1 **Thiols in brewed coffee: assessment by fast derivatization and liquid**

2 **chromatography–high-resolution mass spectrometry**

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17

18   **ABSTRACT**

19   In the present paper, we present a simple, reliable, selective and sensitive method for the

20   identification and quantification of volatile thiols at trace levels in coffee brews. A

21   simultaneous derivatization/extraction procedure followed by liquid chromatography–

22   electrospray high-resolution mass spectrometry is proposed and adapted to coffee brew

23   matrix and the performance of the method is evaluated. The linearity, sensitivity, recovery and

24   both the intra-day and inter-day accuracy were all satisfactory. According to established

25   identification criteria, seven target and ten non-target thiols were identified and quantified in

26   coffee brew samples. Several of them are reported here for the first time in coffee brews, and

27   our results are in agreement with previously reported results for coffee powder analyzed using

28   similar analytical approach.

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31   **Keywords:** Thiols; Brewed coffee; Derivatization; High resolution mass spectrometry

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1. INTRODUCTION

Coffee quality is defined mainly by its taste and odor, and most of the volatile compounds responsible for its sensory characteristics are formed during the roasting of coffee beans (López-Galilea, Fournier, Cid, &Guichard, 2006; Caprioli, Cortese, Cristalli, Maggi, Odello, Ricciutelli et al., 2012; Sunarharum, Williams, &Smyth, 2014). Among these compounds, those containing sulfur are some of the most significant for coffee flavor. In particular, volatile thiols are extremely influential on the sensory profile of coffee, despite their low concentrations, due to their low odor thresholds (Sunarharum et al., 2014). Some of them, such as methanethiol (MT), 2-furanmethanethiol (2FMT), 3-mercapto-3-methylbutanol (3MMB) and 3-mercapto-3-methylbutyl formate (3MMBF), have been described as key impact aroma compounds, often correlated to roasty, sulfurous and coffee notes (Sunarharum et al.,, 2014; Mayer, Czerny, &Grosch, 2000; Sanz, Czerny, Cid, &Schieberle, 2002; Kumazawa, &Masuda, 2003). Moreover, the depletion of thiols during coffee storage has been associated with a decrease of its sensory quality (Holscher, &Steinhart, 1992; F. Mayer, &Grosch, 2001; Hofmann, &Schieberle, 2002; Charles-Bernard, Roberts, &Kraehenbuehl, 2005). For these reasons, the assessment of volatile thiols is crucial for the determination of coffee quality.In particular, the aromatic profile of brewed coffee is better correlated with sensorial assays than that of coffee powder, since brewed coffee is the mode of consumption (Caprioli et al, 2012).Therefore, the identification and quantification of volatile thiols in brewed coffee are necessary for objective evaluation of both coffee brewing conditions and the sensory quality of coffee.

The analytical assay of volatile thiols in coffee is hindered by the complexity of the matrix, and by both their high reactivity and their typically low concentrations. The additional difficulty in analyzing coffee brew rather than coffee powder, comes from the reported instability of thiols after brewing, due to chemical interactions with other coffee components in aqueous solution (Hofmann et al., 2002; Charles-Bernard et al., 2005; Kumazawa, & Masuda, 2003b).Current methods to assess thiols in brewed coffee require relatively large samples, they are in general laborious and time-consuming, and some of them involve sample manipulation steps during

60 which volatile thiols can be lost or undergo degradation. These circumstances together have

61 led to the need for a rapid and simple method that would enable the immediate extraction of

62 thiols and their protection from degradation. Recently, a simple, reliable, selective and

63 sensitive method involving a simultaneous derivatization/extraction step followed by liquid

64 chromatography–electrospray high-resolution mass spectrometry (LC-ESI-HRMS) was

65 proposed to assess volatile thiols in coffee powder (Vichi, Jerí, Cortés-Francisco, Palacios, &

66 Caixach, 2014). The fast stabilization of thiols by derivatization, and their simultaneous

67 extraction from the matrix, could make of this method the appropriate solution for thiols

68 analysis in unstable products like brewed coffee. Nevertheless, the existing method was

69 developed for the analysis of solid coffee matrix and it cannot be directly applied to an

70 aqueous solution such as brewed coffee. For this purpose, the extraction conditions must be

71 adapted to the coffee brew matrix and the performances of the new method should be

72 evaluated to verify its suitability.

