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FULL PAPER

Quality assurance of tea leaf and its products using selected major chemical markers

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Abstract

Camellia sinensis or locally called the tea plant is one of the important food crops in Malaysia besides other popular crops such as coffee and cocoa. Since the earliest generations, tea has become one of the most popular beverages consumed globally. The tea drinks popularity has been inherited by current generations together with traditional knowledge of its benefits to human health. Recently, many types of tea products available in local markets. In order to achieve the expected quality, the quality assurance of the products must be in place. In this study, analysis method for detection and determination of the selected major chemical markers which include catechin, epicatechin, caffeine, theanine and theaflavin has been developed via High-Performance Liquid Chromatography (HPLC). Evaluation of the tea products in the local market showed variability in the level of chemical markers content. The content of catechin, epicatechin, caffeine, theaflavin and theanine varies from 2.7-14.9, 2.7 - 30.3, 3.2 - 43.6, 0.28 -15.5, 29.1-57.7 mg/g, respectively. Quality assurance monitoring based on chemical markers is crucial in order to produce high-quality tea-based products. The main advantage of this developed method is all the targeted constituents could be detected within a single running experiment. These could speed up analysis work and increase productivity. Consequently, the scientific study data was also found to agree with the traditional claims. The previous study demonstrated that tea leaves are rich with antioxidant properties especially catechin which is reported to possess the powerful antioxidant capability, besides epicatechin, caffeine, theanine and theaflavin.

1. Introduction

Tea or the scientific name, *Camelia sinensis* is a popular food crop in Malaysia, other than cocoa and coffee. Statistically, the arable land for tea plantations in Malaysia is around 2000 hectors. In Peninsula Malaysia, especially at Cameron Highland and Selangor contribute to 1254 hectors of tea plantation followed by Sabah, which contributed 365 hectors. These areas produce 6621 tons of tea products for Malaysia (Department of Agriculture, Malaysia, 2019). Tea is sold in the form of loose powder or sachets in the market. Many brands and types of tea are in the market such as black tea, green tea and flavoured tea. In order to evaluate Standard Operating Procedures in the processing, a technique for quality assessment of tea products shall be developed. Therefore, an analytical method was developed using High-performance Liquid Chromatography.

For generations, tea has been one of the most popular daily drinks around the world. This development has been driven by the community's traditional awareness of its benefits to human health as well as its delicious taste. Since the majority of society in the past was involved in heavy work, especially agriculture, tea is believed to be able to cool down body temperature (Chen, 2013). Apart from that, tea is also traditionally believed to relieve fatigue and refresh the body. Now, advances in medical and health sciences have confirmed the nutritional value of tea drinks. Based on research, tea drinks have various benefits in improving human health. Some studies demonstrated the main nutrients in tea products promoting good bioactivities such as antioxidant, anti-inflammatory, anti-carcinogenic, anti-microbial and anti-thyroxine (Reygaert, 2017; Thitimuta *et al.*, 2017).

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The main chemical compounds of tea such as catechins, caffeine, theaflavins and amino acids, were known to promote better antioxidant activities and contributed to the taste of tea drinks. Good quality tea needs a balance of bitterness, astringency and sweetness, as well as a refreshing aftertaste. The major nutrients that bring the taste of the tea are catechins, amino acids and caffeine (Chaturvedula and Prakash, 2011). The most important is the balancing between catechins and amino acids which involved bitterness and umami tastes. Umami taste is often described as the taste of "meaty" (Shallenberger, 1993) or "brothy" savoury deliciousness that deepens the flavour. Catechin and epicatechin are a family of polyphenols that are known to promote antioxidant activities (Tong *et al.*, 2019). Epicatechin is known to have a bitter taste, but less astringency taste. Amino acid controls the balance of bitterness and sweetness. Twenty different kinds of amino acids included in green tea contributed to the taste of tea. However, 50% of total amino acids consist of theanine as a major amino acid (Balentine, 1997; Kaneko, 2006). Caffeine is a known major natural alkaloid in tea leaves, contributing to the bitterness of tea drinks (Zhang *et al.*, 2020). This constituent is released in high-temperature water and the hotter temperature of water more caffeine will be released; thus, the bitterness increases. Theaflavins were reported to have inhibition of tumour proteasome activity which contribute to tumour growth-inhibitory effects (Mujtaba and Dou, 2012). Due to the major existence in the tea leaves and the bioactivities the constituents reported, five major constituents were adopted as a chemical marker in this experiment. The constituents are catechin, epicatechin, caffeine, theaflavin and theanine.

