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SIFAT-SIFAT OPTIK DAN PENENTUAN KONTEN RELATIF KAFEIN DALAM SEDUHAN TEH HITAM

OPTICAL PROPERTIES AND RELATIVE CONTENT DETERMINATION OF CAFFEINE IN BLACK TEA LIQUOR

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Abstrak

Kafein merupakan salah satu parameter kualitas utama pada teh, dikenal karena khasiatnya untuk meningkatkan ketajaman perhatian, dan mempunyai rasa yang sedikit pahit. Sebagai parameter kualitas, kandungan kafein juga menentukan profil cita rasa dan aroma teh yang nantinya akan berpengaruh pada harga teh di pasar global. Terdapat banyak faktor yang mempengaruhi senyawa kimia dalam teh, salah satunya adalah ketinggian tumbuh tanaman teh. Penelitian ini bertujuan untuk mengkarakterisasi spektrum kafein dari seduhan teh menggunakan spektrofotometer UV-Vis yang nantinya digunakan untuk menghubungkan kandungan teh dengan ketinggian tumbuh sampel teh di perkebunan. Transisi optik kafein dari daun teh yang diseduh diukur dari berbagai sampel teh hitam yang diproduksi sesuai dengan standar kualitas pasar global. Sebanyak 63 sampel teh hitam dari empat kelas yang berbeda (FANN, PekoFANN, FANN2, dan Dust) dari 12 perkebunan teh yang berbeda di Indonesia telah direplikasi dalam transisi optiknya dan diklasifikasikan berdasarkan kandungan kafein. Hasil penelitian memperlihatkan bahwa kandungan kafein dalam teh hitam bervariasi bergantung pada ketinggian tanam tanaman teh. Kelas FANN secara umum mempunyai kandungan kafein yang lebih tinggi dibandingkan kelas yang lain sementara sampel teh yang berasal dari perkebunan yang lebih tinggi umumnya mempunyai kandungan kafein yang lebih tinggi juga.

Kata kunci: kafein, ketinggian tanam, spektrofotometer UV-Vis, teh hitam

Abstract

Caffeine is one of main quality parameter for teas, known for its ability to increase alertness, and has a slightly bitter flavor. As quality parameter, caffeine content will later determine flavor and aroma profile for teas that affect the price in global market. There were many factors that affected chemical compounds in tea, one of it was growth altitude. This research aimed to characterize caffeine spectra from tea liquor using UV-Vis visible which later can be used to relate caffeine content with the altitude of tea sample plantation. The optical transition of caffeine from brewed tea leaves liquor was measured from a variety of black tea products produced according to quality standards for global market. A total of 63 tea samples from four different grades (FANN, PekoFANN, FANN2, and Dust) and from 12 different tea estates in Indonesia have been replicated in their optical transitions and classified on the basis of caffeine content. It was found that caffeine content in tea products may vary depending on the grade and origin of the tea plantation. FANN grade has higher caffeine content than other grades while products from highland tea plantation generally have more caffeine content for all tea grades.

Keywords:altitude, black tea, caffeine, spectrophotometer UV-Vis

Introduction

Tea is the most consumed beverage in the world after water [1]. Tea is not only consumed as a drink but also used as a traditional medicine that has many healthy effect from its antioxidant properties. The chemical components in tea include alkaloids (theobromine, caffeine, theophylline), polyphenols (catechins, flavonoids), amino acids, polysaccharides, volatile acids, vitamins, lipids as well as inorganic elements [2]. Chemical composition in tea become important factor that affect its flavor and color from tea liquor. From previous study, climatic conditions, horticultural practices, soil, growth altitude, plucking season, sorting, grading, processing, extraction, storage and drying affected the chemical composition of tea [3,4]. Caffeine is one chemical compound contained in tea which is essential and affect important parameter for commercial tea evaluation, especially in flavor and color. Caffeine itself has a slightly bitter flavor, and determine the color that precipitated during tea leaves infusion process [2] which it will later determine the price of tea in

global market. There are three types of tea based of fermentation process, green tea (unfermented product), oolong tea (partially fermented), and black tea (fully fermented tea).

In fact, fermentation process involves browning reaction which are catalyzed by polyphenol oxidase [4]. During fermentation process, polyphenol compounds are enzymatically oxidized and polymerized to theaflavins and thearubigins thus simultaneous increase caffeine and sugar content in tea [5,6]. Theaflavins, thearubigins, cathechins and caffeine are known to be responsible for black tea quality, where green tea quality depends more on the content of amino acids, cathecins, and caffeine [3]. The quality and price of tea has been and continue to be established through the judgment by professional tea tasters, but there is growing concern to find assessment method to assess tea quality by measurement method. To determine important compounds in tea, previous research has been done and focused on chromatography method [7]. Chromatography method has good separation advantage, but is expensive, time consuming, and labor intensive [8].