73 The present study therefore aimed to optimize a novel, fast, simple, reliable and sensitive

74 method for the analysis of thiols in brewed coffee, on the basis of the method recently

75 proposed for coffee powder. To this end, the extraction solvent was modified and analytical

76 conditions were adjusted for this matrix. The analytical performance of the modified method

77 was then evaluated in the different target areas. Finally, the method was also applied to

78 several brewed mono-origin coffee samples, which were expected to present different

79 profiles.

80 **2. MATERIALS AND METHODS**

81 **2.1. Chemicals and reagents**

82 Mass spectrometry grade dichloromethane and methanol (MS SupraSolv®) were purchased by

83 Merck (Darmstadt, Germany). Water was of ultrapure milli-Q grade. Ammonium formate was

84 from Sigma. Nitrogen (Alphagaz, purity 99.999%, Air Liquid) was used in the Orbitrap-Exactive

85 as the nebulization and fragmentation gas.

86 Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one, purity 98%), ethylenediaminetetraacetic

87 acid (EDTA) (purity 98,5%);4-methoxy-α-toluenethiol (IS, purity 90%); 4-mercapto-1-butanol

88 (4MB) (purity 95%); 3-mercapto-3-methylbutan-1-ol (3MMB) (purity 98%); 2-methyl-3-

89 tetrahydrofuranthiol (2MTHFT) (purity 97%); 3-mercaptohexanol (3MH) (purity 98%);

90 ethanethiol (ET) (purity 97%); 3-mercapto-3-methylbutylformate (3MMBF) (purity 97.5%) and

91 2-furanmethanethiol (2FMT) (purity 97%) were purchased by Sigma-Aldrich (St Louis, MO,

92 USA). Molecular structures of the reference thiols are shown in **Fig. 1**.

93 **2.2. Coffee samples**

94 Freshly roasted arabica coffee bean samples were purchased by a local producer. Mono-origin

95 coffee samples were from distinct geographical origins: South East Asia (Indonesia, Papua and

96 southern India) (*n*=4), Central America (El Salvador, Panama, Guatemala, Colombia, Honduras

97 and the Dominican Republic) (*n*=9) and Africa (Ethiopia, Kenya and Tanzania) (*n*=7). A total of

98 twenty coffee samples were analyzed in duplicate.

99 A commercial roasted coffee sample provided by a local retail outlet was used in the assays for

100 the optimization of the extraction conditions and to assess the method performance.

101 To obtain the coffee brew, 10 g of roasted coffee beans was ground for 1 minute in a domestic

102 grinder, then 5 g of the resulting coffee powder was placed on a filter (pore size 12-15 μm) and

103 100 mL of MilliQ water at 90ºC-95ºC was poured over the coffee powder. Each coffee brew

104 was analyzed immediately after preparation.

105 **2.3. Derivatization/extraction conditions**

106 The derivatization and extraction conditions were optimized in a real coffee brew matrix,

107 prepared from commercial coffee. Different volumes of both the sample and extraction

108 solvent, as well as different concentration of the derivatization agent, ebselen, were tested.

109 The most suitable conditions were determined by comparing absolute peak areas. The most

110 suitable agitation was also evaluated between a vortex and orbital shaker. The final

111 derivatization/extraction conditions were as follows: 10 mL of fresh coffee brew was placed

112 into a screw-cap tube, 200 μL of EDTA 30 mg/mL was added and this was then spiked with 4-

113   methoxy-α-toluenethiol (IS) in methanol to a final concentration of 10 ng/L. 4 mL of ebselen

114   0.1 mM in dichloromethane was finally added and the mixture was mixed by an orbital shaker

115   (400 rpm) for 1 min. During all this process, the sample was maintained under a nitrogen

116   atmosphere. After centrifugation (4000 rpm, 10 min), 1 mL of the organic phase was collected,

117   dried under nitrogen and redissolved in 100 μL of methanol. The sample was vortex mixed and

118   directly injected into the HPLC-HRMS system.

