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Determination of caffeine content of Nensebo coffee beans Southern Ethiopia, using ultra violet-visible (UV/V) is and high performance liquid chromatography (HPLC) methods in Ethiopia

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The main objective of the study was to determine caffeine content of coffee beans (*Coffee arabica*) collected from Nensebo district, West Arsi Zone, Oromia Region using ultra violet-visible and high performance liquid chromatography methods. The coffee beans were purchased from coffee supplying farmers' cooperatives in Nensebo district (namely Tulu Gola, Habera, Refisa, Nensebo Chebi and Melka Dembi) and local coffee markets (in Hawassa and Shashemene). The coffee samples collected from the selected sites were mixed (homogenized). The same was done for coffee samples from coffee markets (in Hawassa and Shashemene cities). The samples were roasted and ground using grinding machine. The resulting powders were boiled in distilled water and extracted with dichloromethane. The dichloromethane extracts were subjected to high performance liquid chromatoraphy and ultra violet-visible analyses. The data obtained from spectra the methods used in the study revealed that the caffeine content of the extract homogenized coffee beans of the selected sites in % (w/w) was found to be 1.03 ± 0.001 by high performance liquid chromatography and 1.17 ± 0.01 by ultra violet-visible analysis. Similarly, the caffeine content of Nensebo coffee samples purchased from coffee markets was found to be 1.14 ± 0.01 by high performance liquid chromatoraphy analysis. The findings showed that the caffeine contents of the coffee samples used in the study was within reported standards, and also relatively lower than reported caffeine contents of coffee beans growing in other parts of Ethiopia. Further studies are recommended to determine the levels of other chemicals constituents and minerals exist in the coffee beans in order to fully determine the properties (quality) of Nensebo coffee.

Key words: Caffeine content, high performance liquid chromatography (HPLC) analysis, ultra violet-visible (UV/Vis), *Coffea arabica*, liquid-liquid extraction, roasted coffee beans

INTRODUCTION

Coffee is one of the natural products, and also the most important commodity in tropical regions that is traded worldwide (Pay, 2009). It has many health benefits that include its use as stimulant, painkiller, antioxidant activity

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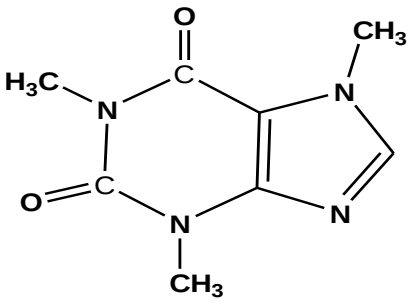

Figure 1. The chemical structure of caffeine (1,3,7-trimethylxanthine).
Source: Nuhu, 2014

and diuretics that could be attributed to the bio-active compounds such as caffeine (1,3,7-trimethylxanthine) (Figure 1) existing in its beans (Nuhu, 2014; Klang et al., 2002; Karacan et al., 1976; Svilaas et al., 2004; Belete and Solomon, 2015; Smith, 2005; Illy, 2002; Glade, 2010; Higdon and Frei, 2006). There are, however, adverse pharmacological effects associated to high caffeine concentration (level) in coffee or excess consumption of coffee. These include health problems such as headaches, fatigue, high blood pressure, decreased attention, depression, poor work performance, heart irregularities, reduce blood flow to brain by causing the brains blood vessels to constrict, increased gastric secretion and poor liver function, etc. (Nehlig, 1999; Francis and Roberts, 1999; Klang et al., 2002; Karacan et al., 1976; Svilaas et al., 2004; Belete and Solomon, 2015; Yukawa et al., 2004). The aforementioned problems have led many consumers to look for caffeine–free or coffee products with low caffeine contents. This is the reason why caffeine content is considered as on indicator of the quality of coffee beans and also prompted many countries to take regulatory actions on products containing caffeine (Fung, 1985; Naegele, 2003; Ahsan* and Bashir, 2019; Antonella and Bettina; 2019; Dobraca et al., 2016; Deepak et al., 2017; Andreia and Bianca, 2014).

Reports revealed that there are over 80 coffee species. Among these large number of coffee species, *Coffea arabica* and *Coffea robusta* are the two widely available varieties in market (Gopinandhan et al., 2014; Farah, 2009, 2012; Heckman et al., 2010). *Coffea arabica* is considered to be of a higher quality coffee. It is well known for its complex aroma, flavours and low caffeine levels (0.8 to 1.4%) (Adepoju et al., 2017; Gray, 1998).

