

The hunt for hoppy compounds

Formation and flavor-activity of hop-derived sesquiterpene oxidation products

Tatiana Praet, Filip Van Opstaele, Guido Aerts and Luc De Cooman



Master Brewers Association of the Americas
Dedicated to the technology of brewing.
MBAA Annual Conference



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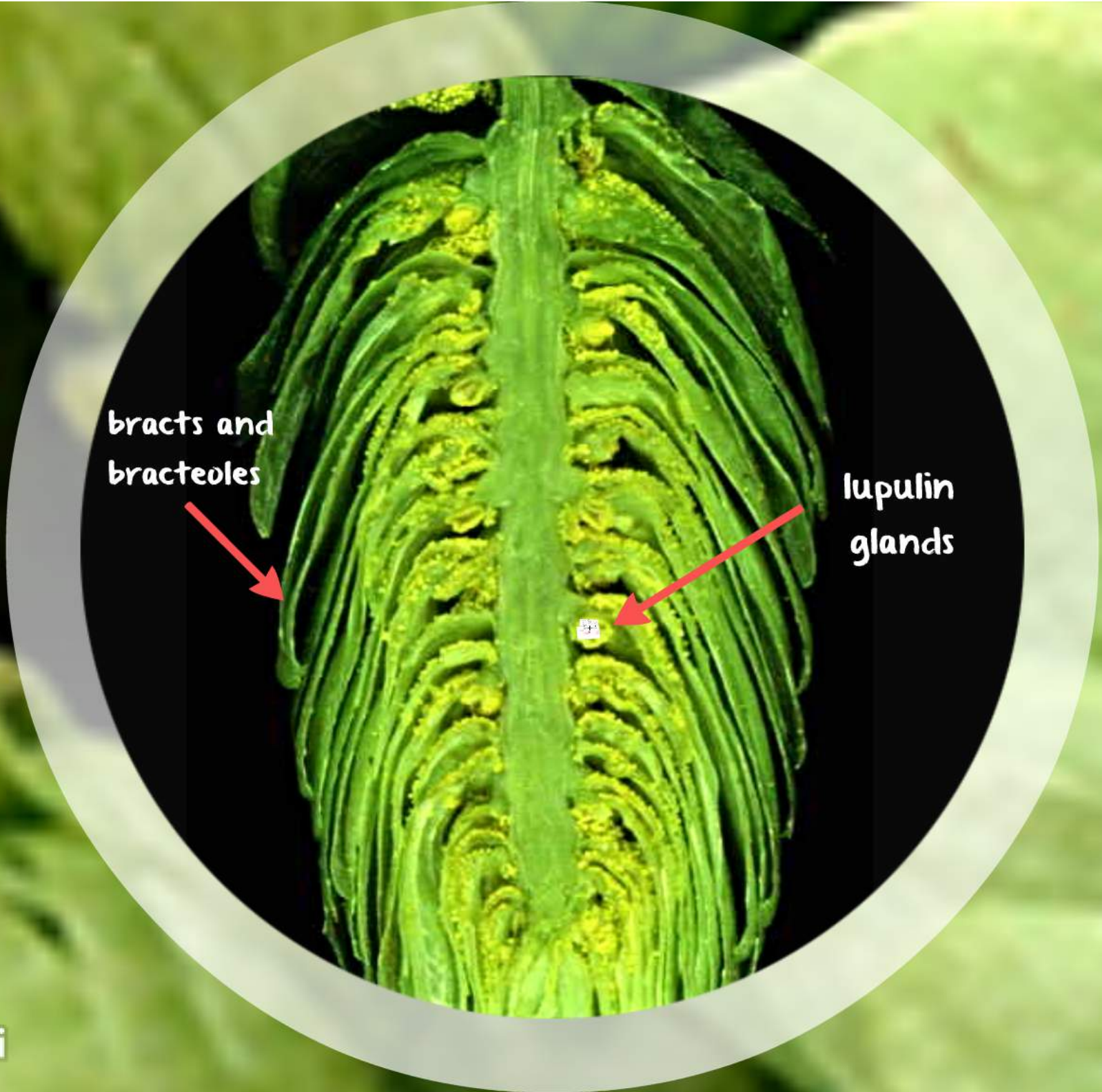


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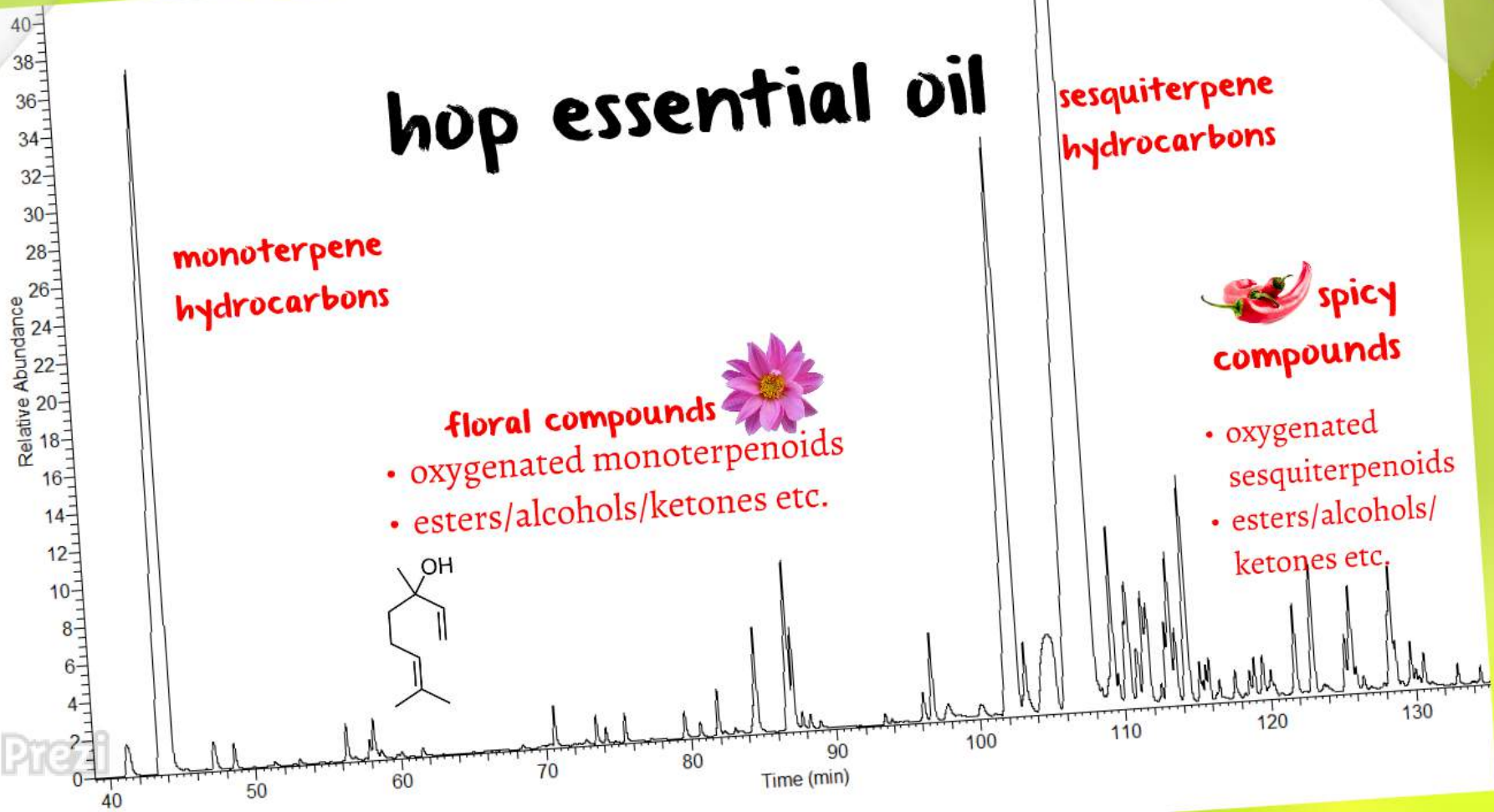
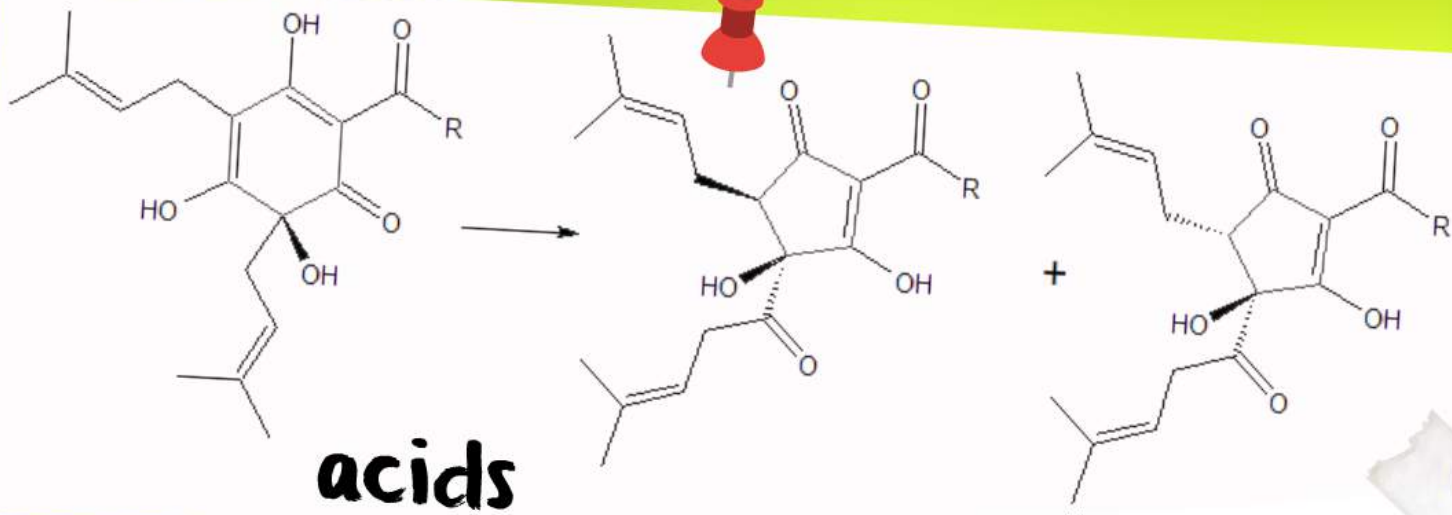
RAW MATERIALS





bracts and
bracteoles

lupulin
glands



Prezi

the brewing process

kettle hopping

- addition of hops to wort in late
- increases and extends
- head of foam
- reduces bitterness
- enhances aroma
- improves mouthfeel
- improves stability
- improves clarity
- improves color
- improves head retention
- improves head retention
- improves head retention



dry hopping

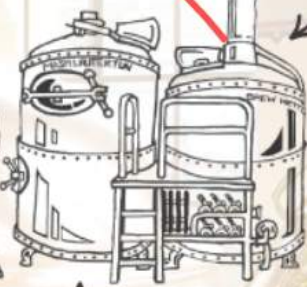
- addition of hops to wort late in the brewing process
- adds to aroma
- treated hops of hop compounds
- no formation of isohumulone products
- increases head retention
- improves mouthfeel
- improves clarity
- improves head retention
- improves head retention
- improves head retention



