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Factors affecting the levels of catechins and caffeine in tea beverage: estimated daily intakes and antioxidant activity

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Abstract: Increased public awareness of health benefits of green tea is generally based on the high polyphenol content of tea leaves and the resulting beverage. A number of factors, such as species, season, agronomic condition and age of the leaves, are known to affect the composition of commercial teas. In the present study the effects of factors associated with domestic preparation and analytical methods, such as brewing time, concentration, solvent and type of tea product, on levels of catechins and caffeine, antioxidant activity and estimated daily intakes were investigated. There were large variations in the levels of total catechins: 43 and 117 mg g^{-1} dry matter (DM) (brewed for 30 s and 5 min respectively); 72 and 161 mg g^{-1} DM (extracted in boiling water and 50% acetonitrile respectively); 72 and 117 mg g^{-1} DM (a tea bag and tea leaves respectively). The effects on caffeine content were comparatively smaller. These variations consequently led to considerable variations in estimated daily intakes based on three cups (600 ml), ranging between 538 and 2014 mg g^{-1} DM of total catechins and between 103 and 466 mg g^{-1} DM of caffeine. The antioxidant activity was highest (26 680 μmol g^{-1} DM) for tea leaves brewed for 5 min and lowest (10 110 μmol g^{-1} DM) for a tea bag product brewed for 1 min.

Keywords: green tea; tea catechins; EGCG; caffeine; daily intake; antioxidant activity; tea polyphenols

INTRODUCTION

Tea (*Camellia sinensis*) is the second most popular beverage, after water, in the world. In addition to its attractive aroma and good taste, tea has been recognised to have health-promoting properties, including antioxidant activity[1,2] and anticarcinogenic[3,4] and antihypertensive[5] effects. The beneficial effects of tea have been reported to be due to its polyphenolic composition and content, especially that of its major catechins, namely (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epicatechin (EC). In general, green tea contains higher amounts of catechins than black tea. Two epidemiological studies have shown an inverse relationship between tea consumption and concentration of serum triacylglycerols and cholesterol[6,7] and a decreased risk of coronary heart disease.[8–10] Other experimental studies, based largely on isolated cells and animal models, have shown cardioprotective and anticarcinogenic activities that are generally associated with the antioxidant effects (free radical scavenging and metal chelation) of catechins. Although such findings are not wholly supported by epidemiological studies, a significant body of research is directed towards elucidating the association between dietary consumption of tea and other dietary polyphenols derived from plants and reduction in the incidence of cancer and heart disease.[11,12] One requirement of such research is an estimation of dietary intakes based upon compositional data on tea beverage prepared and consumed by individuals.

Caffeine (CF), another major component in tea extract, has been studied intensively for its physiological effects on human health in terms of behaviour/mood[13,14] and as a diuretic[15] and weak bronchodilator.[16] Recently it was reported by the Food Standards Agency[17] that pregnant women should limit their intake of caffeine to less than 300 mg day^{-1} in the light of research indicating that caffeine intakes above this may be associated with low birth weight and, in some cases, miscarriage. Therefore it is important to estimate the level of CF consumed from food and drinks.

Tea plants are mainly cultivated in India, China, Japan, Taiwan, Sri Lanka and Indonesia and in Central Africa, notably Kenya. Hundreds of teas are now produced and sold all over the world. The composition of tea varies with species, season, age of the leaves, agronomic condition, manufacturing process and storage. In addition to these production factors, several other variables associated with blending at industrial

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level (brands) and domestic preparation of 'a cup of tea' may significantly affect the composition and expected health benefits of the drink.

In some cases, published data would not seem to be relevant to dietary consumption; for example, research on extraction of tea in organic solvents or on levels of polyphenols extracted from tea after 30 min.[2,18–20] A current study from this laboratory (unpublished) showed that most individuals ($n = 34$) spend less than 1 min in preparing 'a cup of tea'.

The present study was therefore conducted to investigate the effects of extraction time/brewing, dry matter (DM) concentration, type of tea, brand and different solvents on levels of major catechins and caffeine and their associated antioxidant activity in order to examine the extent of compositional variation and see how this may affect subsequent estimation of individual dietary intakes.

MATERIALS AND METHODS

Tea samples

Commonly consumed green tea products, ie tea leaves, powdered tea and tea bags, were purchased from supermarkets or specialised tea outlets in Tokyo, Japan. Powdered tea is a type of tea which is ground to a fine powder, normally after drying; it is most famously used for the tea ceremony.

Chemicals

Pure standards of (+)-catechin, (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC) and caffeine (CF) were purchased from Sigma-Aldrich (Poole, UK). Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), ferric chloride, 2,4,6-tripyridyl-*s*-triazine (TPTZ) and ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) were also purchased from the same source. Hydrochloric acid and acetic acid were obtained from Fisher Scientific (Loughborough, UK). All chemicals and solvents were of HPLC grade or analytical grade.

Tea preparation

Boiling distilled water (100 ml) was added to the tea product (1 g) in a flask and the mixture was shaken at fixed intervals (1, 2, 4 and 5 min) for 10 s each time. The concentration of dry matter used was 1–4% w/v. The effect of time on catechin extraction was studied by varying the extraction time (30 s, 1, 2, 4 and 5 min). The tea extract was strained, cooled to room temperature and the pH was adjusted to 3.2 with 1 M citric acid in order to maximise detection and stabilise the catechins during analyses. The diluted extracts were again filtered (0.2 μm filter) before being analysed by reverse phase high-performance liquid chromatography (RP-HPLC). All extracts were prepared in duplicate, and two analyses were carried out on each extract.

