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In vitro assessment of antioxidant capacity of Sri Lankan black tea *(Camellia sinensis L.)*, during storage and its relation to phenolic content

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Abstract

Black tea is reported to contain natural antioxidants mainly with polyphenolic compounds. In this study, the effect of storage on antioxidant capacity and total polyphenols content of black tea (stored at 25±2 ^{0}C and 50±5% RH) from major agroclimatic elevations in Sri Lanka were studied for 36 months at 6 months intervals. The antioxidant capacity measured by DPPH assay had shown significant ($P < 0.05$) and time dependent decrease

over the experimental period. Fresh black tea had 38% (high grown), 41% (mid grown) and 83% (low grown) greater antioxidant capacity than tea stored for 36 months. According to FRAP assay, rapid time dependent reduction was evident up to 18 months: by 14% in high grown, 14% in mid grown and 15% in low grown, and thereafter the decline was slow up to 36 months. Interestingly, a time dependent decrease was observed in the total polyphenols content by 9% in high grown, by 12% in mid grown and by 22% in low grown over the experimental period. In conclusion, a time dependant reduction of antioxidant capacity was evident in black tea in this study and it may be due to depletion of total polyphenols content during storage.

1. Introduction

Manufactured Sri Lankan black tea (Camellia sinensis L.), contains up to about 30% polyphenolic flavonoids on a dry weight basis and they exhibit high antioxidant activity [1]. Polyphenolic compounds in plant sources could undergo changes during storage and thereby the antioxidant capacity could also be reduced depending on the storage conditions [2]. In view of the above facts, in the present study was undertaken to investigate the changes in antioxidant capacity and the polyphenols content of Sri Lankan black tea during storage for 36 months, under controlled environmental conditions.

2. Materials and Methods

Black tea samples (1 kg) of BOPF grade were collected from randomly selected tea factories from low grown (382m, amsl), mid grown (820m, amsl) and high grown (1382m, amsl). The total polyphenols content (control values) and the water activity were measured in each sample on the day of sample collection. Then, each of samples was divided into mini packs (100g) in triple laminated aluminium foil bags, sealed, labeled and kept in stores under controlled environment (temperature at 25 ± 2 ^{0}C and relative humidity at 50 ± 5%). All chemicals used were of analytical reagent grade and distilled water was used throughout the experiments.

Assessment of antioxidant capacity in black tea:

The DPPH radical scavenging assay was performed as described by Brand–Williums et. al., [3]. The ability of tea solution to scavenge DPPH free radicals compared to Trolox (reference drug) were expressed as the percentage of DPPH radical scavenged.

The ferric reducing antioxidant power (FRAP) was performed as described by Benzie & Szeto [4]. The combined (total) ferric reducing antioxidant capacity (as FRAP value) in the samples were expressed as μmol/g, compared to ascorbic acid (reference drug).

Estimation of total polyphenols in black tea:

The total polyphenols (TPP) content was determined as described in ISO/14502-1(2004). The total polyphenol content was expressed as gallic acid equivalents (GAE) in milligrams per milliliter in tea infusion of black tea.

Statistical analysis:

The results (n = 3) are expressed as mean ± SEM. $P \leq 0.05$ was considered as significant.

3. Results

The results of the DPPH assay and FRAP assay at 0, months and at 6 months intervals for 36 months are shown in Fig. 1a and Fig. 1b, respectively. As shown, the fresh black tea had antioxidant capacity: 88.5±8.2%, 75.7±6.3% and 69.40±8.2% in high-, mid- and low grown, respectively. A significant and time dependent (r^2 = 0.963, 0.879 and 0.989, respectively) decrease of antioxidant activity was observed over the experimental period. Fresh black tea had 38% (high grown), 41% (mid grown) and 83% (low grown) greater antioxidant capacity than 36 months stored tea. Highest loss of antioxidant activity was evident with low grown tea. With the FRAP assay, antioxidant activity in fresh black tea were: 484±25, 391±15 and 317±19μmol/g in high-, mid- and low grown, respectively. Further, a rapid time dependent reduction of antioxidant activity was seen up to 18 months: by 14% in high grown (r^2 = 0.964), 14% in mid grown (r^2 = 0.896) and 15% in low grown (r^2 = 0.867) and thereafter the decline was slow up to 36 months.

The results of the TPP content of black tea, measured in fresh black tea (0, months) and at 6 months of time intervals for 36 months is shown in Fig. 2. A time dependent decrease was observed in the TPP content by 9% in high grown (r^2 = 0.823), by 12% in mid grown (r^2 = 0.927) and by 22% in low grown (r^2 = 0.984) up to 36 months.

4. Discussion

This study, examined the antioxidant capacity and TPP content of Sri Lankan black tea using BOPF grade stored for 36 months in triple laminated aluminium foil bags at 25 ± 2 ^{0}C and 50 ± 5% RH. Before the commencement of storage trials, the content of TPP and water activity in tea samples were determined. This is because water activity greater than 0.65% is known to accelerate the degradation of TPP [2]. Our results showed that water activity was lower than 0.65%, indicating minimal loss of TPP due to water activity during storage. The TPP content varied with the agroclimatic elevations and a significant time dependant reduction of TPP content was evident with storage. It is suggested that TPP content in black tea may be one of the major reasons related to the observed antioxidant ability.

