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Determination of the antioxidant capacity of samples of different types of tea, or of beverages based on tea or other herbal products, using a superoxide dismutase biosensor

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Abstract

Research was performed to experimentally evaluate the antioxidant capacity of different plant products sold by herbalists (ginger, dog rose, ginseng and camomile) and of several types of tea (ordinary tea, green tea, detheinated tea, lemon and peach flavoured tea) using a superoxide dismutase (SOD) biosensor recently developed by the present authors. Measurements were carried out by comparing biosensor response to the superoxide radical produced in solution using the xanthine–xanthine oxidase system, both in the presence and absence of the antioxidant sample considered. Precision of antioxidant capacity measures for herbal products and for non diluted samples was good, generally with a R.S.D.% $\leq$ 10% and a LOD value about 0.1 for relative antioxidant capacity. Also a "pool" of polyphenols from different tea samples was measured using a tyrosinase biosensor (LOD $\approx$ 2 μM).
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Keywords: Antioxidant capacity; Determination; Tea; Superoxide dismutase biosensor

1. Introduction

Diet is increasingly considered a decisive factor of protection against even such serious diseases as cardio- and cerebro-vascular disorders and tumours [1]. A number of substances contained in foodstuffs, for instance, antioxidant compounds, seem to exert an effective protective action [2]. In addition to fresh vegetables, also other foods contain antioxidants: this is true of tea, above all green tea (particularly rich in flavonoids), as well as of several other herbal products [3], such as ginger, dog rose and ginseng. Some tests were, therefore, run to determine the antioxidant capacity of different types of tea: green tea, ordinary tea, detheinated tea, as well as commercially available tea-based beverages, such as lemon and peach flavoured tea. A comparative test was also performed on the antioxidant capacity of a common cup of tea, with or without the addition of lemon or milk. In addition, the antioxidant capacity of herbal products such as ginger, dog rose, ginseng and camomile was also measured.

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The antioxidant capacity was measured using a biosensor recently developed in our laboratory [4]. This superoxide dismutase (SOD) biosensor for the determination of the superoxide radical had already proved suitable for determining the antioxidant capacity of various different types of synthetic and natural substances [5,6]. In the present research, the emphasis was laid not so much on biosensor development, which has already been amply described in previous works [4–6], as on the definitive development of the method for measuring antioxidant capacity and on its widespread application to products of considerable topical interest in the beverages and herbal sectors.

Analysis of the tea and camomile samples was performed on both cold and hot beverages, and that of the other products only on cold infusions, both homogenised and homogenised and then centrifuged. In the case of hot teas also different infusion times were studied.

Lastly also the polyphenol content of the different tea samples, in view of the important role played by polyphenols among the substances contained in the tea, as well as in many herbal products [7–10], was measured using a tyrosinase biosensor described in a previous article [11], and thus related to the data referring to the antioxidant capacity of the various samples tested.

2. Experimental

2.1. Chemicals

Xanthine (2,6-dihydroxy purine) sodium salt, ethylene diamino tetracetic acid (EDTA), SOD 4980 U mg^{-1}, dialysis membrane (D-9777), Tris buffer, tyrosinase 6050 U mg^{-1}, were supplied by Sigma (Milan); acetone RPE, cyclohexanone RPE, anhydrous dibasic potassium phosphate RPE, analytical grade methanol RPE, phenol, were supplied by Carlo Erba (Milan); xanthine oxidase 0.39 U mg^{-1}, cellulose acetate, kappa-carrageenan, were supplied by Fluka AG, Buchs (Switzerland); polyvinylacetate was supplied by Aldrich (Germany); potassium chloride was supplied by Riedel-de Haen (Seelze, Germany).

2.2. Apparatus

Mod. 4000-1 electrode, supplied by Universal Sensor Inc. New Orleans, LA, USA, connected to a mod. 551 Amel (Milan) potentiostat, coupled to an Amel mod. 631 differential electrometer and an Amel mod. 868 analogue recorder; thermostable glass cell coupled to a Julabo 58 thermostat, supplied by Labospital (Rome); ultra-Turrax homogeniser, mod. T8, supplied by Ika Labortechnik (Germany); model F20ST magnetic stirrer, supplied by Falc Instrument (Lugano, Bergamo, Italy).

2.3. Samples

a) Different types of tea: Chinese green tea, purchased at the herbalist's; ordinary tea in bags, purchased at the supermarket; tea with lemon and peach flavoured tea, conserved in plastic bottles, purchased at the local supermarket; detheinated tea in bags, purchased at the local supermarket.
b) Different herbal products: Ginseng, fragments of root, purchased at the herbalist's; ginseng, fragments of root in bags, treated with honey, purchased at the drugstore; dog rose, fragments of leaves and berries, purchased at the herbalist's; ginger, powder, purchased at the herbalist's; camomile, in bags, purchased at the local supermarket.

