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Effect of Heat Treatment on the Lipophillic Pigments of Fresh Green Tea Liquor

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Abstract Changes in lipophillic pigments concentration and its relation to color of fresh green tea liquor during heat treatment were studied. The results showed liquor greenness decreased markedly with extension of incubation time at 55°C, while the brightness and yellowness changed a little. Significant increase in 'a' and 'b' values of tea liquor was observed at 95°C. Color change of liquor at 55°C was accompanied by a decrease in the level of chlorophylls, lutein and neoxanthin, and an increase in the pheophytins and β-carotene levels. However, all pigments except β-carotene decreased with time extension at 95°C. Significant correlation was found between pigments and color difference index. The browning of fresh green tea liquor was attributed to vicissitudes of lipophillic pigments during heat treatment, especially to the change of chlorophylls/pheophytins ratio. Result also showed addition of Zn^{2+} at 1.6 μmol/L could partially alleviate the decrease in greenness during heat treatment.

Keywords: fresh green tea liquor, heat treatment, lipophillic pigment, color difference, metal ion addition

Introduction

Catechins, the main components in tea products, were proved to possess multitude of health benefits, such as antioxidation (1,2), antimutagenic effect (3), anti-cancer (4,5), and reducing risk of cardiovascular diseases (6). Ready-to-drink (RTD) tea has become popular around the world, especially in Japan and China, as it can be consumed conveniently and, more importantly, contains these functional compounds (7-9).

Unfortunately, green tea beverage is an unstable solution system, which is quite sensitive to heat treatment, such as extraction and pasteurization or sterilization. Thus, production of green tea extract in cans or bottles is more problematic than that of black tea or *ulong* tea extracts (10). Thermal processing improves the extraction efficiency and extends the shelf life of tea beverage, but it often leads to unexpected changes in beverage color. The color of green tea beverage is the first quality factor that the consumer appreciates and has a remarkable influence on its acceptance. Thus, keeping the original color of the green tea beverage throughout the processing and storage is a major and difficult goal for researchers and processors (7,9,11). Manzocco *et al.* (11) reported that pasteurization and storage caused an increase in the optical density at 390 nm of the tea beverages. When changes in color of green tea liquor during thermal processing and storage were expressed as 'L', 'a' and 'b' values, a decrease in 'L' value and increases in 'a' and 'b' values indicated the development of a brown color (9). During pasteurization, with increase in temperature from 85 to 120°C, the green tea liquor became darker and less green, but deeper yellow in color (7).

Compared to the extraction from dried made green tea, the fresh green tea beverage processed from steam-denatured tea leaves is much greener in color and therefore, more prone to color browning. Kim *et al.* (7) reported that epigallocatechin gallate, epigallocatechin, epicatechin, and epicatechin gallate partially epimerized and concentration of total catechins decreased in normal green tea beverage during pasteurization. The decrease in total phenolic content was mainly due to the fact that the phenolic compounds in green tea liquor were oxidized or polymerized during processing and storage (9). It was suggested that the brown color of green tea beverage was probably resulted from the catechins oxidation and epimerization. Research also showed that the greenish-yellow color of edible oils was due to various lipophillic pigments, i.e., chlorophylls, pheophytins, and carotenoids, though these compounds presented in minute amount (12). Degradation of these pigments after illumination exposition would cause the color change (12,13). In orange juice, browning reaction was mainly due to the variation of carotenoid pigments (14). But up to present, very little information about browning mechanism of fresh green tea beverage during heat treatment, such as heat extraction was available. In this work, we studied about the changes of lipophillic pigments in fresh green tea liquor and its relationship with color variation. Metallo-chlorophyll complexes are similar in color to natural chlorophylls, however, they survive heat treatment as observed in the food processing industry (15-17). Therefore, attempt was also made to preserve the color of fresh green tea by addition of metal ion.

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Materials and Methods

Materials Chlorophylls (*Chl*) (Wacay Intl Inc., Rowland Heights, CA, USA), β-carotene (*Car*), and lutein (*Lut*) (Sigma-Aldrich, St Louis, MO, USA) were dissolved in acetone as references for high performance liquid chromatography (HPLC). Highly pure neoxanthin (*Neo*) was prepared in lab by thin layer chromatography (TLC) according to method described as Rodriguez-Amaya (18). Pheophytins (*Phe*) were obtained by adding suitable hydrochloric acid (0.1 mol/L) into chlorophyll solution. The other chemical reagents used were of HPLC grade (Tedia Company Inc, Fairfield, OH, USA), except where stated otherwise.

Tea shoots with 3 or 4 leaves and a bud (*Camellia sinensis* cv. Zhenong 139) were harvested from tea garden (Zhejiang University Tea Research Institute, Huaji Campus, Hangzhou, China), and 'fixed' by steam at 95-100°C for 45-60 sec to inactivate enzymes, grinded with TJ-12 rotor grinder (Henglian Food Machinery Factory, Fanou, Guangdong, China) and stored at –20°C until use.

