Stabilization of Seal Blubber and Menhaden Oils with Green Tea Catechins

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ABSTRACT: Catechins, namely (–)epicatechin (EC), (–)epigallocatechin (EGC), (–)epicatechin gallate (ECG) and (–)epigallocatechin gallate (EGCG), were isolated from commercial Chinese green tea leaves. The antioxidant activity of isolated catechins was compared with those of α-tocopherol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ), all at 200 ppm, in refined, bleached and deodorized seal blubber oil and menhaden oil. The study was carried out under Schaal oven test conditions at 65°C over a 144-h period, except for weight gain measurements, which were continued for up to 200 h. Progression of oxidation was monitored by measuring changes in weight gain and values of peroxide, conjugated diene, and 2-thiobarbituric acid-reactive substances. Oils treated with tea catechins showed excellent oxidative stability as compared with samples that contained commonly used antioxidants, such as α-tocopherol, BHA, BHT, and TBHQ. The potency of catechins in prevention of oxidation of marine oils was in the decreasing order of ECG > EGCG > EGC > EC; ECG was slightly more effective than TBHQ in systems studied.

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KEY WORDS: Antioxidant activity, marine oils, menhaden oil, natural antioxidants, oxidative stability, seal blubber oil, synthetic antioxidants, tea catechins.

Lipids that are rich in polyunsaturated fatty acids (PUFA) play an important role in human health and nutrition. This is particularly true for marine lipids, which have attracted much attention because of their high content of long-chain n-3 PUFA, especially eicosapentaenoic (EPA, 20:5n-3), docosapentaenoic (DPA, 22:5n-3), and docosahexaenoic (DHA, 22:6n-3) acids. These fatty acids are considered essential because they cannot be synthesized by humans and must be acquired through the diet. The beneficial effects of n-3 PUFA have been ascribed to their ability to lower serum triacylglycerol and cholesterol (1). While DHA is essential for proper functioning of the eye and may have a structural role in nerve and brain tissues, EPA serves as a precursor to eicosanoid compounds (2). EPA also has been recognized as having therapeutic benefits in treatment of human cardiovascular diseases (1,3). On the other hand, n-3 fatty acids have been shown to possess beneficial effects in prevention or possible treatment of coronary heart diseases, diabetes, high blood pressure, and autoimmune diseases (4). Although increased consumption of seafoods and marine oils is encouraged due to their health benefits, the highly unsaturated nature of their fatty acids renders them more sensitive to oxidative deterioration (5,6).

Hydroperoxides are the primary products of lipid oxidation. Degradation of highly unsaturated fatty acids, *via* a free-radical chain mechanism, results in changes in odor and flavor of fats and oils and/or lipid-containing foods (7). Chemical reactions involved in oxidative processes require low activation energies, and their rates are not changed significantly by lowering the storage temperature (7,8). Therefore, to overcome this problem, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and/or *tert*-butylhydroquinone (TBHQ) are often incorporated into fats and oils or lipid-containing foods (9). However, there is a decline in the use of synthetic antioxidants (10), perhaps due to consumers' preference for natural ingredients.

The choice of natural antioxidants to stabilize marine oils for human consumption is restricted to a few substances, with α-tocopherol (or its synthetic analog) being the most frequently used. Although tocopherols are considered as safe natural antioxidants, they do not always provide effective protection against oxidation, especially when oils are contaminated with trace amounts of metal ions, such as Fe^{++} or Cu^{++} (11). Therefore, research on other natural antioxidants has gained momentum as they are considered to pose no health risk to consumers.

Naturally occurring antioxidative components in foods include flavonoids, phenolic acids, lignans, terpenes, tocopherols, phospholipids, and polyfunctional organic acids (12,13). Catechins are naturally occurring flavonoids and constitute common components of human diet. In particular, green tea leaves contain relatively large amounts of (–) epicatechin (EC), (–)epicatechin gallate (ECG), (–)epigallocatechin (EGC) and (–)epigallocatechin gallate (EGCG) (Figure 1; 14–16). In recent years, these catechins have attracted much attention in relation to their physiological potential, such as antimutagenic and antitumorigenic activities (17). Epidemiological studies also have suggested that tea polyphenols are effective in cancer prevention (18). Furthermore, cat-

