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Antioxidant and pro-oxidant activity of green tea extracts in marine oils

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The activity of green tea extracts (GTE) on the oxidation of refined, bleached and deodorized (RBD) seal blubber oil (SBO) and menhaden oil (MHO) was examined under Schaal oven conditions at 65°C. Progression of oxidation was monitored using weight gain, peroxide value (PV) and 2-thiobarbituric acid-reactive substances (TBARS) data. GTE exhibited a pro-oxidant effect in both oils examined, perhaps due to the catalytic effect of their chlorophyll constituents. Therefore, in follow-up experiments, a column chromatographic technique was employed to remove chlorophyll from GTE. The resultant dechlorophyllized green tea extract (DGTE) was applied to both SBO and MHO at 100, 200, 500 and 1000 ppm levels. The antioxidant activity of DGTE was compared with the effects of the commonly-used antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) at 200 ppm and α-tocopherol at 500 ppm. DGTE at ≥200 ppm exhibited excellent antioxidant activity in both oils and its efficacy was higher than that of BHA, BHT and α-tocopherol, but less than that of TBHQ.

INTRODUCTION

Marine oils are rich in polyunsaturated fatty acids (PUFA), especially those of the ω3 family such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Shahidi *et al.*, 1997). During the last two decades interest in the dietary effects of ω3-PUFA has increased because of their ability to lower serum triacylglycerols and cholesterol and in their conversion to eicosanoids which are known to reduce thrombosis (Kinsella, 1986). In addition, these fatty acids play an important role in the prevention and possible treatment of coronary heart disease, hypertension, arthritis, and other inflammatory and autoimmune disorders. Therefore, increased consumption of marine lipids has been suggested in order to provide adequate intake of ω3 fatty acids in our diet (Simopoulos, 1991). However, despite their health benefits, PUFA are highly sensitive to oxidative deterioration and thus pose practical problems (Cho *et al.*, 1987).

Degradation of PUFA, via a free radical chain mechanism, results in changes in odour and flavour (rancidity) of edible oils, and lipid-containing foods (Frankel, 1980). Chemical reactions involved in oxidative processes require low activation energies (4–14 kcal mol^{-1}) and their rates are not changed significantly at lower storage temperatures (Labuza, 1971). Therefore, it is necessary to delay the onset of oxidation of marine oils in order to maintain their bland favour (Wanasundara and Shahidi, 1997). Phenolic compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ), have been widely used as antioxidants in food lipids. However, possible toxicity and/or mutagenicity of these antioxidants has been a subject of study for many years (Branen, 1975). There are several recent reports on the effect of BHA on conversion of ingested material into toxic substances or carcinogens due to increased secretion of microsomal enzymes of liver and extra-hepatic organs, such as the lungs and gastrointestinal tract mucosa (Ito *et al.*, 1982; Wattenberg, 1986; Barlow, 1990). Therefore, at the present time, the Food and Drug Administration (FDA) in the United States is examining possible removal of BHA from the GRAS (generally recognized as safe) list. Furthermore, Ito *et al.* (1985) reported that BHT is carcinogenic in rats; this antioxidant is also in the process of being carefully scrutinized. In addition, TBHQ has not been approved for food use in Europe, Japan and Canada. Thus, natural antioxidants have gained popularity in recent years.

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)

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and its mixed counterparts being most frequently used. Although tocopherols are considered as safe natural antioxidants, they do not always provide effective protection against *in vitro* oxidation (Frankel, 1980). Therefore, research on other natural antioxidants has gained momentum as they are considered, rightly or wrongly, to pose no health risk to consumers (Wanasundara and Shahidi, 1994*a*; Wanasundara *et al.*, 1994). Naturally-occurring antioxidative components in foods include flavonoids, phenolic acids, lignan precursors, terpenes, mixed tocopherols, phospholipids, polyfunctional organic acids and also plant extracts such as those of rosemary and sage (Schuler, 1990; Wanasundara *et al.*, 1997).

