ISSN: 2347-467X, Vol. 06, No. (1) 2018, Pg. 35-40

Current Research in Nutrition and Food Science

Journal Website:www.foodandnutritionjournal.org

Antioxidant Activity of Different Forms of Green Tea: Loose Leaf, Bagged and Matcha

SUMAYA FAROOQ and AMIT SEHGAL*

Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, India.

Abstract

Green tea is commercially available in three forms: loose leaf, bagged and powdered. The objective of this study was to compare the radical scavenging capacity of different forms of green tea like loose leaf (3), bagged (2) and powdered matcha (2) of various brands. The green tea forms were prepared at 95-100 °C for 5 min., to mimic conditions usually used for tea preparations at home. The comparison of combined IC_{50} values of different green tea forms (loose leaf, bagged and matcha) showed no significant difference in their radical scavenging activity except bagged tea that exhibited slightly more DPPH radical scavenging potential as compared to matcha. Individually, the Bud white loose leaf demonstrated highest antioxidant activity followed by Laplant bag, Lipton bag, Laplant loose, Gourmet matcha, Wow matcha and Lipton loose. These findings revealed that on the basis of form, it may not be possible to generalize which form of tea whether loose leaf, bagged or matcha, is more effective in scavenging free radicals.

Article History

Received: 13 November 2017
Accepted: 20 March 2018

Keywords

Antioxidant,
Camellia sinensis,
Free radical,
Green tea,
Matcha.

Introduction

Green tea is obtained from the dried leaves of tea plant *Camellia sinensis* and is one of the drinks gaining popularity throughout the world[1]. It is manufactured from newly picked leaves that are exposed to heat, and then pan fried or steamed prior to rolling or shaping and drying[2]. It is widely consumed, due to its refreshing taste, aroma and various other properties like antioxidant, anticarcinogenic, antimutagenic and antihypertensive[3-10]. All the above mentioned properties are mainly attributed to high polyphenolic content present in the green tea[11]. The catechins constitute the major part of the polyphenolic content in green tea, main catechins and its derivatives present are: (-) – epigallocatechin (EGC), (-) - epigallocatechin 3- gallate (EGCG), (-) - epicatechin gallate (ECG), (-) - gallocatechin gallate (GCG) and (-) – epicatechin (EC)[12-15]. It is reported that EGCG is one of the major and biologically effective catechin of green tea[16].

CONTACT Amit Sehgal sehgalamitres@gmail.com Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara 144411, India.

To link to this article: http://dx.doi.org/10.12944/CRNFSJ.6.1.04

Green tea is commercially available in the market in three forms: Loose leaf, bagged and powdered (matcha). There have been few comparative studies conducted on different forms of tea and their association with antioxidant potential. It was demonstrated that antioxidant capability of different forms of tea (bagged and loose leaf) was similar at 80 ^{0}C/5'17 but another study showed that maximum extraction efficiency for green tea bioactive compounds also depends on steeping time at constant temperature for different tea types i.e. 5' (powder), 15' (bagged) and 30' (loose leaf) at 80 ^{0}C[18]. The powdered form of green tea revealed greater scavenging effect on the production of reactive oxygen species (ROS) *in vitro* compared with the same amount of loose leaf tea[19]. Matcha is a powdered form of green tea that is produced from shade grown tea leaves that are briefly steamed after harvesting and then grinded in a stone mill[19,20]. It is found to be effective in quenching reactive oxygen species, inhibition of blood glucose accumulation and stimulation of lipid metabolism[21]. The aim of this study was to assess and compare the effect of different forms of green tea such as loose leaf (Bud white, Lipton and Laplant loose), bagged (Lipton and Laplant bag) and powdered (Gourmet and Wow matcha) on their antioxidant potential at conditions usually used for tea preparations at home.

Materials and Methods

Chemicals and Reagents

Methanol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). All these chemical are of analytical grade and procured from Himedia (Mumbai, India).

