

Antioxidative Activity of Green Tea Catechin Extract Compared with That of Rosemary Extract

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ABSTRACT: This study compared the antioxidative activity of green tea catechin (GTC) extract with that of rosemary extract in canola oil, pork lard, and chicken fat. The GTC extract was obtained from jasmine and longjing green teas and mainly consisted of four epicatechin isomers including (–)epigallocatechin gallate (EGCG), (–)epigallocatechin (EGC), (–)epicatechin (EC), and (–)epicatechin gallate (ECG). The oxidation was conducted at $100 \pm 2^\circ\text{C}$ by monitoring oxygen uptake. The oxygen consumption test demonstrated that GTC extract was much more effective than the rosemary extract against lipid oxidation in canola oil, pork lard, and chicken fat under the conditions of the present study. Together with our previous study which showed that GTC extract was more protective than butylated hydroxytoluene as an antioxidant, these results suggest that GTC as a mixture of EGCG, EGC, EC, and ECG may serve as an antioxidant in processed foods. *JAACS* 75, 1141–1145 (1998).

KEY WORDS: Canola oil, chicken fat, epicatechin, green tea, jasmine tea, longjing, pork lard, rosemary.

Tea is the most widely consumed beverage in the world and is prepared from the leaves of the *Camellia sinensis* plant. Green tea catechins (GTC) are a mixture of epicatechin isomers mainly consisting of (–)epigallocatechin gallate (EGCG), (–)epigallocatechin (EGC), (–)epicatechin (EC), and (–)epicatechin gallate (ECG). Consumption of green tea has been shown to be associated with a decrease in serum total cholesterol and triacylglycerols (1,2). It has been suggested that oxidative modification of low-density lipoprotein (LDL) may play a role in the development of atherosclerosis (3–6). The Zutphen Elderly Study by Hertog *et al.* (7) showed an inverse association between tea consumption and coronary disease mortality after adjustment for age, diet, and other risk factors. In fact, the ingestion of both black and green tea produced a significant increase in human plasma antioxidant capacity *in vivo* (8). Furthermore, the GTC extract and its epicatechin isomers are effective agents to protect human LDL from oxidative modification *in vitro* (9–11).

GTC may also offer an alternative in protecting fats and oils in foods from oxidative rancidity (12–13). We previously

examined the antioxidative activity of ethanol tea extract and found that green tea and white tea exhibited a stronger inhibition of oxidation of canola oil than did black tea and dark-green tea (14). In another study, we examined the antioxidative activity of individual epicatechin isomers and found that EGC was most effective followed by EGCG, EC, and ECG in canola oil at 95°C (15). In addition, all four isomers were shown to be more protective than butylated hydroxytoluene (BHT) against oxidation of heated canola oil at the concentration of 200 ppm.

The extract of rosemary leaves has been one of the most effective antioxidants among the spices (16,17), and its active compounds have been purified and identified elsewhere (18–23). However, there have been no reports comparing the antioxidative activity of rosemary extract with that of GTC extract. Therefore, the present study was conducted to examine the relative antioxidative activity of rosemary extract and GTC extract from Chinese longjing and jasmine tea in canola oil, pork lard, and chicken fat using the oxygen consumption test.

MATERIALS AND METHODS

Preparation of chicken fat and pork lard. Fresh white pork and chicken adipose tissues were purchased from the local market. After homogenization (Polytron, Brinkmann, Rexdale, Ontario, Canada), the fatty tissues were then placed in a hot pan (approximately 100°C) for 10 min. The liquid pork and chicken fats were filtered through cotton wool, cooled to room temperature, and then saved at -20°C until use. Canola oil without addition of any synthetic antioxidants was obtained from Lam Soon Marketing Service Ltd. (Kowloon, Hong Kong). The rosemary extract (brand name: Pristene RO) was obtained from Kemin Industries PTE Ltd., Singapore). According to the supplier, Pristene RO is a natural rosemary oleoresin extracted from rosemary leaves (*Rosemarinus officianalis*). It is proposed to be used as a natural seasoning and stabilizer in a wide range of materials such as lard, tallow, fish oils, processed poultry, meat, salad dressings, sauces, minced fish, seasonings, snacks, nuts, soup bases, pet foods, and other products.

