THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF FOOD SCIENCE

EFFECTS OF PHENOLIC COMPOUNDS ON THE MECHANISMS OF PYRAZINIUM RADICAL GENERATION IN THE MAILLARD REACTION: TRAPPING OF REACTIVE IMINE INTERMEDIATES

QING BIN
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ABSTRACT

Previous studies have demonstrated the generation of pyrazinium radical cation in the Maillard reaction and its significant role as a precursor to the non-enzymatic Maillard browning. In this study, effects of polyphenol chemistry on the mechanisms of pyrazinium radical generation in the Maillard reaction were investigated. Aqueous models of glyxoal–alanine (GO–Ala), glycolaldehyde–alanine (GA–Ala), and glucose-alanine (Glu–Ala) were treated with a concentration gradient of phenolic compounds (epigallocatechin-3-gallate, catechin, and 4-methylcatechol, respectively), and quantitative analysis of the pyrazinium radicals in these models was performed using electron paramagnetic resonance (EPR) spectroscopy. Catechins were reported to alter pyrazinium radical generation with the A-ring determined as the main reactive site. Enhancement of radical formation was found in GO–Ala model, with the maximum enhancement reached at catechin concentration being 1/10 of GO concentration; suppression of radical formation was found in GA–Ala and Glu–Ala model. Studies on the mechanism of the effect of catechins on the radical formation by LC/MS revealed the reactive imine-trapping by catechins in these models, which was suggested to directly control the generation and quenching of radical precursor enaminol, thus affecting the radical yield. LC/MS/MS analysis provided the detection and identification of these imine adducts of EGCG. This is the first reported mechanism including the reactive imine-trapping by catechins and its linkage to radical formation in the Maillard systems.

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Chapter 1

Introduction and Literature Review

The Maillard reaction, initiated by the condensation between carbonyl and amino function groups, is ubiquitously found in food and under biological conditions. The following complex array of reactions involves the formation of many types of compounds (pyrazines, furans, carbonyls, melanoidin-brown polymers, etc.) responsible for several changes in the flavor, aroma, color, nutritional value and toxicity of foods[1].

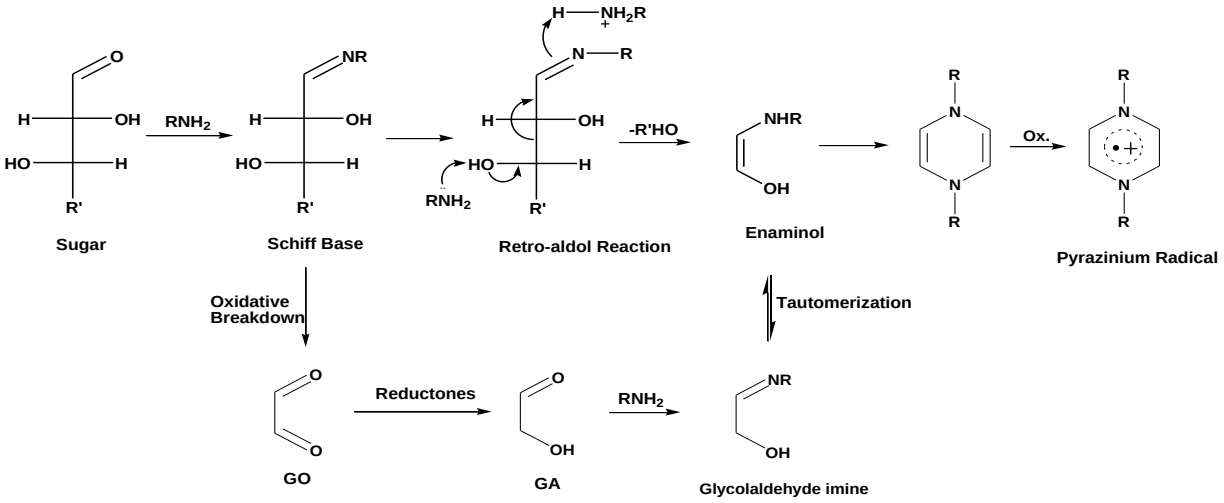

Figure 1-1. Mechanism of the pyrazinium radical cation formation in the Maillard reaction[2-3].

In 1980s, pyrazinium radical cation formation during the early stage of the Maillard reaction was detected by electron paramagnetic resonance (EPR) spectroscopy[2] and model study has elucidated its role as a key precursor to the non-enzymatic Maillard browning[3]. As proposed by Namiki and Hayashi, the mechanism (**Figure 1-1**) of this radical formation involves the

generation of glycolaldehyde imine (in equilibrium with its tautomer enaminol) that is dimerized and oxidized to the symmetrical, resonance stabilized 1,4-dialkylpyrazinium radical cation[2]. They indicated that the glycolaldehyde imine was formed either from the fragmentation of Schiff base, or from glycolaldehyde–amino acid condensation, where the glycolaldehyde (GA) was reported as the most effective reagent in yielding the pyrazinium radical. Recent years Hofmann et al. conducted a series of time-course study investigating the changes (e.g. glycolaldehyde/glyoxal concentrations, reducing substance, color development) accompanying this radical formation during the early stage of the Maillard reaction[3]. They have demonstrated that glyoxal (GO) is generated abundantly prior to radical formation, and with the increasing concentration of the reducing substances in solution, the amount of GA increases due to the reduction of GO, resulting in the sequent increasing of radical formation. In the same study, the role of the pyraizinium radical cation as a color precursor was investigated by using synthesized 1,4-diethylpyrazinium diquaternary salt (turned color immediately upon dissolving in aqueous solution) and studying the redox reactions between the diquaternary salt and pyrazinium radical by LC/MS and EPR. A mechanism of disproportionation reaction of the radical cation leading to the duiquat was proposed that elucidates the role of the pyrazinium radical in assisting non enzymatic browning in aqueous Maillard models[3].

Although with its significant role as a precursor to the browning of the Maillard systems, this pyrazinium radical formation has been much less studied comparing to other pathways of the Maillard reaction. Surprisingly few studies have given attention to the influence of other food constituents on this radical formation. In fact, many minor food constituents can significantly affect the chemistry in food systems due to their specific chemical properties. Recently, a series of studies by Peterson and Totlani have demonstrated the alternation of Maillard pathways by epicatechin, which inhibits the generation of several volatile compounds (e.g. pyrazines, furans) in Maillard models and food processing[4]. Epicatechin function as trapping agents of sugar

fragments/carbonyl compounds. C^{13} and N^{15} labeling study has proved the trapping of C_2 (e.g. glyoxal), C_3 (e.g. methylglyoxal, glyceraldehyde), and C_4 (erythrose) sugar fragments by epicatechin and formation of EC-sugar fragment adducts. The structure EC-MGO adduct was elucidated by 2D ^{1}H NMR, revealing the mechanism of trapping being a typical electrophilic aromatic substitution occurring at the highly activated A ring (**Figure 1-2**)[4d]. Trapping of reactive dicarbonyls by catechins has also been studied under physiological conditions. Sang et al. reported the trapping of methylglyxoal and glyxoal by epigallocatechin-3-gallate (EGCG) under 37 ℃ at neutral condition, functioning through the same mechanism[5]. In model wine systems, the trapping of aldehydes (e.g. glyoxylic acid, acetaldehyde, benzylaldehyde) by (epi)-catechin has been reported, and it has been indicated to increase the color development in wine[6].

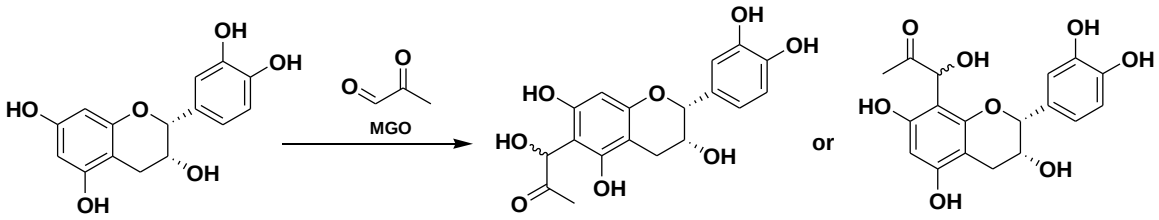

Figure 1-2. Epicatechin carbonyl-trapping reaction and formation of EC-MGO adduct[4d].

In the current study, it was hypothesized that reactive carbonyl-trapping by catechins may affect the radical formation in the Maillard reaction. The purpose of the study is to explore the linkage between these two seemingly independent Maillard pathways. Since glyoxal (GO) and glycolaldehyde (GA) are the two key C_2 fragments participated in the pyrazinium radical formation (**Figure 1-1**), and additionally they have been studied extensively as being important carbonyls connecting many pathways of the Maillard reaction and other food/wine reactions, it was to my great interest to study the aqueous GO–Ala and GA–Ala models. Electron

paramagnetic resonance (EPR) spectroscopy and LC/MS were used to investigate how polyphenol chemistry alters the mechanism of pyrazinium radical formation in these two models.

