

Bioactive compounds distribution in tea leaves from different maturity stages and altitude in Malaysia

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Abstract

High altitude young tea leaves are the most preferred raw material for high quality tea production while old tea leaves were considered low quality. This study examined the chemical compounds in tea leaves such as (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), caffeine, gamma aminobutyric acid (GABA) and total phenolics content (TPC) in different maturity stages of tea leaves from highland (HP01, HP02 and HP03) and lowland (LP) tea plantation in Malaysia. The results showed that young tea leaves from samples HP01, HP02 and LP contain high GABA, caffeine and TPC as compared to old tea leaves. However, with the exception of caffeine, old tea leaves from sample HP03 contained higher TPC and GABA than young tea leaves. In addition, the highest accumulation of GABA was found in a bud from HP01. However, in general, the accumulation of GABA was high in LP (lowland) tea leaves from bud until 4th leaves when compared to highlands tea leaves. Furthermore, the number of catechins with a high concentration in a very young leaf was HP01>LP01>HP02>HP03. The major catechins were identified as EGC (49.02 mg/g) and the minor was ECG (1.17 mg/g). Overall, the accumulation of catechins, caffeine and phenolics content was high in tea leaves at high altitudes whereas GABA content was high in tea leaves at low altitudes. This finding provides information on bioactive metabolites distribution in different maturity stages of tea leaves from high- and lowland tea plantations in Malaysia.

1. Introduction

Tea beverage prepared by steeping *Camellia sinensis* leaves in boiling or hot water is the most widely consumed beverage throughout the world. High quality tea is normally produced from a young leaf which consists of a bud and two leaves cultivated at high altitudes. In addition, the quality of tea depends on the plucking season as it influences the chemical compositions and the taste of tea. Tea harvested in spring contains a high level of amino acid content while the level of flavan-3-ol, dimeric catechins and flavone-C-glycosides (FCG) was high in the summer and autumn seasons (Dai *et al.*, 2015).

Tea is rich in polyphenolic compounds which are known to have multiple health benefits such as catechins.

There are five major catechins: (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) have been identified in tea leaves. The distribution of catechins in tea varies in different parts of the plant and maturity stages. Young tea leaves are rich in C, EC, EGC (non-galloylated catechins) while old tea leaves are high in ECG and EGCG (galloylated catechins) (Lee, Lee, Hwang *et al.*, 2011; Zhang *et al.*, 2016; Samanta *et al.*, 2017; Liu *et al.*, 2020). Tea is also rich in caffeine, particularly in young tea leaves (Sanyal, 2011).

Gamma-aminobutyric acid (GABA) is an amino acid with four carbon backbone, but it is not classified as a protein. It is naturally present in tea plants in small quantities which is less than 1 mg/g (Wu *et al.*, 2018).

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GABA is getting more attention as it was reported to have many health benefits. The latest findings showed the benefits of GABA in reducing stress (Hinton *et al.*, 2019), giving a calming and relaxing effect (Yamatsu *et al.*, 2015) and helping against insomnia (Yu *et al.*, 2020).

Malaysia is one of the tea producers in the world with the largest tea plantation located in Cameron Highlands (Pahang, highland tea), followed by the foothills of Mount Kinabalu (Sabah, highland tea) and Banting (Selangor, lowland tea). The main tea produced in Malaysia is black tea, mostly consumed by Malaysians. Despite being one of the tea producers in the world, there is still a lack of study and information on the chemical composition of tea leaves from high- and lowland which can be used as a reference in the preparation of tea products with different taste quality and health benefits. The antioxidant activity and polyphenols content in a mixture of tea leaves with different maturity stages from high- and lowland in Malaysia have been studied earlier (Chan *et al.*, 2007; Nor Qhairul Izzreen and Mohd Fadzelly, 2013; Nor Qhairul Izzreen *et al.*, 2018). However, the information reported is insufficient and more studies are needed to investigate the accumulation of bioactive compounds in individual tea leaves from different maturity stages and altitudes. Thus, the objective of this study was to measure the changes in catechins, caffeine, polyphenolic compounds and GABA content in individual tea leaves, collected from different maturity stages and the influence of altitude on the production of these compounds is examined also.