GTC extraction and high-performance liquid chromatography (HPLC) analysis. The method described by Agarwal

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et al. (24) was modified and used to extract total GTC from longjing tea (Huangshan Forestry Farm, Xiaoshan, Zhejiang, China) and jasmine tea (Cheong Hing Tea Co. Ltd., Hong Kong). In brief, 10 g of dry tea leaves were soaked three times with 150 mL of hot distilled water (80°C). The infusion was cooled to room temperature, filtered, and then extracted with an equal volume of chloroform to remove caffeine and pigment. The GTC in the remaining aqueous layer was then extracted twice with an equal volume of ethyl acetate. After removing ethyl acetate using a vacuum rotary evaporator, the resulting crude GTC extract was dissolved in 10 mL of distilled water and freeze-dried overnight.

The individual epicatechin isomers in longjing and jasmine tea extracts were separated using a Shimadzu LC-10AD HPLC (Tokyo, Japan) equipped with a ternary pump as we previously described (9,15). Five milligrams of longjing or jasmine GTC extract were dissolved in 10 mL distilled water. An aliquot of the solution (0.4 mL) was mixed with 0.1 mL of the internal standard solution containing 0.5 mg/mL (+) catechin (Sigma, St. Louis, MO). 15 μ L of the resulting mixture was then injected onto a column (Hypersil ODS, 250 \times 4.6 mm, 5 μ m; Alltech, Deerfield, IL) via a rheodyne valve. An eluting mixture of H₂O containing 0.05% H₂SO₄, acetonitrile, and ethyl acetate (86:12:2, vol/vol/vol) was used at a flow rate of 1 mL/min. The separated GTC isomers were monitored and quantified using an ultraviolet (UV) detector at 280 nm (UVIS-205, Alltech).

Oxygen consumption test. The method as we previously described (14,25) was used to monitor oxygen consumption. The rosemary extract is soluble, whereas the GTC extract is poorly soluble in oil and fat. To overcome the problem of poor solubility in oil and fat, we have used ethanol as a solvent to deliver the GTC extract into canola oil, pork lard, and chicken fat, followed by evaporation of the ethanol. In the case of the rosemary extract, hexane was used as a solvent instead. In brief, 1 mL of hexane containing 200 mg of canola oil or pork lard or chicken fat was placed in a glass tube (150 \times 16 mm, o.d.). One milliliter of ethanol containing 0.04 mg of the GTC extract or 1 mL of hexane containing 0.04 mg of the rosemary extract was also delivered to the reaction tube using a pipette. The components were mixed thoroughly on a mixer. The hexane and ethanol were removed under a gentle stream of nitrogen at 45°C. The final concentration of GTC extract or rosemary extract was set at 200 ppm in the three oils and fats tested because a maximum 200 ppm of a single or a mixture of antioxidants is generally permitted as an additive to fats and oils in many countries (26). After the reaction tube was flushed with air, a rubber stopper obtained from an evacuated blood collection tube (100 \times 16 mm, o.d., Becton-Dickinson, Rutherford, NJ) was used to seal the reaction tube. Oxidation was conducted at 100 \pm 2°C, and 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 \times 6 inch stainless-steel column packed with Molecular Sieve 5A (60/80 mesh) and a thermal conductivity detector. The headspace oxygen concen-

tration was determined from the ratio of the oxygen to nitrogen peak (14).

Fatty acid analysis. To examine the effect of rosemary extract and longjing and jasmine GTC extract on oxidation of polyunsaturated fatty acids in three fats and oils tested, another set of reaction tubes were similarly prepared and heated for 20 h. Fatty acids of heated canola oil, pork lard, and chicken fat with or without addition of the rosemary extract, longjing and jasmine GTC extract were then converted to the corresponding methyl esters with a mixture of 14% BF₃ in methanol (Sigma) and toluene (1:1, vol/vol) under nitrogen at 90°C for 45 min. Fatty acid methyl esters were analyzed on a SP 2560 column (100 m \times 0.25 mm i.d.; Supelco, Bellefonte, PA) in an HP 5890 Series II gas-liquid chromatograph, equipped with a flame-ionization detector. Column temperature was programmed from 180 to 220°C at a rate of 1°C/min and then held for 20 min. Injector and detector temperatures were set at 250 and 300°C, respectively. Hydrogen was used as the carrier gas at a head pressure of 20 psi.

Statistics. Data are expressed as means \pm SD of 4–5 replicates. Analysis of variance was used for statistical evaluation of significant differences between treatments.

