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# Metabolomics fingerprint of Philippine coffee by SPME-GC-MS for geographical and varietal classification

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**Abstract**

Volatile metabolites of Philippine Arabica and Robusta coffee beans in the both forms standard (not-eaten by the Asian palm civet) and civet coffee grown in different Philippine regions were identified using the hyphenated technique headspace-solid phase microextraction-gas chromatography-mass spectrometry. A great number of volatile metabolites with a wide variety of functional groups were extracted and forty-seven prominent compounds were identified.

The volatile metabolomics (volatilomics) fingerprint of Arabica coffees considerably differed with Robusta coffee and geographical origin slightly altered the fingerprint profile of coffee samples. Chemometric analysis such as principal component analysis (PCA) displayed a good classification between Arabica and Robusta coffee samples. Although, Arabica coffee samples from different geographical origins were clustered separately from each other, the proximity of clusters between Arabica coffee samples which can be classified into one large group, indicated their close similarity of headspace metabolites. PCA also identified several key volatile metabolites for the distinction of this group from Robusta coffees which is attributed to the higher amount of acetic acid, furfural, 5-methylfurfural, 2-formylpyrrole, and maltol, and lower concentration of 4-ethylguaiacol and phenol in all Arabica samples. These discriminating metabolites could be useful quality markers to differentiate Arabica with Robusta coffee. Results revealed that the headspace metabolites in coffee provide significant information on its inherent aroma quality. Also, the findings suggested that the overall quality of Philippine coffee is variety and region specific.

**Keywords:** Volatile metabolites, Volatilomics, Civet coffee, Asian palm civet, Arabica, Robusta, Geographical origin, HS-SPME-GC-MS, Discriminant markers

[1]*Abbreviations*

[1] *Abbreviations:* AC, Asipulo Civet; AR, Asipulo Robusta; CA, Cordillera Arabica; CC, Cordillera Civet; GC, Gas chromatography; HS, headspace; i.d., Internal diameter; KC, Kalinga Civet; KR, Kalinga Robusta; MA, Matutum Arabica; MC, Matutum Civet; MS, Mass spectrometry; MW, Molecular weight; PC, Principal Component; PCA, Principal Component Analysis; SPME, Solid phase microextraction

## 1. Introduction

Coffee aroma is the result of the multiplicity of volatile compounds present in roasted coffee beans (*Coffea* spp.). The complex balance of the most important volatile compounds in coffee has a relative contribution to its overall aroma quality (Bernard, Roberts, & Kraehenbuehl, 2005). So far, more than eight hundred volatile compounds belonging to a wide range of chemical classes have been identified in roasted coffee (Mayer & Grosch, 2001; Rocha, Maetzu, Barros, Cid & Coimbra, 2003), including aliphatic volatile metabolites (carbonyl-containing compounds, sulfur-containing compounds), alicyclic compounds (including several ketones), benzenoids (phenols); heterocyclic compounds (furans, hydrofurans, pyrroles, pyridines, quinolines, pyrazines, quinoxalines, indoles, thiophens, thiophenones, thiazoles, oxazoles) (Clarke, 1986).

Nowadays, coffee drinking is the best social lubricant and people are becoming more discriminating in their preference for coffee. The aroma of coffee is one of the most important consumer's preference vectors due to its contribution to the palatability and appreciation of overall coffee quality. This has recently given rise to a fast growing demand for specialty coffee or commonly referred to gourmet or premium coffee produced from special geographic microclimates beans with unique flavor profiles (Teuber, 2019).

Among the specialty coffees, civet coffee ranks as the most expensive and best coffee in the world due to its unique aroma and taste (Lee, 2006). It is made from coffee cherries which have been eaten and passed through the digestive tract of the (Asian palm) civet. Civets naturally select and consume the ripest and sweetest coffee cherries, and excrete the undigested inner beans. The passage of the beans through the digestive tract of civet adds flavor to the coffee by partially breaking down the proteins, thus modulating the coffee bitter taste (Marcone, 2004).

Civet coffee is produced in only few countries from Far East including Philippines, where it has been recognized as one of the important indigenous export products of the country (Yulia & Suhandy, 2017). Philippine civet coffee is derived mainly from the beans of Arabica and Robusta coffee trees found in the forests where the Asian palm civet thrives, particularly those in the mountains of the Cordillera region, Batangas, Davao, and Cotabato. The different aroma characteristics of Philippine Arabica and Robusta (not eaten and eaten by the Asian palm civet) and their inherent attributes are still a puzzle and require deeper understanding of their chemical nature.

The need to identify reliable method that can determine the volatile compounds responsible for the aroma quality of Philippine coffee varieties and geographical origin is therefore of crucial relevance.

Some studies have recently used a metabolomic approach to ascertain the authenticity of far Eastern civet coffees. They focused on non-volatile compounds, such as organic and phenolic acids, carbocyclic sugars, and their ratios (Jumhawan, Putri, Marwani, Bamba, & Fukusaki, 2013; Jumhawan, Putri, Bamba, & Fukusaki, 2016). In particular, inositol to pyroglutamic acid ratio was selected as a chemical marker to discriminate the authenticity of civet coffee. This index makes sense, as pyroglatamic acid derives from the degradation of two amino acids, glutamine and glutamic acid (Montevecchi, Masino, & Antonelli, 2010), which could originate from the enzymatic action of Asian palm civet on protein structures of the green coffee.

