TITLE: Contribution of volatile compounds to the antioxidant capacity of coffee

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ABSTRACT

Heterocyclic volatile compounds present in coffee have been proposed as potent antioxidants, but their contribution to the antioxidant capacity of coffee is still unclear and controversial. The aim of this study was to assess the actual contribution of the main volatile compounds to the overall antioxidant capacity of coffee. A total of sixty-two and sixty-four volatile compounds were identified and quantified in Arabica and Robusta coffee, respectively, by static headspace-gas chromatography-mass spectrometry (SH-GC-MS). ABTS (2,2'-Azino-bi(3-ethylbenzo-thiazonile-6-sulfonic acid) diammonium salt) and DPPH (2,2-Diphenyl-1-picrylhydrazyl) antioxidant activity of the most abundant volatile heterocyclic compounds (7 furans (Fu), 3 pyrroles (Py) and 2 thiophenes(Th)), aldehydes (5) and diketones (2) was evaluated in model systems at different concentrations including those found in coffee. The model system with all the heterocyclic volatiles (Fu-Py-Th) was the most active followed by pyrroles and furans. Thiophenes were ineffective as radical scavengers at all concentrations including 100-fold, and aldehydes and ketones showed negligible activities in comparison to heterocyclic volatiles. In addition, only furans exhibited linear concentration dependent ABTS antioxidant activity and individual volatiles model systems showed that only 2-methyl-tetrahydrofuran-3-one and pyrrole for ABTS, and also 1-methylpyrrole for DPPH, were the main volatile compounds responsible for the coffee antioxidant activity. However, the contribution of the heterocyclic volatile compounds to the overall antioxidant capacity of a filter coffee brew was almost insignificant, even at 100-fold concentrated Fu-Py-Th model system, accounting only for up to 3.3%.

KEYWORDS: antioxidants; aroma; coffee; GC/MS; volatiles

1 INTRODUCTION

Coffee is well known as a good source of antioxidants (Esquivel & Jimenez, 2012). The presence of (poly)phenolic compounds, mainly chlorogenic acids, and other major coffee compounds, such as caffeine and melanoidins, and their contribution to the antioxidant capacity of coffee have been widely studied during last decades (Crozier, Jaganath & Clifford, 2009; Delgado-Andrade, Rufian-Henares & Morales, 2005; Ludwig, Sanchez, Caemmerer, Kroh, de Peña & Cid, 2012). However, coffee contains many other minor compounds, such as volatile compounds, that might play a role in the antioxidant capacity of coffee.

Aroma is one of the most valuable properties of coffee. A great variety of volatile compounds, most of them Maillard reaction products (MRP), are generated during roasting of coffee beans at high temperatures. Besides their contribution to aroma and flavor, some typical volatile heterocyclic compounds found in coffee have been investigated for their antioxidant properties. Some authors (Fuster, Mitchell, Ochi, & Shibamoto, 2000; Yanagimoto, Lee, Ochi, & Shibamoto, 2002) analyzed individually the inhibitory effect of isolated volatile compounds on hexanal oxidation and reported considerable antioxidant activity for some pyrrols, furans and thiophenes whereas thiazoles and pyrazines were ineffective antioxidants at all concentrations tested. Later they suggested that some of these volatile compounds were mainly responsible for the antioxidant activity exhibited by a dichloromethane extract of coffee brew (Yanagimoto, Ochi, Lee, & Shibamoto, 2004). Furthermore, typical volatile compounds formed in Maillard reaction model systems have been reported to inhibit oxidation of lipids (Elizalde, Bressa, & Rosa, 1992; Osada & Shibamoto, 2006). Also, other authors observed that certain volatile compounds produced in the roasting process of almonds displayed an antioxidant effect (Severini, Gomes, De Pilli, Romani, & Massini, 2000).

Maillard reaction products may contribute to a higher degree than chlorogenic acids to the overall antioxidant capacity in dark roasted coffee (Smrke, Opitz, Vovk, & Yeretzian, 2013). Although Maillard reaction products include volatiles and non-volatiles, the latter are the most abundant, with melanoidins accounting for up to 25% of coffee dry matter (Belitz, Grosch, & Schieberle, 2009). In a previous study of our research group, we found that coffees with higher antioxidant capacity showed lower amounts of volatile compounds due to the influence of the botanical variety (Arabica or Robusta) and the roasting process (conventional or torrefacto)

(Lopez-Galilea, de Peña, & Cid, 2008b). The application of multivariate studies (Pearson correlations and Principal Component Analysis) to the results also showed that some selected constituents, including heterocyclic volatile compounds, were significantly but negatively correlated with radical quenching activity suggesting a prooxidant capacity. When coffee brew preparation was studied, coffee pressure-brewing procedures, such as espresso and mocha, extracted more volatiles and showed higher antioxidant capacity than plunger and filter procedures, but no significant correlations between volatiles and antioxidant activity were found (Lopez-Galilea, de Peña, & Cid, 2007). Despite the different approaches in the reported studies, the results might not be considered as contradictory if it is taken into account that some compounds proposed as antioxidants may act as pro-oxidants at different doses (Andueza, Manzocco, de Peña, Cid & Nicoli, 2009; Halliwell, 2008) and that most of the works which study the antioxidant activity of volatiles have been carried out using standard compounds at concentration levels higher than those present in coffee. Thus, these results cannot be directly transferred to the knowledge of the contribution of volatiles to the antioxidant capacity of coffee. For the reasons outlined above, the aim of this study was to assess the actual contribution of the main volatile compounds, especially those proposed as potential antioxidants like heterocyclic ones, to the overall antioxidant capacity of coffee. This will reveal whether or not the coffee volatile compounds, and consequently coffee aroma, have a relevant role in the antioxidant capacity of coffee.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

ABTS (2,2'-Azino-bi(3-ethylbenzo-thiazonile-6-sulfonic acid) diammonium salt), potassium persulfate, DPPH· (2,2-Diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, and pure reference standards for dimethyl sulfide, dimethyl disulfide, 2-furanmethanethiol, acetaldehyde, propanal, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, hexanal, furan, 2-methylfuran, 2,5-dimethylfurane, 2-methyl-tetrahydrofuran-3-one, furfural, 2-furfurylacetate, 5-methylfurfural, furfuryl alcohol, 2,3-butanedione, 2,3-pentanedione, 2-methyl-1-propanol, thiophene, 2-methylthiophene,1-methylpyrrole, pyrrole, 2-

formyl-1-methyl-pyrrole, 2-methylpyrazine, phenol, and 1,3-pentadiene were obtained from Sigma-Aldrich (Steinheim, Germany). The methanol was from Panreac (Barcelona, Spain). Pure reference standards of 2-methyl-2-butenal, 3-methylfuran, 2-furfuryl methyl sulfide, 2-acetylfuran, 2-propanone, 2-butanone, 3-penten-2-one, pyridine, pyrazine, and 2,5-dimethylpyrazine were purchased from Acros Organics (Springfield, NJ).

