



It's a kind of magic...

Leandro Meiners

**BREWING SUMMIT 2022**

Providence, Rhode Island | August 14-16



# About Me...

## Head Brewer / Co-Host / Author

- PLACEBO BREWING
- BIRRATECNIA (PODCAST)
- ZYTHOLOGIA (BLOG)
- SKEPTICAL BREWING (ZYMURGY COLUMN)



# AGENDA

- Different types of *biotransformation*, and what is **KNOWN** for each one of them:
  - Monoterpenes
  - Glycosides
  - Esters
  - Thiols
- *If not... then what?*
- *Key takeaways*



# BIOTRANSFORMATION: Intro & Types

Transformation of a compound (present in the wort or beer), by means of a microorganism, which has an impact in the taste / aroma.

1



2



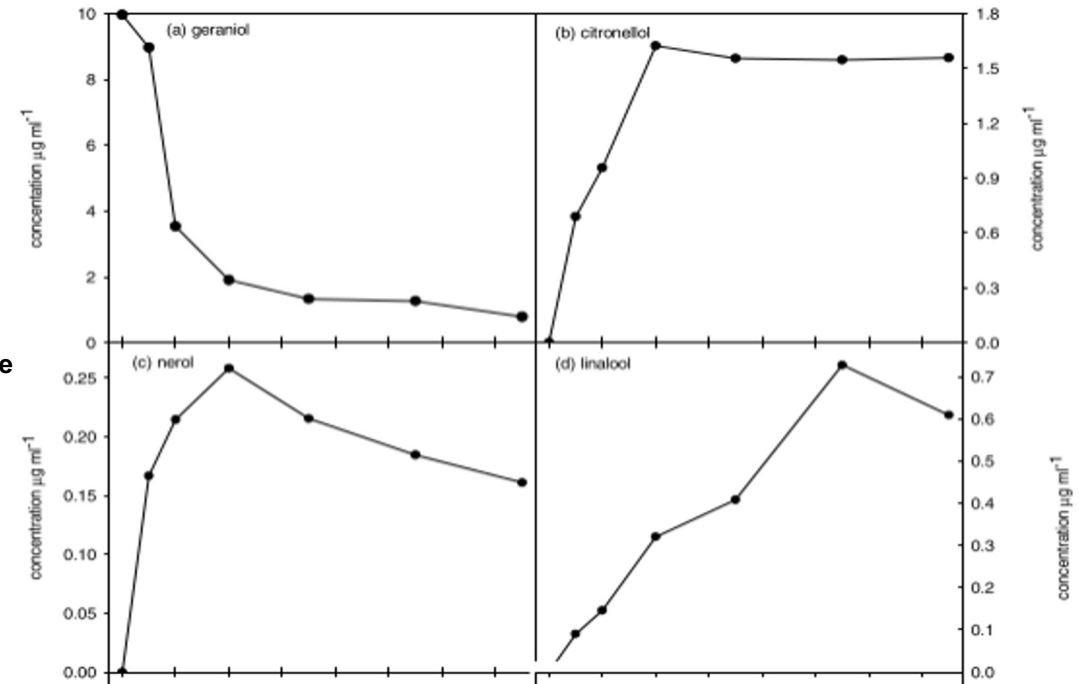
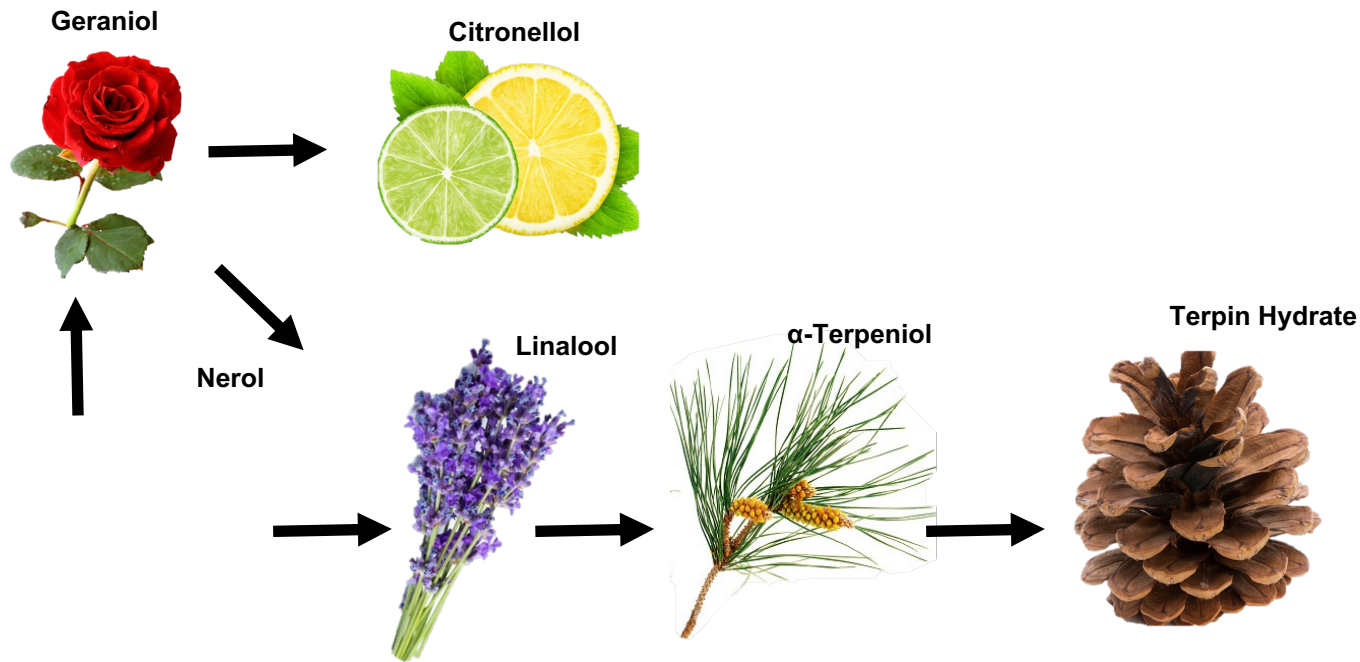
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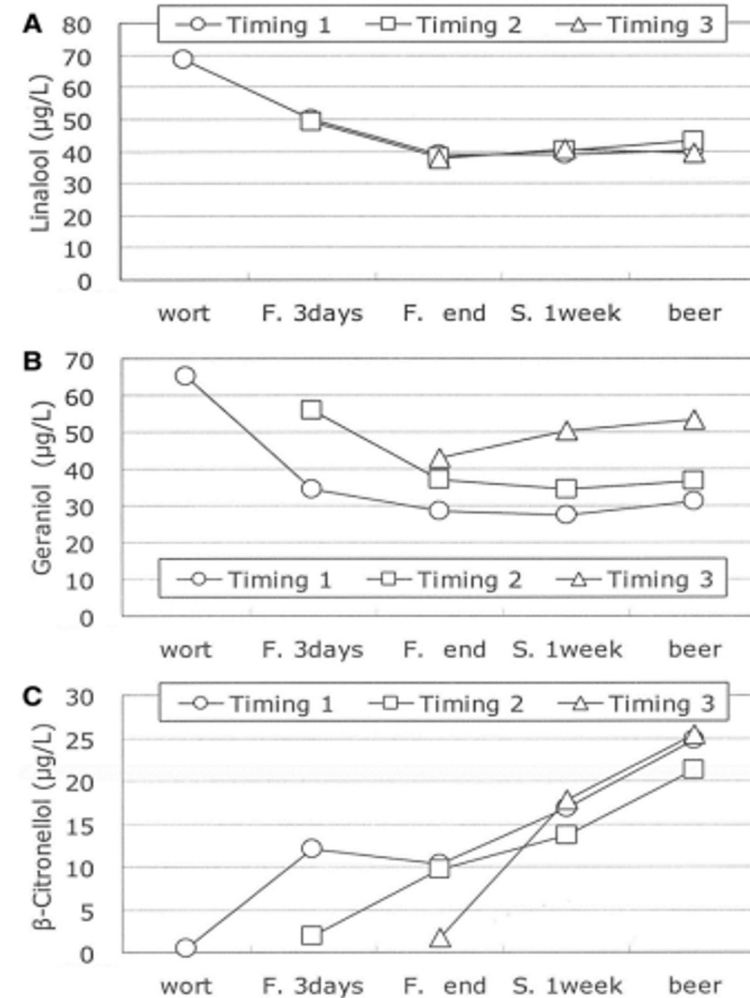
# BIOTRANSFORMATION: Monoterpenes



