JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Tea Creaming in Nonfermented Teas from *Camellia sinensis* and *Ilex vomitoria*

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ABSTRACT: Tea creaming is the development of a cloudy or hazy appearance in tea and ready-to-drink tea products on cooling and is highly undesirable in the tea beverage industry. Commonly associated with fermented black or oolong teas, the objective of this study was to investigate the physicochemical mechanism of the formation of tea cream in nonfermented green tea (*Camellia sinensis*) and a caffeine-containing botanical tea from yaupon holly (*Ilex vomitoria*) that is free of catechin-based polyphenolics. Four tea-creaming activators (phenolics, soluble protein, caffeine, and metal ions) were added to tea infusions as well as decaffeinated teas created by chloroform extraction. Tea-creaming activators increased the weight and turbidity of both teas with the exception of soluble protein addition (as bovine serum albumin) to green tea, whereas the greatest increase in turbidity occurred with the addition of metal ions in green tea. Tea creaming was equally developed at three incubation temperatures (4, 25, and 40 °C) in both teas, but tea-creaming compositions in each tea were different at the incubating temperatures. The antioxidant capacity of each tea was lowered after creaming due to the loss of antioxidants that participated in tea cream formation.

KEYWORDS: *ready-to-drink tea, tea cream, green tea, yaupon holly, polyphenols, chemical complex, precipitation, turbidity, antioxidant capacity, haze*

INTRODUCTION

Chemical complexes formed by interactions among caffeine, polyphenolics, proteins, metal ions, carbohydrates, and/or other reactive agents in tea infusions are known as "tea cream". Especially troublesome in refrigerated teas, creaming gives a cloudy or hazy appearance to ready-to-drink (RTD) tea beverages and adversely affects consumer quality. "Chill haze" is formed in beer, wine, and fruit juices, and it makes the products appear cloudy, so the products become less attractive to customers. Chill haze should be differentiated from tea cream because it is formed mostly only by interactions between haze-active proteins and proanthocyanidins.[1] The consequences of tea cream formation affect not only physical attributes but also potentially biological activities and sensory attributes with altered levels of astringency, aroma, color, and taste.[2,3] The turbidity of RTD teas can be reduced by physical filtration methods; however, loss of bioactive polyphenolics and organoleptic properties are potential concerns. Previous studies have used both chemical and physical means to remove tea cream from black tea infusions without decreasing concentrations of bioactive compounds. Wright[4] used tannase treatments to reduce tea cream in black tea by hydrolyzing gallated polyphenolics, but compounds were degraded that resulted in a loss of astringency and overall quality. Metal chelators such as EDTA were able to inhibit tea cream by up to 50%, but re-formation of the cream was subsequently observed during storage.[5] Other efforts to reduce tea cream have included calcium removal using a two-stage extraction at different temperatures, but resulted in only partial success followed by re-formation of the tea cream.[2,6] Thus far, no studies have shown complete removal of tea cream without loss of organoleptic traits or lowered concentrations of bioactive compounds. The formation of black tea cream was shown to be the result of hydrophobic interactions and/or hydrogen bonding between caffeine and functional groups on the causative chemical constituents.[7] For example, hydroxyl groups of polyphenols, peptide groups of proteins, and the keto-amide group of caffeine can interact to form poorly soluble complexes in black tea infusions. Most studies have reported tea cream formation in either black tea or oolong tea, because their oxidized polyphenolics during fermentation are more prone to tea cream formation;[2,4−9] however, no binding mechanisms from green tea or botanical tea infusions have been reported.

Botanical teas are a growing market segment, and only a few have been extensively studied for their chemical composition and potential for augmenting human health. Most botanical teas are free of natural caffeine with the exception of certain *Ilex* species, namely, *Ilex vomitoria* (yaupon holly) and *Ilex paraguariensis* (yerba maté), that possess significant amounts of caffeine[10] and therefore have the potential to form tea cream on cooling. Yaupon holly is native to the southern and southeastern United States and has historically been used as a caffeine-containing beverage by Native Americans along the Gulf of Mexico and South Atlantic coasts; it contains high concentrations of chlorogenic acid (3-*O*-caffeoylquinic acid)

Received: August 14, 2012
Revised: October 12, 2012
Accepted: November 12, 2012
Published: November 12, 2012

dx.doi.org/10.1021/jf303555f | *J. Agric. Food Chem.* 2012, 60, 11793−11799

and its isomers (4 and 5-*O*-caffeoylquinic acid) that were reported to bind with caffeine, protein, and metal ions in a reaction similar to black tea creaming.[10−14] To date, studies into tea creaming relate only to teas from *Camellia sinensis*, and most have focus on fermented teas. These studies investigated the chemical mechanism for tea creaming in nonfermented teas including green tea and yaupon holly and evaluated chemical and physical changes caused by tea creaming. Also, changes in antioxidant capacity in both green tea and yaupon holly by tea creaming were reported.

