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Albumin causes a synergistic increase in the antioxidant activity of green tea catechins in oil-in-water emulsions

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Abstract

Model oil-in-water emulsions containing epicatechin (EC) and epigallocatechin gallate (EGCG) showed a synergistic increase in stability in emulsions containing added albumin. EGCG showed a stronger synergy (35%) with ovalbumin than did EC. Oxidation of the oil was monitored by determining peroxide values and hexanal contents. The effect of bovine serum albumin (BSA) on model oil-in-water emulsions containing each of the green tea catechins [epicatechin gallate (ECG), EGCG, EC and epigallocatechin (EGC)] was studied during storage at 30 °C. The green tea catechins showed moderate antioxidant activity in the emulsions with the order of activity being ECG ≈ EGCG > EC > EGC. Although BSA had very little antioxidant activity in the absence of phenolic antioxidants, the combination of BSA with each of the catechins showed strong antioxidant activity. BSA, in combination with EC, EGCG or EGC, showing the strongest antioxidant activity with good stability after 45 days storage. Model experiments with the catechins stored with BSA in aqueous solutions confirmed that protein–catechin adducts with antioxidant activity were formed between the catechins and protein. The antioxidant activity of the separated protein–catechin adducts increased strongly with storage time and was stronger for EGCG and ECG than for EC or EGC.

Keywords: Antioxidant; Bovine serum albumin; Catechins; Emulsion; Green tea

1. Introduction

Green tea is a widely consumed beverage. The antioxidant activity of green tea (GT) has been extensively studied, because tea represents a rich source of dietary antioxidants (Henning et al., 2004). Many *in vitro* experiments have demonstrated their strong antioxidant activity. Components of green tea have antioxidant power because they are able to quench free radicals. In addition, flavonoids have often been associated with decreased risk of developing several diseases. Green tea has developed a high reputation as a health-promoting dietary component, with most activity ascribed to the antioxidant activity of epigallocatechin gallate (EGCG) (Elbling et al., 2005), its main polyphenolic constituent. Also, tea polyphenols have been extensively studied as cancer chemopreventive agents. However, the effect of green tea extracts depends on the concentration: evidence is increasing that tea constituents can damage cells and be pro-oxidant themselves. These effects have been ascribed to spontaneous H_2O_2 generation by polyphenols in solution.

Alleged health-promoting effects of flavonoids are usually attributed to their powerful antioxidant activity, but evidence for *in vivo* antioxidant effects of flavonoids is limited and equivocal, mainly because flavonoid metabolites tend to have decreased antioxidant activity (Halliwell, Rafter, & Jenner, 2005).

Green tea extracts have been compared with other antioxidants in a number of studies. Green tea, together with extracts of blueberry, curcuma and particularly grape seeds, were identified, from a wide range of plant extracts, as being interesting antioxidants for use as natural additives for foods, using the trolox equivalent antioxidant

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capacity (TEAC) assay (Marc, Brisbarre, Davin, Baccaunaud, & Ferrand, 2004).

Green tea extracts were shown to have higher antioxidant activities than extracts of aerial parts of Otostegia persica (Burm.) Boiss., and Labiateae, using β-carotene bleaching and lipid peroxidation models (Yassa, Sharififar, & Shafiee, 2005).

Stewart, Mullen, and Crozier (2005) investigated the antioxidant potential of individual tea phenolics using an on-line high-performance liquid chromatography (HPLC) method, in conjunction with the analysis of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) radical-scavenging ability. EGCG was identified as the most potent antioxidant with a TEAC value of 3.0, contributing approximately 30% of the total antioxidant capacity of green tea. Theaflavins had antioxidant capacities similar to that of (−)-epicatechin monomers whilst conjugated flavonols did not contribute significantly to the antioxidant capacity.

Apak, Guclu, Ozyurek, and Karademir (2004) developed a method to measure the total antioxidant activity of vegetable extracts by determining the dietary polyphenols antioxidant capacity index, utilizing the copper(II)-neocuproine [Cu(II)-Nc] reagent as the chromogenic oxidizing agent (CUPRAC). ECG, EGCG, EC and catechin were shown to have the highest capacities in the CUPRAC method, in accordance with theoretical expectations. This study demonstrated that the antioxidant capacity of mixtures of compounds was a weighted average of that of the individual components.

