

Looking for Unknown Molecules in Arabica and Robusta Coffee Brews by High Performance Liquid Chromatography Coupled to High Resolution Mass Spectrometry and Differentiating both Coffee Species by this Technique

Evandro A. Nascimento

Sérgio A. L. Morais

Roberto Chang

Francisco J. T. Aquino

Institute of Chemistry
Federal University of Uberlândia
Uberlândia/MG
Brazil

Blyeny H. P. Alves

Federal Institute of Goiás
Itumbiara/GO
Brazil

Robson J. C. F. Afonso

Department of Chemistry
Federal University of Ouro Preto
Ouro Preto/MG
Brazil

Abstract

The chemical composition of coffee brews originating from four varieties of Coffea arabica cv. (Yellow Catuai, Mundo Novo, Topázio and Acaiá) and one variety of Coffea canephora cv. (robusta) was evaluated by high performance liquid chromatography coupled to mass Spectrometry with high resolution ((HPLC/HRMS) at different roasting degrees. The results obtained in the present study demonstrate that there are still many unknown components that can influence the coffee flavor, in addition to those reported as such. They deserve been investigated. Further, more than different constituents, the different concentrations of the same constituents are crucial for distinguishing the flavor of arabica and robusta coffee brews.

Keywords: coffee, coffee beverage, coffee flavor, coffee analysis, *Coffea arabica*, *Coffea canephora*

1. Introduction

Brewed coffee is one of the most appreciated beverages in the world. Its aroma causes a pleasant feeling of well-being that makes it irresistible. The coffee trade is very important for the world economy and especially for Brazil, the largest producer and exporter, and the second largest consumer of coffee in the world (Campanhaet *al.*, 2010; Muriel & Arauz, 2010). *Coffea arabica* cv. and *Coffea canephora* c.v. (robusta) are the most important cultivars in Brazil, and their species are associated with beverages of better quality because they possess more equilibrated sensorial attributes after roasting. Robusta coffee is mainly used in coffee blends to improve the “body” of the final beverage and to reduce costs (Campanhaet *al.*, 2010; Flament, 2002).

The makeup of the flavor and aroma of brewed coffee is intrinsically linked to the roasting process, in which Maillard and Strecker reactions occur (Flament, 2002). These reactions produce characteristic substances that give the coffee a heady aroma and a pleasant bitterness. The degree of roasting is selected according to beverage type (espresso, percolated, etc.).

Light roasting is normally used for sorting coffee, since the bean defects are better detected; medium roasting is commonly used in espresso coffee; and dark or strong dark roasting is used for percolated infusion according to the preference of the regional market.

The diversity of compounds responsible for the flavor and aroma of coffee makes coffee chemical research very complex (Flament, 2002; Farah & Donangelo, 2006). Most chemical studies on green and roasted arabica and robusta coffees are related to chlorogenic acids (Martin *et al.*, 1998; De Maria *et al.*, 1995), caffeine, trigonelline, nicotinic acid and sucrose (Perrone *et al.*, 2008 - 7), sugar (Rogers *et al.*, 1999; Amorim *et al.*, 2009), lipids (Kurzrock & Speer, 2001), triacylglycerol (Segal *et al.*, 2005], vitamins (Alves *et al.*, 2009), alkaloids (Koshiro *et al.*, 2006], volatiles (Amorim *et al.*, 2009; De Maria *et al.*, 1999; Moreira *et al.*, 2000; Nascimento *et al.*, 2002, 2003; Morais *et al.*, 2007) and metal profiles (Martin *et al.*, 1998; Amorim *et al.*, 2009). The volatile constituents of coffee have been well studied and models for reproducing the aroma of coffee from different geographic regions have been also proposed (Semmelroch & Grosch, 1996; Mayer *et al.*, 2000). However, investigations into coffee flavor have been somewhat delayed due to the great difficulties in identifying the unknown small and large molecules remaining in the complex coffee infusions.

The use of high performance liquid chromatography (HPLC) has enabled some progress in the study of coffee composition (Flament, 2002; Trugo & Macrae, 1984). De Maria *et al.* (1995, 1996) have obtained better results using high performance gel filtration chromatography (HPGF) when compared with reverse-phase HPLC and they also concluded that roasting promoted the extensive degradation of trigonelline, sucrose, amino acids and arabinogalactan. Discrimination between arabica and robusta green coffee varieties was performed by Martin *et al.* (1998). In this study, they selected chlorogenic acids, caffeine, trigonelline, amino acids and polyphenols as chemical descriptors, which differentiate the *Arabica* from *Canephora* species. Perrone *et al.* (2010) developed good models for weight loss as well as chlorogenic acid degradation during coffee roasting process. Looking for a correlation between chemical composition and coffee classification Farah *et al.* (2006) found a good correlation between trigonelline and 3,4-dicaffeoylquinic acid contents in green and roasted high quality coffee and, to a lesser extent, caffeine. On the other hand, caffeoylquinic acids and feruloylquinic acids are correlated with low quality coffee.

Therefore, due to the high complexity of the coffee matrix, many liquid chromatographic techniques combined with ultra-high resolution and mass accuracy mass spectrometers have been introduced to investigate secondary metabolites in coffee (Flament, 2002). Recently, Rodrigues & Bragagnolo (2013), using HPLC/MS, identified different levels of bioactive compounds in brews of roasted ground coffee and soluble coffee, reporting the presence of caffeoylferuloylquinic acid isomers, and cinnamoyl-amino acid conjugates. In addition, Alonso-Salces *et al.* (2009) presented also an exhaustive work on the profile of polyphenols and methylxanthines.

