Antioxidant Activity of Tea Theaflavins and Methylated Catechins in Canola Oil

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ABSTRACT: The present study examined the antioxidant activity of black tea theaflavins and catechin derivatives in canola oil. Oxidation was conducted at 95°C by monitoring the oxygen consumption and decreases in the linoleic and α-linolenic acids of canola oil. All were tested at a concentration of 0.5 mM. Catechins, including (–)-epicatechin, (–)-epicatechin gallate, (–)-epigallocatechin, and (–)-epigallocatechin gallate (EGCG), were more effective than theaflavins, namely, theaflavin-1, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-digallate (TF_3), against the lipid oxidation of canola oil. Among the four theaflavins, TF_3 was the most effective, whereas among the four catechins, EGCG was the most potent. Under the same conditions, all theaflavins and catechins were more powerful than BHT as an antioxidant in heated canola oil. Little or no difference in antioxidant activity was observed between each catechin and epimer pair. Methylation of the 3′-OH led to a significant loss of antioxidant activity of the catechins.

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KEY WORDS: Catechins, methylated catechins, oxidation, theaflavins.

Catechins, including (–)-epicatechin (EC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC), and (–)-epigallocatechin gallate (EGCG), are the principal components in green tea (Scheme 1). The fermentation process in black tea leads to dimerization and polymerization of catechins, forming brown products, namely, theaflavins (TF) and thearubigins. The TF comprise mainly four compounds, namely, theaflavin-1 (TF_1), theaflavin-3-gallate (TF_2A), theaflavin-3′-gallate (TF_2B), and theaflavin-3,3′-digallate (TF_3) (Scheme 1). Heat treatment in manufacturing commercial tea drinks produces an additional group of catechin epimers, including (–)-catechin (C), (–)-catechin gallate (CG), (–)-gallocatechin (GC), and (–)-gallocatechin gallate (GCG) (1). The methylated catechins present in different tea varieties have also been found to vary with seasons and processing conditions (2). Extensive investigations have demonstrated that both catechins and TF possess anticarcinogenic, hypolipidemic, and antioxidant activity (3–7). However, little information is available on the biological activity of methylated catechins and epimers (8).

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Food manufacturers use antioxidants to prevent the development of rancidity in foods. However, the safety of the traditional synthetic antioxidants BHT and BHA is of concern to the public (9,10). Therefore, research has focused on the development and utilization of antioxidants derived from natural sources. We have previously studied the antioxidant activity of various tea extracts (11) and individual catechins in heated canola oil (12) and found them to be very effective in preventing lipid oxidation. The present study was carried out to further investigate the antioxidant activity of individual TF, catechin epimers, and methylated catechins as compared with their precursor catechins and the synthetic antioxidant BHT.

MATERIALS AND METHODS

Isolation and purification of tea catechins. Individual catechins were isolated from Longjing green tea as described previously (13). In brief, Longjing green tea (100 g) was soaked in hot water (150 mL) three times, and the infusion was extracted first with an equal volume of chloroform and then with ethyl acetate. The individual catechins in the ethyl acetate extract were separated using a Shimadzu LC-10AD high-performance liquid chromatograph (HPLC) equipped with a semipreparative column (Hypersil ODS, 250 × 4.6 cm, 5 μM; Alltech, Deerfield, IL), eluted with a 29% methanol solution in H_2O at a flow rate of 0.7 mL/min. All solvents used were of HPLC grade (BDH Laboratory Supplier, Poole, United Kingdom).

Isolation of purification of TF. Individual TF were isolated as we described previously (5). Keemun black tea was first extracted three times with a 70% ethanol solution. After the removal of ethanol in a rotary evaporator, the remaining water solution was extracted using chloroform, ethyl acetate, and butanol. The ethyl acetate extract was then applied onto a silica gel column (80 × 6.5 cm, i.d.; silica gel 60 M, 230–240 mesh). The total TF fraction was obtained when the column was eluted with a mixture of chloroform and ethyl acetate (1:1, vol/vol), after which the ratio of chloroform to ethyl acetate was increased to 4:1 (vol/vol). The total TF fraction was then applied onto a Sephadex LH-20 column (50 × 6.0 cm, i.d.) and eluted with 15 L of 70% ethanol to obtain crude fractions of TF_1, TF_2A, TF_2B, and TF_3. TF_1 was purified in a Sephadex LH-20 column B (50 × 2.5 cm, i.d.) and eluted using 4 L of 30% acetone in water containing 2% acetic acid.

