Degradation of Green Tea Catechins in Tea Drinks

Zhen-Yu Chen,*,† Qin Yan Zhu,† David Tsang,† and Yu Huang‡

Departments of Biochemistry and Physiology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, The People's Republic of China

Green tea cateachins (GTC). namely (−) epicatechin (EC), (−) epicatechin gallate (ECG), (−) epigallocatechin (EGC), and (−) epigallocatechin gallate (EGCG), have been studied extensively for their wide-ranging biological activities. The goal of the present study was to examine the stability of GTC as a mixture under various processing conditions. The stability study demonstrated that GTC was stable in water at room temperature. When it was brewed at 98 °C for 7 h, longjing GTC degraded by 20%. When longjing GTC and pure EGCG were autoclaved at 120 °C for 20 min, the epimerization of EGCG to (−) gallocatechin gallate (GCG) was observed. The relatively high amount of GCG found in some tea drinks was most likely the epimerization product of EGCG during autoclaving. If other ingredients were absent, the GTC in aqueous solutions was pH-sensitive: the lower the pH, the more stable the GTC during storage. When it was added into commercially available soft drinks or sucrose solutions containing citric acid and ascorbic acid, longjing GTC exhibited varying stability irrespective of low pH value. This suggested that other ingredients used in production of tea drinks might interact with GTC and affect its stability. When canned and bottled tea drinks are produced, stored, and transported, the degradation of GTC must be taken into consideration.

Keywords: *Catechin; epigallocatechin gallate (EGCG); gallocatechin gallate (GCG); stability; tea drinks*

INTRODUCTION

Green tea catechin (GTC) derivatives, namely (−) epicatechin (EC), (−) epigallocatechin (EGC), (−) epicatechin gallate (ECG), and (−) epigallocatechin gallate (EGCG), have recently received much attention as protective agents against cardiovascular disease and cancer (*1*−*5*). GTC is also believed to have a wide range of other pharmaceutical activities including being antihypertensive (*6*), antioxidative (*7*−*10*), and hypolipidemic (*11*−*13*).

Canned and bottled tea drinks are getting popular worldwide (*14*). Although total catechins in green tea, oolong tea, and black tea have been reported, information on the catechin composition of canned and bottled tea drinks is limited (*15* and *16*). We have analyzed fourteen brands of canned and bottled tea drinks and eleven brands of green, black, and oolong teas which are mostly consumed in Hong Kong. The green teas analyzed had total catechins ranging from 8 to 15 g/100 g dry leaves, whereas canned tea drinks contained only 0.3−35 mg total catechins per 100 mL. It was found that (−) gallocatechin gallate (GCG) only accounted for less than 1.5% of total catechins in green tea. But GCG could reach as much as 50% of total catechins in some tea drinks. We speculate that the relatively high GCG level in canned and bottled tea drinks is derived from thermal conversion of EGCG. It is particularly important to protect GTC from degradation before it is consumed. There has been no study to date examining the long-term stability of GTC in canned tea drinks during their manufacture, storage, and transport. The goal of the present study was, therefore, to examine (i) conversion of EGCG to GCG during the sterilization (autoclave) step in production of canned and bottled tea drinks; (ii) thermal stability of GTC as a mixture under various processing conditions; and (iii) long-term stability of GTC in drinks during their storage.

MATERIALS AND METHODS

HPLC Analysis of Tea GTC Extracts. The method described by Agarwal et al. (*17*) and modified by us (*10*) was used to extract total GTC from eleven brands of dry tea leaves available in the Hong Kong market. The individual catechin derivatives in tea extracts were quantified using a Shimadzu LC-10AD HPLC (Tokyo, Japan) equipped with a ternary pump delivery system as described previously (*18*). In brief, tea extracts (10 μL, 0.5 mg/mL) were injected onto the column (Hypersil ODS, 250 × 4.6 mm, 5 m, Alltech, Deerfield, IL) via a rheodyne valve (20-L capacity, Shimadzu, Tokyo, Japan). An eluting mixture of H_2O containing 0.05% H_2SO_4, acetonitrile, and ethyl acetate (86:12:2, vol/vol/vol) was used at a flow rate of 1 mL/min. The individual catechin derivatives were monitored using a UV detector at 280 nm (UVIS-205, Alltech, Deerfield, IL), quantified and calibrated using (+) catechin as an internal standard. Identification of each catechin derivative was confirmed by comparison of retention time and co-chromatography with authentic standards of EC, EGC, ECG, EGCG, and GCG, which were obtained from Kurita Industrial Co., Ltd, Tokyo, Japan.

Catechin Composition of Canned or Bottled Tea Drinks. Fourteen brands of canned or bottled tea drinks were purchased from several local supermarkets in Hong Kong. After the drinks were shaken vigorously, 10-ml aliquots of each were mixed with 0.1 mL of (+)-catechin internal standard solution (3 mg/mL). The mixture was then extracted twice with an equal volume of ethyl acetate. The ethyl acetate phase was saved and subjected to HPLC analysis.

* To whom correspondence should be addressed. Telephone: (852) 2609-6382. Fax: (852) 2603-5123. E-mail: zhenyuchen@cuhk.edu.hk.

† Department of Biochemistry.

‡ Department of Physiology.

