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Decaffeination of fresh green tea leaf (*Camellia sinensis*) by hot water treatment

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Abstract

Hot water treatment was used to decaffeinate fresh tea leaf in the present study. Water temperature, extraction time and ratio of leaf to water had a statistically significant effect on the decaffeination. When fresh tea leaf was decaffeinated with a ratio of tea leaf to water of 1:20 (w/v) at 100 °C for 3 min, caffeine concentration was decreased from 23.7 to 4.0 mg g^{-1}, while total tea catechins decreased from 134.5 to 127.6 mg g^{-1}; 83% of caffeine was removed and 95% of total catechins was retained in the decaffeinated leaf. It is considered that the hot water treatment is a safe and inexpensive method for decaffeinating green tea. However, a large percentage of tea catechins was lost if rolled leaf and dry tea were decaffeinated by the hot water treatment and so the process is not suitable for processing black tea.

Keywords: Decaffeinated tea; Green tea; Black tea; Caffeine; Catechins; EGCg; HPLC; Polyphenol oxidase

1. Introduction

Many attempts have been made to remove caffeine from tea, to produce decaffeinated teas and tea extracts. Chloroform or methylene chloride is an effective solvent for isolating caffeine from tea leaf. However, it is not widely accepted by consumers because of its toxicity (Sakanaka, 2003). Decaffeination using supercritical carbon dioxide is effective and leaves no solvent residues (Chang, Chiu, Chen, & Chang, 2000, 2001), but it needs expensive equipment. Sawdust lignocellulose columns can be used to separate caffeine from tea extracts (Sakanaka, 2003), but they are difficult to use for decaffeination of tea leaf. Searching for inexpensive and safe decaffeination methods, which leave no solvent residue, is now an important topic in the tea processing field.

In this present paper, hot water was used to isolate caffeine from tea leaf so as to develop a new method for processing decaffeinated tea.

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2. Materials and methods

2.1. Materials

Fresh tea shoots with a bud and 4–5 leaves were plucked in November 2005 from the Experimental Tea Farm of the Zhejiang University Tea Research Institute, Hangzhou. HPLC reference compounds of (+)catechin (C), (−)epicatechin (EC), (+)gallocatechin (GC), (−)epigallocatechin (EGC), (+)catechin gallate (Cg), (+)gallocatechin gallate (GCg), (−)epicatechin gallate (ECg), and (−)epigallocatechin gallate (EGCg) were provided by Dr. Takeda from the National Tea Research Institute, Japan. Caffeine was bought from Sigma Company. The other chemical reagents used were of HPLC grade (Jinmei Biotech Corporation, Tianjin, China), except where stated otherwise.

2.2. Methods

2.2.1. Experimental design

Extraction temperature, extraction time, and ratio of tea leaf to water are important factors affecting extractability

Table 1
Orthogonal test design with three factors and three levels each factor

Treatment number	Temperature (°C)	Time (min)	Ratio of leaf to water (w/v)
1	50	1	1/20
2	50	3	1/15
3	50	5	1/10
4	75	1	1/15
5	75	3	1/10
6	75	5	1/20
7	100	1	1/10
8	100	3	1/20
9	100	5	1/15

of tea (Liang & Xu, 2003) and three levels each of the three factors were tested in the present paper. Orthogonal matrix design with nine treatment combinations (Table 1) was carried out on software of the SAS System for Windows (version 8.01; SAS Institute Inc., 1999–2000). Samples of 100 g fresh tea shoots were extracted in a 3 l glass beaker with distilled water. Volume of distilled water, extraction temperature, and time of the nine treatments are shown in Table 1.

The extracted tea leaf was removed from the hot water and dried at 80 °C for 4 h. To check the original chemical composition of the sample, fresh tea leaf (100 g) was blanched by steam for 30 s, a process known as fixing, and dried at 80 °C for 4 h as a control sample (Liang, Lu, & Shang, 1996).

Chinese green tea is manufactured through the processes of pan fixation, rolling and drying. To investigate the effect of hot water treatment on decaffeination of the processed tea, the fresh tea leaf was used to manufacture green tea, using conventional methods, and samples of the fixed leaf, rolled leaf and dried tea were collected for decaffeination and HPLC analysis. The experimental conditions were the same as for treatment No. 8 (Table 1).

