DETERMINATION OF CATECHINS IN BREWED GREEN TEA AND READY-TO-DRINK GREEN TEA BEVERAGES

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PURPOSE

To determine the content of eight kinds of catechins and caffeine in brewed green tea or ready-to-drink green tea beverage.

MATERIALS AND METHODS

1. Standards

- (–)-Epigallocatechin gallate (EGCG) Kurita Water Industries, Ltd. (Tokyo, Japan)
- (–)-Epigallocatechin (EGC) Kurita Water Industries, Ltd. (Tokyo, Japan)
- (–)-Epicatechin gallate (ECG) Kurita Water Industries, Ltd. (Tokyo, Japan)
- (–)-Epicatechin (EC) Kurita Water Industries, Ltd. (Tokyo, Japan)
- (–)-Gallocatechin gallate (GCG) Kurita Water Industries, Ltd. (Tokyo, Japan)
- (–)-Gallocatechin (GC) Kurita Water Industries, Ltd. (Tokyo, Japan)
- (–)-Catechin gallate (CG) Kurita Water Industries, Ltd. (Tokyo, Japan)
- (–)-Catechin (C) Kurita Water Industries, Ltd. (Tokyo, Japan)
- Caffeine Nacalai Tesque, Inc. (Kyoto, Japan)

2. Green tea samples

2.1. Ready-to-drink green tea beverage

- Brand name: Tea's Tea-Pure Green

2.2. Leaf green tea

- Brand name: O-I O-CHA 1000
- Brand name: O-I O-CHA 800

3. Regents

- Milli-Q water Nihon Millipore Ltd. (Tokyo, Japan)
- Methanol, HPLC grade Wako Pure Chemical Industries, Ltd. (Osaka, Japan)
- Ethyl acetate, HPLC grade Wako Pure Chemical Industries, Ltd. (Osaka, Japan)
- Phosphoric acid Wako Pure Chemical Industries, Ltd. (Osaka, Japan)
- L-ascorbic acid Wako Pure Chemical Industries, Ltd. (Osaka, Japan)

4. Apparatus

4.1. HPLC system

- Pump two LC-10AD pumps for high-pressure gradient elution (Shimadzu Co., Kyoto, Japan)
- Detector an SPD-10Avp UV detector (Shimadzu Co., Kyoto, Japan)
- Column a Wakosil-II 5C18 HG (3.0 mm I.D. × 150 mm) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) equipped with a guard column of a Mightysil RP18GP (2.0 mm I.D. × 5 mm) (Kanto Kagaku, Tokyo, Japan)
- Software a V-Station data analysis software (GL Sciences Inc., Tokyo, Japan)

4.2. Others

- Analytical balances

- Volumetric flasks
- Teapot
- Stainless-steel pot
- Beakers
- Sample vials
- Disposable syringes for sample filtration
- 0.45-μm acrylic copolymer filters, Ekicrodisc type 13 (Nihon Pall Ltd., Tokyo, Japan)

5. HPLC conditions

5.1. Mobile phase

A: water/methanol/phosphoric acid (85/15/0.1, w/w/w)
B: water/methanol/ethyl acetate/phosphoric acid (85/15/1/0.1, w/w/w)

5.2. Elution program

Time (min)	A%	B%	Flow rate (ml/min)
0	100	0	0.30
12	100	0	0.30
13	100	0	0.45
19	100	0	0.45
20	0	100	1.00
40	0	100	1.00
41	100	0	1.00
50	100	0	1.00
51	100	0	0.30

5.3. Detection wavelength

280 nm

6. Standards

6.1. Preparation of stock solution

- Using calibrated analytical balance (±0.1 mg), weigh approximately 10 mg of eight kinds of catechins and caffeine.
- Bring these standards into a 100-ml volumetric flask.
- Add 100 ml of 0.5% (w/v) L-ascorbic acid solution to give concentrations of 100 mg/l.
- Store this stock solution at –20°C until use.

6.2. HPLC injection

- When used, thaw the standard solution at room temperature and filter through a 0.45-μm filter.
- Inject 2, 5 and 10 μl of standard mixture (approximately 100 mg/l) into HPLC system.

7. Preparation of green tea samples

7.1. Bottled green tea beverage

- Open up the bottle of green tea beverage.
- Dilute 1 ml of green tea sample with 4 ml of distilled water.

- Filter through a 0.45-μm filter before HPLC injection.
- Inject 10 μl of sample solutions into HPLC system.
- Calculate the concentrations of catechins and caffeine in the sample solutions.