Sample Preparation

Different forms of green tea such as loose [Bud white (BW), Lipton loose (LL), and Laplant loose (LAL)], bagged [Lipton bag (LB) and Laplant bag (LB)] and matcha [Gourmet matcha (GM) and Wow matcha (WM)] of various brands were evaluated in this study. Various tea samples were prepared by adding 0.2 g of tea in 10 ml of distilled water and steeped for 5 minutes at 95-100 ^{0}C. The hot water samples were filtered using Whatman's filter paper and the filtrate was used for further investigations[22].

Antioxidant Potential

DPPH Free Radical Scavenging Assay

The scavenging activity of different tea extracts against stable DPPH free radical was examined spectrophotometrically. DPPH solution (0.3 mM) was prepared by dissolving DPPH in methanol. The optical density (OD) of DPPH solution was set between 0.8-1 by diluting it with 50% methanol. Different concentrations of tea extracts were added separately to 2 ml of DPPH solution[23]. After 30 minutes of incubation, the discoloration of the purple to yellow color was observed at 520 nm. Methanol was taken as blank and 2 ml of DPPH solution was taken as control. The test was carried out in triplicates. Radical scavenging potential was determined applying the following relationship:

% Scavenging activity = $A_{520}(c) - A_{520}(s) / A_{520}(c)$ x 100

Where, A(c) = absorbance of control and A(s) = absorbance of sample.

ABTS Free Radical Scavenging Activity

This assay depends on the ability of different substances to scavenge ABTS [2, 2'- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)]. The radical cation was generated by reacting ABTS stock solution (7 mM) with potassium persulfate (2.4 mM) in 1: 1 ratio. The reaction mixture was kept in dark for 16 hours at room temperature. The optical density (OD) of ABTS solution was set between 0.8-1 by diluting it with 50% methanol. Different concentrations of tea extracts were added to every 2 ml of ABTS solution[24]. After 30 minutes of incubation, absorbance of respective samples was taken at 745 nm. The scavenging potential of the test samples was determined by following equation:

% Scavenging activity = $A_{745}(c) - A_{745}(s)/A_{745}(c) \times 100$,

Where, A(c) = absorbance of control and A(s) = absorbance of sample.

Statistical Analysis

The data was expressed as mean ± S.D for triplicate readings. The inter group comparisons were performed by one way analysis of variance (ANOVA) succeded by Tukey's honestly significant difference test using SPSS software (version 18).

The observations were considered statistically significant when the p-values are 0.05 or less.

Results and Discussion

Antioxidant activity of different forms of green tea was determined using DPPH and ABTS tests as shown in Figure 1 and 2.

DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of different forms of green tea were ranked upon IC_{50} values as followed: Lipton loose, LL (30.48 µg/ml) > Wow matcha, WM (29.93 µg/ml) > Gourmet matcha, GM (27.55 µg/ml) > Laplant loose, LAL (26.26 µg/ml) > Lipton bag, LB (24.42 µg/ml) > Laplant bag, LAB (20.38 µg/ml) > Bud white loose leaf, BW (18.93 µg/ml) as depicted in Figure 1. Lesser the IC_{50} value, greater is the antioxidant potential. It was observed that BW and LAB are significantly more effective in scavenging DPPH radical followed by LB, LAL, GM, WM and LL.

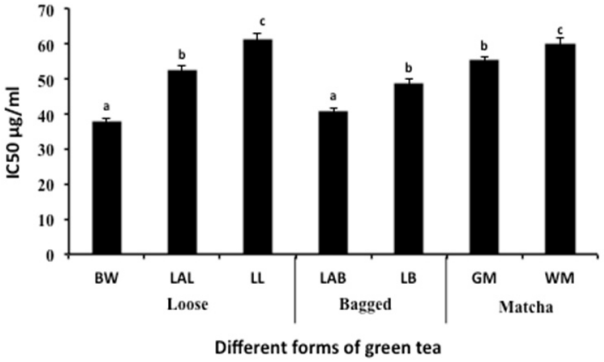

Fig. 1: DPPH radical scavenging activity of different brands and forms of green tea (mean ± sd, n=3). Different alphabets symbolize significant variation between different tea brands. Bud white (BW), Gourmet matcha (GM), Laplant bag (LAB), Laplant loose (LAL), Lipton bag (LB), Lipton loose (LL) and Wow matcha (WM) are various brands of different forms of green tea