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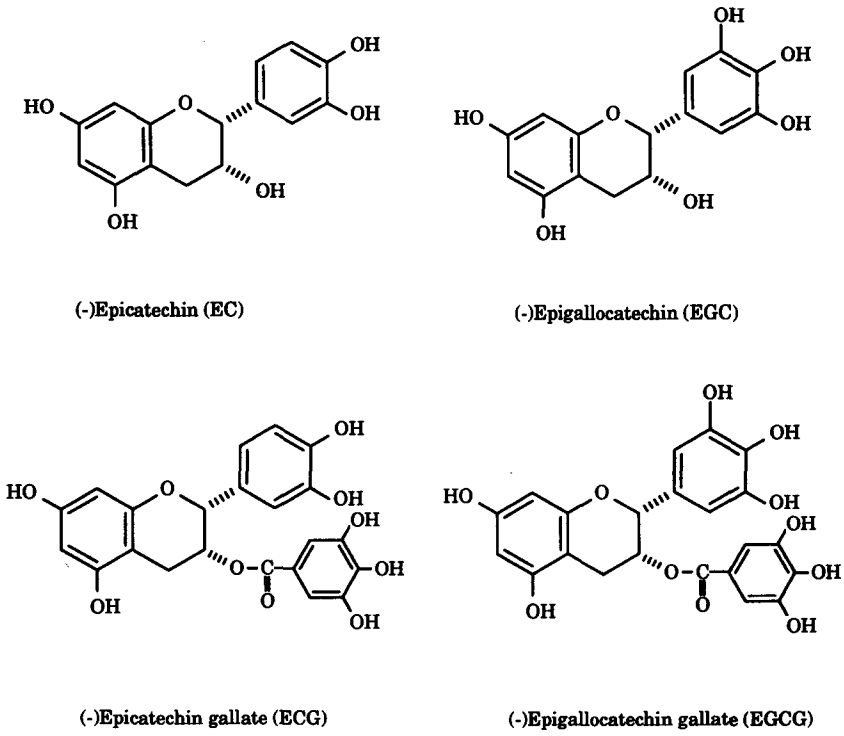

FIG. 1. Chemical structures of catechins from green tea leaves.

echins and other flavonoids have been recognized as efficient antioxidants by scavenging oxygen radicals and chelating metal ions (19–25). Hara (26) has evaluated the antioxidative potency of crude extracts of green tea and individual catechins in lard by the Active Oxygen Method. Crude tea catechins reduced the formation of peroxides more effectively than α-tocopherol or BHA. The antioxidant potency of individual catechins, evaluated in the same manner, was in the order of EGCG > EGC > ECG > EC. Recently, Amarowicz and Shahidi (16) found that ECG possessed the strongest and EGC the weakest antioxidative effect in a β-carotene–linoleate model system. However, antioxidative activity of individual tea catechins in highly unsaturated marine oils has not been investigated.

The objective of this study was to examine the effect of individual tea catechins on the oxidative stability of refined–bleached and deodorized seal (marine mammal) blubber and menhaden (fish) oils. The efficacy of these catechins was compared with those of commonly used food antioxidants, such as α-tocopherol, BHA, BHT, and TBHQ.

MATERIALS AND METHODS

Freshly-prepared, refined, bleached, and deodorized (RBD) menhaden oil, devoid of any additives, was obtained from Zapata Protein (USA) Inc. (Reedville, VA). Blubber of harp seal was obtained from local sources in Newfoundland, and the extraction, refining, and bleaching of the oil was carried out as described elsewhere (6). The refined–bleached seal blubber oil was deodorized in a laboratory-scale vacuum steam distillation apparatus. The oil was heated to 190°C with steam, while under vacuum. Volatile compounds were then recovered during the deodorization process over a 5-h period and retained for further studies. The resulting RBD-seal blubber oil was stored at −60°C until used. Commercial antioxidants, namely TBHQ, BHA and BHT and α-tocopherol, were obtained from Sigma Chemical Company (St. Louis, MO). Fatty acid methyl esters (FAME) were purchased from either Supelco (Mississauga, Ontario, Canada) or Nu-Chek-Prep Inc. (Elysian, MN). All other chemicals used in this study were of ACS-grade or better quality. The fresh RBD-seal blubber and RBD-menhaden oils were analyzed by determining their iodine, peroxide and acid values (27; method numbers Cd 1-25, Cd 8-53 and Cd 3a-63, respectively), and fatty acid composition. FAME were prepared (28) and analyzed on a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Mississauga, Ontario, Canada), equipped with a flame-ionization detector and split/splitless injector. A Supelcowax 10 column 0.25 mm × 30 m; 0.25 μm film thickness (Supelco) was used. Chromatographic parameters were set as follows: injector and detector temperatures, 250°C; oven temperature programming: held 10.25 min at 220°C, then ramped to 240°C at 30°C/min, followed by a hold period of 9 min. Total run time was 19.92 min. Helium was used as the carrier gas. FAME were identified by comparison of their retention times with reference standards. The content of fatty acids was calculated from their corresponding integration data.

