

Interaction of Environmental Moisture with Powdered Green Tea Formulations: Relationship between Catechin Stability and Moisture-Induced Phase Transformations

JULIETA ORTIZ,[†] UMESH S. KESTUR,[‡] LYNNE S. TAYLOR,[‡] AND LISA J. MAUER^{*,†}

[†]Department of Food Science, Purdue University, 745 Agriculture Mall Drive, West Lafayette, Indiana 47907, and [‡]Department of Industrial and Physical Pharmacy, Purdue University, 575 Stadium Mall Drive, West Lafayette, Indiana 47907

This study investigated the effect of phase transformations of amorphous and deliquescent ingredients on catechin stability in green tea powder formulations. Blends of amorphous green tea and crystalline sucrose, citric acid, and/or ascorbic acid were analyzed by X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), dynamic water vapor sorption, water activity measurements, and high-performance liquid chromatography (HPLC) after storage for up to 12 weeks at 0–75% relative humidity (RH) and 22 °C. The glass transition temperature (T_g) of green tea was reduced to below room temperature (<22 °C) at 68% RH. Dissolution of deliquescent ingredients commenced at RH values below deliquescence points in blends with amorphous green tea, and these blends had greater water uptake than predicted by an additive model of individual ingredient moisture sorption. Catechin degradation was affected by T_g of green tea powder and both dissolution and deliquescence of citric and ascorbic acids.

KEYWORDS: Catechins; water vapor sorption; phase behavior; chemical stability; deliquescence

INTRODUCTION

The presence of moisture in a powdered product can influence the rate of chemical degradation in the solid state, dispersibility, wetting, powder flow, lubricity, powder compactibility, compact hardness, dissolution rate, etc. (1). It has been established that organic solids degrade when exposed to moisture, but the factors which govern reactivity and the mechanisms by which certain compounds are more sensitive to degradation at high relative humidities are not well understood (2). Moisture may interact with a solid by adsorption on the surface (as mono- and multi-layers), absorption into the bulk phase (typical of amorphous materials), capillary condensation, crystal hydrate formation, and deliquescence (3). Of these, the formation of crystal hydrates, the absorption of water into amorphous solids, and deliquescence are the most critical in affecting the properties of the solid (3).

Deliquescence is a first-order phase transformation in which crystalline solids undergo dissolution when the environmental relative humidity (RH) exceeds the critical relative humidity, RH_o , of the solid (4). The condensation of atmospheric humidity onto the surface of the deliquescent crystalline solid induces its dissolution. When different deliquescent solids are in contact, deliquescence lowering takes place, and dissolution occurs at a lower relative humidity (RH_{mix}) than the individual RH_o s (5). Deliquescence is affected by multiple ingredient interactions and can lead to degradation and losses of physiologically important compounds during storage (6, 7).

The three-dimensional long-range order characteristic of crystalline substances is absent in amorphous materials, and hence the physical properties of these solid states are different (8). Some processing techniques used to obtain dry powders may lead to partially or fully amorphous products (8, 9), which are usually more chemically unstable than their crystalline counterparts (10, 11). Water acts as a plasticizer when absorbed by amorphous substances and increases the molecular mobility (12, 13). Exposing amorphous substances to high relative humidities results in moisture sorption, plasticization, and often a decrease in physical and chemical stability, particularly if the storage temperature exceeds the glass transition temperature, T_g (8).

The addition of water changes the composition of a product and may induce phase transformations (14). Most powdered food products contain a mixture of different ingredients, and it is important to understand the behavior of blends of amorphous and crystalline ingredients to prevent undesired changes in the properties of the product. Sucrose, citric acid, and ascorbic acid are common crystalline ingredients used in bottled and canned tea beverages (15). They all deliquesce: sucrose at 85% RH, citric acid at 75% RH, and ascorbic acid at 97% RH at 25 °C (5, 7). In diluted green tea systems (beverage solutions) monitored during short-term storage, acidic pH values enhanced catechin stability (16); however, in long-term storage of tea drinks (up to 6 months) acidified products were more susceptible to catechin degradation (15). Therefore, coformulation of green tea with citric or ascorbic acid could be problematic. Information on the stability and properties of amorphous green tea powder

*Corresponding author [e-mail mauerl@purdue.edu; telephone (765) 494-9111; fax (765) 494-7953].

alone and in combination with common crystalline ingredients is limited (7).

Because water is a key mediator of both chemical and physical instabilities in crystalline and amorphous powdered blends (17), it is 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 and temperature. A previous study investigated the effects of storage RH and formulation on catechin stability in green tea powder formulations containing sucrose, citric acid, and/or glucose (7). Increasing storage RH and the presence of citric and ascorbic acids were related to catechin degradation (7). However, the underlying mechanisms of water–solid interactions and phase transformations were not addressed. Further understanding of the physicochemical properties of all ingredients in the blends, as well as the interactions of the powder mixtures with moisture, would lead to better formulation strategies directed toward improving stability.

The objective of this study was to determine the effects of phase transformations (deliquescence, dissolution, and glass transition) on the chemical stability of catechins in green tea powder formulations. It is hypothesized that the presence of a water-soluble amorphous ingredient (green tea powder) will alter the water sorption behavior of deliquescent ingredients, allowing them to dissolve at RH values lower than their RH_0 values. Likewise, the presence of deliquescent ingredients may destabilize amorphous green tea catechins by inducing rapid moisture uptake, and hence catechin dissolution, at lower RH values than expected. It is also hypothesized that a phase transformation, whether deliquescence, glass transition, or dissolution of any ingredient in the blend, will enhance catechin degradation.

