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Catechin Content of 18 Teas and a Green Tea Extract Supplement Correlates With the Antioxidant Capacity

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Abstract: *Our literature review of currently available data in the area of tea and cancer prevention demonstrated that there is more conclusive evidence for the chemopreventive effect of green tea compared with black tea. We suggest that this is due to a large variation of the flavanol content in tea, which is not taken into consideration in most of the epidemiological studies. It was the purpose of this study to determine the flavanol content of various teas and tea products and to correlate it with their radical scavenging activity. A modified oxygen radical absorbance capacity (ORAC) assay at pH 5.5 was utilized. The total flavavol content varied from 21.2 to 103.2 mg/g for regular teas and from 4.6 to 39.0 mg/g for decaffeinated teas. The ORAC value varied from 728 to 1686 trolox equivalents/g tea for regular teas and from 507 to 845 trolox equivalents/g for decaffeinated teas. There was a significant correlation of flavanol content to ORAC value (*r = *0.79,* P = *0.0001) for the teas and green tea extract. The large variation in flavanol content and ORAC value among various brands and types of tea provides critical information for investigators using tea in studies of nutrition and cancer prevention.*

Introduction

Tea is one of the most popular beverages in the world and is consumed by over two-thirds of the world's population. Tea (*Camellia sinensis*) is manufactured as black (78%), green (20%), or oolong tea (2%). The consumption of tea has been associated with anticarcinogenic, antimutagenic, and cardioprotective effects based on experimental studies using cell culture and animal models. Epidemiological studies, however, are not as conclusive (Table 1). The consumption of tea has been associated with a decreased risk of developing cancer of the stomach, colorectum, esophagus, lung, and prostate as well as a decreased risk of atrophic gastritis, coronary heart disease, and incidence of stroke in some studies (1). Other studies, however, do not support the protective effect of tea against cancer (Table 1). Based on a summary including epidemiological studies with more than 200 cases (Table 1) we concluded that there is stronger evidence for the chemopreventive potential of green tea in Asian countries, whereas studies of the chemopreventive effect of black tea in smaller quantities are less convincing (Table 1).

The biological benefits of tea are due to their flavanol content. Tea flavanols are a group of natural polyphenols found in green and black tea. Four flavanol derivatives are found in tea: (–)-epicatechin (EC), (–)-epigallocatechin (EGC), EC gallate (ECG), and EGC gallate (EGCG) (Fig. 1). Their biological benefits are due to their strong antioxidant and anti-angiogenic activity as well as their potential to inhibit cell proliferation and modulate carcinogen metabolism (1).

Flavanols account for 6–16% of the dry green tea leaves (2). During the manufacturing process of black and oolong teas, tea leaves are crushed to allow polyphenol oxidase to catalyze the oxidation and polymerization of flavanols to polymers called theaflavins (2–6%) and thearubigins (20%) (3). These polymers contribute to the characteristic bright orange-red color of black tea. Three to 10% of the flavanols remain in black tea. The major fraction of black tea polyphenols is composed of high molecular weight compounds called thearubigins, which have been poorly characterized thus far (4).

Tea is usually prepared by infusing green or black tea leaves in hot water. A typical cup of tea in Western society is prepared by brewing one tea bag (1.8–2.4 g tea) in 200–250 ml of hot water for 3–5 min. Decaffeinated green tea extract dietary supplements are also available to provide the consumer with a convenient way to benefit from the health benefits of tea flavanols without ingesting caffeine.

Chen et al. demonstrated that the flavanols in tea drinks are stable in aqueous solutions with low pH (5). Even after a 7-h brew at 98°C, only 20% of the green tea flavanols degraded. Previous measurements of the antioxidant capacity of foods and beverages have been performed using the classical oxygen radical absorbance capacity (ORAC) assay with a phosphate buffer pH 7 (6). Because most flavanols are unstable at pH 7, the results from the classical ORAC assay may have underesti-

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Table 1. Tea consumption and Cancer

Ref.	Intervention/Location of Study	Cancer Site/Outcome	No. of Cases/Controls
		Beneficial effects of tea consumption against cancer	
7	10 cups green tea, Japan	Delay in onset in all sites RR = 0.57	384/8,552
8	Green tea in female, nonsmoker, China	Lung cancer RR = 0.65	649/675
9	>2 cups of black tea, male nonsmoker, Uruguay	Lung cancer RR = 0.34	427/428
10	10 cups of Okinawa tea, Japan	Lung cancer RR = 0.38	333/666
11	Green tea consumption, China	Stomach cancer RR = 0.53 and chronic gastritis RR = 0.49	299/433
12	>7 cups of green tea, Japan	Stomach cancer RR = 0.69	1706/21,128
13	300 g/mo of tea, China	Colon, rectum, and pancreas, RR = 0.82, 0.72, 0.63	931,884,451/1,552
14	>2 cups of tea/day, postmenopausal women, Iowa	Digestive and urinary tract, RR = 0.68, 0.4	2,936/35,369
15	Green tea, China	Stomach cancer, RR = 0.71	711/711
16	Green tea, Shanghai, China	Esophageal cancer, RR = 0.43	734/1,552
17	>5 cups, Japan	Recurrence of breast cancer stage I and II, R = 0.56	472/8,552
18	>10 cups of green tea	Chronic atrophic gastritis, R = 0.64	636/—
19	Green tea, China	Gastric cancer	272/544
20	>1 cup hot tea, Arizona	Squamous cell carcinoma RR = 0.63	234/216
21	3–4 cups tea, The Netherlands	Bladder cancer RR = 0.8	569/3,123
22	Green tea, China	Stomach cancer RR = 0.77	1,124/1451
		No association of tea consumption with cancer	
23	>5 cups of green tea, Japan	Gastric cancer, R = 1.1	419/26,311
24	>5 cups of black tea, The Netherlands	Breast, colorectal, stomach, and lung cancer	2,264/121,043
25	2–3 cups of black tea, Sweden	Breast cancer, R = 1.1	1,271/59,036
26	>5 cups of green tea, Japan	Cancer of all sites	4,069/38,540
27	Meta-analysis, 37 studies	Urinary tract cancer	
28	>4 cups of tea, Canada	Prostate cancer	1,623/1,623
29	>2 cups of tea, postmenopausal women, Iowa	Cancer of the colon and rectum	685/2,434
30	Tea, Italy	Cancer of the oral cavity, esophagus, stomach, bladder, kidney, and prostate	6,277/6,147
31	>2.6 cups tea, Iowa	Bladder and kidney cancer	1,452,406/2,434
32	Black tea, Sweden	Colon cancer	460/61,463
33	Tea, Canada	Bladder, colon, and rectal cancer	927,991,825/2118
34	>4 cups of tea, Italy	Ovarian cancer	1,031/2,411
35	>1 cup of tea, Italy	Cancer of the colon and rectum	3,530/7,057
14	>2 cups of tea, postmenopausal women, Iowa	Melanoma, non-Hodgkins lymphoma, cancer of the pancreas, lung, breast, uterine corps, and ovaries	6,277/35,369

