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A COMPARATIVE STUDY ON ANTIOXIDANT CONCENTRATION IN ORTHODOX AND CTC TEA CAMELLIA SINENSIS L

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ABSTRACT

It is now well established that tea has antioxidant properties. In the present study the antioxidant concentrations of two common types of teas, viz. Orthodox and CTC (Crush Tear Curl) were compared. The comparison was done with teas manufactured from two different clones. Samples were collected from six Estates of Upper Assam, where homogenous areas of plantations of the two clones TA-17 (Tinali 17/1/54) & S3A/3 were available. The samples were from the commercially manufactured product with special segregation of the clones to maintain the homogeneity during manufacture. Antioxidant activity measured the highest in the Orthodox teas as compared to the CTC teas in both the clones. The results analysed on one way ANNOVA test also corroborated this ($p<0.05$) finding.

KEYWORDS: Polyphenol; antioxidant activity; antioxidant concentration, CTC, clones, DPPH Assay.

INTRODUCTION

Brewed tea is not only a beverage but nature's low calorie wonder drink, second only to water which is a product of the processed leaf of the plant *Camellia sinensis* L. The common commercial varieties of tea are Green tea, Oolong tea and Black tea. The raw material for all the three types is the same, it is the differences in the manufacturing processes which results in the three different types: non-fermented green tea, semi-fermented oolong tea and fermented black teas.[1] Black tea are of two types – Orthodox and CTC. While orthodox consists mainly of the large leaf, CTC constitutes smaller grains manufactured by the *Crush-Tear-Curl* (CTC) process.

Tea is known to have beneficial effects on human health which has been corroborated by current research, epidemiological studies and clinical evaluations all across the globe. These beneficial effects are attributed to the antioxidant activity, e.g., free radical scavenging and metal chelating abilities of the beverage. The tea flavonoids, in both green tea and black tea demonstrate antioxidant activity.[2,3]

More and more research has been going on recently on the benefits of tea on human health for various types of cancer,[4,5] reducing of blood pressure[6] or for general health and wellbeing.[7,8] The growing popularity of the beverage is for similar reasons and all research confirms that the benefits are due to the antioxidant activities of the polyphenols. However, the antioxidative activity decreased from non-fermented (green tea) to fermented tea (black tea).[9] Yang *et al.*[7] reported that black tea contained high levels of theaflavins and thearubigins, which accounted for most of the antioxidant potential in this type of tea.

Carloni et al.[10] found that the antioxidant profile decrease from Green tea to White tea to Orthodox black tea to CTC tea. Lin et al.[11] again reported from Taiwan, that the antioxidant activity reduced from Green tea to Oolong to Black tea. Pal et al,[12] also reported that the antioxidant activity of Black Orthodox teas were higher than that of the CTC teas analysed from the commercially available products.

The objective of this study was to see if there was any difference in the antioxidant activity/concentration between Orthodox and CTC teas from samples drawn from a few factories in Assam.

MATERIALS AND METHODS

Samples for the present study were collected from six Estates of Upper Assam, four for CTC and two for orthodox. The samples were homogenous being from Tinali -17/1/54 and S3A/3 clones. The samples consisted of bulks (non-graded) of Orthodox and CTC teas collected and analysed from the same estates over a period of four years. The antioxidant activity was determined spectrophotometrically by Burits & Bucar's[13] method and the concentration per gram of made tea was calculated subsequently.

Chemicals

2, 2 Diphenyl 1picryl hydrazyl (DPPH) was obtained from Sigma chemicals.

Samples

The samples were collected from six different Estates of Upper Assam. Of these, four estates produced only CTC and two estates produced only Orthodox. A total of two clonal varieties, viz. Tinali-17/1/54 (TA-17) and S3A/3 were selected. The samples analysed were bulk (non-graded) teas of each clone from the six Estates collected during all the flushes for four years.

