Full Length Research paper

Determination of caffeine content of tea and instant coffee brands found in the Kenyan market

H. N. Wanyika*, E. G. Gatebe, L. M. Gitu, E. K. Ngumba and C. W. Maritim

Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62 000 – 00200, Nairobi, Kenya.

Caffeine (1, 3, 5-trimethylxanthine), a mild addicting drug though used for medicinal purposes is the active ingredient that makes tea and coffee valuable to humans. In this study, the levels of caffeine in certain coffee (nescafe, africafe, dormans) and tea (chai mara moja, kericho gold, sasini, finlays premium) brands found in the Kenyan market were determined using high performance liquid chromatography (hplc) and UV/ Vis spectrophotometric methods. The levels of caffeine in all the tea and coffee brands were found to be within the documented range. The order of caffeine concentration in tea samples was found as follows: chai mara moja > finlays premium > kericho gold > sasini. In coffee it was found that the caffeine content of africafe > nescafe > dormans. Generally, higher concentration of caffeine in all the samples were realized with the UV/ Vis spectrophotometric method compared to hplc method indicating that acidified water was a better caffeine extractor than pure water.

Key words: Caffeine, tea, coffee, high performance liquid chromatography, UV/ Vis spectrophotometry.

INTRODUCTION

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 (Aurnaud, 1987). Its structural formula is as shown below.
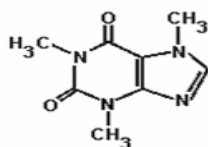

Structure of caffeine

Caffeine is a pharmacologically active substance and depending on the dose, can be a mild central nervous system stimulant. Caffeine does not accumulate in the body over the course of time and is normally excreted within several hours of consumption (Barone and Roberts, 1996).

Other naturally occurring methylxanthines include theobromine and theophyline. Methylation of theobromine forms caffeine. Caffeine and related compounds contain an imidazole ring fused to a pyrimidine ring. Their structures are given below:
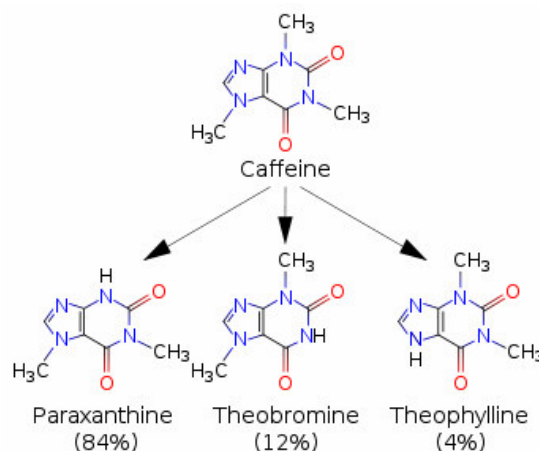
*Corresponding author.E-mail:eujed@yahoo.com. Tel: +254-723-470213. Fax: +254-67-52164.

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 (http://www.ico.org/caffeine.asp), even beans within a given bush can show variations in concentration. In general, one serving of coffee ranges from 40 milligrams, for a single shot (30 milliliters) of arabica-variety espresso, to about 100 milligrams for a cup (120 ml) of drip coffee. Generally, dark-roast coffee has less caffeine than lighter roasts because the roasting process reduces the bean's caffeine content (http://coffeefaq.com/site/node/15, http://www.jeremiahspick.com/caffeine-e-13.html).
Arabica coffee normally contains less caffeine than the robusta variety (http://www.ico.org/caffeine.asp). Coffee also contains trace amounts of theophylline, but no theobromine. Tea is another common source of caffeine. Although tea contains more caffeine than coffee, a typical serving contains much less, as tea is normally brewed much weaker. Besides strength of the brew, growing conditions, processing techniques and other variables also affect caffeine content. Certain types of tea may contain somewhat more caffeine than other teas. Tea contains small amounts of theobromine and slightly higher levels of theophylline than coffee. Preparation and many other factors have a significant impact on tea and color is a very poor indicator of caffeine content (http://www.nobleharbor.com/tea/caffeine.html).

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 (Bolton et al., 1981). These tablets are commonly used by students studying for their exams and by people who work or drive for long hours (Pullicino, 2008).

Uses of caffeine

Caffeine which is found in tea and coffee imparts bitterness and also acts as a flavor constituent (Leo, 1992). It is a mild nervous stimulant towards drowsiness and fatigue. In this respect, is used by athletes to enhance performance since it mobilizes fats from stores a process that normally does not become maximal until intense activity is underway (Eva, 1988).

