Identification of the Maillard reaction intermediates as divalent iron complexes in alanine/glucose/FeCl₂ model system using ESI/qTOF/MS/MS and isotope labelling technique

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1. Introduction

In the thermal processing of food, Maillard reaction intermediates (MRIs), resulting from the degradation of sugars and Amadori rearrangement products (ARPs) are considered important precursors for the development of color, flavor, and thermally generated toxicants (Yaylayan, 1997). Analysis of the MRIs and sugar degradation products (SDPs) has been achieved through the use of a range of time-consuming analytical techniques (Yaylayan and Huyghues-Despointes, 1994). High-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy have also been employed for structural elucidation (COSY, and 2D nuclear Overhauser enhancement spectroscopy (COSY), and 2D nuclear Overhauser enhancement spectroscopy (COSY), and 2D nuclear Overhauser enhancement spectroscopy (COSY). Furthermore, infrared spectroscopy has been used to study the effect of environmental factors, such as pH and temperature, on the concentration of the keto form (Wnorowski and Yaylayan, 2003).

Due to their high reactivities and short half-lives, the detection of Maillard reaction intermediates is relatively difficult to achieve in a single analytical run. In this study, the formation of Maillard reaction intermediates from heated alanine/glucose mixtures (110 °C for 2 h) was investigated through their complexation with divalent iron using electrospray ionization/quadrupole time-of-flight mass spectrometry and isotope labelling. Analysis of the mixtures indicated that this approach allows the simultaneous detection of many important labile and reactive Maillard reaction intermediates along with unreacted alanine and glucose in various other Maillard reaction products, such as glyceraldehyde, erythrose, ribose, acetol, glycolaldehyde, fructosamine, glucosone, osones, deoxyosones, and Amadori products. Some osones and deoxyosones also formed their corresponding Schiff bases with alanine. The above mentioned Maillard reactions intermediates were detected either as binary metal complexes with alanine or with other enediol generating species including self-complexation adducts and they formed positively charged ions such as [M + H]⁺, [M + Na]⁺, [M + K]⁺, [M + Fe²⁺Cl]⁺, and [M + Fe³⁺Cl]⁺, that can be detected using the positive ionization mode.

Research Paper

https://doi.org/10.1016/j.crf.fs.2021.04.003
Received 5 March 2021; Received in revised form 7 April 2021; Accepted 8 April 2021
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However, rapid analytical procedures for the simultaneous profiling of MRIs and SDPs have yet to be reported in the literature. In a previous study (Kim and Yaylayan, 2020), a convenient analytical procedure for profiling of SDPs through complexation with divalent metal ions combined with ESI/qTOF/MS was developed and applied for the analysis of honey. Here we demonstrate the utility of this technique to detect iron (II) catalyzed Maillard reaction intermediates of alanine and glucose using a methodology that most researchers already utilize, the qTOF/LC/MS with additional step of adding metal salts to the solution being analyzed. This step facilitates the detection of not only hard-to-identify and labile products but at the same time enhances the detection of nitrogen containing MRIs due to the ability metal ions to coordinate equally with nitrogen and oxygen atoms.

2. Materials and methods

2.1. Materials and reagents

L-alanine (98%), D-glucose, copper(II) chloride (CuCl₂) (99.9%) and iron(II) chloride (FeCl₂) (99%) were purchased from Sigma-Aldrich Chemical Co. (Oakville, Ontario, Canada). Alanine-3-¹³C (¹³C₆H₁₂O₆) (99%) and glucose-¹³C-¹⁵N (¹⁵N₆C₆H₁₂O₆) (99%) were purchased from Cambridge Isotope Laboratories (Andover, MA). Liquid chromatography-mass spectrometry (LC-MS)-grade water and methanol (OmniSolv, Bremen, Germany) operated in positive-ion mode. Samples (1 μL) were injected directly into ESI/qTOF/MS. Instrument calibration was performed using sodium formate clusters. The electrospray interface was from VWR International (Mississauga, Ontario, Canada).