Electron paramagnetic resonance (EPR) was used to study the synergistic antioxidant mechanism of α-tocopherol (vitamin E) in mixtures with green tea polyphenols, (EC, EGC, ECG, EGCG) and gallic acid (GA) (Zhou, Miao, Yang, & Liu, 2005; Zhou, Wu, Yang, & Liu, 2005). The green tea polyphenols could reduce α-tocopheroxyl radical to regenerate α-tocopherol with this effect being strongest for catechins with gallate ester groups.

Besides having excellent free radical-scavenging activity, green tea extract, catechin and EC possessed good antimutagenic activity (Geetha, Garg, Chopra, & Kaur, 2004). EC was more effective than were the other two agents.

There are several intervention studies to indicate the type and magnitude of the physiological effects of consumption of green tea catechins by humans on the basis of short-term changes in biomarkers.

Monomeric catechins (found at especially high concentrations in tea) have effects on plasma antioxidant biomarkers and energy metabolism. Procyanidins (oligomeric catechins) have pronounced effects on the vascular system, including, but not limited to, increasing plasma antioxidant capacity. The procyanidins can be formed by polymerisation of catechin during incubation. Compared with the effects of polyphenols *in vitro*, the effects demonstrated *in vivo*, although significant, are more limited. It has been suggested that the length of human intervention studies should be increased, to more closely reflect the long-term dietary consumption of polyphenols (Williamson & Manach, 2005).

The aim of this study was to determine the influence of albumin on the total antioxidant activity of different catechins, determined by various methods, including a model food emulsion. In recent years we have shown that the polyphenols of green tea have antioxidant activity in an emulsion (Roedig-Penman & Gordon, 1997) and that the total antioxidant activity increases nearly linearly with concentration.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), EGCG, EGC, ECG, EC, BSA, ferric chloride, potassium persulfate, methyl linoleate, polyoxyethylene sorbitan monolaurate (Tween-20) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), were purchased from Sigma–Aldrich Company Ltd. (Gillingham, UK). Hexanal was purchased from Sigma, Poole, UK. Ovalbumin was isolated in our laboratory. Refined sunflower oil, of a brand known to lack added antioxidants, was purchased from a local retail outlet.

2.2. Removal of tocopherols from sunflower oil

Tocopherols were removed from sunflower oil by column chromatography, using activated alumina, as described by Yoshida (1993).

2.3. Emulsion preparation

Oil-in-water emulsions (20.2 g) were prepared by dissolving Tween-20 (1%) in acetate buffer (0.1 M, pH 5.4), either with or without protein, namely BSA or ovalbumin (0.2%), and catechins (5×10^{-4} M). The emulsion was prepared by the dropwise addition of oil (methyl linoleate or sunflower oil) to the water phase, cooling in an ice bath with continuous sonication with a Vibracell sonicator (Sonics and Materials, USA) for 5 min.

2.4. Emulsion oxidation

All emulsions were stored in duplicate in 100 ml glass beakers in the dark (inside an oven). Two aliquots of each emulsion (0.005–0.1 g, depending on the extent of oxidation) were removed periodically for peroxide value (PV) and conjugated dienes (CD) determinations and 1 ml was removed periodically for analysis of hexanal by gas chromatography (GC).

2.5. Analytical methods

PV was determined by the ferric thiocyanate method (Frankel, 1998) (after calibrating the procedure with a series of oxidized oil samples analyzed by the AOCS Official

Method Cd 8-53). Data from the PV measurements were plotted against time. The times to reach 40 meq/kg PV were determined for each stored sample. Conjugated diene (CD) content was determined by a method based on AOCS Official Method Ti 1a-64, but using ethanol as a solvent.