Extraction with two different organic solvents, 80% methanol and 50% acetonitrile, was also employed to compare with the extraction efficiency in boiling water. However, it is important to note that extraction of tea in organic solvent is not used in practice.

Analytical methods

Five major catechins, (+)-catechin, EC, ECG, EGC and EGCG, were determined by the method of Khokhar *et al*[21] using a Merck-Hitachi (Darmstadt, Germany) model D-7000 HPLC with System Manager (HSM, version 3.1) software. A Prodigy ODS-2 column (150 mm × 4.6 mm; Phenomex, Macclesfield, UK) fitted with a C18 guard column, L-7100 pump, L-7300 column oven, L-7200 autosampler and L-7450 diode array detector was employed. Tea extracts were injected directly without additional pretreatment, and detection was carried out at 278 nm. All peaks were plotted and integrated using the dedicated software. Peak identification was carried out by comparing retention times and UV spectra obtained by diode array detection with those of pure standards.

In summary, separations were carried out with 5% acetonitrile (eluant A) and 25% acetonitrile (eluant B) in phosphate buffer (0.025 M, pH 2.4). The gradient employed was: 0–5 min, 15% B; 5–20 min, linear gradient 15–80% B; 20–23 min, 80% B; 23–25 min, linear return to 15% B; 25–37 min, 15% B. The flow rate was 1 ml min^{-1} (10 μl injection volume), the column oven being set at 30 °C. Calibration solutions were freshly prepared for each series of analyses in methanol from stock solutions. Stock solutions of pure standards of (+)-catechin, EC, ECG, EGCG, EGC and CF (1 mg ml^{-1} in methanol containing 8 mg ml^{-1} citric acid) were stored at 4 °C Calibration curves were constructed by linear regression of the peak area against concentrations of the calibration solutions (10–100 μg ml^{-1}).

Total phenolic content

The total phenolic content of teas was determined using the Folin–Ciocalteu assay as described by Singleton *et al*;[22] the reagent is reduced by phenolic compounds with concomitant formation of a blue complex. The intensity measured spectrophotometrically at 760 nm increases linearly with the concentration of phenols in the reaction medium. Catechin was used as standard in the range 0–1000 μg ml^{-1}. The phenolic contents of the teas were determined from calibration equations and expressed as catechin equivalents (mg CtE g^{-1} DM).

Antioxidant activity

The FRAP (ferric–reducing antioxidant power) assay was performed according to Benzie and Strain[23] as modified by Pulido *et al*.[24] In brief, reductants (antioxidants) in the sample reduced the Fe^{3+}/tripyridyltriazine complex (present in excess) to its blue-coloured ferrous form with an increase in absorbance at 593 nm. Antioxidant power (FRAP value) results are expressed as μmol ferrous g^{-1} DM.

Recovery and stability

The recovery of catechins was calculated by comparing catechin levels in tea with those determined in tea spiked with known amounts (25 and 50 μg) of standard compounds. The standard mixture solution was run along with every four samples.

The stability of tea samples was studied because, during routine analysis, individual tea samples could remain in the autoinjector at room temperature for up to 24 h. The peak areas of the first four samples in a run were compared with the peak areas of the same samples analysed after 24 h. Data from four runs were pooled and a two-sided paired *t*-test was used for statistical analysis.

Estimated dietary intake

The daily intake of EGCG, total catechins and caffeine was calculated using compositional data (see Table 1) and on the basis of three cups (600 ml) of tea of variable strength (1–4% w/v), brewing time (1 and 5 min), type of tea (tea leaves, powdered tea and tea bags) and brand. The published data available on green and black teas are mainly taken from extractions in organic solvents. Comparisons were therefore also made between different solvents popularly used for such extraction (acetonitrile, methanol and water). It is reported by Imai *et al*[12] that medium-size (180 ml) tea cups were used by 70% of their study subjects (survey on living habits of the general population

Table 1. Effect of brewing time on catechin and caffeine contents (mg g^{-1} DM)

