Estimation of Caffeine Content in Coffee of Malaysian Market Using Fourier Transform Infrared (FTIR) Spectroscopy

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ABSTRACT

This study reports the caffeine content in seven locally available coffee. The caffeine was extracted with chloroform and analysed using Fourier Transform Infrared (FTIR). The method reports an average recovery of 101% with the limit of determination established at 0.1%. The absorption band at 1654 cm^{-1} was used to construct the calibration curve for quantification of caffeine where the regression was fitted with satisfactory linearity. An average of 0.55% of caffeine was detected in the seven coffee products with Arabica coffee demonstrating lower caffeine concentration. The study evidenced that caffeine content in coffee is determined by the coffee types. The caffeine content found in the local coffee products was relatively lower likely due to the solvent types, extraction procedure and analytical method used.

Keywords: Arabica coffee, decaffeinated, chloroform extraction, Robusta coffee

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is an alkaloid of the methylxanthine family which is widespread in plants. It is a stimulant that acts on the central nervous system to give off numerous effects, primarily to boost the physical and mental performance (Glade, 2010). Caffeine is most commonly found in coffee bean, cocoa bean, tea leaves and soft drinks (Frary, Johnson & Wang, 2005). Typically, coffee beans contain 0.8-2.8 % caffeine contributing to bitter taste of coffee brew (Eggers & Pietsch, 2001). The caffeine level depends on the species and origin of coffee beans as well as the brewing methods (Alves, Casal & Oliveira, 2007). The caffeine content in brewed coffee was found the highest, in the range of 56 – 100 mg/100 mL, followed by instant coffee and tea (20-73 mg/100 mL) (Nawrot *et al*., 2003).

Coffee comes from the genus of *coffea* from *Rubiaceae* family. In the global market, there are three most widely traded coffee species. They are *coffea arabica*, *coffea canephora* (Robusta) and *coffea liberica* of which the varieties of robusta (10%) and liberica (90%) are commercially grown in Malaysia (Mohammad Nor & Abd Wahap, 2016). Essentially, there is a marked positive growth of 30% in the coffee industry in Malaysia annually (The Malaysian Reserve, n.d.). Many coffee chains are seen to spring up in the street mainly as a result of the changes in lifestyle, boosted by the young consumers and professionals.

The coffee culture penetrates the society of Malaysia rapidly; a survey shows that Malaysians drink an average of 2.38 cup of coffee daily (Essays, 2013). The amount of caffeine consumed is not normally of interest. As a matter of fact, excessive caffeine intake could lead to toxicity, cardiovascular effects, alteration in calcium balance, changes in behaviour, increased risk of cancer and effects on male fertility. As recommended, a daily intake of up to 400 mg of caffeine/day is considered safe for an adult (Nawrot *et al*., 2003).

According to Malaysian Standard MS 1360 (1994), all coffee species are expected to contain averagely 0.9% caffeine nevertheless, the caffeine content in most local coffee products are undeclared. Hence, it is the objective of the study to determine the caffeine content in the coffee products available locally using the rapid Fourier Transform Infrared (FTIR) spectroscopic method. The method has been widely used for quantification of caffeine with promising accuracy (Garrigues, Bouhsan, Garrigues & de la Guardia, 2000; Paradkar & Irudavaraj, 2002; Weldegebreal, Redi-Abshiro & Chandravanshi, 2017). Garrigues *et al*. (2000) reports the detection limit of FTIR for caffeine at 3 mg/L with recovery between 94.4 – 100.1%. Singh, Wechter, Hu and Lafontaine (1998) on the other hand reports at sensitivity of 5 mg/L.

MATERIALS & METHODS

Samples

A total of seven coffee products were obtained from the local market as shown in Table 1. The coffee was used for analysis without any pre-treatment.

Table 1. The seven coffee sample obtained from the local market and their corresponding coffee type.

Brand	Coffee bean type
Boncafe Mocha (BM)	Arabica coffee
Cap Tangan (CT)	Arabica and Robusta mixture
Decaffeinated Nescafe (DC)	Robusta
Indocafe (IN)	Arabica and Robusta mixture
Kopi Luak (KL)	Arabica
Nescafe Gold (NG)	Arabica and Robusta mixture
Tenom Cafe (TC)	Robusta

Standard Preparation

A stock caffeine standard of 5000 ppm was prepared by weighing 0.05 g of pure caffeine into a 10 mL volumetric flask and made up to mark with chloroform. The stock solution was diluted into caffeine standards at 1000, 2000, 3000 and 4000 mg/L which is 0.1%, 0.2%, 0.3% and 0.4%. The standard solutions were subjected to analysis with Fourier Transform Infrared (FTIR) (Thermo Scientific Nicolet iS10) equipped with a diamond Attenuated Total Reflectance (ATR).