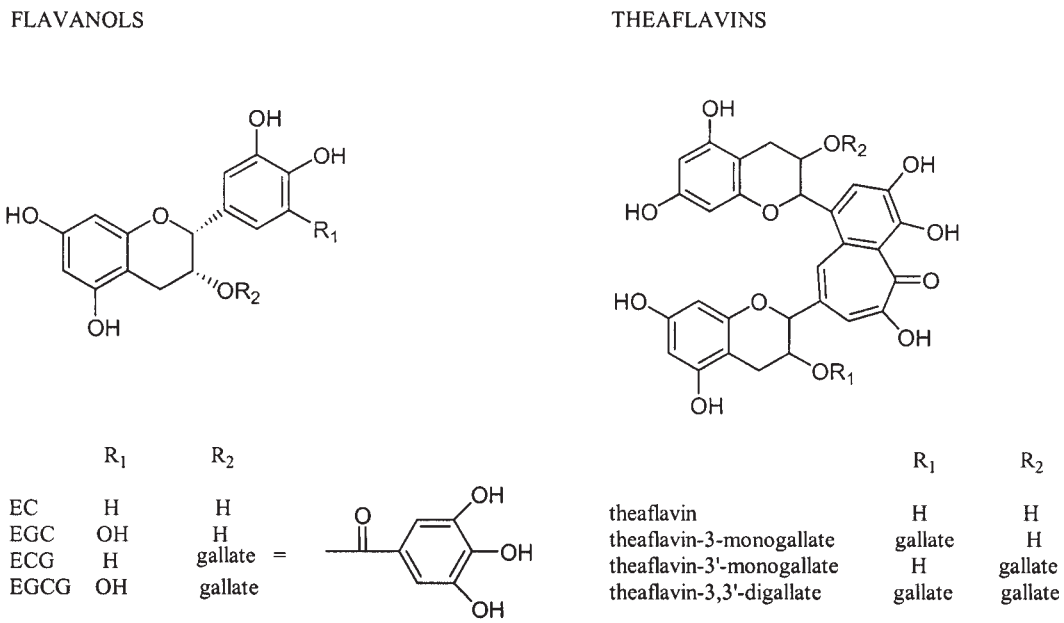

Figure 1. Chemical structures of EC, ECG EGC, EGCG, theaflavin, theaflavin-3-monogallate, theaflavin-3′-monogallate, and theaflavin-3,3′-digallate.

mated the antioxidant capacity of the flavanols. The purpose of this study was to measure the flavanol and theaflavin content of various green tea, black tea, iced tea beverages, and one green tea extract supplement. In addition, the ORAC values of these teas and tea products were measured using a modified ORAC assay at pH 5.5 and correlated to the flavanol and theaflavin content of the teas and green tea supplement.

Results of this study provide important data for epidemiological studies by demonstrating the importance of collecting more detailed information about the type of tea (decaffeinated or regular, black or green). The results also will assist consumers to choose the tea product that provides the most health benefits.

Materials and Methods

Chemicals

β-Phycoerythrin (β-PE) from porphyridium cruentum, gallic acid, (–)-catechin, (–)-catechin gallate, EC, EGC, ECG, (–)-gallocatechin gallate, EGCG, caffeine, and a theaflavin mixture called black tea extract containing four theaflavins were purchased from Sigma (St. Louis, MO). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals, Inc. (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). HPLC solvents were purchased from Fisher Scientific (Pittsburgh, PA).

Teas

Eighteen different green and black tea bags and two brands of iced tea were purchased in local supermarkets. Pharmanex generously provided the green tea extract supplement.

Sample Preparation

Tea leaves from each tea bag (1.5–2.4 g) were removed, weighed, and used for tea brewing in 100 ml boiling deionized water for 3 min. Tea brews were filtered through a coffee filter to remove tea leaves. The catechin content of the filtered tea brews was analyzed by high-performance liquid chromatography (HPLC), and aliquots were frozen at –20°C for ORAC analysis. Tea brews prepared to test the difference in flavanol content among different lots of Uncle Lee's Green Tea, Lipton Green Tea, and Bigelow Darjeeling Blend were brewed for 5 min. The flavanol content of Tegreen capsules was analyzed by dissolving one capsule in 100 ml of boiling water. Aliquots were frozen at –20°C and analyzed by HPLC. All determinations were performed in duplicates.

pH Stability Test

Flavanol stock solutions (6 mM) were prepared in methanol and stored at –70ºC. Twenty- to 60-fold dilutions were prepared in phosphate buffers (0.5 M), pH 3–7, at room temperature. Samples were placed into the autosampler immediately, and their flavanol concentrations were determined using HPLC analysis.

ORAC Assay

The ORAC assay was performed as described by Cao and Prior (6) except that a sodium acetate buffer (75 mM, pH 5.5) was used to prevent degradation of the flavanols. In the final mixture of 0.2 ml, β-PE (3.39 mg/l) was used as a target of free radical attack and AAPH (8 mM) was used as a peroxyl radical generator at 37°C. Trolox (10 μM) was used as a standard control. The decrease of PE fluorescence was determined by reading the fluorescence (excitation 535 nm, emission 595 nm) every 2 min for 70 min in a Perkin Elmer HTS BioAssay Reader (Norwalk, CT). The ORAC value was evaluated as an area under curve (AUC) and calculated by taking into account the Trolox reading using the following equation: $(AUC_{sample} - AUC_{buffer})/(AUC_{Trolox} - AUC_{buffer}) \times$ dilution factor of sample × initial Trolox concentration (μM). Brewed tea was diluted 1:250 with sodium acetate buffer (75 mM, pH 5.5) and flavanol and other flavonoid standard solutions were prepared in methanol (3 mM) and diluted 1:150 to 1:600 in the same buffer. Tea samples were analyzed in triplicate and flavanol standards were measured in six replicates.

