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ABSTRACT

The first aim of our study was to improve characterisation of the volatile fraction of aromatic caramel by applying heart-cutting multidimensional gas chromatography coupled to mass spectrometry and olfactometry (MDGC–MS–O) on targeted odorant fractions. The second aim was to compare the volatile composition of two caramel samples, which differed in terms of their carbohydrate composition and cooking process. MDGC analyses enabled identification of 37 compounds (17 with the addition of pure standard) in the burnt sugar caramel, 20 of which were reported for the first time in caramel. Fifteen compounds were identified as odour-active and described using a range of attributes such as *floral*, *roasted*, *spicy* and *almond*. Furans, lactones and acids resulting from the thermal breakdown of sugars predominated in the volatile fraction of the burnt sugar caramel, due to the harsher cooking conditions. Finally, these results have enabled a clearer understanding of aromatic caramel as well as the identification of new compounds which might make an important contribution to its aroma.

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1. Introduction

Aromatic caramel results from a non-enzymatic browning reaction that involves the dehydration of sugars during heat treatment under specific conditions, either dry or in a concentrated solution, either alone or with additives. The non-volatile fraction of caramel accounts for 90–95% of the mass and is composed of high molecular weight compounds such as oligosaccharides and brown polymers. The remaining 5–10% of the mass represents the volatile fraction which is made up of hundreds of low molecular weight compounds mainly responsible for the typical caramel odour. Because odour is one of the main factors that contributes to consumer acceptability of food products, the identification of volatile compounds is an important concern for the food industry.

Volatile compounds obtained from the thermal degradation of carbohydrates were studied extensively in the 1960s (Fagerson, 1969) but surprisingly, only a few more recent studies have dealt with caramel itself (Cottier, Descotes, Neyret, & Nigay, 1989;

Goretti, Liberti, & Dipalo, 1980; Pons, Garrault, Jaubert, Morel, & Fenyo, 1991). In 2012, the composition of the volatile odorant fraction of four aromatic caramels was investigated using gas chromatography coupled either to olfactometry (GC–O) or mass spectrometry (GC–MS) (Paravisini et al., 2012). Seventy-six zones on the chromatograms appeared to be odour-active according to the GC–O study. Unfortunately, only 49 odorant compounds could be identified by GC–MS analysis, due to the complexity of the caramel volatile fraction which cannot be solved with one-dimensional GC analysis. The number of compounds was too high for a single column peak capacity so the separation was highly compromised. Moreover, the presence of numerous oxygenated heterocyclic compounds such as furans and furanones exhibiting similar interactions with the column stationary phase, led to numerous co-elutions, so that the perceived odour could not be related to a single identified compound.

Multidimensional gas chromatography (MDGC) could be an appropriate technique to overcome identification issues in complex food samples such as caramel. MDGC enables the selection of a targeted fraction on the first dimension. The fraction is then re-separated on a second column that exhibits a different stationary phase. Another advantage of this technique is the use of

cryo-trapping between the two dimensions that can enhance sensitivity by focusing analytes prior to the second separation (Tranchida, Sciarrone, Dugo, & Mondello, 2012).

During the past ten years, the MDGC technique has been applied successfully to the aroma characterisation of complex samples such as alcoholic beverages (Mac Namara, Howell, Huang, & Robbat, 2007), coffee (Miyazato, Nakamura, Hashimoto, & Hayashi, 2013) and fruits (Darriet et al., 2002). However, to the best of our knowledge, the application of heart-cutting MDGC to the volatile fraction of aromatic caramel has not yet been reported.

The principal purpose of this study was therefore to investigate the volatile composition of caramel by applying MDGC to targeted fractions according to the results of a previous GC/O study. As odour properties are closely related to the cooking properties of caramel, this study also focused on two samples which differed to a considerable extent in terms of their carbohydrate composition and cooking process.

2. Materials and methods

2.1. Samples and chemicals

Caramel samples were supplied by Nigay SAS (Feurs, France). Caramel A was a standard blond aromatic caramel prepared from sucrose. Caramel B was a burnt sugar type caramel prepared from a mixture of sucrose and glucose under harsher cooking conditions.

The analytical grade (>99% purity) dichloromethane used for the extraction procedure was purchased from Carlo-Erba (Val de Reuil, France) and was re-distilled prior use to ensure the highest possible purity. The chemical standards used to validate identification were purchased from Sigma–Aldrich (Saint Quentin-Fallavier, France).

2.2. Extraction of volatile compounds

Caramel samples (20 g) were mixed with 100 ml deionised water (Milli-Q®, Bedford, MA, USA). Two hundred microlitres of internal standard solution (pentan-2-ol, 78.5 ng μl^{-1} in water) were added to check the repeatability of the extraction procedure. This mixture was introduced into the SAFE (Solvent Assisted Flavour Evaporation) apparatus (Engel, Bahr, & Schieberle, 1999), and vacuum distillation (10^{-3} mbar) was performed for two hours. The thermostat of the head and legs of the apparatus was fixed at 35 °C. The same temperature was used to heat the distillation vessel (500 ml) by means of a water bath. The aqueous distillate was then extracted with dichloromethane (1×20 ml then 2×10 ml). The organic phase was collected, filtered through glass wool and dried over anhydrous sodium sulphate. The extract was concentrated to 400 µl with a Kuderna-Danish apparatus in a 70 °C water bath, and then to 50 µl under a nitrogen stream. Samples were stored at −20 °C prior to chromatographic analysis.

