

Total Antioxidant Capacity of Teas by the Ferric Reducing/Antioxidant Power Assay

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This study aimed to compare in vitro antioxidant power of different types of tea (*Camellia sinensis*). The ferric reducing/antioxidant power (FRAP) assay was used to measure the total antioxidant power of freshly prepared infusions of 25 types of teas. Results showed that different teas had widely different in vitro antioxidant power and that the antioxidant capacity was strongly correlated ($r = 0.956$) with the total phenolics content of the tea. Expressed as μmol of antioxidant power/g of dried tea leaves, values ranged as 132–654 $\mu\text{mol/g}$ for black (“fermented”) teas, 233–532 $\mu\text{mol/g}$ for Oolong (“semifermented”) teas, and 272–1144 $\mu\text{mol/g}$ for green (“nonfermented”) teas. One cup of tea of usual strength (1–2%), therefore, can provide the same potential for improving antioxidant status as around 150 mg of pure ascorbic acid (vitamin C).

Keywords: Antioxidant; FRAP; polyphenolics; tea

INTRODUCTION

Tea (*Camellia sinensis*) is the most widely consumed beverage worldwide and has become an important agricultural product (Balentine, 1992). The type and quantity of tea taken varies in different countries and races (Weisburger, 1996; Kohlmeier, 1997). Black (“fermented”) tea is popular in the West; “semifermented” Oolong-type tea is commonly drunk in Taiwan and parts of China; green (“nonfermented”) tea is favored in the rest of China, Northern Africa, and Japan (Weisburger, 1996). Tea contains large amounts of polyphenolic compounds with antioxidant properties, and these may prevent oxidative damage of DNA (Zhao et al., 1989; Scott et al., 1993; Cook and Samman, 1996; Rice-Evans et al., 1996; Wiseman et al., 1997; Zhang and Shen, 1997) and inhibit the peroxidation of low-density lipoprotein (Miura et al., 1994; Ishikawa et al., 1997; Luo et al., 1997). Chemical changes such as oxidative damage and peroxidation are related to mutagenesis, to increased risk of cancer (Emerit, 1994; Halliwell, 1996), and to atherogenesis and cardiovascular disease (Maxwell and Lip, 1997). Regular intake of tea may, therefore, improve antioxidant status in vivo and, thereby, help lower risk of certain types of cancer and coronary heart disease (Stensvold et al., 1992; Weisburger, 1996).

Tea antioxidants can protect against strong mutagens in animal models (Yamane et al., 1991; Hasegawa et al., 1995; Leanderson et al., 1997). Moreover, lower incidence of cancer in association with high consumption of tea has been reported in some epidemiological studies (La Vecchia et al., 1992; Baron et al., 1994; Gao et al., 1994; Yu et al., 1995). However, results are not consistent and a protective role for antioxidants in tea has not been clearly demonstrated (Goldbohm et al., 1996; Kohlmeier et al., 1997). This may be due to the form in which tea is taken (Kohlmeier, 1997). Different teas have different antioxidant compositions, and some tea

antioxidants may be more active and/or more easily absorbed than others (Graham, 1992; Weisburger, 1996; Cook and Samman, 1996; Rice-Evans et al., 1996; Van Acker et al., 1996; Hollman, 1997; Kohlmeier, 1997). The aim of this study was to measure and compare in vitro antioxidant activities of different types of tea to assess their relative potential for improving in vivo antioxidant status. The relationship of total phenolics content to antioxidant capacity in tea infusions was also studied, and the stability of antioxidant potential in infusions of tea and the effect of dilution on antioxidant potential were investigated.

MATERIALS AND METHODS

Total antioxidant power of a freshly prepared, cooled, filtered infusion (5 g of dry tea leaves/100 mL of boiling, distilled water) of each of 25 different teas was measured using the ferric reducing/antioxidant power (FRAP) assay (U.S. patent pending) (Benzie and Strain, 1996; Benzie and Strain, 1999). Teas were all purchased locally and measured in quadruplicate, after additional dilution in distilled water as appropriate. The FRAP assay was performed as previously described (Benzie and Strain, 1996) using a Cobas Fara centrifugal analyzer (Roche Diagnostics Ltd., Basel, Switzerland). In the FRAP assay, reductants (“antioxidants”) in the sample reduce Fe^{3+} /tripyrityldiazine complex, present in stoichiometric excess, to the blue colored ferrous form, with an increase in absorbance at 593 nm. The ΔA is proportional to the combined (total) ferric reducing/antioxidant power (FRAP value) of the antioxidants in the sample. The FRAP assay has a limit of detection of $<2 \mu\text{mol/L}$ reducing/antioxidant power and precision is excellent; within- and between-run CVs are $<0.5\%$ and 1.0% , respectively, between 500 and 2000 $\mu\text{mol/L}$, $n > 8$ in each case.

In vitro antioxidant power results are expressed as μmol of ferric reducing/antioxidant power (the FRAP value)/g of dry tea leaves, and as μmol of FRAP estimated to be contained in a typical serving (150 mL) of tea in the strength commonly drunk (1.5% w/v). The antioxidant power of freshly prepared, aqueous solutions of ascorbic acid (*D*-ascorbic acid, extra pure crystals; Merck, Darmstadt, Germany) was also measured, and the calculated antioxidant power of 1 g of pure ascorbic acid is given as reference.

