The Effect of Reaction Conditions on the Origin and Yields of Acetic Acid Generated by the Maillard Reaction

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ABSTRACT: The effect of the reaction conditions on the origin and yields of acetic acid from glucose was studied in the system containing equimolar concentrations of $^{13}$C-labeled glucose and glycine. Acetic acid was quantified by GC-MS using isotope dilution assay. The $\beta$-dicarbonyl cleavage of 1-deoxy-hexo-2,4-diulose is proposed to be a major pathway leading to the formation of acetic acid in the glucose-based Maillard reaction systems under food processing conditions. Acetic acid was built up from all six carbon atoms of glucose. The relative distribution of acetic acid was independent of the reaction time. Temperature and pH had only small effects.

KEYWORDS: Maillard reaction; acetic acid; D-$[^{13}$C]-glucoses; mechanism; $\beta$-dicarbonyl cleavage

INTRODUCTION

The formation of acetic acid by sugar degradation during the Maillard reaction has been known for several decades. Most recent data indicate that acetic acid is formed in high levels from free and protein-bound Amadori compounds.\(^1\)\(^-\)\(^3\) Depending on the reaction conditions, the yield of acetic acid may reach up to 60 mol%.\(^2\) Despite this fact, the formation mechanism is not yet fully understood. About 50 years ago, Hodge had already postulated 1-deoxy-2,3-diuloses as a possible precursor of acetic acid. The proposed mechanism included retroaldolization of 1-deoxy-2,3-diuloses followed by the metasaccharinic rearrangement of the arising glycolaldehyde.\(^4\) The recent kinetics studies and labeling experiments confirmed the 1-deoxy-2,3-diulose as an efficient precursor of acetic acid; however, currently not retroaldolization but the direct degradation of 1-deoxy-2,3-diulose via $\alpha$-dicarbonyl cleavage is proposed as a mechanism leading to acetic acid.\(^1\)\(^-\)\(^3\)\(^5\)\(^6\) Apart from this mechanism other pathways must exist, as acetic acid was also reported to originate from C$_5$-C$_6$ carbon atoms of glucose.\(^7\) Almost no data are available concerning the effect of pH, temperature, or reaction time on the origin of acetic acid.
To obtain more precise insight into the formation mechanism of acetic acid and to better understand how its origin is affected by reaction conditions, the formation of acetic acid was quantified from $^{13}$C-labeled glucoses and 3-deoxyglucosone by GC-MS using isotope dilution assay.

**MATERIALS AND METHODS**

Glycine and D-(+)glucose were from Fluka (Buchs, Switzerland); monosodium dihydrogenphosphate and disodium monohydrogenphosphate were from Merck (Darmstadt, Germany); D-[1-$^{13}$C]glucose (99%), D-[2-$^{13}$C]glucose (99%), D-[3-$^{13}$C]glucose (99%), D-[4-$^{13}$C]glucose (99%), D-[5-$^{13}$C]glucose (99%), and D-[6-$^{13}$C]glucose (99%) were from Cambridge Isotope Laboratories (Andover, MA). [2$^{2}$H$_{3}$]Acetic acid was from Isotec (Miamisburg, OH). 3-Deoxyglucos-2-ulose was from Toronto Research Chemicals (Ontario, Canada). N-(1-Deoxy-D-Fructos-1-yl)glycine (Fru-Gly) was synthesized as reported by Staempfli *et al.*$^8$ All the chemicals were analytical grade.

**Model Reactions**

A solution of glycine (0.35 mmol) and D-glucose (0.35 mmol) in phosphate buffer (3.5 mL, 0.2 mol/L) was dispatched (0.4 mL/vial) into 1.5 mL screw-cap vials (Infochroma, Zug, Switzerland) and thermally treated at 120°C in a silicone bath for a defined period of time. The experiments were performed at pH 4, pH 6, and pH 8. Similar experiments were carried out using, respectively, [1-$^{13}$C]glucose, [2-$^{13}$C]glucose, [3-$^{13}$C]glucose, [4-$^{13}$C]glucose, [5-$^{13}$C]glucose, and [6-$^{13}$C]-glucose instead of unlabeled glucose.