The dried sample was ground using a EUPA TSK-927S grinder (Cankun Co., Ltd., Shanghai, China) and sifted through 40 meshes per inch before extraction for HPLC analysis.

2.2.2. HPLC analysis

Three hundred milligrams of the ground tea sample were extracted in 50 ml of 50% ethanol solution (v/v) at room temperature for 45 min. The extract was filtered through "Double-ring" No. 102 filter paper (Xinhua Paper Industry Co., Ltd., Hangzhou, China) and then centrifuged at 13,000 rpm and 4 °C for 10 min and finally filtered through a 0.22 μm Millipore filter before analysis by HPLC.

HPLC was carried out under the conditions described in our previous papers (Liang, Lu, Zhang, Wu, & Wu, 2003; Liang, Ma, Lu, & Wu, 2001), using a Shimadzu SCL-10A HPLC system (Shimadzu Corporation, Tokyo, Japan):

Injection volume	10 μl
Column	5 m-DiamonsilTM C18, 4.6 × 250 mm
Column temperature	40 °C
Mobile phase	Solvent A: acetonitrile/acetic acid/water (6:1:193, by volume); Solvent B: acetonitrile/acetic acid/water (60:1:139, by volume)
Gradient	100% solvent A to100% solvent B by linear gradient during first 45 min and then 100% solvent B until to 60 min
Flow rate	1 ml min^{-1}
Detector	Shimadzu SPD ultraviolet detector, 280 nm

2.3. Data statistics

All tests in the present paper were carried out in duplicate and the mean values were presented, based on dry weight of tea leaf. Statistics was carried out on software of the SAS System for Windows (version 8.01; SAS Institute Inc. 1999–2000).

3. Results

3.1. Decaffeination of fresh tea leaf by hot water treatment

Table 2 showed that caffeine concentration differed between the nine decaffeination treatments. Caffeine concentrations of treatments No. 8 [100 °C, 3 min and 1:20 (w/v) of leaf to water], No. 9 [100 °C, 5 min and 1:15 (w/v) of leaf to water] and No. 7 [100 °C, 1 min and 1:10 (w/v) of leaf to water] were significantly lower than those of treatment Nos. 1–6. Also, treatment Nos. 8–9 had significantly lower caffeine concentrations than that of treatment No. 7. Compared to the original caffeine concentration in fresh leaf (the control sample, Table 3 and Fig. 1A), 83 and 75% of caffeine were removed in treatments No. 8 (Fig. 1B and Table 2) and No. 9, respectively (Table 2). When we looked at the combinations of treatment factors, it is found that the treatment Nos. 7–9, with low caffeine concentrations, were all extracted at 100 °C, though their extraction time and ratio of leaf to water varied (Tables 1 and 2). It suggests that temperature is the most important of the tested factors.

Table 4 listed the mean values of tested chemical compositions related to each level of the three tested factors. It showed that the mean caffeine concentration in samples extracted at 100 °C was 7.2 mg g^{-1}, being the lowest among the tested levels of the three factors. Furthermore, caffeine concentration decreased with increasing extraction temperature and at 100 °C was significantly lower than at 50 and 75 °C (Table 4). This confirmed that extraction

Table 2
Effect of hot water treatment on chemical composition of fresh tea leaf[a]

Treatment No.	Caffeine (mg g^{-1})	Tea catechins (mg g^{-1})								
		GC	EGC	C	EC	EGCg	GCg	ECg	Cg	Total
1	24.4A	8.9A	34.4A	2.1A	2.7B	68.9A	0.6D	11.2A	0A	128.8A
2	23.8AB	7.3A	30.6A	1.9A	2.3B	64.6A	0.6D	10.4A	0.1A	117.8A
3	22.9AB	6.6A	31.3A	1.4A	2.6B	63.5A	0.7DC	10.3A	0.1A	116.5A
4	21.0AB	5.9A	37.4A	2.1A	2.9B	69.3A	0.9DC	11.4A	0.1A	130.0A
5	20.4B	5.4A	37.0A	1.7A	2.7B	69.8A	1.1BCD	11.0A	0.1A	128.8A
6	16.6C	5.3A	34.9A	1.2A	5.2A	70.7A	1.5ABC	11.5A	0.1A	130.4A
7	11.8D	8.2A	34.9A	1.7A	5.4A	74.0A	1.6ABC	11.0A	0.1A	136.9A
8	4.0E	5.1A	32.9A	1.4A	4.9A	71.1A	2.0A	10.0A	0.2A	127.6A
9	5.9E	5.3A	32.0A	1.0A	4.8A	70.6A	2.0AB	10.2A	0.2A	126.1A

