Characterization of volatile and non-volatile fractions of spices using evolved gas analysis and multi-shot analytical pyrolysis

Marco Mattonai, Atsushi Watanabe, Erika Ribechini

Department of Chemistry and Industrial Chemistry, University of Pisa, Via G. Moruzzi 13, 56124 Pisa, Italy

Frontier Laboratories Ltd., 4-16-20 Saikon, Koriyama, Fukushima, 963-8862 Japan

Abstract

In the present work, Evolved gas analysis-mass spectrometry (EGA-MS) and analytical pyrolysis-GC/MS (double-shot Py-GC/MS) were used to characterize both the volatile and non-volatile fractions of six commercially available spices with the aim to exploit the potential of such techniques in performing authentication studies and establish the botanical origin of spices EGA-MS allowed us to establish thermal degradation regions, and double-shot Py-GC/MS was used to obtain compositional information on each region separately. Analyses are usually carried out by collection of the headspace components. This study demonstrates that EGA-MS and Py-GC/MS provide the same advantages of solid-phase micro extraction (SPME), mainly reported in the literature for the analysis of spices volatile components, and increase the range of detectable products by performing high-temperature desorption and degradation of the non-volatile fraction of spices. our approach provided both qualitative and semi-quantitative data that could be used in the future to improve authentication studies.

Keywords: Spices; Lignocellulose; Evolved gas analysis; Analytical pyrolysis; Mass spectrometry

Highlights

- Characteristic gas evolution profiles for six spices were obtained
- Thermal desorption and pyrolysis were discriminated based on gas evolution profiles
- Volatile compounds were detected by thermal desorption at 250 °C
- Carbohydrates and lignin derivates were detected by pyrolysis-silylation at 550 °C
- Compositional data could be used to improve authentication analyses

1. INTRODUCTION

Spices have been used worldwide since ancient times, not only as food ingredients, but also as medicinal plants [1-3] and even as dyeing agents [4]. The global production of spices has risen by more than twofold in the last two decades [5]. This market growth has raised the need for accurate and fast characterisation techniques, which can be used to assess the botanical origin of spices and to detect adulterations [6-8].

Characterization of spices is usually carried out by the analysis of their volatile fraction, which is mainly composed of terpenes, terpenoids and light phenols [9,10]. The general aim of these analyses is to obtain a chemical fingerprint that is characteristic of a certain spice deriving from a specific plant variety. The most widely used technique for this purpose is headspace solid-phase micro-extraction followed by gas chromatography-mass spectrometry (SPME-GC/MS) [9,11-14], which offers the advantages of no sample preparation, no use of solvents and short sampling times. Solvent extraction and supercritical fluid extraction can also be used before chromatographic analysis [15,16].

While there are numerous papers dealing with volatiles of spices, in the literature there is very little information regarding the heavier fractions of these materials. This is likely due to the complexity of the lignocellulosic matrix, which requires intense sample pre-treatments to be analysed with conventional chromatographic techniques.

Evolved gas analysis-mass spectrometry (EGA-MS) and analytical pyrolysis-GC/MS (Py-GC/MS) are powerful tools for the characterisation of complex materials, and they have been extensively used to characterize natural products in many research fields [17,18]. The advantages of analytical pyrolysis are that very little sample amount is required, and that sample preparation is virtually absent. *In situ* derivatisation can also be used when dealing with lignocellulose pyrolysis products to reduce their polarity and improve their chromatographic behaviour [19,20].

Double-shot analytical pyrolysis [21] is a technique that allows to perform thermal desorption and high-temperature pyrolysis in two separate steps on the same sample. This technique could be used to obtain the characterisation of both the volatile and non-volatile fractions of the same spice sample, while retaining the advantages of SPME such as no sample preparation and no solvents required. Despite this potential, however, no literature references are available reporting a study of spices using this technique.

In the present work, we evaluate the suitability of EGA-MS and double-shot Py-GC/MS with *in situ* derivatisation for the study of five commercially available spices: black pepper, cinnamon, ginger, turmeric and cloves. EGA-MS is used as screening technique to determine the temperature intervals for the thermal desorption or degradation of the different fractions of the samples. Both the volatile and non-volatile fractions are then characterised for each spice using multi-shot analytical pyrolysis. To the best of our knowledge, this is the first work reporting the use of evolved gas analysis and analytical pyrolysis for the characterisation of spices.

2. MATERIALS AND METHODS

2.1 Samples and materials: Black pepper (*Piper nigrum*, grains), cinnamon (*Cinnamomum verum*, dry sticks), ginger (*Zingiber officinalis*, powder), turmeric (*Curcuma longa*, powder), saffron (*Crocus sativus*, powder) and cloves (*Syzygium aromaticum*, dry flower buds) were acquired from local companies in Italy. Spices in powder form were not processed further. Spices in other forms were ground to a fine dust using a Pulverisette 23 laboratory-scale vibratory ball-mill (Fritsch, Germany), which was operated at 50 Hz and at ambient temperature. Before analysis, each sample was filtered on 120-mesh nets to obtain a homogenous powder. Hexamethyldisilazane (HMDS, ReagentPlus grade, 99.9%, Sigma-Aldrich, USA) was used as derivatising agent.

2.2 Evolved gas analysis-mass spectrometry (EGA-MS): Experiments were carried out with an EGA/PY-3030D micro-furnace pyrolyser (Frontier Laboratories Ltd., Japan) coupled to a 7890N gas

chromatograph (Agilent Technologies, USA) equipped with a split/splitless injector and a 5975C mass spectrometer (Agilent Technologies, USA). Approximately 100 µg of sample were used in each experiment. During the experiment, the pyrolysis furnace temperature was increased from 50 °C to 700 °C at 10 °C/min, while the interface temperature was kept 100 °C above the furnace temperature, up to a maximum of 300 °C. The injector was operated in split mode at 250 °C with a 10:1 ratio. An UADTM-2.5N deactivated stainless steel capillary tube (3 m x 0.15 mm, Frontier Laboratories Ltd., Japan) was used to connect the injector to the mass spectrometer. Helium (1 mL/min) was used as carrier gas. The tube was kept at 300 °C inside the GC oven, and the transfer line temperature was set to 300 °C. The mass spectrometer was operated in EI positive mode (70 eV, *m/z* range 50-500). The ion source temperature was 230 °C, and the quadrupole temperature was 150 °C.

2.3 Double-shot analytical pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS):

Analytical pyrolysis experiments were performed with the same instrumentation used for EGA-MS experiments. In this case, an UltraALLOY^{+}-1 stainless steel capillary column (30 m x 0.25 mm, film thickness 0.25 µm) to achieve chromatographic separation. Approximately 200 µg of sample were used in each experiment. Each double-shot experiment is composed of two consecutive stages. The first shot, corresponding to the thermal desorption, was performed at 250 °C for 10 min, and the evolved compounds were collected at the head of the chromatographic column by means of a liquid nitrogen trap. During this time, the GC injector was operated at 250°C and with a 25:1 split ratio. After the thermal desorption, evolved compounds were separated using the following oven temperature gradient: 40 °C isothermal for 1 min; 10 °C/min up to 280 °C, then isothermal for 20 min. The detected *m/z* range in this run was 29-500. At the end of the first chromatographic run, before the second shot, 2 µL of HMDS were added to the sample. The second shot, corresponding to the pyrolysis step, was performed at 550 °C for 0.2 min, and the injector was operated at 250°C and with a 20:1 split ratio. The following oven temperature gradient was used: 50 °C isothermal for 1 min; 10 °C/min up to 100 °C, then isothermal for 2 min; 4 °C/min up to 190 °C, then isothermal for 1

106 min; 30 °C/min up to 280 °C, then isothermal for 20 min. The detected *m/z* range in this case was 50-
107 800.

108 **2.4 Data processing:** Mass spectra from the EGA-MS thermograms and Py-GC/MS pyrograms were
109 interpreted based on the comparison with reference mass spectra libraries (Wiley and
110 NIST/EPA/NIH) and with literature publications [9,19,22,23]. Reproducibility of the EGA-MS and Py-
111 GC/MS experiments was evaluated by performing triplicate analysis of each spice. Peaks were
112 integrated and normalized by the sample amount. Relative standard deviations were always lower
113 than 10% for EGA-MS experiments, and lower than 15% for Py-GC/MS experiments.

114

115 ## 3. RESULTS AND DISCUSSION

116 **3.1 EGA-MS:** The thermograms of all six spices are presented in Figure 1. All samples provided
117 unique thermal degradation profiles, but some common traits can be observed. All thermograms can
118 be divided into two regions. The first region, up to 250 °C, corresponds to the desorption of the low
119 molecular weight fractions of the sample. This includes all the low molecular weight extractives of
120 the sample, including the volatile compounds responsible for the aroma of each spice. After 250 °C,
121 the high molecular weight fractions of the samples undergo pyrolysis processes. A brief description
122 of all the thermograms will be presented in the next paragraphs.

123 **3.1.1 Black pepper:** The first region of the thermogram of black pepper presented two small peaks
124 before 150 °C. The mass spectra of these peaks showed signals at *m/z* 204, 189, 161, 133, 105 and
125 93, which are characteristic of sesquiterpenes. Sesquiterpenes such as β-caryophyllene are known to
126 be among the main components of the headspace of black pepper [9]. The high peak centred at
127 220 °C showed signals at *m/z* 115, 173, 201 and 285 in the mass spectrum, which can be ascribed to
128 piperine [24]. In the second region of the thermogram, the characteristic profile of lignocellulose
129 pyrolysis can be observed [22,25]. The mass spectrum of the peak at 300 °C presented signals at *m/z*
130 57, 69, 85 and 98, which can all be ascribed to pyrolysis products of the polysaccharide fraction of

lignocellulose. Finally, the mass spectrum of the broad shoulder peak at high temperatures presented signals at *m/z* 77, 91 and 107, which can be attributed to secondary pyrolysis products of lignin.

3.1.2 Cinnamon: The broad peak in the thermal desorption region shows signals at *m/z* 51, 77, 103 and 131, which are characteristic of cinnamaldehyde, a major component of the aroma of this spice [26]. The broadness of the peak can be likely attributed to a strong interaction of this compound with the lignocellulosic matrix. The second region of the thermogram showed a similar profile to the one observed for black pepper, with the main signals in the mass spectra belonging to pyrolysis products of lignocellulose.

3.1.3 Ginger: The first region in the thermogram of ginger presents two peaks. The first peak, centred at around 75 °C, showed the same signals of sesquiterpenes that were found in the thermogram of black pepper at low temperatures. The second peak, which is centred at around 175 °C, showed *m/z* signals at 137, 194, 205 and 276, which can be attributed to (6)-gingerol and to a derivate molecule obtained from gingerol dehydration, (6)-shogaol [27]. The pyrolysis region of the thermogram of ginger showed the same signals of lignocellulose that were found for black pepper and cinnamon.

3.1.4 Turmeric: The thermal degradation region of turmeric was dominated by a peak centred at approximately 80 °C, whose mass spectrum can be attributed to ar-turmerone [6]. As for pepper, cinnamon and ginger, the pyrolysis region showed the characteristic profile and mass spectra of pyrolysis products of lignocellulose.