MATERIALS AND METHODS

Materials. The ingredients used in dry blend formulations were powdered green tea (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 ratio of ingredients was 20:1:1:0.3 of sucrose, green tea, citric acid, and ascorbic acid, respectively, to simulate sweet/sour taste and flavor profiles of green tea and to provide the recommended daily intake of vitamin C (60 mg). The salts used to provide selected relative humidities in environmental chambers were anhydrous calcium sulfate (W. A. Hammond Drierite Co. Ltd., Xenia, OH); potassium acetate, sodium bromide, and copper chloride (Fisher Scientific); phosphorus pentoxide, potassium carbonate, magnesium chloride, cobalt chloride, sodium chloride, and potassium chloride (Mallinckrodt-Baker); lithium chloride (EMD Chemicals Inc., Gibbston, NJ); and magnesium nitrate (Sigma Aldrich, St. Louis, MO).

Preparation of Samples. Triplicate samples of green tea and 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 (A), levels representative of typical concentration ratios in a 1 cup single-strength beverage, were prepared and stored for up to 12 weeks in environmental chambers at 22 °C with RH controlled by saturated salt solutions at 0, 11, 25, 33, 43, 54, 59, 68, and 75% RH. For the differential scanning calorimetry (DSC) analysis, triplicate samples of GT and GTC were also placed in the environmental chambers. After a plateau in water uptake was reached (after 14 days of storage), the samples were removed. About 3–5 mg of the samples was placed in DSC pans and hermetically sealed.

X-ray Powder Diffraction. A Shimadzu LabX XRD-6000 (Kratos Analytical, Chestnut, NY) diffractometer operating in Bragg–Brentano configuration and equipped with fine-focus Cu K α rated at 1500 W operating at 40 kV/30 mA was used to obtain diffractograms. Prior to measuring the sample, calibration was done using Si. The sample was scanned from 5 to 60 degrees 2 θ at 4 degrees per minute, with a 0.1 degree

step size. The sample holders used were low-background sample holders with Si inserts. The samples were run in duplicate.

Differential Scanning Calorimetry. The onset of the glass transition temperature (T_g) was measured in triplicate using a TA Q10 DSC equipped with a refrigerated cooling accessory (TA Instruments, New Castle, DE). Operating in standard mode, the instrument was calibrated for temperature using benzophenone (Sigma-Aldrich Inc.A) and indium (Perkin-Elmer Corp., Norwalk, CT), and the enthalpic response was calibrated using indium. Nitrogen, 50 mL/min, served as the purge gas, and reference and sample pans were matched for weight to within 0.01 mg. The samples were cooled to –20 °C and then heated at 20 °C/min after erasing thermal history by heating the samples to 20 °C above the T_g and cooling to 50 °C below the T_g before actual T_g measurements were done.

Gravimetric Moisture Sorption. Dynamic moisture sorption was conducted using a Symmetrical Gravimetric Analyzer (SGA-100) (VTI Corp., Hialeah, FL) coupled with a 3-CCD color camera model HV-030 (Hitachi Kakusai Electric, Woodbury NY). Physical mixtures or individual components were prepared in duplicate, and 10–20 mg was analyzed. Samples were dried at 60 °C using an equilibrium criterion of 0.01% w/w with a maximum drying time of 30 min. Temperature was then reduced to 25 °C, and samples were exposed to increasing RH (0–95%) using an equilibrium criterion of 0.001% w/w with a maximum step time of 60 min. RH_0 and RH_{mix} were measured from the vapor sorption isotherms by extrapolating the linear parts of the isotherm before and after the deliquescence event. RH_0 and RH_{mix} were also determined by measuring, in duplicate, the water activity (a_w) of the samples at 25 °C using an Aqua Lab 3TE (Decagon, Pullman, WA) as described by Salameh et al. (5). Predicted isotherms for the different blends were calculated from the experimental isotherms of each individual ingredient by adding their contributions to moisture sorption at each RH. The predicted moisture sorption isotherms of the mixtures were then compared to those obtained experimentally for the same mixture, and the difference was plotted against RH.

Water Activity Measurements. To measure water activity (a_w), saturated solutions were prepared by mixing approximately 2 g of sample and adding ~300–600 μ L of double-distilled water. The a_w values of individual and multicomponent saturated solutions were measured in duplicate at 25 °C using an Aqua Lab 3TE (Decagon, Pullman, WA). The a_w was determined by using dew point and sample surface temperatures.

Determination of Catechin Concentrations. Catechin concentrations were determined by high-performance liquid chromatography (HPLC) using the method described by Ortiz et al. (7, 18). 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. \times 100 mm) reverse phase column (Milford, MA) with a guard column packed with the same stationary phase were used. Detection and tentative identification of catechins were done using in-line PDA data between 220 and 600 nm, and total catechins were calculated as the sum of all measured individual catechins.

