



ASSESSING BIOCHEMICAL AND SENSORY CHANGES OF TEA DURING STANDARDIZATION OF A MODERN GREEN TEA PROCESSING UNIT FOR MANUFACTURING QUALITY GREEN TEA

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Abstract

A programmable logic controller (PLC) based modern green tea factory was recently launched at Bangladesh Tea Research Institute to meet the demand of some good quality green tea for the people of the country and also to strengthen research in this area to support the tea industry. This necessitated to carry out research with this processing unit to standardize the manufacturing technique for the production of quality green tea. Different parameters were changed during processing according to the treatments and the product green tea samples were analyzed biochemically for the determination of quality. From the results, it was observed that total polyphenols and caffeine contents decreased significantly with the increase of withering period from 1 hour to 3 hours. If the fixation temperature increased from 250°C to 270°C, the caffeine content almost remained unchanged but the total polyphenols content increased, though not significantly. The total polyphenols and caffeine contents increased as the rolling time increased from 45 minutes to 55 minutes. Immediate after rolling, application of high temperature to dry the leaves was found detrimental for the final green tea quality and thus lowering the roll-drying temperature from 220°C to 200°C significantly enhanced the quality. It was observed that after cooling the roll-dried leaves, increase of temperature in the dryer from 100°C to 120°C enhanced tea quality. From the findings of the present study, it can be suggested that 1 hour Withering → Fixation at 270°C → 55 min Rolling → Roll Drying at 200°C → Final Drying at 120°C can be set as a common procedure for manufacturing quality green tea using the newly established green tea factory of Bangladesh Tea Research Institute.

Keywords: Green tea, Processing, Polyphenols, Caffeine, Sensory

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Introduction

Green tea is a popular non-alcoholic beverage that originated in China and is now consumed all over the world. The young tender leaves of the plant *Camellia sinensis* are used to make green tea. Green tea contains higher quantities of bioactive compounds than other types of teas (Oliveira, 2012). It provides a high concentration of antioxidants called polyphenols (Amin et al., 2023). The growing health awareness level among the households has led to the increasing popularity of green tea in recent times (Adhikary et al., 2023). Green tea is widely recognized for its many health benefits, such as boosting metabolism, improved digestion, decrease type II diabetes, immune system enhancement, enhancing mental alertness, reducing the risk of cardiovascular diseases, and lowering the risk of certain types of cancer (Chacko et al., 2010). Most of these beneficial effects of green tea are mainly attributed to the presence of high contents of polyphenols (Hsu et al., 2011).

Green tea is a nonoxidized tea that is produced by steaming or pan-firing the fresh leaves to inactivate the enzyme polyphenol oxidase which is responsible for the oxidation of tea polyphenols and cause the formation of the brown or black color (Tadesse et al., 2015). Different methods of processing are used for producing green tea. After plucking, according to Chinese tradition, a small amount of indoor withering is permitted to remove the moisture from the leaves but in Japanese style no withering is done. Withering allows to develop some flavor compounds in tea (Singh et al., 2014). After withering, the leaves are promptly steamed or pan-fired to inactivate the enzymes responsible for breaking down the color pigments in the leaves and thus this process preserves the tea's green colour during the subsequent rolling and drying operations. Rolling is also an important process in

green tea manufacturing. The objective of rolling is to sufficiently break the cell walls of the tea leaves so that some interior components can interact with the air. This process increases flavor and aroma. Additionally, rolling controls the release of natural substances and flavor during steeping in the cup. Rolling also allows the leaves to be shaped (Anonymous, 2020). After rolling is completed, the leaves are dried by gently applying heat that reduces the moisture content of the tea leaves to less than 5%. This drying process finalizes the tea, by locking in fragrance and flavor, and enables the leaves to be stored at room temperature. In green tea processing, only minor transformation takes place during the fixing, rolling and drying steps and therefore the majority of biochemical constituents that are present in green leaf are retained in the finished product (Bortolini et al., 2021). Thus, the processing technology of green tea allows to preserve natural polyphenols with respect to the health-promoting properties (Chacko et al., 2010). In addition to polyphenols, green tea also contains the stimulating agent caffeine which is attributed to a reduction in mental fatigue (Joseph et al., 2011).

