



CONTENT OF 4(5)-METHYLIMIDAZOLE, CAFFEINE AND CHLOROGENIC ACID IN COMMERCIAL COFFEE BRANDS

Thi Thanh Dieu Phan, Miroslava Bittová, Kamil Mikulášek, Vlastimil Kubáň, Stanislav Kráčmar, Pavel Valášek, Blanka Svobodová

ABSTRACT

Content of 4(5)-methylimidazole (4-MeI), caffeine and chlorogenic acid in commercial coffee brands were determined using high-performance liquid chromatography (HPLC) with UV DAD and MS detectors. Positive ion ESI mass spectra of the 4-MeI standard yielded intense signals corresponding to $[M+H]^+$ (83.0604) and $[2M+H]^+$ ions (165.1115). Also, adducts of 4-MeI with acetonitrile from mobile were detected – $[M+ACN]^+$ ions (124.0849). The LOD of 2.5 ng mL^{-1} and LOQ of 8.4 ng mL^{-1} were calculated according to the following formulas: $\text{LOD} = 3.SD/S$, and $\text{LOQ} = 10.SD/S$, where S is the slope of the calibration curve and SD is the standard deviation of the noise. The caffeine content was compared to the results of the standard addition, 1st derivative and liquid-liquid extraction spectrophotometry. 4-MeI was in tens $\mu\text{g g}^{-1}$ in the Vietnamese coffees while in units $\mu\text{g.g}^{-1}$ in all Czech and Brazilian coffees ($<2.4 \mu\text{g.g}^{-1}$ and $<4.9 \mu\text{g.g}^{-1}$, respectively). The results for caffeine were within the documented range (0.31 – 2.20%) in all coffee samples. The lower content of caffeine and chlorogenic acid was observed in Vietnamese coffees. All the methods used for determination of caffeine in the Czech and Brazilian coffees gave acceptable precision and accuracy. However, there were significant differences in the results in Vietnamese coffees. The caffeine extractability (100 °C, 3 min brewing) almost reached 100% in Czech and Brazilian coffees, while it was less than 90% in Vietnamese coffees. The Czech and Brazilian coffees tend to produce more caffeine in brews than the Vietnamese coffee because of the different composition of blends and the particle size degree.

Keywords: coffee; spectrophotometric methods; HPLC; caffeine extractability

INTRODUCTION

The study of 4(5)-methylimidazole (4-MeI) and 2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)-imidazole (THI) has recently become an important aspect of food chemistry. In many studies regarding the identification of the causes of carcinogenesis, 4-MeI has been a factor, which could be associated with cancer risks (NTP, 2007; OEHHA, 2010). 4-MeI can induce alveolar/bronchiolar adenoma and carcinoma in male and female mice (Chan et al., 2008). It can also inhibit the cytochrome P450 isoenzyme, which catalyzes the oxidation of many known or suspected carcinogens of low molecular mass in the human liver (Hargreaves et al., 1994). Thus, this compound has been classified as a group 2B compound “probably carcinogenic to humans” by the International Agency for Research on Cancer (IARC, 2011). 4-MeI and THI are undesired byproducts formed by heating carbohydrates (glucose, sucrose, invert sugar, etc.) in the presence of ammonia (Hodge, 1967; Kort, 1971; Tomasik et al., 1989; Gobin a Phillips, 1991; Bradbury et al., 1996). These compounds can be present in caramel color products such as soy sauce, caramel colors, carbonated soft drink, Worcestershire sauce, canned coffee, dark beer etc. (Casal et al., 2002; Cunha et al., 2011; Yamaguchi and Masuda, 2011). Because roasted coffee beans are used for brewing coffee, the levels of 4-MeI can be considered to be significant. During the

roasting process (commonly 210 – 230 °C), the coffee beans composition dramatically changes as a consequence of pyrolysis, caramelization, Maillard and Strecker reactions. The color of the beans is changing from light brown to almost black, depending on cultural and personal preferences; and the characteristic aroma of roasted coffee is formed (Nicoli et al., 1997; Paul, 2009). However, potential carcinogenic compounds may be also formed at this temperature (Tressl et al., 1998). In recent years, several researchers have focused on the determination of 4-MeI and THI contents using liquid chromatography coupled to mass spectrometry (LC-MS). Both analytes were determined in samples in canned coffee purchased from Japanese local shops (Yamaguchi and Masuda, 2011) or in caramel, dark beers and roasted coffees (Klejdus et al., 2006) after solid phase extraction (SPE). Methods of gas chromatography with flame ionization or mass spectrometric detection (GC-FID and GC-MS, respectively) after ion-pair extraction were applied for 4-MeI determination in roasted coffee beans from Brazil and Ivory Coast coffees (Casal et al., 2002) or in ground roasted coffee purchased from Czech local company using HPLC/ESI-MS via supercritical fluid (SFE) extraction (Lojková et al., 2006).

Caffeine (1,3,5-trimethylxanthine) is known as a central nervous system (CNS) stimulant. It is naturally present in leaves, seeds and/or fruits of at least 63 plant species

worldwide, such as coffee and cocoa beans, tea leaves and kola nuts (Barone and Roberts, 1996; Frary et al., 2005). Coffee beans contain between 0.8 and 2.8% caffeine, depending on species and origin. It contributes to 10 – 30% of the bitter taste of coffee brews (Burmester and Eggers, 2010). The caffeine content in *Coffea canephora* (Robusta) is about two times that of *Coffea arabica* (Arabica) (Monica, 1998). It is not significantly changed during coffee roasting (Farah, 2009). However, the amount of caffeine in coffee brewing substantially varies according to the type of product (Arabica, Robusta, or their ratio in blending), the grinding degree, brewing methods and the serving size (Bell et al., 1996; Petracco, 2001). Robusta coffee tends to produce inferior quality and aroma compared to Arabica coffee, but Robusta coffee is cheaper; as a result, more Robusta coffee are mixed into the blending products.

