JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY

M.SC. THESIS

EXTRACTION KINETICS, ANALYSIS OF CAFFEINE AND TOTALACID CONTENTS OF COFFEE AT DIFFERENT ROASTING CONDITIONS

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LOQ	Limit of Quantification
LLE	Liquid –Liquid Extraction
LOD	Limit of Detection

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Abstract

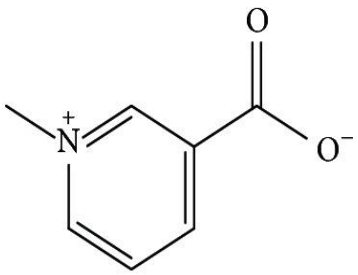
Green coffee beans contain important components like total acids that were labile of temperature effects and can be affected by roasting. So, it is important to investigate effects of coffee roasting conditions classifying as light, medium and dark roasting on the caffeine and total acid contents of coffee from Jimma, Ethiopia. To investigate the effect of roasting, coffee samples were weighed properly and roasted at three different roasting conditions adapting traditional coffee roasting and classified as light, medium and dark. The roasted coffee beans were evaluated for quality attribute at all degree of roasting by five professional cuppers and by the so called regular users (four in number). During the experiment, the amount of total acidis determined by titration method whereas caffeine content was measured using UV-Visible spectrophotometer. The extraction efficiency of caffeine was performed from extraction kinetics study. Based on cupping evaluation, medium roasted was selected as very important roasted condition and assumed as a common roasted coffee type in Ethiopia. The light roasted coffee was found to have higher concentrations of total titratable acids than that medium and dark. The caffeine content of the three different types of roasted coffee (light, medium and dark) ranged from 1.1 – 1.15% (w/w). For kinetic study, the experimental data fitted first order kinetics with linear regression equation of (R2 =0.944). The caffeine extraction rate decreased with increasing extraction time.

Keywords: Caffeine, Extraction kinetics, Roasting, Total acids

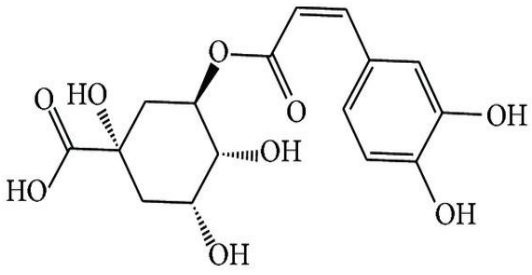
1. Introduction

Today, coffee is one of the most consumed beverages in the world mainly due to its stimulating effects, characteristic taste, and richness of coffee aroma which makes it a unique beverage. There has been identified more than 80 species of coffee in the world, but only two main coffee species: *Coffea*(Arabica), which stands for about 60% of the world coffee market, and *Coffeacanephora* (Robusta) about 40%.Minor cultivated species include *C.* (Liberica) and *C. excelsa*account for only 1-2% of global production. Arabica and Robusta have a very distinct chemical composition and Arabica coffee is milder, more aromatic and contains less caffeine than Robusta coffee[1]. However, Robusta coffee tree is stronger and more resistant than Arabica tree in various aspects [2]. The different varieties of coffee show differences in the size and shape of the coffee bean but, on average, beans are approximately 9.5 mm long and 7 mm wide. The weight of a parchment seed at 9%moisture content is about 0.15 g for Arabica and 0.16 g for Robusta. The bean sizes are also influenced by environmental conditions and husbandry (nutrition, moisture, care etc.) [2].

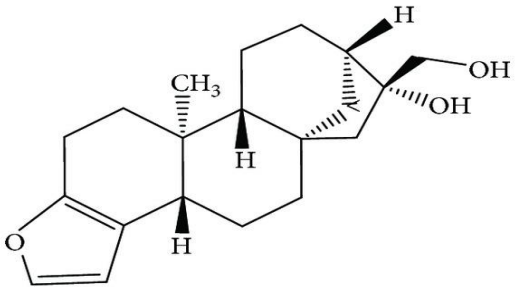
Coffee is a complex mixture of thousands of chemical compounds responsible of its flavor and aroma, carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids, and phenolic compounds [3]. Coffee is rich in many bioactive substances (trigonelline, chlorogenic acid, diterpenes, caffeine), and its consumption has been associated with many beneficial effects [4]. Coffee chemistry is determined by numerous factors, including the varietal of bean, region of origin, growing conditions, roasting process, grind size distribution, water chemistry, and temperature of water used during extraction [5].

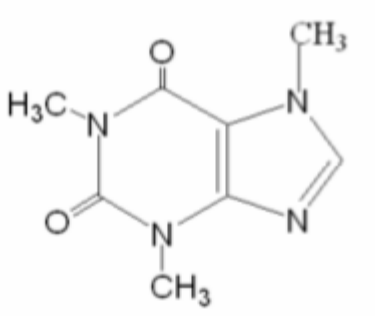


Trigonelline



Chlorogenic acid





Diterpenes Caffeine

Scheme 1 Chemical structure of bioactive compounds found in coffee

Caffeine (1, 3, 7-trimethylxanthine) is known as a Central Nervous System (CNS) stimulant. It is a naturally found in the leaves, seeds and/or fruits of at least 63 plant species worldwide. The most commonly known sources of caffeine are coffee, cocoa beans, kola nuts and tea leaves[6]. Caffeine is consumed most frequently in beverage such as coffee (71%), soft drinks (16%), and tea (12%)[7].Coffee beans contain between 0.8 and 2.8% caffeine, depending on species and origin. It contributes to 10-30% of the bitter taste of coffee brews[8]. The caffeine content in *C. canephora*(Robusta) is about two times that of *C. Arabica* (Arabica) [9]. Caffeine content of coffee bean is not significantly changed during coffee roasting [2],

Green coffee beans contain 5–14% of the reported major components, chlorogenic acids (CGAs)[2] and these compounds are significantly reduced after roasting [9].Literature data indicated an inverse relation between roasting time and antioxidant activity due to the degradation of chlorogenic acids and other phenolic compounds [10]. Increase roasting time found to decrease both the anti-oxidant and anti-inflammatory potential of different coffee extracts[12]. However, the effect of roasting in caffeine content is controversial. Many articles indicated that caffeine contents are higher in the darker grades of coffee subjected to longer roasting time than the light grades and raw coffee[13]. Other researchers found that both phenolic and caffeine contents decrease in the darker grades of coffee [14]. Increases in roasting temperatures correlate to a decrease in extractable chlorogenic acid concentrations and to an increase in caffeine concentrations[8]. Thus, further study in the area need to be

investigated. Hereby, the effect of roasting on caffeine and total acids, extraction kinetics, and quantitative analysis of caffeine and total acids were evaluated for Ethiopian coffee samples collected from Jimma town. On top of that, sensory attribute evaluation has been made under different roasting temperatures and optimizes roasting temperature of coffee samples.

1.1. Statement of Problem

The stimulant effect ,its pleasant taste, aroma and health benefits of coffee makes it one of the most popular beverages in the world is due to the presence of caffeine (1, 3, 7-trimethylxanthine) and total acids. However, high doses of these compounds may produce negative effects in some sensitive individuals, including anxiety, tachycardia and insomnia. In addition to this, some of these compounds are thermally unstable and degraded during roasting and change the coffee's test and aroma. Therefore, it is important to study the effect of coffee roasting conditions on the quality of coffee beans collected from Jimma town.

