CRAFT BREWERS CONFERENCE
& BrewExpo America

Development of Thiols and Thiol Precursors in different Hop varieties during Hop Harvest and their impact on beer flavor

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BREWERS ASSOCIATION

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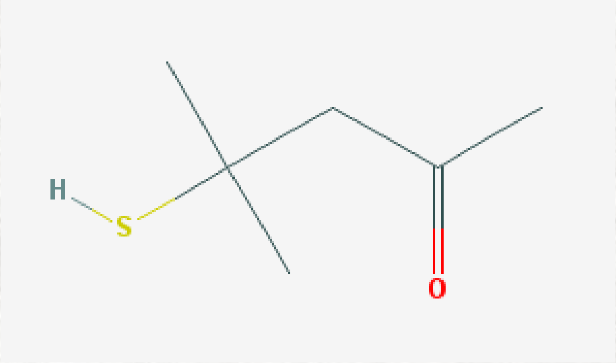
Background of this study

- Dry hopped craft beers, especially very hop intensive beers, are very successful, brewers have no means to control the hop aroma
- Many of the today used hop varieties are breeded varieties with intensive fruit characters
- **Many of these fruit characters are caused by thiols in hops**
- Varieties like Citra, Mosaic, Cascade are famous for these fruit characters
- For other varieties like European land varieties these fruit characters are absent
- Thresholds of those fruity thiols are about a factor of 1000 (and more) lower than e.g. threshold of linalool

What are thiols and why do they matter?

- Thiols (R-SH) and mercaptans are organosulfur compounds that contain a sulfur-hydrogen bond
- The -SH group is an acid and a nucleophile for attacks on saturated carbon
- Polyfunctional thiols are thiols with other functional groups, e.g. alcohol
- Oxidisable and convert to disulfides, hydrolized to thiols – reactive
- Mercapto relates to the ability to bind mercury (II) ions (capturing Mercury)
- Aromatic and volatile compounds in wine, milk, cheese, vegetables, fruits…hops
- Thiols (R-SH) are powerful odorant volatiles participating to the hoppy character of beer.
- Hops represents a significant source of thiol precursors either as free or bound forms

Polyfunctional thiols

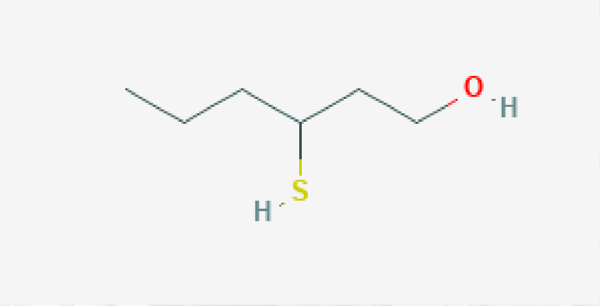


4-Mercaptomethylpentan-2-one= **4MMP**=4S4M2Pone **4ng/L**




Citra, Mosaic,
Cascade, Simcoe,
Sorachi Ace

Range in wines (ng/L): 4-40

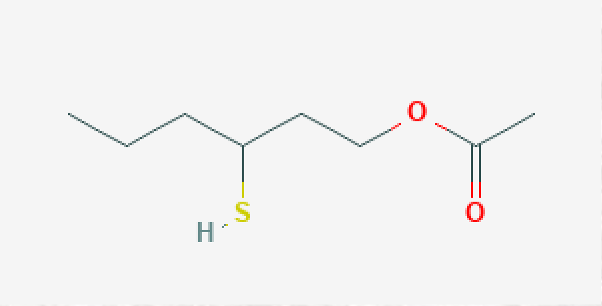


3-Mercaptohexan-1-ol= **3MH** = 3SHol **60ng/L**



Citra, Cascade,
Herkules,Tradition,
Perle, Saaz

Range in wines (ng/L): 26–18,000



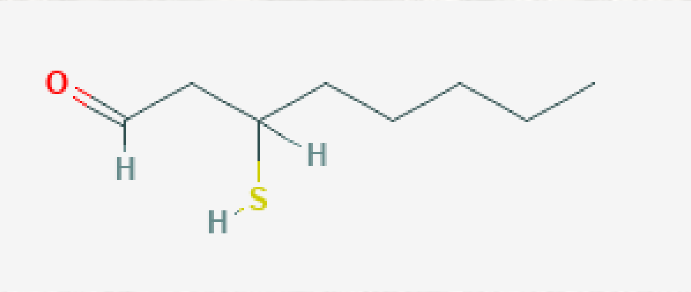
3-Mercaptohexylacetate = **3MHA**=3SHA **0.8-4 ng/L**




