Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 88 (2004) 447–451
Food Chemistry
www.elsevier.com/locate/foodchem

The effect of ascorbic acid on total antioxidant activity of black and green teas

Dorota Majchrzak *, Sabine Mitter, Ibrahim Elmadfa

Institute of Nutritional Sciences, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Received 18 July 2003; received in revised form 12 January 2004; accepted 12 January 2004

Abstract

The beneficial effects of green and black tea are generally attributed to the antioxidant activity of their phenolic compounds. Tea is commonly used with milk or lemon. Milk proteins might complex with tea polyphenols and reduce their antioxidant activity. Lemon contains vitamin C (ascorbic acid) which has antioxidative properties and can positively influence the antioxidant potential of tea.

The present study aimed to compare, in vitro, the antioxidant activities of different commercially available types of tea, prepared by commonly used domestic methods and to evaluate the possible effects of different doses (5–40 mg/100 ml) of vitamin C (ascorbic acid) on the total antioxidant capacity (TAC) of tea. The antioxidant activity of tea extracts was determined by the photometric method, according to Rice-Evans and Miller [Methods Enzymol. 234 (1994) 279], measuring the formation of the radical cation ABTS. The values of antioxidant activity of teas prepared in the same way as when consumed were in similar ranges, from 13.3 to 21.6 mmol TE (TE = Trolox equivalents) in green tea and 10.4–17.6 mmol TE in black tea. The experiment in which ascorbic acid was added to teas showed that TAC in black tea extracts increased in a linear manner between 5 and 20 mg ascorbic acid/100 ml tea solution ($r = 0.984$; $p < 0.01$) and in green tea extracts up to 30 mg ascorbic acid/100 ml tea solution ($r = 0.959$; $p < 0.01$).

Keywords: Green and black tea; Total antioxidant capacity; Ascorbic acid

1. Introduction

Tea is the most widely consumed beverage worldwide and is rich in polyphenolic compounds known as the tea flavonoids. Green tea, which is the favourite type consumed in Japan and China, has been increasingly used also in Western countries in recent years. Green tea is prepared from fresh tea leaves and contains mainly catechins. Black tea is popular in the West and India and it is rich in thearubigins and theaflavins, which are dimers of catechins, formed by enzymatic oxidation during the manufacture of black tea. Tea flavonoids (such as catechins, theaflavins and thearubigins) possess strong antioxidant properties, i.e. they may protect the body from damage caused by free radical-induced oxidative stress. Therefore, the consumption of tea has been associated with reduced risk of major diseases, including coronary heart disease, stroke and cancer (Benzie & Szeto, 1999; Langley-Evans, 2000; Leenen, Roodenburg, Tijburg, & Wiseman, 2000; Ramarathnam, Osawa, Ochi, & Kawakishi, 1995; Robinson, Maxwell, & Thorpe, 1997). The considerable antioxidant potential of tea has long been recognized and it is dependent on many factors involved in tea preparation. It has been shown that green tea extracts have higher antioxidant capacity than black tea solutions and the total antioxidant potential correlates strongly with the total phenolic contents of tea of all types (Benzie & Szeto, 1999; Henn & Stehle, 1998; Langley-Evans, 2000). The findings of the in vitro experiments suggest that maximum antioxidant capacity is associated with the drinking of green tea prepared at high temperatures (90 °C) and with long infusion times. Black tea should ideally be prepared, between 70 and 90 °C, from leaves

* Corresponding author. Tel.: +43-1-4277-54901/54948; fax: +43-1-4277-9549.
E-mail address: dorota.majchrzak@univie.ac.at (D. Majchrzak).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.01.058

