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SHORT COMMUNICATION

Effect of infusion time and consecutive brewing on antioxidant status of black tea infusion

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ABSTRACT: Time duration for which the black tea leaves are left in contact with the water i.e. infusion time and consecutive brewing showed variation in the antioxidant status of in-cup tea infusion. In the tea tested, an optimum duration of 10 min of infusion time is essential to achieve maximum polyphenol content and antioxidant activity. The results also evidence a significant ($P < 0.05$) decrease in polyphenol content due to consecutive brewing method.

KEYWORDS: *Black tea; Antioxidant; Infusion; Polyphenol; Consecutive brewing*

Introduction

Tea (*Camellia sinensis*) is one of the most frequently consumed beverages. About 20% of the world production is consumed as green tea, an extract from heated and dried tea leaves, whereas 80% is consumed as black tea, which is produced from leaves by enzymatic oxidation. Dried tea extract contains 25–40% polyphenols; in green tea, these are flavonols (catechins), of which epigallocatechin gallate (EGCG) is the most prevalent compound.[1,2] In black tea, most of the catechins are oxidized to thearubigens and theaflavins, which give the extract its characteristic red-brown colour. Worldwide consumer observations and questionnaire studies on tea preparation habits have shown wide variations among countries and among individuals within countries in the way they make their tea, e.g. the amount of tea leaves taken, the amount of water added to the leaves, the amount of agitation used to assist infusion, the length of time tea leaves are left in contact with the water, and the use of additional ingredients etc. Effects of these differences in tea preparation on the in-cup chemical composition of tea infusions is of interest because the quality and health properties of the consumed drink are associated with the chemical components (in particular, the polyphenols) extracted from tea leaves.

The health effects of tea consumption have, for example, been the subject of a number of epidemiological and intervention studies, and evidence is emergence of a relationship between tea consumption and a reduced risk of cardiovascular disease and cancer.[3,4] Black tea extract have also been found to play a pivotal role in radioprotection.[5,6] Health benefits of tea are attributed to tea polyphenols or flavonoids, which have been demonstrated to have strong antioxidant effects *in vitro*.[7–9]

Often "number of cups of tea per day" is the only measure of tea consumption. Little consideration has been given to different brands and varieties of tea consumed or to methods of preparation. We have already reported about different brands of Indian tea in our previous work.[10] Consequently, the current investigation considered two potential sources of variation *viz.*, infusion time and consecutive brewing practice, and their contribution to the in-cup antioxidant composition.

Methodology

Chemicals

2,2-diphenyl-1-picryl hydrazyl (DPPH) was obtained from Sigma Chemical Co., St. Louis, MO, USA. Ferrous sulphate, potassium sodium tartrate tetrahydrate were purchased from Himedia, Mumbai, India.

Tea sample

Lipton Darjeeling tea (leafy variety) was used for all the experiments and purchased from a local market of Kolkata, India.

Preparation of Tea Infusion for Time-dependent Study

Each infusion was prepared by adding 3 g of tea leaves to 150 ml of boiled distilled water (100°C). Such nine

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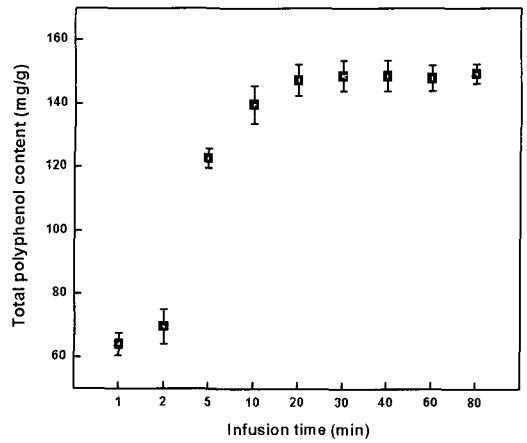

Figure 1: Changes in total polyphenol content with increasing infusion time (1–80 min).

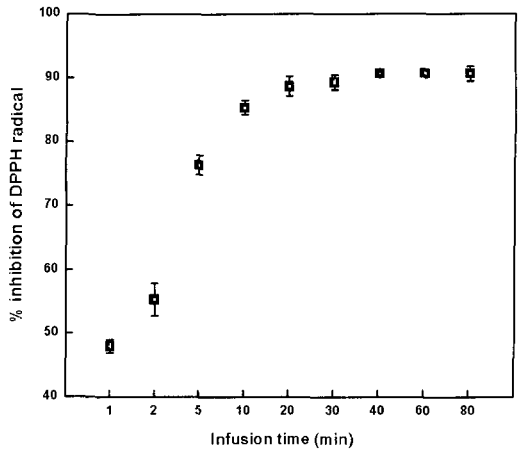

Figure 2: Changes in antioxidant activity with increasing infusion time (1–80 min).

infusions were then allowed to stand for 1, 2, 5, 10, 20, 30, 40, 60, and 80 min, respectively. After required time interval, each infusion was separated from contact of tea leaves and filtered for further studies.

Preparation of Tea Infusion for Consecutive Brewing Study

Tea leaves (3 g) was infused with 150 ml of boiled distilled water (100°C) for 5 min. Total 100 ml liquid were filtered off and cooled to room temperature under running water; a 2nd infusion was made by adding a further 100 ml boiling distilled water to the tea leaves for 5 min, and filtering off 100 ml which was cooled to room temperature under running water; a 3rd and 4th infusions were made using the same procedure.

Spectrophotometric Analysis

All the spectrophotometric analyses were performed at room temperature using matched quartz cells of 1-cm path length with the help of Varian UV-Visible Spectrophotometer (CARY 100 Bio, USA).

