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Research Article
Determination of Caffeine Content in Robusta Roasted Coffee (*Coffea canephora*) by RP-UHPLC-PDA

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Abstract

Background and Objective: *Coffea canephora* (named Robusta coffee) represent around the 30% of the production worldwide of coffee. This specie is used as coffee beverage. Their content of caffeine is important in their quality. The aim of this study was to determine the caffeine content in robusta coffee (*Coffea canephora*) variety obtained from 8 cultivars (Piedra Grande, Sabanetillas, Pitiamby, Industria Tablas de Florida, Yatuví, Pueblo Nuevo and Paraiso) of 2 Cantons (Caluma and Echeandía) of the Bolívar province from Ecuador. **Materials and Methods:** Caffeine content of robusta coffee (*Coffea canephora*) from Ecuador was calculated using the reverse phase ultra-high-performance liquid chromatography RP-UHPLC-PDA with the help of caffeine standard using a C18 column and lineal gradient of 0-70% B for 12 min with an absorbance of 274 nm (solvent B: Acetonitrile and solvent A: Water). Caffeine content of robusta coffee from Ecuador was also evaluated with the extraction soxhlet method. **Results:** It was found that three of these Ethiopian plants (designated here as UEB-F, UEB-Y and UEB-P in honor to their localization) had an almost low caffeine content as the *Coffea canephora* content. This *Coffea canephora* plant was detected during the analysis of ultra- high-performance liquid chromatography (UHPLC-PDA) of chloroform extracts of seeds. **Conclusion:** Robusta coffee (*Coffea canephora*) cultivated in the Echeandía canton present an extremely low value of caffeine content. These values of caffeine allow this coffee variety to be used to produce decaffeinated coffee. Caluma canton coffee presents caffeine content according to the robusta coffee variety.

Key words: *Coffea arabica*, *Coffea canephora*, caffeine, robusta coffee, Soxhlet method

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus *Coffea* L. has more than 100 species but only around 25 species are used to produce coffea fruits with commercial value in the world for the food industry. Only 4 species of coffea are used by the food industry to produce the coffee used for the preparation of the most popular beverage in the world after tea infusion[1,2].

Coffee fruit is the more important agricultural crop export around in the world. *Coffea arabica* (named Arabica coffee) represents 70% of the world coffee production and the remainder consists mainly of *Coffea canephora* (named robusta coffee). These 2 species are the more used in the elaboration of coffee beverage[3,4] Coffea species grow in tropical and subtropical areas, especially in the Equatorial region at an altitude of 200-1200 m and at 18-22°C. The range from 1300-1600 m has been identified as the optimum altitude for wild coffee[5,6]. The chemical composition of green coffee is characterized by the presence of caffeine that can range from 1.45-2.38% in *Coffea arabica* and in *Coffea canephora*, respectively[7]. Caffeine content in roasted coffee is much higher in *Coffea canephora* (1.7-4.0%) than in *Coffea arabica* (0.8-1.4%). Caffeine (1,3,7-trimethylxanthine) is a naturally occurring alkaloid which represents a mild stimulant for the central nervous system, muscle, heart and circular systems of the human body. Due to its effects, caffeine may improve alertness, learning capacity and exercise performance when moderately consumed[8-10]. Some studies have focused on the application of different analytical techniques able to monitor the different compounds of the food matrixes, among them the coffee matrix. Infrared spectroscopy[11-13] and GC-MS[14,15] have been applied with the view of possible species differentiation of coffee, hyperspectral imaging technique[16], ESI-MS[17], HPLC, HPLC-MS and UHPLC[18,19], electrophoresis, capillary electrophoresis high-performance thin layer chromatography (HPTLC)[20,21] and supercritical fluids[22] have been applied to study the content of caffeine in coffee and coffee drinks. Caffeine content was calculated using the RP-UHPLC analysis. It allows characterizing the composition of the crops of the Andean region of Ecuador knowing its caffeine content allows establishing its possible uses in the food industry either for beverage preparation or other purposes. The aim of this study was to determine the content of caffeine in robusta coffee cultivated in different regions of Ecuador using two methods of extraction liquid-liquid extraction and soxhlet methods.

MATERIALS AND METHODS

Study area: The preparation of samples was conducted in the laboratory of the Bolívar State University (Guaranda-Ecuador). The analysis of caffeine by RP-UHPLC was conducted in the laboratory of Functional Foods, Faculty of Foods Science and Engineering, Technical University of Ambato (Ambato-Ecuador). The assays were conducted in 2016 from May to October.

