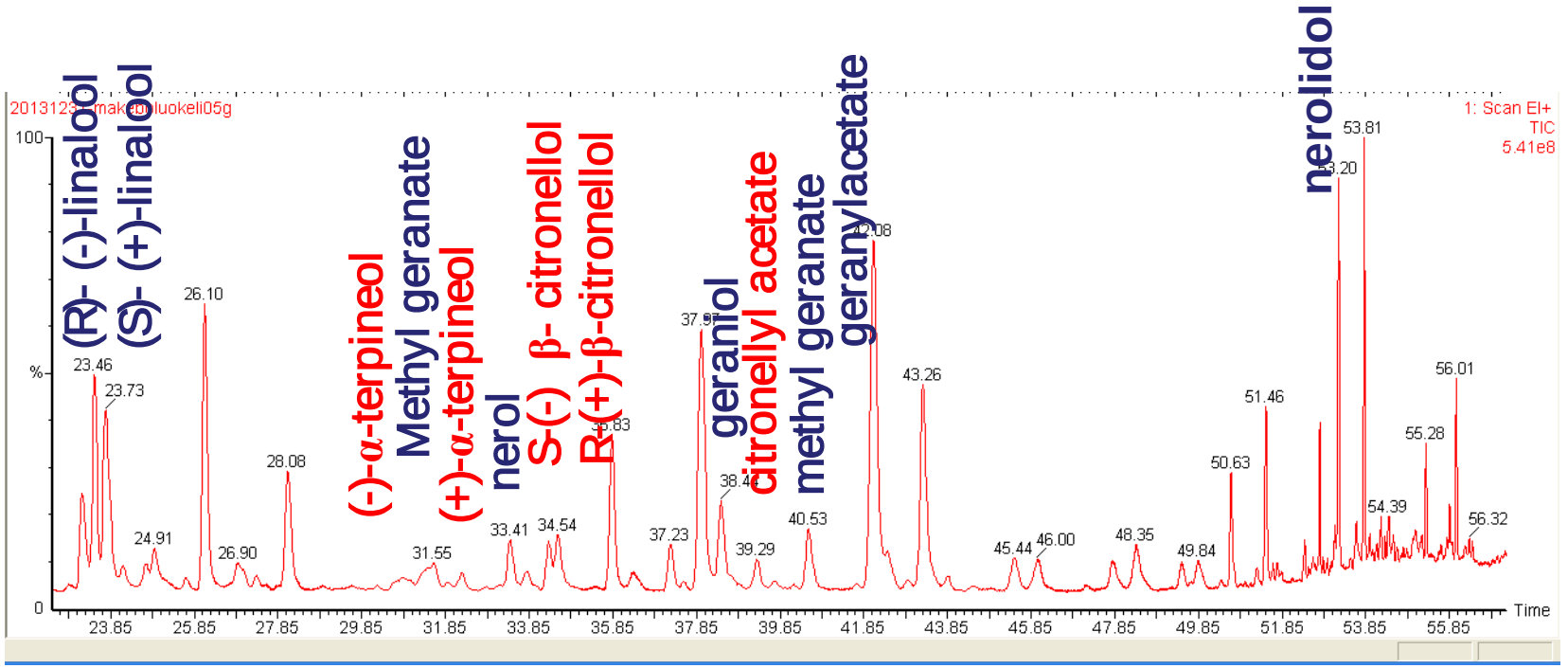
Comparison

Items	Existing Method	Our Method
1. Cost	High	Low
2. GC-MS	High resolution	Quadrupole
3. Column	Two columns	One column
4. Pretreatment	Complicated	Simple
5. Isomer Group	Single	Multiple
6. Introduction	Difficult	Easy

Spectrum of the Standard for Beers by GC-MS



Spectrum of Terpenol and Isomers in Hops by GC-MS



Trace concentration in hops

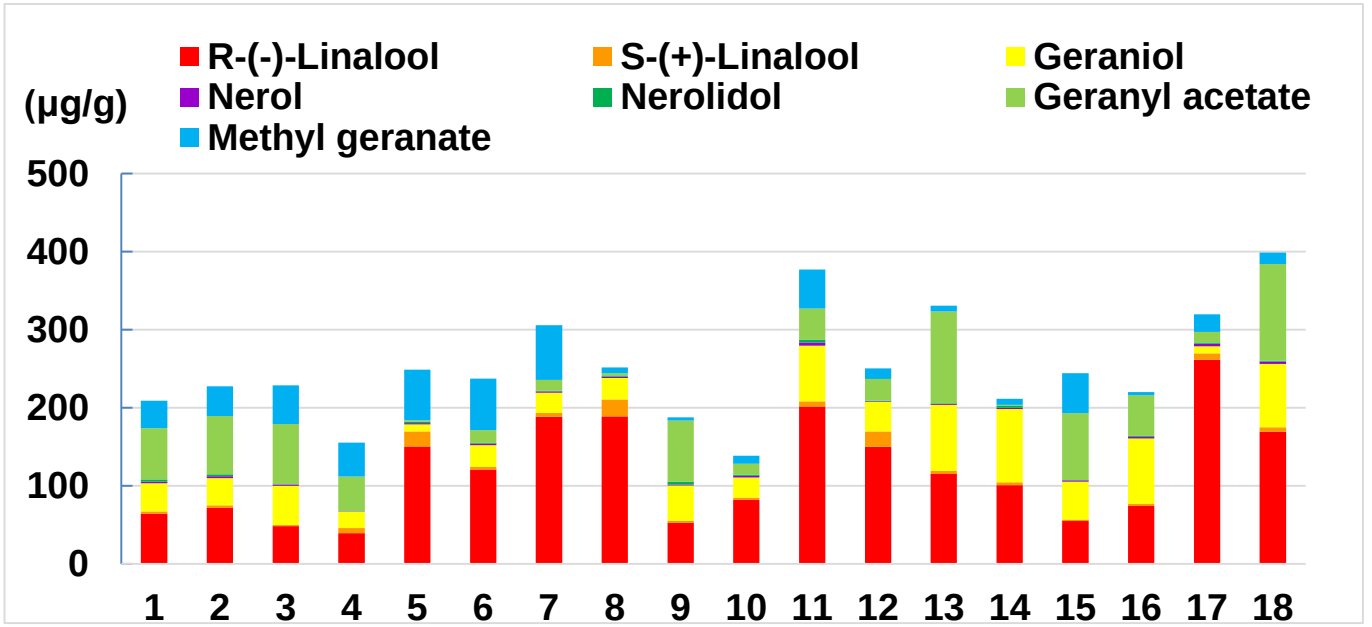
(R/ S)-β-citronellol, citronellyl acetate, (+/ -)-α-terpineol

