

# The Impact of Packaging Materials on the Antioxidant Phytochemical Stability of Aqueous Infusions of Green Tea (*Camellia sinensis*) and Yaupon Holly (*Ilex vomitoria*) during Cold Storage

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**ABSTRACT:** Ready to drink (RTD) teas are a growing segment in the beverage category, brought about by improvements in the flavor of these products and healthy market trends driven by consumers. The presented results evaluated the antioxidant phytochemical stability of RTD teas from aqueous infusions of traditional green tea (*Camellia sinensis*) and a botanical tea from yaupon holly (*Ilex vomitoria*) as influenced by packaging materials during cold storage. Two common packaging materials for RTD products are glass and polyethylene terephthalate (PET) and have been compared to a retortable pouch (RP), an emerging packaging material for various types of food since it is durable, inexpensive, lightweight, and easy to sterilize. Storage stability was then evaluated for each aqueous infusion prepared at 10 g/L at 90 °C for 10 min and evaluated at 3 °C in the absence of light over 12 weeks. Analyses included quantification and characterization of individual polyphenolics by high-performance liquid chromatography—photodiode array and liquid chromatography—electrospray ionization—mass spectrometry as well as changes in total antioxidant capacity. For green tea, concentrations of the three major flavan-3-ols, epigallocatechin gallate, epigallocatechin, and epicatechin gallate were better retained in glass bottles as compared to other packages over 12 weeks. In yaupon holly, chlorogenic acid and its isomers that were the predominant compounds were generally stable in each packaging material, and a 20.6-fold higher amount of saponin was found as compared to green tea, which caused higher stability of flavonol glycosides present in yaupon holly during storage. The antioxidant capacity of green tea was better retained in glass and PET versus RP, whereas no differences were again observed for yaupon holly. Results highlight the superiority of oxygen-impervious glass packaging, but viable alternatives may be utilizable for RTD teas with variable phytochemical compositions.

**KEYWORDS:** Tea, green tea, yaupon holly, phytochemical, polyphenolics, stability, packaging, glass bottle, PET, retortable pouch, ready to drink tea, antioxidant capacity

## INTRODUCTION

Next to water, tea is the most consumed beverage and is enjoyed by two-thirds of the world's population for its characteristic flavors and association to reduced risk of several major diseases such as coronary heart disease, stroke, and cancer.<sup>1</sup> Green tea (*Camellia sinensis*) is especially popular in Asian countries and includes a significant amount of flavan-3-ols (also known as tea catechins) such as (+)-catechin (C), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin gallate (EGCG), (–)-gallocatechin gallate (GCG), and (–)-gallocatechin (GC) that contribute to its antioxidant capacity and organoleptic properties.<sup>2,3</sup> Conversely, yaupon holly (*Ilex vomitoria*) is a dioecious (having male and female reproductive organs in separate plants) shrub that commonly grows wild in pine and hardwood forests surrounding the Gulf of Mexico and the southern Atlantic coast and can reach heights up to 25 feet tall.<sup>4</sup> The small and leathery leaves have historically been used to make stimulating aqueous infusions, most notably by Native Americans and early European colonists, due to their moderately high concentrations of methylxanthines including caffeine and theobromine.<sup>5–7</sup> Yaupon holly shares striking similarities in

phytochemical composition to South American yerba maté (*Ilex paraguariensis*), and its use by Native Americans as a medicinal tea was recorded the early 1500s.<sup>4,8</sup> Only limited information is available regarding the chemical and biological activities of yaupon holly, but its moderate caffeine content and elevated polyphenolic content are advantageous in beverage industries that prize these attributes from natural sources.

Ready to drink (RTD) beverages are commonly sold in glass, plastic, steel, or aluminum containers with little regard to potential adverse effects that these packages may have on the phytochemical stability or overall product quality including browning, flavor, and nutrient losses during storage.<sup>9,10</sup> The chemical changes from various packagings are mostly caused by oxygen contact and light transmission through them.<sup>11</sup> In these studies, the phytochemical stability of RTD infusions of green tea and yaupon holly was evaluated over time in glass, polyethylene terephthalate (PET), and a retortable pouch (RP) due to their

