International Journal of Food Science and Nutrition
ISSN: 2455-4898, Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 2; Issue 2; March 2017; Page No. 69-74

Influence of preparation methods on antioxidant profile and phytochemical constituents of commercial tea (*Camellia sinensis*) infusions

Pallabi Roy, Sanchita Bhattacharjee, *Dr. Sauryya Bhattacharyya

Department of Food & Nutrition, Sarada Ma Girls' College, Talikhola, Barasat, Kolkata, India

Abstract
Tea (*Camellia sinensis*) is the most consumed beverage, also known for its medicinal value. However, due to fast life, different tea infusion preparation methods have been utilized nowadays, the present study was undertaken to find out whether variation in preparation processes might alter the antioxidative qualities or bioactive contents of the infusions. Infusions were prepared by traditional soaking in hot water as well as by microwave heating of six different commercial samples. The assays performed included DPPH radical decolorization assay and estimation bioactives like of total polyphenols, tannin, monomeric anthocyanin, total flavonoid and ascorbic acid. It was observed that there were non-significant differences in the levels of bioactives like polyphenols, tannins, flavonoids and monomeric anthocyanins. However, DPPH radical scavenging abilities were lower in commercially packaged samples indicating lower levels of non-polar antioxidants in the samples, which might not affect the acceptability of the samples as the consumable infusions are prepared in water. The study also divulged that the total phenolic content did not differ significantly between the sources of the tea samples. Even after heat treatments, the amounts did not change significantly, indicating possible balance between the stabilization and decomposition of the bioactives. There was also a tendency of conservancy of the bioactives by soaking method of infusion preparation compared to the microwave method.

Keywords: Tea, Antioxidant, flavonoids, polyphenols, tannins

1. Introduction
Tea is the second most popular beverage worldwide as its' popularity surpasses coffee, soft drinks, beer or wine [1, 2]. The economic and social interest of tea is vast and its consumption is part of daily routine of several populations as a stimulant and as a therapeutic abet in many adverse physiological conditions [3]. It grows well in tropical and subtropical areas such as South and South East Asia, Africa and the Middle East [4]. Tea cultivated and processed in Darjeeling hill in India is one of the most popular teas having exquisite aroma with proven therapeutic potential [5]. Flavonoids in tea are reported to be the most important therapeutically, as it was proved that an intake level of flavonoids of 1 gm per day could fend off deleterious systemic free radicals effectively [6]. As tea is one of the most popular beverages, it serves as an important source of such polyphenolic constituents which produce exciting therapeutic possibilities.

Two botanical variants have been identified for tea – the original Chinese variant, *Camellia sinensis* and the Indian variant, *Camellia assamica* [7], members of Theaceae family. Tea beverage is an infusion of the dried and processed leaves of both of the above shrubs. Green tea is prepared from the fresh tea leaf and widely consumed in Japan, China, Korea and Morocco. Black tea is preferred in Western cultures, whereas oolong tea is popular in China and Taiwan [8, 9]. Consumption of green tea is especially popular in different human cultures and races, and its association with anti-inflammatory, anti-proliferative and anti-atherosclerotic activities has led to the inclusion of green tea extracts in dietetic supplements, nutraceuticals and functional foods as well [10]. Green tea is characterized by its high flavonoid content, mainly catechins (20-30% of the dry weight). Condensed tannins are also an important component of tea which are transformed products of flavan-3-ols or flavan-3,4-diols [11]. The other important bioactives are – caffeine, theophylline, teanine (a typical amino acid), proteins and carbohydrates [12]. The leaves have known to possess anti-carcinogenic, anti-obesity, anti-diabetic, anti-bacterial, anti-arthritic, anti-allergic and anti-caries effects. However, sometimes harmful effects of tea overconsumption (black or green) were observed [13].

The concentrations of tea bioactives solely depend upon the industrial processes through which the tea leaves undergo during processing [14, 15]. Traditionally, tea is infused only before drinking. During preparation of infusions for consumption in different populace, the leaves are heated with different intensities, in burners as well as in micro-wave ovens. In some countries like Iran and India, the infusions are even consumed with milk [13]. Use of different processes for infusion preparation not only governs the taste or bioactive concentrations, but also is a demand of the modernized fast life of the people.

