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Stability of tea theaflavins and catechins

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Abstract

Green tea catechins (GTC) and theaflavins (TF) possess a variety of biological activities. The present study focused on stability of GTC and TF in various solutions and drinks. It was observed that both GTC and TF were vulnerable to degradation caused by elevation of temperature and pH of incubation media. In general, GTC was more stable than TF. When boiled in water, four GTCs showed similar rates of degradation, but in sodium phosphate buffer (pH7.4) at room temperature, four GTCs demonstrated varying stability, with epigallocatechin gallate (EGCG) and epigallocatechin (EGC) being completely degraded in 6 h of incubation, while epicatechin (EC) and epicatechin gallate (ECG) were only degraded by less than 35%. Four TFs also demonstrated varying stability, with theaflavin-3,3′-digallate (TF3) and theaflavin-3′-gallate- B (TF2B) in general being more stable than theaflavin-1 (TF1) and theaflavin-3-gallate-A (TF2A) in either boiling water or alkaline sodium phosphate buffer. When incubated in various solutions and soft drinks, both GTC and TF had poor long-term stability and decayed by at least 50% during the first month of storage at room temperature.

Keywords: Catechins; Theaflavins; Stability; Tea drinks

1. Introduction

Green tea catechins (GTC) are the principal components in tea leaves and comprise mainly four compounds namely (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG). During the production of black tea, extensive enzymatic oxidation of the leaf catechins leads to brown products such as theaflavins (TF) and thearubigins (TR). The major TF in black tea are theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3′-gallate (TF2B), and theaflavin-3,3′-digallate (TF3). Both GTC and TF have been subjects of extensive research for their anticarcinogenic, hypolipidemic and antioxidant activities (Ahmad & Mukhtar 1999; Chan, Fong, Huang, Ho, & Chen, 1999; Leung, Su, Chen, Zhang, Huang, & Chen, 2001).

Stability of GTC and TF in tea beverage has not received much attention. Although GTC and TF have many beneficial effects, their decomposition must be prevented when tea drinks are produced, stored and transported. Our previous study demonstrated that stability of GTC, as a whole, is pH-dependent; in alkaline solution, it is very unstable and decomposes in a few minutes, whereas, in acidic solution, it is relatively stable (Zhu, Zhang, Tsang, Huang, & Chen, 1997). It was also observed that the four catechins showed varying stability with EGCG and EGC being more unstable than EC and ECG, irrespective of pH. Another study also analysed the composition of bottled and canned tea drinks available in the market and found that the four catechins were present in low quantities, having been mainly converted to their corresponding epimers (Chen, Zhu, Tsang, & Huang, 2001). However, there is no study to date that has examined the stability of theaflavins in black tea. The objective of the present study was to study the degradation of TF compared with that of GTC under various conditions.

