LWT - Food Science and Technology 44 (2011) 67–74

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Phytochemical stability in dried apple and green tea functional products as related to moisture properties

Mark E. Corey [a], William L. Kerr [a,*], Jake H. Mulligan [a], Vera Lavelli [b]

[a] Department of Food Science and Technology, University of Georgia, Athens, GA 30602, USA
[b] Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

ARTICLE INFO

Article history:
Received 2 December 2009
Received in revised form
6 July 2010
Accepted 7 July 2010

Keywords:
Apple
Green tea
Differential scanning calorimetry
Nuclear magnetic resonance
Water activity

ABSTRACT

In this study, apple products made with and without added green tea extract were freeze-dried and stored for up to 45 days at 30 °C in low and intermediate moisture environments (water activity, a_w, 0.11, 0.22, 0.32, 0.57, and 0.75). Kinetic models were developed for the changes in color and decreases in contents of selected green tea and apple monomeric and polymeric flavanols and ascorbic acid. Moisture isotherms were developed for each product. At various moisture levels, the glass transition temperature (T_g) was measured by DSC and water mobility by ^{1}H NMR. Chemical changes were related to T_g, a_w, and water mobility in the products.

Phytochemical degradation occurred more rapidly at higher moisture contents, except for caffeine which was stable. In the product containing apple with green tea, the content of monomeric flavan-3-ols decreased by 34% and 39% after 45 days of storage at a_w of 0.56 and 0.75, respectively. Phytochemical degradation correlated with increasing a_w, T_g, and water mobility. This study showed that, in general, storage at a_w 0.75 most affected phytochemical stability and color.

1. Introduction

There is growing interest in processed functional foods that contain one or more food ingredients that promote health. For example, components of green tea (*Camellia sinensis*) have been found to have anti-oxidative, anti-mutagenic, anti-inflammatory, anti-cariogenic, anti-diabetic, anti-bacterial, and anti-viral properties, and may reduce the risk of coronary heart disease or cancer (Cabrera, Artacho, & Gimenez, 2006; Zaveri, 2006). Green tea is high in polyphenolic compounds, which are comprised mostly of monomeric and polymeric flavan-3-ols. The predominant monomeric flavan-3-ols of green tea catechins (GTC) include catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG). The antioxidant and processing stability of green tea catechins in beverages has been extensively studied (Chen, Zhu, Tsang, & Huang, 2001; Chen, Zhu, Wong, Zhang, & Chung, 1998; Labbe, Tetu, Trudel, & Bazinet, 2008; Wang, Zhou, & Jiang, 2008; Yang, Hwang, & Lin, 2007). Ortiz, Ferruzzi, Taylor, and Mauer (2008) evaluated the storage stability of GTC in dry beverage powder at different relative humidities.

Apples are also another important dietary source of nutrients and phytochemicals. They are consumed fresh, dried, pureed, or in juice (Deng & Zhao, 2008; Guyot, Marnet, Sanoner, & Drilleau, 2003; Oszmianski, Wolniak, Wojdylo, & Wawer, 2008; Van Der Sluis, Dekker, Skrede, & Jongen, 2002). Phytochemicals in apples also have anti-oxidative, anti-hypercholesterolemic, and anti-carcinogenic properties, and may reduce the risk of developing coronary heart disease, diabetes, or asthma (Boyer & Liu, 2004). The polyphenolic content of apples includes procyanidins, hydroxycinnamic acids, dihydrochalcones, flavonols, flavan-3-ols, ascorbic acid, and anthocyanins in the peel of red varieties (Guyot et al., 2003; Khanizadeh et al., 2008).

In dried products, moisture properties influence antioxidant stability and also affect their color, flavor, and nutritional content (Bell, 2007). Studies on dried products as a function of moisture content or water activity have allowed the development of mathematical models that predict changes in product chemical or physical properties over time (Venir, Munari, Tonizzo, & Maltini, 2007; Welti-Chanes et al., 1999). Differences in moisture content determine water mobility and the degree of plasticization of larger food molecules, which also affects the rates of chemical reaction (Bell, 2007; Labuza & Altunakar, 2007).

Dried products are often amorphous materials that exist in a glassy state below their glass transition temperature (T_g) and in a rubbery, less solid-like state at temperatures greater than T_g.

* Corresponding author. Tel.: +1 706 542 1085; fax: +1 706 542 1050.
E-mail address: wlkerr@uga.edu (W.L. Kerr).

0023-6438/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2010.07.005

At temperatures greater than T_g, there is also greater molecular mobility and lower viscosity. A plasticizer such as water can decrease the T_g (Welti-Chanes et al., 1999). Thermal analysis, including differential scanning calorimetry (DSC), has been used to determine T_g values in food systems, which can then be related to moisture properties. This helps in understanding how water affects the physical state of a material, which can then be related to other physicochemical attributes (Deng & Zhao, 2008).

The state of water in a system may also be evaluated using nuclear magnetic resonance (NMR) relaxation techniques based on spin magnetization relaxation. NMR may differentiate water as low- or high-molecular mobility based on relaxation properties (Choi & Kerr, 2003). This method of analysis allows for the evaluation of moisture mobility as related to chemical or physical changes in a food product, such as in bread staling (Van Nieuwenhuijzen et al., 2008) or in pharmaceuticals (Yoshioka & Aso, 2007).

Previous studies (Corey, 2009) have shown the benefits of apple products fortified with green tea (GT-apple) as a novel functional product with a more complete antioxidant profile. The objectives of this study were to evaluate phytochemical stability in dried apple and GT-apple samples stored at different relative humidity, and determine how these are related to moisture-related physical properties. Samples of dried apple and GT-apple were prepared and stored at 30 °C at various relative humidities for up to 45 days. Over time, samples were assayed to determine levels of several important phytochemicals. To better understand the role of water in product stability, moisture isotherms were developed, glass transition data were determined by DSC, and proton NMR studies were conducted to assess moisture mobility.

2. Materials and methods

2.1. Materials

Reagents for maintaining constant relative humidity included lithium chloride, potassium acetate, magnesium chloride, sodium bromide, and sodium chloride and were purchased from Sigma Aldrich (Milan, Italy). Analytical standards of ascorbic acid and caffeine were purchased from Sigma Aldrich (Milan, Italy). The standards of procyanidin B2, EC, C, EGC, ECG, and EGCG were purchased from Extrasynthese (Lyon, France).

