

Evaluation of Antioxidant Status in Cold Brewed Tea with Respect to Hot Decoction: Comparative Study Between Green and Black Varieties

Chandrima Das, Sirshendu Chatterjee*

Department of Biotechnology, Techno India University, West Bengal, EM-4, Sector V, Salt Lake, Kolkata: 7000091, India

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ABSTRACT

Tea is known as nature low calorie wonder drink. Consumption of hot tea decoction became popular from the ancient times. Currently researchers observed that many secondary metabolites that are heat sensitive might get destroyed on heating. Moreover people, who are permanently live at higher altitude or the members of high altitude expedition team, are deprived of various tea brewing facilities like electricity, fuel etc and hence they have to depend on brewing tea at cold condition only for consumption. In this backdrop present study aims at analysis of antioxidants like polyphenols, flavonoids and free radical scavenging activity of cold brewed tea and compared with its hot decoction. The study highlighted that cold brewed green tea harbour more polyphenolics and flavonoids with respect to its hot decoction. However no significant change being observed between hot and cold decoction of black tea. It is also observed that mg Ascorbic Acid Equivalent per cup of cold and hot tea brew has no significant difference and hence both the brews can be consumed for replenishment of antioxidant requirements.

Keywords: Antioxidant; Black tea; Cold tea brew; Green tea; Flavonoids; Polyphenolics.

INTRODUCTION

Tea decoction is the most preferred beverage in the world next to water¹. Tea decoction is generally prepared by brewing tea in hot water or milk or both. Among the several commonly available varieties, green and black teas are preferred most throughout the world. Green and black teas are preferred for aroma, taste, brew colour and various positive physiological functions². Health benefits of tea consumption have been well known to human civilization for centuries. Since the early days hot tea infusions was very much popular for improving mood, cognition ability, blood flow, detoxification and prevention of different diseases³. Phytochemical screening of tea leaves have shown the presence of more than 700 chemicals viz. polyphenols, flavonoids, alkaloids, tannins, volatile constituents and amino acids as the main active ingredients having potent antioxidant activities, anti-ulcer, anti-cancer and hepato-protective activities⁴⁻⁹. Among the several bioactive components of tea, polyphenols and flavonoids play an important role in maintaining health and wellness by boosting up the body's antioxidant system. Antioxidant is a group of compounds that help to protect the cells in from free radical damage, and thereby controlling stress and the rate of ageing. Scot et al showed that tea decoction can reduce fatigue of the team members during their high altitude expedition. During their journey hot brewed tea formed a major part of the required fluid intake where brewing tea at hot was possible, but at unfavourable condition for tea brewing, tea was excluded

from their diet¹⁰ because from the time unknown drinking hot tea decoction becomes the only popular way to consume the tea. Recent research shows that heating sometime deactivates or destroys several important heat sensitive bioactive molecules mainly flavonoids class of compounds present in the tea or other green vegetables or fruits¹¹. It is also observed that people of several high altitudes or extreme environments are devoid of all such facilities to prepare hot tea brew. In this backdrop finding an easiest low cost method become essential to serve tea and its bioactive components to all without any bar. Hence, the aim of this study was to screen total polyphenolics content (TPC), flavonoids content and free radical scavenging property of cold water decoction of tea and to compare with the traditional hot water decoction.

MATERIAL AND METHODS

Instruments and Apparatus

Analytical digital weighing balance (Wensor), UV-Visible Spectrophotometer (Sytronics117), Centrifuge (Remi PR 23) was used for the experiments.

Chemicals and Reagents

For quantitative estimation of polyphenols content, flavonoids content and their anti-oxidants activity in tea samples the following reagents and chemicals were used: Gallic Acid standard (Sigma), Ascorbic acid (Sigma), Sodium carbonate (Merck), Sodium Hydroxide (Merck), Anhydrous Sodium Nitrite (Merck), Anhydrous

Table 1: Tea Samples used for the present Study.

