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# Quality characteristics of infusion and health consequences: a comparative study between orthodox and CTC green teas†

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The present study compares the effects of two green tea processing techniques, viz. orthodox and CTC (curl, tear and crush) on the quality parameters and sensory profiles under the geographical and climatic conditions of Assam, India. The results showed that CTC green tea infusions had 13.3, 7.5, 7.1, 9.8, 5.4, 17.3, 17.1 and 18.6% more total polyphenol, total catechin, (–)-epigallocatechin-3-gallate (EGCG), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC), (–)-epicatechin (EC), water extract and theanine level, respectively than the infusions prepared from orthodox green tea. The sensory evaluation preferred the orthodox over CTC processing mode. Risk assessment with daily consumption of five cups (10 g) of green tea reveals that the EGCG level is free from the risk of hepatotoxicity and caffeine will not inflict any health hazard.

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## 1. Introduction

The potential health beneficial activities have made green tea widely popular. The popularity and demand for green tea in India are increasing at a high rate after Asian countries like China and Japan. Food and nutritional sciences have given in-depth attention to a better understanding of its chemical constituents and their benefits.<sup>1</sup> Many researchers have demonstrated the beneficial effects of green tea consumption on human health over many diseases. Epidemiological study reveals direct relationship of green tea consumption with enhanced human leukocyte activity,<sup>2</sup> reduced risk of Parkinson's disease<sup>3</sup> and Alzheimer's disease.<sup>4</sup> The most dominant green tea catechin (–)-epigallocatechin-3-gallate has been demonstrated effective against viral infection,<sup>5</sup> various types of cancers in a number of *in vitro* studies.<sup>6,7</sup> Clinical studies on green tea demonstrate its effectiveness in cognitive dysfunction,<sup>8</sup> modulation of brain activity.<sup>9</sup> These medicinal effects can be attributed to its flavanol (also called catechin) contents

which are the major group of polyphenolic compounds present in tea. The principal flavanols present in green tea are (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG) and (–)-epigallocatechin-3-gallate (EGCG).<sup>10</sup> The polyphenolic compounds account for 24–36% of total biochemical contents in tea.<sup>10</sup> Furthermore, green tea is an unfermented tea and its infusion has higher content of polyphenols than other types of fermented teas such as black and oolong tea.<sup>11</sup> Fermented teas are reasonably expected to have a similar level of polyphenols as that of unfermented teas. However, the complex and polymeric nature of most of the polyphenols, formed during the fermentation stage, has prevented chemical characterization.<sup>12</sup> The taste of green tea infusions primarily comprises astringency, bitterness and umami.<sup>13</sup>

Several researchers reported green tea processing with some modifications in the conventional orthodox processing method, and their effect on biochemical constituents.<sup>10,14,15</sup> In orthodox processing of green tea, first deactivation of endogenous enzymes, mainly polyphenol oxidase and peroxidase is carried out. This deactivation of fresh leaf enzymes is the key step in green tea processing which prevents the oxidation of catechins. This could be achieved either by steaming or pan firing of green leaves.<sup>16</sup> However, these enzyme deactivation processes drastically affect the quality of green tea, particularly the aroma and physical appearance. Typically, in the steaming process, freshly plucked leaves are subjected to hot steam of water at around 102 °C for a certain amount of time (1–2 min).<sup>15</sup> On the other hand, in the pan firing process, freshly plucked leaves undergo withering for 2–3 h, where endogenous enzymes affect the chemical composition of the leaves,<sup>15,16</sup> followed by pan-fired at

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220 °C for 5 minutes.<sup>14–16</sup> The steamed or pan-fired leaves are then rolled for 30–60 minutes for rupture of the leaf tissues as well as to have the desired shape and finally dried at 90–110 °C for 5–15 minutes.

CTC is widely used in black tea manufacturing process. The process involves five different steps consisting of withering, rolling, CTC, fermentation and drying. In the CTC step, the rolled leaves are subjected to crushing, tearing and curling in a CTC machine. The advantages of CTC black tea manufacturing are lower fermentation time and higher extraction efficiency. Very limited literatures are available where CTC step has been introduced for green tea processing. Barbora and Saha introduced CTC after the initial step of steaming or pan firing for green tea processing.<sup>17</sup> The authors reported more cuppage (also known as number of cups) and shorter processing period for CTC green tea over the orthodox. However, no biochemical data was reported by the authors. Kilel *et al.* also introduced the CTC step in green tea processed from purple colored tea clones of Kenya.<sup>18</sup> To the best of our knowledge, report on the comparative study of orthodox and CTC green tea processing is very limited in terms of biochemical constituents and their subsequent transfer into the infusion. Moreover, this aspect in the scenario of Assam's geographical and climatic condition has never been addressed. Therefore, the present investigation was aimed to determine the change in biochemical content on introducing CTC during green tea manufacturing over conventional orthodox processing and transfer rate of bioactive compounds from tea to its infusion. Organoleptic evaluation (also known as sensory evaluation) of both types of tea and the risk assessment of the daily intake level of caffeine and EGCG were also carried out.

## 2. Materials and methods

### 2.1. Chemicals

Gallic acid monohydrate ( $\geq 98.0\%$ ), caffeine (anhydrous, 99%), (–)-epigallocatechin-3-gallate ( $\geq 95\%$ ), (–)-epicatechin-3-gallate ( $\geq 95\%$ , HPLC), (–)-epigallocatechin ( $\geq 95\%$ , HPLC), (–)-epicatechin ( $\geq 90\%$ , HPLC), (+)-catechin ( $\geq 95\%$ , HPLC) and L-theanine ( $\geq 98\%$ , HPLC) were procured from Sigma Aldrich, India. Folin–Ciocalteu reagent (LR) was purchased from Himedia (HiMedia Laboratories, India). Acetic acid (HPLC Grade), acetonitrile (HPLC Grade), sodium carbonate and all other chemicals were obtained from Merck KGaA, Darmstadt, Germany.

### 2.2. Instrumentation

A Dionex, Ultimate 3000 UPLC system fitted with Luna 5  $\mu$  phenylhexyl phenomenax column (4.5 mm  $\times$  250 mm) maintained at  $25 \pm 0.5$  °C and UV-vis detector was used for determination of catechin, caffeine and theanine. The detector wavelength was set at 278 nm for catechin and caffeine quantification. For theanine, it was set at 210 nm. For UV-vis spectroscopy, Varian, Cary 50 Conc was used. Millipore Milli-Q Synthesis water purification system from Merck, Germany was used for Milli-Q water in all experiments. A fluid bed drier (FBD)

from Teacraft, United Kingdom was used for enzyme deactivation and drying of green tea. A CTC roller with 10 TPI (teeth per inch) was used for CTC green tea processing. A Thermo Scientific, Orion Star A214 ion selective electrode (ISE) meter was used for quantification of fluoride and chloride. For flame photometry, a Systronics India Ltd flame photometer (Model 128) was used.

