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Characterisation of polyphenols in green, oolong, and black teas, and in coffee, using cyclic voltammetry

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Abstract

Phenolic compounds are important for their astringent taste and as antioxidants in health. Green, oolong and black tea and an instant coffee were each diluted 50 times in a pH 7.0 phosphate buffer and, along with phenolic standards, were analysed by cyclic voltammetry at a carbon electrode. Standards with a pyrogallol group (gallocatechins) were strong reducing agents and produced a peak at 90–120 mV, while those with a catechol or gallate group peaked at 180–220 mV. The response of green and oolong teas was dominated by epigallocatechin gallate, and levels determined by HPLC were consistent with the electrochemical response. Black tea behaved like a theaflavin extract, and coffee like 5-*O*-caffeoylquinic acid, both with peaks at around 230 mV. The level of phenolics increased with water temperature from 20 to 100 °C, while lower levels were obtained for repeat infusions. The addition of milk (skim or full-fat alike) lowered the response more in the teas than in the coffee.

Keywords: Tea; Coffee; Polyphenols; Cyclic Voltammetry; Antioxidants; HPLC

1. Introduction

Tea and coffee are popular beverages which provide a significant source of phenolic compounds in the diet. While polyphenols have long been of interest for their contribution to the astringent taste of teas (Ding, Kuhr, & Engelhardt, 1992), research into the health benefits of tea has been actively pursued only in more recent times (Dufresne & Farnworth, 2001; McKay & Blumberg, 2002). While results are mixed for the relationship between tea and health in epidemiological and clinical studies, in vitro studies point to a range of mechanisms by which tea phenolics may help to offset chronic diseases such as cancer and cardiovascular disease (Kris-Etherton & Keen, 2002).

The major phenolic compounds in tea belong to the catechin family, also known as flavan-3-ols, which constitute up to 30% of the tea solids by weight, while various flavonols are also present (up to 4%). Structures of the catechin monomers found in high levels in green and oolong teas are shown in Fig. 1, along with the chlorogenic acid, 5-*O*-caffeoylquinic acid (5-CQA), which is the major phenolic in coffee, accompanied by various related compounds (Shahidi & Naczk, 1995). A general structure is also provided for the theaflavins produced when green tea is fermented through to black tea, which make up the bulk of the phenolics in black tea, along with the less well defined thearubigin fraction (Harbowy & Balentine, 1997).

It is well known that the extraction of catechins or theaflavins from teas is greater with stirring, a longer infusion time, and a higher water temperature (Robertson & Hall, 1989; Robinson, Maxwell, & Thorpe, 1997; Langley-Evans, 2000; Astill, Birch, Dacombe, Humphrey, & Martin, 2001; Khokhar & Magnusdottir, 2002). On the other hand, the use of higher temperature infusion can also lead to the loss of labile polyphenolic components after standing for 10–15 min (Robertson & Hall, 1989; Robinson et al., 1997). The effect of milk in altering the antioxidant activity of teas has produced mixed results, with some in vitro tests indicating that phenolics interact with the lipid fraction (Robinson et al., 1997; Langley-Evans, 2000) or with casein proteins (Luck et al., 1994; Arts et al., 2002) or that milk will

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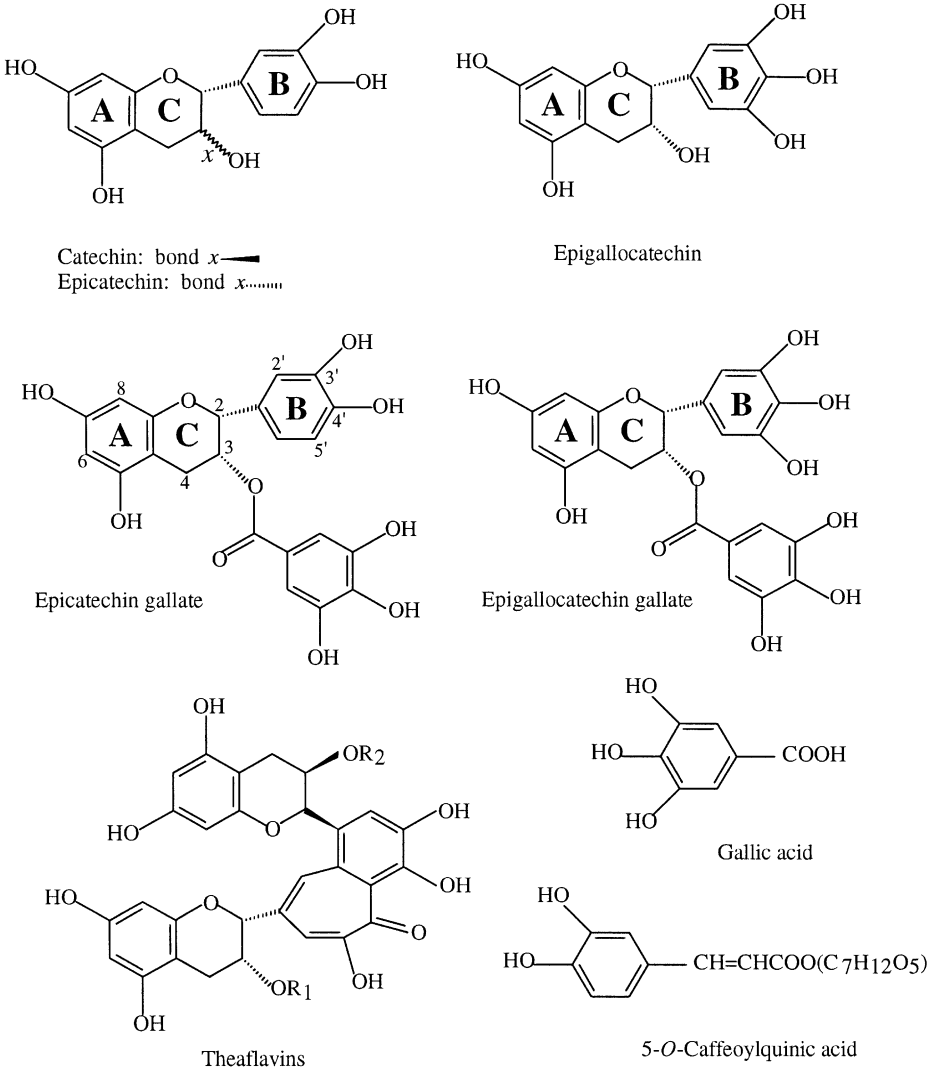

Fig. 1. Structures of phenolic compounds discussed in this report.

lower the antioxidant activity (Langley-Evans, 2000) or produce no change at all (Richelle, Tavazzi, & Offord, 2001). The effects of tea consumption in increasing phenolic antioxidant levels in human plasma have also been reported to be either inhibited (Serafini, Ghiselli, & Ferro-Luzzi, 1996) or unaffected (Van het Hof, Kivits, Weststrate, & Tijburg, 1998; Hollman, van het Hof, Tijburg, & Katan, 2001; Leenen, Roodenburg, Tijburg, & Wiseman, 2000) by the addition of milk to tea.

