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Influence of Epicatechin Reactions on the Mechanisms of Maillard Product Formation in Low Moisture Model Systems

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The influence of the polyphenolic compound epicatechin on Maillard chemistry was investigated under simulated roast conditions (10% moisture at 220 °C for 10 min). Quantitative gas chromatography (GC) analysis indicated that the addition of epicatechin to glucose or fructose/glycine model systems significantly reduced the generation of hydroxyacetone, 2-methylpyrazine, 2,3,5-trimethylpyrazine, furfural, 2-acetylfuran, 5-methylfurfural, 2(5*H*)-furanone, 2-acetylpyrrole, and furfuryl alcohol. These analytes were reported to be primarily generated from intact C_2, C_3, C_4, C_5, and C_6 sugar fragments based on gas chromatography/mass spectrometry quantitative isotopomeric analysis of a 1:1 $^{13}C_6$:$^{12}C_6$ hexose sugar/glycine model system. Liquid chromatography/mass spectrometry qualitative isotopomeric analysis of a 1:1 $^{13}C_6$:$^{12}C_6$ hexose sugar/glycine/epicatechin model systems confirmed epicatechin reacted with Maillard reactants in the model systems; two main reaction products were reported, epicatechin−C_5 and −C_6 sugar fragment adducts. In addition, LC/MS analysis of a model system consisting of only 3-deoxy-2-hexosulose and epicatechin identified 3-deoxy-2-hexosulose as a precursor of the epicatechin−C_5 and −C_6 sugar fragment adducts reaction products. These results imply that epicatechin quenched 3-deoxy-2-hexosulose (a key source C_6 to C_1 sugar fragments) and consequently inhibited Maillard product formation.

KEYWORDS: Epicatechin; glycation; Maillard reaction; 3-deoxy-2-hexosulose; inhibit; carbonyl trapping

INTRODUCTION

The Maillard reaction is well-known for impacting food color, flavor, and nutritional value during processing and/or storage (*1*); it also plays an important role in the chemistry of regulatory biology (*2*). Mechanistically, the reaction is initiated by the combination of a carbonyl group (i.e., reducing sugar) with an amine group (i.e., amino acid) to form an unstable Schiff's base (imine) that rearranges to a more stable aminoketose (Amadori rearrangement product) (*3*). The degradation of Amadori products results in the production of deoxyosuloses or key α-dicarbonyl Maillard reaction transient intermediates, which generate a plethora of compounds via several concurrent mechanisms. These α-dicarbonyl compounds, mainly 1-deoxy-2,3-hexosulose and 3-deoxy-2-hexosulose, are well-established to further generate more reactive short chain mono- and dihydroxy carbonyl compounds via retroaldol or *β*-elimination (i.e., glyceraldehyde, methylglyoxal, hydroxyacetone, etc.) (*4*). The generation of short chain sugar fragment carbonyl compounds is known to propagate the reaction via numerous mechanisms involved in the generation of color and flavor compounds; for example, hydroxyacetone (acetol) is considered a well-known precursor of substituted pyrazine whereas methylglyoxal and glyceraldehyde are more prone to participate in browning mechanisms (*5*). In biology, 3-deoxy-2-hexosulose, glyoxal, and methylglyoxal are considered key Maillard reaction precursors responsible for the formation of advanced glycated end products (AGEs), which have been associated with diabetic complications and age-related pathology (*6*). Consequently, understanding the fate of these key carbonyl sugar fragment Maillard reactants has been the subject of numerous investigations.

Recently, Totlani and Peterson (*7*, *8*) reported that epicatechin (EC) in aqueous glucose/glycine Maillard model systems functioned as a carbonyl trapping agent of C_2, C_3, and C_4 sugar fragments or key transient precursors of the Maillard reaction (i.e., glyoxal, methylglyoxal, acetol, erythrose, etc.). These authors suggested that EC underwent electrophilic aromatic substitution reactions with these reactive carbonyl compounds. This reaction mechanism was further supported by nuclear magnetic resonance (NMR) analysis of a EC−methylglyoxal adduct product, which indicated that the C6 and/or C8 position of the flavanol (A ring) and the carbonyl-bearing carbon of the aldehyde compound were the bonding sites (*8*). Furthermore, as anticipated, the addition of EC in model food and Maillard reaction systems has been reported to reduce the generation Maillard type aroma compounds (*10*−*12*).

EC−carbonyl trapping reactions of key Maillard intermediate products have been previously investigated using aqueous Maillard model systems (*7*, *8*) and have not been reported under

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Table 1. Low Moisture Maillard Model Reactions[a]

	reactant (mmol)							
	hexose				amino acid			Maillard intermediate product
	glucose		fructose		glycine		phenolic	
model	unlabeled	labeled [$^{13}C_6$]	unlabeled	labeled [$^{13}C_6$]	unlabeled	labeled [$^{13}C_2$, ^{15}N]	EC	3-deoxy-2-hexosulose
1	3				3			
2	3				3		1	
3[b]	1.5	1.5			3			
4	1.5	1.5			3		1	
5	3				1.5	1.5	1	
6			3		3			
7			3		3		1	
8[b]			1.5	1.5	3			
9			1.5	1.5	3		1	
10			3		1.5	1.5	1	
11[c]							1	0.06

[a] Reactants were mixed with 1.5 g of water and 15 g of sand and heated at 220 °C for 10 min. [b] CAMOLA technique adapted from ref *9*. [c] Reactants were directly reacted as described in the Materials and Methods.

