



WATER SOLUBLE ANTIOXIDANT CAPACITY OF DIFFERENT TEAS

Determination by Photochemiluminescence

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INTRODUCTION

Tea is one of the most widely consumed foods in form of infusion from the dried leaves, and more recently as a food supplement in form of extracts. It has recently gained increasing attention because of its relevant content of antioxidant components. Aside from differences in cultivation, differences in teas are largely due to the extent to which enzymatic processes are halted within the tea leaf, resulting in green tea, or, when left to proceed, result in oolong and black teas.

There are many types of tea, and they differ in their ingredient profile. The primary antioxidant potential of tea is attributed to catechins, but tea antioxidant contents may vary largely in function of type, origin, methods of preparation of the dry leaves. Green tea, obtained from the steamed or pan-fried leaves of *Camellia sinensis*, contains polyphenolic components which have been postulated to be protective against cancer. Tea is particularly rich in catechins, of which epigallocatechin-3-gallate (EGCG) is the major polyphenolic constituent (1), but it also contains a large number of bioactive chemicals which are also responsible of biological effects. However, catechins and their derivatives are thought to be the substances most responsible for the beneficial effects ascribed to tea. Indeed, tea catechins and polyphenols are effective scavengers of reactive oxygen species *in vitro* and *in vivo* and may also function indirectly as antioxi-

dants through their effects on transcription factors and enzyme activities (2).

Several *in vivo* and *in vitro* experiments have been carried out either on green tea or EGCG. Green tea has been demonstrated to have antimicrobial, immunostimulant and anti-inflammatory activities (3). In addition, green tea and EGCG have been shown to have protective effects against cardiovascular disease and preventive/curative effects against various kinds of cancer (4,5). Although some of the mechanisms of the antimicrobial, immunostimulant and anti-inflammatory effects have been identified and attributed to specific tea components, the molecular mechanisms of the chemopreventive effects of green tea are still uncertain. Catechin present in green tea, black tea and white tea (6) have been reported to possess antioxidant property in various pathological conditions, i.e. carcinoma (7), inflammation states such as acute lung injury (8), and in the treatment of cataract (9) or glomerular and renal dysfunction

Key words

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(10). However, reports on antioxidant capacities and epidemiologic evidence regarding the effects of tea consumption on cancer and cardiovascular disease risk are conflicting, mainly because tea types greatly differ in their functionality depending on species, region of cultivation, manufacture.

Leung *et al* (11) reported that drinking black tea has benefits equal to those of drinking green tea in terms of their antioxidant capacities, because theaflavins (TF) present in black tea possess at least the same antioxidant potency as catechins present in green tea. However, the concentration of diverse antioxidants in the different teas should be first considered before drawing any conclusions about overall health benefits of teas (12). In fact, each tea differs both in terms of composition and concentration of antioxidant compounds. As reported previously (13), black teas have low amounts of TF (2–6% of extracted solids) and high thearubigen (20%) contents, whereas green teas have much higher catechin contents (30–42%), particularly EGCG, which is the most abundant catechin. It is known that, due to the different structures, these substances show a different pattern of activity against the different free-radical species. The dietary phytophenolics have been recognized largely as beneficial antioxidants that can scavenge harmful active oxygen species including $O_2^{\bullet-}$, H_2O_2 , OH^{\bullet} , and $^1O_2^{\bullet}$, but they can also act as pro-oxidant in some conditions.

The combined effects of the different antioxidants contained must be taken into account when considering the health benefit of a food. The total antioxidant capacity of tea is not related to a particular

kind of polyphenol but to the combined activity of diverse antioxidants, including phenolic acids and polyphenols. In this regard, we have very recently shown that molecular combination of synergistic antioxidants exert much higher activity, thus demonstrating that antioxidants work within a complex pathway which involves concerted activity of synergistic antioxidants in counteracting the negative effects of free-radical species (14,15).

The antioxidant capacity of tea mainly relies on its water-soluble components, because these represent the principal antioxidant molecules in a tea. The lipid soluble antioxidants are represented by carotenoids which are present in very limited concentration. Thus, apparently, most or all beneficial properties of tea have been ascribed to antioxidant capacity, thus referring to the so-called 'non-nutrient' antioxidant phytochemicals (16). However, to the best of our knowledge, very few reports in the literature (17) have dealt with the comparison of antioxidant capacities of teas from different origin, thus highlighting possible differences in place of growth, methods of preparation, species, etc.

In this study, we have compared the antioxidant capacity of different tea infusions deriving from fermented tea, green tea, white tea, red tea and other specialties, chosen from those available on the market. In order to gain better insight into the antioxidant and scavenging capacities of tea, we examined their antioxidant capacity, intended as their ability to counteract reactive oxygen species (ROS), by means of photochemiluminescence (PCL). The aim was to better understand the complex pattern of activities reported on teas by determining if differences exist between special teas and supermarket products, different preparation methods, origin and species.

