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Simulated intestinal digestion of green and black teas

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Abstract

Previous studies have shown that significant changes to green tea catechins occur as a result of changes in pH similar to those found in the gastrointestinal tract. In this study we have demonstrated that the sum of the antioxidant activities attributable to the four major catechins in brewed green and black tea samples was less than the total measured antioxidant activity, although there was a high degree of correlation between antioxidant activity and total measured polyphenol concentration. In addition, incubation of either form of tea at acid pH (as found in the stomach) had little effect of the concentration of individual catechins. However, incubation at slightly alkaline pH, similar to that found in the small intestine, resulted in a rapid decline in the concentrations of both green and black tea catechins, but with a lesser reduction in antioxidant activity and polyphenol concentration.

Keywords: Green tea; Black tea; Antioxidants; Polyphenols; Digestion

1. Introduction

Throughout the world two main types of tea are consumed, green tea which is largely consumed in China, Japan and regions of Africa and the Middle East, and black tea which is mainly consumed in India, Sri Lanka and European countries. Both teas are prepared from the leaf of *Camellia sinensis*, either *var assamica* or *sinensis*. In preparation of green tea, the leaves are dried relatively rapidly after plucking and withering to minimise chemical and enzymatic reactions, whereas black tea is prepared from leaves that have been withered and undergone a polyphenol oxidase-catalysed oxidation of catechins prior to drying. The differences between the two processes result in differences in the polyphenol profiles between green and black teas. Detailed discussions of the production of teas are described elsewhere (Wilson & Clifford, 1992). In some populations tea also provides the greatest single source of polyphenolic antioxidants in the diet (Hertog, Hollman, Katan, & Kromhout, 1993; Hertog, Sweetnam, Fehily, Elwood, & Kromhout, 1997). There is increasing interest in the potential health benefits of green tea, and its epidemiological and experimental association with a reduction in the morbidity and mortality from a number of degenerative diseases such as cardiovascular disease and certain cancers (Chen & Han, 2000; Tijburg, Mattern, Folts, Weisgerber, & Katan, 1997). The beneficial effects of green tea have been attributed to the presence of antioxidant flavonoids, however evidence is accumulating that consumption of black tea has also been associated with positive health benefits (Blot, McLaughlin, & Chow, 1997). Indeed it has been demonstrated that the antioxidant activity of the black tea, as well as increases in antioxidant activity in plasma following black tea consumption are similar to green tea (Benzie & Strain, 1999; Leenen, Roodenburg, Tijburg, & Wiseman, 2000).

Despite the epidemiological evidence that consumption of various teas is associated with positive health benefits, there is little information on the fate of tea components in the gastrointestinal tract. Zhu, Zhang, Tsang, Huang, and Chen (1997) reported that (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin (EGC) were relatively unstable at modestly alkaline pH, whereas (−)-epicatechin (EC) and (−)-epicatechin

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gallate (ECG) were comparatively stable. Yoshino, Suzuki, Sasaki, Miyase, and Sano (1999) studied the oxidative dimerisation of EGCG in simulated intestinal juice and in mouse plasma and reported that these dimers (referred to as P1, P2, and P3) had similar or greater antioxidant activity to EGCG. In addition they reported that EGC, EGCG and ECG were rapidly degraded at alkaline pH, as exists in the small intestine, however, EC was relatively resistant. In this study we have taken extracts of both green and black teas as normally consumed and subjected them to a simulated gastric and intestinal digestion in vitro and monitored the fate of individual catechins during this process. In addition we have also monitored the antioxidant activity and the total phenolic concentration in the same fractions.

2. Materials and methods

Green and black tea bags (Madura Tea Estates, Murwillumbah, NSW, Australia) were purchased at a local supermarket. The tea bags contained 1-g tea and were described as a blend of local and imported teas. Infusions were prepared by immersing a tea bag in 50ml water purified by reverse osmosis that had just been brought to the boil. The tea bag was then left for up to 4 min, removed from the water, lightly squeezed and discarded. The resultant infusions were analysed as soon as practical after preparation.