2.2. Preparation of samples and storage conditions

GT extract was prepared by extracting 25 g of dried tea leaves (Java Green Tea, Twinings, London, UK) in 500 mL of pre-heated 85 °C deionized water for 5 min. The extract was immediately cooled in an ice water slurry and filtered through Whatman No. 4 filter paper. A portion of the GT extract was removed for HPLC analysis of phenolics. Fresh apples (*Malus domestica*) of the Golden Delicious cultivar were purchased at a supermarket in Milan, Italy. The apples were peeled, cored, and quartered. Apple quarters were randomly designated for either the control or experimental batches. Each batch consisting of 2 kg of quartered apple pieces was blanched for 4 min in 100 °C deionized water. Apple pieces were immersed in ice water and drained over paper towels, then pureed in a K 3000 Braun Multisystem food processor (Braun, Kronberg, Germany) for 3 min.

The amount of GT extract used was chosen so that a single serving of the fortified product provided an equivalent amount of flavan-3-ols as that present in one cup of GT infusion. (or about 4 cups of green tea). A half-batch of the apple puree had 200 g of solids, corresponding to 4 dried single serving portions. The concentration of catechins in the GT extract was known and preliminary trials showed that approximately 80% of GT catechins were retained after freeze drying. Based on this information, one half-batch of apple puree was added to 384 mL of GT extract.

The apple and GT-apple samples were spread in a 1 cm thick layer onto stainless steel trays and dried in a Lyoflex SO4 freeze dryer (BOC Edwards, Crawley, UK). Samples were dried for 8 h at −45 °C, 24 h at −20 °C, 24 h at 0 °C and 10 h at 10 °C. Freeze-dried materials were ground in a food processor and sieved (800 μm). Powders were then weighed into Petri dishes (0.141 g of powder/cm^2) in duplicate, and stored in desiccators at 30 °C suspended over saturated salt solutions having different water activity levels: lithium chloride ($a_w = 0.11$), potassium acetate ($a_w = 0.22$), magnesium chloride ($a_w = 0.32$), sodium bromide ($a_w = 0.56$), and sodium chloride ($a_w = 0.75$).

2.3. Moisture sorption isotherms

The a_w of saturated salt solutions were checked using an Aqualab water activity meter (Decagon Devices, WA, USA). After samples reached equilibrium, their moisture content was determined after drying in a vacuum oven at 70 °C and 50 torr for 18 h (AOAC, 1998).

Plots of moisture content versus a_w were fit to the Guggenheim-Anderson-de Boer (GAB) model (Labuza & Altunakar, 2007):

$$m = \frac{m_o k c a_w}{(1 - k a_w)(1 - k a_w + c k a_w)} \quad (1)$$

where m_o is the monolayer moisture content, c is a factor associated with surface enthalpy, and k represents a multilayered moisture component.

2.4. Differential scanning calorimetry and glass transitions

About 10–15 mg of equilibrated apple or GT-apple were hermetically sealed into an aluminium DSC pan, and analyzed by a DSC 1 differential scanning calorimeter (Mettler-Toledo, Inc., Columbus, OH, United States). DSC data was analyzed with StarE software, which uses first-derivative analysis to calculate the glass transition temperature including onset (T_{go}) and midpoint temperatures (T_{gm}). The thermal scan consisted of equilibration of samples at 30 °C for 2 min, cooling to −50 °C at 5 °C per min, holding at −50 °C for 5 min, and then heating to 80 °C at 5 °C per min. Scans were conducted under a nitrogen gas flush.

The Gordon and Taylor equation was used to model the relationship between glass transition temperatures and moisture properties of samples (Bell, 2007):

$$T_g = \frac{w_s T_{gs} + k w_w T_{gw}}{w_s + k w_w} \quad (2)$$

where T_g is the observed glass transition temperature, T_{gs} the glass transition temperature of amorphous dry solid, T_{gw} the glass transition temperature of amorphous water, k a constant, w_s is the weight fraction of solids, and w_w is the weight fraction of water. For water, T_{gw} was taken as −135 °C (Welti-Chanes et al., 1999).

2.5. Nuclear magnetic resonance studies

^{1}H NMR analysis was conducted using a 20 MHz ^{1}H NMR spectrometer (Resonance Instruments, Whitney, U.K.). Each equilibrated sample was removed from its relative humidity chamber and transferred into 18 mm diameter NMR glass tubes to a height of less than 25 mm, and then immediately sealed.

The methods of Choi and Kerr (2003) were followed with modifications. The transverse spin–spin relaxation (T_2) of protons

was analyzed using the free induction decay (FID) and the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequences. The acquisition parameters consisted of a 90° pulse set at 4.1 μs and a 2 s recycle delay. The pulse length was 60 μs for the spacing at 90°–180° (τ). The analysis was conducted at 22 °C. FID curves were fit to a Gaussian model, while those from CPMG studies were fit with a multiple-exponential model.

2.6. Analysis of phenolic compounds

For phenolic evaluation, samples stored at different a_w levels were extracted in duplicate at 0, 7 and 24 days of incubation and in quadruplicate at 45 days. Extraction of samples was performed in 10 mL centrifuge tubes using 0.5 g of sample in 10 mL of methanol (Van Der Sluis et al., 2002).

The method of Tomas-Barberan et al. (2001) was used to determine the concentrations of phenolic compounds. Methanolic extracts (20 μL on a Rheodyne loop) were injected on a 250 × 4.6 mm i.d., 5 μ, Symmetry C18 reverse-phase column (Waters, Vimodrone, Italy), and detected by a model 2996 photodiode array (Waters, Vimodrone, Italy). Mobile phase stock solutions of water and methanol were acidified with formic acid (95:5, v/v) and consisted of: solution A of water:methanol (95:5, v/v),solution B of water:-methanol (88:12, v/v), solution C of water:methanol (20:80, v/v), and solution D of methanol only. The gradient elution (at 1.0 ml/min) was adjusted as follows: (1) 100% of A for 0–5 min, (2) a linear gradient to reach 100% of B from 5 to 10 min, (3) 100% of B from 10 to 13 min, (4) a linear gradient to reach 75% of B and 25% of C from 13 to 35 min, (5) a linear gradient to reach 50% of B and 50% of C from 35 to 50 min, (6) a linear gradient to reach 100% of C from 50 to 52 min, (7) 100% of C from 52 to 57 min, and (8) 100% of D from 57 to 60 min. The analysis was conducted at 22 °C. The standards of C, EGC, ECG, EGCG, EC, phloridzin (phloretin 2′*O*-glucoside), and caffeine were used for identification of peaks by retention times and UV–vis spectra, and were used to build calibration curves. Quantification was based on the calibration curves and relative response factors (Wang, Provan, & Helliwell, 2003). The peak for phloretin 2′*O*-xyloglucoside was identified on the basis of UV–vis spectra and literature data.