King & Dickinson, (2003)

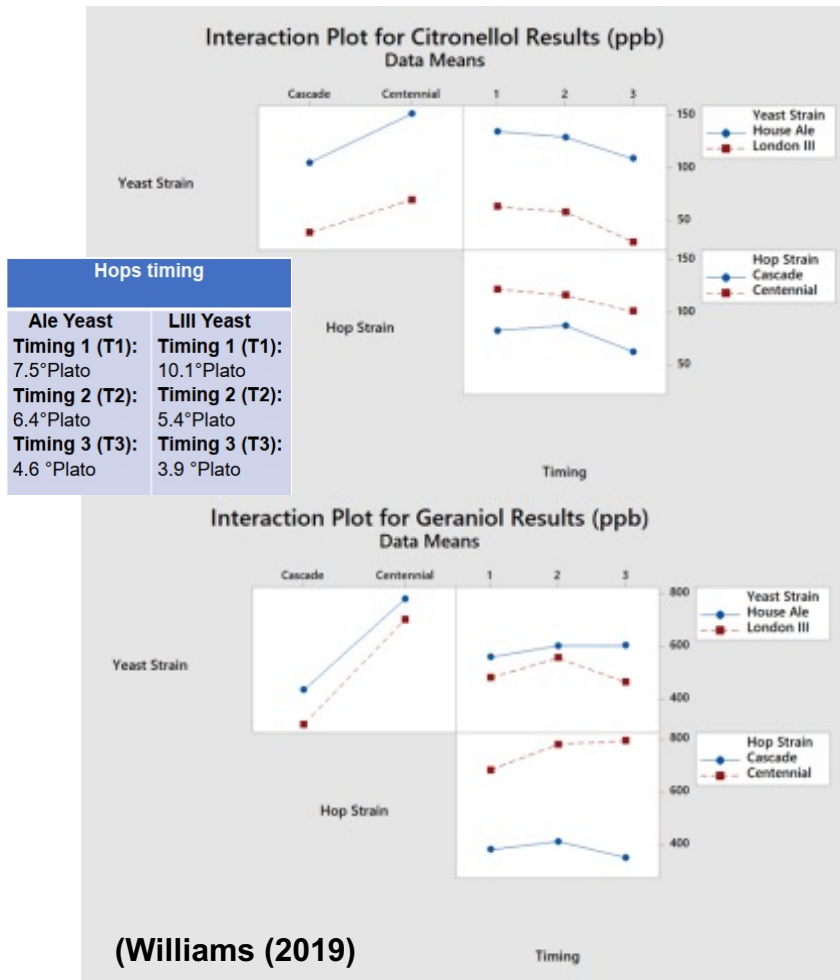
# BIOTRANSFORMATION OF MONOTERPENES: Impact of Hop Addition Timing