1.1.1 Research Questions

This study was answered the following questions:

1. Does coffee roasted at different conditions contain variable concentration of caffeine and total acid?
2. At which roasting condition the quantity of caffeine and total acids is high?
3. What is quantify of caffeine and total acids in coffee samples?

1.2. Objectives

1.2.1. General Objective

- The general objective of this study is to investigate the caffeine and total acids of coffee samples at different roasting conditions

1.2.2. Specific Objectives

- To optimize roasting conditions of coffee samples
- To study the extraction kinetics of caffeine in optimized roasting conditions
- To determine total acids at different roasting conditions in coffee

- To determine the caffeine content of coffee using UV-Vis spectrophotometer

1. 3.Significance of the Study

The outcome of this study would have significant contribution to filling the current research gap on the effects of coffee roasting conditions on the caffeine and total acids content of coffee beans. In addition, the results obtained from this study can be used by coffee traders, coffee processors and researchers as useful inputs when purchasing, using and studying coffee roasting conditions.This data will be used as baseline information to advance research knowledge in similar areas.

2. ReviewLiterature

Coffee is most popular drink around the world. Different people of the world consume coffee in different forms. Historically, consumption of coffee had been related to unhealthy behaviors, such as smoking, large amount alcohol drinking. But recently it becomes popular due to its beneficial activities[15]. All cultivated species of coffee have their origin in Africa. The name coffee is derived from the name of the province "*Keffa" in* Ethiopia, where shepherds discovered the coffee beans in the 6 centuries[15]. The Arabs introduced coffee from Ethiopia to Yemen during the 13^{th} century, where the habit of drinking coffee was developed in the 15^{th} century. This habit gradually spread to the rest of the world, leading to the increased interest of some countries to produce coffee as a commodity on a large scale. Coffee has enormous commercial and social importance and is the most important traded commodity in the world after oil[16]. Global output is expected to reach 7.0 million tons by 2010. World consumption of coffee is projected to increase by 0.4% annually from 6.7 million tons in 1998–2000 to 6.9 million tons in 2010[16]. It is also very important commodity crop for many developing countries, once contributing over 10–11 billion US $ annually and providing a source of income for thousands of small-scale farmers, as well as being a significant source of employments[17]. The production of coffee beans is the base of the economy of several tropical countries, such as Mexico, Guatemala, El Salvador, Nicaragua, Costa Rica, Panama, Venezuela, Colombia, Ecuador, Peru, Bolivia, Paraguay, Brazil, Ethiopia, Kenya, Tanzania, Zambia, and Mozambique. [17]. More than 50 countries, in which 25 of them in Africa, depend on coffee for their foreign exchange Ethiopia is the third largest coffee producer in Africa after Uganda and Ivory Coast [18]. It covers 2.5% of the world's coffee trade [19]Coffee covers about 60% of Ethiopia's export [19]. It also accounts for 5% of gross national product, 42% of taxes from foreign trade. In the country 24.5 Kg coffee is consumed annually per household and 4.5 Kg per capita [19].Coffee grows in most part of Ethiopia. Oromia and SNNP regions comprise largest coffee cultivated areas such as Wolega, EluAbaboura, Jimma, BenchMaji, Sidamo, Gedeo, Guji, East and West Hararge, South and north Omo. About 204500to 683600 hectares of land is under coffee cultivation [20]. This is a small part of areas, which is suitable for coffee production. Around 25% of the country's population directly or indirectly depends on coffee [18]. About 95% of

the coffee output is grown by small farmers, most of whom work less than half a hectare of land. An average yield is between 540 to 490 Kg per hectare[20].

2.1. Coffee Constituents

Coffee contains a multitude of substances, many of which are potentially biologically active. The chemical composition of coffee varies for different reasons such as the species and variety of coffee beans and to a lesser extent other factors such as agricultural activities, degree of maturation and storage conditions[19]. In the order of their abundance, coffee contains phenol polymers 8%, polysaccharides 6%, chlorogenic acids 4%, and minerals 3%, water 2%, and caffeine 1%, organic acids 0.5%, sugar 0.3%, lipids 0.2% and aroma 0.1% [18]. Organic acids such as oxalic, succinic, fumaric, malic, tartaric, citric and quinic acids are considered to play an important role in coffee flavor [21].Coffee has the highest and the most variable caffeine content among dietary products, which contain this alkaloid. The value differs from 30-175 mg of caffeine per cup (150 mL) coffee. The standard value has been suggested to be 85 mg of caffeine per cup of ground roasted coffee [22]. Coffee is also enriched with many other ingredients that may contribute to its biological activities such as niacin, potassium, magnesium and antioxidants such as tocopherols, phenols and chlorogenic acids. The two Di- terpinoids, *cafestol*and *kahweol,* are also found in significant level. They are a natural constituent of green coffee beans, are realized from roast, and ground coffee by hot water [21].

2.2. Caffeine

Caffeine (1, 3, 7-trimetylxanthine) is one of the main alkaloid found in various kinds of foods and drinks that we consume in daily life [23].It is naturally found in leaves, seeds or fruits of 63 plant species [23]. The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves. The chemical formula for caffeine is $C_8H_{10}N_4O_2$. Pure caffeine occurs as odorless, white powder. It has molecular weight of 194.19 g, melting point of 236 ^0C, and sublimation point of 178^0C and pH values in the range of 6 to 9 [22].

Figure 1Caffeine in coffee

Based on the data reviewed, it can be concluded that low to moderate caffeine intake (300 mg/day or less) is generally associated with improvements in alertness, learning capacity, exercise performance and perhaps mood. In addition, caffeine metabolites, especially 1-methylxantine and 1-methylurate have exhibited antioxidant activity. Antioxidants have been widely linked to a number of potential health benefits including protection against heart disease and cancer. However, high doses may produce negative effects in some sensitive individuals, including anxiety, tachycardia and insomnia [24]. A study of Dr. Pollack [25] recommended upper limits for caffeine: healthy adults should consume below 300-500 mg daily, pregnant women must stay below 150-200 mg daily and children should stay below 50 mg daily.