MATERIALS AND METHODS

Tea Infusions and Pretreatments. Green tea leaves were harvested and hot air-dried at 70 °C in Hwagae, Korea, and yaupon holly leaves were wild-harvested in southeastern Texas, USA, and hot air-dried at 70 °C for 4 h. Tea infusions were prepared from finely powdered leaves crushed with a mortar and pestle and Milli-Q water (Billerica, MA, USA) at 90 °C poured directly onto the leaves at 5% w/v. After 30 min of infusion with constant agitation, the slurry was filtered through cheesecloth and allowed to cool to 25 °C for final filtrations through Whatman no. 4 filter paper followed by a 1 cm bed of prewashed diatomaceous earth (MP Chemicals; +150 mesh, 6.6%, and permeability, 2112) under a slight vacuum to ensure a particle-free infusion. For both green tea and yaupon holly, the original infusions were aliquoted for individual trials for tea cream development. Modifications included the addition of soluble protein (100 mg/100 mL of bovine serum albumin (BSA)), caffeine (100 mg/100 mL), gallic acid (100 mg/100 mL), and calcium chloride ($CaCl_2$) as a metal ion source (100 mg/100 mL), as well as decaffeination by three extractions with chloroform (1:1 ratio) in a separatory funnel; all were compared to a nonmodified control. BSA was chosen as the soluble protein due to its high solubility in the aqueous tea infusions and is widely used as a reference protein for tannin interactions and protein quantification. Each tea was held at the same temperature (4 °C) for 12 h to allow tea cream formation in one trial, whereas a second trial investigated the effects of short-term tempering of the teas by holding aliquots at 4, 25, and 40 °C for 12 h. The studies were overlapped at the 4 °C holding temperature, and treatments in each trial were conducted in triplicate.

Tea Cream Determination. Tea cream weight was determined gravimetrically according to the method of Nagalashmi et al.[15] with modification. First, 15 mL of tea infusion from each treatment was dried at 85 °C in an aluminum pan for 12 h using a laboratory-scale oven to determine total tea solid. Second, after a12 h incubation for each tea creaming trial and holding temperature, 15 mL aliquots were centrifuged at 4000*g* at 7 °C for 30 min to separate the supernatant from insoluble tea cream sediments. The separated supernatants (postcream infusion) were transferred to a preweighed aluminum pan and dried as described above. Both dried tea materials from the initial tea infusion and cleared supernatant were carefully weighed using an analytical scale. The amount of tea cream was the difference of the dried weight between the initial tea infusion and the cleared supernatant. Subsequently, concentrations of polyphenolics, caffeine, protein, and metal ions in tea cream were determined by difference between the original infusions and the supernatant after centrifugation as described by Chao and Chiang[9] and Smith.[16]

Turbidity. Turbidity was measured using a Micro TPW portable field turbidimeter (HF Scientific, Inc., Fort Myers, FL, USA) after a 12 h incubation of each treatment. Tea infusions were diluted 5-fold with Milli-Q water, transferred to a glass sample cuvette, and measured three consecutive times following manual mixing to ensure homogeneity with data expressed as nephelometric turbidity units (NTU).

Polyphenolics and Caffeine. Individual polyphenolics were analyzed by HPLC as described by Lee and Talcott,[17] with a slight modification. Each infusion was diluted 3-fold with deionized water and filtered through a 0.45 μm PTFE filter (Whatman, Clifton, NJ, USA) prior to injection. Polyphenolic separations were conducted on a Waters 2695 alliance HPLC system using a Waters 996 photodiode array (PDA) detector with a Dionex 250 × 4.6 mm Acclaim 120-C_{18} column run at 0.8 mL/min. A gradient mobile phase for both green tea and yaupon holly consisted of phase A (100% H_2O) and phase B (60% methanol and 40% H_2O), each adjusted to pH 2.4 using orthophosphoric acid. The gradient for green tea infusion started by running 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. The column was equilibrated with 100% phase A for 2 min prior to the next sample injection. The polyphenolic separation for yaupon holly infusion was done with the same mobile phases by running 0−50% phase B in 10 min, 50−70% phase B in 5 min, and 70−100% in 15 min, for a total run time of 30 min. The column was also equilibrated by running 100% phase A for 2 min before the next injection. Phenolic compounds were detected and quantified at 280 nm against external standards of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin gallate, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-gallocatechin, caffeoylquinic acid, caffeine, kaempferol, rutin, and quercetin, all procured from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA).

