FROM CONCEPT TO FUNCTIONAL TOOL:
HOW BOUND-STATE ALDEHYDES CAN INFLUENCE BEER FLAVOR INSTABILITY

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• In fresh beer: Minimal aldehyde concentrations due to yeast’s reducing activity
• During beer storage: Aldehyde concentrations increase, associated with typical off-flavors (e.g. papery, caramel, bread, almond)
> 60% of Belgian beer production is exported

Market globalization requires improved beer flavor stability, otherwise risk of consumer rejection

Need for more conscious transportation and storage conditions to prolong shelf life

Need for more fundamental insights and solutions
• **Origin** of the increases in aldehyde concentrations during aging?

*De novo* formation during storage?

*Upstream formation and release from a bound state* during storage?

**Literature:** Fatty acid oxidation, Strecker Degradation, Maillard reactions, ...

**Literature:** bisulfite adducts, imines

Inconclusive, contradictions

Likelihood of contribution to flavor instability?
• **Bound state: Bisulfite Adducts**
  – SO$_2$ is excreted by yeast during fermentation
  – SO$_2$ can be added as food additive

```
\[ \text{aldehyde} \xrightarrow{\text{HSO}_3^-} \text{α-hydroxy sulfonate} \]
```

• **Bound state: Imines** (‘Schiffs bases’)
  – Aldehydes can bind to amino acids, peptides and proteins

```
\[ \text{aldehyde} + \text{amino group} \xrightarrow{\text{H}_3\text{O}^+} \text{imine} \]
```
Methodology

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

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

+ aldehyde + aldehyde + binding agent

GC-MS

HIGH concentration

GC-MS

LOW concentration
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
**Bound state: Thiazolidine carboxylic acids**

- Thiol group of cysteine attacks carbonyl function, stabilization by cyclization

![Chemical structures](image)

- Cysteine + Aldehyde → Hemithioacetal → 2-substituted thiazolidine-4-carboxylic acid
Methodology

• How to release aldehydes from these thiazolidines?

\[ \text{cysteine-bound aldehyde} + \text{competitor} \rightarrow \text{cysteine-competitor} + \text{free aldehyde} \]

4- vinylpyridine (4VP)
Methodology

- + aldehyde
- + 4VP
- + binding agent
- GC-MS

HIGH concentration

- + aldehyde
- + 4VP
- GC-MS

HIGH concentration
**Experiment**

- **Aim:** Assess the release of bound aldehydes by addition of 4VP as competitor

- **Setup:**
  - Phosphate buffer (0.05 M)
  - Nonanal and \((E)-2\)-nonenal (1 µM)
  - Cysteine (500 µM)
  - \(SO_2\) (500 µM)
  - 4-vinylpyridine (500 mM)
Binding and release in models

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

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

Similarly:
- furfural
- benzaldehyde

Similarly:
- furfural (binding)
Conclusions from models

• **Bound state: Imines** (‘Schiffs bases’)
  - Formation not confirmed

• **Bound state: Bisulfite Adducts**
  - **A higher pH yields more SO₂ binding**
    - SO₂ only present after fermentation (at beer pH)
    - SO₂ depletion can shift equilibria from SO₂ adducts to free SO₂ 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

![Chemical structures and reactions](image)
Conclusions from models

- **Bound state**: Thiazolidine carboxylic acids
  - 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
Methodology

Cysteine spiking

kept at 0°C

aged at 30°C

fresh

fresh 4VP

90d 30°C

90d 30°C 4VP
Aldehyde release from beer

EXPERIMENT

• **AIM:**
  o Assess aldehyde release by addition of 4VP as competitor in beer
  o Assess the influence of cysteine content in fresh beer on flavor stability

• **SETUP:**
  o Fresh commercial pale lager beer
  o Unspiked, and spiked with cysteine (100 mg L\(^{-1}\))
  o Forced aged at 30°C for 90 days
  o 4-vinylpyridine (500 mM)
Aldehyde release from beer