119   **2.4. High performance liquid chromatography-high resolution mass spectrometry (HPLC-**

120   **HRMS) analysis**

121   **2.4.1. High performance liquid chromatography (HPLC)**

122   The HPLC system consisted of a Surveyor MS Plus pump coupled to an Accela Open automatic

123   sampler (Thermo Fisher Scientific, San Jose, California) equipped with 10 μL loop. The

124   chromatographic separation was performed on a Luna C18 (150 × 2.1 mm, 5 μm) analytical

125   column (Phenomenex, Torrance, CA). Elution was performed at a flow rate of 200 μL/min,

126   using water (solvent A) and methanol (solvent B), both containing ammonium formate 10 mM.

127   The solvent gradient changed according to the following conditions: 50% (A)-50% (B) during 2

128   min, to 100% (B) in 18 min; 100% (B) during 13 min, then to 50% (A)-50% (B) in 1 min, followed

129   by 6 min of maintenance.

130   **2.4.2. High resolution mass spectrometry (HRMS)**

131   Mass spectrometric analyses were carried out with an Orbitrap-Exactive-HCD (Thermo Fisher

132   Scientific, Bremen, Germany) equipped with an electrospray source (H-ESI II). The ionization

133   conditions in positive mode were according to Vichi et al. (2014): spray voltage 3.75 kV,

134   capillary voltage 25 V, skimmer voltage 16 V, tube lens voltage 80 V. Sheath gas flow rate was

135   set at 40 arbitrary units (au), auxiliary gas flow rate was 10 au, capillary temperature was 275

136   ºC, and heater temperature was 30 ºC. The mass range was set to m/z 50-1200. The automatic

137   gain control was used to full fill the C-trap and gain accuracy in mass measurements (high

138   dynamic range mode, $3x10^6$ ions). Maximum injection time was 500 ms. High resolving power

defined as R: 50,000 (*m/z* 200, full width half maximum-FWHM), 2 Hz, was set. High energy

Collision Dissociation (HCD) voltage was fixed at 25 eV. In a single injection, the Orbitrap mass

analyzer alternated full scan mode and "All Ion Fragmentation" (AIF) mode at a resolution

power of 50,000 (*m/z* 200, FWHM). Mass accuracies better than 2 ppm were achieved for

molecular and product ions, always working with external calibration.

The molecular formulae calculation was carried out with Xcalibur 2.1 (Thermo Fisher Scientific,

Bremen, Germany). In order to obtain an accurate list of possible candidate formulae from a

mass measurement, restrictive criteria were set to generate reliable elemental formulae: C ≥

13, H ≥10, O ≥ 1, N=1, Se=1, S=1, Na≤1 and RDB ≥ 8.5. The mass peaks considered were single

positive charged ions with absolute intensity>10^4 area counts.

2.5. Quantification and method performances

2.7. Quantification

Quantification was carried out by internal standard and by applying the response factors (RFs)

calculated in coffee matrix on the basis of a standard addition curve results.

Non-target thiols were quantified by applying a RF=1, and expressed as ng equivalents of IS/kg.

2.7.1. Standard addition curve

The standard addition curve was constructed by analyzing the commercial coffee sample

fortified with increasing amounts of reference thiols (0, 0.9, 1.8, 4.5, 9, 27 ng/kg for ET, 4MMP,

3MH, 4MMB, 2FMT, 2MTHFT, 4MB and 3MHA; 0, 9, 18, 45, 90, 270 ng/kg for 3MMB, 3MMBF

and 3MBT).The point "0" was obtained by analyzing a commercial coffee sample as previously

described, while fortified samples were prepared by adding the reference thiols dissolved in

dichloromethane to the coffee-hexane suspension, prior to centrifugation and derivatization.