Fresh green tea liquor preparation One-hundred-and-twenty g steam-denatured leave samples were put into beaker with 3 L distilled water (40°C). The beaker was placed in a SK250HP ultrasonic washer (Shanghai Cany Precision Instrument Co., Ltd., Shanghai, China) to perform ultrasound-assisted extraction at 59 kHz, 100 W, and 40°C for 10 min. Infusion was filtered through a cotton wool. The filtrate was then centrifuged at 5,478×g and 4°C for 20 min (Beckman J2 HS; Beckman Instruments Inc., Fullerton, CA, USA). The supernatants were collected carefully and stored at 4°C.

Heat treatment The fresh green tea liquor (25 mL) was transferred into a 25-mL volumetric bottle and then incubated at 55, 75, and 95°C for 15, 30, 45, 60, and 120 min in water bath. The treated liquor was cooled to room temperature and its volume was readjusted to 25 mL with distilled water. The liquor without incubation was used as control and the experiments were repeated 3 times.

Color preservation test Forty-nine mL fresh green tea liquor was transferred into 50-mL volumetric bottles, followed by adding different amount of Zn^{2+} stock solution ($ZnCl_2$, 800 μmol/L), and final volume was made-up to 50 mL with distilled water. The final Zn^{2+} concentration in liquor samples were 0, 0.2, 0.4, 0.8, 1.6, and 3.2 μmol/L, respectively. The liquors were then incubated at 95°C for 30 min and then cooled to room temperature. After incubation and cooling as previously, the total volume of the liquor was readjusted to 50 mL. The experiments were repeated 3 times.

Analysis of pigments HPLC (Model LC20A; Shimadzu Co, Kyoto, Japan) was used to estimate chlorophylls and carotenoids in liquor. The HPLC analysis conditions were as follows: injection volume, 20 μL; column, TC-C_{18} 5 μ, 4.6×150 mm (Agilent Technologies Inc., Santa Clara, CA, USA); oven temperature, 35°C; mobile phase A, acetonitrile/acetic acid/water (6:1:193); mobile phase B, acetonitrile/methanol/chloroform (75:15:10); gradient elution, 80% mobile phase B to 100% mobile phase B by linear gradient increasing during the early 20 min and holding at 100% mobile phase B for further 15 min; flow rate, 1 mL/min; detector, Shimadzu SPD-20A ultraviolet-visible detector (UV-VIS) at 450 nm (Shimadzu Co, Kyoto, Japan). Pigments were identified by comparing the retention time and absorption spectra with that of the authentic standard. The operation details were described in previous paper (19).

Measurement of color change Transmission color difference in various treatments was measured by using Hunterlab Colorquest XE (Hunter Associates Laboratory Inc., Reston, VA, USA). The detail operation was carried out as described as previous paper (20).

Data analysis and reaction model fitness The tests in the present paper were in triplicate, and the mean±standard deviation (SD) of the triplicate tests was presented. Statistics was carried out on Version 8.1 SAS System for Windows (SAS Institute Inc, Cary, NC, USA) software.

Three different kinetic models, i.e., zero-order ($c_1=c_0+k_0t$, where c_1 is observation value when treatment time is 't', c_0 is the initial observation value when time is 0, k_0 is the rate constant of zero-order model, t is the treatment time), first-order ($\mathrm{Ln}\ c_1=\mathrm{Ln}c_0+k_1t$, where k_1 is the rate constant of first-order model) and second-order ($1/c_1=1/c_0+k_2t$, where k_2 is the rate constant of second-order model), were tried for describing the changes of color index and pigments concentration during heat treatment. The rate constants of the 3 models were calculated by least squares estimation from the observed dataset, and the most suitable model was selected and presented based on its *p* value.

Results and Discussion

Liquor color changes after heat treatment Table 1 showed that no significant difference in 'L' value was obtained in liquor before and after incubation at 55°C. The value 'a' increased significantly with extension of the incubation time. However, at the end of incubation, 'a' value was still negative, which implied the color of liquor still remained green after incubation at 55°C for 120 min. The value 'b' increased gradually with prolongation of incubation time, and it was significantly higher after incubating for 60-120 min than that of control. During incubation at 75°C, 'a' and 'b' values of the liquor increased significantly with treatment time, while the 'L' value decreased significantly after 30 min of incubation. Compared to the control, significant decrease in 'L' value and significant increase in 'a' and 'b' values were observed when liquor was incubated at 95°C. When total color difference [$\Delta E=\{(L_i-L_0)^2+(a_i-a_0)^2+(b_i-b_0)^2\}^{1/2}$, where L_i, a_i, b_i: the L, a, b value of the treatment liquor; L_0, a_0, b_0: L, a, b value of the control liquor] of the liquor was calculated, it increased with extension of incubation time at the tested temperatures, but the change of ΔE was much quicker at higher incubation temperatures than those at lower temperatures (Table 1). These results were consistent with the previous studies (7,9,11). After incubation at 95°C for more than 30 min, 'a' value shifted from negative to positive scale, which implied the color of liquor became

Table 1. Changes of color difference index during heat treatment of fresh green tea liquor[1)]

