Original Article

Caffeine extraction from Arabic coffee: The role of brewing and roasting

Saad Mohammed AlShareef

Department of Medicine, College of Medicine, Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia

Abstract

Objectives: The global population's primary intake of caffeine comes from the consumption of coffee. Arabic coffee is traditionally brewed and served using a unique process. The objective of this study was to examine the plausibility of two widespread myths regarding Arabic coffee; that the longer the cooking time, and/or the more roasted the coffee, the higher is the amount of caffeine extracted per kilo-gram of raw coffee (CE).

Materials and Methods: A total of 12 different samples of traditionally brewed Arabic coffee (with correction of lost volume due to evaporation) were directly analyzed for their caffeine concentration using the ultra-high performance liquid chromatography. The amount of caffeine extracted per kilogram of raw coffee (CE) where then calculated. Comparisons were then made between the CE from three types of raw coffee beans; Yemeni Bari, Yemeni Kulani, and Ethiopian Harrari. They were each roasted to two different grades (light vs. medium-dark) and each grade was cooked for a different duration of time (15 min vs. 30 min).

Results: The type of coffee bean used was shown to significantly affect the amount of CE from raw coffee ($P = 0.011$). The highest amount of caffeine was extracted from raw Ethiopian Harrari coffee bean, followed by the Yemeni Kulani bean ($P = 0.020$ and $P = 0.027$, respectively). A longer cooking time significantly decreased the amount of CE from raw coffee as compared to a shorter time ($P = 0.041$). Medium-dark roasting was observed to cause a slight but nonsignificant decrease in amount of CE compared to light roasting ($P = 0.178$).

Conclusions: The type of coffee bean used in brewing Arabic coffee is the main determinant of the amount of caffeine extracted from raw coffee. Longer cooking time and a darker roast both decrease the amount of extracted caffeine in the final brew, rendering the old myths nonplausible.

Keywords: Arabic coffee, caffeine, cooking time, roasting, Saudi coffee

Address for correspondence: Dr. Saad Mohammed AlShareef, Department of Medicine, College of Medicine, Imam Mohammad Ibn Saud Islamic University, PO Box 7544, Riyadh 13317-4233, Saudi Arabia.
E-mail: drsaad321@hotmail.com

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INTRODUCTION

Coffee, the primary human source of caffeine intake, is considered the most widely consumed beverage worldwide, with an estimated global consumption of more than 2.25 billion cups/day.[1] Coffee beans are obtained from the small berry-like fruit of the coffee plant which belongs to genus Coffea, a member of the *Rubiaceae* family.[2]

Out of the 90, or possibly more, different varieties of coffee, two major types are *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta variety).[3] The Arabica coffee

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bean accounts for over 60% of the coffee produced worldwide, with its profile including higher lipid content and lesser caffeine.[4] In contrast, the Robusta coffee bean is much cheaper, with a bitter taste profile and twice the caffeine content of Arabica coffee.[5,6]

Personal preference remains the most important determining factor in coffee processing. Despite the rising popularity of cold brewing methods,[1] hot brewing is still the most used method for coffee preparation.[7,8] Hot brewing methods include boiling whole ground-roasted coffee, or pouring hot water onto beans, and using a filter, percolator, or French press to serve only the soluble brewed product, or by mixing the freeze-dried or agglomerate instant coffee preparations with hot water.[9]

The taste of coffee is influenced by the degree of bean roasting, which affects its original features. An increase in roasting reduces acidity and increases bitterness, and the roasted beans darken due to Millard reaction. The degree of roasting (light, medium, medium-dark, or dark) is generally defined by the color of the roasted beans, and this distinguishes the coffee of different countries from each other.[10]

Arabic coffee is the primary ingredient of Levantine coffee, which is made with medium-dark to dark roasted ground Arabic coffee beans, whereas Peninsular coffee uses lighter-roasted beans. The Peninsular coffee kind is made by freshly roasting green coffee beans to a light, medium, medium-dark, and dark grade. The beans are then coarsely ground in a metal mortar, mixed with 50 g of ground coffee in boiling water and cooked for an average of 15–30 min over a moderate flame. A number of spices may be added, namely cardamom, clove, and saffron. Rezk *et al*. concluded that the added spices did not significantly affect the caffeine content.[11] The process is completed by serving the coffee from a "Dallah," a traditional coffee pot, into a "Finjan," a small specialized cup. An adult Saudi may consume 2–10 cups in one sitting.[12] The United Nations Educational, Scientific, and Cultural Organization now recognize this coffee as an intangible aspect of human cultural heritage. Data show that the Kingdom of Saudi Arabia (KSA) imported more than 247 million US dollars of coffee in 2019. The most commonly used coffee beans in Saudi Arabia are the Ethiopian Harrari, the Bari Yemeni, and the Kulani Yemeni.