7.2. Extraction from leafy green tea as commonly consumed

- Bring distilled water to a boil in a stainless-steel pot.
- Put accurately 4 g of green tea leaves per 170 ml of boiling water into the teapot.
- Cover the teapot with its lid and steep the green tea leaves for 1 min.
- Pour the green tea decoction from the teapot into the beakers, and weight this green tea decoction.
- Dilute 1 ml of green tea decoction with 4ml of distilled water.
- Filter through a 0.45-μm filter before HPLC injection.
- Inject 10 μl of sample solutions into HPLC system.
- Calculate the concentrations of catechins and caffeine in the sample solutions.

Volume of green tea decoction extracted from leafy green teas.

	Volume of green tea decoction (ml)	
	O-I Ocha 1000	O-I Ocha 800
Trial 1	140 ml	146 ml
Trial 2	147 ml	146 ml
Trial 3	144 ml	145 ml

8. Calculations

8.1. Calibration curves of standards

Prepare calibration curves for eight kinds of catechins and caffeine using their peak areas *versus* quantities using a linear least-squares analysis.

8.2. Catechin concentrations in green tea sample solutions

$$\text{Concentration (ng/10 μl)} = \frac{5 \times (\text{peak area} + \text{y-intercept})}{\text{slope}}$$

* where **5** is the dilution factor.
* where **ng/10 μl** was converted into **0.1 mg/l** or **ppm**

RESULTS

1. Calibration curves of standards

<Analysis date: Feb.24. 2005>

	Conc. of stock soln. (mg/l)	Injection volume (μl)	Injection quantity (ng)	Peak area	Calibration curve
Gallocatechin	96.6	2	193.2	74571	y = 346 x + 9328
(GC)		5	483	179010	
		10	966	342599	
Epigallocatechin	101.6	2	203.2	75414	y = 336 x + 8848
(EGC)		5	508	182011	
		10	1016	348919	
Catechin	98.2	2	196.4	224329	y = 1102 x + 8853
(C)		5	491	551290	
		10	982	1090253	
Caffeine	101.8	2	203.6	588514	y = 2897 x + 5470
		5	509	1490670	
		10	1018	2950298	
Epigallocatechin gallate	99.0	2	198	316817	y = 1613 x + 6289
(EGCG)		5	495	818589	
		10	990	1597542	
Epicatechin	88.4	2	176.8	111428	y = 619 x + 2367
(EC)		5	442	276492	
		10	884	549252	
Gallocatechin gallate	96.8	2	193.6	143139	y = 733 x + 1200
(GCG)		5	484	355845	
		10	968	710677	
Epicatechin gallate	94.6	2	189.2	175937	y = 913 x + 3345
(ECG)		5	473	435171	
		10	946	866677	
Catechin gallate	100.2	2	200.4	208328	y = 969 x + 11006
(CG)		5	501	491618	
		10	1002	983994	

<Analysis date: Mar.25. 2005>

	Conc. of stock soln. (mg/l)	Injection volume (µl)	Injection quantity (ng)	Peak area	Calibration curve
Gallocatechin	96.6	2	193.2	78272	y = 365 x + 7933
(GC)		5	483	184676	
		10	966	360588	
Epigallocatechin	101.6	2	203.2	79815	y = 354 x + 8152
(EGC)		5	508	188119	
		10	1016	367335	
Catechin	98.2	2	196.4	224303	y = 1105 x + 4883
(C)		5	491	543504	
		10	982	1091327	
Caffeine	101.8	2	203.6	607176	y = 2967 x – 1823
		5	509	1500561	
		10	1018	3021586	
Epigallocatechin gallate	99.0	2	198	317322	y = 1627 x – 574
(EGCG)		5	495	811477	
		10	990	1607483	
Epicatechin	88.4	2	176.8	106700	y = 585 x + 2060
(EC)		5	442	258816	
		10	884	520062	
Gallocatechin gallate	96.8	2	193.6	143535	y = 738 x – 1715
(GCG)		5	484	351311	
		10	968	713696	
Epicatechin gallate	94.6	2	189.2	176105	y = 917 x – 815
(ECG)		5	473	427629	
		10	946	868915	
Catechin gallate	100.2	2	200.4	212817	y = 967 x + 10529
(CG)		5	501	481502	
		10	1002	984679	