2.4. Pre-treatment of samples

The ginger sample consisted of a homogeneous powder with the same consistency as flour; the dog rose sample, consisting of fragments of dried berries, was finely ground up before being analysed; both the ginseng samples were finely ground up before being analysed; the ordinary tea, green tea and camomile samples were analysed as is.

a) Cold infusions: 0.5 g of each sample was taken and weighed. Each weighed sample was then homogenised (in a homogeniser at 10 000 rpm for 5 min) in 3.0 ml of phosphate buffer (5×10^{-2} M at pH 7.5); 0.5 ml were then sampled for the analysis.The homogenate was then

centrifuged at 3000 rpm for 15 min; 0.5 ml of the supernatant was then taken for the analysis.

b) Hot infusions: 0.5 g of ordinary tea, green tea, detheinated tea or camomile, still sealed in their filter bags, was placed in 3.0 ml of boiling distilled water; after a prearranged infusion time, 0.5 ml was then taken, allowed to cool to room temperature and then analysed.0.5 g of tea (or camomile), still sealed in their filter bags, were placed in 30.0 ml of boiling water (''diluted'' tea sample, corresponding to the quantity of tea generally used to prepare a common tea infusion); the rest of the procedure was the same as described above.Also in the case of polyphenol measures in tea and camomile carried out after hot infusion of the sample, the infusion and the required subsequent cooling before measurement at room temperature were carried out exactly as described above.

c) The samples of bottled tea were tested as is, adding 0.5 ml of each beverage to 15.0 ml of phosphate buffer contained in the measuring cell, and then proceeding with analysis by SOD biosensor.

3. Methods

3.1. The superoxide dismutase biosensor (SOD)

The biosensor we used to determine the superoxide radical was obtained by coupling a transducer (an amperometric electrode for hydrogen peroxide, with platinum anode maintained at a constant potential of +650 mV with respect to an Ag/AgCl/Cl^- cathode) and the SOD enzyme immobilised in a gel-like Kappa-carrageenan membrane. The gel containing the enzyme was sandwiched between a cellulose acetate membrane and a dialysis membrane (Fig. 1). The whole assembly was fixed to the head of the electrode by means of a rubber O-ring.

The preparation of the Kappa-carrageenan membrane and the immobilisation of the SOD enzyme in the gel membrane, was described in detail in a previous paper [11,12].

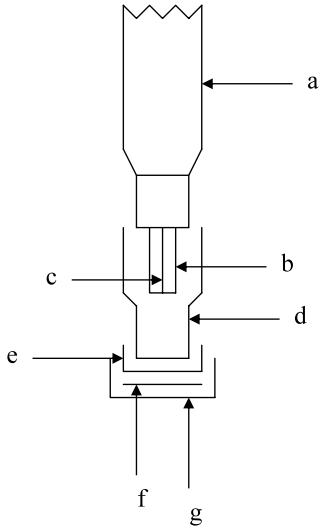

Fig. 1. SOD biosensor assembly, using amperometric hydrogen peroxide electrode as transducer. (a) Electrode body; (b) Ag/AgCl/Cl^- cathode; (c) Pt anode; (d) electrode plastic cap filled with buffer solution; (e) cellulose acetate membrane; (f) kappa-carrageenan membrane entrapping SOD enzyme; (g) dialysis membrane.

3.2. Measurement of antioxidising capacity using the SOD biosensor

3.2.1. Principle of the method

The superoxide radical is produced by the oxidation in aqueous solution of the xanthine to uric acid in the presence of the enzyme xanthine oxidase:

$$\text{xanthine} + H_2O + O_2 \xrightarrow{\text{xanthine oxidase}} \text{uric acid} + 2H^+ + O_2^{\bullet -} \quad (1)$$

the disproportion reaction of the superoxide radical, catalysed by the SOD, immobilised on the H_2O_2 electrode, releases oxygen and hydrogen peroxide:

$$O_2^{\bullet -} + O_2^{\bullet -} \xrightarrow{\text{superoxide dimutase}} H_2O_2 + O_2 \quad (2)$$

the H_2O_2 is monitored by the amperometric sensor for hydrogen peroxide.

The hydrogen peroxide released is oxidised at the anode, that is, it generates an amperometric signal variation (of the order of tens of nA) that is proportional to the concentration of superoxide radical in solution.

The addition of a sample possessing antioxidant properties produces a decrease in signal strength as the antioxidant species react with the superoxide radical, thus reducing its concentration in solution. There is a consequent decrease in the H_2O_2 released and thus also in the intensity of the amperometric signal.

3.3. Performance of the measures

The electrode is placed in a glass cell thermostatted at 25 °C, containing 15.0 ml of phosphate buffer 5×10^{-2} M at pH 7.5 and allowed to stabilise under constant magnetic stirring. After the addition of a fixed quantity of the enzyme xanthine oxidase (1.2 mg), a series of further additions of 200 μl of the xanthine solution 1×10^{-2} M is performed, waiting for the signal to stabilise between additions before proceeding to read off the current. The graph (Fig. 2) shows the successive signal variations recorded on the analogue recorder.

