Study on selected tea cultivars of North Bengal for their suitability in green tea production

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ABSTRACT

This study aims towards selection of suitable cultivars from Dooars and Terai regions of North Bengal for green tea production. Green teas were processed by steaming the freshly plucked leaves of different cultivars under three inactivation times. Cultivars belonging to the TV-series, TRA/Garden series and seed stocks were selected for the study. Results showed marked variation in taster’s score for green teas produced from different inactivation period. Significant differences were observed in biochemical quality compositions of green teas processed from selected cultivars along with variation in organoleptic scores irrespective of inactivation time. These findings have pointed towards the basis of cultivar categorization that can be useful for commercially suitable green tea manufacture under North Bengal condition.

Keywords: Biochemical; Categorization; Cultivar; Green tea; Steaming

INTRODUCTION

Tea manufacturing is an operation in which leaves from the plant Camellia sinensis undergoes a series of circumspectly modulated steps to produce different types of tea [1], and based on the degree of fermentation are broadly classified as green teas, oolong teas and black teas. The main difference in green tea manufacturing is the omission of oxidation stage, so as to retain green colour of the end product. The enzyme activities are inhibited immediately after plucking by arresting oxidation through steaming or pan frying. The constituents which impart their effect on taste, flavour and colour characteristics of green tea are mainly polyphenolic bodies, caffeine, amino acids, peptic substances, minerals, chlorophyll etc [2]. In the recent years, green tea has garnered a lot of research attention as a nutraceutical due to diverse health benefits associated with it [3,4]. Green tea contains numerous bioactive compounds and is one of the major dietary sources of polyphenols, flavan-3-ols (also known as catechins) being the main subclass present in tea, which constitutes up to 30-40% on a dry weight basis, is water-soluble and can easily be extracted in infusions. The amount of catechins extracted depends upon the genetic characteristics of the cultivars, the manufacturing style and the temperature of infusion [5]. Emerging evidence suggests that polyphenols acts as antioxidants, anti-carcinogens, anti-microbial, anti-viral, cardio-protecting agents and may play a relevant role in maintaining neurological health [6].

There are mostly two varieties viz. Camellia sinensis var. sinensis and Camellia sinensis var. assamica commonly used for making tea. Depending on genetic characters of plant and processing techniques, the biochemical compositions of green tea exhibit wide variation. Green tea manufactured in the Darjeeling hills has got potential market because of the areas’ unique agro climatic condition and phenomenal making style irrespective of diverse planting materials. But, in spite of the agro-climatic limitations, estates in Dooars and Terai have started producing green tea which may prove to be an alternative and profitable proposition in view of the increasing demand for green tea in Indian market.
Keeping the above perspective in mind, the main objectives of the present study included:

- to categorize cultivars suitable for green tea production in Dooars and Terai regions
- standardize the steaming operation to obtain desirable quality constituents of green tea
- to determine changes in the levels of polyphenols, catechins, caffeine, chlorophyll a and b and soluble solids in the final product and
- to corroborate findings with organoleptic evaluation by the tea taster.

MATERIALS AND METHODS

Selection of cultivars

Tocklai clones TV-1, TV-9, TV-20, TV-25, TV-26; TRA/Garden series clone TeenAli-17/1/54 and Tocklai Stock TS-462, TS-463, TS-491 and TS-520 of similar age group from North Bengal Regional R&D Centre experimental plots were used for green tea manufacturing. Leaf quality (fine percentage of 60 % or above) was maintained for all processing trials and green tea was manufactured from May to October 2013.

Manufacturing of green tea

Manufacturing process of green tea was followed as practised in North East India. In brief, as a small scale manufacturing, 400 gm of freshly plucked shoots were inactivated by steaming to prepare green tea. The basic steps of the manufacturing procedure were simplified as follows-

- Steaming of the leaves in a perforated chamber above 100°C for fixed time regime of 4, 6 and 8 minutes separately.
- Cooling and removal of surface water by blowing air over steamed leaf for 15-20 min.
- Rolling of the surface-dried leaf in conventional roller for 25-30 min.
- Final drying of the rolled leaf in a conventional drier maintaining drier temperature at 104°C for 35-40 min to achieve moisture content of final product within 3 percent.

