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**Title:**

Quantitative determination of volatile compounds using TD-GC-MS and isotope standard addition for application to the heat treatment of food.

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**Keywords:**

Thermal desorption, on-line sampling, aroma, process-induced reactivity, newly formed compounds, Maillard reaction

**Highlights:**

- A novel quantitative method to determine volatile compounds in gaseous samples
- The assay combines volatile trapping, isotope standard addition and GC-MS analysis
- Markers with different chemical properties were quantified from ng to mg levels
- The method was applied to sample and measure newly formed compounds during baking
- This method is transferable to monitoring kinetics, other processes and VOCs

**Abstract:**

For many foods that undergo thermal treatment, the generation of numerous process-induced compounds has strong implications for food quality and safety. Today, increasing attention is paid to the generation and occurrence of particular classes of newly formed compounds in processed foods for their potential health implication. It is therefore of the utmost importance to monitor the process with fit-for-purpose methods that are appropriate for use in quality control or in research and innovation. Since many quality-related compounds are volatile, there is a need for robust methods that can quantify a broad range of volatile markers and are applicable to on-line monitoring.

To meet this need, an original and reliable method based on thermal desorption has been developed for the quantitation of volatile compounds sampled on-line by sorbent tubes. For the first time, this method combines in-tube calibration and deuterated standard addition. Ten volatile compounds that are likely to form during heat processing of food (i.e. baking of cereal products), were chosen as target analytes for their relevance to food quality and their different physicochemical properties: 3-methylbutanal, pyrazine, 2-methylpyrazine,

2,5-dimethylpyrazine, 2,6-dimethylpyrazine, acetic acid, furfural, 5-methylfurfural, furfuryl alcohol and 5-hydroxymethylfurfural. The key steps in the analytical procedure were optimized and carefully characterized in terms of recovery, repeatability and reliability. The TD-GC-MS method displayed good linearity over extended ranges for all compounds ( $R^2$ : 0.9950 to 0.8880) with low limits of quantification (*LOQs*) ranging from 0.0141 to 11.5 ng. The matrix effect was negligible for most compounds, except for 5-hydroxymethylfurfural (21.5%), the most polar and least volatile compound. The method was applied to determining process-induced compounds generated during the baking of a model cake and sampled from baking vapors at three different times during the heat treatment. Of all the compounds extracted, the target analytes exhibited concentrations spread over very broad ranges. This highly sensitive method could therefore be used for the early quantification of relevant markers during the processing of food matrices, for quality or mitigation purposes. Quantitative TD-GC-MS with in-tube calibration and isotope standard addition is particularly well-suited for applications where an accurate determination is required of both trace level and major volatile compounds over time. This method may therefore be relevant for monitoring either industrial or domestic food processes (e.g. baking, frying, roasting), for multi-residue analyses linked to quality and safety, or reaction kinetics for multi-response modeling. It can also be transferable to emerging non-food applications.

## 1 Introduction

The thermal treatment of food can trigger the formation of a large number of volatile compounds that affect quality. Indeed, food products contain reactants (sugars, amino acids and lipids) which may participate in the heat-sensitive reactions caused by heat treatments (drying, pasteurizing, baking or roasting). The impact of physical and chemical variables on the profile of volatile compounds has been studied extensively during recent decades, particularly in cereal and bakery products (Birch et al., 2014; Cho & Peterson, 2010; Hansen & Schieberle, 2005; M.A. Pozo-Bayón et al., 2006; Rega et al., 2009; Salim-ur-Rehman et al., 2006) from the angles of both flavor and food safety-related issues (Cepeda-Vázquez et al., 2018; Ozolina et al., 2011). The volatile compounds in bakery products mostly result from the Maillard reaction, caramelization and lipid oxidation (Grosch & Schieberle, 1997; Maire et al., 2013), with ingredients and process conditions being the main factors that affect their generation (Ait Ameer et al., 2008; Birch et al., 2014; Cepeda-Vázquez et al., 2019; Cho & Peterson, 2010; Maria Angeles Pozo-Bayón et al., 2007; Zhou et al., 1999). However, from a more comprehensive standpoint, thermally induced volatile compounds have been studied as markers of the degree of processing and more recently as markers of reaction kinetics (Srivastava et al., 2018). Whatever the purpose of the research (e.g. controlling final concentrations or understanding the phenomena that underpin the generation of process-induced markers) the analytical strategies to determine volatile compounds from foods remain a challenge because of their low concentrations, their different physicochemical properties and the variability of matrices and processes (Elmore, 2015; Xu et al., 2016; Zhou et al., 1999).

The most widely applied solvent-free extraction methods for volatile compounds include headspace (HS) techniques such as SPME in headspace sampling mode (Elmore, 2015; Sgorbini et al., 2019). Static Headspace

74 is a rapid and simple sampling method (Paik & Venables, 1991) and provides a very good odor representation  
75 of extracts (Rega et al., 2003), but, it often lacks sensitivity compared to dynamic HS and HS-SPME (Crews  
76 & Castle, 2007). HS-SPME has been widely used for the screening and qualitative analysis of a large number  
77 of volatile compounds from solid and liquid products and it is also used extensively for foodomics applications  
78 due to its high throughput characteristics (Moreira et al., 2019; Pico, Antolín, et al., 2018; Pico, Tapia, et al.,  
79 2018). However, this method is poorly applicable to the assay of a large number of volatile compounds during  
80 processing, because their partition coefficients between the headspace and the fiber differ and are impacted by  
81 many factors such as polarity, volatility and molecular weight of the compounds (Zhang et al., 2018).  
82 In the case of specific applications such as kinetic studies and kinetic modeling, the HS and HS-SPME methods  
83 have limitations when quantitative measurements are required (Fehaili et al., 2010; Parker, 2013; van Boekel,  
84 2001). Recently, HS trap extraction coupled to isotopic dilution was developed for the quantification of two  
85 reaction markers (furan and furfural) from sponge cakes (Cepeda-Vázquez et al., 2016; Srivastava et al., 2018).  
86 Despite the fact that this technique is highly sensitive (ppb levels) and robust, it is not applicable to a large  
87 number of volatile markers and to determining numerous experimental points for kinetics studies since it is  
88 destructive and time-consuming.

89 Many studies have indicated the potential of the on-line sampling of baking vapors in order to identify and  
90 follow for kinetic purposes a large number of volatile markers throughout thermal processing (Fehaili et al.,  
91 2010; Poinot et al., 2010; Zanin et al., 2020). However the quantification of volatile compounds has remained  
92 a bottleneck with these methods.

93 There is therefore a need for high throughput methods that can both sample on-line and quantitatively analyze  
94 volatile compounds during thermal processing for the purposes of monitoring and modeling kinetics.