Antioxidant capacity was determined as described by Benzie and Strain (1999) and expressed as μmol Fe^{2+} reducing equivalents. Total polyphenol concentrations were determined with the Folin method (Folin & Denis, 1915). Caffeine and individual catechins were determined by high performance liquid chromatography (HPLC) following the method described by Wang, Helliwell, and You (2000) with slight modifications. A Shimadzu LC 10 HPLC fitted with an autosampler and SPD-M10Avp photodiode array detector with a class LC 10 chromatography workstation was used for analysis of the prepared samples. The column used was a Rainin (4.6 mm ID X 250 mm length) C18 (5 μm spherical particles) reverse phase column. The mobile phase was water/methanol/orthophosphoric acid (79.9:20:0.085) with a flow rate of 1.5 ml/min at 30°C. Absorbances at 210 nm and 280 nm were monitored throughout each run.

Simulated gastric digestion was carried out essentially as described by Yoshino et al. (1999). Samples of tea beverages were incubated at 37°C for 1 h after acidification with HCl to pH 2.0. The samples were then brought to pH 7.5 with sodium bicarbonate and the incubation continued for up to 60 min.

Catechins, 2,4,6 tripyridyl-s-triazine and gallic acid were obtained from Sigma (St Louis, Mo, USA). Caffeine, other reagents and HPLC solvents were obtained from BDH, Poole, Dorset, UK).

3. Results

Individual catechins, gallic acid (GA) and caffeine were examined for their antioxidant activity (Fig. 1). On a molar basis ECG had the greatest activity, followed by EGCG and GA then EGC and EC. An equimolar mixture of the epicatechins and GA was also analysed. The measured antioxidant activity of the mixture was found to be 12.5±0.8 mmol Fe^{2+} equivalents/l, and the calculated activity from the sum of the measured antioxidant activities was 11.6 mmol Fe^{2+} equivalents/l indicating that there was no synergism between the antioxidants.

Hot-water extraction of GA, catechins, caffeine and antioxidant activity from green and black tea bags with time is presented in Table 1. It can be seen that, in most cases, the concentration of the individual catechins was

Table 1
Extraction of tea catechins, caffeine and antioxidant activity from green or black tea bags by boiling water[a]

Brewing time (seconds)	GA (μmol/l)	EGC (μmol/l)	EGCG (μmol/l)	EC (μmol/l)	GCG (μmol/l)	ECG (μmol/l)	Total phenolics (mmol/l)	Caffeine (mmol/l)
Green tea								
30	54±7	344±34	296±24	85±7	3±0	69±4	0.85±0.07	0.49±0.04
60	190±24	794±44	716±71	213±21	7±1	174±21	2.09±0.18	1.09±0.10
90	289±90	1150±328	1060±304	317±92	13±4	258±74	3.10±0.02	1.56±0.44
120	325±46	1230±125	1220±127	365±44	16±3	306±34	3.41±0.04	1.77±0.19
240	456±45	1470±156	1560±131	505±52	23±3	435±41	4.44±0.42	2.23±0.24
Black tea								
30	132±37	105±41	128±29	90±21	9 ±1	78±15	0.54±0.07	1.35±0.27
60	198±18	188±59	182±16	134±12	11±1	119±10	0.83±0.11	1.86±0.13
90	276±28	226±32	260±29	185±18	20±4	168±22	1.13±0.13	2.41±0.18
120	357±14	309±25	350±18	244±12	22±2	209±11	1.49±0.06	2.98±0.09
240	481±51	458±161	493±130	331±85	31±1	287±69	2.08±0.16	3.65±0.88

[a] Values are means ± S.E. of three independent extractions.