Total Saponins. Saponin analysis was conducted as described by Gnoatto et al.[18] with minor modification. Tea infusions were prepared by brewing 15 g of each powdered tea leaf sample with 200 mL of distilled water at 90 °C for 30 min and filtered as previously described. Infusions were divided into three aliquots of 20 mL each, treated with 3 mL of 32% hydrochloric acid solution, and hydrolyzed for 2 h at room temperature. The saponin fraction was extracted three times with an equal volume of chloroform in a separatory funnel and evaporated to dryness. Sapogenins (saponin aglycones) were redissolved in 10 mL of acetonitrile and diluted 3-fold in deionized water prior to HPLC analysis. Sapogenin separation was conducted on a Waters 2695 alliance HPLC system using a Waters 996 photodiode array (PDA) detector with a Dionex 250 × 4.6 mm Acclaim 120-C_{18} column run at 0.8 mL/min for 60 min. The mobile phase consisted of 70% of acetonitrile and 30% of purified water. Total saponin (sapogenin) in each infusion was detected and quantified at 203 nm against external standards of ursolic acid procured from Sigma-Aldrich.

Soluble Protein and Metal Ions. Soluble protein in tea infusions was determined using the biuret assay as described by Gornall et al.[19] Filtered and diluted tea infusions were reacted with biuret reagent (Ricca Chemical Co., Arlington, TX, USA) against a standard curve of BSA and allowed to react for 20 min for absorbance reading at 540 nm on a Helios gamma UV−vis spectrometer (Thermo Scientific, Waltham, MA, USA). Metal content was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) as described by Fernández-Cáceres et al.[20] using an ICP spectrometer (ARL Fisons 3410 with Minitorch).

Antioxidant Capacity. The antioxidant capacity of each tea infusion was measured using the oxygen radical absorbance capacity (ORAC) assay run according to Talcott and Lee[21] and quantified against a standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Data are reported in micromoles of Trolox equivalents (TE) per milliliter of tea infusion.

Statistical Analysis. Data obtained from green tea and yaupon holly analyzed in triplicate were analyzed by ANOVA using JMP software, version 5,[22] and mean separation determined by the LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

Induced Changes in Green Tea Creaming. Early work on tea creaming in black tea (*C. sinensis*) reported complex formation by the binding action of caffeine or protein to oxidized polyphenolics with galloyl esters such as theaflavin and thearubigin.[6,23] The role of metal ions may be secondary to the initial tea cream formation, binding to preformed tea cream complexes that increase the mass and radius of the cream and also attracting polyphenols due to their binding affinity to the metals.[2,8,9,24] In these studies, the amount of tea cream formed

in the control green tea infusion, without the addition of tea cream activators, was 536.8 mg/L (Figure 1A). The weight of

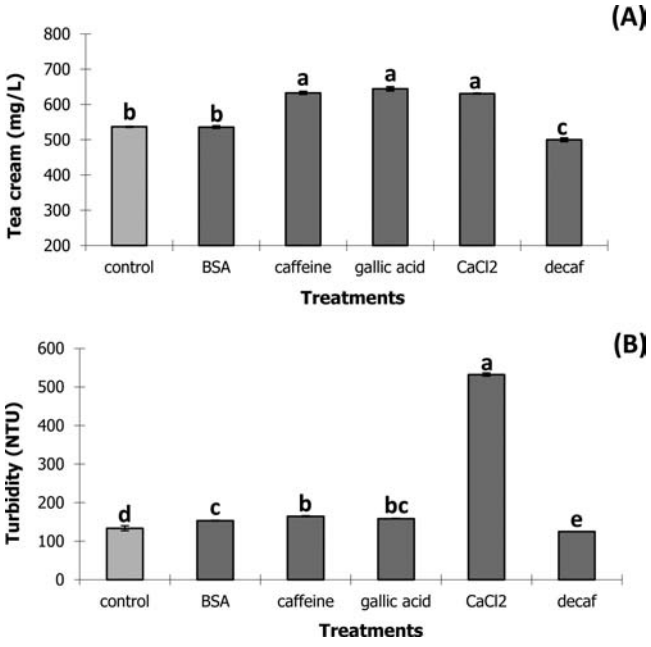

Figure 1. (A) Amount of green tea cream (mg/L) and (B) turbidity (NTU) of green tea infusions caused by the addition of BSA, caffeine, gallic acid, and $CaCl_2$ (100 mg/100 mL each) or decaffeination at 4 °C for 12 h. Bars with different letters for each treatment represent significant difference between triplicated samples (LSD test, $P < 0.05$).