Bangladesh is an important tea producing country. However, majority of the tea planters of Bangladesh are still unfamiliar about the standard green tea processing technique. Due to lack of proper knowledge about green tea processing, planters are not being able to produce green tea; and those who are trying to produce some, are not able to produce good quality green tea. As the demand for domestically produced green tea has shown an upward trend, the Bangladesh Tea Board has recently launched a programmable logic controller (PLC) based modern green tea factory at Bangladesh Tea Research Institute (BTRI) not only to meet the demand of some good quality green tea for the people of the country but also to strengthen research in this area to support the industry. Therefore, the present study was carried out to standardize the green tea processing technique through the newly launched green tea production unit by assessing the biochemical and sensory properties of the final product. The objectives of the study were - (i) to observe the effect on green tea quality by changing different parameters at the critical stages of processing, and (ii) to establish a standard procedure of production of quality green tea through this processing unit.

Materials and Methods

Experimental site

The site of the study was the newly launched green tea factory of BTRI, Srimangal, Moulvibazar.

Description of the newly established green tea processing unit

The whole processing unit was purchased from the Yaojiangyuan Mechanics Co., Ltd., Ningbo, Zhejiang, China. The machineries of the unit are controlled by PLC (programmable logic controller). The main processing stages in this unit are Withering → Fixation → 1st Re-humidification → Rolling → Roll Drying → 2nd Re-humidification → Final Drying. The machineries used for these stages are shown in the Figure 1.