HPLC Tea Flavanol Analysis

After mixing the brewed tea with mobile phase 1:1 v/v and filtering the mix through a 0.2-μm PVDF acrodisc syringe filter (Gelman, Ann Arbor, MI), tea flavanols were analyzed by HPLC. Filter discs were washed with 200 μl methanol and the wash solution was also analyzed for flavanols by HPLC. The flavanol content eluted from the filter disc was added to the data from the tea analyses. The flavanol analysis was performed by HPLC with a Waters NovaPak C18 (150 × 3.9 mm, 4 μm) HPLC column and an Alltech Macrosphere RP 300 C18 5U guard column. Mobile phase A was composed of acetonitrile and mobile phase B was composed of 960 ml 0.1% acetic acid (pH 3.5) + 20 ml acetonitrile + 20 ml tetrahydrofuran. Flavanols were eluted with the following gradient: at time 0 min, 100% B; at time 45 min, 40% B; and at time 47 min, 100% B. The equilibration period was 8 min. An Agilent Technologies (San Diego, CA) 1050 HPLC system was used with a Shimadzu (Cole Scientific Inc., Moorpark, CA) SPD-6AV, UV-VIS spectrophotometer (260 nm). Peak areas were integrated using the Agilent Technologies 2D ChemStation Rev. A.0701. Final concentrations were calculated in comparison with a known standard response.

Statistical Analysis

For each tea analysis, two samples were analyzed and the mean values obtained. ORAC values were determined in six replicates and mean values obtained. The Pearson correlation

coefficient for the tea flavanol content and ORAC values was analyzed with the SAS program.

Results

Tea Flavanol Content

The four most common flavanols in green and black tea are EGCG, EGC, EC, and ECG (Figs. 1 and 2). The flavanol, gallic acid, and caffeine content of the teas, tea beverages, and green tea extract supplement are shown in Table 2. The green tea flavanol content ranged from 59.3 to 103.2 mg/g tea in regular teas and from 26.7 to 52.2 mg/g in decaffeinated teas. The flavanol content of regular black tea varied from 21.2 to 68.3 mg/g tea and from 4.6 to 5.4 mg/g decaffeinated tea (Table 2). The tea content per tea bag ranged from 1.6 to 2.4 g of tea per tea bag. Black tea contained less flavanols than green tea due to the fermentation process that generates the epicatechin polymers known as theaflavins and thearubigins and their gallate derivatives (Fig. 1). The theaflavin content of regular black tea varied from 3.5 to 8.3 mg/g tea for regular teas and from 0.9 to 1.2 mg/g decaffeinated black tea. In general, decaffeinated teas contained less flavanols and theaflavins compared with regular teas. The flavanol content of the green tea extract supplement was equivalent to the flavanol content of one cup of the green tea with the highest flavanol content. Iced tea beverages did not contain any flavanols (Table 2a). Variations of flavanol content in tea bags from different lots purchased at different times and different stores (Table 3) were smaller compared with differences in teas from different brands (Table 2a,b).

Flavanol pH Stability

The stability of flavanols in different conditions such as pH and temperature is an important factor to consider in the determination of their biological activity. As shown in Figs. 3 and 4, the pH stability varies among different flavanols. At pH 7, catechin, epicatechin, and ECG are still relatively stable, whereas EGC, EGCG, and GCG are completely degraded (Fig. 3). After 2 h at pH 7 only 34% of EGC and 61% of EGCG remained (Fig. 4). After 7 h at pH 7 EGC and EGCG were completely degraded. This shows the importance of performing the measurements of the antioxidant capacity at a lower pH where all the flavanols are stable.

ORAC Values of Individual Flavanols and Flavonoids

The intra-assay coefficient of variation (CV) in the ORAC assay was 0.9–3.7% for buffer and 1.3–3.2% for the Trolox standard. The interassay CV was 8.0% for buffer and 5.4% for the Trolox standard. The ORAC values of the individual flavanol standard solutions as determined with the modified ORAC assay are shown in Table 4. If expressed in Trolox equivalents/μmol flavanol the following order of antioxidant capacity was observed: ECG > EGCG > EC = catechin > EGC > mixed theaflavins > gallic acid. To validate the modified ORAC assay, the ORAC values of ascorbic acid and other flavonoids such as quercetin, kaempherol, and naringenin were determined (1.2, 6.7, 2.6, and 2.4 μmol TE/μmol). The ORAC values of these antioxidants were consistent with the data from other investigators (9).

ORAC Values of Individual Teas and Tea Products

The ORAC values of the individual teas and tea products were also determined with the modified ORAC assay. The standard and samples were diluted with the 75-mM sodium acetate buffer (pH 5.5). ORAC values varied from 728 to 1,372 Trolox equivalents/g tea for regular black tea and 507–618 for decaffeinated black tea. Regular green tea ORAC values varied from 1,239 to 1,686 trolox equivalents/g tea, and the ORAC values for decaffeinated green tea varied from 765 to 845 trolox equivalents/g tea (Table 5). Fig. 5 shows the correlation between the ORAC value and the catechin content of individual teas with $r = 0.79$ ($P = 0.0001$). The ORAC value of the green tea extract supplement was higher than all the green or black tea brews, whereas the iced teas showed the lowest ORAC values (Table 5).

Discussion

The antioxidant capacity of polyphenols in vivo is due to several factors: 1) radical scavenging activity, 2) metal ion-chelating effect, 3) stability of the resulting radical formed after scavenging, 4) pH sensitivity, and 5) solubility in the lipophilic phase (36). As shown by Van Acker et al. (37), the free radical scavenging activity is related to the electrochemical oxidation potential of the flavonoids. Flavonoids with the lowest electrochemical potential showed a high radical scavenging activity (36). Measurements of the structure-activity relationship by other investigators (36,37) showed that the radical scavenging activity is highest in flavonoids with either a catechol or pyrogallol group in the B ring. The additional double bond between C2-C3 and the 3-OH group enhanced the scavenging activity. The metal ion-chelating activity also depended on the catechol structure as well as the hydroxyl group in position 3 (36). In addition, Cao et al. (36) pointed out that an increase in the number of OH substitutions in the A- and B-ring corresponded to a stronger antioxidant response as determined by the ORAC assay.