Chapter 2

Materials and Methods

Chemicals. Glyoxal (40% in aqueous solution), glycolaldehyde, D-glucose, L-alanine , catechin hydrate were obtained from Sigma Aldrich Co. (St. Louis, MO). 4-Methylcatechol (95%) was obtained from Fluka. All other chemicals and solvents were of analytical or HPLC grade. Water was purified through a Millipore Q-Plus (Millipore Corp., Bedford, MA) purification train.

Effect of Phenolic Compounds on the Pyrazinium Radical Formation in the GO–Ala Model. Glyoxal (0.2 M) and L-Alanine (0.5 M) were treated with 0, 5, 10, 20, 50, and 100 mM of EGCG respectively (or catechin, 4-methylcatechol, respectively) in phosphate buffer (0.1 M, pH = 7.0) (only up to 50 mM of catechin was obtained due to its limited solubility in solution at 25 ℃). In each vial, the solution was bubbled with N_2, and then an aliquot of the mixture was sealed into a 50 μL VWR micropipette and incubated at 25 ℃ (the micropipette was used to minimize the impact of oxygen on the radical formation and to apply directly into EPR tube for measurement). EPR spectra were recorded for each sample at time points indicated in **Figure 3-1** to track the formation and decay of pyrazinium radical in the mixture.

Effect of Phenolic Compounds on the Pyrazinium Radical Formation in the GA–Ala Model. Glycolaldehyde (0.1 M) and L-Alanine (0.5 M) were treated with 0, 5, 10, 20, 50, and 100 mM of EGCG respectively (or catechin, 4-methylcatechol, respectively) in phosphate buffer (0.1 M, pH = 7.0) (only up to 50 mM of catechin was obtained due to its limited solubility in solution at 25 ℃). In each vial, the solution was bubbled with N_2, and then an aliquot of the mixture was sealed into a 50 μL VWR micropipette and incubated at 25 ℃ (the micropipette was used to minimize the impact of oxygen on the radical formation and to apply directly into EPR

tube for measurement). EPR spectra were recorded for each sample at time points indicated in **Figure 3-10** to track the formation and decay of pyrazinium radical in the mixture.

Effect of Phenolic Compounds on the Pyrazinium Radical Formation in the Glu–Ala Model. Glucose (0.5 M) and L-Alanine (0.5 M) were treated with 0, 5, 10, 20, 50, and 100 mM of EGCG respectively (or catechin, 4-methylcatechol, respectively) in phosphate buffer (0.1 M, pH = 7.0) (only up to 50 mM of catechin was obtained due to its limited solubility in solution at 25 ℃). In each vial, the solution was bubbled with N_2, and then an aliquot of the mixture was sealed into a 50 μL VWR micropipette and heated at 100 ℃ for 30min. After cooled down to room temperature, EPR spectra were recorded for each sample at time points indicated in **Figure 3-13** to track the decay of pyrazinium radical in the mixture. The t = 0 stands for the first measurement after 30 min of heating.

Determination of Key Intermediates in the Reaction Mixture of GO-Ala-EGCG by LC/MS Analysis. Glyoxal (0.2 M) and L-Alanine (0.5 M) were treated with 20 and 100 mM of EGCG respectively in phosphate buffer (0.1 M, pH = 7.0). After 1 h, an aliquot (0.5 mL) of the reaction mixture was diluted by 0.1% formic acid in water (2.5 mL) and 0.1 % formic acid in methanol (2 mL). The solution was filtered through 0.45 μm PTFE tip filter using a 3 mL syringe (Becton Dickonson) and analyzed by LC/MS.

Determination of Key Intermediates in the Reaction Mixture of GA-Ala-EGCG by LC/MS Analysis. Glycolaldehyde (0.1 M) and L-Alanine (0.5 M) were treated with 20 mM of EGCG in phosphate buffer (0.1 M, pH = 7.0). After 0.5 h, an aliquot (0.5 mL) of the reaction mixture was diluted by 0.1% formic acid in water (2.5 mL) and 0.1 % formic acid in methanol (2 mL). The solution was filtered through 0.45 μm PTFE tip filter using a 3 mL syringe (Becton Dickonson) and analyzed by LC/MS.

LC/MS Method. Sample Analysis was conducted with Shimadzu HPLC system (Shimadzu, Columbia, MD) coupled with water ZMD 2000 mass spectrometer (Waters, Milford,

MA) via an electrospray ionization probe. The HPLC system consisted of a binary pumping system (LC-10ADvp), a degasser (DGU-14A), an autosampler (SIL-10vp), a column oven (CTO-10Avp), and a UV/vis detector (SPD-10Avp). 10 μL of injection was separated on a reverse phase C-18 column (2.1 mm × 150 mm, 5 μm packing column) maintained at temperature of 25^{o}C using a binary solvent system of 0.1% formic acid in water (A) and 0.1 % formic acid in methanol (B). All of the solvents were filtered with 0.45 μm Nylon 66 membrane filter. The mobile phase started at 10% B in A (0-2 min), linearly increasing to 80% B in A (2-40 min). The flow rate was 200 μL/min, and the UV/vis detector was set at λ = 280 nm. Mass spectrometric ionization conditions were as follows: ES^{-} mode; source temperature, 110 ℃; desolvation temperature, 300 ℃; capillary voltage, 3.0 kV. The structural information of analytes was obtained by using LC/MS/MS with the collision energy to be 20-30V.

EPR Method. EPR spectra were recorded on a Bruker eScan X-band spectrometer. The experimental parameters were as follows: modulation amplitude: 0.69 G; sweep time: 2.62 s; frequency: 9.78 GHz; gain: 1 × 10^{3}.

Chapter 3

Results and Discussion

Effect of Polyphenol Chemistry on the Pyrazinium Radical Formation in GO-Ala Model

Glyoxal (GO) is the smallest dicarbonyls possible. As GO being an important reactive dicarbonyls generated in food and in vivo, the reaction between GO and amino acids or proteins is ubiquitously found in many ways (Strecker Degradation, Protein modification).

The effect of EGCG on the generation and fate of pyrazinium radical in GO-Ala model was studied by EPR. The GO-Ala model was treated by EGCG with a concentration gradient from 0 to 100 mM, and strong effect of EGCG on the radical formation was observed from this model (**Figure 3-1a**). With the absence of EGCG, only little radical intensity was gained through the entire time-course. This was anticipated since GO is not a direct precursor to the pyrazinium radical. With the EGCG concentration climbing up through 5, 10, to 20 mM, there was a significant increasing enhancement of radical formation observed. Maximum enhancement of radical generation was seen at 20 mM EGCG (10% GO concentration). However, when the EGCG concentration continued going up through 50, to 100 mM EGCG, the trend went down as there was a weakening enhancement of radical formation. The EPR spectra (**Figure 3-2**) were included to help to visualize this effect.

