TRACKING HOP AROMA COMPOUNDS WITHIN HOP VARIETIES AND BEER

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PRESENTATION OVERVIEW

- Hop Aroma Compounds
- Hop Aroma Compound Extraction and Analysis Techniques
- Analysis Instrumentation for 2018 Harvest
- 2018 Harvest Hop Aroma Data
- Beer Analysis Data
- Conclusions
- New Analysis Instrumentation for 2019 Harvest

CURRENTLY REPORTED

- Total oil 0.5 – 3.0% reported as ml/100 g
- Reported as % Area of Chromatogram
  - Qualitative representation of the oil composition by GC
  - β-Pinene – 0.1-2.0%
  - Myrcene-most abundant 20-60%
  - Linalool – 0.1 – 1.0%
  - Caryophyllene – 5-20%
  - Humulene- 5-20%
  - Farnesene – 0-10%
  - Geraniol – 0.1-1.0%

HOP AROMA

- Total Oil Content in Hops 0.2 - 3%
- Hop Aroma
  - Perceived Hop Aroma depends on oil composition
    - Hundreds of compounds contribute to hop aroma
  - Factors that affect hop aroma composition
    - Variety (genetics)
    - Growing Region
    - Agronomics
    - Ripeness
    - Kilning
    - Processing
    - Storage

HOP AROMA IN BEER

- Hop aroma perceived in beer is not the same as hop aroma perceived in hops
- Factors that affect extraction of hop aroma compounds in beer include:

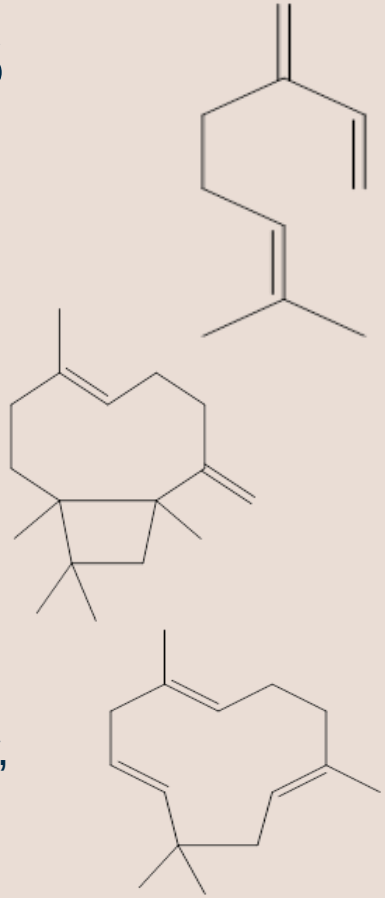
- Contact with the liquid
- Chemical Structure of the Hop Compound
  - Polar and nonpolar
- When the hop addition occurs
  - Kettle
  - Late kettle or whirlpool
  - Dry hopping
- Beer Style
  - Yeast
  - pH
  - Gravity
  - Alcohol
  - Haze
  - Temperature

COMPOSITION OF HOP AROMA

- Nonpolar compounds – Hardest to extract
  - Terpenes (Hydrocarbons) 80% of hop aroma
    - Monoterpenes (C10's)
    - Sesquiterpenes (C15's)
- Aliphatic Hydrocarbons <1% (straight chains nonaromatic rings)
- Polar compounds – Easier to extract
  - Esters 15%
  - Carboxylic acid 1%
  - Terpene Alcohols1%
  - Sesquiterpene Oxides 1%
  - Aldehydes and Ketones 1%
  - Thiols (sulfur containing compounds)

MAJOR TERPENES

- Monoterpenes
  - Myrcene (most abundant in hops)
  - Aroma profile: herbal, spicy, geranium, woody, carrot
- Sesquiterpenes
  - β-Caryophyllene
    - Aroma profile: cedar, spicy, floral, turpentine
  - Humulene
    - Aroma profile: grassy, floral, spicy, woody, herbal



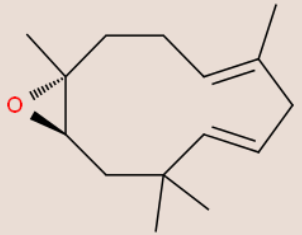
Dry Hopping extracts these compounds (pine, woody)

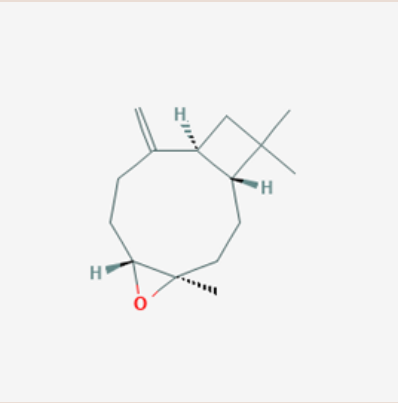
Late kettle and whirl pool very small amounts survive, some evaporate, some convert/oxidize to other compounds.

Lost in fermentation, from CO_2 Scrubbing or lost in biomass

SESQUITERPENE OXIDES

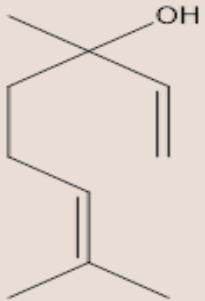
- Humulene Oxide
  - Aroma profile: musty, floral, spicy, woody, herbal
- Caryophyllene Oxide
  - Aroma profile: musty, floral, spicy, woody, dry
- Both compounds form during hop storage and are a sign of oxidized hops
  - Late kettle, whirl pool additions and dry-hopping known to bring spicy or noble character to beer

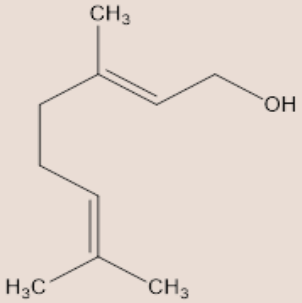


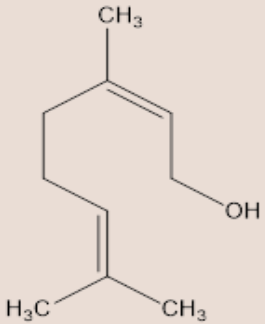


TERPENE ALCOHOLS

- Linalool
  - Aroma profile: floral, fruity, citrus, sweet
  - First hop oil discovered in beer
  - Survives the brewing process – kettle, whirl pool and dry hopping
- Geraniol
  - Aroma profile: geranium, floral, lemon, lime
  - Survives the brewing process
  - Involved in biotransformation
- Nerol
  - Aroma profile: floral, lemon, lime

