

# Antioxidant Activity of Green Teas in Different Lipid Systems

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**ABSTRACT:** Different commercial green teas from Japan, China, and India, were compared in different lipid systems. Green teas were active antioxidants in bulk corn oil oxidized at 50°C but were prooxidant in the corresponding oil-in-water emulsions. Green teas also were active antioxidants in soybean lecithin liposomes oxidized at 37°C in the presence of cupric acetate as catalyst. At 50°C, however, three of the samples of green tea were active antioxidants in the absence of copper catalyst, and two samples showed prooxidant activity in the presence of copper catalyst. The marked variation in activity among green tea samples may be due partly to differences in their relative partition between phases in different lipid systems. The improved antioxidant activity observed for green teas in lecithin liposomes compared to corn oil emulsions can be explained by the greater affinity of the polar tea catechin gallates for the polar surface of the lecithin bilayers, thus affording better protection against oxidation. Liposomes may thus be appropriate lipid models to evaluate antioxidants for foods containing phospholipids.

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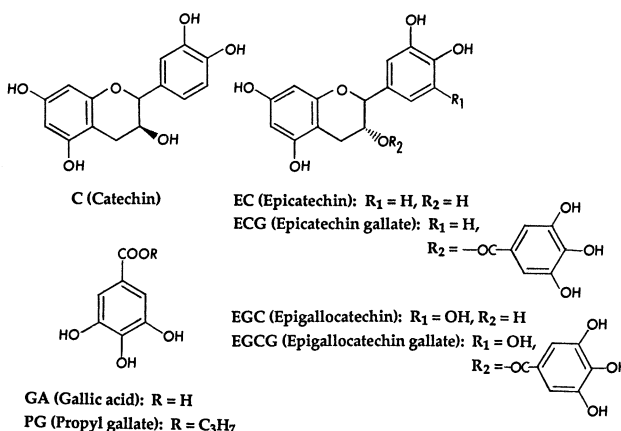
**KEY WORDS:** Antioxidant mechanism, antioxidants, catechin, emulsion, green tea, hexanal, hydroperoxides, liposome, prooxidants, tea catechins.

Green tea catechins have attracted much attention because of their physiological effects, including antimutagenic and antitumorogenic activities (1). The hydrophilic catechol-type flavonoids of green tea were more effective in inhibiting oxidation of liposomes composed of egg phosphatidylcholine than was the lipophilic  $\alpha$ -tocopherol (2). However, kinetic studies showed that the flavonoids had lower activity for the inhibition of oxidation of methyl linoleate solution than did  $\alpha$ -tocopherol. The marked differences in activity between the hydrophilic catechins and the lipophilic  $\alpha$ -tocopherol may be due to their relative partition between the water phase and the surface environment of the phospholipid bilayers. This interfacial phenomenon was used to explain the contrasting properties of polar and nonpolar antioxidants in bulk oil vs. oil-in-water emulsion systems (3).

Green tea extracts had antioxidant activity in vegetable oils and animal fats (4–6). The major antioxidant components

of green tea extracts included (+)-catechin, (+)-gallocatechin, (–)-epicatechin (EC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), and (–)-epigallocatechin gallate (EGCG) (Scheme 1). Crude green tea catechin powders were more effective than  $\alpha$ -tocopherol and butylated hydroxyanisole in lard under conditions of the active oxygen method at 97.8°C (5). The antioxidant indexes of green tea extracts correlated with their EGCG contents based on induction time measured by the Rancimat method with a ternary mixture containing lecithin, tocopherols, and propyleneglycol in chicken fat (7). The ethanol extracts of green teas strongly inhibited the oxidation of canola oil at 100°C, as shown by measuring oxygen consumption, whereas extracts of black teas showed little or no antioxidant activity (8).

The conditions of accelerated oxidation used in these studies evaluating the effectiveness of tea extracts and catechins in edible oils and fats have been too drastic and may be questionable because of the elevated temperatures used, and the degree of oxidation measured may not be relevant to oxidative deterioration in food systems (9,10). Also, the lipid systems used as substrates contained natural antioxidants or tocopherols which interfered with the antioxidant evaluations of tea catechins. Extracts of rosemary, sage, thyme, and green tea prepared by a mechanical procedure with propylene glycol as a carrier were shown to be effective as antioxidants when added in a concentration of 1% and evaluated by the Rancimat method at 110°C in different fats, and by an oxygen electrode method at 37°C in



SCHEME 1

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oil-in-water emulsions (11). We showed previously that natural antioxidants varied in activity in different lipid systems because they were affected by their physical state (3,12,13), by different lipid substrates (14,15), and by pH (16), and according to the methods used to evaluate lipid oxidation (3,13). It is therefore important to use different lipid systems and more than one method to evaluate natural antioxidant activity (9,10).

