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Proceeding Paper

Determination of 4(5)-Methylimidazole in Sugar-Amino Acid Aqueous Model Systems by UPLC-Q-ToF-MS †

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Abstract: 4(5)-Methylimidazole (4(5)MEI) can be formed during the caramelization procedure and the Maillard reaction and has been classified as possibly carcinogenic to humans. Data concerning the formation of 4(5)MEI by the reaction between amino acids and sugars are still scarce. In this study, an Ultra-high Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UPLC-Q-ToF-MS) method was developed for the determination of 4(5)MEI in aqueous model systems, containing sugars (fructose and glucose) and amino acids (proline, phenylalanine, tyrosine and lysine), after thermal processing at 100 °C. The results showed that the 4(5)MEI was formed in all model systems, with the highest concentrations to be determined in fructose-proline (3.5 μg mL^{-1}) and fructose-tyrosine (3.0 μg mL^{-1}) aqueous model systems.

Keywords: 4(5)-methylimidazole; honey; Maillard reaction; liquid chromatography; mass spectrometry

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1. Introduction

4(5)-Methylimidazole (4(5)MEI) is formed in food matrices during their thermal processing as a result of the Maillard reaction and has been classified from International Agency for Research on Cancer (IARC) as potentially carcinogenic to humans [1]. This compound is also produced during the preparation of ammonia caramel colorant additives, by the caramelization procedure [2].

Early research reports have led to the hypothesis that 4(5)MEI can be produced from the reaction between ammonia and α-dicarbonyl compounds [3]. Therefore, ammonia has been mainly used as a nitrogen source for the study of 4(5)MEI in Maillard reaction systems, in combination with sugars (L-rhamnose, D-glucose) or methylglyoxal [4]. However, studies employing amino acids in Maillard model systems for the investigation of 4(5)MEI formation are still scarce. Amino acids could be a source of nitrogen-containing compounds in foods from the Strecker degradation, and exist in significant amount in honey [5]. The possibility of honey adulteration with caramel color raises concerns about the existence of 4(5)MEI under specific conditions, apart from the addition of caramel

colorants [6]. 4(5)-Methylimidazole is a highly polar molecule, characterized by the absence of chromophores, hence, liquid chromatography-mass spectrometry methods (LC-MS/MS) have been mainly applied for its determination in foods [7–10]. Nevertheless, the complexity of food matrices suggests the need for the development of accurate and sensitive analytical methods for the quantification of 4(5)MEI. Quadrupole Time-of-Flight (Q-ToF) mass analyzer offers rapid food analysis, providing high resolution, sensitivity and selectivity. To the best of our knowledge, there is no literature report employing liquid chromatography in combination with a Q-ToF mass analyzer for the quantification of 4(5)MEI. In line with the information presented above, the aim of this study is the development of an Ultra-high Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UPLC-Q-ToF-MS) method in order to determine 4(5)MEI in sugar/amino acid model systems, after thermal processing.

2. Materials and Methods

2.1. Reagents

Glucose, fructose, proline, phenylalanine, tyrosine, lysine, 4(5)MEI, and ammonium acetate were supplied by Sigma-Aldrich (St Louis, MO, USA). Formic acid, hydrochloric acid, ammonium hydroxide, methanol (MeOH) (HPLC grade) and acetonitrile (ACN) (LC-MS grade) were purchased from Fisher Scientific Co. (Chicago, IL, USA). Ultra high purity water was produced using a Genie Water System from RephiLe Bioscience Ltd. (Shanghai, China). Solid Phase Extraction (SPE) cartridges (Bond Elut SCX, 500 mg) were supplied by Agilent Technologies (Santa Clara, CA, USA).

2.2. Standard Solutions

Stock solution (1000 mg L^{-1}) of 4(5)MEI was prepared using ACN and stored in dark glass vial at −20 °C. Calibration curve of 4(5)MEI was constructed using the standard concentrations of 10.0, 8.0, 5.0, 3.0, 1.0, 0.8, 0.5, 0.3, 0.1 mg L^{-1} via dilution with ACN.

2.3. Preparation of Aqueous Model Systems

Eight model systems were prepared by mixing the appropriate amount of sugar and amino acid (Table 1). Subsequently, water was added in order to reach the quantity of 100 g for each model system. The aqueous model systems were heated at 100 °C for 60 h in a Tv10b heating oven (Memmert, Germany).

Table 1. Aqueous model systems of sugars-amino acids analyzed by UPLC-Q-ToF-MS and the concentration of 4(5)MEI after their thermal treatment.

