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Kinetic Study of Catechin Stability: Effects of pH, Concentration, and Temperature

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Supporting Information

ABSTRACT: The degradation behaviors of catechins in dilute aqueous systems, including tea beverages and catechin solutions, have been documented; however, their reaction kinetics in green tea concentrated solutions, and impacts of pH, concentration, and temperature thereon, have not yet been established. In this study, reactions were conducted at pH levels ranging from 1.5 to 7, concentrations ranging from 1 to 1666.7 mg/mL, and temperatures ranging from 25 to 120 °C. Catechin contents were determined using high-performance liquid chromatography. Catechins were found to be more stable at high concentrations around pH 4. An empirical model for catechin content was established as a function of pH and temperature and showed good correlation between green tea concentrated solutions and previous reports of catechin stability in powder systems. These results provide useful approaches for shelf life calculations and catechin loss predictions at given temperature and pH conditions in green tea concentrates.

KEYWORDS: *EGCG, stability, pH, kinetic model, shelf life, tea*

INTRODUCTION

Tea, derived from the plant *Camellia sinensis* (L.) O. Kuntze, is one of the most widely consumed beverages in the world today, second only to water. The green tea catechins, primarily (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC), have been associated with a series of health benefits and as a result are increasingly attracting attention from industry and consumer groups. Uses of tea concentrates in the beverage industry are increasing due to their ease of reconstitution in the manufacturing and retail sales environments. However, tea catechins are susceptible to degradation with important rate-determining parameters, including temperature, pH, and moisture.[1−5] In addition to catechin loss, degradation is often associated with generation of negative sensory attributes such as off color, limiting the application of green tea catechins in beverage products, fortified foods, dietary supplements, and pharmaceutical products. Therefore, the ability to understand the behavior of catechin degradation in different systems under environmental stress and to preserve the stability of green tea catechins has become very important.

In food powders and concentrates, different ingredients which are present in the product may significantly affect the pH of the system.[6] Previous studies have shown that catechin stability is pH dependent in dilute solutions, and kinetic models for such systems have been established;[1,4,7−10] however, the effect of pH on the kinetics of catechin loss in concentrated solutions and powder systems remains, as of yet, unreported. Mathematical models developed from dilute solutions and beverage systems might not be applicable to concentrates and powders due to the increased levels of concentrations and altered molecular mobility. Ortiz et al.[3] discussed the impact of different formulations on the stability of catechins in powder systems exposed to varying levels of environmental relative humidity. However, one must consider the fact that reactions might be affected not only by the pH of the system, which is altered by different formulations, but also by the synergistic and/or pro-antioxidant activities of selected ingredients.

This study aims to investigate the impact of pH and temperature on the kinetics of catechin loss in a variety of concentrated and solution systems. By comparing these results with those derived from previous studies of powder systems,[5] this study will provide a practical approach to simulate the microenvironments of powder and solution systems along with stability data such that the shelf life calculations and catechin degradation predictions at different temperature and pH conditions cover a range of catechin concentrations relevant to a variety of food products.

MATERIALS AND METHODS

Materials. The green tea powder (Nestlé Choladi TCTG GT) was a tea extract prepared from fresh tea leaves (*C. sinensis* (L.) O. Kuntze, Theaceae) provided as a generous gift from the Nestlé Research and Development Center (Marysville, OH). Catechin standards, including (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG), (−)-catechin gallate (CG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), and caffeine, were all purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Methanol (HPLC grade), acetonitrile (HPLC grade), hydrochloric acid (HCl), and glacial acetic acid were purchased from Mallinckrodt Baker (Phillipsburg, NJ). pH conditions were adjusted using 1 mol/L sodium hydroxide (NaOH, purchased from Sigma-Aldrich) and 2

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mol/L HCl solutions. All other chemicals were purchased from Sigma-Aldrich. High-temperature heat treatments (100 and 120 °C) were performed using a digital heat block (VWR International LLC, Bristol, CT). Other storage experiments were conducted using Forma Scientific model 3187 temperature-controlled incubators (Marietta, OH). The temperature and water activity conditions were verified by thermometers and an AquaLab 3TE water activity meter (Decagon Devices Inc., Pullman, WA).

Moisture Sorption. Two approaches were adopted to determine the moisture sorption profiles of the green tea (GT) powder. First, sorption isotherms from 0% to 95% RH with intervals of 5% RH, an equilibrium criterion 0.001% (w/w) change in 5 min, and a maximum interval time of 720 min were generated for 10 mg samples using an SGA-100 symmetrical gravimetric analyzer (VTI Corp., Hialeah, FL) at 25 °C.[5] Moisture sorption isotherms were also prepared by adding different amounts of water (10−100%, w/w) to green tea powders in water activity cups (Decagon Devices Inc., Pullman, WA) and measuring their water activities (a_w) using the AquaLab 3TE water activity meter after a 24 h equilibration. The moisture content was calculated by adding the amount of water introduced to the system and the initial water content in the green tea powder determined by an oven-drying method (100 °C for 4 h). The moisture sorption isotherm was obtained by plotting the moisture content versus water activity. Consistent results were obtained by both approaches (data not shown). By adding 300 μL of water into 0.5 g of green tea powder, the resulting a_w was 0.95. This was chosen to represent a green tea concentrated solution system and to simulate green tea powder systems stored at the corresponding RH condition (95%) and could also be representative of a plasticized powder green tea matrix above its glass transition temperature, T_g.[5]