The caramel samples were extracted in triplicate and their repeatability was checked by GC–MS analyses prior to the MDGC experiments. After validation, the three extracts were pooled.

2.3. Multidimensional Gas Chromatography–Mass Spectrometry–Olfactometry (MDGC–MS–O)

The separations were performed on a MDGC system equipped with two independent gas chromatographs. Columns were interconnected by means of a Gerstel multi-column switching device (MCS, Gerstel, Germany).

The ^{1}D separation device was a HP 6890 chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a Flame Ionisation Detector (FID) system kept at 250 °C and a polar DB-Wax

fused silica column (30 m length × 0.32 mm i.d., 0.5 µm film thickness, Agilent J&W, USA). Sample injections of 2 µl were made using a 7683 Series autosampler (Agilent Technologies, Santa Clara, USA). Injections were carried out in a Split/Splitless injector kept at 250 °C. The split valve was opened for 30 s after the injection, with a purge flow of 44 ml min^{-1}. Helium was used as the carrier gas at a constant flow of 1.5 ml min^{-1}. The ^{1}D GC oven temperature started at 40 °C and was then raised at 3 °C min^{-1} up to 240 °C and kept isothermal for 10 min. The ^{1}D column outlet was connected to the ^{2}D system by means of the MCS switching device. A deactivated fused silica column transferred 10% split from the MCS device to the FID in order to monitor the eluent from the ^{1}D column. Additional permanent inlet and outlet capillary lines connected the MCS device to a mass flow controller and a pressure regulator module (EPC) which provided a constant inlet flow of 10 ml min^{-1} through the MCS device, thus allowing the heart-cut procedure. This counter-current flow was switched off during the transfer of a selected heart-cut in the ^{2}D system. Heart-cut fractions were trapped in the first 50 cm of the ^{2}D column across a cryo trap system (CTS, Gerstel) and then cooled at −150 °C with liquid nitrogen. After each heart-cut, the current flow was switched on and the CTS was heated to 250 °C at a rate of 20 °C s^{-1}. The EPC module ensured programmed pressure at the head of the ^{2}D column in order to maintain a constant flow of 1.5 ml min^{-1}.

The ^{2}D system was an HP 7890A chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a non-polar DB-5MS fused silica column (30 m length × 0.32 mm i.d., 0.5 µm film thickness, Agilent J&W, USA). The ^{1}D GC oven temperature started at 40 °C, and was then increased to 240 °C at a rate of 4 °C min^{-1}. The eluent of the ^{2}D column was split 2:1 between an olfactometric detection port (ODP3, Gerstel, Germany) and a 5975C mass spectrometric detector (Agilent Technologies, Santa Clara, USA). The ODP transfer line was kept at 250 °C. An additional humidified air flow was added to the ODP for the comfort of the panellists. Indeed, two experienced panellists who had participated in the previous GC–O analyses were asked to give a qualitative description when an odorant was perceived. The MS conditions were: transfer line at 150 °C, ion source at 250 °C and EI voltage at 70 eV. Data were recorded in full scan mode (*m/z* range: 29–350 amu).

2.4. Selection of the heart-cutting fractions

Based on a previous GC–O study performed on the same caramel samples (Paravisini et al., 2012), relevant odorant zones for which no compound had been identified because of co-elution or a poor FID signal were selected. The 12 heart-cutting fractions selected, their corresponding odour descriptions and their detection frequencies are shown in Table 1. For each fraction, the heart-cutting times (start and end) were based on the retention indexes (RI) of the GC–O study on the same DB-Wax fused silica column.

2.5. Identification of compounds

Compounds were identified on the basis of their RI on the DB-Wax and DB5MS columns, their mass spectra and the injection of pure standard when available. A series of n-alkanes (C_{10}–C_{30}) was analysed to establish RI values using the Van Den Dool and Kratz formula (Van den Dool & Kratz, 1963), which were compared with data from the literature. The mass spectra were compared with those from three databases: NIST 2.0, WILEY 138 and INR-AMASS (internal database developed using standard compounds).

Table 1
Summary of the targeted fractions of the ^{1}D chromatogram according to the previous GC/O study for caramel samples A and B.

Heart-cut No	RI[a] Start	RI[a] End	Compound identified by GC/MS	Odour description GC/O	Detection frequency (%)[b] Caramel A	Detection frequency (%)[b] Caramel B
1	1290	1315	Octanal + 3-hydroxybutan-2-one	Citrus floral mushroom	39	17
2	1517	1541	Benzaldehyde + 3-methylcyclopent-2-en-1-one	Caramel roasted green	0	44
3	1573	1591	5-Methyl-2-furfural + 2-propionylfuran	Almond floral	0	83
4	1645	1682	Acetophenone + 2,5-dihydro-3,5-dimethylfuran-2-one	Coffee roasted	17	6
			2-Methylbutanoic acid + 3-methylbutanoic acid	Cheese	44	78
5	1698	1714	ni	Green roasted hazelnut	61	78
6	1729	1750	Pentanoic acid + cyclohex-2-en-1,4-dione[*]	Hazelnut chemical cheese	28	39
7	1830	1876	ni	Roasted	44	72
			ni	Caramel lactone	78	39
			ni	Chemical pharmaceutical	0	67
8	1905	1929	nd	Lactone	22	39
9	2070	2082	nd	Lactone floral	33	0
10	2158	2192	nd	Floral lactone spicy	72	61
			nd	Floral lactone roasted	11	39
11	2486	2512	5-(Hydroxymethyl)-2-furfural + dodecanoic acid	Animal sharp	22	28
12	2543	2608	nd	Chocolate vanilla	44	61
			nd	Caramel	50	17
			nd	Tobacco	11	11

ni: non identified.
nd: non detected.
[a] RI on DB-WAX column (30 m length × 0.32 mm i.d., 0.5 μm film thickness).
[b] Detection frequency obtained from GC/O analyses (Paravisini et al., 2012)
[*] Tentatively identified.

