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#ElevateBeer

A role of harvest time on aroma characteristics and related compounds in Saaz hop.

Takako Inui, Hiroo Matsui*, Nobuyuki Fukui*, and Kaneo Oka

Suntory Beer Ltd. Osaka Japan

***Suntory Global Innovation Center Ltd. Kyoto Japan**

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- ✔ *Introduction and Objectives*
- ✔ *Experimental design and analytical methods*
- ✔ *Results*
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- ✔ ***Introduction and Objectives***
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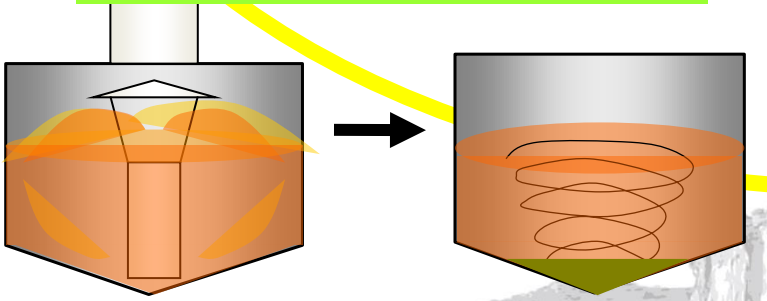
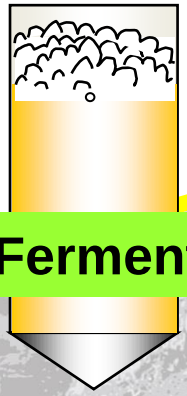
The factors that control hop aroma qualities in beer during the brewing process

Hop varieties and their qualities

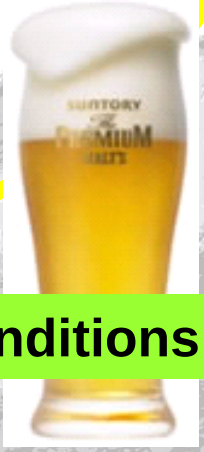
Hop processing and storage conditions

Hop boiling conditions





Fermentation conditions

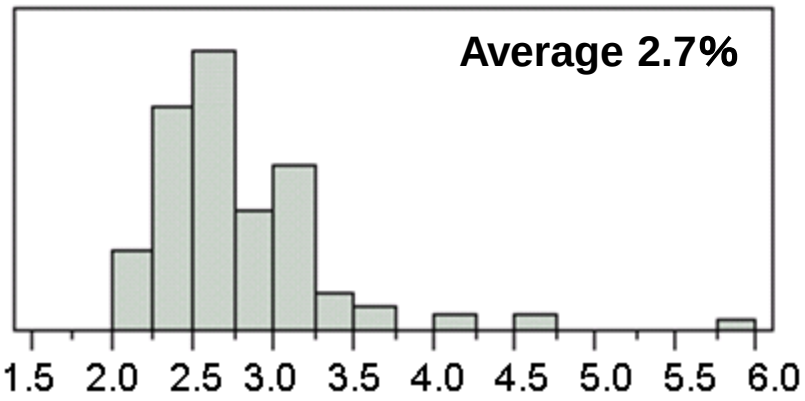


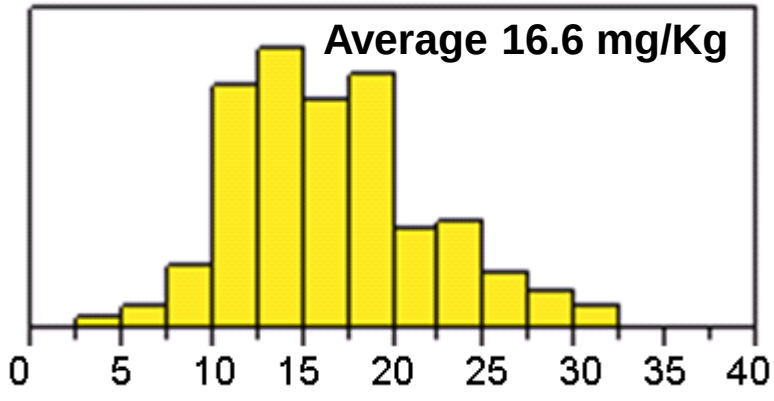
Humulone and linalool distribution in Czech Saaz hops

Humulone

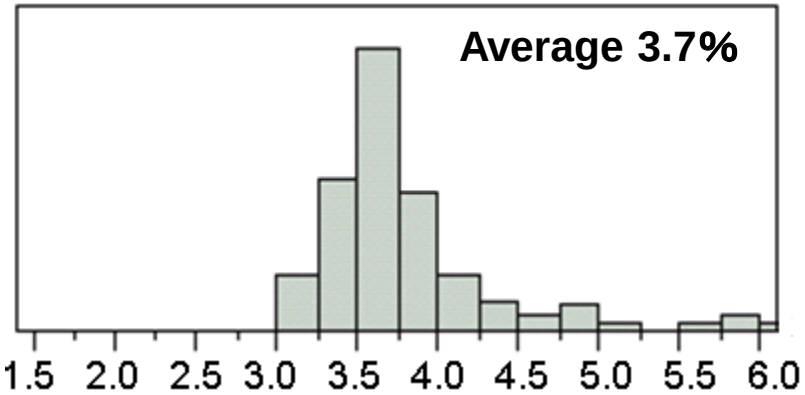
Linalool

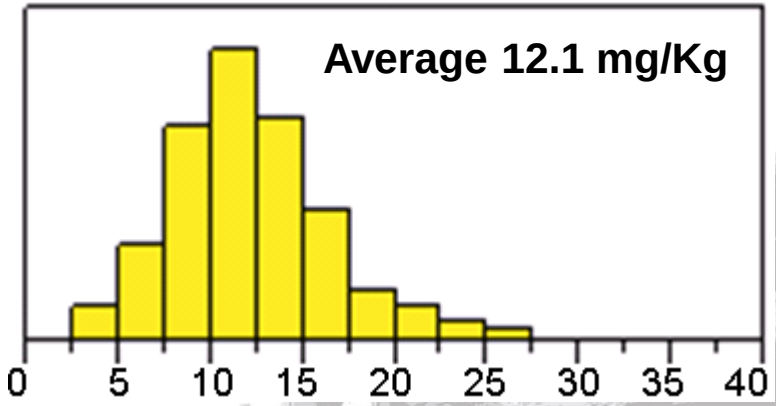
2010 (*N*=120)