**Received:** December 14, 2010

**Accepted:** March 24, 2011

**Revised:** March 16, 2011

**Published:** March 24, 2011

**Table 1.** Initial Concentrations (mg/L), Retention Time ( $t_R$ ), and  $\lambda_{\max}$  (280 nm) of Major Polyphenolics in Green Tea Infusion Identified by HPLC-PDA and Mass Spectral Characteristics Determined by LC-ESI-MS<sup>a</sup>

compound	initial content (mg/L green tea infusion)	$t_R$ (min)	$\lambda_{\max}$	$[M - H]^-$	MS <sup>2</sup>	MS <sup>3</sup>
gallic acid	1.62 ± 0.12 <sup>a</sup>	14.8	277.5	N/I <sup>b</sup>		
EGC	214.81 ± 7.84	32.2	272.8	305	179, 221, 261	135, 151, 164
caffeine	445.19 ± 10.10	40.8	272.8	N/I		
EGCG	946.75 ± 29.20	41.2	277.5	457	169, 305, 331	135
EC	46.23 ± 1.60	42.1	277.5	289	179, 205, 245	125
GCG	96.74 ± 3.78	43.1	277.5	457	169, 305, 331	
ECG	225.94 ± 4.73	46.0	277.5	441	289	
myricetin-3-glycoside	20.70 ± 0.66	47.7	263.4, 357.5	479	316	
quercetin-3-rutinoside (rutin)	78.93 ± 2.21	48.3	258.6, 357.5	609	301	151, 179
quercetin-3-glycoside	23.25 ± 1.78	49.9	258.6, 357.5	463	485, 303	
kaempferol-3-glycoside	13.14 ± 0.69	52.0	268.1, 348.7	755	285	
kaempferol-3-rutinoside	18.39 ± 1.00	55.4	268.1, 349.7	593	285	162

<sup>a</sup>Data are expressed as means ± standard deviations of  $n = 3$ . <sup>b</sup>N/I, not ionized under current HPLC-MS conditions.

inherent differences in function and oxygen permeability. The present study on the stability of antioxidant polyphenolics provides an understanding of packaging and storage conditions that lead to retention of those compounds responsible for the quality and potential health benefits of these products.