Brand name	Extraction time (min)	Total catechins	EGC	C	EC	EGCG	ECG	Caffeine
Tea leaves								
Ban-cha	0.5	42.7 ± 2.8	25.3 ± 1.5	ND	2.8 ± 0.3	12.4 ± 1.2	2.3 ± 0.2	15.8 ± 0.3
	1	43.7 ± 7.9	25.6 ± 3.5	ND	3.0 ± 0.5	12.6 ± 3.5	2.5 ± 0.6	16.4 ± 2.6
	2	62.2 ± 10.0	34.2 ± 5.8	ND	4.3 ± 0.6	19.7 ± 2.9	4.1 ± 0.8	18.9 ± 3.3
	4	73.3 ± 6.8	41.2 ± 4.2	ND	4.7 ± 0.2	22.9 ± 2.2	4.5 ± 0.4	18.2 ± 1.3
	5	83.6 ± 2.9	45.7 ± 2.3	ND	5.3 ± 0.1	27.3 ± 0.4	5.4 ± 0.1	20.3 ± 1.4
Fukamushi-cha	0.5	56.1 ± 2.4	35.6 ± 1.4	ND	4.0 ± 0.3	13.8 ± 0.8	2.8 ± 0.1	12.6 ± 0.6
	1	59.1 ± 2.2	37.3 ± 1.7	ND	4.5 ± 0.2	14.5 ± 0.3	2.9 ± 0.2	13.5 ± 0.2
	2	69.0 ± 9.5	43.8 ± 5.0	ND	5.0 ± 0.8	16.8 ± 3.1	3.5 ± 0.6	14.6 ± 0.9
	4	72.9 ± 2.4	46.0 ± 2.5	ND	6.0 ± 1.3	17.3 ± 0.3	3.6 ± 0.2	14.6 ± 0.5
	5	78.9 ± 4.6	51.0 ± 3.2	ND	5.4 ± 0.3	18.6 ± 1.5	3.8 ± 0.3	14.9 ± 0.9
Yame-cha	0.5	66.6 ± 5.9	37.0 ± 3.4	1.2 ± 0.5	4.6 ± 0.3	19.7 ± 1.4	4.2 ± 0.4	16.6 ± 0.8
	1	78.2 ± 2.3	44.0 ± 1.5	1.5 ± 0.3	5.2 ± 0.2	22.5 ± 0.8	4.9 ± 0.2	18.2 ± 0.1
	2	90.1 ± 1.5	48.8 ± 2.1	1.9 ± 0.4	6.0 ± 0.1	27.4 ± 0.6	5.9 ± 0.3	20.5 ± 0.1
	4	107.8 ± 6.3	58.9 ± 3.4	1.6 ± 0.7	7.2 ± 0.3	32.9 ± 2.2	7.2 ± 0.4	23.4 ± 0.2
	5	105.3 ± 4.5	56.6 ± 3.0	1.6 ± 0.4	7.4 ± 0.2	32.7 ± 1.1	7.1 ± 0.2	22.2 ± 0.8
Uji-cha	0.5	77.3 ± 4.8	43.5 ± 3.2	1.6 ± 0.4	5.3 ± 0.3	22.2 ± 1.2	4.6 ± 0.2	22.0 ± 0.3
	1	76.4 ± 0.7	42.5 ± 0.8	0.8 ± 0.6	5.4 ± 0.2	23.4 ± 0.3	4.4 ± 0.2	22.4 ± 0.1
	2	99.7 ± 13.2	53.6 ± 7.6	1.7 ± 0.2	6.6 ± 0.7	31.3 ± 4.0	6.4 ± 0.9	26.7 ± 1.7
	4	112.0 ± 5.4	60.0 ± 3.2	1.4 ± 0.4	7.4 ± 0.5	35.9 ± 1.8	7.3 ± 0.4	28.3 ± 1.7
	5	105.8 ± 8.5	57.3 ± 3.0	1.3 ± 0.9	6.8 ± 0.3	33.5 ± 3.8	7.0 ± 0.9	27.3 ± 2.7
Sayama-cha	0.5	70.1 ± 4.0	38.1 ± 2.5	1.6 ± 0.4	4.7 ± 0.1	21.5 ± 1.0	4.3 ± 0.2	17.8 ± 1.1
	1	89.7 ± 6.5	49.2 ± 3.4	1.6 ± 0.6	5.9 ± 0.6	27.4 ± 2.9	5.5 ± 0.7	20.2 ± 0.8
	2	99.9 ± 7.2	54.3 ± 5.7	1.6 ± 0.4	6.3 ± 0.1	31.4 ± 1.5	6.4 ± 0.3	21.4 ± 0.2
	4	115.2 ± 5.9	61.4 ± 3.5	1.9 ± 0.2	7.5 ± 0.6	37.0 ± 1.4	7.4 ± 0.2	24.6 ± 1.5
	5	116.6 ± 3.7	62.5 ± 2.2	2.0 ± 0.4	7.5 ± 0.2	37.2 ± 1.4	7.4 ± 0.3	24.3 ± 0.4
Powdered tea								
Uji-matt-cha	0.5	68.1 ± 15.9	38.3 ± 7.8	ND	4.7 ± 1.0	20.1 ± 5.9	4.6 ± 1.3	14.9 ± 2.3
	1	95.9 ± 4.3	52.9 ± 2.7	ND	6.8 ± 0.3	29.6 ± 1.3	6.6 ± 0.2	20.9 ± 0.9
	2	92.1 ± 3.8	52.6 ± 2.4	ND	6.1 ± 0.4	27.4 ± 1.4	6.0 ± 0.3	19.1 ± 0.7
	4	97.8 ± 5.3	55.5 ± 3.6	ND	6.8 ± 0.3	29.0 ± 1.3	6.4 ± 0.3	20.7 ± 1.2
	5	91.9 ± 7.7	54.1 ± 5.1	ND	6.0 ± 0.3	25.9 ± 1.9	6.0 ± 0.6	19.8 ± 1.1
Gyokuro-cha	0.5	94.9 ± 3.5	53.1 ± 4.5	ND	6.0 ± 0.2	29.9 ± 0.7	5.9 ± 0.2	24.6 ± 0.2
	1	95.0 ± 13.8	51.2 ± 7.3	ND	6.2 ± 0.9	31.5 ± 4.7	6.2 ± 1.0	25.1 ± 2.8
	2	105.7 ± 6.0	56.9 ± 3.8	ND	6.9 ± 0.4	34.9 ± 1.7	7.0 ± 0.3	25.9 ± 1.7
	4	111.8 ± 1.9	60.0 ± 1.4	ND	7.3 ± 0.2	37.2 ± 1.1	7.3 ± 0.1	26.0 ± 0.2
	5	110.5 ± 3.1	60.4 ± 2.7	ND	6.9 ± 0.2	35.9 ± 1.0	7.3 ± 0.2	25.8 ± 0.9
Tea bag								
Sen-cha	0.5	42.0 ± 11.3	20.8 ± 5.1	ND	3.3 ± 0.5	12.9 ± 5.5	2.9 ± 0.8	9.8 ± 0.3
	1	44.7 ± 1.3	21.9 ± 0.7	ND	3.8 ± 0.2	14.6 ± 0.5	3.0 ± 0.2	10.3 ± 0.1
	2	63.5 ± 12.6	32.6 ± 4.7	ND	5.8 ± 1.1	17.6 ± 6.0	4.9 ± 0.1	15.9 ± 0.1
	4	67.2 ± 5.5	34.3 ± 2.7	ND	7.1 ± 0.5	21.5 ± 2.2	5.1 ± 0.3	16.2 ± 0.3
	5	72.2 ± 5.3	36.8 ± 3.3	ND	7.9 ± 0.3	23.6 ± 2.8	5.4 ± 0.2	17.3 ± 0.2