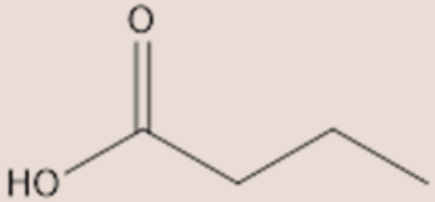


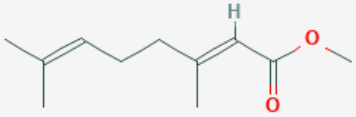




ESTERS AND CARBOXYLIC ACIDS

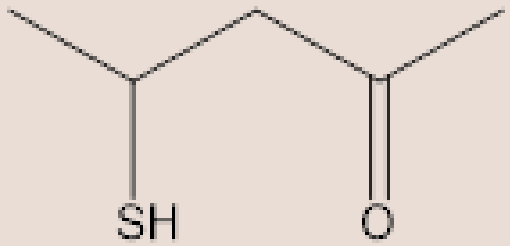
- Esters-Third most abundant compounds in hops15%
  - Aroma profile: fruity
    - Methyl geranate
  - Survive brewing process, but can thermally degrade
- Carboxylic acids
  - Aroma profile: cheesy
    - Butanoic acid
  - Can survive brewing process, but during fermentation can be turned into ethyl esters





THIOLS

- Sulfur containing compounds that generally cause off odors like onion, garlic, cheesy, and over cooked vegetable.
- 4-Mercapto-4-methylpentan-2-one (4MMP)
  - At low concentrations (1.5 ppt) it has a pleasant aroma of black currant or muscat.
  - At high concentrations, it has an offending aroma of very bad body odor or cat urine.

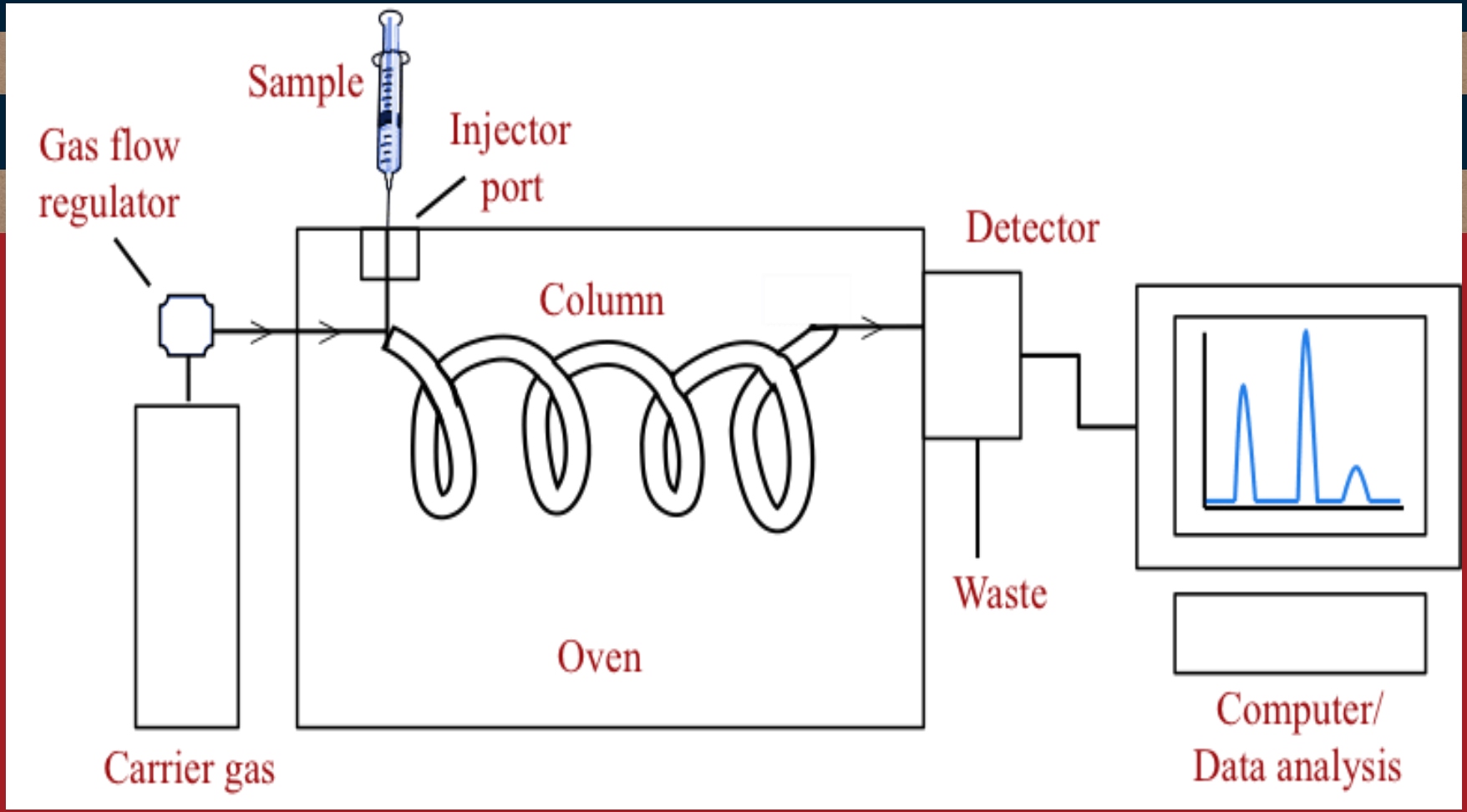


ANALYSIS TECHNIQUE FOR AROMA COMPOUNDS

Gas Chromatography: Generally considered the best instrumental analysis technique for the analysis volatile compounds.

- Chromatography – is a separation technique that consists of a mobile phase and a stationary phase, and the analytes are separated by their individual affinities/attraction for the mobile phase and stationary phase. Each analyte will take a different amount of time to travel through the stationary phase, which is called the retention time.
    - Mobile phase is the medium used to carry analytes through the stationary phase
    - Stationary phase is a medium that the compounds interact depending on their affinity for the medium

- Gas Chromatography – uses gas as a mobile phase called a carrier gas usually helium, so the analytes have to heated into their gaseous state to be carried through the stationary phase. The stationary phase is called a column that can are either packed (filled with material) or capillary (open tubular).


