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Discrimination between defective and non-defective Brazilian coffee beans by their volatile profile

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Abstract

The coffee roasted in Brazil is considered to be of low quality, due to the presence of defective coffee beans that depreciate the beverage quality. In view of the fact that coffee flavour is directly related to the volatile compounds produced during roasting, the objective of the present study was to perform a comparative evaluation of the volatile fraction of defective (black, immature, sour) and healthy coffee beans, in order to find possible chemical markers for detection of defective coffee beans in roasted coffee. Volatiles extraction and concentration was performed by solid phase micro-extraction (SPME) of the roasted coffee headspace, using a triple phase (divinylbenzene/carboxen/polydimethylsiloxane) fiber. Analysis of the volatile profiles was performed by GC–MS. The results obtained showed that the proposed methodology was adequate for extraction, concentration and analysis of the coffees volatile profile. Several substances were identified as possible markers for differentiating black, sour and immature beans from healthy coffee beans. Statistical analysis of the data by principal components (PCA) demonstrated that the volatile profile enables the differentiation of healthy and defective coffees. The data were separated into two major groups, one represented by immature and black beans and the other by healthy and sour coffee beans. Such results indicated that black and sour beans can be associated to fermentation of immature and of healthy beans, respectively.

Keywords: Coffee; Defective beans; Volatiles profile; Roasting; SPME; GCMS; PCA analysis

1. Introduction

Flavour is one of the most important quality evaluation criteria employed for coffee commercialization and consumption, being directly affected by the presence of defects. The term defect is employed in reference to the occurrence of defective (black, sour or brown, immature, bored, broken, etc.) beans and also of extraneous matter (husks, twigs, stones, etc.) in a given coffee sample. The most important types of defect are represented by black, sour or brown and immature beans. The defects black and sour are associated to bean fermentation, and have been reported as quite relevant in downgrading coffee flavour (Clarke, 1987). Black beans result from coffee fruits that fall on the ground by action of rain or over-ripening (Mazzafera, 1999) or from dead beans within the coffee cherries (Clarke, 1987). Sour beans are those that have fermented due to improper processing conditions, such as 'overfermentation' during wet processing, improper drying or picking of overripe cherries. Immature beans are those that come from immature fruits (Franca, Oliveira, Mendonça, & Silva, 2005; Vasconcelos, Franca, Glória, & Mendonça, 2007). Even though defects are known to negatively affect coffee flavour, the counting of defects alone cannot be used to accurately predict cup quality (Smith, 1985).

Defective coffee beans are usually present in the coffee produced in Brazil, due to the strip-picking harvesting and processing practices adopted by the coffee producers (Oliveira, Franca, Mendonça, & Barros-Júnior, 2006).

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Such beans are separated from the healthy ones prior to export and dumped in the Brazilian internal market, being employed by the Brazilian roasting industry in blends with healthy ones. This practice results on a low grade roasted and ground coffee being consumed in Brazil (Vasconcelos et al., 2007). Currently there are no analytical methodologies that allow for detection and quantification of defective beans in roasted coffee, and thus an assessment of chemical attributes that could allow for differentiation between defective and non-defective beans is of relevance.

Only a few studies have addressed the physical and chemical attributes of defective coffee beans in comparison to non-defective ones (Mazzafera, 1999; Franca et al., 2005; Oliveira et al., 2006; Vasconcelos et al., 2007). Such studies have shown that there are physical and chemical differences between defective and non-defective coffee beans prior to roasting but have not been able to provide the means to allow for discrimination after roasting. To the authors knowledge, the volatile profile of defective coffee beans has not been previously described in the literature. Considering the direct impact of defective beans on coffee flavour and aroma, one could expect that the volatile profile could be a valuable tool for the differentiation of defective and non-defective beans in roasted coffee.

A considerable amount of research has been carried out on the characterization of flavour related substances in coffee, with over 850 substances being identified on the volatile fraction of roasted coffee (Grosch, 2001; Rocha, Maeztu, Barros, Cid, & Coimbra, 2003). The most common classes of compound reported in the headspace of this matrix include acids, alcohols, aldehydes, alkanes, alkenes, benzenic compounds, esters, furans, ketones, lactones, oxazoles, phenolic compounds, pyridines, pyrazines, pyrroles, sulfur compounds, thiazoles, and thiophenes (Dart & Nursten, 1985; Sanz, Ansorena, Bello, & Cid, 2001; Yeretzian, Jordan, & Lindinger, 2003; Mondello et al., 2004; Ryan et al., 2004). Some of the recent studies on the volatile profile of roasted coffee have focused on the comparison of Arabica and Robusta coffees and also of coffees from different origins (Costa Freitas & Mosca, 1999; Costa Freitas, Parreira, & Vilas-Boas, 2001; Rocha et al., 2003; Zambonin, Balest, De Benedetto, & Palmisano, 2005. The study performed by Costa Freitas & Mosca (1999) described the use of purge and trap method coupled to gas chromatography analysis for the determination of volatile patterns of green and roasted coffees from Arabica and Robusta varieties. Hierarchical cluster analysis (HCA) was applied to the reduced set of variables obtained with PCA. Clustering of roasted coffees showed a grouping by variety (Arabica and Robusta) and within the variety by geographical origin. Most recent studies showed that gas chromatography–mass spectrometry analysis (GC–MS) of the volatile components of the coffee headspace concentrated by solid-phase micro-extraction (SPME) in combination with principal component analysis (PCA) also allowed for discrimination of Arabica/Robusta blends having different geographical origins (Costa Freitas et al., 2001; Zambonin et al., 2005). Rocha et al. (2003) employed SPME/GC–MS to analyse the volatile profiles of espresso and plunger coffee brews prepared from Arabica and Robusta/Arabica blends. According to this study, the volatile profiles were dependent mostly on the botanical variety rather than on the brew preparation method. PCA of the results provided discrimination between Arabica and Robusta by the first two components (PC1 and PC2) and between brew preparation method by the first and third components (PC1 and PC3). According to the studies developed by Mondello et al. (2004) and Ryan et al. (2004), Arabica and Robusta coffee present quite similar pyrazine compositions, with higher concentrations in Arabica samples. Arabica coffees are characterized by higher amounts of maltol, furfurylpyrroles and lower amounts of phenols and alkylpyrroles in comparison to Robusta coffees.