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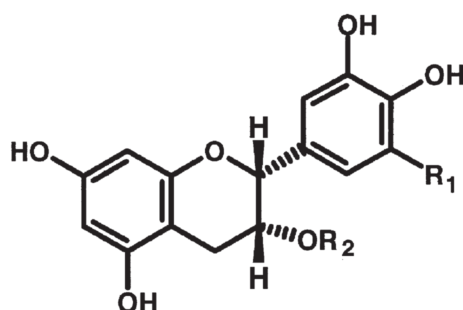
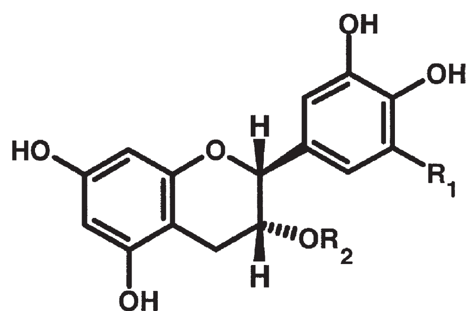
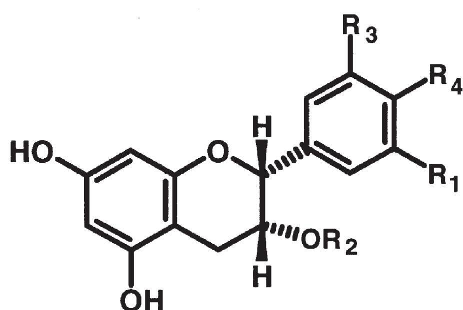


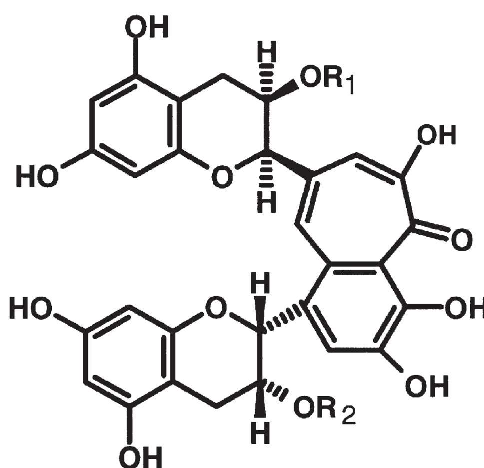
EG = Epicatechin R_1 = H R_2 = H
ECG = Epicatechin gallate R_1 = H R_2 = galloyl
EGC = Epigallocatechin R_1 = OH R_2 = H
EGCG = Epigallocatechin gallate R_1 = OH R_2 = galloyl



C = Catechin R_1 = H R_2 = H
CG = Catechin gallate R_1 = H R_2 = galloyl
GC = Gallocatechin R_1 = OH R_2 = H
GCG = Gallocatechin gallate R_1 = OH R_2 = galloyl



3′-M-EC = 3′-*O*-Methyl epicatechin
R_1, R_2 = H R_3 = OCH_3 R_4 = OH
4′-M-EC = 4′-*O*-Methyl epicatechin
R_1, R_2 = H R_3 = OH R_4 = OCH_3
3′-M-ECG = 3′-*O*-Methyl epicatechin gallate
R_1 = H R_2 = galloyl R_3 = OCH_3 R_4 = OH
3′-M-EGC = 3′-*O*-Methyl epigallocatechin
R_1, R_4 = OH R_2 = H R_3 = OCH_3
3′-M-EGCG = 3′-*O*-Methyl epigallocatechin gallate
R_1, R_4 = OH R_2 = galloyl R_3 = OCH_3