All the samples were also spiked with IS (12.5 ng/kg).The concentration of each thiol in the

coffee matrix was then determined on the basis of the intercept of the curve on X-axis.

163 Quantification was performed using an internal standard and by applying the response factors

164 (RFs) obtained by matrix-matched calibration curves. Thiols for which a reference compound

165 was not available (non-target thiols) were quantified as ng equivalents of IS/L.

166 As coffee matrix in not free of thiols, the matrix-matched calibration curves were constructed

167 by adding up the background concentration and the spiked amount of each thiol.

168 The background concentration of each thiol was extrapolated from a standard addition curve,

169 as previously described (Vichi et al., 2014). The standard addition curve was constructed by

170 fortifying a coffee brew with increasing amounts of reference thiolsin the range 0.5-80 ng/L for

171 ET, 4MMP, 3MH, 4MMB, 2FMT, 2MTHFT, 4MB and 3MHA, and 5-800 ng/L for 3MMB, 3MMBF

172 and 3MBT). Spiked matrix solutions were derivatized and extracted as previously described for

173 coffee samples.

174 Linearity within these ranges was evaluated by the correlation coefficient, r^2.

175 The precision, expressed as intra-day and inter-day relative standard deviation (RSD) was

176 determined by analyzing the same commercial coffee sample. The intra-day RSD was

177 calculated over six samples analyzed on the same day; whereas the inter-day RSD was

178 determined from five samples analyzed on different days.

179 Limits of quantification (LOQ) in coffee matrix were extrapolated from calibration curve, by

180 assuming minimum areas of 10^4 and 10^3 for the identification and confirmation ions (isotope

181 ions containing ^{80}Se and ^{78}Se, respectively).

182 **2.6. Statistical analysis**

183 *SPSS* Statistic software was used to the statistical treatment of the results obtained in the

184 present study. A one-factor ANOVA test was carried out to compare the mean thiol

185 concentrations of coffee samples with different origin.

186 **3. RESULTSANDDISCUSSION**

187 **3.1. Derivatization and extraction conditions**

188   The aim of the present study was to propose a simple, reliable, selective and sensitive method

189   to identify and quantify volatile thiols in coffee brews. A single-step procedure was applied to

190   simultaneously extract and derivatize the volatile thiols contained in relatively small amounts

191   of coffee brew, by means of the rapid formation of a Se-S bond between the free thiol group (-

192   SH) and the Se-N bond of ebselen (Xu et al., 2010). The reaction of thiols with ebselen has two

193   main advantages: it protects the free thiol group from oxidation and it enables the ESI-HRMS

194   responses of the derivatives to be increased due to the presence of the easily ionizable aminic

195   nitrogen atom, thereby improving the sensitivity of the method (Vichi, Cortés-Francisco,

196   Caixach, 2013). Moreover, the typical selenium isotopic pattern contributes to the selectivity

197   of the method. The overall extraction and derivatization procedure is performed in 1 min, and

198   if it is carried out immediately after the coffee is brewed, it allows volatile coffee thiols to be

199   recovered, preventing most thiol depletion due to interaction with other coffee components

200   (Hofman et al., 2002; Charles-Bernard et al., 2005; Kumazawa et al., 2003b).

201   Assays for the optimization of the extraction conditions were performed in order to maximize

202   the sensitivity, using a commercial coffee sample. With the aim of revealing any limiting effect

203   of the amount of the derivatizing reagent, different concentrations of ebselen were tested in

204   the extraction solvent. No statistically significant differences were found between the results

205   obtained using concentrations of ebselen from 0.1 mM to 0.3 mM (Supplementary

206   information, **Fig. S1a**), and 0.1 mM was the concentration finally used in the

207   derivatization/extraction of the coffee brew samples.