2.2. Sample preparation

Test model systems were prepared by heating glucose (18 mg), alanine (9 mg), and FeCl₂ (6.4 mg) in methanol or water (1 mL) in tightly closed stainless-steel reactors at 110 °C for 2 h. Control model systems were prepared by heating glucose (18 mg) and alanine (9 mg) with or without CuCl₂ (5.6 mg) in methanol at 110 °C for 2 h. All samples were analyzed at least in two replicates, as indicated in Table 1.

2.3. ESI/qTOF/MS

The dry reaction mixtures were dissolved in liquid chromatography (LC)-grade methanol to a concentration of 1 mg/mL. The samples were then diluted 10-fold in 10% methanol prior to analysis by ESI/qTOF/MS in positive mode. The ESI/qTOF/MS system was comprised of a Bruker Maxis Impact quadrupole-time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany) operated in positive-ion mode. Samples (1 μL) were injected directly into ESI/qTOF/MS. Instrument calibration was performed using sodium formate clusters. The electrospray interface settings were the following: nebulizer pressure, 0.6 bar; drying gas, 4 L/min; temperature, 180 °C; and capillary voltage, 4500 V. The scan range was from m/z 90 to 1000. Molecular formulae were assigned to all the observed peaks based on their exact m/z values by using the online software “ChemCalc” (Institute of Chemical Sciences and Engineering, Lausanne, Switzerland) (Patiny and Borel, 2013). ESI/qTOF/MS/MS was carried out in the multiple reaction monitoring (MRM) mode using a collision energy of 10.0 eV for the ions at m/z 252, 342, and 395.

2.4. Structural elucidation

Evidence for the proposed structures was provided through ESI/ qTOF/MS analysis of their elemental composition, MS/MS analysis, and isotope-labeling. Furthermore, the incorporation of chlorine and copper in the identified complexes was also confirmed through the detection of their specific isotopic signatures; for chlorine the (M + 2) peaks accounted for ~ 25% of the peak intensity for the M ions, while for copper, the (M + 2) peaks accounted for ~ 30% of the peak intensity. Isotope labelling techniques was also used to generate the corresponding isotopically-labelled counterparts from [¹³C⁻¹⁵N]-labelled glucose and [¹³C⁻³]-labelled alanine. The proposed structures represent only one possible isomeric form out of many possible forms for a particular nominal molecular weight, and are based on the most commonly reported structures in the literature.

3. Results and discussion

Sugar degradation products formed during the Maillard reaction are normally more amenable for analysis under negative ionization mode when analyzed by mass spectrometry (Figure S1). However, the addition of metal ions prior to analysis allows these mixtures to be analyzed in the positive ionization mode (Kim and Yaylayan, 2020), where nitrogen-containing MRPs are also readily detectable (Figure S2). Furthermore, the formation of metal complexes can prevent the degradation or further reactions of these reactive intermediates, while at the same time providing structural features for the development of the positive charge necessary for their detection under the positive ionization mode of the ESI/qTOF/MS system (Kim and Yaylayan, 2020). In the presence of metal ions, sugars, amino acids, MRIs (i.e., ARPs), and SDPs (i.e., 3-deoxyglucosone (3-DG), α-hydroxyl carbonyl, and α-dicarbonyl compounds) have the ability to undergo self- or random complexation to generate various metal-centered binary complexes, as listed in Table 2. In this study, the alanine/glucose model system was heated at 110 °C for 2 h in the presence of metal ions (FeCl₂ or CuCl₂) in water or methanol, and analyzed by ESI/qTOF/MS in the positive ionization mode. The heating of glucose and alanine in the absence of metal ions was also performed as a control, and it was found that the control system also produced alanine Amadori compound with glucose as the dominant product, but with reduced formation of other MRPs, as analyzed under positive ionization mode.