Crude catechins were extracted from commercial Chinese green tea leaves as described by Price and Spitzer (29). Pu-

rification of individual catechins, namely EC, EGC, EGCG, and ECG, was achieved as described elsewhere (16). Purified catechins (200 ppm) and commercial antioxidants BHA, BHT and TBHQ (200 ppm), and α-tocopherol (500 ppm) were dissolved in a minimum volume of absolute ethanol and added to RBD-seal blubber oil and RBD-menhaden oil and mixed for 10 min in an ultrasonic water bath. Samples, containing the same amount of ethanol, were used as controls for comparative studies. The weight-gain data were collected according to the procedure of Olcott and Einset (30) up to a 200-h period at 6-h intervals, with minor modifications. Two grams of each sample, prepared as given above, were placed in glass petri dishes (of 60 mm diameter and 15 mm height), and traces of water in samples were removed by placing them in a vacuum oven at 35°C for 12 h. Storage under accelerated oxidation conditions was carried out in an oven at 65°C. The time required for a 0.5% weight increase for each sample was taken as an index of oil stability (30). Each sample (20 mL) was stored separately in the oven at 65°C for 144 or 200 h in small open glass containers (of 30 mm diameter and 60 mm height) for other chemical analyses. Treated samples were removed after 0, 24, 48, 84, and 144 h, flushed with nitrogen for 30 s, covered with aluminum foil-parafilm and stored at −20°C for further analyses (usually within 10 d).

Chemical analyses of oils, subjected to accelerated oxidation, included determination of peroxide value (PV) (27; method number Cd 8-53), conjugated dienes (CD) as reflected in ultraviolet (UV) absorbance at 234 nm (31; method number 2.505) and percentage inhibition of formation of 2-thiobarbituric acid-reactive substances (TBARS) by the classical TBA procedure (27; method number Cd 19-90) as given in the following equation:

$$\% \text{ inhibition of TBARS formation} = \left(1 - \frac{\text{TBARS content of treated sample}}{\text{TBARS content of control}}\right) \times 100 \quad [1]$$

All experiments and/or measurements were replicated three times; mean values ± SD are reported for each case. ANOVA and Tukey's studentized range test (32) were performed on the Statistical Analysis System (33) to evaluate the significance of differences among different mean values.

RESULTS AND DISCUSSION

RBD-seal blubber oil and RBD-menhaden oil had initial iodine values of 145.4 and 171.8 g iodine/100 g oil, respectively, and possessed low PV of 1.09 and 3.05 meq/kg oil and acid values (free fatty acid contents) of 0.04 and 0.07 mg KOH/g oil, respectively (Table 1). The higher PV of menhaden oil may be due to its higher content of total PUFA (33.6%) as compared to seal blubber oil (22.6%); this results in the formation of more hydroperoxides as the former samples were received two weeks after their production. Fatty acid composition of oils showed that menhaden oil had a higher amount of EPA (13.2%) and DHA (10.0%) as compared to seal blubber oil, but the latter had a higher content of DPA (4.7%), which is less abundant in fish oils (2.4% in menhaden oil). Furthermore, seal blubber oil contained a significantly higher amount of monoenes (59.9%) as compared to menhaden oil (27.3%).

TABLE 1
Characteristics and Fatty Acid Composition of Refined, Bleached, and Deodorized Seal Blubber and Menhaden Oils[a]