Experiments with 3-deoxyglucos-2-ulose: A solution of glycine (0.05 mmol) and 3-deoxyglucos-2-ulose (0.05 mmol) in phosphate buffer (0.5 mL, 0.2 mol/L) was placed into a 1.5 mL screw-cap vial and thermally treated at 120°C in a silicone bath for 4 hours. The experiments were performed at pH 4, pH 6, and pH 8.

**Quantification of Acetic Acid**

Acetic acid was quantified by isotope dilution assays (IDA)$^9$ using [2$^{2}$H$_{3}$]acetic acid as an internal standard. After cooling down by cold water, an aliquot of the reaction mixture was spiked with a defined amount of [2$^{2}$H$_{3}$]acetic acid in water. Samples were acidified with phosphoric acid (85%) and analyzed by solid-phase microextraction (SPME) in combination with gas chromatography coupled to mass spectrometry (SPME-GC-MS).

**Gas Chromatography-Mass Spectrometry**

The fiber [polydimethylsiloxane-divinylbenzene (PDMS-DVB), Supelco] was exposed for 30 min at 25°C to the headspace above the samples in the glass vials. The volatile compounds on SPME fiber were desorbed for 5 min in an injector port heated at 250°C. GC-MS analyses were performed on a GC 6890A coupled to an MSD 5973 (both Agilent, Palo Alto, CA) using a DB-Wax capillary column (30 m × 0.25 mm × 0.25 µm, J&W Scientific). After insertion of the SPME device into the
injector, the oven temperature program was started, and the temperature raised at 6°C/min from 20 to 240°C and held for 10 min isothermally. Mass spectra in the electron impact mode (EI) were generated at 70 eV and at a scan range from m/z 28 to 350. Quantification of acetic acid by IDA was performed in the EI-MS mode by measuring the molecular ions of analyte and labeled internal standard at m/z 60 and m/z 63, respectively.

RESULTS AND DISCUSSION

The effect of pH, temperature, and reaction time on the origin and yields of acetic acid from glucose was studied in the system containing equimolar concentrations of 13C-labeled glucose and glycine (0.1 mol/L) dissolved in phosphate buffer. Preliminary experiments with unlabeled glucose indicated that at 120°C the concentration of acetic acid increased with pH and with reaction time (Fig. 1). When glucose and glycine were replaced by corresponding Amadori compound Fru-Gly, a similar effect was observed; however, the yields of acetic acid arising from Fru-Gly were higher than those form glucose. For example, at pH 8 the final yields of acetic acid from Fru-Gly and glucose/glycine were 56 mol% and 38 mol%, respectively. The fi-

FIGURE 1. Formation of acetic acid from glucose/glycine (solid lines) and from the Amadori compound Fru-Gly (dotted lines) in phosphate buffer at 120°C at pH 4 (circles), pH 6 (squares), and pH 8 (triangles).
nal yields of acetic acid obtained by degradation of Fru-Gly at 120°C were comparable to those obtained at 90°C. These observations are well in line with the formation of acetic acid via 1-deoxy-2,3-diulose. Currently, α-dicarbonyl cleavage is proposed as the mechanism leading to acetic acid (Fig. 2A).