Coffee is a rich source of caffeine, antioxidants, and anti-inflammatory compounds.[13] Caffeine naturally occurs in cocoa, coffee, tea, and cola nuts.[14] It is toxic for several herbivorous animals and insects, and the plants use it as a tool to defend themselves. Thus, it is considered to be a co-evolutionary protecting agent.[15] This provides an explanation as to why Robusta coffee, with its doubled caffeine content,[5,6] is more resistant to diseases.

In humans, caffeine is considered to be the most commonly used psychostimulant worldwide.[16] According to the American Food and Drug Administration (FDA), caffeine is a multipurpose food substance which is generally recognized as safe, when used within permissible limits. Such limits differ from one country to another and are also dependant on the type of beverage. The US allows up to 200 mg/L, while Australia permits 145 mg/L.[17] Current regulations do not require the marketers of dietary supplements to state caffeine levels on product labels.[16]

Caffeine is chemically related to the adenine and guanine bases of deoxyribonucleic acid and ribonucleic acid.[18] When ingested, caffeine is quickly and completely absorbed by the gastric and small intestinal mucosa and distributed to all tissues, including the brain. Biologically, caffeine acts as an adenosine receptor antagonist (A1 and A2A).[19] It achieves a stimulatory reaction by blocking the inhibitory effect of the major endogenous neuromodulator, adenosine. Some of its primary actions include central nervous system stimulation, raising the metabolic rate, acute elevation of blood pressure, and dieresis.[20] It also acts as an antioxidant against lipid peroxidation induced by reactive oxygen species.[21]

Caffeine intake has both positive and negative consequences, such as disrupted sleep.[22,23] Researchers found, in subjects consuming caffeine later in the day, a significant increase in sleep onset latency, decreased sleep efficiency, and decreased total sleep time.[24] Such effects were more evident among adolescents as they consumed caffeine throughout the day.[25] In adolescents aged 12–15 years, higher caffeine use was also associated with increased interruption in sleep and daytime sleepiness.[26] Although caffeine is often consumed by university students looking to enhance their cognitive performance, lower caffeine use is generally associated with better GPA scores.[27] Rasheed and Al-Sowielem recommended that women with premenstrual syndrome should eliminate caffeine-containing beverages, particularly coffee, from their diet.[28]

El Shabrawy Ali and Felimban showed that the serum total cholesterol concentration was significantly higher among coffee drinkers.[12] On the contrary, Ismail proved that different doses of Arabic coffee improved serum lipid profile, uric acid, and liver enzymes in experimental rats.[29] Further studies proved that the two constituents of ground coffee, cafestol and kahweol, were responsible for the cholesterolemic action of boiled coffee, which was found to be minimal in filtered coffee preparation.[30]

The main peripheral effect of caffeine is mediated by its impact on the sympathetic nervous system and cardiovascular system. Caffeine at a dose of 250 mg significantly elevated blood pressure in nonhabitual drinkers but not in habitual ones; however, it stimulated the sympathetic nervous system in both.[31] Therefore, depending on the response system assessed, tolerance to the peripheral effects of caffeine may be variable.[15] Due to its significant impact on the physical activity, it was listed under prohibited substances up until 2004 by the International Olympic Committee. Professional athletes who tested positive for more than 12 μg/L of caffeine in their urine were banned from the Olympic Games.[15]

Alternatively, epidemiological studies have shown a link between chronic caffeine intake and a significantly lower risk of late-life dementia and Alzheimer's disease.[32] In the experimental models, this link was explained by caffeine's ability to prevent amyloid-beta production and the memory deficits of Alzheimer's disease.[33,34] Caffeine prevents, or even restores, memory impairment by affecting brain homeostasis.[35] However, caffeine's cognition-enhancing properties remain controversial.[36,37] It is noteworthy, however, that only low or nonconsumers of caffeine can eventually benefit from acute administration.[38]

In regard to Parkinson's disease, caffeine's role in decreasing the motor symptoms and preventing the loss of dopaminergic neurons has been reported in both epidemiological studies[39] and experimental models.[40,41] Consuming 2–3 cups of coffee daily has been associated with lower erectile dysfunction among males.[42] This was also proven to be the case among obese and hypertensive men but not among diabetics.[43] Caffeine increases testosterone levels[44,45] and initiates a series of pharmacologic reactions that lead to the relaxation of the cavernous smooth muscle and improve blood supply through penile arteries.[46]

Caffeine decreases the synthesis of prostaglandin E_2 and inhibits COX-2 protein synthesis, which are check points along the pain pathway.[47] Therefore, caffeine acts as an adjuvant to analgesics (paracetamol and ibuprofen) and is an approved medication).[48] Caffeinism is a state of chronic toxicity as a direct consequence of excessive consumption of caffeine. It is a recognized clinical syndrome in the Diagnostic and Statistical Manual of Mental Disorders-5 and in the WHO's International Classification of Diseases-10.[48] The common features include insomnia, anxiety, restlessness, tachycardia, psychomotor agitation, tremors, gastrointestinal disturbances, and death.[48]

The varying levels of caffeine content, along with the type of coffee used, the degree of roasting, the brewing process, additives (if any), cup size, and the number of servings typically consumed by each particular population need to be assessed. In this study, two widespread myths in KSA regarding the degree of roasting and the cooking time's effecting the amount of caffeine extracted from raw coffee, were assessed.

