Antioxidant Properties of Catechins and Green Tea Extracts in Model Food Emulsions

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Extraction of green tea with hot water provided an extract that was highly effective as an antioxidant for an oil-in-water emulsion at pH 5.5 during prolonged storage (40 days). Other components besides epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) made important contributions to the antioxidant activity of the extract in an oil-in-water emulsion, since ECG, when pure, had no significant antioxidant activity ($p > 0.05$) and EGCG was completely oxidized within 15 days. Myricetin had a greater antioxidant effect in this system than EGCG or ECG at a concentration of 10^{-4} M in the absence of ferric ions, but all three flavonoids exhibited prooxidant effects in the presence of ferric ions.

Keywords: *Antioxidants; epigallocatechin gallate; epicatechin gallate; green tea; emulsions*

INTRODUCTION

Green tea extracts are of great interest as food antioxidants with possible beneficial effects on human health in terms of anticarcinogenic (Ito *et al.*, 1992) and antimutagenic effects (Price, 1994). Many studies have shown that green tea extracts have strong antioxidant effects, with the catechins epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), and epicatechin (EC) being considered the only significant antioxidant components by many authors, although Amarowicz and Shahidi (1995) showed that a crude extract from green tea had a greater antioxidant activity than a reconstituted catechin mixture. The antioxidant activity of these catechins has been demonstrated in heated oils (Wanasundara *et al.*, 1996), in a β-carotene/linoleate emulsion (Amarowicz and Shahidi, 1995), in tests of radical-scavenging activity with radical initiators (Rice-Evans *et al.*, 1996; Hara, 1995; Chen and Ho, 1995), in superoxide-scavenging tests (Chen and Ho, 1995), in oxidation accelerated by heat and oxygen supply (Lunder, 1992; Matsuzaki and Hara, 1985), and in enzyme-catalyzed oxidation (Yeo *et al.*, 1995). However, all of these studies involved rapid investigations in which oxidation is studied over a short time period. The aim of this paper is to report a study of the antioxidant properties and stability of tea catechins and green tea extracts in model emulsions stored for longer periods, which are more relevant to normal food systems.

MATERIALS AND METHODS

Sodium acetate and acetic acid were supplied by Merck Ltd., Lutterworth, U.K. Tween 20 was supplied by Sigma-Aldrich Co. Ltd., Poole, U.K., and myricetin by Aldrich Chemical Co., Gillingham, U.K. Rosemary extract was supplied by W. Janitz, Agricultural University of Poznan, Poland. ECG was donated by Unilever plc, Sharnbrook, U.K. EGCG was isolated by column chromatography from a methanolic tea extract according to the method of Bailey and Nursten (1993). The purity of the ECG was determined as 95% and the purity of the EGCG was determined as 98% by HPLC. UV spectra of solutions in acetate buffer (pH 5.5) showed peaks at 273 and 216.6 nm for EGCG and at 275.7 and 218.2 nm for ECG. There were no peaks in the visible region of these spectra.

Gunpowder green tea from Twinings plc was used for extraction. The aqueous tea extract was prepared by extracting the tea leaves (100 g) in water (500 mL) for 8 min at 94 °C, vacuum filtration through filter paper No. 1, and freeze-drying of the filtrate. A methanolic tea extract was prepared according to the method of Ho *et al.* (1992).

Emulsions, pH 5.5, were prepared with sunflower oil (30%) and water (70%) containing acetate buffer (0.1 M) and Tween 20 (1.0%) by sonication on a 25 g scale. Emulsions (50 g) were stored in 100 mL beakers covered with cling film, and samples were removed periodically during storage at 30 °C. No mold growth or physical separation of samples was observed during storage. All storage experiments were performed in duplicate. Isolation of oil from emulsions for analysis was by freezing, thawing, and centrifugation. Progress of oxidation was monitored by determination of the peroxide value (PV) (AOCS Official Method Cd 8-53) and conjugated dienes (AOCS Official Method Ti 1a-64). Headspace analysis of volatiles by gas chromatography was performed on selected samples according to a method based on that of Warner *et al.* (1989), with the modification that a sample heating temperature of 150 °C was used.

Tocopherol analysis by HPLC was performed according to IUPAC Method 2.432.

HPLC analysis of catechins was performed with a HIPBR (base-deactivated) column (25 cm × 6 mm i.d.) (Hichrom, Reading, U.K.) and UV detection at 280 nm. The mobile phase contained citric acid (1%) adjusted to pH 2.8 with sodium hydroxide, with a linear gradient starting with acetonitrile/water (8:92), changing to acetonitrile/water (31:69) over a period of 50 min with a flow rate of 1 mL/min. Authentic samples of ECG and EGCG were used as external standards for calibration in the range 0−0.5 mg/mL.

