



**WORLD BREWING CONGRESS**

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#ElevateBeer



# Identification of a precursor of 2-mercapto-3-methyl-1-butanol in beer

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**Asahi**



Source: Demand Media

...and identifying  
the precursor of this  
off-flavor.

# Main topics

1. Why did we focus on the precursor?

2. How was the precursor identified?

3. How can onion-like off-flavor be reduced?

# 2M3MB (2-mercapto-3-methyl-1-butanol)

Odor

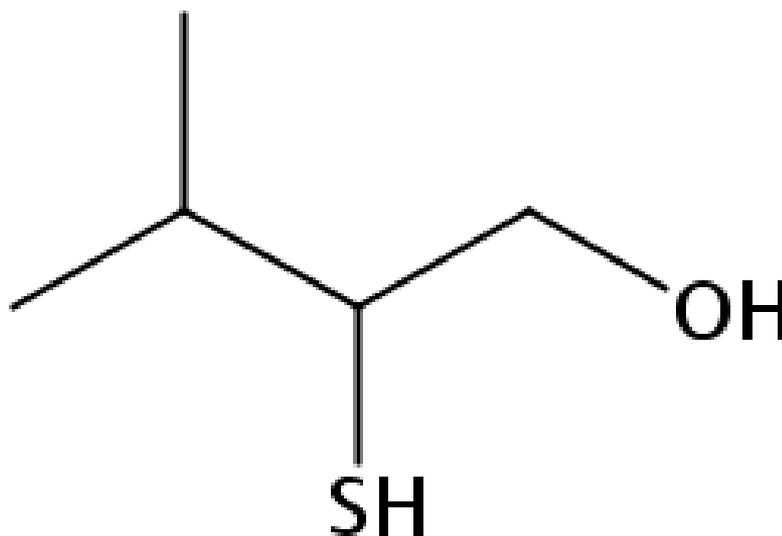
Onion, Sweat

Threshold

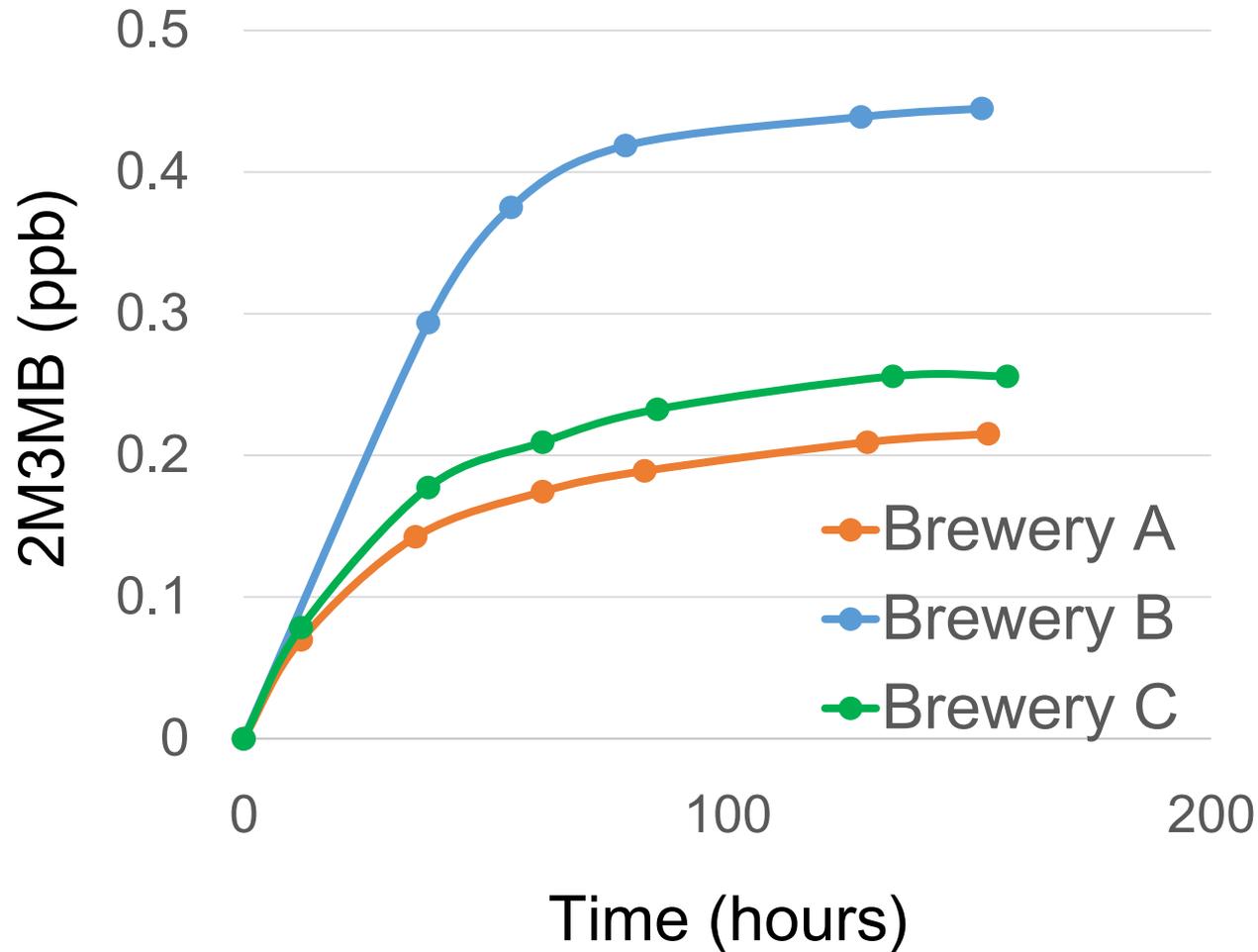
0.13 ppb

Origin

Hops



# 2M3MB formation during fermentation

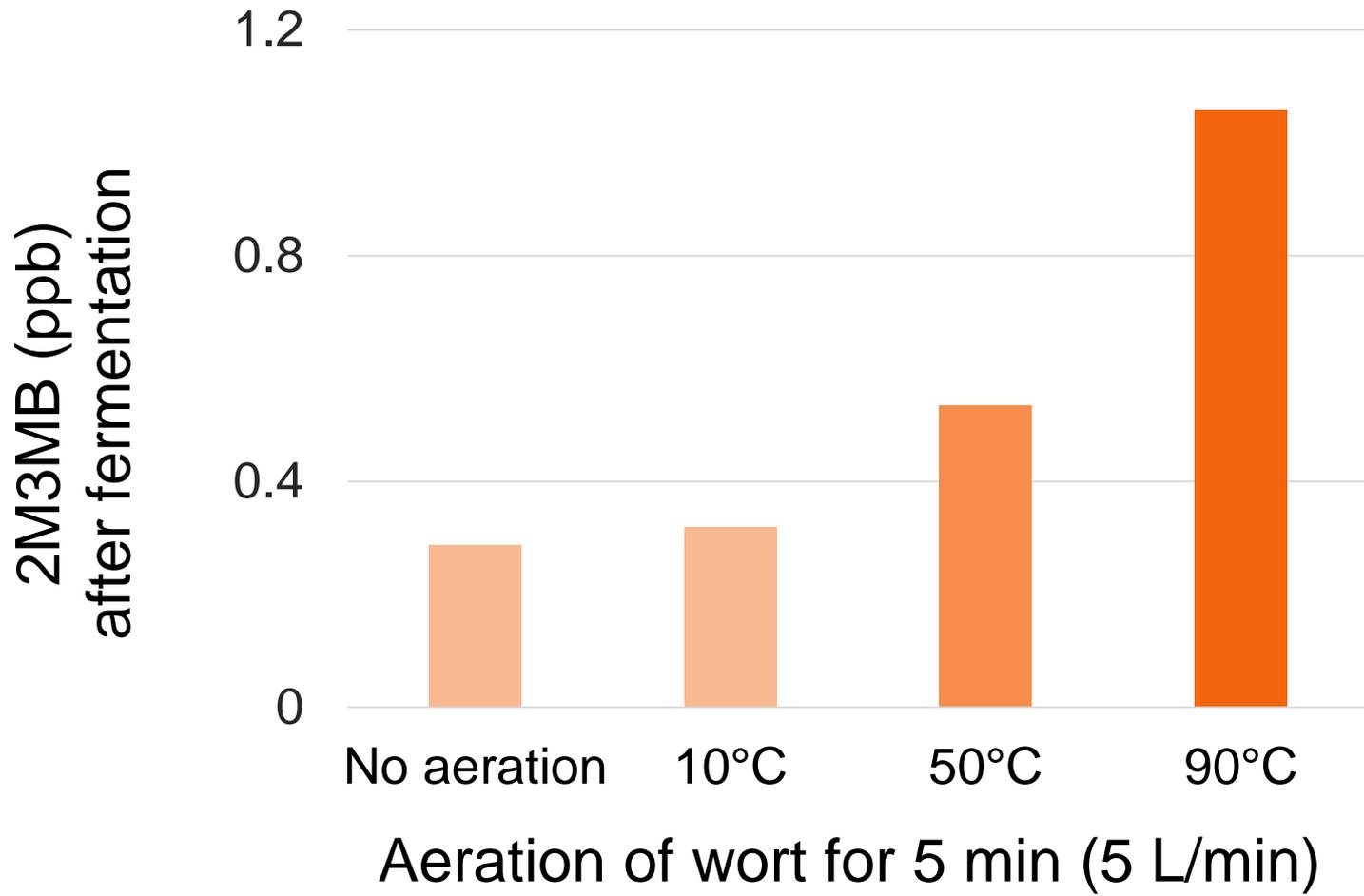




- **Hot aeration** of wort has been proposed as a cause of the onion-like odor in beer.