Another method that can be used is spectrometry method using UV-Visible spectrophotometer. Spectrometry method has many advantages including simple, fast, inexpensive, and generally available in laboratories related with food analysis. As previously mentioned, there were many factors that affected the chemical composition in tea, so we can also conclude that different tea origin will produce its own distinctive characters of tea. In Indonesia, there were many state enterprises that engaged in tea plantations. This tea planting place spread around Indonesia, such as Sumatera, Java, Borneo, and Sulawesi. Determination of caffeine content from tea leaves samples from Indonesia were studied by many researcher before but no research was found which relate caffeine content with growth altitude of tea planting site. So, this research aims to characterize caffeine spectra from tea liquor using UV-Vis visible which later can be used to relate caffeine content with the altitude of tea sample plantation.

Material and Methods

Samples

A total of 63 blacks tea samples with different grades (FANN, PekoFANN, FANN2, and Dust) were taken from different locations in Indonesia with different altitude (800-1850 m). The selection was done on the basis of growth altitude. The grades of samples used in this research categorized as fine tea (small grades) which the tea leaves particles had been sorted using 20-mesh sieve.

Materials and Chemicals

UV-Visible spectrometer (Spectronic M105), analytical scales for measuring tea leaves mass, 20-mesh sieve, beakers, thermometer, hot plate, distilled water, Whatmann no. 42 filter paper.

Methods

Preparation of Tea Liquor

Tea liquor from each sample is prepared according to following steps [9–11]: 150 ml of aquades heated to 85 ± 1^{o}C and then poured onto 3 gr dried tea leaves sample, allowed to stand for 3 minutes at room temperature. After that, the tea liquor was filtered using Whatman no. 42 filter paper. The tea leaves filtered sample cooled at room temperature then be used as subsequent analysis.

Absorbance Measurement

The measurement of tea liquor spectra was done using UV-Vis spectrophotometer. First of all, tea liquor was diluted by taking 1 ml of sample and diluted with 30 ml of aquades. The absorbance measurements were performed at wavelength of 200 – 500 nm [12,13].

Data Analysis

Data obtained was analyzed chemometrically by Matlab. Each spectrum was smoothed by Savitzky-Golay smoothing algorithm using polynomial order 3 at 9 frames sizes. All spectrum was then normalized (0-1) to maximum absorbance. Caffeine content were monitored at caffeine absorbance maxima. Data obtained were expressed as means ± standard deviation. The data were also subjected to variance analysis using SPSS Statistics 20. The significance level of $P<0,05$ was considered significantly different.

Results and Discussion

Preparation of tea liquor was done by extracting tea leaves sample using water as solvent that was heated to 85^{o}C. The choice of extraction temperature was due to consideration of best extraction yield of caffeine [14] and best perception overall flavor of tea liquor [9]. Caffeine is an

important component of tea which is essential for the efficiency and other taste characteristics of commercial tea and regarded as an important parameter for commercial tea evaluation. The black tea quality is strongly associated with the caffeine content amount for colored precipitates formation during infusion process [2]. Absorption spectra from various black tea liquor measured using UV-Visible spectrometer and data obtained from absorbance measurement can be seen at Figure 1.

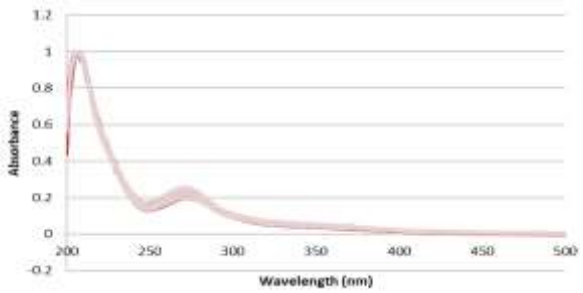

Figure 1. Absorbance from various tea liquor

The data shown on Figure 1 were normalized data so that the difference between peak absorbance at wavelength that indicates caffeine (250 – 300 nm) [13] can be determined. From UV-Visble spectra, the optical transition properties of caffeine in black tea liquor can be compared using the Beer-Lambert law. The Beer-Lambert law can be seen from equation as below:

$$\ln\left(\frac{Io}{I}\right) = A = \varepsilon(v)cl \qquad [15]$$

where $\varepsilon(v)$ is the molar decadic absorption coefficient, c is the concentration of the absorbing compound, A is the dimensionless quantity called absorbance and I/I_o is transmittance (T). From Beer-Lambert law, we know that absorbance is directly proportional to other parameters (concentration, molar absorbtivity, and the path length of the sample). Since caffeine content in tea samples cannot be determined directly by UV-Visible spectrometer due to the matrix effect of UV absorbing substance, data obtained at Figure 1 can be used as caffeine content assumption contained in the tea liquor as it represented color. The higher peak absorbance data obtained was assumed as the higher caffeine content as the color and concentration of tea liquor got thicker.

