

Antioxidative Effect of Ethanol Tea Extracts on Oxidation of Canola Oil

Z.Y. Chen*, P.T. Chan, H.M. Ma, K.P. Fung, and J. Wang

Department of Biochemistry, The Chinese University of Hong Kong Shatin, New Territories, Hong Kong

ABSTRACT: There is an increasing interest in the biological effects of natural antioxidants present in teas on formation of *in vivo* free radicals, carcinogenesis, and atherogenesis. Teas are traditionally classified into six major groups, namely, green, yellow, white, black, dark-green, and oolong teas. The present study examined the antioxidative activity of ethanol extracts from these six major groups of teas against oxidation of heated canola oil. The oxidation was conducted at 100°C by monitoring oxygen consumption and changes in linoleic and linolenic acids in canola oil. The ethanol extracts of green, yellow, and white teas strongly inhibited oxidation of canola oil compared to butylated hydroxytoluene, probably due to the presence of natural polyphenols. In contrast, oolong teas examined exhibited only moderate antioxidative activity because of the partial destruction of natural polyphenols by semifermentation. The ethanol extracts of black, dark-green, and ginseng teas studied showed little or no protection to canola oil from lipid oxidation, probably due to the complete destruction of natural polyphenols by fermentation during manufacturing processes. *JAACS* 73, 375–380 (1996).

KEY WORDS: Antioxidants, black teas, canola oil, dark-green teas, ginseng teas, green teas, oolong teas, tea.

Tea, one of the most popular beverages in the world, has been traditionally classified into six categories based on distinct manufacturing processes, namely, green tea, black tea, dark-green tea, oolong tea, white tea, and yellow tea (1). Green teas are mostly consumed in Asian countries, while black teas are more popular in Western countries. The green teas generally refer to nonfermented products, while black teas are fermented products in which browning reactions are catalyzed by polyphenol oxidase. In contrast to black teas, the dark-green teas are fermented products in which the browning reaction is nonenzymatic. The oolong teas are semifermented products; the white teas are unfermented products and are started with the tender leaves or unopened leaf buds. Yellow teas are made similarly to green teas except that they are slightly fermented, and nonenzymatic browning reactions occur to a lesser extent.

Teas are believed to have a wide range of physiological properties, including being stimulants, antidepressants, and antiinflammatory, antihypertensive, antiatherosclerotic and

hypcholesterolemic agents (2,3). Teas are also excellent dietary resources for natural antioxidants known as polyphenolic compounds (4). Ingestion of such natural antioxidants has been shown to be inversely related to risk of coronary heart disease and perhaps to development of cancers (5,6). This is probably because an assortment of compounds, including superoxide anions, cholesterol oxide, hydroxyl radicals, and lipid peroxy radicals, may trigger lipid oxidation *in vivo* and accelerate the atherosclerosis process (7), whereas natural antioxidants, such as polyphenolic compounds of dietary origin, may inhibit lipid oxidation and attenuate the progress of atherosclerosis and thrombosis (8,9).

Epidemiological studies on the relationship between tea consumption and cancer so far have been controversial. In a study covering 20 countries, there was a significant inverse relationship between tea drinking and stomach cancer (10). Kono *et al.* (11) and Oguni *et al.* (12) have shown that the green tea drinkers tended to have a lower risk of cancer. However, Kinlen *et al.* (13) made an opposite observation, showing a significant positive correlation between tea consumption and stomach cancer in London. Although a few reports suggested that tea extracts may promote carcinogenesis (14,15), several studies have shown an anticarcinogenic activity of tea or tea components in animals (16,17).

Butylated hydroxyanisole and butylated hydroxytoluene (BHT) are still widely used as antioxidants in foods because of high stability, low cost, and efficacy, although they may have negative health consequences and consumers may reject synthetic food additives (18–22). Tea extracts may offer an alternative in protecting fats and oils in foods from oxidation. However, they are unmerited due to low solubility in oils. We found that ethanol may serve as a carrier to make tea extracts soluble in oil. Although antioxidative activity of green and black tea has been reported (23,24), information on white, yellow, ginseng, slimming (commercial tea products claiming to reduce lipogenesis), and dark-green teas is needed. The present study was to examine the antioxidative effects of various Chinese teas on oxidation of heated canola oil.

MATERIALS AND METHODS

Canola oils. Canola oil without addition of any synthetic antioxidants was obtained from the local market in Hong Kong. Five brands of green teas (ji-ping-long-jing, long-jing, xiang-

*To whom correspondence should be addressed.

pian, gou-gu-nao, and ling-zhi-cha), two brands of white teas (shou-mei-cha and bai-hao-yin-zhen), three brands of yellow teas (jun-shan-yin-zhen, wen-zhou-huang-cha, and meng-ding-huang-cha), two brands of oolong teas (tie-guan-yin and wuyi-oolong), four brands of black teas (da-hong-bao, fuzhou-hong-cha, qi-hong-cha, and lizhi-hong-cha), two brands of dark-green teas (xijiao-yun-wu and pu-er-cha), two brands of slimming teas (wulong-anti-adiposis and Chinese-kipling-keepfit), and two brands of ginseng teas (Korean ginseng and American ginseng tea) were purchased from tea shops in Hong Kong or other parts of China.

