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Research article

ANTIOXIDANT CONCENTRATION OF CTC GRADED TEA (*CAMELLIA SINENSIS* L.) DIFFERING WITH SIZE OF TEA PARTICLES IN THREE DIFFERENT CLONES.

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ABSTRACT: It is well known that tea has antioxidant properties and is a natural source of polyphenols. The polyphenol contents of various teas from China, Japan are well documented. Some reports are also available from Turkey and India, on the antioxidant activity of different types of teas. However, a study of the antioxidant concentration vis-à-vis the particle size of the same type of tea has not been documented in India. In the present study, the antioxidant concentrations were compared with special reference to the sizes ie. a Broken grade (larger size), a Fanning (medium size) and a Dust (small size) of CTC (Crush Tear Curl) teas from three different clones. Samples were collected from two Estates in Upper Assam where homogenous areas of plantations of three clones TV-1, TA-17 (Tinali 17/154) & S3A3 were available. These were from the commercially manufactured product with special segregation of the clones to maintain the homogeneity during manufacture. Antioxidant activity of all clones, determined by DPPH Assay, measured the highest in the larger grade, followed by the medium grade ie. Pekoe Fanning (PF) and decreased further in the Dust grades. 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

Tea is the processed leaf of the plant *Camellia sinensis* L. Black Tea is manufactured by complete oxidation on withered leaf unlike Green Tea where oxidation is totally avoided. This product of *Camellia sinensis*, is a unique non-alcoholic drink that cares, cures and cheers. Tea helps the human body to keep its glow, and remain beautiful over and above helping the internal defence system to remain active and strong. No other beverage can compare so closely with tea, as far as its multifunctional appeals and effects are concerned [1].

The therapeutic values of tea has been known for ages and current research, epidemiological studies and clinical evaluations have all established the superior abilities of tea compounds in preventing and curing a wide range of human diseases from simple ailments as stomach trouble to as complex ones as cancer. The major bioactive compounds responsible for the health benefits of tea are suggested to be Phenolics - tea catechins and polyphenols [2]. Tea is known to have antioxidant properties. This property of tea is due to the presence of polyphenols called “flavonoids”. These phenols and polyphenols are compounds found naturally in plants. Natural Tea flavonoids, also called catechins, are of 30 different types. The total antioxidant capacity of tea is not related to a particular kind of polyphenol but to the combined activity of diverse antioxidants, including phenolic acids and polyphenols [3]. There has been extensive documentation on the polyphenolic contents of various teas from China and Japan. Of late, work in this field is also being carried out in India.

Investigations showed that the tea polyphenols possess a potent scavenging effect on free radicals thus displaying its antioxidative activity [4]. Yen & Chen [5] carried out an experiment on the antioxidative activity of various tea extracts. Research showed that all tea extracts exhibit antioxidative activity markedly and in excess over most vegetables [6], another source of naturally available antioxidants. Green tea displayed the highest antioxidative activity followed by black tea and Oolong tea [7,8]. Twelve types of commercially available teas were studied for their antioxidative activity in Kenya also [9]. It has also been reported that Black tea prepared using tea bags had significantly lower antioxidant capacity than black leaf tea [10]. Here, the size of tea in the tea bags were smaller than the normal leaf tea. It was then thought that tea bag materials may prevent some extraction of flavonoids into the tea solution.

Barua et al, [11], reported the antioxidant activity of TV-1 samples of Black Tea to be 74.52% while that of Black soluble tea as 71.28%. Here, black soluble tea was a powder tea while black tea was from normal manufacture, the grains being larger than the spray-dried powder. Srestha et al, [12], also reported that Green tea had higher antioxidant activity than Orthodox, which was higher than CTC teas from samples collected from different parts of Kathmandu Valley, Nepal. Similarly, Serpen et al, [13], reported on the antioxidant activity of seven different grades of tea from Turkey, where it was also seen that the bolder grades had higher antioxidant activity than the smaller grades. Anesini et al [14] similarly reported about teas from Argentina. The objective of this study was to see if there was any difference in the antioxidant activity / concentration between the larger and the smaller grades of CTC teas and in three different clones commonly used in Assam.

