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# Simultaneous determination of advanced glycation end products and heterocyclic amines in roast/grilled meat by UPLC-MS/MS

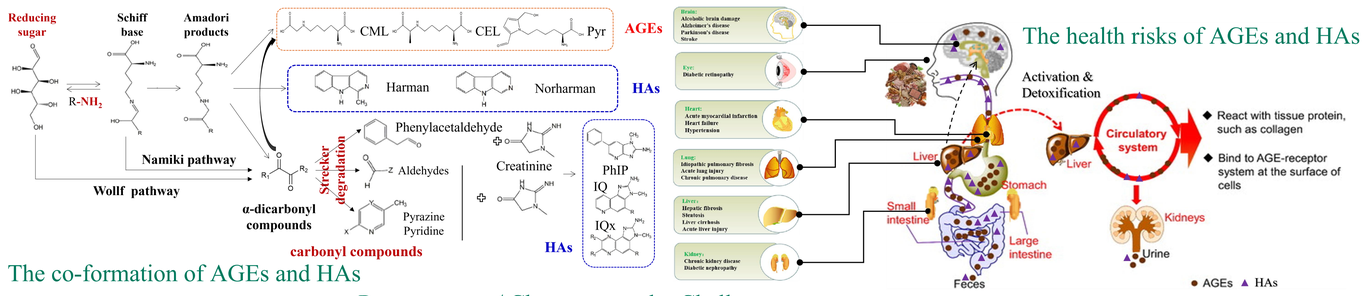
Mingyu Li[a,b], Chunjiang Zhang[a], Zhenyu Wang[a], Na Liu[c], Ruiyun Wu[a], Jiajing Han[a], Wenhan Wei[a], Christophe Blecker[b], Dequan Zhang[a,*]

[a] Integrated Laboratory of Processing Technology for Chinese Meat Dishes, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing, China. dequan_zhang0118@126.com

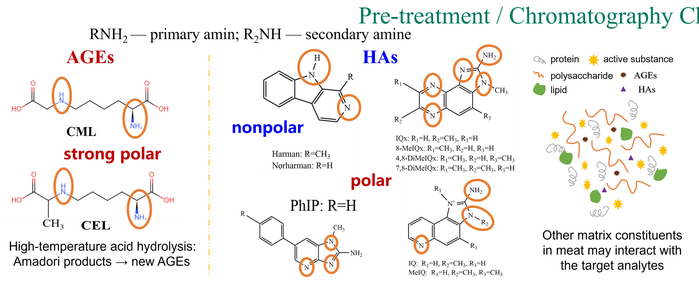
[b] Gembloux Agro-Bio Tech, Unit of Food Science and Formulation, University of Li`ege, Avenue de la Facult´e d'Agronomie 2, Gembloux, Belgium

[c] Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China

## Introduction




Pre-treatment / Chromatography Challenge







AGEs and HAs are hamful Maillard reaction products in thermally processed meats;
Current methods analyze them separately, doubling time/cost and introducing sample variability;
No validated protocols for simultaneous quantification in roast/grilled meats.

## Objective

The main objective of this study was to establish an accurate, robust and efficient analytical method for the simultaneous determination of AGEs and HAs in roast/grilled meat.

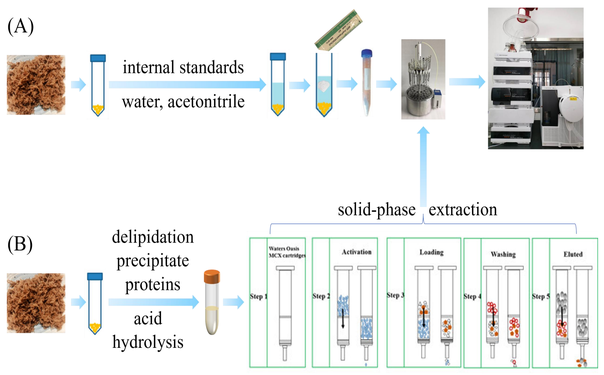
## Materials and Methods

**1 Preparation of standard solution**

5 mg of $d_4$-CML was added to a 5 mL volumetric flask, and 10 mg of CML, CEL, PYR, IQ, MeIQ, IQx, MeIQx, 4,8-DiMeIQx, 7,8-DiMeIQx, PhIP, Harman, Norharman, $d_4$-CEL and 4,7,8-TriMeIQx were transferred to 14 volumetric flasks (10 mL), then a stock solution (1 mg/mL) was prepared with ultrapure water. Dilute the stock solution with ultrapure water to prepare a mixed standard solution.

