Degradation Kinetics of Catechins in Green Tea Powder: Effects of Temperature and Relative Humidity

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Ⓢ *Supporting Information*

ABSTRACT: The stability of catechins in green tea powders is important for product shelf life and delivering health benefits. Most published kinetic studies of catechin degradation have been conducted with dilute solutions and, therefore, are limited in applicability to powder systems. In this study, spray-dried green tea extract powders were stored under various relative humidity (RH) (43−97%) and temperature (25−60 °C) conditions for up to 16 weeks. High-performance liquid chromatography (HPLC) was used to determine catechin contents. Catechin degradation kinetics were affected by RH and temperature, but temperature was the dominant factor. Kinetic models as functions of RH and temperature for the individual 2,3-*cis*-configured catechins (EGCG, EGC, ECG, and EC) were established. The reaction rate constants of catechin degradation also followed the Williams−Landel−Ferry (WLF) relationship. This study provides a powerful prediction approach for the shelf life of green tea powder and highlights the importance of glass transition in solid state kinetics studies.

KEYWORDS: EGCG, solid state, stability, kinetic model, shelf life, glass transition

■ INTRODUCTION

Green tea polyphenols, also known as green tea catechins, derived from the plant *Camellia sinensis* (L.) O. Kuntze, are receiving increasing attention worldwide due to their perceived health-promoting effects, including antioxidant, anti-inflammatory, antithrombogenic, and anticarcinogenic activities.[1] The four main green tea catechins are (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC). These 2,3-*cis*-configured compounds can undergo reversible epimerization to their corresponding 2,3-*trans* epimers: (−)-gallocatechin gallate (GCG), (−)-gallocatechin (GC), (−)-catechin gallate (CG), and (−)-catechin (C), respectively (Figure 1). EGCG, which is the most abundant antioxidative component in green tea, in particular has been widely investigated as a marker of green tea catechins in kinetic and stability studies.[2−4] It is important to understand the stability of catechins in tea products and ingredients in order to deliver these compounds to consumers.

Catechins are susceptible to degradation by many environmental factors, which have greatly limited their dietetic and pharmaceutical applications. Green tea catechin degradation patterns have been well characterized in dilute green tea extracts or purified EGCG in aqueous solutions and tea beverages under common processing conditions.[2−7] The degradation of catechins was reported to follow first-order or pseudo-first-order kinetics in dilute solutions of 20 mg/L EGCG,[3] 80 mg/L EC,[5] 1% (w/w, tea leaves/water) green tea infusions,[5] and 500 mg/L EGCG solution[6] in different studies. Temperature and pH have been identified as two critical factors affecting catechin stability in aqueous solutions.[5−7] Temperature has been reported to not only affect the reaction rate constant but also alter the reaction mode if several types of reaction occur simultaneously.[2] Arrhenius behavior has been observed for green tea catechin degradation

kinetics.[2,3,5,6] Zimeri et al.[3] reported a log−linear relationship between pH and rate constant for EGCG degradation in dilute aqueous solution. Catechins were found to be susceptible and extremely unstable in alkaline solutions, whereas acidic pH conditions (pH <4[7] and pH <5[5]) helped retain catechin stability. Other important factors affecting catechin stability in powdered products are water activity (or relative humidity) and formulation. According to Ortiz et al.,[8] significantly higher losses of catechins in green tea powder were observed with increasing relative humidity (RH) conditions at ambient temperature, whereas catechin chemical stability was maintained at RH conditions below 43%.

Although many tea products are in the leaf or beverage form, powdered tea extracts are widely used in manufacturing, delivery, and retail sales due to their light weight, ease of reconstitution, and long shelf life. In food powder systems, moisture is often a key mediator affecting chemical and physical stability. Water absorbed into amorphous powder systems, acting as a plasticizer and possible reactant, accelerates chemical reactions by enhancing molecular mobility as well as changing the local environment.[8] Water activity as a non-negligible factor affecting catechin stability remains insufficiently understood in kinetic models of catechin degradation. To our knowledge, there is no information about the reaction kinetics of green tea catechins in the solid state. Although kinetic models have been developed in different dilute aqueous solutions,[2,3,5,6] these models may not apply to powder systems due to increased concentration, reduced molecular
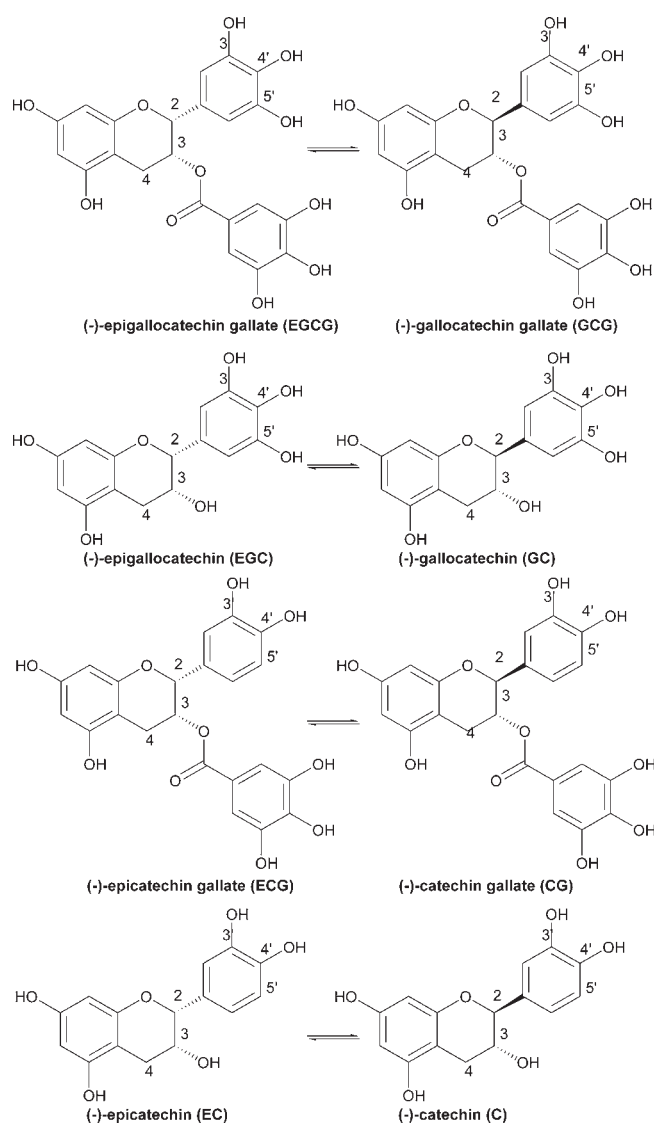
Figure 1. Chemical structures of the major catechins in green tea.

mobility, and potentially altered microenvironments. Therefore, the objective of this study was to investigate the kinetics of catechin loss in spray-dried green tea powders under various RH and temperature conditions.

