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Reaction Kinetics of Degradation and Epimerization of Epigallocatechin Gallate (EGCG) in Aqueous System over a Wide Temperature Range

RONG WANG, WEIBIAO ZHOU,* AND XIAOHUI JIANG

Food Science and Technology Programme, Department of Chemistry, National University of Singapore, Science Drive 4, Singapore 117543

(–)-Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea, which has been linked with many health benefits. To ensure the conceivable health benefits from thermally processed products, a kinetic study on the stability of (–)-EGCG in aqueous system was carried out using a HPLC-UV system and Matlab programming. Simultaneous degradation and epimerization of (–)-EGCG were characterized during isothermal reactions at low temperatures (25–100 °C) combined with previously conducted experimental results at high temperature (100–165 °C); the degradation and epimerization complied with first-order reaction and their rate constants followed Arrhenius equation. Mathematical models for the stability of (–)-EGCG were established and validated by the reactions at 70 °C and with varied concentrations from different catechin sources. Two specific temperature points in the reaction kinetics were identified, at 44 and 98 °C, respectively. Below 44 °C, the degradation was more profound. Above 44 °C, the epimerization from (–)-gallocatechin gallate (GCG) to (–)-EGCG was faster than degradation. When temperature increased to 98 °C and above, the epimerization from (–)-GCG to (–)-EGCG became prominent. Our results also indicated that the turning point of 82 °C reported in the literature for the reaction kinetics of catechins would need to be re-examined.

KEYWORDS: Epigallocatechin gallate; kinetic model; stability; epimerization; degradation

INTRODUCTION

Being revealed as anticancer, antidiabetic, antibacterial, anti-inflammatory, and antiaging agents in various biological and pharmaceutical studies, green tea has attracted more and more attention in recent years. The beneficial effects of green tea are attributed to its polyphenol components, namely tea catechins, which hold strong antioxidative activity in relation to the protection against various diseases such as coronary heart diseases (CHD) and cancers associated with oxidative stress (*1, 2*).

The main green tea catechins have been identified as epicatechins, including (–)-epigallocatechin (EGC), (–)-epigallocatechin gallate (EGCG), (–)-epicatechin gallate (ECG), and (–)-epicatechin (EC). These cis compounds can convert to their epimers that are non-epicatechins, i.e., (–)-gallocatechin (GC), (–)-gallocatechin gallate (GCG), (–)-catechin gallate (CG), and (±)-catechin (C), respectively (*3–7*) (**Figure 1**). This epimerization between pair catechins is reversible. The chemical structures of epicatechins and non-epicatechins only differ between 2*R*,3*R* (2,3-*cis*, epi- form) and 2*S*,3*R* (2,3-*trans*, nonepi-form) (**Figure 2**).

The antioxidative activity of tea catechins is structure-dependent. The three adjacent hydroxyl (OH) groups at positions C-3′, -4′, and -5′ on the B ring of (–)-EGCG, (–)-EGC, (–)-GCG, and (–)-GC are more effective in scavenging free radicals than the two adjacent OH groups at C-3′ and -4′ in (–)-ECG, (–)-CG, (–)-EC, and (±)-C, respectively. Moreover, catechins with additional gallate moiety at C-3 generally hold stronger scavenging effects than non-gallate catechins, i.e. (–)-ECG > (–)-EC, and (–)-EGCG > (–)-EGC (*3, 5, 8, 9*). Meanwhile, it was also reported that some *trans*-catechins, e.g., (–)-GCG, (–)-GC, and (+)-C are more effective in scavenging singlet oxygen ($^1O_2$) and inhibiting in comparison with their *cis*-catechins, i.e., (–)-EGCG, (–)-EGC, and (–)-EC (*5, 10*).

The free radical scavenging capability of tea catechins is radical-dependent. In the singlet oxygen ($^1O_2$) induced system, the quenching capability of tea catechins decreased in the order of (–)-EGCG/(–)-GCG > (–)-EGC/(–)-GC > (–)-EC/(+)-C (*5*). In the radical ABT^{*+} induced aqueous system, the scavenging capability of tea catechins was in the order of (–)-ECG > (–)-EGCG > (–)-EGC > (–)-EC ≈ (±)-C (*8*). And, in the peroxynitrite radical ($ONOO^-$) induced system, the order was as follows: (–)-EGCG > (–)-GCG > (–)-ECG > (–)-EGC > (–)-EC > (+)-C (*11*).

The antioxidant activity of tea catechins also differ in lipid and emulsion systems. In canola oil, the activity decreased in the following order: (–)-EGC > (–)-EGCG > (–)-EC > (–)-ECG (*3*), whereas in lard, the order was (–)-EGCG > (–)-EGC

* Author to whom correspondence should be addressed. Telephone: (65) 6516-3501. Fax: (65) 6775-7895. E-mail: chmzwb@nus.edu.sg.

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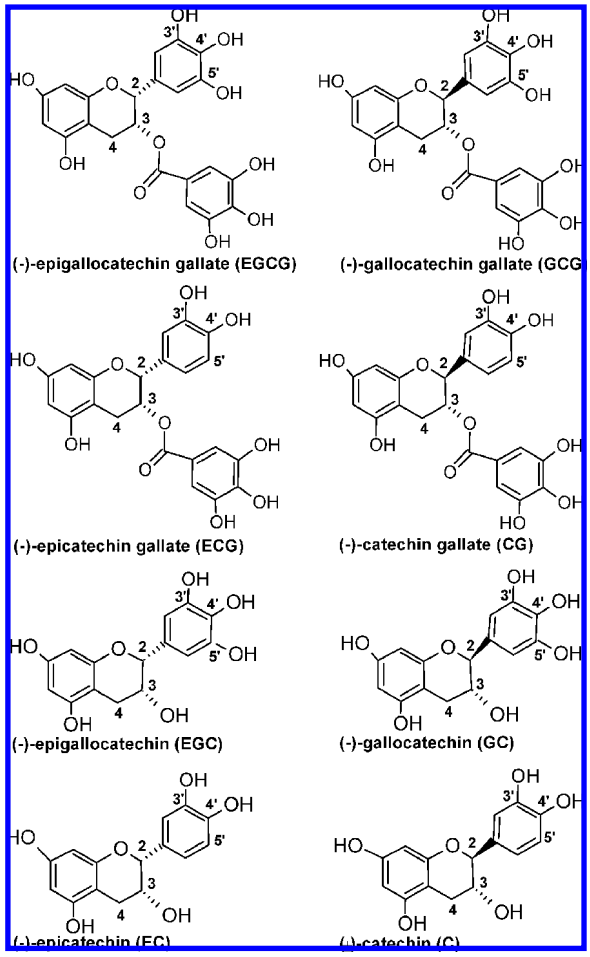

