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Oxidative Stability of (−)-Epigallocatechin Gallate in the Presence of Thiols

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ABSTRACT: Polyphenols are attractive ingredients due to their purported health benefits, but their addition to foods is limited by their chemical instability, as they are rapidly oxidized under many conditions. This oxidation not only compromises the potential biological activity of the phenolic compound, but can also affect the chemical stability of the surrounding food matrix. Polyphenols bearing catechol or gallate groups, when oxidized to their benzoquinone forms, are strong electrophiles capable of reacting with nucleophilic thiols *via* 1,4-Michael addition reactions. These reactions are known to proceed in foods during processing and storage, and can profoundly affect the quality and biological efficacy of polyphenols when they are added as functional food ingredients. The stability of (−)-epigallocatechin gallate (EGCG) in the presence of three thiol-containing species [cysteine (Cys), glutathione (GSH), 3-mercaptohexan-1-ol (3SH)] was followed under both neutral and acidic conditions. Both Cys and GSH increased the rate of EGCG oxidation at pH 4. At pH 7, only Cys was found to increase the rate of EGCG oxidation. On the basis of these results, the reactivity of thiols toward EGCG follows the trend: Cys > GSH > 3SH, which is consistent with observed thiol-quinone adduct formation rates. Contrary to the results observed for Cys and GSH, 3SH was observed to inhibit EGCG oxidation.

KEYWORDS: *polyphenols, oxidation, thiols, quinones*

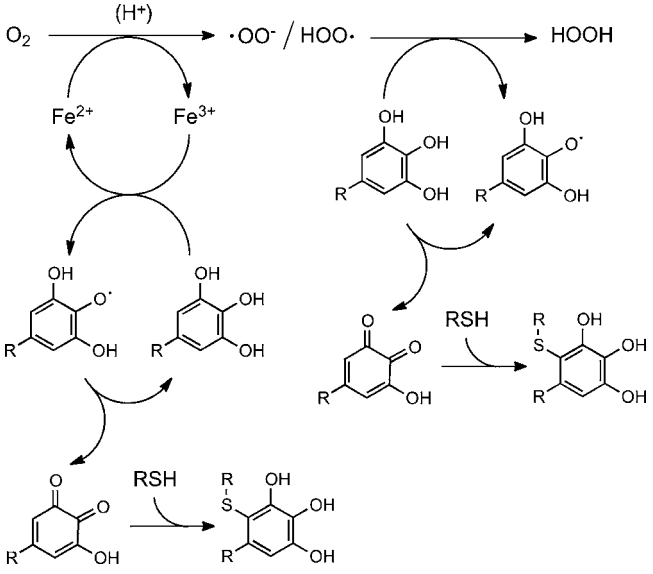
INTRODUCTION

The biological activity of polyphenols has been widely established, and, as such, these compounds have become attractive functional food ingredients. (−)-Epigallocatechin gallate (EGCG), in particular, has been the focus of much attention in recent years, due primarily to its potential pro-health effects, which include antioxidant activity,[1] anticancer,[2] and antiobesity activities.[3] Unfortunately, phenolic compounds, such as EGCG, bearing two or more vicinal hydroxyl groups on their B- or D-rings, are particularly labile to oxidation (Scheme 1), thus complicating their addition to formulated foods. This oxidative stability of EGCG is greatly influenced by the surrounding matrix. For example, the stability of EGCG is well-known to decrease as a function of increasing pH,[4] thus making the incorporation of this compound within low acid foods difficult. The presence of minor and major food components may also affect the stability of phenolics, including trace levels of transitions metals and nucleophilic compounds capable of reacting with phenolic oxidation products.

Polyphenol oxidation is thought to proceed *via* transition metal catalysis (e.g., Fe, Cu).[5] This reaction (Scheme 1) occurs concomitantly with the reduction of oxygen to superoxide, or its protonated form, the hydroperoxyl radical.[6−8] The pK_a of the hydroperoxyl radical is 4.88; therefore, at pH 7, the vast majority (ca. 99.25%) of this species is present in its anionic form (superoxide).[9] Superoxide and hydroperoxyl radicals are eventually reduced further to hydrogen peroxide,[10] which decomposes by the Fenton reaction to hydroxyl radicals that can oxidize virtually all food components at diffusion controlled rates.

The initial oxidation product of EGCG is its semiquinone radical, which is thought to rapidly disproportionate to its electrophilic benzoquinone form (Scheme 1). Hagerman et al. directly observed EGCG and (−)-epigallocatechin (EGC) semiquinone radical formation using electron paramagnetic resonance spectroscopy (EPR), and the corresponding spectra

Scheme 1. Proposed Mechanism of Thiol-Quinone Covalent Adduct Formation



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of the Zn(II)-stabilized semiquinone radicals were mainly assigned to the gallyl radical and the gallyl anion radical, with only ca. 10% of the signal assigned to a radical from the galloyl ester.[11] EPR spectroscopy has been used to demonstrate that the hydroxyl groups of green tea polyphenols undergo "auto-oxidation" to their semiquinone forms under alkaline (pH 13) conditions.[12] Similar reactions have also been shown to occur at physiological pH (7.4).[13] A study with caffeic acid showed that the rate of oxidation increased with increasing pH (range 4 to 8) such that a good correlation was found between pH and phenolate ion concentration.[14] Therefore, it was proposed that the reaction was initiated by single electron transfer from phenolate ions directly to oxygen, producing the semiquinone and superoxide, which then reacted further to yield a quinone and hydrogen peroxide.[14] However, the direct reaction between oxygen (triplet state) and polyphenols (singlet state) is not favorable; therefore nonenyzmatic phenolic oxidation most likely proceeds by a different mechanism. Danilewicz et al. suggested that the increase in the rate of oxidation is due to the much reduced stability of the quinone which, by decomposing with increasing rapidity as pH increases, draws the reaction forward.[4]

