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Chemistry

KEY WORDS: Caffeine determination; HPLC; Raw coffee beans, Dark roasted and light roasted coffee beans; caffeine content

COMPARISON OF CAFFEINE CONTENTS OF *YIRGACHEFFE* AND *HARAR* COFFEE BEANS USING HPLC ANALYSIS

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ABSTRACT

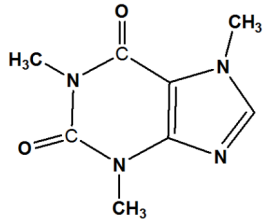
The objective of the present study was to determine the caffeine levels of *Arabica Yirgacheffe* and *Harar* coffee beans using HPLC analysis. The coffee beans were obtained from *Yirgacheffe Coffee* Farmers' Cooperative Union and Harar coffee supplier, Addis Ababa. The raw and roasted beans were ground, boiled in distilled water, and extracted with dichloromethane. The HPLC analysis revealed that caffeine contents *Yirgacheffe raw, light roasted* and *dark roasted* coffee sample extracts to be 1.23 ± 0.011%, 1.29 ± 0.025% and 1.24 ± 0.015%, respectively. Whereas the caffeine levels were 1.30 ± 0.021%, 1.35 ± 0.018% and 1.33 ± 0.016% for *raw*, *light roasted* and *dark roasted coffee* samples of *Harar*. The findings of the study indicated that *Yirgacheffe* coffee samples generally have lower caffeine contents than *Harar* coffee samples. The caffeine contents of the coffee samples used in the study are within the range of reported standards of Ethiopia.

INTRODUCTION

Coffee is one of the most popular beverages in the world as well as the most traded commodity that takes the second rank next to oil (**Fujioka & Shibamoto, 2008**). Coffee plant was originally found and cultivated in *Keffa* province of Ethiopia in the 6th century. Then Arab traders took its seeds from the place of its origin (Ethiopia), and started the first coffee plantation in Arabian region. Then it was distributed to the whole Europe **(Heran, https:// open.bu.edu/bitstream/handle)**. Not only distributed to different regions of the world but also it became one of the most widely consumed beverages throughout the world due to its pleasant taste, aroma, stimulant effect and health benefits (**Schenker *et al.*, 2002; Camargo, Toledo & Farah, 1999**). Coffee beans (seeds) are the most important parts of the plant in this regard. In addition to compounds that are responsible for all observed properties (stimulant and aroma effects and health benefits), the seeds are also known to contain carbohydrates, proteins, lipids, minerals and vitamins (**Higdon & Frei, 2006; Singh, 2006**)

There are about sixty different coffee species. However, only two species namely Coffea arabica and Coffea robusta have commercial values or economical importance (**Higdon & Frei, 2006**). For instance, 66% of the world production mostly comes from Coffea arabica and 34% comes from Coffea robusta (**Singh, 2006**). These coffee species are different in their chemical compositions mainly in their caffeine levels. Coffea arabica is considered to be of higher quality bean, prized for its complex aroma, flavors and low caffeine levels (0.8 to1.4%). It is the most expensive species in the world coffee market (**Adepoju *et al.*, 2017; Gray, 1998**).

Coffee quality refers to a desirable appearance, attractive flavor, good cup taste and level of caffeine. Coffee quality is very critical in coffee industry/market (**Adepoju *et al.*, 2017; Abrar & Nugussie,2013;Heran, https:// open.bu.edu/ bitstream/ handle; https:// www. kew.org/ sites/ default/ files/Coffee Farmin**). In addition to caffeine level, there are other factors that affect the quality of coffee. Some of the factors are the genotype, age of coffees, climatic conditions, soil characteristics, agricultural practices, harvesting methods and post-harvest processing techniques such as drying system, storage conditions, roasting levels, preparations of the beverage and girding size (systems) (**Heran,https://open.bu.edu/bitstream/handle;https://www.kew.org/sites/default/files/Coffee Farmin**). Though there are health benefits of caffeine (**Shirisha & Varalakshmi, 2016; Higdon & Frei, 2006; Svilaas et al., 2004; Klang *et al.*, 2002**), there are also reports that associate higher caffeine contents with low quality of coffee (**Farah *et al.*, 2006**) and/or harmful health effect (**Suter *et al.*, 1993**). Therefore, high concentration of caffeine in coffee needs to be decaffeinated before it is supplied to consumers or markets. This, in turn, incurs cost to coffee industries (**Farah *et al.*, 2005**). This is the reason why people need to consume coffee with low caffeine levels or decaffeinated coffee. This makes determination of caffeine levels of coffee beans produced in different countries before marketing in order to meet consumer preferences or to fix market price.