Source: production during fermentation.

Parameters of Terpenols and Isomers for Beers by GC-MS

Substance	Molecular formula	Quantitative ion	Qualitative ion	Range (μg/ L)	Regression equation	R^2	Detection limits (μg/ L)	RSD (%)	Recovery (%)
(R)- (-)-Linalool	$C_{10}H_{18}O$	71	71,93,121	0~200	y=0.26x	0.9932	0.41	4.66	114.90
(S)- (+)-Linalool	$C_{10}H_{18}O$	71	71,93,121	0~200	y=0.32x	0.9946	0.33	2.26	118.70
2-Nonanol (IR)	$C_9H_{20}O$	69	45,55,69	——	——	——	——	——	——
(-)-α-Terpineol	$C_{10}H_{18}O$	93	69,93,121	0~50	y=0.07x	0.9911	0.51	5.42	81.10
(+)-α-Terpineol	$C_{10}H_{18}O$	93	69,93,121	0~50	y=0.15x	0.9988	0.23	4.79	99.24
Nerol	$C_{10}H_{18}O$	69	41,69,93	0~50	y=0.28x	0.9960	1.18	4.55	89.97
S-(-)-β-Citronellol	$C_{10}H_{20}O$	123	93,95,123	0~50	y=0.25x	0.9900	0.38	7.31	90.15
R-(+)-β-Citronellol	$C_{10}H_{20}O$	123	93,95,123	0~50	y=0.30x	0.9904	0.21	7.64	118.52
Geraniol	$C_{10}H_{18}O$	69	69,93,123	0~50	y=0.34x	0.9993	0.22	5.08	90.58
Citronellyl acetate	$C_{12}H_{22}O_2$	69	69,95,123	0~50	y=0.16x	0.9930	0.85	5.98	92.83
Methyl geranate	$C_{11}H_{18}O_2$	69	69,114,123	0~50	y=0.40x	0.9975	0.31	10.93	93.94
Geranyl acetate	$C_{12}H_{20}O_2$	69	69,93,136	0~50	y=0.40x	0.9940	0.28	11.35	87.03
Nerolidol	$C_{15}H_{26}O$	93	69,93,107	0~50	y=0.41x	0.9930	0.25	4.03	94.94

