**Coffee aroma: Chemometric comparison of the chemical information provided by three different samplings combined with GC–MS to describe the sensory properties in cup**

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Abstract: This study is part of a wider project aiming to correlate the chemical composition of the coffee volatile fraction to its sensory properties with the end-goal of developing an instrumental analysis approach complementary to human sensory profiling. The proposed investigation strategy compares the chemical information concerning coffee aroma and flavor obtained with HS-SPME of the ground coffee and in-solution SBSE/SPME sampling combined with GC-MS to evaluate their compatibility with the cupping evaluation for quality control purposes. Roasted coffee samples with specific sensory properties were analyzed. The chemical results obtained by the three samplings were compared through multivariate analysis, and related to the samples' sensory attributes. Despite the differences between the three sampling approaches, data processing showed that the three methods provide the same kind of chemical information useful for sample discrimination, and that they could be used interchangeably to sample the coffee aroma and flavor.

## HIGHLIGHTS

- Three different sampling of Coffee Aroma were tested simulating the SCAA cupping
- Chemometric comparison of the sampling approaches shows their interchangeability
- The discrimination role of the aroma compounds in each sampling depends on their properties
- Sample sensory description agrees with chemical output

# Coffee aroma: chemometric comparison of the chemical information provided by three different samplings combined with GC-MS to describe the sensory properties in cup

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**ABSTRACT**

This study is part of a wider project aiming to correlate the chemical composition of the coffee volatile fraction to its sensory properties with the end-goal of developing an instrumental analysis approach complementary to human sensory profiling. The proposed investigation strategy compares the chemical information concerning coffee aroma and flavor obtained with HS-SPME of the ground coffee and *in*-solution SBSE/SPME sampling combined with GC-MS to evaluate their compatibility with the cupping evaluation for quality control purposes. Roasted coffee samples with specific sensory properties were analyzed. The chemical results obtained by the three samplings were compared through multivariate analysis, and related to the samples' sensory attributes. Despite the differences between the three sampling approaches, data processing showed that the three methods provide the same kind of chemical information useful for sample discrimination, and that they could be used interchangeably to sample the coffee aroma and flavor.

*Keywords: aroma and flavor, HS-SPME, SBSE, GC-MS, chemometric, sensory properties, coffee*

## 1. INTRODUCTION

The quality of a cup of coffee and its distinctive sensory properties depend on the entire production chain. Some of the major factors influencing the final product are: geographical origin, climate, species, harvesting methods, technological processing (mainly roasting and grinding), storage conditions, and last but no less important, the brewing method (International Trade Centre, 2011; Sunarharum, Williams, & Smyth, 2014).

Aroma and flavor are undoubtedly important hedonic aspects of a good coffee (Sunarharum et al., 2014), and thus these two aspects should be carefully considered in coffee classification during coffee-bean selection, in addition to their physical aspects, such as size, color and defective beans (http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=47950S). The Cupping Protocol of the Specialty Coffee Association of America (SCCA) (http://www.scaa.org/PDF/resources/cupping-protocols.pdf) provides an international standard for cup evaluation that, besides aroma and taste, also considers kind of roasting, equipment, and cupping preparation, among other factors. Assessment of sensory attributes consists of scoring the aroma, by smelling the dry milled sample and water infusion (Steps 1 and 2) and the flavor plus other attributes, such as aftertaste, acidity, body, and balance, by tasting the brew (Step 3).

A number of studies, some of them involving molecular sensory science, have been carried out to understand the chemistry behind the overall sensory perception given by a cup of coffee, in order to identify and define key aroma and flavor compounds (Blank, Alina, & Grosch, 1992; Czerny & Grosch, 2000; Flament, 2002; Frank, Zehentbauer, & Hofmann, 2006; Nebesny & Budryn 2006; Nebesny, Budryn, Kula. & Majda, 2007; Budryn, Nebesny, Kula J., Majda & Krysiak, 2011; Sunarharum et al., 2014). Different analytical platforms have been used to study coffee aroma; gas-chromatography mass spectrometry and/or olfactometry (GC-MS, GC-O) were the analytical techniques of choice. Conversely, several sampling approaches were used to extract and concentrate the flavor components directly from the ground coffee (powder) and/or from the coffee brew, including steam distillation (SD), solvent extraction (SE), fractionation of solvent extracts, simultaneous distillation–extraction (SDE), supercritical fluid extraction (SFE), pressurized-fluid extraction, Soxhlet extraction, solvent-assisted flavor evaporation (SAFE), microwave-assisted hydrodistillation (MAHD), headspace (HS) techniques, and solid-phase microextraction (SPME) (Picó,2012). Whatever the approach, sample preparation is still the bottle-

neck of the analytical process, since it must provide a consistent and meaningful picture of the sensory-informative components. An effective sample preparation technique requires some key requisites, including (a) the possibility of tuning extraction selectivity by modifying physico-chemical characteristics of extractants and sampling conditions; (b) use of methods involving mild interactions to limit artifact formations (e.g. partition (sorption) *versus* adsorption as extraction mechanism); (c) the possibility of full automation, and of integrating the extraction step with the analytical system.

However, both compositional data and sensory information alone do not fully explain the importance of key compounds, nor indicate which of them cause distinct sensory attributes. Recently, Dunkel et al. (2014) considered more than 10,000 volatiles detected in food, and determined that the specific odor code of a food is due to between 3 and 40 key odorants. Moreover, flavor implies a multisensory process involving distinct sensory properties (mainly odors and tastes) that are closely integrated and reinforce one another (Chiralertpong, Acree, Barnard, & Siebert, 2008; Köster & Mojet, 2007). These interactions may be due to different compounds that mutually influence the perceived flavor, involving interactions between odorants (odor synesthesia) and/or odorants and tastes (chemesthesis) (Prescott, 2015). An important contribution to clarifying how our sense of olfaction deconvolves a complex food odor at the molecular level has been made by the genetic codification of the olfactory receptors, and the exploration of the chemistry-biology synergism of olfaction (Dunkel et al., 2014; Sunarharum et al., 2014). Very recently, Geithe et al. demonstrated that a recombined butter aroma, resulting from four odor-active compounds, each tested on *in vitro* class-I odor receptors, showed different and concentration-dependent patterns of activation (Geithe, Andersen, Malki, & Krautwurst, 2015).

Although several studies have sought to clarify the link between sensory properties and chemical composition, including through multivariate data analysis (MVA) (Bhumiratana, Adhikari, & Chambers, 2011; Liberto et al., 2013; Michishita et al., 2010; Ribeiro, Augusto, Salva, & Ferreira, 2012; Ribeiro, Augusto, Salva, Thomaziello, & Ferreira, 2009; Ruosi et al., 2012; Science, Pérez-Martínez, Sopelana, de Peña, & Cid, 2008; Sunarharum et al., 2014), the challenge of explaining the pleasure of a coffee-experience at the molecular level still remains, mostly because of the limits of the strategies used to collect information (number and kind of samples, standardization of the samples, precision and accuracy) (Ongo et al., 2012).