3.1.5 Saffron: The thermogram of saffron was considerably different from the other samples. A high peak in the desorption region showed the characteristic *m/z* signals of safranal [28]. However, the pyrolysis region presented a unique profile, and a high signal intensity was detected even at 250 °C. As these peaks are not resolved, specific mass spectra could not be obtained. The average mass spectrum in the region 250-325 °C showed the signals of the pyrolysis products of polysaccharides,

while the peak at higher temperatures showed signals at 77, 91, 105 and 119, which are characteristic of aromatic compounds. Aromatic compounds could be obtained from secondary pyrolysis reaction of carbohydrates [29]. This result suggests that the broad peak at high temperatures could correspond to secondary pyrolysis of polysaccharides, taking place after the first pyrolysis process at lower temperatures.

3.1.6 Cloves: The peak centred at 75 °C in the thermal degradation region of cloves showed signals at *m/z* 77, 91, 103, 131, 149, 164, which can be attributed to eugenol [9]. The height of this peak suggests that the volatiles content of cloves is very high compared to the non-volatile fraction. The pyrolysis region presented two peaks at low intensity. Both these peaks presented signals due to lignocellulose pyrolysis products, as well as signals at m/z 203 and 248, which are characteristic of oleanolic acid, a triterpene found in many essential oils [30].

3.1.7 Desorption time and weight loss: To obtain an estimation of the time required for a quantitative thermal desorption of the volatile compounds, six samples of black pepper were heated in the pyrolysis furnace at 250 °C at six different times from 0 to 10 min. The furnace temperature was then lowered, and EGA-MS was performed on the residues. Figure 2 shows the resulting thermograms. Each thermogram was normalized by the height of the peak corresponding to the pyrolysis of lignocellulose, which was not affected by thermal desorption. The peaks of sesquiterpenes disappeared after only 0.5 min of heating, while 10 min were required for piperine to be completely desorbed. This result suggests that 10 min are enough to achieve a complete desorption of all the low-molecular weight compounds in black pepper. As piperine is the low-molecular weight compound evolving at the highest temperature among all spice samples, we assumed 10 min to be enough for a complete desorption of all the volatiles for all spices.

Following this conclusion, the weight fraction of low-molecular weight compounds was estimated for all spices by weighting triplicate samples before and after heating at 250 °C for 10 min. Table 1 summarises the results . The weight fractions accounted for approximately 25% in black pepper,

cinnamon, ginger and turmeric, while it accounted for 50% in saffron and cloves. This result is consistent with the EGA profiles of saffron and cloves, which showed high signal intensities at lower temperatures.

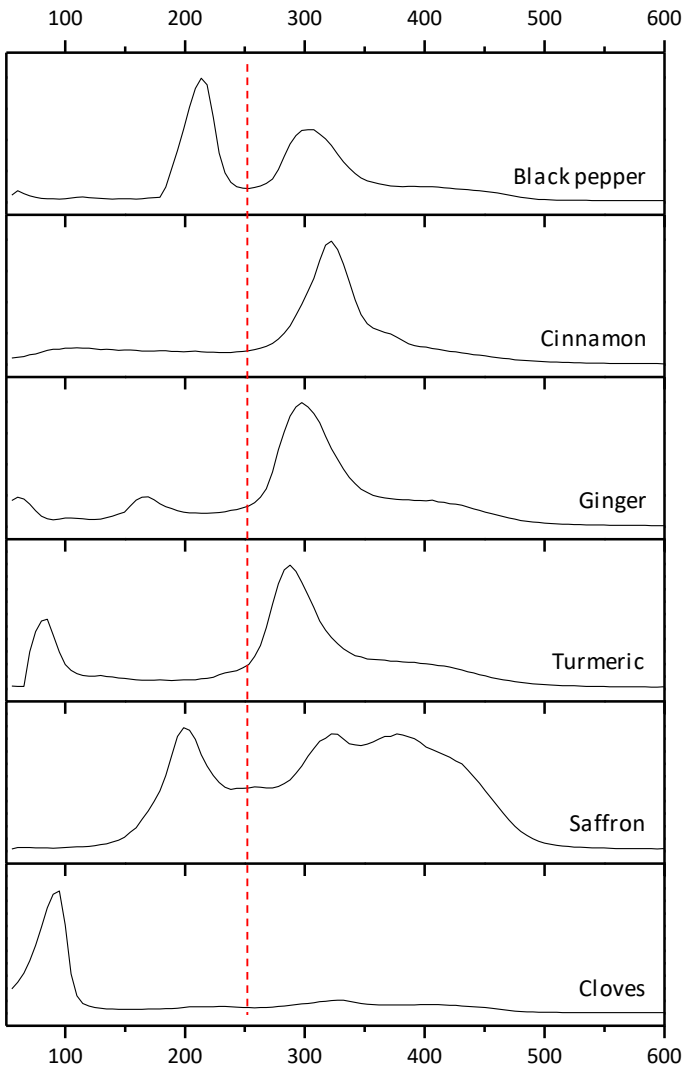


Figure 1: EGA-MS profiles of the six analysed spices. The dotted line at 250 °C highlights the separation between the thermal desorption zone at low temperatures and the pyrolysis zone at high temperatures.

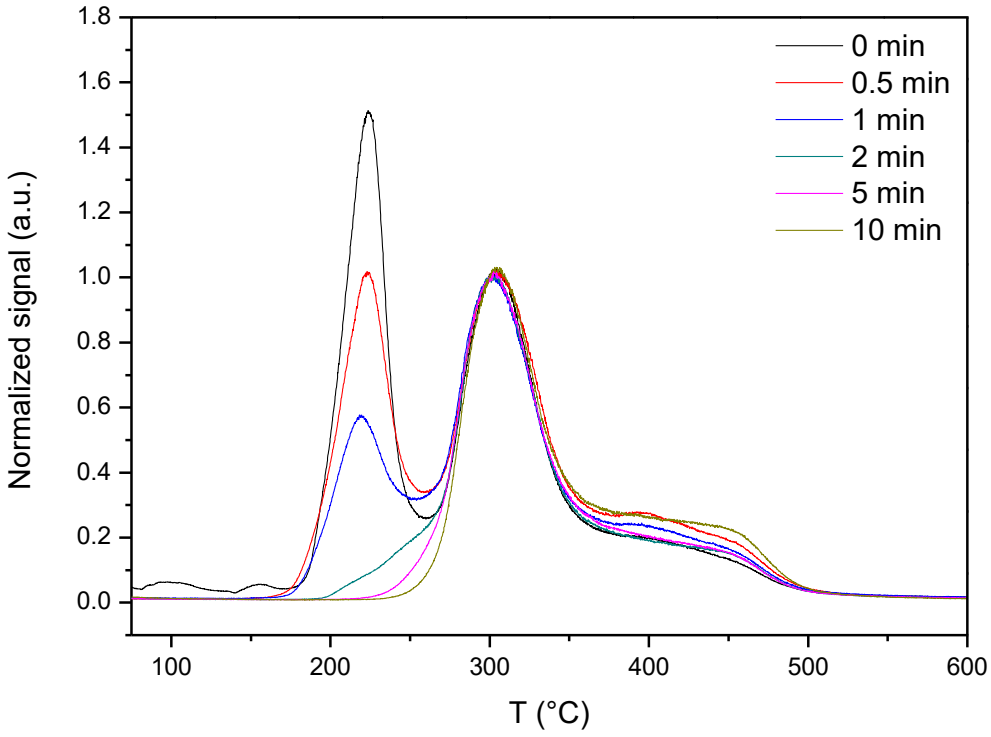


Figure 2: Thermograms for black pepper obtained after various thermal desorption times at 250 °C. Each thermogram has been normalized by the height of the peak at 300 °C, which corresponds to lignocellulose pyrolysis.

Table 1: Weight fractions of the volatile compounds for each spice. Errors are expressed at a 5% significance level (n = 3, t = 4.303).

Spice	Weight fraction of volatiles (%)
Black pepper	26 ± 4
Cinnamon	25 ± 6
Ginger	23 ± 3
Turmeric	26 ± 7
Saffron	50 ± 10
Cloves	51 ± 3

3.2 Double-shot Py-GC/MS: Following the results obtained from the EGA-MS analyses, double-shot analytical pyrolysis experiments were performed to obtain information on both the volatile and non-

volatile fractions of spices. The first pyrolysis step was performed at 250 °C, to obtain the volatilisation of the low-molecular weight compounds, while the second step was performed at 550 °C, to achieve the thermal degradation of the solid residue. The double-shot approach for the analysis of these samples is required for two main reasons. The first reason is that both fractions generate very rich chromatographic profiles, and therefore their separate analysis can prevent co-elution, reducing the complexity of the results and allowing thorough identification of the desorption/pyrolysis products. The second reason is the different polarity of the compounds eluted in the two steps. The compounds in the first shot have low polarity, and can be efficiently retained by the stationary phase of the GC column. On the contrary, the compounds in the second shot are highly polar, especially those that are obtained from the pyrolysis of carbohydrates and lignin. Derivatisation of these compounds is therefore required to improve the chromatographic quality.

More than 120 compounds were detected in the pyrograms of the first shot (Figures 3 to 8), and a full list is presented in Table 2. More than half of these compounds could be categorized as terpenoids. Terpenoids are the most abundant compounds in the headspace of spices, and they are the compounds which are most commonly detected using conventional SPME techniques [6,9]. The other compounds showed a high variability in their structures, and their further classification was not straightforward and beyond the aim of the study. Most of these other compounds were characteristic of only one spice sample.

The use of a high thermal desorption temperature in our experiments allowed us to detect a series of compounds which were eluted at high retention times, and which are not usually detected by SPME or solvent extractions. A more detailed description of these compounds will be provided in the discussion of the corresponding spice.

Table 2: List of all identified compounds in the first pyrograms of the spice samples. Numbers refer to the peak numbering in the chromatograms of Figures 3-8. Retention time, category and main *m/z*

226 signals are displayed for each compound. Underlined *m/z* values indicate the base peak. N = non

227 terpenoids, T = terpenoids, S = sesquiterpenoids.