rather than tea bags, and infusion times should not exceed 1–2 min to produce maximum antioxidant potential (Langley-Evans, 2000). Moreover, due to the flavonoid-binding capacity of milk proteins, the addition of milk can decrease the antioxidant potential of black tea preparations (Langley-Evans, 2000; Robinson et al., 1997). An increase of plasma antioxidant capacity was observed in humans following tea consumption without milk (Serafini, Ghiselli, & Ferro-Luzzi, 1996). It has been suggested that milk/polyphenol complexes resist gastric breakdown, rendering the polyphenols unavailable for absorption (Serafini et al., 1996). However, this is not consistent with more recent findings (Leenen et al., 2000; Van het Hof, Kivits, Weststrate, & Tijburg, 1998), which have shown that adding milk to black tea did not influence the absorption of tea catechins and antioxidant capacity in human plasma. The addition of milk to tea is common practice in the United Kingdom. In other countries it is popular to take tea with lemon. Lemon contains vitamin C (about 50 mg ascorbic acid/100 g), which also has antioxidative properties. Ascorbic acid, in vitro, protects some flavonoids, such anthocyanins, against oxidative degradation during processing and storage of juices (Kaack & Austed, 1998). Around 150 mg of pure ascorbic acid can provide the same antioxidant potential as one cup of green tea of usual strength (1.5%) (Benzie & Szeto, 1999). Studies in human volunteers showed intake of ascorbic acid and green tea consumption to have inhibiting effects on endogenous formation of *N*-nitroso compounds (Vermeer, Moonen, Dallinga, Kleinjans, & van Maanen, 1999). This indicates the importance of investigating the antioxidant potential of teas with addition of vitamin C. The present study has been designed to compare the antioxidant activity of different commercially available types of tea in vitro, prepared following the instructions provided on the package, and to evaluate the possible effects of different doses (5–40 mg/100 ml tea solution) of vitamin C (ascorbic acid) on the total antioxidant capacity (TAC) of the teas.

2. Materials and methods

2.1. Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and ABTS (2,2′-azino-bis-[3-ethylbenzthiazoline-6-sulfonic acid]), required for the measurement of TAC, were purchased from Fluka (Buchs, Switzerland) and Sigma-Aldrich (Vienna, Austria), respectively. L-ascorbic acid was obtained from Sigma-Aldrich (Vienna, Austria). All other chemicals were supplied by Riedel-de Haen (Seelze, Germany). The ascorbic acid – enzyme test was from Boehringer Mannheim (Darmstadt, Germany).

2.2. Tea samples

Seven commercially available types of green and six black teas (leaf and bagged) were purchased from retail outlets. All tea extracts were prepared by pouring 200 ml deionised water at 90 °C into a glass with tea bags (1.75–2 g leaves) or 2 g dry tea leaves and brewed according to the instructions on the package for 2–3 min. The volume of 200 ml was selected to represent the typical quantity consumed by tea drinkers. An aliquot of each tea extract was removed for immediate measurement of antioxidant activity.

2.3. Determination of total antioxidant capacity

The antioxidant capacity of fresh tea extracts was determined, after cooling to 20 °C, by measuring the formation of the radical cation ABTS, according to the photometric method of Rice-Evans and Miller (1994). In the presence of H_2O_2 and metmyoglobin, ABTS is oxidized to the ABTS cation. In this reaction, metmyoglobin acts as a peroxidase. The radical cation formed is relatively long-lived and shows an absorption maximum at 734 nm. All black and green teas were diluted individually prior to assay. Total antioxidant capacity of samples was determined against a Trolox standard curve. The results were expressed in mmol Trolox equivalents (mmol TE) as mean ± SD of three consecutive measurements of the same sample. The intra-assay variation was 5.3%.

2.4. Experiment with vitamin C

2.4.1. Effect of different doses of ascorbic acid on the antioxidant activity

To investigate the effect of different doses of vitamin C (5, 10, 15, 20, 25, 30, 35, 40 mg ascorbic acid/100 ml tea extract) on antioxidant potential, two types of tea with similar antioxidant potential were studied, Inter Spar green tea and black Ceylon tea bags. Two extracts of each tea type were prepared by adding 200 ml water at 90 °C and infusing for 2–3 min. At the end of the infusion period, an aliquot of 100 ml was removed and mixed with one of the doses of ascorbic acid.