2. Materials and methods

2.1 General

Methanol was purchased from BDH Chemicals, water was obtained from Milli-Q Ultrapure, with a conductivity of 18.2 MΩ.cm, formic acid and acetonitrile were purchased from Merck. Chemical standards used in the study such as catechin, epicatechin, caffeine, theaflavine and theanine were purchased from Sigma-Aldrich. HPLC Agilent 1200 was used for analysis completed with a quaternary pump, column oven and photodiode array detector (PDA).

2.2 Preparation and extraction of samples

A total of seven samples of commercial tea products were purchased from local markets and labelled as BH, DL, AH-G, AH-E, CM-T, CM-T and LT. The two samples from MARDI's tea plantation plot were labelled as Dried Tea MARDI and Dust Tea MARDI.

Sample of dried tea leaves or tea products were ground to small sizes using a grinder with a 2 mm particle filter to enhance extraction efficiency. The samples were purchased from a local market or collected from MARDI's Tea plotted area at MARDI Cameron Highland, Pahang. Approximately 1 g of sample was macerated with 7 mL of boiled water for 10 mins followed by 3 mL of methanol and sonicated for 10 mins (Rana *et al.*, 2015). Sonicated samples were filtered with a nylon syringe filter of 0.45 µm.

2.3 Preparation of standards solution

The standards of catechin, epicatechin, caffeine, theaflavin, and theanine were purchased from Sigma-Aldrich. The standards were weighed accurately at 5 mg into a 10 mL volumetric flask. The stock was diluted to a series of working standard solutions such as 1000 ppm, 500 ppm, 250 ppm, 125 ppm, 50 ppm and 0.5 ppm.

2.4 HPLC condition

In this analysis, HPLC was used column Kinetex 5µ C18, with dimension 250 mm length × 4.6 mm ID. The flow rate used was 1 mL/min and the sample injection volume was 10 µL. The mobile phase used was a mixture of solvent A and the other was solvent B. Mixture of solvent A was a mixture of distilled water from Milli-Q Ultrapure (18.2MΩm) and methanol (BDH Chemicals) with a ratio of 70:30 with 0.1% formic acid (Merck). Solvent B was acetonitrile purchased from Merck with 0.1% Formic acid. The method used the solvent gradient system based on the time program as follows: The system starts with 100% solvent A and changes to 80% solvent A at the 10^{th} minute gradually. At the 15^{th} minute to the 20^{th} minute, 20% solvent A and finally 21^{st} minute to 26^{th}-minute system back to 100% solvent A. Detection system was set at 210nm wavelength to detect catechin, epicatechin, caffeine, theaflavin and theanine (Theppakorn *et al*., 2014).

2.5 Quantification of major chemical markers

In general, the amount of chemical marker detected is based on a comparison of the area of chemical standard signal with an area of analyte signal from the sample. Determination of chemical marker content in samples was carried out by using a plotted calibration curve. A typical example of a calibration curve for a chemical marker is shown in Figure 1. Each standard of the chemical marker was injected at five different concentrations and analysed using HPLC. Area of peak signal was recorded and a graph of the area against the concentration of chemical standards was plotted. The calculation was made by comparing the area of peak signal from samples with an area of peak signal from chemical markers standards.

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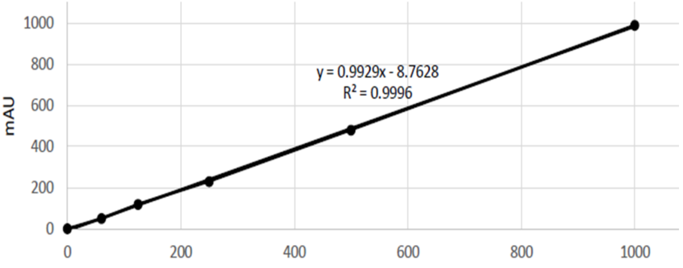