**2 Preparation of sample**

1. Sample weighing
2. Defatting / deproteinization
   - Add 5 mL n-hexane, vortex 30 s.
   - Centrifuge 4 800 r/min, 10 min, 4 °C.
   - Discard supernatant; repeat 3×.
   - Dry residue under $N_2$ stream.
3. Reduction reaction
   - Add 1.5 mL sodium borate buffer (0.2 M, pH 9.2).
   - Add 1 mL $NaBH_4$ (1 M).
   - Incubate 4 °C, 8 h (prevents AGEs regeneration).
4. Acid hydrolysis
   - Add 2.5 mL HCl (12 M).
   - Seal tube; hydrolyze 110 °C, 24 h.
5. Clarification & dilution
   - Cool, filter hydrolysate.
   - Transfer to 25 mL volumetric flask; make up to mark with ultrapure water.
6. Solid-phase extraction (Oasis MCX, 3 cc/60 mg)
   a) Conditioning: 1 mL MeOH, 1 mL 0.1 M HCl.
   b) Loading: 1 mL hydrolysate + 100 µL mixed IS (1 µg/mL, $d_4$-CML, $d_4$-CEL, 4,7,8-TriMeIQx).
   c) Wash: 1 mL 0.1 M HCl, 1 mL 5 % MeOH.
   d) Elute: 2 mL 5 % $NH_4OH$ in MeOH.
7. Concentration & reconstitution
   - Evaporate eluate under $N_2$ at 40 °C.
   - Re-dissolve in 1 mL MeOH.
   - Transfer to LC vial for UPLC-MS/MS.




**3 UPLC-MS/MS analysis**

- Instrument
  Agilent 1290 Infinity II UPLC + 6470 triple-quadrupole MS/MS (Agilent, USA)
- Chromatographic column
  ACQUITY UPLC HSS T3, 2.1 × 100 mm, 1.8 µm (Waters, USA)
- Mobile phase
  A: aqueous solution containing 0.1% (*v/v*) formic acid
  B: methanol
  Gradient elution
- Injection volume: 2 µL
- Flow rate: 0.2 mL/min
- ESI positive mode
- Dynamic multiple reaction monitoring (D-MRM)

**4 Method verification**

- Linearity range: 10, 20, 50, 100, 200 ng/mL (concentration levels)
- Sensitivity: S/N = 3 (LODs); S/N = 10 (LOQs)
- Matrix effects (ME):
  $$\text{ME}\ (\%) = \frac{Response\ in\ the\ matrix}{Response\ in\ pure\ solvent} \times 100$$
- Recovery: spiked with low, medium and high concentration standards (20, 100 and 200 µg/kg)
  $$\text{Recovery}\ (\%) = \frac{amount\ of\ analytes\ added - amount\ of\ analytes\ tested}{amount\ of\ analytes\ in\ sample \quad matrix - amount\ of\ analytes\ tested} \times 100$$
- Precision (RSD): n = 6 / level, RSD ≤ 15 %

**References**

1 Barzegar, F., Kamankesh, M., & Mohammadi, A. (2019). Food Chemistry, 280, 240-254.
2 Zhu, Z., Huang, M., Cheng, Y., Khan, I. A., & Huang, J. (2020). Trends in Food Science & Technology, 98, 30-40.