After addition of a measured amount of GT extract to apple puree (384 mL in 2 kg of puree), phenolic analysis revealed that the recovery of GT catechins from apple puree was 100% and an average loss lower than 20% occurred during the freeze–drying process.

Peak concentrations (C) for each analyte were determined over time and fit to a pseudo-first-order kinetic model (Wang et al., 2008):

$$C = C_o e^{-kt} \quad (3)$$

The reaction rate coefficient (k) was then calculated for each analyte at each storage relative humidity.

2.7. Ascorbic acid determination

For ascorbic acid evaluation, samples stored at different a_w levels were extracted in duplicate at 0, 3, 5, 14, 28 and 41 days of incubation. Extraction of samples was performed in 10 mL centrifuge tubes using 0.5 g of sample in 5 mL of 60 g/L metaphosphoric acid (containing 1 g/L of sodium metabisulphite) (Vrhovsek, Rigo, Tonon, & Mattivi, 2004). The determination of ascorbic acid content was conducted following the methods of Lavelli and Vantaggi (2009). Samples were injected on a Waters HPLC using a 300 × 7.8 mm i.d. Bio-Rad Fruit Quality Analysis column. An isocratic mobile phase of 1 mmol/L sulfuric acid at 1 mL/min flow rate was used. Analyses were conducted at 22 °C, and ascorbic acid was detected at 245 nm. A calibration curve of ascorbic acid was made with pure standard. Changes in ascorbic acid concentrations over time were also fit to a pseudo-first-order kinetic model.

2.8. Total procyanidin content

For procyanidin evaluation, samples stored at different a_w levels were extracted in duplicate at 0, 7 and 24 days of incubation and in quadruplicate at 45 days of incubation. Extraction of samples was performed in 10 mL centrifuge tubes using 0.5 g of sample in 10 mL of acetone:water (70:30, v/v) (Vanzani et al., 2005).

Total procyanidin content of samples was determined as follows. Aliquots of 0.25 mL of 70:30 acetone:water (v/v) extracts were dried under nitrogen gas. Samples were then reconstituted in 1 mL of 0.1 mol/L phosphate buffer, pH 7.0 and purified with 500 mg Sep-Pak C-18 cartridges (Waters, Vimodrone, Italy). The retained components were eluted with 1 mL of methanol (Vrhovsek et al., 2004). For extracts containing green tea polyphenols, the removal of chlorophyll was also necessary. GT-containing extract was mixed with hexane (1:1, v/v). Once two phases had separated, the upper phase was discarded and the lower phase analyzed for procyanidin content.

The determination of the total procyanidin content of samples was conducted following the vanillin assay (Sun, Ricardo-da-Silva, & Spranger, 1998). The reaction mixture consisted of 0.5 mL of Sep-Pakpurified extracts from 70:30 acetone:water (v/v) or standard dissolved in methanol, 1.25 mL of vanillin:methanol (1:99, w/v), and 1.25 mL of 9 mol equ/L sulfuric acid dissolved in methanol. The reaction mixture was held at 25 °C until the maximum absorbance occurred at 500 nm. The results were reported as milligrams of catechin equivalents per kilogram of dry product, based on the development of a calibration curve of catechin standard. Changes in total procyanidin concentrations over time were also fit to pseudo-first-order kinetic models (Dallas, Hipolito-Reis, da Silva, & Laureano, 2003).

2.9. Determination of enzymatic activity

Polyphenoloxidase (PPO) and peroxidase activity (POD) were analyzed in duplicate in the freeze-dried samples. For enzyme extraction, 100 mg of sample was added to 1 mL of buffer, which consisted of 0.03 mol/L acetic acid, 0.14 mol/L dipotassium phosphate, 1 mol/L sodium chloride, and 5% (w/w) polyvinylpolypyrrolidone, pH 6.5. Extracts were then centrifuged (10,000 × g for 10 min) and filtered through Whatman No. 1 filter paper.

PPO activity was determined following the methods of Alvarez-Parrilla et al. (2007). The filtered supernate (100 μL) was transferred into 900 μL of 10 mM chlorogenic acid in buffer. The reaction mixture was then incubated at 25 °C. The absorbance was measured and monitored at 400 nm. A linear increase in absorbance was equated to the reaction rate.

The determination of POD followed the methods of Ahn, Paliyath, and Murr (2007). Filtered supernate (200 μL) was added to a reaction mixture containing 3.6 mL of 100 mmol/L phosphate buffer (pH 6.5), 100 μL of 640 mM guaiacol, and 100 μL of 400 mmol/L hydrogen peroxide. The reaction mixture was then incubated at 25 °C, and the absorbance was continuously measured at 470 nm. The reaction rate was measured from the linear increase in absorbance.

2.10. Color measurements

Color evaluation of samples stored at different a_w levels was performed in triplicate at 0, 7, 5, 14, 24, 31, 39 and 52 days of incubation. Color was measured with a SL-2000 Chromameter (Labo

scientifica, Parma, Italy), which provides the Hunter L^*, a^*, and b^* coordinates, representing: lightness and darkness (L^*), redness ($+a^*$), greenness ($-a^*$), yellowness ($+b^*$), and blueness ($-b^*$). The chromameter was calibrated with a white standard. To study the variation in color over time, color difference, namely ΔE was calculated:

$$\Delta E = \left[(a^* - a^*_0)^2 + (b^* - b^*_0)^2 + (L^* - L^*_0)^2\right]^{1/2} \quad (4)$$

where a^*_0, b^*_0, and L^*_0 are the values of the colorimetric parameters of the sample at the beginning of storage and a^*, b^*, and L^* are the colorimetric parameters at a given time (Soliva-Fortuny, Grigelmo-Miguel, Odriozola-Serrano, Gorinstein, & Martin-Belloso, 2001).

2.11. Statistical analysis

Results represent the average values ± standard deviations of at least duplicate determinations. Analysis of variance was conducted using the Statgraphics 5.1 software (STCC Inc., Rockville, MD); Tukey's HSD ($p < 0.05$) was used to discriminate among the means. 2-D regression of data was also conducted using the Statgraphics 5.1 software, whereas 3-D regression of data was carried out with TableCurve 3D (Jandel Scientific, Chicago, IL).

