ECRONICON OPEN ACCESS

EC NUTRITION
Research Article

# Flavanols and Flavonols Content of *Camellia sinensis* with Different Maturity Stage Planted at Cameron Highland and Sabah Tea Plantation in Malaysia

**Nor Qhairul Izzreen MN[1]*, Sridaran A[1], Kueh TTP[1], Mohd Fadzelly AB[2], Amir H[1], Umi Hartina MR[1] and Rabiatul Amirah R[1]**

[1]*Faculty of Food Science and Nutrition, University Malaysia Sabah, Jalan UMS, Kota Kinabalu, Sabah, Malaysia*

[2]*Food Technology Panel, Department of Technology and Heritage, Faculty of Science, Technology and Human Development, Universiti Tun Hussein Onn Malaysia (UTHM), Batu Pahat Malaysia*

***Corresponding Author**: Nor Qhairul Izzreen MN, Faculty of Food Science and Nutrition, University Malaysia Sabah, Jalan UMS, Kota Kinabalu, Sabah, Malaysia.

**Received:** September 21, 2018; **Published:** December 28, 2018

## Abstract

This study was done to determine and compare the nutritional composition of ash, crude protein, crude fat and fiber, as well as the content of flavanols (EGCG, EGC, ECG and EC) and flavonols (quercetin and kaempferol) in tea planted in Cameron Highland (CH) and Sabah tea Plantation (ST) with different maturity stages (young, matured and old leaves). Young tea leaves in both CH and ST had the highest content of crude fat, protein and fiber and the values decreased as the leaves aged. Interestingly, crude protein content in all maturity stages of CH and ST were higher than in commercial tea (13.81%) with the range of 15.41 - 16.35% and 14.20 - 15.32% respectively. Meanwhile, ash content in ST (8.59 - 13.49%) was higher compared to CH (5.06 - 5.14%) and values decreased from young to old leaves. CH leaves had the highest moisture content (8.18 - 8.55%) followed by ST (5.23 - 9.20%), and commercial tea (5.7%). The order of flavanol in young leaves of CH and ST, and commercial tea leaves was ECG > EGCG > EGC > EC but for mature and old leaves the order was ECG > EGC > EGCG > EC with the only difference was in EGC and EGCG. The content of flavonol quercetin and kaempferol in CH were 3.51 mg/g and 4.05 mg/g respectively. Meanwhile, in ST leaves the values were 1.79 mg/g and 3.35 mg/g respectively, and both CH and ST showed that the highest content of flavonol was observed in young leaves and decreased as leaves aged.

***Keywords:*** *Flavanol; Flavonol; Camellia sinensis; Cameron Highland; Sabah Tea*

## Introduction

Tea is one of the most popular and widely consumed beverages worldwide due to its health benefit for human consumption. Tea is majorly classified into green and black tea. Green tea is a non-fermented tea used as the main beverage in China and Japan, while black tea is more popular in North America and Europe. Amongst the benefits of tea is that it can prevent tumour cells growth, reduce cardiovascular disease, reduce cholesterol and induce body weight loss. Those health benefit are mostly contributed by the presence of polyphenols that enable it to scavenge free radicals especially those classified as flavanol and flavonol. Tea flavanols are potent antioxidants and make up 2-3% of the water-soluble solids from tea leaves. Tea flavanols such as (+)- catechin, (-)- epicatechin (EC), (-)- epigallocatechin (EGC), (-)- picatechin3-gallate (ECG) and (-)- epigallocatechin-3-gallate (EGCG) were reported to be abundant in fresh leaves and green tea as fermentation process would deteriorates flavanols which was in contrast with flavonols such as quercetin and kaempferol that are more stable [1]. The contents of flavanol and flavonol however, varies according to the differences in varieties, geographic locations and agricultural practices [2].

Taste and aroma are the main factors impacting consumers' preferences with regard to tea of their choice; on the other hand consumers less frequently pay attention to the chemical composition and nutritional value of tea. Malaysia has only two important plantation sites; Cameron Highland and Sabah Tea, and both are considered as highland plantation. In Malaysia, black tea are most popular, however, the consumption of green teas are increasing due to their fine taste and beneficial health effects.

