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Antioxidant and antimicrobial activities of tea infusions

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Abstract

Tea polyphenols, especially the catechins, are potent antimicrobial and antioxidant agents, with positive effects on human health. White tea is one of the less studied teas but the flavour is more accepted than that of green tea in Europe. The concentrations of various catechins in 13 different kinds of infusion were determined by capillary electrophoresis. The total polyphenol content (Folin–Ciocalteu method), the trolox equivalent antioxidant capacity (TEAC value determined with the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation) and the inhibitory effects of infusions on the growth of some microorganisms were determined. Five different infusions (black, white, green and red teas and rooibos infusion) were added to a model food system, comprising a sunflower oil-in-water emulsion containing 0% or 0.2% bovine serum albumin (BSA), and the oxidative stability was studied during storage at 37 °C. Oxidation of the oil was monitored by determination of the peroxide value.

The highest radical-scavenging activity observed was for the green and white teas. Emulsions containing these extracts from these teas were much more stable during storage when BSA was present than when it was not present, even though BSA itself did not provide an antioxidant effect (at 0.2% concentration). Rooibos infusion did not show the same synergy with BSA. Green tea and white tea showed similar inhibitions of several microorganisms and the magnitude of this was comparable to that of the commercial infusion 2 (C.I.2), "té de la belleza". This tea also had an antioxidant activity comparable to green tea.

Keywords: Antioxidant activity; Antimicrobial; Bovine serum albumin; Emulsions; Polyphenols; Tea

1. Introduction

1.1. General

The importance of diet for the prevention of some diseases is well recognized (Sur, Chandhuri, Vedasiromoni, Gomes, & Ganguly, 2001; Wongkham et al., 2001; Yao, Tan, Zhang, Su, & Wei, 1998) Antioxidant components are most important in foods because of their ability to reduce free radical-mediated degradation of cells and tissues in an organism (Jin, Hakamata, Takahashi, Kotani, & Kusu, 2004; Wongkham et al., 2001). Vegetables, legumes and whole-grain cereals (Karakaya & Kavas, 1999; Nihal, Ahmad, Mukhtar, & Wood, 2005) are good sources of antioxidants but there are many other food sources. Herbal infusions (specially tea) are also important sources (Marongiu et al., 2004; Wu, Ng, & Lin, 2004). The average estimated consumption of tea in the United Kingdom is 1 l/person/day (Karakaya & Kavas, 1999; Yanagimoto, Ochi, Lee, & Shibamoto, 2003).

Black tea is the most popular drink in the West, and the consumption of green tea is less (18–20%). Less than 2% of tea consumed corresponds to oolong tea, which is very common in China and Taiwan (Karakaya & Kavas, 1999; Wheeler & Wheeler, 2004; Yao et al., 1998; Yen & Chen, 1995).

Many investigators have studied tea properties, especially their health-related properties, including antimicrobial and

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antioxidant effects, either in homogeneous solution or in biphasic lipid. (Frei & Higdon, 2003; Karakaya & Kavas, 1999; Nihal et al., 2005).

One of the most important beneficial effects of tea is the antioxidant activity and free radical-scavenging ability of the polyphenol components (Frei & Higdon, 2003).

Polyphenols are the most important constituents of tea leaves (Karakaya & Kavas, 1999; Nihal et al., 2005). Fresh green tea leaves are rich in monomeric flavanols, known as catechins (Chattopadhyay et al., 2004; Frei & Higdon, 2003; Lau, He, Dong, Fung, & But, 2002; Wheeler & Wheeler, 2004) and (−)-epigallocatechin gallate (EGCG) is the most abundant green tea catechin (Frei & Higdon, 2003; Rietveld & Wiseman, 2003). Its chemical structure is shown in Fig. 1. Catechins are present at levels of 30–40% of the dry weight of fresh green tea leaves (Karakaya & Kavas, 1999; Nihal et al., 2005; Wheeler & Wheeler, 2004).

Tea composition is affected by the fermentation process. There are three kinds of teas: not fermented (green and white tea), partially fermented (red and oolong tea) and completely fermented (black tea). During fermentation of fresh tea leaves, some catechins are oxidized or condensed to larger polyphenolic molecules (dimer or polymer) such as theaflavins (3–6%) and thearubigins (12–18%). These polymers are responsible for black tea's bitter taste and dark colour (Chattopadhyay et al., 2004; Karakaya & Kavas, 1999; Nihal et al., 2005; Pelillo et al., 2004; Rietveld & Wiseman, 2003; Wheeler & Wheeler, 2004).

One of the mechanisms of antioxidant action (which is believed to be important in the activity of tea catechins) is to scavenge free radicals, thereby inhibiting oxidation. This mechanism is also the explanation for antimicrobial activity in cells and cell membranes (Frei & Higdon, 2003).

