JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Stability of Tea Catechins in the Breadmaking Process

RONG WANG AND WEIBIAO ZHOU*

Food Science and Technology Program, Department of Chemistry, National University of Singapore, Science Drive 4, Singapore 117543

A green tea extract (GTE) was incorporated into bread as a source of tea catechins. The stability of tea catechins in the breadmaking process including unfrozen and frozen dough was studied. A method was developed for the separation and quantification of tea catechins in GTE, dough, and bread samples using a RP-HPLC system. The separation system consisted of a C18 reversed-phase column, a gradient elution system of water/methanol and formic acid, and a photodiode array UV detector. Tea catechins were detected at 275 nm. GTEs at 50, 100, and 150 mg per 100 g of flour were formulated. The results obtained showed that green tea catechins were relatively stable in dough during freezing and frozen storage at −20 °C for up to 9 weeks. There were no further detectable losses of tea catechins in bread during a storage of 4 days at room temperature. It was also revealed that (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin (EGC) were more susceptible to degradation than (−)-epicatechin gallate (ECG) and (−)-epicatechin (EC). (−)-EGCG and (−)-ECG were normally selected as the quality indices of green tea catechins, and their retention levels in freshly baked bread were ca. 83 and 91%, respectively. One piece of bread (53 g) containing 150 mg of GTE/100 g of flour will provide 28 mg of tea catechins, which is ∼35% of those infused from one green tea bag (2 g).

KEYWORDS: Tea catechins; tea polyphenols; green tea extract; epimerization; stability; bread; breadmaking; HPLC

INTRODUCTION

Tea antioxidants have drawn increased attention in recent years because of their potential health benefits, not only as an antioxidant agent but also as antiarteriosclerotic, anticarcinogenic, and antimicrobial agents. They may contribute to reducing risks of chronic diseases and cancer, promoting oral health, and prolonging shelf life of food products without damage to their organoleptic or nutritional qualities (*1*−*5*). These various biological properties are believed to be due to the functions of tea polyphenols. This group of polyphenols is called tea catechins, present in oolong tea, black tea, and green tea. Easily oxidized during the fermentation process with the aid of enzyme, tea catechins are mainly retained in green tea, which is a nonfermented product (*6*).

Commonly there are four main tea catechins in green tea leaves or green tea extract (GTE), identified as (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin (EC), and (−)-epicatechin gallate (ECG) (*6*). (−)-ECG and (−)-EGCG are the most abundant catechins (*7*, *8*), having similar gallate structures and efficient radical scavenging abilities (*9*). Thus, (−)-EGCG and (−)-ECG were accepted as markers of the quality of GTE (*10*). The chemical structures of tea catechins have a common backbone, with variations in the substituents at the C-3 and C-5′ positions (**Figure 1**).

As a rich source of tea catechins, green tea or green tea extract has nowadays been applied in a wide range of products, such as toiletries, cosmetics, foods, and beverages. Several studies have examined the stability of tea catechins in tea drinks under either direct brewing or industrial canning processes (*8*, *11*−*13*). It was found that the stability of tea catechins was pH-dependent. Under acid condition (pH <4) tea catechins were stable, whereas they were relatively unstable in neutral or alkaline condition (pH >6). The stability of tea catechins was also subject to heating temperature (*11*, *13*−*15*). A turning point temperature of 82 °C was found to vary the stability of tea catechins (*11*). On the other hand, in the breadmaking process, bread dough requires oxidants to aid the formation of disulfide bonds (SS), which form a major cross-linking structure in the gluten network so that a desired bread volume can be achieved. In such a case, tea catechins, as antioxidants, seem to be in conflict with this demand. As there has been no systematic report on the interaction between tea catechins and dough matrices, a study of tea catechins in the breadmaking process seems to be necessary and interesting. The aim of this work is to provide some insights on the effects of tea catechins in bread as well as their stability during bread production and storage.

MATERIALS AND METHODS

Materials. (−)-Epigallocatechin (EGC), caffeine, (−)-epigallocatechin gallate (EGCG), (−)-epicatechin (EC), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG), and (−)-catechin gallate (CG)

* Author to whom correspondence should be addressed [telephone (65) 6874 -3501; fax (65) 6775-7895; e-mail chmzwb@nus.edu.sg].