3.1. Identification of the Maillard reaction intermediates through complexation with iron(II) using ESI/qTOF/MS and ESI/qTOF/MS/MS analysis

The Maillard reaction intermediates obtained (see Tables S1 and S2) in all the model systems heated at 110 °C for 2 h could be categorized into four groups (1) metal complexes with free amino acids/and or intact sugars, (2) ARPs and their corresponding metal complexes, (3) amino sugars and their corresponding metal complexes, and (4) reactive SDPs and their metal complexes. Table 2 shows selected examples of the above complexes. Previously (Kim and Yaylayan, 2020), we demonstrated that SDPs acting as bidentate ligands were converted into stable metal complexes, and were easily profiled by ESI/qTOF/MS in the positive ionization mode. In this study, the alanine/glucose model system was reacted in the presence or absence of FeCl₂ or CuCl₂ as metal catalysts to enhance the formation of MRPs, and at the same time to provide the metal ions needed for the formation of stable binary complexes for detection by ESI/qTOF/MS in positive ionization mode.

### Table 1

| Model System | Composition of the model systemsa |
|--------------|----------------------------------|
| Control Model | Alanine was added to glucose solution and heated in the absence of metal ions - Ala/Glu |
| System | Alanine was added to glucose solution and heated in the presence of CuCl₂ - Ala/Glu/CuCl₂ |
| Test Model | Alanine was added to glucose solution and heated in the presence of FeCl₂ - Ala/Glu/FeCl₂ |
| Isotope Labelling | Alanine was added to glucose-¹³C-U solution and heated in the presence of FeCl₂ - Ala/¹³C-U/FeCl₂ |
| Model System | Alanine-3-¹³C was added to glucose solution and heated in the presence of FeCl₂ - Ala/¹³C-³/FeCl₂ |

* All the Model systems were prepared in 1.1 M ratio and heated at 110 °C for 2 h in water or methanol by using a sealed stainless-steel reactor and analyzed in at least two replicates.
3.1.1. Detection of intact amino acid and intact sugar metal complexes
The amino acid metal complexes were detected as mono(alaninate)- and bis(alaninate)iron(II) complexes and were observed as [M⁺] ions at m/z values of 143.9744 (C₃H₆FeNO₂) and 233.0224 (C₆H₁₃FeN₂O₄), respectively. These structures were confirmed by observing the incorporation of one or two carbon atoms from [13C-3] alanine, but no carbon atoms from [13C] glucose. Furthermore, mono(alaninate)iron(II) was found to conjugate with glucose to give a signal as [M + H⁺] at m/z 324.0378 (C₉H₁₆NO₆) that was found to incorporate six carbon atoms from [13C-CU] glucose and one C-3 atom from [13C-3] alanine. The ions corresponding to the free alanine or glucose were observed as [M + H⁺] ions at m/z 90.055 (C₆H₁₄NO₂) or [M + K⁺] ions at m/z 219.0266 (C₉H₁₄KO₂) not shown in Tables 2 and S1.

