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Interaction of Environmental Moisture with Powdered Green Tea Formulations: Effect on Catechin Chemical Stability

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Green tea and tea catechins must be stable in finished products to deliver health benefits; however, they may be adversely affected by tea processing/storage conditions and the presence of other components. The objective of this study was to determine the effects of storage relative humidity (RH) and addition of other ingredients on catechin stability in simulated dry beverage mixtures. Samples of green tea powder alone and mixed with sucrose, citric acid, and/or ascorbic acid were prepared and stored in desiccators at 22 °C and 0−85% RH for up to 3 months. Epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate were determined by high-performance liquid chromatography (HPLC). Formulation and the interaction of formulation and RH significantly promoted catechin degradation ($P < 0.0001$). The chemical degradation of total and individual catechins in green tea powder formulations was significantly increased ($P < 0.0001$) by exposure to increasing RH, and the degradation was exacerbated at ≥58% RH by the presence of powdered citric acid and at ≥75% RH by the presence of ascorbic acid. Catechins degraded the most in formulations containing both acids. Although catechin chemical stability was maintained at ≤43% RH in all samples stored at 22 °C for 3 months, caking was observed in samples at these realtive humidities. These results are the first to demonstrate that addition of other dry components to tea powders may affect catechin stability in finished dry blends and highlight the importance of considering the complex interplay between a multicomponent system and its environment for developing stable products.

KEYWORDS: Green tea; catechin stability; degradation; powder; humidity

INTRODUCTION

Epidemiological evidence linking increased consumption of tea to reduced chronic disease risk has stimulated interest in tea polyphenols as potential disease preventative agents. Tea (*Camellia sinensis*) is a rich source of flavan-3-ols, also known as catechins, which constitute 6−16% of the dry green tea leaves and approximately 80% of the total flavonoids (*1, 2*). There are four main catechins in green tea: (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG) (**Figure 1**). Catechins from tea possess diverse biological properties, including antioxidant (*3*), anti-inflammatory (*4*), and anticarcinogenic (*5*) activities, as well as protection against neurodegenerative (*6*) and cardiovascular diseases (*7*).

The proposed health benefits associated with tea have increased interest in including catechin-rich tea extracts in

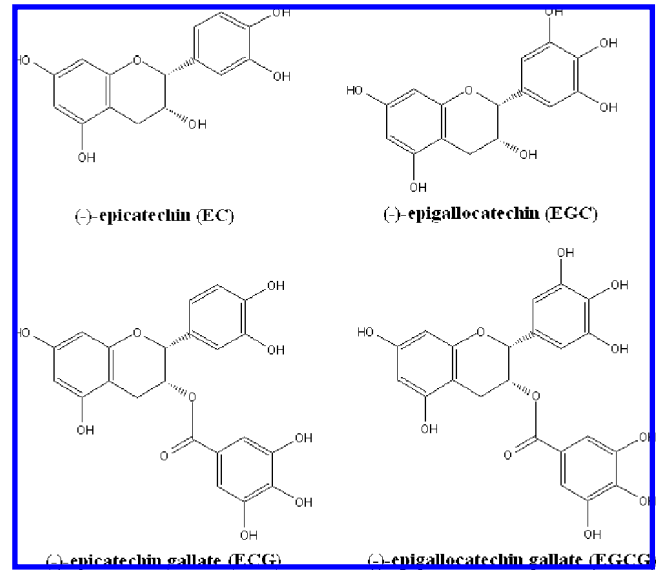

Figure 1. Structures of the major catechins in green tea.

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powdered food and beverage systems. A critical component for the delivery of these apparent benefits is the stability of biologically active catechins in food and beverage formulations. Degradation of catechins has been characterized in dilute aqueous systems exposed to environmental and processing-induced stresses (*1, 8*). Oxygen, heat, and pH have been identified as independent factors affecting the degradation of native biologically active catechins (*9*). Combined, these factors typically result in product shelf-life failure through the generation of excessive brown color. However, perhaps more critically, these reactions in aqueous systems consume native biologically active catechins, thereby limiting their availability for subsequent absorption and, by extension, physiological activity.

Sucrose, citric acid, and ascorbic acid are common ingredients used in bottled and canned tea beverages (*10*) and powder premixes. In buffered solutions (pH 7.4), ascorbic acid increases catechin stability, whereas citric acid has no effect over a 24 h period (*8*). In longer storage studies of autoclaved solutions of green tea catechins (up to 6 months), ascorbic acid decreases catechin degradation during the first month but increases it during longer storage; citric acid increases catechin degradation, and sucrose has shown no significant effect (*10*). Catechins are unstable in alkaline solutions (pH >7) but may be stable in acidic solutions. Zhu et al. (*1*) found catechins to be stable at pH <4; however, Tu et al. (*11*) found significant catechin degradation in solutions of pH 2.6 and 3.6.

Although most tea is distributed in leaf form, powdered extracts are commonly used in manufacturing instant beverages and in premixes for liquid beverage processing. Although the degradation of catechins in single-strength solutions is documented (*8, 10*), the chemical stability of catechins in powdered formulations has not been reported, perhaps due to the common assumption that dry products are inherently stable. However, water is a key mediator of both chemical and physical instabilities in powder blends (*12*). It is therefore anticipated that the stability of catechins in powdered formulations will be dependent not only on the physicochemical properties of each ingredient but also on the interaction of the blend with atmospheric moisture. The objective of this study was to determine the effect of environmental moisture and the presence of secondary ingredients on catechin stability in model dry tea blends composed of green tea powder and ingredients commonly associated with tea beverage products (sucrose, citric acid, and/or ascorbic acid).