2011 (*N*=141)





Cultivation factors affecting hop qualities

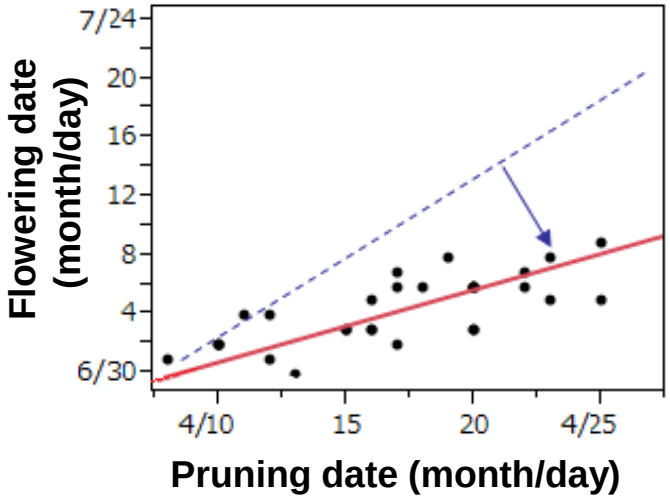
Characteristics of stock	Root age
	Clone
	Viral infections
Natural environment	Climate
	Soil
Cultivation conditions	Pruning date
	Harvest time
	Fertilization
	Vein hoisting

Saaz hop cultivation stages throughout the year

Pruning for new growth | **Flowering** | **Formation of hop cone** | **Harvest**

Early-Apr~ 20 days | **Early-July ~ 18 days** | **Mid-Aug ~ 25 days**

Relationship between pruning and flowering dates for Czech Saaz hops grown in 2012

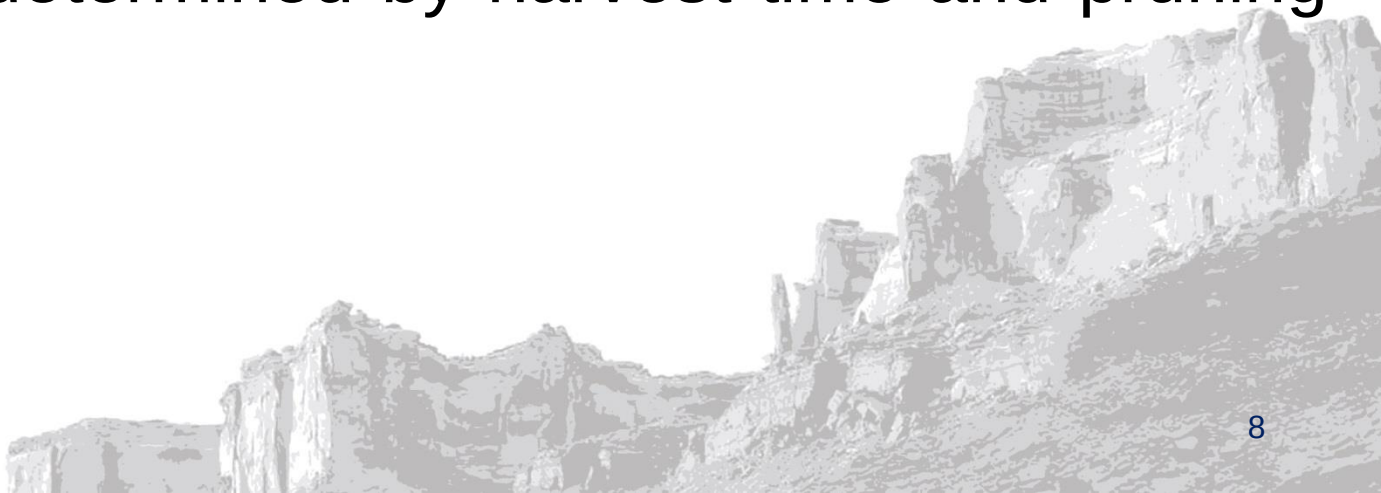


“ *Harvest time* “ is defined as: number of days from flowering to harvest.

The objectives of this work

- ✔ To examine the changes in hop aroma characteristics as determined by harvest time and pruning date.

- ✔ To identify the compounds that are responsible for hop aroma characteristics and their chemical profiles as determined by harvest time and pruning date.



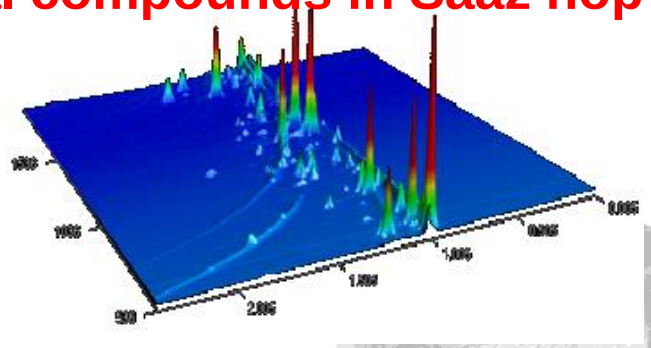
Experimental procedure in this study

Pruning date **Flowering** **Harvest time**

Formation of hop cone

How does “ harvest time and pruning date “ affect the aroma characteristics of hop teas and the chemical compounds in Saaz hop?