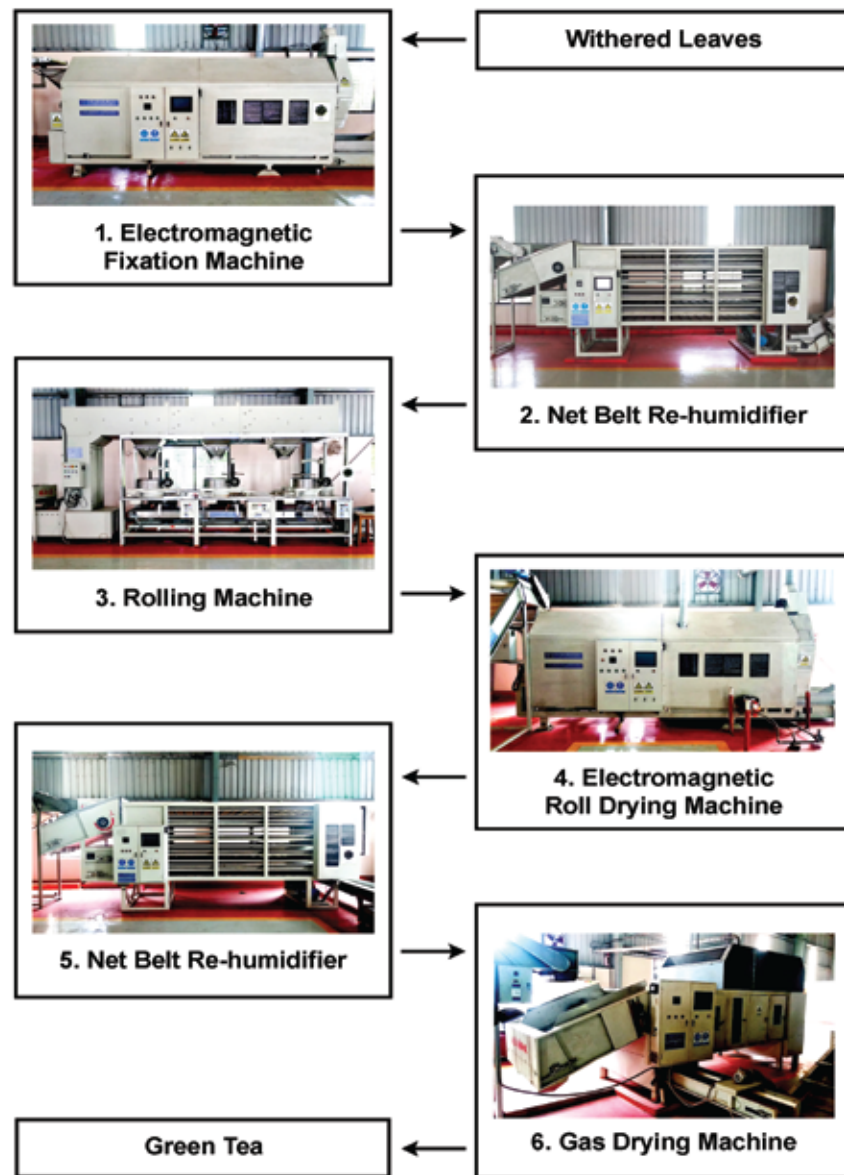


Fig. 1. Flow diagram of green tea manufacturing (sequentially the main machineries used) at the BTRI green tea factory.

For the processing of green tea using this unit, the fresh tea leaves are first withered for a certain period over a tarpaulin laying on the floor and blowing air over the leaves by fans. When withering is completed, the leaves are then ready to go for fixation to kill the enzymes of the leaves. Fixation is done by the Electromagnetic Fixation Machine. After fixation, the 1st Re-humidification is done by the 1st Net Belt Re-humidifier for cooling and softening the leaves. The softened leaves are then transferred to the Rolling Machine to roll the leaves for rupturing the cells of the leaves. After rolling, the leaves are roll dried in the Electromagnetic Roll Drying Machine. The roll dried leaves are then transferred to the 2nd Net Belt Re-humidifier for 2nd Re-humidification. Finally, the leaves are sent to the Gas Drying Machine for final drying.

Green leaf quality and tea manufacturing

Young tea shoots, mostly comprising of single leaf and a bud and two leaves and a bud, were plucked from the BTRI Main Farm and Bilashcherra Experimental Farm of BTRI for all of the processing trials carried out during July to September 2021. Freshly plucked green leaves were then immediately transferred to the factory for manufacturing. Among the processing stages of the newly launched processing unit, five stages i.e. withering, fixation, rolling, roll-drying and final drying were identified as the critical stages of manufacturing that can affect the final green tea quality. Therefore, the tentative relevant time, temperature and duration for withering, fixation, rolling, roll-drying and final drying were pre-fixed based on the manufacturer's instruction and by few processing trials with green leaves and assessing the quality characteristics of processed green teas. Then different treatments were set for all of the mentioned critical stages; and thus, there were five sets of treatments. When all are set, starting from the withering stage, different treatments were employed one by one. When the treatments of a specific stage were employed, the parameters of other stages were remained unchanged to get the treatment effect of that particular stage only. All of the mentioned processing trials were conducted in a similar environment.

In brief, according to the treatments, the fresh tea leaves were first withered for 1-3 hours and then passed through the fixation machine at 250-270°C (for five minutes). After cooling the fixed leaves through the 1st Net Belt Re-humidifier, they were transferred to the rolling machine. The leaves were then rolled in the roller for 45-55 minutes. After rolling, the leaves were discharged from the roller and the balls or lumps developed during the rolling process were broken with hands to separate the shoots. Then the leaves were passed through the electromagnetic roll drying machine at 200-220°C (for five minutes) to partially dry the leaves. After cooling the roll-dried leaves through the 2nd Net Belt Re-humidifier, they were transferred to the final drying machine. In the drying machine, the leaves were dried at 100-120°C, for 17 minutes. After cooling the dried green tea samples, they were packed in airtight bags. The five sets of treatments used in this study are summarized below-

- a) Withering periods-
 - W1 : 1 hour
 - W2 : 2 hours
 - W3 : 3 hours
- b) Fixation temperatures-
 - F1 : 250 °C
 - F2 : 260 °C
 - F3 : 270 °C
- c) Rolling periods-
 - R1 : 45 minutes
 - R2 : 50 minutes
 - R3 : 55 minutes
- d) Roll drying temperatures-
 - RD1 : 200 °C
 - RD2 : 210 °C
 - RD3 : 220 °C
- e) Final drying temperatures-
 - FD1 : 100 °C
 - FD2 : 110 °C
 - FD3 : 120 °C

Data collection

Only one set of treatments were employed during manufacturing in a single processing day due to the limited amount of raw materials. Different treatments were employed one by one and the product green tea samples were collected and preserved in the refrigerator within airtight bags for further biochemical and sensory analysis. The samples were biochemically analyzed in the biochemistry lab of BTRI. Sensory (organoleptic taste) assessment was done in the miniature tea factory and tea tasting room of BTRI.