1. The grain mill



2. The mash tun



3. The kettle



4. Fermentation



5. Conditioning



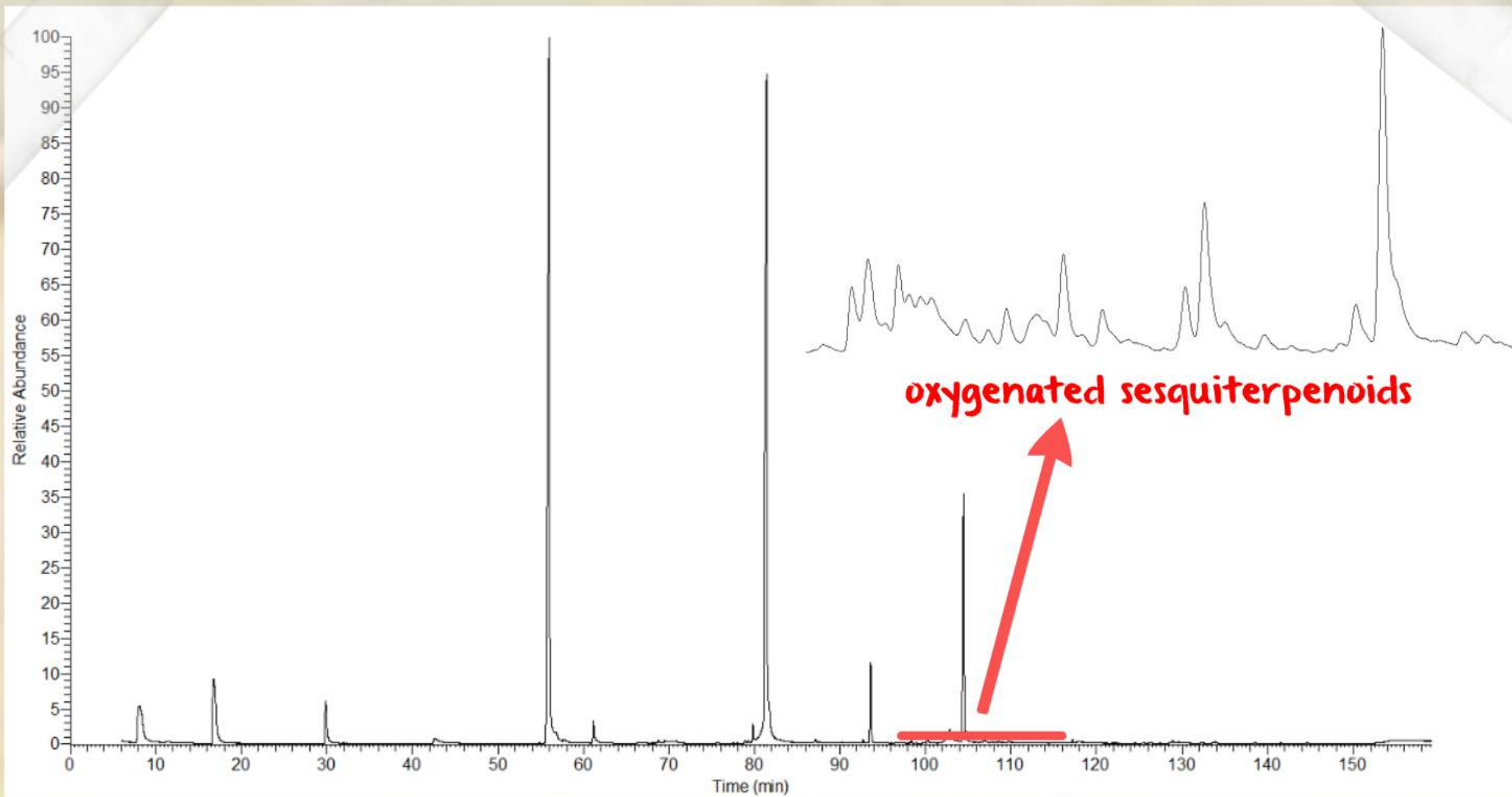
kettle hopping



- addition to **kettle** (early vs late)
- bitterness and **aroma**
- **loss of hop oil volatiles** by stripping
- **conversion** terpene hydrocarbons into **oxygenated terpenoids** by oxidation
- oxygenated derivatives have a **higher probability to survive the brewing process**: are found in the final beer
- kettle hop or noble/spicy/European hop aroma, linked with noble European varieties
- especially important in **LAGER BEERS**



GC-MS profile of kettle hopped commercial lager beer



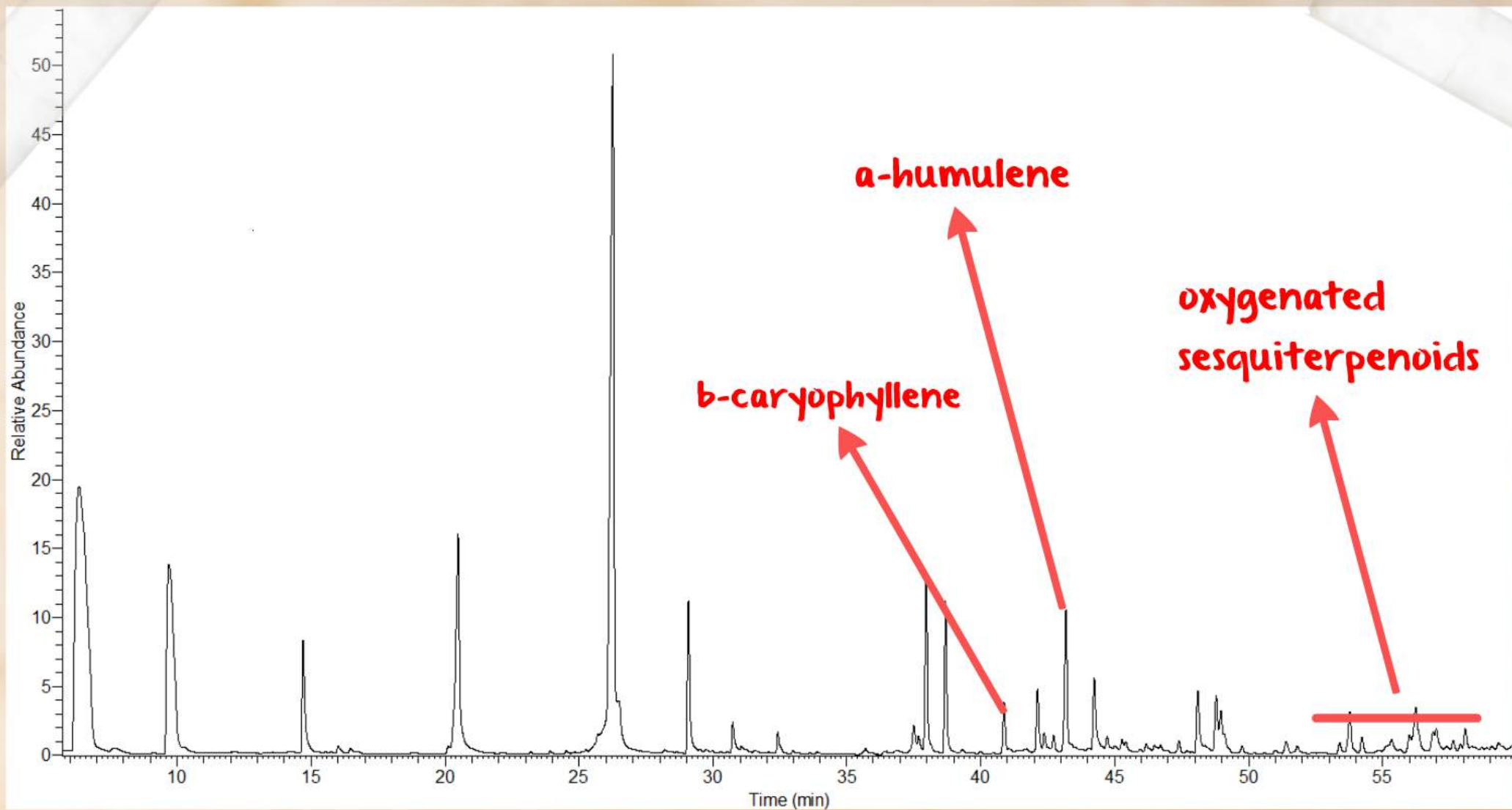
- fermentation derived esters and alcohols
- oxygenated sesquiterpenoids (correlation hoppy/spicy aroma)

dry hopping



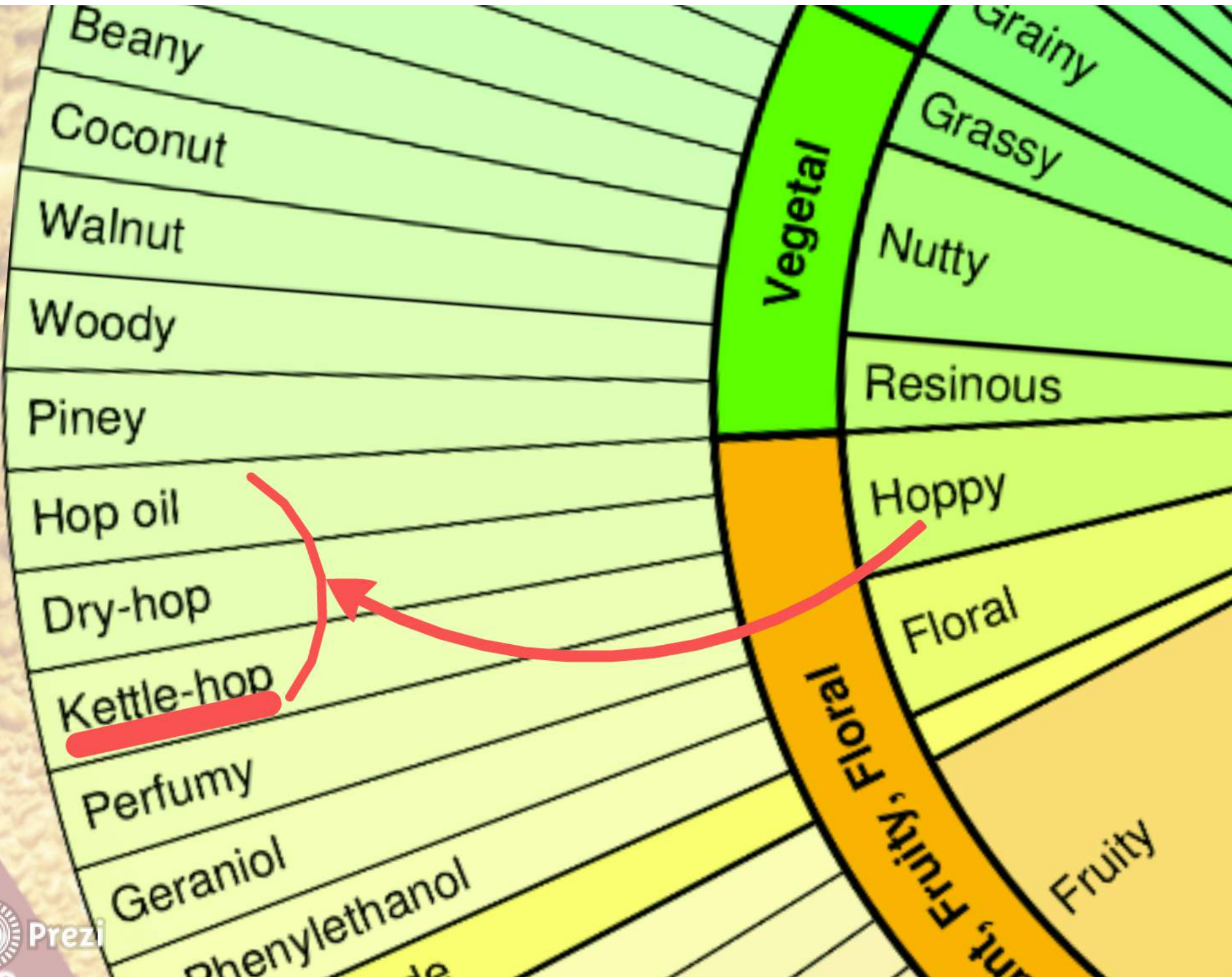
- addition **end of primary fermentation** or later in the brewing process
- only for **aroma**
- limited losses of hop oil compounds
- **no** formation of **oxidation** products
- the **variety** used will determine the dry hop aroma: important for **diversification** of USA craft beers
- aroma reminiscent of aroma of hops (fruity, citrus, even exotic!)
- important in **ALES**