#	t(min)	Name	Cat	*m/z*
1	2.8	Acetic acid	N	60, 45, 43
2	3.4	Hydroxyacetone	N	74, 43, 31
3	6.6	2-hydroxymethylfuran	N	98, 81, 69, 53, 41
4	7.1	Dihydro-2-furanone	N	84, 55, 39
5	7.6	2-hydroxy-2-cyclopenten-2-one	N	98, 69, 55, 42
6	8.3	Thujene	T	136, 121, 105, 93, 91, 79, 77
7	8.4	α-pinene	T	136, 121, 105, 93, 91, 79, 77
8	8.8	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	N	144, 101, 73, 55, 43
9	9.0	m-cymene	T	134, 119, 91
10	9.0	Sabinene	T	136, 121, 93, 91, 79, 77
11	9.1	β-pinene	T	136, 121, 107, 93, 91, 79, 77, 69, 41
12	9.3	β-myrcene	T	93, 69, 41
13	9.6	α-phellandrene	T	136, 93, 91, 77
14	9.7	D-3-carene	T	136, 121, 105, 93, 91, 79, 77, 41
15	9.8	p-cymene	T	134, 119, 91
16	10.0	D-limonene	T	136, 121, 107, 93, 91, 79, 77, 68, 53, 39
17	10.4	2,5-dimethyltetrahydrofuran-3,4-dione	N	150, 121, 105, 91, 79
18	10.4	γ-terpinene	T	136, 121, 105, 93, 91, 79, 77
19	10.5	4-thujanol	T	154, 139, 121, 111, 93, 71, 43
20	10.9	Linalool	T	93, 80, 71, 69, 55, 43, 41
21	11.0	2,6,6-trimethylcyclohexa-1,4-dienecarbaldehyde (safranal isomer)	T	150, 135, 121, 107, 91, 79
22	11.1	Isophorone	N	138, 82
23	11.2	N-formylpiperidine	N	113, 98, 84, 70, 56, 42, 29
24	11.3	2,6,6-trimethyl-2-cyclohexen-1,4-dione	N	152, 137, 124, 109, 96, 68
25	11.4	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	N	144, 115, 101, 72, 55, 43
26	11.6	2-hydroxy-3,5,5-trimethylcyclohex-2-enone	N	154, 139, 126, 111, 98, 83, 70, 55
27	11.6	Benzenepropanal	N	134, 115, 103, 91, 77, 51
28	11.8	2-hydroxy-4,4,6-trimethylcyclohexa-2,5-dienone	N	152, 137, 124, 109, 91, 79
29	12.0	Endo-borneol	T	139, 136, 121, 110, 95, 79, 67, 55, 41
30	12.1	2-methylbenzofuran	N	131, 103, 77, 51
31	12.1	2,4-dimethylbenzaldehyde	N	134, 133, 105, 91, 77
32	12.2	Terpinen-4-ol	T	154, 136, 111, 93, 71
33	12.4	α-terpineol	T	136, 121, 93, 81, 59
34	12.4	Safranal	T	150, 135, 121, 107, 105, 91, 79, 77
35	12.5	cis-sabinol	T	151, 134, 119, 109, 92, 91, 81, 79
36	12.5	Z-cinnamaldehyde	N	131, 103, 77, 51
37	12.7	4-methyleneisophorone	N	150, 135, 122, 107, 91, 79, 66
38	12.8	2-hydroxy-4-oxoisophorone	N	168, 153, 140, 126, 84, 69, 56, 41
39	13.1	p-allylphenol	N	134, 133, 107, 105, 91, 77, 51
40	13.2	Geraniol	T	136, 123, 111, 93, 69, 53, 41

41	13.3	E-cinnamaldehyde	N	131, 103, 77, 51
42	13.7	4-hydroxy-3,5,5-trimethylcyclohex-2-enone	N	154, 139, 112, 98, 70, 42
43	13.8	Bornyl acetate	T	154, 136, 121, 95, 80, 67, 55, 43
44	14.0	4-vinylguaiacol	N	150, 135, 107, 77
45	14.3	4-hydroxy-2,6,6-trimethyl-3-oxocyclohex-1-enecarbaldehyde	N	182, 153, 125, 111, 107, 69, 55, 43
46	14.7	Eugenol	N	164, 149, 131, 121, 103, 91, 77
47	14.8	δ-elemene	S	204, 189, 161, 136, 121, 105, 93, 77
48	14.8	Hydrocoumarin	N	148, 120, 106, 91, 78, 63, 51, 39
49	14.9	α-cubebene	S	204, 161, 119, 105, 91
50	15.0	Geranyl acetate	S	154, 136, 121, 107, 93, 80, 69, 53, 43
51	15.0	4-hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	N	180, 165, 152, 137, 123, 109, 91, 79, 55, 39
52	15.3	4-hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde	N	168, 153, 150, 135, 121, 107, 91, 79, 55, 41
53	15.3	Copaene	S	204, 189, 161, 119, 105, 91, 77
54	15.5	Coumarin	N	146, 118, 89, 63
55	15.8	Levoglucosan	N	57, 60, 73, 98, 144
56	15.9	β-caryophyllene	S	204, 189, 175, 161, 147, 133, 120, 105, 93, 79, 69, 55, 41
57	16.0	2,2,6-trimethyl-4-oxocyclohexanecarbaldehyde	N	168, 138, 123, 111, 97, 79, 67
58	16.4	Humulene	S	204, 147, 121, 93, 80
59	16.5	Ar-curcumene	S	202, 159, 145, 132, 119, 105, 91, 77, 69, 55, 41
60	16.6	Cadina-1(6),4-diene	S	204, 189, 161, 145, 134, 115, 105, 81
61	16.6	γ-cadinene	S	204, 161, 145, 133, 119, 105, 91, 79
62	16.7	Eugenol acetate	N	206, 164, 149, 131, 121, 103, 91, 77
63	16.7	Zingiberene	S	204, 133, 119, 105, 93, 77, 69, 56, 41
64	16.8	β-eudesmene	S	204, 189, 175, 161, 147, 133, 120, 105, 93, 79, 69, 55, 41
65	16.8	α-farnesene	S	204, 189, 161, 133, 119, 107, 93, 79, 69, 55, 41
66	16.8	α-muurolene	S	204, 119, 105, 91, 77
67	16.9	α-selinene	S	204, 189, 175, 161, 147, 133, 120, 105, 93, 79, 69, 55, 41
68	16.9	β-bisabolene	S	204, 189, 133, 121, 109, 93, 79, 69, 53, 41
69	17.1	β-sesquiphellandrene	S	204, 161, 133, 120, 109, 93, 77, 69, 55, 41
70	17.1	cadina-1(10),4-diene	S	204, 189, 161, 134, 119, 105, 91, 81, 41
71	17.2	Cubenene	S	204, 161, 119, 105, 91, 77, 41
72	17.2	Dodecanoic acid	N	200, 171, 157, 129, 101, 85, 73, 60, 43
73	17.6	Ar-turmerol	S	203, 160, 119, 91
74	17.8	7-methoxymethyl-2,7-dimethylcyclohepta-1,3,5-triene	N	216, 161, 132, 119, 105, 91
75	17.9	Caryophyllene oxide	S	220, 205, 177, 149, 121, 109, 91, 79, 69, 55, 41
76	18.1	Zingerone	N	194, 151, 137, 119, 91, 77, 43
77	18.1	Turmerone isomer I	S	218, 200, 185, 157, 119, 105, 85
78	18.2	Humulene epoxide	S	138, 123, 109, 96, 93, 81, 67, 55, 43
79	18.3	Turmerone isomer II	S	218, 203, 187, 120, 105, 91, 77, 55, 43
80	18.3	Isospathulenol	S	220, 205, 177, 159, 147, 119, 105, 91, 79, 43
81	18.4	1,10-diepicubeol	S	204, 179, 161, 119, 105, 95, 82, 55
82	18.4	11,11-dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	S	187, 177, 159, 149, 136, 131, 117, 107, 91, 79, 69, 55, 41

83	18.5	δ-cadinol	S	204, 189, 161, 119, 105, 95, 79, 43
84	18.5	Ar-turmerone	S	216, 201, 132, 119, 105, 91, 83
85	18.6	Turmerone	S	218, 200, 120, 111, 105, 91, 83, 77, 55
86	18.6	Cyclocopacamphenol	S	220, 204, 189, 161, 145, 131, 119, 105, 91, 81, 55, 41
87	18.7	Trimethoxyacetophenone	N	210, 195, 177, 152, 137 ,43
88	18.8	Caryophyllene oxide isomer	S	220, 205, 177, 149, 121, 109, 91, 79, 69, 55, 41
89	18.9	Zingiberenol	S	204, 189, 137, 119, 109, 93, 84, 69, 55, 41
90	19.0	Cedrenol	S	159, 118, 109, 91, 79, 69, 55, 41
91	19.0	β-turmerone	S	218, 203, 120, 105, 91, 83, 77, 55
92	19.5	Bisabolone	S	220, 205, 177, 137, 135, 123, 110, 95, 82, 69, 55, 41
93	19.5	Tetradecanoic acid	N	228, 185, 171, 129, 73, 60, 43
94	19.7	γ-atlantone	S	214, 199, 149, 131, 119, 114, 105, 91, 77
95	19.8	Atlantone	S	218, 203, 135, 123, 107, 91, 83, 67, 55
96	19.9	Nootkatene	S	202, 187, 159, 145, 131, 119, 105, 91, 77, 55, 43
97	19.9	Hydroxy-dehydroatlantone	S	234, 216, 201, 136, 125, 109, 95, 91, 83, 67, 55
98	20.8	Dehydroturmerone	S	136, 118, 83, 55
99	21.0	Dehydro-β-turmerone	S	234, 219, 151, 137, 121, 110, 95, 83, 55
100	21.2	(E,E)-N-isobutyl-2,4-decadienamide	N	223, 208, 151, 110, 96, 81
101	21.6	Palmitic acid	N	256, 213, 185, 171, 157, 129, 115, 97 ,83, 73, 60, 55, 43
102	23.2	Linoleic acid	N	280, 150, 136, 123, 109, 95, 81, 67, 55, 43
103	23.3	(E,E)-N-isobutyl-2,4-dodecadienamide	N	251, 236, 179, 152, 96, 81, 55, 41
104	23.3	Oleic acid	N	282, 264, 123, 111, 97, 83, 69, 55, 41
105	23.5	Octadecanoic acid	N	284, 241, 185, 129, 97, 83, 73, 60, 55, 43, 29
106	24.0	(6)-isoshogaol	N	276, 179, 151, 137, 122, 91
107	24.1	(6)-gingerone	N	278, 179, 151, 137, 119, 91, 57
108	24.6	(6)-shogaol	N	276, 205, 151, 137, 119, 91, 55
109	24.9	(6)-gingerdione	N	292, 150, 137, 122, 91
110	25.4	Gingerol	N	294, 276, 205, 194, 179, 150, 137, 122, 91
111	26.4	(8)-shogaol	N	304, 205, 151, 137, 119, 91, 55
112	26.6	(6)-gingerdiol-3,5-diacetate	N	380, 320, 260, 189, 175, 163, 150, 137, 131, 43
113	27.6	Piperanine	N	287, 202, 174, 135, 105, 77
114	27.9	(10)-isoshogaol	N	332, 179, 151, 137, 122, 91
115	28.8	(10)-shogaol	N	332, 205, 151, 137, 119, 91, 55, 41
116	28.9	Piperine isomer I	N	285, 201, 173, 143, 115, 84
117	29.1	Piperine isomer II	N	285, 201, 173, 143, 115, 84
118	29.4	(10)-gingerdione	N	348, 179, 150, 137, 122, 91, 43
119	29.5	(E,E,E)-N-isobutyloctadeca-2,4,6-trienamide	N	333, 304, 261, 180, 152, 115, 95, 81, 67, 55, 41
120	29.7	(E,E)-N-Isobutyloctadeca-2,4-dienamide	N	335, 320, 263, 152, 113, 96, 81, 67, 55, 41
121	30.7	Piperyline	N	271, 201, 173, 135, 115, 81
122	31.6	Piperine isomer III	N	285, 201, 173, 143, 115, 84
123	31.7	Piperoleine A	N	315, 230, 174, 140, 127, 103, 84
124	32.3	(2E,6E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)hepta-2,6-dien-1-one	N	313, 161, 131, 103, 77

125	36.5	Piperoleine B	N	343, 258, 208, 182, 140, 127, 103, 84

More than 100 compounds were identified in the pyrograms of the second shot (Figures 3 to 8). A complete list is displayed in Table 3. Note that some compounds did not achieve a quantitative derivatisation and were found both as derivatised and un-derivatised in the pyrograms. An example of such compounds is 1,2-dihydroxybenzene (#18' and 37').