Temperature (°C)	Time (min)	L	a	b	ΔE[2)]
Control		96.73±0.06g	-2.70±0.07a	10.81±0.11a	/
55	15	96.82±0.07g	-2.39±0.07b	10.90±0.05a	0.35±0.06a
55	30	96.76±0.07g	-1.94±0.10c	10.90±0.05a	0.77±0.09b
55	45	96.74±0.09g	-1.56±0.06d	11.02±0.09ab	1.16±0.07c
55	60	96.71±0.03g	-1.42±0.10e	11.09±0.12bc	1.41±0.14de
55	120	96.53±0.06g	-1.25±0.07f	11.16±0.08bc	1.51±0.07de
75	15	96.65±0.16g	-2.27±0.11b	10.89±0.05a	0.46±0.13a
75	30	96.20±0.15f	-1.72±0.07c	11.21±0.13cd	1.19±0.12cd
75	45	95.99±0.10f	-1.44±0.09de	11.37±0.08d	1.57±0.03ef
75	60	95.61±0.08e	-1.18±0.04f	11.85±0.09f	2.16±0.06g
75	120	95.22±0.40d	-0.61±0.08g	14.27±0.18g	4.33±0.27i
95	15	96.04±0.09f	-0.50±0.09g	11.68±0.10e	2.46±0.13h
95	30	95.49±0.05de	0.09±0.04h	14.85±0.13h	5.09±0.17j
95	45	94.91±0.23c	0.83±0.06i	17.00±0.15i	7.35±0.19k
95	60	93.69±0.16b	1.86±0.07j	20.90±0.04j	11.49±0.05l
95	120	91.24±0.27a	3.31±0.12k	26.30±0.09k	17.50±0.18m

[1)]Data showed as mean±SD, and the different letter in column indicated significant difference at $p=0.05$.
[2)]$\Delta E=[(L_i-L_0)^2+(a_i-a_0)^2+(b_i-b_0)^2]^{1/2}$, where L_i, a_i, b_i : the L, a, b values of the treatment liquor; L_0, a_0, b_0: L, a, b values of the control liquor.

Table 2. Rate constants (k_0) by zero-order reaction in color change of fresh green tea liquor[1)]

	L	a	b	ΔE
55°C	-0.0027	0.0097	0.0027	0.0100
	$p<0.0001$	$p=0.0003$	$p=0.0006$	$p=0.0001$
75°C	-0.0127	0.0145	0.0330	0.0362
	$p<0.0001$	$p<0.0001$	$p<0.0001$	$p<0.0001$
95°C	-0.0464	0.0365	0.1365	0.1431
	$p<0.0001$	$p<0.0001$	$p<0.0001$	$p<0.0001$

[1)]Three different reaction models were tried, and zero-order reaction ($c_1=c_0+k_0t$) showed good fitness; rate constant k_0 and significance (p value) were presented.

red. When heating for 120 min, 'L' value decreased by 5.7%, 'a' and 'b' value increased by 222.6 and 143.3% respectively, compared to the control. Therefore, relatively less color change was observed at 55°C, and liquor color remained slight greenish-yellow at the end of the treatment. However, when the liquor was incubated at 95°C, the color varied dramatically and turned deeper yellow and red from greenish-yellow. It was suggested that the green colored pigments were much more sensitive to heating than the yellow colored pigments, or degradation of compounds with green color and formation of compounds with yellow and red color were simultaneously occurred in liquor during heat treatment.

Analysis also showed that changes in color difference index followed zero-order reaction kinetics at 3 different incubation temperatures, and negative rate k_0 was obtained in change of 'L' value, while positive k_0 in change of 'a', 'b', and ΔE. Moreover, absolute value of k_0 for 'L', 'a', 'b', and ΔE increased with incubation temperature (Table 2). These results were consistent with study focusing on the change of salmon color during thermal processing (21).

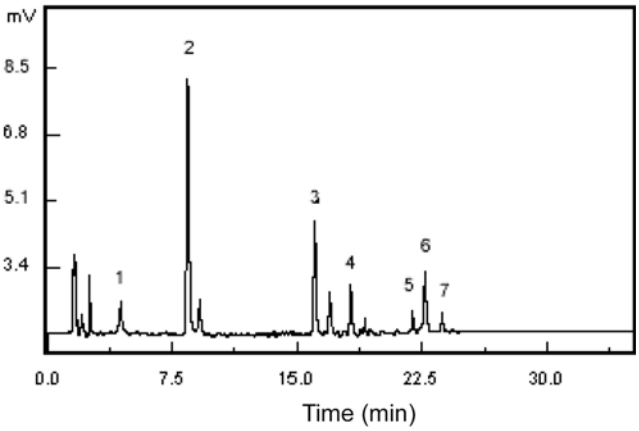

Fig 1. HPLC separation of pigments in fresh green tea liquor. Peak 1, neoxanthin; peak 2, lutein; peak 3, chlorophyll *b*; peak 4, chlorophyll *a*; peak 5, pheophytin *b*; peak 6, ß-carotene; peak 7, pheophytin *a*.