Sample preparation: Coffee bean samples of 8 different cultivars were collected from 2 major productions in 2 cantons of Andean region. Canton Caluma 79°13'55.56 S,1°39'21.6 W in 4 recintos (Piedra Grande, Sabanetillas, Pitiamby and Industria) in the canton Echeandia region 79°17'5.628 S, 1°26'30.492 W in four recintos (Tablas de Florida, Yatuví, Pueblo Nuevo and Paraiso) in the province of Bolívar of Ecuador including *Coffea* robusta. All coffee beans samples were ground and roasted and 20 randomly selected coffee beans of each cultivar were used as one sample for caffeine content measurement using the RP-UHPLC method. An accurately weighed amount of coffee 300 mg, was dissolved in 200 mL of distilled water. The solution was stirred for 1 h using a magnetic stirrer and heated gently to remove caffeine easily from the solution. In addition, the solution was filtered using a paper filter (Whatman N°40) to get rid of particles from the solution.

Liquid-liquid extraction of caffeine: Liquid-liquid extraction with chloroform was used to extract caffeine of coffee beans. The coffee solution prepared above (under coffee sample preparation) was mixed with dichloromethane by a volume ratio (30:30 mL) for the extraction of caffeine from coffee. First, a mixture of the solution was stirred for 10 min. The extraction of caffeine was performed 4 times with 30 mL chloroform at each round[23].

Soxhlet extraction of caffeine: For comparison purposes extract of coffee grounds were firstly obtained by Soxhlet. An amount of 300 mg of robusta coffee was placed inside the Soxhlet apparatus and treated with 300 mL of analytical grade chloroform for 4 h. At the end of the process, the solvent was evaporated to dryness and the extract was weighed. The content of caffeine was calculated with the help of a standard of caffeine. The extraction was performed on a triplicate basis[24].

Analysis of caffeine by Liquid chromatography: Extract of coffee *Coffea canephora* were analyzed using RP-UHPLC on Agilent 1200 infinity series UHPLC System (Agilent Technologies, Waldbronn, Germany). The variable wavelength detector was 274 nm. The column used was EC C18 (Agilent Poroshell 120, 4.6×50 mm×2.7 µm of particle size). Samples were eluted at 1.0 mL min^{-1} with a linear gradient from 0-70% of solvent B (acetonitrile, CAN) in solvent A (Milli-Q water) for 10 min. The injection volume was 10 µL for each duplicated sample[25]. Caffeine concentration was calculated with the help of a standard curve, standard caffeine (Sigma-Aldrich, St Louis, USA). All samples were analyzed in duplicate in 2 independent runs.

Standard solutions of caffeine were prepared in methanol at the concentration of 1 mg mL^{-1} and diluted (1:10, v/v) with a mobile phase. Standard stock solutions of caffeine for quantitative analysis were prepared separately in methanol at the concentration of 1 mg mL^{-1} and stored at 4°C. Working solutions of each standard at 5, 10, 20 and 50 µg mL^{-1} were prepared by diluting the stock solutions with the mobile phase A:B (95:5, v/v). Calibration samples were prepared in triplicate and analyzed in duplicate in two independent runs. Peak integration was performed at $\lambda = 274$ nm for caffeine. Calibration curves of caffeine was calculated with equal weighted least squares linear regression analysis of peak area against standard nominal concentration ($R^2 = 0.9995$). The content of caffeine is the mean of 2 samples[26].

RESULTS

Robusta coffee from 8 cultivars of the 2 cantons (Caluma and Echeandía) of Bolívar province in Ecuador was used in this study to determine their caffeine content using the RP-UHPLC method and the extraction with the soxhlet method. The calibration curve of caffeine was calculated with a standard of caffeine (Fig. 1a-d). Good calibration was obtained with the equation of lineal regression (Y = 16.593*x-1.2848) with a correlation coefficient of $R^2 = 0.9995$. With the help of standard caffeine, it was possible to observe the retention time of caffeine with an average value of 0.575 min in the conditions of the study and the concentration of caffeine ranged from 3.99-31.92 ppm (Table 1).