2.2 Coffee samples

Roasted coffee from Colombia (*Coffea arabica*, named Arabica, L* = 24.82±0.81, a*=12.70±0.13, b*=15.11±0.40) and Vietnam (*Coffea canephora* var. robusta, named Robusta, L* = 25.40±0.71, a*=11.84±0.36. b*=15.65±0.56) were provided by a local roasting company (Unión Tostadora, S.A.). Three samples of each coffee were analyzed. The L*, a* and b* values were analyzed by means of a tristimulus colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan) using the D65 illuminant and CIE 1931 standard observer. The instrument was standardized against a white tile before sample measurements. Ground roasted coffee was spread out in a 1 cm Petri plate and the L*, a* and b* values were measured in triplicate on the CIELab scale. Coffee beans were ground for 20 s using a grinder (model Moulinex super junior “s”, París, France).

Filter Coffee Brew was prepared from 36 g of ground roasted coffee for a volume of 600 mL of tap water (pH=7.0, 48-56 mg Ca/L), using a filter coffee machine (model Avantis 70 Aroma plus, Ufesa, Spain). Extraction took approx. 6 min at 90 °C.

2.3 Volatile Compounds Analysis

Volatile compounds were analyzed according to the method described by Maeztu, Sanz, Andueza, de Peña, Bello, & Cid (2001) using static headspace-gas chromatography-mass spectrometry (SH-GC-MS). Six mL of coffee or volatile compounds standards solution was introduced into a 20 mL vial, which was immediately sealed with a silicone rubber Teflon cap. Each vial was equilibrated at 40 °C for 15 min in the headspace sampler (model 7694E, Agilent Technologies, Palo Alto, CA), pressurized with carrier gas for 12 s, and 1 mL of the headspace sample was injected into an HP-Wax glass capillary column (60 m × 0.25 mm × 0.5 μm film thickness) in an HP 7890 gas chromatograph (Agilent Technologies). The injector temperature was 60 °C, and the carrier gas was helium (1 mL/min linear speed). The oven temperature was maintained at 40 °C for 3 min and then raised at 2.5 °C/min to 205 °C and maintained for 10

min. Mass spectrometry analysis was performed with a mass selective detector (model 5975C, Agilent Technologies) operating in the electron impact ionization mode (70 eV), with a scan range of 30-160 amu. Ion source temperature was set at 230 °C. Each sample was analyzed in triplicate.

The volatile compounds were identified by comparing their mass spectra with those of pure reference compounds and also by comparing their Kovats indices with those of standard compounds. The Kovats indices were calculated according to the method of Tranchant (1982). Peak areas were measured by calculation of each volatile total area based on integration of a single ion. The ion used for area quantification of each volatile compound and the obtained areas are given in **Table 1.** Volatile compounds selected for model systems were additionally quantified by calibration curves to determine their concentrations in coffee. Aqueous solutions of each standard volatile compound were analyzed at different known concentrations and chromatographic areas obtained using selective ion monitoring (SIM) were plotted against concentration. Equations for each compound obtained by linear regression included the areas found in the coffee samples in all cases. Coefficients of linearity for the calibration curves were typically R^2>0.99.

2.4 Volatiles model systems

Twelve volatile heterocyclic compounds (7 furans, 3 pyrroles, and 2 thiophenes) identified in roasted coffee by SH-GC-MS and previously reported as potential antioxidants (Fuster et al., 2000; Yanagimoto et al., 2002, 2004) were selected to prepare 4 model systems: Furans (Fu), Pyrroles (Py), Thiophenes (Th) and Furans-Pyrroles-Thiophenes (Fu-Py-Th). Furthermore, five volatile aldehydes identified among the most abundant and previously reported as Maillard reaction products, and two diketones were selected to prepare other 2 model systems: Aldehydes and Ketones. Pure reference standards were dissolved in deionized water (pH=6.0) at concentrations equivalent to those found in coffee (**Tables 2 and 3**). When it was required, a previous solubilization with the minimum amount of methanol was made. Additionally, 10- and 100-fold concentrated model systems were prepared to analyze dose dependent antioxidant activity.

2.5 Antioxidant activity by ABTS assay

The antioxidant activity measured with ABTS was carried out according to the method described by Re, Pellegrini, Proteggente, Pannaia, Yang & Rice-Evans (1999) with some modifications. The $ABTS^{\cdot+}$ radicals were generated by the addition of 2.45 mM potassium persulfate to an 7 mM ABTS solution prepared in phosphate-buffered saline (PBS, pH 7.4) and allowing the mixture to stand in darkness at room temperature for at least 12 h before use. The $ABTS^{\cdot+}$ stock solution was adjusted with PBS to an absorbance of 0.7 (±0.02) at 734 nm in a 1 cm cuvette at 25 °C (Lambda 25 UV, VIS spectrophotometer, Perkin Elmer Instruments, Madrid, Spain). An aliquot of 50 µL of coffee sample diluted with demineralized water (15:1000) or 50 µL of each model system was added to 2 mL of $ABTS^{\cdot+}$ reagent and the absorbance was measured spectrophotometrically at 734 nm after exactly 18 min at 25 °C. Calibration was performed with Trolox solution (a water-soluble vitamin E analogue) and total antioxidant activity was expressed as milimoles (mmol) of Trolox per liter of coffee brew or model system.

2.6 Antioxidant capacity by DPPH assay.

The antioxidant capacity was measured using the DPPH decolorization assay (Brand-Williams, Cuvelier, & Berset, 1995). A 6.1x10–5 M DPPH• methanol solution was prepared immediately before use. The DPPH· solution was adjusted with methanol to an absorbance of 0.7 (±0.02) at 515 nm in a 1 cm cuvette at 25 ºC (Lambda 25 UV, VIS spectrophotometer, Perkin Elmer Instruments, Madrid, Spain). Fifty microliters of appropriate diluted coffee sample (3:100) or 50 µL of each model system was added to DPPH· solution (1.95 mL). After mixing, the absorbance was measured spectrophotometrically at 515 nm after exactly 18 min at 25 ºC. Calibration was performed with Trolox solution and total antioxidant capacity was expressed as milimoles (mmol) of Trolox per liter of coffee brew or model system.

2.7 Statistical analysis

Each parameter was analyzed in triplicate. Results are shown as means ± standard deviations. Student's t-test was applied to volatile compounds of Arabica and Robusta coffee samples. Linear regresion was applied to evaluate dose dependent increase in antioxidant activity. Statistical analyses were performed using the SPSS v.15.0 software package.