TF_1 = Theaflavin-1 R_1 = R_2 = H
TF_2A = Theaflavin-3-gallate-A R_1 = galloyl R_2 = H
TF_2B = Theaflavin-3′-gallate-B R_1 = H R_2 = galloyl
TF_3 = Theaflavin-3,3′-digallate R_1 = R_2 = galloyl

SCHEME 1

TF_2A, TF_2B, and TF_3 were similarly isolated and purified. The four purified TF were subjected to structural verification by using the m.p. test, UV spectrometry, LC–MS, and ^1H NMR spectrometry. The results were in agreement with those reported by Nonaka *et al.* (14).

Isolation and purification of catechin epimers. Longjing green tea (700 g) was used to prepare individual catechin epimers. The dry tea leaves were soaked in 4 L of boiling water for 30 min, and the infusion was reduced in a rotary evaporator to 1.8 L. The reduced infusion was autoclaved at 121°C for 20 min. The HPLC analysis showed that about 45% of catechins were converted to their corresponding epimers. For decaffeination, an equal volume of chloroform was added, which removed caffeine from the aqueous phase. The remaining aqueous phase was extracted with an equal volume of ethyl acetate. After removal of the ethyl acetate in a rotary evaporator, the crude extract (30 g) was loaded onto a glass column (60 × 6.0 cm, i.d.) packed with 500 g of Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) and eluted with a mobile phase of 90% ethanol. Each catechin and its corresponding epimer, as a mixture, were eluted in the following order, EC/C, EGC/GC, EGCG/GCG, and ECG/CG. Separation of each catechin and its epimer was then accomplished by using a semipreparative HPLC column (250 × 22 mm, i.d., Econosil; Alltech), eluted with a mobile phase containing 10% acetonitrile and 2% acetic acid at a flow rate of 4 mL/min. The individual catechins and epimers were monitored by using a UV detector at 280 nm. The structures of the purified catechin epimers were confirmed by checking the retention times of the HPLC chromatograms and the UV absorption spectra with those of standards obtained from Sigma (St. Louis, MO).

Preparation and purification of methylated catechins. Methylated catechins were synthesized and identified as described by Donovan *et al.* (15). In brief, 100 mg of each cate-

chin was mixed with 200 mg K_2CO_3 and 0.4 mL of methyl iodide in 5 mL of acetone in an ultrasonic bath for 2.5 h. The reaction mixture was then dried under a gentle stream of nitrogen gas, followed by addition of 5 mL of distilled water and three extractions with 10 mL of ethyl acetate. The ethyl acetate extracts were pooled and evaporated under vacuum in a rotary evaporator at 60°C. The residue containing methylated derivatives was then dissolved in 2 mL of methanol.

Methylated derivatives were separated from their corresponding catechins by using a semipreparative column (Econosil C18, 250 × 10 mm, 10 µm; Alltech). In brief, the sample (3–5 mg) in 250 µL of methanol was loaded onto the column each time *via* a rheodyne valve. A gradient of solvent A (2% acetic acid in HPLC water) and solvent B (acetonitrile) was used at a flow rate of 5 mL/min and monitored at 280 nm. After injection, solvent B was increased from 8 to 15% over 20 min, to 31% over an additional 20 min, held at 31% for 5 min, and then changed back to 15% over an additional 5 min. Each methylated derivative was repurified in the same column once its purity reached 98%. The structures of the purified methylated catechins were confirmed by checking their LC–MS and ^{1}H NMR spectra (15).