Figure 1. Typical calibration curve to analyse caffeine

3. Results and discussion

The content of major constituents in tea products contributes to the final taste, aroma and most important is the health benefits from a cup of tea. Therefore, the profile of chemical markers may reflect the quality of tea products. Based on the data, five selected chemical markers were analysed simultaneously at retention time between 5.097 mins to 11.417 mins using 210 nm detection wavelength as shown in Figure 2. The chemical markers were significantly detected at retention times 5.097, 5.687, 6.175, 6.868 and 11.417 mins for theanine, catechin, epi-catechin, caffeine and theaflavin, respectively. The chromatogram in Figure 2 showed theanine was the earliest (5.097 mins) analyte that came out from the column, indicating that it is the most polar analyte among all five chemical markers. This is in agreement with a previous study by Peng *et al.* (2008), the shorter time to elute out from the column, the higher polarity of the chemical marker would be.

Nine tea samples were collected from the local market and the MARDI tea plantation. Figure 3 shows 9 samples that were analysed included 2 samples from MARDI's tea plotted area at row 5^{th} and 10^{th} which were Dried Tea MARDI and Dust Tea MARDI. The rest of the samples were commercial tea from the local market.

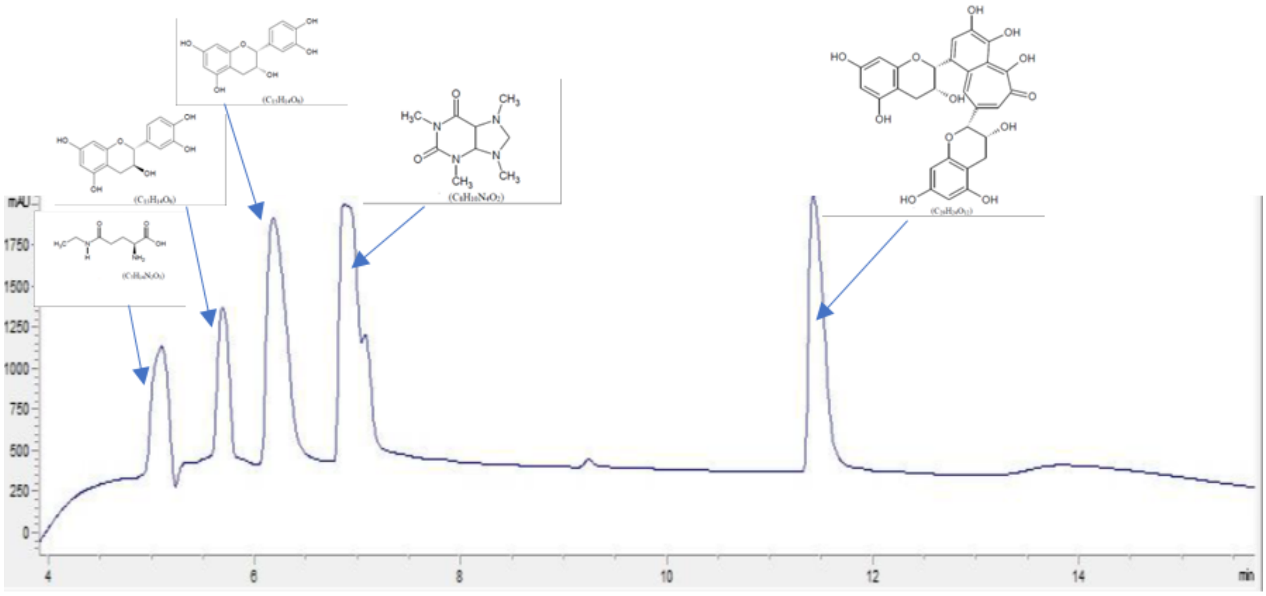

Figure 2. Chromatogram of analysis selected chemical markers theanine, catechin, epicatechin, caffeine and theaflavin were detected at 210 nm wavelength