Hexanal levels were monitored by headspace solid-phase microextraction (HS-SPME) (Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003). A manual SPME fibre holder unit and 30 μm DVB-CAR-PDMS fibres (Supelco Bellefonte, PA, USA) were used to adsorb volatiles from the emulsion in a closed vial at 60 °C with a sampling time of 12 min. Volatiles were thermally desorbed (240 °C for 12 min) in the injection port of the gas chromatograph. Before use for the first time, the fibre was conditioned by heating at 240 °C for time >2 h in the GC injection port.

GC analyses were performed with a Hewlett Packard 5890 series II gas chromatograph equipped with FID detector and split/splitless injector. Chromatographic separation was carried out using a BPX5 fused silica column (25 m length, 0.22 mm i.d., and 0.25 μm film thickness; SGE, Milton Keynes, UK). The oven temperature was 50 °C for 5 min, followed by temperature programming to 100 °C at 4 °C/min, and then increased to 150 °C at 10 °C/min. Helium was used as carrier gas, split ratio 1:50. The FID temperature was 260 °C and the injection port was held at 240 °C. Hexanal was identified by comparison of its retention time with that of an authentic standard (Sigma, Poole, UK).

2.6. SPME sampling conditions

A 1.00 (±0.02) g aliquot of emulsion was weighed in a 20 ml vial. A magnetic follower was added and the vial was capped with a Teflon™-faced rubber septum and aluminium cap (Fisher, Loughborough, UK). It was stored (−20 °C) prior to analysis. The vial was placed in a water bath on a magnetic stirrer and the sample was equilibrated for 10 min at 60 °C. The septum was manually pierced with the SPME needle and the fibre was exposed for 15 min to the emulsion headspace and transferred to the gas chromatograph where the volatiles were desorbed in the injection port. The desorption time in the injection port was 5 min.

2.7. Incubation of the catechins with protein and isolation of the protein fraction

In order to study antioxidant binding by protein, aqueous solutions containing ovalbumin or BSA (0.2% w/w) and catechins (5×10^{-3} M) were incubated at 50 and 30 °C, respectively. Samples were removed periodically during 10 days of storage. Aliquots were stored at −20 °C prior to analysis.

Incubated aliquots were treated by passing them (1 ml) through a micro Bio-Spin P-6 column (Bio-Rad, Richmond, CA) equilibrated with water. The protein fractions were separated from free antioxidant by elution with water. The protein fraction (2.7 ml) was collected and the fluorescence due to the tryptophan groups was determined by excitation at 280 nm with emission at 331 nm (Perkin–Elmer LS 3B, Beaconsfield, UK). Solutions containing individual antioxidants were stored in triplicate and duplicate determinations were performed for each sample.

The radical-scavenging activity of the protein fraction was also determined by the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and ferric radical antioxidant power (FRAP) method.

2.8. Preparation of the $ABTS^{\cdot+}$

The method used was based on that of Re et al. (1999). Preparation of the $ABTS^{\cdot+}$ was as described previously (Almajano & Gordon, 2004). Solutions containing protein (including any protein–catechin adducts formed during storage) (≈0.07%) isolated by gel filtration (20 μl) were added to $ABTS^{\cdot+}$ solution (2 ml) with a blank as control sample. After mixing, the absorbance at 734 nm was measured immediately, and then every minute for 6 min. Duplicate determinations were made for triplicate samples. The percentage inhibition was calculated from the absorbance values at 5 min, as described previously (Almajano & Gordon, 2004).

2.9. FRAP

The FRAP assay was performed as described previously (Benzie & Strain, 1996). The FRAP reagent was freshly prepared each day by mixing together 10 mM TPTZ and 20 mM iron(III) chloride in 0.25 M acetate buffer (pH 3.6). Each test component (100 μl) was added to FRAP reagent (3 ml), and the absorbance was read at 593 nm (Perkin–Elmer UV/Vis Lambda Bio 20), after incubation at room temperature for 15 min, using the FRAP reagent with distilled water as a blank. Data were expressed relative to values obtained for trolox from a calibration line and expressed as trolox equivalents.

2.10. Preparation of green tea extract

The green tea extract was prepared by stirring green tea (20 g) and boiled water (100 ml) continuously for 5 min. The mixture was filtered under vacuum and washed with boiling water (50 ml). The final solution was freeze-dried to provide 2.35 g extract.