MATERIALS AND METHODS

Materials. The ingredients used in dry blend formulations were green tea powder (a commercial spray-dried, low-yield hot water extract of green tea leaves used in ready to drink beverage formulations, a gift from Nestlé Research and Development Center, Marysville, OH), sucrose and citric acid monohydrate (Mallinckrodt-Baker, Phillipsburg, NJ), and ascorbic acid (Fisher Scientific, Fair Lawn, NJ). The salts used to provide selected relative humidities (RHs) in environmental chambers were anhydrous calcium sulfate (W. A. Hammond Drierite Co. Ltd., Xenia, OH); potassium acetate, sodium bromide, and copper chloride (Fisher Scientific); potassium carbonate, cobalt chloride, and potassium chloride (Mallinckrodt-Baker); and sodium nitrite (EMD Chemicals Inc., Darmstadt, Germany). All solvents used were of certified HPLC and ACS grade including methanol, acetonitrile, and glacial acetic acid (Mallinckrodt-Baker) and trifluoroacetic acid (TFA) (Sigma-Aldrich Inc., St. Louis, MO). Catechin standards (>98%) including epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG) as well as caffeine were purchased (Sigma-Aldrich).

Storage. Triplicate mixtures of 0.2 g of green tea powder (GT), 4 g of sucrose (S), 0.2 g of citric acid (C), and/or 0.06 g of ascorbic acid, levels representative of typical concentration ratios in single-strength beverages, were prepared and stored for 12 weeks at 22 °C in environmental chambers. Relative humidity was controlled by anhydrous calcium sulfate (0% RH) or saturated salt solutions of potassium acetate (25% RH), potassium carbonate (43% RH), sodium bromide (58% RH), sodium nitrite (65% RH), cupric chloride (69% RH), 3.0 molal solution of cobalt chloride [75% RH (*13*)], potassium chloride (85% RH), and potassium sulfate (98% RH). The RHs of all chambers were verified by digital hygrometer (traceable humidity/temperature/dew point meter, Control Co., Friendswood, TX) or water activity (Aqual Laboratory 3TE, Decagon Devices Inc., Pullman, WA) measurements. Samples stored at 75 and 85% RH were also analyzed at 0, 3, 6, 9, and 12 weeks to determine catechin degradation as a function of time. After storage, samples were weighed (Mettler Toledo AG 204, Columbus, OH) and immediately frozen at −70 °C. Prior to the HPLC assay, double-distilled (dd) water was added to the samples, the slurry was transferred to volumetric flasks and diluted to 50 mL, and aliquots were stored in 1.5 mL polypropylene tubes (Life Science Products, Inc., Frederick, CO).

Determination of Individual and Total Catechins. Catechin concentrations were determined by high-performance liquid chromatography (HPLC) using the method described by Neilson et al. (*14*) with minor modifications. A Waters 2695 HPLC system equipped with a model 2996 photodiode array (PDA) detector and a Waters Xterra C-18 (3.8 mm i.d × 100 mm) reverse phase column (Milford, MA) with a guard column packed with the same stationary phase were utilized. Detection and tentative identification of catechins were done using in-line PDA data between 220 and 600 nm. Individual catechin quantification was accomplished by construction of multilevel calibration curves from response at 280 nm resulting from injection of authentic standards of EC, EGC, EGCG, and ECG. Total catechins were calculated as the sum of all measured individual catechins. Results are reported as milligrams of catechin per gram of green tea powder.

Moisture Sorption. The difference in mass before and after storage was used to determine the amount of water incorporated into each sample. Moisture sorption was expressed as percent moisture on a w/w basis.

Determination of Ascorbic Acid. Ascorbic acid was determined by HPLC using the method described by Heudi et al. (*15*) with minor modifications. A Waters 510 HPLC system (Milford, MA) equipped with a model 490E programmable multiwavelength detector and an XTerra RP C-18 column (3.9 mm i.d × 100 mm) (Milford, MA) were used. Resolution of ascorbic acid was achieved using isocratic elution at 1 mL/min with 0.025% TFA in dd water (pH 2.6) as the mobile phase. Detection was performed at 240 nm.

pH Determination. To obtain an estimated pH value of all formulations at the dissolution point, saturated solutions of all ingredients in all formulations were prepared and measured using an Orion SA 720 (Orion Research Inc., Boston, MA) potentiometer calibrated from pH 2 to 7.

Statistical Analysis. For the 12 week storage study, a completely randomized two-factor factorial design was used. The data were analyzed using a two-way ANOVA model. The factors were RH and formulation, and the response was catechin concentration. For the kinetics study, a completely randomized three-factor factorial design was used. The data were analyzed using a MANOVA model. The treatments were RH, formulation, and time, and the response was catechin concentration. For the moisture sorption analysis, a completely randomized design and two-way ANOVA model were used. The factors were RH and formulation, and mass gain (percent) was the response. In all of the experiments, individual differences were tested using Tukey's multiple-means comparison procedure. All statistical analysis procedures were conducted using PC SAS system software and a significance level of 0.05.

RESULTS

Effect of Moisture on Catechin Stability in GT. Exposure to environmental relative humidity significantly contributed to catechin degradation ($P < 0.0001$) in the green tea powder