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2. Materials and methods

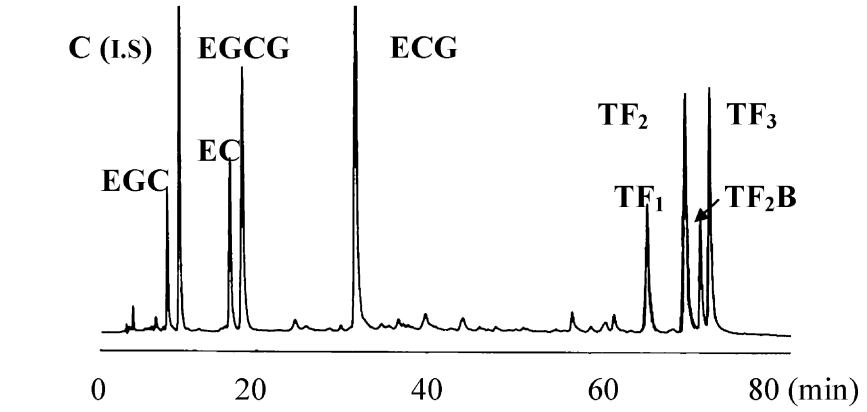
2.1. Preparation of GTC–TF mixture

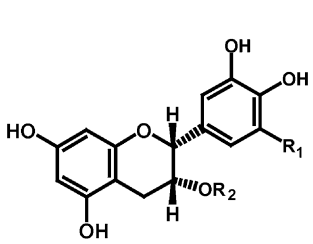
TF extract was prepared with Keemun black tea (Shanghai Tea Import & Export Corporation, China), as previously described (Leung et al., 2001). In brief, the tea leaves (2.25 kg) were firstly extracted three times using 13.5 l of 70% ethanol. The ethanol was then removed in a rotary evaporator. The remaining aqueous solution was extracted subsequently using chloroform (3 l), ethyl acetate (2 l), and butanol (2 l). The ethyl acetate extract was applied onto a silica gel column (30×2 cm i.d.; silica gel 60 M, 230–240 mesh). Total TF fraction was obtained when the column was eluted with 4 litres of chloroform-ethyl acetate (1:1, v/v), followed by 4 l of chloroform-ethyl acetate (4:1,v/v), as previously described (Leung et al., 2001)

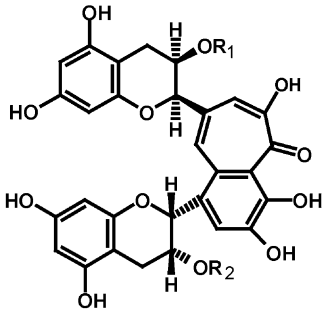
GTC extract was prepared with Longjing green tea (Huangshan Forestry Farm, Xiaoshan, Zhejiang, China), as previously described (Agarwal, Katiyar, Zaidi, & Mukbtar, 1992). In brief, 10 g of dry longjing tea leaves were extracted three times with 140 ml of hot distilled water (80 °C). The infusion was then cooled to room temperature, filtered and extracted with an equal volume of chloroform to remove caffeine and pigments. The remaining aqueous layer was then extracted twice with an equal volume of ethyl acetate. The total GTC extract was obtained after the removal of ethyl acetate in a rotary evaporator. The TF–GTC mixture was prepared by mixing equal amounts of GTC and TF extracts.

2.2. HPLC analysis of catechins and theaflavins

The individual catechins and theaflavins were quantified using a Shimadzu LC-10AD HPLC (Tokyo, Japan), equipped with a ternary pump delivery system, as described previously. In brief, the mixture (10 μl, 0.5 mg/ml) was injected onto column (Hypersil ODS, 250×4.6 mm, 5 m, Alltech, Deerfield, IL, USA) via a Rheodyne valve (20 μl capacity, Shimadzu, Tokyo, Japan). The mobile phase consisted of 2% acetic acid in water (v/v) (Solvent A) and acetonitrile (Solvent B). After the injection of the sample, Solvent B was increased from 8 to 15% over 28 min, to 31% over an additional 52 min and then back to the starting ratio over an additional 5 min. The flow rate was maintained at 1.0 ml/min. The individual catechin and TF were monitored at 280 nm and quantified using (+)-catechin as an internal standard (Fig. 1).







EGC = Epigallocatechin R_1=OH R_2=H
EC = Epicatechin R_1=H R_2=H
EGCG = Epigallocatechin gallate R_1=OH R_2=galloyl
ECG = Epicatechin gallate R_1=H R_2=galloyl

TF_1 = Theaflavin-1 R_1=R_2=H
TF_2A = Theaflavin-3 -gallate-A R_1=galloyl R_2=H
TF_2B = Theaflavin-3'-gallate-B R_1=H R_2=galloyl
TF_3 = Theaflavin-3,3'-digallate R_1=R_2=galloyl

Fig. 1. Structures and HPLC eluting pattern of green tea cateachins and theaflavins.

2.3. Thermal stability of GTC and TF

The GTC–TF mixture (5 mg) was dissolved in 10 ml of doubly deionised water and delivered into each Pyrex tube (10×1.6 cm, i.d.). The stabilities of individual GTC and TF were examined at 24, 70, and 100 °C for 3 h in open air without any agitation. During the incubation, 0.5 ml were periodically sampled and mixed with 0.2 ml (+)-catechin solution (0.5 mg/ml) as an internal standard. The mixture was extracted with 1 ml of ethyl acetate. After the removal of ethyl acetate under a gentle stream of nitrogen, 0.5 ml of distilled water were added to dissolve the GTC and TF. The sample was then subjected to HPLC analysis, as previously described.