Raw green coffee beans are also rich in the content of polyphenols and phenolic acids. Generally, simple phenolic acids contribute to the acidity, bitterness and astringency of the coffee and act in potential biopharmacological properties (Basnet et al., 1996; Tatefuji et al., 1996). Beside the substances, an important group of polyphenolic compounds called chlorogenic acids (CQAs) is present (Clifford, 2000; Perrone et al., 2008). The levels of CQAs vary from approximately 7.88 to 14.4% dry matter for *Coffea canephora* (Robusta) and approximately 3.4 to 4.8% dry matter for *Coffea arabica* (Ky et al., 2001). Higher amount of CQAs in green coffee tends to produce an undesirable flavour because of negative notes of their products of oxidation and degradation prior to and during roasting. The methods for CQAs determination are predominantly based on HPLC (Monteiro and Farah, 2012; Gloess et al., 2013; Mills et al., 2013).

The purpose of this study was to obtain information on content of 4(5)-methylimidazole (4-MeI), caffeine and chlorogenic acid (3-caffeoylquinic acid, CQA) in selected Vietnamese, Czech and Brazilian ground roasted coffees. Chromatographic methods with UV-VIS DAD and MS detection systems were applied for identification and quantitative determination of studied analytes. Further, the caffeine content was compared to the results of the three simple and rapid UV spectrophotometric methods (standard addition, 1st order derivative and liquid-liquid extraction). Transfer efficiency of caffeine in Vietnamese, Czech and Brazilian coffee samples was also compared and discussed.

MATERIAL AND METHODOLOGY

Chemicals and reagents. Chloroform (CHCl₃; 99%), caffeine standard (>99%), chlorogenic acid (>99.5%), sodium carbonate, methanol (for HPLC, >99.9%), were obtained from Sigma Aldrich (Steinheim, Germany). Formic acid (96.5%) was from LachNer (Neratovice, Czech Republic). De-ionized water was from a Milli-Q purification system (Millipore, Bedford, MO, USA). The Vietnamese ground roasted coffee 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 Nguyễn), Origin (Công ty TNHH một thành viên Tín Nghĩa) and Vinacafe (Công ty cổ phần Vinacafe Biên

Hòa) were purchased at Saigon CO.OP, in HoChiMinh Town, in Vietnam. The Czech ground roasted coffee samples, including Dadák (Dadák Ltd., Valašské Meziříčí, Czech Republic), Jacobs Aroma (Kraft Foods CR Ltd., Czech Republic), Marila Standard 100% Robusta (Mokate Czech Ltd., Czech Republic), Jihlavanka (Tchibo Praha Ltd., Jihlava, Czech Republic), Grande 100% Arabica (Grande Ltd., Poland; imported by Kaufland, Czech Republic), were purchased at local market in Zlín in the Czech Republic. Coffee Caboclo Brasilia (D.E. Cafes do Brasil LTDA., Sao Paulo, Brasil) represented Brazilian coffees.

LC/MS determination of 4(5)-methylimidazole. Stock solution of coffee (2.5 g in 45 mL water of 90°C) was filtered and transferred into 50 mL volumetric flasks. Ansys SPEC 3 mL SXC SPE cartridges (30 mg, Agilent Technologies, Palo Alto, CA, USA) were used for solid phase extraction after conditioning with 1 mL of methanol and 1 mL of water. Sample solution (3 mL) containing 1000 µL of the stock solution of coffee, 1980 µL of water and 20 µL of 0.1 M HCl was transferred on the SPE cartridge, washed with 1 mL of methanol and eluted with 2 mL of methanol acidified with 5 M HCl (3:1). The extract was evaporated to dryness and dissolved in 0.5 mL of water. The solution was directly injected into the LC/MS in aliquots of 10 µL.

Sample analysis was performed under gradient elution on a Zorbax Eclipse XDB C-18 (Agilent Technologies, 4.6 x 150 mm, 5 µm) analytical column using mobile phase consisting of 5 mM NH₄OH (phase A) and acetonitrile (ACN, phase B) with following steps: 0 – 9 min: 2 - 20% ACN, 10 - 13 min: 20 – 2% ACN, 13 – 16 min: 2% ACN). Column temperature was set at 25 °C. Signal was monitored using UV detector at 215 nm. MS spectra were recorded with an Agilent 6224 Accurate-Mass TOF mass spectrometer (Agilent Technologies, Wilmington, DE, USA) calibrated in the range 30 – 1700 *m/z* using an ESI tuning solution (G1969-85000, Agilent Technologies). Dual electro-spray ionization working in a positive mode was chosen. The parameters were set as follows: nitrogen flow 6 L min⁻¹ at 350 °C, nebulizer 40 psig, capillary voltage of 4.5 kV, fragmentor voltage of 40 V and skimmer voltage of 65 V. The MS spectra were recorded from 30 to 500 *m/z* and the chromatogram for 4-MeI determination was extracted for *m/z* 83.06.

HPLC measurements. Samples (0.2 g ±0.001 g) of fine ground roasted coffee and 20 mL of boiling distilled water (100 °C) were extracted under reflux for 15 min. Clear solutions were centrifuged for 15 min at 2500 rpm after cooling to ambient temperature. Supernatant was filtered through a membrane filter (45 µm) and diluted with water (1:5) and 20 µL were injected on a chromatographic column.

