Free thiol release from cysteinylated & glutathionylated adducts in beer by SafAle™ yeasts

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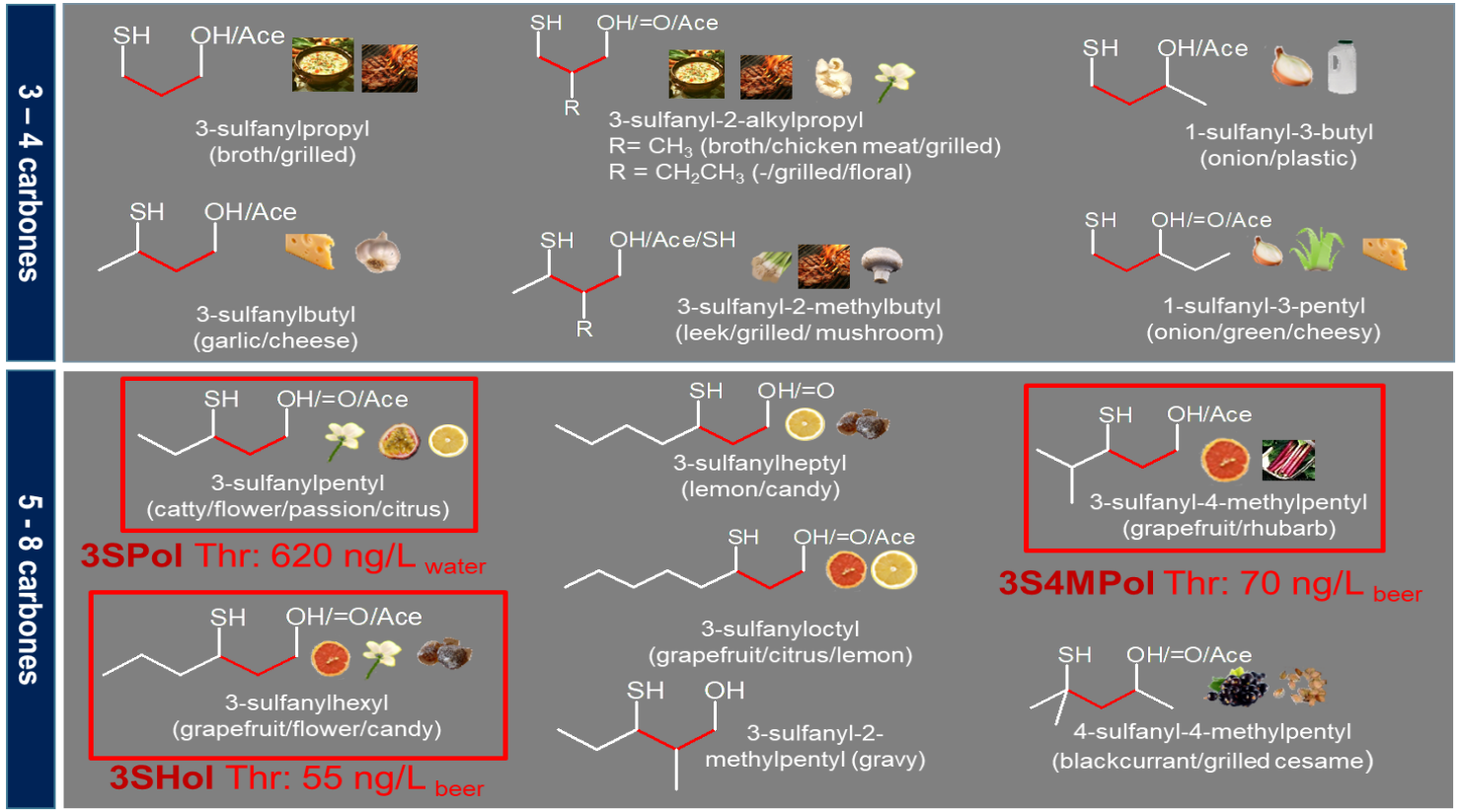
Fermentis by Lesaffre

1. Introduction & Objectives

⇒ Distance between SH et OH/=O/Ace : 3 carbones (exception for MBT)



Free thiols

⇒ **Big organoleptic impact due to very low threshold of thiols**

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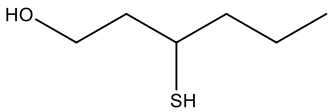
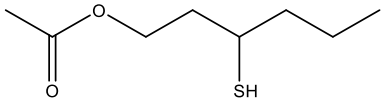
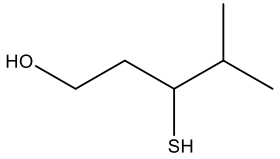
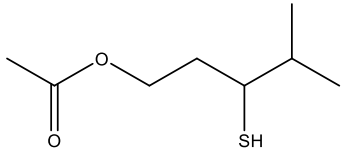
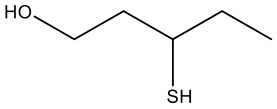
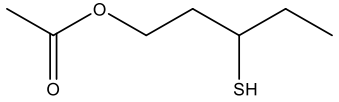
1. Introduction & Objectives

Name	Structure	Aroma	Threshold (in beer)
3-sulfanylhexan-1-ol **3SHol**			55 ng/L
3-sulfanylhexyl acetate **3SHA**			5 ng/L
3-sulfanyl-4-methylpentan-1-ol **3S4MPol**			70 ng/L
3-sulfanyl-4-methylpentyl acetate **3S4MPA**			160 ng/L
3-sulfanylpentan-1-ol **3SPol**			620 ng/L
3-sulfanylpentyl acetate **3SPA**			??