3. Results

The main objectives of this study were to investigate the composition of caramel by applying MDGC to targeted fractions and to identify volatile compounds by means of MS detection. In addition, in some cases, sensory detection by two panellists from the nine who had performed the GC–O analyses provided further insight into the potential odorant activity of the compounds.

Fig. 1 shows the ^{1}D chromatograms of the caramel extracts and the heart cutting times for the 12 selected fractions (Table 1). Six fractions corresponded to a co-elution of compounds (no 1, 2, 3, 4, 6 and 11) while the other six fractions corresponded to unsuccessful identifications on the first dimension.

Fraction no 1 was composed of an odorant zone which was defined as citrus/floral on ^{1}D GC–O, immediately followed by an odorant zone defined as mushroom (Table 1). 3-Hydroxybutan-2-one and octanal, which co-eluted on the polar phase, were identified separately on the non-polar phase. Our first hypothesis was that octanal might be responsible for the citrus/floral odour of the first targeted zone. However, MDGC analyses did not support
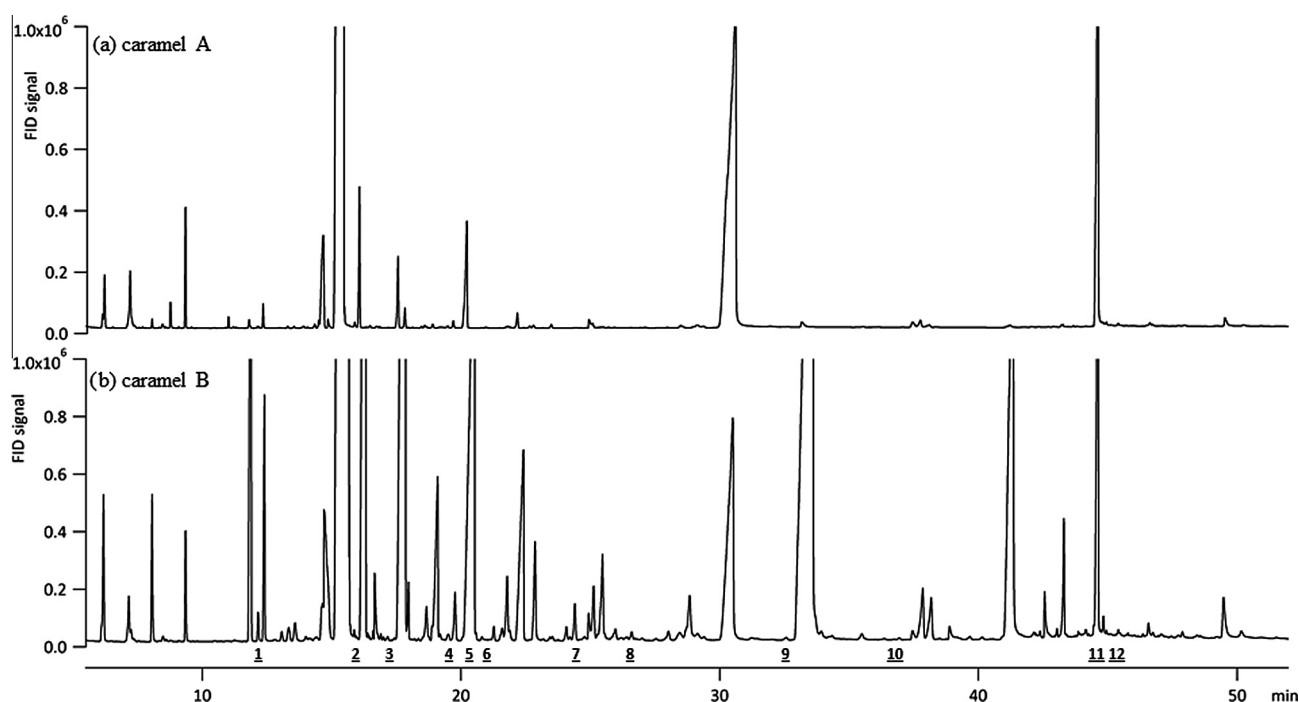

