New Approaches in the Analysis of Amadori Compounds

7TH INTERNATIONAL SYMPOSIUM ON THE MAILLARD REACTION

Kumamoto, October 29 - November 01, 2001

I. Blank, T. Davidek, S. Devaud, N. Clety, H. Schlichtherle-Cerny
Structure of the presentation

• Introduction
  – Analytical methods

• High Performance Anion Exchange Chromatography
  – one detector
  – two detectors

• Application examples
  – Formation of Amadori compounds
  – Degradation of Amadori compounds
    (reversibility of the Amadori rearrangement)
Methods for analysing Amadori compounds

• **Gas separation**
  – Derivatisation
    Wittmann & Eichner (1989)

• **Liquid separation**
  – HPLC (DEAE-Si)
    Reutter & Eichner (1989)
  – HPLC (Waters)
    Huyghues-Despointes & Yaylayan (1994)
  – Capillary electrophoresis
    de Sa et al. (2001)

• **Mass separation**
  – FAB-MS/MS
    Staempfli et al., 1994
Chromatographic separation of Amadori compounds

GC

HPLC

(Eichner et al., 1990)
Multidetector system for monitoring Amadori compounds, sugars and amino acids via HPLC

(Huyghues-Despointes & Yaylayan, Food Chem. 51, 109, 1994)
Analysis of Amadori compounds by anion exchange chromatography

Core
- highly cross-linked
- inert, non-porous
- surface-sulfonated region

Ion exchange surface
- submicron layer
- high density of ion exchange sites

... using quaternary amine phases
Analysis of Amadori compounds by HPAEC-PAD

Simultaneous analysis of glucose and Fru-Gly

Separation of glucose-derived Amadori compounds

Gradient: H₂O + NaOH (500 mM)
Separation of Amadori compounds by HPAEC

Pulse Amperometric Detection

PAD Response (µC)

Time (min)

0.0 40.0 20.0 60.0

0.500

Glucose

Fru-Pro

Fru-Ala

Fru-Val

Fru-Leu & Ile

Fru-Met

Fru-Phe

Na acetate (500 mM)

NaOH (500 mM)
Analysis of Fru-Pro by HPAEC-PAD

- **Reaction conditions**
  - D-Glucose : 0.2 mol
  - L-Proline : 0.2 mol
  - MeOH (250 mL)
  - Reflux (8 h)

- **Clean-up**
  - Evaporation of MeOH
  - Solution in water
  - Filtration (0.45 µm)
  - Analysis
Formation of Fru-Pro from glucose and proline
Column: Carbopac PA1 (strong anion exchange column)
Detection: #1: UV-Detection (DAD) for furans and enol-oxo compounds
          #2: Electrochemical Detector (ECD), integrated amperometry mode for sugars, amino acids, and Amadori compounds

Gradient:

<table>
<thead>
<tr>
<th>Time (mn)</th>
<th>Gradient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 H2O</td>
</tr>
<tr>
<td>30</td>
<td>Column cleaning with NaOH 300mM</td>
</tr>
<tr>
<td>40</td>
<td>Initial conditions</td>
</tr>
<tr>
<td>60</td>
<td>0 NaOH 300mM</td>
</tr>
<tr>
<td>30</td>
<td>100 CH3COONa 300mM</td>
</tr>
</tbody>
</table>
Simultaneous analysis of Maillard intermediates

ECD detection

DAD Detection
<table>
<thead>
<tr>
<th>Compound</th>
<th>Detection limit (nmol/ml)</th>
<th>Linearity range (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fru-Gly</td>
<td>0.2</td>
<td>0.2 - 100</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.2</td>
<td>0.2 - 200</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.8</td>
<td>0.8 - 200</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.8</td>
<td>0.8 - 200</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.8</td>
<td>0.8 - 100</td>
</tr>
<tr>
<td>HMF</td>
<td>0.03</td>
<td>0.03 - 200</td>
</tr>
<tr>
<td>Furaneol</td>
<td>0.2</td>
<td>0.2 - 200</td>
</tr>
<tr>
<td>Maltol</td>
<td>0.03</td>
<td>0.03 - 200</td>
</tr>
</tbody>
</table>
Reaction of glucose and glycine
(pH 9, 90°C, 3 h)
Mechanism of the decomposition of Amadori compounds

(Hodge, 1953; Anet, 1964; Simon & Heubach, 1965)
Decomposition of Fru-Gly (pH 5, 90°C, 7 h)

Electrochemical detection (integrated amperometry)

UV detection at 285nm

UV detection at 355nm
Formation of glucose and mannose via reversed Amadori rearrangement of Fru-Gly
Identification of glucose and mannose by HILIC-MS/MS

Fru-Gly heated in phosphate buffer (pH 6, 90°C, 3 h)

Co-injection of mannose & glucose
Conclusion

- HPAEC is a rapid method for analysing Maillard reaction products
- Simultaneous detection of various types of compounds feasible using DAD and ECD in series
- Low detection limit and wide linearity range
- Particularly suitable for monitoring known compounds

- Identification only by co-injection with reference compound and comparison of UV spectra
- Coupling with MS required for identification