Pigments change in liquor after heat treatment HPLC analysis showed that abundant pigments were *Lut* (755.3 nmol/L), *Phe a* (508.6 nmol/L), *Chl b* (415.0 nmol/L), and *Car* (311.3 nmol/L) in fresh green tea liquor (control), and concentrations of *Chl a*, *Neo,* and *Phe b* were of 143.0, 66.9, and 57.4 nmol/L, respectively (Fig. 1, Table 3). During incubation at 55°C, concentration of *Chl b*, *Chl a*, *Lut,* and *Neo* decreased with the treatment time, but the concentration of *Phe b* and *Car* were increased gradually. *Phe a* increased up to 45 min and then decreased. Statistically, *Chl b*, *Chl a,* and *Neo* concentrations of the liquor incubated at 55°C for 15 min were significantly lower than that of control, while *Phe a* was significantly higher than control. Moreover, significant decrease in *Lut* concentration was observed after 120 min treatment, while significant increase in *Car* concentration was witnessed after 30 min

Table 3. Changes of pigments contents during the heat treatment of fresh green tea liquor (nmol/L)[1)]

Temperature (°C)	Time (min)	*Neo*	*Lut*	*Chl b*	*Chl a*	*Phe b*	*Car*	*Phe a*
Control		66.9±3.4f	755.3±12.3g	415.0±4.0k	143.0±14.2c	57.4±4.1b	311.3±13.0a	508.6±20.7cde
55	15	57.2±0.7e	755.4±10.6g	304.9±9.4j	28.1±6.4b	58.7±3.7b	312.6±10.8a	540.0±9.4f
55	30	56.5±3.3e	752.0±8.8g	237.1±12.3i	27.1±4.5b	97.6±13.5c	398.2±5.9b	539.0±15.9f
55	45	54.1±3.7e	741.0±11.0fg	210.4±3.6h	0.0a	132.4±2.7e	449.4±8.5c	572.5±11.4g
55	60	54.2±0.3e	736.6±4.1efg	156.8±4.1g	0.0a	143.6±5.7e	482.9±4.1efg	516.8±2.5def
55	120	53.4±1.1e	729.7±3.5def	102.7±6.5d	0.0a	162.5±7.2g	490.7±6.0fg	504.9±16.7cde
75	15	56.8±2.2e	746.3±13.2fg	144.5±6.8f	0.0a	141.7±3.9f	467.2±6.7d	529.3±8.7def
75	30	55.0±4.9e	744.3±2.7fg	120.7±10.4e	0.0a	140.0±17.9ef	476.5±8.1de	521.9±36.9def
75	45	38.8±2.4c	743.6±10.7fg	86.4±5.4c	0.0a	128.0±5.1e	478.2±1.5def	505.0±15.6cde
75	60	36.4±2.2c	725.4±9.6cde	83.0±3.4c	0.0a	111.8±4.0d	479.7±11.5def	502.0±12.9cd
75	120	30.6±5.9b	721.3±15.1bcde	0.0a	0.0a	101.2±3.1cd	494.3±8.0g	481.1±30.7c
95	15	47.2±0.8d	705.0±10.3b	99.8±0.8d	0.0a	139.5±9.7ef	481.5±8.7efg	533.6±6.0ef
95	30	38.5±0.6c	720.7±13.8bcde	17.3±7.0b	0.0a	139.8±4.3ef	518.9±3.0h	527.4±15.4def
95	45	33.4±1.6b	716.1±4.1bcd	0.0a	0.0a	127.8±1.8e	522.9±2.3h	391.4±11.1b
95	60	32.8±2.2b	709.9±9.5bc	0.0a	0.0a	93.9±6.0c	518.8±10.1h	379.1±24.6b
95	120	14.1±5.2a	568.8±7.5a	0.0a	0.0a	13.5±9.0a	311.9±7.2a	98.1±9.5a

[1)] *Neo*=neoxanthin, *Lut*=lutein, *Chl b*=chlorophyll *b*, *Chl a*=chlorophyll *a*, *Phe b*=pheophytin *b*, *Car*=β-carotene, *Phe a*=pheophytin *a*. Data showed as mean±SD, and the different letter in column indicated statistically significant difference at $p=0.05$.

treatment. *Chl a* disappeared after 45 min of treatment, meanwhile, *Phe a* in liquor reached to the peak value, as 112.6% high as the control. Compared to control, *Phe b* and *Car* increased by 183.1 and 57.6% at the end of treatment, respectively.

Incubation at 75°C showed a similar pattern of pigment changes with that of 55°C treatment, i.e., continually declining in *Neo*, *Lut,* and *Chl b* concentrations while increment in *Car* were observed with prolongation of treatment time (Table 3). Concentrations of *Phe a* and *Phe b* reached to the peak value after incubation at 75°C for 15 min, which increased by 146.9 and 4.1% compared to the control, while *Chl a* was below detection level at that time. *Chl b* also disappeared from liquor after 120 min treatment.

Variation trend of pigments concentration in green tea liquor incubated at 95°C was similar to that of 55 and 75°C incubation, but the range of variation was much severe (Table 3). Compared to the control, concentrations of *Neo*, *Lut,* and *Chl b* in liquor after incubation at 95°C for 15 min decreased by 29.5, 6.7, and 76.0%, meanwhile, *Phe b*, *Car,* and *Phe a* increased by 143.0, 54.7, and 4.9%, respectively. However, with further extension of treatment time concentration of all pigments decreased significantly.