2.11. Quantification of the catechins in freeze-dried green tea power by RP-HPLC analysis

HPLC analysis was carried out on a Dionex Summit quaternary pump system (Dionex plc, Camberley, England) equipped with an ASI-100 autosampler and a PDA-100 photodiode array detector. The column used was from Kromasil (100-5C-18, 3.2 mm i.d × 250 mm; 5 μm particle size) fitted with a guard column (50 mm) of

an identical phase. Elution was performed at 30 °C using a 1 ml/min flow rate. A mixture of water/glacial acetic acid (96:4, v/v) (solvent A) and methanol/acetonitrile (40:60, v/v) (solvent B) was used as a mobile phase. The composition of the mobile phase changed as follows: gradient for 30 min, was 95% A–5% B till 60% A–40% B, and finally gradient, for 10 min, to the initial conditions (95% A–5% B). Data acquisition was carried out at 240 and 280 nm. Quantification was performed at 280 nm (general absorption wavelength for catechins and for the caffeine) and also at 240 nm, after doing the calibration curve for the pure standards.

2.12. Statistical analysis

Antioxidant capacity by the $ABTS^{\cdot+}$ test and FRAP values, as well as fluorescence values and PV induction times (times to reach 40 meq/kg) were analyzed by one-way analysis of variance (ANOVA) to determine the pooled standard deviation (using SPSS statistical software). The mean values within each test were compared by a two-sample *t*-test by using the pooled standard deviation to determine significant differences.

3. Results and discussion

3.1. Effect of ovalbumin on the antioxidant activity of EC and EGCG in Sunflower oil-in-water emulsions

Emulsions containing ovalbumin (0.2%) were stored with EC or EGCG (0.5 mM) or green tea extract (GT, ≈0.5 mM total catechins) at 50 °C and compared with emulsions without protein. Oxidation was followed by PV determination (Fig. 1) and hexanal analysis (Fig. 2). The order of stability, according to the PV determination, was EGCG-ovalbumin^{d} > EC-ovalbumin^{c}, GT-ovalbumin^{c}, EGCG^{bc}, EC^{b}, ovalbumin^{b} > control^{a} (Table 1). The hexanal content at 19 days of incubation confirmed that the antioxidant activity was in the order GT > EGCG > EC > ovalbumin > control. The samples containing both catechin and protein had very low hexanal levels at this time (Fig. 2). Samples containing EGCG, EC or GT plus ovalbumin were more stable than samples without protein (at 19 days and 26 days of incubation), with the sample containing EGCG plus ovalbumin having the lowest concentration of hexanal at 37 days. The emulsion containing green tea extract contained total catechins at 0.5 mM concentration, but it was clear that, at this concentration, oxidation was starting to proceed at 37 days. The catechins in the green tea extract were mainly EGC, EGCG and EC (Table 2), with the composition being similar to that reported by other workers (Arts et al., 2002; Atoui, Mansouri, Boskou, & Kefalas, 2005; Zhao, 2003). The antioxidant activity in the emulsion can be ascribed mainly to EGCG, since EGCG is a major component and EGCG is much more effective at stabilising emulsions than are

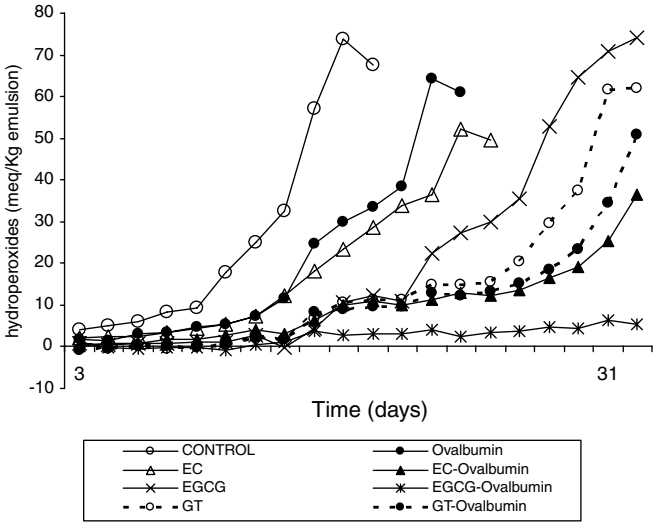


Fig. 1. Changes in peroxide value of emulsions containing antioxidants during storage at 50 °C.