green tea was contributed by polyphenol (21.5%), caffeine (10.2%), protein (15.8%), and metal ions (9.5%) when the infusion was stored at 4 °C. In the present study, a total of 12 phenolic compounds including phenolic acids, tea catechins, and flavonol glycosides were detected, and they all participated in green tea creaming (Table 1; Figure 2A). In green tea, only the most prevalent metal ions (potassium and calcium) participated in creaming (Table 2). Known but unidentified creaming compounds in the present study such as saponin, amino acids, and carbohydrates might make up the rest of green tea cream weight.[25,26] The presence of saponin in green tea infusion was also proven in this study, and its concentration was 50.0 mg/L.

The addition of known creaming activators caffeine, gallic acid, and metal ions caused significant increases in green tea cream by 15.2, 16.7, and 15.0%, respectively, when compared to the control. Green tea cream was not increased when BSA was added to the infusions, and caffeine removal decreased tea cream by 18.4% (Figure 1A). Similar to fully fermented black tea, unfermented green tea cream was likely related to interactions between caffeine and catechins as binding among keto-amino groups or the basic nitrogen of caffeine and gallyl and/or galloyl group of tea catechins results in insoluble particle formation. Data indicated that when caffeine was added to green tea infusions, significantly more catechins were found in the green tea cream. Caffeine addition resulted in 1.9-, 3.9-, 8.9-, 2.7-, and 8.6-fold increases in epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin (EC), gallocatechin gallate (GCG), and epicatechin gallate (ECG) in the green tea cream compared to the controls. Overall, gallo-flavanols were found at significantly higher concentrations in green tea cream (55.5% of total phenolic compound), whereas

Table 1. Concentrations of Polyphenolic Compounds and Caffeine in Original Green Tea Infusion and Postcream Infusion (Clear Extract) after 12 h of Holding at 4 °C and Concentration Difference Present in Insoluble Tea Cream[a]

compound	original infusion (mg/L)	postcream infusion (mg/L)	tea cream (mg)
1. gallic acid	26.3 ± 2.8	3.73 ± 0.3	22.6
2. (−)-epigallocatechin (EGC)	128 ± 2.2	102 ± 5.1	26.0
3. caffeine	603 ± 9.8	549 ± 12.5	54.6
4. (−)-epigallocatechin gallate (EGCG)	435 ± 8.1	397 ± 15.8	37.9
5. (−)-epicatechin (EC)	84.8 ± 4.0	84.2 ± 1.1	0.6
6. (−)-gallocatechin gallate (GCG)	19.5 ± 0.7	17.0 ± 0.9	2.5
7. (−)-epicatechin gallate (ECG)	99.2 ± 4.2	97.2 ± 1.8	2.0
8. myricetin 3-glycoside-1	29.8 ± 2.0	22.2 ± 0.9	7.6
9. myricetin 3-glycoside-2	39.8 ± 2.6	32.4 ± 2.3	7.4
10. quercetin 3-rutinoside (rutin)	119 ± 4.1	89.0 ± 2.8	29.8
11. quercetin 3-glucoside	21.3 ± 2.8	21.3 ± 0.1	0.1
12. kaempferol 3-glucoside	19.1 ± 0.6	19.0 ± 1.0	0.1
13. kaempferol 3-rutinoside	26.3 ± 0.9	25.2 ± 1.0	1.1

[a]Listed compounds 8−13 are expressed as mg/L rutin equivalents.

