Master Brewers Association of the Americas
Dedicated to the technology of brewing.
MBAA Annual Conference

A SPRINGBOARD TO UNDERSTANDING BEER FLAVOR STABILITY: THE ROLE OF BOUND-STATE ALDEHYDES

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KU LEUVEN
KAHO SINT-LIEVEN

Case Study

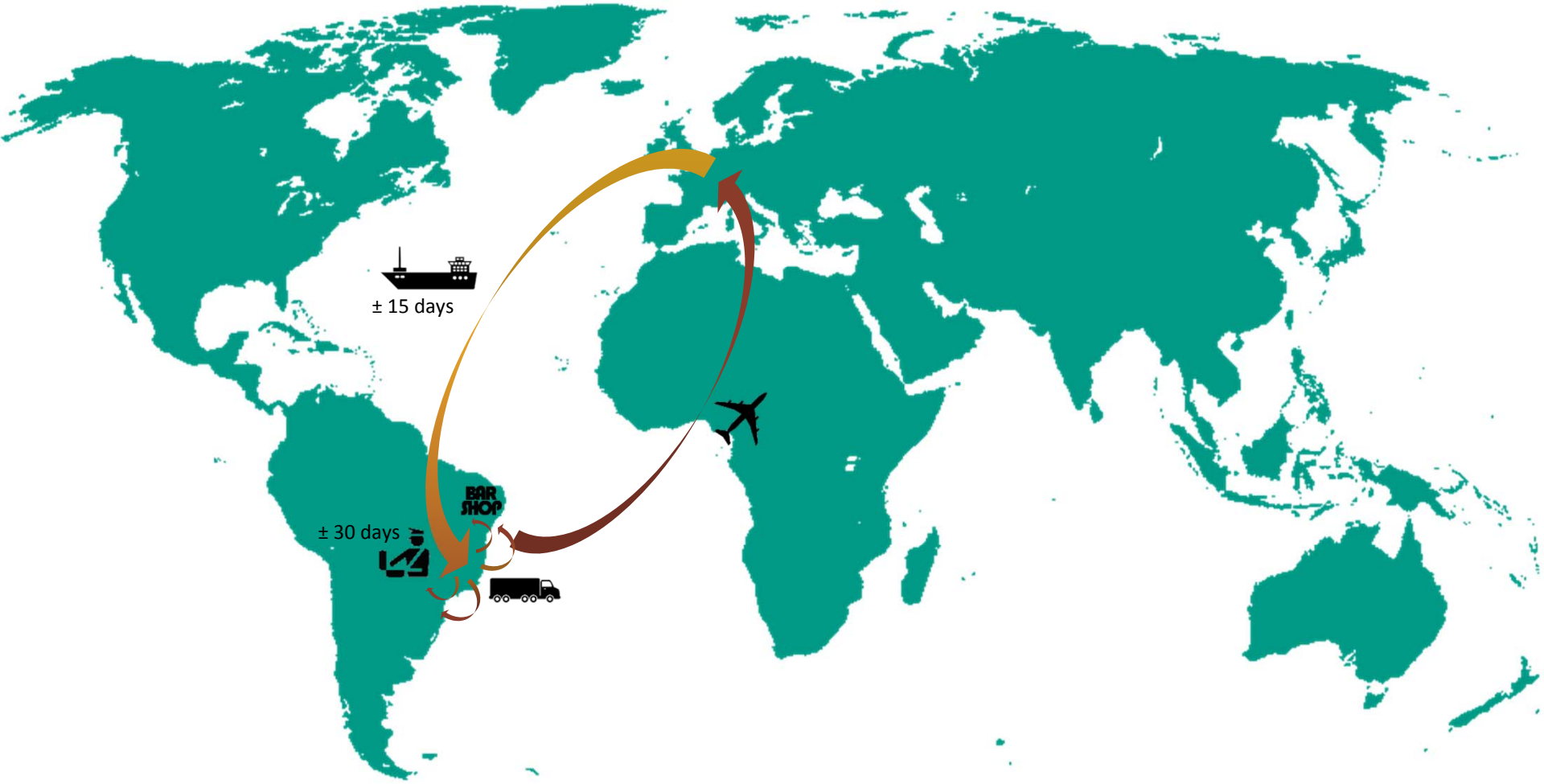
- > 60% of Belgian beer production is exported
  - Mostly pale lager beers
  - Also many specialty beers
- Common belief:

  Flavor richness of specialty beers masks aging flavors
- Case Study:

  European specialty beers as sold on the Brazilian market

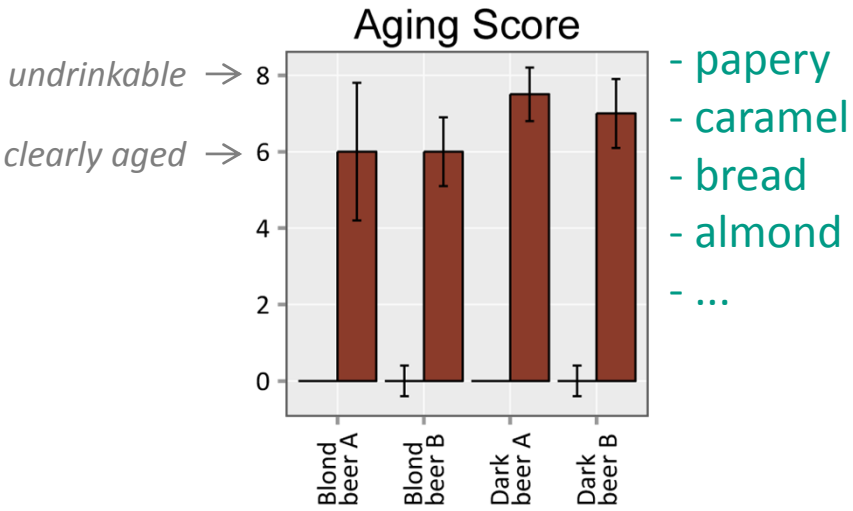
→ Why is beer flavor stability so important?