- Geraniol  content in beer can be **increased by dry-hopping later in the fermentation process**
- Linalool  levels are **constant** regardless of the **timing of hop addition**
- $\beta$ -citronellol  content (produced by biotransformation) **does not depend on the time of hop addition** and continues to occur even after packaging



(Takoi et al., 2014)

# BIOTRANSFORMATION OF MONOTERPENES – Is There Consensus?



## Final Concentration of Monoterpene affected by:

<b>Hop Variety</b>	
<b>Yeast Strain</b>	
<b>Timing of the addition</b>	

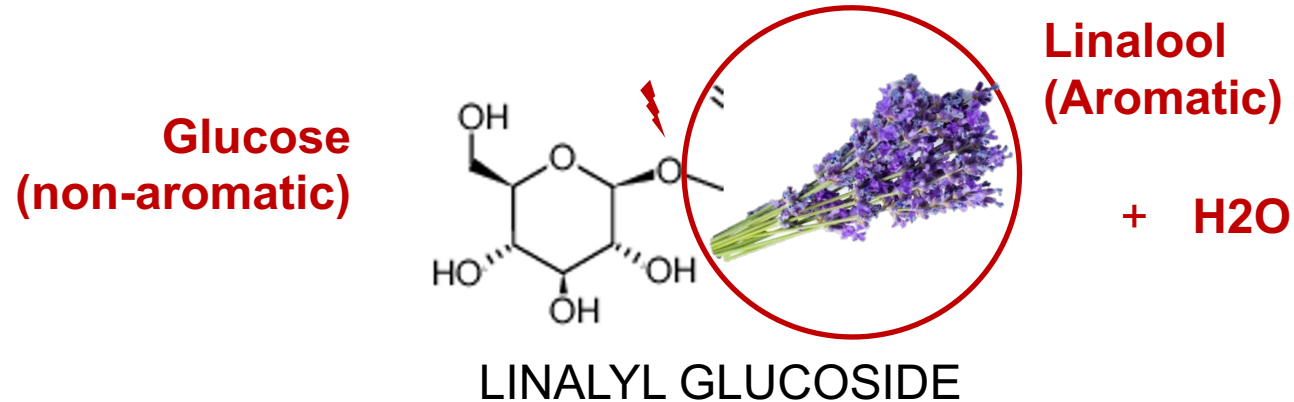
**BUT...**

**the effect depends on the yeast strain!**

# BIOTRANSFORMATION: GLYCOSIDES

Glycosides are *non-aromatic molecules* where a *sugar* is *linked* to *another functional group* (plants generate them to store and transport energy)

For example:

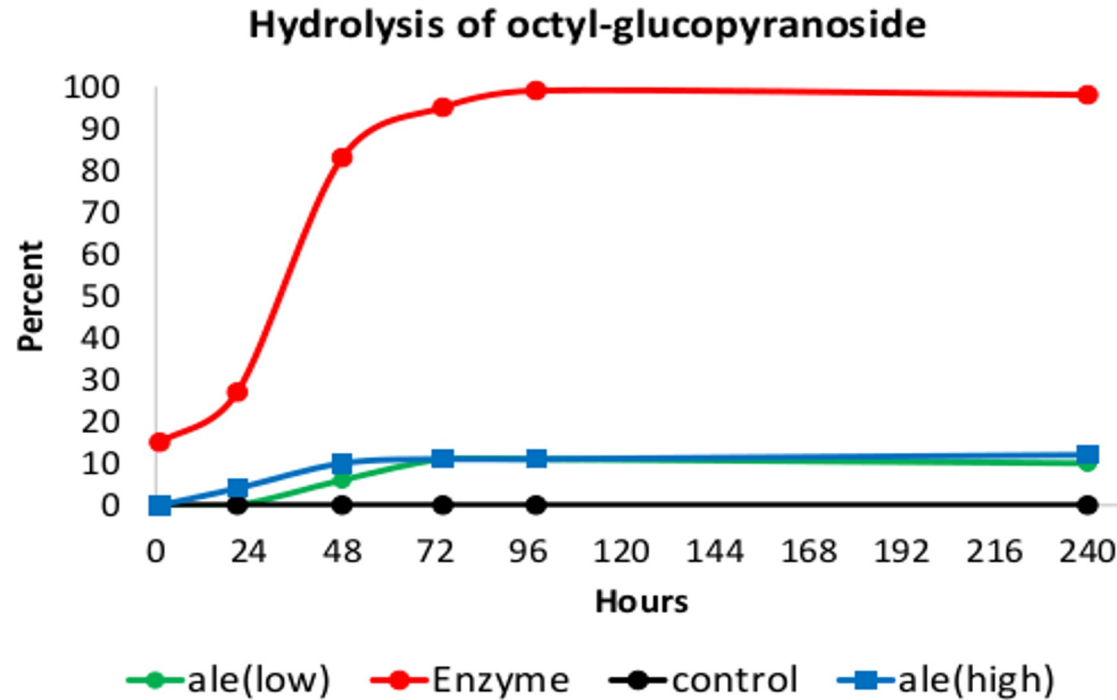


The functional group can be released by the action of the *yeast* (or *enzyme*)



# BIOTRANSFORMATION OF GLYCOSIDES – Can Yeast Do It Without Extra Help?

Tracking the release, of an “*artificial*” (aka not present in beer) *glycoside* during *fermentation*:



(Sharp, Steensels, & Shellhammer, 2017):