Citra, Cascade,
Herkules,Tradition,
Perle, Saaz

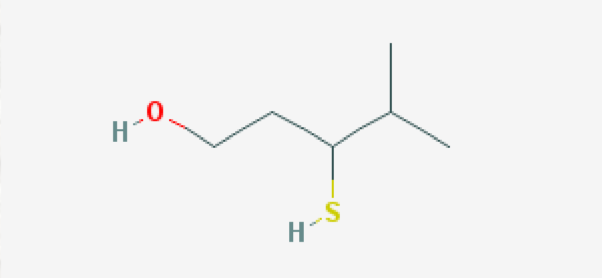
Range in wines (ng/L): 0–2500

Polyfunctional thiols



3-Mercapto-octanal**= 3MO** = 3SOal

Found in Tomahawk/Nelson Sauvin

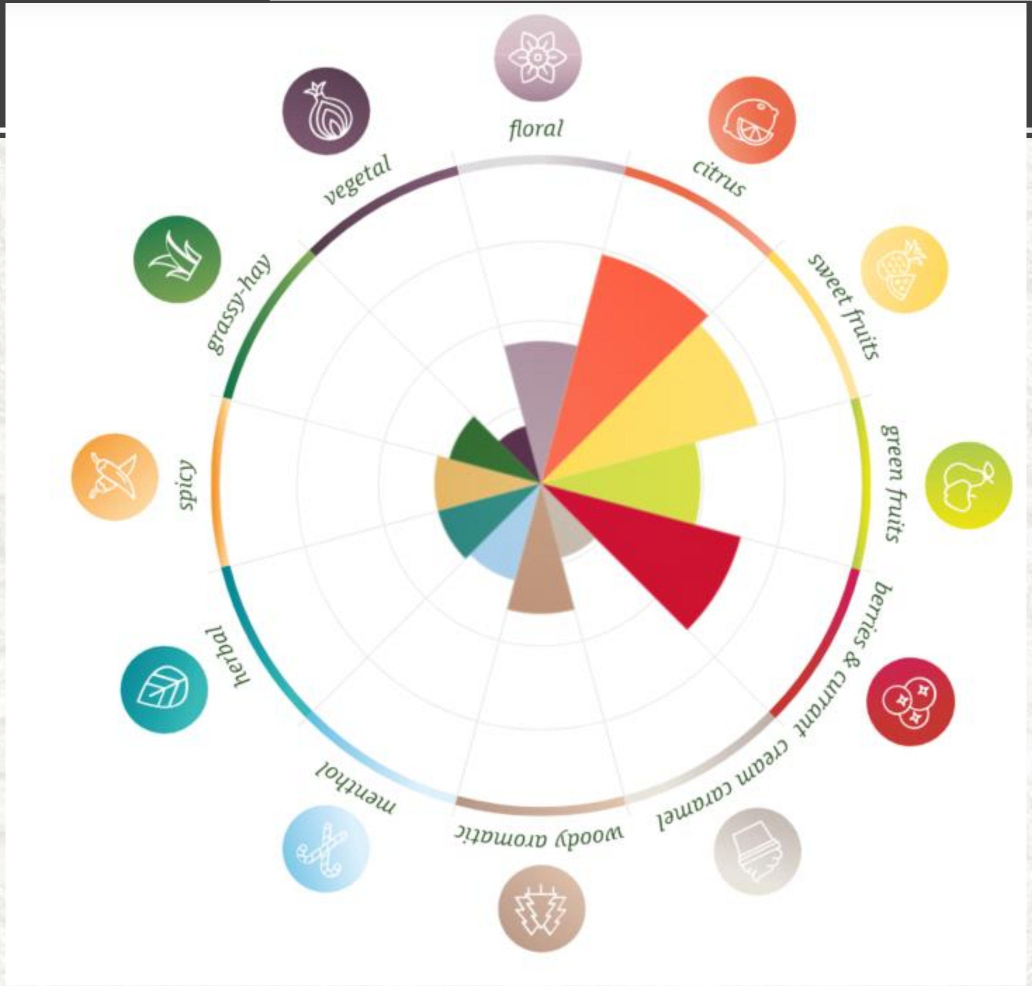


3-Mercapto-4-methylpentan-1-ol=**3M4MP**=3S4MPol

70 ng/L

Mosaic, Amarillo, H. Blanc, Nelson Sauvin

Sensory analysis for hops – Hopsessed ® Example Citra



Assesment of hop varieties with 12 categories:

Intesnity rating 1-10, naming of specific attributes

citrus
Grapefruit, Orange, Lime, Lemon, Bergamot, Lemon Grass, Ginger, Tangerine, Pomelo

green fruits
Pear, Apple, Quince, Gooseberry, White Wine Grapes

cream caramel
Butter, Chocolate, Yoghurt, Honey, Cream, Caramel, Toffee, Coffee, Tonka Bean, Vanilla, Coconut

woody aromatic
Tobacco, Cognac, Barrique, Leather, Woodruff, Incense, Myrrh, Resin, Cedar, Pine, Earthy

sweet fruits
Banana, Watermelon, Honeydew Melon, Peach, Apricot, Passion Fruit, Lychee, Dried Fruit, Plum, Pineapple, Cherry, Kiwi, Mango, Guava

herbal
Marjoram, Tarragon, Dill, Parsley, Basil, Fennel, Coriander, Rosemary, Thyme, Green Tea, Black Tea, Mate Tea, Oregano

menthol
Mint, Lemon Balm, Sage, Camphor, Menthol, Wine Yeast, Eucalyptus

vegetal
Celeriac, Leek, Onion, Artichoke, Garlic, Wild Garlic, Radish

berries & currant
Cassis, Blueberry, Raspberry, Blackberry, Strawberry, Red Currant, Black Currant, Wild Strawberry, Cranberry, Mulberry

grassy-hay
Green Grassy, Fresh Cut Grass, Hay, Tomato Leaves, Green Pepper, Nettle, Cucumber, Bamboo Leaves

floral
Elderflower, Camomile Blossom, Lily Of The Valley, Jasmine, Apple Blossom, Rose, Geranium, Carnation, Lily, Lilac, Lavender, Osmanthus

spicy
Lovage, Pepper, Chilli, Curry, Juniper, Aniseed, Liquorice, Fennel Seeds, Clove, Cinnamon, Gingerbread, Coriander Seeds, Nutmeg

Connection Sensory & Analytics

Descriptors	Possible Key Compounds
Floral	Geraniol, Citronellol, 2-Decanone, a-Ionone, a-Terpineol, Rose Oxide
Citrus	Limonene, Linalool, Myrcene, Ethyl-2-methylbutanoate, α-Pinene 3M4MP, 3M4MPA, 3MHA, 3MH, 4MMP
Sweet Fruits	3MH, 3MOal, 4-MMP, ethyl 3-(methylthio)-propionate
Green Fruits	b-Damascenone, 2-Methylbutyl 2-methylpropanoate
Red Berries	4MMP, 4-(4-Hydroxyphenyl)-2-butanone, a-Ionone, b-Damascenone
Cream Caramel	Lactones, Vanillin
Woody	Myrcene, 8-Acetoxylinalool, a-Cadinene, a-Calacorene, a-Ionone, a-Terpineol, Benzaldehyde, Eudesmol, Farnesen
Menthol	4,4-Dimethyl-2-buten-4-olide, 3-Methyl-2-butanone, 3-Nopinenone
Herbal	Humulene, Humulenol epoxides, Rose Oxide, a-Cadinene
Spicy	Oxygenated sesquiterpenoids, b-Caryophyllene, Caryophyllene epoxide
Green Grassy	2 Dodecanone, Myrcene disulfide, E,Z-2,6-Nonadienal, Cis-3-Hexenol
Vegetal	Polyfunctional thiols, 1-Octen-3-ol, DMS, 3MBT

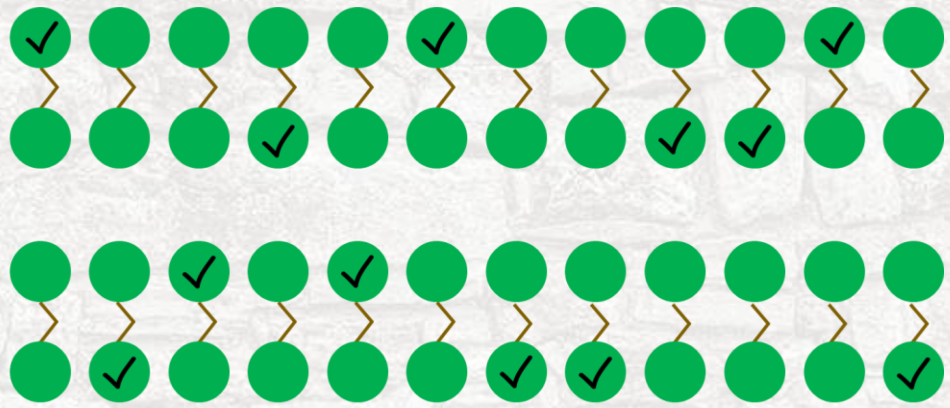
Table 15 — The list of passion fruit VSCs reported in the literature.