Green tea leaves contain up to 36% polyphenols, on a dry weight basis; however, their composition varies with climate, season, variety and state of maturity (Lunder, 1989). Catechins are the predominant group of green tea polyphenols and are comprised of (−)epicatechin (EC), (−)epicatechin gallate (ECG), (−)epigallocatechin (EGC), (−)epigallocatechin gallate (EGCG), (+)catechin and (+)gallocatechin (GC) (Huang *et al.*, 1992; Shahidi *et al.*, 1992; Ho *et al.*, 1994; Amarowicz and Shahidi, 1995). In recent years, catechins have been shown to possess physiological potential with respect to their antimutagenic and antitumorigenic activities (Hara *et al.*, 1989; Conney *et al.*, 1992; Hara, 1994). Epidemiological studies have also suggested that tea polyphenols are effective in cancer prevention (Kim *et al.*, 1994). Furthermore, catechins and other flavonoids have been recognized as efficient antioxidants for scavenging oxygen radicals and chelating metal ions (Sorata *et al.*, 1984; Husain *et al.*, 1987; Chen *et al.*, 1990; Shahidi *et al.*, 1991, 1993; Shahidi and Wanasundara, 1992; Wanasundara and Shahidi, 1994*b*; Chen and Ho, 1995). Hara (1994) 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. However, recently Yen *et al.* (1997) have shown that water extracts of various tea leaves markedly stimulated the oxidation of deoxyribose in the presence of Fe^{+3} and H_2O_2. Nonetheless, the pro-oxidative effect of these extracts was system-dependent.

The objectives of this study were to examine the effect of green tea extracts on the oxidative stability of refined-bleached and deodorized seal blubber and menhaden oils. The efficacy of these extracts was also compared with those of the commonly-used food antioxidants, such as α-tocopherol, BHA, BHT and TBHQ.

MATERIALS AND METHODS

Freshly-prepared, refined-bleached and deodorized (RBD) menhaden oil (MHO), 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 were carried out as described elsewhere (Shahidi *et al.*, 1994). The refined-bleached seal blubber oil (SBO) was deodorized using a laboratory scale vacuum steam-distillation apparatus. The oil was heated to 100 ± 5°C using steam, while under vacuum. Volatile compounds were then recovered during the deodorization process over a 5 h period and the resulting RBD-SBO was kept under a blanket of nitrogen at −60°C until used. Commercial antioxidants namely BHA, BHT, TBHQ and α-tocopherol were obtained from Sigma Chemical Company (St Louis, MO). Fatty acid methyl esters (FAMEs) were purchased from either Supelco (Oakville, ON) or NU-Chek-Prep Inc. (Elysian, MN). All other chemicals used in this study were of ACS-grade or better quality. The fresh RBD-SBO and RBD-MHO were analyzed by determining their iodine, peroxide and acid values (AOCS, 1990; method numbers Cd 1-25, Cd 8-53 and Cd 3a-63, respectively) and fatty acid compositions. FAMEs were prepared (Wanasundara and Shahidi, 1996) and then analyzed on a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Mississuaga, ON) equipped with a flame-ionization detector and split/splitless injector. A Supelcowax 10 column (0.25 mm × 60 m; 0.25 μm film thickness, Supelco, Oakville, ON) was used for separation of FAMEs. Chromatographic parameters were set as follows: injector and detector temperatures, 250°C; oven temperature programming: hold 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 a carrier gas at flow rate of 15 ml/min. FAMEs were identified by comparison of their retention times with those of the reference standards. The content of fatty acids was calculated from their corresponding integration data.

The dried green tea leaves were obtained from Anhui Province of China and were then ground using a Moulinex coffee grinder. Preparation of green tea extracts was carried out as given in Fig. 1. The ground tea leaves (10 g) were homogenized with 50% aqueous ethanol (100 ml) at room temperature using a Polytron homogenizer (PT-3000; Brinkmann, Rexdale, ON). The homogenate was then filtered through a Whatman No 1 filter paper. The collected filtrate was mixed with an equal volume of chloroform and allowed to separate. The upper aqueous layer was extracted three times with an equal volume of ethyl acetate. The ethyl acetate extracts were combined and evaporated to dryness under vacuum at 40°C, using a rotary evaporator (BÜCHI RE 111, Büchi Laboratoriums-Technik, Flawil, Switzerland). The yield of dried green tea extracts (GTE) from the original tea leaves was 30–35%.