X



Sensory profile of hop tea by QDA (quantitative descriptive analysis)

Aroma component profile by GC×GC/TOF-MS

Multivariate analysis (ANOVA,PLS analyses)

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Design of experiment: Trial I

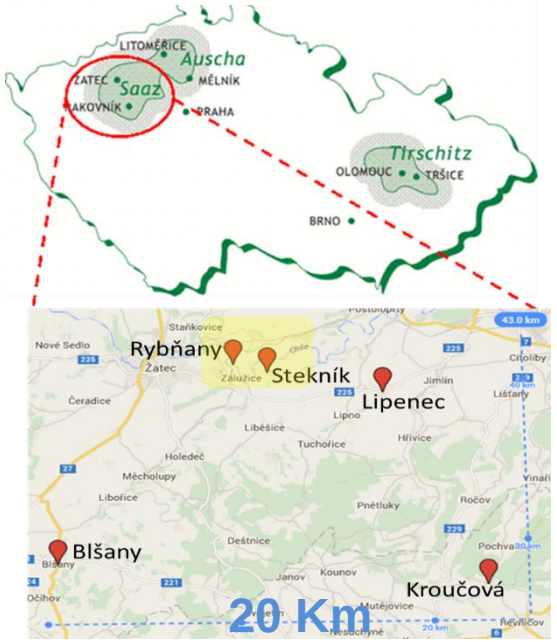
Harvest year (2011–2014) **X** **Harvest time** (8/15–9/21)

Harvest year	2011				2012				2013				2014			
Farm	Rybnany				Rybnany				Steknik				Steknik			
Pruning date	April 15				April 16				April 20				April 15			
Harvest time(days)	22	29	50	57	23	32	51	60	26	37	53	61	29	40	57	64

Other conditions

✔Virus free
✔Same clone
✔Same type of soil (Brown)
✔Root age (10–20 years old)

Growing Area



Design of experiment: Trial Ⅱ

Harvest year: 2012

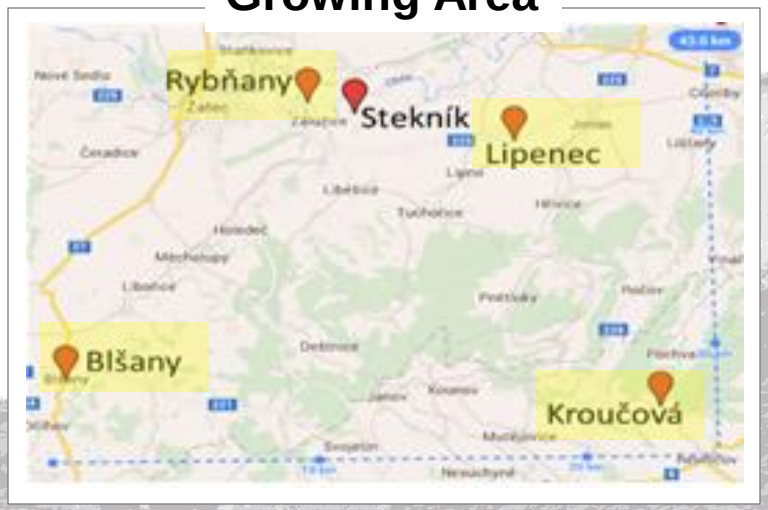
Growing area (4 farms) X **Pruning date** (4/4–4/25) X **Harvest time** (8/15–9/21)

Harvest year	2012																											
Farm	Lipenec								Krouvcova								Rybnany								BIsany			
Pruning date	April 4				April 14				April 4				April 24				April 6				April 16				April 25				April 14			
Harvest time	41	49	69	78	30	38	58	67	30	38	58	67	16	25	44	53	30	39	58	67	23	32	51	60	16	25	44	53	38	45	63	72

Other conditions

- ✔Virus free
- ✔Same clone
- ✔Root age (10–20 years old)

Growing Area



Organoleptic evaluation of Hop tea

Preparation of hop tea

Pulverized hop was added to hot citric acid buffer solution (pH 5.3) and left to stand for 5 min.

After filtration, the hop solution was diluted with water.

Selection of panelists

A panel of 7 well-trained individuals were selected for the evaluation task.

Sensory descriptors

4 generic hop aroma characteristics:

Floral, Fruity, Citrusy, and Hay-like

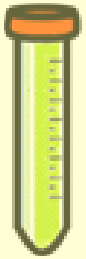
Scoring

Each panelist discriminated the 4 listed characteristics, and scored (0-3) each beer accordingly.

The scores were normalized to remove potential bias from each panelist's score.

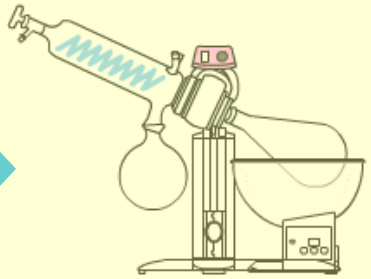
Extraction of hop aroma compounds and quantitative analysis

Pulverizing hop

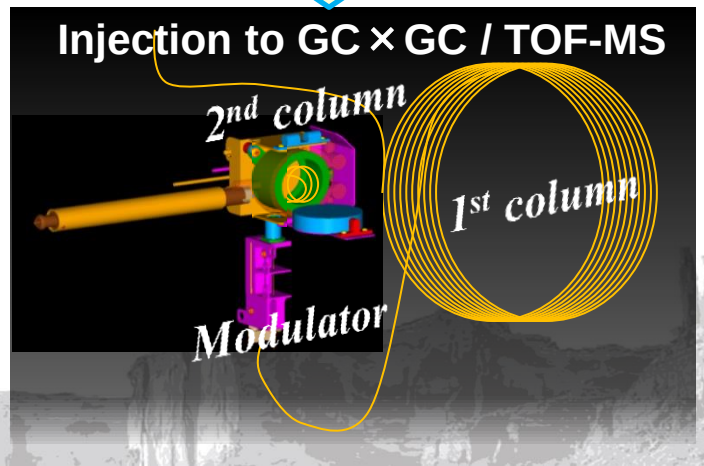


Extraction at 100 ℃ using pressure tight tube

Solid phase extraction (Sep Pak C18)



