Antioxidant Activity of Green Tea and Its Catechins in a Fish Meat Model System

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The antioxidant activity of ground green tea (GGT) and commercial tea extracts, namely Polyphenon 25 (P-25), Polyphenon 30 (P-30), Polyphenon 60 (P-60), and Nikken Polyphenon 60 (NPP-60), as well as green tea extracts (GTE) prepared on laboratory scale and pure tea catechins, namely, (−)-epicatechin (EC), (−)- epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epigallocatechin gallate (EGCG), in a fish meat model system was evaluated. Results so obtained were compared with those of samples containing commonly used antioxidants such as α-tocopherol, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and *tert*-butylhydroquinone (TBHQ). The ground white muscle of mackerel (model system) was cooked at 75 °C and stored at 4 °C for 7 days. Progression of oxidation was monitored by measuring changes in the 2-thiobarbituric acid-reactive substances and selected and/or total volatile contents of samples. The samples treated with GGT leaves, tea extracts, and pure catechins showed excellent oxidative stability as compared with samples that contained α-tocopherol, BHT, BHA, and TBHQ. The potency of catechins in the prevention of oxidation in fish meat was in the decreasing order EGCG ≈ ECG > EGC ≫ EC. However, EGCG was more effective ($p < 0.05$) than TBHQ, as reflected in total volatile and propanal contents in the system studied.

Keywords: *Antioxidants; green tea; tea extracts; catechins; fish meat; oxidative stability*

INTRODUCTION

Lipid autoxidation is a major cause of quality deterioration and off-flavor development in muscle foods (Kanner et al., 1991). Refrigeration of cooked products and use of adequate packaging technologies may delay the onset of oxidation. However, antioxidants may also be used to protect food quality by preventing oxidative deterioration of its lipids. Therefore, antioxidants play an important role in the manufacture, packaging, and storage of lipid-containing foods.

The most common antioxidants used in the food industry are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ) (Sherwin, 1976). However, the use of some antioxidants such as BHA in food has come under attack due to their potential action as a promoter of carcinogenesis (Sherwin, 1990) as well as general rejection of synthetic food additives by consumers (Marshall, 1974). Tocopherol and ascorbic acid derivatives that are being used as alternatives to BHA, BHT, and TBHQ are much less effective antioxidants in foods. Therefore, the development and utilization of more effective antioxidants of natural origin are desired.

Naturally occurring antioxidative components in foods include phenolic acids, flavonoids, lignans, terpenes, tocopherols, phospholipids, and polyfunctional organic acids (Dugan, 1980; Shahidi and Wanasundara, 1992; Wanasundara et al., 1994). Catechins belong to the flavonoid family of compounds and are common components of the 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; Amarowicz and Shahidi, 1995, 1996; Ho et al., 1994, 1997). In recent years, catechins have attracted much attention in relation to their physiological potential as antimutagenic and antitumorigenic agents (Wang et al., 1989). Epidemiological studies have also suggested that tea polyphenols are effective in cancer prevention (Kim et al., 1994). Furthermore, catechins have been recognized as efficient antioxidants by scavenging oxygen radicals and chelating metal ions (Husain et al., 1987; Chen et al., 1990; Shahidi et al., 1992). Hara (1994) has evaluated the antioxidative potency of crude extracts of green tea and individual catechins in lard by the active oxygen method and found that crude tea catechins reduced the formation of peroxides more effectively than did α-tocopherol or BHA. The antioxidant potency of individual catechins was in the order EGCG > EGC > ECG > EC. Amarowicz and Shahidi (1995) found that ECG possessed the strongest antioxidative effect in a β-carotene/linoleate model system when used at 200 mg/kg. Recently, Wanasundara and Shahidi (1996) investigated the antioxidative activities of tea catechins in highly unsaturated marine oils. They found that tea catechins exhibited an antioxidative activity similar to or better than that of BHA, BHT, and TBHQ. The order of their potency, at 200 ppm, in marine oils was ECG > EGCG > EGC > EC. Similar results were obtained when tea catechins were applied to a pork model system, which was subsequently cooked and stored for 14 days (F. Shahidi and D. M. Alexander, unpublished results).