In order to remove chlorophyll from the crude GTE, a column (1.25 cm internal diameter and 20 cm height) packed with TOYO PEARL HW-40 (Bioseparation Specialists, Montgoneyvile, PA) was used. Crude

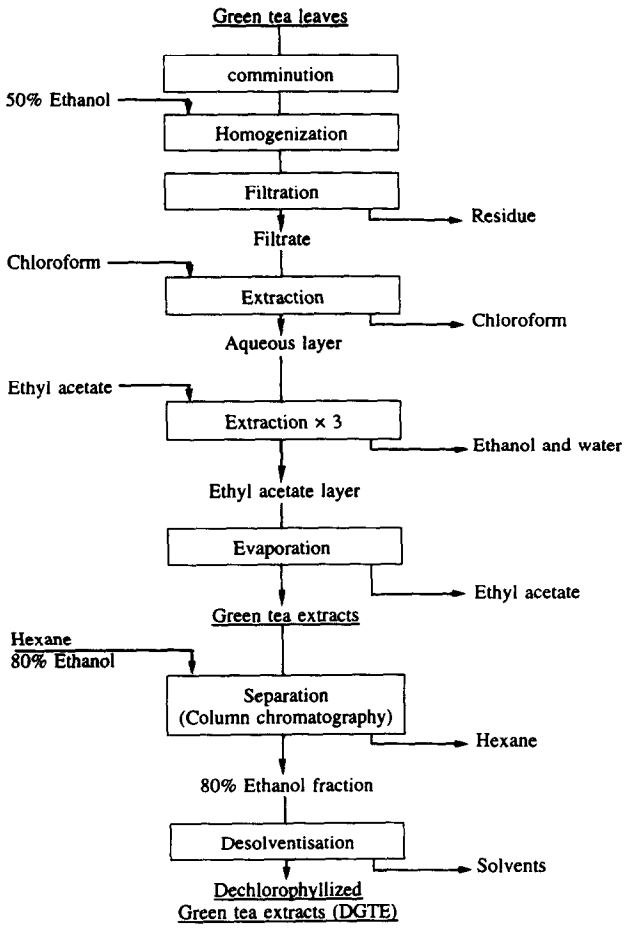

Fig. 1. Flowsheet for preparation of dechlorophyllized green tea extracts (DGTE).

extracts (2 g) were dissolved in 5 ml of 80% (v/v) aqueous ethanol and subsequently introduced onto the column. The column was eluted with hexane until all the residual green colour had disappeared. The column was then washed with 80% (v/v) aqueous ethanol to recover the dechlorophyllized green tea extracts (DGTE). Ethanol was removed by evaporation under vacuum at 40°C, using a rotary evaporator and the residual water was removed by lyophilization (Labconco 5 freeze dryer, Labconco Co. Kansas City, MO) at −60°C and 0.01 torr pressure. The yield of DGTE from the original tea leaves was 15–20%. The extracts so obtained were transferred into air-tight glass vials and stored at −20°C until used.

GTE (500 and 1000 ppm), DGTE (100, 200, 500 and 1000 ppm) and commercial antioxidants BHA, BHT and TBHQ (200 ppm), as well as α-tocopherol (500 ppm), were dissolved in a minimum volume of absolute ethanol and added to RBD-SBO and RBD-MHO 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 described elsewhere (Wanasundara and Shahidi, 1996). Two grams of each sample, prepared as given above, were placed in glass Petri dishes (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 using a forced-air oven (Thelco, Precision Scientific Co, Chicago, IL) at 65°C. The time required to attain a 0.5% weight increase for each sample was taken as an index of oil stability (Wanasundara and Shahidi, 1994*a*). Each sample (20 ml) was stored separately in the oven at 65°C for 144 h (GTE-treated samples were stored up to 84 h only) in small open glass containers (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 aluminium foil-parafilm and stored at −60°C for further analyses (usually within 10 days).

Chemical analyses of oils, subjected to accelerated oxidation, included determination of peroxide value (PV) (AOCS, 1990; method number Cd 8-53) and 2-thiobarbituric acid-reactive substances (TBARS) using the classical TBA procedure (AOCS, 1990; method number Cd 19-90). All measurements were replicated three times; mean values ± standard deviations were reported for each case. Analyses of variance (ANOVA) and Tukey's studentized range test (Snedecor and Cochran, 1980) were performed on Statistical Analysis System (SAS, 1980) to evaluate the significance of differences among mean values.