2.4. pH stability of GTC and TF

The pH stability of GTC and TF was assessed by incubating 5 mg GTC-TF mixture in 10 ml of 60 mM sodium phosphate buffer at different pH values, varying from 5.0 to 7.4, in a Pyrex test tube (10×1.6 cm, i.d.) in open air without any agitation. An aliquot (0.5 ml) of the incubation mixture was periodically sampled and 0.2 ml of (+)-catechin solution (0.5 mg/ml) were added, followed by extracting twice with 1 ml ethyl acetate. After evaporation, the GTC and TF were dissolved into 0.5 ml water and subjected to HPLC analysis.

2.5. Long-term stability of GTC and TF

To study the long-term stability of GTC and TF in tea drinks, The GTC–TF mixture (0.5 mg/ml) was incubated in sodium phosphate buffer solutions with varying pH (4–5), a sucrose solution (0.15 g/ml), a sucrose-citric acid solution (0.15 g sucrose/ml, 2 mg citric acid/ml), and four kinds of commercial soft drinks, namely, Coca cola, 7 UP, Pepsi and Sprite. Each solution containing GTC–TF mixture was autoclaved at 120 °C for 20 min, sealed in a sterilized Pyrex tube (10×1.6 cm, i.d.) and placed in the dark at room temperature for six months. An aliquot (4 ml) of the solution was sampled monthly and mixed with 1 ml of 0.4 mg/ml (+)-catechin solution as an internal standard. The sample was extracted twice with 4 ml of ethyl acetate. After the removal of ethyl acetate under a gentle stream of nitrogen, the GTC-TF was dissolved in 0.5 ml of distilled water and subjected to HPLC analysis, as described previously.

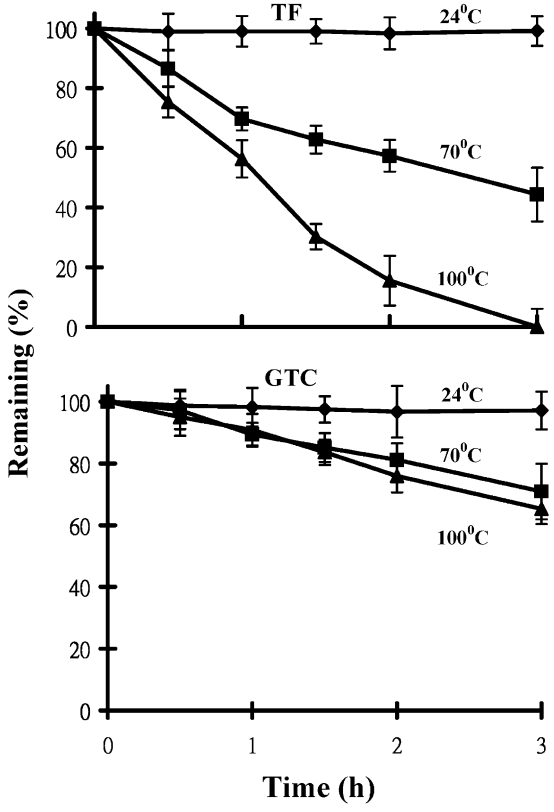


Fig. 2. Thermal stability of green tea catechins (GTC) and theaflavins (TF) in water solutions at 24, 70 and 100 °C. GTC is the sum of EGCG, EGC, ECG and EC. TF is the sum of TF1, TF2A, TF2B and TF3. Data are expressed as means ±S.D. of $n=3$ samples.