Ethiopia is the major coffee producer among coffee producing countries in Africa. It produces and exports more genetically diverse strains of coffee (that is, *C. Arabica*) (Abu and Teddy, 2014). The country earns millions of US dollars annually that contributes around a quarter its total export earnings (Mekuria et al., 2004; Adepoju et al., 2017; Hassen, 2015; Jima, 2020). For instance, the country reported 854.21 million USD annual incomes from coffee export trade from July 9 2019 to July 2020 (https://allafrica.com/stories/202007210598.html). In addition to its economic importance, coffee also has a strong historical, cultural and social importance in Ethiopia. The main coffee producing areas in Ethiopia are west and south west, southern, eastern, and central regions (Melkamu, 2015; Taye et al., 2011; Jima, 2020).

The coffee beans produced in different regions of the country (Ethiopia) are sold at national and international markets in different brands and prices. One of the regions known for coffee production is Nensebo district that is found in West Arsi, Oromia regional state, Ethiopia. The *Arabica coffee* produced in the district is sold with a locally known brand name "*Werka coffee*". The authors made a market survey and found that the from the district coffee is sold in relatively higher prices as compared to other coffee products obtained from other coffee producing regions, and sold in Hawassa and Shasmene cities. The coffee also has high consumer preference. This might be attributed to low caffeine content. But, the caffeine content of the coffee beans from the study areas needs to be determined/quantified (Abrar and Nigussie, 2013; Personal communication with Nensebo coffee and tea development and marketing Authority, June, 2018).

It is a well-known fact that consumption coffee products with high caffeine levels results in adverse pharmacological and physiological effects on consumers. Thus, it is important to determine caffeine levels of coffee beans, to get information about their caffeine contents. This, in turn, will help in assessing qualities of coffee beans in order to deal on market prices of the products and also to attract consumer/buyers. Therefore, it is a mandatory to determine its level using all available methods/tools for coffee bean quality control. So far, many methods have been developed for the determination of this important constituent of coffee beans and other-coffee-based products. Some of the commonly used methods are Ultra violet-visible (UV/Vis) (Phan et al., 2012; Abebe et al., 2008; Demissie et al., 2016; Belay and Gholap, 2009), gas chromatography (GC) (Gopinandha, 2004; Liew et al., 2001; Sereshti and Samadi, 2014; McCusker et al., 2003, 2006), fourier transform infrared (FTIR) (Gopinandhan, 2014; Singh et al., 1998; Zhang et al., 2013; Fox et al., 2013), mass spectroscopy (MS) (Bae, 2013, Gopinandhan, 2014; Choi et al., 2013), high performance liquid chromatography (HPLC) (Suraj et al., 2016; Ali et al., 2012; Liew et al., 2001; Pandurang, 2012; El-Sayed et al., 2013), electrochemical methods (Olbana et al.; 2019; Ivana et al., 2011), and fluorescence spectroscopy (Weldegebreal et al., 2017) methods. Despite their importance, these methods have some limitations. These include (i) requirement of a large amount of sample and undergoing interferences with the determination, (ii) some methods need expensive equipment to be used in small industrial laboratories (e.g., chromatographic methods) and (iii)

some methods require long analysis time and suffer interferences from electrochemical impurities (e.g., polarography). To overcome limitations, two or more methods can be used together. As reports indicates, UV/Vis and HPLC methods are the two most commonly used techniques (together) for the determination of caffeine contents of coffee products using dichloromethane (Rofti, 1971; Belay, 2010; EI Sayid et al., 2013). Similar reports from Ethiopia also revealed the use of UV-Vis (Fisseha and Gosaye, 2018; Tsegaye, 2009; Zewdu et al., 2016), HPLC (Zerihun and Aman, 2019; Tamiru et al., 2018; Mesfine et al., 2018) and combination of the two methods (Mulu et al., 2018) to determine caffeine contents coffee beans grown in different parts of the country. Though it has widespread popularity in national and international markets; there are no reports on determination of caffeine content of Nensebo coffee. Therefore, this study was initiated to determine caffeine contents of roasted coffee bean samples obtained from selected site of the study area and coffee bean samples purchased from local markets. Determination of the caffeine contents was conducted using Uv/Vis and HPLC methods.

MATERIALS AND METHODS

Chemicals

The chemicals used in the experiment were standards caffeine powder, methanol dichloromethane, Na_2SO_4 and Na_2CO_3. All reagents used were of analytical grade and were purchased from Ranchmen Co. Ltd. agents, Adds Ababa, Ethiopia.