RESULTS AND DISCUSSION

The initial iodine values of RBD-SBO and RBD-MHO were 145 and 172 g iodine per 100 g oil, respectively. The corresponding peroxide values of the oils were 1.09 and 3.05 meq kg^{-1} oil and they had acid values (free fatty acid contents) of 0.04 and 0.07 mg KOH g^{-1} oil, respectively (Table 1). The higher PV of MHO compared to SBO is not surprising considering its higher content of total PUFA (33.6%) as compared to that of SBO (22.6%), as these samples were received two weeks after their production. Fatty acid composition of oils showed that MHO had higher amounts of EPA (13.2%) and DHA (10.0%) than SBO, but the latter had a higher content of docosapentaenoic acid (DPA; 4.7%) which is less abundant in fish oils (2.4% in MHO). Furthermore, SBO contained a significantly higher amount of monoenes (59.9%) than MHO (27.3%).

Table 2 shows the effect of addition of GTE on oxidative stability of both oils, as reflected in their PV and TBARS values during storage at 65°C. The PV and TBARS of all treated samples were higher than those of their control counterparts, thus reflecting the pro-oxidant effect of GTE. This pro-oxidative effect of GTE may be due to the presence of chlorophyll. Endo *et al.* (1985) have also reported that chlorophylls and their derivatives promote oxidation of lipids during storage.

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^{-1} oil)	145 ± 0.35	172 ± 3.01
Peroxide value (meq kg^{-1} oil)	1.09 ± 0.03	3.05 ± 0.20
Acid value (mg KOH g^{-1} oil)	0.04 ± 0.00	0.07 ± 0.01
Alpha tocopherol (mg 100 g^{-1} oil)	2.40 ± 0.09	6.90 ± 0.21
Fatty acids (area %)		
C14:0	3.73 ± 0.08	8.32 ± 0.12
C14:1ω9	1.09 ± 0.04	0.38 ± 0.01
C15:0	0.23 ± 0.00	0.71 ± 0.02
C16:0	5.98 ± 0.03	17.1 ± 0.25
C16:1ω7	18.0 ± 0.04	11.4 ± 0.14
C17:0	0.92 ± 0.00	2.45 ± 0.12
C17:1	0.55 ± 0.02	1.86 ± 0.03
C18:0	0.88 ± 0.00	3.33 ± 0.02
C18:1ω9	20.8 ± 0.06	6.68 ± 0.12
C18:1ω11	5.22 ± 0.03	3.46 ± 0.07
C18:2ω6	1.51 ± 0.02	1.42 ± 0.09
C18:3ω3	0.59 ± 0.00	1.82 ± 0.00
C18:4ω3	1.00 ± 0.02	2.90 ± 0.05
C20:0	0.11 ± 0.00	0.20 ± 0.01
C20:1ω9	12.2 ± 0.02	1.44 ± 0.06
C20:2ω6	0.16 ± 0.00	0.21 ± 0.00
C20:3ω6	0.14 ± 0.00	0.46 ± 0.03
C20:4ω6	0.46 ± 0.01	0.83 ± 0.02
C20:5ω3	6.41 ± 0.08	13.2 ± 0.18
C22:0	–	0.12 ± 0.00
C22:1ω11	2.01 ± 0.04	0.12 ± 0.05
C22:2	–	0.02 ± 0.00
C22:4ω6	0.11 ± 0.01	0.19 ± 0.03
C22:5ω3	4.66 ± 0.01	2.40 ± 0.03
C22:6ω3	7.58 ± 0.02	10.1 ± 0.11

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

However, He and Shahidi (1997) have recently shown that GTE, despite the presence of chlorophyll, had an antioxidant effect when applied to white muscles of mackerel. Therefore, it is evident that the antioxidant/pro-oxidant activity of GTE is system-dependent.