2.2.1. Absorption and Metabolism

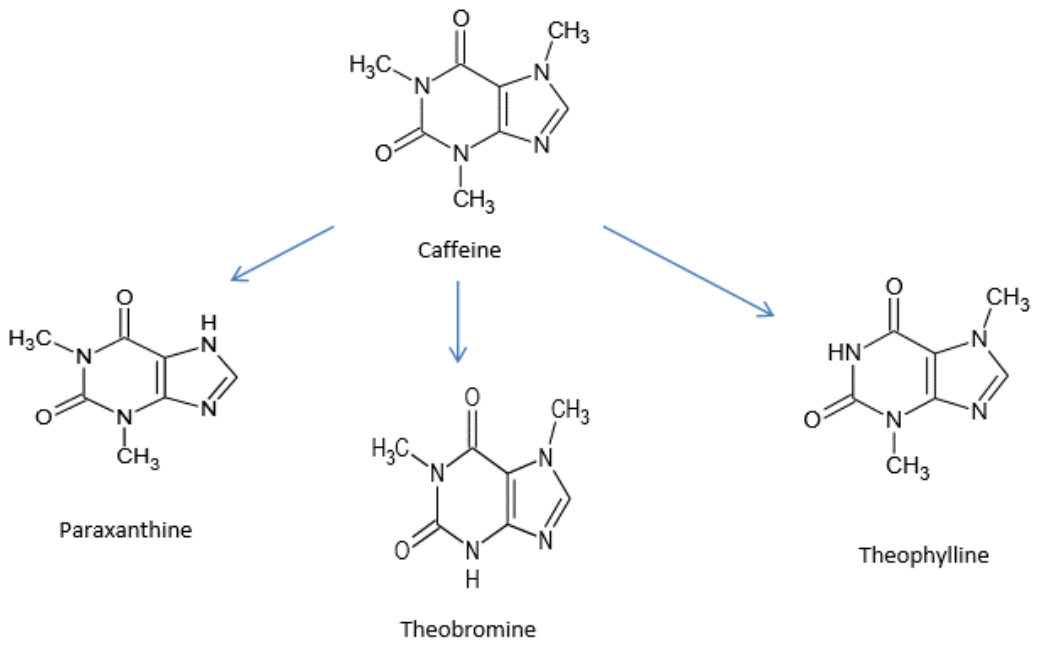
Caffeine enters into human body through different sources such as food stuffs, beverage and medications. Caffeine absorption from the gastrointestinal tract is rapid and virtually completed about 45 minutes after ingestion. The absorbed caffeine is readily disturbed throughout the entire body. It passes across the blood brain barriers, through the placenta into amniotic fluid and the fetus, and into the breast milk [26]

Caffeine is metabolized by *demethylation*and *oxidation*. It is metabolized in the liver through a series of *N- demethylation*and *purine ring oxidation* reaction to yield a mixture of mono and dimethylxanthines and methyl uric acids. The main dimethyl xanthine metabolites are *theophylline* (4 %), *theobromine*(12 %) and *Para xanthine*(*1, 7-dimethylxanthine*) (80 %).

Para-xanthine is not found in foods but it is the main metabolite of caffeine; *1, 3, 7, trimethyl uric acid* and *6-amino-5(N-formylmethylamino)-1,3dimethyluracil* together account for 15% of caffeine elimination. The metabolism is slowed during pregnancy and in women taking oral contraceptives. On the other hand, the metabolism rate of caffeine is greater in smokers than non-smokers [26].

The half – life of caffeine, the time required for the body to eliminate one-half of the total amount of caffeine consumed at a given time varies widely among individuals according to such factors as response, age, sex, liver function , pregnancy, some concurrent medications, smoking and the level of enzymes in the liver needed for caffeine metabolism. Caffeine has a half - life which is faster than theobromine. It is completely metabolized by human body; only 1-5 % of ingested caffeine is recovered unchanged in the urine. Infant up to the age of 8-9 months have a greatly reduced ability to metabolize caffeine, excreting about 85 % of the administered caffeine in the urine unchanged [27].

The half- life in the newborn child range from 5-100 hrs; But it gradually approaches that of an adult. By 6 months of age caffeine's half- life is about 20-30 % shorter in females than in males. The half-life in females using oral contraceptive steroids is approximately twice that observed for ovulatory females. Pregnancy slows down the metabolism of caffeine. The metabolic half –life increases steadily from 4 hrsduring the first trimester to 18 hrs during the third trimester. Obesity also slows down caffeine metabolism. Smoking is another factor which affects the metabolism of caffeine. It accelerates the rate at which caffeine is eliminated [27].



Scheme 2: metabolism of caffeine in the liver

2.2.2. Beneficial Effects of Caffeine

Caffeine is a plant based alkaloid which stimulates the central nervous system. The stimulatory effects of caffeine usually results in an increased ability for mental activity for sweets by simulating the production of those adrenal hormones that cause blood sugars to be increased. Stimulatory effects of caffeine results in increased capacity for mental activity and muscular work, the weakness, depression and discomfort from excess of alcohols can be nullified with black coffee or hypodermic injections of caffeine. Insensibility from hashish is believed to be ended by the use of caffeine medication. Even the dullness and sense of depression from a little too much tobacco is helped by coffee [28]

The other benefits of caffeine include reduced risks of Parkinson's disease, colon cancer, diabetes; decrease in exercise induced myocardial flow reserve and increase and in both sexual motivation and locomotors activity on female mating behavior. Caffeine expands blood vessels and consequently the brain receives more oxygen. It helps in preventing apositive energy balance and obesity. Caffeine is also an accepted drug for intra muscular application to treat arterial hypotension [29]

2.2.3. Physiological Effects of Caffeine

Caffeine has many physiological effects such as, gastric and secretion, diuresis and stimulation of the central nervous and the cardio vascular system. In addition, the caffeine interference with the uptake and storage of calcium by the sarcoplasmic reticulum, i also increase the respiratory rate and causebronchodilation and stimulatelypolgysis [30].

The stimulatory action of caffeine involves antagonism of adenosine receptors which are present in brain, blood vessels, kidneys, heart, the gastro intestinal tract and the respiratory hierarchy [31]. Adenosine is an adenine molecules attached to a ribose or deoxyribose sugar molecules. The similarity in chemical structure between the adenine portion of adenosine and the caffeine molecules is the key to how caffeine works [32]. Adenosine when bound to receptors of nerve cells, slow down nerve cell activity during sleep. Caffeine, being structurally similar to adenosine, has the potential to occupy adenosine receptors sites. When the caffeine molecules binds to the receptors doesn't cause the cells to slowdown; instead, the caffeine blocks the receptors and there by blocks the regulatory functions adenosine and produces the stimulatory effect [33].

2.2.4. Methods of Analysis

Due to above mentioned facts many chemical and physical methods have been developed for the determination of caffeine in coffee and other beverages. The most widely used methods for the determination of caffeine in beverages are based on UV-Vis Spectrophotometry and partial least square [30], UV-Vis Spectrophotometry [34], derivative spectrophotometry, HPLC [21]. Fourier Transform Infrared (FTIR) Spectroscopy [35] NIR Reflectance Spectrometry [36], Raman Spectroscopy [37] and Capillary Electrophoresis [5] are very commonly used techniques.

The spectrophotometric method is fast, simple, accurate, reproducible and inexpensive procedure as compared to other methods, but it is not possible to determine caffeine directly in coffee beans by conventional UV-Vis absorption measurement due to the spectral overlap of UV absorbing substances in the sample [19]. Derivative spectrophotometry is relatively easy, but; it is not reliable for the small concentration of caffeine in samples. By HPLC

methods many caffeine contents were determined in various foods using different procedures since it provides the most reliable method.

2.2.5. Liquid-Liquid Extraction Kinetics

Liquid-liquid extraction (LLE) is one of the most utilized techniques for sample preparation in various types of chemical analysis processes.Liquid–liquid extraction (LLE), also referred to as solvent extraction or partitioning, is a method for separating chemical entities based on their selective affinities for one of the phases in a twophase system, typically an aqueous and an organic phase. Due to its attractive characteristics such as versatility and scalability,LLE has been implemented in a variety of applications at industrial scale including metal extraction [38] and organic synthesis [39] in various sample pre-treatment processes (e.g., purification of biomolecules [40], ultra-sensitive measurement of analytes [41], pesticide analysis, and in analytical applications, e.g., for the determination of the lipophilicity during drug discovery and development [42].