Figure 1. Chemical structures of green tea catechins.

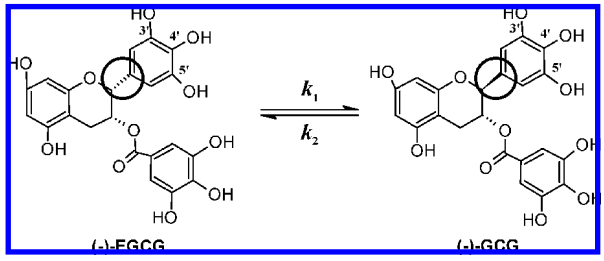

Figure 2. Epimerizations between the pair catechins, (–)-EGCG and (–)-GCG: k_1, rate constant of the epimerization from (–)-EGCG to (–)-GCG; k_2, rate constant of the epimerization from (–)-GCG to (–)-EGCG.

> (–)-ECG > (–)-EC (*12*); on the other hand, in the system of lipoxidase-induced lipid peroxidation of lecithin, the order was (–)-ECG > (–)-EGCG >> (–)-EGC > (–)-EC (*13*).

From the above studies, it is clear that the antioxidant activity of tea catechins depends on factors such as reactive oxygen species, free radicals, and media systems. However, the stability of tea catechins is pH and temperature dependent. Tea catechins in aqueous solutions are very stable when pH is below 4, whereas they are unstable if presented in solutions with pH > 6 (*9, 14–17*). In addition, storage temperature affects the stability of tea catechins significantly even at ambient conditions (*6, 9, 14–16, 18, 19*).

The degradation of tea catechins probably leads to oxidation, dimerization, and polymerization (*20, 21*). Reference *14* reported that the degradation and epimerization of tea catechins could occur simultaneously in thermal processes. Degradation of tea catechins was found to follow first-order kinetics. The first-order kinetics of degradation could be disturbed when other competing reactions such as epimerization were also present. As a result, the reaction kinetics would exhibit different modes beyond a turning point at 82 °C. However, "due to difficulties of qualification and complexity of the kinetics" (*14*), no mathematical model accounting for simultaneous degradation and epimerizations was established. The dominant reactions beyond the turning point were indistinct. Subsequently, the occurrence of epimerization among catechins was further confirmed by many researchers (*4, 6, 16*). It was found that while tea catechins underwent epimerization, superoxide-mediated autoxidation occurred simultaneously in the presence of active oxygen (*22*). The oxidation of catechins followed a pseudo-first-order reaction (*15*). However, inconsistent results of activation energy (E_a) for the degradation of catechins were reported by different research groups. E_a of (–)-EGCG degradation in the presence of oxygen was reported as 18.7 kcal/mol in ref *15*, which is different from either 4.7 kcal/mol (<82 °C) or 37.9 kcal/mol (>82 °C) in ref *14*. The difference could be due to the facts that the epimerization from non-epicatechin to epicatechin was not considered in ref *14*, and the epimerizations between pair catechins were not included in ref *15*.

Besides the degradation and epimerization commonly observed at high temperatures (*4, 19*), tea catechins were also found to degrade slowly at low temperatures. Reference *14* reported that there was ca. 29% reduction of tea catechins after a storage of 3 h at 70 °C (*9*). On the other hand, approximately the same amount (29.3%) of total catechins was lost when tea infusion was kept at 40 °C for 6 months (*6*). In comparison, it was reported that more than 90% of tea catechins were destroyed when they were stored in ambience for the same amount of time, i.e. 6 months (*16*). In ref *18*, (–)-EGCG and (–)-EC degraded completely in 15 and 30 days in an oil-in-water emulsion. Thus, it can be seen that the stability behaviors of tea catechins varied in different studies.

To resolve the above-mentioned inconsistent stability results in aqueous systems, mathematical models accounting for the simultaneous degradation and epimerization of tea catechins at high temperatures (HT), i.e., 100–165 °C in aqueous system were developed previously using a microwave reactor (*19*). However, the models based on HT conditions were not validated at low temperatures (LT) where the reactions of tea catechins also inevitably occurred. Meanwhile, the use of microwave reactor for the model development at HT conditions may be questioned as it is not a popular heating method in actual food processes. Therefore, it is necessary to carry out a kinetic study that can well describe change in tea catechins over a wide rang of temperature regardless the heating method.

(–)-EGCG is the most abundant catechin (4.7–10.4%, dry basis) in green tea and also the most active antioxidant among its homologues (*2, 16*). Moreover, (–)-EGCG is the catechin naturally occurring only in tea species, while other catechins can be found in some fruits and vegetables (*23*). Thus, (–)-EGCG is usually considered as a quality indicator of green tea products (*24*). This study focused on the stability behaviors of tea catechins using (–)-EGCG as an indicator for mathematical modeling at LT conditions. Reactions were conducted in aqueous system for relatively longer durations compared to those at HT conditions. The designated temperatures ranged from 25 to 100 °C. Quantification of (–)-EGCG and (–)-GCG was carried out in a HPLC-UV elution system. Mathematical models were established which were validated by reactions at 70 °C. To further prove the validity of the models established at LT conditions, the kinetic parameters were compared with those at HT conditions.