Local and Imported Hops in the Market

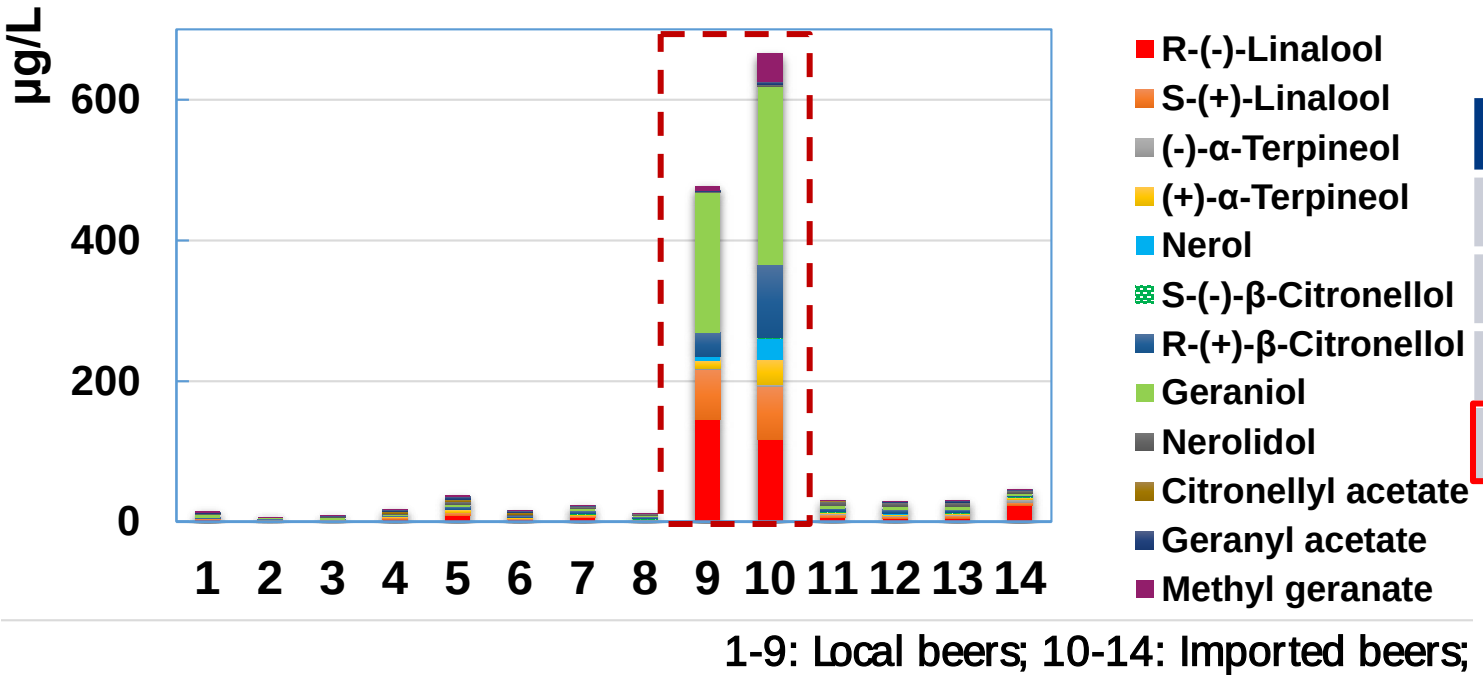


1-5: local aroma hops; 6-11: Imported aroma hops;
12-16: local bitter hops; 17-18: Imported bitter hops.

- R-(-)-Linalool: 39.4μg/ g~261.55μg/ g, ratio of R-linalool：85~97%;
- Geraniol: 8.92μg/ g~ 93.65μg/ g;
- Geranyl acetate: 0.75μg/ g~ 123.79μg/ g;
- Methyl geranate: 4μg/ g~ 69.98μg/ g;

Significant difference observed among hops.

Local and Imported Beers in the Market



Main Compounds
R-(-)-linalool
Geraniol
R-(+)-β-citronellol
S-(+)-linalool

1-9: Local beers; 10-14: Imported beers;

How to transform for isomers during brewing?

Outline

1. Sensory Threshold Test
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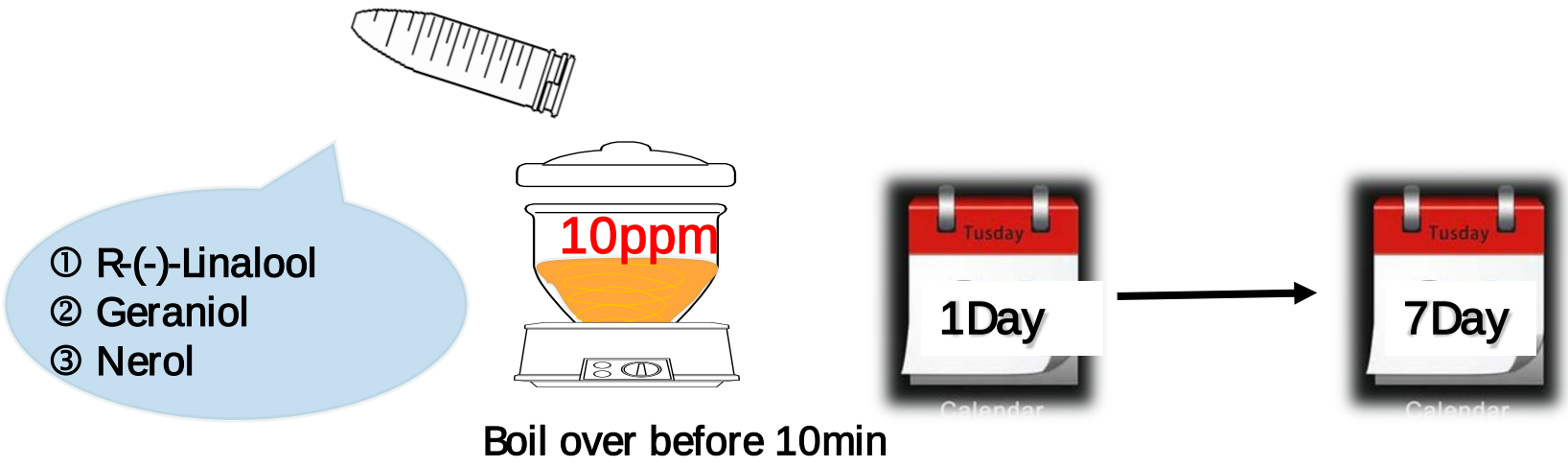
Biotransformation of Terpenol Isomers ?

