Food Sci. Biotechnol. Vol. 18, No. 4, pp. 932 ~ 938 (2009)

Food Science and Biotechnology

Effects of Temperature, Illumination, and Sodium Ascorbate on Browning of Green Tea Infusion

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Abstract Browning of tea infusion is an obstructive factor influencing shelf life of ready-to-drink green tea. Effects of temperature and illumination on the browning of green tea infusion were investigated. It was shown that both elevated temperature and illumination led to the browning of green tea infusion, but temperature had greater effect on infusion color and level of catechins than illumination. The levels of unoxidized catechins such as (-)-epigallocatechin gallate (EGCg), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), (-)-epicatechin (EC), and total catechins remaining in the tea infusion were significantly correlated to color parameters of the tea infusion. Sodium ascorbate inhibited the infusion browning by suppressing the oxidation of tea catechins and it is considered to be a more suitable preservative for prolonging shelf life of ready-to-drink green tea than ascorbic acid because it has less effect on tea taste. The effects of temperature and illumination on the epimerization of catechins were also discussed.

Keywords: *Camellia sinensis*, shelf life, quality control, polyphenol, infusion color

Introduction

Ready-to-drink green tea has been increasingly accepted by consumers, especially in Asian countries due to its high level of tea catechins which were confirmed to have bioactivities (1,2). Except for its healthy benefits, freshly prepared ready-to-drink green tea attracts consumers owing to its greenish color and fragrant flavor. However, the bottled ready-to-drink green tea is gradually become brown in color during shelf life, resulting in decline of sensory acceptance (3). Color is one of the most important sensory attributes of food and holds a preeminent position in overall food quality (4,5). The oxidation of tea polyphenols, especially catechins was shown to be probable cause leading to the browning during pasteurization and shelf life (3,6). Epimerizarion of natural tea catechins such as (-)-epicatechin (EC), (-)-epicatechins gallate (ECg), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCg) took place under action of heating during processing and storage (7-9), which might lead to changes in bioactivities of tea catechins.

Anti-browning agents were used to control the browning of ready-to-drink green tea products, including ascorbic acid, sulfites, citric acid, and cysteine (10-12). Sulfites had been used to control browning of green tea infusion, but they had adverse effects on health, especially for the sensitive individuals with steroid-dependent asthmatics. The effect of citric acid was controversial (11,12). Both citric acid and ascorbic acid bring a lower pH of tea infusion, resulting in low sensory acceptance. The browning of green tea was shown not to be enzymatic and Maillard reactions, but the oxidation of tea polyphenols under the action of heat (3,13). During storage and shelf life, the products were under the action of light and heat, both of which accelerated the oxidation of catechins. The browning was partially controlled by using colored polyethylene terephthalate (PET) bottles as tea infusion container to shield the light action (14). During summer season in tropics and subtropics where the ready-to-drink tea has been widely consumed, warehouse temperature is as high as above 40°C. However, little information on the differences in effects of light and heat on tea infusion browning has been available. Investigation on the differences in effects of temperature and illumination on the browning of green tea infusion will be helpful to further understand the browning mechanism of ready-to-drink tea infusion and to develop methods to control its browning.

The present work was undertaken to investigate the effects of elevated temperature and illumination on browning and oxidation of catechins in green tea infusion. Sodium ascorbate, a substitute of ascorbic acid which has less effect on infusion pH, was used as anti-browning agent in the test.

Materials and Methods

Materials The tea used in the tests was first grade green tea supplied by Daming Tea Co., Ltd. (Zhangzhou, China). Sodium ascorbate was product of Zhengzhou Chemical Industry (Zhengzhou, China). Acetonitrile and acetic acid (HPLC grade) were purchased from Siyou Biotech Co., Ltd. (Tianjin, China).

