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Epicatechin Adducting with 5-Hydroxymethylfurfural as an Inhibitory Mechanism against Acrylamide Formation in Maillard Reactions

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ABSTRACT

This study aimed to investigate the inhibitory mechanism of epicatechin (EC) on the formation of acrylamide in Maillard reaction. Glucose+asparagine model is a typical chemical system used to investigate the acrylamide formation. 5-Hydroxymethylfurfural (HMF) is an important carbonyl intermediate in Maillard reaction and can also react with asparagine to form acrylamide. Time courses showed that EC inhibited more HMF than acrylamide in glucose+asparagine model heated at 180 °C. The reduction of EC on the acrylamide formation in HMF+asparagine model was about 70% while that in glucose+asparagine model was about 50%. Moreover, HMF decreased significantly faster when it was heated in the presence of EC. Liquid chromatography−mass spectrometry analysis revealed the formation of adducts between EC and HMF, and the dimeric adducts were verified in fried potato chips. These results suggested that the condensation of EC and HMF was one of the key steps leading to the inhibition of acrylamide. UV-visible spectra analysis showed that some polymerization products had absorption in the visible region and contributed to the development of browning, which was underestimated in the past.

KEYWORDS: epicatechin (EC), Maillard reaction, acrylamide, 5-hydroxymethylfurfural (HMF), browning

INTRODUCTION

Acrylamide has neural, genetic and reproductive toxicities.[1] It was classified as a probable carcinogen to humans (Group 2A) by the International Agency for Research on Cancer.[2] In April 2002, researchers from Swedish first reported the considerable levels of acrylamide in common heat processing foods.[3-4] Since then, numerous studies have been carried out regarding the mechanism of acrylamide formation and elimination.

Acrylamide is mainly generated from the Maillard reaction between asparagine (Asn) and carbonyl compounds.[3-5] In the first step, the carbonyl compounds and the Asn react to the Schiff base.[6] Then the Schiff base decarboxylates to form azomethine ylide, which can directly change to acrylamide or through a 3-aminopropionamide route.[7] The carbonyl compounds include reducing sugars, hydroxycarbonyls, dicarbonyls, alkadienals, 5-hydroxymethylfurfural (HMF), and so on.[8-10] Among them, reducing sugars especially glucose (Glc) are major carbonyls in food ingredients.[11-12] They can turn into various reactive carbonyls under high temperature, and all of them trigger acrylamide formation (Figure 1).

The role of plant polyphenols in acrylamide formation has attracted many attentions in recent years.[13-15] Epicatechin (EC) was reported to reduce acrylamide formation in Glc+Asn model Maillard reaction systems by several researchers.[16-18] A decrease of acrylamide content was also observed when EC was added in fructose+Asn model systems or biscuits.[15] However, the study of the inhibitory mechanism is limited. Totlani and Peterson reported that EC quenched C_2, C_3 and C_4 sugar fragments in aqueous Glc +glycine Maillard system and adducted with 3-deoxyglucosone (3-DG) in low-moisture Glc +glycine Maillard model.[19-21] It indicated that EC may trap carbonyl intermediates in Maillard reaction, and consequently, mitigate acrylamide

formation. Study of the detailed inhibitory pathway of EC on acrylamide formation in Glc+Asn model, a typical system which was widely used to investigate the formation of acrylamide, is necessary. According to the difference of moisture content, there were three kinds of models: aqueous, low-moisture, and wet-to-dry. [9, 17, 22] The first two models were usually performed in sealed containers. Reactants were dissolved in several milliliters of solvents, or mixed with solid matrix and a few hundred microliters of solvents, respectively. In wet-to-dry models, the containers were not sealed, and water escaped during heating. As water evaporation happens during frying, toasting and baking, wet-to-dry model system involving moisture loss is closer to food thermal processing and was adopted in this study.

HMF, an indicative compound in Maillard reaction and caramelization, is formed via dehydration of hexoses or Amadori products.[23] It has been used to evaluate the degree of nonenzymatic browning in food processing, or the quality deterioration of products during storage.[24-25] HMF contains a reactive aldehyde group and has multifunctions. The health concern of HMF is mainly related to the toxicity of 5-sulfoxymethylfurfural which is converted from HMF in vivo.[26-27] Recent studies showed that EC reduced HMF formation in Maillard reaction model and caramelization system, but the molecular mechanism remained not clear.[28-29] Researchers presumed that it might be associated with the trapping activity of EC on 3-DG, a precursor of HMF. As mentioned above, the adduct of EC and 3-DG in a Maillard reaction model had been reported.[21] The polymerization between HMF and catechins under the Maillard reaction or caramelization condition is barely considered, although their condensation in acidic hydroalcoholic medium has been demonstrated in literature.[30-34]

This study was carried out to elucidate the effect of EC on the kinetics of acrylamide, 3-DG, and HMF in wet-to-dry Maillard reaction systems. Reaction products were analyzed with LC-MS to get an insight into the inhibition mechanism. Furthermore, the reaction between HMF and EC under high temperature was studied for the first time.

MATERIALS AND METHODS

Chemicals and materials. (-)-Epicatechin, 5-hydroxymethylfurfural, acrylamide, and [D_3]-acrylamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-Deoxyglucosone was purchased from Toronto Research Chemicals (Ontario, Canada). D-(+)-Glucose, L-asparagine, and o-phenylenediamine were purchased from J&K Chemical Ltd. (Shanghai, China). HPLC grade solvents were obtained from Fisher Scientific (Pittsburg, PA, USA). Oasis HLB (6 mL, 200 mg) SPE cartridges were supplied by Waters (Milford, MA, USA). Potatoes were bought from a local supermarket in Wuxi, China.