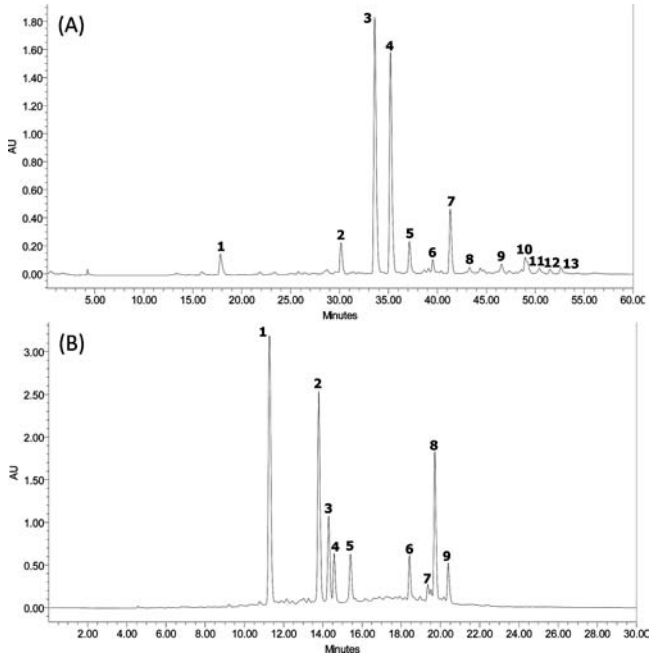

Figure 2. HPLC chromatograms of green tea (A) and yaupon holly (B) at 280 nm. For peak assignment, see Table 1 for green tea and Table 3 for yaupon holly.

catechol-based flavanols were present at only 2.3% of total phenolic compounds. This observation indicated that the number of hydroxyl groups on the B ring has a greater effect on tea creaming than the presence of galloyl moieties on catechins when catechin and caffeine are present in the same medium. However, when caffeine was removed from green tea infusions, the green tea cream had 33.2, 54.5, 84.6, 56.8, and 41.8% lower concentrations of EGC, EGCG, EC, GCG, and ECG, respectively. Caffeine addition and removal trials indicated a

dx.doi.org/10.1021/jf303555f | J. Agric. Food Chem. 2012, 60, 11793−11799

Table 2. Concentrations of Metal Ions Present in Original Infusion, Postcream Infusion, and Tea Cream of Green Tea and Yaupon Holly[a]

	magnesium	calcium	iron	copper	zinc	nickel	sodium	potassium
Green Tea								
original infusion	34	16	<1	<1	<1	<1	28	675
postcream infusion	34	10	<1	<1	<1	<1	27	624
tea cream[b]	<1	6	<1	<1	<1	<1	<1	51
Yaupon Holly								
original infusion	148	57	<1	<1	<1	<1	8	298
postcream infusion	147	57	<1	<1	<1	<1	7	279
tea cream[b]	<1	<1	<1	<1	<1	<1	<1	19

[a]Each metal concentration was determined by ICP-AES analysis and is expressed as mg/L. [b]The concentrations of metal ions in tea cream were calculated as the difference between original and postcream infusions.

clear tendency for caffeine binding to green tea catechins and subsequent tea cream formation. The addition of BSA did not change the weight of green tea cream despite previous reports on the role of proteins in black tea cream formation.[27,28] When BSA was added to green tea infusions, significantly more catechins including EGC, EGCG, EC, and ECG were observed in tea cream by 49.5, 70.1, 90.5, and 85.6%, respectively. However, the concentrations of other tea-creaming compounds such as caffeine and rutin were reduced in tea cream by 29.7 and 36.6%, respectively. These results indicated that catechin−protein binding likely occurs between carbonyl groups on proteins and hydroxyl groups of polyphenols before other tea creaming compounds can bind to protein. Gallic acid was found to be among the most active polyphenolic compounds interacting to form green tea cream with 85.8% of the naturally occurring free gallic acid found in the cream precipitates (Table 1). When additional gallic acid was added to green tea infusions, 43.6% more gallic acid was found in green tea cream while other polyphenolics such as EGC, EGCG, EC, ECG, and caffeine decreased by 49.1, 25.7, 67.6, 11.6, and 81.1%, respectively, indicating its preference in cream formation over other polyphenolics. Addition of $CaCl_2$ to green tea infusion did not demonstrate a clear binding trend with polyphenols in green tea cream formation likely as a result of the diversity of interactions that resulted in green tea cream formation. However, the addition of $CaCl_2$ had the greatest impact on turbidity formation with a nearly 4-fold increase in turbidity (Figure 1B) compared to all other treatments with only minor turbidity changes in relation to the control.