Preparation of Tea Extract

2.5gm of tea was dissolved in 150ml boiling distilled water and the infusion and liquor separated after 5min. The aqueous extract was cooled and filtered through Whatmann 24 filter paper. The filtered extract was diluted with distilled water at 1:4 ratio.

DPPH Assay

The scavenging ability of tea samples on DPPH radical was measured by Burits & Bucar's[13] method. The samples were analysed for their antioxidant contents. To 1ml of the diluted (1:4) extract 1ml of 0.004% methanol solution of DPPH was added and incubated for 30 mins. at room temperature. The violet colour of DPPH changed to yellow during this time. The absorbance was read against a control at 517nm.

Inhibition of free radical, DPPH, in percentage (*%i*) was calculated by the formula

$\% \, i = (C_{OD} - Test_{OD} / C_{OD}) \times 100$

Where

C_{OD} is the control absorbance (1ml DPPH + 1ml Methanol), and $Test_{OD}$ is the absorbance of the test sample.

Tests were carried out in triplicate.

From the standard Trolox curve which was determined earlier, it was found that:-

$\% \, i = (11.49+10.26)$ Conc.

Or Conc. = $(\% \, i - 11.49) / 10.26$

The actual concentration in µmol Trolox Equivalent per gram made tea (*µ*molT E/gm MT) was determined by:-

(Conc.x 4) / 2.5. ... 4 being the dilution factor and 2.5gms of teas brewed in 150ml water.

Statistical Analyses

Statistical analysis was done using SPSS software. ANNOVA test was carried out on the data generated after analysis of the samples.

RESULTS AND DISCUSSION

The characteristics and quality of tea is dependent on the polyphenol content of tea.[14] The health benefits of this beverage is characterised by the functional property of the polyphenols[15] mainly it's ability in scavenging free oxygen radical, making it a potent antioxidant. This is also the characteristic of the beverage on which its main health benefits are attributed to.

On analysis of the samples, it was found that the antioxidant concentration in the orthodox teas were more than the CTC teas. This was found to be so in all the samples drawn from all the Estates for all the flushes and amongst the two clones under review viz. TA-17 and S3A/3. The results were found to be significantly ($p<0.05$) different.

The Orthodox teas showed the highest potency of scavenging activity on DPPH radical. The concentration was finally represented in *µmol Trolox Equivalent* per gram of Made Tea.

Here the comparison of antioxidant concentrations were made flush-wise and clone (variety) wise between the Orthodox and CTC samples (Table 1).

TA17/1/54 CLONE

It was noticed that the orthodox teas had higher antioxidant concentration than the CTC teas in all cases. The general trend was that the highest antioxidant concentrations were during the Rain flush being 10.81 µmolTE/gm and 10.78 µmolTE/gm during the First Year, 12.11 µmolTE/gm and 11.99 µmolTE/gm during the Second Year, 11.12 µmolTE/gm and 11.02 µmolTE/gm in the Fourth Year respectively all in the two orthodox samples. In the third year, highest concentration was recorded during the second flush at 12.01µmolTE/gm and 11.98µmolTE/gm, again both in the orthodox samples.

Table 1: Comparison of Orthodox with CTC for similar clones across different flushes over 4 years

FIRST YEAR		First Flush	Second Flush	Rain Flush	Autumn Flush
ESTATE	CLONE	CONC.	CONC.	CONC.	CONC.
A - CTC	TA 17	7.89 ± 0.001	9.92 ± 0.001	10.01 ± 0.001	6.25 ± 0.001
B - CTC	TA 17	7.98 ± 0.001	9.98 ± 0.001	10.25 ± 0.001	6.04 ± 0.002
C - CTC	TA 17	7.65 ± 0.001	9.83 ± 0.001	10.13 ± 0.002	5.87 ± 0.001
D - CTC	TA 17	7.99 ± 0.001	9.90 ± 0.002	10.50 ± 0.001	6.34 ± 0.001
E – Ortho.	TA 17	8.35 ± 0.001	10.56 ± 0.001	10.78 ± 0.001	6.83 ± 0.001
F – Ortho.	TA 17	8.29 ± 0.001	10.43 ± 0.001	10.81 ± 0.001	6.79 ± 0.001
A - CTC	S3A3	7.51 ± 0.001	8.96 ± 0.001	10.41 ± 0.001	6.01 ± 0.001
B - CTC	S3A3	7.45 ± 0.001	8.63 ± 0.001	10.04 ± 0.001	6.00 ± 0.001
C - CTC	S3A3	7.59 ± 0.002	7.86 ± 0.001	9.91 ± 0.001	5.91 ± 0.001