Volatile metabolomics, or volatilomics, is a novel approach and a useful tool for the assessment of food quality and authenticity. It involves separation and detection of volatile metabolites using a multidisciplinary field of science including analytical chemistry, bioinformatics, statistics, and biochemistry (Bouhifd, Hartung, Hogberg, Kleensang, & Zhao, 2013; Lytou, Panagou, & Nychas, 2019).

The volatilomic analytical platform commonly utilized for the analysis of headspace (HS) metabolites is gas chromatography coupled with mass spectrometry (GC-MS) (Rowan, 2011). Several extraction methods can be employed for HS-GC-MS analysis such as vacuum or steam distillation (Stoffelsma, Sipma, Kettenes, & Pypker, 1968; Kumazawa & Masuda, 2003); purge and trap (Costa Freitas & Mosca, 1999); static headspace (Sanz, Ansorena, Bello, & Cid, 2001; Mayer & Grosch, 2001); sorptive extraction and stir bar sorptive extraction (Bicchi, Iori, Rubiolo, & Sandra, 2002); and finally solid phase extraction (SPE) (Ishikawa et al, 2004). The application of headspace solid phase microextraction (HS-SPME) has been widely recognized because it is a non-destructive and non-invasive method in the determination of volatile and semi-volatile metabolites (Hamm et al., 2003). Also, it is a solvent-free, simple and fast, relatively compact and low cost sampling technique. Moreover, it is highly sensitive, selective and compatible with analytical systems having low detection limits (Pawliszyn, Yang, & Orton, 1997).

The general aim of the project is the characterization of Philippine coffees and the safeguard of their authenticity, in the both forms standard (not-eaten by the Asian palm civet) and civet coffee. Also, the present study aims to outline through the hyphenated technique HS-SPME-GC-MS a volatilomic fingerprint of four types of roasted coffee beans coming from different geographical regions of the

Philippines. The selected samples belong to the two main species of *Coffea* genus (Arabica and Robusta) in their standard form and in their civet version.

## 2. Materials and methods

### *2.1. Sampling*

Samples of *Coffea arabica* (throughout the paper referred to as Arabica) and *C. canephora* (sin. *C. robusta*; throughout the paper referred to as Robusta) roasted beans were acquired from different regions of the Philippines. Arabica and Robusta coffee beans eaten and not-eaten by Asian palm civet (*Paradoxurus hermaphroditus*) were included in the samples.

Four Robusta coffee beans samples were taken from the northern part of the Philippines (Kalinga province and Asipulo district, located in Ifugao province), while four Arabica coffees were obtained from the southern part (Matutum discrict located in South Cotabato province) and the northern part (Cordillera, Mountain province) of the country. A map of the Philippines indicating the sites of the geographic origin of the coffee samples is shown in figure 1. Arabica coffee samples, namely Matutum Arabica (MA), Matutum Civet (MC), Cordillera Arabica (CA), and Cordillera Civet (CC) were compared with four Robusta coffee samples, notably Kalinga Robusta (KR), Kalinga Civet (KC), Asipulo Robusta (AR), and Asipulo Civet (AC). All coffee samples are commercially available and dark roasted between 220 °C and up to 230 °C.

### *2.2. Chemicals and standards*

All high-purity analytical standards were purchased from Sigma-Aldrich (Merck KGaA, Milan, Italy).

### *2.3. Method for the volatiles extraction*

#### *2.3.1. Optimization of the method*

To optimize the protocol of extraction, the effects of sample weight (0.5 g, 1.0 g, and 1.5 g), extraction time (10 min, 20 min, and 30 min) and temperature (60 °C, 70 °C, and 80 °C), desorption time (5 min

and 10 min) were assessed based on the highest number of peaks and highest peak areas. All the optimization analysis was carried out on the same sample of Cordillera Arabica coffee.

*2.3.2. Optimized HS-SPME protocol for the extraction of coffee volatile metabolites*

The roasted coffee beans (1.0 g) were placed in a 20-mL crimped-top-sealed vial. Each vial was heated at 70 °C for 10 min to reach sample headspace equilibrium. The volatile compounds were extracted using a 50/30 μm divinylbenzene–carboxen-polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Merck KGaA, Bellefonte, PA, USA). The fiber was inserted into the vial and exposed to the headspace above the coffee sample for 20 min at 70 °C. After the extraction, the fiber was thermally desorbed into the GC injection port for 5 min. Each coffee sample was analyzed thrice.