2. Materials and methods

2.1 Tea leaves

Fresh tea leaves were harvested from three different tea plantations in Cameron Highland; BOH Plantation Sdn Bhd. (HP01), Cameron Bharat Plantation Sdn Bhd (HP02) and Agro-Technology Park MARDI (HP03) which represent highlands tea leaves. On the other hand, the lowland tea leaves (LP) were collected from BOH Plantation Sdn Bhd located in Bukit Cheeding, Banting. The variety of tea leaves for samples HP01, HP02 and LP was *Camellia sinensis* var *assamica* while sample HP03 was *C. sinensis* var *sinensis*. The tea leaves were kept chilled during transportation from the plantation to the laboratory. Upon reaching the laboratory, the leaves were immediately separated according to maturity stages. The leaves' maturity was separated according to the bud, first leaf, second leaf, third leaf and fourth leaf. Tea leaves were subjected to drying at 50°C, approximately 16 hrs or until the moisture content was reduced to 7.0±1.0%. The dried leaves were ground using an Ultra Centrifugal Mill (ZM 200, Retsch) with

sieve size 0.5 µm, vacuum packed and stored at 4°C for further use.

2.2 Extraction and quantification of GABA

The ground tea leaves were weighed and extracted with distilled water (1:20; w/v) at 90°C for 60 mins in shaking water bath (150 rpm). The extractant containing GABA was then filtered using 0.22 µm nylon syringe filter into a microtube and stored at -20°C before analysis. All extractions were carried out with three replicates. A filtered extract (10 µL) was transferred into an HPLC vial containing 70 µL of AccQ-Tag™ Ultra borate buffer. Both mixtures were vortexed for 1 min before adding 20 µL of AccQ™ Fluor reagent. Then, the mixture was mixed vigorously before heating at 55°C for 10 mins. The separation and quantification of GABA was performed as described by Mohd Danial *et al.* (2015) using the Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system, comprising a binary solvent manager, a sample manager fitted with a 1 µL sample loop and a photodiode array (PDA) detector set at 260 nm. The separation was done using AccQ-Tag™ Ultra C18 Column (2.1 mm. × 100 mm × 1.7 µm) at 55°C with a flow rate and injection volume of 0.7 mL/min and 1 µL, respectively. The mobile phase was water:AccQ-Tag™ Ultra Eluent A (95:5, v/v) (eluent A) and acetonitrile:formic acid (98:2, v/v) (eluent B). The gradient program held eluent A at 99.9% from 0 to 0.54 min before slowly decreasing eluent A to 90.9% in 5.2 min, further decreasing to 78.8% in 2 mins, followed by a rapid decrease to 40.4% in 0.76 min and then remained at this concentration for 0.3 min before rapid increased to 99.9% in 0.1 min. Different concentrations of GABA standards (Sigma Aldrich, Missouri, US) were injected to set up an external calibration curve for GABA quantification. The data were analysed using Waters Empower™ 2 software.

2.3 Extraction and quantification of catechins and caffeine

Catechins and caffeine were extracted with 50% aqueous ethanol. Approximately 50 mL of aqueous ethanol was added to 1 g of ground tea leaves. After being shaken for 60 mins at 30°C and 150 rpm, the mixture was separated by centrifugation at 10,000 rpm for 10 mins at 4°C. The aqueous ethanol containing catechins and caffeine was filtered (0.22 µm nylon syringe filter) into an amber microtube and stored at -20°C before analysis. All extractions were carried out with three replicates. The separation and quantification of catechins and caffeine was done using the Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system with a photodiode array (PDA). The separation was done using Phenomenex Kinetex® LC

C18 Column (2.1 mm × 150 mm × 1.7 μm) at 50°C. The injection volume and flow rate were set at 1.0 μL and 0.35 mL/min, respectively. The mobile phase consists of acetic acid:water (3:97, v/v) (eluent A) and 100% methanol (eluent B). The gradient program was 100% A held for 0.60 min, slowly decreasing to 40% in 11.4 mins before increasing to 100% in 4 mins and held for 2.0 mins. The detection wavelength was 270 nm. Different concentrations of (+)-catechin, (-)-epicatechin (EC), (-)-epicatechin gallate (ECGG), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) and caffeine standards (Sigma Aldrich, Missouri, US) were prepared by dissolving those standards in 50% aqueous methanol (v/v) to build external calibration curve for quantification. The data were analysed using Waters Empower™ 2 software.

