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DETERMINATION OF CAFFEINE IN RAW AND ROASTED COFFEE BEANS OF ILU ABBA BORA ZONE, SOUTH WEST ETHIOPIA

Kebena Gebeyehu Motora, Tamene Tadesse Beyene

Mettu University, Faculty of Natural and Computational Sciences, Department of Chemistry, P.O.Box 318, Mettu (Ethiopia).

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ABSTRACT

The study was done to determine caffeine content of coffee Arabica in Ilu Abba bora. Caffeine is a naturally occurring alkaloid which is found in the leaves, seeds or fruits of over 63 plants species worldwide. The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves. Caffeine is a pharmacologically active substance and depending on the dose, can be a mild central nervous system stimulant, improve cardiac performance, increase brain circulation, and exhibit vasodilator and diuretic effect. It is also increase heartbeat rate, dilate blood vessels and elevate levels of free fatty acids and glucose in plasma. High Performance Liquid Chromatographic (HPLC) method was validated for determination of the levels caffeine in raw and roasted coffee sample of Ilu Abba bora zone. Shim-pack VP-ODS column was used with water: ethanol 65:35 % (v/v) eluent. The detector wavelength was set at 272 nm. Linearity of the method was check from 20-100 ppm and the correlation coefficient was 0.999. The method detection limit was 0.023 ppm and the precision was 1.25% at 40 ppm caffeine concentration. The spiked recoveries for caffeine were 105%, for both Yayo raw coffee and Chora roasted coffee, 102%, for both Chora raw coffee and Mettu raw coffee, 99% and 99.2% for yayo roasted and Mettu roasted coffee bean respectively. The caffeine contents in coffee samples were 57.23 mg/L for Yayo raw coffee, 62.63 mg/L for Yayo roasted coffee, 59.33 mg/L for chora raw coffee, 70.93 mg/L for chora roasted coffee, 64.61 mg/L for Mettu raw coffee and 78.68 mg/L for Mettu roasted coffee. As it can be concluded from the result Mettu rural coffee bean has highest caffeine content followed by Chora coffee and then yayo coffee. Roasting of coffee in all cases also increases the level of caffeine all coffee samples.

Corresponding author
Kebena Gebeyehu Motora
Mettu University, Faculty of Natural and Computational Sciences,
Department of Chemistry,
P.O.Box 318, Mettu (Ethiopia)
kebenagebeyehu@ymail.com

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INTRODUCTION

Today, coffee is the second most valuable product (besides crude oil) exported from developing countries, with a trading volume of approximately 22 billion US $.[1] Quality of coffee depends on its caffeine contents. Caffeine content of a coffee mixture is usually used to indicate its coffee level.[2] The world's primary source of caffeine is the coffee "bean" (which is actually the seed of the coffee plant), from which coffee is brewed. Caffeine content in coffee varies widely depending on the type of coffee bean and the method of preparation used; even beans within a given bush can show variations in concentration. Arabica coffee normally contains less caffeine than the Robusta variety. The caffeine content varies widely from about 100 µg/mL (100 ppm) to over 1000 µg/mL in certain types of coffee. This variation in caffeine content depends on the type of coffee, environment and soil type. Various manufacturers market caffeine tablets, claiming that using caffeine of pharmaceutical quality improves mental alertness. These effects have been borne out by research that shows that caffeine use (whether in tablet form or not) results in decreased fatigue and increased attentiveness. These tablets are commonly used by students studying for their exams and by people who work or drive for long hour.[3] Caffeine is an alkaloid of the methylxanthine family. In its pure state, it is an intensely bitter white powder. Its chemical formula is $C_8H_{10}N_4O_2$, its systematic name is 1, 3, 5-trimethylxanthine and its chemical formula is shown below. [3, 4] Pure caffeine occurs as odorless, white, fleecy masses, glistening needles of powder. Its molecular weight is 194.19g, melting point is 236 ^{0}C, point at which caffeine sublimes is 178 ^{0}C at atmospheric pressure, pH is 6.9 (1% solution), specific gravity is 1.2, volatility is 0.5%, vapor pressure is 760 mm Hg at 178 ^{0}C, solubility in water is 2.17%, vapor density 6.7.[4] Decaffeination is a popular term in present modern world to optimize the caffeine contents in various sources. This is simply use of a solvent, which extract caffeine. For this purpose, the currently available solvents are chloroform, methyl chloride, ethyl acetate, super critical carbon dioxide etc. [4]