pH, Temperature, and Storage Treatments of Tea Concentrates. To investigate the effects of pH on catechin stability, green tea concentrate samples at a constant concentration were used. Green tea concentrates were prepared using 300 μL of water and 0.5 g of tea (this concentration is referred to as 1666.7 mg/mL). A pH meter (IQ Scientific Instrument Inc., San Diego, CA) calibrated from pH 1 to pH 10 was used to measure the pH. The pH of the natural green tea concentrate was measured to be 5.2. The pH of the green tea concentrate systems was adjusted using a fixed amount (300 μL) of NaOH−HCl solutions with different ratios of NaOH to HCl to achieve the following final pH values as shown in the Supporting Information: 1.5, 2, 3, 4, 6, and 7. Samples of 0.5 g of green tea powder were prepared in 20 mL glass vials (VWR International LLC, West Chester, PA), and 300 μL of liquid was added. The samples were then sealed and equilibrated for 24 h prior to storage treatments. All samples with pH values of 1.5−7 (1.5, 2, 3, 4, 5.2, 6, and 7) were stored for up to 35 days at different temperatures (25, 40, 60, 100, and 120 °C). All samples were prepared in triplicate, and at least six time points were chosen to allow sufficient reflection of the reaction kinetics. These pH and temperature parameters were chosen on the basis of common processing conditions in low-acid foods and tea beverage production, as well as human gastrointestinal pH values.[3] After storage treatment, all samples were immediately frozen at −20 °C.

Concentration Treatments. To investigate the impact of the water content on catechin stability, a series of green tea solutions with different concentrations (1, 5, 10, 100, 500, and 1666.7 mg/mL) were prepared and then treated at 80 °C for up to 33 h. These samples were then immediately cooled using an ice−water bath and then diluted for HPLC analysis.

Chemical Stability Determination with HPLC. Prior to analysis, all samples were diluted with 2% acetic acid to 1 mg/mL (GT/H_2O) and then filtered with 0.45 μm membrane syringe filters. HPLC analysis was then performed using a method described previously.[5] Briefly, a Waters 2690 separation module equipped with a Waters 996 photodiode array detector and Masslynx V4.1 software (Waters Corp., Milford, MA) was used. The separation column used was a 100 mm × 3.9 mm i.d., 3.5 μm Waters XTerra RP-18 column. A gradient method was employed using mobile phase A consisting of distilled H_2O, acetonitrile, and trifluoroacetic acid (919/80/1, v/v) and phase B consisting of distilled H_2O, acetonitrile, methanol, and trifluoroacetic acid (699/270/30/1, v/v). An initial ratio of 95/5 (A/B) was used. Subsequently, the solvent composition was changed to 30/70 (10 min, convex) and 1/99 (13 min, convex). Finally, the solvent composition was returned to 95/5 (14−18 min, step, immediate). The flow rate was 0.9 mL/min, and the injection volume was 10 μL. Peak integrations were conducted at 280 nm, except for EGC at 210 nm to obtain better resolution. Catechins and caffeine were verified by the elution time of standards and quantitated by the corresponding standard curves (R^2 = 0.9997−1.0000). The initial concentrations of *cis*-configured catechins were set as 100%, and those of *trans*-configured catechins were set as the percentage relative to the concentrations of the *cis*-configured catechins.

Reaction Kinetics. To establish treatment effects on the kinetics of catechin loss, first-order models were applied to the data collected on the concentration of catechins that remained in the solutions over time, and the Arrhenius equation was used to evaluate the temperature dependence of the reaction rate constants. Our previous work revealed that catechin degradation followed apparent first-order kinetics in green tea powder systems.[5] Thus, catechin contents can be described by

$$\ln \frac{x}{x_0} = -kt \tag{1}$$

where x is the concentration of catechin at time t (min), x_0 is the initial concentration, and k is the reaction rate constant (min^{-1}).

The temperature dependence of the rate constant k can be described using the Arrhenius equation:[5]

$$k = Ae^{-E_a/RT} \tag{2}$$

where k is the rate constant (min^{-1}), A is the frequency factor of collision, E_a is the activation energy (kJ/mol), R is the gas constant (8.3145 J/(mol·K)), and T is the temperature (K).

Statistical Analysis. A completely randomized two-factor factorial design was used to investigate simultaneously the impact of pH and temperature on catechin stability. All data were presented in the form of mean ± standard deviation. The $p < 0.05$ level was used for all significance tests. All statistical analyses and linear regressions were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Catechin Stability in Green Tea Concentrates. A typical degradation profile of green tea catechins (EGCG, EGC, ECG, EC, GCG, and CG) in green tea concentrates (1666.7 mg/mL) at 120 °C is shown in Figure 1. The four *cis*-configured catechins (EGCG, EGC, ECG, and EC) degraded over time in an apparent exponential manner, while the two *trans*-configured catechins (GCG and CG) initially increased and then reached a plateau after 90 min. Such patterns are consistent with a previous study of catechin stability in green tea powders,[5] suggesting that the four *cis*-configured catechins followed apparent first-order degradation not only in green tea powder systems, but also in concentrated solutions. *trans*-Configured catechins were produced under thermal process conditions as isomerization products and were also consumed as reactants in degradation reactions, which would explain the plateau observed in Figure 1.[5] It is likely that prolonged heating would cause a decrease in the *trans*-configured catechins as observed elsewhere.[5] Their unique concentration profiles throughout treatments were likely the combination of several reactions, including epimerization, oxidation, and polymerization.

The apparent first-order reaction kinetics of EGCG in green tea concentrates treated at temperatures from 25 to 120 °C are illustrated in Figure 2. Reactions proceeded faster with temperature increases, as expected. High correlations (R^2 =

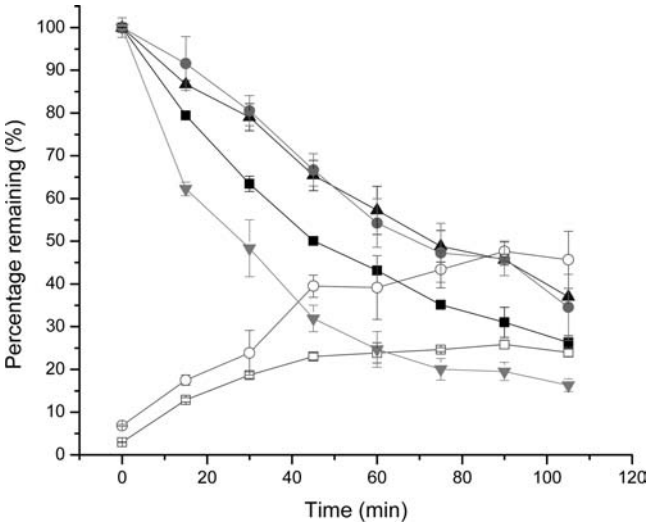

Figure 1. Typical catechin degradation profiles in green tea concentrates at 120 °C: (■) EGCG, (□) GCG, (●) ECG, (○) CG, (▲) EC, (▼) EGC.