An accurate analysis of the phenolics present in teas and coffee is seen as crucial to improving clinical studies on the health benefits of teas (McKay & Blumberg, 2002). For this purpose, reverse phase HPLC is often used as the method of choice (Robb & Brown, 2001; Kris-Etherton & Keen, 2002). However, there is still a need for more rapid and less expensive methods to characterise the phenolics present in beverages such as tea and coffee. We have recently applied an electrochemical method, cyclic voltammetry, to suitably diluted red and white wine samples to provide information about the phenolics present (Kilmartin, Zou, & Waterhouse, 2001; Kilmartin, Zou, & Waterhouse, 2002). The focus of this paper will be to show how cyclic voltammetry can also be used to characterise phenolics in tea and coffee samples.

The electrochemical characteristics of tea catechin standards have been reported previously (Yang, Kotani, Arai, & Kusu, 2001; Kondo, Kurihara, & Fukuhara, 2001; Furuno, Akasako, & Sugihara, 2002), compounds with trihydroxy-substitution on the flavonoid B-ring (pyrogallol) being found to oxidise over 100 mV earlier than catechins with *ortho*-diphenol (catechol) or gallate groups. The polyphenolic content of a green tea extract, in terms of epigallocatechin gallate equivalents, has also been determined by differential pulse voltammetry at a graphite electrode (Romani, Minunni, Mulinacci, Pinelli, & Vincieri, 2000), and the result was in good agreement with quantification using HPLC.

In this report it is intended to show how cyclic voltammetry can be used to indicate the levels of key phenolics

in green, oolong, and black teas and in coffee. This is achieved by adequately diluting the samples in a neutral pH buffer, to avoid excessive contamination of the carbon electrode by more concentrated samples, and by comparison with results obtained for phenolic standards. The effects of water temperature, time, repeat infusions, and milk on the cyclic voltammetry response are also examined.

2. Materials and methods

Three commercial bagged teas and one instant coffee were used in the present study. The average weight of tea in 10 samples, after subtracting the average bag weight (determined for 5 tea bags of each sort), were as follows: Red Seal green tea (Auckland, New Zealand) (1.42±0.04 g), Ever Spring oolong tea (Taipei, Taiwan) (1.81±0.03 g), Dilmah black tea (Auckland, New Zealand) (2.08±0.08 g), and Nescafe granulated instant coffee (Auckland, New Zealand). One tea bag or 2.0 g of coffee was used for each experiment.

Phenolic standards of gallic acid (GA), catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), 5-*O*-caffeoylquinic acid (5-CQA), and an 80% theaflavin extract were purchased from Sigma Chemical Co (St. Louis, MO).

To prepare the extracts, a tea bag was hung in or coffee added to (weighed to±0.0005 g) 200 ml of Milli-Q grade water at a selected temperature in a 400 ml beaker, and stirred with a (8 mm×4 cm) magnetic stirrer bar, set at 250 rpm, for 3 min. For some experiments the tea bags were reused, while in other experiments 0.5 or 2.0 ml of non-fat milk (Anchor, green top), or whole milk (Anchor, blue top), were added to 20 ml samples of the prepared beverages. Each experiment was run in duplicate and the mean result obtained.

Samples were prepared for cyclic voltammetry analysis by diluting 2.00 ml of the extracts, typically 50 times, in a phosphate buffer with 65% w/v 50 mM disodium hydrogen phosphate and 35% w/v 50 mM sodium dihydrogen phosphate at pH 7.0. Using a procedure already described (Kilmartin et al., 2001), cyclic voltammograms were recorded in triplicate with a BioAnalytical Systems (BAS) 100A potentiostat, using a freshly polished (BAS PK-4 kit), 3 mm glassy carbon electrode (BAS M-2012), from −100 to 400 mV at a scan rate of 100 mV s^{-1}. The electrode potential was recorded against a Ag/AgCl reference electrode (+207 mV versus *she*) and the current was taken against the response due to the buffer solution as a blank. The cyclic voltammetry results, which take just a few minutes to obtain, were then normalised to 2.0 g of tea or coffee.

Levels of individual phenolics were determined by the direct injection of 20 μl of the tea extract, after first passing through a 0.45 μm filter (PTFE), onto a Phenomenex Luna reverse-phase C18 column (4.6×250 mm, 5 μm particle size), on a Hewlett-Packard 1100 instrument with diode array detector, as described previously (Kilmartin et al., 2002). A ternary solvent mixture was used, consisting of water (Solvent A), 5% acetic acid in water (Solvent B), and acetonitrile (Solvent C) at a flow rate of 0.8 ml min^{-1}. Beginning with 45% A and 55% B, the eluting solvent was changed to 100% B after 20 min, and from 30 to 105 min a linear gradient moved from 100% B to 55% B and 45% C. External standards (at 1, 4 and 10 mg/l) were used to quantify the level of phenolics, at 280 nm, in the teas for a range of water temperatures. It was found that catechin and epigallocatechin coeluted, and, given the low level expected for catechin (Aucamp, Hara, & Apostolides, 2000), the result for the peak was wholly attributed to epigallocatechin.

3. Results

3.1. Cyclic voltammetry of polyphenolic standards

The various catechins typical of those found in teas and coffee (Fig. 1) were readily oxidised in the potential range from 50 to 300 mV (Ag/AgCl), as shown by the cyclic voltammograms for 0.05 mM standards presented in Fig. 2. In each case the top scan represents the oxidation of the polyphenolic generating a positive (anodic) current, peaking at a particular electrode potential; the lower the potential of oxidation, the more powerful the phenolic is as a reducing agent. On the reverse scan a negative (cathodic) peak is produced where the oxidised form of the phenolic can be reduced back to its original form. For example, the oxidation of catechin can be readily reversed at the carbon electrode, while gallic acid is irreversibly oxidised (Fig. 2).