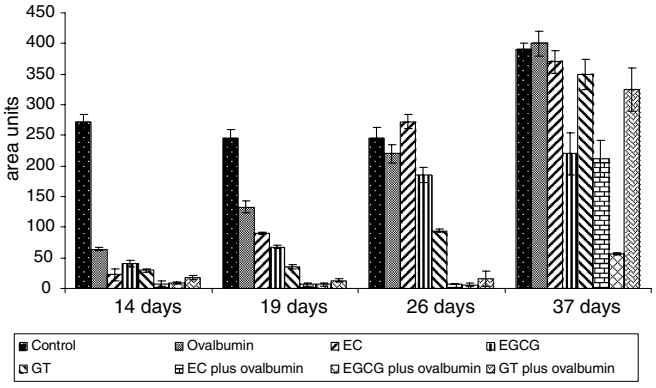


Fig. 2. Changes in hexanal content of emulsions during storage at 50 °C.

Table 1
Times (d) for oil-in-water emulsions (containing additives with or without ovalbumin) stored at 50 °C to reach PV = 40 meq/kg. Values with the same superscript letter are not significantly different ($p < 0.05$) (range of times for duplicate samples)

	No protein	With ovalbumin
Control	11.6^{a}	21.6^{b}
EC	22.7^{b}	32.5^{c}
EGCG	26.3^{b,c}	45^{d}
GT	29.6^{c}	30.8^{c}

Table 2
Freeze-dried green tea extract composition determined by HPLC

Compound	Concentration (%)
EC	10.6
EGC	18.0
EGCG	16.7
ECG	0.9
Caffeine	21.3
Other compounds	32.4

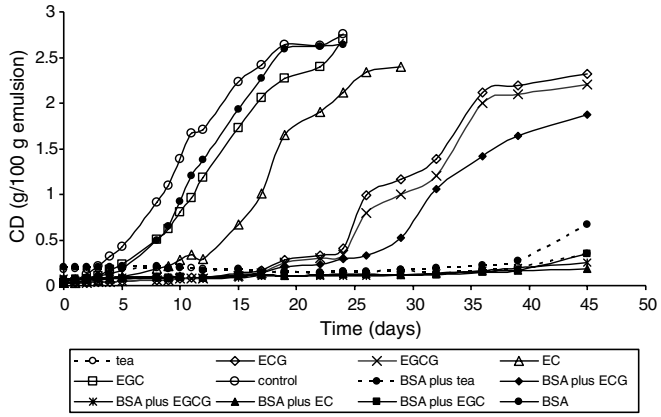


Fig. 3. Changes in conjugated dienes of emulsions during storage at 30 °C.

EGC or EC (Fig. 3). The effects on lipid oxidation are consistent with the reported antioxidant activity of EGCG in emulsions found in an earlier study at 0.1 mM concentration (Roedig-Penman & Gordon, 1997). However, Huang and Frankel (1997) found that the tea catechins were all prooxidant at 5 and 20 μM concentrations. It is clear that the catechins are pro-oxidant at low concentrations, but their antioxidant activity is shown at higher concentrations. The level of metal ions in the emulsions may be very important at low concentrations, because the catechins have been shown to be pro-oxidant in the presence of metal ions (Roedig-Penman & Gordon, 1997).

