



# Mechanism of dimethyl trisulfide formation in stored beer

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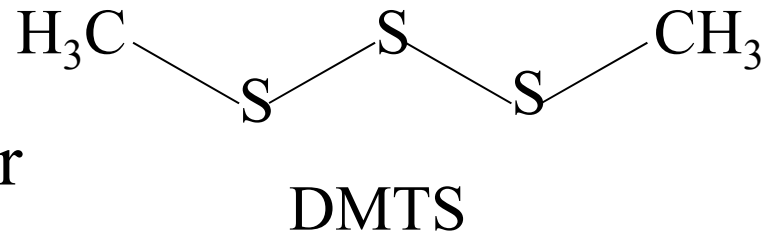
# Outline

- **Introduction**
- **Research objective**
- **Mechanism of DMTS formation in beer storage**
- **Controlling DMTS formation in beer**
- **Conclusion**

# Dimethyl trisulfide (DMTS)

## Taste perception

sulfury, rotten vegetable flavor



## Found in

milk, whiskey, beer, wine and other foods

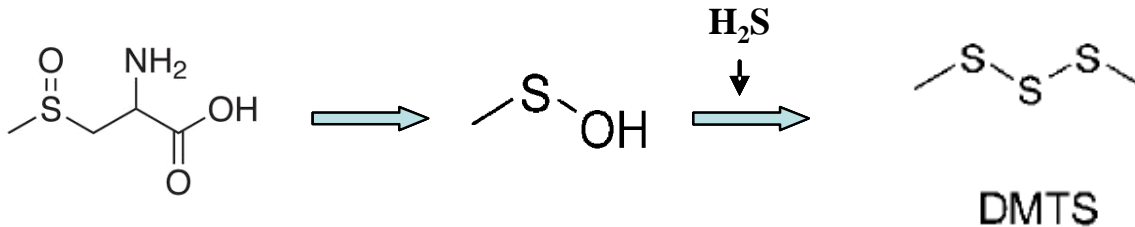
## Flavor threshold

About 10-100 ppt in beer

# DMTS precursors

## 1. S-methylcysteinesulfoxide (SMCS) contained in hop

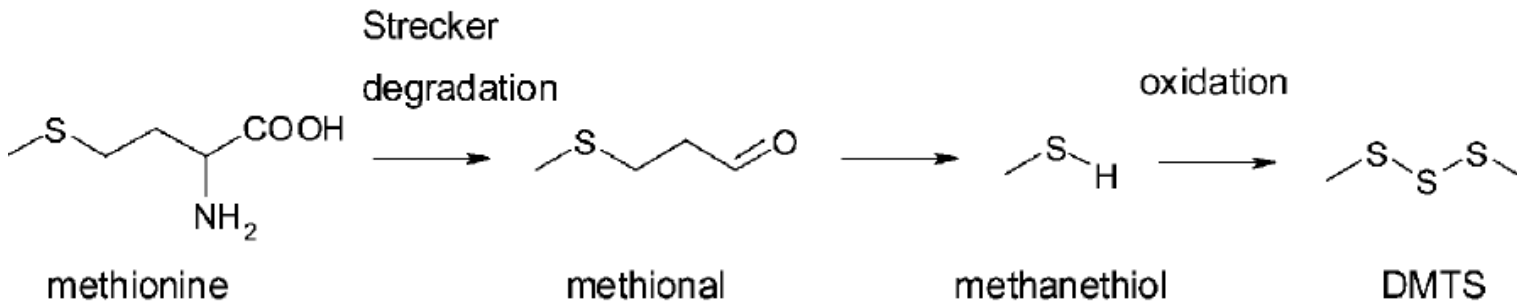
Peppard T.L., *J. Inst. Brew.* (1978)



S-methylcysteinesulfoxide

## 2. Methionine in wort

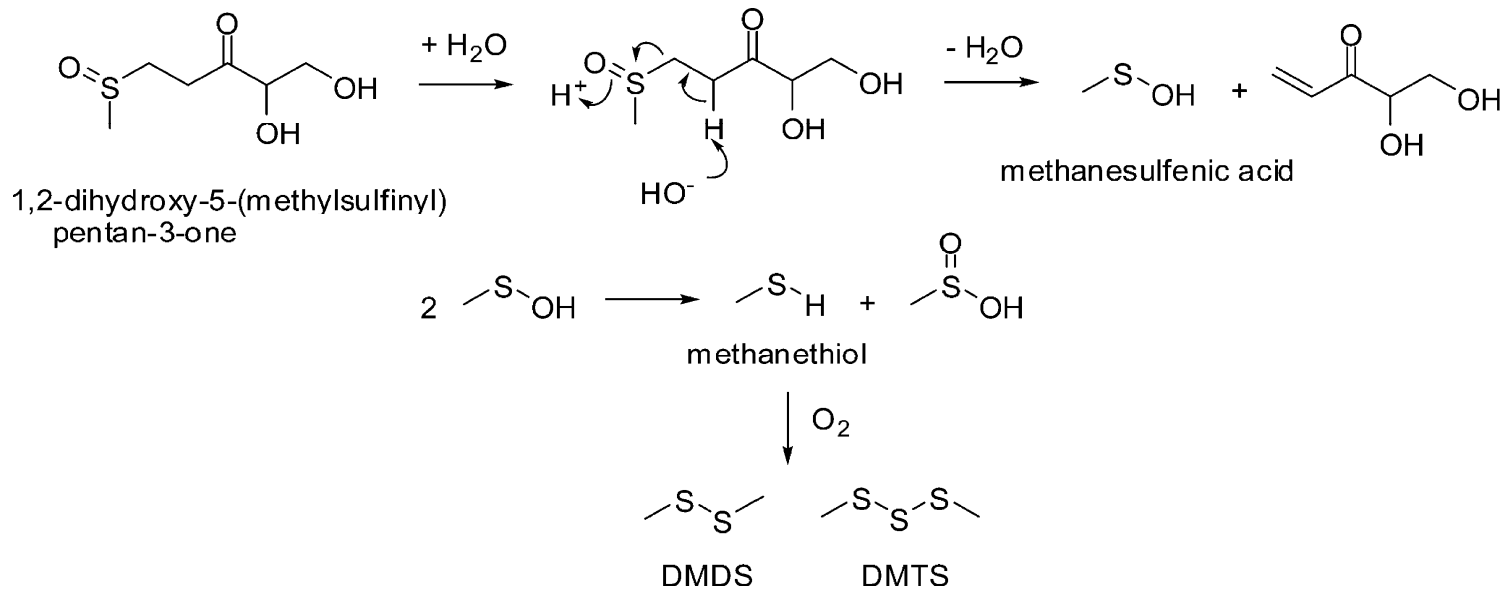
Gijs L., *J. Agric. Food Chem.* (2000)