Preparation of tea infusion Four-hundred g of green tea were extracted in 20 L distilled water at 50°C for 40 min and filtered on 'Double-ring' No.102 filter paper (Xinhua Paper Industry Co., Ltd., Hangzhou, China). The filtrate was centrifuged at 4,000×g at 4°C for 25 min and 17 L of the supernatant was diluted with 34 L distilled water. Tewnty-five L of the diluted tea infusion was heated to 100°C. When the infusion cooled to 90°C, it was filled in

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Received January 6, 2009; Revised March 14, 2009;
Accepted April 8, 2009

350-mL PET bottle and covered with lid. The bottled tea infusion was stood for 5 min and then cooled in cold water.

Treatment with sodium ascorbate To investigate the effect of sodium ascorbate on browning of green tea infusion, sodium ascorbate was dissolved in 25 L of the above remaining diluted tea infusion. The final level of sodium ascorbate was 150 mg/L. The infusion was filled in PET bottle as above.

Treatment of heat and illumination The bottled tea infusion (15 bottles each treatment) was placed in incubators at 40 and 50°C in dark for 30 days. Fifteen bottles of tea infusion for each treatment were placed in illuminated incubators with white light intensity 3,000 and 5,000 lx at 25°C for 30 days, respectively. Two bottles of the infusion were sampled for testing on the 5, 10, 15, 20, and 30th day after treatment.

High performance liquid chromatography (HPLC) analysis The tea infusion was filtered through 0.22-μm Milipore filter before injected into HPLC. Concentration of catechins and caffeine were detected by HPLC (15) and the HPLC condition were as follows:

Injection volume:	10 μL
Column:	5 μm-Diamonsil™ C18 4.6×250 mm
Temperature:	40°C
Mobile phase:	Solvent A: acetonitrile/acetic acid/water (6:1:193, v/v/v) Solvent B: acetonitrile/acetic acid/water (60:1:139, v/v/v)
Gradient:	100%(v/v) solvent A to 100%(v/v) solvent B by linear gradient in 45 min
Flow rate:	1 mL/min
Detector:	Shimadzu SPD UV detector, 280 nm

Analysis of infusion color difference A TC-PIIG automatic color difference meter (Beijing Optical Instrument Factory, Beijing, China) was used to determine Hunter L (lightness-darkness), a (redness-greenness), b (yellowness-blueness), and ΔE (total color difference) as method described as literatures (15).

Statistical analysis The tests in the present paper were carried out in duplicate (2 bottles each sampling) and the mean value of the duplicate tests was presented. Data statistics was carried out on software of SAS 8.0 for Windows.

Results and Discussion

Effect of elevated temperature and illumination on color of tea infusion The 'L' is a parameter of lightness-darkness. The higher the value 'L', the lighter the substance tested. The value 'L' of the tested tea infusions decreased with the elapse of time and differentiated with treatments (Fig. 1A). The 'L' in infusions treated at elevated temperatures, especially that at 50°C decreased more quickly than those of elevated illuminations.

Although greenness-redness parameter 'a' increased with treatment time, treatment under illumination 3,000 lx had negative 'a' at the end of experiment, suggesting its infusion was still green in color. The 'a' of infusion under light intensity 5,000 lx was –0.68 on the 20th and 0.68 on the 30th day, respectively. However, the treatments at 50 and 40°C had positive 'a' values on the 10 and 15th day, respectively (Fig. 1B), showing that the infusions were in the red side on the greenness-redness color scale. The changes in 'b' showed a similar trend as 'a'. On the 30th day after treatment, the tea infusion treated at 50°C had the highest 'b', followed by infusion at 40°C and 5,000 lx. The treatment under illumination of 3,000 lx had the lowest 'b' (Fig. 1C). The study suggests that the effect of elevated temperature on the color of tea infusion was more severe than that of elevated illumination.

The total color difference parameter ΔE of treatment at elevated temperature 50°C increased most quickly, followed by those of 40°C and under illumination 5,000 lx. The change parameter ΔE in sample under illumination 3,000 lx was minimum (Table 1). The time on which ΔE was 6.0 or more was on the zero day for treatment at 50°C and the 20th day for treatment at 40°C. On the 30th day, the ΔE of treatments under the elevated illuminations was still less than 6.0 (Table 1). Our previous study showed that the colors of two objects were distinctly discriminated visually when the ΔE was more than 6 (not published). The present study suggests that there was no significant difference in infusion color between treatments under the elevated temperatures during the experimental period.