Synergy between the catechins and ovalbumin, in retarding oxidation of oil-in-water emulsions, was investigated . Ovalbumin (0.2%) showed some antioxidant activity in emulsions (Fig. 1) which was similar to that of EC (0.5 mM), and this contrasts with the properties of BSA, which did not contribute significant antioxidant properties (Fig. 3, Tables 3 and 4). The time to reach 40 meq/kg for the catechins, with and without ovalbumin, is shown in Table 1. The synergistic increase in stability of the emulsion prepared with ovalbumin containing EGCG was 35% based on the time for the PV to reach 40 meq/kg emulsion, calculated as described previously (Almajano & Gordon, 2004). The emulsion containing EC showed increased stability in the presence of ovalbumin but there was no synergistic increase in time to PV = 40 meq/kg, since ovalbumin itself had antioxidant properties, but the hexanal data showed that some synergistic reduction in volatile formation had occurred. For the green tea extract, where the total catechin concentration was 0.5 mM, the increase in oxidative stability of the emulsion with ovalbumin was not significant according to the PV values but the hexanal values provided evidence of increased effectiveness of the combination with ovalbumin at 26 days.

Table 3
Peroxide values of emulsions containing additives after 22 days of incubation at 30 °C

Sample	PV (meq/kg)
Tea	1.2[a]
ECG	21.1[b]
EGCG	18.0[b]
EC	133.8[c]
EGC	177.4[c]
Control	199.9[c]
BSA plus tea	2.1[a]
BSA plus ECG	2.4[a]
BSA plus EGCG	2.1[a]
BSA plus EC	2.2[a]
BSA plus EGC	2.0[a]
BSA	195.2[c]

Table 4
Time for oil-in-water emulsions stored at 30 °C to reach CD value of 0.5

Sample	Time (d)	Sample	Time (d)
Tea	>45	BSA plus tea	42.5[d]
ECG	24.3[c]	BSA plus ECG	28.7[c]
EGCG	24.7[c]	BSA plus EGCG	>45
EC	13.7[b]	BSA plus EC	>45
EGC	7.9[a]	BSA plus EGC	>45
Control	5.4[a]	BSA	8.1[a]

Values with the same superscript letter are not significantly different ($p < 0.05$) (range of times for duplicate samples).

In order to clarify the effect of catechin structure on antioxidant activity in oil-in-water emulsions containing catechins and protein, combinations of BSA with green tea extract and each of the green tea catechins, ECG, EGCG, EC, EGC, at 0.5 mM concentration, were stored at 30 °C. The oil used was methyl linoleate in order to avoid the necessity to strip tocopherols from the oil. Oxidation was followed by determination of the PV and CD values for 45 days.

The results for the PV and CD were in agreement and both showed that the green tea catechins had moderate antioxidant activity in the emulsions with the order of stability being ECG ≈ EGCG > EC > EGC (Fig. 3 and Tables 3 and 4). This order of antioxidant activity was similar to that reported by Shahidi and Alexander (1998) who assessed the catechins as inhibitors of oxidation of meat lipids. Wanasundara and Shahidi (1996) reported a similar order of activity in a study of the effects of catechins on seal blubber and menhaden oils. It appears that the gallate ester group has an important role in the antioxidant activity of the catechins in these media.

Although BSA had very little antioxidant activity in the absence of catechins, samples containing a combination of BSA with each of the catechins showed good stability and the increase in antioxidant activity was clearly synergistic for all four catechins. After 45 days, the emulsions separated into oil and water layers before samples containing BSA with the catechins were strongly oxidized. However, it appeared that the samples containing BSA in combination with EC, EGCG or EGC were most stable, with samples containing BSA and green tea extract slightly less stable. The sample containing BSA + ECG was significantly less stable than were samples containing BSA with the other three catechins (Fig. 3). It is not possible to calculate the synergy because the emulsion separated before

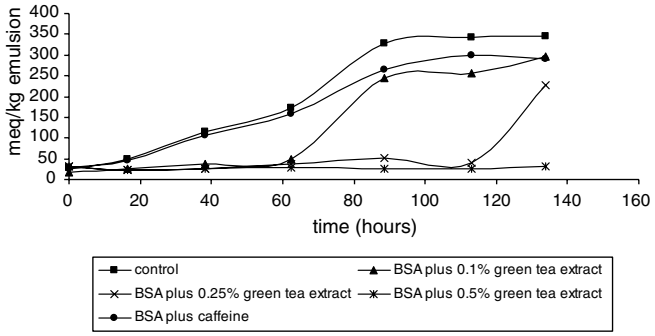


Fig. 4. Changes in peroxide value of emulsions stored at 60 °C, containing different percentage of freeze-dried green tea extract and caffeine.

oxidation had progressed to a level where synergy could be accurately calculated, but the increase of stability was greater than 100% for EC, EGCG and EGC in combination with BSA.