[Arabica (2 standard + 2 civet) + Robusta (2 standard + 2 civet)] x 3 = 24 samples (total)

*2.4. GC-MS analysis*

The analysis was performed using a gas chromatograph Hewlett-Packard (HP) 6890 series instrument (Hewlett-Packard, Waldbronn, Germany) with a split/splitless injection port coupled with a mass spectrometer instrument HP 5973 Mass Selective Detector (Hewlett-Packard, Waldbronn, Germany), equipped with a crossbond acid-deactivated Carbowax-like polyethylene glycol capillary column (Stabilwax-DA 11023, Restek Corporation, Bellefonte, PA, USA), measuring 30 m, having an internal diameter of 0.25 mm and film thickness of 0.25 μm. GC-MS analysis was performed in splitless mode at 250 °C. The oven temperature was set at 60 °C, held for 2 min and increased at 5 °C/min up to 240 °C and finally held for 5 min.

The molecular fragmentation was obtained by electron ionization (EI). The data were obtained in full-scan mode and the mass/charge ratio (*m*/*z*) was recorded between 50 and 550 at 70 eV. Chromatograms were acquired and processed using the software Enhanced Chem Station (G1701AA Version A.03.00, Hewlett Packard).

Identification was carried out by comparing retention times and mass spectrum of all the available pure standards. In the absence of pure standards, the volatiles were identified by comparing their mass spectra with those present in the data system libraries (Wiley 7$^{th}$ Edition Library and NIST-14). Only those compounds with match probabilities above 80% (considered a satisfactory match), and those ones

for which the same identification was matched across several samples and for which a similar mass spectra spectrum was observed, were identified. In cases in which unacceptable confident matches were found through the libraries, the compounds were individually checked and in cases where the compounds showed the same retention time, molecular ion, base ion, and fragmentation patterns in all samples were taken into account and labeled as '*unknown 1-8*' accordingly. The absence of said compounds was verified in blank injections. Whenever it was possible, the identification of volatiles was also verified based on the presence in the literature. A semi-quantitation was carried out by considering the average values of the absolute peak areas.

*2.5. Statistical analysis*

Multivariate analyses, notably principal component analysis (PCA) and cluster analysis, were applied to the whole data set. All tests were performed with Statistica version 8.0 software (Stat Soft Inc., Tulsa, OK, USA).

## 3. Results and discussion

*3.1. Optimization of HS-SPME operating conditions*

The DVB/CAR/PDMS fiber was chosen for HS-SPME due to its high affinity towards a pool of analytes characterized by a wide-range of polarity, including aromatic heterocycles, benzenoids, aliphatic and alicyclic hydrocarbons. In addition, this fiber has already been successfully applied in previous studies (Bicchi et al., 2002; Mondello et al., 2004; Ryan, Shellie, Tranchida, Casilli, Mondello, & Marriott, 2004; Mondello et al. 2005; Toci & Farah, 2008; Franca, Oliveira, Oliveira, Agresti, & Augusti, 2009).

Increasing the sample weight from 0.5 g to 1.0 g, the intensity peaks of most compounds substantially improved. However, 1.5 g of sample did not yield a further increase in the response. This is probably due to a decrease of phase ratio "β" (headspace to sample ratio), and in the retention capacity of the fiber (Kolb & Ettre, 2006). For this reason, 1.0 g was used as a standard sample weight.

Headspace generation was held at 70 °C for 10 min and the extraction temperature was varied from 60 °C to 70 °C and up to 80 °C at the constant extraction time of 20 min. The lowest extraction

temperature of 60 °C generated lower peak areas for most of the semi-volatile compounds. Conversely, the highest extraction temperature of 80 °C resulted in an increase of peak areas of the high boiling compounds, but caused the reduction of the areas of the compounds with a high vapor pressure. This was due to a displacement effect that occurred onto the fiber to the detriment of substances with a high vapor pressure. Extraction temperature of 70 °C was therefore deemed the best condition to achieve the maximum extraction efficiency of volatile metabolites and used for the standard protocol.

Extraction time depends on factors affecting the mass repartition of the volatile metabolites among sample, headspace, and fiber coating. In order to determine the optimum extraction time, extraction temperature was held constant, without sample agitation, and extraction time varied from 10, 20, and 30 min. Results showed that 10-min extraction time yielded high areas of the low boiling volatiles, whereas 30 min were more favorable for some semi-volatile compounds. The finding implied that there was an inverse relationship between the extraction time and the volatility of the analytes. Extraction time of 20 min was considered a good compromise for both volatile and semi-volatile compounds and was adopted as standard procedure.

The complete thermal desorption of volatile metabolites from the fiber coating is necessary to improve chromatographic resolution and prevent carry-over of volatile metabolites to the subsequent extraction process. Desorption of volatile metabolites from the fiber coating was carried out at 250 °C based on previous studies (Toci and Farah, 2008; Oliveira, Oliveira, Franca, & Augusti, 2009; Costa Freitas, Parreira, & Vilas-Boas, 2001). Instead, desorption time was established to achieve the complete purging and cleaning of SPME fiber. The fiber was desorbed in the GC injection port for 5 and 10 min and subjected again to desorption in a subsequent blank run. No peaks appeared during the latter run in both cases, thus indicating that 5 min was a suitable time to prevent carry-over effects.

### *3.3. Identification and semi-quantitation of volatile metabolites*

The list of volatile metabolites extracted and identified is shown in Table 1. IUPAC names are indicated together with the main synonyms. The latter are used throughout the article as they are most commonly used in the literature.