Sample	Model System	Concentration (μg mL^{-1})
1	60 g glucose + 100 mg proline	1.1
2	60 g glucose + 20 mg phenylalanine	0.4
3	60 g glucose + 10 mg tyrosine	0.2
4	60 g glucose + 10 mg lysine	1.3
5	70 g fructose + 100 mg proline	3.5
6	70 g fructose + 20 mg phenylalanine	2.5
7	70 g fructose + 10 mg tyrosine	3.0
8	70 g fructose + 10 mg lysine	0.9

2.4. SPE Procedure

The SPE procedure was performed based on a previous study [11] with some modifications: 10 g of sample were weighted into a glass vial and diluted with 10 mL of water. The solution was acidified with 20 μL of 0.1 M HCl. SCX cartridges were activated with 2 mL of methanol and 2 mL of 1% (*v*/*v*) formic acid solution. Then, the sample solution was loaded into the column under vacuum and impurities were washed out with 4 mL of

methanol and 4 mL of 1% (*v*/*v*) formic acid solution. Elution of 4(5)MEI was achieved using 5 mL of 5% (*v*/*v*) methanolic ammonia solution. Subsequently, the solvent was remove under N_2 gas flow stream and the residue was dissolved with 1 mL of 10% (*v*/*v*) aqueous acetonitrile.

2.5. UPLC-Q-ToF-MS Analysis

An Agilent 6530 Quadrupole Time of Flight system (Q-ToF-MS) with an electron spray ionization (ESI) source was used. The Q-ToF-MS was coupled with Ultra Performance-Liquid Chromatography (UPLC) system (Agilent 1290 Infinity, Agilent Technologies, Santa Clara, CA, USA). The MS experiments were performed in positive and negative ESI mode, and nitrogen was used as the collision gas. The Q-TOF conditions were as follows: fragmentor, 100 V; drying gas, 12 L/min; nebulizer gas, 50 psi; capillary voltage, 3000 V; skimmer, 65 V; gas temperature, 350 °C; acquisition rate, 1 spectra/s (threshold 200 Abs, 0.01% rel.). The MS system was calibrated before each analysis using a calibrant solution. Furthermore, a constant infusion of a solution (Agilent Technologies) with reference ions was also used during the analysis, for mass calibration of the MS system. The Agilent MassHunter software (version B.06.00) was used for the acquisition of data, while data processing was performed using the Agilent MassHunter Qualitative Analysis software (version B.07.00).

2.6. Chromatographic Study

A Nucleoshell Bluebird (RP 18 EC, 2.7 μm particle size, 100 mm length, 4.6 mm i.d.) (Macherey-Nagel, Düren, Germany) column was used for the chromatographic study with a flow rate of 1.0 mL min^{-1}. The mobile phase was: A = water/ammonium acetate 5 mM; mobile phase B = ACN. A gradient elution program was used with the following conditions: 0 min 4% B, 5 min 40% B, 10 min 100% B, 17 min 4% B, 25 min 4% B. The column temperature was 40 °C, with an injection volume of 5 μL.

3. Results and Discussion

3.1. UPLC-Q-ToF-MS Method

4(5)-Methylimidazole was studied in positive and negative ESI mode at different fragmentor (100 V, 120 V, 150 V) and capillary voltage conditions (3000 V, 4000 V). However, in negative ESI mode the $[M-H]^-$ ion was not detected, which confirms previous researchers that determined this compound only at positive ESI [12,13]. Proceeding with the experiments in positive ESI (Figure 1), the optimum abundance of the $[M+H]^+$ ion was observed at fragmentor 100 V and capillary voltage 3000 V. Nevertheless, the $[M+Na]^+$ ion was not observed. 4(5)-Methylimidazole was detected at retention time 1.72 min at *m*/*z* 83.0606 (Δ 2.41 ppm).