The ORAC assay provides an effective way to evaluate the potential antioxidant capacity of various phytochemicals, foods, beverages, or biological samples (38). The assay used in this study measures the capacity of individual compounds or mixtures of compounds to scavenge the peroxyl radicals generated from AAPH at an elevated temperature. The order of antioxidant capacity for the different catechin standard solutions was ECG > EGCG > EC = catechin > EGC > mixed

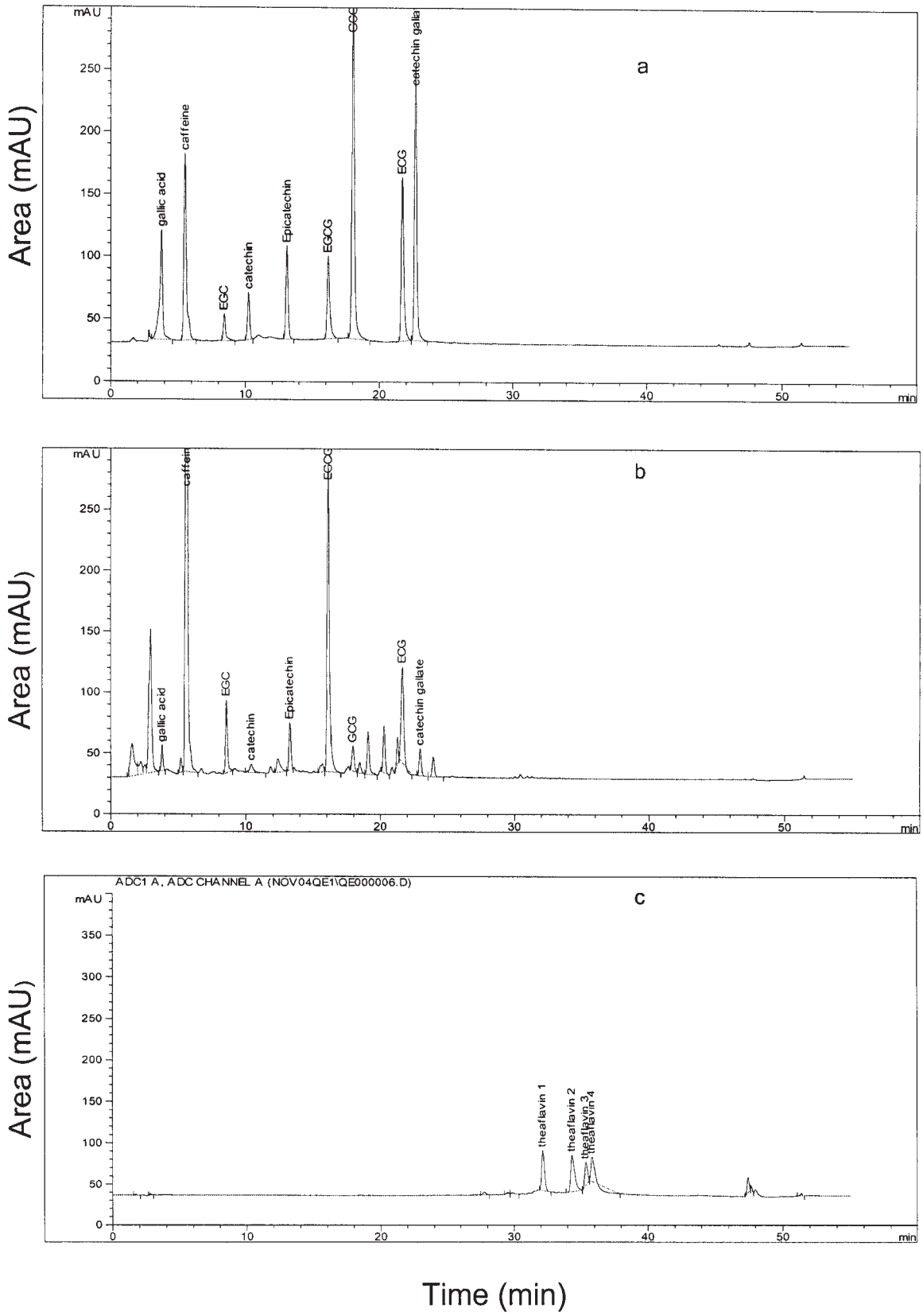

Figure 2. HPLC chromatograms of (A) catechin and caffeine standard mixture, (B) Uncle Lee's Green Tea, and (C) theaflavin standard mixture.

Table 2a. Determination of Catechin Content of 11 Black Teas and 2 Iced Teas[a]

Tea Catechin	Wissotzky Earl Grey	Bigelow Constant Comment	Bigelow English Teatime	Twinings English Breakfast Tea	Bigelow Darjeling Blend	Twinings Irish Breakfast Black Tea	Lipton Black Tea	Twinings Earl Grey Black Tea	Sweet Touch NEE Black Tea	Bigelow Constant Comment Decaf	Bigelow English Tea Time Decaf	Lipton Lemon Iced Tea	Snapple Peach Iced Tea
						mg/100 ml (= teabag)							
Gallic acid	3.3 ± 0.7	3.1 ± 0.1	6.8 ± 0.1	4.5 ± 0.4	6.4 ± 0.1	5.6 ± 0.1	6.5 ± 0.1	5.6 ± 0.2	5.6 ± 0.5	3.0 ± 0.1	4.6 ± 0.2	0.0 ± 0	0.0 ± 0
Caffeine	27.1 ± 5.1	25.3 ± 0.2	51.6 ± 1.3	45.4 ± 1.7	55.1 ± 0.1	39.4 ± 4.4	36.2 ± 1.6	31.5 ± 0.8	38.1 ± 2.3	2.7 ± 0.1	3.4 ± 0.2	2.0 ± 0	6.5 ± 0
EGC	0.0 ± 0	0.0 ± 0	14.8 ± 0.3	0.0 ± 0	11.6 ± 1.0	23.5 ± 10.3	6.2 ± 0.9	4.1 ± 2.1	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
Catechin	8.1 ± 2.0	7.2 ± 0	15.4 ± 0.6	13.1 ± 1.9	16.2 ± 0.1	3.5 ± 0.5	2.7 ± 0.3	4.4 ± 0.8	12.1 ± 0.9	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
Epicatechin	2.9 ± 0.2	4.1 ± 0.1	9.0 ± 0.1	5.2 ± 0.2	5.6 ± 0.2	2.3 ± 0	5.3 ± 0.4	5.2 ± 0.3	1.1 ± 0.3	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
EGCG	3.8 ± 1.0	6.8 ± 0	27.3 ± 0.6	10.9 ± 0.6	74.5 ± 0.8	8.1 ± 2.6	8.9 ± 0.6	10.8 ± 0.5	9.4 ± 0.6	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
GCG	2.3 ± 0.8	3.0 ± 0	2.9 ± 0.1	2.0 ± 0	8.7 ± 0.3	4.8 ± 1.9	4.2 ± 0.5	2.6 ± 0.7	3.6 ± 0.6	2.0 ± 0.2	2.4 ± 0.1	0.0 ± 0	0.0 ± 0
ECG	2.0 ± 0.5	2.7 ± 0.2	9.4 ± 0.2	4.8 ± 0.3	21.3 ± 1.2	3.8 ± 2.0	4.5 ± 0.1	5.4 ± 0.5	1.4 ± 1.7	2.0 ± 0.2	0.0 ± 0	0.0 ± 0	0.0 ± 0
Catechin gallate	3.1 ± 0.7	2.3 ± 0	2.8 ± 0	4.3 ± 0.2	4.3 ± 0	3.3 ± 0.4	3.0 ± 0	1.5 ± 0	3.7 ± 0.2	0.5 ± 0.7	1.2 ± 0.1	0.0 ± 0	0.0 ± 0
Total theaflavin	13.3 ± 4.3	9.0 ± 0.2	10.9 ± 0.7	20.1 ± 3.5	8.8 ± 0.2	10.2 ± 1.3	14.2 ± 1.0	7.3 ± 0	18.1 ± 1.1	2.2 ± 0.3	2.0 ± 0.1	0.0 ± 0	0.0 ± 0
Total catechin	20.4 ± 3.3	26.0 ± 0.4	81.6 ± 1.9	40.4 ± 0.7	148.7 ± 0.8	49.3 ± 2.9	34.8 ± 1.9	32.6 ± 3.1	31.2 ± 1.0	4.6 ± 0.3	3.6 ± 0.2	0.0 ± 0	0.0 ± 0
Total catechin + theaflavins + gallic acid	38.8 ± 9.8	38.1 ± 0.3	99.4 ± 2.7	65.0 ± 2.4	163.9 ± 0.6	65.2 ± 1.5	55.5 ± 2.8	45.6 ± 3.3	54.8 ± 2.5	9.8 ± 0.1	10.1 ± 0.5	0.0 ± 0	0.0 ± 0
Total catechin + theaflavins + gallic acid/g tea	24.3 ± 6.1	21.2 ± 0.2	43.2 ± 1.2	31.0 ± 1.2	68.3 ± 0.2	31.0 ± 0.7	23.1 ± 1.2	21.7 ± 1.6	23.8 ± 1.1	5.4 ± 0.03	4.6 ± 0.2	n/a	n/a