95 Thermal desorption (TD) could be a good candidate as shown by the rising interest and increasing number of  
96 scientific papers on its applications. Despite the fact that TD has been widely and successfully applied to  
97 environmental analyses of the air (Heavner et al., 1992; Hodgson, 1995; Massold et al., 2005) and soil (Zhao  
98 et al., 2019), potential applications in food control are still rather underexplored. In this field, TD started as a  
99 version of dynamic HS (Purge and Trap) used to enrich headspace extracts before GC analysis (Pillonel et al.,  
100 2002). When disconnected from Purge and Trap, it was sometimes called Direct Thermal Desorption (DTD).  
101 TD has several advantages over the HS methods referred to above: greater sensitivity compared to HS-SPME,  
102 the possibility of being directly coupled to GC (Esteban et al., 1996), the need for very small quantity of sample  
103 and the possibility to reanalyze the same extract several times (Göğüş et al., 2007). Not only it is appropriate  
104 for qualitative analysis because of its rapidity and simplicity, it is also suitable for quantitative analysis because  
105 carefully selected trapping sorbent tubes can retain and further desorb all the target compounds present in the  
106 sampled gas (> 99% desorption efficiency)(Bart, 2001). Moreover, the smaller number of extraction  
107 parameters requiring optimization (type of adsorbent, flow, purge cycles) and the greater robustness due to the  
108 steel equipment, make it more time saving, reproducible and durable when compared to HS-SPME techniques.  
109 In a previous paper (Lee et al., 2020), an on-line sampling method for baking vapors using sorbent tubes  
110 coupled to GC-MS analysis was developed. It proved that TD-GC-MS was an appropriate and rapid technique

111 to analyze a large number of volatile compounds with a wide range of physicochemical characteristics. The  
112 aim of the present work was to develop and optimize a quantitative approach for TD-GC-MS using in-tube  
113 calibration and isotope standard addition, with an application and validation for determining  
114 10 process-induced compounds sampled on-line during the thermal transformation of a food model (baked  
115 model cake).

## 116 2 Materials and Methods

### 117 2.1 Ingredients and reagents

118 3-methylbutanal (> 99%), pyrazine ( $\geq 99\%$ ), 2-methylpyrazine ( $\geq 99\%$ ), 2,5-dimethylpyrazine ( $\geq 99\%$ ),  
119 2,6-dimethylpyrazine ( $\geq 99\%$ ), furfural ( $\geq 99\%$ ), furfuryl alcohol ( $\geq 98\%$ ), 5-methylfurfural ( $\geq 99\%$ ),  
120 5-hydroxymethylfurfural ( $\geq 99\%$ ), and d4-pyrazine ( $\geq 98\%$ ) were purchased from Sigma-Aldrich (St. Louis,  
121 MO, USA). Acetic acid ( $\geq 96\%$ ), methanol ( $\geq 99.9\%$ ), and ethanol ( $\geq 95\%$ ) were purchased from Carlo Erba  
122 (Val de Reuil, France) and d4-furfural ( $\geq 99.7\%$ ) was obtained from CIL Cluzeau, CDN Isotopes  
123 (Pointe-Claire, Canada). Table 1 summarizes the main characteristics of the volatile compounds selected for  
124 the study.

125 The ingredients used for the model sponge cakes were Ultrapure water, produced by an Integral 3 water  
126 purification system from Millipore®, native corn starch (Cargill, Wayzata, MN, USA) with a water content of  
127 12.4% w/w (measured according to NF norm V05 707), and food grade cellulose derivatives (Dow Chemicals,  
128 Midland, MI, USA): methylcellulose (MC) type SGA7C and hydroxypropyl methyl cellulose (HPMC) type  
129 K250M. D-(+)-Glucose was supplied by Roquette Frères (Lestrem, France) and L-Leucine ( $\geq 99\%$ , Food  
130 Grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 131 2.2 Preparation of stock, working and calibration solutions

132 The stock methanolic solution of analytes contained 3-methylbutanal (260 mg.g<sup>-1</sup>), pyrazine (0.260 mg.g<sup>-1</sup>),  
133 2-methylpyrazine (700 mg.g<sup>-1</sup>), 2,5-dimethylpyrazine (0.680 mg.g<sup>-1</sup>), 2,6-dimethylpyrazine (0.300 mg.g<sup>-1</sup>),  
134 acetic acid (26.0 mg.g<sup>-1</sup>), furfural (20.0 mg.g<sup>-1</sup>), 5-methylfurfural (4.80 mg.g<sup>-1</sup>), furfuryl alcohol (2.10 mg.g<sup>-1</sup>)  
135 and 5-hydroxymethylfurfural (4.00 mg.g<sup>-1</sup>). The stock methanolic solution for the deuterated internal standards  
136 (*d*IS) contained d4-furfural and d4-pyrazine at a concentration of 2.55 mg.g<sup>-1</sup>. All stock solutions were stored  
137 at -20°C for up to two months in hermetically sealed amber glass bottles.

138 The working solution of *d*IS d4-pyrazine and d4-furfural (8.00·10<sup>-2</sup> g.L<sup>-1</sup>) was prepared by diluting a proper  
139 amount of stock *d*IS solution in methanol. Likewise, working solutions of analytes at five concentrations were  
140 obtained by diluting the stock solution in methanol. The concentration ranges were as follows: 0.316 to  
141 32.5 g.L<sup>-1</sup> for 3-methylbutanal; 3.94·10<sup>-4</sup> to 0.123 g.L<sup>-1</sup> for pyrazine, 1.09·10<sup>-3</sup> to 0.400 g.L<sup>-1</sup> for  
142 2-methylpyrazine, 1.04·10<sup>-3</sup> to 0.326 g.L<sup>-1</sup> for 2,5-dimethylpyrazine, 4.69·10<sup>-4</sup> to 0.147 g.L<sup>-1</sup> for  
143 2,6-dimethylpyrazine, 0.038 to 12.0 g.L<sup>-1</sup> for acetic acid, 0.030 to 9.44 g.L<sup>-1</sup> for furfural, 7.39·10<sup>-3</sup> to 2.31 g.L<sup>-1</sup>  
144 for 5-methylfurfural, 3.26·10<sup>-3</sup> to 1.02 g.L<sup>-1</sup> for furfuryl alcohol and 6.10·10<sup>-3</sup> to 1.91 g.L<sup>-1</sup> for

145 5-hydroxymethylfurfural. These concentrations were chosen to cover the peak areas found during baking of  
146 model cakes in a previous semi-quantitative study (Lee et al., 2020).  
147 Calibration solutions containing a (constant) concentration of  $8.00 \cdot 10^{-2} \text{ g.L}^{-1}$  d4-pyrazine and d4-furfural and  
148 increasing concentrations of analytes were set at the same analyte concentration levels as previously  
149 mentioned.  
150 The working and calibration solutions were stored at  $-20^{\circ}\text{C}$  for up to one month in hermetically sealed amber  
151 glass bottles.