Statistical analysis to determine significant differences in antioxidant activity involved plotting PV against time to determine times to certain peroxide values and then applying ANOVA one-way analysis to determine the pooled standard deviation. The individual means were compared by a two-sample *t*-test using the pooled standard deviation to determine differences significant at the 5% level.

RESULTS AND DISCUSSION

Tea extracts added to oil-in-water emulsions (pH 5.5) were very effective in stabilizing the emulsion with

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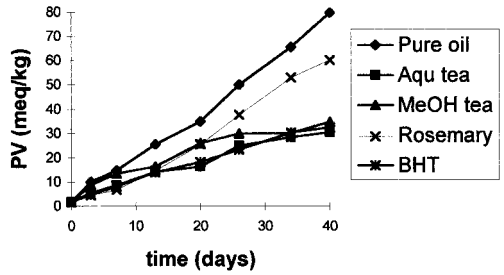

Figure 1. Effect of tea extracts (0.03%), rosemary extract (0.03% overall), and BHT (0.007% i.e. 0.02% in the oil) on the oxidation of sunflower oil-in-water emulsions stored at 30 °C, assessed by the peroxide value.

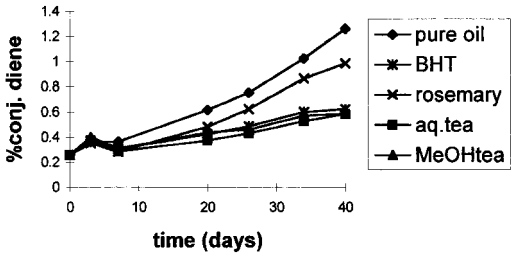

Figure 2. Effect of tea extracts (0.03%), rosemary extract (0.03%), and BHT (0.007% i.e. 0.02% in the oil) on the oxidation of sunflower oil-in-water emulsions stored at 30°C, assessed by the conjugated diene value.

0.03% tea extract being similar to BHT (0.02%) in activity during 40 days of storage at 30 °C (Figure 1). A rosemary extract (0.03%) had moderate antioxidant activity under these storage conditions, whereas a methanolic tea extract (0.03%) had moderate activity similar to that of rosemary in the early stages up to PV = 20 mequiv/kg but was as effective ($p < 0.05$) as the aqueous tea extract at PV = 30 mequiv/kg. Conjugated diene determinations (Figure 2) also showed the good antioxidant activity of the tea extracts, but no significant difference between the aqueous and methanolic tea extracts was evident from this data. The antioxidant activity of tea extracts has been ascribed to the activity of the catechins EGCG, ECG, EC, and EGC. However, HPLC analysis of EGCG, ECG, and EC in stored aqueous solutions of tea extracts (pH 5.5) indicated that EGCG and EC had deteriorated completely in 15 and 30 days, respectively, with the ECG level falling below 20% of the initial concentration after 30 days (Table 1). Storage of aqueous solutions of relatively pure ECG and EGCG confirmed the rate of oxidation of these catechins. Oxidation of tea catechins has been found to proceed within a few minutes under alkaline conditions (Yoshioka *et al.*, 1991), but it appears that oxidation proceeds at a significant rate under acid conditions, too. This suggests that other components in the tea or products formed from the catechins, e.g. dimers, play important roles as antioxidants during the storage of foods containing added tea extracts. The role of the other components is clearly not just one of metal chelation, since the presence of citric acid did not significantly improve the stability of the catechins. When ferric chloride was present in the aqueous phase, peroxide value (Figure 3) and conjugated diene measurements (Figure 4) indicated that the aqueous tea extract still had an antioxidant activity similar to that of BHT, whereas the methanolic tea extract was significantly weaker ($p < 0.05$) as an antioxidant than BHT in the emulsion despite the fact that the methanolic tea extract contained much higher levels of catechins (Table 2) due

Table 1. Stability of Tea Catechins in Aqueous Solutions (pH 5.5) Stored at 30 °C (All Concentrations Expressed as Percent of Initial Concentration)

sample	storage time (days)	EGCG (%)	ECG (%)	EC (%)
tea extract (aqueous, 0.03%)	15	0	47.9	72.2
	30	0	16.2	0
tea extract (aqueous, 0.05%, containing 10^{-4} M EGCG)	15	0	62.2	72.8
	30	0	13.9	nd[a]
tea extract (aqueous, 0.03% + citric acid, 0.01%)	15	0	50.9	76.8
	30	0	18.9	nd
tea extract (aqueous, 0.05%, containing 10^{-4} M EGCG + citric acid, 0.01%)	15	0	57.9	62.1
	30	0	19.1	0
EGCG (10^{-4} M)	15	0		
EGCG (10^{-4} M) + citric acid (0.01%)	15	0		
ECG (10^{-4} M)	15		45.1	
	30		28.3	
ECG (10^{-4} M) + citric acid (0.01%)	15		51.6	
	30		27.8	

[a] nd, not determined.