MATERIALS AND METHODS

A total of 12 samples of Arabic coffee were prepared using three different types of Arabic coffee-Ethiopian Harrari. Bari Yemeni and Kulani Yemeni-which represent the most common types of coffee beans used in KSA. For each type of bean, two different degrees of roasting were used (light and medium-dark). After grinding, each of these six preparations were then cooked for two different durations (15 min and 30 min), to end up with a total of 12 different samples representing the following four varieties for each type of these three beans:

1. Light roasted and cooked for 15 min
2. Light roasted and cooked for 30 min
3. Medium-dark roasted and cooked for 15 min
4. Medium-dark roasted and cooked for 30 min.

Concentration adjustment

For each of the aforementioned 12 samples, 50 g of ground coffee (light or medium-dark) was mixed with boiling water and cooked for 15 or 30 min at a constantly monitored temperature of 98.5°C–100°C. The volume of this boiling water was predefined to allow for the different levels of evaporation due to different cooking durations and were as follows: 1140 ml for samples intended for 15 min cooking time and 1230 ml for the samples intended for 30 min cooking time. This procedure successfully resulted with a final volume of approximately 1 L (981–1002 ml) of brewed coffee for each of the 12 samples despite their different cooking duration.

Analysis of caffeine content in liquid coffee samples

The liquid coffee samples were diluted 1 in 5 with deionized water. This dilution was carried out by taking 5.0 mL of sample and making it up to 25.0 mL with deionized water in a volumetric flask. The diluted samples were then filtered through a 0.22 um nylon syringe filter. Analysis of caffeine content where then determined in this filtrate.

Ultra-high-performance liquid chromatography setting

Analysis of caffeine content where determined using a Thermo Scientific Ultimate 3000 ultra-high-performance liquid chromatography (UHPLC) system with an Ultimate 3000 Diode Array Detector that acquires full ultraviolet-visible spectral data. The analytical column used was a Thermo Fisher Hypersil gold 2.1 mm × 100 mm 1.9 un operated at 30°C. All solvents and reagents used were obtained from Sigma Aldrich. The column employed was a reverse phase C18 column. The injection volume for both analytical standards and samples was 5 μ, the mobile phase was a mixture of mobile Phase A (95% Water/5% ACN) and mobile Phase B (5% Water/95% ACN) with a constant flow rate of0.25 mL/min according to the following gradient [Table 1].

Quantitation was carried out on wavelength 204 nm, with a secondary confirmation wavelength of 274 nm [Figures 1-3], the method had a practical quantitation limit = 0.1 mg/L.

Calibration curve

A stock standard caffeine solution of 1000 mg/L was first prepared using 100 mg of pure caffeine powder (Sigma Aldrich) weighed out on a five-figure analytical balance (AND HR_250AZ), dissolved and made up to a final volume of 100 mL in an A-grade volumetric flask. This 1000 mg/L stock standard was then diluted to produce seven working standards of (1 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 50 mg/L, 100 mg/L, and 200 mg/L). Calibration was performed using the external calibration method on a lin-lin scale. Seven working standards along with blanks where all measured and the area of each of the caffeine peaks (calculated by multiplying peak height at wavelength 204 nm [absorbance in mAU] by its time in min) was then plotted against its concentration, resulting in a linear calibration curve Figure 4, with R^2 value of 0.9963 and a slope of 0.9670.

Validation of the method

A recovery experiment was designed to validated method accuracy and exclude any matrix interference. One of our measured samples (Yemen Bari Coffee Light [roasted 15 min]) was first analyzed for caffeine in duplicate. The results of 102.37 mg/L and 103.21 mg/L were obtained on the instrument (multiplying results by the dilution factor of 5 → 511.85 and 516.05 mg/L, respectively). Two replicates of this Yemen Bari Coffee Light (roasted 15 min) were then spiked with caffeine to end up with a theoretical spike value of 50 mg/L. This was performed by taking 5.0 mL of Yemen Bari Coffee Light (roasted 15 min) sample, adding 1.25 mL of a 1000 ug/mL pure caffeine standard and making up to 25.0 mL with deionized water

