


# Comparative evaluation of the impact of processing methods in determining the levels of health promoting chemical constituents and quality of green tea

Biplab Adhikary<sup>1#\*</sup> , Bishwapran Kashyap<sup>2#</sup>, Bappaditya Kanrar<sup>3</sup>, Romen C. Gogoi<sup>4</sup>, Sam Varghese<sup>5</sup> and Azariah Babu<sup>6</sup>

<sup>1</sup> Department of Biochemistry, North Bengal Regional R & D Centre, Tea Research Association, Nagrakata- 735225, Jalpaiguri, West Bengal, India

<sup>2</sup> Department of Analytical Services, Tocklai Tea Research Institute, Tea Research Association, Cinnamara, Jorhat – 785008, Assam, India

<sup>3</sup> TLabs, Tea Research Association, Kolkata-700 016, WB, India

<sup>4</sup> Department of TP & MA, Tocklai Tea Research Institute, Tea Research Association, Cinnamara, Jorhat-785008, Assam, India

<sup>5</sup> Chief Scientist and Chief Advisory Officer (West Bengal), North Bengal Regional R & D Centre, Tea Research Association, Nagrakata- 735225, Jalpaiguri, West Bengal, India

<sup>6</sup> Director, Tocklai Tea Research Institute, Tea Research Association, Cinnamara, Jorhat-785008, Assam, India

# These authors contributed equally: Biplab Adhikary, Bishwapran Kashyap

\* Corresponding author, E-mail: [biplabnator@gmail.com](mailto:biplabnator@gmail.com)

## Abstract

The initial step of green tea manufacture involves enzyme deactivation by heat application. The present study investigated the effects of various fixing and processing methods viz. steam-roasting (S-6), pan-firing (P-8), blanching (B-2), and CTC cuts after steam-roasting (S-CTC), on the biochemical profiles and organoleptic quality of green teas processed differently into orthodox and CTC types, from region-specific tea cultivars, suitable for green tea production under agroclimatic condition of Dooars, West Bengal, India. Differences in fixing method and processing style showed notable variation ( $p \leq 0.05$ ) in the chemical quality indicators of green tea viz. Total catechin, Polyphenol, Flavonoid and Water Extract content among the differently processed green teas. The most significant finding of the study revealed that when B-2 is employed for deactivation, it resulted in substantial reduction (47%–52%) of caffeine levels without affecting the catechins content and antioxidant potential of green tea samples, when compared to S-6 and P-8 methods. Interestingly, our results demonstrated significantly higher water extract values (42.19% dry weight) in green CTC-teas and lower values in B-2 green tea samples (33.67%), as compared to S-8 and P-8 green teas, which received better taster ratings ( $\geq 7$ ). These findings have highlighted the role of processing method and impact of fixing technique in determining the contents of health-promoting attributes and taste quality of green teas, thus providing diverse choices to tea producers and consumers to opt for specific green tea products and expedited the need to further explore its commercial application in the nutraceutical and pharmaceutical industry.

**Citation:** Adhikary B, Kashyap B, Kanrar B, Gogoi RC, Varghese S, et al. 2024. Comparative evaluation of the impact of processing methods in determining the levels of health promoting chemical constituents and quality of green tea. *Beverage Plant Research* <https://doi.org/10.48130/bpr-0024-0016>

## Introduction

Green tea is an un-aerated product made from the tender shoots of tea plant (*Camellia sinensis* L.). Unlike black tea, the processing technique employed for green tea production omits the oxidation stage by fixing of enzymes, allowing the tea to remain green in colour which leads to an assortment of external and inner qualities<sup>[1]</sup>. Green tea production employ both Chinese and Japanese style processing methods. Steaming is preferred in Japan and India, whereas the pan-firing method is popular in China and Korea. It has been reported earlier that differences in processing methods impart distinguishable changes in the biochemical profile and taste quality of green teas<sup>[2]</sup>. Green tea processed in orthodox form requires hand or machine-assisted rolling of deactivated tea leaves to get relatively bigger sized tea particles, but in the CTC method, the fixing step is followed by complete maceration of the tea leaves to produce smaller sized granular particles. Green tea fixing

methods like steaming, pan-firing and blanching has developed over the years depending on cultural variability, market acceptability and end-user preference<sup>[3]</sup>. The fixing stage of enzyme deactivation along with the rolling step, in which leaves are curled and twisted, are considered important technical parameters to attain desirable quality in green tea. Monitoring of the initial fixing step at elevated temperatures during green tea production is crucial to avoid catechin oxidation that are responsible for the astringency and bitterness of green teas, during storage<sup>[4]</sup>. It has been reported that over-fixing increases the amino acid content due to rapid protein hydrolysis, scorches the leaf and result in smoky taste and higher ratio of broken leaf whereas under-fixing results in browning of green teas upon storage due to residual enzyme activities<sup>[2]</sup>. Research studies suggest that bigger sized orthodox green teas retain more moisture and more likely to get oxidized, reducing its storage life and extraction efficiency, whereas, in contrast, the

CTC teas' increased surface area causes smaller particles to interact with water more during brewing, which increases the extraction efficiency of the final brew and therefore found suitable for ready-to-drink dip tea sachets<sup>[5]</sup>. Production of green CTC tea has been shown to be a simple, better recovery and less time-consuming method<sup>[6]</sup>, wherein, the teas exhibited bright green colour and more cuppage. The blending and mixing of herbs for desirable flavours becomes easier with the smaller sized CTC tea granules. Ready to drink flavoured CTC green tea variants are becoming popular nowadays and presents competitive and customer appealing products in the market. Few researchers have reported that the type and quality of the green tea reflected by its chemical composition and the soluble solids extracted during infusion not only depends upon genetic character and temperature of infusion but also affected by multifarious biochemical changes within the tea leaves during fixing as well as harvesting and subsequent processing conditions<sup>[3,7]</sup>. The primary leaf biochemicals imparting the taste characteristics and health-attributes of green tea include catechin polyphenols, caffeine, free amino acids, chlorophyll and other compounds jointly or separately<sup>[8]</sup>. The water-soluble extract of green tea are the main compounds responsible for bioactivity and the associated medicinal benefits<sup>[9]</sup>.