Caffeine is used as a drug on the basis of its effect on respiratory, cardiovascular and the central nervous system. It is included with aspirin in some preparations for treatment of headaches as it decreases cerebral eye blood flow. It is included with ergotamine in some anti-migrane preparations, the object being to produce a mildly agreeable sense of alertness (Lawrence, 1986).

Caffeine is administered in the treatment of mild respiratory depression caused by central nervous system depressants such as narcotic (Jeanne, 1987).

Caffeine may also be used in the treatment of acute circulatory failure. In either beverage or in nonprescription tablet form, it may be used to relieve fatigue since it increases the amount of urine flow. In fact there are about 2000 non-prescription and about 1000 prescription drugs containing caffeine (Jeanne, 1987).

Effects of caffeine

About 200 mg of caffeine contains pharmacological effect. At this level, it stimulates the central nervous system, decreases fatigue leading to clearer flow of thoughts, sustained intellectual effort and a more perfect association of ideas with a better appreciation of sensory stimuli in man. At this level, it has a diuretic effect on the kidney hence affect fluid balance in the body. It also increase heartbeat rate, dilate blood vessels and elevate levels of free fatty acids and glucose in the plasma. 1 g of caffeine leads to insomnia, nervousness, nausea, ear ringing, flashing of light derillum and tremulousness (Lawrence, 1986).

Caffeine also stimulates the stomach to pour out large amounts of acid. This in turn leads to burning in the pits of the stomach and aggravates peptic ulcers of the stomach and duodenum. It also raises blood sugar level as a result of quickening of respiration. It also reduces blood flow to the brain by causing the brains blood vessels to constrict. It also may induce benign (non cancerous) breast diseases and may worsen pre-menstrual symptoms in women who overuse it.
Caffeine crosses the placenta and enters the fetal circulation and its use at a pharmacological level has been associated with low birth weight. Excessive consumption during lactation may cause irritability and wakefulness in a breast- fed baby (Eva, 1988).

Caffeine has a mild analeptic (respiratory stimulating activity) effect. Other action includes cardiac stimulation which may produce tachycardia dilation of coronary and peripheral blood vessels, constriction of blood vessels and skeletal muscles. It increases the risk of spontaneous abortion in women (Eva, 1988).

An excessive intake of caffeine in some persons appears to augment the sensitivity of the heart to emotional and other factors and so increase the incidence of extra systoles and other arrhythmias. Since caffeine affect the central nervous system conversely, omission of a habitual morning dosage often results in nervousness irritability, drowsiness, poor work performance and headache curable only by taking more caffeine (Stanley et al. 1979). As stated earlier intake of large quantities of caffeine may cause a number of health problems. Coffee and tea are the most popular drinks in Kenya. It is suspected that due to the high frequency of
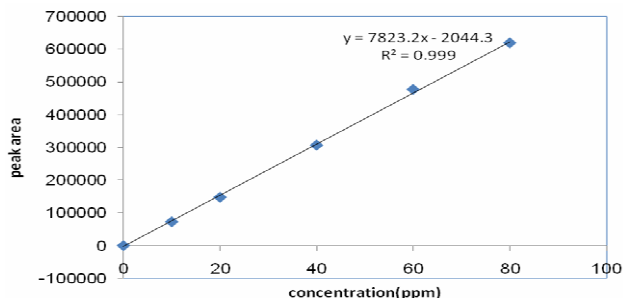
Figure 1. Caffeine calibration curve for HPLC method.

intake, the amount of caffeine consequently taken by people may be significantly high as to cause concern regarding the health problems mentioned.   It was therefore the objective of this study to determine the level of caffeine in coffee and tea beverages available in the Kenyan market in order to establish whether the amount of caffeine in the beverages is so much as to cause adverse health problems. To this end, high performance liquid chromatography (HPLC) and UV/ Vis spectrophotometric methods were employed since they are simple and reliable techniques.

MATERIALS AND METHODS

All the glassware were soaked overnight with chromic acid solution and washed thoroughly with water and detergent, then rinsed with deionised water before use. The chemicals and reagents used in this study were of high quality at least analytical grade and were purchased from Sigma- Aldrich (UK). Three brands of instant coffee samples: nescafe, africafe anad dormans and four brands of tea samples: sasini tea, kericho gold, chai mara moja and finlays tea were obtained from various city supermarkets in Nairobi, Kenya. The coffee and tea samples were kept at room temperature throughout the analysis.

Determination of caffeine in coffee and tea samples by High Performance Liquid Chromatography

Standard solutions

Caffeine stock solution of 1000 ppm was prepared by accurately weighing 100.00 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.