In the follow up experiments, a column chromatographic technique was employed to remove the chlorophylls from GTE (see Fig. 1). The resultant dechlorophyllized green tea extract (DGTE) was then added to both SBO and MHO. The weight-gain data for both oils treated with DGTE and other antioxidants are presented in Fig. 2. All 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, 40, 42, 50, 49, 66, 76 and 112 h for SBO containing α-tocopherol-500, DGTE-100, BHA-200, BHT-200, DGTE-200, DGTE-500, DGTE-1000 and TBHQ-200, respectively, as compared to a 24 h period for the control sample. The time for a 0.5% weight gain for treated MHO with the same antioxidants was 15, 20, 19, 22, 35, 42, 49 and 93 h, respectively, as compared to a 9 h period for the control sample. It has been suggested that each storage day (24 h) under Schaal oven conditions at 65°C is equivalent to one month of storage at ambient temperatures (Evans *et al.*, 1973; Abou-Gharbia *et al.*, 1996). The extension of the induction period of both oils treated with DGTE over 200 ppm was much longer than those containing α-tocopherol, BHA or BHT. Furthermore, DGTE-500 and DGTE-1000 extended the induction period by 2.8 and 3.2 times that of the control for SBO and 4.7 and 5.4 times for MHO, respectively. A gradual increase was noticed in the percentage weight-gain of all samples, reaching a maximum value followed by an eventual decrease during the later stages of storage. The rate of weight-gain was slightly higher for MHO than SBO. This may be due to the fact that rate of addition of oxygen to lipid molecules, to form hydroperoxides, is higher for MHO because of its higher content of PUFA as compared to SBO. Privett and Nickell (1956) have reported that the addition of oxygen to unsaturated fatty acids 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, DGTE delayed the accumulation of oxidative products in both SBO and MHO.

Table 2. Effect of green tea extracts (GTE) on the peroxide value (PV; meq kg^{-1} oil) and 2-thiobarbituric acid-reactive substances (TBARS; μmol g^{-1} oil) value of refined, bleached and deodorized seal blubber oil (SBO) and menhaden oil (MHO) stored under Schaal oven conditions at 65°C[a]

Treatment	After 24 h		After 48 h		After 84 h	
	PV	TBARS	PV	TBARS	PV	TBARS
SBO						
Control	25.2 ± 0.79[a]	8.78 ± 0.23[a]	49.3 ± 1.22[a]	12.3 ± 0.37[a]	119 ± 4.24[a]	19.4 ± 0.54[a]
GTE-500	29.4 ± 1.02[b]	8.99 ± 0.58[a]	63.6 ± 4.21[b]	14.2 ± 1.11[a]	142 ± 4.55[b]	22.7 ± 0.31[b]
GTE-1000	31.3 ± 0.85[b]	10.8 ± 0.34[b]	71.0 ± 2.36[c]	19.1 ± 0.91[b]	174 ± 8.32[c]	23.1 ± 0.33[b]
MHO						
Control	38.1 ± 0.77[a]	17.8 ± 0.36[a]	96.5 ± 1.91[a]	25.8 ± 1.11[a]	283 ± 4.58[a]	42.3 ± 0.41[a]
GTE-500	61.1 ± 1.25[b]	28.1 ± 1.33[b]	135 ± 3.91[b]	45.3 ± 1.35[b]	280 ± 9.11[a]	43.1 ± 1.44[a]
GTE-1000	67.9 ± 1.31[b]	29.3 ± 1.41[b]	138 ± 4.50[b]	51.4 ± 2.01[c]	284 ± 5.11[a]	48.0 ± 2.11[a]

[a]Values in each column for each oil bearing different superscripts are significantly ($p < 0.05$) different.