■ MATERIALS AND METHODS

Materials. Green tea powder (Nestlé TCTG GT) was a gift from the Nestlé Research and Development Center (Marysville, OH). Catechins (EGCG, GCG, ECG, CG, EGC, GC, EC, C) and caffeine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Methanol (HPLC grade), acetonitrile (MeCN) (HPLC grade), and glacial acetic acid were purchased from Mallinckrodt Baker (Phillipsburg, NJ). Drierite was purchased from Fisher Scientific (Pittsburgh, PA). The saturated salt solutions used in environmental chambers to provide different RH conditions were potassium carbonate, sodium bromide, sodium chloride, potassium bromide, potassium chloride, and potassium sulfate, all purchased from Sigma-Aldrich. The selected salts and their RH values at different temperature conditions are shown in the Supporting Information. The selection of RH conditions was based on the glass transition point of green tea powder at the experimental

temperatures. All storage experiments were conducted in the dark in Forma Scientific model 3187 temperature-controlled incubators (Marietta, OH). The temperature and RH conditions were verified by thermometers and an AquaLab 3TE water activity meter (Decagon Devices Inc., Pullman, WA), respectively.

Storage Treatments. To determine the effects of temperature and RH on catechin stability in green tea powders, triplicate samples of 0.5 g of green tea powder were prepared in 20 mL glass vials (VWR International LLC, West Chester, PA) and stored for up to 16 weeks in environmental chambers at various RH (41−97% RH) and temperature (25, 35, 40, 50, 60 °C) conditions (further details are provided in the Supporting Information). These conditions were chosen to encompass what could be encountered in food product preparation, storage, distribution, and/or accelerated shelf life testing, as well as to create conditions above and below the glass transition of the amorphous tea powder. After storage treatment, the glass vials were sealed and samples were immediately frozen at −80 °C.

Chemical Stability Determination with HPLC. Catechin contents were determined by HPLC. Prior to HPLC analysis, all samples were equilibrated to room temperature and then diluted with 2% acetic acid aqueous solution to a final concentration of 1 mg/mL (green tea). All samples were filtered with 0.45 μM membrane filters prior to injection. The elution method follows the approach described by Neilson et al.[9] with minor modifications. Briefly, a Waters Alliance 2690 separation module equipped with a Waters 996 photodiode array detector was used. The column used was a 100 mm × 3.9 mm i.d., 3.5 μm, Waters XTerra RP-18. The wavelength scan used was between 200 and 400 nm. Mobile phase A, comprising ddH$_2$O (distilled water), MeCN, and trifluoroacetic acid (TFA) (919:80:1, v/v), and phase B, comprising ddH$_2$O, MeCN, methanol, and TFA (699:270:30:1, v/v) were used with the following gradient procedure at a flow rate of 0.9 mL/min: 95:5 at 0 min, 30:70 at 10 min (convex), 1:99 at 13 min (convex), and 95:5 from 14 to 18 min (step, immediate), for a total chromatographic run time of 18 min. Integration was conducted at 280 nm, except for EGC for which 210 nm was used to obtain better resolution.

Catechins and caffeine were verified by the elution time of standards (see the Supporting Information) and quantitated by the corresponding standard curves (R^2 = 0.9997−1.0000). Caffeine, which remains stable under the experimental conditions employed, was used as an internal marker to normalize all catechin contents of each sample. The initial catechin contents were 13.2 ± 0.1, 11.9 ± 0.1, 2.3 ± 0.1, and 4.2 ± 0.3% (w/w) for EGCG, EGC, ECG, and EC, respectively, and were 0.4 ± 0.1, 0.2 ± 0.0, and 1.0 ± 0.1% (w/w) for GCG, CG, and C, respectively. The initial relative concentrations of the catechins were determined as follows: each *cis*-configured catechin was set as 100%, and each *trans*-configured catechin was set as the concentration (%) relative to the concentration of its corresponding *cis*-configured catechin. Due to the overlapped elution time with caffeine and the trace amount present in green tea, GC was not quantitated.

Moisture Sorption. Two approaches were used for monitoring moisture sorption of the green tea powder. First, approximately 2.5 g of green tea powder was weighed in aluminum drying pans (i.d., 5 cm; depth, 1.4 cm) and kept in desiccators ranging from 0 to 97% RH at 25 °C. The mass change was monitored over time for up to 100 days for most samples (>200 days for samples that had not reached equilibrium within 100 days) using an Analytic AC 1205 analytical balance (Sartorius, Goettingen, Germany) and expressed as percent moisture on a w/w basis. Moisture sorption isotherms were also measured using an SGA-100 Symmetrical Gravimetric Analyzer (VTI Corp., Hialeah, FL) at 25, 40, and 55 °C. Briefly, about 10 mg of green tea sample was weighed and dried at 60 °C and 0% RH for 360 min prior to analysis. Samples were exposed to 0−95% RH with intervals of 5% RH. The equilibrium criterion was 0.001% w/w change in 5 min, and the maximum time for each step was 720 min.

Differential Scanning Calorimetry (DSC). The glass transition temperatures of green tea powders that had been stored at different RHs were measured using a TA Q2000 DSC with a refrigerated cooling system (TA Instruments, New Castle, DE). The system was calibrated with indium (temperature and enthalpic response) and benzophenone (temperature). Green tea powder samples were prepared in triplicate and preconditioned using two different approaches. Some were kept in desiccators ranging from 0 to 97% RH at 25 °C for 20 days prior to DSC analysis. Other 10 mg samples were stored for 720 min in 0–90% RH conditions created by an RH generator (VTI Corp.). Samples were sealed in hermetic pans and analyzed by cooling to −80 °C and then heating at 10 °C/min to 90 °C. Nitrogen was used at 50 mL/min as the purge gas. The onset temperature of the glass transition was recorded as T_g.