The successful application of chemometrics for discrimination between coffee varieties and geographical origins indicates that this tool should also be evaluated for discrimination between defective and healthy coffee beans. In view of the above, coupled to the already known sensory effect of defective beans on coffee flavour, the objective of the present study was to verify the feasibility of discriminating defective from non-defective coffee beans by their volatile profiles after roasting.

2. Methodology

Arabica green (crude) coffee samples (2004/2005 crop) were obtained from a local producer (Fazenda Samambaia, Santo Antonio do Amparo, Minas Gerais, Brazil). The coffee beans were subjected to selection made by an electronic sorter. The beans rejected by the sorting machine were used in the present study. This mixture of coffee beans consisted of 1% black, 12% immature, 20% sour and 67% non-defective beans in weight. Black, sour, immature and non-defective beans were manually separated from this mixture and divided in sampling lots. Samples of randomly selected 40 beans were separated from each lot and roasted in a convective oven at 200 °C for 30 min (light roast), 60 min (medium roast) and 120 min (dark roast), in duplicates. Roasted coffee samples were stored in sealed containers at ambient temperature for a maximum period of 24 h. Prior to each analysis, the coffee was ground with an electric coffee grinder (C-mill 5679-01US, Bodum, USA) for 30 s.

An SPME triple phase (divinylbenzene/carboxen/polydimethylsiloxane) 50/30 μm fiber (model 57348-U, Supelco Inc., USA) was employed for the extraction of volatiles from the coffee beans headspace. Prior to use, the fiber was conditioned according to manufacturer recommendations. Ground coffee samples (3 mL) were placed in a sealed 5 ml vial (Supelco Inc., USA), and heated for 10 min at 70 °C. The SPME needle was then inserted into the vial, and the fiber exposed to the headspace above the coffee sample for 40 min at 70 °C. After sampling, the

fiber was thermally desorbed in the GC injection port for 10 min at 250 °C.

GC analyses were performed in duplicate using a gas chromatograph (Trace Ultra) coupled to a mass spectrometer (PolarisQ) (ThermoElectron, San Jose, CA), a RTX-5MS column (5% diphenyl, 95% dimethyl polysiloxane) 30 m × 0.25 mm ID (Restek, Ireland) and helium as carrier gas (1 ml/min). The injector temperature was set to 250 °C. The ion source (detector) and interface temperatures were 300 °C and 275 °C, respectively. The GC oven temperature was programmed from 40 °C (5 min) to 180 °C at the rate of 3 °C/min, then to 250 °C (5 min) at 10 °C/min. The GC injector was operated in the splitless mode. Mass spectra were acquired in the electron impact mode at 70 eV and using a m/z range of 50–650 and a scan time of 2 s. Identification of compounds was achieved by comparison of the mass spectra with those of the NIST/EPA/NIH Mass Spectral Library, version 2.0 (available in the instrument software), considering a similarity level (RSI) higher than 800.

For principal component analysis, data matrices were assembled so that each row corresponded to a coffee sample and each column represented the peak area of a specific component. Only the chromatographic peaks with S/N (signal-to-noise ratio) higher than 50 were included in the final data matrix. This choice was based on the extensive number of peaks detected (over 250). Some preliminary tests with smaller S/N ratio values indicated that the amount of detected substances would be prohibitive and increase the degree of redundancy in terms of multivariate statistical analysis, since most of them were present in similar amounts in all chromatograms.

3. Results and discussion

Results for dry matter loss during roasting are shown in Fig. 1, with the dashed lines representing a logarithmic model fit. Average values per roasting degree were 13%, 14% and 16%, corresponding to light, medium and dark roasts, respectively. A comparison of weight loss data between defective and non-defective beans shows that, regardless of roasting conditions, defective beans presented lower weight loss values than non-defective ones. Also, a comparison of the logarithmic model coefficients (3.1 – healthy; 2.8 – immature; 2.5 – sour; 2.2 – black) indicates that healthy coffee beans roast slightly faster than defective coffee beans. These results confirm that defective beans roast to a lesser degree than non-defective ones, under the same processing conditions, as reported in previous studies (Franca et al., 2005; Vasconcelos et al., 2007). In view of such results, the comparison of volatile profiles of healthy and defective beans will be performed under the same roasting conditions and also under different roasting conditions (higher roasting time for defective beans in comparison to healthy ones), in order to simulate situations for which the healthy and defective coffees are roasted together or separately.

Typical chromatograms obtained after 2 h roasting are presented in Fig. 2. It was possible to extract and detect a great number of volatile compounds. Among those, only a few compounds were detected in all defective beans, that were not present in the healthy coffee samples, as shown in Table 1. Such compounds, or at least similar ones, have been previously identified in roasted coffee. Sanz et al.

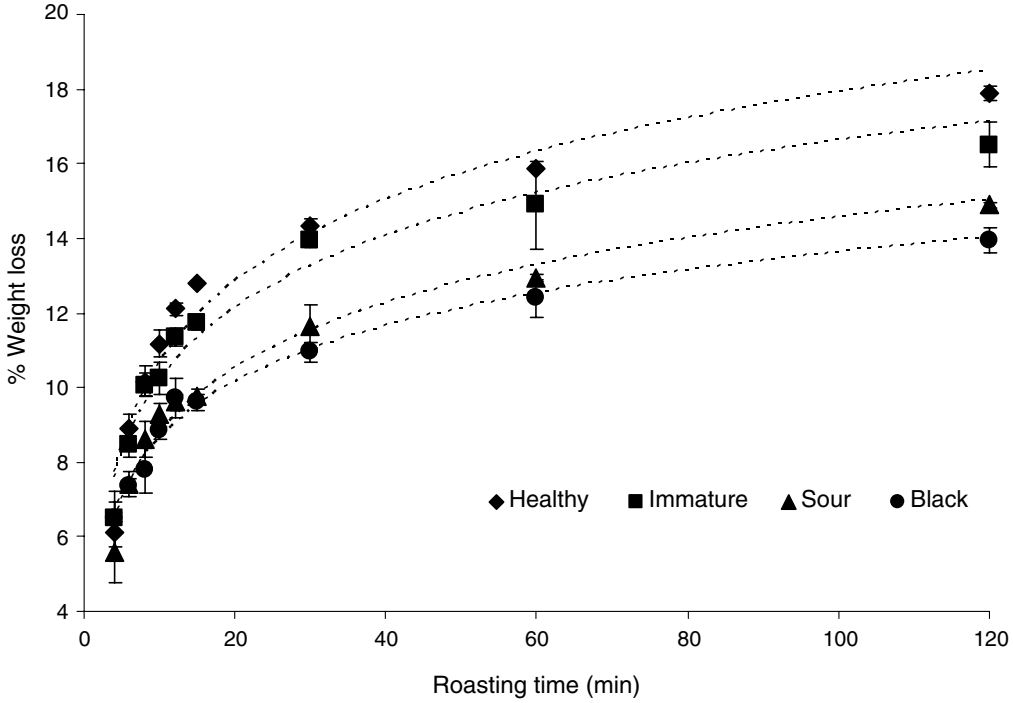

Fig. 1. Dry matter loss during roasting.

