Formation of selected compounds in model systems and food

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Objective:

- Elucidate the formation mechanisms of diketones and cyclic enolones in Maillard reaction systems based on glucose and amino acids as affected by pH (5 / 7), moisture (dry / wet), and amino acid (Gly / Pro)

- Examine the relative importance of different formation pathways by using equimolar mixtures of unlabelled and fully $^{13}$C-labelled glucose (CAMOLA approach)

- Extend the model study to food systems using extrusion conditions (135°C, 20 min) → Poster no. 45 (Davidek et al.)
Carbohydrate module labelling (CAMOLA): Elucidation of formation mechanisms

Examine the relative importance of different formation pathways by using defined mixtures of unlabelled and fully $^{13}$C-labelled sugar

$[^{12}\text{C}_6]$-Glucose + $[^{13}\text{C}_6]$-Glucose

Recombination

$^x$ 1  :  $^{x+3}$ 2  :  $^{x+6}$ 1

Fragment labelling technique (Hofmann and Schieberle, 2001)
Carbon module labelling (Frank and Hofmann, 2002)
Carbohydrate module labelling (Schieberle et al., 2003)
CAMOLA to elucidate the formation of furaneol from glucose/proline

Dry-heating (180°C, 10 min)

- Furaneol formed via acetylformoine exclusively from intact carbon skeleton of the hexose sugar

Aqueous solution (145°C, 20 min)

- Furaneol formed via acetylformoine from:
  - intact hexose sugar (40 %)
  - from C₃-sugar fragments (60 %)

(Hofmann and Schieberle, 2001)
CAMOLA study on formation of selected compounds from glucose

Mechanism of formation:
- from intact sugar skeleton
- recombination of sugar fragments
- recombination of sugar fragments and formaldehyde (generated from glycine)

Impact of: Water content, pH, amino acid co-reactant
**Experimental setup**

- **Aqueous buffer**
  1 ml 0.5 M phosphate buffer pH 5 or 7

- **Dry heating conditions**
  0.061 mmol NaH$_2$PO$_4$ or Na$_3$PO$_4$

- 0.15 mmol $[^{12}$C$_6$]-glucose monohydrate
- 0.15 mmol $[^{13}$C$_6$]-glucose
- 0.1 mmol glycine or proline

- Mixing in 20ml headspace vial

- Heating
  135°C/20min

- Cooling in ice bath

- Dissolution of residue in 1 ml water
  (low moisture system only)

- Addition of 2g anhydr. Na$_2$SO$_4$

- Analysis by SPME-GCxGC/TOFMS
Appearance of mixtures Glc/Gly

Dry with Na$_3$PO$_4$  Buffer pH 5  Buffer pH 7

<table>
<thead>
<tr>
<th>System</th>
<th>pH</th>
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<tbody>
<tr>
<td></td>
<td>Before heating</td>
<td>After heating</td>
<td>Before heating</td>
</tr>
<tr>
<td>Glc/Gly, buffer pH5</td>
<td>5.07</td>
<td>4.71</td>
<td>Glc/Gly, NaH$_2$PO$_4$</td>
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<tr>
<td>Glc/Gly, buffer pH7</td>
<td>6.97</td>
<td>6.23</td>
<td>Glc/Gly, Na$_3$PO$_4$</td>
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<tr>
<td>Glc/Pro, buffer pH5</td>
<td>5.01</td>
<td>5.00</td>
<td>Glc/Pro, NaH$_2$PO$_4$</td>
</tr>
<tr>
<td>Glc/Pro, buffer pH7</td>
<td>7.00</td>
<td>6.45</td>
<td>Glc/Pro, Na$_3$PO$_4$</td>
</tr>
</tbody>
</table>
Glucose + Proline → 2,3-Butanedione

**Acidic**

- **WET**
  - 80% $C_3 + C_1$
  - 6% $C_2 + C_2$
  - 9% Intact skeleton

- **Dry**
  - 44% $C_3 + C_1$
  - 41% Intact skeleton

**Basic**

- **WET**
  - 80% $C_3 + C_1$
  - 20% $C_2 + C_2$
  - No Intact skeleton

- **Dry**
  - 40% $C_3 + C_1$
  - 44% Intact skeleton
Formation of 2,3-Butanedione

From fragments (C$_3$+C$_1$):

Glucose → 1-Hydroxy-2-propanone + Formaldehyde → 2,3-Butanedione

(Schieberle et al., 2003)

From intact skeleton:

2,3-Butanedione

(Weenen, 1998)
Glucose + Glycine → 2,3-Butanedione

<table>
<thead>
<tr>
<th>Wet</th>
<th>Acidic</th>
<th>Basic</th>
</tr>
</thead>
<tbody>
<tr>
<td>86% $C_{3\text{Glc}} + C_1 (70% \text{ Gly} / 16% \text{ Glc})$</td>
<td>&lt;5% Intact skeleton</td>
<td>&lt;5% Intact skeleton</td>
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<tr>
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<th>Acidic</th>
<th>Basic</th>
</tr>
</thead>
<tbody>
<tr>
<td>46% $C_{3\text{Glc}} + C_1 (26% \text{ Gly} / 20% \text{ Glc})$</td>
<td>33% Intact skeleton</td>
<td>&lt;10% Intact skeleton</td>
</tr>
<tr>
<td><img src="image" alt="Graph" /></td>
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Glc/Pro (2,3-B)
Extrusion experiment: Glucose + Gly → 2,3-Butanedione

Contribution of different formation pathways to 2,3-butanedione:

- Recombination of C3 (sugar) and C1 (mainly from Gly) fragments (~70%)
- Close to mainly Wet/Acidic and Dry/Acidic model systems
Formation of Furaneol

(Schieberle et al., 2003; Schieberle, 2005)
Glucose + Proline → Furaneol

**Acidic**

- WET
- 48% Intact skeleton
- 47% ~70% Fragmentation (48% C3Glc + C3Glc)

**Basic**

- M 128
- M+1 129
- M+2 130
- M+3 131
- M+4 132
- M+5 133
- M+6 134

**Dry**

- **Acidic**
  - 48% Intact skeleton
- **Basic**
  - 49% Intact skeleton

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Note: The diagrams depict relative intensity (%) for different samples and conditions.
Glucose + Glycine $\rightarrow$ Furaneol

### Acidic

**WET**

![Graph showing the relative intensity of intact skeleton for Acidic WET conditions.]

- M128: 47%
- M129: 0%
- M130: 46%

**Dry**

![Graph showing the relative intensity of intact skeleton for Acidic Dry conditions.]

- M128: 47%
- M130: 47%

### Basic

**WET**

![Graph showing the relative intensity of intact skeleton for Basic WET conditions.]

- M128: 41%
- M129: 1%
- M130: 2%
- M131: 9%
- M132: 2%
- M133: 2%
- M134: 41%

**Dry**

![Graph showing the relative intensity of intact skeleton for Basic Dry conditions.]

- M128: 44%
- M129: 1%
- M130: 3%
- M131: 6%
- M132: 2%
- M133: 2%
- M134: 44%

~65% Intact skeleton

~70% Intact skeleton
Extrusion experiment: Glucose + Gly → Furaneol

Cyclic enolones mainly formed from intact glucose skeleton
Formation of Furaneol

(Schieberle et al., 2003; Schieberle, 2005)

Model systems:
- Furaneol is mainly formed from intact glucose skeleton (incl. Wet/Acidic)
- Except in Wet/Basic (Pro) where fragmentation is dominating (~70%)

Food system (extrusion):
- Furaneol is almost exclusively formed from the intact glucose skeleton
Conclusions

• Flavour formation mechanisms via Maillard-type reactions depend very much on the reaction conditions
  ▫ Aqueous systems versus dry-heating conditions (molecular mobility)
  ▫ pH (degree of sugar fragmentation / recombination)
  ▫ Results from model experiments cannot be extrapolated to food

• The CAMOLA approach opens new avenues to examine the role of different formation pathways
  ▫ Relative effectiveness of reaction pathways under different reaction conditions (e.g. dry-heating versus aqueous solutions)
  ▫ Possibility to extend approach to other precursors and intermediates
  ▫ Specific labelling experiments in case of small signals (<5%)

• Role of amino acids
  ▫ In the presence of Glycine, the C1 moiety is preferably delivered by Gly
  ▫ In the presence of Pro, more sugar fragmentation observed
  ▫ Need to study more amino acids (source of fragments vs catalysis)
Back-up slides
Semiquantitative data

**HDMF**

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<tr>
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<th>Glucose / Proline</th>
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<tr>
<td>Wet pH 5</td>
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<td>Wet pH 7</td>
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- Higher turnover with Pro

**2,3-butanedione**

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- Glc/Gly: Source of C1 is Gly
- Glc/Pro: Source of C1 is Glc