3 RESULTS AND DISCUSSION

Figure 1 shows the chromatographic profiles of the volatile compounds of roasted Arabica and Robusta coffee. **Table 1** shows the chromatographic areas of the identified compounds in both samples. A total of sixty-two and sixty-four volatile compounds were identified and quantified for Arabica and Robusta coffee, respectively. They comprised 4 sulfur compounds, 8 aldehydes, 6 esters, 15 furans, 8 ketones, 5 alcohols, 2 thiophenes, 6 pyrroles, 2 pyridines, 4 pyrazines, 2 thiazoles, 1 lactone, 2 phenolic compounds, 1 alkene, and 1 ether.

Figure 1

Table 1

Arabica coffee showed a significantly higher total area of volatiles than Robusta ($2.1x10^9$ vs $0.7x10^9$, $p<0.01$), mainly because the most abundant volatile chemical classes (aldehydes, furans, ketones and esters) were significantly higher in Arabica samples ($p<0.01$). Aldehydes and esters are responsible for fruity and malty coffee flavor notes, whereas diketones contribute to the buttery aroma, and furans are considered to be responsible for the typical roasted coffee aroma (Semmelroch & Grosch, 1995; Maeztu et al., 2001; Flament, 2001). Similar results were reported in previous works of our group when volatile compounds of Arabica coffee were compared with those found in Arabica-Robusta coffee blends roasted by conventional or torrefacto techniques (Sanz, Maeztu, Zapelena, Bello, & Cid, 2002; Lopez-Galilea, Andriot, de Peña, Cid, & Guichard, 2008a). In contrast, chromatographic areas of pyrazines and pyridines, and in less proportion thiazoles, were higher in Robusta coffee. Pyrazines are responsible for roasted, earthy, musty and woody flavor notes characteristic of Robusta coffee (Blank, Sen & Grosch, 1991; Semmelroch & Grosch, 1995; Lopez-Galilea, Fournier, Cid, & Guichard, 2006) and pyridines contribute to smoky aroma (Flament, 2001). Also, low molecular weight phenolic compounds, and mainly 2-methoxyphenol (guaiacol) that is a key odorant responsible of phenolic and burnt aroma (Semmelroch & Grosch, 1995; Sanz et al., 2002; Lopez-Galilea et al., 2006), were only detected in Robusta coffee samples at low levels but not in Arabica. Similar results were found by other authors in conventional roasted Arabica and Robusta coffee (Semmelroch & Grosch, 1995; Maeztu et al., 2001; Lopez-Galilea et al., 2008b) and coffee brews (Maeztu et al., 2001).

The concentration of heterocyclic volatile compounds in coffee was firstly measured to further assess their actual contribution to the total antioxidant capacity of coffee at the concentration

usually found in coffee. Seven furans (furan, 2-methylfuran, 2,5-dimethylfuran, 2-methyl-tetrahydrofuran-3-one, furfural, 5-methylfurfural and 2-furfurylacetate), three pyrroles (1-methylpyrrole, pyrrole, and 2-formyl-1-methyl-pyrrole), and two thiophenes (thiophene and 2-methylthiophene) were chosen because both they were previously proposed by other authors as potential antioxidants (Fuster et al., 2000; Yanagimoto et al., 2002, 2004), and their chromatographic areas were among the highest ones in the analyzed coffee samples. Concentrations of these volatile compounds were quantified in both Arabica and Robusta coffees based on the calibration curves of the corresponding standard. Results are shown in **Table 2**. Except in thiophenes with the same concentrations, Arabica coffee exhibited higher concentration in all analyzed compounds, showing considerably higher amounts of 2-methyl-tetrahydrofuran-3-one (more than 5-fold) and 5-methylfurfural (almost 3-fold) than in Robusta coffee. It can also be observed that those volatiles with the highest chromatographic areas, such as 2-methylfuran, were not necessarily the most abundant in coffee in terms of concentration. The same applies in the opposite direction, i.e. some volatiles with the highest concentrations, such as 2-methyl-tetrahydrofuran-3-one, acetaldehyde and propanal, were not necessarily those with the highest chromatographic areas, In fact, the relationship between the chromatographic area and the concentration is characteristic for each volatile compound at the same chromatographic conditions. For that reason, the results of the chromatographic areas of volatile compounds should not be directly considered as equivalent to their concentrations and consequently the quantification by chromatographic areas or by concentration should not be directly comparable.

Table 2

The results of the antioxidant activity of each heterocyclic volatiles model system (furans, pyrroles, thiophenes and Fu-Py-Th) prepared at the mean concentrations of each volatile compound found in Arabica and Robusta coffee **(Table 3)**, and at 10- and 100-fold, are shown in comparison to the antioxidant activity of the coffee brew in **Figure 2**. The antioxidant activity of the coffee brew was calculated as the mean value (and standard deviations) of the Arabica and Robusta filter coffee brews. These results were similar to those reported by other authors in filter coffee brews (Perez-Martinez, Caemmerer, De Peña, Cid, & Kroh, 2010; Sanchez Gonzalez, Jimenez Escrig, & Saura Calixto, 2005; Ludwig et al, 2012).

Table 3

Figure 2

Furans are cyclic ethers present in heated and roasted foods. A great variety of furans are originated during roasting process in coffee as Maillard-reaction products, but they are also the result of thermal oxidation of lipids and thermal degradation of thiamine, nucleotides, terpenes (Flament, 2001) and proteins (Hwang, Chen, & Ho, 2012). In furan model system (Fu), no appreciable antioxidant activity at coffee furan concentration was observed. However, for the 10-fold and 100-fold concentrated furan model systems, ABTS quenching activities equivalent to 0.08±0.01 and 0.85±0.02 mmol Trolox per liter were found, showing a linear dose dependent increase in antioxidant activity (r^2=1), whereas DPPH results were 0.05±0.00 and 0.07±0.00 mmol Trolox per liter, showing a non-linear dose dependent antioxidant increase (r^2<0.6). To ascertain the contribution of each single furan to the overall Fu-model system antioxidant activity, volatile compounds were tested individually. Because the furan model system showed no antioxidant activity at concentration levels actually present in coffee, 10-fold concentrated solutions of each furan were used to measure the ABTS and DPPH antioxidant activity. From the 7 analyzed furans, only 2-methyl-tetrahydrofuran-3-one exhibits antioxidant activity. This activity (0.08±0.01 mmol Trolox per liter for ABTS and 0.05±0.00 mmol Trolox per liter for DPPH) was the same to that of the Fu-model system at the same concentration level (10-fold), showing that the antioxidant activity of the main coffee furans might be mainly attributed to this volatile compound, maybe because 2-methyl-tetrahydrofuran-3-one was by far the most abundant furan in coffee. Although five of the furans analyzed (namely furan, 2-methylfuran, furfural, 5-methylfurfural and 2-furfurylacetate) have been reported as potent antioxidants (Fuster et al., 2000; Yanagimoto et al., 2002, 2004), results obtained in this study show that even at concentrations 10-fold higher than actually present in coffee, only 2-methyl-tetrahydrofuran-3-one exhibited a very limited radical scavenging activity.