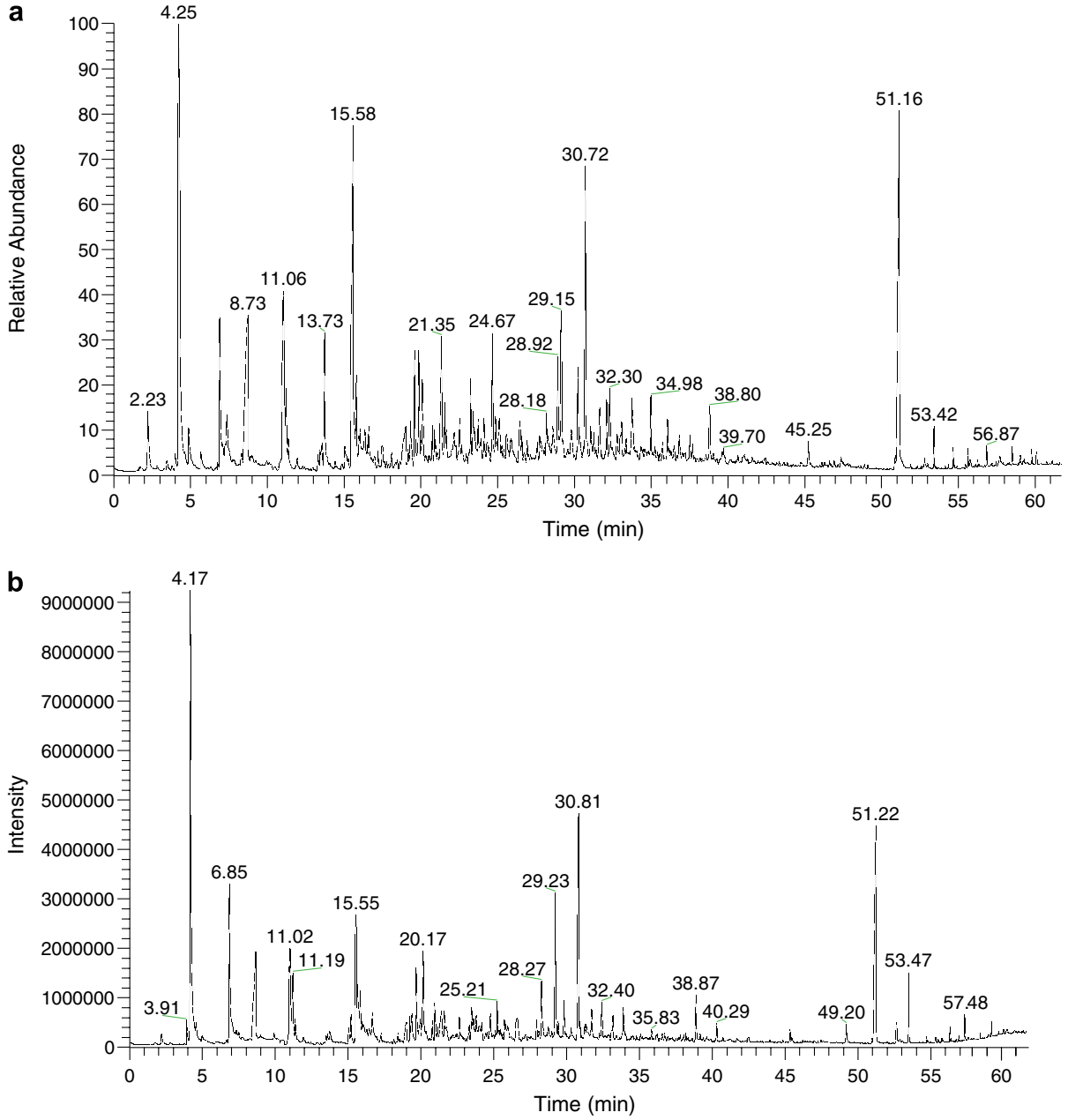

Fig. 2. Typical HS–SPME–GC–MS chromatograms of roasted and ground coffee headspace: (a) healthy; (b) black; (c) sour and (d) immature. Peak identification corresponds to retention time values.

(2001) reported the occurrence of acetic acid-methyl ester, 1H-pyrrole, 2-methylthiazole, and 2-ethyl-6-methylpyrazine, this last compound being also reported by Yeretzian et al. (2003), Mondello et al. (2004) and Zambonin et al. (2005). No reports on the occurrence of 1,5-dimethyl-2-pyrrole carbonitrile were found in the reviewed literature. According to De Maria, Moreira, & Trugo (1999), pyrroles present characteristic sensory properties. These authors reported that alkyl and acyl pyrroles have been associated to off-flavours, but in low concentrations, alkyl and acyl pyrroles present a sweet and slightly burnt flavor. The compounds of such class that have been previously characterized with significant impact on coffee flavour are 2-acetylpyrrole, *n*-furyl-2-methylpyrrole and *n*-ethyl-2-formylpyrrole.