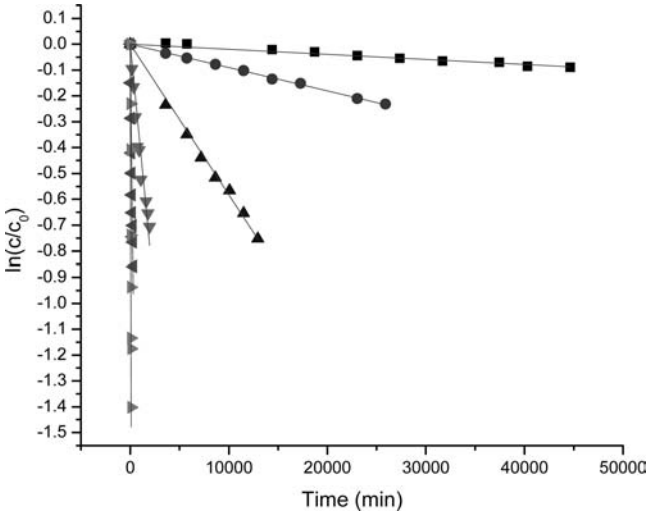

Figure 2. First-order degradation regression lines of EGCG at temperatures from 25 to 120 °C: (■) 25 °C, (●) 40 °C, (▲) 60 °C, (▼) 80 °C, (◀) 100 °C, (▶) 120 °C.

0.972−0.996) were obtained in linear regressions within the experimental conditions. Similar degradation patterns were also observed for the other *cis*-configured catechins (data not shown). These results are in agreement with a previous study,[5] and these data confirm that apparent first-order kinetics occurred not only in green tea powder systems at low temperatures (25−60 °C), but also in concentrated solution systems over a wider temperature range (25−120 °C). Although a first-order kinetic model fits the experimental data with high R^2 values, the actual reaction mechanism might be much more complex. The degradation behaviors of *trans*-configured catechins do not follow the first-order kinetic model, suggesting that multiple reactions are involved. Oxidation, isomerization, and cleavage reactions in EGCG degradation in dilute aqueous solutions during thermal treatment have been reported.[11] However, for this study a simplified multireaction model might not be robust enough to obtain appropriate fitting solutions.

A series of k values were obtained through linear regressions with the use of eq 1 and are listed in the pH 5.2 column of Table 1. At 25 °C, the reaction rate in green tea concentrates (k = 0.222 × 10^{-5} min^{-1}) was similar to that of the green tea powders stored at 97% RH (k = 0.223 × 10^{-5} min^{-1}).[5] At higher temperatures, the k values increased more in the powders stored at 97% RH than with the green tea concentrates. At 60 °C, the k values were 6.669 × 10^{-5} min^{-1} for the powders and 5.586 × 10^{-5} min^{-1} for the tea concentrates. This difference may result from the slightly higher a_w in the powder at 97% RH than that found in the tea concentrate (a_w = 0.95). The k values for the green tea powders at lower storage RHs (43−80% RH) were found to be lower than those for the tea concentrates across all treatment temperatures (25−60 °C).

Effect of pH Levels on Catechin Loss in Green Tea Concentrates. Examples of the effects of pH levels on the reaction of catechins in green tea concentrated solutions (1666.7 mg/mL) after being heated at 120 °C for 30 min are shown in Figure 3. The loss of catechins was dependent on the pH of the system. *cis*-Configured catechins were most stable at pH levels from 4 to 5.2. Above pH 5.2, degradation was accelerated as the pH was raised. At pH levels below 4, catechin loss also occurred faster. Such minima in reaction rate versus pH profiles are common.[12,13] The production of *trans*-configured catechins increased with increasing pH levels, suggesting that isomerization was more favored with increases in pH levels. For *trans*-configured catechins, minimum production was observed at a pH level of 1.5 for CG and GCG. This suggested that isomerization of catechins was inhibited at pH levels below 4, but was enhanced with increasing pH values when the pH level was raised to 4 or above. The pH of samples before and after heat treatments was monitored. No significant difference was found in samples of pH 6 or less (Supporting Information), likely because the concentrated solution is a plasticized semisolid system with limited molecular mobility, which provided a greater buffering capacity than in dilute solutions (Supporting Information). However, in pH 7 samples, the pH value decreased to near 6.25 after 30 min of heating at 120 °C. The production of degradation products such as hydrogen peroxide favored at alkaline conditions likely altered the pH.[14]

Previous studies have reported the stability of catechins in pH conditions ranging from 3.0 to 7.4 on the basis of the pH environment of the human gastrointestinal tract, which varies from a low of pH 1.5 in the stomach to a high of pH 7.4 in the terminal ileum.[1,4,7−9,15,16] The decreasing stability of catechins in tea concentrates above pH 4 is consistent with results reported by Zimeri et al.,[1] where increasing the pH above 5.2 decreased the catechin stability. It has been generally considered that green tea catechins are stable under acidic pH conditions,[4,7,17−19] a fact which could be explained in part by the direct increase in oxidation rates with an increasing pH.[19] The pK_{a1} of EGCG has been reported to be 7.55 ± 0.03.[20] Therefore, the ionization state of EGCG, an indication of its proton-donating ability, is likely a factor contributing to its degradation.[21] Enhanced EGCG oxidation and the formation of semiquinone free radical of EGCG at neutral or alkaline pHs have been reported.[18,22] Komatsu et al. [4] observed very low levels of degradation of catechins at pH levels of 3.0−5.0 after these solutions had been heated for 4.4 min at 121 °C. Zhu et al.[17,18] also reported that green tea catechins were very stable at pH levels below 4. Their conclusions, however, were

