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Original Research

Estimation of Caffeine Intake from Coffee Made From Mixture of Coffee Leaf and Spices

Yitayal A.A[1*] and Tilahun R.D[2]

[1]Department of Chemistry, College of Natural and Computational Sciences, Mizan-Tepi University, P.O.Box: 121, Tepi, Ethiopia

[2 1]Department of Biology, College of Natural and Computational Sciences, Mizan-Tepi University, P.O.Box: 121, Tepi, Ethiopia

Abstract

Coffee prepared from mixture of coffee leaf and spices (here after referred as CCLS)is a drink practiced commonly in the south western part of the southern nations, nationalities and peoples region (Kaffa, Sheka and Bench-Maji zones) in Ethiopia. One purpose of drinking CCLS, as believed by the people in the region, is for the sake of central nervous system stimulation. This study was intended to determine the caffeine content of the drink prepared by three different processes (Roasted, Raw and Mejengir types) in the specified part of the country by UV/VIS spectrophotometry using water and chloroform as solvents for extraction. The study further evaluated the amount of caffeine intake from this drink by the indigenous people in the region. The caffeine content was found to be in the range between 1030.76 ± 61.29 to 1459.10 ± 29.55 and 1157.28 ± 34.96 to 1566.16 ± 35.02 mg/L when water and chloroform, respectively were used for extraction. The caffeine content value of the Roasted type was the highest though the difference was statistically insignificant (P>0.05) with that of the Mejengir type in both solvents. This study results could therefore justify the perception of the society in using the drink for stimulatory purposes. However, the CCLS consumption and therby the caffeine intake custom in the region brought the caffeine content > 340 mg/day which is more than the recommended amount that would affect the health condition of the people in the region.

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***Corresponding Author:**

Yitayal Addis Alemayehu

E-mail:

yituaddis@gmail.com

INTRODUCTION

Caffeine (1, 3, 5-trimethylxanthine), a nervous system stimulant, belongs to a large class of organic compounds called alkaloids. It is naturally present in coffee and is incorporated into many non-alcoholic beverages. Caffeine is found in the leaves, seeds and/or fruits of at least 63 plant species worldwide. Among these the most commonly known sources of caffeine are coffee, cocoa beans, kola nuts and tea leaves (Wanyika *et al.*, 2010).

Coffee is often consumed for its stimulatory effects as caffeine is the most prominent in its composition and it is reported that 70% of caffeine comes from coffee while soft drinks and tea contribute 16% and 12%, respectively (Butt and Sultan, 2011). Coffee beans contain between 0.8 and 2.8% caffeine, depending on species and origin, and it contributes to 10 to 30% of the bitter taste of coffee brews **(**Phan *et al*., 2012). So, Wanyika *et al*. (2010) reported coffee bean, from which coffee is brewed as the world's primary source of caffeine.

Ethiopia is the birth place of the Arabica coffee tree (MOT, 2012) as it originated from montane forests in south and southwest Ethiopia which form part of Eastern Afromontane biodiversity hotspot region (Wiersum *et al.*, 2007). Coffee is vital to the cultural and socio-economic life as it sustains the livelihood for over 15 million people; provides considerable income from casual labor for many poor rural people and contributes more than 25% of the country's foreign exchange earnings (MOT, 2012). Due to these reasons; Petit (2007) reported coffee as the back bone of the nation's economy.

A socio-cultural survey in the south western part of Ethiopia revealed that coffee is prepared not only from coffee bean but also from mixture of coffee leaf and spices (Yitayal and Achame, 2014). In the study it is reported that about 50% of the composition is coffee leaf and the other 50%, excluding water, is the sum of the spices: *Ocimum basilicum, Mentha Piperita, Ruta chalepensis, Coriandrum sativum, Lippia Javanica, Allium sativum, Anethum foeniculum, Zingiber officinale, Capsicum annuum*, *Aframomum corrorima, Allium cepa* and salt (sodium chloride). The report indicated that coffee made from coffee leaf and spices (CCLS) can be prepared in three processes called Roasted, Raw and Mejengir types (Yitayal and Achame, 2014). The findings of the socio-cultural study also pointed that one purpose of drinking CCLS is due to its considerable stimulatory effect (Yitayal and Achame, 2014) , but its caffeine content is not yet determined quantitatively.