Quinones are strong electrophiles capable of reacting with nucleophilic compounds (e.g., thiols) by 1,4-Michael addition reactions. Blanchard et al. demonstrated the Michael-type addition reaction between a catechin quinone and 3-mercapto-1-hexanol (3SH), an important aroma component of wine. 3SH levels were observed to decrease during aging as a result of oxygen addition each time the wine was handled; however, the loss of 3SH did not result from a direct oxidation by oxygen, as the kinetics of its disappearance in oxygenated wine was delayed compared to the kinetics of oxygen consumption.[15] Sang et al. found similar results in mice administered with 200 or 400 mg/kg EGCG *i.p.* Using 2D NMR and MS, the authors showed that EGCG can be oxidized by peroxidase and hydrogen peroxide and then reacted with cysteine or glutathione to form conjugates (two thiol conjugates of EGCG, viz. 2′-cysteinyl EGCG and 2″-cysteinyl EGCG).[16]

The reactivity of quinones and thiols appears to be dependent on the pK_a of the sulfhydryl group, as well as the structural characteristics and accessibility of those groups to quinones. Nikolantonaki et al. showed evidence of several reaction mechanisms in wine between the aroma active thiols 3SH, 2-furanmethanethiol (2FMT), and 4-methyl-4sulfanyl-pentan-2-one (4MSP) and the phenolics (+)-catechin and (−)-epicatechin. The authors demonstrated that iron plays an important catalytic role in these reactions by promoting the formation of quinones, which participate in addition reactions with thiols. The authors showed that 4MSP was relative less reactive toward the quinones compared to 2FMT and 3SH, which may be due to their nucleophilic properties. 2FMT, as a primary thiol, is expected to have the least sterically hindered sulfhydryl group of the three thiols, and thus the greatest reactivity with the quinones.[17] 3SH and 4MSP are secondary and tertiary thiols, respectively, and therefore have increased steric hindrance realtive to 2FMT.[17] A similar finding was reported with coffee thiols, in which the rate constants of thiol loss were observed to increase with increasing nucleophilicity of the thiol.

Contrary to the findings reported above, some studies showed that EGCG was more stable in the presence of some thiols, including *N*-acetyl cysteine and reduced glutathione (GSH), while the opposite was observed in the presence of oxidized glutathione (GSSG).[18] Under wine conditions (pH 3.6), the presence of the nucleophile benzenesulfinic acid was seen to markedly increase 4-methylcatechol oxidation.[19] The authors argued that the benzenesulfinic acid accelerated oxidation of the catechol by reacting rapidly with its oxidation product, the benzoquinone, by a 1,4-Michael-type addition reaction, thus shifting the equilibrium to the right.

In the present study, we show the kinetics of the reactivity of EGCG in the presence of food relevant thiols (Cys, GSH, and 3SH; Figure 1) and oxygen in an aqueous buffered system, both

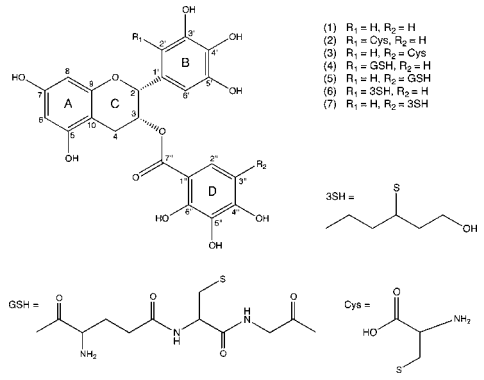

Figure 1. Chemical structures of EGCG (**1**), 2′-cysteinyl EGCG (**2**), 2″-cysteinyl EGCG (**3**), 2′-glutathionyl EGCG (**4**), 2″-glutathionyl EGCG (**5**), 2′-mercapto-1-hexanyl EGCG (**6**), 2″-mercapto-1-hexanyl EGCG (**7**).

under acidic and neutral pH. Cys, GSH, and 3SH represent primary, secondary, and tertiary thiols, respectfully. We also show that the reactivity of thiols toward EGCG depends on pH and the nucleophilicity (related to steric hindrance) of the sulfhydryl groups.

MATERIALS AND METHODS

Materials. Ellman's reagent, 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB; 99% purity), L-(+)-ascorbic acid (≥99% purity) were obtained from Alfa Aesar (Ward Hill, MA). Ethylenediamine-*N*,*N*,′*N*,*N*′-tetraacetic acid (EDTA) (97% purity) was purchased from Sigma (St. Louis, MO). Water used in all experiments and for HPLC-MS was purified through a 0.45 μm Millipore Q-Plus (Millipore Corp., Bedford, MA) purification train. All other chemicals used were of analytical grade or higher.