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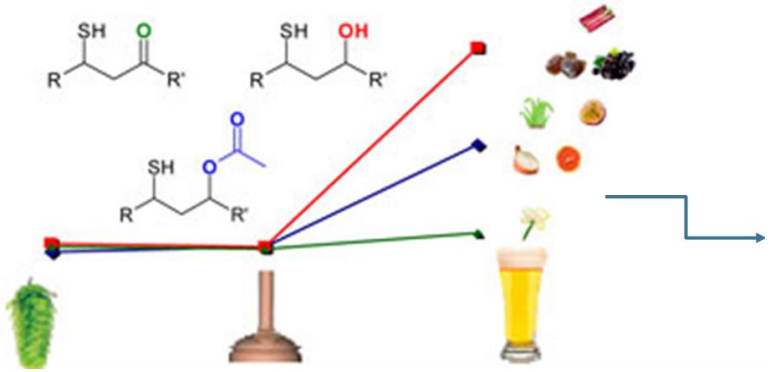
1. Introduction & Objectives

⇒ Evolution during wort boiling and fermentation



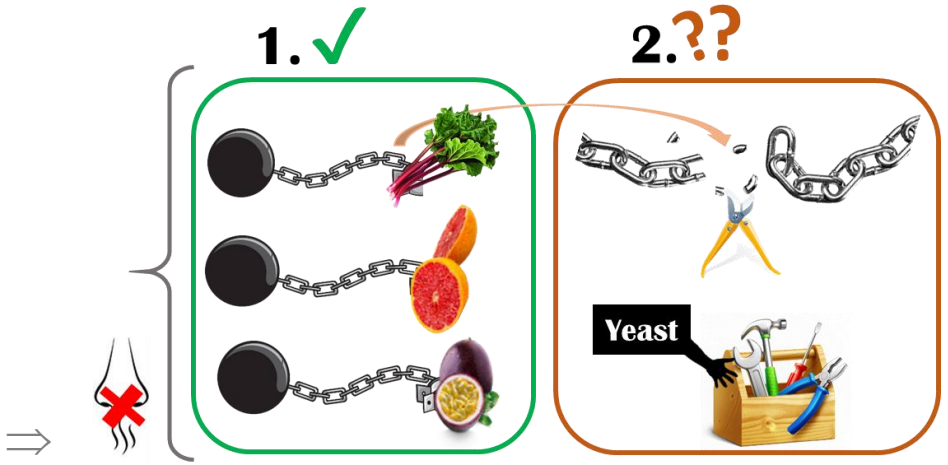
Suspicion of presence of thiol precursors:
Cysteinylated-adducts & Glutathionylated-adducts

mg/Kg of bound forms in hop	Amarillo (USA, 2015)	Citra (USA, 2017)	Hallertau Blanc (Germany, 2015)	Nelson Sauvin (New Zealand, 2018)	Polaris (Germany, 2017)	Saaz (Czech Republic, 2017)
Cys-3SPol	d	d	d	d	0.162	d
G-3SPol	7.5	18.1	3.0	1.4	9.8	2.5
Cys-3SHol + Cys-3S4MPol	2.1	0.3	1.3	0.2	4.9	0.4
G-3SHol	101.0	91.0	77.1	20.1	118.2	95.7
G-3S4MPol	nd	nd	0.3	d	3.6	d

In dual hops
G-thiols > Cys-thiols

1. Introduction & Objectives

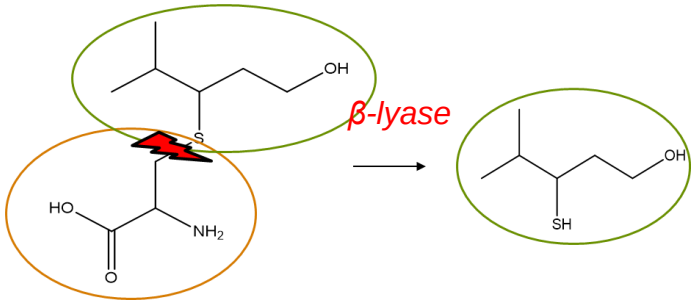
⇒ Yeast activity on Cysteinylated and Glutahionylated adducts?



Does each yeast have the same activity for the libération of free thiols in beer??

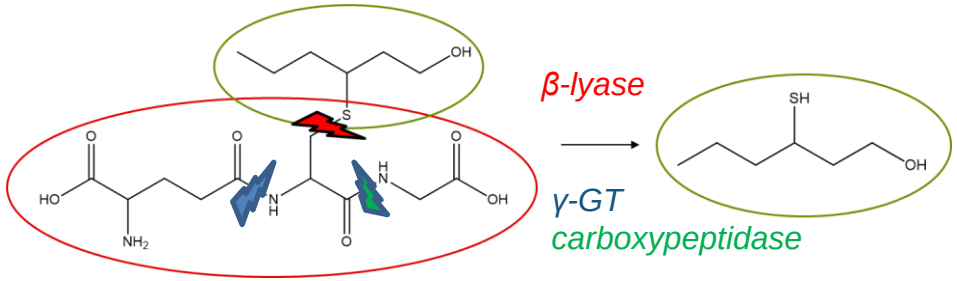
- Cys-adducts (Cys-thiols)

No odor → odor



G-adducts (GluCysGly-thiols)