Table 1. Designated Reaction Conditions for the Kinetic Study of (–)-EGCG Stability at LT Processes

temp, °C	heating duration, min
25	0, 2880 (2 days), 10080 (7 days), 17280 (12 days), 23040 (16 days)
40	0, 60, 240, 480, 960
60	0, 60, 240, 360, 540
70[a]	0, 60, 180, 360, 540
80	0, 60, 180, 360
100	0, 1, 10, 30, 60, 90, 120

[a] The temperature 70 °C was used to validate the models to be established.

MATERIALS AND METHODS

Materials. Catechins (–)-EGCG, (–)-GCG, and ascorbic acid were purchased from Sigma-Aldrich Chemical Co. (USA). Formic acid was purchased from Merck (Germany). HPLC grade of methanol was purchased from Tedia Co. Inc. (USA). HPLC grade of water was produced by a purification system (Millipore Direct-Q, USA). Purified (–)-EGCG powder (PEP) was purchased from DSM Nutritional Products (Switzerland), containing ca. 90% (–)-EGCG, 2% (–)-ECG, and trace amount of (–)-GCG (<0.02%).

Preparation of Catechin Solutions for Reactions. It was reported that the optimum epimerization of tea catechins could be obtained in a solution with pH 5 (*16*). Meanwhile, the natural green tea infusion is around 5.5 (*25*). Thus, the PEP solutions containing a rich amount of (–)-EGCG with a natural pH 5.1 was chosen for the kinetic study. Briefly, the PEP catechin solution was prepared by dissolving PEP powder in deionized water to make a concentration of (–)-EGCG ca. 500 mg/L. This concentration is the average amount of (–)-EGCG (20–130 mg) in 2 g of green tea leaves prepared in 150 mL of water (*3, 26*). A carrier was also prepared to keep tea catechins stable in processed samples, as described in ref *19*. The carrier contained 70% of methanol, 29.7% of water, 0.3% of formic acid, and 0.4% ascorbic acid. Processed or nonprocessed samples were mixed with the carrier at a 1:1 ratio (v/v). The mixture was kept in cold storage (4 °C) until HPLC analysis. Filtration using 0.45 μm membrane was done prior to injection.

Experimental Design for Reactions at LT Conditions. The LT reaction conditions, listed in **Table 1**, embraced the temperatures in ambience (25 °C), incubation (40 °C), pasteurization (70–90 °C), and boiling (100 °C), which are found in common food processes and storage.

Two milliliters of PEP solution was accurately transferred to a reaction vial (Biotage Vial Kit 0.5–2 mL, Sweden), which was attached with disposable septum (Biotage Reseal Septa, Sweden) to ensure an enclosed atmosphere during reaction. Kinetic reactions at temperatures from 40 to 100 °C were conducted in a water bath (Bibby SBS30, UK) without mechanical shaking. For reactions at 25 °C, the PEP solutions were kept in ambience without heat treatment. Kinetic results obtained at 70 °C were used to examine the validity of the models to be established based on the results at other conditions.

Quantification of Tea Catechins Using HPLC-UV System. The catechin (–)-EGCG and its epimer (–)-GCG were analyzed using a HPLC-photodiode array (PDA) detection system, which was equipped with a UV detector (Waters 2695/2696, USA), and a C18 reversed-phase column (Waters 250 × 4.6 mm/5 μm, USA). The separation procedure was described previously (*19*). Briefly, two mobile phases were used for gradient elution. Mobile phase A contained 0.1% formic acid in water and mobile phase B contained 0.1% formic acid in methanol. The following elution gradient plan was adopted: 0–10 min, 10% B; 10–20 min, 10–25% B; 20–30 min, 25–30% B; 30–35 min, 30–40% B; 35–45 min, 40% B; 45–50 min, 40–60% B; 50–55 min, 60–10% B. Post run time was 10 min. Flow rate was at 0.5 mL/min. (–)-EGCG and (–)-GCG were detected at 275 nm.

Statistical Analysis. All kinetic reactions were conducted in at least triplicate. Duplicate of each sample was used for HPLC analysis. Data were expressed as means ± standard deviations.

The software package Matlab 7.1 was used to optimize the reaction kinetic parameters and to model the stability of the pair catechins (–)-EGCG and (–)-GCG in aqueous system. The model parameters were optimized by Marquardt–Levenberg method until the minimum mean squared error (MSE) was achieved between the experimental and modeled values, i.e.,

$$MSE = \sum_{m=1}^{n} (C_m^{\mathrm{Mod}} - C_m^{\mathrm{Exp}})^2/n$$

where C is catechin concentration, m is observation number, and n is the total number of observations. The superscript "Mod" indicates modeled value, "Exp" experimental value. Root mean squared error (RMSE) was taken as a measure of the model quality. Meanwhile, the degree of fitness for linear regression was examined by the coefficient of determination (R^2).

Modeling Approach. Reference *19* on the reactions at HT conditions showed that the degradation and epimerization of tea catechins followed first-order kinetics. The concentration of catechin can be described by

$$\ln(x/x_0) = -kt \quad (1)$$

where x is the retention of catechin at processing time t, min. x_0 is the initial concentration. k is rate constant of a reaction.