### 152 **2.3 Preparation and baking of the model cakes**

153 A model cake was prepared according to the method described in Lee et al., (2020) using a hydrocolloid  
154 solution of HPMC and MC foamed with maize starch and enriched with glucose and leucine prior to baking.  
155 Twenty grams of batter were poured in disposable cylindrical aluminum molds (diameter = 6.6 cm, mold  
156 height = 4 cm, cake height = 1.3 cm) and a total of nine cakes were baked at the same time in an instrumented  
157 pilot oven with precise and uniform control of temperature (Bongard, Wolfisheim, France) (Fehaili et al.,  
158 2010). Baking trials were conducted in triplicate at  $170^{\circ}\text{C}$  and under a high level of convection.

### 159 **2.4 On-line sampling of baking vapors by sorbent tubes**

160 Vapors were sampled during baking according to the method from Lee et al., (2020). An Air Toxics™ sorbent  
161 tube (Perkin Elmer, Waltham, MA, USA), selected for its capacity to trap C3-C12 volatile compounds,  
162 collected the internal atmosphere of the oven through a deactivated and silanized glass column (internal  
163 diameter = 4.5 mm, length = 18 cm) at a flow rate of  $50 \text{ mL.min}^{-1}$  for 4 min by means of a VCP 130 vacuum  
164 pump (VWR, Radnor, PA, USA). The total volume of 200 mL of baking vapor was sufficient to meet the  
165 analytical limits. Prior to any experiments, precautions were taken to prevent external contamination: the oven  
166 was heated at  $300^{\circ}\text{C}$  for 1 hour under the same extraction flow and the glass column was rinsed with ethanol  
167 and dried carefully to eliminate any potential residues.

168 Baking vapors were sampled at three different times (2-6, 8-12, and 86-90 min) representing the very early  
169 and advanced stages of the process and considering that the amounts of the markers were increasing with time  
170 at  $170^{\circ}\text{C}$  (Lee et al., 2020). The sampling was conducted in triplicate.

171 A median time was then attributed to each sampling: 4, 10 and 88 min. After sampling, each sorbent tube was  
172 removed and spiked with  $1 \mu\text{L}$  of the deuterated standard solution (*d*IS) then sealed immediately with a Teflon®  
173 cap, stored at room temperature and analyzed within the day.

### 174 **2.5 Thermal desorption of sorbent tubes**

175 Sorbent tubes were desorbed using an automated TurboMatrix 650 thermal desorber (Perkin Elmer, USA)  
176 adapting the method described by Lee et al., (2020). Firstly, a dry air purge at ambient temperature was applied  
177 for 10 min at  $50 \text{ mL.min}^{-1}$  in order to remove any traces of water from the tube. The volatile compounds were  
178 then desorbed at  $330^{\circ}\text{C}$  for 20 min using helium as carrier gas at a flow rate of  $60 \text{ mL.min}^{-1}$  and with an inlet

split of 8 mL.min<sup>-1</sup>. The compounds were then cryo-focused on an Air Monitoring® cold trap (-20°C) then sent to the GC column by rapidly heating the trap to 330°C at 99°C.s<sup>-1</sup>, with a 15 mL.min<sup>-1</sup> outlet split. In this manner, 7.5% of the volatile compounds desorbed from the sorbent tube were transferred to the GC system at a constant gas flow rate of 1.4 mL.min<sup>-1</sup> via a transfer line heated at 270°C. After each injection, the trap was cleaned by setting the hold time to 45 min at 330°C and the sorbent tubes were systematically purged and conditioned with helium at a flow rate of 50 mL.min<sup>-1</sup> for 40 min at 330°C.

## 2.6 GC-MS analysis and quantification of thermally desorbed volatile compounds

Volatile compounds were analyzed using a Trace 1300 Thermo Scientific Gas Chromatograph (Waltham, MA, USA) equipped with a DB-Wax GC column (60 m × 0.25 mm × 0.5 µm; Agilent, Santa Clara, CA, USA) and coupled with a Thermo Scientific ISQ QD (Waltham, MA, USA) mass spectrometry detector. Helium was used as the carrier gas at a constant flow rate of 1.2 mL.min<sup>-1</sup>. The GC oven started at 100°C and the temperature was raised to 184°C by increments of 3°C.min<sup>-1</sup>, to 240°C by increments of 15°C.min<sup>-1</sup> and then held at that level for 11 min. MS transfer line and ion source temperatures were set at 270°C and 200°C, respectively. The ionization mode was electron impact (EI), 70 eV. Data were acquired in two segments including a full scan mode (from 25 to 250 m/z at 0.1 s per scan) and a selected ion monitoring (SIM) mode, with a dwell time of 0.2 s for each selected ion as shown in Table 1.

Compounds from baking extracts were identified by analyzing the pure standards, performing mass spectral matches with the Wiley 8 and NIST 08 mass spectra databases, and comparison with the Van der Dool retention indices measured on the DB-Wax column.

Chromatographic peak areas for each compound were calculated by extracting the quantifier ions from the SIM mode acquisition data using Quan Browser, Xcalibur 2.1.0 SP1, Build 1160 (Thermo Scientific, Waltham, MA, USA).

Calibration curves for each compound were built using the ratio of the chromatogram peak areas (analyte vs d<sub>4</sub>IS) plotted against the corresponding concentration. d<sub>4</sub>-pyrazine was used for 3-methylbutanal, pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine whereas d<sub>4</sub>-furfural was used for acetic acid, furfural, 5-methylfurfural, furfuryl alcohol and 5-hydroxymethylfurfural.

