Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity

Gow-Chin Yen* and Hui-Yin Chen

Department of Food Science, National Chung Hsing University, 250 Kuokuang Road, Taichung, Taiwan, Republic of China

The relationship between antioxidant activity and antimutagenicity of various tea extracts (green tea, pouchong tea, oolong tea, and black tea) was investigated. All tea extracts exhibited markedly antioxidant activity and reducing power, especially oolong tea, which inhibited 73.6% peroxidation of linoleic acid. Tea extracts exhibited a 65–75% scavenging effect on superoxide at a dose of 1 mg and 30–50% scavenging effect on hydrogen peroxide at a dose of 400 μg. They scavenged 100% hydroxyl radical at a dosage of 4 mg except the black tea. Tea extracts also showed 50–70% scavenging effect on α,α-diphenyl-β-picrylhydrazyl radical. The antioxidant activity and the scavenging effects on active oxygen decreased in the order semifermented tea > nonfermented tea > fermented tea. Tea extracts showed strong antimutagenic action against five indirect mutagens, i.e., AFB_1, Trp-P-1, Glu-P-1, B[*a*]P, and IQ, especially oolong and pouchong teas. The antioxidant effect of tea extracts was well correlated to their antimutagenicity in some cases but varied with the mutagen and antioxidative properties.

Keywords: *Antioxidant activity; antimutagenicity; tea extracts; active oxygen; scavenging effect*

INTRODUCTION

The role of active oxygen and free radicals in tissue damage in various human diseases is becoming increasingly recognized (Halliwell et al., 1992). Active oxygen in the forms superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) is a byproduct of normal metabolism and attacks biological molecules, leading to cell or tissue injury. Active oxygen and free radicals are produced by certain chemical carcinogens and play a role in the carcinogenic process (Cerutti, 1985). Hydrogen peroxide promotes tumors in mouse skin (Slaga et al., 1982). Frenkel and Chrzan (1987) reported that several tumor promoters, such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA), 12-*O*-retinoylphorbol 13-acetate (RPA), and mezerein, induced formation of hydrogen peroxide by human polymorphonuclear leukocytes (PMN) and caused DNA damage. Cunningham et al. (1987) demonstrated that superoxide produced single-strand breaks in DNA in Chinese hamster ovary (CHO) cells in a dose-dependent manner. The number of breaks was decreased on the prior addition of a metal chelator, indicating that some breaks may have been caused by peroxide or hydroxyl radical. The active metabolite of 3-amino-1-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), *N*-OH-Trp-P-2, can generate superoxide in solution and cause single-strand breaks in FM3A DNA by generating intracellular superoxide (Wataya et al., 1988).

Tea has been used as a daily beverage and crude medicine in China for thousands of year. Tea possesses antipyretic and diuretic effects, etc. The pharmacological effects of tea are reviewed, including antioxidative activity (Satoshi and Hara, 1990) and antimutagenic (Kada et al., 1985; Jain et al., 1989; Yen and Chen, 1994) and anticancer effects (Isao, 1990). Concerning the antioxidative and anticarcinogenic effects of tea, Ruch et al. (1989) reported that green tea antioxidant (GTA) had antioxidative activity toward hydrogen peroxide and superoxide and that GTA prevented oxygen radical and hydrogen peroxide induced cytotoxicity and inhibition of intercellular communication in cell culture. In our previous work (Yen and Chen, 1994), the antimutagenic effect of various tea extracts was compared and the antimutagenic activity of tea varied with its fermentation extent. Although some tests indicated that the antimutagenic property of tea may be correlated with its antioxidant activity, further research on the role of the antioxidative ability of tea in the mutagenic process is required.

The objectives of our work were to investigate the scavenging effect of tea extracts on active oxygen and radicals and to find the relationship between antioxidative activity and antimutagenic effect of tea extracts.

MATERIALS AND METHODS

Materials. Teas, including green tea, oolong tea, pouchong tea, and black tea, were purchased at a local market in Taichung, Taiwan. Benzo[*a*]pyrene (B[*a*]P), aflatoxin B_1 (AFB_1), linoleic acid, nitro blue tetrazolium (NBT), α,α-diphenyl-β-picrylhydrazyl (DPPH), and 5,5-dimethyl-pyrroline *N*-oxide (DMPO) were purchased from Sigma Chemical Co. (St. Louis, MO). 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), and 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) were purchased from Wako Pure Chemical Co. (Tokyo, Japan). Dihydronicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) were purchased from E. Merck Co. (Darmstadt, Germany).