Parameter	Seal blubber oil	Menhaden oil
Iodine value (g iodine/100 g oil)	145.4 ± 0.35	171.8 ± 3.01
Peroxide value (meq/kg oil)	1.09 ± 0.03	3.05 ± 0.20
Acid value (mg KOH/g oil)	0.04 ± 0.00	0.07 ± 0.01
Fatty acids (area %)		
14:0	3.73 ± 0.08	8.32 ± 0.12
14:1n-9	1.09 ± 0.04	0.38 ± 0.01
15:0	0.23 ± 0.00	0.71 ± 0.02
16:0	5.98 ± 0.03	17.12 ± 0.25
16:1n-7	18.02 ± 0.04	11.36 ± 0.14
17:0	0.92 ± 0.00	2.45 ± 0.12
17:1	0.55 ± 0.02	1.86 ± 0.03
18:0	0.88 ± 0.00	3.33 ± 0.02
18:1n-9	20.83 ± 0.06	6.68 ± 0.12
18:1n-11	5.22 ± 0.03	3.46 ± 0.07
18:2n-6	1.51 ± 0.02	1.42 ± 0.09
18:3n-3	0.59 ± 0.00	1.82 ± 0.00
18:4n-3	1.00 ± 0.02	2.90 ± 0.05
20:0	0.11 ± 0.00	0.20 ± 0.01
20:1n-9	12.16 ± 0.02	1.44 ± 0.06
20:2n-6	0.16 ± 0.00	0.21 ± 0.00
20:3n-6	0.14 ± 0.00	0.46 ± 0.03
20:4n-6	0.46 ± 0.01	0.83 ± 0.02
20:5n-3	6.41 ± 0.08	13.23 ± 0.18
22:0	—	0.12 ± 0.00
22:1n-11	2.01 ± 0.04	0.12 ± 0.05
22:2	—	0.02 ± 0.00
22:4n-6	0.11 ± 0.01	0.19 ± 0.03
22:5n-3	4.66 ± 0.01	2.40 ± 0.03
22:6n-3	7.58 ± 0.02	10.06 ± 0.11

[a]All values are mean of three replicates ± SD.

Weight-gain data, over a 200-h period, for both oils treated with antioxidants are presented in Figure 2. All antioxidant-treated samples showed a delayed induction period as compared to their control counterparts. The time required to achieve a 0.5% weight increase for samples was 33, 42, 49, 70, 76, 112, 137, and 170 h for seal blubber oil containing α-tocopherol, BHA, BHT, EC, EGC, TBHQ, EGCG, and ECG, respectively, as compared with 24 h for the control sample. The 0.5% weight-gain time for treated menhaden oil with the same antioxidants was 18, 19, 22, 35, 42, 72, 95, and 112 h, respectively, as compared with 9 h for the control sample. It has been suggested that each storage day (24 h) under Schaal oven test conditions at 65°C is equivalent to one month of storage at ambient temperatures (34). The extension of the induction period of oils treated with tea catechins was much longer than those of α-tocopherol-, BHA-, or BHT-treated samples in both oils. Furthermore, EGCG and ECG extended the induction period by 5.7 and 7.1 times that of the control for seal blubber oil and 8.0 and 12.4 times for menhaden oil, respectively. A gradual increase was noticed in the percentage weight gain of all samples, reaching a maximum value,