The kinetics of liquid-liquid extraction of caffeine from aqueous solutions can be a function of both the chemical reactions taking place in the system and the rates of diffusion of the species present in the two phases [43]. The rate of solvent extraction can be characterized by the slow step of the overall reaction mechanism, which can occur either in the bulk (homogeneous reactions) or at the liquid-liquid interface or in a thin volume region very close to it [44]. In addition, diffusion processes can also be rate determining, as the chemical species have to be transferred from one phase to another for the reaction to occur, and the transport of material from the bulk to or from the interface can be significantly slower than the actual reaction.

3. Materials and Methods

3.1. Instruments and Apparatus

Ultraviolet–Visible Spectrometer (SPECORD 200 PLUS - 223E1128F, UK), traditional coffee grinder, digital balance(Mettler Toledo -AL204-ICAmerica),hot plate and magnetic stirrer were utilized during the experiment.

3.2. Chemicals

Dichloromethane and Caffeine werepurchased from (Aldrich-Sigma, Germany), Standardized NaOH was purchased from Sigma-Aldrich (Milwaukee, WI, USA), methanol, distilled water, deionized water and distilled water was used throughout the study. Unroasted Arabica coffee was purchased from the local market in Jimma, Ethiopia.

3.3. RoastingConditions

To investigate the effect of roasting, 20 g coffee samples were weighed properly and roasted at three different roasting conditions and obtained (light, medium and dark) according to appendix I, II and III by traditional coffee roaster at 191 ^{O}C for 4 to 12. The roasted coffee beans were cooled to halt exothermic reaction and it was stayed overnight in order to allow sufficient time for full flavor development. All roasted samples were kept in polyethylene plastic bags until they were selected by judges.

Table 1 illustrates temperature and duration of roasting of coffee samples

Coffee Roasted	**Light roasted**			**Medium roasted**			**Dark roasted**		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Temp (°C)	191	191	191	191	191	191	191	191	191
Time (min.)	4	5	6	7	8	9	10	11	12

3.4. Grinding

The roasted coffee was grinded by traditional coffee grinder and sieved through 250µm sieve to get a uniform texture. All grinded samples were kept in polyethylene plastic bagsas shown in appendix IV until they were found to be analyzed.

3.5. Sensory Evaluation

3.5.1. Brewing

For both tests, coffee beans were ground at medium size before infusion. The amount of coffee used for the infusion preparation during the evaluation was established at 5.5 g per 100 mL of water. This ratio is in accord with the International Standard for the preparation of coffee samples for use in sensory analysis (ISO 6668:2008) that suggests a ratio in a 5–9 g range per 100 mL of water and was the same ratio the Ethiopian cuppers used. Evaluation was conducted in a calm environment, with no interfering aromas in each of the locations. The ground coffee was added into clay coffee pot and Fresh boiled water was poured into the coffee pot.The mixture was left heating for seven minutes. The ground coffees used for each roasting style have the same weightand equal amount of boiled water was used. Finallythe brew was made ready for panelists within 8minutes, for cup test analysis.

3.5.2. Cup Tasting

Three cups per sample in three replications were prepared for each tasting session.The sensory evaluation of eachsample and the cup quality was carried out by a panel of fourcommon coffee drinkers. A spoonful ofthe brew was sucked with air into mouth of a taster and held at the back of the tonguebetween the tongue and the roof of the mouth where the tasting glands are located.It washeld in the mouth and moved around for few (7-10) seconds for sensory evaluation, whichinvolved taste for acidity, body and flavor.

3.5.3. Color Evaluation

The color of each roastedsamples were rated by a panel of five common coffee servers and four common coffee drinkers as light, medium and dark

3.6. Total Titratable Acids

Total acidity in the coffee samples was determined by adding 5g of the coffee sample with 37.5 ml 80% alcohol. Agitation was done using a shaker for 16 hours and the solution was filtered with whatman filter paper. Ten ml of the filtrate was diluted to 100 ml followed by titration with 0.1N Sodium hydroxide using phenolphthalein as an indicator [45]. The titration end point was determined by formation of faint pink color as indicated in appendix IV and PH. 8.2.

Figure 2. A panel of judges (regular users of coffee) during tasting the samples

3.7. Determination of Caffeine by UV-Vis spectroscopy

3.7.1. Standard Preparation

Caffeine stock solution 1000ppm was prepared by dissolving 100 mg of pure caffeine in 100 mL of distilled water and prepared five working solutions of 50ppm, 100 ppm, 150 ppm, 200ppm,250ppm in 50 mL of volumetricflasks. The absorption spectra of 5 solutions were recorded in the wavelength range 200-500 nm to define the maximum absorption (λmax). Thus, after λmax is defined to be 274 nm, each standard was recoded in triplicate, and the

average of each was used to draw calibration curve. Followingthe calibration, samples caffeine content at different optimization has been recorded.

3.7.2. Real Sample Preparation for UV-Vis Spectrophotometry

Roasted coffee bean samples were grounded and screened through 250 μm sieve to get a uniform texture.50 mg of sieved coffee was dissolved in 50 mL distilled water. The solution was stirred for one hour using magnetic stirrer and heated gently (60 ^{0}C) to remove caffeine easily from the solution.The solutionwas filtered by whatman filter paper to get rid of particle from solution. The solution was mixed withdichloromethane by volume ratio (1:1) for the extraction of caffeine from coffee. [46]. First, invert the funnel several times and stayed for 20 min. Then, using separatory funnel caffeine was extracted by dichloromethane from thesolution. The extraction of caffeine was preceded 4 times with 10 mL dichloromethane at each round. The caffeine extracted by dichloromethane at each round was stored in volumetric flasks. Finally, the absorbance of the solutionswere measured by UV/Vis spectrophotometer in the range of 200-400 nm against the corresponding reagent blank [34]

3.6.3. CaffeineContent in a Cup of Coffee

To determinecaffeine content in coffee, six cups of coffee infusion were collected from different coffee servers or baristas. Both coffee infusions were filtered by micro filter and diluted with dilution factor. Then the filtrates were measured with UV-VIS spectrophotometry at maximum wave length of 274 nm, separately.

3.6.4. Liquid-Liquid Extraction

To investigatethe kinetics of extraction of caffeine from coffee, aqueous solution of coffee was added into the separatory funnel withdichloromethane solvent by volume ratio (1:1) [46]. Then invert the funnel several times and extraction was done at different time intervals 20,40,60,80 and 100min.The caffeine content in each extract was measured by spectrophotometric method [36]. Sample spectral absorbance measurements were read at 274 nm.

3.7. Method validation

3.7.2. Accuracy and precision

Accuracy and precision are probably the most often estimated terms to express the extent ofErrors in a given analytical results. Analytical results must be appraised to decide on the bestvalues to report and to attempt to establish the probable limits of errors of these values [47] The analyst will thus be concerned with the question of precision (repeatability of results), that is,the agreement between a set of results for the same quantity; and also with accuracy, that is the difference between the measured value and the true value of the quantity, which is determined [46]. In this study the precision of the results were evaluated by the standard deviation of the results of triplicate samples (n = 3), analyzed under the same condition. Standard deviation is a useful parameter in estimating and reporting the probable size of indeterminate errors.The procedure of spiking was as follows: for the determination of the validity of the developed optimized procedures used for determination of caffeine in roasted coffee bean samples, known concentration of standard solution (that is200 mg/L of caffeine was prepared. From this solution 10ml, 20 mland 30 ml, respectively, were added to 90ml,80ml,70ml sample coffee solution. After spiked samples with standard to the required volume 100ml, they were analyzed with the same procedure followed for the analysis of coffee samples. Triplicate samples were prepared and triplicate readings were obtained.

