

# Impact of heating on chemical compositions of green tea liquor

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Received 11 May 2006; received in revised form 11 August 2006; accepted 13 October 2006

## Abstract

Heating pasteurization changed flavour of green tea liquor and it was a technical barrier in ready-to-drink tea production. The effect of heating on chemical compositions of green tea liquor was investigated by high performance liquid chromatography and gas chromatography–mass spectrometry. With increase of heating temperature from 85 °C to 120 °C, the green tea liquor became darker and less green, but deeper yellow in colour. During the heating, epigallocatechin gallate, epigallocatechin, epicatechin and epicatechin gallate partially epimerized, and concentration of total catechins decreased. Twenty volatiles were identified and concentrations of pentanol, *cis*-3-hexenol, linalool oxide I, linalool oxide II and  $\beta$ -ionone were decreased while phenylacetaldehyde, linalool, linalool oxide III,  $\alpha$ -terpineol and indole increased. The decline of volatiles with pleasant odours and increase of some volatiles with unpleasant odours, such as indole (animal-like) and  $\alpha$ -terpineol (faint ammoniacal), was considered to be responsible for the change in flavour of green tea liquor. Treatment at 85 °C caused fewer changes in liquor colour and concentrations of catechins and volatiles and it is recommended that extraction and pasteurization of canned ready-to-drink green tea should be carried out at 85 °C or less.

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**Keywords:** *Camellia sinensis*; Ready-to-drink tea; Heating processing; Catechins; Volatile constituent; Colour difference; HPLC; GC–MS

## 1. Introduction

Ready-to-drink black and green teas are now increasingly consumed in the world, especially in Japan and China, because of their healthy benefits (Dufresne & Farnworth, 2001; Fujiki, 2005; Huang & Xu, 2004; Liang, Lu, & Zhang, 2002; Xu & Huang, 2004). Creaming down in cooled tea liquors and deterioration in flavour after heating pasteurization were technical barriers for ready-to-drink tea production (Jobstl, Fairclough, Davies, & Williamson, 2005; Kumazawa & Mauda, 2001; Liang & Xu, 2001). Many attempts have been made to investigate the mechanism of creaming down of tea liquors, so as to find techniques to control the tea cream in ready-to-drink tea, and great progress has been made (Chao, 1999; Charlton et al., 2002; Jobstl et al., 2005; Liang & Xu, 2003; Penders, Scollard, Needham, Pelan, & Davies, 1998). Changes in

volatile components before and after heat-processing of black tea were analyzed (Kinugasa & Takeo, 1990). However, comparison is not able to explain the difference in sensory evaluation by the heat-processing (Kumazawa & Mauda, 2001). Kumazawa and Mauda (2001) confirmed that 3-methylbutanal (malty), methional (potato-like),  $\beta$ -damascenone (sweet), dimethyl trisulfide (putrid) and 2-methoxy-4-vinylphenol (clove-like) showed the highest flavour dilution (FD) factors after heat-processing of black tea. Tea liquors had better sensory quality when extracted at lower temperatures (Xia, Shi, & Wan, 2006). Green tea is an unfermented tea and its chemical composition is quite different from black tea. Little information about the effect of heating on changes in flavour of ready-to-drink green tea is available.

In the present paper, the changes in volatile components during heat-processing of green tea liquor are examined. The liquor colour and catechin concentrations were also investigated because they were important quality indicators for ready-to-drink green tea.

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## 2. Materials and methods

### 2.1. Materials

Green tea of ‘Sanbeixiang’ brand, produced in Taishun County of Zhejiang Province in China, was bought from a market and used as tea material in the present paper. Catechins and volatile compound references for high performance liquid chromatography (HPLC) and GC/MS were provided by Dr. Tu from the Department of Tea Science of Zhejiang University, China. The other chemical reagents used were of HPLC grade (Tianjin Shild Biometric Technical Co. Ltd., Tianjin City, China), except where stated otherwise.

### 2.2. Methods

#### 2.2.1. Tea liquor preparation

Thirty grams of the tea sample were extracted in 1800 ml of distilled water in a 60 °C water bath for 15 min and then filtered through a “Double-ring” No. 102 filter paper (Xinhua Paper Industry Co. Ltd, Hangzhou, China). The filtrate was divided into five quotients (300 ml each) which were then transferred into a 500 ml glass beaker, respectively. Four quotients of the tea liquors were heated at 85, 95, 110 and 120 °C for 4 min in a YXQ-LS-50SII autoclave (Shanghai Boxun Industry and Commerce Co., Ltd., Shanghai, China), respectively, followed by cooling to room temperature. The remaining one quotient was not heated and used for a control. The tests were carried out in duplicate.

