Free Radical Research, 2002 Vol. 36 (10), pp. 1127–1135

Black Tea Represents a Major Source of Dietary Phenolics Among Regular Tea Drinkers

A.R. RECHNER[a], E. WAGNER[a], L. VAN BUREN[b], F. VAN DE PUT[b], S. WISEMAN[b] and C.A. RICE-EVANS[a,*]

[a]*Centre for Age-Related Diseases, Guy's, King's and St Thomas's School of Biomedical Sciences, King's College London, Guy's Campus, Hodgkin Building—3rd floor, London SE1 9RT, UK;* [b]*Unilever Health Institute, P.O. Box 114, 3100 AC, Vlaardingen, The Netherlands*

Accepted by Professor B. Halliwell

(Received 16 March 2002; In revised form 10 April 2002)

The phenolic composition and antioxidant activities [TEAC, ORAC, FRAP] of consumer brews (1 tea bag in 230 ml for 1 min) of seven different brands of black tea from the British market were investigated. The main phenolic compounds identified were epigallocatechin gallate, four theaflavins, as well as epicatechin gallate, theogallin (tentative assignment), quercetin-3-rutinoside and 4-caffeoyl quinic acid. Thearubigins represented an estimated 75–82% of the total phenolics. Further, polyphenol fractions were in decreasing order theaflavins, flavan-3-ols, flavonols, gallic acids and hydroxycinnamates. On average, a cup of a consumer brew of black tea is providing polyphenols at the level of 262 mg GAE/serving, of which 65 mg were assigned to individual polyphenols. The antioxidant activity of black tea preparations is higher than that of most reported dietary agents on a daily basis. Correlations were observed between the antioxidant activities and the sum of all quantified polyphenols by HPLC analysis as well as with the total phenolics.

Treatment of the black tea brew with simulated gastric juice resulted in a significant increase of the identified theaflavins implying a partial cleavage of thearubigins in the environment of the gastric lumen.

Therefore, black tea can be considered to be a rich source of polyphenols and/or antioxidants.

Keywords: Black tea; Tea phenolics; Antioxidant activity; HPLC; TEAC; ORAC

INTRODUCTION

Tea is one of the most consumed beverages of the world. In addition, tea consumption is linked to beneficial effects on human health, with the polyphenols as the responsible constituents.[1–4] Tea leaves as well as the resulting beverage tea are known to possess high amounts of polyphenols, especially flavan-3-ols, the so-called catechins.[5–7] Many *in vitro* and *in vivo* effects of tea polyphenols have been reported,[8–13] including antioxidant, anticarcinogenic and hypolipidemic properties. However, there is a great difference in the phenolic composition of black and green tea due to the fermentation process from green tea, rich in flavan-3-ols, to black tea resulting in the formation of condensation and oxidation products such as the thearubigins and the theaflavins.[14] Those two phenolic fractions of black tea represent the majority of phenolic constituents found in black tea.[5,15]

Crucial for the understanding of the *in vivo* bioactivities of the black tea polyphenols in humans is knowledge of the bioavailability, biotransformation and concentration of the black tea polyphenols in the circulation and tissues. The bioavailability and metabolism of individual polyphenols from green or black tea has been studied in humans demonstrating the absorption and elimination of small amounts of flavan-3-ols,[6–19] flavonols[20] and gallic acids.[21] Factors influencing metabolism and conjugation of the tea polyphenols have been reported from human and animal studies as well as from *in vitro* models,[21–29] including glucuronidation/sulphation, O-methylation of catechol groups or the trihydroxymoiety of gallic acids as well as degradation in the large intestine resulting in

*Corresponding author. Address: Antioxidant Research Centre, Wolfson Centre of Age-Related Diseases, Guy's, King's and St. Thomas's School of Biomedical Sciences, King's College London, Guy's Campus, Hodgkin Building—3rd floor, London SE1 9RT, UK. Tel.: +44-20-7848-6141. Fax: +44-20-7848-6143. E-mail: catherine.rice-evans@kcl.ac.uk

ISSN 1071-5762 print/ISSN 1029-2470 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1071576021000006707

the destruction of the flavan structure and the formation of simpler phenolic compounds.

The powerful antioxidant properties of the tea polyphenols are implicated in their proposed health beneficial effects, although findings concerning the effects of black tea consumption on the antioxidant activity of plasma and its components are controversial.[31–33] Interestingly, the addition of milk did not modulate an observed increase in plasma antioxidant activity,[31] consistent with the observation that addition of milk did not affect the bioavailability of black tea phenolics.[34]

The aim of this study was to investigate the content of flavonoids and phenolics in regularly consumed tea brands available on the UK market and the influence of variation in phenolic components on the antioxidant potential of a serving of black tea.

EXPERIMENTAL

Chemicals

Acetonitrile (HPLC grade) was obtained from Rathburn Chemicals Ltd., Walkerburn, Scotland. Hydrochloric acid and Folin–Ciocalteu phenol reagent were from BDH Laboratory Supplies, Poole, England. Chlorogenic acid, quercetin-3-glucoside, quercetin-3-galactoside, kaempferol-3-glucoside and kaempferol-3-rutinoside were obtained from Extrasynthese, Genay, France. Gallic acid, *p*-coumaric acid, quercetin-3-glucoside and simulated gastric juice (without pepsin) were purchased from Sigma Chemical Co., Steinheim, Germany. ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) and β-phycoerythrin were from Sigma–Aldrich, Zwijndrecht, The Netherlands. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from Randox Ltd., Co. Antrim, UK. ABAP (2,2′-azobis (2-methyl-propionamidine dihydrochloride) was from ACROS, Geel, Belgium. TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine) was from Fluka, Sigma–Aldrich, Zwijndrecht, The Netherlands. (+)-Catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, (−)-epigallocatechin gallate and theaflavins were from the Tea Science Unit, Unilever Research Colworth, Sharnbrook, Bedfordshire, UK. All were demonstrated by HPLC to be >95% pure. Seven brands of black tea available in the UK market were purchased as tea bags. The black tea brands studied were: Typhoo, Tesco *standard blend*, Tesco *premium blend*, Sainsbury *red blend*, Sainsbury *gold blend*, PG Tips, Tetley.