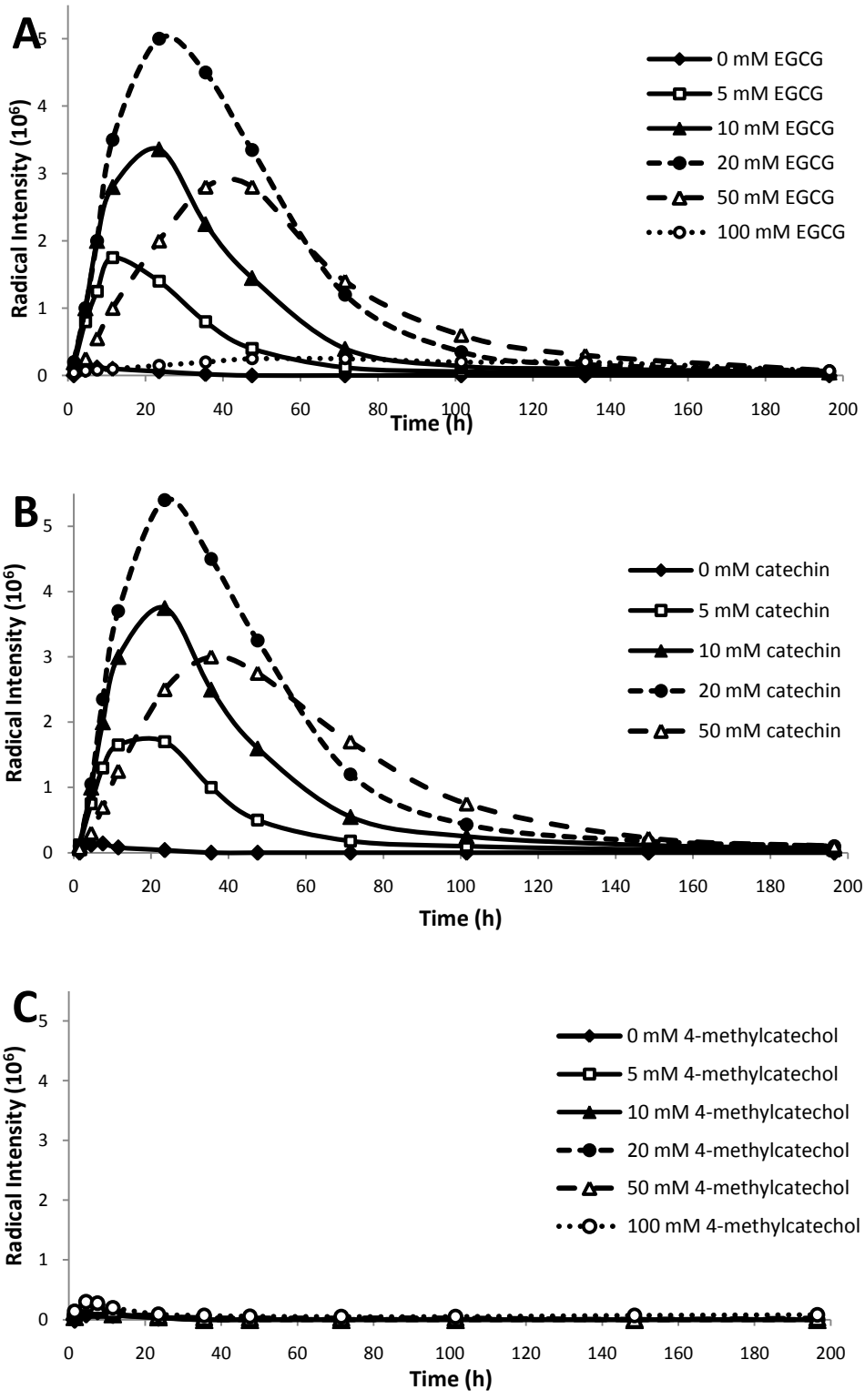

Figure 3-1. Effects of (a) EGCG, (b) catechin, and (c) 4-methylcatechol on the generation and fate of pyrazinium radical in GO–Ala aqueous model at 25 ℃.

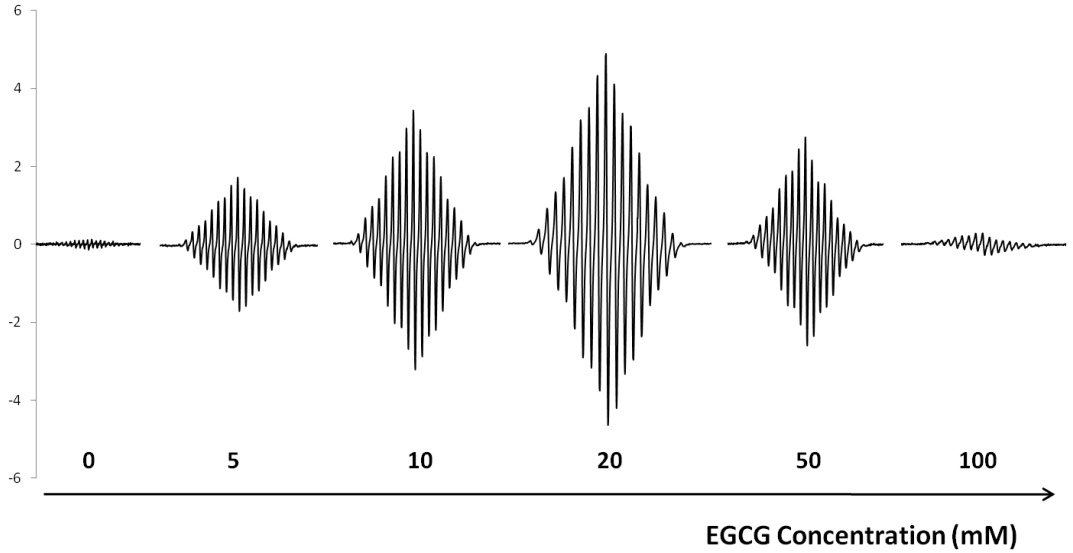

Figure 3-2. EPR spectra showing the effect of EGCG on the radical formation in GO-Ala model. For each concentration, the spectrum was selected for its highest intensity along the time-course of changing.

To investigate the structural role of EGCG that being responsible for this effect discussed above, catechin and 4-methylcatechol were used in the same GO-Ala model (**Figure 3-1b and 3-1c**). The effect of catechin was astonishingly similar to EGCG, but that of 4-methylcatechol was negligible. Inspecting the chemical structure of these three polyphenols: EGCG, catechin, and 4-methylcatechol (**Figure 3-3**), it was not hard to infer that A ring is the active site for this effect on the radical formation. Among those rings that are potentially reactive (A, B, and D rings), only A ring does not change in both EGCG and catechin. B ring is slightly different in these two compounds, and using 4-methylcatchol as an analogue of the catechin B ring eliminates the possibility of the B ring being the active site.

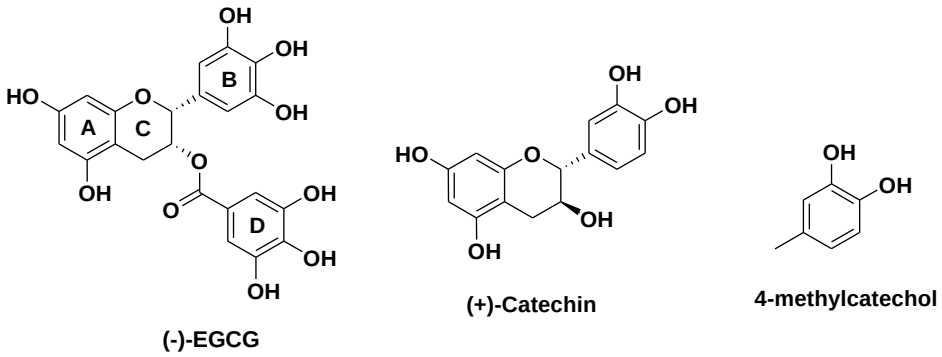

Figure 3-3. Structures of (-)-EGCG, (+)-catechin, and 4-methylcatechol used in this study.

Identification of Key Intermediates Associating with the Mechanism of Enhanced Radical Formation in the GO-Ala-EGCG Model by LC/MS

It intermediately caught our attention with the A ring discovered being the active site for this effect on the radical formation. As introduced above, the A ring of catechins has been reported to trap reactive carbonyls through electrophilic aromatic substitution (**Figure 1-2**). Therefore we were interested to know if there exists any linkage between these two seemingly separate pathways: the carbonyl-trapping by catechins and the pyrazinium radical formation. Or there might exist a similar type of aromatic substitution on the A ring that affect the mechanism of radical formation in the system. To investigate the mechanism of the effect of catechins on radical formation, we studied two GO–Ala–EGCG models (20 and 100 mM of EGCG) using LC/MS. With the concentration of EGCG being at 20 mM, where the maximum enhancement of radical formation was seen, several major molecular ions were observed from the LC/MS chromatogram. The structural information of these major ions was obtained using LC/MS/MS (**Figure 3-4 and 3-5**). On the chromatograms that were obtained from SIM mode of mass scan, multiple peaks were generally seen and the LC/MS/MS spectra of the main peaks were displayed