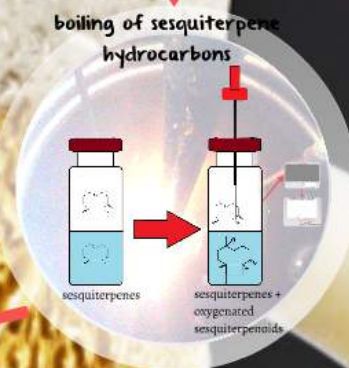
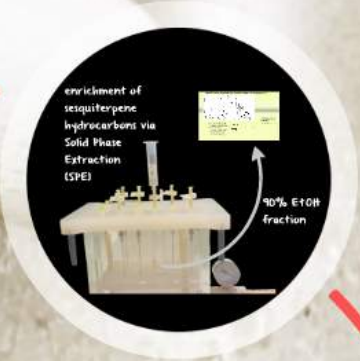
GC-MS profile of dry hopped commercial ale beer



- more fermentation derived esters and alcohols
- dry hopping: less losses hop oil compounds, detection without enrichment







goals

Boiling of sesquiterpene
hydrocarbons:
formation of key aroma
impact compounds,
related to hoppy
aroma?

. fermentation

. flavor activity

. isolation

. identification

. transformation
sesquiterpenes into
oxygenated derivatives

Boiling of sesquiterpene

hydrocarbons:

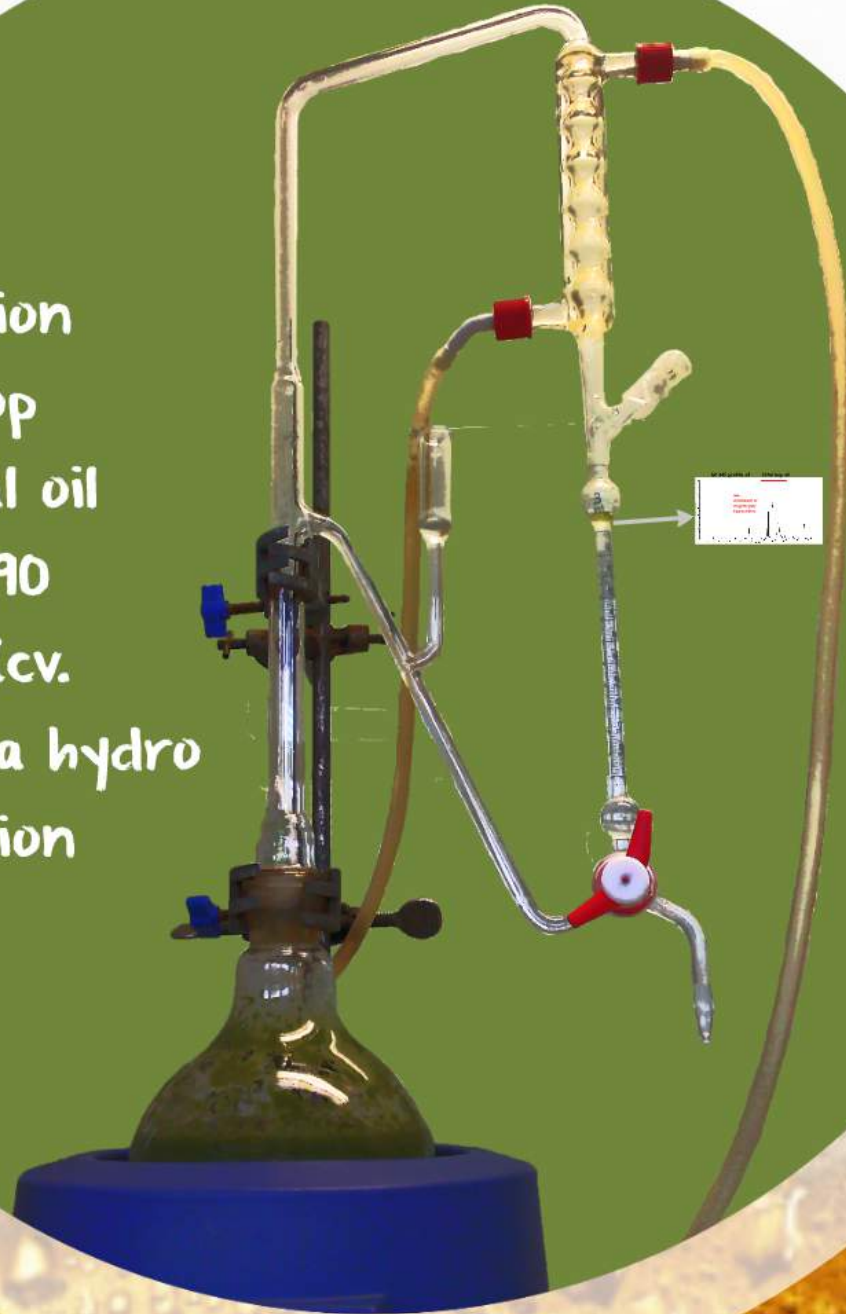
formation of key aroma

impact compounds,

related to hoppy

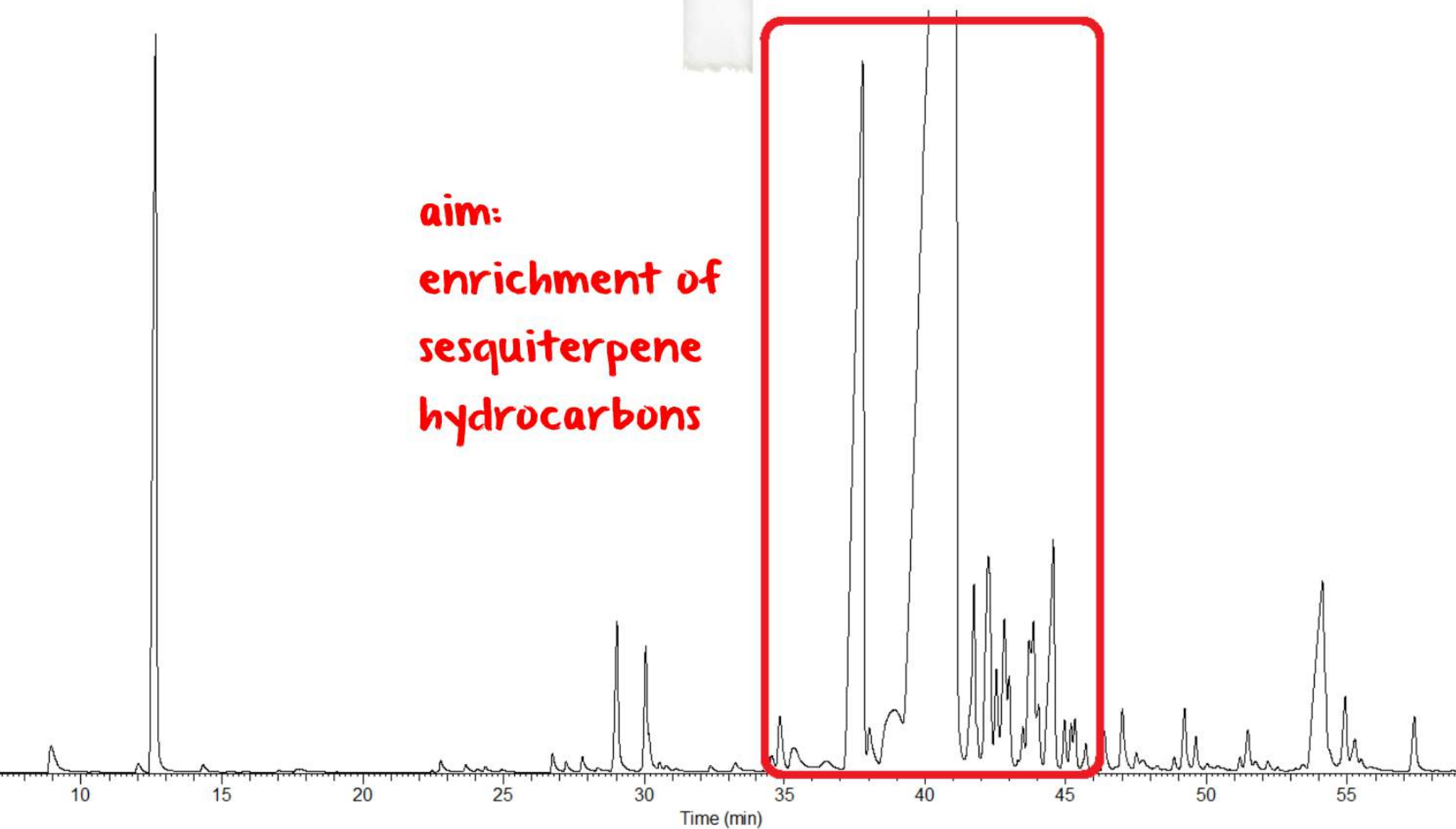
aroma?

extraction
total hop
essential oil
from T90
pellets (cv.
Saaz) via hydro
distillation