The objective of this study was to examine the effect of crude green tea extracts and individual catechins on the oxidative stability of cooked ground white muscle of mackerel. 2-Thiobarbituric acid reactive substance (TBARS) values and volatile aldehyde contents, which are commonly employed for evaluation of oxidative state of muscle foods, were used to monitor the development of oxidation in the samples. The efficacy of these natural antioxidants was compared with those of com-

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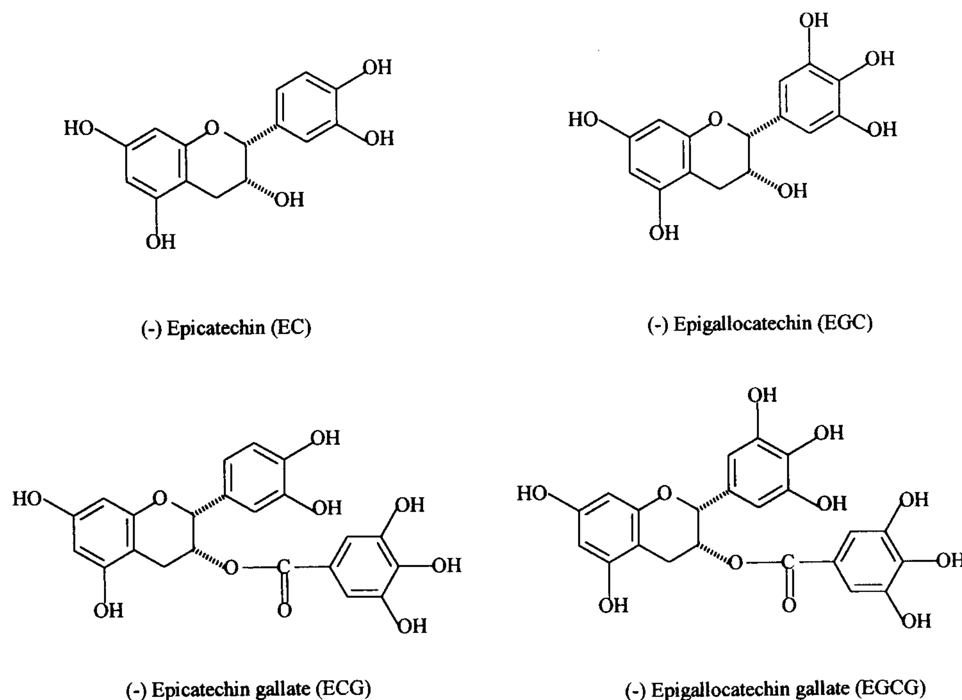

Figure 1. Chemical structures of catechins from green tea leaves.

Table 1. Fatty Acid Composition (Area Percent) of Total Lipids of Ground White Muscle of Mackerel[a]

fatty acid	composition	fatty acid	composition
14:0	3.37 ± 0.02	18:3	0.51 ± 0.02
15:0	0.30 ± 0.01	18:4	1.57 ± 0.09
16:0	12.04 ± 0.49	20:0	0.24 ± 0.01
16:1	9.47 ± 0.45	20:1	11.83 ± 0.50
17:0	0.26 ± 0.01	20:2	1.22 ± 0.07
17:1	0.31 ± 0.01	20:4	0.19 ± 0.01
18:0	2.42 ± 0.09	20:5	8.81 ± 0.40
18:1ω9	11.49 ± 0.48	22:1	13.20 ± 0.83
18:1ω7	4.07 ± 0.19	22:5	1.42 ± 0.04
18:2	0.97 ± 0.03	22:6	8.94 ± 0.41

[a] Values are means of three determinations ± standard deviation.

monly used food antioxidants such as α-tocopherol, BHA, BHT, and TBHQ.

MATERIALS AND METHODS

Materials. Frozen mackerel was acquired from local sources in Newfoundland. The white muscle was separated and homogenized in a Waring Blendor (Model 33BL73, Waring Products, New Hartford, CT). The meat was then vacuum packaged in polyethylene pouches and stored in a freezer (Ultra Low, Revco, Inc., West Columbia, SC) at −60 °C for a week until used.