Quantification of Sulfhydryl Groups. Ellman's reagent (DTNB) was used for the quantitative determination of reactive sulfhydryl groups, according to established methods.[20,21] Briefly, samples (2.0 mL) containing 5−100 μM sulfhydryl were added to DTNB (0.5 mL of 2 mM in 10 mM phosphate, pH 8.1) and Tris-phosphate buffer (0.1 mL of 1 M Tris + 1 M phosphate, pH 8.1). The reaction was allowed to proceed for 30 min at room temperature (∼22 °C).

Kinetic Analysis of Thiol-Quinone Reactions in Model Aqueous Solutions. Solutions of L-cysteine (Cys), glutathione (GSH), and 3-mercaptohexan-2-ol (3SH) were prepared in sodium phosphate buffer (0.1 M; pH 4 or 7) and transferred, in triplicate, to 20 mL rimless Pyrex culture tubes. Reactions were initiated by adding a stock solution of freshly prepared (−)-epigallocatechin-3-gallate (EGCG) at ambient temperature (24 °C) to achieve a final sulfhydryl concentration of 1 mM and 0.4 mM EGCG. Solutions were mixed by vortex and the reactions were allowed to proceed at under air and at room temperature for approximately 120 h in the absence of light. Controls consisted of EGCG, Cys, GSH, or 3SH only, and were prepared and stored under identical conditions. Samples were removed by aliquot 0.5 mL at predetermined time intervals for analysis.

Determination of EGCG Concentration. EGCG oxidation was followed by HPLC analysis. To prevent EGCG oxidation prior to and during analysis, a preservative solution (100 μL) consisting of ascorbic acid (20 wt %) and EDTA (0.1 wt %) in phosphate buffer (0.4 M; pH 3.6) was added to sample aliquots (500 μL) prior to storage at −80 °C.[22] Preservative solutions were prepared daily. Samples was analyzed for monomeric EGCG by HPLC according to a modified method from Hu et al.[23] Chromatographic separation was achieved on a reverse phase Supelcosil LC-18 column (4.6 × 150 mm, 5 μm; Supelco Inc., Bellefonte, PA, USA) using a Shimadzu (Columbia, MD, USA) LC-10ADvp pump with sample introduction by means of a SIL-20ADvp temperature-controlled autosampler set at 4 °C. Samples were filtered over 0.45 μm PTFE filters prior to injection. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). EGCG was eluted by gradient elution according to the following program: 25−45% B in 8.5 min. The injection volume was 20 μL and the flow rate was maintained at 1 mL/min. EGCG was detected at 280 nm using a Shimadzu SPD-M10Avp photodiode array detector. An external standard curve was generated based the area of the chromatographic peak corresponding to EGCG. Quantitation of EGCG in samples was based on this external standard curve.

Determination of Dissolved Oxygen Concentration in Aqueous Solutions. Dissolved oxygen (DO) was measured using a hand-held meter (Orion 4-star, Thermo Fisher Scientific, Beverly, MA) fitted with a rugged dissolved oxygen (RDO) polarographic optical probe (087010MD, Thermo Fisher Scientific, Beverly, MA). The manufacturer specified a lower limit of detection (LOD) of the meter as 0.01 mg O_2/L. Samples were first saturated with atmospheric oxygen by vigorously shaking reaction solutions (1 L) in a closed reagent bottle (5 L) with a large head space. Sample solutions were rapidly transferred to 25 mL amber glass bottles, which were subsequently sealed with threaded stainless steel caps. A hermetic seal was achieved by applying stopcock grease to caps and vial threads. The integrity of the seals was validated by measuring DO in deoxygenated water in capped and greased vials over time. Deoxygenation was achieved by sparging solutions with $N_{2(g)}$ for 5 min. Water samples were observed to maintain their initial DO levels (~0 mg/L) for at least 4 days (data not shown). DO was measured in triplicate samples by quickly removing the vial caps and immersing the meter's probe to ca. 3 mm from the bottom of the bottle, thereby displacing ca.11 mL of the liquid. The meter's reading stabilized within 30 s and remained stable for at least 1 min, indicating that exogenous oxygen was not dissolved in the samples during analysis. Results are plotted as DO concentration (expressed as μM) as a function of time (hours).

Detection and Characterization of Thiol-EGCG Adducts. Oxidation of EGCG during storage and analysis was prevented as described above. Chromatographic separation of thiol-EGCG adducts was achieved on a reverse phase ZORBAX Eclipse Plus C18, narrow-bore column (2.1 × 150 mm, 5 μm; Agilent, Santa Clara, CA). The column temperature was maintained at 30 °C. Samples were filtered over 0.45 μm PTFE syringe filters prior to injection. The HPLC system consisted of a binary pumping system (Shimadzu LC-10ADvp) with high pressure mixing with sample introduction by means of a Shimadzu SIL 10ADvp autosampler. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). Samples were eluted by a gradient according to the following program: 5 - 33% B in 14 min, followed by 33−95% B in 13 min. The injection volume was 15 μL and the flow rate was held at 0.2 mL/min. Mass spectrometry analysis was carried out in negative mode using an electrospray ionization (ESI) interface, and the system was controlled using Masslynx software V4.1 (Waters Laboratory Informatics, Milford, MA). ESI-MS data were recorded using selected ion recording (SIR) mode by monitoring the following: m/z 457 (EGCG), m/z 576.2 (Cys-EGCG), m/z 762.4 (GSH-EGCG), and m/z 589.3 (3SH-EGCG). The MS detector parameters were as follows: ESI-MS, negative polarity; drying gas flow, 650.0 L/min; drying gas temperature, 350 °C; capillary voltage, 3000 V; extractor voltage, 3 V.