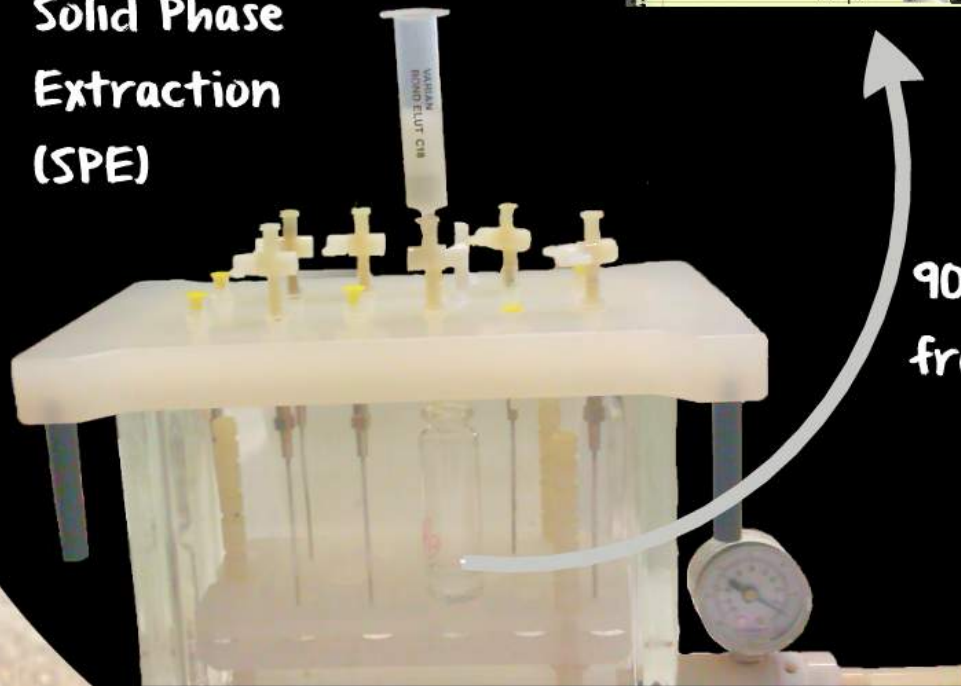
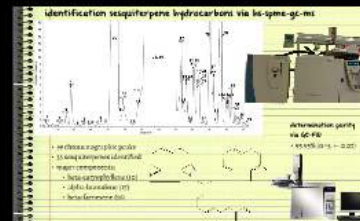


GC-MS profile of total hop oil

aim:
enrichment of
sesquiterpene
hydrocarbons

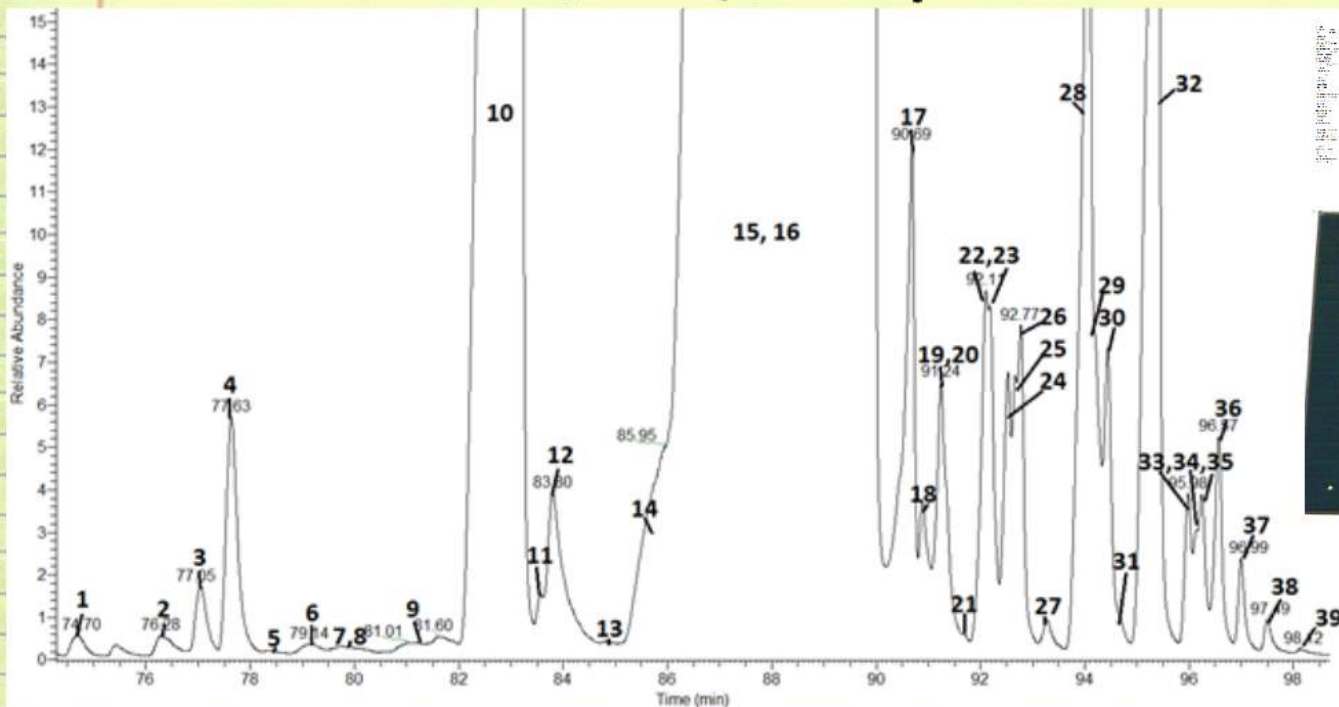


enrichment of
sesquiterpene
hydrocarbons via
Solid Phase
Extraction
(SPE)



90% EtOH
fraction

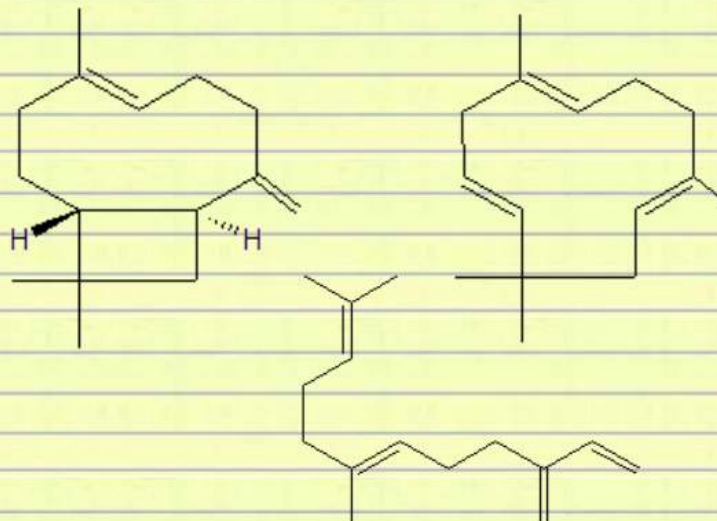
identification sesquiterpene hydrocarbons via hs-spme-gc-ms



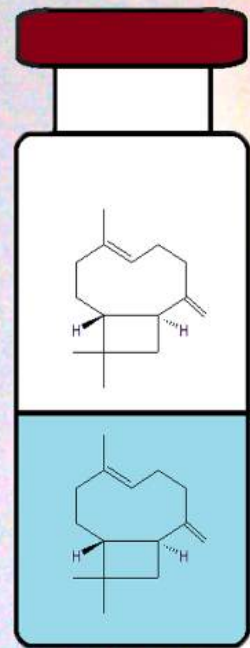
determination purity via GC-FID

• 95.95% (n=3, +/- 0.07)

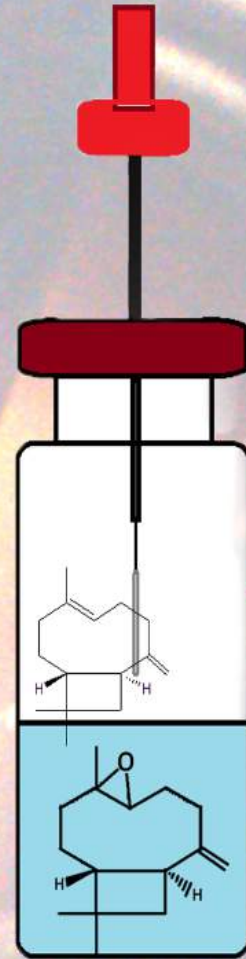
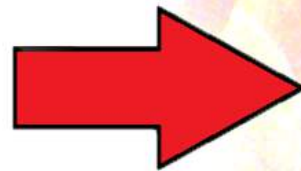
- 39 chromatographic peaks
- 35 sesquiterpenes identified
- major components:
 - beta-caryophyllene (10)
 - alpha-humulene (15)
 - beta-farnesene (16)



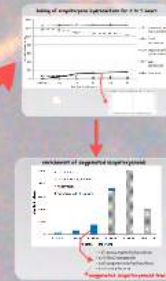
boiling of sesquiterpene hydrocarbons



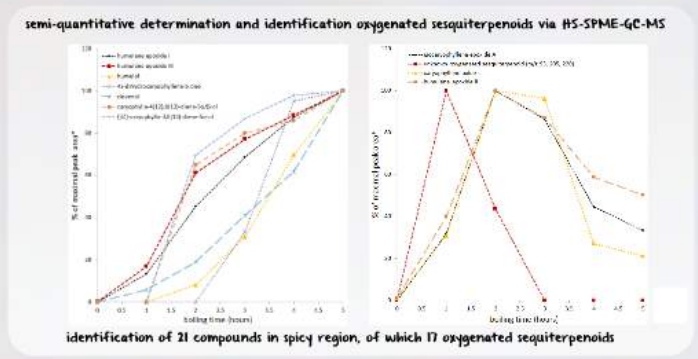
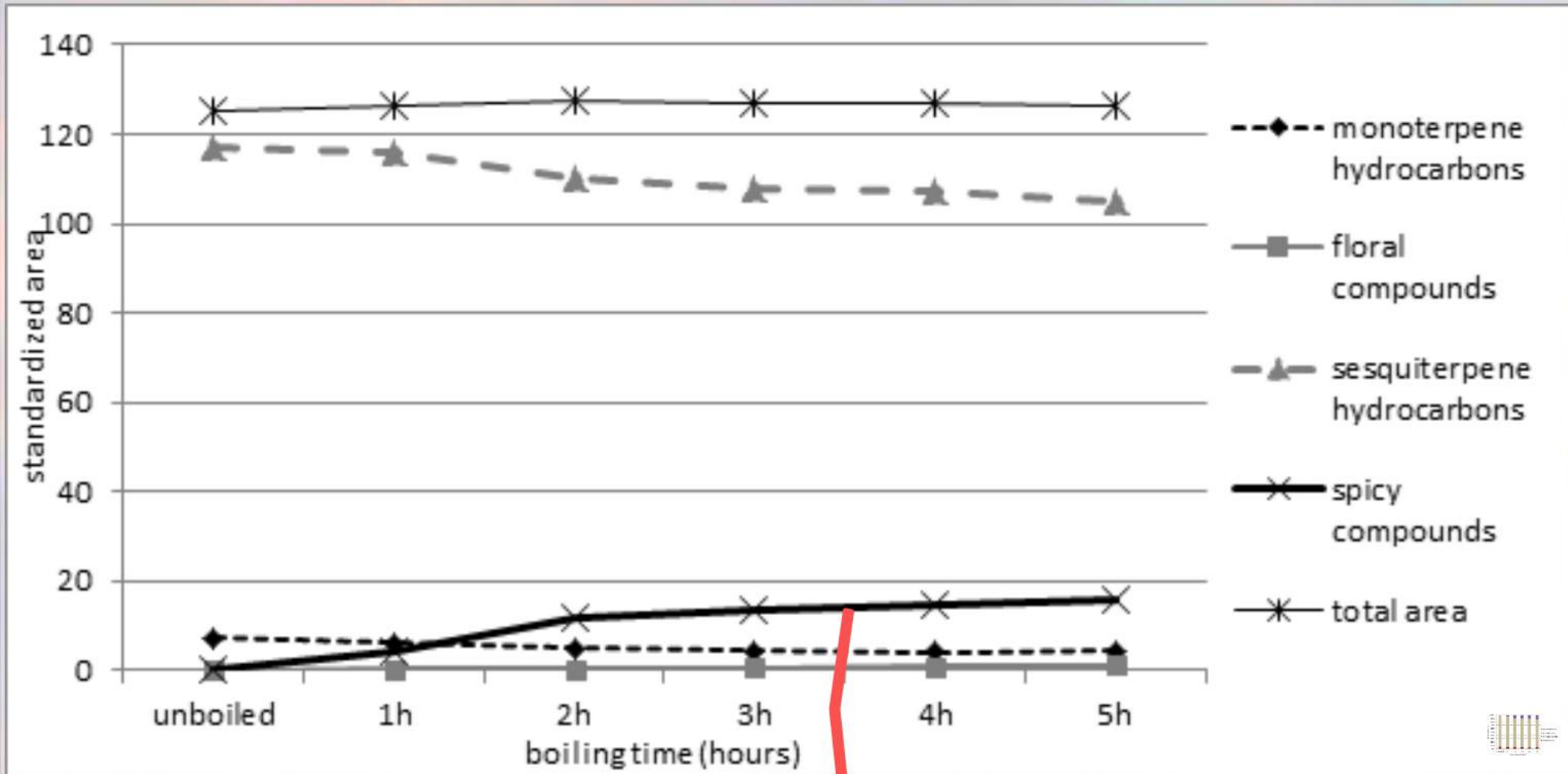
sesquiterpenes