# DMTS precursors

## 3. 1,2-Dihydroxy-5-(methylsulfinyl)pentan-3-one (DMSP) from yeast in Japanese sake

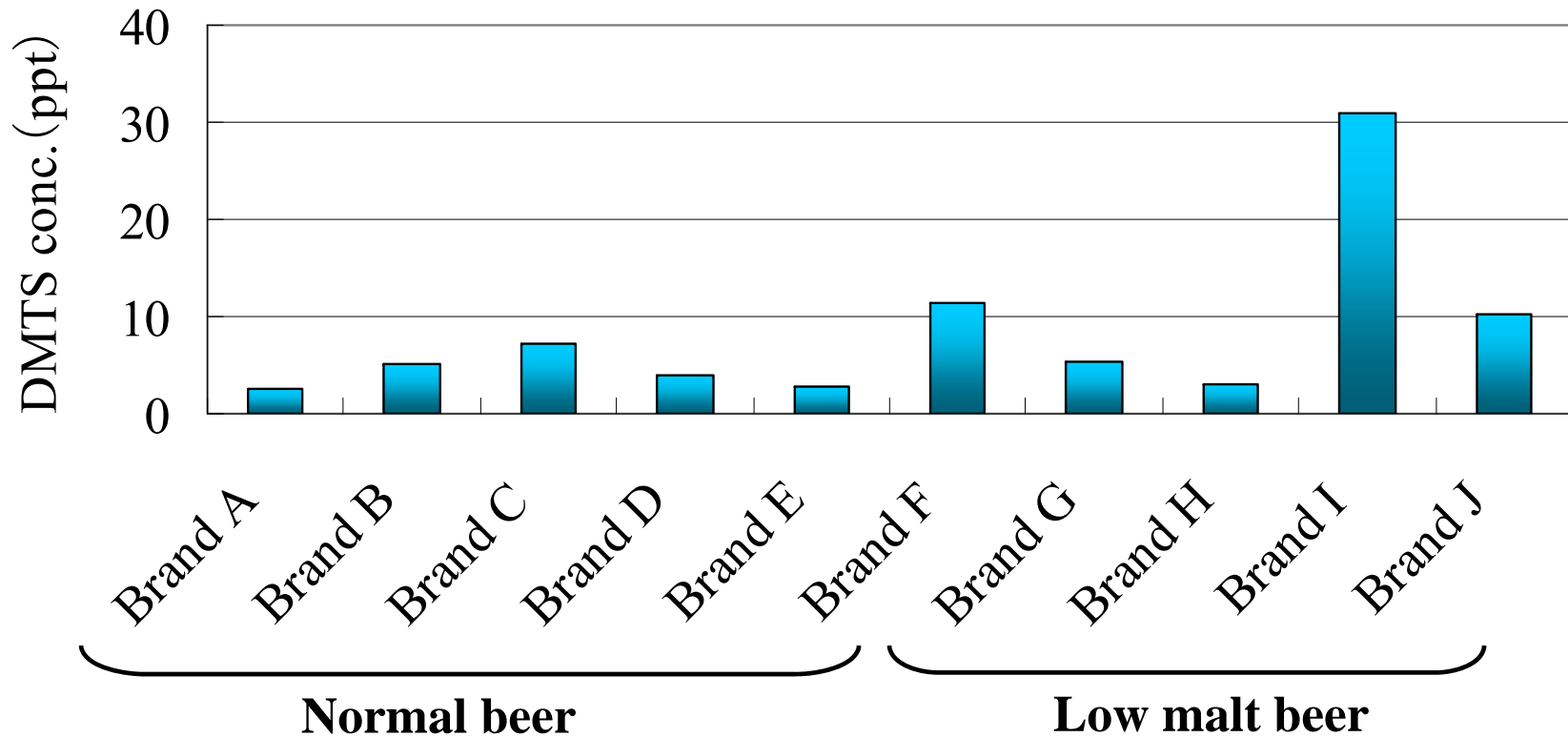
*Isogai A. J. Agric. Food Chem. (2010)*



**DMSP is the main precursor of DMTS in Japanese sake.**

# DMTS amounts in Japanese beers

(after storage for 2 weeks at 37° C)



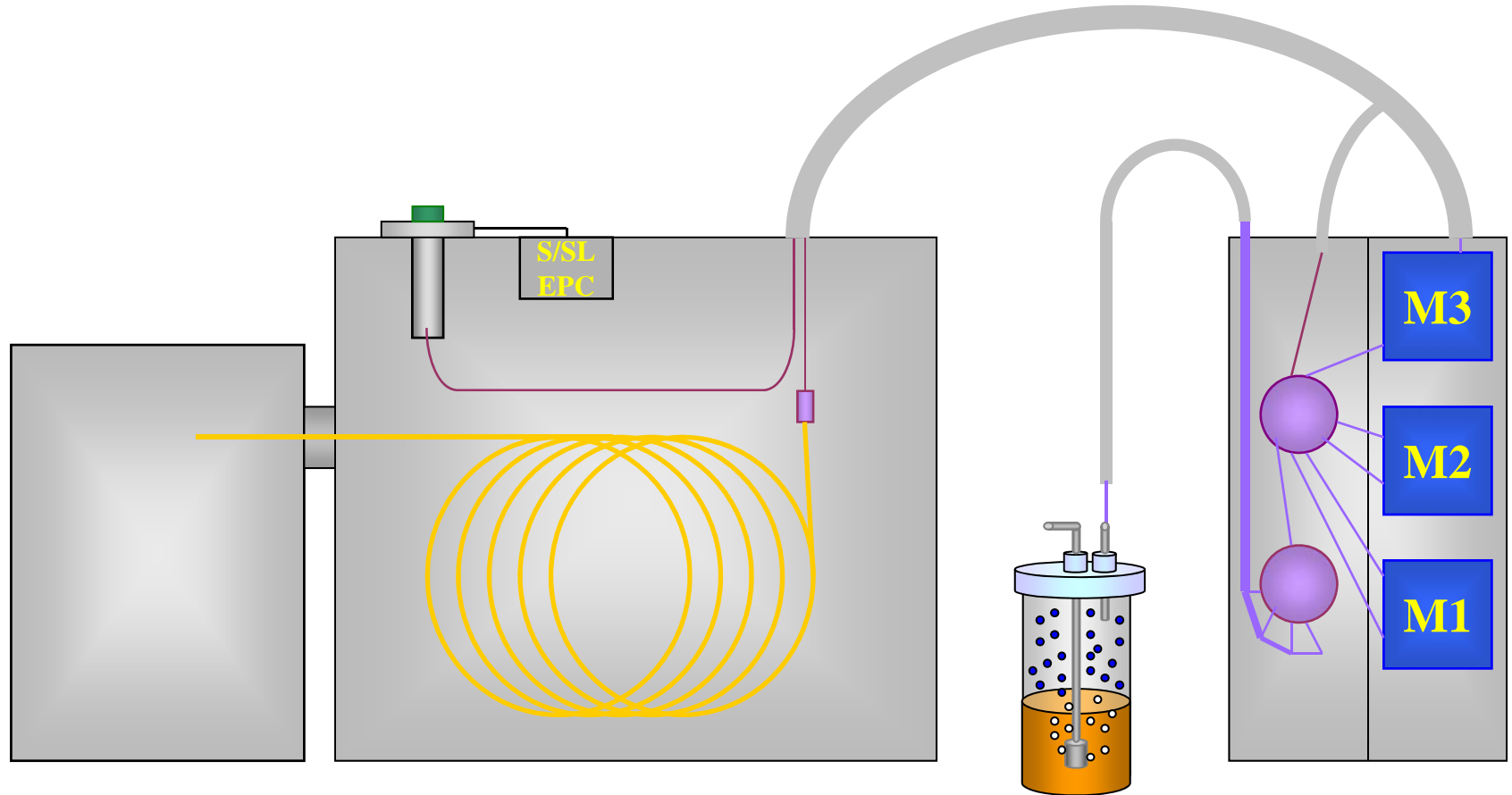
**In this study, we investigated the mechanism of DMTS formation in low-malt beer.**

# Objectives

**To improve the flavor stability of beer, we investigated the factors affecting DMTS formation and methods for controlling its formation during beer storage.**

# DMTS quantification method

( Large Volume Dynamic Headspace - GC/MS )