Collection of coffee bean samples

Coffee bean samples were collected from Nensebo district, West Arsi Zone of Oromia Regional State in Southern part of Ethiopia. The district is located 387 km away from Addis Ababa in southern direction. The district has an altitude of 1450 and 3500 mm above sea level with a total annual rainfall in the range of 741 to 1510 mm. The average temperature was also found to be in the range of 15 to 22°C (Ziyad et al., 2020).

The coffee bean samples (1000 g from each site) were collected from five sites of the district namely Tulu Gola, Habera, Refisa, Nensebo Chebi and Melka Dembi) (Figure 2) in June, 2018. Those areas (sites) were selected due to (i) their high coffee productivity and (ii) their geographical location and agro-climatic condition (altitude, soil, rainfall and climatic condition) variations. Secondly, Nensebo coffee bean samples were also purchased from five coffee traders (in Hawassa and Shashemene cities). One kilogram of coffee bean sample was obtained from each coffee trader**.** The coffee traders were the ones who claimed to possess Nensebo coffee beans. Then the coffee beans were homogenized by mixing 20 gram obtained from each coffee trader.

Sample preparation and extraction

The preparation of coffee samples and their extraction were carried our following procedures used by Kebena et al. (2017). Hundred grams (100 g) of homogenized coffee bean samples (20 g from each coffee sample) was roasted for 25 to 30 min using conventional coffee roasting material (electric roaster). The roasted coffee bean samples were then ground and screened through 250 µm sieve to get a uniform texture (Plate 1). Thirty grams (30 g) of grounded and sieved roasted coffee sample was placed in 250 ml of beaker, and dissolved in 120 ml of distilled water in a temperature range of 80 to 90°C with continuous stirring using magnetic stirrer for 30 min. The coffee solution was allowed to cool to room temperature and then filtered using 0.45 µm filter paper and suction filtration. Small amount of hot water was added to wash the scum left on the filter paper. 3.0 g of Na_2CO_3 was added into the filtrate in order to dissolve tannins and gallic acids in water and to let them remain in the aqueous layer during the extraction process. The filtrate was then subjected to liquid-liquid extraction using 100 ml of dichloromethane in 250 ml separatory funnel. Dichloromethane was chosen as it is known to be the most effective and commonly employed for extraction of caffeine in coffee beans for its low boiling point (39.6°C) and can be used at low temperature (Clarke and Macarae, 1985). The organic phase was separated from aqueous phase and collected in clean and dry flask. The aqueous phase was extracted 3 times with 30 ml of dichloromethane following a literature reported procedure (Clarke, 1980; Belay, 2010). The fraction of organic phases of coffee samples were mixed together and dried with 5 g of anhydrous Na_2SO_4.The solvents were distilled off using rotary vapor to get crude caffeine extract. Similar procedures were employed to extract caffeine from the coffee samples purchased from coffee traders (in Hawassa and Shashemene cities). After the evaporation of dichloromethane, the extracted caffeine was weighed and expressed as percentage.

Preparation of standard caffeine solutions

Caffeine stock solution of 1000 ppm was prepared by dissolving 100 mg of standard caffeine powder with 50 ml of warm ultra-pure water in a 100 ml volumetric flask. Then the flask was filled to the final volume with distilled water (dichloromethane for UV-Vis spectrophotometer) after cooling down to room temperature. 100 ppm of intermediate standard solution was prepared by pipetting 10 ml of stock solution into a 100 ml volumetric flask and brought up to 100 ml mark with ultra-pure water (dichloromethane for UV-Vis analysis). The caffeine working standard solutions of different concentrations (that is, for samples collected from selected sites) of 2, 4, 8 and 16 ppm were prepared from the intermediate solution in a 10 ml volumetric flasks by adding dichloromethane for UV-Vis and ultra-pure water for HPLC analyses. Similarly, standard solutions of 10, 20, 30, 40 and 50 ppm concentrations were prepared by a serial dilution of the stock solution for homogenized Nensebo coffee samples purchased from coffee traders in local coffee markets.