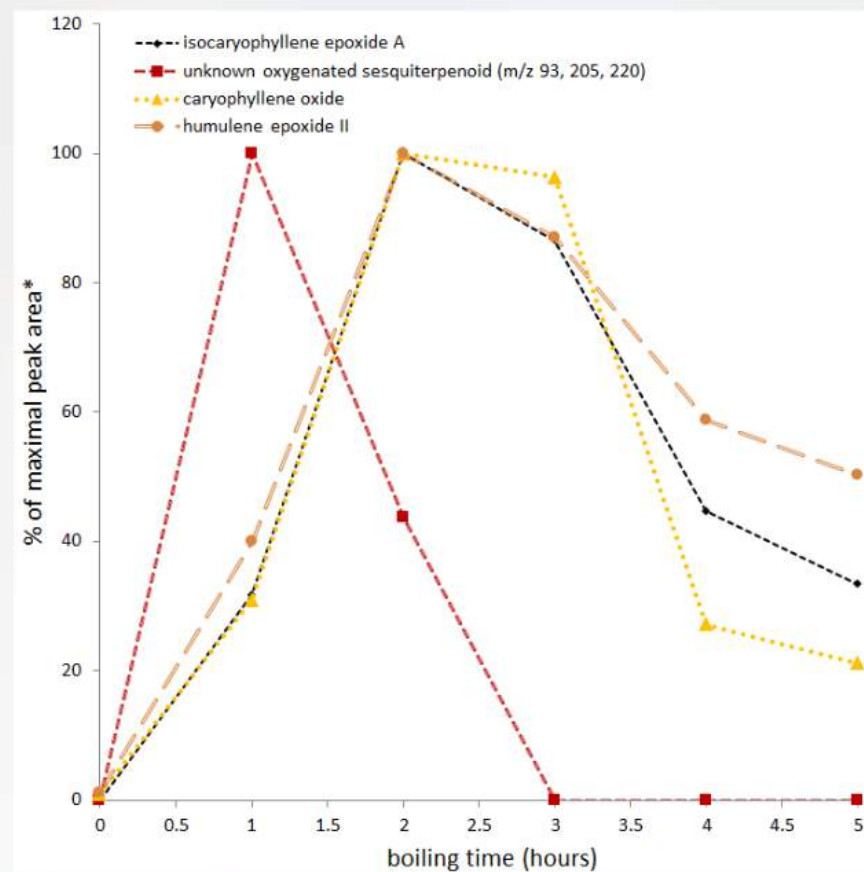
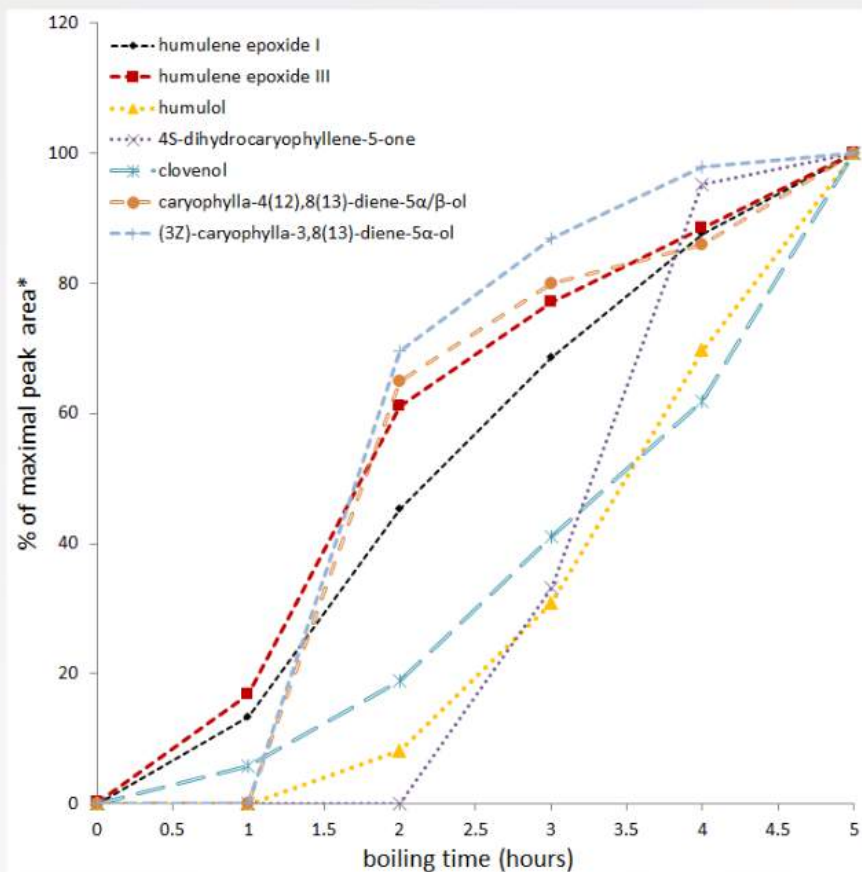
sesquiterpenes +
oxygenated
sesquiterpenoids



boiling of sesquiterpene hydrocarbons for 0 to 5 hours

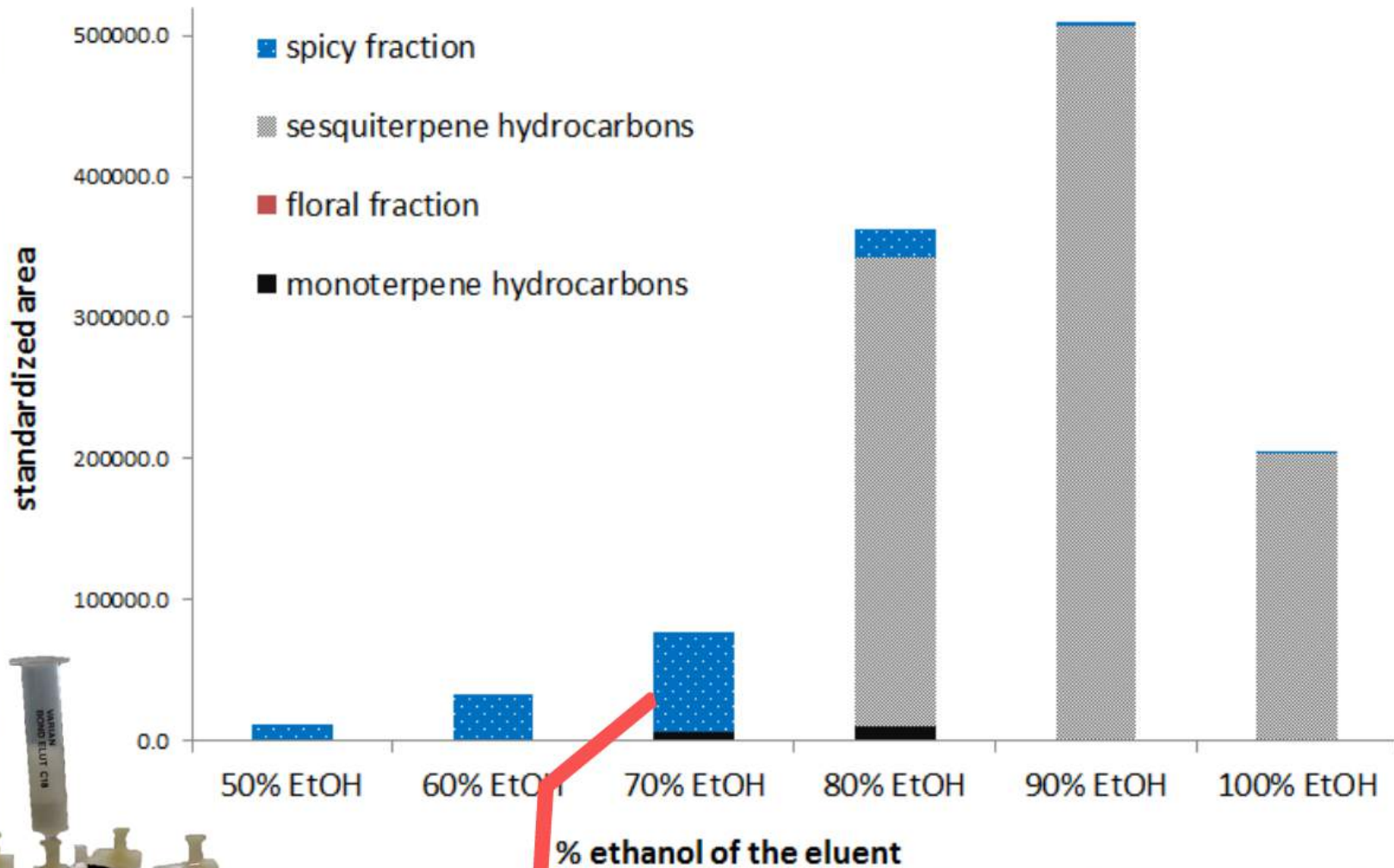


semi-quantitative determination and identification oxygenated sesquiterpenoids via HS-SPME-GC-MS



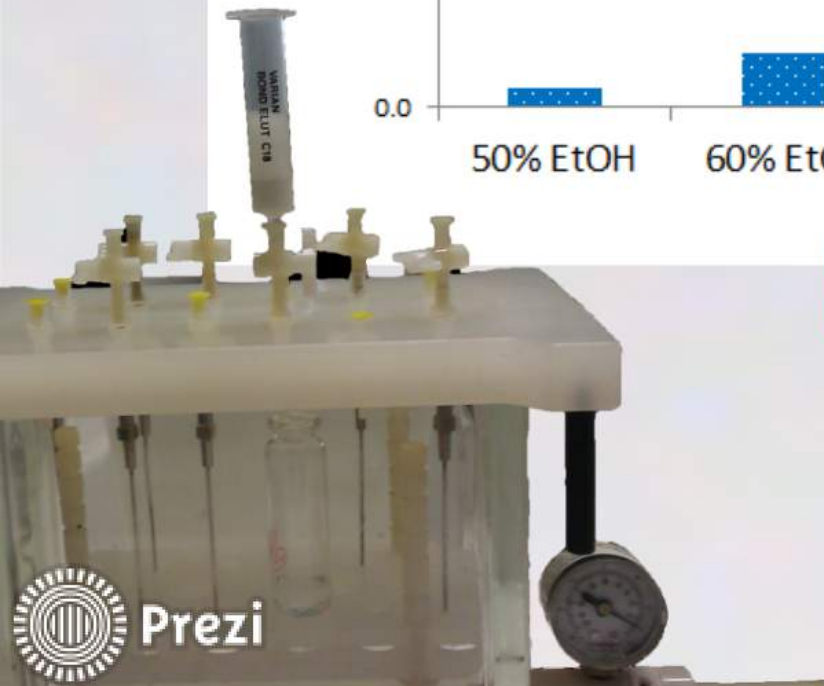
identification of 21 compounds in spicy region, of which 17 oxygenated sesquiterpenoids