Rates of chlorophylls/pheophytins (*Chl/Phe*) and chlorophylls/(carotenoids and pheophytins) (*Chl/Y*) were calculated. Change in *Chl/Phe* with incubation temperature and time was similar to *Chl/Y*. During heat treatment, these rates decreased significantly with extending the treatment time (Fig. 2).

Three different kinetic models, i.e., zero-order, first-order, and second-order, were fitted in changes of pigments during heat treatment. Pigments variations at the different temperature were best described by first-order reaction kinetics (Table 4). This was in line with finding of Chen and Liu (13) who reported the degradation of *Chl a* and *Car* in edible oil fitted the first-order model during illumination

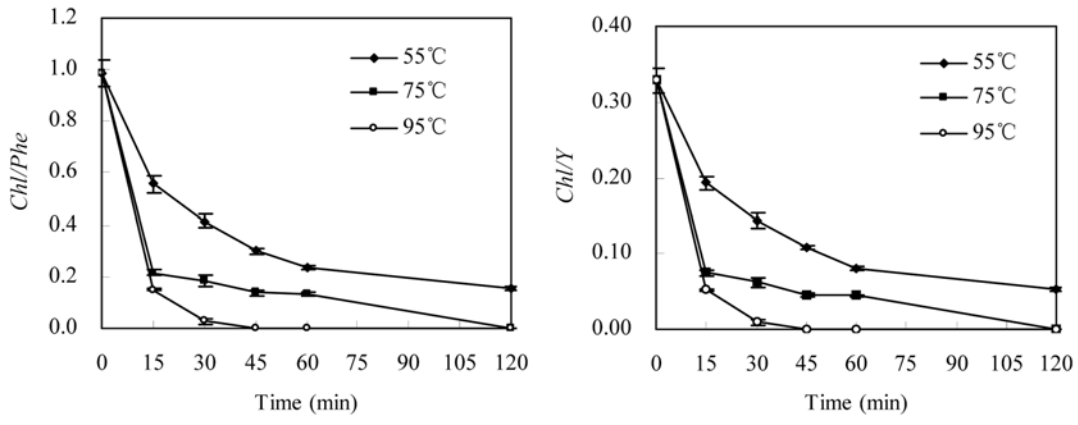

Fig 2. Variation of *Chl/Phe* and *Chl/Y* during heat treatment. *Chl/Phe*=chlorophylls/pheophytins; *Chl/Y*=chlorophylls/(carotenoids+pheophytins).

Table 4. Rate constants (k_1) by first-order reaction in change of pigments concentration[1)]

Temperature	*Neo*	*Lut*	*Chl b*	*Chl a*	*Phe b*	*Car*	*Phe a*	*Chl/Phe*	*Chl/Y*
55°C	-0.0006	-0.0003	-0.0101	-0.0018	0.0081	0.0035	-0.0008	-0.0118	-0.0119
	p=0.0483	p=0.0012	p<0.0001	p>0.05	p=0.0004	p=0.0008	p=0.0161	p<0.0001	p<0.0001
75°C	-0.0061	-0.0004	-0.0133	-	-0.0034	0.0005	-0.0009	-0.0112	-0.0122
	p<0.0001	p=0.0055	p<0.0001	-	p<0.0001	p=0.0006	p=0.0101	p<0.0001	p<0.0001
95°C	-0.0117	-0.0023	-0.1202	-	-0.0256	-0.0047	-0.0169	-0.1196	-0.1216
	p<0.0001	p<0.0001	p=0.0012	-	p<0.0001	p<0.0001	p<0.0001	p=0.0014	p=0.0012

[1)]*Neo*=neoxanthin, *Lut*=lutein, *Chl b*=chlorophyll *b*, *Chl a*=chlorophyll *a*, *Phe b*=pheophytin *b*, *Car*=β-carotene, *Phe a*=pheophytin *a*, *Chl/Phe*=chlorophylls/pheophytins, *Chl/Y*=chlorophylls/(carotenoids+pheophytins). Rate constants k_1 of first-order reaction ($Lnc_1=Lnc_0+k_1t$) and significance (p value) were presented.

