J Food Sci Nutr
Vol 15, p 229～232 (2010)
DOI: 10.3746/jfn.2010.15.3.229

J Food Science and Nutrition

Catechin and Caffeine Concentration Variations in Jeju Green Tea Varieties Harvested Over a Seven-Month Period

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Abstract

Caffeine and catechins from the Yabukita, Yutakamidori, Saemidori, Okumidori, and Fushun varieties of tea leaves picked during different harvesting seasons from April to October were evaluated using HPLC. Total content of catechins increased greatly with the later harvesting time of tea leaves (i.e., picking the leaves in September versus in April) and decreased slightly after September. Yabukita tea leaves picked in August contained 43.1 mg% catechins including EGC, EC, ECG, and EGCG, with the ECGC levels constituting greater than 50% of those four compounds. Yutakamidori and Okumidori varieties picked in September contained the highest catechin values, at 43.6 mg% and 31.0 mg%, respectively. Fushun and Saemidori varieties contained lower catechin concentrations of 14.5 mg% (July) and 11.7 mg% (August) compared to other varieties. The EGCG levels gradually decreased in the late harvesting season, while levels of the other catechins, EC, EGC, and ECG, gradually increased. All varieties of green tea showed a gradual decrease in caffeine content toward the end of our harvesting efforts in October, with levels of 58～68 mg% in April and 28～57 mg% in October. Yabukita, Saemidori, and Okumidori varieties reached their highest caffeine levels in late spring/early summer, with Yabukita and Okumidori varieties reaching a high of 73.4% and 63.5% caffeine, respectively, in May, and Saemidori at 64.0% in June. In particular, Fushun still contained high caffeine of 66.8 mg% (September) during the late harvesting season.

Key words: catechins, caffeine, green tea, HPLC

INTRODUCTION

Green tea leaves are well known as functional ingredients that prevent chronic diseases due to various bioactive compounds, including polyphenolic compounds (1). Green tea leaves contain phytochemicals such as flavonoids, catechins, anthocyanins, theafavin, and anthoxanthin. Catechins, the major component in green tea leaves, have been studied for their biologically active functions (2). The catechins of green tea leaves have a basic structure of C6-C3-C6 substituted with hydroxyl groups. Generally catechins provide the astringent taste in green tea. In green tea leaves, the important catechins consist of (-)epigallocatechin-3-gallate (EGCG), (-)epigallocatechin (EGC), (-)epicatechin (EC), and (-)epicatechingallate (ECG). Catechins are important because the quality and grade of green tea leaves are based on the ratio of each catechin component (3).

Recently, green tea has become popular as a functional ingredient for healthy foods and beverages. Several functional products using green tea leaves are available on the market, and new products are being developed in the functional food industries.

The main components of green tea leaves vary with the variety of the tea plant, the picking method, and the exposure to sunlight (4). In addition, the composition of green tea leaves is dependent upon soil, weather, environment, harvest season, and brewing method (5,6). Green tea leaves picked early in the harvest season (April) have been reported to contain higher amounts of caffeine and amino acids. On the other hand, green tea leaves picked later in the season (August) have a higher content of catechins (5).

Although the functional and biologically active properties of phytochemicals in green tea leaves have been reported, there is less research on the compositional analysis of green tea leaves based on the varieties of green tea plants. Therefore, this research is focused on the analysis of caffeine and catechins in green tea leaves harvested over a seven month period from the five varieties of tea plants cultivated on Jeju Island.

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MATERIALS AND METHODS

Materials and reagents

Green tea leaves (*Camellia sinensis*) of Yabukita, Yutakamidori, Saemidori, Okumidori, and Fushun varieties cultivated on Jeju Island were harvested from April to October. Fresh green tea leaves including the fourth leaves from the top were picked during harvesting and then stored at -70°C.

