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ROASTING EFFECT ON THE CAFFEINE CONTENTS AND ANTIOXIDANT POTENTIAL OF DIFFERENT COFFEE GRADES AVAILABLE IN THE SAUDI MARKET

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Abstract

In the Saudi market different grades of coffee (Coffee arabica) are available based on the degree of roasting. However, some unique grades subjected to very mild roasting are available for the preparation of the popular "Arabic Coffee". Caffeine contents were quantified by HPTLC method in the five available grades of coffee and coffee seed shells "Coffee Husks". Caffeine contents were estimated in both hydro-alcohol and aqueous extracts of the coffee grades using silica gel 60 F_{254} plates and ethyl acetate: methanol 85:15 (%, v/v) as mobile phase. A good linear relationship between peak area and caffeine concentration in the range of 100-600 ng/band was obtained. The proposed HPTLC method was validated following the ICH guidelines. The antioxidant activity using DPPH radical scavenging and Ferric-reducing power assays to explore the effect of roasting on the phenolic contents. The results indicated that increase roasting decrease both caffeine contents and antioxidant activity.

Key words: *Arabic coffee; Husks; Roasting; Caffeine; antioxidant; HPTLC*

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INTRODUCTION:

Caffeine is a natural purine alkaloid found in plants such as coffee, cocoa, tea, cola nuts and guarana. Caffeine is soluble both in water, organic solvents and sublimes at its boiling point [1]. Caffeine is classified by the FDA as safe compound since the toxic doses exceed 10 grams for an average adult. Ordinary consumption has low health risks; moreover consumption of caffeine afford slight protection against some diseases, including Parkinson's [2,3] and heart disease [4] . Caffeine is used as a central nervous system, cardiac, and respiratory stimulant [5]. Caffeine produces vasodilatation of the renal afferent arteriole as it antagonize adenosine action. This effect resulted in a diuretic effect by increasing the glomerular filtration rate. Caffeine also decreases the reabsorption of Sodium ions by the renal proximal tubules [6].

Coffee is considered as one of the rich and highly utilized sources of phenolic antioxidant species, especially chlorogenic acids as well as caffeoylquinic, feruloylquinic and coumaroylquinic acids derivatives [7]. Literature data indicated an inverse relation between roasting time and antioxidant activity due to the degradation of chlorogenic acids and other phenolic compounds [8]. Increase roasting time found to decrease both the antioxidant and anti-inflammatory potential of different coffee extracts [9]. However, the effect of roasting in caffeine contents is controversial. Many articles indicated that caffeine contents are higher in the darker grades of coffee subjected to longer roasting time than the light grades and raw coffee [10-12]. Other researchers found that both phenolic and caffeine contents decrease in the darker grades of coffee [13].

In the Gulf area, in addition to the worldwide coffee habits, a special cultural type of coffee known as “Arabic coffee” is very common. Arabic coffee is lighter than the least roasted grade commonly used worldwide. It is subjected to minimal roasting and with other additives resulted in a pale greenish brown coffee. Due to its unique taste and method of preparation the UNESCO recognized Arabic coffee as one of the Intangible Cultural Heritage of Humanity. It is a symbol of generosity in the Gulf area culture [14].

The current study aims to explore the caffeine contents and antioxidant power of Arabic coffee in comparison with the other coffee grades present in the Saudi market.

MATERIALS AND METHODS:

Standard and chemicals

Standard caffeine (Figure1), DPPH, potassium ferricyanide and trichloroacetic acid were purchased from Sigma-Aldrich, St. Louis, MO, USA. All the solvents used were of HPLC grade.

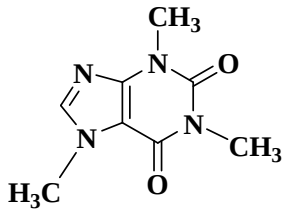

Fig. 1. Chemical structure of Caffeine.

Sample preparations

The different coffee grades (**1**: Raw coffee, **2**: Arabic coffee, **3**: Light grade coffee, **4**: Medium grade coffee, **5**: Dark grade coffee, **6**: Coffee husks) (Figure 2) were purchased from a Super Market series at Al-Kharj city, Riyadh region, Saudi Arabia. The seeds were powdered and 5 gm from each grade were extracted separately by boiling with water for two minutes to give aqueous extracts (AE). Another 5 gm from each grade were extracted separately by boiling with mixture of ethanol/water (1:1) to obtain the hydro-alcohol extract (HAE). The resulted decoctions were filtered and filtrates were transferred to 100 ml volumetric flask. Mixture of ethanol and water were used to complete the volume with final ration of 1:1 ethanol and water.