The peak absorbance data of caffeine then been sorted from the highest to the lowest. As the tea leaf samples came from location with difference altitude, the peak absorbance that been sorted before then connected with the sample's altitude and shown on Figure 2.

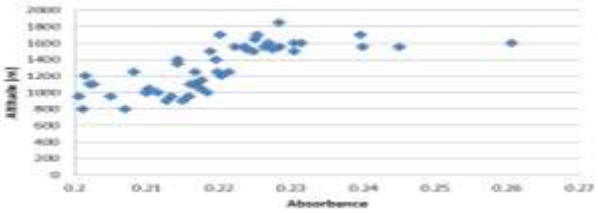

Figure 2. Caffeine content from various tea liquor based on growth altitude of tea sample

To determine if there was significance effect of growth altitude on caffeine content on tea samples, analysis of variance (ANOVA) was done. Descriptive analysis is shown on Table 1 which contained the means, standard deviation and also the maximum and the minimum values. From descriptive analysis, the data showed that the highest caffeine content was tea sample that planted on 1600 m altitude and the lowest was on 800 m – 1300 m range altitude. The analysis of variance (ANOVA) can be seen on Table 2.

Table 1. Descriptive Analysis of Caffeine Content Based on Altitude

Altitude (m)	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
800	3	0.20	0.00	0.00	0.20	0.21
900	3	0.21	0.01	0.01	0.20	0.21
950	7	0.20	0.01	0.00	0.20	0.22
1000	6	0.21	0.01	0.00	0.20	0.22
1050	3	0.21	0.01	0.01	0.20	0.22
1100	6	0.21	0.01	0.00	0.20	0.22
1150	1	0.22	.	.	0.22	0.22
1200	4	0.20	0.01	0.01	0.20	0.22
1250	4	0.22	0.01	0.00	0.21	0.22
1300	2	0.20	0.00	0.00	0.20	0.20
1350	1	0.21	.	.	0.21	0.21
1400	2	0.22	0.00	0.00	0.21	0.22
1500	3	0.22	0.01	0.00	0.22	0.23
1520	2	0.23	0.00	0.00	0.22	0.23
1550	7	0.23	0.01	0.00	0.22	0.25
1600	4	0.24	0.02	0.01	0.23	0.26
1650	1	0.23	.	.	0.23	0.23
1700	3	0.23	0.01	0.01	0.22	0.24
1850	1	0.23	.	.	0.23	0.23
Total	63	0.22	0.01	0.00	0.20	0.26

ANOVA indicated that the value of significance was below 0,05 which means there was a statistically significant difference between the caffeine content in tea sample's liquor with the altitude of tea plantation. From this research, we can conclude that the growth altitude of tea plantation significantly affected the caffeine content, there were also many other factors that also affected the caffeine content on tea sample such as plucking season and climatic conditions. From previous study about caffeine content in tea, caffeine content especially in black tea was affected by clone, stage of plucking, season, geographical locations, late harvesting as well as

more mature leaves for commercial tea production [16,17]. The means of tea samples' caffeine content by absorbance peak shown at Table 3, which showed that the highest caffeine content was on FANN grade but not significantly difference with the other grades.

Table 2. Analysis of Variance (ANOVA)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.01	18	0.00	5.95	0.000
Within Groups	0.00	44	0.00		
Total	0.01	62			

From Table 3, the result of caffeine content in different grades of black tea in this research were well recognized that uncontrolled variables such as soil and climatic condition difference, cultivation practices, post-harvesting handling and processing techniques may affect the chemical compositions. In commercial tea samples, the caffeine content should be controlled fewer than 4% to maintain better quality of the product [18]. However, the data obtained from this research could be used as basic information which can be used as a guideline for establishing chemical standard of black tea in the future. From previous quantitatively study, it was shown that in black tea, the content of caffeine is about 1,5 – 4,3% [7,20] that is not only functioned as quality parameter, but also pharmacologically as health benefits when consumed in lower doses (20-200 mg) [21].

Conclusion

Caffeine content in tea products may vary depending on the grade and origin of the tea plantation. FANN grade has higher caffeine content than other grades while tea products from highland tea plantation generally have more caffeine content for all tea grades. For future research of black tea quality, we suggest consideration of using the proportion of theaflavins concentration to thearubigins concentration.

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