Fig. 1. ^{1}D FID chromatogram of the caramel extracts (a: blond caramel A; b: burnt sugar caramel B).
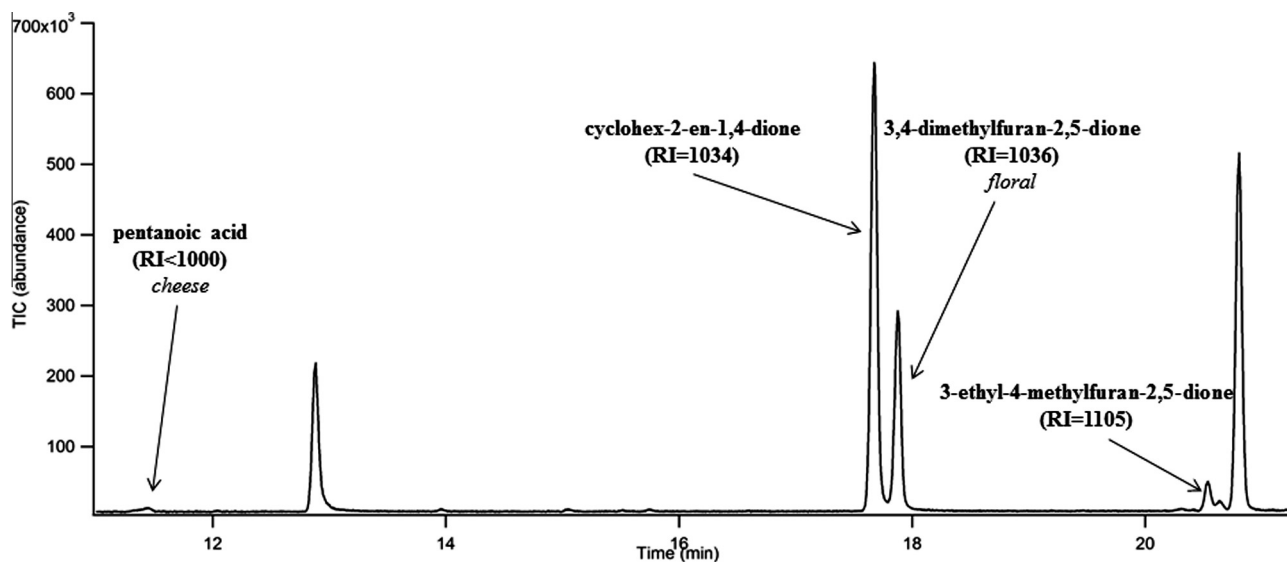
Fig. 2. ^{2}D MS chromatogram of the heart-cut fraction no 6 of the caramel B extract.

this idea as the citrus/floral odour was not reported by the panellists after the ^{2}D separation. It can be assumed that the odour activity of octanal was enhanced while in a mixture with 3-hydroxybutan-2-one but not sufficiently to be detected separately. This type of phenomenon might be due to the existence of perceptive interactions that impact the perception of an odorant compound as a function of context. The mushroom odour of the second targeted zone of fraction no 1 could be clearly associated with the presence of 1-octen-3-one, which was also identified by its mass spectrum.

Finally, another new compound was detected in fraction no 1, but its identification remains uncertain. Its mass spectrum showed a main ion at m/z 86 and two fragment ions at m/z 58 and 29 (26% and 21%, respectively, of the abundance of the main ion). This pattern suggests a short-chain carbonyl compound and the fragment ions suggest the loss of a carbonyl group. Such a structure is typical of the intermediate compounds in the formation pathway of furans during the thermal degradation of sugars, such as 3-hydroxy-2-butenal, which might be involved in the formation of furan from pentoses (Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008).

Fraction no 2 was characterised by a caramel/roasted odour associated with a co-elution of benzaldehyde and 3-methylcyclopent-2-en-1-one on ^{1}D GC–O (Table 1). MDGC analyses enabled the separation of 3-methylcyclopent-2-en-1-one and benzaldehyde and the tentative identification of 3-methylfurane-2,5-dione. According to the olfactometric data, the caramel/roasted odour could be attributed to the presence of 3-methylcyclopent-2-en-1-one.

In fraction no 3, 5-methyl-2-furfural was identified and described as having a nutty odour note, i.e. almond, vanilla and cinnamon. In addition, 2-propionylfuran and 2-acetyl-5-methylfuran were tentatively identified and the panellists reported a floral note for the latter. Here, MDGC analyses produced results that were consistent with the ^{1}D GC–O findings (Table 1) and also enabled the tentative identification of a new compound, 2-acetyl-5-methylfuran.

The heart-cutting of fraction no 4 led to the identification of six compounds, four of which were not identified by the ^{1}D analysis. The 2- and 3-methylbutanoic acids detected as a mixture from the first column were separated on the non-polar phase of the second dimension. After this non-polar separation, the cheese odour of the acids (Table 1) was not reported in MDGC analyses. As seen

in fraction no 1 with octanal, it can be assumed that the odour activity of 2- and 3-methylbutanoic acids was enhanced while in a mixture and was not sufficient if detected separately. 2,5-Dihydro-3,5-dimethylfuran-2-one was described as having a roasted/curry odour note, a description in line with the GC–O description of zone 4 in the GC–O analysis (Table 1). Two furans were also detected: 1-(2-furanyl)-butanone, described as having a plastic/urine/phenolic odour, and 2-methyl-5-propionylfuran.

In fraction no 6, pentanoic acid was detected at t_R = 11.4 min (RI < 1000) and its cheesy odour was reported (Fig. 2). Cyclohex-2-en-1,4-dione, which co-eluted with pentanoic acid on the polar phase, was identified at t_R = 17.8 min (RI = 1034) with no detectable odour under MDGC conditions. This led us to validate the important contribution of pentanoic acid to the odour of this targeted fraction. Two other compounds were also identified by ^{2}D GC–O: 3,4-dimethylfuran-2,5-dione, with a floral odour, and 3-ethyl-4-methylfuran-2,5-dione.

The ^{2}D analysis of fraction no 11 allowed the successful separation of 5-hydroxymethylfurfural (5-HMF) co-eluted with dodecanoic acid on the polar phase. This fraction was characterised as having an animal and sharp odour under ^{1}D GC–O analysis (Table 1), which might have been associated with the presence of 5-HMF. This compound was indeed associated with a musty odour during MDGC analyses, whereas dodecanoic acid was not detected at the sniffing port. The divergence between the ^{1}D GC–O and MDGC descriptors might have been due to co-elution with dodecanoic acid, which might enhance or suppress some of the odorant properties of 5-HMF.