Sample
Injector port
Gas flow regulator
Detector
Column
Waste
Oven
Carrier gas
Computer/ Data analysis

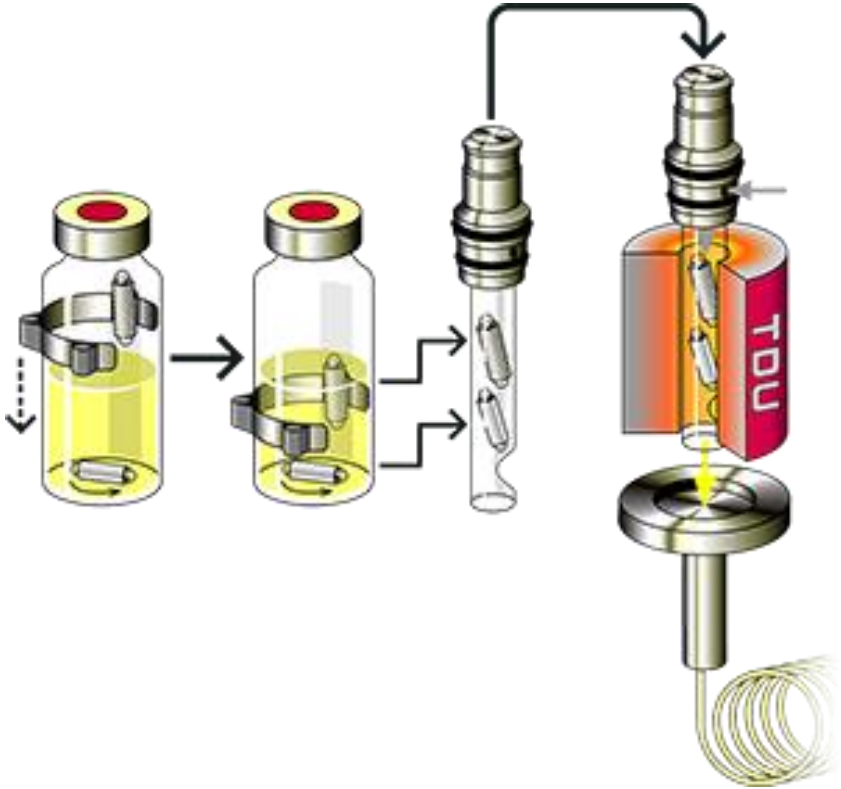
AROMA COMPOUND EXTRACTION TECHNIQUES

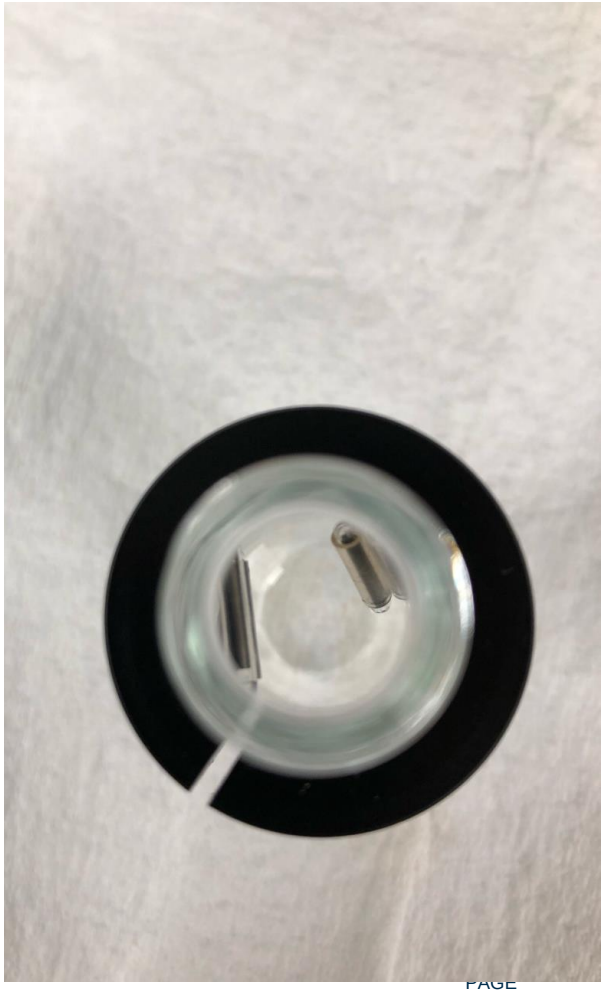
- Distillation
  - Boiling the hops/hop products in water for a set amount of time to distill and collect oil.
- Liquid Injection
  - The oil is mixed with a solvent and then directly injected into the injection port.
- Solid-Phase Microextraction (SPME)
  - A fiber coated with an extracting phase that will extract different analytes from different types of media.
- Headspace (HS)
  - The sample is heated while being agitated and then a syringe will extract the analytes from the headspace above the sample and inject into the injection port.
- Dynamic Headspace (DHS)
  - The sample is agitated and heated while the headspace is purged under a controlled flow of inert gas to provide more efficient extraction conditions.
- Twister Stir Bar Sorptive Extraction (SBSE)
  - A magnetic stir bar covered in a sorbent material (PDMS) that extracts and concentrates analytes.
  - It is solvent-free and more sensitive than SPME.

Liquid injection method

Headspace method
(Syringe method)