To verify, whether acetic acid is formed also via the 1,2-enolization pathway, 3-deoxyglucos-2-ulose was heated in the presence of glycine for 4 hours at 120°C. Only small amounts of acetic acid were found, indicating that the 1,2-enolization pathway does not contribute significantly to the generation of acetic acid (Table 1). On the other hand, the α-dicarbonyl cleavage of 3-deoxyglucos-2-ulose should lead to formic acid instead of acetic acid (Fig. 2B). Surprisingly, the quantities of formic acid

<table>
<thead>
<tr>
<th>pH</th>
<th>Amount of acetic acid (mol/mol 3-DG)</th>
<th>Amount of formic acid (mol/mol 3-DG)</th>
</tr>
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<tbody>
<tr>
<td>pH 4</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>pH 6</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>pH 8</td>
<td>2.4</td>
<td>1.7</td>
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**FIGURE 2.** (A) α-Dicarbonyl cleavage of 1-deoxyhexo-2,3-diulose; (B) 3-deoxyglucos-2-ulose; and (C) β-dicarbonyl cleavage of 1-deoxyhexo-2,3-diulose after isomeration.
were low and comparable to those of acetic acid. Contrary to the hypotheses proposed in the literature, α-dicarbonyl cleavage of 3-deoxyglucos-2-ulse cannot be considered as a major pathway leading to the formation of formic acid from glucose or glucose-derived Amadori compounds. Consequently, the formation of acetic acid via α-dicarbonyl cleavage of 1-deoxy-2,3-diulose is also questionable. On the other hand, acetic acid could be formed via β-dicarbonyl cleavage of 1-deoxyhexo-2,3-diulose after its isomerization to 1-deoxyhexo-2,4-diulose (Fig. 2C). The experiments confirming this hypothesis were done and will be reported elsewhere.

The formation of acetic acid from 13C-labeled glucose and glycine was studied at two temperatures (90°C and 120°C) at pH 6 and 8. As shown in Figure 3, singly labeled acetic acid was formed from all 13C-labeled gluaces at 120°C at pH 8. The major pathway involved incorporation of C-1 and C-2 carbon atoms of glucose (about 67%). The minor pathways involved C-5 and C-6 carbon atoms of glucose (about 20%) and C-3 and C-4 carbon atoms of glucose (<10%). Interestingly, the proportions of acetic acid arising from different carbon atoms of glucose did not change with the reaction time.

The pH had only a negligible effect on the origin of acetic acid from glucose. Although the absolute amounts of acetic acid formed at pH 6 were lower as compared to pH 8, the relative amounts of acetic acid arising from different carbon atoms of
FIGURE 4. Percentage of singly labeled acetic acid arising from $^{13}$C-labeled glucoses under different reaction conditions.

Glucose were similar (Fig. 4) and did not change with the reaction time. Again, acetic acid was mainly formed from C-1 and C-2 carbon atoms of glucose (about 70%), followed by C-5 and C-6 carbon atoms of glucose (about 18%) and C-3 and C-4 carbon atoms of glucose (<8%).

Wnorowski and Yaylayan observed formation of acetic acid only from C-1 and C-2 carbon atoms of glucose (80%) and C-5 and C-6 carbon atoms of glucose (20%). The different results are probably due to different experimental conditions.

The effect of reaction temperature on the origin of acetic acid from glucose was relatively small (Fig. 4). At 90°C compared to 120°C more acetic acid was formed from C-5 and C-6 carbon atoms of glucose, and less acetic acid was formed from C-3 and C-4 carbon atoms of glucose. For example, at pH 6 about 25% of acetic acid originated from C-5 and C-6 carbon atoms of glucose and only about 5% from C-3 and C-4 carbon atoms of glucose. On the other hand, the temperature had almost no effect on the formation of acetic acid from C-1 and C-2 carbon atoms of glucose, which remained the major source of acetic acid also at 90°C.

CONCLUSIONS

Under food processing conditions, all carbon atoms of glucose can serve as a source of acetic acid. The temperature and pH had only small effects, and the reac-
tion time had no effect on the origin of acetic acid from different carbon atoms of glucose. Contrary to the literature, the $\beta$-dicarbonyl cleavage of 1-deoxyhexo-2,4-diulose is proposed to be the major pathway leading to the formation of acetic acid in the glucose-based Maillard reaction systems. 1-Deoxyhexo-2,4-diulose is formed from 1-deoxyhexose-2,3-diulose by isomerization.

REFERENCES