Ethanol extraction. Twenty-five grams of tea were soaked with 200 mL ethanol and then homogenized at full speed in a Brinkmann Polytron (Lucerne, Switzerland), followed by centrifugation. The ethanol phase was pipetted, filtered, and evaporated to a constant weight. The tea extracts were then weighed and diluted to 100 mL with ethanol and stored at -4°C .

Measurement of oxygen consumption. The method described by Bunick (25) and modified by Chen *et al.* (26) was used to monitor oxygen consumption. In brief, 1 mL of hexane, containing 500 mg of canola oil, was placed in a glass tube (150 × 16 mm, o.d.). The ethanol extract obtained from 25 mg tea (equivalent to 0.25–1.5 mg dry weight of tea extract) was added to the reaction glass tube. The components were mixed thoroughly, and the solvent was removed under a gentle stream of nitrogen at 45°C in the light. The reaction tube was then flushed with air and sealed tightly with a rubber stopper obtained from an evacuated blood collection tube (100 × 16 mm, o.d.; Becton-Disckinson, Rutherford, NJ), which usually maintains a vacuum for 2–3 yr. The sealed tube was leak-free verified by filling the tube with nitrogen gas and monitoring by gas chromatography if headspace oxygen concentration decreased. Oxidation was conducted at $100 \pm 2^{\circ}\text{C}$ with constant stirring. The headspace oxygen was sampled periodically with a gas-tight syringe and analyzed in an HP 5890 series II gas-solid chromatograph (Hewlett-Packard, Palo Alto, CA), fitted with a 1/8" × 6' stainless-steel column packed with Molecular Sieve 5A (60:80 mesh) and a thermal conductivity detector. The percentage of oxygen in the headspace was calculated from the ratio of oxygen to nitrogen. After headspace oxygen analysis, the canola oil was extracted with 10 mL chloroform and saved for fatty acid analysis.

The oxygen consumption (mL/tube) of canola oil with or without addition of tea extracts was calculated according to Equation 1:

$$\text{O}_2 \text{ consumption} = (A - B)/A \times C \quad [1]$$

where A = initial % headspace oxygen, B = % headspace oxygen after heating for 7.5, 24, 31, and 48 h, C = total initial volume oxygen (5.04 mL/tube).

Fatty acid analysis. Fatty acids of heated canola oil with or without addition of tea extracts were converted to the corresponding methyl esters with a mixture of 14% BF_3 in methanol (Sigma Chemical Co., St. Louis, MO) and toluene (1:1, vol/vol) under nitrogen at 90°C for 45 min (26). Fatty

acid methyl esters were analyzed on a flexible silica capillary column (SP 2560, 100 m × 0.25 mm, i.d.; Supelco, Inc., Bellefonte, PA) in an HP 5980 Series II gas-liquid chromatograph, equipped with a flame-ionization detector (Hewlett-Packard). Column temperature was programmed from 180 to 220°C at a rate of $1^{\circ}\text{C}/\text{min}$ and then held for 20 min. Injector and detector temperatures were 250 and 300°C , respectively. Hydrogen was used as the carrier gas at a head pressure of 20 psi (27).

Statistics. All experiments were repeated three times. Data were pooled from each experiment in which two to three replicates (total 6–8 reaction tubes/time point) were conducted. Data for headspace oxygen consumption and fatty acid analysis were subjected to the analysis of variance, and the means were compared between treatments by using Duncan's multiple range test (28). This was done by running data on the PC ANOVA software (PC ANOVA for the IBM Personal Computer, Version 1.1, 1985; IBM, Armonk, NY).

RESULTS AND DISCUSSION

As shown in Table 1, the dry weights of ethanol extracts varied with different varieties of teas. There was no consistent pattern to show that the dry ethanol extracts derived from one category of teas weighed more than another. It was, therefore, difficult to quantitate antioxidative effects of different teas on oxidation in canola oil based on the weight of ethanol extracts. For consistency and quantitation, the ethanol extracts obtained from the same amount of tea leaves (25 mg) were added to canola oil instead.

Typical gas-solid chromatograms of headspace air in canola oil with or without addition of green tea ethanol extracts (using long-jing as a typical example) heated at 100°C for 48 h are shown in Figure 1. In the canola oil control, 89% of headspace oxygen was consumed within 48 h (Table 2). In the presence of 200 ppm BHT, the depletion of oxygen was slightly delayed (84%) compared to the canola oil control. In contrast, canola oil with addition of ethanol extracts of four green teas, ji-ping-long-jing, xiang-pian, long-jing, and gou-gu-nao, was remarkably stable through the period examined,

