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Analysis of Caffeine Levels in Packaged Coffee in Indonesia

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ABSTRACT
Coffee is a popular and much-loved drink in Indonesian society to relieve drowsiness or increase stamina when working. This is because in coffee, there is an active compound, namely caffeine. Although it has benefits, in fact, caffeine will have a negative impact on the body if consumed in excess. The Indonesian National Standard 01-3542-2004 has determined that the limit value of caffeine content in food and beverages is 0.45–2% w/w. This study aimed to determine the caffeine content of packaged coffee sold in the community using a UV-Vis Spectrophotometer. The results of the analysis showed that 16 of the 20 samples analyzed had caffeine levels in accordance with the provisions of the Indonesian National Standard, while the other 4 samples had caffeine levels above the specified value. Even sample Q has a caffeine content of 7.12% w/w, far above the threshold value. Based on the results of this study, it can be concluded that there are still packaged coffees containing caffeine above the specified threshold value. So, consumers should pay attention to the amount of caffeine consumed so as not to cause a negative impact on their health.

KEYWORDS
Coffee, Caffeine, UV-Vis Spectrophotometer

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Introduction

Coffee production in Indonesia produces an average of 639 thousand tons per year, making it the fourth largest producer of coffee beans in the world (Sulistya, 2017). One area that is the largest contributor to coffee production is Bali, with a coffee plantation area of 24,721 hectares (Tika, *et al.*, 2017). Compared to other plantation crops, coffee has a relatively high economic value and is a significant source of revenue for the state (Suryani, et al., 2016). Coffee grows in the tropics and is affected by climate change and its treatment (Suryani, *et al.*, 2016). When processed, coffee plants can be used to make drinks that have a high selling value.

Coffee is a type of drink that has a bitter taste but is much favored by people from various circles because of its distinctive aroma (Cheong, *et al.*, 2013). Coffee is produced from coffee beans that are processed and extracted (Suwiyarsa, et al., 2018). Currently, coffee is the second-most consumed beverage worldwide, after mineral water. Coffee enthusiasts can drink it three to four times per day (Aprilia, *et al.*, 2018). Even today, the taste of coffee continues to be developed to meet the tastes of coffee connoisseurs (Tika, *et al.*, 2017).

Coffee has different types in each region. In general, many of these are found in Indonesia, namely robusta, arabica, liberica, and civet coffee (Pracaya, 2019). However, the most famous and popular in Indonesia are Robusta and Arabica coffee. Robusta coffee is famous for its large bean shape and has a bitter taste that tends to be sour, while Arabica coffee has smaller seeds than other coffee beans (Pracaya, 2019). Coffee has various benefits for our bodies, namely as an energy booster during activities and for preventing prostate cancer (Widyotomo and Sri-Mulato, 2007). The chemical content found in coffee naturally includes theophylline, theobromine, cytostepzin, stigmasterin choline, and caffeine (Suryani, *et al.*, 2016). Although coffee has benefits for the body, the presence of caffeine will have a negative impact if consumed in excess.

Caffeine ($C_8H_{10}N_4O_2$) is a natural alkaloid-derived compound (purine) that can be found in coffee beans with the main constituent in the form of protein compounds, namely xanthine purines (Isnindar, *et al.*, 2016; Hesse, 2002; Suryani, *et al.*, 2016; Widyotomo and Sri-Mulato, 2007). Apart from coffee, these alkaloid-derived compounds can be found in tea, cocoa beans, and cola beans (DeBruyn, *et al.*, 2017; Tepsatian and Kittigowittana, 2017; Suryani, *et al.*, 2016). In its pure state, caffeine is a white powder, odourless, and has a bitter taste (Morton, 1984; Sivetz and Desrosier, 1979; Johnson and Peterson, 1974; Clarke and Macrae, 1989). Caffeine has a clinically beneficial effect on stimulating the central nervous system by eliminating fatigue, hunger, sleepiness, and increasing concentration power (Isnindar, *et al.*, 2016; Suwiyarsa, *et al.*, 2018). In low doses, caffeine is able to provide benefits, especially to the immune system (Isnindar, *et al.*, 2016; Goncalves, *et al.*, 2017; Aguilar-Navarro, *et al.*, 2019; Dodd, *et al.*, 1991). In addition to providing benefits, caffeine also has a negative impact on the body if consumed in excess, such as anxiety, tooth colour changes to yellow and, over time, becomes brown and even black, the mouth becomes smelly, increases stress, causes heart attacks, and in men, can cause infertility and disrupt digestion (Isnindar, *et al.*, 2016; Widyotomo