#### 2.2.2. Colour difference analysis of tea liquors

Colour difference indicators ‘L’, ‘a’ and ‘b’ of the heated tea liquors were determined on a TC-PIIG automatic colour difference meter (Beijing Optical Instrument Factory, Beijing, China) according to our previous method (Liang, Lu, Zhang, Wu, & Wu, 2005).

#### 2.2.3. High performance liquid chromatography (HPLC) analysis of tea catechins

HPLC analysis of tea catechins was carried out by the method described in our previous paper (Liang & Xu, 2003). The tea liquors were filtered through a 0.2 µm Millipore filter before being injected into a HPLC (Model Shimadzu LC-2010A, Shimadzu Corporation, Kyoto, Japan). The HPLC conditions were as follows: injection volume, 10 µl; column, 5 µm-Diamonsil™ C18 (4.6 mm × 250 mm); temperature, 40 °C; mobile phase A, acetonitrile/acetic acid/water (6:1:193); mobile phase B, acetonitrile/acetic acid/water (60:1:193); gradient, 100% mobile phase A to 100% mobile phase B by linear gradient during the early 45 min and then 100% mobile phase B up to 60 min; flow rate, 1 ml min<sup>-1</sup>; detector, Shimadzu SPD ultraviolet detector (Shimadzu Corporation, Kyoto, Japan) at 280 nm.

#### 2.2.4. Gas chromatography–mass spectrometry (GC–MS) analysis of volatile constituents

Tea liquor was prepared by the method described in Section 2.2.1. Volatile constituents of 200 ml tea liquor were extracted by the successive distill extraction (SDE) method (Liang, Zhang, & Lu, 2005). An HP6890N GC coupled with an HP 5973 MS [Agilent Technologies Inc. (NYSE:A) Palo Alto, CA, USA] was used for GC–MS analysis. The column was a 30 m × 0.22 mm (i.d.) HP-INNO Wax fused capillary column (Agilent Technologies Inc.) with a film thickness of 0.5 µm. The column temperature was programmed at 50 °C for 5 min, then from 50 °C to 210 °C at a rate of 3 °C min<sup>-1</sup>, remained at 210 °C for 10 min, and finally increased from 210 °C to 230 °C at a rate of 3 °C min<sup>-1</sup>. The injector temperature was 250 °C and injection volume was 2 µl. The flow rate of the helium carrier gas was 1 ml min<sup>-1</sup>. The mass spectrometer was used at ionization voltage of 70 eV and ion source temperature 230 °C.

The identification of the volatile components was done by comparing their Kovats GC retention indices and mass spectra with those of authentic compounds or reported values.

#### 2.2.5. Data analysis and statistics

The tests in the present paper were carried out in duplicate and the mean values of the duplicated tests presented. Tukey’s test was carried out on software of SPSS 10.0 for Windows (SPSS Inc., 1999).

## 3. Results and discussion

### 3.1. Effect of heating processing on colour difference of green tea liquor

Table 1 shows that the colour difference indicator ‘L’ decreased while indicators ‘a’ and ‘b’ increased with increase of heating temperature. The ‘L’ is the indicator of lightness–darkness and the higher it is, the lighter is the liquor. The results suggested that the green tea liquor darkens as heating temperature is raised. The ‘a’, an indicator of greenness (when it is a minus value) and redness (plus value), and ‘b’, an indicator of blueness (minus value) and yellowness (plus value), increased with temperature, suggesting that the tea liquor became less green and deeper

Table 1  
Effect of heating treatments on colour difference of green tea liquor<sup>a</sup>

Treatments (°C)	L	a	b
Control	92.67 ± 0.04a	−2.51 ± 0.09a	10.86 ± 0.02a
85	92.16 ± 0.04ab	−2.33 ± 0.02a	12.09 ± 0.05ab
95	91.76 ± 0.06bc	−2.03 ± 0.09ab	13.02 ± 0.08bc
110	91.19 ± 0.04c	−1.51 ± 0.12b	14.01 ± 0.06c
120	87.80 ± 0.08d	0.70 ± 0.07c	22.85 ± 0.09d

<sup>a</sup> The data marked with different letters in the same column were statistically different at  $p = 0.05$ .

yellow after the heating. These results might be related to the oxidation of tea catechins and degradation of chlorophyll under hot conditions. However, there were no statistically significant differences in the three indicators between the control and 85 °C treatment.