Analyses of fractions no 5, 7, 8, 9, 10 and 12 were more challenging because no compound could be identified under ^{1}D analysis. The MDGC analyses allowed us to identify new compounds in these fractions (Table 2), thus highlighting a greater separation capacity.

Fraction no 5 had previously been characterised by green, roasted and nutty odour notes (Table 1). The co-occurrence of descriptors belonging to different odour categories, such as green and roasted, suggested a co-elution of several unidentified compounds. Six compounds were separated on the ^{2}D MS chromatogram and three were identified: 3-methyl-2(5H)-furanone, γ-hexalactone and 1-(3-hydroxy-2-furyl)ethanone, better known as isomaltol. The three other compounds remain unidentified. While the green, roasted and nutty odours found by the ^{1}D GC/O

Table 2
Results of the MDGC/MS/O analyses performed on the caramel samples A and B.

No	RI non-polar		RI polar		No heart-cut	Compounds[4]	Odour description	Caramel A	Caramel B	Identification[5]
	Exp.[1]	Lit.[3]	Exp.[2]	Lit.[3]						
1	<1000	740	1287	1297	1	3-Hydroxybutan-2-one[b,d]		X	X	MS, RI, std
2	<1000		1290–1315		1	ni			X	
3	<1000	980	1290–1315	1313	1	1-Octen-3-one	Mushroom		X	MS, RI, std
4	1010	1009	1287	1297	1	Octanal		X	X	MS, RI, std
5	<1000	949	1517–1541		2	3-Methyl-2,5-furanedione*			X	MS
6	<1000	975	1532	1513	2	3-Methylcyclopent-2-en-1-one	Caramel roasted		X	MS, RI, std
7	<1000	964	1532	1528	2	Benzaldehyde		X	X	MS, RI, std
8	<1000	957	1578	1575	3	5-Methyl-2-furfural[a,c,b]	Almond vanilla cinnamon	X	X	MS, RI, std
9	1012	1005	1578	1567	3	2-Propionylfuran[a*]			X	MS, RI
10	1050	985	1573–1591		3	2-Acetyl-5-methylfuran*	Urine floral		X	MS
11	<1000	876	1679	1685	4	3-Methylbutanoic acid			X	MS, RI, std
12	<1000	873	1679	1685	4	2-Methylbutanoic acid			X	MS, RI, std
13	1004		1660	1645	4	2,5-Dihydro-3,5-dimethylfuran-2-one*	Roasted curry		X	MS, RI
14	1077	1070	1660	1649	4	Acetophenone			X	MS, RI, std
15	1106		1645–1682		4	1-(2-Furanyl)-1-butanone*	Plastic urine phenol		X	MS
16	1104	1108	1645–1682		4	2-Methyl-5-propionylfuran[a*]			X	MS, RI
17	<1000	983	1698–1714		5	3-Methyl-2(5H)-furanone*		X	X	MS
18	<1000	989	1698–1714		5	1-(3-Hydroxy-2-furyl)ethanone (isomaltol)[a*]	Caramel	X	X	MS
19	<1000		1698–1714		5	ni			X	
20	1048		1698–1714		5	ni			X	
21	1059	1066	1702	1700	5	γ-Hexalactone			X	MS, RI, std
22	1089		1698–1714		5	ni			X	
23	<1000	926	1745	1744	6	Pentanoic acid[c]	Cheese sharp		X	MS, RI, std
24	<1000		1726–1750		6	ni			X	
25	1034	1024	1745	1744	6	Cyclohex-2-en-1,4-dione*			X	MS, RI
26	1036		1726–1750		6	3,4-Dimethylfuran-2,5-dione*	Floral		X	MS
27	1105		1726–1750		6	3-Ethyl-4-methylfuran-2,5-dione*		X	X	MS
28	1108		1726–1750		6	ni			X	
29	1000	1000	1830–1876	1830	7	Hexanoic acid[c,b,*]	Sharp spicy	X	X	MS, RI
30	1040		1830–1876		7	5,6-Dihydro-2H-pyran-2-one*			X	MS
31	1043	1030	1830–1876	1865	7	Benzyl alcohol*		X	X	MS, RI
32	1048	1036	1830–1876		7	4-Methyl-(5H)-furan-2-one*	Roasted	X	X	MS, RI
33	1075		1830–1876		7	ni		X	X	
34	1084		1830–1876		7	ni		X	X	
35	1093	1090	1830–1876	1859	7	Guaiacol*	Pharmaceutical		X	MS, RI
36	1100		1830–1876		7	ni		X	X	
37	1108		1830–1876		7	3,4-Dimethyl-2(5H)-furanone*		X	X	MS
38	1116		1830–1876		7	ni		X	X	
39	1120		1830–1876		7	ni		X	X	
40	1175		1830–1876		7	ni		X	X	
41	1189		1830–1876		7	ni		X	X	
42	1123		1905–1929		8	ni		X	X	
43	1139		1905–1929		8	2,4-Dimethylcyclopentan-1,3-dione*			X	MS
44	1173		1905–1929		8	ni			X	
45	1192		1905–1929		8	ni		X	X	
46	1226		1905–1929		8	ni		X	X	
47	1267	1266	1905–1929	1929	8	γ-Octalactone		X	X	MS, RI, std
48	1130	1136	2066–2082		9	5-Acetyldihydro-(3H)-furan-2-one (solerone)*		X	X	MS
49	1272	1275	2158–2192	2200	10	Nonanoic Acid*	Spicy smoked	X	X	MS, RI
50	1478	1468	2158–2192	2159	10	γ-Decalactone			X	MS, RI, std