10.1021/jf000877h CCC: $20.00
Published on Web 12/20/2000

Table 1. Catechin Composition of Some Green, Oolong, and Black Teas (g/100 g dry tea leaves)[a]

	EGC[b]	EC	EGCG	ECG	GCG	total[c]
			green tea			
longjing	0.59 ± 0.02 (6.03%)[d]	0.53 ± 0.05 (5.42%)	6.47 ± 0.31 (66.15%)	2.05 ± 0.12 (21.00%)	0.14 ± 0.03(1.43%)	9.78 ± 0.58
bi-luo-chun	1.86 ± 0.12 (17.40%)	0.32 ± 0.04 (3.00%)	7.10 ± 0.56 (66.42%)	1.32 ± 0.12 (12.35%)	0.09 ± 0.02 (0.84%)	10.69 ± 0.67
yun-nan-lu-cha	0.16 ± 0.02 (1.26%)	1.41 ± 0.11 (11.11%)	4.70 ± 0.32 (37.04%)	6.30 ± 0.43 (49.65%)	0.12 ± 0.04 (0.95%)	12.69 ± 1.12
xin-yang-mao-jian	0.36 ± 0.05 (2.49%)	0.79 ± 0.10 (5.47%)	10.44 ± 0.71 (72.30%)	2.67 ± 0.20 (18.49%)	0.18 ± 0.04 (1.25%)	14.44 ± 0.92
lu-zhu-cha	0.35 ± 0.10 (4.38%)	0.56 ± 0.14 (7.00%)	5.66 ± 0.31 (70.75%)	1.34 ± 0.12 (16.75)	0.09 ± 0.02 (1.13%)	8.00 ± 0.54
			oolong tea			
wu-yi-wu-long	0.21 ± 0.02 (4.54%)	0.34 ± 0.05 (7.34%)	3.26 ± 0.20 (70.41%)	0.71 ± 0.07 (15.33%)	0.11 ± 0.02 (2.38%)	4.63 ± 0.36
shui-xian	0.16 ± 0.01 (3.86%)	0.37 ± 0.3 (8.94%)	2.80 ± 0.23 (67.63%)	0.73 ± 0.06 (17.63%)	0.08 ± 0.03 (1.93%)	4.14 ± 0.37
guan-yi	0.25 ± 0.02 (5.08%)	0.37 ± 0.03 (7.52%)	3.49 ± 0.32 (70.93%)	0.72 ± 0.10(14.63%)	0.09 ± 0.04 (1.83%)	4.92 ± 0.32
			black tea			
fu-zhou-hong-cha	trace	trace	0.13 ± 0.01 (54.17%)	0.11 ± 0.01 (45.83%)	trace	0.24 ± 0.01
li-zhi-hong-cha	trace	0.05 ± 0.00(13.16%)	0.16 ± 0.02 (42.11%)	0.17 ± 0.02 (44.74%)	trace	0.38 ± 0.10
liu-an-gu	0.01 ± 0.00 (1.96%)	0.11 ± 0.01 (21.56%)	0.31 ± 0.03 (60.78%)	0.08 ± 0.01 (15.69%)	trace	0.51 ± 0.11

[a] Data are expressed as mean ± SD of $n = 6$. [b] EGC, epigallocatechin; EC, epicatechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate; GCG, gallocatechin gallate. [c] Total = sum of EGC, EC, EGCG, ECG, and GCG. [d] Percentage of total catechins. Trace < 0.01 mg/100 g dry tea leaves

Table 2. Composition of Green Tea Catechins in Canned or Bottled Tea Drinks (mg/100 mL)[a]

tea	EGC[b]	EC	EGCG	ECG	GCG	total[c]
Hakuyou oolong	0.25 ± 0.02 (0.77%)[d]	0.17 ± 0.1 (0.53%)	12.97 ± 0.92 (40.15%)	3.05 ± 0.21 (9.44%)	15.86 ± 0.23 (49.10%)	32.30 ± 1.28
Japanese jenmai	0.65 ± 0.10 (2.32%)	0.62 ± 0.03 (2.11%)	10.95 ± 0.81 (39.11%)	3.11 ± 0.11 (11.11%)	12.67 ± 1.34 (45.25%)	28.00 ± 0.97
Japanese	0.78 ± 0.13 (2.18%)	0.42 ± 0.02 (1.18%)	13.03 ± 1.16 (36.48%)	3.63 ± 0.32 (10.16%)	17.86 ± 0.73 (50.00%)	34.17 ± 2.35
Vita-lemon	0.19 ± 0.03 (0.88%)	4.75 ± 0.12 (21.91%)	8.82 ± 0.56 (40.68%)	5.35 ± 0.33 (24.68%)	2.57 ± 0.11 (11.85%)	17.7 ± 1.12
Snapple lemon-flavored iced	trace	0.34 ± 0.0 (19.32%)	0.82 ± 0.10 (46.59%)	0.60 ± 0.10 (34.09%)	trace	1.76 ± 0.12
Refresh lemon-flavored iced	trace	0.18 ± 0.0 (60.00%)	0.12 ± 0.1 (40.00%)	trace	trace	0.30 ± 0.10
Nestea sweet	trace	trace	trace	0.64 ± 0.10 (100)	trace	0.64 ± 0.10
Nestea, peach flavoring	trace	0.03 ± 0.00 (100%)	trace	trace	trace	0.03 ± 0.00
Nestea, natural lemon	trace	0.09 ± 0.0 (100%)	trace	trace	trace	0.09 ± 0.00
Crystal spring honey dew	trace	0.26 ± 0.03 (12.26%)	1.14 ± 0.12 (53.77%)	0.72 ± 0.10(33.96%)	trace	2.12 ± 0.20
Crystal spring apple	0.03 ± 0.00 (1.45%)	0.06 ± 0.00 (2.91%)	58.3 ± 0.13 (58.25%)	0.77 ± 0.14 (37.38%)	trace	2.06 ± 0.21
Crystal spring peach	0.07 ± 0.00 (2.78%)	0.24 ± 0.04 (9.52%)	1.37 ± 0.10 (54.37%)	0.84 ± 0.11 (33.33%)	trace	2.52 ± 0.23
Bo bo	trace	0.14 ± 0.00(28.57%)	0.35 ± 0.10 (71.43%)	trace	trace	0.49 ± 0.10
Hi-C lemon	trace	0.10 ± 0.00 (38.46%)	0.16 ± 0.05 (61.53%)	trace	trace	0.26 ± 0.06