No odor → odor



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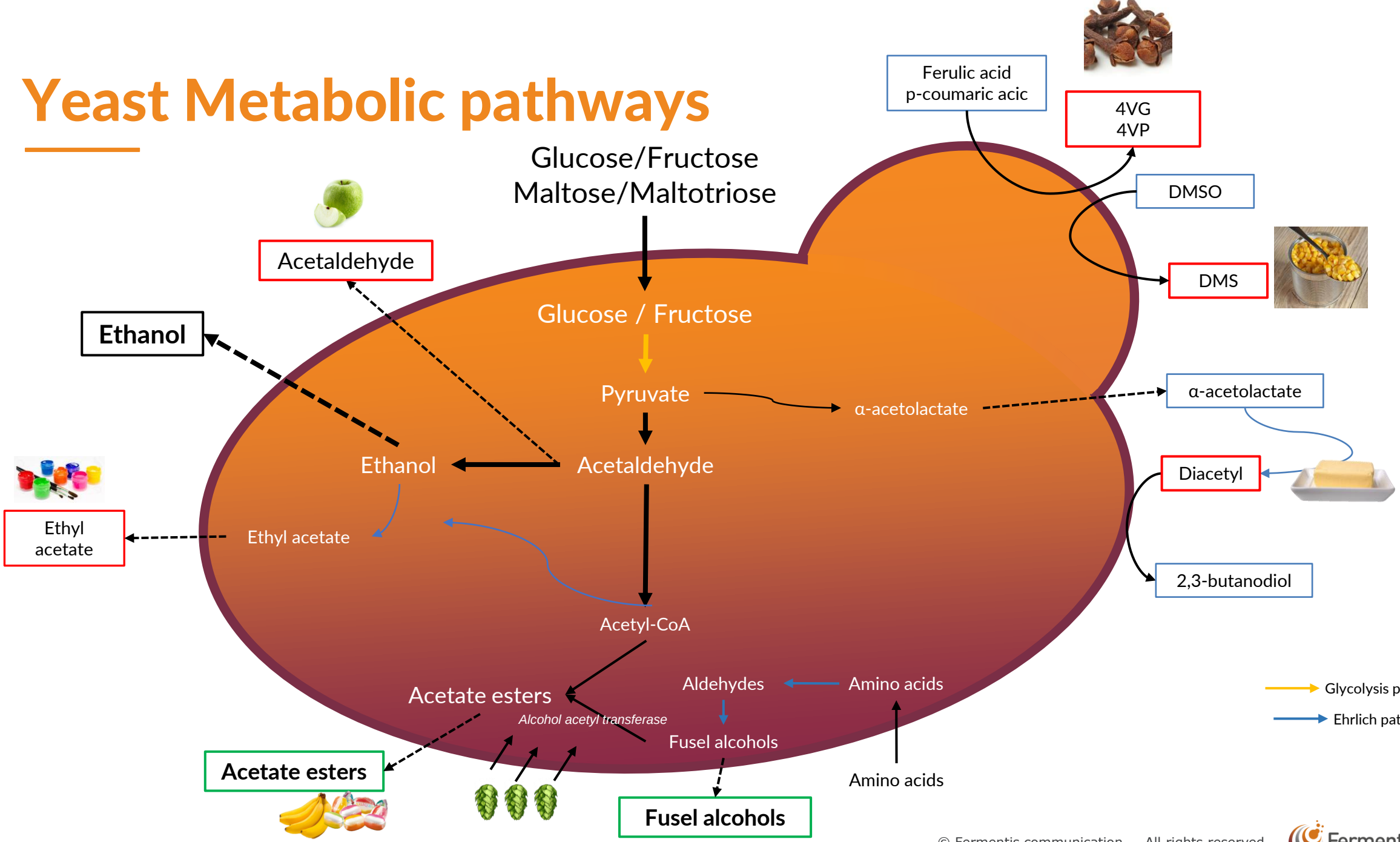
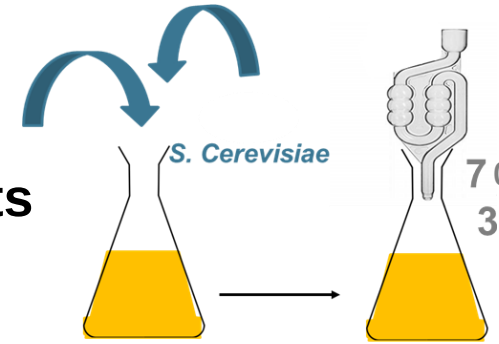
Yeast Metabolic pathways



2. Materials & methods

Cys-adducts
(10ppm)
&
G-adducts
(15ppm)

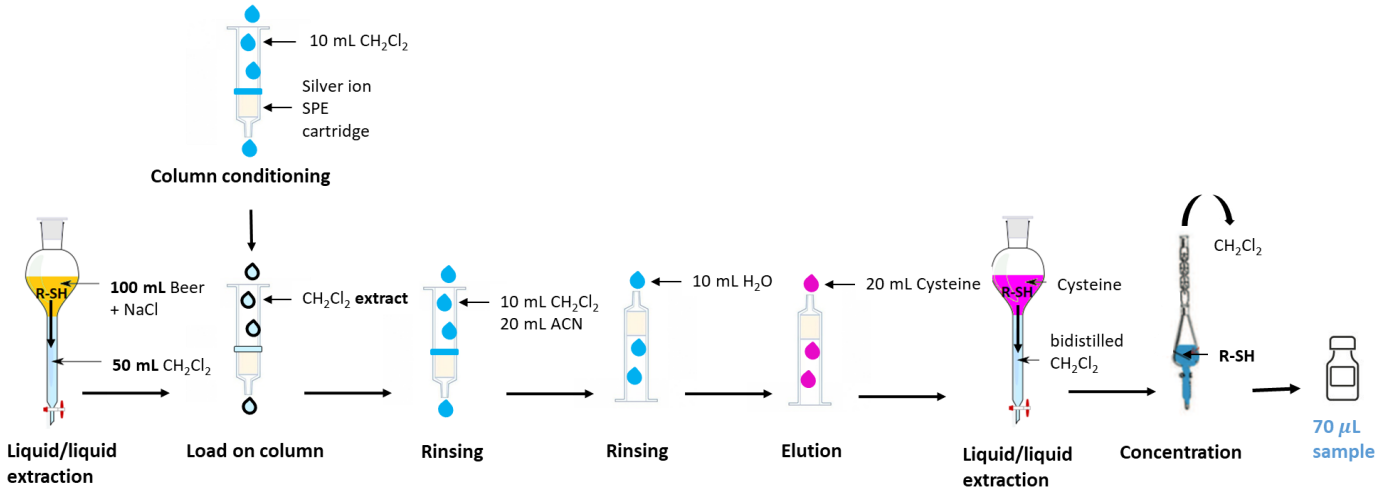


7 days at 24°C
3 days at 4°C

POF- Ale Yeast strains

Fermentation
&
Maturation

The released sulfanylalkyl alcohols and their corresponding acetates were extracted with an Ag-ion SPE cartridge and analyzed by GC-PFPD.