The discovery that lactones formed from chlorogenic acids also have an impact on coffee brew bitterness (Ginz & Engelhardt, 2001; Farah *et al.*, 2005; Perrone *et al.*, 2008) questions the generalized belief that caffeine, trigonelline and roasting degradation products are the principal components responsible for the characteristic bitterness of coffee brews. Thereafter, systematic methodologies based on analytical and sensorial studies allowed for interesting findings (Frank *et al.*, 2006; Frank *et al.*, 2007; Blumberg *et al.*, 2010). These researchers focused directly on coffee water infusions, which were fractionated and submitted to trained panelists in order to detect bitterness. Their studies using HPLC/MS have shown so far that the thermal degradation of chlorogenic and caffeic acids produces quinides and catechol derivatives, which strongly contribute to the characteristic bitterness of coffee, rather than those compounds previously considered. However, and surprisingly, sensory studies have shown that coffee beans roasted for 4 min at 220°C presented no coffee-like bitterness (Blumberg *et al.*, 2010).

A few recent works have used HPLC/MS combined with principal component analysis to discriminate between green and roasted coffees, while providing a “fingerprint” for coffee samples (Amorim *et al.*, 2009; Mendonça *et al.*, 2008, 2009; Garret *et al.*, 2012; Rodrigues-Durán *et al.*, 2014; Aquino *et al.*, 2014). Further, Liquid Chromatography Ion-Trap Time-Of-Flight Mass Spectrometry (LC-IT-TOF-MS) allows for the detection of known molecules with accuracy and it also allows the estimation of the molecular mass of unknown molecules (Alonso-Salces *et al.*, 2009; Frank *et al.*, 2007). Accuracy may be improved using the recently developed technique of Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI FT-ICR MS) (Garret *et al.*, 2012). LC-IT-TOF-MS also enables semi-quantitative comparison of sample composition using the absolute intensity of ions, as long as the samples were prepared under the same conditions.

Accurate quantitative analysis of chlorogenic acids and their quinides using HPLC and HPLC/MS can be found in the papers quoted above (Farah & Donangelo, 2006; De Maria *et al.*, 1996; Perrone *et al.*, 2010; Frank *et al.*, 2007; Blumberg *et al.*, 2010) as well as in more recent articles (Müllen *et al.*, 2011; Chang & Ho, 2014).

This study aims to detect new molecules that can influence the flavor of the coffee drink and to establish differences between drinks of a few varieties of arabica coffee and between drinks of arabica and robusta coffees.

2. Methods

2.1 Samples

Three samples of *Coffea arabica* cv. (Yellow Catuaí, Mundo Novo, and Topázio) were provided by Fazenda Experimental Três Pontas – EPAMIG, southern Minas Gerais (MG) State; two samples of Acaiaí and Yellow Catuaí were provided by Fazenda Rondini, Araguari, Savannah biome of MG State, and one sample *Coffea canephora* cv. Conilon was provided by Cooperativa Agrária dos Cafeicultores de São Gabriel Ltda (COOGABRIEL), southern Espírito Santo State. All selected six samples, two of Yellow Catuaí from different regions (southern and savannah of MG), were harvested in 2007/2008. Two-kg samples of coffee berries, taken at random, were dried to 11–11.5% humidity on an asphalt-coated patio, later dehulled, and selected through a 16/17 mesh sieve. For the trials, a completely randomized design was used, with three replications, totalizing 18 experiments.

2.2 Coffee Beans Roasting

Beans were roasted in a commercial electric bench roaster (Pinhalense, model TC-0), at a temperature of $190 \pm 10^\circ\text{C}$. The roasting degrees of light, medium and dark were achieved in approximately 7.5 ± 1.0 , 9.5 ± 1.0 , and 13.0 ± 1.0 min, respectively. The color of roasting was determined by visual assessment and classified according to the reference system used by the Associação Brasileira da Indústria de Café (ABIC) colorimetric system, which uses the Roast Color Classification System of the SCAA (Agtron scale): light degree # 85, medium # 55 and dark # 35 (Sweet Maria's Coffee, 2010; SCAA Protocols, 2010; Morais *et al.*, 2009). Roasted beans were ground in an electric grinder to a fine particle size (passed through a 24-mesh sieve), packaged, sealed and stored at -20°C until analysis. Three samples were prepared from each of the six coffee cultivars, coming to a total of eighteen samples.

2.3 Preparation of the Standard Coffee Beverage

5.4000 ± 0.0001 g of coffee powder were placed in a filter (no. 4, Melitta) and percolated with 0.1 L of boiling water. The obtained coffee infusion was immediately cooled to room temperature in an ice bath. The filtrate was transferred to a volumetric flask. The volume was made up to 0.1 L with ultrapure water and $5.0 \mu\text{L}$ was immediately injected into the LC/MS equipment. The procedure was carried out in triplicate for each sample.

2.4 HPLC-DAD-ESI/MS Experiments

HPLC was performed using a Shimadzu Prominence system equipped with a high-pressure binary solvent delivery system (LC-20AD), an SIL 20AC auto-sampler and in connection with an SPD-M20A UV/VIS Diode-Array Detector (DAD) system. The injection volume was $5.0 \mu\text{L}$, and chromatography separation was performed on a KINETEX C18 column ($2.6 \mu\text{m} \times 50 \times 2.1$ mm), maintained at 45°C in an oven. The flow rate was 0.13 mL min^{-1} , using water (acidified to 0.3% v/v, with formic acid, mobile phase A) and methanol (mobile phase B). Chromatographic separation was carried out using a gradient method according to the following program: 15% B to 28% B for 10 min; 28% to 30% B for 15 min; 30% to 100% B for 3 min, hold at 100% B for 2 min; reduce to 15% B for 5 minutes. The total run time was 35 min. The UV/Vis DAD spectra were obtained from 190 to 800 nm at 1.2 nm slit width and 40°C microcell temperature. The mass spectrometry detection was performed using a Shimadzu LC-IT-TOF, a tandem quadrupole ion-trap (IT) and time-of-flight (TOF) sequential mass spectrometer. The optimized parameters used to work at high resolution of 10,000 10% FW and high mass accuracy (< 5 ppm) were: ESI ionization at + 4.5 and -3.5 KV and nebulizer gas at 1.5 L min^{-1} ; curved desorption line (CDL) interface at 200°C , and drying gas at 100 KPa. The octapole ion accumulation time was of 10 ms. The total ion chromatograms (TIC) were obtained in one segment containing two events, one in the positive ion mode scan (53 to 1000 m/z range) and other in the negative ion mode scan (50 to 1000 m/z range). High-resolution mass spectra were recorded in both events at 100 ms per scan. Thus, the total TOF ion chromatograms were obtained.

