Equilibration time and temperature were the factors studied to choose the best conditions for analyzing volatiles in roasted ground Arabica coffee by a static headspace sampling extraction method. Three temperatures of equilibration were studied: 60, 80, and 90 °C. A larger quantity of volatile compounds was extracted at 90 °C than at 80 or 60 °C, although the same qualitative profile was found for each. The extraction of the volatile compounds was studied at seven different equilibration times: 30, 45, 60, 80, 100, 120, and 150 min. The best time of equilibration for headspace analysis of roasted ground Arabica coffee should be selected depending on the chemical class or compound studied. One hundred and twenty-two volatile compounds were identified, including 26 furans, 20 ketones, 20 pyrazines, 9 alcohols, 9 aldehydes, 8 esters, 6 pyroles, 6 thiophenes, 4 sulfur compounds, 3 benzenic compounds, 2 phenolic compounds, 2 pyridines, 2 thiazoles, 1 oxazole, 1 lactone, 1 alkane, 1 alkene, and 1 acid.

**Keywords:** Static headspace; roasted coffee; volatile compounds

**EXPERIMENTAL PROCEDURES**

**Materials.** A commercial sample (100% Arabica coffee, roasted) was obtained from a local manufacturer, and just before each analysis, the coffee was ground with a Retsch mill to a particle size of 0.75 mm. Pure reference standards of 2-methylpropanal, 2-methyl-1-propanol, 2-butanone, 3-methylbutanal, 2,3-butanedione, 2,3-pentanedione, and 2-butenal were purchased from Acros; 2-propen-1-ol, 3-penten-2-one, 2-pentanol, 2-methylfuran,
RESULTS AND DISCUSSION

The time and temperature of equilibration are known to be important factors affecting volatile analysis of coffee using SH-GC (10). Volatile analysis by SH-GC was standardized by subjecting 2 g samples of roasted ground coffee to different equilibration temperatures and time periods.

Effect of Temperature of Equilibration. One way of improving the sensitivity of the headspace technique is by raising the temperature of the sample (17–19) so that more volatile compounds are released from the solid matrix.

The total area of chromatograms obtained at three different equilibration temperatures using seven equilibration times was calculated (Figure 1).

A temperature of 90 °C permitted the extraction of a larger quantity of volatile compounds than did 80 or 60 °C at each of the seven times of equilibration studied (Figure 1). Figure 2 shows the chromatograms obtained at 60 °C/30 min and at 90 °C/150 min, and a clear difference in the total area can be observed. However, no differences were found in the qualitative profiles. A temperature of 90 °C was selected to be used in order to study the influence of the equilibration times on the extraction of the individual volatile compounds in roasted ground coffee.

Effect of Equilibration Time. The factors controlling equilibrium in food systems are very complex, which indicate that long equilibration times are needed to reach equilibrium (17). Initially, the total chromatographic area follows a linear relationship with incubation time, after which it reaches a plateau indicating that equilibrium has been established between the matrix and the headspace.

Table 1 shows the percentage of the extraction of each chemical class at 90 °C at the seven equilibration times studied. As can be observed in this table, each chemical class had its maximum extraction rate in one of the seven equilibration times studied: Aldehydes, ketones, furans, and phenolic compounds at 60 min; lactones at 80 min; and pyridines at 100 min. The quantity of sulfur-containing extracted compounds increased when the equilibration time was increased, and this was probably due to their sensitivity to O2. The proportion of extracted thiazoles and alkanes did not depend on the time of equilibration programmed, and

Figure 1. Effect of equilibration temperature on total volatile.

Thiophene, propionaldehyde, and dimethyl sulfide were obtained from Aldrich (Steinheim, Germany), and hexanal was obtained from Sigma (Steinheim, Germany).

Static Headspace Gas Chromatography-Mass Spectrometry (SH-GC-MS). SH-GC analysis was performed with an HP 6890 gas chromatograph (Hewlett-Packard) equipped with a static headspace sampler Hewlett-Packard model 7694.

Ten milliliter vials containing 2 g of roasted ground coffee were immediately sealed with silicone rubber Teflon caps.

Temperature of Equilibration. To choose the optimal temperature of analysis, three different temperatures at seven different equilibration times were tested. Vials were equilibrated at 30, 45, 60, 80, 100, 120, and 150 min using temperatures of 60, 80, and 90 °C.

Time of Equilibration. To select an optimal equilibration time for the extraction of each type of compound, vials were equilibrated at 30, 45, 60, 80, 100, 120, and 150 min using a temperature of 90 °C. Analysis was performed in triplicate.