Liebenow, R. *et al. Mschr Brauerei* 20: 23-24 (1967)

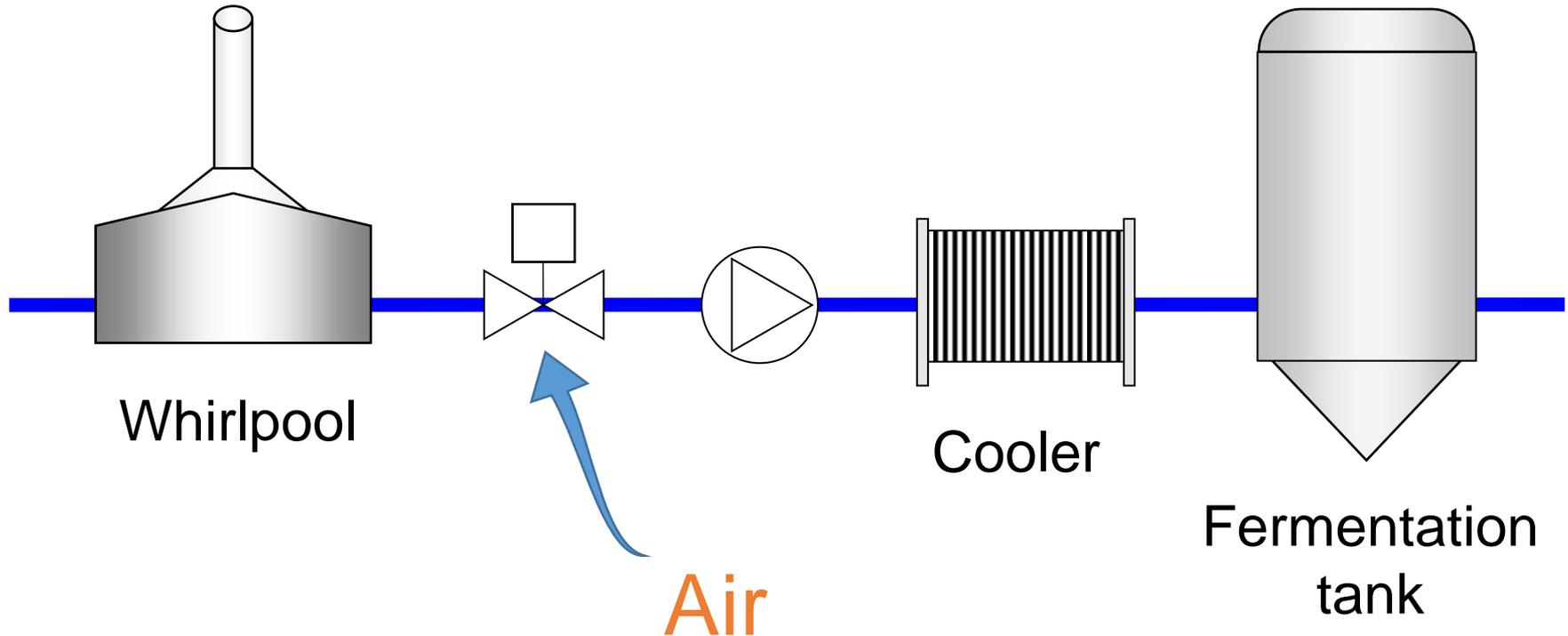




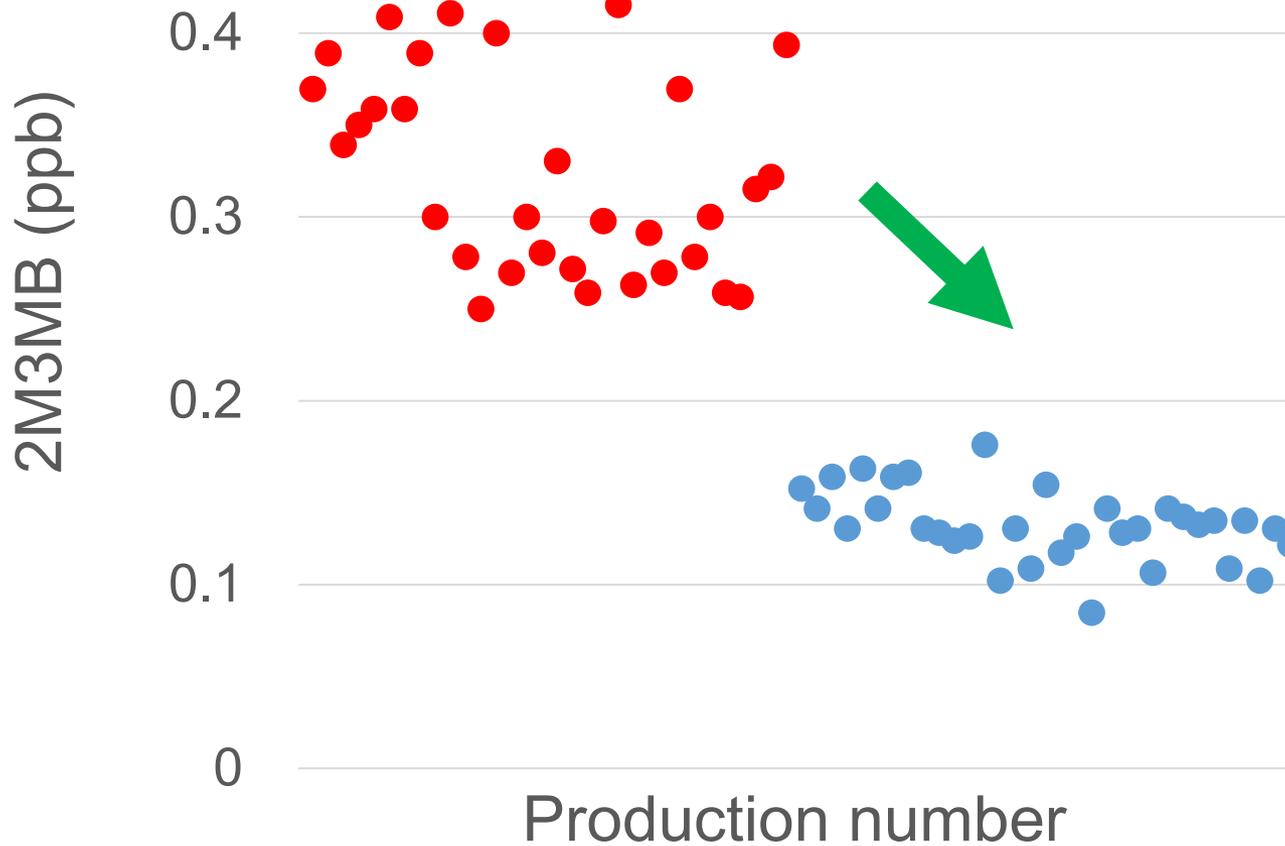
- Wort pumps of inadequate construction or faulty operation may cause onion off-flavors.

Narziss, R. *Brauwelt* 33 (1978)

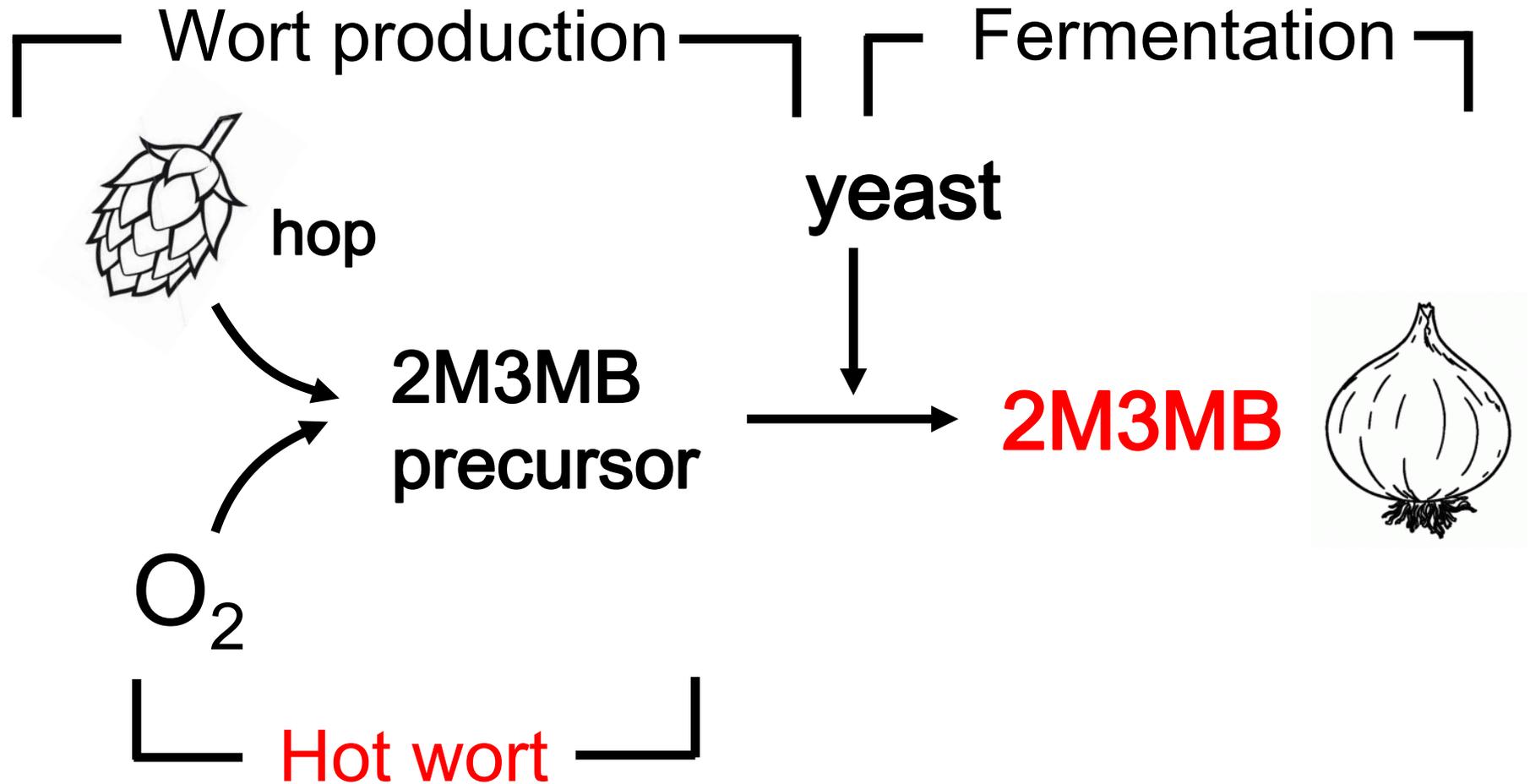
- Our experience:



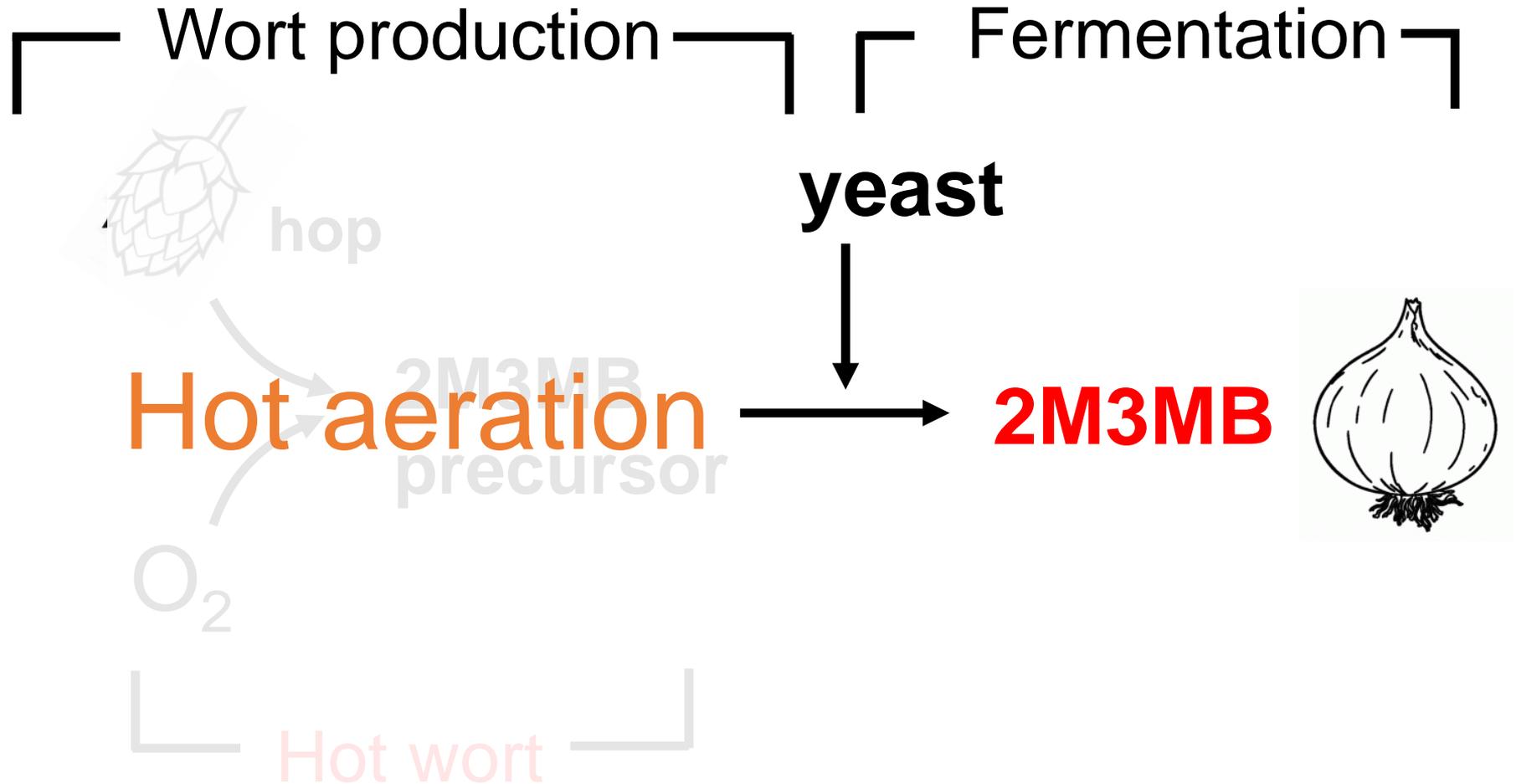
# Hot aeration of wort



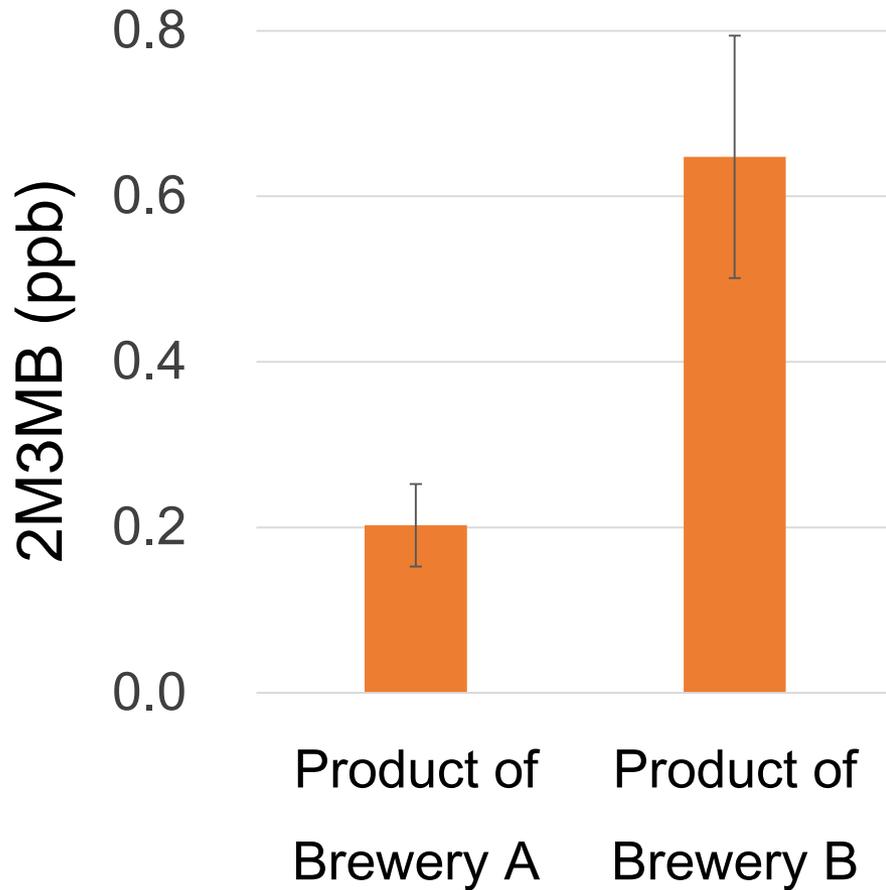
# Hypothetical pathway of 2M3MB formation



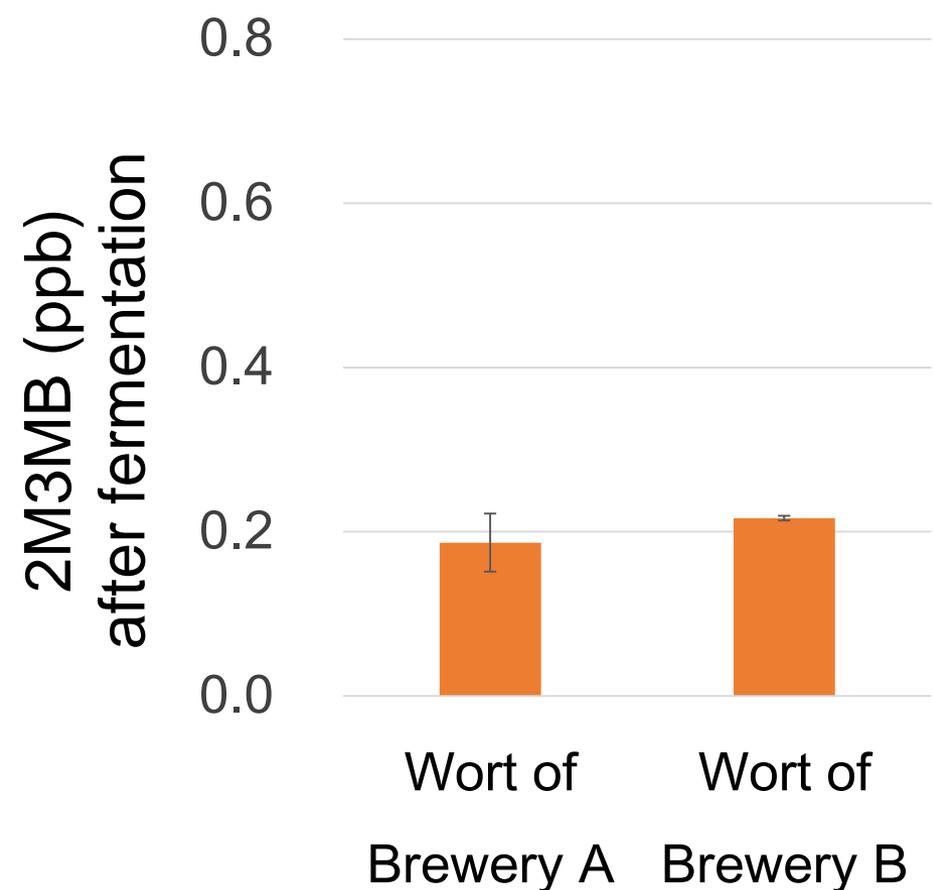
# Hypothetical pathway of 2M3MB formation