Caffeine

Ethiopia is the first (from sub-Saharan Africa) and fifth (from the world) coffee producer (**ICO.www.ico.org**). The coffee produced in Ethiopia is *Coffea Arabica* (**Mekuria *et al.*, 2004**). Coffee contributes the lion share in the national economy (**Abu and Tedy, 2013; http://www.ebc.et/web/ennews/-ethiopia earns-record-866-mln-usd-from-coffee-export**), and also to the livelihood of millions of Ethiopians. Besides its economic importance, coffee has a strong historical, cultural and social importance (**Heran, https://open.bu.edu/bitstream/handle; https://www.kew.org/sites/default/files/Coffee Farmin**).

The major coffee producing regions of the country are Oromia and Southern Nations Nationalities and Peoples of Region (SNNPR). Other region such as Tigray produces coffee in small amounts. Many written documents have discussed four types of coffee production systems of the country. These are forest coffee, semi-forest coffee, garden coffee and semi-modern plantation coffee. Forest coffee, semi-forest and garden coffee and semi-modern plantation account 10%, 70% and 20%, respectively, to the total coffee production of the country (**Abrar & Nugussie, 2013; https://www.kew.org/sites/default/files/Coffee Farmin**).

There are many analytical methods or tools that have been used for determination of caffeine contents in different products including coffee (**Fung *et al.*, 1985; Abebe *et al.*, 2008; Jeffery *et al.*, 2008**). Some the methods have their own limitation such as requiring large amount of sample or unable to use them with

samples complex matrices or interferences from electro chemical impurities, etc. Most literature reports showed that UV/VIS and HPLC methods are the two most commonly used (reported) techniques for the determination of caffeine contents of coffee products (**Tsegaye, 2009**). HPLC is the most recent and relatively reliable method that is used to determine the caffeine contents of different samples/product. It could be used alone or in combination with methods such as UV/VIS, mass spectroscopy and infrared spectrometry (**Sanda *et al.*, 2015; Gopinandhan & Aswini, 2014; Hecimovic et al, 2013; Huck *et al.*, 2005**).

There are reports on the use of above mentioned methods (UV/VIS and HPLC) in determination of caffeine contents of Ethiopian coffee samples obtained from different regions (localities). The caffeine contents of coffee beans grown in Wembera, Goncha, Zegie and Burie localities, North-West Ethiopia, were determined using UV/VIS spectrophotometric method. The report showed that the caffeine contents to be 1.53 ± 0.003%, 1.41 ± 0.040%, 1.29 ± 0.033% and 0.97 ± 0.049% for Wembera, Goncha, Zegie and Burie coffee beans, respectively (**Belete & Solomon; 2015**). Similar method was used to investigate caffeine contents of Coffea arabica beans grown in south western Ethiopia specifically in Bench Maji, Gediyo Yirgacheffe, Tepi and Godere areas, and found the caffeine contents to be 1.1 ± 0.01%, 1.01 ± 0.04%, 1.07 ± 0.02% and 1.19 ± 0.02%, respectively (**Belay *et al.*, 2008**). Similarly, another reports on caffeine content determination of Coffea arabica samples collected from fifteen districts of Hararghe region. The UV/VIS method used for the study showed the amounts of caffeine contents to be 0.60820 ± 0.00277% for Chiro (Dingetie) coffee, 0.71138 ± 0.00544% for Chiro (Gewgewu) coffee, 0.79826 ± 0.00254% for Gurache coffee, 0.90285 ± 0.00601% for Harar Aboker coffee, 0.75884 ± 0.00538% for Harar Zuria-1 coffee, 0.67336 ± 0.00231% for Harar Zuria-2 coffee, 0.67852 ± 0.00499% for Dire Dawa coffee, 0.60098 ±0.00214 for Jarso coffee, 0.51789 ± 0.00231% for Kobo coffee, 0.83628 ± 0.00301% for Kurfacheli coffee, 0.68151 ± 0.00273 % for Girawa coffee, 0.64252 ± 0.00651% for Goru Mute coffee, 0.75053 ± 0.00295% for Hirina Zuria-1 coffee,0.72837 ± 0.00313% for Hirina Zuria-2 coffee and 0.72284 ± 0.00673% for Langie coffee. Harar Aboker coffee was reported to be the highest in its caffeine concentration than all of the other coffee samples used in the study (**Ephrem *et al.*, 2016**). A related report on the caffeine contents determination of Coffea arabica grown in Wolaita Zone of five different districts, namely Boloso Sore, Boloso Bombe, Kindo Koisha, Sodo Zuria and Humbo using UV/VIS spectrophotometer. The results from such study showed that caffeine contents to be 0.50715 mg/ml, 0.56407 mg/ml, 0.50197 mg/ml, 0.55366 mg/ml and for Sodo Zuria coffee for Boloso Sore coffee, Boloso Bomba coffee, Kindo Koisha coffee. 0.55366 mg/ml for Sodo Zuria coffee and 0.52785 mg/ml for Humbo coffee (**Zewdu et al, 2016**). There are also several research reports on the use of HPLC analysis to determine the caffeine contents in coffee bean samples or coffee beverages. For instance, Sanda et al (2015), reported amount of caffeine concentration to be 1.6351 mg/ml and 1.51 mg/ml for green and roasted coffee beans, respectively. Other authors also reported the caffeine concentrations of instant, ground and decaffeinated Coffea arabica samples using HPLC analysis, and found that the amounts of caffeine contents in the samples to be 32.5 mg/g, 13.5 mg/g, and 0.7 mg/g, respectively (**Nogueira & Lago, 2007**). Another author also used the same method, and found that the caffeine contents of coffee beans to increase during the roasting process, from 9.60 to 12.60 mg/g (**Farah et al, 2006**). Similarly, the caffeine contents of green, light roasted, medium roasted and dark roasted of coffea arabica Cioccolatato bean samples have been found to be 1.21 ± 0.11%, 2.24 ± 0.21%, 1.59 ± 0.14% and 1.53 ± 0.14%, respectively (**Hecimovic et al, 2013**).