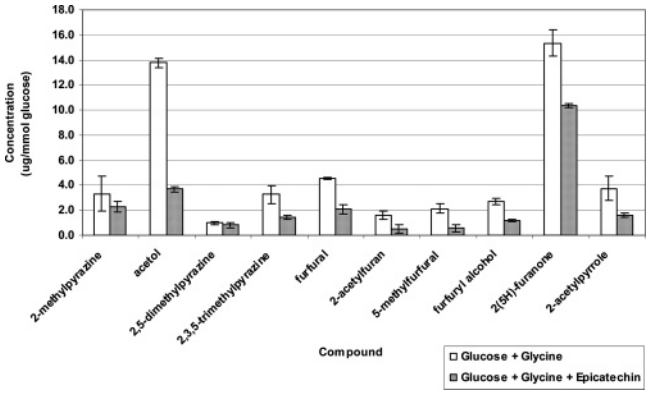

Figure 1. Concentration of main quantitative GC analytes in models 1 and 2; average of triplicate ±95% confidence intervals.

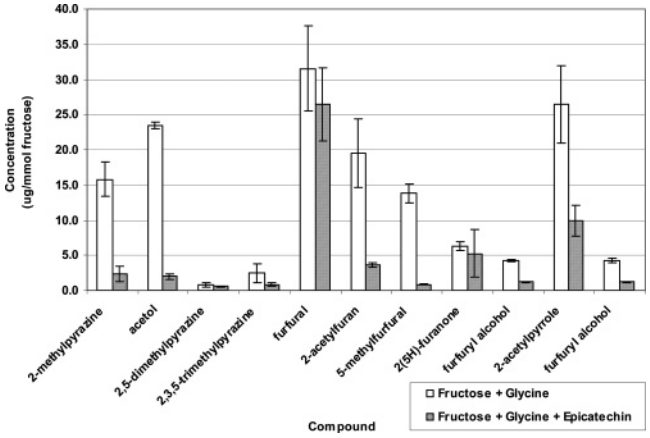

Figure 2. Concentration of main quantitative GC analytes in models 6 and 7; average of triplicate +95% confidence intervals.

simulated "roast" (low moisture−high temperature) conditions. Thermal food processing techniques such as roasting, baking, and frying dehydrate the surfaces of food products, increasing the temperature on the surface, ultimately altering the Maillard reaction kinetics. The objective of this current study was therefore to determine the effect of EC reactions on aroma generation in glucose or fructose/glycine model systems under simulated roast processing conditions.

Table 2. Proportion of Isotopomers from GC-MS Analysis of Model 3 (Glucose)

	retention index[c] on		*m/z*	% of enriched ^{13}C atoms in molecule[a]							
compound[b]	DB-Wax	DB-5ms	(M^+)	0	1	2	3	4	5	6	7
acetol	1274	824	74	51	1	5	43				
2(5*H*)-furanone	1304	661	84	51	0	1	2	46			
furfural	1332	914	96	47	0	2	3	5	43		
furfuryl alcohol	1416	1002	98	46	1	4	2	3	44		
5-methylfurfural	1467	831	110	42	0	1	1	3	7	46	
2-acetylfuran	1512	910	110	40	2	0	0	0	18	40	
2-acetylpyrrole	1581	962	109	50	0	0	0	0	3	47	
2-methylpyrazine	1661	856	94	28	0	26	24	1	21		
2,5-dimethylpyrazine	1770	910	108	28	0	3	45	0	3	21	
2,3,5-trimethylpyr-azine	1985	1068	122	23	2	2	44	4	2	21	2

[a] Corrected for natural abundance of ^{13}C (%1.1) and for loss of hydrogen for M^+ ion in labeled ions by the ratio ($M^+ - 1/M^+$). [b] The compound was identified by comparing it to the mass spectra fragmentation pattern and retention index of authentic compound. [c] Retention index relative to the *n*-alkane ladder.

MATERIALS AND METHODS

Chemicals. D-Glucose, D-fructose, and high-performance liquid chromatography (HPLC)-grade methanol were obtained from EMD Chemicals (Gibbstown, NJ). D-[$^{13}C_6$]Glucose (99% enrichment) and D-[$^{13}C_6$]Fructose (99% enrichment) were purchased from Cambridge isotope laboratories (Andover, MA). (−)(−)-EC (≥98% purity) was purchased from the Zhejiang Yixin Pharmaceutical Co. (Zhejiang, China). Glycine, ($^{13}C_2$,^{15}N) glycine, hydroxyacetone, 2(5*H*)-furanone, furfural, furfuryl alcohol, 5-methylfurfural, 2-acetylpyrrole, 2-acetylfuran, 2-methylpyrazine, 2,5-dimethyl pyrazine , 2,3,5-trimethylpyrazine, and high-purity quartz sand were obtained from Sigma Aldrich (St. Louis, MO). 3-Deoxy-2-hexosulose was purchased from Toronto Research Chemicals (Ontario, Canada).