Table 2 (continued)

No	RI non-polar		RI polar		Compounds[4]	No heart-cut	Odour description	Caramel A	Caramel B	Identification[5]
	Exp.[1]	Lit.[3]	Exp.[2]	Lit.[3]						
51	1236	1224	2496	2507	5-Hydroxymethylfurfural (5-HMF)[a,b,d]	11	Musty old book	X	X	MS, RI, std
52	1564	1560	2496	2505	Dodecanoic acid	11			X	MS, RI, std
53	1196		2543–2608	2596	Dihydro-4-hydroxy-(3H)-furan-2-one	12		X	X	MS,RI, std
54	1406	1410	2543–2608	2589	Vanilline	12		X	X	MS,RI, std

ni: non identified.
[1] Experimental (Exp.), RI on DB-5MS (30 m length × 0.32 mm i.d., 0.5 μm film thickness).
[2] Experimental (Exp.), RI on DB-Wax (30 m length × 0.32 mm i.d., 0.5 μm film thickness).
[3] RI according to literature (Lit.) (Bianchi, Careri, Mangia, & Musci, 2007; Chin et al., 2011), our internal database INRAMASS and Flavornet (http://www.flavornet.org/).
[4] Previous identification in caramel a: (Cottier et al., 1989); b: (Pons et al., 1991); c: (Goretti et al., 1980); d: (Pons, Jaubert, Morel, & Fenyo, 1995).
[5] Identification MS, Mass Spectrum; RI, Retention Index; std, injection of pure standard.
* Tentatively identified.

analyses were not reported in MDGC analyses, isomaltol was described with a typical caramel odour. Once again, the divergence between the odorant descriptions might have been linked to the existence of a co-elution on the first dimension. Although isomaltol is well-known for its sweet caramel odour (Pittet, Rittersbacher, & Muralidhara, 1970), its odorant properties in a mixture with five compounds cannot yet be predicted.

Fraction no 7 was characterised by roasted, caramel/lactone and pharmaceutical odour notes by GC–O analyses (Table 1) but no compound could be identified after GC–MS analysis due to several co-elutions which rendered the mass spectra uninterpretable. The ^{2}D MS chromatogram of this heart-cut fraction from caramel B extract is given in Fig. 3. Thirteen compounds were detected, of which seven were identified by MS and three showed odour activity. Hexanoic acid was described as having a sharp and spicy odour. 4-Methyl-(5H)-furan-2-one (RI = 1048) and guaiacol (RI = 1093) were respectively characterised by roasted and pharmaceutical odour notes.

The ^{2}D analysis of fraction no 8 led to the detection of six compounds, two of which were identified: 2,4-dimethylcyclopentan-1,3-dione and γ-octalactone. In fraction no 9, previously associated with a lactone and floral odour, only 5-acetyldihydro-(3H)-furan-2-one (or solerone) was identified. In fraction no 10, described as being floral, lactone, spicy and roasted after ^{1}D GC–O analyses, only nonanoic acid was described with a spicy and smoked odour by our panellists following ^{2}D GC–O analyses. γ-Decalactone was identified at RI = 1478 in the same fraction but with no detectable odour.

In these fractions no 8, 9 and 10, olfactometric detection did not provide any pertinent information regarding the qualitative properties of the compounds and particularly concerning lactone odours. In this case, the small number of panellists (only two) was probably the cause.

In fraction no 12, described as having chocolate vanilla/caramel/tobacco odours, two new compounds were identified: dihydro-4-hydroxy-(3H)-furan-2-one and vanillin. Neither was detected by our panellists. We can therefore assume that vanillin was the main contributor to the vanilla note, but further analyses are necessary with a larger number of panellists to confirm this finding.