Extraction
&
Concentration

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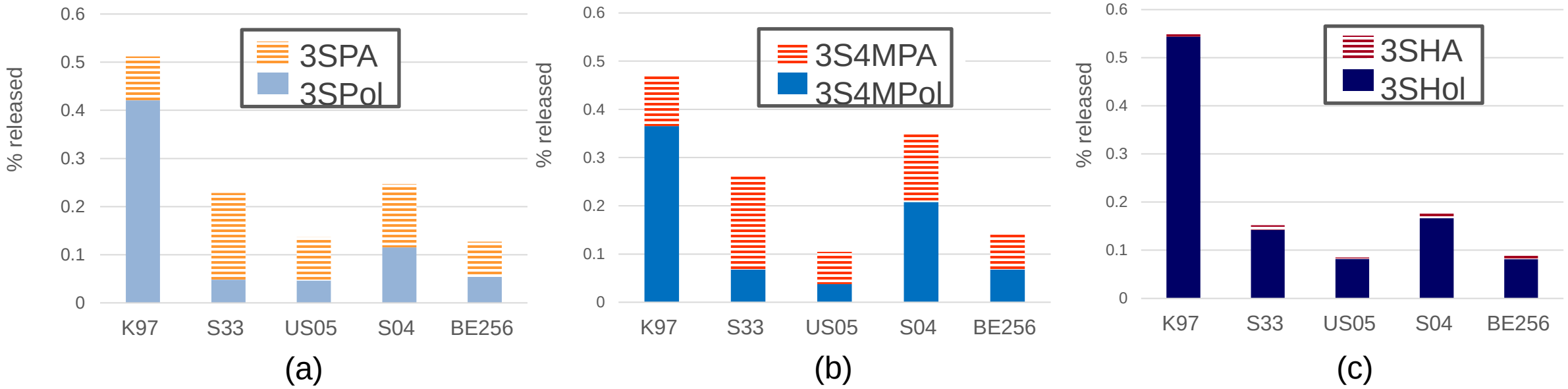
3. Results & Discussions

3.1. Conversion rate from Cys-adducts

From Cys-adducts



⇒ **Same selectivity of release according to thiol nature for the five yeasts:**

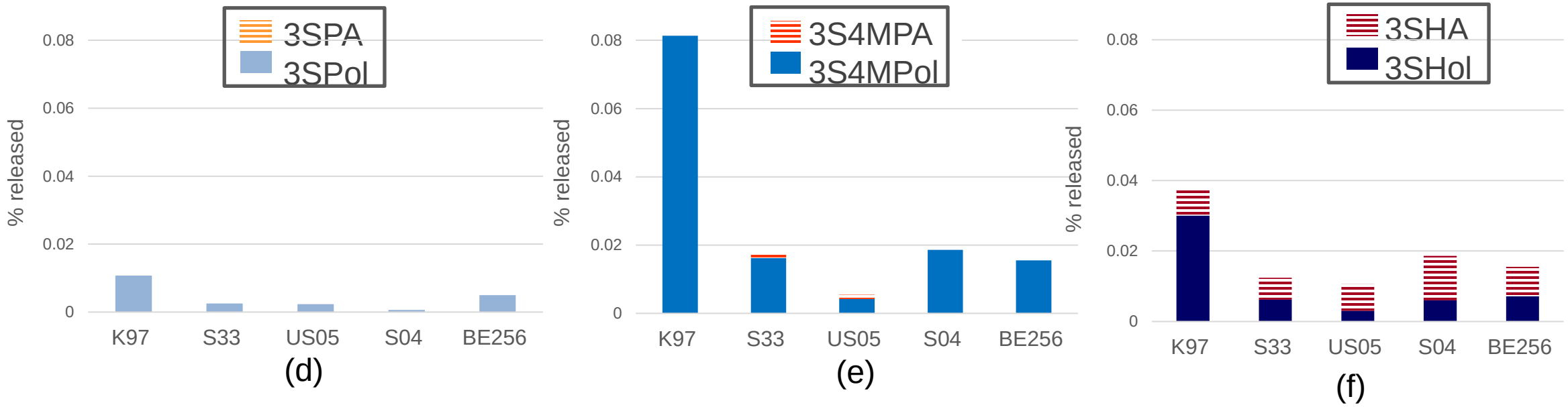
- **High esterification of 3SPol & 3S4MPol resulting in high total (alcohol+acetate) release (except for K-97)**
- **Low esterification of 3SHol**