MATERIALS AND METHODS

In the present study, samples were collected from two Estates in Upper Assam (Dibrugarh and Tinsukia Districts) from TV-1, Tinali -17/154 and S_3A_3 clones. The samples consisted of three grades – BOP (Broken Orange Pekoe), PF(Pekoe Fanning) and Dust. The BOP (Broken Grade) consisted of large particles while PF (Fanning) were of medium size and DUST (Dust Grade) consisted of small particles. The antioxidant activity was determined spectrophotometrically by Burits & Bucar's (2000) 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 collected from two different Estates of Upper Assam were analysed for the antioxidant concentration. A total of three clonal varieties, viz. TV-1, Tinali-17/154 (TA-17) and S_3A_3 were selected. The grades analysed were the bolder grade - BOP (Broken Orange Pekoe), a medium grade - PF (Pekoe Fanning) and a smaller grade - Dust of each clone from the two Estates. The samples were collected during first and second flush in season 2014.

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 (2000) 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 in a calorimeter, 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 (μmolT 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. Z-test was applied to 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 [15] (Atoui et. al., 2005). The health benefits of this beverage is characterised by the functional property of the polyphenols [16] (Dufresne & Farnworth, 2001) 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 content in the BOP (Broken Orange Pekoe) the bolder grade, was more than the medium grade – PF (Pekoe Fanning) and finally, the Dust grade (smaller in size) being lower than the PF. This trend was found to be the same for samples drawn from each Estate for two flushes (first flush and second flush), both intra and inter- specific amongst the three clones viz. TV-1, TA-17 or S_3A_3. The results were found to be significantly ($p<0.05$) different.

The *BOP* (Broken Orange Pekoe) grade showed the highest potency of scavenging activity on DPPH radical. This was followed by the *PF* (Pekoe Fanning) grade and the lowest activity being in the *Dust* grade. The concentration was finally represented in *µmol Trolox Equivalent* per gram of Made Tea.

Table -1 : Table shows the antioxidant concentration of the different grades of CTC tea manufactured from TV-1, TA-17 & S_3A_3 clones during First Flush and Second Flush from two different Estates of Upper Assam.

ESTATE	ESTATE -A						ESTATE - B					
FLUSH	1st Flush			2nd Flush			1st Flush			2nd Flush		
GRADE	BOP	PF	DUST	BOP	PF	DUST	BOP	PF	DUST	BOP	PF	DUST
TV-1	7.16	6.78	6.38	8.46	8.09	7.71	6	5.63	5.24	8.09	7.32	6.03
TA-17	7.71	6.94	6.56	9.98	9.61	9.23	6.73	6	5.63	8.09	6.94	6.03
S3A3	8.46	8.09	6.94	5.53	5.06	4.58	8.09	7.71	7.32	8.09	7.71	6.19

The *TV1* sample of Estate A (Table 1) showed an antioxidant concentration of 7.16 *µmolT.E./gm MT* in the BOP grade in First Flush while the PF showed a concentration of 6.78 *µmolT.E./gmMT* while the DUST grade concentration went down to 6.38 *µmolT.E/gmMT*. In the second flush, the concentration of the BOP grade went up to 8.46 *µmolT.E./gmMT*, the PF showing a concentration of 8.09 *µmolT.E./gmMT* while the DUST grade was 7.71 *µmolT.E./gmMT*. (Fig.1). The difference in concentration was significant ($p<0.05$).

In case of Estate B, (Table1) the TV-1 BOP grade during first flush showed a concentration of 6 *µmolT.E./gmMT* while the PF grade had a concentration of 5.63 *µmolT.E./gmMT* and the DUST grade had a concentration of 5.24 *µmolT.E./gmMT*. During the second flush the antioxidant concentration of the BOP grade increased to 8.09 *µmolT.E./gmMT*, the PF grade to 7.32 *µmolT.E./gmMT* and the DUST to 6.03 *µmolT.E./gmMT*. Here too the difference between the BOP and DUST grades though present, was not very significant in the first flush but was more significant in the second flush (Fig.1).

