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Kinetic Study of the Thermal Stability of Tea Catechins in Aqueous Systems Using a Microwave Reactor

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Tea catechins may undergo complex reactions such as oxidation, polymerization, and epimerization during thermal processing. The thermal stability of tea catechins in an aqueous system, including degradation and epimerization reactions, was investigated using a microwave reactor. Reactions were controlled at high temperatures ranging from 100 to 165 °C with various durations up to 120 min. Three sources of tea catechins containing different levels of (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), and their epimers were studied. Kinetic models for the degradation/epimerization of tea catechins were developed and validated by the reactions at 145 °C. It was shown that the epimerization and degradation of tea catechins followed first-order reactions and the rate constants of reaction kinetics followed the Arrhenius equation. Values of the activation energy (E_a) for the epimerization of EGCG from epi- to nonepi-structures, the epimerization of GCG from nonepi- to epi-structures, and the total degradation of EGCG and its epimer GCG were 117.6, 84.2, and 42.8 kJ/mol, respectively. For ECG and CG, the E_a values were 119.3, 96.2, and 41.6 kJ/mol, respectively. The mathematical models may provide a useful prediction for the loss of tea catechins during any thermal processing.

KEYWORDS: Tea catechins; thermal stability; epimerization; degradation; kinetic model

INTRODUCTION

In recent years, there have been extensive studies on the biological and pharmacological activities of tea catechins for their anticarcinogenic, antiinflammatory, and antimicrobial effects, based on the antioxidative property and inhibitory effect of tea catechins on some enzymes (*1*). Although there is no clear-cut conclusion in pharmaceutical applications, the utilization of tea or tea extracts has been dramatically increased in foods and beverages, toiletries, and cosmetic manufactures (*2*). This has brought into question the stability of tea catechins in industrial processes as the beneficial health effects afforded by these compounds will be lost if they are not retained.

The stability of tea catechins in aqueous systems including bottled and canned tea drinks has been studied by many researchers. The stability is dependent on both temperature and pH. The study in ref *3* indicated that the temperature 82 °C was a turning point between two or more different competing reactions, such as thermal degradation and epimerization, in the green tea infusion process. Tea catechins were found to be very stable when the pH was below 4 and extremely unstable if presented in alkaline solutions (*3*−*7*). However, studies on the degradation kinetics in order to predict the remained amount of tea catechins were not systematic. Temperatures covered in those studies were scattered from room temperature to an autoclave temperature of 121 °C in different liquid systems with varied pH values (*3*, *5*, *6*). In ref *3*, it was pointed out that epimerization of catechins occurred in green tea infusion, accompanied with thermal degradation. However, because of the complexity of the kinetic reactions, independent first-order reactions, which was incapable of handling simultaneous epimerization and degradation, were assumed in ref *3*. A kinetic model was developed in ref *5* that predicted the loss of (−)-epigallocatechin gallate (EGCG) in a liquid model system, where the reaction temperature was up to 100 °C with varied pH values ranging from 4 to 7. The involved oxygen concentration was taken into account for the thermal degradation. Unfortunately, there was lack of consideration for the epimerization reactions of EGCG, i.e., the conversion of EGCG to its epimer (−)-gallocatechin gallate (GCG) and vice versa. In other words, no adequate mathematical model was reported to predict the stability of tea catechins involving both degradation and epimerization during thermal processes. On the other hand, consumers nowadays have realized more and more that eating foods with health benefits is better than taking supplements. Tea and tea-related products are getting popular worldwide; hence, the processing parameters will no longer be limited to the most common canning and bottling conditions, such as 100 and 121 °C. Higher temperatures could be involved in modern productions, for example, UHT and baking processes, etc. Thus, a systematic study on the stability of tea catechins is required to ensure the conceivable benefits after various thermal pro-

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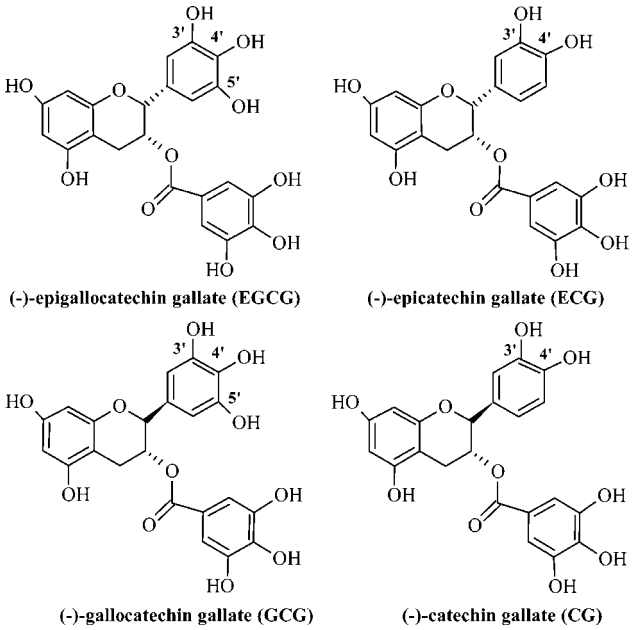

Figure 1. Chemical structures of tea catechins EGCG, ECG, and their epimers GCG and CG.

cesses. The aim of our research presented in this paper was to provide such a systematic study.

EGCG and (−)-epicatechin gallate (ECG) were found to be the most prevalent and effective tea catechins responsible for various biological and pharmacological activities (*1*, *2*). In particular, EGCG was found only in tea products (*8*). Therefore, the amounts of EGCG and ECG are usually accepted as quality indices of the antioxidant activity in tea products (*2*, *9*). In our study, EGCG and ECG were adopted as markers for a kinetic study under various controlled temperatures. Their epimers are GCG and (−)-catechin gallate (CG), respectively. These catechins share a common structure of flavan-3-ol, except an additional hydroxyl group at the 5′-position in EGCG and GCG (**Figure 1**). As microwave reactors are not only capable of providing a rapid and direct heating process but also known to reduce side reactions and improve reproducibility, a microwave reactor was utilized to conduct the thermal processing. To minimize some interference such as ions that may interrupt the stability of tea catechins during reactions (*7*, *10*, *11*), this study used deionized water. It focused on the thermal effect at temperatures ranging from 100 to 165 °C on the degradation and epimerization of tea catechins. Three sources of tea catechins containing different amounts of EGCG and ECG were used for this kinetic study. Mathematical models were developed, and activation energies were calculated.