In the present study, different green teas from Japan, China, and India were compared in bulk tocopherol-stripped corn oil and corresponding oil-in-water emulsions, and in soybean lecithin liposomes oxidized at 50 or 37°C, in the presence or absence of copper acetate as catalyst. The antioxidant effectiveness of green teas was evaluated at different stages of oxidation by measuring the formation of hydroperoxides on the basis of conjugated dienes, and the decomposition of hydroperoxides on the basis of hexanal.

## MATERIALS AND METHODS

**Materials.** Corn oil triglycerides stripped of tocopherols were used as the bulk oil (13). Soybean lecithin and Tween 20 (polyoxyethylene sorbitan monolaurate) were obtained from Sigma Chemical Co. (St. Louis, MO). Nikken tea extract powder (NTEP) from Nikken Foods Co. Ltd. (Tokyo, Japan) and green tea catechin powder (GTCP), a spray-dried aqueous extract of Japanese Sencha green tea prepared according to EP 456 023 (17), were a gift from T.L. Lunder Nestec Ltd. (Lausanne, Switzerland). The tea extracts in liquid form were prepared by a purely mechanical procedure, with propylene glycol as carrier (18), from Chinese green tea (CGTE) (Kentea Ltd., New Rochelle, NY), and Indian green tea (IGTE) (Nestlé India Ltd., Cherambadi, India). Soybean phosphatidylcholine and (±)-catechin, used as standard reference, were obtained from Sigma Chemical Co. Catechin standards for high-performance liquid chromatography analysis were obtained from Fluka Chemie AG (Buchs, Switzerland) and Carl Roth GmbH + Co. (Karlsruhe, Germany).

**High-performance liquid chromatography (HPLC) analyses.** The compositions of tea extracts were determined by HPLC using a Merck L6200 A chromatograph (Merck Co., Geneva, Switzerland), with ultraviolet detection at 280 nm (JASCO UV 970; Jasco Co., Commugny, Switzerland), a TSK gel ODS 80<sup>TM</sup> column: 25 cm × 4.6 mm (Toso Haas, Stuttgart, Germany), particle size 5 µm, and a precolumn: cartridge LiChroCART 4-4, Lichrospher 100 C18-5 endcapped (Merck Co.). Two mobile phases were used: solvent A: 0.5% phosphoric acid in water, and solvent B: 0.5% phosphoric acid in acetonitrile. The following elution profile was used: 0–45 min: linear gradient of 10 to 25% solvent B with solvent A; 45–55 min: isocratic elution with 25% solvent B and 75% solvent A; 55–60 min: linear gradient of 25 to 10% solvent B with solvent A; 60–65 min: conditioning with 10% solvent B and 90% solvent A. Other conditions: flow rate of 1.0 mL/min; temperature 40°C; injection: 20 µL solution. The HPLC analyses of the green tea samples expressed in percentage weight of total sample weight are shown in Table 1. Analyses for chlorophylls

**TABLE 1**  
**Composition of Green Tea Samples Determined by High-Performance Liquid Chromatography (wt%)<sup>a</sup>**

Components	NTEP <sup>b</sup>	GTCP <sup>b</sup>	CGTE <sup>b,c</sup>	IGTE <sup>b,c</sup>
Epigallocatechin + catechin	3.8	5.3	4.5	6.7
Caffeine	8.1	4.4	1.3	1.2
Epicatechin	6.7	6.8	0.4	0.5
Epigallocatechin gallate	23.5	29.7	3.0	3.5
Epicatechin gallate	5.2	5.3	1.2	1.7
Total catechins	39.2	47.1	9.0	12.4

<sup>a</sup>See the Materials and Methods section for description of procedures.

<sup>b</sup>NTEP = Nikken tea extract powder; GTCP = green tea catechin powder; CGTE = Chinese green tea liquid extract; IGTE = Indian green tea liquid extract.

<sup>c</sup>Tea extract/propylene glycol (1:1, w/w).

showed the absence of chlorophyll *a* or chlorophyll *b* in all tea samples, but the liquid extracts contained trace amounts of pheophytin (0.0034 mg/g for CGTE, and 0.0073 mg/g for IGTE) (personal communication, M.H. Benoist, Nestlé Research Center, Lausanne, Switzerland).

**Preparation of bulk oil, emulsion, and liposome samples.** Corn oil samples (6 g) were placed in screw-capped 25-mL Erlenmeyer flasks. Various concentrations of green tea powders (NTEP and GTCP) were added directly, whereas the liquid samples (CGTE and IGTE) were purged under nitrogen before adding to the oil. The green tea samples, and cupric acetate when used as a catalyst, were dissolved in corn oil by heating to 50°C for 10 min.

Oil-in-water emulsions (10%, 30 g) were prepared as described previously (14). To evaluate the effect of metal ions on antioxidant activity, 10 µL of 30 mM cupric acetate was added to some of these emulsion samples to a final concentration of 10 µM.