Table 1. Temperature and pH Effects on Rate Constants and t_{90} Values for EGCG in Green Tea Concentrated Solutions of 1666.7 mg/mL

temp (°C)		pH 1.5	pH 2	pH 3	pH 4	pH 5.2[a]	pH 6	pH 7
25	$k \times 10^5$ (min^{-1})	0.290	0.241	0.132	0.098	0.222	0.193	0.629
	R^2 $(n)^b$	0.922 (19)	0.968 (16)	0.944 (16)	0.893 (19)	0.976 (34)	0.936 (16)	0.914 (17)
	$t_{90}{}^c$ (min)	34832	46210	81330	143142	61731	43302	27797
40	$k \times 10^5$ (min^{-1})	3.361	1.450	1.109	0.553	0.879	1.939	5.146
	R^2 $(n)^b$	0.979 (14)	0.932 (22)	0.884 (18)	0.985 (21)	0.994 (27)	0.950 (21)	0.918 (17)
	$t_{90}{}^c$ (min)	4894	6493	11428	20114	10271	7205	4625
60	$k \times 10^5$ (min^{-1})	16.00	10.12	6.646	4.479	5.586	10.04	11.03
	R^2 $(n)^b$	0.969 (27)	0.933 (22)	0.933 (24)	0.960 (27)	0.996 (24)	0.979 (18)	0.907 (27)
	$t_{90}{}^c$ (min)	470.7	624.4	1099	2057	1208	847.7	544.2
100	$k \times 10^5$ (min^{-1})	1566	669.0	613.0	250.0	347.4	441.0	649.0
	R^2 $(n)^b$	0.945 (27)	0.832 (24)	0.884 (24)	0.926 (25)	0.972 (24)	0.910 (17)	0.888 (23)
	$t_{90}{}^c$ (min)	9.242	12.26	21.58	56.66	33.29	23.35	14.99
120	$k \times 10^5$ (min^{-1})	6660	5482	2595	1538	1278	1794	3542
	R^2 $(n)^b$	0.952 (18)	0.975 (12)	0.941 (24)	0.968 (23)	0.984 (19)	0.966 (17)	0.961 (20)
	$t_{90}{}^c$ (min)	1.748	2.319	4.082	12.37	7.267	5.098	3.273
Arrhenius fittings	slope $\times 10^{-4}$	−1.233	−1.230	−1.228	−1.195	−1.146	−1.093	−1.030
	intercept	28.70	28.09	27.62	26.08	24.90	23.72	22.55
	R^2	0.993	0.994	0.996	0.998	1.000	0.995	0.978

[a]Natural pH of green tea concentrates. [b]Correlation coefficients and numbers of data points for the regression line. [c]Time when 90% of the initial concentration of EGCG is left, calculated on the basis of the models described in Table 2. For pH 4, models were chosen on the basis of the calculated turning pH point.

based upon results obtained in dilute solutions (8 mg/mL[4] and 0.5 mg/mL[18]) and therefore might not be applicable to the concentrated solutions used in this study. Also, for pH adjustment, Komatsu et al.[4] employed 1% aqueous solutions of citric acid, sodium citrate, L-ascorbic acid, and sodium hydrogen carbonate, and Zhu et al. [18] used a sodium phosphate buffer of 60 mM, NaOH solutions, and HCl solutions. Differences in ion species and concentrations of different buffer solutions may also be found to affect the assorted rates of degradation reactions, thus resulting in inconsistencies in trends and results between various studies.

Although it is generally accepted that green tea catechins are stable in acidic pH environments, extremely low pH conditions might enhance catechin loss in both solution and solid states. Tu et al.[23] reported a dramatic decrease in catechin content at a pH of 2.6 when compared to the pH of 3.6−5.6 for citric acid buffer solutions. Ortiz et al.[3] also observed enhanced solid-state loss in green tea powder solid-state samples mixed with citric acid (pH 1.6) and ascorbic acid (pH 2.6). In both studies, catechin loss was found to be more severe at a lower pH (1.6 and 2.6) when compared to less acidic conditions (pH 3.6−5.2).[3,23] These reports are consistent with the results obtained for the green tea concentrates in this work (Figure 3).

Caffeine−catechin interactions can result in precipitation at low pH conditions,[24,25] and increased concentration might favor the formation of these insoluble complexes in more concentrated tea products. Caffeine was stable at the experimental conditions and was used as an internal reference in a previous study conducted at unmodified pH (5.2).[5] In this study, however, the caffeine content declined over time at pH levels from 1 to 3 at temperatures at or above 40 °C (data not shown). This indicated a loss of caffeine due to the formation of insoluble caffeine−catechin complexes. Sedimentation was also observed in these samples. The formation of such insoluble tea cream is neither desired in commercial products nor favored by consumers and could be filtered out of the product; therefore, catechins involved in the insoluble caffeine−catechin complexes were counted as catechin loss due to the physical instability of catechins in this work. The caffeine contents of green tea concentrates at pH levels of 4−7 remained constant at temperatures above 60 °C and all pH values at 25 °C. Hence, at these conditions catechin losses were mainly ascribed to chemical degradation such as isomerization and oxidation. Interestingly, at high pH conditions (6 and 7), a decreasing trend in caffeine contents was also observed at 40 and 60 °C. It has been reported that in a caffeine−methyl gallate crystal structure, hydrophobic interactions and hydrogen bonding stabilize the structure of the cocrystal complex.[26] Therefore, such a decline in caffeine contents might be due to the enhanced hydrophobic interactions between caffeine and catechins at elevated pH values during prolonged storage. Further experiments utilizing LC/MS could be used to better characterize the degradation pathways and products of green tea catechins under various pH conditions. Resolving the insoluble caffeine−catechin complexes to differentiate chemical and physical instability of catechins at low pH conditions might be needed to better describe the effect of pH on catechin loss.