### 2.3. Plucking of tea leaves

The tender two leaves and the terminal bud from cultivars TV1, 9, 18, 20, 22, 23 and 25, Happy Valley 39, RR17/144, Ging186 and 482/12 grown under the identical environmental condition and soil nutrient management were hand-plucked from experimental Tea Estate (T.E.) of Tocklai Tea Research Institute (TTRI), Assam, India which lies between  $26^{\circ}43'14''\text{N}$  and  $94^{\circ}11'54''\text{E}$  and located at an elevation of 96.5 meters above mean sea level with an average precipitation of 2036 mm. Sample from each cultivar consisted of three biological replicates and green tea processing was carried out for each replicate sample.

### 2.4. Green tea processing

The fresh samples were subjected to hot air blow in the FBD set at  $100 \pm 5$  °C for six minutes for enzyme deactivation. The deactivated samples were allowed to cool immediately with the exposure of cold air followed by hand-rolling for 30 minutes. The mass samples were divided into two parts. One part of the rolled samples was subjected to 3 cut CTC and then dried in the FBD at  $90 \pm 2$  °C for twenty minutes to have the CTC green tea. The other part of the rolled leaves was dried under identical conditions to have the orthodox green tea.

### 2.5. Sensory evaluation of tea infusion

Green tea infusions were assessed by a tasting panel consisting of nine members aged between 23 and 55 years. The panellists were trained to quantify and evaluate the intensity of bitterness, sweetness, astringency and umami taste using deionised water solution of caffeine (1 mM), sucrose (50 mM), EGCG (0.8 mM), and sodium glutamate (3 mM), respectively for standardisation of judgments.<sup>19</sup> A 10-point scale was used for scoring, where 8–10 indicates 'extremely strong', 6–8 indicates 'strong', 4–6 indicates 'neutral', 2–4 indicates 'weak' and 0–2 indicates 'extremely weak'.<sup>11</sup> Tea infusions were prepared by brewing 2 g green tea in 150 mL boiled water for 3 minutes. The tea was served in white porcelain bowls at room temperature. For the tasting, 5 mL infusion was held for 20 s in the oral cavity by the panellists and scores for different tastes were drawn. For preventing previous taste interference the panellists cleansed their palates with skimmed milk and flushed oral cavity thrice with water before tasting the next infusion.<sup>19</sup> Three replications were performed for each evaluation on three different days with sample orders being randomised in each day.

Determination protocol of catechin, caffeine, theanine, water extract as well as calibration standard are available in ESI.† Besides these, transfer rate of bioactive compounds and



determination of sodium, potassium, fluoride and chloride in tea infusion are also available in ESI.†

## 2.6. Risk assessment

Estimated daily intake (EDI) of caffeine and catechins on consuming five cups (10 g) of green tea in milligram per kilogram body weight ( $\text{mg kg}^{-1}$  BW) per day was calculated by the following formula

$$\text{EDI} = \frac{C \times M \times T}{\text{BW} \times 100} \quad (1)$$

where  $C$  is the mean concentration of the biochemical compound of interest in green tea ( $\text{mg g}^{-1}$ );  $M$  is the weight of consumed green tea per day (10 g);  $T$  is the transfer rate in percentage and BW is the average body weight for men (67.4 kg) and women (64.9 kg).<sup>20</sup>

## 2.7 Statistical analysis

Analysis of variance (ANOVA) was performed by using SPSS software version 17.00 (SPSS Inc., Chicago, IL). Tukey's multiple comparison test was used to get the differences between means and the differences were considered significant at  $p \leq 0.05$  and  $p \leq 0.01$ . For each sample, all data were reported as the mean  $\pm$  standard error (SE) with three independent replications. Pearson correlations were drawn using SPSS software version 17.00 (SPSS Inc., Chicago, IL) among the different taste attributes and contributing biochemical contents to study the association among them.

# 3. Results and discussion

## 3.1. Total polyphenol

Total polyphenol (TP) refers to flavanols (catechins), catechin gallates, flavonoid glycosides and phenolic acids. The polyphenols impart astringency taste to green tea infusion. The TP contents in CTC and orthodox green teas, in infusions, and their extent of transfer into infusions are presented in Fig. 1(a). TP content varied from  $199.7 \pm 3.4 \text{ mg g}^{-1}$  in RR17/144 to  $258.2 \pm 1.4 \text{ mg g}^{-1}$  in Ging186. The highest TP level in Ging186 observed significant difference ( $p \leq 0.05$ ) from that in TV9, 20, 23, 25 and RR17/144. Available literature reported TP level in green tea in the range from 208 to  $259 \text{ mg g}^{-1}$ ,<sup>18</sup> 182.6 to  $296.4 \text{ mg g}^{-1}$ <sup>21</sup> determined using Folin–Ciocalteu reagent. Wang and Ruan reported TP level in Longjing green tea in the range from 211.2 to  $281.2 \text{ mg g}^{-1}$  using a  $\text{FeSO}_4\text{-NaKC}_4\text{H}_4\text{O}_6$  dyeing solution and phosphate buffer.<sup>22</sup> These observations are in agreement with the current findings. However, Kilel *et al.* reported that two traditionally used clones, Yabukita and Hanlu for green tea manufacturing in Japan and China, had lower levels of TP at  $172 \text{ mg g}^{-1}$  and  $197 \text{ mg g}^{-1}$ , respectively.<sup>18</sup> The CTC step significantly influenced the transfer rate of TP to infusion in all the cultivars. As the introduction of CTC in green tea processing resulted in increased surface area of the leaves that is exposed to the solvent, it facilitated the transfer of bioactive compounds into infusions.<sup>23</sup> An enhanced average transfer rate of 13.3% was observed in CTC green tea. The TP

level in CTC green tea infusion (CGTI) was in the range between  $81.8 \pm 2.1$  and  $120.7 \pm 1.4 \text{ mg g}^{-1}$  (equivalent to 1151–1652  $\text{mg L}^{-1}$  GAE), whereas in orthodox green tea infusion (OGTI) it was between  $53.6 \pm 1.9$  and  $92.4 \pm 1.3 \text{ mg g}^{-1}$  (equivalent to 783–1265  $\text{mg L}^{-1}$  GAE). The highest and lowest TP level was in TV1 and TV9, respectively, for both processing modes. These results support the findings of Liu *et al.* (500–1700  $\text{mg L}^{-1}$  GAE, Folin–Ciocalteu reagent method) where the effect of particle size and brewing condition on TP level in green tea infusions were demonstrated.<sup>23</sup> Farakte *et al.* reported a variation of rate constants from 0.257 to  $0.685 \text{ min}^{-1}$  while preparing tea infusion with a varying particle size of tea.<sup>24</sup> Smaller particle size had higher extraction leading to faster infusion. During the CTC step, massive rupture of cell membranes exposes the biochemical contents. The biochemical components at the exposed surface can easily get dissolved in water. Furthermore, the transfer of compounds from tea to its infusion depends on its concentration in leaf and solubility.<sup>25</sup> The dissolution of TP in water observes different phases of rapid and slow rate. The extraction of TP increases with time. The varying dissolution phases may be attributed to different solubility of the polyphenolic substances present in tea leaves.<sup>25</sup> The current results also support the TP levels in green tea infusions (880–2560  $\text{mg L}^{-1}$  GAE, Folin–Ciocalteu reagent method) reported by Komes *et al.*<sup>26</sup>