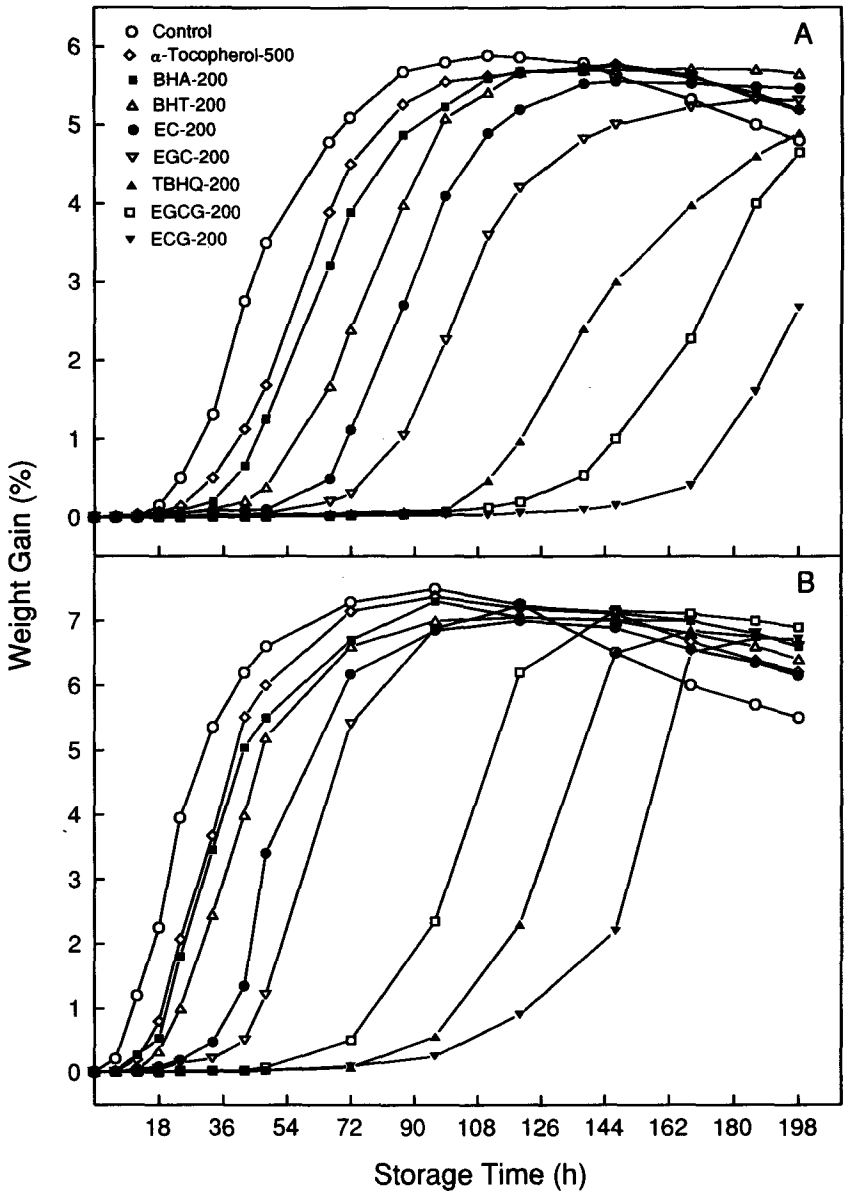

FIG. 2. Effect of tea catechins and conventional antioxidants on the weight gain of refined bleached and deodorized (RBD)-seal blubber oil (A) and RBD-menhaden oil (B) during prolonged storage at 65°C. BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; EC, (–) epicatechin; EGC, (–) epigallocatechin; TBHQ, *tert*-butylhydroquinone; EGCG, (–) epigallocatechin gallate; ECG, (–) epicatechin gallate.

followed by an eventual decrease during the latter stages of storage. The rate of weight-gain and maximum values reached were slightly higher for menhaden oil than seal blubber oil. This may be due to the fact that rate of addition of oxygen to lipid molecules to form hydroperoxides is higher for menhaden oil, which contains a higher PUFA content than seal blubber oil. Farmer *et al.* (35) and Privett and Nickell (36) have reported that the addition of oxygen to lipids to form hydroperoxides is reasonably quantitative during the initial stages of oxidation. The primary purpose of adding antioxidants to lipids is to delay the onset of oxidation and accumulation of oxidative products. Thus, tea catechins delayed the accumulation of oxidative products in both seal blubber and menhaden oils, even though to different degrees.

The PV of seal blubber and menhaden oils treated with tea catechins decreased (at least by 60% even after 144 h storage) significantly ($P < 0.05$) during their storage at 65°C (Tables 2 and 3). However, samples treated with α-tocopherol, BHA, and BHT showed higher PV as compared to tea catechins under similar experimental conditions. Among tea cate-

TABLE 2
Effect of Tea Catechins and Conventional Antioxidants on the Peroxide Value (meq/kg oil) of Refined, Bleached, and Deodorized Seal Blubber Oil Stored at 65°C[a]

Treatment[b]	Storage time (h)				
	0	24	48	84	144
Control	2.09 ± 0.06^{a}	25.2 ± 0.79^{a}	49.3 ± 1.22^{a}	119 ± 4.24^{a}	183 ± 2.34^{a}
EC-200	2.03 ± 0.03^{a}	6.7 ± 0.13^{d,e}	15.8 ± 0.80^{d}	45.9 ± 3.21^{e}	79.3 ± 3.91^{e}
EGC-200	2.19 ± 0.15^{a}	6.2 ± 0.21^{d,e}	10.0 ± 0.31^{e,f}	41.3 ± 0.97^{e}	59.9 ± 1.07^{f}
EGCG-200	2.16 ± 0.01^{a}	5.6 ± 0.60^{d,e}	9.5 ± 0.20^{e,f}	37.9 ± 0.94^{e,f}	51.3 ± 1.70^{f,g}
ECG-200	2.08 ± 0.09^{a}	4.6 ± 0.09^{e}	7.9 ± 0.08^{f}	27.6 ± 0.78^{g}	47.0 ± 2.59^{g}
α-Tocopherol-500	2.18 ± 0.01^{a}	21.5 ± 2.05^{b}	44.5 ± 0.91^{b}	110 ± 0.80^{b}	166 ± 1.23^{b}
BHA-200	2.19 ± 0.02^{a}	13.3 ± 1.27^{c}	42.6 ± 0.01^{b}	71.3 ± 1.38^{c}	124 ± 2.68^{c}
BHT-200	2.11 ± 0.06^{a}	13.3 ± 0.18^{c}	31.6 ± 0.42^{c}	58.8 ± 2.18^{d}	94.9 ± 2.11^{d}
TBHQ-200	2.09 ± 0.07^{a}	8.0 ± 0.24^{d}	10.4 ± 0.31^{e}	29.9 ± 0.86^{f,g}	53.4 ± 1.20^{f,g}

[a]Values in the same column bearing different superscripts are significantly ($P > 0.05$) different.
[b](–)Epicatechin (EC), (–) epigallocatechin (EGC), (–)epigallocatechin gallate (EGCG), (–)epicatechin gallate (ECG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ).