3.1.2. Detection of Amadori rearrangement products
The Amadori product of alanine with glucose, namely N-(1-deoxy-D-fructose-1-yl)-l-alanine, was observed as the dominant peak in both the Ala/Glu and the Ala/Glu/FeCl₂ model systems, being detected in its free form [M⁺] at m/z 252.1074 (C₁₀H₁₉NO₅). It was also detected as [M + Na⁺] at m/z 274.0891 (C₁₀H₁₇NNaO₂) and [M + K⁺] at m/z 290.0662 (C₁₀H₁₇NK₉O₂). All structures were confirmed by observing the incorporation of six carbon atoms from [13C-CU] glucose and one C-3 atom from [13C-3] alanine. The Amadori product was observed to undergo three dehydration reactions generating [M − H₂O]⁺ at m/z 234.0967 (C₉H₁₄NO₅) and [M − 2H₂O]⁺ at m/z 216.0863 (C₉H₁₂NO₃) and [M − 3H₂O]⁺ at m/z 198.0755 (C₉H₁₂NO₂). In addition, the hydrated form [M + H₂O]⁺ appeared at m/z 270.1175 (C₁₀H₂₀NO₇). All three dehydration ions and the hydrated ion were found to incorporate six carbon atoms from [13C-CU] glucose and one C-3 atom from [13C-3] alanine. The Amadori product was observed to undergo three dehydration reactions generating [M − H₂O]⁺ at m/z 234.0967 (C₉H₁₄NO₅) and [M − 2H₂O]⁺ at m/z 216.0863 (C₉H₁₂NO₃) and [M − 3H₂O]⁺ at m/z 198.0755 (C₉H₁₂NO₂). In addition, the hydrated form [M + H₂O]⁺ appeared at m/z 270.1175 (C₁₀H₂₀NO₇). All three dehydration ions and the hydrated ion were found to incorporate six carbon atoms from [13C-CU] glucose and one C-3 atom from [13C-3] alanine. The Amadori product was observed to undergo three dehydration reactions generating [M − H₂O]⁺ at m/z 234.0967 (C₉H₁₄NO₅) and [M − 2H₂O]⁺ at m/z 216.0863 (C₉H₁₂NO₃) and [M − 3H₂O]⁺ at m/z 198.0755 (C₉H₁₂NO₂). In addition, the hydrated form [M + H₂O]⁺ appeared at m/z 270.1175 (C₁₀H₂₀NO₇). All three dehydration ions and the hydrated ion were found to incorporate six carbon atoms from [13C-CU] glucose and one C-3 atom from [13C-3] alanine.

In addition to glucose, smaller sugars, such as glycolaldehyde, glycerol, erythrose, and erythrose were also found to form Amadori products with alanine as either free or as mono(alaninate)iron(II) complexes. More specifically, the free glycolaldehyde Amadori compound was observed as [M + H⁺] at m/z 132.0656 (C₆H₁₀NO₂) and the iron complex was observed as [M⁺] at m/z 185.9848 (C₁₀H₁₄FeNO₃). Both structures incorporated two carbon atoms from glucose and one C-3 atom from alanine. Similarly, the glyceraldehyde and acetal Amadori compounds of mono(alaninate)iron(II) were observed at m/z 215.9958 (C₁₀H₁₄FeNO₃) and m/z 200.001 (C₉H₁₄FeNO₂), respectively, where three carbon atoms from glucose and one C-3 atom from alanine were incorporated in both structures. Moreover, the erythrose Amadori compound of alanine was also observed at m/z 246.0064 (C₁₀H₁₂FeNO₃), which was found to incorporate four carbon atoms from glucose and one C-3 atom from alanine. Interestingly, 3-deoxyerythrose was observed as the mono(alaninate)iron(II) complex of its Schiff base at [M⁺] at m/z 227.9968 (C₉H₁₂FeNO₃), whereas, 3-deoxyerythrose was observed at m/z 230.0117 (C₉H₁₂FeNO₃) most likely as the Amadori compound. These structures were confirmed by detecting the incorporation of four carbon atoms from [13C-CU] glucose and one C-3 atom from [13C-3] alanine. Similar to the case of 3-deoxyerythrose, glycerol (hydroxymethylglyoxal) was also observed as the mono(alaninate)iron(II) complex of its Schiff base at m/z 231.9913 (C₁₀H₁₄FeNO₃), where three carbon atoms from glucose and one C-3 atom from alanine were found incorporated. Furthermore, the Schiff base of glycerol with methyl ester of alanine was detected as [M + H⁺] at m/z 264.1085 (C₁₀H₁₄NO₅) along with its dehydrated form [M + H − H₂O]⁺ at m/z 246.0979 (C₁₀H₁₂NO₃). Both structures incorporated six carbon atoms from glucose and one C-3 atom from alanine.
Table 3: Elemental composition and/or isotope incorporation of the common Maillard reaction intermediates obtained in the Ala/Glu/CuCl₂ and Ala/Glu/FeCl₂ model system in methanol (see Table S1).