MATERIALS AND METHODS

Photochemiluminescence assay

PCL-based methods differ from other procedures for antioxidant evaluation principally because they do not require oxidizing reagents for the production of the radical species. The most widely used methods for measuring antioxidant activity (TEAC I-III, TRAP, DPPH, DMPD) involve the generation of radical species and the presence of antioxidants causing the disappearance of these radicals.

Most of the assays determine the antioxidant activity in the micromolar range, requiring minutes or hours. The photochemiluminescence (PCL) assay presents some advantages: it does not require high temperatures to generate radicals and it is more sensitive (nanomolar range) in measuring, in few minutes, the scavenging activity of antioxidants against the superoxide radical ($O_2^{\bullet-}$) which is one of the most dangerous ROS, also occurring in the human body (17). In the PCL assay, the photochemical generation of free radicals is combined with a sensitive detection method using chemiluminescence. This reaction is induced by optical excitation ($h\nu$) of a photosensitizer S which results in the generation of the superoxide radical $O_2^{\bullet-}$



The free radicals are visualised with a chemiluminescence detection reagent, luminol; this acts as photosensitizer as well as oxygen radical detection reagent.

The PCL method was carried out as described by Popov and Lewin (18b) and can be conducted by two different protocols, ACW and ACL, which permit the measurement of the antioxidant capacity of the water- and lipid-soluble components, respectively. In the water soluble fraction, antioxidants such as flavonoids, ascorbic acid, aminoacids, etc., are detected, while in the lipid soluble fraction tocopherols, tocotrienols, carotenoids, etc., are measured. In this study, measurements were conducted with the ACW kit (AnalytikJena): 1.5 ml of reagent 1 (solvent), 1 ml reagent 2 (water buffer solution, pH 10.5), 25 μ l reagent 3 (photosensitizer) and 10, 15, 20, 25 μ l of standard solution, mixed and measured. In this manner, the conditions were standardised to give comparable results.

ACW calibration and measurements were performed according to the standard kit protocol. The measurements were done by Photochem®.

Measurements were conducted using 10-50 μ l volumes of the sample. Measurement was repeated three times.

One curve corresponding to the calibration was memorized and nmol of ascorbic per sample were then calculated.

A light emission curve was recorded over 130 s, using inhibition as the parameter to evaluate antioxidant potential. The antioxidant capacity was then determined by using the integral under the

curve and was expressed as mmol/l of ascorbic acid used as standard to obtain a calibration curve.

Sample preparation of teas

Green type world wide GDO mint flavoured tea, Bancha, Lung Ching, Italian pods Baobab flavoured tea, world wide GDO Ginseng flavoured tea; *Brown type* Oolong. *Black type* Assam, world wide GDO orange/cinnamon tea; *White type* Pai Mu Tan; *Red tea* Roibos. Teas were purchased in local specialized stores and supermarkets.

3.0 g of each tea were extracted in 150 ml of distilled water by heating for 5 minutes. The temperature of water was kept below 80°C. The mixture of was decanted and filtered over paper filter (No. 2, Whatman, Maidstone Kent, UK). The resulting solutions were centrifuged for 10 min. Samples were diluted (1:100) with R1 solution (ACW-kit, AnalytikJena).

Measurements were accomplished using 10, 30 and 50 μ l volumes of the sample. Measurement were repeated three times.

RESULTS AND DISCUSSION

This study was aimed to quantify and compare the well known antioxidant capacities of teas which are at the base of their therapeutic, nutraceutical and cosmeceutic claims. Indeed, increasing interest in the health benefits of tea has led to the inclusion of tea extracts in dietary supplements and functional foods. Green tea, the major beverage consumed in Asian countries, is not fermented, and for this reason is termed 'virgin tea', while black tea generally refers to the fermented leaves.

Table 1 Water soluble antioxidant capacity of different teas

Tea type	Antioxidant capacity Ascorbic acid equivalent (mmol/L)
Assam Indian black tea	15.27 ± 0.055
Bancha Japanese green tea	30.20 ± 0.035
Lung ching Chinese green tea	23.90 ± 0.057
Oolong, Chinese brown tea	4.27 ± 0.057
Roibos African red tea	4.60 ± 0.01
Pai mu tang Chinese white tea	19.27 ± 0.03
Black tea Italian GDO	18.17 ± 0.07
Black tea Italian GDO	25.60 ± 0.03
World wide GDO green tea, mint flavoured	16.70 ± 0.015
World wide GDO black tea Orange/Cinnamon flavoured	15.00 ± 0.05
Green tea pods, Baobab flavoured	20.60 ± 0.035
World wide GDO green tea ginseng flavoured	20.03 ± 0.04