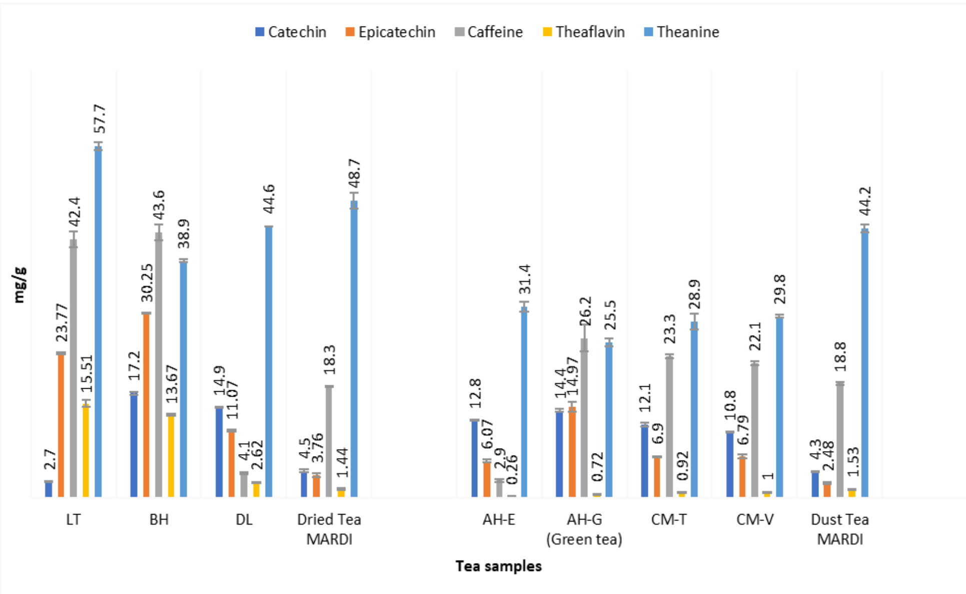

Figure 3. Analysis of catechin, epicatechin, caffeine, theaflavin and theanine from commercial local tea and cultivated tea MARDI Cameron Highland

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In terms of theanine content, the study showed the highest level of theanine in commercial samples LT, Dried Tea MARDI, DL, Dust Tea MARDI and BH, which is important in balancing the bitterness taste and sweetness taste (Kaneko *et al*., 2006; Ochanet, 2017). The caffeine level was found at 18.3 mg/g and 18.8 mg/g in Dried Tea and Dust Tea, respectively. These levels are considered moderate low as compared to LT and BH commercial teas but quite similar with CM-T and CM-V commercial teas which are 23.3 mg/g and 22.1 mg/g. Commercial green tea AH-G was found to be a higher caffeine level than MARDI's tea products which is 26.2 mg/g. Commercial teas AH-G and DL were found the lowest level of caffeine which is 2.9 mg/g and 4.1 mg/g, respectively.

Catechin, epicatechin and theaflavin are polyphenols that are known to have antioxidant activities. Nevertheless, these chemical markers contribute the bitterness and astringency taste to tea products (Tong *et al*., 2019). BH tea showed the highest content of catechin, 17.2 mg/g followed by the DL, AH-G, AH-E, CM-T, CM-T, Dried Tea MARDI, Dust Tea MARDI and LT tea, which is 14.9, 14.4, 12.8, 12.1, 10.8, 4.5, 4.3 and 2.7 mg/g, respectively. BH tea showed the highest content of epicatechin among the others, 30.25 mg/g followed by the LT, AH-G, DL, CM-T, CM-V, AH-E, Dried Tea MARDI and Dust Tea MARDI Which is 23.77, 14.97, 11.07, 6.90, 6.79, 6.07, 3.76 and 2.48 mg/g, respectively. Theaflavin is mainly the product of the fermentation process. This chemical marker found highest in LT tea which is 15.51 mg/g, followed by BH, DL, Dust Tea MARDI, Dried Tea MARDI, CM-V,CM-T, AH-G and AH-E tea which is 13.67, 2.62, 1.53, 1.44, 1.00, 0.92, 0.72 and 0.26 mg/g, respectively.

4. Conclusion

The quality of tea leaves and tea products were determined on the selected five major chemical markers known as catechin, epicatechin, caffeine, theaflavins and theanine. The chemical markers were selected due to the presence of the markers at a significant amount in tea leaves and tea products. The analysis of chemical markers involved two main processes which include extraction using polar solvent and the analysis of the chemical markers using the HPLC system. The determination of the chemical markers in tea leaves and their products were carried out based on the linearity of each standard. The level of selected chemical markers that were found varies between the different brands of tea products and these may influence the taste, aroma, and degree of health benefits.

Conflict of interest

The authors declare no conflict of interest.

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