Effect of elevated temperature and illumination on levels of catechins Total concentration of detected 8 compounds of catechins (total catechins),which were GC, EGC, C, EC, EGCg, GCg, ECg, and Cg, decreased with elapse of time, and the decline was accelerated after the 15th day (Fig. 2A). The total catechins level in tea infusion treated at 50°C decreased from 239.18 mg/L at the beginning to 122.16 mg/L on the 30th day, with a decline of 49% (Fig. 3). The decreases in total catechins of samples at 40°C and under 3,000 and 5,000 lx were 33, 30, and 27%, respectively (Fig. 2A). The change in EGCg, an abundant component of tea catechins, showed a similar tendency as total catechins (Fig. 2B).

It was interesting that concentrations of (+)-gallocatechin gallate (GCg) of treatments at elevated temperature 50°C increased during the earlier 15 days and then decreased, while the other treatments changed in a contrary manner, i.e., decrease in the early stage and increase in the late stage (Fig. 2C). It was confirmed that epimerization of tea catechins took place when catechins were exposed to elevated temperature conditions and EGCg was partially changed into GCg (8,16). The increase in GCg should be ascribed to the epimerization of EGCg and the decrease in GCg be ascribed to its oxidation. GCg might be an intermediate form in the process of EGCg oxidation. At the earlier 15 days, epimerization of EGCg in treatment at 50°C might be more quickly than the oxidation of GCg, resulting in the accumulation of GCg. However, the epimerization of EGCg was slowed down with the decrease in EGCg in the late stage, leading to the decline in level of GCg (Fig. 2C). The delayed increases in GCg level in the other treatments showed that the responses of