The current values (recorded after signal stabilisation) are then recorded in the graph as a function of increasing xanthine concentration, thus obtaining a straight-line calibration curve from which the slope may be measured (Table 1(a)). The same type of measurement is then repeated, but this time adding also the sample to be tested (for instance homogenised green tea) to the cell containing the phosphate buffer and then proceeding as described above. If the sample displays antioxidant properties, the observed signal variation (Fig. 2) will be lower than in the preceding case, in proportion to the decrease in concentration of the superoxide radical in solution; the new calibration curve thus obtained will thus present a lower slope value than in the preceding case (as shown in Table 1(b)). By comparing the values of the two angular coefficients it is possible to determine the total antioxidant capacity of our sample.

Table 1
Analytical data

(*a*)	
Equation of calibration curve:	$Y = (328.9 \pm 6.4)X - (2.3 \pm 1.3)$
(in absence of an antioxidant species)	Y = a.u.; X = [xanthine] (mM)
Confidence interval	$t = 2.23$, $(1-\alpha) = 0.95$
Correlation coefficient (r^2)	0.9996
Linear range (mM)	0.02–2.0
Precision (as R.S.D.%)	≤ 5
Response time (s)	≤ 100
(*b*)	
Equation of calibration curve	$Y = (92.1 \pm 3.5)X - (2.1 \pm 1.4)$
(in presence of an antioxidant sample, i.e. green tea)	Y = a.u.; X = [xanthine] (mM)
Confidence interval	$t = 2.23$, $(1-\alpha) = 0.95$
Correlation coefficient (r^2)	0.9991
Linear range (mM)	0.02–2.0
Precision (as R.S.D.%)	≤ 5
Response time (s)	≤ 100

The value of the relative antioxidant capacity is expressed by the algorithm:

$$\text{Relative antioxidant capacity} = 1 - (m_b/m_a)$$

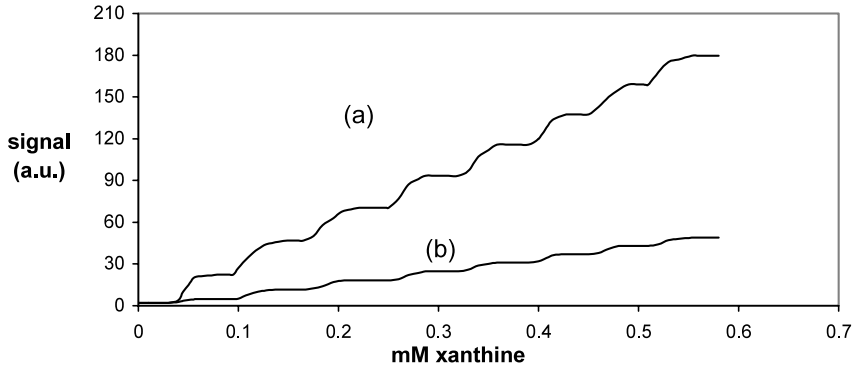

Fig. 2. Typical biosensor responses to SOD towards the superoxide radical after successive additions of 200 μl of xanthine 0.01 M, obtained (a) in the absence and (b) in the presence, of a herbal homogenised sample with antioxidant capacity (green tea).

m_a, slope of the straight line obtained by successive xanthine additions; m_b, slope of the straight line obtained by successive xanthine additions, but in the presence of the sample with antioxidant properties.

All the samples of tea and of the other herbal products were analysed using this method.

3.4. Determination of polyphenols using electrochemical tyrosinase biosensor

The tyrosinase biosensor was constructed by coupling a transducer (an amperometric gaseous diffusion electrode for oxygen) with the enzyme tyrosinase immobilised in Kappa-carrageenan gel [11,12]. The gel containing the enzyme was sandwiched between a Teflon gas-permeable membrane and a dialysis membrane. The internal solution in the electrode consisted of KCl 0.1 M in phosphate buffer 6.0×10^{-2} M at pH 6.6. The electrode had a platinum cathode polarised at -650 mV with respect to an Ag/AgCl/Cl^- anode.

The measuring apparatus was the same as that used in the case of the SOD biosensor; also the tyrosinase immobilisation in kappa-carrageenan was carried out in the same way [11,12].

The polyphenol content of the sample was performed by recording the variation of the amperometric signal related to the reduction in the dissolved oxygen concentration due to the following enzymatic reaction:

$$\text{phenol} + O_2 \xrightarrow{\text{tyrosinase}} 1,2\text{benzoquinone} + H_2O$$

The signal variation due to the O_2 consumed was proportional to the polyphenol concentration in the sample.

The measurements were performed by placing the electrode in a glass cell, thermostatted at 25 °C, containing 15.0 ml Tris buffer 0.1 M at pH 8.5 and left to stabilise under constant stirring, thus allowing the dissolved oxygen concentration in the buffer solution to attain a stationary state, which is reached when the rate of diffusion of atmospheric oxygen into the solution equals the rate of oxygen consumption due to the enzymatic reaction. It was then proceeded to add 200 μl of sample and to record the signal variation. Likewise, 200 μl of a standard solution of phenol 0.1 M was added, also in this case recording the signal variation.