Molecular formulae presented in the tables were selected from a list suggested by the Formula Predictor[®] Software, according to the smallest difference between measured m/z ion and calculated m/z ion (δ), and by the plausibility of chemical existence, double bond equivalence, solubility in water, nitrogen rule, etc. The experiment produces protonated (positive mode) and deprotonated ions (negative mode). Ions with a ratio mass to charge (m/z) that had a large dispersion (> 0.0020 Da from the smallest to the largest value) in all of the 18 studied samples were not taken into account. The selected m/z ion was an average of all those obtained for the 18 samples and mass units were given in Da. The accurate mass calculator is very sensitive and pure compounds present $\delta < 2.0$ mDa; on the other hand, contaminated or mixed compounds of close m/z ion masses are difficult to be separated. Thus, previous comments are necessary here. For instance, deprotonated ions of citric and quinic acids were found with the m/z ion at exactly 191.0207 and 191.0561, respectively. Table 3 registered only one peak, a blend of both ions, whose mass varied from 191.0277 (light roast, more citric acid content) to 191.0414 (dark roast, less citric acid content). Therefore, those acids were not selected with $\delta < 5$ mDa. We selected both acids because they presented different retention times and high intensity in the TIC chromatograms. In turn, many other molecules were suggested. A typical chromatogram for all the samples of *Coffea arabica* studied in the present work is showed in Figure 1.

3. Results and Discussion

3.1 Evaluation of the Degree of Roasting on the Composition of the Coffee Brews

TIC of *Coffea arabica* cv. Yellow Catuaí (from southern MG) coffee brews, in positive and negative modes, for light, medium and dark roasting, can be seen in Figure 1. The chromatographic profiles in the positive mode clearly illustrated the degradation of trigonelline, caffeic acid isomers and others components, except caffeine, which does not degrade with increased roasting (Flament, 2002). Otherwise, the chromatographic profiles in the negative mode showed different behavior of the components during roasting. The behavior of caffeoylquinic acids was not clear until medium roasting, but the concentration of caffeoylquinides increased slightly. However, the increase in quinic acid and caffeoylquinides contents showed the caffeoylquinic acid degradation. Increasing the roasting degree to dark, caffeoylquinic acid and quinic contents visibly decreased. The citric acid content also decreased from medium to dark roast. Peaks of high molecular weight compounds in the chromatographic profiles, such as dicaffeoylquinic acids and dicaffeoylquinides, were not visible.

The differentiation of the six coffee varieties was not possible with liquid chromatography. Surprisingly, the chromatographic profiles of *Coffea canephora* cv. presented the same profile displayed by the *C. arabica* varieties (Figure 2), except for the relatively high content of feruloylquinides, and especially with the medium roasting process (negative mode). Thus, we can conclude that HPLC-DAD-ESI/MS technique can be used to distinguish *C. arabica* from *C. canephora* cv.

A more accurate and broad semi-quantitative analysis can be done using protonated or deprotonated ions, or adduct ions furnished by the high-resolution mass spectrometer. Tables 1 and 2 present the high-resolution mass spectra data of the coffee beverages in positive and negative modes, respectively. There are many m/z ions corresponding to molecules not clearly seen in the total ion current chromatogram (Figures 1 and 2) due to overlapping, even when some of them are present in relatively high concentrations. In positive mode (Table 1), the main m/z ions (content over 10% of the base peak) were: 116.9863 (adduct of E-2-butenedinitrile or 2-methylidenepropanedinitrile + K^+), 17.35 %; 138.0559 (trigonelline + H^+), 19.41 %; 149.0135 ($C_5H_6N_2O + K^+$ as well as a number of other nitrogenated compounds, among them hydroxymethyl-pyrazine), 48.98 %; 163.0397 (caffeic acid - $H_2O + H^+$), 17.75 %; 195.0893 (caffeine + H^+), 100 %; and 236.0718 (no plausible molecule with $\delta < 2$ was suggested by the Formula Predictor[®] Software), 13.37 %). Further, the ion of m/z 133.03454 could be associated to 2-azidoethyl nitrate or 1H-1,2,4-triazol-2-ium nitrate (molecular formula $C_2H_4N_4O_3$), or may result from a compound with the molecular formula [$C_5H_6N_2O + Na^+$] (see ion m/z 149.0135 above). There are a number of typical coffee compounds for the suggested molecular formula corresponding to m/z ions 130.0506 and 301.1414. Some of them have already been reported in coffee brews, and others have not. Further studies are necessary to determine their exact structures. Finally, no plausible molecule was suggested for the ion m/z 214.0895 with $\delta < 2$.