Scientific investigations on tea biochemicals in recent years have justified the ancient belief of health promoting benefits of green tea consumption<sup>[10]</sup>, which is gaining popularity among the wider community. Increased mass media propaganda on the medicinal health promoting effects linked with green tea consumption have created a lucrative market potential. As a popular non-alcoholic beverage, green tea products with diverse physical and chemical quality parameters would be equally beneficial to the tea manufacturers and consumers.

Ensuing the futuristic trend of Indian tea industry, there is a scope for Dooars tea estates to divert attention into these alternative ways of green tea processing as well as its promotion to capture the domestic market domain. Recent findings have generated information on region-specific green tea suitable cultivars<sup>[11]</sup>, however, a detailed scientific study in terms of biochemical constituents and organoleptic taste profiles of differentially fixed and processed orthodox and CTC green teas, has not been addressed under Dooars agroclimatic conditions. Therefore, the present study was conducted to investigate the impact of various fixing-methods viz. steaming, pan-firing and blanching for orthodox and CTC green teas, in determining the anti-oxidative and health promoting biochemical profiles, by keeping minimal variations in cultivars, plucking and other technical aspects of process-parameters.

## Materials and methods

### Chemicals and Machineries

All the chemicals used for this study were of analytical grade. Miniature manufacturing was carried out using a mini-CTC machine (Mesco Equipment) with 10 tpi (tooth-per-inch) roller. UV-VIS spectrophotometer (Cary Bio 50) was used for the purpose of biochemical analysis. HPLC analyses (Agilent 1260 infinity UPLC) for the estimation of catechin fractions and caffeine was carried out.

### Study site and agro-climatic conditions

The Dooars region is situated in the Himalayan foothills and have loamy to sandy loam type and silt clay type of soil. Tea cultivars used in the study were grown in the trial plot of our centre, situated at 26°54' North Latitude and 88°54' East Longitude with an elevation of 226.60 meters. Long-term weather data showed a daily temperature range of 18.7 °C to 28.8 °C, humidity levels of 91.5% (morning) and 64.2% (evening), 5.72 hours of average sunshine, and approximately 3800 mm of annual rainfall. Monsoon commences from May-June and lasts till September, followed by cold winters. During the period of our study, the maximum and minimum temperatures in the month of July, August and September were 31.4 °C, 32.3 °C, 31.3 °C and 21.8 °C, 21.6 °C, 20.5 °C respectively with corresponding precipitation recorded as 867.7 mm, 871.2 and 1058.2 mm. The experimental plot received the balanced fertilizers of Nitrogen, Phosphorus and Potassium in two splits at a ratio of 110:25:110, as recommended for tea cultivation in this region.<sup>[12]</sup>

### Selection of tea cultivars

Locally grown tea cultivars viz. Tocklai Vegetative (TV)-9, TV-20, Teenali (TA)-17/1/54, and Tocklai Stock (TS)-462 of similar age from our experimental plots were used for green tea processing. These cultivars were selected based on previous studies on the suitability of these cultivars for green tea production<sup>[11,13]</sup>. Leaf quality of 50-60% fineness, was maintained. Three sets of green tea samples were processed by different methods, at three separate occasions, during the month of July, August and September respectively, coinciding with monsoon flush and subsequently analyzed for chemical and sensory parameters.

### Green tea processing methods

The leaf samples in equal proportions from each cultivar was mixed and then divided into four parts of 0.5 kg each. Green tea processing of the orthodox and CTC-type was done in a miniature factory set-up by various methods, which included conventional Steam-fixing, Pan-firing, Blanching and Steam-roasting followed by 2 cut CTC, with minor simplified modifications as follows-

#### Steam-roasting (S-6)

Green tea was processed by steam-roasting in a perforated chamber at 105 ± 5 °C for 6-8 min, depending on leaf standard. The steamed leaves were surface dried and cooled by blowing air for 15 min, rolled in a peizy roller for 30 min and finally dried in a cabinet drier at 110 °C for 40 min<sup>[13]</sup>.

#### Pan-firing (P-8)

Fresh leaves were deactivated by pan-frying in an electric panner at 250 ± 10° C for 8-10 min, depending on leaf standard, cooled by blowing air for 15 min, rolled in a peizy roller for 30 min and finally dried in a cabinet drier at 110 °C for 40 min<sup>[11]</sup>.

#### Blanching (B-2)

Fresh leaves were fixed by dipping in boiling distilled water for 1-2 min depending on leaf standard. The subsequent procedure was similar to the conventional steam-roasting method.

#### CTC type (S-CTC)

Fresh leaves were deactivated by steaming in a perforated chamber at 105 ± 5 °C for 6-8 min, depending on leaf standard. The steamed leaves were surface dried and cooled by blowing air for 15 min, rolled in a peizy roller for 30 min prior to cutting

in a mini-CTC machine for two times (2-cut). Final drying was carried out in a cabinet drier at 110 °C for 40 min.

### Individual Catechins, gallic acid and Caffeine

Extraction of green tea catechins was done following the ISO method<sup>[14]</sup>. Samples were finely grounded in a mortar-pestle, and 0.2 g was taken in graded tubes. To the sample, 5 mL of 70% (v/v) pre-heated (70 °C) methanol/water extraction mixture was added and mixed thoroughly on a vortex mixer (Remi). The tubes were put in a water bath at 70 °C (10 minutes), followed by mixing in a vortex mixer after 5 and 10 minutes, respectively, and then cooled to room temperature, before centrifugation (3500 rpm, 10 minutes) (Remi). After decanting the supernatant in a graduated tube, the residue was extracted a second time with 5 mL of extraction mixture. The extracts were then mixed and concentrated to a volume of 10 mL using the methanol/water extraction mixture. For catechin analysis by HPLC, 1 mL of sample extract was mixed with 5 mL of stabilizing solution (10% v/v acetonitrile with 500 µg/mL EDTA and ascorbic acid), and filtered through a 0.45 µm filter and injected.