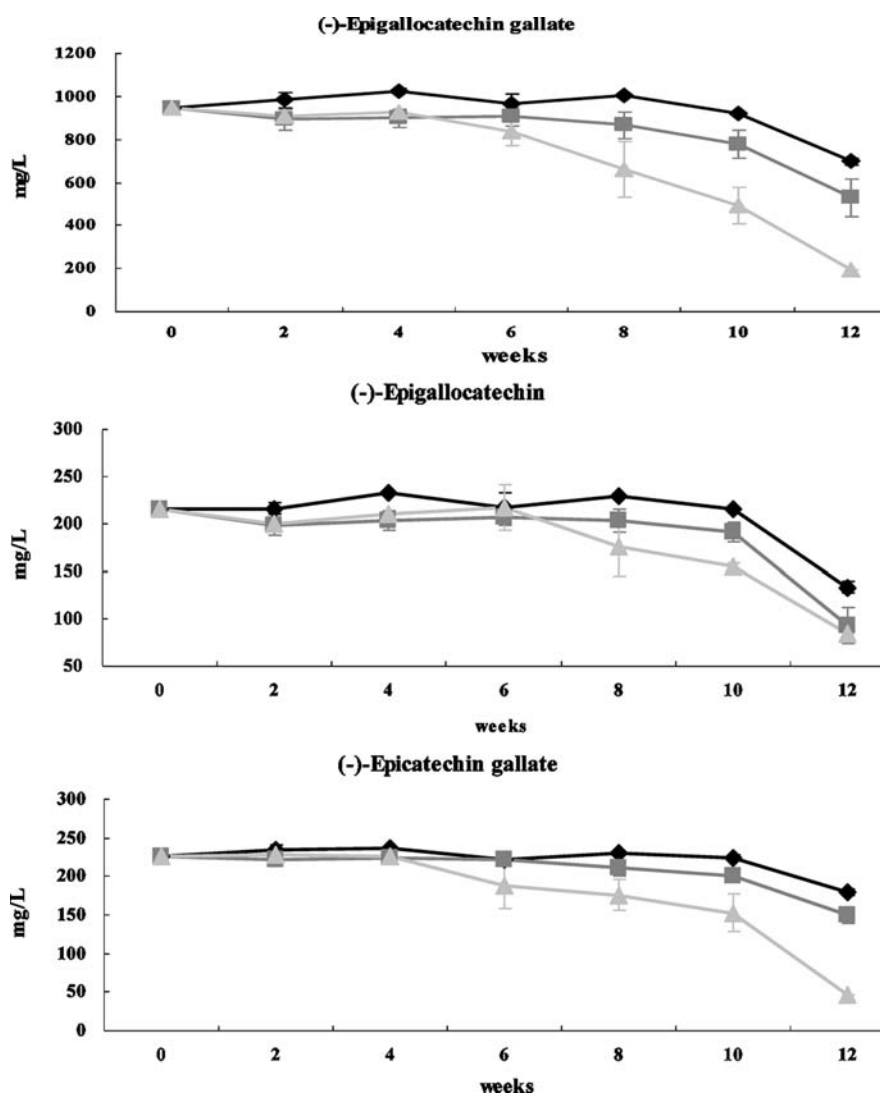
## MATERIALS AND METHODS

**Tea Preparation and Packagings.** Green tea was hand harvested and hot air-dried at 70 °C at Hwagae in South Korea. Likewise, yaupon holly leaves were wild harvested near Lufkin, Texas, and dried at 70 °C for 4 h. Leaves were powdered with a mortar and pestle for efficient polyphenolic extraction prior to hot water infusion, whereby tea infusions were prepared by pouring hot and double distilled H<sub>2</sub>O (90 °C) directly onto the leaves (1:100 ratio) with constant stirring for 10 min, sufficient time to fully extract tea polyphenolics with no degradation. The infusion was immediately filtered through cheesecloth and then allowed to cool to 25 °C prior to filtering through Whatman #4 filter paper followed by a 1 cm bed of diatomaceous earth, prewashed with water, obtained from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO) under a slight vacuum to remove any suspended particles. Sodium azide (50 mg/L) was added to each tea infusion to retard microbial growth during storage. Tea infusions were prepared in triplicate and divided into three equal portions for transfer to three different packaging materials that included glass (soda lime), PET, and a RP and then moved to storage chambers at 3 °C in the absence of light. Glass bottles (125 mL) with a rubber septum under the polytetrafluoroethylene (PTFE)-lined screw cap and PET bottles (250 mL) with formed PE-lined closures were 1.40 and 0.50 mm thick, respectively, and were obtained from Fisher Scientific (Fisher Scientific, Pittsburgh, PA). RP (4 in. × 6 in.) made from PET, polypropylene, and polyester was 0.12 mm thick and obtained from Sung-il packaging (Han-yang Inc., Daejeon, Korea). Prior to filling, each package was presterilized with 100 ppm of chlorine solution for 30 s at room temperature and extensively washed with sterile water to retard microbial growth during storage. For glass and PET bottles, the fill volumes were maximized to minimize headspace, while the RPs were flushed with nitrogen immediately prior to heat sealing. Tea infusions were stored for 3 months in the absence of light and analyzed biweekly. When sampling, tea infusions were transferred from the original package to test tubes using a sterile transfer needle where the needle pierced a rubber lid on the glass bottles and through the silicon-coated surface of the PET and RP packages to prevent air or microbial intake to the containers.

**Phytochemical Assessment.** Individual polyphenolics were analyzed and quantified by high-performance liquid chromatography (HPLC) as described by Lee and Talcott,<sup>12</sup> with a slight modification. Tea infusions first were diluted 3-fold with deionized water and filtered through a 0.45 μm membrane type PTFE filter (Whatman, Clifton, NJ) prior to injection. Polyphenolic separations were conducted on Dionex HPLC system using a PDA-100 photodiode array detector with a Dionex 250 mm × 4.6 mm Acclaim 120-C<sub>18</sub> column run at 0.8 mL/min. Mobile phases consisted of phase A (100% H<sub>2</sub>O) and phase B (60% methanol and 40% H<sub>2</sub>O) both adjusted to pH 2.4 using *o*-phosphoric acid. A gradient solvent system ran phase B 0% in 1 min, 0–30% in 30 min, 30–80% in 15 min, 80–100% in 15 min, and held for 10 min to equilibrate the column and whole system at a flow rate of 0.8 mL/min. Phytochemicals were detected and quantified at 280 nm against external standards of C, EC, EGCG, ECG, EGC, GCG, chlorogenic acid, caffeine, rutin, kaempferol, and quercetin procured from Sigma Aldrich (Sigma Chemical Co.).

Mass spectrometric analysis was carried out to tentatively identify compounds and to define structural information on individual polyphenolics present in each tea. Compounds were separated using a Finnigan Surveyor HPLC system and a Dionex 250 mm × 4.6 mm Acclaim 120-C<sub>18</sub> column. Mobile phase run phase A (100% H<sub>2</sub>O) and phase B (60% methanol and 40% H<sub>2</sub>O) each were adjusted to pH 2.4 using formic acid (5 mM ammonium formate) at 0.8 mL/min and a gradient elution changed phase B from 0% phase B for 1 min, 0–30% phase B over 30 min, 30–80% phase B in 15 min, and 80–100% phase B in 15 min for a total run time of 60 min and then returned to the original condition in 10 min for the next injection. Mass spectra were evaluated on a Thermo Finnigan LCQ Deca XP Max MS<sup>n</sup> ion trap mass spectrometer (ThermoFisher, San Jose, CA) for major polyphenolics in each tea infusion. The MS was operated in negative ion mode and fitted to an atmospheric pressure electrospray ionization (ESI) source. The electrospray voltage was set to 3300 V with sheath gas flow rate of 60 units/min, capillary gas temperature of 250 °C, auxiliary gas (N<sub>2</sub>), 5 units/min, and capillary voltage of 1.5 V. Mass spectra were collected in full scan mode ( $m/z$  200–2000), and fragment ions (MS<sup>2</sup> and MS<sup>3</sup>) were obtained on the most abundant ions present.

