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Research article

A COMPARATIVE STUDY OF THE CAFFEINE PROFILE OF MATURE TEA LEAVES AND PROCESSED TEA MARKETED IN SONITPUR DISTRICT OF ASSAM, INDIA.

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ABSTRACT: The aim of this investigation was to go for a quantitative estimation of the caffeine content in sufficiently mature tea leaves from some tea gardens of Sonitpur district of Assam by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) and to compare the result with that of the caffeine content of processed tea marketed in the region. The experimental findings confirmed that the caffeine content decreases with the age of tea leaves. Analysis of data using t-test by SPSS concerning percentages of caffeine in the processed tea samples of Tezpur and Gohpur sub-divisions has shown no significant difference between the processed tea samples of the two regions at 0.05 levels. But, the overall caffeine content in the samples of processed tea and mature tea leaves was found to be highly significant at 0.05 levels.
Key words: Caffeine, Reverse Phase-High performance Liquid Chromatography, t-test, SPSS.

INTRODUCTION

Tender tea leaves i.e., the first and second leaf have been found to contain considerable amount of caffeine and as such, use of two such leaves and a bud are recommended for producing high quality tea. Apart from tea and coffee, leaf of Mate is also an important source of caffeine. Mate leaves more than a year old, have about 1.5% caffeine while adult leaves, those more than two years old, contain only about 0.7% caffeine [1].The Assam Tea (*Camellia sinensis* var.*assamica*) from where black teas are usually made, is found to contain relatively higher amount of caffeine than the china variety [2]. Caffeine content of five types of tea 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%) [3]. The average caffeine content of processed black tea samples of Tezpur and Gohpur regions of Sonitpur district, Assam, India has already been determined and was found to be 2.61% [4]. Considering these established findings, attempts have been made to study the caffeine content of sufficiently mature dried tea leaves from some tea gardens of Sonitpur district of Assam after more than one year of storage and to compare the findings with that of the caffeine content of processed tea marketed in the region.

MATERIALS AND METHODS:

Sample collection:

Samples of fully mature green tea leaves from ten gardens, five from Tezpur (Latitude: 26° 36' 34" North, Longitude: 92° 49' 37" East) and the other five from Gohpur (26° 53' 0" North, 93° 38' 0" East) sub-divisions of Sonitpur district, Assam were collected (plucked) randomly (Table 1). Processed tea samples available in the markets of different regions belonging to Tezpur and Gohpur sub-divisions of Sonitpur district, Assam were collected (purchased) randomly. (Table 2).

Table 1. Samples of mature leaves taken for quantitative estimation of caffeine.

Sl.No.	Sample name	Collection region	Retention time (min)	Conc. of caffeine (mg/L)	% of caffeine
1	T-crude 0001	Tezpur sub-division.	2.134	14.35	1.435
2	T-crude 0002	Tezpur sub-division.	2.134	14.04	1.404
3	T-crude 0003	Tezpur sub-division.	2.109	10.87	1.087
4	T-crude 0004	Tezpur sub-division.	2.103	6.51	0.651
5	T-crude 0005	Tezpur sub-division.	2.109	10.87	1.087
6	G-crude 0006	Gohpur sub-division.	2.135	14.88	1.488
7	G-crude 0007	Gohpur sub-division.	2.086	16.44	1.644
8	G-crude 0008	Gohpur sub-division.	2.090	15.74	1.574
9	G-crude 0009	Gohpur sub-division.	2.146	9.45	0.945
10	G-crude 00010	Gohpur sub-division.	2.130	8.59	0.859

Table 2. Samples of processed tea taken for quantitative estimation of caffeine.