Model Maillard Reaction System. Model Maillard reactions were conducted as previously described by Wang (*13*). In brevity, the reaction apparatus consisted of round-bottom flask (500 mL) attached with a vigreux column and a glass stirrer fitted with a Teflon blade (Ace Glass, Vineland, NJ). The reaction vessel was heated by oil bath and connected to a rheostat (PowerStat, The Superior Electric Co., Bristol, CT) for temperature control. The reactants (reported in **Table 1**) were mixed with 15 g of quartz sand (previously cleaned and dried) and 1.5 g of water. For model 11, 6 mmol of 3-deoxyglucosone was directly reacted with EC; all of the other conditions were identical. Preliminary studies indicated that this concentration of 3-deoxyglucosone reported a similar quantity of the EC−C_6 sugar fragment reaction products generated (**Figure 5A**) in comparison to the glucose/glycine system (**Figure 5A**)

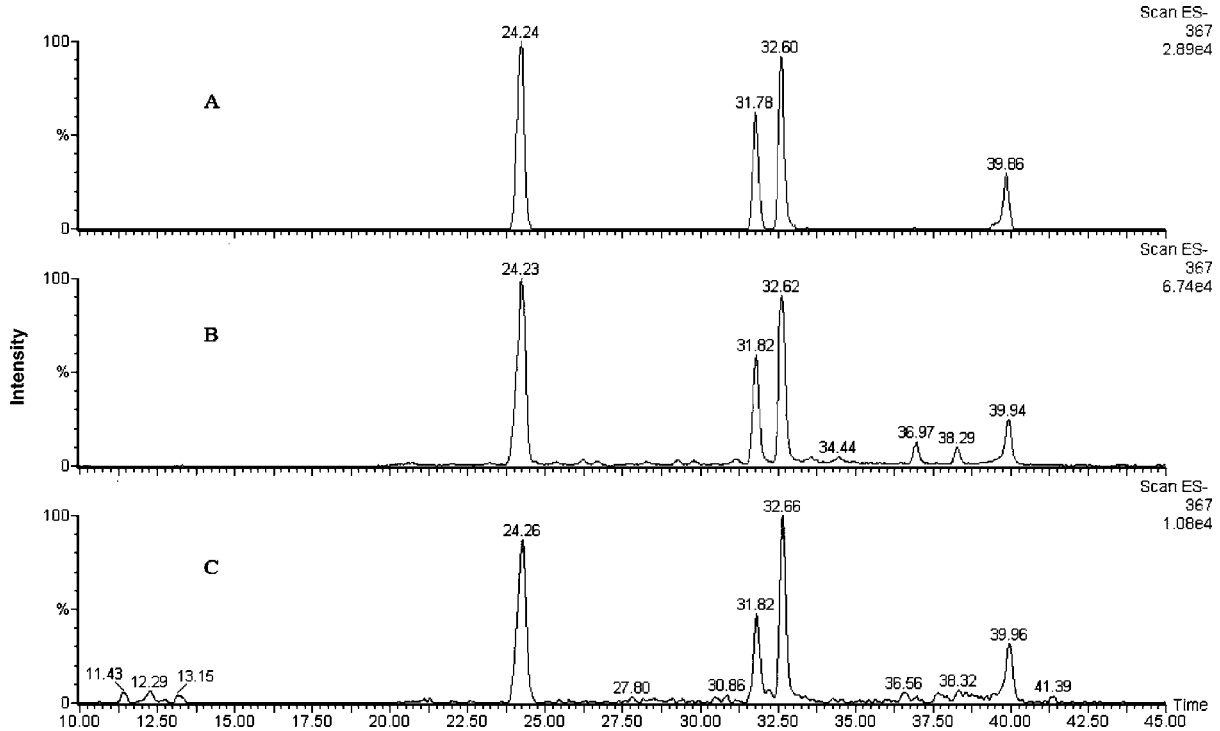

Figure 3. Chromatogram of analyte MW 368 (*m*/*z* 367 [M − 1]$^-$) generated from models: (**A**) Glucose (Glu) ($^{13}C_6$:$^{12}C_6$, 1:1) + glycine (Gly) + EC; (**B**) fructose (Fru) ($^{13}C_6$:$^{12}C_6$, 1:1) + Gly + EC; and (**C**) 3-deoxy-2-hexosulose + EC. All systems were reacted at 220 °C for 10 min.