This study is part of a wider project exploring the correlation between the chemical composition of coffee volatile fraction and the sensory properties of the beverage; the end-goal is to develop an instrumental analysis approach complementary to human sensory profiling (Bhumiratana et al., 2011; Chiralertpong et al., 2008; Lindinger et al., 2008; Michishita et al., 2010). In particular the study compares chemical information related to coffee aroma and flavor obtained with three different sampling approaches, combined in on-line or in off-line mode with GC-MS, taking the SCAA protocols for cup evaluation as reference. Because of the wide range of volatility, water solubility, and concentration of the most significant components of the coffee matrix, three different sampling approaches were tested for the reliability of characterization of the aroma and flavor profiles, and to evaluate their compatibility with the cupping evaluation in coffee selection for quality control. Aroma evaluation (steps 1 and 2 of the SCAA cupping protocol) was associated to Headspace Solid Phase Microextraction (HS-SPME) of roasted coffee powders and the corresponding brews; aroma and taste evaluation (step 3) was combined with *in*-solution sampling of the brew by SBSE (Stir Bar Sorptive Extraction). The ability of each optimized method to discriminate and describe the investigated samples was compared by multivariate analysis, to determine whether it provided consistent and/or complementary information also in connection to the sample sensory properties defined by a trained panel according to SCAA cupping protocols.

## 2. Materials and Methods

**2.1 Reagents and Matrices.** Coffees samples, consisting of roasted coffee ground to suit a coffee-filter machine, were kindly supplied over a period of 9 months by Lavazza Srl (Turin, Italy).

Eight coffee samples with distinctive sensory notes, originating from different countries (Ethiopia, Papua New Guinea, Colombia, Brazil, India, Indonesia, Java, and Uganda), of the species *Coffea Arabica* L. (Arabica) and *Coffea canephora Pierre* (Robusta), were analyzed (Table 1). Each coffee origin was analyzed in five replicates; each replicate was produced by a fresh cycle of roasting and grinding, starting from the same batch of green coffee beans (n=40). The roasting degree of each sample was carefully measured by ground bean light reflectance, with a single-beam Neuhaus Neotec Color Test II instrument (Genderkesee, Germany) at a wavelength of 900 nm on 25-30g of ground coffee. Roasting degree was set at 55°Nh, in order to be close to the international standardization protocol for cupping (SCAA, 2015). Samples were roasted within 24 hours prior to

147 cupping, and left for at least 8 hours to stabilize. For clarity of exposition, samples in the text are
148 labeled with their origins.
149 The coffee brew was prepared from 18g of coffee powder and 300mL of water, using a Lavazza
150 "Xlong" coffee filter machine. Tridecane (*n*-$C_{13}$) in Dibuthylphtalate (DBP), used as internal
151 standard (ISTD), were purchased from Sigma-Aldrich (Milan-Italy).
152
153 **2.2 Sample preparation techniques.** *HS-SPME of the coffee powder*: 1.500 ± 0.010 g of powder
154 were weighed in a septum-sealed gas vial (20mL); the resulting headspace was sampled through
155 the PDMS/DVB SPME fiber for 40 minutes at 50°C with an agitation speed of 350rpm. The internal
156 standard was loaded onto the fiber (Wang, O'Reilly, Chen, & Pawliszyn, 2005) in advance by
157 sampling 5µL of a 1000mg/L solution of *n*-$C_{13}$ in DBP into a 20mL headspace vial for 20 min at
158 50°C, agitation speed of 350rpm.
159 *HS-SPME of the brew*: a volume of 4.5mL of brew in a septum-sealed gas vial (20mL) were sampled
160 through the SPME fiber for 40 min at 50°C with an agitation speed of 350rpm. The internal
161 standard was loaded onto the SPME fiber in advance by sampling 5µL of a 1000mg/L *n*-$C_{13}$ in DBP
162 solution in a 20mL headspace vial for 20 min at 50°C, agitation speed of 350rpm (Wang et al.,
163 2005).
164 *SBSE of the brew*: a volume of 13mL of the brew in a 20mL septum-sealed glass vial were added to
165 5mL of the 1mg/L *n*-$C_{13}$ in water solution and sampled with a PDMS Twister® for 40 min at 50°C.
166 Brew preparation is already described in paragraph 2.1. Each sample was analyzed twice with each
167 of the sampling methods adopted.
168
169 **2.3 Standardization of sampling techniques.** SPME devices and PDMS/DVB fused silica 1 cm long
170 fibers from the same lot were from Supelco (Bellefonte, PA, USA). Before use, all fibers were
171 conditioned as recommended by the manufacturer, and tested to evaluate the consistency of their
172 performance *versus* a reference roasted coffee sample (Bicchi, Cordero, Liberto, Sgorbini, &
173 Rubiolo, 2007). Normalized peak areas collected from the entire set of analyses (three replicates
174 per sample) and from all fibers (n=9) were submitted to analysis of variance (ANOVA). Only fibers
175 that do not showed statistical differences through the one-way ANOVA test (confidence interval
176 95%). The same protocol was applied to SBSE devices (1cm x 0.5mm PDMS coated Twister®,
177 Gerstel GmbH & Co. KG).
178

**2.4 Analysis Conditions.** *HS-SPME analysis* was carried out with a QP2010 GC-MS system (Shimadzu - Milan, Italy) equipped with an autosampler combi-PAL AOC 5000 Autoinjector (Shimadzu - Milan, Italy).

*SBSE sampled* analytes were thermally desorbed from the Twisters® using a thermal desorption system (TDS-2; Gerstel, Mülheim, Germany) installed on an Agilent 6890plus gas chromatograph coupled with a MSD Agilent 5973D. A cooled injection system (CIS-4PTV; Gerstel, Mülheim, Germany) was used to focus the thermally desorbed analytes cryogenically at -50 °C with liquid carbon dioxide.

*HS-SPME-GC-MS chromatographic conditions:* injector temperature: 230°C; injection mode, splitless; carrier gas, helium (2mL/min); fiber desorption time and reconditioning, 5min; column, SGE SolGelwax (100% polyethylene glycol) 30 m x 0.25 mm $d_c$ x 0.25 µm $d_f$ (SGE- Melbourne, Australia); temperature program, from 40°C (1min) to 200°C at 3°C/min, then to 250°C (5min) at 10°C/min. *MS conditions:* ionization mode: EI (70eV); scan range: 35-350 amu; ion source temperature: 200°C; transfer line temperature: 250°C.