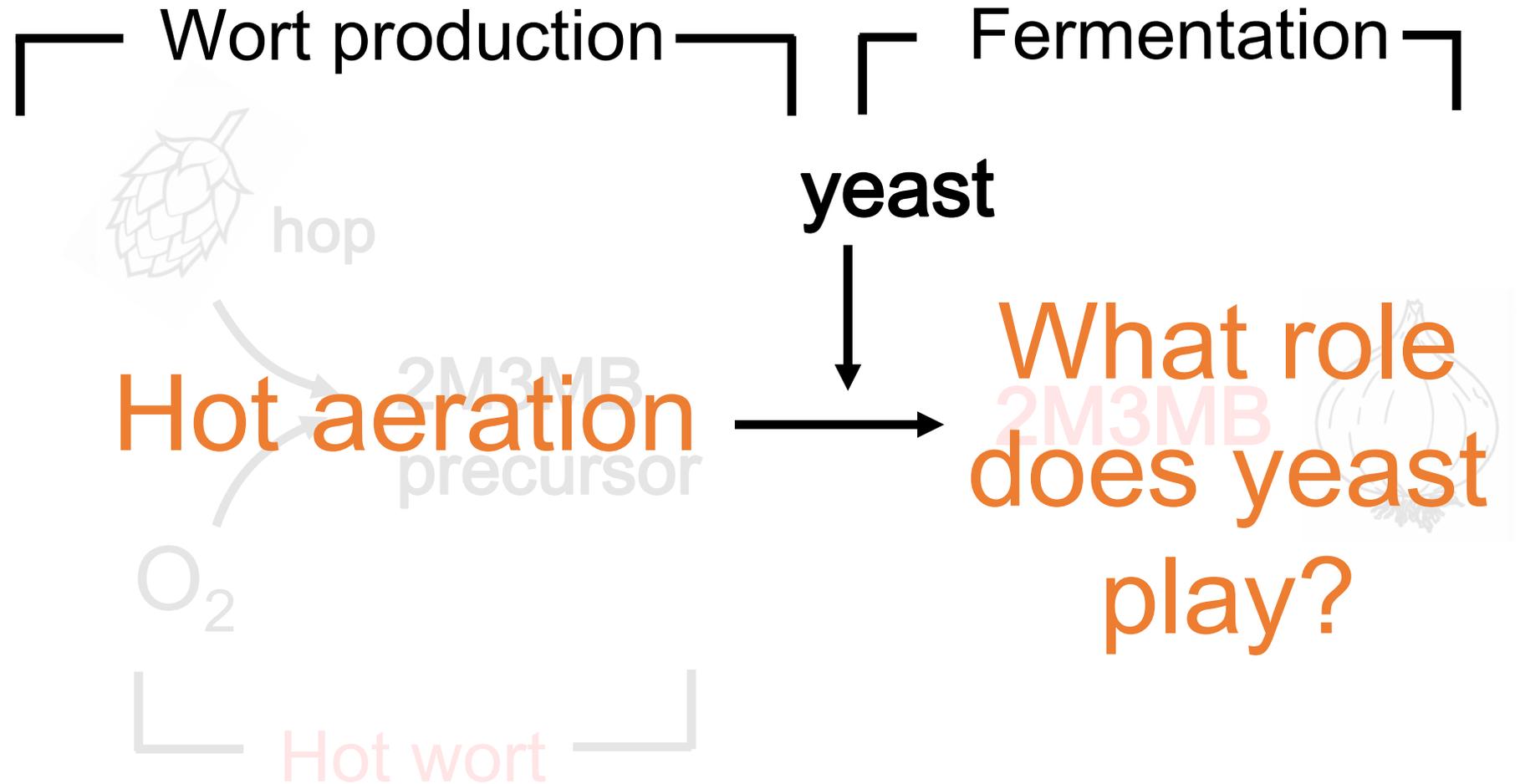
# Brewery



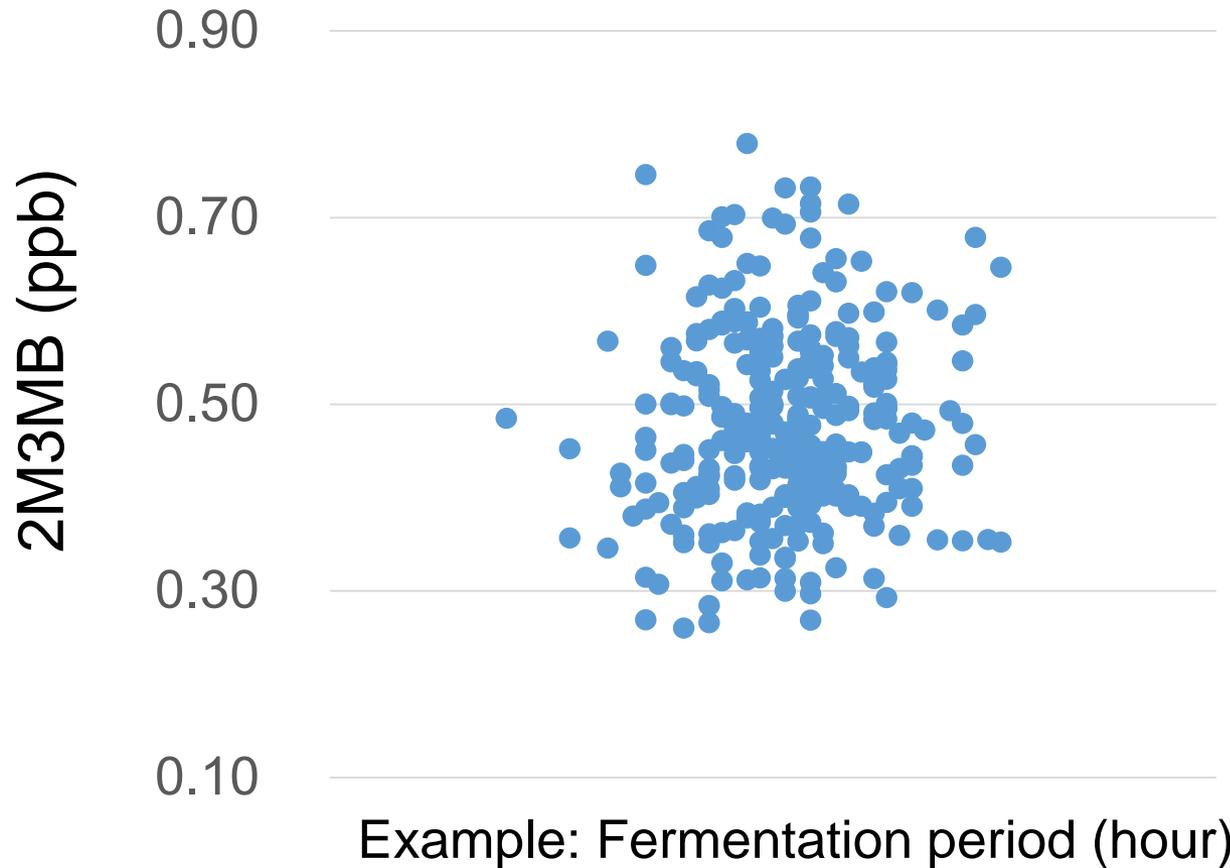
# Laboratory



# Hypothetical pathway of 2M3MB formation

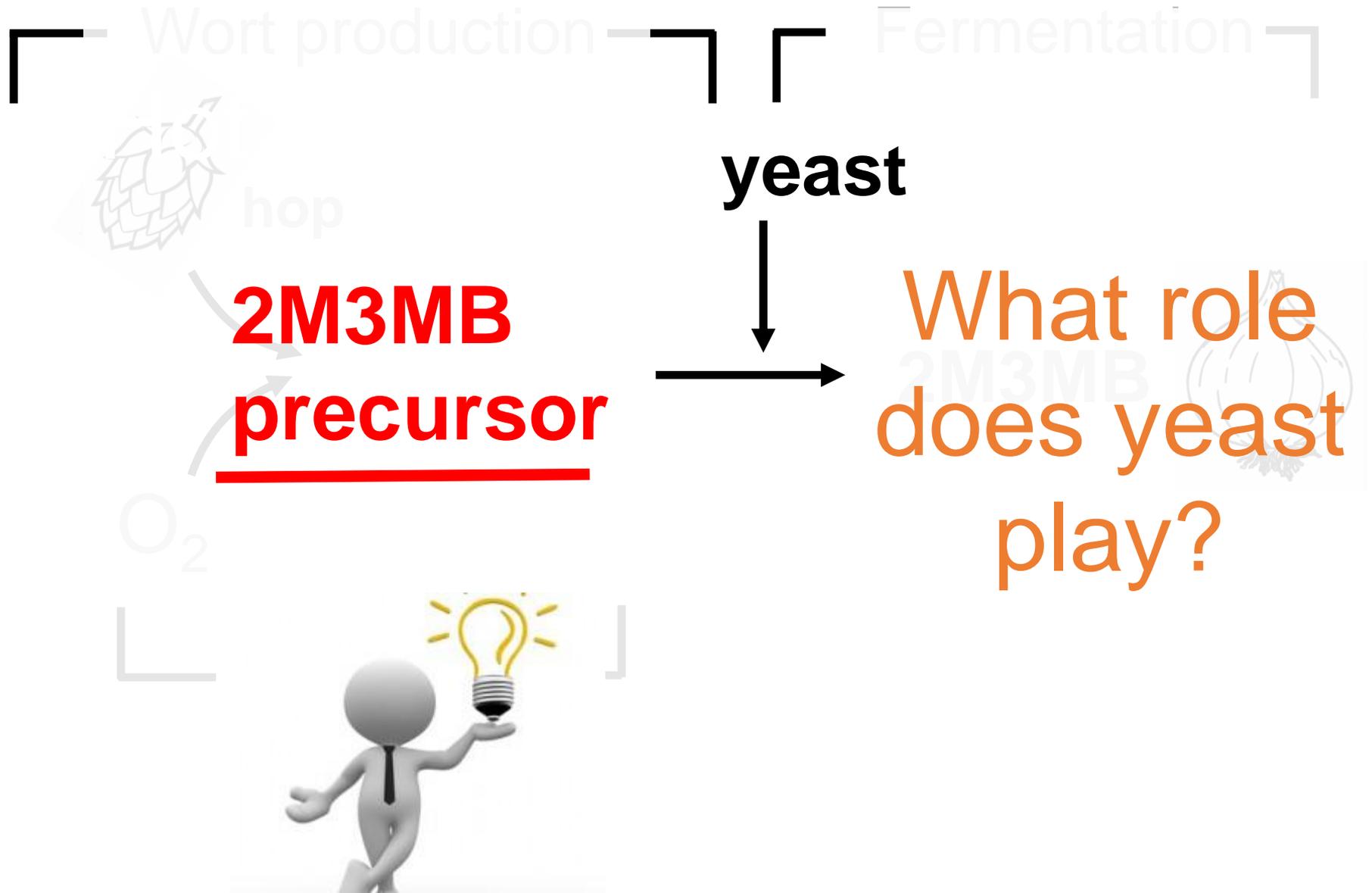


# Regression analysis



✓ No correlation was observed between 2M3MB concentrations and fermentation conditions.

# Hypothetical pathway of 2M3MB formation



# Main topics

1. Why did we focus on the precursor?

2. How was the precursor identified?

3. How can onion-like off-flavor be reduced?

# The difficulty of searching for a precursor

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# The difficulty of searching for a precursor