4. Results and Discussion

4.1. Sensory Evaluation

The analysis and interpretation of the data obtained from common coffee servers that have experience on coffee roasting, and common coffee users/drinkers/ through questionnaires were presented in Table 2 and 3

Table 2 sensory evaluation of coffee samples (T1, T2, and T3) (number of judges, n=9)

Item	Samples	Light	Medium	Dark
How do you rate the sensory feelings of the roasted coffee/categories	T1	5(55.5%)	1(11.11%)	3(33.3%)
	T2	1(11.1%)	7(77.7%)	1(11.1%)
	T3	3(33.3%)	1(11.1%)	5(55.55%)

Roasted coffee beans were rated as Light, Medium and Darkby five professional cuppers and by the so called regular users (four in number). The above table shows that, from three roasting style of light roasting conditions trial one(T1) rated as light roasted by 55.5%,T2 rated next to T3 by 33.3% and T1 rated lastly with 11.11%. From medium roasting condition T2 rated as medium by 77.7%, T1and T3 rated as medium by 11.11%. T3 from dark roasting rated as dark by 55.55%,T1 rated as dark by 33.3and T2rated by 11.1.

Table 3 Sensory evaluation of aroma of roasted coffee (number of judges, n=9)

Which roasting type/condition you assume common in Ethiopia coffee roasting	Light	Medium	Dark
	0	7(77.7%)	2(22.22)

	How do you see the aroma of the roasted coffee you preferred, and assumed to be commonly roasted type in Ethiopia?		
	Less important	Very important	Moderately important
	0	7(77.77 %)	2(22.22 %)

The medium roasted coffee was established as preferred roasting condition by its very important aroma with 77.7% of panelist. And, also it was common roasting condition in Ethiopia with 77.77%, light roasting was not common and dark was least common by 0% and 22.22%, respectively according to the respondents.Other researchers reported the same idea,the higher roasting time and temperature the less desirable the aromawill be and the stronger the bitterness. Conversely,low roasting temperatures with short time fail to fully develop the expected aromas, and acidity. Generally medium roast is where most odorants are fully developed and concentrated [48] .

4.2. TotalTitratble Acidity

Measurements of pH quantify the concentration of aqueous hydrogen ions, providing a metric for the quantity of deprotonated acid molecules in a sample. Total titratable acidity (TA) is a measureof all acidic protons in a sample, includingnon-dissociated protons that can be neutralized through the addition of a strong base [49]. Balzer suggested that phenolic acids deprotonated at pH values greater than 8.0 [50]. Thus, TA titrated to pH 8.2 may be better end point for titration [50]. The distinguished acidity in coffee is an important feature for sensory analysis. The increase in acidity has been associated with coffee making during the roasting process, with an inverse relationship with the quality, the higher the acidity the worse the coffee quality [45]. Total titratable acidity values of different roasting conditions are in the range of 1.062± 0.03565 to 1.37±0.114013,similar results were obtained by [51] who reported titratable acidity of 1.53 to 4.66 mL NaOH.The acidity of all roasted coffee decreased as degree of roast increased, in agreementwithprevious studies [52]. Mean values of total titratable acidityto a pH 8.2 expressedin mL of 0.1 N NaOH per 10 mLof coffee samples studied have been showed in Table 4.

Table 4. Mean content of total titratable acidity n the different roasting conditions of coffee

Roasting conditions	Light	Medium	Dark	Reference
TA(mL of 0.1 N NaOH) of sample	4.66±0.05	3.15±0.06	1.53±0.06	[51]
	4.82±0.13	3.55±0.14	2.06±0.06	[51]
	1.37 ± 0.114013	1.333 ± 0.035501	1.062 ±0.03565	This study

4.3. Determination of Caffeine byUV-Vis Spectroscopy

4.3.1. Calibration Curve of Pure Caffeine in Water

The absorbance of five working standard solutions of purecaffeine in the range of 50ppm and 250 wasmeasured at 274 nm using UV-Vis spectrophotometer. And then the absorbance versus concentration graph (Fig.4 was constructed to authenticate the UV-Vis absorption of caffeine in terms of linearity, sensitivity, precision and for calibration purpose to determine the caffeine content of different roasting condition of coffee samples. From the calibration curve (Fig.4), the calibration equation was: $y = 0.245x + 0.371$, $R^2 = 0.999$, where y is absorbance, x is concentration of caffeine and R^2 is the linear regression coefficient. This equation indicated that the present studies were carried out according to the Beer's law ranges in terms of linearity, sensitivity and precision of the method. Thus, the proposed method was acceptable for determination of caffeine in coffee samples.

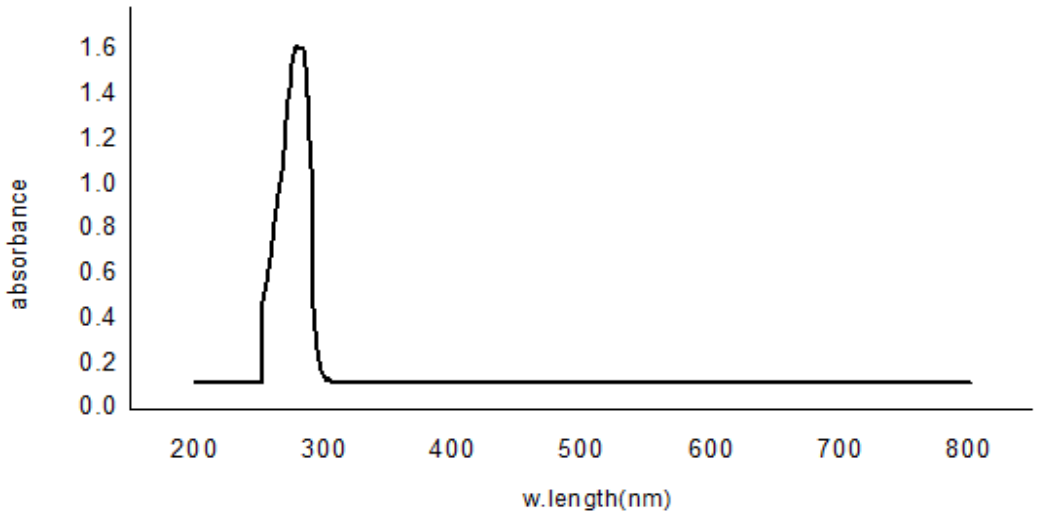


Figure 3 spectra of caffeine standard solution

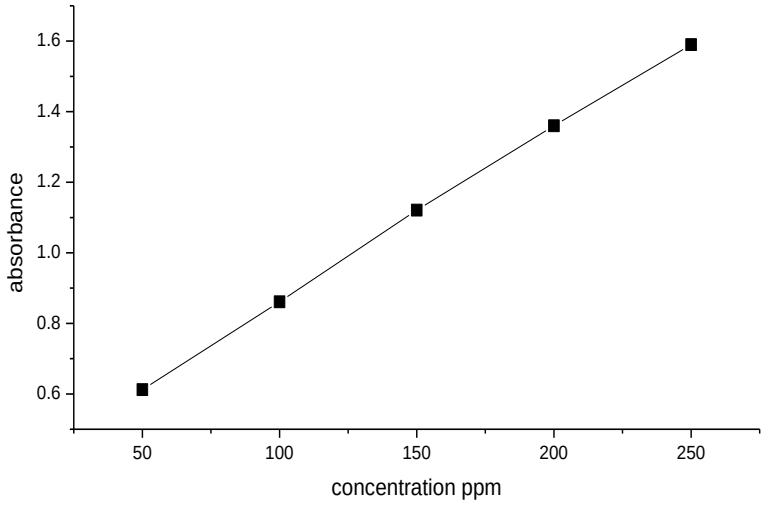


Figure 4 Calibration curve of caffeine standard for UV-visible spectrophotometer analysis