Sample Extraction

The extraction method is according to Samiullah *et al*. (2015) and Singh *et al*. (1998). Five grams of coffee was weighed into a beaker with 50 mL of distilled water and 2 g of sodium carbonate was added. The mixture was stirred with a glass rod and the solution was boiled on a hotplate for 20 mins. The solution was then filtered through filter paper gravitationally followed by 30 mL of distilled water. The filtrate was transferred to a separatory funnel and 8 mL of chloroform was added. The separatory funnel was shook vigorously and left upright for separation. The organic layer was transferred to an Erlenmeyer flask and the process of extraction was repeated with 5 mL of chloroform. The solvent layer from both extractions were combined yielding a total of 13 mL chloroform. A small amount of sodium sulphate was added as the drying agent. The mixture was then filtered gravitationally prior to analysis with FTIR in triplicates.

FTIR

The sample was scanned at a resolution of 4 cm^{-1} in the range of 4000 – 650 cm^{-1}. Each spectrum was ratioed against a fresh background spectrum recorded from the bare ATR crystal. Chloroform was used to clean the ATR crystal. Each sample was scanned in triplicates. The spectra were saved in the format of csv and processed with the automated peak detection algorithm (Sim & Ting, 2012). The peak area of absorption band at 1600 – 1800 cm^{-1} was used to plot the calibration curve. The caffeine concentration in coffee was reported in percentage (% w/w) based on the following equations.

$$\text{Amount of caffeine extracted (mg)} = \frac{x \times \text{volume of solution}}{1000}$$

where x is the concentration (mg/L) determined from the calibration curve

$$\text{Percentage of caffeine in coffee} = \frac{\text{Amount of caffeine extracted (mg)}}{\text{Amount of coffee used for extraction (mg)}} \times 100\%$$

Recovery and Detection Limit

For recovery, a coffee sample was spiked with 2 mL of 2000 mg/L of caffeine and subjected to the above extraction procedure for FTIR analysis in triplicates. A blank of coffee sample was similarly prepared. The recovery performance was calculated according to the following equation.

$$\% \text{ Recovery} = \frac{\text{Experimental concentration - blank}}{\text{Expected concentration}} \times 100\%$$

The limit of determination was examined using standards with various concentrations, starting from 0.01% to 0.02%, 0.03%, 0.05%, 0.07% and 0.1%. The absorption band at 1600-1700 cm^{-1} was studied for each level of concentration tested. The limit of determination is the lowest concentration at which the absorption signal is identified.

Statistical Analysis

Analysis of Variance (ANOVA) was used to evaluate if there is any significant different in caffeine content of seven coffee samples. Tukey's test was performed for multiple comparisons at 95% confidence level.

RESULTS & DISCUSSION

Figure 1 shows the spectral region of 1600 – 1750 cm^{-1} for caffeine standards at various concentrations where two absorption bands at 1654 and 1697 cm^{-1} are identified. These IR bands of caffeine are likewise reported by Singh *et al.* (1998) with no interference from chloroform. The blank chloroform spectrum in figure 1 clearly demonstrates that there is no interfering absorption in this region corroborating the findings of Singh *et al.* (1998). The absorption in this region of 1600-1700 cm^{-1} is commonly used for quantification of caffeine with the band at 1642 cm^{-1} (Abdalla, 2015); 1655 cm^{-1} (Franca & Oliveira, 2011); 1658 cm^{-1} (Ohnsmann, Quintas, Garrigues & de la Guardia, 2002) and 1655 cm^{-1} (Singh *et al.*, 1998).

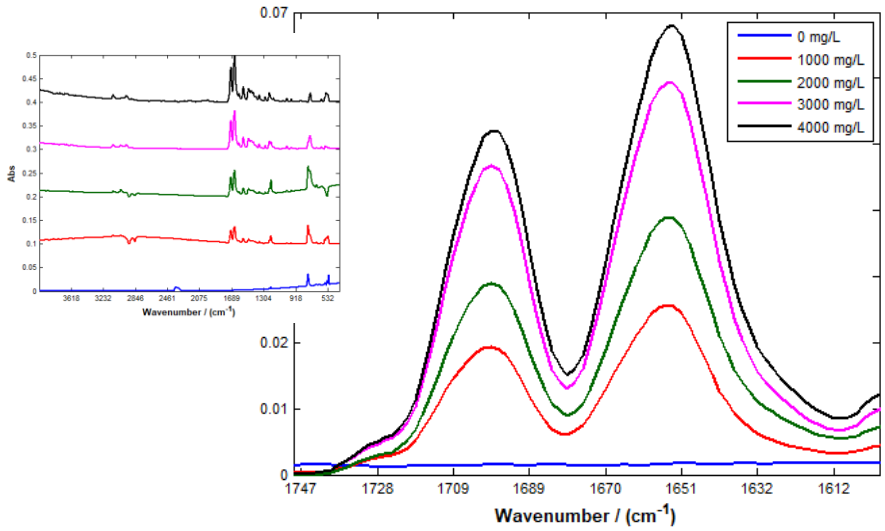

Figure 1. Spectral region of 1600-1750 cm^{-1} for caffeine at various concentrations. The inset illustrates the spectra profile from 400 – 4000 cm^{-1}.