Like searching for a diamond on the beach

- Many similar transparent stones
- Many impurities



# Purification scheme of the precursor

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Isomerized hop extract



Oxidation



Distillation



Liquid-liquid extraction

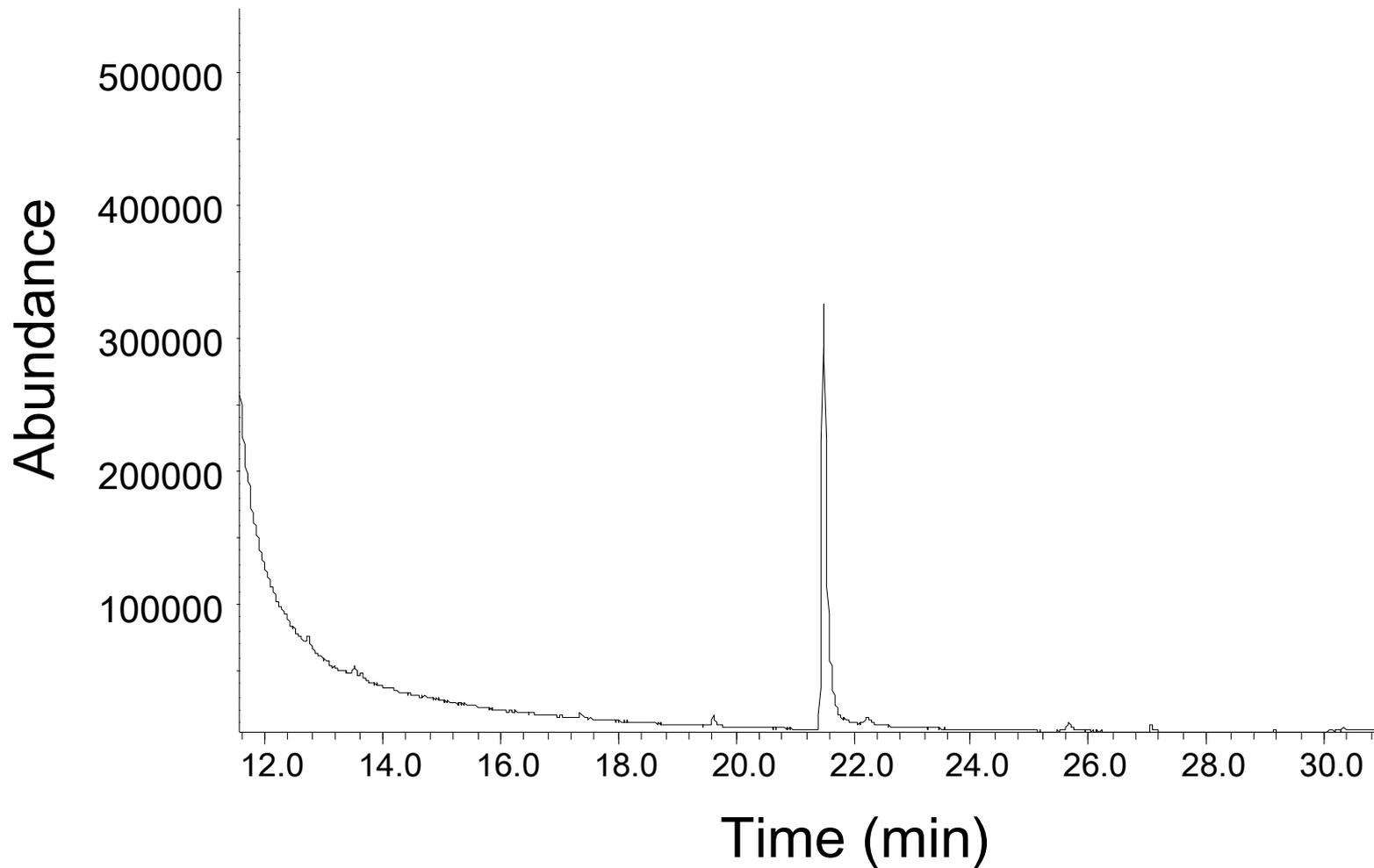


Normal-phase chromatography

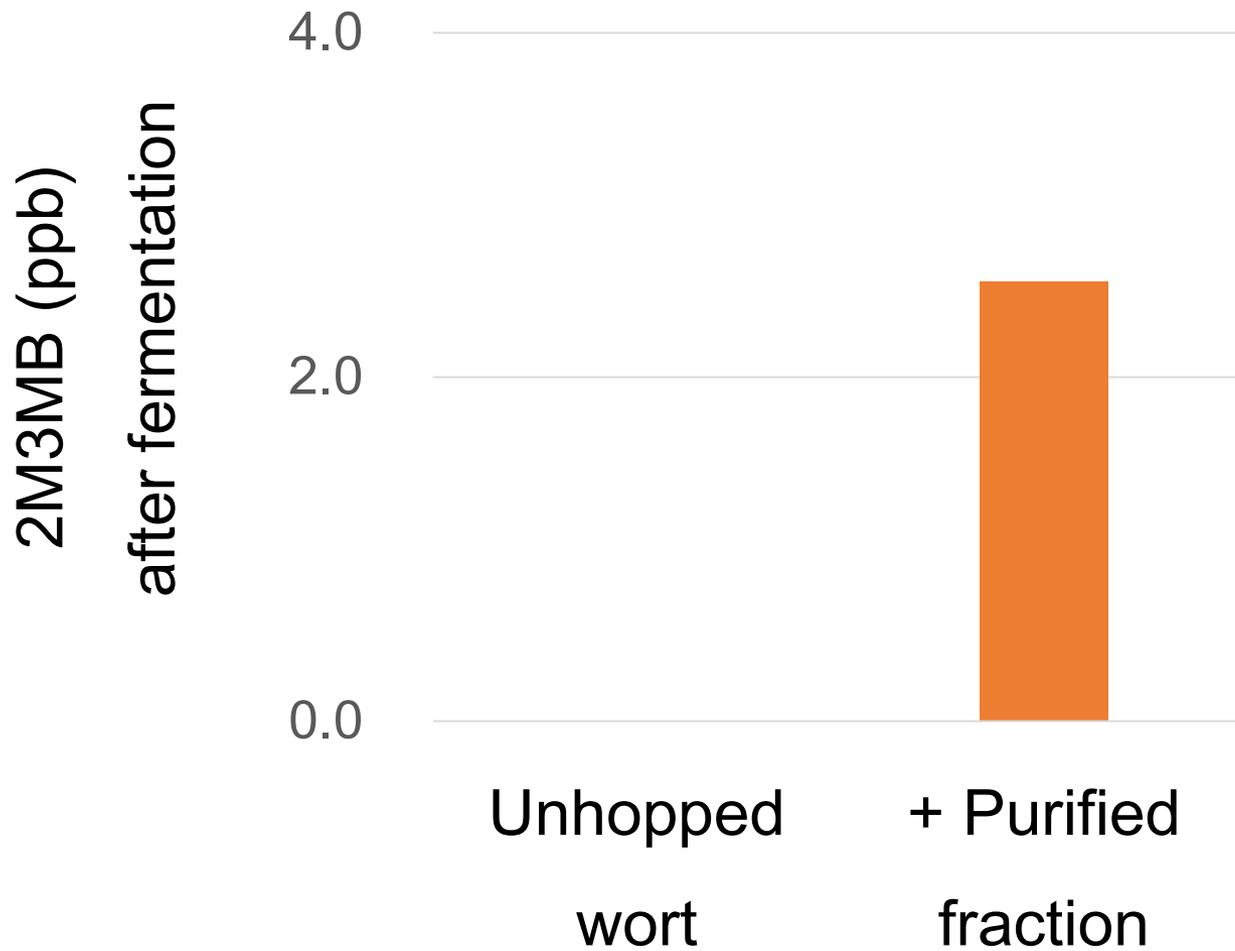


Preparative-GC

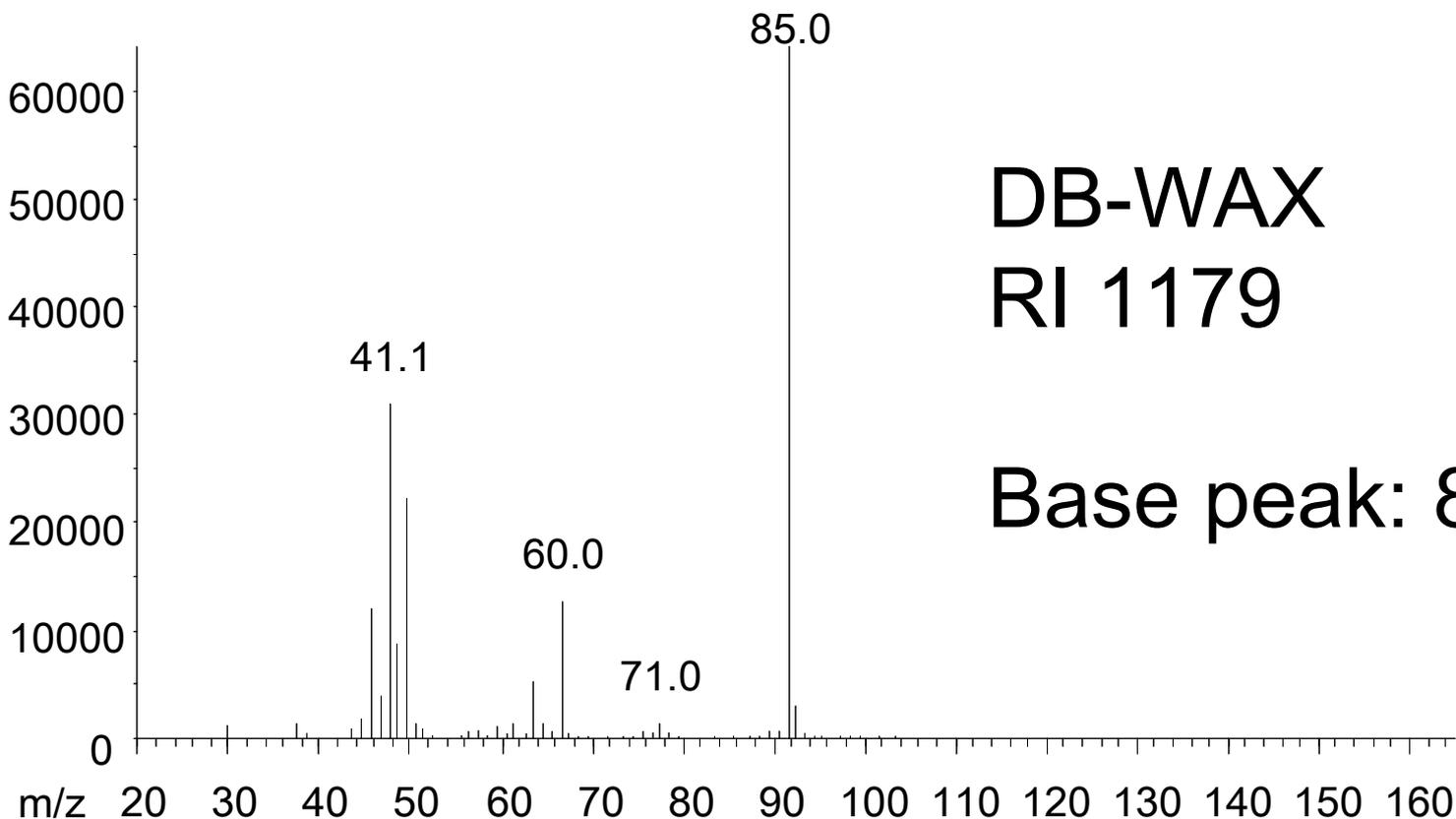
# GC/MS chromatogram of the purified fraction



# Addition test of the purified fraction



# GC/MS spectrum of the purified fraction



- ✓ This compound was not present in the mass spectral library

# Structural analysis



**GC/Q-TOF**  
**(high-resolution GC/MS)**

Calculation of the molecular  
formula



Assembling the structure from  
the molecular formula and  
fragment ions

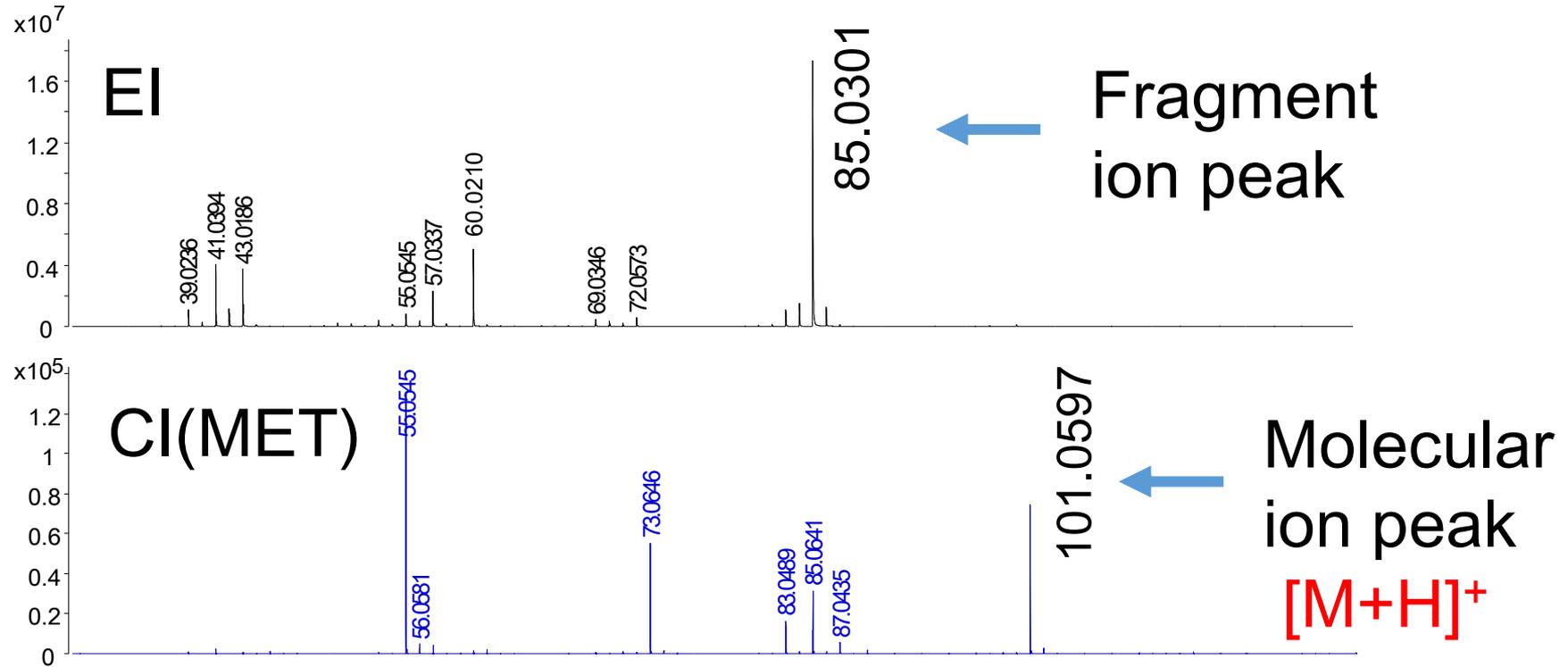
# RULES

- All pieces must be used.
- All pieces must touch.





