

## Determination of caffeine in roasted and irradiated coffee beans with gamma rays by high performance liquid chromatograph

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### Abstract

The present study was carried out to investigate a simple, quick and organic solvent saving procedure for the high performance liquid chromatography determination of caffeine in two different coffee beans (Indonesian and Brazilian) which roasted at two different temperatures (150 & 220 °C) and irradiated at 3, 6, and 9 kGy doses by gamma rays. A linear calibration curve was generated with caffeine concentration ranging from 0.005 to 0.25 mg/g with correlation coefficient ( $R^2 = 0.9995$ ,  $n=4$ ) and relative standard deviation  $\leq 2.1$  %. The developed procedure provided a  $7.3 \times 10^{-9}$  mg/g and  $2.2 \times 10^{-8}$  mg/g limit of detection and limit of quantification, respectively. The developed method was repeatable and could be applied to determine trace amounts of caffeine in popular irradiated coffee beans with three different irradiation doses. Moreover, irradiation treatments at doses up to 9 kGy showed no significant effect on the caffeine content.

**Keywords:** Caffeine determination; High performance liquid chromatography; Coffee bean; Roasting; Gamma rays; Statistical analysis

### 1. Introduction

Caffeine, a naturally occurring alkaloid found in tea leaves, coffee beans, kola nuts, cocoa beans and other plants, is used as a flavoring agent in a variety of beverages, including some soft drinks and energy drinks. The amount of caffeine in soft drinks varies among brands and it is closely regulated by the US Food and Drugs Administration (FDA) to not more than  $200 \text{ mg L}^{-1}$  (Zou & Li, 2006). Caffeine may be the most popular drug in the world. Caffeine was consumed daily in coffee, tea, cocoa, chocolate, some soft drinks and some drugs. Caffeine is also a central nervous system stimulant. In moderate doses, it can increase alertness, reduce fine motor coordination, cause insomnia, headaches, nervousness and dizziness (Tzanavaras & Themelis, 2007). Recently appeared methods (1996–2012) reporting the determination of caffeine in various sample matrices (environmental, biological, plants, food, etc.) cover a broad spectrum of instrumental analysis. These include voltammetry (Aklilu, Tessema, & Redi-Abshiro, 2008; Suw Y. L., 2004), HPLC (Abu-Qare & Abou-Donia, 2001; Antonella A., 2005; Eisei N., 2004; Holland, Godfredsen, Page, & Connor, 1998; Horie, Nesumi, Ujihara, & Kohata, 2002; Huang, Gao, Zhai, Liang, Wang, Bai, et al., 2012; Mar R. B., 2007; Pistos & Stewart, 2004; Schreiber-Deturmeny & Bruguerolle, 1996; Tzanavaras & Themelis, 2007; Zeid A. Al-Othman 2012; Zuo, Chen, & Deng, 2002), electrochemical (Amare & Admassie, 2012; Rajendra N. G., 2011; Sujuan G., 2011; Suw Y. L., 2004), extraction (Chen, Pavelic, Dillon, & Naidu, 2002; Shrivastava & Wu, 2007), spectrophotometric (Ali Reza Khanchi, 2007; Singh & Sahu, 2006; Y. Zhana, 2011) and TLC (Abourashed & Mossa, 2004; Fenske, 2007). Application of ionizing radiation treatment of foods on an industrial scale was started at the beginning of the 1980s after the joint FAO/IAEA/WHO expert committee accepted the application of a 10 kGy overall average dose for foods. The recommended dose levels are: low level at 1 kGy to inhibit insect infestation and delay ripening; medium at 1 to 10 kGy to reduce bacterial load (particularly of pathogens); and high at 10 to 50 kGy for commercial sterilization and elimination of viruses. Gamma irradiation is an effective process for inactivating foodborne pathogens and reducing microbial populations in foodstuffs. The irradiation process is one of the few technologies which address both food quality and safety due to its ability to control spoilage and foodborne pathogenic microorganisms. After many years of research and the development of national and international standards, more than 60 countries have regulations allowing food irradiation of at least one product (Blackburn, 2011). However, the use of radiation to kill pathogens is limited because of radiation-induced adverse effects on

the sensory quality of the food products (M. Lacroix, Jobin, M., Hamel, S., Stahl, V., Gagnon, M., De Couvercelle, C., 1991). New food irradiation technologies have been developed in which combined treatments have been used to reduce the radiation doses required to kill pathogenic bacteria and/or reduce overall microbial load (M. Lacroix, Ouattara, B., 2000). Therefore, the goal of this study was to examine the effectiveness of low gamma irradiation doses in the preservation of coffee without degradation of caffeine at two different roasting temperatures. Also the study aimed to develop an HPLC procedure to determine the caffeine with very LOD.