For quantification, the peak area at 1654 cm^{-1} is used to construct the calibration curve (Figure 2). The regression line is fitted with R^2 value of 0.9062 indicating satisfactory linearity. The limit of detection is established at 1000 ppm (0.1%) as below this concentration, the absorption band at 1600-1700 cm^{-1} is absent. The detection limit reported at 0.0005% (Singh *et al.*, 1998), 0.0003% (Garrigues *et al.*, 2000) and 0.002% (Ohnsmann *et al.*, 2002) are unachievable in this study. Nevertheless, the extraction efficiency based on the recovery of spiked coffee sample is found at an average of 101%. The average of greater than 100% is attributed to the error introduced during extraction and analysis.

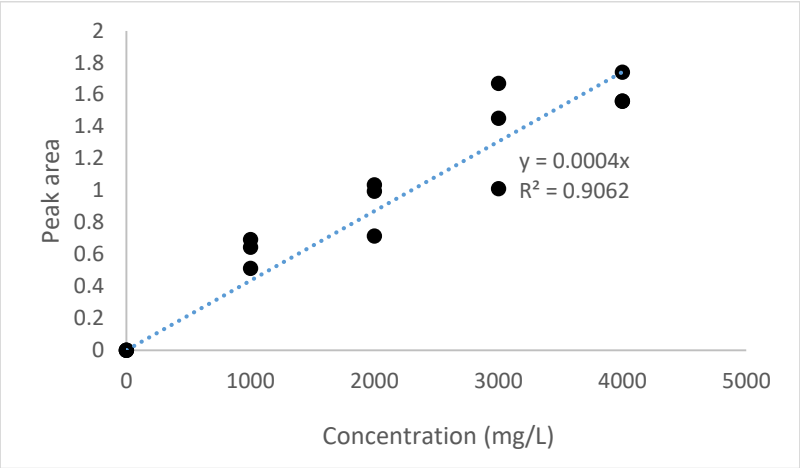

Figure 2. The calibration curve based on the peak area of absorption band at 1654 cm^{-1}.

Figure 3 shows the IR spectra of coffee samples at 1600-1700 cm^{-1}. The band at 1655 cm^{-1} is observably shifted in some samples likely due to the matrix interference. For the decaffeinated sample of DC, the band is shifted to higher frequency of 1658 cm^{-1} whilst for the sample of IN, the band is found at 1650 cm^{-1}.

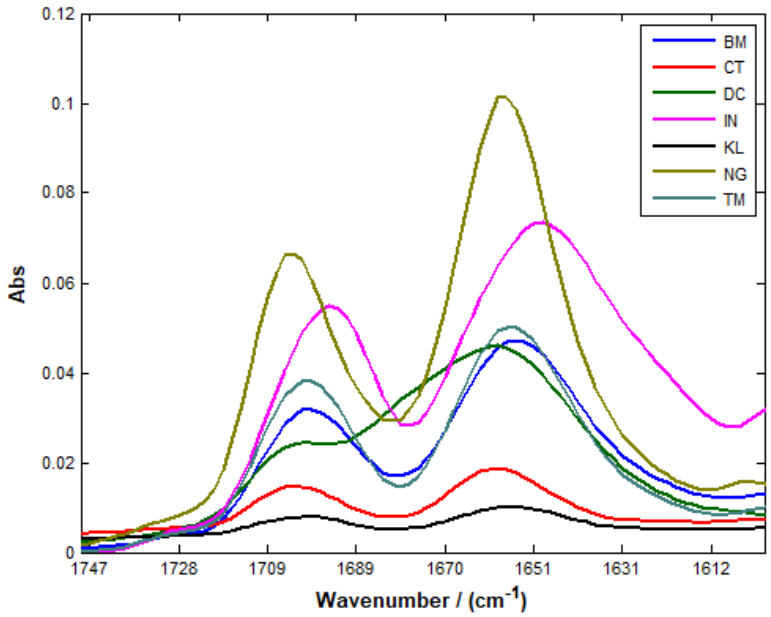

Figure 3. The IR spectra of coffee samples at 1600-1700 cm^{-1}.