D - CTC	S3A3	7.64 ± 0.002	9.00 ± 0.001	10.53 ± 0.001	6.21 ± 0.001
E – Ortho.	S3A3	8.01 ± 0.001	10.53 ± 0.001	10.86 ± 0.001	6.69 ± 0.001
F – Ortho.	S3A3	7.98 ± 0.001	10.48 ± 0.001	11.00 ± 0.001	6.75 ± 0.001
SECOND YEAR					
A - CTC	TA 17	8.10 ± 0.002	10.12 ± 0.001	10.31 ± 0.001	6.36 ± 0.001
B - CTC	TA 17	8.87 ± 0.001	10.06 ± 0.002	10.86 ± 0.001	6.54 ± 0.001
C - CTC	TA 17	7.76 ± 0.001	9.83 ± 0.002	10.15 ± 0.001	5.78 ± 0.001
D - CTC	TA 17	8.89 ± 0.001	10.13 ± 0.001	10.94 ± 0.001	6.61 ± 0.002
E – Ortho.	TA 17	9.02 ± 0.001	10.86 ± 0.001	12.11 ± 0.002	6.87 ± 0.001
F – Ortho.	TA 17	8.98 ± 0.002	10.73 ± 0.001	11.99 ± 0.001	6.80 ± 0.001
A - CTC	S3A3	7.68 ± 0.001	10.48 ± 0.001	10.6 ± 0.001	6.06 ± 0.001
B - CTC	S3A3	7.37 ± 0.001	10.24 ± 0.001	10.67 ± 0.002	6.17 ± 0.001
C - CTC	S3A3	7.42 ± 0.001	10.15 ± 0.001	10.27 ± 0.002	6.18 ± 0.001
D - CTC	S3A3	7.84± 0.002	10.53 ± 0.001	10.60 ± 0.002	6.29 ± 0.001
E – Ortho.	S3A3	8.24 ± 0.002	10.73 ± 0.001	11.78 ± 0.001	6.85 ± 0.001
F – Ortho.	S3A3	8.16 ± 0.001	10.62 ± 0.001	11.59 ± 0.001	6.77 ± 0.001
THIRD YEAR					
A - CTC	TA 17	8.85 ± 0.002	11.36 ± 0.002	11.00 ± 0.002	6.56 ± 0.002
B - CTC	TA 17	8.14 ± 0.001	10.78 ± 0.001	10.89 ± 0.001	6.64 ± 0.001
C - CTC	TA 17	8.12 ± 0.001	10.52 ± 0.001	10.63 ± 0.001	5.87 ± 0.001
D - CTC	TA 17	8.95 ± 0.002	11.47 ± 0.001	11.25 ± 0.001	6.74 ± 0.001
E – Ortho.	TA 17	9.21 ± 0.001	12.01 ± 0.001	11.87 ± 0.001	7.35 ± 0.001
F – Ortho.	TA 17	9.15 ± 0.001	11.98 ± 0.001	12.01 ± 0.002	7.18 ± 0.001
A - CTC	S3A3	8.93 ± 0.001	11.13 ± 0.001	10.91 ± 0.001	5.95 ± 0.001
B - CTC	S3A3	8.82 ± 0.001	11.29 ± 0.001	11.32 ± 0.001	5.51 ± 0.001
C - CTC	S3A3	7.91 ± 0.001	10.34 ± 0.001	10.50 ± 0.001	5.43 ± 0.001
D - CTC	S3A3	7.84 ± 0.001	11.30 ± 0.001	11.59 ± 0.001	5.86 ± 0.001
E – Ortho.	S3A3	9.05 ± 0.001	11.86 ± 0.001	11.89 ± 0.001	6.31 ± 0.001
F – Ortho.	S3A3	9.10 ± 0.001	11.69 ± 0.001	11.76 ± 0.001	6.22 ± 0.001
FOURTH YEAR					
A - CTC	TA 17	6.94 ± 0.001	10.0 ± 0.001	10.01 ± 0.001	6.05 ± 0.001
B - CTC	TA 17	6.00 ± 0.005	8.41 ± 0.001	10.59 ± 0.001	6.55 ± 0.001
C - CTC	TA 17	6.25 ± 0.001	8.22 ± 0.002	10.00 ± 0.001	5.92 ± 0.001
D - CTC	TA 17	7.10 ± 0.001	10.57 ± 0.001	10.98 ± 0.002	6.77 ± 0.001
E – Ortho.	TA 17	8.42 ± 0.001	10.84 ± 0.001	11.02 ± 0.001	7.32 ± 0.001
F – Ortho.	TA 17	8.19 ± 0.001	10.71 ± 0.001	11.12 ± 0.001	7.00 ± 0.001
A - CTC	S3A3	7.32 ± 0.001	8.85 ± 0.001	10.06 ± 0.001	7.38 ± 0.001
B - CTC	S3A3	6.94 ± 0.001	7.71 ± 0.001	10.57 ± 0.001	6.01 ± 0.002
C - CTC	S3A3	7.12 ± 0.002	7.29 ± 0.001	10.42 ± 0.001	6.87 ± 0.001
D - CTC	S3A3	7.45 ± 0.002	8.98 ± 0.001	10.85 ± 0.001	7.47 ± 0.001
E – Ortho.	S3A3	7.95 ± 0.002	10.1 ± 0.001	10.95 ± 0.001	7.56 ± 0.001
F – Ortho.	S3A3	7.68 ± 0.002	9.82 ± 0.001	10.86 ± 0.001	7.19 ± 0.002