[a] Data are expressed as mean ± SD of $n = 6$. [b] EGC, epigallocatechin; EC, epicatechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate; GCG, gallocatechin gallate. [c] Total = sum of EGC, EC, EGCG, ECG, and GCG. [d] Percentage of total catechins. Trace < 0.01 mg/100 mL drinks.

Thermal Stability of GTC Derivatives in Aqueous Solution. Ten ml of longjing GTC water solution (5 mg/mL) was heated in a water bath at 37 °C and 98 °C, or autoclaved at 120 °C for 20 min. The sample (50 μL) was taken and mixed with 50 μL of catechin solution (1 mg/mL) as an internal standard. The change in individual catechin derivatives was monitored using HPLC as described above.

Conversion of EGCG to GCG during Autoclave. Longjing GTC or pure EGCG (50 mg) was dissolved in 100 mL of either citric acid−sodium citrate buffers (0.1 M) with pH 3−4, or NaH_2PO_4−Na_2HPO_4 (0.1 M) buffers with pH 5−6. Each solution was then autoclaved for 20 min at 120 °C. The changes in total GTC and EGCG, and formation of GCG, were monitored using HPLC as described above.

Long-Term Stability of GTC in Soft Drinks. To examine the long-term stability of GTC in canned or bottled tea drinks, longjing GTC (0.5 mg/mL) was dissolved in distilled water (control) or in solutions with varying pH. To examine the effects of common ingredients used in making the soft drinks, longjing GTC was also dissolved in sucrose solutions (0.15 g/mL) with addition of citric acid (2 mg/mL) or ascorbic acid (11 mg/mL). After being autoclaved at 120 °C for 20 min, all samples were sealed in sterilized plastic tubes and placed in the dark at room temperature for 6 months. The remaining GTC was measured every month during the storage. In some cases, longjing GTC (0.5 mg/mL) was dissolved in commercially available soft drinks (Coca-Cola, 7-Up, Pepsi, and Cream Soda) and then sterilized using the membrane filter (0.20 μm, Millipore, Bedford, MA). The soft drinks containing longjing GTC were similarly sealed and stored in the dark for six months.

RESULTS

The content of epicatechin derivatives in different brands of tea is shown in Table 1. Although the total GTC varied with tea type, the composition is relatively consistent in green tea. Among four brands of green tea, namely longjing, bi-luo-chun, xin-yang-mao-jian, and lu-zhu-cha, EGCG was most abundant (>66% of total GTC), followed by ECG (>11%), EC, and EGC. In yun-nan-lu-cha, ECG was most abundant (49.65% of total GTC) instead, followed by EGCG, EC, and EGC. The HPLC analysis showed that the yield of total GTC in green tea was 8.0−14.4 g/100 g dry tea leaves. In contrast, the tested black tea contained very little GTC (0.24−0.51 g GTC/100 g dry tea leaves) and oolong tea had a moderate amount of GTC (4.14−4.92 g/100 g dry tea leaves).

Many canned or bottled tea drinks are available in the local markets. Fourteen types of tea drinks produced locally or imported from different countries have been analyzed for their content of GTC. As shown in Table 2, the content of GTC in these tea drinks differed

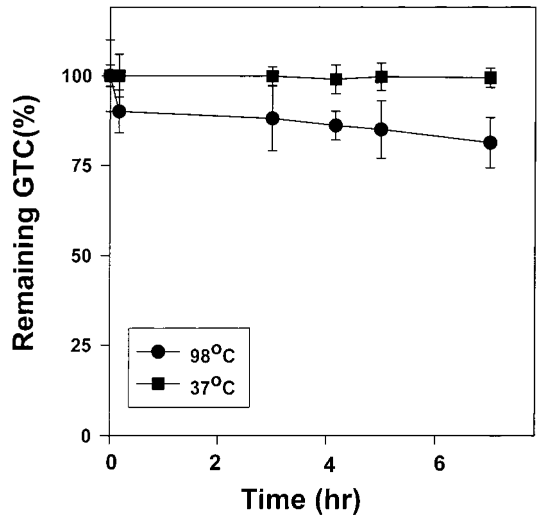

Figure 1. Stability of green tea catechins (GTC) in distilled water heated at 37 °C and 98 °C. Data are expressed as means ± SD of $n = 6$ samples.