Case Study

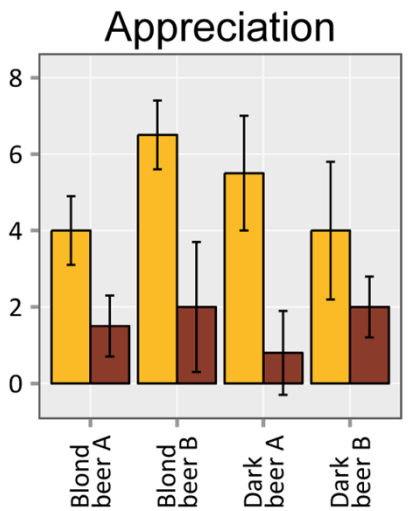


Case Study

- Sensory evaluation:



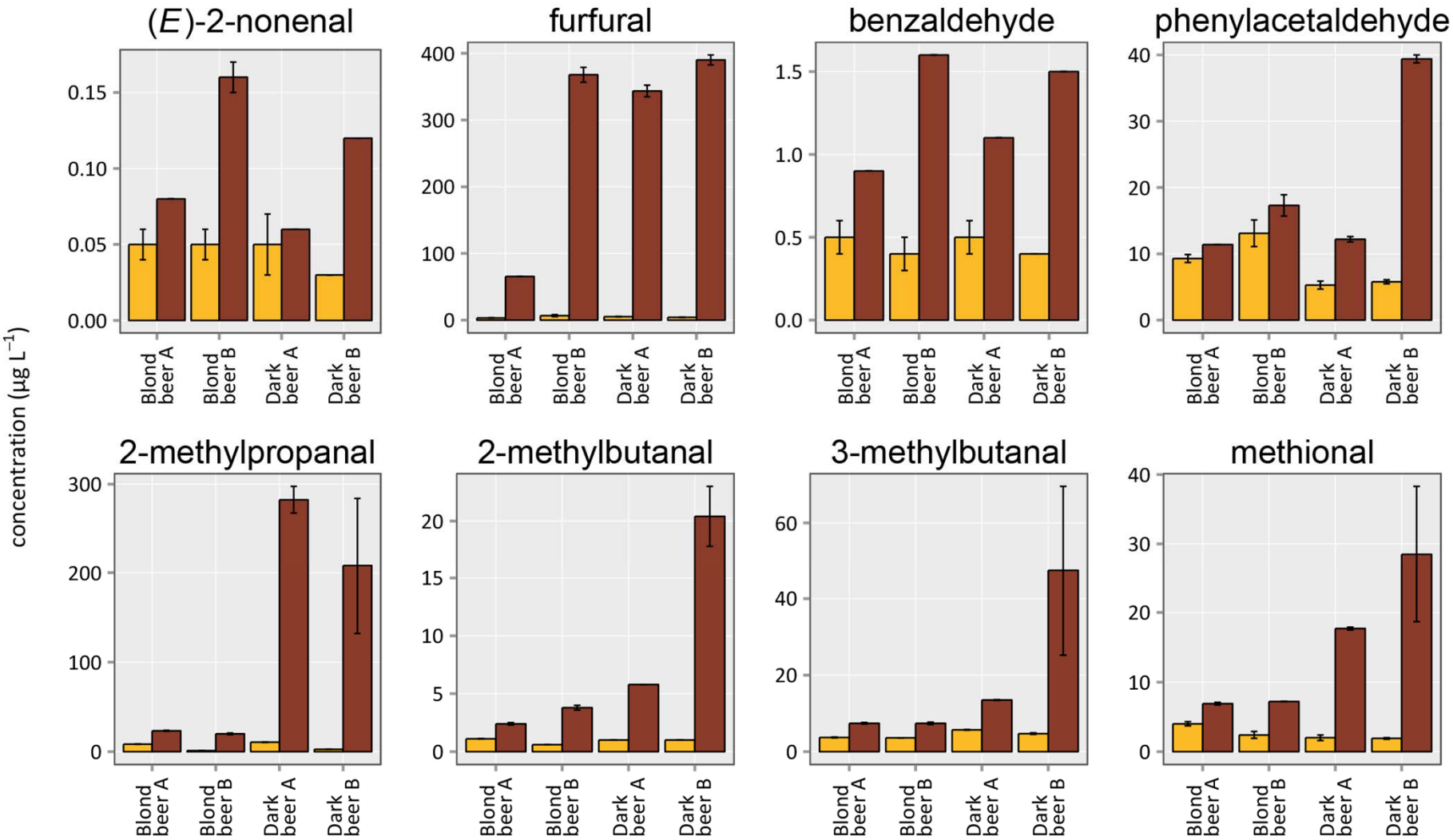
- papery
- caramel
- bread
- almond
- ...



- Beers as sold in Brazil:
  - Clearly aged, both blond and dark beers
  - Typical aldehyde-related off-flavors
  - Strong decrease in panel's appreciation

Case Study

- Aldehyde concentrations:



Montandon, G., Quality of imported beers in Brazil. *Proceedings of the 11th Trends in Brewing* **2014**, IL.03.

Case Study

- Despite masking flavors, even specialty beers deteriorate intensively
- Market globalization requires improved beer flavor stability, otherwise risk of consumer rejection
- Need for more conscious transportation and storage conditions to prolong shelf life
  - Refrigeration
  - Reduced agitation
  - High stock turnover
- **Need for more fundamental insights and solutions**

Chemistry background

- In fresh beer: minimal aldehyde concentrations due to yeast's reducing activity
- **Origin** of the increases in aldehyde concentrations during aging?

and/or

***De novo* formation**

during storage?

Literature: *Fatty acid oxidation, Strecker degradation, Maillard reaction,…*

Inconclusive, contradictions

Upstream formation and

release from a bound state

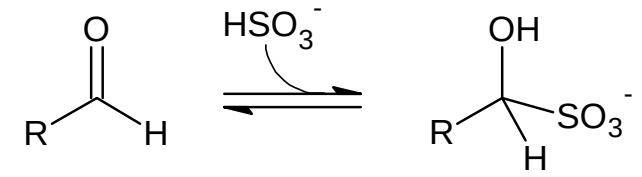
during storage?

Literature: - ***bisulfite adducts***
- ***imines***

Likeliness of contribution to flavor instability?