The antioxidant capacity using the oxygen radical absorbance capacity (ORAC) was determined by the method described by Talcott et al.<sup>13</sup> on a 96-well Molecular Devices fmax fluorescent microplate reader (Sunnyvale, CA) against a standard of Trolox with data expressed in μmol Trolox/mL.



**Figure 1.** Changes of EGCG, ECG, and EGC concentrations (mg/L) in green tea infusions packaged in glass, PET plastic bottle, and RP in the absence of light at 3 °C during 12 weeks of storage. Error bars represent the standard error of the mean ( $n = 3$ ). Glass, black diamond; PET bottle, gray square; and RP, light gray triangle.

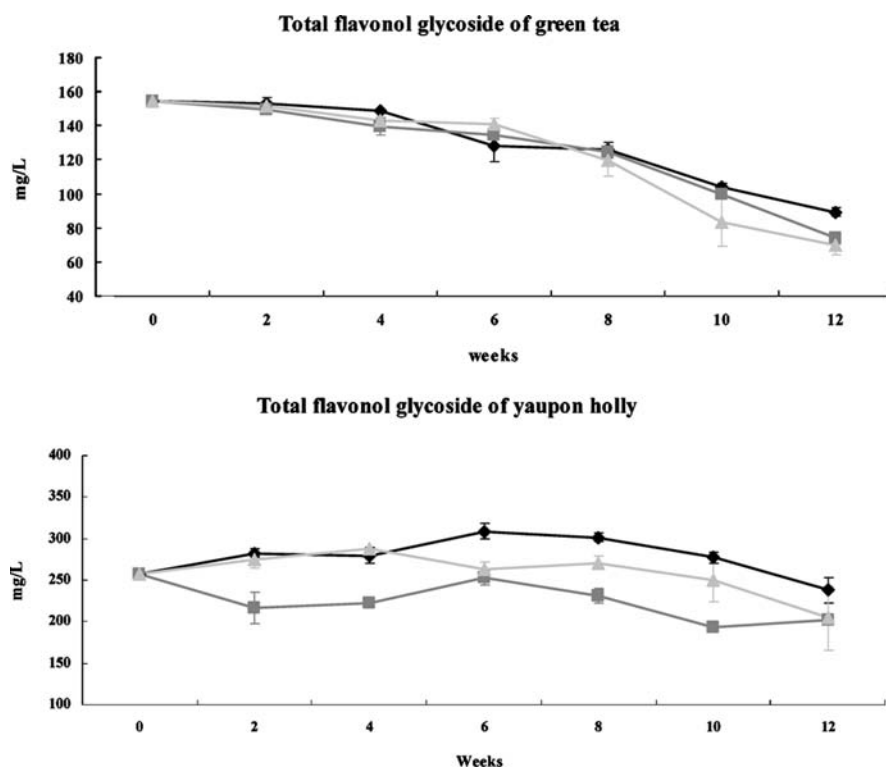
Saponin analysis was conducted as described by Gnoatto et al.<sup>14</sup> with a minor modification. Each tea infusion was prepared by brewing 15 g of powdered tea leaves with 200 mL of distilled water at 90 °C for 30 min followed by filtering through cheesecloth and then allowed to cool to 25 °C before final filtrations. First, the infusion went through Whatman #4 filter paper followed by a 1 cm bed of diatomaceous earth under a slight vacuum to remove suspended particles. The obtained tea extract was divided into three groups containing 20 mL of infusion and treated with 3 mL of chloridric acid to yield an acid concentration of 4 mol/L prior to hydrolysis for 2 h. The saponin fraction was extracted three times with an equal volume of chloroform and evaporated, and the sapogenins (saponin aglycone) were redissolved in acetonitrile for HPLC analysis. Separations were conducted on a Waters 2695 alliance HPLC system using a Water 996 photodiode array (PDA) detector with a Dionex 250 mm × 4.6 mm Acclaim 120-C<sub>18</sub> column run at 0.8 mL/min. The pH of the mobile phase (70% acetonitrile) was adjusted to pH 2.4 using *o*-phosphoric acid, and it was run for 60 min at 0.8 mL/min. Total saponin (sapogenin) in each infusions was detected and quantified at 280 nm against an external standard of ursolic acid (Sigma Chemical Co.).