⇒ **K-97 = the highest thiol release efficiencies**

⇒ **S-33 & S-04 = greater ability to esterify the alcohol forms**

3.1. Conversion rate from G-adducts

From G-adducts



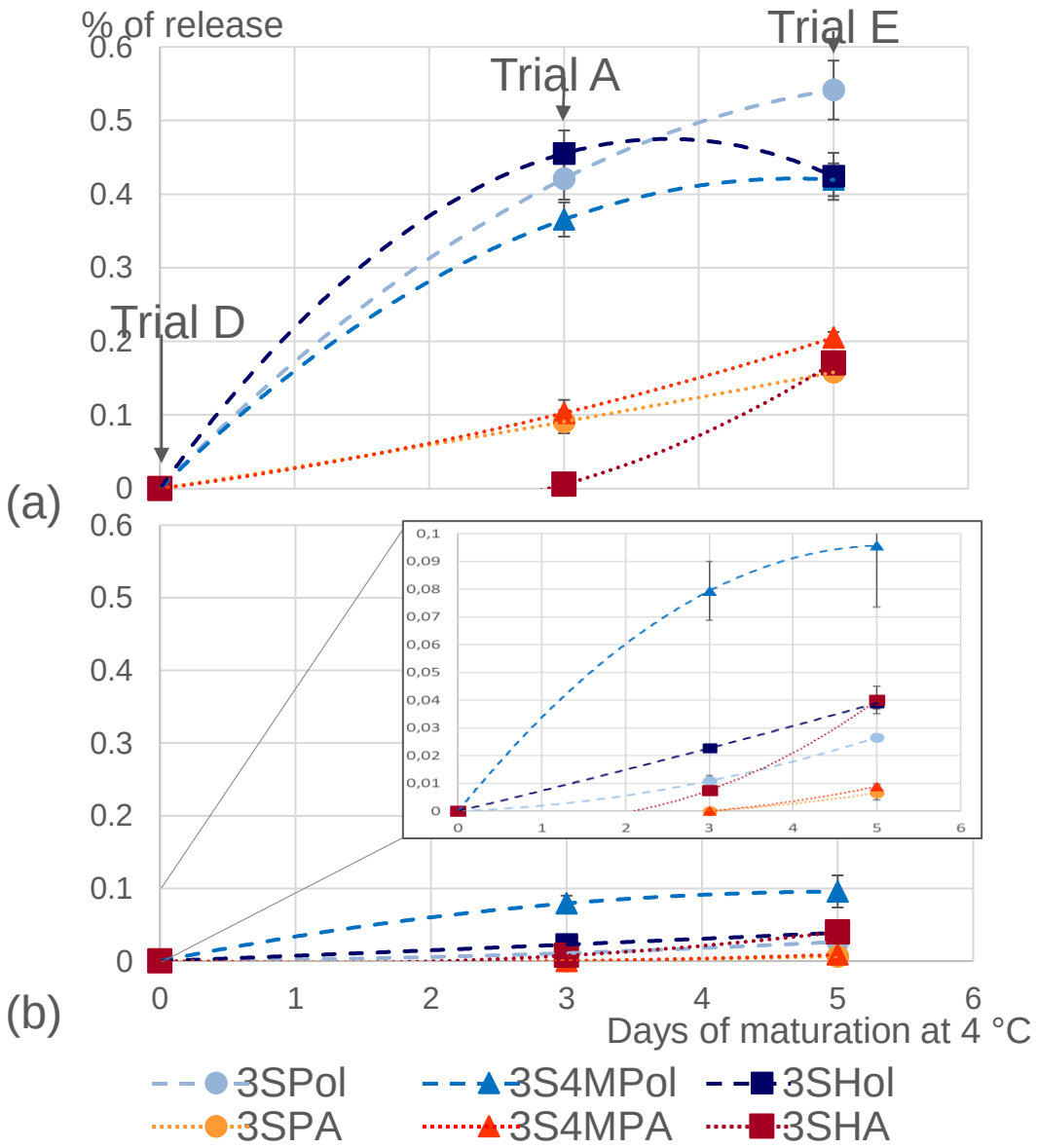
⇒ **Same selectivity of release according to thiol nature for the five yeasts:**

- **Low esterification of 3SPol & 3S4MPol**
- **Higher esterification of 3SHol resulting in high total (alcohol+acetate).**