As for the identified compounds from the first-shot chromatograms, all pyrolysis products were divided into five categories.The first two categories are carbohydrate and lignin pyrolysis products, which were identified based on previous literature publications dealing with analytical pyrolysis of wood [19,23,25,31]. More than 60 compounds were found in total belonging to these categories. The wide variety of lignocellulose pyrolysis products is due to an extremely complex reaction mechanism of these substrates, with hundreds of parallel and competitive reactions. Carbohydrate pyrolysis starts with cleavage of the glycosidic bonds to give dehydrated monosaccharides [32]. The most characteristic pyrolysis products of carbohydrates are anhydrosugars, which are obtained from the monomers by the formation of a C-O-C bridge. As the pyrolysis process unfolds, more water molecules are lost and poly-unsaturated compounds such as furans and pyrans are obtained [33]. Cyclopentenones can also be obtained by multiple dehydration reactions following rearrangement of the furan or pyran ring of monosaccharides. Lignin pyrolysis also starts with depolymerisation and formation of the two main monomers, coniferyl- and synapyl-alcohol [34,35]. These monomers then undergo further degradation mainly involving the alkyl side chain and the methoxy group on the aromatic ring. Disproportionation reactions can also take place between two free lignin monomers, generating both oxidised and reduced versions of the original molecule.

The pyrolysis of carbohydrates and lignin can also lead to the formation of small molecules (1 to 3 carbon atoms) and aromatic compounds such as hydroxybenzenes. Since these two compound categories can originate from both fractions of lignocellulose, they were considered as separate categories.

A fifth compound category was defined to include all compounds that could not be assigned to any of the other categories. The main components of this category are aliphatic carboxylic acids, including long-chain fatty acids. These compounds most likely derive from the lipid fraction of the spices. Five- and six- carbon atoms alcohols and carboxylic acids were also identified in the pyrograms (#25', 40', 64', 66', 76', 78'). The origin of these compounds is unclear. A likely hypothesis is that these compounds are obtained from acid sugars with five and six carbon atoms, that were present as oxidised carbohydrates in the spice matrix. However, since the presence of these compounds in spices has never been evaluated, we assigned these compounds to the fifth category.

Finally, some peaks were found in the chromatograms that could be attributed to side-products of the derivatization process. As these peaks do not bear any information, they were not included in the compounds list. They have been marked with an asterisk in the chromatogram figures.

A more in-depth description of the results obtained for each spice sample is provided in the following paragraphs.

Table 3: Identified compounds in the second shot pyrograms of spices. Numbers refer to the peak numbering in the chromatograms of Figures 3 - 8. Retention time, originating polymer, compound category and main *m/z* signals in the mass spectrum are displayed for each compound. Underlined *m/z* values indicate the base peak. The number of trimethylsilyl groups is also indicated for each derivatised compound. Aro = aromatics, Smo = small molecules, Car = carbohydrate pyrolysis products, Lig = lignin pyrolysis products, Oth = other compounds.

#	t(min)	Name	Cat	*m/z*
1'	10.4	Phenol (TMS)	Aro	166, <u>151</u>
2'	10.9	2-hydroxypropanoic acid (2TMS)	Smo	219, 191, 147, 117, <u>73</u>
3'	11.0	Guaiacol	Lig	124, <u>109</u>, 81
4'	11.2	Hydroxyacetic acid (2TMS)	Smo	220, 205, 161, <u>147</u>, 73
5'	11.6	3-hydroxymethylfuran (TMS)	Car	170, <u>155</u>, 81
6'	12.5	2-furancarboxylic acid (TMS)	Car	184, 169, <u>125</u>, 95
7'	12.7	2-hydroxymethylfuran (TMS)	Car	170, <u>155</u>, 81

8'	13.0	5-oxopentanoic acid (TMS)	Oth	173, 160, 143, 131, 116, 101, 75, 73, 71
9'	13.4	3-hydroxypropanoic acid (2TMS)	Smo	219, 177, 147, 73
10'	13.4	p-cresol (TMS)	Aro	180, 165, 91
11'	13.4	3-hydroxy-(4H)-pyran-4-one (TMS)	Car	184, 169, 95, 73
12'	13.9	2,5-dimethylbenzaldehyde	Aro	134, 133, 105, 91, 77, 63, 51
13'	14.0	3-hydroxycyclopenta-1,2-dione (TMS)	Car	186, 171, 143, 115, 101, 73
14'	14.2	4-methylguaiacol	Lig	138, 123, 95
15'	14.2	2-hydroxycyclopenta-1,3-dione (TMS)	Car	171, 143, 101, 75, 73
16'	14.6	5-hydroxy-2H-pyran-4(3H)-one (TMS)	Car	186, 171, 143, 129, 101, 75
17'	14.6	2-hydroxy-(4H)-pyran-4-one (TMS)	Car	184, 169, 95, 77
18'	14.9	1,2-dihydroxybenzene (TMS)	Aro	182, 167, 151, 91, 73
19'	15.0	2-hydroxymethyl-3-methylcyclopentenone (TMS)	Car	198, 183, 73
20'	15.1	2-methylcyclopenta-1,3-dione, enolic form (TMS)	Car	184, 169, 139, 117, 73
21'	15.2	3-methylcyclopenta-1,2-dione, enolic form (TMS)	Car	184, 169, 125, 97, 73
22'	15.6	Guaiacol (TMS)	Lig	196, 181, 166, 151, 103, 73
23'	15.8	1,3-dihydroxyacetone (2TMS)	Smo	219, 189, 147, 103, 73
24'	16.7	Unknown aliphatic alcohol (TMS)	Oth	173, 131, 116, 101, 75
25'	16.8	Z-2-penten-1-ol (TMS)	Oth	158, 143, 129, 73
26'	17.1	3-hydroxy-6-methyl-(2H)-pyran-2-one (TMS)	Car	198, 183, 168
27'	17.1	Unknown aliphatic alcohol (TMS)	Oth	173, 158, 129, 103, 73
28'	17.3	2-methyl-3-hydroxymethyl-2-cyclopentenone (TMS)	Car	198, 183, 153, 111, 97, 83, 69, 55
29'	17.4	4-vinylphenol (TMS)	Aro	192, 177, 161, 151, 135, 115, 91, 77
30'	17.5	3-hydroxymethylphenol (TMS)	Aro	196, 180, 165, 149, 105, 75
31'	17.9	4-hydroxymethylphenol (TMS)	Aro	196, 180, 165, 149, 105, 75
32'	18.0	2,3-dihydrofuran-2,3-diol (2TMS)	Car	246, 231, 147, 73
33'	18.0	4-vinylguaiacol	Lig	150, 135, 107, 77
34'	18.1	5-hydroxymethyl-2-furaldehyde (TMS)	Car	198, 183, 169, 109 ,73, 53
35'	18.2	Glycerol (3TMS)	Smo	218, 205, 191, 177, 147, 133, 117, 103, 73
36'	18.6	4-methylguaiacol (TMS)	Lig	210, 195, 180, 73
37'	19.0	1,2-dihydroxybenzene (2TMS)	Aro	254, 239, 151, 73
38'	19.1	1:4,3:6-anhydro-α-D-glucopyranose (TMS)	Car	170, 155, 145, 129, 103, 81, 73
39'	19.3	3-hydroxycyclopenta-1,2-dione, enolic form (2TMS)	Car	258, 243, 230, 169, 147, 73
40'	19.5	E-2-penten-1-ol (TMS)	Oth	158, 143, 129, 73
41'	19.5	Eugenol	Lig	164, 149, 131, 121, 103, 91, 77, 65, 55
42'	20.6	2-hydroxymethyl-3-hydroxytetrahydropyran (2TMS)	Car	217, 191, 147, 129, 103, 73
43'	21.0	1,3-dihydroxybenzene (2TMS)	Aro	254, 239, 147, 73
44'	21.1	Syringol (TMS)	Lig	226, 211, 196, 181
45'	21.1	4-ethylguaiacol (TMS)	Lig	224, 209, 194, 179
46'	21.2	5-formyltetrahydrofuran-2-carboxylic acid (TMS)	Car	173, 143, 129, 73
47'	21.2	Dmethylnaphthalene	Aro	156, 141, 128, 115, 77
48'	21.4	4-methylcatechol (2TMS)	Lig	268, 253, 180, 73
49'	21.6	Arabinofuranose (4TMS)	Car	230, 217, 147, 129, 73
50'	21.7	1,4-dihydroxybenzene (2TMS)	Aro	254, 239, 147, 73
51'	21.8	Arabinofuranose isomer (4TMS)	Car	230, 217, 147, 143, 129, 73