Statistical Analysis. A completely randomized two-factor factorial design with $n = 3$ was used for the catechin stability study. The data were analyzed using two-way ANOVA models. The factors were RH and formulation, and the responses were catechin concentration remaining and mass gain (%). 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 AND DISCUSSION

Green Tea Powder Physical Characterization. The X-ray diffractogram of GT showed a halo characteristic of an amorphous substance (Figure 1). This result was expected because the green tea powder was spray-dried. Spray-drying leads to rapid dehydration and frequently results in an amorphous powder that can be hygroscopic (8, 19).

The onset glass transition (T_g) temperatures of green tea samples equilibrated at 0, 11, 25, 33, 43, 54, 59, 68, and 75% RH are presented in Figure 2. The onset of T_g was reduced to the sample storage temperature (22 °C) at some point between 59 and

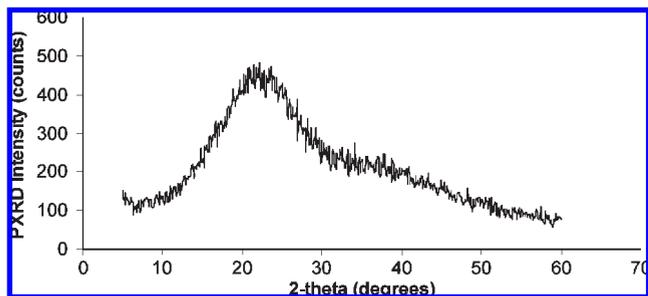


Figure 1. X-ray powder diffraction pattern of green tea powder showing a characteristic amorphous halo.

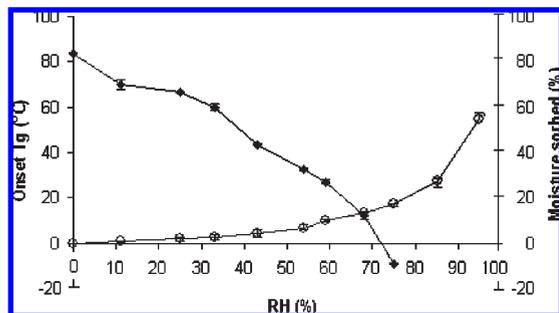


Figure 2. Onset of glass transition temperature, measured using differential scanning calorimetry, for green tea powder equilibrated at 0, 11, 25, 33, 43, 54, 59, 68, and 75% RH and moisture sorbed (% w/w) at each RH: (◆) onset of T_g ; (○) moisture sorbed. Error bars show standard deviation where $n = 3$.

69% RH, estimated by interpolation of the data to be approximately 64% RH (**Figure 2**). At 59% RH, the onset of T_g was 27 °C, close to the storage temperature, and at 68% RH it was 12.1 ± 1.4 °C. After storage at 59% for 12 weeks, GT appeared to have caked and the coloration had changed from green to brown. The transformation of the GT powder into a viscous mass occurred during storage at 68% RH, and above this RH the material became noticeably more liquid-like, suggesting a decrease in viscosity. Although caking is often observed in amorphous samples above the T_g , it is possible for caking and crystallization to occur below the T_g if storage time is extended (20, 21).

Moisture Sorption Behavior of Individual Ingredients and Green Tea Blends. As expected, storage RH had the largest effect on moisture sorption, based on comparison of mean square errors (MSE) in the ANOVA table generated from the study of the effects of RH, ingredient formulation, and their interaction on moisture sorption of the tea blends. The largest MSE value corresponded to the effect of RH. Additionally, both formulation and the interaction of formulation and RH significantly affected moisture sorption ($P < 0.0001$). After 12 weeks of storage, water uptake was significantly larger ($P < 0.0001$) above the glass transition event in GT (68% RH versus 59% RH) (**Figures 3** and **4**). However, in all blends with other ingredients (GTC, GTA, GTCA, and GTSCA) a significantly larger ($P < 0.0001$) amount of moisture was sorbed at 59% RH than at 43% RH, suggesting an increased sensitivity to RH.

The water vapor sorption isotherms of the individual ingredients used in this study (GT, S, C, A) are shown in **Figure 3**. The green tea powder was hygroscopic and had an isotherm similar to that published for freeze-dried green tea (22). The water uptake profile was characteristic of an amorphous compound in that it continually increased as a function of RH (23). Sucrose and citric acid deliquesced at 76 and 86% RH, respectively, at 25 °C

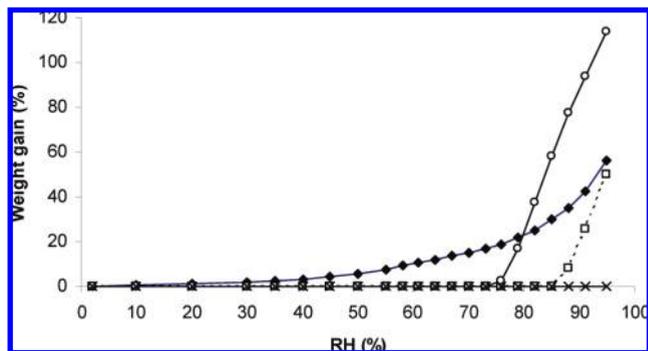


Figure 3. Dynamic moisture sorption isotherms of green tea (GT, ◆), sucrose (S, □), citric acid (C, ○), and ascorbic acid (A, ×) exposed to 0–95% RH at 25 °C.