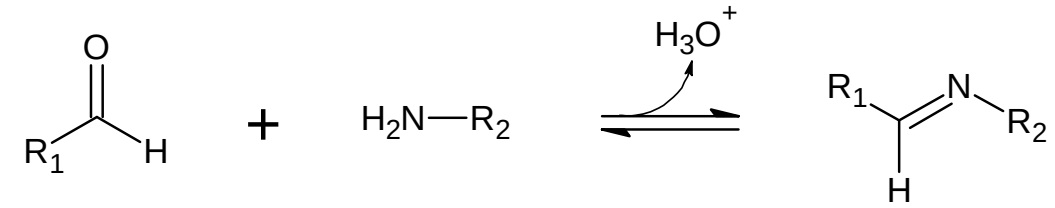
Chemistry background

- Bound state: **Bisulfite Adducts**
  - SO_2 is excreted by yeast during fermentation
  - SO_2 can be added as food additive



aldehyde ***α -hydroxy sulfonate***

- Bound state: **Imines** ('Schiffs bases')
  - Aldehydes can bind to amino acids, peptides and proteins

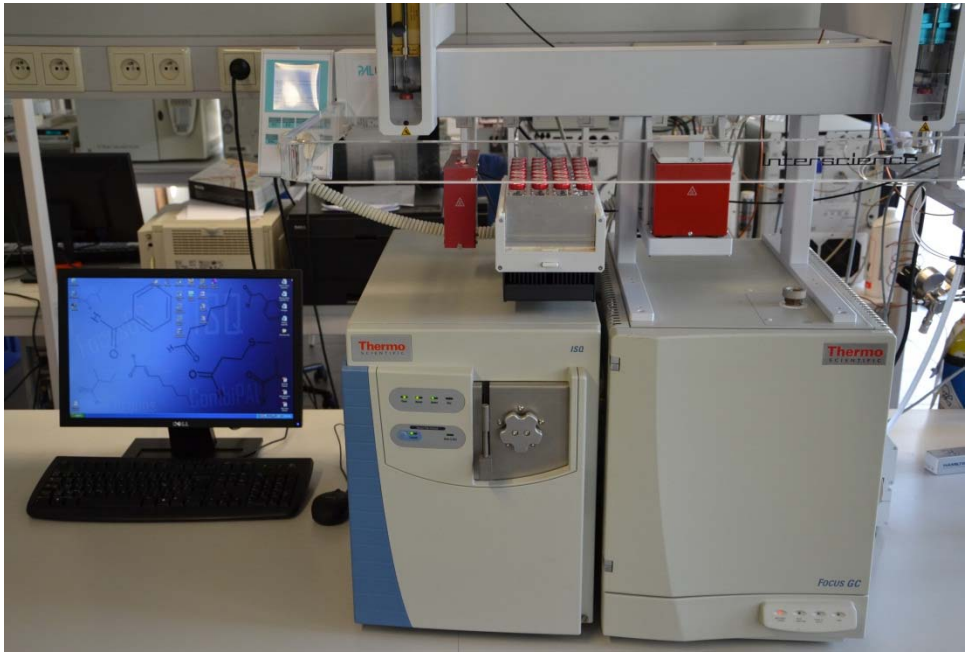


aldehyde ***amino group*** ***imine***

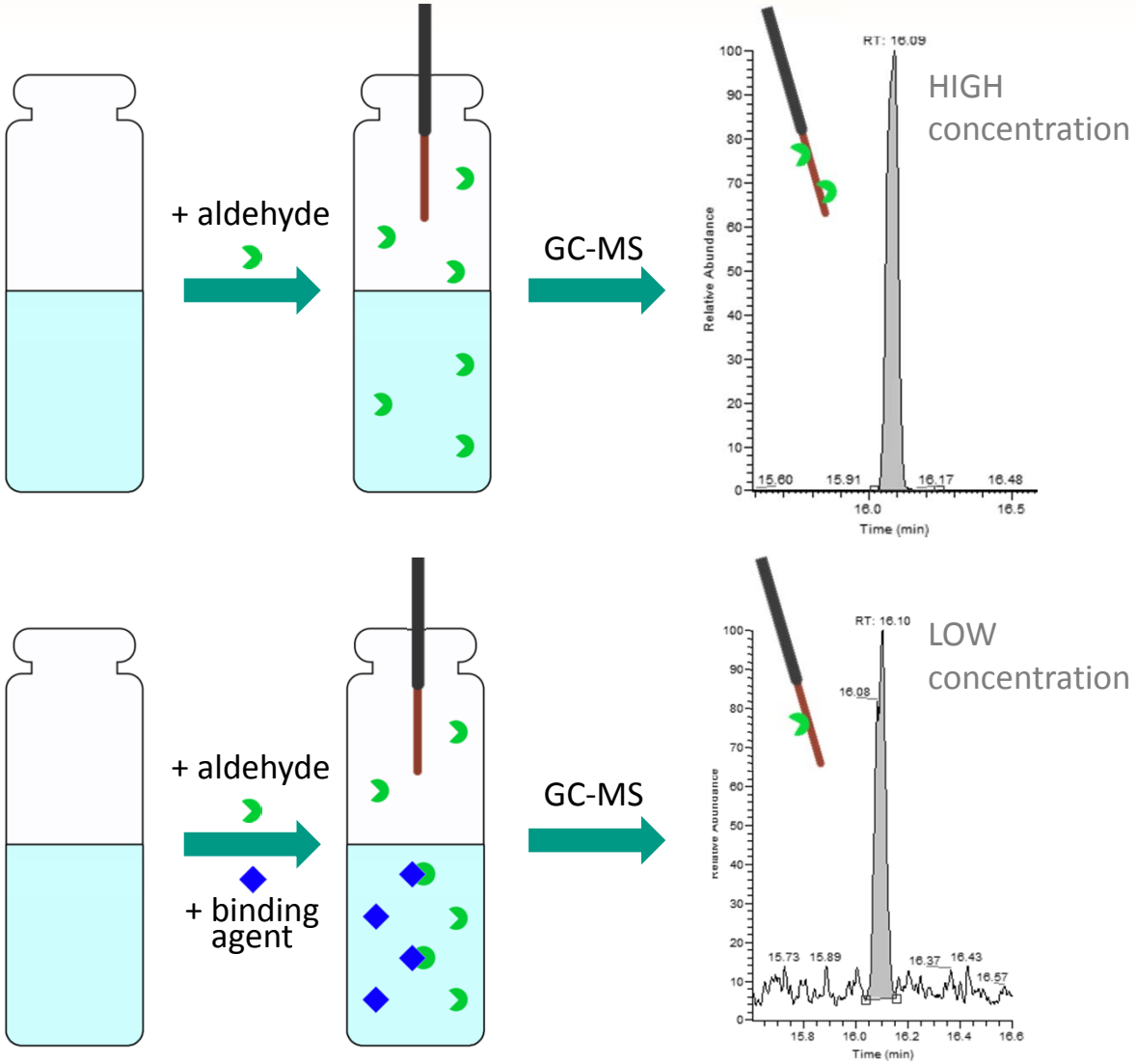
Methodology

- Method for direct measurement of bound aldehydes unavailable
- Free aldehyde quantification by