Kinetic results obtained at HT conditions also revealed that the rate constant of degradation was similar between pair catechins, i.e., $k_{y(\mathrm{EGCG})} \approx k_{y(\mathrm{GCG})}$, and followed Arrhenius equation (*19*). Thus, a mathematical model to predict the stability of (–)-EGCG at LT conditions in aqueous system (pH 5.1) can be described by

$$y = (y_0 + z_0)k_2/(k_1 + k_2)e^{-k_y t} + (y_0 k_1 - z_0 k_2)/(k_1 + k_2)e^{(-k_1 - k_2 - k_y)t} \quad (2)$$

Similarly, a model for the concentration of (–)-GCG can be written as

$$z = (y_0 + z_0)k_1/(k_1 + k_2)e^{-k_y t} + (z_0 k_2 - y_0 k_1)/(k_1 + k_2)e^{(-k_1 - k_2 - k_y)t} \quad (3)$$

where k_y is the rate constant for the degradation, k_1 is of the epimerization from (–)-EGCG to (–)-GCG, and k_2 is of the epimerization from (–)-GCG to (–)-EGCG. y is the concentration of (–)-EGCG, and z is the concentration of the epimer (–)-GCG. To eliminate inevitable variations in individual preparations of catechin solutions, the initial concentration of (–)-EGCG was normalized to 100. It means that at $t = 0$, $y_0 = [\mathrm{EGCG}]_0 = 100$. For the epimer (–)-GCG, $z_0 = [\mathrm{GCG}]_0 = 0$. At time t, $y = ([\mathrm{EGCG}]/[\mathrm{EGCG}]_0) \times 100$, and $z = ([\mathrm{GCG}]/[\mathrm{EGCG}]_0) \times 100$. Hence, the models corresponding to the stability of (–)-EGCG and (–)-GCG can be simplified to the following equations:

$$y = 100k_2/(k_1 + k_2)e^{-k_y t} + 100k_1/(k_1 + k_2)e^{(-k_1 - k_2 - k_y)t} \quad (4)$$

$$z = 100k_1/(k_1 + k_2)e^{-k_y t} - 100k_1/(k_1 + k_2)e^{(-k_1 - k_2 - k_y)t} \quad (5)$$

RESULTS AND DISCUSSION

Stability of Tea Catechins at LT Conditions. A chromatograph profile of tea catechins in the PEP solution before and after a thermal processing is shown in **Figure 3**. It clearly shows that after 6 h of thermal processing at 80 °C in water bath, (–)-EGCG decreased while its epimer (–)-GCG increased significantly. As the initial concentration of (–)-ECG was very low (ca. 2%) in the PEP solution, the accuracy and reliability of the measurements of its epimer (–)-CG could be questionable, and thus no modeling was carried out for (–)-ECG and its epimer (–)-CG in the present study.

Results in **Figure 4** show that during reactions at LT conditions, catechin (–)-EGCG decreased with increases of temperature and time, while its epimer (–)-GCG first increased to a maximum, then decreased with increasing time. The stability profile of (–)-EGCG and (–)-GCG in the PEP solutions at LT

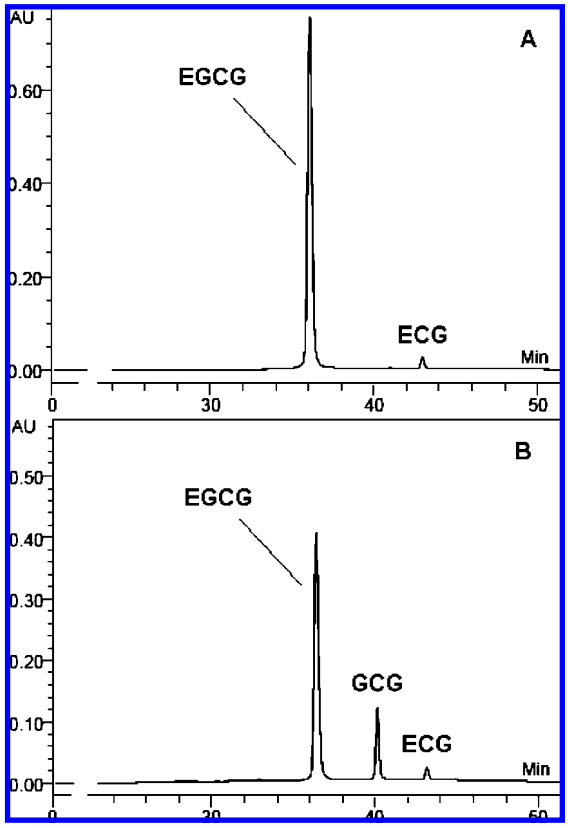

Figure 3. Chromatograph profile of tea catechins in the PEP solutions by HPLC analysis: (**A**) PEP solution before heat treatment; (**B**) PEP solution was heated at 80 °C for 6 h in water bath.

conditions were in the same trend as those in ref *9* and *19*, suggesting that the degradation and epimerization of tea catechins occurred simultaneously not only at high temperatures (100–165 °C), but also at low temperatures (25–100 °C).

Rate Constant k_y of Degradation at HT and LT Conditions. According to eq 1, a series of k_y at different temperatures can be obtained by plotting $\ln(x/x_0)$ vs t, where x is the total concentration of the pair catechins, i.e., [EGCG + GCG]. Thus, straight lines are shown in **Figure 5**. It was found that k_y increased with increases of temperature, similar to that observed during reaction at HT conditions (*19*).

High values of coefficients of determination (R^2, 0.94–0.99) were obtained for all regressions, indicating the validity of the assumption that the degradation of tea catechins at temperatures from 25 to 100 °C indeed followed the first-order kinetics, and the rate constant k_y was similar between the pair catechins (–)-EGCG and (–)-GCG.

It was shown in our earlier study that the rate constant k_y of degradation at HT conditions followed Arrhenius equation (*19*). Hence, k_y can be described as

$$k_y = Ae^{-E_a/RT} \quad (6)$$

where R is the ideal gas constant, T is temperature in kelvin. E_a and A (frequency factor) can be calculated through linear regression by transforming eq 6 to a linear equation.