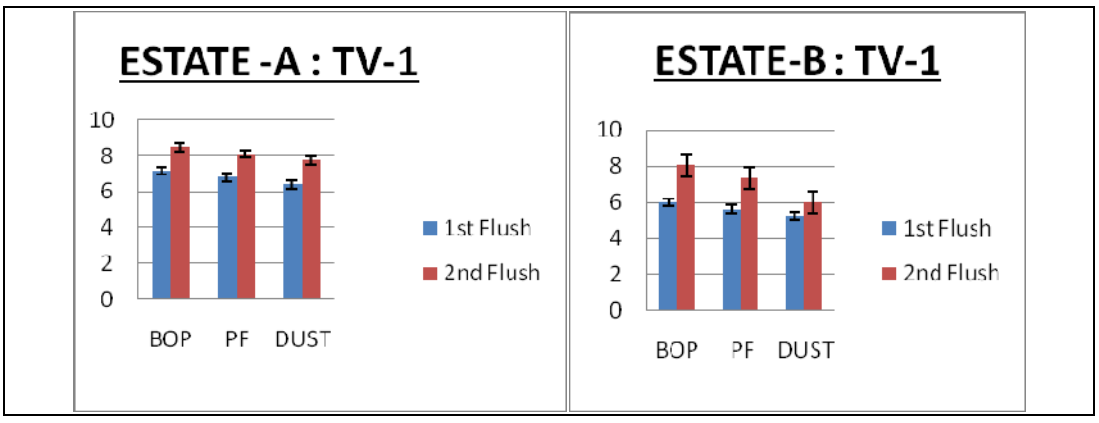

Fig.1: Graphs showing antioxidant concentration of BOP, PF, Dust grades from TV-1 clone of the two Estates for First and Second flush.

The *Tinali 17/154* (TA-17) samples, showed a similar trend (Table 1) with Estate A's first flush BOP sample having a concentration of 7.71 *µmolT.E./gmMT*, the PF sample with 6.94 *µmolT.E./gmMT* and the DUST with 6.56 *µmolT.E./gmMT*. During the second flush the antioxidant concentration in the BOP grade increased to 9.98 *µmolT.E./gmMT*, the PF grade to 9.61 *µmolT.E./gmMT* and the DUST to 9.23 *µmolT.E./gmMT*. (Table1). From Fig.2 it can be seen that the difference was significant ($P<0.05$).

In case of Estate B, the first flush BOP grade showed an antioxidant concentration of 6.73 *µmolT.E./gmMT*, while the PF grade showed 6.0 *µmolT.E./gmMT* and the DUST grade had 5.63 *µmolT.E./gmMT* (Table-1). During second flush, the BOP grade's concentration increased to 8.09 *µmolT.E./gmMT*, the PF grade's to 6.94 *µmolT.E./gmMT* and the DUST grade's to 6.03 *µmolT.E./gmMT*. Here too the antioxidant concentration decreased from the larger grade BOP, to the middle grade PF and finally to the smallest grade DUST (Fig.2).

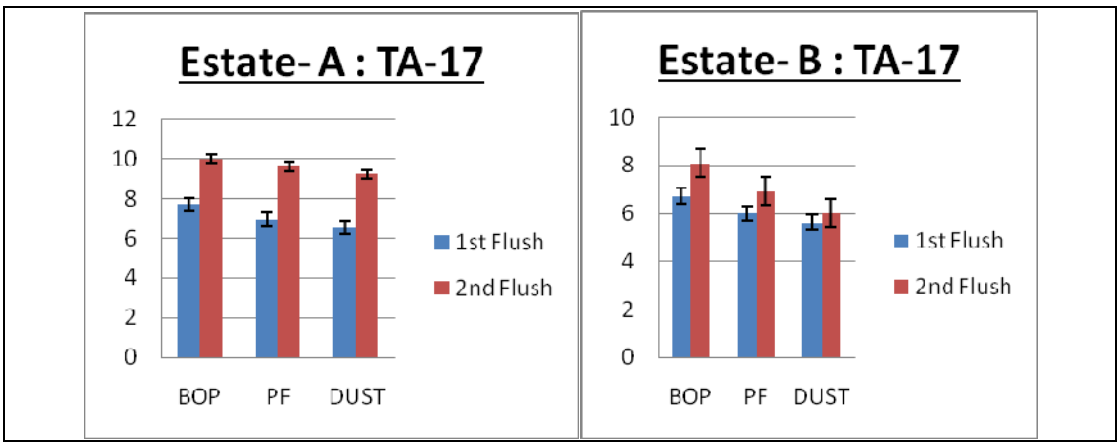

Fig.2 : Graph showing antioxidant concentration of BOP, PF, Dust grades from TA-17 clone of the two Estates for First and Second flush.