Unspiked beer samples

(E)-2-nonenal

<table>
<thead>
<tr>
<th></th>
<th>fresh</th>
<th>4VP</th>
<th>90d 30°C</th>
<th>90d 30°C 4VP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µg L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E)-2-nonenal</td>
<td>0.01</td>
<td>0.15</td>
<td>0.10</td>
<td>0.15</td>
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</table>

Furfural

<table>
<thead>
<tr>
<th></th>
<th>fresh</th>
<th>4VP</th>
<th>90d 30°C</th>
<th>90d 30°C 4VP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µg L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furfural</td>
<td>0</td>
<td>200</td>
<td>400</td>
<td>600</td>
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</table>
Aldehyde release from beer

Unspiked and cysteine-spiked beer samples

Similar trends:
- 2-methylpropanal
- 2-methylbutanal
- 3-methylbutanal
- hexanal
- methional
Methodology

- 2-(furan-2-yl)-1,3-thiazolidine-4-carboxylic acid (interaction product of furfural and cysteine) quantification in beer by

Direct injection in an ultra performance liquid chromatography (UPLC) device with UV detection
Thiazolidines in beer

EXPERIMENT

• **AIM:**
  o Assess the variation in thiazolidine content in pale lager beers
  o Assess potential changes in thiazolidine content in aged pale lager beer

• **SETUP:**
  o Fresh commercial pale lager beer (5 brands)
  o Fresh pale lager beers produced in our pilot brewery (6 beers)
  o Forced aged at 30°C for 90 days
Thiazolidines in beer

2-(furan-2-yl)-1,3-thiazolidine-4-carboxylic acid

Cys ~ thiazolidine

thiazolidine ~ furfural$_{90d 30^\circ C}$

Cys ~ (furfural$_{90d 30^\circ C}$)$^{-1}$

Commercial pale lager

- beer A
- beer B
- beer C
- beer D
- beer E

Graphs showing concentrations of compounds in beer samples.
Conclusions from beer

- Fresh beer clearly contains a bound aldehyde pool, remaining constant or increasing (strongly) upon forced aging.
- Spiking fresh beer with cysteine strongly reduces the free aldehydes concentrations in forced aged samples.
  - Increased flavor stability!
- Fresh beer clearly contains furfural-derived thiazolidines, increasing upon forced aging.
- Cysteine and thiazolidine content in fresh beer are clearly linked to each other and to the free furfural content in forced aged beer samples.
  - Thiazolidine involvement in flavor stability!
• Predicting beer flavor instability from fresh beer analyses (assessed on 11 samples)
  – Input variables:
    • Free aldehydes concentrations
    • 4VP-released aldehyde concentrations
    • Furfural-derived thiazolidine content
    • Cysteine concentration
    • SO₂ content
    • Total amino acid content
    • pH
  – Output variables:
    • Free aldehydes in aged beer
  – Two approaches:
    • Partial Least Square Regression (PLSR)
    • Artificial Neural Network (ANN)
Instability prediction tool

R² values for the **predicted** aldehyde concentrations vs. **observed** aldehyde concentrations:

<table>
<thead>
<tr>
<th>PLSR</th>
<th>ANN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.95</td>
<td>0.83</td>
</tr>
<tr>
<td>0.91</td>
<td>0.82</td>
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<tr>
<td>0.82</td>
<td>0.61</td>
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<tr>
<td>0.54</td>
<td>0.93</td>
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<td>0.66</td>
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<tr>
<td>0.75</td>
<td>0.89</td>
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<tr>
<td>0.05</td>
<td>0.86</td>
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<tr>
<td>0.21</td>
<td>0.70</td>
</tr>
<tr>
<td>0.04</td>
<td>0.62</td>
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</tbody>
</table>

Trustworthy predictions, especially with more extented sample set!
• What can the **flavor instability prediction tool** potentially do for brewers?
  → Evaluation of export feasibility
  → Brewing parameter evaluation
  → Raw material evaluation
  → ...
THANK YOU

FOR YOUR KIND ATTENTION