Preparation of Tea Extracts. Tea extracts were prepared according to the method described in our previous work (Yen and Chen, 1994). In brief, each tea (20 g) was extracted with boiled water (400 mL) for 5 min and the filtrate was freeze-dried. The yields of crude tea extracts for green tea, pouchong tea, oolong tea, and black tea were 3.96, 4.14, 5.11, and 3.89 g, respectively.

Antioxidant Activity. The antioxidant activity of tea extracts was determined according to the thiocyanate method (Mitsuda et al., 1966). Each sample (500 μg) in 0.5 mL of distilled water was mixed with linoleic acid emulsion (2.5 mL,

* Author to whom correspondence should be addressed (fax 886-4-261-2962).

0021-8561/95/1443-0027$09.00/0

0.02 M, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0) in a test tube and placed in darkness at 37 °C to accelerate oxidation. The peroxide value was determined by reading the absorbance at 500 nm with a spectrophotometer (Hitachi U-2000) after coloring with $FeCl_3$ and thiocyanate at intervals during incubation.

Reducing Power. The reducing power of tea extracts was determined according to the method of Oyaizu (1986). Tea extracts (10−1000 μg) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%); the mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Scavenging of Superoxide. The influence of tea extracts on the generation of superoxide was measured by means of spectrophotometric measurement of the product on reduction of nitro blue tetrazolium (Nishikimi et al., 1972). Superoxide was generated in a nonenzymic system. The reaction mixture, which contained 1 mL of tea extracts (10−1000 μg) in distilled water, 1 mL of PMS (60 μM) in phosphate buffer (0.1 M, pH 7.4), 1 mL of NADH (468 μM) in phosphate buffer, and 1 mL of NBT (150 μM) in phosphate buffer, was incubated at ambient temperature for 5 min, and the color was read at 560 nm against blank samples.

Scavenging of Hydrogen Peroxide. The ability of tea extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution (4 mM) of hydrogen peroxide was prepared in phosphate-buffered saline (PBS, pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm using the molar absorptivity 81 M^{-1} cm^{-1} (Beers and Sizer, 1952). Tea extracts (20−400 μg) in 4 mL of distilled water were added to the hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing tea extracts in PBS without hydrogen peroxide.

Hydroxyl Radical Scavenging Activity. The hydroxyl radical reacts rapidly with nitrone spin trap DMPO; the resultant DMPO−OH adduct is detectable with an electron paramagnetic resonance (EPR) spectrometer (Rosen and Rauckman, 1984). The EPR spectrum was recorded 2.5 min after tea extracts (1−4 mg, 0.2 mL) were mixed with DMPO (0.3 M, 0.2 mL), Fe^{2+} (10 mM, 0.2 mL), and H_2O_2 (10 mM, 0.2 mL) in a phosphate buffer solution (pH 7.2) using an EPR spectrometer (Bruker ER 200D 10/12) set at the following conditions: receiver gain, 2×10^5; modulation amplitude, 1.0 G; scan time, 200 s; field, 3461.3 ± 50 G; time constant, 0.5 s (Shi et al., 1991).

Scavenging Effect on DPPH Radical. The effect of tea extracts on DPPH radical was estimated according to the method of Hatano at al. (1988). Tea extracts (40−2000 μg) in 4 mL of distilled water were added to a methanolic solution of DPPH (1 mM, 1 mL). The mixture was shaken and left to stand at room temperature for 30 min; the absorbance of the resulting solution was measured spectrophotometrically at 517 nm.

Antimutagenicity Assay. The antimutagenic effect of tea extracts was assayed according to the Ames test using *Salmonella typhimurium* strains TA98 and TA100 (Maron and Ames, 1983). The mutagens used were IQ (0.1 μg/plate for TA98 and 0.5 μg/plate for TA100), B[*a*]P (10 μg/plate for TA98 and 5 μg/plate for TA100), AFB_1 (0.5 μg/plate for TA98 and TA100), Trp-P-1 (0.025 μg/plate for TA98 and 0.5 μg/plate for TA100), and Glu-P-1 (0.5 μg/plate for TA98 and 2 μg/plate for TA100). Tea extracts (0.7 mg/plate) were added to overnight-cultured *S. typhimurium* TA98 or TA100 (0.1 mL), mutagen (0.1 mL), and S9 mix (0.5 mL). The entire mixture was preincubated at 37 °C for 20 min before molten top agar (2 mL) was added; the mixture was poured onto a minimal agar plate. The His^+ revertant colonies were counted after incubation at 37 °C for 48 h. Each sample was assayed using triplicate plates. The calculation of percent inhibition follows that described by Ong et al. (1986): inhibition (%) = [1 − (number of revertants in the presence of tea extracts/number of revertants in the absence of tea extracts)] × 100 %. The number of spontaneous revertants was subtracted from the numerator and denominator.