In negative mode (Table 2), the main m/z ions (content over 10% of the base peak) were: 91.0044 (2,2-dihydroacetic acid), 17.87%; 112.9875 (acetylenedicarboxylic or squaric acid), 15.63%; 133.0157 (malic acid), 14.81%; 146.9731 (unidentified compound), 13.68%; 191.0329 (citric + quinic acids), 100%; 197.9632 (unidentified compound), 27.04%; 248.9596 (unidentified compound), 19.43%; 335.0776 (caffeoylquinides), 60.60%; 353.0884 (caffeoylquinic acids), 67.36%; and 367.1042 (feruloylquinic acids), 20.78%. Further interesting observations were found. Acid compounds were dominant, and chlorogenic acids and their quinide structures were well represented in the Yellow Catuaí coffee brew. A few carboxylic acids were found for the suggested molecular formula corresponding to the ion of m/z 219.0514. For the m/z ion 233.0670 two molecular formulas were suggested, both with $\delta = 0.2$: $C_9H_{14}O_7$ and $C_8H_{12}O_5$, to which many compounds of different functions can be assigned. The m/z ion 449.0714 was suggested as the molecule $C_{20}H_{18}O_{12}$, for which two chromen-4-ones were found. For many other compounds, no molecular formula was proposed due to the great dispersion of the ion masses, and for a few no plausible molecule was found. Finally, a carbohydrate ($C_{14}H_{24}O_{12}$) was suggested for the molecule corresponding to m/z ion 383.1194, although it usually appears as a potassium or sodium adduct.

Most of the main compounds presented in Tables 1 and 2 are well-known in coffee brews (Flament, 2002), but only dicaffeoylquinic acids were detected as representative of high molecular weight compounds in the Yellow Catuaí coffee brew, although they are present in low concentrations and only after the light roasting process (0.25% of the base peak, complete table not shown). Catechol molecules derivatives from caffeic acid of molar masses 272, 274 and 408 Da were not detected in the dark roasted samples. These findings are in agreement with the results obtained by Frank et al.(2007) who only detected these compounds after prolonged roasting over 240°C.

Certainly, some of these unknown compounds presented in Tables 1 and 2 contribute to coffee flavor and, thus, they deserve further investigation.

A comparison of the absolute intensities shown in Tables 1 and 2 reveals in detail the findings presented in Figure 1. Caffeine concentration decreased after medium roasting (from 49693 to 38178) and increases to 41332 after dark roasting. On the other hand, the citric and quinic acid contents remained constant. Chlorogenic acid contents (caffeoylquinic acids, feruloylquinic acids and coumaroylquinic acid) decreased as expected, and chlorogenicquinide contents (caffeoylquinides and feruoylquinides) increased after medium roasting and decreased after dark roasting.

In this work, the general trend observed for *Coffea arabica* cv. Yellow Catuaí from southern MG was typical for all the arabica cultivars studied. Differences in component concentrations from one cultivar to another can explain the characteristic flavor of each brew. It is, however, difficult to know which components and/or synergic effects among them are responsible for their flavor nuances.

3.2 Differentiating Coffee Brews (*Coffea Arabica* versus *Coffea Canephora*) Using Medium Roasting

In order to differentiating arabica from robusta coffee brews, the absolute intensity obtained from the medium roasting process (Tables 1 and 2) was used. The medium roasting process had been selected as the parameter of analysis because it produced the most appreciated flavor of coffee brews.

The data comparison showed marked differences between Arabica and robusta coffee brews. The high-resolution mass spectra in positive mode (Table 1) detected an outstanding difference in caffeine content, which was found at high levels (absolute intensity of 77992) in the robusta coffee brew, almost double the quantity usually present in arabica infusions. Furthermore, with the exception of the protonated compound with m/z ion 236.0718, concentrations of the components in the robusta coffee brew were meaningfully higher than those seen in the arabica coffee brew. Certainly, this difference contributes to the differentiation of the brews.

On the other hand, the data from the negative mode (Table 2) allowed for the detection of many other interesting differences. First, contrary to the positive mode, in the negative mode the concentrations of some components were greater for arabica coffee, with a few exceptions. For example (and as expected): a – the contents of feruloylquinic acids and their quinides were higher in the robusta brew; b – the intensity of the m/z ions 219.0514 and 383.1201 was also greater in robusta. For the former ion, the molecular formula $C_8H_{12}O_7$ was suggested with $\delta = 0.4$, which corresponds to a few carboxylic acids. The second ion was assigned to carbohydrates.

The content of the caffeoylquinides in the robusta coffee brew was high (absolute intensity of 18645) in spite of the unpleasant taste of the beverage. This is a proof of the existence of other potent flavorings in coffee brews, as

well as the presence of components intherobusta brew that can smother the pleasant taste and the coffee-like bitterness of the chlorogenicquinides compounds.

4. Conclusions

The above results demonstrate that there are still many unknown components that can influence the coffee flavor, in addition to those reported as such. They deserve been investigated. Further, more than different constituents, the different concentrations of the same constituents are crucial for distinguishing the flavor of arabica and robusta coffee brews.

5. Acknowledgements

The authors wish to thank FAPEMIG and the post-graduate program in chemistry of the Federal University of Uberlândia for their financial support, EPAMIG, Fazenda Rondini, Araguari – MG and COOGABRIEL – ES for the coffee samples, Daniela Daniel (Shimadzu do Brazil) for her valuable discussion, and FINEP-CT-INFRA for financing the LC-IT-TOF equipment.