The objective of this study was therefore; to determine the caffeine content of CCLS prepared in the aforementioned three processes and then compare the results obtained from these three processes one another and with the recommended caffeine intake from the literature.

MATERIALS AND METHODS

Amount of CCLS Intake

Semi-structured interview, critical participant observation and on site volume measurement were the instruments applied to collect data on the amount consumed for the three types of CCLS in the sampled areas: Gesha (Damo and Yofo Kebeles) and Gimbo (Mision sefer) in Kaffa zone, Yeki woreda (Addisalem Kebele for Mejengir participants and Kobito Kebeles) in Sheka zone and Aman (Shishika Kebele) and Shay Bench (Kashita Kebele) in Bench-Maji zone.

Experimental

Caffeine Determination

The caffeine content in CCLS was determined by UV/Vis Spectrophotometry by combining the procedures followed by Suteerapataranon (2010), Wanyika *et al*. (2010) and Maidon *et al*. (2012). For the extraction of the caffeine from CCLS two solvents were used, water and chloroform. The former was used as the society uses water for CCLS preparation thereby indicate the concentration of caffeine that would commonly available in CCLS drinks prepared by the three processes and might differ based on the boiling time, and the latter was chosen to determine the maximum possible caffeine concentration available in CCLS as chloroform is the best selective solvent due to its polarity properties and good ability to dissolve caffeine (Maidon *et al*., 2012).

Materials

The ingredients: leaves of *Coffea Arabica, Ocimum basilicum, Mentha Piperita, Ruta chalepensis, Coriandrum sativum, Lippia Javanica, Anethum foeniculum, Zingiber officinale, Capsicum annuum*, *Aframomum corrorima* were collected from the garden of selected three women who have the experience of preparing three different types of CCLS (one woman for one type of CCLS). *Allium sativum, Allium cepa* and salt (sodium chloride) were purchased from TEPI MARKET.

Chemicals

Lead (II) acetate, hydrochloric acid and sulfuric acid (all obtained from Sigma-Aldrick (UK) through Mizan-Tepi University, Ethiopia) were employed to remove tannins which could interfere with the analysis by spectrophotometry (Suteerapataranon *et al*., 2009). In addition, pure caffeine (99.6%) also obtained from Sigma-Aldrick (UK) through Jimma University, Ethiopia was used as a standard.

Calibration Standards

1000 ppm caffeine stock solution was prepared by dissolving 100 mg of pure caffeine in 100 ml of distilled water. 5, 10, 15 and 20 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 Determination

Following the processes and proportions used by the indigenous people, Kaffa, Sheka and Bench-Maji in Ethiopia, each of the three types of CCLS were prepared separately by adding 87.65 g raw leaf (22.92 g in dry weight basis), 32.15 g roasted leaf (26.77 g in dry weight basis) and other 36.61 g roasted leaf (31.22 g in dry weight basis) per litre of water for the preparation of the Raw, Roasted and Mejengir type CCLS, respectively. 0.5 ml of each of CCLS samples were accurately measured and dissolved in water and made to the net volume of 40 ml with distilled water as sample solution. The sample solution was pipetted to 250 ml flask and 15 ml 0.01 mol/L hydrochloric acid; 3 ml of 2 M basic lead acetate solution was then added and made to the mark with distilled water, shaken up and filtered to clarify. 50 ml filtered solution was pipetted and added to 100 ml flask, 0.3 ml of 5 M sulphuric acid was 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 (T-80) at 273 nm using 10 mm quartz quvette. The distilled (deionized) water was used as a blank and the caffeine level of the samples were calculated from the regression equation of the best line of fit of the standards. The test was done in triplicate and the same process was repeated for chloroform. The reason for the selection of the method was due to UV/Vis spectrophotometer availability in most laboratories, being simple and fast.