**Statistical Analysis.** Data represent the mean triplicate analysis using ANOVA (analysis of variance) with JMP 5 statistical software

(SAS Institute., Cary, NC). Mean separation was conducted using the LSD (least significant difference) test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

**Phytochemical Changes in Green Tea Infusions.** Initial concentrations of 12 predominant polyphenolics in green tea are reported in Table 1, and ESI-MS fragmentation patterns were in agreement with Del Rio et al.<sup>15</sup> and Atoui et al.<sup>16</sup> EGCG, EGC, and ECG together accounted for 90% of the total phenolics in green tea sharing a common chemical structure of 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone) (Figure 1). These compounds were also reported to possess the highest antioxidant capacity among phytochemicals present in green tea.<sup>17–19</sup> During 12 weeks, the concentration of EGCG was reduced by 26, 44, and 80% in glass, PET, and RP, respectively, whereas EGC and ECG followed the same decreasing trend with 38, 43, and 57% and 11, 34, and 79%, respectively, from oxidative degradation. Because the stability of polyphenolic compounds is highly pH-dependent and flavan-3-ols were shown to exhibit good storage



**Figure 2.** Changes in total flavonol glycosides (mg/L) in green tea (top) and yaupon holly (bottom) infusions packaged in glass, PET plastic bottle, and RP in the absence of light at 3 °C during 12 weeks of storage. Error bars represent the standard error of the mean ( $n = 3$ ). Glass, black diamond; PET bottle, gray square; and RP, light gray triangle.

stability under acidic conditions, reductions in green tea catechins are largely attributed to oxidative conditions.<sup>2,17</sup> The pH of the green tea infusion was slightly acidic (pH 5.09); yet, commercially the addition of citric acid would further reduce the pH prior to packaging. No differences in concentrations were observed for these predominant compounds over the first 6 weeks of storage in each package ( $P < 0.05$ ), but subsequently, the degradation was enhanced for RP and PET as compared to glass. The concentrations of EGCG, EGC, and ECG were significantly higher in glass than PET and RP at the end of storage ( $P < 0.05$ ). Packaging in gas impervious glass bottles was therefore critical to slow oxidative conditions relative to PET and RP with their higher oxygen permeability. The oxygen transmission rates of PET bottles and RP used in the present study were 0.3 and 52 cc/pkg/day at room temperature (23 °C) and 0% relative humidity according to the manufacturers, while glass is known as oxygen impermeable packaging material.<sup>20</sup> Because more oxygen could pass through RP during tea storage, more degradation of EGCG, EGC, and ECG was anticipated in this package as compared to PET, and data trends supported this effect as more oxidative degradation was observed for EGCG and ECG in RT than PET packaging during storage. However, there were no significant differences for EGC between PET and RP packages.

Oxidative changes generally occurred slowly in packaged green tea as evidenced by the 6 week delay in measured polyphenolic changes. During oxidation of flavan-3-ols, a hydrogen atom is detached from hydroxyl group, and it reacts with an unpaired electron from oxygen to form a semiquinone radical.<sup>21</sup> Flavan 3-ols are generally divided into catechol and gallo-flavanols based on the number of hydroxyl groups on the B rings. ECG and

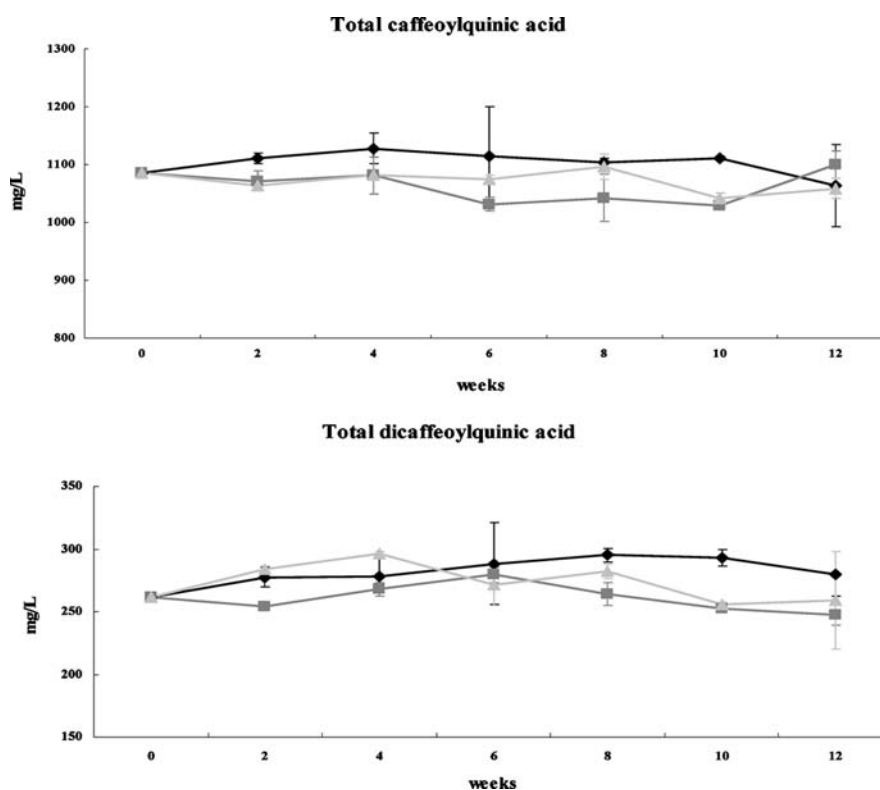
EC, which have two hydroxyl groups on their B rings, are called catechol flavanols, while EGCG and ECG are called gallo flavanols due to three hydroxyl groups attached to their B rings.<sup>21</sup> According to Yoshioka et al.,<sup>22</sup> gallo flavanols are more susceptible to oxidation since radicals are more easily formed on the ring containing three hydroxyl groups. Likewise, in the present study, gallo flavanols were determined as the most unstable flavanols to oxidation among green tea catechins since it has three hydroxyl groups on its B rings. This tendency was in agreement with Yoshioka et al.<sup>22</sup> and Miyazawa et al.,<sup>23</sup> and this susceptibility to semiquinone free radical formation in relation to other tea catechins makes gallo flavanols a good marker compound for green tea quality and stability. However, gallo flavanols are superior antioxidant compounds than catechol flavanols because they are oxidized more rapidly, and it results in higher hydrogen-donating ability to quench free radicals.<sup>21,24</sup> Overall, these data indicate the importance of oxidative changes to RTD green tea, where dissolved oxygen can accelerate destruction of polyphenolics responsible for antioxidant capacity, color, and flavor.