10.1021/jf048655x CCC: $27.50
Published on Web 12/08/2004

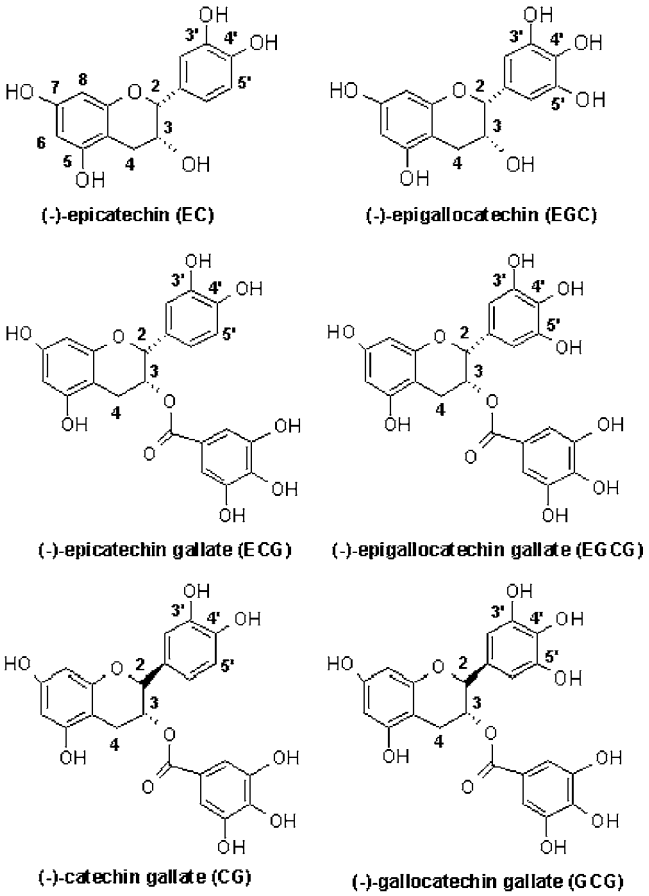

Figure 1. Chemical structures of green tea catechins: (–)-epigallocatechin (EGC); (–)-epigallocatechin gallate (EGCG); (–)-epicatechin (EC); (–)-gallocatechin gallate (GCG); (–)-epicatechin gallate (ECG); (–)-catechin gallate (CG).

were purchased from Sigma-Aldrich Chemical Co. HPLC grade methanol and *n*-hexane were purchased from Tedia Co. Inc. Ascorbic acid was purchased from Sino Chemical Inc. Formic acid was purchased from Merck. Bread flour (14% moisture) was obtained from Prima Ltd. (Singapore). Fine sugar was purchased from SIS (Singapore). Instant dry active yeast (*Saccharomyces cerevisiae*) was obtained from Algict Bruggeman N.V. Shortening and table salt (sodium chloride) were purchased from Phoon Huat & Co. Pte Ltd. (Singapore). Green tea extract was purchased from Pure Herbal Remedies Pte Ltd. (Singapore), which was made from green tea (*Camellia sinensis*) leaves harvested in Guangxi, China.

Preparation of Bread and Dough with Tea Catechins. Bread and dough were prepared using a no-time breadmaking process with slight modification (*16*), with ingredients as follows (1 kg, flour basis): flour, 100; water, 59; sugar, 4; shortening, 3; salt, 2; instant dry active yeast, 1; ascorbic acid, 0.01. GTE was added as a source of tea catechins at levels of 50, 100, and 150 mg per 100 g of flour. Ingredients were slowly mixed for 1 min followed by an intense mixing for 7 min, and then the dough was manually divided and rounded. After a resting period of 15 min, dough was separated into four groups. Group 1 proceeded to the unfrozen dough process with a proofing at 40 °C for 70 min under 95% relative humidity (RH) and a baking at 215 °C for 11 min. Groups 2−4 were taken for studying the effect and stability of tea catechins in the frozen-dough process. Blast freezing took place at −18 °C for 45 min, and then the dough pieces were stored at −20 °C for 1, 5, and 9 weeks. Upon the completion of a frozen storage time, dough was thawed at 2 °C for 16 h and then continued in the breadmaking process under the same condition as that for unfrozen dough. Crumb and dough were freeze-dried and vacuum-sealed in aluminum laminated bags and then stored at −20 °C until HPLC analysis. Type T thermocouples were employed for temperature monitoring during the baking process.

Preparation of GTE Solution. The GTE was specified by the manufacturer as having total catechins ≥55% (w/w, HPLC determination), polyphenol ≥90% (w/w, HPLC determination), and, as a quality marker of GTE, (−)-EGCG ≥22%. Approximately 10 mg of green tea extract was dissolved in 100 mL of a solution with HPLC grade water/formic acid (99.7/0.3, volume fraction). The solution was filtered at 0.45 μm prior to injection.

Preparation of Standard Solutions. HPLC grade pure standards of (−)-EGC, caffeine, (−)-EGCG, (−)-EC, (−)-GCG, (−)-ECG, and (−)-CG were freshly prepared in aqueous solution containing 70% methanol, 29.7% water, and 0.3% formic acid. Five concentrations of each standard solution were made at levels of 1, 5, 10, 20, and 50 ppm, respectively. Calibration curves were linear when forced through the origin, with the coefficients of determination (R^2) close to unity: 1.000 for (−)-EGC, caffeine, (−)-EGCG, and (−)-ECG; 0.9996 for (−)-EC; 0.9999 for (−)-GCG; and 0.9989 for (−)-CG.