6. References

- Alonso-Salces, R. M., Guillou, C., &Berrueta, L. A. (2009). Liquid chromatography coupled with ultraviolet absorbance detection, electrospray ionization, collision-induced dissociation and tandem mass spectrometry on a triple quadrupole for the on-line characterization of polyphenols and methylxanthines in green coffee beans. *Rapid Communication in Mass Spectrometry*, 23: 363-383.
- Alves, R. C., Casal, S., & Oliveira, M. B. P. P. (2009). Determination of vitamin E in coffee beans by HPLC using a micro-extraction method. *Food Science and Technology International*, 15: 57-63.
- Amorim, A. C. L., Hovell, A. M. C., Pinto, A. C., Eberlin, M. N., Arruda, N. P., Pereira, E. J., Bizzo, H. R., Catharino, R. R., Filho, Z. B. M., &Rezende, C. M. (2009). Green and roasted arabica coffees differentiated by ripeness, process and cup quality via electrospray ionization mass spectrometry fingerprinting. *Journal of the Brazilian Chemistry Society*, 20: 313-321.
- Aquino, J. F. T., Augusti, R., Alves, J. O., Diniz, M. E. R., Morais, S. A. L., Alves, B. H. P., Nascimento, E. A., &Sabino, A. A. (2014). Direct infusion electrospray ionization mass spectrometry applied to detection of forgeries: roasted coffees adulterated with their husks. *Microchemical Journal*, 117: 127-132.
- Blumberg, S., Frank, O., & Hofmann, T. (2010). Quantitative studies on the influence of the bean roasting parameters and hot water percolation on the concentrations of bitter compounds in coffee brew. *Journal of Agricultural and Food Chemistry*, 58: 3720-3728.
- Campanha, F. G., Dias, R. C. E., &Benassi, M. T. (2010). Discrimination of coffee species using kahweol and cafestol: effects of roasting and of defects. *Coffee Science*, 44: 87-96.
- Chang, K. L. &Ho, P. C. (2014). Gas chromatography time-of-flight mass spectrometry (GC-TOF-MS)-based metabolomics for comparison of caffeinated and decaffeinated coffee and its implications for Alzheimer's disease. *PlosOne* 9(8): e104621.
- De Maria, C. A. B., Trugo, L. C., Moreira, R. F. A., &Petracco, M. (1995). Simultaneous determination of total chlorogenic acid, trigonelline and caffeine in green coffee samples by high performance gel filtration chromatography. *Food Chemistry*, 52: 447-449.
- De Maria, C. A. B., Trugo, L. C., Neto, F. R. A., Moreira, R. F. A., & Alviano, C. S. (1996). Composition of green coffee water-soluble fractions and identification of volatiles formed during roasting. *Food Chemistry*, 55: 203-207.
- De Maria, C. A. B., Moreira, R. F. A., &Trugo, L. C. (1999). Volatile components in roasted coffee. Part I: heterocyclic compounds. *Química Nova*, 22: 209-217.
- Farah, A., De Paulis, T., Trugo, L. C., & Martin, P. R. (2005). Effect of roasting on the formation of chlorogenic acid lactones in coffee. *Journal of Agricultural and Food Chemistry*, 53: 1505-1513.
- Farah, A. &Donangelo, C. M. (2006). Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology*, 18: 23-36.
- Flament, I. (2002). *Coffee Flavor Chemistry*. West Sussex, John Wiley & Sons.
- Frank, O., Zehentbauer, G., & Hofmann, T. (2006). Bioresponse-guided decomposition of roast coffee beverage and identification of key bitter taste compounds. *European Food Research and Technology*, 222: 492-508.

- Frank, O., Blumberg, S., Kunert, C., Zehehtbauer, G., & Hofmann, T. (2007). Structure determination and sensory analysis of bitter-tasting 4-vinylcatechol oligomers and their identification in roasted coffee by means of LC-MS/MS. *Journal of Agricultural and Food Chemistry*, 55: 1945-1954.
- Garret, R., Vaz, B. G., Hovell, A. M., Eberlin, M. N., & Rezende, C. M. (2012). Arabica and Robusta coffees: identification of major polar compounds and quantification of blends by direct-infusion electrospray ionization-mass spectrometry. *Journal of Agricultural and Food Chemistry*, 60: 4253-4258.
- Ginz, M. & Engelhardt, U. H. (2001). Analysis of bitter fractions of roasted coffee by LC-ESI-MS - new chlorogenic acid derivatives. *Colloque Scientifique International sur le Café*, 19th: 248.
- Koshiro, Y., Zheng, X. Q., Wang, M. L., Nagai, C., & Ashihara, H. (2006). Changes in content and biosynthetic activity of caffeine and trigonelline during growth and ripening of *Coffea arabica* and *Coffea canephora* fruits. *Plant Science*, 171: 242-250.
- Kurzrock, T. & Speer, K. (2001). Diterpenes and diterpene esters in coffee. *Food Reviews International*, 17: 433-450.
- Martin, M. J., Pablos, F., & Gonzalez, A. G. (1998). Discrimination between arabica and robusta green coffee varieties according to their chemical composition. *Talanta* 46, 1259-1264.
- Mayer, F., Czerny, M., & Grosch, W. (2000). Sensory study of the character impact of aroma compounds of a coffee beverage. *European Food Research and Technology*, 211: 272-276.
- Mendonça, J. C. F., Franca, A. S., Oliveira, L. S., & Nunes, M. (2008). Chemical characterisation of non-defective and defective green arabica and robusta coffees by electrospray ionization-mass spectrometry (ESI-MS). *Food Chemistry*, 111: 490-497.
- Mendonça, J. C. F., Franca, A. S., Oliveira, L. S., & Afonso, R. J. C. F. (2009). Application of electrospray ionization-mass spectrometry to the discrimination of green and roasted coffees by species and quality. In: LANG J. K. (Org.), *Handbook on mass spectrometry: instrumentation, data and analysis, and applications*. New York: Nova Publishers, v. 17.
- Morais, S. A. L., Aquino, F. J. T., Nascimento, E. A., Chang, R., Oliveira, G. S., & Santos, N. C. (2007). Chemical analysis of arabica coffee (*Coffea arabica* L.) and defective beans submitted to different degrees of roasting. *Coffee Science*, 2: 97-111.
- Morais, S. A. L., Aquino, F. J. T., Nascimento, P. M., Nascimento, E. A., & Chang, R. (2009). Bioactive compounds and antioxidant activity of conilon coffee submitted to different degrees of roasting. *Química Nova*, 32: 327-331.
- Moreira, R. F. A., Trugo, L. C., & De Maria, C. B. (2000). Volatile components in roasted coffee. Part II. Aliphatic, alicyclic and aromatic compounds. *Química Nova*, 23: 195-203.
- Müllen, W., Nemzer, B., Stalmach, A., Hunter, J., Clifford, M. N., & Combet, E. (2011). The antioxidant and chlorogenic acid profiles of whole coffee fruits are influenced by the extraction procedures. *Journal of Agricultural and Food Chemistry*, 59: 3754-3762.
- Muriel, P. & Arauz, J. (2010). Coffee and liver diseases. *Fitoterapia*, 81: 297-305.
- Nascimento, E. A., Morais, S. A. L., Chang, R., & Aquino, F. J. T. (2002). Volatile and volatilizable constituents of roasted coffee from cerrado and the effect of harvest and irrigation on its composition. *Revista Ceres*, 49: 295-297.
- Nascimento, E. A., Morais, S. A. L., & Rocha, R. S. (2003). Volatile constituents of soft and special Arabian coffees from Brazilian "cerrado" in function of the roasting grade. *Ciência e Tecnologia de Alimentos*, 23: 282-284.
- Perrone, D., Donangelo, C. M., & Farah, A. (2008). Fast simultaneous analysis of caffeine, trigonelline, nicotinic acid and sucrose in coffee by liquid chromatography-mass spectrometry. *Food Chemistry*, 110: 1030-1035.
- 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*, 106: 859-869.
- Perrone, D. R., Donangelo, C. M., & Farah, A. (2010). Modelling weight loss and chlorogenic acids content in coffee during roasting. *Journal of Agricultural and Food Chemistry*, 58: 12238-12243.