Interestingly, we measured the pH levels of several commercial green tea powder beverage formulations that were reconstituted by mixing 2 g of powders with 1200 μL of water and found that, although the pH values may vary between brands, most values fall within the range of 1.78−2.95 (data not shown). In addition to green tea powder, these formulations contained the following acidic ingredients: citric acid, ascorbic acid, and malic acid. Our results indicate that if these products were rehydrated to a concentrated solution, these low pH levels would destabilize the catechins more rapidly than if the pH were modified to 4.

Impact of both pH and Temperature Levels on Catechin Stability in Green Tea Concentrates. The rate constants k for EGCG in green tea concentrates stored under different pH and temperature conditions are summarized in

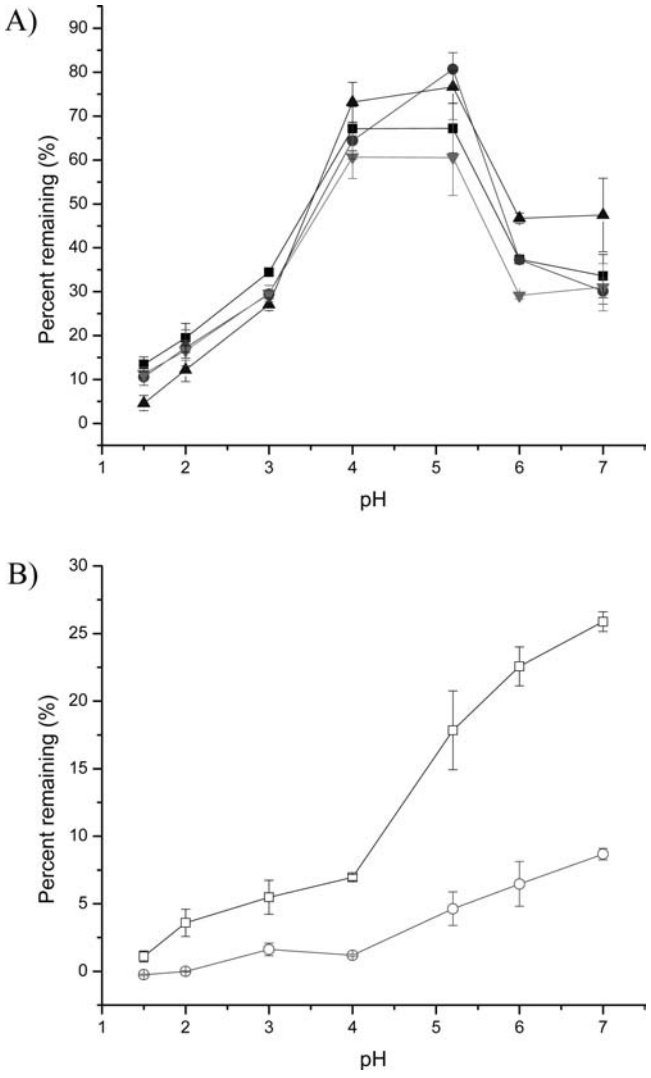

Figure 3. Impact of pH on catechin degradation in green tea concentrates after 30 min of heating at 120 °C: (A) (■) EGCG, (●) ECG, (▼) EGC, and (▲) EC, (B) (□) GCG and (○) CG.

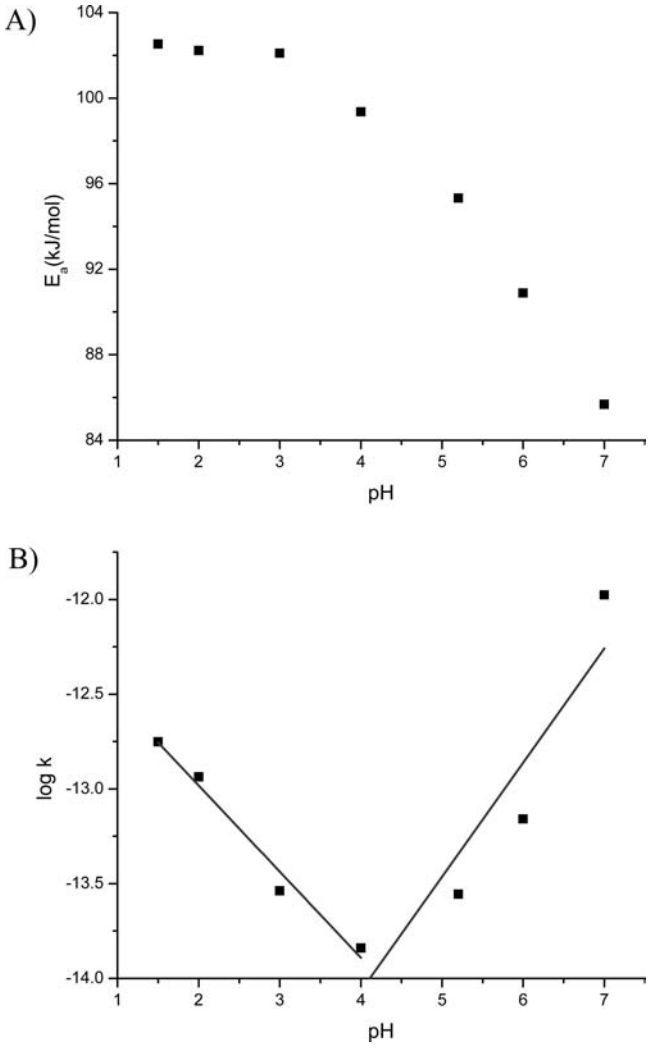

Figure 4. Impact of pH on the catechin degradation kinetics: (A) reaction activation energy as a function of pH in green tea concentrates, (B) log-linear relationship between the rate constant k and pH at 25 °C. Key: (■) experimental values, (—) predicted values.

Table 1. The reactions were affected by both the pH and temperature. A minimum k value was reached at approximately pH 4, followed by increasing k values with increasing pH. Thus, the pH level around 4 was an approximate minimum for the pH dependency, in agreement with the catechin loss profiles as shown in Figure 3.