MATERIALS AND METHODS

Materials. Catechin standards EGCG, GCG, ECG, CG, and ascorbic acid were purchased from Sigma-Aldrich Chemical Co. (United States). Formic acid was purchased from Merck (Germany). Purified EGCG powder (PEP) was obtained from DSM Nutritional Products (Switzerland). Green tea extract (GTE) and green tea polyphenols (GTP) were purchased from Pure Herbal Remedies Pte Ltd. (Singapore), which were made from green tea (*Camellia sinensis*) leaves harvested in Guangxi (China).

Preparation of Tea Catechin Solutions. To eliminate the interference of metal ions, deionized water was used and chemical buffers were avoided in this study. Three sources of tea catechins containing different levels of EGCG were used for the study (**Table 1**). Catechin solutions were prepared to have the same concentration of EGCG (ca. 500 mg/L). The designated concentration was selected from our previous study (*12*), in which the level of EGCG ranged from 200 to 610 mg/L in the aqueous phase of bread dough. The concentrations of ECG varied from 11.7 to 431.8 mg/L, GCG varied from 0 to 212.2 mg/L, and CG varied from 0 to 142.2 mg/L, respectively, in the three sources.

Table 1. Contents of EGCG, ECG, and Their Epimers in the Three Sources of Tea Catechins (wt %)

catechins	PEP	GTE	GTP
EGCG	89.55 ± 1.63 (100)[a] (500 mg/L)[c]	26.14 ± 0.44 (100)[a] (500 mg/L)[c]	19.66 ± 0.24 (100)[a] (500 mg/L)[c]
GCG	(0)[b] (0 mg/L)[c]	6.24 ± 0.15 (24)[b] (119.4 mg/L)[c]	8.34 ± 0.12 (42)[b] (212.2 mg/L)[c]
ECG	2.10 ± 0.05 (100)[a] (11.7 mg/L)[c]	14.47 ± 0.22 (100)[a] (276.7 mg/L)[c]	16.98 ± 0.15 (100)[a] (431.8 mg/L)[c]
CG	(0)[b] (0 mg/L)[c]	3.47 ± 0.09 (24)[b] (66.4 mg/L)[c]	5.59 ± 0.07 (33)[b] (142.2 mg/L)[c]

[a] Data in blankets are the initial relative concentration of epi-structured catechins, set as 100, i.e., $[EGCG]_0 = 100$; $[ECG]_0 = 100$. [b] Data in parentheses are the initial percentages of nonepi-structured catechins relative to the concentrations of the corresponding epi-structured catechins. [c] Data in parentheses are the concentrations of catechins relative to 500 mg/L of EGCG in the freshly prepared and nonheated catechin solutions.

Table 2. Designated Heating Temperatures and Times for Catechin Solutions in the Microwave Reactor

temp (°C)	duration of heating (min)
100	1, 10, 30, 60, 90, 120[a]
121	1, 10, 20, 40, 60
135	1, 5, 15, 30, 45
165	1, 3, 5, 10, 20
145[b]	1, 5, 8, 15, 30

[a] A total of 120 min of heating at 100 °C were applied to the GTE solutions only. [b] A temperature of 145 °C was used to validate the models for catechins in both PEP and GTE solutions.

A carrier with 70% methanol, 29.7% water, 0.3% formic acid, and 0.4% ascorbic acid was developed to stop all reactions in the catechin solutions immediately after a thermal processing. Both carrier and catechin solutions were freshly prepared before use. As samples might be kept in cold storage (4 °C) for more than 2 days before high-performance liquid chromatography (HPLC) analysis, it is necessary to examine the efficiency of the carrier. Experiments on the stability of tea catechins in the carrier under cold storage were conducted. Nonheated and heated samples were mixed with the carrier at a 1:1 volume ratio and then stored at 4 and −20 °C for various lengths of time before they were analyzed. The amounts of catechins detected in the stored samples were compared with those originally present in the freshly prepared and nonheated solutions.

Reactions at the Designated Temperatures and Durations. The thermal processing was conducted in a microwave reactor (Initiator EXP, Sweden). The reaction temperatures were designated to simulate the various thermal processes, including 100 (pasteurization), 121 (sterilization), 135 (UHT), and 165 °C for baking processes where the crust temperature could easily be above this level for a substantial period in a continuous industrial oven (*13*). To exclude the possibility of a kinetic model being overfitted to the experimental results, reactions at a temperature different from those for model development were conducted to validate the kinetic models to be developed. A reaction temperature of 145 °C was chosen for the validation, as it is within the range of 135−165 °C for which very limited study can be found in the literature. The employed various heating times are listed in **Table 2**. Typical temperature profiles in the microwave reactor are shown in **Figure 2**. The temperature was brought to 100 °C within 60 s and 165

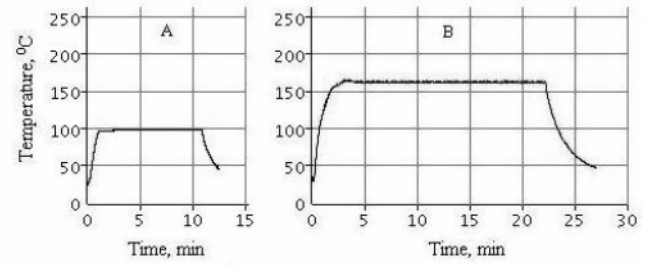

Figure 2. Typical temperature profiles in the microwave reactor. (**A**) Heating at 100 °C for 10 min; (**B**) heating at 165 °C for 20 min.

°C within 150 s. Cooling toward 50 °C was done using compressed air. A solution at 100 °C was able to be cooled to 50 °C within 2 min, while for a 165 °C solution it took ca. 5 min to cool to 50 °C. After it was autocooled, the reaction tube was immediately placed in an ice bath for 10 min, and then, prechilled carrier was added at a 1:1 volume ratio. The solution was kept in cold storage at 4 °C until HPLC analysis.