The compounds that were present in only one or two types of defects in comparison to healthy coffee under the same or milder roasting conditions, are listed in Tables 2–4. Even though the specific compounds listed in Tables 2–4 were not detected in healthy coffee, most of them belong to classes that have been identified in previous studies, such as aldehydes, furans, pyrazines, pyridines, pyrroles, etc. (De Maria et al., 1999; Sanz et al., 2001; Mondello et al., 2004 ; Zambonin et al., 2005). An evalua-

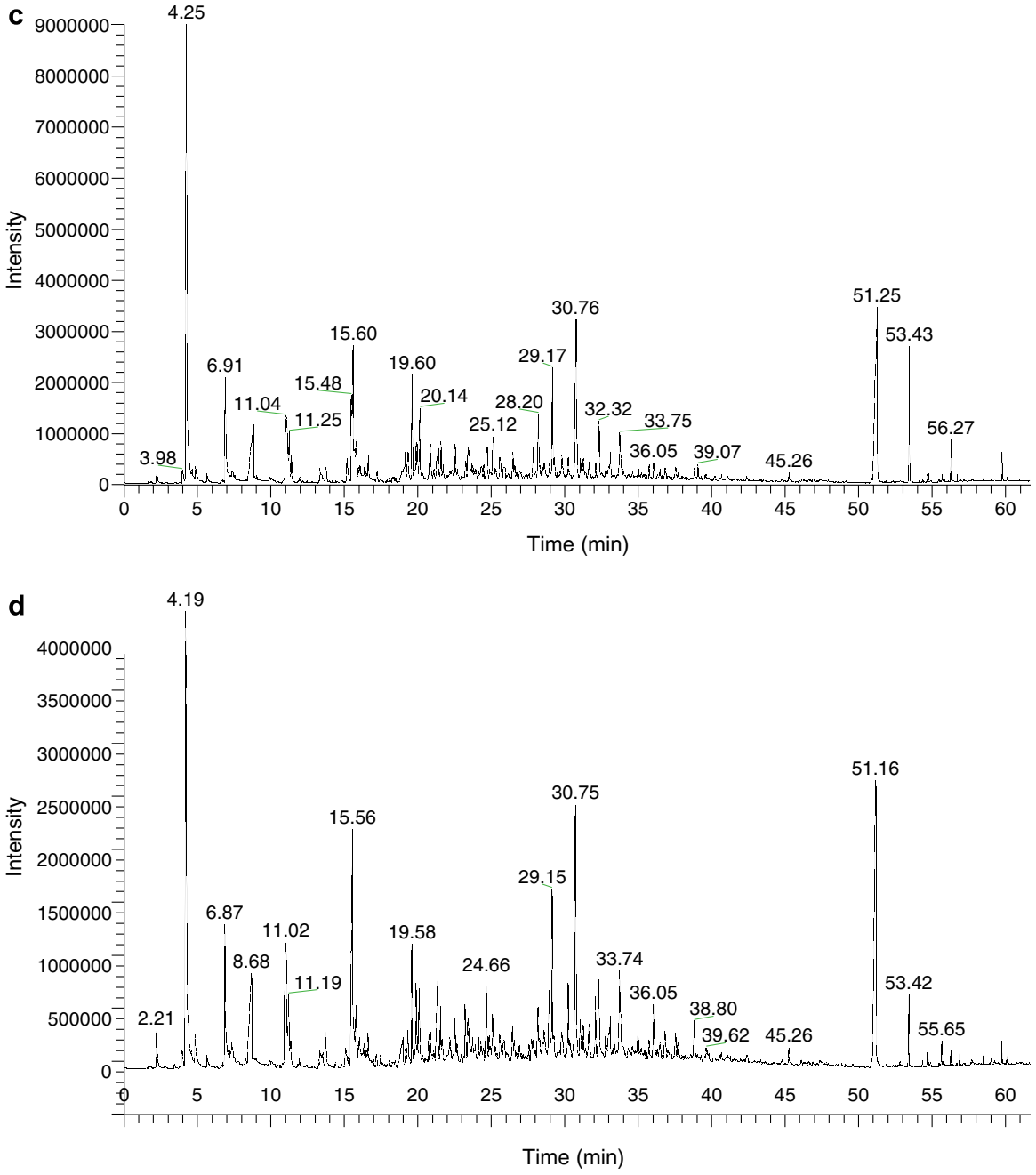

Fig. 2 (*continued*)

tion of the data presented in Tables 2–4 shows that sour beans presented the highest amount of substances not detected in healthy coffee, followed by black and immature beans. This could be an indication that some of those compounds are associated to coffee fermentation. Both black and sour coffees presented four pyrazines compared to only one detected in immature beans. Among those, only 2-ethyl-5-methyl-pyrazine, present in black beans, has been reported in recent studies (Sanz et al., 2001; Yeretzian et al., 2003; Zambonin et al., 2005). Pyrazines are products of Maillard reactions known to be abundant in coffee, and over 80 of such compounds have been previously detected in coffee (Grosch, 2001). Many pyrazines have been reported to contribute to roasted aromas of cooked foods, and in the case of coffee, such compounds have been associated to burnt and roasted flavours. Some recent studies have pointed out pyrazine derivatives as key components for differentiation between Arabica and Robusta coffees (Rocha et al., 2003; Zambonin et al., 2005). Pyrroles were also detected in greater quantity in both black and sour coffee beans in comparison to immature ones. Such compounds have been associated to medicine and cereal flavours (Rojas Monroy, 2005). Among those listed in Tables 2–4, only 1-(2-furanyl methyl)-1H-pyrrol and 1-

Table 1
Tentative identification of compounds that were detected in the three types of defective beans that were not detected in the healthy coffee beans

Retention time (min)	S/N[a]			Compound	*m/z* of the most intense ions (relative abundance %)
	Black	Immature	Sour		
60 min roasting					
24.5	104	116	213	Benzene acetic acid, methyl ester	91(100), 140(42), 65(17)
27.9	315	310	521	2-Isoamyl-6-methylpyrazine	108(100), 121(32), 147(30)
120 min roasting					
4.6	226	80	122	1H-pyrrole	67(100), 68(8), 52(5)
6.6	90	195	246	4-Methylthiazole	71(100), 99(76), 72(75)
20.9	837	592	863	1,5-Dimethyl-2-pyrrolecarbonitrile	119(100), 120(72), 78(30)

[a] S/N = signal-to-noise ratio.

(1H-pyrrol,2-yl)-ethanone have been previously reported in roasted coffee (Zambonin et al., 2005). Among the substances detected only in defective beans, aldehydes were present only in the black and sour coffee samples. Such compounds have been previously reported in association with fermented flavours (Rojas Monroy, 2005).