- Rodrigues-Durán, L. V., Ramíres-Coronel, M. A., Aranda-Delgado, E, Nampoothiri, K. M., Favela-Torres, E., Aguilar, C. N., & Saucedo-Castañeda, G. (2014). Soluble and bound hydroxycinnamates in coffee pulp (*Coffea arabica*) from seven cultivars at three ripening stages. *Journal of Agricultural and Food Chemistry*, 62: 7869-7876.
- Rodrigues, N. P. & Bragagnolo, N. (2013). Identification and quantification of bioactive compounds in coffee brews by HPLC-DAD-MSⁿ. *Journal of Food Composition and Analysis*, 32: 105-115.
- Rogers, W. J., Michaux, S., Bastin, M., & Bucheli, P. (1999). Changes to the content of sugars, sugar alcohols, myo-inositol, carboxylic acids and inorganic anions in developing grains from different varieties of Robusta (*Coffea canephora*) and Arabica (*C. arabica*) coffees. *Plant Science*, 149: 115-123.
- SCAA Protocols (2010). Cupping specialty coffee. Published by the Specialty Coffee Association of America (SCAA) Revised: November 21, 2009. Available at: <<http://scaa.org/PDF/PR%20%20CUPPING%20PROTOCOLS%20V.21NOV2009A.pdf>>. (November 24, 2010).
- Semmelroch, P. & Grosch, W. (1996). Studies on character impact odorants of coffee brews. *Journal of Agricultural and Food Chemistry*, 44: 537-543.
- Segall, S. D., Artz, W. E., Raslan, D. S., Jham, G. N., & Takahashi, J. A. (2005). Triacylglycerol composition of coffee beans (*Coffea canephora* p.) by reversed phase high-performance liquid chromatography and positive electrospray tandem mass spectroscopy. *Journal of Agricultural and Food Chemistry*. 53: 9650-9655.
- Sweet Maria's Coffee (2010). Using sight to determine degree of roast. Available at: <<http://www.sweetmarias.com/roasting-VisualGuideV2.php>>. (November 28, 2010).
- Trugo, L. C. & Macrae, R. (1984). A study of the effect of roasting on the chlorogenic acid composition of coffee using HPLC. *Food Chemistry*, 15: 219-227.

Figure 1: TIC chromatograms of *Coffea arabica* Yellow Catuai (YC) beverages. (1) Positive Mode and (2) Negative Mode; (L), (M), and (D): Light, Medium, and Dark Roast Degrees, Respectively