TABLE 3
Effect of Tea Catechins and Conventional Antioxidants on the Peroxide Value (meq/kg oil) of Refined, Bleached, and Deodorized Menhaden Oil Stored at 65°C[a]

Treatment	Storage time (h)				
	0	24	48	84	144
Control	4.53 ± 0.41^{a}	38.1 ± 0.77^{a}	96.5 ± 1.91^{a}	283 ± 4.58^{a}	346 ± 5.82^{a}
EC-200	4.05 ± 0.44^{a}	14.2 ± 1.10^{c}	47.6 ± 3.03^{d}	97.4 ± 2.26^{e}	133 ± 3.54^{d}
EGC-200	4.14 ± 0.20^{a}	12.1 ± 1.42^{c,d}	31.7 ± 0.95^{e}	92.0 ± 2.05^{e}	133 ± 3.57^{d}
EGCG-200	4.10 ± 0.16^{a}	9.2 ± 0.32^{d,e}	14.8 ± 0.97^{f}	81.1 ± 1.60^{f}	113 ± 3.77^{e}
ECG-200	4.09 ± 0.13^{a}	7.6 ± 0.52^{e}	9.7 ± 0.26^{g}	75.9 ± 1.31^{f}	101 ± 2.10^{f}
α-Tocopherol-500	4.10 ± 0.12^{a}	37.3 ± 0.42^{a}	66.3 ± 0.50^{b,c}	227 ± 5.59^{b}	280 ± 6.10^{b}
BHA-200	4.27 ± 0.06^{a}	36.1 ± 1.44^{a,b}	71.3 ± 0.94^{a,b}	135 ± 4.90^{c}	163 ± 3.60^{c}
BHT-200	4.43 ± 0.01^{a}	32.9 ± 1.63^{b}	63.3 ± 1.18^{c}	120 ± 0.82^{d}	137 ± 2.70^{d}
TBHQ-200	4.22 ± 0.02^{a}	15.1 ± 1.30^{c}	15.1 ± 0.14^{f}	42.8 ± 1.57^{g}	101 ± 1.02^{f}

[a]Values in the same column bearing different superscripts are significantly ($P > 0.05$) different. See Table 2 for abbreviations.

TABLE 4
Effect of Tea Catechins and Conventional Antioxidants on the Conjugated Diene Value of Refined, Bleached, and Deodorized Seal Blubber Oil Stored at 65°C[a]

Treatment	Storage time (h)				
	0	24	48	84	144
Control	8.4 ± 0.01^{a}	10.9 ± 0.06^{a}	12.6 ± 0.50^{a}	17.1 ± 1.01^{a}	28.3 ± 0.02^{a}
EC-200	8.1 ± 0.10^{a}	8.4 ± 0.08^{d}	9.3 ± 0.05^{c}	12.0 ± 0.02^{c}	17.5 ± 0.61^{c}
EGC-200	8.0 ± 0.54^{a}	8.3 ± 0.48^{d,e}	9.0 ± 0.20^{c,d}	10.9 ± 0.56^{c,d}	15.3 ± 0.23^{d}
EGCG-200	8.1 ± 0.77^{a}	8.1 ± 0.71^{d,e}	8.4 ± 0.14^{d}	10.0 ± 0.17^{d}	13.2 ± 0.66^{e}
ECG-200	8.0 ± 0.01^{a}	8.1 ± 0.04^{e}	8.3 ± 0.56^{d}	8.4 ± 0.02^{e}	10.6 ± 0.63^{f}
α-Tocopherol-500	8.3 ± 0.08^{a}	8.1 ± 0.04^{b}	12.4 ± 0.13^{a}	16.3 ± 0.54^{a}	24.6 ± 0.46^{b}
BHA-200	8.3 ± 0.17^{a}	9.3 ± 0.11^{b}	10.2 ± 0.05^{b}	14.8 ± 0.17^{b}	18.5 ± 0.25^{c}
BHT-200	8.2 ± 0.27^{a}	8.7 ± 0.05^{c}	9.2 ± 0.12^{c}	14.7 ± 0.15^{b}	18.2 ± 0.05^{c}
TBHQ-200	8.0 ± 0.28^{a}	8.2 ± 0.16^{d,e}	8.6 ± 0.30^{d}	9.8 ± 0.38^{d}	11.5 ± 0.17^{f}

