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Hot vs. cold water steeping of different teas: Do they affect antioxidant activity?

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ABSTRACT

A new popular way of making tea, especially in Taiwan, is to steep leaves in cold water. Here we investigate whether antioxidant activity of teas may be affected by hot or cold water steeping and if this correlates with their polyphenol content and metal-chelating activity. A set of five loose tea samples, consisting of unblended and blended teas, was analysed following their infusion in either hot water (90 °C, 7 min) or cold water (room temperature, 2 h). Antioxidant activity, measured as hydrogen-donating ability, using the ABTS· and DMPD assays, showed no significant differences among hot or cold teas, except in the case of white tea, where significantly higher values were obtained after cold water steeping, a recurrent finding in this study. The antioxidant activity of the teas correlates well with their total phenolic content and metal-chelating activity. Cold teas were, however, generally better inhibitors of *in vitro* LDL conjugated diene formation and of loss in tryptophan fluorescence. The results of this study contribute to gaining further knowledge on how the potential health benefits of this popular beverage may be maximised by the different methods of preparation.

1. Introduction

Tea, second only to water, is the most highly consumed beverage worldwide and its medicinal and health properties, long known to early Chinese civilizations, have been widely explored (McKay & Blumberg, 2002). It is prepared from the young, tender leaves of *Camellia sinensis* (L.) (family *Theaceae*) which undergo different manufacture procedures to give various types of teas: black, green, white, and oolong. In addition, the growing season, geographical region, processing of the leaves (rolling; cut-tear-curl (CTC)), and whether teas are blended or unblended, create the vast selection available commercially and contribute to each tea's uniqueness (Balentine, 1992). The most popular types of tea are green and black: withering, steaming, rolling and drying of the leaves produce green tea; additional crushing/tearing of leaves and fermentation (i.e. enzymatic oxidation), without steaming, gives rise to black tea. Instead, oolong tea undergoes partial fermentation before drying. During fermentation, the enzymatic oxidation of tea polyphenols takes place, which partially converts catechins into theaflavins and thearubigins, responsible for the characteristic aroma and colour of black and oolong teas (Obanda, Owuor, & Mang'oka, 2004). White tea is an unfermented tea made from the new growth buds and young leaves of the plant that are sometimes shielded from sunlight during growth to reduce the formation of chlorophyll and, unlike black, green and oolong teas, are not withered, rolled or crushed but steamed rapidly and air-dried.

Preference (regarding the consumption) for these different teas varies considerably. Black tea is consumed worldwide while green and oolong teas are consumed mainly in Asia and North Africa. Less consumed but also highly appreciated is white tea. The methods of preparing the beverage also vary worldwide: in China, tea leaves are steeped in hot water (70–80 °C for green tea, 80–90 °C for oolong and 100 °C for black) for 20–40 s, and the same tea leaves are usually repeatedly steeped (seven times). The Japanese usually prepare green tea by steeping leaves in hot water for about 2 min and using them for 2–3 infusions. In the United Kingdom, Ireland and in Canada, black tea is mostly prepared using boiling water and consumed with milk and often sugar. Americans are large consumers of iced tea which is made from hot tea cooled with ice. In recent times in Taiwan, especially in summer, cold water (4 or 25 °C) steeping is a new popular way for making tea. Apparently, tea prepared using cold water contains lower amounts of caffeine, reduced bitterness and higher aroma (Yang, Hwang, & Lin, 2007). For its preparation, tea leaves are steeped in water at 25 °C for at least 2 h before consumption.

Considering the increasing interest in the health properties of tea, unrelated in part to its antioxidant activity (Wiseman, Balentine, & Frei, 1997), and the different types of teas and

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methods of making a cup of tea in different countries and cultures, the present study aimed to discover, whether the antioxidant activity, total phenolic content and metal-chelating activity of different teas could be affected by steeping leaves in hot or cold water.

2. Materials and methods

2.1. Chemicals and equipment

All reagents, including ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt], DMPD (*N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ferrozine [4,4′-[3-(2-pyridinyl)-1,2,4-triazine-5,6-diyl]bisbenzenesulphonic acid], AAPH [2,2′-azobis(2-methylpropionamidine)dihydrochloride] and solvents of the highest purity available, were purchased from Sigma–Aldrich Chemical Co. (Milan, Italy) and used as received. Ultrapure water was used throughout and obtained from a Milli-Q system from Millipore (Milford, MA), except for preparation of teas, where bottled mineral water, purchased from local retail shops, was used.

Spectrophotometric measurements were recorded on a UV Kontron 941 spectrophotometer or on a microplate reader (Synergy HT, Biotek, Winooski, VT, USA).