for 24 hr. Table 4 showed that k_1 for *Neo*, *Lut*, *Chl b*, *Phe a*, *Chl/Phe,* and *Chl/Y* was negative at three different temperature, while the k_1 for *Phe b* and *Car* was positive at low treatment temperature and negative at high temperature. Absolute value of rate constant k_1 increased with incubation temperature. It indicated that high temperature would accelerate reaction speed. During incubation at 95°C, the order of the absolute k_1 for different pigments could be described as: *Chl b*>*Phe b*>*Phe a*>*Neo*>*Car*>*Lut*. These results indicated that *Chl* were greatly unstable and easily broken down by removal of Mg^{2+} during heat treatment, therefore, *Phe* concentration increased at the early incubation stage. But *Phe* were also prone to destroying under continual incubation condition. Although the concentration of *Lut* and *Neo* also declined with increasing of incubation temperature and extension of treatment time, their variations were obviously less than *Chl*. It was suggested that heat sensitivity of carotenoids was lower than *Chl*. This result was consistent with some studies in vegetable storage (22,23). Cichelli and Pertesana (12) reported that the *Chl b*, *Phe,* and carotenoids were always detectable except *Chl a* in edible oils although concentration of *Chl a* was much higher than *Chl b* in raw materials, which revealed that *Chl a* was more unstable than other pigments (13). In present work, result also showed that *Chl a* was more susceptible than *Chl b* because *Chl a* disappeared earlier than *Chl b* and other pigments during heat treatment. Unlike *Chl*, *Phe,* and other carotenoids pigments, concentration of *Car* increased with incubation time. It was suggested that *Car* was relatively insensitive to heating incubation, and that there might be some combined precursors or isomers in liquor which could be transformed to *Car* after heat treatment. Lessin *et al.* (24) also found that canning increased the percentage of some isomers of carotenoids having provitamin A activity in several fruits and vegetables. Because of relative stability of carotenoids (yellow and orange color), instability of *Chl* (green color) and formation of *Phe* (dark brown or yellow brown color), browning was occurred in fresh green tea liquor after heat treatment. This result was in concert with the previous color difference measurement.

Correlation between color difference index and pigments concentration Under 55°C incubation condition, *Chl b*, *Chl a*, *Chl/Phe*, *Chl/Y*, *Lut,* and *Neo* were negatively and significantly correlated with 'a', 'b', and ΔE, and positively with 'L' value. *Phe b* and *Car* was positively correlated with 'a', 'b', and ΔE, and negatively with *L* value (Table 5), whilst no significant relation between *Phe a* and color difference was found. It was suggested that liquor with higher *Chl*, *Chl/Phe,* and *Chl/Y* and lower *Phe b* and *Car* showed smaller value of 'a' and 'b' and bigger 'L' value, and in consequence, liquor looked much green and clear, less yellow in appearance. Therefore, the change of liquor color at low incubation temperature might directly result from the vicissitudes of lipophillic pigments. A similar significant correlation between pigments and color

Table 5. Correlation between color index and pigments concentration during heat treatment[1)]

		Neo	*Lut*	*Chl b*	*Chl a*	*Phe b*	*Car*	*Phe a*	*Chl/Phe*	*Chl/Y*
55°C	L	0.410	0.556*	0.817**	0.590**	-0.684**	-0.630*	0.381	0.777**	0.778**
	a	-0.553*	-0.785**	-0.942**	-0.892**	0.954**	0.972**	-0.340	-0.974**	-0.972**
	b	-0.701**	-0.575*	-0.813**	-0.767**	0.776**	0.746**	-0.451	-0.810**	-0.813**
	ΔE	-0.568*	-0.787**	-0.947**	-0.893**	0.956**	0.969**	-0.357	-0.976**	-0.974**
75°C	L	0.773**	0.565*	0.887**	-	0.814**	-0.706**	0.710**	0.867**	0.879**
	a	-0.866**	-0.651**	-0.949**	-	-0.851**	0.791**	-0.668**	-0.926**	-0.941**
	b	-0.726**	-0.650**	-0.958**	-	-0.802**	0.780**	-0.594*	-0.966**	-0.963**
	ΔE	-0.795**	-0.654**	-0.980**	-	-0.842**	0.798**	-0.661**	-0.976**	-0.980**
95°C	L	0.938**	0.874**	0.601*	-	0.974**	0.811**	0.964**	0.601**	0.600*
	a	-0.944**	-0.797**	-0.683**	-	-0.944**	-0.720**	-0.953**	-0.684**	-0.681**
	b	-0.947**	-0.786**	-0.715**	-	-0.938**	-0.707**	-0.946**	-0.715**	-0.713**
	ΔE	-0.946**	-0.811**	-0.678**	-	-0.952**	-0.737**	-0.955**	-0.679**	-0.677**

[1)]*Neo*=neoxanthin, *Lut*=lutein, *Chl b*=chlorophyll b, *Chl a*=chlorophyll a, *Phe b*=pheophytin b, *Car*=β-carotene, *Phe a*=pheophytin a, *Chl/Phe*=chlorophylls/pheophytins, *Chl/Y*=chlorophylls/(carotenoids+pheophytins); *p<0.05, **p<0.01; n=15.

Table 6. Changes of color difference index in green tea liquor after Zn^{2+} addition and incubation at 95°C for 30 min[1)]

Zn^{2+} (μmol/L)	L	a	b	ΔE[2)]
0	95.01±0.03c	0.09±0.03d	14.95±0.15ab	/
0.2	95.20±0.06c	-0.22±0.03c	14.82±0.07a	0.40±0.03a
0.4	94.77±0.15c	-0.37±0.11bc	15.30±0.22bc	0.64±0.20a
0.8	94.69±0.09bc	-0.46±0.05b	15.54±0.11c	0.87±0.13a
1.6	93.84±0.41b	-0.88±0.04a	16.24±0.17d	2.01±0.34b
3.2	92.80±0.10a	-0.77±0.23a	15.23±0.15bc	2.39±0.15b