2-Thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (TMP), (±)-α-tocopherol, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were obtained from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid (TCA) was purchased from Fisher Scientific (Nepean, ON). *tert*-Butylhydroquinone (TBHQ) was acquired from Aldrich Chemical Co. (Milwaukee, WI). Green tea extracts, namely Polyphenons 25, 30, and 60 (P-25, P-30, and P-60, respectively) and Nikken Polyphenon 60 (NPP-60), were obtained from Mitsui Norin Co., Ltd. (Fujieda, Japan) and Nikken Foods (Tokyo, Japan), respectively. Green tea leaves were obtained from Anhui Province, People's Republic of China, and GTE were prepared as described by Price and Spitzer (1993). Purification of individual catechins, namely EC, EGC, ECG, and EGCG, was achieved as described elsewhere (Amarowicz and Shahidi, 1996). Helium, hydrogen, and compressed air were obtained from Canadian Liquid Air Ltd. (St. John's, NF).

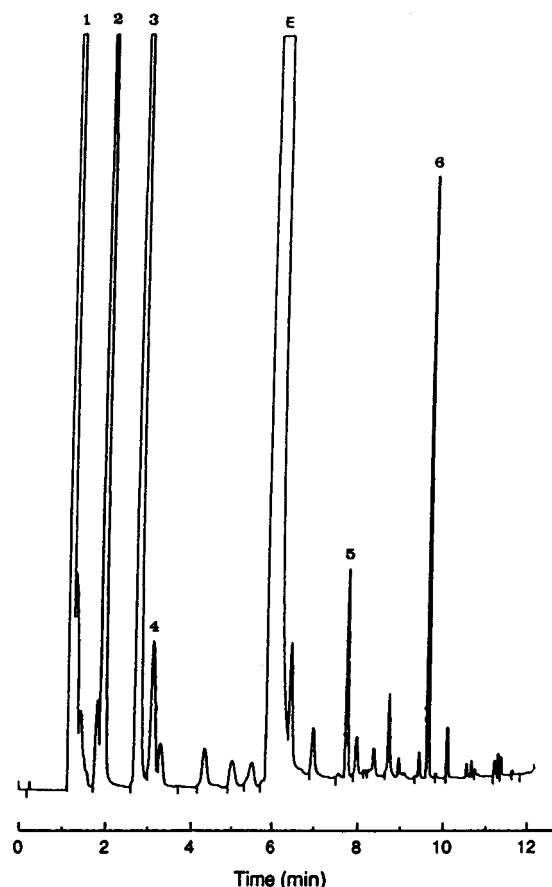

Figure 2. Gas chromatogram of the headspace volatiles of an untreated cooked white muscle of mackerel sample (control) after 3 days of storage at 4 °C. Peaks: (1) formaldehyde; (2) acetaldehyde; (3) propanal; (4) isobutyl alcohol; (5) hexanal; (6) heptanal; (E) ethanol (solvent used to dissolve additives).

Proximate Composition. Determination of moisture, crude protein, and ash contents of white muscle of mackerel was carried out according to AOAC (1990) methods. Total lipids were extracted and quantified according to the method of Bligh and Dyer (1959). The fatty acid composition of total

Table 2. Effect of Different Antioxidants on the TBARS Values (Milligrams of Malonaldehyde Equivalents per Kilogram of Sample) of Cooked White Muscle of Mackerel Stored at 4 °C[a]