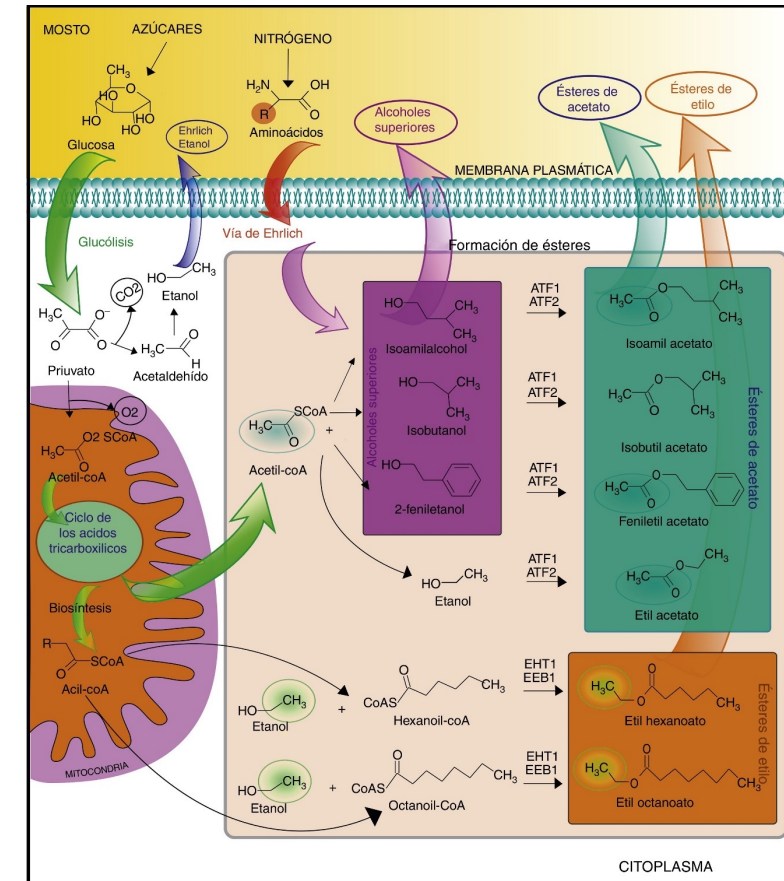
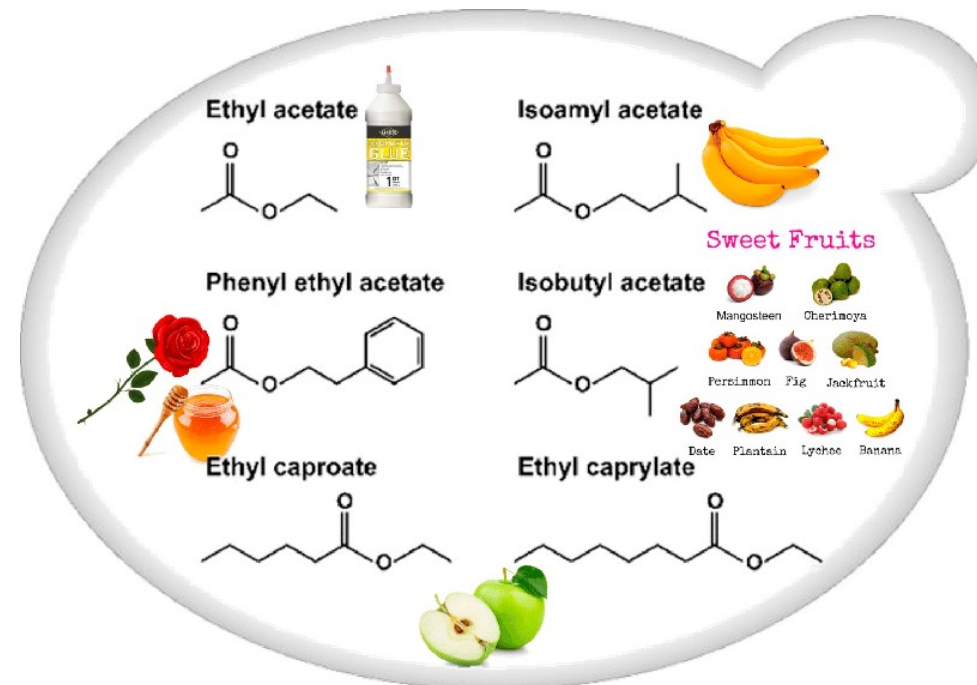
- **Conventional yeasts** (regardless of high or low  $\beta$ -glucosidase activity) could **NOT** release **more** than **10%** of the **glycoside**
- **Only way to significantly “release” was using added enzymes**