*SBSE-GC-MS chromatographic conditions:* injector temperature: 250°C; injection mode, splitless; carrier gas, helium (1mL/min); column, SGE SolGelwax (100% polyethylene glycol) 30 m x 0.25 mm $d_c$ x 0.25 µm $d_f$ (SGE- Melbourne, Australia); temperature program, from -30°C (0min) to 40°C (1min) at 60°C/min, then to 200°C (0min) at 3°C/min, then to 250°C (5 min) at 10°C/min.

*MS conditions:* ionization mode: EI (70eV); scan range: 35-350 amu; ion source temperature: 230°C; transfer line temperature: 280°C.

*TDS temperature program:* from 30°C to 250°C at 60°C/min; hold time at final temperature: 10min; delay time: 0min; initial time: 1 min.

*CIS temperature program:* from -50°C to 250°C at 12°C/s; hold time at final temperature: 5min; equilibration time: 0.1min; initial time: 0 min.

**2.5 Identification of Volatile Components.** Aroma compounds sampled from headspace of powder and from brew were identified by comparing their calculated linear retention indices and their mass spectra to those of authentic samples or, tentatively, to those collected in homemade or commercial libraries (Wiley *7N* and Nist 05 ver 2.0 Mass Spectral Data) or reported in the literature.

**2.6 Sensory analysis**

The fourty samples were submitted to a sensory evaluation by a panel of five experts using 18 g of roasted and ground coffee in 300 mL of hot water according to the SCAA protocols (SCAA, 2014). The protocol implies three tasting steps after roasting to a fixed color (55-60° Nh) and eight hours of sample stabilization: i) evaluation of the aroma by sniffing the dry grounded coffee, ii) evaluation of the aroma by sniffing the brew three minutes after its preparation and stirring, and iii) 8-10 minutes after flavor evaluation. Other attributes such as aftertaste, acidity, body, and balance are evaluated by tasting the brew by spraying it in the mouth to maximize retro-nasal vapors. The cup quality was assessed for several attributes, among them this study considered: flavor (floral, fruity, woody, nutty, spicy), acidity, bitterness, body (mouthfeel), astringency, and overall quality. The quality and intensity of each attribute were evaluated simultaneously by using a scale varying from 1 to 10.

**2.7 Data processing.** Data were collected with a Shimadzu GCMS Solution 2.5SU1, and an Agilent ChemStation D.02.00.275. Principal Component Analysis (PCA) was used to visualize sample groups and to compare information provided by each sampling. PCA based on Pearson correlation coefficient was carried out on normalized ISTD data. Statistical analysis one-way ANOVA and PCA were done by XLSTAT (version 2015.5.01.23164) copyright Addinsoft 1995-2015. non polar

## 3. RESULTS AND DISCUSSION

The objective evaluation of coffee quality, by correlating chemical analysis and sensory properties, requires an analytical platform that provides information appropriate to describing the human sensory experience. Coffee powder and brew, evaluated through SCAA protocols, were thus analyzed with three different sampling methods, each combined with GC-MS; this resulted in chemical information describing the coffee aroma and flavor that was in line with that employed for cup evaluation. In the following, for short, the analytical platform will be identified by the sampling used, its on-line or off-line combination with GC-MS being implicit.

### 3.1 Samplings comparison

A total of 117 compounds were identified (or tentatively identified) (20 compounds were unknown or not identified unequivocally) with the above platforms. **Table 1 SM** (supplementary material) reports the list of the compounds identified with each sampling with their Linear

242 Retention Indices ($I^T$s). The highest number of compounds (96) were identified in the headspace of
243 the coffee powder, followed by HS-SPME (72) and SBSE (53) of the brew.
244 The chemometric approach (PCA) was used to obtain as much information as possible from the
245 three sampling methods: each sample (observations) is described by different compounds
246 (variables), with their own analytical response. **Figure 1** reports the PCA score plots of a) HS-SPME
247 of coffee powder, b) HS-SPME of coffee brew, and c) SBSE of coffee brew. The comparison of the
248 PCA results from the brews sampled by HS-SPME (b) and SBSE (c) shows a similar distribution of
249 the samples on the score plot. Similar discrimination of samples is also obtained by the HS-SPME
250 of the powder (a); this means that independently of the sampling approach applied, the
251 information derived from the chemical profiles of the samples is the same, as it is also evident
252 from the total explained variance obtained with PCA elaborations. Two large groups were
253 recognizable along the PC2 that, as expected, were chiefly characterized by species, i.e. Arabica or
254 Robusta. INDIA samples were the only exception, being close to Robusta samples although
255 classified as Arabica. Analysis of Robusta sample profiles showed that specimens from Indonesia
256 (INDO) can clearly be discriminated from the two other origins (JAV and UGA) on the first two PCs
257 (**Figure 2**). PCA analysis on Arabica samples showed similar distribution for the three different
258 sampling approaches (**Figure 1 SM)**.
259
260 **3.2 Investigation on discriminant aroma compounds with the different sampling approaches**
261 The volatiles directly responsible for discrimination of the Robusta samples deriving from the
262 vector projections of the original variables on PC1 and PC2 (variable $\cos^2$) are listed in **Table 2,**
263 together with their odor description. For the sake of clarity, these components will henceforth be
264 indicated as Direct Discriminant Compounds (DDCs). PCA determined different DDCs for each
265 sampling method, partly because the methods are based on different principles, employ different
266 sampling materials (PDMS/DVB SPME fibers for headspace, and PDMS Twisters® for *in*-solution
267 sampling), and are applied to different matrices (coffee powder and brew) (**Table 2**). Further,
268 compounds directly responsible for sample discrimination in SBSE sampling of the brew, which
269 may be considered the most representative sampling technique for flavor evaluation, cannot be
270 the same as those for HS-SPME sampling of the coffee powder, because the intrinsic physical-
271 chemical properties of those compounds influence their recovery. The relationship between the
272 role of each compound in sample discrimination and their physico-chemical properties (EPI Suite
273 v3.10 developed by the EPA's Office of Pollution Prevention Toxics NS Syracuse Research

Corporation (SRS) 2000 U.S.) was thus studied, to investigate in greater depth why different compounds may play the same roles in sample discrimination, independently of the technique adopted. Most of the DDCs with SBSE of the brew are slightly soluble in water and relatively non polar, i.e. with high $k_{o/w}$ (**Table 2**). Conversely, DDCs in the HS-SPME volatile fraction of the coffee powder generally present high volatility (expressed as Vapour Pressure, VP) and low $k_{o/w}$ (below 1) (**Table 2**). Similarly to SBSE, HS-SPME of the brew includes compounds extracted during brewing whose relatively high water solubility has less influence on the composition of the headspace, since they are retained in the aqueous phase (Mestdagh, Davidek, Chaumonteuil, Folmer, & Blank, 2014; Sgorbini et al., 2012). Moreover, the coffee powder may be considered a fatty matrix, and thus polarity may also influence migration into the headspace, and non polar compounds (high $k_{o/w}$ values) may undergo a more severe matrix effect.