Total flavonol glycoside (sum of five flavonol glycosides including myricetin 3-glycoside, quercetin 3-rutinoside, quercetin 3-glycoside, kaempferol 3-glycoside, and kaempferol 3-rutinoside) decreased by 42, 52, and 55% in glass, PET, and RP, respectively, during 12 weeks of storage (Figure 2), and the presence of these flavonol glycosides in green tea infusion is in agreement with Del Rio et al.<sup>15</sup> and Atoui et al.<sup>16</sup> The concentration of total flavonol glycosides was about 10% of total phenolic compounds (sum of gallic acid, five flavanols, and five flavonol glycosides determined by HPLC analysis) in a tea infusion, which is significantly lower as compared to the concentrations of total

**Table 2.** Initial Concentrations (mg/L), Retention Time ( $t_R$ ),  $\lambda_{\max}$  (280 nm) of Major Polyphenolics in Yaupon Holly Tea Infusion Identified by HPLC-PDA<sup>1</sup>, and Mass Spectral Characteristics Determined by LC-ESI-MS<sup>2</sup>

compound	initial concentration (mg/L yaupon holly infusion)	$t_R$ (min)	$\lambda_{\max}$	$[M - H]^-$	MS <sup>2</sup>	MS <sup>3</sup>
3-caffeoylquinic acid	256.18 ± 9.52 <sup>a</sup>	38.2	324.9	353	179, 191	
5-caffeoylquinic acid	542.62 ± 16.91	41.8	329.6	353	179, 191	127
4-caffeoylquinic acid	279.36 ± 10.20	42.1	329.6	353	179, 191	135
3,4-dicaffeoylquinic acid	45.10 ± 3.75	43.0	329.6	515	353	135, 179, 191
3,5-dicaffeoylquinic acid	216.85 ± 15.20	44.2	329.6	515	353	135, 179, 191
quercetin-3-rutinoside (rutin)	237.44 ± 13.89	45.7	256.4, 363.8	515	353	135, 179, 191
keampferol-3-rutinoside	19.93 ± 3.80	50.4	268.1, 348.7	593	285	

<sup>a</sup>Data are expressed as means ± standard deviations of  $n = 3$ .