Statistical Analysis. All experiments were performed in triplicate and results are reported as mean (M) ± standard deviation (SD). Two-way ANOVA with Bonferroni's post test was performed using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, California, USA). Differences were considered statistically significant when $p < 0.01$.

RESULTS AND DISCUSSION

EGCG Stability in the Presence of Thiols Under Acidic Conditions. Three thiol compounds were assessed for their ability to accelerate EGCG oxidation in aqueous solution (pH 4). The metal-catalyzed oxidation of EGCG is thought to be coupled to the reduction of oxygen to superoxide or its protonated form, the hydroperoxyl radical, which is further reduced to hydrogen peroxide. Therefore, the depletion rate of dissolved oxygen (DO) is related stoichiometrically to the rate of EGCG oxidation.

Cys and GSH were observed to markedly accelerate DO consumption compared to the EGCG-only control at pH 4, whereas 3SH apparently inhibited EGCG oxidation (Figure 2).

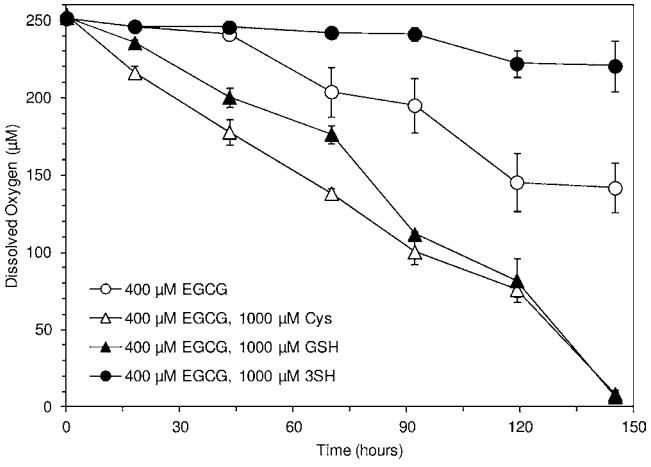

Figure 2. Consumption of dissolved oxygen in aqueous pH 4 buffered system for reactions between EGCG and thiols (Cys, GSH and 3SH) at 24 °C. The control consisted of EGCG only (i.e., no thiol).

The contribution of thiol autoxidation to total oxygen uptake was controlled for by following thiol consumption in solutions containing only the test thiols (i.e., EGCG was excluded). At pH 4, Cys, GSH, and 3SH were oxidatively stable and DO levels were found to be stable within the time frame of the study (data not shown). Therefore, DO consumption was attributed solely to EGCG oxidation at pH 4. The rate of EGCG oxidation was relatively slow under the acidic conditions employed in this study, which is consistent with previous studies.[19] Despite the accelerating effect that Cys and GSH had on EGCG oxidation and, thus, DO consumption, ca. 140 h of storage was required for all of the initial DO (~250 μM) to be depleted. Interestingly, 3SH was observed to inhibit DO consumption compared to the EGCG only control.

EGCG concentrations were followed by HPLC at pH 4 in order to support the results obtained by DO consumption studies. Cys and GSH were again observed to markedly accelerate EGCG oxidation (Figure 3). These results are consistent with previous reports, wherein bisulfite — itself a strong nucleophile — was shown to accelerate the oxidation of (+)-catechin, (−)-epicatechin, gallic acid, caffeic acid and pyrogallol in model wine solution (pH 3.6, 12% ethanol).[24]

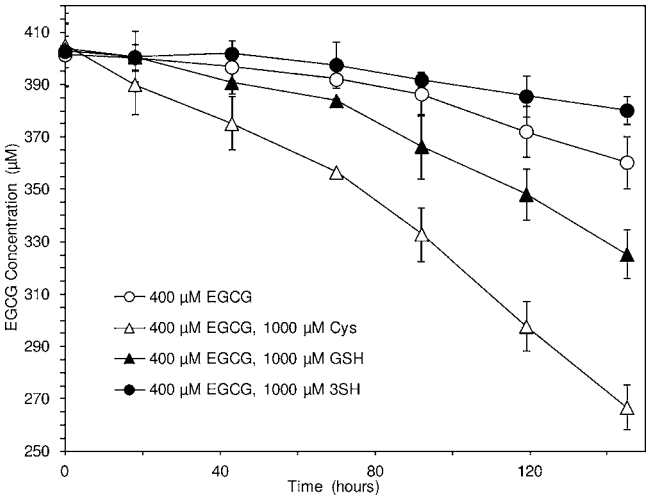

Figure 3. Kinetics of consumption of EGCG in aqueous pH 4 buffered system for reactions between EGCG and thiols (Cys, GSH, 3SH) at 24 °C.