VSC	Reference
1,1-bis-(methylthio)-2-methylpropane	[147]
2-(methylthio)-ethyl acetate	[147]
2-methylbutyl 3-(methylthio)-propionate	[147]
3-(1-acetoxy-3-hexyldithio)-hexyl acetate	[147]
3-(1-acetoxy-3-hexyldithio)-hexyl butyrate	[147]
3-(1-acetoxy-3-hexyldithio)-hexyl hexanoate	[147]
3-(1-butyryloxy-3-hexyldithio)-hexyl butyrate	[147]
3-(1-butyryloxy-3-hexyldithio)-hexyl hexanoate	[147]
3-(1-hydroxy-3-hexyldithio)-hexanol	[147]
3-(1-hydroxy-3-hexyldithio)-hexyl acetate	[147]
3-(1-hydroxy-3-hexyldithio)-hexyl butyrate	[147]
3-(methylthio)-hexanol	[141,142,144,145,147]
3-(methylthio)-hexyl acetate	[144,147]
3-(methylthio)-hexyl butyrate	[144,147]
3-(methylthio)-hexyl hexanoate	[144,147]
3-(methylthio)-propanal (methional)	[147]
3-(methylthio)-propionate	[144]
3-(methylthio)-propionic acid	[14]
3-(methylthio)-propyl acetate	[147]
3-(methylthio)-propyl butyrate	[147]
3-(methylthio)-propyl hexanoate	[147]
3-mercapto-3-methylbutanol	[157]
3-mercapto-3-methylbutyl acetate	[157]
3-mercaptohexanol (3MH)	[14,144,145,147,157]
3-mercaptohexyl acetate (3MHA)	[14,144,146,147,157]
3-mercaptohexyl butyrate	[14,144,146,147,157]
3-mercaptohexyl hexanoate	[14,144,147]
3-mercaptohexyl pentanoate	[147]
3-methylbutyl 3-(methylthio)-propionate	[147]
4-methyl-5-vinylthiazole	[140,144,147]
butyl 3-(methylthio)-propionate	[147]
(Z)-2-methyl-4-propyl-1,3-oxathiane	[141,142,143,144,146,147]
(Z)-3-hexenyl 3-(methylthio)-propionate	[147]
diethyl disulfide	[147]
diisopropyl disulfide	[147]
diisopropyl trisulfide	[147]
ethyl 2-(methylthio)-acetate	[147]
ethyl 3-(methylthio)-(Z)-2-propenoate	[147]
ethyl 3-(methylthio)-propionate	[14,141,142,143,144,146,147]
ethyl 3-(methylthio)-(E)-2-propenoate	[141,142,143,144,146,147]
ethyl 3-mercaptobutyrate	[14]
hexyl 3-(methylthio)-propionate	[147]
hexyl 3-(methylthio)-(E)-2-propenoate	[147]
isobutyl 3-(methylthio)-propionate	[147]
methyl 2-methylbutyl disulfide	[147]
methyl 3-(methylthio)-(Z)-2-propenoate	[147]
methyl 3-(methylthio)-propionate	[147]
methyl 3-(methylthio)-(E)-2-propenoate	[147]
pentyl 3-(methylthio)-propionate	[147]
propyl 3-(methylthio)-propionate	[147]
propyl 3-(methylthio)-(E)-2-propenoate	[147]
sec. butyl 3-(methylthio)-propionate	[147]
s-methyl acetothioate	[147]
(E)-2-methyl-4-propyl-1,3-oxathiane	[141,142,143,144,146,147]

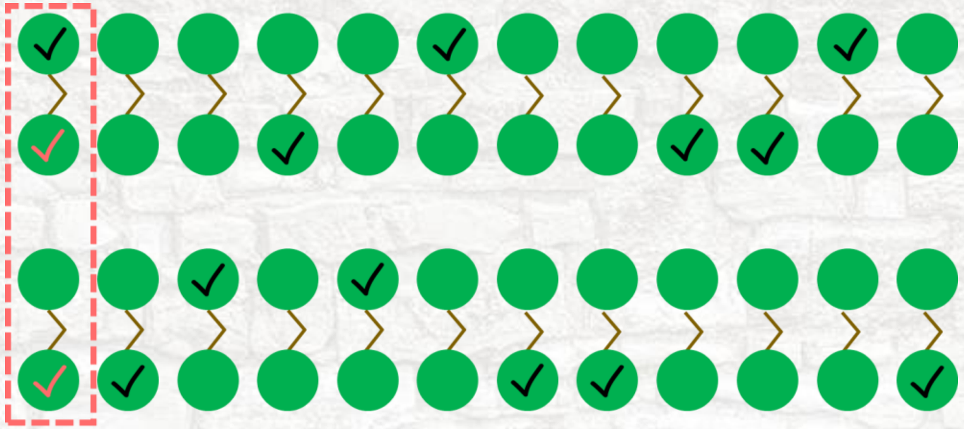
Timeline of project

- Hop samples were collected in harvest 2019
- Hop samples arrived in Germany/France in October
- Analysis ran from Nov 19 to Feb 20
- Sensory profiles of hop samples are being compiled (Compusense, Hopsessed)
- Due to pandemic concept brewery had to be closed until May 1st.
- Brewing will start in June 20

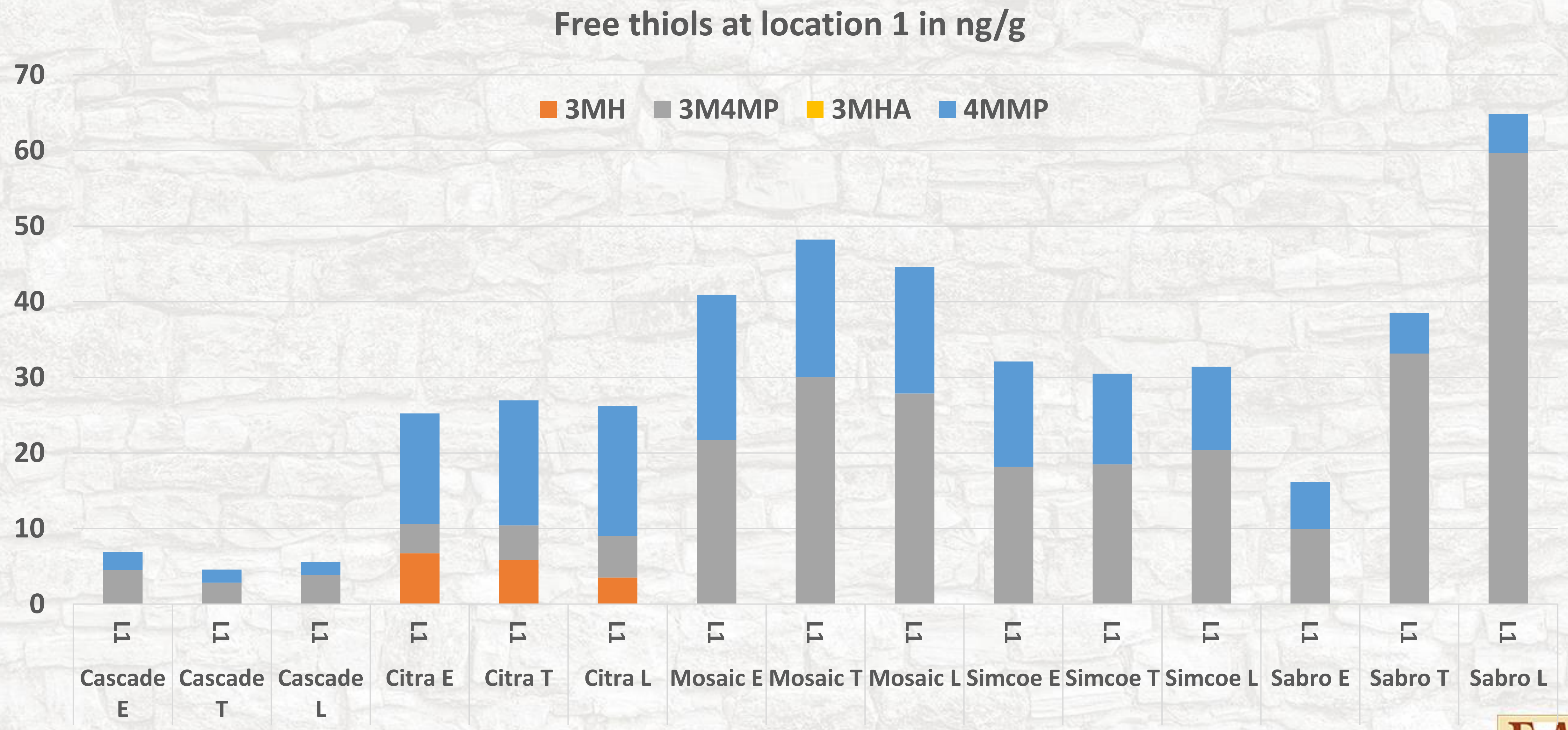
- Select Hop varieties (6)
- Determine harvest dates (early, typical, late)
- Determine harvest locations
- Determine harvest and kilning procedures (same for all samples)
- 30 Samples sent to Germany
- Samples split between France and Germany
- Analysis in France (Nyseos)
- Brewing Trials in Nuremberg (Barth Haas)