Pyrroles are formed during roasting process. Pyrrole and 1-methyl-pyrrole are formed in the pyrolysis of proline and threonine alone or combined with glucose or sucrose, and in the pyrolysis of trigonelline (Flament, 2001). 2-formyl-1-methylpyrrole is formed from 1-methylpyrrole and also when D-xylose reacts thermally with various amines or amino acids (glycine, alanine, beta-alanine, leucine). In Pyrrole model system (Py), no appreciable

antioxidant activity was found at concentration levels equivalent to coffee. The 10-fold and 100-fold concentrated pyrrole systems exhibited antioxidant activity equal to 0.32±0.01 and 0.81±0.01 mmol Trolox per liter, respectively, for ABTS and 0.07±0.00 and 0.09±0.00 mmol Trolox per liter, respectively, for DPPH showing a non-linear dose dependent antioxidant activity increase (r^2<0.9). In comparison with furans, pyrroles showed a 4 times higher ABTS radical quenching activity at 10-fold concentrations, but similar antioxidant activity at 100-fold concentrations. These results suggest a higher effectiveness of pyrroles at lower concentrations, as proposed by other authors (Fuster et al., 2000) but still undetectable at coffee concentration. To assess the contribution of each pyrrole, the three compounds were analyzed separately at 10-fold concentration by the ABTS and DPPH assays. Results reveal that the ABTS antioxidant activity measured for the Py model system might be totally attributed to pyrrole (0.32±0.01 mmol Trolox per liter), whereas 1-methylpyrrole and 2-formyl-1-methylpyrrol seem to be ineffective in quenching ABTS radicals at the tested concentrations. However, when DPPH assay was applied, both 1-methylpyrrole and pyrrole model systems at 10-fold concentration had similar antioxidant activity than Py model system (0.07±0.00 mmol Trolox per liter for pyrrole and 0.06±0.00 mmol Trolox per liter for 1-methylpyrrole). Also, Yanagimoto et al (2002) observed higher inhibition of hexanal oxidation by pyrrole than by 1-methylpyrrole, but the inhibition was quite low for both volatile compounds (<10% at 10 μg/mL for pyrrole and <3% at 5-20 for μg/mL for 1-methylpyrrole). However, 2-formyl-1-methylpyrrole seems to be more effective as a lipophilic antioxidant inhibiting hexanal oxidation in dichloromethane solutions (Yanagimoto et al., 2002) than as a hydrophilic antioxidant, quenching radicals in aqueous solutions similar to coffee brews.

Thiophenes present in roasted coffee can be formed by pyrolysis of sulfur amino acids as methionine or cysteine and cystine alone, or by browning reactions in the presence of sugars (Flament, 2001). Although some authors (Fuster et al., 2000; Yanagimoto et al., 2002) reported that both thiophenes found in coffee exhibit antioxidant activity, the results obtained in this study did not show radical quenching activity at any analyzed concentration level (coffee, 10-fold and 100-fold). This could probably be due to the very low amounts of thiophenes used in this study to evaluate the ABTS and DPPH antioxidant activity even at the highest concentration (100-fold, with 10 μg/mL for tiophene and 2 μg/mL for 1-methylthiophene). Actually, these compounds are

present in coffee in very low amounts and therefore, although their antioxidant capacity was demonstrated at high concentrations (more than 50 µg/mL), tiophenes barely contribute to the antioxidant capacity of coffee.

When the antioxidant activity of a model system containing all the 12 selected heterocyclic compounds (furans, pyrroles and thiophenes) was analyzed, no radical quenching activity was detected at concentration levels similar to coffee brew. At 10-fold concentrated sample ABTS and DPPH quenching activities were 0.35±0.02 and 0.08±0.00 mmol Trolox per liter for DPPH, respectively. One hundred-fold concentrated sample showed radical quenching activities of 0.90±0.02 for ABTS and 0.09±0.00 mmol Trolox per liter for DPPH were observed, respectively. Thus, the radical quenching activity of the Fu-Py-Th model system was slightly higher than the maximum value showed for furans at 100-fold in ABTS and for pyrroles at 10-fold and 100-fold concentrations in both ABTS and DPPH assays, but not the sum. These results suggest antagonistic effects among furans, pyrroles and thiophenes. Moreover, when the radicals quenching activity of model systems was compared to the overall antioxidant capacity of a filter coffee brew, the results clearly showed the almost insignificant contribution of these heterocyclic volatile compounds to the antioxidant activity of coffee, even at the 100-fold concentrated Fu-Py-Th model system, which exhibited the highest ABTS and DPPH quenching activity, accounting only for up to 3.3% of the overall antioxidant capacity of a filter coffee brew (**Figure 2**).

The most abundant volatile compounds in coffee are aldehydes and ketones and some of them are also Maillard reaction products. For those reasons, we decided to test the antioxidant activity of two new model systems, one with aldehydes and another with ketones. In terms of chromatographic areas (**Table 1**), 2-methylpropanal, 2-methylbutanal and 3-methylbutanal (Strecker degradation products of valine, isoleucine, and leucine), were the most abundant aldehydes followed by acetaldehyde and propanal that are formed by pyrolysis of alanine and serine, and/or sugar. However, acetaldehyde and propanal were present in significantly higher concentrations than Strecker aldehydes in both coffee brews (**Table 2**). Also, two diones (2,3-butanedione and 2,3-pentanedione) were selected for quantification and further evaluation of their antioxidant activity in a model system. All aldehydes and ketones were present in significantly higher amounts in Arabica coffee than in Robusta one, in agreement with other

studies (Grosch, 1996). As in the previous model systems, mean concentrations of the selected aldehydes and ketones found in Arabica and Robusta coffee were used to prepare the aldehyde and the ketone model systems **(Table 3)**. The antioxidant activity of each model system at three different concentration levels (coffee, 10-fold and 100-fold) was assessed using the ABTS and DPPH radicals quenching assays. **Figure 3** shows that, even at the highest concentration, the antioxidant activity of the aldehydes and ketones were negligible in comparison to that of the coffee brew and also to those of the heterocyclic volatiles model systems.

