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Research article

A STUDY OF THE VARIABILITY OF CAFFEINE PROFILE OF PROCESSED BLACK TEA MARKETED IN CERTAIN REGIONS OF ASSAM WITH REFERENCE TO THE CAFFEINE CONTENT OF TENDER TEA LEAVES AND ORGANIC TEA.

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ABSTRACT: The aim of this investigation was to go for an in-depth comparative study of the variability of caffeine profile of processed black CTC tea marketed in the Tezpur and Gohpur regions of Sonitpur district of Assam with reference to the caffeine content of tender tea leaves and handmade organic tea by Reverse Phase-High performance Liquid chromatography method. HPLC analysis of different processed CTC tea samples has shown that the caffeine content ranges from 2.21 to 3.05%.The average caffeine percentage in black tea samples analyzed was found to be 2.61%. HPLC analysis has also shown a negligible regional variation in caffeine percentage of the tea samples of Tezpur and Gohpur sub-divisions of the district. The caffeine content of dried tender tea leaves was found to be highest (3.07%) as expected while handmade organic black tea was found to contain 2.56 % caffeine. Variation in the average caffeine content of the processed CTC tea samples was due to a number of factors such as stages of leaves used for tea production, manufacturing processes adopted in the gardens in general and Jats and Clones of tea plants in particular.
Key words: Caffeine profile, CTC tea, Reverse Phase-High performance Liquid Chromatography, Organic black tea

INTRODUCTION

Nature has ungrudgingly blessed Assam with a congenial climate for tea cultivation. Nevertheless, widespread occurrence of deadliest tea pests like Tea mosquito bug (*Helopeltis theivora),* Red spider mite (*Oligonichus coffeae*) and looper caterpillar (*Buzura suppressaria*) has compelled the tea planters of the region to resort to application of different categories of pesticides [1].Caffeine is an active ingredient in all types of tea chemically which is a bitter, white crystalline xanthine alkaloid, 1, 3, 7-tri methyl xanthine and is the world's most widely consumed psychoactive drug. In humans, caffeine acts as a stimulant for CNS that temporarily wards off drowsiness [2]. Caffeine is also known to have a quenching effect on the production of hydroxyl radicals as well as on oxidative DNA breakage by hydroxyl radicals [3]. Tea products, both CTC and orthodox types widely used as a beverage by almost all sections of people of Assam are reported to contain a relatively higher caffeine content in comparison to the processed tea of other countries like China, Japan, Indonesia, Sri Lanka and so on [4].Earlier studies revealed that caffeine content is associated to origin, genetic and environmental variability, harvest time and processing manner of plant material [5]. Caffeine content of various tea brands determined by the HPLC analyses is found to decrease in the following order: White tea (3.62%) > yellow tea (3.18%) > black tea (2.79%) > oolong tea (2.77%) > green tea (2.35%) > roasted mate tea (1.13%) > mate tea (1.02%) [5]. Studies have shown that the caffeine content of Ethiopian green tea leaves was greatest of all the Ethiopian teas while black lion tea has the least. [6].The caffeine content of certain tea brands of Kenyan market is reported to be in the following order chai mara moja > finlays premium> kericho gold > sasini [7].

Considering the usefulness of caffeine in optimum concentration in human body, and keeping in view its wide spread consumption in the state of Assam, attempts had been made to go for an in-depth comparative study the caffeine content of processed tea marketed in different regions of Sonitpur district of the state with reference to the caffeine content of tender tea leaves and handmade organic tea grown without using chemical pesticides and fertilizers.

MATERIALS AND METHODS

Processed tea samples available in the markets of different regions belonging to Tezpur (Latitude: 26° 36' 34" North, Longitude: 92° 49' 37" East) and Gohpur (26° 53' 0" North, 93° 38' 0" East) sub-divisions of Sonitpur district, Assam were collected (purchased) randomly. Tender tea leaves were collected from a local tea garden and organic black tea sample was procured from Meen Mohan Tea Garden, Madhupur village, Lakhimpur, Assam, a reknown producer and exporter of organic tea.