Standard reagents of (-)epigallocatechin (EGC), (-)epicatechin (EC), (-)epigallocatechin-3-gallate (EGCG), (-)epicatechingallate (ECG), and caffeine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The standard reagents were dissolved in ethyl acetate and used for determining a standard curve. All other reagents used in analysis were HPLC grade.

Extraction of caffeine and catechins

Five varieties of green tea leaves were used for extraction of catechins and caffeine. The bud, first, second, and third leaves of green tea plants were separately picked and grouped. Each group of fresh green tea leaves (1 g) was homogenized using an ACE homogenizer (Nissei AM, Nihonseiki Kaisha LTD) at 10,000 rpm for 3 min and then extracted twice with 20 mL hot water (80°C) for 5 min. The water extract was filtered with a filter (Whatman No. 1) and then filtrate was transferred to a separating funnel filled with 40 mL of ethyl acetate. The ethyl acetate fractionation step was repeated three times and the extracts combined (7).

Analysis of caffeine and catechins

Caffeine and catechins were analyzed using HPLC (High Performance Liquid Chromatography) equipment with Spectra-Physics (spectra SYSTEM), P4000 pump (Spectra-Physics Analytical, Inc, CA, USA), UV1000 uv/vis detector (Spectra-Physics Analytical, Inc), and AS3500 Auto-sampler (TSP Inc., CA, USA). The separation of compounds was accomplished with C18 reverse phase column (μBondapak^{TM} 3.9×300 mm, particle size 10 μm, Waters Chromatography, Milford, MA, USA). The mobile phase was prepared by mixing with acetonitrile (130 mL), acetic acid (5 mL), methanol (20 mL), and water (862 mL) and then filtering with a micro membrane filter (0.5 μm PTFE, ADVANTEC MFS, Inc, CA, USA). Sample (2 μL) was injected and then measured at 280 nm (8).

The standard curve for calculating contents of caffeine and catechins was constructed with caffeine and catechins including (-)epigallocatechin, (-)epicatechin, (-)epigallocatechin-3-gallate, and (-)epicatechingallate as standard reagents. The mean value of analysis data determined from each group of fresh green tea leaves was calculated.

RESULTS AND DISCUSSION

Concentrations of caffeine and catechins in fresh green tea leaves of Yabukita, Yutakamidori, Okumidori, Saemidori, and Fushun varieties

The concentrations of caffeine and catechins from five different varieties of green tea were determined using HPLC. The retention time of each catechin showed a similar pattern compared to previous analysis results (5, 9). Contents of caffeine and catechins of fresh green tea leaves differed greatly, depending on the time they were picked, as shown in Table 1. By delaying the time to pick the leaves, the concentration of catechins of Yabukita green tea leaves was gradually increased from 5.9 mg% (April) to 43.1 mg% (August), but the levels of caffeine decreased from 68.4 mg% (April) to 28.4 mg% (October). In particular, the content of catechins was drastically increased in tea leaves picked in August, with 43.1 mg% catechins, and, after that, the content of catechins decreased slightly. EGCG catechin was greatly increased in tea leaves picked in August, accounting for more than 50% of the total catechin concentration. ECG catechin had the lowest value among the four catechins. The EGCG catechin levels decreased slightly throughout the picking season, but contents of other catechins gradually increased. In the analysis of polyphenol content in dried green tea leaves, EGCG was the highest, with a value of 67.5% (10). Although the contents of catechin in this study were lower than a previous study because the catechin was extracted from fresh leaves, it is concluded that EGCG catechin is one of the major components in green tea leaves.

The functional properties of catechins have been well reported for the prevention of chronic disease because of their anti-oxidant and anti-cancer properties, as well as their ability to lower blood pressure (11,12). Recently, it has been reported that a major catechin component in green tea leaves showed the negative role in regulating blood pressure (unpublished results). Therefore, the content of each catechin in the different varieties of green tea plants can provide useful information for preparing healthy and functional foods. It has been reported that EGCG catechin in Korea and Yamamoto (Japan) green tea leaves were predominant with 58% and 62.9%, respectively (7). The content of caffeine in green tea leaves of Yabukita decreased during the course of picking the leaves over the seven month period, from 68.4 mg% (April) to 28.4 mg% (October).