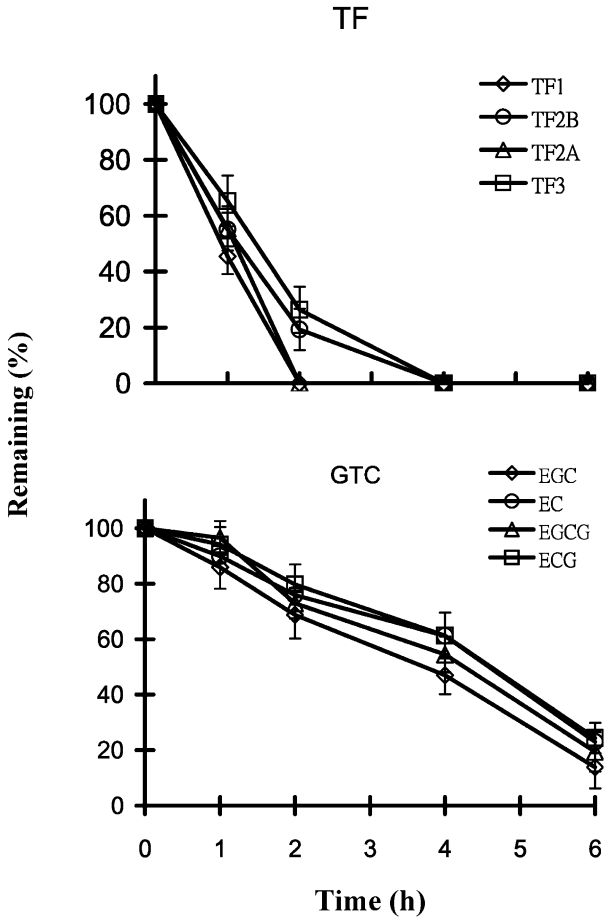


Fig. 3. Thermal stability of individual green tea catechins (GTC) and theaflavins (TF) in boiling water. Data are expressed as means ±S.D. of $n=3$ samples.

3. Results and discussion

3.1. Thermal stability of GTC and TF

GTC and TF demonstrated different thermal stabilities. As shown in Fig. 2, GTC as a sum of EGCG, EGC, ECG and EC was more stable than TF, which is a sum of TF1, TF2A, TF2B and TF3. Heating at 100 °C for 3 h led to 25% degradation of GTC. In contrast, TF was completely degraded (Fig. 2). Heating at 70 °C for 3 h degraded TF 56%, whereas it only destroyed 29% of GTC. It is concluded that GTC in green tea, as a whole, is more stable than TF in black tea.

When individual TFs were compared with those of GTCs, the former were more susceptible to thermal destruction than the latter (Fig. 3). The four GTC derivatives appeared to have similar rates of degradation at 100 °C. Among the four TF derivatives, TF3 and TF2B had relatively slower rates of destruction than the other two (Fig. 3).

Traditional preparations of tea drinks in a porcelain teapot do not degrade very much of the GTCs. In China, tea beverage is simply brewed by pouring 300–400 ml of boiling water onto 4–5 g of dry tea leaves in a tea cup. After approximately 5 min, the tea leaves are saturated and sink to the bottom; the infusion is then ready for serving. As shown in Fig. 2, heating at 100 °C for 30 min degraded only about 5% of the GTC. This observation is similar to that previously reported by Chen, Zhu, Tsang, & Huang (2001), namely, that about 10% of the GTC was lost after 10 min in boiling water. However, destruction of TF reached 25% under the same conditions.

3.2. pH-Dependent stability of GTC and TF

The effect of pH on the stability of GTC and TF was significant at room temperature. As illustrated in Fig. 4, both GTC and TF demonstrated pH-dependent stability; the lower the pH value of the sodium phosphate buffer, the greater the stability. When incubated in buffer of the same pH, TF was much more unstable than GTC. For example, incubation in sodium phosphate buffer of pH 7.4 for 6 h led to 95% destruction of TFs but only to 65% GTC degradation. We have measured the pH values of several bottled and canned tea drinks available on the Hong Kong market. It was found that the pH value of tea drinks varied with brands, ranging from 3.3 to 6.5. As shown in Fig. 4, GTC and TF had