The present study was conducted to evaluate phytochemical constituents and antioxidant capacities of six different types of tea infusions prepared by method which is not practiced normally in the households. The method, which uses microwave oven, is followed in corporate sectors of Kolkata to minimize the infusion preparation time. The study would also help to understand about the antioxidant capacity including bioactive contents of different types of tea samples sold in the city, and to provide new information on the antioxidant function of these beverages for nutritionists and the general populace, when consumed.

2. Materials and methods
2,2′-Diphenyl-1-picryl hydrazyl (DPPH) were obtained from

Himedia, India. Folin-Ciocalteau reagent, quercetin, gallic acid, tannic acid and ascorbic acid were obtained from Merck, India. All other reagents and chemicals used were of analytical grade procured from SRL, India. Deionized distilled water was used in the entire study.

2.1 Collection of samples

All the six tea samples were purchased from local markets of Kolkata. Three samples were packaged samples, sold in sealed pouches by renowned industrial houses. These samples were designated as C1-C3.The other three samples were retailed samples, sold in open non-packaged form in the market. They are designated as N1-N3. The samples were kept in tight glass containers and extracted within seven days of purchase.

2.2 Preparation of infusions

Tea infusions are prepared and designated in the following manner –

AM – Methanol extracts of the samples were obtained by a reported procedure with minor modifications [7]. 1 gm sample was extracted with 20 ml aqueous methanol (60%, v/v) for 30 min in an orbital shaker at 70ºC in the dark. The mixture was filtered through Whatman No.1 and the volume was made up again to 20 ml with aqueous methanol (60%, v/v). This sample was prepared to adjudicate the maximum antioxidant capacity that would be provided by the samples.

BL – Tea sample (1gm) were soaked into boiled hot distilled water (20ml) for 5 minutes. Then the mixture was centrifuged and the supernatant was made up to the desired volume (*ca*. 20ml) with distilled water.

MW – Tea sample (1gm) were heated in a microwave at moderate power with distilled water (2 ml) for 90 seconds. Then the mixture was centrifuged and the supernatant was made up to the desired volume (*ca*. 20ml) with distilled water.

2.3 DPPH radical decolorization assay

The DPPH assay was performed using a previously described procedure [16]. 1 ml DPPH solution (3 mg in 25 ml ethanol) was mixed with 0.5 ml sample solution and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). Gallic acid was used as positive control and the results were expressed as Gallic acid equivalents (µg/gm sample).

2.4 Estimation of total phenolic contents

Total phenolic compound contents were determined by the Folin-Ciocalteau method [17]. The samples (0.5 ml) were mixed with Folin-Ciocalteau reagent (5 ml, of 1:10 diluted sample with distilled water) for 5 min and aqueous sodium carbonate (4 ml, 1 M) was then added. The absorbance of the reaction mixture was then measured at 765 nm with a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid was used as standard. The results were expressed in terms of mg gallic acid equivalent/gm sample.

2.5 Estimation of tannin contents

Total tannin contents were determined by a published method [18]. Briefly 5 ml aliquot of the extract was mixed with 12.5 ml of indigo carmine solution and 375 ml of distilled water. This mixture was titrated against standardized $KMnO_4$ solution when the blue colour of the indigo carmine changed to yellow with a faint pink tint at the rim. To determine the volume of $KMnO_4$ used to titrate non tannin (related) compounds, another aliquot of extract was treated with gelatin solution. The mixture was shaken for 15 minutes and filtered through Whatman no. 1 filter paper. 12.5 ml of the filtrate was mixed with same volume of indigo carmine solution and 375 ml of distilled water. This mixture was again titrated against $KMnO_4$ solution until colour changed to faint pink as earlier. Tannin content was determined in mg/ml infusion by taking the difference of the above two titer values and standardizing the $KMnO_4$ solution with 0.1N oxalic acid.