⇒ **K-97 = the highest thiol release efficiencies, confirmed**

⇒ **S-04 & BE-256 = good ability to esterify the alcohol forms**

3.1. Conversion rate of precursors



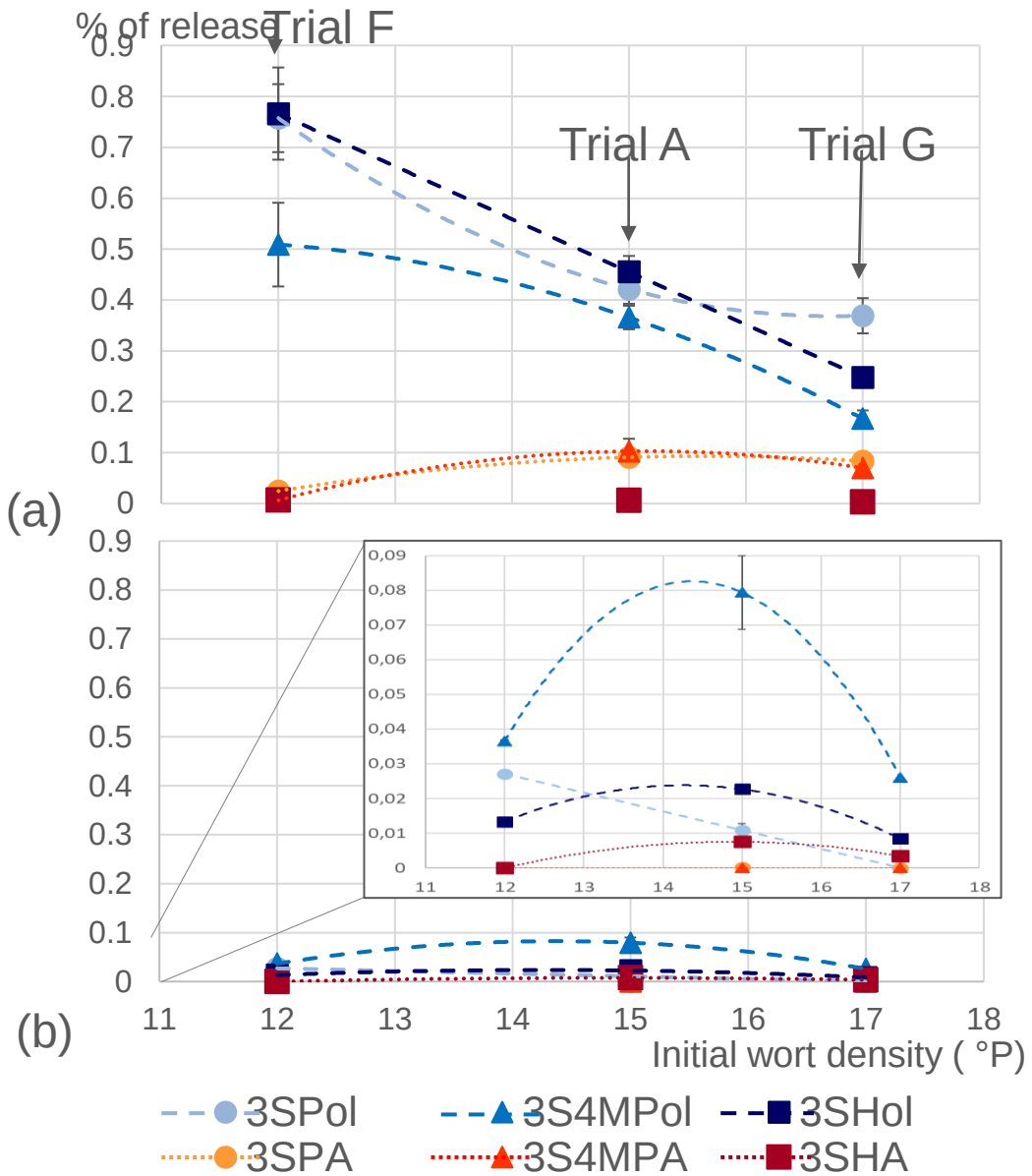
Maturation process

Release in function of maturation time at 4°C

⇒ **No thiol release observed if no maturation**

⇒ **3 days of maturation = good compromise**

3.1. Conversion rate of precursors



Wort production

Release in function of the original gravity (12-18°P)

⇒ **Higher release of alcohol forms at 12 °P**

⇒ **Higher esterification at 15-17 °P;**

⇒ **15 °P = good compromise**

3.2. Expression of Results in Odor Units (OU) with different hops: theoretical compilation

Theoretical experimental conditions

- Brewing wort 15 °P
  - Composition: 100% pils malt
  - Brewing diagram: 65°C/50min + 73°C/20min
  - Simulation of Dry hopping with 400 g/hl:
    - Citra
    - Hallertau blanc
    - Polaris
  - Fermentation of 7 days at 24 °C
  - Maturation of 3 days at 4 °C

Results expression:

- Using the content of free and bound thiols
- Using the specific yeast conversion rates
- Using the specific threshold of each thiols

*Calculation of results in odor units for each thiol**

$$OU = \frac{\text{Quantity of each thiol (estimation)}}{\text{Specific threshold for each thiol}}$$

** Assuming that 100% of each precursor is solubilized in the beer*

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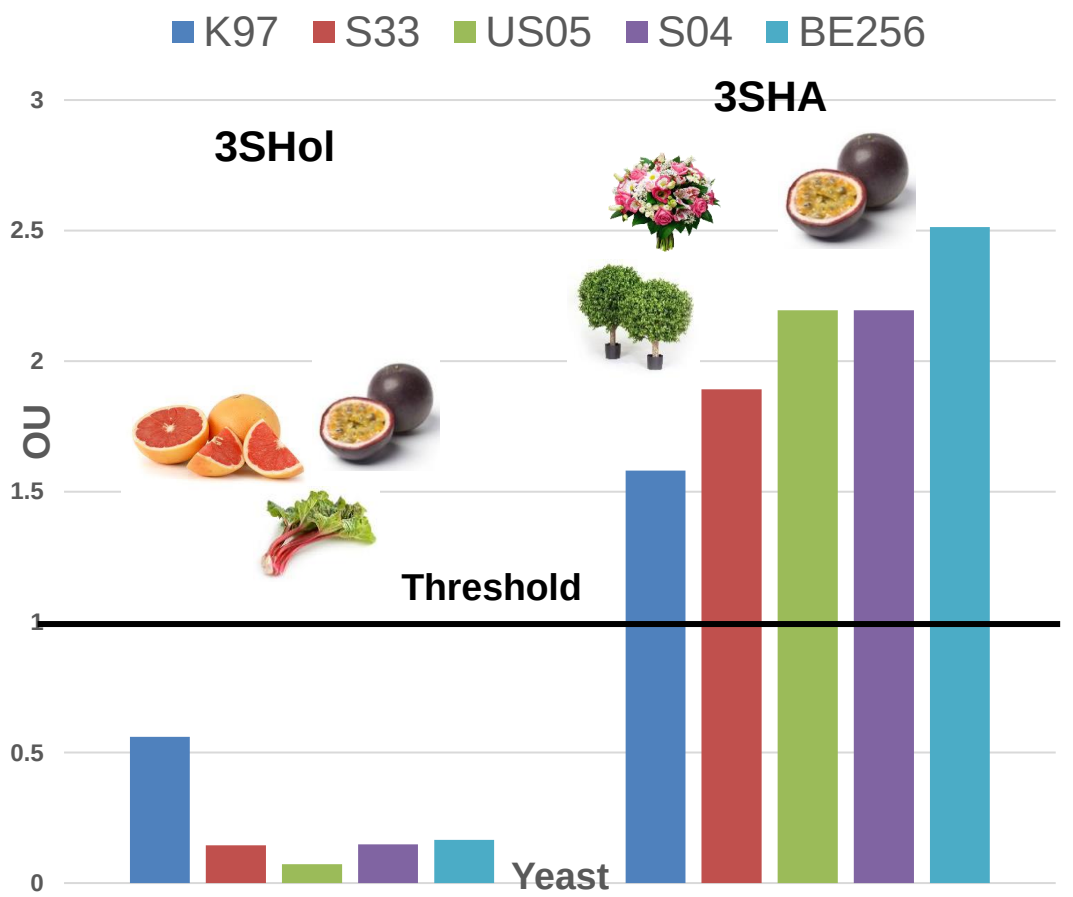
3.2. Expression of Results in Odor Units (OU) Citra (Cys- & G-adducts)