Liposome samples containing 10% lecithin were prepared with or without various concentrations of tea extracts, and 10 µM cupric acetate. Lecithin (2.4 g) was suspended in deionized water at a concentration of 8 mg/mL by stirring with a glass rod and sonicating for 5 min (Bransonic bath-type sonicator, Model 12; Branson Ultrasonic Corporation, Danbury, CT). The particle size of liposome was between 0.03–0.1 µm (Microtrac ultrafine particle analyzer; Leeds & Northrup, North Wales, PA). All liposome samples (30 mL) were sonicated for 2 min before adding 10 µL of 30 mM cupric acetate.

**Oxidation.** Oxidations were carried out in a shaker oven (Lab-Line Instrument, Inc., Melrose Park, IL) at 50°C. The liposome samples also were oxidized in the presence of copper acetate at 37°C in a shaker water bath (New Brunswick Scientific Co., Inc., Edison, NJ). The oxidative stability of these samples was determined by measuring conjugated diene hydroperoxides spectrophotometrically and hexanal by headspace gas chromatography (GC). All analyses were done in duplicate, and the results were calculated by one-way analysis of variance (19).

**Measurement of conjugated diene hydroperoxides.** Conjugated dienes were determined in oil samples according to the same procedures described previously (3). For emulsions and liposomes, samples (0.1 g) were dispersed in 5 mL methanol.

**TABLE 2**  
**Inhibition of Hydroperoxide and Hexanal Formation by Green Teas in Bulk Corn Oil Triglycerides at 50°C in the Absence or Presence of Copper<sup>a,b,c</sup>**

Samples <sup>d</sup>	Hydroperoxides		Hexanal	
	(6 d)	(8 d)	(6 d)	(8 d)
Control	0.0 ± 1.3 e	0.0 ± 0.7 d	0.0 ± 0.4 d	0.0 ± 7.2 c
NTEP (98 ppm)	-28.4 ± 0.6 f	-8.0 ± 0.8 e	7.1 ± 14 c, d	9.6 ± 0.3 b, c
GTCP (112 ppm)	46.2 ± 0.8 c	37.3 ± 0.5 b	29.2 ± 10 b, c	4.2 ± 3.5 c
CGTE (120 ppm)	31.3 ± 1.9 d	22.2 ± 0.8 c	44.1 ± 3.0 b	35.4 ± 4.5 b
IGTE (155 ppm)	52.2 ± 1.5 b	38.4 ± 0.5 b	3.1 ± 9.3 c, d	5.6 ± 18 c
Catechin (125 ppm)	88.4 ± 0.3 a	92.9 ± 0.5 a	29.2 ± 1.6 a	92.2 ± 1.1 a
	(4 d)	(6 d)	(4 d)	(6 d)
Control + Cu <sup>2+</sup>	0.0 ± 1.2 d	0.0 ± 0.8 e	0.0 ± 3.3 e	0.0 ± 4.9 e
NTEP (98 ppm)	-11.3 ± 2.1 e	7.6 ± 0.8 d	36.1 ± 1.2 d	22.9 ± 1.7 d
GTCP (112 ppm)	-44.4 ± 1.1 d	-1.4 ± 0.6 e	47.9 ± 0.6 c	22.4 ± 1.7 d
CGTE (120 ppm)	52.6 ± 0.1 c	26.5 ± 0.4 c	67.6 ± 2.0 b	42.5 ± 3.3 c
IGTE (155 ppm)	61.7 ± 0.4 b	40.7 ± 0.2 b	65.9 ± 0.5 b	62.4 ± 2.6 b
Catechin (125 ppm)	93.0 ± 0.3 a	96.0 ± 0.2 a	92.8 ± 0.1 a	94.9 ± 0.5 a

<sup>a</sup>Values represented as mean percentage inhibition ± SD, where percentage inhibition = [(C - S)/C] × 100, and C = hydroperoxide or hexanal formed in control and S = hydroperoxides or hexanal formed in sample. Negative values represent prooxidant activity. SD, calculated for n = 2.

<sup>b</sup>Values within each column followed by the same letter are not significantly different (P < 0.05).

<sup>c</sup>10 μM cupric acetate.

<sup>d</sup>Total catechin concentrations in ppm. See Table 1 for composition of teas and abbreviations.

The absorbance was measured at 234 nm, and results were calculated as hydroperoxides in mmole/kg of oil.

*Measurement of hexanal by static headspace GC.* The same procedure was used for hexanal measurements as described previously (3), except that the samples were equilibrated at 60°C for 15 min before GC analyses.

## RESULTS

*Bulk corn oil triglycerides.* EGCG is a major catechin in the four tea extracts used (Table 1). The green tea powders NTEP and GTCP were compared at 250 ppm, whereas the liquid green teas CGTE and IGTE were compared at 1250 ppm because they contained lower amounts of total catechins. Pure (±)-catechin was tested at 125 ppm and used as a reference.