By applying the Arrhenius equation, eq 2, to the k values of samples stored at each pH condition, good linear fits (R^2 = 0.978−1.000) were obtained, as displayed in Table 1. Both the measured activation energy and the frequency factor of collision, i.e., E_a and A, reached their maximum values at a pH level of 1.5. The relationship between pH and the activation energy is shown by plotting E_a as a function of pH (Figure 4A). As an indicator of temperature sensitivity, E_a decreased as the pH was increased from 1.5 to 7. At pH levels of 1.5, 2, 3, 4, 5.2, 6, and 7, the E_a for catechin loss was 102.54 ± 4.29, 102.23 ± 3.81, 102.11 ± 3.29, 99.36 ± 2.24, 95.32 ± 0.00, 90.88 ± 3.24, and 85.68 ± 6.36 kJ/mol, respectively. There were no significant differences among E_a values at pH 1.5−3 for catechin loss at these conditions, but the E_a values at pH levels of 5.2, 6, and 7 were significantly lower compared to those at pH values below 4. The extent of physical instability and chemical degradation pathways of catechins were altered due to elevated pH levels, and this seems to be reflected in the E_a data. By comparing the E_a (95.32 ± 0.00 kJ/mol) at a pH of 5.2 with the average E_a (95.69 ± 4.67 kJ/mol) obtained for green tea powder systems,[5] there is no significant difference. Thus, the kinetic models developed from green tea concentrated solution systems and green tea powder systems could possibly be interchangeable, a factor with essential relevance for both calculating shelf life and predicting percent catechin remaining at given temperature and pH conditions for green tea powders and concentrates.

An example of the log-linear relationship between reaction rate constants and pH is shown in Figure 4B, and a turning point of pH 4 is observed. A mathematical model correlating the effect of temperature and pH is described below:[1]

$$\ln k = \ln A - \frac{E_a}{R}\frac{1}{T} + C(\mathrm{pH}) \quad (3)$$

where k is the rate constant, A is the frequency factor of collision, E_a is the activation energy, R is the gas constant, T is the temperature, and C is a pH coefficient. This equation was developed for an EGCG dilute solution system with a

concentration of 0.02 mg/mL,[1] but the equation had not previously been applied to more concentrated systems. Thus, we applied it to our data for tea concentrates.

Multiple linear regressions were performed to obtain the average activation energy and pH coefficient for each catechin within two pH ranges (1.5−4 and 4−7). The results are shown in Table 2. The pH level of 4 was taken as a turning point by

Table 2. Semiempirical Kinetic Models Relating the Temperature and pH of Catechin Degradation in Green Tea Concentrated Solutions of 1666.7 mg/mL

catechin	pH	kinetic model	R^2
EGCG	1.5−4	$\ln k = -12215(1/T) - 0.5653(\mathrm{pH}) + 29.1086$	0.996
	4−7	$\ln k = -11163(1/T) + 0.4432(\mathrm{pH}) + 21.8551$	0.991
EGC	1.5−4	$\ln k = -12009(1/T) - 0.4723(\mathrm{pH}) + 28.3330$	0.995
	4−7	$\ln k = -10833(1/T) + 0.4461(\mathrm{pH}) + 21.3114$	0.980
ECG	1.5−4	$\ln k = -11928(1/T) - 0.5094(\mathrm{pH}) + 28.2254$	0.993
	4−7	$\ln k = -10995(1/T) + 0.3750(\mathrm{pH}) + 21.8252$	0.990
EC	1.5−4	$\ln k = -11985(1/T) - 0.4636(\mathrm{pH}) + 28.2155$	0.983
	4−7	$\ln k = -10084(1/T) + 0.3467(\mathrm{pH}) + 19.3410$	0.985

which to divide the pH dependency into two linear regions (above and below that specific pH level of 4). High correlations were then obtained (R^2 = 0.980−0.996). Therefore, it is proposed that these semiempirical kinetic models could be applied to predict the effects of temperature and pH on the shelf life of catechins in green tea concentrated solution systems.

For EGCG, the p values of the regression coefficients of $1/T$ and pH are all less than 0.05, suggesting that both temperature and pH have a significant impact on EGCG loss in green tea concentrates. The semiempirical models assume a constant activation energy over the specified pH range. Therefore, the average E_a values obtained from these models for EGCG in the pH 1.5−4 and 4−7 ranges were 101.56 ± 2.25 and 92.81 ± 3.08 kJ/mol, respectively. The E_a values are significantly different, in agreement with the results shown in Figure 4A.

Theoretical pH turning points for EGCG stability (indicative of the pH level at which the catechin was most stable) were obtained by calculating the points of intersection of the two models (Table 2). These were established to be pH levels of 3.69, 3.86, 4.06, 4.40, and 4.54 at temperatures of 25, 40, 60, 100, and 120 °C, respectively. Differences could be because interactions between caffeine and catechins were enhanced at low pH values at elevated temperatures, leading to higher pH turning points at higher temperatures. Similar pH shifts of the turning point for catechin loss were also obtained for EGC, ECG, and EC, suggesting that the optimum pH for catechin stability increases slightly with an increasing temperature. The t_{90} (the time by which 10% catechin loss is reached) values of EGCG in green tea concentrates stored under different temperatures and pH conditions were then calculated on the basis of the mathematical models (Table 2), and these values are shown in Table 1. The shortest shelf life was obtained at a pH of 7 at 25 and 40 °C and a pH of 1.5 at temperatures above 40 °C, while the longest shelf life was calculated to be pH 4 at temperatures ranging from 25 to 120 °C.

At a pH below 4, it can be calculated that, to compensate for the impact of a 1 unit pH decrease, the temperature needs to be lowered by 4.17 and 7.29 °C at a temperature of 25 and 120 °C, respectively. At a pH level above 4, the temperature decreases are 3.49 and 6.04 °C at temperatures of 25 and 120 °C to compensate for a 1 unit pH increase. In the mathematical models (Table 2), the coefficients of pH have larger absolute values at pH levels below 4 than those at pH levels above 4, suggesting that the pH has an increasingly significant impact on catechin loss when the pH is lower than 4.