Extraction of Tea Catechins from Bread Crumb and Dough. One gram of lyophilized and ground sample was accurately weighed and defatted in 30 mL of hexane at 70 °C for 20 min. The hexane fraction was decanted. The defatted sample was extracted in 40 mL of an aqueous solution with 70% methanol, 29.7% water, and 0.3% formic acid. The extraction was carried out in a water bath at 70 °C for 45 min with mechanical shaking. The aqueous layer was obtained by vacuum filtration, and its volume was made up with the same solvent to 50 mL. Filtration at 0.45 μm was done prior to HPLC analysis.

Recovery Rate. The recovery rate of the analytical method was assessed by comparing the catechin content in blank samples with those spiked with a known amount of GTE. Approximately 3−5 mg of GTE was added to 1 g of bread crumb or dough sample. The extraction procedure was the same as previously described. The solution was filtered at 0.45 μm prior to injection.

HPLC Analysis. Green tea catechins were assessed by the HPLC system, which consisted of a HPLC-PDA with UV detector (Waters 2695/2696) and a C18 reversed-phase column (250 × 4.6 mm/5 μm, Waters). It was equipped with an autoinjector. As sample extracts could remain in the autoinjector for up to 24 h during a routine analysis, the storage temperature was set at 4 °C. Mobile phases consisted of 0.1% formic acid in water (eluent A) and 0.1% formic acid in methanol (eluent B). A gradient system was adopted as follows: 0−10 min, 10% B; 10−28 min, linear gradient from 10 to 30% B; 28−35 min, linear gradient from 30 to 45% B; 35−45 min, linear gradient from 45 to 60% B; 45−50 min, 60% B; 50−55 min, linear gradient from 60 to 10% B. Postrun time was 5 min. Sample injection volume was 20 μL. The flow rate was 0.5 mL/min. Column temperature was set at 23 ± 2 °C. Tea catechins and caffeine were detected at 275 nm. Identification of each catechin in GTE was made by comparing the retention time and spectrum with those of the standards of (−)-EGC, caffeine, (−)-EGCG, (−)-EC, (−)-GCG, (−)-ECG, and (−)-CG. To confirm each tea catechin, an internal standard analysis was applied for peak location. In brief, GTE solution was mixed proportionally with a known concentration of individual authentic standard, respectively. To determine the catechin content in bread crumb and dough, the eluting peaks of the two most abundant catechins (−)-EGCG and (−)-ECG were identified and compared as the indices of the stability of tea catechins in the breadmaking process.

Experimental Design and Data Analysis. All baking tests and chemical determinations were made in at least triplicate, and the results reported are the means of those determinations with the corresponding standard deviations. Analysis of variances and pairwise comparisons were examined by ANOVA single-factor test at the $P < 0.05$ confidence level. Evaluation of the relationships among variables was carried out by computing the relevant correlation coefficients at the $P < 0.05$ confidence level.

RESULTS

Tea Catechin Content in GTE. The HPLC analysis showed that the green tea extract used in this study contained ∼73% green tea catechins (**Table 1**). Of the seven green tea compounds verified, (−)-EGCG, (−)-ECG, (−)-EGC, and (−)-EC were the four main tea catechins, making up ∼62% of GTE. (−)-EGCG

Table 1. Tea Polyphenols in Green Tea Extract (GTE)[a]

green tea polyphenols	% in GTE
(–)-epigallocatechin gallate (EGCG)	24.1 ± 0.4
(–)-epicatechin gallate (ECG)	18.4 ± 0.8
(–)-epigallocatechin (EGC)	12.7 ± 0.7
(–)-epicatechin (EC)	7.1 ± 0.2
(–)-gallocatechin gallate (GCG)	5.9 ± 0.2
(–)-catechin gallate (CG)	2.7 ± 0.2
caffeine	2.2 ± 0.1
total tea catechins	73.0 ± 0.4

[a] Data are expressed as mean ± standard deviation of eight samples.

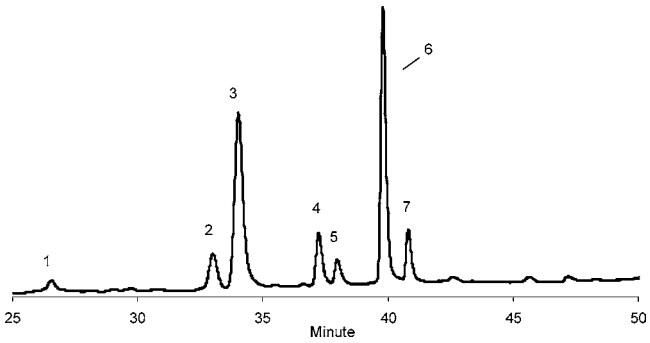

Figure 2. HPLC profile of tea catechins in green tea extract: 1, (–)-EGC; 2, caffeine; 3, (–)-EGCG; 4, (–)-EC; 5, (–)-GCG; 6, (–)-ECG; 7, (–)-CG.