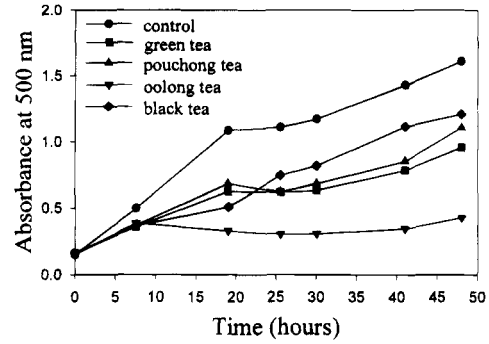

Figure 1. Antioxidative activity of various tea extracts.

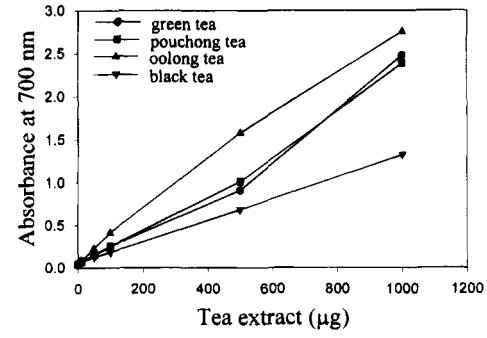

Figure 2. Reducing power of various tea extracts.

Statistical Analysis. The correlations between the antimutagenicity and the antioxidation of tea extracts were calculated as prescribed by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Antioxidant Activity and Reducing Power of Tea Extracts. The effects of various tea extracts on the peroxidation of linoleic acid are shown in Figure 1. In general, the oxidative activity of linoleic acid was markedly inhibited by any tea extract compared with the control assay. Among the four tea extracts, the greatest antioxidant activity was observed in oolong tea, which exhibited 73.6% inhibition of linoleic acid peroxidation. Peroxide formation was decreased about 40% by green tea and pouchong tea, and no significant difference ($p > 0.05$) was found between these two tea extracts. Polyphenols are the most abundant group of compounds in tea leaf, and the catechins constitute the major component and seem to be responsible for the antioxidant activity. Lunder (1992) reported that there was a good correlation between the antioxidant activity and the epigallocatechin gallate (EGCg) content. In general, the content of catechins in tea is related to the degree of fermentation of tea during manufacture; therefore, the content of catechins in various teas is in the order green tea > pouchong tea > oolong tea > black tea. In our results, oolong tea extracts showed stronger antioxidant activity than other tea extracts; thus, the variable in antioxidant activity of these four tea extracts may not be completely attributed to the content of catechins.

As shown in Figure 2, the reducing powers (absorbance at 700 nm) of green tea, pouchong tea, oolong tea, and black tea were 2.47, 2.38, 2.75, and 1.32 at a dose

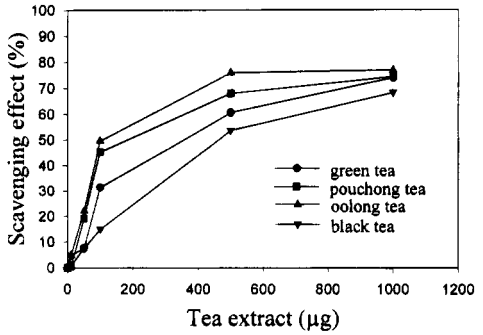

Figure 3. Scavenging effect of various tea extracts on superoxide.

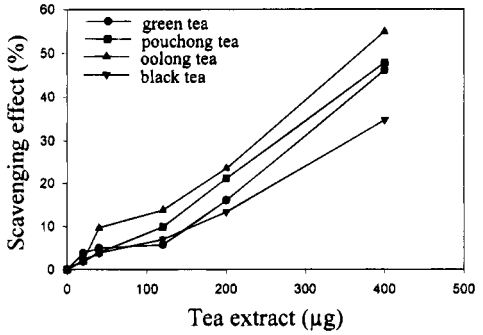

Figure 4. Scavenging effect of various tea extracts on hydrogen peroxide.

of 1 mg extracts, which is equivalent to 5.05, 4.83, 3.91, and 5.14 mg tea samples, respectively. The greatest reducing power was observed in oolong tea relative to the other teas. Therefore, tea extracts were electron donors and can react with free radicals to convert them to more stable products and terminate radical chain reaction.