In the RP-UHPLC analysis, the retention time of caffeine of the 8 samples of robusta coffee presented a value in average of 0.575 min of retention time at 274 nm of absorbance (Fig. 2a-d, 3a-d). The software of this instrument identified the peaks of caffeine. The content of caffeine was low in the 4 cultivars of the Echeandía region with values

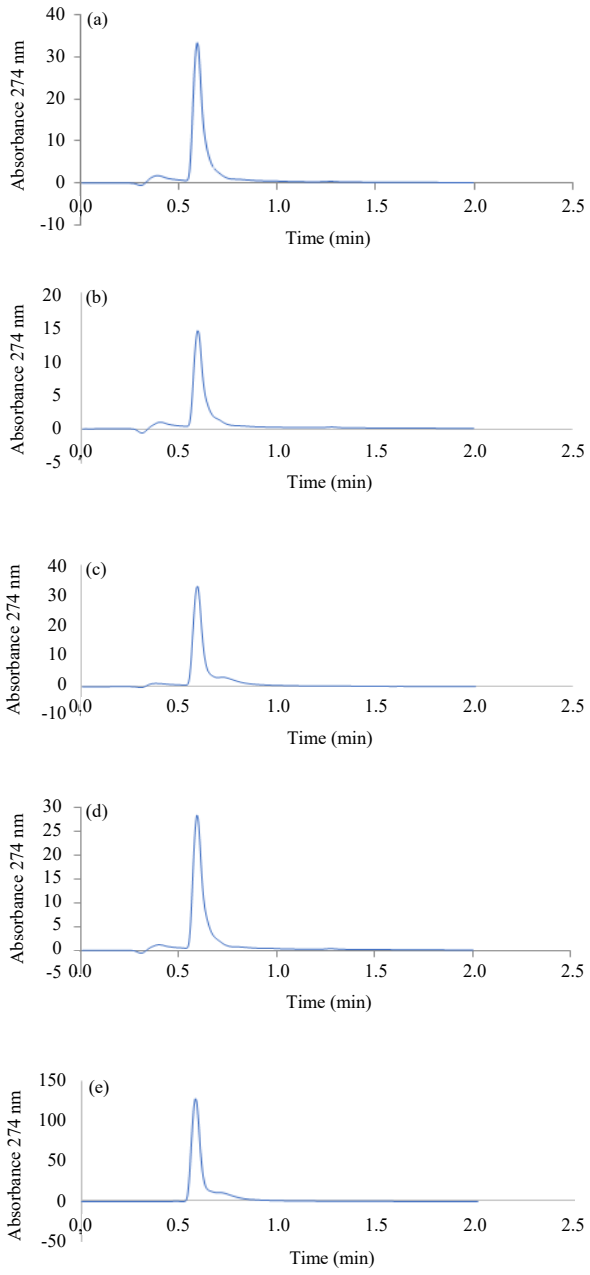

Fig. 1(a-e): Standard of caffeine analyzed by RP-UHPLC-PDA, (a) standard at 5 µg mL^{-1}, (b) Standard at 10 µg mL^{-1}, (c) Standard at 20 µg mL^{-1} and (d) Standard at 50 µg mL^{-1}

Table 1: Concentration of caffeine calculated with standard caffeine

Samples	Concentration (ppm)	Area	Retention time
Standard 1	3.99±0.02[a]	65.5±0.02[a]	0.577
Standard 2	7.98±0.02[b]	135.2±0.02[b]	0.576
Standard 3	15.96±0.03[c]	256.7±0.03[c]	0.576
Standard 4	23.94±0.015[d]	399.3±0.015[d]	0.575
Standard 5	31.92±0.010[e]	530.7±0.010[e]	0.575

Different letters show statistical difference between the groups ($p<0.05$) ANOVA and Tukey's test

