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Determination of the Caffeine Contents of Guji Zone Coffee Beans in Southern Ethiopia Using Ultra Violet-Visible (UV-Vis) Spectrometer Analysis

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Abstract: The determination of the caffeine content of coffee beans is the most popular way to solve the problem related to the level caffeine in coffee. In the present study, the caffeine level of Guji coffee beans was determined using the Ultra- Violet visible spectrometer Analysis Method. The coffee samples were collected from the coffee supplier farmer cooperatives union in Bule Hora. The sample was roasted and grinding using grinding for caffeine extraction. The obtained data by the study were compared with the caffeine levels that determined by different researchers from different selected sites, and indicates that the values of the results ranged from (1.04 ±0.0010 to 1.31±0.0021)% using ultraviolet-visible. The levels of caffeine from Hambella Wammana (1.31 ±0.0020)% was high, and that of Kercha (1.04 ±0.0010)% is low, and claimed that the caffeine levels of coffee beans of the same species that growth on different regions were varied due to different vital factors that influences amount caffeine in coffee beans. Generally, the study demonstrated that the caffeine level from selected areas of Guji Zone confirms the caffeine level of arabica coffee informed by the literature.

Keywords: Coffee Bean, Caffeine, Guji Zone, Ultra-Violet Visible Spectrometer

1. Introduction

The word coffee is originated from the name of the province Kaffa where shepherds from Abyssinia/Ethiopia [1] discovered the coffee beans in the 6th century [2, 3]. The coffee bean is obtained from the fruit of the coffee plant, a small evergreen shrub, belonging to the genus *Coffea arabica* and Robusta coffee) [4] are economically important [5], and are different in their chemical composition including their caffeine level. Among these, arabica coffee is a higher quality bean, prized for its complex aroma, and the most expensive one in the world market [3]. Compared with Robusta coffee it is grown in about 80 tropical and sub-tropical countries, and the majority of those countries supply the product to the world market. Coffee is not only distributed to the different provinces of the

world, but it is the most daily consumed beverage throughout the world owing to it is pleasant taste, aroma, and stimulant effects [6, 7] and healthy benefits [8, 9].

Ethiopia is also a country that uses coffee as international trade for foreign exchanger suppliers, as an element of cultural beverage, and as another imperative raw material inside international trade [10-12].

Arabica coffee is a unique coffee bean variety that still growing in the country with its distinct flavor profiles [3]. The coffee beans produced in different regions of Ethiopia are sold at national and international markets at different trademarks and prices. One of the regions known for coffee production is the Guji zone which is found in, the Oromia regional state, of Southern Ethiopia. Guji Zone is one of the coffee-producing zones in the Oromia Regional State, and the total area of land covered by coffee in the zone is about

114,000 hectares, which includes small-scale farmers' holdings as well as state and privately owned plantations [10].

The excellence of coffee denotes desirable appearance, pretty flavor, noble cup taste, and amount of caffeine. Numerous factors may affect the quality of coffee beans. Amid factor caffeine content is a vital influence [13, 14]. Several studies have stated that caffeine has healthy benefits and physiological effects if it is taken in moderate quantity and, adversative effects if it is taken in higher amounts [12]. Several methods have been claimed to be used in caffeine level investigation of coffee, and further confined and industrial beverages. Therefore, the accuracy of techniques may differ from method to method demonstrating that an assortment of suitable analytical methods is essential for the accurate determination of caffeine in samples. Higher-performance liquid chromatography (HPLC) and ultraviolet-visible spectrometer (UV-Vis) methods are the most frequently used techniques in the determination of the caffeine level of coffee products [3]. However, owing to its requirement of costly equipment and demands for operator care the application of HPLC in lesser industrial laboratories where only a rare is achieved in the daytime [15].

On the contrary, UV-Vis spectrometer methods are fast, avail in most laboratories, cheap, and easy are used for the determination of caffeine contents of coffee beans. Yet, it has prevalent popularity in national and international markets, there are no sufficient documents on status analysis of caffeine contents coffee beans in studies area. Hence, this study was commenced to determine the caffeine contents of coffee beans obtained from selected sites of Guji woreda using UV/Vis methods.