52'	22.0	2-(1,2-dihydroxyethyl)furan (2TMS)	Car	272, 257, 183, 169, 147, 73
53'	22.4	4-vinylguaiacol (TMS)	Lig	222, 207, 192, 177, 162
54'	22.6	2-hydroxycyclopenta-1,3-dione, enolic form (2TMS)	Car	243, 73
55'	22.7	3-hydroxy-2-hydroxymethyl-2-cyclopentenone (2TMS)	Car	272, 257, 147, 73
56'	22.9	3-deoxypentofuranose (3TMS)	Car	157, 147, 129, 103, 73
57'	23.5	Eugenol (TMS)	Lig	236, 221, 206, 179
58'	23.6	Methylhydroquinone (2TMS)	Aro	282, 268, 253, 237, 179, 163, 119, 73
59'	23.7	4-methylsyringol (TMS)	Lig	240, 225, 210, 195, 167
60'	23.8	1,6-anhydro-β-D-glucopyranose (TMS C4)	Car	155, 145, 129, 103, 73
61'	23.9	1,6-anhydro-β-D-glucopyranose (TMS C2)	Car	155, 145, 129, 116, 101, 73
62'	24.0	3-methoxy-1,2-benzenediol (2TMS)	Lig	284, 269, 254, 239, 196, 169, 153
63'	24.2	3,5-dihydroxy-2-methyl-(4H)-pyran-4-one (2TMS)	Car	271, 199, 128, 73
64'	24.3	4-hydroxy-5-oxopentanoic acid (2TMS)	Oth	276, 261, 233, 147, 129, 117, 103, 73
65'	24.6	2-hydroxypropiophenone (TMS)	Aro	224, 207, 193, 163, 133, 91, 75
66'	24.8	3-hydroxy-5-oxopentanoic acid (2TMS)	Oth	276, 261, 233, 147, 129, 117, 103, 73
67'	25.9	1,4-dihydroxy-2-methoxybenzene (2TMS)	Lig	284, 269, 254, 239, 73
68'	26.3	1,2,3-trihydroxybenzene (3TMS)	Aro	342, 327, 239, 73
69'	26.3	E-isoeugenol (TMS)	Lig	236, 221, 206, 179, 73
70'	26.5	4-hydroxybenzoic acid (2TMS)	Lig	282, 267, 223, 193, 151, 135, 73
71'	26.8	2-(4-hydroxyphenyl)-ethanol (2TMS)	Aro	282, 267, 193, 179, 149, 103, 73
72'	26.9	1,6-anhydro-D-galactopyranose (2TMS)	Car	204, 189, 161, 145, 129, 101, 73
73'	27.0	4-phenyl-6-hydroxyhexanal (TMS)	Aro	264, 249, 174, 146, 131, 119, 91, 73
74'	27.1	4-vinylsyringol (TMS)	Lig	252, 237, 222, 179, 73
75'	27.2	2-hydroxymethyl-5-hydroxy-2,3-dihydro-(4H)-pyran-4-one (2TMS)	Car	288, 273, 183, 155, 147, 129, 73
76'	27.5	5-hydroxy-6-oxohexanoic acid (2TMS)	Oth	290, 275, 247, 203, 157, 147, 129, 116, 101, 75, 73
77'	27.8	1,4-anhydro-β-D-glucopyranose (2TMS)	Car	217, 157, 145, 129, 103, 73
78'	27.9	4-hydroxy-6-oxohexanoic acid (TMS)	Oth	290, 275, 247, 203, 157, 147, 129, 116, 101, 73
79'	28.0	1,2,4-trihydroxybenzene (3TMS)	Aro	342, 327, 239, 73
80'	28.2	1,6-anhydro-β-D-glucopyranose (2TMS)	Car	217, 204, 191, 147, 129, 116, 101, 73
81'	28.8	3-hydroxy-2-hydroxymethylcyclopenta-2,4-dienone (2TMS)	Car	270, 255, 133, 73
82'	29.1	1,3,5-trihydroxybenzene	Aro	342, 327, 268, 147, 133, 73
83'	30.1	1,4-anhydro-β-D-galactopyranose (3TMS)	Car	332, 243, 217, 191, 157, 147, 129, 117, 103, 73
84'	30.7	2,3,5-trihydroxy-(4H)-pyran-4-one (3TMS)	Car	360, 345, 330, 270, 255, 147, 133, 103, 73
85'	30.7	Propenylsyringol (TMS)	Lig	266, 251, 236, 205, 73
86'	31.0	1,6-anhydro-β-D-glucopyranose (3TMS)	Car	333, 217, 204, 147, 129, 73
87'	31.2	1,4-anhydro-β-D-glucopyranose (3TMS)	Car	332, 217, 204, 191, 157, 147, 73
88'	31.6	Acetosyringone (TMS)	Lig	268, 253, 238, 223, 193
89'	32.0	1,6-anhydro-β-D-glucofuranose (3TMS)	Car	319, 243, 217, 191, 147, 116, 73
90'	32.5	Arabinoic acid γ-lactone (4TMS)	Car	292, 246, 205, 147, 129, 103, 73
91'	33.0	Z-coniferyl alcohol (TMS)	Lig	252, 235, 221, 204, 181, 162, 131, 103, 73
92'	33.0	3-vanillylpropanol (2TMS)	Lig	326, 311, 236, 221, 206, 179, 149, 73
93'	33.2	Z-confieryl alcohol (2TMS)	Lig	324, 309, 293, 235, 219, 204, 73
94'	34.3	E-coniferyl alcohol (2TMS)	Lig	324, 309, 293, 235, 219, 204, 73
95'	34.4	Palmitic acid	Oth	256, 213, 185, 171, 157, 129, 115, 83, 73, 60

96'	34.5	Z-synapyl alcohol (2TMS)	Lig	354, 339, 323, 293, 265, 234, 204, 73
97'	34.6	Gallic acid (4TMS)	Aro	458, 443, 355, 281, 179, 147, 73
98'	34.7	3,4-dihydroxycinnamyl alcohol (3TMS)	Lig	382, 355, 293, 205, 179, 147, 73
99'	35.1	Palmitic acid (TMS)	Oth	328, 313, 145, 132, 129, 117, 75, 73
100'	35.3	E-synapyl alcohol (2TMS)	Lig	354, 339, 323, 293, 265, 234, 204, 73
101’	35.5	Linoleic acid	Oth	280, 123, 109, 95, 81, 67, 55
102’	35.6	Oleic acid	Oth	264, 125, 111, 97, 83, 69, 55
103’	36.1	Linoleic acid (TMS)	Oth	352, 337, 145, 132, 129, 117, 75, 73
104’	36.2	Oleic acid (TMS)	Oth	354, 339, 145, 132, 129, 117, 75, 73

3.2.1 Black pepper: Both chromatograms obtained for black pepper are displayed in Figure 3. The chromatogram of the first shot can be roughly divided into three regions. The first region, from 8 to 13 min, showed peaks with mass spectra that could be ascribed to monoterpenes. The main peaks in this region were attributed to sabinene, D-3-carene and D-limonene (#10, 14 and 16). These compounds were already addressed in previous publications as the main monoterpenes in the headspace of black pepper [9]. The second region, from 14 to 20 min, was dominated by peaks that could be attributed to sesquiterpenes. The main peak in this region was assigned to β-caryophyllene (#56), which is a known major component of the aroma of black pepper [36,37]. The last region, from 20 to 35 min, showed peaks that were attributed to compounds with high boiling points. Due to their low volatility, these compounds are not usually found in headspace analysis of black pepper. Two groups of compounds can be distinguished in this region. The first group is composed by piperine and its derivates. Piperine (#122), which is a characteristic alkaloid of black pepper, provided the highest peak in the chromatogram at 31.6 min. Two peaks belonging to piperine isomers were also found at lower pyrolysis times (#116 and 117). It is likely that these compounds correspond to piperine molecules with different double-bond configurations. In addition, five piperine derivates were also identified (#113, 121, 123, 124 and 125). These compounds have been recently investigated for their nutraceutical properties [38]. The second group is composed by long-chain, poly-unsaturated N-isobutylamides (#100, 103, 119 and 120). Aliphatic amides have already

been isolated from black pepper, and they have also been investigated for their potential biological activity [39,40].

The chromatogram of the second shot was richer than the one of the first shot, reflecting the complex pyrolysis mechanism of the solid matrix. The main peaks of the chromatogram belonged to small molecules, namely 2-hydroxypropanoic and hydroxyacetic acid (#2' and 4'). 2-hydroxymethylfuran (#7') also showed high peak intensity. Peak heights tended to decrease with the increase of retention time, suggesting that the pyrolysis conditions used in our experiments favoured an extensive degradation of the substrate and a high yield of the lightest products. Anhydrosugars (#60', 61' and 80') provided the highest peaks at high retention times, while the peaks of lignin monomers were very low, suggesting a high carbohydrates content in comparison with the lignin content.

3.2.2 Cinnamon: The chromatograms obtained for cinnamon are displayed in Figure 4. The chromatogram of the first shot was dominated by the peak of cinnamaldehyde (#41). The top of this peak has been cut from Figure 4 to ease the labelling of the other peaks. The second highest peak belongs to coumarin (#54). Coumarin is a known component of a specific variety of cinnamon, *Cinnamomum aromaticum*, also known as cassia, while *Cinnamomum verum* (true cinnamon) contains only trace amounts of coumarin [26]. *C. aromaticum* has a lower price than *C. verum*, and is replacing true cinnamon in the food market. An interesting publication regarding the Italian market of cinnamon showed that a significant percentage of the commercially available cinnamons are either pure *C. aromaticum*, or a mixture of the two varieties [26]. The commercialization of *C. aromaticum* has raised concerns due to its high content in coumarin, which has been shown to possess cytotoxic properties [41]. The maximum coumarin content allowed in food has been regulated in the European Union since 2008 [42]. This result suggests that analytical pyrolysis-GC/MS could be used as a fast screening tool to detect the presence of coumarin in cinnamon.

316 The chromatogram of the second shot of cinnamon provided the most complex profile among all
317 samples. In addition to the characteristic peaks of lignocellulose that were also found in black
318 pepper, cinnamon showed a high yield of aromatic compounds. 1,2-dihydroxybenzene (#18') was
319 the highest peak in the pyrogram. High peaks of arabinofuranose and anhydrosugars (#49', 72' and
320 80') were also detected, suggesting that, as for black pepper, the holocellulose content of this
321 sample is higher than the lignin content. The chromatogram also showed a peak with significant
322 intensity that was attributed to 1,3,5-trhydroxybenzene (#82'). This compound has been reported as
323 a marker for condensed tannins in lignocellulose [20], and in fact catechin oligomers have been
324 extracted from cinnamon in previous studies [43].

325 **3.2.3 Ginger:** The chromatograms of ginger are displayed in Figure 5. As with black pepper, the
326 chromatogram of the first shot can be divided in two regions. In the first region, up to 20 min, the
327 characteristic volatile compounds of ginger are eluted. The main peaks in this region were attributed
328 to ar-curcumene, zingiberene, α-farnesene, β-bisabolene and β-sesquiphellandrene (#59, 63, 65, 68
329 and 69), in agreement with literature results [10,14]. The second region showed another group of
330 peaks, which were attributed to gingerol (#110) and its derivatives. These derivatives of gingerol are
331 obtained during heating, drying or long-term storage of ginger [27]. The main peak in this region was
332 attributed to (6)-shogaol (#108), which is obtained by dehydration of gingerol. (8)-shogaol and (10)-
333 shogaol (#111 and 115), which are homologues of (6)-shogaol, were also among the main peaks in
334 this region. The presence of these compounds in the chromatogram is most likely due to
335 dehydration reactions that took place in the pyrolysis cup during the desorption step at 250 °C.

336 The chromatogram of the second shot showed a similar profile to the one of black pepper, with high
337 peaks at low retention times belonging to small molecules (#2' and 4') and to 2-hydroxymethylfuran
338 (#7'). Ginger showed particularly high yields of 4-hydroxy-5-oxopentanoic acid and 3-hydroxy-4-
339 oxpentanoic acid (#64' and 66'). The high yields of these compounds could be associated with the
340 high content of pentoses in ginger root, which can be up to 7.6% [44].