### 3.2. Effect of heat-processing on concentrations of tea catechins

Tea catechins are important functional components of tea and green tea contains more tea catechins than does black tea because there is no fermentation process during green tea manufacture. It is also important to control the oxidation of tea catechins during ready-to-drink green tea processing. Table 2 shows that concentrations of epimers of catechins, such as epigallocatechin gallate (EGCg), epigallocatechin (EGC), epicatechin (EC) and epicatechin gal-

late (ECg), decreased but the isomers of these catechins, such as gallicocatechin gallate (GCg), gallicocatechin (GC), catechin (C) and catechin gallate (Cg) increased as the heating temperature increased. These results suggest that epimerizations of tea catechins took place under the heating conditions. There was a declining trend in total catechins with increase of temperature. This suggests that some of the catechins were oxidized (besides the epimerization) and they might be responsible for the changes in the liquor colour. Except for C and Cg, the differences in catechins were not significant statistically between the control and 85 °C treatment.

### 3.3. Effect of heating processing on concentrations of volatiles

Twenty volatiles were identified by the GC–MS (Table 3 and Fig. 1). With the increase of heating temperature, concentrations of pentanol, *cis*-3-hexenol, linalool oxide I, linalool oxide II and  $\beta$ -ionone decreased while those of phenylacetaldehyde, linalool, linalool oxide III,  $\alpha$ -terpineol and indole increased. Concentrations of some volatiles, such as 1,2-dimethyl benzene, benzaldehyde, benzyl alcohol, nonanal, phenethyl alcohol, 2,4-di-*tert*-butylphenol, diisobutyl phthalate, dibutyl phthalate and phytol, increased when the tea liquors were heated at 85 °C or 95 °C, but declined as the temperature was further increased. Aroma quality of some volatiles is shown in Table 4. Kanani, Nikhade, Balakrishnan, Singh, and Pangarkar (2003) showed that *cis*-3-hexenol and  $\beta$ -ionone made a significant contribution to tea aroma. The decline in concentrations of *cis*-3-hexenol and  $\beta$ -ionone might be

Table 2  
Changes in concentrations of catechins of green tea liquor (mg ml<sup>-1</sup>)<sup>a</sup>

Treatments (°C)	Control	85	95	110	120
EGCg	0.624a	0.611a	0.595b	0.503c	0.373d
GCg	0.0037a	0.0055a	0.0079b	0.0905c	0.243d
EGC	0.234a	0.232a	0.229b	0.2200b	0.195c
GC	0.316a	0.320a	0.325a	0.335b	0.362c
EC	0.119a	0.118a	0.117a	0.116b	0.0448c
C	0.0176a	0.0205b	0.0237b	0.0339c	0.0561d
ECg	0.108a	0.108a	0.103a	0.101a	0.0859b
Cg	0.0160a	0.0212b	0.0289c	0.0353d	0.0607e
Total	1.44a	1.44a	1.43b	1.43a	1.42b

<sup>a</sup> The data marked with different letters in the same row were statistically different at  $p = 0.05$ .

Table 3  
Changes in concentrations of volatiles of green tea liquors<sup>a</sup>

Peak no.	Volatile components	Control	85 °C	95 °C	110 °C	120 °C
1	Pentanol	0.0733a	0.0420b	0.0389bc	0.0333d	0.0238e
2	<i>cis</i> -3-Hexenol	0.0538a	0.0513a	0.0447b	0.0454b	0.0347c
3	1,2-Dimethyl benzene	0.0331a	0.0168d	0.0341ab	0.0277c	0.0317bc
4	Benzaldehyde	0.0328e	0.0587bc	0.0606b	0.0632a	0.0522d
5	Benzyl alcohol	0.119c	0.194a	0.176b	0.165b	0.207a
6	Phenylacetaldehyde	0.193c	0.229bc	0.238b	0.233b	0.2710a
7	Linalool oxide I	0.0669a	0.0571b	0.0592b	0.0562b	0.0486c
8	Linalool oxide II	0.0557a	0.0512b	0.0478c	0.0492bc	0.0452c
9	Linalool	0.248b	0.2742b	0.303a	0.307a	0.324a
10	Nonanal	0.0475d	0.0858c	0.145a	0.0742c	0.111b
11	Phenethyl alcohol	0.0752b	0.112a	0.0666b	0.107a	0.104a
12	Linalool oxide III	0.0706b	0.0884a	0.0816a	0.0831a	0.0869a
13	$\alpha$ -Terpineol	0.0418c	0.0510b	0.0513b	0.0526b	0.0689a
14	Geraniol	0.133c	0.137b	0.135c	0.138b	0.141ab
15	Indole	0.0497c	0.0586b	0.0520b	0.0553b	0.0968a
16	$\beta$ -Ionone	0.0652a	0.0622a	0.0625a	0.0592b	0.0503c
17	2,4-Di- <i>tert</i> -butylphenol	0.142b	0.148b	0.212a	0.162b	0.214a
18	Diisobutyl phthalate	0.0754a	0.0770a	0.0658ab	0.0655ab	0.0585b
19	Dibutyl phthalate	0.0621c	0.0714bc	0.105a	0.0793b	0.0979a
20	Phytol	0.0444ab	0.0486a	0.0415b	0.0266c	0.0091d
Total		1.68c	1.93ab	2.02a	1.88b	2.08a

<sup>a</sup> The data are presented as the ratio of the peak area of each volatile to the peak area of the internal standard reference decanoic acid ethyl ester, and those marked with different letters in the same row were statistically different at  $p = 0.05$ .