In the case of Yutakamidori green tea leaves, the content of catechins increased from 29.5 mg% (April) to 43.7 mg% (September), and the caffeine levels decreased throughout the picking season. The content of catechin

Table 1. Catechin and caffeine contents in green tea leaves of each cultivar picked from different seasons

Plucking time	Component		Contents (mg%)				
			Yabukita	Yutakamidori	Okumidori	Saemidori	Fushun
April	Catechins	EGC	1.542	3.640	0.589	1.720	0.561
		EC	1.486	8.542	0.670	0.845	0.591
		EGCG	1.384	14.133	0.354	1.098	0.279
		ECG	1.539	3.176	0.555	1.138	0.715
	Caffeine		68.382	66.079	58.684	62.974	67.937
May	Catechins	EGC	0.242	0.264	0.256	0.223	1.220
		EC	0.902	0.884	0.720	0.887	4.182
		EGCG	0.203	0.244	0.205	0.189	4.325
		ECG	0.043	0.165	0.055	0.041	0.830
	Caffeine		73.406	60.427	63.508	49.348	37.459
June	Catechins	EGC	0.262	0.292	0.393	0.187	0.933
		EC	0.875	0.752	0.762	0.862	4.299
		EGCG	0.354	0.198	0.243	0.189	1.836
		ECG	0.050	0.028	0.054	0.028	0.404
	Caffeine		44.628	40.046	40.795	64.021	25.675
July	Catechins	EGC	0.295	3.724	0.462	0.282	3.309
		EC	0.590	9.890	1.442	0.762	1.020
		EGCG	0.555	11.170	1.743	0.269	8.794
		ECG	0.436	2.128	0.719	0.557	1.356
	Caffeine		53.088	53.088	56.159	55.483	46.525
August	Catechins	EGC	7.458	6.520	2.490	1.258	0.343
		EC	7.993	9.452	4.731	3.143	2.093
		EGCG	25.056	19.101	7.265	5.890	0.378
		ECG	2.636	2.791	1.389	1.430	0.435
	Caffeine		38.536	33.438	40.517	44.814	50.902
September	Catechins	EGC	6.727	9.360	7.141	0.684	0.192
		EC	6.037	13.249	6.132	2.798	0.697
		EGCG	17.445	18.094	16.169	1.380	0.332
		ECG	1.819	2.954	1.606	1.069	0.043
	Caffeine		31.904	45.163	43.625	45.624	66.851
October	Catechins	EGC	6.734	6.243	5.755	1.024	0.251
		EC	11.822	14.986	7.493	2.659	1.328
		EGCG	10.376	7.132	13.454	3.771	0.387
		ECG	1.429	1.262	1.319	0.856	0.453
	Caffeine		28.395	28.879	36.779	55.252	57.847

Values are the mean of three replicates.
EGC: (-)epigallocatechin, EC: (-)epicatechin, EGCG: (-)epigallocatechin-3-gallate, ECG: (-)epicatechingallate.

in Yutakamidori green tea leaves drastically increased in September with 43 mg% catechin and then decreased drastically to 29.6 mg%. In particular, Yutakamidori green tea leaves contained a higher content of catechin (66.1 mg%) in April. The content of caffeine also greatly decreased to 22.9 mg% in October.

In Okumidori, the concentration of catechins drastically increased to 31 mg% in September from 2.2 mg% (April), and then decreased in October. In particular, the content of EGCG catechin as a major component was more than 50% among total catechins in Okumidori tea leaves. In contrast to EGCG, other catechins including EC, EGC, and ECG slightly increased from July through October. The content of caffeine in green tea leaves greatly decreased from 58.7 mg% in April to 36.8 mg% in October. In particular, the content of catechins in Okumidori was lower than those of Yabukita and Yutakamidori, but the content of caffeine was similar.