2. Contents of catechins and caffeine in green tea samples

2.1. Tea's Tea-Pure Green (Lot. PRD001105)

	Peak area	Concentration (mg/l)	Diluted factor	Original Concentration (mg/l)	Per serving size* (mg)
GC	136251	36.69	5	183	44
EGC	61056	15.56	5	78	19
C	81703	6.61	5	33	8
Caffeine	695510	23.82	5	119	29
EGCG	284725	17.28	5	86	21
EC	26718	3.93	5	20	5
GCG	148096	20.04	5	100	24
ECG	31969	3.14	5	16	4
CG	31128	2.08	5	10	2
Total catechin					**127 mg**

* serving size 240 ml

2.2. Brewed green tea

<Brand name : O-I O-CHA 1000>

		Peak area	Conc. (mg/l)	Diluted factor	Original conc. (mg/l)	Volume of decoction (ml)	Per serving size* (mg)
GC	Trial 1	30629	6.21	5	31	140	4
	Trial 2	25037	4.68	5	23	147	3
	Trial 3	37854	8.19	5	41	144	6
					32		**5**
EGC	Trial 1	307627	84.68	5	423	140	59
	Trial 2	261323	71.59	5	358	147	53
	Trial 3	342430	94.52	5	473	144	68
					418		**60**
C	Trial 1	3334	-0.14	5	–		
	Trial 2	2861	-0.18	5	–		
	Trial 3	4026	-0.07	5	–		
Caffeine	Trial 1	1776611	59.94	5	300	140	42
	Trial 2	1480786	49.97	5	250	147	37
	Trial 3	2030895	68.51	5	343	144	49
					297		**43**
EGCG	Trial 1	1080565	66.45	5	332	140	47
	Trial 2	912522	56.13	5	281	147	41
	Trial 3	1276172	78.48	5	392	144	57
					335		**48**
EC	Trial 1	98469	16.48	5	82	140	12
	Trial 2	82169	13.69	5	68	147	10
	Trial 3	110319	18.5	5	93	144	13
					81		**12**
GCG	Trial 1	8173	1.35	5	7	140	1
	Trial 2	7699	1.28	5	6	147	1
	Trial 3	8495	1.39	5	7	144	1
					7		**1**
ECG	Trial 1	96172	10.58	5	53	140	7
	Trial 2	80532	8.88	5	44	147	7
	Trial 3	109987	12.09	5	60	144	9
					53		**8**
CG	Trial 1	3277	-0.7	5	–		
	Trial 2	1618	-0.87	5	–		
	Trial 3	3394	-0.69	5	–		
Total catechin							**133 mg**

<Brand name : O-I O-CHA 800>

		Peak area	Conc (mg/l)	Diluted factor	Original conc. (mg/l)	Volume of decoction (ml)	Per serving size* (mg)
GC	Trial 1	38525	8.38	5	42	146	6
	Trial 2	37401	8.07	5	40	146	6
	Trial 3	28998	5.77	5	29	145	4
					37		**5**
EGC	Trial 1	271810	74.55	5	373	146	54
	Trial 2	263269	72.14	5	361	146	53
	Trial 3	293048	80.56	5	403	145	58
					379		**55**
C	Trial 1	2692	-0.2	5	–	146	
	Trial 2	2598	-0.2	5	–	146	
	Trial 3	2872	-0.18	5	–	145	
Caffeine	Trial 1	1740355	58.72	5	294	146	43
	Trial 2	1707406	57.61	5	288	146	42
	Trial 3	1772544	59.8	5	299	145	43
					294		**43**
EGCG	Trial 1	1003501	61.72	5	309	146	45
	Trial 2	968436	59.56	5	298	146	43
	Trial 3	1036684	63.76	5	319	145	46
					308		**45**
EC	Trial 1	92696	15.49	5	77	146	11
	Trial 2	94013	15.72	5	79	146	11
	Trial 3	98316	16.45	5	82	145	12
					79		**12**
GCG	Trial 1	4666	0.87	5	4	146	1
	Trial 2	4528	0.85	5	4	146	1
	Trial 3	5115	0.93	5	5	145	1
					4		**1**
ECG	Trial 1	93548	10.3	5	52	146	8
	Trial 2	89464	9.85	5	49	146	7
	Trial 3	100959	11.1	5	56	145	8
					52		**8**
CG	Trial 1	1873	-0.84	5	–	146	
	Trial 2	1427	-0.89	5	–	146	
	Trial 3	1741	-0.86	5	–	145	
Total catechin							**125 mg**

REFERENCES

Nishitani, E. and Sagesaka, Y. M. (2004) Simultaneous determination of catechins, caffeine and other phenolic compounds in tea using new HPLC method. *Journal of Food Composition and Analysis* **17**, 675-685.