2. Materials and Methods

2.1. Chemicals

The chemicals used in experiments were standard caffeine powder, dichloromethane, Na_2SO_4, and Na_2CO_3. All the chemicals were analytical of grade and purchased from Fine Chemical PLC Addis Ababa, Ethiopia.
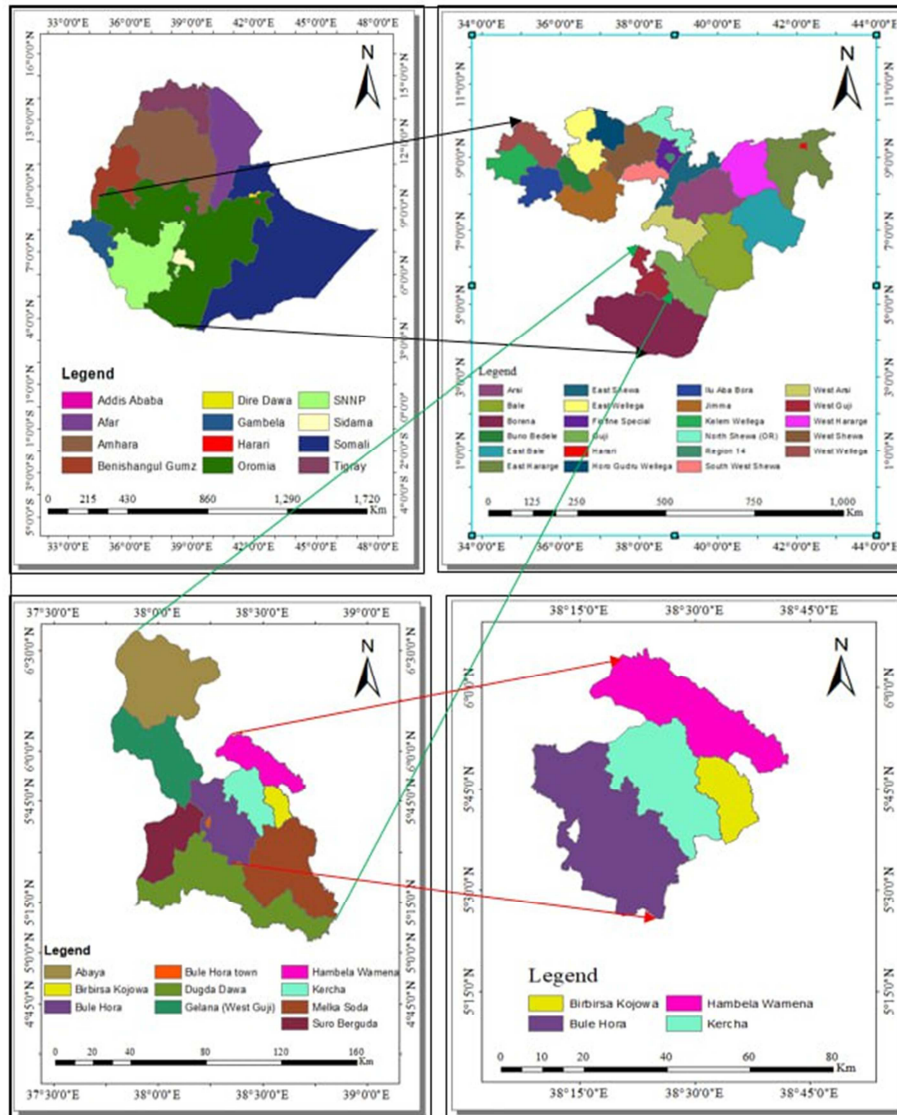

Figure 1. *Map and geographical location of selected areas of Guji zone, Oromia regional state Ethiopia.*

2.2. Sample Collection

Arabica coffee beans samples (1000g) of Bule hora woreda, Qerca woreda, Hambella Wammana woreda, and Birbissa Kojowa woreda were obtained from farmers' unions and coffee suppliers. The areas were selected as they are the major coffee production zones. The coffee production of Kercha woreda covers 90277.54 tons, Bule Hora woreda covers 72914.84 tons, Hamballa Wammana woreda covers 46869.35 tons and Birbirsa Kojowa woreda covers 38500.8 tons [16]. Figure 1 shows the map and geographical location of selected areas of the Guji zone, Oromia regional state, Ethiopia.

2.2.1. Sample Preparation for Caffeine Extraction

Preparations of coffee beans were carried out using the procedure followed by [14]. 50g of each of the four selected areas of raw coffee samples were obtained from the farmer union and roasted using the conventional coffee roasted method. The roasted coffee beans were ground and screened through 250μm to acquire a uniform texture. Twenty-five grams (25gm) of grounded and sieved roasted coffee sample was kept in 500ml of a beaker, dissolved in 120ml of deionized water in a temperature range of 80 to 90°C, and stirred with a magnetic stirrer for 30min. Then the coffee solution was permitted to cool to room temperature and filtered using filter paper. The left on the filter paper was washed by adding a small amount of hot water. 3.0 g of Na_2CO_3 was added into the filtrate to dissolve tannins gallic acids in water to lease them persist in the aqueous layer during extraction. The filtrate was then subjected to liquid-liquid extraction using 100ml of dichloromethane in a 250ml separator funnel.