4.3.2. Determination of Caffeine in Roasted Coffee Beans

The degree of roast has no significance effect on caffeine level of the roasted coffee beans. The difference in caffeine contents among same species of coffee beans whichare roasted at different temperatures are due to removal of some compounds from coffeebeans. The caffeine content for coffee roasted at medium temperature greater than light, thisis because water and carbon dioxide which account about 20 % of the total coffee beanswere removed at this temperature. On the other hand a decrease in the caffeine content was observed at the dark, where it is expected that the caffeine contents might melt and removed by evaporation [34].

Due to the temperature of sublimation (178°C) [10], it would be expected that the loss of caffeine would occur to a higher extent when this temperature is reached. Macrae [27] reported that these phenomena could be related with porosity and the internal pressure created into the beans that may cause some difficulties for the sublimation of caffeine. Nevertheless, in a model system, where caffeine is probably free of chemical and physical linkages, a similar gradual decrease of its content occurs [53]. Moreover, important microstructural changes occurring during roasting can drive an additional loss in caffeine [54]. The high temperature reached during roasting causes bursts accompanied by popping sounds [22]. During popping phenomena, caffeine is easily detectable inthe roasting gas;because it is

emitted during seed fracturing popping is a consequence of the accumulation of inorganic gases formed into the closed pores of beans, during the paralysis of several compounds. When the pressure reaches a critical limit, the seedscrack and the entrapped gases are abruptly released. Under these conditions, darker roastingdegrees could present less caffeine amount.

The caffeine content in the literature ranged between 0.7 and 1.6 g/100 g of roasted coffees [52]. In this study the caffeine content in different roasting conditions wereranged between 0.75 and 1.15 (w/w%) the same with the range in the literature. The caffeine content inlight, medium, and dark roasted coffee were presented in table 5

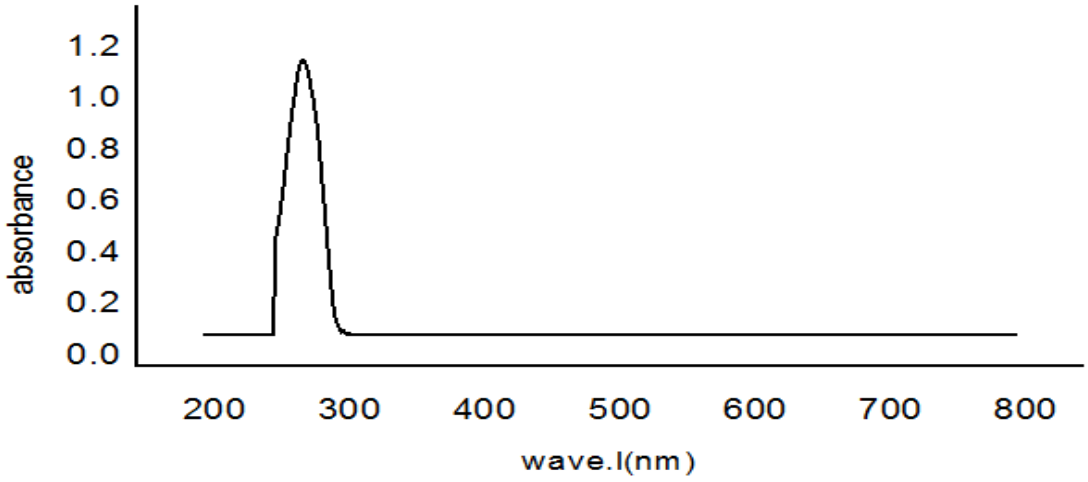


Figure 5 spectra of caffeine extracted from coffee sample for Uv-visible spectrophotometer analysis

Table 5 comparison of caffeine content in different roasting conditions with other reports

Roasting	Caffeine Content (%)	Reference

Condition		
Light	0.69 ± 0.02	
Medium	0.78 ± 0.00	[55]
Dark	0.79 ± 0.05	
Light	1.33± 0.10	
Medium	1.39± 0.10	[55]
Dark	1.43± 0.12	
Light	1.8± 0.3	
Medium	-	
Dark	1.0 ± 0.1	[12]
Light	1.12±0.4128	
Medium	1.15±0.0178	Present study
Dark	1.1±0.0393	

4.3.3. Determination of Caffeine in a Cup of Coffee

4.3.3.1. Calibration Curve of Caffeine Standard

The absorbance of five standard solutions were measured and the absorbance versus concentration was plotted. The calibration give the equation $y= 0.0044x + 0.0186$ and $R^2= 0.9988$. Then caffeine content in a cup of coffee was calculated from calibration curve.

The coffee beverage is now an important item in the lives of billions of people which is traditionally used to complement meals, as well as for hedonistic and psycho stimulant purposes. Epidemiological data support the view that habitual coffee consumption has several health benefits because of its content of bioactive compounds and caffeine, which can exert physiological and healthy effect. The consumer preferences in terms of the sensorial properties of coffee are ejected by different factors, such as culture, lifestyle, social behaviors, habits, and economic aspects. More recently, the attention of consumers is focused on the outcomes of coffee intake on health and well-being of specific components, such as caffeine and bioactive compounds. In this contest, brewing methods and the extraction conditions are

essential to obtain the desired chemical, sensorial, and healthy properties of coffee in cup. Due to the wide spread consumption of caffeine, it is important to collect precise information of their content in foods.

Therefore the caffeineconcentrations of six coffee brews prepared by coffee makers in Jimma town wereanalyzed. Thecaffeinecontent in thesesamples ranged between 17.7-53.4 mg/100mland the average concentration of them was 41.02 mg/100ml as shown in table 6which is in the same range with caffeine content in the literatures indicated in table 7. Different results were obtained from all six samples taken from coffee makers. That variationmay be influenced by the roasting degree, brewing method and origin of coffee.