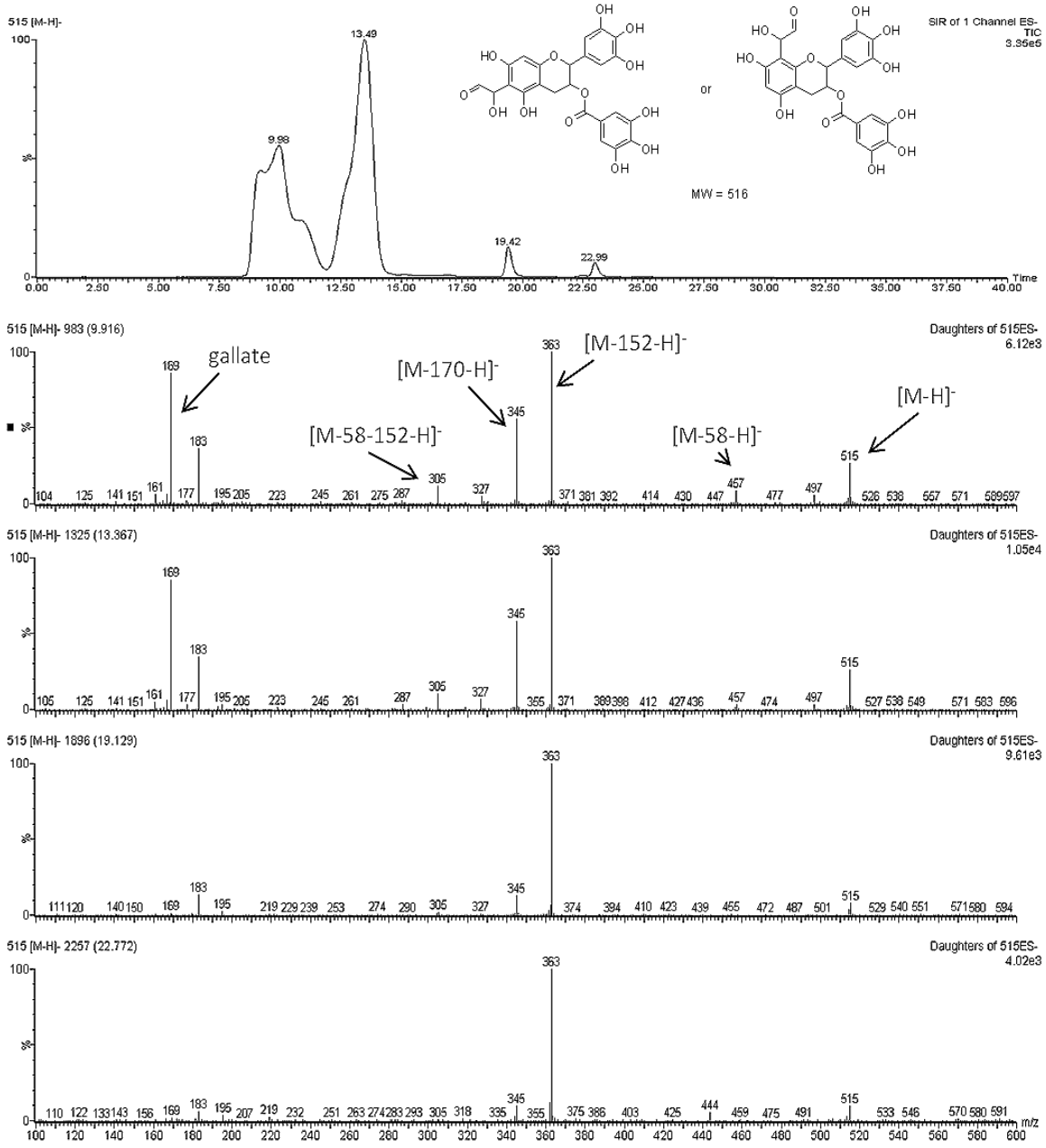

Figure 3-4a. Chromatogram and LC/MS/MS spectra of mono-substituted EGCG–GO adduct generated from GO–Ala–EGCG model.

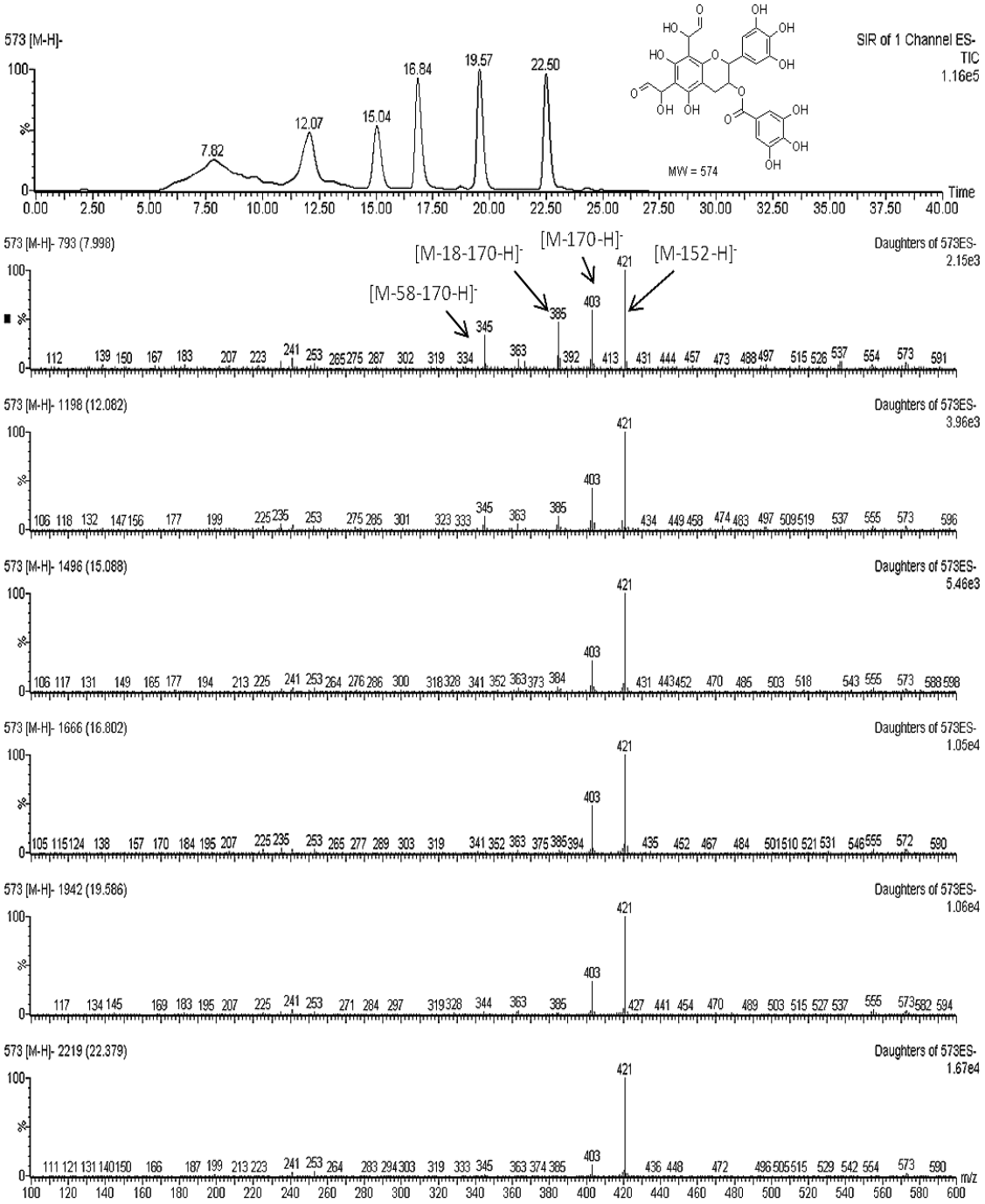

Figure 3-4b. Chromatogram and LC/MS/MS spectra of di-substituted EGCG–GO adduct generated from GO–Ala–EGCG model.

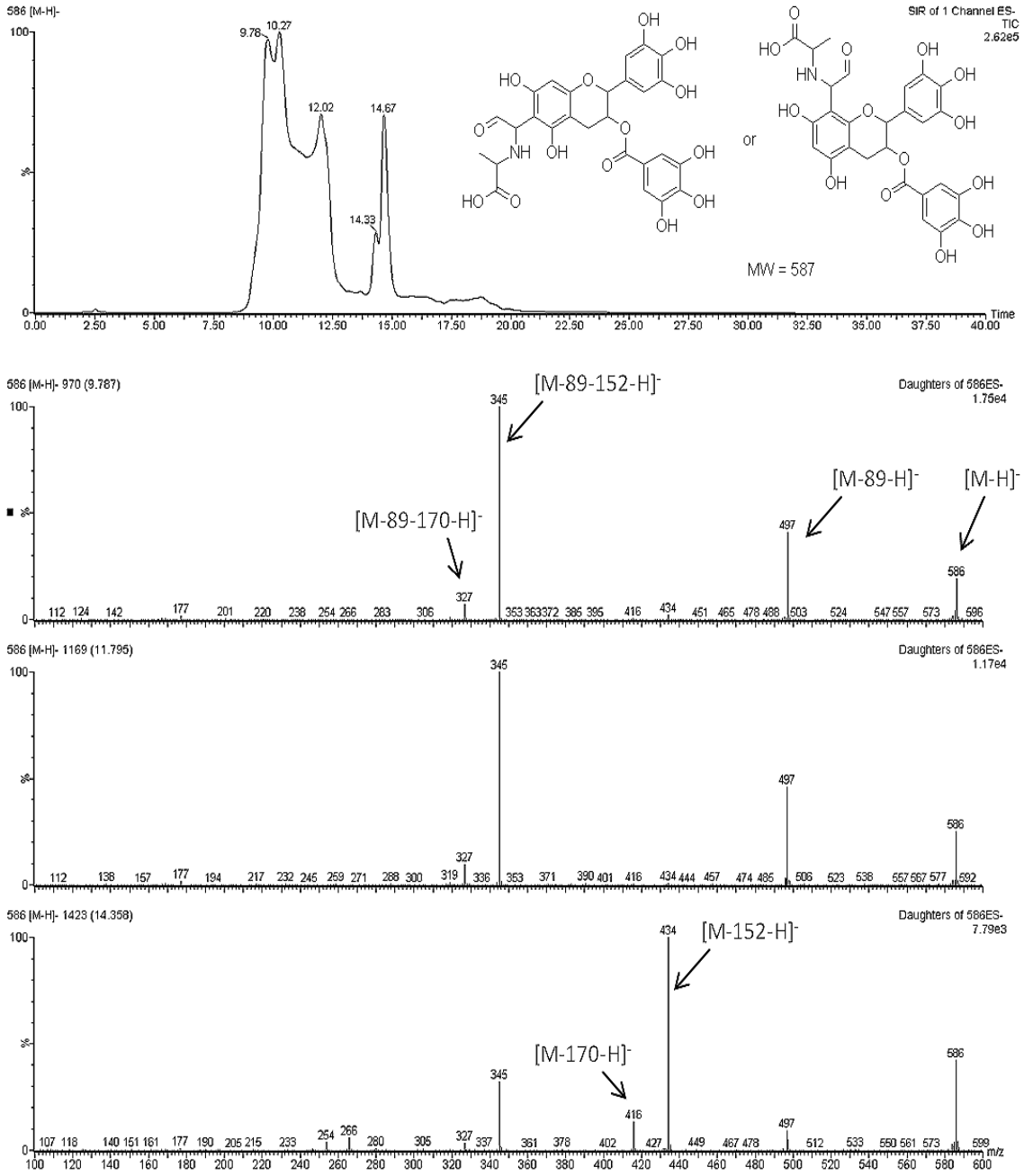

Figure 3-5a. Chromatogram and LC/MS/MS spectra of analyte MW 587 (586 [M-H]$^-$) generated from GO–Ala–EGCG model. The EGCG concentration was at 20 mM.