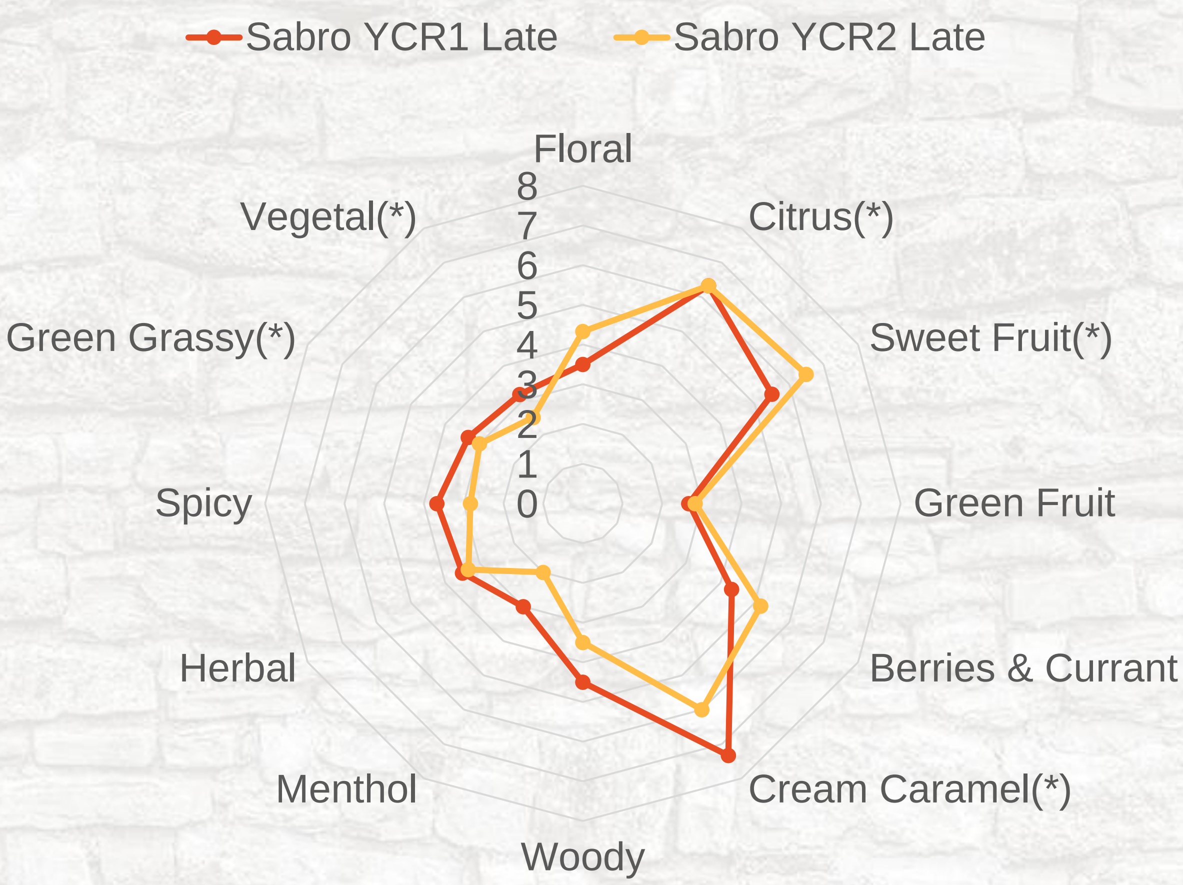
Identify 12 random bines for each variety/location for Early Harvest
1 bine = 500g dry cone

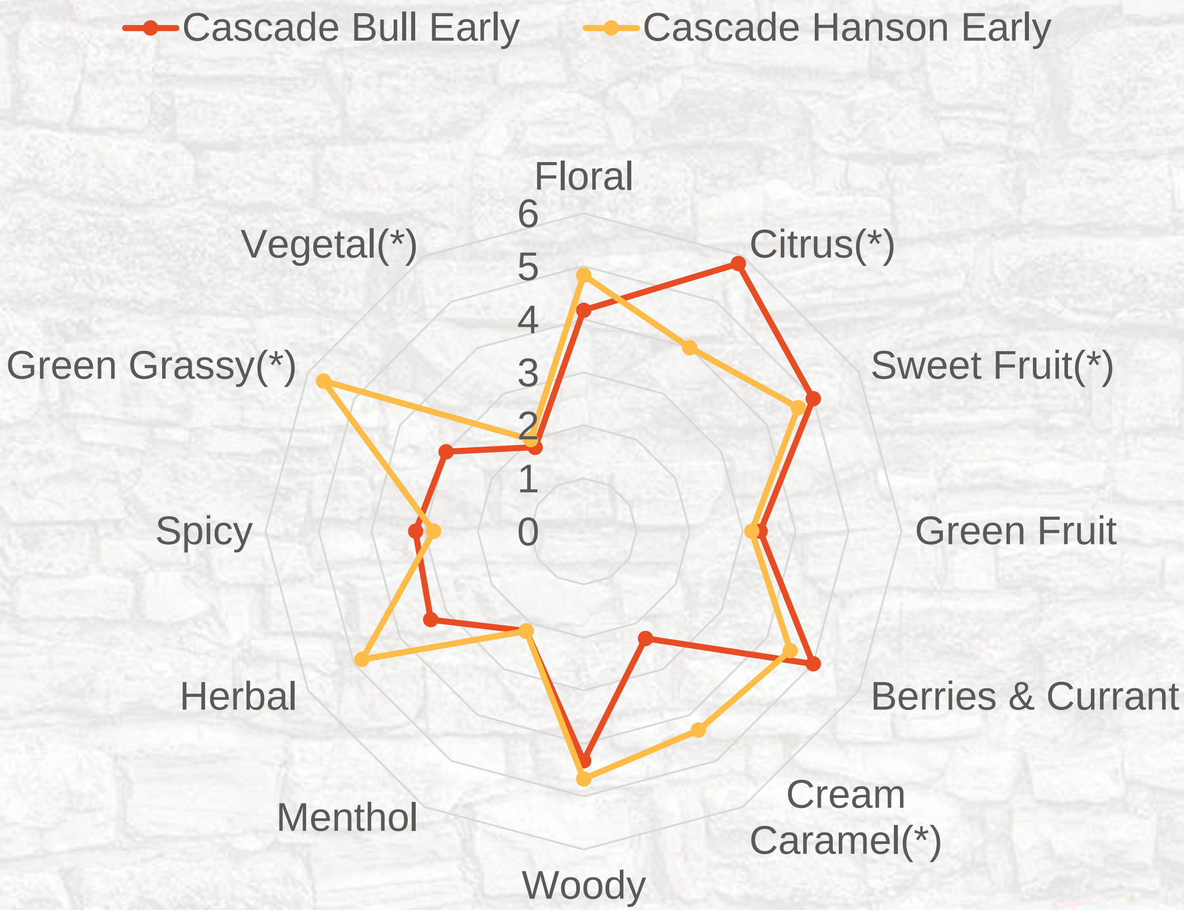


For Typical and Late Harvest, select randomly from three remaining bines in same rows, ensuring broadest selection for each harvest, across the whole plot