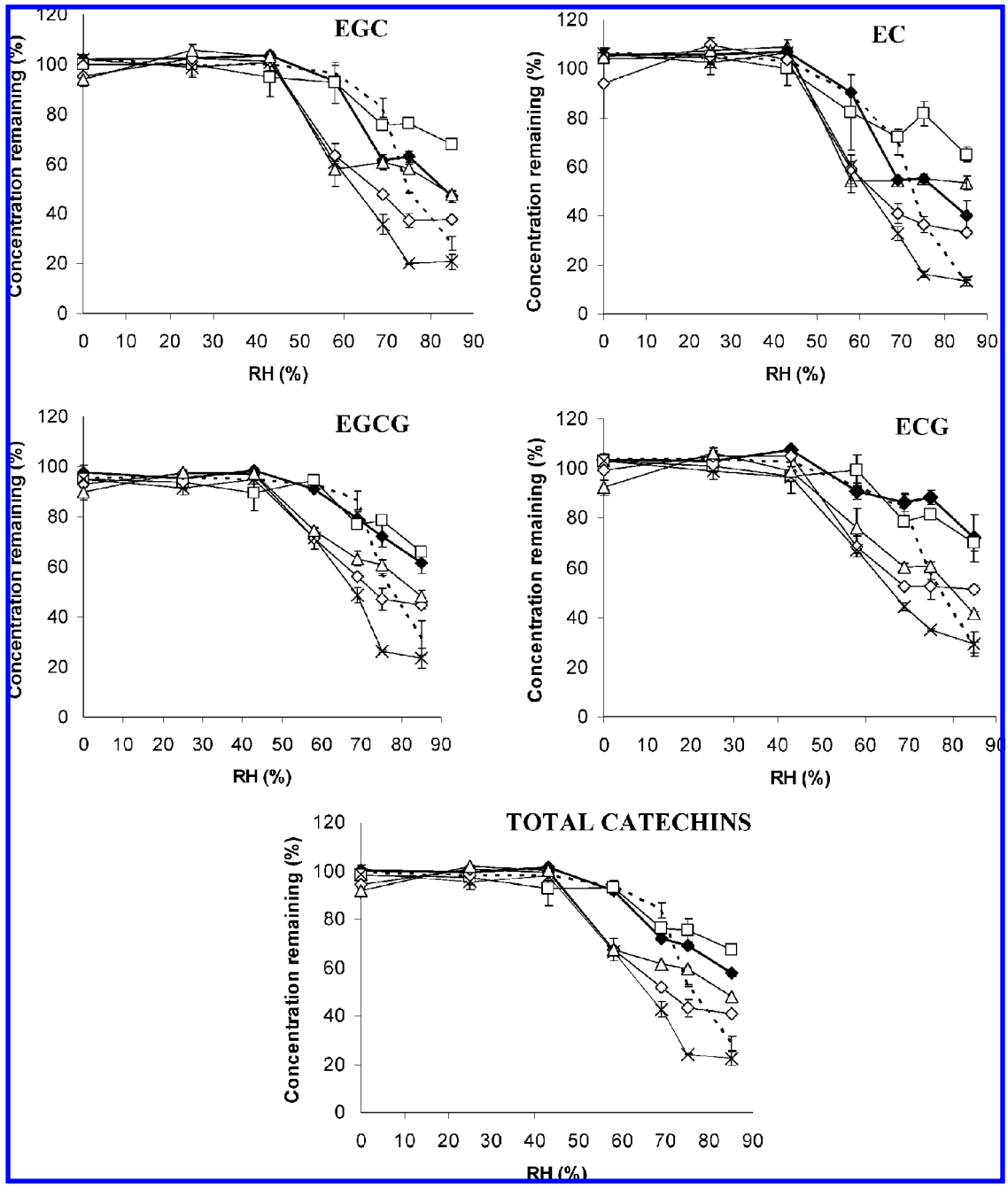

Figure 2. Percent (w/w) total and individual (EGC, EC, EGCG, and ECG) catechin concentrations remaining in green tea powder formulations after 12 weeks of storage at 22 °C and 0, 25, 43, 58, 69, 75, or 85% RH. The powder formulations used were GT (green tea), GTS (green tea + sucrose), GTC (green tea + citric acid), GTA (green tea + ascorbic acid), GTCA (green tea + citric acid + ascorbic acid), and GTSCA (green tea + sucrose + citric acid + ascorbic acid). Data points and trend lines for these formulations are shown by ◆ (GT), □ (GTS), ◇ (GTC), dashed line (GTA), × (GTCA), and △ (GTSCA).

(**Figure 2**). The concentrations of EGCG, EGC, ECG, and EC in the initial green tea were 166.7 ± 9.7, 113.7 ± 3.7, 26.7 ± 0.7, and 24.8 ± 2.9 mg/g tea, respectively, yielding 331.9 ± 4.9 mg of total catechins/g of tea. Mold growth was observed in most samples stored at 98% RH, and these samples were discarded prior to analysis. Total catechin losses were 28.1 ± 0.2, 31.0 ± 1.3, and 42.2 ± 0.8% at 69, 75, and 85% RH, respectively. Significant degradation ($P < 0.0001$) of EGC, EC, and EGCG in GT took place following 12 weeks of storage at ≥69% RH but not at lower storage RHs. ECG in GT powder was stable at all RH levels tested (≤85% RH). At ≥69% RH, EGC and EC degraded more than EGCG (with maximum losses at 85% RH of 57.5 ± 3.0, 59.5 ± 0.9, and 33.5 ± 0.7% w/w, respectively).

The HPLC profiles of all catechins in the control (initial GT powder) and GT powder exposed to 85% RH for 12 weeks are presented in **Figure 3**. The elution times of EGC, EC, EGCG, and ECG were approximately 3.6, 4.8, 5.9, and 7.3 min, respectively. After storage at 85% RH, a clear decrease in peak areas for native catechins was observed. Interestingly, a new peak (2.0 min) was found to increase proportionally to native catechin loss in degraded GT samples.