Automated headspace (HS) solid-phase microextraction (SPME) coupled to gas chromatography – mass spectrometry (GC-MS)



Methodology

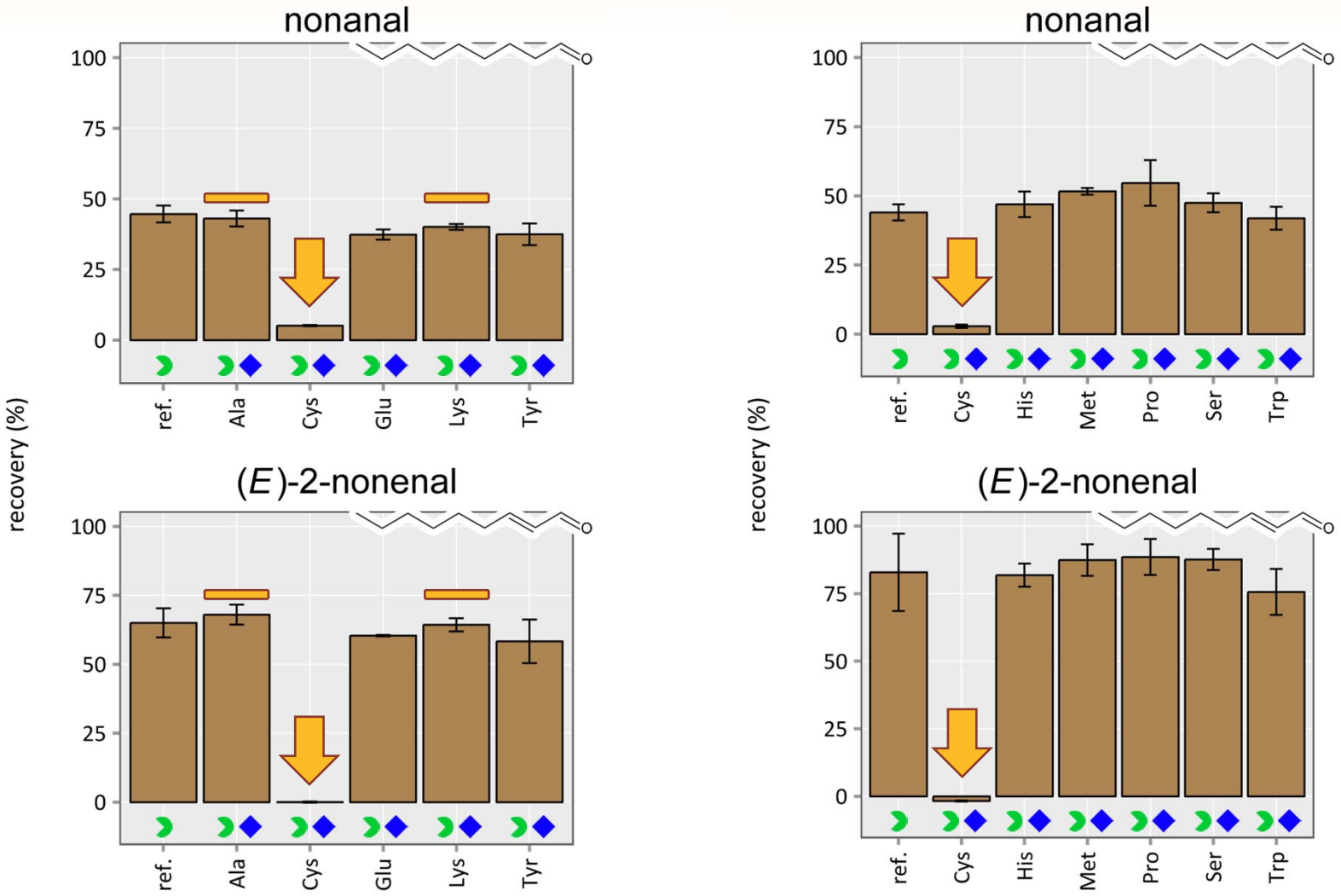


Aldehyde binding in models

EXPERIMENT

- AIM: Assess aldehyde binding to different amino acids
- SETUP:
  - Phosphate buffer (0.05 M, pH 6.0)
  - Nonanal and (*E*)-2-nonenal (1 µM)
  - Individual amino acids (1 mM)

Aldehyde binding in models



Aldehyde binding in models

EXPERIMENT

- AIM: Assess the influence of concentration and pH on aldehyde binding
- SETUP:
  - Phosphate buffer (0.05 M)
  - Nonanal and (*E*)-2-nonenal (1 µM)
  - Alanine (0 - 10 mM)

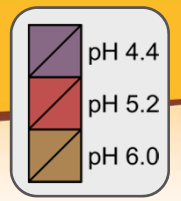
or

  - Cysteine (0 - 1 mM)

or

  - SO_2 (0 - 1 mM)

Aldehyde binding in models



Alanine

Cysteine

SO_2

nonanal

(*E*)-2-nonenal

recovery (%)