Young leaves are discovered to have higher antioxidant activity than mature leaves. According to Gadow., *et al*. [3], Chan., *et al.* [4] and Pilar., *et al*. [5], green tea was found to contain higher antioxidant activity than black tea, as they determined by total phenolic content, DPPH (2,2-diphenyl-1- picrylhydrazyl) free radical scavenging activity, ferric reducing antioxidant power (FRAP) and ABTS [2,2'-azino-bis-(3-ethylbenzothiazoneline-6-sulfonic acid)] decolorization assays. Also, Nor and Fadzelly [6] found that shoot tea leaves had relatively higher content of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity compared to young and mature leaves. This was in line with the values of tea as white teas, obtained from young buds and undeveloped leaves, are the most exclusive, refined and expensive group of products as reported by Czernicka., *et al* [7]. Interestingly, according to Farhoosh., *et al*. [8] the old tea leaves still contain a significant antioxidant activity.

## Purpose of the Study

The purpose of the study was to examine and compare the chemical compositions, flavanol and flavonol content of tea leaves planted in Cameron highland and Sabah tea. The influence of leaf age is of importance as current commercial practices in Malaysia is to discard the mature leaves or to turn them into agricultural compost. Also, tea compositions vary depending on the geographic locations and this is our interest to compare between the two plantation sites.

## Experimental

### Sample

Tea leaves with three different maturity stages (young, mature and old) were plucked from two different plantation site: Cameron Highland Plantation and Sabah Tea Plantation. Fresh shoots (leaf bud and two youngest leaves; yellowish green), young leaves (third to fifth leaves from the top; light green) and mature leaves (sixth to eighth leaves; dark green) [6].

### Chemicals

(+)- catechin hydrate, (-)- epicatechin (EC), (-)- epicatechin gallate (ECG), (-)- epigallocatechin (EGC), (-)- epigallocatechin gallate (EGCG), quercetin, and kaempferol were purchased from Sigma Chemical Co. Acetonitrile, methanol and ethanol were of HPLC quality and purchased from Fisher Scientific (Essex, UK). HCl and $KH_2PO_4$ were of analytical grade, and also purchased from Fisher Scientific (Essex, UK). The water used in HPLC and sampling was prepared with a Super Purity Water System (Purite Ltd, England) with a resistivity over 17.5 MΩ cm.

### Sample preparation

**Green tea:** Freshly picked leaves were steam blanched for 10 minutes to deactivate enzymes in the leaves. Then, the leaves were dried and ground into smaller particles [9].

### Nutritional compositions

Dry matter (DM), ash, crude protein (CP), crude fat (CF) and fiber content of dried leaves were determined according to the standard AOAC methods [10]. The nitrogen content was estimated using micro-Kjeldhal techniques and the protein content was calculated as N x 6.25.

### Sample extraction

### Flavanol

Sample extraction were prepared according to method by Sharma., *et al.* [11] with some modifications. In brief, grinded samples of 0.5g of dried tea leaves were extracted with 5 ml of 70% aqueous methanol for 10 minutes on an orbital shaker. The mixture was centrifuged at 8500g for 10 minutes. The pellets were re-extracted with the same conditions and the supernatants was combined, filtered through a 0.45 µm membrane and stored at -18°C prior to the injection for HPLC analysis.

### Flavonols

Sample extraction were prepared according to method by Huafu Wang and Helliwell [2] with some modifications. In brief, 1g of dried tea leaves were extracted with 40 ml of 70% aqueous methanol and 6M HCl. The mixture was heated in reflux at 95°C for 2 hours. The hydrolyzed solution was filtered and volume was made up to 50 mL with 70% aqueous methanol. 1 ml of diluted solution was allowed to cool under running water and filtered through a 0.45 µm membrane and stored at -18°C prior to the injection for HPLC analysis.