Effect of Concentrations on Catechin Degradation. The degradation profiles of green tea solutions at concentrations ranging from 1 to 1666.7 mg/mL stored at 80 °C are shown in Figure 5. Catechin degradation proceeded much more rapidly with more dilute solutions, and the reaction rate decreased with increasing concentration. For example, after a 360 min heat treatment, the percentage of EGCG remaining in solutions which initially contained green tea concentrations of 1666.7, 500, 100, 10, and 5 mg/mL was measured to be 85.33 ± 1.27%, 77.87 ± 1.82%, 77.06 ± 2.60%, 56.87 ± 2.43%, and 41.42 ± 1.98%, respectively. For the most dilute solution (1 mg/mL), only 1.08 ± 0.14% EGCG remained after a 300 min heating period.

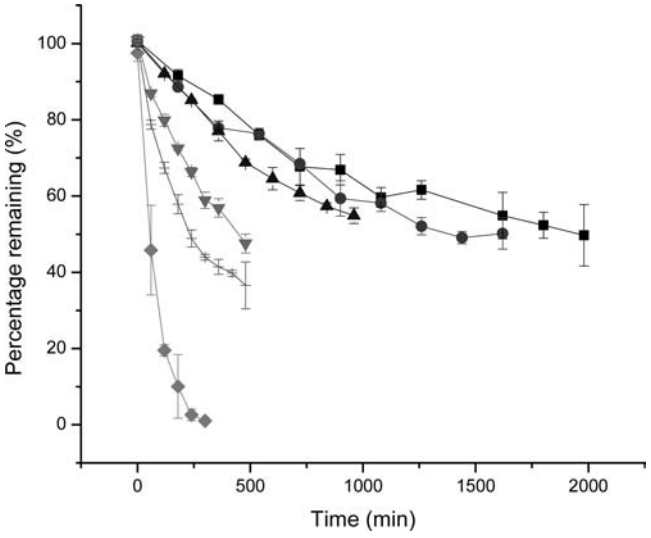

Figure 5. Degradation profiles of EGCG in green tea solutions at different concentrations: (■) 1666.7 mg/mL, (●) 500 mg/mL, (▲) 100 mg/mL, (▼) 10 mg/mL, (+) 5 mg/mL, (◆) 1 mg/mL.

A set of 1 mg/mL green tea solutions were treated at different temperatures, ranging from 25 to 120 °C (note that solutions stored at 25 °C were discarded due to mold growth within 48 h). Similar apparent first-order degradation profiles were obtained (data not shown) for these dilute 1 mg/mL solutions as compared to the concentrated 1666.7 mg/mL solutions (Figure 2). The k values were calculated to be 23.166 × 10^{-5} and 76.260 × 10^{-5} min^{-1} at 40 and 60 °C for the 1 mg/mL solutions. When compared with the green tea concentrates, the reaction rates in the dilute solutions were 26.35 and 13.65 times higher at temperatures of 40 and 60 °C, respectively. It is worth noting that this concentration dependence of catechin degradation might suggest different reaction kinetics consisting of multiple degradation pathways. However, the apparent first-

order kinetic model was only used for mathematic modeling purposes, and a good agreement between calculated and experimental values was obtained.

These results are in agreement with the concentration dependency of EGCG degradation reported by Sang et al [27] in green tea extract solutions at concentrations of 0.05%, 0.2%, and 0.6%, where EGCG was more stable at the higher concentrations. Similar results were also observed in purified EGCG solutions (0.025%, 0.1%, and 0.32%) in nanopure water.[27] However, the reasons for such an interesting phenomenon, whereby degradation proceeds faster at lower concentrations, remained unknown according to this report.[27]

According to other studies,[1,4] increases in dissolved oxygen content and pH could accelerate catechin degradation. As the catechin concentration decreases, the increased amount of water enables an increased amount of dissolved oxygen to enhance catechin oxidation. The pH values in our study varied with concentration, increasing from 5.19 ± 0.01 to 5.79 ± 0.17 as the green tea concentration decreased from 1666.7 to 1 mg/mL. Another possible factor is the dramatically reduced molecular mobility in concentrated solutions, an element which could act as the rate-limiting factor. Therefore, it is not surprising that reaction rates increased as more water was present in the system, which in turn increased levels of dissolved oxygen, pH, and molecular mobility. These results highlight the importance of controlling catechin concentration in maintaining the stability of catechins in tea products and suggest that green tea concentrates would be a better form to maintain catechin stability for product storage and transportation than dilute solutions. Further study is needed to better explain the effects of water content on catechin degradation mechanisms and reaction kinetics and to differentiate the contributions of pH, molecular mobility, and dissolved oxygen. LC/MS and NMR techniques could be used to investigate possible differences in reaction mechanisms.

Degradation Kinetics of Green Tea Catechins. The increases in the reaction rate constant k with increasing temperature followed the Arrhenius equation. By plotting the natural log of k against the inverse of temperature, straight lines were obtained (Figure 6A). Reactions proceeded much faster in 1 mg/mL green tea solutions within the experimental conditions than in more concentrated solutions. However, such differences became smaller with increases in temperature. A crossing point in the Arrhenius plot was observed at approximately 120 °C. Interestingly, extrapolations to temperatures higher than 120 °C suggest that reactions would probably proceed faster in green tea concentrated solution systems than in dilute solutions at these elevated temperatures. Due to equipment limitations, experiments at temperatures above 120 °C were not performed. Although scientifically interesting, it is unlikely that tea products would be processed or stored at temperatures above 120 °C.