Sample preparation and analyte determination

2.00 g of tea and coffee samples were weighed in triplicate and put 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 278 nm, pressure, 150 khf/cm$^{2.}$, mobile phase, water, acetic acid, methanol (79.9, 0.1 and 20) 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.

Determination of caffeine in coffee and tea samples by UV/ Vis Spectrophotometry

Calibration standards

Caffeine stock solution (1000 ppm) was prepared by dissolving 100.00 mg of pure caffeine in 100 ml of distilled water. 0, 10, 20, 40, 60 and 80 ppm caffeine working solutions were prepared by serial dilution of the stock in 25 ml volumetric flasks with addition of 1.0 ml hydrochloric acid before topping to the mark with distilled water.

Sample preparation and analytic determination

0.25 g sample were accurately weighed and dissolved in water and made to the net volume of 20 ml with distilled water as sample solution. 20 ml sample solution were pipetted to 250 ml flask and 10 ml 0.01 mol/l hydrochloric acid, 2 ml basic lead acetate solution were then added and made to the mark with distilled water, shaken up and filtered to clarify. 50 ml filtered solution were pipetted and added to 100 ml flask, 0.2 ml 4.5 mol sulphuric acid were added and again made to the net volume with distilled water, shaken up and filtered. The absorbance of the working standards and samples were measured on a UV/ Vis spectrophotometer (Shimadzu) at 274 nm using 10 mm quartz quvette. The caffeine levels of the samples were calculated from the regression equation of the best line of fit of the standards.

RESULTS AND DISCUSSION

The calibration curves (Figures 1 and 2) were obtained using a computer algorithm and they illustrate a positive linear relationship between the instrumental signal and the concentration of the caffeine standards for both the HPLC and the UV/ Vis spectrophotometric methods. The results indicate that the levels of caffeine obtained by UV/ Vis spectrophotometric method were much higher than those obtained by HPLC (Table 1 and Figure 3). This shows that acidified water is a more efficient extractor of caffeine. This proposition makes sense theoretically since caffeine been basic because of the lone pair of electrons on one of the nitrogen forms the conjugate acid salt under acidic conditions. This gives it increased water solubility as a cation. Furthermore, making the solution basic by addition of lead acetate solution facilitated the precipitation of insoluble tannin salts and thus the removal of interfering compounds which would have minimized the caffeine signal.

However, the amount of caffeine that enters the human system through drinking of tea and coffee beverages would better be determined by the HPLC method since the said beverages are prepared using pure water

$$y = 0.009x$$
$$R^2 = 0.999$$
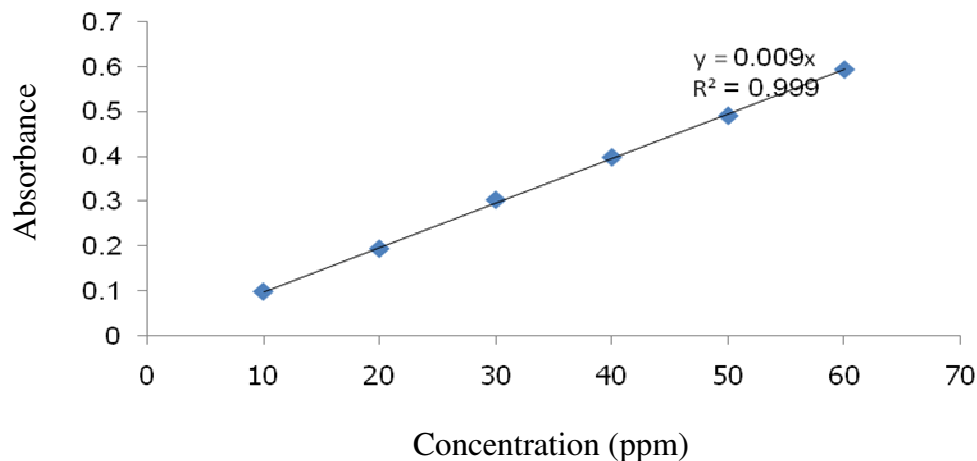
Figure 2. Caffeine calibration curve for UV/ Vis Spectrophotometric method.

Table 1. Caffeine concentration (ppm) of tea and coffee brands in the Kenyan market.