Oxygen consumption test. The uptake of oxygen by canola oil was used as an index of oxidation. As described previously (16), 220 mg of canola oil (Parknshop, Shatin, Hong Kong) was placed in a glass tube (150 × 16 mm, o.d.), followed by the addition of 1 mL of acetone containing 0.5 mM of the individual TF, catechins, and epimers. The solvents were evaporated under a gentle stream of nitrogen at 45°C. The reaction tube was then flushed with air using an air pump and sealed tightly with a rubber stopper obtained from an evacuated blood collection tube (100 × 16 mm, o.d.; Becton-Dickinson, Rutherford, NJ). The reaction tubes were then placed in a heating block at 95°C, with a variation of ±3°C. The fluctuation in temperature leads to an inconsistent oxygen consumption rate over time. Therefore, we compared the antioxidant activity of only the samples that were placed in the same heating block and heated at the same time. The headspace air (50 µL) was sampled every 4–6 h with a gas-tight syringe for a total of 40 h and injected into an HP 5890 Series II gas–solid chromatograph (Hewlett-Packard, Palo Alto, CA) fitted with a 1/8″ × 6′ stainless-steel column packed with Molecular Sieve 5A (60/80 mesh) and a thermal conductivity detector. The percentage of oxygen in the headspace was calculated from the ratio of oxygen to nitrogen.

FA analysis. TAG of heated canola oil with or without the addition of individual TF, cataechins, and epimers were converted to their corresponding methyl esters with a mixture of 14% BF_3 in methanol (Sigma) and toluene (1:1, vol/vol) under nitrogen at 90°C for 30 min (11). FAME were analyzed on a flexible silica capillary column (Innowax 19091N-213, 30 m × 0.32 mm, i.d.; J&W Scientific, Folsom, CA) in an HP 5980 Series II gas–liquid chromatograph equipped with an FID (Hewlett-Packard). Column temperature was programmed from 180 to 230°C at a rate of 2°C/min and then held for 5 min. Injector and detector temperatures were set at 250 and 300°C, respectively. Helium was used as the carrier gas at a head pressure of 15 psi. Identification of each FAME was made by comparison of its retention time with that of an authentic standard (Sigma).

Statistics. Data are expressed as means ± SD of five replicates. Data for headspace oxygen consumption and the FA analysis were subjected to ANOVA, and the means were compared among treatments by using Duncan's multiple range test. The difference was considered significant when the *P* value was less than 0.05.

RESULTS

The antioxidant activity of TF_1, TF_2A, TF_2B, and TF_3 was first compared with that of EC, ECG, EGC, and EGCG at a concentration of 0.5 µM by using oxygen consumption as an index of oxidation (Fig. 1). The 0.5 mM of EGCC (the major catechin in tea) was approximately equivalent to 200 ppm. The rationale for choosing this concentration was that a maximum of 200 ppm of a single antioxidant or a mixture of antioxidants is generally permitted in fats or oils by many countries. As shown in Figure 1, the headspace oxygen concentration of the samples to which catechins had been added decreased at a slower rate than that of the samples to which TF had been added. This clearly demonstrated that catechins from green tea exhibited a significantly stronger protection of canola oil from lipid oxidation than TF isolated from black tea. Compared with BHT, the four catechins and four theaflavins were much more effective against the lipid oxidation of canola oil at 95°C.

The protective activity of TF and catechins on canola oil was also assessed by monitoring changes in the FA composition of canola oil (Table 1). In general, the results are in agreement with those of the oxygen consumption test. The more headspace oxygen was consumed, the more α-linolenic and linoleic acids were oxidized. As shown in Table 1, α-linolenic acid in the sample without any antioxidant decreased from 5.9