## 2. Experimental

### 2.1. Materials

Standard caffeine powder was provided by Sigma (St. Louis, MO, USA). 500  $\mu\text{g mL}^{-1}$  standard solutions were prepared in solution of methanol and water (70:30) and stored under refrigeration and protected from light. Caffeine standard stock solution was stable for at least 3 weeks. Coffee samples (Indonesian and Brazilian) were purchased from local market.

### 2.2. Roasting process

The roasting process was performed at 150 °C for 10 min and 220 °C for 20 min according to (Carla I. Rodrigues, 2007).

### 2.3. Irradiation process

Three bags from each of coffee beans (Indonesian and Brazilian) were gamma irradiated at 3, 6, and 9 kGy doses using cobalt-60 gamma chamber (1.367 kGy/h) in Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Inshas, Cairo, Egypt. The control non-irradiated samples were left at room temperature during irradiation of the other samples for uniformity of conditions.

### 2.4. Sample preparation

Coffee bean samples were ground in a coffee grinder for 5 min. then, 40 mg of dry powder sample were added to 0.5 mL solution of methanol and water (70:30, v/v) and shaken for 5 min. then centrifuged at 13000 rpm / min for 5.0 min. and separated the filtrate. The precipitate was added to 5.0 mL of the same solution and follows the same previous steps until separate the filtrate and this step was repeated another time. The three filtrates were collected in one tube to remove the solvent by nitrogen gas. The samples were stored at freezing at -20 °C until HPLC analysis.

### 2.5. HPLC procedure

Samples were filtered through PTFE (0.45  $\mu\text{m}$ ) membrane syringe filters. HPLC analysis was carried out in a Sykam EZ CHROM elite system with S 1122 model pump, with a 20  $\mu\text{L}$  sample loop, S 3240 UV-Visible detector. The analysis was carried out using Column Thermo Controller S4011 C18 column (4.6 mm i.d  $\times$  25 cm) in the reversed phase connected through a guard column of C18. The mobile phase used was methanol: water (30:70, v/v) with a flow rate of 1 ml/min. The UV-Visible detector was set at 275 nm.

### 2.6. Statistical analysis

Four different replicate trials were conducted in this study and analysis was performed using duplicate pouches per each replicate trial. Data were then statistically analyzed by using ANOVA procedure.

## 3. Results and Discussion

### 3.1. Study of the chromatographic variables

The effect of the flow rate and composition of the mobile phase on (a) the retention time ( $t_R$ , min) of the analyte, (b) the width at half peak ( $w_{1/2}$ , min) and (c) the number of theoretical plates (N), was studied using an aqueous caffeine standard having a mass concentration of 10  $\text{mg L}^{-1}$ . The sample injection volume was set at 20  $\mu\text{L}$  and the column temperature at 25 °C. The flow rate and the polarity of the mobile phase had the most effective parameter on the separation of caffeine in HPLC. The combination of 1.0  $\text{mL min}^{-1}$  and methanol:water (30:70, v/v) was selected as the optimum for high separation efficiency of caffeine. The retention time was found to be 7.123 min.

### 3.2. Validation of the HPLC assay

The developed HPLC assay was validated in terms of linearity, Fig. [1], limit of detection (LOD) and quantification (LOQ), precision, selectivity, robustness and ruggedness. An additional series of experiments was carried out to validate the suitability of external calibration versus the standard addition approach for real sample analysis.