TABLE 1
Dry Weight of Ethanol Tea Extracts^a

Tea	Weight (mg/g tea)	Tea	Weight (mg/g tea)
Jing-ping-long-jing	48.5 ± 1.8	Da-hong-bao	22.2 ± 0.2
Long-jing	43.3 ± 0.8	Fuzhou-hong-cha	24.2 ± 0.2
Xiang-pian	28.4 ± 2.4	Qi-hong-cha	11.4 ± 0.2
Gou-gu-nao	23.80 ± 0.4	Lizhi-hong-cha	10.3 ± 0.2
Ling-zhi-cha	23.2 ± 0.3	Xijiao-yun-wu	42.7 ± 0.8
Shou-mei-cha	63.2 ± 0.2	Fu-er-cha	10.5 ± 0.2
Bai-hao-yin-zhen	44.9 ± 0.2	Wulong-anti-adiposis	18.6 ± 0.2
Jun-shan-yin-zhen	22.5 ± 0.5	Chinese-Kipling-keepfit	11.6 ± 0.1
Wen-zhou-huang-cha	24.7 ± 0.2	Korean ginseng tea	36.0 ± 0.2
Meng-ding-huang-cha	34.4 ± 0.3	American ginseng tea	41.2 ± 0.2
Tie-guan-yin	49.7 ± 0.8		
Wuyi-oolong	24.9 ± 0.2		

^aData are means ± SD/n = 3.

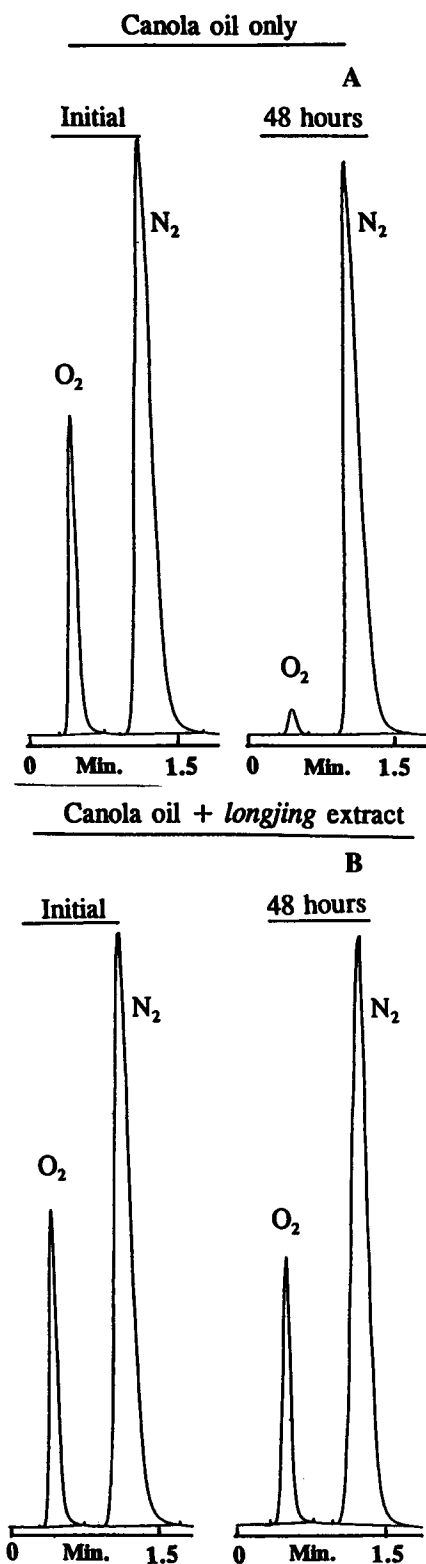


FIG. 1. Gas-chromatographic traces of headspace oxygen (O_2) and nitrogen (N_2) in reaction tubes containing (A) canola oil or (B) canola oil + long-jing ethanol extracts heated at 100°C for 48 h.

and only 15–35% headspace oxygen was consumed at 100°C for 48 h of heating (Table 2). The ethanol extract of ling-zhi-cha, however, was less protective in oxidation of canola oil than to the other four green teas examined ($P < 0.01$). The oxygen con-

sumption test indicated that all green teas examined, except for ling-zhi-cha, showed stronger antioxidative activity than BHT, in the order of ji-ping-long-jing, xiang-pian, long-jing, and gou-gu-nao, under the conditions described.

Oxygen consumption tests also indicated that the ethanol extracts of white teas, shou-mei-cha and bai-hao-yin-zhen, exhibited strong protection to canola oil from lipid oxidation ($P < 0.01$; Table 2), with the former white tea being more effective. Although BHT inhibited oxidation of lipids in canola oil, it was much less effective than these white tea extracts under the conditions described ($P < 0.01$). At 100°C for 31 h, shou-mei-cha and bai-hao-yin-zhen were equally protective as two green teas, ji-ping-long-jing and xiang-pian. When canola oil was heated at 100°C for 48 h, these two white teas were even more protective than long-jing, gou-gu-nao, and ling-zhi-cha against lipid oxidation in canola oil ($P < 0.01$; Table 2).

The effects of three yellow tea ethanol extracts on oxidation of canola oil were also examined under the same conditions. As for the green teas and white teas, three yellow teas exhibited stronger antioxidative activity than BHT ($P < 0.01$; Table 2), with jun-shan-yin-zhen being the most effective and meng-ding-huang-cha the least. When compared with the green teas tested, jun-shan-yin-zhen was less protective than ji-ping-long-jing and equally as protective as xiang-pian, but it was more protective than long-jing, gou-gu-nao, and ling-zhi-cha. When compared with two white teas examined, jun-shan-yin-zhen was equally protective as bai-hao-yin-zhen, but it was less protective than shou-mei-cha. In contrast, wen-zhou-huang-cha and meng-ding-huang-cha showed less antioxidative activity than the green teas and white teas examined except for ling-zhi-cha, long-jing, and gou-gu-nao at 100°C for 48 h ($P < 0.01$; Table 2).