3. Results and discussion

3.1. Moisture content, a_w and glass transition temperatures

The moisture content, a_w and glass transition temperature (T_g) of apple and GT-apple samples are shown in Table 1. As expected, moisture content increased with a_w, and there were no significant differences between moisture content of the apple and GT-apple samples at a given a_w. Moisture adsorption isotherms were developed for the apple and GT-apple products, and the data fit with the GAB model (Fig. 1). From the GAB model, the factors m_o, c and k for the apple and GT-apple were $m_o = 10.7$ and 10.2 g of water/100 g of dry product, respectively; $c = 2.28$ and 2.45; and $k = 0.89$ and 0.90, respectively. The isotherms were closest to a Type II isotherm, suggesting a product with limited swelling and capillary effects. The a_w values corresponding to the monolayer moisture (m_o) contents were $a_w = 0.437$ and 0.423 for apple and GT-apple, respectively. Moraes, Rosa, and Pinto (2008) found m_o for fresh apples at 50–70 °C ranged from 16.8 to 10.7 g of water/100 g of solids.

Table 1
a_w, moisture content, and glass transition temperatures of dried apple and GT-apple at several a_w levels ($n = 3$).

a_w	Sample	Moisture content (g water/100 g product, d.w.)	Onset temp T_{go}	Midpoint temp T_{gm}
0.113	Apple	2.81 ± 0.03	8.72 ± 1.64	12.85 ± 0.70
	GT-Apple	2.63 ± 0.26	10.28 ± 1.39	13.67 ± 0.09
0.216	Apple	4.50 ± 0.07	−2.89 ± 0.36	−0.295 ± 2.15
	GT-Apple	4.76 ± 0.12	−3.05 ± 1.49	0.17 ± 0.10
0.324	Apple	6.87 ± 0.25	−15.15 ± 1.90	−11.77 ± 1.71
	GT-Apple	6.95 ± 0.23	−13.12 ± 0.76	−9.23 ± 0.57
0.560	Apple	14.95 ± 0.42	−38.76 ± 0.87	−34.67 ± 0.46
	GT-Apple	14.69 ± 0.18	−39.15 ± 1.67	−34.41 ± 0.19
0.751	Apple	26.44 ± 0.07	n.d.	n.d.
	GT-Apple	26.09 ± 0.19	n.d.	n.d.

n.d. = not detectable.
No significant differences ($p \leq 0.05$) existed between apple and GT-apple products within a_w for moisture content, T_o, or T_m when analyzed using Tukey's HSD.

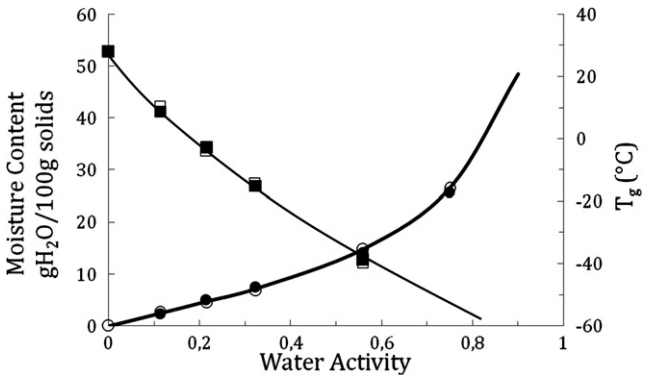

Fig. 1. Adsorption isotherm (○ – apple, ● – GT-apple) and onset glass transition temperature (T_g) (□ – apple, ■ – GT-apple) as a function of water activity ($n = 3$). Apple a_w data fit by GAB model, T_g data fit by Gordon–Taylor model.

Labuza and Altunakar (2007) provided a detailed discussion on moisture sorption isotherms. When the equilibrium moisture content is below the m_o, then the bulk of water present is bound or adsorbed to solids. The rate of chemical reactions is often the lowest at moisture contents near the m_o. At equilibrium moisture contents greater than m_o, additional bulk phase water is present. This provides a flowing solvent medium in which chemical reactants can interact. For reactions that involve water, the reactant solubility may also increase, affecting the reaction rate. Greater solubility is attained until a critical moisture content is reached above which the reactants are completely hydrated. At higher moisture contents, dilution of the reactants occurs and the reaction rate decreases. As equilibrium moisture contents change at different a_w levels, the effects of moisture mobility on plasticization of components in a material may also affect the physical state of the material.

Glass transition onset (T_{go}) and midpoint (T_{gm}) temperatures at each a_w are reported in Table 1. Values for apple and GT-apple were not substantially different at a given a_w. As moisture content and a_w increased, T_{go} decreased from 8.72 (apple) and 10.28 °C (GT-apple) at 0.11 a_w to −38.76 (apple) and −39.15 °C (GT-apple) at 0.56 a_w. Decreasing T_{go} is often associated with increasing water content as the water serves to increase the relative free volume for motions of food molecules. At the highest a_w (0.751), a distinct T_g was not measured most likely because the transition was below −50 °C. Acevedo, Schebor, and Buera (2006) found similar T_g values for freeze-dried apple products stored at 70 °C and equilibrated to similar relative humidity levels.

T_{go} values were well fit ($r^2 > 0.98$) by the Gordon–Taylor equation (Eq. (3)) with $k = 4.49$ and 4.77 for the apple and GT-apple, and intercept = 24.58 and 27.72 °C, respectively (Fig. 1).

All T_g values were below the storage temperature of 30 °C, indicating that none of the samples were truly in the glassy state. However, the temperature difference (30 °C – T_g) determines how much the material exhibits solid-like behavior, and may be a measure to which chemical reactions are controlled by diffusional limits.

4. Nuclear magnetic resonance

Equilibrated samples were analyzed by ^{1}H NMR using FID and CPMG pulse sequences. Curves for the FID runs are shown in Fig. 2. CPMG pulse sequences were only successful for samples at a_w of 0.56 or 0.75. FID curves were fit to a Gaussian. At $a_w \leq 0.32$, the

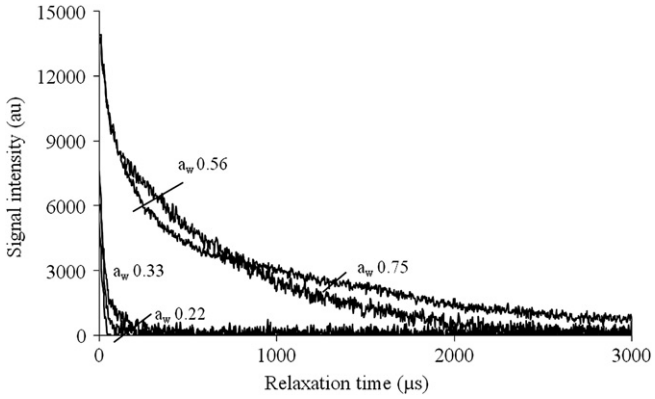

Fig. 2. ^{1}H NMR free induction decay (FID) curves for GT-apple products at various a_w levels.

signal decayed rapidly in under 50–100 μs. Samples at $a_w \leq 0.32$ were well fit with a single component (Table 2). For apple, the single T_2^* values were 16.3, 23.6 and 31.6 μs at $a_w = 0.11$, 0.22 and 0.32. For GT-apple, T_2^* values were 12.7, 25.7 and 32.3 μs. While the very short decay times are indicative of solid relaxation, the increase in T_2^* does suggest that those solid components were plasticized by the increasing presence of water.