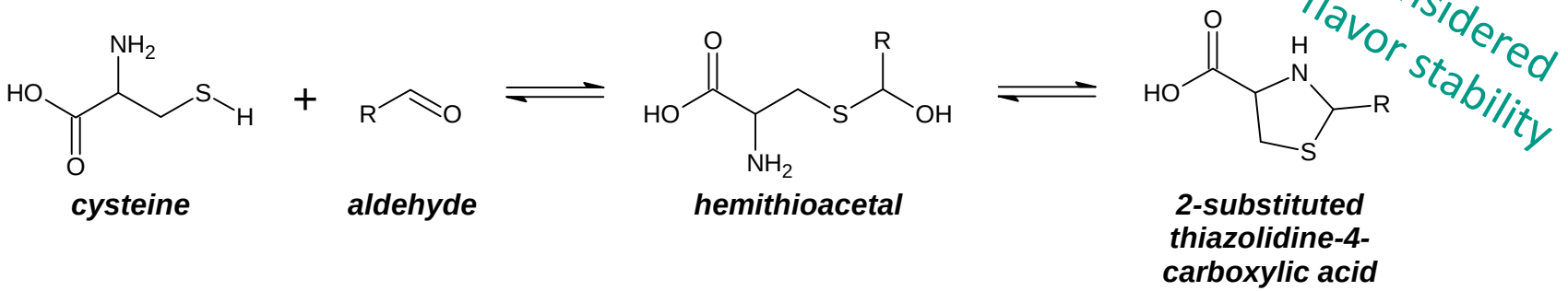
Ala concentration (µM)

Cys concentration (µM)

SO_2 concentration (µM)

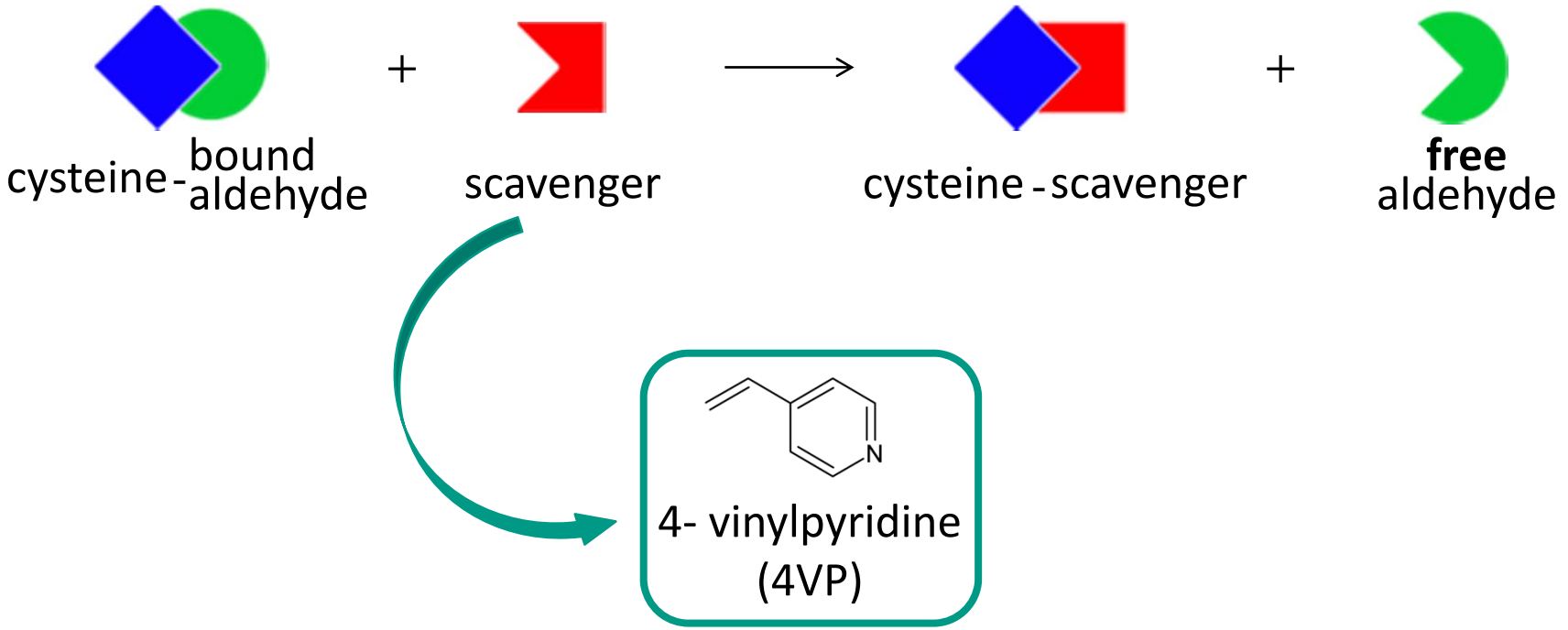
Chemistry background

- Bound state: **Thiazolidine carboxylic acids**
  - Thiol group of cysteine attacks carbonyl function, stabilization by cyclization