Figure 2. Photograph of different coffee grades.

Chromatographic Conditions

Glass-backed plates coated with 0.2 mm layers of silica gel 60 F_{254} (E-Merck, Germany) 10 × 20 cm were used for the HPTLC densitometric analysis. Samples application on the TLC plates in the form of 6 mm bands by a Camag Automatic TLC Sampler 4 (ATS4) sample applicator (Switzerland) equibbed with a Camag microlitre syringe. Application rate was 150 nl/s. Plates were developed to a distance of 80 mm using ethyl acetate/methanol 85:15 (%, *v/v*) as mobile phase in a Camag Automatic Developing Chamber 2 (ADC2) saturated with mobile phase for 30 min at 22 °C.

Standard solution was prepared by dissolving 10 mg of caffeine in 100 ml of 1:1 EtOAc/H2O mixture. A volume of 1, 2, 3, 4, 5, 6 ml were applied on silica gel plates to obtain the calibration curve.

Method Validation

The HPTLC method was validated according to the guidelines adopted by international conference on harmonization [15]. The method was linear between 100 and 600 ng/spot of caffeine where concentration was plotted against peak area.

Accuracy

The standard addition method was used to access accuracy, as recovery. Pre-analyzed caffeine samples (200 ng/spot) were enriched with extra caffeine standard (0, 50, 100, and 150%) and the mixtures were reanalyzed. Percentage recovery and relative standard deviation (RSD, %) were calculated for each concentration level.

Precision

Precision of the method were determined by measuring repeatability and intermediate precision. Repeatability was determined as intra-day variation while intermediate precision was determined by determination of inter-day variation for analysis three different caffeine samples (300, 400, and 500 ng/spot) in six replicate.

Robustness

Robustness of this method was proved by inducing small deliberate changes in the chromatographic conditions. Small changes to ratio of mobile phase components, volume of mobile phase, mobile phase saturation time and pre-activation of HPTLC plates during the quantification were applied.

Limit of detection (LOD) and limit of quantification (LOQ)

Standard deviation (SD) method using the slope of the calibration (S) curve, SD of the blank sample and applying the following equations:

$$LOD = 3.3\times SD / S$$

$$LOQ = 10\times SD / S$$

Were used to determine the limit of detection (LOD) and limit of quantification (LOQ).

Specificity

Both the R_f values and spectra of the spot corresponding to caffeine in AE and HAE in comparison with the standard caffeine confirmed the specificity of the system.

Antioxidant evaluation

DPPH radical scavenging assay

The DPPH radical scavenging of different coffee grades was done using the method of Yusufoglu et al [16] with some modifications. The DPPH radicals showed a strong absorption at 517 nm, color changed from purple to yellow, the absorbance decreased with reduction by an antioxidant compound (s). A portion (1 mL) of each of the different concentrations (10-250 µg mL–1) of the AE, HAE or ascorbic acid (standard) was added to 1 mL of 1 mmol L–1 DPPH in methanol. The reaction mixtures were mixed and

incubated in the dark for 30 min, after that the absorbance was measured against control (DPPH having 1 mL of methanol in place of the extract). The experiment was carried out in triplicate. Percentage radical scavenging activity of DPPH was calculated using the following formula:

$$DPPH\ scavenging\ effect\ (\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} X\ 100$$

EC_{50} of the DPPH scavenging effect of the AE and HAE of all coffee grades were calculated.

Ferric-Reducing power assay (FRA)

This was determined according to the described method [16]. One milliliter of each of AE and HAE of different coffee grades or standard with different concentrations (10-250 µg mL–1) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and cooled. Trichloroacetic acid (10%, 2.5 mL) was added to the mixture and the content was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with $FeCl_3$ (0.1%, 0.5 mL) and distilled water (2.5 mL). The absorbance was measured at 700 nm in a UV/visible spectrophotometer. The increasing, reducing power was indicated by increasing absorbance of the reaction mixture. EC_{50} of the FRA of the AE and HAE of all coffee grades were calculated.