In Saemidori, the catechin content was about 11 mg% in August, the lowest value compared with other varieties of tea. The caffeine content decreased slightly from 62.9 mg% in April to 55.3 mg% in October. In particular, the caffeine content was higher than in the other varieties of tea plant. This shows that the catechin content of Saemidori was also affected by the picking season, but the caffeine content was slightly changed indicating the similar value in April and October.

The caffeine and catechins contents in Fushun gradu-

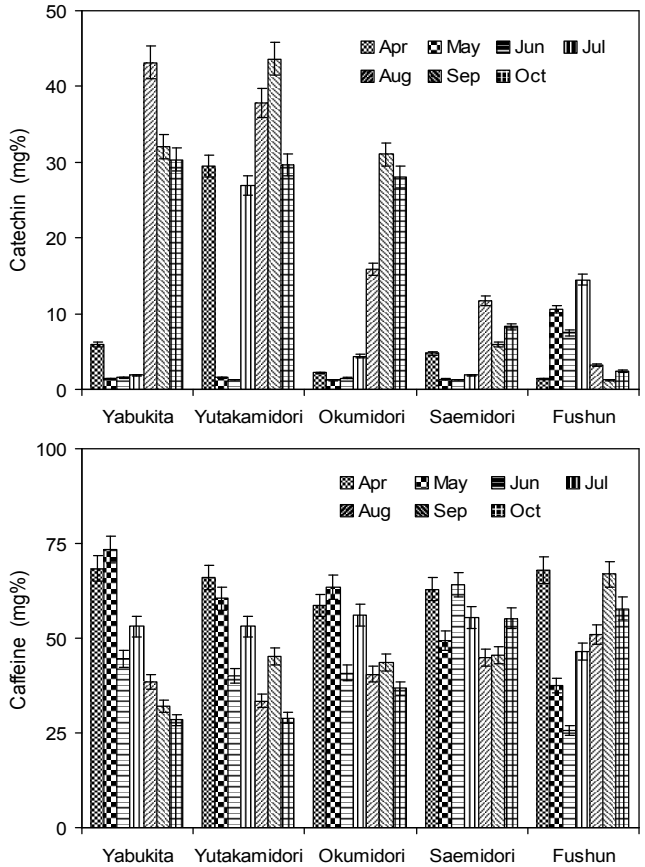

Fig. 1. Changes in total catechin and caffeine contents in green tea leaves of each variety picked in different seasons.

ally increased over the course of the picking season. The maximum content of catechin was about 14 mg% in July. It was observed that Fushun green tea leaves contained lower catechin, similar to that of Saemidori. Caffeine content in Fushun showed a higher value with 67.9 mg% in April, and then greatly decreased to 25.7 mg% in June. Unlike the other varieties, the caffeine content in Fushun gradually increased throughout the picking season, reaching 66.8 mg% in September. These results indicate that green tea leaves in Fushun showed lower catechin and higher caffeine contents later in the picking season.

Five varieties of green tea plants cultivated on Jeju Island produce catechins, such as EGCG, EC, EGC, and ECG. The maximum content of catechin was determined in the later months of the picking season, such as August or September (Fig. 1). In particular, EGCG catechin was a dominant component, making up more than 50% of total catechins which decreased later in the picking season (Table 1). The caffeine content gradually decreased over the course of the seven months of this study. Among the five varieties, Saemidori and Fushun showed higher caffeine and lower catechin contents during all the months of the study. EGCG catechin was shown to be a dominant component in green tea leaves from all five varieties of green tea plants. The contents of caffeine and catechin in green tea plants cultivated on Jeju Island varied with the types of tea plants and the date of picking. Phytochemicals in green tea leaves, including caffeine and catechins, provide valuable information about how to best use green tea leaves in food and functional food industries.

ACKNOWLEDGEMENTS

This work was supported by the research grant of the Cheju National University in 2006.

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(Received February 22, 2010; Accepted July 20, 2010)