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SIMULTANEOUS DETERMINATION OF ADVANCED GLYCATION END PRODUCTS AND HETEROCYCLIC AMINES IN ROAST/GRILLED MEAT BY UPLC-MS/MS

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I. INTRODUCTION

Meat is a vital source of high-quality protein, vitamins, and minerals in the human diet, essential for maintaining good health. However, the modern meat industry is under increasing scrutiny, particularly concerning the safety and sustainability of meat production and consumption. In an era when the meat sector is often criticized by some with unscientific arguments, it is crucial to emphasize the importance of meat as a natural and nutritious product and to address these concerns through scientific research and innovation.

The Maillard reaction, a series of complex chemical reactions that occur during the thermal processing of meat, imparts desirable flavours and colours but also generates potentially harmful by-products, such as advanced glycation end products (AGEs) and heterocyclic amines (HAs). These compounds have been associated with chronic diseases, including diabetes, cardiovascular diseases, and cancer, raising significant concerns about their presence in roast/grilled meat products. However, simultaneously quantifying these compounds in roast/grilled meat is challenging due to their different polarities, low levels in food, and the complex food matrix. Existing methods for detecting AGEs and HAs often require separate procedures due to differences in pre-treatment and instrument parameters, which increases testing time and cost.

The objective of this study was to develop an accurate, robust, and efficient analytical method for the simultaneous determination of AGEs and HAs in roast/grilled meat using UPLC-MS/MS by optimizing instrument parameters and pre-treatment methods. This research is highly relevant to the "Real Meat, Real Care" theme of the conference, as it focuses on ensuring the safety of meat products, which is a critical aspect of caring for consumers' health. Additionally, it contributes to the sustainable development of the meat industry by providing scientific insights into product quality improvement and supporting the industry's commitment to preserving tradition while igniting innovation for a sustainable future.

II. MATERIALS AND METHODS

Chemicals & Materials: PYR (99%), d_4-CML (96%), d_4-CEL (96%), CML, CEL, IQ, Harman, Norharman, MeIQ, MeIQx, 7,8-DiMeIQx, IQx, PhIP, 4,8-DiMeIQx, and 4,7,8-TriMeIQx (98%) were purchased from Toronto Research Chemicals Inc. Solid phase extraction cartridges (Oasis MCX, 3 cc, 60 mg) were purchased from Waters Co.

AGEs & HAs Detection: 50 mg of the crushed sample was taken, deproteinized with 5 mL of hexane, centrifuged at 4800 r/min for 10 min and repeated three times. The residue was dried, and reduced with 1.5 mL sodium borate buffer (0.2 M, pH 9.2) and 1 mL sodium borohydride (1 M) at 4 °C (8 h), followed by hydrolysis with HCl (12 M, 2.5 mL) at 110 °C for 24 h. The hydrolysate was filtered into a 25 mL volumetric flask and made constant volume with ultrapure water. The above hydrolysate (1 mL) and mixed internal standard (100 µL, 1 µg/mL) of d_4-CML, d_4-CEL and 4,7,8-TriMeIQx were spiked, loaded onto the MCX column and eluted with 5% methanol-ammonium. The eluate was dried under a nitrogen stream and then dissolved in 1 mL methanol for UPLC-MS/MS (Agilent 1290-6470) analysis. An injection volume of 2 µL was employed, while the flow rate used was 0.2 mL/min. The mobile phase employed consisted of methanol (B) and an aqueous solution containing 0.1% (*v/v*) formic acid (A). The gradient elution started with 95% A (0 min) and decreased from 0 ~ 2.0 min to 85% A, 3.0 min to 80% A, then 20% A every minute, directly to 10% A at 6.0 min, to 0% A at 7.0 min and finally returned to 95% A at 7.1 min, equilibrating to 8 min. Nitrogen was used as the nebulizer gas; the drying gas temperature was set at 280 °C; the drying gas flow rate was adjusted to 12 L/min; the nebulizer pressure was set at 25 Psi; and a capillary voltage of 3500 V was applied. The method was validated according to SANTE/2020/12830 guidelines.

Statistical Analysis: All experiments were conducted independently at least three times. Significant differences ($p < 0.05$) were analyzed using SPSS 17.0 statistical analysis software. GraphPad Prism (version 8.0) was used for all data plots.