2.6 Estimation of total flavonoid contents

Total flavonoid content was determined according to a published colorimetric method [19] with some modification. Briefly 0.5 ml sample was mixed with 2 ml of distilled water and 0.15 ml of aqueous sodium nitrite solution ($NaNO_2$, 5% w/v), allowed to stand for 6 min, 0.15 ml aqueous aluminium trichloride solution ($AlCl_3$, 10% w/v) was added and allowed to stand again for 6 min, followed by addition of 2 ml of aqueous sodium hydroxide (NaOH, 4% w/v) solution. The final volume was made up to 5 ml by distilled water. The reaction mixture was mixed thoroughly and allowed to stand for another 15min. The absorbance of the reaction mixture was then measured at 510nm with a UV-Vis spectrophotometer (model – Systronics 2202). Quercetin was used as standard. The results are expressed in terms of quercetin equivalent (µg/gm sample).

2.7 Estimation of monomeric anthocyanin contents

Determination of monomeric anthocyanin content was conducted by pH-differential method [20]. Total monomeric anthocyanins were expressed as cyanidin-3-glucoside. Sample absorbance was read against a blank cell at 700nm and 510nm and at pH 1.0 and 4.5. The absorbance (A) of the sample was then calculated according the following formula:

$$A = (A_{510} - A_{700})_{pH\ 1.0} — (A_{510} - A_{700})_{pH\ 4.5}$$

where A is the net absorbance of samples at the wavelengths mentioned in the subscript. The monomeric anthocyanin pigment content in the sample will be calculated according the following formula:

$$\text{Anthocyanin content (mg/L)} = (A \times MW \times DF \times 1000) / (\varepsilon \times l)$$

Where DF was dilution factor, MW was molecular weight of cyanidin-3-glucoside (449.2), l is the path length and ε was molar absorptivity (26,900). The anthocyanin content in the sample solution was converted into □g per gram of fresh sample and expressed.

2.8 Determination of ascorbic acid contents

The assay was done following a standard procedure [20]. Stock iodine solution was standardized by titrimetric method using standard (N/100) sodium thiosulphate solution followed by addition of 1% starch solution, when the solution turned blue and continue the titration until the blue color just discharged. The sample extractives were then titrated with the standard iodine solution. In a similar procedure, standard ascorbic acid solution was also titrated. Comparing the titers, the results were expressed as mg ascorbic acid/l infusion.

2.9 Statistical analysis
Experimental results are expressed as mean ± SD of three individual samples. The statistical analysis was done by using the software 'SPSS Statistics 17.0' (IBM Corporation, USA).

3. Results & Discussion
Tea has always influenced human health by its vast content of antioxidany bioactives [21]. A plethora of evidences suggest that tea components including bioflavonoids and tannins are highly effective in scavenging free radicals. Since bioflavonoids like catechin and its derivatives have remarkable antioxidant potential and since tea has reported health promoting benefits [22], the present study was undertaken specifically to observe whether the quality of tea, sold in different packaged forms in the market, are markedly different or not. To answer the above objective, six assays were chosen specifically as a single assay would not be sufficient for such assessment for a natural product [23].

The results of DPPH radical scavenging assay indicated that antioxidant activities of the non-packaged samples (viz. N1-N3), purchased from local markets, were greater than the packaged samples (viz. C1-C3) as observed in Fig. 1. Gallic acid equivalent values of the packaged samples were 453.63±39.00, 514.78±43.93 and 552.88±41.45 µg/gm sample, whereas the values of the non-packaged samples were 617.35±49.98, 824.22±28.92 and 759.14±46.69 □g/gm sample. Radical scavenging abilities of all the six samples were decreased after preparation of the infusions as compared to the aqueous-methanolic extract (Fig. 1). If one goes carefully through the figure, it can easily be observed that microwave treatment during preparation of the infusions retained the DPPH radical scavenging abilities of the six samples in comparison to the infusions prepared by soaking.

The *in vitro* radical scavenging activities like DPPH assay are generally used to indicate antioxidant potential of plant extracts. The assay is based on non-aqueous less polar medium [16], which was suitable for estimation of antioxidant capacities of the mostly non-polar tea bioactives. Since tea leaves contain non-polar bioactives like flavones (e.g. apigenin, vitexin) [24], the above assay was performed in the present study. Higher antioxidant potential of the non-packaged samples might be due to the preservance of bioactives in such samples, as the packaged samples usually undergo several treatment procedures before dispatch to the retailers.