Concentrating via vacuum evaporation



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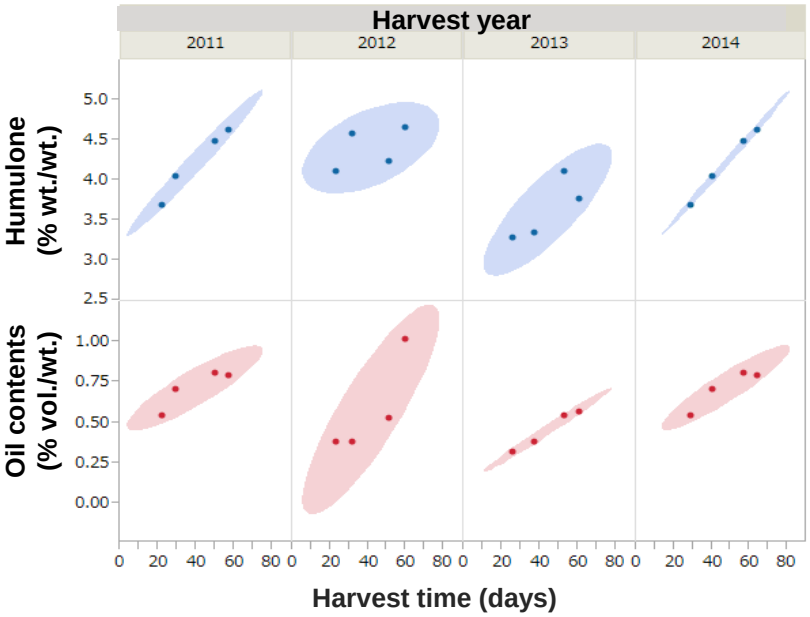
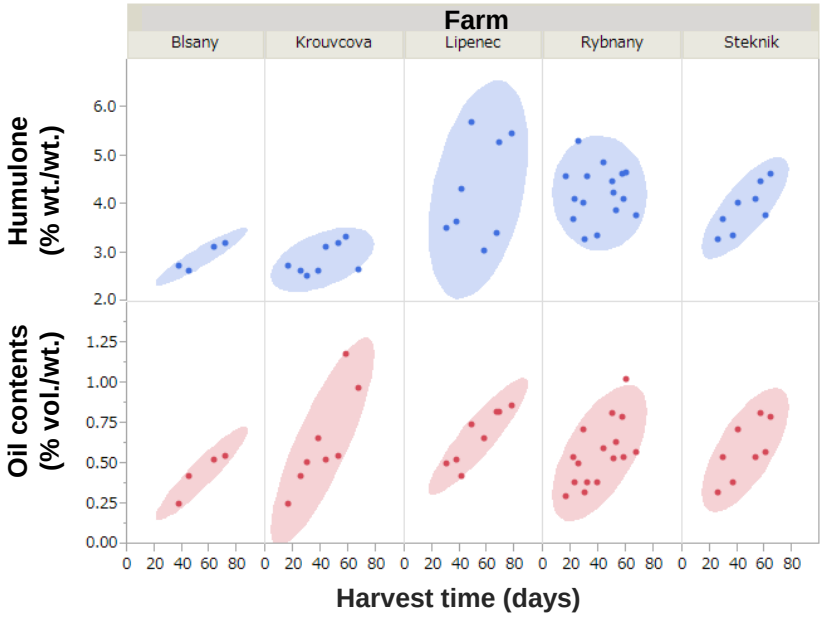
Humulone and oil contents according to harvest time

Trial Ⅰ



Trial Ⅱ



Trial Ⅰ

Compounds	Harvest time	Harvest year	Harvest time X Harvest year
Humulone (% wt./wt.)	30.45[c]	12.12[b]	1.45
Oil contents (% vol./wt.)	23.69[b]	5.56[a]	1.29

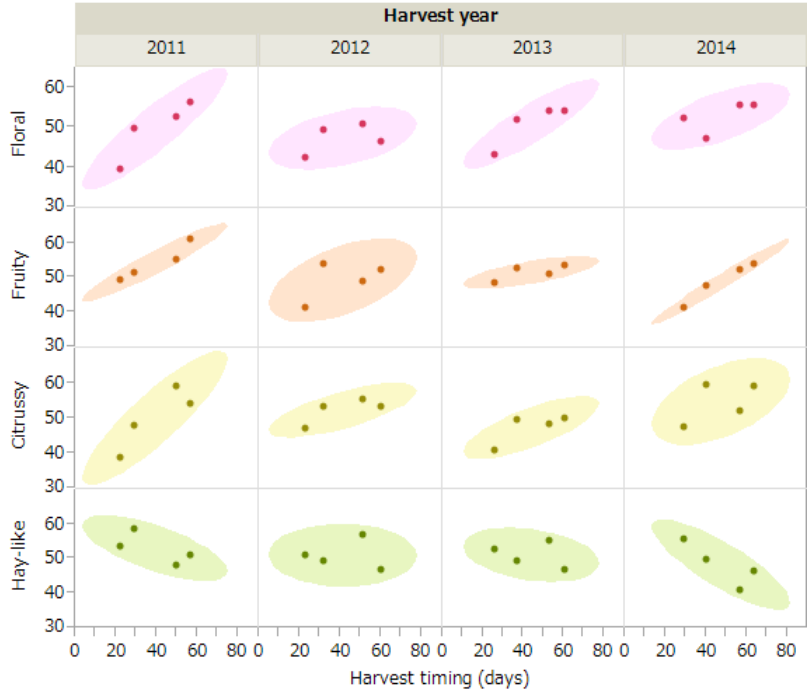
[a] F value at the 95.0% significance level.
[b] F value at the 99.0% significance level.
[c] F value at the 99.9% significance level.

Trial Ⅱ

Compounds	Harvest time	Farm	Pruning date	Harvest time X Farm	Harvest time X Pruning date
Humulone (% wt./wt.)	1.59	8.78[c]	0.07	0.35	3.21
Oil contents (% vol./wt.)	27.24[c]	3.89	0.07	0.95	0.21

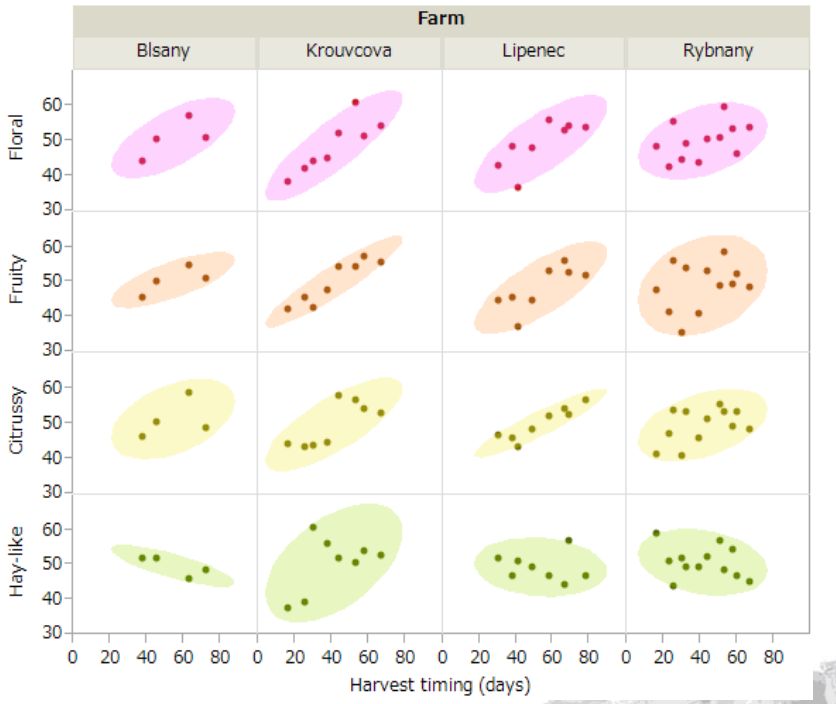
Organoleptic evaluation of hop tea according to harvest time