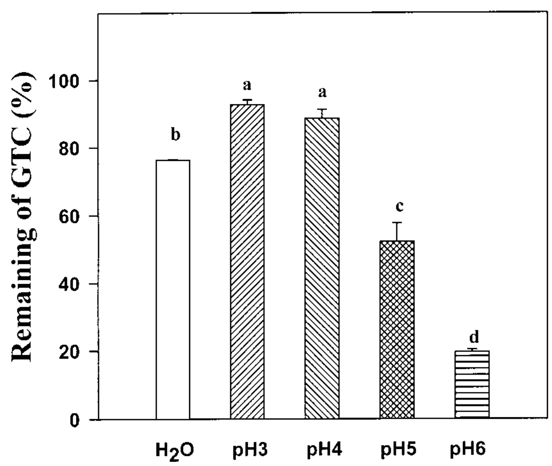

Figure 2. Stability of green tea catechins (GTC) in buffer solutions with varying pH during autoclaving at 120 °C for 20 min. Data are expressed as means ± SD of $n = 8$ samples. Means with different letters (a−d) differ significantly at $p < 0.01$.

significantly. Three brands of canned or bottled tea drinks from Japan, namely Hakuyou oolong tea, Japanese genmai tea, and Japanese tea, contained relatively greater amounts of GTC (>28 mg/100 mL) as compared with other tea drinks. The percentage composition of individual catechins in these three drinks was different from that in green tea. Green tea contained mostly EGCG. In contrast, the three Japanese tea drinks contained mostly GCG (>45%), followed by EGCG (36−40%), EGC (0.7−2.3%), and EC (0.5−2.2%).

Thermal stability of longjing GTC was examined in the present study. The time course of remaining GTC is shown in Figure 1. About 20% of GTC was lost when GTC was heated in water for 7 h at 98 °C. In contrast, GTC in water remained unchanged for the same period at 37 °C (Figure 1).

Effect of the autoclave process used for sterilization in manufacture of tea drinks on the stability of longjing GTC or pure EGCG was also studied in the present work . After being autoclaved for 20 min at 120 °C, only 76% of GTC remained in water (Figure 2). The stability of GTC in solutions during autoclaving was pH-dependent. As shown in Figure 2, GTC was relatively stable at pH 3 and 4, but it degraded readily at pH 5 and 6 during autoclaving. As shown in Figure 2, 80% of longjing GTC in a solution of pH 6 was lost during autoclaving for 20 min.

When longjing green tea was heated at 80 or 95 °C in water, HPLC analysis demonstrated that a HPLC peak following that of EGCG was gradually becoming bigger with heating (Figure 3). This peak was later identified as GCG. A 50-mg portion of GCG was collected and purified from the heating solution of longjing GTC using a semipreparative C-18 column (Spherisorb ODS-1, 250 × 4.6 mm, 10 uM, Isco, Inc., Lincoln, NE). The structure of GCG was confirmed using ^{1}H NMR and ^{13}C NMR as previously demonstrated by Seto et al. (*19*). The ^{1}H NMR spectrum of GCG was similar to that of EGCG, except for differences in the chemical shifts and coupling constants at 2-, 3-, and 4-H. The ^{13}C NMR chemical shifts of GCG were close to those of EGCG, but the slight differences were observed in the shifts at C-2, C-3, and C-4. As a result, GCG (2S:3R) is an epimerization product of EGCG.

Effect of temperature on formation of GCG was examined in the present study. The results showed that the higher the temperature, the greater the epimerization of EGCG to GCG (Figure 4). The formation of GCG from EGCG was pH-sensitive. As shown in Figure 5, the formation of GCG was most efficient at pH 5 when EGCG was autoclaved at 120 °C for 20 min.

A six-month evaluation of long-term stability of GTC in canned or bottled tea drinks was conducted. As shown in Figure 6, 23% of GTC was degraded at 6 months after sterilization if it was dissolved in distilled water. For the same period, 55% of GTC was degraded if it was dissolved in the pH 4 buffer, whereas more than 90% of GTC was lost when pH was increased to 5. It was confirmed that stability of GTC was pH-sensitive. Sucrose, citric acid, and ascorbic acid are commonly used ingredients in canned and bottled tea drinks. When longjing GTC was dissolved in sucrose solution (0.15 g/ml, pH 4.0), it degraded similarly to GTC which was dissolved in distilled water (Figure 7). When longjing GTC was dissolved in a solution containing 0.15 g/mL sucrose and 2 mg/mL citric acid (pH 3.02), it degraded faster than GTC which was dissolved in distilled water or the less-acidic 0.15 g/ml sucrose solution (Figure 7). Ascorbic acid was protective to GTC for the first month after sterilization. Afterward, it accelerated degradation of longjing GTC (Figure 7). When added to three popular soft drinks, longjing GTC was degraded up to 45% in 7up (pH 3.23) for 6 months, whereas it was completely degraded in Pepsi (pH 2.60) and Coca-Cola (pH 2.61) only for four months (Figure 8).