Quantification of Caffeine in different grades of coffee seeds

Chromatograms of the AE and HAE of different coffee grades were obtained under the same conditions used for caffeine standard. The areas of the bands corresponding to the R_f value of standard caffeine was quantified for caffeine contents from the regression equation of the calibration plot.

2.7. Moisture contents determination

Moisture contents were determined by thermogravimetric approach via measuring the weight loss of the samples after 24 hours stable heating at 105 ^{0}C in oven [17]. Then the water loss percent was calculated by:

$$moisture\ content = \frac{initial\ weight - final\ weight}{initial\ weight} X\ 100\ \%$$

Statistical Analysis

Results are expressed as mean ± standard error (SE) of mean. Statistical analyses were performed, using one-way analysis of variance (ANOVA). Statistically significant ($P < 0.05$) when the F-value was found, further comparisons were conducted by using Dunnett's multiple comparisons test. SPSS software 17.0 (Released Aug. 23, 2008), Chicago, USA was used for all the statistical analyses.

RESULTS AND DISCUSSION:

The mobile phase composition was optimized to establish a suitable and accurate densitometric HPTLC method for analysis of caffeine. The mobile phase ethyl acetate: methanol 85:15 (%, *v/v*) resulted in a compact, symmetrical, and well resolved peak at R_f value of 0.38 ±0.01 (Figures 3-4). UV spectra measured for the bands showed maximum absorbance at approximately 275 nm (Figure 5).

The calibration plot of caffeine standard was linear between 100-600 ng/spot (Figure 6) and the linearity was confirmed from the linear regression data of the plot (Table 1). The correlation coefficient (R^2) was 0.9984 indicating the high data significance ($P<0.05$). The obtained linear regression equation: $Y = 14.174x + 698.93$, where Y represents the UV absorption and X correspond to caffeine concentration (Figure 6).

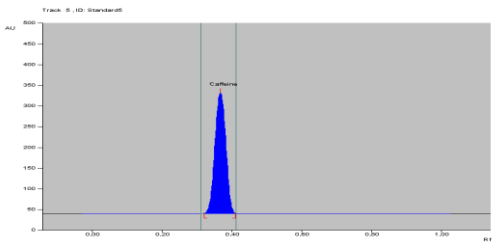

Figure 3. HPTLC Chromatogram of standard Caffeine.

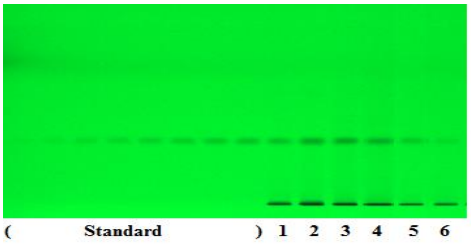

Figure 4. HPTLC Chromatogram of standard Caffeine and AE of different coffee grades.

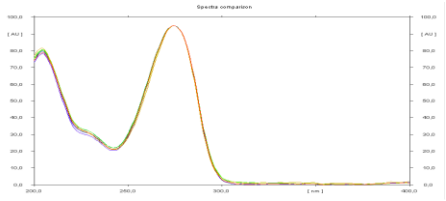

Figure 5. Overlaid UV absorption spectra of standard caffeine and different grades of coffee.

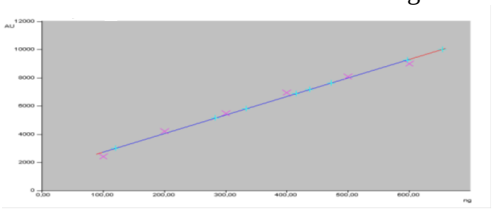

Figure 6. Linearity graph of caffeine.

Table 1. Linear regression data for the calibration curve of caffeine (n=6)

Linearity range (ng/spot)	100-600
Regression equation	Y = 14.174x + 698.93
Correlation coefficient	0.9984
Slope ± SD	4.8171 ± 0.1894
Intercept ± SD	913.6 ± 73.76
Standard error of slope	0.07735
Standard error of intercept	3.12
95% confidence interval of slope	4.613 – 5.043
95% confidence interval of intercept	830- 997.2

Accuracy was expressed as percentage recovery(Table 2). The accuracy of the method, as recovery, was 98.00-99.53 %, with RSD values in the range 0.24-1.42. These results indicated that the quantification is accurate.