## 2.7 Analytical strategy for development of the quantitative TD-GC-MS method

Figure 1 summarizes the global analytical strategy (calibration and sampling), including the parameters tested and the performance criteria chosen for optimization. Firstly, different desorption times (10, 20, 30 and 60 min) were compared. The tubes were analyzed twice and the quantity recovered from the first run was compared to the sum of those of first and second runs ( $\text{recovery} = \text{Area}_{1\text{st run}} / (\text{Area}_{1\text{st run}} + \text{Area}_{2\text{nd run}})$ ). It was concluded that 20 min was the most appropriate time, with average recoveries higher than 99.5% except for acetic acid (97.4%) and 5-HMF (91.1%) (n = 5; see supplementary data). Secondly, two spiking modes for introducing the analytes and the d<sub>4</sub>IS into the tubes were compared. Liquid spiking consisted in the direct injection of 1 µL of the working solution (calibration or analyte solution) into the top of the sorbent tube using an electronic

214 eVOL XR 5  $\mu\text{L}$ -precision syringe (SGE Analytical Science Europe Ltd, Milton Keynes, UK). Vapor spiking  
 215 consisted in introducing the same amount (1  $\mu\text{L}$ ) of working solution in a vaporized form by means of a GC  
 216 injector port (300°C, constant helium flow rate 50  $\text{mL}\cdot\text{min}^{-1}$ , splitless mode) connected to the sorbent tube *via*  
 217 a deactivated silica column (internal diameter = 0.25 mm, length = 25 cm, 300°C). The criterion of choice was  
 218 the repeatability of spiking ( $\text{RSD}_{\text{chromatographic response}} < 15\%$  for all analytes and at all concentration levels). Any  
 219 eventual breakthrough of this spiking mode was assessed by vapor spiking a solution of 3-methylbutanal,  
 220 2,5-dimethylpyrazine, acetic acid, furfural, furfuryl alcohol and 5-hydroxymethylfurfural at the highest  
 221 concentration level into two sorbent tubes in series and checking for any eventual carry-over into the second  
 222 tube. The quantity recovered from the first tube was thus compared to the sum of those of first and second  
 223 tubes ( $\text{carry-over} = \text{Area}_{2\text{nd tube}} / (\text{Area}_{1\text{st tube}} + \text{Area}_{2\text{nd tube}})$ ). It was concluded that the overall breakthrough was  
 224 negligible, with average carry-overs lower than 0.3% except for acetic acid (15.7%) and 5-HMF (5.0%) ( $n = 5$ ;  
 225 supplementary data).

226 Finally, the reliability of the in-tube *d*IS introduction method was assessed. Although the calibration tubes  
 227 contained both the analytes ( $A_i$ ) and the *d*IS, the sampling tubes (i.e. the sorbent tubes used for on-line  
 228 extraction) always required a further addition of isotope standard (Figure 1). To achieve quantitation without  
 229 any bias, it was evaluated whether there was any potential difference in the responses of the analytes and those  
 230 of the *d*IS between calibration and sampling. In order to simulate vapor sampling, working solutions of analytes  
 231 were spiked into the sorbent tubes. The *d*IS areas and  $A/d$ IS responses between the calibration and sampling  
 232 tubes were compared using Tukey and Fisher tests ( $p > 0.05\%$ ).

## 233 2.8 Performance evaluation

234 Linearity, *LOD*, *LOQ* and the matrix effect were evaluated according to Aszyk, Woźniak, Kubica, Kot-Wasik,  
 235 & Wasik, 2019; Cepeda-Vázquez et al., 2016. The analytical blanks used to validate the method came from  
 236 the empty oven atmosphere sampled under the same heating and extraction conditions as previously presented  
 237 in section 2.4.

238 Linearity was assessed using four- or five-point calibration curves built in quadruplicate with the mean peak  
 239 area ratio ( $\text{Area}_{\text{analyte}} / \text{Area}_{d\text{IS}}$ ) of the blank ( $n = 6$ ) used as the intercept. *LOD* and *LOQ* values for each analyte  
 240 were determined using the mean peak area ratio ( $\text{Area}_{\text{analyte}} / \text{Area}_{d\text{IS}}$ ) of blanks with a signal-to-noise ratio of  
 241 3 and 10, respectively.

242 The analytical blanks were spiked with the calibration solutions so a matrix-matched calibration was obtained.  
 243 The sorbent tubes were spiked with calibration solutions so that the calibration curve was obtained. The matrix  
 244 effect (%) was evaluated by comparing the slopes of these two curves according to Equation (1).

$$245 \text{ Matrix effect (\%)} = \left( \frac{\text{Slope}_{\text{Matrix-matched calibration}}}{\text{Slope}_{\text{Calibration}}} - 1 \right) \times 100 \quad (1)$$

## 246    **3    Results and Discussion**

### 247    **3.1    Spiking method for 10 volatile analytes over a broad range of concentrations**

248    A robust, reliable and reproducible method to introduce calibration solutions into the sorbent tubes is of  
249    paramount importance in order to develop a quantitative method.

250    A previous study by Liaud, Nguyen, Nasreddine, & Le Calvé, (2014) had found liquid spiking to be more  
251    time-saving and easier to implement than vapor spiking because it does not require any solvent evaporation.  
252    However, this conclusion was reached when studied analytes (benzene, toluene, ethylbenzene, mpp-xylene  
253    and o-xylene) and the types of sorbent tubes used differed from those in the present study. On the other hand,  
254    vapor sampling may better mimic the conditions of on-line sampling applications. Therefore, in the present  
255    study, we compared the two spiking techniques over a range of 10 specific process-induced VOCs and a broad  
256    range of concentrations.

257    Table 2 shows the responses obtained for two sets of analytes using both methods. Liquid spiking proved to  
258    be more repeatable than vapor spiking for 2,5-dimethylpyrazine, furfural, furfuryl alcohol and  
259    5-hydroxymethylfurfural, and over the chosen range of concentrations. On the other hand, vapor spiking  
260    globally produced higher RSD whatever the level of concentration. This result could be explained by the larger  
261    number of sources of variability induced by the experimental device (gas flow, inlet and column temperature,  
262    injected volume, eventual leakage, etc.) when compared to liquid spiking which requires fewer steps. These  
263    results therefore confirmed the trends observed by Liaud, Nguyen, Nasreddine, & Le Calvé, (2014).  
264    Nevertheless, the results for 3-methylbutanal and acetic acid presented poorer repeatability for with both  
265    spiking techniques when low concentration levels where applied. This may be explained by the fact that the  
266    two compounds were the most volatile of the evaluated analytes (Table 1). Liquid spiking was therefore tested  
267    further on the second set of volatile analytes, i.e. key volatile markers for bakery products such as pyrazine,  
268    2-methylpyrazine, 2,6-dimethylpyrazine and 5-methylfurfural. The results confirmed good repeatability, with  
269    RSD values lower than 15% for almost all the concentration levels for all compounds. The liquid spiking mode  
270    was therefore chosen for all subsequent analyses.

271

### 272 3.2 Reliability of the in-tube spiking method for deuterated internal standards

273 As mentioned above, a further step was necessary for the sampling tubes, consisting in spiking the *d*IS solution  
274 just after trapping the volatile compounds from the gaseous phase (Figure 1).

275 Figure 2 shows the responses of the two *d*IS in both cases: calibration tubes where the analytes and *d*IS were  
276 present in the same solution so simultaneously spiked, and sampling tubes where the analytes were spiked first  
277 followed by the *d*IS in a second solution. The responses of d4-pyrazine and d4-furfural did not differ  
278 significantly for the calibration and sampling tubes (Tukey and Fisher tests,  $p > 0.05\%$ ), suggesting that the *d*IS  
279 were adsorbed in the same manner in both cases. Whether the *d*IS were introduced at the same time as the  
280 analytes or later did not alter their chromatographic responses.