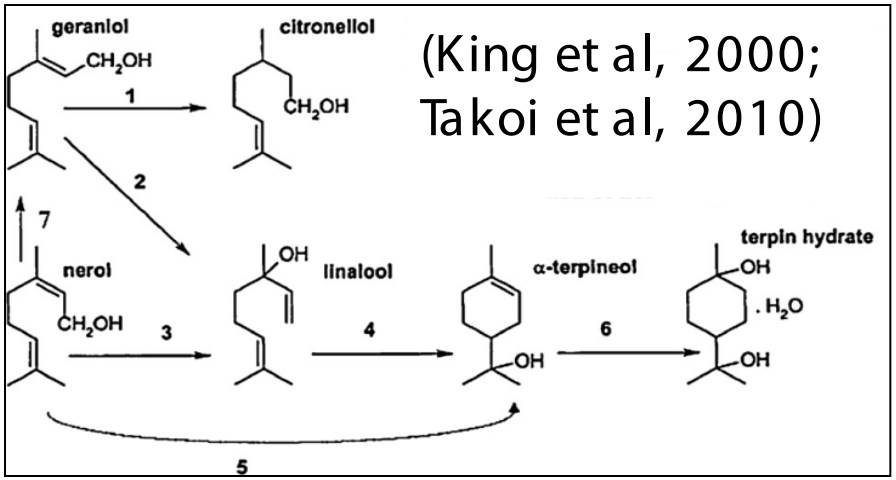


Boil over before 10min

Standard of each compound

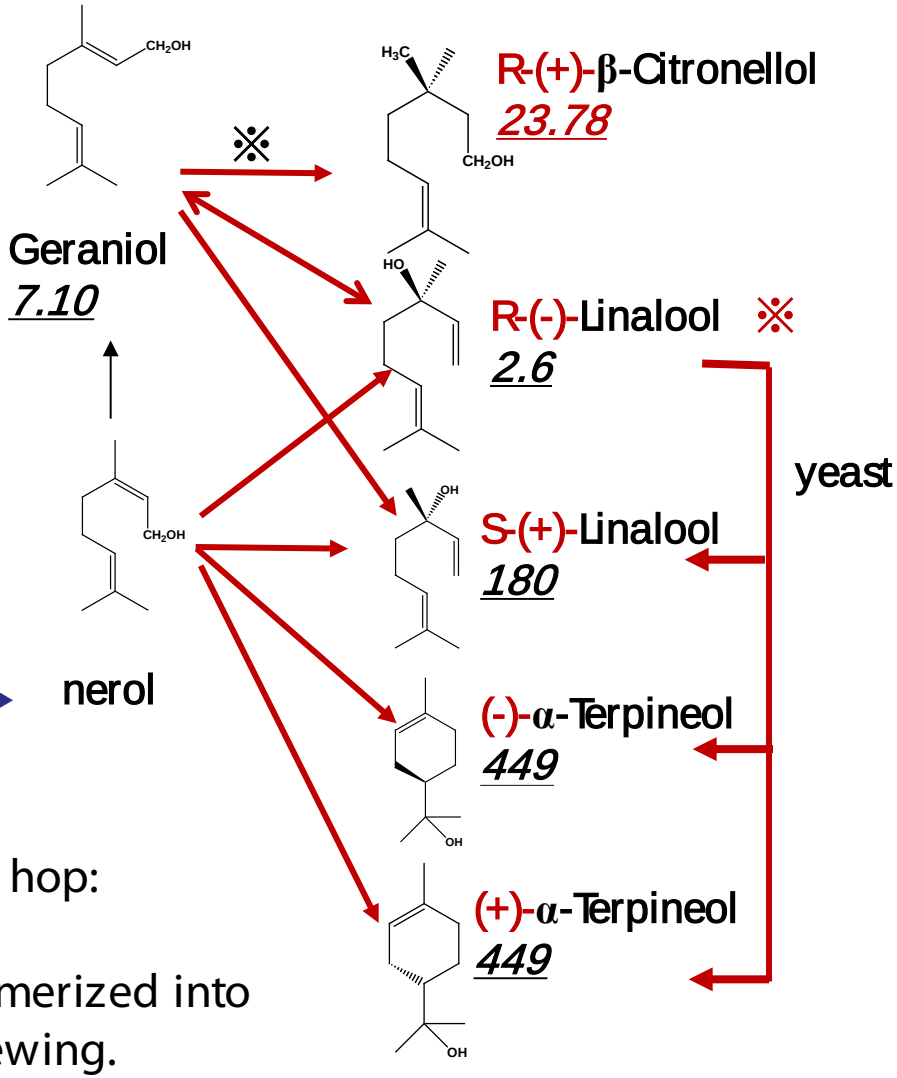
Simulate the hop addition process:
add 10 ppm of those compounds in wort, respectively
and study on the bio-transformation reactions

Biotransformation of Terpenol Isomers



(King et al, 2000; Takoi et al, 2010)

Transformation of isomers



Hop flavor intensity decreases after adding hop:
1. Volatilization under high temperature;
2. Terpinols with low thresholds in hops isomerized into compounds with high thresholds during brewing.

Biotransformation Comparison

Items	Existing Research	Our Research
Isomer	No	Yes
Geraniol	β-citronellol	R-(+)-β-citronellol
Geraniol	Linalool	R and S- linalool
Nerol	Geraniol, Linalool, Terpineol	Geraniol, R/ S-linalool, (+/ -)-Terpineol
R-(-)-Linalool	No	S-linalool, (+/ -)-Terpineol