As shown in ref *19*, E_a is independent of catechin concentration in the medium environment where the reaction takes place. In other words, E_a should be the same in catechin solutions with varied concentrations. Linear regression with constraints using Matlab 7.1 was conducted on the experimental results at LT and HT conditions among various catechin solutions, where the solutions of green tea extract (GTE) and green tea polyphenol (GTP) used in ref *19* contained different levels of (–)-EGCG. Four parallel lines are showed in **Figure 6A**, which illustrate the change of rate constant (k_y) with temperature. A common activation energy (E_a = 43.09 kJ/mol) was obtained from the slope of the lines. It was noted that this E_a was close to 42.78 kJ/mol, which was obtained at HT conditions in ref *19*.

It can be seen that the R^2 values were all above 0.98, and the root mean squared error (RMSE) ranged from 0.08 to 0.18 between the modeled and experimental values among the catechin solutions. These results indicate the validity of the assumption that activation energy (E_a) for the degradation remains unchanged not only in aqueous system with different levels of catechins but also at temperatures in a wide range of 25–165 °C.

On the other hand, the frequency factor (A) in the Arrhenius equation refers to the frequency of collision between molecules in the proper orientation for reaction to occur. Assuming that A also remained the same for reactions in the PEP solutions at both LT and HT conditions, a linear regression with constraints was carried out on the overall data sets obtained from both conditions. A common value of frequency factor (2.70×10^3) was obtained with a relatively high R^2 value (0.9948) and a low RMSE value (0.18), as shown in **Figure 6B**. This suggests that the frequency factor for the degradation of catechins in the same solutions remains unchanged under both LT and HT conditions, regardless the heating method.

Rate Constant k_1 of Epimerization from Epicatechin to Non-epicatechin. Epimerization of tea catechins was reported to follow the first-order kinetics at temperatures between 100 and 165 °C (*19*). Similar to that for degradation, the activation energy E_a of the epimerizations from (–)-EGCG to (–)-GCG could be assumed to remain the same in various catechin solutions. Four parallel best-fit lines were therefore obtained for reactions at LT and HT conditions as shown in **Figure 7A**. It can be seen that the values of R^2 were relatively high in the range of 0.9829–0.9966, and the values of RMSE were relatively low in the range of 0.22–0.60, suggesting that the activation energy (E_a) indeed remained unchanged. Furthermore, the epimerization from epi-structured (–)-EGCG to non-epi-structured (–)-GCG at low temperatures, i.e., 25–100 °C, also followed first-order kinetics.

On the other hand, it was observed in **Figure 7A** that there was only a very slight deviation between the two parallel lines for the PEP solutions at LT and HT conditions, respectively. As described earlier for the degradation, frequency factor (A) shared the same value in the same catechins solutions between LT and HT conditions. Thus, it is reasonable to postulate that A for the epimerization should also behave the same. Using Matlab 7.1, a constrained linear regression was made over the combined data of ($\ln(k)$, T) at both conditions in the PEP solutions. As shown in **Figure 7B**, a single line was obtained for the rate constant k_1 with a high value of R^2 (0.9921) and a low value of RMSE (0.54), indicating the validity of the assumption that frequency factor remained unchanged in the same catechin solution. In other words, the reaction kinetics, i.e,. activation energy and frequency factor, remain unchanged in the same catechin solutions at reaction temperatures ranging from 25 to 165 °C in an aqueous system.

Rate Constant k_2 of Epimerization from Non-epicatechin to Epicatechin. Similarly, the rate constant k_2 for the epimerization from (–)-GCG to (–)-EGCG is illustrated in **Figure 8A**. It is observed that the four parallel lines had relatively high values of R^2 (0.9154–0.9980) and low values of RMSE (0.29–1.08). These results indicate that the activation energy (E_a) of the epimerization from (–)-GCG to (–)-EGCG remained

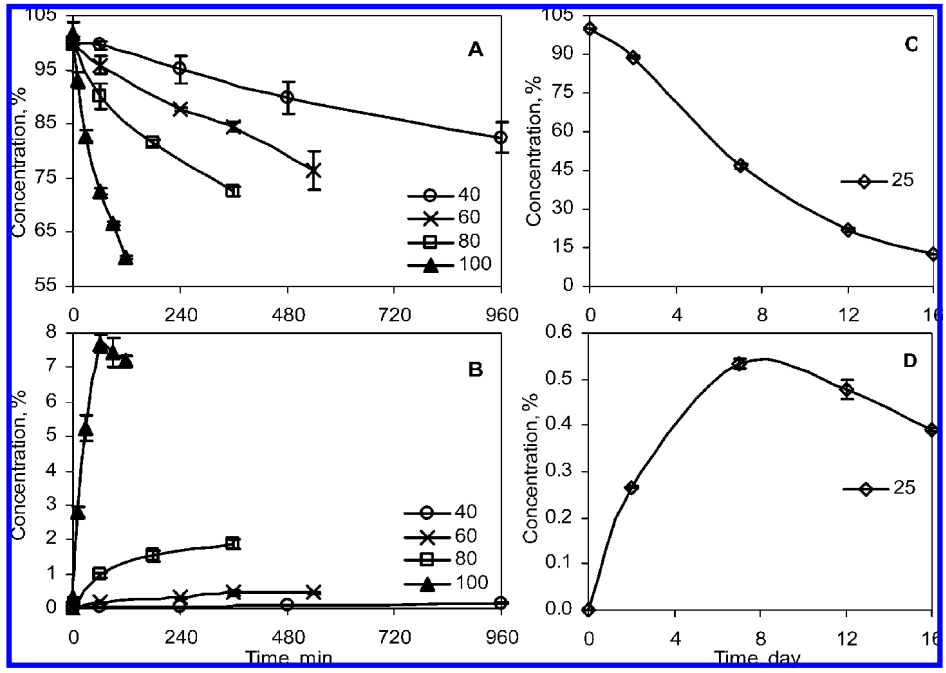

Figure 4. Stability results of tea catechins at LT, 25–100 °C: (**A** and **C**) (–)-EGCG; (**B** and **D**) (–)-GCG.