Induced Changes in Yaupon Holly Tea Creaming. The weight of yaupon holly tea cream in the untreated control was 506.6 mg/L, and the tea cream formation trends in the presence of externally added activators were similar to observations with unfermented green tea. Exceptions were noted with the addition of BSA, which enhanced creaming to the same extent as caffeine, gallic acid, and $CaCl_2$ addition by 7% on average (Figure 3A). The known components of yaupon holly tea cream were polyphenols (20%), caffeine (3%), protein (35%), and potassium (4%), indicating that protein is the major contributor to tea cream in yaupon holly (Tables 2 and 3). Other potential tea-creaming compounds present in yaupon holly included saponins (1032 mg/L of original infusion), and related compounds such as carbohydrates and amino acids could make up to 38% of total yaupon holly tea cream. A total of 8 phenolic compounds (6 caffeoylquinic acids and 2 flavonols) were detected in yaupon holly infusions (Figure 2B). Dicaffeoylquinic acids were negligible in yaupon holly tea cream compared to monocaffeoylquinic acids, in agreement

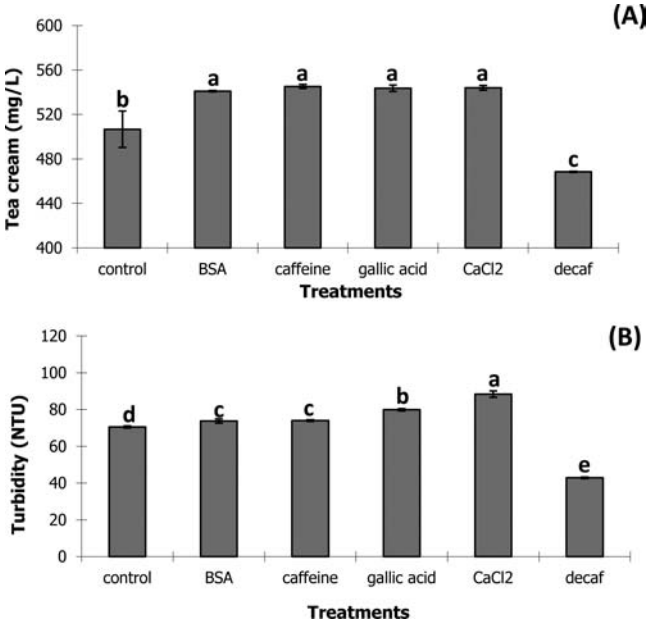

Figure 3. (A) Amount of tea cream (mg/L) and (B) turbidity (NTU) of yaupon holly infusions caused by the addition of BSA, caffeine, gallic acid, and $CaCl_2$ (100 mg/100 mL each) or decaffeination at 4 °C for 12 h. Bars with different letters for each treatment represent significant difference between triplicated samples (LSD test, $P < 0.05$).

with Sondheimer et al.,[14] who described a lower reacting ability with other tea-creaming compounds such as caffeine, protein, and metal ions. Among phenolic compounds found in yaupon holly tea cream, rutin (quercetin 3-rutinoside) was detected at the highest concentration (22.0% of total) followed by 3-*O*-caffeoylquinic acid (21.5%), 5-*O*-caffeoylquinic acid (20.2%), and 4-*O*-caffeoylquinic acid (8.8%).

As observed in green tea creaming, all of the treatments generally increased the turbidity of yaupon holly infusion, whereas decaffeination lowered turbidity (Figure 3B). Tea cream formation in yaupon holly infusion was affected by the presence of caffeine because caffeine removal resulted in an 8.0% decrease compared to the control, whereas caffeine addition increased yaupon holly tea cream weight by 7.0%. Caffeine addition also changed the composition of the cream as caffeine and rutin were increased by 23.9 and 36.2%, respectively, and three caffeoylquinic acids were increased by 20.5% (3-caffeoylquinic acid), 18.9% (5-caffeoylquinic acid), and 31.7% (4-caffeoylquinic acid). This observation is in agreement with previous studies that reported caffeoylquinic acids formed an insoluble complex with caffeine via hydrogen