# Calculation of molecular formula

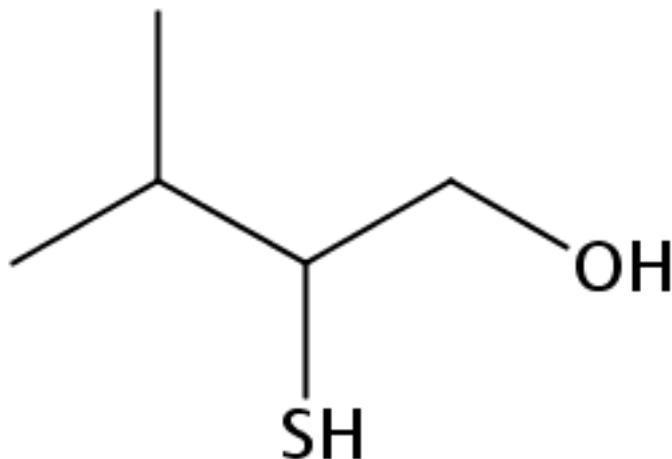


$$[M+H]^+ = m/z \ 101.0597$$

$$\text{Molecular weight (M)} = 100.0524$$

**Molecular formula**  $C_5H_8O_2$

# Prediction of the structure



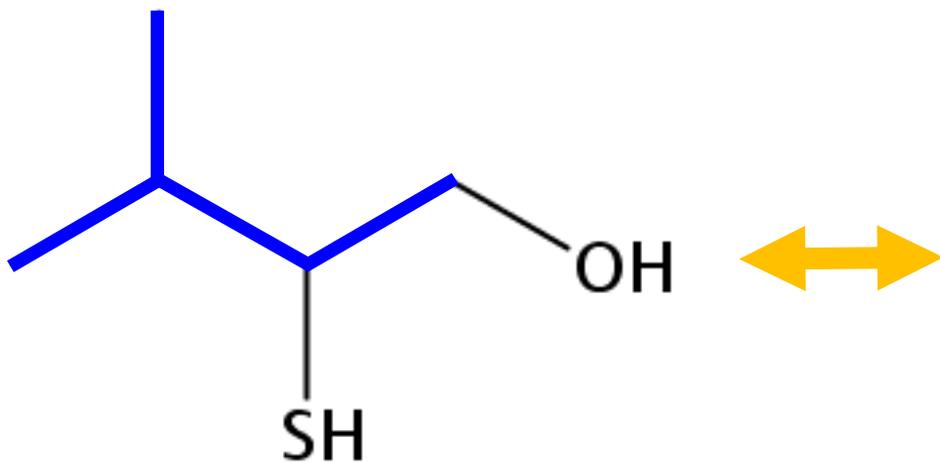
2M3MB (C<sub>5</sub>H<sub>12</sub>OS)



Unknown (C<sub>5</sub>H<sub>8</sub>O<sub>2</sub>)

## RULES

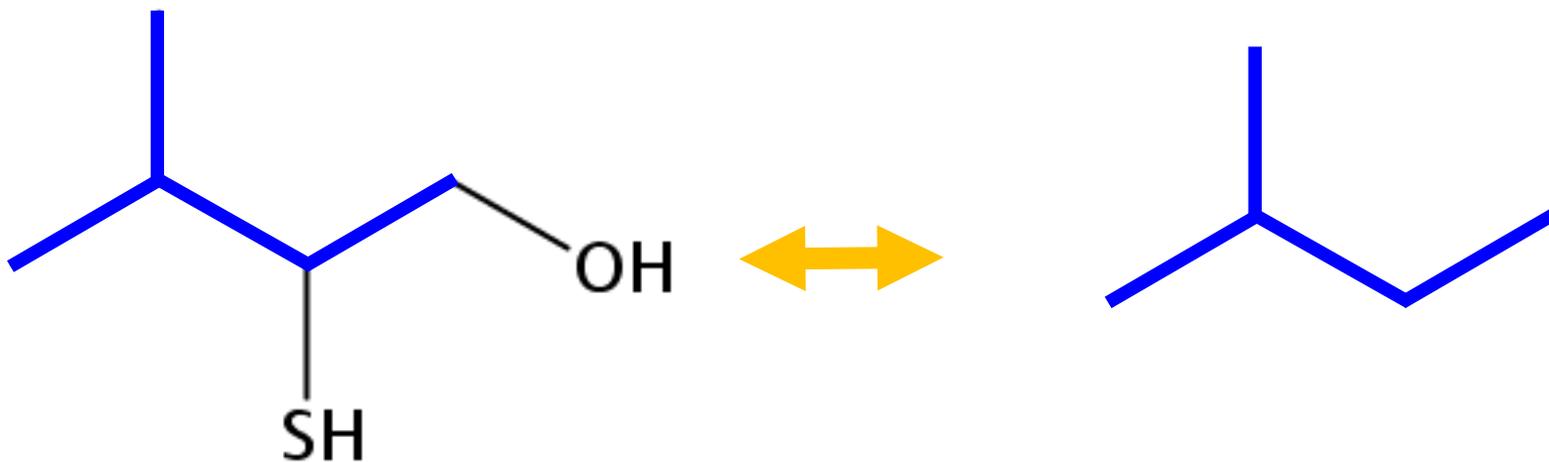
- All pieces must be used.
- All pieces must touch.
- Guess the shape.



2M3MB (C<sub>5</sub>H<sub>12</sub>OS)



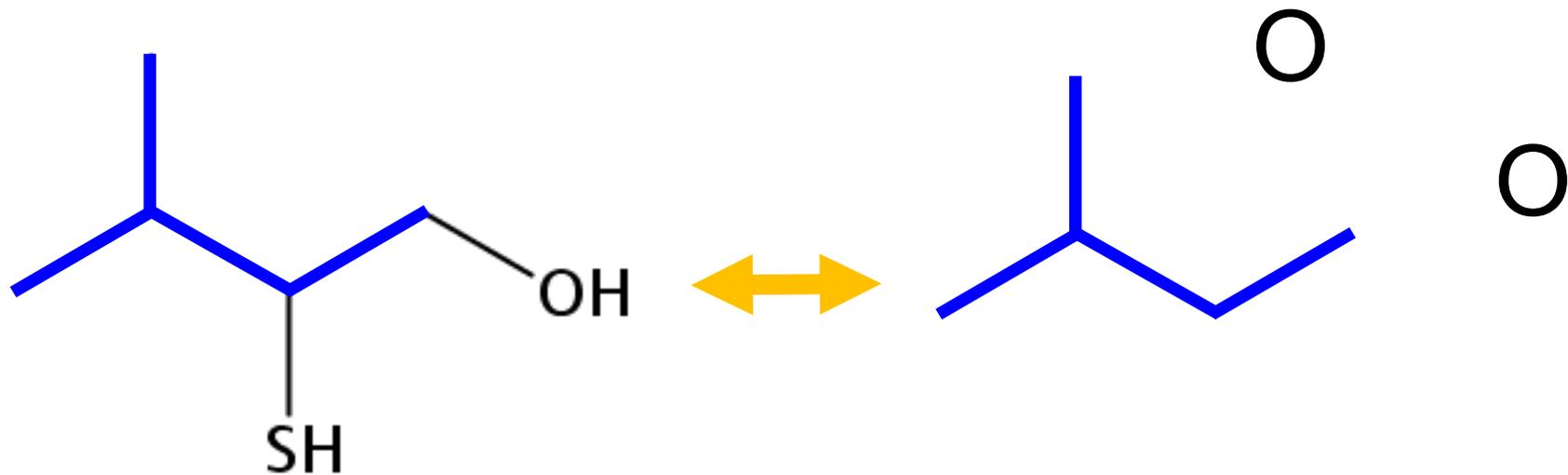
Unknown (C<sub>5</sub>H<sub>8</sub>O<sub>2</sub>)



2M3MB ( $C_5H_{12}OS$ )

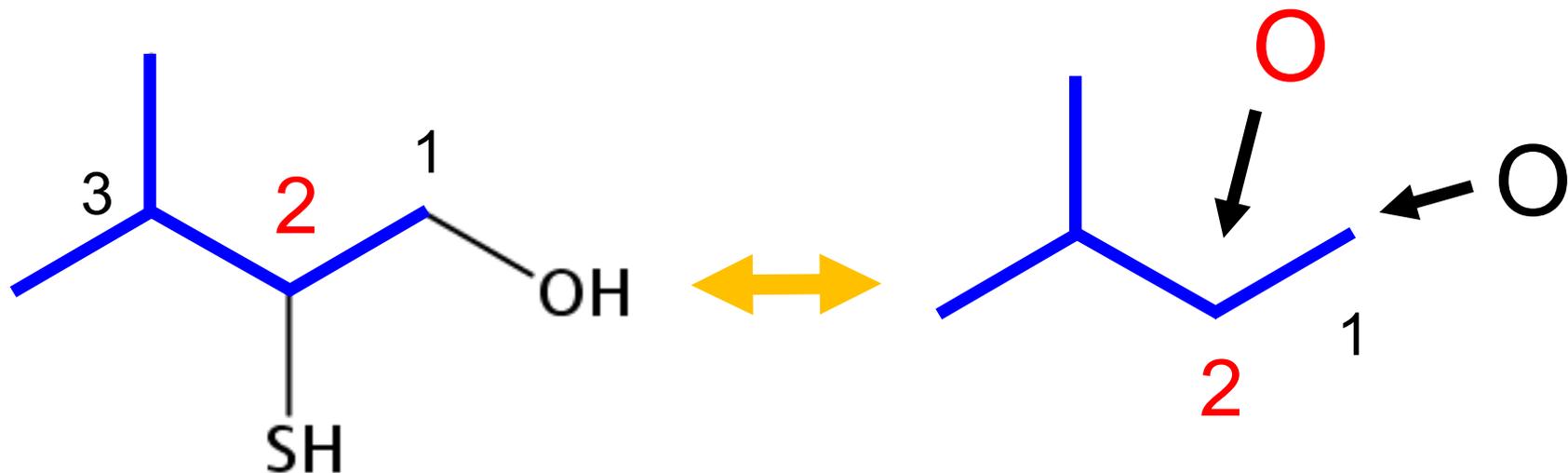
Unknown ( $C_5H_8O_2$ )

✓ Guess: the same carbon skeleton



2M3MB (C<sub>5</sub>H<sub>12</sub>OS)