2.2. Tea samples and preparation

A set of five loose tea samples was analysed, which included four unblended teas: black tea (chinese Lapsang Souchong), white tea (chinese Pai Mu Tan), green tea (China Special Gunpowder), oolong tea (from Fujian Province – China), and one blended black tea (Lyons Gold Brand which is a blend of African and Indian black teas) denominated Lyons in the figures. All were purchased from local retail shops. Prior to tea preparation, the teas were ground using a pestle and mortar to obtain a homogeneous fine powder for each kind of tea. Cold tea infusions were prepared by placing 0.5 g of tea in 50 ml of mineral water at room temperature and gently agitating for 2 h under magnetic stirring. Hot tea infusions were prepared by placing 0.5 g of tea in 50 ml of mineral water at 90 °C and gently agitating under magnetic stirring for 7 min. Both hot and cold infusions were then filtered through Whatman paper filters (43–38 μm) and diluted appropriately with water according to each specific assay. Throughout the paper, the terms hot tea and cold tea will now be used to express the two types of infusions.

2.3. Total phenol content (TPC)

Total phenol content of the different tea samples was determined using the Folin–Ciocalteu reagent (Singleton, Orthofer, Lamuela-Raventos, & Lester, 1999). Briefly, to 0.025 ml of each tea sample previously diluted (2.5×), 1.5 ml of water were added, followed by 0.125 ml of Folin–Ciocalteu reagent and mixed. After 1 min, 0.375 ml of 20% Na_2CO_3 and 0.475 ml of water were added to reach a final volume of 2.5 ml. After mixing, the samples were left for 2 h at room temperature in the dark. The absorbance was then read at 760 nm against a blank containing water instead of tea. For determining the TPC, standard concentrations of gallic acid (0–80 μM) were used for constructing the calibration curve (mM gallic acid vs. absorbance). Gallic acid stock solution was prepared in ethanol at a concentration of 26.5 mM. The results are expressed as mM gallic acid equivalents (GAE).

2.4. Metal-chelating assay

The metal-chelating activity of the different teas was determined on ferrous ions according to Chua, Tung, and Chang (2008). Briefly, 0.2 ml of tea (previously diluted 10×) was added to 0.74 ml, 0.1 M acetate buffer pH 5.25. To the above, 0.02 ml of 2 mM $FeSO_4$ dissolved in acetate buffer was also added and mixed. The reaction was initiated by addition of 0.04 ml of 5 mM ferrozine, mixed, and the whole left to stand for 10 min in the dark at room temperature. The absorbance was then read at 562 nm against a blank containing the acetate buffer instead of tea and $FeSO_4$. For determining the metal-chelating activity, a 50 mM solution of EDTA in 0.1 mM acetate buffer, pH 5.2, diluted in the range 0–40 μM, was used as standard. This was used for constructing a calibration curve by plotting the absorbance at 562 nm as a percentage of the absorbance of the iron (II)-chelated ferrozine solution according to the equation:

$$\text{Inhibition of } A_{562}\ (\%) = (1 - A_c/A_0) \times 100$$

where A_c is the absorbance of the samples and A_0 is the absorbance of the control containing the acetate buffer instead of tea.

The results are expressed as mM EDTA equivalents, using the inhibition percentage value obtained from the EDTA calibration curve.

2.5. ABTS assay

For measuring the antioxidant activity of the different teas, the ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt] assay was performed according to the method described by Pellegrini, Ke, Yang, Rice-Evans, and Lester (1999). The radical cation ($ABTS^{+\cdot}$) was prepared by mixing an aqueous ABTS solution (final conc. 7 mM) with an aqueous solution of potassium persulfate (final conc. 2.45 mM), used as oxidising agent, and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Prior to use, the $ABTS^{+\cdot}$ stock solution was diluted 80× with water in order to obtain an absorbance at 734 nm ranging from 0.6–0.8. To 2.5 ml of this diluted $ABTS^{+\cdot}$ solution, 0.025 ml of tea (previously diluted 20×) was added. As control, 0.025 ml water was used instead of tea. The mixtures were left for 30 min in the dark at room temperature, after which absorbance was read at 734 nm against water.

For determining antioxidant activity, a 0.5 mg/ml methanol trolox solution, the water-soluble vitamin E analogue, was used as standard to construct a calibration curve in the range 0–18 μM (dilution with water). Antioxidant activity was expressed as mM TEAC equivalents (trolox equivalent antioxidant capacity), using the inhibition percentage value obtained from the trolox calibration curve, as described above for the metal-chelating assay.