281 On the other hand, Table 3 highlights the responses for all the analytes evaluated at the three different  
282 concentration levels in the calibration and sampling tubes. Differences in (*A/d*IS) ratios were negligible in the  
283 majority of cases (70%) and no concentration dependency was detected. Therefore, the addition of the *d*IS did  
284 not remove the analytes that were already trapped. A notable discrepancy was seen regarding the acetic acid  
285 response at the highest concentration level. Due to the high repeatability of measurements, some minor  
286 differences were found for five other compounds, but in these cases no systematic bias was evident (no effect  
287 of the injection mode or level of concentration). We can reasonably conclude that spiking the *d*IS into a  
288 sampling tube already containing the analytes did not affect the response of most of the analytes whose  
289 responses were highly consistent with those found for the calibration tubes.

290 Finally, these results validate the in-tube spiking method for deuterated internal standards after the on-line  
291 sampling step.

292

### 3.3 Performance of the quantitative TD-GC-MS analysis

The performance characteristics of the TD-GC-MS method outlined above are summarized in Table 4. The ratios ( $A/dIS$ ) of the analytical blanks were low (0.00010-0.0175). These values were used as intercepts. Good linearity was observed throughout the concentration range, and the linearity range was defined as that between  $LOQ$  and the upper concentration.  $LOD$  and  $LOQ$  were calculated in ng for the 10 analytes.  $LOQ$  ranged from 0.041 ng (2,5-dimethylpyrazine) to 11.5 ng (3-methylbutanal) which was very satisfactory. The data thus obtained were not very comparable to those found in the literature where  $LOD$  and  $LOQ$  were expressed as concentrations, but a comparison could be made for furfural: the present method produced an  $LOQ$  which was 10 times lower than that obtained using a TD-GC-MS/MS method developed for e-cigarette aerosol by Aszyk et al., (2019) ( $LOQ_{\text{furfural}} = 4.7$  ng and 19 g, respectively).

The pyrazine group displayed remarkably stable responses, explaining the excellent linearity observed ( $R^2$  within the range 0.9874-0.9950) and low  $LOD$  and  $LOQ$  (down to picogram levels). Acetic acid and furfurylic compounds also had very good linearity for very broad ranges of concentration.

Despite the fact that 5-hydroxymethylfurfural was the least volatile and most polar compound compared to the other analytes, a low  $LOQ$  (0.14 ng) was determined but with moderate linearity ( $R^2 = 0.8880$ ) and a matrix effect value higher than the threshold usually chosen for a significant matrix effect (+/- 20%).

Finally, for all the other analytes, no matrix effect was apparent, meaning that any suppression or enhancement of the signal due to the sample matrix could be disregarded. This method is therefore globally well suited to quantifying intense variations in the concentration of the pool of volatile markers that are likely to occur during an industrial process.

### 3.4 Application to analyzing process-induced volatile compounds in the baking vapors of a model cake

This quantitative TD-GC-MS method was then applied to the targeted analysis of 10 process-induced compounds released and sampled during a baking operation. A model food product (model cake) was used as described in Lee et al., (2020), to generate specific compounds issued from Maillard reaction and caramelization between one sugar and one amino acid, in order to study reaction mechanisms. These volatile compounds are linked to sensory, safety and quality in terms of aroma and potential toxicity (Birch et al., 2014; Cha et al., 2019; Crews & Castle, 2007).

In order to evaluate the suitability of the method to follow the progress of the thermal reactions throughout an entire baking process, the ten targeted analytes were analyzed at three different baking times which included a very early (4 min) and very late baking stage (88 min) thus producing very different concentrations. Having an on-line sample that lasts a known length of time at a given flow rate, it is possible to calculate the volume of gas passing through the tube, enabling expression of the concentration of each analyte present in the oven in  $\text{ng.mL}_{\text{air}}^{-1}$  by hypothesizing that the air in the oven is perfectly stirred when the oven is at its highest convection rate. Considering the potentially wide range of duration of a cake baking (up to 60 min depending

on the targeted intensities), it appears that a sampling duration of 4 min was short enough to obtain multiple different samples at all stages of the process, enabling a detailed description of the reaction dynamics. Figure 3 groups the 10 process-induced compounds based on their chemical class and the concentrations found in the sampled vapors. It is worth noting that thanks to the high sensitivity of the TD-GC-MS method, it was possible to detect the compounds even at the earliest step of processing (at 4 min baking, meaning sampling from 2 to 6 min baking).

Pyrazine and substituted pyrazines (Figure 3A) are important process-induced compounds which play a major role in the aroma of baked foods (Grosch & Schieberle, 1997; Maire et al., 2013), even though they are found at trace quantities in the food matrices. TD-GC-MS enabled quantification of two of these strong odor-active compounds in the baking vapors, even after 4 min of baking, although at very low levels (6.88 ng for 2-methylpyrazine and 1.12 ng for 2,5-dimethylpyrazine). These trace compounds displayed a global increase during baking of up to 26.1 ng (corresponding to 0.13 ng.mL<sub>air</sub><sup>-1</sup>) for 2-methylpyrazine after 88 min of baking. These results were consistent with those obtained by Rega et al., (2009) using a real sponge cake; using on-line SPME-GC-MS analysis they showed that pyrazine levels increased in line with baking time.

Likewise, furanic compounds (furfural, 5-methylfurfural, furfuryl alcohol, and 5-hydroxymethylfurfural) increased significantly during baking (Figure 3B). These compounds were found in quite large amounts compared to pyrazines, particularly after a longer baking time, which was consistent with data on different cereal products (Ait Ameer et al., 2008; Petisca et al., 2013; Rega et al., 2009; Srivastava et al., 2018). Among these, furfural reached the highest concentration in the baking vapors (788 ng corresponding to 4 ng.mL<sub>air</sub><sup>-1</sup> at 88 min). These compounds could be closely followed for mitigation purposes (Capuano & Fogliano, 2011; Rannou et al., 2016), so this highly sensitive method could be used for their early detection and quantification in both the atmosphere and food matrices during processing.