Data Analysis

The caffeine content values obtained from the three processes of CCLS preparation were compared using SPSS version 20 software. Data collected were analyzed by ANOVA, while significant differences among the mean were determined using least significant difference (LSD) multiple comparison test and results were considered statistically at $P<0.05$. The results were then presented as mean ± SD.

RESULTS AND DISCUSSION

The caffeine contents in Roasted, Raw and Mejengir types of CCLS in water and chloroform were studied. The results showed that each type of CCLS contains considerable caffeine and the Roasted type of CCLS contained the highest concentration of caffeine followed by the Mejengir type (Table 1).

The results of caffeine content of this study was significantly higher than the caffeine contents of coffee infusions which ranges from 20.00 ± 0.360 to 53.00 ± 0.300 mg/L (Phan *et al*., 2012); caffeine concentrations in tea infusions 260.8 ± 0.81 and 220.3 ± 5.55 mg/L for ground and non-ground samples, respectively (Suteerapataranon *et al*., 2009); and Coca cola 170 mg/L and Pepsi cola 180 mg/L indicated in jenway. However, the current study results were in agreement with the study on two coffee samples which are 1571.47 ± 2.53 and 1528.54 ± 5.05 mg/L and significantly lower than study results of tea valued as 3196.46 ± 11.01 mg/L as reported by Wanyika *et al.* (2010), and black tea and green tea values of 24,700 and 34,500 mg/L in chloroform, respectively (Komes *et al*., 2009). Generally, the caffeine content of CCLS was comparable to other caffeine rich liquors.

Table 1: Caffeine content of CCLS in water and chloroform solvents

CCLS Types	Water as a solvent		Chloroform as a solvent	
	Caffeine content (mg/L)	Regression Equation	Caffeine content (mg/L)	Regression Equation
Roasted CCLS	1491.05 ± 11.00	0.0338x + 0.18	1566.16 ± 35.02	0.026x + 0.42
Raw CCLS	1030.76 ± 61.29	0.0335x + 0.19	1157.28 ± 34.96	0.025x + 0.39
Mejengir CCLS	1459.10 ± 29.55	0.0332x + 0.13	1526.61 ± 14.16	0.024x + 0.41

Caffeine content results were in mean ± SD; SD=standard deviation

The difference of the caffeine contents of the roasted type with that of the Raw one is statistically significant (P<0.05), but insignificant (P>0.05) with that of the Mejengir type of CCLS type as shown in Table 2.

Table 2: Multiple comparisons (LSD) for caffeine content of CCLS types in two solvents

Dependent Variable	(I)Types of CCLS	(J)Types of CCLS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Water as a solvent	Roasted CCLS	Raw CCLS	555.75000*	36.38389	0.000	466.7218	644.7782
		Mejengir CCLS	27.40667	36.38389	0.480	-61.6215	116.4348
	Raw CCLS	Roasted CCLS	-555.75000*	36.38389	0.000	-644.7782	-466.7218
		Mejengir CCLS	-528.34333*	36.38389	0.000	-617.3715	-439.3152
	Mejengir CCLS	Roasted CCLS	-27.40667	36.38389	0.480	-116.4348	61.6215
		Raw CCLS	528.34333*	36.38389	0.000	439.3152	617.3715
Chloroform as a solvent	Roasted CCLS	Raw CCLS	608.88333*	73.76194	0.000	428.3944	789.3723
		Mejengir CCLS	39.54667*	73.76194	0.611	-140.9423	220.0356
	Raw CCLS	Roasted CCLS	-608.88333*	73.76194	0.000	-789.3723	-428.3944
		Mejengir CCLS	-569.33667*	73.76194	0.000	-749.8256	-388.8477
	Mejengir CCLS	Roasted CCLS	-39.54667*	73.76194	0.611	-220.0356	140.9423
		Raw CCLS	569.33667*	73.76194	0.000	388.8477	749.8256

* The mean difference is significant at the .05 level; SD: Least significant Difference

One reason could be the larger mass of the coffee leaf (dry weight basis) that was taken in the Roasted type. In the Raw type case the mass of the water in the coffee leaf was considered as the mass of the coffee leaf alone which could minimize the net content of the coffee leaf (i.e. the mass of the coffee leaf in dry weight basis was only 22.92 g per liter of water). Second, roasting to optimal temperature could increase caffeine yield as reported for the coffee bean where green coffee been was compared with the roasted one (Alemayehu, 2007 unpublished). Moreover, the Roasted and Mejengir types were similar in the type of coffee leaf used (i.e. both used roasted and dried form) so that the net mass of the coffee leaf was higher relative to the Raw type CCLS.