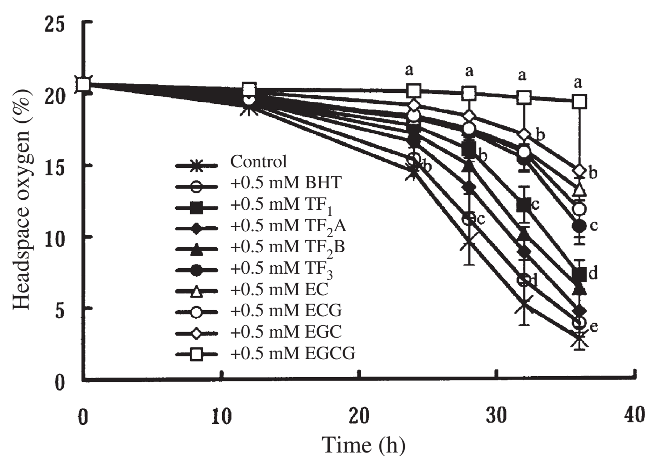


FIG. 1. Effect of 0.5 µM theaflavins and catechins on the oxygen consumption of canola oil heated at 95 ± 3°C. Data are expressed as mean ± SD (n = 5 samples). Means at the same time point with different roman letters (a–e) differ significantly (P < 0.05). TF_1, theaflavin-1; TF_2A, theaflavin-3-gallate; TF_2B, theaflavin-3′-gallate; TF_3, theaflavin-3,3′-digallate; EC, (–)-epicatechin; ECG, (–)-epicatechin gallate; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin gallate.

to 4.4% in canola oil heated for 36 h, but it decreased only to 5.2–5.5% in samples to which EC, ECG, and EGC had been added. When EGCG was added, no change in α-linolenic acid was observed (Table 1). In contrast, the four individual TF also prevented α-linolenic acid from oxidizing, but their protection was less effective than that of the four catechins. Similarly, the four catechins demonstrated stronger protection of linoleic acid than the four individual TF when canola oil was heated at 95°C for 36 h (Table 1).

The antioxidant activity of the catechin epimers, C, CG, GC, and GCG, was compared with that of their corresponding precursors, EC, ECG, EGC, and EGCG, by using the oxygen consumption method (Fig. 2). Catechins and their epimers were more effective than BHT in preventing a headspace oxygen drop. No significant differences in effectiveness were observed between each precursor and epimer pair. The results from the FA analysis were in agreement with those of the oxygen consumption test (Table 1).

The oxygen consumption test showed that methylated catechins were less effective than their corresponding precursors in protecting canola oil from oxidation. As shown in Figure 3, the sample with EGCG added had little consumption of headspace oxygen, whereas that with 3′-*O*-methyl-EGCG added showed a significant drop in headspace oxygen, from 21 to 8%. A similar trend was observed for other pairs of catechins and their methylated derivatives, namely, EGC and 3′-*O*-methyl-EGC, ECG and 3′-*O*-methyl-ECG, and EC and 3′-*O*-methyl-EC. The results of the FA analysis were consistent with those of the oxygen consumption test. The methylation of catechins decreased their ability to protect α-linolenic acid and linoleic acids from oxidation (Table 1).

DISCUSSION

Catechins, namely, EC, ECG, EGC, and EGCG, are abundant in tea leaves. In addition to catechins, TF, namely, TF_1, TF_2A, TF_2B, and TF_3, are also present in black tea. Previous studies have shown that both catechins and TF are effective as antioxidants in various biological systems (6,7,13). The application of catechins as food antioxidants also has been reported (12,17). However, little or no information is available on the application of TF as food antioxidants. The present study is the first to examine the relative potency of individual TF and methylated catechins compared with that of catechins in inhibiting the oxidation of a vegetable oil. First, the results demonstrated that both catechins and TF were more effective than BHT against the lipid oxidation of canola oil under the present experimental conditions. Second, compared with catechins, TF were less potent as antioxidants in heated canola oil. This partially explains the observation that green tea extract is much more efficient in preventing the oxidative rancidity of a vegetable oil than black tea extract (11). Third, the four catechins and the four TF within each group demonstrated varying degress of antioxidant effectiveness, depending on their chemical structures. Fourth, the methylation of one hydroxyl group in the catechins reduced their antioxidative effectiveness. Last, the epimerization of catechins had little or no effect on their antioxidative potency.