Preparation of Consumer Brews of Black Tea

Tea infusions were prepared by adding 230 ml of boiling deionised water to one tea bag. After 15 s delay the brew was stirred for 5 s and left for an additional 40 s resulting in total extraction time of 60 s. The tea bag was than removed, left decanting for 20 s and gently squeezed using a teaspoon. This procedure was performed three times to produce one sample for analysis by HPLC and the determination of total phenolics by the Folin–Ciocalteu assay. For every black tea brand, three samples were generated. The samples were analysed in duplicate.

HPLC Analysis

HPLC analysis was undertaken using a Waters system consisting of controller 600, an autosampler 717 plus, a photodiode array detector 996 and an in-line degasser. Samples were analysed on a Fluofix column (INEOS Co. Ltd, Kobe, Japan), 250 mm × 4.6 mm, with 5 μm particle size and a guard column of the same material, 4.6 × 15 mm. Mobile phase A consisted of water/5N HCl (99.85/0.15 v/v) and mobile phase B of acetonitrile/water/5N HCl (50/49.9/0.1 v/v/v). The gradient applied was as follows: from 0 to 5 min 100% A, from 5–40 min to 60% A and 40% B, from 40–65 min to 100% B, from 65 to 67 min 100% B and from 67.1 min 100% A. Run time was 70 min followed by a 10 min delay prior to the next injection. Injection volume of the samples was 25 μl. Peaks were identified via spiking with authentic standards, retention time and UV-spectra. Tentative assignments were suggested using information from UV-spectra, retention time and reports in the literature. Quantification was undertaken with standard curves or in the absence of a standard with a standard curve of a related compound. Wavelengths used for phenolic quantification were: catechins and theaflavins 280 nm, caffeic acid derivatives 324 nm, *p*-coumaric acid derivatives 310 nm, flavonols 354 nm and gallic acids 270 nm. Integration was performed manually for every peak.

The coefficient of variance appeared to be mainly between 1 and 15%, except for epicatechin (38%), quercetin-3-galactoside (38%) and epigallocatechin gallate (16%) in brand 5, epicatechin (33%) and theaflavin 2 (16%) in brand 4 and chlorogenic acid (20%) in brand 3, which was due to insufficient resolution of the peak in the particular chromatograms.

Gastric Juice Treatment

A representative consumer brew of black tea was diluted 1:10 with simulated gastric juice of pH 1.5 (sample) and with water (control) and incubated at 37°C for 2 h. After cooling, the filtered solutions were analysed by HPLC.

Total Phenolics

Total phenolics were measured by the Folin–Ciocalteu assay, based on the method described by Singleton and Rossi.[35] The values of total phenolics are expressed as gallic acid equivalents (GAE).

In order to validate the results of the assay the analyses were carried out in the two different participating laboratories applying the same methodology and gave consistent results.

Total Antioxidant Activity

For every black tea brand, one sample of three pooled tea infusions was prepared at the Unilever Health Institute, Vlaardingen, The Netherlands. The total antioxidant activity in each sample was measured applying the TEAC (Trolox Equivalent Antioxidant Capacity), the ORAC (Oxygen Radical Absorbance Capacity) and FRAP (Ferric ion Reducing Antioxidant Power) assays.

The TEAC Assay is a spectrophotometric assay that determines the relative efficiency of an antioxidant or a prepared extract to quench the ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation. This value is related to the quenching efficiency of the water-soluble vitamin E analogue Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the TEAC values are expressed as Trolox equivalents. The TEAC assay was undertaken according to Re *et al.*[36] The TEAC value was determined in duplicate for each pooled sample (of three separate brews of each individual tea infusion) and the results presented are the mean values.

The ORAC assay measures the relative efficiency of antioxidants to protect the fluorescent protein β-phycoerythrin against peroxidation. Peroxyl radicals were generated at a constant rate using the thermolabile azo-initiator ABAP (2,2′-azobis (2-methyl-propionamidine dihydrochloride). This value is related to that of the water-soluble vitamin E analogue Trolox. The ORAC assay was performed on the COBAS-FARA II Centrifugal Analyser as described by Cao *et al.*[37] The ORAC value was determined once for each pooled sample (of three separate brews of each individual tea infusion).

The FRAP assay is another chemical assay that determines the antioxidant activity present in extracts. This assay determines the ability of extracts to reduce ferric—2,4,6-tri(2-pyridyl)-*s*-triazine complex (Fe^{3+}–TPTZ complex) into the ferrous form. The ferrous–TPTZ complex has an intense blue colour and has an absorption maximum at 593 nm. The FRAP activities were measured using a COBAS-FARA II Centrifugal Analyser (Roche Diagnostics, Almere, The Netherlands). The method is essentially the same as that described by Benzie and Strain,[38] except that the incubation had a duration of 7.5 min. Data are expressed as Fe^{2+}-equivalents and are normalised to the amount of material used. The FRAP value was determined in duplicate for each pooled sample (of three separate brews of each individual tea infusion) and the results presented are the mean values.

RESULTS AND DISCUSSION

Tea infusions were prepared and subjected to HPLC analysis. The elution profile revealed a complex polyphenol pattern including flavan-3-ols, flavonols, hydroxycinnamates, gallic acids and theaflavins (Fig. 1). Twenty phenolic compounds were identified, 16 confirmed using data from retention time, spiking with authentic standards and UV-spectra. For four compounds a tentative assignment was suggested based on the relationship between retention time, UV-spectra and reports in the literature.[5,39]

The quantitative results for a serving of a consumer brew prepared from seven different brands of black tea bags are summarised in Table I. As shown, the total flavan-3-ols constituted between 19 and 26% of the total structurally identified polyphenols by HPLC, the major one being (−)-epigallocatechin gallate. The total theaflavins accounted for 27–37% and the total gallic acids with theogallin being the major one for 13–17% of the total structurally identified polyphenols by HPLC. The total flavonols were detected at a proportion of 15–24% of the total structurally identified polyphenols with the major constituents being quercetin-3-rutinoside and kaempferol-3-rutinoside. A significant proportion of hydroxycinnamates, the major ones being 4-caffeoyl and 4-*p*-coumaroyl quinic acid, were also measured in the tea brews with a variation of 8–10% of the total structurally identified polyphenols.