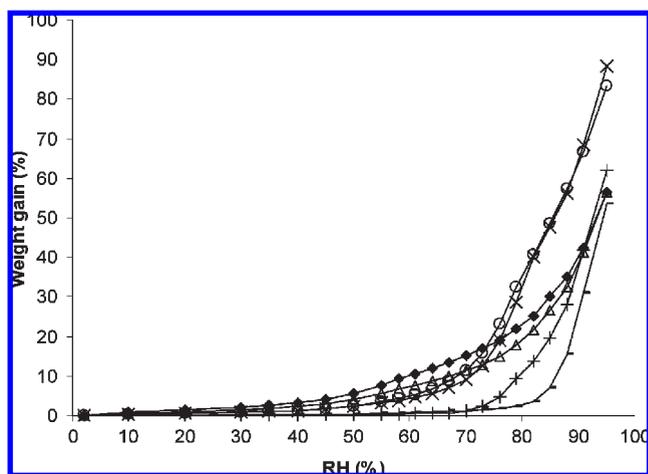


Figure 4. Dynamic moisture sorption isotherms of green tea (GT) powder and mixtures containing sucrose (S), citric acid (C), and/or ascorbic acid (A) exposed to 0–95% RH at 25 °C. Formulations are shown as follows: (◆) GT; (-) GTS; (○) GTC; (△) GTA; (×) GTCA; (+) GTSCA.

(**Figure 3**). These values were in close agreement with those obtained by measuring the a_w of a saturated solution of the ingredient and multiplying this a_w by 100 (76.9 ± 0.6 for citric acid and 85.0 ± 0.1 for sucrose, shown in **Table 1**). Ascorbic acid did not deliquesce under the experimental conditions (0–95% RH) in the moisture sorption balance. The RH₀ obtained for ascorbic acid by the measurement of the a_w of its saturated solution was 96.6 ± 0.1 (**Table 1**).

The moisture sorption profiles for the blends of green tea and sucrose, citric acid, and/or ascorbic acid are shown in **Figure 4**. As hypothesized, the presence of amorphous green tea powder altered the water sorption behavior of citric acid, ascorbic acid, and sucrose, allowing enhanced water sorption below their RH₀ values. This is shown in **Figure 5**, where the difference between the experimental and predicted moisture sorption at each RH is plotted.

Obviously changing ingredient ratios would be expected to alter the moisture uptake of the blends containing both amorphous and crystalline powders, and moisture sorption can either be additive (i.e., only dependent on the amount of the individual components and their individual hygroscopic behaviors (24)) or synergistic, whereby more (or less) water is sorbed by the blend than would be anticipated on the basis of composition (25). It is interesting to note that all of the experimental isotherms of GT blends had enhanced moisture sorption compared to the amount predicted by consideration of the water uptake of the individual

Table 1. Values of a_w at 25 °C^a

sample	a_w at 25 °C	Ross eq	RHo	RHomix	total wt gain at 95% RH
GT	0.770 ± 0.011				55.6 ± 1.0
S	0.850 ± 0.001		86		49.7 ± 1.0
C	0.769 ± 0.006		76		113.5 ± 1.4
A	0.966 ± 0.001		>95		0 ± 0.0
GTS	0.803 ± 0.008				51.7 ± 1.8
GTC	0.626 ± 0.003				84.9 ± 2.6
GTA	0.764 ± 0.013				56.5 ± 1.1
SC	0.550 ± 0.009	0.654		65	
SA	0.819 ± 0.021	0.821		85	
CA	0.751 ± 0.004	0.742		74	
GTSC	0.633 ± 0.003				
GTSA	0.774 ± 0.018				
GTCA	0.614 ± 0.014				87.9 ± 0.4
SCA	0.594 ± 0.022	0.632		0.7	
GTSCA	0.606 ± 0.001				59.5 ± 2.4

^a Values predicted from the Ross equation: $(a_w)_{\text{mix}} = (a_w)_1(a_w)_2(a_w)_i$; experimental RHo values and RHomix and total weight gain due to moisture sorption at 95% RH and 25 °C. Data for Ross equation, RHo, and RHomix shown only for deliquescent ingredients and their blends.

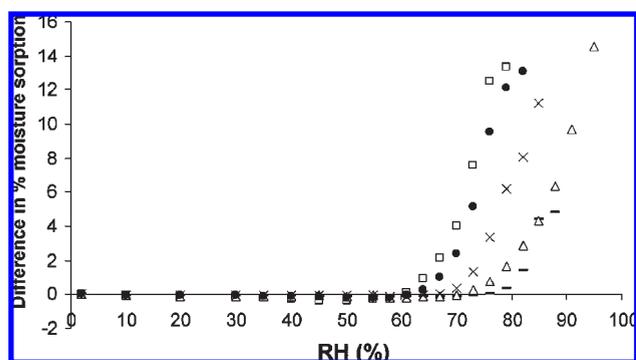


Figure 5. Difference in percent moisture sorption between experimental and predicted moisture sorption isotherms of green tea + sucrose (GTS), green tea + citric acid (GTC), green tea + ascorbic acid (GTA), green tea + citric acid + ascorbic acid (GTCA), and green tea + sucrose + citric acid + ascorbic acid (GTSCA) powder blends exposed to 0–95% RH at 25 °C. Predicted isotherms for the different blends were calculated from the experimental isotherms of each individual ingredient (Figure 3) by adding their contributions to moisture sorption at each RH. The predicted moisture sorption isotherms of the mixtures were then compared to those obtained experimentally for the same mixture, and the difference was plotted against RH. Different formulations are presented as (-) GTS, (□) GTC, (△) GTA, (●) GTCA, and (×) GTSCA.