Sl. No.	Sample name	Collection region	Retention time (min)	Conc. of caffeine (mg/L)	% of caffeine
11	T-001	Tezpur Town.(Tezpur sub-division)	2.193	22.1	2.21
12	T-002	Dekargaon.(Tezpur sub-division)	2.171	23.9	2.39
13	T-003	Binduguri.(Tezpur sub-division)	2.185	28.4	2.84
14	T-004	Rongapara. .(Tezpur sub-division)	2.193	26.0	2.60
15	T-005	Goroimari.(Tezpur sub-division)	2.193	30.1	3.01
16	T-006	Balipara.(Tezpur sub-division)	2.193	30.5	3.05
17	T-007	Dhekiajuli.(Tezpur sub-division)	2.207	23.0	2.30
18	T-008	Majbat.(Tezpur sub-division)	2.207	23.5	2.35
19	T-009	Chariduar.(Tezpur sub-division)	2.244	25.0	2.50
20	T-010	Missamari.(Tezpur sub-division)	2.251	27.7	2.77
21	G-001	Gohpur town- Daily Bazar	2.185	29.0	2.90
22	G-002	Kalabari. (Gohpur sub-division)	2.193	23.2	2.32
23	G-003	Halem. (Gohpur sub-division)	2.193	28.4	2.84
24	G-004	Borongabari.(Gohpur sub-division)	2.200	24.0	2.40
25	G-005	Boroi.(Gohpur sub-division)	2.200	27.3	2.73
26	G-006	Balijan .(Gohpur sub-division)	2.193	26.4	2.64
27	G-007	Hawajan.(Gohpur sub-division)	2.200	24.0	2.40
28	G-008	Ghahigaon.(Gohpur sub-division)	2.207	29.6	2.96
29	G-009	Dubia.(Gohpur sub-division)	2.185	23.6	2.36
30	G-010	Magoni (Gohpur sub-division)	2.244	26.1	2.61

Pretreatment of mature tea leaves:

The tea leaves collected were placed in between pieces of news paper sheets. The leaves thus arranged were moderately sun dried for three days and then dried in shade for another four months to ensure complete loss of moisture content. The dried leaves were crushed in a mortar with pestle and kept in airtight containers.

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 mobile phases

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

For analysis of samples of processed tea, standard–I was prepared by dissolving 2.71 mg. of pure caffeine in 5 ml. of mobile phase in a 5 ml volumetric flask. For mature leaves, the amount taken for preparation of standard-I was 2.95 mg. Standard-II and III were prepared by serial dilution of standard-I in each case. Calibration curves for HPLC analysis of processed tea and mature leaves were obtained from the chromatograms of the three standard solutions.

Sample preparation procedure adopted for processed tea and mature tea leaves

For quantitative estimation of caffeine content in the samples of processed tea and mature leaves, 30 mg of each sample was accurately weighed and kept in 10 ml volumetric flasks. Each sample was then mixed with 5 ml. of mobile phase, kept in ultrasonic bath for thirty minutes and left overnight. 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 estimation of caffeine content in the samples of processed tea and mature tea leaves was performed using PerkinElmer series 200 HPLC equipped with Total chrom software (version:6.2.0.0.0:B27).The calibration curves (Figures 3 and 4) were used for quantitative estimation of caffeine in the samples of mature tea leaves and processed tea. 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.

RESULTS

The caffeine content of the samples of dried mature tea leaves collected from the gardens of Tezpur and Gohpur regions of the district was found to be in the range of 0.65 % to 1.64%. On the other hand, caffeine content of the processed black tea samples collected from the markets of Tezpur and Gohpur regions of the district was found to be in the range of 2.21 to 3.05 %. The average caffeine content of the samples of processed tea and mature tea leaves analyzed was found to be 2.61% and 1.22% respectively. The statistical analysis of HPLC data using t-test by SPSS for retention time, concentration of caffeine (mg/L) and percentages of caffeine in the processed tea samples of Tezpur and Gohpur sub-divisions of Sonitpur district (Table 3) has shown no significant difference between the processed tea samples of the two regions at 0.05 levels. On the contrary, t-test carried out for the same parameters in the samples of processed tea and mature tea leaves has shown that the difference in overall caffeine content of these two groups of samples was highly significant at 0.05 level (Table 4).

Figure 1.Some samples of dried and crushed mature tea leaves.