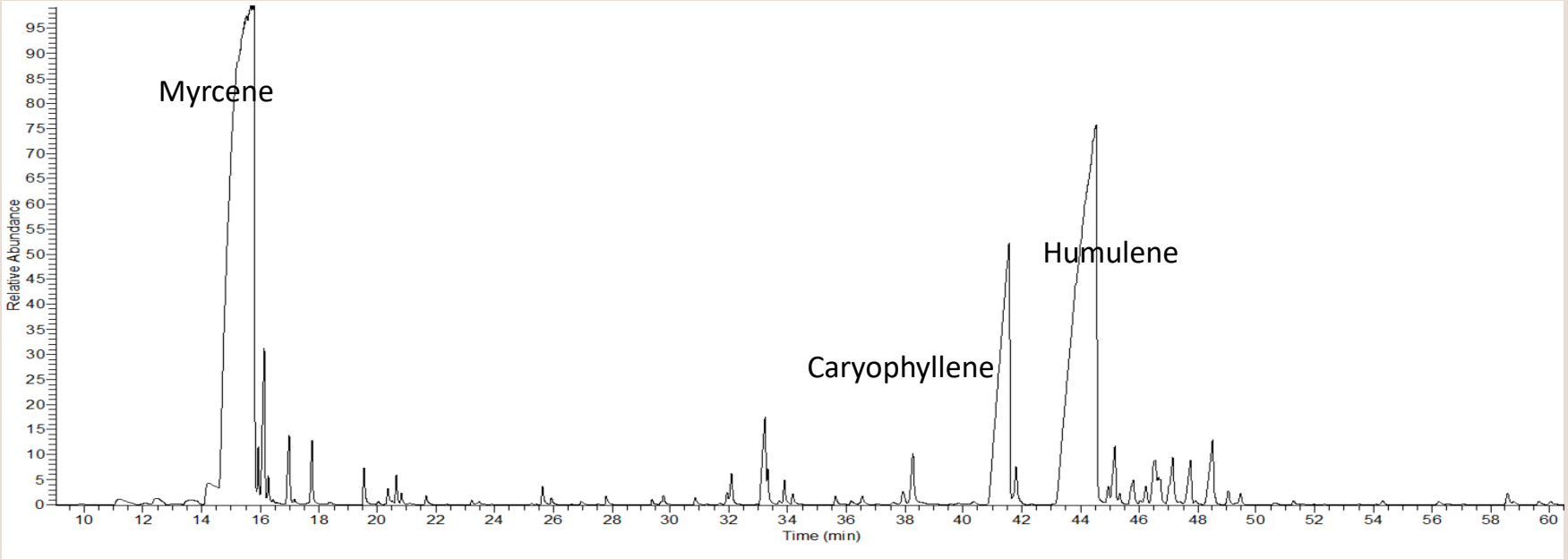
SPME method





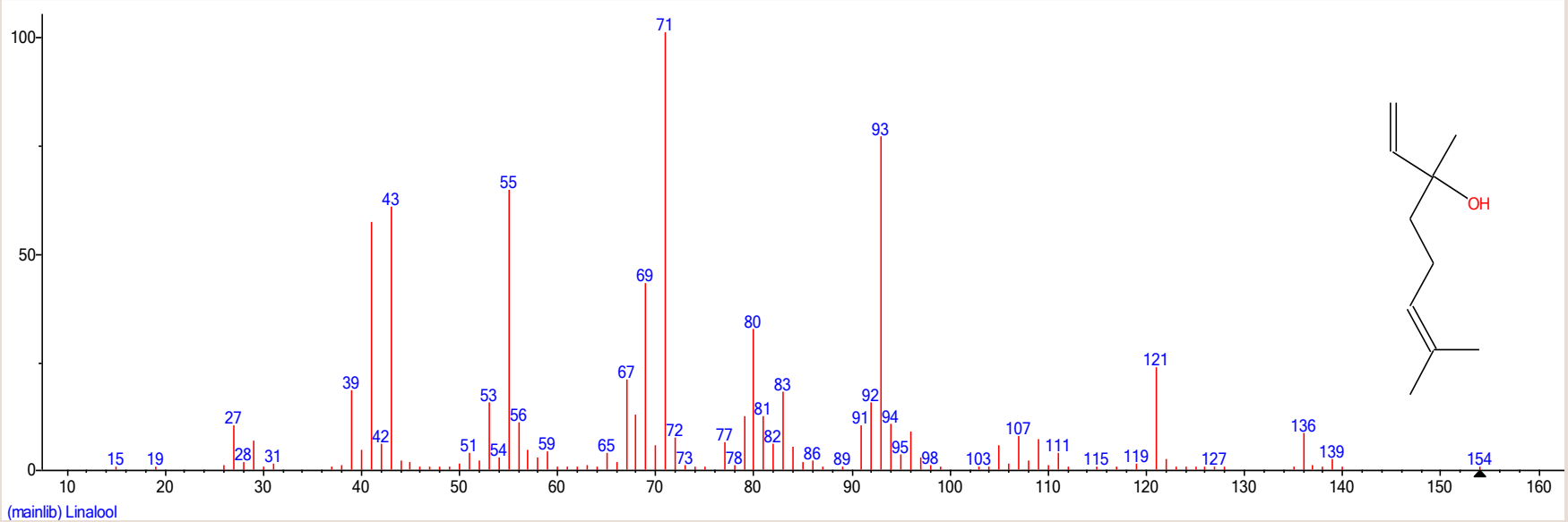
GC DETECTORS

Flame Ionization Detector (FID): A universal detector that combust the analytes once the pass through the column. Identification of compounds is restricted by retention time, and verified by referenced standards.



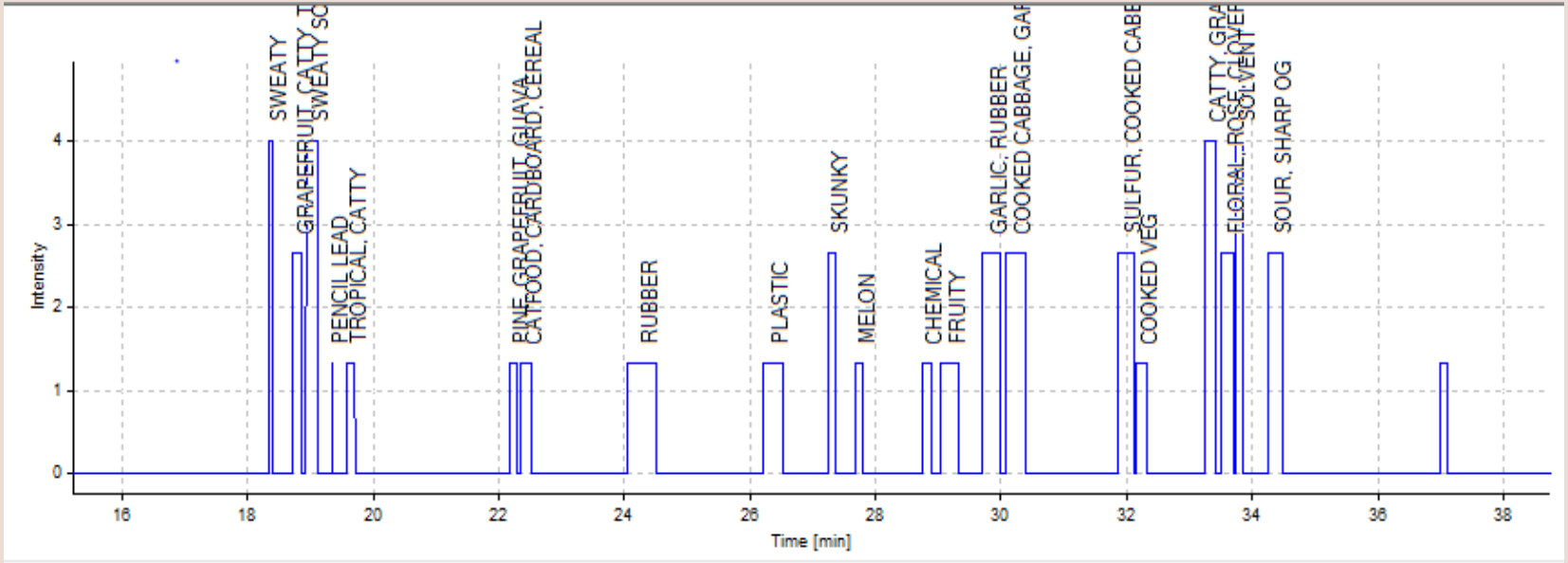
GC DETECTORS

Mass Spectrophotometer (MS): A precise and accurate detector. Once the compounds have passed through the column they are fragmented by applying a electron voltage. The fragmented ions create a Mass Spectra (finger print) of the compound that can be compared against national data bases. Identification is completed by retention time, mass spectra libraries and reference standards.