Questions

1. Why the hop flavor is not obvious in beers even after adding lots of hops?

√

Volatilization and isomerization

2. Why the hop flavor from different brewing process is significantly different in intensity?

Outline

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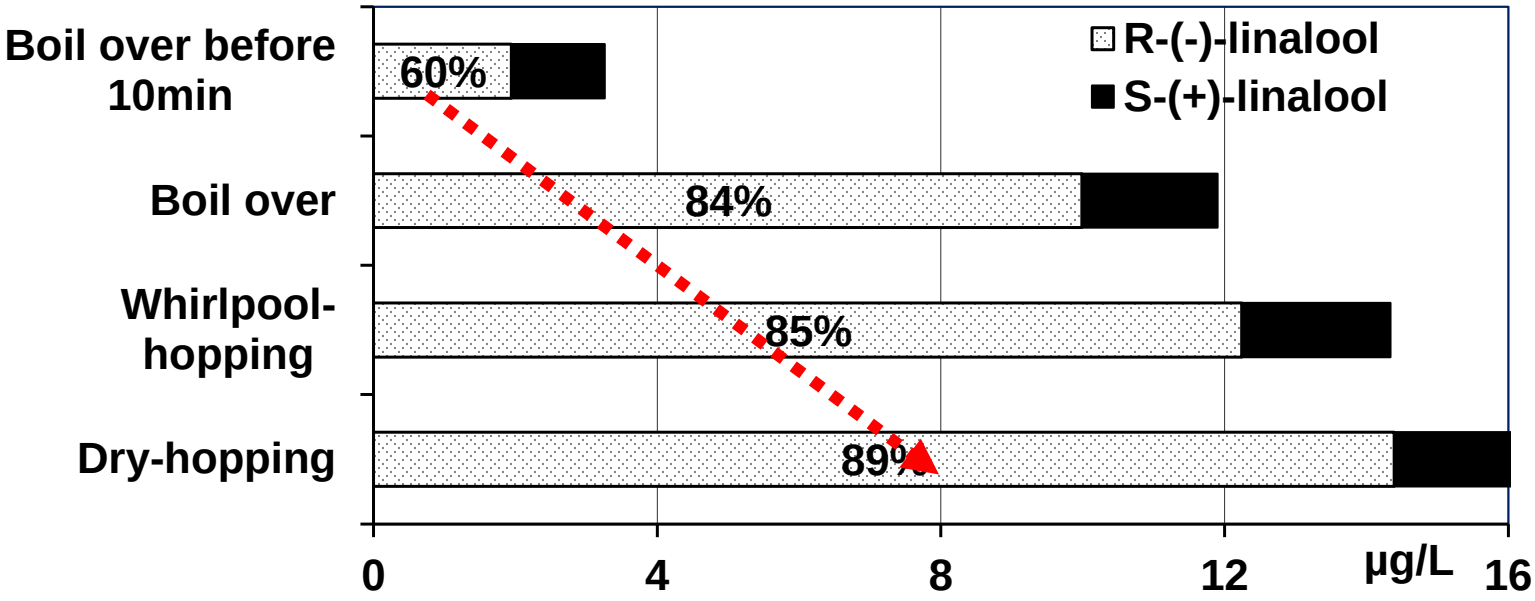
Four Hop Additions in Beers

Concentration (odour activity value(OAV))

(unit: μg/ L)

Compound	Boil over before 10min	Boil over	Whirlpool-hopping	Dry-hopping	Sensory thresholds
R-(-)-Linalool	**2.29(0.88)**	**11.80(4.54)**	**14.46(5.56)**	**17.00(6.54)**	**2.6**
S-(+)-Linalool	1.32(<0.01)	1.91(0.01)	2.1(0.01)	1.74(<0.01)	180
(-)-α-Terpineol	0.43(0.01)	0.73(0.02)	0.84(0.02)	0.73(0.02)	40
(+)-α-Terpineol	0.31(<0.01)	0.31(<0.01)	0.42(<0.01)	0.21(<0.01)	449.00*
Geraniol	12.07(1.70)	11.64(1.64)	12.99(1.83)	14.34(2.02)	**7.1**
R-(+)-β-Citronellol	2.91(0.12)	3.52(0.14)	3.86(0.16)	6.79(0.29)	23.78*
S-(-)-β-Citronellol	1.49(0.21)	0.85(0.12)	0.98(0.14)	0.66(0.09)	7.00*
Nerolidol	6.81(0.32)	5.34(0.25)	5.34(0.25)	5.86(0.27)	21.44*
Nerol	0.64(<0.01)	0.82(0.01)	1.09(0.01)	0.86(0.01)	70
Methyl geranate	1.39(0.06)	1.96(0.09)	2.45(0.11)	2.80(0.13)	21.44*
Geranyl acetate	1.08(0.08)	1.31(0.09)	1.36(0.10)	1.05(0.07)	14.14*
Citronellyl acetate	N.D.	N.D.	0.04(<0.01)	0.01(<0.01)	16.25*

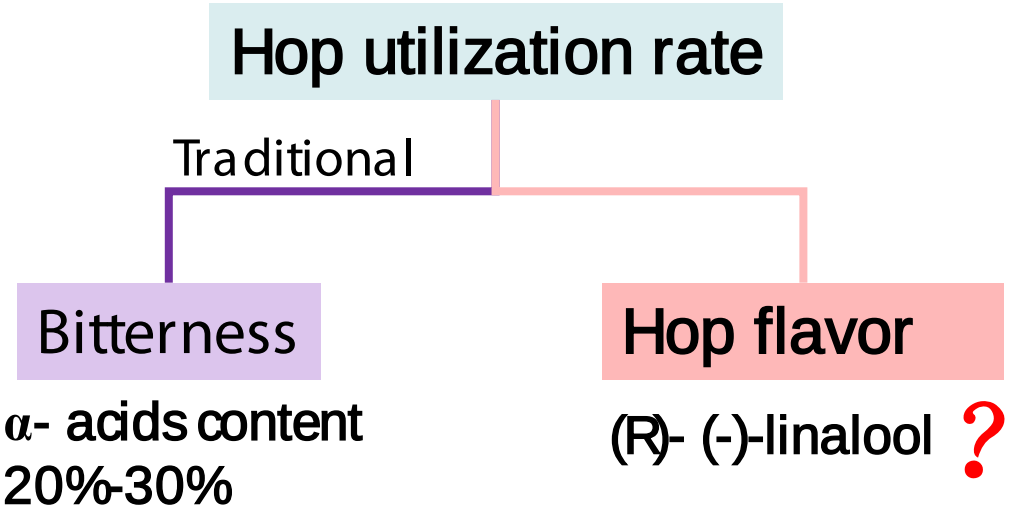
Four Hop Additions in Beers



R-(-)-linalool

- *60~89%*
- *40%~ 11% isomerization*
- *OAV: 0.88~ 6.54*
- This is the key hop flavor