Comparison between the signal due to the addition of sample and that due to the phenol allows the polyphenol content of the test sample to be determined.

4. Results and discussion

As previously stated, the aim of this research is to determine the antioxidant capacity of different kinds of sample (herbal products sold by herbalists and/or pharmacies for their antioxidant and radical scavenging properties and samples of different quality teas).

All these samples were analysed using the SOD biosensor, starting from the herbalist's plant samples. The results obtained for the antioxidant capacity of the latter are shown in Fig. 3 in which the numerical values of the antioxidant capacity for the various herbal samples are reported and their trend shown. The white colour in the histogram represents the homogenised and centrifuged samples, while black refers only to homogenised samples.

As shown by the results, the homogenised herbal samples display an antioxidant capacity that is generally higher than the homogenised and centrifuged samples. This kind of result was only to be expected if we consider that the centrifuged samples were obtained by centrifuging the same,

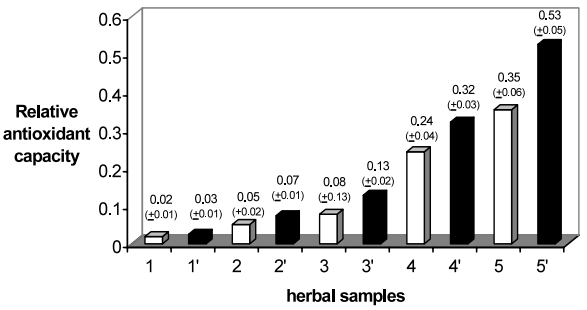

Fig. 3. Trend of antioxidant capacity of herbal samples: (1, 1′) camomile; (2, 2′) ginseng with honey; (3, 3′) ginger; (4, 4′) dog rose; (5, 5′) ginseng. S.D. values in brackets. (□) Homogenised and centrifuged samples, (■) homogenised samples.

previously homogenised, samples and that the analysis of the centrifuged samples is performed on the supernatant; it is, therefore, likely that some antioxidant compounds, in suspension in the homogenate and retained in the precipitate after centrifuging, are excluded from the supernatant. The highest antioxidant capacity among the examined herbal samples was displayed by ginseng and dog rose, according to literature data [13].

It is also interesting to observe how the ginseng samples treated with honey possess a much lower antioxidant capacity than that displayed by the samples of virgin ginseng root.

Different types of tea were then analysed. First, ordinary tea in bags and herbalist's green tea were analysed. For the purpose of regular comparison with the results obtained for herbal samples purchased at the herbalist's, samples of ordinary tea, green tea and detheinated tea, either simply homogenised or homogenised and centrifuged, were analysed. The results obtained for antioxidant capacity of the latter and the relative trends, represented in a histogram, are shown in Fig. 4, respectively.

The white coloured portions of the histogram represent the centrifuged sample and the dark colour the homogenised sample.

The trend of the antioxidant capacity of the various different kinds of tea was similar to that found in the plant samples already analysed and subjected to the same type of pre-treatment: the homogenised samples were found to have a higher antioxidant capacity than those homogenised and centrifuged, possibly for the same reasons as those already put forward in the case of herbal products.

Using the tyrosinase biosensor [11] also the polyphenol content of the same teas was analysed in order to compare it with the value of their antioxidant capacity.

The results obtained in the polyphenol analysis are shown in Fig. 5; their trend is shown in the histogram of the same figure, in which the dark colour represents the homogenised samples and the white colour the centrifuged ones.

Comparing the antioxidant capacity trend in Fig. 4 with that of the polyphenol content in Fig. 5, the polyphenol value found both in ordinary tea and in green tea is seen to follow about the same trend as the antioxidant capacity. This type of result is to be expected in view of the fact that polyphenol substances possess a high antioxidant capacity and clearly, a high value of the latter is consistent with a simultaneous high concentration of polyphenol in the sample and thus also in the solution of the extract.

For example, the higher antioxidant capacity found in green tea compared with ordinary tea may be accounted for, at least in part, by the higher polyphenol content found in green tea.