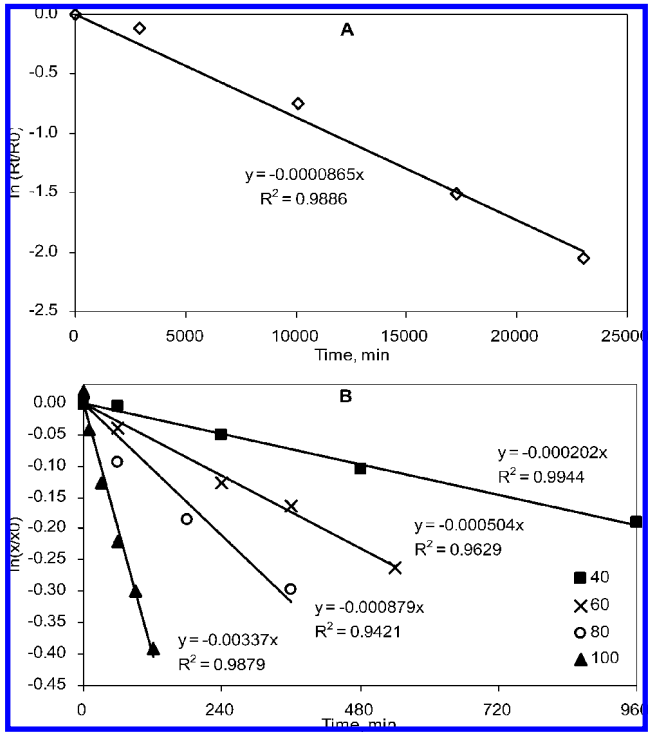

Figure 5. Apparent first-order kinetics of the epimers [EGCG + GCG] degradation at LT conditions in the PEP solutions: (**A**) [EGCG + GCG] degradation at 25 °C; (**B**) [EGCG + GCG] degradation at 40–100 °C.

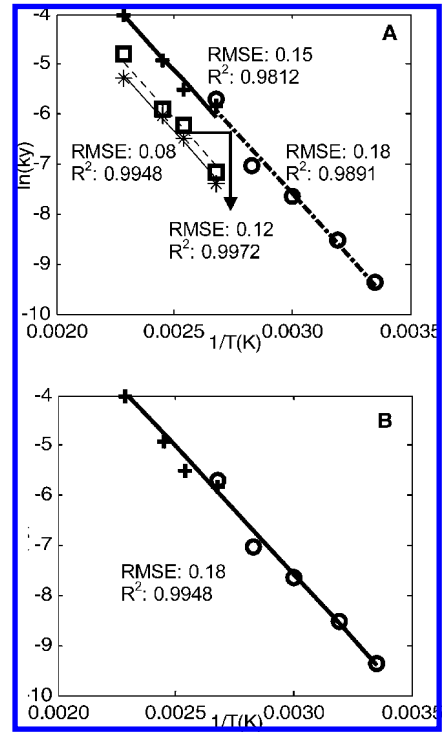

Figure 6. Arrhenius plots of rate constant (k_y) for the degradation of [EGCG + GCG] in aqueous system. (**A**) A common E_a obtained by linear regression: +, HT reactions in the PEP solutions; ○, LT reactions in the PEP solutions; □, HT reactions in the GTE solutions (pH 3.9); ∗, HT reactions in the GTP solutions (pH 3.7). (**B**) A common frequency factor (A) from linear regression based on reactions at both HT and LT conditions in the PEP solutions. Data of the degradation at HT conditions were adopted from ref *19*.

unchanged in aqueous system over the wide temperature range of 25–165 °C. Meanwhile, when a constrained linear regression was made over the combined data of the PEP solutions at both LT and HT conditions, a single line was obtained and accompanied with a relatively low value of RMSE (1.01) and a high value of R^2(0.9555) (**Figure 8B**). These results evidently prove that E_a of the epimerization from nonepi- to epicatechin remained the same in various catechin solutions at reaction temperatures of 25–165 °C, while for the same catechin solutions the same frequency factor also remained unchanged in aqueous system over the temperature range.

Specific Points in the Reaction Kinetics of Tea Catechins. As shown in the Arrhenius plots **Figures 6–8**, high R^2 (0.95–0.99) and low RMSE (0.18–1.01) values were obtained from the linear regressions over the entire data set at LT and HT conditions, evidently indicating there is no apparent turning point exhibited in any individual reaction. However, when the

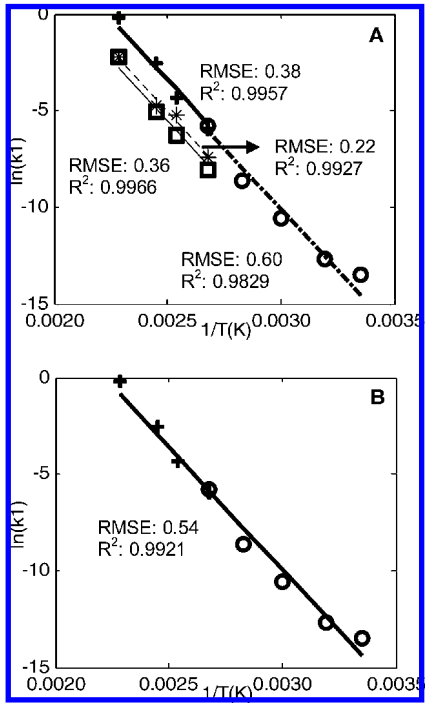

Figure 7. Arrhenius plots of rate constant (k_1) for the epimerization from (–)-EGCG to (–)-GCG in an aqueous system. (**A**) A common E_a obtained by linear regression: +, HT reactions in the PEP solutions; ○, LT reactions in the PEP solutions; □, HT reactions in the GTE solutions (pH 3.9); ∗, HT reactions in the GTP solutions (pH 3.7). (**B**) A common frequency factor (A) from linear regression based on reactions at both HT and LT conditions in the PEP solutions. Data of the epimerization at HT conditions were adopted from ref *19*.