HPLC Analysis of Tea Catechins. Analysis of tea catechins was performed in a HPLC-photodiode array detection with a UV detector (Waters 2695/2696) and a C18 reversed-phase column (250 mm × 4.6 mm/5 μm, Waters). It was equipped with an autoinjector. Elution of tea catechins was referred to a method described in ref *12* with slight modifications. Mobile phases consisted of 0.10% formic acid in water (eluent A) and methanol (eluent B), respectively. A gradient system was applied as follows: 0−10 min, 10% B; 10−20 min, linear gradient from 10 to 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 to 10% B. The postrun time was 10 min. The sample injection volume was 20 μL. The flow rate was 0.5 mL/min. Detection of tea catechins was at 275 nm.

Kinetics Study. Microsoft Excel program was used for linear regression analysis, where the degree of fit was examined by the coefficient of determination (R^2). Matlab 7.1 was used to model the epimerization reaction kinetics between the pairs of {EGCG, GCG} and {ECG, CG}, obtaining the rate constants of the epimerizations. To validate the developed models, reactions at 145 °C in the PEP and GTE solutions were conducted to examine the accuracy of the models. Experiments with the GTP solutions were conducted to examine the effect of initial concentration of tea catechins in the reaction kinetics. The root-mean-squared error (RMSE) between the experimental values and the model predicted values was taken as a measure of the model quality. Mathematical models for the stability of catechins in the three solutions were compared.

Statistical Analysis. All thermal reactions were performed in at least triplicate, and duplicate samplings were made to every reacted sample for HPLC elution. Data were presented as means ± standard deviations. An analysis of variance single factor test was used to examine the significance at the $P < 0.05$ confidence level.

Modeling Approach. As the degradation of EGCG was reported to follow first-order kinetics (*3*, *5*), the decrease of catechins could be assumed exponentially with time. The concentration of catechins can be described as

$$x = x_0 e^{-kt} \tag{1}$$

or

$$\ln(x/x_0) = -kt \tag{2}$$

where x is the concentration of a catechin at time t, x_0 is the initial concentration of the catechin, and k is the rate constant of the degradation or epimerization of the catechin. As the chemical structures of epi- and nonepicatechins only differ between 2*R*,3*R* (2,3-cis,epi-form) and 2*S*,3*R* (2,3-trans,nonepi-form) (**Figure 1**), an assumption was made that the rate constants of the degradations of epi- and nonepi-structured catechins were similar. Hence, the rate constant k_y of the degradation of the total epi- and nonepicatechins, e.g., EGCG and GCG, could be obtained from the gradient of a best-fitted straight line if ln-([EGCG] + [GCG])/([EGCG]$_0$ + [GCG]$_0$) was plotted against time, t. Assume k_y follows the Arrhenius equation, i.e.,

$$k_y = A\, e^{-E_a/RT} \tag{3}$$

where A is the frequency factor, E_a is the activation energy, R is the ideal gas constant, and T is the temperature in Kelvin. E_a and A could be obtained by linear regression from the (k_y, T) data.

In our kinetic modeling, both degradation and epimerization were taken into account, because they occurred simultaneously (*3*). As a nonepicatechin such as GCG increased with time exponentially in green tea infusion, first-order kinetics can be assumed for the corresponding epimerization reaction (this assumption will be examined with the experimental data in the next section). As the initial concentration of catechins might vary in different preparations, normalization of the concentration was necessary. The concentration of EGCG and its epimer GCG in any processed sample was always normalized by the corresponding initial concentration of EGCG in the catechin solutions, denoted as y and z, respectively, i.e., $y = ([\text{EGCG}]/[\text{EGCG}]_0) \times 100$; $z = ([\text{GCG}]/[\text{EGCG}]_0) \times 100$. The kinetic models can be described as follows:

$$y' = -(k_1 + k_y)y + k_2 z \tag{4}$$

$$z' = -(k_2 + k_y)z + k_1 y \tag{5}$$

where y' and z' are the rate of change of y and z, respectively. k_1 is the rate constant of epimerization from EGCG to GCG, and k_2 is the rate constant of epimerization from GCG to EGCG. Denoting $a = k_1 + k_y$, $b = k_2$, $c = k_2 + k_y$, and $d = k_1$, the following equation can be derived from eq 4:

$$z = (y' + ay)/b$$

therefore,

$$z' = (y'' + ay')/b$$

Substituting the above two equations to eq 5 results in

$$y'' + (a + c)y' + (ac - bd)y = 0$$

This is a second-order ordinary differential equation. Its solution can be written as:

$$y = C_1 e^{-k_y t} + C_2 e^{(-k_1 - k_2 - k_y)t} \tag{6}$$

$$y' = -C_1 k_y e^{-k_y t} + C_2(-k_1 - k_2 - k_y)e^{(-k_1 - k_2 - k_y)t} \tag{7}$$

where C_1 and C_2 are constants to be determined by the initial conditions. Note that $y(0) = 100$ and $z(0) = ([\text{GCG}]_0/[\text{EGCG}]_0) \times 100$. For the PEP solutions, $z(0) = 0$ (**Table 1**). From eq 6, it is easy to obtain

$$C_1 + C_2 = 100$$

$$C_2 = 100 - C_1$$

Substituting the above to eqs 4 and 7 and taking $t = 0$, the two constants can be derived as:

$$C_1 = 100k_2/(k_1 + k_2) \tag{8}$$

$$C_2 = 100k_1/(k_1 + k_2) \tag{9}$$

The activation energy (E_a) of the respective degradation and epimerization was calculated according to eq 3 for k_y, k_1, and k_2. Substituting eqs 8 and 9 to eq 6, a mathematical model for predicting the concentration of EGCG in the PEP solutions is obtained

$$y = 100k_2/(k_1 + k_2)e^{-k_y t} + 100k_1/(k_1 + k_2)e^{(-k_1 - k_2 - k_y)t} \tag{10}$$

Similarly, a model for the concentration of GCG in the PEP solutions is

$$z = 100k_1/(k_1 + k_2)e^{-k_y t} - 100k_1/(k_1 + k_2)e^{(-k_1-k_2-k_y)t} \quad (11)$$

Because the initial concentration of the catechin ECG (2%) was dramatically lower in the PEP solutions as compared to that of EGCG in the GTE and GTP solutions, the accuracy and reliability of the measurements of CG, the epimer of ECG, became questionable. Consequently, modeling could not be carried out for ECG and its epimer CG in the PEP solutions. The major catechin epimers EGCG and GCG were the focus of the modeling in the PEP solutions.