# BIOTRANSFORMATION: ESTERIFICACION

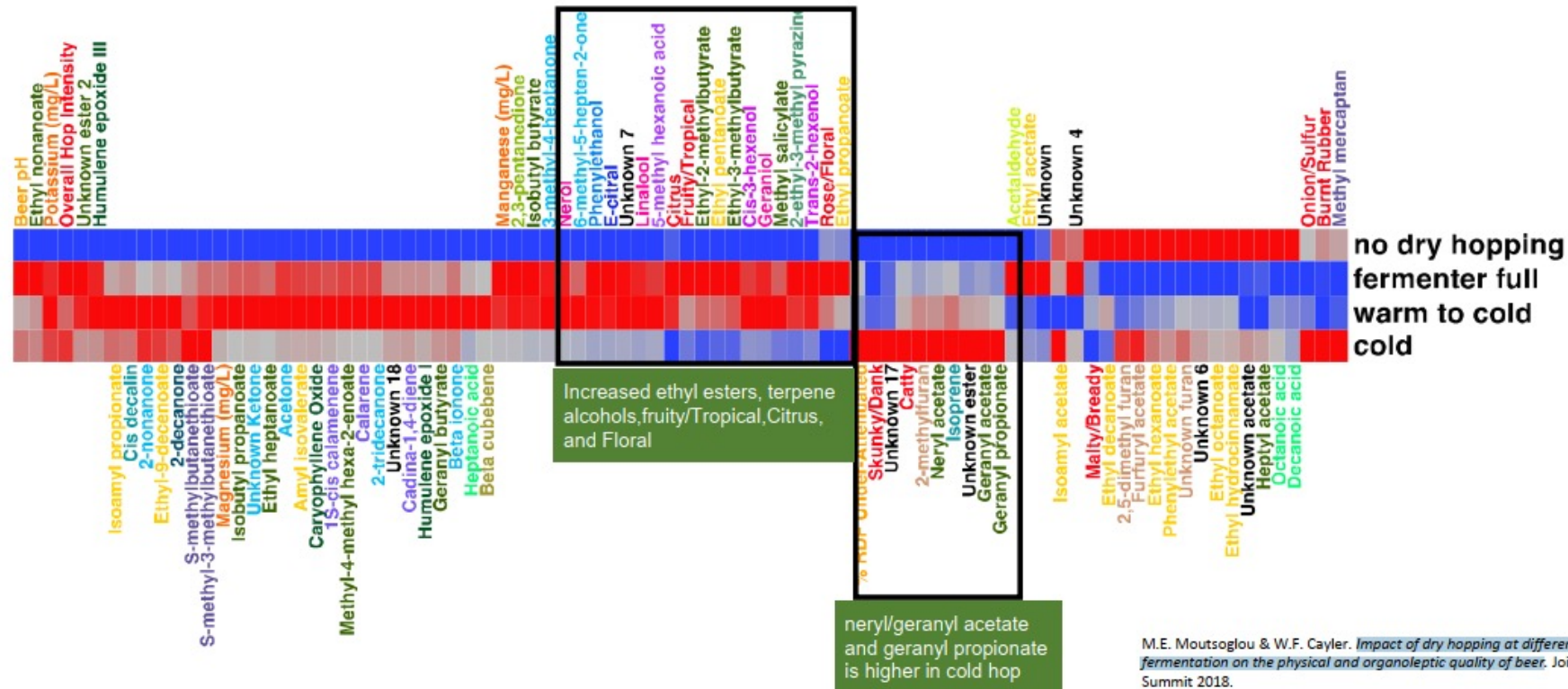
**Yeast-generated esters** are produced by *metabolizing* an organic **acid** and an **alcohol**.

**Two major classes of esters** generated by yeast secondary metabolism :

- **Ethyl esters**
- **Acetate esters**



# BIOTRANSFORMATION OF ESTERS – Impact Due To Timing Of Hop Addition



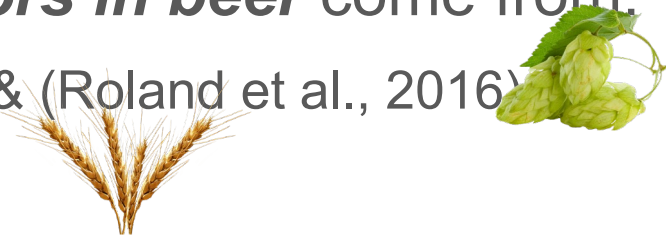
- ✓ **Ethyl esters:** *higher* concentration when *dry hopping on transfer* to fermenter (aka “dip-hopping”)
- ✓ **Geranyl esters** higher concentration when *dry hopping cold* (post-fermentation)

# BIOTRANSFORMATION: THIOL PRECURSORS

**Thiol precursors are non-aromatic**

Identified **thiol precursors in beer** come from:

- **Hops:** (Gros et al., 2012) & (Roland et al., 2016)
- **Malt:** (Dagan et al., 2016)



**Thiols** have extremely **low perception thresholds:** (Swiegers & Pretorius, 2007)



**3MH**



**3MHA**



**4MMP**

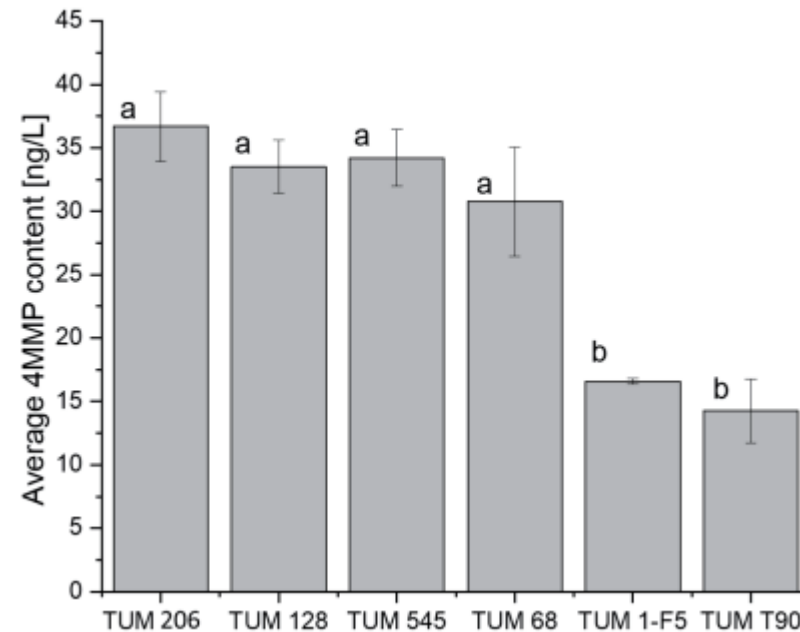
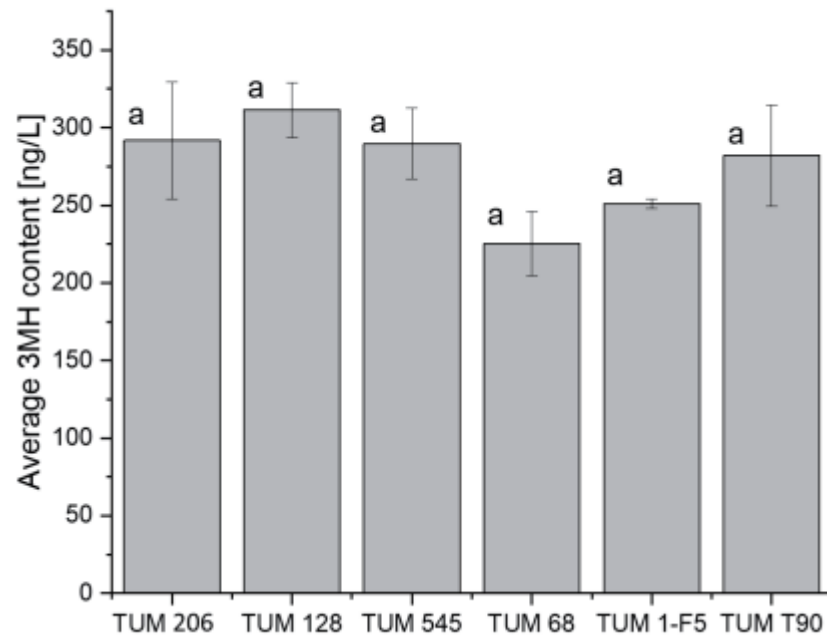
**0.06 ppb**  
**0.004 ppb**  
**0.0008 ppb**

