Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.10 No.1, 2018

Chemistry of Caffeine in Coffee and Its Determination Using UV/Vis Spectrophotometer: A Review Article

Fisseha Gebrewold Gosaye Geletu
Madda Walabu University, College of Natural and Computational Sciences, Department of Chemistry, Bale-Robe, Ethiopia; P.Box: 247

Abstract
This review is all about the chemistry of caffeine and some methods applied to determine the concentration of caffeine in coffee. Coffee is the major sources of caffeine. In pure form, caffeine is a crystalline white powder moderately soluble in water and in a wide range of organic solvent such as ethanol, ethyl acetate, methanol, benzene etc. When it is removed from the source plants and reduced to its most natural state, it forms a white powder. Consequently, the ingestion of caffeine, an important ingredient of coffee as well as tea, has become a daily habit for many persons. Among different methods used to determine the concentration of caffeine in coffee, spectrophotometric method is an effective method used for the determination of caffeine in Coffee raw. The method was found to be fast, simple, cost effective and environmental friendly for the determination of caffeine in aqueous solution of coffee with satisfactory results.
Keywords: Coffee, caffeine, UV-Viscible spectrophotometer

INTRODUCTION

Coffee

It is believed that coffee tree was first originated in the Southern Nation Nationalities People Region (SNNPR) of *Kaffa*zone, in the area known today as “Mankira”. Its name also believed to be derived from the name of this zone where shepherds discovered the coffee beans in the 6^{th} century. Then, the Arabs introduced coffee from Ethiopia to Yemen during the 13th century; whereas the habit of drinking coffee was developed in the 15th 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 (Kolayli *et al.,* 2004).

The growth of popular coffee houses, which became favorite meeting places for both social and business purpose, spread from the mid-17th century to other European countries including Austria, France, Germany, Holland and England. Coffee is now one of the valuable to primary commodities in the world, often second in value only to oil as a source of foreign exchange to developing countries. Millions of people around the world earn their living from it (Fujioka *et al.,* 2008).

Caffeine an alkaloid of the methylxanthine family is a naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide. The most commonly known sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves. 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 (MJ, 1987). Its structural formula is as shown below.

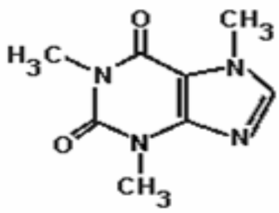

Fig.1. Structure of caffeine

In the manufacturing of decaffeinated coffee the manufacturer needs to know how much caffeine is in the coffee to ensure products quality. One of the requirements of a good coffee is whether it contains a reasonable moderate quantity of caffeine or not. Therefore quantitative determination of the amount of caffeine in green coffee is necessary.

Hence the objective of this review is to discuss some chemistry of caffeine in coffee as well as to give insight about simple, rapid, cost-effective, and environmentally friendly method for the quantitative determination of caffeine in aqueous solution of coffee.

The Chemistry of selected components of coffee

Coffee contains several biologically active substances, which have some capability of causing physiological effects. The chemical composition of coffee varies for different reasons such as species and variety of coffee beans and to lesser extent other factors such as agricultural activities, degree of maturation and storage conditions. The beans of the most economically important and cultivated species of coffee. *Arabica*, contains such as 8 % phenol polymers, 6 % polysaccharides, 4% hydroxycinnamic acids, 2 % trigonelline, 3 % minerals, 2 % water, 1 % caffeine, 0.5% Carboxylic acids, 0.3 % sugar and 0.2 % lipids (Mehari, 2008).

Carbohydrate

Only traces of free mono and disaccharides in green coffee remain after roasting. Cellulose, hemicelluloses, arabinogalactan and pectins play important roles in the retention of volatiles and contribute to coffee brew viscosity. It is reported that in espresso coffee, the foam stability is related to the amount of galactomannan and arabinogalactan (Combria *et al*., 1997).

Acids

Acids are responsible for acidity, which together with aroma and bitterness is a key contributor to the total sensory impact of a coffee beverage. Carboxylic acids, mainly citric, malic and acetic acids are responsible for acidity in brewed coffees. Arabica coffee brews are more acidic (pH 4.85-5.15) than Robusta brews (pH 5.25-5.40) (Vitzthum, 1975).