GC DETECTOR

Olfactory Detection Port (ODP): This detectors requires individuals that have a good nose. As the compounds pass through the column one identifies the compounds by smell. This is generally in conjunction with one of the above detectors

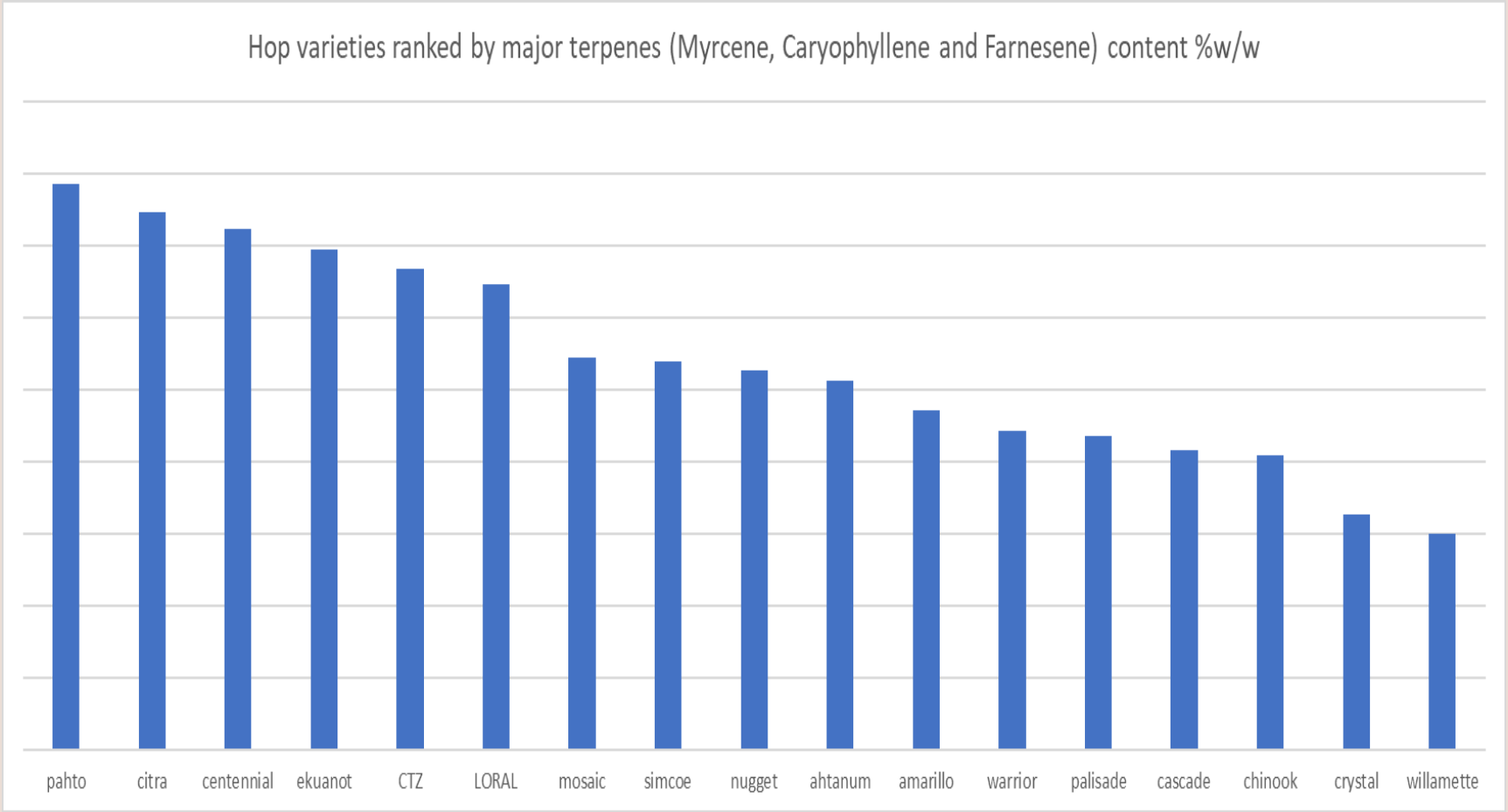


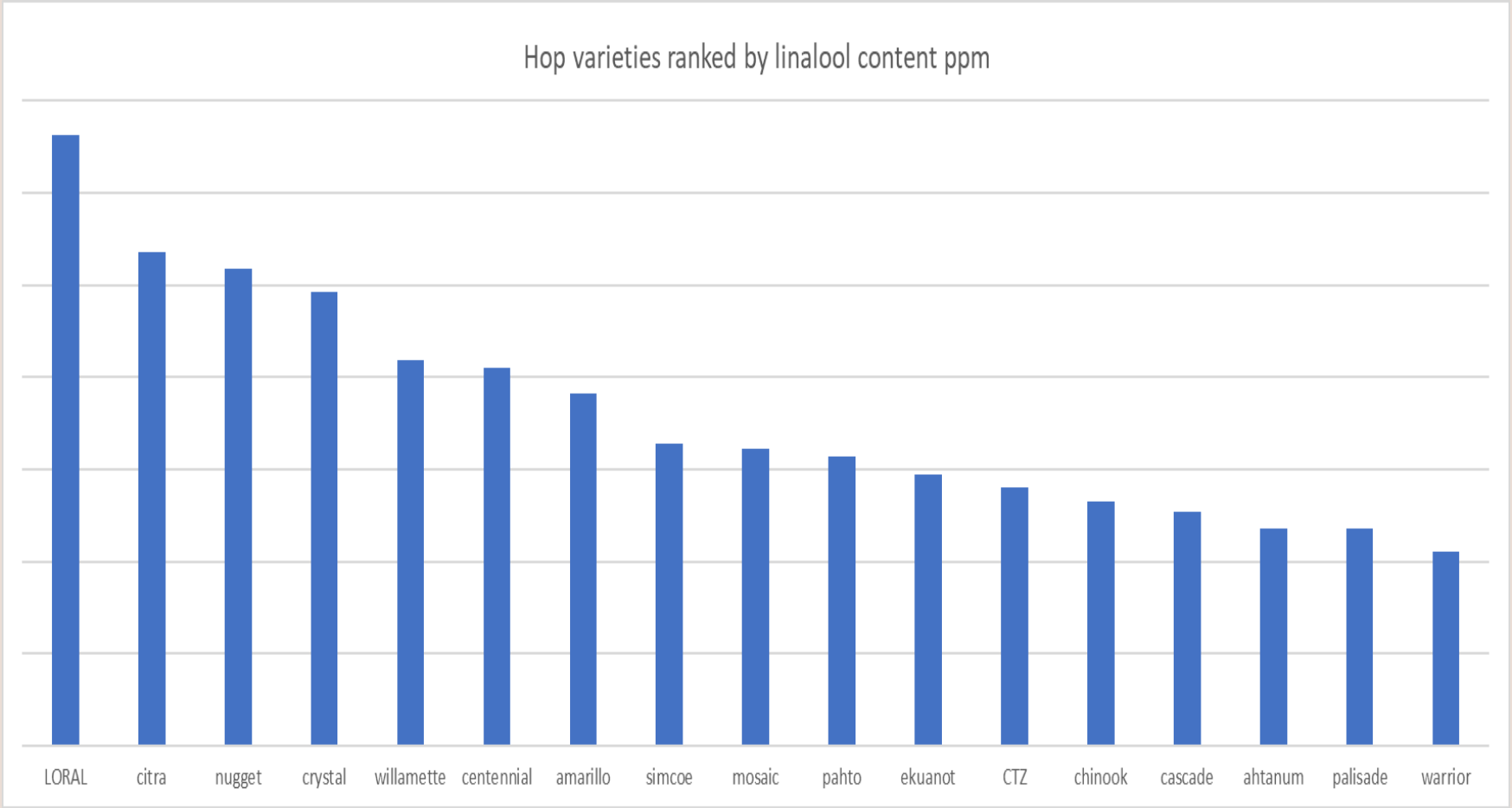