**Thiols** can also **reduce** other **aromatic compounds perception thresholds** (Takoi et al., 2016)

# BIOTRANSFORMATION OF THIOL PRECURSORS - Yeast Selection Based On Genetic Profiling

Conventional yeast strains:

- Interact differently with different thiols
- Low conversion rates for thiol precursors



(Michel et al., 2019)

# BIOTRANSFORMATION: How Much of an Impact Can it Really have

TYPE OF BIOTRANSFORMATION	SENSORY THRESHOLD LEVELS	QUANTITY OF PRECURSORS	CONVERSION LEVELS
<b>MONOTERPENES</b>	Geraniol ~ 53 ppm Linalool ~ 9 ppm Citronellol ~ 25 ppm	Citra: (Late Hopping @ 1 g/L) Linalool ~ 75 ppm Geraniol ~ 16 ppm Citronellol ~ 18 ppm	Unknown %
<b>GLYCOSYDES (MONOTERPENES)</b>	Nerol ~ 500 ppm	Simcoe: (In spent hops @ 50 g/L) Linalool ~ 18 ppm Geraniol ~ 25 ppm Citronellol ~ 1 ppm	~ 10 %



# BIOTRANSFORMATION: How Much of an Impact Can it Really have

TYPE OF BIOTRANSFORMATION	SENSORY THRESHOLD LEVELS	QUANTITY OF PRECURSORS	CONVERSION LEVELS
<b>ESTERS</b>	Ethyl 3-methyl butyrate ~ 7 ppm (Ethyl Ester Formation) Geraniol ~ 53 ppm (Ester Hydrolysis) geranyl isobutyrate ~ 45 ppm	Isobutyric acid ~ 4-8 ppm (commercial lager beers) Geranyl isobutyrate ~ 1.5% hop oil (Cascade) – For ref. linalool was 0.85%. Geranyl acetate ~ 170 ppm/L (DH with Cascade)	<b>Geranyl to Geraniol ~ 15%</b>
<b>THIOLS</b>	4MMP ~ 4 ppb 3MH ~ 55 ppb	Mosaic: [C3MH] ~ 170 [CG3MH] ~ 510 [G3MH] ~ 3400 For comparison: [3MH] ~ 25 ppb/g	<b>~ 0.1–0.5%</b>



# BIOTRANSFORMATION: Is It Responsible For The Profile Change Of Mid-fermentation Dry Hopping

TYPE OF BIOTRANSFORMATION	SENSORY THRESHOLD LEVELS	CONTRIBUTION OF THIS BIOTRANSFORMATION TYPE TO BEERS TROPICAL PROFILE	IMPACT OF TIMING OF HOP ADDITION (HINT, HINT: PROCESS CHANGE)
MONOTERPENES	MODERATE/HIGH	LOW	NO IMPACT
GLYCOSYDES (MONOTERPENES)	MODERATE/HIGH	VERY LOW	NO STUDIES
ESTERS	MODERATE/HIGH	MEDIUM TO LOW	NO IMPACT
THIOLS	EXTREMELY LOW	HIGH	NO STUDIES





# SO... EARLY/MID-FERMENTATION DRY HOPPING DOES NOTHING?



# BIOTRANSFORMATION: If Not, What Else ?

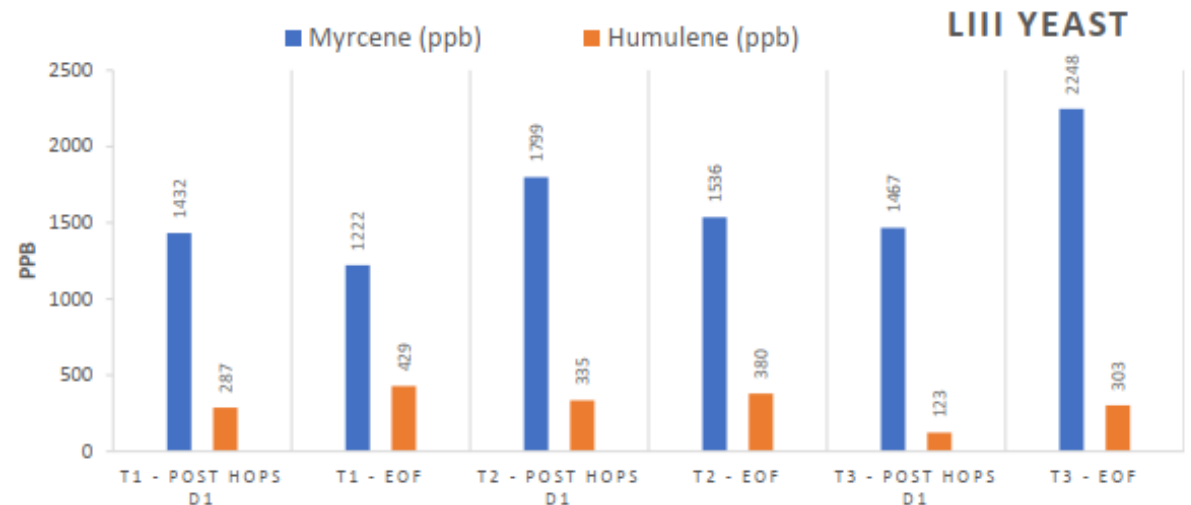
**Removal of highly volatile compounds** (generally with a herbal profile), due to:

- CO<sub>2</sub> evolution (Haefliger, 2013)
- Absorption on yeast's cell walls (Kishimoto, 2013)

**Non-yeast mediated hydrolysis of esters** (Forster, 2014)

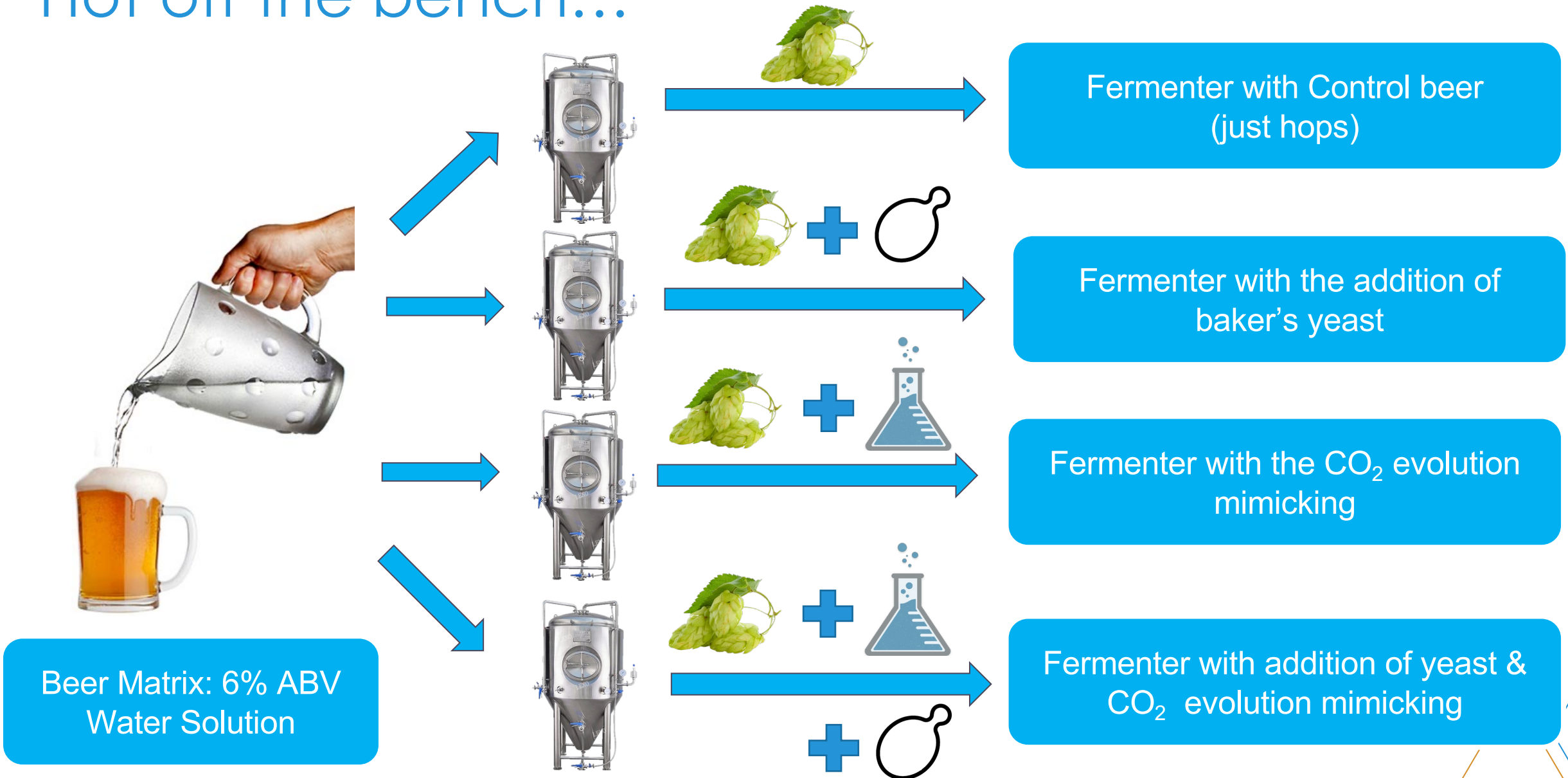
(Noro, 2015): Showed the use of “**dead**” yeast to remove compounds with an herbal profile

The **earlier** the dry hopping, the **greater** the removal of Myrcene:



(Williams, 2018)

# Hot off the bench...



# Triangle Test Results

	<b>Yeast Tasting</b>	<b>CO<sub>2</sub> tasting</b>	<b>Yeast + CO<sub>2</sub> Tasting</b>
<b># Participants</b>	28	27	28
<b># Correct answers</b>	17	24	23
<b>Significant?</b>	YES (value p = 0.003)	YES (value p = 0.000000003)	YES (value p = 0.000000015)
<b>Preference (only for correct answers)</b>	Control = 0 Yeast = 9 No Preference = 8	Control = 11 CO <sub>2</sub> = 3 No Preference = 10	Control = 3 CO <sub>2</sub> + Yeast = 12 No Preference = 8

# Conclusions

- Both **Yeast** and **CO<sub>2</sub>** make an **impact on resulting hop expression**
- Most tasters **prefer** samples with **Yeast**
- Common reasons for preferring samples with yeast (just yeast and Y+CO<sub>2</sub>) were:
  - More “tropical“
  - Fruiter (“sweet fruit“)
  - “Fresh hop“ aroma
  - More intense aroma
- For Control vs CO<sub>2</sub>, preference was with Control reasons were more herbaceous, floral, “balanced“ aroma. Yet the few who chose CO<sub>2</sub> stated more “tropical“ and fruitier.