A liquid chromatographic system 10 AVP consisting of two LC – 10 ADVP chromatographic pumps, a GT – 154 degasser, a CTO – 10 ASVP column thermostat, and a UV DAD detector SPD – M10AVP was controlled by a SCL – 10A unit with a Class – VP 5.02 software (all from Shimadzu, Tokyo, Japan). An analytical chromatographic column Luna C18 (250 x 3.0 mm, particle size 5 µm) with a Luna C18 Security Guard

column (4 x 2.0 mm, both Phenomenex, Torrance, CA, USA) were for caffeine and chlorogenic acid determination using mobile phase consisting of 3% aqueous formic acid (phase A, pH 2.05) and methanol (phase B) at flow rate 0.5 mL min⁻¹ and under linear gradient elution (0 – 10 min: 0 – 30% B; 10 – 20 min: 30 – 40% B; 20 – 30 min: 40 – 100% B; 35 – 38 min: 100% B; 38 – 41 min: 100 – 0% B and finally up to 50 min at 0% B). Caffeine was detected at 270 nm while chlorogenic acid was determined at 320 nm. Injection volume was 20 µL. Column temperature was set to 25 °C.

Standard addition UV spectrophotometric measurement. Working standard solutions (10.0 – 16.0 µg mL⁻¹) for the preparation of calibration curves were prepared by dilution of caffeine stock solution (100 µg mL⁻¹ in water). UV spectrum was recorded against water over the wavelength range of 190 – 300 nm. The concentration of caffeine in coffee sample was obtained from the calibration curve ($y = 0.0503x + 0.2584$, $r^2 = 0.998$) in which y is absorbance of sample and x is concentration of caffeine in the samples.

First derivative UV spectrophotometric measurement. The coffee solution (0.05 g in 50 mL of boiling water) was filtered and the cold filtrate was diluted to 100 mL by water. The first order derivative absorption spectra of caffeine solutions (6.0, 8.0, 10.0 and 12.0 µg mL⁻¹) were recorded (see Figure 1) against distilled water in range of 190 and 300 nm. Peak-to-peak measurements (Alpdogan et al., 2002) of two neighboring peaks of 260 nm (minima) and 287 nm (maxima) were used for preparation of calibration curve. The concentrations of caffeine were calculated from the regression equation ($y = 0.0483x + 0.0107$, $r^2 = 1$) in which y is peak-to-peak

amplitude of the first order spectra at extreme of each sample and x (µg mL⁻¹) is concentration of caffeine in samples. The % caffeine in coffee samples was obtained by the following formula: (caffeine content/cake mass) x 100, (cake mass: 0.05 g).

Isolation of caffeine from the coffee brews. Na₂CO₃ (0.2 g) was added to 10 ml of the filtrate (coffee solution) to remove non-caffeine solids (Heilmann, 2001). The filtrate was extracted 3 times for 10 minutes with 10 mL CHCl₃ in each run. The combined extracts were diluted by CHCl₃ to 50 mL. The absorbance of the standard solutions (10, 20, and 30 µg mL⁻¹) and the sample solutions were measured at 276.2 nm against CHCl₃ (AOAC method 979.11). The concentrations of caffeine were calculated from the regression equation ($y = 0.0489x$, $r^2 = 0.9999$) in which y is absorbance of the sample and x is concentration of caffeine in samples. The % of caffeine in coffee samples was obtained by the above-mentioned formula.

Statistical evaluation. All results were statistically evaluated using the variation statistics (ANOVA, StatSoft, Prague, Czech Republic). Correlation matrices and regression functions were calculated using the statistical package Unistat, v. 5.5 (Unistat Ltd., England).

RESULTS AND DISCUSSION

LC/MS determination of 4(5)-methylimidazole. The extracted ion chromatogram for 4-MeI (in concentration of 1 µg mL⁻¹) and MS spectrum are shown in the Figure 2. The retention time was 13.5 min. Positive ion ESI mass spectra of the 4-MeI standard yielded intense signals corresponding to [M+H]⁺ (83.0604) and [2M+H]⁺ ions (165.1115). Also, adducts of 4-MeI with acetonitrile from mobile were detected - [M+ACN]⁺ ions (124.0849).

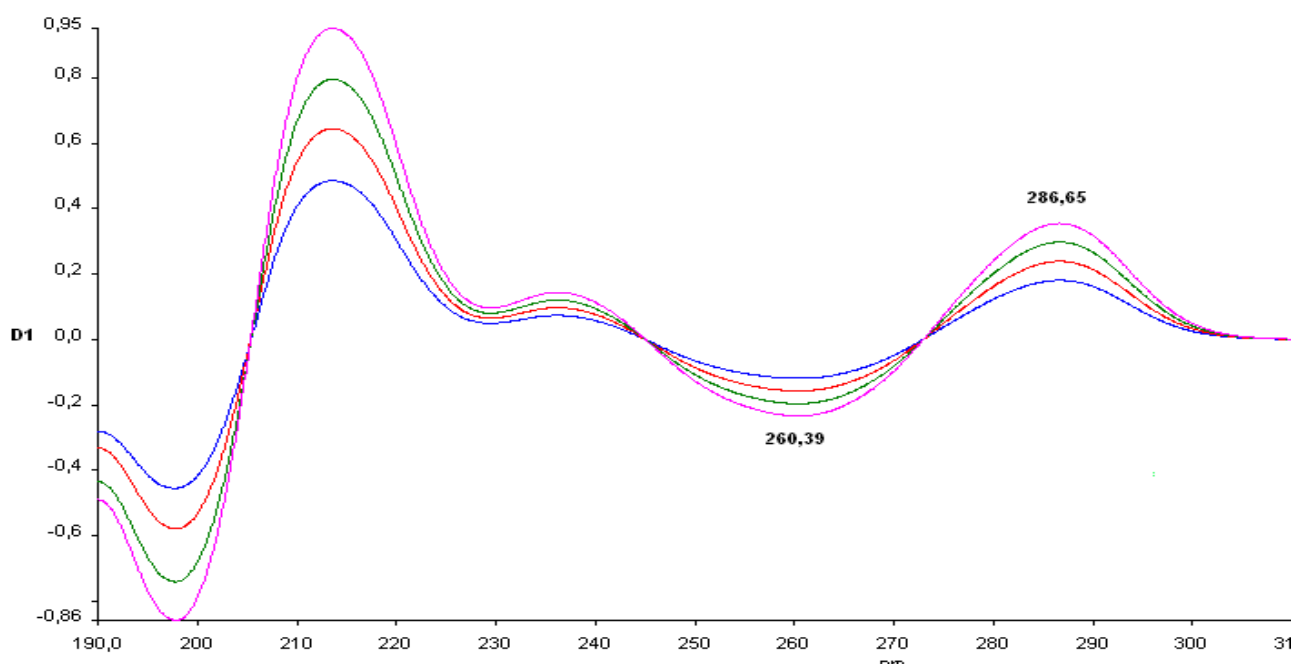


Figure 1 First derivative spectra of caffeine standards (6.0, 8.0, 10.0 and 12.0 µg mL⁻¹), reference: water.