Use of UV/Vis for determination of caffeine

Quantitative analyses of caffeine levels in the coffee samples were determined by measuring their absorbance using a double-beam Uv-Vis spectrophotometer (model PGT180). It was interfaced with a computer using 2 nm resolutions in a 0.5 cm path length quartz cell. The λ_{max} was determined by scanning the standard solution from 200 to 400 nm and the obtained results were given as absorption spectra, which were characterized by a single intensive absorption band located in the Uv range at λ_{max} = 272 nm. Calibration curve was made using the data obtained from the prepared standard solutions (Table 1) until they showed a linear relationship between the absorbance and concentrations. This curve was constructed using absorbance versus concentration to validate the UV-Vis absorption of caffeine in terms of linearity, sensitivity, precision and

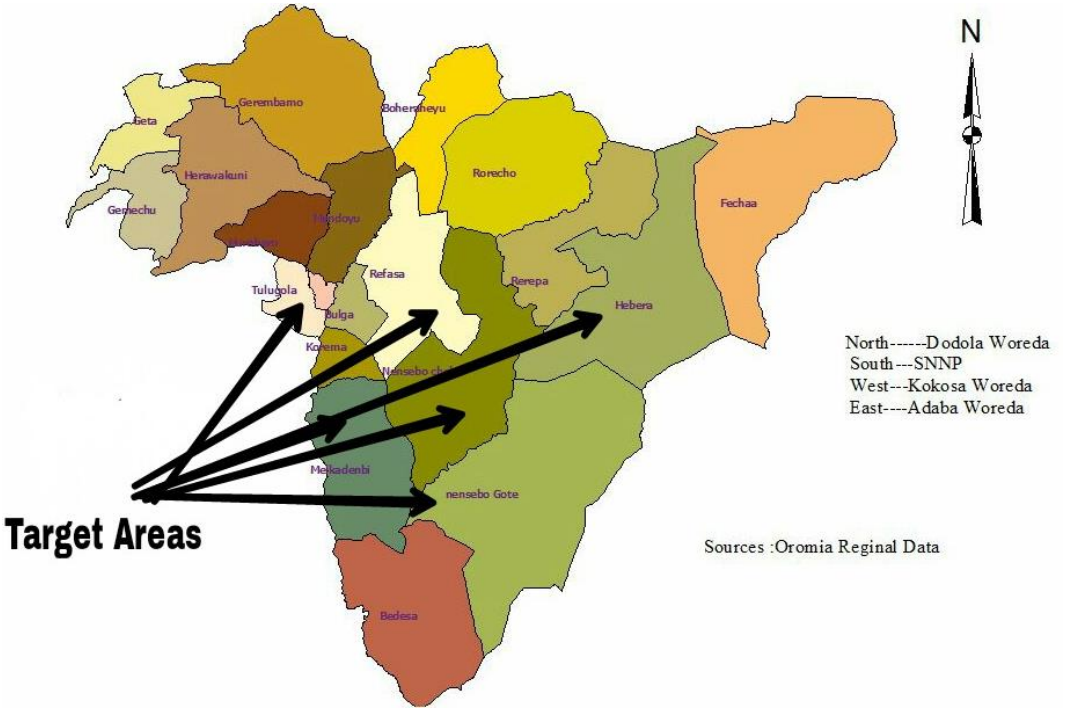

Figure 2. The administrative map of Nensebo district, Oromia Regional State, Ethiopia **(**Map of Nensebo Districts Oromia Regional data**).**
Source: Nensebo District Agriculture Office, June, 2018

Plate 1. The physical appearance of Sun dried coffee, green coffee, roasted and ground coffee bean samples.
Source: Photo by Abera T, June, 2018.

Table 1. The Uv-Vis data of blank solution and standard caffeine solutions.

S/N	ID	Type of solution	Concentration (ppm)	Absorbance
1	S0	Blank	0.0000	0.000
2	S1	Standard	2.0000	0.143
3	S2	Standard	4.0000	0.258
4	S3	Standard	8.0000	0.608
5	S4	Standard	16.0000	1.202

Source: Experimental data of this study.