Biochemical analysis

1. Total Soluble Solids in green tea was determined following the method of ISO9768:1994 [7].
2. The Total polyphenol content in tea sample was determined using F-C reagent by ISO/CD 14502-1-2: 2001 method [8].
3. Caffeine content in the sample was determined using the method of [9].
4. Chlorophylls of green tea shoots were estimated following the method of [10].

Statistical analysis

The differences between mean values of each parameter were analyzed using Duncan’s Multiple Range Test. Principal component analysis was performed to determine the grouping of cultivars using SPSS 16.0.

RESULTS AND DISCUSSION

Method of fixing/steam inactivation during manufacture

By definition, green tea is light greenish yellow in colour retaining the natural bitterness and astringency, umami aroma and a more delicate (sweet/mellow/brothy) taste. The first step of manufacture involves inactivation or fixing of enzymes present in leaf shoots viz. polyphenol oxidase, catalase, peroxidase, ascorbic acid oxidase and chlorophyllase, which exhibit high activity after plucking. So it is essential to prevent oxidation for retaining green colour [12,13] of the end product and this is done generally by steaming or pan frying of the plucked leaves. But the extent of heat exposure to the leaves has been reported to be critical in view of make style, chemical composition and taster quality [14]. Inadequate inactivation can result in reddening of leaves due to retained enzyme activity and over-steaming may hydrolyze leaf proteins resulting in yellow liquor and grassy flavor. It was therefore essentially felt to establish the optimum steaming time for green tea manufacture with desired quality and taste. Three steaming time
were adopted in this experiment that showed very significant differences in taster’s quality, presented in Figure 1, however, not much differences were observed in chemical compositions.

Figure 1: Variation in taster quality (TQ) due to steaming duration.

Multiple regression analysis of data taking time as dependent variable showed that 54% variance took place in different biochemical constituents and TQ due to time of inactivation or the amount of steam applied for enzyme fixation.

The following equation defines relationship between steaming time (T) and quality parameters with acceptable goodness of fit ($r^2$).

$$T\text{(time)}=3.587-0.089\text{TSS}-0.9\text{CA}+0.047\text{TP}-0.607\text{C}+0.357\text{EC}^*+0.357\text{EGC}^*+0.714\text{TC}+1.24\text{TQ}^*.\quad (r^2=0.54, \quad F=2.612^*)$$

Where TSS (total soluble solid % dry weight); CA (caffeine % dry weight); TP (total polyphenol % dry weight); C (catechin % dry wt.); EC (epicatechin % dry wt.); EGC (epigallocatechin % dry weight); EGCG (epigallocatechingallate % dry wt.); TC (total catechin % dry wt.); TQ (taster’s quality, score in the scale of 10) ($^*$ is significant at 0.05% and $^{**}$ is significant at 0.01%).

Choice of cultivar

The cultivars grown in Dooars and Terai are predominantly of Assamica type, Assam-Cambod hybrids and a few seed stocks [15,16]. Green tea is most often sourced from the small-leaf varieties because of sweeter taste than the broad-leaf varieties which are mainly preferred for black tea production and more astringent in nature. The TV clones like TV-1 (Assam-China hybrid), TV-9, TV-20, TV-25, TV-26 (Cambod variety), TRA/Garden series clone Teen Ali (TA)-17/1/54 (Assam-China variety) and seed stocks TS-462, TS-463, TS-491, TS-520 (Cambod characteristics) are used in general for CTC black tea manufacture in North Bengal. However, some varieties like TV-9, TV-26 and Teenali-17/1/54 were found ideal for green tea manufacturing [17] under North Bengal condition. In this study ten above mentioned varieties were selected for green tea manufacturing and evaluated for their quality characteristics.