Finally, the compounds found at the highest levels are shown in Figure 3C. Acetic acid may form as a result of Maillard or caramelization reactions during baking and was found at considerable concentrations (9242 ng corresponding to 46 ng.mL<sub>air</sub><sup>-1</sup>) after 88 min of baking. On the other hand, 3-methylbutanal was already found at a very high level after only 4 min of baking, with the highest level quantified at 10 min (9541 ng corresponding to 48 ng.mL<sub>air</sub><sup>-1</sup>). This very high level was due to the presence in the cake formula of a specific precursor (leucine) which reacts during baking to generate 3-methylbutanal *via* Strecker degradation (Lee et al., 2020). Although both acetic acid and 3-methylbutanal are characterized by high volatility, they display different polarities. Nevertheless, their different occurrence at the three baking times is mainly related to their different generation pathways during the heat treatment.

On-line sampling coupled to TD-GC-MS with isotope standard addition therefore stands out as a highly appropriate method to monitor a broad range of concentrations of process-induced compounds with different generation and release behaviors during the baking of a food matrix. In particular, this method is sufficiently sensitive to be suitable for these types of reaction dynamics.

Furthermore, the extraction technique using sorbent tubes is capable of trapping multiple compounds including those derived from lipid oxidation during baking of sponge cakes and they can be identified by GC-MS (Lee

et al., 2020). In the present study, the choice was made to quantify 10 compounds among them, however, the developed method could be applied to quantify other compounds by establishing calibration curves with their standards.

## 4 Conclusion

During this work, an original and reliable TD-GC-MS method was developed for the quantitation of volatile compounds sampled on-line on sorbent tubes. For the first time, this method combines both in-tube calibration and deuterated standard addition. Ten volatile compounds that are likely to form during the heat processing of food were chosen as targeted analytes for their relevance with respect to food quality and for their different physicochemical properties. The key steps in the analytical procedure were optimized and carefully characterized in terms of recovery, repeatability and reliability.

The performance of the method was characterized over broad ranges of concentration. All analytes could be quantified with high sensitivity (*LOQ* at ng level), making it possible to express the concentrations in ng.mL<sup>-1</sup> of air. Quantitative TD-GC-MS with isotope standard addition therefore stands out as accurate and competitive when compared to existing methods for on-line applications.

This method was successfully applied to the on-line analysis of newly formed compounds during the baking of a model cake. For this application, reproducible results were found for the target analytes within very broad ranges of concentration between 0.15 ng and 0.63 ng, at three different baking times.

The high extraction capacity and robustness of the sampling method are a clear advantage compared to other extraction techniques previously used for on-line sampling such as SPME. This advantage, combined with the good linearity and high sensitivity of the method, means that quantitative TD-GC-MS is highly appropriate for the on-line extraction throughout a unit operation such as baking and could be relevant for food analysis applications such as the multi-residue analysis of volatile compounds linked to quality and safety as well as to reaction kinetics for multi-response modeling.

The quantitative approach presented here is transferable to determining other volatile markers (e.g. aldehydes, VOCs, furans) during other industrial or domestic processes (e.g. frying, drying) and in other types of food or emerging non-food applications.

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## Conflict of interest

The authors have declared no conflict of interest.

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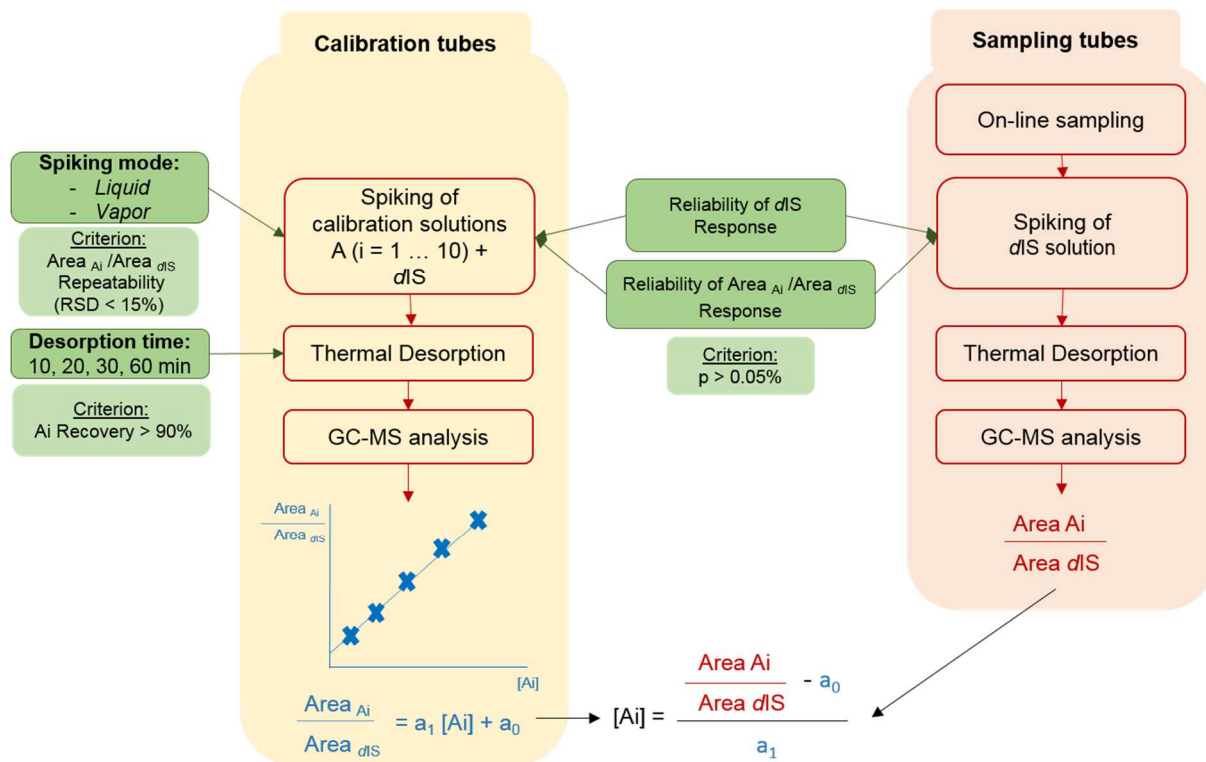
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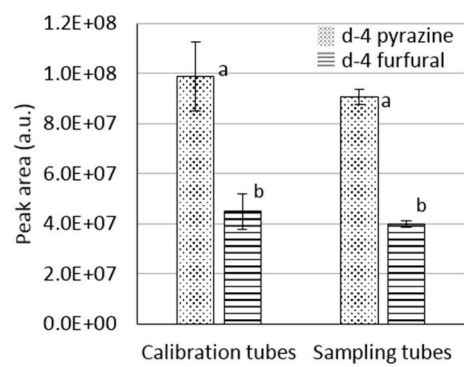
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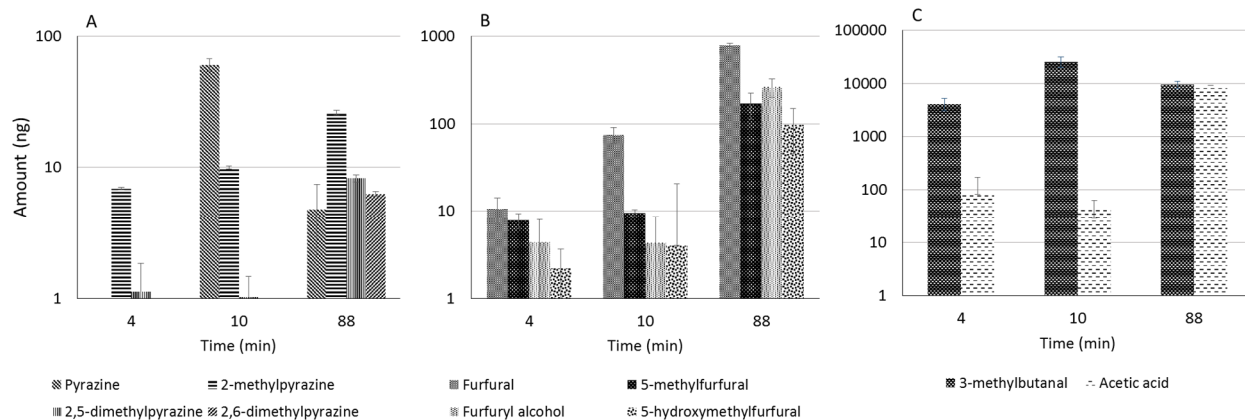
Figure 1. Analytical strategy applied for development of the quantitative TD-GC-MS method using in-tube spiking: analytical steps are indicated in red for calibration and sampling tubes while the tested parameters and responses are in dark green on the side and the corresponding performance criteria chosen for optimization are in light green on the side.