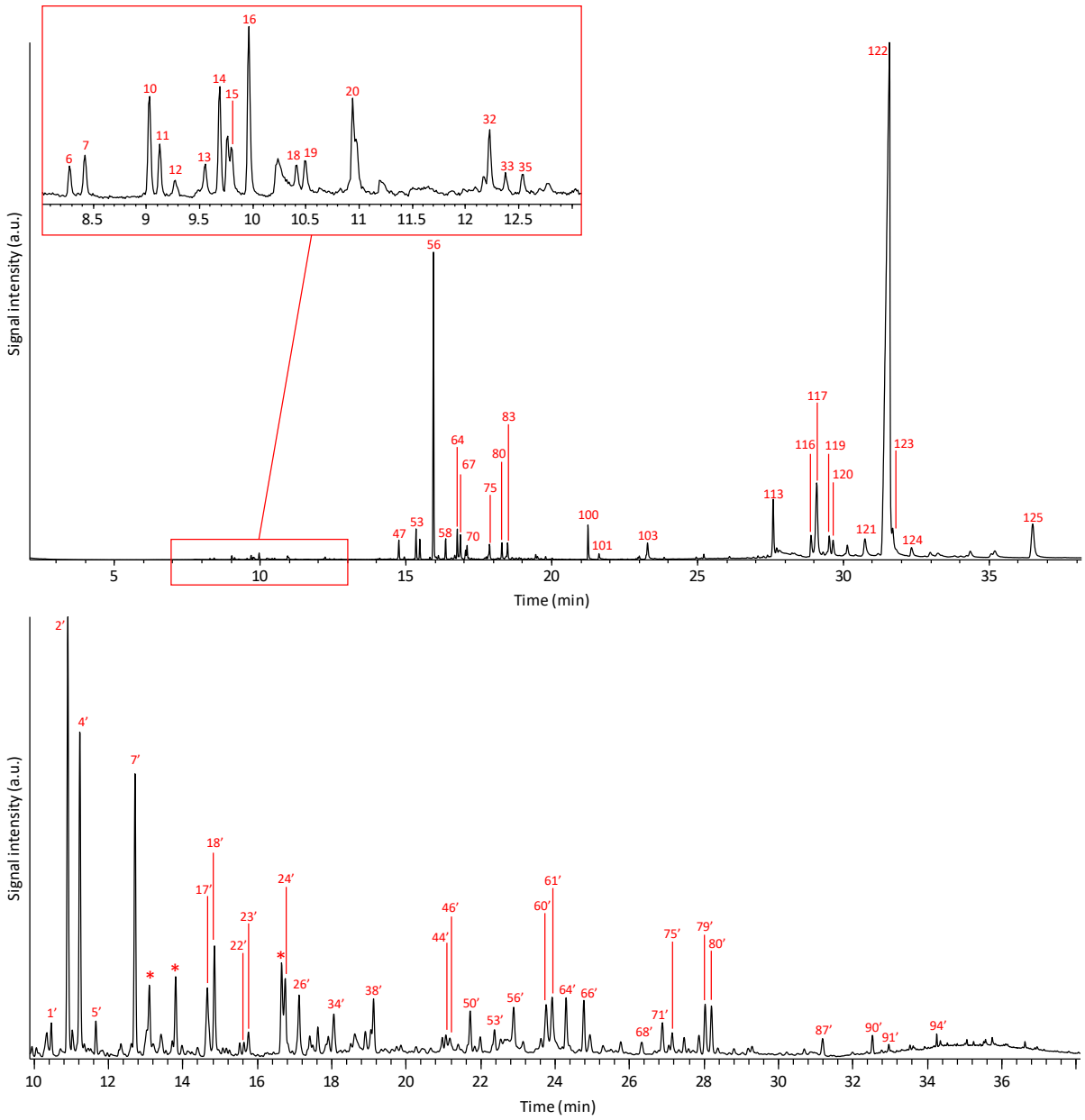
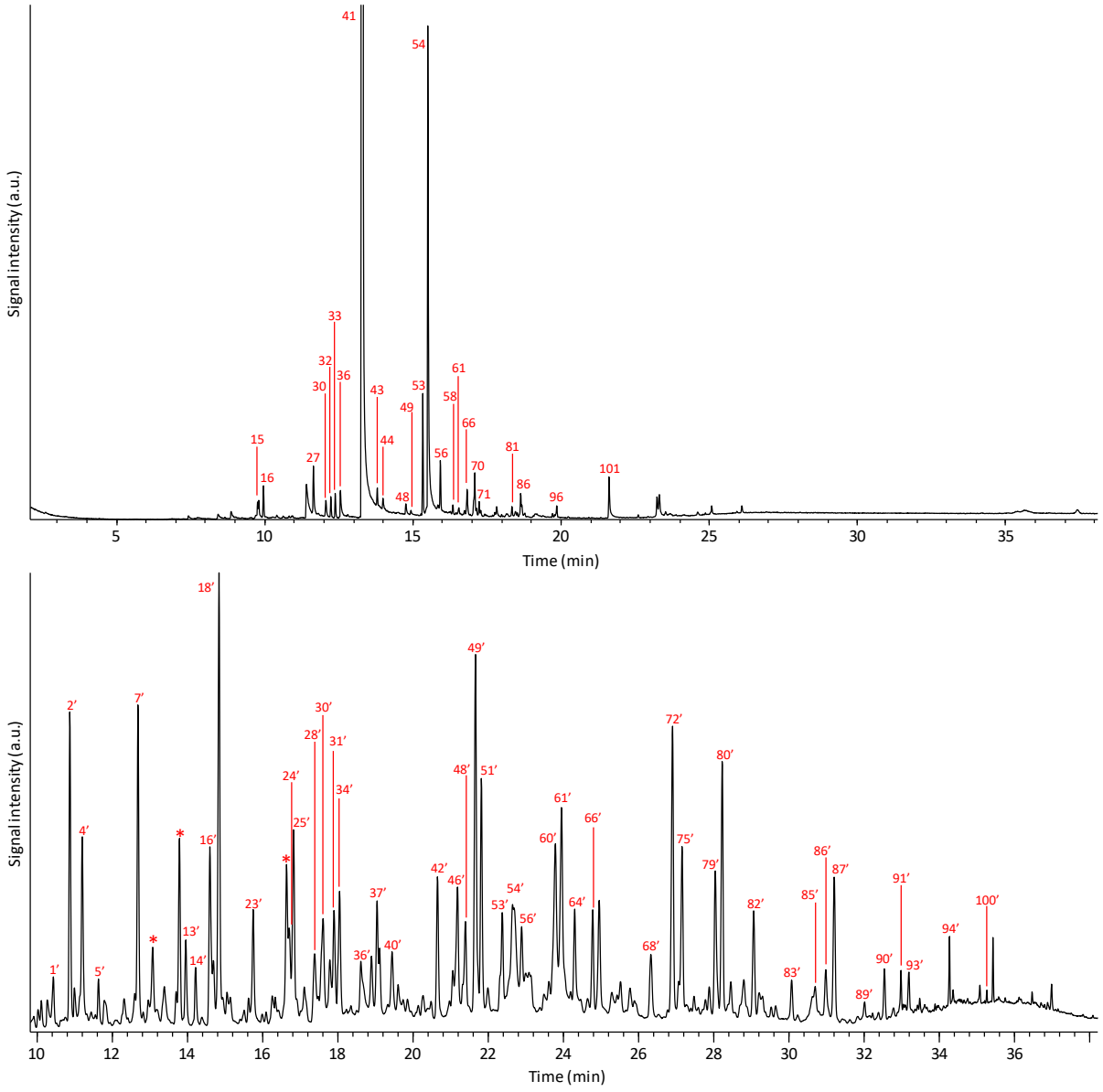


Figure 3: Chromatograms for the first shot (top) and second shot (bottom) of black pepper. The main peaks are labelled according to Table 2 (top) and Table 3 (bottom). Peaks labelled with an asterisk are side-products of the derivatisation process.



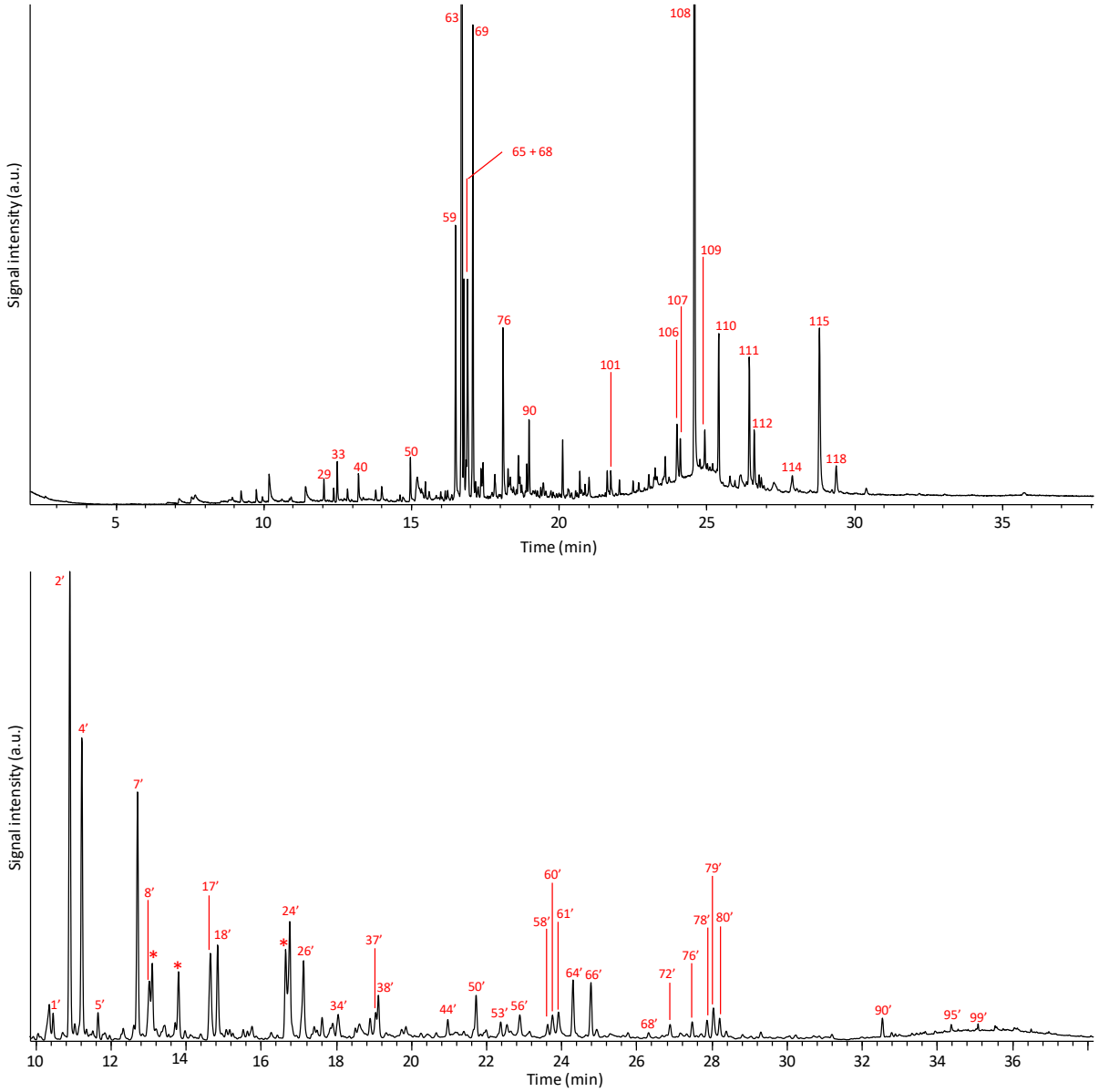
347

348 ***Figure 4:*** Chromatograms for the first shot (top) and second shot (bottom) of cinnamon. The main

349 peaks are labelled according to Table 2 (top) and Table 3 (bottom). Peaks labelled with an asterisk

350 are side-products of the derivatisation process.

351



352

353 ***Figure 5:*** Chromatograms for the first shot (top) and second shot (bottom) of ginger. The main peaks

354 are labelled according to Table 2 (top) and Table 3 (bottom). Peaks labelled with an asterisk are side-

355 products of the derivatisation process.