For samples held at a_w of 0.56 and 0.75, the FID curves were better fit by a 2 component model. The fast decaying component (T_{2a}^*) was 44.2 and 60.4 μs for apple (at 0.56 and 0.75 a_w), and 45.1 and 51.9 μs for GT-apple. Again, this can be attributed to relaxation processes in the solid component with enhanced mobility due to the plasticization of water. The slower decaying component (T_{2b}^*) was 305.6 and 526.1 μs for apple (at 0.56 and 0.75 a_w), and 255.2 and 834.2 μs for GT-apple. This second component can be attributed to more mobile water not closely associated with larger molecules. However, this water is still close enough to diffuse to and exchange molecular spins with those molecules within the relaxation time. Thus, the relaxation times, in the range of 255–834 μs, are still much shorter than the T_2^* times of 1–2 s that are typical of bulk water.

The ^{1}H NMR relaxation data can be related to the moisture isotherms. GAB models of the isotherms showed that a_w values corresponding to monolayer moisture (m_o) were 0.437 and 0.423 for apple and GT-apple, respectively. At a_w less than 0.4, the NMR data showed a relaxation process indicative of only a solid component. At $a_w > 0.4$, additional components were present associated with a more mobile water phase. According to the GAB theory, at $m < m_o$, no more than a single layer of water is sorbed at molecular surfaces. Above m_o, multiple layers of water exist that are not sorbed. Above the m_o, greater molecular mobility and solubility of analytes can be expected due to the presence of excess water as solvent, until a dilution effect prevails once analytes are completely solubilized (Bell, 2007). While the NMR data does not confirm the existence of monolayer or multilayer water, it does suggest that at low a_w the water is very closely associated with food macromolecules, while at higher a_w an additional aqueous phase exists with enhanced mobility.

4.1. Phytochemical degradation

Table 3 shows the initial levels of antioxidants in apple and GT-apple. Apples had substantial levels of EC, C and procyanidins, as well as ascorbic acid. GT-apple also had substantial levels of EGC, EGCG, ECG and GCG, as well as caffeine. Concentrations of total monomeric flavan-3-ols, oligomeric flavanols and ascorbic acid were measured over 45 days of storage and fitted to a pseudo-first-order kinetic model to determine the degradation rate constant k. The first-order plot of monomeric flavan-3-ols degradation in GT-apple is shown in Fig. 3 as an example. Wang et al. (2008) also found that green tea catechins followed a pseudo-first-order kinetic model.

The dependence of the reaction rate constant k on a_w is shown in Fig. 4. For monomeric flavan-3-ols, k increased with increasing a_w, both for apple and for GT-fortified apple. The rate of degradation, however, was more rapid in the apple samples, suggesting that the green tea provided enhanced stability. Ortiz et al. (2008) found that catechins did not degrade in dried GT beverage powders stored at less than 43% relative humidity at 22 °C for 3 months, or put another way, catechin levels did not change when samples were held below the onset T_g. They also showed that catechin was affected by the presence of sugars, ascorbic acid, or citric acid, all of which are found in apple. They suggested that degradation proceeds at higher moisture conditions when there is greater mobility of reactants, dissolution or deliquescence of organic acids. Interaction with other phytochemicals or food additives may also affect chemical stability of flavan-3-ols (Chen et al., 1998; Chen et al., 2001; Ortiz et al., 2008). For example, when ascorbic acid is present, it can exert anti-oxidative properties (Chen et al., 1998), but then become a pro-oxidant during storage (Zhu, Hammerstone, Lazarus, Schmitz, & Keen, 2003).

Procyanidins from apples include dimers such as procyanidin B2 (Hagen et al., 2007) and larger complexes with greater degree of polymerization (Hamauzu, Yasui, Inno, Kume, & Omanyuda, 2005). In this study, both the dimeric procyanidin B2 present in methanolic extracts and total procyanidin content present in the

Table 2
One and two component fits to ^{1}H NMR FID curves for dried apple and GT-apple ($n = 3$).

a_w	%	T^*_{2a} (μs)	%	T^*_{2b} (μs)
Apple				
0.113	100	16.3		
0.216	100	23.6		
0.324	100	31.6		
0.560	48	44.2	52	305.6
0.751	33	60.4	67	526.1
GT-Apple				
0.113	100	12.7		
0.216	100	25.7		
0.324	100	32.3		
0.560	55	45.1	45	255.2
0.751	48	51.9	52	834.2

Table 3
Initial concentrations of antioxidants and caffeine in apple and GT-apple products ($n = 2$).

Antioxidant	Apple (mg/kg, d.w.)	GT-fortified apple (mg/kg, d.w.)
EC	$277^a \pm 18$	$860^b \pm 42$
C	$56^c \pm 4$	$301^d \pm 17$
EGC	n.d.	$2066^e \pm 157$
EGCG	n.d.	$2317^f \pm 63$
ECG	n.d.	$548^g \pm 28$
GCG	n.d.	$542^h \pm 32$
Procyanidin B2	$498^i \pm 5$	$507^i \pm 6$
Total procyanidins	$1505^j \pm 257$	$2438^k \pm 89$
Caffeine	n.d.	$1429^l \pm 61$
Ascorbic acid	$118^m \pm 4$	$120^m \pm 3$

Different letters within the same row indicate a significant difference ($p \leq 0.05$) using Tukey's HSD.