On the basis of hydroperoxide formation at 50°C, IGTE at 1250 ppm (155 ppm as total catechins) and GTCP at 250 ppm (112 ppm as total catechins) had the same antioxidant activity and were more effective than CGTE at 1250 ppm (120 ppm as total catechins) (Table 2). In contrast, NTEP at 250 ppm (98 ppm as total catechins) acted as a prooxidant by promoting hydroperoxide formation. Pure (±)-catechin at 125 ppm was the most active antioxidant. After 6 d of oxidation, IGTE was significantly more active than GTCP in inhibiting hydroperoxides, but after 8 d of oxidation they were not significantly different. Although GTCP contained a lower concentration of total catechins than IGTE, their antioxidant activities were similar and better than that of CGTE at comparable concentrations. In the presence of the copper catalyst, IGTE was the most effective antioxidant, followed by CGTE, NTEP and GTCP (Table 2). However, GTCP promoted hydroperoxide formation initially

during the first 6 d of oxidation, and NTEP during the first 4 d of oxidation.

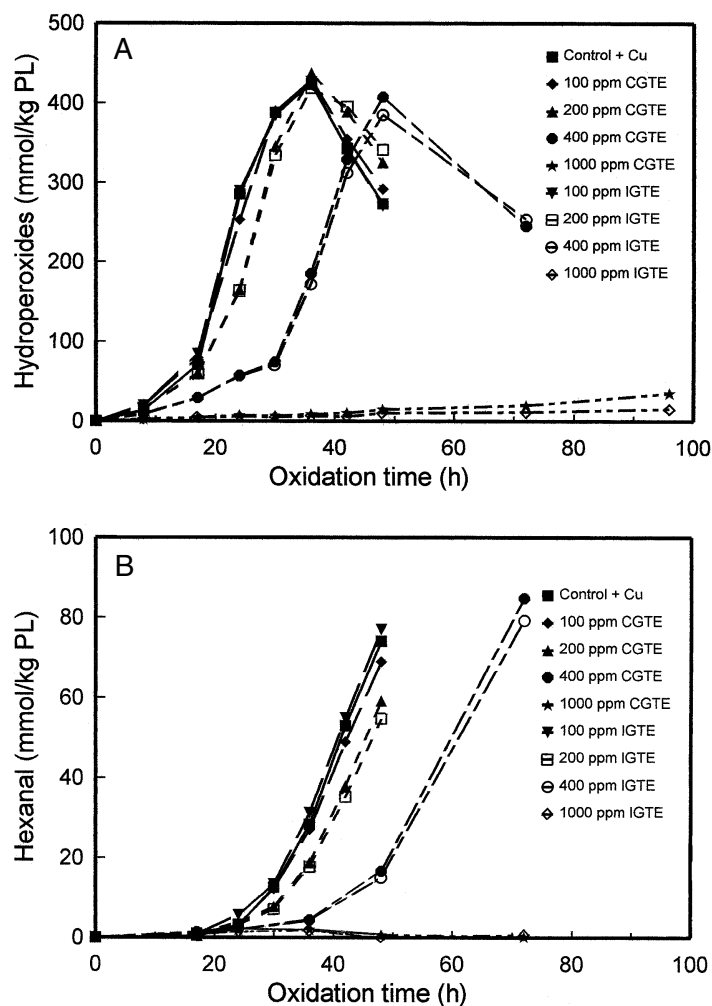
On the basis of hexanal formation, CGTE was the most active tea sample during the entire period of oxidation whereas GTCP showed the lowest activity, which decreased significantly after 8 d of oxidation. Pure (±)-catechin was the most effective inhibitor of hexanal. Tea samples IGTE and NTEP showed no significant activity, and IGTE increased hexanal formation after 8 d of oxidation. After 6 d of oxidation, the following trend was obtained on the basis of hexanal formation: CGTE > GTCP > NTEP > IGTE. In the presence of copper

**TABLE 3**  
**Inhibition of Hydroperoxide and Hexanal Formation by Tea Extracts in Oil-in-Water Emulsions at 50°C in the Absence or Presence of Copper<sup>a,b,c</sup>**