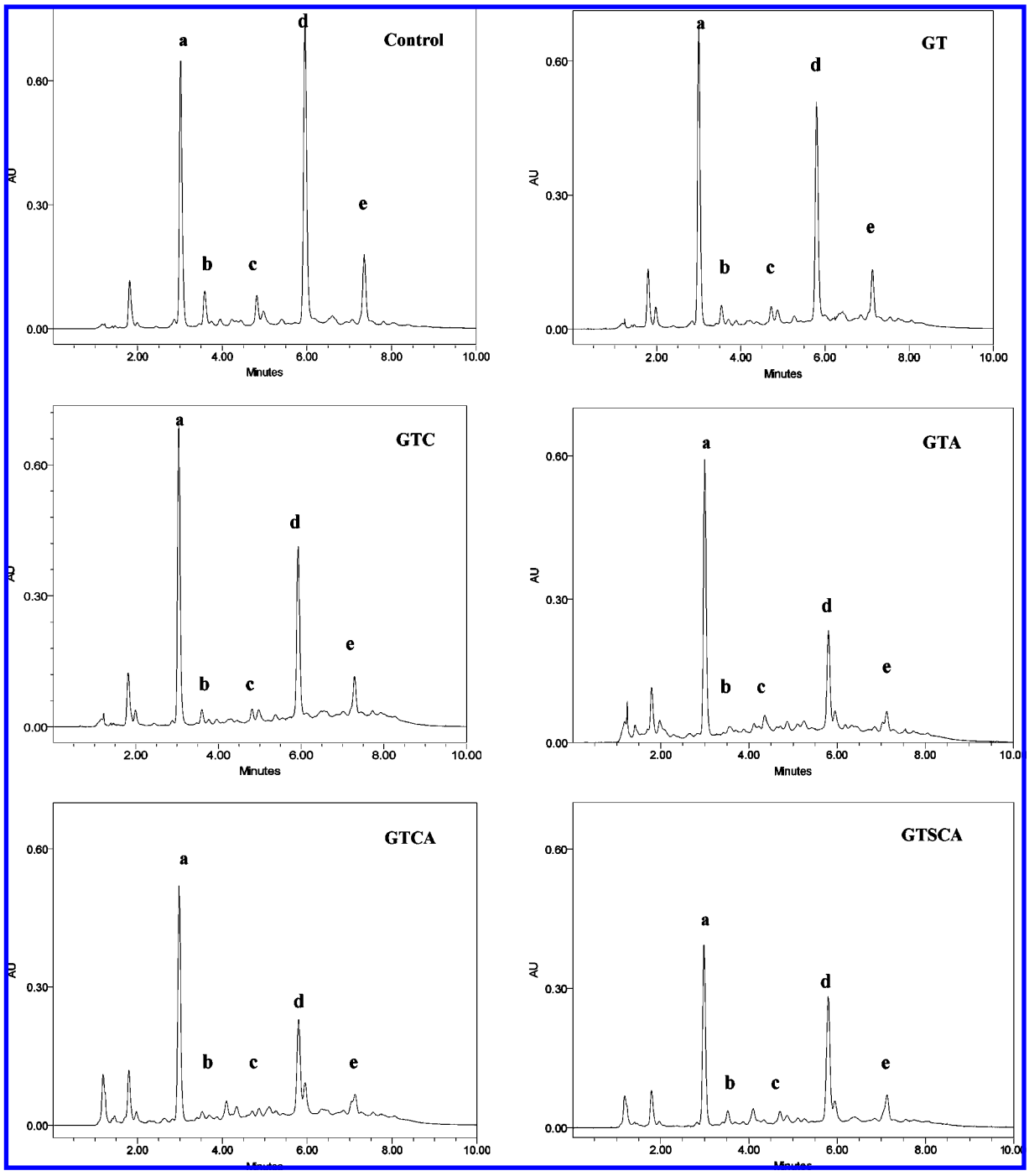

Figure 3. HPLC profiles of green tea powder formulations stored at 85% RH for 12 weeks. The formulations analyzed were GT (green tea), GTC (green tea + citric acid), GTA (green tea + ascorbic acid), GTCA (green tea + citric acid + ascorbic acid), and GTSCA (green tea + sucrose + citric acid + ascorbic acid). The peaks eluting were (a) caffeine, (b) EGC, (c) EC, (d) EGCG, and (e) ECG. Degradation products formed during storage are evidenced by peaks eluting at 1.3, 1.50, 2.0, 4.0, 4.4, and 6.0 min.

The mass gain due to moisture sorption by GT powder after 12 weeks of storage from 0 to 85% RH is shown in **Figure 4**. Moisture sorption increased with increased storage RH, from 7.5 ± 0.7 at 58% RH to 17.1 ± 0.9 at 85% RH. Although significant moisture uptake in GT powder occurred at ≥58% RH, the degradation of catechins in GT powder did not proceed until the amount of water sorbed was about 13.5 wt %/wt (at 69% storage RH). GT powder caked after 12 weeks of storage at 43% RH, turned brownish green at 58% RH, and changed from a caked powder to a plastic mass at 69% RH.

Effect of Formulation on Moisture Sorption. The mass gain in all of the powdered GT formulations due to moisture sorption during 12 weeks of storage at 0−85% RH is shown in **Figure 4**. Ranked from highest to lowest moisture sorption at 85% RH, GTSCA, GTS, GTC, GTCA, GT, and GTA formulations sorbed 77.2 ± 2.5, 71.9 ± 2.0, 46.7 ± 3.3, 35.9 ± 0.8, 17.1 ± 0.9, and 14.3 ± 1.8% of water, respectively. Formulations, with the exception of GTA, showed significantly greater moisture sorption relative to GT at 85% RH.

Effect of Formulation on Catechin Stability. Formulation and the interaction of formulation and RH significantly promoted

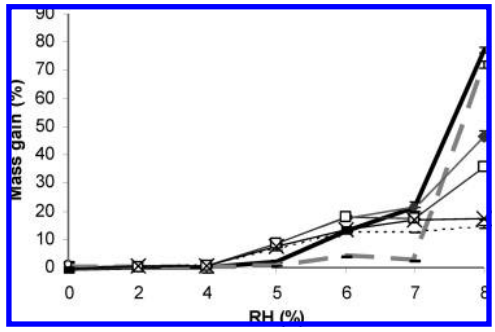

Figure 4. Moisture sorption of green tea (GT) powder and mixtures containing sucrose (S), citric acid (C), and/or ascorbic acid (A) stored at 0−85% RH for 12 weeks. The formulations are noted as follows: heavy black line, GTSCA; heavy dashed gray line, GTS; ◆, GTC; □, GTCA; ×, GT, and dashed line, GTA.

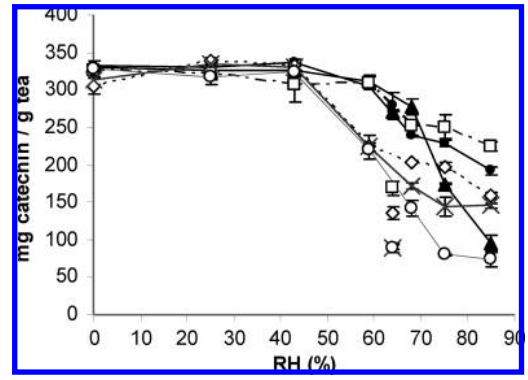

Figure 5. Total catechin concentrations (mg/g tea) in green tea powder formulations stored for 12 weeks in environmental chambers with 0−85% RH. Data from the 65% RH chamber using sodium nitrite are included in this figure only, and trend lines were drawn on this figure excluding the outlier data at 65% RH, likely created by an interaction with the sodium nitrite environment. The formulations are shown by □ (GTS), ● (GT), ◇ (GTSCA), × (GTC), ▲ (GTA), and ○ (GTCA).

catechin degradation ($P < 0.0001$) (**Figure 2**). In general, catechins were most stable in GTS samples and least stable in GTCA samples. The HPLC profiles of all catechins in the different formulations exposed to 85% RH for 12 weeks are presented in **Figure 3**. Caffeine (retention time of 3.0) did not degrade at any storage RH (the decreases in peak heights in GTCA and GTSCA are due to dilution factors taken into account in the calculations), but the degradation of the individual catechins is apparent both in the decrease in the peak areas for the catechins and in the emergence of several new peaks that were tentatively characterized as potential catechin degradation products.