Given that an extensive number of compounds were detected and identified only in headspaces of specific defective coffee beans and the fact that similar compounds were identified on healthy coffee, multivariate statistical analysis (PCA and clusters) was performed in order to verify the possibility of discrimination between defective

Table 2
Tentative identification of compounds that were detected exclusively in the black coffee beans at a specific retention time and roasting degree

Retention time (min)	S/N[a]	Compound	*m/z* of the most intense ions (relative abundance %)
60 min roasting			
15.65	4812	2-Ethyl-5-methylpyrazine	121(100), 122(37), 123(7)
16.28	65	2-Carboxaldehyde-1H-pyrrole	94(100), 95(87), 66(42)
16.69	349	3,6-Dimethyl-2(1H)-pyridinone	80(100), 94(75), 123(35)
18.15	66	Tricyclo[2.2.1.0.2,6] heptan-3-one, oxime	94(100), 106(95), 123(80)
20.35	433	1-Methyl-3-piperidinone	84(100), 108(71), 113(53)
21.29	790	2,3-Dihydro-1H-indole	118(100), 117(37), 91(27)
21.63	387	2-Methyl,5-propyl-pyrazine	108(100), 136(37), 93(31)
25.49	114	2-Pentyl-pyridine	93(100), 106(44), 120(34)
38.05	77	1-(4-Hydroxy-3-methoxyphenyl)-ethanone	151(100), 166(37), 127(23)
120 min roasting			
12.57	59	2-(Methyl amino)-benzoic acid	106(100), 107(81), 77(41)
16.33	88	2-Dodecyl-1-methyl-pyrrolidine	84(100), 94(20), 66(12)
17.62	109	2-Cyclopenten-1-one, 3,4-dimethyl	110(100), 93(67), 95(32)
18.40	381	*n*-Buthylbenzene	91(100), 92(54), 65(21)
19.22	407	Isopropenyl-pirazine	119(100), 78(21), 120(15)
21.61	343	2-Methyl-6-propyl-pirazine	108(100), 136(36), 93(34)
22.20	73	3-(4-Methyl-5-cis phenyl-1,3-oxazolidin-2-yl)-furan	122(100), 123(59), 94(25)
22.36	170	(Unidentified)	126(100), 133(57), 98(46)
23.11	72	4-Methyl-5-ethyltiazole	127(100), 112(62), 71(39)
23.22	751	1-Isopropyl-3,4-dimethyl 2-pyrazoline	125(100), 140(14), 69(11)
23.29	182	1,4-Diisopropyl ciclohexane	69(100), 55(79), 83(63)
24.19	247	2-One-5,9-dimethyl-, (E)-5,8-decadien	107(100), 122(41), 77(41)
24.75	520	1-(2-Furanyl methyl)-1H-pyrrole	81(100), 147(83), 53(43)
25.44	389	2-Pentyl-pyridine	93(100), 106(39), 120(29)
26.65	540	3-Methyl, 2-furanylmethyl ester butanoic acid	81(100), 98(57), 53(46)
27.49	126	1,5-Dimethyl-2-pyrrole carbonitrile	119(100), 120(71), 108(23)
27.92	449	2-Buthy-l,3-methylpirazine	108(100), 121(33), 107(31)
34.75	97	3-(3,4-Dihydro-2H-pyrrol-5-yl)-pyridine	146(100), 145(56), 104(35)
35.81	178	(Unidentified)	173(100), 174(84), 145(23)
37.79	218	3-Amino-4-methyl-6-methoxyquinoline	145(100), 188(63), 159(16)
46.34	80	(1,1,3,3-Tetramethylbutyl)-phenol	135(100), 107(43), 136(11)
46.99	75	4-Nonylphenol	135(100), 107(77), 212(50)
47.99	50	Hexestrol (phenol,4,4′-[1,2-diethyl-1,2-etanediyl] bis-	134(100), 107(45), 136(10)

[a] S/N = signal-to-noise ratio.

Table 3
Tentative identification of compounds that were detected exclusively in the immature coffee beans at a specific retention time and roasting degree

Retention time (min)	S/N[a]	Compound	*m/z* of the most intense ions (relative abundance %)
60 min roasting			
13.54	127	1-Methoxy-2-methyl-benzene	122(100), 107(81), 77(75)
16.96	64	2-Cyclohexen,1-ol,-3-methyl-6-(1-methylethyl)-, cis-	84(100), 112(88), 55(51)
25.41	152	5-ol-1H-Indol	133(100), 104(22), 77(21)
28.93	544	3,4-Dihydroxyacetophenone	137(100), 152(67), 81(33)
32.10	507	4-Methyl-2(1H)-quinolinone	130(100), 159(48), 131(18)
33.97	80	4-Hydroxy,3-methoxy-benzonitrile	134(100), 149(64), 106(33)
35.22	87	1-Ethyl-1H-benzaimidazole	146(100), 131(94), 147(23)
37.22	60	Furfuryl, isothiocyanate	81(100), 53(38), 154(32)
39.15	90	1-(4-Methylphenil)-1H-pyrrole	157(100), 115(42), 159(39)
120 min roasting			
6.69	70	3-Carbonitrile-1H-pyrrole	65(100), 92(84), 78(21)
16.34	144	2-Acetonitrile,1-methyl-1H-pyrrole	120(100), 119(84), 71(22)
16.95	79	1,3-Cyclohexanedione	84(100), 112(89), 55(44)
19.13	408	Carbamic acid, phenil, phenil ester	119(100), 94(89), 109(37)
20.33	59	Benzaldehyde	106(100), 105(53), 51(40)
20.51	54	1H-inden-1-ol-2,3-dihydro-	133(100), 57(59), 134(58)
22.94	78	1H-pyrrole,2-carboxaldehyde	95(100), 94(63), 133(29)
23.64	995	2,3,5-Trimethyl-6-ethylpyrazine	149(100), 150(46), 121(42)
24.86	162	2,5-Tiophenedicarboxaldehyde	140(100), 109(58), 139(39)
28.03	98	1,6-Dimethyl-5-oxo,1,2,3,5-tetrahydroimidazol[1.2-a] pyrimidine	165(100), 164(89), 94(75)
34.32	68	2-Naphthalenol,3-methoxy-	131(100), 174(46), 159(22)
40.55	670	[1,1′Biphenil]-4-ol acetate	170(100), 171(42), 151(29)

[a] S/N = signal-to-noise ratio.