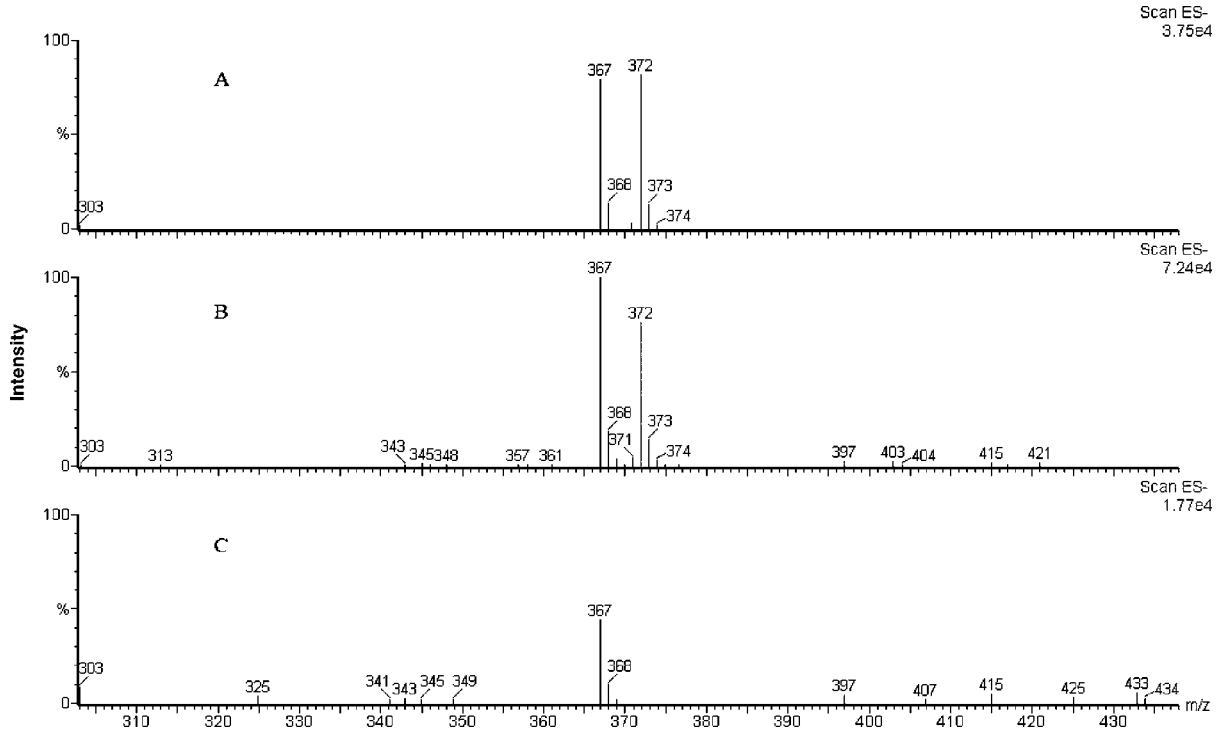

Figure 4. Mass spectrum of analyte MW 368 (*m*/*z* 367 [M − 1]$^-$) at a retention time of 24.2 min generated from models: (**A**) Glucose (Glu) ($^{13}C_6$:$^{12}C_6$, 1:1) + glycine (Gly) + EC; (**B**) fructose (Fru) ($^{13}C_6$:$^{12}C_6$, 1:1) + Gly + EC; and (**C**) 3-deoxy-2-hexosulose + EC. All systems were reacted at 220 °C for 10 min.

based on the peak intensity (liquid chromatography/mass spectrometry, LC/MS). The reactant mixture was mixed at 40−60 rpm in the round-bottom flask for an interval of 2−3 min. The apparatus was then placed in an oil bath maintained at 220 °C, and the reaction was conducted for 10 min, immediately removed for the oil bath, and prepared for further analysis.

Sample Preparation for Gas Chromatography (GC) Analysis. The reaction mixture was extracted (3 × 30 mL) with diethyl ether spiked with internal standard *n*-dodecane (2.75 μL/1000 mL) and subsequently concentrated to 0.5 mL by spinning band distillation (model 800, B/R Instruments, Easton, MD) prior to GC and gas chromatography−mass spectrometry (GC-MS) analysis.

GC-MS. The extracts were analyzed by an Agilent 6890 gas chromatograph connected to an Agilent 5973 mass spectrometer operating in EI mode (Agilent Technologies, Palo Alto, CA). Sample introduction was performed using a liquid autosampler (model A200SE, CTC Analytics, Carrboro, NC). All analyses were performed on a DB-Wax and DB-5 MS capillary column (Agilent Technologies; 30 m ×