### HPLC conditions

The analysis of flavanol and flavonol in tea was carried out by the following HPLC method. A HP 1100 series liquid chromatograph system, comprising vacuum degasser, quaternary pump, auto-sampler, thermostated column compartment, and diode array detector, was used. For the determination of flavanoids, HPLC analysis was performed using Waters HPLC (water Breeze System) with 250 mm x 4.6 mm SymmetryShield® RP18 column at 25°C. Mobile phase consisted of 30% acetonitrile in 0.025 M $KH_2PO_4$ buffer solution (v/v); the pH of the mobile phase was adjusted with 6M HCl to 2.5. The flow rate was 1.0 ml/min and the column was operated at 30°C. The sample injection volume was 20 µl. UV spectra were recorded from 200 to 400 nm, and peak areas were measured at 370 nm using Waters 2487 Dual λ Absorbance Detector. For determination of flavonol, the same HPLC system was used except the column was a C18 reversed phase 3 µm C18 (150 × 4.6 mm) (SupelcosilTM LC-DABS UK). The UV spectra obtained for each peak, after subtraction of the corresponding UV base spectrum, were computer normalized and the plots were superimposed.

### Identification and quantification

Chromatogram peaks were considered to be chromatographically pure when there was exact coincidence to their corresponding UV spectra. Chromatographic peaks in the samples were identified by comparing their retention time and UV spectra with those of the reference standards. Working standard solutions (5 - 80 µl) were injected into the HPLC, and peak area responses were obtained. A standard graph for each component was prepared by plotting concentration versus area. Quantification was carried out from integrated peak areas of the sample against the corresponding standard graph. Each sample was analyzed in triplicate.

### Statistical analyses

All experiments were carried out in triplicate and presented as mean ± standard deviation of mean (SD) using SPSS version 15.0. The data were statistically analysed by one-way ANOVA. When ANOVA showed significant differences, post- hoc tukey-test was computed (95% significance level) to describe the effects of the parameter tested.

## Results and Discussions

### Nutritional composition of tea leaves

The nutrient composition of a different maturity stage of tea leaves planted at Cameron Highland (CH) and Sabah tea (ST) plantation are presented in table 1. Similar pattern of fat content in tea leaves planted in CH and ST was observed as the young shoot in both tea leaves had the highest fat content and the values decreased as the leaves matured. Tea leaves planted in Cameron highland however, showed a relatively higher fat content (6.83 - 7.55%) compared to ST leaves (2.34 - 5.40%). Fat content in commercial tea leaves (5.60%) was similar with young ST as fat content in both leaves were not differed significantly. In contrast, protein content increased in CH and ST from young to old tea leaves with the range of 15.41 - 16.35% and 14.20 - 15.32% respectively, and the content of crude protein in commercial tea was much lower (13.81%). High percentage of protein and fat contents in CH and ST may be due to no fermentation during processing and short processing preparation compared to commercial leaves as it must undergone several processing stages. Also, protein built up in older leaves as the leaves mature and might be due to the growth and structural development if the leaves itself.

| Tea leaves | Young | | Mature | | Old | | Commercial |
|---|---|---|---|---|---|---|---|
| Content (%) | CH | ST | CH | ST | CH | ST | |
| Crude fat | 7.55 ± 0.41$^{a}$ | 5.40 ± 0.89$^{c}$ | 7.14 ± 0.07ab | 5.23 ± 0.06$^{c}$ | 6.83 ± 0.03$^{b}$ | 2.34 ± 0.05$^{d}$ | 5.60 ± 0.04$^{c}$ |
| Crude Protein | 15.41 ± 0.41$^{b}$ | 14.20 ± 0.02$^{b}$ | 16.02 ± 0.12$^{a}$ | 14.95 ± 0.12$^{b}$ | 16.35 ± 0.08$^{a}$ | 15.32 ± 0.07$^{b}$ | 13.81 ± 0.08 |
| Crude fibre | 27.71 ± 0.28$^{b}$ | 22.81 ± 1.80$^{cd}$ | 28.13 ± 0.12$^{ab}$ | 24.74 ± 0.91$^{c}$ | 28.89 ± 0.35$^{a}$ | 24.80 ± 2.18$^{c}$ | 29.36 ± 0.41$^{a}$ |
| Ash | 5.06 ± 0.11$^{d}$ | 13.49 ± 0.06$^{a}$ | 5.14 ± 0.07$^{d}$ | 8.59 ± 0.34$^{b}$ | 5.09 ± 0.19$^{d}$ | 8.82 ± 0.55$^{b}$ | 6.74 ± 0.25$^{c}$ |
| Moisture | 8.18 ± 0.03$^{b}$ | 9.20 ± 0.27$^{e}$ | 8.55 ± 0.08$^{a}$ | 7.51 ± 0.76$^{c}$ | 8.23 ± 0.19$^{ab}$ | 5.23 ± 0.65$^{d}$ | 5.70 ± 0.18$^{d}$ |