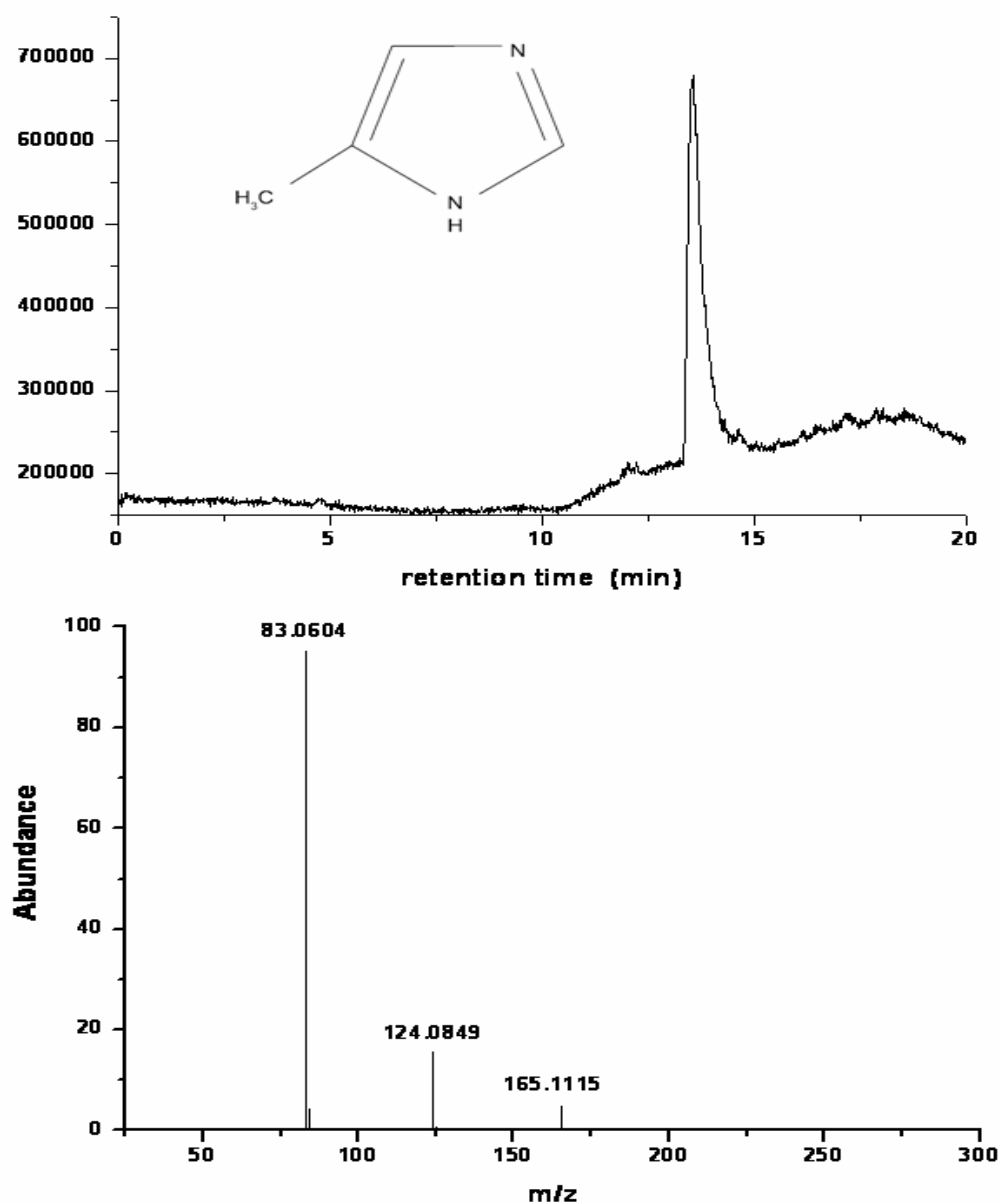


Figure 2 Extracted ion chromatogram (m/z 83.06) and ESI negative spectrum of 4-MeI.

The content of 4-MeI in the coffee samples was quantified using calibration curve ($y = 21210 \cdot 442x + 5098.331$, $r^2 = 0.998$) constructed from the peak area of the extracted ion chromatogram at m/z 83.06. The calibration was linear in the range 5 – 100 $\text{ng} \cdot \text{mL}^{-1}$. The LOD of 2.5 $\text{ng} \cdot \text{mL}^{-1}$ and LOQ of 8.4 $\text{ng} \cdot \text{mL}^{-1}$ were calculated according to the following formulas: $\text{LOD} = 3 \cdot \text{SD}/S$, and $\text{LOQ} = 10 \cdot \text{SD}/S$, where S is the slope of the calibration curve and SD is the standard deviation of the noise. The determined amount of 4-MeI varied in tens of $\mu\text{g} \cdot \text{g}^{-1}$ (13.1 – 58.1 $\mu\text{g} \cdot \text{g}^{-1}$) in the Vietnamese coffees (except of Vinacafe 5.1 $\mu\text{g} \cdot \text{g}^{-1}$) while in units $\mu\text{g} \cdot \text{g}^{-1}$ in Czech coffees (1.8 – 2.4 $\mu\text{g} \cdot \text{g}^{-1}$) and Brazilian coffee

(4.9 $\mu\text{g} \cdot \text{g}^{-1}$). All analyzed Vietnamese samples were characterized by intensive, suave, non-typical coffee aroma and differed from Czech and Brazilian samples in consistency. Therefore, more than ten times higher 4-MeI values observed in Vietnamese coffee samples relate with different ways of roasting or processing and with different composition of blends. Corn and soya, essential oils and even butter are also used for blending because of benefit. In some previous studies, observed values of 4-MeI in roasted coffees were also lower and ranged between 0.31 – 1.24 $\mu\text{g} \cdot \text{g}^{-1}$ (Casal et al., 2002) and 0.39 – 2.05 $\mu\text{g} \cdot \text{g}^{-1}$ (Klejduš et al., 2006).