Arabica and Robusta coffees showed a high number of volatile metabolites belonging to a wide variety of chemical classes, notably aromatic heterocycles (furans, pyranes, pyrazines, pyridines, pyrroles), aliphatic and alicyclic hydrocarbons, phenols, aldehydes, ketones, alcohols, esters, lactones, and fatty

234 acids. Forty-seven volatile metabolites were considered in total, 27 of which were confirmed using pure
235 reference standards, while other 12 were tentatively identified based on MS-libraries matching. Eight
236 peaks were included in the list as unknown compounds, since their presence was verified in most of the
237 samples.
238 Figure 2 presents the volatiles composition of the complete samples set. The volatile that showed by far
239 the highest concentrations was furfuryl alcohol, followed by furfuryl acetate, 5-methylfurfural, and 3-
240 acetylanisole. Furfuryl alcohol has a very mild, slightly caramel-like, warm-oily smell and is well
241 correlated with the undesirable burnt and bitter note of dark-roasted coffees (Flament, 2002).
242 The comparison between Robusta and Arabica samples showed that the latter had higher amounts of
243 acetic acid, furfural, 2-acetylfuran, 5-methylfurfural, furfuryl alcohol, 3-methylcyclopentane-1,2-dione,
244 maltol, and 2-formylpyrrole. Conversely, Robusta samples showed higher amounts of 3-ethyl-2,5-
245 dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, guaiacol, phenol, 4-ethylguaiacol, and 3-
246 acetylanisole. Pyrrole and 2-ethyl-3,5-dimethylpyrazine were not detected at all in Arabica samples and
247 only found in small concentrations in Robusta samples.
248 In general, the concentrations of furanic compounds in Arabica and of pyrazine compounds in Robusta
249 stood out. A marked prevalence of furanic derivatives in Arabica samples, as well as a concomitant
250 slighter prevalence of pyrazine volatile metabolites in Robusta samples, has already been described
251 (Mondello et al., 2005). Furthermore, Ryan et al. (2004) reported that maltol was significantly higher in
252 Arabica samples, as well as phenol was significantly lower, in comparison with Robusta samples.
253 However, phenol has a medicinal odor and does not contribute to the pleasantness of coffee flavor
254 (Dorfner, Ferge, Kettrup, Zimmermann, & Yeretzian, 2003). Robusta coffees showed also higher
255 content of phenolic compounds. In particular, guaiacol is an important character impact volatile that
256 provides a smoky peaty phenolic note (Semmelroch, Laskawy, Blank, & Grosch, 1995).
257 Acetic acid must be considered separately. Unlike many other volatiles, the concentration of this
258 compound decreases with increasing degree of roasting (Somporn, Kamtuo, Theerakulpisut, &
259 Siriamornpun, 2011). Although acetic acid may represent a valid chemical marker for the degree of
260 roasting, its concentration in Arabica coffee was generally higher than that of Robusta once roasted
261 under the same conditions (Caporaso, Whitworth, Cui, & Fisk, 2018).
262 At least 22 compounds identified in Arabica (coming from El Salvador, Costarica, and Brazil) and
263 Robusta (coming from Togo, India, and Vietnam) coffees (Mondello et al., 2005) were also present in
264 Philippine coffees, notably pyridine, pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2,3-

dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, furfural, 2-acetylfuran, pyrrole, furfuryl acetate, 5-methylfurfural, furfuryl alcohol, γ-butyrolactone, furfurylpyrrole, guaiacol, 2-acetylpyrrole, furfuryl ether and 2-formylpyrrole.

AR samples showed a lack of volatile substances compared not only to their corresponding civet samples but also to all other samples, being its average volatiles sum from one third to one fifth lower. The cause of it is unknown and might be due to the specific lot of sample. This behavior has drastically affected a correct comparison of this sample within the characterization of all other Philippine coffees.

Roasting time and temperature of coffee cause extensive chemical modification on green beans (Franca et al., 2009). Non-enzymatic browning reactions are responsible of the formation of a very high number of volatile compounds, most of them belonging to aromatic heterocycles, such as furans, ketones, and pyrazines. Furans partly come from the dehydration of sugars that occurred during the sugar caramelization (Montevecchi, Masino, Chinnici, & Antonelli, 2008), while ketones and pyrazines were produced through Maillard-like reactions between sugars and amino acids (Knoch & Baltes, 1992). Grinding size and brewing methods are equally relevant in the coffee-flavor expression. However, the quali-quantitative variations in volatile metabolites observed in roasted coffee beans can also be attributed to the specific species/variety. Aside from the genotypic traits, the sensory properties of roasted coffee are particularly affected by other factors, such as growing region, altitude, macro- and micro-climatic conditions, and different cherries-fermentation processes (dry or wet) (Illy & Viani, 2005). In addition, for Philippine coffees must be also considered whether or not the cherries were passed through the gastrointestinal apparatus of the Asian palm civet (Ongo et al., 2012; Ongo et al., 2015).

Based on the present results, it was not possible to make general observations on the different composition in volatiles between civet and non-civet coffees. As for Robusta civet coffees, KC showed an average increase (ratio 1.4) in volatile amount in comparison to its standard coffee. In particular, the volatiles that showed the highest increase were pyrazines (in particular ethyl and isopropenyl substituted), furanic derivatives, phenolic compounds, maltol, and other minor volatiles.