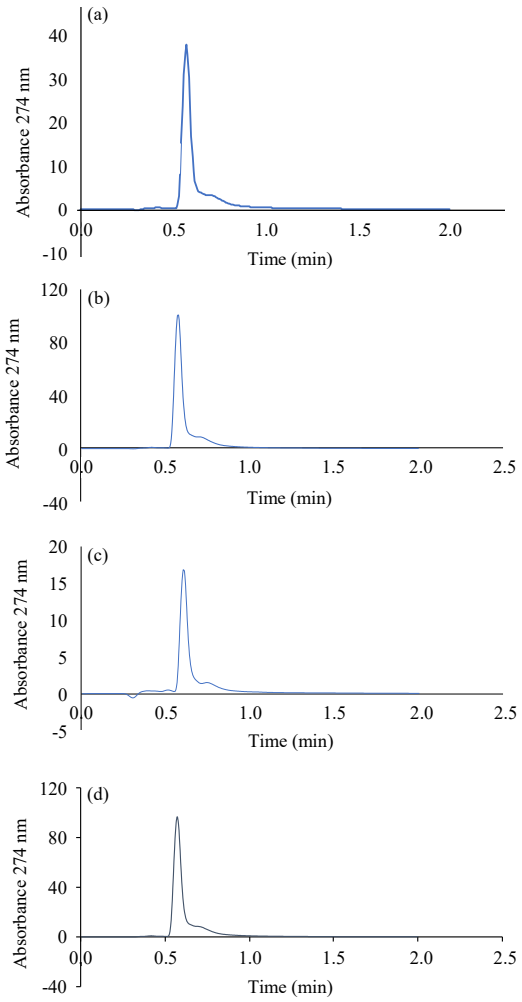

Fig. 2(a-d): Analysis of robusta coffee samples from Caluma canton of Bolívar Province from Ecuador by RP-UHPLC-PDA, (a) Piedra Grande, (b) Sabanetillas, (c) Tablas de Florida and (d) Pitiamby

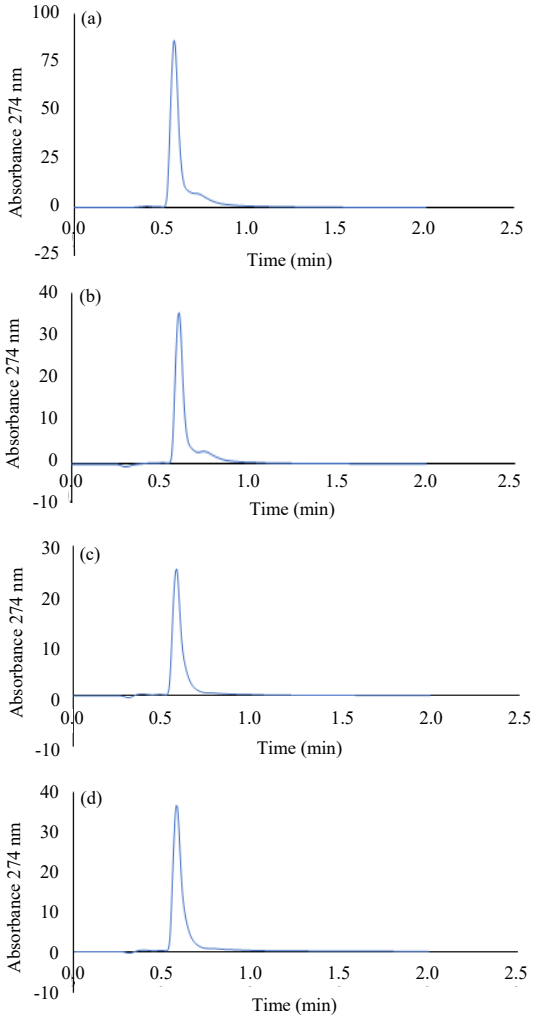

Fig. 3(a-d): Analysis of robusta coffee samples from Echeandía canton of Bolívar Province from Ecuador by RP-UHPLC-PDA, (a) Industria, (b) Yatuví, (c) Pueblo Nuevo and (d) Paraíso

Table 2: Content and percentage of caffeine of robusta coffee by RP-UHPLC-PDA

Cantón	Recintos	Caffeine (mg L^{-1})	Caffeine (%)
Caluma	Piedra Grande	10.312±0.001[a]	0.859±0.001[a]
	Sabanetillas	26.193±0.002[b]	2.182±0.002[b]
	Pitiamby	25.234±0.001[c]	2.100±0.001[c]
	Industria	22.788±0.040[d]	1.899±0.040[d]
Echeandía	Tablas de la Florida	5.341±0.025[a]	0.445±0.025[a]
	Yatuví	7.763±0.035[b]	0.646±0.035[b]
	Pueblo Nuevo	6.998±0.011[c]	0.583±0.011[c]
	Paraíso	10.083±0.023[d]	0.840±0.023[d]

Different letters show statistical difference between the groups ($p<0.05$) ANOVA and Tukey's test

ranging between 0.445-0.840% of caffeine content. On the other hand, the cultivars (Industria, Yatuví, Pueblo Nuevo and Paraiso) of Caluma region presented a higher value ranging between 0.859-2.182% of caffeine content (Table 2). When using the extraction Soxhlet method, the content of caffeine was also low, in the Echeandía region, with values ranging between 0.625-1.375% of caffeine content. The Caluma region presented the highest value with a range of 1.033-2.150% of caffeine content (Table 3).