These considerations are clearly explained by the comparison of normalized percent areas of some DDCs obtained with the three sampling approaches. 3-Ethyl pyridine and furfural (i.e. two DDCs with similar physico-chemical characteristics) are differently recovered by SBSE, 3-ethyl pyridine predominating because of its higher $k_{o/w}$, while furfural, being more polar, is less retained by the fatty matrix and more easily released into the headspace. Conversely, by comparing HS-SPME of the brew to SBSE, the more polar furfural is less recovered than does 3-ethyl pyridine from the headspace of the brew and recovered to a greater extent by SBSE **(Figure 2SM).** Acetoxyacetone is highly concentrated in the headspace of coffee powder, and is recovered better by SBSE than by HS-SPME from the brew, because of its high solubility in water. 1-H-Pyrrole-2-carboxaldehyde contributes similarly to HS-SPME from coffee powder and brew, but having a medium-low $k_{o/w}$, good water solubility and low VP, its accumulation in the headspace is limited.

Moreover, DDCs from SBSE can also be correlated to other compounds from the HS-SPME sampling, “indirect markers” or CDCs (Correlative Discriminant Compounds), which are indirectly involved in the discrimination of the coffee powder by HS-SPME. CDCs can be defined through the Pearson correlation coefficient (r), used here to assess the degree of linear association between variables (peak area vectors) defined by the different samplings, r values > 0.8 were taken as cut-off point. From the chemometric standpoint, variables with high r values with DDCs, within the PCA elaboration of the HS-SPME of coffee powder, are redundant for the purpose of explaining sample behavior with this approach. Therefore some of them may be eliminated without lacking in quality of discrimination, because they are dependent variables and provide the same information of DDCs, in terms of sample definition.

306 The consistency between the three samplings were confirmed by including DDCs of the SBSE in the
307 data correlation matrix of the HS-SPME; resulting in a close correlation with 56 compounds
308 identified in the HS-SPME of the powder. Twenty-four of them were also HS-SPME DDCs, while the
309 remaining 32 were CDCs of this method. **Table 3** reports the compounds identified in the HS-
310 SPME-GC-MS profile of the coffee powder having high r (> 0.8) with SBSE DDCs. This means that
311 DDCs from *in*-solution SBSE sampling, direct (DDCs) or indirect markers (CDCs) of the HS-SPME of
312 the coffee powder, provide chemical information for sample differentiation that is related to the
313 sample different chemical processing and sensory characteristics, and, as a consequence, to their
314 chemical pathways of formation. In other words, a compound that is highly soluble in water may
315 not play a direct role in the discrimination of coffee powder headspace but, thanks to its solubility,
316 it may be solubilized during brewing in large amounts, and thus play an important role in the
317 discrimination of beverages. Conversely, a CDC may have different physico-chemical properties
318 but provides the same kind of chemical information as a DDC in the discrimination of samples with
319 different sensory characteristics***.*** Similar observations can be made for the role played by SBSE
320 DDCs in samples discrimination obtained by the HS-SPME of the brews (**Table** 2 **SM)**. These
321 considerations resulted also valid for the analysis of INDIA Arabica samples (data not reported).
322 The similarity of the sample discrimination achieved by the three sampling approaches indicates
323 not only that they provide complementary data, but also that they may be used interchangeably
324 to discriminate the chemical profiles of a set of samples, and can thus be applied to the problem
325 under study. This can be explained in two complementary ways: a) the first is related to the
326 physico-chemical properties of the components referred to as DDCs, depending on the sampling
327 approach under study; b) the second is due to the (r) value, which correlates compounds
328 indicative of the same change(s) in sample discrimination, and, as a consequence, of a common
329 chemical pathway of formation.
330 This correlation is also clear from the chemical standpoint, if the behavior of groups of compounds
331 of different nature (e.g. guaiacoles, pyridines, pyrazines and furans) is examined. The statistical
332 analysis shows that these compounds are in all cases correlated with one another, irrespective of
333 the sampling used. The comparison of data from the three approaches shows that different
334 classes of compounds change as one, moving in the same direction, and that they always play a
335 role in sample discrimination, irrespective of which component(s) is involved in the discrimination
336 of a specific sampling. The formation pathways of these groups of components are induced by
337 roasting, but also depend on the processing of the green beans. Pyrazines (generally having nutty,

earthy, roasted, and green aromas) and pyridines (fishy note), principally arise from the Maillard reaction of amino acids and sugars, direct pyrolysis of amino acids and degradation of trigonelline. The roasting pathways for guaiacoles (spicy notes), for example, involve the decarboxylation of phenolic carboxylic acids and the thermal degradation of lignin; however, their formation (or concentration) in coffee aroma also depends on bacterial, fungal, and yeast enzymes, and on glyosidic reactions occurring in the green beans (Flament, 2002; Sunarharum et al., 2014). Furans, responsible for malty, caramel, and sweet-roast notes, are formed during the roasting process through the Maillard reaction of carbohydrates, thermal oxidation of lipids, and degradation of thiamine. The discriminant furanic compounds differ with the different sampling methods, but are in any case involved in the discrimination of INDO samples within Robusta, and INDIA samples for Arabica.

### 3.3 Relationship between chemical results and sensory cupping data

A Lavazza-trained panel determined the sensory description of the set of investigated coffee samples. The panel considered the following sensory characteristics: acid, bitter, aromatic intensity, floral, fruity, woody, nutty, spicy, together with body and astringency. Each sensory attribute was classified by the panelists on a scale from 0 to 10, where 0 signified no attribute and 10 a strong sensory attribute. **Figure 3** reports the PCA scores (top) and loading plots (bottom) of the sensory evaluation of the Robusta (left) and Arabica (right) samples. Within the Robusta set, INDO samples were characterized by woody, spicy, and bitter notes; JAVA samples were slightly acid and nutty, and INDO and UGA samples were more spicy and aromatic than those from JAVA. In the Arabica set, INDIA samples were markedly woody and spicy, similarly to Robusta INDO, and presented a bitter note and strong body. BRA samples were astringent and nutty, while KAFA were the most fruity samples, also characterized by stronger acid and floral notes, followed by COL and PNG.

Most DDCs resulting from the chemical investigation in the different sampling approaches are known to be connected with these notes. In a chemometric investigation on Arabica samples, Ribeiro et al. showed that several compounds can be responsible for more than one sensory attribute. For instance, 3-ethyl pyridine may be responsible for acidity, flavor, and bitterness, or 4-vinyl guaiacol for flavor and body. However, when considered as such, their sensory attributes are not always associable to the above characteristics (Ribeiro et al., 2012). In particular, DDCs from the chemometric analysis of INDO and INDIA respectively for Robusta and Arabica samples include

components with sensory notes that can be related directly to the sensory characteristics highlighted for these samples (**Table 1 and 2**). However, the peculiar odor and flavor of these samples are not only related to the presence or absence of some compounds, but also closely depend on their relative concentrations and odor thresholds, which together are responsible for their synergistic or antagonistic effect at the receptorial level, in eliciting the sensory experience. All sampling approaches, even if with different DDCs related together to the sampling peculiarity and compound physico-chemical characteristics, are coherent with the discrimination obtained with sensory evaluation. However, the direct HS-SPME sampling of the powder requires a limited sample manipulation since it does not include the brewing step, avoids possible water interference with the GC analysis, and results in a quicker analytical screening because of automation and shorter sampling procedure.