Samples <sup>d</sup>	Hydroperoxides <sup>e</sup>	Hexanal <sup>e</sup>
NTEP (9.8 ppm)	-42 ± 1 b	-23 ± 3 b
NTEP (19.6 ppm)	32 ± 1 a	35 ± 1 a
GTCP (11.8 ppm)	-135 ± 2 e	-113 ± 1 f
GTCP (23.6 ppm)	-45 ± 6 b	-46 ± 1 c
CGTE (11.3 ppm)	-171 ± 8 f	-120 ± 9 f
CGTE (22.5 ppm)	-66 ± 2 c	-53 ± 1 d
IGTE (15.5 ppm)	-145 ± 2 e	-93 ± 2 e
IGTE (31 ppm)	-101 ± 3 d	-47 ± 2 c
NTEP (9.8 ppm) + Cu <sup>2+</sup>	-555 ± 9 b	-735 ± 39 f
NTEP (19.6 ppm) + Cu <sup>2+</sup>	-88 ± 5 a	-29 ± 2 a
GTCP (11.8 ppm) + Cu <sup>2+</sup>	-636 ± 7 c	-517 ± 12 d
GTCP (23.6 ppm) + Cu <sup>2+</sup>	-75 ± 7 a	-115 ± 1 b
CGTE (11.3 ppm) + Cu <sup>2+</sup>	-783 ± 19 e	-641 ± 25 e
CGTE (22.5 ppm) + Cu <sup>2+</sup>	-642 ± 23 c	-331 ± 33 c
IGTE (15.5 ppm) + Cu <sup>2+</sup>	-722 ± 24 d	-553 ± 3 d
IGTE (31 ppm) + Cu <sup>2+</sup>	-554 ± 23 b	-346 ± 3 c

<sup>a-d</sup>See corresponding footnotes in Table 2.

<sup>e</sup>Determinations on day 2.



**FIG. 1.** Effect of tea extracts CGTE and IGTE on oxidative stability of soybean lecithin liposomes in the presence of 10  $\mu$ M cupric acetate at 37°C: (A) hydroperoxides and (B) hexanal. Abbreviations: CGTE, Chinese green tea extract; IGTE, Indian green tea extract. Units: mmol/kg phospholipids (PL).

catalyst, all green tea samples were effective antioxidants in inhibiting hexanal formation. After 4 and 6 d of oxidation IGTE and CGTE were better antioxidants than NTEP and GTCP.

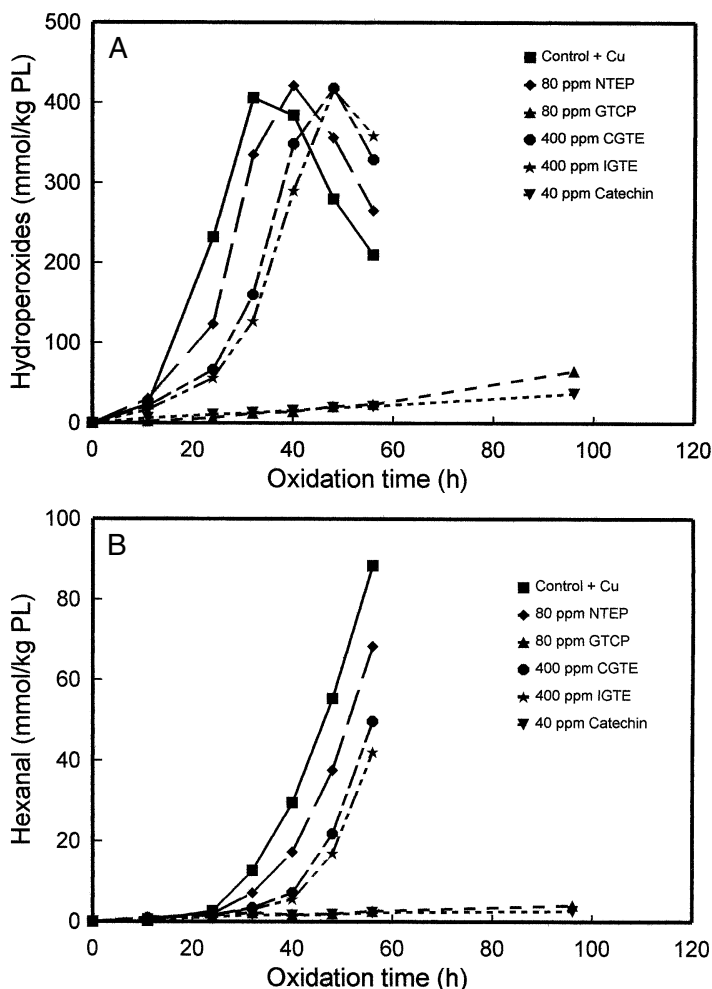
**Oil-in-water emulsions.** On the basis of both hydroperoxide and hexanal formation at 50°C, NTEP showed weak antioxidant activity at 50 ppm (30% inhibition) but acted as a prooxidant at 25 ppm (Table 3). Tea samples GTCP also showed prooxidant activity at 25 and 50 ppm, CGTE and IGTE at 125 and 250 ppm. In the presence of a copper catalyst, all the tea extracts showed prooxidant activity, which decreased with increased concentration. The concentration effects of NTEP and GTCP were greater than those of CGTE and IGTE.

**Soybean lecithin liposomes.** At 50°C, NTEP and GTCP had similar hydroperoxide-inhibiting activity at 4 ppm (1.6 ppm NTEP and 1.9 ppm GTCP as total catechins) after 4 and 7 d of oxidation (Table 4). Their inhibition was greater than that of ( $\pm$ )-catechin at 1.6 ppm but lower than that of IGTE at 20 ppm (2.5 ppm as total catechins) after 7 d of oxidation. Sample

CGTE acted as a prooxidant at 20 ppm (1.8 ppm of total catechins). In the presence of a copper catalyst, green tea samples were less effective and only NTEP inhibited hydroperoxide formation after 6 h of oxidation. Pure ( $\pm$ )-catechin had no activity in liposomes in the presence of copper catalyst.