In addition to the cold infusions, also a number of other hot infusions in distilled water, with

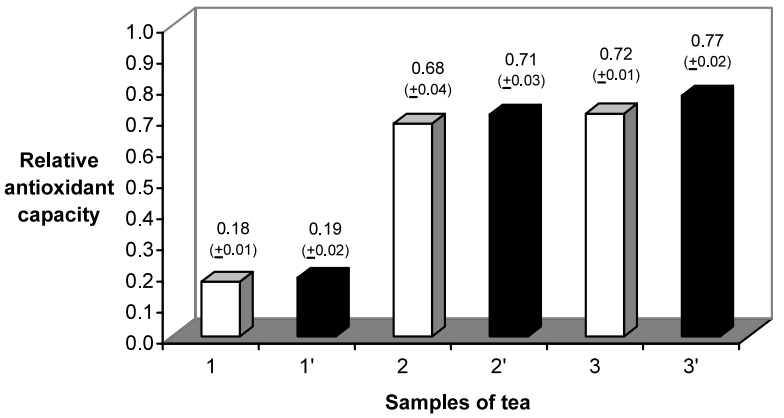

Fig. 4. Trend of antioxidant capacity, for samples of: (1, 1′) detheinated tea, (2, 2′) ordinary tea, (3, 3′) green tea, homogenised, or else homogenised and centrifuged. S.D. values in brackets. (□) homogenised and centrifuged samples, (■) homogenised samples.

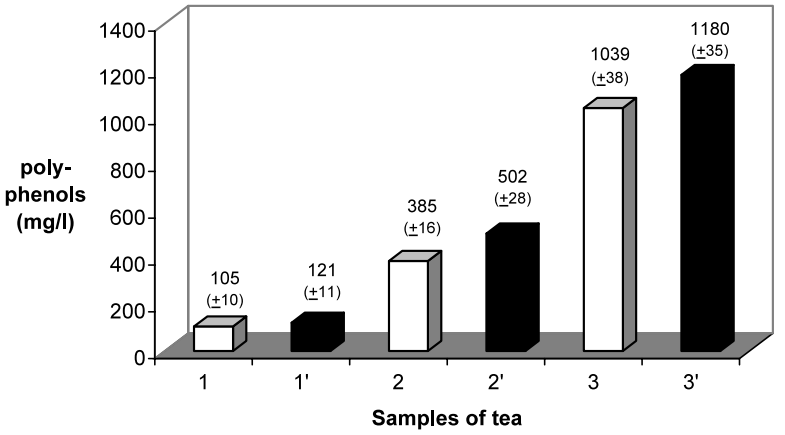

Fig. 5. Trend of polyphenol content, determined using the tyrosinase biosensor, for samples of: (1, 1′) detheinated tea, (2, 2′) ordinary tea, (3, 3′) green tea, homogenised, or else homogenised and centrifuged (values expressed in mg l^{-1} of phenol). S.D. values in brackets. (□) Homogenised and centrifuged samples, (■) homogenised samples.

various different infusion times, were performed for ordinary tea and for green tea.

Also the polyphenol content of these teas was assayed for different hot infusion times. The values of antioxidant capacity and polyphenol content obtained using ordinary tea and their trends are shown in the histograms in Figs. 6 and 7, respectively.

The analysis carried out on ordinary tea bags shows that hot infusion for a period of 5 min produces the greatest antioxidant capacity. Of course, initially, with increasing infusion time also the quantity of antioxidant substances contained in the tea increases. These substances are extracted by the hot distilled water and reach a maximum value after 5 min infusion. After a

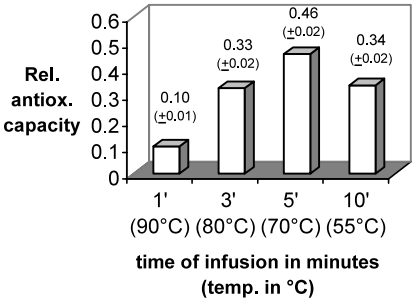

Fig. 6. Trend of antioxidant capacity of ordinary tea, at different infusion times, in hot distilled water. S.D. values in brackets.

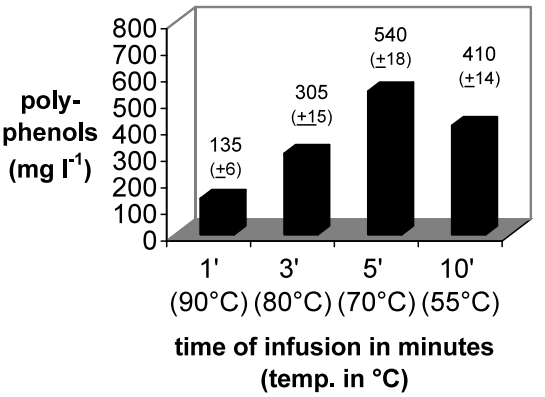

Fig. 7. Trend of polyphenol content of ordinary tea, at different infusion times, in hot distilled water (values expressed as mg l^{-1} of phenol). S.D. values in brackets.

further 5 min, the antioxidant capacity seems to decrease. The polyphenol content obtained using the tyrosinase biosensor displays a trend that is very similar to that of antioxidant capacity.