All the selected varieties are known to have different physical and chemical characteristics. In congruence, the green tea processed from these cultivars expectedly showed marked variation in their chemical compositions, presented in Table 1. This study revealed that all varieties used to produce green tea have acceptable taster’s quality irrespective of their variation in chemical components suggesting that green tea quality was not solely dependent on the genetic characteristics of the cultivars. Amount of polyphenolic compounds varies among the cultivars and higher catechins are found in Assam varieties (20-25%) as compared to china and cambod varieties. Polyphenols exhibit therapeutic properties and their concentration in the water infused extracts of green tea varies greatly due to processing [18]. The water soluble polyphenols and their derivatives contribute to flavor, colour and mouth feel of tea and have potential use singly or in combination with other active principles in food, pharmaceuticals and cosmetic industry [19]. The obtained data presented in Table 1 revealed that TV-1, TV-25, TV-26, TS-491 have significantly higher total polyphenol (TP) over other cultivars. Individual catechins too varied widely among the cultivars. Caffeine, a
secondary metabolite which acts as a defense compound against pathogens and predators [20] also showed marked variation. Caffeine is an antioxidant component of green and black tea which is not present in most herbal teas, and is thus responsible for their higher antioxidant activity [21]. Caffeine contributes to green tea’s briskness and stimulant properties, is less sensitive to heat and does not undergo considerable reduction during processing. Since tea tasters’ evaluation for quality of tea is associated significantly with briskness of its liquor [22], caffeine is therefore an important quality attribute of tea. It varied significantly among the cultivars as shown in table 1. TV-9 was found to have the highest caffeine content, and relatively lower values were obtained for TV-25 and TV-26.

Chlorophylls are the predominant photosynthetic pigments present in the tea leaves which contributes to the greenness of green tea [23]. Varietal and seasonal variation in chlorophyll a and b in Assam, China and Cambod tea was reported by [24]. Significant variation in chlorophyll content was also noted in this study, highest total chlorophyll was recorded in TV-25 while TS-520 has lowest (Table 1).