Table 1 Content (mean ±SD; in µg g⁻¹) of 4(5)-methylimidazole (4-MeI) and content (mean ±SD; in mg 100 g⁻¹) of chlorogenic acid (CQA) in selected samples of Vietnamese, Czech and Brazilian coffees (n = 3).

Analyte	Vietnamese coffees				Czech coffees			Brazilian coffee
	Vina-cafe	Di Linh	Dak Tin	Origin	Nam Nguyen	Marila Standard	Jacobs Aroma	Caboclo Brasilia
	Content (mean±SD; in µg g ⁻¹)							
4-MeI	5.1 ±0.3	58.1 ±1.5	38.7 ±1.8	13.1 ±0.3	29.1 ±0.4	1.8 ±0.1	2.4 ±0.1	4.9 ±0.1
	Content (mean±SD; in mg 100 g ⁻¹)							
CQA	202.4 ±22.1	28.6 ±9.0	2.2 ±0.8	59.9 ±3.7	59.6 ±0.1	683.4 ±46.7	n.d.	n.d.

n.d. - not detected

Table 2 Caffeine content (Cont.; mean ±S.D.; in % w/w; n = 3) in coffee samples and transfer (T; in % w/w; n = 3) into coffee brewing at 100 °C and for 3 minutes.

Sample	Standard addition		Extraction using CHCl ₃		1 st derivative measurement ^a		HPLC/DAD	
	Cont. (%)	T (%)	Cont. (%)	T (%)	Cont. (%)	T (%)	Cont. (%)	T (%)
Dadák	6.51 ±0.06	93 ±2	2.11 ±0.04	97 ±1	2.12 ±0.01	94 ±1	2.13 ±0.02	95 ±3
Jihlavanka	8.03 ±0.07	91 ±1	2.20 ±0.02	96 ±2	2.20 ±0.04	99 ±1	2.19 ±0.03	97 ±2
Grande	9.98 ±0.08	99 ±1	1.98 ±0.01	95 ±1	2.11 ±0.01	100 ±2	2.03 ±0.05	98 ±2
Jacobs Aroma	6.22 ±0.06	97 ±2	1.93 ±0.02	100 ±1	1.83 ±0.00	92 ±1	2.01 ±0.02	96 ±1
Marila Standard	7.04 ±0.10	97 ±2	2.10 ±0.1	99 ±2	2.11 ±0.02	95 ±1	2.13 ±0.01	99 ±1
Dak Tin	2.90 ±0.06	65 ±5	0.31 ±0.01	64 ±4	0.57 ±0.00	76 ±6	0.63 ±0.01	60 ±5
Di Linh	4.10 ±0.16	68 ±6	0.57 ±0.01	70 ±4	1.05 ±0.04	73 ±5	0.68 ±0.05	68 ±5
Nam Nguyen	4.50 ±0.01	64 ±3	1.03 ±0.02	77 ±3	1.40 ±0.01	85 ±5	1.13 ±0.04	75 ±6
Origin	5.55 ±0.11	66 ±4	1.45 ±0.01	90 ±4	1.80 ±0.00	86 ±4	1.43 ±0.03	89 ±7
Vinacafe	6.62 ±0.01	91 ±6	2.10 ±0.06	100 ±2	2.10 ±0.01	98 ±4	2.13 ±0.02	99 ±5

^a measured at 265 and 289 nm for Dadák, Grande and Vinacafe, 268 and 290 nm for Jihlavanka, 266 and 289 nm for Jacobs Aroma, 268 and 289 nm for Marila Standard, 265 and 284 nm for Dak Tin, 264 and 285 nm for Li Linh, 264 and 287 for Nam Nguyen, 263 and 284 nm for Origin

HPLC determination of caffeine and chlorogenic acid. A five points calibration curve ($y = 103858x + 170.42$, $r^2 = 0.9999$) was constructed as a peak area vs. caffeine concentration for 2.5, 5, 10, 25 and 50 µg.mL⁻¹ of caffeine with LOD = 1.56 µg.mL⁻¹ and LOQ = 5.20 µg.mL⁻¹. The calibration curve for chlorogenic acid was constructed similarly with the concentration of 0.5, 1, 2, 10 and 20 µg.mL⁻¹ ($y = 16340x + 1.478$, $r^2 = 0.9999$) with LOD = 0.08 µg.mL⁻¹ and LOQ = 0.25 µg.mL⁻¹. The contents of caffeine and chlorogenic acid (see Tables 1 and Table 2) were lower in Vietnamese coffees in comparison

with Czech coffee Marila Standard. Even in case of Dak Tin sample, the found caffeine content was less than 3 µg.g⁻¹. While the content of caffeine is not so much influenced by roasting time and relates mainly with the coffee cultivars, the level of chlorogenic acid is roasting time dependent and decrease with roasting duration (Farah, 2009). Comparison of 4-MeI, caffeine and chlorogenic acid content is illustrated in Figure 3. Our findings indicate that Vietnamese coffee samples were roasted longer than Czech samples which results in higher content of 4-MeI and lower level of chlorogenic acid.