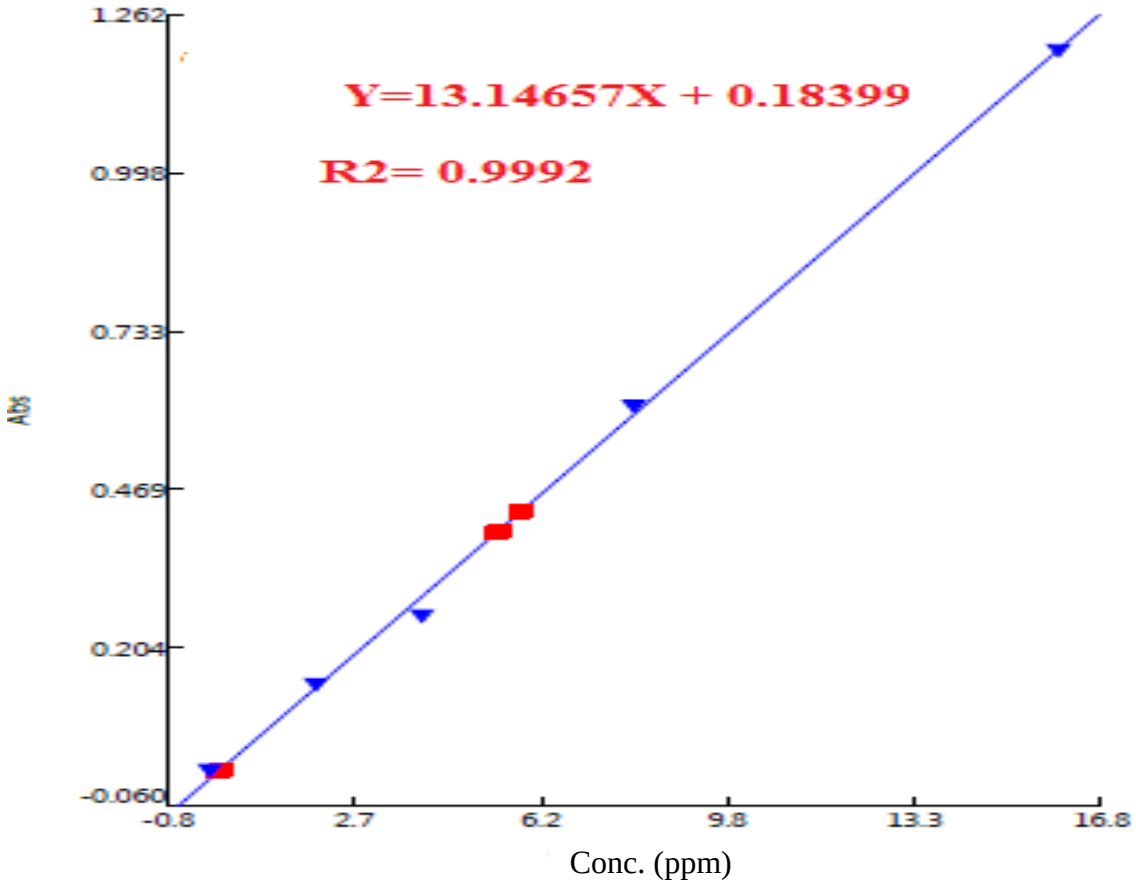

Figure 3. The calibration curve used for validation of UV-Vis measurement of caffeine level in the coffee sample collected from the selected sites of the study area.
Source: Experimental data of this study.

for calibration purpose to determine the caffeine content of the coffee beans (Figure 3). Finally, the quantitative amount of caffeine in samples (in %) was then determined using the standard curve. The analysis was carried out at JIJE Analytical testing Service Laboratory, Addis Ababa, Ethiopia (Doc. No. JATSL/F510-3).

Use of HPLC for determination of caffeine

HPLC machine (Agilent 1260) was used for the determination of caffeine levels of coffee samples obtained from selected sites of the study area. It was equipped with G1310B pump, G1316A column compartment, G1329B auto sampler and G4286B detector (VWD). To determine the caffeine level of the coffee samples, HPLC analysis was also used in the present study. Prior to the analysis, the method was validated using blank solution and standard caffeine solutions. The concentrations of standard solutions used in experiment were 2, 4, 8 and 16 ppm (for samples collected from selected areas). The solutions were then injected into the HPLC machine following the chosen chromatographic conditions (Table 2).

After setting the conditions, the chromatograms of the blank solution and the standard solutions were generated. The blank sample did not give any peak. On the other hand, the standard

Table 2. HPLC conditions at JIJE Analytical testing Service Laboratory during the experiments (for samples collected from selected sites).

S/N	Parameter	Value
1	Mobile phase	Solutions of water and methanol (75:25, v/v)
2	Flow rate	0.5 ml/min
3	Elution condition	Isocratic, 25% methanol
4	Limit of detection (LOD)	0.0018 mg/L
5	Limit quantification (LOQ)	0.0055 mg/L
6	Column type	Agilent Poroshell-C_{18}, 2.1×100 mm and 2.7 µm particle diameter
7	Injection volume	20 µL
8	Column temperature	30°C
9	Detector	272 nm and data rate at 10 Hz
10	Concentration accuracy	5.163 mg/1 or 1.03±0 0.01%
11	Concentration precision	0.01% at 5.163 mg/L

Source: Experimental data of this study.