In the GTE and GTP solutions, mathematical models for all four catechins covering various concentrations were developed. Noting that $[GCG]_0 = 24$ and $[CG]_0 = 24$ in the GTE solutions (**Table 1**), the models for EGCG and ECG in the GTE solutions could be obtained as follows:

$$y = 124k_2/(k_1 + k_2)e^{-k_y t} + (100k_1 - 24k_2)/(k_1 + k_2)e^{(-k_1-k_2-k_y)t} \quad (12)$$

For their epimers GCG and CG in the GTE solutions, the models are

$$z = 124k_1/(k_1 + k_2)e^{-k_y t} + (24k_2 - 100k_1)/(k_1 + k_2)e^{(-k_1-k_2-k_y)t} \quad (13)$$

In the GTP solutions, $[GCG]_0 = 42$ (**Table 1**). The models for EGCG and GCG are

$$y = 142k_2/(k_1 + k_2)e^{-k_y t} + (100k_1 - 42k_2)/(k_1 + k_2)e^{(-k_1-k_2-k_y)t} \quad (14)$$

$$z = 142k_1/(k_1 + k_2)e^{-k_y t} + (42k_2 - 100k_1)/(k_1 + k_2)e^{(-k_1-k_2-k_y)t} \quad (15)$$

Similarly, as $[CG]_0 = 33$ (**Table 1**), the models for ECG and CG in the GTP solutions are

$$y = 133k_2/(k_1 + k_2)e^{-k_y t} + (100k_1 - 33k_2)/(k_1 + k_2)e^{(-k_1-k_2-k_y)t} \quad (16)$$

$$z = 133k_1/(k_1 + k_2)e^{-k_y t} + (33k_2 - 100k_1)/(k_1 + k_2)e^{(-k_1-k_2-k_y)t} \quad (17)$$

RESULTS AND DISCUSSION

Efficiency of the Carrier on the Stability of Tea Catechins during Storage. Considering that the catechin isomers after a thermal processing could degrade slowly at room temperature (*6*, *10*, *14*), 0.4% ascorbic acid was adopted in the carrier to immediately stop the degradation in processed solutions after heating and keep it until HPLC elution (*14*). The residual ascorbic acid in the final solution at the time of HPLC analysis was around 0.2%. Results on the efficiency of the carrier on the stability of catechins are listed in **Table 3**, showing that tea catechins were very stable in the solutions with the carrier added (pH 3.0) for up to 10 days at 4 °C, regardless if the solutions were processed in the microwave reactor or not.

Verification of the First-Order Kinetics for the Degradation of Tea Catechins. It is worthy to note that a holding time of 1 min at a designated temperature between 100 and 165 °C was considered too short for the catechins in the GTE and GTP solutions to carry out significant degradation or epimerization. There was no significant loss of catechins, i.e., $[EGCG]_{t=0}$ was approximately equal to $[EGCG]_{t=1}$. This could be due to the characteristics of the microwave reactor and the favorable pH range of 3.5−3.7. To establish the actual kinetics for the reactions of tea catechins in the GTE system, $t' = 1$ min was taken as the effective starting point for modeling the reactions of catechins in the GTE and GTP solutions. This phenomenon was not found in the PEP solutions where the pH was 5.1; therefore, the amounts of tea catechins starting from $t = 0$ were used in modeling the kinetics of catechins in the PEP solutions.

Table 3. Stability Test of Tea Catechins in the PEP Solutions Mixed with the Carrier at 1:1 Ratio

catechins solutions	shelf storage	EGCG (%)[a]
nonheated PEP	ambience, 0 day	99.6 ± 0.0
nonheated PEP	4 °C, 10 days	102.0 ± 2.1
nonheated PEP	−20 °C, 10 days	100.2 ± 0.3
heated PEP	4 °C, 10 days	100.5 ± 1.4
heated PEP	−20 °C, 6 days	99.0 ± 0.3

[a] The percentages are relative to the concentration of EGCG at the beginning of storage.

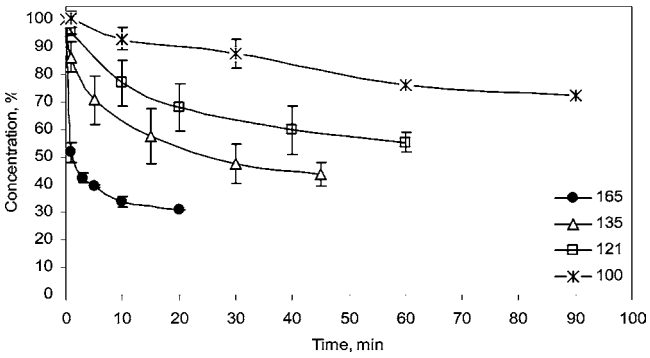

Figure 3. Concentration profile of EGCG in the PEP solutions in the microwave reactor at different temperatures, $n = 6$.

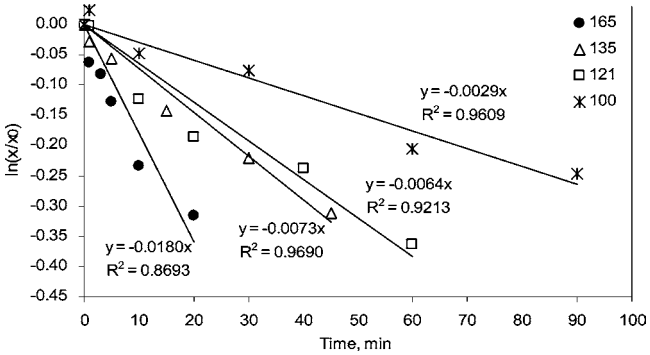

Figure 4. Arrhenius plot of apparent first-order degradation of EGCG and GCG in the PEP solutions at different temperatures in the microwave reactor, $n = 6$.