Preparation of Chemical Model Systems. The reactants in chemical models are listed in Table 1. Glc, Asn, and HMF were dissolved in ultrapure water at a concentration of 20 mM. EC was prepared as 5 mg/mL. One hundred microliter Glc (or HMF), 100 µL Asn (or water), and 50 µL water (or EC) were added into glass tubes (10 × 100 mm). Then, the tubes without caps were placed in an oil bath maintained at 180 °C. As the water evaporated, the tubes dried at about 3.5 min.

For the kinetic analysis, samples were withdrawn from the oil at 1, 2, 3, 4, 5, 7, and 10 min. To the tubes were added 0.25 mL [D3]-acrylamide solutions (2 µg/mL). Then more water was added to make the total volume of solvent in each tube reach to 1 mL. The mixtures were

extracted for 10 min with the help of sonication. Afterwards, the samples were filtered through 0.45 μm filters. Concentration of acrylamide, HMF, and 3-DG were determined.

For the adducts analysis and color analysis, tubes were removed from oil bath at 5 min. The dry residues were diluted in 200 μL methanol. Finally, the samples were subject to determination of browning intensity or filtration before UPLC-MS analysis.

Preparation of Fried Potato Chips. Potatoes were cut into thickness of 2.0 ± 0.1 mm and diameter of 30 mm. Then the slices were blanched in water at 95 °C for 5 min. Afterwards, the potato chips were immersed in an EC solution (1 mg/mL) for 15 min at room temperature, while water was used as control. Next, excess water on the surface of the chips was wiped and the samples were fried in palm oil at 175 °C for 5 min using an oil bath device (DF-101S, Lichen Technology Co., Ltd, Shanghai, China). The fried potato chips were ground into fine pastes using mortars and pestles.

Ground potato chips (1.50 g) were weighed and defatted with hexane before extracted with water for three times (10 mL each). The mixtures were sonicated for 30 min and then centrifuged at 30 000 *g* for 10 min. The supernatants were combined and treated with Carrez I and Carrez II reagents (500 μL each). Part of the prepared samples were filtered through 0.45 μm filters prior to HMF analysis. Other samples were spiked with [D3]-acrylamide before cleaned up with Oasis-HLB cartridges for acrylamide analysis.[18]

Some of the defatted samples (3.0 g) were extracted with 20 mL methanol by sonication for 30 min. The extraction was repeated twice followed by centrifugation at 30,000 *g* for 10 min. Afterwards, the supernatants were concentrated to about 5 mL under vacuum (BUCHI rotary

evaporator, BUCHI Shanghai Ltd., China). The samples were filtered through Nylon filters and analyzed with UPLC-MS in multiple reaction monitored (MRM) mode for the verification of adducts in fried potato chips.

Analysis of Acrylamide. Acrylamide was determined similar to a published procedure.[18] Quantification was performed with an ACQUITY UPLC system coupled to an ACQUITY triple quadrupole (TQ) detector (Waters, Milford, MA, USA). The chromatographic separations were conducted on an HSS T3 Column (2.1 × 100 mm, 1.8 µm) at 35 °C. Acrylamide was eluted using 0.1% formic acid in ultrapure water at a flow rate of 0.2 mL/min. The electrospray ionization (ESI) conditions were as follows: positive mode; capillary voltage of 3.0 kV; source temperature of 120 °C; desolvation gas temperature of 400 °C. Data were acquired in MRM mode. The parent to daughter m/z were 72.1 to 55 for acrylamide, and 75 to 58 for [D3]-acrylamide, respectively.

Analysis of HMF. HMF quantification was performed according to previous method with some modifications.[28] A Shimadzu LC-20AT system equipped with a SPD-20A UV-VIS detector and a Symmetry C18 Column (4.6 × 250 mm, 5 µm) was used. Elution was conducted at a flow rate of 1 mL/min with isocratic 10% aqueous methanol as a mobile phase at 30 °C. HMF was detected at 284 nm and quantified using external standard method.

Analysis of 3-DG. Analysis of 3-DG was conducted according to the method described by Zhang et al.[35] Samples and 3-DG (as external standard) were derivatized with o-phenylenediamine (OPD) and then measured using HPLC-UV.

Analysis of Adducts. Samples from the chemical models were analyzed on an ACQUITY UPLC system interfaced with a SYNAPT quadropole time-of-flight (qTOF) instrument (Waters,