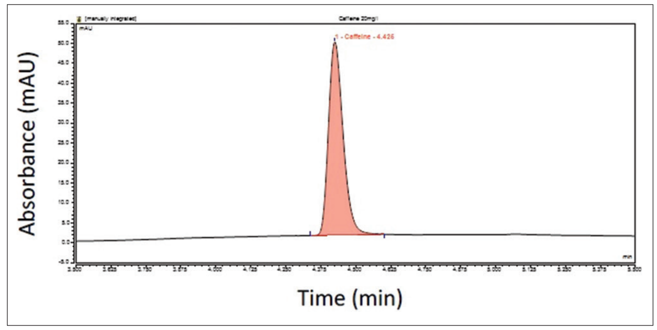


Figure 1: Chromatogram: Caffeine peak at 4.425 min

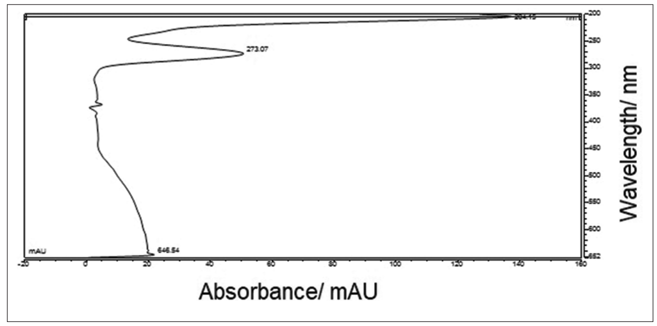


Figure 2: Ultraviolet-visible spectra taken from apex of caffeine peak

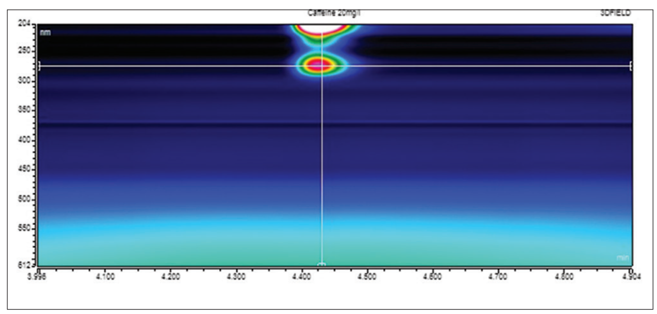


Figure 3: Three-dimensional data produced by the diode array detector. Time versus wavelength versus intensity

in volumetric flask, mixed, and finally filtered through a 0.22um nylon syringe fitter. The spiked samples were then analyzed and results of 140 and 146 mg/l were obtained on the instrument. The accuracy of spiked replicates was then calculated from the formula:

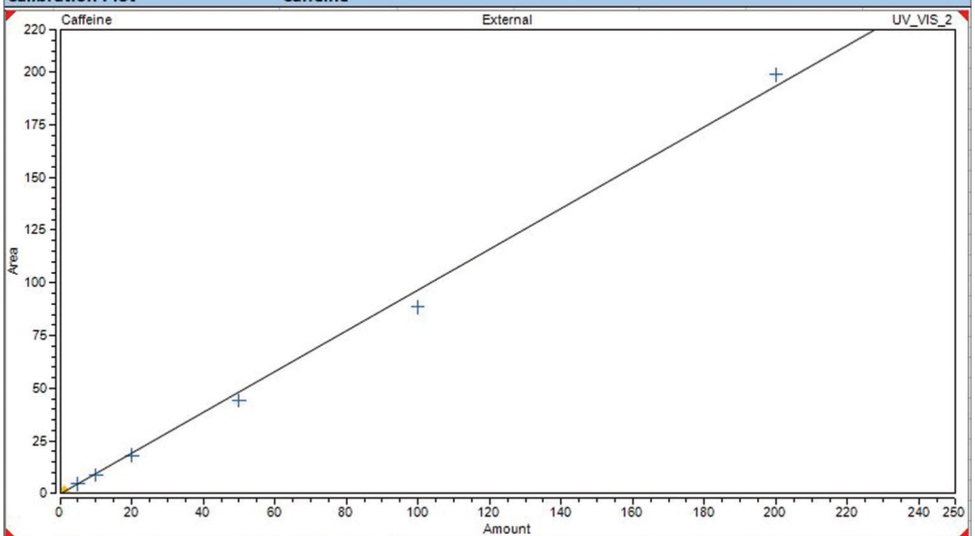
Acc% = (calculated concentration/nominal concentration) ×100.

- Where: Calculated concentration = Calculated spike concentration = Result of spiked sample-the result of un-spike sample (i.e., the initial content of caffeine in un-spiked samples)
- Nominal concentration = Theoretical spike value.
- Spike 1: 139.69 mg/L–102.37 mg/L = 37.32 mg/L. (37.32/50) ×100 = 74.6%
- Spike 2: 146.25 mg/L–103.21 mg/L = 43.04 mg/L. (43.04/50) ×100 = 86.1%.