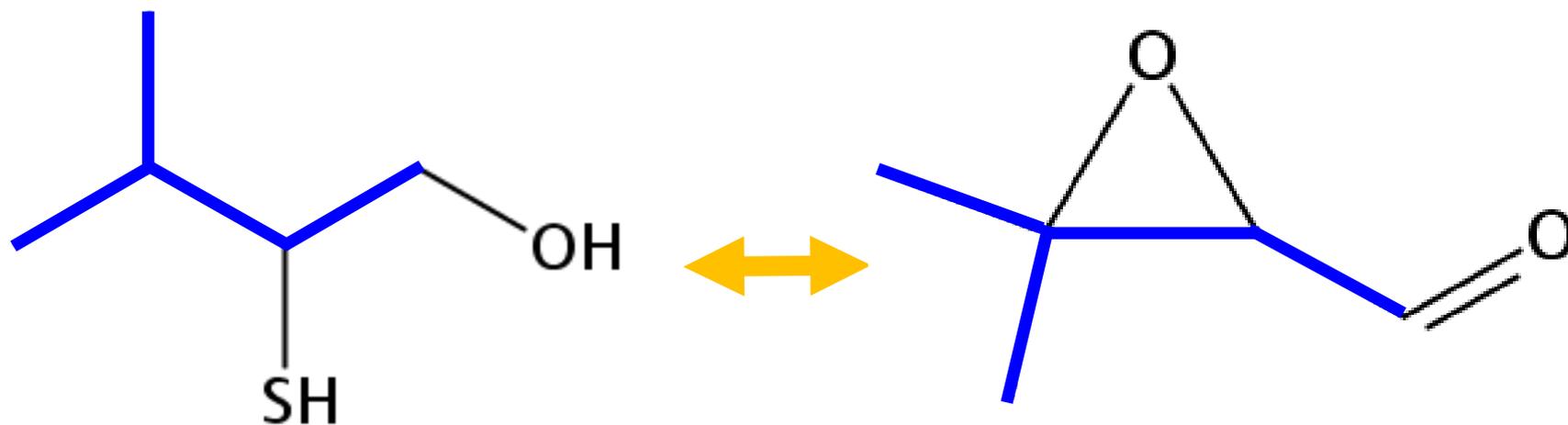
Unknown (C<sub>5</sub>H<sub>8</sub>O<sub>2</sub>)



2M3MB (C<sub>5</sub>H<sub>12</sub>OS)

Unknown (C<sub>5</sub>H<sub>8</sub>O<sub>2</sub>)

- ✓ Guess: **O** has higher reactivity  
→ epoxide ring



2M3MB ( $C_5H_{12}OS$ )

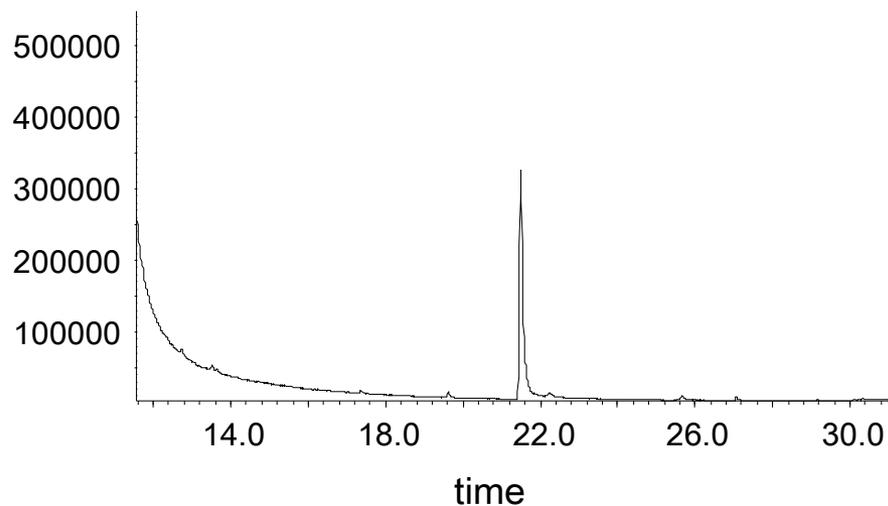
Unknown ( $C_5H_8O_2$ )

- ✓ Putative structure:  
2,3-epoxy-3-methyl-butanal (EMB)

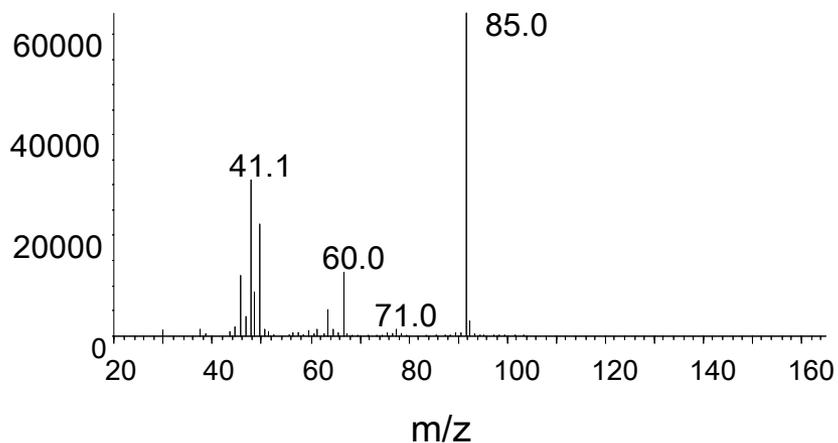
# Identification of 2,3-epoxy-3-methyl-butanal

Purified

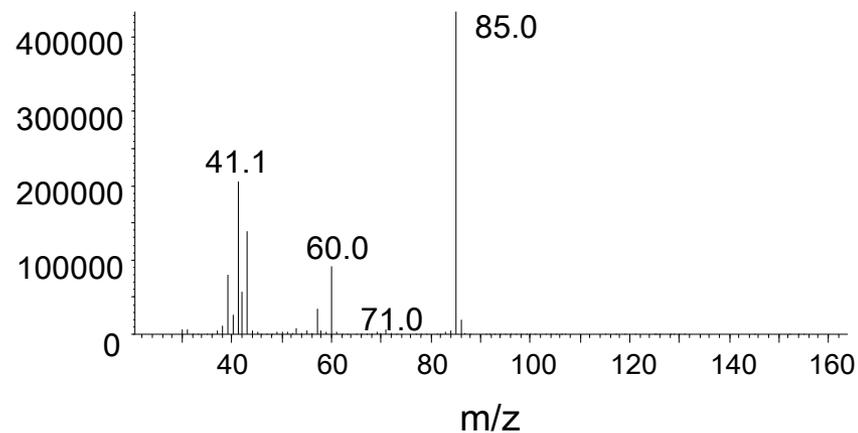
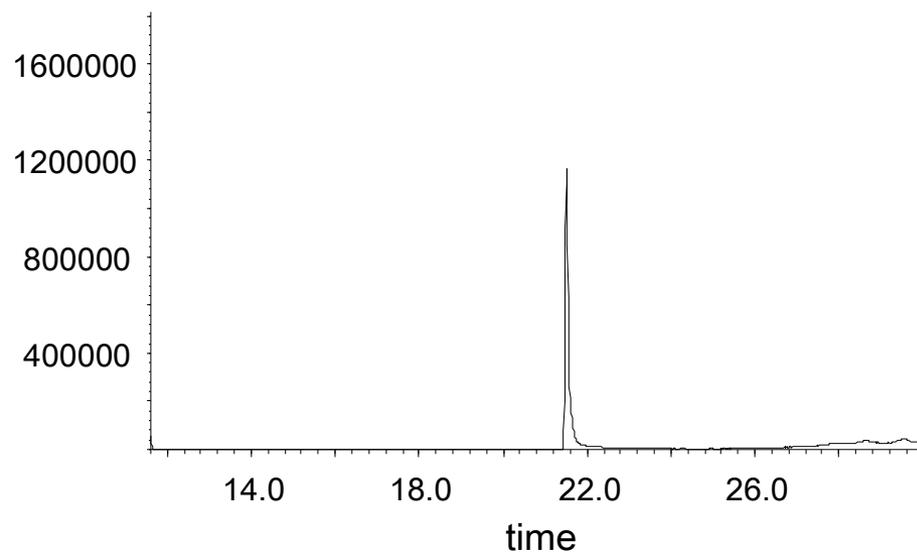
GC/MS chromatogram



GC/MS spectrum



Synthesized EMB



# Short Summary

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- ✓ Unknown compound purified from iso-hop extract was a potential 2M3MB precursor.
- ✓ Unknown compound was presumed to be 2,3-epoxy-3-methyl-butanal (EMB).
- ✓ GC/MS data of the unknown compound and synthesized EMB were identical.