Analysis and separation of the thearubigin fraction, complex polymeric products deriving from polyphenols during the fermentation process of green tea to black tea, was impossible, but the "hump" on which the individual peaks are sitting in the chromatogram is interpreted as chromatographically irresolvable thearubigins. The approximate thearubigin fraction was calculated by subtraction of the total structurally identified polyphenols (from the HPLC analysis) from the total phenolic content (Folin–Ciocalteu).[5] Of a percentage of 72–79% of total phenols, the thearubigins are estimated as representing the major fraction of the polyphenols in the consumer brews of black tea, although this method has limited validity if many unidentified phenolics remain, which is highly probable. Consequently, due to the incomplete identification

of the polyphenols in consumer brews of black tea, this estimate is likely to be too high. The seven brands of black tea differed significantly in their total phenolic content ranging from 219 to 288 mg GAE/230 ml and total sum of individual polyphenols HPLC ranging from 49 to 78 mg/230 ml. The results reveal that a cup of a consumer brew of black tea is providing an average of 262 mg GAE/serving of polyphenols. The polyphenolic content of one cup (230 ml) of these brands is approximately twice as high as world market brands investigated by other researchers.[5] The flavan-3-ols, namely (−)-epicatechin, (−)-epigallocatechin, (−)-epigallocatechin gallate, (−)-epicatechin gallate, and the theaflavins, four different compounds, were the main groups of phenolic compounds in the tea brews followed by the flavonols, namely quercetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-galactoside, kaempferol-3-rutinoside, kaempferol-3-glucoside, the gallic acids, namely gallic acid and an other gallic acid derivative (possibly theogallin) and the hydroxycinnamates, namely chlorogenic acid, 4-caffeoylquinic acid, 3-caffeoylquinic acid, 4-*p*-coumaroylquinic acid, 3-*p*-coumaroylquinic acid (Fig. 2).

The average composition of the six identified classes of polyphenols, namely thearubigins, theaflavins, catechins, flavonols, hydroxycinnamates, gallic acids, in all consumer brews of black tea is shown in Fig. 3. Thearubigins are the major fraction in all seven brands, followed in decreasing order by the theaflavins, the catechins, the flavonols, the gallic acids and finally the hydroxycinnamates.

Taking the annual imports of tea for consumption (134,077 t in 2000; International Tea Committee, Ltd., Annual Bulletin of Statistics; Tea Brokers' Publications, London (2001)), the consumption of one cup of tea per day (which approximates to a usage of 800 g tea per year) and the population in the UK (60 million), average daily tea consumption per capita in the UK equates to 2.8 cups. Taking this estimation and the contents of polyphenols in one cup, found in this study, black tea contributes approximately 840 mg of total polyphenols, 48 mg of flavan-3-ols, 56 mg of theaflavins, 20 mg of flavonol aglycones, 8 mg of hydroxycinnamates and 20 mg of gallic acid to the daily intake of polyphenols in the UK. For the flavonols these data are consistent with reports of other researchers.[5] Khokhar and Magnusdottir[40] have produced an estimate based on the analysis of black tea leaves and a proposed average consumption of three cups per day containing 1% w/v tea leaves, which led to the calculation of the total intake of catechins (flavan-3-ols) from black tea in the UK to be 92.7 mg/day, which is slightly