A number of parameters can be extracted from the cyclic voltammetry curves to characterise the phenolics as reducing agents, and these values are recorded in Table 1. During the positive scan, we obtain the anodic peak potential ($E_{p,a}$) and anodic peak current ($I_{p,a}$), which are used to calculate the potential at half the peak height ($E_{p/2}$). Two estimates of the formal potential, used to quantify the reducing power of the phenolic antioxidants (Kilmartin, 2001), are given by the potential mid-way between the anodic and cathodic peaks (E_{mid}), and by the potential halfway between $E_{p/2}$ and $E_{p,a}$, which can be used even in the absence of a return cathodic peak, as for gallic acid. The phenolics in Table 1 are ranked in order of reducing strength. The tea gallocatechins had formal potentials of 74–109 mV, up to 100 mV lower than the catechins containing a catechol or gallate group, which appeared at 165–188 mV, pointing to the higher reducing strength of the

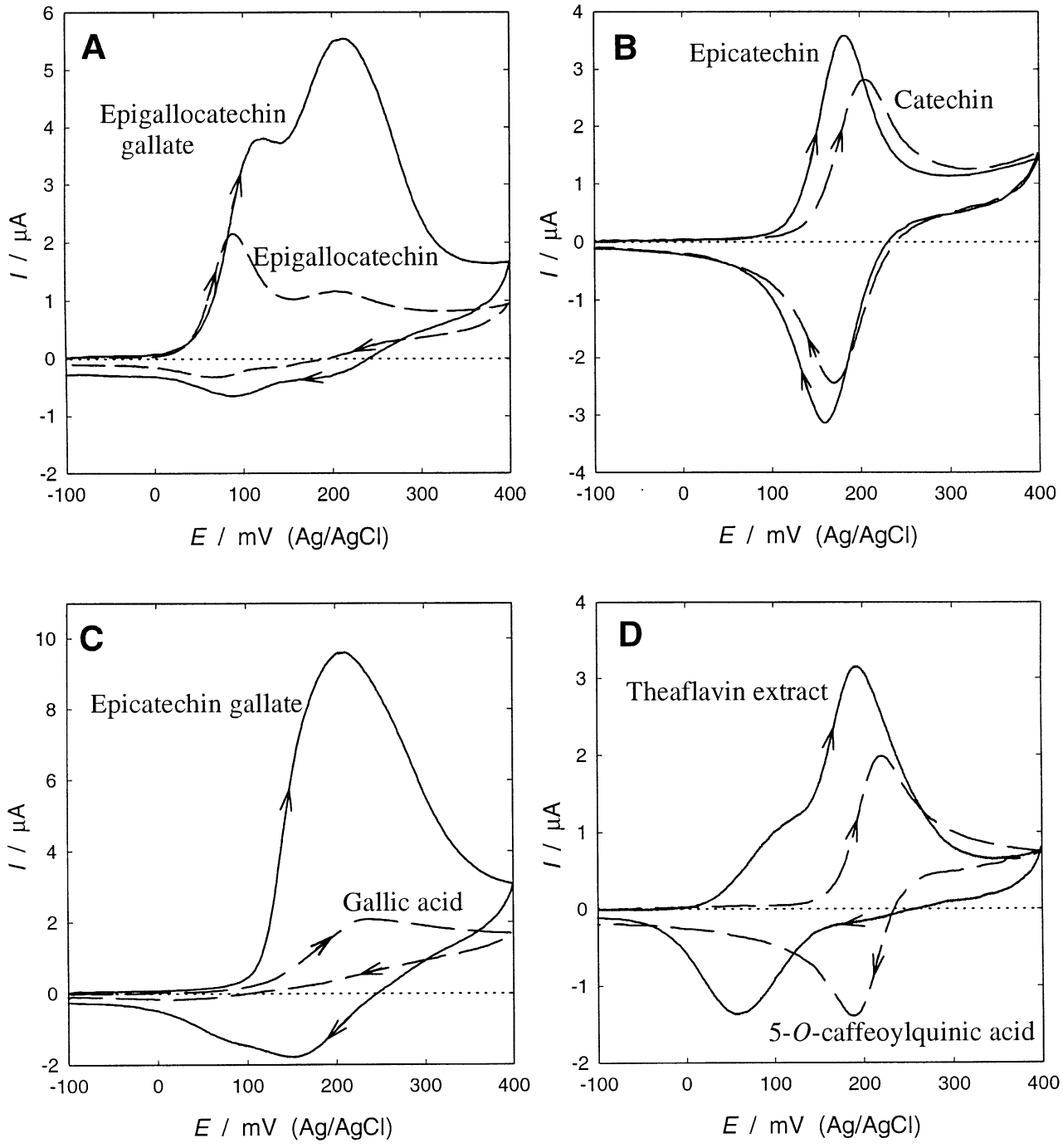

Fig. 2. Cyclic voltammograms of 0.05 mM phenolic standards measured at a 3 mm glassy carbon electrode at 100 mV s^{-1} in pH 7.0 phosphate buffer (the background response due to the buffer solution has been subtracted from each curve).

pyrogallol group (triphenol on the flavonoid B-ring). The potential values obtained are consistent with previous reports on the electrochemistry of tea catechins using more concentrated solutions (Yang et al., 2001; Kondo et al., 2001; Furuno et al., 2002).

Epigallocatechin gallate, containing both a pyrogallol and a gallate group, produced peaks in both potential ranges, while epicatechin gallate, with both a catechol and a gallate group, generated a very high current response at around 200 mV (Fig. 2C). The variation in the peak current ($I_{p,a}$) for catechins of the same concentration is due to a variety of factors, such as the diffusion coefficient of the phenolic in the solution (Kilmartin, 2001). The exceptionally high current response for epicatechin gallate has been seen previously (Yang et al., 2001). While the peak current is expected to increase linearly with concentration, the results in Table 1 show that the current for the catechins at 0.05

Table 1
Cyclic voltammetry parameters for 0.05 and 0.01 mM phenolic standards in a pH 7.0 phosphate buffer, measured at a 3 mm glassy carbon electrode at 100 mV s^{-1}

E in mV (Ag/AgCl)	Conc. (mM)	$(E_{p,a}+E_{p/2})$ 2 (mV)	E_{mid} (mV)	$E_{p,a}$ (mV)	$E_{p,a}-E_{p/2}$ (mV)	$I_{p,a}$ (μA)	Q_{400} (μC)	$I_{p,c}/I_{p,a}$
Epigallocatechin	0.05	74	76	87	26	2.15	4.4	0.16
	0.01	77	79	90	27	0.79	2.8	0.29
Epigallocatechin gallate	0.05	102	105	123 (213)[a]	43	3.8 (5.5)[a]	11.4	0.12
	0.01	105	109	122 (216)[a]	35	1.56 (1.9)[a]	3.8	0.15
Epicatechin	0.05	166	171	182	32	3.6	4.9	0.89
	0.01	165	173	178	26	1.12	1.28	0.99
Theaflavin extract	0.05	171	124	193	45	3.14	4.8	0.44
	0.01	168	135	195	53	0.93	1.60	0.74
Epicatechin gallate	0.05	174	182	205	63	9.5	17.3	0.18
	0.01	168	178	191	46	4.8	7.6	0.18
	0.005	167	180	184	34	2.61	4.1	0.17
Catechin	0.05	188	187	205	34	2.8	3.5	0.87
	0.01	183	188	201	36	1.29	1.33	0.94
Gallic acid	0.05	202	—	236	67	2.08	4.62	—
	0.01	184	—	218	68	0.44	1.09	—
5-*O*-Caffeoyl-quinic acid	0.05	205	204	221	31	1.9	2.7	0.72
	0.01	204	202	219	30	0.36	0.57	0.90

The following abbreviations are used: $E_{p,a}$ (anodic peak potential); $E_{p/2}$ (potential at half the peak height); E_{mid} (potential midway between the anodic and cathodic peak potentials); $I_{p,a}$ (anodic peak current); $I_{p,c}$ (cathodic peak current); Q_{400} (charge passed to 400 mV).