125 Milford, MA, USA). The UPLC system contained a binary pump, an autosampler, a column
126 thermostat, a photodiode array (PDA) and a BEH C18 Column (2.1 × 100 mm, 1.7 μm). Mobile
127 phase consisted of acetonitrile (solvent A) and 0.1% formic acid aqueous solution (solvent B).
128 Elution gradient were 0-15 min, 5-25% A; 15-20 min, 25-40% A; 20-22 min, 40-80% A; 22-24
129 min, 80-100% A, followed by washing and re-equilibrating of the column. The flow rate was
130 0.3 mL/min and the column temperature was set at 45 °C. Two μL samples were injected. UV-
131 visible spectra were recorded from 250 to 600 nm. Mass data were acquired in negative ion
132 mode. The parameters of ESI source were as follows: capillary voltage 3 kV, cone voltage 20
133 V, source temperature 100 °C; desolvation temperature 400 °C, collision energy 6 eV, and
134 scan range m/z 50-1500. For MS/MS measurements, the cone voltage and collision energy
135 were 30 V and 20 eV, respectively. In order to get more accurate masses of the adducts, a
136 Vanquish UHPLC system coupled with an Exactive Plus Orbitrap mass spectrometry (Thermo
137 Fisher Scientific, Waltham, MA, USA) was used. This mass spectrometer was operated with
138 the following parameters: ESI^- mode, spray voltage 2.8 kV, capillary temperature 325 °C,
139 sheath gas flow rate 35 (arbitrary units) and aux gas flow rate 15 (arbitrary units).
140 In order to verify the adducts in fried potato chips, the chip isolates and the sample of HMF+EC
141 (model IIIB, as reference) were analyzed on an ACQUITY UPLC system coupled to an ACQUITY
142 TQ detector (Waters, Milford, MA, USA). The chromatographic conditions were the same as
143 that stated in the last paragraph. Injection volume was increased to 10 μL. The operational
144 parameters of the mass spectrometer were ESI^- mode, capillary voltage 3 kV, source
145 temperature 100 °C, and desolvation temperature 400 °C. Data were collected in MRM mode
146 with traces as follows: dimeric adducts, m/z 687.2 > 289.1 and 687.2 > 397.1, cone 30 V,

collision 20 eV; trimeric adducts, m/z 1085.3 > 397.1 and 1085.3 > 795.2, cone 30 V, collision 25 eV**.**

Determination of Browning Intensity. The color intensities of the models of HMF+EC and EC (model IIIB and model IV) after heating were measured by using a UV-vis spectrophotometer (PGeneral, Beijing, China) with a path length of 1 cm at 420 nm. Samples were appropriately diluted and the browning intensities were calculated as the absorbance values multiplied by the dilution factors.[36]

Statistical Analysis. All reactions were performed in triplicates. Data were presented as mean ± standard deviation. Statistical comparisons with the corresponding controls were made using t-test. Statistical comparisons among three groups were made using analysis of variance with Tukey's test. Analysis were conducted using SPSS 21.0 (SPSS Inc., Chicago, IL, USA), and statistical differences were considered with $p<0.05$.

RESULTS AND DISCUSSION

Effect of EC on the Formation of Acrylamide, HMF and 3-DG in the Glc+Asn model (model I). Model I containing Glc and Asn with or without EC were heated for various time to obtain the kinetic behaviors of acrylamide, HMF and 3-DG. Kinetic approaches involving intermediates can provide a better understanding of the reaction processes. Figure 2A illustrates the formation of acrylamide. At 0-3 min, the temperatures of the aqueous reaction systems were below 100 °C, and few acrylamide was detected. Acrylamide production increased at 3-7 min and then became a plateau at 7-10 min. Adding of 0.86 μmol EC in the system resulted in a reduction of acrylamide from 24.9 nmol to 11.8 nmol at 10 min. This

inhibition rate is in agreement with the study of dose-response relationship under similar conditions performed by Xu et al.[17]

Different from the gradual accumulation of acrylamide, HMF concentration in the system had an increase followed by a decrease trend (Figure 2B). It is clear that HMF is an intermediary of the Maillard reaction products and subject to further reactions after formation. The curves showed that, the presence of EC significantly mitigated the HMF but did not affect the time needed to reach the maximum yield. The percentage of reduction compared at the highest point was 72%. The observed inhibitory effect was consistent with previous research.[28]

As for 3-DG, another transient intermediate, its concentration rose quickly within three minutes and then dropped to near zero within one minute. At the same time (between 3 and 4 min), the system turned from wet to dry and reached 180 °C. This phenomenon hinted that 3-DG was potentially vulnerable to degradation under high temperature. The amount of 3-DG formed in the Glc+Asn+EC model (model IB) was 1.87 times higher than that in the Glc+Asn model (model IA) when heated for 3 min.

Effect of EC on the Formation of Acrylamide and Consumption of HMF in the HMF+Asn model (model II). Based on the observations from the Glc+Asn model (model I), it was hypothesized that EC inhibited the formation of acrylamide through reducing the content of HMF in Maillard reaction. So, the HMF+Asn model (model II) was built. Results summarized in Figure 3A showed the trend of acrylamide formation in HMF+Asn model during heating. Compared with Glc+Asn model, acrylamide appeared a little later in this model. Afterwards, its content quickly reached to a maximum of 26.1 nmol at 7 min and then remained stable. The amount of acrylamide generated in HMF+Asn model (model IIA) is comparable to that in

Glc+Asn model (model IA) after heating for 10 min, which is different from the results in other papers.[9, 22] For example, Gökmen et al. reported that HMF converted 2.27 times more Asn to acrylamide than Glc when heated for 10 min.[9] The different results were likely due to the different reaction conditions. Previous studies were carried out in closed tubes with 0.1 mL[9] or 4 mL[22] water inside, whereas open tubes were employed here and water escaped from the system. It has been acknowledged that moisture content is an important factor for Maillard reaction progress and acrylamide formation.[10, 37] The reduction (about 70%) of EC on the acrylamide formation in HMF+Asn model (model II) was more efficient than that (about 50%) in Glc+Asn model (model I). This phenomenon supports the involvement of HMF in the inhibition mechanism of EC on acrylamide formation in Maillard reaction. In Glc+Asn model, Glc turns into many reactive carbonyls which react with Asn to further form acrylamide. HMF is one of the carbonyl compounds. So, the inhibition of EC in Glc+Asn model should be lower than that in HMF+Asn model.