2.6. DMPD assay

Antioxidant activity of the different teas was also evaluated using the DMPD assay as an alternative means, according to the method described by Fogliano, Verde, Randazzo, and Ritieni (1999). The coloured radical cation ($DMPD^{+\cdot}$) was obtained by adding 0.1 ml of a 75 mM ferric chloride solution in water (final concentration 0.15 mM) to 0.5 ml of a 100 mM DMPD solution in water (final concentration 1 mM). The resulting solution was then diluted to a final volume of 50 ml with 0.1 M acetate buffer, pH 5.25, and left to stand in the dark at room temperature for 2 min prior to use. To 2.5 ml of $DMPD^{+\cdot}$ solution, 0.125 ml of tea (previously diluted 20×) was added. As control, 0.125 ml water was used instead of tea. The samples were left to stand in the dark at room temperature for 10 min, after which absorbance at 505 nm was measured against acetate buffer.

For determining antioxidant activity, a calibration curve in the range 0–40 μM (dilution with water), using a 0.5 mg/ml methanol trolox solution, was plotted and antioxidant activity, expressed as mM TEAC, as described above for the ABTS assay.

2.7. LDL isolation and oxidation

LDL particles were isolated from pooled fresh human blood obtained from volunteers after overnight fasting. Blood was collected in heparin-containing vacutainers from which plasma was obtained by centrifugation at 3000 rpm for 15 min. LDL (density between 1.025 and 1.063 g/ml) was isolated by single vertical spin gradient ultracentrifugation on a Sorvall RC-150GX ultracentrifuge equipped with a Sorvall RP120VT fixed-angle rotor, following the procedure described by Chung et al. (1986). After dialysis at 4 °C for 24 h against 5 mM PBS pH 7.4, protein concentration was determined by the method of Bradford (1976).

To determine the effects of tea samples on the inhibition of LDL oxidation, conjugated dienes (a measure of lipid oxidation) and tryptophan fluorescence (a measure of protein oxidation) were monitored in LDL samples incubated in the presence and absence of tea samples in a 96-well microplate reader. The volume of each well was adjusted with deionized water to reach the final volume of 0.2 ml. Briefly, to 0.02 ml of LDL (100 μg protein/ml), 0.01 ml of tea (previously diluted 125×) was added. The oxidation reaction was initiated by adding 0.01 ml of 0.1 mM Cu^{2+} (final concentration 5 μM) to each well and conjugated dienes were monitored at 234 nm for 4 h at 37 °C. The lag phase was then determined graphically. Formation of conjugated dienes could not be assessed in samples when AAPH was used because its strong absorbance interferes with the assay. For monitoring tryptophan fluorescence, the method of Ivanov, Carr, and Frei (2001) was used. Briefly, to 0.014 ml of LDL (125 μg protein/ml), 0.02 ml of tea (previously diluted 125×) was added. The oxidation reaction was initiated by adding 0.01 ml of 0.1 mM Cu^{2+} (final concentration 5 μM) or 0.04 ml AAPH (final concentration 10 mM) to each well and tryptophan fluorescence (280 nm excitation/331 nm emission) was monitored for 1 h at 37 °C. The residual tryptophan fluorescence was expressed as a percentage of the unexposed control's initial fluorescence.

Appropriate controls were carried out throughout all the experiments described above. The data reported represent average values from at least three independent experiments, each performed in duplicate. Differences were regarded as statistically significant using the Student's *t*-test, where *p* values are <0.05 (*) and <0.01 (**).

3. Results

3.1. Levels of total tea polyphenols

The total phenol content levels in hot or cold teas using Folin–Ciocalteu's reagent, are summarised in Fig. 1. In general, TPC is always higher in hot teas than in cold teas, although this is significant only for green tea. The exception is with white tea, where TPC is significantly higher in the cold infusion than in the hot one. In addition, TPC, in white tea prepared with cold water steeping, is significantly higher than in all other teas prepared in the same way. Among the hot teas, the highest TPC was observed in the blended Lyons black tea while the lowest was observed in the unblended black tea.

3.2. Metal-chelating activity

The chelating activity of the tea infusions measures how effective the compounds in them can compete with ferrozine for ferrous ion. The iron–ferrozine complex has maximum absorbance at 562 nm and a decrease in this absorbance is an indication of chelating activity: the greater the decrease, the stronger the chelating power. Fig. 2 shows that chelating activity is, in general, always higher in hot teas than in cold teas, although this is significant only in the case of oolong tea and white tea. White tea appears to have the highest chelating power, compared to the other teas considered in this study, regardless of whether they are prepared with hot or cold water. Chelating power then decreases in the order: Lyons black > oolong > green > black Lapsang Souchong.