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and Sri-Mulato, 2007). Caffeine consumption can also affect bone mass (Bonura, 2009; Khwanchuea, *et al.*, 2012). In addition, if a person has problems related to the hormone metabolism of uric acid, the entry of caffeine into the body will trigger the formation of high uric acid (Burnham, 2001). In the body, caffeine is absorbed by the intestines and within minutes, it is spread by the blood to all organs and tissues. Caffeine can stay active by tricking the body into increasing blood pressure and increasing urine output (Novita and Aritonang, 2017).

One of the processed coffee beans that many people like is packaged coffee. Packaged coffee is a coffee drink that is processed in such a way and mixed with other ingredients to produce an instant coffee drink. The standard level of caffeine contained in beverages and food has been set by the Indonesian National Standard, which is 0.45-2.00% (w/w) (Suwiyarsa, *et al.*, 2018; Indonesian National Standard 01-3542-2004, 2004). But the fact is that there are still many packaged coffee drinks that have high levels of caffeine. This is in accordance with research conducted by Suwiyarsa, Nuryanti, and Hamzah on the description of caffeine levels in local ground coffee circulating in Palu City, Indonesia, using the UV-Vis spectrophotometric method using six samples of ground coffee. Of the six samples, two of them did not comply with the caffeine standard set by the Indonesian National Standard, where the two samples had a caffeine content of 2.06% and 2.63% (Suwiyarsa, *et al.*, 2018).

There are many methods to determine caffeine content, one of which is UV-VIS spectrophotometry (Novita and Aritonang, 2017). It is the superior method because most of these methods are reported to have problems with their detection (Susanti, *et al.*, 2019). This instrument has a principle based on the Lambert-Beer law, namely, when monochromatic light passes through a medium or solution, the light will be partially absorbed, partially transmitted, and partially reflected (Miconos, 2021). The UV-VIS spectrophotometer has the advantage that it is easy to operate and can analyze solutions with very small concentrations (Dewinto, 2014). For this reason, researchers intend to analyze and describe the levels of caffeine contained in packaged coffee. As for calculating the caffeine content in the packaged coffee, the UV-Vis spectrophotometer method was used.

Method

This research uses a descriptive method. A descriptive method is a research method that describes the population or phenomenon being studied. Usually, this method focuses on the research subject (Hayati, 2021). This research was conducted to determine the caffeine content in packaged coffee using the UV-Vis Spectrophotometry method. In general, the steps in this study can be seen in Figure 1.

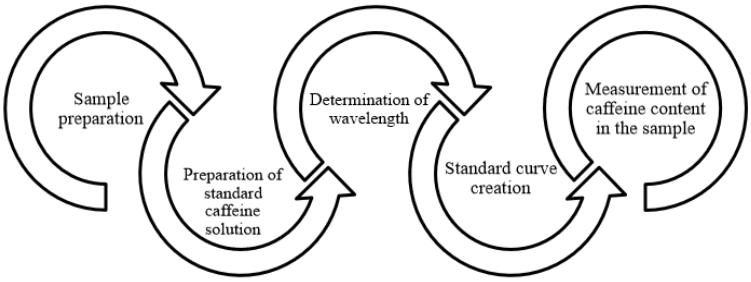

Figure 1. Research working mechanism

Sample Preparation

The number of samples used in this study was 20 brands of packaged coffee. The initial stage is carried out by taking as much as 2 grams of each packaged coffee brand and putting them into 20 beakers. Then added 150 mL of distilled water was to dissolve the coffee. Filtration is carried out to separate the solution from the precipitate using filter paper. The filtrate was homogenized after being mixed with 1.5 grams of $CaCO_3$. The solution was put into a separating funnel and the extraction process was carried out three times. The chloroform phase at the bottom was taken and heated in a bath until it evaporated.

Preparation of caffeine standard solution

The standard solution was made by making a caffeine stock solution with a concentration of 200 ppm. A dilution technique made a series of standard caffeine solutions with concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm from the stock solution.