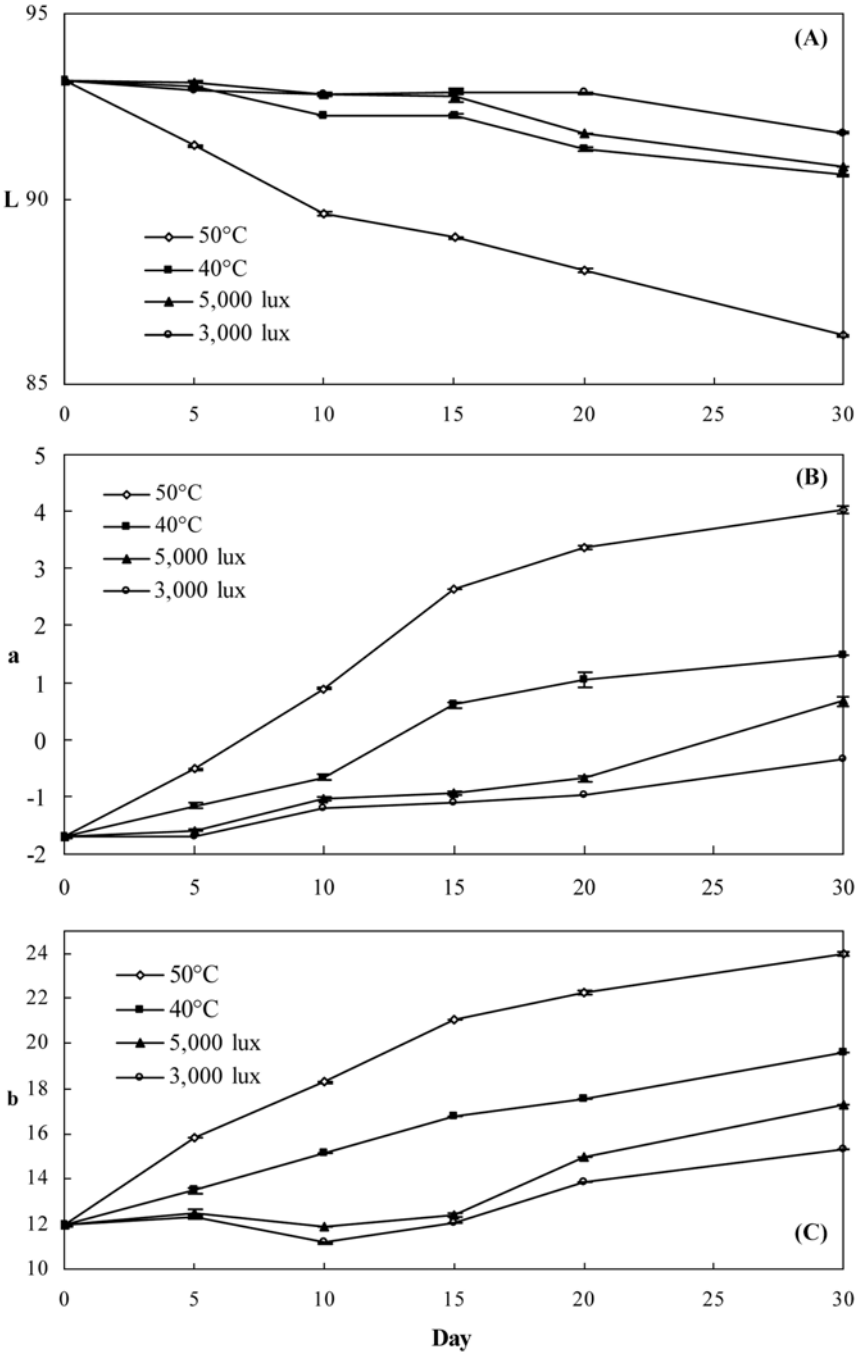

Fig. 1. Effect of treatments and duration on color parameters. A, lightness-darkness parameter L; B, redness-greenness parameter a; C, yellowness-blueness parameter b.

Table 1. Effect of treatments and duration on total color difference parameter ΔE of tea infusion

Day[1)]	50°C	40°C	5,000 lx	3,000 lx
0	0	0	0	0
5	4.20±0.12	1.52±0.06	0.31±0.05	0.57±0.13
10	7.27±0.09	3.35±0.11	0.07±0.02	0.01±0
15	10.29±0.15	5.02±0.08	0.52±0.05	0.15±0.03
20	11.84±0.16	6.00±0.19	3.28±0.17	1.93±0.06
30	14.18±0.13	8.25±0.21	5.82±0.25	3.61±0.14

[1)]The time when the experiment started was defined as 0 day. The ΔE was the difference in total hue value 'E' between the day of sampling and the day when experiment started.