lower in the black tea extract than that obtained from green tea. The exceptions were GA, which was similar in both teas and (−)-gallocatechin gallate (GCG), which was lower in the green tea extracts. Caffeine concentrations were lower in the green tea extract at each time-point than in the corresponding black tea extract. The sum of GA and catechin concentrations calculated from HPLC measurements in the black and green tea extracts is also presented in Table 1. At each time point examined the total catechin content as measured by HPLC was less in the black tea extract than in the green tea infusions. Summation of the calculated and measured antioxidant activities of the individual components at each time-point is presented for comparison in Table 2. Antioxidant activity was lower in the black tea than in green tea extracts (Table 2). There was a high degree of correlation between the measured antioxidant activity and the molar sum of the individual antioxidant phenols ($r^2 = 0.93$ for green tea and 0.91 for black tea, respectively). Comparison Table of the calculated and measured antioxidant activities shows that the calculated antioxidant activity accounted for by the measured catechins decreased with infusion time in green tea from 91% after 30 s to 73% after 4 min. In contrast, in the black tea infusion, accountable antioxidant activity increased marginally from 35–36% in the first 1.5 min to 45% after 4 min (Table 2).

The observed concentrations of the analytes following simulated gastric digestion are presented in Table 3. Incubation of the tea solutions at pH 2.0, the approximate pH of the stomach showed variable losses under these conditions. GA, EC and ECG were either unaffected or declined slightly, however EGC, EGCG and GCG decreased by over 50% in the black tea samples,

Table 2
Comparison of calculated and measured antioxidant activities of green or black tea[a]

Brewing time (s)	Antioxidant activity measured (mmol/l Fe^{2+} equivalents)	Antioxidant activity calculated (mmol/l Fe^{2+} equivalents)
Green tea		
30	4.00±0.11	3.6±0.08
60	10.7±0.45	9.0±0.20
90	15.4±0.22	13.3±0.04
120	20.0±0.08	15.1±19.6
240	26.9±0.41	19.6±0.46
Black tea		
30	6.77±0.59	2.5±0.10
60	10.7±0.51	3.7±0.11
90	14.3±0.77	5.1±0.14
120	16.8±0.55	6.7±0.07
240	20.6±0.46	9.3±0.22

[a] Values are means ± S.E. of three independent extractions.

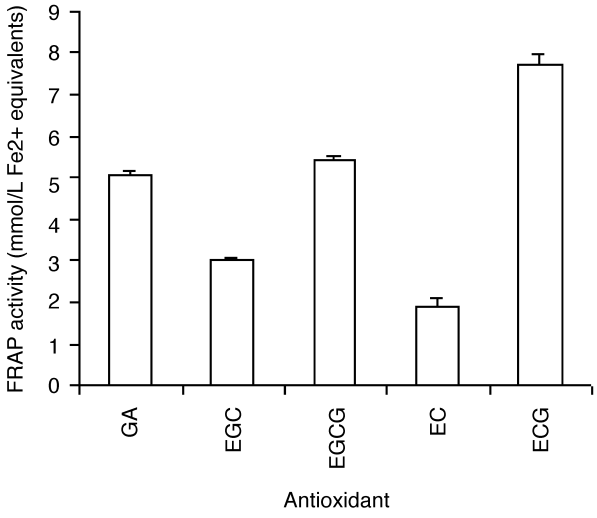

Fig. 1. Comparative antioxidant activity (mmol Fe^{2+} equivalents/l) of gallic acid (GA), epigallocatechin EGC, epigallocatechin gallate (EGCG), epicatechin (EC) and epicatechin gallate (ECG). Each antioxidant was present at a final concentration of 1 mmol/l. Values are means ± S.E. of 3 individual determinations.