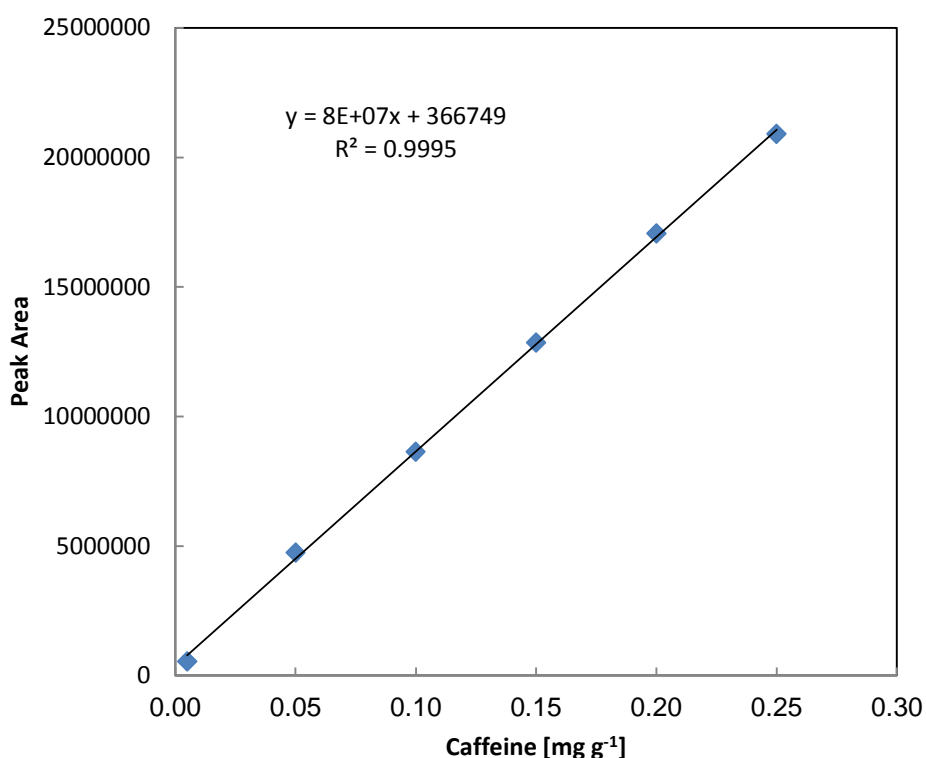
#### 3.2.1. Linearity, LOD and LOQ

The developed assay was found to be linear in the range 0.005–0.25  $\text{mg/g}$  caffeine with correlation coefficient ( $R^2$ ) of 0.9995 where peak area was used for signals evaluation, Fig. [1] and Table 1. The limit of detection (LOD) and limit of quantification (LOQ) were determined (J. C. Miller, 1993) and found to be  $7.3 \times 10^{-9}$   $\text{mg/g}$  and  $2.2 \times 10^{-8}$   $\text{mg/g}$  respectively.

### 3.2.2. Robustness and ruggedness

The ruggedness of the method assay against small but deliberate variations ( $\pm 4\%$ ) of the composition (25–35% Methanol) and the flow rate ( $1.90\text{--}3.00\text{ mL min}^{-1}$ ) of the mobile phase was validated by calculating the percent recoveries of a  $0.05\text{ mg g}^{-1}$  caffeine standard solution. A 6-point calibration curve constructed under the optimal chromatographic conditions was used for this purpose. The findings verified the ruggedness of the procedure since the percent recoveries were satisfactory, varying in the range of 99.70–131 in all cases.

The robustness of proposed method is measured of its capacity to remain unaffected by small, but deliberate variation in the method parameters and provides an indication of its reliability during usage. The most significant variables of system [temperature and flow rate] which modified in the range  $\pm 7.0\%$  (excluding pH, which was modified in the range  $\pm 5.0\%$ ) from their optimum values. Errors lower than 3.0 % were observed in all cases. Thus, the proposed method was found to be robust for routine determination of caffeine in different real sample.



**Fig. 1.** Calibration curve of standard caffeine using mobile phase of water/methanol (70:30, v/v) with ultraviolet (UV) detection

**Table 1**

Chromatographic and method calibration parameters of caffeine determination

<b>characterizations of HPLC separation</b>	
Caffeine retention time (min)	7.123
Capacity factor (k)	46.297
<b>HPLC calibration curve</b>	
Range (mg/g)	0.005-0.250
Slop	$8.0 \times 10^7$
Correlation coefficient ( $R^2$ ) n=4	0.9995
LOD(mg/g)	$7.3 \times 10^{-9}$
LOQ(mg/g)	$2.2 \times 10^{-8}$
Recovery(%) standard (n = 3)	131