## 4. Conclusion

Coffee samples were analyzed with three sampling approaches (HS-SPME of the coffee powder, HS-SPME of the brew, and *in*-solution SBSE of the brew) coupled with GC-MS; each sampling can be considered as a part of the sensory experience perceived during cupping coffee evaluation. Despite the differences between the three sampling approaches, data processing showed that the three methods provide the same kind of chemical information useful for sample discrimination, and that they could be used interchangeably to sample the coffee aroma and flavor. Comparison of the multivariate analysis of the sensory data with the chemical fingerprint of the investigated samples showed that: a) sensory and chemical data are in good agreement, and b) sensory evaluation can be related to the different chemical composition of the samples investigated. The choice of sampling technique used for this purpose may thus be guided by factors such as simplicity, sensitivity, reliability, and possibility of automation. As a consequence, HS-SPME of the coffee powder is the approach providing the most satisfactory performance, because: a) the direct sampling of coffee powder does not require further operations, while the brewing process may be a source of variability, b) HS-SPME affords full and easier automation of the analytical procedure, and c) HS-SPME of the coffee powder provides the largest number of identified (or tentatively identified) components.

Further in-depth studies will be necessary to correlate groups of compounds to a specific sensory note characterizing coffee samples, and to enable the development of a predictive model to support sensory panels in their sensory evaluation of coffee samples. In addition, knowledge on

the odor active compounds correlated to a characteristic note, the concentrations of these compounds and their interactions, may open new perspectives in understanding the biological mechanisms underlying the pleasure related to the aroma and flavor of coffee.

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**Further Reading**

http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=47950S [Retreived October 2015]

http://www.scaa.org/PDF/resources/cupping-protocols.pdf [December 2015]

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485 **Tables**

486 **Table 1** List and characteristics of the coffee samples used in this study.

487

488 **Table 2** DDCs extracted from processing Robusta samples. Brews sampled by SBSE or HS-SPME
489 and HS-SPME of the powder, with their relative odor descriptors and physico-chemical properties.
490 Letters near the name indicate the sampling approaches where each compound was recovered:
491 SBSE: A; HS-SPME pow: B; HS-SPME brew: C. * The Good Scents Company, [&] http://www.iso.org, [+]
492 Blank et al.

493

494 **Table 3** Compounds present in HS-SPME of the powder that are closely correlated with DDCs of
495 SBSE. The DDCs in common between the two sampling techniques are in bold type. Compounds
496 with a direct discriminant role in SBSE or HS-SPME of coffee powder are marked with an X; indirect
497 markers (CDCs) are in italics.

498

499 **Table 1 SM** List of identified and *tentatively identified compounds in all sampling methods.

500

501 **Table 2 SM** Compounds present in HS-SPME of the brew that are closely correlated with DDC of
502 SBSE. The DDCs in common between the two sampling techniques are in bold type. Compounds
503 with a direct discriminant role in SBSE or in HS-SPME of the brew are marked with an X; HS-SPME
504 brew indirect markers (CDCs) are in italics.

505

506

507 **Figures**

508 **Figure 1** PCA score plots of a) HS-SPME of the coffee powder; b) HS-SPME of the brew; c) SBSE of
509 the brew. Autoscale pre-processing. Legend: BRA: □; COL: ◊; JAV: Δ; UGA: X; PNG: *; INDIA: -;
510 INDO: ◯; KAFA: +

511

512 **Figure 2** Robusta PCA score plots: a) HS-SPME of the coffee powder b) HS-SPME of the brews; c)
513 SBSE of the brews. Autoscale pre-processing Legend: JAV: Δ; UGA: X; INDO: ◯

514

515 **Figure 3** PCA scores (top) and loading plots (bottom) of the sensory evaluation of Robusta (left)
516 and Arabica (right) samples Legend: BRA: □; COL: ◊; JAV: Δ; UGA: X; PNG: *; INDIA: -; INDO: ◯;
517 KAFA: +. • active variables attributes sensory scores, ▲supplementary variables origin

518

519 **Figure 1 SM** Arabica PCA score plots: a) HS-SPME of the coffee powder b) HS-SPME of the brews;
520 c) SBSE of the brews. Autoscale pre-processing Legend: : BRA: □; COL: ◊; PNG: *; INDIA: -; KAFA: +

521

522 **Figure 2 SM** Comparison between normalized percentage contributions of the common direct
523 discriminant compounds in the three sampling approaches under study.

524

**Table(s)**

**Table 1** List and characteristics of the coffee samples used in this study.

| ***Sample acronym*** | ***Sample Name*** | ***Species*** | ***Treatment*** | ***Sensorial Attribute*** |
|---|---|---|---|---|
| *BRA* | BRAZIL LA2 | Arabica | Natural | Nutty, quite acid, rich |
| *COL* | COLOMBIA CL1 | Arabica | Washed | Flowery, Acid |
| *JAV* | JAVA WB1 MB | Robusta | Washed | Nutty |
| *UGA* | UGANDA STD | Robusta | Natural | Spicy |
| *PNG* | PAPUA NG Y | Arabica | Washed | Fruity |
| *INDIA* | INDIA ARAB CHERRY | Arabica | Natural | Astringent, quite bitter |
| *INDO* | INDONESIA EK1 | Robusta | Natural | Woody, Bitter |
| *KAFA* | ETIOPIA KAFA GR. 3 | Arabica | Natural | Flowery/Fruity, rather Acid |

**Table 2** DDCs extracted from processing Robusta samples. Brews sampled by SBSE or HS-SPME and HS-SPME of the powder, with their relative odor descriptors and physico-chemical properties. Letters near the name indicate the sampling approaches where each compound was recovered: SBSE: A; HS-SPME pow: B; HS-SPME brew: C. * The Good Scents Company, [&] http://www.iso.org, [+] Blank et al.