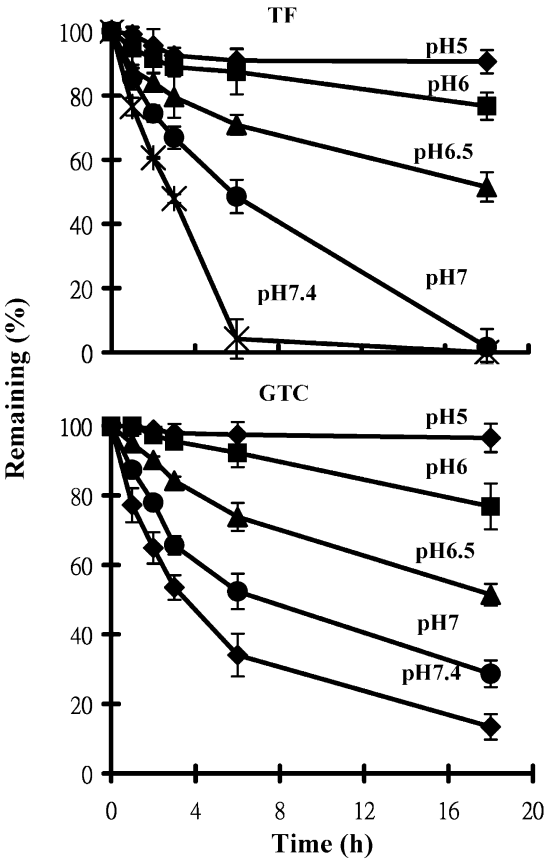


Fig. 4. Stability of green tea catechins (GTC) and theaflavins (TF) in sodium phosphate buffer of various pH. Data are expressed as means ± S.D. of $n = 3$ samples.

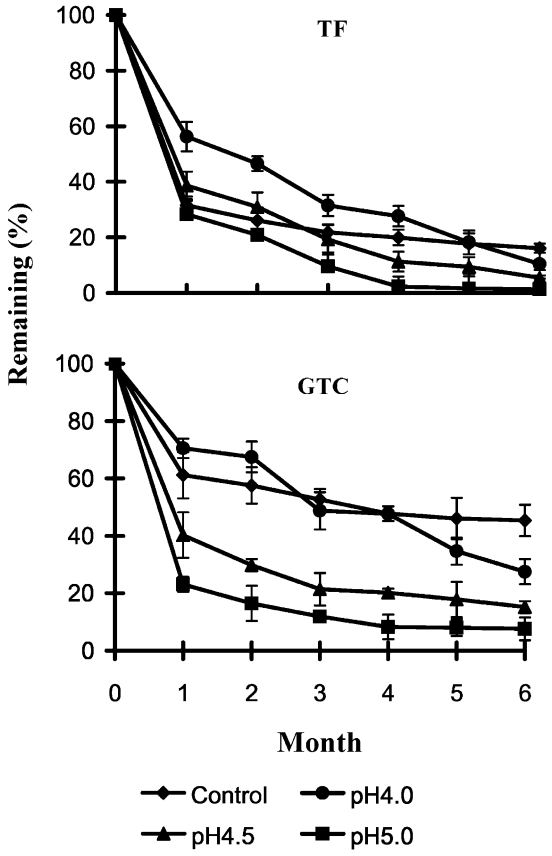


Fig. 5. Long-term stability of green tea catachins (GTC) and theaflavins (TF) in distilled water (control) and sodium phosphate buffer solution with pH 4.0–5.0. Data are expressed as means ± S.D. of $n = 3$ samples.

short-term stability in the tea drinks with pH 5 or less. However, the long-term stability of GTC and TF was not promising. As shown in Fig. 5, 45–73% of TF was lost when incubated in sodium phosphate buffers with pH 4–5 for 1 month. Similarly, incubation for one month led to 30–78% of GTC destruction. The observed pH-dependent stability of GTC was in agreement with the reports by Chen, Zhu, Wong, Zhang, and Huang (1998), Chen, Zhu, Tsang, and Huang (2001), Zhu, Zhang, Tsang, Huang, and Chen (1997), Suematsu, Hisanobu, Saigo, Matsuda, Hara, & Komatsu (1992), and Komatsu (1991). The present study is the first to report that TF also exhibit a pH-dependent stability. The results suggest that the pH-dependent stability has to be taken into consideration when commercial canned green or black tea drinks are produced.