Chemicals and reagent

Folin-ciocalteu's reagent (FCR), Sodium carbonate anhydrous pure (99.5%), Gallic acid (98%) and Methanol HPLC grade (99.8%) used in this study were purchased from the Sisco Research Laboratories Ltd., India.

Biochemical analyses

The polyphenols and caffeine are the most important compounds present in tea which are of considerable pharmacological significance (Tadesse et al., 2015). The polyphenols in tea include mainly flavanols, hydroxyl-4-flavanols, flavones, flavonols, anthocyanins and phenolic acids (Mukhtar et al., 2000). The important tea flavanols are catechins (flavan-3-ols), of which the major ones are (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (+)-catechin (C), and (+)-gallocatechin (GC) (Chen et al., 2003). The levels of polyphenols in tea has been regarded as a quality indicator of tea (Obanda et al., 1997). Caffeine also has pharmacological effects on central nervous system, heart, and respiratory system. Caffeine is known to decrease fatigue and improve performance (Altimari et al., 2000). Therefore, in this study, we tried to optimize the process with respect to maximal polyphenols and caffeine contents as an indicator of tea quality.

Analysis of total polyphenols content

Total polyphenols content (TPC) was assessed by following the method described by International Organization for Standardization (ISO, 2005) and Anesini et al. (2008). In a mortar and pestle, green tea samples were finely ground, and 0.2 gm was then transferred to graded extraction tubes. Then 5 mL of a hot, 70% v/v methanol/water solution was added, capped, and vortexed. The tubes were heated to 70°C in a hot water bath for 10 min, vortexed after 5 and 10 min, and then cooled to room temperature for 10 min before being centrifuged at 3500 rpm. A second extraction of the residue was performed using 5 mL of a hot, 70% methanol/water solution. The extracts were then mixed together and made up to a volume of 10 mL using cold methanol/water solution. This extracted solution was further diluted by placing 1 mL of extract into a 100 mL volumetric flask and topping it up with distilled water. Gallic acid stock solution @ 1 mg/ml was made by dissolving gallic acid monohydrate into distilled water. Gallic acid standard solutions were made by transferring 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL of stock solutions to 100 mL volumetric flasks and making the volume up to the mark with distilled water which corresponds to 10, 20, 30, 40, and 50 µg/ml gallic acid. One mL of the diluted tea samples and gallic acid standards were placed into separate tubes and 5 mL (10% v/v) Folin-Ciocalteu phenol reagent was added to each tube. They were mixed by vortexing and 4.0 mL (5.7% w/v) sodium carbonate solution was added to each tube. After that, the tubes were left to stand in the dark for 60 minutes at room temperature. A UV-VIS spectrophotometer (Hitachi, U-2900) set to 765 nm against water was used to measure the absorbance values of the standards and samples. Using the gallic acid standard curve, the total amount of polyphenols in a sample was quantified as mg/g dry matter basis.

Determination of caffeine

Caffeine content was estimated by following the method as described by Shrestha et al. (2016). Caffeine was analysed using an HPLC system (Hitachi-chromaster) equipped with a C18 Column (Column Size: 4.6×250 mm, Pore size: 5µm). The temperature of the column and the UV detector were set to 40°C and 275 nm, respectively. For each sample, a 10 µL volume was injected. A flow rate of 1 mL/min was used using 40% methanol (methanol: water = 40: 60) as a mobile phase. In a mortar and pestle, green tea samples were finely ground. A 300 mg grounded tea sample was taken into a conical flask and 200 ml of distilled water was added. The flask was then placed on a hot water bath at 100°C for half an hour. After cooling to room temperature, the solution was filtered using Whatman filter paper. Using distilled water, the solution was diluted to a volume of 250 ml. The solution was further diluted by taking 1 ml of that solution into a 10 ml volumetric flask and made the volume up to the mark by adding distilled water. The solution then filtered through 0.2 µm micro-filter and filled the vial for HPLC analysis. Working standard caffeine solutions were prepared by serial dilutions with HPLC grade water to make different concentrations (2, 4, 6, 8 and 10 ppm); which were used for standard calibration curve preparation (peak area vs concentration). The caffeine content was calculated from the regression equation. Figure 2 illustrates a representative chromatogram of Caffeine for a green tea sample.