Each value represents mean ± standard deviation (SD) of four individual determinations. ND, not detectable.

in Saitama Prefecture, Japan). Daily consumption of three cups by an adult above the age of 10 years in the UK[25] may be considered to be the equivalent of three to five Japanese cups of green tea.

RESULTS

The recovery rate for individual catechins and caffeine ranged between 79 and 115% (EGC, 115%; (+)-catechin, 111%; ECG, 99%; EGCG, 96%; EC, 83%; caffeine, 79%). The stability of tea samples was examined using a two-sided paired *t*-test; the results showed no significant differences in peak area between the first four samples and the same samples analysed after 24 h, with all the *p* values being in the range 0.65–0.95. It may thus be concluded that there was no degradation of the individual catechins during the analysis and that the samples were stable for at least 24 h at room temperature in the autosampler.

Effect of brewing/extraction time

Composition

The composition of individual catechins and caffeine is presented in Table 1. The time of extraction of all teas was selected from 30 s to 5 min. Regardless of extraction time, EGC and EGCG predominated (comprising more than 80% of total catechins). The overall ranking of catechins (EGC > EGCG > EC > ECG) brewed in boiling water was similar in all green teas and was not affected by extraction time.

In general, the levels of all catechins except (+)-catechin increased with increasing brewing time from 30 s to 4 min, after which they did not increase substantially (Fig 1). In spite of the increase in brewing time, (+)-catechin was detected in only three brands. In other brands, (+)-catechin may be present in negligible amounts below the detection limit. It is also know that Ban-cha, Fukamushi-cha and tea bags contain lower grades of Japanese green tea and are less carefully handled during processing, which could lead to losses. Although this trend was observed in all green teas, the extraction efficiency of individual catechins varied most notably at shorter extraction times (30 s, 1 and 2 min).

Assuming that maximum (100%) extraction was achieved at 5 min, the effect of brewing time on caffeine was smaller, showing the highest extraction efficiency at 1 min (84%) followed by 30 s (78%). On average the caffeine level of tea leaves increased from 16.9 $\pm$ 3.4 mg g^{-1} DM at 30 s to 21.8 $\pm$ 4.6 mg g^{-1} DM at 5 min. In comparison, the extraction efficiencies of EGCG and ECG were much lower (61 and 59% respectively) at 30 s. The average EGCG level increased from 17.9 $\pm$ 4.5 to 29.8 $\pm$ 7.2 mg g^{-1} DM with time. It appears that catechins which possess galloyl moieties have a lower extraction efficiency over the shorter time period. The extraction efficiency of major tea components was found to be in decreasing order of caffeine > EGC > EC > EGCG > ECG.

Dietary intake

Increase in individual catechins with increased extraction time leads to substantial variations in the estimated dietary intake of catechins (411 $\pm$ 106 and 589 $\pm$ 97 mg day^{-1} when tea leaves are brewed in water for 1 and 5 min respectively). In reality, most individuals brew tea for less than 1 min (unpublished data from this laboratory). Calculation of dietary catechin intake obtained from longer periods of brewing (5 min) would overestimate this value.

Variations in daily intake of caffeine after 1 and 5 min brewing time of tea leaves (109 $\pm$ 21 and 131 $\pm$ 28 mg day^{-1} respectively) were smaller.