**Agilent 5973 inert  
MSD**

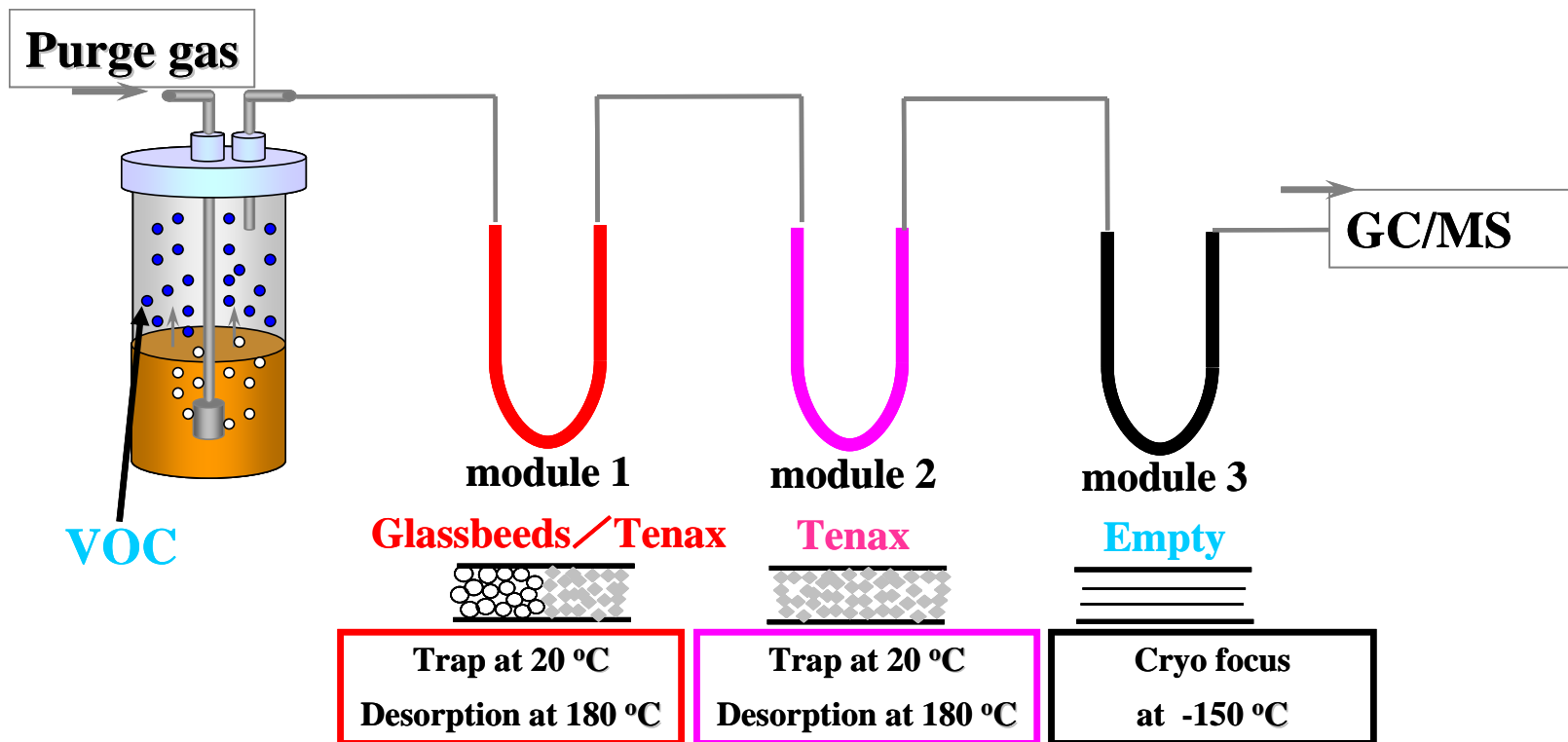
**Agilent 6890N  
GC**

**Entech 7100A  
3 stage concentrator**



# DMTS quantification method

( Large Volume Dynamic Headspace - GC/MS )



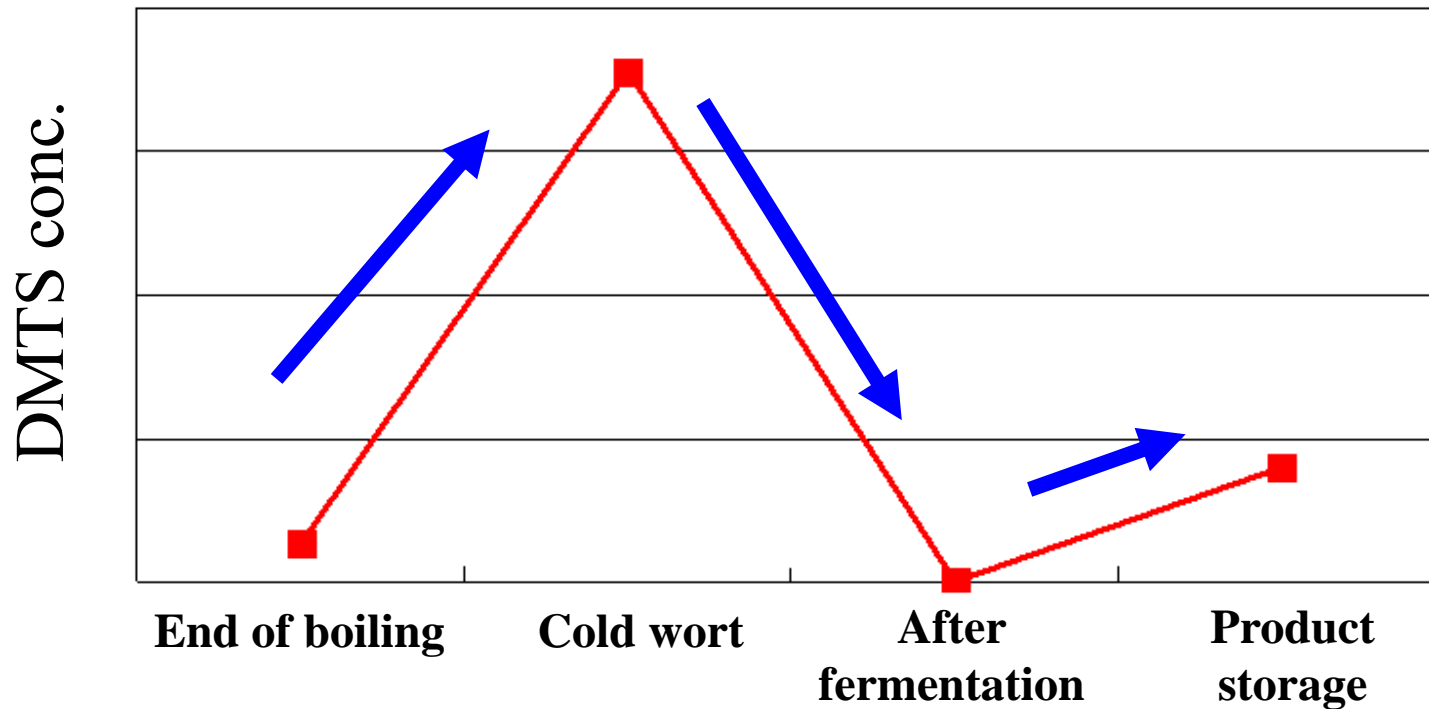
## GC/MS conditions

Column: DB-1 60 m × 0.32 mm (0.4 um F.T.)

Program: 40 °C (5 min) – 10 °C /min -120 °C (7min)- 300 °C (5 min)

Transfer line temperature: 280 °C Injection: Pulsed Split

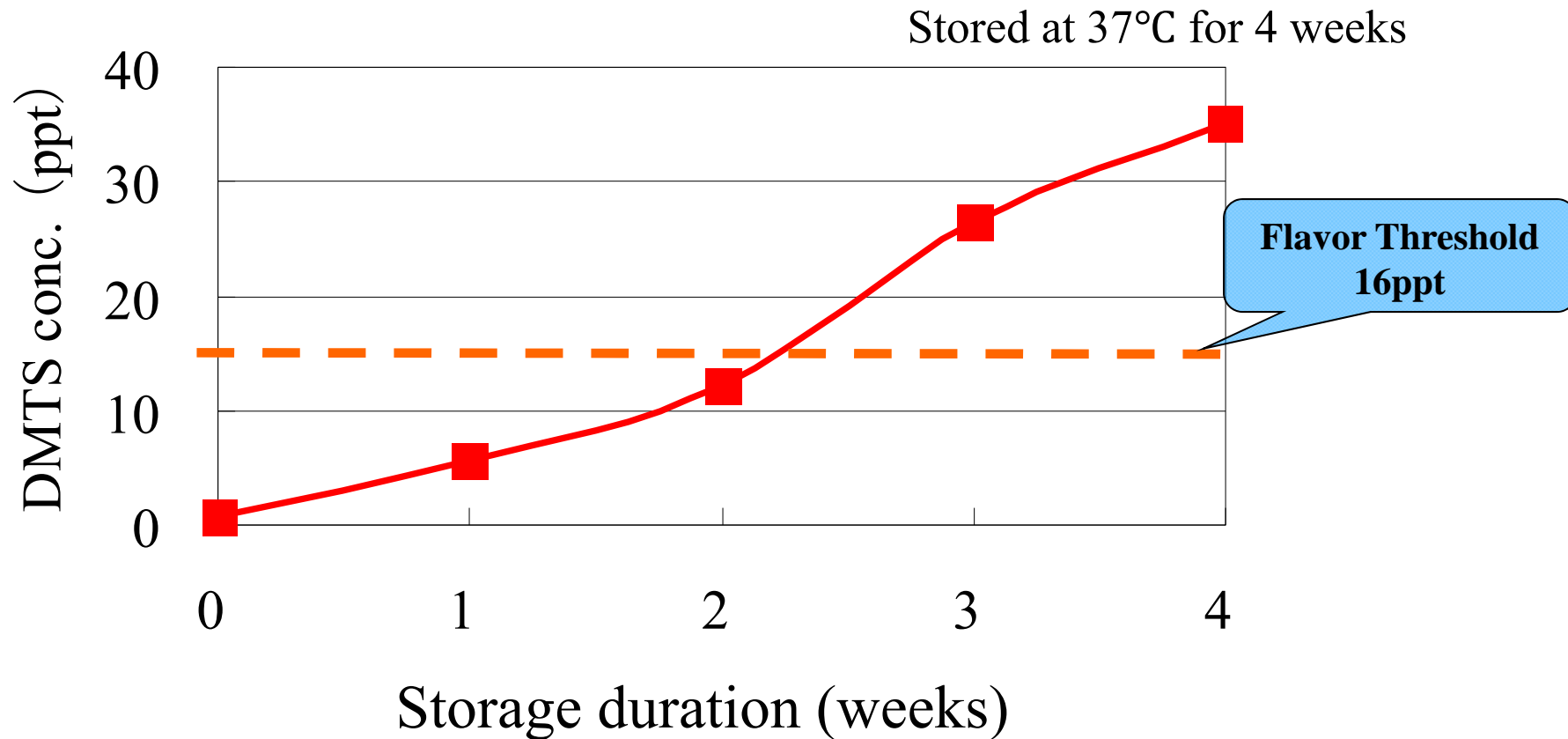
# Behavior of DMTS concentration during the beer brewing process