HMF concentrations in HMF+Asn model (model IIA) and HMF+Asn+EC model (model IIB) were monitored to examine the effect of EC on HMF degradation (Figure 3B). A rapid consumption was observed between 3-4 min when all water in the system evaporated and the temperature reached 180 °C. It illustrates that high temperature is necessary to trigger the reaction between HMF and Asn. When EC was present, the residual of HMF was significantly lower. EC played this role probably through promoting the reaction between HMF and Asn, enhancing the degradation of HMF, or directly adducting with HMF. The following experiments verified the third pathway.

Effect of EC on the Residual of HMF During Heating. In order to confirm the trapping of HMF

by EC, HMF alone or binary mixtures of HMF and EC were heated in the oil bath (model III). Determination of the residual amounts of HMF revealed that after 10 min of heating, 85% of the initial HMF was still present in model IIIA (containing HMF alone). The presence of EC in the system did significantly enhance the decline of HMF. Increasing EC:HMF molar ratio from 0.86:2 to 8.6:2, the residual of HMF dramatically decreased from 60% to 18% (Figure 4).

Identification of EC-HMF Adducts. Reaction mixtures were characterized with UPLC MS/MS. The total ion chromatogram (TIC) of HMF+EC model (model IIIB) displayed the existence of unreacted HMF and EC, and the neo-formed catechin. The epimerization between EC and catechin at high temperature has been well established in former studies.[38] It was observed that there were many newly formed peaks on the chromatograph, suggesting the formation of new compounds after heating. Mass spectrometric analysis revealed that majority of the peaks gave rise to molecular ion of m/z 687.2 and had similar product ion spectra (Figure 5A). The molecular weight (688 Da) matched with the adducts composed of two catechin/EC units linked through an HMF bridge. The detected $[M-H]^-$ m/z ranging from 687.17109 to 687.17136 accorded with the theoretical m/z value of 687.17193 ($\Delta < 1.2$ ppm). The produced m/z 397.1 and 289.1 ions were proposed to be derived from the Quinone-Methide cleavage of the bridge, and the ion of 535.1 was from the retro Diels-Alder fragmentation of the heterocyclic ring in the flavan-3-ol (Figure 5A). In addition, several compounds with parent ions of $[M-H]^-$ m/z 1085.3 and daughter ions of 397.1, 289.1, 687.2 and 795.2, corresponded to a sandwich-type structure consisting of three catechin/EC bridged by two HMF molecules. The measured m/z having error less than 0.7 ppm compared with the theoretical value of 1085.27210, verified the proposed structures. Their mass spectra were also alike and the fragmentation

234 pathways are shown in Figure 5B. It should be noted that the isomers with different linkage
235 position and stereoisomerism at C-3 site could not be distinguished by mass spectrometer.
236 There is an upward baseline shift and a non-resolved group of peaks at around 17 min in the
237 TIC chromatogram. It may be caused by the overlapping of the isomers and the generation of
238 numerous more polymerized compounds.
239 The condensation between polyphenols and carbonyl compounds is an important reaction
240 during wine aging. So, works concerning these interactions were usually performed in models
241 with low pH at room temperature.[30-33] In particular, when catechin was incubated with HMF
242 at pH 2.2, four dimeric adducts with m/z 687 were detected.[34] It was supposed that the acid
243 catalyzed the protonation of HMF. Then the nucleophilic site of EC (C6 or C8 position in A ring)
244 adducted with the formed carbocation, resulting four positional isomers linked with C8-C8,
245 C8-C6, C6-C8, or C6-C6 bonds (carbon at bridge of C8-C6 linkage was chiral, so C8-C6 and C6-
246 C8 are different).[34] In the present study, the reaction was triggered by the elevated
247 temperature. The combination of HMF and EC treated at high temperature yielded more
248 complicated products than those treated under acidic hydroalcoholic solutions at room
249 temperature. It agreed with the fact that EC coexists with catechin at 180 °C. Taking into
250 account the four C-C linkage and two epimers, sixteen isomeric patterns of dimeric adducts
251 and 128 trimers were yielded. Due to the difference of steric effect, C6 and C8 position had
252 different approachability in nucleophilic substitution, resulting in the unequal production of
253 the isomers.[39]
254 Ions with m/z 687.2 were the richest ions of adducts detected by MS in HMF+EC model (model
255 IIIB). Majority of them were derived from the ionization of dimeric adducts and others were