The antioxidant effectiveness depends on the tea variety and the content of EGCG is very important. Oolong and green tea has high levels of EGCG and (−)-epigallocatechin (EGC), but the content in black tea is much lower (Katalinic, Milos, Kulisic, & Jukic, 2006). The antioxidant abilities of catechins, assessed by the diphenylpicrylhydrazyl (DPPH) method, were found to be: EGCG > (−)-epicatechin gallate (ECG) > EGC > (−)-epicatechin (EC) > catechin (C) (Katalinic et al., 2006; Luczaj & Skrzydlewska, 2005).

1.2. Antimicrobial activity

Most polyphenols also show antimicrobial activity. Some studies have investigated the effects of polyphenols on intestinal pathogens, although there is some disagreement over precisely which bacterial species are inhibited by antioxidants. Catechins, the polyphenols in tea, proanthocyanidins and hydrolysable tannins show antimicrobial activity. The tolerance of bacteria to polyphenols depends on the bacterial species and the polyphenol structure (Campos, Couto, & Hogg, 2003; Taguri, Tanaka, & Kouno, 2004). Polyphenols can inhibit growth of clostridia

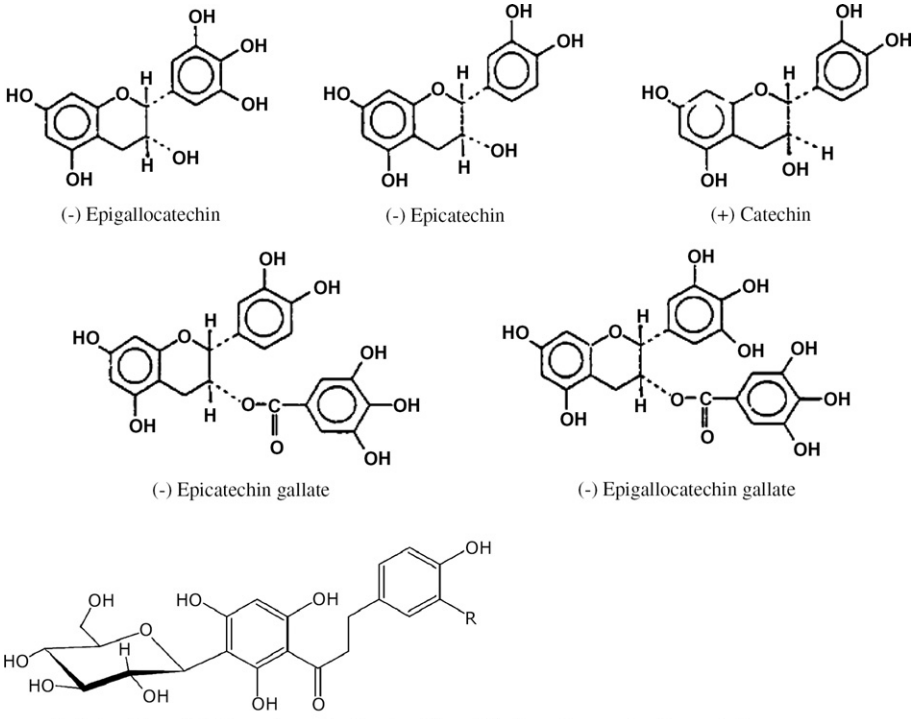

Fig. 1. The structures of the main catechins occurring in *Camelia Sinensis* and polyphenols in Rooibos.

and *Helicobacter pylori* but not of some intestinal lactic bacteria (Gramza & Korczak, 2005).

The use of natural antioxidants as preservatives in food has great potential because consumers request additive-free, fresher and more natural-tasting food. However, it is necessary to maintain microbiological safety and minimize the number of food-borne microorganisms. In this respect, there are studies that show that tea extracts act as inhibiters of food pathogens, including *Staphylococcus aureus*, *Shigella disenteriae*, *Vibrio cholerae*, *Campylobacter jejuni*, *Listeria monocytogenes*, etc. (Negi, Jayaprakasha, & Jena, 2003; Taguri et al., 2004). However, not all catechins from tea extracts have antibacterial activity. Some workers have found that green tea extract was not effective against *Escherichia coli*, although EGCG at 10–100 μM reduced growth by approximately 50% (Nazer, Kobilinsky, Tholozan, & Dubois-Brissonnet, 2005).