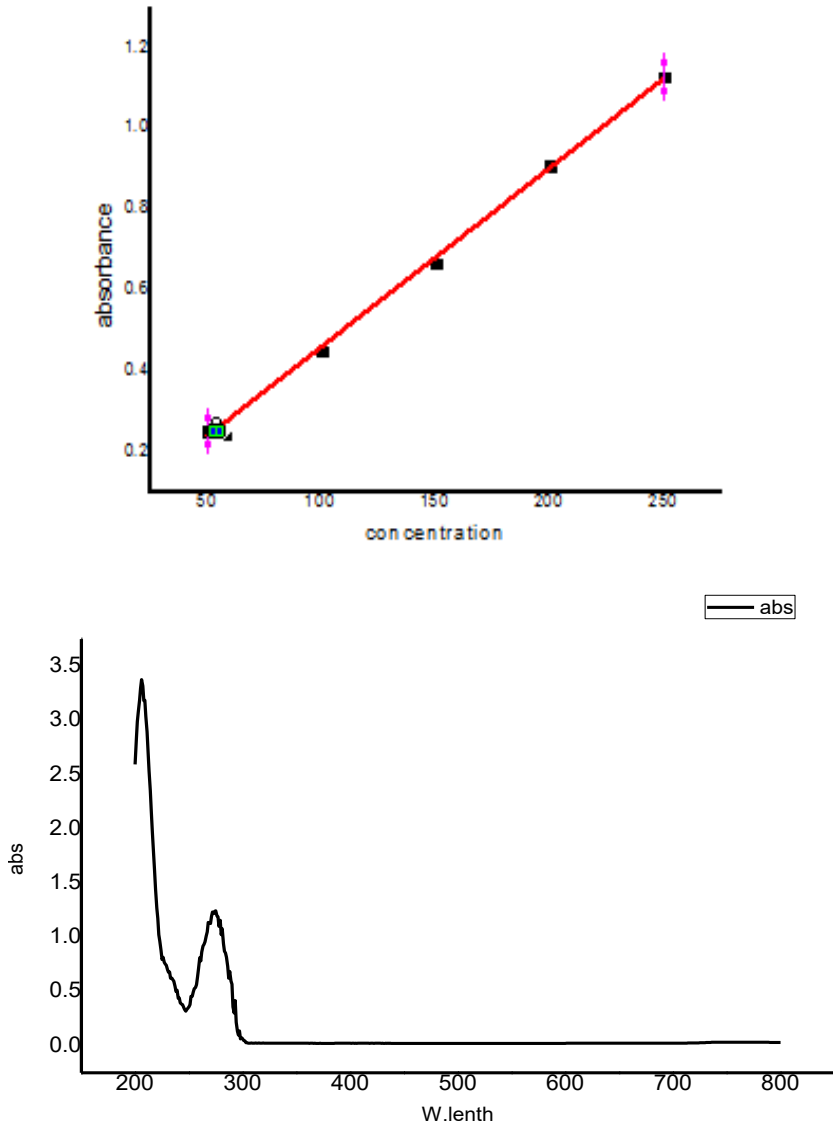


Figure 6 calibration curves for caffeine standard for determination of caffeine in a cup coffee and its spectra

Table 6experimental and calculated concentration of caffeine in a cup of coffee

Sample	Concentration(mg/L)	Concentration (mg/100ml
1	1771	17.7
2	229.2556	45.85
3	189.6833	37.94
4	227.5926	45.52
5	249.0396	53.54
6	228.0921	45.62
average		41.02

Table 7.Comparison of Concentration of Caffeine in a Cup of Coffee with Other Reports

	Concentration mg/100ml	Reference	
	50.8	[56]	
	39.7	[57]	
	43.2	[24]	
	44.0	[9]	
	39.0	[58]	
	39.0	[59]	
	41.02	**This study**	

4.3.4. Extraction kinetics

The total Caffeine content in extract was measured by spectrophotometric with the same procedure for determination of caffeine performed in the above experiment.Samplespectral absorbance measurements were read at274 nm. The caffeine concentration was calculatedfrom the calibration curve.To show the extraction kinetics different models can be found in the literature. In this study first order kinetic was fitted with experimental data with R^2 =0.944.Figure 5 shows that when increasing extraction time the rate of extraction was linearly decreasing. Other model which cannot fit with the experimental data was shown in appendix 6.

Table 8 Experimental data for extraction kinetics of caffeine

Co(mg/L)	Time	Ce(mg/L)	Ct(mg/g)	ln(Ce-Ct)
5049.2	20	1293.462	751.1476	6.295845907
5049.2	40	2226.153	564.6094	7.415502329
5049.2	60	3114.497	386.9406	7.911161396
5049.2	80	4025.918	204.6564	8.248335909
5049.2	90	4954.262	18.9876	8.504163553

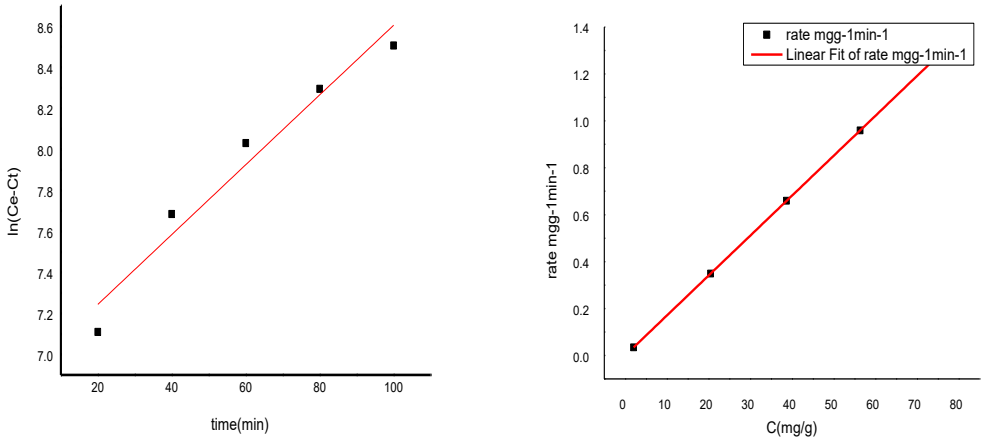


Figure 7 graph of ln (Ce-Ct) vs. time and rate vs. concentration

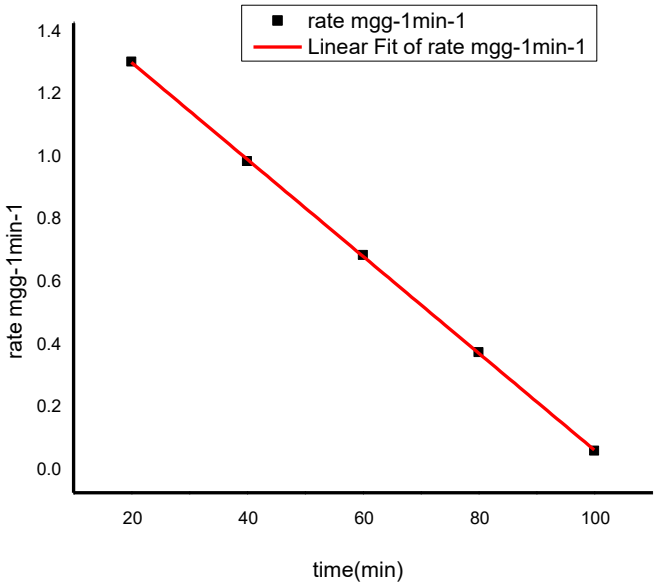


Figure 8 shows effect of time on rate of caffeine extraction

4.4. Accuracy

The accuracy of the method used for determination of caffeine was evaluated by analyzing the concentration of spikedSamples. The results are given in Table 7. The recoveries of caffeine in the spiked coffeesamples were 88.3% to 97.9% indicating the accuracy of the method for the analysis of the caffeine content.