Lipids

Lipids are constituted of green coffee. The *diterpenes*(cholesterol-like) *kahweol*and *cafestol* are specific to coffee. The content of lipids can reach a few percent in coffee preparation or when ground coffee is percolated and not filtered but levels are in significant in filtered and instant coffees (Olcina *et al*., 2006).

Trigonelline

Trigonelline comprise about 2% of the dry weight of green coffee but does not survive roasting, breaking into nicotinic acid, pyridine and other volatile compounds. It is also found in various plants and in some animal species including sea urchins and jelly fish. It also appears in mammalian urine after administration of nicotinic acid. It is one of the responsible components for bitter tasting in coffee brew. It is also thermally unstable and converted nicotinic acid and to certain flavor compounds during roasting (Mumin *et al*., 2006).

Hydroxycinnamic acids

They are one of the major classes of phenolic compounds. They are present in a large variety of fruits and vegetables. The major representative of hydrocinnamic acid in food is caffeic acid. It is largely occurs conjugated with quinicacid as chlorogenic acid. Coffee, one of the most widely consumed beverages in the world, is the major dietary source of chlorogenic acid and caffeic acid is produced from chlorogenic acid by hydrolysis at elevated temperature (Asihara, 2006; Seid, 2008).

Chlorogenic acid has antioxidant properties showed by its ability to scavenge various free radicals when tested. Moreover, chlorogenic acid reduces glucose uptake by favoring the dissipation of the Na^+ electrochemical gradient and inhibits the activity of hepatic glucose-6-phospates which is implicated in glucose homeostasis. When it is ingested coffee form, it increases the plasma antioxidant capacity (Seid, 2008).However, among these components of coffee, trigonelline and hydroxycinnamic acids arethe major variables that affect the determination of caffeine in coffee sample.

The presence ofthese acids in green coffee extracts, they affect the quantitative determination of caffeine incoffee using Uv/vis spectrophotometer. From the literature report these coffee componentsabsorb with λmax 271, 275 and 330nm for trigonelline, caffeic acid and chlorogenicacid, respectively (Mehari, 2008). Developing suitable method for removing these acids from caffeine is the firstwork in direct absorption method for the determination of caffeine.

Caffeine in coffee

Caffeine

Caffeine is a naturally occurring alkaloid which belongs to a class of organic compounds called methylxanthines. Chemically speaking caffeine belongs to the alkaloid family and the methylxanthine group. Caffeine or *1,3,7-trimethylxanthine* is a xanthine molecule with methyl groups replacing all of the three hydrogen's bound to nitrogen in xanthine. The closely related substances theophylline or 1, 3-dimethylxanthine and theobromine or 3, 7-dimethylxanthine are members of *N*-methyl derivates of xanthine (Asihara, 2006; Venadai, 1998).

In pure form, caffeine is a crystalline white powder moderately soluble in waterand in a wide range of organic solvent such as ethanol, ethyl acetate methanol, benzene etc. When it is removed from the source plants and reduced to its most natural state, it forms a white powder. This powdered form of caffeine is actually the scientific definition of bitter, that is why many beverages containing caffeine also contains copious amounts of sugar or other sweeteners. Its molecular weight is 194.19 g/mol.with chemical formula $C_8H_{10}N_4O_2$, melting point is 236 °C, which sublimes at 178 °C at atmospheric pressure and its specific gravity is 1.2(Olcina *et al*., 2006).

Occurrence

Caffeine occurs naturally in over 60 plant species of which the most well-known are coffee, tea and cocoa-beans, all of which have been consumed for many years. It is produced commercially by extraction from natural sources. Coffee is the major sources of caffeine. Coffee is one of the most popular consumed beverages in the world. Consequently, the ingestion of caffeine, an important ingredient of coffee as well as tea, has become a daily habit for many persons. A large amount of caffeine is found in coffee seeds (mainly in endosperms). Caffeine is found in coffee associated to trigonelline and chlorogenic acids. Coffee has both the highest and the mostvariable caffeine content among dietary products containing this alkaloid (Venadai, 1998; Maria Inez de Godoy, 2008).