The concentration of the catechin EGCG appeared to decrease exponentially with time in the PEP solutions (**Figure 3**) as well as in the GTE and GTP solutions. The concentration of EGCG + GCG, i.e., the total of the two epimers, also decreased exponentially with time in the three solutions. A series of k_y at different temperatures were obtained from plotting $\ln(x/x_0)$ vs t, where x is the total concentration of EGCG and GCG (**Figure 4**). The rate constant k_y increased with an increase of temperature.

According to eq 3, plotting $\ln(k_y)$ against inversed temperature ($1/T$), a straight line should be obtained. **Figure 5** shows the straight lines in the Arrhenius plots of the degradation kinetics of catechins in the three solutions. These results confirmed the findings in refs *3* and *5* that the rate constant of the degradation of catechins in aqueous system followed the Arrhenius equation.

Moreover, it is well-known that the activation energy (E_a) refers to the change in the potential energy of a chemical system that is required to convert reactants into products by a reaction.

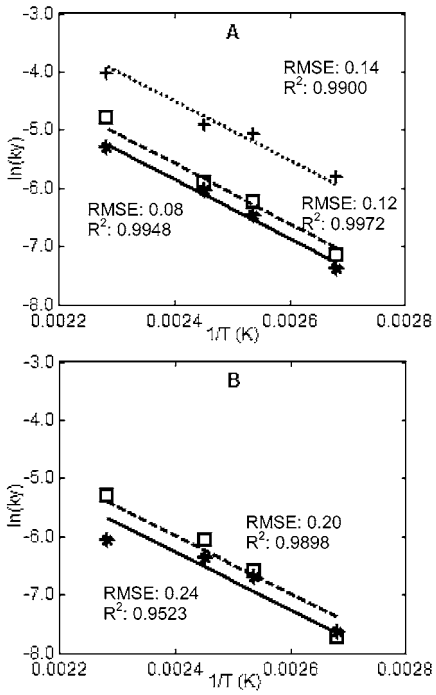

Figure 5. Arrhenius plot of the rate constant k_y of tea catechins in the three catechins solutions. (**A**) The total of EGCG and GCG; (**B**) the total of ECG and CG. Key: +, in the PEP solutions; □, in the GTE solutions; and *, in the GTP solutions.

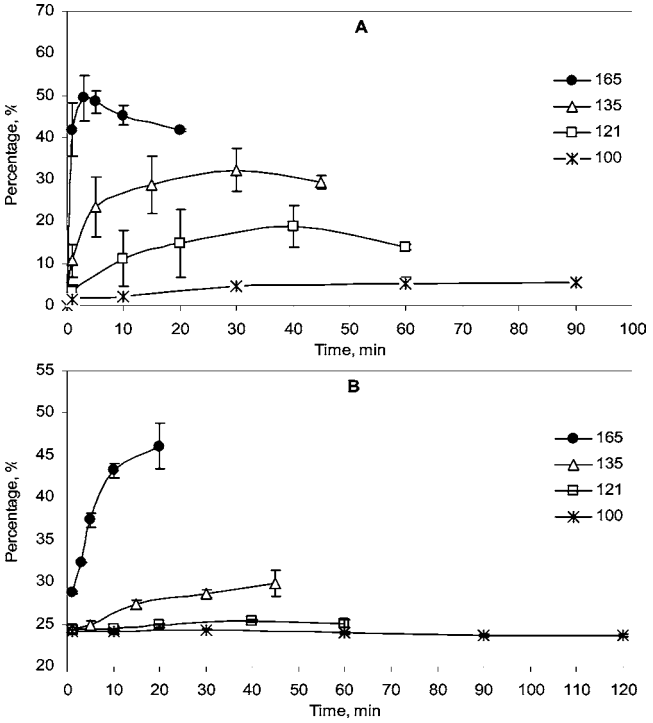

Figure 6. Concentration profile of nonepi-structured catechins at different temperatures in the microwave reactor, $n = 6$. (**A**) GCG in the PEP solutions; (**B**) CG in the GTP solutions.

In other words, E_a is independent of the medium environment where the reaction proceeds. Hence, the E_a of the thermal degradation of the paired catechins, i.e., {EGCG + GCG} and {ECG + CG}, should remain unchanged in the three solutions. Three parallel lines were thus obtained, as shown in **Figure 5**, by linear regression with constraints using Matlab 7.1. The coefficients of determination (R^2) were all above 0.95, and the RMSE ranged from 0.08 to 0.24 between the modeled data and the experimental values of catechins in the three solutions, indicating the validity of not only the assumption that the degradation of catechins followed first-order kinetics but also the assumption that the rate constants of degradation, k_y, for all epimer pairs, i.e., {EGCG, GCG} and {ECG, CG}, were similar. GCG shares the same chemical formulas with EGCG and has a very similar structure to it (**Figure 1**). It was reported in ref *15* that GCG in 2,3-trans form is more stable than EGCG in 2,3-cis form. On the other hand, it was also reported in the same study that GCG is a more effective scavenger to free radicals in comparison with EGCG when both were at a low concentration of 0.1 mmol/L, while there were no significant differences in the scavenging ability when both concentrations were from 0.3 to 0.5 mmol/L. In our present study, the initial concentration of EGCG was 1.1 mmol/L in the three catechin solutions, and that of GCG was 0, 0.26, and 0.46 mmol/L in the PEP, GTE, and GTP solutions, respectively. Overall, the difference in the degradation kinetics between GCG and EGCG could be very little. In other words, the assumption that the rate constant k_y of EGCG was similar to that of GCG was reasonable. For the pair of ECG (2,3-cis form) and CG (2,3-trans form), they also have similar structures, and the assumption that ECG and CG have similar rate constants k_y is also valid.