Sample No.	Types of Tea Sample	Brands
T1	Black Tea	Roasted Darjeeling Tea
T2	Black Tea	Brooke Bond Taj Mahal
T3	Black Tea	Tetley Tea Bags
T4	Black Tea	Castleton Vintage Darjeeling Tea
T5	Black Tea	Goodrick Thurbo Darjeeling
T6	Green Tea	Barnesbeg Organic Darjeeling
T7	Green Tea	Eco valley Pure Long Leaf
T8	Green Tea	Te-A-me Natural Teas
T9	Green Tea	Tetley Tea Bags
T10	Green Tea	Dilmah Natural Tea

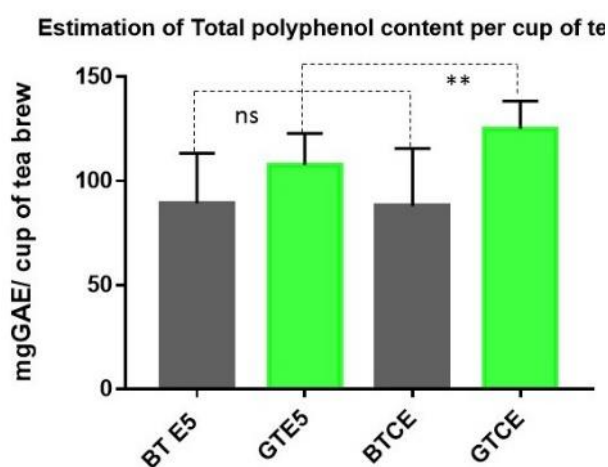


Figure 1: Comparative analysis of Total polyphenols contents in hot and cold decoction of green (n=5) and black tea (n=5). Here BTE5 and GTE5 denote black tea hot decoction and green tea hot decoction (5 min incubation at 90 degreeC) whereas BTCE and GTCE denote black tea cold decoction and green tea cold decoction (6 hr incubation at 4 degreeC). Here 'ns' signifies non-significant $P > 0.05$ and '**' signifies significant having p value = 0.0026.

Table 2: TPC in Hot and Cold decoctions of black and green varieties of tea. Here 5 black teas and 5 green teas were used for this experiment.

Tea Type	TPC in Hot Decoction (mg GAE/ cup of tea)	TPC in Cold Decoction (mg GAE/ cup of tea)
Black Tea	89.31 ± 23.99	87.97 ± 27.69
Green Tea	107.63 ± 15.17	125.10 ± 13.37

Aluminium Chloride(Merck), methanol (Merck), catechins (Sigma), Follin-ciocalteu reagent (Merck) and 2,2 diphenyl-1-picrylhydrazyl DPPH (Merck). All chemicals were analytical graded. HPLC grade water (Merck) was used throughout the experiment.

Sample Collection

Different tea samples were collected from the locally situated shop in Kolkata, West Bengal. Name of the tea brands are summarized in the table 1:

Preparation of tea infusion

Prior to extraction, tea samples were grinded by using mortar pestle and pass through a sieve in order to obtain a homogeneous fine powder. For preparing hot infusion 100 ml boiling water was added into 2 gm of powdered tea in 250ml conical flask and kept in 90 degree C temperature for 05 min, then clarified through Whatman filter paper (Grade 1). Similarly, cold tea brew was prepared by adding 100 ml water (at room temperature) into 2 gm of powdered tea in 250ml conical flask and kept in 4°C for 06 hr with intermittent shaking, then clarified through Whatman filter paper (Grade 1). The clear flow through was collected in a 100 ml volumetric flask, volume was made up with water and used for subsequent assay¹².

Determination of Total Polyphenolics Content (Follin-Ciocalteu Assay)

Tea infusions prepared from different samples were used to determine the total polyphenolics content using standard method¹³ with slight modification. Follin-Ciocalteu (1:10) reagent was added to each samples of both experiments followed by addition of 7.5% Sodium Carbonate. This reaction was incubated for 2 h. The absorbance was recorded at 765nm. The calibration curve was prepared using Gallic acid as standard^{12,13}.