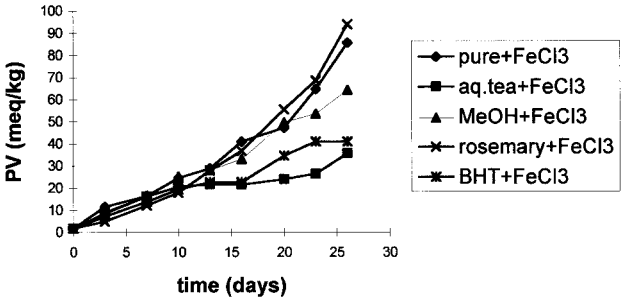

Figure 3. Effect of tea extracts (0.03%), rosemary extract (0.03%), and BHT (0.007% i.e. 0.02% in the oil) on the oxidation of sunflower oil-in-water emulsions containing ferric chloride (5×10^{-5} M) stored at 30 °C, assessed by the peroxide value.

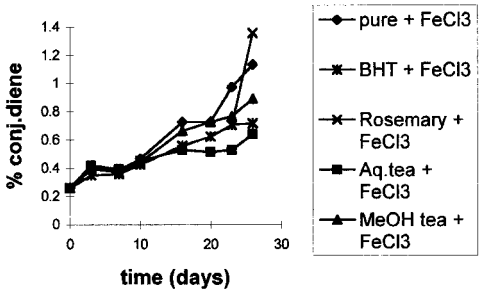

Figure 4. Effect of tea extracts (0.03%), rosemary extract (0.03%), and BHT (0.007% i.e. 0.02% in the oil) on the oxidation of sunflower oil-in-water emulsions containing ferric chloride (5×10^{-5} M) stored at 30 °C, assessed by the conjugated diene value.

Table 2. Catechin Composition of Tea Extracts

extraction method	ECG[a] (mg/g of extract)	EGCG[a] (mg/g of extract)
aqueous	22.7 ± 0.7	88.7 ± 2.3
methanolic	89.9 ± 3.4	309.9 ± 1.2

[a] Mean and range of duplicate determinations.

to a longer extraction time and removal of more of the non-catechins by washing with hexane and chloroform.

When ECG and EGCG were assessed as antioxidants in an emulsion using measurements of peroxide value (Figure 5) and conjugated dienes (Figure 6), EGCG

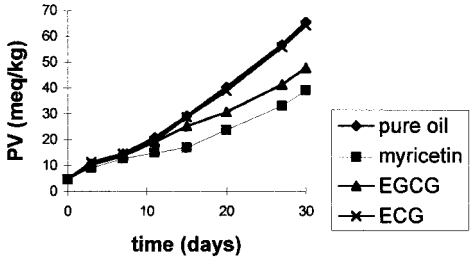

Figure 5. Effect of ECG, EGCG, and myricetin (all 10^{-4} M) on the oxidation of sunflower oil-in-water emulsions stored at 30 °C, assessed by the peroxide value.

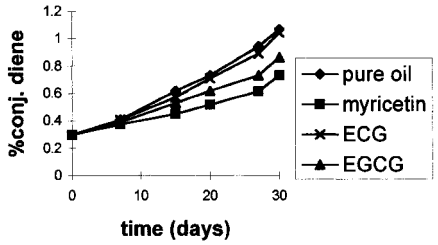

Figure 6. Effect of ECG, EGCG, and myricetin (all 10^{-4} M) on the oxidation of sunflower oil-in-water emulsions stored at 30 °C, assessed by the conjugated diene value.