Table 1: Biochemical quality composition of green tea manufactured from different cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>CHa</th>
<th>CHb</th>
<th>CHt</th>
<th>TSS</th>
<th>CA</th>
<th>TP</th>
<th>(+)C</th>
<th>(+)EC</th>
<th>(+)EGC</th>
<th>(+)EJC</th>
<th>(+)EGCG</th>
<th>TC</th>
<th>TQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV-1</td>
<td>0.22 ± 0.03ab</td>
<td>0.15 ± 0.01a</td>
<td>0.37 ± 0.04a</td>
<td>44.07 ± 0.80a</td>
<td>3.84 ± 0.03cd</td>
<td>24.29 ± 0.38ab</td>
<td>0.21 ± 0.02ab</td>
<td>1.15 ± 0.01b</td>
<td>3.85 ± 0.15ab</td>
<td>1.64 ± 0.01e</td>
<td>7.77 ± 0.20ab</td>
<td>14.62 ± 0.32ab</td>
<td>5.73 ± 0.23a</td>
</tr>
<tr>
<td>TV-9</td>
<td>0.26 ± 0.04ab</td>
<td>0.15 ± 0.02ab</td>
<td>0.41 ± 0.05b</td>
<td>43.60 ± 1.44b</td>
<td>4.17 ± 0.05a</td>
<td>20.90 ± 0.04ab</td>
<td>0.13 ± 0.04ab</td>
<td>0.76 ± 0.04ab</td>
<td>2.74 ± 0.05ab</td>
<td>1.99 ± 0.10ab</td>
<td>9.90 ± 0.11bc</td>
<td>15.62 ± 1.71al</td>
<td>6.43 ± 0.26a</td>
</tr>
<tr>
<td>TeenAli-17/154</td>
<td>0.25 ± 0.01a</td>
<td>0.15 ± 0.01a</td>
<td>0.42 ± 0.03ab</td>
<td>45.33 ± 0.68b</td>
<td>3.71 ± 0.07a</td>
<td>19.47 ± 0.10ab</td>
<td>0.23 ± 0.11ab</td>
<td>1.23 ± 0.03ab</td>
<td>2.06 ± 0.03ab</td>
<td>4.11 ± 0.15a</td>
<td>9.40 ± 0.11cd</td>
<td>17.03 ± 0.24ab</td>
<td>5.90 ± 0.32a</td>
</tr>
<tr>
<td>TV-20</td>
<td>0.27 ± 0.03ab</td>
<td>0.16 ± 0.01a</td>
<td>0.43 ± 0.04ab</td>
<td>47.85 ± 0.35a</td>
<td>3.52 ± 0.07a</td>
<td>22.32 ± 0.68ab</td>
<td>0.07 ± 0.02a</td>
<td>0.91 ± 0.08ab</td>
<td>2.63 ± 0.04ab</td>
<td>2.67 ± 0.12ab</td>
<td>9.98 ± 0.21bc</td>
<td>16.26 ± 0.20bc</td>
<td>6.37 ± 0.20a</td>
</tr>
<tr>
<td>TV-25</td>
<td>0.36 ± 0.03a</td>
<td>0.17 ± 0.01a</td>
<td>0.55 ± 0.03a</td>
<td>44.55 ± 0.73b</td>
<td>3.11 ± 0.04a</td>
<td>23.73 ± 0.76ab</td>
<td>0.15 ± 0.05ab</td>
<td>0.98 ± 0.02ab</td>
<td>3.28 ± 0.04ab</td>
<td>2.29 ± 0.03ab</td>
<td>11.25 ± 0.18ab</td>
<td>17.96 ± 0.27a</td>
<td>5.87 ± 0.26a</td>
</tr>
<tr>
<td>TV-26</td>
<td>0.25 ± 0.03ab</td>
<td>0.17 ± 0.01a</td>
<td>0.42 ± 0.03ab</td>
<td>45.69 ± 0.32ab</td>
<td>3.04 ± 0.05b</td>
<td>24.49 ± 0.11ab</td>
<td>0.12 ± 0.01ab</td>
<td>0.84 ± 0.01bc</td>
<td>3.15 ± 0.05ab</td>
<td>1.96 ± 0.02ab</td>
<td>11.64 ± 0.17a</td>
<td>17.17 ± 0.23a</td>
<td>6.13 ± 0.18a</td>
</tr>
<tr>
<td>TS-462</td>
<td>0.29 ± 0.01ab</td>
<td>0.17 ± 0.02a</td>
<td>0.46 ± 0.02ab</td>
<td>45.80 ± 0.46b</td>
<td>4.10 ± 0.02b</td>
<td>20.08 ± 0.24cd</td>
<td>0.27 ± 0.14a</td>
<td>1.24 ± 0.04ab</td>
<td>4.05 ± 0.12ab</td>
<td>1.83 ± 0.09ab</td>
<td>8.66 ± 0.36cd</td>
<td>16.05 ± 0.30bc</td>
<td>6.33 ± 0.22a</td>
</tr>
<tr>
<td>TS-463</td>
<td>0.33 ± 0.04ab</td>
<td>0.18 ± 0.01a</td>
<td>0.51 ± 0.04ab</td>
<td>44.56 ± 0.86a</td>
<td>3.79 ± 0.07cd</td>
<td>20.15 ± 0.56a</td>
<td>0.21 ± 0.07ab</td>
<td>1.11 ± 0.06b</td>
<td>4.06 ± 0.12ab</td>
<td>1.74 ± 0.06ab</td>
<td>8.90 ± 0.17bc</td>
<td>16.02 ± 0.32bc</td>
<td>6.07 ± 0.18a</td>
</tr>
<tr>
<td>TS-491</td>
<td>0.30 ± 0.05ab</td>
<td>0.19 ± 0.02a</td>
<td>0.49 ± 0.07ab</td>
<td>47.83 ± 0.65a</td>
<td>3.96 ± 0.04ab</td>
<td>23.96 ± 0.22ab</td>
<td>0.11 ± 0.03ab</td>
<td>1.19 ± 0.06b</td>
<td>3.05 ± 0.08ab</td>
<td>2.57 ± 0.16ab</td>
<td>9.23 ± 0.28ab</td>
<td>16.15 ± 0.59bc</td>
<td>6.40 ± 0.21a</td>
</tr>
<tr>
<td>TS-520</td>
<td>0.22 ± 0.02a</td>
<td>0.15 ± 0.01a</td>
<td>0.37 ± 0.02b</td>
<td>47.82 ± 0.61a</td>
<td>3.87 ± 0.09cd</td>
<td>23.15 ± 0.40ab</td>
<td>0.13 ± 0.04a</td>
<td>1.19 ± 0.03ab</td>
<td>3.59 ± 0.23ab</td>
<td>2.57 ± 0.19bc</td>
<td>10.16 ± 0.21bc</td>
<td>17.63 ± 0.18ab</td>
<td>6.33 ± 0.28a</td>
</tr>
</tbody>
</table>