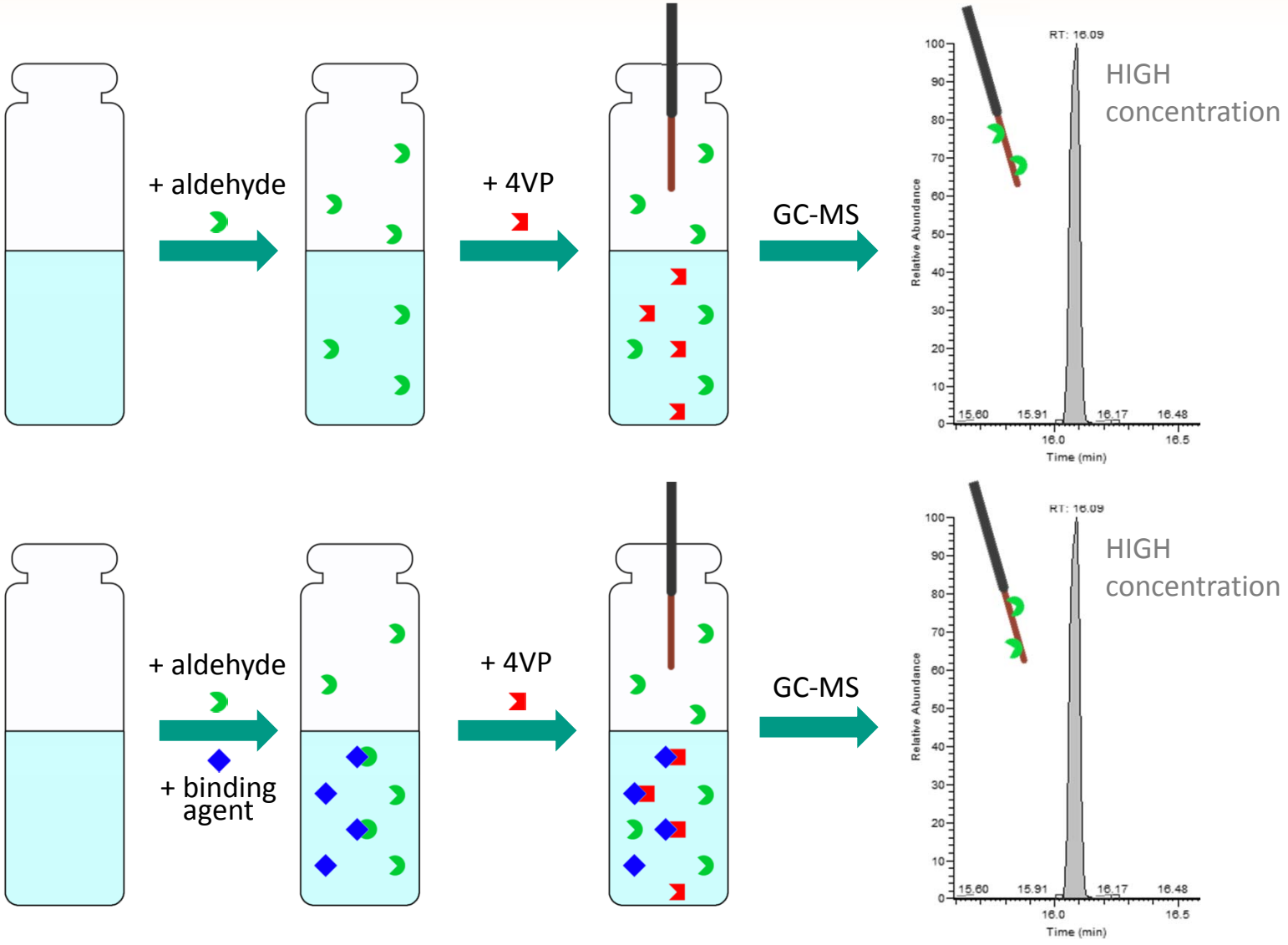


Methodology

- How to release aldehydes from these thiazolidines?



Methodology



Aldehyde binding and release in models

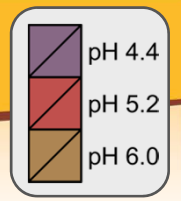
EXPERIMENT

- AIM: Assess the release of bound aldehydes by addition of 4VP as scavenger
- SETUP:
  - Phosphate buffer (0.05 M)
  - Nonanal and (*E*)-2-nonenal (1 µM)
  - Cysteine (500 µM)

or

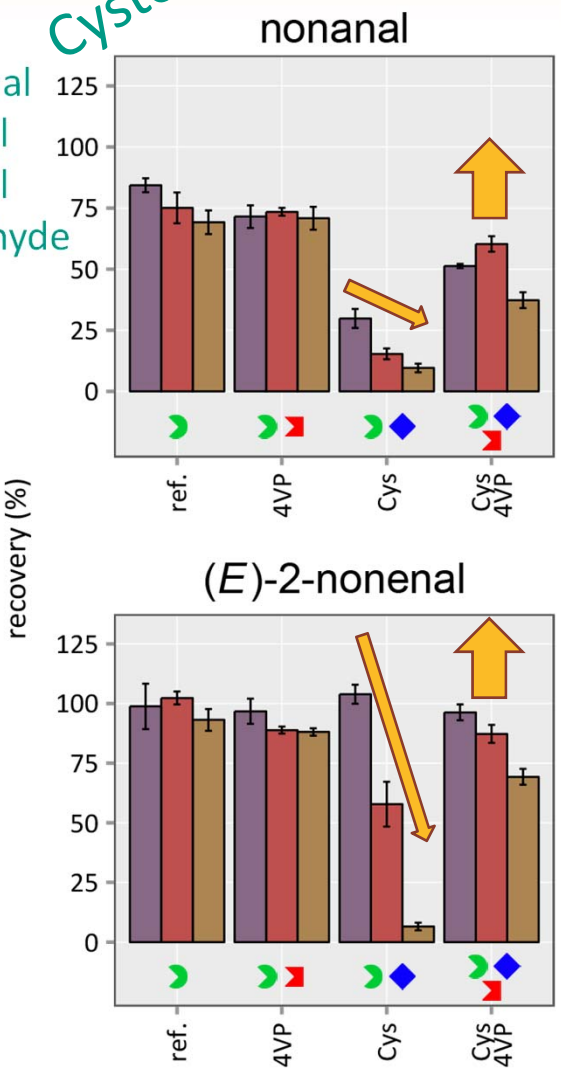
  - SO_2 (500 µM)
  - 4-vinylpyridine (500 mM)

Aldehyde binding and release in models



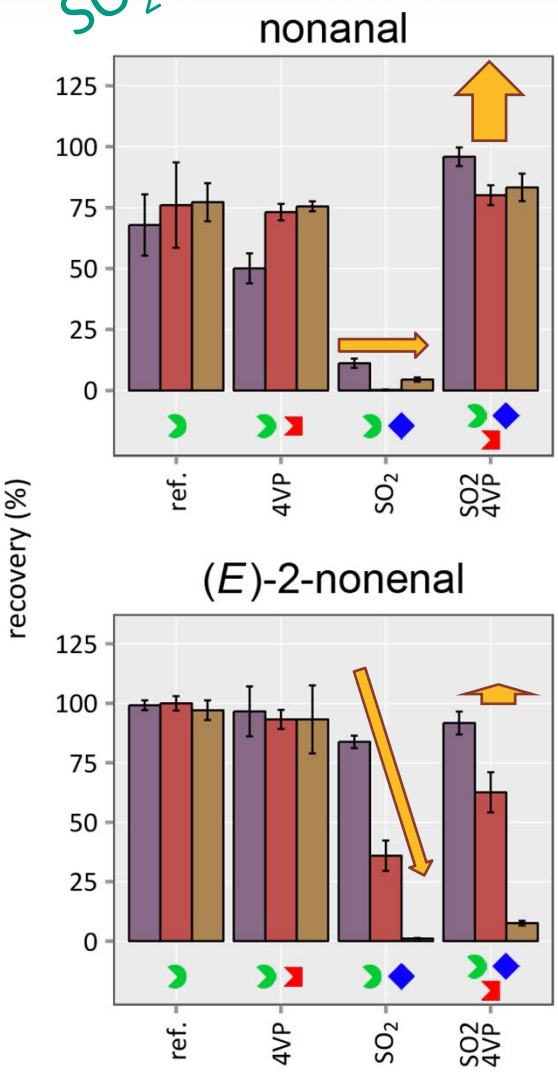
Cysteine

Similarly:
- 2-methylpropanal
- 2-methylbutanal
- 3-methylbutanal
- phenylacetaldehyde
- methional