3.3. Antioxidant activity

The antioxidant activity of the tea infusions was assessed using two independent assays, ABTS and DMPD, which both work on the same principal. As the odd electron of the coloured radical cation becomes paired off in the presence of a hydrogen donor, its absorbance decreases. Therefore, the extent of decolorization is directly related to the antioxidant capacity of the solution being investigated. This reaction has been widely used to assess the ability of compounds to act as hydrogen donors and to evaluate their antioxidant activity (Lee, Lee, Kim, Shetty, & Kim, 2008; Morales & Babbel, 2002; Oliveira, Ferreira, de Pinho, & Silva, 2008; Re et al., 1999). Fig. 3 shows the results obtained using the ABTS assay. Antioxidant activity was generally higher in hot teas than in cold teas, although this is significant only in the case of oolong tea. The

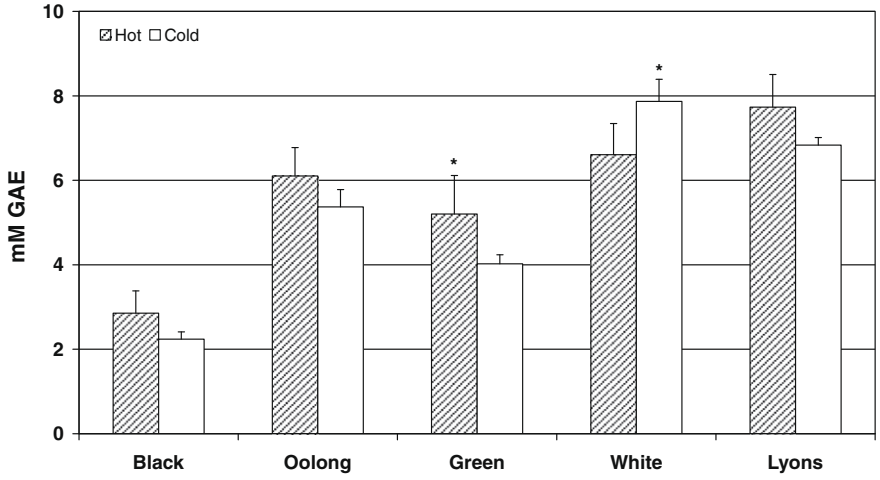

Fig. 1. Total phenol content (expressed as mM gallic acid) of the tested teas prepared either in hot water or cold water, as described in the Section 2, determined using the Folin–Ciocalteu reagent. * = hot vs. cold tea significant ($p < 0.05$).

exception is once again white tea, which surprisingly showed a higher significant antioxidant activity when leaves were steeped in cold water than in hot water and the highest antioxidant activity overall. This trend is similar to the one observed for the TPC assay (compare Fig. 1 with Fig. 3) and, in fact, a good correlation was found between the ABTS assay and TPC assay ($r^2 = 0.8487$). Comparable results in antioxidant activity were also obtained using the DMPD assay, such that the correlation found between the ABTS and DMPD values was $r^2 = 0.9807$. With the exception of white tea, one can observe that the results of Figs. 2 and 3 are similar and, in fact, a strong correlation was found between the ABTS and ferrozine values, $r^2 = 0.9054$ (excluding cold white tea) whereas the correlation is lower when cold white tea is included ($r^2 = 0.726$). Therefore from the above results, one can deduce that antioxidant activity in the various teas tested, regardless of whether they are prepared using hot or cold water, is correlated with their total phenolic content and metal-chelating activity.

3.4. Inhibition of LDL oxidation

The effects of the different tea infusions in suppressing the oxidation of LDL, i.e. a test of antioxidant functionality, were also evaluated. As an example, Fig. 4 shows typical kinetic curves of conjugated diene formation at 234 nm in LDL obtained in the absence and presence of white tea. In the presence of antioxidants, LDL oxidation is delayed, as characterised by the increase in lag time (Zieden, Wuttge, Karlberg, & Olsson, 1995), whereas the rate of oxidation, as well as the maximum production of conjugated dienes, remains constant. The lag times obtained from monitoring conjugated diene formation in the presence or absence of the tested teas are shown in Fig. 5. From this figure, one can observe that, in general, cold teas significantly suppress copper-induced LDL oxidation. White tea more than doubled the lag time, similarly to oolong, whereas the remaining teas all prolonged the lag time by 15–20 min (52–69% increase). Differences between hot and cold teas in suppressing this oxidation were significant only in the case of white tea and Lyons black tea. Furthermore, the only hot teas which significantly prolonged the lag time compared to the control (no tea) were white tea and green tea. With regard to loss of tryptophan fluorescence, due to protein oxidation, one can observe (in Fig. 6A and B) that there is a remarkable decrease in tryptophan fluorescence in oxidised LDL in the absence of tea compared to the control, regardless of whether LDL was oxidised with copper or AAPH. All teas significantly suppressed this loss in fluorescence with respect to the negative control (oxidised LDL). Significant differences between hot and cold teas were not observed, except in