and healthy coffee beans. From the chromatograms obtained, the peaks that presented signal-to-noise ratios above 50, regardless of substance identification, were selected to be used as variables for principal component analysis, resulting in a 18×251 data matrix of three healthy and six defective coffee samples (and respective replicates). The first two principal components (PCs) explained 73.2% and 13.5% of the chromatographic variance, respectively. The biplot of the samples under investigation on the first two PCs is presented in Fig. 3. Such results show that the black and immature samples constitute a broad group spread along the negative values of PC1 (x-axis) whereas the healthy and sour samples constitute another group located on the positive side of the x-axis (see dashed lines in Fig. 3). PCA loading data indicated that the aroma components that determine the scores on PC1 correspond to the retention times of 4.20 and 4.26 min. The first one (unidentified compound, mass spectrum displayed in Fig. 4a) contributing to the negative value of PC1, is characteristic of black and immature beans, and the second one (also unidentified, mass spectrum shown in Fig. 4b) contributing to the positive value of PC1, is characteristic of healthy and sour coffee. While the PC1 allowed for distinction between black/immature and healthy/sour, the combined information from both the first and second PC values could be applied to discriminate between fermented/non-fermented samples and also between roasting degrees. PC2 values increased in absolute value as roasting progressed, for all samples. This could be attributed to the increase in volatiles concentration as roasting time increases. To verify similarity between the samples and to single out some classes, Hierarchical cluster analysis (HCA) was applied to the set of variables employed for PCA. The resulting dendrogram is displayed in Fig. 5. Black beans submitted to 120 min roasting were well separated from the other samples (0% similarity), indicating that such type of defect presents the most distinct volatiles profile in comparison to healthy coffee and other types of defective beans. This is in agreement with the fact that such defect has been known to have a significant downgrading effect on coffee flavour and it is considered the most important and worst type of intrinsic defect (Clarke, 1987). Clustering also showed two distinct groups of immature/black and sour/healthy. Within each group, the effect of roasting degree can be observed. The similarity between samples inside each major group indicates that black beans can be associated to fermentation of immature ones, whereas sour beans correspond to fermentation of healthy coffee. It is noteworthy to mention that a correlation of the results obtained in the present study with previous studies from the literature on volatile components in roasted coffee is not possible, since in those studies no references to the presence of defective beans or to the cup quality of the employed coffee samples was made.

4. Conclusions

The coffee headspace volatiles profile was evaluated for defective coffee beans in comparison to non-defective ones, after roasting at light, medium and dark degrees. It was

Table 4
Tentative identification of compounds that were detected exclusively in the Sour coffee beans at a specific retention time and roasting degree

Retention time (min)	S/N[a]	Compound	*m/z* of the most intense ions (relative abundance %)
60 min roasting			
6.72	50	3-Carbonitrile-1H-pyrrole	65(100), 92(91), 78(21)
9.02	58	3,4-Dimethyl-1H-pyrrole	94(100), 95(48), 93(28)
12.06	88	2-Ethyl,4-methyl-1H-pyrrole	94(100), 109(32), 93(16)
13.44	263	Benzaldehyde	105(100), 106(79), 77(76)
15.19	424	2,7-Dioxatryciclo[4.4.0.0(3,8)] dec-4-ene	94(100), 66(59), 65(44)
18.05	72	*N*,*N*-Dimethyl-2-pyridinamine	106(100), 123(96), 94(79)
18.36	87	Bicyclo[3.1.0]hex-2-ene,4-methylene-1-(1-methylethyl)-	91(100), 92(52), 119(25)
18.49	73	3-Methyl-1H-pyrrole	80(100), 81(74), 137(18)
20.27	199	2-Methyl-5-propyl-pyrazine	108(100), 84(58), 107(42)
20.35	321	Benzaldehyde	106(100), 105(65), 51(46)
21.56	609	2-Acetyl-3-methylpyrazine	108(100), 136(34), 93(33)
22.34	129	2-Isobutyl-3-methylpyrazine	108(100), 107(26), 135(24)
23.64	254	2,3,5-Trimethyl-6-ethylpyrazine	149(100), 150(58), 122(28)
24.19	355	4-(2-Methylpropyl) phenol	107(100), 77(32), 122(16)
24.26	161	1,2,3,4-Tetrahydro quinoline	133(100), 109(94), 132(77)
25.29	284	2,3,5-Trimethyl-6-ethylpyrazine	150(100), 107(87), 81(59)
25.59	328	2H-Cyclopenta[d]pyridazine	118(100), 91(29), 63(23)
25.72	324	5,6,7,8-Tetrahydroquinoxaline	133(100), 134(99), 130(53)
27.19	48	5-Amino,2-methoxyphenol	124(100), 139(65), 67(51)
27.31	286	2,3-Dimethyl-5-(1-propenyl),-(Z)-pyrazine	147(100), 148(40), 94(31)
28.30	200	2-Methyl,5-propyl-pyrazine	108(100), 146(46), 121(38)
29.67	395	2-Isopropenyl-3,6-dimethylpirazine	148(100), 147(93), 133(36)
32.87	303	3-(2-Methoxypyridin-6-yl-)1-propanol	124(100), 167(52), 94(36)
34.06	165	*N*,*N*-Diethyl benzenamine	134(100), 149(62), 106(26)
36.32	74	(Unidentified)	174(100), 173(90), 98(21)
39.62	93	1,3,5-trimethyl-2-ciclohexylbenzene	159(100), 202(45), 167(37)
46.28	50	Hexestrol (phenol,4,4′-(1,2-diethyl-1,2-ethanediyl) bis-	135(100), 107(38), 136(11)
55.66	135	1,5,6,7-Tetramethyl-3-phenil bicyclo[3.2.0] repta-2, 6-diene	209(100), 252(34), 210(19)
56.71	96	Octadecanoic acid, metil ester	143(100), 199(96), 255(77)
120 min roasting			
1.84	51	Dimethyl acetal aminoacetaldehyde	75(100), 74(17), 59(10)
6.71	61	3-Methyl-pyridine	93(100), 65(66), 92(50)
11.20	831	Ethylpyrazine	107(100), 108(27), 80(16)
11.28	969	Acetylcholine, chloride	58(100), 71(43), 56(26)
16.20	162	1-(1H-pyrrol,2-yl)-ethanone	94(100), 109(23), 17(95)
17.63	51	4,5-Dihydro-1,4-dimethyl 1H-tatrazaborole	98(100), 54(66), 97(52)
18.49	102	3-Methyl-1H-pyrrole	80(100), 81(80), 54(18)
20.78	929	1-One,4,5-dimethyl-2-cyclohexen	82(100), 124(75), 67(57)
21.98	51	(Unidentified)	139(100), 81(31), 140(26)
22.95	66	2,3-Dimethyl-1H-pyrrole	94(100), 95(99), 80(22)
23.25	237	(Unidentified)	81(100), 70 (69), 150(57)
24.87	80	3,4,5-Trihydroxy fenetyl amine	140(100), 109(53), 94(48)
25.39	397	2-Propyl-pyridine	93(100), 120(29), 106(26)
26.46	478	(Unidentified)	135(100), 93(99), 133(58)
31.57	221	5-Carboxaldehyde,2,3-dihydro,1-methyl 1H-indole	161(100), 112(77), 132(74)
33.66	535	(Unidentified)	146(100), 145(76), 127(56)