| [M + X] | Elemental Composition | Error PPM | [M + X] | Elemental Composition | Error PPM |
|---------|----------------------|-----------|---------|----------------------|-----------|
| 127.0386 | C₅H₇O₃                | 7.235     | 127.0389 | C₅H₇O₃                | 4.873     |
| 180.0862 | C₅H₇NO₃               | 5.539     | 180.0867 | C₅H₇NO₃               | 2.763     |
| 162.0757 | C₅H₇NO₄               | 5.756     | 162.0761 | C₅H₇NO₄               | 2.671     |
| 144.0645 | C₅H₇NO₅               | 10.885    | 144.0656 | C₅H₇NO₅               | 4.638     |
| 126.0545 | C₅H₇NO₆               | 7.961     | 126.0543 | C₅H₇NO₆               | 3.994     |
| 202.0701 | C₅H₇NO₇O₄             | 4.74      | 202.0701 | C₅H₇NO₇O₄             | 9.194     |
| 206.1018 | C₅H₇NO₈               | 5.083     | 206.1002 | C₅H₇NO₈               | 4.113     |
| 188.0914 | C₅H₇NO₉O₄             | 4.694     | 188.0917 | C₅H₇NO₉O₄             | 3.099     |
| 240.0159 | C₅H₇NO₉O₄[¹³C]       | 5.137     | 233.0224 | C₅H₇NO₉O₄[¹³C]       | 0.318     |
| 242.0132 | C₅H₇NO₉O₄[¹³C][¹⁷C]   | 9.669     |         |                       |           |

| [M + X] | Elemental Composition | Error PPM | [M + X] | Elemental Composition | Error PPM |
|---------|----------------------|-----------|---------|----------------------|-----------|
| 127.0389 | C₅H₇O₃                | 4.873     | 133.0585 | [¹²C]C₅H₇O₃          | 6.829     |
| 180.0864 | C₅H₇NO₃               | 8.170     | 133.0584 | [¹²C]C₅H₇NO₃         | 6.829     |
| 162.0758 | C₅H₇NO₄               | 4.929     | 126.0543 | C₅H₇NO₄              | 2.671     |
| 144.0647 | C₅H₇NO₅               | 7.977     | 144.0647 | C₅H₇NO₅              | 11.579    |
| 126.0547 | C₅H₇NO₆               | 4.157     | 126.0546 | C₅H₇NO₆              | 8.754     |
| 180.0869 | C₅H₇NO₇O₄             | 5.265     | 180.0869 | C₅H₇NO₇O₄            | 5.053     |
| 202.0702 | C₅H₇NO₈               | 8.203     | 202.0702 | C₅H₇NO₈              | 9.669     |
| 188.0917 | C₅H₇NO₉O₄             | 7.789     | 189.0939 | C₅H₇NO₉O₄            | 9.19      |
| 240.0160 | C₅H₇NO₉O₄[¹³C]       | 4.873     | 235.0253 | C₅H₇NO₉O₄[¹³C]      | 16.524    |

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**a** All of the ions listed in Table S1 are included in this table.