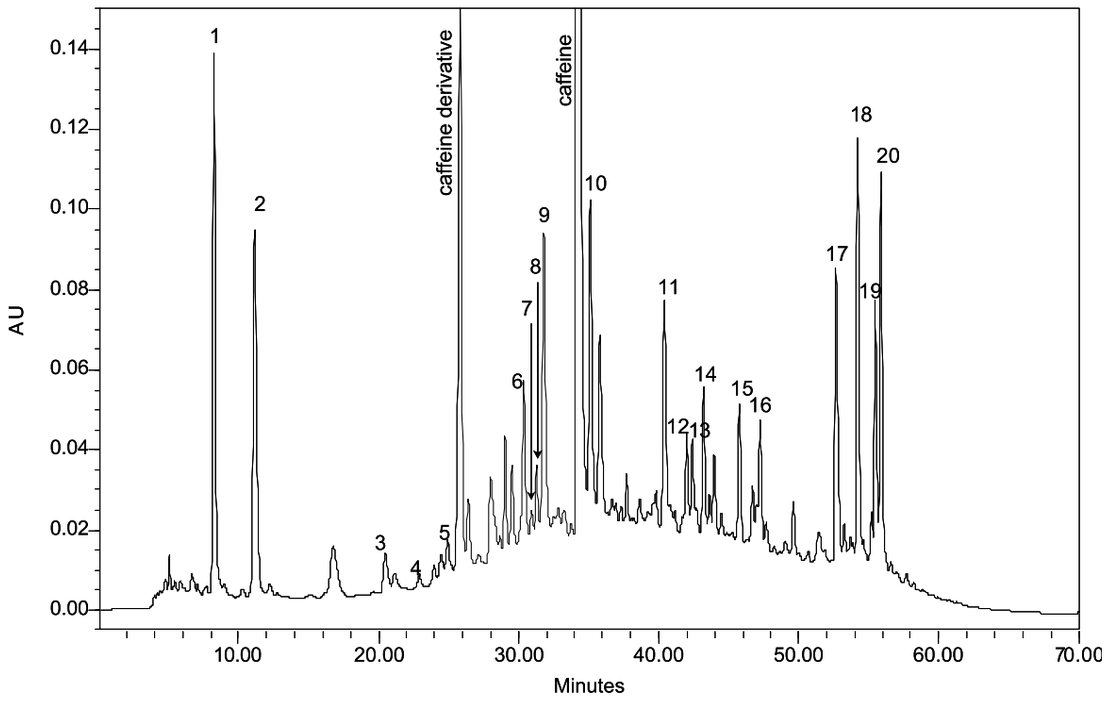

FIGURE 1 Representative chromatogram of a consumer black tea brew at 280 nm (1) gallic acid [RT: 8.2 min], (2) gallic acid derivative [RT: 11.3 min], (3) 3-caffeoyl quinic acid* [RT: 20.5 min], (4) epigallocatechin [RT: 22.9 min], (5) 3-*p*-coumaroyl quinic acid* [RT: 25.0 min], (6) 4-caffeoyl quinic acid* [RT: 30.2 min], (7) chlorogenic acid [RT: 30.9 min], (8) epicatechin [RT: 31.8 min], (9) epigallocatechin gallate [RT: 32.0 min], (10) 4-*p*-coumaroyl quinic acid* [RT: 34.7 min], (11) epicatechin gallate [RT: 40.4 min], (12) quercetin-3-rutinoside [RT: 41.8 min], (13) quercetin-3-galactoside [RT: 42.3 min], (14) quercetin-3-glucoside [RT: 43.0 min], (15) kaempferol-3-rutinoside [RT: 45.8 min], (16) kaempferol-3-glucoside [RT: 47.1 min], (17) theaflavin 1 [RT: 52.7 min], (18) theaflavin 2 [RT: 54.2 min], (19) theaflavin 3 [RT: 55.0 min], (20) theaflavin 4 [RT: 55.7 min] *tentative assignments.

TABLE I Amounts of single polyphenols in consumer brew black tea ($n = 3$) in mg/230 ml (mg/serving)

Compound	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6	Brand 7
Epicatechin	2.89 ± 0.27	0.35 ± 0.01	1.72 ± 0.10	2.99 ± 0.98	3.43 ± 1.30	1.94 ± 0.08	1.20 ± 0.04
Epigallocatechin	3.04 ± 0.21	1.33 ± 0.08	1.50 ± 0.16	3.60 ± 0.23	4.51 ± 0.20	1.63 ± 0.03	1.49 ± 0.03
Epigallocatechin gallate	7.95 ± 0.51	4.70 ± 0.17	5.63 ± 0.66	8.51 ± 1.02	11.81 ± 1.86	7.15 ± 0.09	5.79 ± 0.36
Epicatechin gallate	5.85 ± 0.04	2.77 ± 0.34	3.34 ± 0.32	8.11 ± 0.04	8.08 ± 0.45	5.75 ± 0.15	3.71 ± 0.28
Total catechins	19.73 ± 0.49	9.15 ± 0.58	12.19 ± 1.30	23.21 ± 2.41	27.83 ± 3.78	17.99 ± 0.30	12.19 ± 0.68
Theaflavin 1	6.51 ± 0.10	3.88 ± 0.09	5.58 ± 0.60	4.87 ± 0.18	5.33 ± 0.38	4.10 ± 0.04	4.76 ± 0.18
Theaflavin 2	7.46 ± 0.56	5.11 ± 0.67	7.01 ± 0.79	6.68 ± 1.08	6.53 ± 0.97	6.47 ± 0.47	7.35 ± 0.51
Theaflavin 3	3.53 ± 0.41	2.46 ± 0.09	3.01 ± 0.23	3.40 ± 0.11	3.46 ± 0.14	2.94 ± 0.12	3.05 ± 0.31
Theaflavin 4	5.18 ± 0.14	4.38 ± 0.11	4.55 ± 0.52	5.14 ± 0.08	5.54 ± 0.30	5.16 ± 0.17	5.47 ± 0.25
Total theaflavins	22.68 ± 0.40	15.83 ± 0.75	20.15 ± 2.12	20.09 ± 1.41	20.86 ± 1.65	18.67 ± 0.77	20.63 ± 1.20
Quercetin-3-rutinoside	3.41 ± 0.18	3.54 ± 0.41	2.60 ± 0.27	2.47 ± 0.24	2.53 ± 0.34	3.27 ± 0.08	3.18 ± 0.10
Quercetin-3-galactoside	1.46 ± 0.14	1.16 ± 0.04	1.60 ± 0.15	1.23 ± 0.07	1.05 ± 0.40	1.44 ± 0.03	1.82 ± 0.06
Quercetin-3-glucoside	2.72 ± 0.10	2.15 ± 0.17	2.65 ± 0.30	2.97 ± 0.04	2.81 ± 0.15	2.91 ± 0.06	2.98 ± 0.10
Kaempferol-3-rutinoside	3.68 ± 0.19	3.24 ± 0.08	3.65 ± 0.37	3.02 ± 0.04	3.17 ± 0.22	3.64 ± 0.07	3.65 ± 0.13
Kaempferol-3-glucoside	2.23 ± 0.06	1.68 ± 0.03	1.92 ± 0.19	2.01 ± 0.12	2.07 ± 0.09	1.95 ± 0.02	1.69 ± 0.02
Total flavonols	13.5 ± 0.16	11.77 ± 0.16	12.42 ± 1.28	11.7 ± 0.59	11.63 ± 1.18	13.21 ± 0.26	13.32 ± 0.40
Chlorogenic acid	0.67 ± 0.04	0.30 ± 0.00	0.31 ± 0.06	0.71 ± 0.08	0.65 ± 0.03	0.55 ± 0.02	0.34 ± 0.01
4-caffeoyl quinic acid*	3.64 ± 0.05	1.79 ± 0.03	2.27 ± 0.31	3.75 ± 0.12	3.53 ± 0.22	2.53 ± 0.04	1.92 ± 0.05
3-caffeoyl quinic acid*	0.78 ± 0.01	0.42 ± 0.01	0.45 ± 0.06	0.79 ± 0.03	0.65 ± 0.04	0.64 ± 0.01	0.46 ± 0.01
4-*p*-coumaroyl quinic acid†	2.32 ± 0.03	1.60 ± 0.03	1.84 ± 0.22	2.19 ± 0.19	1.98 ± 0.28	1.79 ± 0.02	1.87 ± 0.09
3-*p*-coumaroyl quinic acid†	0.34 ± 0.01	0.16 ± 0.01	0.38 ± 0.04	0.35 ± 0.01	0.33 ± 0.03	0.33 ± 0.00	0.35 ± 0.01
Total hydroxycinnamates	7.75 ± 0.03	4.27 ± 0.06	5.25 ± 0.69	7.79 ± 0.42	7.14 ± 0.60	5.84 ± 0.07	4.94 ± 0.15
Gallic acid	4.43 ± 0.06	3.98 ± 0.07	4.03 ± 0.42	5.07 ± 0.16	4.71 ± 0.33	5.04 ± 0.05	4.25 ± 0.13
Gallic acid derivative‡	5.70 ± 0.08	3.53 ± 0.05	3.56 ± 0.41	6.48 ± 0.24	5.88 ± 0.32	5.75 ± 0.03	4.13 ± 0.15
Total gallic acids	10.13 ± 0.13	7.51 ± 0.12	7.59 ± 0.83	11.55 ± 0.37	10.59 ± 0.65	10.79 ± 0.07	8.38 ± 0.28
Sum of quantified polyphenols	73.78 ± 0.37	48.53 ± 1.20	57.67 ± 6.10	74.32 ± 5.03	78.08 ± 7.74	64.98 ± 1.39	59.46 ± 2.65
Total polyphenols (Folin-Ciocalteu)	267.5 ± 34.5	219.0 ± 43.0	270.5 ± 29.5	288.0 ± 48.0	283.5 ± 36.5	248.0 ± 48.0	255.5 ± 44.5
Thearubigins¶	193.7	170.5	212.8	213.7	205.4	183.0	196.0