## 3.2. Total catechin

As evident all green tea samples proved to be a rich source of phenolic compounds. The total catechin (TC) contents in CTC and orthodox green teas, in infusions, and along with their transfer rate into infusion are presented in Fig. 1(b). The highest TC content of green teas was detected in Ging186 ( $234.5 \pm 1.2 \text{ mg g}^{-1}$ ) which is significantly different ( $p \leq 0.05$ ) from all others except 482/12 and the lowest TC was detected in TV1 ( $172.9 \pm 3.2 \text{ mg g}^{-1}$ ). Unlike our findings, Kilel *et al.* reported TC content in green tea processed from purple leaf colored cultivars of Kenya in the range between 52 and  $120 \text{ mg g}^{-1}$  using HPLC.<sup>18</sup> They also demonstrated two standard green tea clones, Yabukita and Hanlu with TC contents of 94.1 and  $123.0 \text{ mg g}^{-1}$ , respectively. The catechin (flavanol) content in fresh tea leaves is affected by sunlight intensity, temperature, rainfall, harvesting season, and other environmental factors. Therefore, the geographical origin of the growing region of tea cultivar plays a critical role in its biochemical contents which is reflected in the quality of processed tea.<sup>27,28</sup> With increasing sunshine hour biosynthesis of flavanol increases to protect the plant from UV rays' damage.<sup>29</sup> Moreover, the genetic diversity of tea plant is another important factor which affects its biochemical contents. However, the environment of the growing region is predominant in controlling the biochemical composition over that of genetic characteristics of the cultivar.<sup>29</sup>

The CTC step significantly influenced the transfer rate of TC into infusion in all the cultivars. The average transfer rates of TC into infusion were found to be 25.8% and 18.3% for CTC and orthodox green tea, respectively. TC content in CGTIs ranged from  $34.8 \pm 0.8 \text{ mg g}^{-1}$  in TV1 to  $84.9 \pm 1.1 \text{ mg g}^{-1}$  in RR17/144



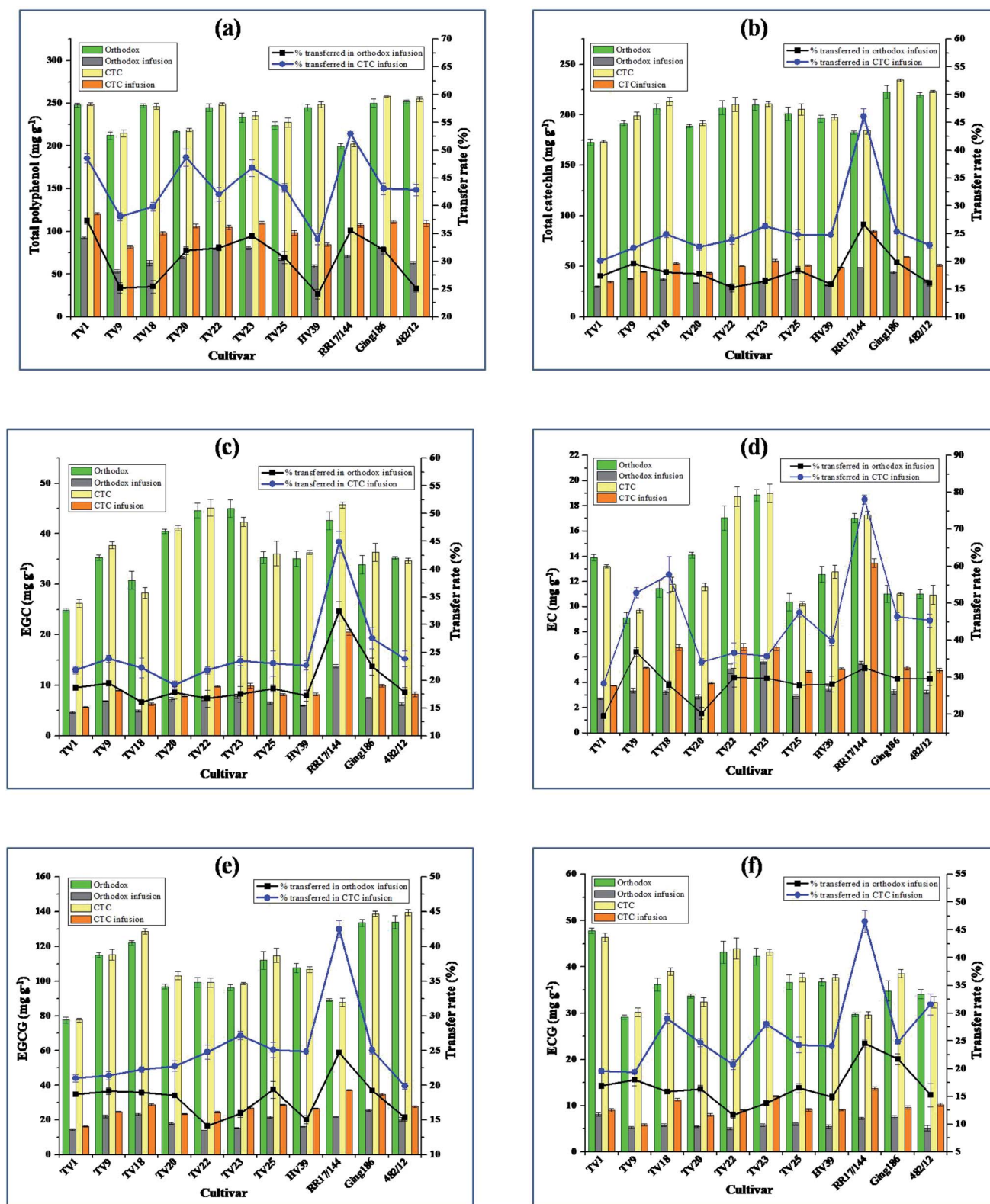


Fig. 1 Average content (mg g<sup>-1</sup>) of total polyphenol (a), total catechin (b), EGC (c), EC (d), EGCG (e), and ECG (f) in CTC and orthodox green tea, in infusion, and their per cent transfer into infusions (error bars specify the standard error).

(equivalent to 463.9–1133.1 mg L<sup>-1</sup>), whereas in OGTIs it was from 29.9 ± 0.9 mg g<sup>-1</sup> in TV1 to 48.6 ± 0.3 mg g<sup>-1</sup> in RR17/144 (equivalent to 399.1–647.6 mg L<sup>-1</sup>). The present results of TC

content are comparable with other documented results such as 350.45–1073.15 mg L<sup>-1</sup>,<sup>26</sup> 398–1127 mg L<sup>-1</sup>,<sup>30</sup> 571–1416 mg L<sup>-1</sup><sup>31</sup> determined using HPLC. From infusion kinetics



study of CTC tea with varying particle size, Farakte *et al.* demonstrated that infusion prepared with tea (particle size 0.36 mm) observed 90% extraction in 3 min indicating its very fast extraction rate whereas tea with particle sizes 0.63, 0.83 and 1.17 mm had around 80% extraction in 3, 4 and 5 min, respectively.<sup>24</sup> The diffusion of biochemical compounds from the complex tea matrix affects the infusion rate. This diffusion process becomes easier with reduced size of tea granules as the diffusion path gets shortened.