[a] Data with different letters in a same column differed significantly at $p = 0.05$.

Table 3
Concentration of caffeine and tea catechins in fresh tea leaf (control)[a]

Caffeine (mg g^{-1})	Tea catechins (mg g^{-1})								
	GC	EGC	C	EC	EGCg	GCg	ECg	Cg	Total
23.7	10.4	34.1	2.7	2.7	72.3	1.5	10.7	0.1	134.5

[a] Control sample preparation: Fresh tea leaf was fixed by steam for 30 s and dried at 80 °C for 4 h.

temperature was the main factor influencing decaffeination of fresh leaf.

Extraction time and the ratio of leaf to water also affected the decaffeination of fresh leaf though they were not as important as temperature. Caffeine concentrations in samples extracted for 3–5 min did not significantly differ but they were significantly lower than at 1 min (Table 4). This suggests that the extraction time should not be less than 3 min. Treatments where the ratio of tea leaf to water was 1:20 had a significantly lower caffeine concentration than those where the ratio was 1:15 and 1:10, suggesting that the ratio of leaf to water should not be less than 1:20.

3.2. Effect of fresh leaf decaffeination on catechins concentration

Tea catechins affect tea quality and possess numerous health benefits (Dufresne & Farnworth, 2001; Liang, Liu, Xu, & Hu, 1990; Liang et al., 2001, 2003). It is important to investigate whether tea catechins are retained in the tea leaf or not during the decaffeination process. Table 2 and Fig. 1 showed that the total concentration of tea catechins did not differ significantly between the treatments and more than 95% of total catechins was retained using treatment No. 8, compared to the original concentration of total catechins in fresh leaf (Tables 2 and 3; Fig. 1). Furthermore, the major components of tea catechins were EGCg and EGC in the original leaf and the treated samples (Tables 2 and 3; Fig. 1), suggesting that the composition of major tea catechins did not significantly change during the decaffeination process.

Tables 2 and 3 showed that total concentrations of catechins in treatments No. 8 (127.6 mg g^{-1}) and No. 9 (126.1 mg g^{-1}) were 95 and 94% of that in fresh leaf (134.5 mg g^{-1}) although a large percentage of caffeine was removed. It means that most tea catechins remained in the fresh leaf during the decaffeination process.

3.3. Decaffeination of leaves sampled at various stages of green tea processing

During green tea processing, fresh tea leaf is fixed by heat in a hot pan or by steam, so as to inactivate polyphenol oxidase enzyme and control oxidation of tea catechins. The fixed leaf is then rolled in a rolling machine for about 30 min. Green tea is obtained when the rolled leaf is dried at 90–110 °C. In order to investigate the effect of hot water treatment on concentrations of caffeine and catechins in materials at various stages of green tea processing, the above fresh leaf was used to process green tea using conventional green tea manufacturing techniques. Samples (100 g) of pan fixed leaf, rolled leaf and dry tea were collected for decaffeination, using treatment No. 8 (Table 1).