1.3. Activity against lipid systems

Catechins scavenge free radicals generated in an aqueous environment preventing them from interacting with lipids in a membrane (Shimada et al., 2004). However, although antioxidants have been frequently studied in oils, emulsions and foods, such as meat, there have been few reports of how proteins, which are commonly present, may affect the activity of antioxidants in foods. Most antioxidants of interest for foods have one or more phenolic hydroxyl groups and there are several studies demonstrating that molecules with this structure may bind to proteins. Polyphenols may associate with proteins through hydrophobic interactions and hydrogen bonding (Oda, Kinoshita, Nakayama, & Kakehi, 1998) and a range of phenolic antioxidants has also been shown to bind to bovine skin proteins (Wang & Goodman, 1999). Plant phenols have been shown to react with whey proteins at pH 9 (Rawel, Rohn, Kruse, & Kroll, 2002), but such a high pH is not commonly encountered in foods. Bovine serum albumin (BSA) is a minor whey protein with molecular weight 66 kDa. It is well characterized (Carter & Ho, 1994; Peters, 1985) and is commercially available in high purity. It has surface active properties and has been used to stabilize model food emulsions (Rampon, Lethuaut, Mouhous-Riou, & Genot, 2001). It is known that caffeic acid can bind to BSA (Bartolome, Estrella, & Hernandez, 2000) and chlorogenic acid reacts with BSA at alkaline pH to form an adduct (Rawel et al., 2002). Also, in previous studies (Almajano & Gordon, 2004), we found a synergistic effect between BSA and antioxidants, which depended on the antioxidant structure.

The objective of this work was to estimate the phenolic content, determine the catechin profile and evaluate the antioxidant and antimicrobial activities of different extracts of teas and herbal infusion. This paper describes, also, a study of the influence of bovine serum albumin (BSA) on the effectiveness of five different infusions as antioxidants in model food emulsions and the relationship between this behaviour and the tea composition.

2. Materials and methods

2.1. Plant material

Thirteen kinds of tea were studied. These comprised four pure teas (white, green, red and black), three pure herbal infusions (peppermint, nettle and rooibos) and six mixed teas. All teas were purchased from a commercial supplier (Sara Lee Southern Europe S.L., Mollet del Vallés, Barcelona, Spain).

2.2. Chemicals

(−)-Epigallocatechin gallate (EGCG) from green tea, 95%, (−)-epicatechin gallate (ECG) from green tea, 98%, (−)-epigallocatechin (EGC) from green tea, 95%, (−)-epicatechin (EC), 90%, gallic acid (GA) purity not specified, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), bovine serum albumin (BSA), 95%, ferric chloride, Tween-20 and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma–Aldrich Company Ltd. (Gillingham, UK). Refined sunflower oil of a brand known to lack added antioxidants was purchased from a local retail outlet.

2.3. Microorganism strains

The microorganism strains used in this study were *Bacillus cereus* (CECT 5144), *Micrococcus luteus* (CECT 5863), *E. coli* (CECT 99), *Pseudomonas aeruginosa* (CECT 108), *Lactobacillus acidophilus* (CECT 362) and *Candida albicans* (CECT 1002). They were obtained from the Culture Collection and Research Centre, University of Valencia, Spain.

2.4. Infusion preparation

Each plant (1.5 g) was mixed with 100 ml of boiling water for 5 min, with constant shaking and the samples were then filtered through Whatman No. 1 filter paper (Whatman® Schleicher & Schuell®, Castelldefels, Spain).

Infusions for the TEAC and TP assays were treated in three different ways:

1. Direct extraction to obtain the infusion.
2. Freeze-drying the direct infusion and keeping the solid at −20 °C until analysis.
3. Freezing (−20 °C) the direct infusion and analyzing after 2–5 weeks.

For each procedure, every experiment was performed in duplicate, that is six measures for each infusion. Analysis of samples that had been previously packed and unpacked, were compared with samples that were freshly packed. The results from these three sets are treated together, because significant differences were not found.

2.5. Determination of antimicrobial activity

For microbiological tests, the infusion was concentrated to a fifth of the volume on a rotary evaporator. The prepared samples were filtered through 0.45 μm membranes and kept in small sterile bottles and stored at −20 °C prior to analysis.

All microorganisms were grown at 30 °C for 18 h in nutrient broth, except *Lactobacillus* which was grown in MRS for 48 h. Suspensions of microorganisms, adjusted to 4–5 log cfu/ml, were placed in flasks containing 5 ml of sterile nutrient or MRS agar at 45–46 °C. The mix was poured into Petri plates with 10 ml of basal medium. When the agar was solidified, sterile discs (6 mm) were inserted into the plates. Finally, 50 μl of solution of each extract were transferred to the discs. The plates were incubated at 30 °C for 48 h and read at 8, 24 and 48 h.

At the end of the incubation period, inhibition zones formed in the medium were measured. All tests were performed in triplicate.