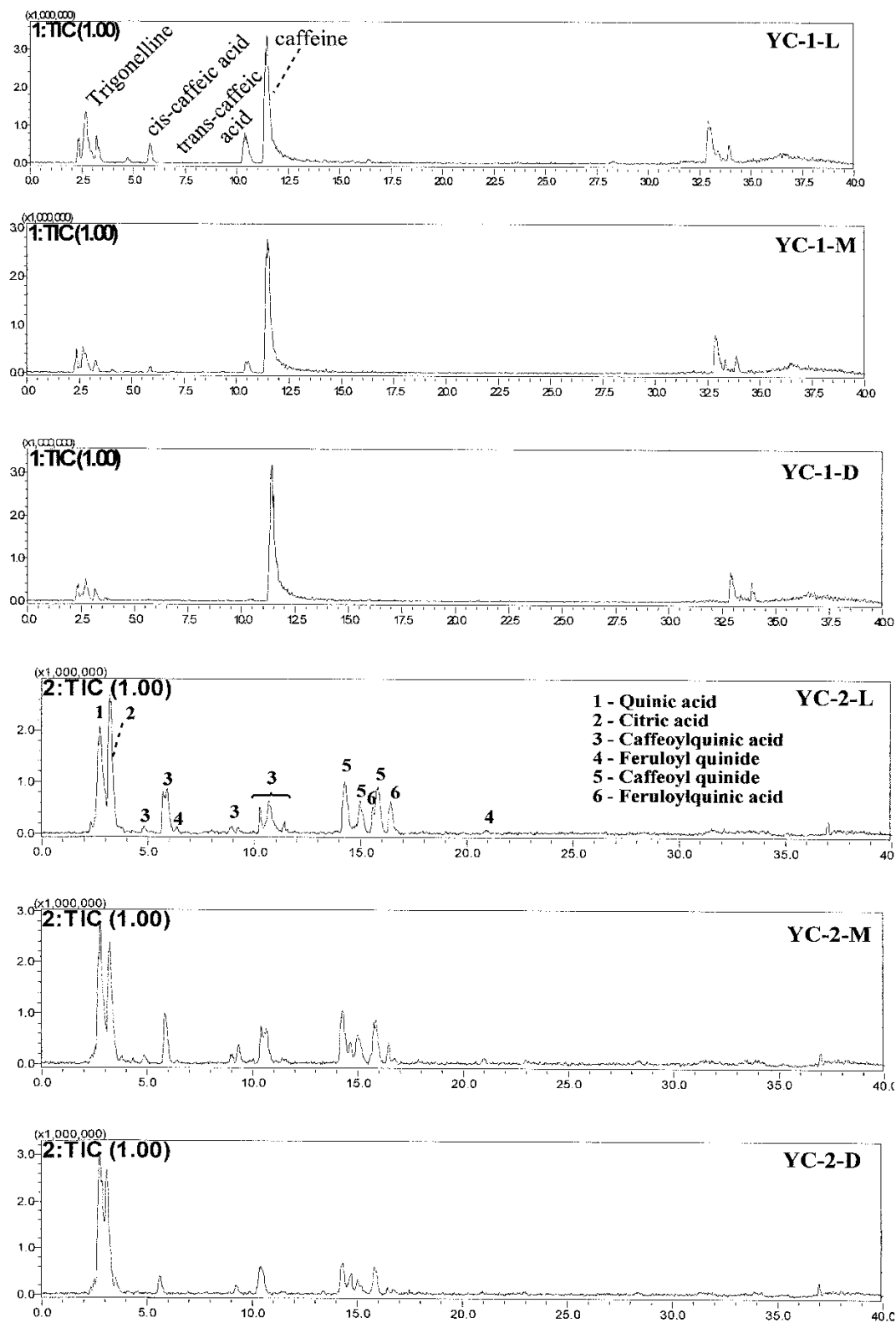


Figure 2: TIC Chromatograms of *Coffea Canephora* Conilon (CC) Beverages. (1) Positive Mode and (2) Negative Mode; (L), (M), and (D): Light, Medium, and Dark Roast Degrees, Respectively