Individual catechins, gallic acid and caffeine was analyzed using Agilent 1260 infinity series HPLC equipped with Zorbax Eclipsed plus Phenyl-Hexyl column (4.6 x 250 mm, 5 µm) and Agilent Zorbax Eclipsed Plus Phenyl-Hexyl guard column (4.6 x 12.5 mm, 5 µm), in accordance with ISO method<sup>[14]</sup>. Different ratios of Water, acetonitrile, and acetic acid solvents were employed for mobile phase A (89:9:2) and mobile phase B (18:2:80). Both the mobile phases received an addition of EDTA (20 µg/mL). The injection volume was 20 µL. Flow rate, column temperature and detection wavelength were set to 1 mL/min, 35 ± 0.5 °C, and 278 nm respectively. A binary gradient condition was initiated with 100% mobile phase A for 10 minutes, followed by a linear gradient over a period of 15 minutes to 68% mobile phase A and 32% mobile phase B, which was run and maintained for 10 minutes. The system was then reset to 100% mobile phase A and given 10 minutes to equilibrate before the next injection. Catechin and caffeine peaks were identified by comparing retention times from sample chromatograms with those obtained from the mixed standard solutions under the same chromatographic conditions.

Quantification of individual catechins, gallic acid and caffeine was done by using the Relative Response Factors (RRFs) values for catechins and gallic acid with respect to caffeine and comparing the peak area of individual components to the caffeine calibration graph (Supplementary Table S1) as described<sup>[14]</sup>.

### Total Polyphenol content (TPC)

The Folin-Ciocalteu reagent was used to estimate the total polyphenol content by ISO method<sup>[15]</sup>. Approximately, 0.2 g of well-ground green tea powder was extracted using a methanol-water (70-30) mixture, and the extract was then diluted to 100 times with water. To 1 mL of the diluted extract, 3% v/v Folin-Ciocalteu reagent was added, followed by Sodium Carbonate solution (7.5% w/v), which was thoroughly mixed. The mixture was left to stand at room temperature for 1 hour. The result of the absorbance measurement at 760 nm was reported as a weight percentage by the gallic acid standard calibration curve values (Supplementary Table S1).

### Total Flavonoid content (TFC)

TFC was estimated following the method described<sup>[16]</sup>.

Powdered green tea sample (0.2 g) was extracted with methanol-water (70-30) mixture. 1 mL of this extract was diluted with 3 mL of methanol. To this diluted solution 0.2 ml of Aluminium Chloride (AlCl<sub>3</sub>, 1 M), 0.2 mL potassium acetate solution (10% w/v) and 5.6 mL distilled water were added and mixed well. Similarly, sample blank was prepared by replacing AlCl<sub>3</sub> with water. The absorbance reading was taken at 415 nm and result was obtained by quercetin standard calibration curve values (Supplementary Table S1).

### Water Extract (WE) content

The water extract content in green tea samples was determined using ISO method<sup>[17]</sup>, with minor modifications, to evaluate the effect of process type in determining the water-soluble extract content. Briefly, the non-grounded orthodox and CTC green tea samples (2.0 g) were put in in a 250 mL flask, and 200 mL hot distilled water was added and refluxed over low heat for 1 hour, rotating the flask occasionally. The flask was repeatedly washed out with hot distilled water transferring all the insoluble residue into a pre-weighed crucible. Finally, the residue was washed with 200 mL of hot water. Residue was dried by suction. The crucible and its contents were heated in an oven at 103 ± 2 °C, for 16 hours. The dessicator cooled crucible with its content was weighed. Result was expressed as a weight% on a dry mass basis, as mentioned<sup>[17]</sup>.

### Organoleptic evaluation and Tasters Quality Score (TQS)

ISO 3103:2109<sup>[18]</sup> was followed in the preparation process of tea infusion and liquor. This method entails extracting soluble substances from 2.8 ± 0.2 g of dried tea leaf contained in a porcelain cup using 150 ml of freshly heated water, that is covered with a lid for 4-5 minutes for brewing. The liquor is then poured into the bowl through the serration in the lid cover to keep the infused leaf in the pot. The lid was removed and then inverted and the infused leaf placed on it. The tea taster used a randomized method for the tasting, concealing the sample details in accordance with ISO 3163<sup>[19]</sup> and examined the infused leaf and liquor organoleptic qualities. Once the tea is cool enough, a tea taster uses a large spoon to noisily slurp the liquor into the lips, which guarantees a consistent taste profile by allowing adequate oxygen and tea to travel through the tongue's taste receptors<sup>[20]</sup>. The liquid was spit subsequently in a spittoon, before the next sample. The parameters of tea quality (colour, strength, briskness, and brightness), manufacturing defect parameters, and field-related agro-practices has been described by FAO<sup>[21]</sup>. After taking these findings into account, the taster evaluated the tea's organoleptic quality in Hedonic scale ratings ranging from 0 to 10<sup>[11,22]</sup>.

### Antioxidant assays

#### 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The DPPH antioxidant activity assay was carried out following a reported method<sup>[23]</sup>, with slight modifications. Briefly, the powdered tea sample (0.2 g) was extracted with methanol-water (70-30) mixture. DPPH solution (0.1 mM in methanol, 4 mL) was added to 2 mL each of the different concentration of the extract (2.5, 5, 7.5, 10, 12.5, 15 and 20 µg/mL). The reaction mixture was incubated at room temperature (30 min) and absorbance readings were taken at 517 nm, with ascorbic acid (100 µg/mL) as positive control and methanol as blank. The inhibition ratio (i.e., the concentration of the tea extract

required to reduce the absorbance of DPPH by 50%) of the sample was obtained from the following equation.

Inhibition ratio (%) =  $\{(Ac - As)/Ac\} \times 100$ ; where, Ac = Absorbance of the control and As = Absorbance of the sample

A plot of inhibition ratio against concentration gives a straight line from which  $IC_{50}$  was calculated.