RESULTS AND DISCUSSION

Green tea polyphenols are mainly derivatives of epicatechin with EGCG being most abundant followed by ECG (Table 1). The HPLC analysis showed that the yield of longjing and jasmine GTC extract was 10.1 and 7.5 g/100 g dry tea leaves, respectively (Table 1). These compounds obtained from tea possess strong antioxidant activity under the experimental conditions evaluated (12,27). The antioxidative properties of various tea ethanol extracts were previously examined and green and white tea (which is the product of unfermented tender or unopened leaf buds) extract was found to exhibit a stronger inhibition on lipid oxidation in canola oil than did BHT (14). In contrast, the extract from black and dark-green tea showed little or no antioxidative activity. The varying protection of tea extract against lipid oxidation may be attributed to the amount of GTC, because green and white tea are non-fermented products in which GTC is mostly preserved, whereas GTC in black tea and dark-green tea are oxidized to form brown polymers either by polyphenol oxidase or non-

TABLE 1
Composition of Catechins Extracted from Jasmine and Longjing Tea^a

	Absolute (g/100 g tea)		Relative (% of total)	
	Longjing	Jasmine	Longjing	Jasmine
Epigallocatechin gallate	6.5	4.7	64.3	62.7
Epicatechin gallate	2.1	1.4	20.8	18.7
Epicatechin	0.5	0.4	5.0	5.3
Epigallocatechin	0.1	0.6	1.0	8.0
Others	0.9	0.4	8.9	5.3
Total	10.1	7.5	100	100

^aData are expressed as an average of three samples.

enzymatic browning reactions (28). In a separate study that examined the relative antioxidative activity of four epicatechin isomers at 200 ppm in canola oil heated at 95°C, it was found that EGC was most effective as an antioxidant followed by EGCG, EC, and ECG. We analyzed 33 different kinds (products from different manufacturers) of green teas available in the retail market and found that the total green tea catechins as a mixture of EGCG, EGC, EC, and ECG accounted for 6.5–12.5% of the dry tea leaves (data not shown).

In the present study, crude extract from both longjing and jasmine tea at 200 ppm exhibited strong antioxidative activity against lipid oxidation in canola oil, pork lard, and chicken fat, compared with those samples with addition of the same concentration of the rosemary extract (Figs. 1–3). The oxygen consumption test showed that the GTC extract slowed the oxygen uptake of canola oil and pork and chicken fat more effectively than the rosemary extract under the present experimental conditions. Fatty acid data were generally consistent with the oxygen consumption test, suggesting longjing and jasmine GTC extract was more effective than rosemary extract against oxidation of linoleic acid and linolenic acid (Table 2). The extract of rosemary leaves is generally believed to be one of the most effective antioxidants among spices and has wide application in processed foods (16,17). However, the GTC extract has not found much application in foods, probably owing to its poor solubility in fat and oil. The present study was the first report to directly compare the antioxidative activity of the rosemary extract added to canola oil, pork lard, and chicken fat with that of the GTC extract,

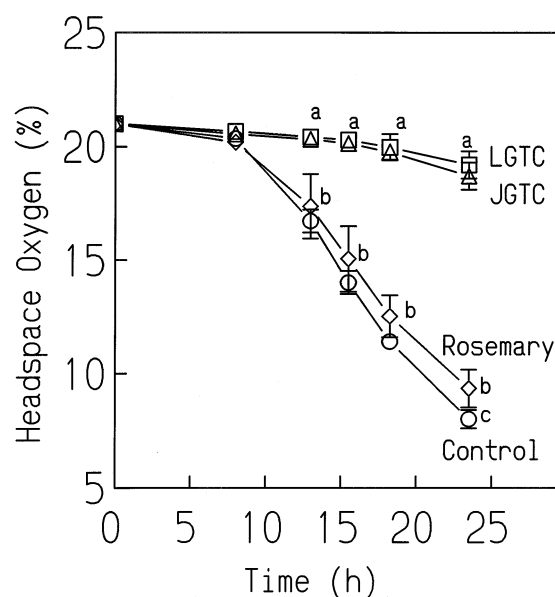


FIG. 2. Effect of rosemary extract, LGTC, and JGTC on oxidation of pork lard heated at 100°C. Each value is the mean \pm SD/ $n = 4-5$ samples. (○) Lard; (◇) lard + 200 ppm rosemary extract; (△) lard + 200 ppm JGTC; and (□) lard + 200 ppm LGTC. Means at the same time point with different letters (a,b,c) differ significantly at $P < 0.05$. See Figure 1 for abbreviations.

although the technique and method used were simple and straightforward.