The Caffeine content of coffee beans is mainly expressed as percentage of dry weight. The bean of most cultivars of Arabica coffee (Coffea Arabica) contains up to 1.0% caffeine, but Robusta coffee (Coffeacanephora) contains about 2.0% caffeine. Although these species are not commonly used for beverages, Coffeadewevrei (1.2%) and Coffealibberica (1.4%) also contain high concentration of caffeine (Groisser, 1978).

Nowadays, coffee is one of the most popular drinks across the world and the total annual worldwide production of green coffee is estimated to be about six million tons and the coffee industry is second next to oil with associated revenue of $60 billion worldwide. The roasting of green coffee beans yields the apparently bitter and dark coffee beverage, in which caffeine is responsible for the mild stimulating properties and to a particular proportion of its apparent to bitterness. Coffee is grown in different parts of the world and differs genetically in their composition; as a result the amount of caffeine per gram of the coffee bean varies depending on the species of beans, geographic areas, and the preparation process (Venadai, 1998; Emara, 2004).

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 foetus, and into the breast milk (Angelov *et al.*, 2007).

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 methyluric acids. The main dimethyl xanthine metabolites are *theophyline*(4 %), *theobromine*(12 %) and *paraxanthine*(*1, 7-dimethylxanthine*) (80 %). *Paraxanthine*is not found in foods but it is the main metabolite of caffeine. Formation of the other metabolites; *1,3 7- trimethyluric acid* and *6-amino-5 (N-formylmethylamino)-1,3-dimethyluracil* together account for 15% of caffeine elimination. The metabolism is slowedduring pregnancy and in women taking oral contraceptives. On the other hand, themetabolism rate of caffeine is grater in smokers than non-smokers (Singh, 2006).

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 **(**Safvi *et al.*, 2008)

Beneficial Effects of Caffeine

Caffeine is a plant based alkaloid which stimulates the central nervous system. The stimulatoryeffects of caffeine usually results in an increased ability for mental activity for sweets bysimulating the production of those adrenal hormones that cause blood sugars to beincreased. Stimulatory effects of caffeine results in increased capacity for both mental activityand muscular work. The weakness, depression and discomfort from excess of alcohols can benullified 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 ofdepression from a little too much tobacco is helped by coffee (Alpdoğan *et al.*, 2002).

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 a positive energy balance and obesity. Caffeine is also an accepted drug for intramuscularapplication to treat arterial hypotension (Tavallali *et al.*, 2009; Cauli, 2002).

Side effects of Caffeine

There is no requirement for caffeine in the diet. A moderate caffeine intake, 50-200 mg of caffeine, is generally sufficient for a mild stimulation. Excessive Caffeine intake can lead to a fast heart rate, dieresis (excessive urination), nausea and vomiting, restless, anxiety, depression tremors. Over doses of caffeine may result in hypertension, hypotension, tachycardia, fever, delusions, hallucination, and cardiac arrest (Leong way, 1985; Hassan, 2008).

Caffeine is thought to increase alertness, reduced sleep tendency and produce adverse effects in the central nervous system such as insomnia (difficulty in getting to sleep or staying asleep). One of the negative effects of caffeine is its ability to disrupt sleep cycles, causing less deep sleeps , particularly when intake caffeinated beverage within a few hours of bedtime. Caffeine can create panic attacks and interfere with medications taken to calm the system. Caffeine does reduce dopamine, a chemical produced by the brain that affects the brain pleasure centers. This can create more depressions and anxiety (Quiroz-Neto, 2001; Kaye, 2007, Pavel *et a*l., 2003).

For people with high blood pressure or high blood sugar levels (diabetes) the negative effects of caffeine should be understood. Caffeine can raise these levels. Any type of heart problems can be affected by caffeine. The negative effects of caffeine is increasing heart rate can create problems for people with heart conditions and in high doses; caffeine can induce irregular heartbeats in healthy people. Caffeine increase acidic responses in the stomach which may lead to excusive bowel movements and is associated with stomach discomfort (Pavel *et a*l., 2003).