208   Moreover, the volumes of sample and extraction solvent were adjusted in order to maximize

209   the extraction of thiols and to allow a sufficient volume of derivatized extract to be separated

210   from the sample matrix (Supplementary information, **Fig. S1b**).

211   **Fig. 1** shows the chromatograms of derivatized thiols present in a brewed commercial coffee

212   sample analyzed under the optimized conditions. The chromatogram was obtained by

213   selecting the exact mass of each thiol derivative from the ESI + full scan analysis.

3.2. Quantification and method performances

As previously reported for the identification and quantification of volatile thiols in other matrices (Vichi et al., 2013; 2014), the identification of target thiols was performed in full scan mode, using the following identification criteria: the presence of the quantification ion (which corresponds to the presence of the ^{80}Se isotope) and the confirmation ion (which corresponds to the presence of the ^{78}Se isotope) of the derivatized thiol; the exact mass (R > 20,000) of the derivatized thiol with a mass error tolerance < 2 ppm; and the corresponding chromatographic retention time. Non-target thiols were detected by means of the diagnostic ion corresponding to the ebselen molecule (*m/z* 275.9922, $[C_{13}H_{10}ONSSe]^+$) present in the fragmentation spectrum, which conserves the typical selenium pattern. The identification should be confirmed by the presence of a precursor ion in full scan mode, which satisfies the previous identification criteria and complies with the molecular formula restrictions.

According to the above criteria, ten derivatized thiols were identified in the commercial coffee brew (**Tab. 1**), comprising seven target and three non-target compounds. They were considered in order to assess the method performance.

Target thiols were quantified by constructing calibration curves in the ranges reported in **Table 1**. Linearity within these ranges, evaluated by correlation coefficient (r^2), was always > 0.97 (**Table 1**).

The LOQ extrapolated from the calibration curve were lower than 1 ng/L (**Table 1**), and far below thiols' odor thresholds in water, as reported in the literature (**Table 2**).

The precision of the proposed method, expressed as intra-day and inter-day RSD, was calculated for each target and non-target thiol by analyzing the same commercial coffee sample. As shown in **Table 1**, intra-day RSD was in general around 10%, except for 4MB (19%); while inter-day RSD was always below 29%. These values are acceptable, given that they are referred to concentration at the ng/L level (Horwitz, 1982).

3.3. Analysis of coffee brews samples

240   The composition of volatile thiols was assessed in coffee samples of different origin (South East

241   Asia, Africa and Central America) and they were expected to present different aroma profiles.

242   Our aim here was to assess the capacity of the method to characterize thiols in coffee brews.

243   The thiols identified and quantified in the coffee brews are reported in **Table 2**.

244   Seven target thiols (4MB, 3MMB, 2MTHFT, 3MH, ET, 3MMBF and 2FMT) were identified and

245   quantified, and ten non-target thiols, including 3MMBA and MT, were tentatively identified

246   and quantified as IS equivalents. MT, 2FMT, 3MMBF and 3MMBA had been previously

247   reported in coffee brews (Mayer et al., 2000; Kumazawa et al., 2003a; 2003b), while the rest of

248   the compounds were reported here for the first time in this product. Nevertheless, all the

249   thiols reported in **Table 2** had been detected in coffee powder using the present analytical

250   approach (Vichi et al., 2014), thus corroborating their presence in coffee and their possible

251   contribution to coffee brew aroma. In contrast, 3-methylbutenthiol and some non-target thiols

252   reported in coffee powder (Vichi et al., 2014) were not detected here in the coffee brews. This

253   could be due to the different composition of the samples analyzed in each study. Nevertheless,

254   the present sampling was wide enough to ensure that the lack of these thiols was not sample-

255   dependant; it is most probably a consequence of the low extraction efficiency of the brewing

256   procedure. These results may be useful in furthering understanding of the real contribution of

257   thiols to coffee brew sensory characteristics.