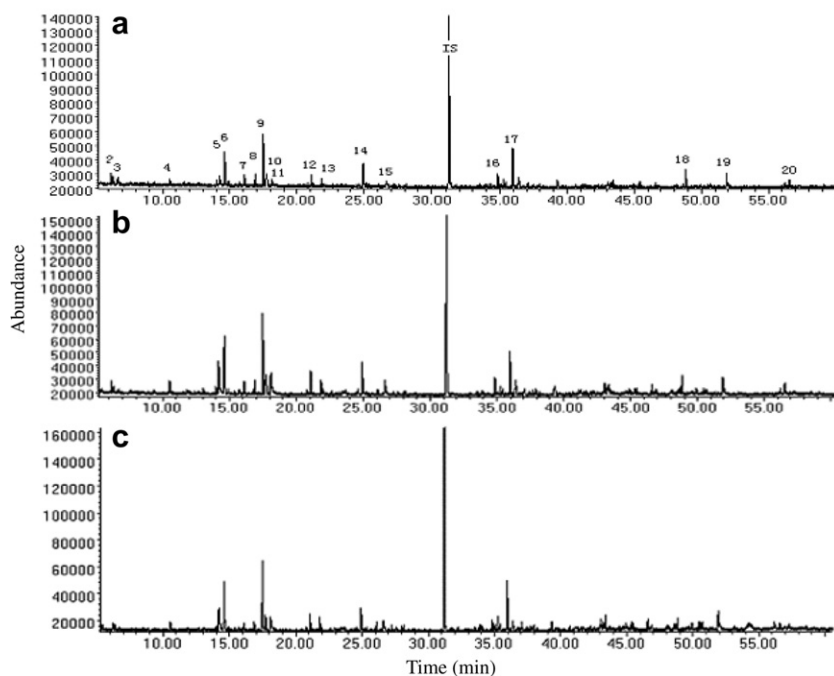


Fig. 1. Total ion current chromatographs of volatiles in green tea liquors: (a) control; (b) 85 °C; (c) 120 °C; (IS) internal standard (decanoic acid ethyl ester); and volatiles of each peak are shown in Table 3.

Table 4  
Aroma quality of partial volatiles

	Aroma quality	Increase or decrease by heating
Pentanol	Choking odour	Decrease
<i>cis</i> -3-Hexenol	green	Decrease
Linalool oxide I,	Floral	Decrease
Linalool oxide II	Floral	Decrease
$\beta$ -Ionone	Violet-like	Decrease
Phenylacetaldehyde	Vanilla-like	Increase
Linalool	Citrus; floral	Increase
Linalool oxide III	Floral	Increase
$\alpha$ -Terpineol	Faint ammoniacal	Increase
Indole	Animal-like	Increase

related to the flavour deterioration after heat-processing of tea liquor. Of course, the increase of some volatiles with unpleasant odours, such as indole (animal-like) and  $\alpha$ -terpineol (faint ammoniacal), should also be responsible for the changes in flavour during the heating. The importance of a volatile to food flavour depends, not only on its actual concentration, but also on its odour threshold. Flavour dilution (FD) factor, a ratio of actual concentration to odour threshold, is always used to evaluate the contribution of a volatile compound to the food flavour. It was confirmed that indole was one of the volatiles that showed the highest FD factors in green tea (Kumazawa & Mauda, 1999). The odour threshold limit value of  $\beta$ -ionone is very low ( $0.007 \mu\text{g l}^{-1}$ ) (Kanani et al., 2003). Therefore, the changes in concentrations of indole and  $\beta$ -ionone may be important factors responsible for the changes in flavour of green tea liquor during heating. Compared to treatments

at 95 °C or above, fewer changes in volatile concentrations were found during the heat-processing at 85 °C and there were no statistically significant differences in concentrations of *cis*-3-hexenol, phenylacetaldehyde, linalool,  $\beta$ -ionone, diisobutyl phthalate or phytol between the control and treatment at 85 °C (Table 3).

Based on the effects of heating temperature on colour and concentrations of catechins and volatile components of green tea liquor, extraction and pasteurization of canned ready-to-drink green tea are recommended to be carried out at 85 °C or less.

#### Acknowledgements

The authors thank Dr. Y. Tu for offering the reference compounds for the HPLC and GC–MS analysis. Contract/grant sponsor: National Natural Science Foundation of China and Natural Science Foundation of Zhejiang Province.

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