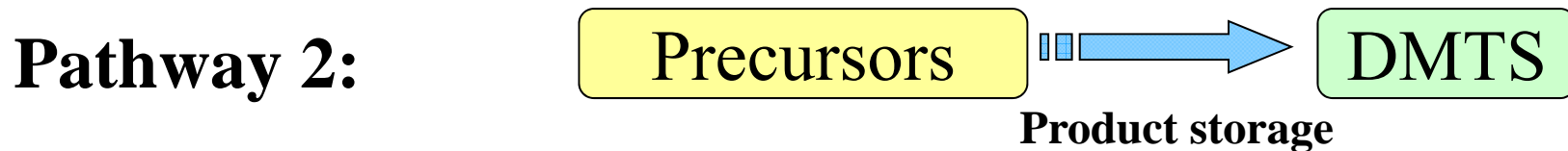
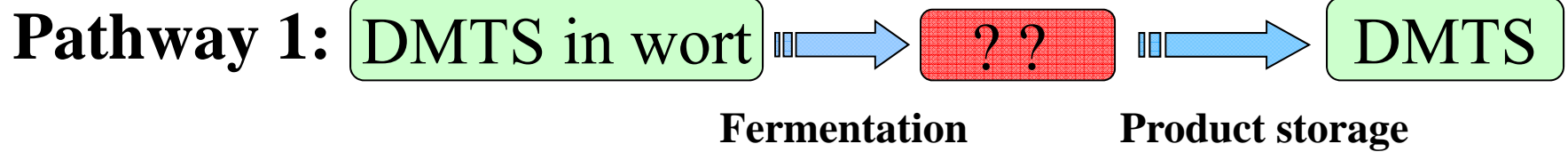
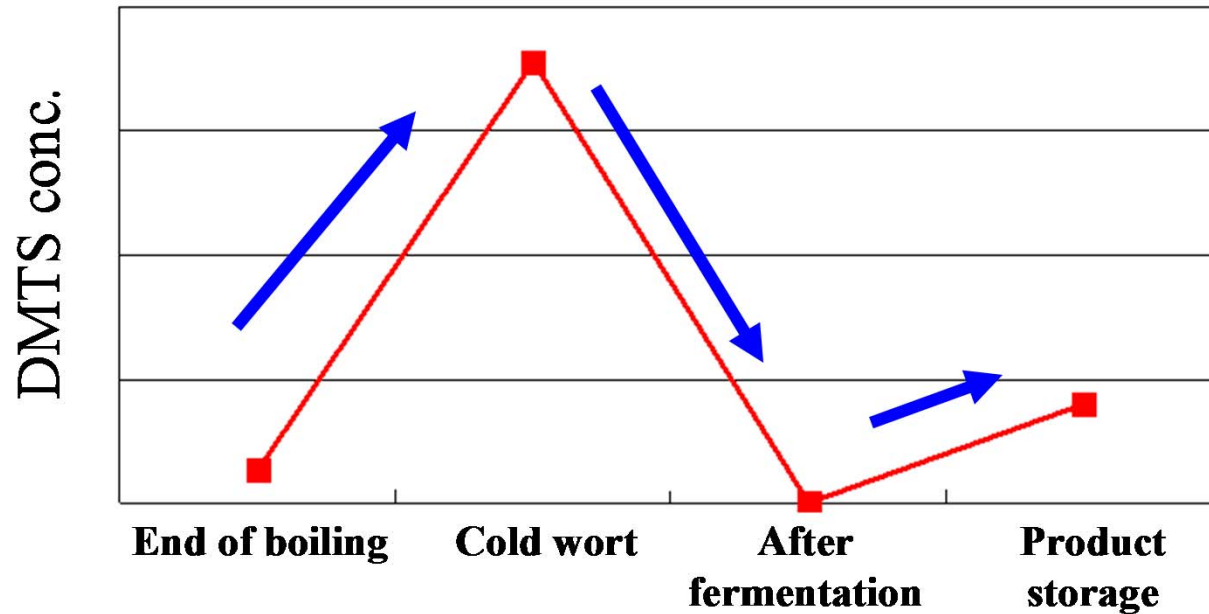
DMTS increased after boiling and significantly decreased during fermentation, but increased again during storage.

# Factors promoting DMTS formation during product storage

# Behavior of DMTS concentration during product storage

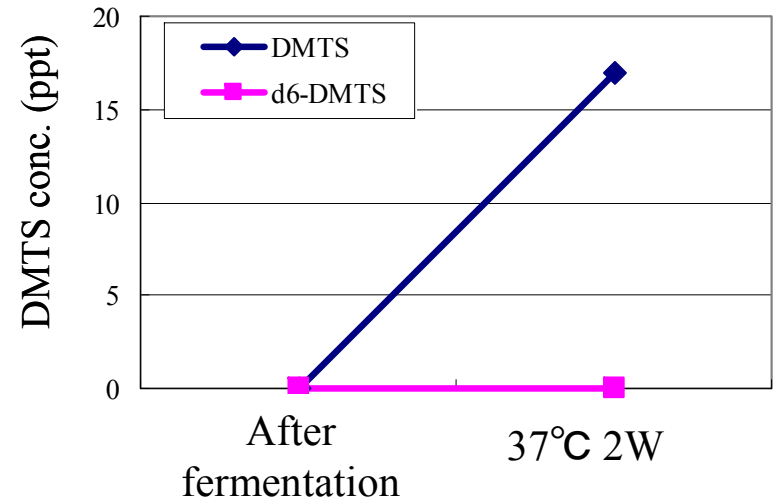
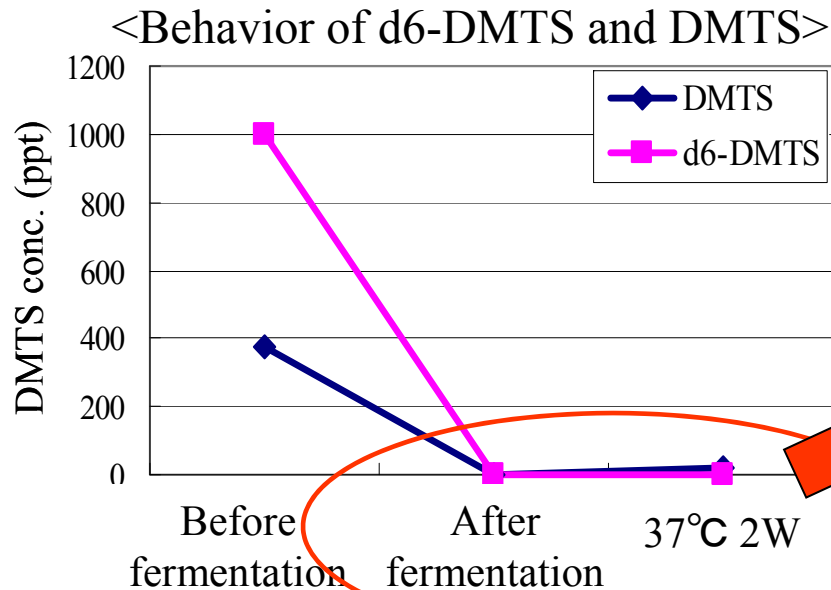


# Hypotheses for DMTS formation in stored beer



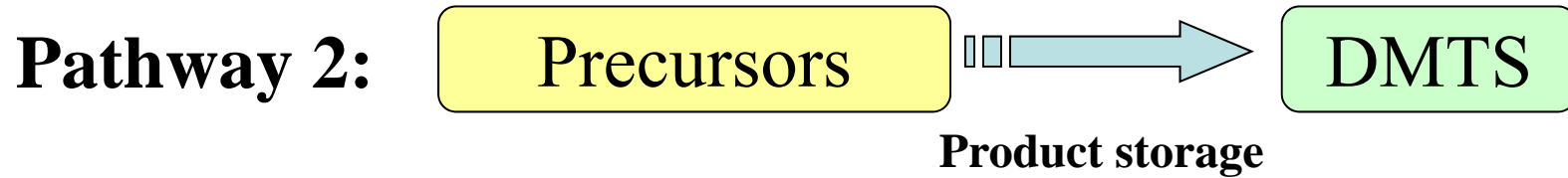
# Relation between DMTS in wort and finished product

Before fermentation, d6-DMTS (stable isotope) was spiked at 1000ppt in the wort.