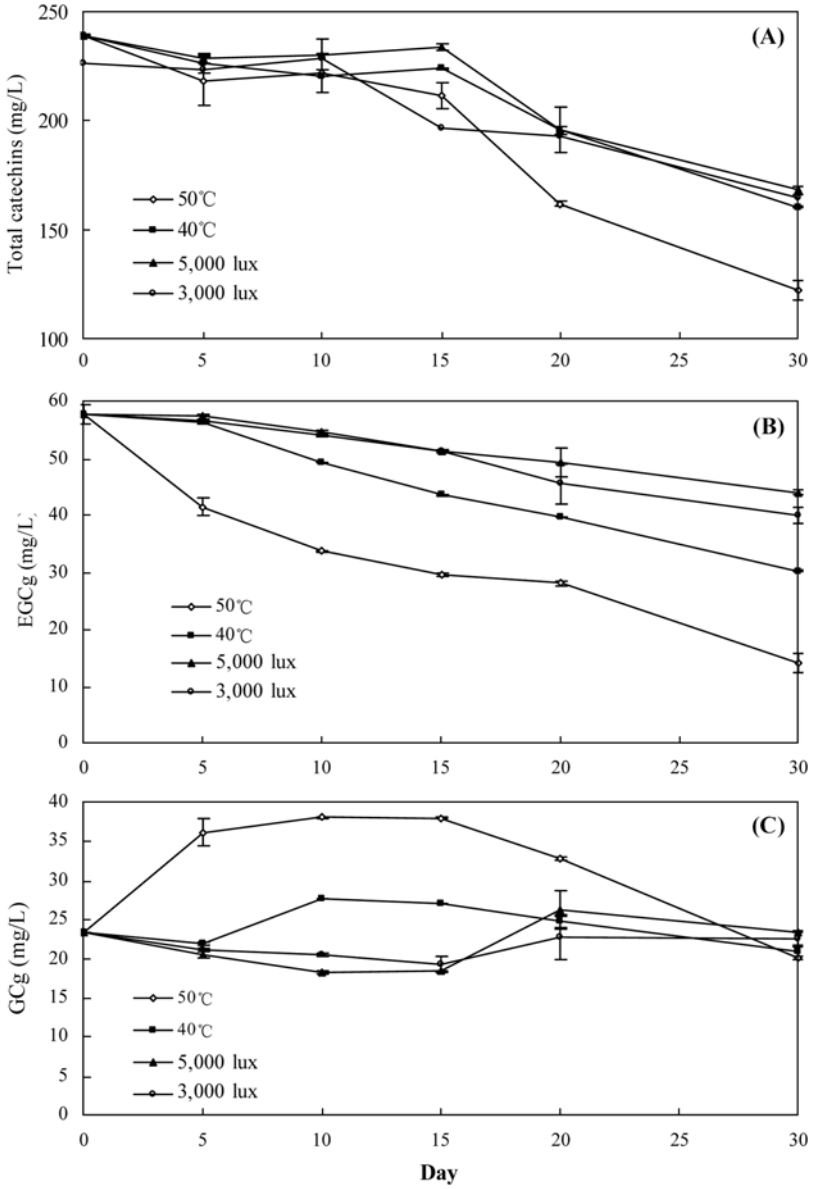

Fig. 2. Effect of treatments and duration on total tea catechins (A), EGCg (B), and GCg (C). EGCg, (-)-epigallocatechin gallate; GCg, (+)-gallocatechin gallate.

EGCg epimerization to lower temperature (40°C) and illumination were tardier. This study suggests that both heat and illumination had effects on the epimerization of EGCg, but the effect of elevated temperature was stronger than elevated illumination.

Relation of color parameters with level of catechins

The oxidation of phenolic compounds were important contributors to color of tea infusion (7,14). During storage and shelf life, the browning of ready-to-drink green tea infusion might be owing to the accumulation of colored oxidation products of tea catechins. The relationship between color parameters and levels of unoxidized catechins showed that the level of total catechins remaining in the tea infusion was positively correlated to the lightness-darkness indicator 'L', but negatively correlated to greenness-redness 'a' and blueness-yellowness 'b' (Table 2). These suggest that the tea infusion became darker, deeper yellow and red in color with the decrease in level of total catechins. The oxidation of tea catechins led to the decrease in the unoxidzied catechins and increase in colored pigments in the tea infusion. The major oxidation products of tea catechins are theaflavins in orange or orange-red color and thearubigins in red-brown or dark brown color (17,18). Level of theaflavin was positively correlated to value 'L', but negatively to values 'a', 'b', and 'ΔE'. It is important to control the oxidation of tea catechins so as to maintain infusion color stable.

The relationships between tea infusion and levels in single component of tea catechins differentiated. The relationship between color parameters and levels of EGC, EC, EGCg, and ECg, which are present in fresh tea leaf, showed the same tendency as total catechins. The levels of the 4 catechins were significantly correlated to color parameters 'L', 'a' and 'b'. However, there were no statistically significant relations of color parameters to levels in (+)-