2018 HARVEST HOP AROMA DATA

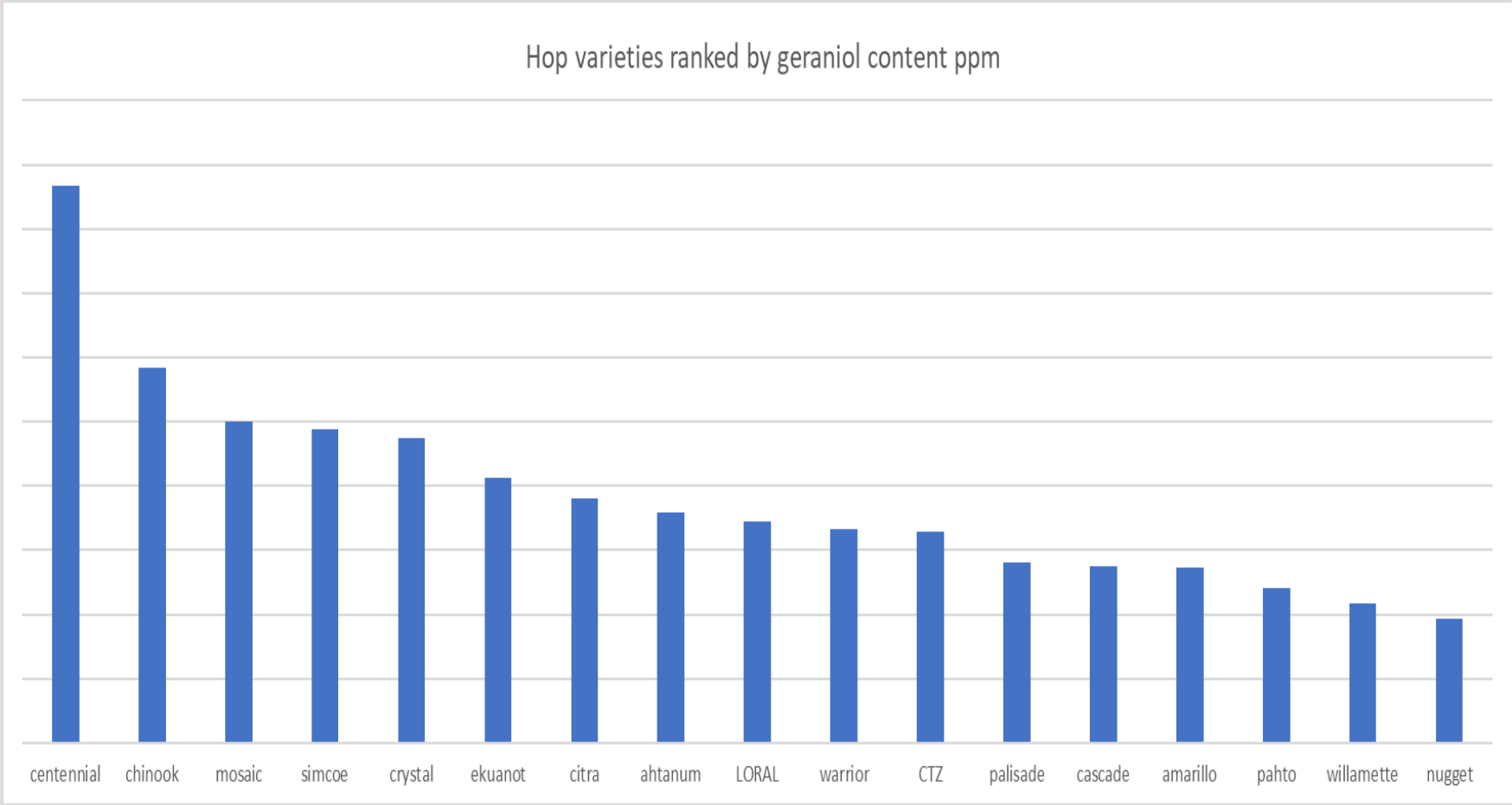
- Analysis completed on the GC-MS, each variety had a minimum of 9 lots tested.
- Twister SBSE extraction method used.
- Major Terpenes (content %w/w)
- Linalool (ppm)
- Geraniol (ppm)
- Linalool and Geraniol (ppm)
- Relative total ester content (area counts)