The degree of protection varied among the ethanol extracts of oolong teas (Table 2). Tie-guan-yin was more effective than wuyi-oolong against oxidation of canola oil ($P < 0.01$; Table 2). Compared with the green teas and white teas examined, tie-guan-yin showed a similar effect to xiang-pian and bai-hao-yin-zhen in inhibiting lipid oxidation of canola oil heated at 100°C for 48 h. However, wuyi oolong demonstrated less antioxidative activity than the white teas and green teas examined except for ling-zhi-cha ($P < 0.01$; Table 2). Both oolong teas showed stronger protection than BHT against oxidation of canola oil ($P < 0.01$; Table 2).

All ethanol extracts of black teas tested exhibited only slight protection in canola oil against oxidation, except for da-hong-bao, which was more effective than BHT after 31 h heating at 100°C ($P < 0.01$; Table 2). All black teas tested showed much less antioxidative activity than the green teas (except for ling-zhi-cha), white teas, and yellow teas examined under the conditions described. They also were less protective than oolong tea, tie-guan-yin ($P < 0.01$; Table 2).

The ethanol extracts of two dark-green teas possessed little or no antioxidative effect on oxidation of canola oil compared with those of the green teas, white teas, and yellow teas tested (Table 2). Xijiao-yun-wu was equally as effective as BHT at 100°C for 31 h heating, whereas pu-er-cha did not show any protection to canola oil against oxidation (Table 2).

TABLE 2
Effect of Ethanol Tea Extracts on Oxygen Consumption (mL/tube) of Canola Oil Heated at 100°C in Sealed Reaction Tubes^a

	Oxygen consumption (mL/tube) ^b			
	7.5 h	24 h	31 h	48 h
Canola oil	0.16 ± 0.01 ^b	1.78 ± 0.15 ^{a,b}	3.30 ± 0.38 ^a	4.47 ± 0.69 ^a
Canola oil + BHT	0.25 ± 0.01 ^a	1.35 ± 0.04 ^c	2.55 ± 0.27 ^{b,c}	4.24 ± 0.38 ^a
Canola oil + green tea extract				
+ Ji-ping-long-jing	0.04 ± 0.01 ^c	0.18 ± 0.01 ^h	0.28 ± 0.01 ^h	0.79 ± 0.01 ^e
+ Long-jing	0.08 ± 0.01 ^c	0.24 ± 0.01 ^g	0.48 ± 0.01 ^{f,g}	1.67 ± 0.04 ^c
+ Xiang-pian	0.03 ± 0.01 ^c	0.17 ± 0.01 ^h	0.33 ± 0.01 ^h	1.29 ± 0.01 ^d
+ Gou-gu-nao	0.07 ± 0.01 ^c	0.24 ± 0.01 ^g	0.47 ± 0.01 ^{g,f}	1.78 ± 0.17 ^c
+ Ling-zhi-cha	0.10 ± 0.01 ^{b,c}	0.72 ± 0.01 ^d	1.31 ± 0.05 ^d	3.90 ± 0.29 ^a
Canola oil + white tea extract				
+ Shou-mei-cha	0.04 ± 0.01 ^c	0.16 ± 0.01 ^h	0.27 ± 0.01 ^h	0.74 ± 0.01 ^e
+ Bai-hao-yin-zhen	0.07 ± 0.01 ^c	0.28 ± 0.01 ^g	0.39 ± 0.01 ^g	1.12 ± 0.01 ^d
Canola oil + yellow tea extract				
+ Jun-sha-yin-zhen	0.25 ± 0.01 ^a	0.39 ± 0.02 ^{f,g}	0.54 ± 0.02 ^{f,g}	1.24 ± 0.05 ^d
+ Wen-zhou-huang-cha	0.31 ± 0.02 ^a	0.47 ± 0.02 ^f	0.70 ± 0.02 ^f	1.76 ± 0.02 ^c
+ Meng-ding-huang-cha	0.26 ± 0.01 ^a	0.55 ± 0.01 ^e	0.92 ± 0.02 ^e	2.49 ± 0.03 ^b
Canola oil + oolong tea extract				
+ Tie-guan-yin	0.11 ± 0.01 ^{b,c}	0.27 ± 0.01 ^g	0.36 ± 0.01 ^g	1.18 ± 0.03 ^d
+ Wuyi-oolong	0.07 ± 0.01 ^c	0.66 ± 0.01 ^d	1.34 ± 0.27 ^d	3.67 ± 0.65 ^a
Canola oil + black tea extract				
+ Da-hong-bao	0.15 ± 0.01 ^b	0.78 ± 0.03 ^d	1.53 ± 0.12 ^d	4.07 ± 0.60 ^a
+ Fuzhou-hong-cha	0.11 ± 0.01 ^b	1.25 ± 0.02 ^c	2.66 ± 0.09 ^{b,c}	4.42 ± 0.19 ^a
+ Qi-hong-cha	0.18 ± 0.01 ^b	1.38 ± 0.03 ^c	2.94 ± 0.03 ^b	4.45 ± 0.31 ^a
+ Lizhi-hong-cha	0.14 ± 0.02 ^b	1.68 ± 0.04 ^b	3.04 ± 0.14 ^b	4.40 ± 0.31 ^a
Canola oil + dark-green tea extract				
+ Xijiao-yun-wu	0.26 ± 0.01 ^a	1.26 ± 0.06 ^c	2.54 ± 0.03 ^c	3.98 ± 0.10 ^a
+ Pu-er-cha	0.07 ± 0.01 ^c	1.98 ± 0.01 ^a	3.41 ± 0.05 ^a	4.54 ± 0.65 ^a
Canola oil + slimming tea extract				
+ Wulong-anti-adiposis	0.07 ± 0.01 ^c	0.33 ± 0.02 ^g	0.61 ± 0.02 ^f	2.62 ± 0.72 ^b
+ Chinese-kipling-keepfit Tea	0.09 ± 0.01 ^{b,c}	0.59 ± 0.02 ^e	1.05 ± 0.04 ^e	3.36 ± 0.67 ^{a,b}
Canola oil + ginseng tea extract				
+ Korean ginseng tea	0.10 ± 0.01 ^{b,c}	0.84 ± 0.02 ^d	2.48 ± 0.15 ^{b,c}	4.27 ± 0.33 ^a
+ American ginseng tea	0.29 ± 0.01 ^a	1.37 ± 0.03 ^c	2.81 ± 0.39 ^b	4.44 ± 0.55 ^a