Measurements of the contents of ascorbic acid and antioxidant activity were carried out in duplicate, after cooling to 20 °C. There were 8 different levels of addition of vitamin C plus the control (without vitamin C), making 9, each in duplicate to give the 18 samples. Significance for linearity was done by the Pearson-test and was accepted as statistically significant at $p < 0.05$. Ascorbic acid was not detected (detection limit was 0.3 mg ascorbic acid/l) in any sample investigated prior to the addition of this vitamin.

3. Results

3.1. Antioxidant activity of commercially available black and green teas

The values of antioxidant activity of both black and green teas, prepared following the instructions provided on the package, were in similar ranges, 13.3–21.6 mmol TE in green tea and 10.4–17.6 mmol TE in black tea and showed only small SDs (see Table 1). The maximal antioxidant potential was measured in Java green tea and the lowest in Ceylon black tea leaf. Darjeeling black tea (Queen's Blend), Assam Bherjan green tea and Green Blend (Sencha + Green Darjeeling) had nearly the same second highest antioxidant capacity among the investigated samples. This indicates that there need not be significant differences between green and black types of tea. Comparing black leaf and black bagged teas, no significant difference was observed between the same type (Ceylon) of tea (leaf tea, 10.4; bagged tea, 12.2 mmol TE).

3.2. Experiment with vitamin C

3.2.1. Effect of different doses of ascorbic acid on the antioxidant activity of black tea

In the experiment in which ascorbic acid was added to black tea extracts (Black Ceylon Tea in bags), antioxidant activity increased in a linear manner for the samples containing between 5 and 20 mg ascorbic acid/100 ml tea solution ($r = 0.984$; $p < 0.01$). At a concentration of 20 mg ascorbic acid/100 ml, black tea extract had a 20% higher TAC (15.2 mmol TE) than the extract without vitamin C (12.6 mmol TE). A higher dose of between 25 and 40 mg ascorbic acid/100 ml tea solution did not improve the antioxidant capacity of tea. The TAC value decreased at 25 mg ascorbic acid/100 ml to 13.6 mM TE and remained nearly constant to the end of the experiment (Fig. 1).

Table 1
TAC (mean ± SD) of commercially available black and green teas (mmol TE)

	TAC
Bagged black teas	
Darjeeling (Queen's Blend)	17.6 ± 0.06
Assam special (Indian Blend)	14.0 ± 0.03
Inter Spar Blend	13.5 ± 0.04
Ceylon	12.2 ± 0.02
Black tea with lemon concentrate	11.7 ± 0.04
Leaf black teas	
Ceylon	10.4 ± 0.02
Bagged green teas	
Java	21.6 ± 0.02
Green blend (Sencha, Green Darjeeling)	18.3 ± 0.04
Green tea with lemon	15.3 ± 0.01
Inter Spar Green Tea	13.4 ± 0.14
Lemon flavoured green tea	13.3 ± 0.08
Leaf green tea	
Assam Bherjan	18.1 ± 0.23
Yunnan	16.9 ± 0.33

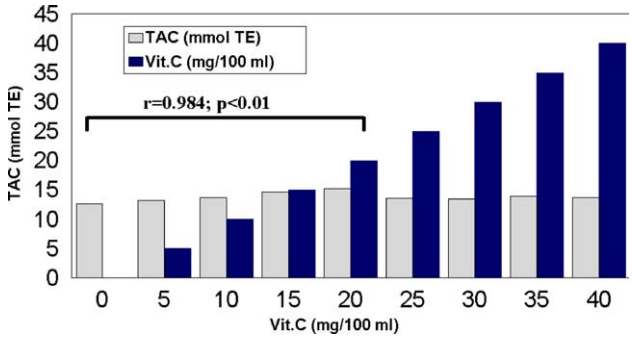

Fig. 1. Effect of different doses of ascorbic acid on the antioxidant activity of black tea.