Trial Ⅰ ***N*=7**



Attribute	Harvest time	Harvest year	Harvest time X Harvest year
Floral	13.32[b]	1.14	1.04
Fruity	15.85[b]	4.14[a]	0.93
Citrusy	9.87[a]	1.63	0.86
Hay-like	3.48	0.22	0.78

Trial Ⅱ ***N*=7**

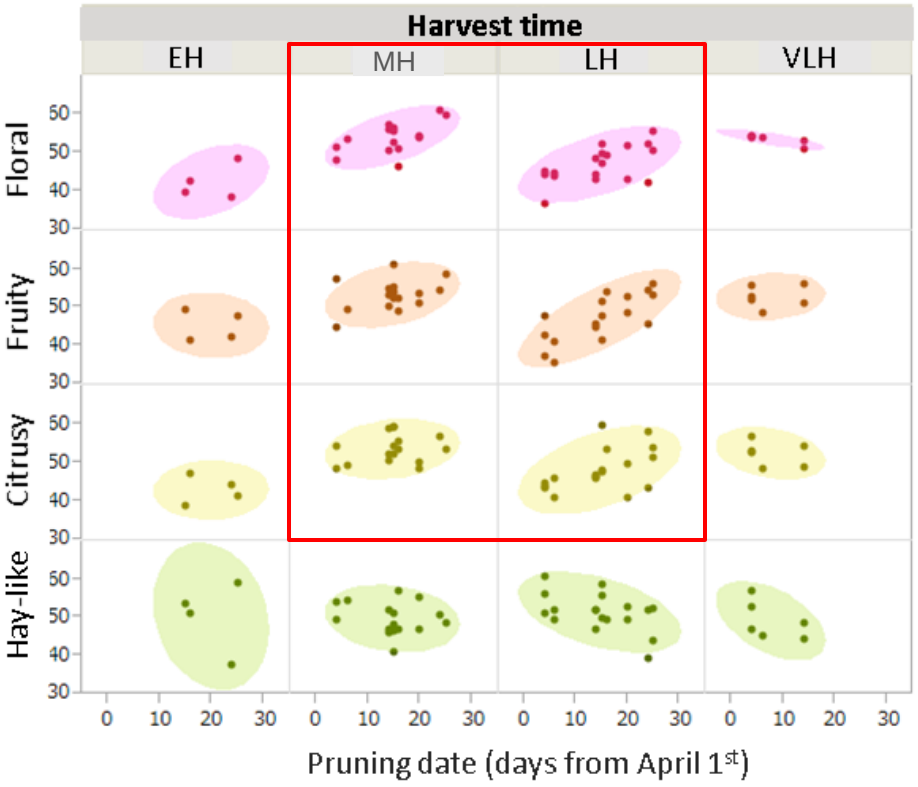


Attribute	Harvest time	Farm	Harvest time X Farm
Floral	34.43[c]	0.55	2.12
Fruity	34.08[c]	1.52	1.98
Citrusy	30.19[c]	0.53	1.44
Hay-like	0.32	0.53	1.76

[a] F value at the 95.0% significance level.
[b] F value at the 99.0% significance level.
[c] F value at the 99.9% significance level.

Organoleptic evaluation of hop tea according to pruning date

Trial Ⅰ and Ⅱ ***N*=7**



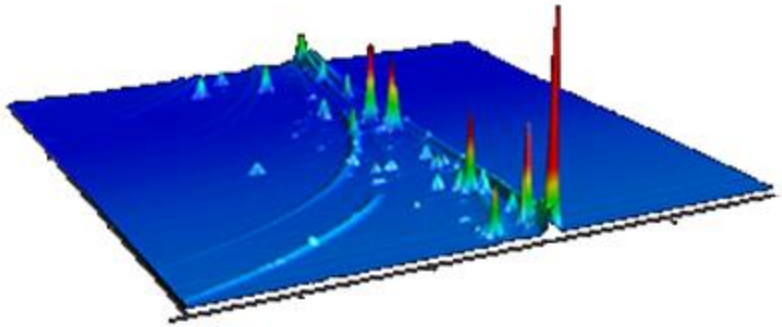
EH: early harvest
MH: middle harvest
LH: late harvest
VLH: very late harvest

Attribute	Farm	Pruning date	Harvest time X Pruning date
Floral	0.55	12.02[b]	0.13
Fruity	1.52	22.14[c]	0.36
Citrusy	0.53	14.38[b]	0.46
Hay-like	0.53	3.57	1.72

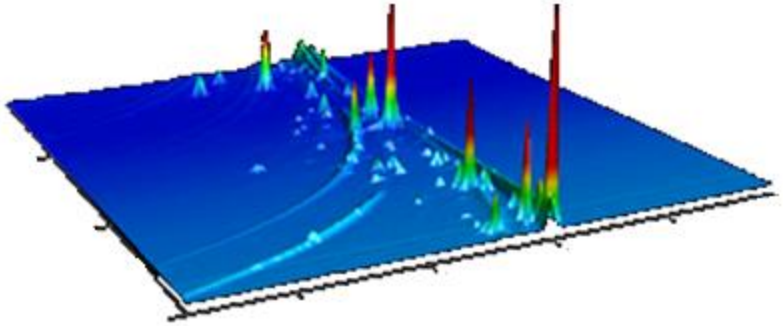
[b] F value at the 99.0% significance level.
[c] F value at the 99.9% significance level.