#### Ferric reducing antioxidant potential (FRAP) Assay

The FRAP assay was performed following a reported method<sup>[24]</sup>, with some modifications. Preparation of FRAP reagent was done by mixing 10 mM 2,4,6-Tripyridyl-S-triazine (TPTZ) in 40 mM HCl with 20 mM Ferric Chloride ( $FeCl_3$ ) in 300 mM acetate buffer. For the assay, 200  $\mu$ L of tea extract, 1.8 mL of water and 4 mL FRAP reagent was mixed and the solution was incubated in the dark for half-an-hour. Then, the absorbance was taken at 593 nm using water as blank. The result was obtained by comparison with Ferrous Sulphate ( $FeSO_4$ ) standard graph.

#### Statistical analysis

The data of each experimental analysis that was performed in triplicate was analyzed by one-way analysis of Variance (ANOVA). Mean values of the biochemical parameters were separated using Duncan's multiple test range ( $p \leq 0.05$ ). All values are represented as mean  $\pm$  standard error (SE). Statistical analysis and correlations among the biochemical quality parameters were calculated by Pearson's correlation coefficient test by means of SPSS software (version 16.0., SPSS < Chicago, IL, USA).

## Results and Discussion

### Catechins, Caffeine and gallic acid content in green tea samples

The content of individual catechin fractions and total catechins, caffeine, gallic acid in green tea samples processed differently are presented in Table 1, and Fig. 1 respectively.

The data indicates that processing method exhibits a significant impact on the chemical composition of green tea samples especially caffeine. The content of Epigallocatechin was found to be marginally higher in pan-fired and blanched- green teas than steamed orthodox and CTC-mode of processing. Although insignificant, the amounts of Epigallocatechin gallate and Epicatechin gallate were found to be notably higher in S-6, P-8 and B-2 as compared to S-CTC samples. However, significant differences were observed between the Total Catechin contents of S-CTC type green tea as compared to other process-type green teas, which may be attributed to the loss of

gallated catechin fractions viz. Epigallocatechin gallate and Epicatechin gallate due to frictional heat generation at the crushing-tearing and curling step, that is congruent with a previous report that the level of green tea catechins is reduced due to epimerization and degradation during processing<sup>[25]</sup>, and storage conditions such as temperature and relative humidity<sup>[2]</sup>. Contrary to our findings, higher Total Catechin content for CTC green tea than orthodox type has been detailed<sup>[26,27]</sup>, the difference can be attributed to the diversity in tea cultivars, agroclimatic variation in the study and smaller size of CTC granules enhancing the extraction efficiency. It was noted that deactivation by blanching (B-2) reduced the caffeine content by 40–50% as compared to other green tea processing type viz. S-6, P-8 and S-CTC respectively (Fig. 1). Representative HPLC chromatographs of the mixed standards and green tea samples processed by different methods is mentioned in Fig. 1a.

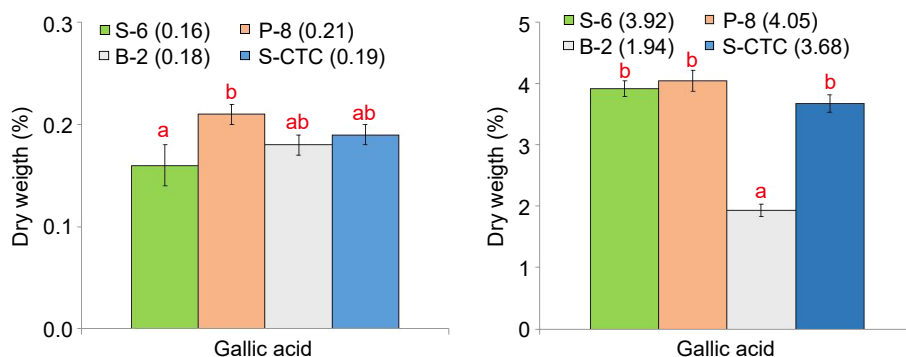
Our finding of significantly lower caffeine content of B-2 orthodox green tea (1.94%) as compared to other green tea types, is consistent with a previous report<sup>[27]</sup>, where it was conferred that blanching tea leaves for 3 minutes eliminated 83% of the caffeine while retaining 95% of the catechins. It has been reported that the solubility of caffeine is very low in water at room temperature (2.2% w/w), however, in boiling water, the solubility of caffeine increases greatly (66.7% w/w). Specific removal of significant amount of caffeine as compared to catechins, from fresh tea leaves during blanching, may be attributed to the higher solubility of caffeine in hot water and its lower molecular weight (21.7gL<sup>-1</sup>, 194.2 kDa) than the catechins (~ 5 gL<sup>-1</sup>, 290–458 kDa), that allows caffeine molecules to diffuse through the cell membrane and hence, during the blanching step, a large amount of caffeine goes out of the leaf resulting in lower caffeine content in the green tea<sup>[28]</sup>. Our data also indicated significant variation ( $p \leq 0.05$ ) in gallic acid content of S-6 and P-8 orthodox green teas that could have occurred due to wet and dry mode of heat application and resulted in greater loss of gallic acid in steaming by wet-heat as compared to pan-firing.

The combination of catechins with caffeine and gallic acid is often associated with green tea taste. Although caffeine intake has some proven health benefits, higher intake of caffeine can have negative impact on the human central nervous system and therefore contraindicated for children and pregnant women<sup>[29,30]</sup>. Moreover, studies have linked consumption of caffeine containing beverages with irritation of the gastrointestinal tract and sleeplessness<sup>[31]</sup>. There is persistent market demand for decaffeinated versions of tea and coffee beverage,

**Table 1.** Content (% dry weight) of non-gallated catechins viz. Catechin (+)C, Epicatechin (EC) and Epigallocatechin (EGC), Gallated catechins viz. Epicatechin gallate (EGCG) and Epigallocatechin gallate (EGCG), Caffeine (CAFF) and Gallic acid (GA) in green tea samples processed by different methods.