[a] The values in brackets refer to the second anodic peak.

mM was less than five times the response for the 0.01 mM solutions. Some contamination of the electrode is likely to occur due to oxidation products adsorbing onto the electrode surface, even during the few seconds in which the peak is produced, thus lowering the current response for the more concentrated solutions. For repeat runs, the peak current varied by up to 10%, while the peak potential was more consistent and varied by only 2–3 mV as the standards were retested.

The main peak for the 80% theaflavin extract was located at 193 mV (Fig. 2D), typical of catechol-containing phenolics. However, an early current feature at around 100 mV pointed to the presence of some pyrogallol-containing compounds in the extract, which may include residual gallocatechins. 5-*O*-Caffeoylquinic acid, the major chlorogenic acid in coffee, oxidised at a potential 20 mV higher than catechin, a trend seen previously with related hydroxycinnamic acids containing a catechol group, such as caffeic and caftaric acids (Kilmartin et al., in press). The increase in peak current was close to 5-fold in moving from 0.01 to 0.05 mM solutions for the two non-flavonoids, gallic acid and the chlorogenic acid, pointing to less electrode contamination than for the catechin standards.

Further parameters included in Table 1 are the differences between $E_{p/2}$ and $E_{p,a}$, which characterise the sharpness of the peak. This value approaches $56.5/n$ mV at 25 °C for a rapidly occurring and reversible process, with n being the number of electrons passed in the reaction. In the case of epicatechin and catechin ($n=2$), the values obtained in the 26–36 mV range are close to the theoretical value of 28 mV, and point to rapid kinetics for the oxidation of these phenolics. A further measure of reversibility is the ratio of $I_{p,a}$ to the cathodic peak current ($I_{p,c}$), which approaches a value of 1.0 for a fully reversible process. A value less than 1.0 may indicate that the oxidised form is gradually converting to some other chemical form, such as a dimer, or simply that the reverse process does not readily occur at the carbon electrode. The value of $I_{p,c}/I_{p,a}$ was close to 1.0 for the dilute catechin and epicatechin solutions, but was less than 0.3 for the gallocatechins and gallates, and close to zero for gallic acid (Table 1). The Q_{400} value is obtained as the integral of the current to 400 mV, and is an alternative measure of concentration to $I_{p,a}$, and one which is more suitable for samples with several overlapping and broad peaks (Kilmartin et al., 2001).

3.2. Cyclic voltammetry of green, oolong and black teas, and instant coffee

During a cyclic voltammogram of a tea or coffee solution, the phenolics present will be oxidised as the electrode potential is scanned in a positive direction. The overall response will be the sum of the various species present. While any other oxidisable components present in the beverages will also contribute at some potential, it is only the phenolics that are expected to produce a current at potentials less than 400 mV.

Cyclic voltammograms of a green, oolong and black tea, and an instant coffee, extracted into 200 ml of boiling water for 3 min, and then diluted 50 times in the pH

7.0 phosphate buffer, are shown in Fig. 3. With the green and oolong teas, the cyclic voltammograms were very similar. An initial current peak was evident at about 125 mV, followed by a larger peak at 230 mV. For the black tea there was only little additional current at 125 mV, beyond that due to the leading edge of the main 230 mV peak. In the case of a black tea, it is expected that most of the pyrogallol groups would have been oxidised by polyphenol oxidase during tea fermentation. The oxidation process for all of the teas showed little reversibility, and only a small current response was seen on the reverse scan. With the coffee sample, a single, moderately reversible peak was seen at about 235 mV. The electrochemical parameters for these cyclic voltammograms are given in Table 2.

The position of the cyclic voltammetry peaks reveals a large number of phenolics with catechol and gallate groups (230 mV peak) in all of the samples. A significant