The estimation of caffeine content in the collected tea samples was performed using Perkin Elmer series 200 HPLC equipped with Total chrom software (version:6.2.0.0.0:B27) running under Microsoft Windows 2000 which is shown in the figure: 1

Figure 1. Perkin Elmer series 200 HPLC.

HPLC conditions

HPLC Column: SPHERI- 5 RP (Reversed-phase) column C18 (PerkinElmer, USA) with particle size 5 µm and dimension 4.6 mmx250mm.

Detector: PDA (Photo Diode Array).

λ_{max} =255nm.

Mobile phase:

A: Ammonium acetate buffer.

B: 1, 4-dioxane and Acetonitrile

Flow: A: B=80:20 at 2 ml/min.

Experimental Procedure

Preparation of mobile phase A

0.75% ammonium acetate buffer (w/v) was prepared by mixing 7.5 gm of ammonium acetate in 1000 ml HPLC grade water. The P^H of the solution was adjusted at 5.8 by adding ammonia and acetic acid. The solution was then filtered using micro filter.

Preparation of mobile phase B

HPLC grade 1, 4-dioxane and acetonitrile were mixed in the ratio of 1:1.The total volume taken was 200 ml (100 ml 1, 4 dioxane and 100 ml acetonitrile).

Preparation of caffeine standards

Three caffeine standards were prepared as under:
Standard–I: 2.71mg of accurately weighed pure caffeine was dissolved in 5 ml. of mobile phase in a 5 ml volumetric flask. Standard-II and III were prepared by serial dilution of standard-I as follows:
Standard –II: 2 ml. of standard-I was mixed with 2 ml of mobile phase.
Standard –III: 2 ml. of standard-II was mixed with 2 ml. of mobile phase.

Calibration of the apparatus

After preparation of standard caffeine solution, the apparatus was calibrated by using the above solutions.Calibration curve was obtained from the chromatograms of the three standard solutions of known concentration.

Preparation of sample

30 mg of each sample was accurately weighed in duplicate and kept in 10 ml volumetric flask. Each sample was mixed with 5 ml. of mobile phase with the help of micropipette. Sample solutions were kept in ultrasonic bath for thirty minutes and left overnight to ensure complete decaffeination.The solutions were then filtered through micro filter and filtrates were injected into the HPLC system for analysis. All analyses were repeated three times.

Quantitative analysis of caffeine:

The calibration curve was used for quantitative analysis of caffeine in different tea samples. A comparison of the caffeine peak areas in the tea samples (extracts) with that of the standards was made for quantitative determination of caffeine content. The caffeine levels of the samples were calculated from the regression equation of the best line of fit of the standards.

RESULTS

HPLC analysis for caffeine level of the black processed tea samples collected from the two regions of the district has shown that the caffeine content ranges from 2.21 to 3.05 % (Table 1and 2). The average caffeine percentage in black tea samples analyzed was found to be 2.61. HPLC analysis has also shown a negligible regional variation in caffeine percentage of the tea samples of Tezpur and Gohpur sub-divisions of the district; the mean caffeine content of the former being 2.60 ± 0.29 % while that of the latter was found to be 2.62 ± 0.23 % (Table 3) .
On the other hand, the caffeine content of dried tender tea leaves (t-crude-001) and handmade organic tea was found to be 3.07 % and 2.56 % respectively (Figures 5 and 6).

Table: 1. Percentage of caffeine in the processed CTC tea samples collected from Tezpur sub-Division (Standard Deviation = 0.29).