Table 3. Peroxide Values and Tocopherol Content of Oil Isolated from Emulsions after 30 Days of Storage at 30 °C

sample	tocopherol content (μg/g of oil)	% of initial tocopherol content	peroxide value (mg/kg)
no additive	535.1	82.3	65.5
EGCG	561.3	86.3	47.7
ECG	532.3	81.9	64.2

showed moderate antioxidant activity despite the fact that the catechin was oxidized during the first 15 days of storage. However, the antioxidant activity of EGCG was smaller than that of the flavonol myricetin. Headspace analysis of pentane levels after 30 days of storage confirmed that the order of antioxidant activity was myricetin > EGCG > ECG with headspace pentane levels of 0.31, 0.47, and 0.79 relative to the levels in an emulsion lacking added antioxidants. One possibility that was considered was that the catechins function partly by assisting retention of tocopherols during the first part of the storage period, with the consequence that oxidation of the emulsion occurs at a slower rate in samples containing added catechins even after the catechins have been oxidized. Analysis of the residual tocopherols after 30 days showed that 82.3%, 86.3%, and 81.9% of the initial tocopherol content remained in the emulsions containing no additive, EGCG, and ECG, respectively (Table 3). The higher tocopherol retention in the presence of EGCG is consistent with this catechin assisting retention of tocopherols, but the difference is rather small and within experimental error and it is likely that there is another explanation for the antioxidant activity of the EGCG. Possibly, products from the oxidation of EGCG, e.g. dimers, are themselves active as antioxidants. The presence of ferric ions in emulsions stored at pH 5.5 produced a prooxidant effect with myricetin, EGCG, and ECG (Figures 7 and 8), and with cupric ions prooxidant effects of the flavonoids were weaker but became significant ($p < 0.05$) after 30 days for ECG (Figures 9 and 10). The relative headspace pentane levels after 30 days were 10.6, 8.8. and 6.6 for emulsions containing ferric chloride together with myricetin, EGCG, and ECG, respectively, relative to the

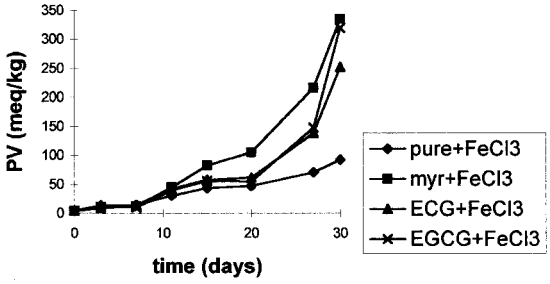

Figure 7. Effect of ECG, EGCG, and myricetin (all 10^{-4} M) on the oxidation of sunflower oil-in-water emulsions containing ferric chloride (5×10^{-5} M) stored at 30 °C, assessed by the peroxide value.

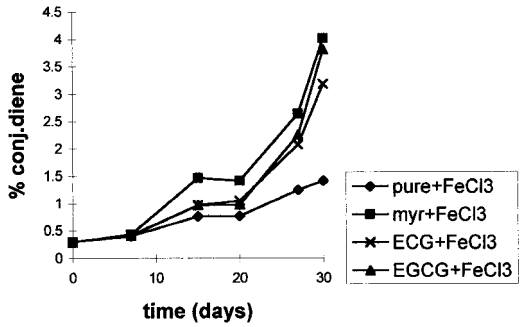

Figure 8. Effect of ECG, EGCG, and myricetin (all 10^{-4} M) on the oxidation of sunflower oil-in-water emulsions containing ferric chloride (5×10^{-5} M) stored at 30 °C, assessed by the conjugated diene value.

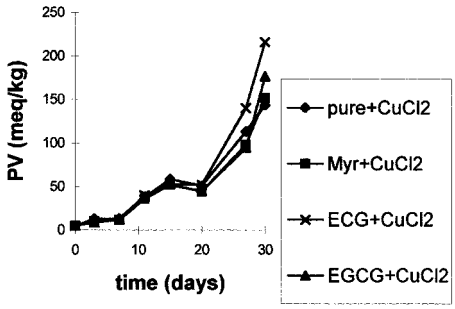

Figure 9. Effect of ECG, EGCG, and myricetin (all 10^{-4} M) on the oxidation of sunflower oil-in-water emulsions containing cupric chloride (5×10^{-5} M) stored at 30 °C, assessed by the peroxide value.

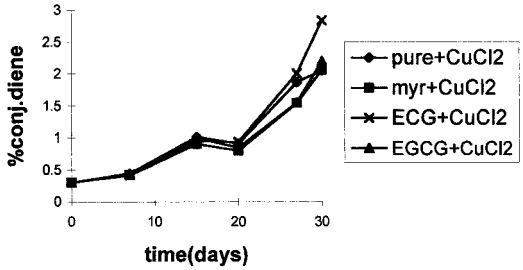

Figure 10. Effect of ECG, EGCG, and myricetin (all 10^{-4} M) on the oxidation of sunflower oil-in-water emulsions containing cupric chloride (5×10^{-5} M) stored at 30 °C, assessed by the conjugated diene value.

emulsion containing no added antioxidants, and the relative pentane levels were 0.95, 1.2, and 1.5 for the analogous series of emulsions containing cupric chloride. Prooxidant effects of catechins have been observed previously in the presence of iron (Hiramoto *et al.*, 1996).