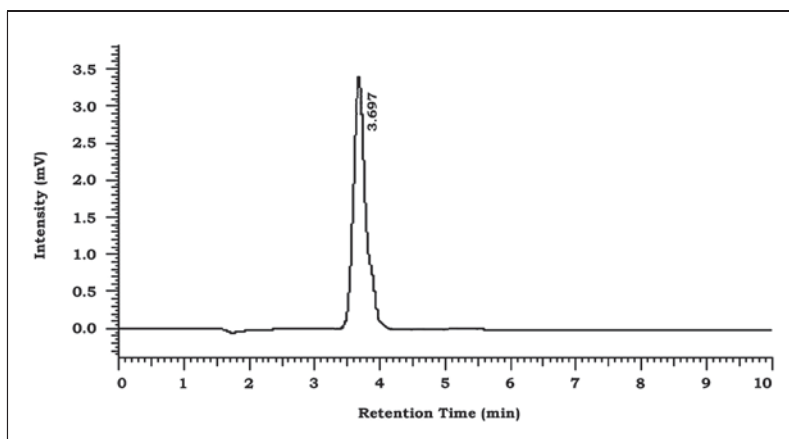


Fig. 2. A representative chromatogram of Caffeine of a green tea sample.

Sensory (organoleptic taste) assessment

Sensory assessment of the product green tea samples was done by conventional organoleptic tasting method and scored numerically on the basis of liquoring characteristics (Hossain et al., 2017). The liquor was prepared by pouring boiling water in a mug of a capacity of 142 ml in which 2.5 g tea was contained. After 3 minutes of brewing, the liquor was poured into a bowl and the taster evaluated the liquor. Liquoring characteristics were assessed by scoring numerically out of 50 points against Liquor color (10 points), Strength (10 points), Brightness (10 points), Bitterness (10 points) and Astringency (10 points). All the quality scores were recorded and they were analyzed statistically.

Statistical analysis

Data were analyzed statistically using the software package SPSS (SPSS, Inc., Chicago, IL, USA). For the comparison of means, one-way ANOVA was used. DMRT was used to test for significant differences at 5% level of probability.

Results and Discussion

Effects of different withering periods

It was noted that total polyphenols and caffeine contents were significantly highest when withering was done for one hour (Figure 1). The total polyphenols and caffeine levels gradually declined significantly as withering periods were extended. According to Kosińska & Andlauer (2004), development of aroma and partial oxidation occurs due to the breakdown of cell walls caused by moisture loss during withering. This suggested that some polyphenols undergo oxidation process during withering that reduces the total polyphenols content. So, longer periods of withering might increase the partial oxidation of polyphenols and thus reduces the total polyphenols contents in the product green tea. In this study, caffeine contents also decreased with extending the withering periods; which is not in agreement with Thombs et al. (1997) who reported that caffeine content increased during withering. The aim of withering is to remove moisture to such an extent that the leaves become softened and ready for further rolling. From sensory analysis, significant changes were noticed in liquor color and brightness due to different withering periods (Appendix 1). When the withering periods were increased, the liquor color and brightness points were decreased. Similarly, total points have been found to be significantly decreased with the increase of withering time. These results imply that, assuming all other variables remain constant, 1 hour of withering is sufficient and preferable to 2 to 3 hours of withering for the production of high-quality green tea.