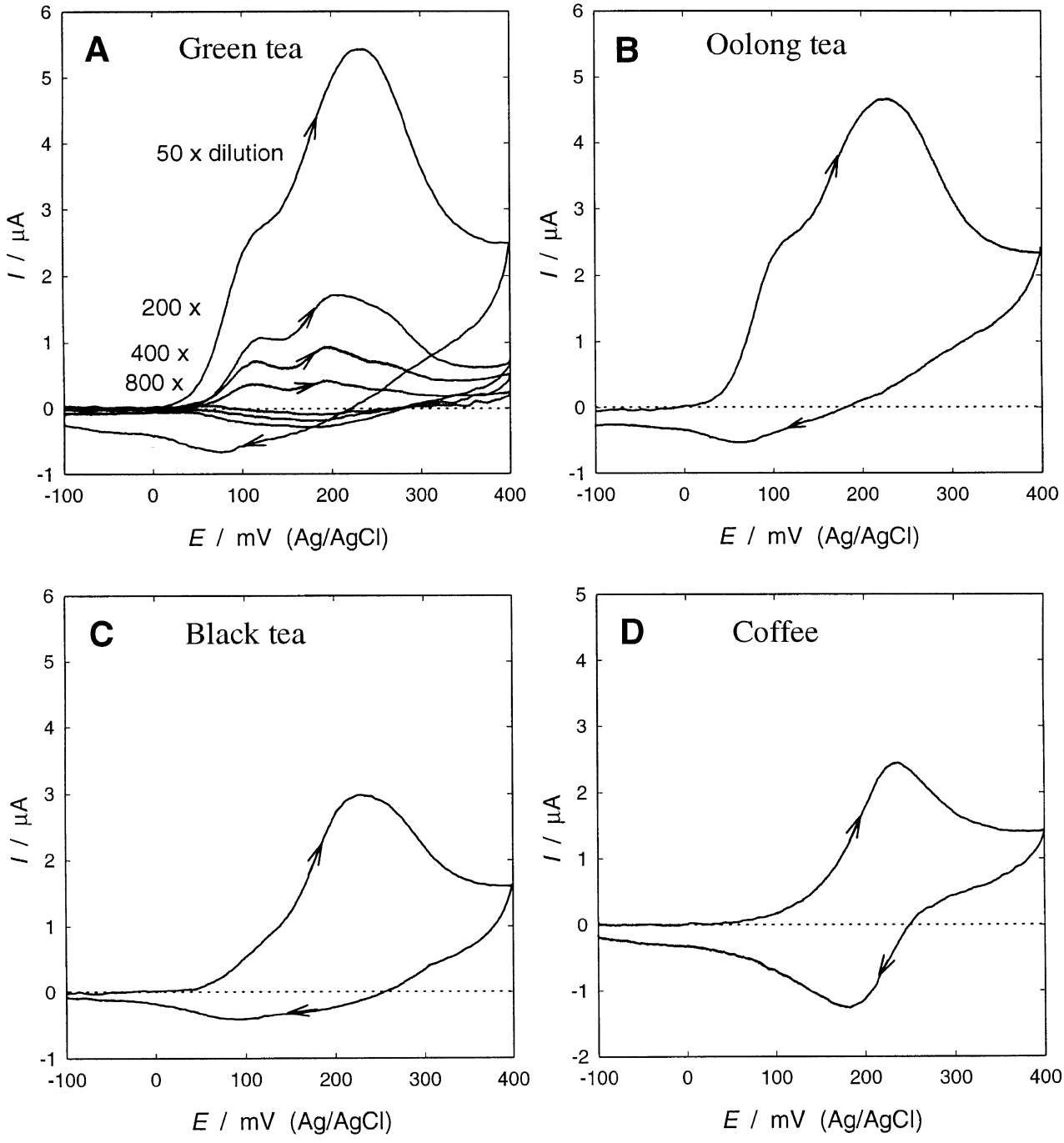

Fig. 3. Cyclic voltammograms of the green, oolong, and black teas and of instant coffee, treated for 3 min in 200 ml of water at 100 °C, and diluted in pH 7.0 phosphate buffer, recorded at a 3 mm glassy carbon electrode at 100 mV s^{-1} (the background response due to the buffer solution has been subtracted from each curve).

Table 2
Cyclic voltammetry parameters for the peaks seen with green, oolong, and black teas, and for instant coffee, treated for 3 min in 200 ml water at 100 °C, in a pH 7.0 phosphate buffer, measured at a 3 mm glassy carbon electrode at 100 mV s^{-1}. The $I_{p,a}$ and Q_{400} values are normalised for 2.0 g of tea or coffee

E in mV (Ag/AgCl)	Dilution	$(E_{p,a}+E_{p/2})$ 2 (mV)	$E_{p,a}$ (mV)	$E_{p,a}-E_{p/2}$ (mV)	$I_{p,a}$ (μA)	Q_{400} (μC)	$I_{p,c}/I_{p,a}$
Green tea	50	109	126 (228)[a]	42	2.5±0.1 (5.5)[a]	10.8±01	0.06
	200	104	121 (205)[a]	35	1.06±0.09 (1.7)[a]	3.48±0.02	0.16
	400	99	115 (196)[a]	33	0.71±0.08 (0.92)[a]	2.11±0.25	0.14
	800	98	115 (194)[a]	34	0.36±0.04 (0.43)[a]	1.06±0.02	—
Oolong tea	50	98	129 (228)[a]	43	2.6±0.2 (4.6)[a]	9.6±0.4	0.12
Black tea	50	195	228	66	3.0±0.3	6.2±0.3	0.14
Coffee	50	209	236	55	2.4±0.2	4.3±0.4	0.52

The same abbreviations are used as in Table 1.
[a] The values in brackets refer to the second anodic peak.

level of pyrogallol groups (125 mV feature) was seen in the green and oolong teas, a much smaller amount in the black tea, and none in the coffee sample. As might be expected, the cyclic voltammograms for the green and oolong teas resembled that of epigallocatechin gallate, expected to be the major phenolic present. Likewise the black tea resembled the theaflavin extract (except for a much lower reversibility in the case of the tea). Glycosides of the flavonol quercetin, present in lower levels than the major catechins (Shahidi & Naczk, 1995), are also likely to contribute at potentials a little higher than the 230 mV peak, a trend seen with other flavonol glycosides (Kilmartin et al., 2002). The additional current evident at around 260 mV in the green teas diluted 200 and 400 times (Fig. 3A) may well be due to quercetin glycosides.

On the other hand, the coffee sample behaved in a manner similar to the chlorogenic acid standard. In this case the current response of the sample diluted 50 times is equivalent to 0.063 mM of 5-CQA standard, or 3.2 mM in the undiluted 200 ml of coffee, which equates to 223 mg (11%) of the 2.0 g of the instant coffee. This value lies at the upper end of CQA values reported previously (Clifford, 1999).

Our earlier studies on diluted wines have shown that a peak current less than 3 μA is generally required to reach a range in which the current is directly proportional to the concentration of phenolics present (Kilmartin et al., 2001). This is necessary because of the contamination of the carbon electrode by phenolic oxidation products which can occur during the cyclic voltammogram itself. Initial studies with 2 g of teas in 200 ml of water showed that dilution by 50 times was required to reach a current less than 3 μA for the first anodic peak, and this level of dilution was used for most of the experiments reported in this paper. Given the increased tendency of catechins to contaminate the electrode, compared, for example, with hydroxycinnamic acids (Kilmartin et al., 2001), the cyclic voltammetry response of the green tea sample, diluted as far as 800 times, was obtained more recently, and the results are also presented in Table 2. It can be seen that the anodic peaks at 115–125 and 195–230 mV, and the Q_{400} value, were only fully linear with concentration corresponding to 400-fold dilution or greater. In this case, the peak currents were less than 1 μA and of a similar size to the background current due to the phosphate buffer, which may be a problem with less sensitive potentiostats. The choice of dilution will thus be a compromise between signal intensity and achieving a current response which will provide a more quantitative measure of the phenolics present, and for this purpose a 400-fold dilution would be recommended. When comparing the responses of different tea solutions, the main requirement will be the use of a consistent dilution step, and a 50-fold dilution is well suited when less concentrated tea solutions are expected, as when a lower water temperature is used.

3.3. Effect of water temperature monitored by cyclic voltammetry and HPLC

The three teas were extracted for 3 minutes with 200 ml of water at temperatures ranging from room temperature (20 °C) to 100 °C. The tea samples were either diluted 50 times in the pH 7.0 phosphate buffer for analysis by cyclic voltammetry or filtered (0.45 μm) prior to direct injection onto the HPLC column. The results of the cyclic voltammetry scans and the levels of individual phenolics by HPLC are given in Table 3. The change in the cyclic voltammetry response for the oolong tea at the five temperatures is also illustrated in Fig. 4, where the background response due to the buffer solution (the blank) has been included.