After the vials had been pressurized with carrier gas for 12 s, 3 mL of the coffee headspace sample was injected into a capillary column HP-Wax (60 m × 0.25 mm × 0.5 μm film thickness; Hewlett-Packard).

The injector temperature was set at 180 °C, and helium (10 mL/min linear speed) was the carrier gas. The oven temperature was maintained at 40 °C for 6 min and programmed to 190 °C at 3 °C/min.

Mass spectrometry analysis was carried out using a Hewlett-Packard mass selective detector model 5973 coupled to the gas chromatograph. The mass spectrometer operated in the electron impact ionization mode (70 eV), with a scan range of 33 to 300 amu. The ion source temperature was set at 230 °C.

Identification of the Volatile Compounds. Volatile compounds were identified by comparing their spectra to those of the Wiley library and also by comparison of their GC Kovats index and retention time to those of standard compounds and data from literature.

Quantitative Measurements. The total content of the volatile of each headspace analysis was defined by integrating the peak areas of the 122 compounds identified. The relative percentages of individual compounds were calculated from the total content of volatile on the chromatograms.

Statistical Analysis. The data were subjected to one-way analysis of variance (ANOVA) and a Tukeys posteriori test using SPSS 9.0 for Windows. A significance level of p ≤ 0.05 was used for all mean evaluations.
Pyrazines showed an irregular extraction when these times were varied. No significant differences relating to the total quantity of the volatile compounds extracted were found among the different equilibration times studied.

Volatile Compounds Identified. One hundred and twenty-two volatile compounds were identified by headspace analysis in roasted ground coffee (Table 2): 26 furans, 20 ketones, 20 pyrazines, 9 alcohols, 9 aldehydes, 8 esters, 6 pyrroles, 6 thiophenes, 4 sulfur compounds, 3 benzenic compounds, 2 phenolic compounds, 2 pyridines, 2 thiazoles, 1 oxazole, 1 lactone, 1 alkane, 1 alkene, and 1 acid. Ninety-nine of them have been previously identified in coffee (11, 13–15, 20–25).

Figure 2. Chromatograms obtained at 60 °C/30 min and at 90 °C/150 min.

Table 1. Percentages of Extraction of Each Chemical Class with Regard to the Total Area at 90 °C