### 3.3. Non gallated catechins

Non gallated catechins comprise of (–)-epigallocatechin (EGC) and (–)-epicatechin (EC). The EGC and EC contents in green tea, in infusion, and its extent of transfer into infusion are presented in Fig. 1(c) and (d), respectively. Non-gallated catechins impart less astringency as compared to its gallated counterpart. EGC contributes a slight sweet after taste, even at a concentration level as low as 0.1% (w/v).<sup>18</sup> It was found that RR17/144 ( $45.7 \pm 0.6 \text{ mg g}^{-1}$ ) and TV22 ( $45.2 \pm 1.7 \text{ mg g}^{-1}$ ) had the highest content of non-gallated catechin EGC, while TV1 ( $24.9 \pm 0.4 \text{ mg g}^{-1}$ ) had the lowest. Green tea processed from cultivar TV23 had the highest EC content with  $19.0 \pm 0.7 \text{ mg g}^{-1}$  and TV9 had the lowest with  $9.1 \pm 0.4 \text{ mg g}^{-1}$ . In line with our results, Kilel *et al.* reported EGC and EC levels of green tea processed from Kenyan tea cultivars in the range between 4.0 and  $61.2 \text{ mg g}^{-1}$ ; and between 2.0 and  $18.0 \text{ mg g}^{-1}$ , respectively.<sup>18</sup> Dai *et al.* found EGC and EC level in Huangshan Maofeng green tea much lower than the current study at  $11.32$  and  $8.68 \text{ mg g}^{-1}$ , respectively using HPLC.<sup>21</sup> CGTIs had higher EGC levels in the range between  $5.7 \pm 0.1$  and  $20.5 \pm 0.6 \text{ mg g}^{-1}$  (equivalent to  $76.1$ – $273.3 \text{ mg L}^{-1}$ ) than OGTIs in the range between  $4.7 \pm 0.2$  and  $13.7 \pm 0.3 \text{ mg g}^{-1}$  (equivalent to  $62.0$ – $183.2 \text{ mg L}^{-1}$ ). EC levels in CGTIs varied from  $3.7 \pm 0.1$  to  $13.5 \pm 0.4 \text{ mg g}^{-1}$  (equivalent to  $49.9$ – $179.6 \text{ mg L}^{-1}$ ) whereas in OGTIs it was from  $2.7 \pm 0.1$  to  $5.6 \pm 0.2 \text{ mg g}^{-1}$  (equivalent to  $36.3$ – $74.8 \text{ mg L}^{-1}$ ). These results are consistent with previously published EGC and EC levels in tea infusion in the range from  $69.71$  to  $279.87 \text{ mg L}^{-1}$  and  $48.80$  to  $169.16 \text{ mg L}^{-1}$ , respectively.<sup>26</sup> The data reported here also supports the earlier documented EGC ( $34.9$ – $291 \text{ mg L}^{-1}$ ) and EC ( $19.3$ – $154 \text{ mg L}^{-1}$ ) levels in green tea infusions using HPLC.<sup>23</sup>

The processing mode had a significant influence on the transfer rate of non-gallated catechins into the infusion. The higher extraction level of non gallated catechins in CGTIs is probably due to reduced pore diffusion path of the tea matrix in CTC green tea. Green tea processed from cultivar RR17/144 had the highest EGC transfer rate with  $44.9 \pm 1.9$  and  $32.4 \pm 1.8\%$  for CTC and orthodox mode, respectively. The highest EC transfer rate ( $78.1 \pm 1.3\%$ ) was observed for CTC green tea processed from RR17/144.

### 3.4. Gallated catechins

Gallated catechins consist of (–)-epigallocatechin-3-gallate (EGCG) and (–)-epicatechin-3-gallate (ECG). In addition to astringency, ECG can contribute to a bitter after taste. The levels of EGCG and ECG in green tea, in infusion, and their transfer

rate into infusion are presented in Fig. 1(e) and (f), respectively. EGCG level in green teas varied from  $77.5 \pm 1.8 \text{ mg g}^{-1}$  in TV1 to  $139.5 \pm 1.8 \text{ mg g}^{-1}$  in cultivar 482/12. Green tea processed from TV1 had the highest level of ECG with  $47.8 \pm 0.6 \text{ mg g}^{-1}$ . The EGCG and ECG levels from the present study are at variance with some available literature such as  $19$ – $62 \text{ mg g}^{-1}$  (EGCG) and  $10$ – $30 \text{ mg g}^{-1}$  (ECG);<sup>18</sup>  $70.76 \text{ mg g}^{-1}$  (EGCG) and  $27.08 \text{ mg g}^{-1}$  (ECG),<sup>21</sup> where the levels are much lower. The effect of processing method had a strong influence in content of these catechins in infusions. The cultivar RR17/144 had the highest water extraction efficiency of gallated catechins for both modes of processing. EGCG level in CGTIs it was in the range between  $16.3 \pm 0.4$  and  $37.3 \pm 0.3 \text{ mg g}^{-1}$  (equivalent to  $217.1$ – $497.2 \text{ mg L}^{-1}$ ), while in OGTIs it was between  $14.0 \pm 0.2$  and  $25.8 \pm 0.7 \text{ mg g}^{-1}$  (equivalent to  $186.9$ – $343.5 \text{ mg L}^{-1}$ ). ECG levels were in the range between  $5.9 \pm 0.2$  and  $13.7 \pm 0.4 \text{ mg g}^{-1}$  (equivalent to  $78.0$ – $182.9 \text{ mg L}^{-1}$ ); and between  $5.0 \pm 0.2$  and  $8.1 \pm 0.3 \text{ mg g}^{-1}$  (equivalent to  $66.9$ – $107.7 \text{ mg L}^{-1}$ ) in CGTIs and OGTIs, respectively. The present results are in conformity with literature available EGCG and ECG levels in green tea infusion in the range between  $94.54$  and  $357.07 \text{ mg L}^{-1}$ ; and between below detectable limit (BDL) and  $310.80 \text{ mg L}^{-1}$ , respectively.<sup>26</sup> Liu *et al.* reported a much wider range of EGCG ( $201$ – $1289 \text{ mg L}^{-1}$ ) and ECG ( $48.5$ – $435 \text{ mg L}^{-1}$ ) in Xihu Longjing green tea infusions in different brewing conditions and with different particle sizes.<sup>23</sup> The highest EGCG transfer rate was observed in RR17/144 with  $42.5 \pm 1.2\%$  and  $24.7 \pm 0.3\%$  for CTC and orthodox green tea, respectively, while for ECG it was  $46.5 \pm 2.0\%$  and  $24.6 \pm 0.8\%$  for respective modes of processing in the same cultivar. The gallated catechins contributed  $60$ – $78\%$  to total catechin in green tea infusions. This observation is in agreement with Xu *et al.* who reported  $75.17\%$  contribution of gallated catechin towards total catechin concentration.<sup>31</sup> A thorough disruption of cell membranes in the CTC process efficiently exposes the catechins.<sup>32</sup> The higher surface area for smaller tea particles enhances the infusion rate owing to dissolution of compounds on surface.