TABLE 1
Effect of Theaflavins, Catechins, and Their Derivatives on Change in the FA of Canola Oil (wt% of total) Heated at 95°C [a]

	Linoleic	α-Linolenic	Oleic	Palmitic	Stearic	Other
Unheated canola oil	53.3 ± 2.0^{a}	$5.9 \pm 0.6^{a,b}$	$22.4 \pm 1.3^{d,e}$	$11. \pm 0.5^{e,f}$	$4.4 \pm 0.3^{f,g}$	2.2 ± 0.1^{e}
Heated canola oil	48.7 ± 0.2^{c}	4.4 ± 0.1^{e}	25.1 ± 0.1^{a}	13.9 ± 0.1^{b}	5.2 ± 0.1^{a}	2.8 ± 0.1^{b}
+BHT	48.9 ± 0.1^{c}	4.4 ± 0.1^{e}	$25.0 \pm 0.2^{a,b}$	$13.8 \pm 0.1^{b,c}$	5.1 ± 0.1^{b}	2.8 ± 0.1^{b}
+TF_1	49.9 ± 0.1^{b}	4.7 ± 0.1^{d}	24.4 ± 0.1^{c}	13.3 ± 0.1^{c}	4.9 ± 0.1^{c}	2.7 ± 0.1^{c}
+TF_2A	$49.1 \pm 0.6^{b,c}$	4.5 ± 0.1^{e}	24.8 ± 0.1^{b}	$13.7 \pm 0.2^{b,c}$	5.0 ± 0.1^{b}	2.8 ± 0.1^{b}
+TF_2B	$49.5 \pm 0.8^{b,c}$	$4.6 \pm 0.2^{d,e}$	$24.9 \pm 0.5^{a,b}$	13.4 ± 0.3^{c}	4.9 ± 0.1^{c}	$2.6 \pm 0.1^{c,d}$
+TF_3	$50.7 \pm 0.4^{a,b}$	5.0 ± 0.1^{c}	23.8 ± 0.4^{d}	12.9 ± 0.1^{d}	4.7 ± 0.1^{d}	2.9 ± 0.1^{b}
+EC	$51.9 \pm 0.3^{a,b}$	5.3 ± 0.1^{b}	23.3 ± 0.2^{d}	12.4 ± 0.1^{e}	4.6 ± 0.1^{f}	$2.5 \pm 0.1^{c,d}$
+ECG	$51.4 \pm 0.2^{a,b}$	$5.2 \pm 0.2^{b,c}$	23.5 ± 0.3^{d}	12.7 ± 0.2^{d}	4.7 ± 0.1^{d}	$2.6 \pm 0.1^{c,d}$
+EGC	$52.3 \pm 1.7^{a,b}$	$5.5 \pm 0.5^{a,b}$	22.9 ± 1.1^{d}	12.4 ± 0.4^{e}	4.5 ± 0.2^{f}	2.4 ± 0.2^{d}
+EGCG	54.2 ± 0.1^{a}	6.1 ± 0.1^{a}	21.8 ± 0.1^{e}	11.6 ± 0.1^{f}	4.3 ± 0.1^{g}	2.1 ± 0.1^{e}
+C	$51.3 \pm 0.6^{a,b}$	$5.2 \pm 0.2^{b,c}$	23.5 ± 0.3^{d}	$12.7 \pm 0.3^{d,e}$	4.7 ± 0.1^{d}	2.7 ± 0.1^{c}
+CG	$51.8 \pm 1.4^{a,b}$	$5.3 \pm 0.4^{a,b}$	23.3 ± 0.9^{d}	$12.5 \pm 0.6^{d,e}$	4.6 ± 0.2^{f}	$2.6 \pm 0.1^{c,d}$
+GC	$52.1 \pm 0.4^{a,b}$	$5.5 \pm 0.4^{a,b}$	22.9 ± 0.9^{d}	12.3 ± 0.5^{f}	4.9 ± 0.2^{c}	$2.5 \pm 0.1^{c,d}$
+GCG	53.8 ± 0.5^{a}	6.0 ± 0.2^{a}	22.0 ± 0.2^{e}	$11.9 \pm 0.4^{e,f}$	4.3 ± 0.1^{g}	2.2 ± 0.1^{e}
+3′-M-EC	$48.5 \pm 0.3^{c,d}$	4.4 ± 0.1^{e}	25.1 ± 0.2^{a}	$13.8 \pm 0.1^{b,c}$	5.1 ± 0.1^{b}	3.1 ± 0.1^{a}
+4′-M-EC	48.2 ± 0.2^{d}	4.3 ± 0.1^{f}	25.3 ± 0.1^{a}	14.1 ± 0.1^{a}	5.2 ± 0.1^{a}	2.9 ± 0.1^{b}
+3′-M-ECG	$48.5 \pm 0.1^{c,d}$	4.4 ± 0.1^{e}	25.1 ± 0.1^{a}	$13.8 \pm 0.1^{b,c}$	5.1 ± 0.1^{b}	3.1 ± 0.1^{a}
+3′-M-EGC	$50.6 \pm 0.1^{a,b}$	5.0 ± 0.1^{c}	$24.0 \pm 0.1^{c,d}$	12.9 ± 0.1^{d}	$4.8 \pm 0.1^{c,d}$	2.8 ± 0.1^{b}
+3′-M-EGCG	49.9 ± 0.3^{b}	4.8 ± 0.1^{d}	24.4 ± 0.1^{c}	$13.1 \pm 0.1^{c,d}$	4.9 ± 0.1^{c}	2.9 ± 0.1^{b}