On the basis of hexanal formation, GTCP was more active than NTEP at 4 ppm after 4 d of oxidation, but not after 7 d (Table 4). At 4 d, tea samples NTEP and GTCP were better at 4 ppm than pure ( $\pm$ )-catechin at 1.6 ppm, but at 20 ppm IGTE was the best antioxidant and CGTE promoted hexanal formation. In the presence of copper ions, only NTEP was active in inhibiting hexanal formation at 4 ppm, and at 6 and 11 h, sample GTCP had no activity, but the other tea samples and pure ( $\pm$ )-catechin promoted hexanal formation at the concentrations tested.

At 37°C in the presence of cupric acetate, CGTE inhibited hydroperoxide and hexanal formation in a dose-dependent way from 100 to 1000 ppm (9 to 90 ppm as total catechins) (Fig. 1 and Table 5). Tea sample IGTE was not active at 100



**FIG. 2.** Effect of tea extracts on oxidative stability of soybean lecithin liposomes in the presence of 10 μM cupric acetate at 37°C. (A) Hydroperoxides and (B) hexanal. Abbreviations: NTEP, Nikkon tea extract powder; GTCP, green tea catechin powder. See Figure 1 for other abbreviations.

ppm, but showed increasing activity between 200 and 1000 ppm (25 and 124 ppm as total catechins). The difference in activity between both extracts was small at 400 ppm (36 ppm CGTE and 50 ppm IGTE as total catechins). Initially, the activity of GTCP at 80 ppm (38 ppm as total catechins) was similar to that of pure (±)-catechin at 40 ppm, but decreased after 56 h (Fig. 2). Tea sample NTEP was a weaker antioxidant at 80 ppm (31 ppm as total catechins) compared to other tea samples at the concentrations tested.

In the presence of copper catalyst at 50°C, green teas and pure catechin were effective antioxidants in bulk corn oil but not in liposomes. On the other hand, at 37°C the tea samples and pure catechin were effective antioxidants in the presence of a copper catalyst.

## DISCUSSION

To clarify the action of green teas as antioxidants, several test systems were used in this study to evaluate their activity. The

antioxidant activity of green teas, like other natural antioxidants, were very system-dependent, and a wide range of activities was observed according to the lipid systems used as substrates.

The four tea extracts were less effective in inhibiting the formation of hydroperoxides and hexanal than was pure (±)-catechin in corn oil. The antioxidant activity of the different tea extracts tested was not related to their concentrations of total catechins. Thus, although sample GTCP contained a lower concentration of total catechins than IGTE, their inhibition of hydroperoxides was similar in the absence of copper, but GTCP showed prooxidant activity in the presence of copper. The lower activity of tea extracts than pure catechin may be due to other substances in the extracts or to possible antagonistic effects of other tea components. Although the tea extracts had no chlorophyll, they contained trace amounts of pheophytins that may act as a photosensitizers. The presence of traces of metals can initiate lipid oxidation. The effect of caffeine in the tea extracts is unknown.

**TABLE 4**  
**Inhibition of Hydroperoxide and Hexanal Formation by Green Teas in Soybean Lecithin Liposomes at 50°C in the Absence or Presence of Copper<sup>a,b,c</sup>**

Samples <sup>d</sup>	Hydroperoxides		Hexanal	
	(4 d)	(7 d)	(4 d)	(7 d)
Control	0.0 ± 3.0 c	0.0 ± 2.5 d	0.0 ± 0.9 d	0.0 ± 2.6 c
NTEP (1.6 ppm)	59.7 ± 0.8 a	80.1 ± 0.4 b	14.6 ± 0.1 b	86.4 ± 0.1 a
GTCP (1.9 ppm)	59.1 ± 0.1 a	79.6 ± 0.4 b	29.2 ± 10 a,b	4.2 ± 3.5 b
CGTE (1.8 ppm)	-79.6 ± 3.0 d	n.d.	-39.4 ± 1.2 f	19.6 ± 3.5 d
IGTE (2.5 ppm)	66.1 ± 0.8 a	89.3 ± 0.3 a	36.7 ± 1.0 a	91.5 ± 0.1 a
Catechin (1.6 ppm)	37.1 ± 2.3 b	37.0 ± 1.2 c	-5.6 ± 1.0 e	69.1 ± 0.3 b
	(6 h)	(11 h)	(6 h)	(11 h)
Control + Cu <sup>2+</sup>	0.0 ± 2.7 b	0.0 ± 0.6 b,c	0.0 ± 1.4 b	0.0 ± 0.8 b,c
NTEP (1.6 ppm)	13.9 ± 2.1 a	-0.8 ± 1.6 b,c	21.6 ± 1.6 a	20.2 ± 1.8 a
GTCP (1.9 ppm)	-4.6 ± 0.8 b,c	-2.2 ± 0.6 c	-6.1 ± 0.4 c	3.6 ± 1.6 b
CGTE (1.8 ppm)	-8.9 ± 1.1 c	2.3 ± 2.4 a,b	-35.8 ± 1.0 e	-13.9 ± 1.3 d
IGTE (2.5 ppm)	-3.3 ± 1.2 b,c	3.2 ± 0.3 a,b	-19.8 ± 0.1 d	-2.2 ± 1.7 c
Catechin (1.6 ppm)	0.2 ± 3.4 b	6.3 ± 0.1 a	-47.1 ± 2.5 e,f	-15.5 ± 0.3 d