Table 2. Absolute Retention Rate of (–)-EGCG and (–)-ECG in Bread Crumb[a]

extraction time	(–)-EGCG, %	(–)-ECG, %
30 min[b]	79.6 ± 0.1	79.5 ± 0.1
45 min[b]	83.0 ± 0.1	83.0 ± 0.3
60 min[b]	80.6 ± 0.1	81.8 ± 0.2
90 min[b]	80.5 ± 0.1	82.2 ± 0.2
5 h[c]	77.3 ± 1.0	80.1 ± 1.2
15 h[c]	66.2 ± 8.5	72.1 ± 9.7

[a] Data are expressed as mean ± standard deviation of triplicate samples. [b] Extraction was carried out at 70 °C. [c] Extraction was carried out at room temperature (22–23 °C).

(24%) was the most abundant component in the GTE, which was in agreement with the study in ref *10*. In addition, (−)-GCG and (−)-CG made up ∼8.6% of GTE. These results of the total tea catechins and (−)-EGCG are comparable with the specifications provided by the producer. A chromatogram of tea catechins is shown in **Figure 2**.

Optimum Extraction Temperature and Time for Bread Crumb and Dough. It was reported that the tea catechins were relatively stable at 120 °C for 20 min in a medium with pH <4 (*8*). In addition, a turning point temperature of 82 °C was reported to alter the stability of tea catechins (*10*). Thus, an extraction temperature of 70 °C was carefully chosen. Experiments carried out at 70 °C for 45 min obtained the highest extraction rates for (−)-EGCG and (−)-ECG (**Table 2**).

Recovery Rate. The recovery rate varied from 92 to 94% for (−)-EGCG and (−)-ECG (**Table 3**). There was no significant difference between tea catechins in dough and in bread crumb. The average recovery rate of tea catechin was ∼93%.

Retention of Tea Catechins in the Breadmaking Process. There was no significant difference ($P < 0.05$) in the amount of tea catechins detected between unfrozen and frozen dough with all three levels of GTE (**Table 4**). Averages of 94% (−)-EGCG and 94.7% (−)-ECG were detected in both frozen and unfrozen dough. In other words, tea catechins are relatively stable during the freezing and frozen period for up to 9 weeks. There was a ∼6% loss of tea catechins compared to the original dose of GTE in dough. However, this might be due to the analysis method because the recovery rate by this analytical procedure was ∼93%.

Table 3. Recovery Rates of (–)-EGCG and (–)-ECG in Bread Crumb and Dough[a]

	(–)-EGCG	(–)-ECG
crumb	91.7 ± 2.4	93.4 ± 3.8
dough	94.1 ± 4.9	94.0 ± 4.8

[a] Data are expressed as mean ± standard deviation of six samples.

Table 4. Retention Rate (Percent) of Tea Catechins in Unfrozen and Frozen Dough[a]

	unfrozen	frozen for 1 week	frozen for 5 weeks	frozen for 9 weeks
(–)-EGCG	94.3 ± 2.4	92.9 ± 1.7	94.4 ± 5.9	94.5 ± 1.1
(–)-ECG	95.8 ± 3.3	95.3 ± 2.4	95.2 ± 4.7	93.2 ± 2.2

[a] Data are expressed as mean ± standard deviation of 12 samples.

Table 5. Relative Retention Rate of Tea Catechins and Caffeine in Bread[a]

component	bread with 50 mg of GTE/100 g of flour, %	bread with 100 mg of GTE/100 g of flour, %	bread with 150 mg of GTE/100 g of flour, %
(–)-EGCG	80.6 ± 3.0	86.2 ± 4.9	82.6 ± 2.5
(–)-ECG	93.3 ± 2.9	90.9 ± 4.1	90.1 ± 4.4
(–)-EGC	66.8 ± 7.1	67.8 ± 5.2	62.8 ± 7.3
(–)-EC	93.6 ± 3.4	95.9 ± 2.4	97.1 ± 3.9
(–)-GCG	83.2 ± 5.4	84.8 ± 4.2	85.9 ± 4.0
(–)-CG	94.3 ± 2.8	94.9 ± 3.1	99.6 ± 4.3
caffeine	95.0 ± 4.6	95.7 ± 5.1	96.5 ± 5.5
total catechins	83.7 ± 3.8	85.8 ± 4.2	83.7± 3.2

[a] Data are expressed as mean ± standard deviation of 12 samples.

Apart from the 94% of the two tea catechins obtained in dough, average retentions of 83% (−)-EGCG and 91% (−)-ECG were determined in freshly baked bread (**Table 5**). There was no further detectable loss of these two catechins in bread during storage of 4 days at room temperature. Results in **Figure 3** show that the loss of (−)-EGCG and (−)-ECG in bread increased proportionally with an increased level of GTE in dough. A highly linear relationship was found between the loss and addition level of GTE in bread dough. The percentage of loss from a nonproofed dough to a baked bread ranged from 14 to 20% for (−)-EGCG and from 10 to 14% for (−)-ECG. In other words, the stability of (−)-EGCG was lower than that of (−)-ECG in the breadmaking process. Furthermore, it can be seen from **Table 5** that the stabilities of (−)-EC, caffeine, and (−)-CG, having relatively higher retention rates (>93%) in all breads with three levels of GTE, are superior to those of the other catechins, that is, (−)-ECG, (−)-EGCG, (−)-GCG, and (−)-EGC.

