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Quantitative Estimation of γ-Glutamylethylamide in Commercially Available Made Teas [*Camellia sinensis* (L.) O. Kuntze, Theaceae] in Kenya

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Abstract

A study was carried out to quantitatively estimate the L-theanine content in 19 teas commercially available in the Kenyan market by High Performance Liquid Chromatography (HPLC). The test tea samples analyzed were green (*n* = 4), black (*n* = 8) and flavored (*n* = 7) teas from different origins *viz.*, Kenya (*n* = 4), Uganda (*n* = 2), Tanzania (*n* = 5), Rwanda (*n* = 4), Cameroon (*n* = 1) and Sri-Lanka (*n* = 2) commercially available in the Kenyan market. The estimated Limit of Detection (LOD) of the current method was 0.01% L-theanine. The L-theanine content ranged from below the detection limit (<0.01% L-theanine) to 1.60% L-theanine on a dry weight (d.w) basis. Statistically significant differences ($p < 0.05$) were observed in the L-theanine contents of black, green and flavoured teas. Rwandan green tea contained the highest L-theanine content with 1.60% d.w. whereas six of the seven flavoured teas had very low theanine levels (<0.01%) that could not be quantified by the current method.

Keywords

Food Analysis, Food Composition, HPLC, L-Theanine, Non-Protein Amino Acids, Tea

1. Introduction

Tea, the most widely consumed plant-based beverage in the world [1], is processed from young and tender

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shoots of the tea plant (*Camellia sinensis*, family Theaceae) and historians have linked its consumption to almost 5000 years back [2]. Tea can be broadly classified according to the processing methods as; un-aerated tea (green tea), semi-aerated tea (Oolong tea), fully aerated tea (black tea) or post-aerated tea (pu-erh tea) [3]. The beverage has over time gained popularity as a "health drink" due to the numerous beneficial medicinal properties that have been attributed to its polyphenolic content as evidenced by *in vitro* and animal studies [4]-[6]. Indeed, a growing body of research describing many putative benefits of regular tea consumption such as antibacterial [7], antimicrobial [8] [9], anti-diabetic [10], antioxidant [11]-[14], anti-viral effects [15]-[17] among others have been reported. Based on how the young tender shoots of the tea plant (raw material for tea manufacture) are handled during the manufacture process, different types of tea products with different biochemical profiles can be obtained [18]. This is because the nature and quality of a given tea product is mainly dependent on the chemical composition of the young tea shoots and the reactions they undergo during the manufacture process [19]. Further, several research findings have shown that tea contains a myriad of compounds, a portion of which end up in the tea liquor during the tea brewing process. Such compounds include; flavonoids, proteins, amino acids, enzymes, vitamins and a number of trace elements such as iron, zinc, copper and fluoride [20]-[27].

Theanine (γ-glutamylethylamide), a non-protein amino acid, is a glutamic acid analog commonly identified in tea. It constitutes between 1% and 2% of the dry weight of the tea leaves and about 50% of total free amino acids [28]. It is the major "umami" (good taste) component of tea [29] and its favorable physiological effects on mammals have been reported; influence on the functionality of the brain [30], mitigation of mental and physical stress due to its ability to cross the blood-brain barrier [31]-[33] and boosting of immunity against infection by enhancing the disease-fighting ability of gamma delta T cells [34]. Besides being a major tea producer of tea globally, data on the L-theanine of Kenyan tea as well as that from other countries commercially available locally is scarce. Thus, the objective of this study was to establish the L-theanine contents of different types of tea commercially available in the Kenyan market. Data obtained could be an important source of information with regard to quality, standards and nutrition.

2. Materials and Method

2.1. Samples and Chemicals

The study targeted processed (made) teas commercially available in the Kenyan market. The Mombasa tea auction, being the second largest tea selling point in the world after Colombo, was chosen as the best sampling point. 19 tea samples constituting of green, black and flavored teas were collected in triplicates. Random sampling was done and based on the availability of the samples at that time. The teas obtained were from; Kenya (n = 5), Uganda (n = 2), Tanzania (n = 5), Rwanda (n = 4), Cameroon (n = 1) and Sri-Lanka (n = 2) of which 4, 8 and 7 were green, black and flavored teas respectively as depicted in **Table 1**.