^aData are expressed as mean ± SD/n = 6–8 tubes.^bThe initial headspace oxygen was 5.04 mL/tube. Means in the same column with different superscripts (a–h) differ significantly ($P < 0.01$). BHT, butylated hydroxytoluene.

The effects of two popular slimming teas, wulong-anti-adiposis tea and Chinese kipling-keepfit tea, on oxidation of canola oil also were tested. These slimming teas were more protective against oxidation of canola oil than was BHT ($P < 0.01$, Table 2); however, they were less effective than the green teas (except for ling-zhi-cha), the yellow teas (except for meng-ding-huang-cha), and the white teas tested at 100°C for 48 h ($P < 0.01$; Table 2).

Korean ginseng tea and American ginseng tea had less antioxidative activity than the green (except for ling-zhi-cha), yellow, and white teas tested. However, the antioxidative effect of both ginseng teas was similar to that of BHT when canola oil was heated at 100°C for 31 h (Table 2).

To generalize the data obtained in the oxygen consumption test, the antioxidative activity of ethanol extracts tested were in the order of the white teas ≥ green teas ≥ yellow teas > oolong teas ≥ slimming teas > ginseng teas ≥ black teas = dark-green teas.

Fatty acid data from fatty acid methyl esters were generally consistent with the oxygen consumption test (Table 3). The more headspace oxygen was consumed, the more the

linoleic and linolenic acids were oxidized. Addition of ethanol extracts from the green teas (except for ling-zhi-cha), white teas and yellow teas (except for meng-ding-huang-cha) significantly prevented loss of linoleic and linolenic acids in canola oil heated at 100°C for 48 h ($P < 0.05$; Table 3). Oolong teas and slimming teas exhibited little protection against oxidation of linoleic and linolenic acids. Under the same conditions described, the black teas, dark-green teas, and ginseng teas tested showed no protection to linoleic and linolenic acid in canola oil from oxidation. It was noticed that fatty acid analysis was less sensitive than the oxygen consumption test (Tables 2 and 3). Not all significant differences detected by the oxygen consumption test between the tea extracts of different varieties were demonstrated by the fatty acid analysis.

The protective effect of tea extracts may be attributed to the total content of natural polyphenols present in tea leaves (23,24,29). The phenolic composition of teas has been analyzed previously (24,29). Xie *et al.* (23) reported that total flavanols (catechin, epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate) account for 25 and 31% of total dry extracts in green teas and oolong teas, re-

TABLE 3
Effect of Tea Ethanol Extracts on Fatty Acids of Canola Oil (wt% of total fatty acids)