Similarly:
- furfural
- benzaldehyde

SO_2



Similarly:
- 2-methylpropanal
- 2-methylbutanal
- 3-methylbutanal
- phenylacetaldehyde
- methional
- benzaldehyde
- furfural (release)

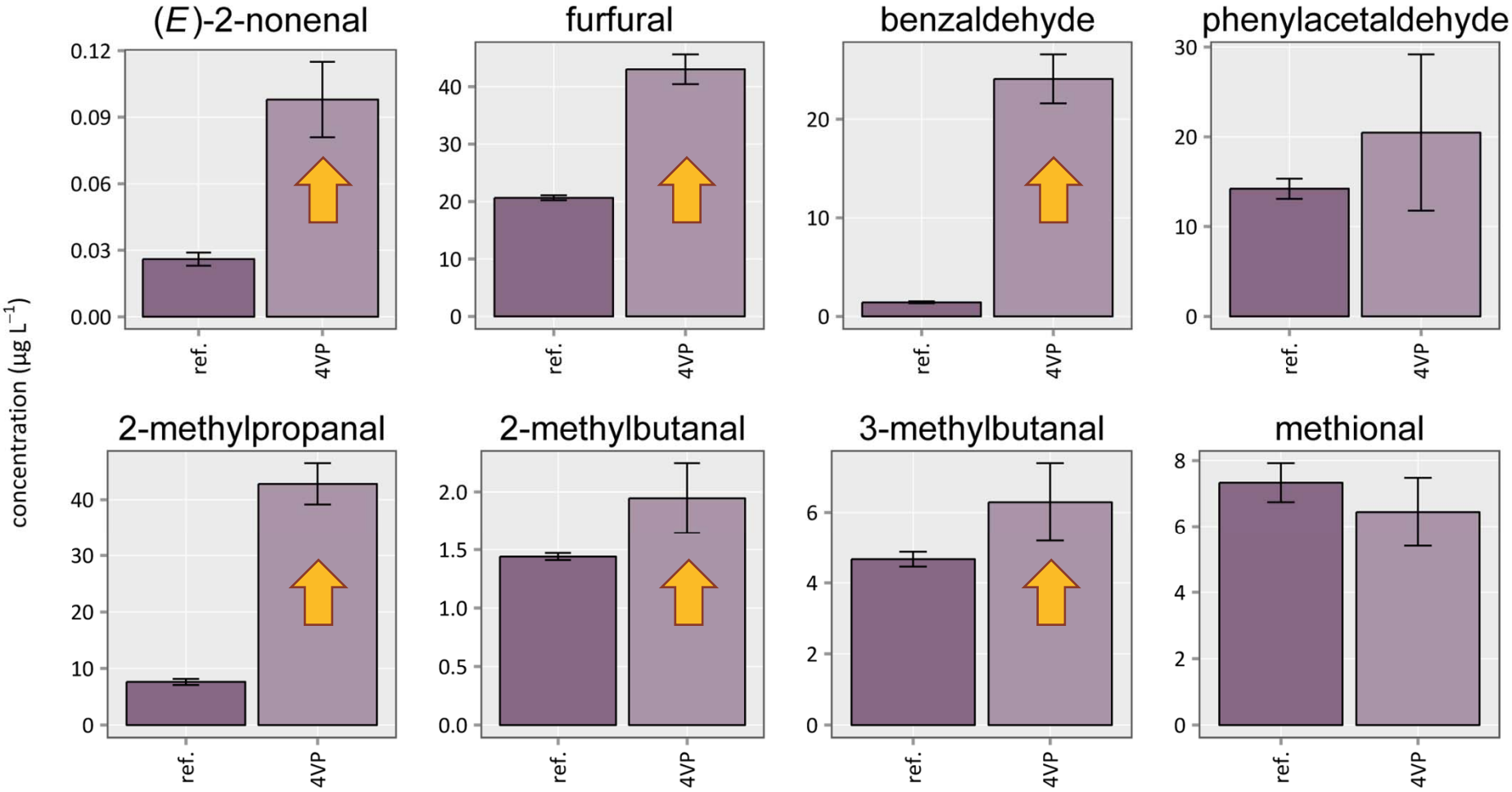
Similarly:
- furfural (binding)

Aldehyde release from beer

EXPERIMENT

- AIM: Assess the release of bound aldehydes by addition of 4VP as scavenger in fresh pale lager beer
- SETUP:
  - Fresh commercial pale lager beer
  - 4-vinylpyridine (500 mM)

Aldehyde release from beer

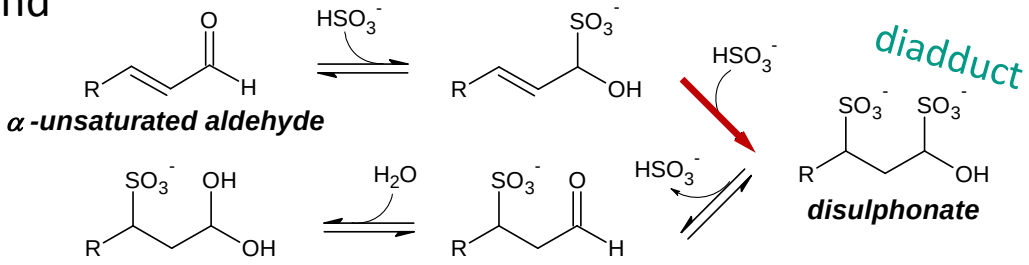


Strong link with aldehyde increases during aging!