Brand name	Mean ± sd (HPLC method)(n=3)	Mean ± sd (UV/ Vis spectrophotometric method)(n=3)
Sasini tea	360.86 ± 14.27	738.19 ± 2.53
Chai mara moja tea	1471.73 ± 196.92	3196.46 ± 11.01
Kericho gold tea	474.89 ± 10.98	768.43 ± 2.53
Finlays premium tea	1074.34 ± 78.32	2343.10 ± 1.46
Dormans coffee	327.80 ± 2.40	717.79 ± 2.68
Africafe coffee	684.56 ± 24.35	1528.54 ± 5.05
Nescafe coffee	624.53 ± 2.64	1571.47 ± 2.53
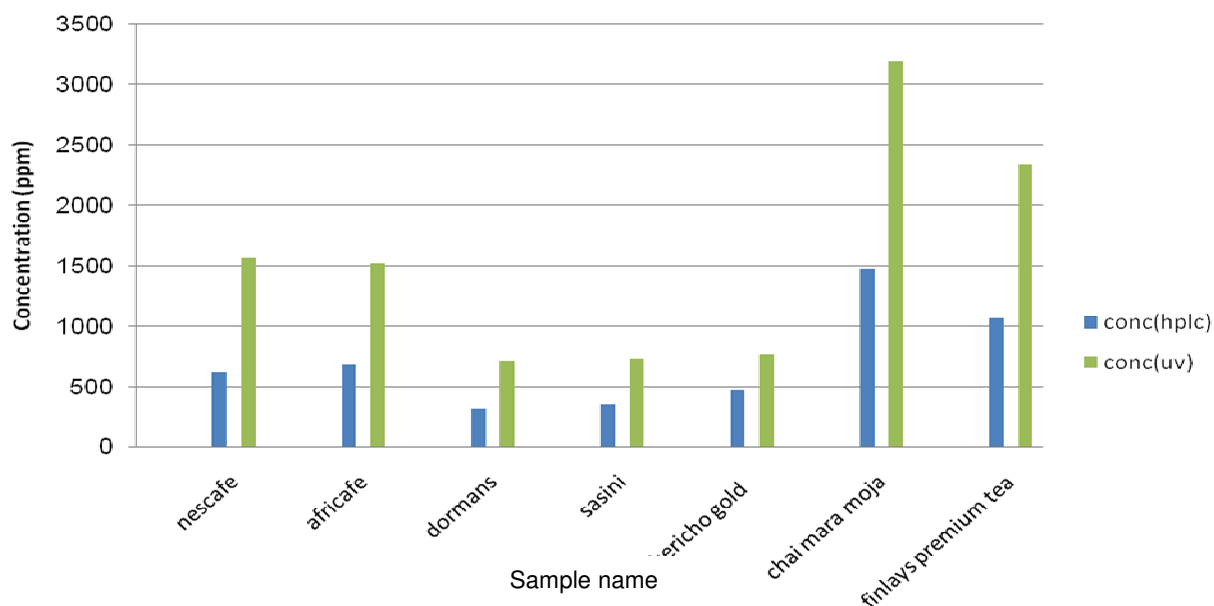
Figure 3. Comparison of Caffeine levels (ppm) obtained by hplc and UV/ Vis spectrophotometric.

Table 2. Percentage levels of caffeine in the tea and coffee samples.

Sample	Classification	Caffeine(% w/w) (hplc method)	Caffeine (% w/w) (UV/Vis spectrophotometric method)
Sasini tea	Black tea	1.80 ± 0.07	5.91 ± 0.02
Chai mara moja tea	Black tea	7.36 ± 0.98	25.57 ± 0.09
Kericho gold tea	Black tea	2.37 ± 0.05	6.15 ± 0.02
Finlays premium tea	Instant tea	5.37 ± 0.39	18.74 ± 0.01
Dormans coffee	Instant coffee	1.64 ± 0.01	5.74 ± 0.02
Africafe coffee	Instant coffee	3.42 ± 0.12	12.23 ± 0.04
Nescafe coffee	Instant coffee	3.12 ± 0.01	12.57 ± 0.02

as the solvent. (Table 1), the highest caffeine concentration was obtained in tea sample chai mara moja (black tea) which gave a concentration of 1471.73 ± 196.92 ppm and 3196.46 ± 11.01 ppm by the HPLC method and the UV/ Vis spectrophotometric method respectively; this was followed by finlays premium tea (instant tea) which gave a concentration of 1074.34 ± 78.32 ppm and 2343.10±1.46 ppm. The least was obtained in dormans coffee (instant coffee) which gave a concentration of 327.80±2.40 ppm and 717.79± 2.68 ppm by the two methods. The current findings demonstrate that tea contains higher level of caffeine than coffee. This is in agreement with previous work reported by Kaplan et al. (1974). As stated by Kaplan et al. (1974) that growing conditions, processing conditions and other variables affect caffeine content and that certain types of tea contains somewhat more caffeine than other teas, this is in agreement with the caffeine content obtained using different samples of tea. Although coffee contains less caffeine than tea (dry weight basis), some coffee solutions contain higher concentration of caffeine than tea solutions. This is normally because coffee is ground extremely fine and more ground coffee than tea is used to make the solutions. Other reasons could be because of high quality coffee beans used in terms of caffeine content or due to comparison with low quality tea.