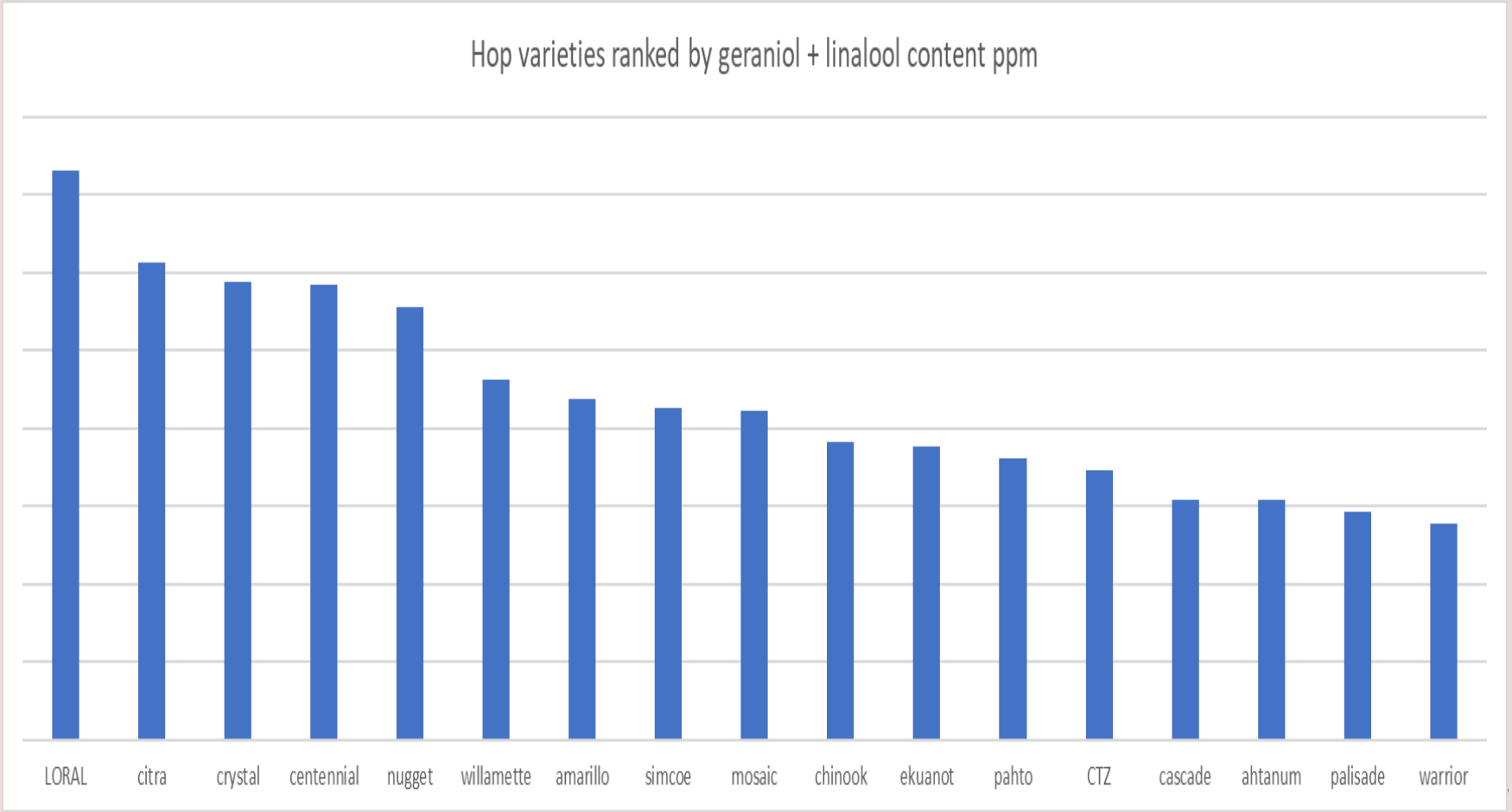
2018 HARVEST INSTRUMENTATION

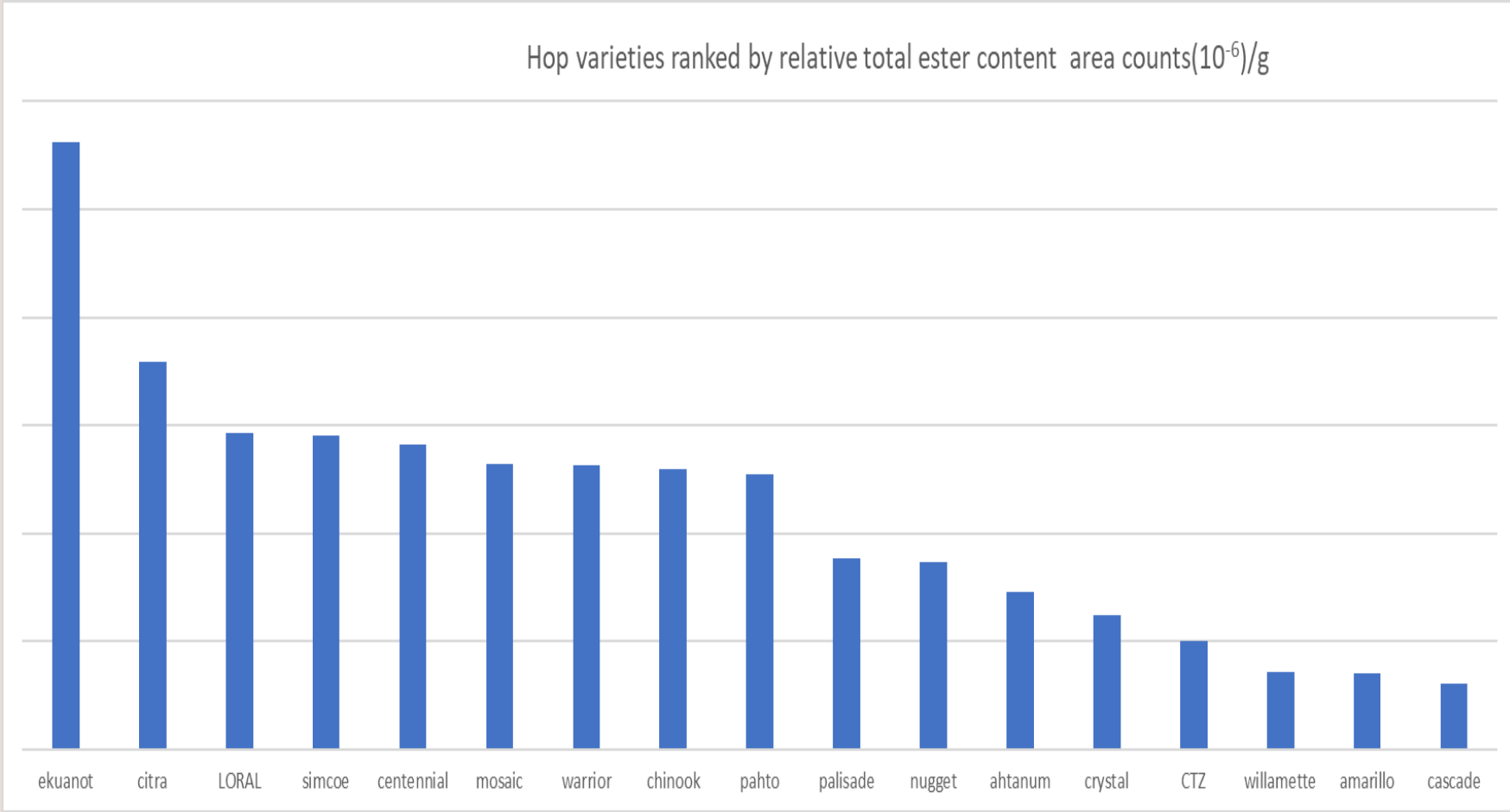
- Agilent 5975B GC/MSD
- Gerstel MPS
- Gerstel Olfactory Detection Port (ODP)


Hop varieties ranked by major terpenes (Myrcene, Caryophyllene and Farnesene) content %w/w
pahto
citra
centennial
ekuanot
CTZ
LORAL
mosaic
simcoe
nugget
ahtanum
amarillo
warrior
palisade
cascade
chinook
crystal
willamette


Hop varieties ranked by linalool content ppm
LORAL
citra
nugget
crystal
willamette
centennial
amarillo
simcoe
mosaic
pahto
ekuanot
CTZ
chinook
cascade
ahtanum
palisade
warrior