The caffeine content in the seven coffee samples is summarized in Table 2. According to Hodgson, Randell and Jeuendrup (2013), the average caffeine in coffee is approximately 3.4% whilst Dunayar (2008) reported 1-2% w/w dry weight. In this study, the average caffeine estimated in seven coffee samples is 0.55% with the concentration ranging between 0.14% and 0.98%. Apparently, the coffee of Arabica (BM: 0.37% and KL: 0.17%) contains lower caffeine than Robusta coffee (DC: 0.60% and TC 0.65%). Typically, Robusta coffee comprises higher caffeine than Arabica with the concentration reported at 2.2% and 1.2%, respectively (Khapre, Kyamuhangire, Njoroge & Khaturima, 2017). For the mixture coffee of Arabica and Robusta, the caffeine content may vary depending on the proportion of two

different coffee types. The results suggest that coffee CT and KL contain significantly lower caffeine with the concentrations arranged in decreasing order of NG > IN > TC > DC > BM > KL > CT. Among the seven samples, coffee NG and DC are produced by the same manufacturer with the latter decaffeinated. As shown in Table 2, the decaffeinated sample of DC demonstrates lower caffeine than NG. Essentially, decaffeinated coffee does not guarantee caffeine free. As reported by McCusker, Fuehrlein, Goldberger, Gold and Cone (2006), the caffeine content in decaffeinated coffee may range from undetected to 13.9 mg in 16 oz of coffee. Chalmers and Cossey (2016) further revealed 39 mg of caffeine in the decaffeinated coffee of Blackbird for each serving.

Table 2. The percentage of caffeine in seven coffee samples.

Brand	Coffee bean type	% caffeine in coffee
BM	Arabica	0.37 ± 0.05^{b}
CT	Arabica and Robusta mixture	0.14 ± 0.03^{a}
DC	Robusta	0.60 ± 0.17^{c}
IN	Arabica and Robusta mixture	0.97 ± 0.07^{d}
KL	Arabica	0.17 ± 0.08^{a}
NG	Arabica and Robusta mixture	0.98 ± 0.06^{d}
TC	Robusta	0.65 ± 0.08^{c}

The same letter in the column of % caffeine indicates no significant different ($p > 0.05$)

The caffeine content determined in this study is compared against the literature findings as summarized in Table 3. Generally, the caffeine content identified is probably lower likely due to the solvent types, extraction procedure and analytical method used. According to Wanyika, Gatebe, Gitu, Ngumba and Maritim (2010), analysis of caffeine using ultraviolet-visible (UV-Vis) spectroscopy tends to produce higher measurements than high performance liquid chromatography (HPLC). Liew, Nik Daud and Hassan (2001) likewise compares the strategy and analytical method for determination of caffeine. Gas chromatography (GC) (75.7%) was reported with lower recovery performance than HPLC (92.9-98.6%) and spectrophotometry method (91.4%). The extraction strategy, with and without defatting step, further contributes to variation in the method efficiency with the latter yielding higher recovery percentage.

Standard MS 1360 assumes that all coffee species comprises 0.9% caffeine. The caffeine content determined in the coffee products of this study (0.14 – 0.97%) matches well with the expected concentration. There is no minimal safe limit of caffeine for coffee however the consumption of 300-400 mg per day is recommended for a healthy adult (which is equal to four cups of coffee per day) (Gera, Kalra & Gupta, 2016).

Table 3. Comparison of caffeine content determined in this study and the literature values.

Coffee type	Caffeine content (%)		
	Arabica	Robusta	Mixture of Arabica and Robusta or others
This study	0.17-0.37	0.60 – 0.65	0.14 – 0.97
Farah (2012)	0.9 – 1.3		1.2-1.5 (Cenophora)
Gichimu, Gichuru, Mamati, and Nyende (2014)	1.72		1.35 (Liberica) 0.2 (Paracoffea)
IARC (1991)	1.1	2.2	
Khapre *et al*. (2017)	1.2	2.2	
Illy (2002)	< 1.5		

CONCLUSION

Caffeine in seven locally available coffee products was extracted with chloroform and analysed using ATR-FTIR. The method demonstrated recovery at an average of 101% with the limit of determination established at 0.1%. The seven coffee samples demonstrated average caffeine of 0.55% with Arabica coffee exhibiting lower caffeine than Robusta coffee. Besides, the decaffeinated coffee was found with some caffeine (0.60%) but the amount significantly lower than the caffeinated product from the same manufacturer. Evidently, the caffeine content in coffee was determined by the coffee types. The solvent type, extraction strategy and analytical method used will contribute to the method efficiency leading to over or underestimated caffeine content.

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