Caffeine is a pharmacologically active substance and depending on the dose, can be a mild central nervous system stimulant, improve cardiac performance, increase brain circulation, and exhibit vasodilator and diuretic effect. It is also increase heartbeat rate, dilate blood vessels and elevate levels of free fatty acids and glucose in plasma.[4, 5] Caffeine is added to soft drinks as a flavoring agent, it is part of the overall profile of soft drinks, which consumers enjoy for refreshment, taste and hydration.

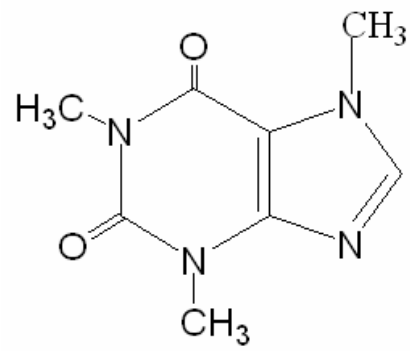

Figure 1: Caffeine (1, 3, 5-trimethylxanthine).

Chemically coffee is one of the most complex consumable that contains many other chemicals in addition caffeine. One of such compounds is cafestol. Chemically, cafestol belongs to the group of diterpenes, which naturally occur in coffee beans.[6]

Decaffeination is a popular term in present modern world to optimize the caffeine contents in various sources. This is simply use of a solvent, which extract caffeine. For this purpose, the currently available solvents are chloroform, methyl chloride, ethyl acetate, super critical carbon dioxide etc. Methylene chloride is used to decaffeinate a high proportion of conventional coffee.[7] As a solvent, methylene chloride is highly effective, but also potentially dangerous under certain circumstances. It can cause faintness, dizziness, and headache if inhaled at high concentrations. Ethyl acetate is another compound used to extract caffeine from coffee. It removes caffeine from tealeaves effectively; it can also extract other chemical components as well. Studies on green tea decaffeinated with ethyl acetate have shown the potential for up to 30% of epigallocathechin gallate (EGCG-considered to be the primary beneficial component in green tea) and other beneficial antioxidant compounds to be extracted along with the caffeine. [8, 9]

Different studies show that spectrophotometric and HPLC with the defatting step methods showed satisfactory accuracy and precision as well. Comparison of the two HPLC methods showed that HPLC without the defatting step was better. In this study samples of coffee of different type were taken and its caffeine and cafestol content were analyzed by using high performance liquid chromatography.[10]

Ilu Abba bora zone which has area of 1.633 million square kilometer including Buno Bedele has land covered by coffee plant of 221, 635(two hundred twenty one thousand six hundred and thirty five) square kilometer and the land covered by coffee from total land is 14 % . This zone supplied 81, 014 (Eighty one thousands and fourteen) ton of coffee in 2008 E.C for the national market. The coffee this Zone is being sent to the national market by name of Jimma B. The major coffee producing woredas are: Chora woreda which holds first rank in the zone, Yayo woreda which holds second rank in the zone and Mettu rural woreda which hold third rank according to data obtained from Ilu Abba bora Agricultural and development office. Out of total population of the Ilu Abba bora zone 70% are participating in the production of coffee and they also use it as major source of income. Although coffee is the major product and means of income for this much percent of population, there is no any research conducted on the caffeine content the coffee being produced which major ingredient to assign level of coffee for the national market.

MATERIALS AND METHODS

Sample collection

Coffee beans were collected from Mettu rural, Chora and Yayo woreda agricultural and rural development offices and the collected coffee was coffee Arabica as they have told us depending up on their species analysis. And it was taken to Jimma University where the experimental work of the research was done.