	Linoleic	Linolenic	Oleic	Polyunsaturated ^a	Monounsaturated ^b	Saturated ^c
Unheated canola oil	21.47 ± 0.01 ^a	7.85 ± 0.02 ^a	54.43 ± 0.04 ^b	29.32 ± 0.03 ^a	57.31 ± 0.05 ^b	7.71 ± 0.08 ^{a,b}
Heated canola oil	19.50 ± 0.50 ^b	6.01 ± 0.26 ^b	57.37 ± 0.38 ^a	25.21 ± 0.75 ^b	61.85 ± 0.36 ^a	8.12 ± 0.43 ^a
Canola oil + BHT	19.96 ± 0.29 ^b	6.21 ± 0.15 ^b	57.21 ± 0.29 ^a	26.17 ± 0.44 ^b	60.96 ± 0.37 ^a	7.43 ± 0.03 ^{a,b}
Canola + green tea extracts						
+ Ji-ping-long-jing	21.29 ± 0.16 ^a	7.48 ± 0.09 ^a	54.98 ± 0.25 ^b	28.77 ± 0.24 ^a	58.56 ± 0.23 ^b	7.08 ± 0.06 ^b
+ Long-jing	21.25 ± 0.24 ^a	7.43 ± 0.20 ^a	54.94 ± 0.37 ^b	28.68 ± 0.44 ^a	58.20 ± 0.46 ^b	7.64 ± 0.08 ^{a,b}
+ Xiang-pian	21.20 ± 0.22 ^a	7.31 ± 0.20 ^a	55.61 ± 0.22 ^b	28.51 ± 0.42 ^a	58.99 ± 0.22 ^b	7.61 ± 0.30 ^{a,b}
+ Gou-gu-nao	20.71 ± 0.21 ^{a,b}	7.16 ± 0.10 ^a	55.42 ± 0.32 ^b	27.92 ± 0.25 ^a	58.69 ± 0.35 ^b	6.96 ± 0.16 ^b
+ Ling-zhi-cha	20.06 ± 0.02 ^b	6.17 ± 0.05 ^b	57.03 ± 0.09 ^a	26.22 ± 0.06 ^b	60.66 ± 0.12 ^a	7.60 ± 0.06 ^{a,b}
Canola oil + white tea extracts						
+ Shou-mei-cha	21.12 ± 0.10 ^a	7.38 ± 0.08 ^a	54.82 ± 0.12 ^b	28.50 ± 0.18 ^a	58.49 ± 0.13 ^b	7.22 ± 0.05 ^b
+ Bai-hao-yin-zhen	20.91 ± 0.34 ^{a,b}	7.21 ± 0.41 ^a	55.15 ± 0.57 ^b	28.12 ± 0.62 ^a	58.80 ± 0.68 ^b	7.14 ± 0.17 ^b
Canola + yellow tea extracts						
+ Jun-shan-yin-zhen	21.25 ± 0.25 ^a	7.40 ± 0.11 ^a	54.91 ± 0.32 ^b	28.65 ± 0.24 ^a	58.52 ± 0.33 ^b	7.10 ± 0.12 ^b
+ Wen-zhou-huang-cha	20.95 ± 0.24 ^a	7.24 ± 0.16 ^a	55.51 ± 0.33 ^b	28.20 ± 0.25 ^a	58.74 ± 0.35 ^b	7.28 ± 0.17 ^b
+ Meng-ding-huang-cha	20.43 ± 0.18 ^{a,b}	6.65 ± 0.14 ^{a,g}	56.22 ± 0.22 ^{a,b}	27.08 ± 0.25 ^b	59.64 ± 0.28 ^{a,b}	7.28 ± 0.15 ^b
Canola oil + oolong tea extracts						
+ Tie-guan-yin	21.25 ± 0.17 ^a	7.45 ± 0.03 ^a	54.81 ± 0.18 ^b	28.70 ± 0.19 ^a	58.27 ± 0.21 ^b	7.28 ± 0.11 ^b
+ Wuyi-ooling	20.03 ± 0.17 ^b	6.36 ± 0.08 ^b	57.01 ± 0.22 ^a	26.20 ± 0.11 ^b	60.63 ± 0.28 ^a	7.45 ± 0.07 ^{a,b}
Canola oil + black tea extracts						
+ Da-hong-bao	19.97 ± 0.04 ^b	6.37 ± 0.09 ^b	57.09 ± 0.16 ^a	26.34 ± 0.13 ^b	60.76 ± 0.20 ^a	7.55 ± 0.07 ^{a,b}
+ Fuzhou-hong cha	19.74 ± 0.08 ^b	6.14 ± 0.06 ^b	57.02 ± 0.06 ^a	25.88 ± 0.13 ^b	60.64 ± 0.22 ^a	8.18 ± 0.12 ^a
+ Qi-hong-cha	19.56 ± 0.06 ^b	6.03 ± 0.05 ^b	57.14 ± 0.14 ^a	25.59 ± 0.10 ^b	61.29 ± 0.13 ^a	7.80 ± 0.02 ^{a,b}
+ Lizhi-hong-cha	19.70 ± 0.11 ^b	6.03 ± 0.06 ^b	57.61 ± 0.20 ^a	25.73 ± 0.17 ^b	61.25 ± 0.21 ^a	7.57 ± 0.08 ^{a,b}
Canola oil + dark-green tea extracts						
+ Xijiao-yun-wu	19.97 ± 0.08 ^b	6.28 ± 0.15 ^b	57.17 ± 0.06 ^a	26.11 ± 0.23 ^b	60.73 ± 0.15 ^a	7.55 ± 0.11 ^{a,b}
+ Pu-er-cha	19.40 ± 0.45 ^b	6.03 ± 0.22 ^b	57.01 ± 0.23 ^a	25.25 ± 0.48 ^b	61.21 ± 0.38 ^a	8.12 ± 0.37 ^a
Canola + slimming tea extracts						
+ Wulong-anti-adiposis	20.54 ± 0.35 ^{a,b}	6.68 ± 0.23 ^{a,b}	56.25 ± 0.43 ^{a,b}	27.22 ± 0.58 ^{a,b}	59.80 ± 0.56 ^{a,b}	7.34 ± 0.16 ^b
+ Chinese-kipling-keepfit	20.14 ± 0.06 ^b	6.27 ± 0.03 ^b	56.92 ± 0.13 ^a	26.41 ± 0.09 ^b	60.49 ± 0.22 ^a	7.64 ± 0.07 ^{a,b}
Canola + ginseng tea extracts						
+ Korean ginseng tea	19.84 ± 0.21 ^b	6.16 ± 0.15 ^b	57.23 ± 0.36 ^a	26.00 ± 0.24 ^b	60.95 ± 0.38 ^a	7.51 ± 0.19 ^{a,b}
+ American ginseng tea	19.86 ± 0.02 ^b	6.07 ± 0.14 ^b	57.16 ± 0.06 ^a	25.93 ± 0.16 ^b	60.76 ± 0.12 ^a	7.61 ± 0.21 ^{a,b}