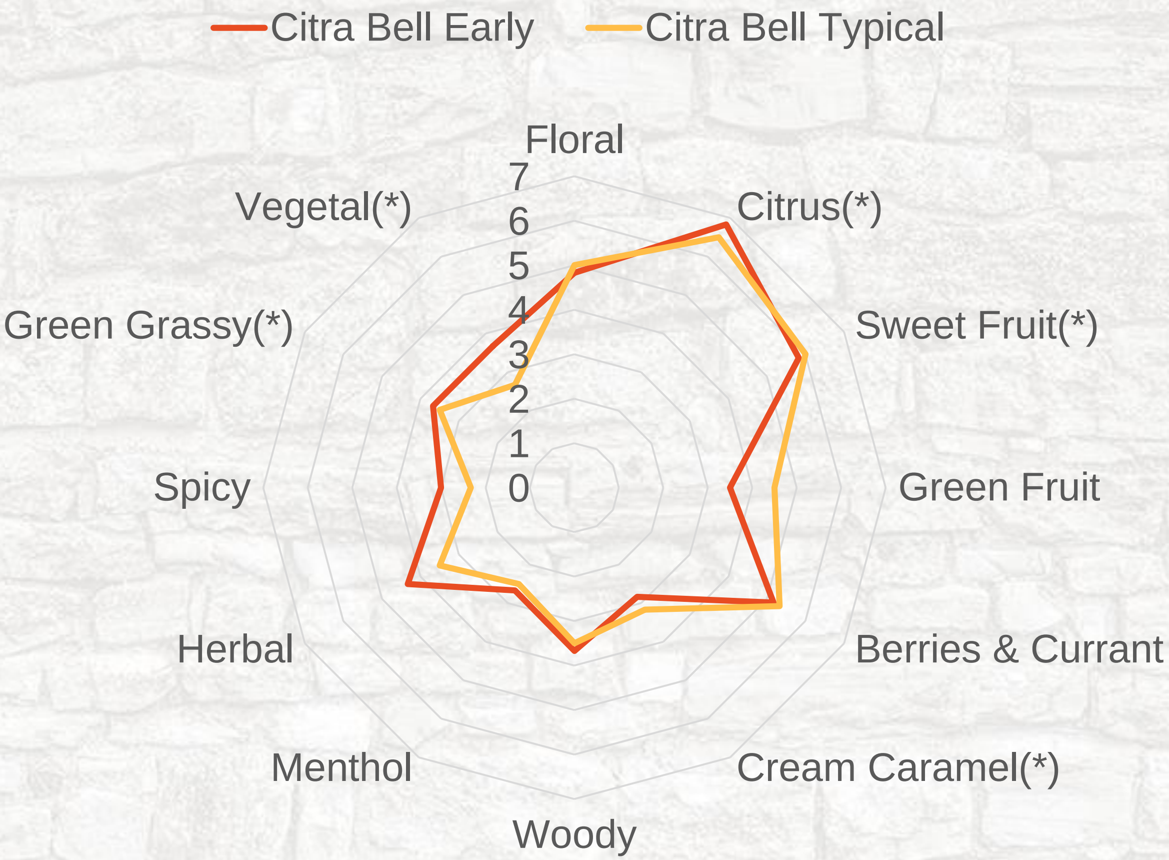

Free thiols at location 1 in ng/g
3MH
3M4MP
3MHA
4MMP
70
60
50
40
30
20
10
0
L1
Cascade E
Cascade T
Cascade L
Citra E
Citra T
Citra L
Mosaic E
Mosaic T
Mosaic L
Simcoe E
Simcoe T
Simcoe L
Sabro E
Sabro T
Sabro L

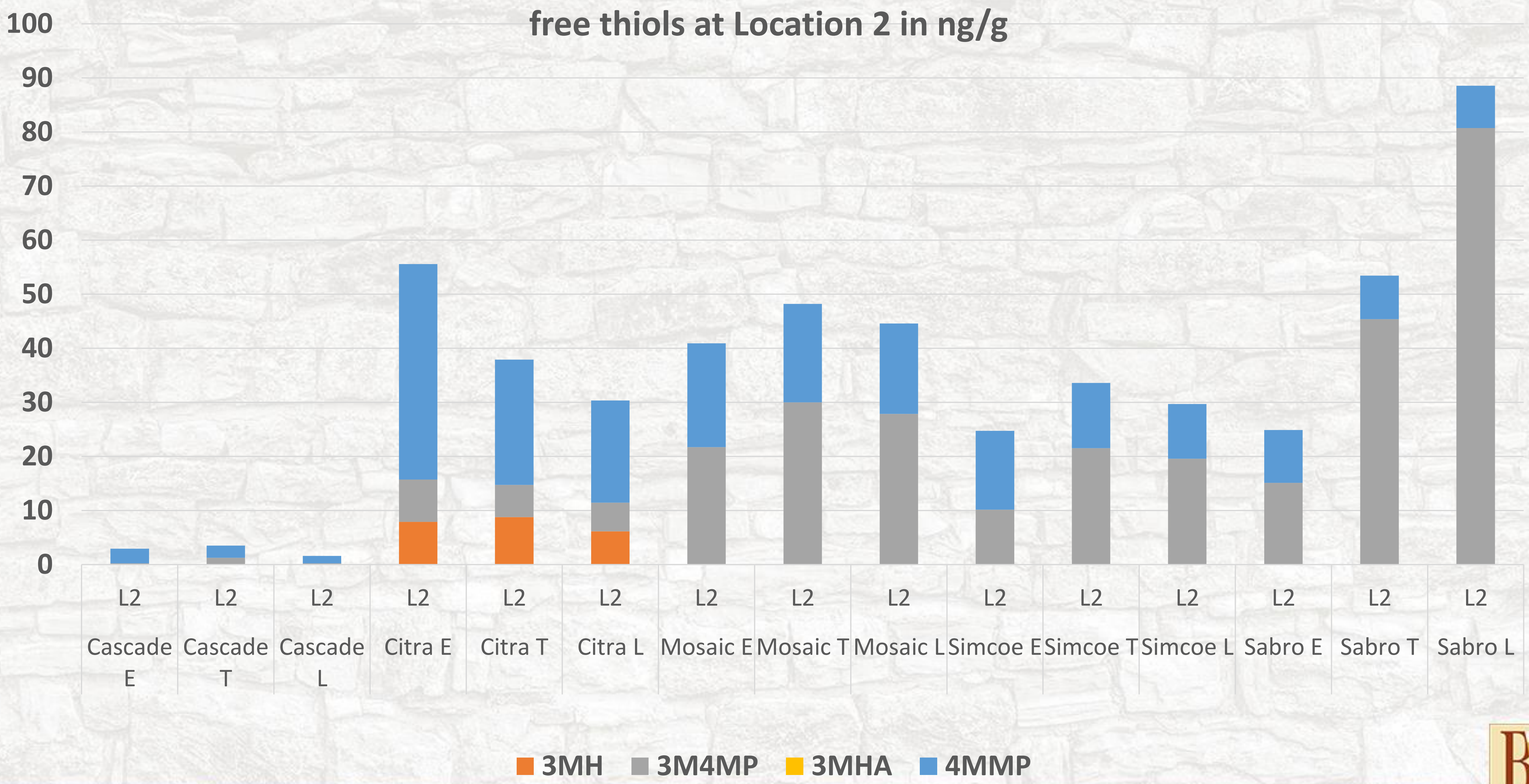
Sensory Profiles same time/diff. location