Table 2. Accuracy of the proposed method (n=6)

Excess drug added to analyte (%)	Theoretical content (ng)	Conc. found (ng) ± SD	% Recovery	% RSD
0	200	196.17 ± 2.14	98.08	1.09
50	300	294.00 ± 2.97	98.00	1.01
100	400	394.33 ± 5.61	98.58	1.42
150	500	497.67 ± 1.21	99.53	0.24

Precision was expressed as percentage coefficient of variation (% CV) of measured concentrations for each calibration level. Results from determination of repeatability and intermediate precision, are expressed as SD (%). RSD ranged between 0.41-0.57 for repeatability and 0.47-0.73 for intermediate precision (Table 3). These low values indicated that the method is precise.

Table 3. Precision of the proposed method.

	Repeatability (Intraday precision)			Intermediate precision (Interday)		
Conc. (ng/spot)	Area ± SD (n = 6)	Standard error	% RSD	Area ± SD (n = 6)	Standard error	% RSD
300	4891.60 ± 27.94	11.41	0.57	4882.40 ± 35.64	14.64	0.73
400	6538.00 ± 29.23	11.93	0.45	6537.60 ± 30.50	12.45	0.47
500	7644.20 ± 31.09	12.69	0.41	7634.20 ± 36.96	15.09	0.48

Low values of % RSD (0.36-0.39) were obtained after introducing small deliberate change into the mobile phase composition (Table 4) indicating that the methods robust. Calculated LOD and LOQ of the proposed method were 5.16 and 15.87 ng/spot, respectively (Table 1). These values give indication about the wide range of caffeine that can be detected and quantified by the method.

Table 4. Robustness of the proposed HPTLC method.

	Mobile phase composition (Ethyl acetate: methanol)			Results		
Conc. (ng/spot)	Original	Used		Area ± SD (n = 6)	% RSD	R_f
		8.4:1.6	+0.1	6537.80 ± 30.26	0.46	0.39
400	8.5:1.5	8.5:1.5	0.0	6533.60 ± 21.59	0.33	0.38
		8.6:1.4	-0.1	6540.40 ± 27.32	0.42	0.36

The peak purity of caffeine in coffee samples were assessed by comparing the overlaid UV spectra of the spot with that of the standard (Figure 5).

Caffeine peaks from AE and HAE of different grades of coffee seed were identified by comparing their single spot at R_f = 0.38 ± 0.01 values with corresponding spots of caffeine under same chromatographic comditions. The Caffeine content in AE and HAE of different grades of coffee were obtained using the linear regression equation. A clear relation could be observed between the caffeine contents and the roasting time (Table 5). The darker grade subjected to longer roasting time contains the least amount of caffeine among the other brands. These results are logic, as part of caffeine may be lost during roasting, and supported by the findings of Fuller et al [13]. Other groups [11, 12, 14] published data where caffeine contents apparently increased in the darker grades with more roasting time. Among these studies, only Ludwig et al [11] accessed the moisture contents of the different coffee grades. As roasting time increase the moisture contents will decrease and that leads to “apparent” higher caffeine percentage. Considering the moisture contents in calculating caffeine contents prove that the “actual” percentage decreases as the coffee roasted for longer time. Caffeine percentage in green raw coffee did not follow the same concept as it is always low even if moisture contents are considered. This fact can be explained by suggesting that caffeine exist in green coffee partially in a complex form that dissociate as roasting starts. However, this suggestion needs further investigation. Coffee husks contain much less caffeine than coffee seeds. The HAE is slightly higher in caffeine than AE except in case of light coffee grade. However, the differences are very small indicating that solvent effect is not significant.