The organic phase was separated from the aqueous phase and collected in a clean and dry flask. The aqueous phase was extracted 3 times with 30 ml of dichloromethane following a literature. The fraction of organic phases of coffee samples were mixed and dried with 5 g of anhydrous Na_2SO_4. The solvents were distilled off using rotary vapor to get crude caffeine extract.

2.2.2. Preparation of Caffeine Standard Solution

1000ppm of caffeine stock solution was prepared by dissolving 100mg of pure caffeine in 100ml of distilled in a 250ml volumetric flask. Then 10.15, 20, and 25ppm caffeine working solutions were prepared by serial dilution of stock in 25ml volumetric flask calibration solution were prepared. The absorption spectra of the solution were recorded at the wavelength of 272nm.

2.3. Determination of Caffeine Using Ultra Violet Visible Spectrophotometer

The quantitative analysis of the amount of caffeine in coffee samples was determined by measuring their absorbance using the UV-vis spectrophotometer model JENWAY6300. The maximum absorbance (λmax) was determined by scanning the standard solution. The absorption spectra of the solution were recorded at the wavelength of 272nm. The tests were repeated three times, and a Calibration curve was made using the data obtained from the prepared standard solutions. This curve was constructed using absorbance versus concentration to validate the UV-Vis absorption of caffeine in terms of linearity, for calibration purposes to determine the caffeine content of the coffee in samples. The caffeine level of the coffee was calculated from the regression equation of the best line of the fit the standards. The reason for the selection of UV-vis was due to it is most available in laboratories, being easy, fast, and economical.

3. Results and Discussion

In this study, it was envisioned to determine the amount of caffeine in coffee samples selected from major coffee production areas of the Guji zone, using a UV-vis spectrophotometer.

3.1. Percentage of Crude Caffeine Extract

The result of an experiment indicates that the caffeine levels in coffee samples collected from different areas are comparable to each other. The percentage of caffeine samples was calculated using the following formula.

$$\% \text{ yield of crude caffeine} = \frac{mass \text{ of crude caffeine}}{mass \text{ of coffee powder used extraction}} \times 100 \tag{1}$$

3.2. Quantitative Determination of Caffeine Using UV-Vis Spectrophotometer

In this research, the caffeine level of some selected areas of Guji zone coffee beans was determined by quantifying the intensity of the absorption of a series of concentration standard caffeine solutions in dichloromethane. Therefore, the absorbance of the working standard of caffeine was measured at a wavelength of 272nm using a UV-Vis spectrometer. Thus, as revealed in Figure 2. The absorbance versus concentration graph was developed. From this calibration curve (figure 2) the calibration equation y = 0.0788x + 0.0078, R = 0.99837, where y is absorbance, x is the concentration of caffeine and R is the linear regression coefficient was obtained.

Owings to the matrixes of coffee beans UV-Vis spectrometer cannot directly determine the level in the samples. In mandate to overwhelm this effort, the samples were dissolved in water and then the caffeine was removed from the solution using dichloromethane. Dichloromethane is stated to be the greatest appropriate organic solvent that extracts caffeine from coffee beans as it is free of matrices interfering with measurement [6].
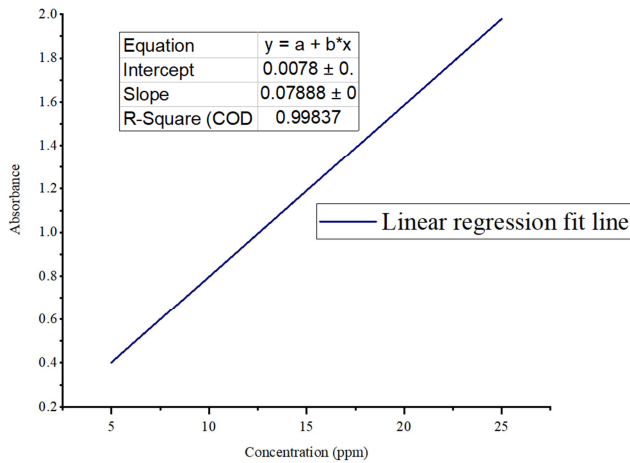
Figure 2. *Calibration curve caffeine for standard work.*