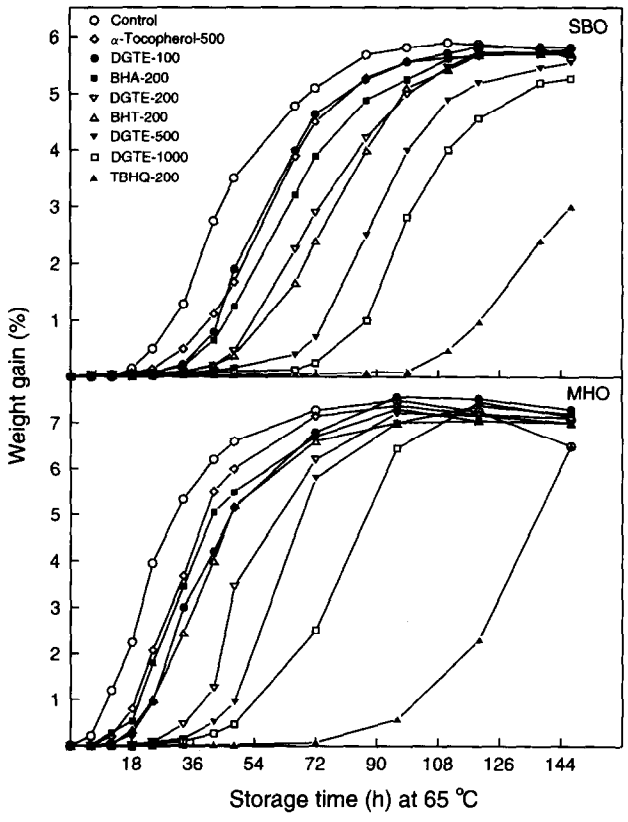

Fig. 2. Effect of dechlorophyllized green tea extracts (DGTE) and conventional antioxidants on the weight-gain of RBD-seal blubber oil (A) and RBD-menhaden oil (B) during prolonged storage at 65°C (standard deviation for each point was within ±1% of the mean value; $n=3$). Refer to Table 3 for abbreviations.

Peroxide values (PV) of SBO and MHO samples containing DGTE, α-tocopherol and synthetic antioxidants are presented in Table 3. Addition of DGTE at >200 ppm to both SBO and MHO significantly ($p<0.05$) decreased their PV under accelerated oxidation conditions. However, samples treated with α-tocopherol, BHA and BHT showed higher PV as compared to DGTE-treated oils under similar experimental conditions. Furthermore, the effect of DGTE on the reduction of PV was dose-dependent in both oils. For up to 144 h, the PV of the control SBO sample increased from 2.09 meq kg^{-1} (fresh oil) to 183 meq kg^{-1} (oxidized oil) and that of MHO from 4.53 meq kg^{-1} (fresh oil) to 348 meq kg^{-1} (oxidized oil). These values for oils treated with DGTE-500 and DGTE-1000 changed from 2.18 to 115 and from 2.21 to 85.0 meq kg^{-1} in SBO and from 4.80 to 160 and from 4.71 to 148 meq kg^{-1} in MHO, respectively. DGTE was quite effective at 1000 ppm and gave a much lower PV than the controls and oils treated with α-tocopherol, BHA, BHT and other levels of DGTE. Among these treatments, TBHQ remained the most effective and gave the lowest PV in both SBO and MHO. However, it should be noted that the present results do not take into account the existing differences in the molecular weight of the antioxidants employed.

Addition of DGTE, α-tocopherol and synthetic antioxidants to both SBO and MHO had a significant ($p<0.05$) effect in lowering the formation of TBARS as compared to those for the control samples (Table 4). Among these additives, TBHQ was most effective in retarding TBARS formation at 200 ppm. The ability of TBHQ to lower TBARS values of stored marine oils

Table 3. Effect of dechlorophyllized green tea extracts (DGTE) and conventional antioxidants on peroxide values (meq kg^{-1} oil) of refined-bleached and deodorized seal blubber oil (SBO) and menhaden oil (MHO) stored under Schaal oven conditions at 65°C[a]