^aHeated at 100°C for 48 h. Data are expressed as means for n = 6–8 samples. Polyunsaturated = linoleic acid + linolenic acid; BHT, butylated hydroxytoluene. Means at the same column with different superscripts (a,b) differ significantly ($P < 0.05$).

^bMonounsaturated = oleic (18:1n-9) + 18:1n-7.

^cSaturated = myristic acid + palmitic acid + stearic acid + arachic acid.

spectively, whereas total flavanols only account for 10% of total dry extracts in black teas. They also demonstrated that these flavanols exhibited strong antioxidative activity against lipid oxidation in lard. Lunder (24) has shown that the antioxidant activity of green teas is positively related to the content of epigallocatechin. We are currently using high-performance liquid chromatography to purify and quantitate individual polyphenols and browning products in various Chinese teas and to examine their individual contribution to antioxidation.

The varying protection of tea ethanol extracts against lipid oxidation may be due to the distinct manufacturing processes. Green and white teas are nonfermented products in which the polyphenols are mostly preserved during processing. In contrast, the polyphenols in black teas are extensively oxidized by polyphenol oxidase while those in dark-green teas undergo nonenzymatic oxidation to form browning polymers, such as theaflavin and thearubigens (24,29). These browning compounds were reported to account for 17% in black tea extracts and 28% in oolong tea extracts, but they were quantitatively minor in green teas (23,29). Yellow teas are also subjected to

nonenzymatic oxidation but to a lesser extent than dark-green teas. Oolong teas are products in which the polyphenols present in the rim area of tea leaves are enzymatically oxidized, whereas those in the inner areas of leaves remain unoxidized. In fact, inhibitory effects of the oolong tea extracts on lipid oxidation in canola oil were between the green teas and black teas tested in the present study (Tables 2 and 3). It appears that the content of polyphenols varies with different varieties of teas and manufacturing processes.

In summary, we examined the antioxidative properties of 22 teas that are commonly consumed in China and Hong Kong. The ethanol extracts of white teas, green teas, and yellow teas contained powerful antioxidants, which are believed to be natural polyphenols. Due to the destruction of polyphenols by fermentation, nonenzymatic and/or enzymatic browning reactions, black teas and dark-green teas had little or no antioxidative activities against lipid oxidation in canola oil. The ethanol extracts of green teas, white teas, and yellow teas were even more protective than BHT against lipid oxidation in canola oil under the conditions described. It will be of in-

terest to purify and identify the active components in various teas and assess their individual contribution to antioxidation. Furthermore, an understanding of the mechanism involved, and the factors that influence antioxidative activity and the thermal stability of these antioxidants, would be of significant value in development of natural antioxidants to control lipid oxidation in foods.

ACKNOWLEDGEMENT

We thank the University Grant Council (Hong Kong) for financial support.