a: $n = 2$.

Table 2b. Determination of Catechin Content of 8 Green Teas and 1 Green Tea Extract Supplement[a]

Tea Catechin	Bigelow Green Tea	Celestial Seasoning Green Tea	Uncle Lee's Green Tea	Salada Green Tea Earl Green	Lipton Green Tea	Stash Premium Green Tea Decaf	Salada Green Tea Decaf	Celestial Seasoning Decaf Green Tea	Green Tea Supplement	Green Tea Supplement
					mg/100 ml				*per capsule*[b]	*per g powder*
Gallic acid	1.5 ± 0.1	0.6 ± 0	1.0 ± 0.1	0.8 ± 0.1	1.2 ± 0	0.7 ± 0.1	2.0 ± 0	1.8 ± 0.1	9.6 ± 0.5	27.4 ± 1.4
Caffeine	23.6 ± 1.5	33.6 ± 0.2	29.4 ± 2.7	21.8 ± 1.8	33.1 ± 0.7	5.8 ± 0.6	3.8 ± 0	0.7 ± 0	5.7 ± 0.2	16.3 ± 0.6
EGC	30.9 ± 1.5	79.7 ± 1.0	49.2 ± 2.3	38.7 ± 2.9	76.4 ± 1.8	22.0 ± 1.5	23.8 ± 0.3	22.2 ± 0.4	7.6 ± 1.5	21.7 ± 4.3
Catechin	0.0 ± 0	4.4 ± 0.1	3.6 ± 0.5	0.0 ± 0	5.8 ± 0.9	0.0 ± 0	3.4 ± 0.5	0.0 ± 0	4.7 ± 0.1	13.4 ± 0.3
Epicatechin	6.5 ± 0.4	13.3 ± 0.1	15.4 ± 1.2	7.0 ± 0.6	11.9 ± 0.1	0.0 ± 0	4.1 ± 0	2.9 ± 0	6.9 ± 0.3	19.7 ± 0.9
EGCG	42.5 ± 2.5	99.3 ± 1.8	65.0 ± 7.1	49.8 ± 3.6	83.9 ± 2.8	20.7 ± 1.8	46.3 ± 0.7	37.7 ± 0.8	100.5 ± 3.4	285.1 ± 9.7
GCG	4.1 ± 0.2	5.4 ± 0.3	4.3 ± 0.4	3.1 ± 0.3	1.1 ± 0.1	3.6 ± 0.6	6.2 ± 0.1	3.4 ± 0	52.8 ± 2.2	150.9 ± 6.3
ECG	3.6 ± 0	4.0 ± 1.6	15.9 ± 1.5	9.5 ± 0.8	13.7 ± 0.3	6.1 ± 0.6	2.0 ± 0.1	5.2 ± 0.3	25.2 ± 0.8	72.0 ± 2.3
Catechin gallate	0.0 ± 0	10.0 ± 1.2	2.4 ± 0.2	0.3 ± 0.5	3.1 ± 0.1	0.4 ± 0.5	1.1 ± 0	0.9 ± 0	7.7 ± 0.2	22.0 ± 0.6
Total catechin	87.5 ± 4.6	216.2 ± 0.5	155.7 ± 13.2	108.5 ± 8.6	196.6 ± 5.2	52.7 ± 5.0	86.8 ± 0.7	72.3 ± 0.7	205.4 ± 5.5	584.8 ± 15.7
Total catechin + gallic acid	89.0 ± 4.6	216.7 ± 0.5	156.8 ± 13.3	109.3 ± 8.7	197.8 ± 5.2	53.3 ± 5.0	88.8 ± 0.7	74.1 ± 0.6	214.9 ± 6.0	612.2 ± 17.1
Total catechin + gallic acid/g tea	59.3 ± 3.1	103.2 ± 0.3	78.4 ± 6.6	60.7 ± 4.8	82.4 ± 2.2	26.7 ± 2.5	52.2 ± 0.4	39.0 ± 0.3		

a: $n = 2$.
b: 350 mg teasolids per capsule.