Process type	Catechin					Total Catechins (TC)	CAFF	GA
	C	EC	EGC	EGCG	EGCG			
S-6	0.99 $\pm$ 0.07	1.30 $\pm$ 0.06	3.87 $\pm$ 0.33	10.49 $\pm$ 0.57	2.84 $\pm$ 0.25	19.48 $\pm$ 0.44 <sup>ab</sup>	3.92 $\pm$ 0.13 <sup>b</sup>	0.16 $\pm$ 0.02 <sup>a</sup>
P-8	0.93 $\pm$ 0.03	1.46 $\pm$ 0.08	4.35 $\pm$ 0.31	10.84 $\pm$ 0.50	2.70 $\pm$ 0.14	20.26 $\pm$ 0.47 <sup>b</sup>	4.05 $\pm$ 0.17 <sup>b</sup>	0.21 $\pm$ 0.01 <sup>b</sup>
B-2	0.95 $\pm$ 0.05	1.39 $\pm$ 0.07	4.47 $\pm$ 0.40	10.79 $\pm$ 0.34	2.74 $\pm$ 0.25	20.33 $\pm$ 0.27 <sup>b</sup>	1.94 $\pm$ 0.10 <sup>a</sup>	0.18 $\pm$ 0.01 <sup>ab</sup>
S-6,2-C	0.94 $\pm$ 0.05	1.43 $\pm$ 0.07	3.91 $\pm$ 0.21	9.84 $\pm$ 0.53	2.58 $\pm$ 0.21	18.69 $\pm$ 0.42 <sup>a</sup>	3.68 $\pm$ 0.14 <sup>b</sup>	0.19 $\pm$ 0.00 <sup>ab</sup>

All values are represented as mean  $\pm$  SE. Values within a column with different letters are significantly different by ANOVA with Duncan multiple test range at  $p < 0.05$ . C, (+)-catechin; EC, (-)-epicatechin; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate; ECG, (-)-epicatechin gallate; TC, Total catechins; GA, Gallic acid; CAFF, Caffeine. S-6, steaming for 6 minutes; P-8, Panning for 8 mins; B-2, Blanching for 2 minutes; S-CTC, steaming for 2 minutes followed by 2 CTC cuts.



**Fig. 1** Content (% dry weight) of Gallic acid and Caffeine of green tea samples processed by different methods. Values are represented as mean  $\pm$  SE. Different lowercase letters on top of the bars indicate significant difference by ANOVA with Duncan multiple test range at  $p < 0.05$ ; S-6, steam-roasting for 6 minutes; P-8, Panning for 8 mins; B-2, Blanching for 2 minutes; S-CTC, steam-roasting for 2 minutes followed by 2 CTC cuts.

therefore, caffeine-reduction in the green tea manufacturing process is often desirable. Based on these findings, it can be suggested that fixation by blanching is one of the simple, non-toxic and low-cost process of decaffeinating green tea without removal of the catechin antioxidants.

### Total Polyphenol and Flavonoid Content

The TPC and TFC in green teas processed by different methods is presented in Fig. 2. Significant variation in polyphenol content was observed for green tea samples processed by different fixing- methods and types.

The conventional steam-roasting process (S-6) of orthodox green tea production retained the maximum polyphenols. Green teas (S-6, P-8 and S-CTC) exhibited higher polyphenol content (23.65%, 22.97%, 22.50%) as compared to B-2 (21.90%). Similar data was obtained for TFC, wherein significant differences ( $p \leq 0.05$ ) were obtained for S-CTC and B-2 type green tea. The extraction rate of green tea polyphenols is influenced by shape, size of tea leaves and degree of destruction during

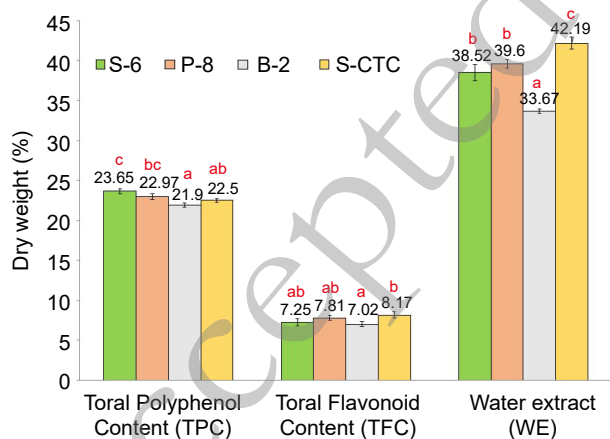
the fixing of leaves and usually the longer time of fixation during steaming and panning method causes more destruction of leaves yielding more small particles compared to the shorter duration blanching process. The bi-directional rolling after fixation step aids the juices to spread out in steam-roasted green teas as compared to other methods. The CTC step after steaming leads to loss of polyphenolic compounds because of prolonged processing<sup>[1]</sup>. Our results on differences of TPC and TFC content due to process-variation are in congruence with previous finding<sup>[32]</sup>, where the researchers inferred that thermal treatment by blanching resulted in transformation, as well as, loss of phenolic and flavonoid compounds due to leaching in water and therefore reduction in the phenolic and flavonoid content. Likewise, variation in polyphenol levels in the water infused extracts of green tea due to processing has also been described<sup>[33]</sup>.

Green tea polyphenols and flavonoids are key compounds conferring the antioxidative and therapeutic properties of tea consumption and also imparts astringency and bitter taste to green tea infusion. The water-soluble polyphenols and flavonoids have potential use singly or in combination with other active principles in food, pharmaceuticals, and cosmetic industry<sup>[10]</sup>. The health value of green tea beverages is determined, among others, by the content of polyphenolic substances, therefore, in summary, the conventional methods of steam-roasting and pan-firing are best suited to conserve polyphenols and flavonoids in green tea products.

### Water Extract content and Tasters Quality Score

Water Extract content is a quality indicator that constitutes the phenolics, alkaloids, amino acids, and many minor water-soluble substances extracted from the tea samples which determines the quality and cuppage of the tea, and employed in the tea industry<sup>[17]</sup>. Data presented in Fig. 2, showed the average water extract contents to be 38.52%, 39.60%, 33.67% and 42.19% for S-6, P-8, B-2 and S-CTC type- green teas respectively.