Table 3. Concentrations of Polyphenolic Compounds and Caffeine in Original Yaupon Holly Tea Infusion and Postcream Infusion (Clear Extract) after 12 h of Holding at 4 °C and Concentration Difference Present in Insoluble Tea Cream[a]

compound	original infusion (mg/L)	postcream infusion (mg/L)	tea cream (mg)
1. 3-*O*-caffeoylquinic acid (chlorogenic acid)	423 ± 2.6	399 ± 3.8	23.7
2. 5-*O*-caffeoylquinic acid (neochlorogenic acid)	318 ± 0.3	293 ± 1.9	25.1
3. 4-*O*-caffeoylquinic acid (cryptochlorogenic acid)	125 ± 0.3	115 ± 1.0	10.3
4. caffeine	140 ± 0.5	123 ± 0.4	16.9
5. 3,4-dicaffeoylquinic acid	67.7 ± 0.2	66.1 ± 1.1	1.64
6. kaempferol 3-glycoside	102 ± 9.9	98.2 ± 3.9	3.93
7. 3,5-dicaffeoylquinic acid	18.9 ± 0.2	16.8 ± 0.6	2.14
8. quercetin 3-rutinoside (rutin)	392 ± 0.1	367 ± 0.4	25.7
9. 4,5-dicaffeoylquinic acid	52.1 ± 0.4	44.6 ± 0.6	7.48

[a]Listed compounds 6 and 8 are expressed as mg/L rutin equivalents.

bonds and hydrophobic interactions in various types of beverages.[13,14,29,30] Formation of a caffeoylquinic acid−caffeine complex were also reported by Hamidi and Wanner,[31] who illustrated the complex might be formed during the extraction process.

The caffeoylquinic acid and protein complex also had a significant impact on yaupon holly tea cream formation and accounted for 55% of the total weight of yaupon holly tea cream. Such interactions have been previously reported when present in the same medium.[32,33] When BSA was added to yaupon holly infusion, 3-, 5-, and 4-caffeoylquinic acids were elevated in tea cream by up to 46.2, 29.2, and 50.9%, respectively, whereas the amounts of both caffeine (51.6%) and rutin (17.5%) were reduced compared to the control. When gallic acid was added to yaupon holly infusion, the concentrations of three caffeoylquinic acids were either not changed or reduced in tea cream. The 3-, 5-, and 4-caffeoylquinic acids decreased by 1.4, 55.2, and 62.4%, respectively, and other tea-creaming compounds such as caffeine and rutin were also reduced by 55.3 and 71.2%. This is because gallic acid might have bound to other tea-creaming compounds before caffeoylquinic acids did by taking their binding sites due to its superior creaming ability as observed in green tea creaming. By adding calcium chloride to yaupon holly infusion, tea cream formation increased by 7.0% and the increase mostly came from calcium−rutin or calcium−protein complex because no change was observed in caffeine and caffeoylquinic acids. Only rutin increased by 18% in tea cream among all phenolic compounds when calcium chloride was added to yaupon holly infusion. It was reported that caffeine does not bind to metals including Ca, Mg, Fe, Zn, Pb, Mn, Co, and Cr due to very low interaction between them,[34] whereas metal−protein binding was reported by several studies.[35,36]

Temperature Dependence of Tea Cream Formation. Tea creaming is associated with noncovalent bonds among tea-creaming agents from hydrogen bond and/or hydrophobic interaction that may vary with storage temperature. The RTD teas were incubated at three storage temperatures to determine the bulk effects on tea cream formation, simulating possible handling conditions with handling and storage.[37,38] Storage temperatures (4, 25, and 40 °C) did not have an effect on tea cream for either green or yaupon holly teas. The total weight of green tea cream was 536.8, 535.2, and 538.8 mg/L and 506.6, 508.7, and 511.8 mg/L for yaupon holly tea cream at 4, 25, and 40 °C, respectively. Due to lowered hydrogen bond strengths at higher temperatures,[39] the refrigerated conditions were hypothesized to induce greater tea cream formation, and tea creaming is generally reported following cold storage. However, compositional differences were noted in the tea creams, with significantly more tea catechins and caffeine detected in green tea cream formed at 4 °C but more rutin and protein observed in tea cream formed at 25 and 40 °C (Figure 4A). Likewise,