3.3. pH-stability of individual catechin and theaflavin

Individual TFs and GTCs demonstrated differences in stability (Fig. 6). To simplify the presentation, only data for pH 7.4 have been shown. Among the four TFs, TF2B appeared to be more stable for the first three hours of incubation, while TF3 was more resistant to degradation from 3 to 6 h in sodium phosphate buffer (pH 7.4). Among the four GTCs, EGCG and EGC were most unstable, while EC and ECG were relatively stable. EGC was almost completely degraded when incubated for 3 h at pH 7.4, while EGCG was completely destroyed at the end of the 6 h incubation. Under the same incubation conditions, ECG and EC were decreased by 20 and 5%, respectively, after 3 h. The reason that EGCG and EGC were more unstable than ECG and EC is almost certainly due to the three vicinal hydroxyl groups at positions 3′, 4′ and 5′ in EGCG and EGC being more vulnerable to destruction (producing semiquinone free radicals) than the two vicinal hydroxyl groups at positions 3′ and 4′ in ECG and EC (Yoshioka, Sugiura, Kawakara, Fugita, Makino, Kamiya, & Tsuyumu 1991). It was noteworthy that the degradation pattern of the four GTCs in boiling water was different from that in sodium phosphate buffer (pH 7.4) (Fig. 3). In the boiling water, they had similar rates of degradation while, in sodium phosphate buffer (pH 7.4), EGCG and EGC were destroyed much faster than

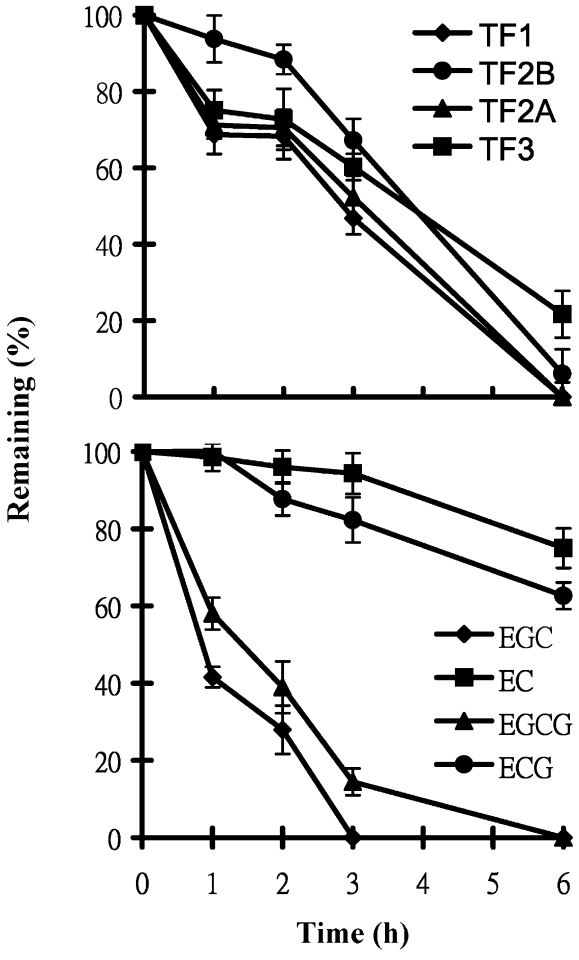


Fig. 6. Stability of individual green tea catechins (GTC) and theaflavins (TF) in sodium phosphate buffer (pH 7.4). Data are expressed as means ±S.D. of $n=3$ samples.