In the S_3A_3 variety, the concentration of the antioxidant followed a similar trend as in the case of the *TV1* & *TA17* varieties (Table 1).

In case of Estate A, the first flush BOP grade antioxidant concentration showed 8.46 *µmolT.E./gmMT*, the PF grade showed 8.09 *µmolT.E./gmMT* and the DUST grade showed 6.94 *µmolT.E./gmMT*. During the second flush, though the concentration decreased to 5.53 *µmolT.E./gmMT* in case of BOP, 5.06 *µmolT.E./gmMT* for PF and 4.58 *µmolT.E./gmMT* for DUST (Fig.3), the difference was significant ($p<0.05$).

In case of Estate B here (Table-1), the concentration of antioxidant in the BOP grade for both first and second flush was same at 8.09 *µmolT.E./gmMT*, and for PF grade at 7.71 *µmolT.E./gmMT*, the DUST grade during first flush showed 7.32 *µmolT.E./gmMT* and during second flush 6.19 *µmolT.E./gmMT*. However, the difference between the bolder grade BOP and the decrease to the Dust grade was noticeable (Fig.3).

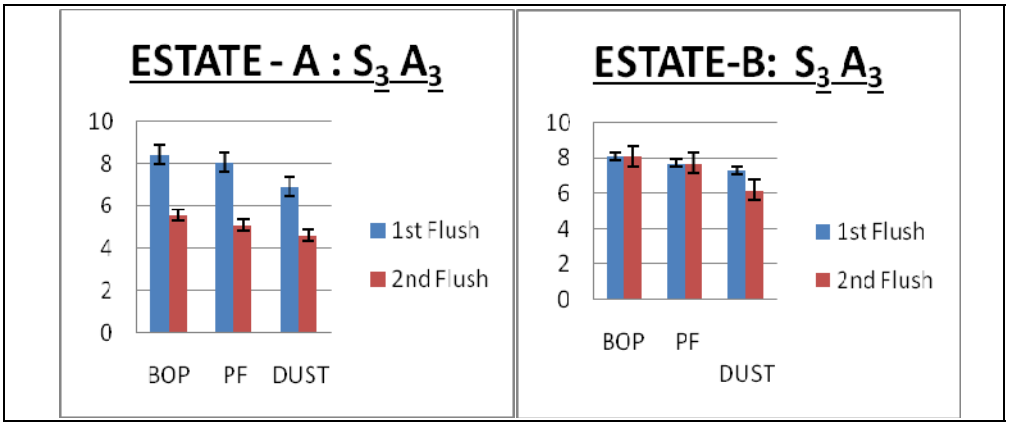

Fig.3: Graph showing antioxidant concentration of BOP, PF, Dust grades from S_3A_3 clone of the two Estates for First and second flush.

CONCLUSION

From this study it can be seen that the antioxidant concentration of the larger grade, Broken Orange Pekoe (BOP) was higher than the medium grade Pekoe Fanning (PF) which was higher than the smallest grade which was DUST in our experiment. The trend was the same for the three different clones which were tested and from two different Estates from where the samples were collected. It has been reported earlier pertaining to teas from Nepal [12] (Sreshtha et al, 2010), [13].

Turkey (Serpen et al 2012) that the antioxidant activity differed with the size of tea, the larger being more active than the smaller grades being sold there. Pal et al, 2012[17], also reported that the antioxidant activity of Black Orthodox teas were higher than that of the CTC teas which they analysed from the commercially available products from Kolkata (India). However, this initial effort can be taken forward by analysing all grades of orthodox as well as CTC, independently, and for different areas of tea Districts of North & South India.

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