Based on our findings, all the analyzed green tea samples complied with the ISO requirement with regard to WE content, implying the presence of adequate extractable substances. However, significant variation was observed between orthodox and CTC type green tea samples. The WE content was higher in case of S-6, 2-CTC (42.19%) and lower in B-2 type processed orthodox green teas (33.67%). It has been reported



**Fig. 2** Content (% dry weight) of Total Polyphenol, Total Flavonoid and Water Extract of green tea samples processed by different methods. Values are represented as mean  $\pm$  SE. Different lowercase letters on top of the bars indicate significant difference by ANOVA with Duncan multiple test range at  $p < 0.05$ ; TPC, Total Polyphenol content; TFC, Total Flavonoid content; WE, Water Extract; S-6, steam-roasting for 6 minutes; P-8, Panning for 8 mins; B-2, Blanching for 2 minutes; S-CTC, steam-roasting for 2 minutes followed by 2 CTC cuts.

that the water extract of tea depends on tea and water ratio, temperature of the tea brew, type, and size of made tea particles<sup>[34]</sup>. Our findings emphasized that the CTC cut facilitated smaller particle size thereby presenting a larger surface area of tea granules exposed to water and enabling effective extraction of soluble constituents in water during brewing as compared to the orthodox tea type, whereas, the soluble solid content of B-2 process was lower compared to other fixing methods which can be attributed to the draining-out of water-soluble components during the blanching process<sup>[35]</sup>.

The benefit of CTC type green teas consisting of fannings and dust grades is that these are readily packaged in tea bags for easy marketability and a lesser time is required for brewing out the extractable bioactive compounds, than the leafy orthodox green teas, however, the smaller sized particles of CTC green teas are more influenced by oxidative processes during storage than whole tea leaves, because a larger surface area is exposed to oxygen and light<sup>[7]</sup>.

The TQS of steamed (S-6) and pan-fired green teas (P-8) showed significant difference with blanched (B-2) and steamed-CTC (S-6, 2-C) type with lesser bitterness component in orthodox green tea liquor that CTC type (Fig. 3), that indicates the preference of tea tasters towards conventionally processed orthodox type green teas<sup>[11,13]</sup>. Representative pictures of dry leaf, infused leaf and liquor appearance of green teas processed differently are shown in Fig. 4.

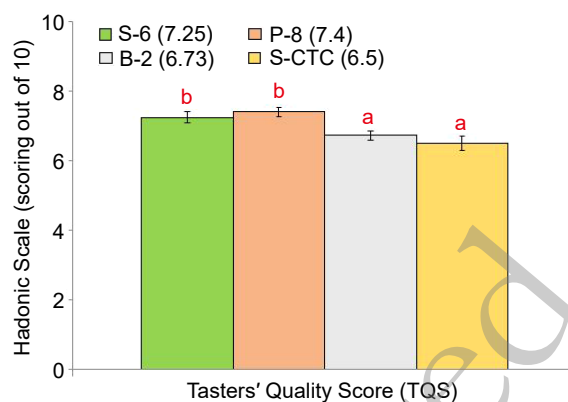
### Anti-oxidant activity

The antioxidant activity serves as an indicator for the proportion of antioxidant substances in green tea. Since, it is not always possible to characterize the antioxidant potential of tea by a single assay because the majority of naturally occurring antioxidants found in tea have multiple functions, hence, we used the DPPH and FRAP assays in the current study to describe the antioxidant activity of green tea samples processed in different ways. Under the conditions described in this manuscript, no significant differences were observed in the  $IC_{50}$  values of green tea samples processed by different methods (Fig. 5).

The B-2 and P-8 type processed green teas exhibited the maximum and minimum  $IC_{50}$  value of 18.21 and 16.34  $\mu\text{g/ml}$  respectively in the DPPH assay, which may be attributed to the loss of water-soluble antioxidants during blanching and negligible loss during deactivation by dry heat application or pan-firing. Likewise, in the FRAP assay, the values of S-6 and P-8 green teas were significantly higher than B-2 and S-CTC methods, wherein, the variance noted in the FRAP values can primarily be attributed to the loss of anti-oxidative polyphenols during the blanching and CTC steps of green tea processing<sup>[23]</sup>.

### Correlation Coefficient Matrix analysis

Pearson correlation was used to evaluate the relationship among the various chemical quality parameters and tasting scores for the green teas processed by four different methods (Table 2), wherein, a strong positively significant correlation was obtained between EGC and EC, TC and also between C and ECG, TFC. Additionally, Caffeine content was positively and significantly correlated with TPC, WE, TQS and likewise, TFC with EC; TC with EGCG; TQS with ECG, WE also displayed significant positive correlation. However, a negative correlation was observed between EGC and C, ECG, WE, TQS; C and EGCG, TC; EC, ECG with EGCG; TC with ECC, TFC, WE, TQS as reported



**Fig. 3** Taster Quality Score (TQS) of green tea samples processed by different methods. Values are represented as mean  $\pm$  SE. Different lowercase letters on top of the bars indicate significant difference by ANOVA with Duncan multiple test range at  $p < 0.05$ ; TQS, Tasters' Quality Scores. S-6, steam-roasting for 6 minutes; P-8, Panning for 8 mins; B-2, Blanching for 2 minutes; S-CTC, steam-roasting for 2 minutes followed by 2 CTC cuts.



**Fig. 4** Representative pictures of dry leaf appearance, infused leaf and liquor colour of green tea processed by different processing methods and used for organoleptic evaluation.

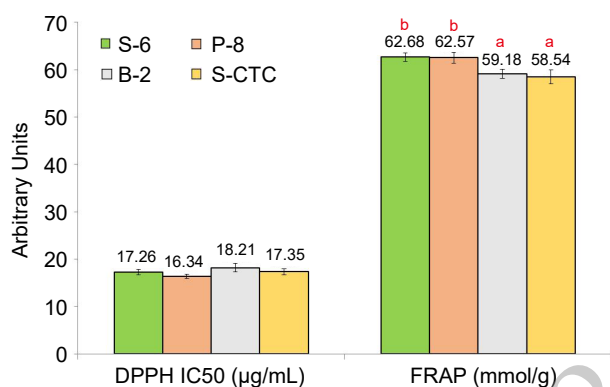
earlier<sup>[36]</sup>. These results confirm our above discussed results that some of the green tea chemical constituents are corre-

## Impact of processing methods on the green tea quality constituents

**Table 2.** Correlations between gallic acid (GA), epigallocatechin (EGC), catechin (C), caffeine (CAFF), epicatechin (EC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), total catechin (TC), total polyphenol content (TPC), total flavonoid content (TFC), water extract (WE) and tasters' quality score (TQS) in green tea samples processed by different methods.