There are also some reports on the caffeine content determination of Ethiopian coffee varieties using HPLC method. For example, the caffeine contents of Coffea arabica beans grown in Kaffa and Illubabor areas were determined using this method. The results of HPLC analysis of the author showed that the caffeine contents were found to be in the range of 0.46-2.82% (an average value of 1.18%) for Kaffa coffee and 0.42-2.90% (an average value of 1.10%) for Illubabor coffee beans (**Maria *et al.*, 2000**). Similar analyses were employed on 42 Ethiopian green coffee bean samples grown at Finote Selam area, Ethiopia, showed that the amount of caffeine contents of the coffee samples used in the study to be 1.10 % (on average) and in the range of 0.91 ± 0.01 % to 1.32 ± 0.06 % (**Yigzaw, et al., 2007**).

As discussed above, the major coffee producing regions are found in Western, Southern and Eastern regions (**https:// www.kew.org/ sites/default/files/Coffee Farmin**). The coffee beans from these different regions have different qualities (caffeine contents along with other chemicals). These differences could have implication in consumer preference and market prices. Therefore, the present study aims on caffeine content determination of coffee bean samples obtained from Yirgacheffe and Harar coffee samples using HPLC method.

2.0. Experimental section

2.1. Plant material collection

Yirgacheffe and Harar coffee bean samples were obtained from Yirgacheffe Coffee Farmers Cooperative Union (from Addis Ababa) and Harar (Asebe Teferi) (from Addis Ababa in the month of August 2017).

2.2. Sample preparation and extraction

A total of six coffee samples (*Arabica Yirgacheffe raw, Arabica Yirgacheffe light roasted, Arabica Yirgacheffe dark roasted, Arabica Harar raw, Arabica Harar light roasted and Arabica Harar dark roasted)* were prepared for the experiments (**Supplementary Fig. 1**). In both cases, 30 g of raw, light roasted and dark roasted coffee bean samples were ground using grinding machine. Then, the powders were screened through 250 µm sieve to get a uniform texture.

Procedures reported in literature (**Sanda *et al.*, 2015; Gopinandhan & Ashwini, 2014; Hecimovic *et al*, 2013**) were used to carry out the caffeine extraction. An accurately 0.5 g of each ground and sieved coffee powders (samples) were placed in six 250 ml beakers that contain 100 ml of distilled water. The mixtures were boiled in a temperature range of 80-90 ^{0}C for 30 minutes using hot plate with magnetic stirrer. The hot solutions were cooled to room temperature, and filtered in conical flasks using suction filtration setup to get rid of the suspended particles. To each of the filtrates, 2 g of Na_2CO_3 was added in order to dissolve tannins and gallic acids in water and to let them remain in the aqueous layer during the extraction process. The filtrates were subjected to liquid-liquid extraction by adding 100 ml of dichloromethane.

The mixtures were transferred into separatory funnels and swirled gently and allowed to stand until clear boundaries were observed between the organic and aqueous phases. The organic phase was separated from aqueous phase and collected in clean and dry flasks. The aqueous phase (in each case) was extracted 4 times with each 20 ml of dichloromethane. Few grams of anhydrous Na_2S0_4(drying agent) were added to the organic phases to remove traces of water molecules from the extracted caffeine. The organic solvent (dichloromethane) that was used for the extraction was removed under reduced pressure (or using rotary evaporator). The dried crude caffeine extracts were weighted and kept in refrigerator until used for further HPLC analysis.

2.3. Preparation of standard solutions

To determine quantity of caffeine in the coffee samples, standard solutions were prepared by dissolving known quantity of standard caffeine in known volume of solvent. The standard solutions were used to determine retention time and caffeine content of the extracts. In the present study, standard 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 and filled to the final volume with distilled water 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 volume with ultra-pure water. The caffeine working standard

solutions of different concentrations (0, 5, 10 and 25) ppm were prepared from the intermediate solution in a 10 ml volumetric flasks with ultra-pure water. A calibration curve of peak area versus concentration of the standards was plotted using standard solutions of different concentrations mentioned above.