4. Discussion

Multidimensional gas chromatography appears to be an efficient way to overcome identification issues in complex food samples such as caramel. In particular, heart-cutting is a suitable approach to investigate the composition of targeted fractions. This study focused on 12 odorant fractions in which compounds potentially making an important contribution to aroma could not be identified by ^{1}D GC–MS analyses. Olfactometric detection at the end of ^{2}D GC was performed by two experienced panellists in order to obtain a qualitative description of the odorants. The results revealed some divergences from the ^{1}D GC–O results, probably due to the smaller number of panellists in the MDGC study. Indeed, one of the main disadvantages of characterisation technique such as heart-cutting, is the large number of analyses required for just one sample. Then, for practical reasons, it is practically impossible to perform a quantitative GC–O study with a dozen panellists. Comprehensive two-dimensional gas chromatography (GC×GC) has recently been the subject of intense development and has now become one of the most powerful techniques in terms of resolution (Seeley & Seeley, 2013). This technique enables the full characterisation of a sample in two orthogonally stationary phases with only one run. However, it also necessitates the use of "fast" detectors such as Time of Flight-MS (TOF-MS) or Flame Ionisation Detector (FID) because of the very high separation speed on the
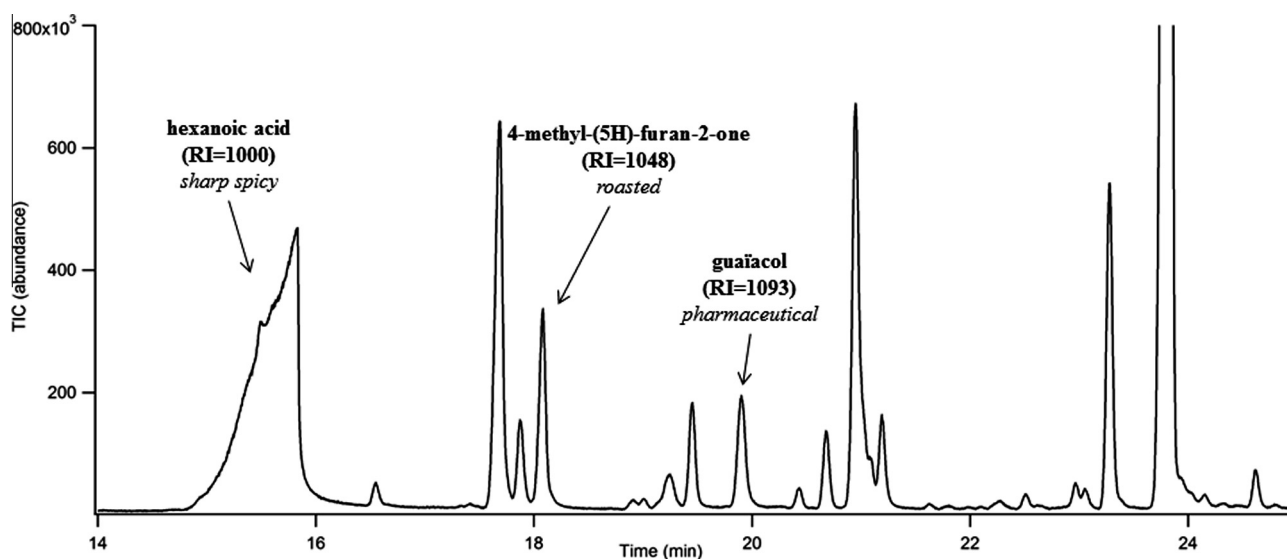
Fig. 3. ^{2}D MS chromatogram of the heart-cut fraction no 7 of the caramel B extract.

second column. Thus combining GC×GC with olfactometric detection is not possible because of the slow breathing rate of humans.

Another putative explanation for the discrepancy found between odour descriptions concerns the existence of synergistic effects between the co-eluted compounds. For example, according to our previous results, numerous odorant zones were characterised by fruity and lactone descriptors. However, only a few lactones were detected by the nose after ^{2}D GC–O analyses. The odour activity of lactones may be enhanced by the presence of other compounds in co-elution, so that the mixture may be detected but not each compound separately. Miyazawa et al. demonstrated the synergy that occurs between carboxylic acids and maple lactone (or 2-hydroxy-3-methylcyclopent-2-ene-1-one) (Miyazawa, Gallagher, Preti, & Wise, 2008). The presence of acetic and butyric acids at low concentrations rendered the odour of maple lactone more easily detectable. We can assume that a similar phenomenon occurs when lactones are co-eluted with other compounds after ^{1}D separation.

Nevertheless, the use of MDGC enabled the MS detection of 54 compounds (Table 2) from the heart cutting of only 12 fractions. Seventeen compounds remained unidentified due to non-indexed mass spectra and the presence of numerous isomers hampering their identification. To our knowledge, 20 compounds were reported for the first time in caramel, which thus constituted the added value of this study as a significant contribution to scientific knowledge on caramel. Most of the volatile compounds were furans and furan derivatives, carboxylic acids and lactones. We also counted four aromatic compounds, two ketones and one aldehyde. Furans and furan derivatives are the principal compounds that arise from the thermal transformation of sugars, so most have already been widely reported in caramel. In line with the literature, isomaltol (1-(3-hydroxy-2-furyl)ethanone) was identified and described as having a caramel odour (Pittet et al., 1970). This compound can easily be obtained from the thermal degradation of sucrose in aqueous solution at 120 °C (Ito, 1977) and has already been reported in caramel (Cottier et al., 1989). However, 2-acetyl-5-methylfuran and 1-(2-furanyl)-butanone were reported for the first time in caramel, with urine, floral and phenolic odour notes. Most of these compounds have previously been reported in food products undergoing a thermal treatment, such as coffee (Chin, Eyres, & Marriott, 2011). Major differences were observed between the two caramel samples in terms of their furan

composition. Among the seven furans identified, only three of them: 5-methylfurfural, isomaltol, and 5-HMF, were detected in the blond caramel A. We can assume that their formation was favoured by the cooking process used for caramel B.

Six carboxylic acids were identified in the burnt sugar caramel B: 2- and 3-methylbutanoic, pentanoic, hexanoic, nonanoic and decanoic acids. Some of these (methylbutanoic, pentanoic and hexanoic acids) had previously been reported in caramel (Goretti et al., 1980; Pons et al., 1991), but the presence of nonanoic and decanoic acids is reported here for the first time. Acids are well-known to contribute to food aromas; for example in dairy products, where they directly contribute to the lactic and cheesy odour (Thomsen et al., 2012). The hexanoic acid odour was described as being sharp and spicy. This odorant is known to occur in many food products such as chocolate where it has a pungent odour (Schnermann & Schieberle, 1997), and in wine where its odour is described as tobacco, floral and toasty (Miranda-Lopez, Libbey, Watson, & McDaniel, 1992). Recently, it was shown that carboxylic acids constitute one of the main chemical classes identified in the volatile fraction of caramel and are responsible for the pungent odour notes of burnt sugar type caramels (Paravisini et al., 2012). This supports our findings regarding the identification of only two out of six acids in the blond caramel A.