ingredients (Figure 5). In particular, upon the addition of citric acid to GT in GTC and GTCA formulations, the moisture uptake was greatly increased when the RH was close to the deliquescent RHo or RHomix (Figures 4 and 5). In GTC at 67, 70, and 73% RH (below the RHo of citric acid), the differences between the experimental and predicted moisture gain were 2.2, 4.0, and 7.6%, respectively. In GTA at 82, 85, 88, 91, and 95% RH (below the RHo of citric acid), the moisture gains were 3, 4.3, 6.7, 10.1, and 14.7% more than predicted, respectively. This trend was also observed in GTCA, GTSCA, and GTS formulations below their respective RHomix values. Therefore, the mixture of amorphous and deliquescent ingredients induced higher moisture uptake than expected below the deliquescence RH values. Another study found that the presence of liquid impurities had an effect on decreasing the RHo at which solid-solution phase transformations occur and that moisture sorption was enhanced below RHo to a larger extent than what would be predicted by the

sum of the moisture sorptions of the individual ingredients (26). In the present study, we observed that an amorphous material, plasticized above the T_g , can have a similar effect.

In deliquescent ingredient blends, the RHomix can be predicted using the Ross equation (5):

$$(a_w)_{\text{mix}} = (a_w)_1(a_w)_2(a_w)_i$$

Values for RHomix of the blends of the deliquescent ingredients used are reported in Table 1. The Ross equation has been shown to be less accurate in predicting RHomix when more than two deliquescent ingredients are present (5, 6). Added to this, it is apparent that the presence of amorphous green tea affected the interaction of the deliquescent ingredients with moisture (Figure 5). The photographs taken in the moisture sorption apparatus showed some dissolution of deliquescent ingredients at RH values below their deliquescence points; for example, citric acid crystals underwent some dissolution at 60% RH in a GTSCA mixture (Figure 6).

The results presented here are very important because they indicate that amorphous and deliquescent ingredients may interact synergistically to promote higher moisture uptake and at lower RH values than expected. Our results provide evidence that the plasticized amorphous material, in this case GT, leads to partial dissolution of crystalline substances at RH values at which no dissolution would occur for the pure substances, and this leads to enhanced moisture uptake. The enhanced moisture uptake in the presence of additional dissolved solute can be theoretically rationalized by consideration of the Gibbs–Duhem relationship at constant temperature and pressure, where ideality is assumed for all solutes (2, 27):

$$\text{RH} = \exp(-M_w \sum_s m_s)$$

M_w is the molecular weight of water and m_s is the molality of each solute, s . From this equation it can be seen that if an additional solute dissolves at a given and fixed RH (in this case the deliquescent solid that is partially dissolving prior to RHo), the total molality must remain constant. Given that molality is the number of moles of solute per kilogram of solvent, an increase in moisture content is thus necessary to remain a constant molality. In other words, if the number of moles of total dissolved solute increases, then the Gibbs–Duhem equation predicts that the mass of absorbed water must also increase.

Catechin Stability. Catechin stability decreased as moisture sorption increased in all formulations (Figure 7). RH had the largest effect on catechin stability, based on comparison of mean square errors in the ANOVA table generated from the study of the effects of RH, ingredient formulation, and their interaction on catechin stability. The largest MSE value corresponded to the effect of RH. Formulation and the interaction of formulation and RH also significantly affected catechin stability ($P < 0.0001$). These results are consistent with a previous report of storage RH and formulation effects on total and individual catechin stability in powdered green tea blends (7).

Effect of Glass Transition on Green Tea Catechin Stability. No significant degradation of the catechins in GT powder alone took place during storage for 12 weeks at $\leq 59\%$ RH and 22 °C, but catechins in GT degraded at $\geq 68\%$ RH (7). From the DSC measurements, the onset of T_g at 59% RH was 27 °C (Figure 2); therefore, the glass transition had not occurred in the samples stored at 59% RH and 22 °C. The onset T_g at 68% RH was 12.1 °C, below the storage temperature. The results indicate that catechins significantly degraded in GT at 22 °C only when the sample was stored above the onset T_g . In many