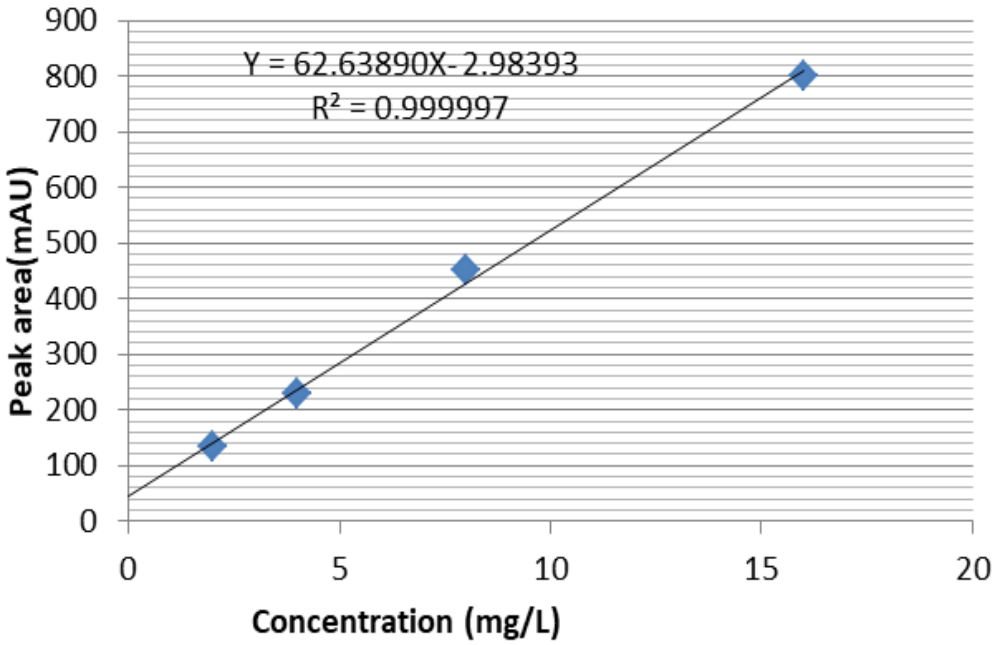

Figure 4. The calibration curve that was used in the HPLC analysis
Source: Experimental data of this study.

solutions (2, 4, 8 and 16 ppm) gave single peak at retention time of 2.887, 2.900, 2.956 and 2.897 min, respectively. A calibration curve was also constructed for the peak areas against concentration of working caffeine standards to validate the HPLC quantification of caffeine in the coffee samples in terms of linearity, sensitivity, precision and for calibration purpose. The curve showed good linear relationship between the peak area and concentrations of the standard solutions. Its equation was derived as Y = 62.63890X - 2.98393 and calibration curve of standard (R^2 = 0.999997) (Figure 4) where Y is peak area (mAU), X is concentration of caffeine (mg/L) and R is the linear correlation factor. The validated method was used to determine caffeine analysis in the coffee samples. The HPLC analyses were carried out at JIJE Analytical testing Service Laboratory, Addis Ababa, Ethiopia (Doc. No. JATSL/F510-3).

Similarly, HPLC machine (ES ISO 20481) was determined to determine the caffeine level of Nensebo coffee sample purchased from local coffee markets (in Shashemene and Hawassa cities) (Table 3). The standard caffeine solutions with concentrations of 10, 20, 30 and 40 ppm were used to validate the method.

The calibration curve with equation Y = 2.66642X - 0.565167 and R^2 = 0.999816) was constructed where Y is peak area (mAU), X is concentration of caffeine (mg/L) and R is the linear correlation factor. The curve showed good linear relationship, and the method was taken as suitable and reproducible for the quantitative determination of caffeine extracted from the coffee samples used in the study (Figure 5). This analysis was carried out at Bless Agri

Table 3. The HPLC conditions at Bless Agri Food Laboratory Services during the experiments (for samples collected from coffee markets).

S/N	Parameter	Value
1	Mobile phase	water and methanol (76:24, v/v)
2	Flow rate	1 ml/min
3	Elution condition	Isocratic, 24% methanol
4	Limit of detection (LOD)	0.0013 mg/L
5	Limit quantification (LOQ)	0.0036 mg/L
6	Column type	C_{18} , 2.1×100 mm and 2.7µm particle diameter
7	Injection volume	10 µL
8	Column temperature	25°C
9	Detector	272 nm and data rate at 10 Hz=
10	Concentration accuracy	1.14 ±0 0.01%
11	Concentration Precision	0.01%

Source: Experimental data of this study.