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3.2.4 Turmeric: The chromatograms of turmeric are displayed in Figure 6. The chromatogram of the first shot was dominated by peaks belonging to sesquiterpenoids. The three main peaks were attributed to ar-turmerone, turmerone and β-turmerone (#84, 85 and 90). Characteristic peaks were also found at lower retention times, belonging to ar-curcumene, zingiberene and β-sesquiphellandrene (#59, 63 and 69) as well as at higher retention times, belonging to bisabolone, atlantone and dehydroturmerone (#92, 95 and 98). These results agree with the available literature on headspace analysis of turmeric [6,45].

The profile in the second chromatogram was very similar to the one of ginger. This was expected as both these samples are obtained from the root of two plants of the same family (*Zingiberaceae*), and so they are likely to show similar composition for the solid matrix. However, the intense peak of guaiacol (#3') at low retention times suggests that the lignin content of turmeric is slightly higher than that of ginger.

3.2.5 Saffron: The chromatograms of saffron are displayed in Figure 7. The chromatogram of the first shot was dominated by the peak of safranal (#34). High peaks of hexadecenoic acid and linoleic acid (#101 and 102) were also detected at high retention times. Some peaks were found that could be attributed to pyrolysis products of holocellulose, including 2-hydroxymethylfuran, 2-(5H)-furanone, 2-hydroxycyclopenten-2-one and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (#3, 4, 5 and 25). These compounds are likely obtained from a partial pyrolysis of carbohydrates at 250 °C, which is consistent with the EGA/MS profile showing an overlap of the desorption peak of safranal and the pyrolysis peak of the solid matrix. The other peaks in the chromatogram were attributed to safranal derivates. Interestingly, no peak was found that could be attributed to crocin and crocetin, which are known carotenoids responsible for the colour of saffron flowers [46-48]. It is possible that these compounds underwent degradation at the desorption temperature.

The chromatogram of the second shot provided the same peaks belonging to holocellulose and lignin of the other samples, but with very low abundances. This result is consistent with the higher

386 weight ratio of volatile compounds in saffron and cloves that was found from the EGA-MS
387 investigations. As the amount of solid sample was lower, the excess of derivatising agent was higher,
388 and the peaks belonging to side-products of the derivatisation process showed high intensities.
389 Another difference of this chromatogram with those of the other samples is the presence of peaks
390 belonging to aromatic compounds, including dimethylbenzaldehyde and dimethylnaphthalene (12'
391 and 47'). The presence of these compounds supports the hypothesis of secondary pyrolysis reactions
392 being favoured in this sample, as already hypothesised from the EGA/MS results.

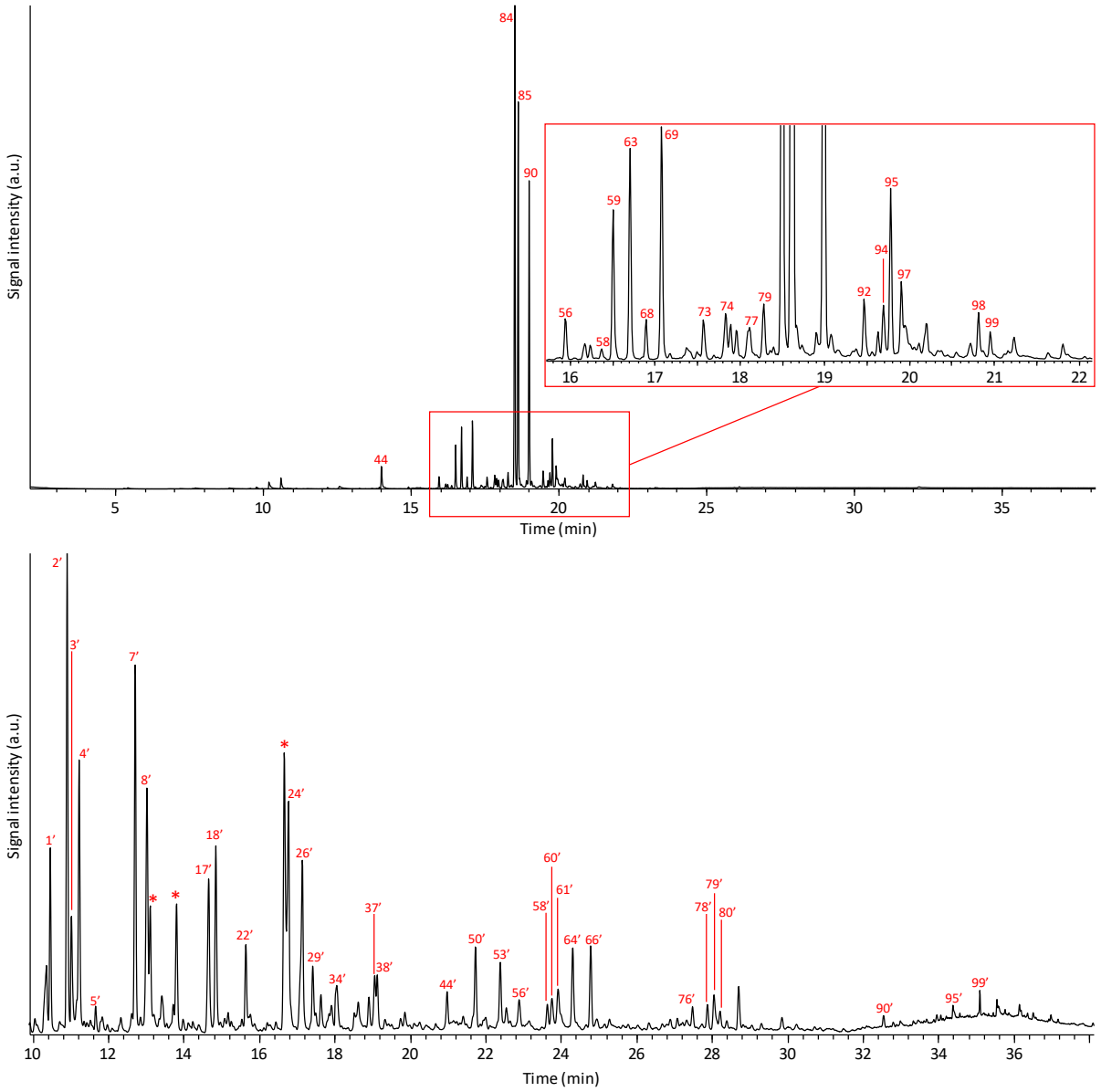
393 Finally, high peaks of fatty acids were detected in this sample at long retention times. Palmitic,
394 linoleic and oleic acids were found both in their underivatized and derivatized forms (#95', 99', 101',
395 102', 103' and 104'). This result agrees with the available literature [49], in which linoleic, linolenic
396 and palmitic acids were found to be the main fatty acids in saffron.

397 **3.2.6 Cloves:** The chromatograms of cloves are displayed in Figure 8. The chromatogram of the first
398 shot contained the least number of peaks among all the observed spices. Two of the main peaks in
399 the chromatogram were attributed to eugenol and eugenol acetate (#46), in agreement with
400 literature results showing eugenol as one of the main components of cloves essential oil [9,37,50].
401 The other main peak in the chromatogram was attributed to β-caryophyllene, which has also been
402 reported in the literature [9]. The other peaks in the chromatogram belonged to minor
403 sesquiterpenoids.

404 The chromatogram of the second shot provided similar result to that of saffron, as the weight
405 fraction of volatiles in these two spices were similar. The most interesting find in this chromatogram
406 was gallic acid (#97'), which is a marker for the presence of hydrolysable tannins [20].

407 Surprisingly, no peak was found that could be attributed to oleanolic acid, although its *m/z* signals
408 were detected in the EGA thermograms. This could be due to either the compound undergoing
409 pyrolysis, or to its retention being inefficient.

410



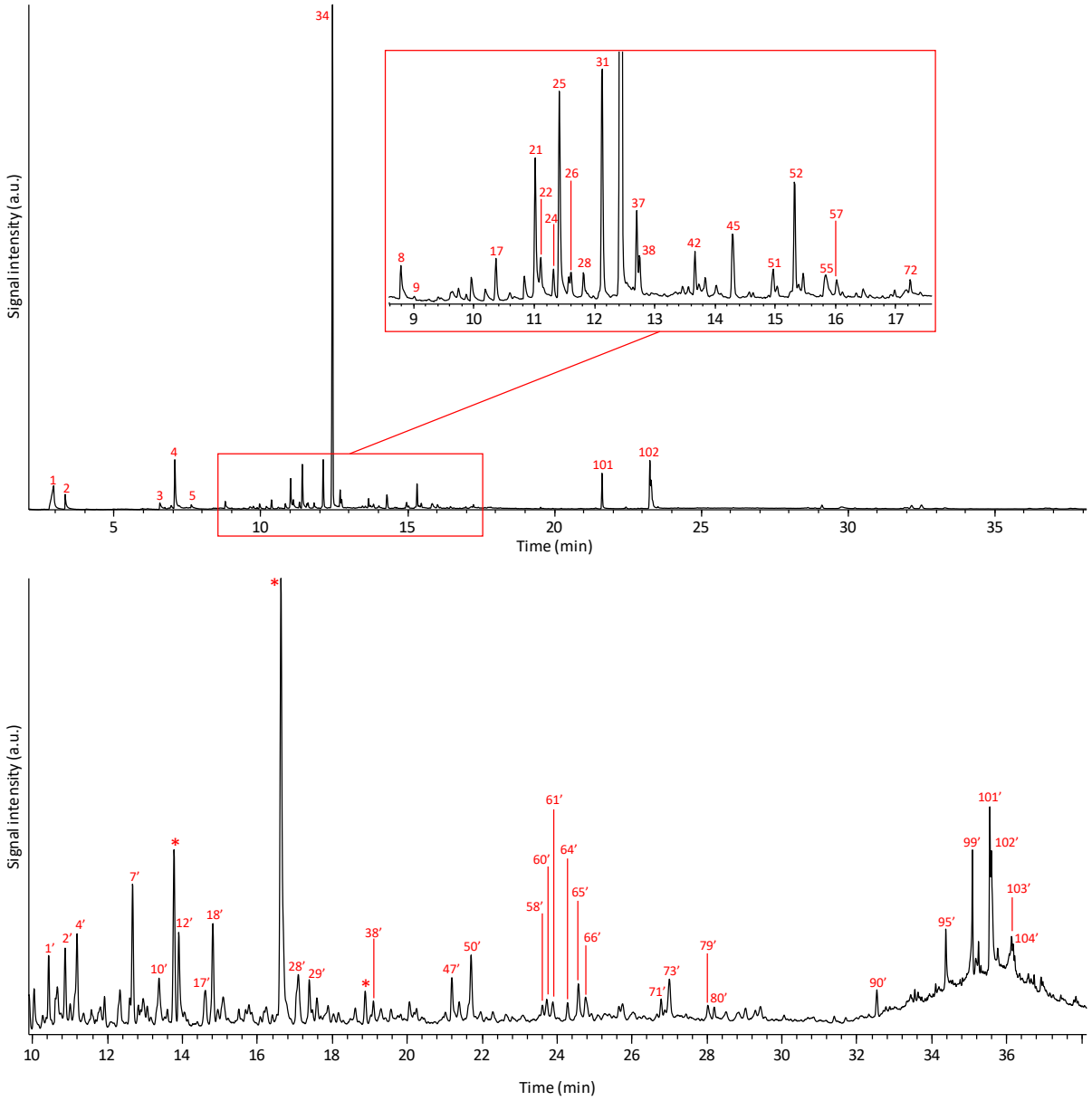
411

412 ***Figure 6:*** Chromatograms for the first shot (top) and second shot (bottom) of turmeric. The main

413 peaks are labelled according to Table 2 (top) and Table 3 (bottom). Peaks labelled with an asterisk

414 are side-products of the derivatisation process.