### 3.5. Caffeine

Green tea has been a rich source of caffeine.<sup>26</sup> Caffeine level in tea leaf decreases with increasing maturity.<sup>18</sup> The caffeine content in CTC and orthodox green teas, in infusions, and its extent of transfer into infusions are presented in Fig. 2(a). The caffeine content in green teas was in the range between  $30.0 \pm 0.1 \text{ mg g}^{-1}$  in Ging186 and  $55.0 \pm 0.6 \text{ mg g}^{-1}$  in TV9. Caffeine content in TV9 green tea was significantly different ( $p \leq 0.05$ ) from all others except TV18. The caffeine level in the teas being reported here is higher than some earlier literature cited levels determined using HPLC.<sup>18,21,22,33</sup> Dai *et al.* found caffeine content in Huangshan Maofeng, a famous Chinese green tea, at  $32.88 \text{ mg g}^{-1}$ .<sup>21</sup> The caffeine level in Ziyang green tea, a selenium enriched Chinese green tea, was demonstrated in the range between  $26.71 \text{ mg g}^{-1}$  and  $38.06 \text{ mg g}^{-1}$ .<sup>33</sup> In Kenya, green teas processed from purple color cultivars had caffeine level in the range between  $11.6$  and  $22.0 \text{ mg g}^{-1}$ .<sup>18</sup> The authors also reported that Chinese variety Hanlu origin green tea had



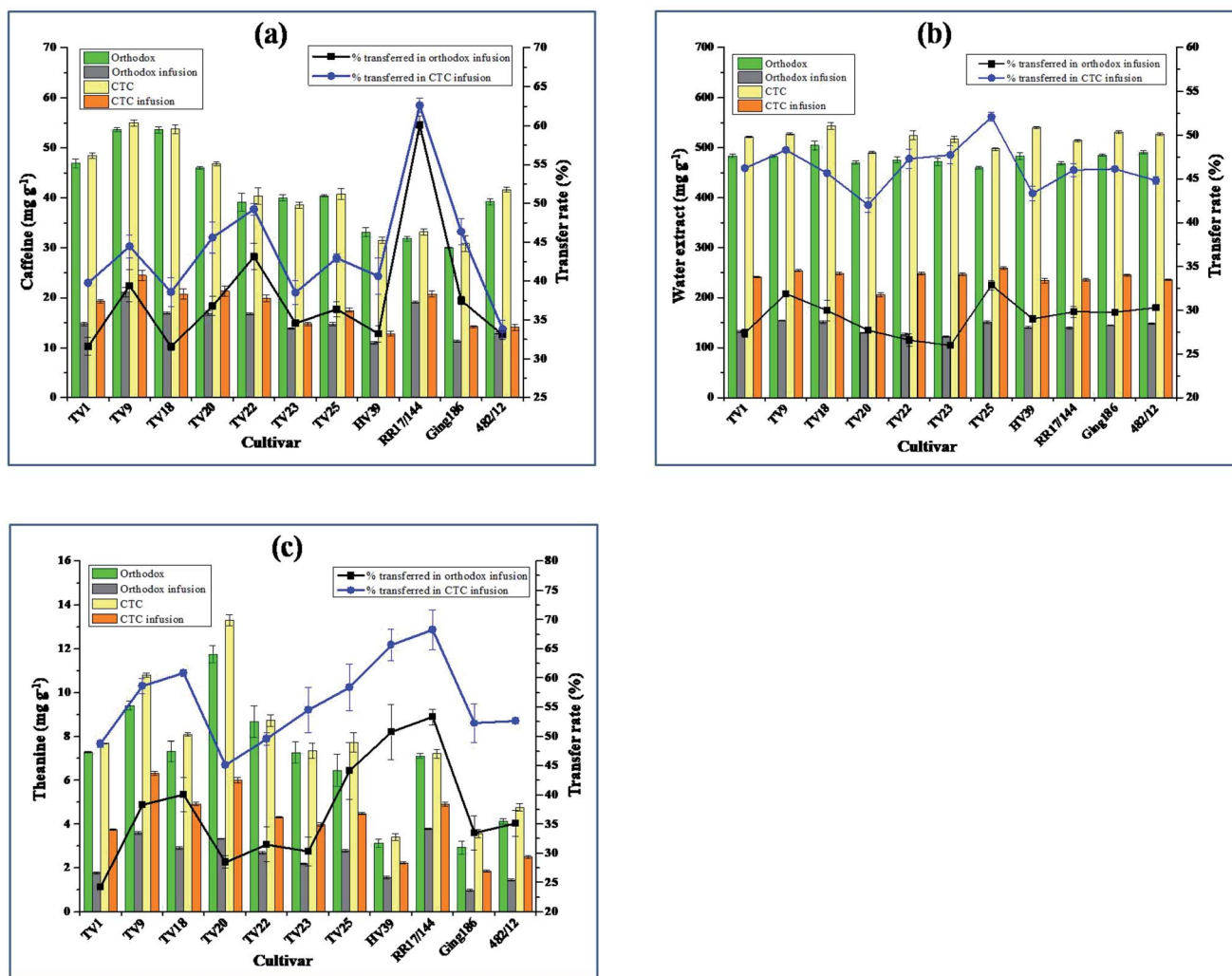


Fig. 2 Average content ( $\text{mg g}^{-1}$ ) of caffeine (a), water extract (b), and theanine (c) in CTC and orthodox green tea, in infusion, and their per cent transfer into infusions (error bars specify the standard error).

24.2  $\text{mg g}^{-1}$  caffeine. Longjing green tea has caffeine content in the range from 26  $\text{mg g}^{-1}$  to 38.50  $\text{mg g}^{-1}$ .<sup>22</sup> The caffeine levels in CGTIs and OGTIs did not represent any significant difference. The level of caffeine in CGTIs was between  $12.8 \pm 0.5$  and  $24.5 \pm 1.1 \text{ mg g}^{-1}$  (equivalent to 170.1–326.5  $\text{mg L}^{-1}$ ) whereas in OGTIs it was between  $11.0 \pm 0.2$  and  $21.1 \pm 0.9 \text{ mg g}^{-1}$  (equivalent to 146.4–281.6  $\text{mg L}^{-1}$ ). The caffeine contents in infusions are strongly supported by earlier reports such as 99.21–300.0  $\text{mg L}^{-1}$ ;<sup>26</sup> 141–338  $\text{mg L}^{-1}$ ;<sup>30</sup> 139–823  $\text{mg L}^{-1}$ <sup>23</sup> determined using HPLC.