DISCUSSION

Tea is one of the most widely consumed beverages in the world. Recently, canned and bottled tea drinks are getting popular in China. It is generally believed that catechin derivatives are active components responsible for beneficial effects associated with drinking tea. The present study measured the amount of GTC in fourteen selected canned and bottled tea drinks. Surprisingly, it was found that GTC content was very low when compared with that of tea traditionally prepared in a porcelain cup or teapot. In China, drinking tea is an everyday pleasure, and it is simply prepared by pouring hot water (400 mL, 90−100 °C) onto 4−5 g of dry tea leaves in a tea cup. After the tea leaves are soaked and

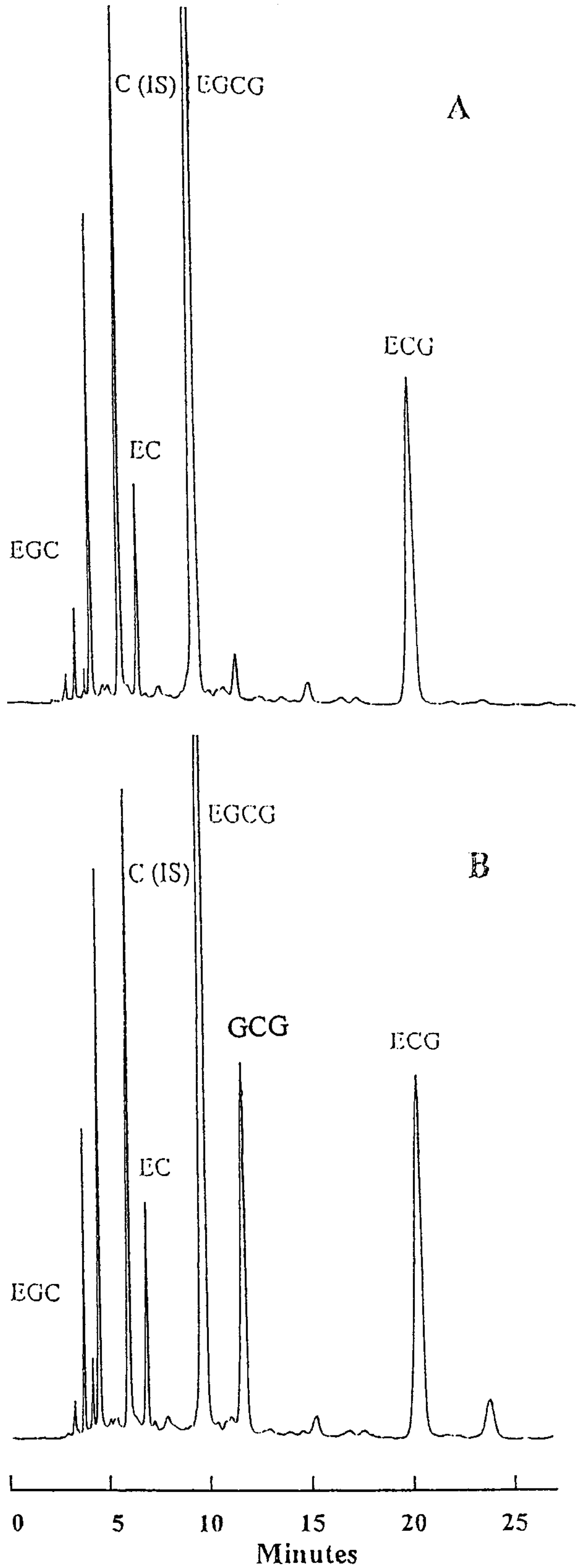

Figure 3. HPLC profile of longjing green tea catechins (GTC). A, before heating at 98 °C; B, 7 h after heating at 98 °C in distilled water. Peak identification: EGC, epigallocatechin; C, catechin (internal standard); EC, epicatechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate; and GCG, gallocatechin gallate.

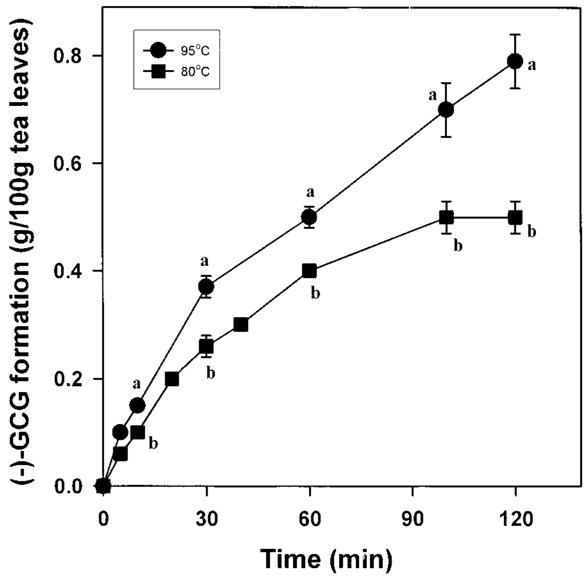

Figure 4. Formation of (−) gallocatechin gallate (GCG) when longjing green tea leaves were heated in distilled water at 80 and 95 °C. Data are expressed as mean ± SD of $n = 5$ samples. Means at the same time point with different superscript letters (a, b) differ significantly at $p < 0.01$.