Table 3. Catechin Content in Tea With Different Lot Numbers[a]

Tea Catechin	Uncle Lee's Green Tea	Lipton Green Tea	Bigelow Darjeeling Blend
Gallic acid	1.0 ± 0.2	1.3 ± 0.61	6.2 ± 0.4
Caffeine	46.5 ± 3.2	29.0 ± 2.4	67.0 ± 3.9
EGC	79.8 ± 14.5	80.2 ± 6.2	16.8 ± 0.9
Catechin	4.5 ± 1.6	4.9 ± 1.7	4.3 ± 0.4
Epicatechin	18.9 ± 0.9	16.3 ± 1.7	5.1 ± 0.4
EGCG	97.2 ± 13.0	83.3 ± 14.9	96.0 ± 7.2
GCG	7.6 ± 1.6	3.3 ± 0.9	8.0 ± 0.4
ECG	19.4 ± 3.4	10.5 ± 3.2	21.7 ± 1.6
Total catechins	230.4 ± 28.9	201.4 ± 27.2	151.9 ± 10.5

a: $n = 2$.

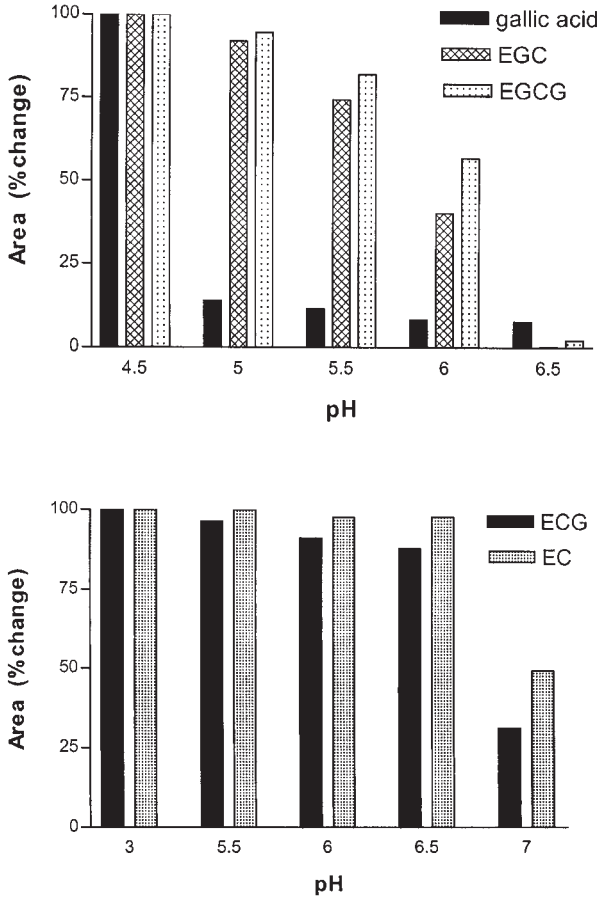

Figure 3. HPLC peak area of gallic acid, EGC, EC, EGCG, and ECG exposed to pH 3–7.

theaflavins > gallic acid. This is in good agreement with the structure-activity analysis by Van Acker et al. and Cao et al. (36,37) and with results by Salah et al. (39). The results from our study, however, indicate that epicatechin and catechin have a stronger radical scavenging potential than EGC and gallic acid. This is possibly due to the pH stability of epicatechin and catechin. As shown in Fig. 3, catechin and epicatechin are more stable in the pH range from 5 to 7 than EGC and gallic acid. The antioxidant capacity of theaflavins

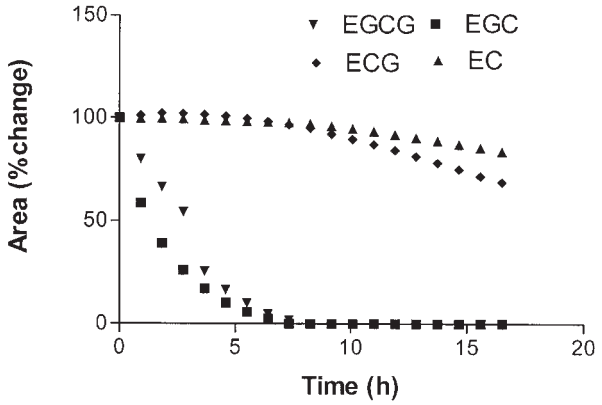

Figure 4. Kinetic change of HPLC peak area of EGC, EC, EGCG, and ECG at pH 7.

Table 4. ORAC Value of Different Catechins and Flavonoids[a]

Catechins	ORAC (mmol /mmol)	ORAC (mmol /mg)
Gallic acid	2.7	15.9
Epigallocatechin	4.6	15.0
Epicatechin	6.7	23.1
Catechin	6.1	21.0
Epicatechin gallate	10.4	23.5
Gallocatechin gallate	6.4	14.0
Epigallocatechin gallate	8.2	17.9
Quercetin	6.7	22.2
Kaempherol	2.6	9.1
Naringenin	2.4	8.8
Ascorbic acid	1.2	6.8
Caffeine	0.4	2.1

a: ORAC values are expressed as means of two determinations.

and their gallate esters has also been evaluated by Miller et al. (40) and Leung et al. (41). In these studies, however, the antioxidant capacity was measured via Cu^{2+}-mediated LDL oxidation, which is an indication of the metal ion-chelating capacity rather than their radical scavenging activity. In our study, the black tea extract theaflavin mix purchased from Sigma was ranked low compared with the other flavanols. Due to the lack of purified individual theaflavin standards, we were unable to determine the ORAC value for individual theaflavins.

The tea flavanol analysis (Tables 2a and b) showed large variations among teas from different brands. This variation was larger than the standard deviation of flavanol concentrations determined in teas of the same brand but different lot numbers (Table 3). Therefore, we concluded that the difference among brands (Tables 2a and b) is due to different production conditions and technologies of the tea companies rather than differences in production lots, shelf life, and storage conditions. The flavanol contents determined in our analyses compared well with flavanol contents published by Khokhar and Magnusdottir (47). They also found that Darjeeling tea contained a large amount of flavanols compared with other black teas.

Table 5. ORAC Values of Different Teas and a Green Tea Extract[a]

Tea Brand	Trolox Equivalent (mmol/g tea)	Total Catechin (mg/g tea)
Green Tea Supplement[b]	3461 ± 66	204.7
Celestial Seasonings Authentic Green Tea	1686 ± 47	105.7
Bigelow Green Tea	1477 ± 24	58.6
Uncle Lee's Green Tea	1477 ± 4	76.7
Lipton Black Tea	1372 ± 24	22.8
Salada Green Tea Earl Green	1250 ± 26	59.7
Lipton Green Tea	1239 ± 47	84.3
Wissotzky Earl Grey	1205 ± 58	23.6
Bigelow English Tea Time	1189 ± 42	43.8
Bigelow Darjeeling Blend	1079 ± 50	68.1
Sweet Touch NEE Black Tea	967 ± 39	23.5
Twinnings English Breakfast Tea	935 ± 33	30.0
Celestial Seasonings Decaf Mandarin Orange	845 ± 24	39.9
Twinning Irish Breakfast	811 ± 54	30.0
Snapple Peach Ice Tea[c]	790 ± 36	0
Stash Premium Green Tea Decaf	765 ± 14	26.4
Bigelow Constant Comment	757 ± 53	21.7
Twinnings Earl Grey Black Tea	728 ± 35	21.6
Bigelow Constant Comment Decaf	618 ± 33	5.4
Lipton Lemon Ice Tea[c]	609 ± 15	0
Bigelow English Tea Time Decaf	507 ± 46	4.7

a: ORAC values are expressed as means ± SD of $n = 6$ samples. Catechin concentrations are means of two measurements.
b: ORAC value per capsule (350 mg teegreen extract powder).
c: ORAC value per 100 ml of iced tea.