In summary, this study measured the content of epicatechin isomers of longjing and jasmine GTC extract and found that the antioxidant activity of these two tea extracts was

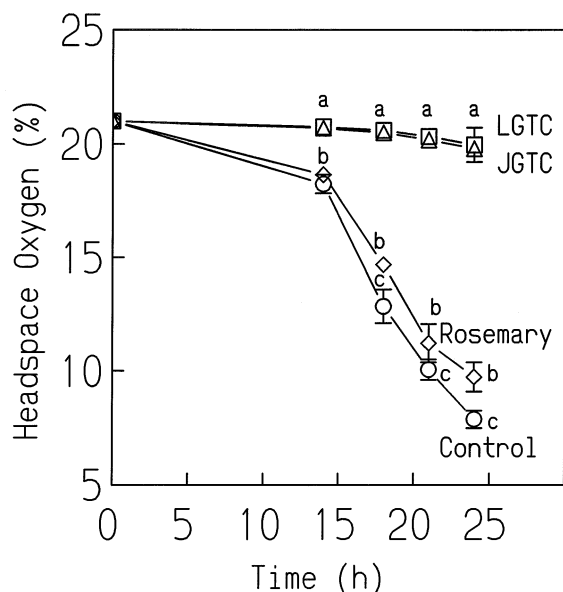


FIG. 1. Effect of rosemary extract, longjing green tea catechin extract (LGTC), and jasmine green tea catechin extract (JGTC) on oxidation of canola oil heated at 100°C. Each value is the mean \pm SD/ $n = 4-5$ samples. (○) Canola oil; (◇) canola oil + 200 ppm rosemary extract; (△) canola oil + 200 ppm JGTC; and (□) canola oil + 200 ppm LGTC. Means at the same time point with different letters (a,b,c) differ significantly at $P < 0.05$.

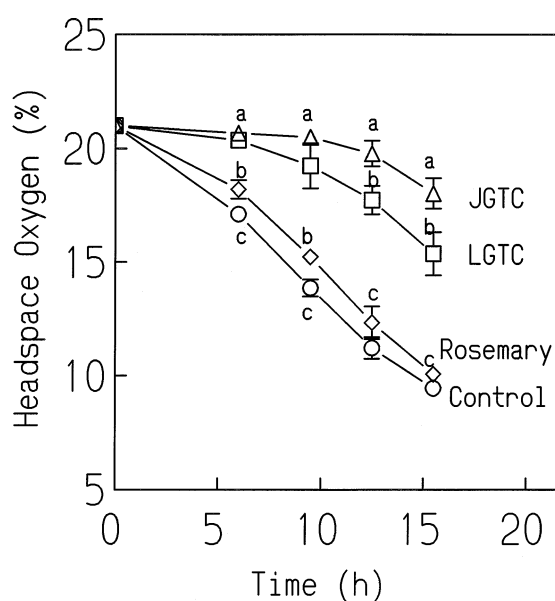


FIG. 3. Effect of rosemary extract, LGTC, and JGTC on oxidation of chicken fat heated at 100°C. Each value is the mean \pm SD/ $n = 4-5$ samples. (○) Chicken fat; (◇) chicken fat + 200 ppm rosemary extract; (△) chicken fat + 200 ppm JGTC; and (□) chicken fat + 200 ppm LGTC. Means at the same time point with different letters (a,b,c) differ significantly at $P < 0.05$. See Figure 1 for abbreviations.

TABLE 2
Effect of Rosemary Extract, Longjing Green Tea Catechin (LGTC), and Jasmine Green Tea Catechin (JGTC) Extract on Changes in Fatty Acids of Canola Oil, Pork Lard, and Chicken Fat (wt% of total fatty acids)^a