2.4 Measurement of total phenolic content

Total phenolic content was measured as described by Okmen *et al.* (2009). A mixture of 1 mL ethanol extract of the sample, 5 mL of Folin-Ciocalteu reagent (Merck, Kenilworth, NJ, USA) and 4 mL of 7.5% aqueous sodium carbonate (w/v) was left to react in dark condition for 2 h. The presence of phenolic compounds was measured using UV-VIS spectrophotometer (VARIAN, Cary 50, Agilent) at 765 nm. Different concentration of gallic acid standard (0-200 ppm) was prepared by diluting in distilled water. The results were expressed as gallic acid equivalent (GAE). All experiments were carried out with three replicates.

2.5 Statistical analysis

The general influence of tea leaves maturity on GABA, catechins and TPC was analyzed using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$. Tukey's multiple comparison test was further applied to compare the mean of each sample. GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) was used for statistical analyses.

3. Results and discussion

3.1 Total phenolic content

Total phenolic content (TPC) is a measurement of phenolic compounds, an important secondary metabolite produced by plants. The phenolic compounds in tea plant included phenolic acids, O-glycosylated flavonols, acylated O-glycosylated flavonols, proanthocyanidins, C-glycosylated flavones and flavanols (Lin *et al.*, 2008; Jiang *et al.*, 2013).

The changes in total phenolics content in different parts of tea leaves (bud to 4th leaf), collected from high- (HP01, HP02 and HP03) and lowland (LP) plantation

areas were shown in Figure 1. The changes of TPC in tea leaves were found to be variable. As for the HP01 tea sample, the highest TPC was detected in a bud (118.6 mg GAE/g) and it showed a significant decrease ($p < 0.05$) as the leaves mature particularly the 1st leaf which contains the lowest concentration of TPC. The concentration was then increased slowly from the 2nd leaf until the 4th leaf but the TPC was still significantly ($p < 0.05$) lower than a bud. A similar trend was also found in HP02 tea leaves. The TPC was significantly decreased from a bud to a 2nd leaf but then significantly increased in 3rd leaf before dropping again in 4th leaf. Meanwhile the LP tea sample, with the exception of the 2nd leaf, the TPC was similar in young and old tea leaves. However, the lowest phenolics content (40.7 mg GAE/g) was found in a bud of the HP03 tea sample before increasing to the highest level (85.5 mg GAE/g) in 3rd leaf and then decreased when the leaves got older. The accumulation of phenolic content was found relatively high at low altitude tea plantations. Total accumulation of phenolics (from a bud to 4th leaf) in tea leaves collected from LP, HP02, HP01 and HP03 was 504.8, 469.7, 416.9 and 306.8 mg/g, respectively.