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Table 1. In Vitro Antioxidant Power of Different Teas Measured Using the FRAP^a Assay

type of tea	name	FRAP value ($\mu\text{mol/g}$ of dry tea leaves; mean (SEM)) ^b	antioxidant power, as μmol of FRAP, estimated to be contained in 1 cup of tea ^c
	<i>pure ascorbic acid (vitamin C)</i>	11 364	
		$\mu\text{mol/g}$	
black	China Black Tea	379 (39)	853
	Rose Black Tea	378 (16)	851
	Yunnan Tuocha	358 (33)	806
	Marks and Spencers Extra Strong Breakfast Tea	654 (47)	1472
	Pu Li Tea (brand 1)	309 (33)	695
	Pu Li Tea (brand 2)	225 (10)	506
	Pu Li Tea (brand 3)	238 (23)	536
	Pu Li Tea (brand 4)	380 (15)	855
	Premium Yun-Nan Puerh Tea	132 (9)	297
oolong	Oolong Tea (brand 1)	532 (23)	1197
	Oolong Tea (brand 2)	233 (18)	524
	Iron Buddha Tea (brand 1)	472 (26)	1062
	Iron Buddha Tea (brand 2)	340 (25)	765
	Iron Buddha Tea (brand 3)	289 (29)	650
green	China Green Tea (brand 1)	1144 (6)	2574
	China Green Tea (brand 2)	699 (77)	1573
	Jasmine Tea (brand 1)	629 (55)	1415
	Jasmine Tea (brand 2)	526 (29)	1184
	Jasmine Tea (brand 3)	602 (40)	1355
	Jasmine Tea (brand 4)	698 (55)	1571
	Lok On Ngan Jum (brand 1)	727 (48)	1636
	Lok On Ngan Jum (brand 2)	525 (15)	1181
	Shou Mei Tea	386 (20)	869
	Shui Sin Tea (brand 1)	522 (45)	1175
	Shui Sin Tea (brand 2)	272 (5)	612
	Yunnan Green Tea	996 (68)	2241
	Japanese Green Tea	700 (54)	1575

^a U.S. patent pending. ^b Mean (SEM) of two or three different infusions, prepared on separate days, each infusion measured in quadruplicate. ^c 150 mL of a 1.5% infusion of tea.

For the stability study of antioxidant in tea infusion, tea infusions were kept at 4 °C for 48 h. The infusions were then re-assayed in duplicate and the results compared to those obtained on fresh samples.

For the relationship between antioxidant power and levels of tea polyphenols, FRAP values and the total phenolics content of infusions of 26 different teas were compared in a separate experiment. Total phenolics content was measured by the Folin–Ciocalteu method (Theis and Benedict, 1924; Das, 1971; Singleton et al., 1999).

RESULTS

Different types of tea had widely different antioxidant activities, ranging from 132 $\mu\text{mol/g}$ for one type of black tea ("Premium Yun-Nan Puerh Tea") to 1144 $\mu\text{mol/g}$ for one of the green teas ("China Green Tea") tested (Table 1). From these results it is estimated that a typical cup of green tea contains an amount of antioxidant power similar to that found in 100–200 mg of pure ascorbic acid (vitamin C), highlighting the enormous potential of tea as a dietary source of antioxidant power.

There was no significant change observed in the FRAP values after 48 h storage of tea infusions at 4 °C (paired *t*-test), indicating that antioxidants in tea are stable. There was a linear relationship between the strength of the tea infusion and the FRAP value (Figure 1) over a wide range of values, with close agreement

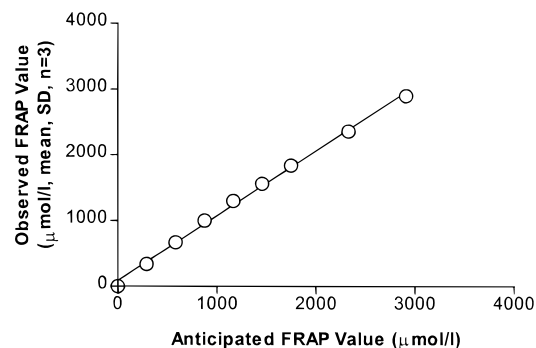


Figure 1. FRAP values of infusion of green tea at different dilutions. Result shows good agreement ($r = 0.998$, $p < 0.0001$) between the anticipated and the observed (measured) FRAP value, indicating no change in antioxidant at different concentrations. Each point represents the mean of triplicate measurement, and while 1 SD error bars are included, reproducibility was high and error bars do not show.