415



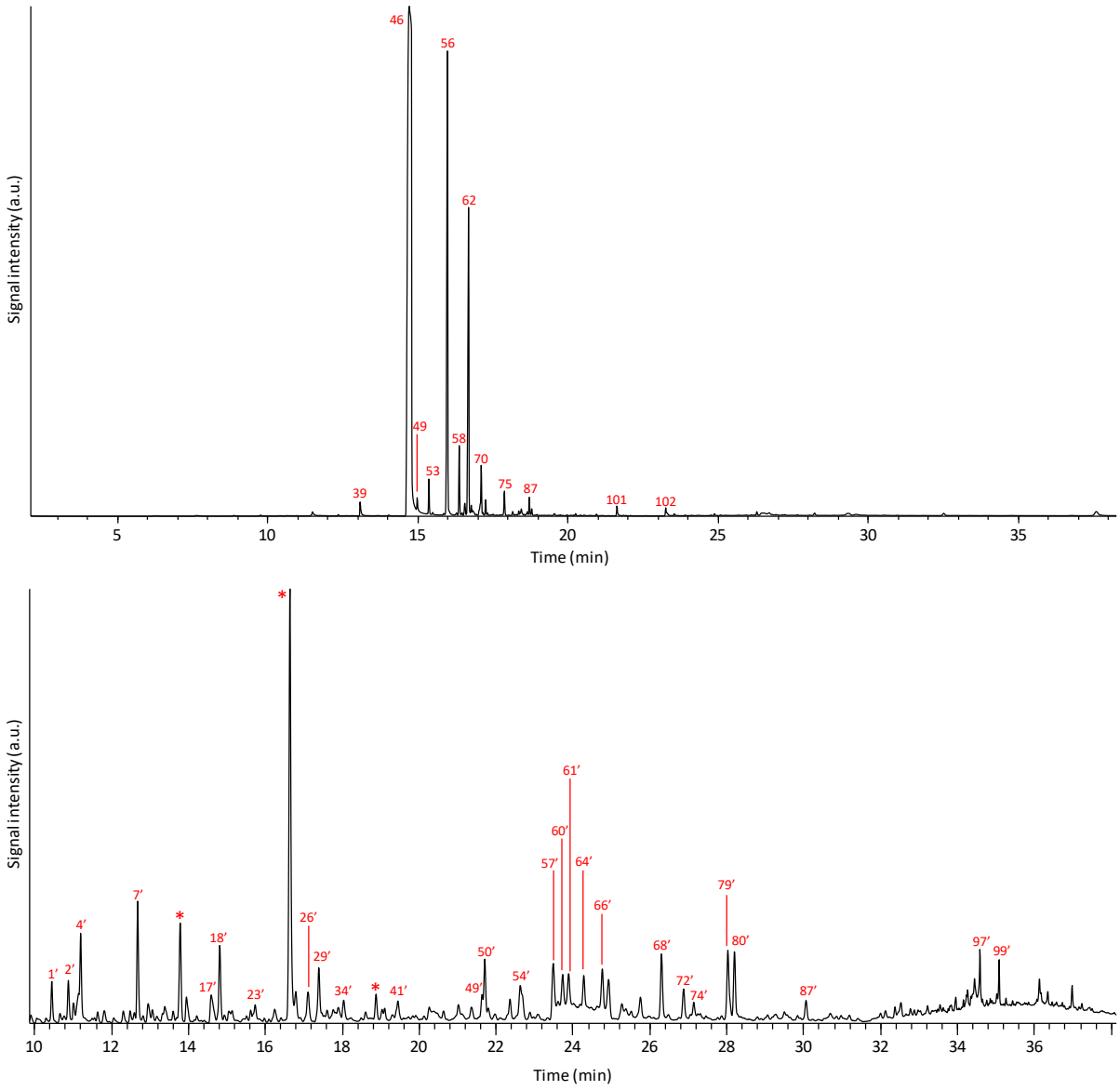
416

417 ***Figure 7:*** Chromatograms for the first shot (top) and second shot (bottom) of saffron. The main

418 peaks are labelled according to Table 2 (top) and Table 3 (bottom). Peaks labelled with an asterisk

419 are side-products of the derivatisation process.

420



421

422 ***Figure 8:*** Chromatograms for the first shot (top) and second shot (bottom) of cloves. The main peaks

423 are labelled according to Table 2 (top) and Table 1 (bottom). Peaks labelled with an asterisk are side-

424 products of the derivatisation process.

425

426 **3.2.7 Category yields:** To obtain additional information on the composition of both the volatile and

427 non-volatile fractions of the six spices, percentage category yields were calculated for the first and

428 second shot chromatograms by adding together the percentage areas of compounds belonging to

429 the same category. Figure 9 shows the results.

Non-terpenoid compounds were the main categories in the chromatogram obtained after the first shot of pyrolysis of black pepper, cinnamon and cloves, as high yields of characteristic components (piperine, cinnamaldehyde and eugenol respectively) were detected in these spices. A high yield of non-terpenoids was also observed for ginger, due to the presence of shogaol, and in saffron, due to the peaks belonging to pyrolysis products of carbohydrates.

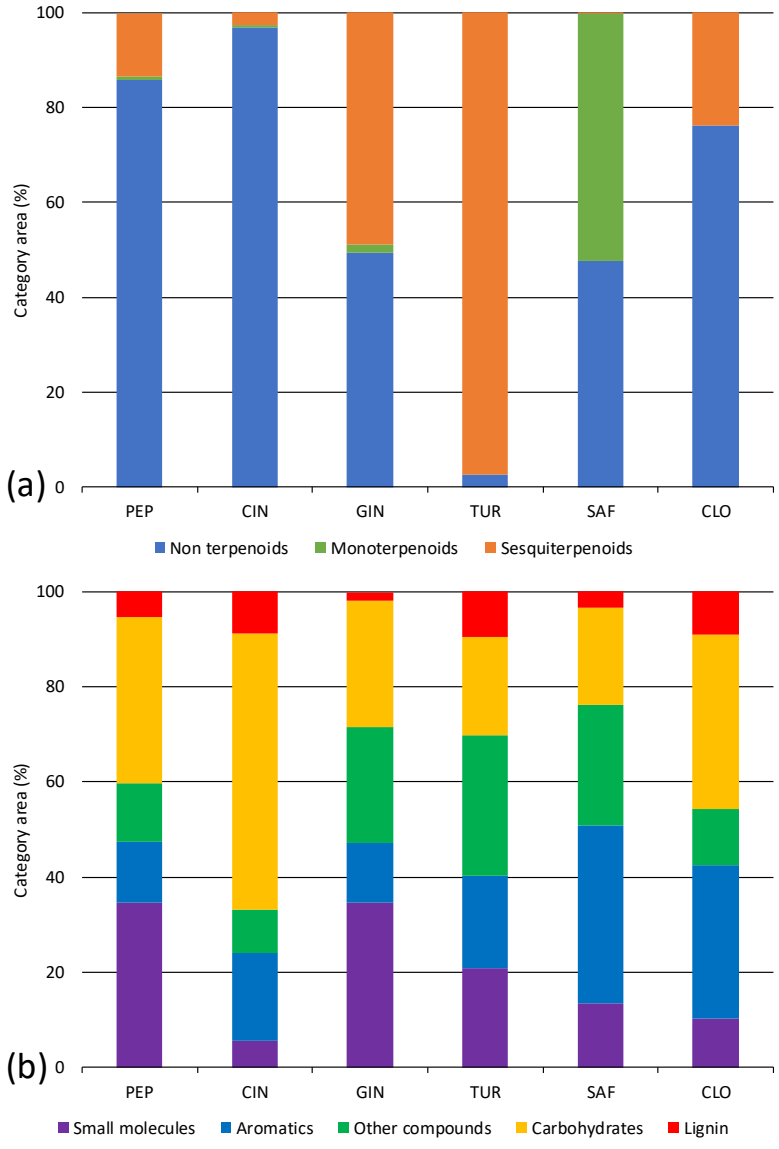


Figure 9: Percentage category yields for (a) the first shot and (b) the second shot of all spice samples.

Monoterpenoids showed low category yields in most of the spices, the only exception being saffron in which safranal was the most abundant peak. Sesquiterpenoids were the main components in the chromatogram of turmeric, as turmerones provided the most abundant peaks. Monoterpenoids provided lower yields than sesquiterpenoids in all spices except saffron. This result is in apparent contrast with the data available in the literature regarding the headspace analysis of the same spices, especially black pepper, turmeric and cloves, in which the relative amount of monoterpenoids is higher [6,9]. However, this discrepancy is due to the intrinsically different experimental conditions. SPME uses higher sample amounts, and the absorption efficiency of each compound on the solid phase depends on its volatility at the sampling temperature, which is usually 60 °C or lower. For this reason, SPME-GC/MS chromatograms will be richer in the most volatile compounds. On the other hand, desorption in the present work is performed on a lower sample amount, and the high temperature ensures that the evolution of the volatiles is quantitative. This is confirmed by the absence of volatile compounds in the second-shot chromatograms. We can conclude that, while SPME provides reliable information on the headspace composition, thermal desorption-GC/MS provides information on the content of volatiles in the bulk of the sample. The two techniques provide complementary information, and they are both required to achieve a detailed knowledge on the composition of a spice sample.

Additional information can also be obtained from the composition of the second shot chromatograms. Carbohydrates pyrolysis products showed significant yields in all samples, especially in cinnamon, where they accounted for more than 50% of the total chromatogram area. An interesting result was obtained from the comparison of ginger and turmeric. Although both samples were obtained from the roots of a plant of the *Zingiberaceae* family, their composition was significantly different. Turmeric showed a higher content in lignin pyrolysis products, while ginger was richer in low-molecular weight pyrolysis products. Finally, a high yield of aromatic compounds was obtained from both saffron and ginger.

4. CONCLUSIONS

The present work shows the results of an extensive study of spices pyrolysis. Preliminary analysis by EGA-MS allowed us to establish 250 °C as the discriminating temperature between thermal desorption of light compounds, and pyrolysis of the heavier compounds. Using this result, double-shot analytical pyrolysis experiments were designed to obtain information on both fractions separately.

The chromatograms obtained from the first shot provided peaks belonging to the most abundant terpenoids, as well as the most characteristic components of all six spices. In the case of black pepper and ginger, additional compounds that are not usually detected in headspace analyses were found at high retention times. As previously discussed, these results demonstrate that SPME and thermal desorption-GC/MS provide complementary results.

The chromatograms of the second shot provided peaks that could be attributed to carbohydrates and lignin, as well as to additional compounds such as polyphenol pyrolysis products and fatty acids. Each spice provided a characteristic set of percentage yields both in the first and second chromatograms.

We believe that both the qualitative and semi-quantitative data obtained from double-shot analytical pyrolysis-GC/MS could be used in the future as additional tools for authentication studies, while retaining the same advantages of SPME such as no sample preparation and short analysis time.

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