Concentrations of caffeine in the three samples ranged from 1.2 to 3.4 mg g^{-1}(Table 5), being even lower than that in fresh leaf samples from treatments No. 8 (4.0 mg g^{-1}) and No. 9 (5.9 mg g^{-1}) (Table 2). Hence, the pan fixed leaf, rolled leaf, and dry tea were effectively decaffeinated by the hot water treatment. However, the concentration of total catechins and major tea catechin components, such as EGCg, decreased markedly in the processed samples. Compared to fresh leaf, 16, 49, and 68% of total catechins was removed from the pan fixed leaf, rolled leaf and dry tea, during the hot water treatment (Tables 3 and 5). Fig. 1 shows the HPLC profiles of fresh leaf (A), decaffeinated fresh leaf (B) and rolled leaf (C). A large proportion of

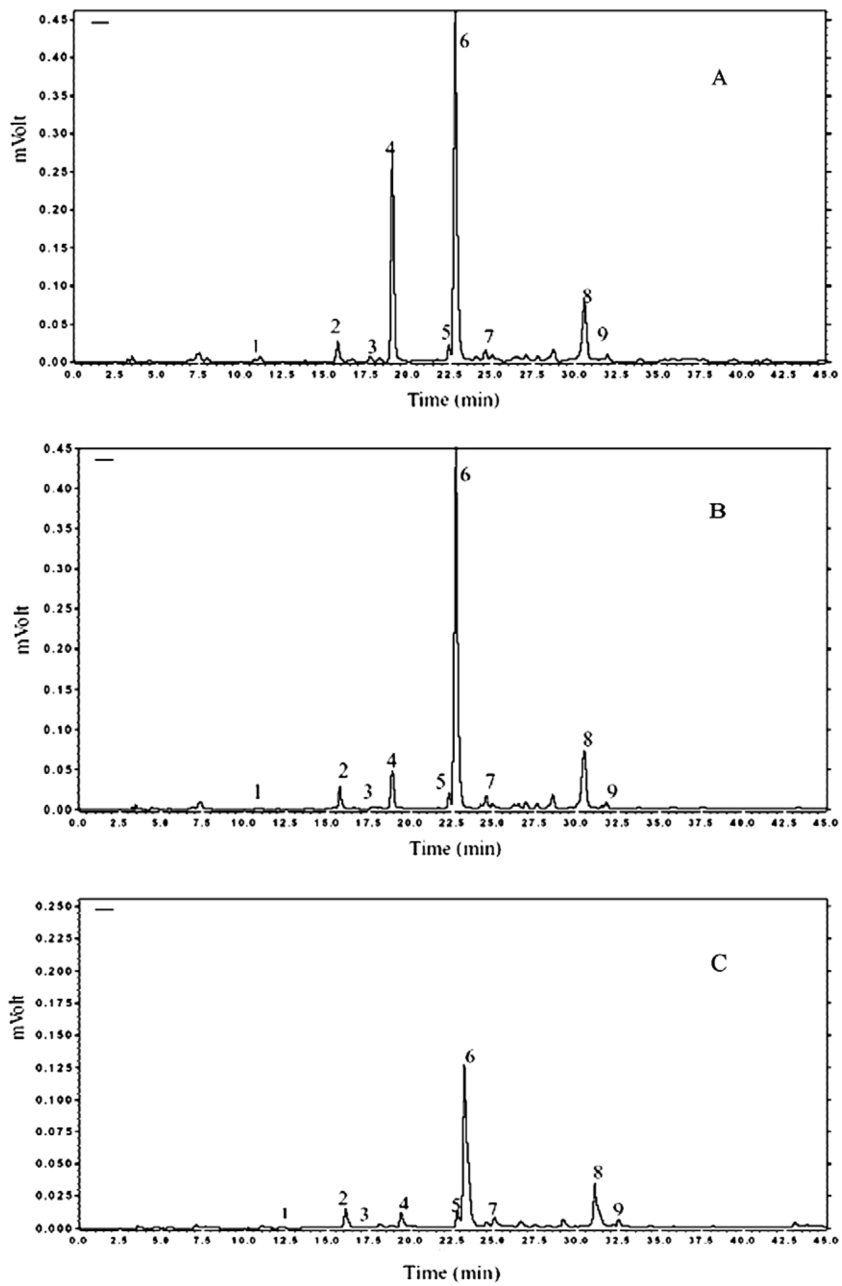

Fig. 1. HPLC profiles of control sample (A), treatment No. 8 (B), and decaffeinated sample of rolled leaf (C) 1: GC; 2: EGC; 3: C; 4: Caffeine; 5: EC; 6: EGCg; 7: GCg; 8: Ecg; 9: Cg.

the tea catechins were removed when the rolled leaf and dry tea were decaffeinated by hot water. Hence, the hot water treatment was not suitable for decaffeination of rolled leaf and dry tea.