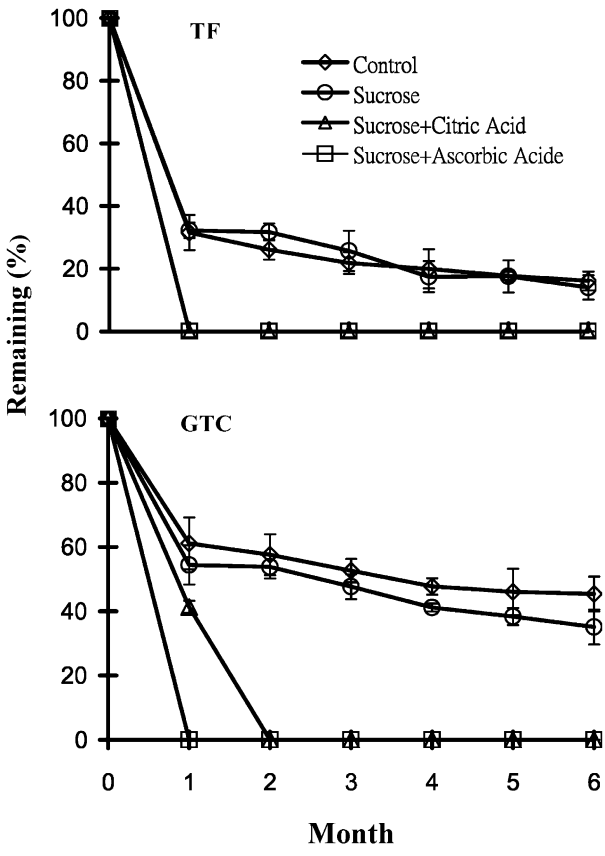


Fig. 7. Long-term stability of green tea catachins (GTC) and theaflavins (TF) in distilled water (control), sucrose solution (0.15 mg/ml), sucrose solution containing citric acid (2 mg/ml) and sucrose solution containing ascorbic acid (11 mg/ml). Data are expressed as means ±S.D. of $n=3$ samples.

ECG and EC. It would be of interest to study the decomposition pathways of the two types of GTC to better understand the behaviour of these active tea ingredients in various in vitro and in vivo media.

3.4. Stability of GTC and TF in drinks

A 6-month assessment of stability of GTC and TF was conducted in various solutions containing sucrose, citric acid and ascorbic acid, that are commonly added to the tea drinks. Compared with the control water solution, addition of sucrose had no or little effect on stability of either GTC or TF (Fig. 7). Addition of citric acid and ascorbic acid destabilised GTC and TF. When the GTC–TF mixture was added to the four soft drinks, they showed a faster degradation than those in the control water solution (Fig. 8). Regardless of the types of solutions and soft drinks, TF was less stable than GTC (Figs. 7 and 8).

There is limited information on long-term stability of GTC and TF in canned and bottled tea drinks. To assure the quality and shelf-life of tea drinks, it is a must to assess the long-term stability of GTC and TF. In addition to being pH-dependent, GTC and TF had poor stability in drinks. The results clearly demonstrate that GTC and TF were degraded by at least 50% within the first month of storage, in either water–sucrose solution or other soft drinks. This may partially explain the low content of GTC in the commercial tea drinks (Chen, Zhu, Tsang, & Huang, 2001). There has been no report on decomposition of GTC and TF except that Zhu, Huang, Yu, LaVoie, Yang, & Ho (2000) isolated and identified three oxidation products when EGCG and EGC interacted with H_2O_2. No study has addressed the possible fates of ECG, EC and TF during heat treatment, except for our previous report (Chen, Zhu, Tsang, & Huang, 2001) which demonstrated that half of the GTC was converted to the corresponding epimers if GTC was heated at 120 °C for 20 min. We are currently studying the decomposition pathway of each GTC and TF and examining whether these decomposition products have any biological functions such as those of their precursors.