## Results

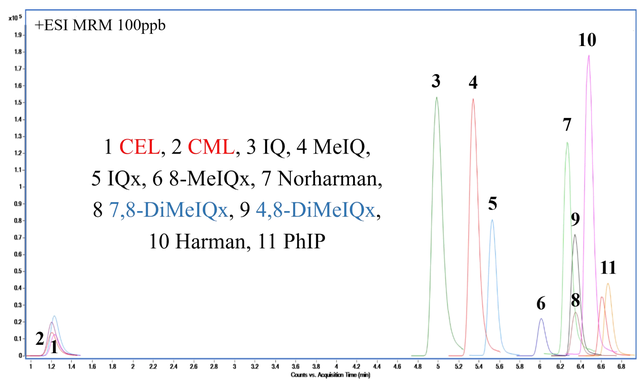
### 1. Optimization of MS/MS conditions

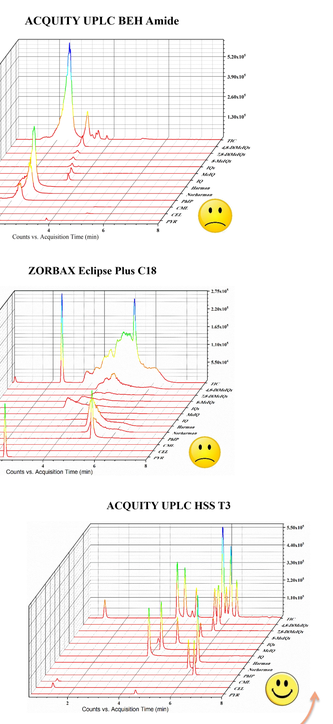
Table 1 MS/MS parameters and retention times of 11 analytes and 3 internal standards

| Compounds | Precursor ion | Product ion[a] | Fragmentor (V) | Collision Energy (eV) | Retention time |
|---|---|---|---|---|---|
| 4,7,8-TriMeIQx[b] | 242.1 | 201.2 (C) | 100 | 32 | 6.630 ± 0.5 |
| 4,7,8-TriMeIQx[b] | 242.1 | 145.1 (Q) | 100 | 40 | 6.630 ± 0.5 |
| 4,8-DiMeIQx | 228.1 | 213.1 (Q) | 128 | 32 | 6.358 ± 0.5 |
| 4,8-DiMeIQx | 228.1 | 187.1 (C) | 128 | 28 | 6.358 ± 0.5 |
| 7,8-DiMeIQx | 228.1 | 187.2 (C) | 100 | 32 | 6.366 ± 0.5 |
| 7,8-DiMeIQx | 228.1 | 131.1 (Q) | 100 | 40 | 6.366 ± 0.5 |
| PhIP | 225.1 | 210.1 (Q) | 90 | 32 | 6.687 ± 0.5 |
| PhIP | 225.1 | 140.1 (C) | 90 | 40 | 6.687 ± 0.5 |
| $d_4$-CEL[b] | 223.0 | 134 (C) | 70 | 12 | 1.239 ± 0.5 |
| $d_4$-CEL[b] | 223.0 | 88.0 (Q) | 70 | 28 | 1.239 ± 0.5 |
| CEL | 219.1 | 130.3 (C) | 80 | 12 | 1.239 ± 0.5 |
| CEL | 219.1 | 84.1 (Q) | 80 | 20 | 1.239 ± 0.5 |
| 8-MeIQx | 214.1 | 173.2 (C) | 100 | 24 | 6.032 ± 0.5 |
| 8-MeIQx | 214.1 | 131.1 (Q) | 100 | 40 | 6.032 ± 0.5 |
| MeIQ | 213.1 | 198.1 (Q) | 85 | 32 | 5.362 ± 0.5 |
| MeIQ | 213.1 | 145.2 (C) | 85 | 28 | 5.362 ± 0.5 |
| $d_4$-CML[b] | 209.1 | 134.0 (C) | 70 | 10 | 1.206 ± 0.5 |
| $d_4$-CML[b] | 209.1 | 87.8 (Q) | 70 | 20 | 1.206 ± 0.5 |
| CML | 205.2 | 130.1 (C) | 55 | 12 | 1.206 ± 0.5 |
| CML | 205.2 | 84.0 (Q) | 55 | 20 | 1.206 ± 0.5 |
| IQx | 200.1 | 185.1 (Q) | 103 | 32 | 5.552 ± 0.5 |
| IQx | 200.1 | 132.1 (C) | 103 | 40 | 5.552 ± 0.5 |
| IQ | 199.1 | 184.1 (Q) | 60 | 32 | 4.994 ± 0.5 |
| IQ | 199.1 | 131.1 (C) | 60 | 24 | 4.994 ± 0.5 |
| Harman | 183.1 | 115.2 (Q) | 55 | 40 | 6.496 ± 0.5 |
| Harman | 183.1 | 89.1 (C) | 55 | 40 | 6.496 ± 0.5 |
| Norharman | 169.1 | 115.1 (Q) | 55 | 40 | 6.287 ± 0.5 |
| Norharman | 169.1 | 89.1 (C) | 55 | 40 | 6.287 ± 0.5 |

[a] Q, quantification ion; C, confirmation ion; [b] internal standard.