	Palmitic	Stearic	Oleic	Vaccenic	Linoleic	Linolenic	Others
Canola oil							
Unheated	4.29 ± 0.01 ^c	2.06 ± 0.01	57.85 ± 0.05 ^c	3.25 ± 0.01	19.37 ± 0.05 ^a	7.01 ± 0.01 ^a	6.17 ± 0.10
Heated	4.50 ± 0.01 ^a	2.15 ± 0.02	59.79 ± 0.03 ^a	3.32 ± 0.03	18.17 ± 0.17 ^b	5.94 ± 0.09 ^c	6.13 ± 0.25
+ Rosemary extract	4.40 ± 0.03 ^{a,b}	2.10 ± 0.01	58.94 ± 0.11 ^{a,b}	3.31 ± 0.01	18.82 ± 0.03 ^c	6.45 ± 0.03 ^b	6.00 ± 0.08
+ LGTC extract	4.31 ± 0.02 ^{b,c}	2.07 ± 0.01	58.07 ± 0.08 ^{b,c}	3.27 ± 0.01	19.36 ± 0.02 ^a	6.94 ± 0.02 ^a	5.99 ± 0.05
+ JGTC extract	4.24 ± 0.01 ^{b,c}	2.08 ± 0.01	58.07 ± 0.04 ^{b,c}	3.26 ± 0.01	19.35 ± 0.02 ^a	6.93 ± 0.02 ^a	6.08 ± 0.06
Lard							
Unheated	23.80 ± 0.29 ^b	8.82 ± 0.07 ^b	35.01 ± 0.19 ^b	4.00 ± 0.04 ^b	17.34 ± 0.11 ^a	0.71 ± 0.01 ^a	10.33 ± 0.17 ^b
Heated	27.05 ± 0.22 ^a	9.98 ± 0.10 ^a	36.38 ± 0.03 ^a	4.18 ± 0.02 ^a	11.24 ± 0.20 ^b	0.35 ± 0.02 ^b	10.81 ± 0.10 ^a
+ Rosemary extract	26.48 ± 0.89 ^{a,b}	9.90 ± 0.20 ^a	36.32 ± 0.08 ^a	4.17 ± 0.01 ^a	12.30 ± 1.36 ^b	0.40 ± 0.07 ^b	10.44 ± 0.25 ^{a,b}
+ LGTC extract	23.85 ± 0.07 ^b	8.82 ± 0.01 ^b	35.00 ± 0.03 ^b	4.02 ± 0.01 ^b	17.34 ± 0.04 ^a	0.71 ± 0.01 ^a	10.27 ± 0.05 ^b
+ JGTC extract	23.89 ± 0.03 ^b	8.79 ± 0.02 ^b	34.94 ± 0.01 ^b	4.00 ± 0.02 ^b	17.33 ± 0.01 ^a	0.70 ± 0.01 ^a	10.33 ± 0.09 ^b
Chicken fat							
Unheated	25.77 ± 0.46 ^b	6.13 ± 0.05 ^b	36.04 ± 0.28 ^b	1.88 ± 0.07	19.35 ± 0.14 ^a	0.79 ± 0.01 ^a	10.02 ± 0.28 ^b
Heated	29.07 ± 0.29 ^a	6.97 ± 0.07 ^a	37.54 ± 0.20 ^a	1.91 ± 0.06	13.11 ± 0.18 ^c	0.35 ± 0.01 ^b	11.05 ± 0.22 ^a
+ Rosemary extract	28.95 ± 0.71 ^a	6.88 ± 0.04 ^a	37.03 ± 0.39 ^a	1.86 ± 0.52	13.85 ± 0.52 ^b	0.39 ± 0.03 ^b	11.05 ± 0.37 ^a
+ LGTC extract	26.03 ± 0.04 ^b	6.15 ± 0.01 ^b	35.95 ± 0.01 ^b	1.81 ± 0.01	19.28 ± 0.01 ^a	0.78 ± 0.01 ^a	10.01 ± 0.08 ^b
+ JGTC extract	26.25 ± 0.47 ^b	6.11 ± 0.08 ^b	35.80 ± 0.36 ^b	1.81 ± 0.02	19.27 ± 0.22 ^a	0.78 ± 0.02 ^a	9.98 ± 0.27 ^b

^aSamples were heated at 100°C for 20 h. Data are expressed as means for $n = 4$ samples. Means at the same column with different superscript letters (a,b,c) in the same oil differ significantly ($P < 0.05$).

stronger than that of the rosemary extract under the experimental conditions evaluated. It is recognized that the conditions used in this study had some limitations. The advantages of using the tea extracts as antioxidants over rosemary extract or other antioxidants should be evaluated under various realistic conditions including varying temperatures, light, emulsions, and different food processes before final conclusions can be made.

ACKNOWLEDGMENTS

We thank the Hong Kong Research Grant Council and the Wei-Lun Foundation for partial support of this research. Zesheng Zhang is from Tianjin Institute of Light Industry and Li-Ya Wang is from Luoyang Normal College, China.

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[Received November 17, 1997; accepted May 2, 1998]