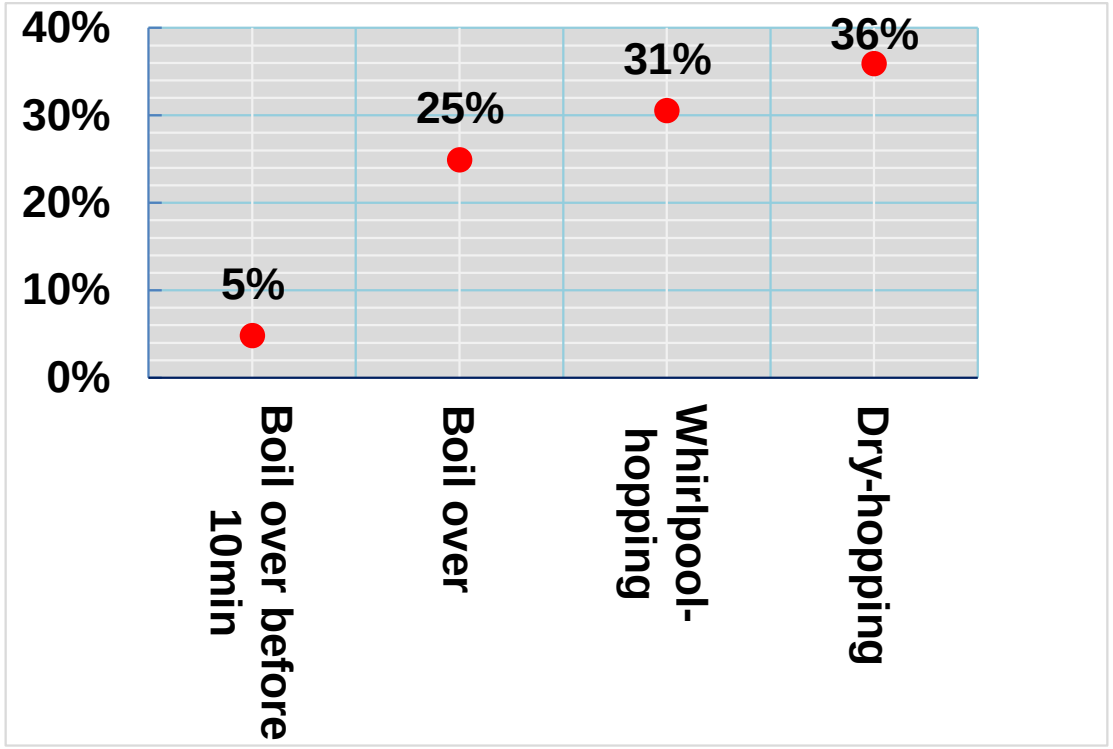
Utilization Rate of R-linalool



Utilization rate for **R-(-)-linalool**

$$\text{Utilization rate} = \frac{\text{R-(-)-linalool in beer}}{\text{R(-)-linalool in hop}}$$

Utilization rate of R-(-)-linalool



Dry-hopping > Whirlpool-hopping > Boil over-hopping> Boil over before 10 min

Questions

2

- Why the hop flavor from different brewing process is significantly different in intensity?

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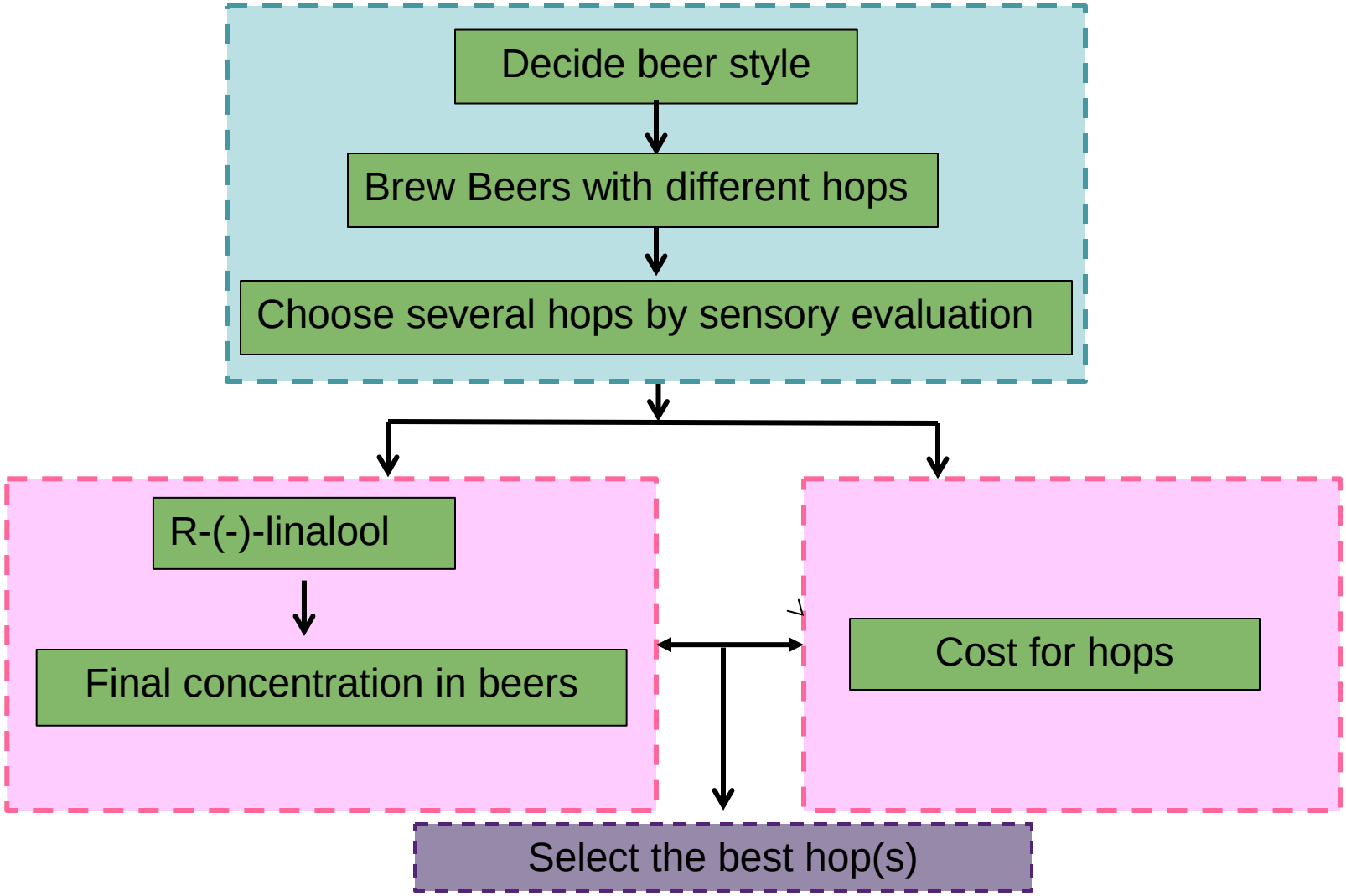
Loss under high temperature;
Different degree of isomerization.