<sup>a-d</sup>See corresponding footnotes in Table 2. Abbreviation: n.d., not determined.

The green teas were active antioxidants in bulk corn oil and in lecithin liposomes oxidized at 37°C in the presence of a copper catalyst, but were prooxidant in corn oil-in-water emulsions oxidized at 50°C. The green teas were, however, less effective at 50°C in the presence of copper. In contrast to bulk corn oil triglycerides, one green tea sample (GTCP) tested was as effective as pure (±)-catechin in the lecithin liposome system.

The improved antioxidant activity observed for green teas in lecithin liposomes compared to corn oil emulsions can be explained by the greater affinity of the polar catechin gallates (Scheme 1) with the polar surface of the lecithin bilayers, thus affording better protection against oxidation. Liposomes may thus be appropriate lipid models to evaluate antioxidants for both foods and lipoprotein particles containing phospholipids.

It is now becoming evident that the antioxidant activity of natural antioxidants is very system-dependent, and a wide

range of activities can be observed according to the lipid systems used as substrates (10,14,15). Green tea extracts behaved like other very hydrophilic antioxidants such as Trolox (a water-soluble analog of  $\alpha$ -tocopherol) and ascorbic acid in showing poor antioxidant activity in oil-in-water emulsions, but very good antioxidant activity in bulk oils (3). (±)-Catechin was shown to be more hydrophilic than Trolox in the absence of buffers (20). In emulsions, Trolox partitioned into the water phase, oil-water interfaces, and Tween 20 micelles, and its concentration in the oil phase was reduced to protect lipids from oxidation (14). Similarly, the hydrophilic tea catechins in green teas would also be expected to partition into the water phase in emulsions and become less protective. In the present study, we showed that green teas have good antioxidant activity in lecithin liposomes. We can explain these results by the interaction of the polar tea catechins with the polar environment of lecithin liposomes, which thus affords better protection against oxidation. However, the antioxidant activity of some tea samples was not directly related to their catechin composition determined by HPLC. More information is needed on the noncatechin components of tea extracts to better explain their relative antioxidant activities.

**TABLE 5**  
**Inhibition of Hydroperoxide and Hexanal Formation by Green Teas in Soybean Lecithin Liposomes at 37°C in the Presence of Copper<sup>a,b,c</sup>**

Samples <sup>d</sup>	Hydroperoxides	Hexanal
	(24 h)	(32 h)
Control + Cu <sup>2+</sup>	0.0 ± 0.1 e	0.0 ± 1.2 e
100 ppm CGTE	11.5 ± 0.4 d	4.8 ± 1.9 d
200 ppm CGTE	42.2 ± 0.3 c	34.1 ± 1.4 c
400 ppm CGTE	80.0 ± 0.2 b	84.8 ± 0.2 b
1000 ppm CGTE	97.6 ± 0.2 a	92.9 ± 0.1 a
100 ppm IGTE	-1.4 ± 0.6 e	-10.4 ± 1.5 f
200 ppm IGTE	42.7 ± 0.5 c	37.3 ± 0.2 c
400 ppm IGTE	80.3 ± 0.1 b	84.6 ± 0.4 b
1000 ppm IGTE	98.0 ± 0.1 a	94.1 ± 0.1 a
Control + Cu <sup>2+</sup>	0.0 ± 0.7 e	0.0 ± 1.3 e
80 ppm NTEP	46.7 ± 0.1 d	44.4 ± 0.3 d
80 ppm GTCP	97.1 ± 0.3 a	82.9 ± 0.6 b
400 ppm CGTE	71.5 ± 0.5 c	73.7 ± 0.1 c
400 ppm IGTE	76.0 ± 0.4 b	76.0 ± 0.5 c
400 ppm Catechin	95.5 ± 0.4 a	86.3 ± 0.2 a

<sup>a-d</sup>See corresponding footnotes in Table 2.