Antioxidant activity

Antioxidant activity (FRAP) and total phenolic content increased with increasing extraction time in a similar manner as for total catechin levels (Table 2). There was a strong correlation between antioxidant activity and total phenolic content ($R^2 = 0.99$). This strong antioxidant activity may be a result of the majority of phenolics being present as major catechins

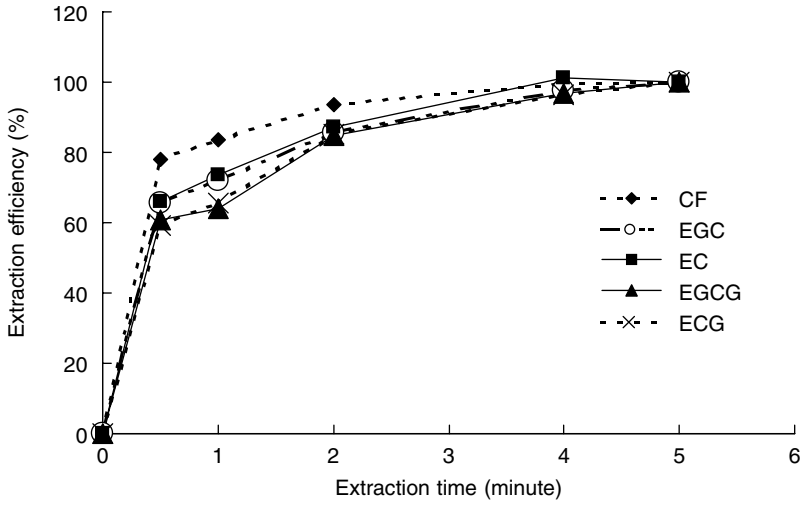

Figure 1. Effect of brewing time on levels of individual catechins and caffeine.

(82–100% in tea leaves, 83–95% in powdered teas and 68–79% in tea bags; Table 2). A previous study has also shown similar findings.[26] The average FRAP value increased from $15\,063 \pm 432\,\mu mol\,g^{-1}$ DM (1 min) to $26\,681 \pm 535\,\mu mol\,g^{-1}$ DM (5 min). When compared with the antioxidant activity of selected fruits, tea brewed for 1 min exhibited a similar activity to that of 1 kg of fresh fruit (kiwi and pear).[27] According to Szeto *et al*,[28] strawberry and lemon possess the highest FRAP values (15 940 and $10\,400\,\mu mol\,kg^{-1}$ fresh weight respectively); however, much of these activities may have been due to ascorbic acid rather than polyphenols, as is the case for tea.

Effect of dry matter concentration

The effect of leaf concentration on catechin levels was determined by brewing 1, 3 and 4% w/v of Sayama tea leaves for 1 and 5 min. As expected, the amount of catechins present in the tea extract was linearly related to its dry matter concentration (Fig 2), indicating that stronger brews will provide higher dietary intakes (EGCG, 154–533 and 538–1756 mg day^{-1} of total catechins brewed for 1 min) than longer brewing time (EGCG, 179 and 588 mg day^{-1} of total catechins when brewing 1% w/v tea leaves for 5 min).

Table 2. Effect of brewing time and product type on antioxidant activity (FRAP value) and total phenols

	FRAP value ($\mu mol\,g^{-1}$ DM)	Total phenols (mg CtE g^{-1} DM)
Tea leaves		
1 min	15 062.5 ± 432.3	88.7 ± 5.7(101.3%)[a]
2 min	18 725.0 ± 231.2	100.5 ± 1.5(97.6%)
5 min	26 680.6 ± 535.3	142.6 ± 25.7(81.9%)
Powdered tea		
1 min	18 215.0 ± 233.2	100.5 ± 2.7(94.6%)
2 min	20 187.5 ± 98.0	113.8 ± 13.6(93.0%)
5 min	25 902.8 ± 264.0	132.5 ± 8.8(83.5%)
Tea bag		
1 min	10 116.7 ± 265.0	62.6 ± 1.3(68.4%)
2 min	13 000.0 ± 536.9	80.3 ± 6.0(79.0%)
5 min	15 354.2 ± 108.3	95.3 ± 2.7(75.8%)

Results are expressed as mean ± SD of 4–12 determinations. Tea leaves (average of Fukamushi-cha, Uji-cha and Sayama-cha), powdered tea (Uji-matt-cha and Gyokuro-cha) and tea bag (Sen-cha) were extracted for 1, 2 and 5 min in boiling water.
[a] Percentage contribution of total catechins to total phenolic content.

Effect of type of tea

Composition

Levels of total catechins were found to be similar for leaf and powdered teas (78–117 and 92–112 mg g^{-1} DM respectively) but significantly ($p = 0.003$) lower for tea bags ($72 \pm 5\,mg\,g^{-1}$ DM) when compared at 5 min extraction. For a shorter extraction time, powdered tea showed a much higher extraction efficiency (95%) than tea leaves (69%) (Fig 3). This may be a result of the powder being more easily wetted, resulting in faster extraction into the liquid due to diffusion. There are several possible reasons for the low level of catechins in tea bags. The bag may act as a barrier, leading to slow diffusion; in addition, leaf and powdered teas are generally of superior quality and so contain higher amounts of catechins.[26] There was no difference in the caffeine content of these teas according to extraction time.

Antioxidant activity

Increase in extraction of catechins was directly related to antioxidant activity (Fig 4), with tea bags having a significantly lower FRAP value compared with leaf and powdered teas. When considering the average time of tea preparation, powdered tea may be considered a better choice because of its higher extraction efficiency and higher antioxidant activity at comparatively shorter extraction time (1 min).