treatment	concn (mg/kg of sample)	storage time			
		0 days	1 day	3 days	7 days
control		8.8 ± 0.4[k]	9.4 ± 0.5[h]	9.7 ± 0.5[h]	11.1 ± 0.5[i]
BHA	124	3.0 ± 0.1[a]	3.1 ± 0.2[a]	3.8 ± 0.2[abc]	3.8 ± 0.2[abc]
BHT	152	4.3 ± 0.02[fgh]	5.5 ± 0.4[ef]	6.1 ± 0.4[f]	6.1 ± 0.4[efg]
TBHQ	115	3.0 ± 0.2[a]	3.2 ± 0.1[ab]	3.7 ± 0.2[ab]	4.0 ± 0.2[abc]
α-tocopherol	296	7.3 ± 0.4[j]	7.9 ± 0.4[g]	8.1 ± 0.5[g]	8.9 ± 0.4[h]
EC	200	5.8 ± 0.3[i]	6.1 ± 0.2[f]	6.5 ± 0.4[f]	6.6 ± 0.4[g]
ECG	304	3.4 ± 0.1[abc]	3.4 ± 0.1[ab]	3.9 ± 0.2[abc]	3.7 ± 0.2[ab]
EGC	211	3.9 ± 0.1[cdef]	4.1 ± 0.2[d]	4.4 ± 0.2[bcd]	4.2 ± 0.2[abc]
EGCG	316	3.3 ± 0.2[ab]	3.6 ± 0.2[abcd]	3.9 ± 0.1[abc]	3.6 ± 0.2[a]
P-25	1265	4.0 ± 0.1[defg]	3.9 ± 0.2[cd]	3.8 ± 0.2[abc]	3.8 ± 0.2[abc]
P-30	1053	3.6 ± 0.1[bcde]	3.6 ± 0.1[abcd]	3.6 ± 0.2[a]	3.7 ± 0.2[ab]
P-60	527	3.7 ± 0.1[bcde]	3.9 ± 0.1[cd]	3.9 ± 0.2[abc]	3.9 ± 0.2[abc]
NPP-60	527	3.5 ± 0.1[abcd]	3.9 ± 0.2[cd]	3.9 ± 0.1[abc]	4.2 ± 0.2[abc]
GTE	485	3.7 ± 0.1[bcde]	3.8 ± 0.2[bcd]	3.9 ± 0.1[abc]	3.9 ± 0.2[abc]
GGT	1265	4.1 ± 0.2[efgh]	4.0 ± 0.2[cd]	4.1 ± 0.2[abc]	4.2 ± 0.2[abc]
P-25	200	4.6 ± 0.2[h]	5.1 ± 0.3[e]	5.8 ± 0.4[ef]	6.4 ± 0.4[fg]
P-30	200	4.5 ± 0.2[gh]	5.1 ± 0.3[e]	5.3 ± 0.3[e]	5.6 ± 0.4[ef]
P-60	200	3.8 ± 0.2[bcdef]	3.9 ± 0.1[cd]	5.1 ± 0.3[de]	5.3 ± 0.3[de]
NPP-60	200	4.0 ± 0.1[defg]	4.1 ± 0.2[d]	4.4 ± 0.2[bcd]	4.5 ± 0.2[bcd]
GTE	200	5.4 ± 0.4[i]	6.1 ± 0.4[f]	6.3 ± 0.3[f]	6.7 ± 0.4[g]
GGT	200	3.7 ± 0.2[bcde]	4.1 ± 0.2[d]	4.5 ± 0.2[cd]	4.6 ± 0.2[cd]

[a] Values are means of three determinations ± standard deviation. Values followed by different letters are different ($p < 0.05$) from one another. The concentrations provided as milligrams per kilogram are equivalent to 0.68 mmol/kg of sample (200 mg of EC equiv/kg).

Table 3. Correlation Coefficients between Storage Time and Selected Volatile Contents[a] of Cooked White Muscle of Mackerel Treated with Different Antioxidants[a]

treatment	propanal	hexanal	treatment	propanal	hexanal
control	0.99	0.99	P-25	0.99	0.95
BHA	0.98	0.97	P-30	0.97	0.99
BHT	0.99	0.79	P-60	0.99	0.99
TBHQ	0.99	0.60	NPP-60	0.90	0.99
α-tocopherol	0.98	0.97	GTE	0.99	0.99
EC	0.99	0.46	GGT	0.96	0.94
ECG	0.93	0.63			
EGC	0.99	0.89			
EGCG	0.99	0.41			

[a] Antioxidants were used at 0.68 mmol/kg of sample.

lipids extracted was determined by their transmethylation and subsequent gas chromatographic analysis, as described elsewhere (Wanasundara and Shahidi, 1996).