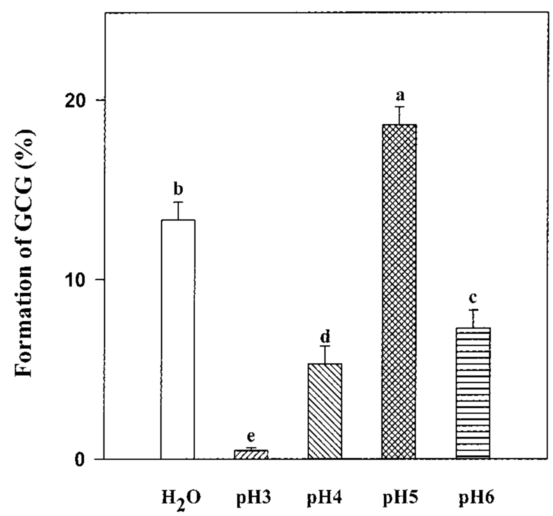

Figure 5. Effect of pH on the formation of (−) gallocatechin gallate (GCG) when epigallocatechin gallate (EGCG) was autoclaved at 120 °C for 20 min. Data are expressed as means ± SD of $n = 8$ samples. Means with different letters (a−e) differ significantly at $p < 0.01$.

have precipitated into the bottom of the tea cup, the infusion is ready for service. The present analysis showed that dry green tea contains approximately 10% GTC. Thus, one will ingest 400−500 mg GTC if one cup of tea drink is brewed traditionally. In contrast, one will ingest only 3−60 mg GTC when one canned or bottled tea drink (250 mL) is consumed. The reasons that canned and bottled tea drinks available in the commercial market have such low content of GTC are unclear. Perhaps, the starting tea materials are responsible, because a high amount of GTC is expected if green or oolong tea is used in making the canned or bottled tea drinks. In addition, the possibilities of thermal degradation of GTC during processing and loss of GTC during storage cannot be eliminated.

The thermal stability of GTC in brewed tea drinks has not been thoroughly examined. When pure longjing GTC was heated at 98 °C for 15 min, a 10−15% loss of GTC was observed for the first half hour (Figure 1). Heating for an additional 6 h led to only an additional 5% loss of GTC. Perhaps the initial oxygen concentra-

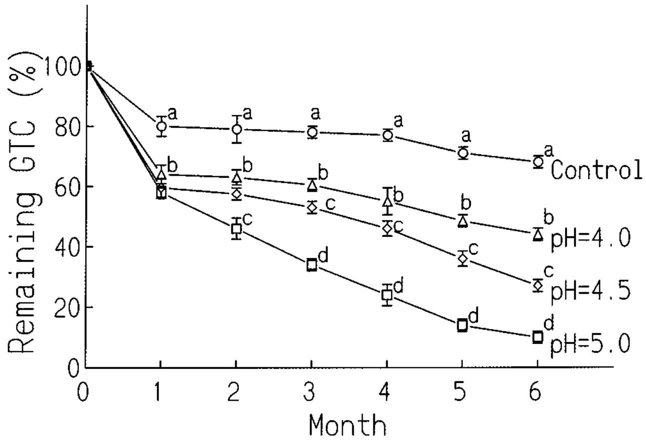

Figure 6. Long-term stability of longjing green tea catechins (GTC) in distilled water (control) and buffer solutions with varying pH after autoclaving at 120 °C for 20 min. Data were expressed as mean ± SD of $n = 6$ samples. Means at the same time point with different superscript letters (a−d) differed significantly at $p < 0.01$.

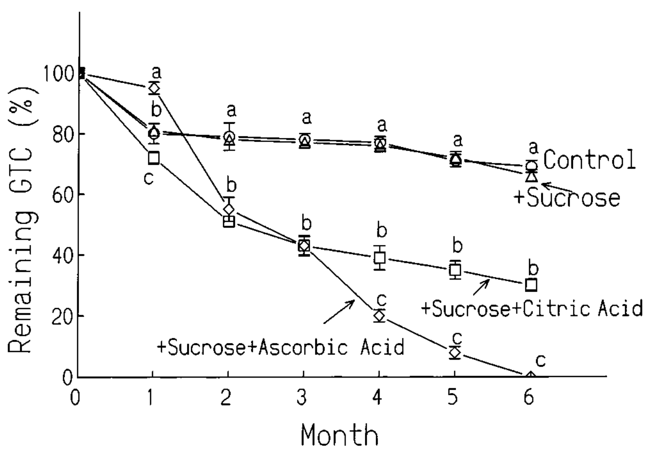

Figure 7. Long-term stability of longjing green tea catechins (GTC) in (1) distilled water (control), (2) sucrose solution (0.15 g/ml, pH 4.0), (3) sucrose solution containing citric acid (2 mg/mL, pH 3.02), and (4) sucrose solution containing ascorbic acid (11 mg/mL, pH 2.55). All solutions were autoclaved at 120 °C for 20 min. Data were expressed as means ± SD of $n = 6$ samples. Means at the same time point with different superscript letter (a−c) differed significantly at $p < 0.01$.

tion was higher in the tea solution so that oxidation of GTC was extensive. Additional heating for several hours and water vapor generated upon heating would decrease the oxygen concentration in the solution and reduce the contact or reaction between oxygen and GTC.

Temperature is one of the important parameters in determination of GTC stability. In contrast to that of heating at 98 °C, no loss of GTC could be observed when longjing GTC solution was maintained at 37 °C for 7 h. This implies that traditional preparation of tea drinks using freshly boiled water does not degrade very much GTC; probably less than the 15% identified on the basis of the present observation (Figure 1). The present results suggest that GTC is stable in aqueous solution at room temperature.