Effect of extraction solvent

Although extraction data obtained on all catechins and caffeine for organic solvents do not reflect real tea preparation, such solvents were included in this study to compare our results with published data;

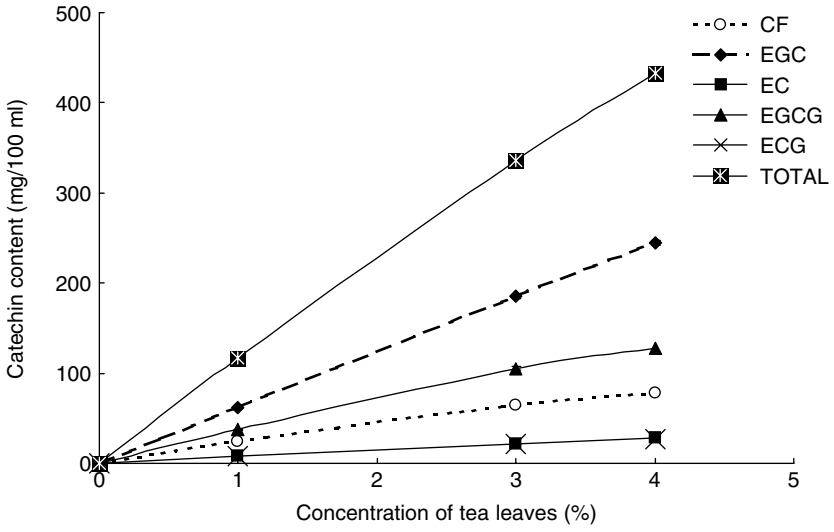

Figure 2. Effect of dry matter concentration (strength of brew) on levels of individual catechins extracted in 100 ml of water.

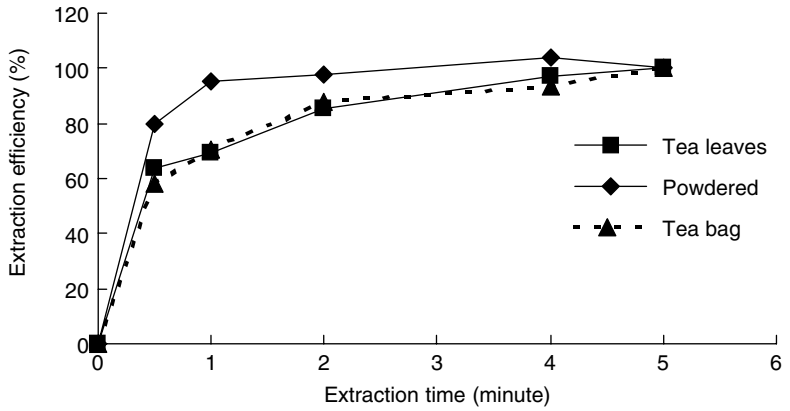

Figure 3. Effect of brewing time on levels of catechins in different tea products.

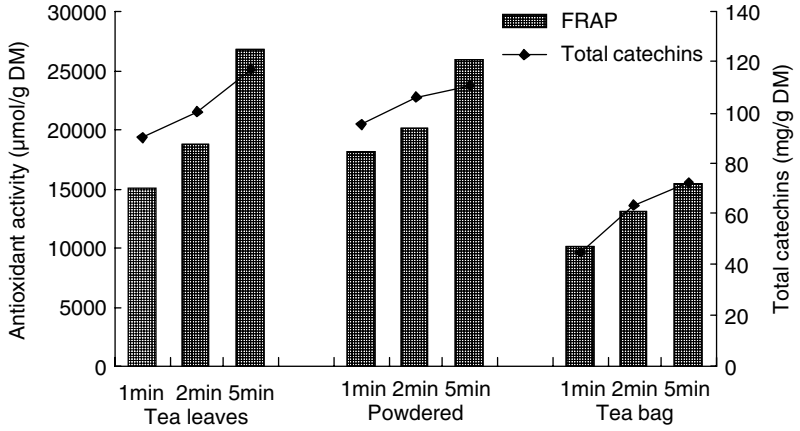

Figure 4. Variations in antioxidant activity (FRAP value) and total catechin content among different tea products.

80% methanol and 50% acetonitrile were selected because they have been widely used for quantitative extraction of catechins from green tea.[18,20,29] In addition, Suematsu *et al*[29] have previously suggested 50% aqueous acetonitrile to be an effective extraction solvent for tea catechins.

Acetonitrile (50%) showed the highest extraction efficiency for all individual catechins as well as caffeine (Fig 5). The amounts of EGCG and ECG extracted by this solution were significantly ($p < 0.001$) higher (74.7 ± 10.8 and $13.5 \pm 2.4\,mg\,g^{-1}$ DM respectively) than those extracted by boiling water (32.1 ± 4.8 and $5.9 \pm 1.0\,mg\,g^{-1}$ DM respectively). EGC, EC and CF were extracted by 50% acetonitrile slightly more than by boiling water, although the difference was not significant. EGCG was the most abundant of the major catechins when extracted with acetonitrile, whereas EGC was present in the highest amount when extracted with boiling water. This contributed to significantly ($p < 0.001$) higher levels of total catechins ($161 \pm 25\,mg\,g^{-1}$ DM) in acetonitrile as compared with boiling water ($102 \pm 14\,mg\,g^{-1}$ DM) and methanol ($113 \pm 2\,mg\,g^{-1}$ DM). Lin *et al*[30] have reported more EGCG in tea samples extracted in 65–75% ethanol than occurred with boiling water. Khokhar and Magnusdottir[26] reported that water was a better solvent for black tea catechins than either aqueous ethanol (70%) or aqueous methanol (80%). It is considered that the lower extraction efficiency of boiling water compared with unheated organic solvents may be due to epimerisation of catechins, since Xu *et al*[31] have shown that EGCG, EGC, ECG and EC were epimerised to (−)-gallocatechin gallate (GCG), (−)-gallocatechin (GC), (−)-catechin gallate (CG) and (+)-catechin when heated at 120 °C for 20 min.