***Table 1:*** *Proximate analysis of different maturity stage of tea leaves planted in cameron highland and sabah tea plantation.*

**CH: Cameron Highland; **ST: Sabah Tea*

*Results are expressed as the mean of three replicated measurements and standard error of the mean ( ± SEM).*

*All measurements were performed in duplicate and error margins indicate standard deviations. Values with the different letters within the same row are significantly different ($p < 0.05$).*

Fiber content is an important quality parameter. High fiber content was observed in young tea leaves of CH and the percentage increased from young to old leaves. Similar pattern was observed with ST but in general CH possessed relatively higher percentage than ST. However, commercial tea had the highest percentage of fiber among all tested samples (29.36). These results were expected as old tea leaf contains more fiber than young leaf due to the differences of structural rigidity such as cellulose that might determine the content of fiber in the leaf. Therefore, young leaves are softer and more susceptible to damage from post-harvest handling. Also, crushing, tearing and curling process might destroy the leaf structure that might have effect on fiber content. High fiber content in commercial tea samples may be due to the use of impurities like stems during processing. In addition, the processing of commercial tea might include mature tea leaves which are usually between 4 to 6th leaves, thus lower the quality of the tea. Previous researchers also indicated positive association between fiber content and keeping quality of the tea and proposed fiber content of less than 16.5% in order to maintain high quality of tea during storage [12,13].

Meanwhile, ash content in ST (8.59 - 13.49%) was higher compared to CH (5.06 - 5.14%) and the values decreased from young to old tea leaves. In CH, no significant differences was observed on the values of ash in all tea leaves. The ash content in commercial tea was slightly higher than CH but much lower when compared to ST. Ash content of tea is also an important quality parameter. According to Adnan., *et al.* [14] ash content is inversely proportion to moisture content. However, this was not applied to this study as there was no pattern between ash and moisture content of tea leaves. Less ash content in commercial tea might be due to adulteration using extracted raw material for the production of tea which lead to the inferior quality of tea.

Moisture content in commercial tea was the lowest (5.7%) followed by ST (5.23 - 9.20%), and CH has the highest moisture content (8.18 - 8.55%). Adnan., *et al.* [14] suggested that moisture percentage should be controlled between 2.5 - 6.5% for better quality of the product and this was in line with the values of moisture in commercial tea and old tea leaves of ST. The rest of the leaves had higher percentage than the suggested. One of the reasons of high moisture content in tea is the use of packaging material to maintain a constant moisture level during storage as moisture might be absorbed by the leaves. High moisture percentage up to 8% could have negative effect on shelf life of the product.

## Content of flavanol in tea leaves

Flavanol content in tea leaves planted in Cameron highland and Sabah tea was shown in table 2. The content of EGC was the highest in the old leaves (37.37 mg/g) followed by mature (36.61 mg/g) and young leaves (36.05 mg/g). The same trend was observed for Sabah tea leaves as EGC content increased with increasing maturity. Interestingly, EGC content in ST was double the amount of CH where in the highest was 72.60 mg/g in old tea leaves and the lowest was 70.27 mg/g in young leaves. Unlike other catechins, EGC was the only catechin that was the highest in old leaves. This shows an indication that EGC is stable and might accumulates throughout the leaves' age and said to be one of the major catechins in tea leaves [15,16]. Commercial leaves had the lowest EGC amongst all leaves with the value of 34.61 mg/g which may be due to through tea processing such as firing at high temperature that lead to substantial degradation of EGC [17].