2.4. HPLC analysis of caffeine

All HPLC analyses were carried out at JIJE Analytical Testing Service Laboratory, Addis Ababa. The standards and the samples were run in the HPLC system using the HPLC machine (Agilent 1260 equipped with G1310B pump, G1316A column compartment, G1329B auto sampler and G4286B detector (VWD)).The following table (**Table 1**) shows the parameters or the HPLC conditions that have been used during the experiments.

Table 1. The HPLC conditions used during in the experiment

Parameters	Values
Mobile phase	Solutions of water and methanol (75:25, v/v)
Flow rate	1.0 ml/min
Elution condition	Isocratic, 25% methanol
Limit of detection (LOD)	0.0018 mg/L
Limit quantification (LOQ)	0.0055 mg/L
Column type	Agilent Zorbax Eclipse XDB C18 4.6 x75 mm and 5µm particle diameter
Injection volume	20 µL
Column temperature	25 °C
Detector	272 nm and data rate at 10 HZ
Concentration accuracy	101.25 % at 33.22 mg/L
Concentration precision	0.19 % at 33.22 mg/L

3.0. Results and Discussion

3.1. Caffeine yield

Caffeine was extracted from raw, light roasted and dark roasted coffee beans using dichloromethane. A yellowish-white solid (crude) product was obtained. The results/data indicated that raw coffee sample gave the highest amount of crude caffeine (8.17 mg or 1.63 ± 0.017 %) followed by light roasted and dark roasted that were found to give 8.00 mg (1.60 ± 0.016 %) and 7.00 mg (1.40 ± 0.015%) crude caffeine, respectively. Similarly, the masses of the crude caffeine of *Harar coffee* samples were found to be 11.33 mg (2.27 ± 0.023%), 10 mg (2 ± 0.021%) and 9.73 mg (1.96 ± 0.018 %) for raw, light roasted and dark roasted coffee samples, respectively (**Table 2**). The values (masses or percentages) of *Harar coffee* samples were relatively higher than the corresponding values for *Yirgachefe* coffee samples. Moreover, in both cases, the masses of the crude caffeine extracts decreased in the order of raw, light roasted and dark roasted coffee samples (Table 1). This result was in a good agreement with literature report showed that for both cases the masses of extracted crude caffeine obtained from raw coffee samples were higher than that of roasted coffee samples because weight loss increased with increasing temperature or degree of roasting due to release of water, CO_2 and emission of volatile organic compounds (**Niya 2012**).

Caffeine is a white solid compound. The observed yellowish-white color of the product could be attributed to the presence of some other compounds along with the extracted caffeine. The decreased in masses of the crude caffeine extracts on roasting may also suggest possible existence of other compounds such as chlorogenic acids that could be extracted together with caffeine. Such compounds may be decomposed upon roasting. This could be the reason to obtain lowest masses of crude caffeine extracts from the roasted coffee samples. This observation was also consistent with literature reports indicated that temperature or degree of roasting decreases amounts of crude caffeine contents of coffee samples (**Antonio *et al*, 2011; Weinberg, 2001**).

3.2. Quantitative determination of caffeine using HPLC method

3.2.1. Method validation

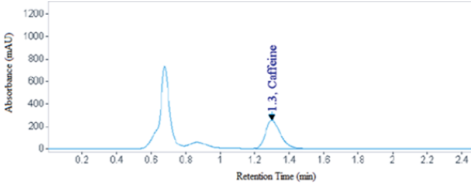
The method of the study can be validated using standard solutions of different concentrations (0, 5, 10 and 25 ppm). The standard solutions were injected into the HPLC machine following the chosen chromatographic conditions (Section 2.4). The solution with 0 ppm did not give any peak (**Supplementary Fig 2**). On the other hand, the rest standard solutions (5, 10 and 25 ppm) showed single caffeine peak at retention time of 1.305, 1.299 and 1.304 minutes, respectively (**Supplementary Fig 3**(**A-C**)). Moreover, the peak areas were found to increase with concentration. The peak areas were found to be 288.518, 547.941 and 1342.375 for 5, 10 and 25 ppm solutions, respectively. A calibration curve for peak area against concentration of working caffeine standards was constructed to validate the HPLC quantification of caffeine in terms of linearity, sensitivity, precision and for calibration purpose to determine the caffeine contents of various coffee samples. The curve showed good linear relationship between the peak area and concentrations of the standard solutions. Its equation was derived as Y = 52.8X + 22.9 and calibration curve of standard (R^2 = 1.0000) (**Supplementary Fig. 4**), where Y is peak area, X is concentration of caffeine (mg/L) and R is the linear correlation factor. Hence, the chosen method was taken as suitable and reproducible for the quantification of caffeine extracted from the coffee bean samples used in the experiment.