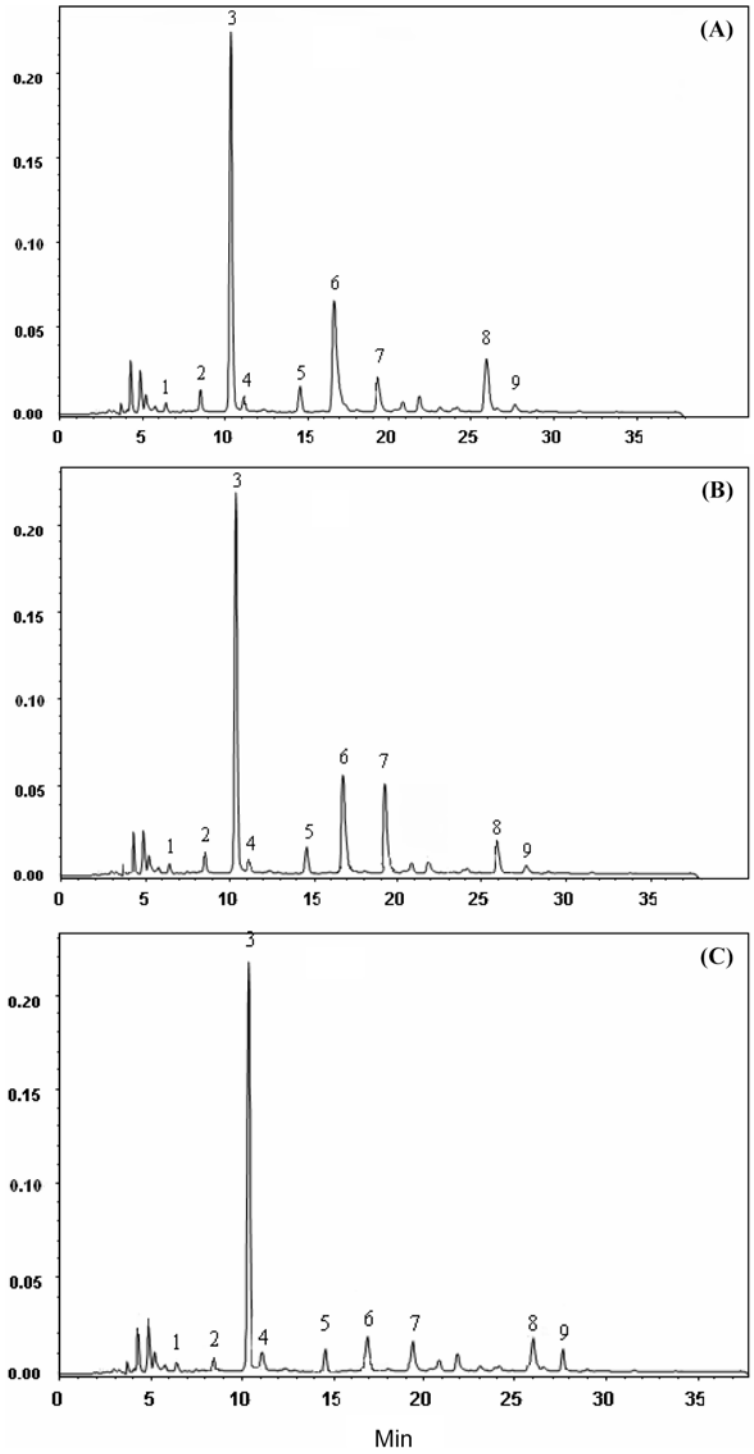

Fig. 3. Effect of treatments and duration on tea catechins. A, HPLC profile of catechins without any treatment; B, HPLC profile of catechins under the treatment of 50°C in dark for 10 days; C, HPLC profile of catechins under the treatment of 50°C in dark for 30 days; 1, GC; 2, EGC; 3, Caffein; 4, C; 5, EC; 6, EGCg; 7, GCg; 8, ECg; 9, Cg.

gallocatechin (GC), (+)-gallocatechin gallate (GCg), and (+)-catechin gallate (Cg) which are not present in fresh tea leaf but are formed during processing due to epimerization of corresponding isomers, except for relation of GC to 'b' in the illuminated treatments (Table 3). The levels of EGC, EC, EGCg, and ECg were continuously declined because of oxidation by action of heat and illumination. But the levels of GC, GCg, and Cg depended on the rate of epimerization of their isomers and oxidation of themselves. This explains why levels of GC, GCg, and Cg fluctuated during treatment and were not significantly correlated to color parameters.