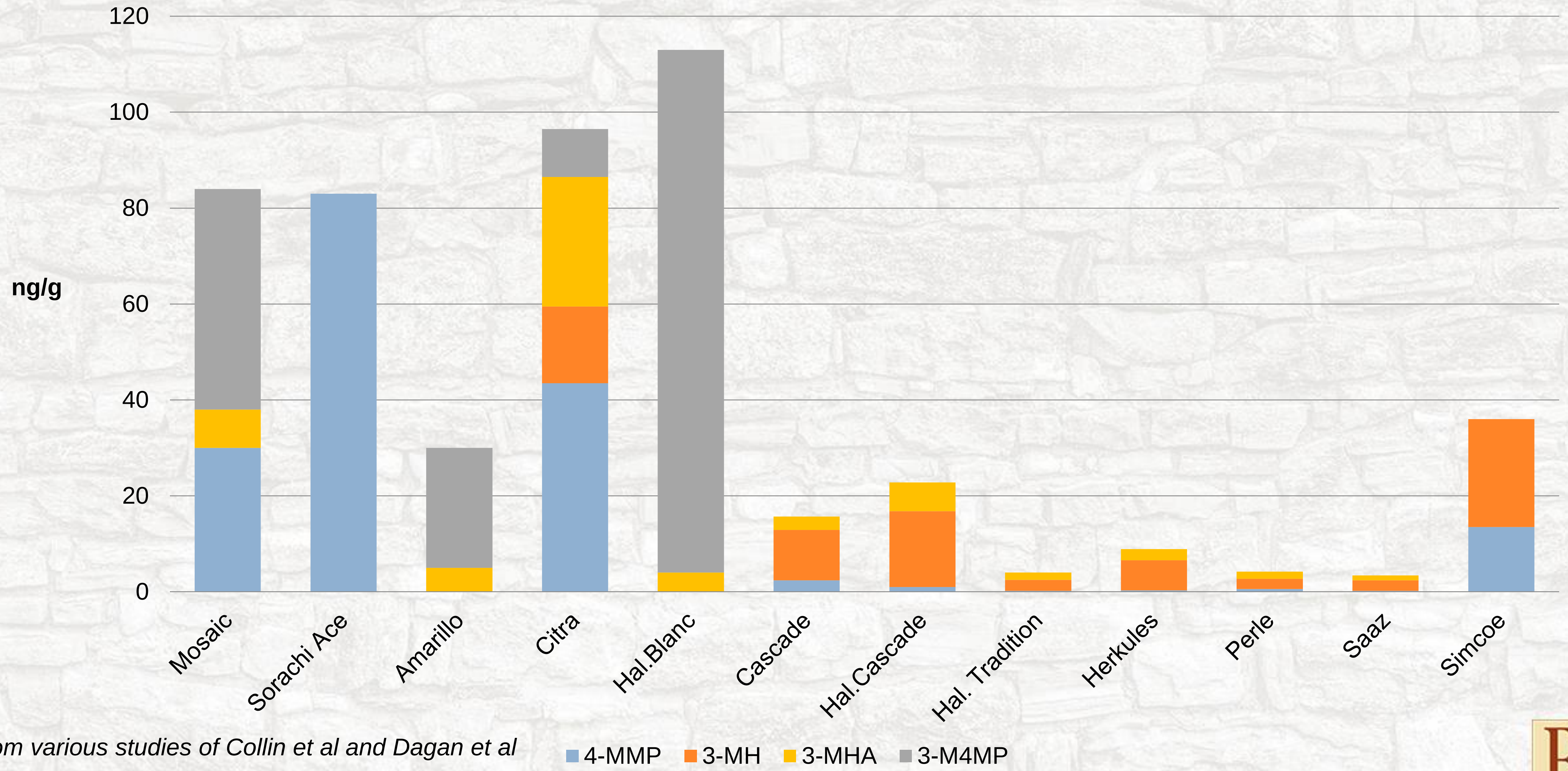


Sensory Profiles same loc/diff. time



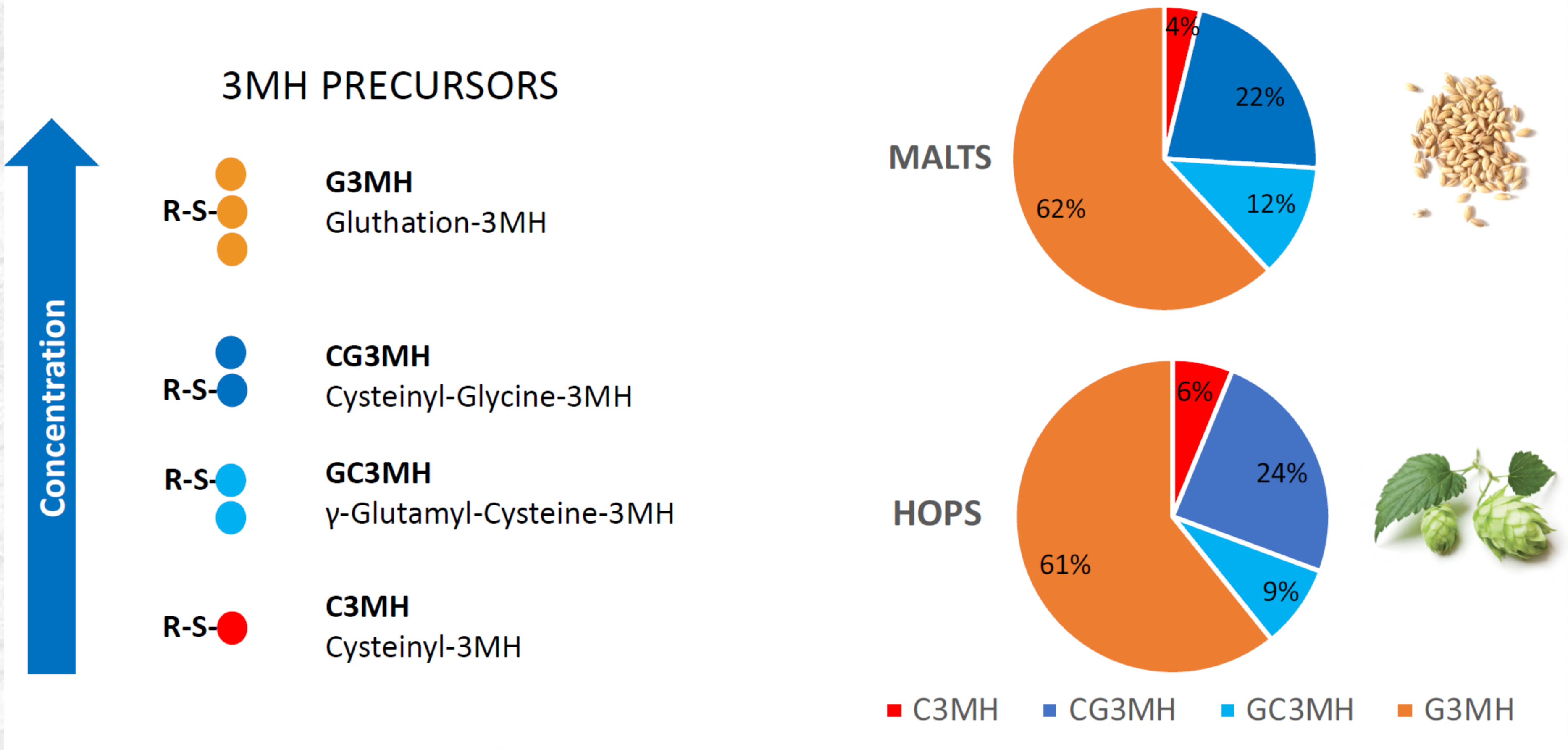

free thiols at Location 2 in ng/g
100
90
80
70
60
50
40
30
20
10
0
L2 Cascade E
L2 Cascade T
L2 Cascade L
L2 Citra E
L2 Citra T
L2 Citra L