Table 2. The mean mass and percentage of crude caffeine for three independent measurements extracts obtained from raw and roasted coffee samples

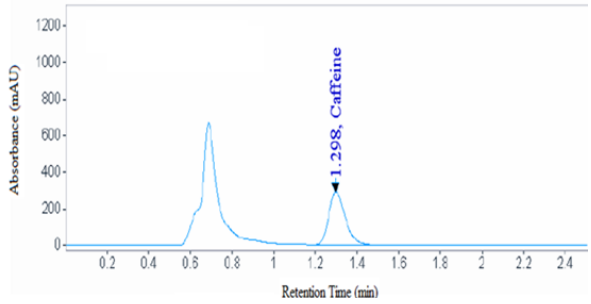
Coffee bean sample used for extraction	*Yirgachefe coffee*		*Harar coffee*	
	Mass of extracts (mg)	Yield of extracts (%)	Mass of extracts (mg)	Yield of extracts (%)
Raw	8.17 ± 0.009	1.63 ± 0.017	11.33 ± 0.003	2.27 ± 0.023
Light roasted	8.00 ± 0.002	1.60 ± 0.016	10.00 ± 0.007	2.00 ± 0.021
Dark roasted	7.00 ± 0.005	1.40 ± 0.015	9.80 ± 0.003	1.96 ± 0.018

3.2.2. Determination of caffeine contents of coffee samples

HPLC methods are the most common, reliable methods for the determination caffeine in complex samples. Very low concentration of caffeine can also be determined with high accuracy and precision using HPLC (**Patil, 2012; Joon & Yoo, 2009**). The validated method (of the experiment) 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. The first samples injected into the machine were the solutions of Yirgacheffe and Harar raw coffee samples. The result (chromatogram) showed that the crude extracted from raw coffee sample gave caffeine peak at retention time of 1.30 minutes (**Fig 1.** (A)). The caffeine content obtained from Yirgacheffe raw coffee sample was 28.51 mg/L or 12.30 mg/g or 1.23 ± 0.011% (**Table 3**). In the case of crude caffeine solution which was extracted from Harar raw coffee sample, the chromatogram showed caffeine peak at retention time of 1.298 minutes (Fig 1. (B)). Thus, the caffeine content of this extract was 13.08 mg/g or 30.20 mg/L or 1.30 ± 0.021% (**Table 3**). Therefore, the chromatograms indicated that the caffeine content of Harar raw coffee sample was generally higher than that of *Yirgacheffe coffee* sample. Moreover, the caffeine content of raw Yirgacheffe coffee beans was comparable reported for Gediyo area [46].



(A)

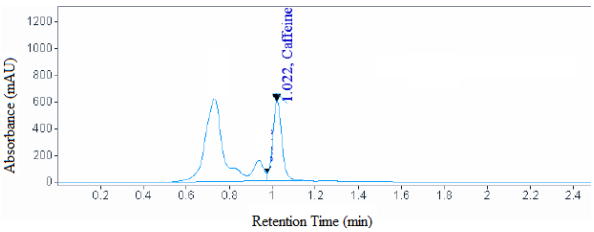


(B)

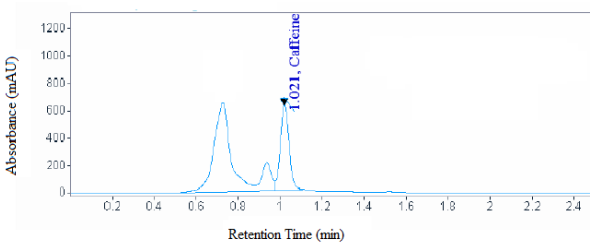
Fig 1. The HPLC chromatogram of *arabica YirgaCheffe raw coffee* sample (A) and *arabica Harar raw coffee* sample (B)

Similar procedures were employed to investigate the caffeine contents of crude caffeine extracts obtained from light roasted coffee samples. The results showed the appreance of peaks at retention times of 1.022 minutes (Fig 2 (A)) and 1.021 minutes (Fig 2. (B)) for *Yirgacheffe and Harar light roasted coffee* samples, respectively. The caffeine contents of *light roasted Yirgacheffe coffee* sample (12.95279 mg/g or 30.97mg/L or 1.29 ± 0.025%) was slightly lower than that of Harar coffee (13.59 mg/g or 33.22 mg/L or 1.35 ± 0.018%) light roasted coffee sample (Table 3). But the caffeine contents of light roasted coffee samples of both *Yirgacheffe* and *Harar* were higher than their *raw* and dark roasted coffee samples (Table 3).

These results were in an agreement with literature reports on HPLC analysis of caffeine contents of *coffea arabica Minas* and *Cioccolatato* bean samples (Hecimovic et al, 2013). The amount of caffeine contents in green, light roasted, and dark roasted of *coffea arabica Minas* samples were found to be 0.66 ± 0.04, 1.07 ± 0.03, and 0.86 ± 0.01%, respectively. Earlier reports on the caffeine contents of green, light roasted, and dark roasted of *coffea arabica Cioccolatato* bean samples indicated in the range of 1.21 ± 0.11, 2.24 ± 0.21 and 1.53 ± 0.14%, respectively (Hecimovic et al, 2013). The detection of caffeine in this study was slightly smaller than literature reports. This could be due to difference in: coffee species, geographical origin, harvesting methods and post harvesting process techniques (such as roasting process, brewing process, grinding size, etc).