Scavenging of Active Oxygen by Tea Extracts. Figure 3 shows the scavenging of superoxide anions by tea extracts. The scavenging effect of tea extracts increased with increasing concentration of tea extracts, and superoxide anions (65–75%) were inhibited by tea extracts at a dose of 1 mg. The scavenging effect of four tea extracts decreased in the order oolong tea > pouchong tea > green tea > black tea. Ruch et al. (1989) reported that green tea antioxidant (GTA) was capable of scavenging superoxide produced in an enzymic system. The addition of GTA (50 μg/mL) to the xanthine/xanthine oxidase system reduced superoxide production from 0.064 to 0.05 nmol of superoxide mL^{-1} min^{-1}. Robak and Gryglewski (1988) indicated that antioxidative properties of several flavonoids such as quercetin, myricetin, and rutin were effected mainly via scavenging of superoxide anions.

The tea extracts were also capable of scavenging hydrogen peroxide in a manner dependent on concentration (Figure 4). They exhibited scavenging effect (30–50%) on hydrogen peroxide at the dose 400 μg. Among the four tea extracts, oolong tea showed the strongest scavenging effect on hydrogen peroxide. Ruch et al. (1989) reported that the hydrogen peroxide concentration was reduced from 4 to 0.5 mM with GTA (50 μg/mL). Typical application of the polyphenol fraction of green tea inhibited TPA-induced hydrogen peroxide formation in mouse epidermis; this factor may be important for the inhibitory effect of the polyphenol fraction of green tea on TPA-induced inflammation and tumor promotion.

To test the reaction of hydroxyl radical (•OH) with tea extracts, we used the Fenton reagent ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$) as a source of hydroxyl radical. As shown in Figure 5, the reaction of Fe^{2+} with H_2O_2 in the presence of spin trapping agent DMPO generated a 1:2:2:1 quartet with hyperfine coupling parameters (a^n and a^h = 14.9 G). Table 1 shows the effects of tea extracts on the EPR signal intensity of DMPO–OH adducts. The addition of tea extracts to the reaction system resulted in dose-dependent inhibition on the EPR signal intensity of DMPO–OH adducts. All tea extracts showed 100% scavenging effect on hydroxyl radical at a dosage of 4 mg except black tea. Shi et al. (1991) demonstrated that caffeine effectively scavenged hydroxyl radical, and this mechanism may be responsible for the effect of caffeine on chemical carcinogens. Husain et al. (1987) indicated that flavonoids such as myricetin, quercetin, and rhamnetin were scavengers of hydroxyl radical and that the scavenging effect increased with increasing number of hydroxyl groups substituted in the aromatic B-ring.

Active oxygen and free radicals are related to various physiological and pathological events, such as inflammation, immunization, aging, mutagenicity, and carcinogenicity (Namiki, 1990). Wataya et al. (1988) reported that the mutagenicity of Trp-P-2 is considered to be due to DNA damage induced by active oxygen

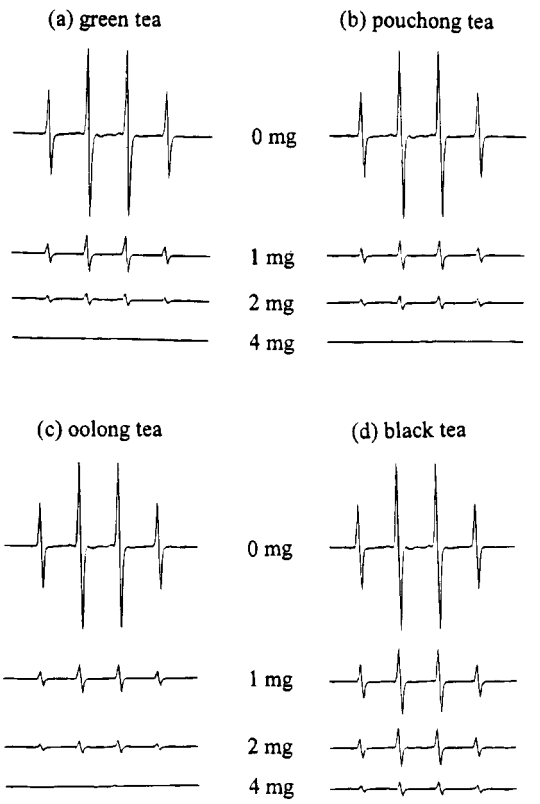

Figure 5. EPR spectrum recorded 2.5 min after mixing in a phosphate buffer solution (pH 7.2) of 0.075 M DMPO with 2.5 mM Fe^{2+}, 2.5 mM H_2O_2, and tea extracts.