Modeling of Moisture Sorption. The moisture sorption experimental data were fitted using the Guggenheim–Anderson–de Boer (GAB) equation as follows:[10]

$$\frac{m}{m_m} = \frac{Cka_w}{(1 - ka_w)[1 + (C - 1)ka_w]} \tag{1}$$

m is the moisture content (g of H_2O/g of dry solids), m_m is the monolayer moisture content (g of H_2O/g of solids), a_w is the water activity (RH = $a_w \times 100\%$), and C and k are constants.

Modeling of Glass Transition Temperature. According to previous studies,[11] the relationship between moisture content and glass transition temperature in amorphous powder systems follows the Gordon–Taylor model:[12,13]

$$T_g = \frac{w_1 T_{g1} + Kw_2 T_{g2}}{w_1 + Kw_2} \tag{2}$$

w_1 is the fraction of dry solids (w/w), T_{g1} is the glass transition temperature of the sample at zero moisture content, w_2 is the fraction of moisture (w/w) ($w_2 = 1 - w_1$), T_{g2} is the glass transition temperature for glassy water, and K is a constant. A T_{g2} of −138 °C was used for water.[14] The Gordon–Taylor model was applied to the green tea experimental data.

Reaction Kinetics. Despite the complexity of the reaction mechanisms, as discussed later, catechin degradation has been reported to follow first-order or pseudo-first-order kinetics.[2,3,5,6] Thus, the following equation was applied to experimental data to evaluate the reaction rate constants:

$$\ln \frac{x}{x_0} = -kt \tag{3}$$

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

The Arrhenius equation was used to evaluate the temperature dependence of the experimental reaction rate constants:[15]

$$\ln k = \ln A - \left(\frac{E_a}{R}\right)\left(\frac{1}{T}\right) \tag{4}$$

k is the reaction rate constant, 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).

The Williams–Landel–Ferry (WLF) type temperature dependence of reaction rate constants described in the equation below was also used to evaluate the data. The relationship between ln$k$ and T_g was evaluated by

$$\ln(k_{ref}/k) = -C_1(T - T_{ref})/[(C_2 + (T - T_{ref}))] \tag{5}$$

where T_g is commonly used as the reference temperature (T_{ref}), k_{ref} is the reaction constant at T_g, and C_1 and C_2 are empirical constants.[16]

Statistical Analysis. A completely randomized two-factor factorial design was used for the storage study. Data were presented as the mean
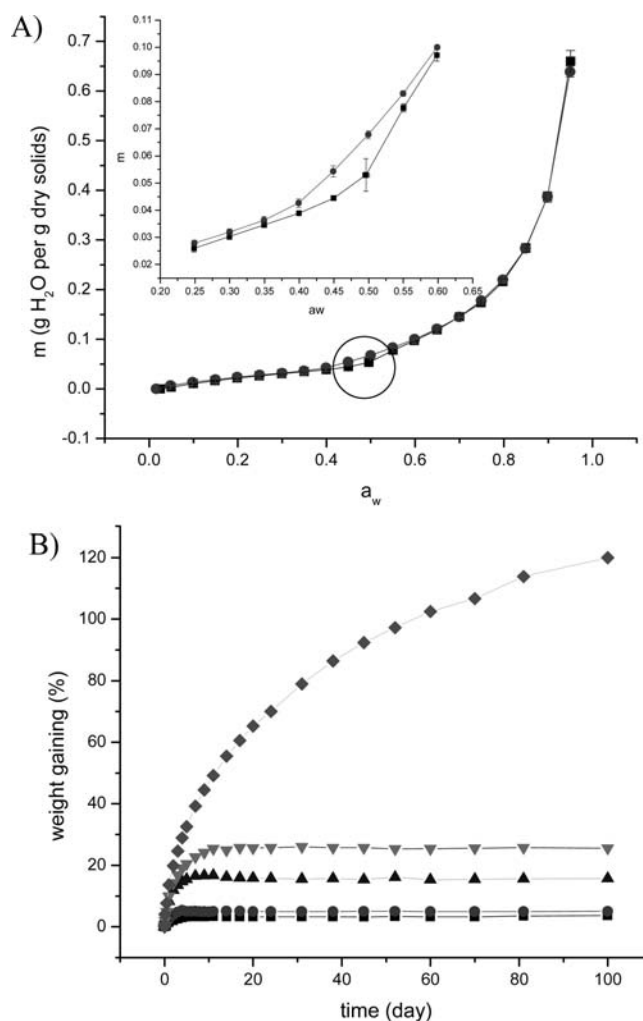

Figure 2. Moisture sorption behaviors of green tea powder: (A) moisture sorption isotherms of green tea powder as a function of a_w and moisture content at (■) 25 °C and (●) 40 °C. (B) Kinetic moisture sorption profiles of green tea powder stored at select RH values and monitored over time at (■) 43% RH, (●) 58% RH, (▲) 75% RH, (▼) 81% RH, and (◆) 97% RH.

± standard deviation. Paired comparisons were conducted using t tests, and pariwise comparisons were conducted using Tukey's range test. The significance tests were adopted at the $p < 0.05$ level. All statistical analyses and linear regressions were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC). Nonlinear regression was performed with Matlab R2009a (The MathWorks Inc., Natick, MA).

■ RESULTS

Moisture Sorption of Green Tea Powder. The relationship between water activity (a_w) and moisture content (m) of the green tea powder is shown in Figure 2A. The experimental data were fitted using the GAB model. Estimates of GAB constants were calculated and are listed in Table 1. The kinetic moisture sorption profiles of the green tea powder are shown in Figure 2B. Samples stored at 97% RH had a much higher weight gain compared with others stored at lower RH values. There was a time period before equilibrium was reached for each sample, the length of which increased with increasing RH. Likewise, the moisture sorption increased with increasing storage RH. After