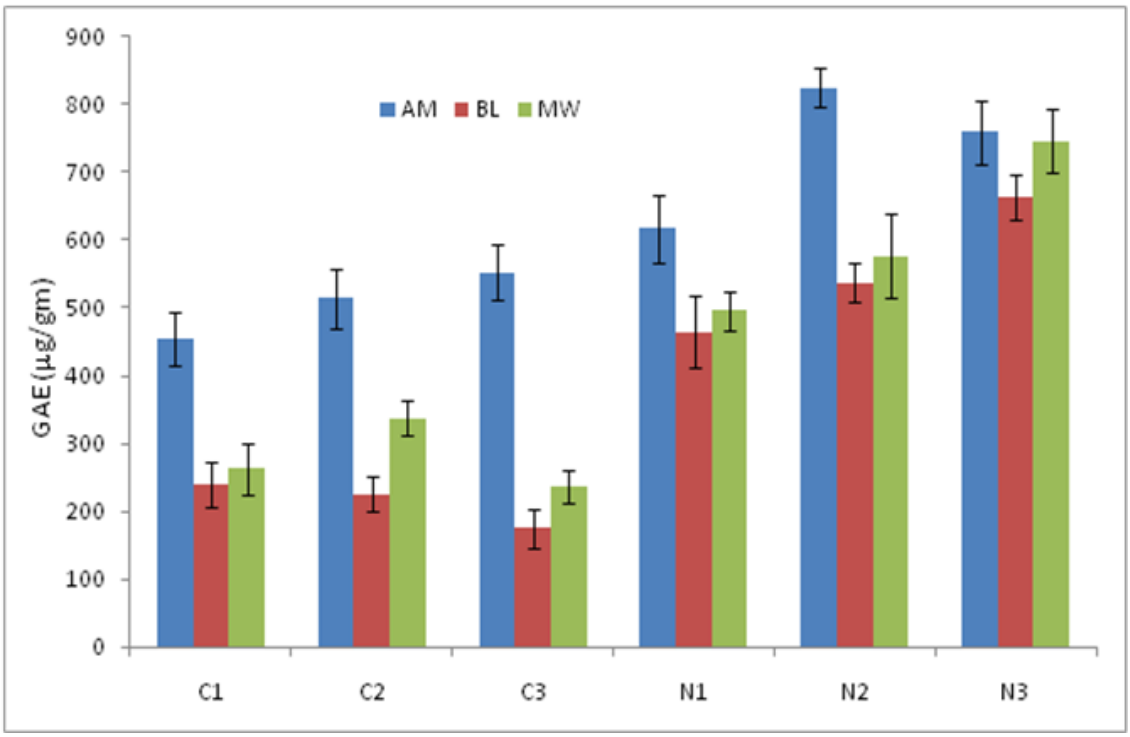

Fig 1: Comparative DPPH radical scavenging potential of commercial tea (*Camellia sinensis*) samples collected from local markets. Data are Mean ± SD (*n*=3), GAE = gallic acid equivalent (µg gallic acid equivalent/gm sample). C1-C3 = packaged samples, N1-N3 = non-packaged loose samples. AM = Aqueous-methanol extract, BL = Infusion prepared by soaking, MW = Infusion prepared by microwave exposure

Total phenolics contents of the packaged samples (viz. C1-C3), purchased from local markets, were greater than the non-packaged samples (viz. N1-N3) as observed in Fig. 2. Gallic acid equivalent values of the packaged samples were 1.78±0.12, 1.56±0.09 and 1.47±0.11 mg/gm sample, whereas the values of the non-packaged samples were 1.49±0.11, 1.16±0.08 and 1.34±0.10 mg/gm sample. Total phenolics contents of all the six samples were decreased after preparation of the infusions (Fig. 2). If one goes carefully through the figure, it can easily be observed that microwave treatment during preparation of the infusions retained the total phenolics contents of the six samples in comparison to the infusions prepared by soaking.