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## REFERENCES

- Hara, Y., Prophylactic Functions of Tea Polyphenols, in *Food Phytochemicals for Cancer Prevention II. Teas, Spices, and Herbs*, edited by C.-T. Ho, T. Osawa, M.T. Huang, and R.T. Rosen, American Chemical Society, Washington, DC, 1994, pp. 34-50.
- Terao, J., M. Piskula, and Q. Yao, Protective Effect of Epicatechin, Epicatechin Gallate, and Quercetin on Lipid Peroxidation in Phospholipid Bilayers, *Arch. Biochem. Biophys.* 308:278-284 (1994).

3. Frankel, E.N., S.-W. Huang, J. Kanner, and J.B. German, Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils vs. Emulsions, *J. Agr. Food Chem.* 42:1054–1059 (1994).
4. Das, D.N., J.J. Ghosh, K.C. Bhattacharyya, and B.C. Guha, Tea. II. Pharmacological Aspects, *Indian J. Appl. Chem.* 28:15–40 (1965).
5. Matsuzaki, T., and Y. Hara, Antioxidative Activity of Tea Leaf Catechins, *J. Agric. Chem. Soc. Jap.* 59:129–134 (1985).
6. Balentine, D.A., Manufacturing and Chemistry of Tea, in *Phenolic Compounds in Food and Their Effects on Health I*, edited by C.-T. Ho, C.Y. Lee, M.T. Huang, and C.Y. Huang, American Chemical Society, Washington, D.C., 1992, pp. 102–117.
7. Lunder, T.L., Catechins of Green Tea. Antioxidant Activity, in *Phenolic Compounds in Food and Their Effects on Health II. Antioxidants and Cancer Prevention*, edited by M.T. Huang, C.-T. Ho, and C.Y. Lee, American Chemical Society, Washington, D.C., 1992, pp. 114–120.
8. Chen, Z.Y., P.T. Chan, H.M. Ma, K.P. Fung, and J. Wang, Antioxidative Effect of Ethanol Tea Extracts on Oxidation of Canola Oil, *J. Am. Oil Chem. Soc.* 73:375–380 (1996).
9. Ragnarsson, J.O., and T.P. Labuza, Accelerated Shelf-Life Testing for Oxidative Rancidity in Foods—A Review, *Food Chem.* 2:291–308 (1977).
10. Frankel, E.N., In Search of Better Methods to Evaluate Natural Antioxidants and Oxidative Stability in Food Lipids, *Trends Food Sci. & Technol.* 4:220–225 (1993).
11. Aeschbach, R., and P. Rossi, Novel Approach for Antioxidant Extraction, in *Oils–Fats–Lipids 1995, Proceedings of the 21st World Congress of the International Society for Fat Research*, The Hague, October 1995, vol. 2, edited by W.A.M. Castenmiller, P.J. Barnes, Bridgwater, 1996, pp. 331–332.
12. Frankel, E.N., S.-W. Huang, R. Aeschbach, and E. Prior, Antioxidant Activity of a Rosemary Extract and Its Constituents, Carnosic Acid, Carnosol, and Rosmarinic Acid, in Bulk Oil and Oil-in-Water Emulsion, *J. Agric. Food Chem.* 44:131–135 (1996).
13. Huang, S.-W., E.N. Frankel, and J.B. German, Antioxidant Activity of  $\alpha$ - and  $\gamma$ -Tocopherols in Bulk Oils and in Oil-in-Water Emulsions, *Ibid.* 42:2108–2114 (1994).
14. Huang, S.-W., A. Hopia, K. Schwarz, E.N. Frankel, and J.B. German, Antioxidant Activity of  $\alpha$ -Tocopherol and Trolox in Different Lipid Substrates: Bulk Oils vs. Oil-in-Water Emulsions, *Ibid.* 44:444–452 (1996).
15. Hopia, A.I., S.-W. Huang, K. Schwarz, J.B. German, and E.N. Frankel, Effect of Different Lipid Systems on Antioxidant Activity of Rosemary Constitutes Carnosol and Carnosic Acid With and Without  $\alpha$ -Tocopherol, *Ibid.* 44:2030–2036 (1996).
16. Huang, S.-W., E.N. Frankel, K. Schwarz, and J.B. German. Effect of pH on Antioxidant Activity of  $\alpha$ -Tocopherol and Trolox in Oil-in-Water Emulsions, *Ibid.* 44:2496–2502 (1996).
17. Lunder, T.L., Procédé d'Obtention de Complexes de Catechines, European Patent 456 023 (1991).
18. Aeschbach, R., and P. Rossi, Procédé d'Extraction d'Antioxydants de Matière Végétale, European Patent 728 420 (1995).
19. Wagner, S.F., Analysis of Variance, in *Introduction to Statistics*, Harper Perennial, New York, 1992, Chapter 11.
20. Schwarz, K., E.N. Frankel, and J.B. German, Partition Behaviour of Antioxidative Phenolic Compounds in Heterophasic Systems, *Fett/Lipid* 98:115–121 (1996).

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