Figure 3

In summary, volatile compounds present in coffee contribute very little to the antioxidant capacity of coffee in comparison to other coffee antioxidants, such as phenolics and melanoidins, even if other minor non-tested volatiles could also contribute to the overall antioxidant capacity. . However, because antioxidants may act by several mechanisms of action in both coffee brews and human cells, further studies should be needed to deepen in the role of coffee volatiles as antioxidants or in health-properties of coffee. Additionally, the results of the present study also indicate that, although some volatile compounds may act as antioxidant in high doses, it is necessary to evaluate their capacity at the concentrations in food samples to know their actual contribution to the antioxidant capacity.

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FIGURE CAPTIONS

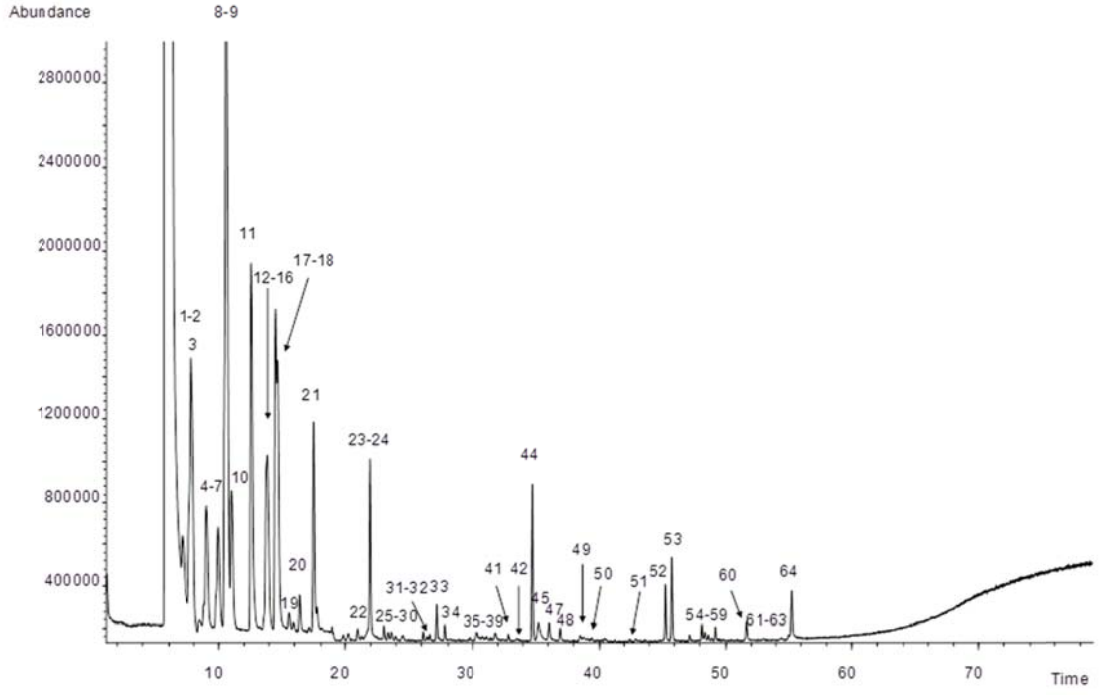
Figure 1. SH-GC-MS chromatograms of Arabica **(a)** and Robusta **(b)** coffee. For peak identification see **Table 1**.

Figure 2. Antioxidant activity (ABTS and DPPH) of heterocyclic volatiles model systems and filter coffee brew.

Figure 3. Antioxidant activity (ABTS and DPPH) of aldehydes and ketones model systems and filter coffee brew.

Figure 1. SH-GC-MS chromatogram of Arabica **(a)** and Robusta **(b)** coffee. For peak identification see **Table 1**.

(a) Arabica



(b) Robusta

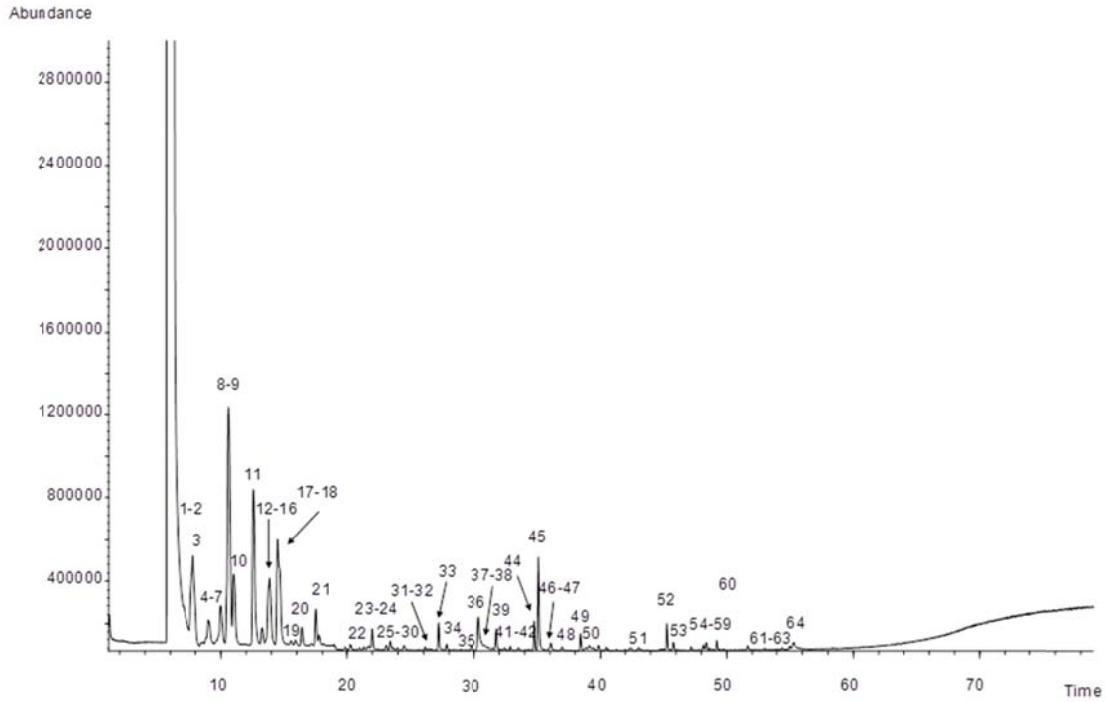
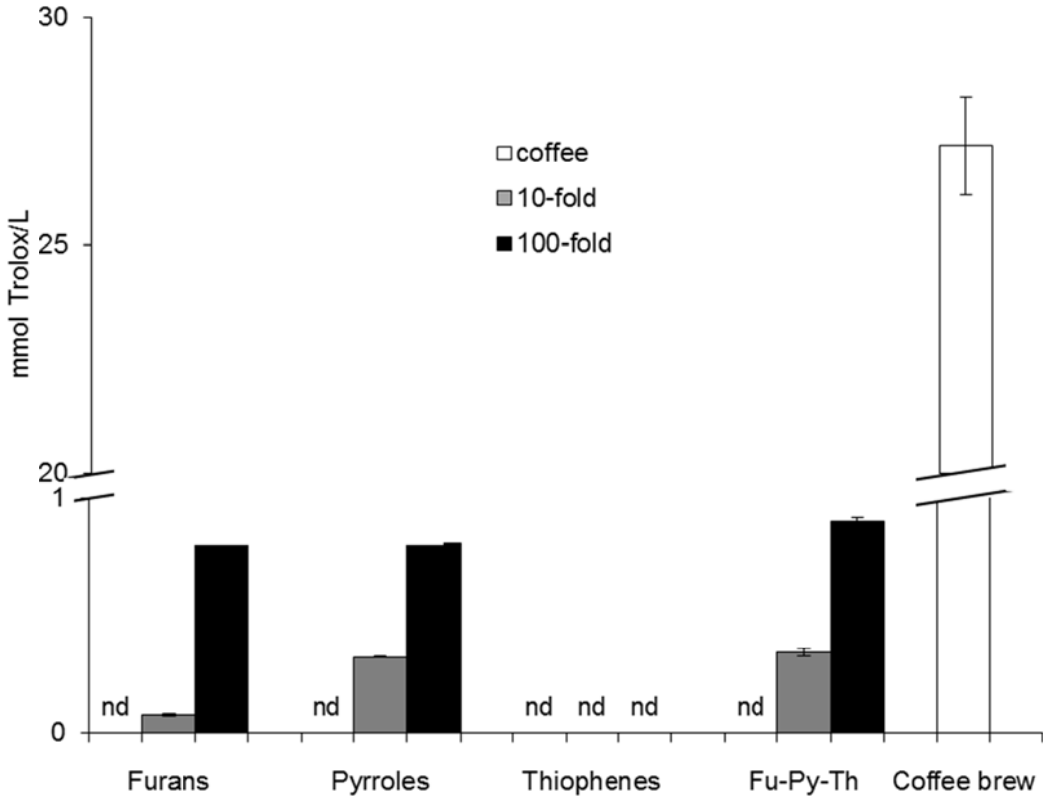