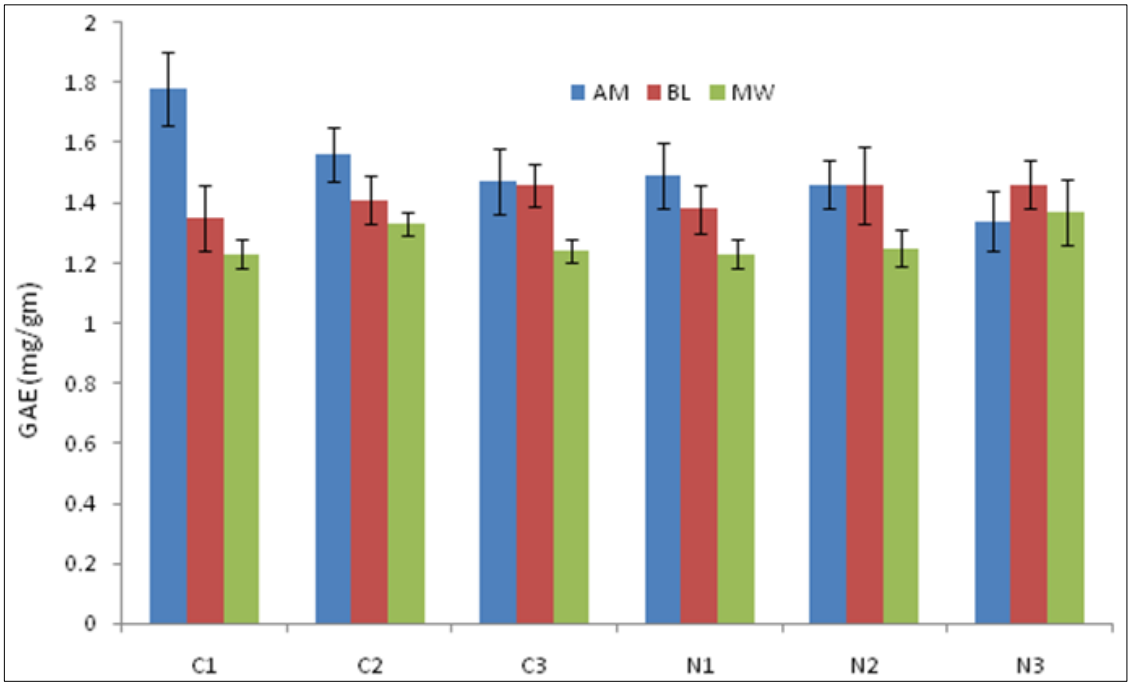

Fig 2: Comparative total phenolics contents of commercial tea (*Camellia sinensis*) samples collected from local markets. Data are Mean ± SD (*n*=3), GAE = gallic acid equivalent (mg gallic acid equivalent/gm sample). C1-C3 = packaged samples, N1-N3 = non-packaged loose samples. AM = Aqueous-methanol extract, BL = Infusion prepared by soaking, MW = Infusion prepared by microwave exposure

Tannin contents of the packaged samples (viz. C1-C3), purchased from local markets, were greater than the non-packaged samples (viz. N1-N3) as observed in Fig. 3. Tannic acid equivalent values of the packaged samples were between *ca.*48 to 50 mg/l infusion, whereas the values of the non-packaged samples were in a range of 38 and 39 mg/l infusion with one sample having 53 mg/l. The contents of all the six samples were decreased after preparation of the infusions (Fig. 3). Carefully scrutiny of the figure signified that traditional soaking method retained the tannin contents of the six samples in comparison to the infusions prepared by microwave exposure.

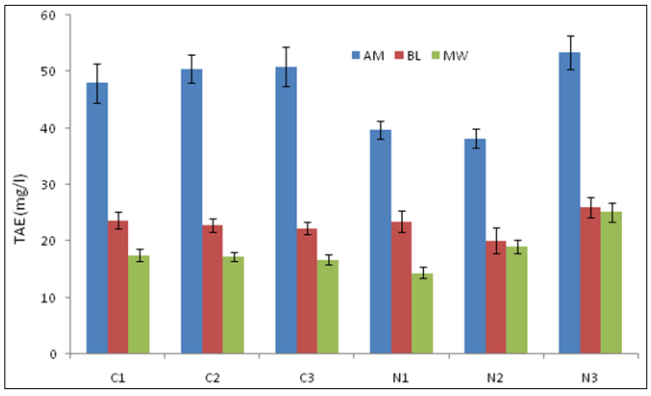

Fig 3: Comparative tannin contents of commercial tea (*Camellia sinensis*) samples collected from local markets. Data are Mean ± SD (*n*=3), TAE = tannic acid equivalent (mg tannic acid equivalent/l infusion). C1-C3 = packaged samples, N1-N3 = non-packaged loose samples. AM = Aqueous-methanol extract, BL = Infusion prepared by soaking, MW = Infusion prepared by microwave exposure