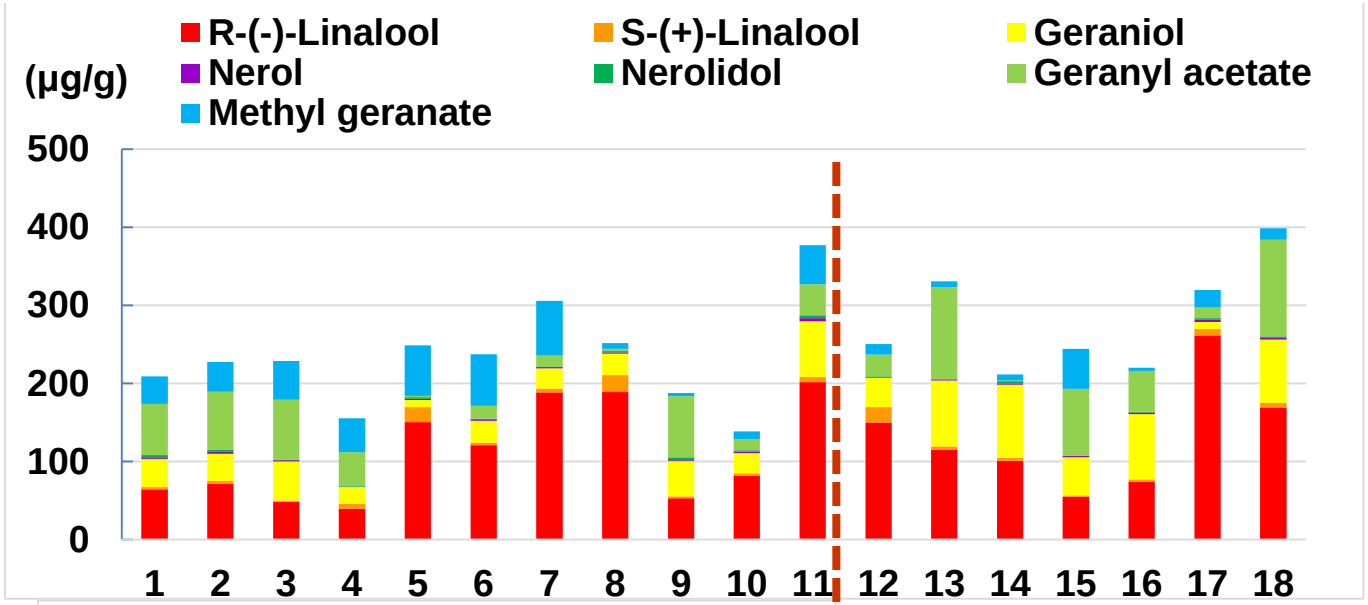
3

- How to choose aroma hops?

Process to Choose Hops



Beers Made From Different Hops

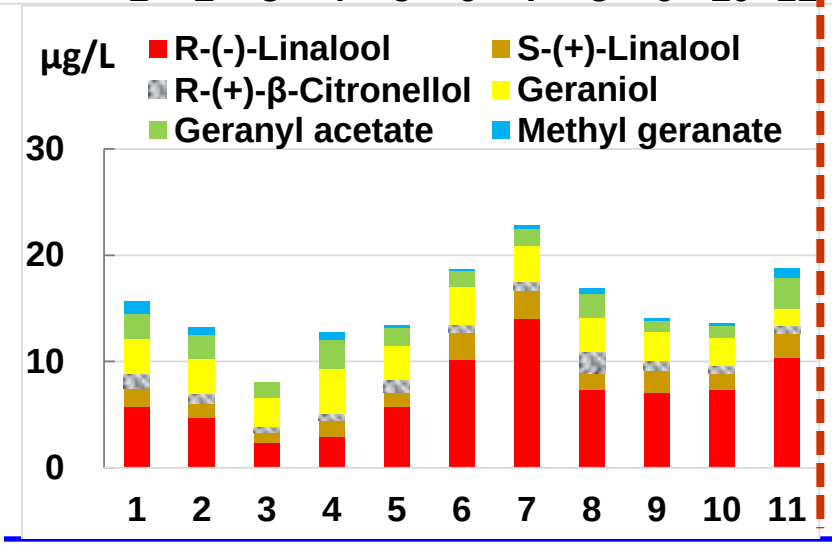


1-5: local aroma hops;