From the results, chai mara moja which is classified as black tea had the highest concentration of caffeine compared to any other brand of tea. On the other hand, the concentration of caffeine in coffee samples was such that africafe had the highest concentration while dormans had the lowest. According to theory, black tea has the highest amount of caffeine content of caffeine amongst tea, although, there are some factors that can have an effect on the amount of caffeine content. Such factors include strength of the brew, growing conditions, processing techniques and other variables such as soil chemistry, altitude and position of leaf on the tea bush, type of plant and cultivation practices (http://www.nobleharbor.com/tea/caffeine.html). Caffeine content in coffee also varies widely depending on the type of coffee bean and the method of preparation used (http://www.ico.org/caffeine.asp).

The amount of caffeine in tea and coffee samples ranged between 1.64 ± 0.01% to 7.36 ± 0.98% by HPLC method and 5.74 ± 0.02% to 25.57 ± 0.09% by UV/ Vis spectrophotometric method (Table 2). Within experimental error, the values generally agree well with literature quoted values of 2-5% (http://www.polaris.nova.edu).

Conclusion

The order of caffeine concentration in tea brands was: chai mara moja > finlay premium tea > kericho gold tea > sasini while for coffee samples it was: africafe > nescafe> dormans. The highest amount of caffeine in samples analyzed was found in tea sample (chai mara moja) while the lowest was found in coffee sample (dormans).The amounts of caffeine obtained by UV/ Vis spectrophotometric method were higher than those obtained by HPLC method. The caffeine content of the tea and coffee brands analyzed was not found to be alarming since it correlated well with documented values.

As stated by 'Barone, J.J. and Roberts, H.R.' in their book 'caffeine consumption' that caffeine is a pharmacological active substance and depending on the dose, can be a mild central nervous system stimulant. It is noted that caffeine is not food but a drug working through nervous system. Excessive amount should be avoided since caffeine consumed in large amounts has adverse health effects. In particular, people suffering from high blood pressure should be advised to avoid use of caffeine containing beverages since caffeine is known to increase the blood pressure. In addition those with coronary heart disease should avoid such beverages as caffeine disrupts normal heart rhythm.

Department, JKUAT for his technical assistance.

REFERENCES

Aurnaud MJ (1987). The pharmacology of caffeine. Prog. Drug 31: 273.

Barone JJ, Roberts HR (1996). Caffeine consumption. Food chem. toxicol., 34:119.

Bolton, Sanford, Gary Null (1981). Caffeine: Psychological Effects, Use and Abuse. Orthomolecular Psychiatry, 10(3): 202 – 211.

Eva MH (1988). Nutrition. West publishing company, 4(7): 351.
http://coffeefaq.com/site/node/15. Coffee and caffeine FAQ: Does dark roast coffee have less caffeine than light roast? Retrieved on 2007 – 01 – 02.

http://www.ico.org/caffeine.asp.   Caffeine.   International   coffee organization. Retrieved on 2006-08-21.

http://www.jeremiahspick.com/caffeine-e-13.html.   All   about   coffee: Caffeine level. Jeremiah's pick coffee company. Retrieved on 2007-06 - 14.

http://www.nobleharbor.com/tea/caffeine.html. Caffeine in tea versus steeping time. Retrieved on 2006 – 08 – 12.

http://www.polaris.nova.edu/shanbhag/chemistry/oc1labs/caffeine.pdf retrieved on 2010 - 03 – 14.

Jeanne CS. (1987). Introductory clinical pharmacology. J. B. Uppimcott Company, 3(19): 122-125.

Kaplan E, Holmes JH, Sapeika N (1974). Caffeine content of tea and coffee. S. Afr. Med. J., 48: 510-511

Lawrence EL (1986). What you need to know about food and cooking for health. Viking Company, 5 (235): 254-258.

Leo ML (1992). Food analysis by HPLC. Marcel Decker Inc., 17: 656-659.

Pullicino I (2008). Altasterol caffeine pro+ and mental alertness.
http://www.articlesbase.com/supplements-and-vitamins-articles/altasterol-caffeine-pro-and-mental-alertness-435631.html.

Stanley D, Passmore R, Brock JF (1979). Human nutrition and dietetics. Longman publishers, 2 (11): 200.