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REGULAR ARTICLE

DETERMINATION OF CAFFEINE CONTENTS OF COFFEE BRANDS IN THE VIETNAMESE MARKET

Thi Thanh Dieu Phan, Vlastimil Kuban, Stanislav Kráčmar*

Address: Tomas Bata University in Zlin, Faculty of Technology, Department of Biochemistry and Food Analysis, nám. T.G. Masaryka 275, 762 72 Zlín, Czech Republic

***** Corresponding author: phan_dieu@yahoo.com

ABSTRACT

In this study, the caffeine contents in five certain Vietnamese coffee (Dak Tin, Di Linh, Nam Nguyen, Origin and Vinacafe) found in the Vietnamese market were determined using UV/vis spectrophotometry. The quantification of caffeine sample was calculated by standard addition method. Our results showed that the caffeine contents in coffee brewing were influenced by temperature of water used to brew, time of brewing, and independent on the volume of water, respectively. In general, higher concentrations of caffeine were found in all samples prepared at temperature 100°C for 5 minutes. The order of caffeine contents in coffee samples was Dak Tin, Di Linh, Nam Nguyen, Origin and Vinacafe, respectively. This study can contribute to a better knowledge of caffeine contents in Vietnamese coffee of Vietnamese consumers.

Keywords: Vietnamese coffee, caffeine, standard addition method, UV/Vis Spectrophotometry

INTRODUCTION

Caffeine is an alkaloid of the methylxanthine family known as a central nervous system stimulant through its adenosine antagonist action (**Eggers *et al.*, 2001**). Caffeine is a naturally found in the leaves, seeds and/or fruits of at least 63 plant species worldwide. The most commonly known sources of caffeine are coffee, cocoa beans, kola nuts and tea leaves (**Barone *et al.*, 1996; Frary *et al.*, 2005**). 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 (**Eggers *et al.*, 2001**). The amount of caffeine in coffee beverage varies depending upon the serving size, the type of product, and brewing method (**Alves *et al.*, 2009**). Based on the data reviewed (**Dorea *et al.*, 2005**), that epidemiological and experimental studies have shown positive effects of regular coffee drinking on various aspects of health, such as psychoactive responses (alertness, mood change), neurological condition (infant hyperactivity, Parkinson's disease), metabolic disorders (diabetes, gallstones), and gonad and liver function. However, high doses may produce negative effects in some sensitive individuals, including anxiety, tachycardia and insomnia (**Nehlig, 1999; Ogita *et al.*, 2003; Farah *et al.*, 2006)**. **Pollak (2003)** recommended upper limits of caffeine for healthy adults below 300-500 mg daily, pregnant women must stay below 150-200 mg daily and children should stay below 50 mg daily. Amounts exceeding 700 milligrams of caffeine can be dangerous. Coffee is the most popular drink in Vietnam. Most commercial brands of coffee in Vietnam are made up of both Arabica and Robusta beans. Therefore, it will be interesting to determine the level of caffeine in coffee available in Vietnamese market in order to establish whether the amount of caffeine in the beverages is so much as to cause adverse health problem.

The purpose of this research was to determine caffeine contents of Vietnamese coffee purchased at supermarket in Viet Nam (Dak Tin, Di Linh, Nam Nguyen, Origin and Vinacafe) at different temperature (80 °C, 90 °C and 100 °C), at different brewing times (1 min, 3 min and 5 min) and at different volume of water (30 ml, 70 ml and 150 ml) by UV/vis spectrophotometry, which is available in most laboratories. Although spectrophotometry is a fast and simple method, it is not possible to determine caffeine directly in coffees by conventional UV absorption measurement due to the spectral overlap (**Zhang *et al.*, 2005**). Standard addition method can be used instead of a calibration curve to eliminate the interference of matrices. The methods are easy, fast and cheap for the determination of the caffeine content in coffee beverages.

MATERIALS AND METHODS

Chemicals

Caffeine standard (>99%) was obtained from Sigma-Aldrick (UK). Caffeine stock solution of 100 $\mu g.ml^{-1}$ was prepared by dissolving 10 mg of caffeine in 100 ml of distilled water. The caffeine solution was then diluted to provide the necessary working concentration 16 $\mu g.ml^{-1}$ to obtain standard solutions for the preparation of standard addition calibration curves.