The representative triplicate test samples were transported to the Tea Research Institute (TRI) laboratories situated at Kericho (latitude 0˚22'S, longitude 35˚21'E, altitude 2180 m above mean sea level). Here, the samples were finely milled using an electric blade grinder (Moulinex AR1043, China) for particle size reduction and homogenization. Sieving of the test tea samples was not done since the teas were already graded. The test samples were then stored in well labeled tightly sealed aluminium-lined sachets awaiting analysis.

Table 1. The nature, number and origin of the made tea in the Kenyan Market.

	Type of tea		
Country of origin	**Green tea**	**Black tea**	**Flavored tea**
Kenya	-	1	4
Rwanda	2	2	-
Uganda	-	2	-
Tanzania	1	1	3
Cameroon	-	1	-
Sri-Lanka	1	1	-

An authentic commercial standard of L-theanine with a purity ≥99.3% and HPLC grade acetonitrile (CH_3CN; Purity ≥99.93%) were procured from Sigma Aldrich (UK) via Kobian Kenya Ltd., Nairobi. All dilutions of standards and test samples were done using double distilled water purified by Distinction Water Still-D4000 (England) water distillation system.

2.2. Sample Analysis

2.2.1. Dry Matter (DM) Content Estimation

2.0 ± 0.01 g of the sample was put into aluminium dishes and heated in an oven at 103˚C ± 2.0˚C for 8 hours to constant weight; when all the moisture in the sample had been lost. The dry matter content was then computed and expressed as a percent as follows;

$$\frac{\Delta W}{IW} \times 100\% = \%\ DM$$

where ΔW is the change in weight, IW is the initial weight whereas % DM is the percent dry matter. DM for the test tea samples ranged between 93% - 98%.

2.2.2. Preparation of Standards and Test Tea Samples

A standard stock solution was prepared by dissolving 0.05 ± 0.001 g of an authentic commercial L-theanine standard in a 50 ml volumetric flask using double distilled water with the aid of sonication in an ultrasonic bath (Grant XB14, England). Standard working solutions in the concentration range between 20 - 80 $\mu g \cdot mL^{-1}$ were prepared by serial dilution of the standard stock solution using double distilled water and described by [35].

1.0 ± 0.01 g of a finely ground sample was weighed into a clean and dry 200 mL beaker, into which 100 mL boiling double distilled water was added. The sample was then allowed to brew while being constantly agitated for 5 minutes on a hot plate stirrer (Corning PC-351, USA). The mixture obtained was allowed to cool to room temperature, made up to volume with double distilled water and then filtered through a 0.45 µm membrane into sample vials prior to injection.

2.2.3. Chromatographic Estimation of L-Theanine

The L-theanine contents in the various tea taste solutions were estimated by High Performance Liquid Chromatography (HPLC). The chromatograph used was a Shimadzu LC 20 AT make fitted with an SIL 20A auto sampler, two LC-20 AT pumps, a DGU $20A_{5R}$ degasser and an SPD-20 UV-Visible detector set at 210 nm, operated with a class LC 10 solution workstation, manufactured in Kyoto, Japan, as described by [35]. The L-theanine peak was identified by comparing the retention time of the test tea solutions peaks against those obtained from the authentic commercial L-theanine standard analysed under similar conditions. L-theanine quantitation was done using the regression equation of the L-theanine calibration curve obtained by plotting the concentration of L-theanine in the working solutions against theanine peak area. The theanine content was computed and expressed as a percentage by mass on a dry matter basis using the relation;

$$\%\ \text{L-theanine} = \left[\left(A_{sample} - b_{intercept}\right) \times V_{sample} \times d \times 100\right] \Big/ \left[m_{std} \times M_{sample} \times 10000 \times DM\right]$$

where A_{sample}: is the peak area of the test tea solution,

$b_{intercept}$: is the y intercept of the calibration curve,

V_{sample}: is the volume of sample injected during the chromatographic analysis,

m_{std}: is the slope of the calibration curve,

M_{sample}: is the mass in grams of the sample,

d: is the dilution factor and

DM: is the dry matter content, expressed as a mass fraction in percent, of the test sample.