### 3.2.3. Selectivity and precision

The selectivity of the developed method was evaluated by examining the separation efficiency of the

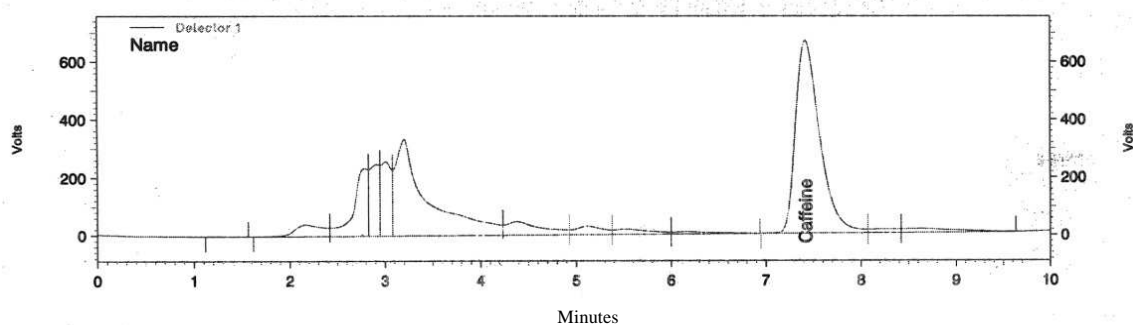
chromatographic system under the optimum conditions, Fig. [2]. The separation was excellent since there was no overlap between peaks in the chromatogram. The repeatability of the method was evaluated by calculating the confidence limit of four consecutive injections at 95% confidence level; the respective values were in narrow range 0.001- 0.0613 mg/g of caffeine.

### 3.3. Caffeine content (mg/g) of raw and roasted Indonesian and Brazilian coffee samples

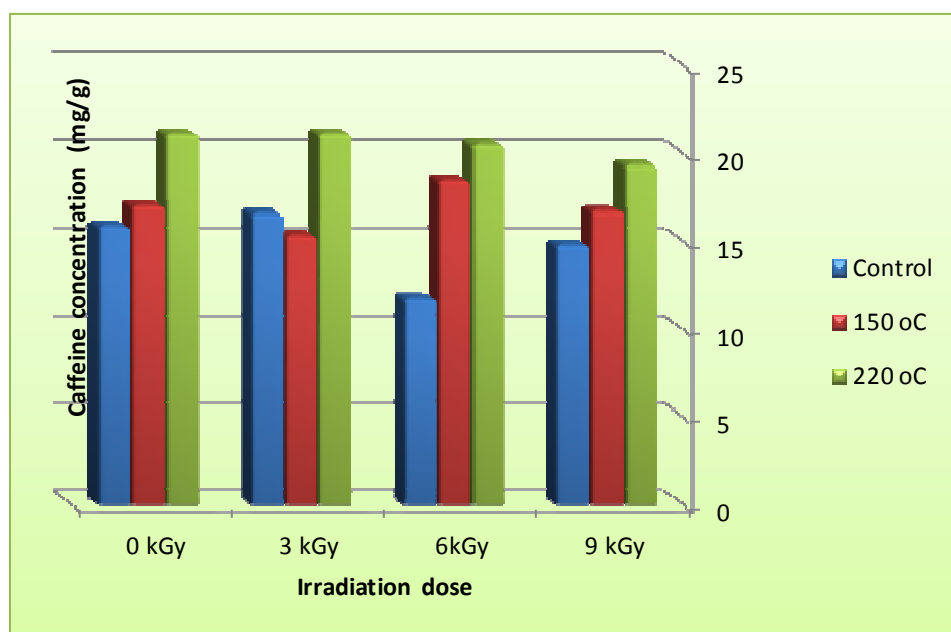
Also the effects of two different roasting temperatures on the caffeine content in two different kinds of coffee beans were shown in Fig. [3] and recorded Table [2]. The experimental results showed that there is an increase in the caffeine content by raising temperatures in Indonesian coffee and this increase has a significant difference. So increasing the temperature up to 220 °C was recommended in case of long shelf life for this type of coffee, whereas in case of Brazilian coffee, increasing temperatures causes slight increase in caffeine content with no significant difference.