The caffeine contents of the CCLS types when chloroform was used were higher than the contents when water was used (Table 1). This indicated that the society could utilize the caffeine content in the second and third rounds of the drink as water would not extract the full content at a time and depends on the time and temperature given for boiling (Phan *et al*., 2012). This in part could justify why the community drink the second and third round CCLS prepared for a single ceremony. The least significant difference (LSD) test result showed that the difference within the Roasted CCLS of the two solvents and the Mejengir type CCLS is significant ($p<0.05$) (Table 2), and insignificant for the Raw one.

Though the caffeine content of CCLS was comparable to other caffeine rich beverages, the amount of CCLS daily consumed by the society was so high that the commulative caffeine intake could reach to the level that would affect its health. The survey result on the daily CCLS consumption show that , the CCLS consumption of the indigenous people ranges from 295 to 330, 420 to 520 and 530 to 584 ml for children, teenagers and adults, respectively (Table 3).

The Roasted type of CCLS was found to provide the maximum amount of caffeine daily consumed which was 447, 626 and 790 mg/day for the different age groups, respectively. It is reported, in other studies, that the moderate daily consumption of up to 400 mg of caffeine has been considered safe for healthy adults and non-pregnant/non-lactating women (Nawrot *et al*., 2003) and caffeine in moderate doses up to 300 mg can improve cognitive performance in rested, sleep-deprived, and fatigued individuals (Lieberman *et al*.,2002) which is half and only one-third of this study results, respectively. Moreover, doses of caffeine over 600 mg/day can cause significant side effects including tachycardia, tremors, insomnia, nervousness, upset, chest pain, and arrhythmias (Lieberman *et al*., 2002). In addition, moderate caffeine consumption for most individuals, including sensitive populations such as pregnant women and children, is about 200 mg per day (Nour *et al*., 2010)

Table 3: CCLS and caffeine consumption rate by the indigenous people

Types of CCLS	Age Groups (year)	Daily intakes of CCLS (ml)	Daily intakes of caffeine from CCLS (mg)*
Roasted CCLS	5-10	300±13	447
	11-18	420±18	626
	19 and above	530±23	790
Raw CCLS	5-10	330±12	340
	11-18	520±27	536
	19 and above	570±29	587
Mejengir CCLS	5-10	295±11	431
	11-18	414±16	604
	19 and above	527±27	768

*calculated based on table 1

which is by far lower than 447 mg/day. Generally, the results of this study revealed that the daily caffeine intake from CCLS alone was more than 600 mg for adults except the Raw type of CCLS users. Together with CCLS, the society would consume other caffeine source beverages especially coffee there by maximize the total caffeine intake and would exacerbate the side effects of caffeine in the region. Therefore, CCLS users should minimize the amount intake to adequate levels (up to 250 to 400 ml/day) except for children, non-pregnant and non-lactating women who require caffeine below 200 ml/day.

CONCLUSION

One purpose of drinking coffee made from coffee leaf and spices by the people in the south western part of the southern nations nationalities and peoples region (Kaffa, Sheka and Bench-Maji zones) in Ethiopia is for its stimulatory effect. This study could assure the indigenous people perception and practice on CCLS use as central nervous system stimulation because CCLS was found to contain considerable amounts of caffeine (especially the Roasted one). CCLS is therefore a drink that could be used as an alternative for coffee and other caffeine source liquors to provide caffeine for the rest of the people in and out of Ethiopia. Using CCLS more than 300 ml/day (more than the adequate caffeine in it) should be lowered to avoid its side effects.

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Conflict of Interest

Conflict of interest none declared.

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