Table 1. Effect of Various Tea Extracts on EPR Signal Intensity of DMPO–OH Spin Adduct[a]

tea extract: (mg)	relative EPR signal intensity			
	green tea	pouchong tea	oolong tea	black tea
0	100.0	100.0	100.0	100.0
1	23.0	18.7	22.0	40.8
2	8.1	8.5	7.7	22.9
4	0.0	0.0	0.0	13.6

[a] Treatment and other details are the same as in the legend of Figure 5.

Table 2. Inhibitory Effect of Tea Extracts on the Activity of Mutagens[a] to *S. typhimurium* TA98 and TA100

tea extract	His$^+$ revertants/plate[b] (% of inhibition)[c]				
	IQ	B[a]P	AFB_1	Trp-P-1	Glu-P-1
		TA98			
control[d]	1739 ± 116[a f]	380 ± 31[a]	448 ± 27[a]	1737 ± 72[a]	911 ± 81[a]
green tea	909 ± 76[b] (49.1)	155 ± 16[b] (68.0)	64 ± 8[b] (96.2)	867 ± 46[b] (51.5)	134 ± 76[b] (90.1)
pouchong tea	930 ± 55[b] (47.9)	104 ± 10[c] (83.4)	60 ± 8[b] (97.2)	736 ± 82[c] (59.3)	160 ± 12[b] (87.1)
oolong tea	1079 ± 82[c] (39.1)	94 ± 6[c] (86.4)	80 ± 5[b] (92.2)	642 ± 76[c] (64.9)	241 ● 22[c] (77.7)
black tea	1206 ± 34[c] (31.5)	178 ± 17[b] (61.0)	153 ± 8[c] (73.9)	1018 ± 59[d] (42.6)	258 ± 16[c] (75.8)
spontaneous revertants[e]	49 ± 6				
		TA100			
control	911 ± 109[b]	797 ± 42[a]	388 ± 40[a]	534 ± 43[a]	596 ± 18[a]
green tea	183 ± 6[b] (95.3)	253 ± 15[bc] (83.7)	161 ± 28[b] (85.9)	321 ± 5[b] (55.0)	274 ± 23[b] (77.7)
pouchong tea	346 ± 41[c] (74.0)	234 ± 32[bc] (86.6)	155 ± 14[b] (96.7)	358 ± 30[bc] (45.5)	237 ± 37[bc](80.0)
oolong tea	267 ± 14[bc] (84.3)	211 ± 3[c] (90.2)	170 ± 8[b] (90.5)	243 ± 6[d] (68.2)	223 ± 31[c] (83.1)
black tea	493 ± 8[d] (54.7)	283 ± 21[b] (79.1)	178 ± 13[b] (87.1)	379 ± 11[c] (40.1)	345 ± 23[d] (55.9)
spontaneous revertants	147 ± 10				

[a] Mutagen was preincubated with tea extracts (0.7 mg/plate) at 37 °C for 20 min before antimutagenicity assay. [b] Data are means ± SD of three plates. [c] Inhibition (%) = [1 − (number of His$^+$ revertants in the presence of tea extracts/number of His$^+$ revertants in the absence of tea extracts)]. The number of spontaneous revertants was subtracted from the numerator and denominator. [d] The number of control was determined without tea extract. [e] The number of spontaneous revertants was determined without tea extract and mutagen. [f] Data bearing different superscript letters in the same column were significantly different ($p < 0.05$).

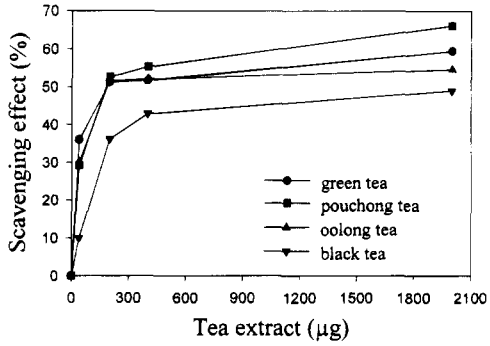

Figure 6. Scavenging effect of various tea extracts on DPPH radical.

generated by oxidation of N-OH-Trp-P-2, which is the active form of Trp-P-2. Kim et al. (1991) and Ueno et al. (1991) indicated that mutation induced by various mutagens was reduced by active oxygen scavengers. As the results show above, tea extracts had a strong activity of scavenging superoxide, hydrogen peroxide, and hydroxyl radical. Therefore, the scavenging of active oxygen species by tea extracts seems to explain how the antioxidative and antimutagenic activities function.