*Tentative assignment, quantified relative to chlorogenic acid (5-caffeoyl quinic acid). †Tentative assignment, quantified relative to *p*-coumaric acid. ‡Tentative assignment, quantified relative to gallic acid. ¶Total polyphenols (Folin–Ciocalteu)—sum of quantified polyphenols HPLC.

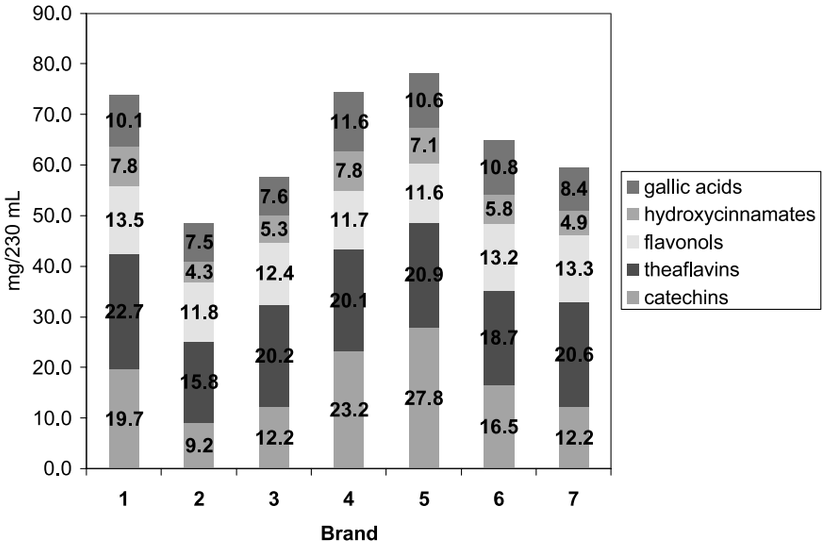

FIGURE 2 Amounts of catechins, theaflavins, flavonols, hydroxycinnamates and gallic acids (expressed in mg per serving) in consumer brews of seven different brands of tea bags from the UK market.

higher than the estimate reached in this study. An epidemiological study in the Netherlands determined an average daily intake of 72 ± 47.8 mg/day catechins with black tea being the major source contributing 87% (63 mg) to this total daily intake.[41] This result is broadly consistent with findings herein.

Due to the high content of polyphenols the consumer brews of black tea possessed high antioxidant activities (Table II). The brands with the highest values (brand 4 and 5) provide approximately 50% more antioxidant activity than the brand with the lowest values (brand 2) mirroring the differences in phenolic content of the seven tea brands investigated here. The three assays used for the determination of the antioxidant activity delivered relatively consistent results, even though their principles of measurement, ABTS radical or oxygen radical scavenging or iron reducing abilities, were different. Correlations between each of the assays were $R = 0.93$ for TEAC and FRAP, $R = 0.90$ for TEAC and ORAC and $R = 0.89$ for ORAC and FRAP. The antioxidant activity determined with these three assays correlated well with the total phenolics assay (Folin–Ciocalteu) as well as the sum of identified polyphenols ($R > 0.81$). Other studies have observed consistent correlations between antioxidant activity assays for a variety of food and beverages including the three assays used here.[42,43] The limitations and differences of most *in vitro* methods for the determination of the antioxidant activity[42,44] results also in the recommendation that at least two different assays should be used to determine the antioxidant activity for food matrices, such as beverages, as proposed by Schlesier *et al.*[42] The determined values of antioxidant activity of the consumer tea brews as well as the correlations between the total phenolics and the antioxidant activity were consistent with the ranges and results of other researchers.[12,42,45,46]

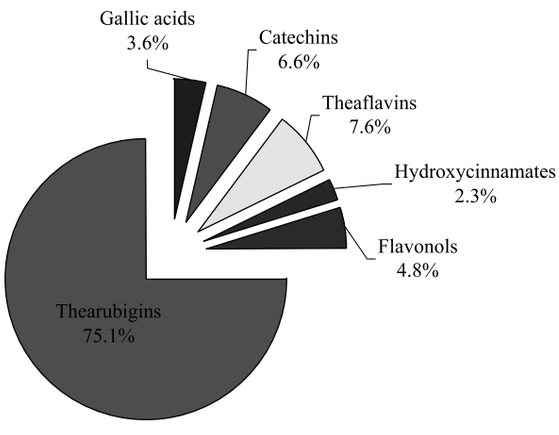

FIGURE 3 Mean percentile composition of different classes of polyphenols in consumer brews of different black teas ($n = 7$).