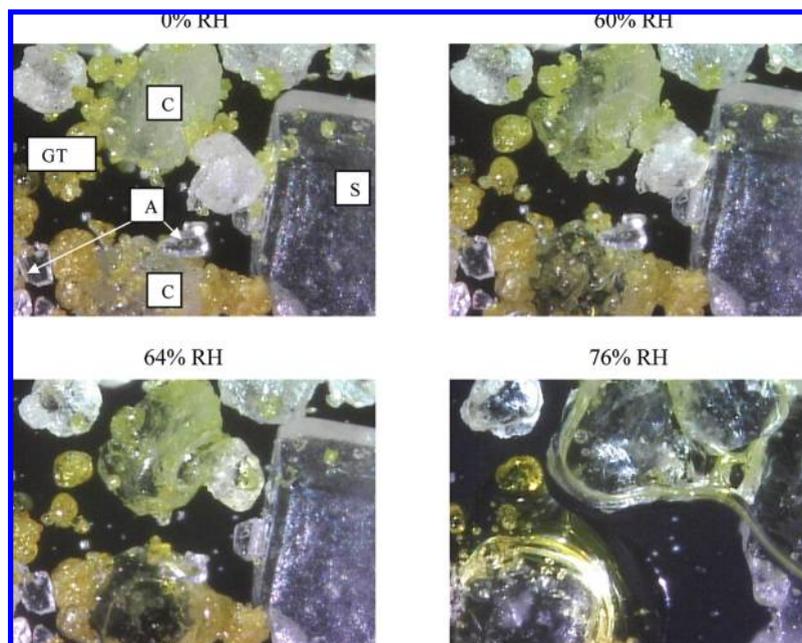


Figure 6. Photographs taken during moisture sorption isotherms of green tea + sucrose + citric acid + ascorbic acid (GTSCA) at 0, 60, 64, and 76% RH and 25 °C. When in contact with green tea, citric acid dissolution occurred at and above 60% RH and ascorbic acid dissolution at and above 76% RH.

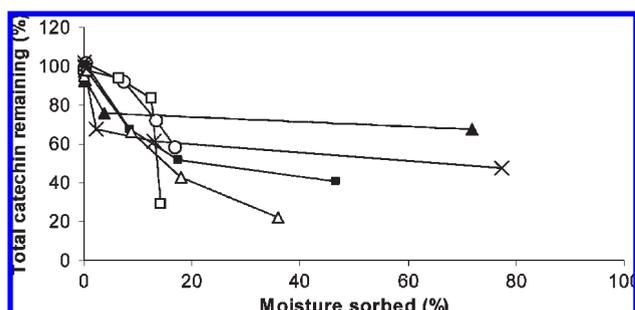


Figure 7. Relationship between moisture sorption and catechin stability in powdered green tea formulations stored for 12 weeks at 0–75% RH and 22 °C. Each point corresponds to the averages of catechin concentration and total moisture sorbed at each RH. Formulations are shown as follows: (○) GT; (▲) GTS; (■) GTC; (□) GTA; (△) GTCA; (×) GTSCA.

amorphous materials, it has been demonstrated that chemical stability decreases above T_g , especially in diffusion-limited processes (28).

It has been reported that the presence of crystalline compounds is not expected to affect T_g unless some dissolution of the crystalline material in the amorphous phase occurs, which has a plasticization effect that ultimately results in a decrease in T_g and storage stability (29). This appears to have happened in the GTC blends. DSC measurements of equilibrated GTC samples (data not shown) found that at 59% RH the onset of T_g occurred at 18.0 ± 2 °C, which was below the storage temperature (22 °C). In GT alone, the onset of T_g occurred at 27 ± 1 °C (above the storage temperature). GTC samples at 59% RH were no longer a caked powder, but had transformed into a fused viscous mass.

Effects of Phase Transformations of Deliquescent Ingredients on Catechin Stability. Phase transformations of deliquescent ingredients significantly influenced catechin stability. When the values of concentration of total catechins remaining in GTC and GTCA below and above the RH where C and CA undergo deliquescence (76 and 74%, respectively, **Table 1**) were compared, catechins degraded significantly more ($P < 0.05$) above the RH_0 or RH_{mix} of the deliquescent ingredients compared to samples

stored below these RH values. In GT, catechins were stable for 12 weeks at 22 °C at $\leq 59\%$ RH; however, in GTC catechin degradation occurred at 59% RH but not at $\leq 43\%$ RH (7). Additionally, the enhancement of catechin degradation was significant when GTC blends below and above the RH_0 of citric acid (68 vs 85% RH) were compared, indicating that deliquescence played an important role in further promoting catechin degradation and confirming the effect of the additional ingredients on chemical stability, which is much more subtle below RH_0 .

Although citric acid by itself did not adsorb detectable amounts of moisture below 75% RH (**Figure 3**), the hygroscopic nature of green tea powder allowed significant amounts of moisture to be incorporated into GTC mixtures between 50 and 60% RH (**Figure 4**). Partial dissolution of citric acid in GTC was observed in photographs of the sample subjected to water vapor sorption at 60% RH (similar to those presented **Figure 6**), which was related to catechin degradation. Thus, a partial phase transformation of citric acid from the crystalline solid to solution took place at RH values below RH_0 in the presence of amorphous green tea. It appears that the water-plasticized amorphous green tea promoted citric acid dissolution at RH values lower than its RH_0 , although the amount of moisture sorbed was less than at the deliquescence event (**Figure 3**). This dissolution differs from the deliquescence process because it is mediated by water already present in the amorphous matrix. GTC blends stored at 59% RH for 12 weeks sorbed $8.43 \pm 0.6\%$ water. On the basis of the solubility of citric acid (30) and assuming that its dissolution is independent from that of green tea powder, this amount of water would result in a maximum of 19.70 ± 1.6 mg citric acid dissolved. This observation is important for two reasons. First, the dissolved acid would be expected to influence the microenvironment of the catechins, thereby influencing degradation rates. Second, the dissolved citric acid will result in more water being absorbed into the system and accounts for the discrepancy in the experimentally observed and theoretically predicted moisture sorption values shown in **Figure 5**. As a result, the glass transition event occurred below the storage temperature (verified by DSC results for GTC samples), accounting for the increase in catechin degradation at a lower RH than for GT. As the RH increased, allowing more