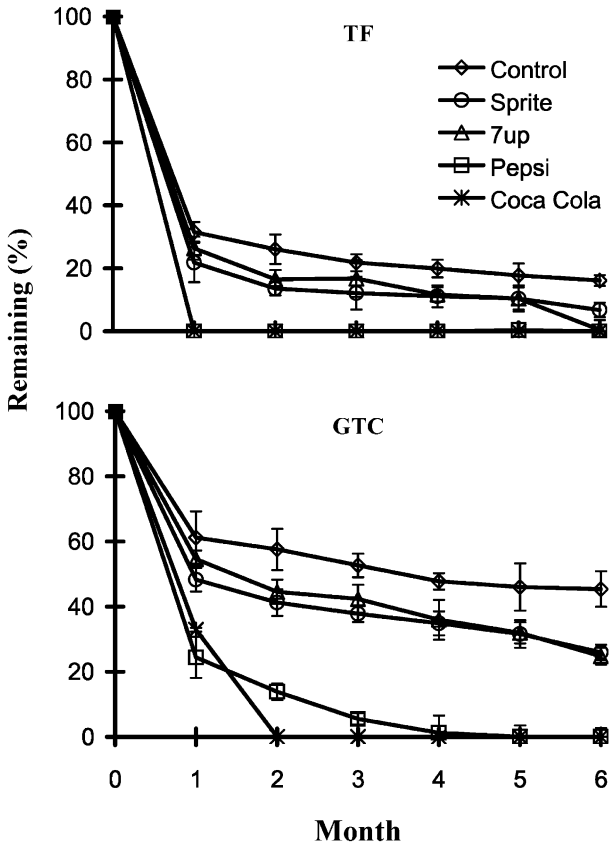


Fig. 8. Long-term stability of green tea catachins (GTC) and theaflavins (TF) in distilled water (control), and soft drinks. Data are expressed as means ±S.D. of $n=3$ samples.

4. Conclusion

Four catechins in green tea and four theaflavins in black tea are believed to be the active ingredients that possess a range of beneficial effects. The present study examined the stability of GTC and TF. In general, TF as a whole is more susceptible to degradation than GTC in boiling water and alkaline solutions. The four GTCs showed varying stability in alkaline pH solution with EGCG and EGC being unstable and ECG and EC being relatively stable. In boiling water, the four GTCs showed similar patterns of destruction. Regarding the four TFs, TF3 and TF2B appeared to be relatively more stable than TF1 and TF2A in both boiling water and alkaline solutions. When GTC and TF were added to various drinks, they were degraded by at least 50% within the first month of storage at room temperature. The observed behaviour of GTC and TF derivatives should be taken into consideration when bottled and canned tea drinks are produced.

Acknowledgements

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References

Agarwal, R., Katiyar, S. K., Zaidi, S. I. A., & Mukbtar, H. (1992). 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 Research*, *52*, 3582–3588.

Ahmad, N., & Mukhtar, H. (1999). Green tea polyphenols and cancer: biological mechanisms and practical implications. *Nutrition Review*, *57*, 78–83.

Chan, P. T., Fong, W. P., Huang, Y., Ho, W. K. K., & Chen, Z. Y. (1999). Jasmine green tea epicatehins are hypolipidemic in hamster

(*Mesocricetus auratus*) fed a high fat diet. *Journal of Nutrition*, *129*, 1094–1101.

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

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

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

Leung, L. K., Su, Y., Chen, R., Zhang, Z., Huang, Y., & Chen, Z. Y. (2001). Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *Journal of Nutrition*, *131*, 2248–2251.

Suematsu, S., Hisanobu, Y., Saigo, H., Matsuda, R., Hara, K., & Komatsu, Y. (1992). 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*, *39*, 178–182.

Yoshioka, H., Sugiura, K., Kawakara, R., Fugita, T., Makino, M., Kamiya, M., & Tsuyumu, S. (1991). Formation of radicals and chemiluminescene during the autoxidation of tea catechins. *Agriculture, Biology and Chemistry*, *55*, 2717–2723.

Zhu, Q. Y., Zhang, A., Tsang, D., Huang, Y., & Chen, Z. Y. (1997). Stability of green tea catechins. *Journal of Agriculture and Food Chemistry*, *45*, 4624–4628.

Zhu, N., Huang, T. C., Yu, Y., LaVoie, E. J., Yang, C. S., & Ho, C. T. (2000). Identification of oxidation products of (−)-epigallocatechin gallate and (−)-epigallocatechin with H_2O_2. *Journal of Agriculture and Food Chemistry*, *48*, 979–981.