2.6. Removal of tocopherols from sunflower oil

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

2.7. Preparation of emulsions

Oil-in-water emulsions (20.2 g) were prepared by dissolving Tween-20 (1%) in water containing lyophilized tea (0.1% in weight, final concentration) and BSA (0% or 0.2% in weight, final concentration). The control was prepared in the same way, without tea or BSA. The oil was added dropwise to the aqueous sample cooled in an ice-bath while sonicating for 5 min in total.

2.8. Storage and sampling of emulsions

All emulsions were stored in triplicate in 50 ml glass beakers in the dark (inside an oven). Two aliquots of each (0.005–0.1 g, depending on the extent of oxidation) were removed periodically for determination of the peroxide value (PV).

2.9. Analytical methods

2.9.1. Peroxide value (PV)

PV was determined by the ferric thiocyanate method (Frankel, 1998) after calibrating the procedure with a series of oxidised oil samples analyzed by the AOCS Official Method Cd 8-53.

2.9.2. TEAC assay

The radical-scavenging activity of the infusions was also analyzed by the 2, 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay (TEAC).

The method used was based on that of Re et al. (1999). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and potassium persulfate (7 mM ABTS and 2.45 mM potassium persulfate, final concentration) were separately dissolved in water and then, the mixture was made up to volume in a 10 ml volumetric flask. The mixed solution was transferred to an amber bottle, covered with aluminium foil and allowed to stand at room temperature for 12–16 h in the dark. The $ABTS^{\cdot+}$ solution was diluted with phosphate buffer solution (PBS, pH 7.4, 1:100) to an absorbance of 0.7 (±0.02) at 734 nm in a 1 cm cuvette equilibrated at 30 °C.

PBS (pH 7.4) was used as blank. After mixing, the absorbance at 734 nm (Hewlett Packard 8452A Diode Array Spectrophotometer) was measured immediately, and then every minute for 5 min. Duplicate determinations were made for triplicate samples (six determinations for each sample). The percentage inhibition was calculated from the absorbance values at 5 min.

The relative change in sample absorbance, ΔA_{sample}, was calculated according the following equation to correct for the solvent:

$$\Delta A_{\text{sample}} = \frac{A_{t=0(\text{sample})} - A_{t=5(\text{sample})}}{A_{t=0(\text{sample})}} - \frac{A_{t=0(\text{solvent})} - A_{t=5(\text{solvent})}}{A_{t=0(\text{solvent})}}$$

Percent inhibition values were obtained by multiplying ΔA_{sample} values by 100. The TEAC value (trolox equivalent antioxidant capacity) was determined by comparing the values with the standard calibration curve.

2.9.3. Total polyphenol (TP) and catechin content

The TP content of the samples, determined in duplicate for triplicate samples with the Folin–Ciocalteu assay (scaled down to a 10 ml final volume), was quantified in terms of gallic acid.

Determination of individual catechins in the samples was conducted by capillary electrophoresis (CE) (Barroso & van de Werken, 1999). A calibration curve for each compound was prepared. Analyses were carried out in duplicate for triplicate samples.

2.10. Statistical analysis

Data from the PV measurements were plotted against time. The times to reach 10 meq/kg (PV) were determined for each stored sample. Antioxidant capacities, by the $ABTS^{\cdot+}$ test, TP and PV induction times, were analyzed by one-way analysis of variance (ANOVA) to determine the pooled standard deviation. 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. Antioxidant activity

The codes used and compositions of the commercial teas are given in Table 1. Infusions were prepared from each

Table 1
Codes and compositions of commercial tea samples

Mixed teas	Commercial teas	% Red tea	% Green tea	% Black tea	% Darjeeling tea	% White tea	Rooibos	Other
C.I.1	Tea assam			80				20
C.I.2	Tea belleza	10	10		10	40		30[a]
C.I.3	Tea citrus		75					25
C.I.4.	Tea classic			80				20
C.I.5	Tea infudefensas							100
C.I.6.	Tea bienestar						88	12

Other: elder flower, eucalyptus, thyme, mint, grapefruit aroma, lemon aroma, lemon rind, "escaramujo", hibiscus, vanilla, cinnamon, cardamom, ginger, lemongrass.
[a] Mainly vanilla and hibiscus.

tea, in duplicate, as fresh infusion, frozen infusion and freeze-dried extract, but the compositions and antioxidant activities were not significantly different for the different treatments, so the total phenolics (by the Folin–Ciocalteu method), TEAC values and the ratios between them are reported as the means of at least six measurements. The results are in Table 2. The catechin composition is given in Table 3.