L2 Mosaic E
L2 Mosaic T
L2 Mosaic L
L2 Simcoe E
L2 Simcoe T
L2 Simcoe L
L2 Sabro E
L2 Sabro T
L2 Sabro L
3MH
3M4MP
3MHA
4MMP

Published data on free thiols in hops



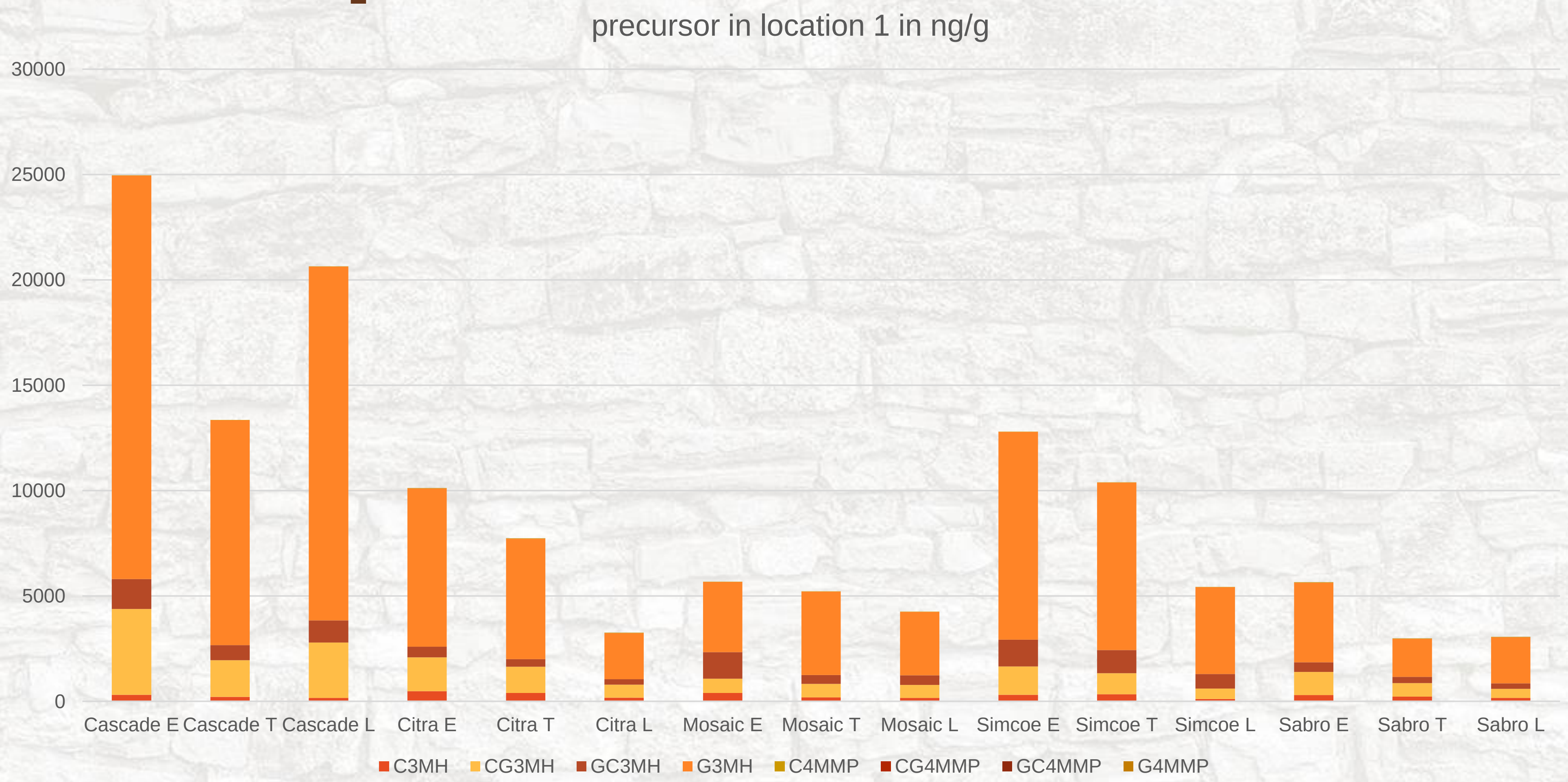
Data from various studies of Collin et al and Dagan et al