### 2. Optimization of UPLC conditions

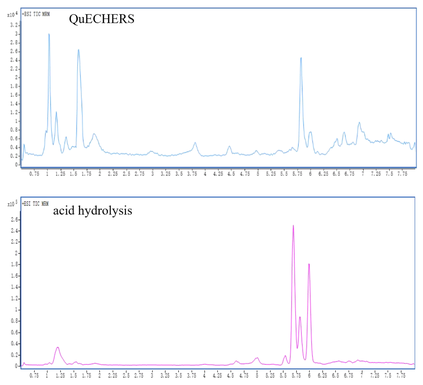


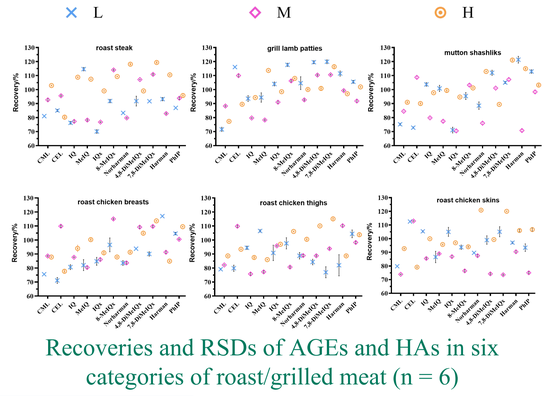

UPLC-MS/MS chromatograms of analytes on different columns

UPLC-MS/MS chromatograms of analytes and internal standards

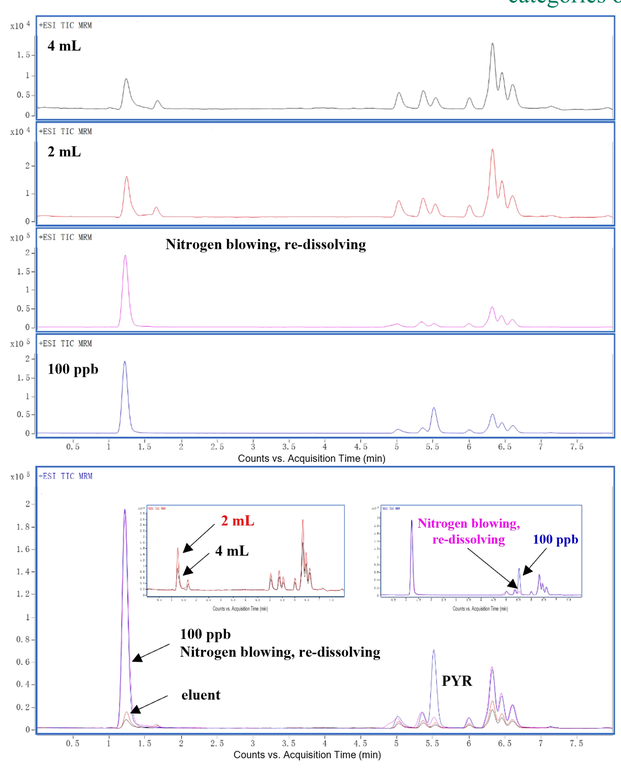
### 3. Optimization of sample preparation




### 4. Method validation




Recoveries and RSDs of AGEs and HAs in six categories of roast/grilled meat (n = 6)




UPLC-MS/MS chromatogram of solid phase extraction optimization

- Linearity: $R^2 > 0.99$
- LODs: 0.01 - 0.27 ng/mL
- LOQs: 0.04 - 0.91 ng/mL
- recovery: 71–121 %
- ME: 0.6 - 1.4
- RSD: 0.05 % - 7.58%

### 5. Analysis of real samples

Content: µg $g^{-1}$ level

Roast chicken skin > roast chicken thigh > mutton shashliks > grill lamb patties > roast chicken breast > roast steak

## Conclusions and Perspectives

- ❑ Set up a UPLC-MS/MS method was developed for the simultaneous extraction and purification of 11 kinds of AGEs and HAs in roast/grilled meat by acid hydrolysis and SPE
- ❑ Had good linearity, sensitivity, precision, stability and recoveries
- ❑ Greatly reduces the time and cost of detecting AGEs and HAs, respectively
- ➢ Future: expanding to plant-based meat, modeling dietary exposure