### 3.6. Water extract

Water extract (WE) level was significantly influenced by the processing methods. The CTC green teas contain 17.12% (on average) more WE than their orthodox counterparts. WE levels in CTC and orthodox green teas, in infusions, and their transfer rate into infusion are summarized in Fig. 2(b). WE in CTC green teas varied from  $490.7 \pm 2.5 \text{ mg g}^{-1}$  in TV20 to  $544.0 \pm 6.8 \text{ mg g}^{-1}$  in TV18. WE content in TV18 CTC green tea was significantly different ( $p \leq 0.05$ ) from TV1, 20, 23, 25 and RR17/144. In

orthodox green teas, WE was highest for TV18 ( $504.5 \pm 9.3 \text{ mg g}^{-1}$ ) and lowest for TV25 ( $459.5 \pm 2.6 \text{ mg g}^{-1}$ ). The highest content was significantly different ( $p \leq 0.05$ ) from TV20, 22, 23, 25 and RR17/144. WE levels in the present study are much higher than the minimum limit of 320  $\text{mg g}^{-1}$  as defined by Food Safety and Standard Authority of India.<sup>34</sup> The WE levels in the present study are higher than that reported in Longjing green tea (372.10–446.70  $\text{mg g}^{-1}$ ) determined using ISO 9768:1994 method.<sup>22</sup> The infusions from both types of teas were also estimated for the WE contents. The level of WE in CGTIs were in the range between  $206.2 \pm 4.5$  and  $259.2 \pm 2.3 \text{ mg g}^{-1}$  (equivalent to 2749–3455  $\text{mg L}^{-1}$ ), whereas in OGTIs it was between  $122.6 \pm 1.6$  and  $154.1 \pm 0.7 \text{ mg g}^{-1}$  (equivalent to 1634–2055  $\text{mg L}^{-1}$ ). The higher surface area for CTC green tea particles led to higher extraction rate which is due to dissolution of biochemical compounds on surface.<sup>24,35</sup>

### 3.7. Theanine

Theanine was found in all green tea samples and their contents in green tea, in infusion, and its transfer rate into infusion are



presented in Fig. 2(c). Theanine content in green teas varied from  $2.9 \pm 0.2 \text{ mg g}^{-1}$  in Ging186 to  $13.3 \pm 0.3 \text{ mg g}^{-1}$  in TV20. The cultivar TV20 had significantly higher ( $p \leq 0.05$ ) theanine content from all other cultivars. In line with our results, Too *et al.* reported theanine level in the range between 1.4 and  $15.0 \text{ mg g}^{-1}$  in green tea manufactured from Kenyan tea cultivars using HPLC.<sup>36</sup> Kilel *et al.* reported a higher level of theanine ( $15.0\text{--}21.8 \text{ mg g}^{-1}$ ) in green tea processed from purple tea cultivars of Kenya.<sup>18</sup> Theanine content in Longjing green tea varied between 5.68 and  $17.36 \text{ mg g}^{-1}$ .<sup>22</sup> Theanine contents in infusions were positively influenced by CTC step during processing. The CGTIs had 19% (on average) more theanine content than OGTIs. Theanine levels in CGTIs were in the range between  $1.9 \pm 0.1$  and  $6.3 \pm 0.1 \text{ mg g}^{-1}$  (equivalent to  $24.7\text{--}84.4 \text{ mg L}^{-1}$ ), whereas in OGTIs it was between  $1.0 \pm 0.1$  and  $3.8 \pm 0.1 \text{ mg g}^{-1}$  (equivalent to  $12.9\text{--}50.5 \text{ mg L}^{-1}$ ). The stripping of the leaf epidermis and maceration during CTC step led to microstructural changes in cells which efficiently exposed its components.<sup>37</sup> The findings in the present work support the reported theanine level ( $2.58\text{--}4.23 \text{ mg g}^{-1}$ ) in twenty-seven famous commercial teas of UK.<sup>38</sup> Liu *et al.* reported higher levels of theanine ( $38.5\text{--}272 \text{ mg L}^{-1}$ ) in green tea infusions determined by derivatization of infusion using 2,4-dinitrofluorobenzene, and detected at 360 nm using HPLC.<sup>23</sup>

### 3.8. Sensory evaluation

The overall taste of green tea is characterized by the basic tastes comprising bitterness, astringency and umami,<sup>39</sup> with astringency and bitterness being the primary ones.<sup>11</sup> The overall acceptability of the CTC green teas (score: 6.1–8.0) was slightly lower than its orthodox counterpart (score: 6.5–8.6) (Table 1). The difference in taste profile can be attributed to the difference in biochemical constituents contributing to taste. The gallated and non-gallated catechins had a profound effect on the intensities of astringency, bitterness and sweetness as reported by Cao *et al.*<sup>40</sup> The intensities of astringency ( $R^2 = 0.89$ ,  $p \leq 0.01$  for both orthodox and CTC); and bitterness ( $R^2 = 0.78$ ,  $p \leq 0.01$

for orthodox and  $R^2 = 0.72$ ,  $p \leq 0.01$  for CTC) were positively correlated with the concentration of gallated catechins. Non gallated catechins had positive correlation with sweetness ( $R^2 = 0.88$ ,  $p \leq 0.01$  for orthodox and  $R^2 = 0.71$ ,  $p \leq 0.01$  for CTC) and overall acceptability ( $R^2 = 0.85$ ,  $p \leq 0.01$  for orthodox and  $R^2 = 0.77$ ,  $p \leq 0.01$  for CTC). The scores for umami taste had strong positive correlation ( $R^2 = 0.98$ ,  $p \leq 0.01$  for orthodox and  $R^2 = 0.96$ ,  $p \leq 0.01$  for CTC) with theanine content. Previous studies also reported a profound effect of free amino acids present in infusion on umami taste.<sup>11</sup>

### 3.9. Fluoride and chloride content

Fluoride prevents dental caries, although excessive exposure to high levels may lead to skeletal and dental fluorosis.<sup>41</sup> The CGTIs had higher levels of fluoride than OGTIs (please see ESI Table S1†). Fluoride content ranged from 0.5 to  $1.1 \text{ mg L}^{-1}$  (mean  $0.83 \text{ mg L}^{-1}$ ) in CGTIs, whereas it was from 0.4 to  $0.9 \text{ mg L}^{-1}$  (mean  $0.63 \text{ mg L}^{-1}$ ) in OGTIs. Based on World Health Organisation (WHO) guideline on fluoride in drinking water ( $1.5 \mu\text{g mL}^{-1}$ ) all the green tea samples are safe for human consumption with respect to fluoride content.<sup>42</sup> The fluoride content, determined using fluoride selective electrode, in infusions of green tea available in Portugal was much higher ( $0.80\text{--}2.0 \text{ mg L}^{-1}$ , mean  $1.20 \text{ mg L}^{-1}$ ) than the results being reported here.<sup>30</sup> In a study conducted in Republic of Ireland, Waugh *et al.* reported mean fluoride content of  $3.3 \text{ mg L}^{-1}$  in commercially available black tea samples demonstrating the risk of health hazards from fluoride exposure.<sup>43</sup> The mean chloride contents were determined to be 19.02 and  $14.24 \text{ mg L}^{-1}$  in CGTIs and OGTIs, respectively (please see ESI Table S1†). The current results are in disparity with previously documented work which reported much higher chloride content ( $86.0 \text{ mg L}^{-1}$ ) in green tea infusions in Romania using ion chromatography.<sup>44</sup>