Sl. nos.	Sample name:	Retention time (min)	Conc. of caffeine (mg/ml)	% of caffeine
1.	T-001	2.193	0.0221	2.21 ± 0.29
2.	T-002	2.171	0.0239	2.39 ± 0.29
3.	T-003	2.185	0.0284	2.84 ± 0.29
4.	T-004	2.193	0.0260	2.60 ± 0.29
5.	T-005	2.193	0.0301	3.01 ± 0.29
6.	T-006	2.193	0.0305	3.05 ± 0.29
7.	T-007	2.207	0.0230	2.30 ± 0.29
8.	T-008	2.207	0.0235	2.35 ± 0.29
9.	T-009	2.244	0.0250	2.50 ± 0.29
10.	T-010	2.251	0.0277	2.77 ± 0.29

Table: 2. Percentage of caffeine in the processed CTC tea samples collected from Gohpur sub-Division (Standard Deviation = 0.23).

Sl.nos.	Sample name:	Retention time (min)	Conc. of caffeine (mg/ml)	% of caffeine
11.	G-001	2.185	0.0290	2.90 ± 0.23
12.	G-002	2.193	0.0232	2.32 ± 0.23
13.	G-003	2.193	0.0284	2.84 ± 0.23
14.	G-004	2.200	0.0240	2.40 ± 0.23
15.	G-005	2.200	0.0273	2.73 ± 0.23
16.	G-006	2.193	0.0264	2.64 ± 0.23
17.	G-007	2.200	0.0240	2.40 ± 0.23
18.	G-008	2.207	0.0296	2.96 ± 0.23
19.	G-009	2.185	0.0236	2.36 ± 0.23
20.	G-010	2.244	0.0261	2.61 ± 0.23

Table: 3. Overall caffeine content in processed black CTC tea in the two sub-divisions of Sonitpur district.

Sample type:	Collection region:	HPLC- % of caffeine (Mean)	Range
Black (CTC)tea	Tezpur and its adjoining areas (markets).	2.60 ± 0.29	2.21-3.05
Black (CTC)tea	Gohpur and its adjoining areas (markets).	2.62 ± 0.23	2.32-2.96