Comparison of the spectrophotometric methods for caffeine determination. The contents of caffeine

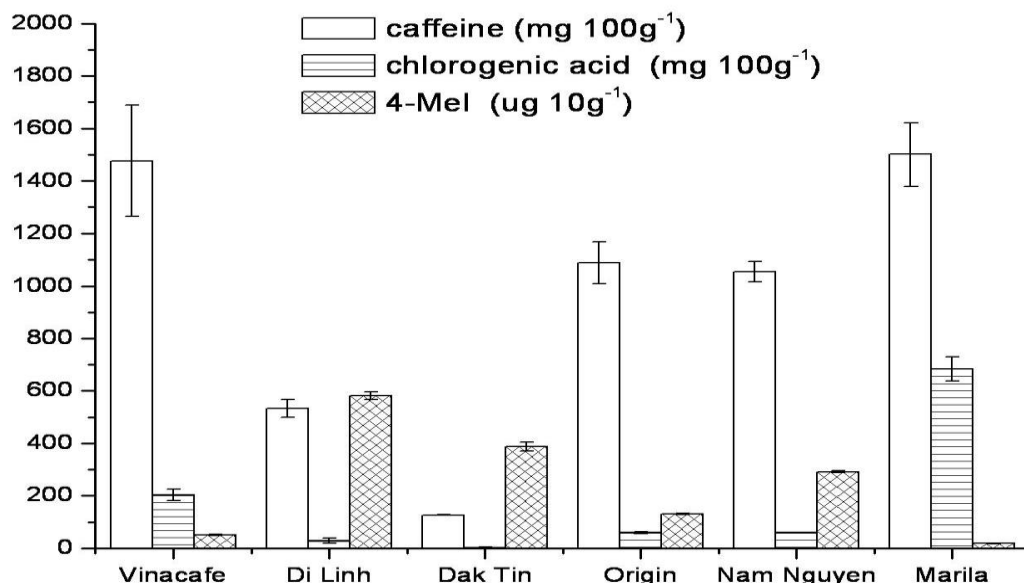


Figure 3 Comparison of caffeine, chlorogenic acid and 4(5)-methylimidazole content in Vietnamese and Czech coffee samples.

determined by liquid chromatographic, 1st derivative standard addition, and extraction spectrophotometric methods were found to be within the documented range (0.31 ± 0.01 and $2.20 \pm 0.04\%$) in all the coffee samples. There were no significant differences among the results in all the Czech coffees. However, there were significant differences in standard addition and other methods in all the Vietnamese samples (1.2 – 1.8 times), except of the Vinacafe sample ($2.10 \pm 0.06\%$ and $2.10 \pm 0.01\%$, respectively). This may be explained by a more significant interference of matrices and different composition of blends in the Vietnamese samples compared to the Czech ones (see above). The caffeine contents of the Czech coffees were higher than those of the Vietnamese coffees in all samples. The caffeine extractability at 100 °C and 3 min of brewing (commonly used time for brewing coffee) almost reached 100% in Czech coffees, while it was less than 90% in Vietnamese coffees (see Table 2). The particle size of coffee powders is probably major cause of the differences in caffeine extractability between Czech and Vietnamese coffee brands. Previous studies have also shown that caffeine content in coffee was influenced by grinding techniques (Bell et al., 1996). The results showed that the caffeine contents of Grande (100% Arabica) and Marila Standard (100% Robusta) were $1.98 \pm 0.01\%$ and $2.10 \pm 0.1\%$, respectively. However, they did not agree with the previous results published, 1.1 – 1.3% for Arabica and 2.4 – 2.5% for Robusta (Belitz et al., 2009). This may be due to deception in blending commercial ground roasted coffee.

CONCLUSION

The determination of 4(5)-methylimidazole, chlorogenic acid and caffeine content in Czech, Vietnamese and Brazilian ground roasted coffee brands illustrate significant differences in the amount of the analytes. They also indicate variant procedures in roasting and coffee processing. The contents of caffeine in five Czech and five Vietnamese coffees were also compared to the results of the 1st derivative, standard addition and extraction spectrophotometry applying CHCl_3 as an extraction solvent. The Czech coffee tends to produce more caffeine than the Vietnamese coffee because of the different composition of blends and the particle size degree. All methods can be successfully used for determination of caffeine. However, there were significant differences in the results among the standard addition and the other methods in the case of the Vietnamese coffee brands. The first derivative UV spectrophotometry gives 1.2 – 1.8 times higher results compare to the extraction spectrophotometric method.

REFERENCES

- Alpdogan, G., Karabina, K., Sungur, S. 2002. Derivative spectrophotometer determination of caffeine in some beverages. *Turkish Journal of Chemistry*, vol. 26, no. 2, p. 295-302. [cit. 2014-10-16] Available at: <http://dergipark.ulakbim.gov.tr/tbtkchem/article/download/500028831/5000029068>
- Barone, J. J., Roberts, H. 1996. Caffeine consumption. *Food Chemistry and Toxicology*, vol. 34, no. 1, p. 119-129. [http://dx.doi.org/10.1016/0278-6915\(95\)00093-3](http://dx.doi.org/10.1016/0278-6915(95)00093-3)