Scavenging of Free Radicals by Tea Extracts. The carcinogenic property of polyaromatic hydrocarbons (PAH) may be due in part to the in situ generation of PAH cation free radicals (Lesko et al., 1982). Antioxidants have therefore been reported to possess antimutagenic activity because they could scavenge a free radical or induce antioxidative enzymes (Hochstein and Atallah, 1988). The scavenging effects of four tea extracts on the DPPH radical decreased in the order pouchong tea > green tea > oolong tea > black tea (Figure 6) and were 66.1, 59.4, 54.6, and 49.0% at a dose of 2 mg, respectively. Wang et al. (1989) assessed the perylene free radical scavenging potential of green tea polyphenols; EGCg showed the strongest scavenging effect among four catechins.

Antimutagenic Effect of Tea Extracts. The antimutagenic activities of tea extracts on five indirect mutagens, including IQ, B[a]P, AFB_1, Trp-P-1, and Glu-P-1, were evaluated (Table 2). The tea extracts from green tea exhibited the strongest inhibitory effect on IQ toward strains TA98 and TA100. The antimutagenic effect of four tea extracts on B[a]P, AFB_1, and Trp-P-1 decreased in the order semifermented tea (pouchong tea and oolong tea) > nonfermented tea (green tea) > fermented tea (black tea). The extract of pouchong tea showed the greatest inhibitory effect on AFB_1 to either TA98 or TA100, whereas oolong tea showed the strongest inhibitory effect on B[a]P and Trp-P-1. The extracts from green tea and oolong tea exhibited the stronger inhibitory effect on Glu-P-1 toward strains TA98 and TA100, respectively. Kojima et al. (1989) also reported that the antimutagenic effect of oolong tea against B[a]P is greater than that of green tea. For strains TA98 and TA100, black tea exhibited the weakest inhibitory activity to five mutagens studied. The antimutagenic activity of tea extracts varied with the extent of fermentation of tea during the process of manufacture. In general, the antimutagenic activity of semifermented tea was greater than that of other teas for some mutagens. In addition, semifermented teas, especially oolong tea, showed higher extraction yield and greater antioxidant activity, reducing power, and scavenging effects on active oxygen and free radical. It has been suggested that compounds which possess antioxidant activity can inhibit mutation and cancer because they can scavenge a free radical or induce antioxidative enzyme (Hochstein and Atallah, 1988). Katiyar et al. (1993) also indicated that oral feeding of green tea extracts in drinking water to mice showed significant enhancement in the activity of glutathione *S*-transferase in liver, stomach, and lung. The antioxidative properties of tea extracts have therefore played an important role with regard to their antimutagenicity.

Correlation between Antimutagenicity and Antioxidant Activity. Calculated coefficients of correlation between antimutagenic and antioxidative properties of various tea extracts are shown in Table 3. The antimutagenic effect of tea extracts on IQ toward TA98 and TA100 was significantly correlated with their reducing power and scavenging effect of hydroxyl radical ($p < 0.05$). For *S. typhimurium* TA98 and TA100, significant correlations were observed between the antimutagenic activity of tea extracts on mutagenesis induced by B[a]P and the antioxidative properties ($p < 0.05$). The same trends were observed in the correlation of inhibition by tea extracts of mutagenesis induced by Trp-P-1 and the antioxidative activity, reducing power, and the scavenging effects of tea extracts on active oxygen. The antimutagenic effect of tea extracts on Glu-

Table 3. Correlation between Antimutagenicity toward *S. typimurium* TA98 and TA100 and Antioxidative Activity, Reducing Power, Scavenging Effect of Superoxide, Hydrogen Peroxide, Hydroxyl Radical, and DPPH Radical of Tea Extracts