Sample preparation

All samples both for raw and roasted coffee was prepared for analysis in Jimma university chemistry department research laboratory of Analytical chemistry and determination of the caffeine contents the three coffee samples for both raw and roasted were also done at the same place.

Roasting of coffee beans

Randomly selected coffee beans of the three samples were placed into a pan and then roasted by using a muffle roaster. Each time the pan was positioned in the same place of the muffle roaster in an effort to ensure uniform roasting conditions. After roasting, the beans were cooled immediately using an electric fan. The roasting temperature for all samples were 165 º C which was established based on weight loss measurements and visual inspection of the external color of beans, which is the most widely used in the coffee roasting industry.

Grinding and storage

Roasted coffee samples were stored in sealed containers at ambient temperature for a maximum period of 24 h. Just before each analysis, roasted coffee beans were finely ground with an electric coffee grinder (Multi-purpose disintegrator MJ-04) at 3.5 screen size (0.30 mm) and keep refrigerated in sealed plastic bag prior to analysis.

Chemicals and Reagents

All reagents used in this study were of analytical or HPLC grade and all solutions were prepared by using distilled water. All the glassware were soaked overnight with chromic acid solution and washed thoroughly with water and detergent, then rinsed with deionized water before use. Ultrapure water was purchased from Milli-Q System (Millipore Corp., Milford, MA, USA). The mobile phases were filtered in HAWP and HVWP membranes, respectively, for aqueous and organic solvents (47 mm diameter and 0.45-mm pore size, Millipore Corp., Milford, MA, USA).

Standard solutions preparation

Caffeine stock solution of 100 ppm was prepared by accurately weighing 10.00 mg of pure caffeine and quantitatively transferring it into 100 ml volumetric flask and making it to the mark with the mobile phase. Working standards of 10, 20, 40, 60, 80 ppm were prepared by serial dilution of the stock solution with the mobile phase.

Caffeine analysis

Raw and roasted coffee samples preparation:

2.00 g of raw and roasted coffee samples were weighed and transferred into 250 mL beakers.100 mL of boiling distilled water was added and let to stand for five minutes with stirring; the solution was cooled and filtered into conical flasks. 5 ml of the filtrate were pipetted into clean 50 ml volumetric flasks and made to the mark with the mobile phase. The standards and the samples were run in the HPLC system. The following were the HPLC conditions: Column, Reverse phase – ODS, 250 × 4.6 mm, flow rate, 1 ml/min, detector, photodiode array set at 272 nm, pressure, 150 khf/cm^2. Mobile phase, water and methanol (65 and 35) and sample volume, 10 µl. A calibration curve of peak areas versus concentration of the standards was plotted. The caffeine level of the various samples was calculated using the regression equation of the best line of fit.

Instrumentation:

The HPLC system used in this study was isocratic Waters HPLC, which consisted of a model 1515 isocratic pump, vacuum degasser and 2996 PDA detector (USA). The injector was a model 7725i Rheodyne injector with injection loop 10 µL. The analytical column used was Shim-pack VP-ODS with internal diameter 4.6 mm and length 250 mm (Shimadzu Corporation Tokyo, Japan). All chromatographic results was acquired and processed by Empower software (Waters Corporation). The experimental conditions are mentioned below.

Experimental Condition

Table 1: experimental condition.

Item	Conditions
Mobile phase composition	Water : methanol (65:35)
Flow rate	1 mL/min
Injection volume	10 µL
Column temperature	25 ºC
Wave length	272 nm

Table 2: Method detection and quantification limits for caffeine [a].