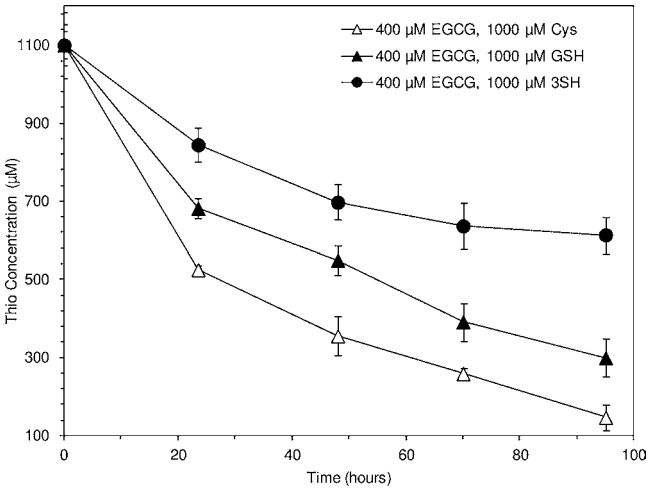

Figure 4. Residual thiol concentration in aqueous pH 4 buffered system containing EGCG and thiols (Cys, GSH, 3SH).

The author argued that bisulfite accelerated phenolic oxidation by rapidly quenching their corresponding quinones, thereby shifting the equilibrium to the product side. It could be argued that a similar acceleration is occurring in the present study, as Cys and GSH quench EGCG quinones.

3SH was not observed to accelerate EGCG oxidation and, paradoxically, appeared to inhibit EGCG oxidation after 150 h. These findings are inconsistent with previous studies by Nikolantonaki et al., who showed that 3SH accelerated (−)-epicatechin oxidation under acidic conditions, although (+)-catechin loss was less affected.[17] The slow oxidation of EGCG in the presence of 3SH could be due to 3SH's sterically hindered sulfhydryl group, which may be relatively less accessible compared to Cys and GSH.[19] 3SH may exert a protective, antioxidant effect with respect to EGCG under these conditions by scavenging reactive oxygen intermediates (e.g., hydroperoxyl radicals) that may promote EGCG oxidation; however, this warrants further study.

The reactivity of the test thiols with EGCG quinones was followed by monitoring the loss of sulfhydryl groups over time. Cys was consumed at the highest rate in the presence of EGCG, with ca. 90% of the thiol lost within 90 h (Figure 4). GSH was the second most reactive thiol tested, followed by 3SH. Control solutions consisting of each thiol without EGCG were analyzed in parallel for free sulfhydryl content in order to account for any thiol losses due to auto-oxidation. Under these acidic conditions (pH 4), all test thiols were oxidatively stable by themselves (data not shown), but were consumed once EGCG was added, presumably as reactants in 1,4-Michael addition reactions.

Finally, thiol-quinone adducts were identified and quantified by LC-MS in order to give direct and unambiguous evidence that EGCG quinones and the test thiols reacted *via* 1,4-Michael addition processes, and demonstrating that the observed loss of EGCG and thiols were not simply due to auto-oxidative reactions. Sample mass spectra for EGCG and its corresponding Cys, GSH, and 3SH covalent adducts are shown in Figure 5. At pH 4, the rate at which the various thiols formed adducts followed the trend: Cys > GSH > 3SH (Figure 6). This is most likely due to differences between the test thiols in terms of nucleophilicity, as was previously documented for coffee thiols.[25]

EGCG Stability in the Presence of Thiols at Neutral pH. The effect that Cys, GSH, and 3SH have on EGCG stability was investigated at pH 7. Under these neutral conditions, the reactivity of the thiols toward EGCG quinones again followed the same trend in decreasing order of reactivity: Cys > GSH > 3SH; only here, the rates of oxygen depletion were considerably higher than those observed at pH 4 (Figure 7). Furthermore, EGCG control solutions were found to consume DO at a higher rate than GSH and 3SH treatments, with only the Cys treatment accelerating EGCG oxidation. The pK_a of a typical thiol group is roughly 8.3; therefore, the fraction of thiols in their anionic thiolate forms is naturally expected to be greater at pH 7 (4.77% thiolate) vs pH 4 (0.005% thiolate), which are expected to be more reactive with quinones compared to their protonated forms.[26,27]

Under these conditions, nearly all (ca. 400 μM) of the EGCG was lost after 47 h at 24 °C for the EGCG control, Cys + EGCG and GSH + EGCG treatments (Figure 8). The rate of EGCG oxidation followed the following trend in increasing order of stability: EGCG < Cys + EGCG < GSH + EGCG < 3SH + EGCG. As expected, and as was also observed in DO consumption studies, EGCG oxidation rates were markedly higher under neutral pH conditions. It has been well-established that the oxidative stability of polyphenols decreases as a function of increasing pH. Classically, this has been attributed to the fact that the anionic forms of phenols (i.e., phenolate ions) are more reactive toward oxygen; however, an alternative explanation is that the stability of quinones decreases as a function of increasing pH. For example, the estimated half-life of 1,2-benzoquinone was 30 min, 8 min and 9 s at pH 2.7, 4.6, and 7.6 respectively.[4] Recently, Danilewicz[4] proposed that the increase in rate of oxidation of polyphenols with increasing pH is due to the increasing instability of the quinones, the loss of which displaces the redox equilibrium. It was argued that the rate of catechin oxidation at pH 6.97 was extremely high relative to the reaction between nucleophiles (e.g., benzenesulfonic acid) and the catechin benzoquinone.