Table 3
Extraction of tea catechins, caffeine and antioxidant activity from green or black tea bags following simulated gastric and intestinal digestion[a]

Treatment	GA (μmol/l)	EGC (μmol/l)	EGCG (μmol/l)	EC (μmol/l)	GCG (μmol/l)	ECG (μmol/l)	Caffeine (μmol/l)
Green tea	610±107	1560±67	1880±78	624±23	19±2	279±2	2350±55
1h at pH 2.0	542±56	1261±58	1500±81	585±22	24±1	246±2	2260±46
15 min at pH 7.5	515±7	538±79	943±91	556±27	22±4	233±11	2240±80
30 min at pH 7.5	490±19	175±73	454±118	540±31	15±4	213±10	2240±68
60 min at pH 7.5	429±19	102±2	21±0	414±35	9±3	146±18	2430±121
Black tea	430±34	521±41	585±70	429±64	26±8	154±14	3410±458
1h at pH 2.0	541±121	244±101	250±101	467±151	12±2	148±47	4110±1300
15 min at pH 7.5	462±129	91±17	34±12	398±122	16±6	118±39	4200±1250
30 min at pH 7.5	429±115	91±18	32±11	315±90	5±0	88±24	4220±1270
60 min at pH 7.5	327±104	ND	ND	224±106	ND	69±43	4240±1370

[a] Values are means ± S.E. of three independent extractions.

Table 4
Comparison of calculated and measured antioxidant activities and polyphenol concentrations of green or black tea[a]

Treatment	Measured antioxidant activity (mmol/l Fe^{2+} equivalents)	Calculated antioxidant activity (mmol/l Fe^{2+} equivalents)	Measured polyphenol concentration (mmol/l)	Calculated polyphenol concentration (mmol/l)
Green tea	22.1±0.64	20.5±0.4	16.0±0.3	4.97±0.11
1 h at pH 2.0	19.8±0.94	16.9±0.4	14.4±1.2	4.16±0.10
15 min at pH 7.5	19.5±1.19	11.4±0.8	13.9±0.8	2.81±0.20
30 min at pH 7.5	17.1±0.52	7.3±1.1	14.7±1.0	1.89±0.25
60 min at pH 7.5	16.1±2.50	3.6±0.4	11.5±1.0	1.06±0.01
Black tea	21.0±0.71	8.1±0.8	20.0±2.11	2.15±0.21
1 h at pH 2.0	17.6±0.95	6.2±2.1	17.4±2.0	1.58±0.01
15 min at pH 7.5	17.8±1.13	2.1±1.4	18.8±1.6	1.07±0.53
30 min at pH 7.5	16.6±1.31	3.1±1.1	16.5±2.4	0.92±0.01
60 min at pH 7.5	15.0±1.25	2.0±1.0	12.8±2.3	0.67±0.34

[a] Values are means ± S.E. of three independent extractions.

with lesser losses from the green tea. Caffeine concentrations were unaffected. Total antioxidant activity was reduced by 10% in the green tea and 16% in the black tea following incubation at acid pH. However, subsequent incubation at pH 7.5, approximately equal to that found in the proximal small intestine, resulted in the loss of almost all the detectable EGC and EGCG in both teas and reduced the concentrations of all the other catechins. Despite these losses, the total antioxidant activity declined by only 27% in the green tea and 29% in the black tea as a result of both treatments. The theoretical sum of antioxidant activity was calculated by summation of the contributions from the individual measured phenolic components and was found to be consistently less than that measured in the tea samples (Table 4). After 1 h at pH 7.5 the calculated antioxidant activity accounted for only 22% of the measured antioxidant activity of the green tea digest and 13% of that of the black tea.

The total phenolic concentration declined by 28% in the green tea and 36% in the black tea (Table 4), however the sum of GA and the catechins decreased by 79 and 69% in the green and black teas, respectively.