III. RESULTS AND DISCUSSION

1. Optimization Results

MS/MS Conditions: Positive electrospray mode was used for detection as target compounds and internal standards have amine groups that form [M + H] molecular ions. By directly injecting standards, MS/MS parameters like fragmentor and collision energy were optimized. The dynamic multiple reaction monitoring (D-MRM) was chosen to avoid interference from isomers, ensuring accurate quantification.

UPLC Conditions: Three chromatographic columns were compared. The ACQUITY UPLC HSS T3 column showed sharp and symmetrical peak shapes for AGEs and HAs, unlike the other columns. It was selected due to its compatibility with polar and non - polar compounds and balanced retention of different molecules. An 8 min gradient elution was developed, separating each target analyte into distinct peaks (Figure 1).

Sample Preparation: Comparing acid hydrolysis + SPE and QuEChERS methods, acid hydrolysis + SPE was chosen. Although acid hydrolysis can convert Amadori products into AGEs, adding $NaBH_4$ corrected this overestimation. The acid hydrolysis + SPE method had better extraction efficiency and response. Activation and washing with 0.1 M HCl improved recoveries, and the optimal elution volume was 2 mL.

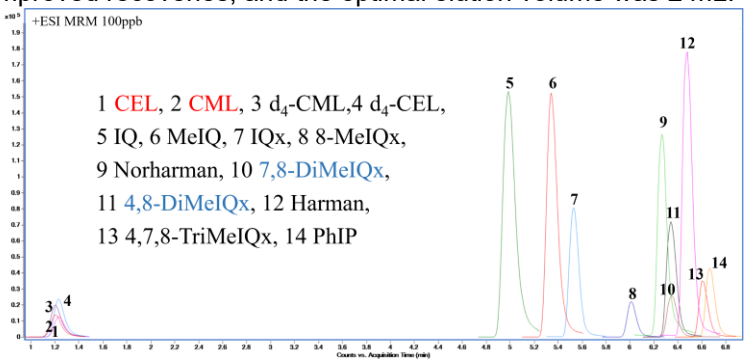


Figure 1. UPLC-MS/MS chromatograms of analytes and internal standards

2. Method Validation

Linearity and Sensitivity: Standard curves for AGEs and HAs showed good linearity (R^2>0.99) in the 10 - 200 ng/mL range. LODs ranged from 0.01 - 0.27 ng/mL and LOQs from 0.04 - 0.91 ng/mL, indicating high sensitivity.

Precision and Recovery: The method had recoveries ranging from 71.59% - 102.92% for different analytes, and precision (RSDs) ranging from 0.10% - 5.50%. Matrix effects were acceptable considering the complex food matrix.

Comparison with Previous Studies: For AGEs, the developed method had higher LODs and LOQs than some previous methods but offered advantages like shorter elution time and better separation. For HAs, its LODs and LOQs were comparable to some methods, and recoveries were generally better.

3. Analysis of Real Samples

The total levels of AGEs and HAs varied among different roast/grilled meat samples. AGEs levels were higher than HAs levels in all samples. Roast chicken skins had the highest levels and roast steaks the lowest. These results show that processing technology significantly impacts AGEs and HAs formation, which is crucial for understanding meat quality and safety in the context of "Real Meat, Real Care". It helps the meat industry make informed decisions to balance tradition and innovation for sustainable production.

IV. CONCLUSION

In this study, we successfully developed a UPLC-MS/MS method for the simultaneous determination of 11 AGEs and HAs in roast/grilled meat. By optimizing instrument parameters and the pre-treatment method, we achieved good separation and high - sensitivity detection within 8 min. The method showed excellent linearity (R^2>0.99) in the range of 10 - 200 ng/mL), acceptable precision, recovery, and matrix effects. This study significantly advances food safety in the meat industry by offering a time- and cost-efficient method to simultaneously detect AGEs and HAs, crucial for assessing meat quality and health risks. It also counters unfounded meat criticism with scientific data, supporting sustainable meat production and consumption.

Future research could focus on: 1) investigating how different cooking conditions (e.g., heat sources and durations) affect AGEs and HAs formation; 2) clarifying the interaction mechanisms of AGEs and HAs in meat during cooking and digestion to understand their health impacts; 3) exploring ways to reduce AGEs and HAs formation during meat processing without compromising traditional flavours and qualities, such as using natural inhibitors or modified techniques. This would enhance food safety and align with the meat industry's sustainability goals.

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