[a]Values in the same column bearing different superscripts are significantly ($P > 0.05$) different. See Table 2 for abbreviations.

chins tested, ECG served best in lowering peroxide formation in seal blubber oil, even to a greater extent than TBHQ, which is the most effective antioxidant used by the food industry. However, menhaden oil samples that contained ECG maintained lower PV as compared to those containing TBHQ for up to 48 h of storage, after which the TBHQ-treated oil had significantly ($P > 0.05$) lower PV than all other treatments. The potency of ECG was 5.6 and 6.8 times that of α-tocopherol and 5.4 and 7.4 times that of BHA in seal blubber oil and menhaden oil after 48 h of storage, respectively. Changes in CD values of treated oils as a function of time are shown in Tables 4 and 5. Similar to PV data, CD values reflect the formation of primary products during lipid oxidation, and samples that contained tea catechins (both seal blubber and

TABLE 5
Effect of Tea Catechins and Conventional Antioxidants on the Conjugated Diene Value of Refined, Bleached, and Deodorized Menhaden Oil Stored at 65°C[a]

Treatment	Storage time (h)				
	0	24	48	84	144
Control	15.4 ± 0.11[a]	20.6 ± 0.48[a]	24.6 ± 0.24[a]	40.5 ± 0.51[a]	53.0 ± 2.25[a]
EC-200	14.9 ± 0.10[a]	16.7 ± 0.31[c]	18.4 ± 0.11[d,e]	25.2 ± 0.48[d]	32.0 ± 0.21[d]
EGC-200	14.8 ± 0.10[a]	16.3 ± 0.02[c]	18.0 ± 0.07[e]	28.6 ± 0.01[c]	29.6 ± 0.70[d,e]
EGCG-200	14.9 ± 0.04[a]	15.2 ± 0.20[d]	12.6 ± 0.62[e,f]	22.9 ± 0.12[e]	25.0 ± 0.17[f]
ECG-200	14.8 ± 0.01[a]	14.9 ± 0.06[d]	16.4 ± 0.52[f]	22.5 ± 0.13[e,f]	24.0 ± 0.10[f]
α-Tocopherol-500	15.5 ± 0.65[a]	19.3 ± 0.03[b]	21.0 ± 1.00[b]	37.2 ± 0.60[b]	47.5 ± 0.70[c]
BHA-200	15.0 ± 0.50[a]	16.9 ± 0.26[c]	20.6 ± 0.60[b,c]	27.5 ± 0.74[c]	38.2 ± 1.61[c]
BHT-200	15.3 ± 0.55[a]	16.9 ± 0.10[c]	19.6 ± 0.11[c,d]	27.5 ± 0.83[c]	38.0 ± 1.59[f]
TBHQ-200	15.3 ± 0.46[a]	16.5 ± 0.18[c]	17.9 ± 0.71[e]	20.8 ± 0.08[f]	27.1 ± 0.51[f]

[a]Values in the same column bearing different superscripts are significantly ($P > 0.05$) different. See Table 2 for abbreviations.

menhaden oils) exhibited significantly ($P < 0.05$) lower CD values as compared to the control and oils containing α-tocopherol and BHA. For up to 144 h of storage at 65°C, the CD values of the control seal blubber oil sample increased from 8.4 to 28.2. Corresponding values for seal blubber oil treated with EC, EGC, EGCG, and ECG increased from 8.0–8.1 to 17.5, 15.3, 13.2 and 10.6, respectively. A similar trend was observed for catechin-treated menhaden oil samples.