One of the important steps in manufacturing canned and bottled tea drinks is sterilization for longer shelf life. The stability of longjing GTC during autoclaving was therefore examined. It was clear that degradation up to 23% occurred when longjing GTC was simply dissolved in water and then autoclaved at 120 °C for 20 min (Figure 2). However, the degradation was pH-dependent. The higher the pH value of the medium, the greater the percentage of longjing GTC that degraded. When longjing GTC was dissolved in pH 6 buffer, degradation of the longjing GTC was extensive: up to more than 80% (Figure 2). The observed pH-dependent stability was in agreement with the reports by Chen et al. (*20*), Zhu et al. (*18*), Suematsu et al. (*21*), and Komatsu et al. (*14*).

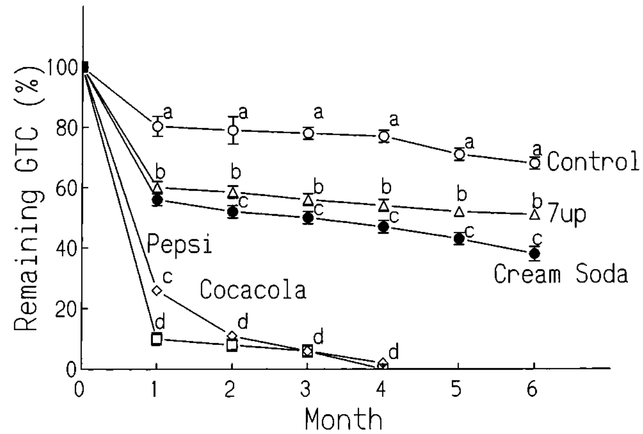

Figure 8. Long-term stability of longjing green tea catechins (GTC) added in distilled water (control) and soft drinks. The soft drinks containing longjing GTC were sterilized using Millipore 0.20-μm filter. Data were expressed as mean ± SD of $n = 6$ samples. Means at the same time point with different superscript letters (a−d) differed significantly at $p < 0.01$.

The long-term stability of GTC in canned and bottled drinks is currently unknown. To assess the quality and shelf life of canned and bottled drinks, it is particularly important to examine the long-term stability of GTC. The present study clearly demonstrated that the stability of GTC was pH-dependent in the absence of other ingredients. The higher the pH, the more unstable the GTC (Figure 6). For the drinks with pH below 4.5, half of the GTC would remain for at least 3 months. The effects of some common drink ingredients, including sucrose, citric acid, and ascorbic acid, on the stability of GTC were not consistent. Sucrose appeared to have no effect, but citric acid accelerated the degradation of GTC (Figure 7). Ascorbic acid was unique: it protected at the first month and then promoted the degradation of longjing GTC. The present observation supported the view that ascorbic acted as both antioxidant and prooxidant (*22* and *23*). The short-term protective effect of ascorbic acid was in agreement with the previous report by Chen et al. (*20*). When added to Coca-Cola (pH 2.6) and Pepsi (pH 2.6), longjing GTC exhibited poorer stability than it did in 7up (pH 3.23). This suggests that other ingredients in tea drinks may interact with GTC and affect its stability. To maximize the shelf life of GTC in canned and bottled tea drinks, the formulation of ingredients has to be carefully optimized. We are currently studying the effect of individual ingredients in tea drinks on the stability of GTC.

It should be noted that the relative percentage of GCG accounted for only 0.84−1.43% of the total GTC in green tea, whereas in some canned or bottled tea drinks (namely Hakuyou oolong tea, Japanese jenmai tea, and Japanese tea), it increased to more than 45% of the total GTC (Table 2). This led us to speculate that thermal sterilization would convert EGCG to GCG. To demonstrate this, we were able to show the formation of GCG when longjing GTC as a mixture, or pure EGCG, was autoclaved at 120 °C for 20 min (Figures 3 and 4). The result was in agreement with that of Seto et al. (*19*), who studied the epimerization reactions of EGCG, EGC, ECG, and EC. It was found that EGCG, ECG, EGC, and EC were susceptible to epimerization and converted to their corresponding epimers, namely GCG, (−)-catechin gallate (CG), (−)-gallocatechin (GC), and (−)-catechin at 120 °C for 30 min. It was therefore concluded that GCG present in canned and bottled tea drinks was produced during the sterilization step in their manufacture. However, there were no or little amounts of CG, GC, and (−)-catechin detected in these canned or bottled tea drinks because their corresponding epimers GCG, EGC, and EC in tea leaves were relatively minor as compared with EGCG.

In conclusion, the content of GTC varies among green tea, black tea, and oolong tea. Green tea, which is a nonfermented product, has the greatest amount of GTC, whereas black tea has the lowest content of catechins because it was extensively oxidized during fermentation. Oolong tea has moderate amount of GTC because it was a partially fermented product (*15*). The stability study demonstrated that GTC was stable in water at room temperature. HPLC analysis revealed that about 10−15% GTC would be lost if tea was prepared and brewed in boiling water. The present results showed that GCG in tea drinks was most likely an epimerization product of EGCG during autoclaving. The long-term stability of GTC during storage was governed by several factors including pH and the other ingredients used in production of the tea drinks. Although GTC might have many beneficial effects, the prevention of its degradation must be taken into consideration when tea drinks are produced, stored, and transported.