ABTS Radical Scavenging Activity

The results obtained from the ABTS test shown in Figure 2. The IC50 values of different forms of green tea based upon their scavenging activity are as followed: LL (13.62 µg/ml) > WM (11.95 µg/ml) > GM (11.44 µg/ml) > LAL (11.16 µg/ml) > LB (10.89 µg/ml) > LAB (9.75 µg/ml) > BW loose leaf (8.84 µg/ml). It was demonstrated that BW was significantly more effective in radical scavenging activity followed by LAB, LB, LAL, GM, WM and LL.

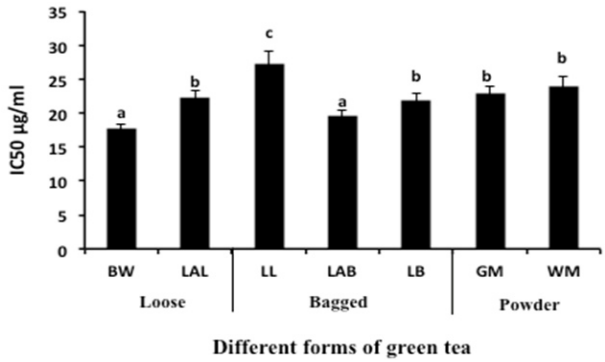

Fig. 2: ABTS radical scavenging activity of different brands and forms of green tea (mean ± sd, n=3). Different alphabets symbolize significant variation between different tea brands. Bud white (BW), Gourmet matcha (GM), Laplant bag (LAB), Laplant loose (LAL), Lipton bag (LB), Lipton loose (LL) and Wow matcha (WM) are various brands of different forms of green tea

The fluctuations in antioxidant activity of green tea from various brands were reported. This is in agreement with previous studies, which concluded that phytochemical content of tea is affected by the cultivation conditions, horticultural practices, cultivar, age of leaf, grade, geographical area, storaging and type of processing[25-27].

The combined IC_{50} values of different forms of green tea such as loose (Bud white, Lipton and Laplant loose), bagged (Lipton and Laplant bag) and matcha (Wow and Gourmet matcha) showed no statistical significant difference in both the antioxidant assays except in case of DPPH radical scavenging, bagged tea demonstrated a small but significant difference in radical quenching ability as compared to matcha as depicted in Table 1.

Table 1: Average values of combined IC_{50} (µg/ml) of loose, bagged and powdered form of green tea (mean ± sd, n=3)

Green tea form	DPPH	ABTS
Bag tea (Lipton and Laplant bag)	22.40 ± 2.26	10.32 ± 0.77
Loose tea (Bud white green tea, Lipton and Laplant loose)	25.45 ± 5.12	11.21 ± 2.10
Matcha tea (Wow and Gourmet matcha)	28.74 ± 1.57 *	11.70 ± 0.65

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2' azino- bis (3- ethylbenzothiazoline-6-sulfonic acid) (ABTS). * Represents a significant difference as compared to bagged tea at $p \leq .05$

A previous study on different forms of green tea such as powdered (Matcha), loose leaf (Kukicha, Gyokuro, Longjing, Sencha J, Bancha, Yunnan, Sencha CH, Gunpowder and Rose of the Orient) and bagged (Twinings of London, Taylors of Harrogate and Franck) demonstrated that antioxidant potential of different forms of tea varies with extraction time and temperature. The same study also showed that antioxidant potential and total phenolic content was found to be maximum for all green tea forms at 100 ^{0}C/3', the bagged tea possess highest scavenging activity followed by powdered and then loose leaf tea[18]. *In vivo* studies demonstrated that regular consumption of green tea exerts protection against benzo(a)pyrene mediated toxicity in mice model[28,29]. In the present work, no significant difference was found between the radical quenching ability of different forms of tea except bagged tea, which exhibited slightly more scavenging potential in case of DPPH radical. Interestingly, earlier studies also revealed that antioxidant capability of different forms of green tea were similar for steeping time of 5' and 10' at 80 ^{0}C[17-18]. It was also suggested that high water temperature and short steeping time is best for extraction of tea bioactive compounds[18,30,31]. Our investigation was concentrated on short steeping time (5') and high water temperature (95-100 ^{0}C) as these conditions mimic the household method of tea preparation.