Figure 2. Antioxidant activity (ABTS and DPPH) of heterocyclic volatiles model systems and filter coffee brew.

(a) ABTS



(b) DPPH

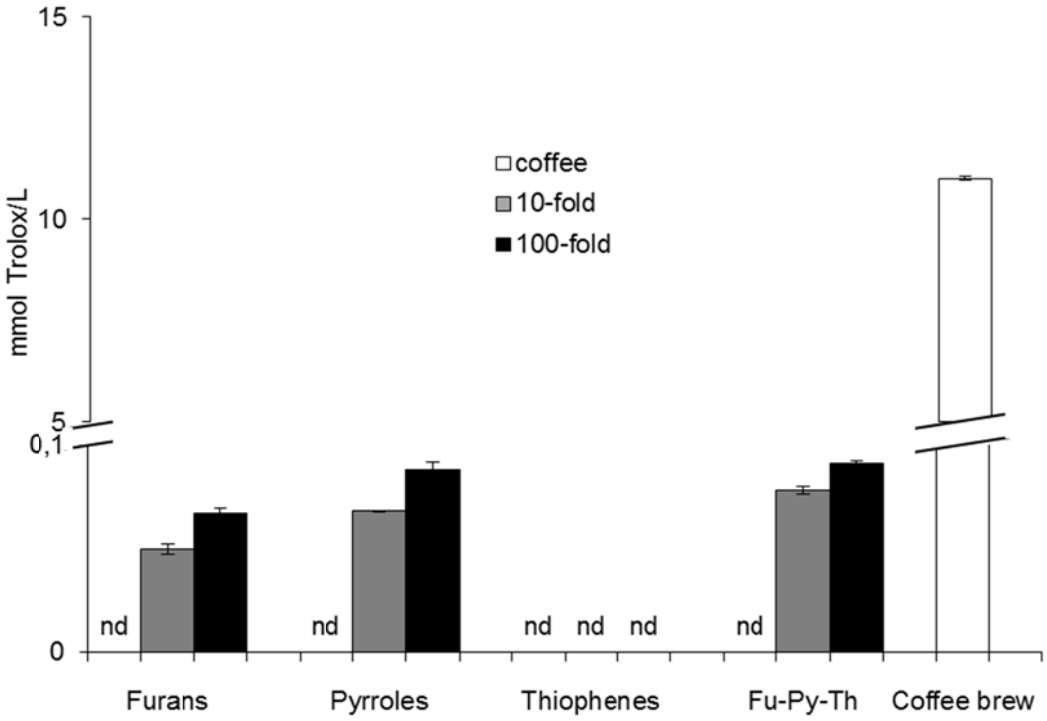
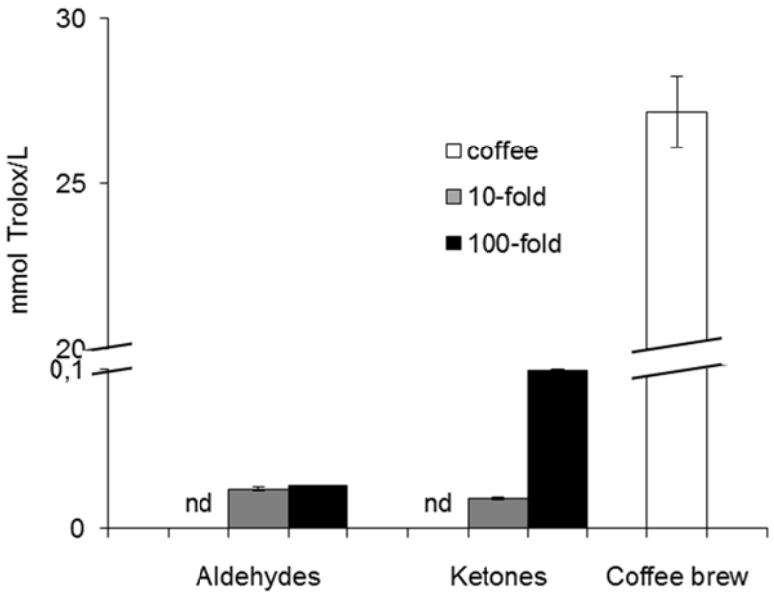


Figure 3. Antioxidant activity (ABTS and DPPH) of aldehydes and ketones model systems and filter coffee brew.