Meat Model Systems. Meat model systems were prepared as described by Shahidi and Pegg (1990). Ground white muscle of mackerel (80 g) was mixed with 20% by weight of deionized water in a Mason jar. Antioxidants were dissolved in 2 mL of 100% ethanol and added to meat at different levels. The control sample containing 2 mL of 100% ethanol, without any antioxidant, was also prepared. Meat systems were thoroughly homogenized and cooked at 75 ± 2 °C in a thermostated water bath for 30 min with occasional stirring with a glass rod. Samples were cooled to room temperature, homogenized in a precooled Waring Blendor for 30 s, transferred into plastic bags, and then stored for 7 days at 4 °C. Samples were analyzed for TBARS according to the method of Siu and Draper (1978) as described by Shahidi and Hong (1991). TBARS values were calculated by multiplying absorbance readings by a factor of 3.4 determined from a standard line prepared using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde.

Headspace Analysis. A Perkin-Elmer 8500 gas chromatograph and an HS-6 headspace sampler (Perkin-Elmer Corp., Montreal, PQ) were used for analysis of volatiles in cooked samples. A high-polarity Supelcowax 10 fused silica capillary column (30 m × 0.32 mm internal diameter, 0.10 μm film,

Table 4. Content of Propanal (P) and Total Volatiles (TV) (Milligrams per Kilogram of Sample) of Cooked White Muscle of Mackerel Treated with Different Antioxidants at 0.68 mmol/kg of Sample[a]

treatment	day 0		day 1		day 3		day 7	
	P	TV	P	TV	P	TV	P	TV
control	17.1 ± 0.7[f]	67.0 ± 3.2[f]	20.1 ± 1.0[g]	74.1 ± 3.9[g]	23.1 ± 1.1[h]	79.8 ± 4.0[g]	25.4 ± 1.2[h]	87.8 ± 4.3[h]
BHA	3.2 ± 0.1[c]	14.6 ± 0.7[b]	4.0 ± 0.2[d]	35.9 ± 1.6[d]	4.6 ± 0.2[de]	30.7 ± 1.7[d]	5.6 ± 0.2[cd]	40.2 ± 2.0[e]
BHT	7.8 ± 0.3[de]	36.0 ± 1.8[e]	8.9 ± 0.4[ef]	39.4 ± 2.1[f]	10.0 ± 0.5[f]	43.6 ± 2.0[e]	11.5 ± 0.5[f]	61.5 ± 3.3[f]
TBHQ	1.0 ± 0.1[a]	13.4 ± 0.7[b]	4.0 ± 0.2[d]	19.8 ± 1.1[c]	5.5 ± 0.3[e]	28.7 ± 1.5[d]	6.5 ± 0.3[d]	33.9 ± 1.8[d]
α-tocopherol	8.4 ± 0.4[e]	26.8 ± 1.3[d]	9.7 ± 0.5[f]	32.3 ± 1.4[e]	13.1 ± 0.7[g]	48.8 ± 2.6[f]	14.1 ± 0.6[g]	80.0 ± 4.1[g]
EC	7.7 ± 0.3[d]	27.6 ± 1.4[d]	8.2 ± 0.4[e]	31.5 ± 1.5[e]	9.5 ± 0.5[f]	40.0 ± 1.9[e]	10.3 ± 0.5[e]	38.4 ± 2.0[d]
ECG	2.3 ± 0.1[b]	13.3 ± 0.7[b]	2.8 ± 0.1[bc]	13.3 ± 0.7[b]	3.0 ± 0.2[c]	13.3 ± 1.0[b]	3.2 ± 0.1[ab]	14.3 ± 0.7[b]
EGC	3.1 ± 0.1[c]	18.6 ± 0.9[c]	3.3 ± 0.1[cd]	19.6 ± 1.1[c]	3.8 ± 0.2[cd]	19.2 ± 1.0[c]	4.6 ± 0.2[c]	24.3 ± 1.4[c]
EGCG	2.0 ± 0.1[b]	11.7 ± 0.6[b]	2.2 ± 0.1[b]	13.7 ± 0.7[b]	2.7 ± 0.2[bc]	13.9 ± 0.5[b]	3.4 ± 0.2[b]	14.4 ± 0.7[b]
P-25	1.0 ± 0.1[a]	3.9 ± 0.2[a]	1.1 ± 0.1[a]	4.6 ± 0.4[a]	1.5 ± 0.1[a]	6.6 ± 0.3[a]	2.1 ± 0.2[a]	9.6 ± 0.4[ab]
P-30	0.6 ± 0.0[a]	3.3 ± 0.2[a]	0.9 ± 0.0[a]	4.5 ± 0.8[a]	1.4 ± 0.0[a]	6.7 ± 0.3[a]	2.6 ± 0.1[ab]	10.9 ± 0.5[ab]
P-60	0.6 ± 0.0[a]	2.8 ± 0.1[a]	1.1 ± 0.1[a]	4.4 ± 0.4[a]	1.5 ± 0.1[a]	7.3 ± 0.3[a]	2.3 ± 0.1[ab]	10.3 ± 0.4[ab]
NPP-60	0.7 ± 0.0[a]	3.3 ± 0.2[a]	0.8 ± 0.1[a]	4.4 ± 0.4[a]	0.9 ± 0.0[a]	6.5 ± 0.4[a]	2.4 ± 0.2[ab]	11.0 ± 0.5[ab]
GTE	0.8 ± 0.1[a]	2.9 ± 0.2[a]	1.1 ± 0.1[a]	4.4 ± 0.3[a]	1.4 ± 0.1[a]	6.6 ± 0.4[a]	2.2 ± 0.1[a]	9.8 ± 0.4[ab]
GGT	1.0 ± 0.1[a]	4.1 ± 0.2[a]	1.1 ± 0.1[a]	5.2 ± 0.4[a]	1.6 ± 0.1[ab]	5.8 ± 0.3[a]	2.5 ± 0.2[ab]	8.6 ± 0.3[a]