In Fig. 8 the correlation between antioxidant capacity values and polyphenol content is extremely clear. In the figure, the dark coloured part of the histogram represents the polyphenol content, (expressed as mg l^{-1} of phenol and multiplied by a factor of 3), the white colour represents the value of the antioxidant capacity of the tea samples (multiplied by a factor of 100). The histogram trend shown in Fig. 8 indicates that the polyphenol

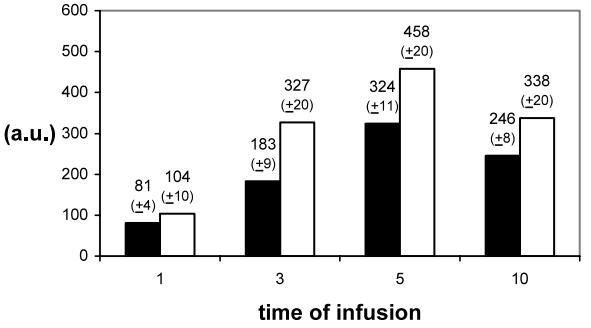

Fig. 8. Correlation between polyphenol content and antioxidant capacity in ordinary tea, at different infusion times, in hot distilled water. S.D. values in brackets. (□) antioxidant capacity, (■) polyphenol content.

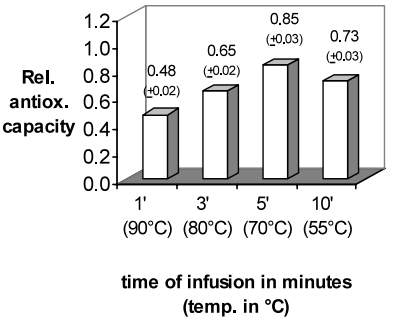

Fig. 9. Trend of antioxidant capacity of green tea, at different infusion times, in hot distilled water. S.D. values in brackets.

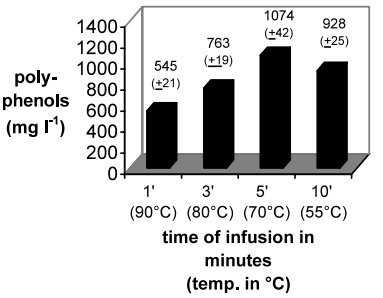

Fig. 10. Trend of polyphenol content of green tea, at different infusion times, in hot distilled water (values expressed as mg l^{-1} of phenol). S.D. values in brackets. (□) antioxidant capacity, (■) polyphenol content.

concentration found in ordinary tea and the value of the antioxidant capacity for hot infusions attain their maximum value after an infusion time of about 5 min. This may be accounted for by the fact that the hot water's capacity for extracting the antioxidant substances contained in the tea initially increases with increasing contact time. After 5 min, however, several antioxidant substances probably begin to precipitate out or begin to form micelles in solution, which is favoured also by the gradual cooling; these substances were thus excluded from the analysis sample. From a macroscopic point of view, a darkening and gradual cloudiness of the tea infusion may actually be observed after this period of time. Another possible explanation is that the high temperature causes the partial thermal degradation of certain antioxidant substances, which causes the antioxidant capacity of the entire mixture to decrease after an infusion time exceeding 5 min. However, this would seem less likely. We actually verified experimentally that, at least in the case of homogenised sample obtained at room temperature, the antioxidant capacity, measured about every 15 min, remained constant, within the range of experimental error, for at least 3 h after preparation of the sample.

Also for green tea both antioxidant capacity and polyphenol content were determined for increasing hot infusion time. The results obtained are shown in Figs. 9 and 10, respectively, while the trends in these values, reported in the histogram, are shown in the same figures.

In sum, the results of the tests performed on green tea at different hot infusion times display an similar trend to that obtained for ordinary tea. The reasons for this behaviour, the same as already observed also for ordinary tea, are probably the same as those previously described.

The relationship between antioxidant capacity and polyphenol content for green tea at different hot infusion times is shown in Fig. 11; in the figure, the dark-coloured portion of the histogram represents the polyphenol content of the tea samples (expressed as mg l^{-1} of phenol and divided by a factor of 2), the white-coloured portion represents the antioxidant capacity of the tea samples (multiplied by a factor of 1000). The histogram trend indicates how, even in the case of green tea, the value found for the polyphenols is in

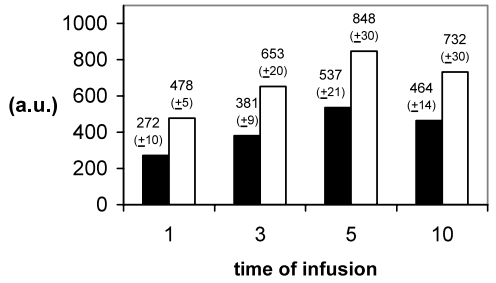

Fig. 11. Correlation between polyphenol content and antioxidant capacity in green tea at different infusion times in hot distilled water. S.D. values in brackets. (□) antioxidant capacity, (■) polyphenol content.

good agreement with the antioxidant capacity trend. Furthermore, in green tea, the increased antioxidant capacity compared with ordinary tea, may be accounted for by the higher polyphenol content that is always found. This emerges clearly from the histograms shown in Fig. 12, in which sample 1 represents ordinary tea, sample 2 green tea, while the dark-coloured histogram represents the polyphenol content of the tea samples (expressed as mg l^{-1} of phenol and divided by a factor of 2), the white colour represents the antioxidant capacity of the tea samples (multiplied by a factor of 1000).