For the bread produced in this study, the amount of tea catechins provided in one piece of bread (53 g) ranged from 13 to 35% of those provided by the infusion (150 mL) of a typical commercial 2 g tea bag (**Table 6**). The calculation was based on the results in ref *17* on green tea infusion, which gave 78.2 mg/100 mL total catechins from the infusion of 3 g of tea leaves. The infusion was prepared by having the tea leaves in 150 mL of boiling distilled water for 5 min.

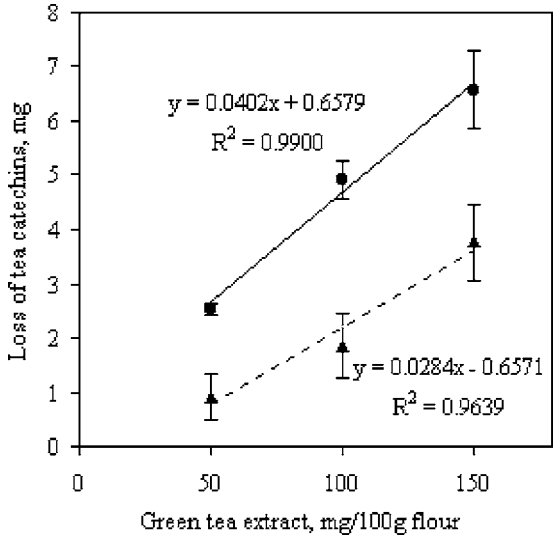

Figure 3. Losses of tea catechins in bread: ▲, (–)-EGCG; ●, (–)-ECG; number of replications, $n = 12$.

Table 6. Total Tea Catechins in Final Bread (53 g)[a]

	bread with 50 mg of GTE/ 100 g of flour	bread with 100 mg of GTE/ 100 g of flour	bread with 150 mg of GTE/ 100 g of flour
amount, mg	10.2 ± 0.5	20.9 ± 1.0	27.6 ± 1.1
percentage[b]	13.0 ± 0.6	26.8 ± 1.3	35.3 ± 1.4

[a] Data are expressed as mean ± standard deviation of 12 samples. [b] Percentage of the total catechins infused from 2 g tea bag.

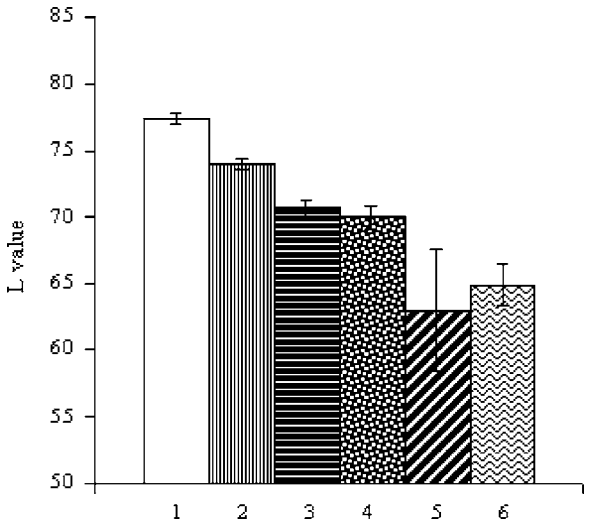

Figure 4. Color comparison between GTE bread and market products: 1, control bread; 2, bread with 50 mg of GTE/100 g of flour; 3, bread with 100 mg of GTE/100 g of flour; 4, bread with 150 mg of GTE/100 g of flour; 5, market whole meal bread; 6, market soft meal bread (fiber enriched).

Color of the GTE Bread. As a rich source of tea catechins, the GTE used is in fine powder form with brown color, which was expected to cause a color change in bread. A color comparison between the GTE bread and two types of commercial bread is shown in **Figure 4**. The *L* value of all GTE bread was higher than that of the market products, although it was slightly grayish when compared to the control sample. It is worth noting that the lighter the color, the higher the *L* value. Therefore, no acceptability problem would be expected with bread containing GTE up to a level of 150 mg/100 g of flour. The effect of GTE on various other quality attributes of bread including specific volume, dough rheology, and bread texture will be reported separately.

DISCUSSION

The HPLC analysis revealed that tea catechins (−)-EC, (−)-ECG, and (−)-CG were relatively more stable than (−)-EGCG, (−)-EGC, and (−)-GCG during the breadmaking process. As shown in the previous section, ∼16% of total tea catechins were lost during the breadmaking process. Averages of 17% (−)-EGCG and 34% (−)-EGC were lost in bread when compared with their corresponding amount in dough. In contrast, averages of 9% of (−)-ECG and 4% of (−)-EC were lost in bread. The relative stability of tea catechins and caffeine in bread can be ranked as caffeine, (−)-CG, (−)-EC > (−)-ECG > (−)-GCG, (−)-EGCG > (−)-EGC.