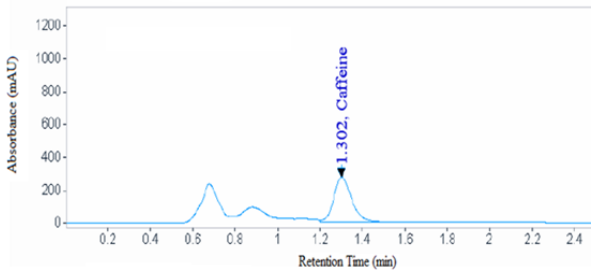
(A)



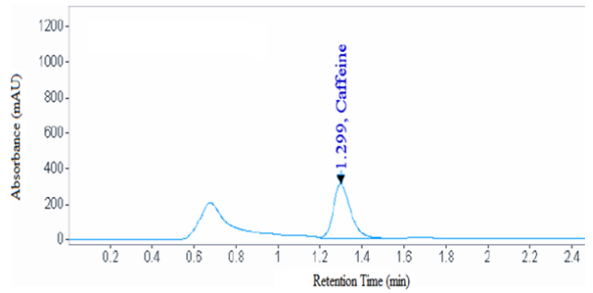
(B)

Fig 2. The HPLC chromatogram of *light roasted arabica Yirgacheffe coffee* sample (A) and *light roasted arabica Harar coffee* sample

Similar procedures that were employed on dark roasted coffee extracts, showed caffeine peaks at retention times of 1.302 and 1.299 minutes for *Yirgacheffe* (Fig 3. (A)) and *Harar* (Fig 3. (B)) *coffee* samples, respectively. The concentration of caffeine contents in the extracted crude of dark roasted *Yirgacheffe* and *Harar coffee* samples were found to be 12.37 mg/g or 30.05 mg/L or 1.24 ± 0.015% and 13.39 mg/g or 32.73 mg/L or 1.33 ± 0.016%, respectively **(Table 3)**. The findings of this study suggested that the caffeine contents of dark roasted *Yirgacheffe coffee* to be lower than that of *Harar* dark roasted *coffee* sample.



(A)



(B)

Fig 3. The HPLC chromatogram of dark roasted arabica *Yirgacheffe coffee* sample (A) and dark roasted *Arabica Harar coffee* sample **(B)**

Table 3. Summary of results obtained from HPLC chromatograms

Coffee Variety	Degree of roasting	Mass of caffeine (mg/g)	Caffeine concentration (mg/L)	Percentage (%w/w) of caffeine
Yirgacheffe	Raw	12.30112	28.51	1.23 ± 0.011
	Light roasted	12.95279	30.97	1.29 ± 0.025
	Dark roasted	12.36543	30.05	1.24 ± 0.015
Harar	Raw	13.07816	30.20	1.30 ± 0.021
	Light roasted	13.58940	33.22	1.35 ± 0.018
	Dark roasted	13.38855	32.73	1.33 ± 0.016

In both cases, when the caffeine contents of raw and roasted coffee samples were compared to each other, the caffeine contents of roasted coffee samples were slightly higher than that of their raw coffee samples (**Tabe 3**). This result was in line with literature reports showed that the caffeine contents of arabica green coffee bean was slightly lower (0.9-1.2 %) than its roasted coffee bean (1.1-1.3%) (**Patil, 2012; Joon & Yoo, 2009; Belay *et al.*, 2008**). Moreover, the caffeine contents of light roasted coffee samples were higher than that of green and dark roasted coffee samples (**Table 3**). This is consistent with litrature report (**Andrews *et al.*, 2007**) that showed HPLC analysis of showed amount of caffeine in green, light roasted, medium roasted and dark roasted of Coffea arabica to be 0.66 ± 0.04%, 1.07 ± 0.03%,0.82 ± 0.03%, and 0.86 ± 0.01%, respectively.

The findings of this study also showed that the caffeine contents of *Yirgacheffe* and *Harar coffee* beans were slightly higher than the reported caffeine contents of other Ethiopian coffee beans such as

Kaffa (1.18%) and *Illubabor* (1.10%), *that were* determined using HPLC analysis (**Belay *et al*., 2008; Maria et al., 2000**). The variation in caffeine levels of coffee samples may be due to geographical origins which might have different altitude, soil type, rain fall, agricultural practices, environmental conditions, variety of coffee species, harvesting methods, post harvesting processing techniques such as roasting process, grinding size, brewing process, caffeine extraction method from coffee beans and matrix effect which absorbed by instruments (**Heran, https:// open.bu.edu/bitstream/handle; https:// www. kew. org/sites/default/files/Coffee Farmin**).