[a] Values are means of three determinations ± standard deviations. Values followed by different letters in each column are different ($p < 0.05$) from one another. The concentrations provided as milligrams per kilogram are equivalent to 0.68 mmol/kg of sample (200 mg of EC equiv/kg).

Supelco Canada Ltd., Oakville, ON) was used. Helium was the carrier gas employed at an inlet column pressure of 120.6 kPa and a split ratio of 7:1. The oven temperature was maintained at 40 °C for 5 min, raised to 200 °C at 20 °C/min, and held at 200 °C for 5 min. The injector and flame ionization detector (FID) temperatures were adjusted to 280 °C and held at this temperature throughout the analysis (Shahidi and Pegg, 1993).

For headspace analysis, 2.0 g portions of homogenized fish samples were transferred to 10 mL glass vials, capped with Teflon-lined septa, crimped, and then frozen at −60 °C until used. To avoid heat shock after removal from storage, frozen vials were tempered at room temperature for 30 min and then preheated in the HS-6 magazine assembly at 90 °C for a 40 min equilibration period. Pressurization time of the vial was 6 s, and the volume of the vapor phase drawn was around 1.5 mL. Chromatograph peak areas were expressed as integrator count units. Individual volatile compounds were tentatively identified by comparing relative retention times of GC peaks with those of commercially available standard. Quantitative determination of dominant aldehydes was accomplished using 2-heptanone as an internal standard (Shahidi and Pegg, 1993). All experiments in the present study were replicated, at least, two times.

Stastical Analysis. One-way analysis of variance (ANOVA) and Tukey's studentized range test (Snedecor and Cochran, 1980) based on data collected from triplicate determinations were carried out. Significance was established at $p \leq 0.05$.

RESULTS AND DISCUSSION

White muscle of mackerel contained 63.85 ± 0.32% moisture, 19.80 ± 0.06% crude protein, 10.12 ± 0.04% total lipids, and 1.43 ± 0.08% ash. The fatty acid composition of total lipids of white muscle of mackerel is given in Table 1. Results in this table demonstrate the highly unsaturated nature, and hence oxidative susceptibility, of lipids from white muscle of mackerel.