Calibration

Calibration Details	Caffeine		
Calibration Type	Lin	Offset (C0)	0.0000
Evaluation Type	Area	Slope (C1)	0.9670
Number of Calibration Points	7	Curve (C2)	0.0000
Number of disabled Calibration Points	0	R-Square	0.9963

Calibration Plot Caffeine



Calibration Results Caffeine

No.	Injection Name	Calibration Level	X Value UV_VIS_2 Caffeine	Y Value UV_VIS_2 Caffeine	Y Value UV_VIS_2 Caffeine	Area mAU*min UV_VIS_2 Caffeine	Height mAU UV_VIS_2 Caffeine
2	0.1 mg/L	1	0.1000	n.a.	n.a.	n.a.	n.a.
3	1 mg/L	2	1.0000	0.8788	0.8788	0.879	9.030
4	5 mg/L	3	5.0000	4.5764	4.5764	4.576	48.237
6	20 mg/L	5	20.0000	18.0392	18.0392	18.039	194.836
7	50 mg/L	6	50.0000	44.1450	44.1450	44.145	488.021
8	100 mg/L	7	100.0000	88.5130	88.5130	88.513	976.964
9	10 mg/L	4	10.0000	8.9814	8.9814	8.981	96.275
27	200 mg/L	8	200.0000	198.7184	198.7184	198.718	2222.663

Figure 4: Calibration curve

Averaging both results, our recovery experiment returned an overall average accuracy of 80.4%.

Conversion and dilutional factors

According to the described preparation steps, all results of caffeine concentration (CC) determined in the final prediluted filtrate were multiplied by a dilution factor of 5 to give the actual concentration of caffeine extracted in liquid coffee samples. Since each liquid coffee sample was first prepared by cooking 50 g of raw coffee in a total volume of one liter of water, all results of measured concentration of caffeine in mg/L of liquid coffee extract were multiplied by 20 to get results in mg_{caf}/kg_{raw} (where mg_c = milligrams of extracted caffeine and kg_r = kilograms of raw coffee beans) then divided by 1000 to get our final results in g_{caf}/kg_{raw} used in our statistical analysis (where g_c = grams extracted caffeine and kg_r = kilograms of raw grinded coffee of predefined (type, degree of roasting, and cooking time). Accordingly, the practical quantitation limit of our UHPLC based method of = 0.1 mg/L of the injected diluted filtrate extracted sample can now by translated to 0.01 g_{caf}/kg_{raw}.

Statistical analysis

Statistical analysis was performed using the (Statistical Package of Social Sciences (SPSS) version 22 software (SPSS Inc., Chicago, IL, USA). The amount of caffeine extracted from kg of raw coffee (CE) (in g_{caf}/kg_{raw}) was summarized as mean (95% confidence interval [CI]), standard deviation (SD), median, interquartile range, minimum, and maximum. The Shapiro test was used for testing normality. The mean CE was compared using ANOVA (*F*-test), followed by pair-wise comparison across each two types using the Student's *t*-test with Bonferroni adjusted significance. The paired-*t*-test was used to compare the mean CE from each sample of raw coffee either roasted to two different degrees (light vs. medium-dark) or cooked for two different cooking durations (15 min vs. 30 min). Box-plots were used to display the results across different subgroups. All tests were two tailed, and a $P \leq 0.05$ was considered statistically significant.

RESULTS

Our results revealed the amount of caffeine extracted per kg of raw coffee (CE) in all 12 samples to have a mean (SD) of 10.65 (0.6) g_{caf}/kg_{raw} (95% CI = 10.27–11.03 g_{caf}/kg_{raw}), ranging from a minimum of 9.6 g_{caf}/kg_{raw} for a sample prepared from Yemeni Bari ground beans, medium-dark roasted and cooked for 30 min, to a maximum 11.8 g_{caf}/kg_{raw} for a sample prepared from light roasted Yemeni Kulani ground beans cooked for 15 min [Figure 4 and Table 2].