The losses could be due to the combined effect of oxidation, isomerization/epimerization, and degradation of tea catechins during the various breadmaking stages including mixing, thawing, proofing, and baking. However, it is well-known that yeast immediately assimilates oxygen during the mixing process; in other words, the oxidation of tea catechins induced by active oxygen must have a very minor opportunity to take place. In contrast, the isomerization, for example, epimerization reaction between the tea catechins and their isomers would be relatively easier to conduct under the stated processing conditions.

Many researchers have found that tea catechins could convert to their corresponding epimers in traditionally brewed tea infusion and canned tea drinks during brewing, production, and storage (*8*, *11*−*15*, *18*). The epimerization of tea catechins depends strongly on pH and temperature. The higher the pH (>5) and the temperature, the less stable the tea catechins. It was reported in ref *14* that tea catechins could decrease in a filler bowl (95 °C) during the processing of canned green tea drink. In ref *11* it was stated that the critical heating temperature for the epimerization of tea catechins was ∼82 °C. These findings were supported by the study in ref *13*. In the same study, the formation of (−)-gallocatechin gallate (GCG), which is the epimer of (−)-EGCG, was found to reach a maximum level (∼2.7 times its original concentration) after 20 min of heating when the green tea infusion was heated at 100 °C. Meanwhile, nearly 90% of (−)-EGCG decreased and the concentration of (−)-EGCG reduced from the initial 13.2 to 1.34 mg/100 mL at the end. Moreover, (−)-EGCG had its maximum level of epimerization when the pH was 5 (*15*); and the epimerization can be accelerated at pH 5 during a sterilization process (120 °C/20 min) (*8*). In the present study, the operation parameters coincided partially with those repeated epimerization conditions. It appears likely for green tea catechins to carry out a similar epimerization in the breadmaking process. First, bread dough was baked at 215 °C for 11 min. The actual core temperature of the dough remained between 80 and 101 °C for 8−9 min (**Figure 5**), which could provide sufficient energy for catechin epimerization. Second, the pH of bread dough before and after proofing ranged from 5 to 6, in which tea catechins were able to produce their epimers. Finally, the concentration of (−)-EGCG in dough ranged from 20 to 61 mg/100 mL of water, which was higher than that in the study in ref *13*. Thus, the epimerization of individual tea catechins could not be ruled out, although the amount of epimers, for example, (−)-GCG and (−)-CG, did not increase as (−)-EGCG and (−)-ECG decreased, which they should in a typical epimerization process.

However, the sum of (−)-EGCG and (−)-GCG reduced in bread when compared with that in the corresponding dough.

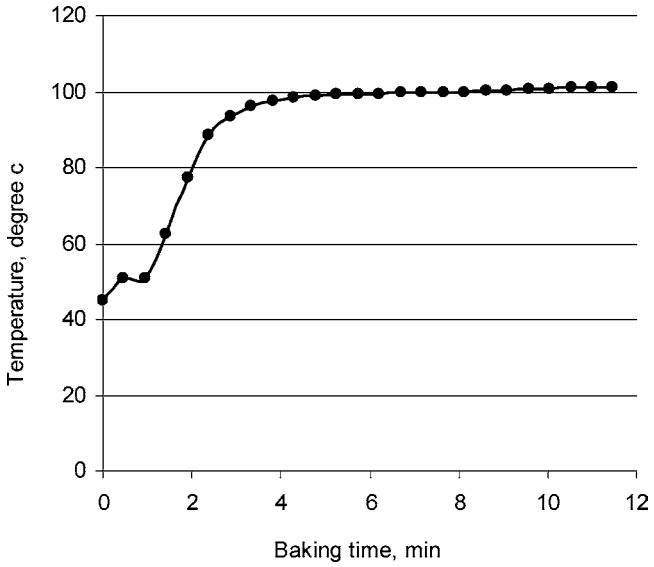

Figure 5. Core temperature profile of bread dough (60 g) baked at 215 °C for 11 min.

The same trend was also obtained for the sum of (−)-ECG and (−)-CG. Thus, the detected loss of tea catechins must be due to some other reactions during processing.

Besides the unknown possibility of a thermal degradation of tea catechins during the breadmaking process, there are possible interactions between tea catechins and wheat proteins during dough preparation, during which the gluten network was developed into a cross-linking structure by disulfide bonds (SS) through the interchange reaction of thiol group SH to SS. It has been pointed out that there were free radicals GS• initiated by the broken SS bonds in dough during mixing, and the free radicals were rapidly scavenged in the first hour by the added antioxidants such as BHA and BHT (*19*, *20*). Therefore, BHA and BHT participated in the SH−SS interchange reaction and increased its rate. Green tea catechins are a group of polyphenol compounds as well as scavengers of free radicals. They might have a capability similar to that of BHA and BHT in the dough matrix. On the other hand, as reported in ref *12*, green tea catechins can easily initiate their correspondent semiquinone free radicals at neutral or alkaline pH, and (−)-EGCG is more pliable to the formation of a semiquinone free radical than (−)-ECG. Thus, with the combined effect of being free radical scavengers and initiators, tea catechins could interact with GS• and be involved in the SH−SS interchange reaction. This postulation may be supported by the study in ref *21*, which found that the quantity of SH in dough increased significantly with the addition of GTE, especially with a higher content (59%) of (−)-EGCG. Rheological results obtained in the same study showed that the resistance-to-extension ratio of the dough was greatly decreased by the addition of GTE. It seemed that tea catechins reacted with wheat protein by scavenging free radicals GS• that resulted in changes in dough rheology. However, whether tea catechins interact with wheat proteins and by what mechanisms they react remain unclear.