At pH 7, nearly all Cys is lost to the thiol-quinone reaction within the first 14 h of incubation (Figure 9a). The rates of thiol depletion were observed to be higher at pH 7 than at pH 4, and the rate of thiol loss appeared to be higher during the

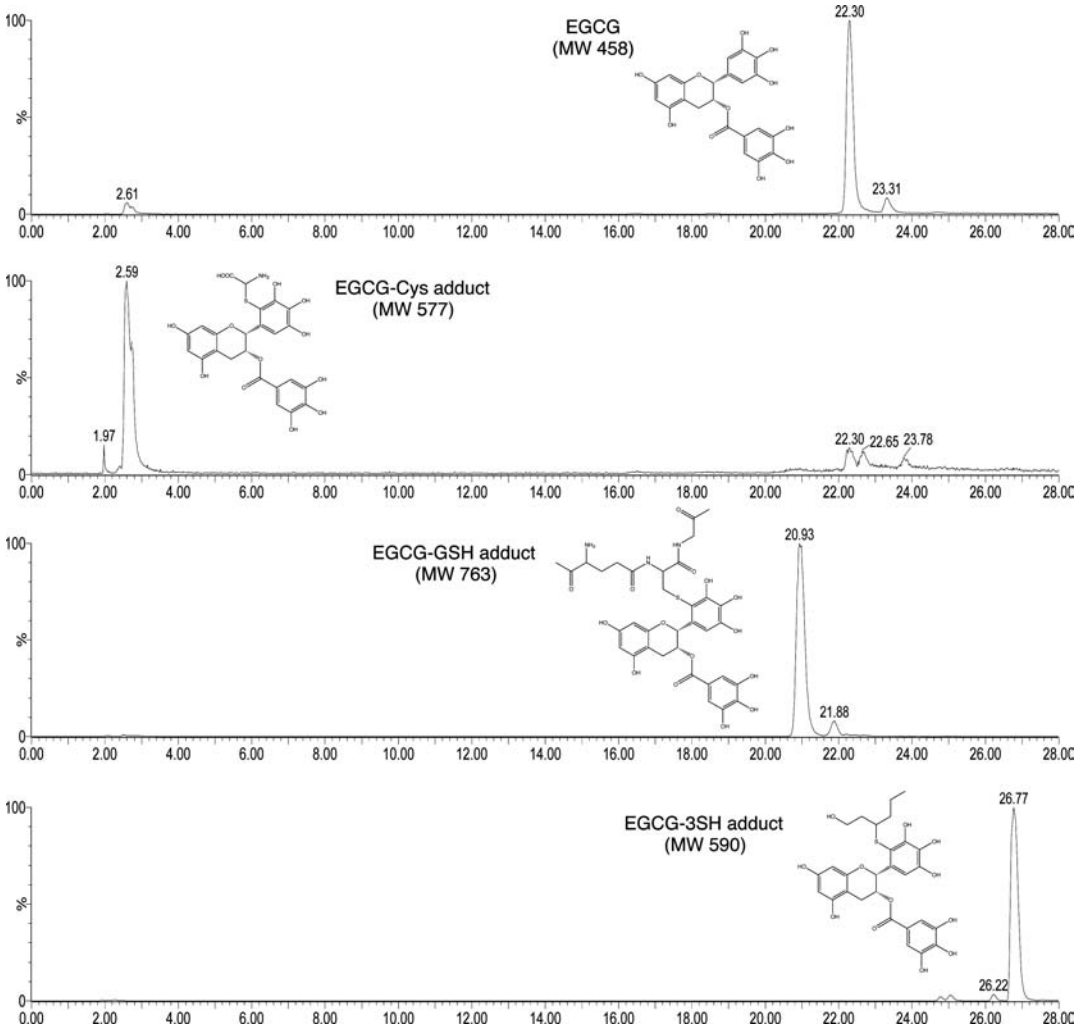

Figure 5. Sample SIR mass spectra showing corresponding peaks for EGCG-thiol adducts.

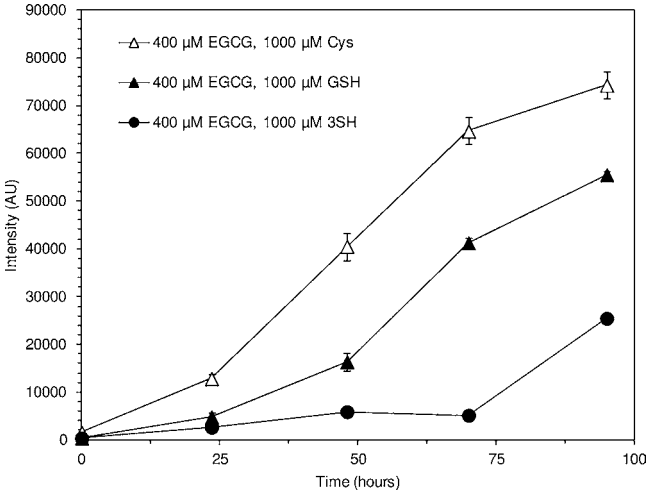

Figure 6. Thiol-EGCG adduct formation in aqueous pH 4 buffered system containing EGCG and thiols (Cys, GSH, 3SH).