As for the Arabica civet samples, MC showed no difference in the comparison (average ratio 1.0) with its standard MA, while CC has even shown an opposite behavior with an average reduction (ratio 0.7) in volatile amount compared to the CA. The only volatile compound that showed an increase in all the civet samples was furfural, a compound that mainly originates from pentose-sugars degradation during the roasting process. This remark consistently leads to confirm a hydrolytic action that occurs in the

digestive tract of the Asian palm civet on polysaccharides rich in pentose sugars, such as arabinogalactan (Bradbury & Halliday, 1990). A similar action on protein constituents with consequent release of amino acids, precursor of nitrogen volatiles, cannot be excluded.

### *3.4. Coffee Classification*

#### *3.4.1. Principal Component Analysis*

Autoscaled data concerning the areas of volatile compounds were chemometrically processed through the principal component analysis (PCA) to evaluate the possibility of discriminating Arabica with Robusta coffee, as well as civet and non-civet coffees, through specific volatilomic fingerprints. The PCA score plot of the whole sample set is shown in Figure 3a.

The clustering among all Arabica samples and the clear separation with Robusta samples was mainly evident on the second principal component (PC2), which explained 30.70% of the total variance. In the negative quadrants of the PC2, the proximity of CC, CA, MC and MA, which can be also clustered into one large group, indicated a close similarity of the volatiles composition of Arabica coffee samples. The distinction of this wide group from Robusta samples, which were set on the positive quadrants of the PC2, was mainly due to the higher amount of acetic acid, furfural, maltol, 2-formylpyrrole and the lower concentrations of phenol and 4-ethylguaiacol showed in all Arabica samples.

Figure 3b depicts the loading plot. The 47 volatile metabolites (for compounds names see Table 1) were all distributed in the negative quadrants of the PC1, except dodecane. This result confirmed that using this data set PCA could separate the samples on the PC2 more than on PC1. Indeed, due to their general scarcity of volatile substances, AR coffees were completely separated from all the other samples. For this reason, different PCAs were run in order to reduce this effect. In particular, civet coffees were subjected alone to a PCA (Fig. 4a), while Arabica (CA and MA) standard coffee samples were compared individually with Asipulo Robusta (Fig. 4b) and Kalinga Robusta (Fig. 4c) standard coffees in two different PCA analysis.

The PCA score plot of all civet coffees successfully discriminated Arabica civet (CC and MC) from Robusta civet (AC and KC) coffees (Fig. 4a). A clear separation between Arabica and Robusta civet coffees was, indeed, observed on PC1, while PC2 discriminated the samples coming from different

regions of production. Likewise, a clear discrimination between Arabica and Robusta samples on PC1 was showed in the figures 4b and 4c.

To determine the volatilomic fingerprints conducive to the discrimination among the different coffees samples, an accurate variable-loading analysis was performed using the loadings with consistent values in the all the three latter PCAs. Variables that exhibit loading values higher than 0.8 (80%) provide a major contribution within each PC and can be considered as discriminating variables. On the contrary, variables associated with very low loading values are considered useless and can be ruled out.

The volatile metabolites primarily accountable for this discrimination (Table 2) were acetic acid, furfural, 5-methylfurfural, 2-formylpyrrole, and 4-ethylguaiacol. Furfuryl alcohol, pyrrole, and maltol could be considered potential discriminating volatile metabolites as well, although they presented some loading value lower than 0.8. The high positive loading values of acetic acid, furfural, 5-methylfurfural, and 2-formylpyrrole on PC1 indicated a higher amount of these volatile metabolites in samples with positive scores on PC1, notably Arabica coffees. On the contrary, 4-ethylguaiacol weighed on PC1 with a negative loading value, thus indicating that the samples with negative scores, notably Robusta coffees, contained a higher amount of it. Similarly, Robusta samples contained higher concentrations of pyrrole and a lower amount of furfuryl alcohol and maltol than Arabica coffees. Furthermore, these findings are consistent with previous reports showing that the higher amounts of furfural, 5-methylfurfural, maltol, and 2-formylpyrrole and the lower concentrations and 4-ethylguaiacol are characteristics of Arabica samples (Blank, Sen, & Grosch, 1991; Semmelroch & Grosch, 1996; Ryan et al., 2004; Mondello et al., 2005; Caporaso, Whitworth, Cui, & Fisk, 2018).

Furfural is produced during the acid hydrolysis or heating of polysaccharides containing pentose (or hexose) sugars (Maarse et al., 1994). It has a characteristic of lightly roasted coffee to give it a flavor similar to that of roasted cereals. Furfural is also described as pungent, but sweet, bread-like, caramel-like, cinnamon-almond-like odor of poor tenacity (Fors, 1983). Maltol is a degradation product of disaccharides (maltose). Its odor is sweet, caramel-like, cotton-candy with fruity overtones (Flament, 2002). 2-Formylpyrrole was found as a product of the reaction of glutamine with ribose (Ho & Chen, 1999) and has a corny, pungent odor (Shibamoto & Russell, 1977). Finally, 4-ethylguaiacol was identified in the thermal decomposition of ferulic acid. It has a smoky and roasted flavor, burnt taste. Likewise, guaiacol is characterized by a smoky aroma (Flament, 2002).