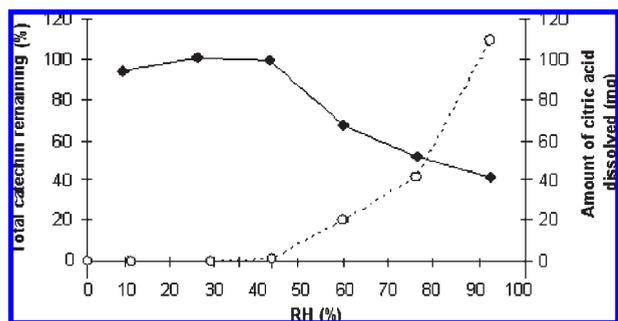


Figure 8. Total catechin remaining and hypothetical amount of acid dissolved in GTC samples stored for 3 months at 0–85% RH and 22 °C. Data are shown as (◆) total catechins remaining and (○) amount of acid dissolved.

moisture to be sorbed, the amount of acid in solution was also expected to increase, thereby further enhancing catechin degradation (**Figure 8**).

The addition of ascorbic acid was related to catechin degradation at $\geq 75\%$ RH (7), where visual dissolution of ascorbic acid in contact with GT also was noted (also seen in **Figure 6**). No significant degradation of catechins occurred below this RH in GTA. Because ascorbic acid did not adsorb moisture below its RH_0 (97%), the dissolution observed at 75% RH must have been due to the moisture introduced into the blend by the green tea. The dissolution of ascorbic acid in the blend at 75% RH coincided with catechin degradation. Sucrose also underwent dissolution at a lower RH than its RH_0 in the presence of green tea, but no catechin degradation occurred in GTS samples even at 85% RH (7). These results indicate that catechin degradation related to solid-solution transformations is affected by the type of deliquescent ingredient present, with organic acids promoting degradation.

In summary, moisture-induced phase transformations (glass transition, dissolution, and deliquescence) were related to catechin chemical degradation. A synergistic effect resulting from the interaction of amorphous and crystalline ingredients and environmental moisture increased the water vapor sorption to higher levels occurring at lower relative humidities than those predicted by a simple additive model of the individual contributions. The water-soluble amorphous nature of spray-dried green tea influenced the deliquescence relative humidity of the crystalline ingredients by facilitating water uptake and dissolution. The physical and chemical characteristics of green tea spray-dried powders are compromised if the products are exposed to relative humidities that reduce the glass transition temperature to (or below) the storage temperature. Higher storage temperatures or formulation with other ingredients can enhance reactivity, and degradation can occur at lower relative humidities. In green tea powder alone, the onset T_g dropped below room temperature between 59 and 68% RH, and catechins were stable at 59% RH but not at 68% RH. For blends with citric acid, the T_g at 59% RH was lowered to 18.0 ± 2 °C and storage at 59% RH compromised catechin stability. Catechins were stable in all samples stored below RH_{mix} and T_g . The combination of temperature, environmental moisture, and type of co-ingredient(s) present needs to be carefully controlled to prevent phase transformations that will detrimentally affect the physical and chemical integrity of green tea powder formulations.

ABBREVIATIONS USED

A, ascorbic acid; a_w , water activity; C, citric acid; DSC, differential scanning calorimetry; GT, green tea powder formulations;

GTA, green tea plus ascorbic acid powder formulations; GTC, green tea plus citric acid powder formulations; GTCA, green 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; T_g , glass transition temperature; XRPD, X-ray powder diffraction.

ACKNOWLEDGMENT

We thank Nestlé Product Technology Center (Marysville, OH) for the donation of green tea powder. We also thank Dr. Mario Ferruzzi and Rodney Green for support in HPLC analyses.