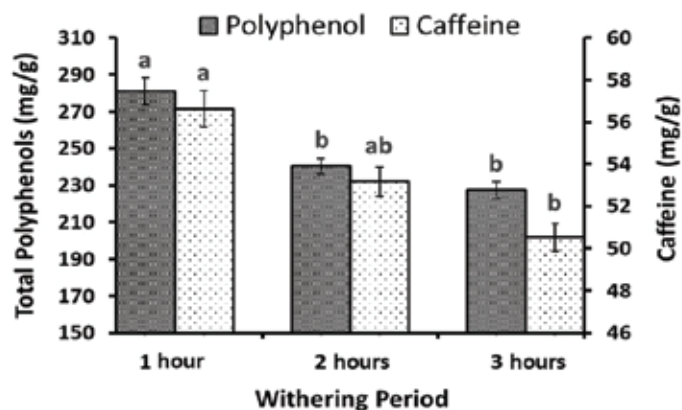


Fig. 3. Effects of different withering periods on the amount of total polyphenols and caffeine in the product green tea.

Effects of different fixation temperatures

To determine the best fixation temperature, three different temperatures were examined. It was observed that the total polyphenols contents increased progressively, but not significantly, when the fixation temperatures were raised from 250 to 270 °C (Figure 4). Caffeine contents did not vary due to different fixation temperatures. Fixation is done to deactivate the enzymes, which initiates oxidation of polyphenols (Kosińska & Andlauer, 2004). Thus it can be assumed that higher temperature deactivated the enzymes efficiently that resulted reduced or no oxidation of polyphenols. However, it is observed that if the fixation temperature is increased further, the leaves become so dry that they break during rolling which adversely affects its physical appearance. These findings suggested that within tolerable limit (so that the leaves do not become friable), higher fixation temperature favored the manufacture of high quality green tea. From sensory analysis, no significant differences on liquor characteristics were observed due to different fixation temperatures (Appendix II).

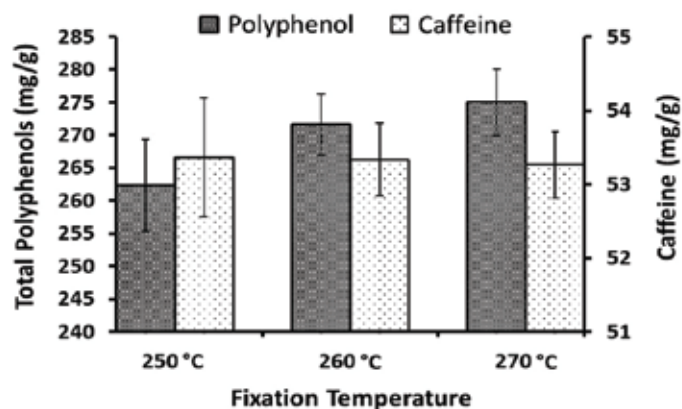


Fig. 4. Effects of different fixation temperatures on the amount of total polyphenols and caffeine in the product green tea.

Effects of different rolling durations

Following fixation, which stops fermentation (oxidation), and allowing the leaves to cool, rolling is the next step in the manufacturing of green tea. In rolling, the leaves are rolled by a rolling machine. Duration of rolling was identified as a crucial factor significantly affecting the green tea quality. When rolling periods were extended from 45 to 55 minutes, there was a significant increase in the amount of

total polyphenols and caffeine (Figure 5). Thus, if all other conditions are constant, 55 minutes rolling performed best in terms of total polyphenols and caffeine concentrations. Sensory analysis also showed significant effect of rolling durations on green tea liquor characteristics (Appendix III). The strength and bitterness as well as the total points were found to be increased with the increase of rolling durations. The purpose of rolling is to sufficiently break down the cell walls of the tea leaves so that some internal components can interact with the air to enhance the flavor and aroma. Rolling also allows the leaves to be shaped (Anonymous, 2023). It is observed that duration of rolling is somewhat dependent on the previous withering and fixation stages. If the optimum temperatures and durations were maintained in the previous stages, 55 minutes rolling gave best result. The newly established processing unit is such a setup that produces whole leaf green tea. If the rolling period is further extended, the rate of splitting of a single shoot into multiple segments increases which adversely affects the appearance of the shoot within the infusion.