(a) ABTS



(b) DPPH

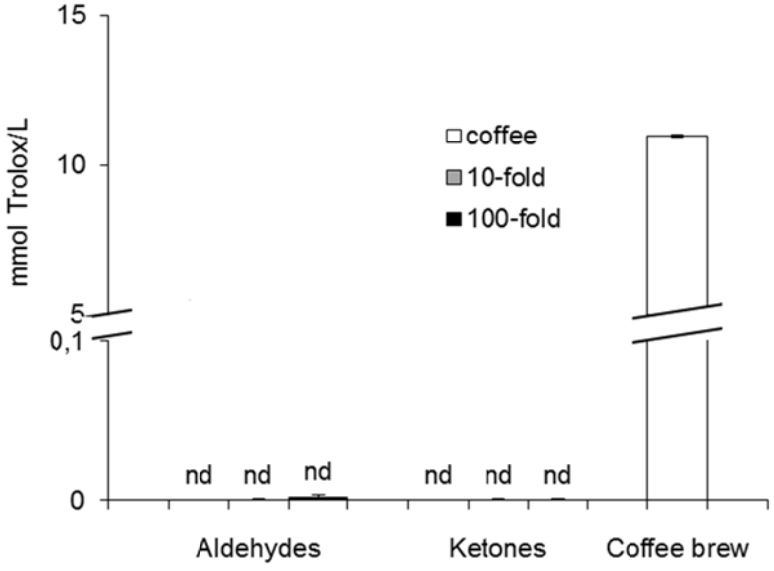


Table 1. Areas (expressed as Areax10^{-3}) of volatile compounds identified in the headspace of Arabica and Robusta coffee.

Peak nr.[b]	*QI*[c]	*ID*[d]	*KI*[e]	*Compounds*	**Arabica**	**Robusta**	**p**[f]
Sulfur compounds							
2	47	B	633	Methanethiol	10639 ± 283	2171 ± 204	.00
4	62	A	673	Dimethyl sulfide	21413 ± 645	4288 ± 506	.00
26	94	A	1079	Dimethyl disulfide	3677 ± 402	4126 ± 432	.26
51	114	A	1432	2-furanmethanethiol (furfurylthiol)	70818 ± 5636	48049 ± 4299	.01
				Total Sulfur compounds	**106547 ± 6972**	**58634 ± 5068**	**.00**
Aldehydes							
3	43	A	649	Acetaldehyde	86612 ± 9108	18058 ± 1704	.00
6	58	A	710	Propanal	23479 ± 2675	4132 ± 477	.00
8	41	A	754	2-methylpropanal	205848 ± 6800	41530 ± 5784	.00
12	72	B	841	Butanal	5007 ± 223	2133 ± 160	.00
17	39	A	883	2-methylbutanal	120962 ± 6328	33608 ± 3009	.00
18	44	A	888	3-methylbutanal	120636 ± 6882	83086 ± 1081	.00
27	56	A	1086	Hexanal	3683 ± 241	1526 ± 305	.00
30	84	A	1103	2-methyl-2-butenal	2283 ± 151	1709 ± 155	.01
				Total Aldehydes	**568510 ± 32408**	**185782 ± 12675**	**.00**
Esters							
5	60	B	682	Formic acid, methyl ester	244615 ± 31288	41513 ± 5307	.00
10	43	B	791	Acetic acid, methyl ester	22410 ± 1352	9909 ± 1081	.00
13	43	B	854	Acetic acid, ethyl ester	traces	638 ± 315	.02
16	57	B	876	Propanoic acid, methyl ester	1592 ± 154	884 ± 91	.00
52	43	B	1484	1-hydroxy-2-propanone acetate	13511 ± 1617	6455 ± 826	.00
58	57	B	1554	1-hydroxy-2-butanone acetate	819 ± 22	366 ± 54	.00
				Total Esters	**282947 ± 34112**	**59765 ± 6022**	**.00**
Furans							
7	68	A	724	Furan	37036 ± 3267	28830 ± 5724	.10
11	82	A	832	2-methylfuran	216408 ± 7745	93177 ± 5589	.00
14	82	A	862	3-methylfuran	8897 ± 807	3219 ± 225	.00
20	96	A	934	2,5-dimethylfuran	16239 ± 635	14123 ± 976	.03
25	94	B	1077	2-vinylfuran	3179 ± 102	1021 ± 159	.00
34	108	B	1162	2-vinyl-5-methylfuran	4656 ± 271	1449 ± 238	.00
44	43	A	1284	2-methyltetrahydrofuran-3-one	52497 ± 2324	9506 ± 991	.00
53	96	A	1491	2-furancarboxaldehyde (furfural)	23557 ± 205	8778 ± 326	.00
54	81	A	1517	2-furfuryl methyl sulfide	148 ± 30	225 ± 105	.29
55	81	B	1520	2-furfuryl formate	645 ± 134	234 ± 70	.01
56	95	A	1537	2-acetylfuran	3726 ± 415	1541 ± 262	.00
59	81	A	1560	2-furfuryl acetate	2895 ± 291	1633 ± 201	.00
60	110	A	1606	5-methylfurfural	1189 ± 62	438 ± 29	.00
61	91	B	1631	2-furfurylfuran	391 ± 71	335 ± 82	.42
64	98	A	1687	Furfuryl alcohol	11148 ± 997	2964 ± 251	.00
				Total Furans	**382611 ± 17194**	**167473 ± 15075**	**.00**
Ketones							
9	58	A	763	2-propanone	297481 ± 24952	107777 ± 27101	.00
15	43	A	869	2-butanone	60667 ± 1544	22195 ± 2774	.00
21	43	A	965	2,3-butanedione	96526 ± 4428	14796 ± 1501	.00
23	57	B	1055	3-hexanone	97686 ± 4562	10822 ± 1047	.00
24	43	A	1060	2,3-pentanedione	66523 ± 3292	6705 ± 1552	.00
31	69	A	1138	3-penten-2-one	1829 ± 139	709 ± 83	.00
32	57	B	1144	3,4-hexanedione	3188 ± 252	643 ± 56	.00
48	43	B	1323	1-hydroxy-2-propanone	230 ± 58	62 ± 10	.01
				Total Ketones	**624130 ± 39029**	**163709 ± 30821**	**.00**
Alcohols							
19	45	B	917	Ethanol	15764 ± 1482	3636 ± 186	.00
28	43	A	1105	2-methyl-1-propanol	259 ± 134	516 ± 69	.04
37	56	B	1221	3-methylbutan-1-ol	1452 ± 223	586 ± 185	.01