**D6-DMTS did not increase after beer storage.**

# Precursors of DMTS tested in this study



## 1. SMCS contained in hop

*Peppard T.L., J. Inst. Brew.(1978)*

## 2. Methionine in wort *Gijs L., J. Agric. Food Chem. (2000)*

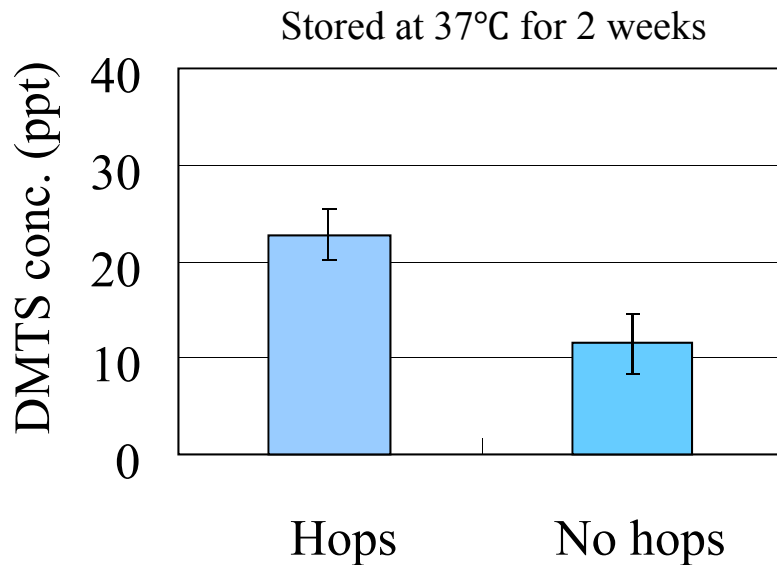
## 3. DMSPO from yeast in Japanese sake

*Isogai A. J. Agric. Food Chem. (2010)*

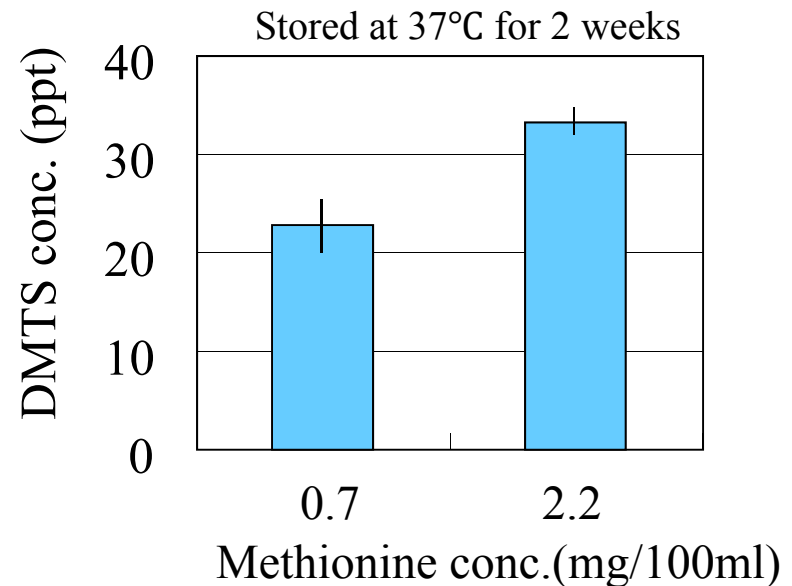
# Effect of hops and methionine on DMTS formation during beer storage

Prepared unhopped wort and wort with added methionine

<Effect of hop on DMTS formation>



<Effect of methionine on DMTS formation>

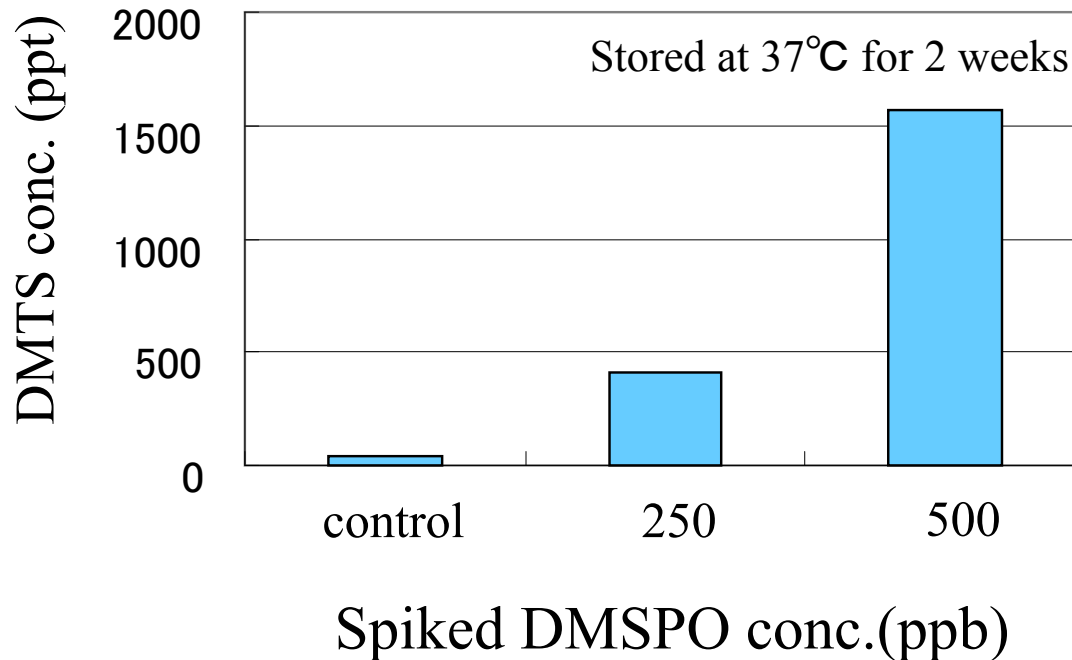


**Both hop and methionine increased DMTS formation during beer storage.**



# Effect of DMSPO on DMTS formation during beer storage

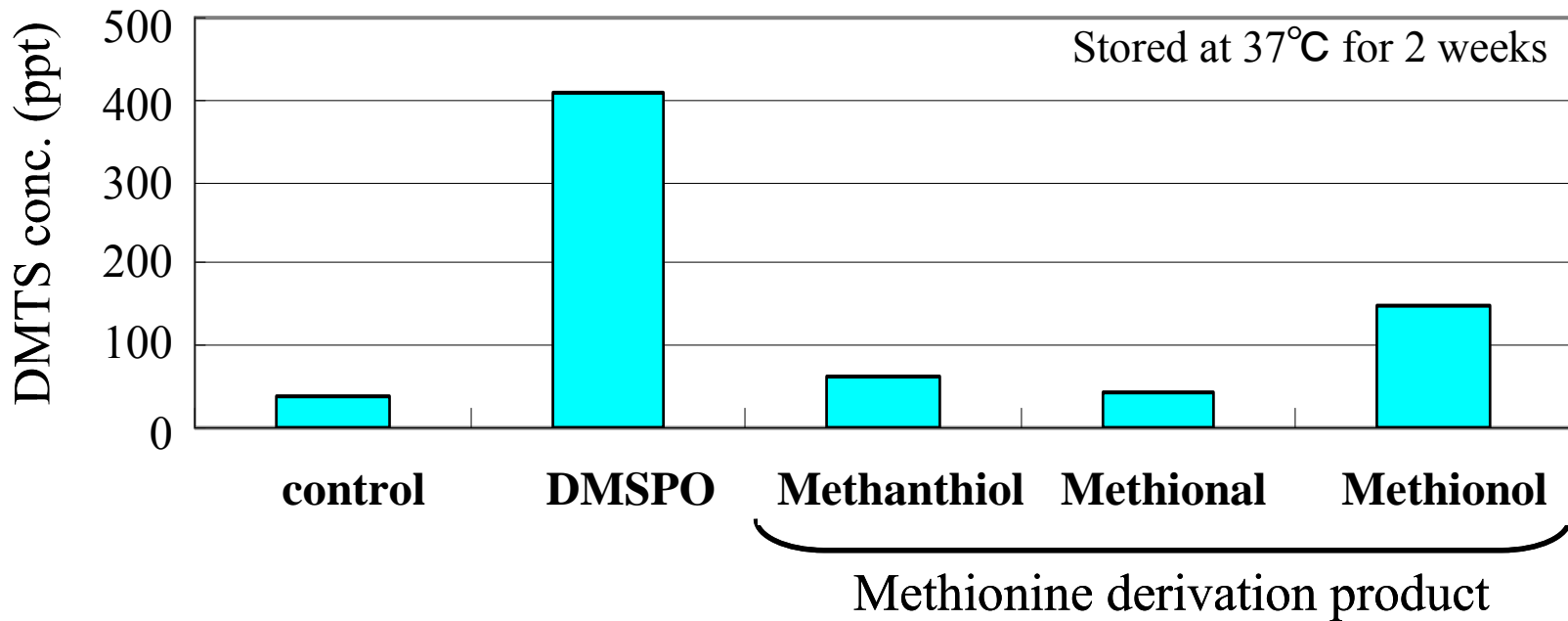
DMSPO was added to beer before storage at a concentration  $\approx 10$  times higher than that found in normal beer.