Treatment[b]	Storage time (h)				
	0	24	48	84	144
SBO					
Control	2.09±0.06^{a}	25.2±0.79^{a}	49.3±1.22^{a}	119±4.24^{a}	183±2.34^{a}
DGTE-100	2.13±0.04^{a}	13.2±0.99^{c}	36.2±0.85^{c}	90.0±4.88^{c}	180±7.14^{a}
DGTE-200	2.20±0.14^{a}	11.7±0.12^{cd}	29.2±0.58^{d}	77.5±5.21^{d}	172±1.86^{ab}
DGTE-500	2.18±0.05^{a}	8.92±0.38^{de}	18.9±0.66^{e}	64.6±5.86^{ef}	115±6.19^{c}
DGTE-1000	2.21±0.11^{a}	8.23±0.47^{e}	11.8±1.48^{f}	40.7±0.76^{gh}	85.0±6.60^{d}
α-Tocopherol-500	2.18±0.01^{a}	21.5±2.05^{b}	44.5±0.91^{b}	110±0.80^{ab}	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^{de}	124±2.68^{c}
BHT-200	2.11±0.06^{a}	13.3±0.18^{c}	31.6±0.42^{d}	58.8±2.18^{f}	94.9±2.11^{d}
TBHQ-200	2.09±0.07^{a}	8.04±0.24^{e}	10.4±0.31^{f}	29.9±0.86^{h}	53.4±1.20^{e}
MHO					
Control	4.53±0.41^{a}	38.1±0.77^{a}	96.5±1.91^{a}	283±4.58^{a}	348±5.82^{a}
DGTE-100	4.65±0.23^{a}	37.9±0.31^{a}	67.6±2.00^{bc}	257±5.82^{b}	280±1.39^{b}
DGTE-200	4.45±0.45^{a}	28.6±0.24^{c}	53.9±0.48^{c}	151±1.32^{d}	166±4.17^{c}
DGTE-500	4.80±0.70^{a}	16.9±1.31^{de}	41.1±0.29^{f}	109±1.67^{fg}	160±1.80^{cd}
DGTE-1000	4.71±0.01^{a}	16.0±1.02^{de}	37.8±0.98^{f}	89.7±2.16^{g}	148±7.70^{d}
α-Tocopherol-500	4.10±0.12^{a}	37.3±0.42^{a}	66.3±0.50^{c}	227±5.59^{c}	280±6.10^{b}
BHA-200	4.27±0.06^{a}	36.1±1.44^{ab}	71.3±0.94^{b}	135±4.90^{de}	163±3.60^{cd}
BHT-200	4.43±0.01^{a}	32.9±1.63^{b}	63.3±1.18^{cd}	120±0.82^{ef}	137±2.70^{d}
TBHQ-200	4.22±0.02^{a}	15.1±1.30^{e}	15.1±0.14^{g}	42.8±1.57^{h}	101±1.02^{e}

[a]Values in each column for each oil bearing different superscripts are significantly ($p<0.05$) different, mean ± SD ($n=3$).
[b]Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ).

Table 4. Effect of dechlorophyllized green tea extracts (DGTE) and conventional antioxidants on 2-thiobarbituric acid-reactive substances (TBARS) values (μmol g^{-1} oil) of refined-bleached and deodorized seal blubber oil (SBO) and menhaden oil (MHO) stored under Schaal oven conditions at 65°C[a]

Treatment[b]	Storage time (h)				
	0	24	48	84	144
SBO					
Control	4.19 ± 0.01^{a}	8.78 ± 0.23^{a}	12.3 ± 0.37^{a}	19.4 ± 0.54^{a}	24.7 ± 0.20^{a}
DGTE-100	4.96 ± 0.91^{a}	6.60 ± 0.09^{bc}	8.24 ± 0.12^{b}	15.3 ± 0.05^{b}	21.6 ± 0.53^{bc}
DGTE-200	4.45 ± 0.28^{a}	4.87 ± 0.10^{e}	8.24 ± 0.60^{b}	12.5 ± 0.17^{cd}	20.1 ± 0.12^{cd}
DGTE-500	4.37 ± 0.25^{a}	4.82 ± 0.15^{e}	6.39 ± 0.20^{c}	10.2 ± 0.28^{de}	18.2 ± 0.54^{de}
DGTE-1000	4.19 ± 0.02^{a}	4.46 ± 0.04^{de}	6.14 ± 0.13^{c}	9.82 ± 0.29^{e}	14.9 ± 0.87^{f}
α-Tocopherol-500	4.32 ± 0.14^{a}	7.67 ± 0.47^{ab}	11.8 ± 0.06^{a}	13.8 ± 0.64^{bc}	22.9 ± 0.20^{ab}
BHA-200	4.55 ± 0.34^{a}	6.89 ± 0.27^{bc}	8.97 ± 0.50^{b}	14.8 ± 0.11^{bc}	20.0 ± 0.32^{cd}
BHT-200	4.59 ± 0.46^{a}	6.35 ± 0.39^{cd}	7.80 ± 0.11^{b}	10.6 ± 0.27^{de}	16.8 ± 0.97^{ef}
TBHQ-200	4.54 ± 0.62^{a}	4.53 ± 0.06^{e}	5.50 ± 0.42^{c}	7.07 ± 0.14^{f}	10.5 ± 0.28^{g}
MHO					
Control	7.65 ± 0.49^{a}	17.8 ± 0.36^{a}	42.3 ± 0.41^{a}	45.2 ± 3.03^{a}	12.6 ± 0.58^{cd}
DGTE-100	7.63 ± 0.33^{a}	25.8 ± 1.11^{a}	30.9 ± 0.78^{b}	31.9 ± 0.48^{cd}	12.4 ± 0.35^{cd}
DGTE-200	7.61 ± 0.26^{a}	21.8 ± 1.15^{bc}	26.1 ± 2.48^{c}	27.2 ± 0.48^{ef}	11.8 ± 0.68^{de}
DGTE-500	7.42 ± 0.31^{a}	19.9 ± 0.17^{c}	20.0 ± 0.66^{d}	24.5 ± 1.16^{ef}	11.5 ± 0.45^{de}
DGTE-1000	7.18 ± 0.14^{a}	17.2 ± 1.59^{d}	18.3 ± 0.33^{d}	23.3 ± 0.33^{gh}	15.0 ± 0.18^{b}
α-Tocopherol-500	6.95 ± 0.06^{a}	16.1 ± 1.14^{d}	33.4 ± 0.78^{b}	36.7 ± 2.21^{b}	14.4 ± 0.36^{b}
BHA-200	7.06 ± 0.01^{a}	25.5 ± 0.21^{a}	25.4 ± 0.54^{c}	31.1 ± 1.61^{cd}	12.5 ± 0.59^{cd}
BHT-200	7.43 ± 0.02^{a}	23.0 ± 0.45^{b}	24.2 ± 0.57^{c}	28.4 ± 0.80^{de}	10.5 ± 0.42^{e}
TBHQ-200	7.61 ± 0.14^{a}	21.7 ± 0.12^{bc}	13.1 ± 0.13^{e}	17.3 ± 1.57^{d}	20.5 ± 0.48^{h}