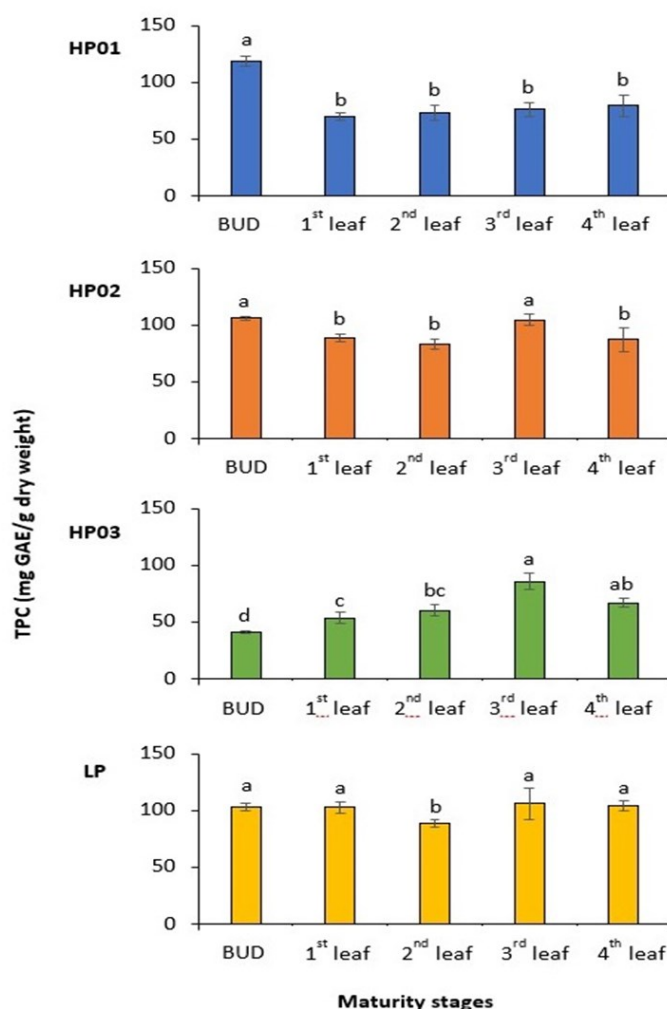


Figure 1. Total phenolics content (TPC) of fresh tea leaves from different maturity stages and altitudes. Error bars indicate the standard deviation of the mean. Bars with different notations are statistically significantly different ($p < 0.05$) within the leaf's maturity.

Past studies have reported that the TPC in tea shoot (bud to second tea leaves), young leaves (third to fifth leaf) and mature tea leaves (sixth to eight leaves) was 76.66, 72.80 and 58.36 mg GAE/g, respectively, which clearly showed a decreasing trend when leaves mature (Chan *et al.*, 2007). The main focus of this study was to target the different stages of matured tea leaves from low - and highland tea. Specific leaves, collected from different tea growth stages were used, in order to explore more detailed information on the TPC changes during tea plant growth. Interestingly, it was noted that the TPC changes trend (from a bud until 4th leaf) for sample HP03 was slightly different from sample tea from HP01, HP02 and LP. These discrepancies could be due to the differences in the tea leaves variety whereby the variety of tea leaves from HP03 was *C. sinensis* var *sinensis* and the other three tea samples were *C. sinensis* var *assamica*. In Paiva *et al.* (2020) study on individual tea leaf (*C. sinensis* var *sinensis*) from bud to a second leaf, the TPC was increased from the bud to a second leaf and this finding tally with the present study on the sample HP03 under the same variety. The results indicated that

young tea leaves particularly a bud contain high phenolic content than mature leaves and could be influenced by the variety of tea leaves. In addition, there is a significant influence of tea plantation altitude on the chemical changes in plant during growth. It was reported that the tea polyphenols were significantly decreased as the altitude was increased from 420 m to 1020 m (Han *et al.*, 2016). In tea, phenolic compounds not only contribute to the flavour of the tea but play an important role in health as it was reported to have many pharmacological activities such as antioxidant (Venkatakrisnan *et al.*, 2018), anti-inflammatory (Su *et al.*, 2017) and antibacterial (Tsai *et al.*, 2016) activities.

3.2 Catechins

The concentration of catechin (C), two galloylated catechins (ECG and EGCG) and two non-galloylated catechins (EC and EGC) in tea leaves from bud to fourth leaf, collected from high and low tea plantations area is shown in Table 1. It was observed that the level of catechin (C) in a bud from HP01 and HP02 was the highest but slowly reduced until 3rd leaf before

Table 1. Catechins in fresh tea leaves from different maturity stages and altitudes.