Lastly, also diluted tea infusions were analysed, that is, hot infusions made by using the quantity of tea that is normally used to prepare a common cup of tea. Diluted tea is thus defined as an infusion of ordinary tea or green tea obtained with a hot infusion time of 5 min, but using a volume of distilled water ten times greater than that used previously, so that the solution obtained is ten

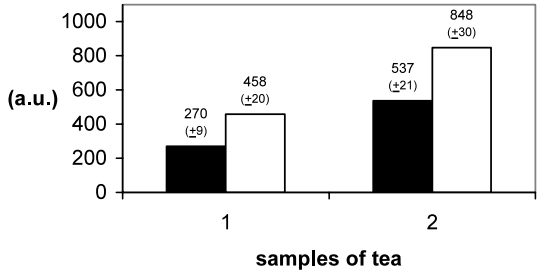

Fig. 12. Comparison between polyphenol content and antioxidant capacity for hot infusions of (1) ordinary tea, (2) green tea. S.D. values in brackets.

times more diluted than the solutions used in the preceding tests. Together with the diluted teas two types of bottled tea ready for consumption were analysed (tea with lemon and tea with peach) as well as hot infusions of ordinary tea prepared like those previously tested, but with the addition of milk or lemon.

Lastly, it was decided to determine whether also hot common infusions of camomile had any antioxidant capacity, as there was little available information on the subject in the literature. The results of the antioxidant capacity obtained for the diluted tea samples and for the bottled tea samples and the relative histogram trend, are displayed in Fig. 13.

The samples of diluted tea show an antioxidant capacity that is of course greatly reduced with respect to the previous samples due to the obvious fact that also the concentration of the antioxidant species in the solution is greatly reduced.

It is in any case interesting to note that, in this case, the antioxidant capacity of a hot infusion of ordinary tea is of practically the same order of magnitude as that of tea sold already prepared in bottles. Hot tea infusions with the addition of milk or lemon result, respectively, in a decrease and in an increase in antioxidant capacity, compared with a simple hot infusion of tea. This is only to be expected in view of the ascorbic acid contained in the lemon added.

It is also interesting to observe how the hot camomile infusions have an extremely low antioxidant capacity.

Lastly, the comparison of some of the data shown in Fig. 4 with those shown in Figs. 6 and 9, may arouse some doubts as the former refer to tea samples obtained by cold infusion and the latter to hot infusions. Nevertheless, in the first case the antioxidant capacity values for ordinary tea are higher than in the second case. This was assumed to be due to the fact that, in the first case, the tea was treated with an aqueous solution of phosphate buffer 5×10^{-2} M at pH 7.5, while in the second case, simply with non decarbonated distilled water at pH 5.6. In order to experimentally verify the validity of this hypothesis the antioxidant capacity of homogenised and centrifuged tea was assayed cold, in phosphate buffer, or with non decarbo-

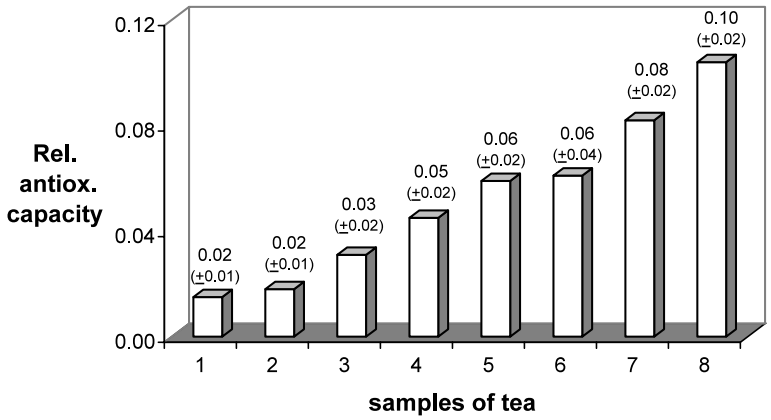

Fig. 13. Trend of antioxidant capacity, measured with the SOD biosensor, for samples of: (1) camomile; (2) detheinated tea*; (3) tea with milk*; (4) bottled tea with peach; (5) bottled tea with lemon; (6) ordinary tea*; (7) tea with lemon*; (8) green tea*. S.D. values in brackets. (*diluted sample).

nated distilled water alone, in addition to tea infusions in distilled water alone, both hot and cold.

The results obtained are set out in Tables 2 and 3. They show how the antioxidant capacity of the infusions obtained actually increases as the temperature of the extracting solution increases, but also how it increases even more (especially in the case of ordinary tea) using phosphate buffer solution rather than distilled water.

This demonstrates that the extractive capacity depends to a great extent not only on the temperature but also on the composition and the pH of the extracting solution. This explains, at least to a significant extent, the apparent contradictions previously observed between antioxidant capacity obtained in cold or hot infusions.