| Tea leaves | EGC | | EC | | EGCG | | ECG | |
|---|---|---|---|---|---|---|---|---|
| | *CH | **ST | CH | ST | CH | ST | CH | ST |
| Young | $36.05 \pm 0.15^{b}$ | $70.27 \pm 0.10^{a}$ | $16.13 \pm 0.23^{c}$ | $12.09 \pm 0.28^{a}$ | $38.58 \pm 0.42^{c}$ | $28.32 \pm 0.41^{b}$ | $55.06 \pm 0.66^{d}$ | $60.53 \pm 0.41^{a}$ |
| Mature | $36.61 \pm 0.05^{bc}$ | $70.64 \pm 0.57^{a}$ | $10.26 \pm 0.23^{a}$ | $12.03 \pm 0.42^{a}$ | $29.52 \pm 0.20^{b}$ | $29.49 \pm 0.21^{b}$ | $41.08 \pm 0.11^{b}$ | $59.54 \pm 0.15^{a}$ |
| Old | $37.37 \pm 0.02^{c}$ | $72.60 \pm 0.07^{b}$ | $12.86 \pm 0.30^{b}$ | $14.14 \pm 0.26^{b}$ | $26.23 \pm 0.26^{a}$ | $35.20 \pm 0.78^{a}$ | $38.65 \pm 0.21^{a}$ | $58.85 \pm 0.15^{a}$ |
| Commercial | $34.61 \pm 0.37^{a}$ | | $17.34 \pm 0.30^{d}$ | | $38.18 \pm 0.18^{c}$ | | $52.26 \pm 0.18^{c}$ | |

***Table 2:*** *Flavanol (mg / g) dry basis content in cameron highland and sabah tea plantation.*

**CH: Cameron Highland; **ST: Sabah Tea.*

*Results are expressed as the mean of three replicated measurements and standard error of the mean ( ± SEM).*

*All measurements were performed in duplicate and error margins indicate standard deviations. Values with the different letters within the same column of the same flavanol are significantly different ($p < 0.05$).*

The levels of EC was higher in CH than in ST (Table 2). No clear pattern was observed in both tea leaves. Young CH tea leaves had the highest EC content 16.13 mg/g and the lowest was in the mature leaves 10.26 mg/g. in contrast with ST leaves, the old leaves had the highest EC content with 14.14 mg/g and the lowest was the same with CH which was in mature leaves 12.03 mg/g. Commercial leaves had the highest EC content amongst others with 17.34 mg/g. EC was the least flavanol in young and mature because ECG was degraded from

young to old leaves and EC would re- occur when the leaf becomes old as seen in the trend. However, some of the uneven results might be due to the interactions between sunlight exposure and the activity of catechin synthesis enzymes [18,19].

Young tea leaves planted in CH had the highest amount of EGCG with 38.58 mg/g and the amount decreased as the leaves matured and old. Contrary with ST, the EGCG content was the highest in the old leaves and the content decreased in mature and followed by young leaves. EGCG is the main polyphenol in tea and theoretically, EGCG content shall be higher in young leaves compared to old leaves as young leaves contain more catechin- synthesis enzymes like phenylalanine ammonia lyase (PAL) and the activity of PAL decrease as the leaves aged. ECGC is affected by the activity of PAL therefore, when the activity of PAL decrease, EGCG will decrease which was in line with CH leaves. Also, EGCG is the most vulnerable to degradation compared to other flavanols [20] and this might be the reason for the results of ST leaves.

The young CH leaves contained the most ECG at 55.06 mg/g while mature 31.08 mg/g and old 38.65 mg/g and they were all significantly differed with each other. Meanwhile young ST leaves contained the highest ECG 60.53 mg/g followed by mature 59.54 mg/g and old 58.85 mg/g and showed no significant different with each types of leaves maturity. ST showed higher ECG content compared to CH and the content of ECG in commercial leaves was 52.26 mg/g which was lower than young CH leaves and ST leaves at all maturity stages. This might be due to the processing practices such as drying and firing of the leaves which could decrease the amount of ECG in the leaves. The trend of ECG and EGCG planted in CH was similar but not ST in which ECG and EGCG was inversed with each other. Yao., *et al*. [20] observed similar pattern of ECG and EGCG as shown by the CH leaves and suggested that both are synthesized from the same metabolic pathways. ECG is among the main flavanols in tea and this was in line with the results in this work.