*3.4.2. Cluster analysis*

Cluster analysis (Fig. 5) confirmed the similarity among coffee varieties. The individual spots (n = 24) of samples were arranged along the bottom of the dendrogram. The similar spots were formed into clusters by joining them together. The clusters that were nearer to the bottom of the dendrogram were considered highly correlated. The left sub-branch of the grouped points of the dendrogram was populated by all Arabica coffees (CA, MC, MA and CC), while the right sub-branch was populated by Robusta coffees (AC, KR, KC, AR).

MC was closely similar to MA, so that the two samples were connected to CA followed by CC. On the other side, all KC samples were linked with two KR samples. The level of similarity between the two samples was less intense as indicated by the distance connecting the two different samples. AC was more similar to Kalinga coffee samples (KC and KR) than to AR samples. Indeed, AR samples were isolated from all the other samples, as already highlighted through the other statistical analysis.

## 4. Conclusions

The classification of volatile metabolites of Philippine Arabica and Robusta coffee roasted beans was successfully carried out using a hyphenated analytical approach to outline specific volatilomic fingerprints through multivariate statistical tools. PCA and cluster analysis allowed the discrimination between Arabica and Robusta samples. The key volatile metabolites responsible for the classification of Arabica and Robusta coffees (both types, standard and civet) were acetic acid, furfural, 5-methylfurfural, 2-formylpyrrole, maltol, phenol and 4-ethylguaiacol. The achieved results suggest that the overall quality of Philippine coffee is variety/species and region specific. The findings revealed that the composition of volatile metabolites in coffee is able to provide significant information on the authenticity like other non-volatile markers already used for the same purpose.

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571 **Figure captions**

572

573 **Figure 1.**

574 Philippine map showing the site of the geographical origin of coffee samples.

575

576 **Figure 2.**

577 Average amounts of the volatiles of the complete samples set.

578 AC, Asipulo Civet; AR, Asipulo Robusta; KC, Kalinga Civet; KR, Kalinga Robusta; CA, Cordillera
579 Arabica; CC, Cordillera Civet; MA, Matutum Arabica; MC, Matutum Civet.

580

581 **Figure 3. a)** PCA score plot (PC1 vs. PC2) of the complete samples set. Robusta samples are in light
582 grey; **b)** PCA loading plot of PC1 vs. PC2. For compounds names refer to Table 1.

583

584 **Figure 4. a)** PCA plot (PC1 vs. PC2) of Arabica and Robusta civet coffees; **b)** PCA plot (PC1 vs. PC2)
585 of Arabica (MA and CA) vs. Asipulo Robusta standard coffees; **c)** PCA plot (PC1 vs. PC2) of Arabica
586 (MA and CA) vs. Kalinga Robusta standard coffees.
587 All Robusta samples are in light grey.

588

589 **Figure 5.**

590 Cluster Analysis (dendrogram) of the complete samples set.

591

**Table 1.**

Volatile compounds detected in Philippine roasted coffee beans and their retention times ($t_R$).