Using the paired *t*-test, we found a significant higher mean CE for samples cooked for 15 min (mean [95% CI] [SD] = 10.87 [10.25–11.48] [0.59] g_{caf}/kg_{raw}) compared to samples cooked for 30 min (mean [95% CI] [SD] =10.43 [9.82–11.05] [0.59] g_{caf}/kg_{raw}), with a mean difference (95% CI) (SD) of mean difference = 0.433 (0.026–0.841) (0.388) g_{caf}/kg_{raw}, test statistic t = 2.735, degrees of freedom = 5, and P = 0.041 [Figure 5]. This significant difference in CE due to different cooking durations was not found when applying the same analysis only across samples light roasted (t[2] = 1.835, P = 0.208), although the mean CE of those light roasted and cooked for 15 min (mean [95% CI] = 11.07 [9.32–12.81] g_{caf}/kg_{raw}) was higher than those light roasted but cooked for 30 min (mean [95% CI] = 10.53 [8.94–12.13] g_{caf}/kg_{raw}). Furthermore, significant difference in CE due to different cooking durations was not found when applying the same analysis only across samples medium-dark roasted (t[2] =1.890, P = 0.199), although the mean CE of those medium-dark roasted and cooked for 15 min (mean [95% CI] = 10.67 [9.42–11.92] g_{caf}/kg_{raw}) was higher than those medium-dark roasted but cooked for 30 min (mean [95% CI] = 10.33 [8.74–11.93] g_{caf}/kg_{raw}).

On the contrary, using the paired-*t*-test, we found no significant difference in mean CE in samples light roasted (mean [95% CI] [SD] =10.8 [10.1–11.5] [0.67] g_{caf}/kg_{raw}) compared to when they were medium-dark roasted (mean [95% CI] [SD] =10.5 [9.93–11.07] [0.55] g_{caf}/kg_{raw}), with a mean difference (95% CI) (SD) of mean difference = 0.3 ([− 0.192–0.792] (0.469) g_{caf}/kg_{raw}, test statistic t = 1.567, degrees of freedom = 5, and P = 0.178 [Figure 6]. However, no significant difference in CE due to different roasting temperature was found when applying the same analysis only across the six samples cooked for 15 min, light roasted (mean [95% CI] [SD] =11.07 [9.32–12.81] [0.7] g_{caf}/kg_{raw}) compared to medium-dark roasted (mean [95% CI] [SD] =10.67 [9.42–11.92] [0.5] g_{caf}/kg_{raw}) than when only applied across the six samples all cooked for 30 min, light roasted (mean [95% CI] [SD] =10.53 [8.94–12.13] [0.64] g_{caf}/kg_{raw}) compared to medium-dark roasted (mean [95% CI] [SD] =10.33 [8.74–11.93] [0.64] g_{caf}/kg_{raw}) [Figure 7].

Table 1: Gradient method

Time (min)	Percentage A	Percentage B
0	95	5
1	95	5
5	0	100
8	0	100
8.1	95	5

DISCUSSION

Coffee consumption is taken as the main measure of an individual's caffeine intake in several epidemiological studies concerned with the impact of caffeine on health. Using the UHPLC, the CC of 12 different samples of traditionally brewed coffee was directly measured, and the amount of caffeine extracted per kilogram of raw coffee (CE) where then calculated and compared.

Our results revealed that the type of coffee beans used in coffee preparation significantly affects CE. The highest CE was that of Ethiopian Harrar (mean = 11.00 g_{caf}/kg_{raw}) followed by Yemeni Kulani (mean = 10.95 g_{caf}/kg_{raw}) and Yemeni Bari (mean = 10.00 g_{caf}/kg_{raw}). Our finding is in accordance with other studies reporting variations in CC with the brands and blending duration.[4] Rodrigues *et al*. reported that the genotype of green coffee is a determinant characteristic of its caffeine content and other bioactive compounds such as chlorogenic and trigonelline.[49] Souza *et al*. suggested that discrimination of commercial roasted and ground coffees can be done according to their chemical content of some thermostable parameters (such as caffeine, kahweol, and cafestol).

Variations in CC have frequently been ignored in the examination of the possible relationship between coffee

Table 2: Comparison of the amount of caffeine extracted per kilogram of different types of raw coffee

Extracted caffeine in (g_{caf}/kg_{raw})	Sample (n=12)	Yemen Bari (n=4)	Yemen Kulani (n=4)	Ethiopian Harrar (n=4)	ANOVA F (df)	P	Pairwise comparison
Mean (95% CI) (SD)	10.65 (10.27–11.03) (0.6)	10 (9.42–10.58) (0.37)	10.95 (10.04–11.86) (0.57)	11 (10.74–11.26) (0.16)	7.776	0.011	P_1=0.027
Median (IQR)	10.7 (0.75)	10 (0.7)	10.7 (0.95)	11 (0.3)			P_2=0.020
Minimum–maximum	9.6–11.8	9.6–10.4	10.6–11.8	10.8–11.2			P_3=1

g_{caf}/kg_{raw}=Grams of extracted caffeine per kilograms of raw coffee, P_1=Bari versus Kulani, P_2=Bari versus Harrar and P_3=Kulani versus Harrar; Df=Degree of freedom and $P \leq 0.05$ was considered statistically significant. CI: Confidence interval, SD: Standard deviation, IQR: Interquartile range consumption and various health problems. Thus, our finding would partially eliminate the potential for the misrepresentation of individual consumption of coffee as a measure of the daily intake of caffeine. Each individual should consider the effects of their brewed coffee according to the type of coffee bean used in its preparation. The weaker Yemeni Bari beans have about 10% less caffeine than Harrar or Kulani coffee.