4. Discussion

The comparative antioxidant activity of tea polyphenols has been examined using a number of methods. Zhang, Chan, Luk, Ho, and Chen (1997) found that EC and EGC were less effective against Cu^{2+} mediated lipid peroxidation than EGC and EGCG, probably because the latter compounds are more hydrophilic than the former two catechins and were more able to chelate copper ions. Previously Chen and Chan (1996) had reported that EC and ECG were more effective against peroxidation of canola oil than the other catechins due to increased lipid solubility. Guo, Zhao, Li, Shen, and Xin (1996) examined the activity of the four main catechins against iron-induced lipid peroxidation and found EGCG > ECG > EGC > EC. However the ability to chelate Fe^{3+} declined in the order EGC > EGCG = ECG > EC, and the ability to scavenge hydroxyl radicals decreased in the order ECG > EC > EGCG > > EGC. In addition they found that the stabilities of the semiquinone free radicals also varied. Salah, Miller, Paganga, Tijburg, Bolwell, and Rice-Evans (1995) used a metmyoglobin inhibition assay to compare the antioxidant activities and found EGC > EGCG > GA > EC = catechin. In the current study we have found decreases in the order ECG > EGCG = GA > EGC > EC. Clearly variations in antioxidant activity are due to the structures of the individual polyphenols, their ability to chelate metals and to scavenge free radicals as well as the chemistry involved in the antioxidant analysis.

Liebert, Licht, Böhm, and Bitsch (1997) found a strong relationship between polyphenol concentrations and antioxidant activity at different brewing times up to 10 min for both green and black teas. In the current study we found a similar relationship, which was also present for the individual phenolic antioxidants. We also observed that the sum of the potential antioxidant activities of the major polyphenols and gallic acid accounted for almost all of the measured antioxidant activity of green tea but less than half of that activity in black tea. The presence of larger catechin polymers such as the theaflavins and thearubigens in black tea and smaller amounts of bisflavanols in green tea (Balentine, Wiseman, & Bouwens, 1997) may explain this. There appeared to be little difference in the extraction rate of the various catechins from the tea samples, whether black or green. In general, the concentrations of the individual catechins were in the same range as those reported by others, however from the literature it is apparent that there are great variations between tea sources.

It has previously been reported (Zhu et al., 1997) that various catechin components of green tea had different stabilities at alkaline pH. EGCG and EGC were unstable and degraded rapidly under these circumstances. The other major catechins EC and ECG were however more stable under these conditions. In contrast, at acid pH all the catechins were stable for several hours. Yoshino et al. (1999) studied the effects of incubating EGCG at alkaline pH and also in mouse plasma. These workers reported the formation of dimers (P1, P2 and P3) as a result of incubating EGCG under these conditions. Furthermore, from their studies it appeared that the products had higher antioxidant activity in vitro and a greater apparent bioavailability than EGCG. One of the components that they identified was theasinensin A, a compound found in fermented (or oxidised) teas such as oolong or black tea. Yoshida, Kiso, and Goto (1999) examined the effects of various brewing conditions on the extraction of catechins from green tea leaves. This group found that extraction at pH 7 or 8 at 80C resulted in an apparent epimerisation of the major catechins (EC, EGC, ECG, and EGCG) to their respective minor catechins. In our studies we found decreases in gallic acid and all catechins measured at alkaline pH with both green and black teas, however we did not detect epimerisation of epicatechins to catechins. Wang and Helliwell (2000) have also reported that epimerisation of the epicatechins occurs only slowly at temperatures less than 40°C.

These studies have shown that incubation of both green and black teas at alkaline pH results in a major reduction in the concentration of catechins. This diminution is not accompanied by a reduction of a similar magnitude in either antioxidant activity or total polyphenolic content, which is probably due to the formation of dimers (P1, P2 and P3) from green tea as suggested by Yoshino et al. (1999). The importance of the formation of these unidentified antioxidants, especially from black tea, to systemic health or to the environment in the gastrointestinal tract is unknown. Clearly the impact of added milk, or consumption of teas in accompaniment to a meal could influence the fate of the catechins, as could the presence of digestive enzymes. In addition Leenen et al. (2000) have reported that the addition of milk to black tea does not adversely affect the plasma antioxidant activity and Serafini, Ghizellia, and Ferro-Luzi (1996) found no effect of milk on tea antioxidant capacity in vitro. This would suggest that binding of polyphenols by other dietary components does not necessarily irreversibly affect their ability to act as antioxidants. In order to understand the mechanisms whereby black tea catechins and catechin condensates exert their physiological effects, further studies are required which will examine the metabolism of these compounds both in vitro and in vivo.

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