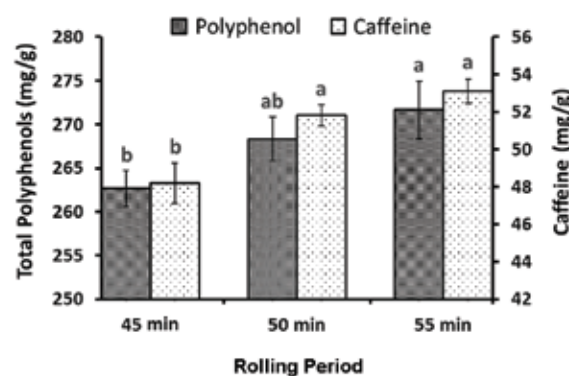


Fig. 5. Effects of different rolling durations on the amount of total polyphenols and caffeine in the product green tea.

Effects of different roll drying temperatures

When rolling is completed, the next task is to dry the leaves gradually. For betterment of final product, removal of moisture and enhancement in flavour, drying is usually repeated several times (Singh et al., 2014). Through this newly established processing unit, drying is completed in two stages i.e. (i) partial drying through the roll drying stage and (ii) complete drying through the final drying stage. Green tea quality was significantly influenced by the roll drying temperature. The roll drying temperature was found inversely correlated with the green tea quality. The total polyphenols and caffeine concentrations dropped when the roll drying temperature increased from 200 to 220 °C (Figure 6). Compared to 200 °C, the decrease was not significant at 210 °C but was significant at 220 °C. Therefore, it can be suggested that the temperature for roll drying shouldn't be more than 210 °C. This result is in accordance with the statements of some researchers in this area who mentioned that if the drying method is improper, heat-sensitive polyphenols may be oxidized and destroyed (Kaur & Singh, 2014; Nistor et al., 2017), resulting in the loss of the majority of the product's antioxidant properties (Hwang & Thi, 2014; Li et al., 2017). Furthermore, Maillard & Berset (1995) and Méndez-Lagunas et al., (2017) described three potential mechanisms that reduce the phenolic content during drying at high temperatures, including the release of bound phenolic compounds, partial degradation of lignin leading to the release of phenolic acid derivatives, and the start of thermal degradation of the phenolic compounds. Hence, the decrease of the phenolic content at high temperature in this study could be due to the above mechanisms. From, sensory analysis, no significant differences on liquor characteristics were noted due to different roll drying temperatures (Appendix IV).

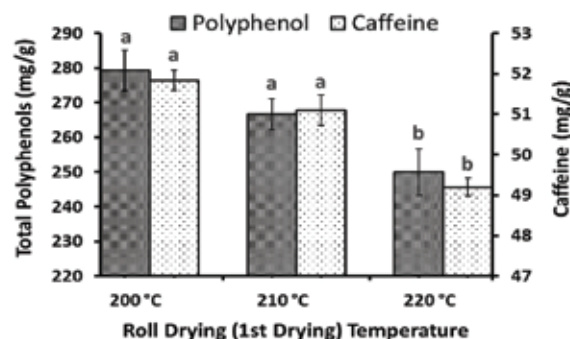


Fig. 6. Effects of different roll drying temperatures on the amount of total polyphenols and caffeine in the product green tea.

Effects of different final drying temperatures

The purpose of drying is to set the color, fragrance, taste and shape in the final product; and to preserve it easily. According to Li et al. (2006), drying procedures, particularly high temperatures and lengthy drying times, may cause some of the phenol components to be destroyed. The duration of final drying is fixed (17 minutes) in the newly established processing unit. In our initial trial, we noticed that drying temperature around 130 °C resulted some burnt smell in the final product. Therefore, comparatively lower drying temperatures (<130 °C) were chosen for this study. It was observed that higher drying temperatures had a favorable impact on the quality of the green tea within the tested range. The total polyphenols and caffeine concentrations were increased as the final drying temperature increased from 100 to 120 °C (Figure 7). The increase was significant for total polyphenols, but insignificant for caffeine. Sensory analysis also showed significant effect of final drying temperatures on green tea liquor characteristics (Appendix V). Liquor color and astringency points as well as the total points were found to be increased with the increase of final drying temperatures. From the finding of the current study, it can be suggested that regardless of other conditions, the final drying temperature can be kept at 120 °C for the manufacture of better quality green tea through the newly established processing unit of BTRI.