The next lower concentrations were during Second flush with the highest of 10.56µmolTE/gm and 10.43µmolTE/gm. for first year, at 10.86µmolTE/gm and 10.73µmolTE/gm during the second year, 10.84µmolTE/gm and 10.71µmolTE/gm during the fourth year, all in the orthodox teas. In the third year the second highest concentrations were in the Rain Flush at 12.01µmolTE/gm and 11.87µmolTE/gm. this too in the orthodox samples.

The next lower antioxidant concentrations were during the First flush with the highest being 8.35 µmolTE/gm, 8.29 µmolTE/gm in the orthodox samples of First year, 8.98 µmolTE/gm and 9.02 µmolTE/gm in the orthodox samples of Second year, 9.21 µmolTE/gm and 9.15 µmolTE/gm in the orthodox samples of third year and 8.42 µmolTE/gm and 8.19 µmolTE/gm in the orthodox samples of Fourth year.

The Autumn Flush concentrations were the lowest amongst all the flushes. However, here too the orthodox samples had higher concentrations than the CTC samples at 6.83µmolTE/gm and 6.79 µmolTE/gm in the First year, 6.87 µmolTE/gm and 6.80 µmolTE/gm in the Second year, 7.35 µmolTE/gm and 7.18 in the Third year and 7.32 µmolTE/gm and 7.00 µmolTE/gm in the Fourth year.