### Content of flavonol in tea leaves

Based on table 3, it can be seen that quercetin level decreased with leaf aged. Quercetin level was the highest for young leaves in ST compared to CH meanwhile, commercial leaves were slightly lower than young leaves of ST and CH perhaps due to manufacturing reasons. This shows that both young leaves of CH and ST are better in providing high quercetin content compared to commercial tea leaves. Also, ST and CH mature leaves had the third highest level of quercetin and old leaves had the least quercetin. Generally, the content of quercetin was higher in young, mature and old leaves of ST compared to all levels of maturity of CH leaves.

| Tea Leaves | Quercetin | | Kaempferol | |
|---|---|---|---|---|
| | *CH | **ST | CH | ST |
| Young | $3.51 \pm 0.06^{b}$ | $4.05 \pm 0.02^{a}$ | $1.79 \pm 0.05^{b}$ | $3.35 \pm 0.03^{a}$ |
| Mature | $2.50 \pm 0.22^{c}$ | $3.75 \pm 0.04^{b}$ | $1.17 \pm 0.07^{b}$ | $3.31 \pm 0.04^{a}$ |
| Old | $1.39 \pm 0.05^{d}$ | $1.75 \pm 0.03^{d}$ | $0.33 \pm 0.02^{c}$ | $0.89 \pm 0.03^{c}$ |
| Commercial | $2.97 \pm 0.00^{c}$ | | $1.28 \pm 0.04^{b}$ | |

***Table 3:*** *Content of quercetin and kaempferol in cameron highland and sabah tea plantation.*

**CH: Cameron Highland; **ST: Sabah Tea.*

*Results are expressed as the mean of three replicated measurements and standard error of the mean ( ± SEM).*

*All measurements were performed in duplicate and error margins indicate standard deviations. Values with the different letters within the same column of the same flavonol are significantly different ($p < 0.05$).*

Similarly, the same trend was observed for kaempferol as it was observed that young leaves of ST had the highest content of kaempferol compared to CH and the content in both ST and CH leaves decreased as the leaves matured. Young leaves of CH and ST had higher content of kaempferol compared to commercial leaves and no significant differences were found between the content of kaempferol in commercial leaves and mature leaves This may indicate that tea processing could drastically reduce kaempferol content of the leaves. According to Bhagwat and Beecher [21], quercetin is the dominant flavonol in tea which was in line with this work as the concentration of quercetin is much higher than kaempferol in all samples.

## Conclusion

Nutritional compositions of CH and ST were basically in the same range except for ash. The content of ash in young leaves of ST was clearly higher compared to CH. As ash is closely associated with minerals, it could be expected that minerals content in young leaves of ST might be higher than CH. The content of crude fibre and crude protein were the most dominant in CH and ST and similarly with the

content of those in commercial leaves, compared to other nutritional compositions. Also, the leaves of ST was superior compared to CH as it contained higher levels of most of the flavanols such as ECG and EGCG as well as quercetin and kaempferol. Even commercial leaves had lower contents of most flavanols compared to the young leaves of both CH and ST. The level of most of the flavanols and flavonols decreased as leaves ages which was expected as most of them could be degraded by prolonged exposure to sunlight, associated with leaf physiological changes and metabolic activity. As both CH and ST are highland plantation, not much differences in terms of nutritional compositions, flavanols and flavonols content that could be associated with the geographical location but might be due to the differences in post-harvest and processing handling, which is not in our scope of study.

## Acknowledgement

The financial support of e-Science fund Research Scheme from Ministry of Science, Technology and Innovation of Malaysia (MOSTI) entitled 'Antioxidant and Anticancer Properties of Tea Waste' is gratefully acknowledged. Special thanks to Sabah Tea Plantation and Cameron Highland Plantation for the leaves supplied. The authors also extended their thanks to School of Food Science and Nutrition; Institute for Tropical Biology and Conservation and Biotechnology Research Institute of Universiti Malaysia Sabah, for the use of laboratory facilities and technical assistance.