From Free & bound thiols

From Bound thiols



- **Most of the aromatic fraction come from the acetate form**
- **No differences between the yeasts for the total thiols**
- **Most of the thiols from Citra are free thiols**

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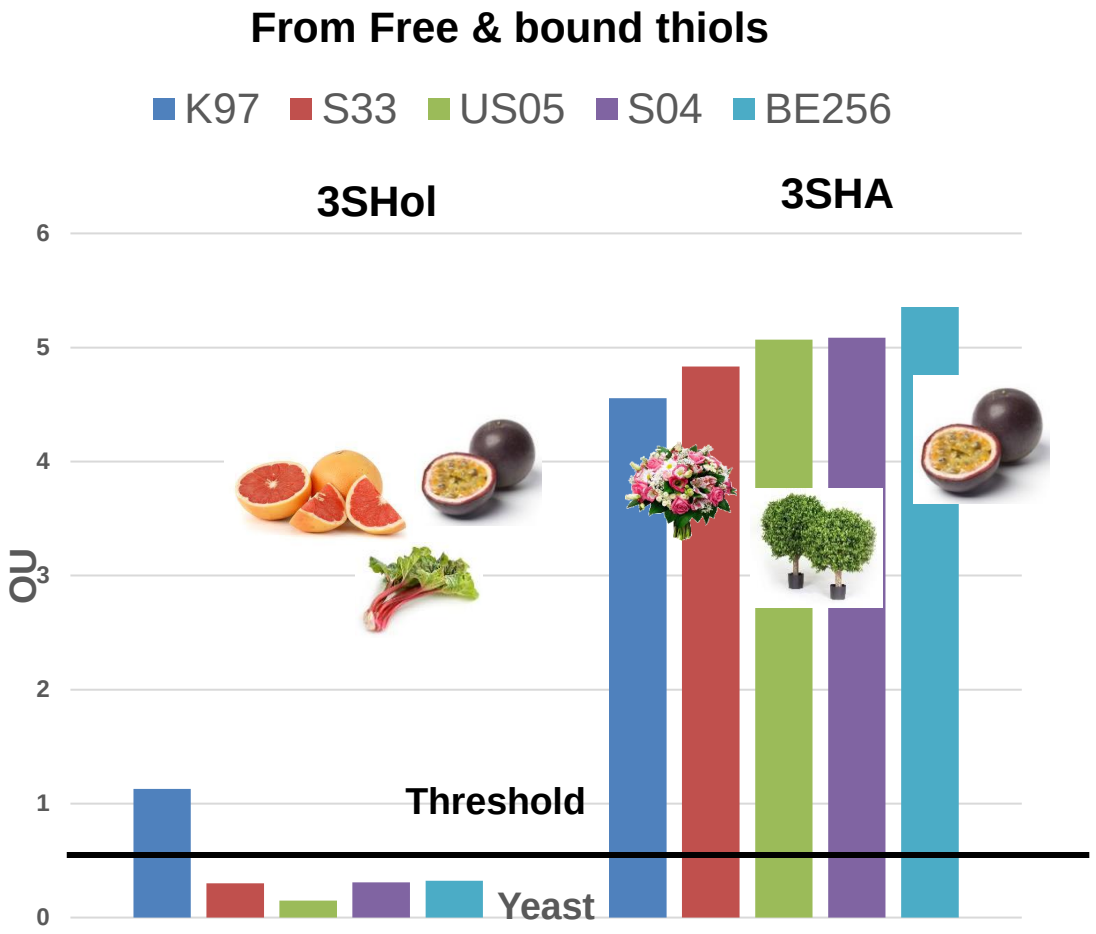
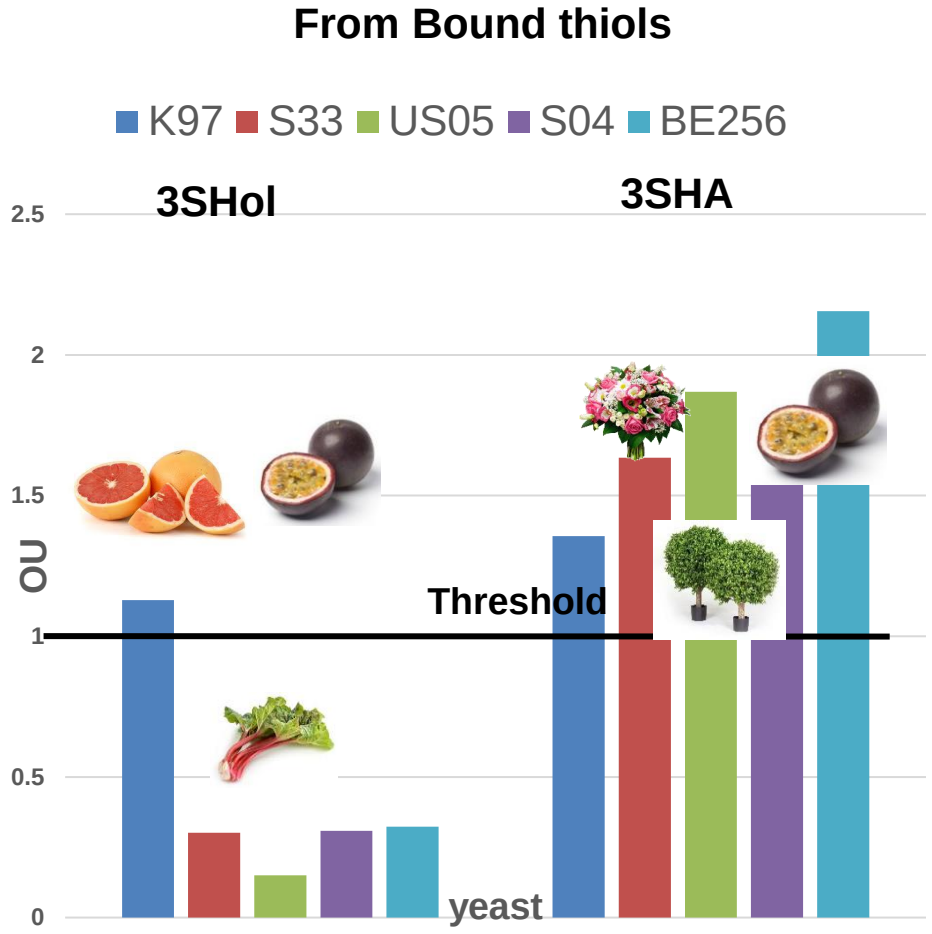
3.2. Expression of Results in Odor Units (OU) Hallertau Blanc (Cys- & G-adducts)