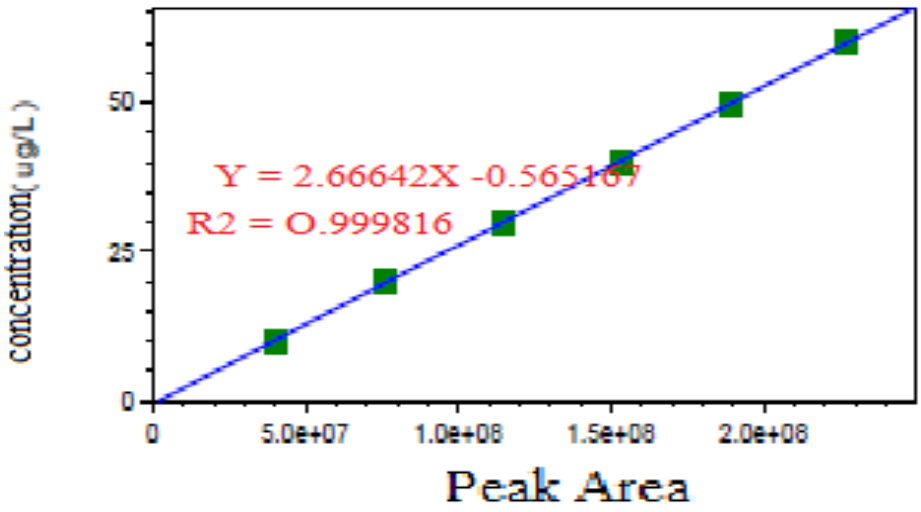

Figure 5. The calibration curve that was use in the HPLC analysis (coffee samples collected from coffee traders in Hawassa and Shashmene cities)
Source: Experimental data of this study.

Food Laboratory Services PLC, Addis Ababa, Ethiopia.

RESULTS AND DISCUSSION

There are different analytical methods/tools that have been used for determination of caffeine contents in different products including coffee. In such reports, the use of one analytical method (such as UV/Vis only) is difficult to check whether the work is done accurately or not (Castro et al., 2010; Abdul et el., 2006; Smith, 2005; Fung, 1985). It is advisable to employ at least two analytical methods. Most literature reports showed that UV/Vis and HPLC methods as the two most commonly reported techniques used together for determination of caffeine contents of coffee products (Castro et al., 2010; Abebe et al., 2008; Huck et al., 2005). In this study, determination of caffeine levels of roasted coffee samples of Nensebo coffee beans were determined using the above two methods. Determination of caffeine level of the raw coffee beans was not attempted for the following two coffee beverages are mainly prepared from roasted coffee beans. reasons; (i) reports show roasting does not affect caffeine level of coffee bean (Tawfik and Bader, 2005) and (ii)

Amount and percent yield of crude caffeine extract

Caffeine was extracted from roasted coffee bean samples

using dichloromethane. A yellowish-white solid product was obtained. The masses crude caffeine products were found to be 300 mg (1.0%) and 200 mg (0.67%) for coffee samples collected from selected sites and local coffee markets, respectively, from 30 g of roasted coffee powder. The data indicates the percent yield of the crude caffeine extracts (1.0 and 0.67%) to be lower than that of roasted coffee bean samples of Bale (1.8 ± 0.002 - 2.0±0014%) (Mesfine et al., 2018) and Harar (1.40 ± 0.015%) and Yirgacheffee coffee samples (1.96±0.018%) (Tamiru et al., 2018). This is in line with higher preferences or prices of coffee beans of Nensebo coffee than Harar and Yirgacheffee coffee beans in markets. The percent yield was calculated using the following formula;

$$\% \text{ yiled of crude caffeine} = \frac{\text{mass of the crude caffiene}}{\text{Mass of coffee powder used for extraction}} \times 100\ \%$$

Quantitative determination of caffeine using UV-Vis spectrophotometer

In this study, the caffeine level of Nenesebo coffee bean sample was determined by measuring the intensity of the absorption of a series of concentrations of standard caffeine solutions in dichloromethane. Dichloromethane is reported to be the most suitable organic solvent caffeine from coffee beans as it is free matrices interfering with the measurement (Rofti, 1971; Clarke, 1980; Abebe et al., 2008). Using the calibrated curve/validated method, the average caffeine level of homogenized coffee beans collected from Nensebo area was found to be 1.17±0.001% (w/w in dry basis) as measured at 272 nm (Showkat et al., 2015; Ephrem et al., 2016). The finding of the study revealed that the mean percentage caffeine contents of Arabic coffee of Nensebo coffee beans to be lower than that of the Ethiopian Coffee arabica produced Wmbera (1.53 ± 0.003%), Goncha (1.41 ± 0.040%) and Zegie (1.29 ± 0.033) (Yigzaw et al., 2007). But comparable with the reported caffeine levels of coffee beans obtained from Kaffa (1.18%), Illu Ababor (1.10%) (Maria et al., 2000), Bench Maji (1.10±0.01%), Yirga chefe (1.01±0.04%), Tepi (1.07±0.02%), Burie (0.97 ± 0.049%) and Godere (1.19±0.02%) areas of Ethiopia (Belay, 2010). The caffeine content reported for Ethiopian *Arabica* coffee concentration ranges from 0.90 to 1.27% by using UV/Vis spectrophotometer (Zewdu et al., 2016; Belay and Cholap, 2009).