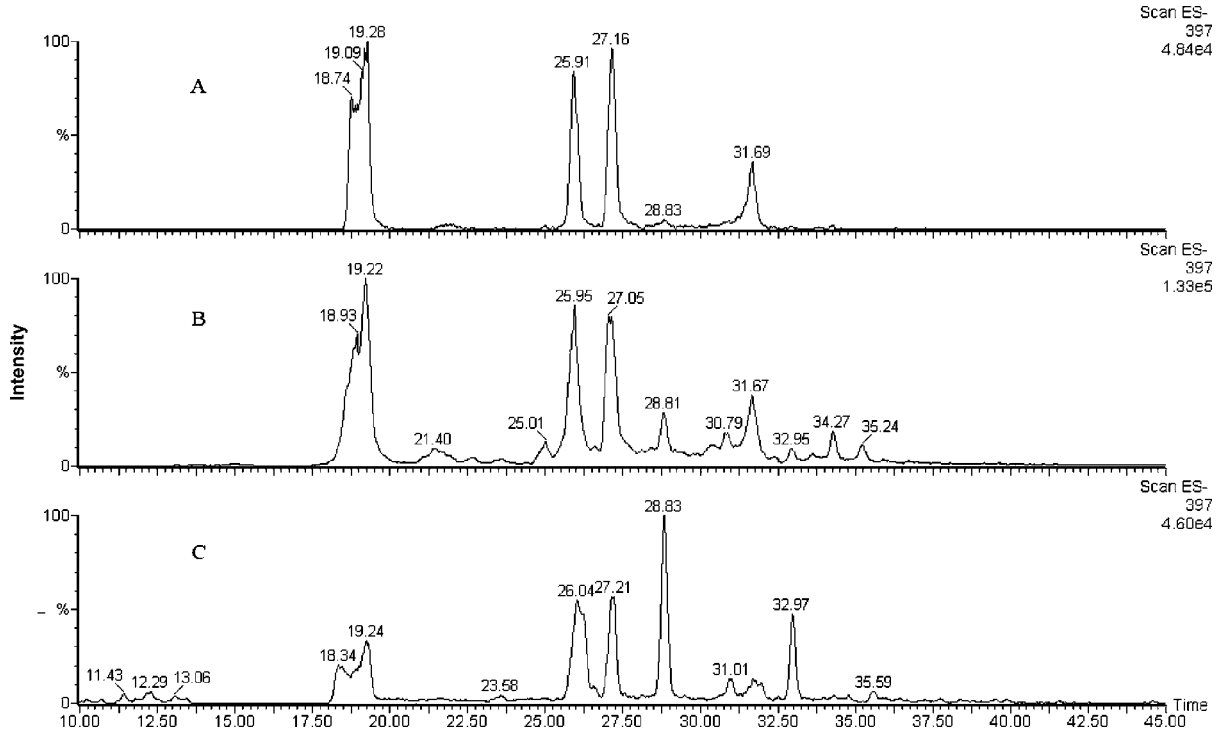

Figure 5. Chromatogram of analyte MW 398 (*m*/*z* 397 [M − 1]$^-$) generated from models: (**A**) Glucose (Glu) ($^{13}C_6$:$^{12}C_6$, 1:1) + glycine (Gly) + EC; (**B**) fructose (Fru) ($^{13}C_6$:$^{12}C_6$, 1:1) + Gly + EC; and (**C**) 3-deoxy-2-hexosulose + EC. All systems were reacted at 220 °C for 10 min.

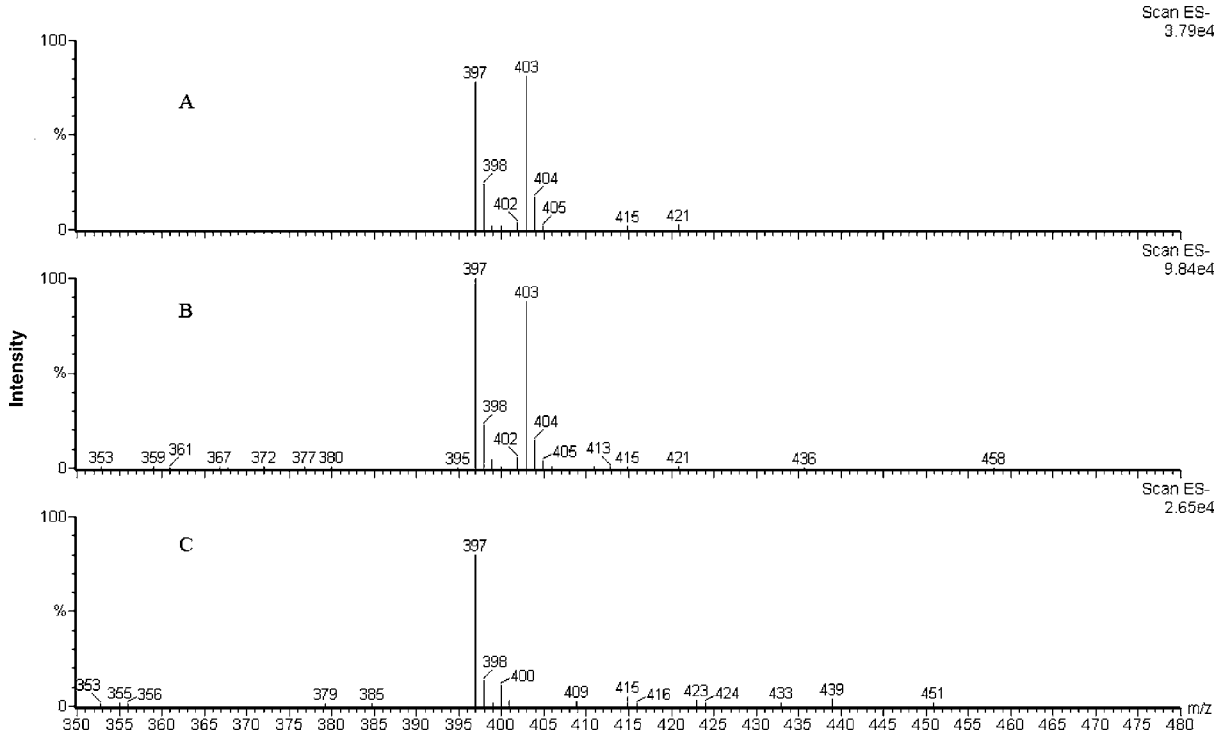

Figure 6. Mass spectrum of analyte MW 398 (*m*/*z* 397 [M − 1]$^-$) at a retention time of 25.9 min generated from models: (**A**) Glucose (Glu) ($^{13}C_6$:$^{12}C_6$, 1:1) + glycine (Gly) + EC; (**B**) fructose (Fru) ($^{13}C_6$:$^{12}C_6$, 1:1) + Gly + EC; and (**C**) 3-deoxy-2-hexosulose + EC. All systems were reacted at 220 °C for 10 min.