The 2-TBARS test, which measures secondary products of lipid oxidation, is frequently used for monitoring stability of marine oils and was also employed in this study. Addition of tea catechins, α-tocopherol, and other antioxidants to both seal blubber oil and menhaden oil showed a significant ($P < 0.05$) effect in lowering the formation of TBARS in comparison with the control sample. The order of potency of these additives in inhibiting the formation of TBARS in both seal blubber oil and menhaden oil was in the decreasing order of ECG > TBHQ > EGCG > EGC > EC > BHT > BHA > α-tocopherol (Fig. 3). Among catechins tested, ECG and EGCG exhibited >50% inhibition of TBARS formation in treated seal blubber oil, whereas for menhaden oil, only ECG-treated sample showed a similar inhibition of oxidation as reflected in the TBARS values. The TBHQ-treated samples also exhibited excellent stability as TBARS production was inhibited by 57.4 and 51.1% for seal blubber oil and menhaden oil, respectively. However, TBHQ is not yet licensed for food use in Canada and in the European countries. The most commonly used synthetic antioxidants, namely BHA and BHT, were not as effective as tea catechins in inhibiting the formation of flavor-active secondary oxidation products. Among

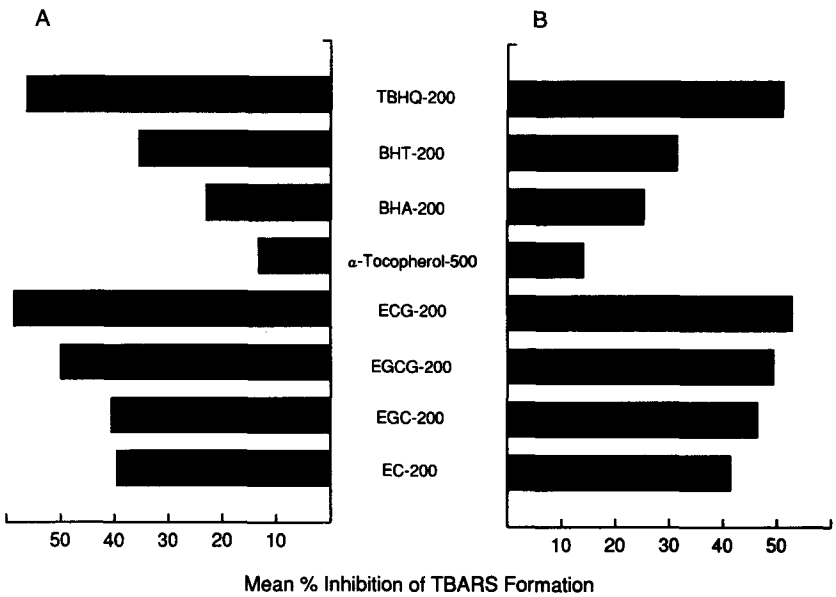

FIG. 3. Percentage inhibition of 2-thiobarbituric acid-reactive substances (TBARS) formation by tea catechins and conventional antioxidants during prolonged storage of RBD-seal blubber (A) and RBD-menhaden oils (B). See Figure 2 for abbreviations.

the catechins tested, ECG was 2.6 and 1.7 times more potent in seal blubber oil and 2.1 and 1.7 times more effective in menhaden oil in inhibiting the formation of TBARS as compared to BHA and BHT, respectively.

The main purpose of using antioxidants in lipids is to delay accumulation of free radicals (to lower the free radical burden) and thus to enhance their oxidative stability. Catechins with free hydroxyl groups may act as free-radical acceptors and could also delay the formation of free radicals. Catechins that possess multiple hydroxyl groups (Fig. 1) exhibited good antioxidant activity and extended the induction period in both oils more effectively than BHA and BHT, which have only one hydroxyl group in their chemical structures. When in *ortho* configuration, multiple hydroxyl groups may participate in chelation of metal ions, in addition to their free-radical scavenging effect.

In summary, tea catechins exhibited excellent antioxidant properties in both seal blubber and menhaden oils, as evidenced by weight-gain, peroxide, CD, and TBARS values. The order of potency of the catechins in marine oils was ECG > EGCG > EGC > EC. Tea catechins may act as primary antioxidants by donating a hydrogen atom and act as free-radical acceptors or chain breakers and may also act as metal chelators. Extensive hydroxylation of catechin molecules would perhaps be the main reason for their strong antioxidative properties as compared with BHA, BHT, or α-tocopherol. Torel *et al.* (37) have demonstrated that catechins suppress lipid peroxidation in biological tissues and subcellular fractions. Hara (26) have illustrated effectiveness of catechins as antioxidants in salad oils, whereas tocopherols and BHA were ineffective. Chen and Ho (38) have tested the inhibitory effect of tea catechins on the production of superoxide and have found that their order of potency is EGCG > ECG > EGC > EC. Our findings have demonstrated that tea catechins may be considered as potential antioxidants for stabilization of highly unsaturated marine oils. Therefore, green tea extracts that contain 49, 14, 11 and 6% of EGCG, ECG, EGC, and EC, respectively (15), may be used as natural antioxidants to stabilize lipids and lipid-containing foods.

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