Table 1. Constants for the GAB Moisture Sorption Model

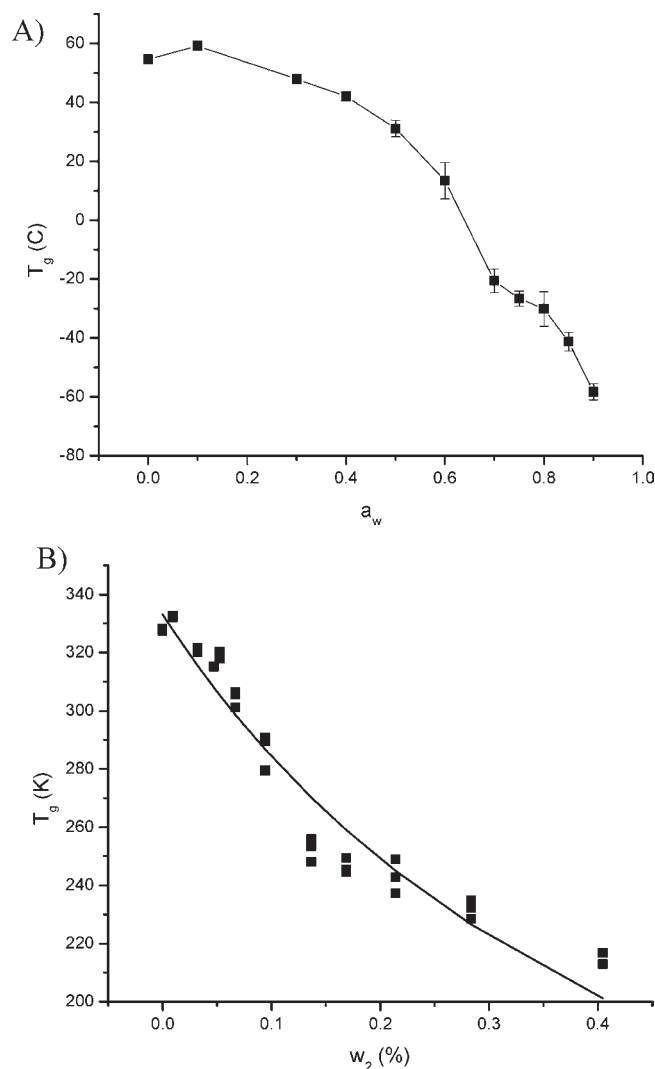| temp (°C)	m_m (g H$_2$O/g dry GT)	k	C	R^2
25	0.06029 ± 0.00553	0.9618 ± 0.00735	1.417 ± 0.262	0.998−0.999
40	0.05964 ± 0.00266	0.9600 ± 0.00697	1.706 ± 0.058	0.999−1.000

A)

B)

Figure 3. Effect of moisture content on the glass transition temperature of green tea powder: (A) onset temperature of glass transition for green tea powder equilibrated at different RH values; (B) Gordon−Taylor relationship between T_g and moisture content ((■) experimental data; (—) Gordon−Taylor fitting curve).
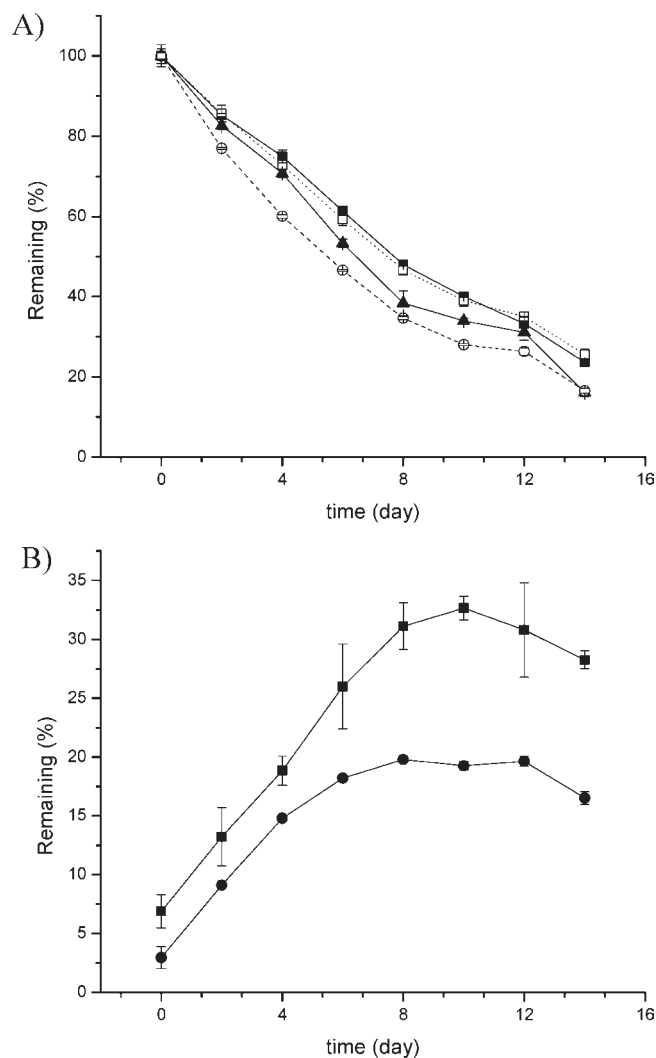
A)

B)

Figure 4. Typical degradation profiles of green tea catechins at 60 °C and 97% RH: (A) degradation profiles of (□) EGCG, (○) EGC, (■) ECG, and (▲) EC; (B) degradation profiles of (●) GCG and (■) CG.

2 weeks of storage at 25 °C, samples stored at 43, 58, 75, and 81% RH had all reached equilibrium, whereas the samples at 97% RH were still absorbing moisture after 200 days of storage.

Glass Transition of Green Tea Powder. The onset glass transition temperatures of green tea powders stored at 43, 58, 75, and 81% RH were 45.8 ± 0.5, 15.9 ± 1.0, −30.6 ± 1.1, and −42.3 ± 5.4 °C, respectively (see the Supporting Information). Due to the plasticization effect of moisture, the T_g of these amorphous powders decreased with increasing moisture content. The T_g profile of samples stored at 0−90% RH is shown in Figure 3A, and the T_g versus the moisture content (calculated using the GAB equation as reported in Table 1) of green tea powder stored

at different RH values is plotted in Figure 3B, with a good fit to the Gordon−Taylor equation ($K = 2.946$, $R^2 = 0.942$).