The CTC samples on the other hand ranged between 10.50µmolTE/gm to 10.01µmolTE/gm during Rain flush in the First year, 10.94µmolTE/gm to 10.15 µmolTE/gm in the Second year, 11.25µmolTE/gm to 10.63µmolTE/gm in Third year and 10.98µmolTE/gm to 10.00µmolTE/gm in the Fourth year. The Second Flush antioxidant concentrations in the CTC teas ranged from 9.98µmolTE/gm to 9.83 µmolTE/gm in the First year, 10.13µmolTE/gm to 9.83 µmolTE/gm in the Second year, 11.47µmolTE/gm to 10.52µmolTE/gm in the Third year and 10.57 µmolTE/gm to 8.22 µmolTE/gm in the Fourth year. The First Flush antioxidant concentrations in the CTC teas ranged from 7.99µmolTE/gm to 7.65µmolTE/gm in the First year, 8.89µmolTE/gm to 7.76µmolTE/gm in the Second year, 8.95µmolTE/gm to 8.12µmolTE/gm in the Third year and 7.10µmolTE/gm to 6.00µmolTE/gm in the Fourth year. The Autumn Flush concentrations of antioxidants in the CTC teas ranged between 6.34µmolTE/gm to 5.87µmolTE/gm in the First year, 6.61µmolTE/gm to 5.78µmolTE/gm in the Second year, 6.74µmolTE/gm to 5.87µmolTE/gm in the Third year and 6.77µmolTE/gm to 5.92µmolTE/gm in the Fourth year.

S3A/3 CLONE

This clone also displayed maximum concentrations during the Rain flush with the next lower being in Second flush followed by First flush and Autumn flush. Here too, in all cases the antioxidant concentrations of the orthodox teas were higher than the CTC teas. In this clone, the Rain flush concentrations for orthodox teas were 11.00µmolTE/gm and 10.86µmolTE/gm in the First year while the CTC concentrations ranged between 10.53µmolTE/gm to 9.91µmolTE/gm. In the Second year, the orthodox tea concentrations were 11.78µmolTE/gm and 11.59µmolTE/gm while the CTC tea concentrations ranged between 10.67µmolTE/gm to 10.27µmolTE/gm. The Third year readings of orthodox teas were at 11.89µmolTE/gm and 11.76µmolTE/gm while the CTC tea concentrations ranged between 11.59µmolTE/gm to 10.50µmolTE/gm. The orthodox tea readings in the Fourth year were at 10.95µmolTE/gm and 10.86µmolTE/gm while the CTC teas ranged between 10.8µmolTE/gm to 10.42µmolTE/gm.

The next lower concentrations were in the Second flush with the Orthodox sample concentration in the First year being 10.53µmolTE/gm and 10.48µmolTE/gm while the CTC concentrations were between 9.00µmolTE/gm and 7.86µmolTE/gm. In the Second year while the orthodox concentrations were 10.73µmolTE/gm and 10.62µmolTE/gm, the CTC ranged between 10.53µmolTE/gm and 10.15µmolTE/gm. In the Third year, the orthodox concentrations during this flush were 11. 86µmolTE/gm and 11.69µmolTE/gm while the CTC concentrations were between11.30 µmolTE/gm and 10.34µmolTE/gm. The Fourth year orthodox readings were 10.1µmolTE/gm and 9.82µmolTE/gm while the CTC readings were 8.98µmolTE/gm to 7.29µmolTE/gm.

The Second flush concentrations were followed by the First flush concentrations. While in the First year the orthodox tea concentrations were 8.01µmolTE/gm and 7.98µmolTE/gm, the CTC tea concentrations were between 7.64µmolTE/gm and 7.45µmolTE/gm, in the Second year the orthodox tea concentrations were 8.24µmolTE/gm and 8.16µmolTE/gm, the CTC concentrations ranged between 7.84µmolTE/gm and 7.37µmolTE/gm. In the Third year the orthodox concentrations were at 9.10µmolTE/gm and 9.05µmolTE/gm while the CTC concentrations were between 8.93µmolTE/gm and 7.84µmolTE/gm. The Fourth year readings were 7.95µmolTE/gm and 7.68µmolTE/gm for the orthodox teas and the range of 7.45µmolTE/gm to 6.94µmolTE/gm for CTC teas.