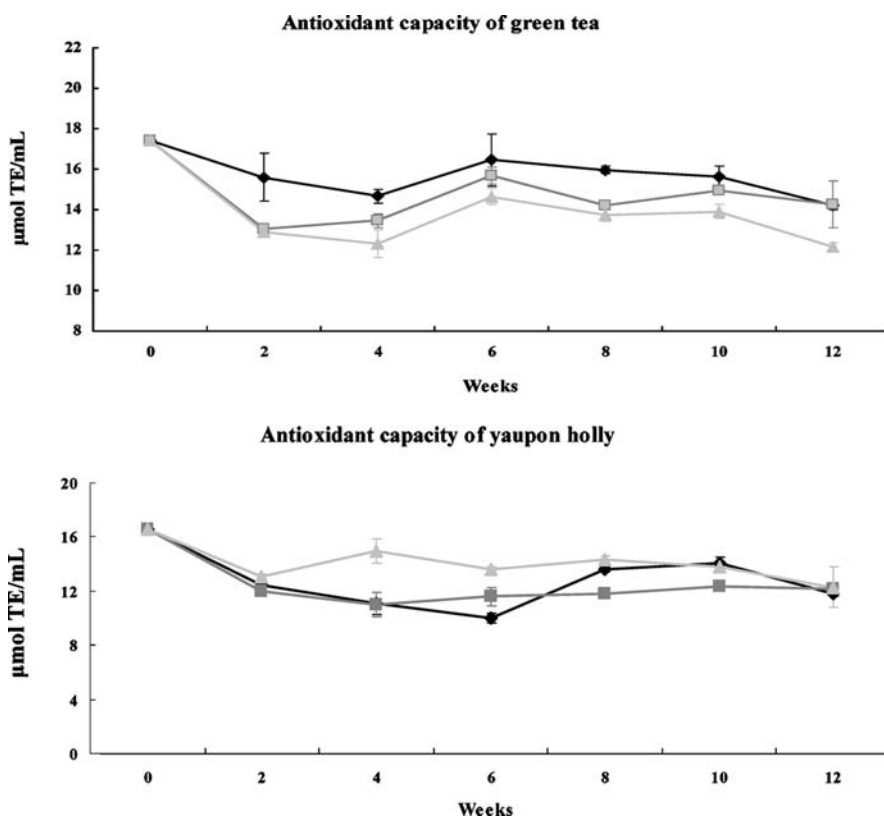
**Figure 3.** Changes in total caffeoylquinic acid (sum of 3-, 4-, and 5-caffeoylquinic acids) and total dicaffeoylquinic acid (sum of 3,4- and 3,5-dicaffeoylquinic acids) (mg/L) in yaupon holly infusion packaged in glass, PET plastic bottle, and RP in the absence of light at 3 °C during 12 weeks of storage. Error bars represent the standard error of the mean ( $n = 3$ ). Glass, black diamond; PET bottle, gray square; and RP, light gray triangle.

flavan-3-ols (sum of five flavanols determined by HPLC analysis). The total flavonol glycoside in all of the packaging materials was not altered for the first 4 week period; however, the total flavonol glycoside was significantly higher in glass bottles by 17 and 21% than that in PET and RP, respectively, at the end of 12 weeks of storage. The oxidative degradation of rutin (49% of total flavonol glycoside at 154.41 mg/L) and its aglycone quercetin was previously reported by Makris and Rossiter,<sup>25</sup> as observed in the present study.

Caffeine (1,3,7-trimethylxanthine) was stable for 12 weeks in all types of packaging materials, and no difference was observed between packaging materials during 12 weeks of storage (data not shown). Caffeine is known to be stable under ordinary tea processing conditions and during storage as observed in this study.<sup>21</sup> There is a significant amount of caffeine in green tea

(445.19 mg/L), and it is a critical compound for bitter flavor and overall quality of tea.

**Phytochemical Changes in Yaupon Holly Infusions.** As an American holly species in the Aquifoliaceae family, yaupon holly was previously reported to have a similar phytochemical composition to the South American botanical tea commonly known as yerba maté.<sup>7,26</sup> Specific polyphenolics present in *I. paraguariensis* were characterized as chlorogenic acid (3-caffeoylquinic acid), chlorogenic isomers (4- and 5-caffeoylquinic acids), 3,4- and 3,5-dicaffeoylquinic acids, and rutin (quercetin 3-rutinoside) with each confirmed to be present in yaupon holly infusions by HPLC-ESI-MS<sup>2</sup> analysis.<sup>26,27</sup> Chlorogenic acid and its isomers were the predominant polyphenolics in yaupon holly infusions accounting for 67% of total polyphenolics present followed by 16% dicaffeoylquinic acids and 14% rutin (Table 2).



**Figure 4.** Antioxidant capacity ( $\mu\text{mol}$  Trolox equiv/mL) changes of green tea (top) and yaupon holly (bottom) infusions packaged in glass, PET plastic bottle, and RP in the absence of light at  $3^\circ\text{C}$  during 12 weeks of storage. Error bars represent the standard error of the mean ( $n = 3$ ). Glass, black diamond; PET bottle, gray square; and RP, light gray triangle.

During first 4 weeks of storage, the concentration of total caffeoylquinic acid (sum of chlorogenic acid and its isomers) and total dicaffeoylquinic acid (sum of 3,4- and 3,5-dicaffeoylquinic acids) did not appreciably change in all packaging materials as compared to the initial concentration, with only minor changes observed throughout the remainder of the study ( $P < 0.05$ ) (Figure 3). This tendency is in agreement with several studies where plant polyphenols such as chlorogenic acid were stable in various beverages and plants during a long-term storage under acidic environments.<sup>17,28,29</sup> The pH values of the yaupon holly infusions were only slightly acidic (pH 4.96) and did not appreciably change during storage among the packaging materials. As compared to green tea infusions, yaupon holly polyphenolics were stable even when packaged into materials with high oxygen permeability. Chang et al.<sup>30</sup> also demonstrated the stability of chlorogenic acid in fruit juice stored for 6 months in oxygen-permeable polyethylene at  $4^\circ\text{C}$  in the dark. Other destabilizing factors such as prooxidant transition metals previously identified in yerba maté infusions may participate in Fenton type reactions to generate reactive oxygen species.<sup>31,32</sup> However, the overall polyphenolic content of yaupon holly infusions was apparently sufficient to inhibit such reactions or to potentially form inactive reduced iron complexes during the course of the 12 weeks of storage.<sup>33</sup>

