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Characterisation of the volatile fraction of aromatic caramel using heart-cutting multidimensional gas chromatography

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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.

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

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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 INRAMASS (internal database developed using standard compounds).