(All values are mean ± SE. Values within a column with different letters are significantly different by ANOVA with Duncun’s post hoc test at p < 0.05.)

CHa, (chlorophyll-a, mg/g); CHb (chlorophyll-b, mg/g); CHt (chlorophyll-total, mg/g); TSS (total soluble solid % dry weight); CA (caffeine % dry weight); TP (total polyphenol % dry weight); C (catechin % dry weight); EC (epicatechin % dry weight); ECG (epicatechin gallate % dry weight); EGCG (epigallocatechin gallate % dry weight); TC (total catechin % dry weight); TQ (taster’s quality, score in the scale of 10).

To categorize the cultivars based on their biochemical parameters and tasters’ quality of individual variety, Principal Component Analyses (PCA) was carried out. Result of the analyses showed two components which were extracted which can explain 100% of variability. According to the loadings of PCA first component has high loadings from TV-1(0.829), TV-9(0.886), TeenAli-17/1/54(0.986), TV-20(0.862), TS-491(0.979) and TS-520(1.000) while second component showed high loadings of TV-25(0.990), TV-26(0.965), TS-462(0.754) and TS-463(0.993). So, depending on chemical composition and tasters quality all ten cultivars could be differentiated in two groups (Figure 2) where quality aspect of each group will differ distinctly from each other.
Apart from cultivar differences green tea quality may also be influenced by factors like cultivation, harvest, processing techniques [25] etc. Several earlier studies have reported that genetic [26] and agro-climatic conditions [27] play an important role on the performance of cultivars and the synthesis of chemical constituents in tea shoots and ultimately influence on the quality of made teas.

**CONCLUSION**

In general, green tea quality is determined by tea variety, cultivation, harvest, processing, storage etc. and is judged on the basis of chemistry, taste, aroma, morphology and bioactivity. Novelty lies in the fact that for the first time, an attempt has been made to screen suitable cultivars for green tea manufacturing from Dooars and Terai region, as estates are gradually adopting green tea manufacturing for sustenance in the competitive market. This study revealed clearly that duration of steaming has an important role in achieving good quality of the green tea. It has been understood that time of steaming should be cultivar specific and also dependant on standard of plucked leaf. However, it is needed to corroborate these findings under commercial factory conditions. Under commercial condition the process parameters should be optimized to improve the quality in terms of total phenolics, catechins, colour and sensory properties of the final green tea product. Since there is high demand for quality green tea in Indian market, it is expected that these findings will be useful to the North Bengal tea industry.

**REFERENCES**