The bioavailability as well as the metabolism of the individual polyphenolic constituents of black tea (flavan-3-ols, flavonols, hydroxybenzoic acids, hydroxycinnamates) in humans has been reported.[16–30] In contrast, no information has been achieved so far about the bioavailability and metabolism of the theaflavins, which are exclusively found in black tea and, as shown in this report and consistent with other researchers,[5] represent the second largest fraction in a consumer brew of black tea. The metabolic fate of the thearubigins is also unknown, which applies also to their precise structure. The theaflavins and thearubigins both derive from fermentation of green tea to black tea. In order to ascertain the potential effects of the acidic

TABLE II TEAC ($n = 2$, pooled samples of three brews), ORAC ($n = 1$, pooled samples of three brews), FRAP ($n = 2$, pooled samples of three brews), total phenolics (Folin–Ciocalteu) and total polyphenols (HPLC) of consumer brews of the seven black tea brands from the British market (mean + SD)

	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6	Brand 7
TEAC (μmol Trolox/ml)	12.5 ± 1.1	9.6 ± 1.0	11.6 ± 0.5	12.4 ± 0.9	13.4 ± 0.7	11.9 ± 1.0	11.3 ± 1.2
ORAC (μmol Trolox/ml)	13.0	7.4	12.4	13.1	12.8	11.9	11.7
FRAP (μmol Fe^{2+}/ml)	10.3 ± 0.7	8.1 ± 0.6	9.8 ± 0.6	10.7 ± 0.6	11.3 ± 0.6	9.7 ± 0.7	10.3 ± 1.3
Total phenolics (Folin) (μg/ml)	1163 ± 150	953 ± 187	1177 ± 129	1252 ± 208	1233 ± 159	1087 ± 208	1111 ± 194
Total identified polyphenols HPLC (μg/ml)	320.8 ± 1.6	211.0 ± 5.2	251.8 ± 26.5	323.1 ± 21.9	339.5 ± 33.6	282.5 ± 6.0	258.5 ± 11.5

environment of the gastric lumen on breakdown of complex polyphenols, a black tea brew was treated with simulated gastric juice. Incubation of a brew of black tea with simulated gastric juice (1 ml tea brew + 9 ml of simulated gastric juice for 2 h at 37°C) resulted in a significant increase in the amounts of theaflavins (approx. 140% increase) while the composition of the other identified compounds did not change (Fig. 4). The observed increase in the amounts of theaflavins after incubation of a black tea brew with simulated gastric juice is consistent with a pH-dependent partial cleavage of thearubigins into the smaller theaflavins, which are more likely to be bioavailable due to their smaller molecule size. Recent studies have demonstrated that it is the acidic component of the simulated gastric juice which is responsible for the cleavage.[47] Evidence that a prolonged colonic metabolism of black tea polyphenols (including theaflavin and thearubigins) might occur has been suggested.[48]

The results reveal that a cup of black tea as usually brewed in the UK is an excellent source of polyphenols providing as much as six different classes of polyphenols and presenting a higher antioxidant activity than other dietary sources. The questions of absorption, metabolic fate and bioactivity of the identified polyphenols and classes of polyphenols remain a task for future investigations.

Acknowledgements

This research was supported by Unilever Research Vlaardingen, The Netherlands.

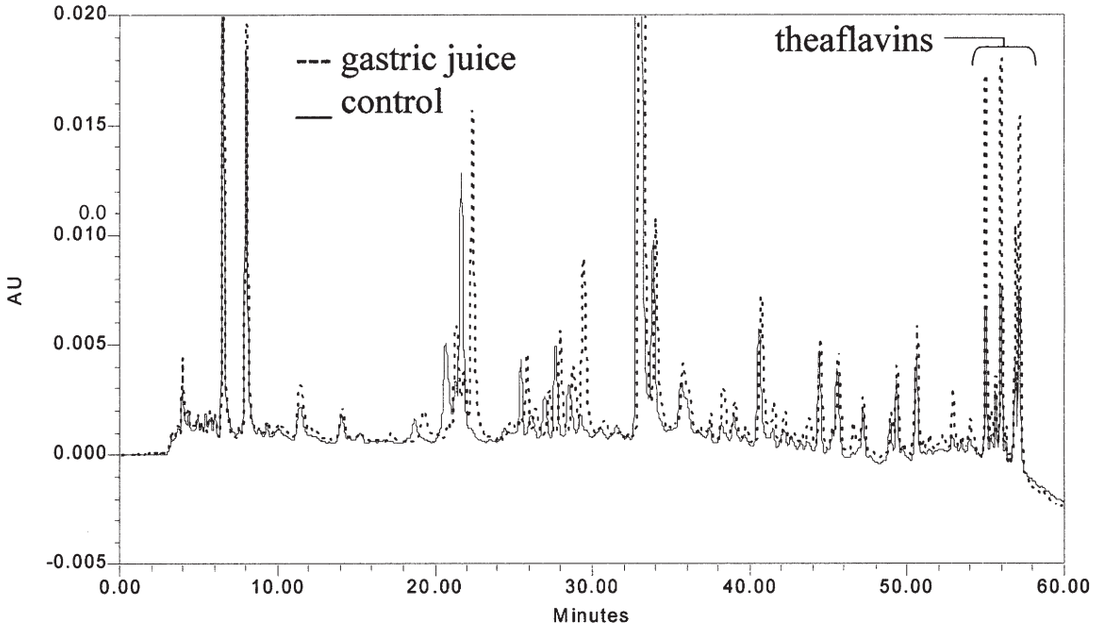

FIGURE 4 Overlay of chromatograms of tea diluted 1:10 in either water (solid line) or gastric juice (dotted line) at 280 nm after 2 h incubation at 37°C.