258   Regarding the characterization of the samples according to the geographical provenance,

259   quantitative, rather than qualitative, differences were observed in the thiol fraction profiles of

260   the coffees of different origin (**Table 2**). However, a comprehensive sampling would be

261   necessary to discern differences for characterization and authentication purposes.

262

263   In conclusion, it can be stated that the method proposed is suitable for the analysis of volatile

264   thiols in brewed coffee, and that it enables minimal sample manipulation and fast

265   extraction/derivatization, which are crucial to prevent thiol depletion after coffee is brewed.

266   Moreover, the identification criteria based on the derivatization step and HRMS analysis

267   permit high selectivity and reliability in the identification. The precision and sensitivity of the

268   method were satisfactory, and the combination of target and non-target approaches allowed

269   several thiols to be determined. Some of them are reported here for the first time in a coffee

270   brew, and these findings are in agreement with results previously reported for coffee powder

271   analyzed using similar analytical approach. These results may be useful in furthering

272   understanding of the real contribution of thiols to coffee brew sensory characteristics.

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340

341 **Figure captions**

342

343 **Figure 1.**

344 Molecular structures of reference thiols analyzeded in the present study: (1) 4-mercapto-1-

345 butanol (4MB); (2) 3-mercapto-3-methylbutan-1-ol (3MMB); (5) 2-methyl-3-

346 tetrahydrofuranthiol (2MTHFT); (6) 3-mercaptohexanol (3MH); (7) ethanethiol (ET); (8) 3-

347 mercapto-3-methylbutylformate (3MMBF); (9): 2-furanmethanethiol (2FMT); IS: 4-methoxy-α-

348 toluenethiol. Numbers are according to **Table 1**.

349

350 **Figure2**

351 ESI + Full scan chromatograms obtained from an Indonesian Arabica brewed coffee, by

352 selecting the exact mass of: (1) 4-mercapto-1-butanol (4MB); (2) 3-mercapto-3-methylbutan-

353 1-ol (3MMB); (3) 3-methoxybutyl mercaptoacetate (3MBMA); (4) methanethiol (MT); (5) 2-

354 methyl-3-tetrahydrofuranthiol (2MTHFT); (6) 3-mercaptohexanol (3MH); (7) ethanethiol (ET);

355 (8) mercapto-3-methylbutylformate (3MMBF); (9) 2-furanmethanethiol (2FMT); (10) 3-

356 mercapto-3-methylbutylacetate (3MMBA).

357

Table 1. Molecular formula, exact mass, chromatographic retention time and precision values, mass accuracy and precision values for ebselen-derivatized target (in bold) and non-target thiols in coffee matrix in positive ESI, setting the R: 50,000 (m/z 200, FWHM). Linearity range evaluated by regression coefficient (r), sensitivity, recovery, method repeatability and reproducibility expressed as intra-day relative standard deviation (RSD) and inter-day RSD, respectively.