In general, the findings of the present study showed that regardles of their degree of roasting, the caffeine contents of *Yirgacheffe* coffee samples are slightly higher than *Harar* coffee samples. These observations are consistent with literture reported of caffeine levels of coffee samples of *Gediyo Yirgachefe* and *Harar* areas **(Belay *et al*., 2008; Tawfik & Bader, 2005)**. Moreover, the caffeine levele of the coffee samples used in the study are in the range of acceptable caffeine contents of coffee beans reported in literature **(Gopinandhan & Ashwini, 2014; Hecimovic *et al*, 2013;; Andrews *et al*., 2007; Farah, *et al*., 2006),** states that the levels of caffeine in coffee ranged from 6.60 to 20.70 mg/g to be very good as mild amounts of caffeine is advised for health. Another report also indicated that an average values of caffeine contents to be less than 1.5% for *Coffea arabica* beans **(Illy, 2002).**

5.0. Conclusion

As discussed in the previous sections, caffeine is one of the main chemical compounds in coffee beans, and has wide variety of health benefits and some harmful effects (if taken in excess). Since it affects coffee quality, determination of the level of caffeine in a given coffee product is very critical for consumers and producers/exporters. In this study, the caffeine levels of coffee samples from *Yirgacheffe* and *Harar* areas, Ethiopia, were successfully determined using HPLC method.

The results of this study revealed that *Harar* coffee bean samples have slightly higher caffeine contents than *Yirgacheffe coffee* beans. Though the caffeine levels show slight variations, the levels of caffeine in the coffee samples of both *Yirgacheffe* and *Harar* areas are in the acceptable range or mild caffeine levels reported in literature **(Gopinandhan & Ashwini, 2014; Hecimovic *et al*., 2013; Illy, 2002)**. Therefore, the coffee products can be consumed directly without further decaffeination.