Conclusion

The study on the antioxidant activity of different forms of commercially available green tea like loose (Bud white, Lipton loose and Laplant loose), bag (Lipton and Laplant bag) and matcha (Gourmet matcha and Wow matcha) prepared at 95-100 ^{0}C and steeped for 5 min, showed no significant difference in the IC_{50} value against DPPH and ABTS radical except bagged tea that displayed slightly more DPPH radical scavenging potential in comparison to matcha. But individually, BW (loose leaf) showed the highest antioxidant activity followed by LAB (bagged), LB (bagged), LAL (loose leaf), GM (powder), WM (powder) and LL (loose leaf). Moreover, this study reported it might not be possible to rank the radical scavenging potential on the basis of green tea form.

Acknowledgments

We acknowledge the financial support by Lovely Professional University to do the necessary research work and to use departmental facilities.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

References

1. Yu Y, Deng Y, Lu B. M, Liu Y. X, Li J, Bao J. K. Green tea catechins: a fresh flavor to anticancer therapy. *Apoptosis;* ***19***(1): 1-18: (2014).
2. Santana-Rios G, Orner G. A, Amantana A, Provost C, Wu S.Y, Dashwood R. H. Potent antimutagenic activity of white tea in comparison with green tea in the Salmonella assay. *Mutation Research;* ***495***(1): 61–74: (2001).
3. Inoue M, Tajima K, Hirose K, Hamajima N, Takezaki T, Kuroishi T, Tominaga S. Tea and coffee consumption and the risk of digestive tract cancers: data from a comparative case-referent study in Japan. *Cancer Causes and Control;* ***9***(2): 209-216: (1998).
4. Skrzydlewsja E, Augustyniak A, Ostrowska J, Luczaj W, Tarasiuk E. Green tea protection against aging – induced oxidative stress. *Free Radical Biology and Medicine;* ***33***: 555: (2002).
5. Chung F. L, Schwartz j, Herzog C. R, Yang Y. M. Tea and cancer prevention: studies in animals and humans. *Journal of Nutrition;* ***133*** (10): 3268S-3274S: (2003).
6. Lambert J. D, Yang C. S. Mechanisms of cancer prevention by tea constituents. *Journal of Nutrition;* ***133*** (10): 3262–3267: (2003).
7. Kumar M, Sharma V. L, Sehgal A, Jain M. Protective effects of green and white tea against benzo (a) pyrene induced oxidative stress and DNA damage in murine model. *Nutrition Cancer;* ***64*** (2): 300-306: (2012).
8. Mittal A, Pate M. S, Wylie R. C, Tollesfsbol T. O, Katiyar S. K. EGCG down regulates telomerase in human breast carcinoma MCF-7 cells, leading to suppression of cell viability and induction of apoptosis. *International Journal of Oncology;* ***24***(3): 703–710: (2004).
9. Negishi H, Xu J. W, Ikeda K, Njelekela M, Nara Y, Yamory Y. Black and green tea polyphenols attenuate blood pressure increases in stroke-prone spontaneously hypertensive rats. *Journal of Nutrition;* ***134*** (1): 38–42: (2004).
10. Yang J, Meyers K. J, Van der Heide J, Liu R. H. Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *Journal of Agriculture and Food Chemistry;* ***52*** (22): 6787-6793: (2004).
11. Hsu Y. W, Tsai C. F, Chen W. K, Huang C. F, Yen C. C. A subacute toxicity evaluation of green tea (*Camellia sinensis*) extract in mice. *Food Chemistry and Toxicology;* ***49***(10): 2624-2630: (2011).
12. Wang H, Provan G. J, Helliwell K. HPLC determination of catechins in tea leaves and tea extracts using relative response factors. *Food Chemistry;* ***81***(2): 307–312: (2003).
13. Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. American *Journal Clinical Nutrition;* ***81***(1): 243S–55S: (2005).
14. Khan N, Mukhtar H. Tea polyphenols for health promotion. *Life Sciences;* ***81***(7): 519–533: (2007).
15. Yang C. S, Lambert J. D, Sang S. Antioxidative and anticarcinogenic activities of tea polyphenols. *Archives of Toxicology;* ***83*** (1): 11–21: (2009).
16. Yang C. S, Hong J. Prevention of chronic diseases by tea: possible mechanisms and human relevance. *Annual Review of Nutrition;* ***33***: 161–181: (2013).
17. Rusak G, Komes D, Likic S, Horzic D, Kovac M. Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. *Food Chemistry;* ***110***(4): 852–858: (2008).
18. Komes D, Horzic D, Belscak A, Ganic K. K, Vulic I. Green tea preparation and its influence