| *Compound Name* | *Odour Description*[*, &, +] | *Water solubility (mg/L)* | *Log $K_{O/W}$* | *VP (mm Hg at 25 °C)* | *Henrys LC (VP/Wsol) (atm-m3/mole)* |
|---|---|---|---|---|---|
| *1-acetyl-1,4-dihydropyridine* ***(C )*** | - | - | - | - | - |
| *1H-Pyrrole-2-carboxaldehyde (**A; B; C)*** | Musty | 3.43E+04 | 0.6 | 0.09 | 3.13E-07 |
| *1-Hydroxy-2-butanone* ***(B )*** | Sweet coffee musty grain malt butterscotch | 7.21E+05 | -0.29 | 0.77 | 1.24E-07 |
| *2,3-Butanedione* ***(B )*** | Buttery | 2.00E+05 | -1.34 | 56.8 | 7.95E-06 |
| *2,3-Pentanedione* ***(B; C )*** | Buttery | 6.16E+05 | -0.85 | 31.1 | 6.65E-06 |
| *2-Butanone, 3-hydroxy-* ***(B )*** | Buttery | 8.33E+05 | -0.36 | 2 | 2.78E-07 |
| *2-cyclopenten-1-one, 2-hydroxy-3-methyl-* ***(C )*** | Caramellic-spicy, maple-like | 8.50E+03 | 1.29 | 0 | 6.68E-08 |
| *2-Furancarboxaldehyde, 5-methyl-* ***(A )*** | Caramel | 2.91E+04 | 0.67 | 1.38 | |
| *2-furfuryl-5-methylfurane* ***(B )*** | - | 6.40E+01 | 1.96 | 2.89 | 1.96E-04 |
| *2-Furfurylfuran* ***(B; C)*** | Roast | 2.14E+02 | 2.99 | 0.26 | 2.36E-04 |
| *2-Oxopropylpropanoate* ***(B )*** | - | 1.10E+04 | 1.2 | 31.5 | 4.02E-04 |
| *2-Propanone, 1-hydroxy-* ***(B )*** | Caramel | 7.44E+01 | -0.78 | 1.74 | 1.70E-07 |
| *2-Vinyl-5-methylfuran* ***(B; C)*** | - | 2.21E+03 | 1.96 | 2.89 | 1.96E-04 |
| *3(2H)-Furanone, 2,5-dimethyl-* ***(B )*** | Fruity, caramellic | 4.63E+04 | 0.43 | 1.66 | 5.29E-06 |
| *4-Ethylguaiacol* ***(A )*** | Spicy | 6.94E+02 | 2.38 | 0.02 | |
| *5 Methyl Furfural* ***(B;C)*** | Caramel | 2.91E+04 | 0.67 | 0.69 | 3.41E-06 |
| *Acetaldehyde* ***(B )*** | Pungent ethereal aldehydic fruity | 2.57E+05 | -0.34 | 910 | 1.72E-04 |
| *Acetic acid* ***(B )*** | sharp pungent sour vinegar | 4.76E+05 | -0.17 | 15.7 | 2.86E-06 |
| *Acetoxyacetone* ***(A; B; C)*** | Fruity | 1.52E+05 | -0.19 | 1.49 | 1.50E-06 |
| *Benzaldehyde* ***(A )*** | Strong sharp sweet bitter almond cherry | 6.10E+03 | 1.71 | 1.01 | |
| *Butanal, 3-methyl-* ***(C )*** | Aldehydic | 1.12E+04 | 1.23 | 51.6 | 5.21E-04 |
| *Difurfuryl ether* ***(C )*** | Coffee, nutty, earthy | 7.11E+02 | 2.22 | 0.02 | 7.48E-06 |
| *Furan, 2-(2-furanylmethyl)-5-methyl-* ***(A )*** | Hearthy, mushroom | 6.41E+01 | 3.53 | 0.07 | |
| *Furan, 2,2'-methylenebis-* ***(A )*** | Roast | 2.17E+02 | 2.99 | 0.26 | |
| *Furfural* ***(A, B; C)*** | sweet woody almond fragrant baked bread | 5.36E+04 | 0.83 | 2.32 | 5.48E-06 |
| *Furfuryl methyl sulphide* ***(A )*** | Vegetable | 1.84E+03 | 2 | 1.37 | |
| *Guaiacol* ***(C )*** | Spicy | 2.09E+03 | 1.88 | 0.06 | 5.16E-06 |
| *4-ethyl-guaiacol* ***(C )*** | Spicy | 6.94E+02 | 2.38 | 0.02 | 7.16E-06 |
| *4-vinyl-guaiacol* ***(C )*** | Woody | 9.26E+02 | 2.24 | 0.01 | 1.64E-06 |
| *Hexanal* ***(B )*** | fresh green fatty aldehydic grass leafy fruity sweaty | 3.52E+03 | 1.78 | 9.57 | 3.58E-04 |
| *Pyridine, 3-ethyl-* ***(A; B; C)*** | Tobacco | 8.48E+04 | 1.84 | 2.53 | 3.29E-06 |

**Table 3** Compounds present in HS-SPME of the powder that are closely correlated with DDCs of SBSE. The DDCs in common between the two sampling techniques are in bold type. Compounds with a direct discriminant role in SBSE or HS-SPME of coffee powder are marked with an X; indirect markers (CDCs) are in italics.

| ***Compounds*** | ***DDCs in SBSE of the brew*** | ***DDCs in HS-SPME of the powder*** |
|---|---|---|
| 1-Hydroxy-2-butanone | | X |
| **1H-Pyrrole-2-carboxaldehyde** | **X** | **X** |
| *1H-Pyrrole-2-carboxaldehyde, 1-methyl-* | | |
| *2-acetylpyrrole* | | |
| *2-butanone* | | |
| 2-Butanone, 3-hydroxy- | | X |
| 2-oxopropylpropanoate | | X |
| *2-Cyclopenten-1-one, 2-hydroxy-3-methyl-* | | |
| 2-furfurylfuran | | X |
| *2-n-propylpyrazine* | | |
| 2-Propanone, 1-hydroxy- | | X |
| 2-Vinyl-5-methylfuran | | X |
| 2,3-butanedione | | X |
| 2,3-pentanedione | | X |
| *2-cyclopenten-1-one 3 methyl+ 3,5-diethyl-2-methylpyrazine* | | |
| 3(2H)-Furanone, 2,5-dimethyl- | | X |
| 5 methyl furfural | | X |
| Acetic acid | | X |
| *Acetone* | | |
| **Acetoxyacetone** | **X** | **X** |
| Acetylfuran | | |
| *Ethanone, 1-(1-methyl-1H-pyrrol-2-yl)- + 2-acetyl-5-methyl pyrrole* | | |
| *Furan, 2-methyl-* | | |
| **Furfural** | **X** | **X** |
| *Furfuryl alcohol* | | |
| *Furfurylformate* | | |
| Furfuryl methyl sulphide | X | |
| *Guaiacol* | | |
| *4-ethyl-guaiacol* | X | |
| Hexanal | | X |
| *Methyl acetate* | | |
| *Pyrazine, (1-methylethenyl)-* | | |
| *Pyrazine, 2-ethyl-3-methyl- + Pyrazyne, trimethyl* | | |
| *Pyrazine, 2-methyl-6-(1-propenyl)-* | | |
| *Pyrazine, 2,3-dimethyl-* | | |
| *Pyrazine, 2,6-diethyl-* | | |
| *Pyrazine, 3,5-diethyl-2-methyl-* | | |
| *Pyridine* | | |
| **Pyridine, 3-ethyl-** | **X** | **X** |
| Unknown 1 | | X |

| | |
|---|---|
| *Furfurylpentanoate + other unknown compounds* | |
| Unknown 12 | X |
| Unknown 13 | X |
| Unknown 14 | X |
| Unknown 17 | X |
| Unknown 2 | X |
| Unknown 21 | X |
| *Difurfuryl ether* | |
| *Unknown 23b* | |
| *(5h)-5-methyl-6,7-dihydrocyclopentapyrazine* | |
| *Unknown 6* | |
| *2-isopropenylpyrazine* | |
| *2,5-dihydro-3,5-dimethyl-2-furanone* | |
| *2-furfuryl-5-methylfurane* | |