MDGC analysis was useful for the identification of lactones, or furanones, in the caramel extracts. Indeed, thanks to a better resolution, 12 lactones were detected and identified rather than just one after ^{1}D analysis. 2,5-Dihydro-3,5-dimethylfuran-2-one and 4-methyl-(5H)-furan-2-one were associated with roasted notes, and the odour of 3,4-dimethylfuran-2,5-dione was described as floral. The other nine lactones were only identified by MS. The occurrence of solerone has already been reported in wine. However its impact on wine aroma is weak, even at its highest natural concentration (Martin, Etiévant, & Le Quéré, 1991). Other authors have reported its odour in dried figs as a weak note of dried fruits (Naef, Jaquier, Boschung, & Lindstroem, 1995). This may explain why this compound was not smelt in our caramel extracts.

Different pathways may explain the formation of lactones, such as the metabolomic pathways of micro-organisms (Feron, Bonnarme, & Durand, 1996) or the thermal degradation of sugars (Beck, Ledl, Sengl, & Severin, 1990). Therefore, lactones occur in many food aromas; for example, γ-lactones are typical compounds of fruity aromas (Guichard, Kustermann, & Mosandl, 1990). But to

the best of our knowledge, the 12 lactones we identified are reported here for the first time in aromatic caramel.

The identification of 2-methoxyphenol (guaiacol) and 4-hydroxy-3-methylbenzaldehyde (vanillin) was one of the main findings of this study. Indeed, in fractions no 7 and 12 respectively, pharmaceutical and vanilla odour notes were detected during the [1]D GC–O study (Table 1), but no compounds could be identified. The RI on the polar phase of these fractions gave some clues as to the presence of these two compounds but their MS ions were not found. The MDGC analysis enabled the identification of guaiacol with both olfactometry and MS detection. Vanillin was only identified through its mass spectrum. Once again, the contribution of these compounds in caramel has never previously been reported, whereas their occurrence in food products resulting from a non-enzymatic browning reaction has been described several times (Dorfner, Ferge, Kettrup, Zimmermann, & Yeretzian, 2003). It should be noted that guaiacol was only detected in the burnt sugar caramel, while vanillin was found in both caramels.

Benzaldehyde and 3-methylcyclopent-2-en-1-one were identified in fraction no 2 but only 3-methylcyclopent-2-en-1-one displayed any odour activity. These compounds had both been reported as being obtained by the thermal degradation of sugars (Baltes & Mevissen, 1988) but their contribution to caramel aroma was still unclear. 3-Methylcyclopent-2-en-1-one was only detected in the burnt sugar type caramel, suggesting its preferential formation under harsher cooking conditions. This compound has previously been reported in Arabica coffee characterised by a roasted note (Sanz, Maetztu, Zapelena, Bello, & Cid, 2002).

Finally, 1-octen-3-one was identified in the heart-cutting analysis of fraction no 1 from the burnt sugar caramel. Despite the detection of a mushroom odour note in the blond caramel A by [1]D GC–O analyses (Paravisini et al., 2012), 1-octen-3-one could not be detected by [2]D GC–O. As 1-octen-3-one was detected in sample B, this lack of detection could not have been due to the panellists' lack of sensitivity. Even if its occurrence in caramel has not previously been reported, 1-octen-3-one is well-known for its powerful odorant properties because of its very low odour threshold ($5 \ \mu g \ l^{-1}$ in water (Buttery, Teranishi, Flath, & Ling, 1990)) and it is present in various food aromas such as cheese (Zehentbauer & Reineccius, 2002) and vinegar (Acena, Vera, Guasch, Busto, & Mestres, 2011).

Our results therefore showed that only 27 compounds in the targeted fractions were detected in the blond caramel A, whereas 54 were detected in the burnt sugars B. No specific compound could be attributed to caramel A, which allows us to theorise that the differences observed were due to a preferential formation of the identified compounds under harsher cooking conditions. Further analyses involving a broad range of caramel samples varying in terms of their initial sugar composition and process now need to be performed in order to validate our findings and help us to better understand their sensory properties.

5. Conclusion

The heart-cutting MDGC technique enabled the identification of new compounds, some of which were reported for the first time in caramel and may be important contributors to its aroma.

The predominance of furans, lactones and acids in the burnt sugar caramel showed that their formation is favoured by harsher cooking conditions. These compounds may originate from the thermal breakdown of sugars. They also result from the degradation of brown high molecular weight (HMW) compounds that are found at much higher concentrations in burnt sugar than in blond caramel.

The [2]D analyses clearly identified the compounds responsible for the odour of the whole fraction. However, some differences were observed between the [2]D and [1]D odour descriptions. There may be several explanations for this. First, such a discrepancy could be explained by a lack of sensitivity of the two panellists involved in the [2]D study when compared to the [1]D study which was carried out with a larger number of panellists, thus compensating for the low sensitivity of some of them. Second, the odour of specific compounds may be enhanced in a mixture with other compounds, whereas they exhibit no odour activity alone. Finally, the odour of a mixture of odorants may differ from that of the individual compounds. These findings could be explained by masking or fusion phenomena resulting from perceptive interactions.

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