Figure 2. Some of the processed tea samples analyzed.

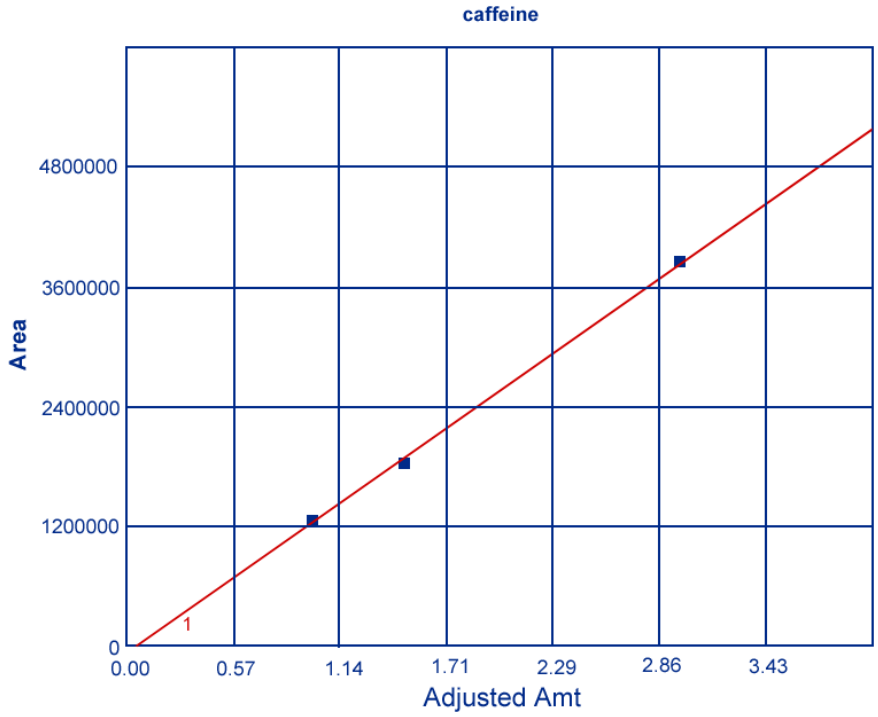
Fit Analysis Output For Method File: D:\DAD200_METHOD\161112 TEA CRUDE-.MTH
Reprocess Number : fsl: 4603
Component Name : caffeine
Date : 12/11/2012 4:00:56 PM

Curve Parameters:

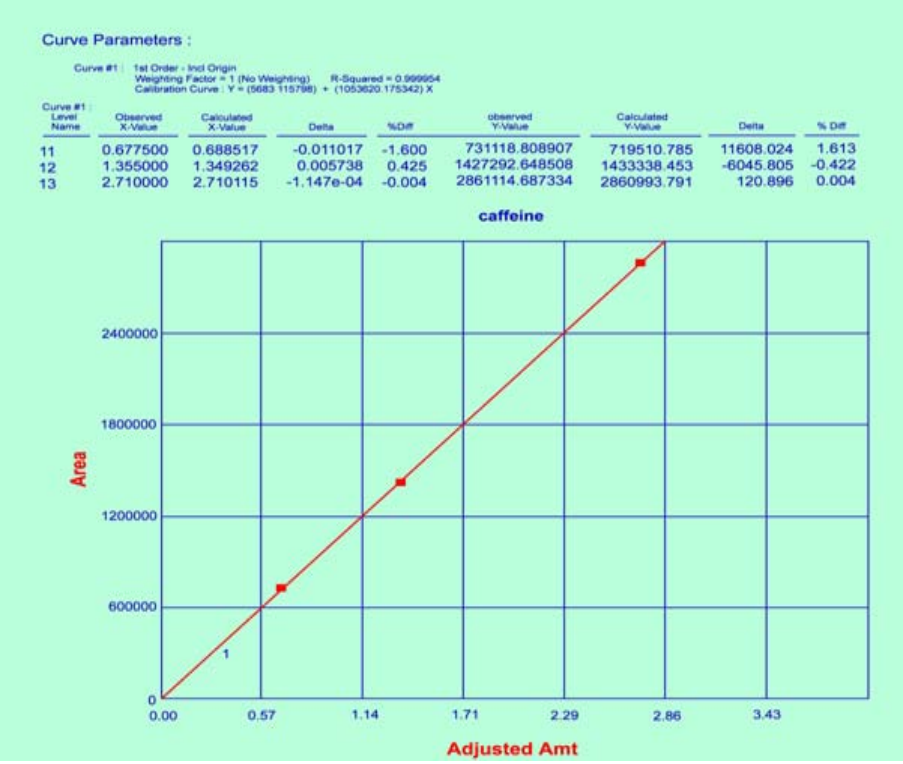
Curve #1 1st Order - Incl Origin
Weighting Factor = 1 (No Weighting) R-Squared = 0.999156
Calibration Curve : Y = (-35251.214722) +(1303944.281619) X