**Figure(s)**

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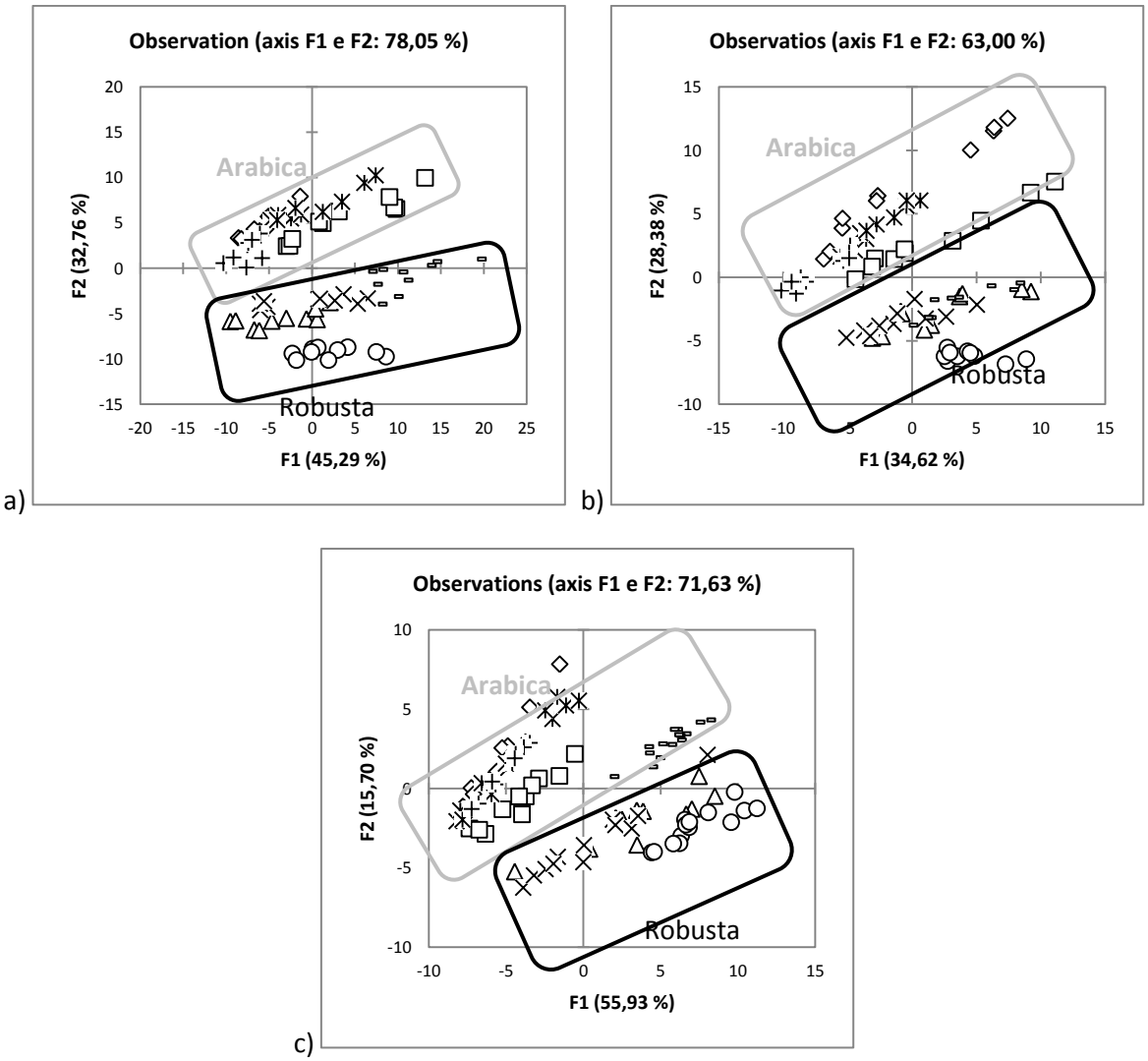