0.25 mm i.d. with a 0.25 μm film thickness). The analysis parameters were as follows: 2 μL of sample was injected in split mode (5:1),the inlet temperature was 200 °C, and the column flow was constant at 1.0 mL/min (helium). The temperature program for the DB-was 35 °C for 2 min, ramped at 5 to 230 °C, and held for 4 min; for the DB-5ms, the temperature program was 35 °C for 2 min, ramped at 3 to 250 °C, and held for 4 min. The MSD operational parameters were as follows: capillary direct interface temperature at 250 °C, source temperature at 150 °C, and mass range of 35−250 amu at 6.35 cycles/min. Positive identifications by GC-MS were determined by comparing the mass spectra fragmentation pattern of analytes with mass spectra of known compounds from the Wiley Database and authentic compounds. Linear retention index values were calculated using an *n*-alkane ladder.

GC. The extracts were analyzed by Agilent 6890 GC (Agilent Technologies) using a flame ionization detector (FID). The volatiles were separated over a DB-wax and DB-5 capillary column (Agilent

Technologies; 60 m × 0.25 mm i.d. with a 0.25 μm film thickness) and a Combi-Pal autosampler (CTC Analytics). The operating conditions for the GC-FID were as follows: 2 μL of sample was injected in split mode (5:1); the inlet temperature was 200 °C; the detector was 250 °C; the oven program was 35 °C for 2 min, then increased at 5 °C/min to 230 °C, and held for 4 min; and the carrier constant flow rate was 1.2 mL/min (helium). All analytes were quantified by internal standard methodology (dodecane). The relative response factor for each analyte was determined by the authentic compound peak area response relative to dodecane (standard and authentic compounds prepared at 5 mg/50 mL diethyl ether).

Sample Preparation for LC/MS Analysis. The reaction extracted with 30 × 3 mL of methanol, filtered and concentrated to 3 ± 1 mL under vacuum (Buchi Rotavapor, Model R110, New Castle, Delaware; 0.1 atm, water bath was maintained at 30 °C). The methanol extract was further concentrated to 1 mL under nitrogen purge and then spiked with 10 μL internal standard butyl paraben (5% solution). The extract was filtered using syringe filter (0.45 μm) PTFE using 1 mL syringe prior to HPLC-MS analysis.

LC/MS. All methanol isolates were analyzed by a HPLC/MS utilizing electrospray ionization (ESI), which consisted of a Shimadzu HPLC system (Shimadzu, Columbia, MD) equipped with two pumps (LC-10AD), degasser (DGU-14A), an autosampler (SIL-10vp), Waters column heater (model TCM, Waters, Milford, MA), and a 4.6 mm × 250 mm, 5 μm packing, RP-18 Pursuit column (Varian Inc., Palo Alto, CA) interfaced to a Waters ZMD 2000 Mass Spectrometer (Waters). HPLC conditions were as follows: The injection volume was 30 μL, the column temperature was 25 °C, binary mobile system A was 10 mM ammonium acetate aqueous solution, pH 5.0 and B was 100% methanol, and column flow rate was 1.0 mL/min. A series of linear gradients were used starting with 10% B into A (0−5 min), then increasing to 80% B into A (5−20 min), then increasing to 99% B into A (20−22 min), and then decreasing to 10% B into A (28−30 min). The total run time was 36 min. The effluent was split 1:4 between the electrospray probe (flow rate, 200 μL/min) and the UV/vis detector (flow rate, 800 μL/min) by a postcolumn splitter (zero dead volume T-splitter, Supelco, Bellefonte, PA). The MS conditions were as follows: a negative ion mode; capillary voltage, 3.0 kV; scan range, 100−1000 Da; source temperature, 110 °C; and probe temperature, 250 °C.

RESULTS AND DISCUSSION

The most abundant volatile compounds detected in the glucose or fructose plus glycine roast model systems with and without EC are illustrated in **Figures 1** and **2**, respectively. Overall, the addition of EC significantly reduced the quantity of acetol, 2,3,5-trimethylpyrazine, furfural, 2-acetylfuran, 5-methylfurfural, 2(5*H*)-furanone, 2-acetylpyrrole, and furfuryl alcohol reported for the glucose model systems and acetol, 2-methylpyrazine, 2,3,5-trimethylpyrazine, 2-acetylfuran, 5-methylfurfural, 2(5*H*)-furanone, 2-acetylpyrrole, and furfuryl alcohol reported for the fructose model system. Furthermore, the level that each compound was inhibited by EC addition was hexose-dependent. For example, in the fructose−glycine model, the average concentrations of 5-methylfurfural and acetol generated were reported to be 16.9- and 12-fold lower in the EC-containing model, respectively, whereas in the glucose−glycine model, the quantity of 5-methylfurfural and acetol generated was 3-fold lower in the EC-containing model (calculations not shown). The observed inhibitory effect of EC addition on Maillard type product generation was consistent with that previously reported by Totlani and Peterson (*7*) in aqueous model systems with the exception that under aqueous conditions 2,5-dimethylpyrazine was reported to be inhibited 113-fold, whereas in the current study (dry roast conditions) the quantity of this compound generated was not significantly impacted (**Figures 1** and **2**).