The lower extraction efficiency of EGCG and ECG in boiling water could be due to their greater hydrophobicity as compared with other catechins (EGC and EC). It is evident from these results that organic solvents have a greater effect on extraction of catechins, especially EGCG and ECG. The former compound is a major component in green tea extract and is the focus of much current study owing to its possible pharmacological benefits.

Estimated dietary intake

Consequently, the effect of organic solvent led to considerable variations in the estimated dietary catechin intake. The estimated intake of EGCG varied enormously between 179 and 448 mg day^{-1} and that of total catechins varied between 588 and 967 mg day^{-1}. The estimated caffeine intake also varied but to a much smaller extent (132–162 mg day^{-1}) (Fig 6). The values obtained by organic solvent may not only lead to overestimation, but also such data will not be relevant

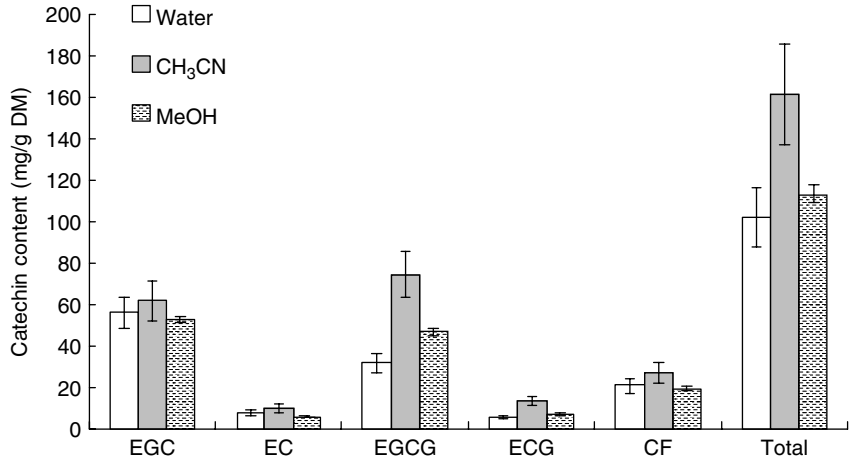

Figure 5. Comparison of boiling water with organic solvents on extraction of individual catechins and caffeine. Sayama-cha was extracted in boiling water, 50% acetonitrile (CH_3CN) and 80% methanol (MeOH) at room temperature for 5 min.

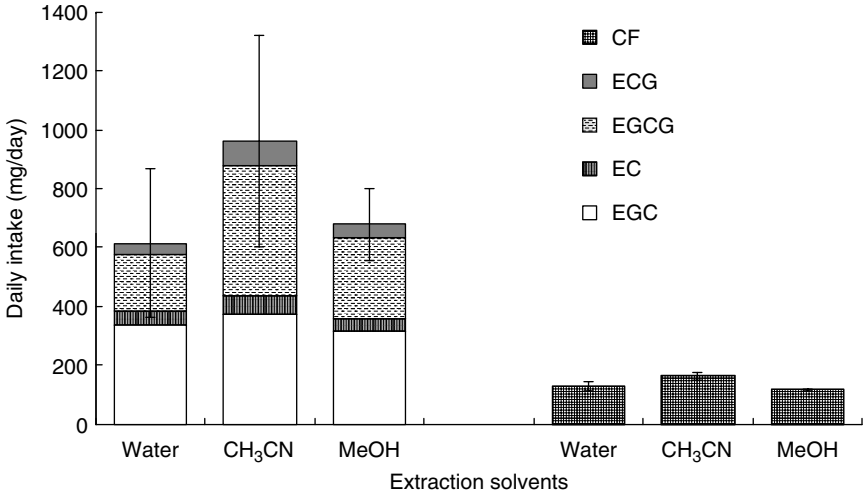

Figure 6. Variations in estimated dietary intake of catechins and caffeine subjected to different extraction solvents: water = boiling water; CH_3CN = 50% acetonitrile at room temperature; MeOH = 80% methanol at room temperature. Data are given on the basis of an average of three cups (600 ml) of Sayama-cha extracted for 5 min.

for estimating dietary intake of catechins and caffeine from beverages. Data obtained on tea composition by extraction in boiling water will represent the domestic preparation of tea beverage.

DISCUSSION

Increased public awareness of the health-protective nature of tea is generally based on the high polyphenol content of tea leaves and the resulting beverage. Previous studies[30,32] have demonstrated that variation in catechin content and composition of commercial teas is a result of a large number of factors, including variety, leaf composition and age, cultivation, husbandry conditions (including location), industrial processing (fermentation and blending) and storage. To these must be added the factors associated with domestic preparation and analytical methods, such as the nature of the solvent, strength, time and temperature of extraction. The wide variation in catechin levels from selected published data is summarised in Table 3. Usage of published data

Table 3. Variations in catechin levels of different tea extracts (published data)

Type of tea	Range of total catechins (mg g^{-1} DM)	Solvent used/time of extraction	Ref
JGT	151–229	AA/40 min	18
JGT	41–114	W/30 min	30
CGT	66–144	AE/30 min	30
CGT	57–74	AM/3 h	2
JGT	89–241	W/10 min	19
CGT	116–227	W/10 min	19
JGT	41–109	AA/40 min	20
BT	16–45	AA/40 min	20
BT	33–39	AM/20 min	32
BT	3–4	W/40–60 s	32
BT	5–48	W/5 min	26

Data have been recalculated as mg g^{-1} DM to enable comparisons with data obtained in the present study. JGT, Japanese green tea; CGT, Chinese green tea; BT, black tea. AA, aqueous acetonitrile solution; W, boiling water; AE, aqueous ethanol solution; AM, aqueous methanol solution.