| # | $t_R$ (min) | Volatiles (IUPAC name) | Synonyms | # | $t_R$ (min) | Volatiles (IUPAC name) | Synonyms |
|---|---|---|---|---|---|---|---|
| **1** | 2.14 | 2-Methylfuran | | **25** | 16.29 | 1-Pyridin-2-ylethanone | 2-Acetylpyridine |
| **2** | 6.20 | Pyridine | | **26** | 16.42 | 2-(Furan-2-ylmethyl)furan | 2-Furfurylfuran |
| **3** | 6.30 | Dodecane | | **27** | 16.60 | 5-Methyl-6,7-dihydro-5*H*-cyclopenta[b]pyrazine | |
| **4** | 6.76 | Pyrazine | | **28** | 16.74 | 1-Methylpyrrole-2-carbaldehyde | |
| **5** | 7.21 | *Unknown 1* | | **29** | 16.97 | Oxolan-2-one | γ-Butyrolactone |
| **6** | 8.05 | 2-Methylpyrazine | | **30** | 17.54 | Furan-2-ylmethanol | Furfuryl alcohol |
| **7** | 9.37 | 2,5-Dimethylpyrazine | | **31** | 18.38 | 1-(6-Methylpyrazin-2-yl)ethanone | 2-Acetyl-6-methylpyrazin |
| **8** | 9.51 | 2,6-Dimethylpyrazine | | **32** | 18.74 | *Unknown 3* | |
| **9** | 9.66 | 2-Ethylpyrazine | | **33** | 18.98 | *Unknown 4* | |
| **10** | 9.98 | 2,3-Dimethylpyrazine | | **34** | 20.25 | *Unknown 5* | |
| **11** | 10.88 | 2-Ethyl-6-methylpyrazine | | **35** | 20.64 | *Unknown 6* | |
| **12** | 11.04 | 2-Ethyl-5-methylpyrazine | | **36** | 20.71 | *Unknown 7* | |
| **13** | 11.37 | 2,3,5-Trimethylpyrazine | | **37** | 21.27 | 3-Methylcyclopentane-1,2-dione | |
| **14** | 12.35 | 3-Ethyl-2,5-dimethylpyrazine | | **38** | 21.32 | 1-(Furan-2-ylmethyl)pyrrole | Furfurylpyrrole |
| **15** | 12.71 | Acetic acid | | **39** | 21.98 | 2-Methoxyphenol | Guaiacol |
| **16** | 12.74 | 2-Ethyl-3,5-dimethylpyrazine | | **40** | 24.13 | 3-Hydroxy-2-methylpyran-4-one | Maltol |
| **17** | 12.94 | *Unknown 2* | | **41** | 24.24 | 1-(1*H*-Pyrrol-2-yl)ethanone | 2-Acetylpyrrole |
| **18** | 12.99 | Furan-2-carbaldehyde | Furfural | **42** | 24.52 | 2-(Furan-2-ylmethoxymethyl)furan | Furfuryl ether |
| **19** | 13.53 | 3,5-Diethyl-2-methylpyrazine | | **43** | 24.72 | *Unknown 8* | |
| **20** | 13.98 | 1-(Furan-2-yl)ethanone | 2-Acetylfuran | **44** | 24.95 | Phenol | |
| **21** | 14.18 | 1*H*-Pyrrole | Pyrrole | **45** | 25.34 | 1*H*-Pyrrole-2-carbaldehyde | 2-Formylpyrrole |
| **22** | 14.66 | Acetic acid;furan-2-ylmethanol | Furfuryl acetate | **46** | 25.41 | 4-Ethyl-2-methoxyphenol | 4-Ethylguaiacol |
| **23** | 15.64 | 5-Methyl-2-furancarbaldehyde | 5-Methylfurfural | **47** | 28.60 | 1-(3-Methoxyphenyl)ethanone | 3-Acetylanisole |
| **24** | 16.12 | 2-Prop-1-en-2-ylpyrazine | Isopropenylpyrazine | | | | |

598 **Table 2.**

599 Loading values of PC1 e PC2 obtained from PCA processings: A) civet coffees alone; Arabica (CA and MA) standard coffee samples
600 compared individually with Asipulo Robusta (AR) (B) and Kalinga Robusta (KR) (C) standard coffees.