Our results suggest that the longer cooking duration increases the CE. We found significantly lower CE in samples cooked for 30 min (mean = 10.43 g_{caf}/kg_{raw}) compared to those cooked for just 15 min (mean = 10.87 g_{caf}/kg_{raw}). However, this significance disappeared when applying the same statistical analysis to the six samples prepared from light roasted beans. Such discrepancy could be explained by the reduction of sample size from a total of 12 to only six after such stratification. However, the mean CE was still numerically higher in any group of samples cooked for 15 min than those cooked for 30 min.

The myth that longer cooking time results in higher CC in the brew may actually come from an apparent false increase in CC due to the water loss from evaporation secondary to longer cooking time. In our study, we corrected this by increasing the volume of water for preparations intended for longer cooking.

Another possible reason for the prevalence of this myth is that many studies reported an actual increase in CC with a longer brewing and boiling time of coffee or tea, but mostly compared only two relatively short boiling durations, 1 min versus 2 or 3 min. Given that caffeine is a stable molecule, easily soluble in hot water, it would quickly reach equilibrium in about 3 min and no significant increase would be noticed with further boiling, even for 45 min, as reported by Saklar *et al.* with tea.[50] Thus, in regard to the traditional method of Arabic coffee brewing and our extended boiling cooking time, no significant difference is expected between cooking for 15 min versus 30 min, provided the temperature is constant.

The results of this study also contradicted the myth that darker roasted coffee beans would result in a brew with a higher CC. No significant difference in CE was found in samples which were light roasted (mean = 10.80 g_{caf}/kg_{raw}) compared to medium-dark roasted (mean = 10.50 g_{caf}/kg_{raw}), and there was no significant difference in CE when the same analysis was applied to six samples. Regarding these findings, it is worth noting that, in accordance with Saudi tradition, only two degrees of roasting were compared in our study (light vs. medium-dark) out of at least six degrees of roasting known worldwide. Second, the mean CE was still numerically higher in any group of samples prepared using light roasted beans than those prepared using medium-dark roasted beans cooked for the same duration, although such a difference did not reach statistical significance properly due to our sample size.

A few studies reported a higher CC in brewed coffee made with medium roast coffees compared to those made with medium-dark roast which was in accordance with our study.[51,52] Others reported that, since caffeine was thermostable, it was not affected by roasting.[53] A third group even reported lower CC in light roasted coffee compared to darker roasted.[54,55]

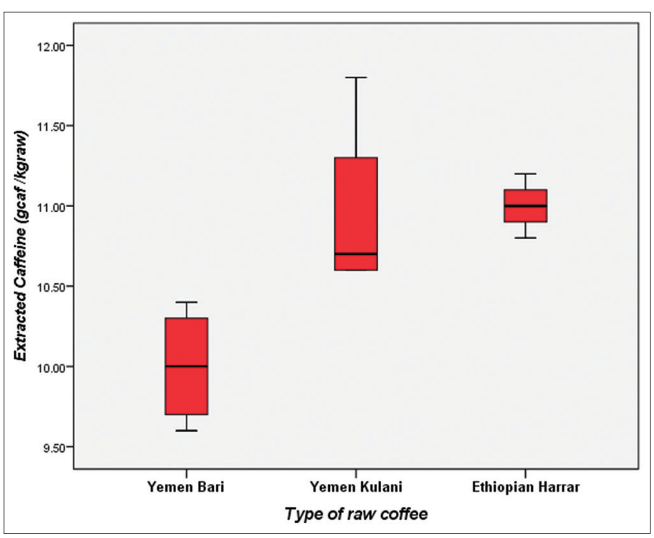


Figure 5: Box plot showing the extracted caffeine in (gcaf/kgraw) in all samples across the three different types of coffee beans included in the study Yemen Bari, Yemen Kulani, and Ethiopian Harrar

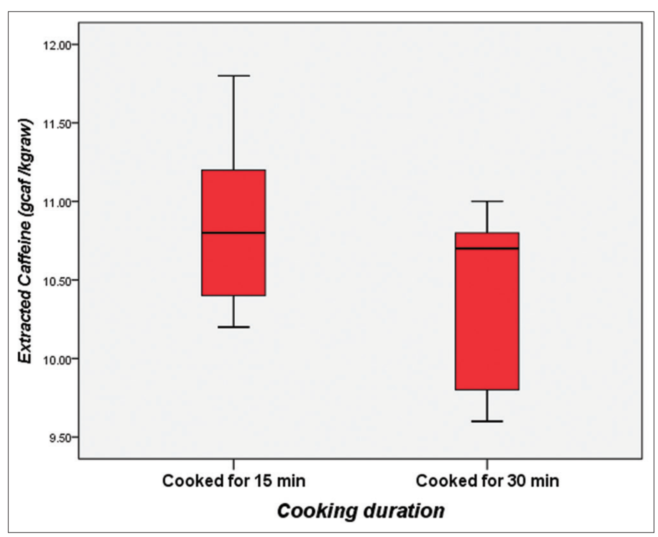


Figure 6: Box plots showing the extracted caffeine in (gcaf/kgraw) in all samples according to two different cooking durations (15 min to the left compared to 30 min to the right)