The total concentration of the two flavonol glycosides in yaupon holly (quercetin 3-rutinoside and kaempferol 3-rutinoside) was reduced by 22 and 21% in PET and RP, respectively, with no change in glass during the 12 weeks of storage (Figure 2). As compared to green tea flavonoids that lost

from 42 to 55% of total in the various packaging materials, yaupon holly flavonoids were comparatively stable during storage. One noted difference in phytochemical composition between the tea types was the presence of water-soluble saponins in yaupon holly infusions (1032 mg/L) as compared to green tea infusions (50.0 mg/L). Even though no direct information is available on the effect of saponins on polyphenol stability, synergistic biological interactions haven't been reported when these two compounds were present in the same medium. Interactions between polyphenols and saponins were previously reviewed by San Martín and Magnunacelaya<sup>34</sup> and Argentieri et al.<sup>35</sup> where a higher biological effect existed against nematodes (unsegmented and colorless worm with elongated round body) when saponins and flavonols were present together in vitro. Moreover, the synergistic interaction was observed when saponins and flavonoids were used in combination to inhibit microbial growth.<sup>36</sup> Thus, it was hypothesized that saponins in yaupon holly infusion may interact with flavonol glycosides and possibly with caffeoylquinic acids resulting in higher polyphenolic stability throughout the storage.

Even though yaupon holly is known as a caffeine-containing plant,<sup>37</sup> caffeine was undetectable by HPLC analysis in this study (data not shown) because the yaupon holly leaves used in this study may not contain caffeine. According to Palumbo et al.<sup>17</sup> and Palumbo et al.,<sup>38</sup> the caffeine content in yaupon holly was 0–1.91% of dry weight because its presence was largely dependent on cultivar, fertilization, gender (different response to fertilization), and exposure to sunlight.

**Antioxidant Capacity Changes in Green Tea and Yaupon Holly.** The antioxidant capacity of green tea infusions decreased

by 18, 14, and 30% in glass, PET, and RP, respectively, during the 12 weeks of storage (Figure 4). Even though the general trend of antioxidant capacity changes during storage was somewhat different from that of tea catechins, it was due to the nature of the nonspecific ORAC assay that detects not only phenolic compounds but also all other antioxidants present in tea including ascorbic acid, caffeine, and saponin. During storage, only small differences in antioxidant capacity were noted at weeks 2 and 8 between glass and PET, but glass was ultimately better at the antioxidant capacity retention than RP during storage ( $P < 0.05$ ). As observed for the individual compounds EGC, EGCG, and ECG in green tea, the antioxidant capacity was higher in glass than in RP throughout storage, but the difference was not significant between glass and PET. A loss of antioxidant capacity in teas containing tea catechins was previously investigated by Naithani et al.<sup>39</sup> as was attributed to decreased polyphenolic concentrations. By comparison, the antioxidant capacity of green tea (17.4  $\mu\text{mol TE/mL}$ ) was higher by 9.48% than yaupon holly (16.5  $\mu\text{mol TE/mL}$ ) because the total phenolic concentration determined by HPLC was higher in green tea (1685 mg/L) by 9.47% than yaupon holly (1596 mg/L), respectively. Despite a few changes in individual polyphenolics during storage, the antioxidant capacity of yaupon holly infusion was reduced from 26 to 29% in all packaging types during storage (Figure 4), presumably from the loss of minor antioxidant constituents.

This study was designed to understand the impact of packaging materials on the retention of phenolic compounds responsible for the quality and potential health benefits of green tea and yaupon holly aqueous infusions. Green tea polyphenolics and antioxidant capacity were directly impacted by packaging materials leading to oxidative loss of polyphenolics, while yaupon holly infusions were less impacted by packaging due to inherent stability differences and radical scavenging ability among the polyphenolics in each tea. Commercial RTD teas generally have a long retail shelf life; therefore, significant losses in polyphenolic compounds or antioxidant capacity are to be expected, even under refrigerated conditions. Packaging materials with different oxygen transmission rates are likely to be a significant shelf life predictor due to the relationship between oxygen permeability and antioxidant polyphenolic degradation observed in these trials.

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## ACKNOWLEDGMENT

We thank you Dr. Kevin Goodner for valuable comments and helpful discussions.

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