256 from the split of trimers. The extracted mass chromatograms (m/z 687.2) of Glc+Asn+EC
257 (model IB) and HMF+Asn+EC (model IIB) were compared with that of HMF+EC (model IIIB) in
258 Figure 6. Overall, the fingerprints of the reaction products generated in these three models
259 were very similar, suggesting that the adducting between EC and HMF was a key step in the
260 intervention mechanism of EC on Maillard reaction.
261 Totlani and Peterson[21] demonstrated that 3-DG might be the most abundant sugar fragment
262 carbonyl compound which was trapped by EC under low-moisture systems. However, in this
263 study, the kinetic study showed that the content of 3-DG decreased to very low when the
264 system turned from wet to dry, and the presence of EC did not reduce the production of 3-
265 DG. On the contrary, the consumption of HMF by EC may cause the equilibrium shifting and
266 the enhance of the Glc dehydration reaction (Figure 1). As a result, more 3-DG and less HMF
267 was determined in Glc+Asn+EC model (model IB) than in Glc+Asn model (model IA) (Figure 2).
268 Therefore, the mitigation effect of EC on Maillard reaction products including acrylamide was
269 due to its polymerization with HMF instead of 3-DG in the current thermal model.
270 **Verification in fried potato chips.** Thermal processed potato products have considerable
271 contents of acrylamide because there are plenty of reducing sugars and asparagine in raw
272 potatoes. Based on this, fried potato chip models were performed to certify the inhibitory
273 mechanism of EC. After the slices were soaked in 0.1% EC solution prior to frying, the
274 formation of acrylamide decreased from 724.5±43.1 to 539.0 ± 44.3 μg/kg, and the HMF
275 contents decreased from 951.3±32.4 to 626.1 ± 15.5 μg/kg. In the chemical model (EC+HMF,
276 model IIIB), the ion abundance of dimers was about 30 times higher than that of trimers. In
277 the potato chip isolates, dimeric adducts between EC and HMF were detected. Trimeric

adducts were not detected because their concentration was too low. These data suggested that EC inhibited acrylamide formation by reacting with HMF directly in fried potato chips.

Analysis of UV-Vis spectra and browning. The UV-visible absorption of the reaction products of EC+HMF was further analyzed (Figure 7). The identified adducts presented spectra similar to that of EC with a maximum absorption at 280 nm. It indicated the retainment of the original flavanol structure and supported the postulated structures. There was a large bump in the end of the chromatogram recorded at 280, indicating the formation of numerous oligomers and polymers. The UV-vis spectrum of the bump recorded between 250 and 600 nm exhibits another absorption peak with maximum around 440 nm besides the one at 280 nm. Moreover, peaks eluted at 11.38, 12.44 and 13.59 min also had absorption at visible wavelength region (Figure 7). The maximum absorption located between 440 and 460 nm matched with the characteristic of yellow xanthylium salts, which were derived from the dehydration and oxidation of the original adducts.[34, 40] Mass analysis detected ions with m/z 669 and 667, corresponding to dehydration intermediates and xanthylium salts, respectively. However, these ions coeluted with others and their intensities were low. Perhaps because of this reason, the absorption peaks at 440-460 nm were lower than that around 280nm.

The obtained visible spectra explained the color development of the reaction between EC and HMF. It was observed that the mixture underwent first yellow then brown and finally precipitated as a dark brown solid in the tubes. Similar darkening progress was also reported in the wine models and was regarded as a reason for the color change of grape-derived foods during aging.[34] However, the reaction of EC and HMF at high temperature was underestimated in the past. It was reported that adding of catechin or EC resulted in an

300 increase of browning intensity in Maillard reaction model, caramelization model, fried potato
301 chips, and heated barley products.[17, 28-29, 41] But the reason was just attributed to the auto-
302 oxidation of flavanols during heating. In this study, the absorbance of the heated EC (model
303 IV) and EC+HMF (model IIIB) at 420 nm were measured as 0.02±0.00 and 1.54±0.09,
304 respectively. It can be proposed that the major browning mechanism is the interaction
305 between EC and HMF, rather than the self-oxidation or self-condensation of EC. As there were
306 relatively higher amounts of HMF formed in fried, baked and toasted foods, the reaction
307 between EC and HMF should be taken into consideration as a potent contributor of browning
308 of thermally processed foods. Furthermore, many acrylamide inhibitors work through
309 suppressing Maillard reaction and have an undesired anti-browning impact on final products.
310 As HMF is an important intermediate in Maillard reaction, EC also blocked Maillard reaction.
311 But the browning promoting effect compensated the color changes. As a result, moderate
312 amount of EC may mitigate acrylamide formation without compromising color properties. On
313 the other hand, excessive EC may cause unwanted browning of thermally processed foods,
314 and it should also be avoided.
315 On the basis of these results, the reaction between EC and HMF explained, at least in part,
316 the inhibition of EC on the generation of acrylamide in Maillard reactions. The adducts were
317 detected in chemical models as well as fried potato chips. The browning effect caused by the
318 condensation of EC and HMF at high temperature should not be neglected. This study
319 provides new understanding on how polyphenols alter Maillard reaction and browning
320 intensity of thermally processed foods.

321 **ASSOCIATED CONTENT**

Supporting Infomation

Figure S1: UPLC-TOF MS/MS total ion chromatogram (TIC) of a mixture of EC and HMF (model IIIB) after heating at 180 °C for 5 min.

Figure S2: General structures of EC-HMF dimers (A, B and C) and trimers (D, E, F and G).

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

Asn, asparagine;

3-DG, 3-deoxyglucosone;

EC, epicatechin;

Glc, glucose;

HMF, 5-hydroxymethylfurfural

MRM, multiple reaction monitored

qTOF, quadropole time-of-flight

TQ, triple quadrupole

ESI, electrospray ionization

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445

FIGURE CAPTIONS:

Figure 1: Formation mechanism of acrylamide from Glc and HMF adapted from Gökmen et al.[9]

Figure 2: Kinetics curves of acrylamide (A), HMF (B) and 3-DG (C) in the Glc+Asn or Glc+Asn+EC model heated at 180 °C (model I). Asterisk (*) denotes significant difference compared at the same time point ($p < 0.05$).