[a]Values in each column for each oil bearing different superscripts are significantly ($p < 0.05$) different, mean ± SD ($n = 3$).
[b]See Table 3 for abbreviations.

has been reported for whale oil (Chihine and MacNell, 1974), mackerel (Ke *et al.*, 1977) and capelin oil (Kaitaranta, 1992). The effect of DGTE (at >200 ppm) was equivalent or slightly higher than that of BHA and BHT in reducing TBARS in both SBO and MHO. At levels of 500 and 1000 ppm, DGTE was able to lower TBARS values more effectively than α-tocopherol, BHA and BHT, even after 144 h storage at 65°C. Percentage inhibition of TBARS formation of samples treated with 100 ppm of DGTE was 49, 50, 49 and 40 for SBO and 36, 38, 57 and 49 for MHO after 24, 48, 84 and 144 h of storage, respectively; inhibition values for BHA and BHT-treated samples were 21 and 28, 27 and 37, 23 and 46, and 19 and 32 for SBO and 19 and 30, 11 and 16, 40 and 43, and 31 and 37, for MHO, respectively.

The 2-thiobarbituric acid reactive substances values represent the content of secondary lipid oxidation products, mainly aldehydes (or carbonyls), that contribute to off-flavours in oxidized oils. Results of this study indicate that DGTE has a marked effect on the inhibition of formation of TBARS in both SBO and MHO. The effect of DGTE (at >200 ppm) in suppressing TBARS formation was better than α-tocopherol (500 ppm), BHA (200 ppm) and BHT (200 ppm), but less than that of TBHQ (200 ppm).

CONCLUSIONS

Results of the present study indicated that aqueous-ethanol extracts of green tea (GTE) have a pro-oxidant effect in edible oils, perhaps due to the catalytic effect of their chlorophylls. However, after dechlorophyllization of the extracts, DGTE exhibited antioxidant activity in both SBO and MHO. Effectiveness of DGTE at 500 and 1000 ppm levels was superior to that of α-tocopherol at 500 ppm and BHA and BHT at 200 ppm, but somewhat less than that of TBHQ at 200 ppm. Apart from being an effective antioxidant, DGTE did not impart any visible colour or perceivable odour change to the treated SBO or MHO. Thus, use of dechlorophyllized green tea extract as a natural source of antioxidant for highly unsaturated marine oils may be of practical value, especially in that they also have other health benefits related to their bioactivity in prevention and treatment of cancer.

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