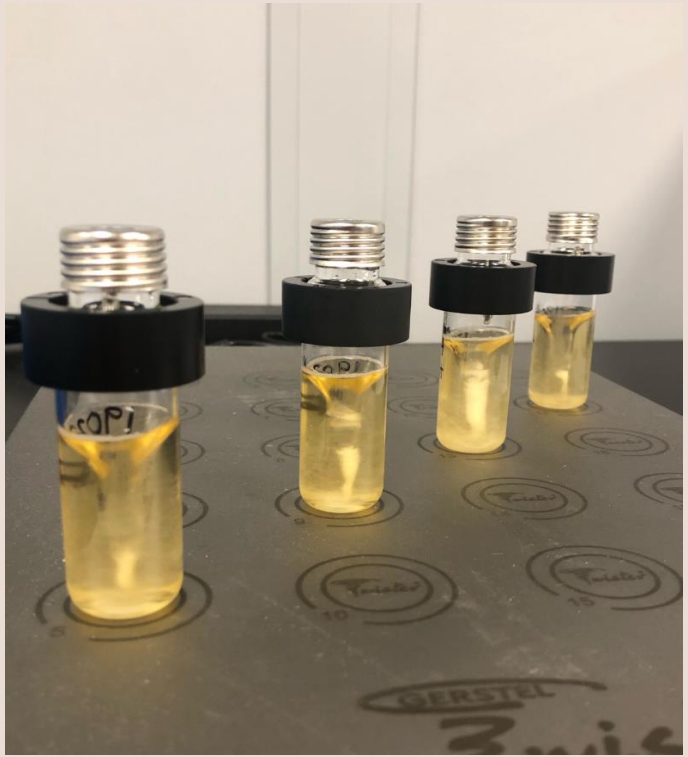

Hop varieties ranked by geraniol content ppm
centennial
chinook
mosaic
simcoe
crystal
ekuanot
citra
ahtanum
LORAL
warrior
CTZ
palisade
cascade
amarillo
pahto
willamette
nugget

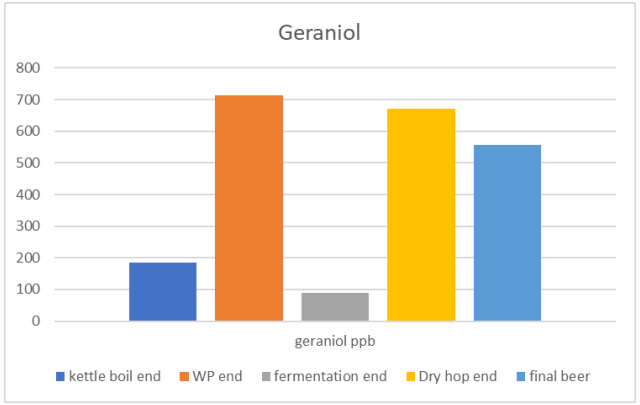

Hop varieties ranked by geraniol + linalool content ppm
LORAL
citra
crystal
centennial
nugget
willamette
amarillo
simcoe
mosaic
chinook
ekuanot
pahto
CTZ
cascade
ahtanum
palisade
warrior

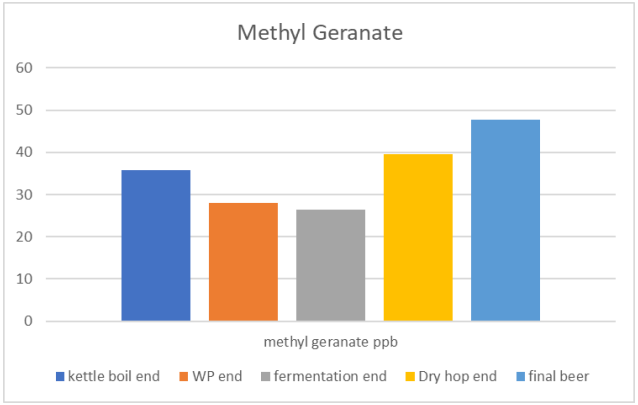

Hop varieties ranked by relative total ester content area counts(10-6)/g
ekuanot
citra
LORAL
simcoe
centennial
mosaic
warrior
chinook
pahto
palisade
nugget
ahtanum
crystal
CTZ
willamette
amarillo
cascade

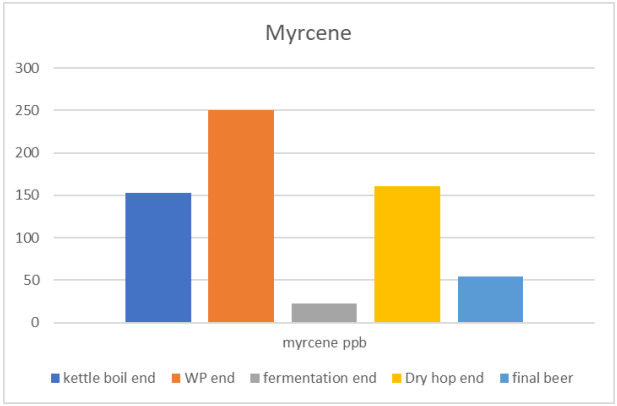
BEER ANALYSIS DATA

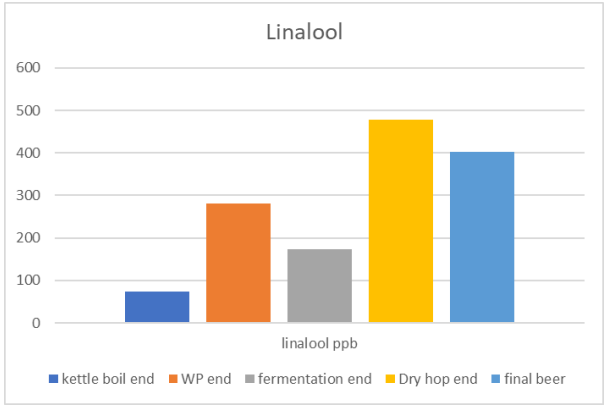
- Data gathered from samples collected throughout the brewing process, focusing on the specific points below.
  - End of the kettle boil
  - End of whirlpool
  - Terminal gravity
  - Post-dry hopping
  - Final Package
- Looking at the most abundant aromatic compounds that make it into the final beer.




Geraniol
800
700
600
500
400
300
200
100
0
geraniol ppb
kettle boil end
WP end
fermentation end
Dry hop end
final beer


Methyl Geranate
60
50
40
30
20
10
0
methyl geranate ppb
kettle boil end
WP end
fermentation end
Dry hop end
final beer


Myrcene
300
250
200
150
100
50
0
myrcene ppb
kettle boil end
WP end
fermentation end
Dry hop end
final beer


Linalool
600
500
400
300
200
100
0
linalool ppb
kettle boil end
WP end
fermentation end
Dry hop end
final beer

CONCLUSIONS

- Hop aroma is not equal to total oil content
  - Composition of the hop aroma
  - Consists of hundreds of compounds
  - Compounds aren’t singular, they work together to create the hop aroma
- Hop aroma in beer is an extraction
  - Depends on the contact with the liquid
  - Depends on the nature of the compounds
  - Before fermentation – polar compounds are best, but biotransformation, oxidation, and degradation occur
  - After fermentation – best place to extract nonpolar compounds

CONTINUED RESEARCH AND ANALYSIS

- Complexity of hop aroma requires several years of research and data collection
- Collect data on each cultivar
  - True average
  - Growing region affects
  - Agronomics
  - Ripeness
- Implementation of new analytical instrumentation
  - GC/MS Q-TOF with sulfur chemiluminescence detector (SCD)

YAKIMA CHIEF -HOPS-

Thank you for listening!

Questions?