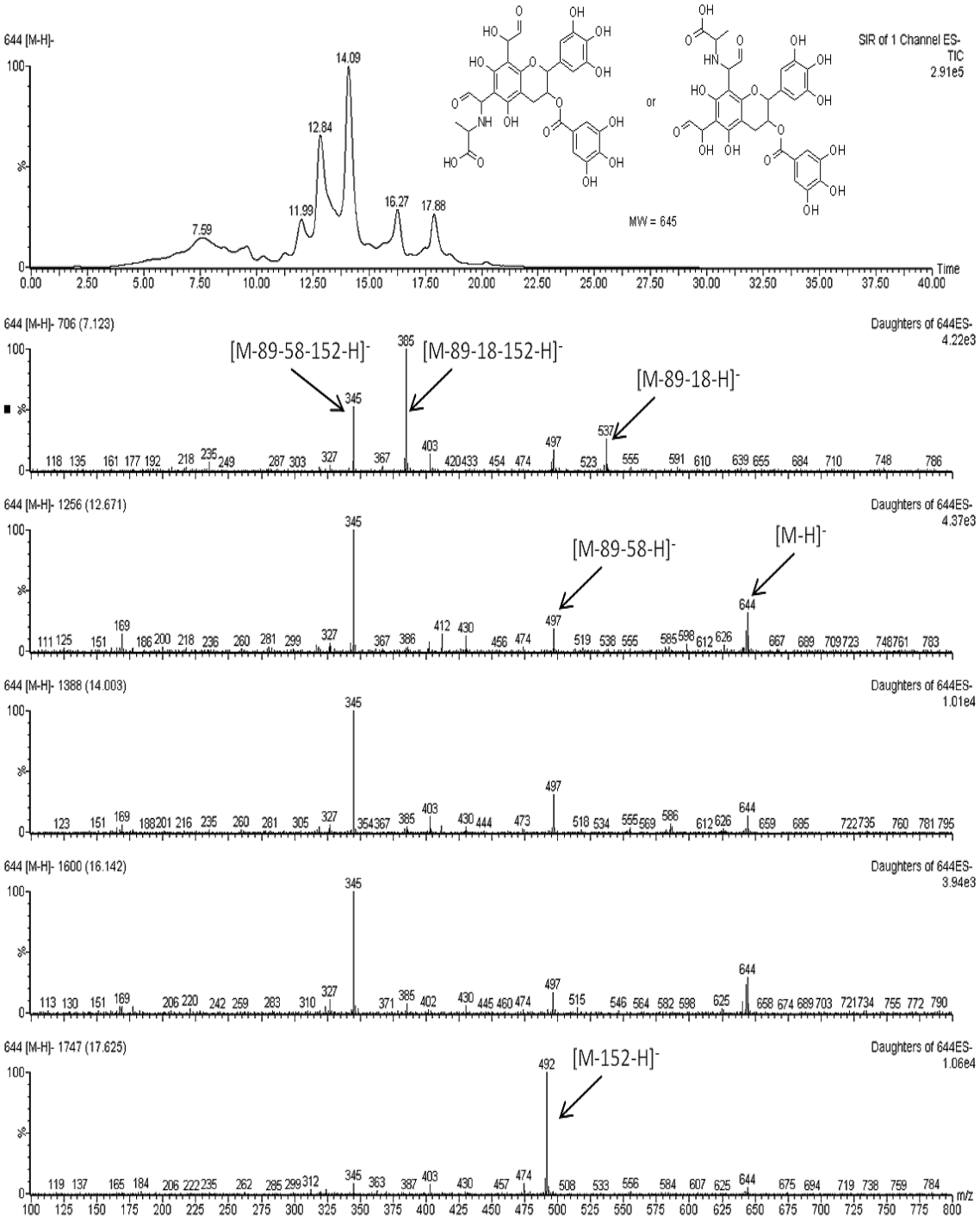

Figure 3-5b. Chromatograms and LC/MS/MS spectra of analyte MW 645(644 $[M-H]^-$) generated from GO–Ala–EGCG model. The EGCG concentration was at 20 mM.

beneath the chromatogram. The molecular ions 515 $[M-H]^-$ and 573 $[M-H]^-$ were mono-GO and di-GO adducts of EGCG (**Figure 3-4**), in agreement with the study reported by Sang et al[5]. In addition, two molecular ions 586 $[M-H]^-$ and 644 $[M-H]^-$ were found with high amount that never been reported before (**Figure 3-5**). For the molecular ion of 586 $[M-H]^-$ (**Figure 3-5a**), fragment 497 $[M-89-H]^-$ was seen in all peaks, indicating the loss of an alanine molecule (MW 89). The loss of galloyl group (MW 152) on the gallate ring, a common fragmentation in EGCG and EGCG adducts, was seen in fragments 434 $[M-152-H]^-$ and 345 $[M-89-152-H]^-$. This MW 587 analyte was identified to be glyoxal imine adduct of EGCG. Its formation involves the prior condensation of GO and Ala, which generated the electrophilic intermediate namely glyoxal imine, and then EGCG underwent electrophilic aromatic substitution on the A ring to trap the glyoxal imine and form the MW 587 adduct (**I**) (**Figure 3-6**). The MW 645 analyte was a di-substituted EGCG adduct, with a GO and a glyoxal imine molecule binding to both 6 and 8 position (**Figure 3-5b**). The loss of 89 (alanine on the bound glyxoal imine), 18 (H_2O on the bound GO), 58 (a GO molecule), and 152 (galloyl group) were observed.

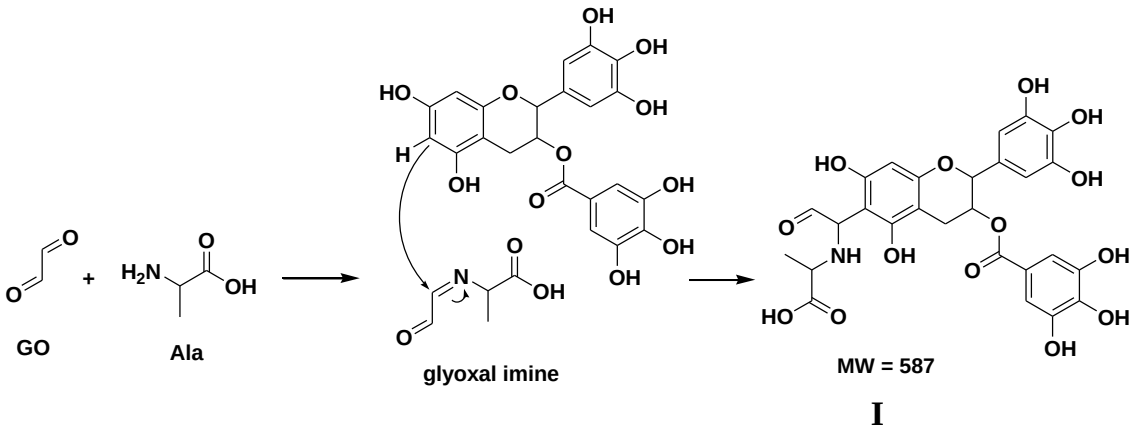

Figure 3-6. Mechanism of formation of analyte MW 587 (**I**) in GO–Ala–EGCG model.

MW 587 adduct (**I)**, as well as MW 645 adduct, was a strong evidence to elucidate the enhancement of pyrazinium radical formation by EGCG in GO-Ala model (**Figure 3-7**). As **I** freely tautomerize to its enaminol form, it is activated to form pyrazinium radical through dimerization and oxidation. We proposed the EGCG finally comes apart from the pyrazine ring of the radical and act as a catalyst to be recycled into this transformation, because the hyperfine structure of the pyrazinium radical from the EPR spectra did not change. We did see the effect of the concentration of EGCG on this mechanism as reported above, as the enhancement of radical formation was increasing with the concentration up to 10% that of GO.