### 3.10. Sodium and potassium content

The CGTIs had a higher content of sodium and potassium than OGTIs (please see ESI Table S1†). Sodium contents were in the

Table 1 Taste attributes of orthodox and CTC green tea infusions<sup>a</sup>

Cultivar	Orthodox					CTC				
	Bitterness	Astringency	Umami	Sweetness	Overall acceptability	Bitterness	Astringency	Umami	Sweetness	Overall acceptability
TV1	$2.30 \pm 0.06$	$5.10 \pm 0.21$	$6.50 \pm 0.23$	$4.10 \pm 0.06$	$6.80 \pm 0.06$	$2.20 \pm 0.06$	$5.20 \pm 0.29$	$5.90 \pm 0.21$	$3.70 \pm 0.12$	$6.50 \pm 0.35$
TV9	$2.30 \pm 0.15$	$6.00 \pm 0.06$	$7.90 \pm 0.35$	$4.80 \pm 0.06$	$6.80 \pm 0.23$	$2.30 \pm 0.06$	$5.90 \pm 0.17$	$7.70 \pm 0.23$	$4.50 \pm 0.23$	$6.30 \pm 0.17$
TV18	$2.40 \pm 0.12$	$6.10 \pm 0.29$	$6.60 \pm 0.40$	$4.60 \pm 0.12$	$6.50 \pm 0.12$	$2.20 \pm 0.17$	$6.20 \pm 0.17$	$6.30 \pm 0.29$	$4.50 \pm 0.12$	$6.20 \pm 0.17$
TV20	$2.20 \pm 0.06$	$5.70 \pm 0.12$	$8.20 \pm 0.12$	$5.90 \pm 0.12$	$8.40 \pm 0.23$	$2.10 \pm 0.06$	$5.80 \pm 0.23$	$8.00 \pm 0.17$	$5.30 \pm 0.23$	$8.00 \pm 0.17$
TV22	$2.20 \pm 0.15$	$5.90 \pm 0.21$	$7.50 \pm 0.49$	$6.20 \pm 0.17$	$7.90 \pm 0.12$	$2.00 \pm 0.12$	$5.80 \pm 0.12$	$7.00 \pm 0.40$	$5.90 \pm 0.23$	$7.70 \pm 0.17$
TV23	$2.20 \pm 0.12$	$5.80 \pm 0.21$	$6.50 \pm 0.17$	$6.60 \pm 0.23$	$8.40 \pm 0.17$	$2.20 \pm 0.17$	$6.00 \pm 0.29$	$6.50 \pm 0.17$	$6.40 \pm 0.17$	$8.00 \pm 0.29$
TV25	$2.40 \pm 0.17$	$6.10 \pm 0.17$	$5.90 \pm 0.15$	$5.70 \pm 0.12$	$7.20 \pm 0.17$	$2.30 \pm 0.12$	$6.30 \pm 0.29$	$5.50 \pm 0.23$	$6.30 \pm 0.29$	$7.00 \pm 0.23$
HV39	$2.40 \pm 0.06$	$6.00 \pm 0.12$	$3.90 \pm 0.06$	$5.80 \pm 0.06$	$7.80 \pm 0.35$	$2.40 \pm 0.12$	$6.20 \pm 0.23$	$3.70 \pm 0.06$	$5.80 \pm 0.29$	$6.90 \pm 0.35$
RR17/144	$2.20 \pm 0.12$	$4.80 \pm 0.06$	$6.20 \pm 0.17$	$6.00 \pm 0.12$	$8.60 \pm 0.35$	$2.10 \pm 0.06$	$5.00 \pm 0.17$	$6.00 \pm 0.23$	$6.10 \pm 0.17$	$7.90 \pm 0.23$
Ging186	$3.50 \pm 0.10$	$6.30 \pm 0.20$	$3.50 \pm 0.06$	$5.00 \pm 0.17$	$6.90 \pm 0.12$	$3.30 \pm 0.06$	$6.40 \pm 0.17$	$3.40 \pm 0.12$	$5.10 \pm 0.06$	$6.10 \pm 0.23$
482/12	$3.90 \pm 0.15$	$6.20 \pm 0.25$	$4.30 \pm 0.12$	$5.70 \pm 0.12$	$7.80 \pm 0.23$	$3.80 \pm 0.12$	$6.30 \pm 0.12$	$4.30 \pm 0.23$	$5.60 \pm 0.17$	$7.50 \pm 0.12$

<sup>a</sup> Values are means ( $\pm$ SE) of three replications.



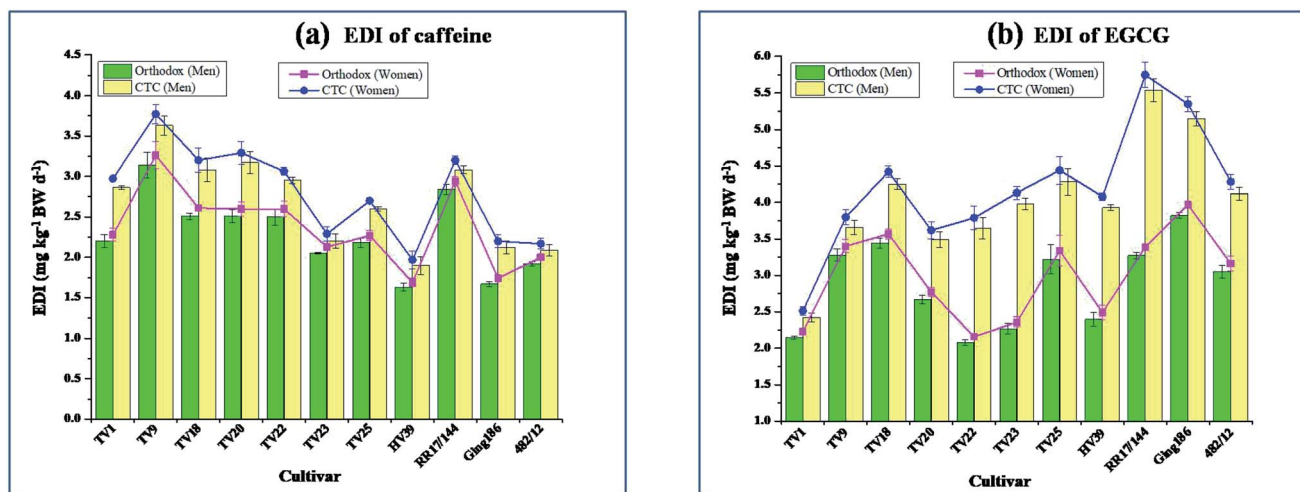


Fig. 3 Estimated daily intake (EDI) of caffeine (a), and EGCG (b) for both men and women on consumption of five cups (equivalent to 10 g) of green tea [average body weight (BW) considered for men and women were 67.4 and 64.9 kg, respectively].