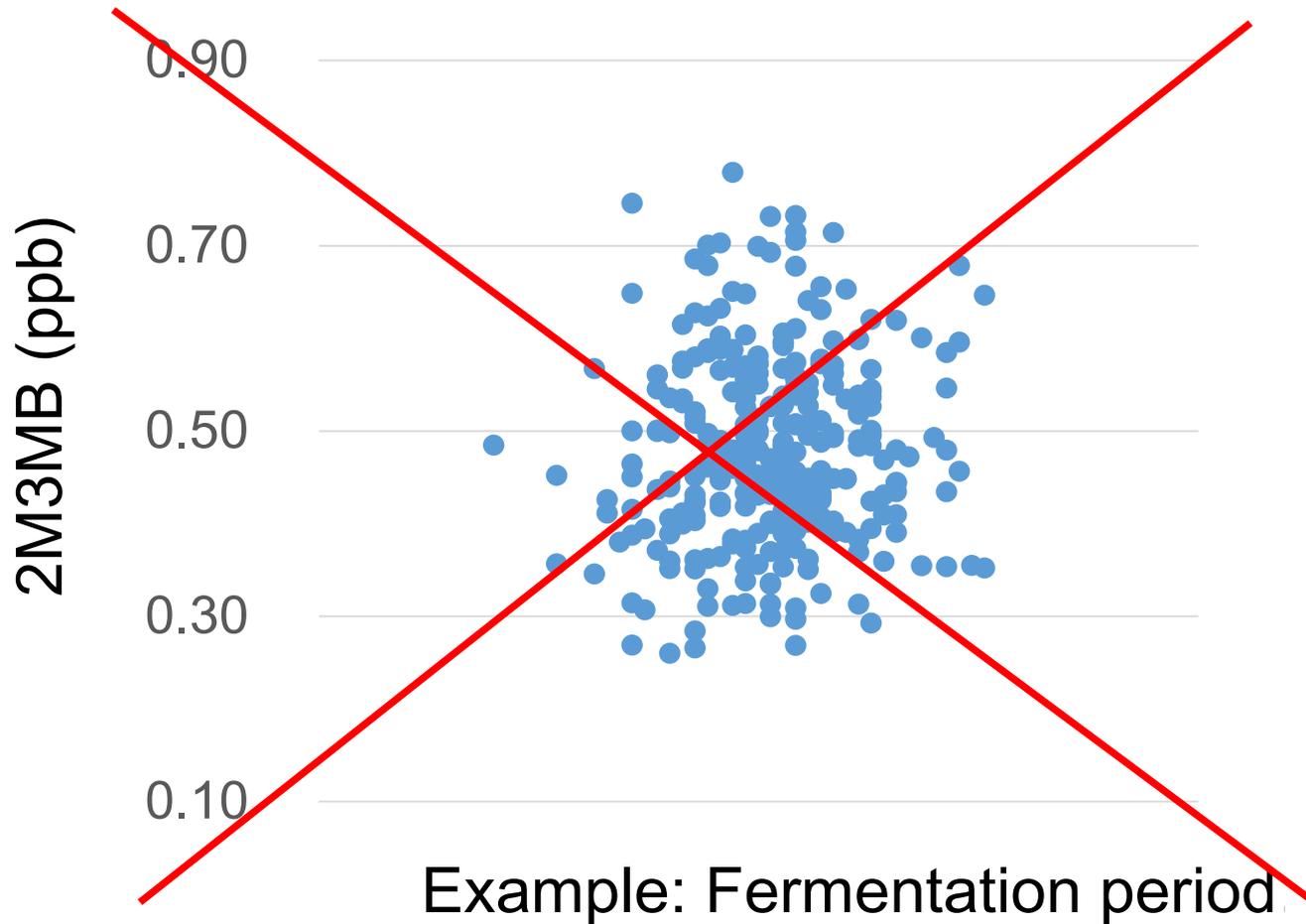
# Main topics

1. Why did we focus on the precursor?

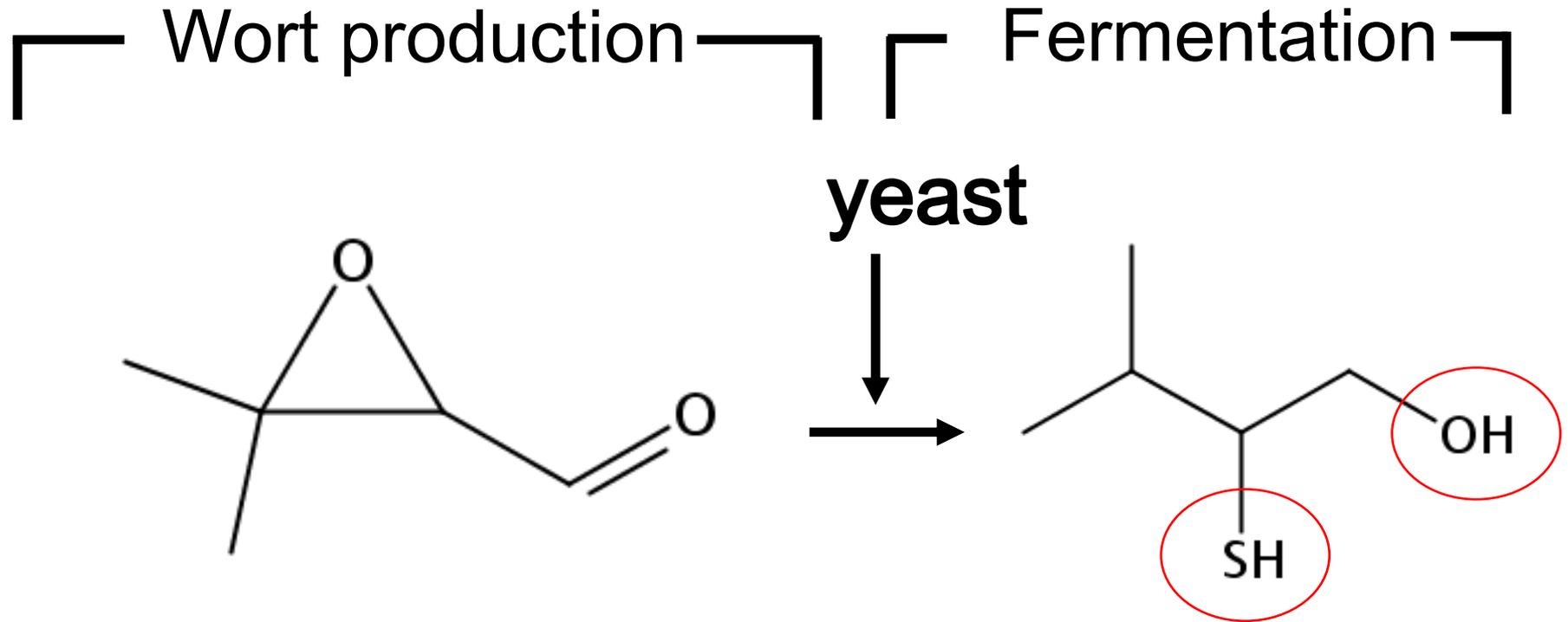
2. How was the precursor identified?

3. How can onion-like off-flavor be reduced?

# Regression analysis

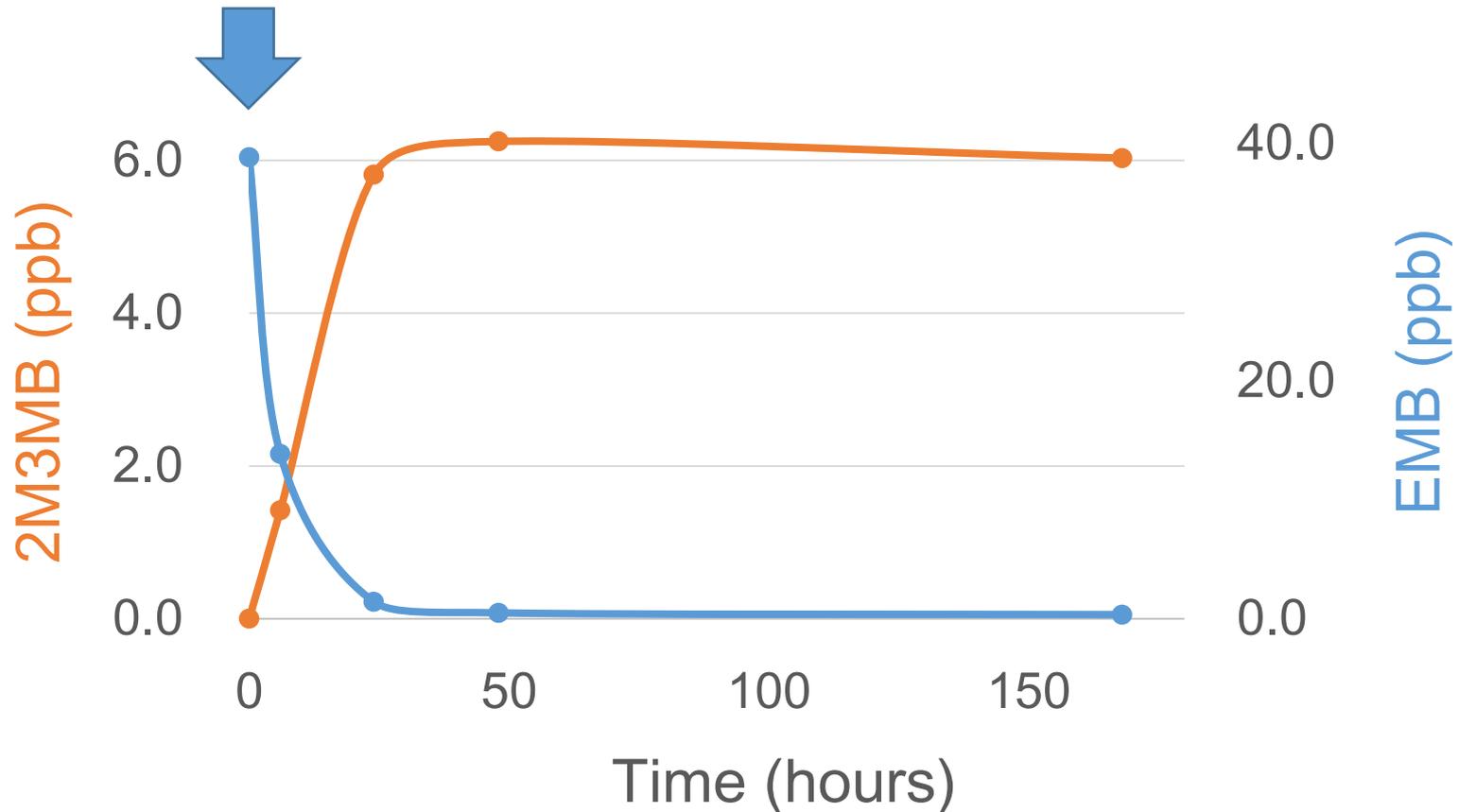


# Hypothetical pathway of 2M3MB formation



The precursor structure indicates how the yeast generates the target compound.

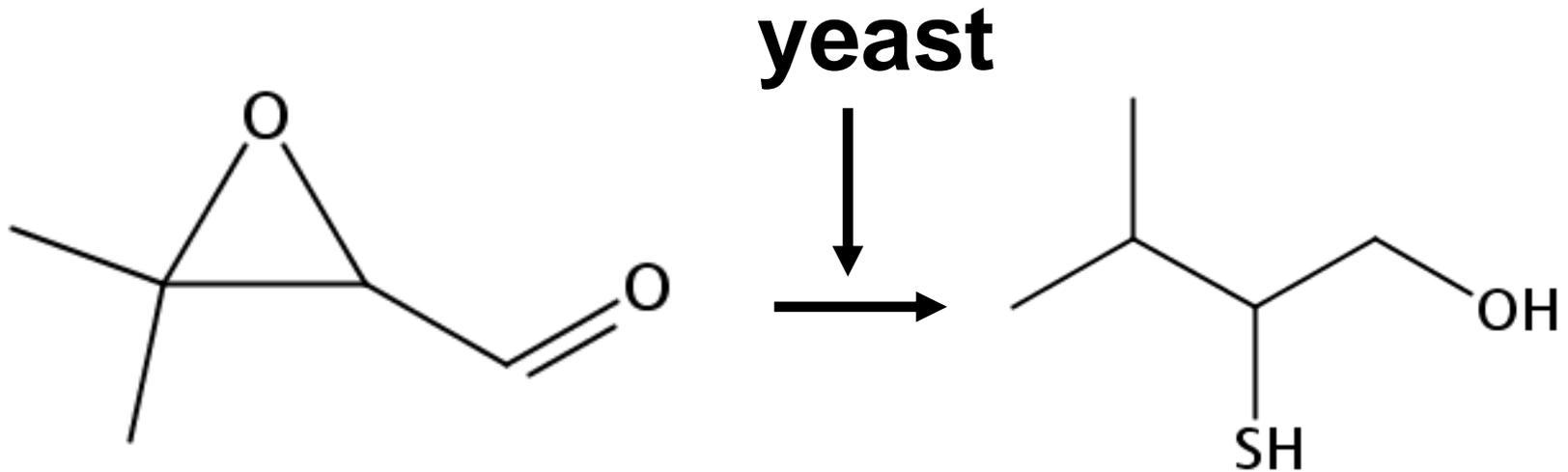
# Addition test of EMB



✓ The behavior of EMB was similar to other aldehyde compounds during fermentation.

# Hypothetical pathway of 2M3MB formation

Wort production      Fermentation



**Which is the bottleneck reaction?**

- Substitution of an SH group?
- Reduction of the terminal aldehyde?



Step 1: Investigate the bottleneck reaction of 2M3MB formation

Step 2: Adjust the fermentation conditions to control the reaction

Maintain the good fermentation process without generating 2M3MB

# Conclusion

- ✓ The precursor of 2M3MB was purified from isomerized hops and identified as 2,3-epoxy-3-methyl-butanal.