Table 4. Effect of brewing time, strength and solvent on estimated daily intakes of EGCG, total catechins and caffeine

Tea type	Brewing time (min)	Conc (%)	Solvent	EGCG intake (mg day^{-1})	Total catechin intake (mg day^{-1})	Caffeine intake (mg day^{-1})
Tea leaves	1	1	W	154.6 ± 17.2	538.1 ± 26.2	121.4 ± 4.6
	5	1	W	179.0 ± 41.1	588.1 ± 99.8	132.0 ± 27.9
	1	3	W	445.5 ± 43.2	1436.1 ± 161.0	316.4 ± 18.0
	5	3	W	631.5 ± 21.7	2014.0 ± 97.4	383.0 ± 25.3
	1	4	W	533.2 ± 39.7	1756.4 ± 178.2	381.8 ± 27.6
	5	4	W	769.4 ± 30.1	2593.8 ± 147.4	466.3 ± 16.6
	5	1	AA	448.1 ± 20.0	967.1 ± 162.1	162.1 ± 9.3
	5	1	AM	282.0 ± 19.4	679.5 ± 35.0	116.8 ± 3.4
Powdered tea	1	1	W	183.2 ± 19.8	572.9 ± 59.3	138.1 ± 17.7
	5	1	W	185.3 ± 33.2	607.1 ± 71.6	136.6 ± 20.1
Tea bag	5	1	W	141.6 ± 17.3	427.6 ± 53.0	103.8 ± 1.0

Each value represents mean ± SD for tea leaves (Sayama-cha), powdered tea (average of Uji-matt-cha and Gyokuro-cha) and tea bag (Sen-cha). The estimated daily intake was calculated assuming that three cups (600 ml) were consumed per day. W, boiling water; AA, 50% acetonitrile aqueous, solution; AM, 80% methanol aqueous solution.

is important for epidemiological and other research studies. However, such data must be treated with caution because of the effect of a wide range of factors on the levels of catechins and caffeine in tea beverages. A range of factors associated with domestic tea preparation have not yet been clearly demonstrated and how these may lead directly to under- or overestimation of dietary intakes. Therefore the results presented here are, for the first time, for the purpose of comparing such factors and to demonstrate variations in the composition of tea beverage leading to considerable variations in the estimated dietary intakes of a population or subgroups of populations.

Dietary estimates are essential in epidemiological and intervention studies and clinical research. The choice of tea and of individual preparation methods varies in different countries; for instance, green tea is normally preferred in Japan and China, whereas black tea is still by far the most consumed beverage in the rest of the world. Recent trends, however, indicate a growing interest in green teas, partially in response to health claims and the use of tea extracts in the food and pharmaceutical industries, modern medicine and natural healing.

The present study shows that time of brewing/extraction, type of tea (tea leaves, powdered or tea bags), brand and strength of tea are critical conditions leading to unexpected variations in the catechin content of 'a cup of tea' (Table 4). Dietary intake calculated from tea leaves (Sayama-cha) brewed for 1 and 5 min varied between 538 and 612 mg day^{-1} respectively. Daily intake of total catechins calculated on the basis of 5 min boiling water extraction was lower (612 mg day^{-1}) than when methanol and acetonitrile were used (679 and 967 mg day^{-1} respectively). The strength of tea (concentration of dry matter used) consumed would obviously affect daily intake (538 and 1756 mg day^{-1} for 1 and 4% w/v respectively on the basis of 5 min extraction). Many individuals will drink tea of variable strength, especially when out of their homes, introducing further variation in their dietary catechin intake from this beverage. In the UK, India and Africa, milk is often added to reduce astringency (an effect brought about by the affinity of casein for polyphenols), and such treatment might even reduce levels of biologically active polyphenols by complexation/precipitation. Therefore the likely range of daily intakes serves to emphasise the need to more effectively relate compositional data to intakes and biological effects in various population groups.

The role of caffeine in the development of certain diseases and conditions has been the subject of extensive research in recent years. The present study shows that the level of caffeine varies depending on the extraction method; however, the variations obtained were relatively smaller than those for catechins.

CONCLUSIONS

The composition of tea beverages (total phenols, major catechins and caffeine) was significantly affected by brewing conditions. During tea preparation, several factors (brand, type of tea product, brewing time, strength of beverage, etc) operate in combination, eg different strengths of different types of tea brewed for a range of times. The results presented here are compared for single as well as multiple variables. The estimated daily intake of total catechins may vary from 538 to 2594 mg day^{-1} depending on the brewing conditions used. These factors will consequently lead to variations in antioxidant activity and dietary intakes, although tea preparation methods are further complicated by individual preferences. These results are of significance if dietary intakes of a population are to be calculated for use in epidemiological, clinical and other research investigations.

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