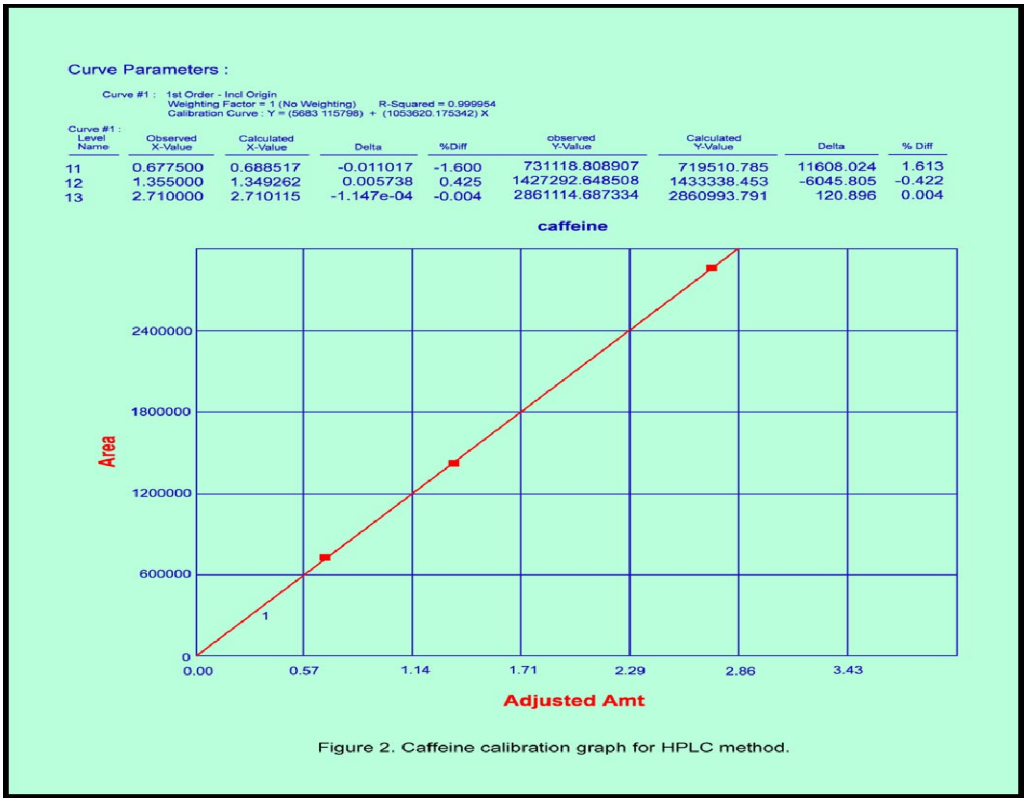
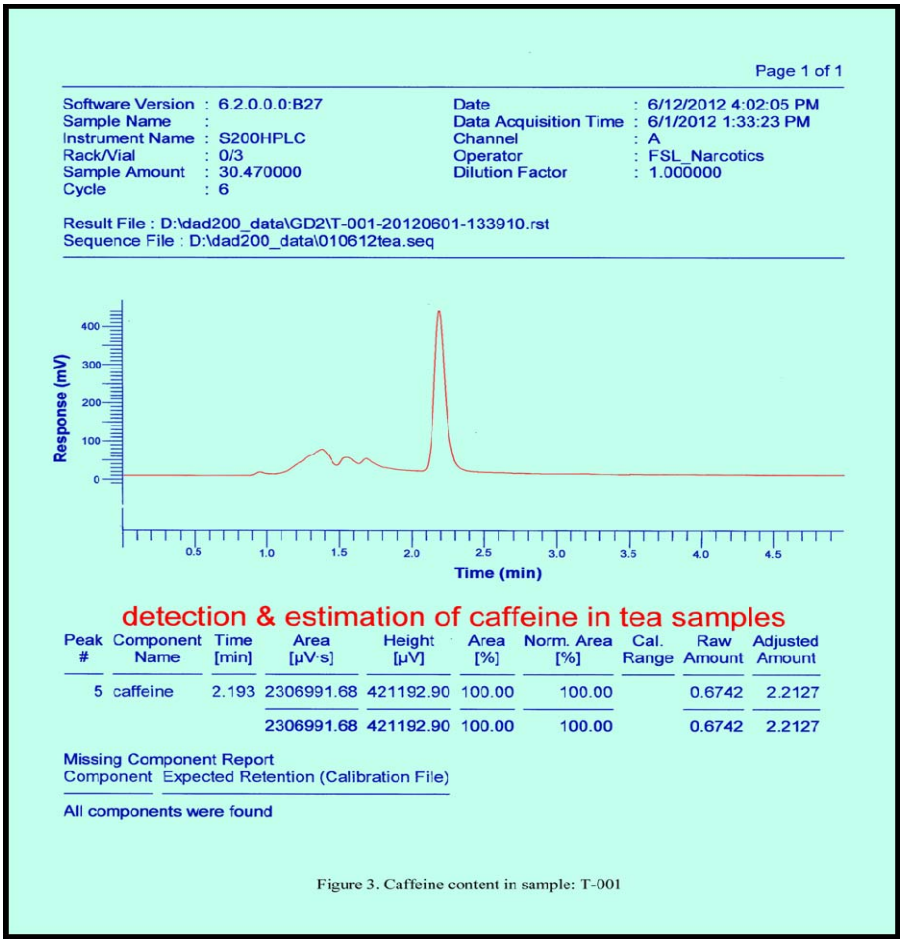


Figure 2. Caffeine calibration graph for HPLC method.