**b** Error (in ppm) in calculating the elemental composition.

**c** nd: not detected.

**d** [M + 2] represents copper isotopes 65Cu.

**e** na: not available.

**f** [M + 2] represents chlorine isotopes 37Cl.
3.1.2.1. MS/MS fragmentations of the Amadori product (m/z 252), the Amadori product-iron complex (m/z 395), and Amadori product of fructosamine (m/z 342) using a collision energy of 10 eV. To further confirm the structures of the glucose/alanine Amadori products, the free ARP, the Amadori product of fructosamine, and the ARP(alaninate)iron(II) complex observed at m/z 252, 342, and 395, respectively, were analyzed using ESI/qTOF/MS/MS, and the MS/MS fragmentations are shown in Fig. 1 that the free ARP and the Amadori product of fructosamine formed with glucose generated a greater number of fragment ions under a 10 eV ionization energy compared to the ARP(alaninate)iron(II) complex (m/z 395), which generated only four fragment ions, thereby indicating the stability imparted by metal ion complexation to the Amadori product (see Table 4). As shown in Fig. 1, the fragment ions are consistent with the proposed structures, and the MS/MS fragmentations of the free ARP (Fig. 1A) generated the expected diagnostic ions at m/z 88 and 97 (Xing et al., 2020) in addition to dehydrated ions at m/z 234 and 216 characteristic of the Amadori compounds in positive ionization mode. (Xing et al., 2020).

3.1.3. Detection of amino sugars and their complexes

Amino sugars, such as fructosamine, are known to be formed in Maillard model systems containing metal ions (Nashalian and Yaylayan, 2015). They originate from the oxidative decarboxylation of amines such as glyoxal and methylglyoxal have been previously reported in the literature (Kerler et al., 2010a; Hodge, 1953), and these compounds are up to 20,000-fold more reactive than glucose (Hofmann, 1999; Usui et al., 2007). As a result, they have been widely studied in model systems (Marceau and Yaylayan, 2009; Yan et al., 2019; Kerler et al., 2010b; Scalone et al., 2015; Thornalley, 2005; Wang and Ho, 2012); however, the profiling of SDPs is complicated due to their high reactivities and their ability to undergo further reactions prior to detection. In this study, the Schiff base formed between fructosamine and the Strecker aldehyde of alanine (acetalddehyde) along with its dehydration product were also observed at m/z 206.102 (C₈H₁₈N₂O₃) and m/z 188.0917 (C₆H₁₀NO₂), respectively. In the iron(II)-containing model systems (see Tables 3 and S1), both of the above ions incorporated six carbon atoms from glucose and one C-3 atom from alanine. In addition, the Amadori product formed between fructosamine and glucose was observed at m/z 342.14 (C₁₂H₂₄N₂O₁₀), along with its three dehydration products at m/z 324.1292 (C₁₀H₁₆N₂O₅), m/z 306.1188 (C₁₀H₁₄N₂O₄), and m/z 288.107 (C₈H₁₂N₂O₂). Moreover, the monohydrated product [M + H + H₂O]⁺ was also detected at m/z 360.1503 (C₁₀H₁₄N₂O₅) (Table S3). All the five ions, including the three dehydrated ions and one hydrated ion, were found to incorporate twelve carbon atoms from glucose and no C-3 atoms from alanine.

### Table 4

| Structure | m/z | Elemental Composition | Error PPM | Glu [13C-U] | Error PPM | Ala [13C-3] | Error PPM |
|-----------|-----|-----------------------|-----------|-------------|-----------|-------------|-----------|
| [M + H]⁺ = 252 | 88.0386 | C₇H₁₄NO₅ | -14.24 | 0 | -14.24 | nd |
| | 90.0547 | C₈H₁₆NO₅ | -8.92 | 0 | -8.92 | 1 | 9.426 |
| | 97.028 | C₈H₁₈O₃ | -9.84 | 5 | -6.16 | 0 | 9.84 |
| | 99.0439 | C₈H₁₆O₂ | -7.11 | Nd | 0 | -7.11 |
| | 102.0546 | C₉H₂₀NO₅ | -8.85 | 1 | 8.329 | 1 | 7.358 |
| | 104.0705 | C₉H₂₂NO₅ | -6.28 | 1 | -26.73 | 1 | 7.694 |
| | 112.0386 | C₁₀H₂₄NO₅ | 10.28 | 0 | -4.89 | 1 | -7.10 |
| | 126.0546 | C₁₀H₂₆NO₅ | 11.92 | Nd | nd |
| | 146.0804 | C₁₀H₂₈NO₅ | -9.02 | 6 | 8.2 | 0 | 8.34 |
| | 168.0651 | C₁₁H₂₈NO₅ | -5.76 | 6 | 24.72 | 1 | 10.332 |
| | 216.0866 | C₁₁H₃₀NO₅ | -2.77 | 6 | 7.774 | 1 | 14.68 |
| | 234.0984 | C₁₁H₃₂NO₅ | 2.72 | 6 | 7.46 | 1 | 7.304 |