References

[1] Riemersma, R.A., Rice-Evans, C.A., Tyrrell, R.M., Clifford, M.N. and Lean, M.E. (2001) "Tea flavonoids and cardiovascular health", *Q. J. Med.* **94**, 277–282.

[2] Yang, C.S. (1999) "Tea and health", *Nutrition* **15**, 946–949.

[3] Mukhtar, H. and Ahmad, N. (2000) "Tea polyphenols: prevention of cancer and optimizing health", *Am. J. Clin. Nutr.* **71**, 1698S–1704S.

[4] Kuroda, Y. and Hara, Y. (1999) "Antimutagenic and anticarcinogenic activity of tea polyphenols", *Mutat. Res.* **436**, 69–97.

[5] Lakenbrink, C., Lapczynski, S., Maiwald, B. and Engelhardt, U.H. (2000) "Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages", *J. Agric. Food Chem.* **48**, 2848–2852.

[6] Balentine, D.A., Wiseman, S.A. and Bouwens, L.C.M. (1997) "The chemistry of tea flavonoids", *Crit. Rev. Food Sci. Nutr.* **37**, 693–704.

[7] Wiseman, S.A., Balentine, D.A. and Frei, B. (1997) "Antioxidants in tea", *Crit. Rev. Food Sci. Nutr.* **37**, 705–718.

[8] Vinson, J.A., Dabbagh, Y.A., Serry, M.M. and Jang, J. (1995) "Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease", *J. Agric. Food Chem.* **43**, 2800–2802.

[9] Vinson, J.A., Jang, J., Yang, J., Dabbagh, Y., Liang, X., Serry, M., Proch, J. and Cai, S. (1999) "Vitamins and especially flavonoids in common beverages are powerful *in vitro* antioxidants which enrich lower density lipoproteins and increase their oxidative resistance after *ex vivo* spiking in human plasma", *J. Agric. Food Chem.* **47**, 2502–2504.

[10] Rice-Evans, C.A., Miller, M.N. and Paganga, G. (1996) "Structure-antioxidant activity relationships of flavonoids and phenolic acids", *Free Radic. Biol. Med.* **20**, 933–956.

[11] Zeyuan, D., Bingyin, T., Xiaolin, L., Jinming, H. and Yifeng, C. (1998) "Effect of green tea and black tea on the blood glucose, the triglycerides, and antioxidation in aged rats", *J. Agric. Food Chem.* **46**, 3875–3878.

[12] Benzie, I.F.F. and Szeto, Y.T. (1999) "Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay", *J. Agric. Food Chem.* **47**, 633–636.

[13] Lin, Y.L., Cheng, C.Y., Lin, Y.P., Lau, Y.W., Juan, I.M. and Lin, J.K. (1998) "Hypolipidemic effect of green tea leaves through induction of antioxidant and phase II enzymes including superoxide dismutase, catalase, and glutathione *S*-transferase in rats", *J. Agric. Food Chem.* **46**, 1893–1899.

[14] Robertson, A. (1992) "The chemistry and biochemistry of black tea production—the nonvolatiles", In: Wilson, K.C. and Clifford, M.N., eds, Tea—Cultivation to Consumption (Chapman and Hall, London).

[15] Kiehne, A. and Engelhardt, U.H. (1996) "Thermospray-LC-MS analysis of various groups of polyphenols in tea. II: Chlorogenic acids, theaflavins and thearubigins", *Z. Lebensm. Unters. Forsch.* **202**, 299–302.

[16] van het Hof, K.H., Wiseman, S.A., Yang, C.S. and Tijburg, L.B. (1999) "Plasma and lipoprotein levels of tea catechins following repeated tea consumption", *Proc. Soc. Exp. Biol. Med.* **220**, 203–209.

[17] Warden, B.A., Smith, L.S., Beecher, G.R., Balentine, D.A. and Clevidence, B.A. (2001) "Catechins are bioavailable in men and women drinking black tea throughout the day", *J. Nutr.* **131**, 1731–1737.

[18] Nakagawa, K. and Miyazawa, T. (1997) "Chemiluminescence-high-performance liquid chromatographic determination of tea catechin (−)-epigallocatechin 3-gallate, at picomole levels in rat and human plasma", *Anal. Biochem.* **248**, 41–49.

[19] Lee, M.J., Wang, Z.Y., Li, H., Chen, L., Sun, Y., Gobbo, S., Balentine, D.A. and Yang, C.S. (1995) "Analysis of plasma and urinary tea polyphenols in human subjects", *Cancer Epidemiol. Biomark. Prev.* **4**, 393–399.

[20] de Vries, J.H.M., Hollman, P.C.H., Meyboom, S., Buysman, M.N.C.P., Zock, P.L., van Staveren, W.A. and Katan, M.B. (1998) "Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake", *Am. J. Clin. Nutr.* **68**, 60–65.

[21] Shahrzad, S., Aoyagi, K., Winter, A., Koyama, A. and Bitsch, I. (2001) "Pharmacokinetics of gallic acid and its relative bioavailability from tea in healthy humans", *J. Nutr.* **131**, 1207–1210.

[22] Li, C., Lee, M.J., Sheng, S., Meng, X., Prabhu, S., Winnik, B., Huang, B., Chung, J.Y., Yan, S., Ho, C.T. and Yang, C.S. (2000) "Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion", *Chem. Res. Toxicol.* **13**, 177–184.

[23] Spencer, J.P.E., Chowrimootoo, G., Choudhury, R., Debnam, E.S., Srai, S.K. and Rice-Evans, C.A. (1999) "The small intestine can both absorb and glucuronidate luminal flavonoids", *FEBS Lett.* **458**, 224–230.