Table 2
Content of total phenolics (by the Folin–Ciocalteu method), TEAC value and ratio of these parameters for the studied infusions (means ± s.d., $n = 6$)

	T.P.[A]	TEAC value[B]	PAC[C]
Black tea	1844 ± 15.7^{e}	3771 ± 21.2^{e}	2.0
Red tea	825 ± 117.2^{c}	1215 ± 91.7^{c}	1.5
Green tea	2083 ± 51.3^{f}	6344 ± 72.8^{h}	3.0
White tea	$2180 \pm 161.6^{f,g}$	4546 ± 54.3^{f}	2.1
Rooibos	881 ± 85.2^{c}	746 ± 12.9^{b}	0.8
C.I.1	1142 ± 133.7^{d}	2488 ± 81.7^{d}	2.2
C.I.2	2247 ± 72.1^{g}	5282 ± 110.4^{g}	2.4
C.I.3	1866 ± 79.1^{e}	$4820 \pm 109.9^{f,g}$	2.6
C.I.4.	1751 ± 109.0^{e}	3884 ± 75.3^{e}	2.2
C.I.5	630 ± 52.1^{b}	383 ± 69.4^{a}	0.6
C.I.6.	$965 \pm 75.7^{c,d}$	1004 ± 37.0^{c}	1.0
Peppermint infusion	315 ± 57.8^{a}	758 ± 22.1^{b}	2.4
Nettle infusion	234 ± 45.2^{a}	170 ± 50.6^{a}	0.7

Different superscript letters, in each column, express significant ($P < 0.05$) differences among results.
[A] T.P.: total phenolics in mg gallic acid equivalent per liter of infusion.
[B] TEAC value: trolox equivalent capacity (mmol of trolox per liter of infusion) determined by the ABTS method.
[C] PAC – phenol antioxidant coefficient, calculated as ratio TEAC/total phenolics.

The total polyphenol content values are consistent with the literature (Peterson et al., 2005). The total polyphenol contents were similar for the green tea and white tea, and the content was lower in the black tea. The TEAC values were also higher in white and green teas than in black tea, though no correlation between the parameters (TP and TEAC value) was found. As for the mixed teas, the polyphenol content was higher in samples C.I.2, C.I.3 and C.I.4 and it ranged from 1750 to 2247 mg l^{-1}. TEAC values were also high for two of these samples (C.I.2. and C.I.3) but not for C.I.4. It is well known that the antioxidant capacity is related to the type and concentration of infusion catechins (Atoui, Mansouri, Boskou, & Kefalas, 2005) and the catechins present were very different.

C.I.1 and C.I.4 had the same percentage of black tea (80%) but analysis of the catechins showed that both commercial samples (mixed tea) had a lower EGCG content than expected from the percentage of black tea present. This can be due to the fact that, during the process of packaging and preparing the infusion, there were more losses with mixed tea than with raw tea, due to the unknown influence of other components. So the polyphenols present would be mainly polymerized catechins, especially theaflavins and thearubigins. Green tea contained the most EGCG, which is known to be the most active antioxidant catechin (Atoui et al., 2005) and the level of this catechin in C.I.3., which has an important amount of green tea in the formulation (Table 1), was also high. The highest TEAC value found was for green tea (Table 2). The order found was: green tea > C.I.2 > C.I.3 > white tea > C.I.4 ≈ black tea > C.I.1 > red tea > C.I.6. The TEAC values for rooi-

Table 3
Content of the main catechins (mg/100 g tea leaves) determined by capillary electrophoresis (mean ± s.d., $n = 6$)

	Caffeine	EGC	EGCG	EC	ECG	Gallic acid
Black tea	447 ± 14.5	94.6 ± 4.8	270 ± 2.0	50.2 ± 2.3	215 ± 11.8	44.3 ± 2.8
Red tea	537 ± 20.1	–	–	23.8 ± 2.0	35.8 ± 15.1	81.5 ± 5.2
Green tea	515 ± 17.8	1028 ± 22.7	2409 ± 62.0	131 ± 4.5	368 ± 33.7	20.3 ± 1.6
White tea	676 ± 31.2	151 ± 15.2	1525 ± 113.4	57.3 ± 2.7	301 ± 29.3	54.8 ± 6.4
C.I.1	537 ± 21.9	51.7 ± 9.1	84.2 ± 9.9	45.3 ± 2.9	38.6 ± 0.4	33.1 ± 3.2
C.I.2	542 ± 17.3	329 ± 33.4	1185 ± 95.7	66.2 ± 3.2	272 ± 19.7	52.6 ± 4.1
C.I.3	563 ± 18.5	518 ± 21.9	958 ± 41.4	81.7 ± 9.1	172 ± 14.1	25.2 ± 0.8
C.I.4	457 ± 29.2	40.1 ± 4.7	99.8 ± 7.6	27.7 ± 1.3	91.4 ± 7.7	41.6 ± 4.2

bos, peppermint, C.I.5 and nettle extracts were very low, compared with the other samples.