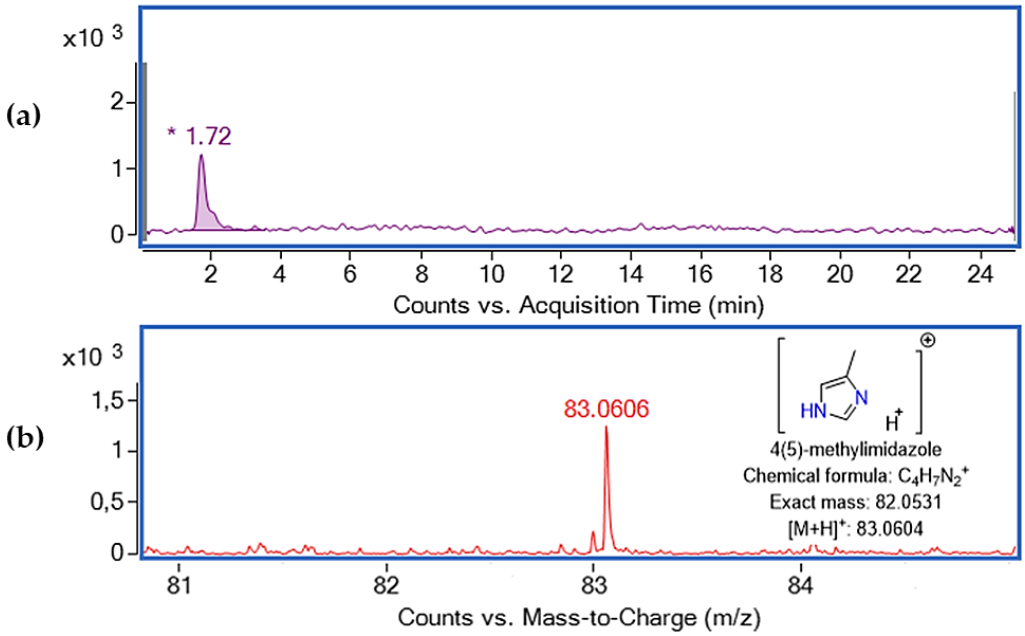

Figure 1. (**a**) Extracted ion chromatogram of 1 mg L^{-1} standard solution and (**b**) mass spectrum of 1 mg L^{-1} standard solution.

The peak area of the extracted ion chromatograms was utilized for the quantification of 4(5)MEI. The linearity of the UPLC-Q-ToF-MS method was determined by the construction of a calibration curve at different concentrations (Figure 2). Limit of detection (LOD) and quantification (LOQ) were 1.7 mg L^{-1} and 5.1 mg L^{-1}, respectively, while linearity was $R^2 = 0.9908$.

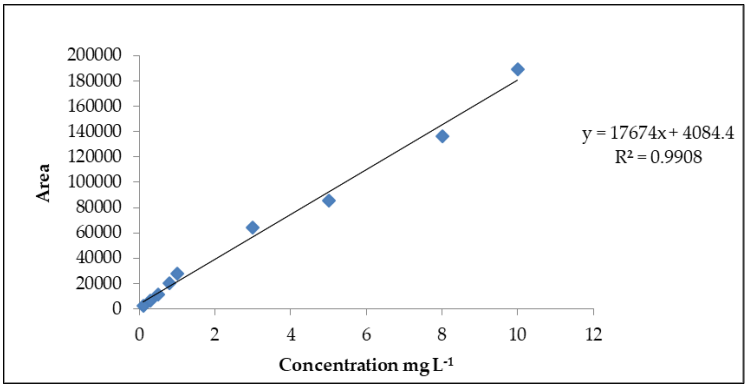

Figure 2. Calibration curve of 4(5)MEI.

3.2. Analysis of Aqueous Model Systems

A prolonged heating of the aqueous model systems was applied at 100 °C for 60 h in order to ascertain the formation of 4(5)MEI. Also, different concentrations of the amino acids added in the model systems were used to test the effect of concentration on the formation of 4(5)MEI). 4(5)-Methylimidazole was detected in all model systems (Table 1). The combination of fructose and proline provided the highest concentration of 4(5)MEI (3.5 μg mL^{-1}). The lowest concentration was detected in the glucose–tyrosine system (0.2 μg mL^{-1}), however, in the fructose–tyrosine system the concentration of 4(5)MEI was significantly elevated (3.0 μg mL^{-1}). In all model systems of amino acids with fructose the concentration of 4(5)MEI was increased, except for fructose-lysine where it was lower (0.9 μg mL^{-1}), compared to glucose-lysine (1.3 μg mL^{-1}), which indicates that the formation of 4(5)MEI is differently affected from each amino acid.

Furthermore, the formation of 4(5)MEI may be affected by the amount of sugar, as in the current study the amount of fructose was increased by 10 g in relation to glucose. These results are in agreement with a research study on glucose-ammonia systems, heated at 150 °C for 2 h, where the authors observed that the concentration of 4(5)MEI increases with increasing glucose concentration [14].

4. Conclusions

An accurate analytical method for the determination of 4(5)MEI was developed employing UPLC-Q-ToF-MS at positive ESI mode. Eight aqueous model systems were prepared with sugar (glucose, fructose) and amino acid (proline, phenylalanine, tyrosine, lysine), which are main components of honey. The model systems were heated at 100 °C for 60 h, in order to study the formation of 4(5)MEI. The results indicate that the formation of 4(5)MEI is differently affected from each amino acid as well as the amount of sugar. The proposed UPLC-Q-ToF-MS method can be used for future applications in other food matrices.

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