**DMSPPO increased DMTS formation during beer storage.**

# What is the main precursor of DMTS in stored beer ?

Each precursor was added at levels 10 times those found normally in beer.

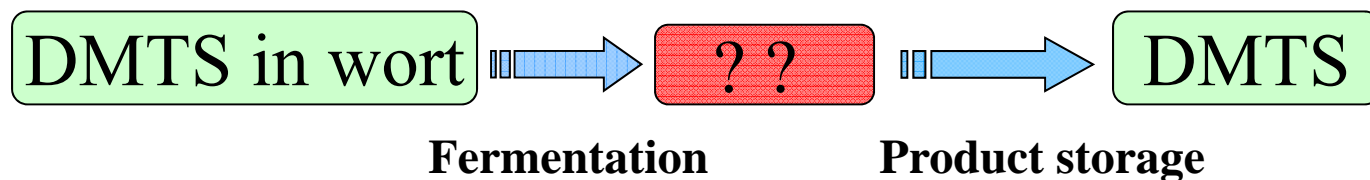


**DMSPPO is the main precursor of DMTS in stored beer.**

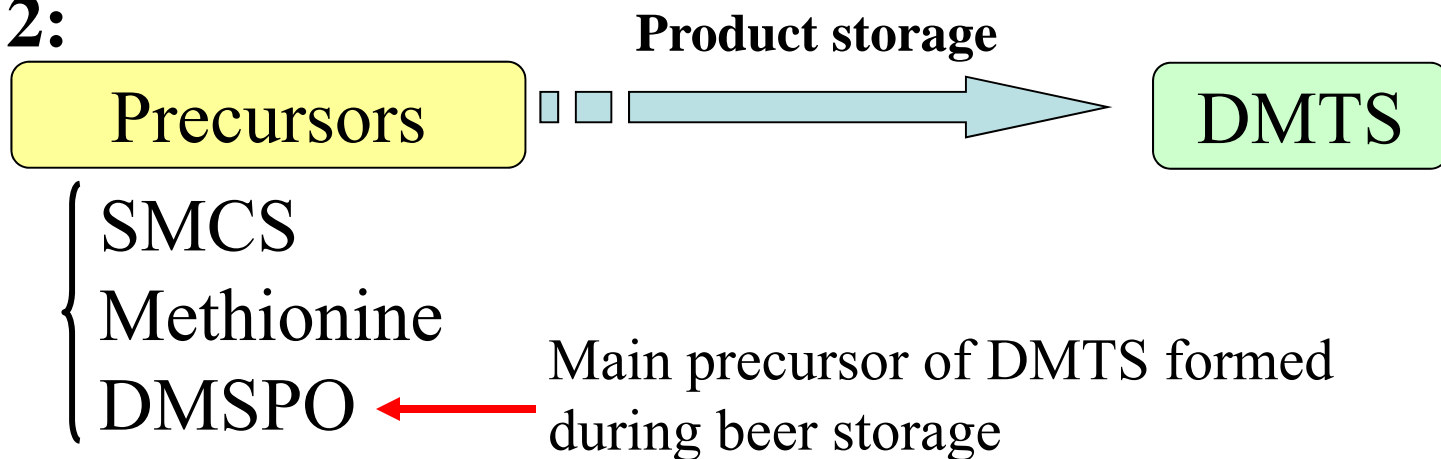
# Short summary

<Possible mechanisms of DMTS formation in stored beer>

## ✗ Pathway 1:

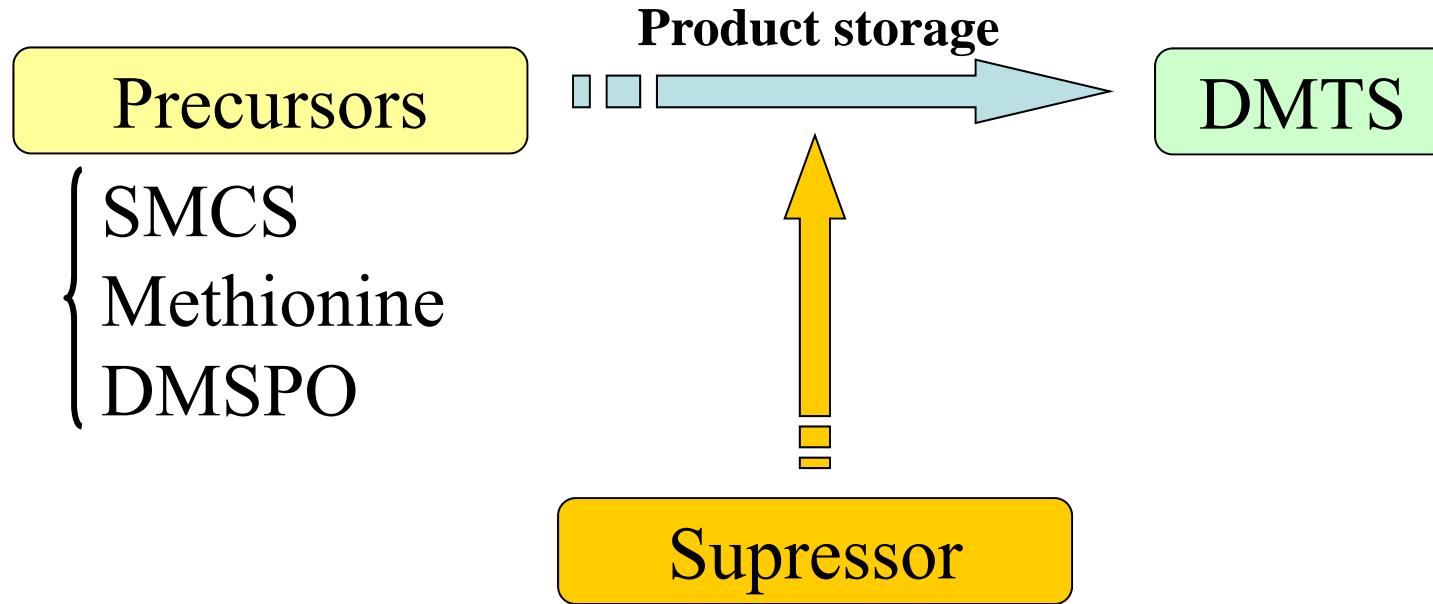


## ✓ Pathway 2:



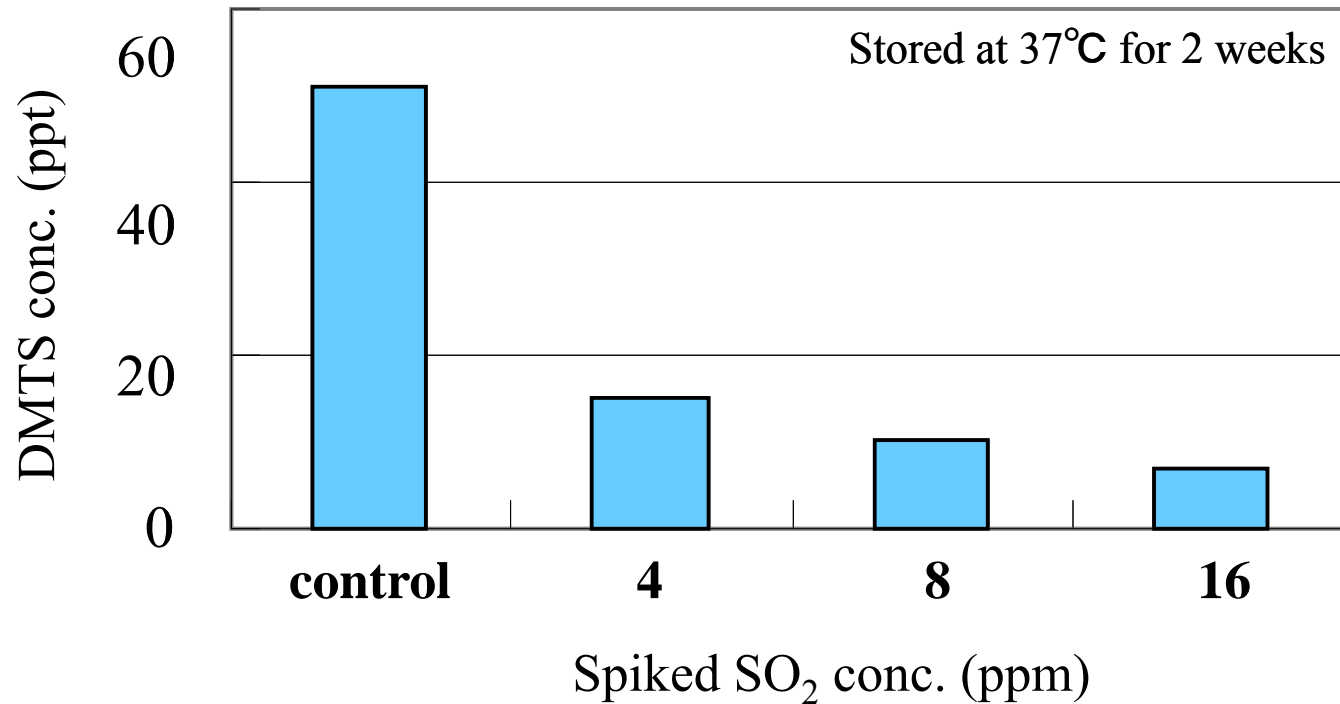
# Factors suppressing DMTS formation during beer storage

# Proposed pathway for DMTS formation during beer storage



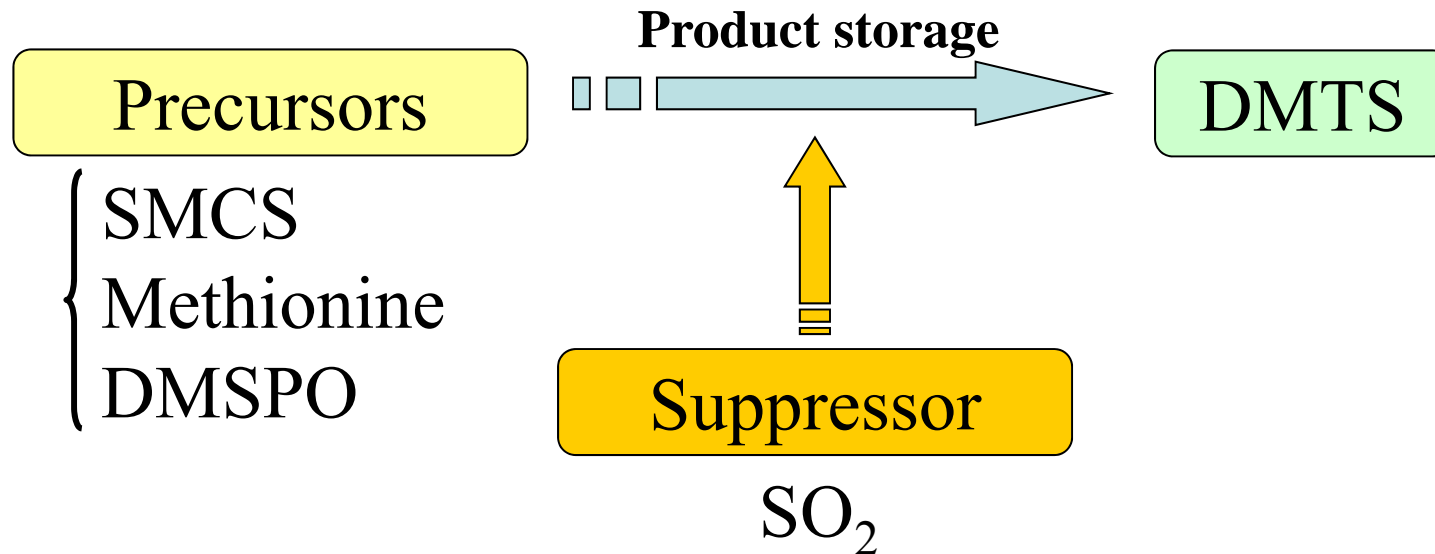