GC ×GC/TOF-MS chromatograms of hop aroma compounds harvested at 4 different harvest times

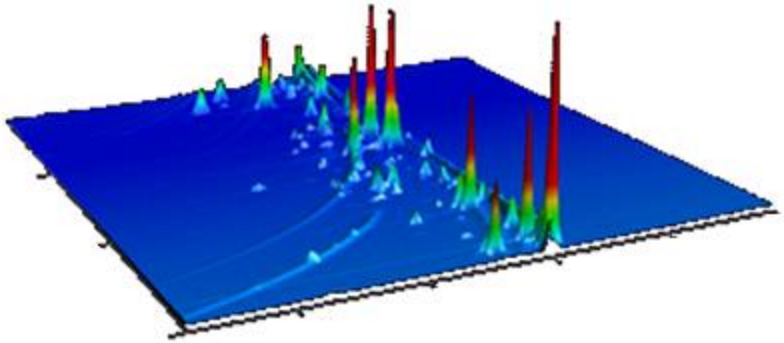
Early



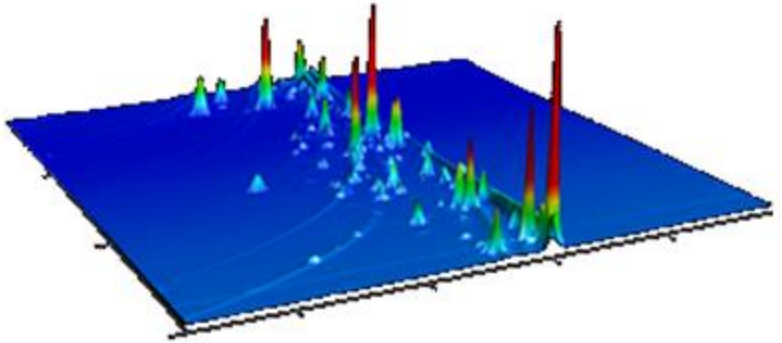
Middle



Late



Very late



F-values of hop aroma compounds and their significance in Trial I: Harvest time X Harvest year

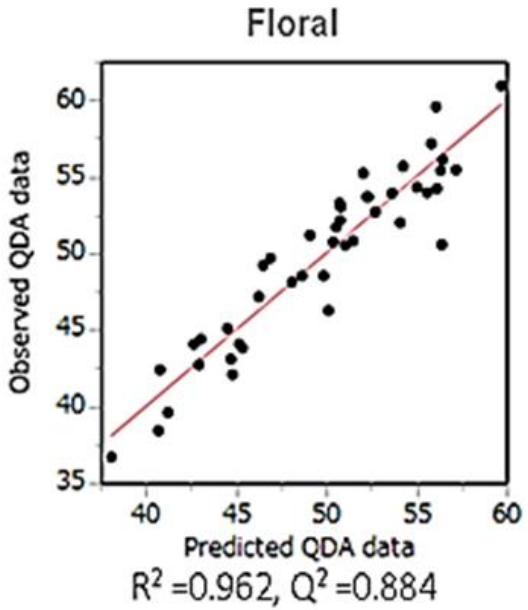
Attribute	Harvest time	Harvest year	Harvest time X Harvest year
4-Methyl-2-pentanone	26.49[c]	44.41[c]	10.53[b]
3-Methyl-2-butenal	0.13	22.72[c]	2.97
2-Hexenal	11.21[a]	12.34[b]	3.21
o-Cymene	1.72	31.83[c]	3.59
Heptanoacid, methyl ester	6.49[a]	11.26[b]	2.32
3-Methyl-2-buten-1-ol	13.23[c]	20.46[c]	4.58[a]
E-3-Hexen-1-ol	16.27[a]	2.66	0.29
Octanoic acid, methyl ester	5.90[a]	8.07[b]	1.06
Nonanal	5.49[a]	20.61[c]	1.89
Octanoic acid, ethyl ester	0.56	5.84[a]	2.63
1-Octen-3-ol	5.03	1.42	1.25
cis−Linalool oxide	51.54[c]	6.89[a]	2.24
trans−Linalool oxide	48.20[c]	2.67	1.31
Nonanoic acid, methyl ester	0.48	4.90[a]	0.15
2-Decanone	3.78	51.55[c]	14.13[b]
Benzaldehyde	0.51	3.84	1.28
Linalool	98.34[c]	22.58[c]	10.14[b]
1-Octanol	2.58	7.69[b]	2.23
2-Undecanone	0.54	20.25[c]	7.56[a]
Decanoic acid, ethyl ester	6.68[a]	8.48[b]	7.75[b]
Z-Citral	29.62[c]	10.04[b]	4.80[a]
Methyl geranate	37.75[c]	9.83[b]	2.91
α-Terpineol	10.83[a]	2.13	0.90
E-Citral	0.77	18.49[c]	13.13[b]
Geraniol	4.61	16.89[c]	2.14
Benzyl alcohol	0.27	0.87	1.04
Phenylethyl alcohol	1.29	17.71[c]	2.78
Heptanoic acid	19.59[b]	17.73[c]	4.52[a]
Caryophyllene oxide	0.33	23.88[c]	2.71
Octanoic acid	6.88[a]	6.10[a]	0.49
Humulene epoxide Ⅱ	2.67	23.84[c]	1.10
2-Methoxy-4-vinylphenol	0.68	1.82	2.00
Decanoic acid	10.47[a]	16.06[c]	1.13

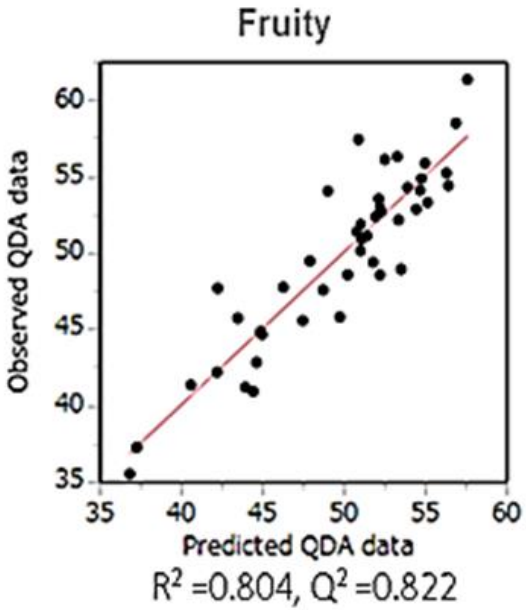
[a] F value at the 95.0% significance level.
[b] F value at the 99.0% significance level.
[c] F value at the 99.9% significance level.