Samples

Five samples including: Dak Tin (Ca phe Dak Tin LTD), Di Linh (CTY CP CHÈ-CAFE DI LINH), Nam Nguyen (CÔNG TY CHẾ BIẾN CÀ PHÊ NAM NGUYÊN), Origin (Công Ty TNHH Một Thành Viên Tín Nghĩa) and Vinacafe (Công ty Cổ phần Vinacafe Biên Hòa). All coffee samples were ground roasted coffee, purchased at Saigon CO.OP in Vietnam.

Brews preparation

Influence of temperature (80 °C, 90 °C and 100 °C): 2.0 g of ground roasted coffee was put in a beaker. After that 100 ml of distilled water at 80 °C, 90 °C and 100 °C was poured into the beaker. After 3 minutes, the mixture was filtered by a paper filter. After cooling, the filtrate was adjusted to 250 ml with distilled water and ready for analysis.

Influence of water volume (30 ml, 70 ml and 150 ml): 2.0 g of ground roasted coffee was put in a beaker. After that 30 ml, 70 ml and 150 ml of distilled water at 90 °C was poured into the beaker. After 3 minutes, the mixture was filtered by paper filter. After cooling, the filtrate was adjusted (diluted) to 250 ml with distilled water and ready for analysis.

Influence of extraction time (1 min, 3 min and 5 min): 2.0 g of ground roasted coffee was put in a beaker. After that 100 ml of distilled water at 90 °C was poured into the beaker. After 1 min, 3 mins and 5 mins, the mixture was filtered by paper filter. After cooling, the filtrate was adjusted (diluted) to 250 ml with distilled water and ready for analysis.

Standard Addition Method (Zellmer, 1998)

The 10 ml of filtrate (coffee sample) was added to each of four 25ml volumetric flask. Then a series of increasing volumes (0, 1, 2 and 3 ml) of caffeine standard solution of 16 $\mu g.ml^{-1}$ were added. Finally, each flask was made up to the mark with distilled water and mixed well. After measuring the response for a series of standard additions, we get the value of the concentration of caffeine in the sample in 25 ml.

$$y = ax + b = 0 \quad (1)$$

$$\rightarrow \quad x = -b/a \ (\mu g.ml^{-1}) \quad (2)$$

Then, concentration of caffeine in coffee sample: $(x*25)/10$ $(\mu g.ml^{-1})$ (3)

UV/Vis spectrophotomectric analysis

The caffeine was quantified using a UV/vis spectrophotometry using Lambda 25 system with UV WinLab V2.85 software and 10 mm quartz cells (all from Perkin-Elmer, USA). The absorbance of the working standards and samples were measured at 273 nm. The caffeine levels of the samples were calculated from the regression equation of the best line of fit of the standards.

RESULTS AND DISCUSSIONS

Table 1 shows the caffeine contents quantified by UV/vis spectrophotometry by standard addition method. At a glance, it can be observed that caffeine content was strongly dependent on temperature of water used to brew, brewing lengths and independent on volume of water (see Table 1). Brewing temperature exerts a predominant effect on yield variability, whilst both contact time and grinding degree have a limited influence and brewing formula only a marginal one (**Nicoli *et al*,. 1990**). Contents of caffeine in coffees brewed by 100 °C are significantly higher than those in coffee brewed by 80 °C. The highest amount of caffeine in samples analyzed was found in Vinacafe (54.30 ± 0.470 $\mu g.ml^{-1}$) at 90 °C with 5 min of brewing length, while the lowest was found in Dak Tin (19.40 ± 0.232 $\mu g.ml^{-1}$) at 90 °C with 1 min of brewing length. Among ground roasted coffee samples, Vinacafe was the finest and Dak tin was the coarsest. The values generally agree well with previous published article, **Leonard (1996)** stated that the longer brew time implies longer contact time between water and coffee grounds leading to more complete caffeine extraction

and caffeine content also depended on the extent of grinding. The contents of caffeine in all the coffee brands were found to be less than those in the documented range, an average caffeine concentration of 60 to 85 mg per cup of instant and roasted and ground coffees, respectively (**Barone and Roberts, 1996**). This was due to using distilled water as the solvent to dissolve coffee sample instead of extracting caffeine by organic solvent.