There was no linear correlation between total polyphenols and TEAC values, which reflects the different activity of the polyphenols present in the samples.

Tea "belleza" (C.I.2) had high antioxidant activity due to the high content of white tea (40%) and some green tea (10%) in its composition. The concentrations of EGCG, EC, ECG and EGC were similar to those of white tea. This shows that products prepared from mixtures of white tea and green tea may be similar in composition and antioxidant properties to green tea but may have a nicer flavour.

The polyphenols contribute to astringency, but they are not responsible for the flavour, although there is evidence that interactions of flavanols in green tea extracts during heat processing and storage cause changes in the tea aroma (Wang, Kim, & Lee, 2002).

Sunflower oil-in-water emulsions (10% of oil), containing 0% or 0.1% of freeze-dried tea extract and 0% or 0.2% of BSA (both 0 is the control sample), were incubated at 37 °C. Oxidation of the oil was monitored by determination of the peroxide value. Pure infusions were used instead of commercial samples, because the aim was to design a better functional food infusion (as antioxidant) and we wanted to know the properties of individual extracts. Peppermint and nettle were not used because their antioxidant activities were not high.

At 37 °C, in the absence of BSA, the different tea extracts showed significant antioxidant activity, with the order of activity being: white tea < red tea ~ black tea < rooibos ~ green tea.

In samples containing BSA, the order was: white tea (+BSA) < red tea (+BSA) ~ rooibos tea (+BSA) < black tea (+BSA) ~ green tea (+BSA). Combining the data for both groups of samples, the order of stability was: white tea < red tea ~ black tea ~ white tea (+BSA) < rooibos ~ green tea ~ red tea (+BSA) ~ rooibos tea (+BSA) < black tea (+BSA) ~ green tea (+BSA) (Fig. 2).

The time required for the emulsions to reach a peroxide value (PV) of 10 meq/kg of emulsion is a suitable measurement of the antioxidant activity (Fig. 3) and it is clear that BSA itself did not have significant antioxidant activity at this concentration (0.2%). However in the presence of BSA, the stability of the samples containing white tea, red tea, and especially black and green teas, increased strongly with the time to PV = 10 meq/kg increasing by 83%, 65%, 194%, >57%, respectively (Table 4). For rooibos infusion, there was no increase in stability in the presence of BSA. In the absence of protein, green tea was the most effective antioxidant (although not significantly different from rooibos). In the presence of BSA, synergy with some components of the infusions caused the green tea extract to be a stronger antioxidant than rooibos infusion (Table 4). There was no significant difference in stability between the samples containing the rooibos infusion, in the presence or absence of BSA. There was a strong synergistic increase in stability in samples containing black tea and BSA, and green tea and white tea also showed some synergy with the protein.

This different behaviour is due to the phenolic components present in the tea extracts. Rooibos infusion does not contain catechins, but contains several compounds, including aspalathin (Fig. 1), isoorientin, orientin and rutin (mainly) and others (in lower concentrations), namely isovitexin, vitexin, isoquercitrin and hyperoside, quercetin, luteolin and chrysoeryol, all with antioxidant activity.

3.2. Antimicrobial activity

Table 5 shows the antimicrobial activities of different teas and infusions. The inhibition zones formed depend on the strain, the kind of extract and the concentration