	IQ	B[a]P	AFB_1	Trp-P-1	Glu-P-1
		TA98			
antiox act.	−0.04[a]	0.60	0.30	0.72	−0.27
	(0.168)[b]	(0.059)	(0.087)	(0.042)	(0.082)
reducing power	0.69	0.80	0.90	0.89	0.47
	(0.049)	(0.045)	(0.029)	(0.027)	(0.076)
$O_2^{\cdot-}$	0.60	0.87	0.85	0.95	0.35
	(0.054)	(0.036)	(0.035)	(0.019)	(0.080)
H_2O_2	0.50	0.90	0.79	0.97	0.24
	(0.058)	(0.033)	(0.042)	(0.015)	(0.083)
·OH	0.82	0.78	0.97	0.85	0.62
	(0.039)	(0.047)	(0.015)	(0.032)	(0.067)
DPPH radical	0.90	0.54	0.86	0.50	0.82
	(0.029)	(0.064)	(0.034)	(0.051)	(0.049)
		TA100			
antiox act.	0.52	0.77	−0.07	0.97	−0.59
	(0.063)	(0.036)	(0.150)	(0.019)	(0.061)
reducing power	0.86	0.91	0.32	0.82	0.99
	(0.037)	(0.024)	(0.094)	(0.043)	(0.012)
$O_2^{\cdot-}$	0.78	0.96	0.40	0.84	0.98
	(0.046)	(0.015)	(0.090)	(0.042)	(0.015)
H_2O_2	0.72	0.98	0.41	0.86	0.96
	(0.051)	(0.011)	(0.090)	(0.038)	(0.023)
·OH	0.85	0.85	0.42	0.68	0.99
	(0.039)	(0.030)	(0.091)	(0.056)	(0.011)
DPPH radical	0.47	0.45	0.69	0.04	0.69
	(0.065)	(0.051)	(0.073)	(0.175)	(0.055)

[a] Correlation coefficients. [b] Probability.

P-1 for TA100 also correlated well with the antioxidative properties of tea extracts ($p < 0.05$). No significant correlation ($p > 0.05$) was observed for the antimutagenicity of tea extracts against AFB_1 to TA100.

Many researchers reported that active oxygen and free radicals are produced by certain chemical carcinogens during the carcinogenic process. Therefore, natural and synthetic antioxidants such as ascorbic acid, tocopherol, ellagic acid, butylated hydroxytoluene, and ethoxyquin inhibit chemically induced carcinogenesis.

Conclusions. Our results indicate clearly that tea extracts show strong antimutagenic activity, antioxidant activity, reducing power, and scavenging effects on active oxygen and free radicals. The antioxidative properties and antimutagenic activity of tea extracts varied with the extent of fermentation of tea during the manufacturing process. Moreover, the antioxidant activity, reducing power, and scavenging effects on active oxygen of semifermented tea were greater than those of fermented tea and nonfermented tea. Semifermented tea extracts have the higher yield and would have more antioxidant and antimutagenic activities than other teas on a gram equivalents basis of tea samples. The antioxidative properties of tea extracts seem to explain the action of antimutagenicity of tea extracts. Further work is required to determine the mechanism involved in the antimutagenic effect of tea extracts, especially for semifermented tea.

ACKNOWLEDGMENT

This research work was partially supported by the National Science Council, Republic of China, under Grant NSC 83-0409-B005-004.

LITERATURE CITED

Beers, R. F.; Sizer, I. W. A spectrofluorometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **1952**, *195*, 133–140.

Cerutti, P. A. Prooxidant states and tumor promotion. *Science* **1985**, *227*, 375–381.

Cunningham, M. L.; Peak, J. G.; Peak, M. J. Single-strand DNA breaks in rodent and human cells produced by superoxide anion or its reduction products. *Mutat. Res.* **1987**, *184*, 217–222.

Duncan, D. B. Multiple range and multiple F test. *Biometrics* **1955**, *11*, 1–42.

Frenkel, K.; Chrzan, K. Hydrogen peroxide formation and DNA base modification by tumor promoter-activated polymorphonuclear leukocytes. *Carcinogenesis* **1987**, *8*, 455–460.

Halliwell, B.; Gutteridge, J. M. C.; Cross, C. E. Free radicals, antioxidants and human disease: where are we now? *J. Lab. Clin. Med.* **1992**, *119*, 598–620.

Hatano, T.; Kagawa, H.; Yasuhara, T.; Okuda, T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chem. Pharm. Bull.* **1988**, *36*, 2090–2097.

Hochstein, P.; Atallah, A. S. The naturee of oxidants and antioxidant systems in the inhibition of mutation and cancer. *Mutat. Res.* **1988**, *202*, 363–375.

Husain, S. R.; Cillard, J.; Cillard, P. Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* **1987**, *26*, 2489–2491.

Isao, T. Preventive effects of tea on cancer. *Fragrance J.* **1990**, Nov, 54–61.

Jain, A. K.; Shimoi, K.; Nakamura, Y.; Kada, T.; Hara, Y.; Tomita, I. Crude tea extracts decrease the mutagenic activity of N-methyl-N′-nitro-N-nitrosoguanidine in vitro and in intragastic tract of rats. *Mutat. Res.* **1989**, *210*, 1–8.

Kada, T. K.; Kaneko, S.; Matsuzaki, T. M.; Hara, Y. Detection and chemical identification of natural bio-antimutagens: A case of green tea factor. *Mutat. Res.* **1985**, *150*, 127–132.