[a] S/N = signal-to-noise ratio.

possible to extract and detect approximately 250 volatile compounds considering all samples, and only five of those were detected in all defective beans and were not present in the healthy coffee samples. Sour beans presented the highest amount of substances not detected in healthy coffee, followed by black and immature beans, which could be an indication that some of those compounds are associated to coffee fermentation. Results from PCA showed that the data were separated into two groups, one containing immature and black beans (negative PC1 values) and the other containing healthy and sour coffee beans (positive PC1 values). While the PC1 allowed for distinction between black/immature and healthy/sour, the combined information from both the first and second PC values could be applied to discriminate between fermented/non-fermented samples and also between roasting degrees. Results from hierarchical cluster analysis confirmed that black beans can be associated to fermentation of immature ones, whereas sour beans correspond to fermentation of healthy coffee.

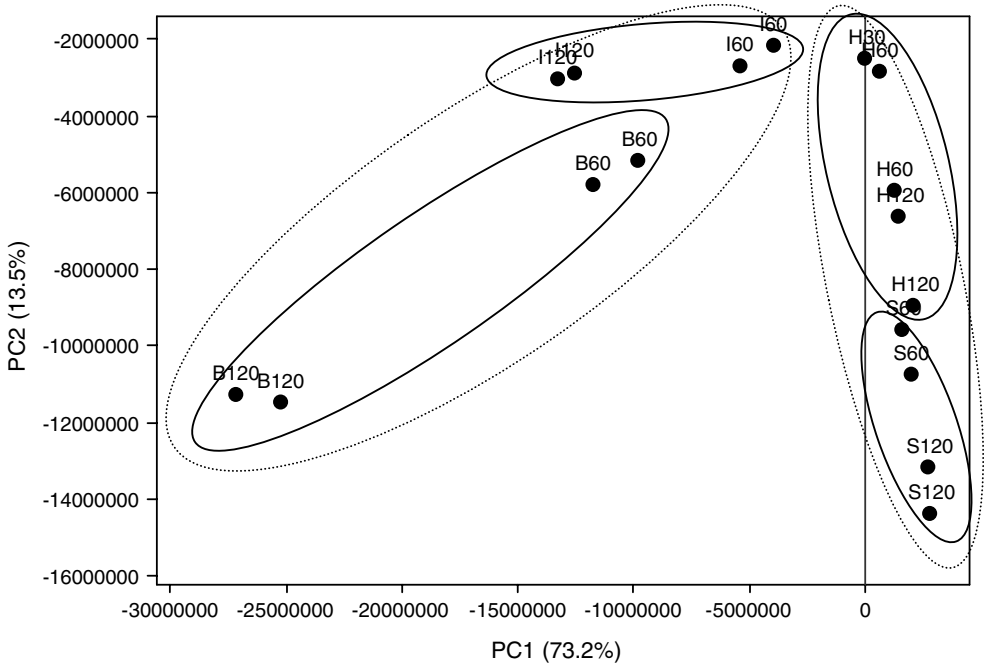

Fig. 3. PCA scores scatter plot of chromatographic SPME areas of coffee volatile compounds (PC1 vs. PC2) – H = healthy, S = sour, B = black, I = immature; numbers correspond to roasting time in minutes.

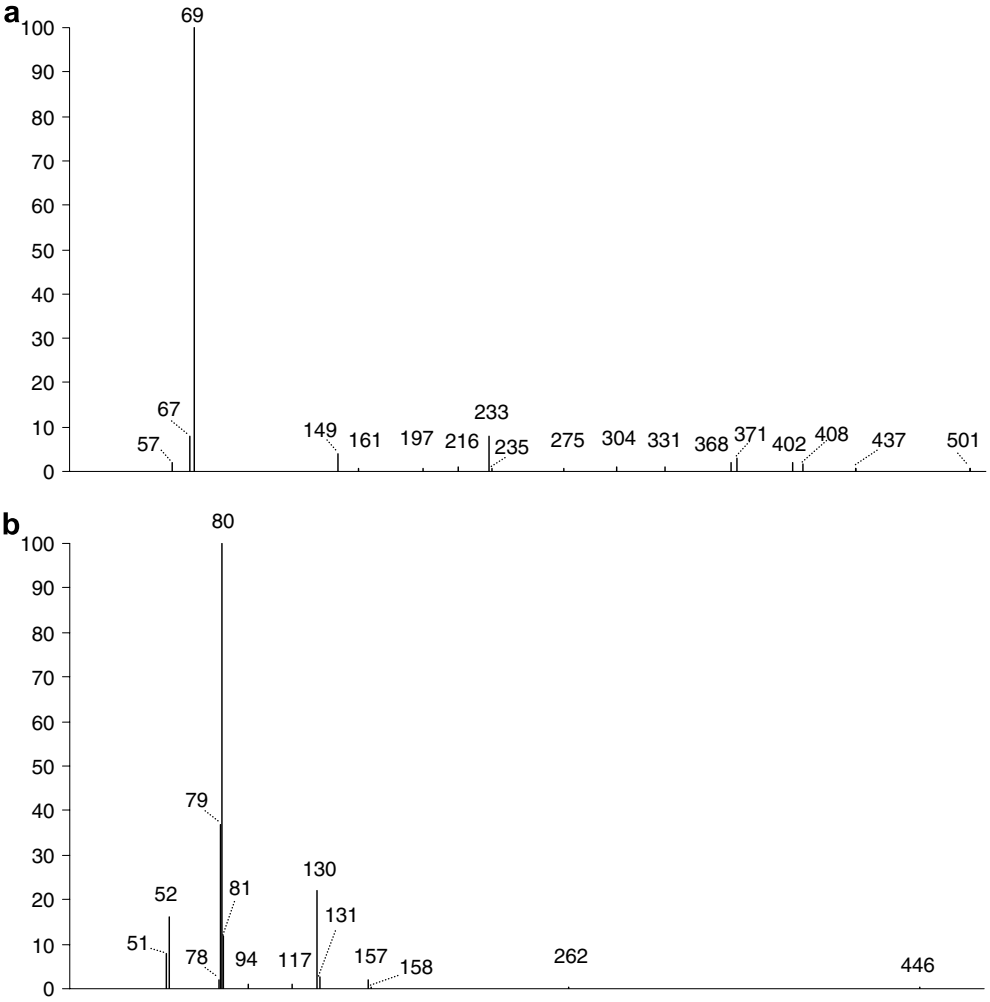

Fig. 4. Mass spectra of the substances that presented the highest influence on PC1 scores: (a) RT = 4.2 min, (b) RT = 4.26 min (RT = retention time).