The addition of sucrose to GT powder (GTS formulation) had no effect on total catechin concentrations compared to GT powder stored at the same RHs; however, differences in individual catechin stabilities were observed. Whereas EGCG and ECG concentrations were not different in GT or GTS, EGC and EC were more stable at ≥75% RH in GTS. GTS powder was partially caked after 12 weeks of storage at 43% RH, turned brown-green at 69% RH, and underwent partial and complete dissolution at 75 and 85% RH, respectively.

The addition of an equivalent mass of citric acid to green tea powder (GTC formulation) resulted in significant degradation of total catechins at ≥58% RH compared to GT powder stored at the same RH (**Figure 2**). Citric acid increased the degradation of EGC, EC, EGCG, and ECG compared to GT at ≥58%RH, except for EGC and EC at 85% RH, when degradation was not significantly different from that in GT alone (**Figure 2**). GTC powder caked after 12 weeks of storage at 43% RH, dissolved and formed a dark brown plastic mass at 58% RH, and turned to a flowable viscous solution at 85% RH.

The addition of ascorbic acid (GTA formulations) had no effect on total catechin degradation compared to GT at 58% RH, reduced degradation at 69% RH, and promoted degradation at 75% RH ($P < 0.0001$). Catechins were more stable in GTA than GTC formulations at 58 and 69% RH. At 75% RH there was no significant difference in total catechin concentration in GTA compared to GTC, whereas at 85% RH, the total catechin concentration was significantly lower in GTA (**Figure 2**). Ascorbic acid reduced the degradation of EGC and EC at 69% RH compared to GT, but it did not influence the degradation of EGC or EGCG. For all individual catechins, the presence of ascorbic acid promoted significant degradation at ≥75% RH ($P < 0.05$). The chromatogram of GTA at 85% RH has a decrease in all catechins compared to GT at the same RH (**Figure 3**), whereas other peaks increased or emerged (1.3, 1.50, 4.4, and 6.0 min). GTA powder caked after 12 weeks of storage at 43% RH, transformed into a plastic mass with undissolved ascorbic acid crystals at 69% RH, turned brown at 75% RH, and dissolved completely at 85% RH.

The largest degradation of catechins at all RHs compared to GT occurred in GTCA formulations, except at 85% RH, at which total and individual catechin losses in GTA and GTCA were similar (**Figures 2** and **3**). All individual catechins in GTCA degraded significantly more ($P < 0.0001$) than in GT at ≥58% RH. GTCA powder caked after 12 weeks of storage at 43% RH, transformed into a brown-green plastic mass with undissolved crystals at 58% RH, turned darker and less viscous at 75% RH, and completely dissolved at 85% RH.

Total catechin concentrations in GTSCA samples were significantly lower than in GT at ≥58% RH. EGCG and ECG concentrations were significantly lower in GTSCA than GT at ≥58% and ≥69% RH, respectively. EGC and EC degraded significantly more at 58% RH, but were not different from GT at ≥69% RH. Compared to GTCA, the presence of sucrose in GTSCA reduced catechin degradation at ≥69% RH. GTSCA powder partially caked after 12 weeks of storage at 43% RH, turned brown and showed partial dissolution at 69% RH, and dissolved completely at 85% RH.

Effect of Sodium Nitrite on Catechin Stability. Sodium nitrite was used to provide a 65% RH environment, but it appeared to adversely affect catechin stability by a mechanism different from moisture sorption. Total catechin concentrations in GT, GTS, GTC, GTA, GTCA, and GTSCA from 0 to 85% RH, including the values from the 65% RH sodium nitrite chamber, are shown in **Figure 5**. Total catechin losses compared to the control in GTS, GTC, GTSC, and GTSCA at 65% RH varied from 41 to 72%, compared to 11−46% at 69% RH and 21−76% at 85% RH. Furthermore, all samples stored in the sodium nitrite chamber developed a yellow (A) or brown-red (GT) discoloration, and a brown-red gas was generated. In contrast, samples containing GT at all other RHs had green-brown to brown colorations, and ascorbic acid alone (A) was white from 0 to 85% RH, excluding 65% RH, and changed in color only when citric acid was present and in solution (75% RH and above). Because the data obtained at 65% RH were outliers form the general trend from 0 to 85% RH (noted by the trend lines in **Figure 5** drawn excluding the 65% RH data points), they were not included in the rest of the results or in the discussion. Sodium nitrite may produce nitric oxide, which

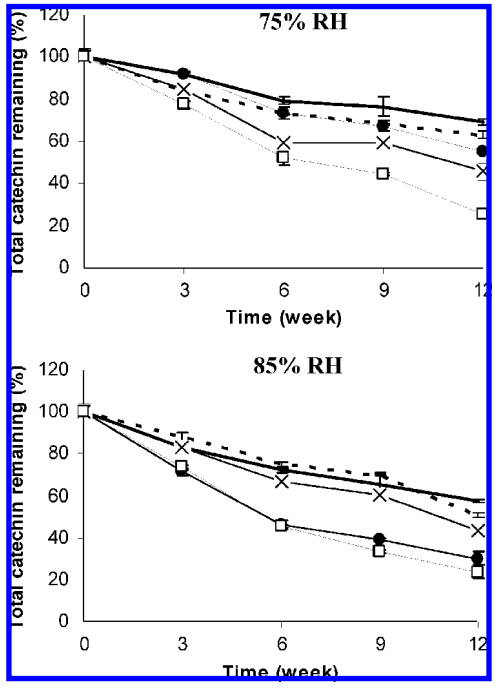

Figure 6. Percent (w/w) total catechin concentration remaining after 0−12 weeks of storage at 22 °C and (**a**) 75% RH and (**b**) 85% RH. The powder formulations used were GT (green tea), GTC (green tea + citric acid), GTA (green tea + ascorbic acid), GTCA (green tea + citric acid + ascorbic acid), GTSCA (green tea + sucrose + citric acid + ascorbic acid). These formulations are shown by a heavy black line (GT), a dashed line (GTSCA), × (GTC), ● (GTA), and □ (GTCA).

upon exposure to environmental oxygen can turn to nitrogen dioxide, a red-brown gas with oxidizing properties (*16*). Apparently, the generation of nitrogen dioxide enhanced catechin oxidation.