[24] Kuhnle, G., Spencer, J.P.E., Schroeter, H., Shenoy, B., Debnam, E.S., Srai, S.K., Rice-Evans, C.A. and Hahn, U. (2000) "Epicatechin and catechin are O-methylated and glucuronidated in the small intestine", *Biochem. Biophys. Res. Commun.* **277**, 507–512.

[25] Rice-Evans, C.A., Spencer, J.P.E., Schroeter, H. and Rechner, A.R. (2000) "Bioavailability of flavonoids and potential bioactive forms *in vivo*", *Drug Metab. Drug Interact.* **17**, 291–310.

[26] Hacket, A.M., Shaw, I.C. and Griffiths, L.A. (1982) "3′-*O*-methyl-(+)-catechin glucuronide and 3′-*O*-methyl-(+)-catechin sulphate: new urinary metabolites of (+)-catechin in the rat and the marmoset", *Experientia* **38**, 538–540.

[27] Donovan, J.L., Crespy, V., Manach, C., Morand, C., Besson, C., Scalbert, A. and Remesy, C. (2001) "Catechin is metabolized by both the small intestine and liver of rats", *J. Nutr.* **131**, 1753–1757.

[28] Scalbert, A. and Williamson, G. (2000) "Dietary intake and bioavailability of polyphenols", *J. Nutr.* **130**, 2073S–2085S.

[29] Scheline, R.R. (1991) Handbook of Mammalian Metabolism of Plant Compounds (CRC Press, Boca Ranton), pp 168–170.

[30] Rechner, A.R., Spencer, J.P.E., Kuhnle, G., Hahn, U. and Rice-Evans, C.A. (2001) "Novel biomarkers of the metabolism of caffeic acid derivatives *in vivo*", *Free Radic. Biol. Med.* **30**, 1213–1222.

[31] Cherubini, A., Beal, M.F. and Frei, B. (1999) "Black tea increases the resistance of human plasma to lipid peroxidation *in vitro*, but not *ex vivo*", *Free Radic. Biol. Med.* **27**, 381–387.

[32] Leenen, R., Roodenburg, A.J., Tijburg, L.B. and Wiseman, S.A. (2000) "A single dose of tea with or without milk increases plasma antioxidant activity in humans", *Eur. J. Clin. Nutr.* **54**, 87–92.

[33] van het Hof, K.H., de Boer, H.S., Wiseman, S.A., Lien, N., Westrate, J.A. and Tijburg, L.B. (1997) "Consumption of green or black tea does not increase resistance of low-density lipoprotein to oxidation in humans", *Am. J. Clin. Nutr.* **66**, 1125–1132.

[34] Hollman, P.C.H., van het Hof, K.H., Tijburg, L.B. and Katan, M.B. (2001) "Addition of milk does not affect the absorption of flavonols from tea in man", *Free Radic. Res.* **34**, 297–300.

[35] Singleton, V.L. and Rossi, J.A.J. (1965) "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents", *Am. J. Enol. Vitic.* **16**, 144–158.

[36] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C.A. (1999) "Antioxidant activity applying an improved ABTS radical cation decolorization assay", *Free Radic. Biol. Med.* **26**, 1231–1237.

[37] Cao, G., Verdon, C.P., Wu, A.H.B., Wang, H. and Prior, R.L. (1995) "Automated-assay of oxygen radical absorbance capacity with the COBAS FARA-II", *Clin. Chem.* **41**, 1738–1744.

[38] Benzie, I.F.F. and Strain, J.J. (1999) "Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration", *Methods Enzymol.* **299**, 15–27.

[39] Kuhr, S. and Engelhardt, U.H. (1991) "Determination of flavonols, theogallin, gallic acid and caffeine in tea using HPLC", *Z. Lebensm. Unters. Forsch.* **192**, 526–529.

[40] Khokhar, S. and Magnusdottir, S.G.M. (2002) "Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom", *J. Agric. Food Chem.* **50**, 565–570.

[41] Arts, I.C.W., Hollman, P.C.H., Feskens, E.J.M., Bas Bueno de Mesquita, H. and Kromhout, D. (2001) "Catechin intake might explain the inverse relation between tea consumption

and ischemic heart disease: the Zutphen Elderly Study", *Am. J. Clin. Nutr.* **74**, 227–232.
[42] Schlesier, K., Harwat, M., Böhm, V. and Bitsch, R. (2002) "Assessment of antioxidant activity by using different *in vitro* methods", *Free Radic. Res.* **36**, 177–187.
[43] Proteggente, A.R., Pannala, A.S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., Van de Put, F., Dacombe, C. and Rice-Evans, C.A. (2002) "The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition", *Free Radic. Res.* **36**, 217–233.
[44] Rice-Evans, C.A. (2000) "Measurement of total antioxidant activity as a marker of antioxidant status *in vivo*: procedures and limitations", *Free Radic. Res.* **33**, S59–S66.
[45] Prior, R.L. and Cao, G. (1999) "Antioxidant capacity and polyphenolic components of teas: implication for altering *in vivo* antioxidant status", *Proc. Soc. Exp. Biol. Med.* **220**, 255–261.
[46] Rechner, A., Patz, C.D. and Dietrich, H. (1997) "Beitrag zur Bewertung der antioxidativen Kapazität verschiedener Getränke", *Flüssiges Obst* **64**, 62–65.
[47] Spencer, J.P.E., Chaudry, F., Pannala, A.S., Srai, S.K., Debnam, E. and Rice-Evans, C.A. (2000) "Decomposition of cocoa procyanidins in the gastric milieu", *Biochem. Biophys. Res. Commun.* **272**, 236–241.
[48] Clifford, M.N., Copeland, E.L., Bloxsidge, J.P. and Mitchell, L.A. (2000) "Hippuric acid as a major excretion product associated with black tea consumption", *Xenobiotica* **50**, 317–326.