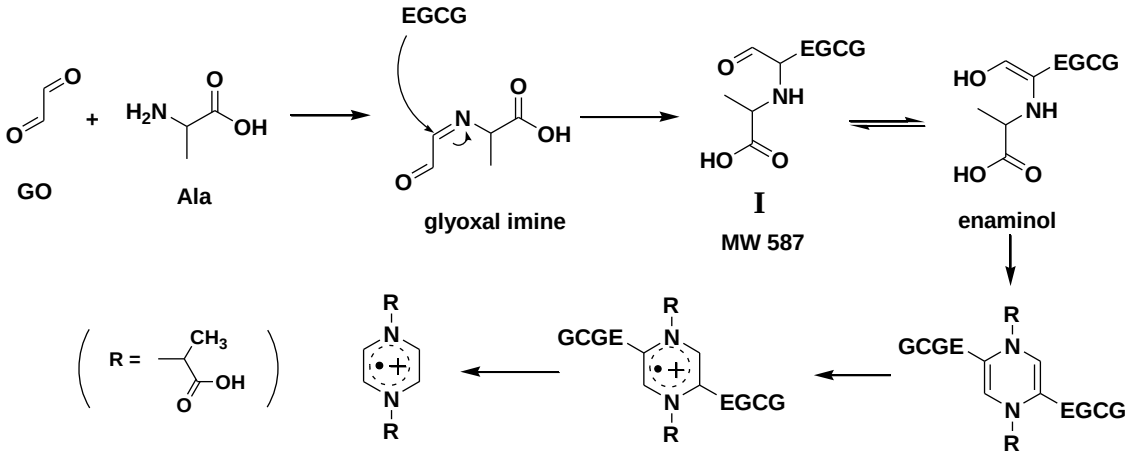

Figure 3-7. Proposed mechanism of the effect of low concentration of EGCG on the pyrazinium radical formation (increasing enhancement) in GO–Ala model.

On the other hand, high EGCG concentration was found to weaken the enhancement of radical formation, as we observed a decrease in radical intensity when the EGCG concentration was climbing up from 10 % to50% of GO concentration. We hypothesized that **I**, radical precursor in this case, can be trapped by another EGCG molecule, especially favorable with the excess of EGCG, and lose its ability to form radical. To investigate this, the GO–Ala model incubated with 100 mM of EGCG was analyzed by LC/MS. We found a comparable amount of molecule ion 1044 $[M-H]^-$, which was not found or found only lightly at lower EGCG

concentrations. LC/MS/MS analysis of this 1044 $[M-H]^-$ ion (**Figure 3-8**) revealed the fragment of 586 $[M-458-H]^-$, indicating a loss of a EGCG molecule (MW 458) from the 1044 $[M-H]^-$ parent ion. Another fragment is 955 $[M-89-H]^-$, indicating a loss of the alanine molecule (MW 89). It was not hard to conclude that this MW 1045 analyte (**II**) was formed from the addition of another EGCG onto the MW 587 adduct (**I**), which confirmed our thoughts. As there is another electrophilic site on **I** available for conjugation, high concentration of EGCG facilitated this reaction. Once bound to another EGCG molecule, **I** lost its structure to freely tautomerize to enaminol, thus lost its ability to form pyrazinium radical (**Figure 3-9**). In other words, high concentration of EGCG quenched some radical precursors by binding to them and depriving their ability to transform to radicals. This mechanism elucidated the effect of high catechin concentration on the radical formation, given that the enhancement of radical formation was weakening with the EGCG concentration exceeding 10% of GO concentration.

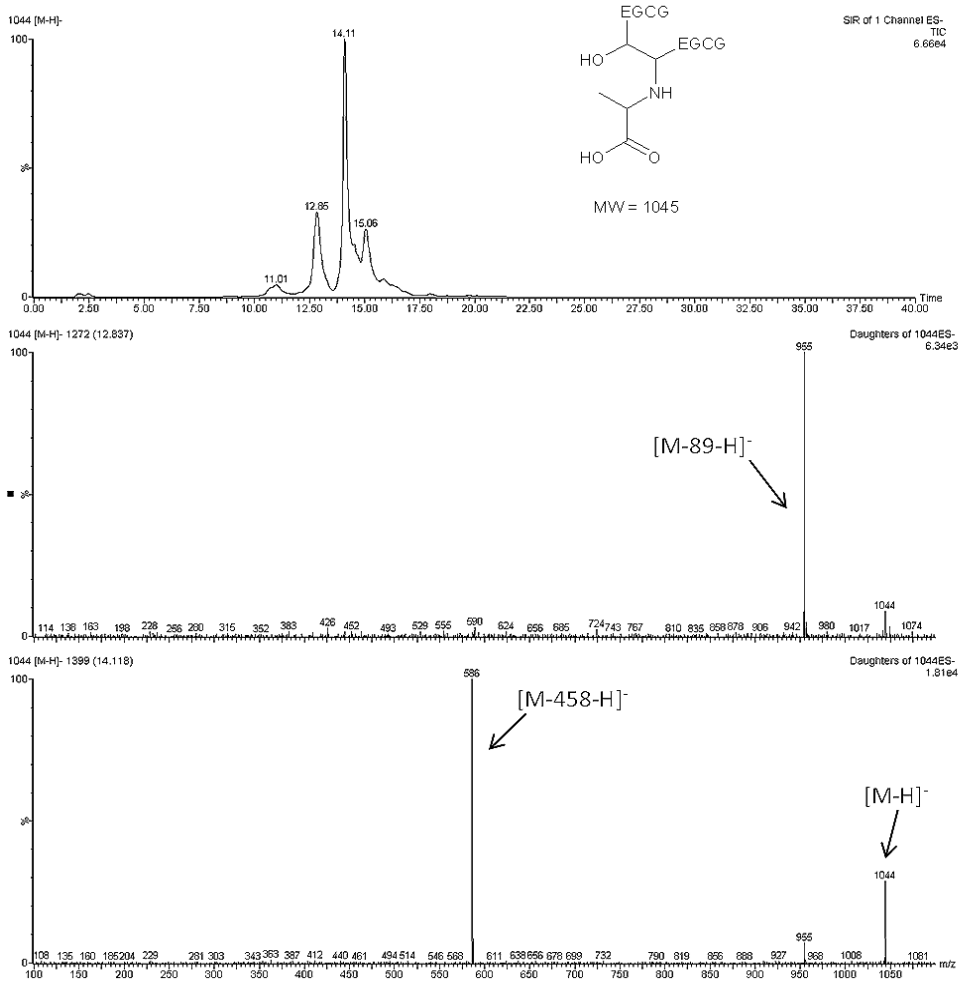

Figure 3-8. Chromatograms and LC/MS/MS spectra of analyte MW 1045 (1044 $[M-H]^-$) generated from GO–Ala–EGCG model. The EGCG concentration was at 100 mM.

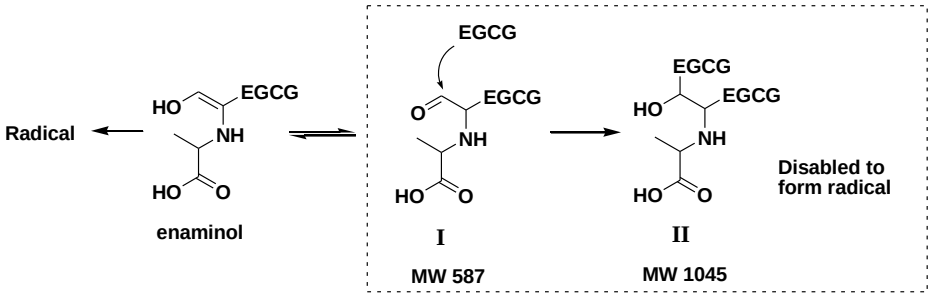

Figure 3-9. Proposed mechanism of the generation of analyte MW 1045 (**II**) and the effect of high concentration of EGCG on the pyrazinium radical formation (weakening enhancement) in GO–Ala model.

Effect of Polyphenol Chemistry on the Pyrazinium Radical Formation in GA-Ala Model

Glycolaldehyde (GA) is the smallest hydroxyl carbonyl possible. GA had expressed significant high activity in generating radicals, as well as in browning. Namiki indicated that the condensation between GA and amino acids generates the direct radical precursor enaminol, which accounts for the strong efficacy of GA in radical formation (**Figure 1-1**)[2].

The effects of EGCG, catechin, and 4-methylcatechol on the radical formation in the GA-Ala model were studied, using the same polyphenol concentration gradient (**Figure 3-10**). Unlike how catechins affected the GO-Ala model, both EGCG and catechin were observed to significantly inhibit the pyrazinium radical formation in the GA-Ala model. 4-methylcatechol, again, did not display comparable effect as these two catechins did on this GA-Ala model.