2.3. Data Analysis

Data obtained from the triplicate determinations of the test teas were subjected to Analysis of Variance (ANOVA) using MSTAT statistical package for windows with the probability limit set at $p \leq 0.05$. The Least Significant Difference (LSD) test was used for mean separation where statistically significant differences were observed.

Graphical representation of the mean L-theanine contents was done using Microsoft® Excel, version 2010.

3. Results and Discussion

L-theanine was eluted at the 6th minute and a sample of chromatogram obtained is as shown in **Figure 1**.

The mean L-theanine content in the test tea samples ranged from below the Limit of Detection (LOD) of the method employed, 0.01% to 1.60% on a dry weight (d.w.) basis as shown in **Figure 2**. Generally, green teas were shown to contain the highest L-theanine contents followed closely by black teas. The mean L-theanine content in Kenyan black tea was 1.02% d.w. and was not significantly different ($p > 0.05$) from the Rwandan (BT1 = 0.98% d.w.; BT2 = 1.19% d.w.) and Ugandan (BT2 = 0.90% d.w.) black teas. However, one of the Ugandan tea samples had L-theanine content lower than the quantitation limit of the current method (<0.01%

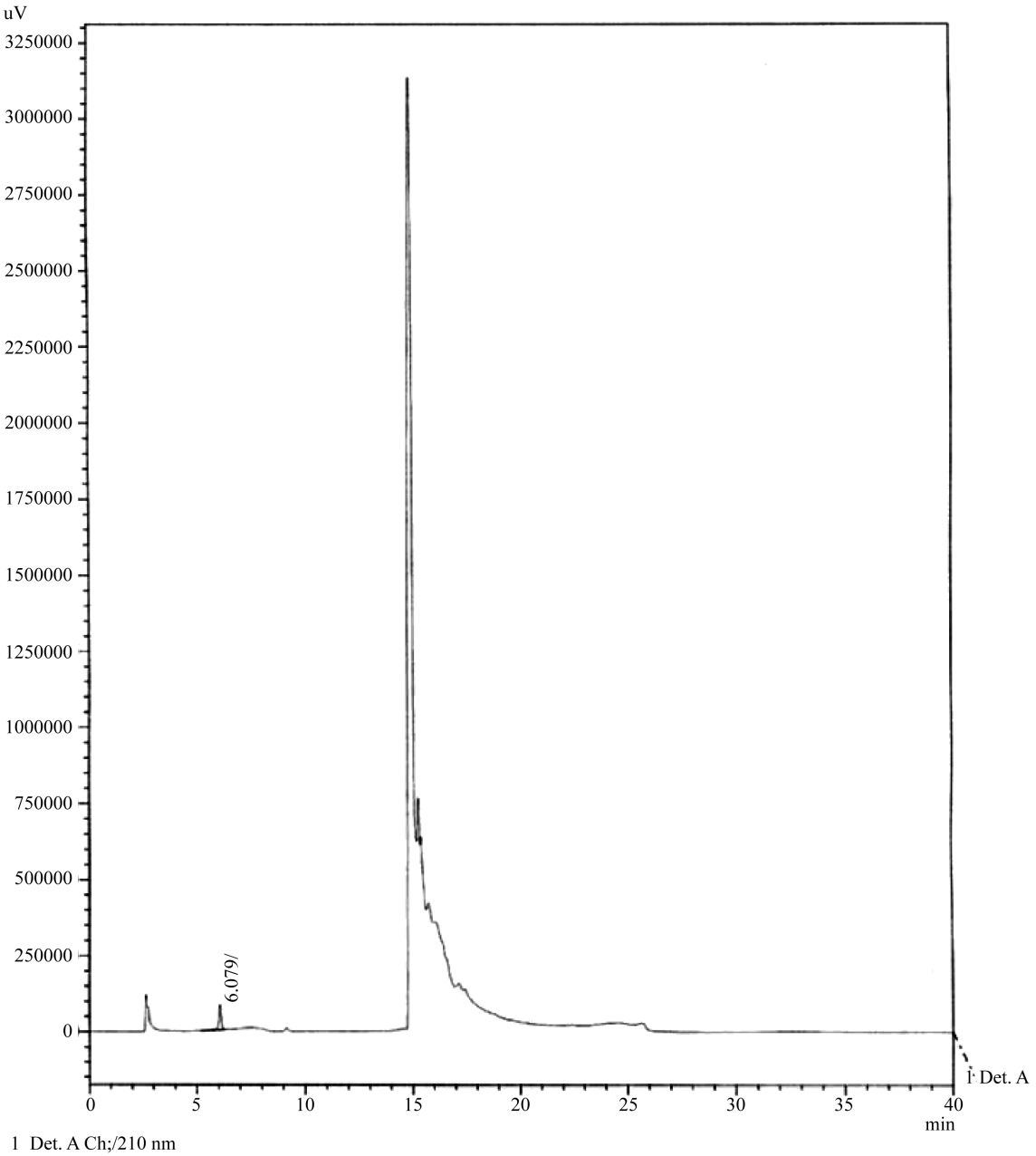

Figure 1. A representative chromatogram of the L-theanine (Kenyan black tea).