Physiological Effects of Caffeine

Caffeine is a powerful central nervous system stimulant; a mild diuretic and has been used as a mild anti-depressant. It has many physiological effects such as, gastric and secretion, dieresis and stimulation of the central nervous and the cardio vascular system. In addition, caffeine, interference with the uptake and storage calcium by the sarcoplasmic reticulum. It also increases the respiratory rate and causes broncho dilation and stimulates lypolgysis. The similarity in chemical structure between the adenine portion of adenosine and the caffeine molecules is the key to how caffeine works. 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 thereby blocks the regulatory functions adenosine and produces the stimulatory effect (Zen *et al*., 1997; Zen *et a*l., 1998).

In addition, caffeine blocks the receptors and thereby disorders the regulatory function of adenosine and produces the stimulatory effect. The resulting effect in turn increases nerve activity that causes the release of hormone epinephrine, which leads to several effects such as higher heart rate, increased blood pressure and release of glucose by the liver (Teshima *et al*., 2001; Perez-Martinez et *al*., 1995; Kuazawa, 1999).

Adenosine inhibits the release of many neurotransmitters including noradrenalin, dopamine, acetylcholine and glutamate. By blocking the receptors, caffeine facilitates the release of neurotransmitters and through this mechanism it also promotes wakefulness. Caffeine enhances muscular performance by lowering threshold for sarcoplamic Ca^{2+} release through theryandoine receptor (Olcina *et al*., 2006). The physiological effects of caffeine associated with cardio vascularsystem are believed to be the result of multiple actions. These include antagonistic effects on adenosine receptors, activation of the sympatic nervous system (release of catecholamine from adrenal medulla), and stimulation of adrenal cortex (release of corticosteroids), renal effects (Diuersis, natriursis and activation of reninangiotensin aldosterone system).

Methods of Determining Caffeine in Coffee

The most commonly used methods for the determination of caffeine in beverages include various analytical methods such as High Performance Liquid Chromatographs (HPLC), Thin Layer Chromatography (TLC), Solid Phase-Extraction And High Performance Liquid Chromatography (SPE-HPLC), Capillary Zone Electrophoresis (CZE), Fourier Transform Infra-red (FT-IR),and Electrochemical methods havebeen reported. Although these methods have been usually used widely used for caffeine determination, the methods require very costly instrumentation, higher skilled technician and complicated and time consuming procedures, (Bouhsain *et al*., 2000; I, 2013; Horie *et al*., 1997; Marcia *et al.,* 2002).

UV/Vis spectrophotometric method became important means in the analyses of caffeine as various methods have been reported for the determination of caffeine in soft drinks, coffee and tea. The method is cost effective, uses fewer reagents, easily performed, is less time consuming and highly accurate. On the other hand, the use of spectrophotometric provides a simple and rapid method for the determination of caffeine in tea and coffee and their products this is because of the characteristics observation peak of caffeine at 271–276 nm (Maidon*et al*., 2012; Redi *et al*., 2008).

CONCLUSIONS

Coffee is one of the most important cash crops grown in southern and eastern part of the country. Among different chemicals that coffee contains, Caffeine is one of the important content of coffee which does have various importances to human body as well as side effects if it is taken to its maximum level. In order to know the quality of coffee, the concentration of caffeine should be determined. There are various methods to determine the concentration of caffeine in coffee. Among various methods of determining caffeine in coffee, UV/Vis Spectrophotometeric method is an important analytical tool for the determination of caffeine in various kinds of samples as it is very simple and is relatively accessible. The aqueous extraction and dilution procedures are simple and require no special technique. The method does not involve use of any special reagent, nor does it include tedious filtration or extraction procedure.