Conclusions

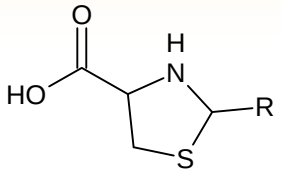
- Bound state: **Imines** ('Schiffs bases')
  - Formation not confirmed

- Bound state: **Bisulfite Adducts**
  - Formation clearly confirmed
  - **A higher pH yields more SO_2 binding**
    - → SO_2 only present after fermentation (at beer pH)
    - → SO_2 depletion can shift equilibria from SO_2 adducts to free SO_2 and free aldehydes
  - **4VP addition yields (almost) full recovery of free aldehydes**
    - Special case: only minor recoveries of (*E*)-2-nonenal due to irreversible binding to double bond



Conclusions

- Bound state: **Thiazolidine carboxylic acids**



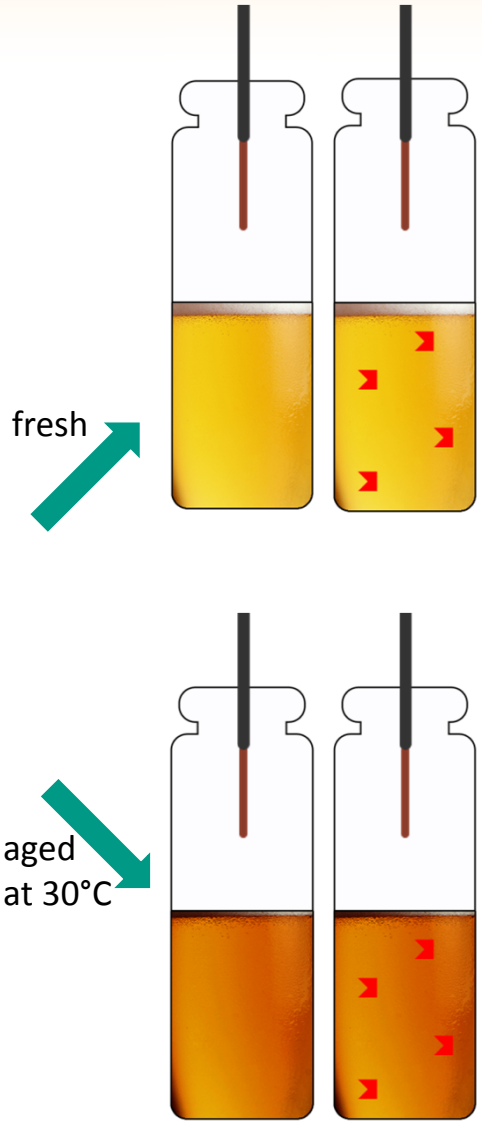
  - Cysteine clearly interacts with aldehydes
  - **A higher pH yields more cysteine binding**
    - → Formation most likely early in the brewing process (wort pH), possibly also during malting (malt pH)
    - → pH control during brewing may affect the thiazolidine content in fresh beer
    - → Free cysteine depletion during aging may shift equilibria from thiazolidines to free cysteine and free aldehydes
  - **4VP addition yields (almost) full recovery of free aldehydes**

- **4VP addition** clearly releases aldehydes from **fresh lager beer**
  - Combination of SO_2-adduct release and thiazolidine release
- EXTRA: Presence of thiazolidine carboxylic acids in fresh lager beer confirmed by liquid chromatography (data not shown)

Future prospects

- Use methodology for **fundamental insights**
  - Beer spiked with (labeled) precursors
    → *de novo*?
  - Beer spiked with labeled aldehydes
    → recovery without and with 4VP?

- Use methodology for more **practical insights**
  - Effect of raw materials
  - Effect of brewing parameters
  - Effect of yeast strain

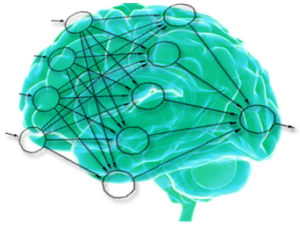


Future prospects

- Use approach as a

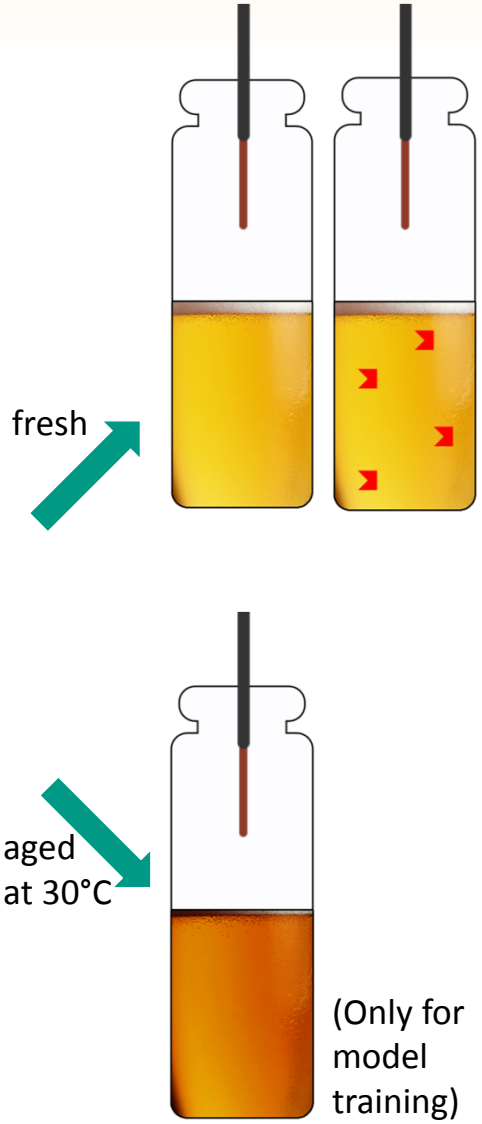
  flavor instability prediction tool

  - Input variables
    - Free aldehydes
    - 4VP-released aldehydes
    - Amino acids incl. cysteine
    - Total thiol content
    - SO_2
  - Artificial neural network

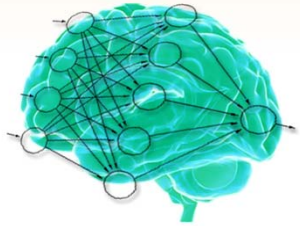


Machine learning method

  - Output variable:
    - Expected free aldehydes in aged beer



Future prospects



- What can the **flavor instability prediction tool** potentially do for brewers?
  - → Raw material evaluation
  - → Brewing parameters evaluation
  - → Evaluation of export feasibility
  - → …

THANK YOU

FOR YOUR ATTENTION

KU LEUVEN

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