**Figure 1**

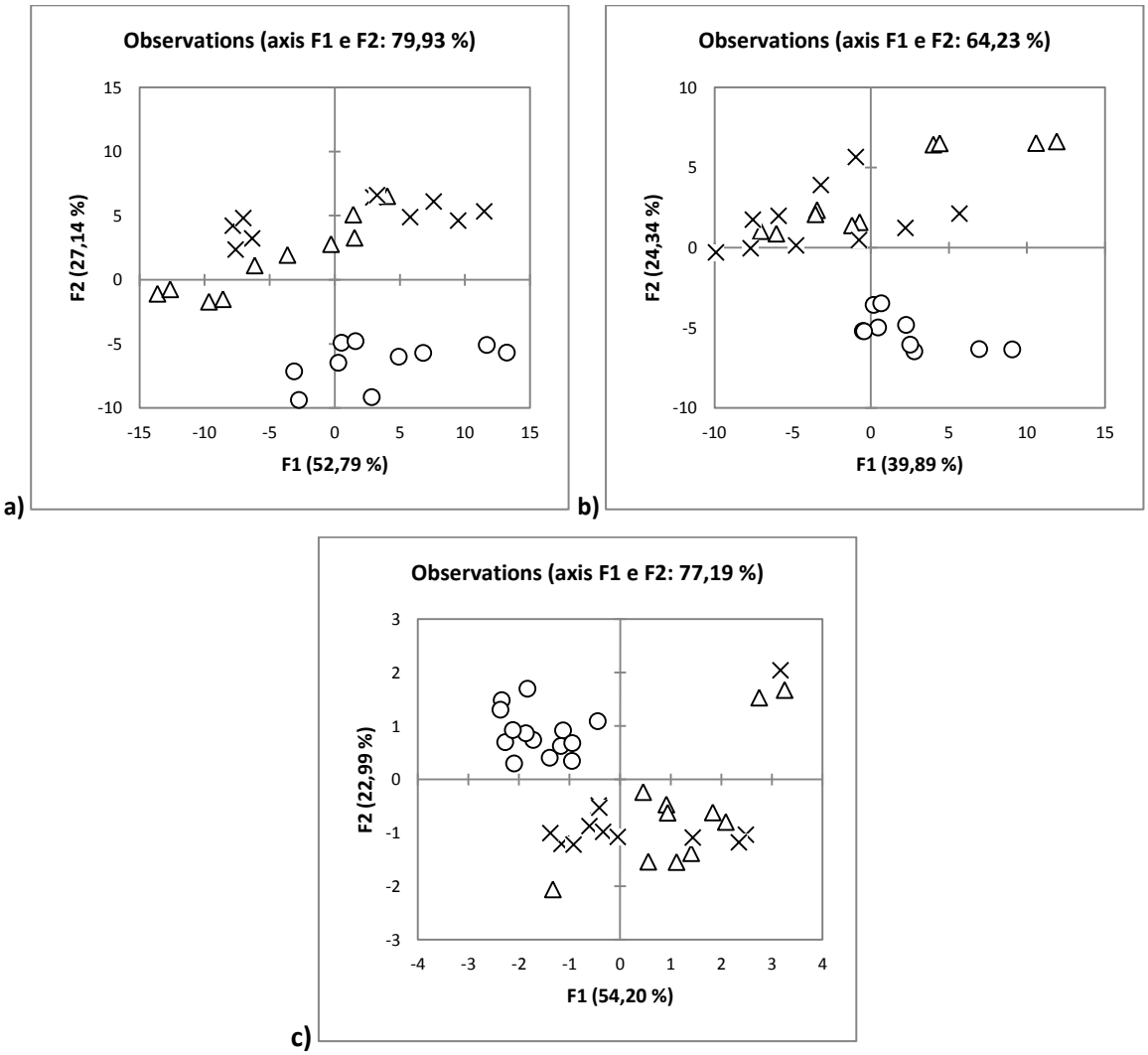
Observations (axis F1 e F2: 79,93 %)
F2 (27,14 %)
F1 (52,79 %)
a)
Observations (axis F1 e F2: 64,23 %)
F2 (24,34 %)
F1 (39,89 %)
b)
Observations (axis F1 e F2: 77,19 %)
F2 (22,99 %)
F1 (54,20 %)
c)

**Figure 2**

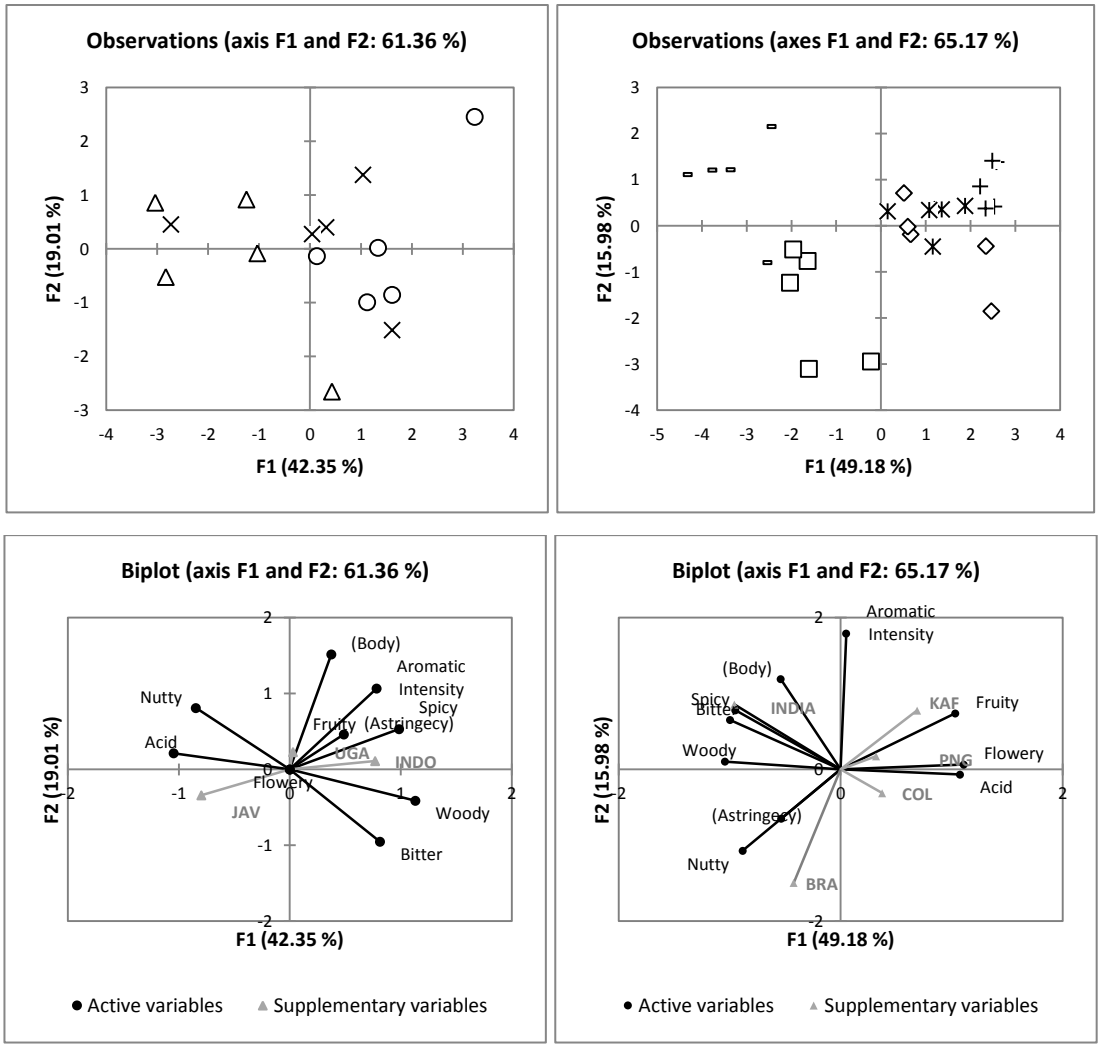
Observations (axis F1 and F2: 61.36 %)
F2 (19.01 %)
F1 (42.35 %)
Observations (axes F1 and F2: 65.17 %)
F2 (15.98 %)
F1 (49.18 %)
Biplot (axis F1 and F2: 61.36 %)
(Body)
Aromatic
Intensity
Spicy
Fruity
(Astringecy)
Nutty
Acid
Flowery
Woody
Bitter
UGA
INDO
JAV
Active variables
Supplementary variables
Biplot (axis F1 and F2: 65.17 %)
Aromatic
Intensity
(Body)
Spicy
Bitter
INDIA
KAF
Fruity
Woody
PNG
Flowery
Acid
COL
(Astringecy)
Nutty
BRA
Active variables
Supplementary variables

**Figure 3**

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