Page 1 of 1

Software Version	: 6.2.0.0.0:B27	Date	: 6/12/2012 4:02:05 PM
Sample Name	:	Data Acquisition Time	: 6/1/2012 1:33:23 PM
Instrument Name	: S200HPLC	Channel	: A
Rack/Vial	: 0/3	Operator	: FSL_Narcotics
Sample Amount	: 30.470000	Dilution Factor	: 1.000000
Cycle	: 6		

Result File : D:\dad200_data\GD2\T-001-20120601-133910.rst
Sequence File : D:\dad200_data\010612tea.seq

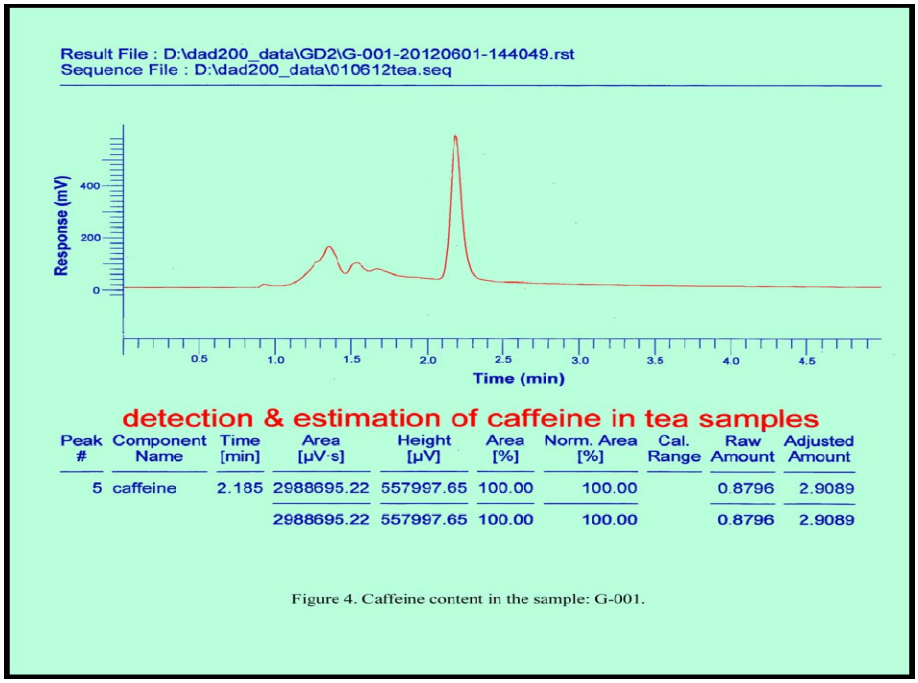
detection & estimation of caffeine in tea samples

Peak #	Component Name	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	Cal. Range	Raw Amount	Adjusted Amount
5	caffeine	2.193	2306991.68	421192.90	100.00	100.00		0.6742	2.2127
			2306991.68	421192.90	100.00	100.00		0.6742	2.2127

Missing Component Report
Component Expected Retention (Calibration File)