In the past decade, the free radical scavenging ability of tea catechins has been studied by many researchers. It was reported that the three adjacent hydroxyl (OH) groups at position C-3′, -4′, and -5′ in (−)-EGCG, (−)-GCG, and (−)-EGC (**Figure 1**) were more effective on scavenging free radicals than the two adjacent −OH groups at C-3′ and -4′ in (−)-ECG, (−)-EC, and (−)-CG (*7*, *9*, *18*, *22*). This may explain the similar result obtained in our present study that there was a lower retention rate of (−)-EGC, (−)-EGCG, and (−)-GCG than of (−)-ECG, (−)-EC, and (−)-CG. Moreover, with the additional gallate group at C-3, catechins with a gallate moiety should have stronger scavenging effects than non-gallate catechins (*9*), that is, (−)-ECG > (−)-EC and (−)-EGCG > (−)-EGC, but this is not in agreement with our findings as (−)-EGC was lost more than (−)-EGCG in the present study. However, there have been inconsistent reports in the literature on the order of scavenging ability of tea catechins. In the aqueous phase mediated by DPPH• radicals, their order was reported as (−)-EGCG = (−)-ECG > (−)-EGC > (−)-EC (*9*), whereas in the aqueous phase induced by ABTS• radicals, a similar order was found: (−)-ECG > (−)-EGCG > (−)-EGC > (−)-EC (*22*). In the lipophilic phase initiated by lipid peroxyl radicals, (−)-ECG = (−)-EGCG = (−)-EC > (−)-GC (*22*), whereas in canola oil (−)-EGC > (−)-EGCG > (−)-EC > (−)-ECG (*7*). On the inhibitory effect of Cu^{2+}-mediated oxidation of low-density lipoprotein (LDL), (−)-EGCG > (−)-ECG > (−)-EC > (−)-EGC (*23*). On the basis of the redox potentials of tea catechins, the order of their antioxidant activity was reported as (−)-EGC > (−)-EGCG > (−)-GCG > (−)-EC > (−)-ECG (*24*). It is clear that the order of tea catechins on the scavenging capacity varies either in aqueous systems or in lipid systems. This may be due to the fact that the scavenging effects of antioxidants could be variable with radical species, polarity, ionization state, and enzyme inhibition (*9*).

Although the stability results in the present study are closer to the order reported in ref *24*, they are not fully consistent with any of the above model systems. Bread dough is a complex matrix containing wheat protein, starch, water, fat, etc. It is also a mixture of two phases, that is, aqueous phase and lipid phase. Hence, the stability of tea catechins may not be simply explained by models of either aqueous or lipid system. With the possibility of combined thermal degradation, epimerization, and radical scavenging effects as well as interaction with protein via hydrogen bonding, the mechanism behind the stability order of tea catechins in the breadmaking process needs to be further investigated.

In conclusion, the tea catechins examined in this study showed their varying stabilities. The relative stability of tea catechins and caffeine after baking under the studied condition is sequenced as caffeine, (−)-CG, (−)-EC > (−)-ECG > (−)-GCG, (−)-EGCG > (−)-EGC. When the GTE was added to dough, it was found that ∼84% of the total green tea catechins were retained in bread after baking and during its shelf life. There was no significant difference in the retention of tea catechins ($P < 0.5$) between unfrozen and frozen dough for up to 9 weeks at −20 °C. There was no acceptability problem in terms of color with bread containing GTE up to a level of 150 mg/100 g of flour.

ACKNOWLEDGMENT

Technical assistance from Guanghou Shui and Yeting Liu is acknowledged.

LITERATURE CITED

(1) Wang, H.; Provan, G. J.; Helliwell, K. Tea Flavonoids: Their Functions, Utilisation and Analysis. *Trends Food Sci. Technol.* **2000**, *11*, 152−160.

(2) Yang, C. S.; Chung, J. Y.; Yang, G. Y.; Chhabra, S. K.; Lee, M. J. Tea and Tea Polyphenols in Cancer Prevention. *J. Nutr.* **2000**, *130*, 472−478.

(3) Yang, C. S.; Landau, J. M. Effects of Tea Consumption on Nutrition and Health. *J. Nutr.* **2000**, *130*, 2409−2412.