The ORAC values of the individual teas, determined in this study, are similar to the values obtained by Cao et al. (38). The regression analysis of the ORAC value in relation to the flavanol content of the individual teas demonstrated that the flavanol content is responsible to a large extent for the antioxidant capacity of tea. However, there are other factors such as the thearubigin and rutin content that can explain the relatively high ORAC value of some black teas with low flavanol and theaflavin content. Iced teas also represented an exception with a zero flavanol content but an ORAC value of 790 and 609 µmol/100 ml of tea. This antioxidant capacity is most likely due to other food additives with antioxidant activity in the iced tea beverages.

The large variation in flavanol content and ORAC value among different teas may be an important factor responsible for the inconsistency of epidemiological studies in regard to cancer prevention (Table 1). It appears that most reports supporting the cancer prevention effects of tea were performed in Asian countries where green tea is predominantly consumed (42). In studies conducted in European countries, where the consumption of black tea is more common, a protective effect was less frequently observed (43). The ORAC values and flavanol contents of the individual teas determined in our study support this observation. Black teas, especially decaffeinated teas, show a much larger variability in catechin content and ORAC value compared with green teas. Because epidemiological studies to this day do not account for the type and flavonoid content of different teas in their assessment of tea consumption, the outcome may differ depending on the characteristics of the particular teas consumed. More studies like the Arizona study are needed, in which tea consumption and the tea preparation were carefully evaluated using a detailed tea questionnaire. In this study a chemopreventive effect of the consumption of >1cup of hot tea in squamous cell carcinoma was determined (20).

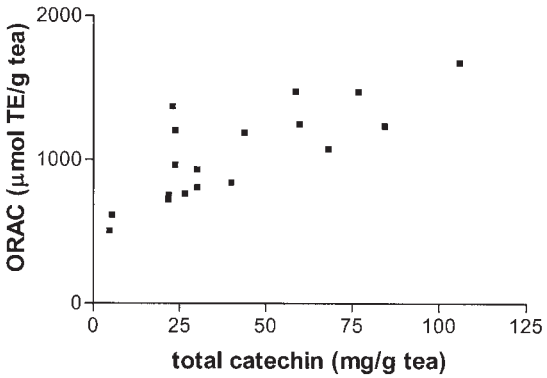

Figure 5. Correlation of ORAC value to catechin content of 18 green and black teas.

Our results confirm that the ORAC value is a good in vitro indicator of the antioxidant capacity of purified compounds and beverages. However, for the in vivo evaluation, the absorption and metabolism of flavanols have to be taken into consideration (44–46).

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References

1. Yang CS, Landau JM, Huang MT, and Newmark HL: Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu Rev Nutr* **21,** 381–406, 2001.
2. Balentine DA, Wiseman SA, and Bouwens LC: The chemistry of tea flavonoids. *Crit Rev Food Sci Nutr* **37,** 693–704, 1997.
3. Subramanian N, Venkatesh P, Ganguli S, and Sinkar VP: Role of polyphenol oxidase and peroxidase in the generation of black tea theaflavins. *J Agric Food Chem* **47,** 2571–2578, 1999.
4. Yang CS, Chung JY, Yang G, Chhabra SK, and Lee MJ: Tea and tea polyphenols in cancer prevention. *J Nutr* **130,** 472S–478S, 2000.
5. Chen Z, Zhu QY, Tsang D, and Huang Y: Degradation of green tea catechins in tea drinks. *J Agric Food Chem* **49,** 477–482, 2001.
6. Cao G and Prior RL: Measurement of oxygen radical absorbance capacity in biological samples. *Methods Enzymol* **299,** 50–62, 1999.
7. Imai K, Suga K, and Nakachi K: Cancer-preventive effects of drinking green tea among a Japanese population. *Prev Med* **26,** 769–775, 1997.