In conclusion, the results show a reduction in antioxidant activity of Sri Lankan black tea with storage and this is attributed to depletion of TPP content.

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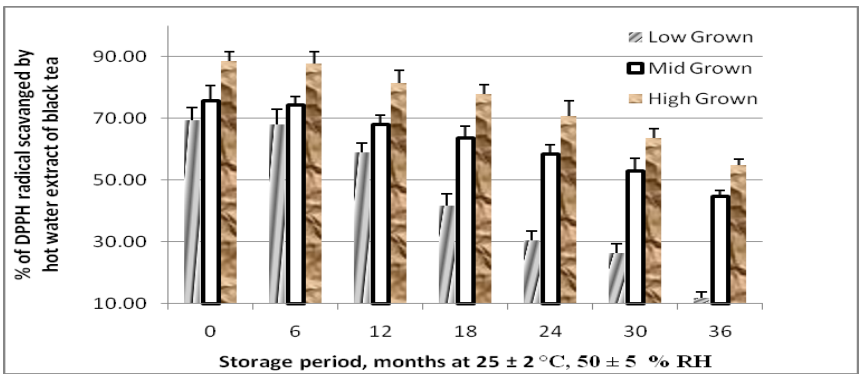

Fig 1a. Decline of antioxidant activity (DPPH assay) in BOPF grade Sri Lankan black tea (*Camellia sinensis* L) from major agroclimatic elevations. (mean ± SEM, n = 3).

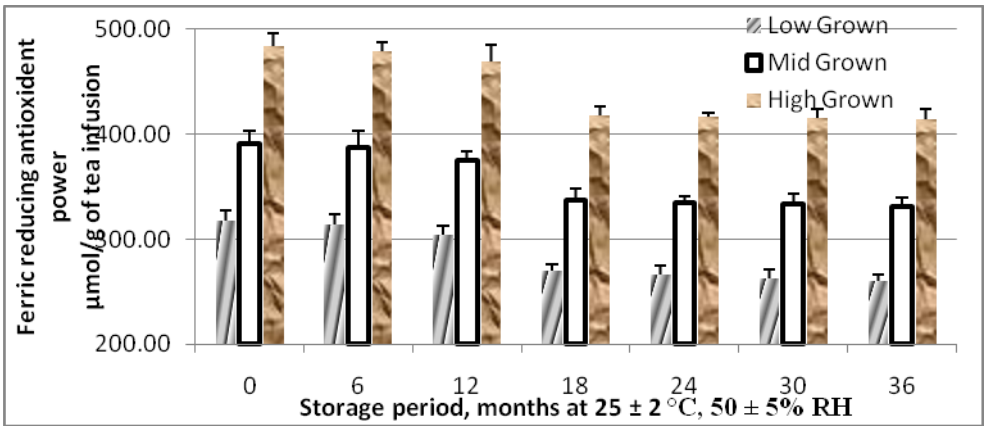

Fig 1b. Decline of antioxidant activity (FRAP assay) in BOPF grade Sri Lankan black tea (*Camellia sinensis* L) from major agroclimatic. (mean ± SEM, n = 3).

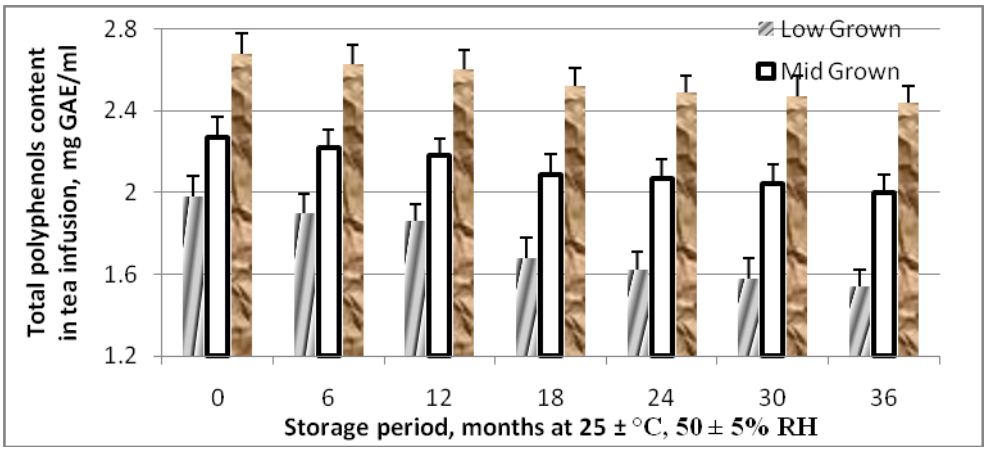

Fig 2. An estimation of the total polyphenols content (as mg of gallic acid equivalent per ml of hot water infusion) in BOPF grade Sri Lankan black tea (*Camellia sinensis* L). (mean ± SEM, n = 3).