Figure 2. Responses of *d*IS for simultaneous spiking in the calibration tubes and spiking after sampling in the sampling tubes (n = 5); the same letter for a same *d*IS means no significant difference ( $p < 0.05$ )

Figure 3. Process-induced volatile compounds quantified by TD-GC-MS from the baking vapor of a model cake. Volatile compounds were sampled on sorbent tubes for 4 minutes at 3 different baking steps (4, 10 and 88 min at 170°C)







**Table 1. Characteristics of the volatile compounds selected for the study**

Compound	CAS No	log K <sub>ow</sub> <sup>a</sup>	Pv (mm Hg at 25°C) <sup>b</sup>	Selected ions (m/z) <sup>d</sup>
<i>Analytes (A)</i>				
3-methylbutanal	590-86-3	1.23	50	<b>58, 71</b>
Pyrazine	290-37-9	-0.2	10.81	<b>53, 80</b>
2-methylpyrazine	109-08-0	0.21	8.06	<b>67, 94</b>
2,5-dimethylpyrazine	123-32-0	0.63	3.18	42, <b>108</b>
2,6-imethylpyrazine	108-50-9	0.54	4.57	42, <b>108</b>
Acetic acid	64-19-7	-0.17	15.7	<b>60</b>
Furfural	98-01-1	0.41	2.21	95, <b>96</b>
5-methylfurfural	620-02-0	0.67	0.610	<b>81, 109, 110</b>
Furfuryl alcohol	98-00-0	0.28	0.609	<b>81, 97, 98</b>
5-hydroxymethylfurfural	67-47-0	-0.6	5.28 × 10 <sup>-3</sup>	97, <b>126</b>
<i>Deuterated Internal standards (dIS)</i>				
d4-pyrazine	1758-62-9	-0.2	41.6	<b>56, 84</b>
d4-furfural	1219803-80-1	0.4	n.d. <sup>c</sup>	99, <b>100</b>

<sup>a</sup>: octanol/water partition coefficients from Pubchem or EPI Suite<sup>TM</sup> v4.0 data bases

<sup>b</sup>: Pv: Vapor pressure from Pubchem database

<sup>c</sup>: Not determined

<sup>d</sup>: Quantifier ions are in bold

n.d.: not determined

Table 1. Chromatographic responses of volatile analytes introduced into the sorbent tubes by liquid or vapor spiking

Compound	Liquid spiking				Vapor spiking					
	Amount <sup>c</sup> (ng)	Response (Area <sub>analyte</sub> /Area <sub>dIS</sub> )			RSD (%)	Amount <sup>c</sup> (ng)	Response (Area <sub>analyte</sub> /Area <sub>dIS</sub> )			RSD (%)
3-methylbutanal <sup>a</sup>	38	0.29	±	0.04		14	91	0.15	±	0.02
	78	0.5	±	0.1		22	181	0.24	±	0.03
	400	2.3	±	0.3		11	923	1.12	±	0.07
	1585	9.5	±	0.6		7	1841	2.06	±	0.05
2,5-dimethylpyrazine <sup>a</sup>	51	1.30	±	0.06		5	99	0.43	±	0.05
	104	2.85	±	0.02		1	197	0.72	±	0.05
	529	9.8	±	0.5		5	1003	3.3	±	0.1
	1053	16.2	±	1.2		7	2000	5.90	±	0.09
Acetic acid <sup>b</sup>	41	0.7	±	0.2		33	94	0.33	±	0.09
	84	1.3	±	0.2		15	187	0.43	±	0.09
	427	4.3	±	0.3		6	953	2.0	±	0.1
	1695	17.6	±	1.0		6	1901	4.3	±	0.2
Furfural <sup>b</sup>	43	1.49	±	0.03		2	95	0.6	±	0.1
	88	2.9	±	0.1		4	188	1.12	±	0.04
	446	12.7	±	0.7		5	956	5.04	±	0.01
	1768	42.1	±	1.1		3	1908	9.4	±	0.8
Furfuryl alcohol <sup>b</sup>	46	1.08	±	0.01		1	101	0.22	±	0.07
	94	2.30	±	0.09		4	202	0.41	±	0.07
	479	12.0	±	0.9		8	1029	2.0	±	0.1
	1901	43.7	±	1.5		4	2053	3.8	±	0.1
5-hydroxymethylfurfural <sup>b</sup>	79	0.77	±	0.07		10	90	0.2	±	0.1
	400	6.04	±	0.01		0.5	179	0.1	±	0.1
	795	11.3	±	0.8		7	914	0.4	±	0.1
	1585	33.9	±	1.1		3	1824	0.8	±	0.4
Pyrazine <sup>a</sup>	46	2.15	±	0.03		1	n.d.	n.d.	n.d.	n.d.
	94	4.3	±	0.3		6	n.d.	n.d.	n.d.	n.d.
	479	19.3	±	0.7		4	n.d.	n.d.	n.d.	n.d.
	954	32.0	±	0.5		1	n.d.	n.d.	n.d.	n.d.
2-methylpyrazine <sup>a</sup>	37	1.43	±	0.04		3	n.d.	n.d.	n.d.	n.d.
	76	2.8	±	0.1		4	n.d.	n.d.	n.d.	n.d.
	386	14.3	±	0.5		3	n.d.	n.d.	n.d.	n.d.
	1530	52.32	±	10.1		19	n.d.	n.d.	n.d.	n.d.
2,6-dimethylpyrazine <sup>a</sup>	39	1.70	±	0.08		4	n.d.	n.d.	n.d.	n.d.
	79	3.2	±	0.2		6	n.d.	n.d.	n.d.	n.d.
	400	16.0	±	0.5		3	n.d.	n.d.	n.d.	n.d.
	795	28.3	±	1.5		5	n.d.	n.d.	n.d.	n.d.
5-methylfurfural <sup>b</sup>	38	1.1	±	0.1		11	n.d.	n.d.	n.d.	n.d.
	78	2.32	±	0.06		2	n.d.	n.d.	n.d.	n.d.
	400	11.7	±	0.7		6	n.d.	n.d.	n.d.	n.d.
	1585	44.3	±	1.6		4	n.d.	n.d.	n.d.	n.d.