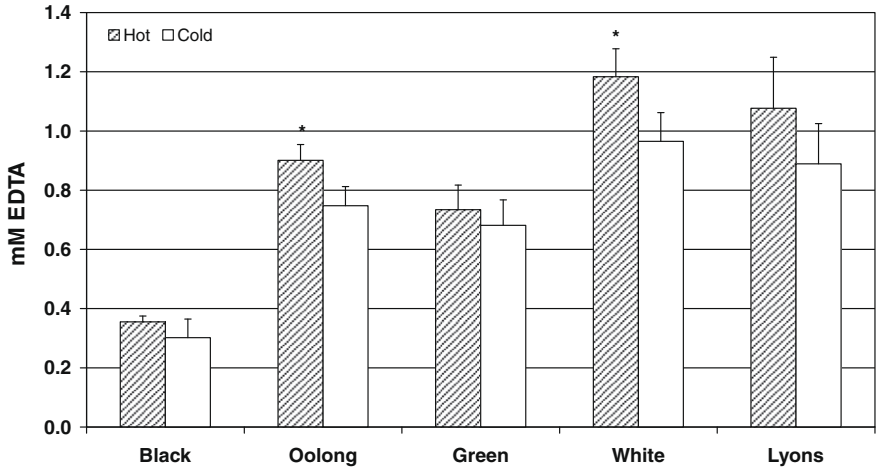

Fig. 2. Metal-chelating activity (expressed as mM EDTA) of the tested teas prepared either in hot water or cold water, determined using the ferrozine assay (see Section 2 for details). * = hot vs. cold tea significant ($p < 0.05$).

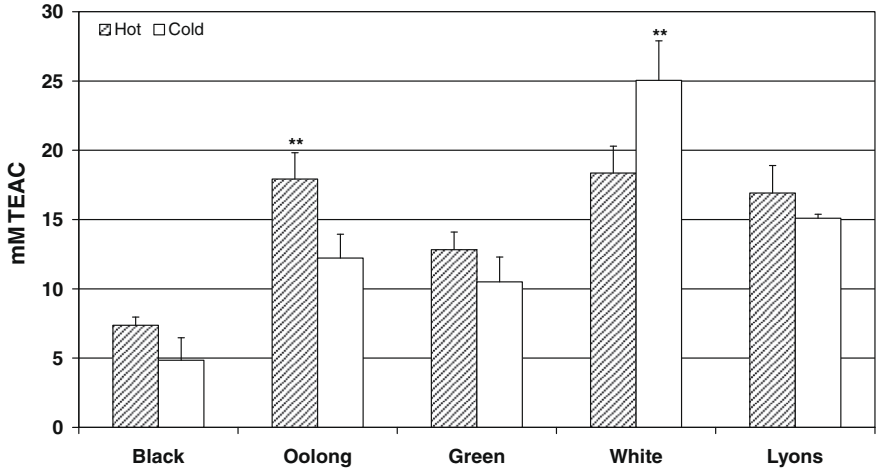

Fig. 3. Antioxidant activity (expressed as mM trolox equivalent antioxidant capacity) of the tested teas prepared either in hot water or cold water, determined using the ABTS assay (see Section 2 for details). ** = hot vs. cold tea significant ($p < 0.01$).