Flavonoid contents of the packaged samples (viz. C1-C3), purchased from local markets were almost similar to that of the non-packaged samples (viz. N1-N3) as observed in Fig. 4. Quercetin equivalent values of the samples were in the range between *ca.* 406 to 466 mg/gm sample with one packaged sample having 575 mg/gm. Like other bioactives, flavonoid contents of all the six samples were decreased after preparation of the infusions (Fig. 4). If one goes carefully through the figure, it can easily be observed that microwave treatment during preparation of the infusions reduced the total flavonoid contents of the six samples in comparison to the infusions prepared by soaking.

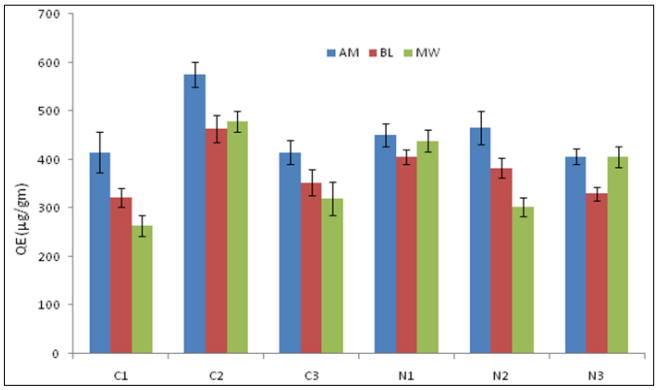

Fig 4: Comparative flavonoid contents of commercial tea (*Camellia sinensis*) samples collected from local markets. Data are Mean ± SD (*n*=3), QE = quercetin equivalent (µg quercetin equivalent/l infusion). C1-C3 = packaged samples, N1-N3 = non-packaged loose samples. AM = Aqueous-methanol extract, BL = Infusion prepared by soaking, MW = Infusion prepared by microwave exposure

Anthocyanin contents of the samples were at par for the all six samples as observed in Fig. 5 with one packaged and one non-packaged sample showing less than average values. The contents of all the six samples were decreased after preparation of the infusions (Fig. 5). Carefully scrutiny of the figure

signified that traditional soaking method retained the tannin contents of the six samples in comparison to the infusions prepared by microwave exposure in general.

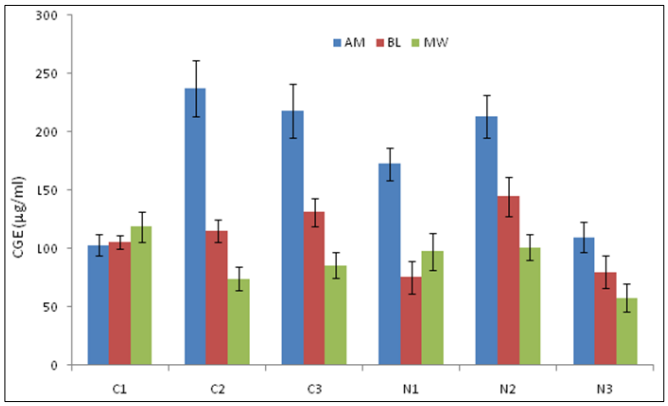

Fig 5: Comparative anthocyanin (total) contents of commercial tea (*Camellia sinensis*) samples collected from local markets. Data are Mean ± SD (*n*=3), CGE = Cyanidin-3-glycoside equivalent (µg CG equivalent/ml infusion). C1-C3 = packaged samples, N1-N3 = non-packaged loose samples. AM = Aqueous-methanol extract, BL = Infusion prepared by soaking, MW = Infusion prepared by microwave exposure

Ascorbic acid contents of the packaged samples (viz. C1-C3), purchased from local markets, were marginally greater than the non-packaged samples (viz. N1-N3) as observed in Fig. 6. Values of the packaged samples were between *ca.*143 to 211 mg/l infusion, whereas the values of the non-packaged samples were in a range of 101 and 151 mg/l infusion. The contents of all the six samples were decreased after preparation of the infusions (Fig. 6). Carefully scrutiny of the figure indicated that infusion preparation methods improved the ascorbic acid contents of the six samples irrespective of their source of purchase.