5. Conclusions

In the first instance, it may be claimed that the electrochemical method involving the use of the SOD biosensor developed in our laboratory proved to be perfectly capable of determining the antioxidant capacity of all the different sample types tested: plant substances sold by herbalists and different tea samples.

Briefly, the method used to measure antioxidant capacity used in the present work is conceptually not very different from the numerous methods proposed in the literature that are based on the reaction of free radicals (very often the superoxide radical) with a specific molecule, such as *N*,*N*-dimethyl-*p*-phenylendiamine dihydrochloride (DMPD) [14], crocin [15], β-phycoerythrin

Table 2
Measurement of antioxidant capacity with the SOD biosensor, for cold samples of ordinary tea and green tea, homogenised, or else homogenised and then centrifuged, in simple distilled water, or else in phosphate buffer

Sample	Reletive antioxidant capacity in phosphate buffer (±S.D.) ($n \geq 5$)	Reletive antioxidant capacity in distilled water (±S.D.) ($n \geq 5$)
(1) Ordinary tea (centrifuged)	0.68±0.04	0.44±0.05
(2) Ordinary tea (homogenised)	0.71±0.03	0.52±0.06
(3) Green tea (centrifuged)	0.72±0.01	0.60±0.03
(4) Green tea (homogenised)	0.77±0.02	0.62±0.07

Table 3
Measurement of antioxidant capacity using the SOD biosensor, for cold or hot samples of ordinary tea and green tea, obtained after 5 min infusion in distilled water

Sample	Reletive antioxidant capacity in hot water (±S.D.) ($n \geq 5$)	Reletive antioxidant capacity in cold water (±S.D.) ($n \geq 5$)
(1) Ordinary tea	0.46±0.02	0.21±0.03
(2) Green tea	0.85±0.03	0.38±0.06

[16,17], 1,1-diphenyl-2-picrylhydrazil (DPPH) [18], 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) [19,20] etc... the absorbance, or fluorescence, or chemiluminescence of which varies, respectively, according to this reaction. In the presence of an antioxidant it thus reduces the free radical, causing a variation in the absorbance, fluorescence or chemiluminescence signal (or else modifying the rate with which they vary in time). The signal variation thus recorded correlates with the antioxidant capacity of the test sample. The advantages of the method used by us are quite apparent. In practice, in our case, the molecule on which the method we proposed is based is a widely available biocatalyst (the SOD enzyme) which catalyses the disproportion reaction of the superoxide radical into oxygen and hydrogen peroxide, but remains unchanged itself. Furthermore, as it is immobilised during the test it can be used for numerous analyses. Conversely, in the methods described above, the molecule on which the method is based can usually be used only once. This is a considerable drawback, as these molecules, as well as being hard to obtain, are often above all extremely expensive (β-phycoerythrin [16], *R*-phycoerythrin [21], etc). Another advantage of the proposed method (compared with those described above) consists in the simplicity and cheapness of the required electrochemical apparatus, which are normally already available in most chemical laboratories and lastly also the fact that this apparatus, being easily transportable, also allows analyses to be performed 'in situ'.

The precision of the measures of antioxidant capacity for the herbal product samples was found to be excellent (R.S.D.% ≤ 8) and the LOD of the method, expressed as relative antioxidant capacity, was found to be about 0.1); for the tea samples precision was generally found to be good (R.S.D% $\leq 10\%$ when less diluted tea samples, extracted both cold and hot, were analysed), and the method proved to be sufficiently robust. As observed also in previous work [5], none of the operating parameters (pH, ionic strength, temperature, etc...) was found to be particularly critical; for instance, comparatively small variations in working pH had a practically negligible effect on biosensor response.

Lastly, it is important to point out that, for example, the ratio between the antioxidant capacity of green tea and black tea, as determined using the SOD biosensor, was found to be $=0.92$, in good agreement with the finds of G. Cao et al. [22] using the ORAC method [16,17] (in this case, in fact, the same ratio was found to be $=0.88$); however, R.J. Ruch et al. [23], using the cytochrome *c* reduction method [23], found that the addition of green tea to the xanthine–xanthine oxidase system reduced the superoxide production by about 78%. This value is in close agreement with the result obtained by us for the antioxidant capacity of homogenised green tea expressed as a percentage value (77%; see Fig. 4). Moreover, also the findings of G.C. Yen et al. employing the spectrophotometric-nitro blue tetrazolium method [24] that the scavenging effect of the superoxide anion in different types of tea extracts ranged from 65 to 75%, is in good agreement with our results. Fig. 4 shows that tea antioxidant capacity, expressed as a percentage value is in the range of 68–77%, taking both the values found for ordinary tea and green tea into account.

All these good correlation data provide effective confirmation of the value of the biosensor method,

with which it is possible in practice to obtain results comparable to those obtained using better known methods [16–27], but that are without doubt more tedious and certainly more expensive.

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