range from 1.2 to 2.6 mg L<sup>-1</sup> (mean 1.67 mg L<sup>-1</sup>) in CGTIs, whereas it was from 1.0 ± 0.1 to 2.1 ± 0.1 mg L<sup>-1</sup> (mean 1.28 mg L<sup>-1</sup>) in OGTIs. Fernandez *et al.* reported a similar level of sodium content (0.4–3.3 mg L<sup>-1</sup>) in twenty green tea samples from different geographical origin using ICP-AES.<sup>45</sup> Reto *et al.* demonstrated a much higher level of sodium (36–69 mg L<sup>-1</sup>) in the infusions of green tea commercialized in Portugal.<sup>30</sup> The variation in concentration of sodium can be attributed to its high extractability and wide range of its content in green leaves. Islam and Ebihara determined sodium and potassium content in Japanese green tea using combined PGA (neutron-induced prompt gamma-ray analysis) and INAA (instrumental neutron activation analysis) technique. The authors reported sodium level in the range 5.16–53.6 μg g<sup>-1</sup> with an extraction efficiency of 59% to the infusion (equivalent to 0.03–0.32 mg L<sup>-1</sup>) which is much lower than the present findings.<sup>46</sup> The potassium contents were in the range between 138.7 ± 4.7 and 281.6 ± 5.9 mg L<sup>-1</sup> (mean 225.79 mg L<sup>-1</sup>) in CGTIs, whereas it was between 89.3 ± 2.7 and 179.5 ± 3.1 mg L<sup>-1</sup> (mean of 152.89 mg L<sup>-1</sup>) in OGTIs. Our findings are in conformity with a previous report of potassium level in the range from 94 to 259 mg L<sup>-1</sup> determined using ICP-AES.<sup>45</sup> Potassium level in Japanese green tea was demonstrated in the range 15 400 to 21 900 μg g<sup>-1</sup> with an infusion transfer rate of 71% (equivalent to 109.34–155.49 mg L<sup>-1</sup>) which is in agreement with the current results.<sup>46</sup> However, Reto *et al.* reported much lower mean potassium content (114.77 mg L<sup>-1</sup>) in nine Portuguese tea samples.<sup>30</sup>

### 3.11. Risk assessment

The EDI results are presented in Fig. 3(a) and (b) for caffeine and EGCG, respectively. The average EDI of caffeine in mg kg<sup>-1</sup> BW was 2.70 and 2.29 for men; 2.80 and 2.37 and for women in CTC and orthodox green tea, respectively. Nawrot *et al.* reported that daily caffeine intake within the level of 6 mg kg<sup>-1</sup> BW for a healthy adult had no adverse health effect like toxicity,

increased cancer risk, cardiovascular effects, decreased male fertility, *etc.*<sup>47</sup> The authors also suggested that women fertility is not affected on consuming caffeine within the level of 4.6 mg per kg BW per day and children should not consume beyond 2.5 mg per kg BW per day. The EDI levels of caffeine in the present study are well within the limit of risk as suggested by Nawrot *et al.*<sup>47</sup>

The average EDI of EGCG in mg kg<sup>-1</sup> BW was 4.04 (equivalent to 272.30 mg per day) and 2.88 (equivalent to 194.11 mg per day) for men; 4.19 (equivalent to 271.93 mg per day) and 2.99 (equivalent to 194.05 mg per day) for women in CTC and orthodox green tea, respectively. EGCG is reportedly suspected as one of the causes for liver toxicity in human.<sup>48</sup> European Food Safety Authority (EFSA) on their study on the association of EGCG intake and hepatotoxicity demonstrated that a small group of population experienced an elevated level of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) on consuming a dose of 800 mg per day for a period extending beyond 4 months.<sup>49</sup> A dose of 316 mg per day did not show any elevation of serum levels of transaminases. The EFSA panel did not find any evidence of ALT level elevation at a dose of 700 mg EGCG per day. Dekant *et al.* proposed an EGCG dose of 300 mg per day as safe for human consumption.<sup>48</sup> The data from the present study revealed the safety of these green teas from the risk of hepatotoxicity.

## 4. Conclusion

This study provides comparative information on the quality components of orthodox and CTC green tea to judge the preferred processing mode with improved quality. The results demonstrated that CTC green teas had significantly high extraction efficiency of bioactive components than orthodox ones which led to a high content of total polyphenol, catechins, theanine and water extract in CTC green tea infusions. The extraction efficiency of total polyphenol, total catechin, theanine and water extract in CTC green tea were 13.3, 7.5, 18.6,





and 17.1%, respectively, higher in comparison to orthodox green tea. This study provides a new fundamental basis of the economic efficiency of CTC green tea in the light of the higher transfer rate of bioactive compounds from tea to its infusion. The study also suggests that EGCG intake from consumption of five cups (10 g) of tea infusion a day is free from the risk of hepatotoxicity and caffeine intake from the same quantity of tea will not inflict any health hazard. Infusion prepared from orthodox green tea had preferred sensory qualities as compared with that of CTC green tea, with lower astringency, and higher umami and overall acceptability score. In spite of the slight low tasters score, the economic efficiency of the CTC green tea, in terms of cuppage, is thought provoking. The current study also found that the cultivars TV20, TV23, and RR17/144 had a high level of overall acceptability tasters score (>8.0).

## Abbreviations

BW	Body weight
CGTI	CTC green tea infusion
CTC	Curl, tear and crush
EC	(–)Epicatechin
ECG	(–)Epicatechin-3-gallate
EDI	Estimated daily intake
EFSA	European Food Safety Authority
EGC	(–)Epigallocatechin
EGCG	(–)Epigallocatechin-3-gallate
FBD	Fluid bed dryer
GAE	Gallic acid equivalent
OGTI	Orthodox green tea infusion
TC	Total catechin
TE	Tea estate
TP	Total polyphenols
TPI	Teeth per inch
TTRI	Tocklai Tea Research Institute
TV	Tocklai vegetative
WE	Water extract

## Author contributions

H. Deka designed the research, collected green leaves from field, processed all green teas, carried out the biochemical analysis, statistical analysis, interpreted the results, and drafted the manuscript. T. Barman analysed the macroelements, fluoride, chloride and actively helped in editing the manuscript. P. P. Sarmah helped in preparing tables, figures and overall improvement of the manuscript. P. Tamuly arranged sensory evaluation of samples. A. Devi retouched the manuscript. R. K. Paul carried out statistical analysis and interpretation of the data set. T. Karak guided in preparing the manuscript, revised it for technical and intellectual content.

## Conflicts of interest

The authors declare no conflict of interest.

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