Curve #1

Level Name	Observed X-Value	Calculated X-Value	Delta	%Diff	Observed Y-Value	Calculated Y-Value	Delta	%Diff
I3	0.983300	0.979615	0.003685	0.376	1242112.053053	1246917.197	-4805.144	-0.385
I2	1.475000	1.425845	0.049155	3.447	1823971.138935	1888066.601	-64095.462	-3.395
I1	2.950000	2.975806	-0.025806	-0.867	3845033.807404	3811384.416	33649.391	0.883



. Figure 3. Caffeine calibration graph for HPLC analysis of mature tea leaves.



. Figure 4.Caffeine calibration graph for HPLC analysis of processed tea samples.

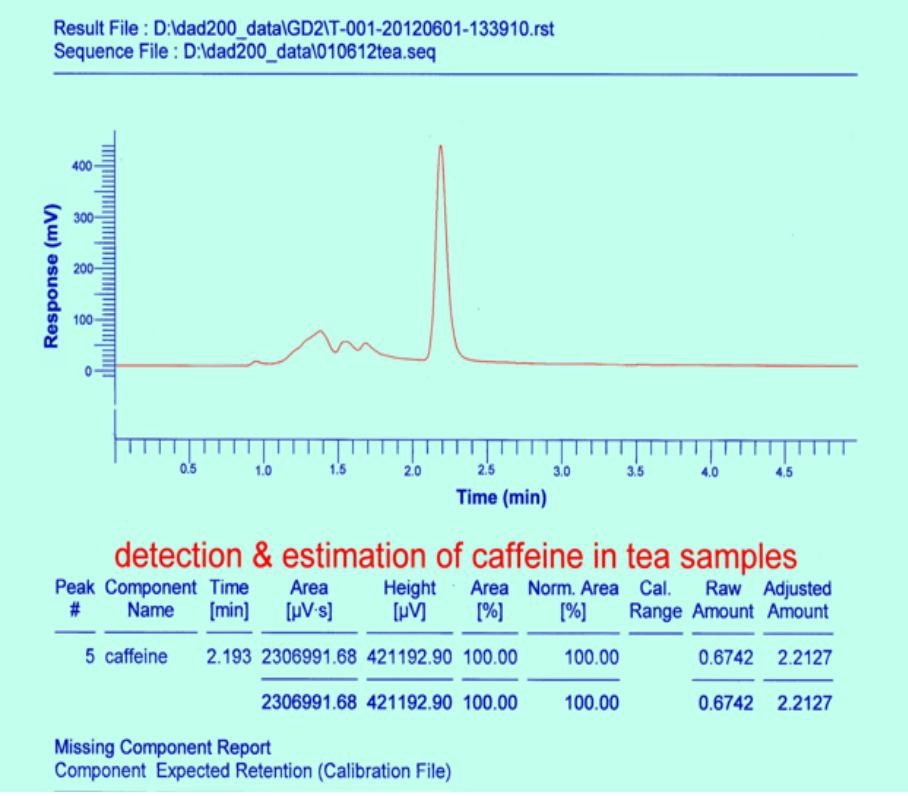
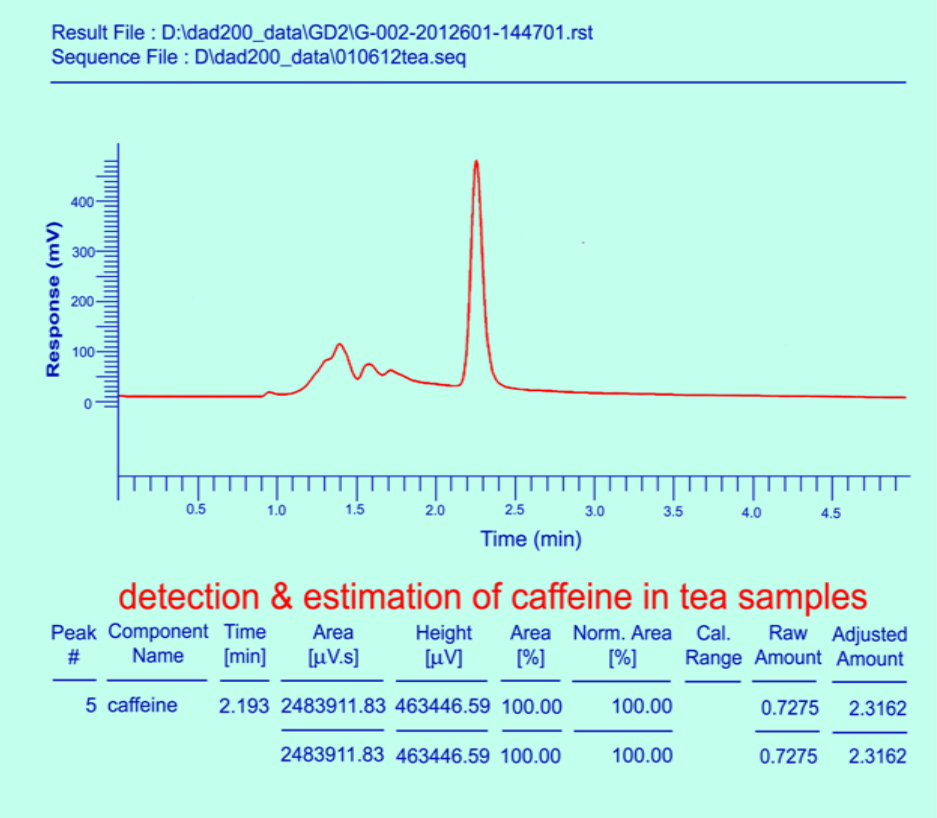
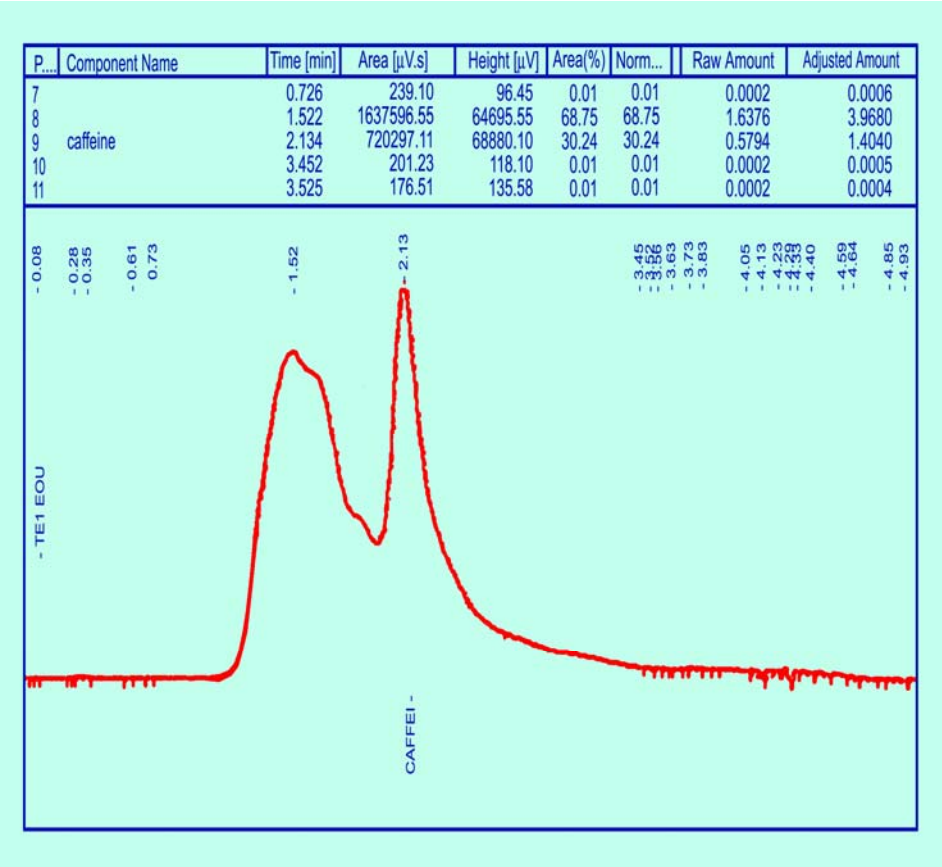