Degradation Profile of Green Tea Catechins. A typical catechin degradation profile of green tea powder stored at 97% RH and 60 °C is shown in Figure 4. Previous studies revealed that catechin degradation in dilute solutions followed first-order or pseudo-first-order reaction kinetics.[2,3,5,6,17] Similar apparent exponential degradation patterns of *cis*-configured catechins (EGCG, ECG, EGC, and EC) were also observed in this study (Figure 4A). The *trans*-configured catechins (CG and GCG) first increased with time to a maximum content at about 10 days and then decreased with prolonged reaction times, without reaching equilibrium within the experimental conditions. This trend was also in good agreement with previous studies.[5,6]
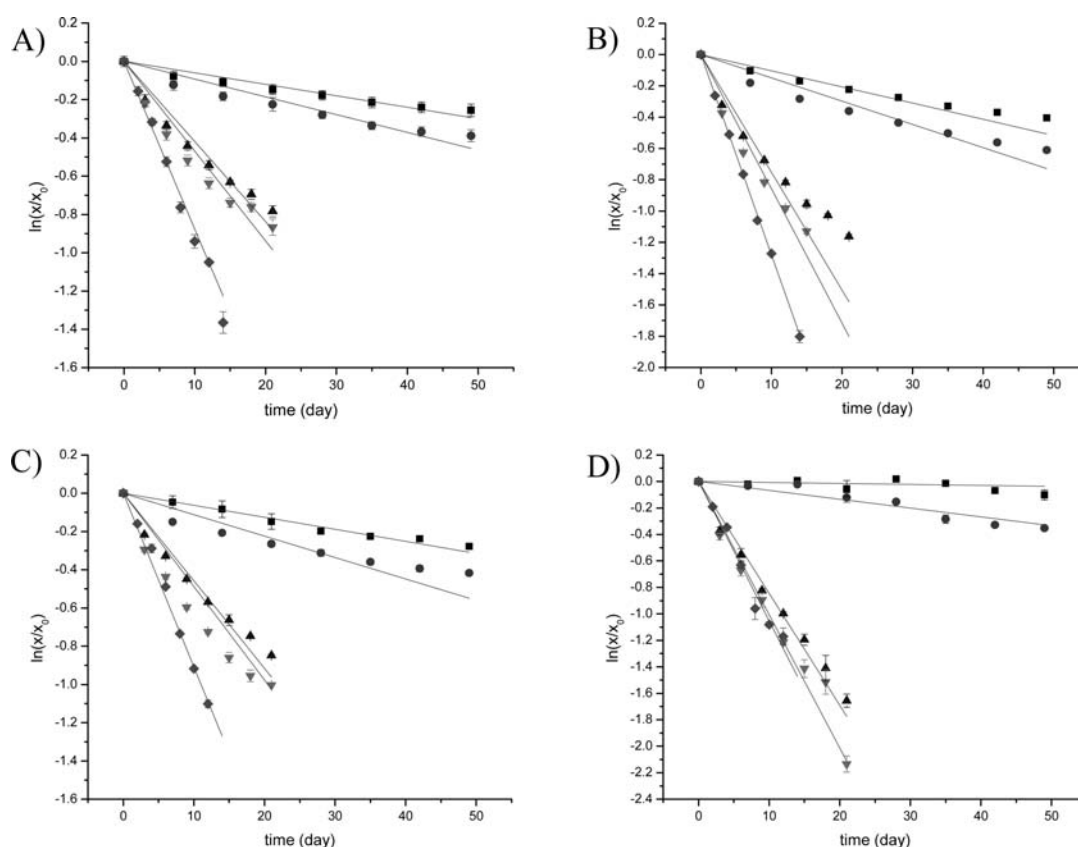
Figure 5. Apparent first-order degradation of catechins in green tea powder stored under various environmental RH values at 60 °C: (A) EGCG; (B) EGC; (C) ECG; (D) EC; (■) 41% RH; (●) 49% RH; (▲) 74% RH; (▼) 79% RH; (◆) 97% RH.

By fitting the data to the first-order reaction kinetics, a series of reaction rate constants k_1 were obtained by plotting $\ln(x/x_0)$ versus time, where x is the concentration of reactant. An example of first-order degradation kinetics of EGCG in green tea powder under various RH conditions at 60 °C is shown in Figure 5A. It was clear that k_1 increased with increases in environmental RH, and high regression coefficients were obtained (R^2, shown in Table 2). Similar apparent first-order degradation patterns were observed for other the *cis*-configured catechins (ECG, EGC, and EC) as shown in Figure 5B–D.

Degradation Kinetics of EGCG. Because EGCG is the most abundant polyphenol component in green tea and is considered as a main criterion in quality assessment, and also the degradation kinetics appeared to be similar among the four *cis*-configured catechins, the degradation kinetics of catechins were illustrated in this study using EGCG as an example. The rate constants and shelf life (t_{90} values, the time by which 10% degradation was reached) of EGCG degradation are summarized in Table 2. The reaction rate constants were affected by both temperature and environmental RH simultaneously. Similar results were obtained for the other *cis*-configured catechins (EGC, ECG, and EC, data not shown).

The degradation rate of catechins decreased with decreasing temperature and RH. Thus, for samples stored at low temperature and low RH conditions (25 °C, and 43 or 58% RH; 35 °C and 43% RH), >95% of the catechins remained after 112 days of storage. Due to low reaction rates and the complex food matrices, high regression coefficients were not obtained for these samples. Short t_{90} values were found at high temperature and high RH

conditions (e.g., 0.84 day at 60 °C and 97% RH, as shown in Table 2), indicating rapid loss of catechins at these conditions. As temperature and RH decreased, t_{90} increased dramatically (e.g., 1300 days at 25 °C and 43% RH). The regression lines fit better when samples underwent further degradation within the experimental space, as shown in Table 2. The effect of temperature on catechin loss was greater than that of RH on catechin loss. For instance, green tea powders stored at 43% RH (T_g = 45.81 °C) had t_{90} values of 192 days at 40 °C and 371 days at 35 °C (below T_g), whereas samples stored at 25 °C and 58% RH (T_g = 15.93 °C) were more stable with a t_{90} of 522 days (above T_g).

RH and Temperature Dependence of Catechin Degradation. The first-order reaction rate constants, plotted against natural log of RH regardless of temperature (Figure 6A) indicated a log–linear increase in rate constant with increasing RH.