	Compound	Formula [M+H]$^+$	Theoretical m/z	RT [a] (min) ±SD	Δ[b] (ppm) ± SD	Range[c] (ng/L)	Linearity[c] (r^2)	LOQ [d] (ng/L)	Recovery (%) (at 8 ng/L)	Intra-day RSD (%) (n=5)	Inter-day RSD (%) (n=5)
1	**4MB-Ebs**	C$_{17}$H$_{22}$O$_2$NSSe	382.0374	13.72±0.08	1.3±0.3	0.1-80	0.996	0.04	71	19	24
2	**3MMB-Ebs**	C$_{18}$H$_{22}$O$_2$NSSe	396.0531	14.35±0.13	1.7±0.2	4.7-800	0.979	0.2	72[e]	10	22
3	3MBMA-Ebs[f,g]	C$_{20}$H$_{24}$O$_4$NSSe	454.0583	14.95±0.12	1.3±0.4	-	-	-	-	10	23
4	MT-Ebs[f,h]	C$_{14}$H$_{14}$ONSSe	323.9956	15.44±0.11	1.2±0.3	-	-	-	-	11	24
5	**2MTHFT-Ebs**	C$_{18}$H$_{20}$O$_2$NSSe	394.0374	15.62±0.12	1.7±0.2	0.9-80	0.977	0.3	107	8	18
6	**3MH-Ebs**	C$_{19}$H$_{24}$O$_2$NSSe	410.0687	16.24±0.03	1.6±0.3	0.7-80	0.977	0.1	111	-[i]	-
7	**ET-Ebs**	C$_{15}$H$_{16}$ONSSe	338.0112	16.50±0.13	1.1±0.2	0.01-80	0.976	0.2	93	13	20
8	**3MMBF-Ebs**	C$_{19}$H$_{22}$O$_3$NSSe	424.0480	16.60±0.12	1.3±0.3	0.1-800	0.988	0.4	72[e]	11	21
9	**2FMT-Ebs**	C$_{18}$H$_{16}$O$_2$NSSe	390.0061	16.79±0.14	1.3±0.3	0.1-80	0.988	0.1	57	12	29
10	3MMBA-Ebs[f,j]	C$_{20}$H$_{24}$O$_3$NSSe	438.0637	17.23±0.12	1.7±0.2	-	-	-	-	8	18

[a]: mean (*n*=5) chromatographic retention time ± standard deviation; [b]: mean (*n*=6) mass error, expressed as root mean square error (RMS error) ± SD; [c]: referred to matrix-matched calibration curves; [d]: limit of quantification extrapolated from standard addition curve equation by assuming minimum areas of 10^4 and 10^3 for the identification and confirmation ions, respectively (isotope ions containing ^{80}Se and ^{78}Se, respectively); [e]: calculated at 80 ng/L; [f]: tentative identification; [g]: 3-methoxybutyl mercaptoacetate (3MBMA); [h]: methanethiol (MT); [i]: not present in the commercial coffee sample; [j]: 3-mercapto-3-methylbutyl acetate (3MMBA).

Table 2. Target (in bold) and non-target volatile thiols identified and quantified in coffee brew samples (mean values according to the origin), statistical significant differences between coffee samples origins (by ANOVA), and aromatic characteristics of volatile thiols.