%Recovery = Spiked-unspiked/con.added *100

Table 9. Analytical results obtained for validation of the optimized procedure

samples	Amount added(ppm)	Spiked sample(ppm)	Unspiked sample(ppm)	%Recovery
1	20	248.15	229.25	94.5
2	40	225	189.68	88.3
3	60	248.38	189.59	97.9

4.5. Limitation of the Study

Roasting of coffee beans were take place by using traditional coffee roaster in open condition. Due to this reason, the cofoundingfactors was difficult to manage roastingtemperature.

5. Conclusions and Recommendations

5.1. Conclusions

During the study, the effect of different types of coffee roasting conditions (light, medium and dark) the cup quality attributes, content of caffeine and total acid were investigatedfrom Jimma coffee Ethiopian. Furthermore, the caffeine content in a cup of coffee in six brew sample taken from coffee cuppers in Jimma town and caffeine extraction kinetics were analyzed. According to the experimental results of the study, medium roasted coffee resulted at the best roasting condition and common roasting coffee in Ethiopia. Thedegree of roast was found to have no significant effect on caffeine contents of the coffee bean samples. Generally, a significant reduction in total acids content of the coffee beans was observed during the roasting process, with darker roasts attaining the least values.From roasting conditions, the highest caffeine level was recorded at medium degree of roast obtaining 1.15% w/w followed by light roast with 1.12 % w/w and dark roast with the least 1.1% w/w.For total acidity, the highest level was recorded at light degree of roast obtaining 1.37% followedmediumwith 1.33% and dark with the least 1.062%. The caffeine content in a cup ranged between 17.7-53.4 mg/100ml coffee. Different results were obtained from all samples taken from coffee makers. That variation may be influenced by the roasting degree, brewing method and origin of coffee.

5.2. Recommendations

- It is very important to extend this study to varieties of coffees, types of coffee roasting technologies, and roasting conditions in order to really pinpoint how different types of roasting machines or technologies and grinding size affect the quality of roasted specialty coffee beans in Ethiopia.
- It is also recommended to promote similar researches in the field of coffee processing technology for successful industrial transformation and economic development of Ethiopia

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Appendixes

Appendix1 Roasted coffee at different condition represented as T1

Dark medium Light

Appendix2 Roasted coffee at different condition represented as T2

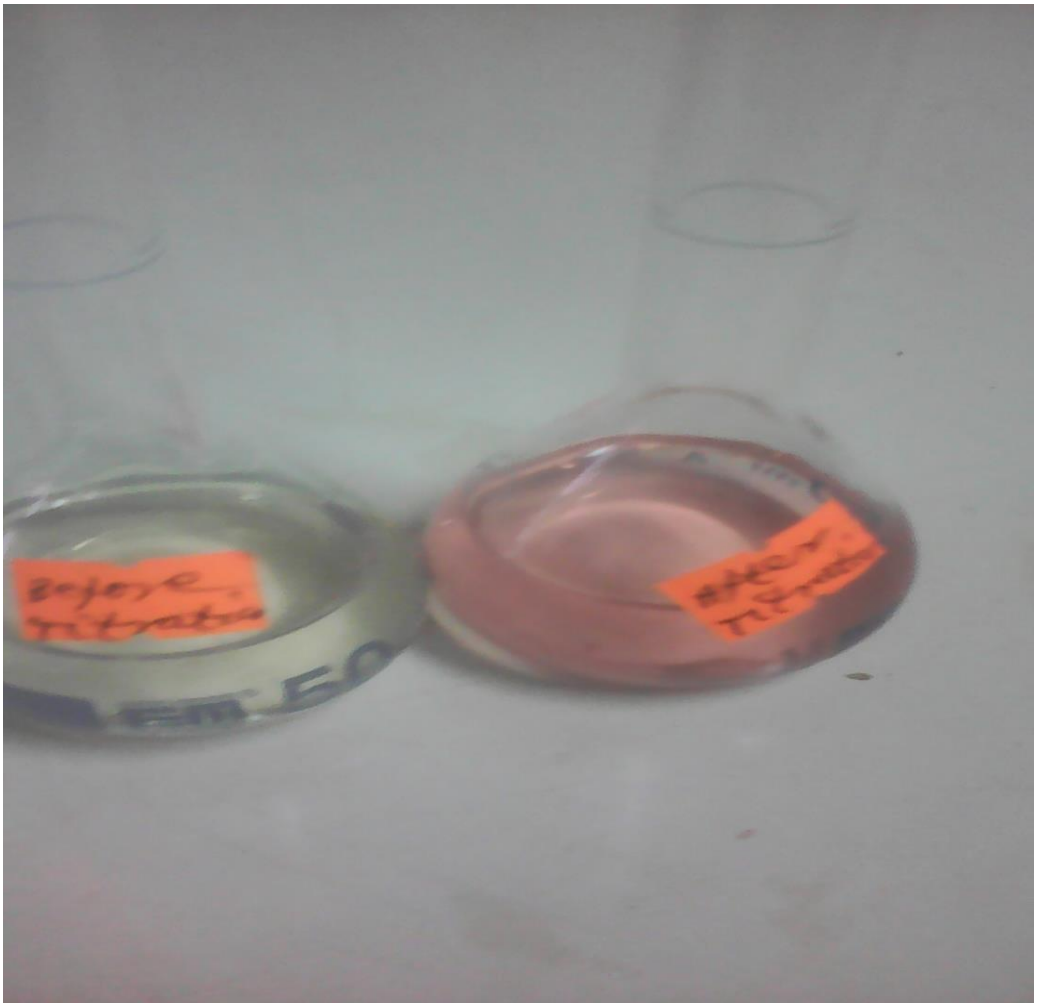
Dark Medium Light

Appendix 3 Roasted coffee at different condition represented as T3

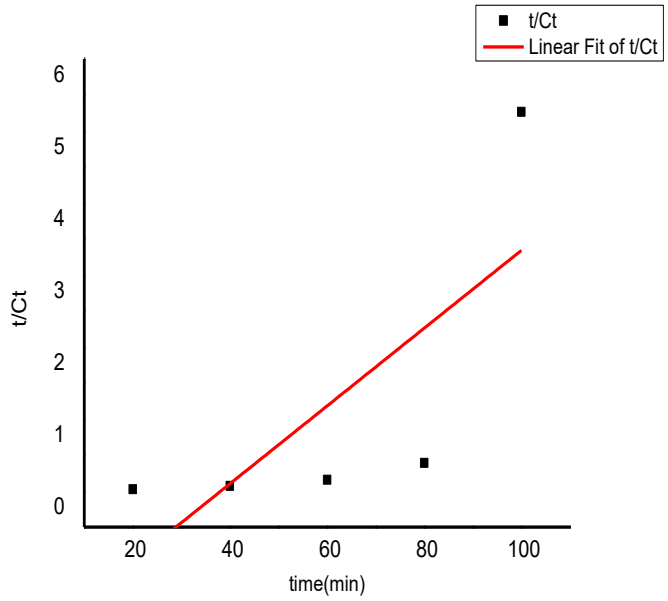
Dark medium Light

Appendixes 4 All grinded coffee samples in plastic bags

Appendix5 Color of coffee solution before titration and after titration



Appendix.6.kinetic model



Kinetic data of second –order

Ce(mg/l)	time(min)	Ct(mg/g)	t/Ct	R^2
1293.462	20	751.1476	0.026626	0.40957
2226.153	40	564.6094	0.070845	
3114.497	60	386.9406	0.155063	
4025.918	80	204.6564	0.390899	
4954.262	100	18.9876	5.266595	