Determination of wavelength

The wavelength was determined using a standard solution of 50 ppm caffeine. The wavelength used is in the range of 200-400 nm. The wavelength can be determined by looking at the data from the largest absorbance measurement. The first absorbance measurement was carried out at wavelengths in multiples of 50 nm. Furthermore,

it is carried out in multiples of 10 nm and finally in multiples of 1 nm. This is done to shorten the measurement time so that it is more effective and efficient.

Standard curve creation

After determining the wavelength, the next step is to make a standard curve by measuring the absorbance of the caffeine standard solution series. The concentration and absorbance data are then plotted into a curve to obtain the linear equation $y = ax + b$ and R^2. This equation can be used to calculate the caffeine content in a sample, where y is the absorbance value and x is the concentration.

Measurement of caffeine content in the sample

The measurement of caffeine content in the sample is carried out by measuring its absorption at the wavelength that has been determined in the previous stage. Calculation of the caffeine content in the sample was carried out using the equation of the line on the standard curve. Furthermore, the calculation of caffeine content in units of % w/w.

Results and Discussion

Determination of wavelength

At this stage, scanning wavelengths is carried out in the range of 200-400 nm. If you look at the value of the wavelength range, it can be said that the measurement of caffeine content was carried out in the UV working area (190 – 380 nm) because the solution was colorless (Suwiyarsa, *et al.*, 2018). Determination of this wavelength was carried out to obtain the wavelength value with the largest absorption, which indicated that the caffeine molecules interacted the most at that wavelength (Kimia FMIPA Universitas Jember, 2017). Scanning was carried out using a standard solution of 50 ppm. Based on the measurement results, the wavelength with a range of 310 – 320 nm gives the largest wavelength measurement results. So measurements with multiples of 1 nm are carried out in this wavelength range. The results of these measurements can be seen in Table 1. The data in Table 1 shows that the maximum absorbance occurs at a wavelength of 314 nm with an absorbance value of 0.049. Thus, these wavelengths were used to construct standard curves and measure the caffeine content of the sample.

Table 1. Absorbance measurement results to determine the maximum wavelength

Wavelength (nm)	Absorbance
310	0.047
311	0.048
312	0.048
313	0.048
314	0.049
315	0.047
316	0.047
317	0.046
318	0.045
319	0.045
320	0.045

Standard curve

The standard curve is created by graphing the standard solution's concentration value against its absorbance value to create a line equation (Sungkawa and Ladika, 2019). The measurement of the absorbance of the caffeine standard solution was carried out at a wavelength of 214 nm, in accordance with the results of determining the maximum wavelength. The results of measuring the absorbance of the caffeine standard solution can be seen in Table 2.

Table 2. The results of measuring the absorbance of caffeine standard solution

Concentration (ppm)	Absorbance
20	0.034
40	0.042
60	0.051
80	0.059
100	0.067

The data in Table 2 is then plotted into a curve to obtain the line equation $y = ax + b$ where the x axis represents the concentration of the solution and the y axis represents the absorbance value. The standard solution curve can be seen in Figure 2.

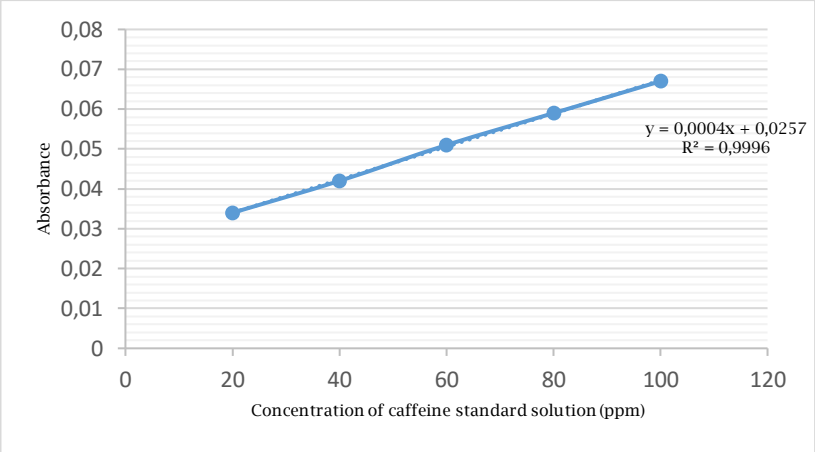

Figure 2. Caffeine standard solution curve

Figure 2 shows that the equation of the line resulting from the data plot of the concentration of the standard solution and its absorbance is y = 0.0004x + 0.0257 with a value of R^2 = 0.9996. This R^2 value shows the level of line linearity, where the linear relationship will be more ideal if the value is close to 1 or -1 (Arwangga, *et al.*, 2016). Through this R^2 value, discrepancies in the process can also be identified, which can occur due to the reagents or tools used (Baehaki, *et al.*, 2020). If you look at the value of R^2 in Figure 1, it can be said that the equation of the line obtained has a very high ideal level and is suitable for calculating the concentration of caffeine in the sample.