Altitude	Maturity	mg/g dry tea				
		C	EC	EGC	ECG	EGCG
HP01	Bud	2.20±0.54 ^a	0.81±0.17 ^{ab}	27.45±4.64 ^a	0.34±0.08 ^b	1.31±0.42 ^a
	1 st leaf	0.99±0.12 ^b	0.62±0.09 ^{bc}	11.39±1.21 ^b	0.48±0.09 ^a	0.49±0.06 ^b
	2 nd leaf	0.59±0.04 ^{bc}	0.52±0.08 ^c	5.29±0.04 ^c	0.39±0.02 ^{ab}	0.34±0.05 ^b
	3 rd leaf	0.30±0.02 ^c	0.69±0.16 ^{bc}	3.24±0.08 ^{cd}	0.21±0.03 ^c	0.26±0.01 ^b
	4 th leaf	0.81±0.06 ^{bc}	0.83±0.16 ^{ab}	1.65±0.16 ^d	0.18±0.04 ^c	0.16±0.02 ^b
	Total		4.89	3.47	49.02	1.60
HP02	Bud	0.89±0.12 ^a	0.95±0.19 ^d	2.66±0.25 ^c	0.82±0.11 ^a	0.92±0.11 ^a
	1 st leaf	0.62±0.04 ^{ab}	1.01±0.08 ^d	1.12±0.03 ^f	0.66±0.08 ^{ab}	0.59±0.08 ^b
	2 nd leaf	0.50±0.06 ^b	1.61±0.07 ^{bc}	3.80±0.18 ^b	0.50±0.03 ^c	0.78±0.06 ^{ab}
	3 rd leaf	0.49±0.05 ^b	1.44±0.20 ^c	3.81±0.47 ^{cd}	0.31±0.05 ^d	0.68±0.11 ^{ab}
	4 th leaf	0.59±0.05 ^{ab}	1.99±0.15 ^a	4.34±0.53 ^{ab}	0.35±0.06 ^{cd}	0.88±0.14 ^a
	Total		3.09	7.00	15.73	2.64
HP03	Bud	0.71±0.06 ^b	0.96±0.11 ^c	3.81±0.33 ^b	0.58±0.06 ^c	0.52±0.09 ^c
	1 st leaf	0.71±0.05 ^b	2.13±0.72 ^b	1.78±0.37 ^c	0.78±0.18 ^{bc}	0.51±0.18 ^c
	2 nd leaf	0.89±0.18 ^b	2.53±0.23 ^{ab}	3.56±0.32 ^b	0.90±0.16 ^b	0.78±0.01 ^c
	3 rd leaf	1.24±0.09 ^a	3.46±0.16 ^a	4.84±0.46 ^a	1.28±0.08 ^a	2.11±0.39 ^a
	4 th leaf	0.91±0.21 ^b	3.01±0.35 ^{ab}	3.16±0.48 ^b	0.67±0.16 ^{bc}	0.92±0.16 ^{bc}
	Total		4.46	12.09	17.15	4.21
LP	Bud	0.69±0.55 ^a	1.61±0.22 ^a	0.90±0.32 ^c	0.28±0.05 ^{ab}	0.59±0.41 ^a
	1 st leaf	0.52±0.06 ^a	1.68±0.42 ^a	1.75±0.48 ^{bc}	0.37±0.09 ^a	0.54±0.33 ^a
	2 nd leaf	0.78±0.08 ^a	1.18±0.15 ^b	1.51±0.49 ^{cc}	0.24±0.07 ^b	0.28±0.05 ^a
	3 rd leaf	0.57±0.01 ^a	1.09±0.08 ^b	1.72±0.09 ^{bc}	0.14±0.01 ^c	0.22±0.01 ^a
	4 th leaf	0.56±0.05 ^a	1.40±0.06 ^a	2.20±0.10 ^{ab}	0.14±0.04 ^c	0.28±0.04 ^a
	Total		2.97	6.96	7.89	1.17

Values are presented as mean±SD of three replicates. Values with different superscripts are statistically significantly different ($p < 0.05$) within the leaf's maturity stage. C: (+)-catechin, EC: (-)-epicatechin, EGC: (-)-epigallocatechin, ECG: (-)-epicatechin gallate, EGCG: (-)-epigallocatechin gallate.

increasing as the leaves mature. However, the reverse trend was observed for the HP03 tea sample with different varieties. There were no differences in catechin content for LP under various tea leaf growth stages. The concentration of EC leaves from HP02 and HP03 significantly increased with the leaf's maturity level. For HP01 and LP, the EC was reduced from bud to 2nd and 3rd leaves, respectively but increased when the leaves began to mature (4th leaf). For EGC, the concentration is reduced significantly ($p < 0.05$) with leaves age with bud containing the highest EGC concentration detected in sample HP01. In contrast, the EGC concentration of HP02 and LP was significantly higher ($p < 0.05$) in old tea leaves than in young tea leaves. A different scenario was observed in the HP03 tea sample, the highest accumulation of EGC was detected in the 3rd leaf and the lowest concentration of EGC was present in the 1st leaf.