- Most of the aromatic fraction come from the acetate forms (except K-97)
- Not much differences between the yeasts for the total thiols
- More than 50% of the thiols from Hallertau Blanc are free thiols

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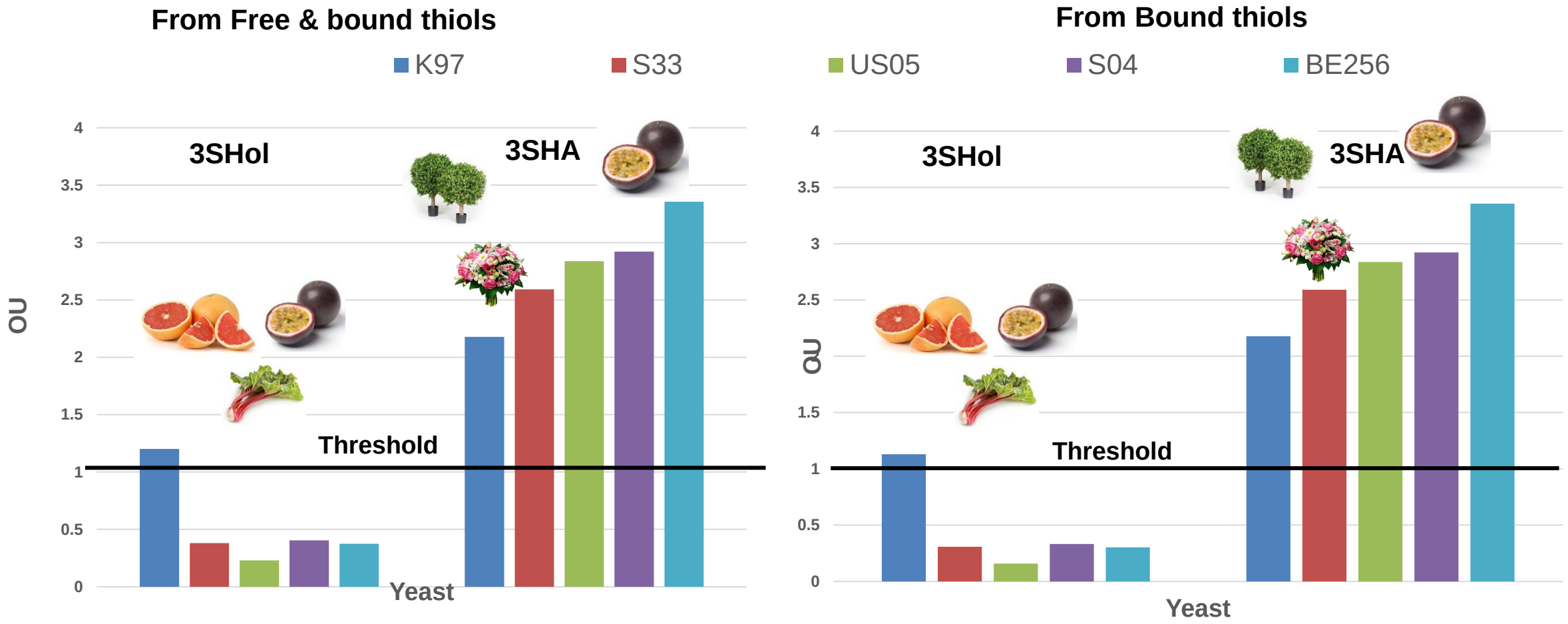
3.2. Expression of Results in Odor Units (OU) Polaris (Cys- & G-adducts)



- Most of the aromatic fraction come from the acetate forms
- Significant differences between the yeasts for the total thiols
- Most of the thiols from Polaris are bound thiols

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4. Conclusions

4. Conclusions

These new results confirm:

- **The ability of SafAle™ yeasts to release free thiols from both cysteinylated and glutathionylated adducts,**
- **SafAle™ K-97 remains the best candidate for its ability to release thiols under alcohol forms (release efficiency up to 0.45% and 0.08% from Cys- and G-adducts, respectively),**
- **SafAle™ S-33 and SafAle™ S-04 emerge as good challengers, for their better esterification efficiency,**
- **Maturation is mandatory to release free thiols in beer, optimum obtained after 3 days at 4°C,**
- **Original gravity of wort plays a role and 15°P is a good compromise,**
- **The flavor contribution of thiols in beer is much bigger with acetate forms of the thiols (after the esterification by the yeast or by using a hop rich in free thiols)**

Acknowledgment

Ph.D. Student Cécile Chenot
&
Prof. Sonia Collin