The TBARS values of antioxidant-treated fish meat samples stored at 4 °C over 7 days are shown in Table 2. Addition of tea catechins at concentrations equivalent to 0.68 mmol/kg of sample resulted in a significant reduction in the formation of TBARS as compared to the control sample. The potency of ECG, EGCG, and EGC in inhibiting the formation of TBARS in fish meat system was significantly ($p < 0.05$) stronger than that of EC and similar to or better than that of BHT, BHA, and TBHQ. At the same concentration α-tocopherol was much less effective than catechins in inhibiting the formation of TBARS in this system.

Crude tea extracts, namely P-25, P-30, P-60, and NPP-60, GTE, and ground green tea (GGT) are assumed to contain approximately 25, 30, 60, 60, and 25% total catechins (mainly EGCG), respectively. We made the assumption that all catechins present in the extracts and tea samples tested were EGCG. Treatment of fish meat samples with 200 mg of extract or GGT/kg of sample resulted in lower TBARS values as compared to the control sample (Table 2). When the crude tea extracts and GGT were added to fish meat at a 200 mg/kg (0.68 mmol/kg of sample) equivalence of EC (equivalent to 316 mg of EGCG/kg), the TBARS values of samples were much lower than those of fish meat treated with only 200 mg of extract or GGT/kg of sample (Table 2). At these levels, the effectiveness of crude tea catechin and GGT to inhibit formation of TBARS was similar ($p > 0.05$) to that of EGCG, ECG, and EGC. It appears that the presence of chlorophyll and other matter in crude tea extracts did not affect their antioxidative activity in the present model system. This is in contrast to what was observed in a bulk oil model system and also when catechins were not fully purified (Shahidi et al., 1997). Therefore, it appears that the antioxidant activity of green tea and its extracts is system dependent and might be markedly influenced by the presence of chlorophyll and other impurities. Antioxidant and pro-oxidant effects of various tea extracts in different systems have recently been reported by Yen et al. (1997).

The content of propanal and hexanal in the volatiles of cooked fish samples treated with different antioxidants was also determined over a 7 day storage at 4 °C (Figure 2). Generally, the concentration of all individual aldehydes increased linearly during storage (Table 3). Propanal was a prominent aldehyde detected. Therefore, propanal may serve as a reliable indicator for assessment of the oxidative status of treated fish meat. Table 4 summarizes the effects of antioxidants listed in lowering the formation of total volatile (TV) aldehydes and propanal (P) content of headspace of samples as compared to that of the control. The inhibitory effect of individual tea catechins on the formation of volatile aldehydes was EGCG ≈ ECG > EGC > EC. Furthermore, EGC, ECG, and EGCG were more effective ($p < 0.05$) than BHT and α-tocopherol in inhibiting the formation of aldehydes. In addition, EGCG and ECG exhibited a somewhat better activity than TBHQ and BHA. The strongest inhibitory effect on aldehyde formation was observed for samples containing crude tea extracts and GGT. As compared to the control sample, these agents decreased the formation of total aldehydes in samples by over 94, 93, 91, and 87% on days 0, 1, 3, and 7, respectively.

CONCLUSIONS

Individual catechins, crude tea extracts, and GGT exhibited excellent antioxidant properties as evidenced by the TBARS values and TV aldehyde and propanal contents of the treated fish meat samples. The order of potency of catechins in mackerel meat system was EGCG ≈ ECG > EGC ≫ EC. Commonly used antioxidants such as α-tocopherol and BHT were not very effective in controlling the oxidative deterioration of fish meat. EGCG showed slightly better antioxidative activity than TBHQ. Extensive hydroxylation of catechin molecules such as EGCG and ECG and changes in the hydrophilicity/hydrophobicity of the molecules involved are perhaps the main reasons for their strong antioxidative properties as compared with other catechins tested. These findings have also demonstrated that tea extracts and GGT leaves containing different amounts of EGCG, ECG, EGC, and EC may be considered as potential natural antioxidants for stabilization of lipid-containing foods. However, dechlorophyllization of crude extracts may be necessary to avail them for application to substrates in which the original color of the crude extracts might be of concern or when chlorophyll might act as a pro-oxidant.

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