Table 3: Content of caffeine and percentage of robusta coffee using Soxhlet method

Region	Canton	Recintos	Caffeine (mg L^{-1})	Caffeine (%)
Andean	Caluma	Piedra Grande	25.800±0.49[a]	2.150±0.49[a]
		Sabanetillas	22.900±0.14[b]	1.908±0.14[b]
		Pitiamby	24.400±0.25[c]	2.033±0.25[c]
		Industria	12.400±0.160[d]	1.033±0.160[d]
	Echeandía	Tablas de la Florida	16.500±0.32[a]	1.375±0.32[a]
		Yatuví	7.500±0.04[b]	0.625±0.04[b]
		Pueblo Nuevo	9.200±0.451[c]	0.767±0.451[c]
		Paraíso	12.400±0.362[d]	1.033±0.362[d]

Different letters show statistical difference between the groups ($p<0.05$) ANOVA and Tukey's test

DISCUSSION

The analysis of caffeine content of two cantons Caluma and Echeandía present values with a range of 0.445-2.182% using the UHPLC method. Values obtained with the Soxhlet method were of 0.625-2.150%, standard deviation value were higher than the ones using the UHPLC method. This indicates that the precision and exactitude of the UHPLC method is more accurate. Robusta coffee from Ecuador present a low caffeine content. Arabica coffee is generally the more appreciated in the markers for their organoleptic properties than robusta coffee. Arabica plants are delicate and are grown at elevations between 600-2000 m. Robusta plants are more hardy, higher yielding and can be grown at lower altitudes[2,27], 200-800 m. Robusta coffee in this study was cultivated in an altitude between 1400-1800 m a.s.l. Concentration of phytonutrients in the plants depend of soil, water and nutrients. These phytonutrients can vary depending on the agronomic and environmental conditions of the cultivar (genetic factors, altitude, temperature, hydric conditions, fertilization and maturation of beans). It is known that parameters as altitude and temperature can affect the caffeine content[28-30]. *Coffea arabica* present a caffeine content of around 0.8-1.4% and *Coffea canephora* present a value around 1.7-4.0% of caffeine content. Campa *et al.*[31] reported analysis of caffeine content from green beans of 21 species of coffee from Africa with values between 0.01-2.6%. Coffea canephora was reported with a caffeine content of 2.6% in green beans of coffee. Hecimovic *et al.*[32] have described the analysis of caffeine content of *Coffee arabica* and *Coffea canephora*. They analyzed four coffee varieties named Minas and Cioccolatato (*Coffea arabica*) and Cherry and Vietnam (*Coffea canephora* syn. Coffea robusta). They compared the HPLC method against the chloroform method. They found higher values using the HPLC method (0.66-2.55%) and (0.56-1.43%) using the chloroform method. In this study, the values of caffeine content reported using the HPLC method were higher than the values obtained with the soxhlet method. In this study, we report coffee canephora (robusta variety) caffeine content with a value of 0.445% in the Tablas de Florida zone, in the canton of Echeandía and 0.583% in the zone of Pueblo Nuevo. This low value of caffeine content allows the use of robusta coffee in the production of decaffeinated coffee in Ecuador, as norms indicate a content lower of 1.0% of caffeine. All samples from Echenadía region present a low caffeine content. The value measured using the RP-UHPLC and soxhlet methods were low in the Echeandía canton, the values measured with the RP-UHPLC method were even lower. This last chromatographic method is very exact for their automatization and the use of standard reactive with a calibration curve. Robusta coffee from Ecuador can be used to elaboration of beverage decaffeinated coffee.

CONCLUSION

Robusta coffee (*Coffea canephora*) grown in Ecuador in the cantons of Caluma and Echeandía present low percentages of caffeine in roasted coffee. Two analytical methods were used to determine the caffeine content from 8 locations in the 2 cantons. The best results were the ones obtained with the UHPLC method due to its high reproducibility and low differences between the results. Robusta roasted coffee had a low concentration of caffeine that suggests that this species could be used to make decaffeinated or low-caffeinated beverages. In the future, sensory analysis work could be carried out to determine the consumer acceptance.