[a]Data are expressed as mean ± SD of five samples. Means in the same column with different superscripts (a–g) differ significantly ($P < 0.05$). TF_1, theaflavin-1; TF_2A, theaflavin-3-gallate; TF_2B, theaflavin-3′-gallate; TF_3, theaflavin-3,3′-digallate; EC, (–)-epicatechin; ECG, (–)-epicatechin gallate; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin gallate; C, (–)-catechin; CG, (–)-catechin gallate; GC, (–)-gallocatechin; GCG, (–)-gallocatechin gallate; 3′-M-EC, 3′-*O*-methyl epicatechin; 4′-M-EC, 4′-*O*-methyl epicatechin; 3′-M-ECG, 3′-*O*-methyl epicatechin gallate; 3′-M-EGC, 3′-*O*-methyl epigallocatechin; 3′-M-EGCG, 3′-*O*-methyl epigallocatechin gallate.

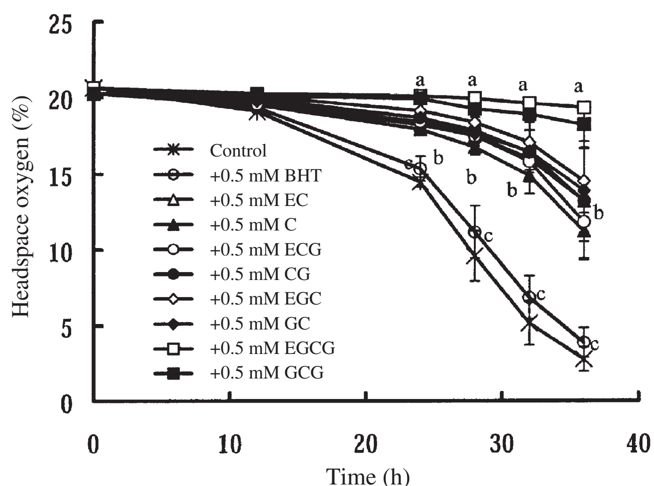


FIG. 2. Effect of catechins and their epimers on the oxygen consumption of canola oil heated at 95 ± 3°C as compared with BHT. Data are expressed as mean ± SD (n = 5 samples). Means at the same time point with different roman letters (a–c) differ significantly ($P < 0.05$). C, (–)-catechin; CG, (–)-catechin gallate; GC, (–)-gallocatechin; GCG, (–)-gallocatechin gallate; for other abbreviations see Figure 1.