All components were found

Figure 3. Caffeine content in sample: T-001

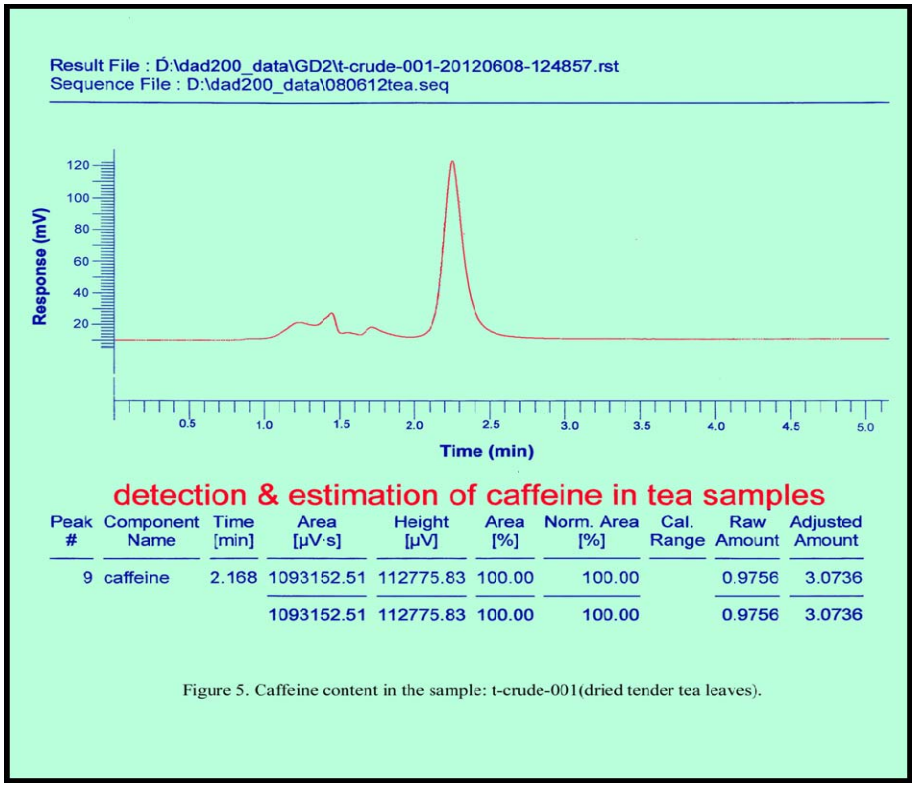


Result File : D:\dad200_data\GD2\G-001-20120601-144049.rst
Sequence File : D:\dad200_data\010612tea.seq

detection & estimation of caffeine in tea samples

Peak #	Component Name	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	Cal. Range	Raw Amount	Adjusted Amount
5	caffeine	2.185	2988695.22	557997.65	100.00	100.00		0.8796	2.9089
			2988695.22	557997.65	100.00	100.00		0.8796	2.9089

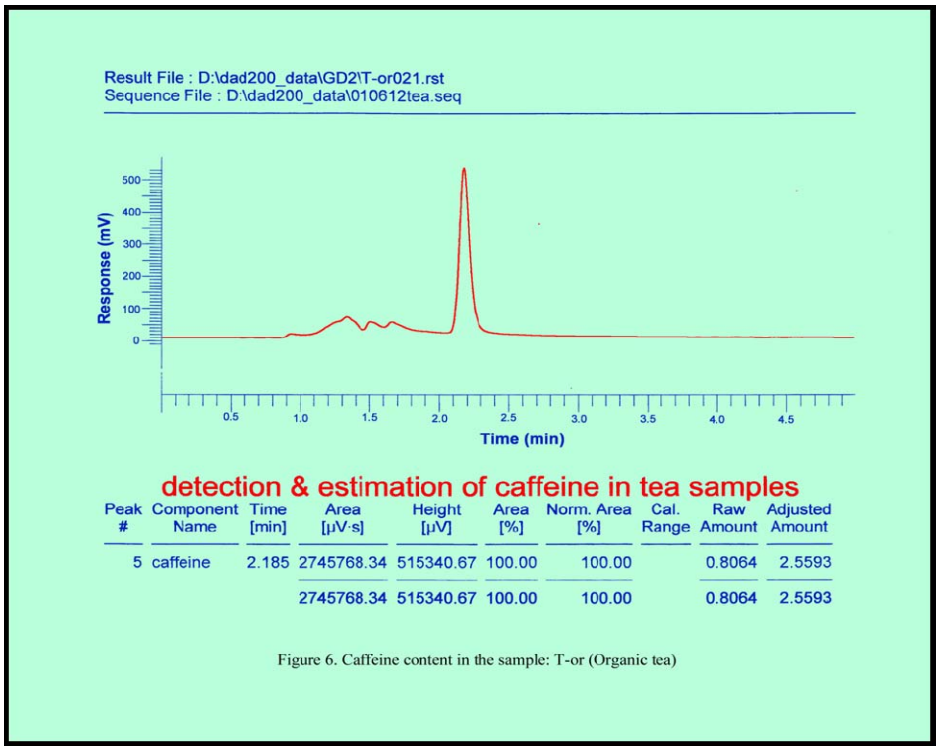
Figure 4. Caffeine content in the sample: G-001.