SIGNIFICANT STATEMENTS

This study describes for the first time in the literature the analysis of the caffeine content in beans of robusta coffee (*Coffea canephora*) by RP-UHPLC-PDA cultivated in Ecuador. The study analyses 3 different geographical levels (region, province and canton), for a total of up to 8 farms. The characterization of the phytonutrients of a food allows the evaluation of its crop. This study helps the researchers to uncover the solution for higher rate growth of robusta canephora species in Ecuador, also to be more appreciated for food purposes, for instance, the preparation of naturally decaffeinated coffee drinks. It is mentionable that this kind of work has not been done before. The samples are very difficult to obtain with the different conditions here studied (geographical origin, season and special varieties).

REFERENCES

1. Cagliani, L.R., G. Pellegrino, G. Giugno and R. Consonni, 2013. Quantification of *Coffea arabica* and *Coffea canephora* var. robusta in roasted and ground coffee blends. Talanta, 106: 169-173.
2. Burns, D.T., L. Tweed and M.J. Walker, 2017. Ground roast coffee: Review of analytical strategies to estimate geographic origin, species authenticity and adulteration by dilution. Food Anal. Methods, 10: 2302-2310.
3. Bhaktha, G., M. Shantaram and S. Nayak, 2016. Beneficial effects of coffee and maintenance of uric acid levels. Int. J. Pharm. Pharmaceut. Sci., 8: 393-395.