Epimerization of Tea Catechins. While the epi-structured catechins appeared to decrease, their nonepi-structured counterparts increased exponentially with time. The concentrations of GCG and CG at different temperatures with various heating times are presented in **Figure 6**. The increase of the nonepi-structured catechins during the thermal processing was consistent with the studies of many researchers (*3*, *6*, *16*, *17*). The rate of increase in the nonepicatechins showed a similar trend to the rate of decrease in the epicatechins in the three solutions, i.e., the epimerization rate increased with increasing temperature. Catechins and their epimers in the three solutions exhibited similar kinetic characteristics. For the epimerization of EGCG at a high temperature of 165 °C in the PEP solutions, the concentration of GCG first increased to a maximum, then decreased due to thermal degradation. It can be seen from **Figure 6A** that the formation of GCG at 121 °C reached a maximum level at around 20−40 min in the PEP solutions, then declined with prolonged reaction to 40−60 min. The epimerization trend was similar to the study in ref *16* reporting that the epimerization of GCG from EGCG at 120 °C reached a maximum level at around 30−60 min but decreased with prolonged reaction from 60 to 90 min at 130 °C. A similar pattern for the epimerization from ECG to CG was also observed in the GTE and GTP solutions (**Figure 6B**). In general, these results were in good agreement with the study in ref *3*, showing that the degradation and epimerization of catechins occurred concurrently during thermal processing.

Mathematical Modeling of the Reaction Kinetics. On the basis of the measurements of tea catechins during thermal processing, first-order reactions for the degradation and epimerization were proposed.

With the results of k_y at different temperatures and the assumption of first-order kinetics, rate constants of the epimerizations k_1 (from epi- to nonepi-structured catechin) and k_2 (from nonepi- to epi-structured catechin) were obtained by nonlinear regression using Matlab 7.1. Similar to the results on thermal degradation as described early, the activation energy E_a of epimerizations between the epi- and the nonepicatechins, e.g., EGCG and GCG, should be the same in different solutions. Plotting ln(k) against the inversed temperature, 1/T, parallel best-

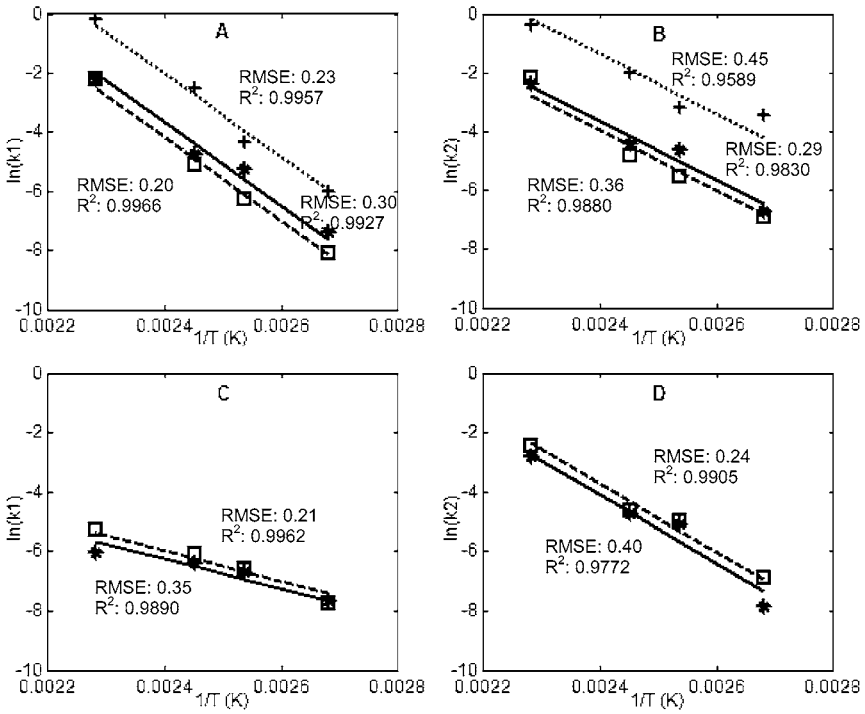

Figure 7. Arrhenius plots of the rate constants of the epimerization reactions. (**A**) Rate constant k_1 for the epimerization from EGCG to GCG, (**B**) rate constant k_2 for the epimerization from GCG to EGCG, (**C**) rate constant k_1 for the epimerization from ECG to CG, and (**D**) rate constant k_2 for the epimerization from CG to ECG. Key: +, in the PEP solutions; □, in the GTE solutions; and *, in the GTP solutions.

fit lines were obtained as shown in **Figure 7**. The corresponding values of R^2 were all above 0.95, and the values of RMSE were between 0.20 and 0.45 for both k_1 and k_2 in all three different solutions, suggesting that the epimerizations of catechins indeed followed first-order reactions and the assumption that the activation energy E_a of epimerizations in different solutions remain unchanged is valid.

Model Validations. Although the developed kinetic models showed good agreement between the model predicted values and the experimental results under all experimental conditions, to examine the validity of the models and exclude the possibility that the models were overfitted to the experimental values, thermal processing of tea catechins at 145 °C for designated heating durations (**Table 2**) was conducted in both PEP and GTE solutions. Modeled and experimental data of epi-structured and nonepi-structured catechins in the PEP and GTE solutions are presented in **Figure 8**. The RMSE between the modeled and the experimental data at 145 °C ranged from 0.60 to 3.81, and the coefficients of determination R^2 were all above 0.96 for the catechins in **Figure 8A**−**F**. It clearly shows that the model predicted values at 145 °C were well in agreement with the experimental results, indicating that the developed models were valid and of high quality.

Activation Energy and Frequency Factor. The activation energy E_a and the frequency factor A of the reaction rate constants of tea catechins, calculated from the Arrhenius plots, are listed in **Table 4**. The E_a of the EGCG degradation was 42.78 kJ/mol, i.e., 10.4 kcal/mol, which was between the literature values of 4.7 kcal/mol in ref *3* and 18.7 kcal/mol in ref *5*. The difference could be due to the fact that only one concentration level of EGCG was studied in ref *3* and the epimerization of catechins was completely ignored in ref *5*.