The increased antioxidant activity of catechins containing a gallate ester group, which was observed for the catechins in the absence of protein, was not observed for samples containing BSA. Also, the differences in stabilities of emulsions containing combinations of either EC or EGCG with ovalbumin were not found for samples containing mixtures of the catechins with BSA.

The emulsion containing the green tea extract was very stable towards oxidation, both with and without BSA. The combination of the different catechins is more effective than predicted from a weighted average of its components. The antioxidant activity of the green tea extract clearly increased with concentration in the presence of BSA (Fig. 4). It was also shown that caffeine (a major green tea component) did not have any significant antioxidant effect in the emulsion (at a concentration of 0.1% m/m).

The catechins studied are polar water-soluble compounds, and it is known that oil-in-water emulsions are more poorly stabilized by polar water-soluble antioxidants than are less polar antioxidants, such as tocopherols, which are present at the oil–water interface, where they are more effective (Porter, Caldwell, & Mills, 1995). This is known as the polar paradox. Since BSA is known to be surface active, as shown by its ability to stabilize emulsions (Kong, Beattie, & Hunter, 2003; Lethuaut, Metro, & Genot, 2002; Rampon, Lethuaut, Mouhous-Riou, & Genot, 2001), the probable mechanism leading to the increase in antioxidant activity of emulsions containing BSA and catechins is that BSA binds the antioxidant and transports it to the oil–water interface, where it is highly effective at reducing the rate of oxidation.

3.2. Study of stored model systems containing BSA and catechins

The catechins were stored with BSA in aqueous solutions at 30 °C to determine the effect of catechin structure on the reaction with BSA. BSA fluoresces strongly, due to tryptophan groups present in the protein, but the fluorescence was reduced to zero on mixing with the catechins. However, when the free antioxidant was removed from the mixture by separation with molecular exclusion cartridges, strong fluorescence was again shown by the protein. Nevertheless, after storage with the catechins, in water, the fluorescence, after separation with molecular exclusion cartridges, was progressively reduced (Fig. 5). This shows that the weak complex formed on mixing catechins with BSA is reversible and the antioxidant is removed from the protein by the molecular exclusion cartridge. However, storage of the catechins with the protein solution leads to the formation of a protein–catechin adduct, as reported for BSA with water-soluble antioxidants in an earlier publication (Almajano & Gordon, 2004). The reduction in fluorescence after storage was strongest for ECG and EGCG. The antioxidant activity of the catechins and the separated protein–catechin adducts was also studied by the ABTS radical-scavenging assay, the ORAC assay and the FRAP assay. The antioxidant activity of the free catechins was in the order ECG ≈ EGCG > EGC > EC for all three assays. The antioxidant activity for BSA alone was very low throughout the storage period but, when solutions containing BSA and catechins were stored, the antioxidant activity of the separated protein, assessed by all three assays, increased, as shown for the ORAC assay (Fig. 6). The changes in separated protein, assessed by the FRAP and ABTS assay, were similar to those of the ORAC assay.

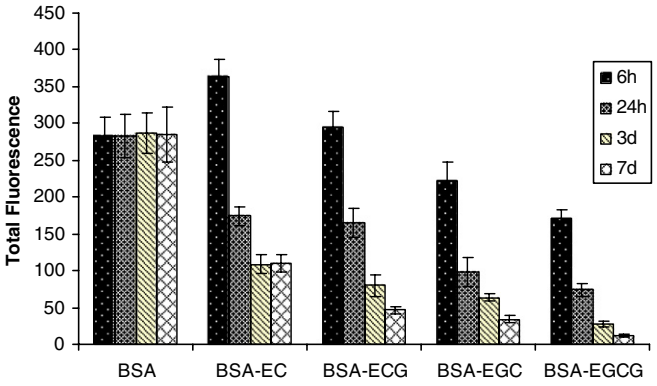


Fig. 5. Change in fluorescence of BSA during storage in the presence of EC, ECG, EGC and EGCG.