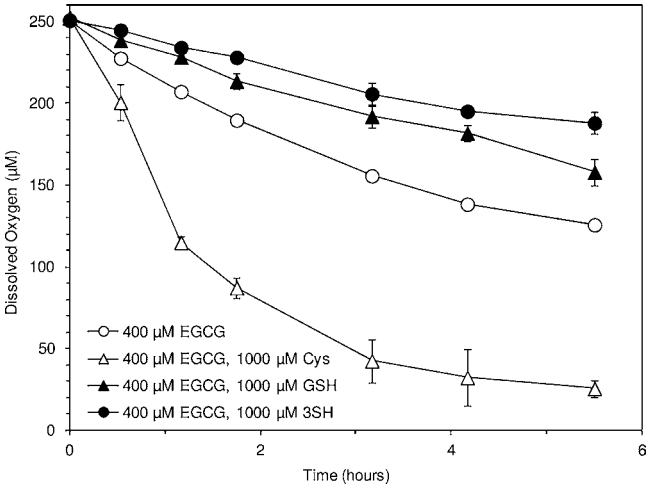

Figure 7. Consumption of dissolved oxygen in aqueous pH 7 buffered system for reactions between EGCG and thiols (Cys, GSH and 3SH) at 24 °C. A control, with only EGCG, was used without any thiol reactant.

initial phase of the reaction (1−21 h) at pH 7. Considerable amounts of GSH (ca. 90%) and 3SH (ca. 50%) were lost over a period of 90 h of storage. The initial rapid decrease in thiol concentration in the presence of EGCG, followed by a slow decrease, is not consistent with our results based on EGCG analysis and DO consumption. Upon studying the rate of thiol losses due to autoxidation (solutions containing only thiols without any added EGCG), 3SH was found to be stable by

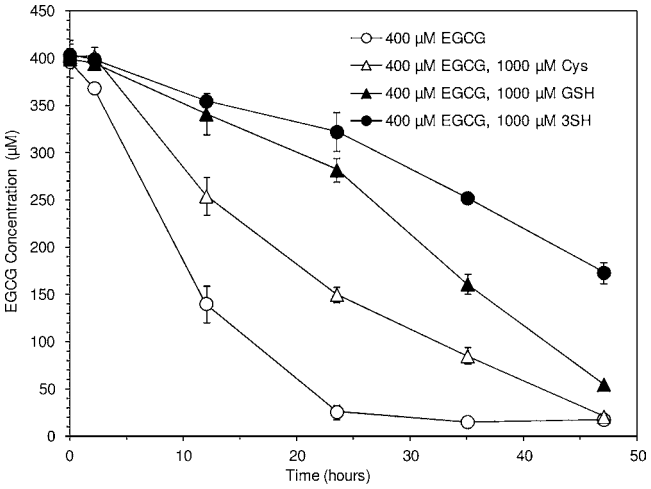

Figure 8. Kinetics of consumption of EGCG in aqueous pH 7 buffered system for reactions between EGCG and thiols (Cys, GSH, 3SH) at 24 °C.

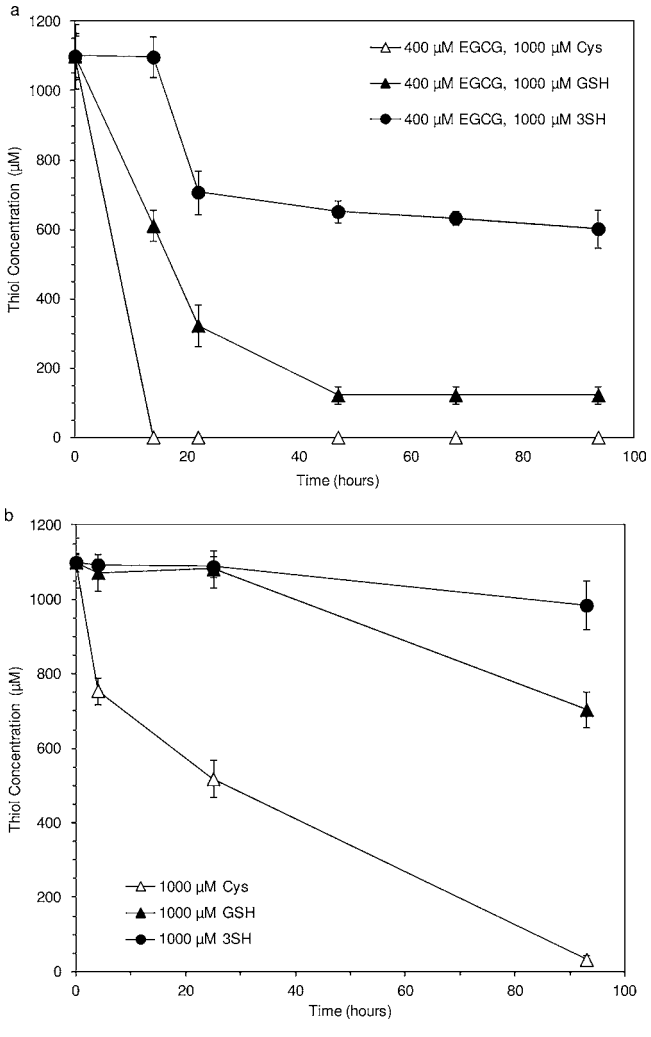

Figure 9. (a) Residual thiol concentration in aqueous pH 7 buffered system containing EGCG and thiols (Cys, GSH, 3SH). (b) Residual thiol concentration in aqueous pH 7 buffered system containing thiols only (Cys, GSH, 3SH).

itself throughout the study; however, only ca. 40% GSH remained, followed by Cys, which was almost completely consumed at the end of 90 h (Figure 9b).