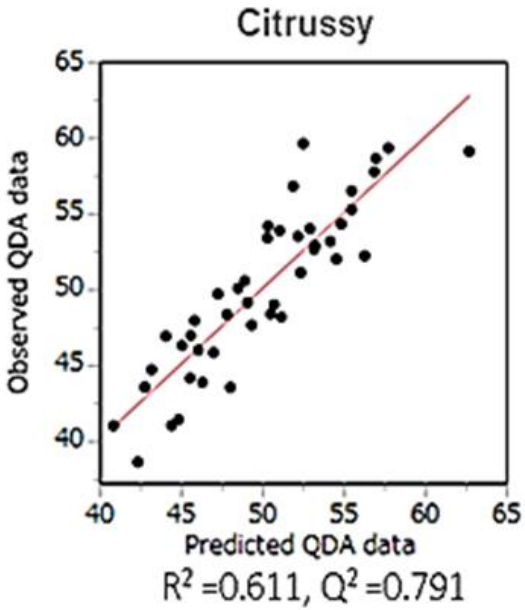
F-values of hop aroma components and their significance in Trial Ⅱ: Harvest time X Farm X Pruning date

Attribute	Harvest time	Farm	Pruning date	Harvest time X Farm	Harvest time X Pruning date
4-Methyl-2-pentanone	0.00	11.72^{c}	4.40^{a}	0.62	0.41
3-Methyl-2-butenal	3.31	14.33^{c}	3.26	2.19	0.10
2-Hexenal	0.41	2.90	0.63	0.43	3.69
o-Cymene	11.31^{b}	30.96^{c}	9.19^{b}	0.63	1.31
Heptanoacid, methyl ester	3.17	4.53^{a}	0.46	2.35	0.56
3-Methyl-2-buten-1-ol	1.83	4.20^{a}	0.90	0.14	0.51
E-3-Hexen-1-ol	7.06^{a}	1.36	3.69	0.94	0.40
Octanoic acid, methyl ester	0.38	4.63^{a}	1.09	1.95	1.13
Nonanal	0.00	5.63^{b}	0.11	1.03	0.02
Octanoic acid, ethyl ester	0.00	1.75	0.71	0.01	0.00
1-Octen-3-ol	7.39^{a}	3.56^{a}	1.95	0.43	0.17
cis−Linalool oxide	17.52^{c}	12.22^{c}	2.42	0.80	0.49
trans−Linalool oxide	5.51^{a}	2.04	5.26^{a}	0.29	0.05
Nonanoic acid, methyl ester	1.41	2.88	6.45^{a}	0.92	0.74
2-Decanone	29.09^{c}	10.50^{c}	1.92	1.85	3.23
Benzaldehyde	0.48	1.23	0.52	0.17	1.14
Linalool	67.40^{c}	9.37^{c}	15.19^{c}	2.76	0.05
1-Octanol	7.71^{a}	5.42^{b}	4.13	0.48	0.07
2-Undecanone	3.62	11.84^{c}	2.35	1.56	4.04
Decanoic acid, ethyl ester	0.44	5.86^{b}	0.47	1.94	0.40
Z-Citral	13.25^{b}	1.62	5.25^{a}	1.63	0.04
Methyl geranate	7.74^{a}	4.68^{a}	3.08	4.60^{a}	0.02
α-Terpineol	1.39	0.14	3.04	0.50	1.64
E-Citral	10.58^{b}	3.02	10.07^{b}	0.63	0.34
Geraniol	4.24	1.32	4.03	2.07	0.25
Benzyl alcohol	0.11	2.10	0.15	1.01	1.07
Phenylethyl alcohol	1.05	0.76	1.37	0.03	1.86
Heptanoic acid	125.45^{c}	11.95^{c}	27.58^{c}	5.78^{b}	0.02
Caryophyllene oxide	2.06	0.94	0.14	2.61	10.53^{b}
Octanoic acid	79.87^{c}	40.14^{c}	21.37^{c}	7.68^{b}	1.88
Humulene epoxide Ⅱ	0.24	1.58	2.54	0.02	0.57
2-Methoxy-4-vinylphenol	1.19	19.37^{c}	4.24	3.07	1.88
Decanoic acid	3.29	1.72	1.43	2.63	0.04

PLS models for predicting QDA data for the floral, fruity, and citrusy characteristics





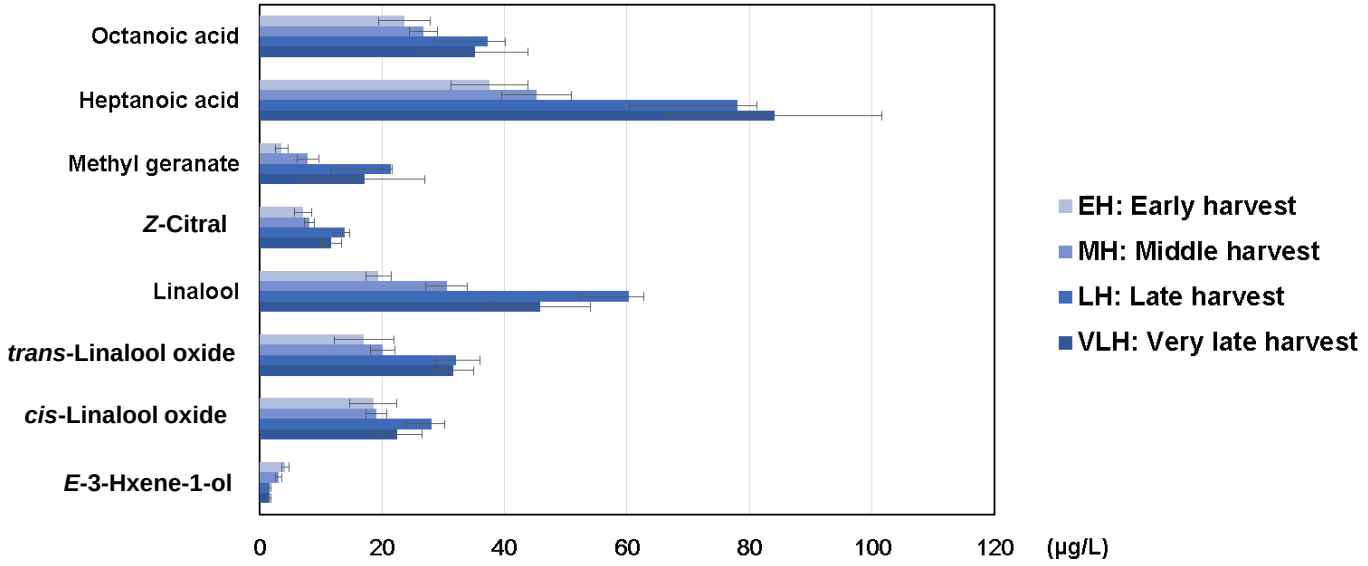


Compounds with a large VIP (>1.0), the regression coefficients, and odor descriptions of each compound