For galloylated catechins, the ECG concentration is high in young tea leaves when compared to old leaves, both plucking from high- and lowland (HP01, HP02 and LP). However, a different phenomenon was observed for HP03 tea leaves, whereby the ECG and EGCG were low in bud but then significantly increased ($p < 0.05$) to 3rd leaf before decreasing when the tea leaves aged. In contrast to the above observation, the EGCG was high in HP01 bud and decreased as the tea leaves matured. For LP and HP02 tea leaf samples, the amount of EGCG at different development stages was relatively constant except for 1st leaf of HP02.

In summary, from the data presented in this study, all the individual catechins (C, EC, EGC, ECG and EGCG) from HP01, two (C and ECG) from HP02 and three (C, EC and ECG) from LP were high in young tea leaves. Whereas, all the catechins from the HP03 tea sample were higher in mature leaves than in young leaves. The non-galloylated catechins from both high- and lowland were relatively higher than galloylated catechins. Among the five catechins, EGC was the major catechin detected in the leaves with a concentration of 7.89 to 49.02 mg/g and the lowest was ECG, ranging from 1.17 to 4.21 mg/g. Furthermore, with the exception of EC from HP01, total individual catechins synthesis in lowland tea leaves was relatively lower than in highland tea leaves.

Catechins are important compounds that exist in tea which contribute to the flavour in green tea and also act as a precursor for theaflavin synthesis in black tea. In the present study, it was shown that there was a variation in catechins distribution in tea leaves with different maturity stages as confirmed by other tea leaves studies as well. Song *et al.* (2012) and Paiva *et al.* (2020) who studied the changes in catechins from bud to second leaves reported an increase in C, EC, EGC, ECG and

EGCG levels as leaves mature. In addition, with the exception of C and ECG. Zhang *et al.* (2016) revealed an increase in EGCG, EGC, EC, ECG, GC and total catechins from bud to 3rd leaf but showed a decrease trend when leaf age (4th leaf). Meanwhile, Samanta *et al.* (2017)'s study reported only EC and EGC levels increase from bud to 3rd leaf but other catechins like C, ECG, EGCG and total catechins were decreased when leaves mature. The variations in the present and the previous studies could be related to the differences in the tea varieties examined. The levels of galloylated catechins and non-galloylated catechins in young and old tea leaves were reported species dependent (Lin *et al.*, 2003). The variations could also be related to the difference in tea plantation altitude. In this study, the total individual catechins concentration of tea leaves from high altitudes were relatively higher than from low altitudes and this finding was consistent with Chen *et al.* (2010) study. It was speculated that low temperature and less sun exposure duration at high altitudes delay the growth of tea leaves and the metabolism process, eventually leading to higher accumulation of secondary metabolites such as galloylated and non-galloylated catechins (Lee, Lee, Chung *et al.*, 2011). Another study by Zheng *et al.* (2008) also revealed short-term exposure to UV-B radiation enhanced the synthesis of tea catechins while longer exposure suppressed the catechins synthesis. In summary, the distribution and level of catechin, galloylated and non-galloylated catechins in tea leaves are influenced by the growth developmental stages and the altitude of the tea plantation area.

3.3 Caffeine

The level of caffeine at different development stages of tea leaves from high and low altitudes was quantified (Figure 2). The level of caffeine in tea leaves plucking from different maturity stages from both high and low altitude tea plantations showed a significant decline trend ($p < 0.05$) as leaves mature. The only exception was the HP03 tea sample where the accumulation was relatively the same and a significant decline was only detected in a very old tea leaf (4th leaf). Overall, with the exception of HP03, the caffeine content in tea leaves (HP01: 7.35-15.6 mg/g and HP02: 9.23-13.81 mg/g) from highland was higher than in lowland (LP: 6.93-10.92 mg/g).