<sup>a</sup>: quantified using d4-pyrazine as dIS<sup>b</sup>: quantified using d4-furfural as dIS<sup>c</sup>: amount corresponding to the injection of 1 µL of working solution

n.d.: Not determined

**Table 1. Responses of analytes with simultaneous spiking in the calibration tubes and spiking after sampling in the sampling tubes (n = 3)**

Compound	Amount (ng)	Response (Area <sub>analyte</sub> /Area <sub>dfs</sub> )* ± SD					
		Calibration tubes			Sampling tubes		
3-methylbutanal	38	0.29 <sup>a</sup>	±	0.04	0.21 <sup>a</sup>	±	0.03
	78	0.5 <sup>a</sup>	±	0.1	0.38 <sup>a</sup>	±	0.02
	400	2.3 <sup>a</sup>	±	0.3	1.93 <sup>a</sup>	±	0.04
2,5-dimethylpyrazine	51	1.30 <sup>a</sup>	±	0.06	1.13 <sup>a</sup>	±	0.09
	104	2.85 <sup>b</sup>	±	0.02	2.17 <sup>a</sup>	±	0.08
	529	9.8 <sup>a</sup>	±	0.5	11.5 <sup>b</sup>	±	0.4
Acetic acid	41	0.7 <sup>a</sup>	±	0.2	0.7 <sup>a</sup>	±	0.1
	84	1.3 <sup>a</sup>	±	0.2	1.5 <sup>b</sup>	±	0.2
	427	4.3 <sup>b</sup>	±	0.3	2.58 <sup>a</sup>	±	0.09
Furfural	43	1.49 <sup>a</sup>	±	0.03	1.5 <sup>a</sup>	±	0.1
	88	2.9 <sup>a</sup>	±	0.1	2.68 <sup>a</sup>	±	0.06
	446	12.7 <sup>a</sup>	±	0.7	12.6 <sup>a</sup>	±	0.2
Furfuryl alcohol	46	1.08 <sup>a</sup>	±	0.01	1.03 <sup>a</sup>	±	0.05
	94	2.30 <sup>b</sup>	±	0.09	2.0 <sup>a</sup>	±	0.1
	479	12.0 <sup>a</sup>	±	0.9	11.2 <sup>a</sup>	±	0.6
5-hydroxymethylfurfural	79	0.77 <sup>a</sup>	±	0.07	0.8 <sup>a</sup>	±	0.1
	400	6.04 <sup>a</sup>	±	0.01	5.6 <sup>a</sup>	±	1.0
	795	11.3 <sup>a</sup>	±	0.8	14.0 <sup>a</sup>	±	1.6
Pyrazine	46	2.15 <sup>b</sup>	±	0.03	1.98 <sup>a</sup>	±	0.05
	94	4.3 <sup>b</sup>	±	0.3	3.88 <sup>a</sup>	±	0.07
	479	19.3 <sup>a</sup>	±	0.7	18.0 <sup>a</sup>	±	0.4
2-methylpyrazine	37	1.43 <sup>a</sup>	±	0.04	1.53 <sup>b</sup>	±	0.04
	76	2.8 <sup>a</sup>	±	0.1	2.87 <sup>a</sup>	±	0.08
	386	14.3 <sup>a</sup>	±	0.5	14.3 <sup>a</sup>	±	0.5
2,6-dimethylpyrazine	39	1.70 <sup>a</sup>	±	0.08	1.7 <sup>a</sup>	±	0.1
	79	3.2 <sup>a</sup>	±	0.2	3.28 <sup>a</sup>	±	0.11
	400	15.9 <sup>a</sup>	±	0.5	16.4 <sup>a</sup>	±	0.6
5-methylfurfural	38	1.1 <sup>a</sup>	±	0.1	1.06 <sup>a</sup>	±	0.06
	78	2.32 <sup>b</sup>	±	0.06	2.1 <sup>a</sup>	±	0.1
	400	11.7 <sup>a</sup>	±	0.7	11.5 <sup>a</sup>	±	0.7

\*: Same letters in a row mean no significant difference (p < 0.05)

**Table 1. Performance characteristics of TD-GC-MS determinations for 10 target analytes**

Compound	Linear regression				Matrix effect (%)	LOD (ng)	Linearity range	
	Slope	Intercept*	R <sup>2</sup>				LOQ (ng)	Upper limit (ng)
3-methylbutanal	0.0007	0.0175 ± 0.0008	0.9792	-4	3.5	11.5		32510
pyrazine	0.0125	0.007 ± 0.004	0.9950	0	0.97	3.25		123
2-methylpyrazine	0.0115	0.0003 ± 0.0001	0.9930	1	0.025	0.085		340
2,5-dimethylpyrazine	0.0077	0.00010 ± 0.00003	0.9874	2	0.013	0.041		326
2,6-dimethylpyrazine	0.0096	0.004 ± 0.001	0.9909	13	0.21	0.67		27
acetic acid	0.0035	0.005 ± 0.003	0.9877	0	2.8	9.4		12014
furfural	0.0074	0.006 ± 0.004	0.9599	2	1.4	4.7		9439
5-methylfurfural	0.0231	0.0015 ± 0.0007	0.9778	1	0.02	0.28		2312
furfuryl alcohol	0.0092	0.0012 ± 0.0009	0.9855	0	0.29	0.97		1901
5-hydroxymethylfurfural	0.0078	0.0002 ± 0.0001	0.8880	22	0.05	0.14		1909

\*: mean of the blanks' area ratio between the analyte and its assigned dIS