601

| # | Volatiles (IUPAC name) | Synonyms | A) Arabica civet (CC, MC) coffees vs. Robusta civet (AC, KC) coffees | | B) CA and MA vs. AR | | C) CA and MA vs. KR | |
|---|---|---|---|---|---|---|---|---|
| | | | PC1 | PC2 | PC1 | PC2 | PC1 | PC2 |
| 1 | 2-Methylfuran | | **-0.84** | 0.23 | **0.97** | 0.21 | -0.53 | **-0.84** |
| 2 | Pyridine | | -0.59 | -0.17 | **0.95** | -0.27 | **0.98** | 0.15 |
| 3 | Dodecane | | 0.58 | -0.64 | -0.02 | **-0.99** | 0.44 | **0.87** |
| 4 | Pyrazine | | -0.24 | 0.52 | **0.99** | 0.02 | -0.56 | **-0.79** |
| 5 | *Unknown 1* | | -0.65 | 0.67 | 0.38 | 0.31 | -0.14 | -0.27 |
| 6 | 2-Methylpyrazine | | -0.57 | -0.65 | **1.00** | 0.04 | **-0.89** | -0.45 |
| 7 | 2,5-Dimethylpyrazine | | -0.60 | -0.66 | **0.99** | 0.06 | **-0.93** | -0.37 |
| 8 | 2,6-Dimethylpyrazine | | -0.58 | -0.72 | **1.00** | 0.02 | **-0.96** | -0.28 |
| 9 | 2-Ethylpyrazine | | -0.72 | -0.49 | **1.00** | 0.09 | **0.97** | -0.24 |
| 10 | 2,3-Dimethylpyrazine | | **-0.91** | -0.36 | **0.99** | 0.10 | **-0.96** | -0.28 |
| 11 | 2-Ethyl-6-methylpyrazine | | **-0.90** | -0.24 | **0.99** | -0.05 | **-0.99** | -0.14 |
| 12 | 2-Ethyl-5-methylpyrazine | | 0.19 | **-0.93** | 0.63 | **-0.77** | -0.72 | 0.66 |
| 13 | 2,3,5-Trimethylpyrazine | | **-0.80** | -0.12 | **0.98** | 0.14 | **-0.96** | -0.25 |
| 14 | 3-Ethyl-2,5-dimethylpyrazine | | **-0.86** | 0.13 | **0.96** | 0.27 | **-0.94** | -0.34 |
| 15 | Acetic acid | | **0.89** | 0.37 | **0.95** | 0.29 | **0.92** | -0.35 |
| 16 | 2-Ethyl-3,5-dimethylpyrazine | | **-0.96** | 0.05 | 0.00 | 0.00 | **-1.00** | -0.08 |
| 17 | *Unknown 2* | | 0.55 | **-0.81** | **0.99** | -0.11 | **1.00** | -0.01 |
| 18 | Furan-2-carbaldehyde | Furfural | **0.94** | 0.12 | **0.96** | 0.02 | **0.93** | -0.21 |
| 19 | 3,5-Diethyl-2-methylpyrazine | | **-0.96** | -0.03 | **0.93** | 0.29 | -0.79 | -0.55 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 20 | 1-(Furan-2-yl)ethanone | 2-Acetylfuran | 0.75 | 0.24 | **1.00** | 0.09 | 0.52 | **-0.85** |
| 21 | 1*H*-Pyrrole | Pyrrole | -0.71 | 0.51 | **-0.97** | 0.20 | **-0.99** | -0.08 |
| 22 | Acetic acid;furan-2-ylmethanol | Furfuryl acetate | -0.53 | -0.71 | **0.99** | -0.05 | **0.84** | -0.47 |
| 23 | 5-Methyl-2-furancarbaldehyde | 5-Methylfurfural | **0.92** | -0.18 | **0.99** | 0.11 | **0.95** | -0.25 |
| 24 | 2-Prop-1-en-2-ylpyrazine | Isopropenylpyrazine | **-0.82** | 0.04 | **0.92** | -0.21 | 0.64 | -0.02 |
| 25 | 1-Pyridin-2-ylethanone | 2-Acetylpyridine | **-0.88** | -0.01 | **0.99** | 0.09 | 0.73 | -0.63 |
| 26 | 2-(Furan-2-ylmethyl)furan | 2-Furfurylfuran | -0.72 | 0.06 | **0.92** | -0.25 | 0.10 | 0.17 |
| 27 | 5-Methyl-6,7-dihydro-5*H*-cyclopenta[b]pyrazine | | **-0.98** | 0.08 | **0.95** | 0.27 | **-0.80** | -0.56 |
| 28 | 1-Methylpyrrole-2-carbaldehyde | | 0.11 | -0.69 | **0.99** | -0.15 | **-0.98** | -0.14 |
| 29 | Oxolan-2-one | γ-Butyrolactone | 0.14 | -0.75 | **0.87** | 0.43 | **0.96** | -0.25 |
| 30 | Furan-2-ylmethanol | Furfuryl alcohol | 0.66 | -0.68 | **1.00** | 0.01 | **0.97** | -0.20 |
| 31 | 1-(6-Methylpyrazin-2-yl)ethanone | 2-Acetyl-6-methylpyrazin | -0.43 | **-0.83** | **0.96** | 0.24 | 0.52 | **-0.80** |
| 32 | *Unknown 3* | | **-0.96** | 0.13 | **0.99** | 0.01 | **-0.97** | -0.21 |
| 33 | *Unknown 4* | | -0.39 | **-0.87** | **0.99** | 0.11 | **0.94** | -0.32 |
| 34 | *Unknown 5* | | 0.05 | **-0.89** | **1.00** | -0.04 | **0.97** | -0.18 |
| 35 | *Unknown 6* | | -0.64 | -0.58 | **0.99** | 0.03 | **0.94** | -0.29 |
| 36 | *Unknown 7* | | 0.33 | -0.17 | **0.99** | -0.06 | 0.79 | -0.38 |
| 37 | 3-Methylcyclopentane-1,2-dione | | 0.46 | **-0.79** | **0.99** | -0.11 | **1.00** | -0.03 |
| 38 | 1-(Furan-2-ylmethyl)pyrrole | Furfurylpyrrole | **-0.94** | -0.24 | **0.99** | -0.12 | **0.98** | -0.12 |
| 39 | 2-Methoxyphenol | Guaiacol | **-0.91** | 0.21 | 0.71 | 0.19 | **-0.97** | -0.16 |
| 40 | 3-Hydroxy-2-methylpyran-4-one | Maltol | 0.77 | -0.30 | **0.91** | -0.34 | **0.94** | 0.19 |
| 41 | 1-(1*H*-Pyrrol-2-yl)ethanone | 2-Acetylpyrrole | -0.51 | -0.21 | **0.99** | -0.14 | **0.98** | -0.06 |
| 42 | 2-(Furan-2-ylmethoxymethyl)furan | Furfuryl ether | **-0.84** | 0.09 | **0.96** | -0.23 | **0.94** | 0.08 |
| 43 | *Unknown 8* | | -0.70 | 0.32 | **0.96** | 0.12 | -0.75 | -0.51 |
| 44 | Phenol | | -0.77 | 0.26 | -0.76 | 0.49 | 0.54 | -0.65 |
| 45 | 1*H*-Pyrrole-2-carbaldehyde | 2-Formylpyrrole | **0.98** | -0.11 | **0.99** | 0.04 | **0.97** | -0.17 |
| 46 | 4-Ethyl-2-methoxyphenol | 4-Ethylguaiacol | **-0.90** | 0.27 | **-0.97** | 0.12 | **-0.99** | -0.06 |
| 47 | 1-(3-Methoxyphenyl)ethanone | 3-Acetylanisole | -0.66 | -0.59 | **0.98** | -0.02 | 0.56 | -0.48 |

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