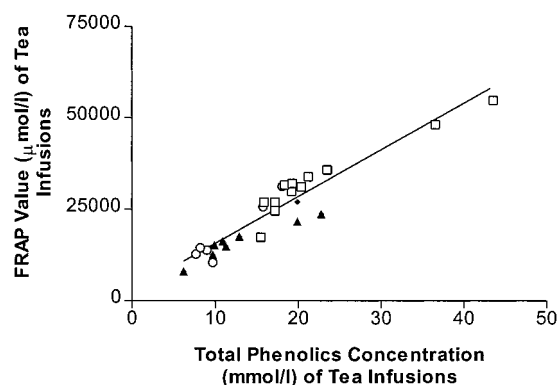


Figure 2. FRAP values and total phenolics content of 26 different tea infusions of 5% (w/v) strength. Green tea infusions are represented by squares, oolong tea by circles, and black tea by triangles. Results showed strong correlation ($r = 0.956$, $p < 0.0001$) between the two parameters. Each point represents the mean of two individual experiments, each experiment with duplicate measurement, i.e., mean of 4 results.

between expected and observed FRAP values ($r = 0.998$, $p < 0.0001$).

There was strong correlation between FRAP value and total phenolics content of the tea infusions tested ($r = 0.956$, $p < 0.0001$), indicating that the number of phenolic hydroxyl groups is a major determinant of antioxidant power of tea (Figure 2).

DISCUSSION

Tea, particularly green tea, is a potentially rich dietary source of antioxidant power. Various studies have demonstrated in vitro radical trapping antioxidant properties of black tea and green tea, of extracts of tea, and of individual polyphenolic compounds found in tea (Zhao et al., 1989; Scott et al., 1993; Lin et al., 1996; Paganga et al., 1996; Rice-Evans et al., 1996; Zhang and Shen, 1997). This current study presents new data comparing the in vitro antioxidant/reducing power of different types of teas, as they would be drunk, using an objective and highly reproducible measure, the FRAP assay for antioxidant/reducing power. It is worth noting that, while tea polyphenolics are known to bind metal ions (Miller et al., 1996; Paganga et al., 1996; Van Acker et al., 1996), this property does not interfere with the measurement of antioxidant power of tea using the FRAP assay. This was demonstrated by a linear re-

sponse when the antioxidant power of different teas was tested over a wide range of concentrations.

Binding of metal ions, such as iron, in vivo is an antioxidant action of itself, preventing metal ion catalyzed generation of reactive species (Emerit, 1994; Halliwell, 1996). However, tea polyphenolics also have electron-donating antioxidant properties, the relative activity of the different polyphenolic compounds being related to the number and location of the hydroxyl groups and the presence of the galloyl moiety (Lin et al., 1996; Miller et al., 1996; Paganga et al., 1996; Rice-Evans et al., 1996). During the process referred to as fermentation, flavanols in green tea leaves, mainly catechins and their gallic esters, undergo a polyphenol oxidase catalyzed oxidative polymerization which turns the leaves black (Graham, 1992; Weisburger, 1996; Nanjo et al., 1996). During several hours, much of the catechin content of green tea is converted to thearubingens and theaflavins, which give black tea its characteristic astringency. Oolong tea is intermediate in composition between green and black tea. Green tea contains 30–42% catechins of total dry mass, while black tea contains 3–10% and oolong 8–20% (Graham, 1992). Almost 80% of the tea consumed throughout the world each year is black; less than 2% is Oolong, and 20% green tea. (Balentine, 1992; Kong, 1993).

This study has confirmed that the antioxidant power of green tea is considerably higher than black tea, while Oolong tea, expected to be intermediate, appeared similar to black tea. Furthermore, the antioxidant power of all teas correlated strongly with the total phenolics content. Previous studies have shown that tea antioxidants with a greater number of phenolic hydroxyl groups have greater antioxidant power, i.e., epigallocatechin gallate (8 groups) > epicatechin gallate (7 groups) > GC (6 groups) > epicatechin (5 groups) > epigallocatechin (6 groups) (Wiseman et al., 1997). Both FRAP values and phenolics content of different types of teas overlapped, however, with a 2–3-fold difference across different brands of teas of the same type, probably reflecting differences in quality, geographical regions of growth, the time of year when the leaves were picked, and varying storage conditions (Weisburger, 1996; Lin et al., 1996). Nevertheless, the potential for all types of tea to contribute significantly to the dietary intake of antioxidant power is high, as one cup of black tea (1–2%) contains around 800 μmol of antioxidant power and several cups of green tea offer the same antioxidant potential as almost 1 g of pure vitamin C. Vitamin C is an antioxidant of established importance, while the bioavailability and role of tea antioxidants are not yet clear. Nonetheless, tea may be an important dietary source of antioxidant power and tea can be ingested frequently and in relatively large volumes without any known harmful effects. Questions then arise regarding the feasibility of realizing this potential in terms of the effect on antioxidant status and the possible long-term benefits to health associated with increased intake of tea antioxidants.

In summary, this study has shown that green teas have the highest antioxidant power and black teas have the lowest values. Oolong teas, expected to be intermediate between green and black teas, demonstrate activities similar to those of black teas. Also antioxidants in tea are stable for at least 48 h stored at 4 °C. The total antioxidant power correlated strongly with the total phenolics content of tea of all types. Further study into

the absorption and effect of tea antioxidants on antioxidant status is needed to evaluate their potential benefit in the maintenance of human health.

ABBREVIATIONS USED

FRAP, ferric reducing/antioxidant power.

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