The reaction rate increased exponentially with increasing temperature regardless of RH, following the Arrhenius relationship, which is typical of catechin degradation.[2,3,5,6,17] By plotting the natural log of k against the inverse of temperature ($1/T$), straight lines were obtained (Figure 6B). The activation energies (E_a) of EGCG degradation derived from the slopes of the individual lines (Figure 6B) were 79.27 ± 7.88, 94.05 ± 2.92, 97.16 ± 3.88, and 92.86 ± 41.46 kJ/mol at 97, 80, 75, and 43% RH, respectively. The large STD at 43% RH is likely due to only three feasible temperature points within the experimental space. There was no statistically significant difference in E_a for green tea stored at 75 and 43% RH, but the E_a was significantly lower at 97% RH ($p < 0.05$) than at 80 and 75% RH.

Table 2. Rate Constants and t_{90} Values for EGCG in Green Tea Powders, upon Storage under Different Conditions

temp (°C)		97% RH[a]	80% RH[a]	75% RH[a]	49−58% RH[a]	43% RH[a]
60	$k \times 10^2$ (day^{-1})	9.604	3.957	3.543	0.758	0.500
	R^2 (n)[b]	0.992 (24)	0.954 (24)	0.970 (24)	0.951 (24)	0.963 (24)
	t_{90}[c] (days)	0.84	2.37	3.41	15.63	25.44
50	$k \times 10^2$ (day^{-1})	5.581	1.634	1.144	0.236	0.077
	R^2 (n)[b]	0.942 (24)	0.988 (24)	0.974 (24)	0.950 (20)	0.867 (19)
	t_{90}[c] (days)	2.45	6.49	9.35	45.53	69.73
40	$k \times 10^2$ (day^{-1})	2.221	0.448	0.357	0.158	0.0577
	R^2 (n)[b]	0.942 (23)	0.955 (23)	0.945 (23)	0.901 (24)	0.864 (18)
	t_{90}[c] (days)	8.12	22.85	29.15	111.29	192.50
35	$k \times 10^2$ (day^{-1})	1.461	0.289	0.164	0.0530	d
	R^2 (n)[b]	0.932 (22)	0.908 (23)	0.908 (20)	0.461 (24)	0.001 (23)
	t_{90}[c] (days)	13.86	39.04	52.93	178.88	371.42[e]
25	$k \times 10^2$ (day^{-1})	0.321	0.0754	0.0628	d	d
	R^2 (n)[b]	0.946 (20)	0.652 (22)	0.623 (23)	0.078 (24)	0.029 (23)
	t_{90}[c] (days)	48.52	128.54	185.23	521.50[e]	1299.88[e]

[a] See the Supporting Information for specific RH values at a certain temperature. [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 according to the model described in Table 3. [d] Calculation of reaction rate constants was not feasible, due to extremely low reaction rates. [e] Samples stored at conditions below T_g.

The combined effect of temperature and RH on the rate constant could be described using the following model:[18]

$$\ln k = \frac{-E_a}{RT} + \ln A + C \times RH \tag{6}$$

k is the reaction rate constant, E_a is the activation energy, R is the gas constant, T is the temperature, A is the frequency factor, C is a constant, and RH is the relative humidity.

Multiple linear regression analysis was conducted on individual catechin stability data to obtain the kinetic model parameters of catechin degradation in green tea powder systems (Table 3). Data at 97% RH were not used for model development due to discrepancies in E_a values. The E_a of individual catechins was in the order EC > EGCG > EGC > ECG.

The kinetic models listed in Table 3 can easily be applied to calculations relating the shelf life of green tea powder to temperature and RH conditions. It is worth mentioning that even though catechins were relatively stable at 58% RH conditions below T_g (92.9% EGCG remaining at 25 °C after 1 year, calculated on the basis of the kinetic model shown in Table 3), undesirable physical changes were observed in the tea powders at this RH, such as caking after about 6 days and slight browning after 16 weeks of storage at room temperature.

Impact of Glass Transition on Catechin Degradation Kinetics. The relationships between the reaction rate constant k of EGCG degradation and $T - T_g$ can be described by the WLF equation. It is clear from this plot that the rate of degradation rapidly increased at temperatures above T_g (see the Supporting Information). The WLF equation is useful for predicting the temperature dependence of viscoelastic properties.[11] The WLF plot and fitting curve (see the Supporting Information) had an R^2 of 0.984 ($C_1 = 8680$, $C_2 = 60730$). The k_{ref} at each RH condition was calculated by using the equation shown in Table 3.

■ DISCUSSION

Moisture Sorption of Green Tea Powder. Green tea powder exhibited a sigmoidal moisture sorption profile (Figure 2A) that is typical of amorphous solids and is consistent with previous green tea powder studies.[8,19] The GAB model fitted reasonably well with the experimental data, also in agreement with previous study.[27] Small differences were observed between moisture sorption isotherms at 25 and 40 °C, noticeable around the a_w at which the glass transition occurred at each temperature (Figure 2A). However, there was no significant difference ($p > 0.05$) between the GAB parameters at 25 and 40 °C.

Degradation Kinetics of Green Tea Catechins. The degradation rates of *cis*-configured catechins (EGCG, EGC, ECG, and EC) decreased with increasing time, following apparent first-order kinetics, which is in agreement with previous studies.[2,6,17] However, even though high R^2 values with linear experimental data fittings were obtained, the reaction mechanism might be more complex if there are indeed curvatures in the first-order reaction fittings (Figure 5). The *trans*-configured catechins do not follow this behavior,[5,6] and more studies on catechin degradation mechanisms, beyond simple loss models, are needed to further understand their degradation kinetics. Due to the difficulties with measuring degradation products, catechin losses were determined to develop the kinetic models in this study. Although this approach makes it difficult to elucidate the reaction mechanisms, it is common in previous studies,[2,3,5,6,17] and is feasible for shelf life model purposes based on catechin loss.