[1)]Data showed as mean±SD, and the different letter in column indicated statistically significant difference at $p=0.05$.
[2)]$\Delta E=((L_i-L_0)^2+(a_i-a_0)^2+(b_i-b_0)^2)^{1/2}$, where L_i, a_i, b_i: the L, a, b values of the treatment liquor with Zn^{2+} addition; L_0, a_0, b_0: L, a, b values of the control liquor without Zn^{2+} addition.

difference index was observed in liquor incubated at 75°C. But unlike to 55°C treatment, *Phe* was negatively correlated with 'a' and 'b', and ΔE, and positively with 'L', significantly. Under 95°C incubation condition, correlation coefficients between pigments concentration and color difference were smaller than that of low incubation temperature, but all the pigments except *Chl a* were significantly correlated with 'L' value positively, and with 'a' and 'b', and ΔE negatively. It indicated that liquor with less lipophillic pigments would display less bright and green, but much yellow. From these results, we might argue that degradation of lipophillic pigments might be responsible for the deterioration of the brightness and greenness in liquor incubated at high temperature, but not be responsible for the increment of yellowness by deeper color materials formation after the degradation of lipophillic pigments. Thus, Maillard reaction, carbonyl compounds formed from L-ascorbic acid degradation and oxidation of phenolic compounds might also be in charge of increasing in 'b' value during 95°C incubation. Because Maillard reaction usually took place in liquid food incubated at high temperature and leaded to forming of nonenzyme browning (25,26). Roig *et al.* (27) reported that degradation of L-ascorbic acid and formation of carbonyl compounds were the major contributors to browning in citrus juice. Although epicatechin and epigallocatechin gallate, 2 main catechins in tea liquor, were able to inhibit formation of Maillard browning compounds in a gluocose/glycine model system and food system (28), catechins themselves in liquor were subjected to epimerization and oxidation (7,9,29) and polymerization with anthocyanins (30) during heat treatment, which would ultimately cause formation of brown-colored macromolecular products (13,30). Therefore, in order to preserve the color of fresh green tea liquor, some measures should be taken simultaneously, such as reducing the degradation of the lipophillic pigments and oxidation of catechins.

Effect of Zn^{2+} on liquor color Table 6 showed that Zn^{2+} addition significantly lessen the 'a' value in the liquor incubated at 95°C for 30 min. 'L' value decreased with Zn^{2+} addition, and significant difference was observed when Zn^{2+} level was higher than 1.6 μmol/L. High dosage of Zn^{2+} would also heighten the yellowness of the liquor. Furthermore, the 'L' ($r=0.9712$, $p<0.0001$), 'a' ($r=-0.7920$, $p=0.0001$), and ΔE ($r=0.9274$, $p<0.0001$) values of liquor were correlated significantly with the level of Zn^{2+} addition. It was well known that chelates of Zn-chlorophylls preserved green color and were more resistant to heat treatment than Mg-chlorophylls (15-17), Zn^{2+} addition could substitute the Mg^{2+} position of chlorophyll molecules and make the liquor much greener than the one without Zn^{2+} addition. Thus, it was proved that chlorophylls degradation by removal of Mg^{2+} was responsible for deterioration of greenness of liquor during heat treatment. However, Zn^{2+} addition could not take green level back to original fresh green tea liquor (without heat treatment), although it could partially alleviate or reverse the decreasing of greenness. It was suggested that other degradations of chlorophylls besides Mg^{2+} removal, such as isomerization, allomerization, and removal of phytol chain (22,31,32), might also occurred in green tea liquor incubated at high temperature.

According to the WHO/FAO document (33), the recommended dietary allowance of Zn^{2+} for adult men is set at 14.0 mg/day, for adult women 9.8 mg/day, therefore, the addition of Zn^{2+} in this work can be accepted because the ingestion of Zn^{2+} by drinking the green tea liquor would be lower than 1 mg/day if the consumption volume is no more than 3 L. However, when addition of Zn^{2+} was used for ameliorating the color of green tea liquor, some care should also be taken on the amount of additive because high level of Zn^{2+} would adversely affect the brightness and yellowness of the liquor.

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References

1. Panza VSP, Wazlawik E, Schütz GR, Comin L, Hecht KC, Silva EL. Consumption of green tea favorably affects oxidative stress markers in weight-trained men. Nutrition 24: 433-442 (2008)
2. Nagle DG, Ferreira D, Zhou YD. Epigallocatechin-3-gallate (EGCG): Chemical and biomedical perspectives. Phytochemistry 67: 1849-1855 (2006)
3. Gupta S, Saha B, Giri AK. Comparative antimutagenic and anticlastogenic effects of green tea and black tea: A review. Mutat. Res. 512: 37-65 (2002)
4. Fujiki H, Suganuma M, Imai K, Nakachi K. Green tea: Cancer preventive beverage and/or drug. Cancer Lett. 188: 9-13 (2002)
5. Khan N, Mukhtar H. Multitargeted therapy of cancer by green tea polyphenols. Cancer Lett. 269: 269-280 (2008)
6. Khan N, Mukhtar H. Tea polyphenols for health promotion. Life Sci. 81: 519-533 (2007)
7. Kim ES, Liang YR, Jin J, Sun QF, Lu JL, Du YY, Lin C. Impact of heating on chemical compositions of green tea liquor. Food Chem. 103: 1263-1267 (2007)
8. Liang YR, Lu JL, Zhang LY. Comparative study of cream in