The mechanism by which catechins and TF demonstrated a stronger antioxidant activity than BHT remains unclear. The effectiveness of an antioxidant is determined by many factors, mainly its activation energy, rate constant, oxidation-reduction potential, stability of its radical intermediate, and solubility. For antioxidants of phenolic types, their activity is conspicuously related to the number and position of aromatic hydroxyl groups. A phenolic antioxidant with a greater number of hydroxyl groups is known to inhibit the chain reaction

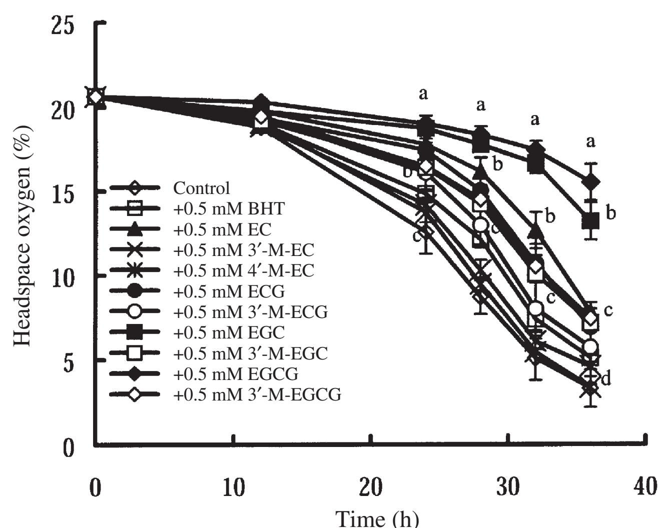


FIG. 3. Effect of catechins and their methylated derivatives on the oxygen consumption of canola oil heated at 95 ± 3°C. Data are expressed as mean ± SD (n = 5 samples). Means at the same time point with different roman letters (a–d) differ significantly ($P < 0.05$). 3′-M-EC, 3′-O-methyl epicatechin; 4′-M-EC, 4′-*O*-methyl epicatechin; 3′-M-ECG, 3′-*O*-methyl epicatechin gallate; 3′-M-EGC, 3′-*O*-methyl epigallocatechin; 3′-M-EGCG, 3′-*O*-methyl epigallocatechin gallate; for other abbreviations see Figure 1.

of lipid oxidation more effectively by acting as a hydrogen donor or a free radical acceptor because it is more vulnerable to donation of a proton or electron and its resulting free-radical intermediate is more stable due to resonance delocalization (18). In this regard, the lower effectiveness of BHT compared with catechins and TF as antioxidants can be explained by differences in their numbers of hydroxyl groups: The former has only one hydroxyl group, whereas the latter have 4–17 hydroxyl groups.

The role of hydroxyl groups in the antioxidant activity of catechins is best illustrated when the antioxidant activity of methylated catechins is compared with that of their corresponding precursors (Fig. 3). All methylated derivatives demonstrated less potency against the lipid oxidation of canola oil than the catechins. Another piece of supporting evidence was that TF_3 was more effective than the other TF against the lipid oxidation of canola oil (Fig. 1) because TF_3 contains two galloyl groups on which there are three hydroxyl groups. A similar deduction was applied to EGCG and the other three catechins (Fig. 1).

The number of hydroxyl groups on an aromatic ring is the major factor contributing to the efficacy of phenolic antioxidants (19). The molecular size of catechins and TF also may play a partial role in antioxidant effectiveness. This is reflected in the observation that TF demonstrated less antioxidant activity than catechins in preventing canola oil from oxidation even though the former have a greater number of hydroxyl groups than the latter. TF are bulkier than catechins, meaning they are less mobile in canola oil.

The solubility of catechins and TF also might influence the efficacy of an antioxidant in a fat or oil. On the one hand, the antioxidant activity of TF increases with an increasing number of hydroxyl groups; on the other, it becomes less potent because the addition of hydroxyl groups (which are hydrophilic) decreases its solubility in canola oil (which are hydrophobic) (19). This might explain why the antioxidant activity of TF_1 was more potent than that of TF_2A and TF_2B even though TF_1 had fewer hydroxyl groups than TF_2A and TF_2B.

The spatial or stereo configuration of hydroxyl groups on the aromatic ring seemed to have little effect on the antioxidant activity of catechins. As illustrated in Figure 2, little or no difference was observed in the pattern of oxygen consumption pattern in the samples with catechin and epimer pairs. Similarly, no differences in the FA profile were observed among samples with the addition of each catechin and its corresponding epimer.

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