Quantitative determination of caffeine using HPLC analysis

Despite its cost/expense and complexity, HPLC is commonly used to determine caffeine contents of coffee beans and coffee products for its high speed, reliability and high accuracy. In the present study, HPLC method was also used along with UV-Vis method (Weinberg, 2001; Perrone et al., 2008; Gopinandhan, and Ashwini, 2014). Thus, in this study, the validated method was used to determine the caffeine contents of the coffee samples by injecting the solutions prepared from crude caffeine extracts of the coffee samples used in the study. Then the analysis of the coffee sample was carried out in triplicates. The result (chromatogram) showed that the crude extract from roasted Nensebo roasted coffee sample was averagely found to be 1.03 ± 0.01%.

Nensebo coffee is well known and has high consumer demand in local coffee market. This made its price higher as compared to other coffee beans growing in the areas such Yirgachefe and Dilla. Thus, to get high prices, it is a common practice by coffee traders (in local market) to adultrate (mix) Nensabo coffee bean with the coffee beans produced in the surrounding areas (Alemayehu, 2014). Similar procedures, were employed to investigate the caffeine contents coffee samples purchased from local coffee markets inorder to investgate whether it is genuine or mixed. The prepared caffeine solution was injected into HPLC machine to determine its caffeine level. The experiment was done in triplicates, and the average values of caffeine content in the crude extract coffee sample purchased from local coffee markets (coffee sellers in local coffee markets) was found to be 1.14±0.01%. The results in both cases indicated that the caffeine contents of Nensebo coffee to be comparable with caffeine contents of Illubabor (an average value of 1.10%), Gedeo Yirga chefe (1.10%), Gomma Limu (1.0%) and Finote selam (an average value of 1.10%) coffee beans that were measured using HPLC analysis (Belay., 2010; Yigzaw et al., 2007; Maria et al., 2000). Moreover, the concentrations of caffeine in coffee collected from Nensebo district (1.03%) and that of coffee beans purchased from local markets (1.14%) were found to be comparable to each other. This suggests that the coffee bean obtained from the local markets were genuine Nensebo coffee beans (not blended with other coffee beans produced in the nearby localities/areas). The data are also in range of caffeine contents export standard Ethiopian coffee beans (that is, 0.46 to 2.82% as determined by HPLC) (Yigzaw et al., 2007; Silvarolla et al., 2004; Maria et al., 2000) and also with the data reported by Illy (2002) states that an average values of caffeine contents to be less than 1.5% for *Coffea arabica* beans. Therefore, the coffee beans of the study area can be used directly without further decaffeination process. This is because its low caffeine content indicates that the coffee beans of Nensebo coffee beans have acceptable (high) quality. However, further studies are recommended to anlyse other constituents such as trigonelline, 3,4-dicaffeoylquinic acid and its mineral contents to get a better information about quality of the Nensebo coffee beans.

Conclusion

The caffeine contents of Nensebo coffee bean samples were successfully determined using the most commonly methods namely HPLC and UV-Vis spectrophotometry. The concentration of caffeine levels of roasted coffee bean samples were found to be 1.17±0.01 and 1.03±0.001% (w/w) as data obtained from UV-Vis spectrophotometry and HPLC methods, respectively. Similarly, the caffeine content of Nensebo coffee bean sample purchased from coffee markets was determined HPLC method (ISO 20481), and the caffeine level was found to be 1.14 ± 0.001% (w/w). These data are in good agreement with the reported caffeine contents of Arabica coffe from many coffee producing regions of the country and are also in the acceptable caffeine level range for good quality coffee beans. However, further studies are recommended to analyze other chemical constituents in order to fully characterize coffee beans of the study area, and also to reach at concrete conclusion on the factors/reasons that made Nensebo coffee beans to be relatively more expensive and preferable than most coffee brands of in local markets in and around the study area.

AVAILABILITY OF DATA AND MATERIAL

Chromatograms of standard and blank solutions are available as supplementary materials

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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