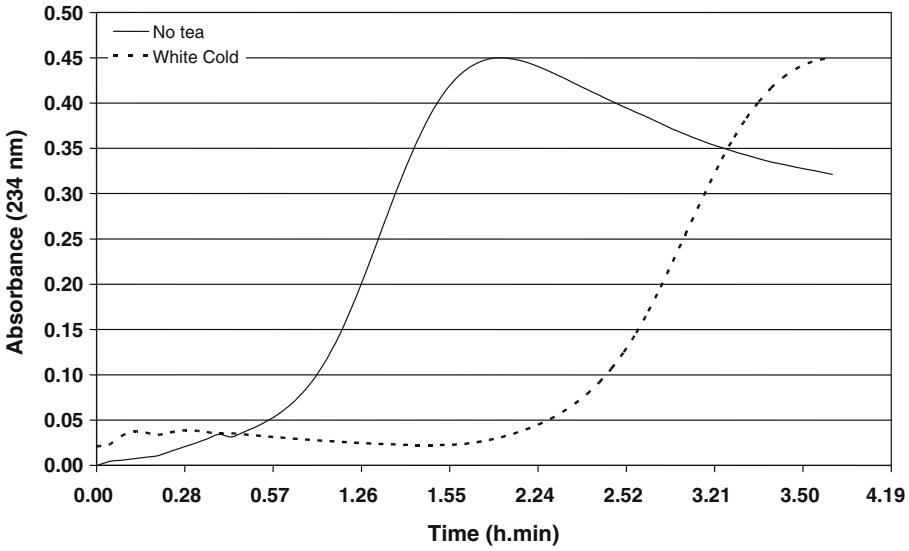

Fig. 4. Kinetic curves of conjugated dienes formation in LDL incubated *in vitro* in the absence and presence of white tea prepared in cold water. LDL (100 μg protein/ml) was incubated with the tea infusion (diluted 125×) and oxidation was initiated by addition of 5 μM Cu^{2+}. Formation of conjugated dienes was determined spectrophotometrically using a microplate reader by recording changes at 234 nm for 4 h at 37 °C.

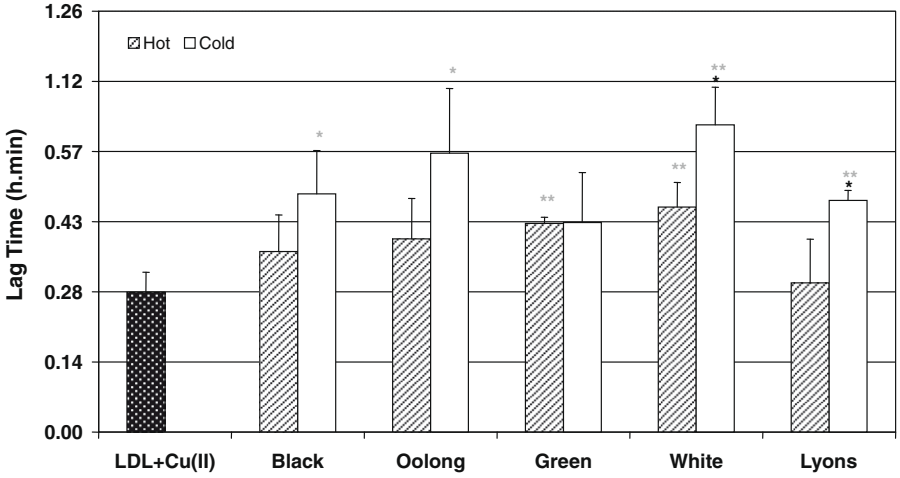

Fig. 5. Antioxidative effects of the tested teas prepared either in hot water or cold water shown as the lag time for formation of conjugated dienes in LDL incubated *in vitro*. LDL (100 μg protein/ml) was incubated with the tea infusion (diluted 125×) and oxidation was initiated by addition of 5 μM Cu^{2+}. Formation of conjugated dienes was determined spectrophotometrically using a microplate reader by recording changes at 234 nm for 4 h at 37 °C. The lag time was calculated from the sigmoidal kinetic curves of LDL oxidation, similar to those shown in Fig. 4. * = hot vs. cold tea significant ($p < 0.05$); ** = significant vs. oxidised LDL ($p < 0.01$), * = significant vs. oxidised LDL ($p < 0.05$).

the case of LDL oxidised with Cu^{2+} (Fig. 6A) where the white tea cold infusion suppressed the loss in tryptophan fluorescence to a greater extent than did the hot one.

4. Discussion

To date, there appear to be no thorough studies on how antioxidant activity of teas may be affected by hot or cold water steeping and how this may be related to their polyphenol content and metal-chelating activity. Since antioxidant activity is one of the principal health attributes of tea, among others, it was of interest to address this issue. In this regard, Lin, Tsai, Tsay, and Lin (2003) recently investigated the effects of different steeping temperatures (4, 25, 70, 85 and 100 °C), durations and numbers of steepings, on caffeine, catechins and gallic acid contents in five types of bag teas (Yang et al., 2007). They observed that catechins, caffeine and gallic acid were released from bag teas as hotter water was used and that these increased with increasing duration. They also report that epigallocatechin (EGC) was the most abundant catechin in cold water infusions, whereas epigallocatechin-gallate (EGCG) was when steeping in hot water. In the present study, the results on the antioxidant activity of the different teas steeped either in cold water or hot water certainly reflect their TPC and the major contributors are the catechins which affect colour, flavour and taste of manufactured tea (Higdon & Frei, 2003). These polyphenols appear to be responsible for the many health benefits attributed to tea, which range from antimutagenic (Hernaez, Xu, & Dashwood, 1997; Santana-Rios et al., 2001), antidiabetic (Venables, Hulston, Cox, & Jeukendrup, 2008), anti-inflammatory (Takano, Nakaima, Nitta, Shibata, & Nakagawa, 2004), antioxidant (Serafini, Ghiselli, & Ferro-Luzzi, 1996), antimicrobial (Gradisar, Pristovsek, Plaper, & Jerala, 2007) and cancer-preventive activities (Chung, Schwartz, Herzog, & Yang, 2003; Siddiqui, Adhami, Saleem, & Mukhtar, 2006; Siddiqui, Saleem, Adhami, Asim, & Mukhtar, 2007) to prevention of cardiovascular