REFERENCES

A. MJ. *The pharmacology of caffeine*.*Prog.Drug*.,1987, 31: 273.
A.Quiroz-Neto, *J.Applied Toxicology*., 2001**,** 21, 229.
A.Safvi, N.Malek, O.Moradlou and F.Tajabadi, *Anal.Bio.Chem*.,2006**,**359,224.
B.Maidon, A.Mansoer, H.Sulistyarti.Study of Various Solvents for Caffeine*:* Determination Using UV Spectrophotometeric.*Journal of Applied Sciences Research.,* 2012*,* 8(5): 2439-2442.
B.Mehari M.Sc. *Thesis*, 2008AAU.
B.Marcia, C.Marcia, L.Heloísa, R.Oliveira, F. Reis, N.J.O. and J.Andrade, B. Simultaneous determination of caffeine, theobromine and theophylline by high-performance liquid chromatography.*J. Chromatographic Sci.*, 2002, 40: 45-48.
B.Venadai, *Bra.J.Med.Bio.Research.,*1998,31,4.
D.Singh and A.Sahu, *Anal.Bio.Chem*.,2006,349,176.
D.Groisser, *Am.J.Clin.Nutr*.,1978, 31, 1721.
E.Leong way, *Ann.Rev.Pharmacol.Toxicol*.,1985**,** 25,769.
F.Combria, M.Duarte*,* Foam Stability, and Chemical Composition of Espresso Coffee As Affected by the Degree of Roast.*Journal of Agricultural and Food Chemistry.,*1997,45(8), 3238-3243.
G. Bouhsain, Z. Garrigues, D. Guardia,FTIR determination of caffeine in roasted coffee sample*s. Fresenius' Journal of Analytical Chemistry.,*2000,366(3), 319-322.31.
G.Alpdoğan , K. Karabina ,and S.Sungar, *J. Turkey.Chem.Soc*.,2002,26,295.
G.Alpdogan,K.Karabina and S.Sungar,*J.Turkey Chem.Soc*.,2002,26,295.
G.Olcina,D.Munoz, R.Timon, M.Caballero, J.Maynar, A.Cordova and M.Maynar, *J.Sport Sci.Medi*.2006, 5,621.
H. Horie,T.Mukai andK.Kohata .Simultaneous determination of qualitatively important components in green tea infusions using capillary electrophoresis*. Journal of Chromatography A.,* 1997, 758:332-5.
H. Asihara,*Braz.J.Plant Physiol*., 2006,18,1.
H.Tavallali and M.Sheikhael, *Afri. J. App. Chem., 2009*, 3, 011.
I.J. Electrochem. Sci., 8 **(**2013**)** 5755 – 5773
I.Pavel, A.Szeghalmi, D.Moigo and S.Cinta, *Biospectroscopy*., 2003, 72, 25.
I.Perez-Martinez,S.Sagardo and M.J.Medina, *Anal.Chim. Acta*.,1995, 304,195.
I.Svancara, K.Vytras, J.Barek and J.Zima, *Critical Reviews in Analytical Chemistry*.,2001**,** 31,311.
J.Zen, Y.Ting and Y.Shih, *Analyst***.,**1998**,** 123, 1145.
J.Zen and Y.Ting, *Anal.Chim.Acta*, 1997, 342,175.
K. Fujioka and T. Shibamato, *Food Chemistry*, 2008,106.217.
K.Seid M.Sc. *Thesis*., 2008**,**AAU.
M.Mumin, K.Akhter, Md.ZainalAbedin and Md.ZakirHossain, *J.Maly.Chem.Soc*.,2006, 8, 45.
M.Redi, A.Belay, K.Ture, and A.Asfaw, *Food Chemistry.,* 2008**,** 108, 310.
N.Teshima, K.Ogawa and T.Sakai, *Anal.Sci*., 2001**,** 17,125.
O.Cauli and M. Morelli, *Psychophamacology.,*2002**,**162,246.
O.G.Vitzthum, Chemie und Bearbeitung des Kaffees.*Berlin Springer*.,1975.
P.Maria Inez de Godoy, *Braz. Arch.Biol.Technl*., 2008,51,447.
S. Kolayli, M. Ocak, M.Kűcűk and R. Abbasoglu, *Food Chemistry.***,**2004,84,383.
S.Emara, *Biomed.Chromatogr*.,2004**,** 18,479.
S.Kaye, *Toxicology of Caffeine*.,2007**,** New York.
T. Angelov and M.Atanassova, *J. Chromatogr. B.Biomed.Sci.Appl.,*2007**,** 2,223.
T.Kuazawa, *Anal.ChimActa*., 1999,387, 53.
W.Hassan, *Am.J.Applied Sci*., 2008**,** 5, 1005.