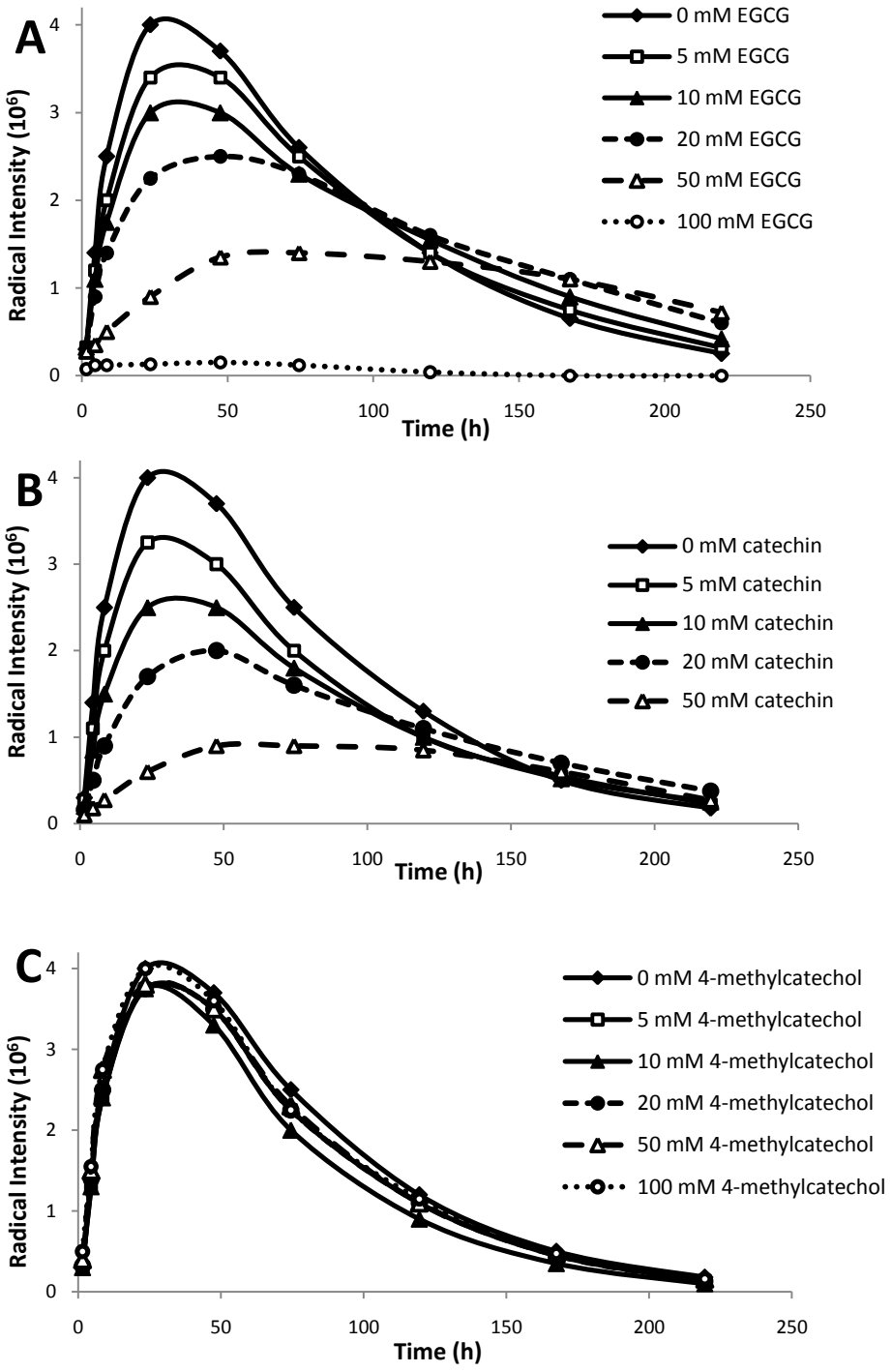

Figure 3-10. Effects of (a) EGCG, (b) catechin, and (c) 4-methylcatechol on the generation and fate of pyrazinium radical in the GA–Ala aqueous model at 25 ℃.

Identification of Key Intermediates Associating with the Mechanism of Inhibited Radical Formation in the GA-Ala-EGCG Model by LC/MS

LC/MS/MS analysis of GA-Ala-EGCG models revealed the similar reactive electrophile trapping by catechins in the GA-Ala. Only two new major molecular ions were found in this model: 588 $[M-H]^-$ and 719 $[M-H]^-$. For the molecular ion of 588 $[M-H]^-$ (**Figure 3-11a)**, it had the fragment 499 $[M-89-H]^-$, indicating the loss of an alanine molecule (MW 89); the fragment 347 $[M-89-152-H]^-$, indicating the loss of both alanine and galloyl group. This MW 589 analyte was identified to be glycolaldehyde imine adduct of EGCG (**III**), structurally similar to the MW 587 analyte (**I**), glyoxal imine adduct of EGCG, found in the GO-Ala-EGCG model. The analyte MW 720 (**Figure 3-11b**) was the di-substituted glycolaldehyde imine adduct of EGCG. The fragment 541 $[M-89-89-H]^-$ indicated the loss of both alanine molecules from the parent 719 $[M-H]^-$ ion.

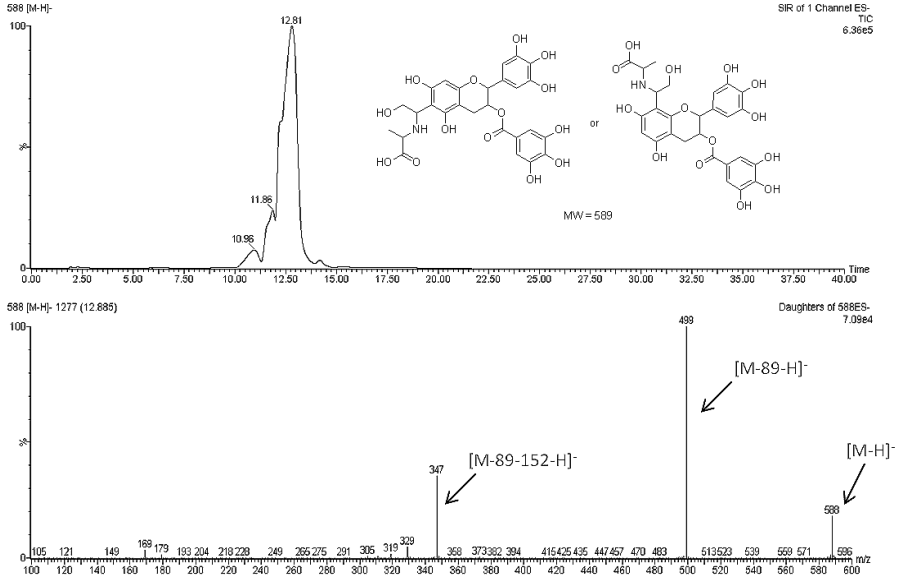

Figure 3-11a. Chromatogram and LC/MS/MS spectrum of analyte MW 589 (588 [M-H]$^-$) generated from GA–Ala–EGCG model.

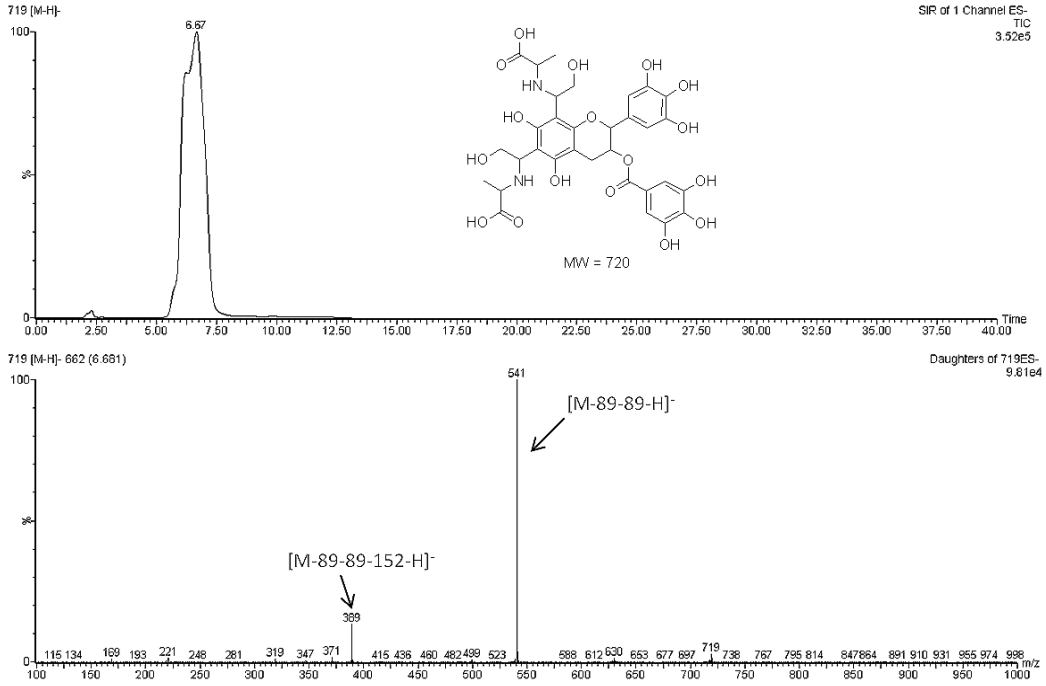

Figure 3-11b. Chromatogram and LC/MS/MS spectrum of analyte MW 720 (719 [M-H]$^-$) generated from GA–Ala–EGCG model.

Likewise, the formation of MW 589 analyte (**III**) involves initial condensation between GA and Ala, which gave the glycolaldehyde imine. Glycolaldehyde imine was trapped by EGCG through electrophilic aromatic substitution on the A ring to form **III** (**Figure 3-12**). Since glycolaldehyde imine, introduced previously, is the direct radical precursor to the pyrazinium radical by freely tautomerizes to enaminol and undergoes dimerization and oxidation, its conjugation with EGCG disabled its ability to form radicals, elucidating the inhibitory effect of catechins on the radical generation in the GA–Ala model. Analyte MW 720 involved the trapping of two glycolaldehyde imine by an EGCG molecule, which further inhibited the radical formation in GA–Ala model.