Katiyar, S. K.; Agarwal, R.; Zaim, M. T.; Mukhtar, H. Protection against N-nitrosodiethylamine and benzo(a)pyrene-induced forestomach and lung tumorigenesis in A/J mice by green tea. *Carcinogenesis* **1993**, *14*, 849–855.

Kim, S. B.; Kim, I. S.; Yeum, D. M.; Park, Y. H. Mutagenicity of Maillard reaction products from D-glucose-amino acid mixtures and possible roles of active oxygens in the mutagenicity. *Mutat. Res.* **1991**, *254*, 65–69.

Kojima, H.; Miwa, N.; Mori, M.; Masahiro, O.; Konish, H. Desmutagenic effect of oolong tea. *J. Food Hyg. Soc. Jpn.* **1989**, *30*, 233–239.

Lesko, S. A.; Ts'o, P. O. P.; Yang, S. U.; Zheng, R. Benzo[a]-pyrene radicals and oxygen radical involvement in DNA damage, cellular toxicity and carcinogenesis. In *Free radicals, lipid peroxidation and cancer*; McBrien, D. C. H., Slater, T. F., Eds.; Academic Press: New York, 1982; pp 400–420.

Lunder, T. L. Catechins of green tea: antioxidant activity. In *Phenolic compounds in Food and their effects on health II. Analysis, Occurrence and Chemistry*; Ho, C. T., Lee, C. Y., Hunag, M. T., Eds.; ACS Symposium Series 506; American Chemical Society: Washington, DC, 1992; pp 114–120.

Maron, D. M.; Ames, B. N. Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* **1983**, *113*, 173–215.

Mitsuda, H.; Yasumoto, K.; Iwami, K. Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyoto Shokuryo* **1966**, *19*, 210–214.

Namiki, M. Antioxidants/antimutages in food. *Crit. Rev. Food Sci. Nutr.* **1990**, *29*, 273–300.

Nishikimi, M.; Rao, N. A.; Yagi, K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 849–853.

Ong, T.; Wong, W. Z.; Stewart, J. D.; Brockman, H. E. Chlorophyllin: a potent antimutagen against environmental and dietary complex mixture. *Mutat. Res.* **1986**, *173*, 111–115.

Oyaizu, M. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* **1986**, *44*, 307–315.

Robak, J.; Gryglewski, I. R. Flavonoids are scavenger of superoxide anions. *Biochem. Pharmacol.* **1988**, *37*, 837–841.

Rosen, G. M.; Rauckman, E. J. Spin trapping of superoxide and hydroxyl radicals. *Methods Enzymol.* **1984**, *105*, 198–209.

Ruch, R. J.; Cheng, S. J.; Klauning, J. E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* **1989**, *10*, 1003–1008.

Satoshi, S.; Hara, Y. Antioxidative activity of tea catechins. *Fragrance J.* **1990**, Nov, 24–30.

Shi, X.; Dalal, N. S.; Jain, A. C. Antioxidant behaviour of caffeine: efficient scavenging of hydroxyl radicals. *Food Chem. Toxicol.* **1991**, *29*, 1–6.

Slaga, T. J.; Klein-Szanto, A. J. P.; Triplett, L. L.; Yotti, L. P.; Trosko, J. E. Skin tumor promoting ability of benzoylperoxide, a widely used free radical-generating compound. *Science* **1982**, *213*, 1023–1025.

Ueno, H.; Nakamuro, K.; Sayato, Y.; Okada, S. Characteristics of mutagenesis by glyoxal in *Salmonella typhimurium*: contribution of singlet oxygen. *Mutat. Res.* **1991**, *251*, 99–107.

Wang, Z. Y.; Cheng, S. J.; Zhou, Z. C.; Athar, M.; Khan, W. A.; Bickers, D. R.; Mukhtar, H. Antimutagenic activity of green tea polyphenols. *Mutat. Res.* **1989**, *223*, 273–285.

Wataya, Y.; Yamane, K. H.; Ohtsuka, Y.; Okubata, Y.; Negishi, K.; Hayatsu, H. Generation of intracellular active oxygens in mouse FM3A cells by 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole, the activated Trp-P-2. *Jpn. J. Cancer Res. (Gann)* **1988**, *79*, 576–579.

Yen, G. C.; Chen, H. Y. Comparison of antimutagenic effect of various tea extracts (green, oolong, pouchong and black tea). *J. Food Prot.* **1994**, *57*, 54–58.

Received for review July 13, 1994. Accepted October 24, 1994.[⊗]

JF940387N

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1994.