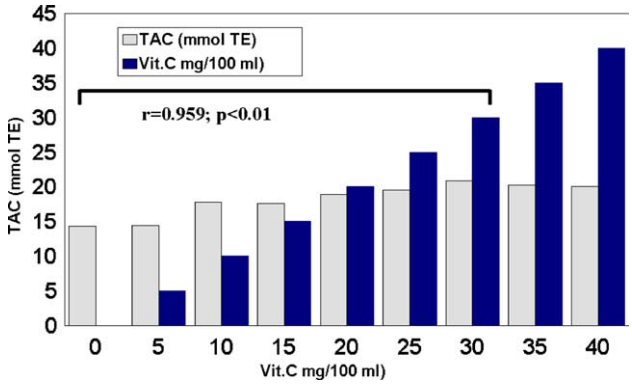

Fig. 2. Effect of different doses of ascorbic acid on the antioxidant activity of green tea.

3.2.2. Effect of different doses of ascorbic acid on the antioxidant activity of green tea

For green tea (Inter Spar Green Tea in bags), addition of up to 30 mg ascorbic acid/100 ml extract resulted in a linear increase of antioxidant capacity up to 20.8 mmol TE ($r = 0.959$; $p < 0.01$), and it remained unchanged in spite of further vitamin C addition. At a concentration of 30 mg ascorbic acid/100 ml, green tea extract had a TAC value about 50% higher than the extract without vitamin C (14.3 mmol TE) (Fig. 2).

4. Discussion

For the green and black tea extracts investigated, the values of antioxidant activity were at a comparable level, which correlates with the results of Henn and Stehle (1998) and Licht, Böhm, and Bitsch (1997). The previously reported (Benzie & Szeto, 1999; Robinson et al., 1997) considerable variations in the antioxidant activities of different teas, were not observed in our study. The

tea varieties on the Austrian market are probably different from those in other countries, e.g. England. However, some of the teas investigated in the present report, e.g. black teas Assam (14.0 mmol TE) and Ceylon (10.4–12.2 mmol TE), had antioxidant capacities similar to that demonstrated by Robinson et al. (1997) (black teas Assam and Ceylon 10.4 and 12.4 mmol TE, respectively) regardless of any differences in preparation. Moreover, we did not observe any statistically significant differences between leaf and bagged black teas of the same type (Black Ceylon, in bags, 12.2 mmol TE; leaf, 10.4 mmol TE). Langley-Evans (2000) reported that tea prepared using tea bags had significantly lower antioxidant capacity than black leaf tea when the temperature of the infusion water lay between 20 and 70 °C but, at an infusion temperature of 90 °C, both of them showed similar TAC values. Most tea consumers routinely prepare tea by adding boiling water to a cold vessel, which will generally produce a brew temperature of approximately 90 °C. Our preparation was at 90 °C and was in accord with the findings of Langley-Evans (2000). Robinson et al. (1997) reported that tea strength, as rated by the manufacturer and linked to taste and flavour, showed an association with its antioxidant activity. The largest difference (60%) in the antioxidant activity among the black teas investigated, between Darjeeling Queen's Blend in tea bags (17.6 mmol TE) and Ceylon leaf tea (10.4 mmol TE), could indicate a relationship between strength of tea and its antioxidant activity. Among green teas, the TAC values of some tea bag and tea leaf extracts were nearly identical. The present findings are not in accord with the report of Robinson et al. (1997) that there is no relationship between the market price of tea and its antioxidant activity in solution. In our study, the cheaper types of tea (e.g. Inter Spar Green Tea, Lemon Flavoured Green Tea, Ceylon Black Tea) had lower antioxidant capacity than the expensive varieties (e.g. Java Green Tea, Green Blend, Darjeeling Black Tea).

Many in vitro studies have considered the effects of infusion time, infusion temperature and the addition of milk on the antioxidant capacity of tea. In the in vitro experiment presented in this report, we tried to evaluate the possible effects of different doses of ascorbic acid on the total antioxidant activity of tea and we demonstrated an increase of the antioxidant potential of both black and green tea by the addition of ascorbic acid. This, indicates that the tea preparation with lemon or lemon juice, which contains ascorbic acid, can improve the antioxidant potential of tea. Green and black teas differ substantially in their composition. Green teas provide approximately twice the total catechin quantity found in black teas. Black teas contain greater proportions of other polyphenols, such as the theaflavins and thearubigins generated by the oxidative processes used in tea production (Van het Hof et al., 1997). These differences in the compositions of teas could be an explanation for our results in the experiment with ascorbic acid, in which antioxidant activity of black tea extracts increased up to 20 mg ascorbic acid/100 ml tea solution and of green tea up to 30 mg ascorbic acid/100 ml extract.