Table 3. Proportion of Isotopomers from GC-MS Analysis of Model 8 (Fructose)

compound[b]	*m/z* (M^+)	% of enriched ^{13}C atoms in molecule[a]							
		0	1	2	3	4	5	6	7
acetol	74	51	1	5	43				
2(5*H*)-furanone	84	51	0	1	2	46			
furfural	96	47	0	2	3	5	43		
furfuryl alcohol	98	46	1	4	2	3	44		
5-methylfurfural	110	42	0	1	1	3	7	46	
2-acetylfuran	110	40	2	0	0	0	18	40	
2-acetylpyrrole	109	50	0	0	0	0	3	47	
2-methylpyrazine	94	28	0	26	24	1	21		
2,5-dimethylpyrazine	108	28	0	3	45	0	3	21	
2,3,5-trimethylpyrazine	122	23	2	2	44	4	2	21	2

[a] Corrected for natural abundance of ^{13}C (%1.1) and for loss of hydrogen for M^+ ion in labeled ions by the ratio ($M^+ - 1/M^+$). [b] The compound was identified by comparing it to the mass spectra fragmentation pattern and retention index of authentic compound.

To investigate mechanistically how EC inhibited the formation of Maillard reaction products, labeling studies consisting of 1:1 ratios of unlabeled to labeled glucose or fructose (**Table 1**; models 3, 4, 8, and 9) and unlabeled to labeled glycine (**Table 1**; models 5 and 10) with and without EC were analyzed for adduct reaction products consisting of EC and sugar fragments, which were key for further Maillard product formation, as previously reported by Totlani and Peterson under aqueous conditions (*7*, *8*). The isotopomeric analyses for the volatile compounds in **Figures 1** and **2** are presented in **Tables 2** and **3**, respectively, and indicated that acetol, 2(5*H*)-furanone, furfural, furfuryl alcohol, 5-methylfurfural, 2-acetylfuran, and 2-acetylpyrrole were generated from intact C_3, C_4, C_5, C_5, C_6, C_6, and C_6 sugar fragment products, whereas the three substituted pyrazines detected, 2-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine, were primarily based of C_2/C_3, C_3/C_3, and C_3/C_3 sugar fragments pairs, respectively. Thus, these sugar fragment products would be considered to be key transient precursors for the generation of each respective compound. For 2,3,5-trimethylpyrazine, which consists of seven carbon atoms, it was presumed that one of the two intact C_3 sugar fragment precursors underwent a chain elongation reaction with the methyl group from the glycine moiety (e.g., generated a C_4 product such as 2,3-butanedione). Similarly, 2-acetylfuran (a six carbon analyte) reported a 12−18% isotopomeric distribution for $M^+ + 5$ (**Tables 2** and **3**) and likewise suggested that the origin of this compounds also resulted from the product of a C_5 sugar fragment and an unlabeled carbon from glycine (e.g., generated a C_6 product such as 1-deoxyhexose). Yaylayan et al. (*14*) demonstrated by stable isotope labeling techniques that the methyl-moiety of glycine can be incorporated into Maillard reaction intermediates/products to generate 2,3-butanedione or trimethylpyrazine and proposed a related mechanism for the transformation of α-ketoaldehydes into methyldiketones, which may explain the isotopomeric patterns reported for 2,3,5-trimethylpyrazine and 2-acetylfuran in this study.

To establish a link indicating that EC reacted with these key sugar fragment Maillard precursors or generated EC−sugar fragment adducts, LC/MS analyses of models 4 and 9 (1:1 $^{12}C_6$:$^{13}C_6$ hexose sugar + glycine and EC) were examined for EC reaction products, which consisted of (i) isotopomers ($M^- + 1$ to $M^- + 6$) and (ii) the parent ion of this EC reaction products were also detected in models 2 and 7 (hexose sugar + glycine and EC) but were not detected in models 1 and 6 (hexose sugar + glycine), at the same peak retention times. Two EC−sugar

moiety adducts were detected in both the glucose and the fructose systems and consisted of intact C_5 or C_6 sugar fragment products with the predicted molecular weights of 368 and 398 (based on pseudomolecular ion $[M - 1]^-$), respectively. The chromatogram and ion spectrum for EC−C_5 sugar fragment adducts are displayed in **Figures 3A**,**B** and **4A**,**B**, whereas the EC−C_6 sugar fragment adducts are displayed in **Figures 5A**,**B** and **6A**,**B** for both glucose and fructose, respectively. The $M^- + 5$ isotopomer detected (**Figure 4**) indicated that this analyte consisted of an intact C_5 sugar fragment, whereas the $M^- + 6$ isotopomer (**Figure 6**) indicated that this analyte consisted of an intact C_6 sugar degradation product.