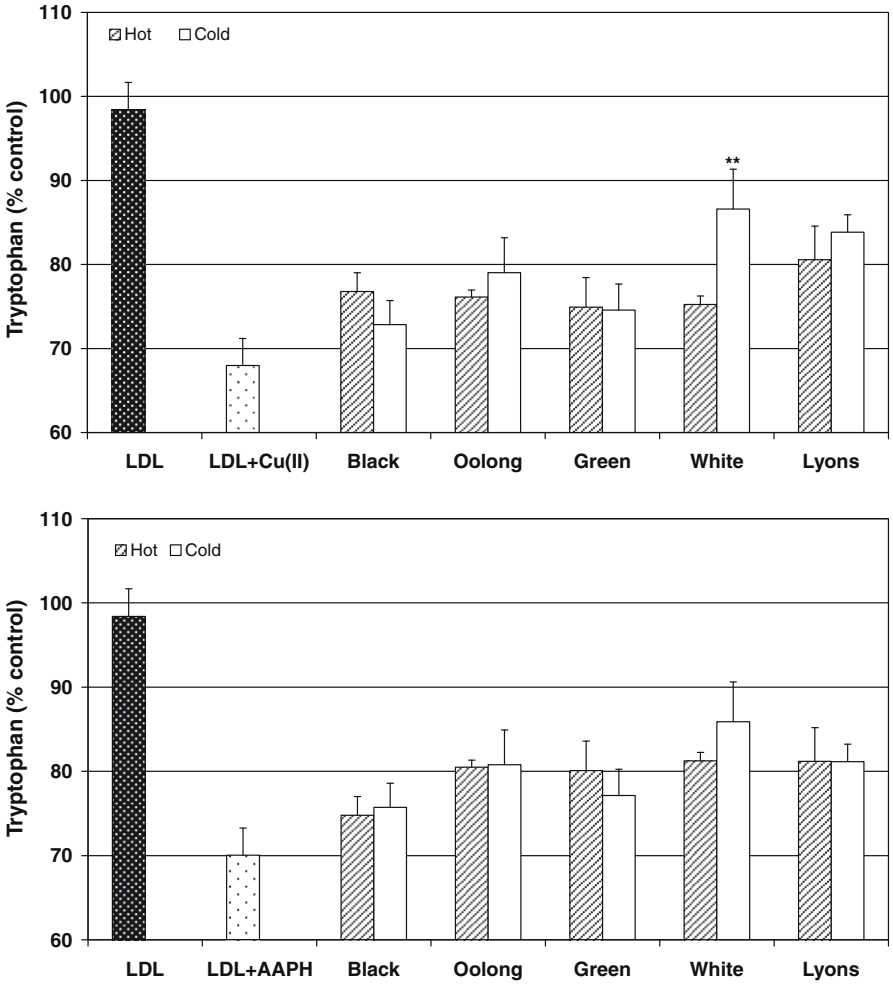

Fig. 6. Antioxidative effects of the tested teas, prepared either in hot water or cold water, shown as percentage of residual tryptophan fluorescence in LDL incubated *in vitro* in relation to the initial fluorescence of the control. LDL (125 μg protein/ml) was incubated with the tea infusion (diluted 125×) and oxidation was initiated by addition of 5 μM Cu^{2+} (top panel) or 10 mM AAPH (bottom panel). Tryptophan fluorescence was measured after incubation for 1 h at 37 °C on a microplate reader. * = hot vs. cold tea significant ($p < 0.05$).

diseases (Stangl, Dreger, Stangl, & Lorenz, 2007) through various mechanisms: radical-scavenging (Wiseman et al., 1997), modification of signal transduction pathways, cell cycle checkpoints, apoptosis, (Ahmad, Feyes, Nieminen, Agarwal, & Mukhtar, 1997; Li et al., 2000; Yang, Liao, Kim, Yurkow, & Yang, 1998) and enzymatic induction (Lee, Liang, & Lin, 1995). The in-cup chemical compounds extracted from the leaf during tea preparation will therefore ultimately affect the above-mentioned health effects of this beverage. In the present study, in general, steeping teas in cold water or hot water does not significantly affect the TPC content. However, for white tea, surprisingly, TPC is higher when it is steeped in cold water instead of hot water. This finding suggests that certain phenolic components, unique to white tea, but not to others, might be broken down or transformed at high temperatures, contributing to the lower content observed. It is also generally reported that TPC is higher in green and white teas than in black teas (Lin et al., 2003; Roginsky, Barsukova, Hsu, & Kilmartin, 2003). However, this statement cannot be generalised to all teas, since in this study, Lyons tea, which is a blend of black African and Indian teas, had TPC levels comparable to white tea and significantly higher than the green tea.