on the content of bioactive compounds. *Food Research International;* ***43***(1): 167-176: (2010).

19. Fujioka K, Iwamoto T, Shima H, Tomaru K, Saito H, Ohtsuka M, Yoshidome A, Kawamura Y, Manome Y. The powdering process with a set of ceramic mills for green tea promoted catechin extraction and the ROS inhibition effect. *Molecular;* ***21***(4): 474: (2016).
20. Yamabe N, Kang K. S, Hur J. M, Yokozawa T. Matcha, a powdered green tea, ameliorates the progression of renal and hepatic damage in type 2 diabetic OLETF rats. *Journal of Medicinal Food;* ***12***(4): 714-721: (2009).
21. Xu P, Ying L, Hong G, Wang Y. The effects of the aqueous extract and residue of matcha on the antioxidant status and lipid and glucose levels in mice fed a high-fat diet. *Food Function;* ***7***(1): 294-300: (2016).
22. Tsai P. J, Tsai T. H, Yu C. H, Ho S. C. Comparison of NO - scavenging and NO-suppressing activities of different herbal teas with those of green tea. *Food Chemistry;* ***103***(1): 181-187: (2007).
23. Mensor L.L, Menezes F.S, Leitao G.G, Reis A.S, Santos T.C.D, Coube C.S. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research;* ***15***(2): 127-130: (2001).
24. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine;* ***26***(9): 1231-1237: (1999).
25. Nicoli M. C, Anese M, Parpinel M, Franceschi S, Lerici C.R. Loss and/or formation of antioxidants during food processing and storage. *Cancer Letters;* ***114***(1-2): 71–74: (1997).
26. Vinson J. A, Dabbagh Y. A. Tea phenols: Antioxidant effectiveness of teas, tea components, tea fractions and their binding with lipoproteins. *Nutrition Research;* ***18***(6): 1067–1075: (1998).
27. Damiani E, Bacchetti T, Padella L, Tiano L, Carloni P. Antioxidant activity of different white teas: Comparison of hot and cold tea infusions. *Journal of Food Composition and Analysis;* ***33***(1): 59-66: (2014).
28. Kumar M, Jain M, Sehgal A, Sharma V.L. Modulation of CYP1A1, CYP1B1 and DNA adducts level by green and white tea in Balb/c mice. *Food and chemical toxicology;* ***50***(12): 4375-4381: (2012a).
29. Kumar M, Sharma V.L, Sehgal A, Jain M. Protective effects of green and white tea against benzo (a) pyrene induced oxidative stress and DNA damage in murine model. *Nutrition and cancer;* ***64***(2): 300-306 (2012b).
30. Friedman M, Kim S. Y, Lee S. J, Han G. P, Han J. S, Lee K. R, Kozukue N. Distribution of catechins, theaflavines, caffeine, and theobromine in 77 teas consumed in the United States. *Journal of Food Science;* ***70***(9): 550-559: (2005).
31. Perva-UzunalicaL A, Skerget M, Knez Z, Weinreich B, Otto F, Gruner S. Extraction of active ingredients from green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine. *Food Chemistry;* ***96***(4): 597–605: (2006).