Caffeine is a purine alkaloid which can be found naturally in plants including tea, coffee and cocoa beans. It is high in young tea leaves and will reduce as the tea leaves mature (Lee, Lee, Hwang *et al.*, 2011; Song *et al.*, 2012). Past studies on tea caffeine postulated that the high concentration of caffeine in the bud and young tea leaves could provide protection to tea plants from pests (Nathanson, 1984), slugs and snail attacks

(Hollingsworth *et al.*, 2003) and pathogenic bacterial infection (Sledz *et al.*, 2015). Previously, the concentration of caffeine in tea leaves was reported to correlate with the altitude. The caffeine concentration was reported to decrease at low altitudes when compared to high altitudes (Ohno *et al.*, 2011). Furthermore, tea planted under less sun exposure accumulates more caffeine than tea that receives a high amount of sunlight (Song *et al.*, 2012). In the present study, the low caffeine content in tea leaves from lowland plantations could be due to the tea plant's exposure to high sunlight intensity. Water stress is another factor that could affect caffeine synthesis. It was reported that there was a significant reduction in caffeine synthesis when the tea plant was exposed to drought-stress conditions (Jeyaramraja *et al.*, 2003).

3.4 Gamma-aminobutyric acid

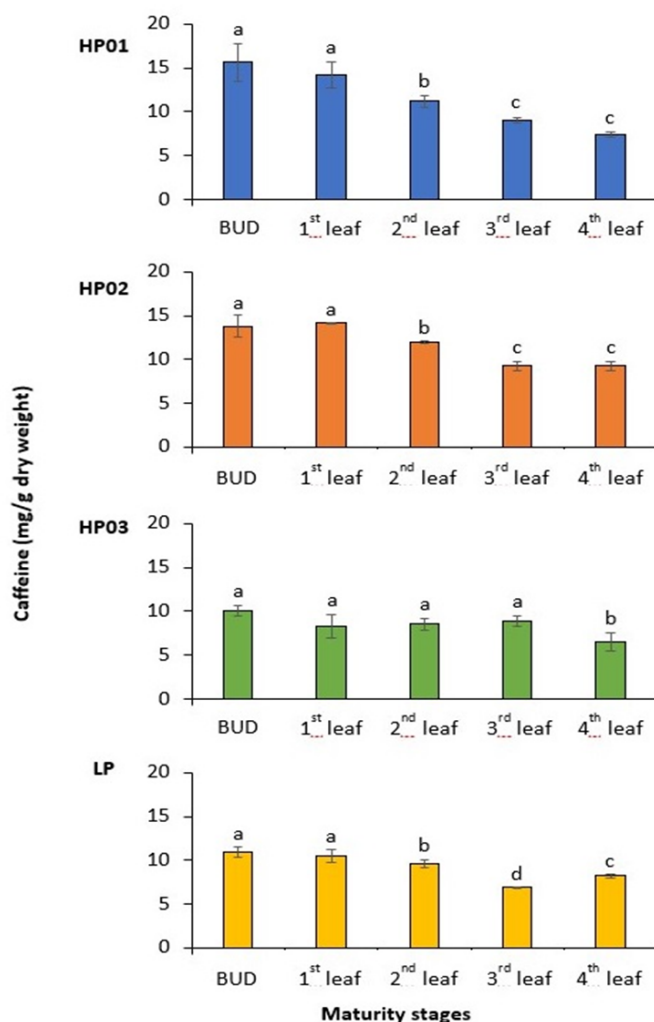


Figure 2. Caffeine in fresh tea leaves from different maturity stages and altitudes. Error bars indicate the standard deviation of the mean. Bars with different notations are statistically significantly different ($p<0.05$) within the leaf's maturity.