The HPLC results show that the major phenolic compound in the green and oolong teas was epigallocatechin gallate (EGCG), with epicatechin gallate (ECG) in second place, an order reversed in the case of the black tea sample, where the levels were also somewhat smaller. Similar levels of phenolics have been reported previously (Aucamp et al., 2000; Shahidi & Naczk, 1995), and some variation is expected, depending upon

Table 3
HPLC levels of individual phenolics (mg/g of the dry tea; average of two runs), and cyclic voltammetry results (normalized to 2 g of tea; average for two tea bags, each run in triplicate), for green, oolong and black teas, using a range of water extraction temperatures

	Temperature (°C)	GA	EC	ECG	EGC (+ C)	EGCG	Predicted $I_{p,a}$ (120 mV) (μA)	Actual $I_{p,a}$ (120 mV) (μA)	Predicted $I_{p,a}$ (220 mV) (μA)	Actual $I_{p,a}$ (220–240 mV) (μA)	Q_{400} (μC)
Green tea	20	0.20	1.59	2.7	1.60	7.3	0.58	0.51±0.07	1.5	0.75±0.10	2.1±0.2
	40	0.52	4.4	9.0	4.6	23.3	1.80	1.64±0.07	4.7	2.68±0.06	6.4±0.2
	60	0.60	5.4	16.3	5.4	38.5	2.9	2.13±0.11	7.7	3.52±0.04	8.6±0.1
	80	0.68	6.0	20.4	6.3	46.0	3.9	2.42±0.04	9.4	4.6±0.1	10.2±0.1
	100	0.88	12.2	38.5	8.0	78.1	5.7	2.90±0.09	16.7	5.5±0.3	12.4±0.4
Oolong tea	20	0.10	0.31	0.46	0.46	1.87	0.16	0.37±0.02	0.33	0.28±0.07	0.89±0.07
	40	0.78	2.4	4.0	2.5	12.1	0.95	1.28±0.07	2.4	1.54±0.13	4.0±0.2
	60	1.11	4.2	9.4	3.6	27.8	2.1	2.19±0.10	5.1	3.1±0.3	7.2±0.4
	80	1.3	5.6	16.2	4.1	39.0	2.9	2.4±0.4	7.7	4.0±0.2	9.1±0.6
	100	1.5	6.7	17.2	4.8	85.7	6.1	2.7±0.1	11.9	4.5±0.3	10.5±0.6
Black tea	20	0.38	–	–	–	–	–	0.08±0.03	–	0.32±0.05	0.68±0.13
	40	1.6	0.78	1.8	0.56	0.71	0.08	0.37±0.06	0.65	1.45±0.06	2.83±0.05
	60	2.1	1.4	5.1	2.3	2.7	0.31	0.52±0.09	1.8	2.1±0.2	4.1±0.3
	80	2.5	5.7	9.9	4.6	7.0	0.72	0.74±0.25	3.8	3.0±0.4	5.8±0.7
	100	3.6	5.8	14.0	5.8	6.8	0.76	0.96±0.22	4.8	3.3±0.4	6.7±0.8

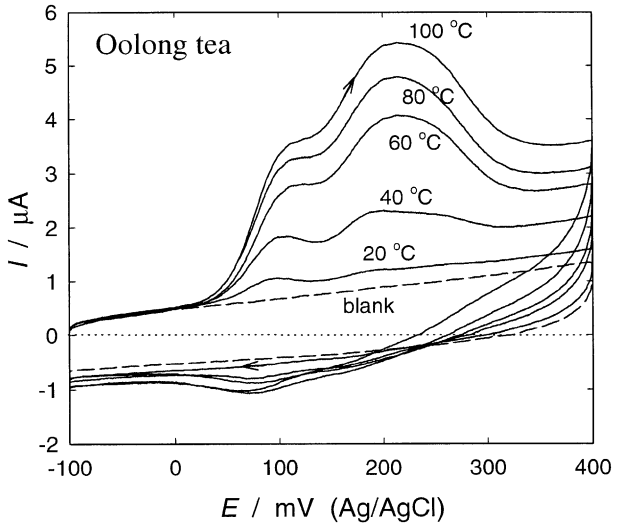

Fig. 4. Cyclic voltammograms of the oolong tea, treated for 3 min in 200 mL of water at various temperatures from 20 to 100 °C, and diluted in pH 7.0 phosphate buffer, recorded at a 3 mm glassy carbon electrode at 100 mV s^{-1} (the response due to the buffer itself, the blank, has been included in this figure).

the particular tea plant, its growing conditions, and subsequent handling. Lower levels of the flavan-3-ol monomers are expected for the fully fermented black tea, while intermediate levels are anticipated for the partially fermented oolong tea. The fact that the level of EGCG was greater in the oolong tea for a 100 °C infusion, than in the green tea, may point to a higher level in the original tea leaves for this particular sample or to different handling conditions. Moderate levels of epigallocatechin (EGC) were also seen, at 5–10% of the EGCG levels, in the green and oolong teas. Between them, EGCG and EGC are expected to determine the height of the first cyclic voltammetry peak at 120 mV. The teas also contained moderate levels of epicatechin, as well as smaller amounts of gallic acid, which were higher in the black tea, where it may have been released during the 'fermentation' (Harbowy & Balentine, 1997).

With increasing water temperature, the levels of individual catechins increased significantly with each 20 °C step from 20 to 100 °C (Table 3). In several cases, the levels at 100 °C were over 10 times higher than for a room temperature extraction. This trend was reflected in the cyclic voltammetry response where the peak currents at 120 and 230 mV, and Q_{400} value, increased several-fold over the same temperature range, as shown for the oolong tea in Fig. 4 (the voltammogram for the 20 °C extraction was only a little larger than the background current due to the phosphate buffer as a blank, and is close to the lower limit for useful analysis). The difference in the electrochemical results presented in Tables 2 and 3, for 50-fold dilution of the same tea prepared in water at 100 °C, varied in some cases by more than 10%. This difference is due to changes in the carbon electrode, particularly its real surface area, for sets of experiments run several weeks apart from each other. Because of this potential variation in electrode response, phenolic standards (such as EGCG) of known concentrations should be run alongside beverage samples to make comparisons legitimate.