Results in **Table 4** show that the E_a of the degradation of EGCG and GCG was similar to that of ECG and CG, while A was ca. two times greater in the former pair than the latter, suggesting that ECG and CG are more stable than EGCG and GCG in thermal processing. These findings are in agreement with those in refs *4* and *7*. It is likely due to the structural property of the catechins. In the past decade, many researchers reported that those catechins with an additional hydroxyl group (OH) at C-5′ in the B ring such as EGCG and GCG (**Figure 1**) were more active than the others with only two adjacent −OH groups at C-3′ and C-4′ such as ECG and CG (*18*). This structural feature plays an important role in their antioxidative activity, leading to EGCG and GCG being more vulnerable to destruction.

In addition, comparing the values of E_a and A of the epimerizations, it was found that E_a of the epimerizations from epi- to nonepi-structure was only 1.2−1.4 times greater than that from nonepi- to epi-structure. However, the frequency factor A of the former was ca. 10^4 greater than that of the latter. These findings reveal that the epimerizations of catechins from epi- to nonepi-structure are the predominant reactions at high temperatures rather than the reversed epimerizations. As the frequency factor indicates how many collisions between the reactants have the correct orientation leading to the products, the lower values of A in the epimerizations from nonepi- to epi-structure could be attributed to the stereostructural property of the catchins. GCG/CG in the 2,3-trans form has a smaller steric hindrance than that of EGCG/ECG in the 2,3-cis form (*10*, *15*) and possibly exhibits a lower rate of collisions during thermal reactions.

Effects of pH on the Rate Constant. Kinetics results have shown that tea catechins in the three solutions were vulnerable to degradation and epimerization, and the rate constants increased with an increase in temperature. Moreover, the rate constants of the degradation and epimerization of EGCG and GCG, i.e., k_y, k_1, and k_2 in the PEP solutions were much greater than those in the GTE and GTP solutions at the same temperature, as shown in **Figures 5** and **7**. These results were consistent with the values of frequency factor (A) in **Table 4**, where A values for the two catechins EGCG and GCG were

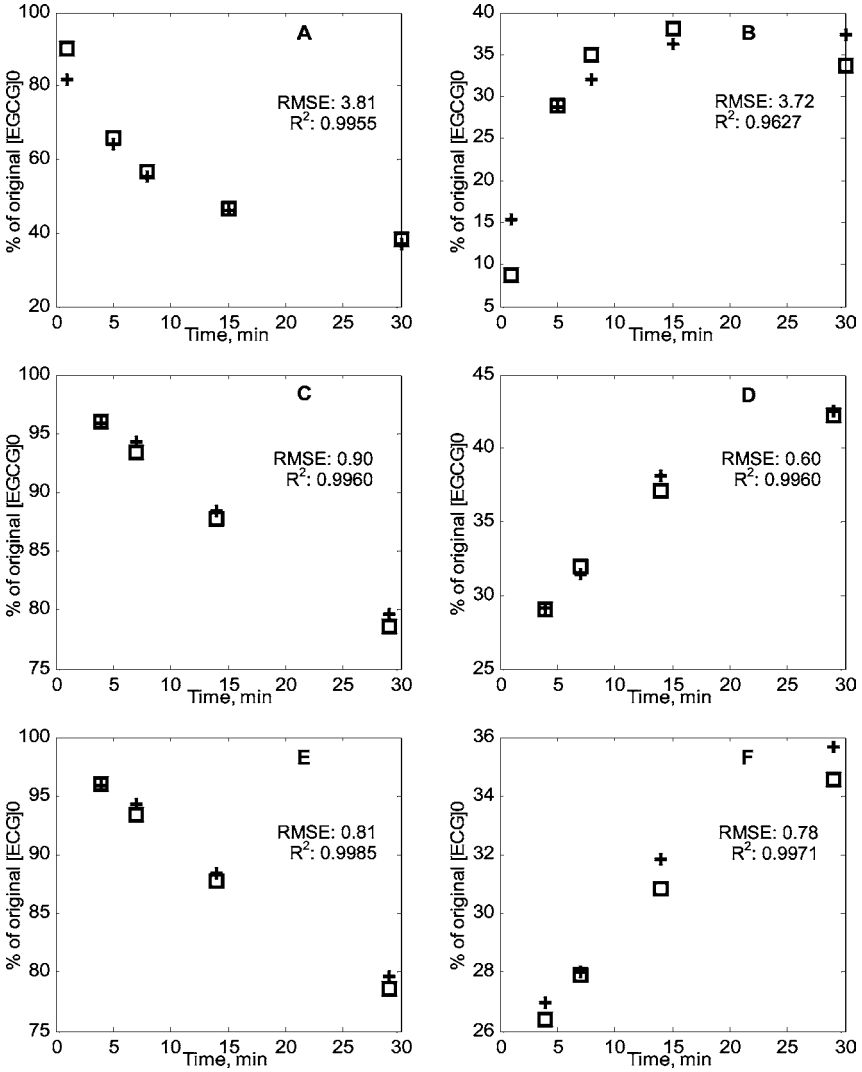

Figure 8. Modeled and experimental values of tea catechins at 145 °C. Key: □, modeled data; +, experimental data. (**A**) EGCG in the PEP solutions, (**B**) GCG in the PEP solutions, (**C**) EGCG in the GTE solutions, (**D**) GCG in the GTE solutions, (**E**) ECG in the GTE solutions, and (**F**) CG in the GTE solutions. The concentration of catechins was expressed as a percentage of the initial concentration of the corresponding epicatechins.

greater in the PEP solution than those in the other two solutions. According to eq 3, the rate constant increases with an increase in the value of frequency factor A when the E_a value remains the same.

The kinetics results were comparable to those in the literature, which were attributed to initial pH and varied heating temperature. It was reported in ref *5* that the rate constant of the degradation of EGCG induced by oxygen could increase 180 and 800% when temperature and pH increased by 10 °C and 1 unit, respectively. Similarly, in our present study, the rate constants including k_y, k_1, and k_2 were significantly increased with the elevation of temperature from 100 to 165 °C in the three solutions. In addition, it was stated in refs *6* and *16* that a pH at 5 resulted in the most efficient formation of GCG from EGCG when autoclaved at 120 °C for 20−30 min and a lower pH at 3−4 significantly decreased this formation. In our present study, the pH of freshly prepared catechin solutions using deionized water was 5.1 for PEP and 3.5−3.7 for GTE and GTP. They differed by up to 1.6 units in pH value. As a combined result, the rate constants k_y, k_1, and k_2 for the catechins EGCG and GCG in the PEP solutions were increased by 2.2−3.8 times, 4.5−13 times, and 3.9−32 times, respectively, as compared to those in the GTE solutions at various temperatures. Hence, it is conceivable that the degradation of the catechins EGCG and GCG in aqueous system with pH 5.1 was faster than that in the GTE and GTP solutions, which were at relatively low pH values of 3.5−3.7.