| Structure | m/z | Elemental Composition | Error PPM | Glu [13C-U] | Error PPM | Ala [13C-3] | Error PPM |
|-----------|-----|-----------------------|-----------|-------------|-----------|-------------|-----------|
| [M + H]⁺ = 342 | 90.0546 | C₈H₁₆NO₅ | -7.81 | 3 | 28.281 | 0 | 8.922 |
| | 104.0703 | C₉H₂₀NO₅ | -8.2 | nd | 0 | 8.202 |
| | 144.0659 | C₁₀H₂₄NO₅ | -1.17 | 6 | 7.977 | 0 | 11.579 |
| | 146.0812 | C₁₀H₂₆NO₅ | -3.55 | 6 | 8.2 | 0 | 8.34 |
| | 162.0762 | C₁₁H₂₈NO₅ | -2.67 | 6 | 9.292 | 0 | 9.458 |
| | 164.0921 | C₁₁H₃₀NO₅ | -1.11 | 6 | -10.06 | 0 | -10.26 |
| | 174.077 | C₁₁H₃₂NO₅ | 2.11 | 7 | -16.66 | 0 | 2.68 |
| | 288.1094 | C₁₃H₂₈NO₇ | 3.72 | 12 | 17.941 | 0 | 11.2 |
| | 306.1207 | C₁₃H₃₀NO₇ | 5.91 | 12 | 6.757 | 0 | 5.526 |
| | 324.1319 | C₁₃H₃₂NO₇ | 7.54 | 12 | 6.587 | 0 | 6.036 |
| | 342.1424 | C₁₃H₃₄NO₇ | 6.95 | 12 | 6.717 | 0 | 5.615 |

| Structure | m/z | Elemental Composition | Error PPM | Glu [13C-U] | Error PPM | Ala [13C-3] | Error PPM |
|-----------|-----|-----------------------|-----------|-------------|-----------|-------------|-----------|
| [M + H]⁺ = 395 | 90.0552 | C₈H₁₆NO₅ | -3.37 | 0 | 8.922 | 1 | 9.426 |
| | 215.9955 | C₁₀H₂₄FeNO₅ | -1.94 | 3 | 3.148 | 1 | 12.81 |
| | 246.0074 | C₁₀H₂₆FeNO₅ | 3.72 | 4 | -7.61 | 1 | -6.63 |
| | 306.0292 | C₁₀H₃₀FeNO₅ | 5.19 | 6 | 7.204 | 1 | 8.383 |

a All of the ions listed in Figure 1 are included in this table.