Determination of Flavonoids Content (Aluminium chloride Assay)

Aluminium chloride colorimetric method¹⁴ was used for determination of flavonoids with slight modification. Tea extract was mixed with 5% sodium nitrate followed by 10% aluminium chloride. After 6 min 1M sodium hydroxide was added to it. Absorbance was recorded at 510nm. The calibration curve was prepared by using Catechin as standard^{12,14}.

DPPH Radical Scavenging Assay

DPPH Scavenging Assay was performed according to standard method¹⁵ with some modification to measure antioxidants properties present in natural products with slight modifications. DPPH (1, 1 - Diphenyl-2-picrylhydrazyl) is a stable free radical. 0.1mM DPPH solution was prepared in methanol solution and its OD was adjusted in between 0.9 to 1.000. 100 µL of each Tea samples (of suitable dilution) were added to DPPH solution and incubated for 30 min in dark. Absorbance was noted at 517 nm. The calibration curve was prepared by using Ascorbic acid as standard¹⁵.

Statistical Analysis

Graph Pad Prism7 has been used for statistical analysis of the data. Paired t test has been performed to study the significant changes among the data < 0.005 .

RESULTS

Total polyphenols content (TPC)

From the present study it was observed that the TPC in black tea has no such significant difference when brewed in cold and hot water. However a significant difference in hot and cold decoction of green tea was observed (Fig.1).

Estimation of Total Flavonoid Content per cup of tea Brew

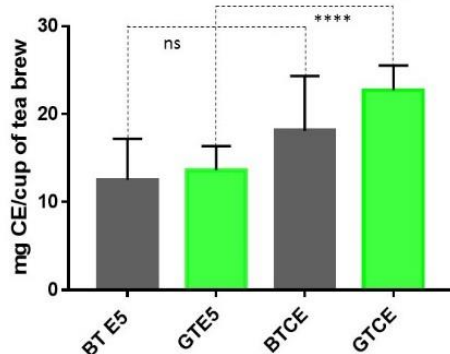


Figure 2: Comparative analysis of Flavonoids contents in hot and cold decoction of green (n=5) and black tea (n=5). Here BTE5 and GTE5 denote black tea hot decoction and green tea hot decoction (5 min incubation at 90 degreeC) whereas BTCE and GTCE denote black tea cold decoction and green tea cold decoction (6 hr incubation at 4 degreeC). Here ns signifies non significant and **** signifies 'highly significant' having p value = 0.0001.

Table 3: FC in Hot and Cold decoctions of black and green varieties of tea. Here 5 black teas and 5 green teas were used for this experiment.

Tea Type	FC in Hot Decoction (mg CE/ cup of tea)	FC in Cold Decoction (mg CE/ cup of tea)
Black Tea	12.5± 4.693	18.09± 6.219
Green Tea	13.6± 2.748	22.68± 2.835

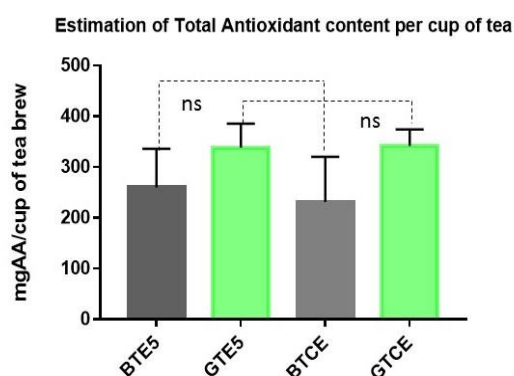


Figure 3: Fig. 1. Comparative analysis of mgAA/cup of hot and cold decoction of green (n=5) and black tea (n=5). Here BTE5 and GTE5 denote black tea hot decoction and green tea hot decoction (5 min incubation at 90 degreeC) whereas BTCE and GTCE denote black tea cold decoction and green tea cold decoction (6 hr incubation at 4 degreeC). Here ns signifies non significant.