Table 5. Caffeine and moisture contents in different coffee grades

Sample no.	Caffeine % w/w		Moisture % w/w	Caffeine % w/w considering moisture content	
	AE	HAE		AE	HAE
1 (Raw Coffee)	0.69	0.71	7.645	0.75	0.77
2 (Roasted Arabic Coffee)	0.91	0.89	5.062	0.96	0.94
3 (Light coffee)	0.89	0.88	3.232	0.92	0.91
4 (Medium dark coffee)	0.82	0.83	3.677	0.85	0.86
5 (Dark coffee)	0.65	0.67	3.284	0.67	0.69
6 (Coffee seeds coat)	0.28	0.25	9.382	0.31	0.28

In agreement with all published data the antioxidant potential of coffee decreased in the darker grades (Fig 3&4) [9- 12]. The best values were obtained in both DPPH and Ferric reducing power assay with sample **1** representing the raw coffee and were very close to the used standard ascorbic acid. Roasting leads to loss in phenolic contents. Another factor significantly affect the antioxidant potential is the solvents used for extraction. In all cases during the course of our study HAE has lower EC_{50} than the AE. In case of sample **1** in the Ferric reducing power assay huge difference was recorded between the EC_{50} of AE (254.88 µg/mL) and HAE (104.95 µg/mL). These variations resulted from more efficient extraction of the antioxidant species when 50% ethanol was used. The antioxidant potential of sample **6** (coffee husks) was comparable to that of sample **4** (Medium grade) but containing about 1/3 of sample **4** caffeine contents.

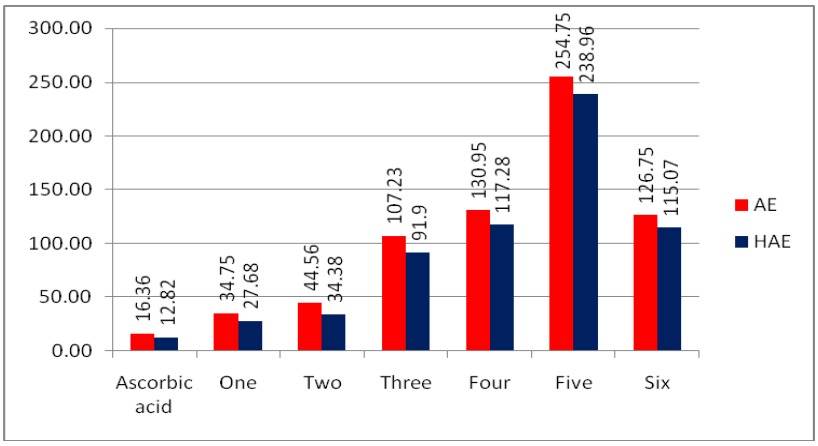

Figure 7. EC_{50} (µg/mL) of AE and HAE of different coffee grades in DPPH scavenging assay.

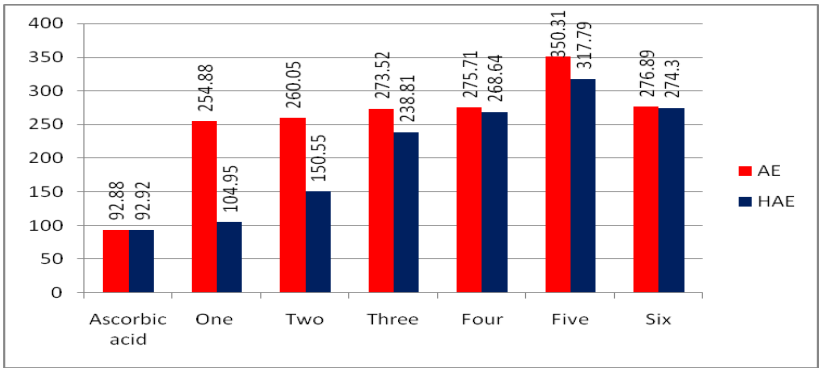

Figure 8. EC_{50} (µg/mL) of AE and HAE of different coffee grades in Ferric-reducing power assay.

CONCLUSION:

Simple, accurate, reproducible and sensitive HPTLC method was developed for quantification of caffeine in different coffee grades. Caffeine contents were calculated without and with considering moisture contents. In addition, antioxidant potential was accessed via DPPH and Ferric reducing power assays *in vitro*. Darker grades of coffee subjected to more roasting process contain less caffeine and lower antioxidant potential due to loss of components under roasting conditions. The calculation of caffeine percentage in coffee samples in some published data with consideration of moisture contents proved that it is actually decrease in the darker grades. Green coffee in all instances contains less caffeine than other grades. Extraction with HA or HAE have little effect on caffeine contents but more on phenolics responsible for the antioxidant activity. Coffee husks can provide users with the benefits of the antioxidant effect with much less caffeine content.

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