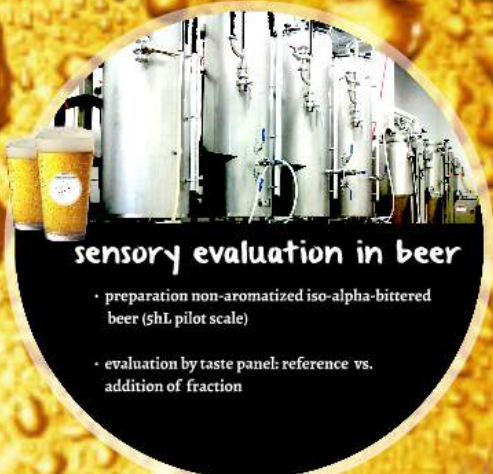
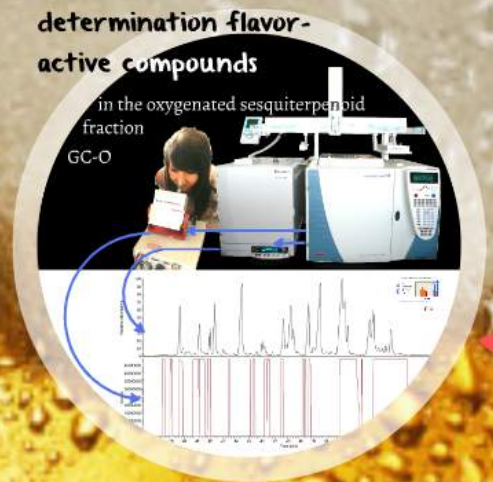
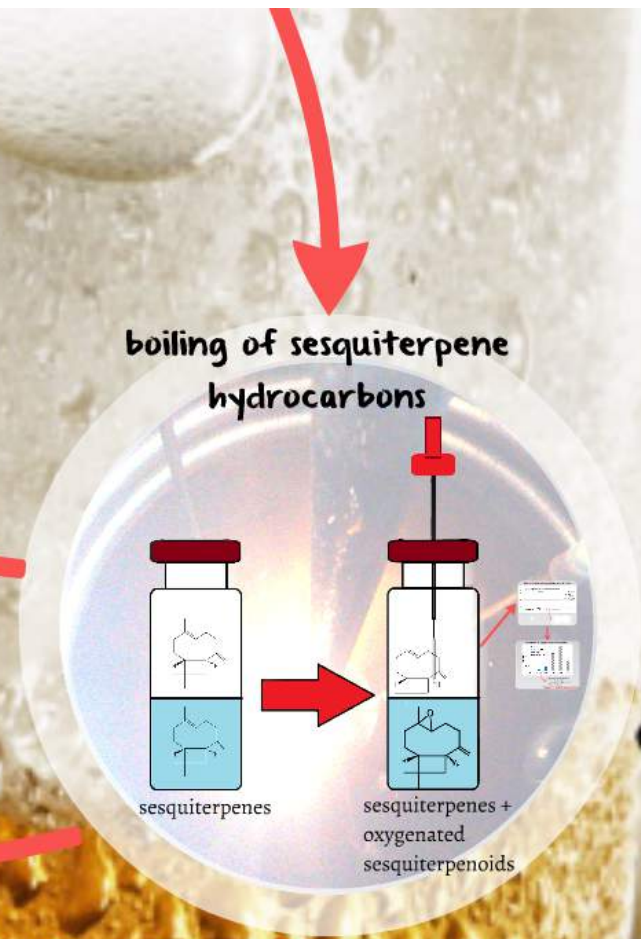
enrichment of oxygenated sesquiterpenoids



- 7.6% monoterpene hydrocarbons
- 0.3% floral compounds
- 0.0% sesquiterpene hydrocarbons
- 92.2% spicy fraction

oxygenated sesquiterpenoid fraction

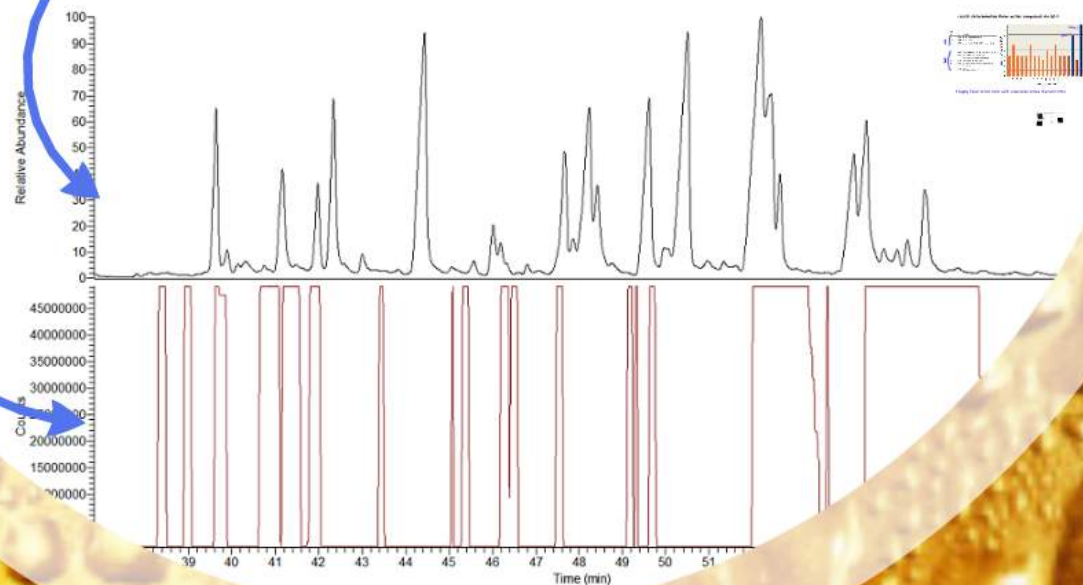




determination flavor-active compounds

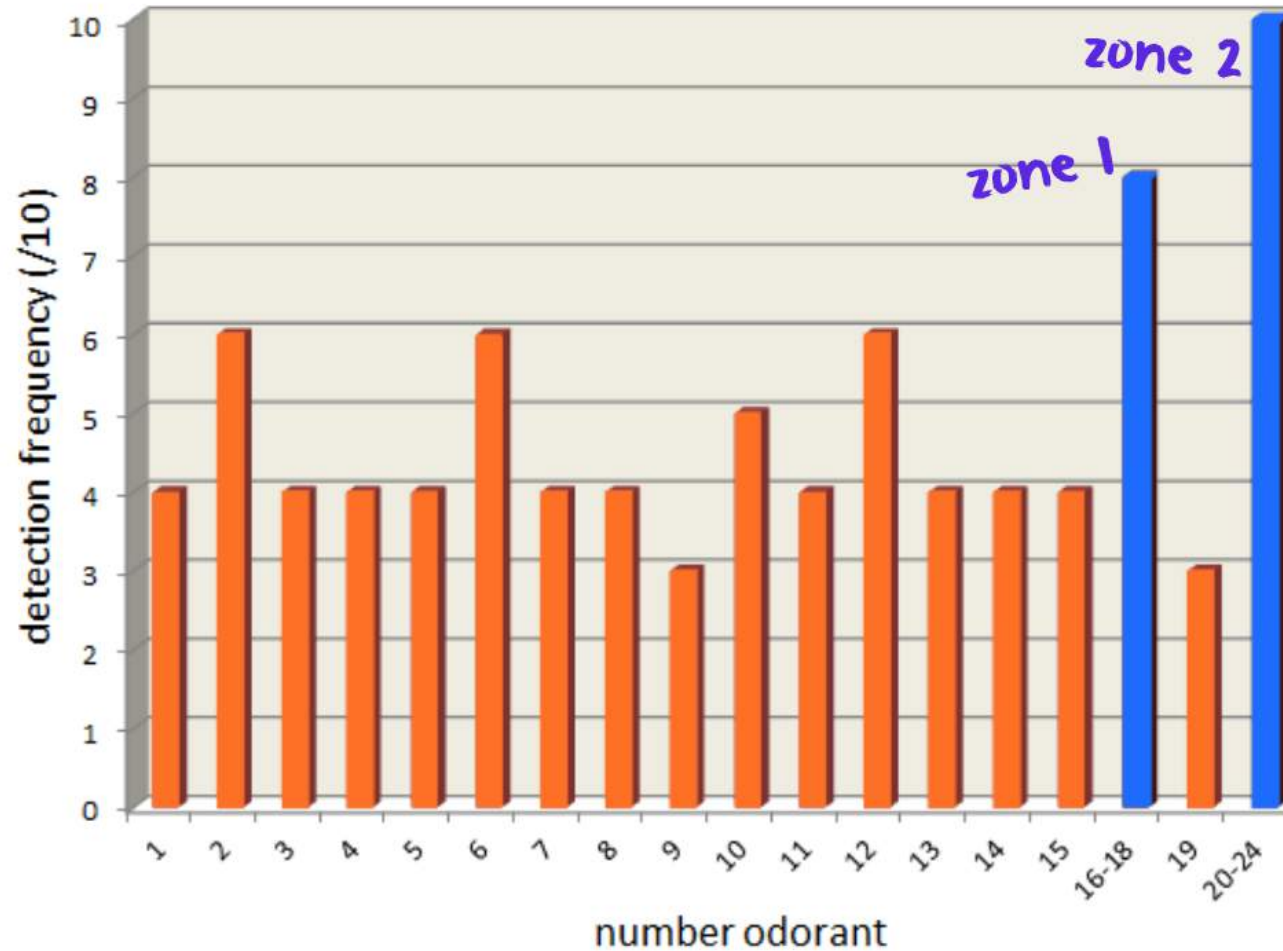
in the oxygenated sesquiterpenoid fraction

GC-O



results determination flavor-active compounds via GC-O

peak n°	RI	odorant
16	1602	humulene epoxide III
17	1604	humulenol II
18	1607	caryophylla-4(12),8(13)-diene-5 α / β -ol
20	1628	(3Z)-caryophylla-3,8(13)-diene-5 α -ol
21	1630	unknown (m/z 79,80,81) 14-hydroxy- β -caryophyllene
22	1639	unknown (m/z 93, 137)
23	1641	(3Z)-caryophylla-3,8(13)-diene-5 β -ol cadalene
24	1644	(6Z)-pentadecen-2-one