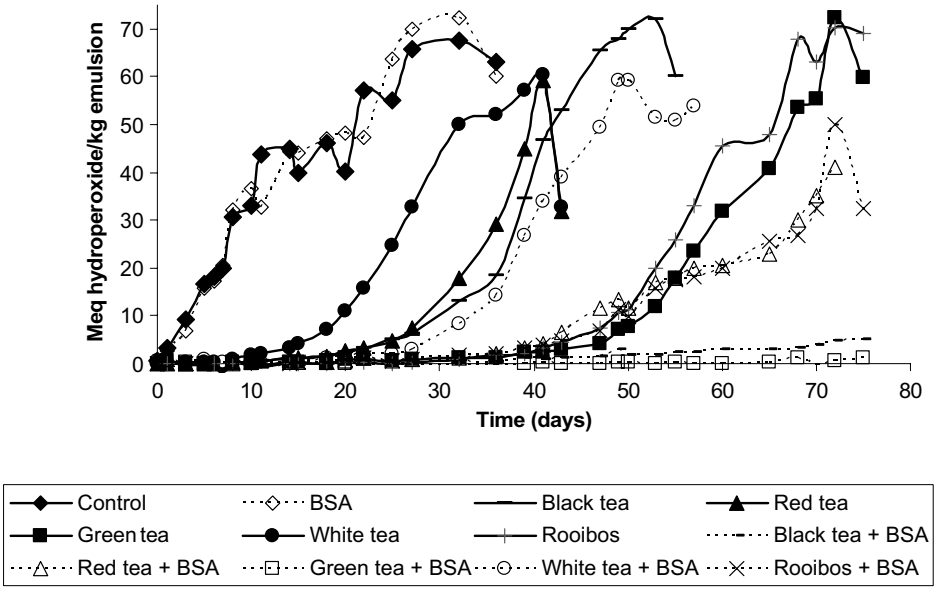

Fig. 2. Changes in peroxide value of emulsions containing freeze-dried infusions during storage at 37 °C.

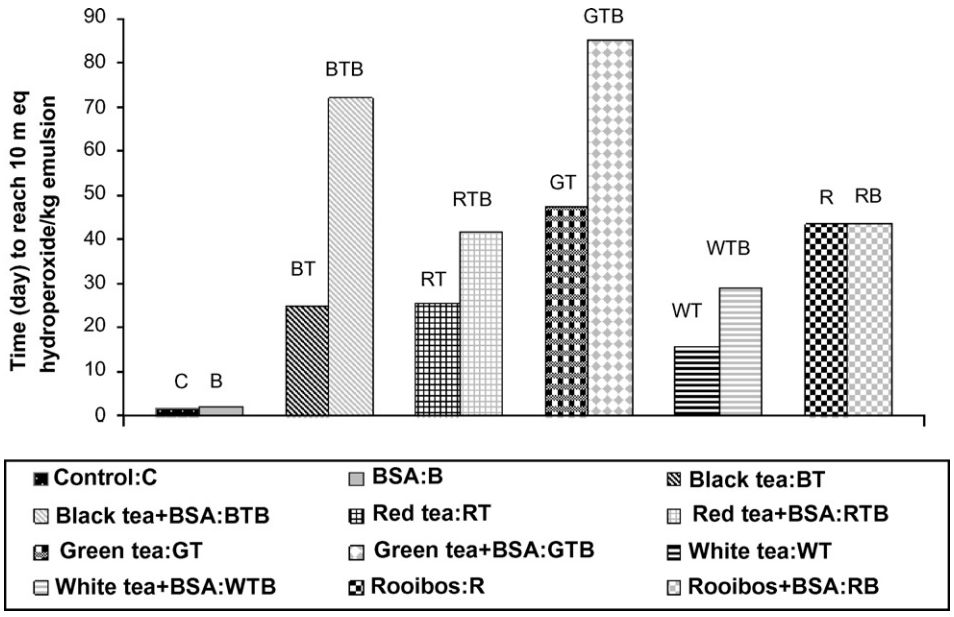

Fig. 3. Times for emulsions to reach PV = 10 meq/kg with and without BSA.

Table 4
Times (in days) for oil-in-water emulsions stored at 37 °C to reach PV = 10 meq/kg, and synergy between BSA and the infusion calculated from this data

	Without BSA	With BSA	% Synergy
Control	1.6[1]	2.2[1]	–
Black tea	25.0[3]	72.1[5]	194
Red tea	25.3[3]	41.7[4]	65.0
Green tea	47.6[4]	>75	>57.0
White tea	15.9[2]	28.9[3]	83.0
Rooibos	43.6[4]	43.3[4]	0

Values with the same superscript number are not significantly different ($P < 0.05$) (mean for triplicate samples).
The induction period (IP) was calculated as the time (in days) required to reaching PV = 10 meq/kg sample (Table 4). Synergy was calculated from the induction period, by modifying the equations of Satué-Gracia, Heinonen, and Frankel (1997) and Alaiz, Hidalgo, and Zamora (1997):

$$\%\ \text{synergism} = 100\frac{[\text{IP(antioxidant + protein)} - \text{IP(control)}] - [(\text{IP}_{\text{antioxidant}} - \text{IP}_{\text{control}}) + (\text{IP}_{\text{protein}} - \text{IP}_{\text{control}})]}{[(\text{IP}_{\text{antioxidant}} - \text{IP}_{\text{control}}) + (\text{IP}_{\text{protein}} - \text{IP}_{\text{control}})]}.$$

Table 5
Effect of extract infusions on antimicrobial activity (diameter of the inhibition zone measured in mm)