5. Conclusion

Following the packet instructions regarding tea preparation, the domestic way of preparation can contribute to the antioxidants needed to protect the body from degenerative diseases. Serafini et al. (1996, 2000) reported that the consumption of tea produced an increase in total plasma antioxidant capacity. The addition of ascorbic acid to tea extract can improve the antioxidant potential of tea in vitro. Further studies are required to investigate whether the consumption of such preparations, e.g. tea with lemon or lemon juice, can influence plasma antioxidant capacity.

References

Benzie, I. F. F., & Szeto, Y. T. (1999). Total antioxidant capacity of teas by the ferric reducing antioxidant power assay. *Journal of Agricultural and Food Chemistry, 47*, 633–636.

Henn, T., & Stehle, P. (1998). Gesamtphenolgehalt und antioxidative Kapazität handelsüblicher Getränke. *Ernährungs-Umschau, 45*, 308–313.

Kaack, K., & Austed, T. (1998). Interaction of vitamin C and flavonoids in elderberry (*Sambucus nigra* L.) during juice processing. *Plant Foods for Human Nutrition, 52*, 187–198.

Langley-Evans, S. C. (2000). Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. *International Journal of Food Sciences and Nutrition, 51*, 181–188.

Leenen, R., Roodenburg, A. J. C., Tijburg, L. B. M., & Wiseman, S. A. (2000). A single dose of tea with or without milk increases plasma antioxidant activity in humans. *European Journal of Clinical Nutrition, 54*, 87–92.

Licht, U., Böhm, V. & Bitsch, R. (1997). The effect of brewing time on total phenolics and antioxidant activity in green and black Darjeeling. In *Proceedings of a European COST concerted action scientific workshop: Polyphenols in food* (pp. 211–214). Aberdeen, Scotland.

Ramarathnam, N., Osawa, T., Ochi, H., & Kawakishi, S. (1995). The contribution of plant food antioxidants to human health. *Trends in Food Science and Technology, 6*, 75–82.

Rice-Evans, C., & Miller, N. J. (1994). Total antioxidant status in plasma and body fluids. *Methods in Enzymology, 234*, 279–293.

Robinson, E. E., Maxwell, S. R. J., & Thorpe, G. H. G. (1997). An investigation of the antioxidant activity of black tea using enhanced chemiluminescence. *Free Radical Research, 26*, 291–302.

Serafini, M., Ghiselli, A., & Ferro-Luzzi, A. (1996). In vivo antioxidant effect of green and black tea in man. *European Journal of Clinical Nutrition, 50*, 28–32.

Serafini, M., Laranjinha, J. A., Almeida, L. M., & Maiani, G. (2000). Inhibition of human LDL lipid peroxidation by phenol-rich

beverages and their impact on plasma total antioxidant capacity in humans. *Journal of Nutritional Biochemistry, 11*, 585–590.

Van het Hof, K. H., de Boer, H. S. M., Wiseman, S. A., Lien, N., Weststrate, J. A., & Tijburg, L. B. M. (1997). Consumption of green or black tea does not increase resistance of low density lipoprotein to oxidation in humans. *American Journal of Clinical Nutrition, 66*, 1125–1132.

Van het Hof, K. H., Kivits, G. A. A., Weststrate, J. A., & Tijburg, L. B. M. (1998). Bioavailability of catechins from tea: The effect of milk. *European Journal of Clinical Nutrition, 52*, 356–359.

Vermeer, I. T., Moonen, E. J., Dallinga, J. W., Kleinjans, J. C., & van Maanen, J. M. (1999). Effect of ascorbic acid and green tea on endogenous formation of *N*-nitrosodimethylamine and *N*-nitrosopiperidine in humans. *Mutation Resarch, 428*, 353–361.