Figure 5. Caffeine content in the processed tea sample T-001.



Peak #	Component Name	Time [min]	Area [µV.s]	Height [µV]	Area [%]	Norm. Area [%]	Cal. Range	Raw Amount	Adjusted Amount
5	caffeine	2.193	2483911.83	463446.59	100.00	100.00		0.7275	2.3162
			2483911.83	463446.59	100.00	100.00		0.7275	2.3162

Figure 6. Caffeine content in the processed tea sample G-002.



P....	Component Name	Time [min]	Area [µV.s]	Height [µV]	Area(%)	Norm...	Raw Amount	Adjusted Amount
7		0.726	239.10	96.45	0.01	0.01	0.0002	0.0006
8		1.522	1637596.55	64695.55	68.75	68.75	1.6376	3.9680
9	caffeine	2.134	720297.11	68880.10	30.24	30.24	0.5794	1.4040
10		3.452	201.23	118.10	0.01	0.01	0.0002	0.0005
11		3.525	176.51	135.58	0.01	0.01	0.0002	0.0004

Figure 7.Caffeine content in T crude-0002.

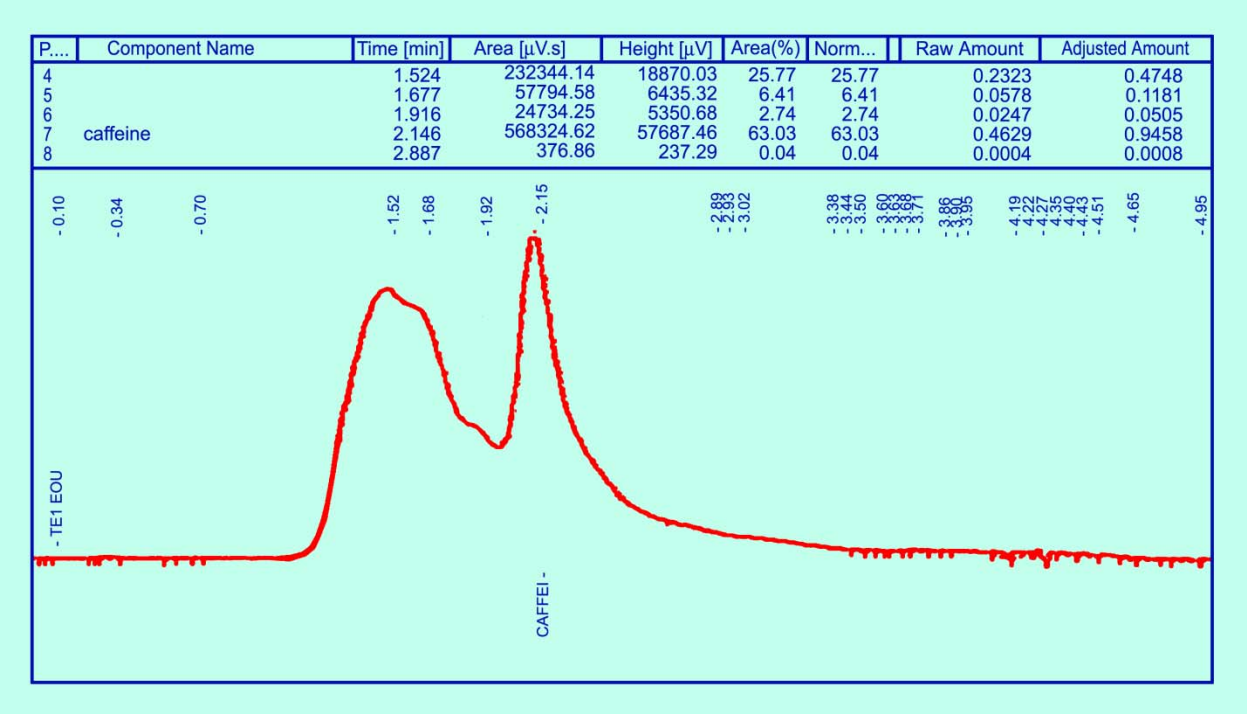


Figure 8. Caffeine content in G crude-0009

Table 3. Statistical analysis of data relating to caffeine content in the processed tea samples.

Parameters	Area	N	Mean	Std. Deviation	t	df	Sig. (2-tailed)	Remarks
Retention time:	Tezpur	10	2.20370	.025298	.384	18	.705	*NS
	Gohpur	10	2.20000	.016938				
Conc. of caffeine(mg/L)	Tezpur	10	26.020	3.0106	-.115	18	.909	* NS
	Gohpur	10	26.160	2.3787				
Percentage of caffeine	Tezpur	10	2.6020	.30106	-.115	18	.909	*NS
	Gohpur	10	2.6160	.23787				

*NS→ Not significant.

Table 4. Results of statistical analysis of data for overall percentages of caffeine in the samples of processed tea and mature tea leaves.

Parameters	Sample types	N	Mean	Std. Deviation	t	df	Sig. (2-tailed)	Remarks
Retention time	Processed tea	20	2.20185	.021039	10.365	28	.000	*HS
	Mature leaves	10	2.11760	.020876				