4. De Carvalho, K., C.L.O. Petkowicz, G.T. Nagashima, J.C.B. Filho, L.G.E. Vieira, L.F.P. Pereira and D.S. Domingues, 2014. Homeologous genes involved in mannitol synthesis reveal unequal contributions in response to abiotic stress in *Coffea Arabica*. Mol. Genet. Genomics, 289: 951-963.
5. Babova, O., A. Occhipinti and M.E. Maffei, 2016. Chemical partitioning and antioxidant capacity of green coffee (*Coffea arabica* and *Coffea canephora*) of different geographical origin. Phytochemistry, 123: 33-39.
6. Dankowska, A., A. Domagala and W. Kowalewski, 2017. Quantification of *Coffea arabica* and *Coffea canephora* var. *robusta* concentration in blends by means of synchronous fluorescence and UV-Vis spectroscopies. Talanta, 172: 215-220.
7. Bicho, N.C., A.E. Leitao, J.C. Ramalho, N.B. de Alvarenga and F.C. Lidon, 2013. Identification of chemical clusters discriminators of Arabica and Robusta green coffee. Int. J. Food Proper., 16: 895-904.
8. Panusa, A., R. Petrucci, R. Lavecchia and A. Zuorro, 2017. UHPLC-PDA-ESI-TOF/MS metabolic profiling and antioxidant capacity of Arabica and Robusta coffee silverskin: Antioxidants vs phytotoxins. Food Res. Int., 99: 155-165.
9. Bresciani, L., L. Calani, R. Bruni, F. Brighenti and D. Del Rio, 2014. Phenolic composition, caffeine content and antioxidant capacity of coffee silverskin. Food Res. Int., 61: 196-201.
10. Alomar, M.J., 2016. Evaluation of caffeine consumption and effect during pregnancy among women in the UAE. Int. J. Pharm. Pharmaceut. Sci., 8: 101-103.
11. Pizarro, C., I. Esteban-Diez and J.M. Gonzalez-Saiz, 2007. Mixture resolution according to the percentage of *Robusta* variety in order to detect adulteration in roasted coffee by near infrared spectroscopy. Anal. Chim. Acta, 585: 266-276.
12. Santos, J.R., M. Lopo, A.O.S.S. Rangel and J.A. Lopes, 2016. Exploiting near infrared spectroscopy as an analytical tool for on-line monitoring of acidity during coffee roasting. Food Control, 60: 408-415.
13. Calvini, R., A. Ulrici and J.M. Amigo, 2015. Practical comparison of sparse methods for classification of Arabica and Robusta coffee species using near infrared hyperspectral imaging. Chemometrics Intell. Lab. Syst., 146: 503-511.
14. Carrera, F., M. Leon-Camacho, F. Pablos and A.G. Gonzalez, 1998. Authentication of green coffee varieties according to their sterolic profile. Anal. Chim. Acta, 370: 131-139.
15. Martin, M.J., F. Pablos, A.G. Gonzalez, M.S. Valdenebro and M. Leon-Camacho, 2001. Fatty acid profiles as discriminant parameters for coffee varieties differentiation. Talanta, 54: 291-297.
16. Wei, X., F. Liu, Z. Qiu, Y. Shao and Y. He, 2014. Ripeness classification of astringent persimmon using hyperspectral imaging technique. Food Bioprocess Technol., 7: 1371-1380.
17. Aquino, F.J., R. Augusti, J.D.O. Alves, M.E.R. Diniz and S.A.L. Morais *et al*., 2014. Direct infusion electrospray ionization mass spectrometry applied to the detection of forgeries: Roasted coffees adulterated with their husks. Microchem. J., 117: 127-132.
18. Pauli, E.D., F. Barbieri, P.S. Garcia, T.B. Madeira and V.R. Acquaro Jr. *et al*., 2014. Detection of ground roasted coffee adulteration with roasted soybean and wheat. Food Res. Int., 61: 112-119.
19. Mehari, B., M. Redi-Abshiro, B.S. Chandravanshi, S. Combrinck, M. Atlabachew and R. McCrindle, 2016. Profiling of phenolic compounds using UPLC-MS for determining the geographical origin of green coffee beans from Ethiopia. J. Food Compos. Anal., 45: 16-25.
20. Vochyanova, B., F. Opekar and P. Tuma, 2014. Simultaneous and rapid determination of caffeine and taurine in energy drinks by MEKC in a short capillary with dual contactless conductivity/photometry detection. Electrophoresis, 35: 1660-1665.
21. Yanes, E.G., S.R. Gratz and A.M. Stalcup, 2000. Tetraethylammonium tetrafluoroborate: A novel electrolyte with a unique role in the capillary electrophoretic separation of polyphenols found in grape seed extracts. Analyst, 125: 1919-1923.
22. De Melo, M.M.R., H.M.A. Barbosa, C.P. Passos and C.M. Silva, 2014. Supercritical fluid extraction of spent coffee grounds: Measurement of extraction curves, oil characterization and economic analysis. J. Supercrit. Fluids, 86: 150-159.
23. McCusker, R.R., B.A. Goldberger and E.J. Cone, 2003. Caffeine content of specialty coffees. J. Anal. Toxicol., 27: 520-522.
24. Adam, D.J., J. Mainwaring and M.N. Quigley, 1996. Soxhlet extraction of caffeine from beverage plants. J. Chem. Educ., 73: 1171-1171.
25. Lara, D., E. Vilcacundo, C. Carrillo, M. Silva, M. Alvarez and, W. Carrillo, 2017. Obtention of protein concentrate and polyphenols from macadamia (*Macadamia intergrifolia*) with aqueous extraction method. Asian J. Pharm. Clin. Res., 10: 138-142.
26. Panusa, A., A. Zuorro, R. Lavecchia, G. Marrosu and R. Petrucci, 2013. Recovery of natural antioxidants from spent coffee grounds. J. Agric. Food Chem., 61: 4162-4168.
27. Burns, D.T. and M.J. Walker, 2019. Critical review of analytical and bioanalytical verification of the authenticity of coffee. J. AOAC Int., (In Press). 10.5740/jaoacint.18-0392.
28. Joet, T., A. Laffargue, F. Descroix, S. Doulbeau, B. Bertrand, A. de Kochko and S. Dussert, 2010. Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. Food Chem., 118: 693-701.

29. Kitzberger, C.S.G., M.B. dos Santos Scholz, L.F.P. Pereira, L.G.E. Vieira, T. Sera, J.B.G.D. Silva and M. de Toledo Benassi, 2013. Diterpenes in green and roasted coffee of *Coffea arabica* cultivars growing in the same edapho-climatic conditions. J. Food Compos. Anal., 30: 52-57.
30. El-Abassy, R.M., P. Donfack and A. Materny, 2011. Discrimination between Arabica and Robusta green coffee using visible micro Raman spectroscopy and chemometric analysis. Food Chem., 126: 1443-1448.
31. Campa, C., S. Doulbeau, S. Dussert, S. Hamon and M. Noirot, 2005. Diversity in bean caffeine content among wild *Coffea* species: Evidence of a discontinuous distribution. Food Chem., 91: 633-637.
32. Hecimovic, I., A. Belscak-Cvitanovic, D. Horzic and D. Komes, 2011. Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. Food Chem., 129: 991-1000.