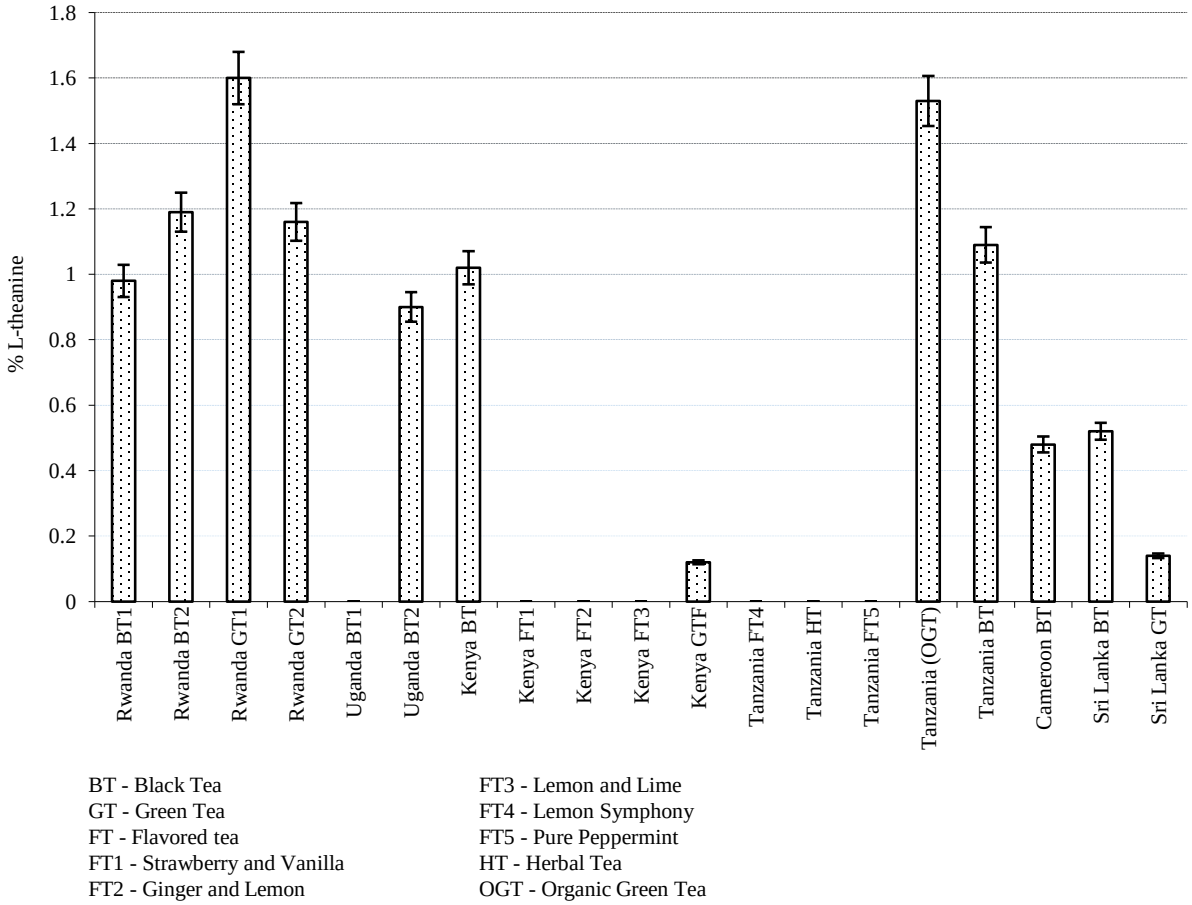

Figure 2. L-theanine contents in the various types of tea from different origins commercially available in the Kenyan Market.

d.w.). On the other hand, black teas from Cameroon and Sri Lanka had L-theanine contents of 0.48% and 0.52% d.w. respectively. These were significantly ($p < 0.05$) lower than those of Kenyan and Ugandan black tea. Rwandan green teas, GT1 (1.60% d.w.) and GT2 (1.16% d.w.) contained the highest L-theanine contents. These mean L-theanine contents were significantly ($p < 0.05$) different and higher than that of Kenyan and Sri-Lankan green teas which contained 0.12% and 0.14% d.w. of L-theanine. Six of the seven flavoured teas analysed had L-theanine contents that could not be quantified using the current method (<0.01%). This possibly implies that these teas majorly contained the flavours and less of tea.

Many studies have reported the L-theanine content in different teas [36]-[38]. In the current study, Kenyan green tea commercially available in the market (GTF) had an L-theanine content of 1.2 mg/g (0.12% d.w.). These levels are in agreement with those reported by [39]. However, as seen in the current studies data, a number of factors may influence the L-theanine content in tea. These include the type of tea, in the current case green or black tea, where green teas have been shown to have higher levels of L-theanine. Indeed, a number of researchers have reported some of the other factors that influence the L-theanine content in tea including; effects of origin, cultivar variety, leaf age, growing area, horticultural practices and plucking season, and the microorganism used for fermentation on metabolites of green tea products [35] [40]-[43].

The L-theanine of black tea in the current study was 10.2 mg/g in this study and was in agreement with those reported by [41] who report a mean L-theanine content of 9.1 mg/g. Thus, with 50 to 200 mg of L-theanine being reported to reduce blood pressure in animal models [44] and comparing to the L-theanine content of Kenyan green tea it means one should take more than 20 cups of tea in a day. This is not practical but with the Rwandan green tea (1.60% d.w.) it means one should take 2 cups of tea to achieve the health benefits of L-theanine. However, a disturbing revelation of the current study's findings is the very low L-theanine content of flavoured

teas. With L-theanine being solely found in tea (*Camellia sinensis*), it should be safe to assume that any tea product should contain it. Thus, the current findings possibly imply that these teas majorly contained the flavours and consumption of these teas will be of little or no health benefit.

4. Conclusion

The levels of L-theanine varied with the type and origin of the tea product studied. Green teas generally contained high levels of L-theanine with flavored teas containing little or no (<0.01%) L-theanine. These data should therefore be used as a basis of setting regulations for the ratios of tea to flavours of "flavoured teas" to ensure that such teas actually contain tea in them. Indeed, this will go a long way in increasing the volumes of tea sold and subsequently consumed.

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Conflict of Interest

The authors declare none.

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