With respect to hot green tea decoction, cold green tea decoction harbour more polyphenolics as seen in the Fig.1. Another interesting point was observed that like hot decoction a cup of cold green and black tea contains a good amount of polyphenolics (Table 2).

Total Flavonoids content (FC)

From the present study it was observed that the FC in black tea has no such significant difference when brewed in cold and hot water. However a significant difference in hot and cold decoction of green tea was observed (Fig.2.). With respect to hot green tea decoction cold green tea decoction contain significantly high amount of flavonoids as seen in the Fig.2.

Interestingly, like TPC hot decoction a cup of cold green and black tea contains a good amount of flavonoids (Table 3).

Total Ascorbic Acid Equivalent Content (TAAEC)

In this experiment TAAEC in different tea decoction has been calculated based on % DPPH radical scavenging assay where Ascorbic Acid was used as standard. Both in the case of green and black tea extracts infused in hot and cold water on average have shown no such significant difference statistically.

Another interesting point was observed that like hot decoction a cup of cold green and black tea contains a good amount of antioxidant (Total ascorbic Acid Equivalent Content).

DISCUSSION

Work stress is one of the most common factors nowadays. Antioxidants, flavonoids and polyphenols are very much needed for proper maintenance of health. Generally, vegetables, fruits and beverages have been suggested for better health as they full of important antioxidants, flavonoids and polyphenols. There are many medicinal plants whose bark, stem are being soaked in water for whole night and the water is drunk in the whole day. Our present work aimed to compare antioxidant status of cold brewed tea with the traditional hot tea infusion.

Tea is chosen for that study because tea is full of good antioxidants and easy to prepare. People of every economical background can procure tea as its price range from very low to high which based on the cultivation process, region, production process, tea cultivar etc. But one thing is true that all kind of tea contains good polyphenols that have huge health promoting activities. In common practice tea is commonly prepared by pouring hot or boiling water over cured leaves of the *Camellia sinensis*¹⁶. But problem occurs when there is scarcity of fuel or electricity. Basically high altitude areas are devoid of all the facilities for preparing tea. Moreover many secondary metabolites that are heat sensitive might get destroyed during hot decoction preparation time¹². In this situation drinking cold brewed tea is only the option. But this method is not popular like hot brewing procedure. So detailed scientific study only can popularize the cold brewed tea to common people. Very few works has been done to date¹⁷⁻¹⁸. Hence in the present have been designed to see the antioxidant status of cold brewed tea in compared to hot. We found that the almost similar amount of polyphenolics, flavonoids and anti-oxidants are released in both the brewing condition. However, it was observed that polyphenols and flavonoids content are significantly higher in cold brewed green tea with respect to hot decoction. However, it was observed that cold brewed tea

Table 4: TAAEC in Hot and Cold decoctions of black and green varieties of tea. Here 5 black teas and 5 green teas were used for this experiment.

Tea Type	TAAEC in Hot Decoction/cup of tea (mg AAE/ cup) of tea	TAAEC in Cold Decoction/cup of tea (mg AAE/ cup) of tea
Black Tea	260.06 ± 76.26	231.35 ± 89.18
Green Tea	338.77 ± 47.93	341.86 ± 32.46

is more or less comparable with hot decoction with respect to antioxidant activity. Ascorbic acid equivalent for cold brewed green and black tea is 341.86 ± 32.46 mg/cup and 231.35 ± 89.18 mg/ cup respectively which is pretty high with respect to average daily intake of vitamin C (107 mg)¹⁹. Therefore, we can say that after brewing the tea overnight in cold condition can also fulfil the antioxidant requirement of our body.

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CONFLICT OF INTEREST

No conflict of interest

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