Table 1 Caffeine contents of coffee infusions at different volume, time and temperature

Sample (n=3)	**Volume (ml)**	µg.mL^{-1} Mean±SD	Regression equation	**Time (min)**	µg.mL^{-1} Mean±SD	Regression equation	**Temperature (°C)**	µg.mL^{-1} Mean±SD	Regression equation
DaK Tin									
	30	20.10 ± 0.140	y = 0.0523x + 0.4201	1	19.40 ± 0.232	y = 0.0518x + 0.4037	80	20.00 ± 0.360	y = 0.0522x + 0.4158
	70	19.64 ± 0.202	y = 0.0515x + 0.4045	3	20.00 ± 0.430	y = 0.0512x + 0.4109	90	23.00 ± 0.110	y = 0.0522x + 0.4737
	150	21.10 ± 0.450	y = 0.0528x + 0.4363	5	22.00 ± 0.272	y = 0.0531x + 0.4661	100	23.80 ± 0.300	y = 0.0521x + 0.4954
Di Linh									
	30	31.30 ± 0.410	y = 0.0522x + 0.6533	1	28.81 ± 0.854	y = 0.0528x + 0.6085	80	26.00 ± 0.940	y = 0.0521x + 0.536
	70	28.02 ± 0.400	y = 0.0529x + 0.593	3	31.00 ± 0.310	y = 0.0521x + 0.645	90	32.50 ± 0.503	y = 0.0534x + 0.6916
	150	31.73 ± 0.240	y = 0.0531x + 0.6739	5	30.20 ± 0.252	y = 0.0508x + 0.6131	100	33.00 ± 0.360	y = 0.052x + 0.6988
Nam Nguyen									
	30	43.12 ± 0.222	y = 0.0534x + 0.921	1	31.02 ± 0.800	y = 0.0517x + 0.6415	80	32.33 ± 0.080	y = 0.0532x + 0.6879
	70	41.52 ± 0.720	y = 0.0523x + 0.8685	3	33.23 ± 0.831	y = 0.0516x + 0.6859	90	35.00 ± 0.591	y = 0.0519x + 0.7263
	150	40.00 ± 0.300	y = 0.0527x + 0.8396	5	35.73 ± 0.420	y = 0.053x + 0.7575	100	36.04 ± 0.664	y = 0.0532x + 0.7669
Origin									
	30	39.73 ± 0.630	y = 0.0524x + 0.8328	1	35.60 ± 0.440	y = 0.0526x + 0.7482	80	33.40 ± 0.800	y = 0.0528x + 0.7064
	70	40.30 ± 0.674	y = 0.0525x + 0.8456	3	40.42 ± 0.561	y = 0.0524x + 0.8472	90	35.00 ± 0.610	y = 0.0537x + 0.7454
	150	39.80 ± 0.465	y = 0.052x + 0.8269	5	43.30 ± 0.354	y = 0.0522x + 0.9042	100	36.10 ± 0.170	y = 0.05x + 0.7217
Vinacafe									
	30	49.20 ± 0.740	y = 0.049x + 0.9634	1	49.64 ± 0.782	y = 0.0496x + 0.9848	80	51.00 ± 0.700	y = 0.0502x + 1.015
	70	49.30 ± 0.590	y = 0.0498x + 0.986	3	53.00 ± 0.184	y = 0.0475x + 1.0056	90	52.00 ± 0.423	y = 0.0532x + 1.0128
	150	50.10 ± 0.564	y = 0.0506x + 1.0134	5	54.30 ± 0.470	y = 0.047x + 1.0202	100	53.00 ± 0.300	y = 0.0483x + 1.019

S.D., standard deviation. All results were evaluated using the variation statistics (ANOVA) in Excel 2010 at $p < 0.05$.

CONCLUSION

The study was to determine caffeine contents in five types of commercial coffee in Vietnamese market (Dak Tin, Di Linh, Nam Nguyen, Origin and Vinacafe) at different temperature, volume of water and brewing lengths. According to the results of UV/vis spectrophotometric analysis, caffeine intake via coffee infusions was mainly dependent on the temperature of water used to brew and brewing lengths. Standard additional method is suitable for determining caffeine contents in beverages because of using distilled water as the solvent. This study can contribute to a better knowledge of the levels of caffeine in commercial coffees for Vietnamese consumers to estimate how many cups of coffee we should have every day. Based on this study, in general moderate coffee consumption ranging from 3 to 5 cups per day is unlikely to be of any health concern.