infusions of black tea and green tea [*Camellia sinensis* (L) O. Kuntze]. Int. J. Food Sci. Tech. 37: 627-634 (2002)
9. Wang LF, Kim DM, Lee CY. Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. J. Agr. Food Chem. 48: 4227-4232 (2000)
10. Yamanishi T, Hara Y, Luo S, Wickremasinghe RL. Special issue on tea. Food Rev. Int. 11: 371-546 (1995)
11. Manzocco L, Anese M, Nicoli MC. Antioxidant properties of tea extracts as affected by processing. Lebensm. -Wiss. Technol. 31: 694-698 (1998)
12. Cichelli A, Pertesana GP. High-performance liquid chromatographic analysis of chlorophylls, pheophytins and carotenoids in virgin olive oils: Chemometric approach to variety classification. J. Chromatogr. A 1046: 141-146 (2004)
13. Chen BH, Liu MH. Relationship between chlorophyll a and β-carotene in a lipid-containing model system during illumination. Food Chem. 63: 207-213 (1998)
14. Cortés C, Esteve MJ, Frígola A. Color of orange juice treated by high intensity pulsed electric fields during refrigerated storage and comparison with pasteurized juice. Food Control 19: 151-158
15. Tonucci LH, Von Elbe JH. Kinetics of the formation of zinc complexes of chlorophyll derivatives. J. Agr. Food Chem. 40: 2341-2344 (1992)
16. LaBorde LF, von Elbe JH. Chlorophyll degradation and zinc complex formation with chlorophyll derivatives in heated green vegetables. J. Agr. Food Chem. 42: 1100-1103 (1994)
17. Canjura FL, Watkins RH, Schwartz SJ. Color improvement and metallo-chlorophyll complexes in continuous flow aseptically processed peas. J. Food Sci. 64: 987-990 (1999)
18. Rodriguez-Amaya DB. A Guide to Carotenoid Analysis in Foods. ILSI Press, Washington, DC, USA. pp. 41-45 (2001)
19. Pan SS, Lu JL, Yang XL, Zheng XQ, Du YY, Devijat B, Liang YR. Study on HPLC method for the analysis of trace pigments in ready-to-drink tea. J. Tea Sci. 27: 343-346 (2007)
20. Liang YR, Lu JL, Zhang LY, Wu S, Wu Y. Estimation of tea quality by infusion color differences analysis. J. Sci. Food Agr. 85: 286-292 (2005)
21. Kong F, Tang J, Rasco B, Crapo C. Kinetics of salmon quality changes during thermal processing. J. Food Eng. 83: 510-520 (2007)
22. Yamauchi N, Harada K, Watada AE. *In vitro* chlorophyll degradation in stored broccoli (*Brassica oleracea* L. var. italica Plen.) florets. Postharvest Biol. Tec. 12: 239-245 (1997)
23. Sankat CK, Maharaj V. Shelf life of the green herb 'shado beni' (*Eryngium foetidum* L.) stored under refrigerated conditions. Postharvest Biol. Tec. 7: 109-118 (1996)
24. Lessin WJ, Catigani GL, Schwartz SJ. Quantification of *cis-trans* isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. J. Agr. Food Chem. 45: 3728-3732 (1997)
25. Lamberts L, Rombouts I, Delcour JA. Study of nonenzymic browning in α-amino acid and γ-aminobutyric acid/sugar model systems. Food Chem. 111: 738-744 (2008)
26. Bozkurt H, Göğüş F, Eren S. Nonenzymic browning reactions in boiled grape juice and its models during storage. Food Chem. 64: 89-93 (1999)
27. Roig MG, Bello JF, Rivera ZS, Kennedy JF. Studies on the occurrence of non-enzymatic browning during storage of citrus juice. Food Res. Int. 32: 609-619 (1999)
28. Schamberger GP, Labuza TP. Effect of green tea flavonoids on Maillard browning in UHT milk. Food Sci. Technol.-Leb. 40: 1410-1417 (2007)
29. Liang HL, Liang YR, Dong JJ, Lu JL. Tea extraction methods in relation to control of epimerization of tea catechins. J. Sci. Food Agr. 87: 1748-1752 (2007)
30. Es-Safi NE, Cheynier V, Moutounet M. Implication of phenolic reactions in food organoleptic properties. J. Food Compos. Anal. 16: 535-553 (2003)
31. Hyvärinen K, Hynninen PH. Liquid chromatographic separation and mass spectrometric identification of chlorophyll *b* allomers. J. Chromatogr. A 837: 107-116 (1999)
32. Eckhardt U, Grimm B, Hortensteiner S. Recent advances in chlorophyll biosynthesis and breakdown in higher plants. Plant Mol. Biol. 56: 1-14, 20 (2004)
33. World Health Organization and Food and Agriculture Organization of the United Nations. 2nd ed. Vitamin and Mineral Requirements in Human Nutrition. Report of a Joint FAO/WHO LC/NLM Classification: QU 145, WHO Press: Geneva, Switzerland. pp. 230-243 (2004)