SD of peak area for seven replicates (ppm)	Calculated MDL (ppm)	Calculated MQL (ppm)
0.0074	0.023	0.07

[a]Standard Concentration was 1 ppm

Caffeine extraction

Two grams of sample was weighed and powdered and 200 ml of distilled water was added to the sample and shaken for 15 minutes using a magnetic stirrer. Sufficient water was added to produce 250 ml and the solution was filtered. To 10 ml of the filtrate, 10 ml of 1N sodium hydroxide (NaOH) was added and extracted immediately with five quantities each of 30 ml of chloroform in a separating funnel. Each extract was washed with 10 ml of water. The chloroform extracts were combined and filtered through a plug of absorbent cotton wool previously moistened with chloroform. The solution was then evaporated to dryness and the residue was dissolved completely in 30 ml of water, warming gently on a water bath. The solution was cooled, made up to 100 ml and filtered. The absorbance of the resulting solution was then measured using a High performance liquid chromatography at 272 nm.

RESULTS AND DISCUSSION

Method validation

Precision:

The analytical precision of the method was assessed from the reproducibility of 6 determinations of 40 ppm caffeine solution and a relative standard deviation of 1.25% was calculated for peak area. The retention time of caffeine was 3.145 min, with a relative standard deviation RSD = 0.5% therefore, in standard solutions, the HPLC method provides stable retention times.

Detection and quantification limits:

Table 2 summarizes the method detection limit (MDL) and Method Quantification Limit (MQL). MDL was estimated as Standard Deviation (SD) of the peak area of seven injections multiplied by 3.14 (at n = 7). MQL was calculated by multiplying SD by 10. [12]

Linearity:

The calibration graph was generated using 10 µl injection loop. Five different concentrations of caffeine from 20 ppm to 100 ppm were analyzed according to experimental conditions. Then the calibration curve was established according to the obtained response (peak area) and the concentrations of caffeine in standard solutions. The results show a good linear relationship. The calibration data was summarized in Table below. The calibration graph is also shown in the Figure below.

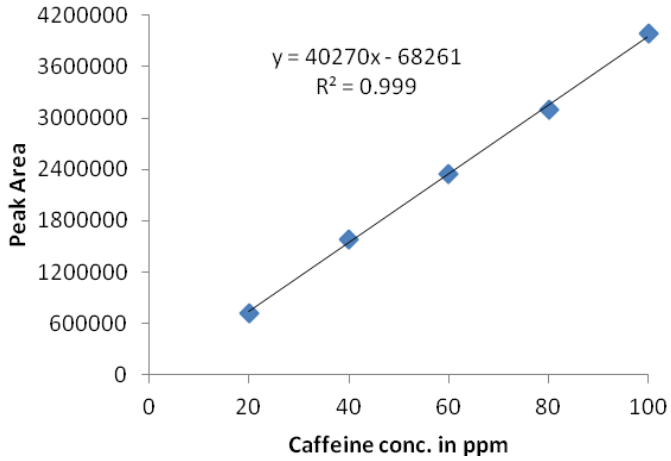

Figure 2: Calibration curve.

Table 3: Regression data and statistical parameters for caffeine calibration curve.

Correlation coefficient R^2	Slope (m)	Y-Intercept	Linear range in (ppm)
0.999	40270	-68261	20 – 100

Table 4: Recoveries of caffeine from spiked coffee samples.

Sample name	Amount[a] of caffeine in the sample (ppm)	Amount of caffeine added (ppm)	Amount of caffeine found (ppm)	Recovery percentage (%)
Yayo raw coffee	18.29±0.48	20	39.30±2.00	105.0
Yayo roasted coffee	18.68±0.40	20	38.47±0.80	99.0
Chora raw coffee	25.24±1.60	20	45.64±0.40	102.0
Chora roasted coffee	28.29 ± 0.48	20	49.30 ± 2.00	105.0
Mettu raw coffee	47.29±0.26	20	67.70±1.50	102.0
Mettu roasted	52.62±0.65	20	72.46±0.35	99.2

[a]Average ± Standard deviation (n = 3)

Table 5: Results of caffeine contents in coffee samples.