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References

1. Abrar, S., & Nugussie, M. (2013). Evaluation of coffee quality of Tigray regional state, Ethiopia. Prudence Journal of Agricultural Research, 2, 3-6.
2. Abu, T., & Teddy, T. (2013). Ethiopian Coffee Annual report. Glob. Agri. Inf. Net. Gain Report.
3. Adepoju, A.F., Adenuga, O.O., & Mapayi, E.F. (2017). Coffee; botany, distribution, diversity, chemical composition and its management. Journal of Agriculture and Veterinary Science, 10, 57-62.
4. Andrews, K., Schweitzer, A., Zhao, C., & Holden, J.M. (2007). The caffeine content of dietary supplements commonly purchased in the U.S. Analytical and Bioanalytical Chemistry, 389, 231-239.
5. Antonio, A.G., Farah, A., Dos Santos, K., & Maia, L.C. (2011). The potential anti carcinogenic effect of coffee. Science against microbial pathogens: Communication Current Research and Technology, 56, 556-564.
6. Belay, A., Ture, K., Redi, M., & Asfaw, A. (2008) Measurement of caffeine in coffee beans using Uv-Vis spectrometer. Food Chemistry, 108, 310-315.
7. Belete, T., & Solomon, L. (2015). Determination of caffeine content and antioxidant activity of coffee using Uv-vsible spectrometer. American Journal of Applied Chemistry, 3, 69-76.
8. Camargo, M. R., Toledo, C.F., & Farah, G. (1999). Caffeine daily intake from dietary sources in Brazil. Food Additives and Contaminants, 16, 79-87.
9. Coffee Farming and Climate Change in Ethiopia Impacts, Forecasts, Resilience and Opportunities Summary Report. https://www.kew.org/sites/default/files/Coffee Farmin. (Downloaded on October,16, 2017)
10. Ephrem, D., Girma, W., & Arayaselassie, A. (2016). Uv-visible spectrophotometer determination of caffeine in green coffee beans from Hararghe, Ethiopia. Chemistry & Chemical Engineering Biotechnology Food Industry, 17, 109-123.
11. Ethiopia earns record 866 million USD from coffee export. http://www.ebc.et/web/ennews/-ethiopia earns-record-866-mln-usd-from-coffee-export.(Downloaded on October 16, 2017).
12. Farah, A., Franca, A.S., & Mendonca, CF. (2005). Composition of green and roasted coffee of different cup qualities. Leben Smitted Wissen Haftund, Technology. 38, 709-715.
13. Farah, A., Monteiro, M.C., & Clado, V. (2006). Correlation between cup quality and chemical attributes of Brazilian coffee. Food Chemistry, 98,373-380.
14. Fujioka, K., & Shibamoto, T. (2008). Chlorogenic acid and caffeine contents in various commercial brewed coffees. Food Chemistry, 106, 217-221.
15. Fung, Y.S., & Luk, S.F. (1985). Caffeine analysis using different methods. Analyst, 110, 201-245.
16. Gopinandhan, T.N., & Ashwini, M.S. (2014). A comparative study on caffeine estimation in coffee samples by HPLC and UV/VIS methods. International journal of current research in chemistry and pharmaceutical sciences, 1, 4-8.
17. Gray, J. (1998). Caffeine, coffee and health. Nutrition and Food Science, 6, 314-319.
18. Hecimovic, I., Belscak, C. A., Horzic, D, & Komes, D. (2011). Comparative study of polyphones and caffeine in different coffee varieties affected by the degree of roasting using HPLC and UV/VIS methods. Food Chemistry, 129, 991-1000.
19. Heran, S.B. Coffee, Culture, and Intellectual Property: Lessons for Africa from the Ethiopian Fine Coffee Initiative .https:// open.bu.edu /bitstream/ handle. (Downloaded on October 16, 2017).
20. Higdon, V., & Frei, B. (2006). Coffee and health: a review of recent human research. Critical Reviews in Food Science and Nutrition, 46, 101-123.
21. Huck, W.C., Guggenbichler, W., & Bonn, G.K. (2005). Analysis of caffeine, theobromine and theophylline in coffee by near infrared spectroscopy (NIRS) compared to HPLC coupled to mass spectrometry. Analytica Chimica, Acta., 538, 195-203.
22. ICO.www.ico.org. (2015). Coffee production data. International coffee organization.
23. Illy, E. (2002). The complexity of coffee. Scientific American, 5, 86-91.
24. Jeffery, H., & Michael, J. (2008). Coyer analysis of total caffeine and other xanthines in specialty coffees using mixed mode solid-phase extraction and liquid chromatography–diode-array. Detection after microwave digestion. Journal of Analytical Toxicology, 32, 65-79.
25. Joon, K.M., & Yoo, H.S. (2009). Role of roasting condition in the level of chlorogenic acid content in coffee beans. Journal of Agricultural and Food Chemistry, 57, 5365-5369.
26. Maria, B., Paulo M., & Marine, M. (2000). Caffeine content determination of Ethiopian coffee. Genetics and Molecular Biology, 23, 213-215.
27. Mekuria, T., Neuhoff, D., & Kopke, U. (2004). the status of coffee production and the potential for organic conversion in Ethiopia. Conference International Agricultural Research Development.
28. Niya, W. (2012). Physicochemical changes of coffee beans during roasting. Food Science. 1, 11-12.
29. Nogueira, T., & Lago, C. (2007). Determination of caffeine in coffee products by dynamic complexation with 3,4-dimethoxy cinnamated and separation CZE. Electrophoresis, 28, 3570-3574.
30. Patil, P.N. (2012). Caffeine in various samples and their analysis with HPLC – A review. International Journal of Pharmaceutical Sciences Review and Research, 16, 76-83.
31. Sanda, B.; Ganea, M.; Moisa, C.; Alina, C. (2015). Method for quantitative determination of caffeine from coffee using HPLC method. Fascicula Science, 14, 39-40.
32. Schenker, S., Heinemann, C., Huber, M., Pompizzi, R., Perren, R., & Escher, F. (2002) Impact of roasting conditions on the formation of aroma compounds in coffee beans, Journal of Food Sciences, 67, 60-66.
33. Shirisha, R.., & Varalakshmi, K.N. (2016) Evaluation of the anticancer potential of coffee beans: An in vitro study. Indian Journal of Traditional Knowledge, 15, 266-271.
34. Singh, D.K. (2006), Spectrophotometric determination of coffee compounds in pure alkaloids and its application in pharmaceutical formulations. Analytical Biochemistry, 349, 176-180.
35. Suter, P.M., & Vetter, W. (1993). Coffee and caffeine. Various selected aspects of every day practice. Schweizerische Rundschau Fur Medizin Praxis, 82, 1122-1128.
36. Svilaas, A., Sakhi, A.K., Andersen, L.F., Svilaas, T., Strom, E.C., Jacobs, J.R., & Blomhoff, L.R. (2004). Intake of antioxidants in coffee; wine and vegetables are correlated with plasma carotenoids in human. Journal of Nutrition, 134, 562-567.
37. Tawfik M.S., & E.L., Bader (2005). Chemical characterization of Harar and Berry coffee beans with special reference to roasting. Journal of Food Technology, 3, 601-604.
38. Tsegaye, G. (2009). Determination of caffeine in coffee using UV/VIS spectrophotometry. A graduate project submitted to the School of Graduate Studies of Addis Ababa University. In Partial Fulfillment of the Requirements for The Degree of Master of Science In Chemistry, MSc Thesis, Addis Ababa, Ethiopia.
39. Weinberg, B.A. (2001).The World of caffeine. The Scientist and culture of the World's most popular drug. Routledge. New York.
40. Yigzaw, D., Labuschagne, M.T., & Osthoff, G. (2007). Variation for green bean caffeine, chlorogenic acids, sucrose and trigonelline contents among Ethiopian Arabica coffee accessions. SINET: Ethiopian Journal Science, 30, 77-82.
41. Zewdu, T., Abdisa, G., & Liboro, H. (2016). Determination of caffeine level of Wolaita Zone using Uv-visible spectrophotometer. American Journal of Applied Chemistry, 4, 59-63.