As expected, catechin losses increased with increasing temperatures and RH values. Even under low RH conditions, for example, 43% RH, exponential degradation patterns ($R^2 = 0.963$) rather than pseudo-zero-order kinetics were observed at 60 °C, suggesting that the first-order kinetics was not altered due to limited moisture contents and excess reactants. Compared with
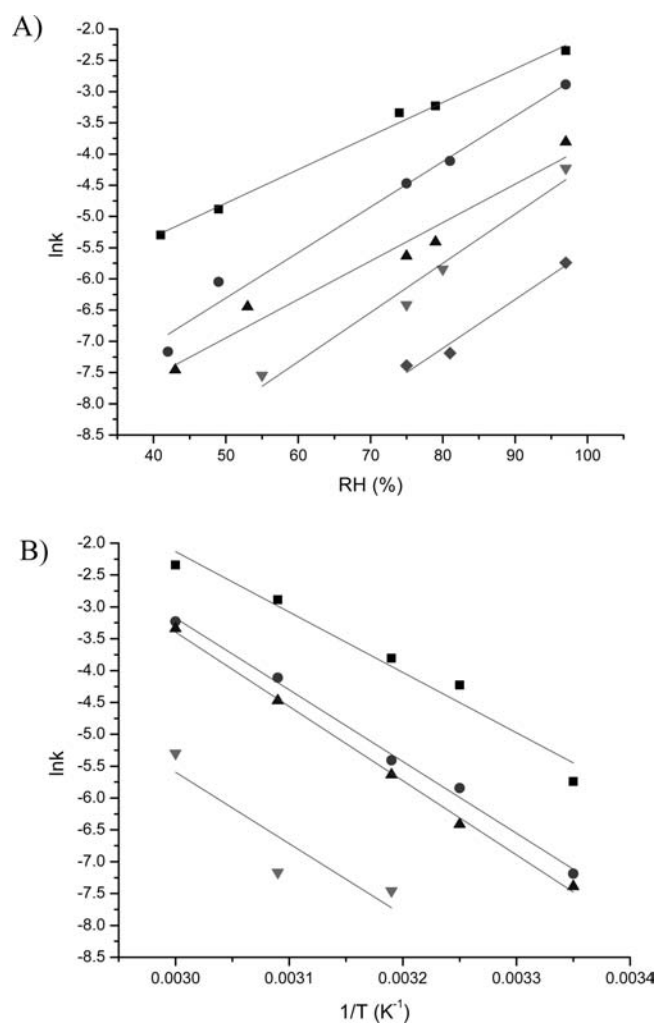
Figure 6. Impact of RH and temperature on degradation kinetics of EGCG in green tea powder: (A) degradation rate constants of EGCG in green tea powder as a function of RH (storage temperatures of (■) 60 °C, (●) 50 °C, (▲) 40 °C, (▼) 35 °C, and (◆) 25 °C); (B) Arrhenius plots of rate constants for EGCG degradation in green tea powder (storage RH values of (■) 97% RH, (●) 80% RH, (▲) 75% RH, and (▼) 43% RH).

Table 3. Kinetic Models Relating Temperature and RH of Catechin Degradation

catechin	kinetic model	R^2
EGCG	$\ln k = -11509.0000\frac{1}{T} + 26.5628 + 6.0888 \times RH$	0.972
EGC	$\ln k = -10490.0000\frac{1}{T} + 24.1493 + 5.7054 \times RH$	0.981
ECG	$\ln k = -9311.5322\frac{1}{T} + 20.7666 + 4.9000 \times RH$	0.975
EC	$\ln k = -13527.0000\frac{1}{T} + 31.5521 + 8.6104 \times RH$	0.982

reported that the degradation pattern of GCG is the combination of simultaneous epimerization and degradation of catechins during thermal processing, by defining degradation as all reactions causing catechin loss other than epimerization. They developed a kinetic model to differentiate between these two kinds of reactions based on the assumption that the rates of degradation for *cis*-configured and *trans*-configured catechins are similar.[2,6] However, this model did not reasonably fit the data obtained in this study of powdered green tea (e.g., $R^2 = 0.747$ at 60 °C and 97% RH). Such discrepancies could possibly be due to different food matrices (pure EGCG vs green tea amorphous powder) and different product states (dilute solution vs powder) used. According to Labuza et al.,[15] at least eight time points are recommended for kinetic studies to get reasonable results considering practical and economical limiting factors. Thus, it is also possible that a lack of data points (usually five time points for model development and four for model validation) in previous studies[2,6] caused a slight overfitting of the kinetic models. The determination that our data could not be fitted in this model revealed that catechin degradation kinetics could not simply be defined as the combination of degradation and epimerization. Much more complex mechanisms are likely involved.

Impact of RH and Temperature on Catechin Stability. A log−linear relationship between RH and reaction rate constant k was revealed for the green tea catechins (Figure 6A). Such log−linear relationships between rate constant and environmental RH have also been reported in pharmaceutical powder systems of nitrazepam[22] and penicillins.[23] In food systems, there are limited studies concerning the RH dependence of reaction rate constants, and log−linear relationships between RH and k are rarely reported. However, this relationship makes it feasible to establish an accurate shelf life model.

Temperature affects the reaction rate constant following the Arrhenius rule for most reactions. Over a narrow temperature range, the activation energy E_a is usually considered as a constant. By generating Arrhenius plots from the experimental data, E_a could be computed from the slope of the linear regression analysis of the data. The E_a remained the same in green tea powder systems stored at low RH conditions (RH ≤ 75%, $p > 0.05$) for the temperature range 25−60 °C studied (Figure 6B). It is worth noting that the E_a of EGCG degradation was significantly lower at 97% RH compared with E_a values at 81 and 75% RH ($p < 0.05$). However, green tea powders stored at 97% RH absorbed much more moisture than those at lower RH conditions (Figure 2B). Moreover, due to the large amount of

previous studies conducted in dilute solution systems, chemical reactions appeared to proceed much more rapidly in the solutions than in the green tea powder systems stored at varies RH values. For instance, about 80% EGCG degradation was observed in 0.05% green tea extract solution after 7 days at room temperature.[4] In purified EGCG solutions of 0.5 mg/mL, about 15% of EGCG remained after 16 days of storage at 25 °C.[2] In the green tea powder systems in this study, 10% degradation of EGCG was reached after 48.5 days at 97% RH and 25 °C (Table 2). The lowered reaction rate could be explained by the substantially limited molecular mobility and diffusion rate in powder systems as compared with dilute solution systems. This illustrates that powder products are more stable than solutions and therefore are likely better choices for long shelf life products.