2 highly flavor active zones with cedarwood aroma characteristics

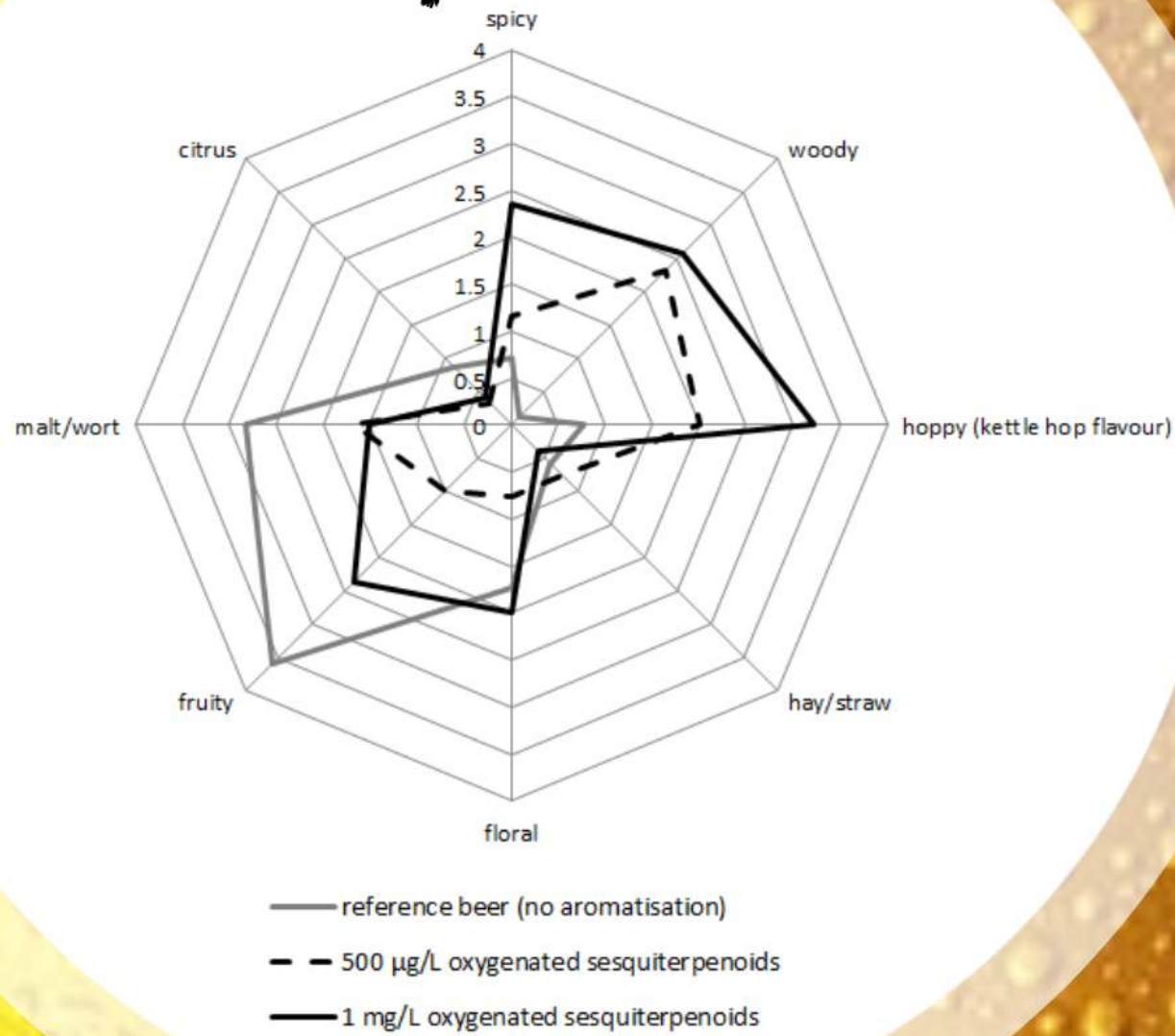


sensory evaluation in beer

- **preparation non-aromatized iso-alpha-bittered beer (5hL pilot scale)**
- **evaluation by taste panel: reference vs. addition of fraction**

results

sensory evaluation

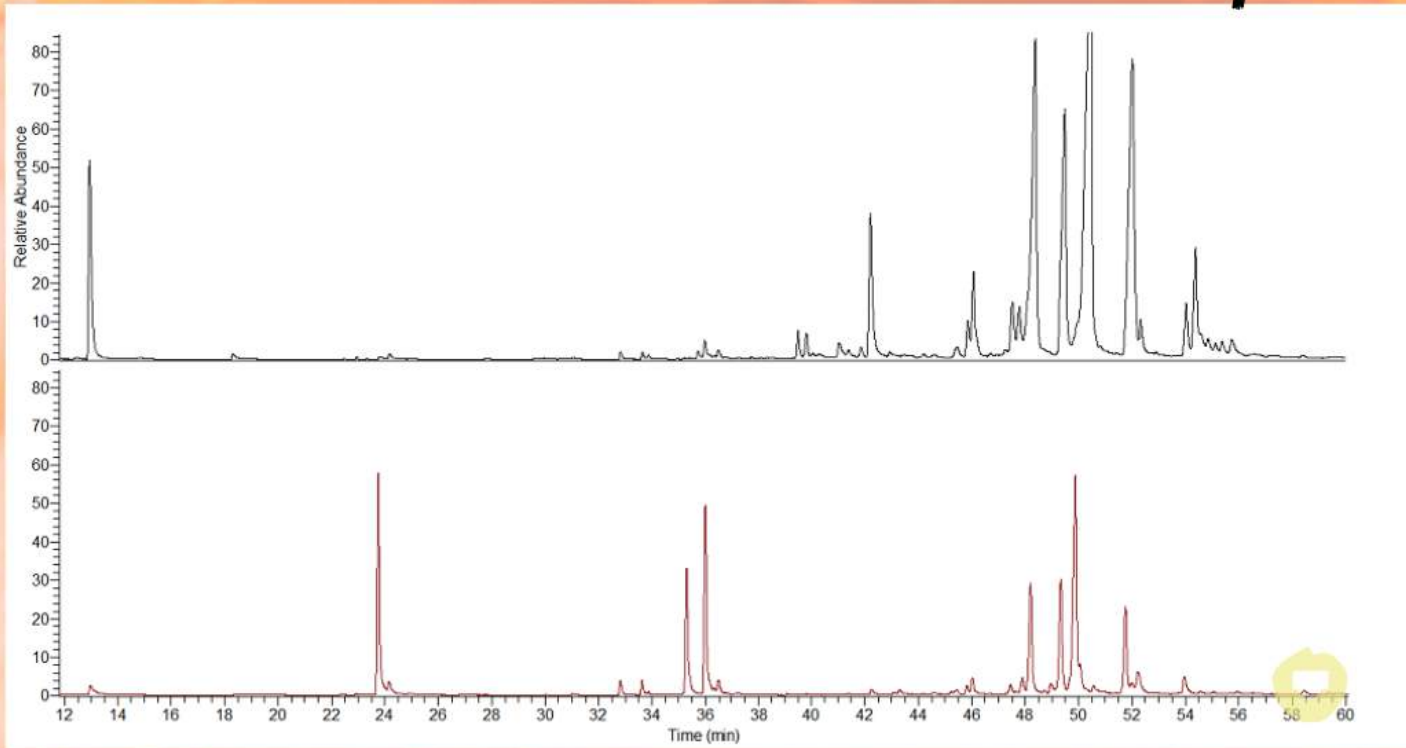


fermentation experiments



- non-hopped wort brewed at pilot scale (5 hL)
- lab scale fermentation in the bottle (bottom fermenting yeast) of pitching wort, spiked with the oxygenated sesquiterpenoid fraction