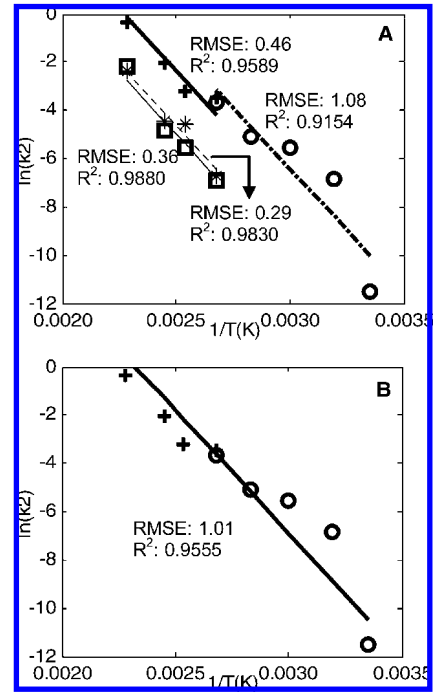

Figure 8. Arrhenius plots of rate constant (k_2) for the epimerization from (–)-GCG to (–)-EGCG in aqueous system. (**A**) Common E_a obtained by linear regression: +, HT reactions in the PEP solutions; ○, LT reactions in the PEP solutions; □, HT reactions in the GTE solutions (pH 3.9); ∗, HT reactions in the GTP solutions (pH 3.7). (**B**) A common frequency factor (A) from linear regression based on reactions at both HT and LT conditions in the PEP solutions. Data of the epimerization at HT conditions were adopted from ref *19*.

three rate constants, i.e., k_y, k_1, and k_2 for the PEP solutions, were presented in one Arrhenius plot, notably, there are two crossing points observed at 44 and 98 °C, respectively, as shown in **Figure 9**.

It can be clearly seen that the reactive speeds of the reactions changed their order at the two crossing points. When the processing temperature was greater than 44 °C, it seems that the epimerization from (–)-GCG to (–)-EGCG was the most predominant reaction. The rate constants k can be ranked as $k_2 > k_y > k_1$. When the temperature increased to 98 °C and above, the rate of the epimerization from (–)-EGCG to (–)-GCG became faster than that of the degradation. The order of the rate constants became $k_2 > k_1 > k_y$ correspondingly. When the reaction temperature was below 44 °C, the degradation, which was probably by oxidation, predominated the changes of (–)-EGCG and (–)-GCG in aqueous system. The rate constants were in the order of $k_y > k_2 > k_1$.

These findings do not support what was reported in the literature in that 82 °C was a turning point in the degradation kinetics of tea catechins (*14*). The earlier reported turning point could be because only degradation was considered in the kinetic model, and the reversible epimerizations were not included in the model in ref *14*.

Model Validation. The mathematical models established under the HT processes (100–165 °C) were validated by the thermal reactions at 145 °C, showing a good agreement between the modeled and the experimental values (*19*). In order to validate the models derived at LT conditions and to exclude the possibilities that the models were overfitted to the experimental values, results from the reactions at 70 °C for various designated heating durations (**Table 1**) were used for model validation.

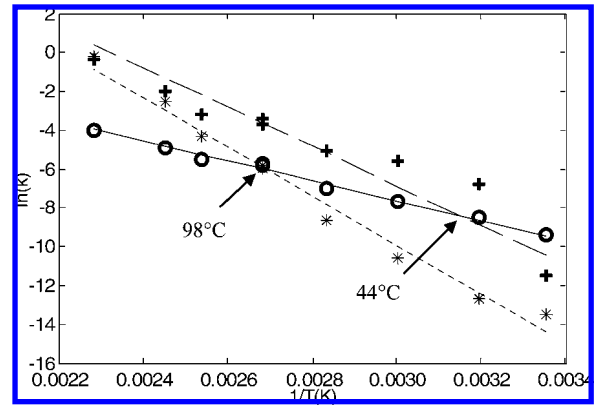

Figure 9. Arrhenius plot of rate constant for the reactions of (–)-EGCG and (–)-GCG in the PEP solutions: ○, rate constant k_y; ∗, rate constant k_1; +, rate constant k_2.

Modeled and experimental data of the catechins (–)-EGCG and (–)-GCG at 70 °C are shown in **Figure 10**. The value of RMSE between the experimental and modeled data was 1.58 for (–)-EGCG with a rather high value of R^2 (0.9917), and 0.05 for (–)-GCG with 0.9878 of R^2. These results clearly showed a good agreement between the modeled and the experimental results, indicating that the derived models were valid and of high quality.

Moreover, it is worth noting that results from the reactions at 25 °C without any heat treatment were involved in the model development in this study. The obtained E_a and A values showed a good agreement between LT and HT reaction kinetics with high values of R^2 and low values of RMSE. These results imply that there is no significant difference from the use of different heating methods. In other words, both microwave reactor and

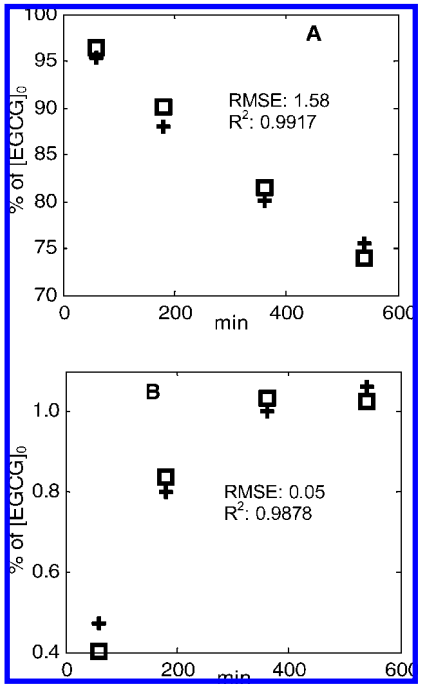

Figure 10. Model predictions and experimental results of the stability of the catechins in the PEP solutions after a thermal process at 70 °C: (**A**) (–)-EGCG; (**B**) (–)-GCG. +, experimental data; □, modeled data.