Effect of Storage Time on Catechin Stability at 75 and 85% RH. The length of storage, as well as the interactions of time and RH, time, and formulation, and all three factors, had a significant effect on catechin degradation ($P < 0.0001$). The effect of storage time (0, 3, 6, 9, and 12 weeks) on the catechin stability in GT, GTC, GTA, GTCA, and GTSCA formulations stored at 75 and 85% RH is presented in **Figure 6**. The total catechin concentration was significantly reduced following 3 weeks of storage at both RHs, and degradation continued as time proceeded. At 75% RH, catechins were least stable in GTCA and GTC. At 85% RH, catechins in GTA degraded more than in GTC. Of the individual catechins, EC had the highest degradation in GTA (**Figure 7**). The rate of catechin degradation was not significantly different at 75 and 85% RH in GTC and GTCA formulations; however, in general, catechins degraded more at 85% RH than at 75% RH in GT, GTA and GTSCA.

Ascorbic Acid Stability in GT Formulations. The percentages (w/w) of ascorbic acid remaining in A, CA, GTA, GTCA, and GTSCA after 12 weeks of storage in chambers ranging from 58 to 85% RH are presented in **Figure 8**. Ascorbic acid alone (A) was stable during storage at 0−85% RH for 12 weeks. However, in formulations with citric acid (CA) and/or green tea powder (GTA, GTCA, and GTSCA), ascorbic acid significantly degraded at ≥75% RH, except in GTSCA, for which significant ascorbic acid degradation did not occur until 85% RH. No ascorbic acid degradation was observed at 58 and 69% RH in any formulation ($P > 0.9754$). At 85% RH, ascorbic acid losses were 37.4 ± 1.4, 44.0 ± 1.4, 60.5 ± 1.6, and 100% in GTSCA, GTCA, CA, and GTA formulations, respectively.

DISCUSSION

GT powder sorbed significantly more moisture during 12 weeks of storage at 58% RH than at lower storage RHs (**Figure 4**), but although it had caked at this RH, no catechin degradation had occurred. At 69% RH the GT powder had fused into a viscous mass. This physical transformation coincided with significantly increased catechin degradation, and above 69% RH catechin losses were enhanced. Significant moisture sorption also occurred in GTC, GTA, and GTCA formulations at 58% RH (**Figure 4**), and degradation of catechins at this RH proceeded only in the formulations containing citric acid. No degradation of catechins occurred at RHs at which moisture sorption was negligible (at and below 43% RH), suggesting that moisture played a significant role in catechin degradation. Whereas EC is generally considered to be the most stable of the four catechins (*1, 17*), in GT alone, EC and EGC degraded the most at ≥69% RH. This enhanced "reactivity" might be explained by the higher solubility of EC and EGC in water compared to their gallated derivatives (EGCG and ECG) (*18*). In GT powders exposed to increasing environmental RH, individual catechin solubility apparently played an important role in enhancing degradation.

The spray-dried green tea powder used in this study was cold water soluble, and it has a catechin profile consistent with that of brewed tea (*2*). Additionally, the polyphenol and methylxanthine profile of this tea powder is equivalent to that of brewed tea (*2*), making findings broadly applicable to common tea powders used in manufacturing. Similar formulation effects on catechin stability were reported in this study and previous studies that used liquid tea beverage systems (*10, 11*). However, the green tea powder used differs from more refined extracts and decaffeinated products, as these have been fractionated to concentrate catechins and other bioactive constituents, and different trends might be found for dried tea leaves, in which the cell structure might influence water-solid interactions.

When additional ingredients (sucrose, citric acid, and/or ascorbic acid) were added to GT, describing catechin stability becomes more complex. The effect of formulation and the interaction of formulation and moisture promoted significant catechin degradation ($P < 0.0001$). The trends in the results for total catechin concentration in GT formulations containing S, C, and/or A (**Figure 2**) are similar to those obtained for green tea aqueous solutions stored for up to 6 months (*10*). Even though sucrose reduced EGC and EC degradation at ≥75% RH compared to GT alone, the total catechin concentrations in GT and GTS were not significantly different at any RH tested. Citric acid significantly promoted catechin degradation at ≥58% RH. Interestingly, ascorbic acid promoted catechin degradation at ≥75% RH but reduced catechin degradation at 69% RH compared to GT. Only a small amount of the ascorbic acid had dissolved at 69% RH, and at intermediate water activities, moisture may prevent oxidation by hydrating metallic catalysts and forming hydrogen bonds with hydroperoxides. As more water is introduced to the system, the mobility of catalysts is enhanced, thus overcoming the antioxidant potential (*19*) of ascorbic acid. In GTA formulations EC degraded the most. EGC did not degrade to the same extent as EC in the presence of ascorbic acid, and because EC and EGC have similar solubility traits, solubility alone cannot be used to predict the degradation of catechins in the presence of other ingredients.