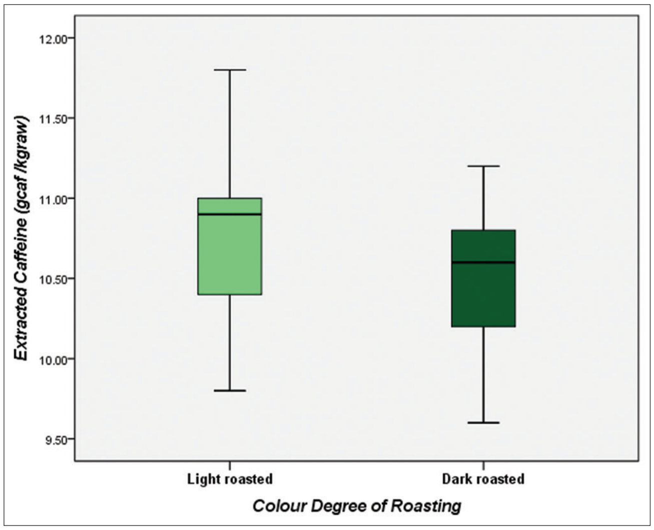


Figure 7: Box plots showing the extracted caffeine in (g_{caf}/kg_{raw}) in samples according to two different roasting degrees (light roasted to the left compared to medium-dark roasted to the right)

Our results are in accordance with the study done by Alqarni *et al.*, who reported that coffee subjected to more roasting will result in the loss of some of its caffeine and moisture and the lowering of its antioxidant potential.[14] In their study, Alqarni *et al.* attributed the contradiction in some published data due to nonadjustment for the decrease in the moisture contents with longer roasting which leads to apparent higher caffeine percentage in the darker grades with more roasting time. They proved that caffeine percentage actually decreases in the darker grades. They also reported that raw unroasted green beans may contain less caffeine.[14]

According to the FDA, caffeine can be a part of a healthy diet for most people, but too much caffeine may pose a danger to your health. Depending on the factors such as body weight, medication, and individual sensitivity, the amount is variable. A higher consumption of caffeine poses serious health risks for pregnant and lactating women, and patients with diabetes, peptic ulcers, hypertension, congestive heart failure, and dysrhythmias.[56]

For healthy adults, the FDA has cited 400 mg daily intake as an amount not associated with dangerous and negative effects. The systematic reviews also supported the recommendation of ≤300 mg/day in pregnant women and ≤2.5 mg/kg/day in children and adolescents. The maximum daily limit for a healthy adult (assumed to intake caffeine solely from coffee) can be translated to about 15 finjan/day, with a limit of ≤11.3 finjan/day for pregnant women.

It is important to consider the total daily intake of caffeine when adopting these limits, which are based on an assumption that the subject is getting all their caffeine only through drinking Saudi coffee. This is difficult in practice, particularly nowadays with many beverages and medications containing caffeine, including energy drinks which have gained more popularity among Saudi adolescents as reported by Musaiger and Zagzoog.[57] Energy drinks are nonalcoholic beverages that typically contain high levels of caffeine (>150 mg/L) and sugar in combination with other ingredients known to have stimulant properties.[58]

In considering the limitations of this study, it may be difficult to generalize our results given that only three types of beans, two roasting levels, and two cooking durations were compared. The temperature was precisely controlled, the precise amount of ground coffee was added and the amount of added water varied according to intended cooking time to adjust for evaporation. Such precautions cannot be guaranteed in practical daily brewing. We calculated the daily safe limits of coffee on the assumption that the person was getting their daily caffeine only solely from drinking coffee, something that is difficult in practices as many beverages and medications contain caffeine. Finally, the limited sample size may decrease the power of this study.

CONCLUSIONS

The type of coffee bean used is the main determinant of the amount of caffeine extracted from raw coffee (CE). The myth that longer cooking time and darker roasting resulted in higher CE were nonplausible, as our results showed that both may decrease CE. This needs further evaluation on a larger sample. Different populations would be required to translate the published results and recommended daily limits of caffeine consumption in accordance with their respective cultural habits, traditional methods of coffee brewing, and consideration of other caffeine sources.

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Conflicts of interest
There are no conflicts of interest.

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