Table 2. Activation Energy (E_a) and Frequency Factor (A) of Tea Catechins in the PEP Solutions

catechins	E_a, kJ/mol	A
Degradation (k_y) of Total Epi- and Nonepi-Structured Catechins		
(–)-EGCG and (–)-GCG	43.09	2.70×10^3
Epimerization (k_1) from Epi- to Nonepi-Structured Catechins		
(–)-EGCG to (–)-GCG	105.07	1.42×10^{12}
Epimerization (k_2) from Nonepi- to Epi-Structured Catechins		
(–)-GCG to (–)-EGCG	84.33	1.70×10^{10}

water bath can be used for studying the kinetics of catechin reactions, with the microwave reactor being particularly useful for temperatures above 100 °C.

Activation Energy and Frequency Factor. Table 2 lists the activation energy (E_a) and frequency factor (A) corresponding to the reaction kinetics of tea catechins, computed according to Arrhenius equation. The values of E_a for the degradation of total catechins [EGCG + GCG], the epimerization from (–)-EGCG to (–)-GCG, and the epimerization from (–)-GCG to (–)-EGCG were 43.09, 105.07, and 84.33 kJ/mol, respectively. The values of A for the degradation of [EGCG + GCG], the epimerization from (–)-EGCG to (–)-GCG, and the epimerization from (–)-GCG to (–)-EGCG in the PEP solutions were 2.70×10^3, 1.42×10^{12}, and 1.70×10^{10} kJ/mol, respectively. These results differed slightly from those obtained at only HT conditions in ref *19*. However, they are applicable in the temperature range of 25−165 °C.

The reaction rates of degradation and epimerization of catechins were shown to increase with an increase of pH (*19*). Similar findings were reported in ref *12* and *14*. It was further shown that frequency factors of the reaction rates could be pH-dependent while activation energies remained unchanged (*19*). For the wide temperature range covered in the present study, further research is needed to confirm if similar conclusions could be drawn on the effect of pH.

Comparison to the Results on the Stability of Tea Catechins in the Literature. On the basis of the established models and reaction kinetics, the stability of tea catechins can be well predicted. A predicted value of 92% was calculated from the models for the retention of (–)-EGCG at 25 °C for 18 h. This result is very close to the literature value of 90% under similar conditions with pH 5.6 (*25*). Moreover, from the models, ca. 94% of (–)-EGCG was predicted to remain at 37 °C for 7 h. This predicted is fairly comparable to the literature result, which was stated as "almost unchanged" in ref *16*. If the PEP solution is heated under boiling for 30 min, the loss of (–)-EGCG will be predicted as 12.4%. This result is well within the range of 10−15%, reported in ref *7*. However, the modeled result of (–)-EGCG is 82.0% at 120 °C for 20 min and 76.5% for 130 °C/20 min, respectively. These modeled results are relatively greater than 50% stated in ref *7* and *27*. The difference might be first due to the metal ions in tea infusion. Metal ions such as ferrous (Fe^{2+}) compound would decrease the stability of tea catechins (*17, 18*). Second, it could be attributed to the conceivable longer cycle of preheating and cooling processes in autoclave before and after reaching the target temperature, compared to that in microwave reactor. As explained in ref *19*, the processing temperature in microwave reactor was promptly raised to the target, e.g., 100 °C within 1 min, or 121 °C within 90 s, and down to 50 °C in around 2 min. As a result, the total effective thermal processing time in the microwave reactor was considerably shorter than that in the autoclave. Thus, less amount of (–)-EGCG remained after an autoclave process.

In conclusion, the stability of tea catechins in aqueous system was successfully modeled, covering a wide range of temperature from 25 to 165 °C. The mathematical models were well validated by results from the reactions at 70 °C, although the models were developed using different heating methods over the wide range of temperature. The uniformity of E_a and A with high values of R^2 and low values of RMSE suggested that the models were valid and of high quality. No significant interference was detected from the use of different heating methods.

Results in this paper substantiated the previous studies that the degradation and epimerization of tea catechins complied with first-order kinetics, and their rate constants followed Arrhenius equation. E_a for the specific reactions remained unchanged in different catechin solutions with varied concentrations, and A remained the same in the same catechin solutions in the temperature range of 25 to 165 °C. Briefly, E_a for the degradation of the catechins [EGCG + GCG], the epimerization from (–)-EGCG to (–)-GCG, and the epimerization from (–)-GCG to (–)-EGCG was 43.09, 105.07, and 84.33 kJ/mol, respectively.

It is worth noting that two specific points were identified at 98 and 44 °C, respectively. We may infer that above 98 °C, the epimerization from nonepi- to epicatechin was faster than other reactions, whereas below 44 °C, the degradation reaction could be more profound compared to the two epimerization reactions. At temperatures between 44 and 98 °C, the epimerization from nonepi- to epicatechin could occur faster, followed by the degradation and then by the epimerization from epi- to non-epicatechin. Based on these specific points, it is possible to manipulate the reaction rates of the epimerizations among tea catechins by adjusting the temperature of a processing so that a desired ratio between epi- and nonepicatechins in the final product may be achieved.

However, these results do not support the earlier reported turning point of 82 °C in the degradation kinetics of (–)-EGCG and (–)-GCG in ref *14*, which might be due to that the reversible epimerization were not considered in the model there.

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