Precursors in malt and hops



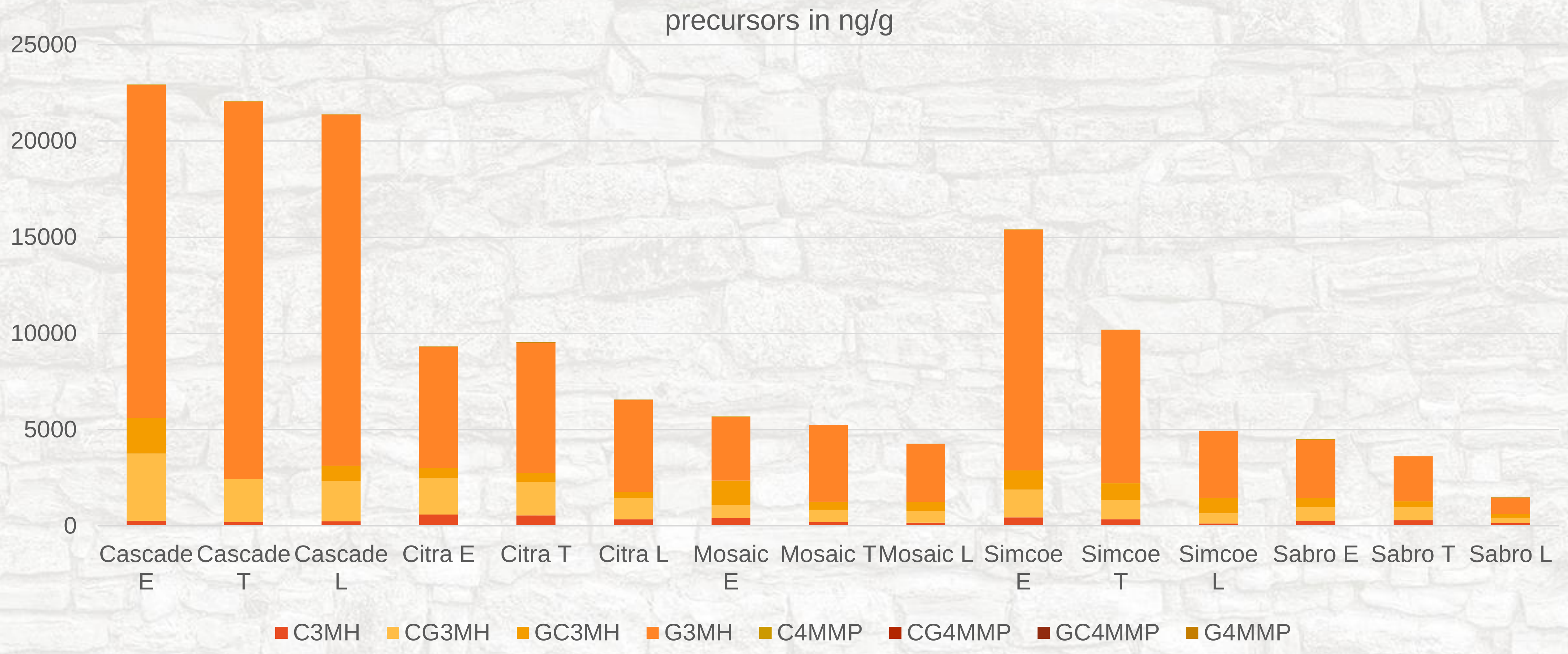
Dagan, L.: Brewing Summit 2018

Amount of precursors of 3MH at Location 1

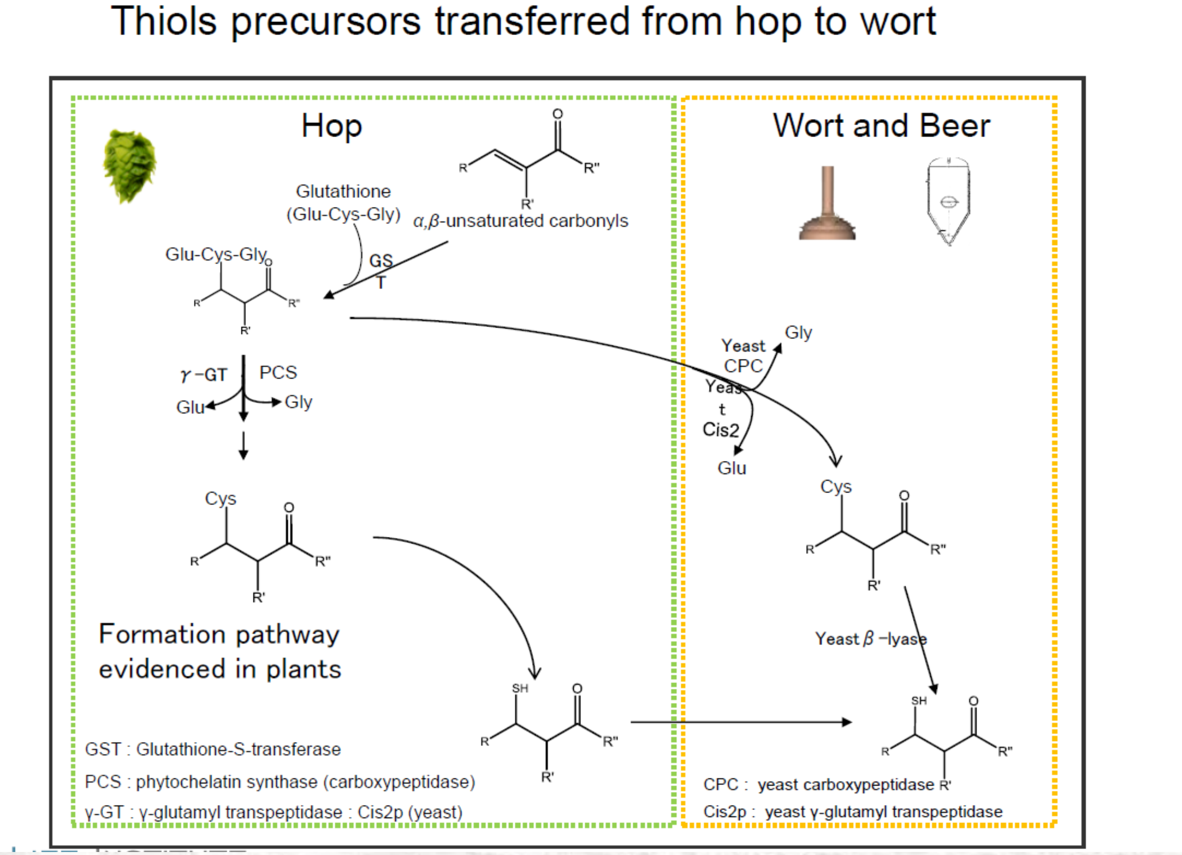


- Factor 1000 between free thiols and Gluthathion precursors
- No or very little precursor structurs of 4 MMP detected

Amount of precursors of 3MH at Location 2



Possibilities for thiol release in brewing



Kankolongo, EBC, 2015

Various enzymes are needed for thiol release:
Glutathion-S-transferase, Carboxypeptidase, Beta lyase
Some precursor structures are present 1000 times the free thiols, high potential for flavour (good and bad)

<u>Sources for enzymes:</u>
Hops
Malt
<u>Commercial Enzyme preparations:</u>
Brewing Yeasts
Wine yeasts
Wild yeasts
Other

Precentage of release from wine research up to 10%
For brewing 0,3% (Dagan)

Questions for Brewing

- How can we release thiols from precursor structures during brewing in a practical/efficient way?
- Under which conditions are the enzymes needed for this process most efficient, how can we translate this to common brewing practice?
- How would this impact hop aroma intensity and quality – will quality change?
- It must be practical for the brewers (work with brewing yeasts, work with enzymes, avoid mixed fermentations, avoid 2nd fermentations)

Conclusion and Outlook

- Location has a small impact on analytical values of thiols
- The sensory profile is influenced by harvest time and location, this is variety dependend
- Weak correlation of 3M4MP and Citrus and 4MMP and Berries & Currants

- Work with hops high in precursors (Cascade) and high in free thiols (Sabro), plus Citra and Mosaic, 4-6 hop samples
- Work with Standard Ale Yeast, with identified Lager yeast from previous trials with TUM (D308), and other Yeasts
- Combination with available commercial enzymes?
- Time point: Dry hop addition at end of main fermentation (1 time point)

Thanks to

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Thanks to the Brewing Solution Team of BarthHaas

Thanks to the John I Haas Team for providing the Hop Samples

Thanks to passionate scientists and brewers that help to discover the power of thiols