The lowest concentrations were during the Autumn flush with orthodox showing higher concentrations than the CTCs. In the First year during this flush the concentration of orthodox teas were 6.75µmolTE/gm and 6.69µmolTE/gm while the concentration of CTC teas were between 6.21µmolTE/gm to 5.91µmolTE/gm. in the Second year, the orthodox concentrations were 6.85µmolTE/gm and 6.77µmolTE/gm while the CTC ranged between 6.29µmolTE/gm and 6.06µmolTE/gm. The Third year trend was similar with concentration of orthodox teas being 6.31µmolTE/gm and 6.22µmolTE/gm while the CTC teas had concentrations between 5.95µmolTE/gm and 5.43µmolTE/gm. Similarly, in the Fourth year, the antioxidant concentrations of the orthodox teas were 7.56µmolTE/gm and 7.19 µmolTE/gm, the concentrations of the CTC teas ranged between 7.47µmolTE/gm to 6.01µmolTE/gm.

In all the cases, it was noticed that orthodox teas had significantly higher concentrations than CTC teas.

Independent Sample T-Test Between CTC and ORTHO

Table 2: Flush wise mean values of CTC and Orthodox teas.

	CTC	**Orthodox**
	Mean	**Mean**
First flush	6.2563 ± 0.001	7.2400 ± 0.002
Second flush	5.0225 ± 0.002	5.9658 ± 0.001
Rain flush	7.7597 ± 0.001	8.3200 ± 0.001
Autumn flush	10.5547± 0.001	11.3492 ± 0.001

Table 3: Flush wise t-test and significance (Sig.).

	Equality of means	
	t	**Sig.**
First flush	(-) 5.664	0.001
Second flush	(-) 4.977	0.001
Rain flush	(-) 2.354	0.023
Autumn flush	(-) 5.040	0.001

From the above table (3), it has been observed that the p-value for First flush corresponding to t-test value -0.5664 is 0.001 which is less than 0.050. Similarly, for Second flush p-value corresponding to t-test value (-) 4.977 is 0.001 which is less than 0.050, for Rain Flush p-value corresponding to t-test value (-) 2.354 is 0.023 which is less than 0.050, for Autumn flush was (-) 5.040 was 0.001, again less than 0.050. Therefore, we can conclude that there is a significant difference between CTC and Orthodox teas across all the flushes.

If we look at the mean column of Table 2, above, we can deduce that the antioxidant concentration of Orthodox teas are higher than the CTC teas.

From this study it can be seen that the antioxidant concentration of the orthodox teas were higher than the CTC teas. The trend was the same for the two clones which were tested. Carloni *et al.*[10] reported that the antioxidant profile decrease from Green tea to White tea to Orthodox black tea to CTC tea. Pal *et al*,[12] also reported the antioxidant activity of Black Orthodox teas to be higher than that of the CTC teas which they analysed from the commercially available products from Kolkata (India). The results of our experiment corroborate with these findings. Laddi *et al*.[16] evaluated the quality of black CTC teas based upon seasonal variations. Their results suggested that quality of tea is dependent upon seasonal variations in terms of chemical changes such as TFs, TRs, which otherwise are also dependent on the ambient temperature during manufacture, according to Asil *et al.*[17] Yao *et al.*[18] found that the phenolic compounds increased during warm months and decreased during the cool month in fresh tea shoots, from plantations in Australia. Our experimental results have also shown that in warmer months, Second flush and Rain flush, the concentrations were the highest and decreased to the First flush which months are cooler than that of the Second and Rain flushes and the antioxidant concentration tapered off to the Autumn flush which period has the lowest temperatures as compared to all the other flushes, thus corroborating with the findings of Asil *et al.*[17] and Yao *et al.*[18]

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