

# Seasonal Variation of L-Theanine Content in Tea: A Study on Darjeeling Black Tea

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## ABSTRACT

To date quality and selling price of a tea mainly depends on its flavour, taste and texture but not on its medicinal properties though a Chinese scientist in 780 AD suggested and acclaimed tea as a divine remedy and supreme gift of God for preserving mental tranquillity. Different studies have been showed that L-theanine is the key component of tea which can make us mentally calm, cool and relaxed. At the same time it can protect us from different ailments. There is a notion that L-theanine is present only in green teas, but that is not accurate. Both black and green teas have been found to contain the compound. However, the L-theanine content varies dramatically from tea to tea. Therefore, in this backdrop we aim at quantization of L-theanine in black tea prepared from five different tea clonal varieties, e.g. AV2, B777, HV39, B157 and P312 of all the four flushes. Our study highlighted that black tea of first flush (Spring flush) have highest L-theanine content (~1.3%) than other three flushes. It was also observed that among the five clonal varieties released for Darjeeling, B777 contain highest amount of L-theanine than other clones under study. Thus L-theanine content in tea along with other bioactive components can be considered as an index of tea quality and price determination in tea auction.

Keywords: Auction; Clonal variety; First flush; L-Theanine; Tea; Quality

## I. INTRODUCTION

L-theanine (2-Amino-4-(ethylcarbamoyl)butyric acid)) is a non-protein derived amino acid found selectively in tea plants (*Camellia sinensis* L.O.Kuntze), constituting 1-2% of the dry weight of tea leaves and accounting for about 50% of the total free amino acids [1-4]. In 1950 theanine was first successfully isolated from shaded green tea (gyokuro leaf). Other than tea plant it can be found in the basidiomycetes mushroom *Boletus badius* and in an Amazonian tree *Ilex guayusa* [5]. Among the 700 chemical constituents present in tea, L-theanine play an important role in development of tea aroma and umami taste [6]. Recent advancement in tea research illuminates the tremendous health importance of L-theanine. It may contribute to a reduction in the risk of cardiovascular disease [5] and certain forms of cancer [8-12], as well as to the promotion other physiological functions such as anti-hypertensive effect, anti-stress/

anti-anxiety [13], hepatoprotective effect [14, 15], beneficial effects in Attention Deficit Hyperactivity Disorder (ADHD) [16] and Schizophrenia [17], improvement of Immune System [18-20] and neuroprotective power [21-23]. Other hand Zarse K et al reported the evidence of L-theanine regarding the promotion of longevity in mammals [24]. Recently we have deciphered its anti-ulcerogenic potential [25].

Till now, there is no evidence regarding the side effects of L-theanine. FDA confirmed L-theanine as Generally Recognized As Safe (GRAS) and the Japanese Ministry of Health and Welfare approved the use of L-theanine for universal consumption [26]. So, this compound can be considered one of the most promising and safe dietary supplement for the prevention and treatment of many diseases. Hence fourth L-theanine becomes the area of interest among the tea researchers. In future L-theanine content may be used as key parameter for fixing the price of black tea in tea auction. So, in the

present study, quantitative estimation and flushwise variation of L-theanine content in black tea samples processed from 5 tea clonal varieties released for Darjeeling were done.

## II. METHODS AND MATERIAL

**2.1. Chemicals :**L-Theanine spectrofluorimetric assay kit from Cayman chemicals (USA). HPLC grade Water was purchased from Merck (Germany). All other reagents used were of HPLC or AR grade and purchased from local suppliers. L-Theanine was used as standard and purchased from Sigma.

**2.2. Tea samples:** Young shoots of five tea clonal varieties viz. AV2, B777, B157, HV39 and P312 were plucked during the four flushes on 2012 and 2013 and orthodox black tea were made in the in-house manufacturing unit at DTR&DC, Kurseong.

### 2.3. Spectrofluorimetric estimation of L-theanine in Darjeeling black tea:

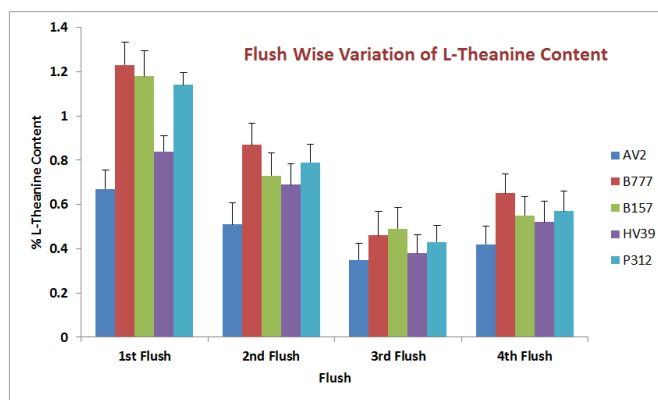
For complete extraction and for tea brew (tea drink) preparation, black tea samples (3 g) were stirred with hot water (150 ml) at 90°C for 30 min. After said incubation period tea extracts were clarified through filtration and the tea solution was allowed to cool to room temperature. A portion of the black tea aqueous extract was diluted 3 times. Now in a 1ml microfuge tube, 25 µL diluted tea extract, 25 µL double distilled water, 50 µL initiator and 50 µL detector (supplied with the kit) were added and fluorescence was read at excitation wavelength 430 nm and emission 485 nm. Concentration of the L-theanine was determined with respect to standard.

## III. RESULTS AND DISCUSSION

Tea, *Camellia sinensis*, is widely grown in the tropical humid climate of South East Asia, and infusion of its leaves (tea) is the most popular ancient non-alcoholic beverage worldwide, besides water. There are several places in India such as Darjeeling, Assam, Niligiri, Kunnur, Munnar etc. are well known for tea production. Among them the Darjeeling tea is unique and world famous for its muscatel flavor. Among different varieties, black tea of Darjeeling origin is preferred for its taste and flavors. Black tea like green tea contains lots of bioactive components e.g. theaflavins, thearubigins, L-theanine etc. In the present study, quantitative estimation

of total L-theanine content and its seasonal variation, if any, have been studied.

From the Fig.1 it was observed that among the five clonal varieties released for Darjeeling, B777 contain highest amount of L-theanine than other clones under study. A seasonal variation of L-theanine content is evident from the Fig.1. The total L-theanine content was highest in first flush (Spring Flush). The L-theanine content gradually decreased from first flush (Spring Flush) to second flush, second flush to third flush. Again it started to rise. L-Theanine content of the fourth flush was higher than third flush. However the monthly rise is insignificant for a particular clone. Therefore L-theanine content in tea along with other bioactive components can be considered as an index of tea quality and price determination in tea auction.



**Figure 1.** Flush wise variation of L-Theanine Content in Darjeeling tea Clonal Varieties

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