Figure 3: Residual HMF (A) and formation of acrylamide (B) in the HMF+Asn model with or without EC during heating at 180 °C (model II). Asterisk (*) denotes significant difference compared at the same time point ($p < 0.05$).

Figure 4: Residual of HMF after heating in the presence or absence of EC for 10 min at 180 °C (model III). Statistical differences compared at the same time point are denoted by different letters ($p < 0.05$).

Figure 5: Product ion mass spectra and fragmentation pathways of dimeric (A) and trimeric (B) adducts between EC and HMF.

Figure 6: Extracted ion current chromatograms of m/z 687.2 generated from EC+HMF model (model IIIB, A), Glc+Asn+EC model (model IB, B), and HMF+Asn+EC model (model IIB, C).

Figure 7: UPLC-DAD chromatogram recorded at 280 nm of a mixture of EC and HMF (model IIIB) after heating at 180 °C for 5 min. Small panels showed the UV-visible spectra of the corresponding peaks.

Figure 1:

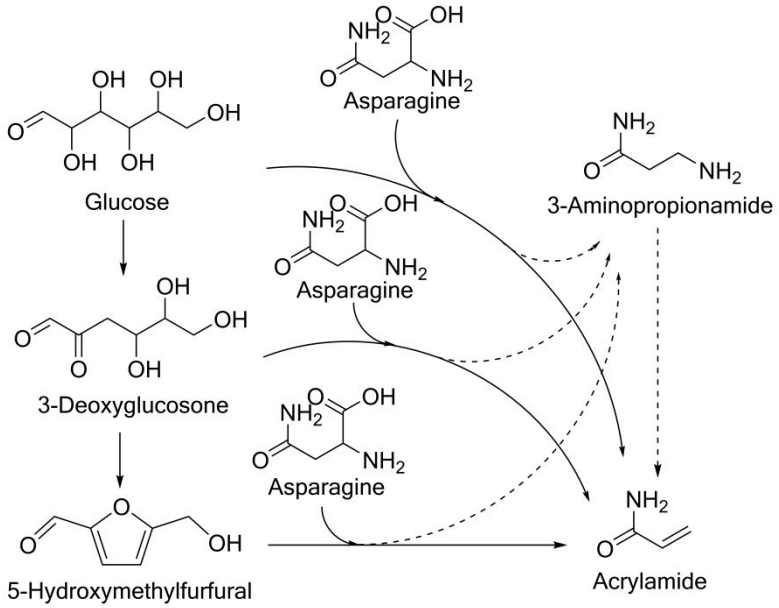
Glucose
3-Deoxyglucosone
5-Hydroxymethylfurfural
Asparagine
Asparagine
Asparagine
3-Aminopropionamide
Acrylamide

Figure 2:

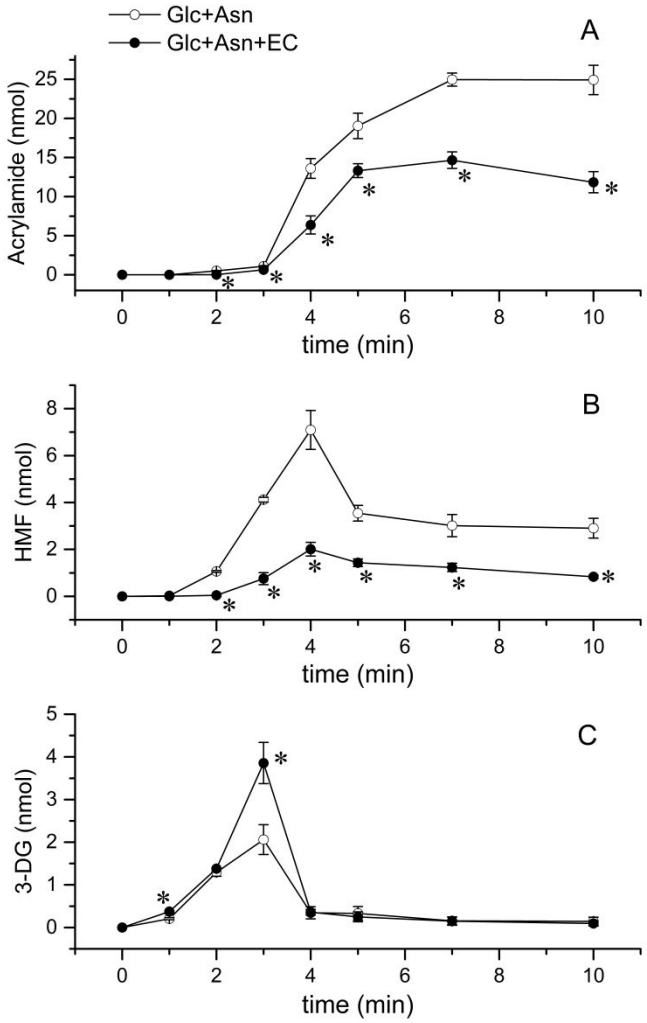
Glc+Asn
Glc+Asn+EC
A
Acrylamide (nmol)
time (min)
B
HMF (nmol)
time (min)
C
3-DG (nmol)
time (min)

Figure 3:

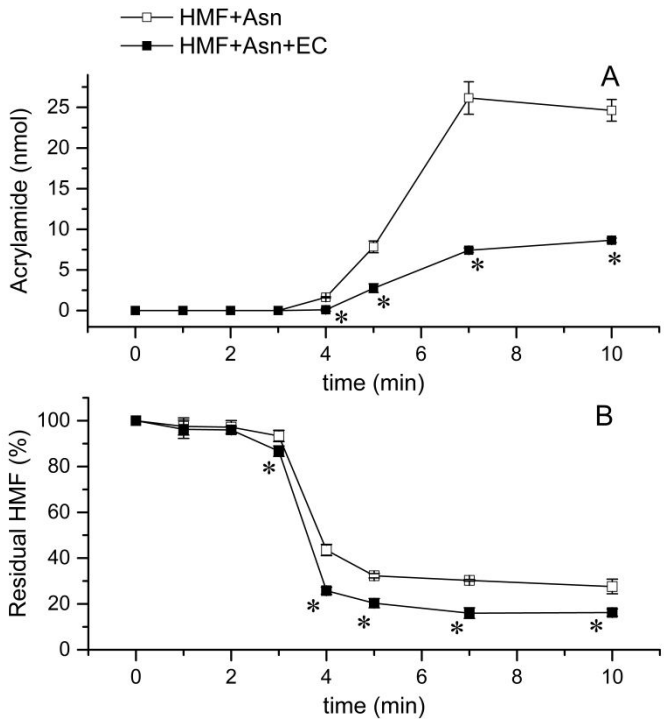
HMF+Asn
HMF+Asn+EC
A
Acrylamide (nmol)
time (min)
B
Residual HMF (%)
time (min)

Figure 4:

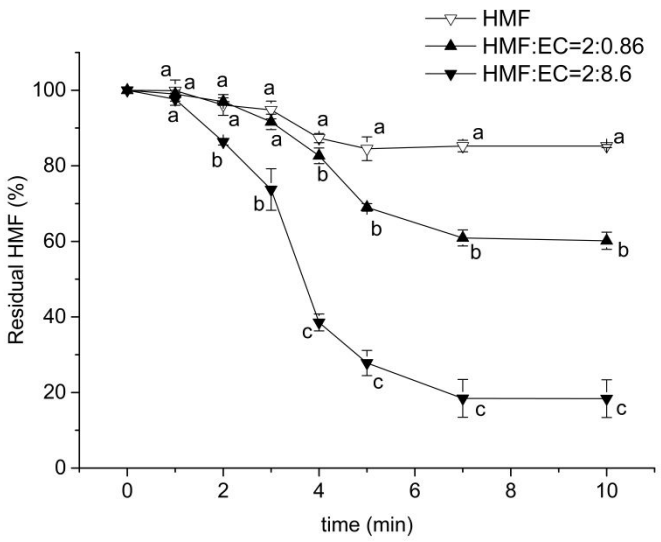
HMF
HMF:EC=2:0.86
HMF:EC=2:8.6
Residual HMF (%)
time (min)
0
20
40
60
80
100
0
2
4
6
8
10

Figure 5:

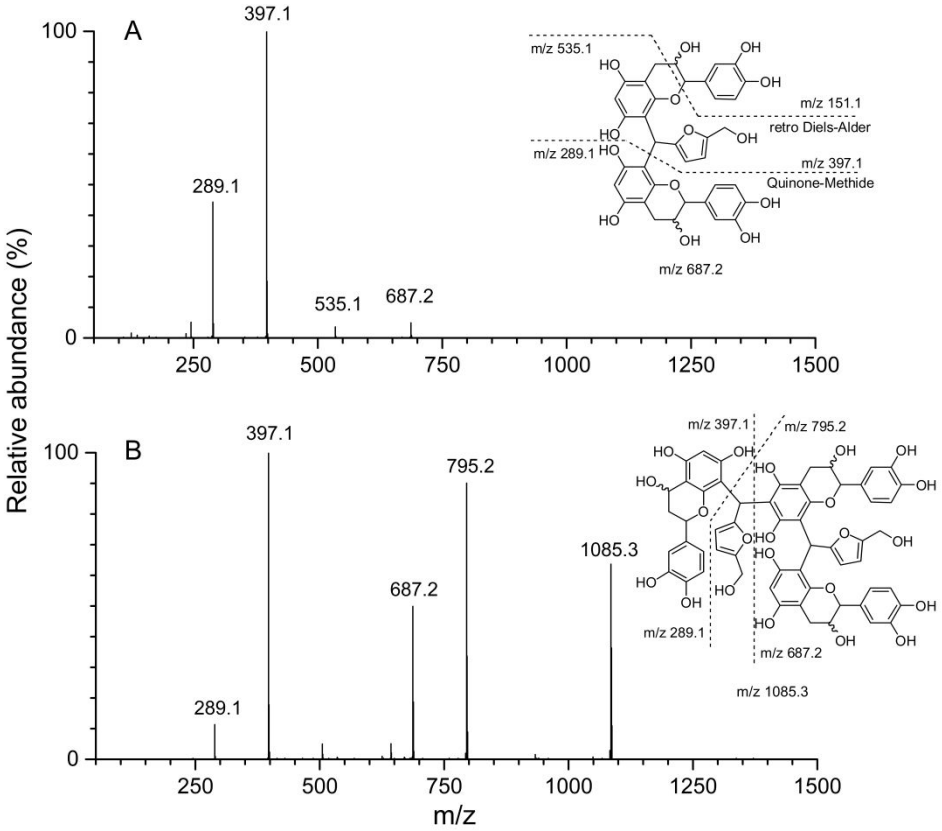
A
100
0
397.1
289.1
535.1
687.2
m/z 535.1
m/z 151.1
retro Diels-Alder
m/z 289.1
m/z 397.1
Quinone-Methide
m/z 687.2
B
100
0
397.1
795.2
687.2
1085.3
289.1
m/z 397.1
m/z 795.2
m/z 289.1
m/z 687.2
m/z 1085.3
Relative abundance (%)
250
500
750
1000
1250
1500
m/z