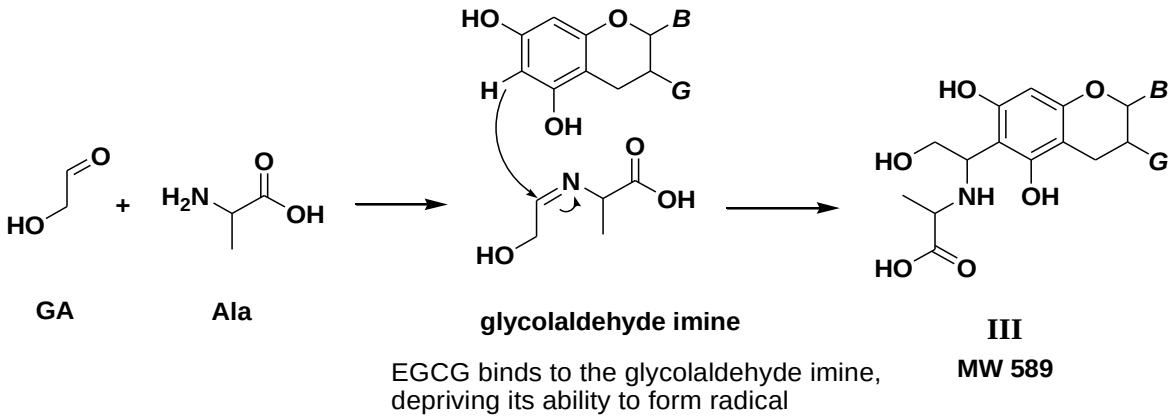

Figure 3-12. Proposed mechanism of generation of analyte MW 589 (**III**) and the inhibitory effect of EGCG on the pyrazinium radical formation in GA–Ala model.

Effect of Polyphenol Chemistry on the Pyrazinium Radical Formation in Glu-Ala Model

D-glucose (Glu) is the one of the most studied sugars in the Maillard reaction. The condensation between glucose and amino acids generates the Schiff base that is thermally unstable and can undergo retro-aldol reaction to give glycolaldehyde imine, where the latter gives pyrazinium radical formation (**Figure 1-1**)[2]. The Schiff base can also undergo oxidative breakdown to glyoxal (GO).

The effects of EGCG, catechin, and 4-methylcatechol on the radical formation in the Glu-Ala model were studied (**Figure 3-13**). EGCG and catechin demonstrated strong inhibitory effect on the radical generation upon heating the mixture, while 4-methylcatechol showed minor effect as well.

Given the findings about the role of catechins in alternating pyrazinium radical generation in GO-Ala and GA-Ala model, the inhibitory effect of EGCG and catechin was attributed to the dominant amount of hydroxycarbonyls generated over dicarbonyls from the condensation between the glucose and alanine. However, the minor effect of 4-methylcathol on the Glu-Ala was not clear as it did not function as trapping reactive imine intermediates, given by its negligible effect on GO-Ala and GA-Ala models. Maillard chemistry is well-known for its complexity of chemical pathways, therefore 4-methylcatechol can affect the radical generation in the Glu-Ala model through other pathways other than trapping of reactive imine intermediates.

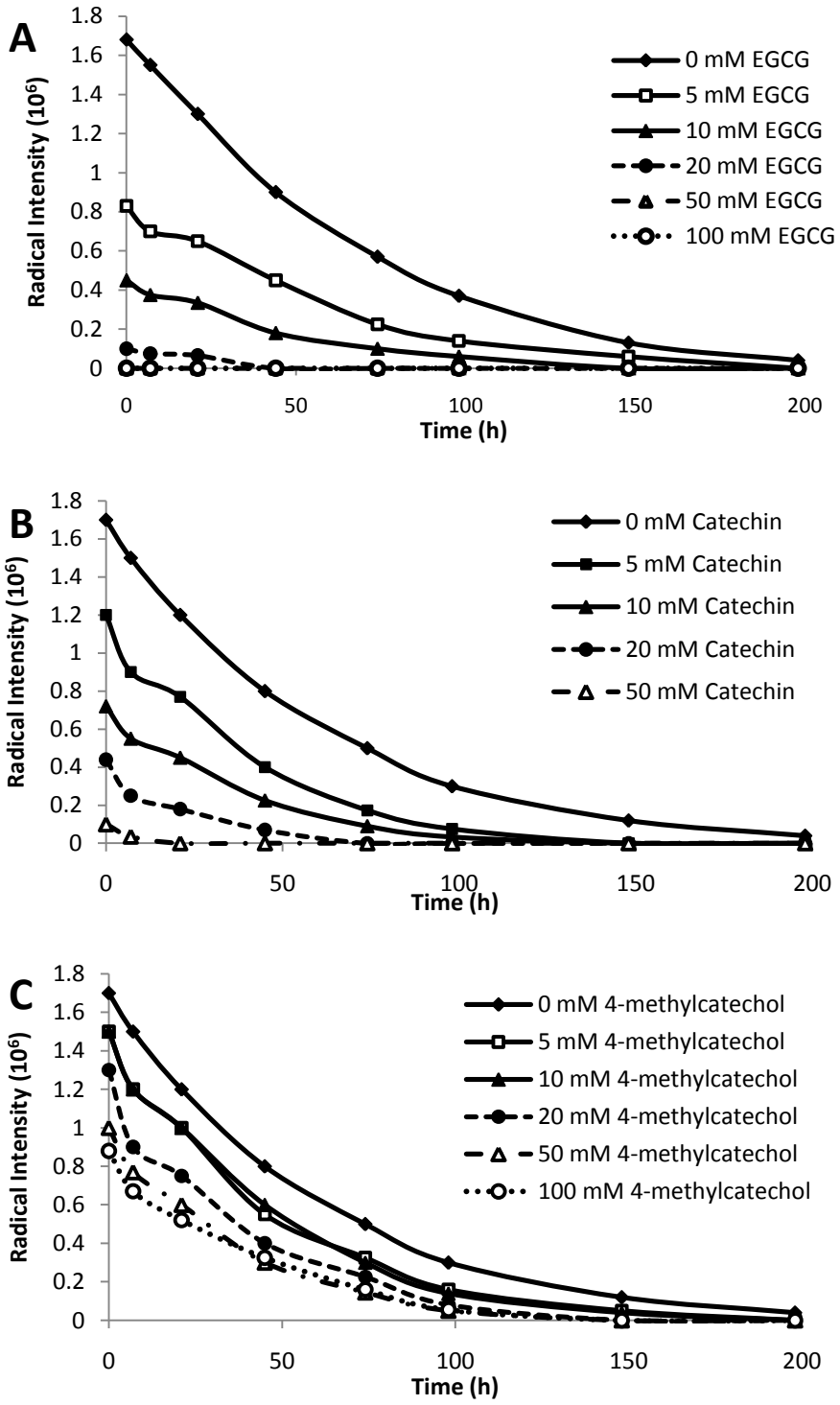

Figure 3-13. Effects of (a) EGCG, (b) catechin, and (c) 4-methylcatechol on the fate of pyrazinium radical in Glu–Ala aqueous model at 25 ℃ after 30min of heating at 100 ℃.

Chapter 4

Conclusions

Catechins were reported to alter pyrazinium radical formation in both GO–Ala and GA–Ala models; the A ring was suggested as the main reactive site. Not only L-alanine, but also glycine was used in this study as another amino acid for comparison, and the same conclusions were drawn. From the EPR analysis, enhancement of pyrazinium radical generation was found in glyoxal-amino acid system, where the maximum enhancement was reached at catechin concentration being 1/10 of glyoxal concentration. Suppression of pyrazinium radical generation was found in glycolaldehyde-amino acid system.

Reactive imine-trapping by catechins was demonstrated by LC/MS analysis to directly control the generation and quenching of radical precursor enaminol through trapping of electrophilic imine intermediates. In the GO-Ala model, low EGCG/catechin concentration facilitated the generation of enaminol through binding to one electrophilic site of the imine intermediate by the catechins; while excess catechins can bind to both electrophilic sites of the imine intermediate, quenching up the enaminol population. In the GA-Ala model, catechins inhibited the generation of enaminol through the same mechanism, depriving its ability to form radicals.

In the Glu-Ala model, catechins demonstrated strong inhibitory effect on the radical generation upon heating the mixture, which was attributed to the dominant amount of hydroxycarbonyls generated over dicarbonyls from the condensation between the glucose and alanine. Minor effect of 4-methylcathol on the radical generation in the Glu-Ala model was found, but was not clear as it did not function as trapping reactive imine intermediates, given by its negligible effect on the GO-Ala and GA-Ala models.

This is the first reported mechanism including the reactive imine-trapping by catechins and its linkage to radical formation in Maillard systems. As pyrazinium radical formation has been strongly linked to the non-enzymatic Maillard browning, the finding provides insights into the coloring in the food/beverage systems.

Chapter 5

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