8. Zhong L, Goldberg MS, Gao YT, Hanley JA, Parent ME, et al.: A population-based case-control study of lung cancer and green tea consumption among women living in Shanghai, China. *Epidemiology* **12,** 695–700, 2001.
9. Mendilaharsu M, De Stefani E, Deneo-Pellegrini H, Carzoglio JC, and Ronco A: Consumption of tea and coffee and the risk of lung cancer in cigarette-smoking men: a case-control study in Uruguay. *Lung Cancer* **19,** 101–107, 1998.
10. Ohno Y, Wakai K, Genka K, Ohmine K, Kawamura T, et al.: Tea consumption and lung cancer risk: a case-control study in Okinawa, Japan. *Jpn J Cancer Res* **86,** 1027–1034, 1995.
11. Setiawan VW, Zhang ZF, Yu GP, Lu QY, Li YL, et al.: Protective effect of green tea on the risks of chronic gastritis and stomach cancer. *Int J Cancer* **92,** 600–604, 2001.
12. Inoue M, Tajima K, Hirose K, Hamajima N, Takezaki T, et al.: Tea and coffee consumption and the risk of digestive tract cancers: data from a comparative case-referent study in Japan. *Cancer Causes Control* **9,** 209–216, 1998.
13. Ji BT, Chow WH, Hsing AW, McLaughlin JK, Dai Q, et al.: Green tea consumption and the risk of pancreatic and colorectal cancers. *Int J Cancer* **70,** 255–258, 1997.
14. Zheng W, Doyle TJ, Kushi LH, Sellers TA, Hong CP, et al.: Tea consumption and cancer incidence in a prospective cohort study of postmenopausal women. *Am J Epidemiol* **144,** 175–182, 1996.
15. Yu GP, Hsieh CC, Wang LY, Yu SZ, Li XL, et al.: Green-tea consumption and risk of stomach cancer: a population-based case-control study in Shanghai, China. *Cancer Causes Control* **6,** 532–538, 1995.
16. Gao YT, McLaughlin JK, Blot WJ, Ji BT, Dai Q, et al.: Reduced risk of esophageal cancer associated with green tea consumption. *JNCI* **86,** 855–858, 1994.
17. Nakachi K, Suemasu K, Suga K, Takeo T, Imai K, et al.: Influence of drinking green tea on breast cancer malignancy among Japanese patients. *Jpn J Cancer Res* **89,** 254–261, 1998.
18. Shibata K, Moriyama M, Fukushima T, Kaetsu A, Miyazaki M, et al.: Green tea consumption and chronic atrophic gastritis: a cross-sectional study in a green tea production village. *J Epidemiol* **10,** 310–316, 2000.
19. Ye WM, Yi YN, Luo RX, Zhou TS, Lin RT, et al.: Diet and gastric cancer: a case control study in Fujian Province, China. *World J Gastroenterol* **4,** 516–518, 1998.
20. Hakim IA, Harris RB, and Weisgerber UM: Tea intake and squamous cell carcinoma of the skin: influence of type of tea beverages. *Cancer Epidemiol Biomarkers Prev* **9,** 727–731, 2000.
21. Zeegers MP, Dorant E, Goldbohm RA, and van den Brandt PA: Are coffee, tea, and total fluid consumption associated with bladder cancer risk? Results from the Netherlands Cohort Study. *Cancer Causes Control* **12,** 231–238, 2001.
22. Ji BT, Chow WH, Yang G, McLaughlin JK, Gao RN, et al.: The influence of cigarette smoking, alcohol, and green tea consumption on the risk of carcinoma of the cardia and distal stomach in Shanghai, China. *Cancer* **77,** 2449–2457, 1996.
23. Tsubono Y, Nishino Y, Komatsu S, Hsieh CC, Kanemura S, et al.: Green tea and the risk of gastric cancer in Japan. *N Engl J Med* **344,** 632–636, 2001.
24. Goldbohm RA, Hertog MG, Brants HA, van Poppel G, and van den Brandt PA: Consumption of black tea and cancer risk: a prospective cohort study. *JNCI* **88,** 93–100, 1996.
25. Michels KB, Holmberg L, Bergkvist L, and Wolk A: Coffee, tea, and caffeine consumption and breast cancer incidence in a cohort of Swedish women. *Ann Epidemiol* **12,** 21–26, 2002.
26. Nagano J, Kono S, Preston DL, and Mabuchi K: A prospective study of green tea consumption and cancer incidence, Hiroshima and Nagasaki (Japan). *Cancer Causes Control* **12,** 501–508, 2001.
27. Zeegers MP, Tan FE, Goldbohm RA, and van den Brandt PA: Are coffee and tea consumption associated with urinary tract cancer risk? A systematic review and meta-analysis. *Int J Epidemiol* **30,** 353–362, 2001.
28. Villeneuve PJ, Johnson KC, Kreiger N, and Mao Y: Risk factors for prostate cancer: results from the Canadian National Enhanced Cancer Surveillance System. The Canadian Cancer Registries Epidemiology Research Group. *Cancer Causes Control* **10,** 355–367, 1999.
29. Cerhan JR, Putnam SD, Bianchi GD, Parker AS, Lynch CF, et al.: Tea consumption and risk of cancer of the colon and rectum. *Nutr Cancer* **41,** 33–40, 2001.
30. La Vecchia C, Negri E, Franceschi S, D'Avanzo B, and Boyle P: Tea consumption and cancer risk. *Nutr Cancer* **17,** 27–31, 1992.
31. Bianchi GD, Cerhan JR, Parker AS, Putnam SD, See WA, et al.: Tea consumption and risk of bladder and kidney cancers in a population-based case-control study. *Am J Epidemiol* **151,** 377–383, 2000.
32. Terry P and Wolk A: Tea consumption and the risk of colorectal cancer in Sweden. *Nutr Cancer* **39,** 176–179, 2001.
33. Woolcott CG, King WD, and Marrett LD: Coffee and tea consumption and cancers of the bladder, colon and rectum. *Eur J Cancer Prev* **11,** 137–145, 2002.
34. Tavani A, Gallus S, Dal Maso L, Franceschi S, Montella M, et al.: Coffee and alcohol intake and risk of ovarian cancer: an Italian case-control study. *Nutr Cancer* **39,** 29–34, 2001.
35. Tavani A, Pregnolato A, La Vecchia C, Negri E, Talamini R, et al.: Coffee and tea intake and risk of cancers of the colon and rectum: a study of 3,530 cases and 7,057 controls. *Int J Cancer* **73,** 193–197, 1997.
36. Cao G, Sofic E, and Prior RL: Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radic Biol Med* **22,** 749–760, 1997.
37. van Acker SA, van den Berg DJ, Tromp MN, Griffioen DH, van Bennekom WP, et al.: Structural aspects of antioxidant activity of flavonoids. *Free Radic Biol Med* **20,** 331–342, 1996.
38. Cao G, Sofic E, and Prior RL: Antioxidant capacity of tea and common vegetables. *J Agric Food Chem* **44,** 3426–3431, 1996.
39. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, et al.: Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch Biochem Biophys* **322,** 339–346, 1995.
40. Miller NJ, Castelluccio C, Tijburg L, and Rice-Evans C: The antioxidant properties of theaflavins and their gallate esters—radical scavengers or metal chelators? *FEBS Lett* **392,** 40–44, 1996.
41. Leung LK, Su Y, Chen R, Zhang Z, Huang Y, et al.: Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *J Nutr* **131,** 2248–2251, 2001.
42. Bushman JL: Green tea and cancer in humans: a review of the literature. *Nutr Cancer* **31,** 151– 159, 1998.
43. Blot WJ, McLaughlin JK, and Chow WH: Cancer rates among drinkers of black tea. *Crit Rev Food Sci Nutr* **37**, 739–760, 1997.
44. Lee MJ, Wang ZY, Li H, Chen L, Sun Y, et al.: Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol Biomarkers Prev* **4**, 393–399, 1995.
45. Warden BA, Smith LS, Beecher GR, Balentine DA, and Clevidence BA: Catechins are bioavailable in men and women drinking black tea throughout the day. *J Nutr* **131,** 1731–1737, 2001.
46. Scalbert A and Williamson G: Dietary intake and bioavailability of polyphenols. *J Nutr* **130,** 2073S–2085S, 2000.
47. Khokhar S and Magnusdottir SGM: Total phenol, catechin and caffeine contents of teas commonly consumed in the United Kingdom. *J Agric Food Chem* **50,** 565–570, 2002.