Total Polyphenol Content

The total polyphenol content was also determined by ferrous tartrate method,[10,11] where absorbance of test samples was recorded at 540 nm, using a blank solution prepared with Milli-Q water replacing tea extract. The content of tea polyphenols was calculated by the following equation:

Total Polyphenols (mg g^{-1}) = $(E_1 - E_2) \times 3.9133 \times 50/1$

where E_1 = Absorbance of the tested solution at 540 nm; E_2 = Absorbance of the control solution at 540 nm; 3.9133 = Constant (polyphenol concentration was 3.9133 mg ml^{-1} when absorbance at 540 nm was 1.0); 50/1 = Constant (1 g of tea sample was extracted in 50 ml water).

Antioxidant Activity

Scavenging ability of tea samples on DPPH radical was measured according to the method reported by Turkmen *et al.* with a slight modification.[11] Absorbance of test samples at 517 nm was measured using methanol as a blank. Antioxidant activity (AA) was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

AA (%) = (Control absorbance – Sample absorbance) / Control absorbance × 100

Consecutive Brewing Method

Tea leaves (3 g) were infused with 150 ml of boiling distilled water for 5 min, 100 ml liquid were filtered off and cooled to room temperature under running water; a 2nd infusion was made by adding a further 100 ml boiling distilled water to the tea leaves for 5 min, and filtering off 100 ml which was cooled to room temperature under running water; a 3rd and 4th infusions were made using the same procedure.

Statistical Analysis

The statistical analysis of the samples was undertaken using Student's *t*-test. All data reported are means ± standard deviations for three independent experiments,

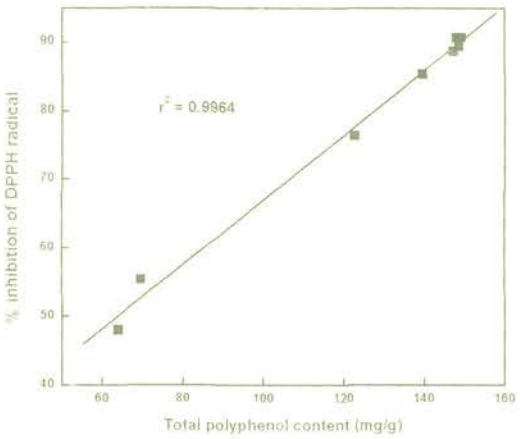

Figure 3: Correlation between total polyphenol content and corresponding antioxidant activity.

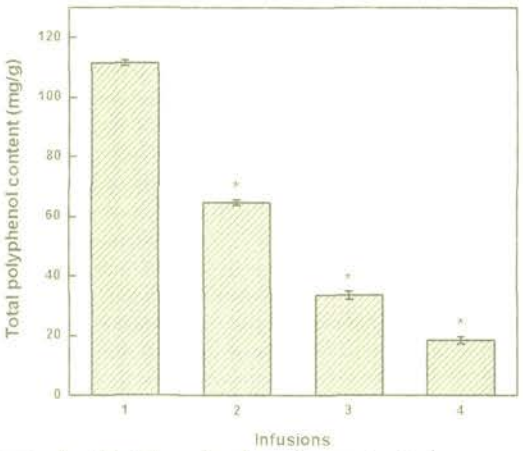

Figure 4: Total polyphenol content in consecutive infusions. (*$P < 0.05$ as compared to 1st infusion).

unless otherwise noted.

Results and Discussion

Tea is potential source natural and dietary antioxidants. Various *in vitro* radical trapping studies have demonstrated antioxidant properties of black tea, green tea, tea extracts, and individual polyphenolic components of tea.[12–17] In the recent study, optimization of antioxidant intake has been demonstrated by simply modifying preparation technique of tea infusion. The total polyphenol content was observed to increase with time (1–80 min) of tea infusion. The total polyphenol content measured was 64.0 ± 3.60, 69.7 ± 5.51, 120.7 ± 3.05, 139.7 ± 6.11, 147.3 ± 4.93, 148.7 ± 4.72, 148.7 ± 4.72, 148.0 ± 4.00, 149.3 ± 3.05 mg g^{-1} for 1, 2, 5, 10, 20, 30, 40, 60, and 80 min, respectively. From the results, a sharp increase (up to 10 min) in total polyphenol content was observed followed by saturation (Fig. 1). The antioxidant activity of the infusions also demonstrated similar trend (Fig. 2). The antioxidant activity measured was 48.0 ± 1.00, 55.3 ± 2.52, 76.3 ± 1.53, 85.3 ± 1.15, 88.7 ± 1.53, 89.3 ± 1.15, 90.7 ± 0.58, 90.0 ± 0.58, 90.7 ± 1.15 % for 1, 2, 5, 10, 20, 30, 40, 60, and 80 min, respectively. A strong correlation (Pearson correlation coefficient, $r^2 = 0.9964$) was observed between total polyphenol content and antioxidant activity in this range of infusion time period (Fig. 3).

Consecutive brewing of same amount of tea leaves led to significant decrease ($P < 0.05$) in polyphenol content up to 2 times in 2nd infusion, 3 times in 3rd infusion, and 5.5 times in 4th infusion, as shown in Figure 4. Hence, it is evident that the practice of consecutive brewing leads to significant decrease in total polyphenol content of the infusion, which in turn deceases the in-cup antioxidant intake.

The results clearly demonstrate that the quality of tea infusion in terms of polyphenol content and antioxidant activity strongly depend on the process of preparation. The preparation process can be optimized for maximum yield of polyphenols by altering the infusion time, which according to our study is around 10 min. The practice of consecutive brewing should be avoided to get maximum health benefits from black tea consumption.

Acknowledgements

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