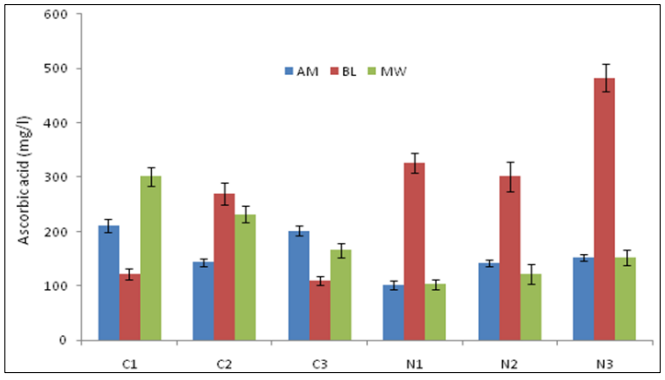

Fig 6: Comparative ascorbic acid contents of commercial tea (*Camellia sinensis*) samples collected from local markets. Data are Mean ± SD (*n*=3). C1-C3 = packaged samples, N1-N3 = non-packaged loose samples. AM = Aqueous-methanol extract, BL = Infusion prepared by soaking, MW = Infusion prepared by microwave exposure

Phenolic compounds of plants having one or more aromatic rings with one or more hydroxyl groups can potentially quench free radicals by forming resonance-stabilized phenoxyl radicals which play a role in their antioxidant properties [25]. The present study revealed that the phenolic content did not differ significantly between the sources of the tea samples. Even after heat treatments, the amounts did not change significantly, indicating possible balance between the stabilization and decomposition of the bioactives. This was substantiated by the fact that tannin contents reduced significantly after heat treatment, whereas ascorbic acid contents increased. However, all the assays indicated that microwave exposure preserved the bioactives more than the soaking treatment, probably due to less exposure time of the bioactives towards heat.

4. Conclusions

The present study concludes that packaged tea samples are inferior to non-packaged samples with respect to their DPPH radical scavenging abilities. This might be due to the deterioration of non-polar bioactives in the packaged samples, as the packaged samples usually undergo several treatment procedures before dispatch to the retailers. The present study also divulged that the total phenolic content did not differ significantly between the sources of the tea samples. Even after heat treatments, the amounts did not change significantly, indicating possible balance between the stabilization and decomposition of the bioactives. This was substantiated by the fact that tannin contents reduced significantly after heat treatment, whereas ascorbic acid contents increased. However, all the assays indicated that microwave exposure preserved the bioactives more than the soaking treatment, probably due to less exposure time of the bioactives towards heat.

5. Acknowledgments

The authors are grateful to Sarada Ma Girls' College authority (under Ramakrishna Vivekananda Mission) for providing financial and infrastructural assistance.

6. References

1. Costa LM, Gouveia ST, Nobrega JA. Comparison of heating extraction procedures for Al, Ca, Mg and Mn in tea samples Analytical Sciences 2002; 18(3):313-318.
2. Rietveld A, Wiseman S. Antioxidant effects of tea: evidence from human clinical trials, Journal of Nutrition. 2003; 133(10):3285S-3292S.
3. Cabrera C, Artacho R, Gimenez R. Beneficial effects of green tea – a review, The Journal of American College of Nutrition. 2006; 25(2):79-99.
4. Ildashti S, Mirzaei A, Abulfathei AA. Comparison of green and black tea antioxidant activity with *Lippia citriodora*, World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(2):942-950.
5. Bhattacharya S, Sen-Mandi S. Variation in antioxidant and aroma compounds at different altitude: A study on tea (*Camellia sinensis*) clones of Darjeeling and Assam, India African Journal of Biochemistry Research. 2011; 5(5):148-159.
6. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols, The Journal of Nutrition. 2000; 130(8S):2073S-2085S.
7. Chakraborty K, Bhattacharjee S, Pal TK, Bhattacharyya S. Evaluation of *in vitro* antioxidant potential of Tea (*Camelia sinensis*) leaves obtained from different heights of Darjeeling Hill, West Bengal Journal of Applied Pharmaceutical Science. 2015; 5(1):63-68.
8. Wu CD, Wei GX. Tea as a functional food for oral health Nutrition 2002; 18:443-444.