	GA	EGC	C	CAFF	EC	EGCG	ECG	TC	TPC	TFC	WE	TQS
GA	1											
EGC	0.018	1										
C	0.099	-0.529**	1									
CAFF	0.090	-0.170	-0.118	1								
EC	0.035	0.510**	0.073	-0.186	1							
EGCG	-0.021	0.093	-0.669**	0.269*	-0.514**	1						
ECG	0.030	-0.679**	0.767**	-0.100	-0.044	-0.430**	1					
TC	0.021	0.523**	-0.630**	0.084	-0.051	0.809**	-0.409**	1				
TPC	-0.167	-0.073	-0.067	0.514**	-0.088	0.175	0.035	0.137	1			
TFC	0.158	0.241*	0.437**	-0.034	0.612**	-0.755**	0.147	-0.443**	-0.097	1		
WE	0.141	-0.495**	0.273*	0.514**	-0.072	-0.326*	0.302*	-0.556**	0.149	0.272*	1	
TQS	0.126	-0.483**	0.243*	0.399**	-0.079	-0.216	0.364**	-0.400**	0.173	0.137	0.856**	1

\*\* Correlation is significant at the 0.01 level (1-tailed). \* Correlation is significant at the 0.05 level (1-tailed). GA, gallic acid; EGC, (-)-epigallocatechin; C, (+)-catechin; CAFF, Caffeine; EC, (-)-epicatechin; EGCG, (-)-epigallocatechin gallate; ECG, (-)-epicatechin gallate; TC, Total catechins; TPC, Total polyphenol content; TFC, Flavonoid content; WE, Water Extract; TQS, Tasters' Quality score. The direction and magnitude of correlation between variables was quantified by the correlation coefficient  $r$ . One-tailed  $p$  value: one asterisk\*,  $p < 0.05$ ; two asterisks\*\*,  $p < 0.01$ .



**Fig. 5** Anti-oxidant activity in terms of DPPH and FRAP assay values of green tea samples processed by different methods. Values are represented as mean  $\pm$  SE. Different lowercase letters on top of the bars indicate significant difference by ANOVA with Duncan multiple test range at  $p < 0.05$ ; DPPH, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging ability; FRAP, Ferric reducing antioxidant power; S-6, steam-roasting for 6 minutes; P-8, Panning for 8 mins; B-2, Blanching for 2 minutes; S-CTC, steam-roasting for 2 minutes followed by 2 CTC cuts.

lated to the different methods of green tea processing.

## Conclusions

The findings of the current study provide practical information about the role of different processing methodology on green tea quality, the composition of health-promoting constituents in green tea extract and also established a trend in which the level of these green tea biochemicals are governed. Phytochemicals and antioxidative properties of orthodox green teas were significantly higher than CTC type. It is reported that blanching method of deactivation results in the production of green tea with significantly lesser amount of caffeine compared to steaming and pan-firing methods. Moreover, significant increase in the content of water extract was observed in CTC-green tea compared to orthodox type. However, the organoleptic evaluation by tasters preferred the orthodox green teas processed by steam and pan-firing than blanched

and CTC-green teas. The detailed scientific and comparative study on variation of chemical constituents due to processing differences of green tea provides relevant information for consumers and professionals from the tea and pharmaceutical industry. Further work on calibration of the green tea process parameters to obtain desirable biochemicals of therapeutic value, under large scale settings, can be attempted.

## Author contributions

B. Adhikary and B. Kashyap contributed equally to this work. B. Adhikary: Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Writing - original draft, review & editing; B. Kashyap: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Writing - original draft, review & editing.; B. Kanrar: Methodology, Resources, Writing - review & editing., R.C. Gogoi: Investigation, Validation, Writing - review & editing.; S. Varghese: Project administration, Supervision, Writing - review & editing; A. Babu: Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing. All authors reviewed and approved the final manuscript.

## Data availability

All data analyzed during this study are included in the published article [and its electronic supplementary information files].

## Acknowledgments

This work was supported by research grant from the Department of Science and Technology and Biotechnology- Government of West Bengal vide Research Grant, Ref: 936 (Sanc.) ST/P/S&T/1G-18/2016 dated 10/01/2017.

## Conflict of interest

The authors declare that they have no conflict of interest.

**Supplementary Information** accompanies this paper at

(XXXXXX)