The caffeine content in package coffee

Measurement of caffeine content was carried out by measuring the absorbance of the sample and performing calculations with the line equation y = 0.0004x + 0.0257 (see Figure 1). The results of measuring caffeine levels in the sample can be seen in Table 3.

Table 3. The results of measuring caffeine levels in packaged coffee samples circulating in the community

Sample	Caffeine level (% b/b)
A	2,60
B	3,10
C	0,40
D	0,35
E	1,20
F	2,70
G	1,50
H	0,35
I	1,25
J	0,45
K	1,35
L	1,05
M	0,45
N	1,00
O	1,70
P	1,35
Q	7,12
R	0,10
S	1,75
T	1,40

The data in Table 3 shows that 16 of the 20 samples analyzed had caffeine levels in accordance with the rules of the threshold value determined by the Indonesian National Standard, namely 0.45% - 2% w/w. Four samples with codes C, D, H, and R had caffeine levels below 0.45% w/w. Meanwhile, samples with codes E, G, I, J, K, L, M, N, O, P, S, and T had caffeine levels in the range of 0.45% - 2% w/w. While the rest have levels above the threshold value determined by the Indonesian National Standard. Even one sample with code Q has caffeine levels far above the threshold value, which is 7.12% w/w. Of the four samples that had caffeine levels above the threshold value, two of them (sample A and Q) were pure coffee, without a mixture of sugar or creamer.

From this data, although most packaged coffees have caffeine content according to the provisions of the Indonesian National Standard, in fact there are still packaged coffees whose caffeine content values are still above the threshold value. Of course, this will be dangerous for consumers because in fact they will consume caffeine in large

enough quantities. Consuming high amounts of caffeine will have negative effects on body health, such as anxiety, yellow teeth, bad breath, increased stress, heart attacks, infertility, digestive disorders, sleep disorders, nervousness, anxiety, and cause cardiovascular problems such as constriction of blood vessels (Isnindar, *et al.*, 2016; Widyotomo and Sri-Mulato, 2007). The narrowing of blood vessels is caused by the formation of plaque in the blood vessels when caffeine is metabolized in the body.

In the body, caffeine is absorbed by the intestines, and within a few minutes, caffeine is spread by the blood to all organs and tissues. Caffeine can stay active by tricking the body into increasing blood pressure and increasing urine output (Novita and Aritonang, 2017). Caffeine will mobilize intracellular calcium, inhibit phosphodiesterase, and block adenosine receptors (Grgic, 2021; Chen, *et al.*, 2019; Bowtell, *et al.*, 2018; Dittrich, *et al.*, 2021; Suryani, *et al.*, 2016). The excitability of the central nervous system is inhibited by adenosine (Fredholm, *et al.*, 2005). Caffeine is a powerful adenosine receptor antagonist while having a structure that is identical to adenosine (Elmenhorst, *et al.*, 2012). Then inhibition of adenosine receptors will activate inhibitory G protein and inhibit cAMP (second messenger *cyclic adenosine monophosphate*) so that caffeine will bind to adenosine receptors in the brain and increase heart activity, blood pressure, adrenaline hormones, and mental activity (Prasetio, 2020). This process causes the loss of sleepiness after consuming caffeine. However, anxiety due to high levels of caffeine consumed is caused by high levels of lactate.

Conclusion

Based on the results of research conducted, it shows that most packaged coffee has caffeine content at the threshold value, in accordance with the provisions of the Indonesian National Standard. However, some brands of packaged coffee still have high levels of caffeine, exceeding the threshold value. This needs to be watched out for and becomes a concern, both for the public as consumers and for the Food and Drug Monitoring Agency of the Republic of Indonesia, because of the negative effects that can be caused when consuming large amounts of caffeine.

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