Sample type	Sample name	Caffeine cont ± sd in ppm(mg/L)
Yayo Coffee	Yayo raw coffee	57.23 ± 0.15
	Yayo roasted coffee	62.63 ± 7.78
Chora coffee	Chora raw coffee	59.33 ± 1.17
	Chora roasted coffee	70.93 ± 2.64
Mettu coffee	Mettu raw coffee	64.61 ± 1.45
	Mettu roasted coffee	78.68 ± 15.26

Average ± Standard deviation (n = 3).

Recovery:

For recovery study one sample of known caffeine concentration form different types of coffee bean samples was spiked with 20 ppm of caffeine standard and recovery was calculated as summarized in Table 4 and the mean recoveries of the obtained results were found to be not significantly different from the value of added caffeine concentration.

Determination of caffeine contents in raw and roasted coffee bean:

The validated method was used to determine the concentration of caffeine in real coffee samples (raw and roasted coffee bean).

Figure 4-6 present the chromatogram obtained for the one of the injections of caffeine standard (60 ppm) and the coffee beans samples, respectively. The highest caffeine concentration in coffee bean samples was obtained in Mettu rural both for raw and roasted coffee bean; this was followed by Chora coffee bean and then by Yayo coffee bean sample. The caffeine contents in Mettu rural coffee samples was in average 64.61 ppm for raw coffee bean and 78.68 ppm for roasted coffee bean, The caffeine contents in Chora coffee samples was in average 59.33 ppm for raw coffee bean and 70.93 ppm for roasted coffee bean and The caffeine contents in Yayo coffee samples was in average 57.23 ppm for raw coffee bean and 62.63 ppm for roasted coffee bean.

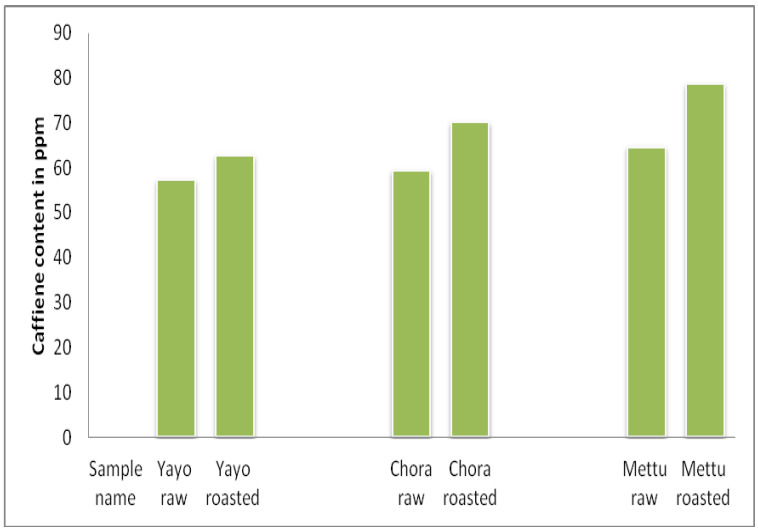

Figure 3: caffeine level of both raw and roasted coffee beans.

Caffeine content (mg/L) of raw and roasted Yayo, Chora and Mettu rural coffee samples

As shown in the table 5 and figure 3 above the experimental results showed that there is an increase in the caffeine content by raising temperatures in all coffee sample and this increase has a significant difference. This indicates that the caffeine content of roasted coffee samples collected from the three woredas is greater than that of raw coffee samples. This is in agreement with literatures findings on effect of raosting on caffeine content of coffee samples. Also, many other studies have reported that caffeine content increased during the roasting process [13-16]