LITERATURE CITED

(1) Ahmad, N.; Mukhtar, H. Green tea polyphenols and cancer: Biological mechanisms and practical implications. *Nutr. Rev.* **1999**, *57*, 78−83.

(2) Buschman, J. L. Green tea and cancer in humans: a review of the literature. *Nutr. Cancer* **1998**, *31*, 51−57.

(3) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007−1011.

(4) Imai, K.; Nakachi, K. Cross sectional study of effects of drinking green tea on cardiovascular and liver disease. *Biochem. Med. J.* **1985**, *310*, 693−696.

(5) Yang, C. S. Tea and health. *Nutrition* **1999**, *15*, 946−949.

(6) Henry, J. P.; Stephens-Larson, P. Reduction of chronic psychosocial hypertension in mice by decaffeinated tea. *Hypertension* **1984**, *6*, 437−444.

(7) Ding, Z. Y.; Chen, Y.; Zhou, M.; Fang, Y. Z. Inhibitory effect of green tea polyphenol and morin on the oxidative modification of low-density lipoprotein. *Chin. J. Pharmacol. Toxicol.* **1992**, *6*, 263−266.

(8) Miura, S.; Watanable, J.; Tomita, T.; Sano, M.; Tomita, S. The inhibitory effects of tea polyphenol (flavan-3-ol derivatives) on Cu^{2+} mediated oxidative modification of low-density lipoproteins. *Biol. Pharm. Bull.* **1994**, *17*, 1567−1572.

(9) Nanjo, F.; Goto, K.; Seta, R.; Suzuki, M.; Sakai, M.; Hara, Y. Scavenging effect of tea catechins and their derivatives on 1,1-diphenyl-2-picryhydrazyl radical. *Free Radical Biol. Med.* **1996**, *21*, 895−902.

(10) 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.

(11) Chan, P. T., Fong, W. P.; Cheung, Y. L.; Huang, Y.; Ho, W. K. K.; Chen, Z. Y. Jasmine green tea epicatechins are hypolipidemic in hamsters fed a high-fat diet. *J. Nutr.* **1999**, *129*, 1094−1101.

(12) Muramatsu, K.; Fukuyo, M.; Hara, Y. Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J. Nutr. Sci. Vitaminol.* **1986**, *32*, 613−622.

(13) Kono, S.; Shinchi, K.; Ikeda, N.; Yanai, F.; Imanishi, K. Green tea consumption and serum lipid profile: a cross-sectional study in Northern Kyush. *Japan. Prev. Med.* **1992**, *21*, 526−531.

(14) Komatsu, Y.; Hisanobu, Y.; Suematsu, S.; Matsuda, R.; Saigo, H.; Hara, K. Stability of functional constituents in canned tea drinks during extraction, processing, and storage. In *Proceedings of the International Symposium on Tea Science*, Shizuoka, Japan; Kurofune Printing Co. Ltd., 1991; pp 571−575.

(15) Ho, C. T., Zhu, N. The Chemistry of Tea. In *Caffeinated Beverages: Health Benefits, Physiological Effects, and Chemistry* Parliament, T. H., Ho, C. T., Schieberle, P., Eds.;, ACS Symposium Series; American Chemical Society: Washington, DC, 1999; pp 316−326.

(16) Zhu, Q. Y., Chen, Z. Y. Isolation and analysis of green tea polyphenols by HPLC. *Anal. Lab.* **1999**, *18*, 70−72.

(17) Agarwal, R.; Katiyar, S. K.; Zaidi, S. I. A.; Mukbtar, H. Inhibition of skin tumor promotor-caused induction of epidermal ornithine decarboylase in SENCAR mice by polyphenolic fraction isolated from green tea and its individual epicatechin derivatives. *Cancer Res.* **1992**, *52*, 3582−3588.

(18) 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.

(19) Seto, R.; Nakamura, H.; Nanjo, F.; Hara, Y. Preparation of epimers of tea catechins by heat treatment. *Biosci. Biotech. Biochem.* **1997**, *61*, 1434−1439.

(20) Chen, Z. Y.; Zhu, Q. Y.; Wong, Y. F.; Zhang, Z.; Chung, H. Y. Stabilizing effect of ascorbic acid on green tea catechins. *J. Agric. Food Chem.* **1998**, *46*, 2512−2516.

(21) Suematsu, S.; Hisanobu, Y.; Saigo, H.; Matsuda, R.; Hara, K.; Komatsu, Y. Effect of pH on stability of constituents in canned tea drinks. Studies on preservation of constituents in canned tea drink. *J. Jpn Soc. Food Sci. Technol.* **1992**, *39*, 178−182.

(22) Podmore, I. D.; Griffiths, H. R.; Herbert, K. E.; Mistry, N.; Mistry, P.; Lunce, J. Vitamin C exhibits pro-oxidant properties. *Nature* **1998**, *392*, 558−559.

(23) Herbert, V.; Shaw, S.; Jayatilleke, E. Vitamin C-driven free radical generation from iron. *J. Nutr.* **1996**, *126*, 1213S−1220S.

Received for review July 17, 2000. Revised manuscript received November 1, 2000. Accepted November 4, 2000. This work was supported by the Hong Kong Research Grant Council and the Wei Lun Foundation.

JF000877H