detection & estimation of caffeine in tea samples

Peak #	Component Name	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	Cal. Range	Raw Amount	Adjusted Amount
9	caffeine	2.168	1093152.51	112775.83	100.00	100.00		0.9756	3.0736
			1093152.51	112775.83	100.00	100.00		0.9756	3.0736

Figure 5. Caffeine content in the sample: t-crude-001(dried tender tea leaves).



detection & estimation of caffeine in tea samples

Peak #	Component Name	Time [min]	Area [µV·s]	Height [µV]	Area [%]	Norm. Area [%]	Cal. Range	Raw Amount	Adjusted Amount
5	caffeine	2.185	2745768.34	515340.67	100.00	100.00		0.8064	2.5593
			2745768.34	515340.67	100.00	100.00		0.8064	2.5593

Figure 6. Caffeine content in the sample: T-or (Organic tea)

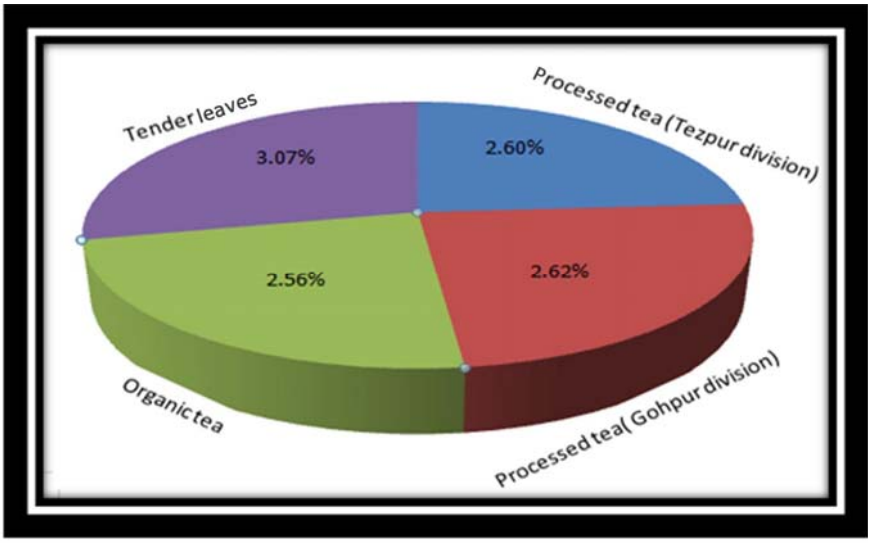


Figure 7 showing average caffeine content of the processed tea samples (black CTC) marketed in Tezpur and Gohpur sub-divisions of Sonitpur district, Assam, India in comparison with the caffeine content of tender tea leaves and handmade organic tea.

DISCUSSION

The presence of 2.21 to 3.05 % caffeine in the processed black CTC grade Assam tea was in agreement with the result obtained by Conrad AStill and his co-workers (2001) who found caffeine in the range of 2.21 to 3.97 % by HPLC analysis of Assam tea. The findings were also in agreement with the result obtained by Komes D.,et al (2009) who found 2.79% caffeine in black tea. However, the average caffeine content in black tea was found to be much less (2.61%) in comparison with what Conrad AStill et.al. found (3.23%) in spite of the fact that the tea leaves of this region contain a relatively higher caffeine content. However, the caffeine content of the samples of dried tender tea leaves was found to be highest (3.07%) amongst all other samples as expected. These caffeine values agree well with literature quoted values of 2-5% ((Wanyika, H.N., et al., 357).

A number of factors have been found to determine the caffeine content of the processed tea. It is an established fact that distribution of caffeine in the tea plant depends on the part of the plant it is derived from. Tea leaves in bud stage contain highest amount of caffeine (4.70%) which is gradually reduced in the subsequent stages of development. Age of the bush is another factor; the quality period is reported to be 15-30 years for most Jats and after 40 years HRP (Height Reduction Pruning) is required to be carried out so as to rejuvenate the tea bushes for production of good quality tea. Caffeine level in tea is also reported to vary greatly with the manufacturing processes.