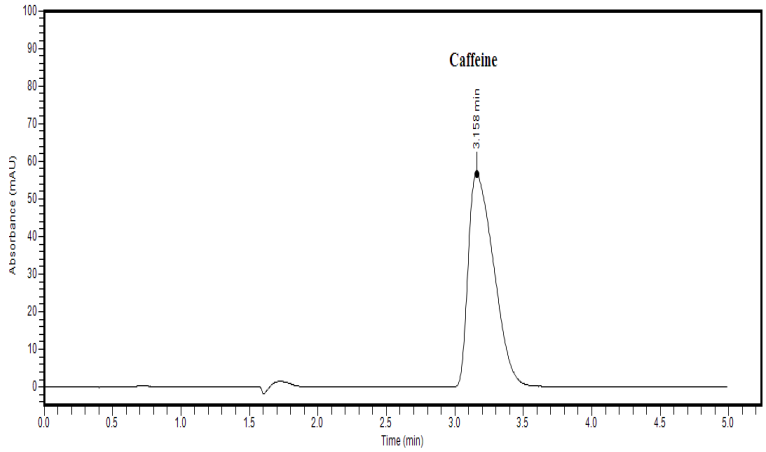

Figure 4:Chromatogram of 60 ppm (mg/L) of caffeine standard.

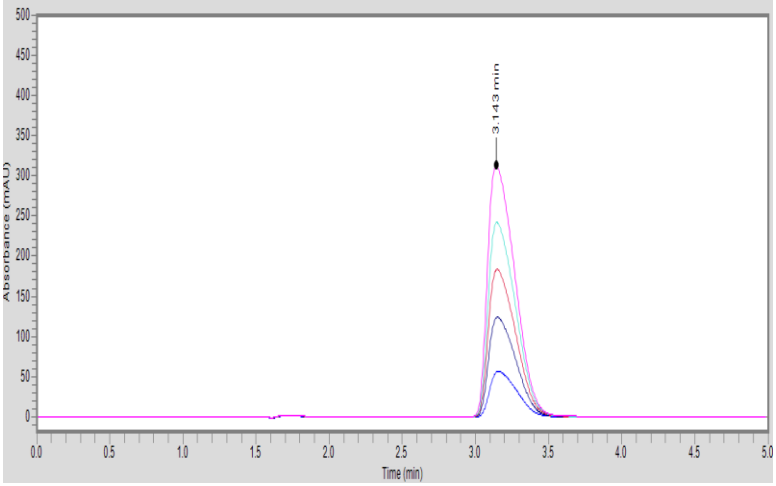

Figure 5: Chromatograms indicating the peaks of caffeine standard concentrations of 20, 40, 60, 80 and 100 ppm).

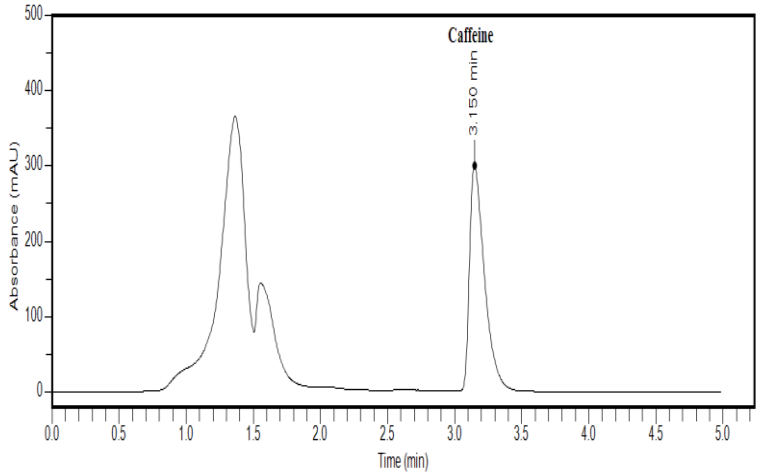

Figure 6: Representative chromatogram of caffeine from Chora raw coffee sample extract.

CONCLUSION

High performance liquid chromatographic method was employed for determination of level of caffeine in coffee samples of Ilu Abba bora zone and also the effect of roasting on caffeine content was studied. The results of this study showed that the caffeine contents of all roasted coffee samples were higher than that of raw coffee beans. According to this study the caffeine content of Mettu rural woreda was higher than that of Chora and again Chora coffee contains higher caffeine level than that of Yayo woreda. The study also showed that roasting coffee bean can increase the caffeine contents of coffee beans.

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The authors declare that there is no conflict of interest regarding the publication of this paper.

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