The stability profiles of *trans*-configured catechins shown in Figure 4B suggested that these catechins are involved as a reactant and a product in two or more reactions, possibly epimerization,[20] oxidation,[21] and polymerization.[4] Wang et al.[2,6]

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bulk water absorbed by the powder stored at 97% RH, no T_g event was observed in DSC scans for these samples (Supporting Information), suggesting that T_g may have occurred below −80 °C (the lowest experimentally accessible temperature possible to begin the DSC scan). Thus, this system can be most likely considered as a concentrated solution rather than in a rubbery state, and therefore the molecular mobility and diffusion rate would be dramatically increased. This assumption is further supported by DSC data, where a melting endotherm for water was present only in the samples stored at 97% RH (Supporting Information), indicating that there is freezable water present; water does not freeze when present only at a low concentration in a highly viscous amorphous matrix.[16] Two possible explanations for the lowered E_a at 97% RH are that (1) the degradation patterns of green tea catechins were altered by excess moisture absorbed in the powder system, that is, degradation mechanisms of green tea catechins are different between powder systems and concentrated solution systems; and (2) the E_a could be lowered due to the dramatically increased molecular mobility, or the temperature dependent changes of mobility, that affect the slope of the Arrhenius relationship in samples at 97% RH.

Activation energy is the energy that must be overcome for a reaction to occur and is an indication of the temperature sensitivity of reaction rate constants. Inconsistent E_a values of EGCG degradation have been reported in previous studies. For example, the E_a values reported for EGCG degradation in dilute solutions include 78.35 kJ/mol (18.7 kcal/mol),[3] 19.69 kJ/mol (4.7 kcal/mol) below 82 °C or 158.80 kJ/mol (37.9 kcal/mol) above 82 °C,[5] and 43.09 kJ/mol.[2] In this study, E_a was found to be 95.69 kJ/mol for EGCG (according to the kinetic model in Table 3). These differences could possibly be due to altered physical systems (dilute solution vs solid state powder, with the powders being more stable); altered reaction mechanisms due to discrepancies in food matrices (differently processed green tea powders, pure EGCG crystalline powder, etc.), and different models employed in different studies.

Impact of Glass Transition on Catechin Stability. The stability of catechins in powder systems is also dependent on the physical state and macrostructure of the matrices.[24] A glass transition is a kinetic transformation that involves a change from a rigid glassy state to a viscous supercooled liquid (sometimes referred to as "rubbery") state. As temperature increases above the glass transition temperature (T_g), amorphous materials undergo a dramatic reduction in mechanical modulus,[25] with a concurrent increase in molecular mobility and a decrease in viscosity. Absorbed moisture will lower the T_g of the solid powder system and, thus, enhance chemical degradation through facilitating mobility. As expected, the T_g of green tea powder stored at different RH values was found to decrease with increasing moisture content due to the plasticizing effect (Figure 3A). The T_g at ambient conditions (25 °C) occurs around 50−58% RH. In food systems, T_g is usually set as a reference temperature to access the relationship between the physical state of the material and chemical, physical, and enzymatic reaction rates: at temperatures below T_g, food products are thought to be stable;[11] at temperatures above T_g, reactions increase substantially (see the Supporting Information).

However, it is worth mentioning that the tea catechins were not stable even at temperatures below T_g for some storage conditions (40 °C at 43% RH). Similar observations were found in studies of hibiscus anthocyanins[11] and saffron carotenoids.[24] These results suggest that there can be sufficient molecular mobility in the glassy state to affect the chemical stability. Three primary factors govern the rate of a chemical reaction at conditions of constant temperature and pressure: diffusion factor D, frequency of collision factor A, and chemical activation energy factor E_a.[25] The T_g affects chemical reactions through changing the diffusion rate. From Table 2, it can be seen that the t_{90} values decreased dramatically as RH and temperature increased. A general recommendation to maintain the stability of green tea powder would be to maintain storage conditions below T_g; the further below T_g, the better for catechin stability.

The diffusion constant D is dependent on both viscosity and the temperature dependence of viscosity, where viscosity is related to $T − T_g$ as described in the WLF equation,[26] and T_g is related to the moisture content according to the Gordon and Taylor model.[12] In this green tea powder system, WLF kinetics ($R^2 = 0.984$, see the Supporting Information) and the Arrhenius equation ($R^2 = 0.972$) described the strong temperature dependence of catechin stability. The degradation kinetics of catechins within the experimental conditions were dependent on both temperature and RH conditions. Temperature affected the reaction rate following the Arrhenius relationship. Increases in RH exerted a strong impact on the reaction rate constant through the possible mechanisms of increasing the molecular mobility and diffusion rate.

It is uncommon for a rubbery system to simultaneously fit both Arrhenius and WLF equations.[27] A WLF relationship is normally applicable to systems in which the ln$k$ against $1/T$ plot is not linear.[16] In previous studies that addressed the chemical stability in food powder systems, the WLF relationship was not supported.[28] In this green tea powder system, large discrepancies were observed if universal constants of C_1 and C_2 ($C_1 = 17.44$ and $C_2 = 51.6$[29]) were applied in the WLF equation. Such a result suggested that the Arrhenius relationship was more applicable to this green tea rubbery system.

In summary, the results of this study generated practical shelf life models of catechin stability in green tea powders and also identified the storage conditions required to maintain high catechin contents in powdered green tea. Catechin stability was affected by both RH and temperature, although temperature was the dominant factor. Storing green tea powder at conditions well below T_g is recommended to maintain catechin stability, for example, at ambient temperature (25 °C) below 43% RH or in a freezer below 60% RH. Apparent first-order degradation was observed under the experimental conditions, and a log−linear relationship between RH and reaction rate constant was found. Catechin degradation followed the Arrhenius relationship throughout the experimental conditions. The catechin degradation in these solid systems is different from previous studies of catechin solutions, and results supported the claim that powders are more stable than solutions. This study provided a good tool for shelf life predictions of catechin stability in green tea powders for the food industry and is an example of a practical application of reaction kinetics to a complex food system.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Experimental conditions (41−97% RH; 25, 35, 40, 50, 60 °C), HPLC chromatograms, DSC profiles of of green tea powder stored at 43, 58, 75, 81, and 97% RH at 25 °C, and WLF fittings of reaction rate constants showing the impact of T_g on degradation kinetics of EGCG in green tea powder. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

a_w, water activity; C, catechin; CG, catechin gallate; E_a, activation
energy; EG, epicatechin; ECG, epicatechin gallate; EGC, epigal-
locatechin; EGCG, epigallocatechin gallate; GAB, Guggenheim—
Anderson—de Boer; GC, gallocatechin; GCG, gallocatechin
gallate; T_g, glass transition temperature; WLF, Williams—
Landel—Ferry.

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