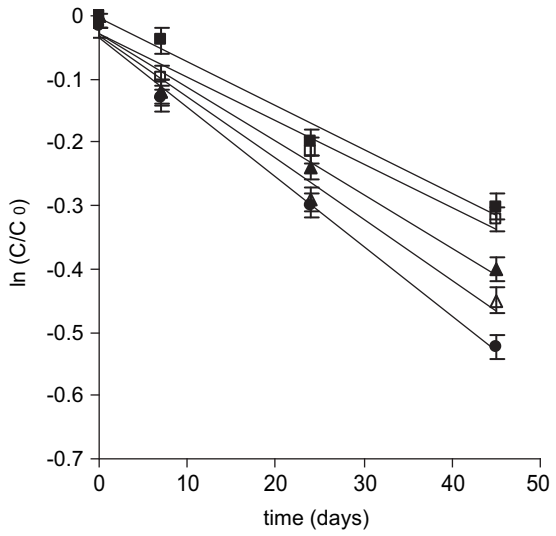

Fig. 3. Degradation of total monomeric flavan-3-ols in GT-apple stored at different a_w levels, at 30 °C (■, a_w 0.11; □, a_w 0.22; ▲, a_w 0.33; △, a_w 0.56; ●, a_w 0.75). Errors bars represent standard deviations ($n = 2$ at 0, 7 and 24 days; $n = 4$ at 45 days). Data fit by pseudo-first-order kinetics.

acetone:water (70:30, v/v) extracts were measured (Table 3). Green tea fortification significantly increased the content of total procyanidins over control apple products, but procyanidin B2 contents were similar. During storage of apples, no change in procyanidin B2 was measured at 0.113 a_w and for GT-apple at either 0.113 or 0.216 a_w (Fig. 4B). In general, k increased with increasing a_w. Degradation rates were higher for apple than GT-fortified apple products.

For total procyanidins, there was similar stability maintained throughout storage (Fig. 4C) for both apple and GT-apple samples. No changes occurred at $a_w = 0.11$ for both GT-apple and apple products. In apple samples, no degradation occurred at a_w below 0.33, but k increased to equivalent levels as in GT-fortified products at $a_w = 0.56$ and 0.75. Neither treatment group showed k to be strongly dependent on a_w. The stability of procyandins composed of polymeric or oligomeric flavan-3-ol compounds was greater than monomeric flavan-3-ol compounds comprised of the catechins. Guyot et al. (2003) postulated that the procyanidins are able to outcompete the catechins for electrophilic attack by *o*-quinones, thus participating to a greater extent in oxidation reactions. The Maillard reaction contributes to non-enzymatic browning and is comprised of a series of reactions that involve reaction of reducing sugars with free amino groups to produce a glycosylamine, which then reacts to form a Schiff base or Amadori product. At pH < 5, polymerization of reactants may occur, forming melanoidins that possess a brown color. In this study, both the apple and GT-apple products developed a brown color during storage (Fig. 5).

Before the addition of green tea extract, the apple portions had undergone a blanching step. This resulted in the denaturation of PPO and POD enzymes, and no activity for either enzyme was found (data not shown). These enzymes may otherwise contribute to enzymatic browning in fruit and vegetable products (Alvarez-Parrilla et al., 2007). Since apples have a high concentration of reducing sugars (50.8 g/100 g solids) (Acevedo et al., 2006), non-enzymatic browning reactions may also occur. In addition to availability and concentration of reactants, the Maillard reaction is also catalyzed by free metal ions. Monomeric and polymeric flavan-3-ol compounds, such as those found in apples and green tea may also chelate and inhibit this reaction. This occurs as flavan-3-ols bind to sugar fragments or to cations such as iron (Totlani & Peterson, 2005).

At low moisture conditions ($a_w \sim 0.1$–0.4), the rate of the Maillard reaction proceeds at a relatively slow rate, but proceeds more rapidly at intermediate moisture conditions ($a_w \sim 0.6$–0.8). At low moisture, the concentration of reactants is very high, but

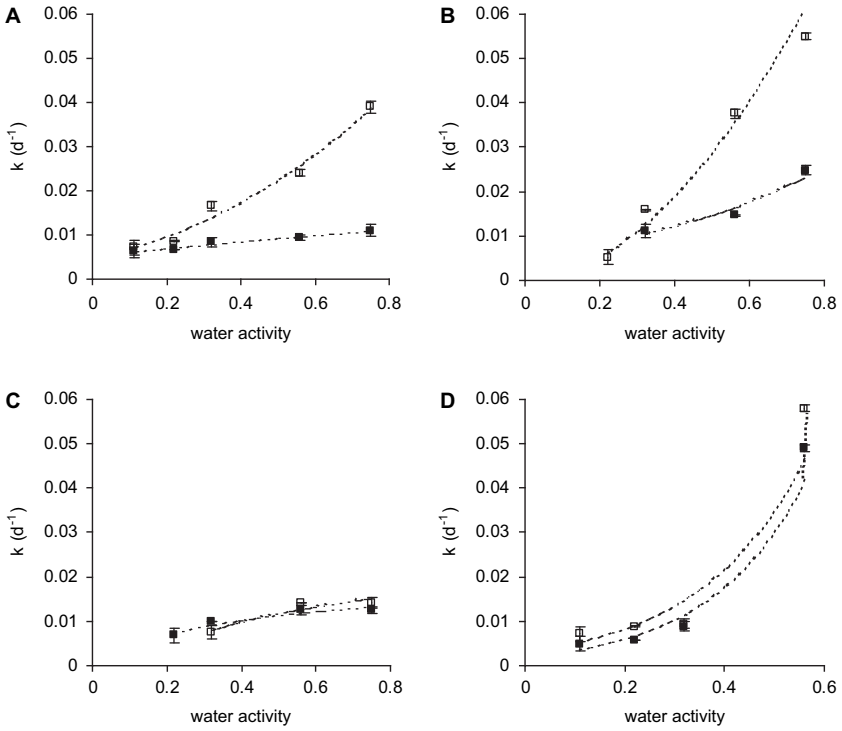

Fig. 4. Reaction rate constants (k) for the degradation of (A) monomeric flavan-3-ols, (B) procyanidin B2, (C) oligomeric procyanidins and (D) ascorbic acid for apple (□) and GT-apple (■) as a function of a_w at 30 °C. Errors bars represent standard deviations ($n = 3$). No changes occurred for procyanidin B2 and total procyanidins at $a_w = 0.11$.