(4) McKay, D. L.; Blumberg, J. B. The Role of Tea in Human Health: An Update. *J. Am. Coll. Nutr*. **2002**, *21* (1), 1−13.
(5) Rietveld, A.; Wiseman, S. Antioxidant Effects of Tea: Evidence from Human Clinical Trials. *J. Nutr*. **2003**, *133*, 3285−3292.
(6) Zhang, A.; Chan, P. T.; Luk, Y. S.; Ho, W. K. K.; Chen, Z. Y. Inhibitory Effect of Jasmine Green Tea Epicatechin Isomers on LDL-Oxidation. *J. Nutr. Biochem*. **1997**, *8*, 334−340.
(7) Chen, Z. Y.; Chan, P. T. Antioxidative Activity of Green Tea Catechins in Canola Oil. *Chem. Phys. Lipids* **1996**, *82*, 163−172.
(8) Chen, Z. Y.; Zhu, Q. Y.; Tsang, D.; Huang, Y. Degradation of Green Tea Catechins in Tea Drinks. *J. Agric. Food Chem*. **2001**, *49*, 477−482.
(9) Nanjo, F.; Goto, K.; Seto, R.; Suzuki, E.; Sakai, M.; Hara, Y. Scavenging Effects of Tea Catechins and Their Derivatives on 1,1-Diphenyl-2-Picrylhydrazyl Radical. *Free Radical Biol. Med.* **1996**, *21*, 895−902.
(10) Pelillo, M.; Biguzzi, B.; Bendini, A.; Gallina-Toschi, T.; Vanzini, M.; Lercker G. Preliminary Investigation into Development of HPLC with UV and MS-Electrospray Detection for The Analysis of Tea Catechins. *Food Chem.* **2002**, *78*, 369−374.
(11) Komatsu, Y.; Suematsu, S.; Hisanobu, Y.; Saigo, H.; Matsuda, R.; Hara, K. Studies on Preservation of Constituents in Canned Drinks. Part II. Effects of pH and Temperature on Reaction Kinetics of Catechins in Green Tea Infusion. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 907−910.
(12) Zhu, Q. Y.; Zhang, A.; Tsang, D.; Huang, Y.; Chen, Z. Y. Stability of Green Tea Catechins. *J. Agric. Food Chem.* **1997**, *45*, 4624−4628.
(13) Wang, H.; Helliwell, K. Epimerisation of Catechins in Green Tea Infusions. *Food Chem.* **2000**, *70*, 337−344.
(14) Suematsu, S.; Hisanobu, Y.; Saigo, H.; Matsuda, R.; Hara, K.; Komatsu, Y. Studies on Preservation of Constituents in Canned Drinks. III. Changes of Some constituents during Processing of Canned Tea Drinks. *Nippon Shokuhin Kogo Gakkaishi* **1993**, *40*, 181−186.
(15) Seto, R.; Nakamura, H.; Nanjo, F.; Hara, Y. Preparation of Empimers of Tea Catechins by Heat Treatment. *Biosci., Biotechnol., Biochem*. **1997**, *61*, 1434−1439.
(16) Gelinas, P.; Lagimoniere, M.; Dubord, C. Baker's Yeast Sampling and Frozen Dough Stability. *Cereal Chem*. **1993**, *70*, 219−225.
(17) Wang, H.; Provan, G. J.; Helliwell, K. HPLC Determination of Catechins in Tea Leaves and Tea Extracts Using Relative Response Factors. *Food Chem.* **2003**, *81*, 307−312.
(18) Su, Y. L.; Leung, L. K.; Huang, Y.; Chen, Z. Y. Stability of Tea Theaflavins and Catechins. *Food Chem.* **2003**, *83*, 189−195.
(19) Hoseney, R. C. Physical Chemistry of Bread Dough. In *Physical Chemistry of Foods*; Schwartzberg, H. G., Hartel, R. W., Eds.; Dekker: New York, 1992; pp 443−456.
(20) Dong, W.; Hoseney, R. C. Effect of Certain Breadmaking Oxidants and Reducing Agents on Dough Rheological Properties. *Cereal Chem*. **1995**, *72*, 58−64.
(21) Achiwa, Y.; Furuichi, Y.; Komiya, T. The Improving Effects of Tea Catechin on Rheological Properties of Wheat Flour Dough. *Bull. Fac. Bioresour., Mie Univ.* **2001**, 25−26, 31−35.
(22) Salah, N.; Miller, N. J.; Paganga, G.; Tijburg, L. Polyphenolic Flavanols as Scavengers of Aqueous Phase Radicals and as Chain-Breaking Antioxidants. *Arch. Biochem. Biophys.* **1995**, *322*, 339−346.
(23) Miura, Y.; Chiba, T.; Tomita, I.; Koizumi, H.; Miura, S.; Umegaki, K.; Hara, Y.; Ikeda, M.; Tomita, T. Tea Catechins Prevent the Development of Atherosclerosis in Apoprotein E-Deficient Mice. *J. Nutr*. **2001**, *131*, 27−32.
(24) Balentine, D. A.; Wiseman, S. A.; Bouwens, C. M. The Chemistry of Tea Flavonoids. *Crit. Rev. Food Sci. Nutr*. **1997**, *37*, 693−704.

Received for review August 10, 2004. Revised manuscript received October 27, 2004. Accepted October 27, 2004.

JF048655X