The HPLC levels for EGCG and EGC were then used to predict the peak current ($I_{p,a}$) at 120 mV, based on the electrochemical response of the standards at 0.01 mM, and these predictions have been included in Table 3 alongside the actual $I_{p,a}$ values determined experimentally. Using a 50-fold dilution for cyclic voltammetry, the predicted and experimental $I_{p,a}$ values were in good agreement for the green tea at 20 and 40 °C, for the oolong tea at 40 and 60 °C, and for the black tea at 80 °C (despite the fact that the majority of the catechin

monomers originally present have been converted to theaflavins and thearubigins). When the predicted $I_{p,a}$ was less than 0.5 μA, the actual $I_{p,a}$ tended to be larger, as for the black and oolong teas at 20 and 40 °C. In this case, the rising curves for components such as ECG, EC and others which peak at around 200 mV (Fig. 2), create some early current at 120 mV. On the other hand, for a predicted $I_{p,a}$ greater than 2.5 μA, the actual $I_{p,a}$ tended to be smaller. Here contamination of the electrode by phenolic oxidation products occurs rapidly enough in the more concentrated solutions to significantly lower the current response.

When a similar prediction was made for the current at the 220 mV peak, using all five phenolics determined by HPLC, the predicted value always greatly exceeded the actual $I_{p,a}$ for the green and oolong teas [e.g., a value of 1.5 μA is predicted for the green tea brewed at 20 °C, while an $I_{p,a}$ of only 0.75 μA was obtained (Table 3)]. Only with the black teas at the lower temperatures was the predicted value lower than the actual value (e.g., 0.65 μA predicted for 40 °C water and 1.45 μA obtained), but here the large contribution of the theaflavins and thearubigins is missing from the predicted value. The quercetin glycosides may have added up to 0.2 μA at around 260 mV, based upon levels typically present in tea extracts, about 25 mg/l (Wang & Helliwell, 2001). It should also be noted that the HPLC levels of the green tea treated with water at 100 °C predict an $I_{p,a}$ value of 0.72 μA for a 400-fold dilution, very close to the value of 0.71 μA obtained experimentally (Table 2). On the other hand, an $I_{p,a}$ of 2.1 μA is predicted at around 200 mV in this case, while only 1.06 μA was produced experimentally.

These results illustrate that an $I_{p,a}$ value between 0.5 and 2.0 μA at 120 mV can be used effectively to estimate the level of EGCG plus EGC in tea samples, which in the case of green and oolong teas can often be approximated as the level of EGCG. However, the second peak at 195–230 mV is always affected by contamination of the electrode by phenolic oxidation products, so this $I_{p,a}$ value can only give a broad indication of the level of phenolics containing *ortho*-diphenol and gallate groups. Similarly, the Q_{400} value can only give a rough indication of total phenolics present with low oxidation potentials.

3.4. Effect of keeping tea extracts at room temperature monitored by cyclic voltammetry

Extracts of each of the three teas, brewed originally at 100 °C for 3 min, were allowed to cool and stand at room temperature, and 2 ml aliquots were taken for dilution in the pH 7.0 phosphate buffer and cyclic voltammetry analysis after 0, 2, 4, 6 and 24 h. Somewhat surprisingly, the electrochemical response was seen to increase by several percent over the first 6 h, less markedly with the black tea, while after 24 h lower values were obtained (Fig. 5). The phenolic compounds are expected to be oxidised under the influence of dissolved oxygen, which will lead to a loss of oxidisable groups with time, and after 24 h this decrease was clearly seen. On the other hand, the increased values over the first 6 h may be due to the release of tea catechins formerly bound to proteins, which may occur more rapidly than loss of phenolics by other mechanisms. Robinson et al. (1997) have suggested that black tea compounds may be readily broken down at higher water temperatures, producing more phenolic groups available for antioxidant activity. Theaflavin degradation in black tea was also noticed after 15 min by Robertson and Hall (1988). The formation of oligomeric products, and the hydrolysis of flavonol glycosides (Wang & Helliwell, 2001), will also affect the response. As shown in Table 1, the current produced by different phenolic standards at the same concentration does vary considerably, with ECG, for example, producing a much higher cyclic voltammetry response than EGCG. It is interesting to note that the decline in levels of individual phenolics over the course of 1 h at pH 7.5 was found to be much greater for the gallocatechins, such as EGCG (over 95% loss), than for other catechins such as ECG (less than 50% loss), while the overall antioxidant activity (as Fe^{2+} reducing equivalents) declined by less than 30% (Record & Lane, 2001). Clearly the composition and state of the phenolics in teas changes rapidly with time, while the overall reducing strength of the solution changes more slowly.

3.5. Effect of repeated extractions on the cyclic voltammetry response

The practice of reusing tea bags is very common and with green teas the first infusion is often discarded due

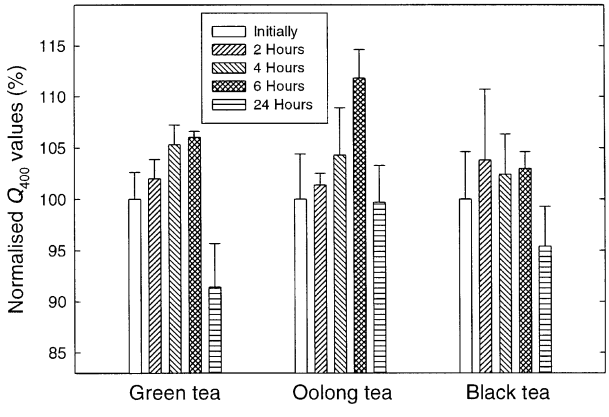

Fig. 5. Normalised peak areas (Q_{400}: the integral to 400 mV) for green, oolong, and black teas, treated for 3 min in 200 ml of water at 100 °C, and then left to stand at room temperature for up to 24 h, with a sample taken periodically and diluted in the pH 7.0 phosphate buffer for analysis by cyclic voltammetry. The means and standard deviations are for two tea bags and with each cyclic voltammetry analysis being run in triplicate.

to excessive astringency (Astill et al., 2002). Cyclic voltammograms were taken of green and black teas extracted three times for 3 min using water at 100 °C and at 60 °C to indicate the levels of phenolics present in each case. The integral area under the cyclic voltammogram peaks (the Q_{400} value) is presented in Fig. 6. As was established above (Table 3), a first extraction at 100 °C generates a Q_{400} value 50% larger than for water at 60 °C, while the level of individual phenolics is close to double that using the higher water temperature. The Q_{400} value for the second extraction at 100 °C was less than half of the first extraction for both green and black teas, while a third extraction produced values only 15% of the first. With water at 60 °C, the drop in levels for repeat infusions was significantly decreased, and the third extract now contained more phenolics than the third extract at 100 °C. As might be expected, water at 100 °C removed more phenolics from the teas leaves, leaving less available for repeat infusions, while the decrease was less marked for repeat extractions using the lower water temperature.