The composition of these EC−sugar moiety adducts was also confirmed not to contain any glycine component as the analogous 1:1 glycine unlabeled to labeled models (5 and 10) and did not report any detectable isotopomers (i.e., M + 1 to M + 3) in these model systems. These results were consistent with our previous findings for aqueous models (*7*, *8*); however, under roast model conditions, the main adduct reaction products reportedly consisted of C_5 and C_6 intact sugar fragment products, whereas under aqueous conditions only C_2, C_3, and C_4 intact sugar fragment reaction products were detected.

On the basis of these observations, it was hypothesized that EC reacted with deoxyhexosuloses (reactive vicinal carbonyl compounds) or subsequent degradation products to generate EC−C_5 or −C_6 sugar moiety adducts. Deoxyosuloses are well-known Amadori degradation products generated in the intermediate stage of the Maillard reaction (*3*, *15*). Furthermore, 3-deoxy-2-hexosulose is also known to undergo C_1−C_2 cleavage to yield formic acid and deoxypentulose (*5*, *16*, *17*), which may explain the source of the reported EC−C_5 sugar fragment adducts in this study. The identity of 3-deoxy-2-hexosulose as a precursor of the C_5 and C_6 sugar moiety for the EC−C_5 and −C_6 sugar fragment reaction products was evaluated by directly reacting 3-deoxy-2-hexosulose with EC (model 11) and comparing it to the pseudomolecular ion $M - 1^-$ 367 (EC−C_5) and 397 (EC−C_6) chromatographic responses (see **Figures 3C** and **5C**) and the mass spectral properties (see **Figures 4C** and **6C**) of those generated for the glucose- and fructose-based systems (**Figures 3**−**6A**,**B**). Overall, the reaction products generated by the 3-deoxy-2-hexosulose and EC model were very similar in chromatographic properties "fingerprints" to those generated for the glucose and fructose systems suggesting that 3-deoxy-2-hexosulose was a key precursor of these EC−sugar fragment reaction products. Based on the molecular weight of the reactants, epicatechin ($C_{15}H_{14}O_6$) and deoxy-2-hexosulose ($C_6H_{10}O_5$), the proposed molecular composition of the m/z $M - 1^-$ 367 (EC−C_5) and 397 (EC−C_6) EC-sugar adducts were $C_{20}H_{16}O_7$ ($C_{15}H_{14}O_6 + C_6H_{10}O_5 - 3H_2O - CO - 2H$) and $C_{21}H_{18}O_8$ ($C_{15}H_{14}O_6 + C_6H_{10}O_5 - 3H_2O$), respectively. It should be noted, however, that the pseudomolecular ion of these analytes may have been complicated by dehydration reactions occurring during the ionization process of mass spectral detection; however, 3-deoxy-2-hexosulose is known to undergo dehydration reactions and cyclizes to form furan type derivative compounds [i.e., hydroxymethylfurfural, 5-methyl-2(3*H*)-furanone, etc.] (*5*).

The observed reactivity between EC and 3-deoxy-2-hexosulose may explain, in part, the noted inhibitory effects of EC on the generation of Maillard reaction products (**Figures 1** and **3**) under "roast" conditions. Deoxyosuloses are a key source of C_1, C_2, C_3, C_4, C_5, and C_6 sugar fragments, the same building blocks that were reported to be key precursors for the generation of the Maillard reaction products listed in **Tables 2** and **3**. Consequently, lower levels of these key Maillard transient intermediates would be anticipated to decrease the generation of the corresponding reaction products.

The noted difference in EC reactivity with sugar fragments under roast conditions (EC−C_5 or −C_6 adducts) vs our previously reported aqueous conditions (EC−C_2, −C_3, or −C_4 adducts) (*7*, *8*) may due differences in the equilibrium constant for the acyclic 3-deoxy-2-hexosulose and the mixture of various hemiacetal and hemiketal cyclic forms of 3-deoxy-2-hexosulose, which are known to occur under aqueous conditions (*5*). Under roast conditions, the low amounts of moisture and high temperature (vs aqueous systems) would be anticipated to result in a higher proportion of acyclic 3-deoxy-2-hexosulose or the α-dicarbonyl isomer. Previously, we have demonstrated that EC−sugar fragment adducts are likely generated by electrophilic aromatic substitution reactions (*8*). Consequently, the acyclic 3-deoxy-2-hexosulose (or the C_5 deoxyribulose fragmentation product) may have been the most abundant reactive sugar fragment carbonyl compound (electrophile) under these reaction conditions and therefore the most analytically detectable EC−sugar fragment reaction products.

The ability of EC to function as a trapping agent of the key Maillard intermediate 3-deoxy-2-hexosulose under roast model conditions to our knowledge has not been previously reported. Consequently, understanding how phenolic chemistry alters the mechanisms of the Maillard reaction may provide an improved understanding of Maillard chemistry in processed food and biology.

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