Conc. of caffeine (mg/L)	Processed tea	20	26.090	2.6417	12.408	28	.000	*HS
	Mature leaves	10	12.174	3.3698				
% of caffeine	Processed tea	20	2.6090	.26417	12.408	28	.000	*HS
	Mature leaves	10	1.2174	.33698				

Group statistics (*HS→ Highly significant.)

DISCUSSION

The Caffeine values of 0.65% - 1.64% in the samples of dried mature tea leaves were close to the caffeine values (1.5 %) of more than a year old mate leaves. Similarly, 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 et al. [2] and Komes D, et al [3]. Thus, a comparison of the caffeine content of dried mature tea leaves with that of the processed tea has shown that the caffeine content in the analyzed mature leaves was much lesser and the difference was found to be highly significant as per results of statistical analysis.

COINCLUSION

The Experimental findings confirmed that the caffeine content decreases with age of tea leaves and that if mature leaves are kept for more than one year, the caffeine content becomes significantly reduced. The age of the mature tea leaves exceeded one and half year at the time of HPLC analysis since the time of their plucking. As such, caffeine content is greatly reduced ranging from 0.65% to 1.64% and overall findings were found to be close to the caffeine values (1.5%) of year old mate leaves. HPLC analysis of the processed CTC tea samples collected from different regions of Sonitpur district, Assam, India has shown a negligible variation in the caffeine content and that caffeine values of these samples also agree well with the literature quoted values of 2-5%.

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