4. Discussion

These results show that caffeine can be specifically isolated from fresh tea leaf by hot water treatment but catechins are also extracted when rolled leaf or dry tea is decaffeinated. The rolling and dry processes may destroy the leaf cells, facilitating loss of catechins. When the pan fixed leaf was rolled, the leaf cells and their membranes were damaged or destroyed by the action of rolling, during which some chemical components, including tea catechins and caffeine, were squeezed out on to the surface of the leaf. In this case, catechins and caffeine were easily dissolved in water during hot water treatment. The leaf cell membranes were further damaged in the subsequent drying process, which would increase the extractability of catechins. This was confirmed by the amount of total catechins remaining in the samples: pan fixed leaf > rolled leaf > dry tea (Table 5). The leaf might be partially bruised by the stirring action during pan fixing process and so catechins in the pan fixed leaf became more easily extracted than in the fresh leaf. Table 5 showed that the decaffeinated pan

Table 4
The grouping mean of tested factors[a]

Factors	Levels	Dependent variable ($mg\ g^{-1}$)									
		Caffeine	GC	EGC	C	EC	EGCg	GCg	ECg	Cg	Total catechins
Temperature	1	23.7A	7.6A	32.1B	1.8A	2.5C	65.7B	0.6C	10.6B	0.1A	121.0A
	2	19.3B	5.5A	36.4A	1.7A	3.6B	69.9BA	1.1B	11.3A	0.1A	129.6A
	3	7.2C	6.2A	33.3BA	1.3A	5.0A	71.9A	1.9A	10.4B	0.2A	130.2A
Extraction time	1	19.1A	7.6A	35.6A	1.9A	3.7A	70.7A	1.0B	11.2A	0.1A	131.8A
	2	16.1B	5.9A	33.5A	1.7A	3.3A	68.5A	1.2AB	10.5B	0.1A	124.7A
	3	15.2B	5.7A	32.7A	1.2A	4.2A	68.2A	1.4A	10.6BA	0.1A	124.1A
Ratio of tea to water	1	15.0B	6.4A	34.1A	1.6A	4.3A	70.2A	1.4A	10.9A	0.1A	129.0A
	2	16.9A	6.2A	33.4A	1.7A	3.3A	68.2A	1.1A	10.7A	0.1A	124.7A
	3	18.4A	6.7A	34.4A	1.6A	3.6A	69.1A	1.1A	10.8A	0.1A	127.4A

[a] Data with different letters in a same column differed significantly at $p = 0.05$.

Table 5
Decaffeination effect of materials sampled at various stages of green tea processing ($mg\ g^{-1}$)[a]

Leaf sample	Caffeine	EGCg	Total catechins
Pan fixed leaf	3.4A	50.2A	113.6 A
Rolled leaf	1.2B	31.7B	69.1 B
Dry tea	2.3BA	17.0C	43.5 C

[a] One hundred grams leaf was extracted in 2000 ml distilled water at 100 °C for 3 min. Data marked with different letters in the same column were statistically different at $p = 0.05$.

fixed leaf had 113.6 $mg\ g^{-1}$ total catechins, 11% lower than in the fresh leaf extracted by treatment No. 8 (Table 2), but 39% higher than in rolled leaf (Table 5). This suggests that leaf damage is an important factor influencing the extractability of tea catechins and although the rolling process is necessary for green tea manufacture, the rolled leaf was not suitable for decaffeination.

Table 5 showed that the decaffeinated dry tea contained a higher concentration of caffeine than decaffeinated rolled leaf. This may be caused by the difference in leaf volume between rolled leaf and dry tea used in the experiment. The volume of dry tea might be bigger than the rolled and pan fixed leaves, because the dry tea has less moisture than the pan fixed and rolled leaves.