Whereas ascorbic acid and catechins generally act as antioxidants, under certain conditions they may promote oxidation. The reported pro-oxidant activity of ascorbic acid is due to its ability to reduce transition metal ions, resulting in the formation

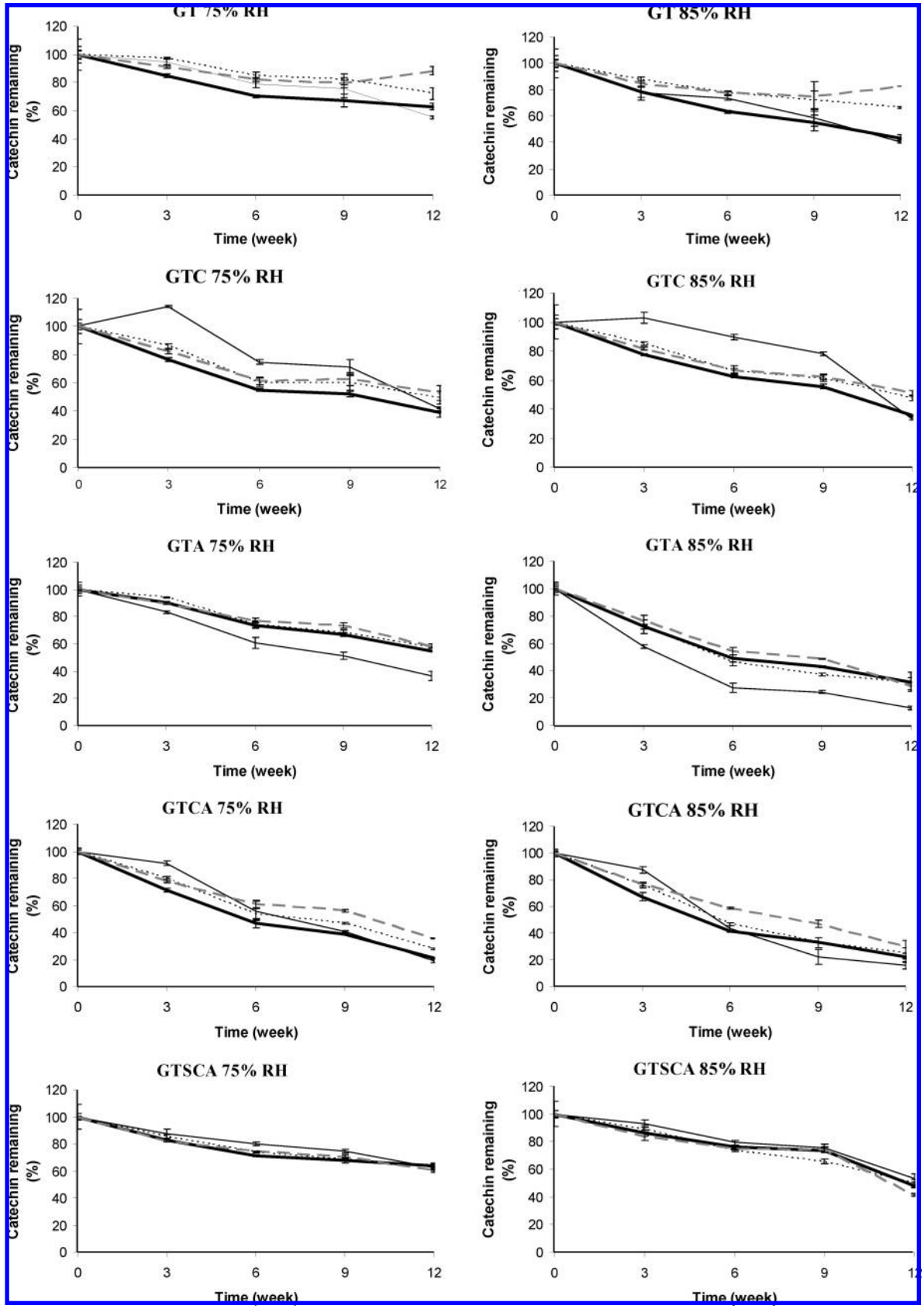

Figure 7. Percent (w/w) individual (EGC, EC, EGCG, and ECG) catechin concentrations remaining in green tea powder formulations after 0−12 weeks of storage at 22 °C and 75% RH (left side) or 85% RH (right side). The powder formulations used were GT (green tea), GTC (green tea + citric acid), GTA (green tea + ascorbic acid), GTCA (green tea + citric acid + ascorbic acid), GTSCA (green tea + sucrose + citric acid + ascorbic acid). The individual catechins are shown by a heavy black line (EGC), a narrower black line (EC), a narrow dashed line (EGCG), and a heavy dashed gray line (ECG).

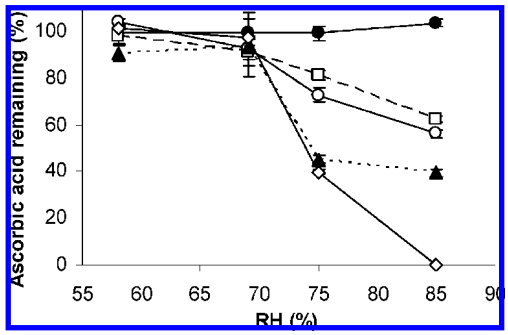

Figure 8. Percent (w/w) of ascorbic acid remaining in the identified formulations after 12 weeks of storage at 22 °C and 58, 69, 75, and 85% RH. Formulations are shown by ● (A), a □ (GTSCA), ○ (GTCA), ▲ (CA), and ◇ (GTA).

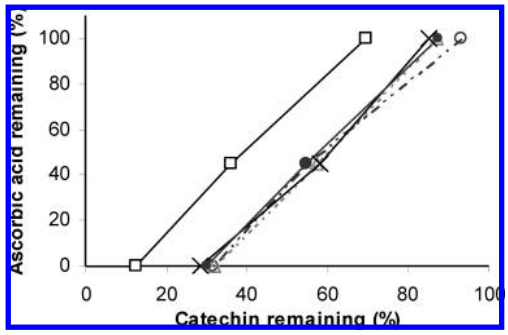

Figure 9. Catechin concentration remaining versus ascorbic acid content remaining in GT formulations after 12 weeks of storage at room temperature in controlled humidity (69−85% RH) environmental chambers. Individual and total catechins are shown by ○ (EGC), □ (EC), △ (EGCG), × (ECG) and ● (total).

of hydrogen peroxide and highly reactive hydroxyl radicals (*20*). Similarly, catechins may act as pro-oxidants (*21*) and generate hydrogen peroxide and/or hydroxyl and superoxide radicals in solution (*21, 22*). Ascorbic acid seems to improve the antioxidant potential of tea in vitro (*23*); however, it may also induce oxidation of (+)-catechin (*24*). It has been suggested that one or more ascorbic acid breakdown products are involved in such oxidation (*24*). Furfural, a degradation product of ascorbic acid, has been found to react with (+)-catechin to produce dimeric adducts having flavonol units linked by furfuryl bridges, which may further oxidize to xanthylium pigments (*25*).