Data are means ±SD of 3 measurements

The major components of green tea leaves are catechins. In black tea they are oxidized and dimerized during fermentation to the yellow-orange 'pigments', theaflavins (TF), or polymerized to the red 'pigments' called thearubigins. Green tea catechins, including (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG) (**Fig 1**) are indeed oxidized and dimerized during the

manufacture of black tea to form TFs as for example theaflavin-3,3-digallate (TF3). Oolong Tea, termed brown tea, is considered intermediate between green and black tea because the tea leaves are partially oxidized. White tea refers to a kind of tea in which the leaves and buds are simply steamed and dried, in this sense it represents the least processed form of tea, since green, oolong and black teas undergo various degrees of oxidation before withering. White tea also contains a higher portion of buds. Red tea is a beverage improperly defined as 'tea' in that it refers to Roibos (*Asphaltus linearis*), a plant originally discovered in the southwestern cape region of South Africa. This 'tea' was also examined in the study because it is claimed to be rich in antioxidant components and because it is the only other product

that undergoes a full fermentation process like black tea (19). Finally, some flavoured teas were also examined, such as mint, orange/cinnamon, ginseng and baobab. The evaluation of the antioxidant efficacy of a given sample is based on the determination of the radical-scavenging activity, thus a very important role is also played by the kind of radical species which is scavenged.

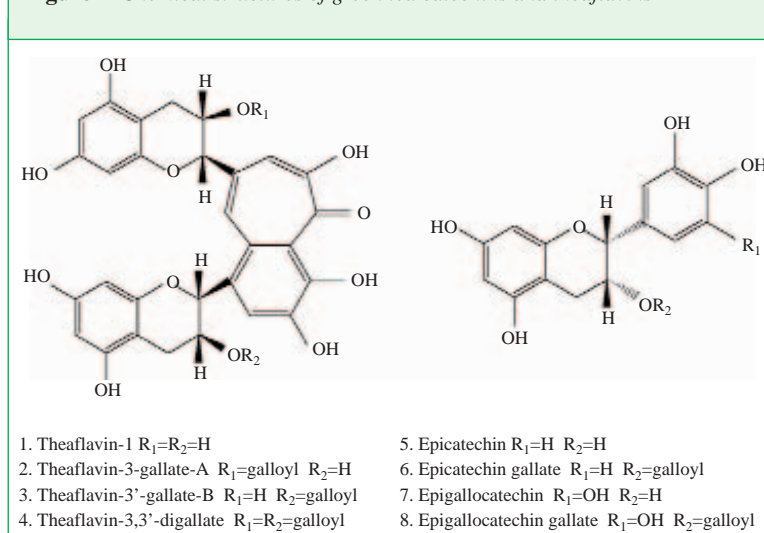
These species vary depending on the method of evaluation selected, thus giving rise to some conflicting opinions on the same kind of product.

As stated above, the PCL method was chosen by us

(19) because the superoxide radical ($O_2^{\bullet-}$) is directly linked to health issues (17).

Indeed, multifactor disease primarily associated with oxidative stress are produced by free radicals, especially ROS species. Moreover, only two assays (LDL, PCL) are able to analyse antioxidant activity in the nanomolar range.

Whereas the PCL assay is ready within minutes, the LDL oxidation assay

Figure 1 Chemical structures of green tea catechins and theaflavins

needs hours. In this study, antioxidant capacity of tea was measured taking into account the water soluble components because they represent the principal antioxidant molecules in a tea.

As may be observed in Table 1 and Figure 2, antioxidant capacity, expressed in terms of ability of a given infusion to counteract ROS (i.e. O₂^{•-}), greatly varied among the examined products, within and among the types. Thus GDO fermented black teas scored better than selected ones (i.e. Assam). Green teas resulted, on average, better than black teas with limited differences between selected high quality teas and GDO teas (i.e. Bancha versus GDO green tea). Apparently, also the species was of importance (i.e. Bancha versus Pai Mu Tang).

Oolong Chinese brown tea, purchased in a specialized shop, showed the worst antioxidant capacity value (4.27 ± 0.057), followed by Roibos red tea (4.6 ± 0.01). As stated above, among green teas, the infusion of Bancha Japanese green tea had the highest antioxidant capacity value (30.20 ± 0.035), which corresponds to more than 30 mmol equivalent of ascorbic acid for each litre of tea infusion.

In other words, one litre of infusion possesses the same antioxidant capacity as 5.28 grams of ascorbic acid. These results compare well with those by Pellegrini *et al* (20) who reported an antioxidant capacity of green tea, determined by three different techniques (TEAC, TRAP and FRAP), considerably higher than that of black tea.