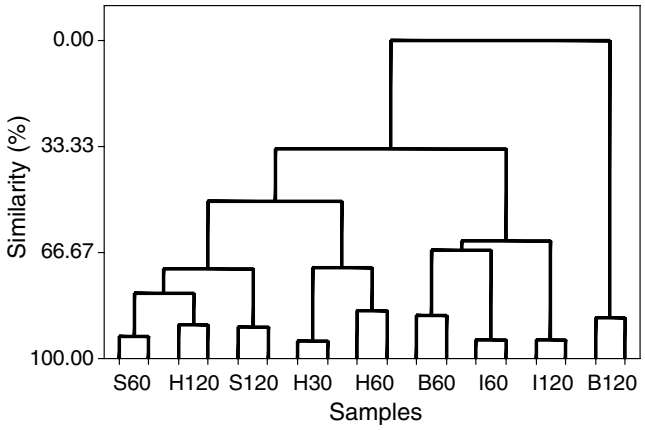

Fig. 5. Cluster analysis (H = healthy, S = sour, B = black, I = immature; numbers correspond to roasting time in minutes).

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References

Clarke, R. J. (1987). Grading, storage, pre-treatments and blending. In R. J. Clarke & R. Macrae (Eds.), *Coffee Volume 2: Technology* (pp. 35–58). London: Elsevier Applied Science.

Costa Freitas, A. M., Parreira, C., & Vilas-Boas, L. (2001). The use of an electronic aroma-sensing device to assess coffee differentiation–comparison with spme gas chromatography–mass spectrometry aroma patterns. *Journal of Food Composition and Analysis, 14*(5), 513–522.

Costa Freitas, A. M., & Mosca, A. I. (1999). Coffee geographic origin – an aid to coffee differentiation. *Food Research International, 32*(8), 565–573.

Dart, S. K., & Nursten, H. E. (1985). Volatile components. In R. J. Clarke & R. Macrae (Eds.), *Coffee Volume 1: Chemistry* (pp. 223–265). London: Elsevier Applied Science.

De Maria, C. A. B., Moreira, R. F. A., & Trugo, L. C. (1999). Componentes voláteis do café torrado. Parte1: compostos heterociclicos (Volatile components in roasted coffee. Part I: heterocyclic compounds). *Química Nova, 22*(2), 209–217 (in Portuguese).

Franca, A. S., Oliveira, L. S., Mendonça, J. C. F., & Silva, X. A. (2005). Physical and chemical attributes of defective crude and roasted coffee beans. *Food Chemistry, 90*(1–2), 84–89.

Grosch, W. (2001). Chemistry III: volatile compounds. In R. J. Clarke & O. Z. Vitzthum (Eds.), *Coffee: Recent Developments* (pp. 68–89). Oxford: Blackwell Science.

Mazzafera, P. (1999). Chemical composition of defective coffee beans. *Food Chemistry, 64*, 547–554.

Mondello, L., Casilli, A., Tranchida, P. Q., Dugo, P., Costa, R., Festa, S., et al. (2004). Comprehensive multidimensional GC for the characterization of roasted coffee beans. *Journal of Separation Science, 27*(5–6), 442–450.

Oliveira, L. S., Franca, A. S., Mendonça, J. C. F., & Barros-Júnior, M. C. (2006). Proximate composition and fatty acids profile of green and roasted defective coffee beans. *Lebensmittel-Wissenschaft & Technologie, 39*, 235–239.

Rocha, S., Maeztu, L., Barros, A., Cid, C., & Coimbra, M. A. (2003). Screening and distinction of coffee brews based on headspace solid phase microextraction/gas chromatography/principal component analysis. *Journal of the Science of Food and Agriculture, 84*, 43–51.

Rojas Monroy, G.M.E. (2005). Caracterización del aroma del café molido de Puerto Rico, mediante la técnica de micro extracción en fase sólida (SPME) y cromatografía de gas acoplada a espectrometría de masas (CG/MS) (Characterization of ground coffee aroma from Puerto Rico using the solid phase microextraction technique (SPME) and gas chromatography coupled to mass spectrometry (GC/MS)). Universidad de Puerto Rico Recinto Universitario de Mayaguez. 156p (MS thesis, Food Science).

Ryan, D., Shellie, R., Tranchida, P., Casilli, A., Mondello, L., & Marriott, P. (2004). Analysis of roasted coffee bean volatiles by using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *Journal of Chromatography A, 1054*(1–2), 57–65.

Sanz, C., Ansorena, D., Bello, J., & Cid, C. (2001). Optimizing headspace temperature and time sampling for identification of volatile compounds in ground roasted Arabica coffee. *Journal of Agricultural and Food Chemistry, 49*, 1364–1369.

Smith, A. W. (1985). Introduction. In R. J. Clarke & R. Macrae (Eds.), *Coffee Volume 1: Chemistry* (pp. 1–41). London: Elsevier Applied Science.

Vasconcelos, A. L. S., Franca, A. S., Glória, M. B. A., & Mendonça, J. C. F. (2007). A comparative study of chemical attributes and levels of amines in defective green and roasted coffee beans. *Food Chemistry, 101*(1), 26–32.

Yeretzian, C., Jordan, A., & Lindinger, W. (2003). Analysing the headspace of coffee by proton-transfer-reaction mass-spectrometry. *International Journal of Mass Spectrometry*, 115–139.

Zambonin, C. G., Balest, L., De Benedetto, G. E., & Palmisano, F. (2005). Solid-phase microextraction-gas chromatography mass spectrometry and multivariate analysis for the characterization of roasted coffees. *Talanta, 66*(1), 261–265.