REFERENCES

- Chen, Z.P., *Encyclopedia of Chinese Teas*, Shanghai Culture Press, Shanghai, 1992, p. 386.
- Stagg, G.V., and D.J. Millin, Nutritional and Therapeutic Value of Tea, *J. Sci. Food Agric.* 26:1439–1459 (1975).
- Graham, H.N., The Plant and Its Manufacture, in *Methyl Xanthine Beverage and Foods: Chemistry, Consumption, and Health Effects*, edited by G. Spiller, Alan R. Liss, New York, 1984, pp. 29–74.
- Zhu, M., P.G. Qiao, P.P. Zhang, and W.H. Lu, Catechins in Green and Black Teas, *J. Chinese Trad. Med.* 17:677–678 (1992).
- Hertog, M.G.L., E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, and D. Kromhout, Dietary Antioxidant Flavonoids and Risk of Coronary Heart Disease, *Lancet* 342:1007–1011 (1993).
- Renaud S., and M. de Lorgeril, Wine, Alcohol, Platelets and French Paradox for Coronary Heart Disease, *Ibid.* 339:1523–1526 (1992).
- Addis, P.B., Coronary Heart Disease, An Update with Emphasis on Dietary Lipid Oxidation Products, *Nutrition Quarterly* 14:43–47 (1990).
- Kinsella, J.E., E. Frankel, B. German, and J. Kanner, Possible Mechanism for the Protective Role of Antioxidants in Wine and Plant Foods, *Food Technology*:85–89 (1993).
- Frankel, E.N., J. Kanner, J.B. German, E. Parks, and J.E. Kinsella, Inhibition *in vitro* of Oxidation of Human Low-Density Lipoproteins by Phenolic Substance in Wine, *Lancet* 341:1–4 (1993).
- Stocks, P., Cancer Mortality in Relation to National Consumption of Cigarettes, Solid Fuel, Tea and Coffee, *Br. J. Cancer* 24:215–225 (1970).
- Kono, S., M. Ikeda, S. Tokudome, and M. Kuratsune, A Case-Control Study of Gastric Cancer and Diet in Northern Kyushu, Japan, *Jpn. J. Cancer Res.* 79:1067–1074 (1988).
- Oguni, I., K. Nasu, S. Yamamoto, and T. Nomura, The Antitumor Activity of Fresh Green Tea Leaf, *Agric. Bio. Chem.* 52:1879–1880 (1988).
- Kinlen, L.J., A.N. Willows, P. Goldblatt, and J. Yudkin, Tea Consumption and Cancer, *Br. J. Cancer* 58:397–401 (1988).
- Kapadia, G.J., B.B. Paul, E.B. Chung, B. Ghosh, and S.N. Pradham, Carcinogenicity of Camellia Sinensis (Tea) and Some Tannin-Containing Folk Medical Herbs Administered Subcutaneously in Rats, *J. Natl. Cancer Inst.* 57:207–209 (1976).
- Bogovski, P., N. Day, M. Chvedoff, and F. Lafaverge, Accelerating Action of Tea on Mouse Skin Carcinogenesis, *Cancer Letter* 3:9–13 (1977).
- Shi, S.T., Z.Y. Wang, T.J. Smith, J.Y. Hong, W.F. Chen, C.T. Ho, and C.S. Yang, Effects of Green Tea and Black Tea on 4-(methylnitrosamine)-1-(3-Pyridyl)-1-Butanone Bioactivation, DNA Methylation and Lung Tumorigenesis in A/J Mice, *Cancer Res.* 54:4641–4647 (1994).
- Wang, Z.Y., M.T. Huang, Y.R. Lou, J.G. Xie, K.R. Reuhl, H.L. Newmark, C.T. Ho, C.S. Yang, and A.H. Conney, Inhibitory Effects of Black Tea, Green Tea, Decaffeinated Black Tea and Decaffeinated Green Tea on Ultraviolet B Light-induced Skin Carcinogenesis in 7, 12-Dimethylbenz(a) Anthracene-Initiated SKH-1 Mice, *Ibid.* 54:3428–3435 (1994).
- Branen, A.L., Toxicology and Biochemistry of Butylated Hydroxyanisole and Butylated Hydroxytoluene, *J. Am. Oil Chem. Soc.* 52:59–63 (1975).
- Ito, N., S. Fukushima, A. Hagiwara, M. Shibata, and T. Ogiso, Carcinogenicity of Butylated Hydroxyanisole in F344 Rats, *J. Natl. Cancer Inst.* 70:343–352 (1983).
- Altmann, H.J., W. Grunow, U. Mohr, H.B. Richter-Reichhelm, and P.W. Wester, Effects of BHA and Related Phenols on the Forestomach of Rats, *Food Chem. Toxic.* 24:1183–1188 (1986).
- Witschi, H., and C.C. Morse, Enhancement of Lung Tumor Formation in Mice by Dietary Butylated Hydroxytoluene: Dose-Time Relationships and Cell Kinetics, *J. Natl. Cancer Inst.* 71:859–866 (1983).
- Linderschmidt, R.C., A.F. Trylka, M.E. Goad, and H.P. Witschi, The Effects of Dietary Butylated Hydroxytoluene on Liver and Colon Tumor Development in Mice, *Toxicol.* 38:151–160 (1986).
- Xie, B., H. Shi, Q. Chen, and C.T. Ho, Antioxidant Properties of Fractions and Polyphenol Constituents from Green, Oolong and Black Teas, Proceedings of the National Science Council, ROC, Part B: *Life Science* 17:77–84 (1993).
- Lunder, T.L., Catechins of Green Tea, in *Phenolic Compounds in Food and Their Effects on Health II*, edited by C-T. Ho, C.Y. Lee, and M.T. Huang, American Chemical Society, Washington, D.C., 1992, pp. 114–120.
- Bunick, F.J., Lipid Autoxidation in Human Red Blood Cell Membrane, Ph.D. Dissertation, University of Massachusetts at Amherst, Massachusetts, 1984.
- Chen, Z.Y., W.M.N. Ratnayake, and S.C. Cunnane, Oxidative Stability of Flaxseed Lipids During Baking, *J. Am. Oil Chem. Soc.* 71:629–632 (1994).
- Chen, Z.Y., G. Pelletier, R. Hollywood, and W.M.N. Ratnayake, Trans Fatty Acids in Canadian Human Milk, *Lipids* 30:15–21 (1995).
- Duncan, D.B., Multiple Range and F-Tests, *Biometrics* 11:1–42 (1955).
- Balentine, D.A., Manufacturing and Chemistry of Tea, in *Phenolic Compounds in Food and Their Effects on Health I*, edited by C.-T. Ho, C.Y. Lee, and M.T. Huang, American Chemical Society, Washington, D.C., 1992, pp. 102–117.

[Received May 22, 1995; accepted December 20, 1995]