<table>
<thead>
<tr>
<th>compound</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>80 min</th>
<th>100 min</th>
<th>120 min</th>
<th>150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>aldehydes</td>
<td>12.56 (2.14)</td>
<td>13.74 (2.48)</td>
<td>13.40 (2.84)</td>
<td>12.61 (2.16)</td>
<td>12.81 (2.23)</td>
<td>13.02 (1.69)</td>
<td>12.69 (1.40)</td>
</tr>
<tr>
<td>ketones</td>
<td>28.40 (3.12)</td>
<td>28.45 (2.01)</td>
<td>29.24 (2.85)</td>
<td>28.04 (2.84)</td>
<td>27.50 (2.60)</td>
<td>27.40 (2.47)</td>
<td>26.79 (2.29)</td>
</tr>
<tr>
<td>alcohols</td>
<td>0.94 (0.26)</td>
<td>1.02 (0.26)</td>
<td>1.01 (0.29)</td>
<td>0.94 (0.22)</td>
<td>0.95 (0.24)</td>
<td>0.95 (0.26)</td>
<td>0.97 (0.27)</td>
</tr>
<tr>
<td>pyrroles</td>
<td>1.47 (0.30)</td>
<td>1.52 (0.21)</td>
<td>1.42 (0.27)</td>
<td>1.23 (0.37)</td>
<td>1.30 (0.27)</td>
<td>1.25 (0.17)</td>
<td>1.25 (0.14)</td>
</tr>
<tr>
<td>pyrazines</td>
<td>7.35 (0.49)</td>
<td>6.05 (1.39)</td>
<td>6.15 (1.46)</td>
<td>7.05 (1.42)</td>
<td>7.15 (0.06)</td>
<td>6.71 (0.62)</td>
<td>6.85 (0.84)</td>
</tr>
<tr>
<td>furans</td>
<td>21.31 (3.00)</td>
<td>21.37 (1.39)</td>
<td>21.58 (2.48)</td>
<td>20.52 (3.23)</td>
<td>21.06 (1.76)</td>
<td>20.77 (1.57)</td>
<td>19.97 (1.72)</td>
</tr>
<tr>
<td>thiazoles</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.00)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.00)</td>
</tr>
<tr>
<td>thiophenes</td>
<td>0.20 (0.05)</td>
<td>0.23 (0.06)</td>
<td>0.20 (0.06)</td>
<td>0.18 (0.06)</td>
<td>0.19 (0.05)</td>
<td>0.19 (0.04)</td>
<td>0.19 (0.03)</td>
</tr>
<tr>
<td>esters</td>
<td>3.76 (0.42)</td>
<td>4.26 (0.04)</td>
<td>4.22 (0.16)</td>
<td>4.10 (0.14)</td>
<td>4.04 (0.12)</td>
<td>4.03 (0.04)</td>
<td>3.99 (0.07)</td>
</tr>
<tr>
<td>oxazoles</td>
<td>0.03 (0.01)</td>
<td>0.04 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.01)</td>
<td>0.03 (0.00)</td>
<td>0.03 (0.00)</td>
<td>0.03 (0.00)</td>
</tr>
<tr>
<td>lactones</td>
<td>0.63 (0.09)</td>
<td>0.44 (0.26)</td>
<td>0.59 (0.14)</td>
<td>0.66 (0.02)</td>
<td>0.61 (0.05)</td>
<td>0.61 (0.02)</td>
<td>0.52 (0.07)</td>
</tr>
<tr>
<td>sulfurous</td>
<td>0.52 (0.13)</td>
<td>0.46 (0.11)</td>
<td>0.39 (0.07)</td>
<td>0.35 (0.09)</td>
<td>0.32 (0.07)</td>
<td>0.30 (0.04)</td>
<td>0.29 (0.05)</td>
</tr>
<tr>
<td>pyridines</td>
<td>4.06 (1.30)</td>
<td>3.04 (1.95)</td>
<td>3.06 (2.25)</td>
<td>3.91 (0.23)</td>
<td>4.14 (1.11)</td>
<td>3.60 (0.47)</td>
<td>4.01 (0.32)</td>
</tr>
<tr>
<td>alkanes</td>
<td>0.10 (0.01)</td>
<td>0.10 (0.01)</td>
<td>0.10 (0.01)</td>
<td>0.08 (0.02)</td>
<td>0.09 (0.02)</td>
<td>0.08 (0.01)</td>
<td>0.08 (0.01)</td>
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<tr>
<td>alkenes</td>
<td>0.43 (0.03)</td>
<td>0.44 (0.06)</td>
<td>0.45 (0.03)</td>
<td>0.45 (0.04)</td>
<td>0.45 (0.04)</td>
<td>0.45 (0.02)</td>
<td>0.44 (0.02)</td>
</tr>
<tr>
<td>phenolic acids</td>
<td>0.07 (0.01)</td>
<td>0.06 (0.01)</td>
<td>0.06 (0.01)</td>
<td>0.06 (0.01)</td>
<td>0.06 (0.01)</td>
<td>0.05 (0.01)</td>
<td>0.06 (0.01)</td>
</tr>
<tr>
<td>acids</td>
<td>7.31 (2.00)</td>
<td>7.56 (3.55)</td>
<td>7.55 (2.51)</td>
<td>8.59 (1.07)</td>
<td>7.88 (1.58)</td>
<td>8.75 (1.51)</td>
<td>8.80 (1.39)</td>
</tr>
<tr>
<td>benzenic acids</td>
<td>0.05 (0.01)</td>
<td>0.06 (0.01)</td>
<td>0.05 (0.01)</td>
<td>0.05 (0.02)</td>
<td>0.05 (0.01)</td>
<td>0.05 (0.01)</td>
<td>0.05 (0.01)</td>
</tr>
<tr>
<td>unidentified</td>
<td>17.16</td>
<td>18.29</td>
<td>17.28</td>
<td>18.78</td>
<td>18.44</td>
<td>19.57</td>
<td>21.00</td>
</tr>
</tbody>
</table>

*a Numbers in parentheses are standard deviations.
The furans are particularly important in quantity and sensory interest. Furfuryl alcohol and 3-methylfuran were the major constituents of the headspace sample (13.8%). Twenty-six furans were identified in this work representing, at 90 °C, between 21.58% of all the compounds extracted at 60 min and 19.97% at 150 min (Table 1).