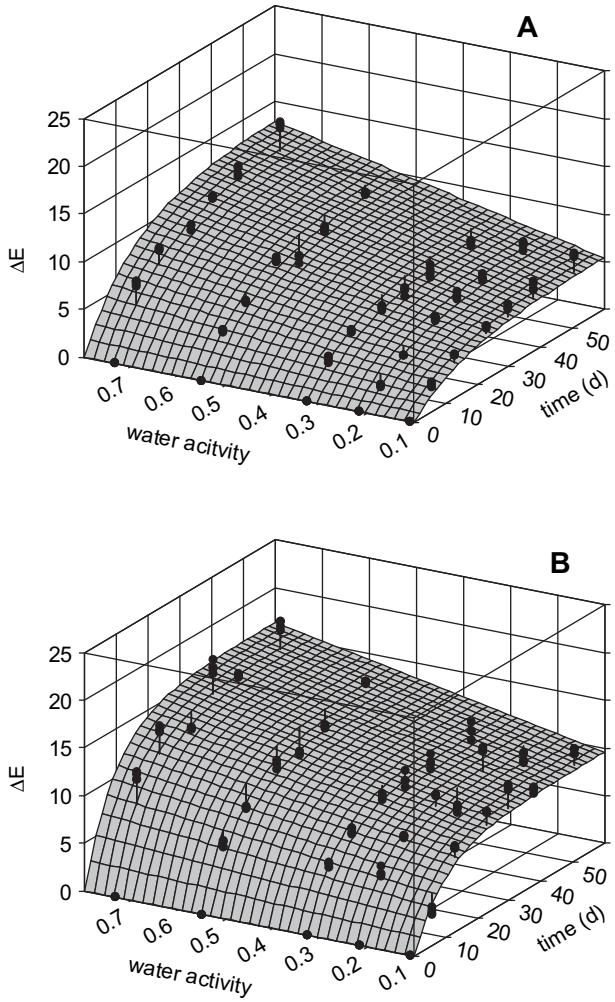

Fig. 5. Color change (ΔE) of GT-apple (A) and apple (B) as a function of a_w and time of storage at 30 °C ($n = 3$).

solute mobility is limited due to limited hydration and low mobility. At higher moisture levels, the availability of water for hydration of reactants is sufficient to increase reaction rates. This also increases diffusion rates for reactants, and may enhance reactant solubility. At even higher moisture levels, a dilution effect on reactants results (Bell, 2007). For freeze-dried apples stored at 70 °C, rates of non-enzymatic browning peaked at 52% equilibrium relative humidity (Acevedo et al., 2006). As non-enzymatic browning is a diffusion-controlled reaction, moisture was thought to enhance the ability of reactants to diffuse and interact. At points above the m_o, structural collapse of samples can also occur, which reduces the number of pores and reaction sites available (Acevedo et al., 2006; White & Bell, 1999). In this study, as samples were blanched, pureed, and freeze-dried, this is not considered to be as significant a factor in antioxidant degradation.

The addition of GT extract to apples also contributed caffeine (1,3,7-trimethylxanthine) to the products (Table 3). The caffeine in GT-apple products showed better stability than most other analytes evaluated (data not shown). In a storage study of green tea powder beverage mix, Ortiz et al. (2008) found that caffeine remained stable through three months of storage at 22 °C.

The initial contents of ascorbic acid in both apple and GT-apple products were similar (Table 3). The rate of ascorbic acid loss increased with a_w for both products, particularly at a_w above 0.324 (Fig. 4). Factors that contribute to ascorbic acid degradation include oxidizers, free metal ions as catalysts, and higher moisture contents (Bell, 2007).

5. Conclusions

This study showed that changes in moisture content or mobility affected phytochemical degradation and color, and that delineation between low and intermediate moisture conditions around the monolayer moisture value had variable effects on phytochemical stability. In a complex product such as apples fortified with green tea extract, multiple factors may affect the antioxidant status. Evaluation of product stability during storage is important when considering the production of consumer food products, as the antioxidant status may be affected. Fortification of apples with green tea extract provided a new type of value-added product, which would provide complementary health benefits from apples and green tea phenolics.

References

Acevedo, N., Schebor, C., & Buera, M. P. (2006). Water-solids interactions, matrix structural properties and the rate of non-enzymatic browning. *Journal of Food Engineering, 77*, 1108–1115.

Ahn, T., Paliyath, G., & Murr, P. D. (2007). Antioxidant enzyme activities in apple varieties and resistance to superficial scald development. *Food Research International, 40*, 1012–1019.

Alvarez-Parrilla, E., De La Rosa, L. A., Rodrigo-Garcia, J., Escobedo-Gonzalez, R., Mercado-Mercado, G., Moyers-Montoya, E., et al. (2007). Dual effect of β-cyclodextrin (β-cd) on the inhibition of apple polyphenol oxidase by 4-hexylresorcinol (hr) and methyl jasmonate (mj). *Food Chemistry, 101*, 1346–1356.

AOAC. (1998). *Official methods of analysis (method 934.06)*. Maryland: Association of Official Analytical Chemists.

Bell, L. N. (2007). Moisture effects on food's chemical stability. In G. V. Barbosa-Canovas, J. Anthony, J. Fontana, S. J. Schmidt, & T. P. Labuza (Eds.), *Water activity in foods: Fundamentals and applications* (pp. 173–198). Ames, Iowa: IFT Press, Blackwell Publishing.

Boyer, J., & Liu, R. H. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal, 3*, 5.

Cabrera, C., Artacho, R., & Gimenez, R. (2006). Beneficial effects of green tea–a review. *Journal of the American College of Nutrition, 25*, 79–99.

Chen, Z.-Y., Zhu, Q. Y., Tsang, D., & Huang, Y. (2001). Degradation of green tea catechins in tea drinks. *Journal of Agricultural and Food Chemistry, 49*, 477–482.

Chen, Z.-Y., Zhu, Q. Y., Wong, Y. F., Zhang, Z., & Chung, H. Y. (1998). Stabilizing effect of ascorbic acid on green tea catechins. *Journal of Agricultural and Food Chemistry, 46*, 2512–2516.

Choi, S.-G., & Kerr, W. L. (2003). ^{1}H NMR studies of molecular mobility in wheat starch. *Food Research International, 36*, 341–348.

Corey, M. (2009). Developing functional food products through novel processing, ingredient, and shelf stability evaluation. Ph.D. Dissertation, University of Georgia. University of Georgia Libraries, Athens, GA.

Dallas, C., Hipolito-Reis, P., da-Silva, J. M. R., & Laureano, O. (2003). Influence of acetaldehyde, pH, and temperature on transformation of procyanidins in model wine solutions. *American Journal Enology and Viticulture, 54*, 119–124.

Deng, Y., & Zhao, Y. (2008). Effect of pulsed vacuum and ultrasound osmopretreatments on glass transition temperature, texture, microstructure and calcium penetration of dried apples (fuji). *LWT – Food Science and Technology, 41*, 1575–1585.