After extraction, the absorbance of the solution was measured with a UV-Vis spectrometer. As shown in Figure 3 the maximum absorbance of caffeine extract was obtained at 273.4nm. Thus the result of the study indicates that the absorption of caffeine in coffee beans grown in Hamballa Wammana is high, and that of Kercha woreda was relatively low when compared to each other.
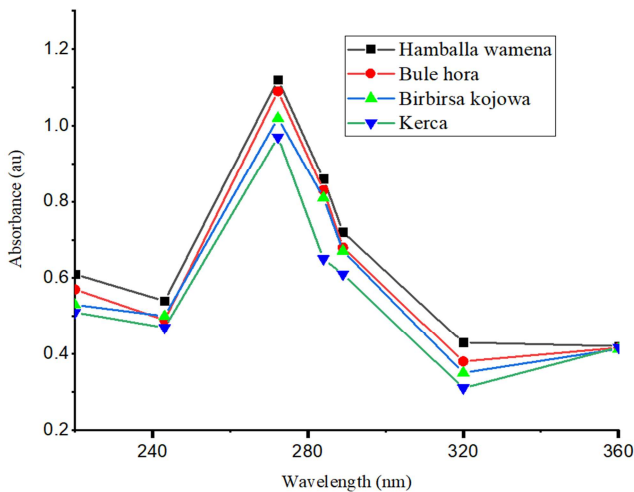

Figure 3. *Absorbance versus wavelength of caffeine in coffee samples.*

The amount of caffeine in samples was calculated using the regression equation of best line fit (Figure 2), of $y = 0.0788x + 0.0078$, where y is the absorbance of caffeine absorbed at 273.4nm, and x is the concentration of caffeine calculated. Besides, the percentage of caffeine was calculated using (Equ.1).

Table 1. *Mass and percentages of caffeine in coffee samples.*

Coffee bean samples (selected area)	caffeine (mg)	caffeine (%w/w)
Bule hora	0.597 ± 0.020	1.2 ±0.0013
Hamballa Wammana	0.652 ± 0.005	1.31 ±0.0020
Birbissa Kojowa	0.592 ± 0.070	1.19 ±0.0040
Kercha	0.517 ± 0.046	1.04 ±0.0010

As shown in Table 1, the average value of caffeine levels

found in Guji coffee beans were (1.20 ±0.0013)% in bule hora, (1.04 ± 0.001)% in kercha, (1.19 ± 0.004)%, in Birbissa Kojowa and (1.31 ± 0.0021)% in Hambla Wammana. There was a significant variance (p < 0.05) in caffeine contents among all coffee samples, which indicated that the caffeine level of coffee beans growing in different geographical locations was different. The result of the study indicates that there is little deviation in caffeine level selected areas. The amount of caffeine in the coffee bean of Hambella Wammana was comparable with each other and greater than the caffeine level of Bule Hora Birbissa Kojowa and Kercha coffee bean. Hence, the caffeine level of coffee beans in selected areas in the Guji zone ranged from (1.04 ± 0.001 - 1.31 ± 0.0021)%. The findings also predict that the percentage contents of arabica coffees in selected woreda of Guji zone is comparable with the reported caffeine level of Nensebo district (1.17±0.001%) [6], Benji Maji (1,10%), Gediyo Yirgachefe (1.10%), and goma limu (1.00%) [15], and lower than, the that of the Ethiopian arabica coffee produced from Wembera (1.53 ± 0.003)%, Goncha (1.41 ±0.04) %, Zegie (1.29 ± 0.033)% [17]. The results also indicate that the Ethiopian arabica produced in the Guji zone was greater than the arabica coffee produced in Sebat bet gurage (0.75)%, Asendabo (0.86)% and wolaita sodo (0.74)% [3]. Therefore, the deviation of caffeine levels in coffee beans of the same species was also stated by researchers. This variation may be due to, geographical location, soil, altitude, rainfall, and environmental conditions [2, 7, 18] According to [6], the standards of caffeine contents of exported Ethiopian coffee beans ranged from 0.46 to 2.82%, and the average caffeine content of Arabica coffee beans is less than 1.5%, which was confirmed by the data acquired by this study.

4. Conclusion

In the current study, the determination of the caffeine content of coffee beans selected from Guji zone areas using an Ultraviolet-visible spectrometer was effectively achieved. The comparison of the caffeine level of coffee beans from selected areas has been investigated, and the caffeine level obtained from Hambella Wamena (1.31 ± 0.0021)% is higher than other selected areas. Generally, this study validated that, the caffeine level from selected areas of Guji Zone coffee beans confirms the caffeine level of arabica coffee informed by the literature.

Author Contribution

All authors paid to research and experimental work. The research work was conceptualized by Mr. Gadisa Tesfaye and Dawit Alemu. Material preparation, data collection, laboratory work, and analyses were performed by Mr. Gadisa Tesfaye, Mr. Dawit Alemu, Mr. Bekele Kuma, and Mr Ashenafi Shiferaw. The first draft of the manuscript was written by Mr. Gadisa Tesfaye, and all authors stated on previous versions of the manuscript. All authors read and

approved the final manuscript.

Data Availability and Materials

The datasets generated during and or analyzed during the current study are not publicly available.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the other authors.

Informed Consent

It was obtained from all individual participants included in the study.

Conflicts of Interest

The authors declare no conflict of interest.

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