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REFERENCES

ALVES, R. C. - SOARES, C. - CASAL S. - FERNANDES, J. O. - OLIVEIRA M. B. P. P. 2007. Factors influencing the norharman and Harman contents in espresso coffee. In *Journal of Agricultural and Food Chemistry*, vol. 55, 2007, p. 1832-1838.

BARONE, J. J. - ROBERTS, H. 1996. Caffeine consumption. In *Food Chem Toxicol*, vol. 34, 1996, p. 119-129.

BELITZ, H. D. - GROSCH, W. - SCHIEBERLE, P. 2004. *Food Chemistry*, 6th Ed. Springer-Verlag, Heidelberg, 2004, p.157–242.

BELITZ, H. D. - GROSCH W. - SCHIEBERLE, P. 2009. Coffee, Tea, Cocoa. In *Food Chemistry*, vol. 21, 2009, p. 937-951.

DOREA, J. G. - DA COSTA, H. M. 2005. Is coffee a functional food? In *British Journal of Nutrition*, vol. 93, 2005, p. 773-782.

EGGERS, R. - PIETSCH, A. 2001. *Coffee Recent Development*. Iowa State University Press, Iowa, 2001, p. 33-46.

FARAH, A. - DE PAULIS, T. - TRUGO, L C. - MARTIN, P. R. 2006. Chlorogenic acids and lactones in regular and water-decaffeinated arabica coffee. In. *J. Agric Food Chem*., 2006, vol. 54, p. 374–381. doi: 10.1021/jf0518305.

FRARY, C.D. - JOHNSON, R. - WANG, M.Q. 2005. Food sources and intakes of caffeine in the diets of persons in the United States. *JADA,* vol. 1052005, p. 110-113.

FRANCA, A. S. - OLIVEIRA, S. L. - OLIVEIRA, R. C. S. - AGRESTI, P. C. M. - AUGUSTI, R. 2009. A Preliminary Evaluation of the Effect of Processing Temperature on Coffee Roasting Degree Assessment. In *Journal of Food Engineering*, vol. 92, 2009, p. 345-352.

KNIGHT, C.A. - KNIGHT, I. - MITCHELL, D.C. - ZEPP, J. E. 2004. Beverage caffeine intake in US consumers and subpopulations of interest: Estimates from the Share of Intake Panel survey. In *Food Chem. Toxicol,* vol. 2, 2004, p. 1923-1930.

LEONARD, N.B. - CLINTON, R.W. - ALEXANDRA, N.G. 1996. Caffeine content in coffee as influenced by grinding and brewing techniques. In *Food Research International,* vol. 29, 1996, n. 8, p. 785-789.

NEHLIG, A. 1999. Exploring biotechnology. In *Chemtech*., vol. 29, 1999, p. 30–35.

NICOLI, M. C. - DALLAROSA, M. - LERICI, R. C. 1990. Influence of some processing conditions on solid-liquid extraction of coffee. In *Lebensmittel-Wisssen. Technol*., vol. 23,1990, p. 386-389.

NIKOLOVA-DAMYANOVA, B. - VELIKOVA, R.- JHAM, G.N. 1998. Lipid Classes, Fatty Acid Compositions and Triacylglycerol Molecular Species in Crude Coffee Beans Harvested in Brazil. In *Food Research International,* 1998, vol. 31, p. 479-486.

OGITA, S. - UEFUGI, H. - YAMAGUCHI, Y. - KOIZUMI, N. - SANO, H. 2003. Producing decaffeinated coffee plants. In *Nature,* vol. 423, 2003, p. 823.

PAUL, P. 2009. Coffee as a functional food. In *Functional and Specialty Beverage Technology,* vol. 15, 2009, p. 370-395,

ZELLMER, D. L. 1998. Department of Chemistry, California State University, Fresno 1998, http://www.green-tea-health-news.com/too-much-caffeine.html

ZHANG, Q. L. - LIAN, H. Z. - WANG, W. H. - CHEN, H. Y. Separation of caffeine and theophylline in poly (dimethylsiloxane) micro channel electrophoresis with electrochemical detection. In *Journal of Chromatography A,* vol. 1098, 2005, p. 172-176.