b Error (in ppm) in calculating the elemental composition.
Fig. 1. Proposed MS/MS fragmentation pathways of (A) Amadori products (m/z 252), (B) Amadori product conjugated (alaninate)iron(II) their derivatives (m/z 395), and (C) glucose conjugated amino sugar (m/z 342) in the Ala/Glu/FeCl2 model system.
it was found that these reactive intermediates, when generated in the presence of metal ions, can act as bidentate ligands and be converted into stable metal complexes that can be easily probed by ESI/qTOF/MS. Furthermore, the ability of various SDPs to undergo self- or random complexation with other SDPs can generate multiple metal-centered binary complexes of the same SDPs; for example, 3-DG was found to form metal complexes with alanine and with itself, thereby providing several opportunities for their identification. In the absence of metal ions, no SDPs were detected due to their high reactivities. A total of 37 degradation products of the MRRs (including their dehydration products) were detected, as confirmed by isotope labelling experiments (see Tables 3 and S2). In this context, alanine was able to form iron(II) complexes with SDPs, such as glycolaldehyde, acetal, glyceraldehyde, 3-deoxyxerythrose, and erythrose, which were observed at m/z 205.9955 (C₉H₁₇FeO₈NO, m/z 218.0111 (C₉H₁₂FeNO₅), m/z 248.0247 (C₉H₁₄FeNO₅), and m/z 264.0162 (C₉H₁₄FeNO₅), respectively. Supporting evidence for these structures were provided by observing the incorporation of expected number of carbon atoms from [¹³C]-U glucose and [¹³C]-alanine. For example, the binary complex of glycolaldehyde with alanine was found to incorporate two carbon atoms from glucose and one C-3 atom from alanine, while acetal and glyceraldehyde complexes incorporated three carbon atoms from glucose, and 3-deoxyxerythrose and erythrose complexes incorporated four carbon atoms from glucose with one C-3 atom originating from alanine. Glyceraldehyde and erythrose were also observed as their respective iron(II) complexes. More specifically, the glyceraldehyde complex was detected as [M+H]+ at m/z 144.9585 (C₆H₇FeO₄) and erythrose was observed in the form of [M]+, [M+35Cl]++, and [M+37Cl]+++ at m/z 174.969 (C₆H₇FeO₄), 210.9473 (C₆H₈[35Cl]FeO₄) and 212.9434 (C₆H₈[37Cl]FeO₄), respectively. All structures were confirmed by detecting the incorporation of expected number of carbon atoms from [¹³C]-U glucose. For example, glyceraldehyde and erythrose incorporated three and four carbon atoms from [¹³C]-U glucose, respectively, but no C-3 carbon atom from alanine. Other SDPs, such as dideoxypentosone, erythritol, 3-deoxy-glucosone, n-5-one, rhamnose, and ribose were also observed as their corresponding dehydrated form [M+H]+ at m/z 295.0117, respectively. The peak intensity of HMF in the Ala/Glu/FeCl₂ system. The monohydrated 3-DG iron(II) complex was detected as [M+H]+ at m/z 284.9825 (C₇H₁₄[35Cl]FeO₅) and [M+35Cl]++ at m/z 286.9816 (C₇H₁₄[35Cl]FeO₅) for example, the iron(II) complex was detected also as alanine-iron(II) complex [M+H]+ at m/z 306.0274 (C₇H₁₂Fe(NO₃)), in addition to its corresponding dehydrated product [M+H]+ at m/z 280.0174 (C₇H₁₂Fe(NO₃)) and [M+H++3H₂O]++ at m/z 270.0067 (C₇H₁₂Fe(NO₃)). Furthermore, 3-DG conjugated with mono(Alaninate)iron(II) was also observed at m/z 342.0044 and m/z 344.0026, corresponding to [M+Fe³⁵Cl]++ (C₇H₁₂Fe[35Cl]NO₃) and [M+Fe³⁷Cl]++ (C₇H₁₂Fe[37Cl]NO₃), respectively (Table S2). All the five ions corresponding to 3-DG conjugated with mono(Alaninate)iron(II) were found to incorporate six carbon atoms from glucose and one C-3 carbon atom from alanine.

4. Conclusions

The addition of metal ions to Maillard model systems before heating not only enhances the reaction and generates metal specific products, such as fructosamine, but also stabilizes many of the reactive enediol-containing moieties through the formation of binary metal complexes, which renders them easily detectable by electrospray ionization/quadrupole time-of-flight mass spectrometry (ESI/qTOF/MS).

CRediT authorship contribution statement

Eun Sil Kim: Data curation, Formal analysis, Methodology, Validation, and, Writing – original draft. Varoujan Yaylayan: Supervision, Conceptualization, Project administration, Writing – review & editing, and, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge funding for this research from Natural Sciences and Engineering Research Council of Canada (NSERC) and McGill University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crf2.2021.04.003.

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