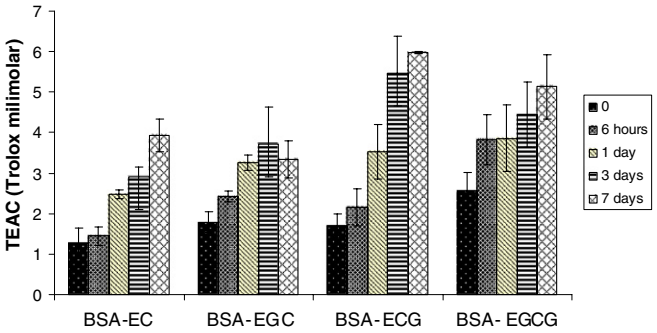
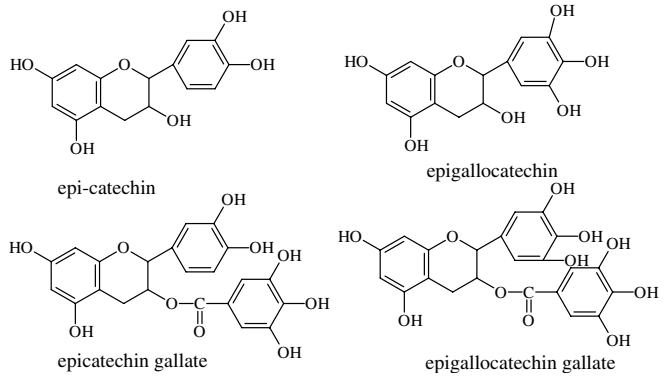


Fig. 6. Change in antioxidant activity assessed by the ORAC assay for the protein isolated from mixtures with EC, ECG, EGC and EGCG during storage at 30 °C.



Scheme 1. Catechin structures.

The progressive increase in antioxidant activity was strongest for ECG and EGCG stored with BSA. Mixtures of BSA with EGC and EC also showed increases in the antioxidant activity during storage, but the activity of the separated protein was significantly less than for the mixtures with ECG and EGCG. EGC appeared to reach a maximum value at 3 days, but the values had fallen significantly by 7 days. The total protein collected was at a concentration of about 0.07% after separation with the desalting column, but the proportion of the protein that was modified is unknown. However, the protein concentration required for the antioxidant assays was about 100 times that required when the free catechins were studied. This suggests that the BSA-catechin adduct was present at low concentration, although it is also possible that the antioxidant activity was low compared with the free catechins.

Since the protein incorporating bound catechins has radical-scavenging activity, it is clear that the binding of this compound still leaves free phenolic hydroxyl groups, which have antioxidant activity. EGCG and ECG have neighbouring hydroxyl groups on two aromatic rings (Scheme 1) and it is likely that, even if the hydroxyl groups on one of these rings are involved in binding to BSA, the hydroxyl groups on the second ring are still free to allow the molecule to act as an antioxidant.

It is clear that the catechins formed covalent bonds with the protein on storage, and that the protein-bound catechin contributed to the antioxidant activity of these systems. One interesting observation is that emulsions containing BSA in combination with EC, EGCG or EGC were more stable than was the emulsion containing ECG, whereas ECG and EGCG bind to a greater extent to BSA on storage (from the fluorescence measurements) and the BSA-catechin adduct has greater antioxidant activity in the case of the gallates ECG and EGCG (from the model system storage experiment). The reason for the relatively poor activity of ECG in the emulsion containing BSA is not clear.

4. Conclusion

Tea catechins can bind irreversibly to protein on storage, and the effect in an oil-in-water emulsion is that a synergistic increase in antioxidant activity occurs if both protein and catechin are present. This is observed for both ovalbumin and BSA. Emulsions are more stable for mixtures containing BSA and EC, EGCG or EGC than are those containing BSA-ECG and also more stable for ovalbumin mixed with EGCG than for ovalbumin-EC.

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