| RT[a] (min)	Compound	Theoretical m/z	Thiol Formula[b]	Concentration[c] (ng/L)			p [d]	Odor threshold (µg/L)	Odor descriptor
				South-east Asia (n=4)	Africa (n=7)	Central America (n=9)			
13.75	Mercaptophenol[e]	402.0061	C_6H_6OS	0.26 ± 0.27 ab	0.42 ± 0.24 a	0.21 ± 0.10 b	0.017		
13.83	**4MB**	382.0374	$C_4H_{10}OS$	2.3 ± 1.5 a	1.3 ± 0.8 b	0.83 ± 0.21 b	0.001		
14.35	**3MMB**	396.0531	$C_5H_{12}OS$	224 ± 165 a	132 ± 70 b	105 ± 29 b	0.008	-	broth, sulfur, sweet, sweat, onion[f]
14.42	Mercaptobutanone[e]	380.0218	C_4H_8OS	0.12 ± 0.14 a	0.23 ± 0.10 b	0.09 ± 0.04 a	0.000		
14.84	Ethyl 3-mercaptoacetate[e]	396.0167	C_4H_8O2S	0.29 ± 0.16 ab	0.34 ± 0.12 a	0.23 ± 0.10 b	>0.05	0.07[g]	sulfurous, baked beans, herbaceous[g]
14.95	3MBMA [e,h]	454.0583	$C_7H_{14}O_3S$	0.16 ± 0.14 a	0.09 ± 0.05 b	0.06 ± 0.02 b	0.007		
15.44	MT[e,i]	323.9956	CH_4S	7.5 ± 5.03 a	3.7 ± 1.4 b	3.3 ± 0.74 b	0.000	0.2[j]	cabbage-like[k], fresh coffee[f]
15.62	**2MTHFT**	394.0374	$C_5H_{10}OS$	2.2 ± 1.5 a	1.0 ± 0.4 b	0.97 ± 0.23 b	0.000		
15.87	Ethyl 3-mercaptopropanoate[e]	410.0324	$C_5H_{10}O2S$	0.10 ± 0.09 a	0.05 ± 0.04 b	0.02 ± 0.02 b	0.000	0.5[l]	fresh grape, foxy, rhubarb[f], fruity[l]
16.15	4,5-dimethoxy-2-methyl-benzenethiol[e]	460.0480	$C_9H_{12}O_2S$	0.19 ± 0.17 a	0.29 ± 0.09 b	0.15 ± 0.05 a	0.001		
16.24	**3MH**	410.0687	$C_6H_{14}OS$	0.14 ± 0.14	0.29 ± 0.91	0.01 ± 0.01	>0.05	0.06[m]	citrus, tropical fruit[f], grapefruit[m]
16.50	**ET**	338.0112	C_2H_6S	11.1 ± 9.8 a	2.4 ± 1.5 b	2.9 ± 1.5 b	0.000		
16.60	**3MMBF**	424.0480	$C_6H_{12}O_2S$	32 ± 29 a	15 ± 12 b	11 ± 3 b	0.006	0.0035[k]	cat urine, ribes[f], roasty[k,o], blackcurrant[o]
16.79	**2FMT**	390.0061	C_5H_6OS	3.5 ± 1.6 a	2.2 ± 0.9 b	2.3 ± 0.5 b	0.004	0.01[k]	coffee-like[f], roasty[f,k,n], sulfury[f,k]
17.23	3MMBA[e,h]	438.0637	$C_7H_{14}O_2S$	0.58 ± 0.55 a	0.21 ± 0.15 b	0.17 ± 0.06 b	0.002		
17.55	2,5-dimethylfuran-3-thiol[e]	404.0218	C_6H_8OS	0.07 ± 0.02 a	0.04 ± 0.03 b	0.04 ± 0.02 b	0.001		

[a]: chromatographic retention time; [b]: obtained by substracting the ebselen moiety; [c]: mean values ± SD; [d]:significance of the differences between groups; [e]: tentative identification based on molecular formula and quantified as ng IS equivalent/L; [f]: McGorrin (2007); [g]: Nikolantonaki & Darriet (2011); [h]: 3-methoxybutyl mercaptoacetate (3MBMA); [i]: methanethiol (MT); [j]: Guth & Grosch (1994); [k]: Semmelroch & Grosch (1995); [l]: Roland, Schneider, Razungles, & Cavelier, 2011; [m]: Tominaga, Baltenweck-Guyot, Gachons, & Dubourdieu (2000); [n]: Mayer, & Grosch, (2001).
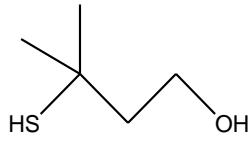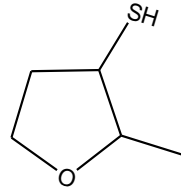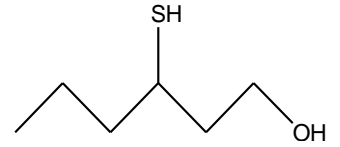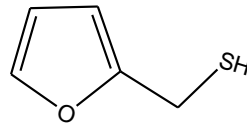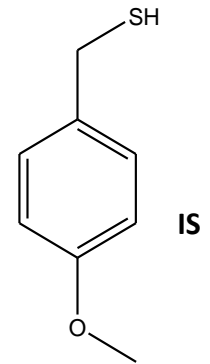
(1) 4MB **(2) 3MMB** **(5) 2MTHFT** **(6) 3MH**
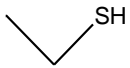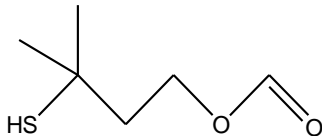
(7) ET **(8) 3MMBF** **(9) 2FMT** **IS**

Figure 1

Figure 2