For the Cys treatment, the thiol losses can be attributed both to the reaction with EGCG and to autoxidation, where ca. 50% of thiol losses were attributed to auto-oxidation at 21 h at pH 7. In the case of GSH, 50% of the thiol losses can be attributed to autoxidation. For the 3SH containing treatment, thiol consumption can be attributed entirely to the reaction with EGCG, since depletion of free sulfhydryl levels of 3SH in the absence of EGCG was insignificant compared to when it was present with EGCG.

The rate of formation of adducts was observed to increase over the course of the experiment at pH 7 (Figure 10), which is

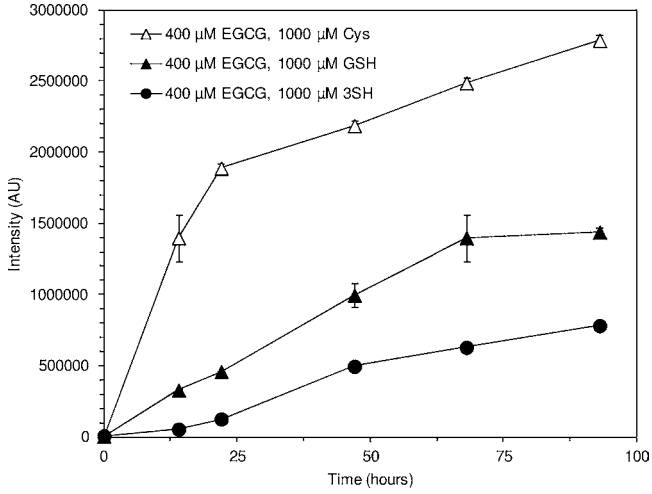

Figure 10. Thiol-EGCG adduct formation in aqueous pH 7 buffered system containing EGCG and thiols (Cys, GSH, 3SH).

consistent with previous reports. Cao et al. characterized the covalent interactions between EGCG and model peptides containing one or more nucleophilic residues (i.e., Arg, Cys, Met, and α -NH_2 of the N-terminus of peptides) under physiological conditions by MS. It was found that EGCG reacts with the thiol groups of peptides to form adducts under physiological conditions (pH 7.4, 37 °C), even in the absence of a peroxidase/hydrogen peroxide system. Besides the thiol groups of peptides, EGCG also reacted with the α-NH_2 of the N-terminus or arginine residues of model peptides to form Schiff base adducts, and the methionine residues of model peptides were easily oxidized by hydrogen peroxide (H_2O_2) generated during the process of EGCG auto-oxidation to form methionine sulfoxide. The preference for the reaction of nucleophlic residues of peptides with EGCG was determined to have the following order: Cys > α-NH_2 of the N-terminus > Arg.[28] Moreover, adducts can be formed at the B- or the D-ring of the EGCG quinone. Adducts formed at both these sites were also reported by Sang et al. for both the GSH and Cys thiols. Two thiol-conjugates of EGCG were identified using 2D NMR and ESI-LC-MS/MS analysis of urine samples of mice fed with EGCG as 2′-cysteinyl EGCG and 2″-cysteinyl EGCG, with similar product formed for GSH.[16] Previous studies by Jongberg et al. had investigated the formation of covalent EGCG adducts of thiols such as cysteine, GSH, bovine serum albumin and myofibrillar protein isolate. The authors found the thiol-quinone adduct to be present in all thiol systems.[29] However, no studies had yet been performed that report the

rate of formation of these thiol-adducts under food conditions. Awad et al. showed that Cys is more reactive with quinones than GSH and *N*-acetylcysteine.[30] A study by Mori et al. further showed that EGCG was relatively stable in the presence of GSH, but unstable with GSSG (the oxidized disulfide form of GSH), as measured by loss of EGCG concentration. The authors also observed the formation of Cys adducts of EGCG and other pyrogallol-type catechins in neutral buffer.

The chemistry of thiol-quinone reactions was studied in the presence of oxygen under acid and neutral pH aqueous systems. The rates of these thiol-quinone reactions were significantly higher at pH 7 compared to pH 4. At pH 4, both Cys and GSH accelerated the oxygen and EGCG consumption, unlike the case at pH 7, where only Cys accelerated DO consumption in presence of EGCG. At pH 4, all of the observed thiol consumption was attributed to the thiol-quinone reaction and none to thiol autoxidation. At pH 7, about 50% of thiol consumption for Cys and GSH containing treatments was attributed to their autoxidation; however, 3SH was consumed only by the thiol-quinone reaction pathway. At both pH 4 and 7, 3SH either inhibited or failed to accelerate the oxidation of EGCG and oxygen consumption, which could possibly be attributed to its hydroperoxyl/superoxide radical scavenging activity. These results are relevant to food systems containing both thiols and oxidatively labile phenolics, and further studies are needed to investigate this chemistry in more complex systems (e.g., emulsions).

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Notes

The authors declare no competing financial interest.

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