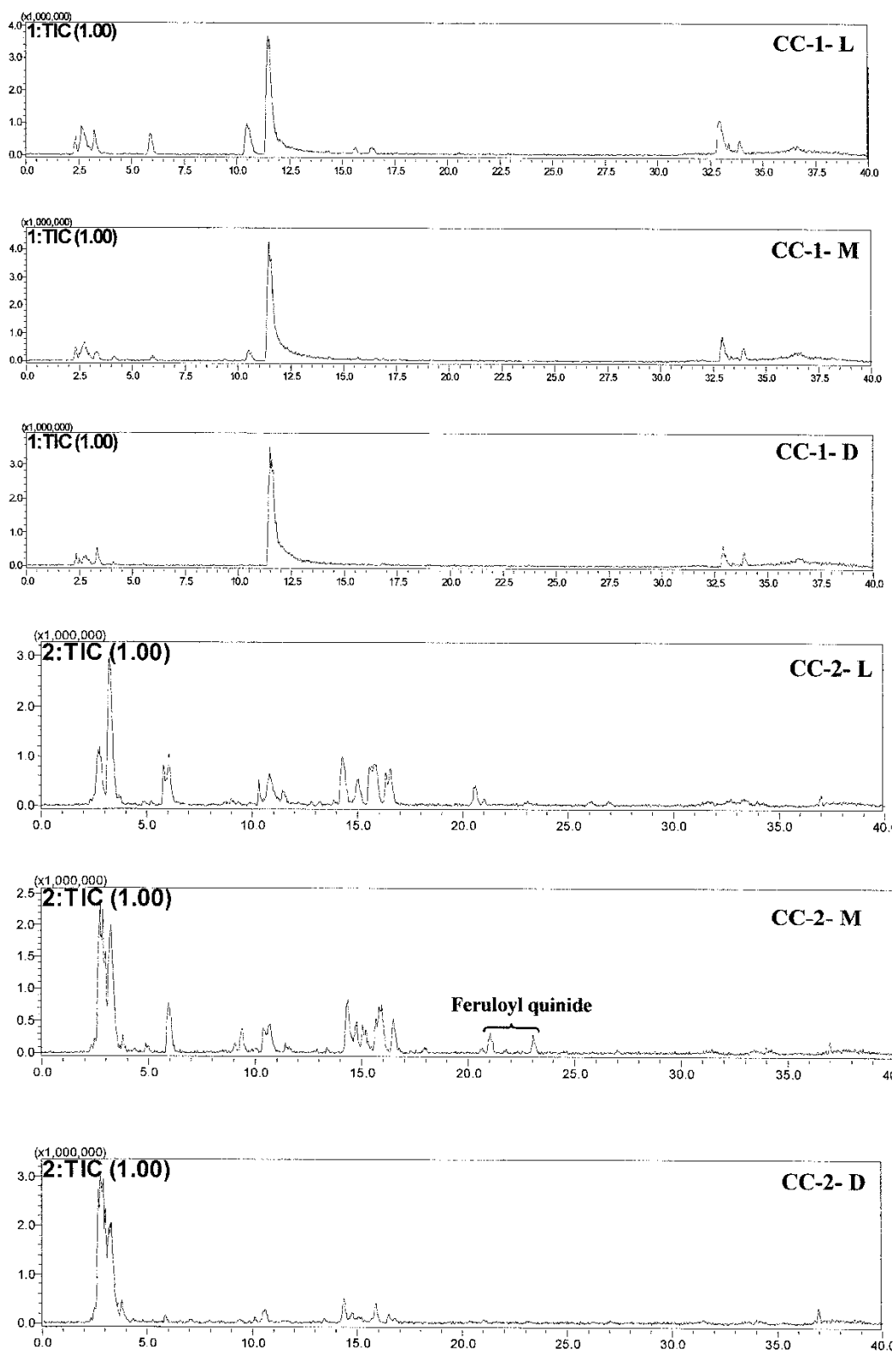


Table 1: High-resolution mass spectra of *Coffea Arabica* vc. Yellow Catuai (YC) beverages and of the *Coffeacanephora*vc. Conilon beverage. Positive mode; (L), (M), and (D): light, medium, and dark roast degrees, respectively; peaks over 2.0% of the base peak for Yellow Catuai and over 1% for Conillon; base peak m/z 195.0885; relative intensities were not shown

Ion m/z	YC-1-L Abs. Inten.	YC-1-M Abs. Inten.	YC-1-D Abs. Inten.	CC-1-M Abs. Inten.	Suggested compound	$\pm \delta$ (mDa)
74.0948	1709	964	1042	2025	Isobutylamine	1.1
100.0777	2793	1922	1796	2735	N-methylpyrrolidin-2-one	1.6
102.0919	1447	813	-	1185	N-methylpyrrolidin-2-ol	1.1
116.9866	8623	6098	5917	9710	Adduct C ₄ H ₂ N ₂ + K ⁺	1.3
122.0589	2686	1688	1781	3702	Adduct N-methylpyrrolidin-2-one + Na ⁺	0.7
130.0506	-	-	1076	-	C ₅ H ₇ NO ₃	0.7
133.0354	1172	777	947	935	C ₂ H ₄ N ₄ O ₃ adduct C ₅ H ₆ N ₂ O + Na ⁺	0.2 1.8
138.0560	9645	3905	1678	4344	Trigonelline	0.9
149.0135	24341	16267	15702	21662	Adduct C ₅ H ₆ N ₂ O + K ⁺	2.0
163.0397	9723	2561	-	4097	Caffeic acid – H ₂ O + H ⁺	0.8
195.0885	49693	38178	41332	77992	Caffeine	0.8
196.0915	2976	2238	2413	4410	C ₁₃ -caffeine	-
214.0895	4707	4358	3766	5206	npm	-
215.0266	1862	991	-	1074	Adducts citric and quinic acids + Na ⁺	-
217.0705	1269	996	1064	1873	Adduct caffeine + Na ⁺	0.0
236.0718	6646	4691	3429	4479	npm	-
301.1414	2129	1629	1754	2346	C ₁₄ H ₁₆ N ₆ O ₂	0.8
355.1024	1965	-	-	-	Caffeoylquinic acid	0.6

npm = no plausible molecule was predicted; C₁₃ = C₁₃ isotope

Table 2: High-resolution mass spectra of *Coffea Arabica* vs. Yellow Catuai (YC) beverages and of the *Coffeacanephora* vs. Conilon beverage. Negative mode; (L), (M), and (D): light, medium, and dark roast degrees, respectively; peaks over 2.0% of the base peak for Yellow Catuai and over 1% for Conillon; base peak m/z 191.0329; relative intensities were not shown

Ion m/z	YC-2-L Abs. Inten.	YC-2-M Abs. Inten.	YC-2-D Abs. Inten.	CC-2-M Abs. Inten.	Suggested compound	± δ (mDa)
91.0044	5630	12021	10882	2730	2,2-dihydroxyacetic acid	0.5
112.9875	4922	8532	8184	1829	Acetilenedicarboxylic or squaric acid	0.9
128.9989	-	760	-	606	gd	-
133.0157	4666	3957	4004	1483	Malic acid	1.6
146.9731	4308	5745	5153	2398	gd	-
158.9872	-	838	-	418	gd	-
174.9785	-	665	-	370	gd	-
179.0551	-	-	722	-	gd	-
180.9760	1075	1401	1078	532	gd	-
191.0329	31499	30506	32603	30845	Citric and quinic acids	-
192.0363	1722	1665	1862	1486	C ₁₃ -citric and C ₁₃ -quinic acids	-
197.9632	8518	9579	-	3982	gd	-
219.0514	-	2239	3179	3541	C ₈ H ₁₂ O ₇	0.4
225.0632	954	-	-	-	gd	-
233.0670	-	735	2265	1350	C ₉ H ₁₄ O ₇ or C ₈ H ₁₂ O ₅	0.2
242.9539	958	845	-	431	gd	-
248.9596	6121	6311	4380	2203	gd	-
289.0177	1452	2126	2039	1726	gd	-
298.0563	1117	-	-	-	gd	-
325.1828	-	962	-	516	npm	-
335.0776	19089	21838	14300	18645	Caffeoylquinides	0.2
336.0803	2159	2564	1190	1841	C ₁₃ -caffeoylquinides	-
337.0922	1687	1376	-	-	Coumaroylquinic acids	0.8
349.0784	1089	19904	-	4057	Feruloylquinides	0.7
353.0884	21218	2047	9588	16162	Caffeoylquinic acids	0.5
354.0914	1864	3221	-	1335	C ₁₃ -caffeoylquinic acids	-
367.1042	6544	3942	1185	6959	Feruloylquinic acid	0.6
383.1194	2419	1319	3745	4870	C ₁₄ H ₂₄ O ₁₂	0.1
398.0718	990	2150	1066	777	npm	-
449.0714	1611	21838	1066	817	C ₂₀ H ₁₈ O ₁₂	1.1

gd = great dispersion of molecular weights; npm = no plausible molecule was predicted; C₁₃ = C₁₃ isotope