Ascorbic acid alone did not degrade at 75 and 85% RH (**Figure 8**), but in the presence of green tea (GTA), it degraded completely after exposure to 85% RH for 12 weeks. Catechins and ascorbic acid in GTA formulations decreased simultaneously at ≥75% RH (**Figure 9**, $R^2 > 0.95$). These results suggest that ascorbic acid and catechins may be interacting and participating in the degradation of one another.

Ascorbic acid degradation increased in the presence of citric acid (CA), with 60.5% degradation after 12 weeks of storage at 85% RH (**Figure 8**). The degradation of ascorbic acid in CA formulations was also evidenced by development of color (ascorbic acid browning, **Figure 5**). The addition of citric acid to systems containing ascorbic acid increases the chromophore development characteristic of ascorbic acid browning (*26*), indicating that citric acid enhances the degradation of ascorbic acid. Catechins were least stable in GTCA formulations in which the presence of citric acid and tea components resulted in the degradation of ascorbic acid, and at the same time the presence of both acids promoted catechin degradation.

The specific mechanism of catechin degradation in a powdered system needs to be elucidated. The results of this study

Table 1. pH of All Green Tea Formulations and Ingredients at Saturation

sample[a]	pH
GT	5.20 ± 0.06
S	6.30 ± 0.35
C	1.13 ± 0.03
A	1.83 ± 0.03
GTS	5.26 ± 0.05
GTC	1.60 ± 0.04
GTA	2.56 ± 0.04
SC	1.30 ± 0.03
SA	1.99 ± 0.06
CA	1.43 ± 0.04
GTSC	1.58 ± 0.03
GTSA	2.66 ± 0.05
GTCA	1.56 ± 0.05
SCA	1.49 ± 0.03
GTSCA	1.60 ± 0.04

[a] GT, green tea; S, sucrose; C, citric acid; A, ascorbic acid.

suggest several potential pathways. Autoxidation reactions are known to result in the formation of catechin homo- and heterodimers (*17*). Preliminary comparison of retention time and electronic absorption spectra of degradation peaks eluting at 1.3, 1.5, 4.4, and 6 min with previous separations by our group (*17, 27*) suggests the possible formation of EGCG and EGC dimers in GT powdered systems (data not shown). The formation of catechin dimers has been reported to proceed through autoxidation mechanisms in solutions at near neutral or greater pH values, at which catechins scavenge O_2. Superoxide ($O_2^{\bullet -}$) is produced, which then forms a semiquinone intermediate that can dimerize with a similar species (*17*). Epimerizations of catechins can also take place, resulting from either strong base or high-temperature treatments (*28*). The conditions in this study were highly concentrated, which could negate the need for higher pH values or temperatures because the proximity of reactants may overcome the suboptimal conditions. Also the pH values in the samples containing citric and ascorbic acids were very acidic compared to GT alone (pH 1.60, 2.6, and 5.2 in GTC, GTA, and GT, respectively; **Table 1**), and at such conditions the degradation of catechins was enhanced. Tu et al. (*11*) also observed higher catechin degradation in tea infusions acidified with citric acid (pH 2.6 and 3.6). In both studies, the general trend was that catechins degraded more at a pH close to 2 and were more stable at a pH close to 5 [**Figures 2**, **3**, and **7**; Tu et al. (*11*)]. Future experiments utilizing LC-MS/MS and NMR are planned to better characterize the nature of these catechin degradation products.

The impact of catechin degradation in the powdered systems investigated in this study on the biological activity of the catechins is not known. Some of the reactions causing reduction of individual catechins (specifically those involving ascorbic acid) were also involved in the formation of other species including dimers and epimers that may also have biological activity (*17, 29, 30*). Furthermore, formulation of green tea with ascorbic acid was recently found to enhance digestive stability to autoxidative reactions and, by extension, bioaccessibility of these biologically active tea catechins (*27*). Whereas results such as these provide justification to coformulate these ingredients to enhance catechin physiological activity, dry blending of green tea powders with acids, such as citric and ascorbic acid, may accelerate the catechin degradation when exposed to environmental moisture.

The findings of this study are of great importance to the food industry and others who manufacture, store, and/or blend powder formulations. The chemical integrity of the ingredients may not be preserved unless the environmental moisture is maintained

below certain levels, emphasizing the importance of both having adequate control of RH and characterizing stability of powder blends of interest. Manufacturing tea products as dry powder formulations does not protect the chemical integrity of the ingredients unless the RH is tightly controlled below certain levels. In this study, the catechins in the cold water soluble green tea powder were most stable at RH ≤ 43%, catechin stability at 58% RH depended on formulation ingredients, and catechin degradation was significant in most green tea formulations stored at ≥69% RH for 3 months at 22 °C. The types of ingredients present in dry mixtures have a great impact on the chemical characteristics of the finished product, resulting in unwanted transformations and loss of stability. Although trends are expected to be similar between water soluble green tea ingredients, formulators should run storage trials using the ingredients of interest to ascertain stability profiles across storage RH conditions. Formulation, storage, packaging with proper barrier materials, and processing conditions need to be carefully controlled to preserve the physical and chemical stability, as well as the potential biological activity, of green tea products.

ABBREVIATIONS USED

A, ascorbic acid; C, citric acid; EC, (−)-epicatechin; ECG, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin gallate; GC, (−)-gallocatechin; GT, green tea powder formulations; GTA, green tea plus ascorbic acid powder formulations; GTC, green tea plus citric acid powder formulations; GTCA, greem tea plus citric acid and ascorbic acid powder formulations; GTS, green tea plus sucrose powder formulations; GTSCA, green tea plus sucrose, citric acid, and ascorbic acid powder formulations; HPLC, high-performance liquid chromatography; RH, relative humidity; S, sucrose.

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