Figure 6:

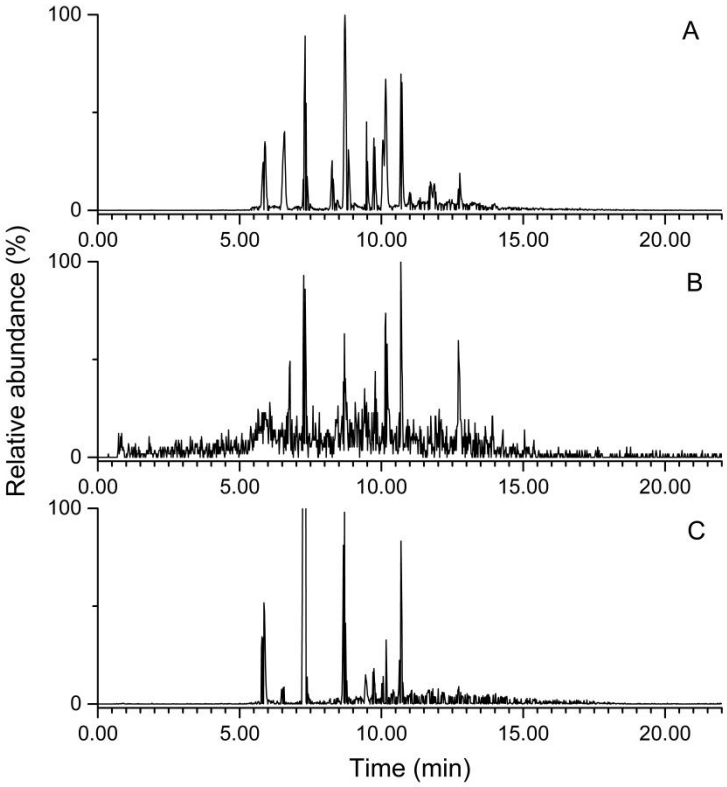

Figure 7:

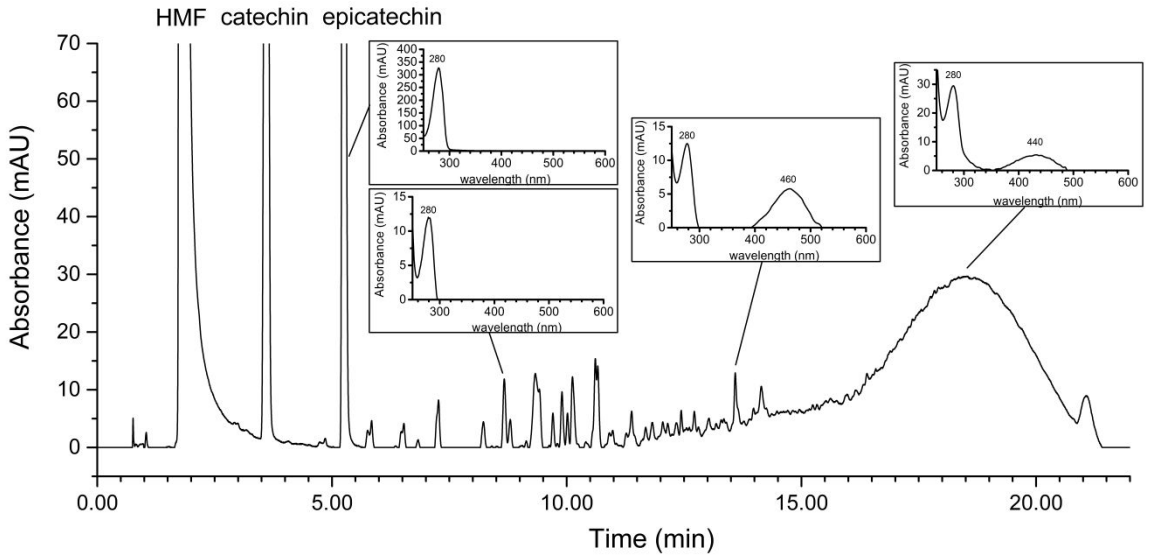
HMF
catechin
epicatechin
Absorbance (mAU)
Time (min)
wavelength (nm)
280
460
440

Table 1. Reactants in Chemical Model Systems[a]

model	reactant (μmol)			
	Glc	HMF	Asn	EC
IA	2		2	
IB	2		2	0.86
IIA		2	2	
IIB		2	2	0.86
IIIA		2		
IIIB		2		0.86
IIIC		0.2		0.86
IV				0.86

[a] Reactants were dissolved in 250 μL water and heated at 180 °C for 1-10 min in open tubes.

Graphic for table of contents