42	41	B	1265	3-methyl-3-buten-1-ol	657 ± 37	858 ± 119	.05
47	71	B	1338	3-methyl-2-buten-1-ol	621 ± 117	714 ± 109	.37
				Total Alcohols	**18753 ± 1578**	**6310 ± 668**	**.00**
Thiophenes							
22	84	A	1023	Thiophene	2324 ± 91	2144 ± 154	.16
29	97	A	1099	2-methylthiophene	1671 ± 29	1599 ± 135	.42
				Total Thiophenes	**3995 ± 120**	**3743 ± 289**	**.24**
Pyrroles							
33	81	A	1151	1-methylpyrrole	13884 ± 602	9870 ± 675	.00
35	80	B	1195	1-ethyl-1*H*-pyrrole	1072 ± 132	1354 ± 245	.15
38	94	B	1226	2,5-dimethylpyrrole	561 ± 59	541 ± 82	.75
57	67	A	1543	1*H*-pyrrole	2719 ± 187	2686 ± 302	.88
62	109	A	1662	2-formyl-1-methylpyrrole	1459 ± 139	539 ± 82	.00
65	81	B	1833	*N*-furfurylpyrrole	482 ± 26	331 ± 17	.00
				Total Pyrroles	**20177 ± 1145**	**15321 ± 1403**	**.01**
Pyridines							
36	79	A	1205	Pyridine	3492 ± 123	25078 ± 75	.00
40	93	B	1240	2-methylpyridine	traces	nd	--
				Total Pyridines	**3492 ± 123**	**25078 ± 75**	**.00**
Pyrazines							
39	80	A	1232	Pyrazine	4902 ± 372	8616 ± 318	.00
45	94	A	1289	2-methylpyrazine	4249 ± 561	20163 ± 1804	.00
49	108	A	1348	2,5-dimethylpyrazine	1500 ± 126	3413 ± 141	.00
50	108	B	1373	2,3-dimethylpyrazine	693 ± 81	1514 ± 400	.03
				Total Pyrazines	**11344 ± 807**	**33706 ± 2663**	**.00**
Thiazoles							
43	85	B	1271	1,3-thiazole	442 ± 26	148 ± 24	.00
46	99	B	1303	4-methylthiazole	nd	391 ± 108	.00
				Total Thiazoles	**442 ± 26**	**539 ± 132**	**.28**
Lactones							
63	42	B	1674	γ-butyrolactone	1589 ± 231	1015 ± 126	.02
				Total Lactones	**1589 ± 231**	**1015 ± 126**	**.02**
Phenolic compounds							
-	94	A	1075	Phenol	nd	traces	--
66	109	B	-	2-methoxyphenol (guaiacol)	nd	62 ± 57	.13
				Total phenolic compounds	**-**	**62 ± 57**	**.13**
Others							
1	67	A	624	1,3-pentadiene	62095 ± 1585	5672 ± 543	.00
41	81	B	1252	2-furfuryl methyl ether	2207 ± 157	1247 ± 115	.00
				Total others	**64302 ± 1742**	**6919 ± 658**	**.00**
				Total compounds	**2088840±135432**	**728056 ± 65437**	**.00**

[a] All values are shown as means ± standard deviations. nd, not detected.[b] Peak number corresponding to chromatograms in **Figure 1**.[c] Ion used for the compound quantification. [d] The reliability of the identification proposal is indicated by the following: A, mass spectrum, KI, and retention time according to standards; B, tentative identification by comparing mass spectrum with Wiley mass spectral database and retention indices with literature data [e] Kovats index calculated for the HP-Wax capillary column. [f]p-value between coffee samples obtained by Student's t-test.

Table 2. Concentration of antioxidant volatile compounds in Arabica and Robusta coffee. All values are shown as mean ± standard deviation (n=3).

Compounds	**Coffee** (µg/mL)		p[a]
	Arabica	Robusta	
Furans			
Furan	0.23 ± 0.02	0.19 ± 0.04	.20
2-methylfuran	1.25 ± 0.06	0.56 ± 0.04	.00
2,5-dimethylfurane	0.24 ± 0.01	0.22 ± 0.01	.07
2-methyl-tetrahydrofuran-3-one	95.72 ± 5.93	18.09 ± 2.53	.00
Furfural	24.23 ± 0.28	10.04 ± 0.44	.00
5-methylfurfural	7.05 ± 0.53	2.50 ± 0.25	.00
2-furfurylacetate	2.93 ± 0.32	1.95 ± 0.22	.01
Pyrroles			
1-methylpyrrole	0.44 ± 0.03	0.30 ± 0.03	.00
Pyrrole	1.17 ± 0.10	1.15 ± 0.16	.90
2-formyl-1-methyl-pyrrole	1.37 ± 0.17	0.56 ± 0.10	.00
Thiophenes			
Thiophene	0.10 ± 0.00	0.10 ± 0.00	.00
2-methylthiophene	0.02 ± 0.00	0.02 ± 0.00	.00
Aldehydes			
Acetaldehyde	95.66 ± 10.03	20.48 ± 1.90	.00
Propanal	92.64 ± 10.56	18.52 ± 2.13	.00
2-methylpropanal	16.91 ± 0.51	4.03 ± 0.56	.00
2-methylbutanal	3.50 ± 0.18	0.82 ± 0.07	.00
3-methylbutanal	4.82 ± 0.17	3.22 ± 0.04	.00
Ketones			
2,3-butanedione	62.48 ± 2.88	8.88 ± 0.92	.00
2,3-pentanedione	32.02 ± 1.60	3.85 ± 0.85	.00

[a]p-value between coffee samples obtained by Student's t-test.

Table 3. Concentrations of volatile compounds present in each model systems

Compounds	Model system (µg/mL)					
	Fu	**Py**	**Th**	**Fu-Py-Th**	**Ald**	**Ke**
Furans (Fu)						
Furan	0.20			0.20		
2-methylfuran	0.90			0.90		
2,5-dimethylfuran	0.20			0.20		
2-methyl-tetrahydrofuran-3-one	55.00			55.00		
Furfural	17.00			17.00		
5-methylfurfural	5.00			5.00		
2-furfurylacetate	2.50			2.50		
Pyrroles (Py)						
1-methylpyrrole		0.35		0.35		
Pyrrole		1.00		1.00		
2-formyl-1-methyl-pyrrole		1.00		1.00		
Thiophenes (Th)						
Thiophene			0.10	0.10		
2-methylthiophene			0.02	0.02		
Aldehydes (Ald)						
Acetaldehyde					58.00	
Propanal					55.50	
2-methylpropanal					10.50	
2-methylbutanal					2.15	
3-methylbutanal					4.00	
Ketones (Ke)						
2,3-butanedione						35.00
2,3-pentanedione						18.00