These studies indicate that components other than EGCG and ECG make major contributions to the antioxidant properties of green tea extracts in stabiliz-

ing oil-in-water emulsions. Other catechins, e.g. EGC and EC, and flavonol glycosides occur in green tea extracts, and these may make significant contributions to the antioxidant activity. In addition, dimers or other oxidation products formed from EGCG may also contribute antioxidant effects. EGC and EC are expected to be less soluble in water than EGCG or ECG and hence may be more effective in emulsions due to an increased concentration at the oil−water interface. The flavonol, myricetin, was a more effective antioxidant than EGCG or ECG in the absence of metal ions (Figures 5 and 6), although it also had a prooxidant effect in the presence of ferric chloride (Figures 7 and 8).

LITERATURE CITED

Amarowicz, R.; Shahidi, F. Antioxidant Activity of Green Tea Catechins in a Beta-carotene-linoleate Model System. *J. Food Lipids* **1995**, *2*, 47−56.

Bailey, R. G.; Nursten, H. E. The Chemical Oxidation of Catechins and other Phenolics: A Study of the Formation of Black Tea Pigments. *J. Sci. Food Agric.* **1993**, *63*, 455−464.

Chen, C-W.; Ho, C-T. Antioxidant Properties of Polyphenols Extracted from Green and Black Teas. *J. Food Lipids* **1995**, *2*, 35−46.

Hara, Y. Action of Tea on Cardiovascular Disease. *Proceedings of the '95 International Tea-Quality-Human Health Symposium*; Tea Society of China: Shanghai, China, 1995; pp 16−31.

Hiramoto, K.; Ojima, N.; Sako, K-i.; Kikugawa, K. Effect of Plant Phenolics on the Formation of the Spin-adduct of Hydroxyl Radical and the DNA Strand Breaking by Hydroxyl Radical. *Biol. Pharm. Bull.* **1996**, *19*, 558−563.

Ho, C.-T. Phytochemistry in Teas. In *Phenolic Compounds in Food and Their Effect on Health II*; Huang, M-T., Ho, C-T., Lee, C. Y., Eds.; ACS Symposium Series 507; American Chemical Society: Washington, DC, 1992; pp 3−19.

Ito, N.; Hirose, M.; Shirai, T. Carcinogenicity and Modification of Carcinogenic Response by Plant Phenols. In *Phenolic Compounds in Food and Their Effect on Health II*; Huang, M-T.; Ho, C-T., Lee, C. Y., Eds.; ACS Symposium Series 507; American Chemical Society: Washington, DC; 1992; pp 269−281.

Lunder, T. L. Catechins of Green Tea. In *Phenolic Compounds in Food and Their Effects on Health II*; Huang, M-T., Ho, C-T., Lee, C. Y., Eds.; ACS Symposium Series 507; American Chemical Society: Washington, DC, 1992; pp 114−120.

Matsuzaki, T.; Hara, Y. Antioxidative Activity of Tea Leaf Catechins. *Nippon Nogeikagaku Kaishi* **1985**, *59*, 129−134.

Price, W. E. Green Tea Flavanols and Cancer. *Agro-Food Industry Hi-Tech*, **1994**, Sept/Oct, 18−20.

Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-Antioxidant Activity Relationships of Flavonoids and Phenolic Acids. *Free Radicals Biol. Med.* **1996**, *20*, 933−956.

Wanasundara, U. N.; Shahidi, F. Stabilization of Seal Blubber and Menhaden Oils with Green Tea Catechins. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1183−1190.

Warner, K.; Frankel, E. N.; Mounts, T. L. Flavor and Oxidative Stability of Soybean, Sunflower and Low Erucic acid Rapeseed Oils. *J. Am. Oil Chem. Soc.* **1989**, *66*, 558−564.

Yeo, S-G.; Ahn, C-W.; Lee, Y-W.; Lee, T-G.; Park, Y-H.; Kim, S-B. Antioxidative Effect of Tea Extracts from Green tea, Oolong tea and Black Tea. *J. Korean Soc. Food Nutr.* **1995**, *24*, 299−304.

Yoshioka, H.; Sugiura, K.; Kawahara, R.; Fujita, T.; Makino, M.; Kamiya, M.; Tsuyumu, S. Formation of Radicals and Chemiluminescence during the Autoxidation of Tea Catechins. *Agric. Biol. Chem.* **1991**, *55*, 2717−2723.

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