**Do antioxidants suppress DMTS formation in beer ?**

# Effect of SO<sub>2</sub> on DMTS formation during beer storage



**SO<sub>2</sub> significantly suppressed DMTS formation.**

# Proposed pathway for DMTS formation during beer storage



**To suppress DMTS formation during product storage, it is important to control DMSPO and SO<sub>2</sub> concentration !**

# Effect of fermentation conditions on SO<sub>2</sub>, DMSPO and DMTS formation



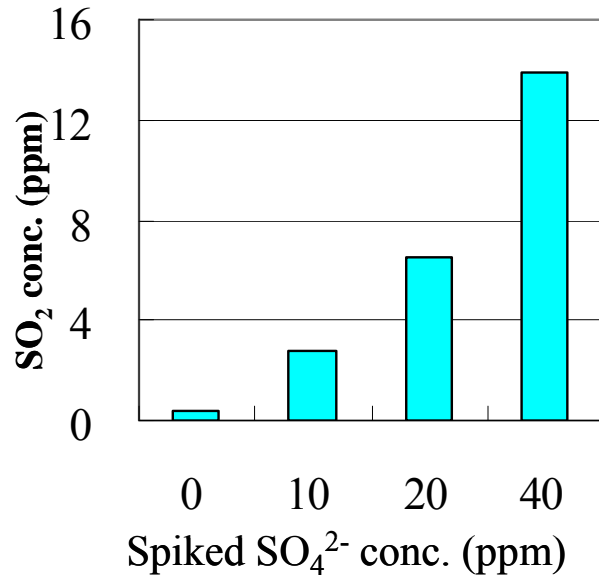
# Effect of $\text{SO}_4^{2-}$ in wort on DMTS formation

The amount of  $\text{SO}_4^{2-}$  in low malt beer was usually lower than normal  
beer.

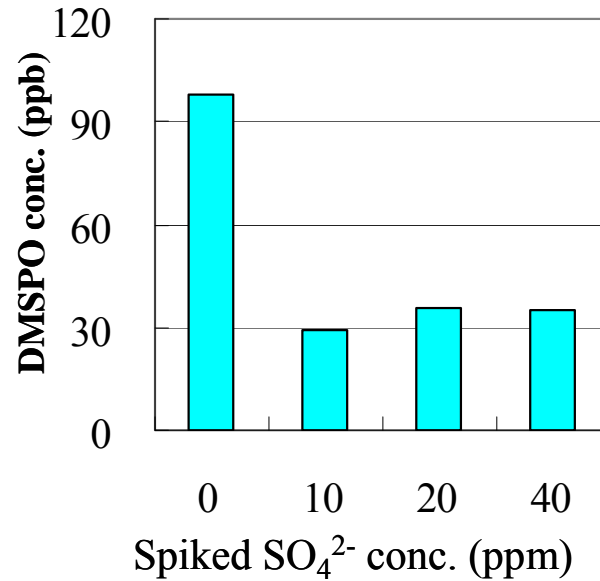


Tested the effect of  $\text{SO}_4^{2-}$  on the  $\text{SO}_2$ , DMSPO and DMTS formation.