Guyot, S., Marnet, N., Sanoner, P., & Drilleau, J.-F. (2003). Variability of the polyphenolic composition of cider apple (*malus domestica*) fruits and juices. *Journal of Agricultural and Food Chemistry, 51*, 6240–6247.

Hagen, S. F., Borge, G. I. A., Bengtsson, G. B., Bilger, W., Berge, A., Haffner, K., et al. (2007). Phenolic contents and other health and sensory related properties of apple fruit (*malus domestica*) borkh., cv. Aroma. *Postharvest Biology and Technology, 45*, 1–10.

Hamauzu, Y., Yasui, H., Inno, T., Kume, C., & Omanyuda, M. (2005). Phenolic profile, antioxidant property, and anti-influenza viral activity of chinese quince (*pseudocydonia sinensis* schneid.), quince (*cydonia oblonga* mill.), and apple (*malus domestica* mill.) fruits. *Journal of Agricultural and Food Chemistry, 53*, 928–934.

Khanizadeh, S., Tsao, R., Rekika, D., Yang, R., Charles, M. T., & Rupasinghe, H. P. V. (2008). Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. *Journal of Food Composition and Analysis, 21*, 396–401.

Labbe, D., Tetu, B., Trudel, D., & Bazinet, L. (2008). Catechin stability of EGC- and EGCG-enriched tea drinks produced by a two-step extraction procedure. *Food Chemistry, 111*, 139–143.

Labuza, T. P., & Altunakar, B. (2007). Water activity prediction and moisture sorption isotherms. In G. V. Barbosa-Canovas, J. Anthony, J. Fontana, S. J. Schmidt, & T. P. Labuza (Eds.), *Water activity in foods: Fundamentals and applications* (pp. 109–154). Ames, Iowa: IFT Press/Blackwell Publishing.

Lavelli, V., & Vantaggi, C. (2009). Rate of antioxidant degradation and color variations in dehydrated apples as related to water activity. *Journal of Agricultural and Food Chemistry, 57*(11), 4733–4738.

Moraes, M. A., Rosa, G. S., & Pinto, L. A. A. (2008). Moisture sorption isotherms and thermodynamic properties of apple fuji and garlic. *International Journal of Food Science and Technology, 43*, 1824–1831.

Ortiz, J., Ferruzzi, M. G., Taylor, L. S., & Mauer, L. J. (2008). Interaction of environmental moisture with powdered green tea formulations: effect on catechin chemical stability. *Journal of Agricultural and Food Chemistry, 56*, 4068–4077.

Oszmianski, J., Wolniak, M., Wojdylo, A., & Wawer, I. (2008). Influence of apple puree preparation and storage on polyphenol contents and antioxidant activity. *Food Chemistry, 107*, 1473–1484.

Soliva-Fortuny, R. C., Grigelmo-Miguel, N., Odriozola-Serrano, I., Gorinstein, S., & Martin-Belloso, O. (2001). Browning evaluation of ready-to-eat apples as affected by modified atmosphere packaging. *Journal of Agricultural and Food Chemistry, 49*, 3685–3690.

Sun, B., Ricardo-Da-Silva, J. M., & Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry, 46*, 4267–4274.

Tomas-Barberan, F. A., Gil, M. I., Cremin, P., Waterhouse, A. L., Hess-Pierce, B., & Kader, A. A. (2001). HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *Journal of Agricultural and Food Chemistry, 49*, 4748–4760.

Totlani, V. M., & Peterson, D. G. (2005). Reactivity of epicatechin in aqueous glycine and glucose maillard reaction models: quenching of c2, c3, and c4 sugar fragments. *Journal of Agricultural and Food Chemistry, 53*, 4130–4135.

Van Der Sluis, A. A., Dekker, M., Skrede, G., & Jongen, W. M. F. (2002). Activity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *Journal of Agricultural and Food Chemistry, 50*, 7211–7219.

Van Nieuwenhuijzen, N. H., Primo-Martin, C., Meinders, M. B. J., Tromp, R. H., Hamer, R. J., & Van Vliet, T. (2008). Water content or water activity: what rules crispy behavior in bread crust? *Journal of Agricultural and Food Chemistry, 56*, 6432–6438.

Vanzani, P., Rossetto, M., Rigo, A., Vrhovsek, U., Mattivi, F., D'amato, E., et al. (2005). Major phytochemicals in apple cultivars: contribution to peroxyl radical trapping efficiency. *Journal of Agricultural and Food Chemistry, 53*, 3377–3382.

Venir, E., Munari, M., Tonizzo, A., & Maltini, E. (2007). Structure related changes during moistening of freeze dried apple tissue. *Journal of Food Engineering, 81*, 27–32.

Vrhovsek, U., Rigo, A., Tonon, D., & Mattivi, F. (2004). Quantitation of polyphenols in different apple varieties. *Journal of Agricultural and Food Chemistry, 52*, 6532–6538.

Wang, H., Provan, G. J., & Helliwell, K. (2003). HPLC determination of catechins in tea leaves and tea extracts using relative response factors. *Food Chemistry, 81*, 307–312.

Wang, R., Zhou, W., & Jiang, X. (2008). Reaction kinetics of degradation and epimerization of epigallcatechin gallate (EGCG) in aqueous system over a wide temperature range. *Journal of Agricultural and Food Chemistry, 56*, 2694–2701.

Welti-Chanes, J., Guerrero, J. A., Barcenas, M. E., Aguilera, J. M., Vergara, F., & Barbosa-Canovas, G. V. (1999). Glass transition temperature (T_g) and water activity (a_w) of dehydrated apple products. *Journal of Food Process Engineering, 22*, 91–101.

White, K., & Bell, L. (1999). Glucose loss and Maillard browning in solids as affected by porosity and collapse. *Journal of Food Science, 64*, 1010–1014.

Yang, D.-J., Hwang, L. S., & Lin, J.-T. (2007). Effects of different steeping methods and storage on caffeine, catechins and gallic acid in bag tea infusions. *Journal of Chromatography A, 1156*, 312–320.

Yoshioka, S., & Aso, Y. (2007). Correlations between molecular mobility and chemical stability during storage of amorphous pharmaceuticals. *Journal of Pharmaceutical Sciences, 96*, 960–981.

Zaveri, N. T. (2006). Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life Sciences, 78*, 2073–2080.

Zhu, Q. Y., Hammerstone, J. F., Lazarus, S. A., Schmitz, H. H., & Keen, C. L. (2003). Stabilizing effect of ascorbic acid on flavan-3-ols and dimeric procyanidins from cocoa. *Journal of Agricultural and Food Chemistry, 51*, 828–833.