Numerous furan compounds in coffee are often responsible for the burnt sugar, burnt, and caramel aromas (1, 13). Furfuryl alcohol, which appears to be the compound with the lowest vapor pressure in a headspace over brewed coffee (12), has been associated with bitter and burnt aromas (13, 28). However, Ho et al. (29) related that alkyl- and alkenyl-substituted furans, such as 3-methylfuran, do not present any sensory interest. Furfuryl alcohol and 3-methylfuran were the most abundant furans identified in our work representing, at 90 °C, between 7.89% of all the volatiles extracted at 30 min and 6.86% at 150 min for furfuryl alcohol and between 6.29% at 45 min and 5.47% at 80 min for 3-methylfuran (Table 3).

Among the aldehydes, which are considered to be some of the most volatile components in coffee (28), acetaldehyde, propanal, and 2-methylpropanal were predominantly present and were related by Semmelroch...
and Grosch (25, 27) to be potent odors of coffee powders and brews. Acetaldehyde constituted between 2.15% of all the extracted compounds at 60 min and 1.95% at 150 min, 2-methylpropanal between 4.36% at 45 min and 4.03% at 30 min, and propanal between 2.49% at 60 min and 2.30% at 30 min (Table 3).

Alcohols were present at relatively high concentrations in freshly roasted coffee, although they are readily lost by volatilization and oxidation. Ethanol was the most abundant alcohol detected in the ground roasted coffee studied, but it represented only between 0.71% at 60 min at 90 °C and 0.64% at 30 min (Table 3).

Four sulfur-containing compounds such as methanethiol, dimethyl sulfide, dimethyl disulfide, and carbon disulfide were detected in roasted ground coffee using headspace gas analysis. Sulfur compounds, which are known to be very O₂ sensitive (14), have very low threshold values and play an important role in the aroma (26). The most important one is methanethiol, which mainly corresponds to the decrease of aroma freshness (14). Due to the O₂ sensitivity of the sulfur-containing compounds, the quantity of this chemical class extracted decreased when the time of equilibration increased, from 0.52% at 30 min to 0.29% at 150 min (Table 1). All four of the sulfur-containing compounds were highly extracted when using 30 min as the equilibration time.

Leino et al. (13) reported that 1-methylpyrrole was the only volatile alkylpyrrole detected with the headspace technique. In this work, five other pyroles were identified: 1-ethyl-1H-pyrrole, 1H-pyrrole, 2-formyl-1-methylpyrrole, 2-acetyl-1-methylpyrrole, and N-furfurylpyrrole. 1-Methylpyrrole was the most abundant pyrrole, representing between 0.78% at 45 min and 0.63% at 80 min (Table 3).

Flament (30) found 79 pyrazines in coffee, and Clarke (28) reported that 81 compounds containing a pyrazine ring have been detected. Twenty pyrazines have been detected by headspace gas analysis in this work. 2-Methylpyrazine was the most abundant one detected in our coffee, representing, at 90 °C, between 2.98% at 60 min and 3.74% at 45 min of all the compounds extracted (Table 3).

Pyrazines are abundant in coffee (13) and are usually associated with the generation of roasted and burnt flavor notes (29), although it may alter depending on the concentration and by synergetic/antagonistic effects among the compounds present (13). Many pyrazines are recognized as the volatiles contributing to roasted aromas of cooked foods (12, 21).

Two pyridines have been detected in roasted ground coffee: pyridine and 2-methylpyridine. Pyridine has bitter and roasty notes, and 2-methylpyridine is astrin- gent (26, 28). Pyridines have been found in only a relatively small number of foods that have been subjected to some heat treatment (31). The presence of pyridines is often associated with off-flavors. Pyridines are described as having green, bitter, astringent, and/or roasty notes.

One phenolic compound was detected in roasted ground coffee. This type of compound, usually present in coffee in low concentrations, has been related to burnt aroma and other aromas such as smoky, spicy, or clove-like and also imparts astringency (26, 28). 2-Methoxyphenol is the unique phenolic compound detected in our work between 0.05% at 80, 120, and 150 min and 0.07% at 30 min (Table 3).

Trace amounts of thiazoles, thiophenes, esters, oxazoles, lactones, alkanes, alkenes, benzenic compounds, and acids were also detected in roasted ground coffee.

In summary, at higher temperature of equilibration, greater extraction of the volatile compounds was obtained and no differences in the qualitative profiles were observed. When the time of equilibration was increased, the quantity of the volatile compounds extracted also increased, but in an irregular way depending on the chemical family considered. The time of equilibration for headspace analysis of roasted ground Arabica coffee should be selected depending on the chemical class or the compound that is being studied: short equilibration times for the analysis of sulfur compounds, aldehydes, pyroles, pyrazines, thiophenes, esters, and phenolic compounds and longer times for pyridines, ketones, and furans.

LITERATURE CITED


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