It was interesting that the level of catechin (C) in treatments of elevated temperatures was significantly correlated to the color parameters in contrary directions to the relationships between total catechins and color parameters,

Table 2. Linear correlation coefficients between levels of catechins and tea infusion color parameters

Treatment	Color parameter	GC	EGC	C	EC	EGCg	GCg	ECg	Cg	Total catechins
Heating[1)]	L	-0.1907	0.9508**	-0.8574**	0.9517**	0.9526**	-0.3996	0.9148**	-0.3562	0.8012**
	a	0.0891	-0.9466**	0.9412**	-0.9061**	-0.9472**	0.2722	-0.9168**	0.3121	-0.8267**
	b	0.1069	-0.9603**	0.9380**	-0.9295**	-0.9754**	0.3134	-0.9568**	0.3207	-0.8386**
Illumination[2)]	L	0.4805	0.6817*	0.2462	0.7092*	0.8338**	-0.5260	0.6658*	-0.3358	0.7162*
	a	-0.5616	-0.7424**	0.1423	-0.2515	-0.9055**	0.1767	-0.8020**	-0.0285	-0.7870**
	b	-0.7683**	-0.8699**	-0.2829	-0.6239*	-0.8870**	0.5398	-0.9228**	0.1343	-0.8596**

[1)]Coefficients calculated from data in experiments of elevated temperatures 40 and 50°C.
[2)]Coefficients calculated from data in experiments of illumination with light intensities 3,000 and 5,000 lx.
*p<0.05; **p<0.01; n=11.

Table 3. Effects of sodium ascorbate on color and catechins of tea infusion treated at 50°C

Sodium ascorbate (mg/L)	Temperature (°C)	L	a	b	GC (mg/L)	EGC (mg/L)	C (mg/L)	EC (mg/L)	EGCg (mg/L)	GCg (mg/L)	ECg (mg/L)	Cg (mg/L)	Total catechins (mg/L)
0	40	94.09a[1)]	-1.45c	8.65c	89.3c	123.4a	18.3a	23.4b	177.4a	12.3b	31.4a	0.5a	476.0a
0	50	90.35b	1.59a	16.47a	102.4a	78.4b	18.3a	28.4a	109.4c	30.4a	27.4b	0.4a	395.1b
150	50	93.84a	-1.08b	11.23b	99.4b	120.3a	11.4a	22.3b	152.4b	38.3a	30.4a	0.5a	475.0a

[1)]Data with different letters in a same column were significantly different at p=0.05.

but the correlations were not statistically significant in treatments of illuminations (Table 2). A statistically negative correlation of 'L' to the level of catechin (C), an epimerization product of EC at elevated temperatures, suggests that the C level was increased during the testing period. This implies that the rate of epimerization of EC was more quickly than the oxidation of C under the heating conditions. Under conditions of illuminations at room temperature, the epimerization of EC was weak, resulting in no significant correlation of C level to the color parameters.

Effects of sodium ascorbate on color and catechin concentration of tea infusion The oxidation of tea catechins was confirmed to be important factor inducing browning of tea infusion and ascorbic acid was shown to be effective to suppress the oxidation of tea catechins (11, 12). However, if ascorbic acid was used as anti-browning agent in ready-to-drink tea, the tea infusion would have a sour taste because of decrease in pH level. When sodium ascorbate was used in the tea infusion at a level of 150 mg/L and tested at 50°C for 30 days, the results showed that there were significantly differences in both in infusion color parameters and catechins levels between control and sodium ascorbate treatment (Table 3). There were no significant differences in parameter 'L' and total catechins between tea infusion stored at 40°C and tea infusion treated with sodium ascorbate. However, levels of EGC, EC, EGCg, and ECg in infusion treated with sodium ascorbate decreased, accompanying the increases in GC, C, GCg, and Cg, compared to the infusion stored at 40°C (Table 3). It suggests that epimerization of tea catechins took place though oxidation of tea catechins was suppressed in the presence of sodium ascorbate.

The present study showed that temperature was a more important factor influencing browning of green tea infusion than light, and sodium ascorbate could prevent catechins from oxidation and enhanced the stability of tea infusion color. Because sodium ascorbate has little effect on sourness of tea taste, it is considered to be good choice for prolonging shelf life of ready-to-drink green tea products.

Acknowledgment

The study was financed by a grant from the Ministry of Agriculture of China (Agricultural Commonweal Project No. 3-35).

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