Tea leaf cells and membranes are not destroyed before rolling. Caffeine and catechins are located in vacuoles and they should diffuse through the cell membranes and dissolve in water during fresh tea decaffeination. Difference in water solubility and molecular weight between caffeine and catechins might also be important reason of specific isolation of caffeine from fresh tea leaf. Water solubility of caffeine is 21.7 $g\ l^{-1}$ while that of EGCg, the most important component of tea catechins, is about 5 $g\ l^{-1}$. The molecular weight of caffeine is 194.2 while that of EGCg is 458.4. When the fresh leaf was extracted in hot water, caffeine could diffuse and dissolve into water more easily than catechins because of its higher solubility in water and smaller molecule size. Table 4 shows that the caffeine concentration of the treated leaves decreased with increasing extraction temperature, but total catechin concentrations were not significantly affected. The diffusion and dissolving of caffeine may be more sensitive to temperature than catechins and temperature is an important factor influencing the decaffeination of fresh leaf.

According to Table 4, extraction time and ratio of leaf to water had an influence on the decaffeination of the fresh tea leaf although the affects were not as great as for temperature. Based on the statistical significance (Table 4), the best treatment combination of the three tested factors is considered to be 100 °C, for 3–5 min and a ratio of tea leaf to water of 1:20 (w/v). Treatment Nos. 8 –9 in Table 3 were the two combinations that gave the best results. Because there was no significant difference in concentrations of caffeine and catechins between 3 and 5 min (Table 4), 3 min extraction will save time and energy and should be recommended.

Hot water treatment is a convenient and inexpensive decaffeination process and leaves no organic solvent residue. During green tea processing, fresh leaf must be first fixed by steam or in a hot pan before rolling and drying. Hot water treatment has the same function as the fresh leaf fixation besides decaffeination. It will be of great interesting if the pan fixation process is replaced by the hot water decaffeination treatment for green tea processing. However, in black tea processing, tea leaf should be rolled or cut before fermentation, during which tea catechins are oxidized by catalysis of polyphenol oxidase and a group of black tea pigments called theaflavins and thearubigins formed. If the fresh leaf was decaffeinated by hot water treatment, the fermentation would be inhibited, as the polyphenol oxidase will be inactivated by the hot water. Caffeine and tea catechins would be extracted simultaneously if the rolled and fermented leaves or dried tea were decaffeinated with hot water treatment. So the hot water treatment is not suitable for processing decaffeinated black tea.

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References

Chang, C. J., Chiu, K. L., Chen, Y. L., & Chang, C. Y. (2000). Separation of catechins from green tea using carbon dioxide extraction. *Food Chemistry, 68*, 109–113.

Chang, C. J., Chiu, K. L., Chen, Y. L., & Yang, P. W. (2001). Effect of ethanol content on carbon dioxide extraction of polyphenols from tea. *Journal of Food Composition and Analysis, 14*, 75–82.

Dufresne, C. J., & Farnworth, E. R. (2001). A review of latest research findings on the health promotion properties of tea. *Journal of Nutritional Biochemistry, 12*, 404–421.

Liang, Y. R., Liu, Z. S., Xu, Y. R., & Hu, Y. R. (1990). A study on chemical composition and quality of two special green teas. *Journal of the Science of Food and Agriculture, 53*, 541–548.

Liang, Y. R., Lu, J. L., & Shang, S. Y. (1996). Effect of gibberellins on chemical composition and quality of tea. *Journal of the Science of Food and Agriculture, 72*, 411–414.

Liang, Y. R., Lu, J. L., Zhang, L. Y., Wu, S., & Wu, Y. (2003). Estimation of black tea quality by analysis of chemical composition and infusion color difference. *Food Chemistry, 82*, 283–290.

Liang, Y. R., Ma, W. Y., Lu, J. L., & Wu, Y. (2001). Comparison of chemical compositions of *Ilex latifolia* Thumb and *Camellia sinensis*. *Food Chemistry, 75*, 339–343.

Liang, Y. R., & Xu, Y. R. (2003). Effect of temperature on cream and extractability of black tea (*Camellia sinensis* L). *International Journal of Food Science and Technology, 38*, 37–45.

Sakanaka, S. (2003). A novel convenient process to obtain a raw decaffeinated tea polyphenol fraction using a lignocellulose column. *Journal of Agricultural and Food Chemistry, 51*, 3140–3143.