## Bibliography

1. Hilal Y and Engelhardt U. "Characterisation of white tea - Comparison to green and black tea". *Journal Fur Verbraucherschutz Und Lebensmittelsicherheit* 2.4 (2007): 414-421.

2. Wang H and Helliwell K. "Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography". *Food Research International* 34.2-3 (2001): 223-227.

3. Gadow VA., *et al*. "Comparison of the antioxidant activity of rooibos tea (Aspalathus linearis) with green, oolong and black tea". *Food Chemistry* 60.1 (1997): 73-77.

4. Chan EWC., *et al*. "Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia". *Food Chemistry* 102.4 (2007): 1214-1222.

5. Pilar AM., *et al*. "Antioxidant and antimicrobial activities of tea infusions". *Food Chemistry* 108.1 (2008): 55-63.

6. Nor Qhairul Izzreen MN and Mohd Fadzelly AB. "Phytochemicals and antioxidant properties of different parts of Camellia sinensis leaves from Sabah Tea plantation in Sabah, Malaysia". *International Food Research Journal* 20.1 (2013): 307-312.

7. Czernicka M., *et al*. "Study of Nutritional Value of Dried Tea Leaves and Infusions of Black, Green and White Teas From Chinese Plantations". *Roczniki Państwowego Zakładu Higieny* 68.3 (2017): 237-245.

8. Farhoosh R., *et al*. "Antioxidant activity of various extracts of old tea leaves and black tea wastes (Camellia sinensis L.)". *Food Chemistry* 100.1 (2007): 231-236.

9. Lena MG., *et al*. "Examination of antioxidant activity of Ginkgo biloba leaf infusions". *Food Chemistry* 82.2 (2003): 275-282.

10. AOAC. "Official methods of Analysis (17th edition)". Washington DC. Association of Official Analytical Chemist (2000).

11. Sharma V., *et al*. "Extractability of tea catechins as a function of manufacture procedure and temperature of infusion". *Food Chemistry* 93.1 (2005): 141-148.

12. Venkatesan S., *et al*. "Quality standards of CTC black teas as influenced by sources of potassium fertilizer". *Journal of the Science of Food and Agriculture* 86.5 (2006): 799-803.

13. Smiechowska M and P Dmowski. "Crude fibre as a parameter in the quality evaluation of tea". *Food Chemistry* 94.3 (2006): 366-368.

14. Adnan M., *et al*. "Chemical Composition and Sensory Evaluation of Tea (Camellia Sinensis) Commercialized in Pakistan". *Pakistan Journal of Botany* 45.3 (2013): 901-907.

15. Fernández PL., *et al*. "HPLC determination of catechins and caffeine in tea. Differentiation of green, black and instant teas". *Analyst* 125.3 (2000): 421-425.

16. Shahidi F and Naczk M. "Phenolics in food and nutraceuticals". New York (2006).

17. Obanda M., *et al*. "Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature". *Food Chemistry* 75.4 (2011): 395-404.

18. Eungwanichayapant PD and Popluechai S. "Accumulation of catechins in tea in relation to accumulation of mRNA from genes involved in catechin biosynthesis". *Plant Physiology and Biochemistry* 47.2 (2009): 94-97.

19. Xq Z., *et al*. "Effect of ultraviolet B irradiation on accumulation of catechins in tea (Camellia sinensis (L) O. Kuntze)". *Journal of Biotechnology* 7.18 (2008): 3283-3287.

20. Yao L., *et al*. "HPLC analyses of flavanols and phenolic acids in the fresh young shoots of tea (Camellia sinensis) grown in Australia". *Food Chemistry* 84.2 (2004): 253-263.

21. Bhagwat S and Beecher G. "Flavonoid composition of tea: Comparison of black and green teas". *Agricultural Research* 15 (2003): 20705.

**Volume 14 Issue 1 January 2019**
**©All rights reserved by Nor Qhairul Izzreen MN., *et al.***