- Basnet, P., Matsushige, K., Hase, K., Kadota, S., Namba, T. 1996. Potent antihepatotoxic activity of dicaffeoyl quinic acids from propolis. *Biological and Pharmacological Bulletin*, vol. 19, no. 4, p. 655-657. [PMid:9132180](#)
- Belitz, H. D., Grosch, W., Schieberle, P. 2009. *Food Chemistry, Coffee, tea, cocoa*. p. 938-970. 4th ed. Springer Berlin Heidelberg, 1070 p. Online ISBN 978-3-540-69934-7 <http://dx.doi.org/10.1007/978-3-540-69934-7>
- Bell, L. N., Wetzel, C. R., Grand, A. N. 1996. Caffeine content in coffee as influenced by grinding and brewing techniques, *Food Research International*, vol. 29, no. 8, p. 785-789. [http://dx.doi.org/10.1016/S0963-9969\(97\)00002-1](http://dx.doi.org/10.1016/S0963-9969(97)00002-1)
- Bradbury, M. G., Doherty, K. V., Parish, C. R., Lyons, A. B. 1996. The immunosuppressive compound 2-acetyl-4-tetrahydrobutyl imidazole inhibits the allogeneic mixed lymphocyte reaction by sequestration of a recirculating subpopulation of T-cells. *Immunology*, vol. 87, no. 1, p. 80-85. [PMid:8666439](#)
- Burmeister, K., Eggers, R. 2010. Heat and mass transfer during the coffee drying process. *Journal of Food Engineering*, vol. 99, no. 4, p. 30-436. <http://dx.doi.org/10.1016/j.jfoodeng.2009.12.021>
- Casal, S., Fernandes, J. O., Oliveira, M. B. P. P., Ferreira, M. A. 2002. Gas chromatographic-mass spectrometric quantification of 4(5)-methylimidazole in roasted coffee after ion-pair extraction. *Journal of Chromatography A*, vol. 976, no. 1-2, p. 285-291. [http://dx.doi.org/10.1016/S0021-9673\(02\)01154-8](http://dx.doi.org/10.1016/S0021-9673(02)01154-8) [PMid:12462620](#)
- Chan, P. C., Hills, G. D., Kissling, G. E., Nyska, A. 2008. Toxicity and carcinogenicity studies of 4-methylimidazole in F344/N rats and B6C13F1 mice. *Archives of Toxicology*, vol. 82, no. 1, p. 45-53. <http://dx.doi.org/10.1007/s00204-007-0222-5> [PMid:17619857](#)
- Clifford, M. N. 2000. Chlorogenic acids and other cinnamates - nature, occurrence, dietary burden, absorption and metabolism. *Journal of Science and Food Agriculture*, vol. 80, no. 7, p. 1033-1043. [http://dx.doi.org/10.1002/\(SICI\)1097-0010\(20000515\)80:7<1033::AID-JSFA595>3.3.CO;2-K](http://dx.doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1033::AID-JSFA595>3.3.CO;2-K)
- Cunha, S. C., Barrado, A. I., Faria, M. A., Fernandes, J. O. 2011. Assessment of 4(5)-methylimidazole in soft drinks and dark beer. *Journal of Food Composition and Analysis*, vol. 24, no. 4-5, p. 609-614. <http://dx.doi.org/10.1016/j.jfca.2010.08.009>
- Farah, A. 2009 *Coffee as speciality and functional beverage*. Paquin, P. Functional Specialty Beverage Technology and Nutrition, vol. 168, p. 370-395.
- 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. 105, no. 1, p. 110-113. <http://dx.doi.org/10.1016/j.jada.2004.10.027> [PMid:15635355](#)
- Gloess, A. N., Schoenbaechler, B., Klopprogge, B., D'Ambrosio, L., Chatelain, K., Bongartz, A., Strittmatter, A., Rast, M., Yeretzian, C. 2013. Comparison of nine common coffee extraction methods: instrumental and sensory analysis. *European Food Research and Technology*, vol. 236, no. 4, p. 607-627. <http://dx.doi.org/10.1007/s00217-013-1917-x>
- Gobin, S. P. J., Phillips, J. A. 1991. Immunosuppressive effects of 2-acetyl-4-tetrahydrobutyl imidazole (THI) in the rat. *Clinical and Experimental Immunology*, vol. 85, no. 2, p. 335-340. [PMid:1864015](#)
- Hargreaves, M. B., Jones, B. C., Smith, D. A., Gescher, A. 1994. Inhibition of p-nitrophenol hydroxylase in rat liver microsomes by small aromatic and heterocyclic molecules. *Drug Metabolism and Disposition*, vol. 22, no. 5, p. 806-810. [PMid:7835233](#)
- Heilmann, W. 2001 Coffee Recent Development. Technology IV: Decaffeination of Coffee: Brewing Trends for the New Millennium, vol. 5, p. 108-124.
- Hodge, J. E. 1967. Origin of flavor in foods nonenzymatic browning reactions. In the Chemistry and Physiology of Flavors; Schultz, H. W. Day, E. A. Libbey, L. M. Eds.; Avi Publishing: Westport, CT, vol. 5, no. 11, p. 472. WOS:A1967A190200018
- IARC International Agency for Research on Cancer 2011. Agents classified by the IARC Monographs, 1 102. [cit. 2014-10-17], Available at: <http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf>
- Klejduš, B., Moravcová, J., Lojtková, L., Vacek, J., Kubáň, V. 2006. Solid-phase extraction of 4(5)-methylimidazole (4MeI) and 2-acetyl-4(5)-(1,2,3,4-tetrahydrobutyl)-imidazole (THI) from foods and beverages with subsequent liquid chromatographic-electrospray mass spectrometric quantification. *Journal of Separation Sciences*, vol. 29, no. 3, p. 378-384. <http://dx.doi.org/10.1002/jssc.200500421>
- Kort, M. J. 1971. Reactions of free sugars with aqueous ammonia. *Advances in Carbohydrate Chemistry Biochemistry* vol. 25, p. 311-349. [http://dx.doi.org/10.1016/S0065-2318\(08\)60431-X](http://dx.doi.org/10.1016/S0065-2318(08)60431-X)
- Ky, C. L., Louarn, J., Dussert, S., Guyot, B., Hamon, S., Noiro, M. 2001. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L. and *C. canephora* P. accessions. *Food Chemistry*, vol. 75, no. 2, p. 223-230. [http://dx.doi.org/10.1016/S0308-8146\(01\)00204-7](http://dx.doi.org/10.1016/S0308-8146(01)00204-7)
- Lojtková, L., Klejduš, B., Moravcová, J., Kubáň, V. 2006. Supercritical fluid extraction (SFE) of 4(5)-methylimidazole (4-MeI) and 2-acetyl-4(5)-(1,2,3,4-tetrahydrobutyl)-imidazole (THI) from ground-coffee with high-performance liquid chromatographic-electrospray mass spectrometric quantification (HPLC/ESI-MS). *Food Additives and Contaminants*, vol. 23, no. 10, p. 963-973. [PMid:16982517](#)
- Mills, C. E., Oruna-Concha, M. J., Mottram, D. S., Gibbon, G. R., Spenser, J. P. E. 2013. The effect of processing on chlorogenic acid content of commercially available coffee. *Food Chemistry*, vol. 141, no. 4, p. 3335-3340. <http://dx.doi.org/10.1016/j.foodchem.2013.06.014>
- Monica, A. S. 1998 The chemical components of coffee. Caffeine, (Ed.) *GENE*, A. S., vol. 6, p. 146-147.
- Monteiro, M. C., Farah, A. 2012. Chlorogenic acids in Brazilian *Coffea arabica* cultivars from various consecutive crops. *Food Chemistry*, vol. 134, no. 1, p. 611-614. <http://dx.doi.org/10.1016/j.foodchem.2012.02.118>
- Nicoli, M. C., Anese, M., Manzocco, L., Leric, C. R. 1997. Antioxidant properties of coffee brews in relation to the roasting degree. *LWT-Food Science and Technology*, vol. 30, no. 3, p. 292-297. <http://dx.doi.org/10.1006/fstl.1996.0181>
- NTP National Toxicology Program. 2007. Toxicology and carcinogenesis studies of 4-methylimidazole (CAS No. 822-36-6) in F344/N rats and B6C3F1 mice (Fze studies); *NTP Technical Report Series*, vol. 535, p. 1-274 [PMid:17342198](#)
- OEHHA Office of Environmental Health Assessment. 2010. Notice of intent to list 4-methylimidazole, [cit. 2014-11-17] Available at: http://oehha.ca.gov/prop65/cmrn_notices/admin_listing/intent_to_list/noilpkg32.html
- Perrone, D., Farah, A., Donangelo, C. M., De Paulis, T., Martin, P. R. 2008. Comprehensive analysis of major and

minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars. *Food Chemistry*, vol. 106, no. 2, p. 859-867.

<http://dx.doi.org/10.1016/j.foodchem.2007.06.053>

Petracco, M. 2001. *Coffee Recent Development. Technology IV: Beverage Preparation: Brewing Trends for the New Millennium*, vol. 7, p. 140-151.

<http://dx.doi.org/10.1002/9780470690499.ch7>

Tatefuji, T., Izumi, N., Ohta, T., Arai, S., Ikeda, M., Kurimoto, M. 1996. Isolation and identification of compounds from Brazilian propolis which enhance macrophage spreading and mobility. *Biological and Pharmacological Bulletin*, vol. 19, no. 7, p. 966-970. PMID:8839971

Tomasik, P., Palasinski, M., Wijek, S. 1989 The Thermal decomposition of carbohydrates. Part I. The decomposition of mono-, di-, and oligo-saccharides. *Advances in Carbohydrate Chemistry and Biochemistry*, vol. 47, p. 203-278.

[http://dx.doi.org/10.1016/S0065-2318\(08\)60415-1](http://dx.doi.org/10.1016/S0065-2318(08)60415-1)

Tressl R., Wondrak G. T., Garbe L. A., Rewicki D., Kruger R. P. 1998, Pentoses and hexoses as sources of new melanoidin-like Maillard polymers. *Journal of Agriculture and Food Chemistry*, vol. 46, no. 5, p. 1765-1776. <http://dx.doi.org/10.1021/jf970973r>

Yamaguchi, H., Masuda, T. 2011. Determination of 4(5)-methylimidazole in soy sauce and other foods by LC-MS/MS after solid-phase extraction. *Journal of Agriculture and Food Chemistry*, vol. 59, no. 18, p. 9770-9775. <http://dx.doi.org/10.1021/jf201736c>

Acknowledgment:

This research was kindly supported by the internal grant of TBU in Zlín Reg. No. IGA/FT/2015/004 funded from the resources of specific university research.

Contact address:

Thi Thanh Dieu Phan, Tomas Bata University in Zlín, Faculty of Technology, Department of Food Technology, Náměstí T. G. Masaryka 275, 762 72 Zlín, Czech Republic, corresponding author, E-mail: phan_dieu@yahoo.com

Miroslava Bittová, Masaryk University, Faculty of Science, Department of Chemistry, Kotlářská 2, 611 37 Brno, Czech Republic, E-mail: bittova@sci.muni.cz.

Kamil Mikulášek, Masaryk University, Faculty of Science, Department of Chemistry, Kotlářská 2, CZ-611 37, Brno, Czech Republic, E-mail: 357352@mail.muni.cz

Vlastimil Kubáň, Tomas Bata University in Zlín, Faculty of Technology, Department of Food Technology, Náměstí T. G. Masaryka 275, 762 72 Zlín, Czech Republic, corresponding author, E-mail: kuban@ft.utb.cz.

Stanislav Kráčmar, Tomas Bata University in Zlín, Faculty of Technology, Department of Food Technology, Náměstí T. G. Masaryka 275, 762 72 Zlín, Czech Republic, E-mail: kracmar@ft.utb.cz.

Pavel Valášek, Tomas Bata University in Zlín, Faculty of Technology, Department of Food Analysis and Chemistry, Náměstí T. G. Masaryka 275, 762 72 Zlín, Czech Republic, E-mail: valasek@ft.utb.cz.

Blanka Svobodová, Tomas Bata University in Zlín, Faculty of Technology, Department of Food Technology, Náměstí T. G. Masaryka 275, 762 72 Zlín, Czech Republic, E-mail: SvobodkaB@seznam.cz.