For the antioxidant activity, measured as hydrogen-donating ability, using the ABTS or DMPD assays, the results here reported suggest that, in general, there are no significant differences in this activity among hot or cold teas, except in the case of white tea, where significantly higher values are obtained after cold water steeping, possibly due to the reason suggested above.

When measuring antioxidant activity in terms of inhibition of *in vitro* LDL conjugated diene formation, overall, teas prepared using cold water are generally more protective than are teas prepared using hot water. This interesting finding may have some important implications if it were to hold true for LDL oxidation *in vivo*, since oxidation of LDL in the vessel wall is thought to be one of the steps involved in atherogenesis (Steinberg, 2009; Steinbrecher, Zhang, & Lougheed, 1990) and high intakes of flavonoids from tea and vegetables appear to be associated with a reduced risk of coronary artery disease (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993). Furthermore, the tea infusions appear to be more protective against protein LDL oxidation than lipid LDL oxidation. An explanation for this might be that the components present in tea infusions are predominantly hydrophilic; hence it is expected that there would be a greater affinity of tea for the hydrophilic compartment of LDL which is the apolipoprotein B 100 protein rather than for the hydrophobic lipid core. Indeed, tea flavonoids may bind to apoB due to their strong protein-binding properties (Haslam, 1974). The presence of tea components in the vicinity of an LDL particle would likely protect the protein portion, probably more efficiently than the lipid portion.

LDL oxidation in the arterial wall is also mediated by the presence of free metal ions and there is evidence that flavonoids are

strong chelators of free metal ions (Morel, Lescoat, Cillard, & Cillard, 1994). For this reason, it was of interest also to determine whether metal-chelating activity of the different teas could be affected by steeping in hot or cold water. As previously mentioned, in general no significant differences are observed among the teas when prepared with hot or cold water and the pattern of results well correlates with those of antioxidant activity and TPC.

However, differences among the tea samples, in terms of antioxidant activity, TPC and metal-chelating activity, were observed, with white tea always scoring as the best and Lapsang Souchong always as the worst. Nevertheless, it is very important to bear in mind that it is not correct to compare these activities among the teas because the cultivar type, growing environment (country, altitude, soil, climate), plucking practices (age of leaf) and manufacturing conditions vary considerably and hence will all ultimately affect the tea leaf and final infusion composition (Astill, Birch, Dacombe, Humphrey, & Martin, 2001). There are many data in the literature that compare the antioxidant activities of different teas and that show green teas to be better than oolong teas, which are better than black teas (Richelle, Tavazzi, & Offord, 2001; Roginsky et al., 2003; von Gadow, Joubert, & Hansmann, 1997). But there are also several reports which show that some black teas are better than green teas (Hoff & Singleton, 1977; Khokhar & Magnusdottir, 2002). In fact, a recent study showed that green, black and white tea products, processed from Kenyan tea cultivars, had significantly higher antioxidant activity than green tea processed from tea cultivars from Japan and China (Karori, Wanyoko, & Ngure, 2007). These controversies could be no better observed than in this study where Lyons black tea antioxidant activity was in most cases comparable to that of white tea and always better than that of green tea, while the other black tea studied, Lapsang Souchong, was always the least potent. Lapsang Souchong's low antioxidant activity is in accordance with another study which showed that, among 33 teas tested, it had the lowest reducing strength, a measure of hydrogen-donating ability (Roginsky et al., 2003). The high antioxidant activity observed for the white tea used in this study may be attributed to the high levels of ECG present in large amounts in the young, fresh leaves of this speciality tea (Stewart, Mullen, & Crozier, 2004). Since antioxidant activity for white tea was, in the majority of cases, always higher when it was steeped in cold water, it is plausible to hypothesise that this catechin might be more susceptible to degradation at high temperatures.

In conclusion, the purpose of this study was not to compare the antioxidant activities of the various teas for the reasons mentioned above, but to collect information on how this activity might be influenced by different drinking habits in specific countries and cultures, in this case, the only variable being tea steeped in hot water or cold water. The results obtained contribute to gaining further knowledge on how the potential health benefits of this popular beverage may be maximised by the different methods of preparation.

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