9. Zuo Y, Chen H, Deng Y. Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and *Pu-erh* teas using HPLC with a photodiode array detector Talanta 2002; 57(2):N5-N7.
10. Kodama DH, Goncalves AEDS, Lajolo FM, Genovese MI. Flavonoids, total phenolics and antioxidant capacity: comparison between commercial green tea preparations. Ciênciae Tecnologia de Alimentos 2010; 30(4):1077-1082.
11. Ashok PK, Upadhyaya K. Tannins are astringent, Journal of Pharmacognosy and Phytochemistry. 2012; 1(3):45-50.
12. Chacko SM, Thambi TP, Kuttan R, Nishigak I. Beneficial effects of green tea: a literature review Chinese Medicine 2010; 5(13):1-9.
13. Mirzaei A, Mirzaei M. The effects of adding milk on antioxidant activities of green and black tea. Bulletin of Environment, Pharmacology and Life Sciences 2013; 2(5): 37-42.
14. Stewart JA, Mullen W, Crozier A. On-line high-performance liquid chromatography analysis of the antioxidant activity of phenolic compounds in green and black tea, Molecular Nutrition and Food Research 2005; 49(1):52-60.
15. Peterson J, Dwyer J, Bhagwat S, Haytowitz D, Holden J, Eldridge AL *et al*. Major flavonoids in dry tea, Journal of Food Composition and Analysis 2005; 18:487-501.
16. Chakraborty A, Bhattacharyya S. Thermal processing effects on *in vitro* antioxidant activities of five common Indian Pulses, Journal of Applied Pharmaceutical Science. 2014; 4(5):65-70.
17. Sarkar S, Saha S, Rai C, Bhattacharyya S. Effect of storage and Preservatives on Antioxidant Status of some Refrigerated Fruit Juices, International Journal of Current Microbiology and Applied Sciences. 2014; 3(7):1007-1013.
18. Khasnabis J, Rai C, Roy A. Determination of tannin content by titrimetric method from different types of tea, Journal of Chemical and Pharmaceutical Research. 2015; 7(6):238-241.
19. Pal TK, Bhattacharyya S, Dey A. Evaluation of antioxidant activities of flower extract (fresh and dried) of *Saraca indica* grown in West Bengal. International Journal of Current Microbiology and Applied Sciences. 2014; 3(4): 251-259.
20. Aich-Roy S, Pal TK, Bhattacharyya S. Effect of thermal processing on *in vitro* antioxidant potential of Capsicum (*Capsicum annuum*) of different ripening stages, Journal of Pharmacy Research. 2014; 8(12):1751-1756.
21. Sarwa KK, Rudrapal M, Debnath M. Extraction of green tea leaves: The use of different methods, their optimization and comparative evaluation. Biosciences Biotechnology Research Asia 2013; 10(1):383-386.
22. Yashin A, Yashin Y, Nemzer B. Determination of antioxidant activity in tea extracts, and their total antioxidant content, American Journal of Biomedical Sciences 2011; 3(4):322-335.
23. Sreeramulu D, Reddy CVK, Raghunath M. Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India, Indian Journal of Biochemistry and Biophysics. 2009; 46:112-115.
24. Jigisha A, Rai N, Kumar N, Pankaj G. Green Tea: A magical herb with miraculous outcomes, International Research Journal of Pharmacy. 2012; 3(5):139-148.
25. Shib M, Saha P, Pal TK, Bhattacharyya S. Thermal processing effects on *in vitro* antioxidant potential of fresh and packaged black pepper (*Piper nigrum*) and Indian red chili (*Capsicum annuum*) Annals of Biological Sciences. 2014; 2(3):72-78.