The GABA content in tea leaves with different maturity stages from high- (HP01, HP02 and HP03) and lowland (LP) is shown in Figure 3. The GABA

concentration of HP01 was significantly decreased ($p<0.05$) from the bud to 4th leaf. Meanwhile, for HP02 and LP tea samples, there was a significant increment of GABA ($p<0.05$) from bud to 2nd leaf but showed a decrease when the tea leaves aged. In contrast, the GABA content in the HP03 leaf was significantly higher ($p<0.05$) in very old leaves (4th leaf). Generally, the accumulation of GABA in tea leaves cultivated at low altitudes was relatively higher than high altitude tea leaves.

GABA can be found in different parts of plants such

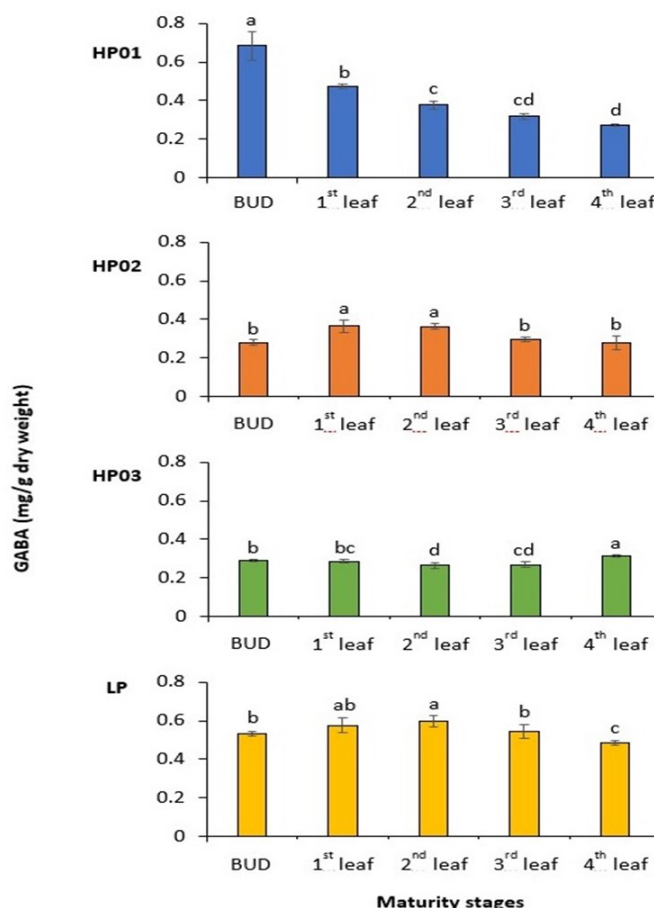


Figure 3. GABA content in fresh tea leaves from different maturity stages and altitudes. Error bars indicate the standard deviation of the mean. Bars with different notations are statistically significantly different ($p<0.05$) within the leaf's maturity.

as the leaves (Wang *et al.*, 2006), fruits (Zhou *et al.*, 2016), flowers (Palanivelu *et al.*, 2003) and roots (Ruperti *et al.*, 2019). It was reported that GABA controls plant growth development and is concentration-dependent. One of the studies showed that low concentration of GABA (up to 10 mM) promotes pollen tube elongation while high concentration inhibits the elongation process (Palanivelu *et al.*, 2003). The finding in this study indicated that the GABA level in tea cultivated in lowland was higher than in highland tea. Higher GABA levels in lowland tea could be partially due to the higher light intensity and temperature that

stimulated GABA synthesis in tea plants as an adaptation to abiotic stress (Balfagón *et al.*, 2021). This is the first study to report on the changes of GABA content in tea leaves from different maturity stages and cultivated at low and high altitudes in Malaysia.

4. Conclusion

This study presents pioneer information on the bioactive metabolites distribution at the different maturity stages of tea leaves, collected from both high- and lowland tea plantations in Malaysia. The findings demonstrated that the levels of catechins, caffeine, GABA and total phenolic content in tea leaves were varied and influenced by the growth development stages and altitude of the growing area. This study provides valuable information for understanding the distribution of bioactive metabolites and also selection of tea leaves for the production of tea with various taste qualities and health benefits, particularly in Malaysia which has both low- and highland tea plantations.

Conflict of interest

The authors declare that they have no conflicts of interest.

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