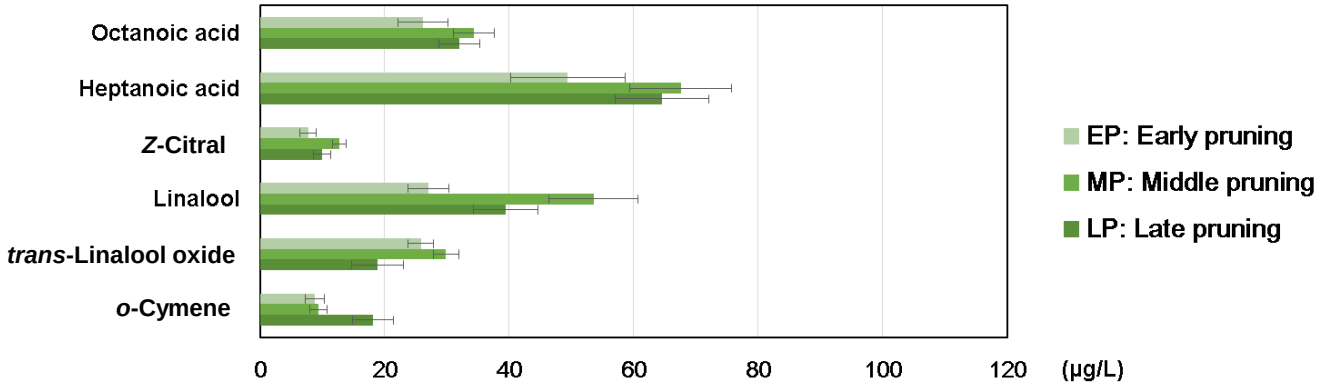
Attributes	Compounds ●Positive ●Negative	VIP	Coefficient	Reference No. / Odor description
Floral	1-Octen-3-ol	1.57	-0.35	1. Mushroom, earthy, green, oily, fungal, raw chicken
	Z-Citral	1.45	0.07	2. Sweet, citral, lemon peel
	Heptanoic acid	1.43	0.16	3. Cheesy, waxy, sweaty, fermented, pineapple, and fruity
	Linalool	1.40	0.21	4. Citrus, orange, floral, terpy, waxy, rose
	Methyl geranate	1.37	0.06	5. Waxy, green, fruity, flower
	Octanoic acid	1.33	-0.15	6. Fatty, waxy, rancid, oily, vegetable, cheesy
	2-Decanone	1.24	0.01	7. Orange, floral, fatty, peach
	α-Terpineol	1.21	0.18	8. Pine, terpene, lilac, citrus, woody, floral
	2-Hexenal	1.21	0.36	9. Sweet, almond, fruity, green, leafy, apple, plum, vegetable
	E-3-Hexen-1-ol	1.20	-0.16	10. Green, grassy, melon, rind-like with a pungent freshness
	trans-Linalool oxide	1.19	-0.19	11. Floral
	cis-Linalool oxide	1.14	0.26	12. Earthy, floral, sweet, woody
	E-Citral	1.05	0.03	13. Citrus, lemon
	Decanoic acid, ethyl ester	1.02	-0.18	14. Sweet, waxy, fruity, apple
Fruity	Heptanoic acid	1.72	0.18	3
	E-3-Hexen-1-ol	1.68	-0.34	10
	1-Octen-3-ol	1.49	-0.31	1
	2-Decanone	1.47	0.04	7
	Z-Citral	1.41	0.03	2
	Octanoic acid	1.39	-0.10	6
	cis-Linalool oxide	1.36	0.28	12
	Methyl geranate	1.29	-0.01	5
	Linalool	1.27	0.08	4
	trans-Linalool oxide	1.07	-0.19	11
	o-Cymene	1.02	0.05	15. Cider-like, clove-like, phenolic, barnyard
Citrusy	*E*-3-Hexen-1-ol	1.73	-0.49	10
	Z-Citral	1.53	0.04	2
	Linalool	1.52	0.11	4
	Heptanoic acid	1.42	0.19	3
	Geraiol	1.29	0.29	16. Sweet, floral, fruity, rose, waxy, citrus
	cis-Linalool oxide	1.29	0.34	12
	Methyl geranate	1.29	0.02	5
	E-Citral	1.28	-0.13	13
	Octanoic acid	1.24	-0.07	6
	trans-Linalool oxide	1.17	-0.16	11
	Phenylethyl alcohol	1.15	0.23	17. Floral, rose, dried rose flower, rose water
	4-Methyl-2-pentanone	1.08	-0.25	18. Sharp, solvent, green, herbal, fruity, dairy, spice
	2-Decanone	1.06	0.05	7

Chemical profiles associated with the floral, fruity, and citrusy characteristics according to harvest time and pruning date

Chemical profile according to harvest time



Chemical profile according to pruning date



Contents

✔ *Introduction and Objectives*

✔ *Experimental design and analytical methods*

✔ *Results*

✔***Conclusion and future work***

Conclusion

- ✔ Harvest time was the most influential factor in determining hop aroma characteristics among the various cultivation factors.
- ✔ Chemical compounds associated with the floral, fruity, and citrusy characteristics are highly dependent on the harvest time or pruning date.
- ✔ Hop aroma quality in beer could be controlled by altering the hop harvest time and pruning date.

Future work

- ✔ To verify that the predicted score of hop teas' aroma characteristics correspond well to the hop aroma characteristics in beer by conducting brewing trials.
- ✔ To examine the comprehensive effects of other essential factors impacting hop aroma characteristics.

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Thank you for your kind attention!