	Bacillus cereus		*Micrococcus luteus*		*Pseudomonas aeruginosa*		*Escherichia coli*		*Lactobacillus acidophilus*		*Candida albicans*	
	Average	s.d.	Average	s.d.	Average	s.d.	Average	s.d.	Average	s.d.	Average	s.d.
White tea	12.5	0.06	12.4	0.28	6.7	0.03	6.4	0.03	–	–	8.5	0.03
Green tea	13.9	0.08	11.2	0.16	10.8	0.17	6.2	0.02	–	–	8.0	0.09
Red tea	7.7	0.09	7.1	0.04	–	0.00	–	–	–	–	6.7	0.12
Black tea	12.0	0.10	10.0	0.00	11.3	0.06	–	–	–	–	–	–
C.I.1	8.7	0.29	6.7	0.06	7.7	0.12	–	–	–	–	–	–
C.I.2	14.0	0.10	9.0	0.20	10.3	0.29	7.0	0.00	–	–	7.7	0.09
C.I.3	14.7	0.06	13.3	0.15	12.0	0.17	–	–	–	–	7.0	0.00
C.I.4	9.7	0.23	7.7	0.12	9.0	0.17	–	–	–	–	–	–
C.I.5	7.0	0.00	7.0	0.00	–	–	–	–	–	–	–	–
C.I.6	7.0	0.00	–	–	–	–	–	–	–	–	–	–
Rooibos	7.0	0.09	6.4	0.03	–	–	–	–	–	–	8.5	0.22

– indicates no inhibition (s.d. standard deviation for $n = 3$).

of extract. Probably a large concentration or volume of infusion added could provide the greatest effect but the limited capacity of the discs did not allow this. Comparing the five bacteria studied, it is clear that the strain *B. cereus* is most sensitive, showing the largest inhibition diameter in the presence of the tea extracts. Extracts from all the teas

studied had an inhibitory effect against this strain. The second most sensitive strain was *M. luteus*, followed by *P. aeruginosa*. In contrast *E. coli* was only inhibited very weakly for some of the extracts studied (white, green tea and C.I.2). In general, Gram negative bacteria are more resistant to polyphenols than Gram positive bacteria, perhaps due to the different cell wall compositions (Negi et al., 2003). *L. acidophilus* showed exceptional resistance to all extracts studied. The results obtained are very interesting because the tea extracts inhibit the food-borne bacteria but not the intestinal bacteria studied. The yeast *C. albicans* was inhibited by extracts from green and white tea and from some commercial teas (C.I.2 and C.I.3). This yeast was more resistant than were the strains of *Bacillus*, *Micrococcus* and *Pseudomonas* assayed.

The antimicrobial activity is higher in non-fermented tea than in semi-fermented or fermented tea. The highest antimicrobial activity corresponds to the highest antioxidant activity (TEAC values) and less to total polyphenol content. For this reason we can claim that not all tea's polyphenols have antimicrobial effects. Our results are in agreement with those of Gramza and Korczak (2005), who studied the effects of individual catechins separately and found that EGCG and EGC are those with highest antioxidant and antimicrobial powers. Our experiments showed that white and green tea extracts and the commercial teas C.I.2 and C.I.3 were the best microbiological inhibitors. They are also the extracts that have the highest EGCG and EGC concentrations. These extracts contain the catechins EGCG + EGC with the sum of the concentrations being higher than 1400 mg/100 g tea leaves. In contrast, semi-fermented and fermented teas have lower concentrations of these catechins. The contents of both EGCG and EGC were less than 365 mg/100 g tea leaves and the antimicrobial activities of these components were less too.

The use of extracts from green and white teas, in combination with other antimicrobial components or methods for stabilizing food products, is an alternative way of maintaining a high flavour quality (Nazer et al., 2005).

The peppermint and nettle infusions did not have antimicrobial activity. These results are consistent with the low total polyphenol content and, moreover, low antioxidant capacity in the nettle infusion. These infusions seem to have insufficient activity to justify their use as natural preservatives in food. However, the rooibos infusion had a low antimicrobial activity against *B. cereus*. Rooibos does not contain catechins, but it contains various others compounds (aspalathin, isoorientin, orientin and rutin) that could contribute to the antioxidant and antimicrobial activities.

4. Conclusions

There is no significant effect of packaging and dry storage time on the contents of total phenolic compounds and on the antioxidant activity. The phenols present in rooibos infusion have a strong antioxidant activity in model food emulsions, but their activity in retarding oxidation of oil droplets in oil-in-water emulsions does not increase in the presence of BSA so there is no synergistic effect.

The antimicrobial activity of non-fermented tea is higher than that of semi-fermented or fermented tea. The highest antimicrobial activity occurs in samples with the highest total polyphenol concentration and antioxidant activity. White and green teas and commercial teas, like C.I.2 and C.I.3, are the best sources of extracts which can act as microbiological inhibitors. The use of these teas in combination with other antimicrobial additives or methods for stabilizing food products represents an alternative way of maintaining a high flavour quality without the use of conventional food preservatives.

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