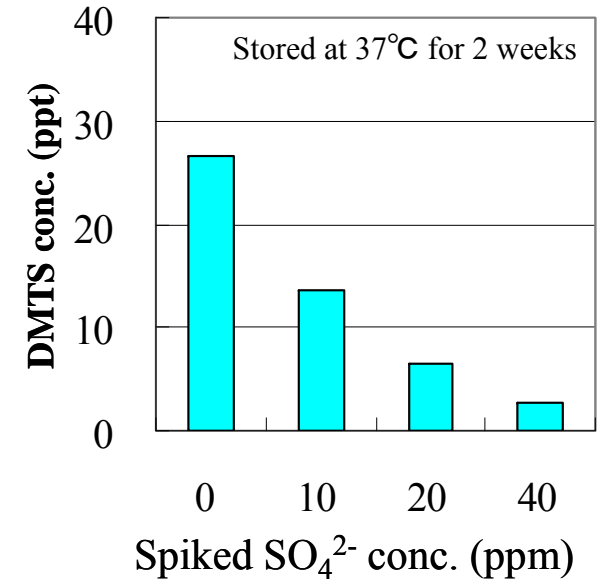
< $\text{SO}_2$  formation>



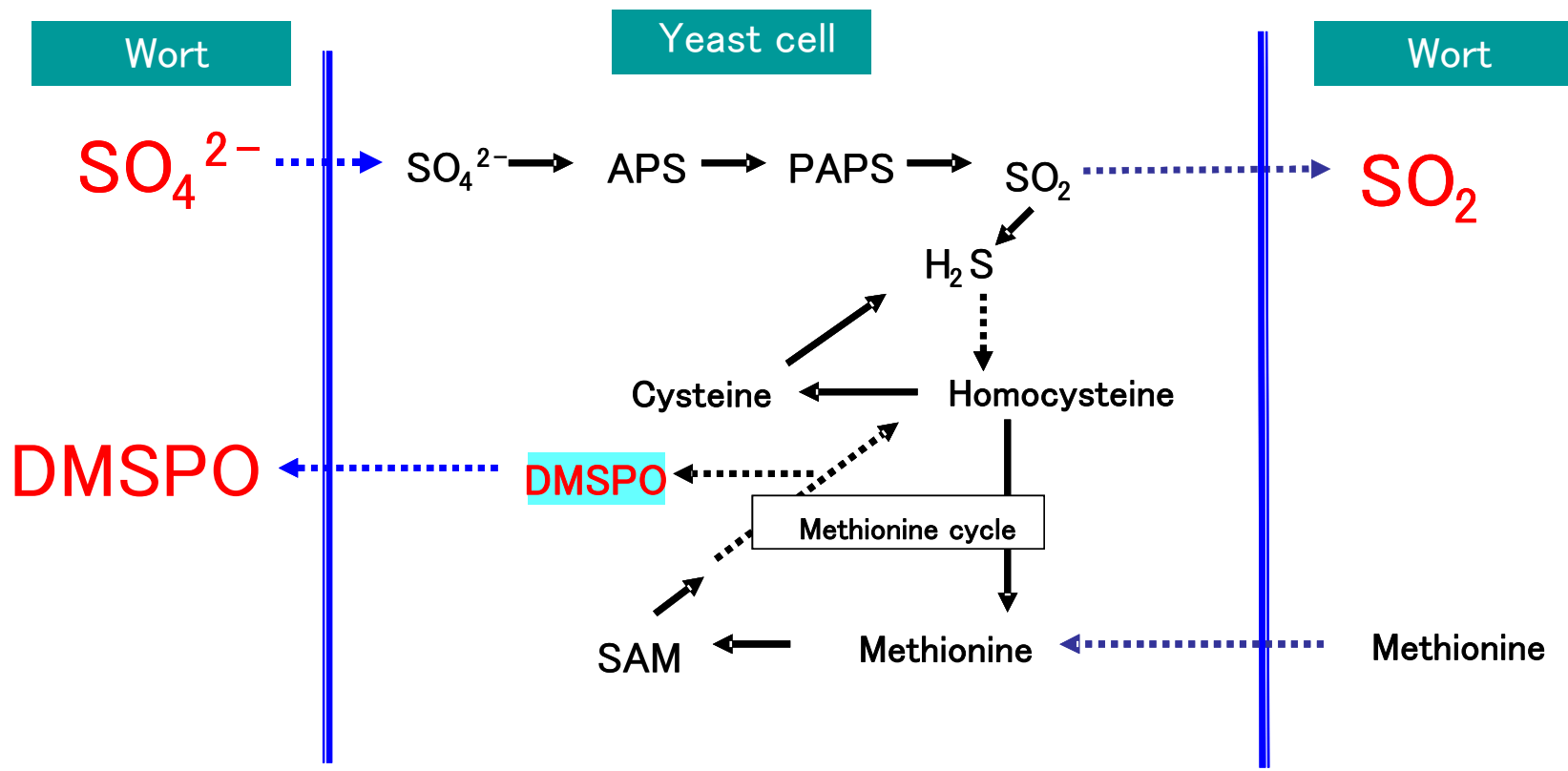
<DMSPO formation>



<DMTS formation>



# Proposed model for SO<sub>2</sub> and DMSP<sub>0</sub> formation during fermentation



**Low  $\text{SO}_4^{2-}$  condition**  $\Rightarrow$  **Activation of methionine cycle**  $\Rightarrow$  **Decrease of  $\text{SO}_2$**   
**Increase of DMSP<sub>0</sub>**

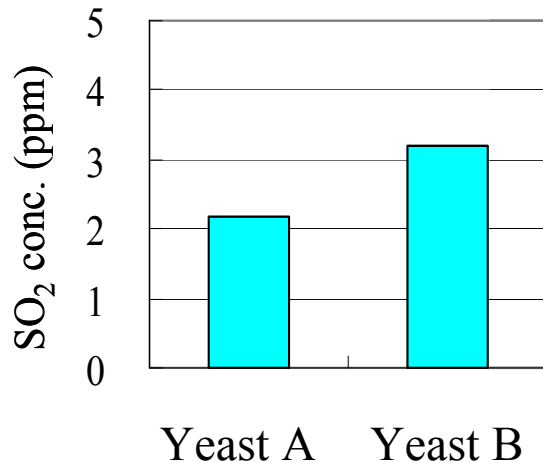
# Effect of yeast strain on DMTS formation

The amount of SO<sub>2</sub> in the beer differed depending on the yeast strains.  
(ASBC fishborn)

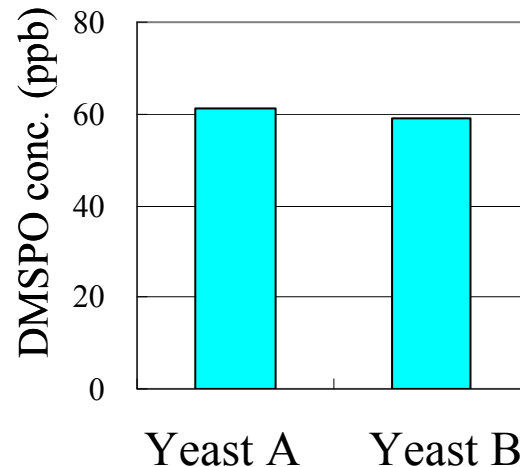


Tested the effect of yeast strains on the SO<sub>2</sub>, DMSPO and DMTS formation.

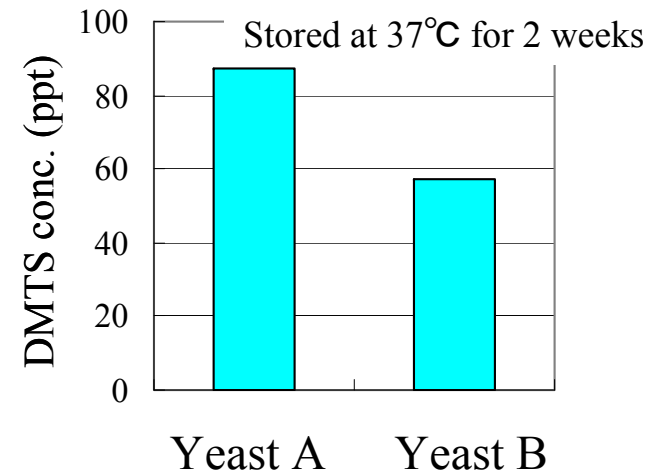
<SO<sub>2</sub> formation>



<DMSPO formation>



<DMTS formation>



# Short summary

- In low  $\text{SO}_4^{2-}$  conditions, DMSPO and DMTS formation can be suppressed by adding  $\text{SO}_4^{2-}$  directly to the wort.
- The amount of DMTS formed in beer varies with the yeast strains.

**It is possible to suppress DMTS formation by changing the fermentation conditions.**

# General summary

**In this study, we showed:**

- 1. DMSPO is the main precursor of DMTS in stored beer**
- 2. SO<sub>2</sub> suppresses DMTS formation**
- 3. DMTS formation can be suppressed by controlling the fermentation conditions**

**Thank you for your kind attention!**

***Asahi***

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*The Science of Beer*