To explore the possible reasons of caffeine variability in the processed tea products of the region, a survey concerning the use of plant parts for tea production and manufacturing processes conducted in the gardens was carried out based on questionnaires and semi-structured personal interviews for managerial staff of the concerned gardens. The study has shown that usually, in most of the gardens, tender leaf i.e. two leaves & a bud containing more chemicals required for good quality tea are used for tea production. However, this is not always uniform and in fact, sometimes the third and occasionally, fourth leaf of the plant are plucked and mixed with the tender leaves for tea manufacture which might affect caffeine content.

The plucking round is usually 7 days during May to July in all the gardens which is found to be economically more viable apart from yield of good quality tea. However, its frequency should vary with weather conditions like seasonal variation in temperature. For instance, from March to April, 8 days of plucking round is preferred while from August to October, 6 days of plucking rounds are suitable. Any increase in plucking round which is not suitable for a particular season leads to deterioration of tea quality due to increase in fiber contents (insoluble solids) in leaves thereby decreasing soluble solid contents like polyphenols and caffeine.

In the process of tea manufacture, chemical withering involves 14-15 hours of treatment while physical withering depends on atmospheric temperature. The fermentation time taken by most of the gardens is judged by the colour and nose of the fermented tea so as to ensure good flavour and aroma in tea products. However, it varies depending on weather conditions. For example, if the temperature is around 75^0 F then 150 minutes of fermentation is required. On an average, the fermentation time ranges from 1 hr. 30 minutes to 1hr.50 minutes depending upon environmental temperature. Changes in caffeine level during various stages of black tea production have already been studied by earlier workers which show that there occurs an initial increase in caffeine content during the short withering stage of black tea production, followed by a decrease during the fermentation and drying stages and that manufacturing of black CTC grade tea involves more fermentation in comparison to the orthodox grade tea production. Variation of oxidation (fermentation) time adopted in different gardens depending on environmental temperature, thus, seems to be an another reason causing variability of caffeine content which is reflected in the processed tea samples marketed in different regions of the district.

The caffeine content which determines the strength of tea is totally dependent on the chemical content of the raw material i.e. green leaf. Again, the chemical content of the green leaf varies from Jat to Jat & Clone to Clone. It is found that normally most gardens are following the combination of variety like Standard clone (50%), Quality clone (30%) and Yielding clone (20%). Jats such as: TS-464, 491, 520, 462 and clones: TV1, TV9, S3A3, Tin-17/1/54, R-94, P-126, TV23, TV25, TV29, TV30 are usually grown in the gardens of Sonitpur region. Of them, all seed jats and the clones particularly TV29, TV90, TV23 and TV9 are high yielding and also economically more viable .However, all gardens do not grow the same Jats and clones.

CONCLUSION AND RECOMMENDATION

HPLC analysis of the processed CTC tea samples collected from different regions of Sonitpur district, Assam, India has shown that the caffeine values of these samples agree well with literature quoted values of 2-5%.Caffeine content of the green leaf which determines the strength of tea varies from Clone to Clone & Jat to Jat. It is also found that tender leaves are sometimes mixed with the 3rd and occasionally 4th leaves for tea production which results in variation of caffeine content as well as flavour and aroma of the tea produced. Moreover, Variation of oxidation (fermentation) time adopted in different gardens depending on environmental temperature plays a major role in determining caffeine content. Thus, it can be concluded that tea products of the surveyed area are suitable enough for consumption in terms of their caffeine content as caffeine values of the analyzed samples matched well with documented values. The variation in the average caffeine content of the analyzed processed tea samples was due to a number of factors such as stages of leaves used for tea production, manufacturing processes adopted in the gardens in general and Jats and Clones of tea plants in particular.

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