## Dates

Received 19 January 2024; Revised ; Accepted 1 April 2024; In press 8 April 2024

## References

1. Astill C, Birch MR, Dacombe C, Humphrey PG, Martin PT. 2001. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. *Journal of Agricultural and Food Chemistry* 49(11):5340–5347
2. Xu N, Chen Z-M. 2002. Green tea, Black tea and semi-fermented tea, *Tea: Bioactivity and Therapeutic potential*. ISBN. 0-203-34636-X. Chapter: 35-57
3. Kim Y-K, Oh Y-J, Chung J-O, Lee S-J, Kim K-O. 2009. Chemical composition of green teas according to processing methods and extraction conditions. *Food Sci. Biotechnol* 18:1212–1217
4. Nagakawa M, Anan T, Ishima N. 1976. The relationship of green tea taste with its chemical composition. *Bull. Natl. Res. Inst. Tea* 17:69–123
5. Sharma V, Gulati A, Rabindranath SD. 2005. Extractability of tea catechins as a function of manufacture procedure and temperature of infusion. *Food Chem* 93:141–148
6. Gulati A, Rawat R, Singh B, Ravindranath SD. 2003. Application of microwave energy in the manufacture of enhanced-quality green tea. *Journal of Agricultural and Food Chemistry* 51(16):4764–4768
7. Wang LF, Kim DM, Lee CY. 2000. Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. *J. Agric. Food Chem* 48:4227–4232
8. Wei K, Wang L, Zhou J, He W, Zeng J, Jiang Y, Cheng H. 2011. Catechin contents in tea (*Camellia sinensis*) as affected by cultivar and environment and their relation to chlorophyll contents. *Food Chem* 125(1):44–48
9. Zhen YS. 2002. *Tea: Bioactivity and therapeutic potential*, Taylor and Francis, CRC Press, London
10. Bag S, Mondal A, Majumder A, Banik A. 2021. Tea and its Phytochemicals: Hidden Health Benefits and Modulation of Signaling Cascade by Phytochemicals. *Food Chem*. doi.org/10.1016/j. foodchem.2021.131098
11. Adhikary B, Kashyap B, Gogoi RC, Sabhapondit S, Babu A, Deka B, Pramanik P, Das B. 2023. Green tea processing by pan-firing from region-specific tea (*Camellia sinensis* L.) cultivars - a novel approach to sustainable tea production in Dooars region of North Bengal. *Food Chemistry Advances* 2: 100181
12. Malakar H, Timsina G, Dutta J, Borgohain A, Deka D, Babu A, Paul RK, Yeasin M, Rahman FH, Panja S, Karak T. 2022. Sick or rich: Assessing the selected soil properties and fertility status across the tea-growing region of Dooars, West Bengal, India. *Front. Plant Sci* 13:1017145
13. Adhikary B, Sen AB, Ghosh JJ, Tamuly P, Gogoi RC, Babu A. 2018. Study on selected tea cultivars of North Bengal for their suitability in green tea production. *Annals of Experimental Biology* 6(2):32–37
14. ISO 14502-2: 2005(E): Determination of substances characteristic of green and black tea - Part 2: content of catechins in green tea - Method using high performance liquid chromatography
15. ISO/CD 14502-1.2 (2001). Tea- Methods for determination of substances characteristics of green and black tea part 1: Determination of total polyphenols in tea- colorimetric method using Folin-Ciocalteu reagent
16. Akbay P, Basaran AA, Undeger U, Basaran N. 2003. In vitro immune modulatory activity of flavonoid glycosides from *Urtica dioica* L. *Phytothera. Res* 17:34–37
17. ISO 9768: 1994. Tea-Determination of water extract
18. ISO 3103: 2019 Tea - Preparation of liquor for use in sensory tests: International Organization for Standardization: Geneva, Switzerland, 1980
19. ISO 3163. Method for Preparation of Infusion for Sensory Evaluation" and "Examples of Pots and Bowls for Preparation of Tea Liquor". Geneva: ISO; (1990)
20. Joshi VK. "Method of sensory analysis and evaluation taste and overall quality," In Sensory Science, Principles and Application in Food Evaluation. Udaipur: Geeta Somani Agrotech Publishing Academy (2006)
21. FAO. Tea Tasting and Tea Tasting Terms In Tea Processing. Rome: FAO; (1974). p. 152–74
22. Gogoi RC, Sanyal S. Tea Tasting Manual. First Edition© ed. Jorhat: Tea Research Association; (2016)
23. Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a radical method to evaluate antioxidant activity. *Lebensm. -Wiss. u. - Technol* 28:25–30
24. Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry* 239:70–76
25. Ananingsih VK, Sharma A, Zhou W. 2013. Green tea catechins during food processing and storage: A review on stability and detection. *Food Research International* 50:469–479
26. Deka H, Barman T, Sarmah PP, Devi A, Tamuly P, Paul RK, Karak T. 2020. Quality characteristics of infusion and health consequences: a comparative study between orthodox and CTC green tea. *RSC Adv* 10:32833–32842
27. Liang H, Liang Y, Dong J, Lu J, Xu H, Wang H. 2007. Decaffeination of fresh green tea leaf (*Camellia sinensis*) by hot water treatment. *Food Chemistry* 101:1451–1456
28. Vuong QV, Roach PD. 2014. Caffeine in Green Tea: Its removal and isolation. *Separation and Purification Reviews* 43:155–174
29. Giannelli M, Doyle P, Roman E, Pelerin M, Hermon C. 2003. The effect of caffeine consumption and nausea on the risk of miscarriage. *Pediatric and Perinatal Epidemiology* 17,4:316–323
30. Kerrigan S, Lindsey T. 2005. Fatal caffeine overdose: two case reports. *Forens. Sci. Int* 153:67–69
31. Chu DC, Juneja LR. 1997. General Chemical Composition of Green Tea and Its Infusion. In: Yamamoto, T., Juneja, L. R., Chu, D. C. and Kim, M., Eds., *Chemistry and Applications of Green Tea*. CRC Press, Boca Raton, 13-22
32. Xua B, Changa SKC. 2008. Effect of soaking, boiling, and steaming on total phenolic content. *Food Chem* 110:1–13
33. Unachukwu UJ, Ahmed S, Kavalier A, Lyles JT, Kennelly EJ. 2010. White and Green Teas (*Camellia sinensis* var. *sinensis*): Variation in phenolic, methylxanthine, and antioxidant profiles. *Journal of Food Science*. 75, 6: C541-C548
34. Yao L, Liu X, Jiang Y, Caffin N, D'Arcy B, Singanusong R, Xu Y. 2006. Compositional analysis of teas from Australian supermarkets. *Food Chem* 94(1):115–122
35. Ahmed S, Stepp JR. 2013. Green tea: The plants, processing, manufacturing and production. *Tea in Health and Disease Prevention*. ed. Victor R. Preedy, Academic Press-Elsevier, Chapter-2: 19-31
36. Bhuyan LP, Hussain H, Tamuly P, Gogoi RC, Bordoloi PK, Hazarika M. 2009. Chemical characterisation of CTC black tea of northeast India: correlation of quality parameters with tea tasters' evaluation. *J Sci Food Agric*. 89:1498–1507



Copyright: © 2024 by the author(s). Published by Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit <https://creativecommons.org/licenses/by/4.0/>.