### 3.4. Caffeine content (mg/g) of raw and irradiated Indonesian and Brazilian coffee samples

The effect of gamma irradiation on two different types of coffee beans (Indonesian and Brazilian) at three different doses was shown in Fig. [3] and Table [2]. The results showed that after irradiation of coffee beans up to 9 kGy, we observed no significant difference in caffeine content despite 3 kGy was the optimum dose in both samples. Tables [3]. So it could be concluded that the results of irradiating multiple compounds together will generally not cause much chemical change in any one of the compound and the chemical changes due to irradiation would be distributed to all food components, though not necessarily evenly. Because the principal nutritional value of proteins food is determined by their amino acids content, this observation is important in connection with the irradiation of foods, and especially the higher limit of 9 kGy that WHO recommended for elimination of pathogens and extended shelf-life, can be used for formulated food preservation without significant losses of amino acids



**Fig. 2.** Chromatograph of extracted coffee bean for determination of caffeine at 25 °C, methanol:water (30:70, v/v) as mobile phase and using UV detector



**Fig. 3.** Caffeine content (mg/g) of roasted and irradiated Indonesian coffee bean

### Conclusions

For the first time, the HPLC technique is successfully applied for investigation and determination of caffeine in two types of coffee beans after roasting and irradiated with gamma rays at three different doses. The present methodology is easy, rapid, sensitive, and requires small sample volumes (10  $\mu$ L) for the separation and preconcentration of caffeine. Also it is a low-cost technique due to use of minimum amount of solvent and reagents for the extraction of caffeine throughout the experiments. The results showed that after irradiation of coffee beans up to 9 kGy, we observed no significant different in caffeine content. The proposed method offers advantages in detection sensitivity, repeatability and simplicity

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**Table 2**

Caffeine content (mg/g) of row, roasted and irradiated Indonesian and Brazilian coffee samples

Roasting	Gamma irradiation doses <sup>a</sup>				Mean SD	F test <sup>b</sup>
	0 kGy	3 kGy	6 kGy	9 kGy		
<b>Indonesian coffee</b>						
Control	15.910 $\pm$ 0.0026	16.588 $\pm$ 0.0062	11.747 $\pm$ 0.0080	14.810 $\pm$ 0.0022	0.140	8.57
150 °C	17.063 $\pm$ 0.0067	15.522 $\pm$ 0.0613	18.331 $\pm$ 0.0066	16.751 $\pm$ 0.0039	0.577	1.98
220 °C	21.144 $\pm$ 0.0010	21.171 $\pm$ 0.0015	20.537 $\pm$ 0.0043	19.364 $\pm$ 0.0123	0.171	5.81
<b>Brazilian coffee</b>						
Control	9.582 $\pm$ 0.0126	11.063 $\pm$ 0.0054	10.998 $\pm$ 0.0014	10.969 $\pm$ 0.0060	0.187	4.80
150 °C	12.705 $\pm$ 0.0026	11.293 $\pm$ 0.0037	11.219 $\pm$ 0.0037	11.904 $\pm$ 0.0067	0.143	8.22
220 °C	10.758 $\pm$ 0.0049	12.252 $\pm$ 0.0052	10.312 $\pm$ 0.0006	11.270 $\pm$ 0.0041	0.161	6.49

<sup>a</sup> Mean  $\pm$  Confidence limit. (n = 4)

<sup>b</sup> theoretical value of F at 95% confidence level is 9.55

**Table 3**

Analysis of variance for Caffeine content of row, roasted and irradiated Indonesian coffee samples

Source of variance	Indonesian coffee					
	SS	df	MS	F	P-value	F crit.
<b>Roasting</b>	68.5232	2	34.262***	11.201	0.009	5.143
<b>Gamma irradiation doses</b>	2.677668	3	0.893 <sup>NS</sup>	0.292	0.830	4.757
<b>Error</b>	18.35302	6	3.059			
Source of variance	Brazilian coffee					
	SS	df	MS	F	P-value	F crit.
<b>Roasting</b>	2.054015	2	1.027 <sup>NS</sup>	1.854	0.236	5.143
<b>Gamma irradiation doses</b>	0.837309	3	0.279 <sup>NS</sup>	0.504	0.693	4.757
<b>Error</b>	3.323249	6	0.553			

NS: Non significant

\*\*\*: Significant

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