6-11: imported aroma hops;

12-16: local bitter hops;

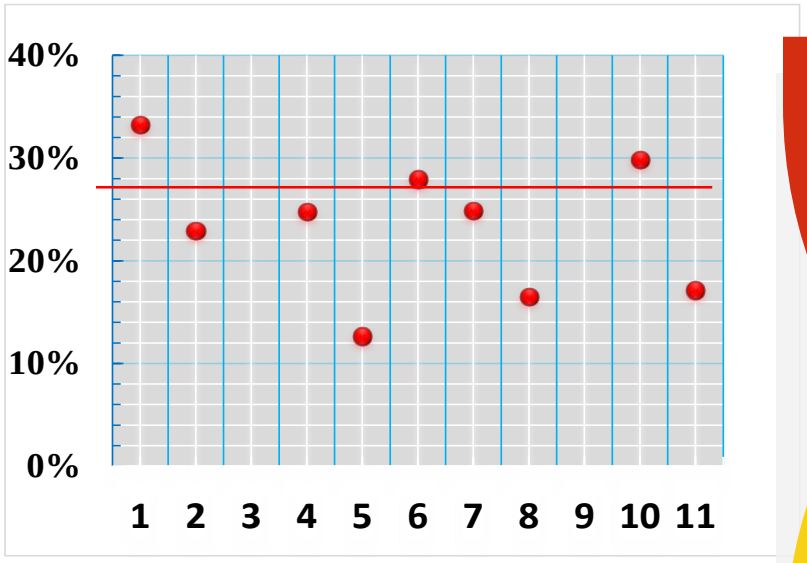
17-18: imported bitter hops.



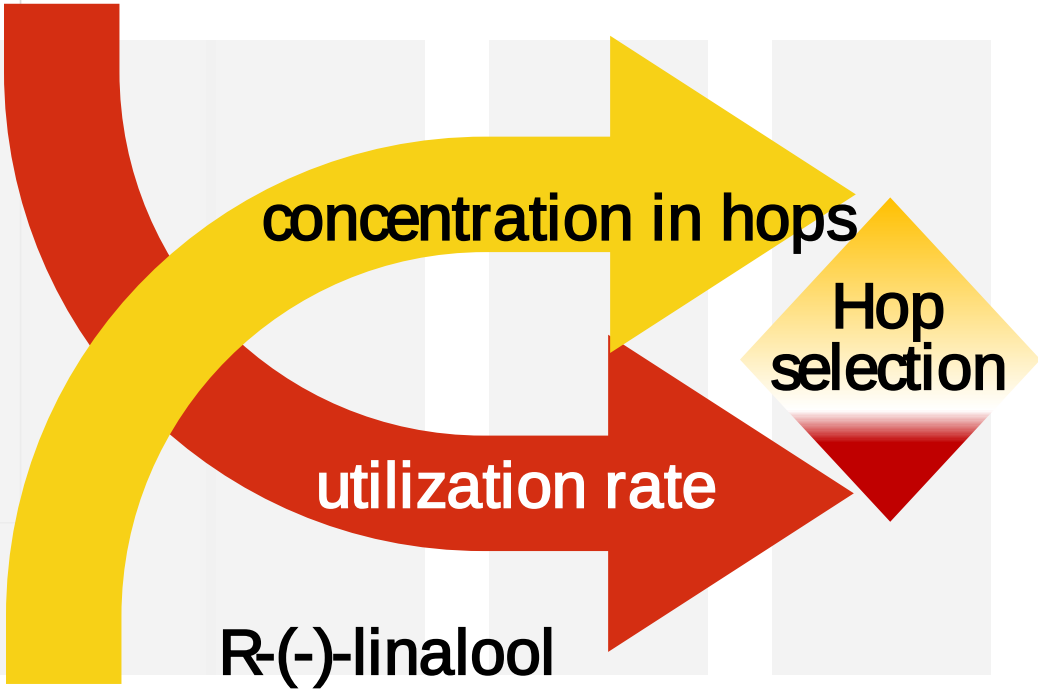
Concentration of R-(-)-linalool in hops:
No. 11>No. 7>No. 8>No. 5

Concentration of R-(-)-linalool in beers:
No. 7>No. 11>No. 6>No. 8

Utilization Rate of R-(-)-linalool in Beers with Different Hops



Average utilization rate: 26.8 %
No. 9>No. 3>No. 1>No. 10



Concentration of R-(-)-linalool in beers:
No. 7>No. 11>No. 6>No. 8

Conclusion

1

- Why the hop flavor is not obvious in beer even after adding lots of hops?

The volatilization on high temperature and isomer forms in beer.

2

- Why the hop flavor from different brewing process is significantly different in intensity?

Volatilization and isomerization.

The less loss and isomerization, the better.

3

- How to choose aroma hops?

Not only the concentration but also utilization rate of R-(-)-linalool should be considered when several hops are all suitable for beers.

Thank you!