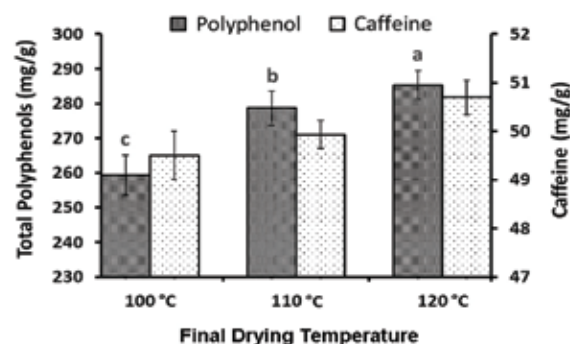


Fig. 7. Effects of different final drying temperatures on the amount of total polyphenols and caffeine in the product green tea.

Conclusion

In this study, a standard procedure for the production of quality green tea through the newly established green tea processing unit of BTRI was established for the main cropping season. Based on the findings, it can be suggested that 1 hour Withering → Fixation at 270°C → 55 min Rolling → Roll Drying at 200°C → Final Drying at 120°C can be set as a common procedure for manufacturing quality green tea. However, slight adjustment is necessary due to the variations in the raw materials and the environmental conditions.

Limitations of this study

The findings obtained and recommendations made in the present study were based on the raw materials collected and the environmental conditions prevailed during the main cropping season (July to September). There will be a variation in shoot quality and environmental conditions during the early and late seasons. Therefore, further studies in early and late seasons may be helpful in standardizing the specific production strategy for those seasons.

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Appendix I. Effects of different withering periods on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
W1	7.54a	6.46	6.55	6.43	5.46a	32.44a
W2	7.23b	6.65	6.22	6.56	5.38ab	32.04b
W3	6.84c	6.84	6.13	6.11	5.17b	31.09c
LSD at 0.05 level of significance	0.18	NS	NS	NS	0.06	0.24

NS- Not significant. Values bearing different letters within a column are statistically different at 5% level of probability.

Appendix II. Effects of different fixation temperatures on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
F1	7.49	6.61	6.49	6.43	5.42	32.44
F2	7.48	6.63	6.48	6.47	5.41	32.47
F3	7.49	6.71	6.53	6.29	5.43	32.45
LSD at 5% level of significance	NS	NS	NS	NS	NS	NS

NS- Not significant. Values bearing different letters within a column are statistically different at 5% level of probability.

Appendix III. Effects of different rolling durations on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
R1	7.43	6.51b	6.43	6.38b	5.39	32.14b
R2	7.45	6.58ab	6.48	6.42ab	5.41	32.34ab
R3	7.48	6.68a	6.53	6.58a	5.55	32.82a
LSD at 5% level of significance	NS	0.06	NS	0.11	NS	0.23

NS- Not significant. Values bearing different letters within a column are statistically different at 5% level of probability.

Appendix IV. Effects of different roll drying temperatures on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
RD1	7.33	6.52	6.46	6.51	5.49	32.31
RD2	7.38	6.48	6.51	6.48	5.48	32.33
RD3	7.28	6.51	6.52	6.47	5.49	32.27
LSD at 5% level of significance	NS	NS	NS	NS	NS	NS

NS- Not significant. Values bearing different letters within a column are statistically different at 5% level of probability.

Appendix IV. Effects of different roll drying temperatures on infusion characteristics of green tea

Treatments	Liquor color	Strength	Astringency	Bitterness	Brightness	Total
	(10)	(10)	(10)	(10)	(10)	(50)
FD1	7.24b	6.53	6.28b	6.54	5.54	32.13c
FD2	7.36ab	6.48	6.49ab	6.47	5.46	32.26b
FD3	7.45a	6.47	6.54a	6.54	5.52	32.52a