Results obtained for the dilute and concentrated tea solutions were compared with results published from a study of temperature and RH effects on green tea powders.[5] In the Arrhenius plot comparing these data, the slope of the green tea concentrated solution is similar to the slopes of the four parallel lines obtained for the green tea powders stored at different RHs (Figure 6A). The E_a values for concentrated solutions and powders stored at 80%, 75%, and 43% RH were 98.52 ± 4.84, 94.05 ± 2.92, 97.16 ± 3.88, and 92.86 ± 41.46 kJ/mol, respectively.[5] There were no statistically significant differences ($p > 0.05$) among E_a values obtained in green tea concentrates and solid powders stored at 80% RH and below. However, the E_a for EGCG degradation in 1 mg/mL green tea solutions (57.34 ± 2.98 kJ/mol) is significantly lower than that in green tea concentrates (98.52 ± 4.84 kJ/mol) ($p < 0.05$). This could be due to altered reaction mechanisms in systems with differences in water content, or the lowered E_a may be caused by increased diffusion rates due to the presence of bulk water.[5]

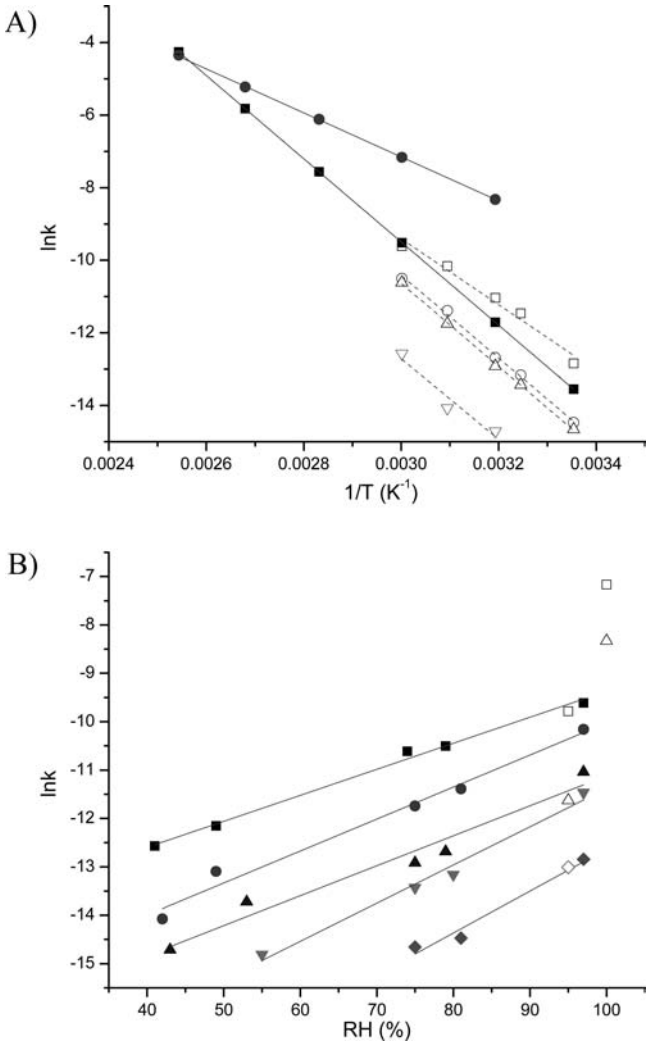

Figure 6. Degradation kinetics of EGCG in green tea powder and solution systems: (A) Arrhenius plots of rate constants (powder, (□) 97%, (○) 80%, (△) 75%, (▽) 43%; solution, (■) concentrated solution of 1666.7 mg/mL, (●) dilute solution of 1 mg/mL), (B) EGCG degradation rate constants as a function of RH (or $a_w \times 100$) (powder, (■) 60 °C, (●) 50 °C, (▲) 40 °C, (▼) 35 °C, (◆) 25 °C; solution, (□) 60 °C, (△) 40 °C, (◇) 25 °C).

RH (or a_w) is another factor that impacts the reaction rate constant following a log-linear relationship.[5,28] The water activity of 1 mg/mL green tea solution was considered to be 1 (RH 100%) and was verified by water activity measurement. When the results in our previous work[5] were compared with those of this study by plotting the natural log of the rate constant against the RH (or $a_w \times 100$), consistent results were obtained, as shown in Figure 6B. This demonstrated that a concentrated solution is a good system by which to simulate and predict the chemical degradation of green tea catechins in powder systems stored at the corresponding RH (or $a_w \times 100$) condition. However, discrepancies were observed in results

obtained from dilute solutions (Figure 6B), suggesting that the kinetic model established on the basis of powder systems is not applicable to 1 mg/mL green tea solutions. Rate constants k obtained in dilute solutions were higher than those in concentrated solutions and powder systems. This could be, in part, due to the altered reaction mechanisms resulting from changes in molecular mobility, dissolved oxygen content, and/or pH level in green tea solution systems.

In conclusion, the results of this study highlight the importance of pH, temperature, and concentration in impacting catechin stability. Shelf life models of catechin stability in green tea concentrated solution systems as functions of pH and temperature were developed. Catechins were more stable as their concentration increased in solutions. In green tea concentrates, the optimum pH for catechin stability was around pH 4. Log-linear relationships between the pH and reaction rate constant k were observed. Catechin degradation in concentrated solution systems followed apparent first-order kinetics. No significant difference in reaction activation energies was found between green tea concentrated solution and powder systems, but the dilute solution system had a much lower activation energy. This study developed useful approaches for shelf life calculations and catechin loss predictions at given temperature and pH conditions in green tea concentrates which can aid in improving both the formulation and storage strategies for high-quality tea products.

■ ASSOCIATED CONTENT

Supporting Information

Designed reaction conditions for the kinetic study at different temperatures, pH changes of green tea concentrated solution heated at 120 °C for 30 min, pH changes of green tea solutions with different concentrations heated at 80 °C for 8 h, and pH profile of green tea concentrates at a_w = 0.95. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

a_w, water activity; CG, catechin gallate; E_a, activation energy; EG, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GCG, gallocatechin gallate; GT, green tea powder; T_g, glass transition temperature

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