This different behaviour could be attributed to the changes occurring during the process of fermentation; as stated above, the flavanols in green tea leaves (mainly catechins and their gallic esters)

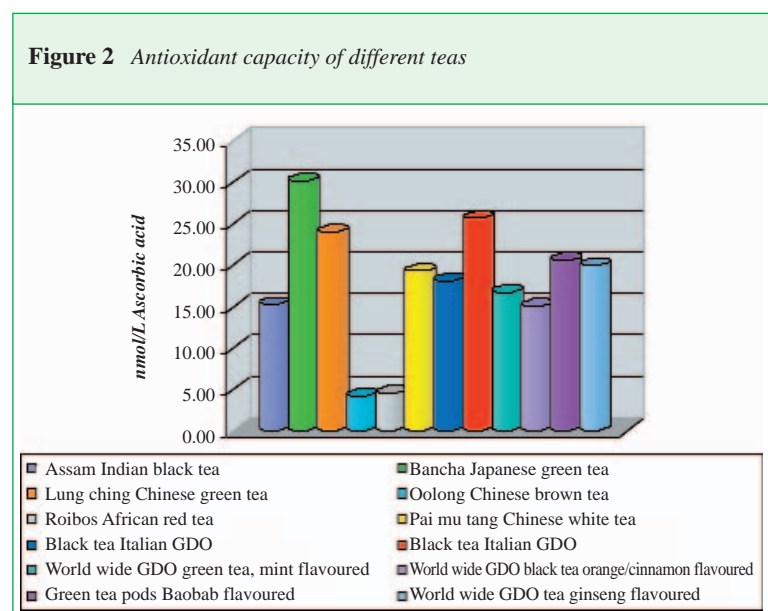
undergo an oxidative polymerization by polyphenol oxidase, which turns the leaves black. During oxidation, much of the catechin content of green tea is converted to oxy-products, such as thearubigens and theaflavins. This occurrence has been associated with the loss of antioxidant capacity, determined by the LDL method, observed in black teas towards green teas (21).

However, conflicting reports have appeared in literature; indeed it has been also demonstrated, again with the LDL method, that the TFs present in black tea possess at least the same antioxidant potency as catechins present in green tea, and that the conversion of catechins to TFs during fermentation in making black tea does not alter significantly their free radical scavenging activity (11). Again, in contrast with these findings, oolong tea that contains a mixture of catechins, TFs and thearubigins (22) was, in our hands, endowed with only poor antioxidant activity.

CONCLUSIONS

The analytical method used in this study, the PCL assay, was chosen because it is rapid, sensitive, relatively simple and reproducible, making it an attractive biomonitoring tool especially for food supplement, nutrition and food technologies. The data obtained add another piece to the complex puzzle represented by the determination of the antioxidant capacity of foods and supplements. As stated above, activity may vary in great extent depending on the

Figure 2 Antioxidant capacity of different teas



evaluation method chosen, because the different methods account for activity against different oxidized and radical species. The PCL method was also chosen because it measures the capability of a given substance to counteract the free radical activity of $O_2^{\bullet-}$, one of the most dangerous species of ROS, and thus it easily reflects the healthy properties of a beverage or food or food supplement.

In our case, antioxidant capacity was expressed as mmol equivalents in activity of ascorbic acid, determined in the best experimental conditions for each sample and indicated the following potencies in descending order: bancha green tea > Italian GDO fermented tea > lung ching green tea > Italian GDO baobab flavoured green tea > Italian GDO ginseng flavoured fermented tea > pai mu tang white tea > Italian GDO fermented tea > worldwide GDO mint flavoured green tea > Assam black tea > worldwide GDO orange/cinnamon flavoured fermented tea >> roibos > oolong brown tea. As can be seen, the bancha tea was the most potent followed by an Italian GDO fermented tea. Lung ching was also endowed with good activity, again followed by an Italian GDO baobab flavoured green tea. This latter result was likely due to the contribution of the antioxidant capacity of the Baobab fruit pulp content (10%). It is interesting to note that the potency of the finest brand of teas was not far superior to low-cost GDO teas.

SUMMARY AND PERSPECTIVES

Tea is one of the most known and consumed traditional beverages. In recent years a growing number of health claims on tea products, with

inclusion of extracts for dietary supplements and functional foods, have appeared in the literature, mainly based on their antioxidant properties. However, these reports, as well as epidemiologic evidence regarding the effects of tea products consumption on cancer and cardiovascular disease risks, are conflicting, mainly because tea types greatly differ in their functionality depending on species, region of cultivation, manufacture and so on. With the aim to add another piece to this complex picture, we have comparatively evaluated the antioxidant capacity of different tea infusions, deriving from fermented tea, green tea, white tea, red tea and other specialties, chosen from those available on the market.

Evaluations were conducted by means of photochemiluminescence (PCL) and expressed as capacity of the given infusion to counteract the superoxide anion $O_2^{\bullet-}$ one of the most dangerous reactive oxygen species.

In this regard, green teas showed the highest antioxidant capacity.

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