As the rate constant increased with an increase of pH while E_a remained the same, it was postulated that the frequency factor increased with an increase of pH. In other words, the rate constant or frequency factor for catechins could be pH sensitive, in particular when pH > 4. The study in ref *19* stated that at neutral or alkaline pH, tea catechins increased their proton-donating potential and became easier to form the corresponding semiquinone free radicals, leading them to be more susceptible to deformation. Although the degradation rate of catechins was found to increase with an increase of pH (*4*−*7*) and the isomerization/epimerization was pH-sensitive (*3*, *6*, *16*), validation of the postulation is required and further investigation in a wide range of pH values covering acidic to alkaline conditions is necessary.

Conversion Rate from EGCG to GCG by Epimerization. It was found that the thermal processing method might play an important role in the results of reaction kinetics. Using the described models, rate constants k_y, k_1, and k_2 of the catechins EGCG and GCG in the PEP solutions were back-calculated to obtain the conversion rate of EGCG to GCG at two selected thermal conditions, which were 100 °C/20 min and 120 °C/30

Table 4. Activation Energy (E_a) and Frequency Factor (A) of the Reaction Rate Constants of Tea Catechins in the PEP, GTE, and GTP Solutions

catechins	activation energy E_a (kJ/mol)	frequency factor A
A: degradation of total epi- and nonepi-structured catechins		
EGCG and GCG in PEP	42.78	2.56×10^3
EGCG and GCG in GTE	42.78	0.88×10^3
EGCG and GCG in GTP	42.78	0.67×10^3
ECG and CG in GTE	41.58	0.41×10^3
ECG and CG in GTP	41.58	0.31×10^3
B: epimerization from epi- structured to nonepi-structured catechins		
EGCG to GCG in PEP	117.59	7.29×10^{13}
EGCG to GCG in GTE	117.59	0.85×10^{13}
EGCG to GCG in GTP	117.59	1.42×10^{13}
ECG to CG in GTE	119.25	1.14×10^{13}
ECG to CG in GTP	119.25	0.96×10^{13}
C: epimerization from nonepi-structured to epi-structured catechins		
GCG to EGCG in PEP	84.15	8.97×10^9
GCG to EGCG in GTE	84.15	0.66×10^9
GCG to EGCG in GTP	84.15	0.93×10^9
CG to ECG in GTE	96.22	28.0×10^9
CG to ECG in GTP	96.22	19.0×10^9

min. The conversion rates of EGCG to GCG were calculated as 4.0 and 17.4% in the PEP solutions at 100 °C/20 min and 120 °C/30 min, respectively. However, much higher values of 34.6 and 56.6% were reported in refs *10* and *16*.

It was reported that for some tea catechins, e.g., EGCG and (−)-epigallocatechin (EGC), several dimers could be formed by coupling the molecules of either the same type or different types of the catechins (*20*, *21*), even in the absence of reagents or oxidative enzyme (*22*). The dimerization reactions of tea catechins were normally subjected to oxidative reaction, triggered by active species such as oxygen, metal ions, and enzymes in the solution (*20*−*22*). Green tea products usually contain various catechins as well as trace amounts of metal ions. Therefore, the dimerization reactions among the catechins are inevitable during thermal processing, which might result in lesser epimerizations as compared to those in the study of refs *10* and *16*, in which the pure standards of catechins were used. However, the reduction of the catechins due to dimerization was part of the overall thermal degradation, which was accounted for by the kinetic models developed in this study.

The difference might also be attributed to the actual processing time in the corresponding thermal processing method. A conceivable longer preheating and cooling time before and after reaching the target temperature is commonly practiced in normal heating processes such as an autoclave at 100 and 120 °C, causing a prolonged effective thermal processing time in total. As a result, the epimerization of catechins would be more intensive, having a higher conversion rate. When a thermal processing was conducted in the microwave reactor, a much shorter period of preheating course was present, as shown in **Figure 2**. Temperature was promptly brought to the target 100 °C within 1 min or 121 °C within 90 s and down to 50 °C in around 2 min. Thus, with the same set of processing temperatures and times, the total effective thermal processing time in the microwave reactor was considerably shorter in comparison with processing in autoclaves, leading to a much smaller conversion rate. Results on the reaction kinetics using microwave reactors are more accurate and reliable.

In conclusion, the thermal stability of tea catechins in aqueous system has been investigated, covering much higher temperature ranges than those in the literature. Mathematical models for predicting the concentration of catechins during thermal processing were developed. The modeled values well-matched the experimental results. It was found that the degradation and epimerization of tea catechins all followed first-order kinetics. The rate constants of reaction kinetics for catechins followed the Arrhenius equation. In the present study, rate constants/frequency factors were pH-sensitive as they were found greater in the PEP solutions (pH 5.1) than those in the GTE and GTP solutions (pH 3.5−3.7). The activation energy of all reactions remained unchanged with varied compositions in the solutions. The values of activation energy (E_a) were 117.6, 84.2, and 42.8 kJ/mol for the epimerization of EGCG to GCG, the reversed epimerization from GCG to EGCG, and the total degradation of EGCG and GCG, respectively. For the catechin pair ECG and CG in the GTE solutions, the corresponding E_a values for the epimerizations and degradation were 119.3, 96.2, and 41.6 kJ/mol, respectively. It is worth pointing out that this study used a microwave reactor for conducting the thermal processing and deionized water as the reaction medium. These conditions are not used in practical food processing. However, the methodology and results of this study may provide useful references for future studies in assessing the thermal stability of tea catechins during thermal processes, particularly those involving high temperatures.

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