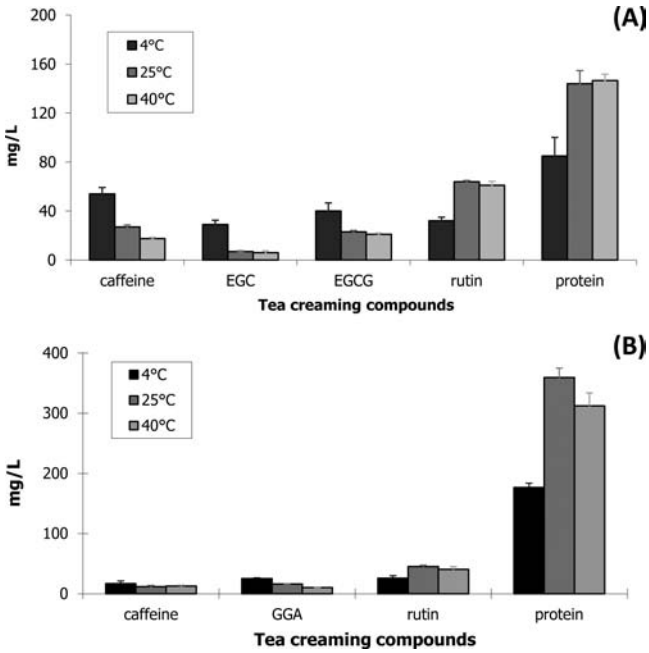

Figure 4. Concentrations of caffeine, epigallocatechin (EGC), epigallocatechin gallate (EGCG), chlorogenic acid (5-caffeoylquinic acid) (CGA), and quercetin-3-rutinoside (rutin) and total soluble protein in tea cream isolated from green tea (A) and yaupon holly (B) infusions as influenced by different storage temperatures (4, 25, and 40 °C) for 12 h. Bars represent standard error of the mean (n = 3).

yaupon holly cream composition was moderately different following variable-temperature storage, with higher amounts of caffeine and caffeoylquinic acids found at 4 °C and higher rutin and protein present after storage at 25 and 40 °C (Figure 4B). In general, results indicated that tea cream complexes at 4 °C were likely influenced by hydrogen bonding at the lower storage temperature, whereas rutin and protein complexes, common to both tea types, were more influenced by hydrophobic interactions at the higher storage temperatures. Also, this finding clearly differentiates tea cream from haze, which is highly temperature dependent.

Changes in Antioxidant Capacity with Tea Cream Formation. The antioxidant capacities of the original tea infusions and postcream tea infusion (clear supernatant) were determined by the ORAC assay to determine the impact of antioxidant losses due to tea cream formation. The ORAC value of the original green tea infusion was 61.3 μmol TE/mL, and it was reduced by 36.7% in postcream tea infusions; likewise, the yaupon holly original infusion was 77.2 μmol TE/mL and also declined by 12.8% in postcreaming due to the loss of antioxidant polyphenolics (Figure 5). Even though the

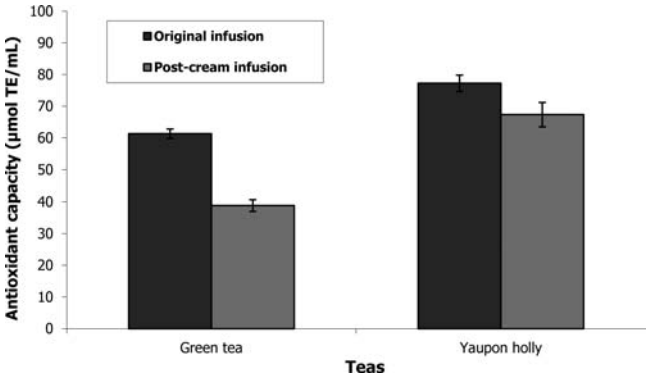

Figure 5. Antioxidant capacity (μmol TE/mL) of original infusion and postcream infusion (clear supernatant) of green tea (A) and yaupon holly (B) determined by ORAC assay. Bars represent standard error of the mean (n = 3). TE, Trolox equivalent.

amount of tea cream formed from green tea was only 5.6% more than cream formed in yaupon holly, a greater impact (56% more) on antioxidant loss was observed for green tea due to tea catechins having higher antioxidant properties compared to the predominance of caffeoylquinic acids found in yaupon holly.[40]

In summary, tea cream formation was observed in both nonfermented teas such as traditional green tea and nontraditional yaupon holly. Known black tea-creaming compounds including polyphenols, caffeine, protein, and metal compounds also played an important role in nonfermented tea creaming, and it was observed that polyphenols and proteins are major contributors to tea cream in both nonfermented teas. No difference was observed in tea cream weight even though tea cream compositions were quite different when teas were stored at various temperatures. The antioxidant capacity of tea infusions decreased when the formed tea cream was removed from the tea infusions due to the loss of the antioxidant polyphenolic compounds attached to tea cream. These findings provide not only a fundamental understanding of the tea-creaming mechanism in nonfermented teas but also important knowledge on antioxidant activity changes by tea cream formation.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; NTU, nephelometric turbidity units; PTFE, polytetrafluoroethylene; ORAC, oxygen radical absorbance capacity; ANOVA, analysis of variance

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