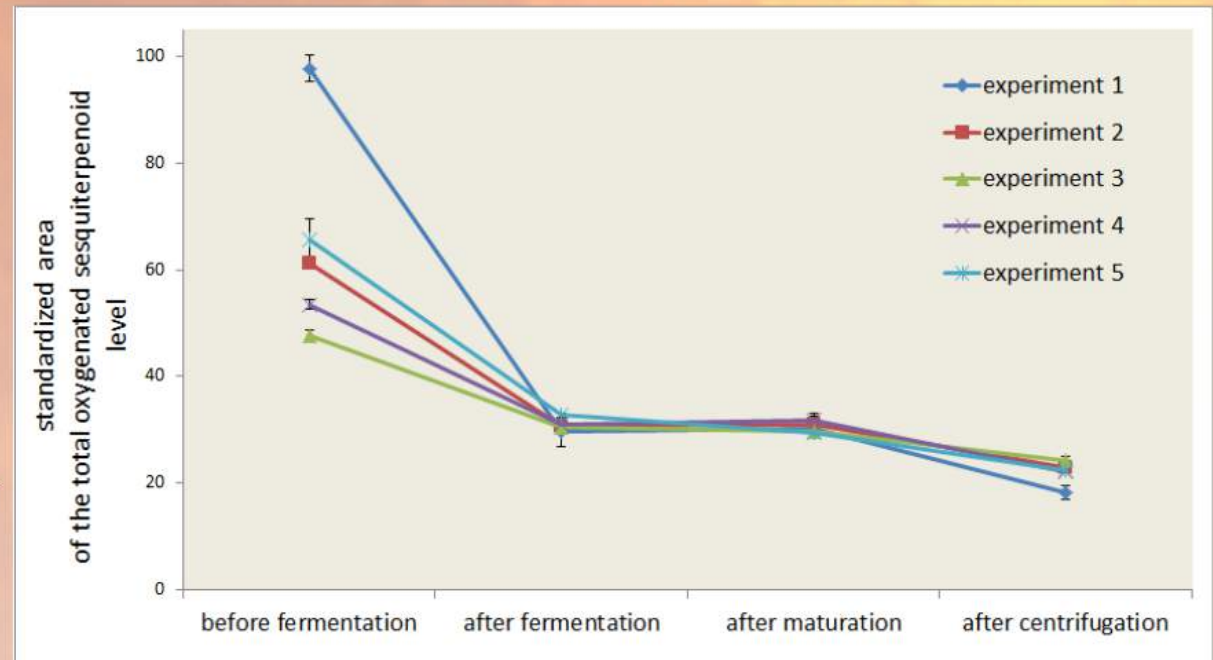
HS-SPME-GC-MS analysis



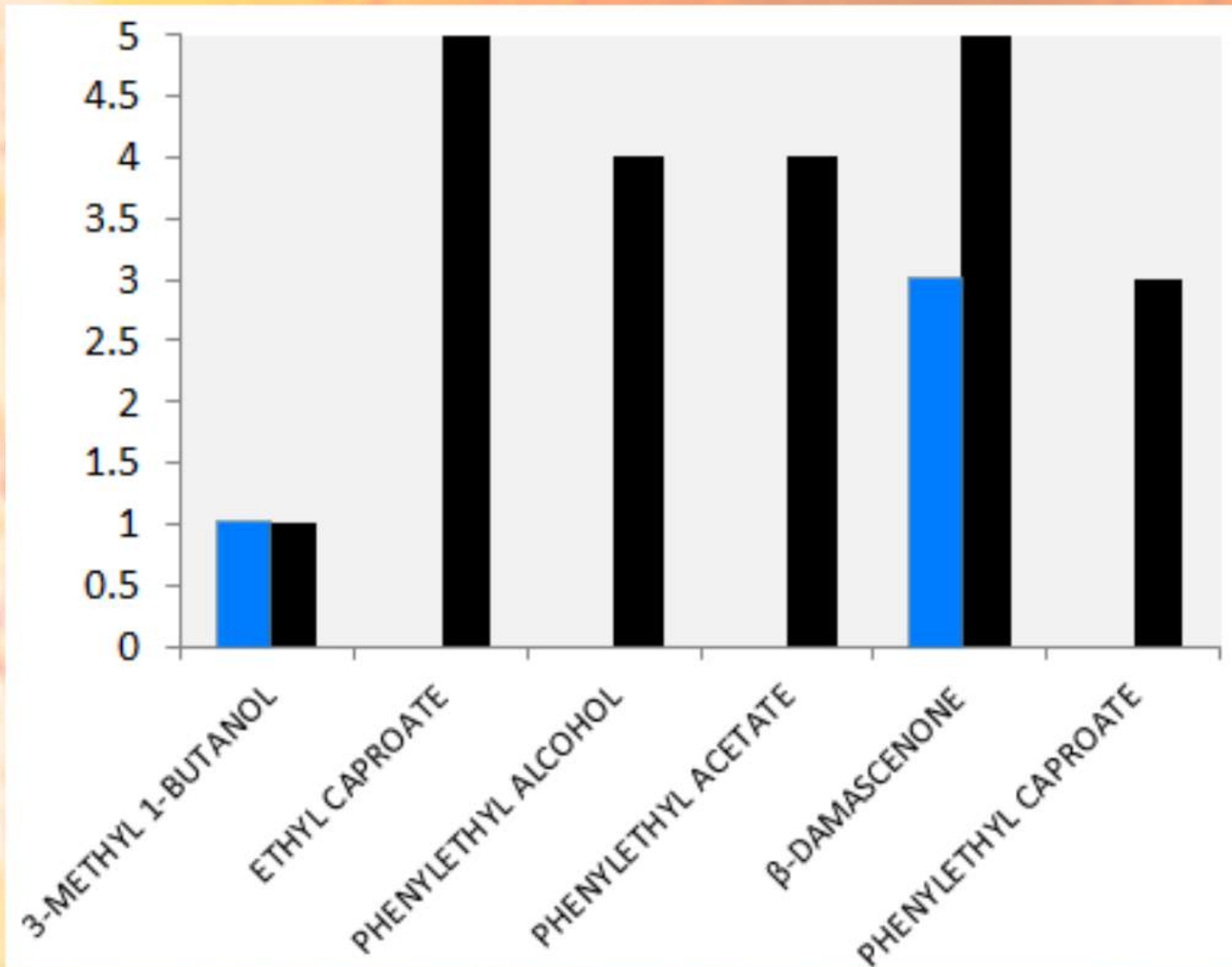
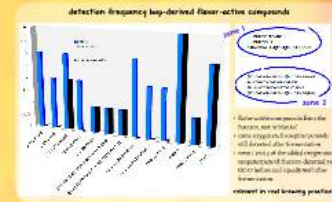
before
fermentation

after
fermentation

- decrease in oxygenated sesquiterpenoid level (adsorption to yeast)
- no new hop-derived compounds detected, except for 1 unknown

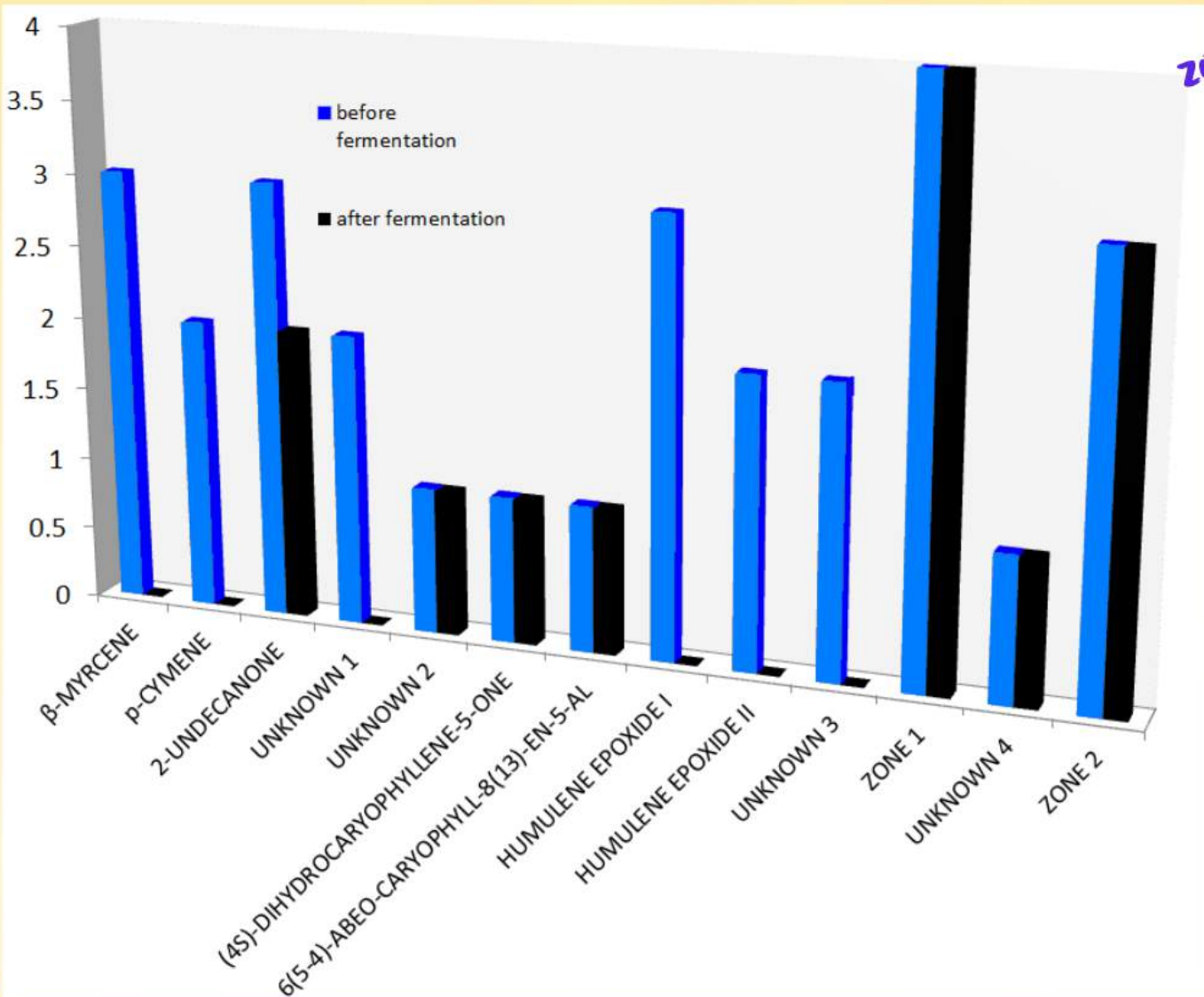


GC-O analysis of samples before vs. after fermentation



- 5 assessors
- sniffing of blanks (without oxygenated sesquiterpenoid fraction): no hop-derived compounds in blanks
- 32 unknowns (detected with GC-O but not with GC-MS)
- flavor-active fermentation products, also found in blanks

detection frequency hop-derived flavor-active compounds



zone 1

HUMULENE EPOXIDE III
HUMULENOL II
CARYOPHYLLA-4(12),8(13)-DIENE-5-OL

(3Z)-CARYOPHYLLA-3,8(13)-DIENE-5 α -OL
14-HYDROXYCARYOPHYLLENE
EPI-14-HYDROXYCARYOPHYLLENE
(3Z)-CARYOPHYLLA-3,8(13)-DIENE-5 β -OL

zone 2

- flavor-active compounds from the fraction, not in blanks!
- some oxygenated sesquiterpenoids still detected after fermentation
- zone 1 and 2 of the added oxygenated sesquiterpenoid fraction detected via GC-O before and equally well after fermentation:

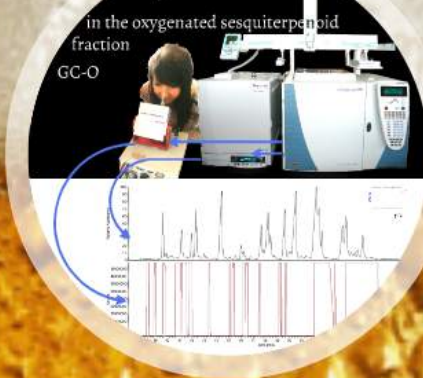
relevant in real brewing practice?

fermentation experiments



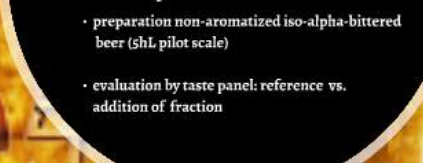
- non-hopped wort brewed at pilot scale (5 hL)
- lab scale fermentation in the bottle (bottom fermenting yeast) of pitching wort, spiked with the oxygenated sesquiterpenoid fraction

determination flavor-active compounds

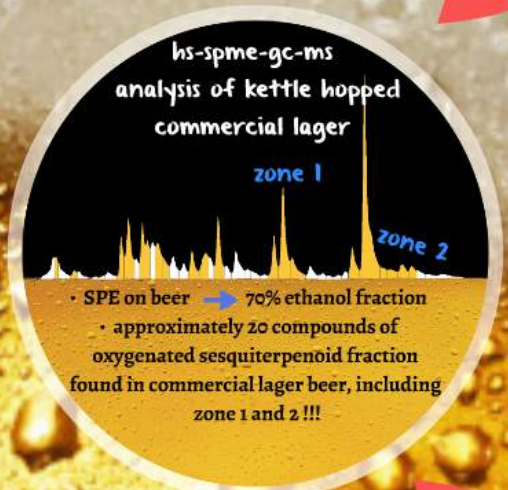


in the oxygenated sesquiterpenoid fraction
GC-O

sensory evaluation in beer



- preparation non-aromatized iso-alpha-bittered beer (5hL pilot scale)
- evaluation by taste panel: reference vs. addition of fraction



hs-spme-gc-ms analysis of kettle hopped commercial lager

zone 1
zone 2

- SPE on beer → 70% ethanol fraction
- approximately 20 compounds of oxygenated sesquiterpenoid fraction found in commercial lager beer, including zone 1 and 2 !!!

hs-spmc-gc-ms
analysis of kettle hopped
commercial lager

zone 1

zone 2

- SPE on beer → 70% ethanol fraction
- approximately 20 compounds of oxygenated sesquiterpenoid fraction found in commercial lager beer, including zone 1 and 2 !!!

conclusions

- formation oxygenated sesquiterpenoids when boiling sesquiterpene hydrocarbons
- series of flavor-active oxygenated sesquiterpenoids in novel fraction
- addition fraction to beer: 'hoppy' 'woody' 'spicy'
- decrease in level during fermentation, but still high flavor-activity of several humulene derivatives and caryophyllene derived alcohols (zone 1 and 2)
- potential for introducing kettle hop aroma after fermentation?

acknowledgements



RESERVE YOUR
SPACE HERE



BARTH-HAAS GROUP

KU LEUVEN



KAHO
SINT-LIEVEN

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Prof. Luc De Cooman
Prof. Guido Aerts

MSc. Nedelina Nikolova
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