LITERATURE CITED

- (1) Giron, D.; Goldbronn, C.; Mutz, M.; Pfeffer, S.; Piechon, P.; Schwab, P. Solid state characterizations of pharmaceutical hydrates. *J. Therm. Anal. Calorim.* **2002**, *68*, 453–465.
- (2) Guerrieri, P. P.; Smith, D. T.; Taylor, L. S. Phase behavior of ranitidine HCl in the presence of degradants and atmospheric moistures—impact on chemical stability. *Langmuir* **2008**, *24*, 3850–3856.
- (3) Zografi, G. States of water associated with solids. *Drug. Dev. Ind. Pharm* **1988**, *14*, 1905–1926.
- (4) Van campen, L.; Amidon, G. L.; Zografi, G. Moisture sorption kinetics for water-soluble substances. I. Theoretical considerations of heat-transport control. *J. Pharm. Sci.* **1983**, *72*, 1381–1388.
- (5) Salameh, A. K.; Mauer, L. J.; Taylor, L. S. Deliquescence lowering in food ingredient mixtures. *J. Food Sci.* **2006**, *71*, E10–E16.
- (6) Hiatt, A. N.; Ferruzzi, M. G.; Taylor, L. S.; Mauer, L. J. Impact of deliquescence on the chemical stability of vitamins B₁, B₆, and C in powder blends. *J. Agric. Food Chem.* **2008**, *56*, 6471–6479.
- (7) Ortiz, J.; Ferruzzi, M. G.; Taylor, L. S.; Mauer, L. J. Interaction of environmental moisture with powdered green tea formulations: effect on catechin chemical stability. *J. Agric. Food Chem.* **2008**, *56*, 4068–4077.
- (8) Hancock, B. C.; Zograf, G. Characteristics and significance of the amorphous state in pharmaceutical systems. *J. Pharm. Sci.* **1997**, *86*, 1–12.
- (9) Saleki-Gerhardt, A.; Ahlneck, C.; Zografi, G. Assessment of disorder in crystalline solids. *Int. J. Pharm.* **1994**, *101*, 237–247.
- (10) Oberholtzer, E. R.; Brenner, G. S. Cefoxitin sodium—solution and solid-state chemical-stability studies. *J. Pharm. Sci.* **1979**, *68*, 863–866.
- (11) Pikal, M. J.; Lukes, A. L.; Lang, J. E.; Gaines, K. Quantitative crystallinity determinations for beta-lactam antibiotics by solution calorimetry—correlations with stability. *J. Pharm. Sci.* **1978**, *67*, 767–772.
- (12) Schmitt, E.; Davis, C. W.; Long, S. T. Moisture-dependent crystallization of amorphous lamotrigine mesylate. *J. Pharm. Sci.* **1996**, *85*, 1215–1219.
- (13) Shalaev, E. Y.; Zografi, G. How does residual water affect the solid-state degradation of drugs in the amorphous state?. *J. Pharm. Sci.* **1996**, *85*, 1137–1141.
- (14) Irzyniec, Z.; Klimczak, J. Effect of temperature on sorption isotherms of Brussels sprout. *Nahrung/Food* **2003**, *47*, 24–27.
- (15) Chen, Z. Y.; Zhu, Q. Y.; Tsang, D.; Huang, Y. Degradation of green tea catechins in tea drinks. *J. Agric. Food Chem.* **2001**, *49*, 477–482.
- (16) Zhu, Q. Y.; Zhang, A. Q.; Tsang, D.; Huang, Y.; Chen, Z. Y. Stability of green tea catechins. *J. Agric. Food Chem.* **1997**, *45*, 4624–4628.
- (17) Ahlneck, C.; Zografi, G. The molecular-basis of moisture effects on the physical and chemical-stability of drugs in the solid-state. *Int. J. Pharm.* **1990**, *62*, 87–95.
- (18) Neilson, A. P.; Green, R. J.; Wood, K. V.; Ferruzzi, M. G. High-throughput analysis of catechins and theaflavins by high performance liquid chromatography with diode array detection. *J. Chromatogr. A* **2006**, *1132*, 132–140.
- (19) Bhandari, B. R.; Datta, N.; Howes, T. Problems associated with spray drying of sugar-rich foods. *Dry. Technol.* **1997**, *15*, 671–684.

- (20) Choong, M. S.; Ruan, R.; Chen, P.; Kim, J. H.; Ahn, T. H.; Baik, C. K. Predicting caking behaviors in powdered foods using a low-field nuclear magnetic resonance (NMR) technique. *Lebensm. Wiss. Technol.* **2003**, *36*, 751–761.
- (21) Yoshioka, M.; Hancock, B. C.; Zografi, G. Crystallization of indomethacin from the amorphous state below and above its glass transition temperature. *J. Pharm. Sci.* **1994**, *83*, 1700–1705.
- (22) Sinija, V. R.; Mishra, H. N. Moisture sorption isotherms and heat of sorption of instant (soluble) green tea powder and green tea granules. *J. Food Eng.* **2008**, *86*, 494–500.
- (23) Swaminathan, V.; Kildsig, D. O. An examination of the moisture sorption characteristics of commercial magnesium stearate. *AAPS Pharm. Sci. Technol.* **2001**, *2*, 73–79.
- (24) Bronlund, J.; Paterson, T. Moisture sorption isotherms for crystalline, amorphous and predominantly crystalline lactose powders. *Int. Dairy J.* **2004**, *14*, 247–254.
- (25) Mackin, L.; Zanon, R.; Park, J. M.; Foster, K.; Opalenik, H.; Demonte, M. Quantification of low levels (<10%) of amorphous content in micronised active batches using dynamic vapour sorption and isothermal microcalorimetry. *Int. J. Pharm.* **2002**, *231*, 227–236.
- (26) Guerrieri, P.; Salameh, A. K.; Taylor, L. S. Effect of small levels of impurities on the water vapor sorption behavior of ranitidine HCl. *Pharm. Res.* **2007**, *24*, 147–156.
- (27) Marcolli, C.; Luo, B.; Peter, T. Mixing of the organic aerosol fractions: liquids as the thermodynamically stable phases. *J. Phys. Chem.* **2004**, *108*, 2216–2224.
- (28) Passot, S.; Fonseca, F.; Barbouche, N.; Marin, M.; Alarcon-Lorca, M.; Rolland, D.; Rapaud, M. Effect of product temperature during primary drying on the long-term stability of lyophilized proteins. *Pharm. Dev. Technol.* **2007**, *12*, 543–553.
- (29) Spyrou, E. Storage stable UV powder coatings. *Focus Powder Coat.* **2005**, *9*, 3–4.
- (30) Schilling, S. U.; Bruce, C. D.; Shah, N. H.; Malick, W. A.; McGinity, J. W. Citric acid monohydrate as a release-modifying agent in melt extruded matrix tablets. *Int. J. Pharm.* **2008**, *361*, 158–168.

Received December 12, 2008. Revised manuscript received April 20, 2009. This research was supported in part by USDA-NRICGP Grant 07-35503-18405.