# MAIN TAKEOUTS

- SOME TYPES OF BIOTRANSFORMATION **DO NOT** HAVE A HIGH IMPACT (MONOTERPENES / GLYCOSIDES )
- EARLY / MID FERMENTATION (HIGH KRAUSEN) DRY HOPPING HAS A SENSORY IMPACT, BUT IT IS **NOT PROVEN** THAT IT IS DUE TO BIOTRANSFORMATION (AND SEEMS **VERY UNLIKELY**)
- **SENSORY CHANGE OF EARLY / MID FERMENTATION (HIGH KRAUSEN) DRY HOPPING IS MORE LIKELY DUE TO YEAST AND CO<sub>2</sub> SCRUBBING.**
- OUR EXPERIENCE ADDS WEIGHT TO THE ANALYSIS THAT BIOTRANSFORMATION IS NOT RESPONSIBLE FOR EARLY DRY HOPPING



# THANK YOU!



# Resources

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HOPS

YEAST



# Resources

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# Resources

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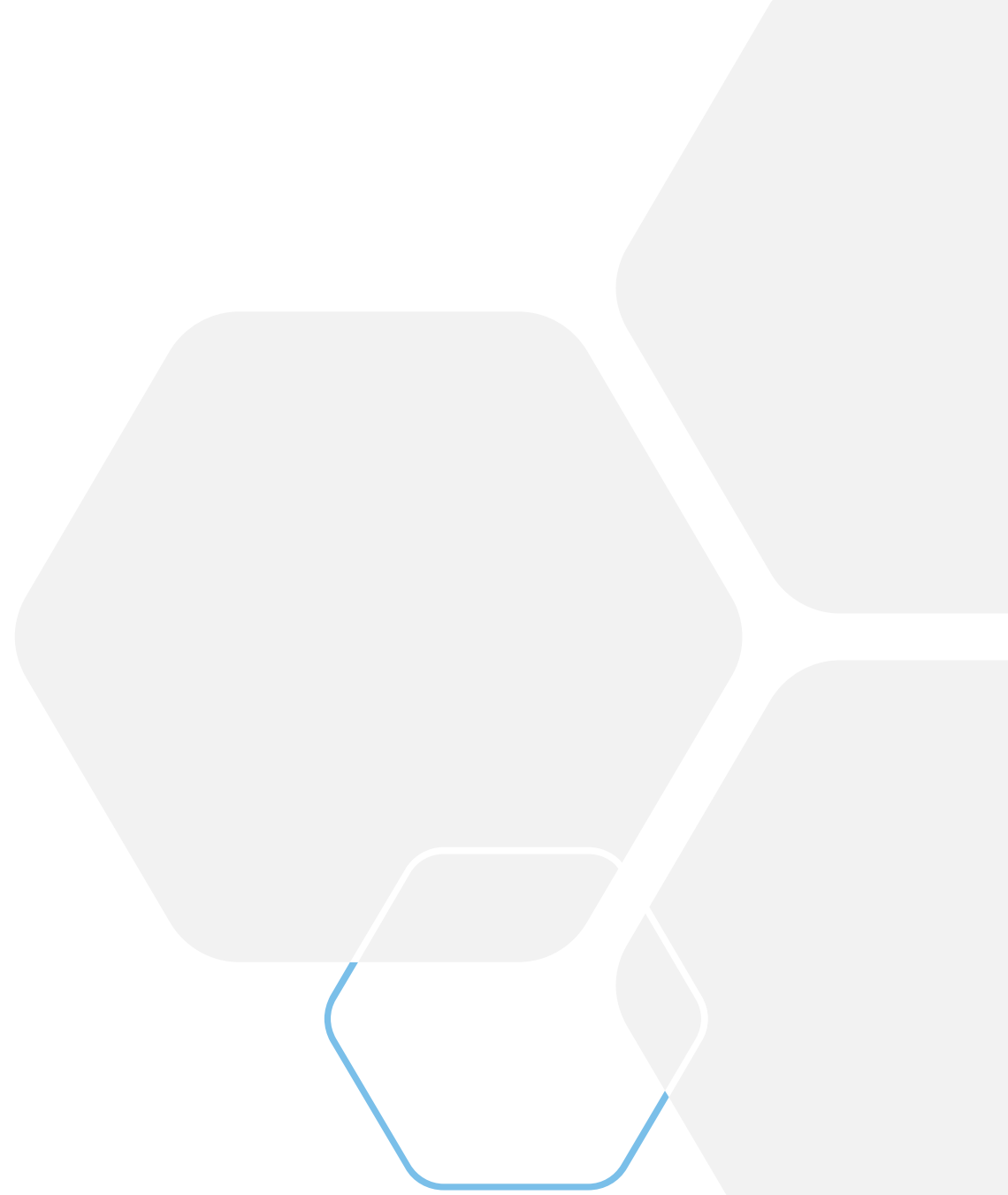
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# /Further Avenues of Research



# Icons

Icons can be recolored using shape fill. Icons can be used with yellow hex shape (center vertically & horizontally with shape then group). To resize icon and/or shape, use the shift key to keep the original proportions.