3.6. Effect of milk addition on the cyclic voltammetry response

The addition of milk to teas, particularly black teas, provides a means of lowering the astringency of the tea brew. As indicated in the introduction, research into the addition of milk to teas has produced mixed results for evaluating the effect on the antioxidant properties of teas. Cyclic voltammetry (Q_{400}) results for 20 ml of green and black tea, and of coffee, extracted at 100 °C, with the addition of 0.5 ml (*c.* 2.5%) or 2.0 ml (*c.* 9%) of whole or non-fat milk are presented in Fig. 7. A significant decrease in the electrochemical response was seen for the addition of milk to the beverages, pointing

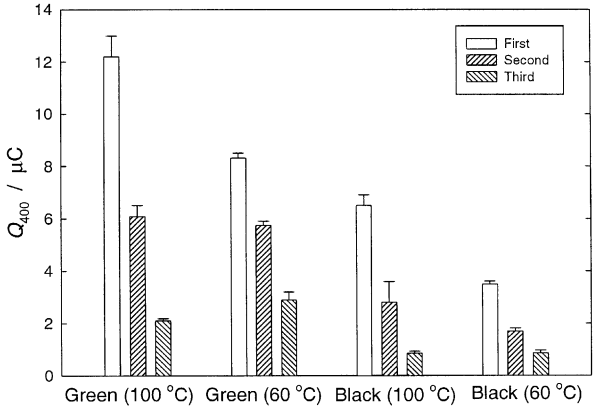

Fig. 6. Peak areas (Q_{400}: the integral to 400 mV) for repeat extractions of the green and black teas, treated for 3 min in 200 ml of water at 100 °C, and diluted in pH 7.0 phosphate buffer, recorded at a 3 mm glassy carbon electrode at 100 mV s^{-1}. The means and standard deviations are for two tea bags and with each cyclic voltammetry analysis being run in triplicate.

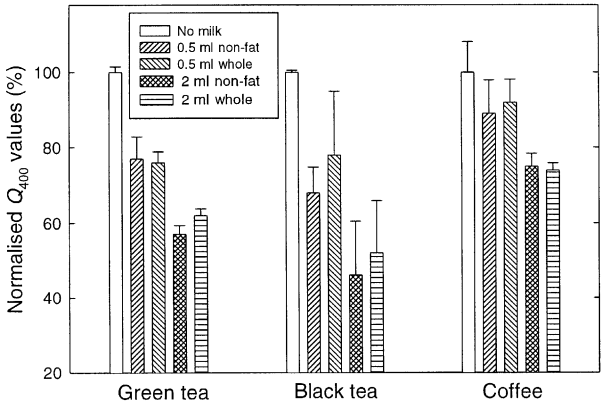

Fig. 7. Normalised peak areas (Q_{400}: the integral to 400 mV) for green and black teas, and instant coffee, treated for 3 min in 200 ml of water at 100 °C, then separated into 20 ml lots for the addition of 0.5 or 2.0 ml of non-fat or whole milk, prior to dilution in pH 7.0 phosphate buffer for analysis by cyclic voltammetry. The means and standard deviations are for two tea bags and with each cyclic voltammetry analysis being run in triplicate.

to a decreased availability of phenolics in solution to be oxidised at the carbon electrode. For the addition of 0.5 mL of milk, a decrease in the response of around 25% was seen in the teas, and of 10% in the coffee (a decrease of only 2.5% was expected due to the dilution, see above). When 2.0 mL of milk were added, the decrease was between 40 and 55% for the teas, and around 25% for the coffee samples (a 9% decrease is expected here due to the dilution). It appears that the tea catechins bind more strongly to milk components than coffee hydroxycinnamic acids with fewer –OH groups. On the other hand, there was no significant difference in the response for full-fat (whole) milk, compared to the non-fat milk. These results point to an interaction between the beverage phenolics and the protein components of the milk, rather than with its lipid fraction. While the removal of phenolics, perhaps into casein micelles, may also lead to changes in some in vitro antioxidant tests, the bioavailability and in vivo antioxidant activity of the phenolics with milk present remains another question.

4. Discussion

Electrochemical analysis of tea and coffee samples, and their phenolic constituents, provides significant information relevant to the astringency and to the antioxidant activity of the beverages. The low electrode potential at which pyrogallol-containing phenolics (EGCG and EGC) are oxidised makes them likely to be excellent scavengers of free radicals, and this applies less strongly to catechol- and gallate-containing phenolics. Other flavonoids with a triphenol group on the B-ring, such as myricetin and delphinidin, show similarly low oxidation potentials and associated high reducing

strength (Kilmartin et al., 2002). It has also been noted that the content of myricetin in black teas is lower than in green teas, while the levels of quercetin are unaffected by polyphenol oxidase and the black-tea fermentation process (Wang & Helliwell, 2001).

However, it has been suggested that the lower oxidation potential of the gallocatechins may lead to the generation of superoxide. Accordingly, the inhibition of lipid peroxidation in phospholipid liposomes provided by pyrogallol-containing catechins was seen to be limited in duration, despite their ability to scavenge radicals quickly (Kondo et al., 2001). On the other hand, a low oxidation potential, combined with good lipophilicity, was shown to lead to greater inhibition of NADPH-dependent lipid peroxidation in microsomes (Yang et al., 2001). Flavonoids with a pyrogallol group were also found to be superior to those with a catechol group in scavenging $O_2^{\bullet -}$ (Furuno et al., 2002). These results indicate that pyrogallol-containing catechins can act as either antioxidants or prooxidants in vitro, depending on the particular experimental conditions.

These points complicate an assessment of the likely antioxidant activity of tea and coffee phenolics in vivo, particularly when many of their major metabolites, and their polyphenolic structures, remain to be identified (Hollman, Tijburg, & Yang, 1997). Key factors which determine the antioxidant activity of these compounds remain their bioavailability, the forms they take when consumed, their solubility in aqueous and non-aqueous media (i.e., where they will be located in the body), and the kinetic factors which describe their interaction with various free radicals and prooxidant sources.

The overall concentration of phenolics in teas, and hence the degree of astringency, can be readily estimated from a cyclic voltammogram of a suitably diluted sample. The initial peak at 120 mV, when brought into the range around 1 μA, provides a good measure of the level of epigallocatechin gallate present in green and oolong teas. The peak at 230 mV similarly provides an indication of the level of phenolics present in black teas, but is less useful for green and oolong teas due to in-run electrode contamination from phenolic oxidation products. In a similar fashion, the 230 mV peak gives an estimate of the total caffeoyl-type chlorogenic acids in a coffee sample. Thus, the cyclic voltammetry method provides a rapid and less expensive alternative to a full HPLC analysis of phenolic levels.

In this study, higher levels of phenolics were obtained in teas prepared with a higher water temperature, for the first infusion, in the absence of milk (although milk-bound phenolics may be released upon digestion). While higher levels of phenolic antioxidants are expected to be beneficial for health, this can also lead to a more astringent beverage, which may not suit all tastes. In a future report, cyclic voltammetry results for a wide range of teas will be compared using a range of antioxidant tests.

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