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Improved determination of flavour compounds in butter by solid-phase (micro)extraction and comprehensive two-dimensional gas chromatography

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Abstract

The practicability and potential of comprehensive two-dimensional gas chromatography (GC × GC) coupled to both conventional flame ionisation (FID) and time-of-flight mass spectrometric (TOF-MS) detection, were compared with those of conventional one-dimensional (1D) GC, with the determination of flavour compounds in butter as an application. For polar flavour compounds, which were collected from the aqueous fraction of butter by means of solid-phase extraction (SPE), it was found that GC × GC dramatically improves the overall separation. Consequently, quantification and preliminary identification based on the use of ordered structures, can be performed more reliably. The improvement effected by replacing 1D-GC by GC × GC is considerable also in the case of TOF-MS detection, as illustrated by the high match factors generally obtained during identification. GC × GC was also used successfully for the characterisation of volatile flavour compounds in the headspace of butter collected by solid-phase microextraction (SPME) and to study the effect of heat treatment on the composition of butter samples in more detail.

Keywords: SPE; SPME; Comprehensive two-dimensional gas chromatography; GC × GC; Flavour compounds; Butter extracts

1. Introduction

Several years ago, we studied the trace-level determination of flavour compounds in butter by means of gas chromatography–mass spectrometry (GC–MS) [1]. The study dealt with the volatile flavours present in the aqueous fraction of butter, and pre-treatment was by means of solid-phase extraction (SPE). Five well-known test analytes were used which represented various classes of compounds, some of which may be found in butter: diacetyl (ketones), furaneol (furanones), maltol (pyranones), sotolon (furanones) and vanillin (benzaldehydes). Generally speaking, the results were quite encouraging: the analytical performance characteristics were fully satisfactory for all target analytes except diacetyl (baseline subtraction and selected ion monitoring required). The effect of heat treatment on sample composition could be demonstrated convincingly, some 20 further compounds could be identified (although there were also a number of distinct failures) and quantification at the 0.1–10 mg/kg level did not cause serious problems.

Today, re-reading of the quoted paper rapidly shows that – next to the problems regarding failed quantification and/or identification already mentioned – the unresolved baseline envelope clearly present in the chromatograms of at least some of the test samples strongly suggests that a more powerful separation technique should be used: comprehensive two-dimensional gas chromatography (GC × GC) with either non-selective flame ionisation (FID) or, if required, selective time-of-flight mass spectrometric (TOF-MS) detection [2–6]. In order to enable a straightforward comparison of the two methods of analysis, conventional (i.e. one-dimensional, 1D) GC and GC × GC, the same general set-up was used as in the earlier study, and the same target analytes and experimental parameters (heat treatment and storage effects) were used. As an extension, the headspace of the butter samples was analysed (by means of solid-phase microextraction, SPME) next to the aqueous fraction.

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2. Experimental

2.1. Analytes and samples

Methyl acetate (J.T. Baker, Deventer, The Netherlands) was freshly distilled and HPLC-grade water was prepared in a Mili-Q (Millipore, Bedford, MA, USA) filtration system. Helium, used as carrier gas (99.999% purity) was supplied by HoekLoos (Schiedam, The Netherlands). Vanillin and diacetyl were from Fluka (Zwijndrecht, The Netherlands), maltol and furaneol from Acros (Geel, Belgium), and sotolon from Aldrich (Brussels, Belgium). Standard solutions of all flavours were prepared at a concentration of 0.1–20 mg/ml by weighing and dissolution in methyl acetate or HPLC-grade water. They were kept in the dark at 4 °C for a period of maximally 4 months.

Two different types of fresh butter, 'grasboter' and 'gezouten roomboter' (Melkunie, Breda, The Netherlands), were purchased from a local supermarket.

2.2. Preparation of butter extracts and SPE procedure

Preparation of butter extracts and SPE-based sample treatment were performed according to the procedure optimized in the earlier study [1]. Briefly, the SPE cartridge (SDB-1; PS–DVB copolymer) was conditioned with 2 ml of methanol and 2 ml of HPLC-grade water. Next, the water phase of the butter sample was loaded on the SPE cartridge via vacuum suction. After washing with 1 ml of water and drying for 15 min at room temperature, the trapped analytes were desorbed with 1 ml of methyl acetate. After drying by adding anhydrous sodium sulphate, 1 μl of the SPE extract was injected for either GC × GC–FID, GC–MS or GC × GC–TOF-MS analysis.

2.3. SPME procedure

The SPME fibres and the manual holder were purchased from Supelco (Bellefonte, PA, USA). Four types of SPME fibre were used: polydimethylsiloxane (PDMS) of 100 μm thickness; Carboxen/PDMS of 75 μm thickness; Stable Flex Carbowax–divinylbenzene (Carbowax/DVB) of 70 μm thickness and Stable Flex divinylbenzene–carboxen–polydimethylsiloxane (DVB/Carboxen/PDMS) of 50/30 μm thickness. Fibres were conditioned prior to use according to the manufacturer's instructions: PDMS was inserted into the GC injector at 250 °C for 1 h; Carboxen/PDMS at 280 °C for 30 min; Carbowax/DVB at 270 °C for 1 h, and DVB/Carboxen/PDMS at 270 °C for 4 h.

For each headspace (HS)-SPME analysis, approximately 8 g of butter sample placed in a 14 ml clean glass vial was smelted at 40 °C or heated at 170 °C for 5 min. The glass vials were sealed with silicone septa and were kept at 40 °C. The SPME needle pierced the septum and the fibre was extended through the needle to bring the stationary phase in contact with the headspace of the sample. The fibre was withdrawn into the needle after the sampling time, which was varied from 1 to 90 min. Finally, the SPME needle was removed from the vial and inserted for 0.5–3 min in the injection port of the gas chromatograph. The extracted compounds were thermally desorbed at 200–270 °C and transferred directly to the (first-dimension) GC column.

2.4. GC–MS and GC × GC systems

For GC–MS, the gas chromatograph was a Hewlett-Packard HP 6890 (Agilent Technologies, Palo Alto, CA, USA) instrument with a split/splitless injector, and a HP-5972 mass-selective detector. A 30 m × 0.25 mm I.D., 0.25 μm BP21 (polyethylene glycol, TPA-treated) column (SGE Europe, Milton Keynes, UK) was used. Conditions were as follows: injector temperature, 250 °C; GC column temperature, 40 °C (2 min), at 5 °C/min to a final temperature of 230 °C (5 min); transfer-line temperature, 280 °C, ion-source temperature, 200 °C; carrier gas, He at 110 kPa. Analyses were performed in the electron ionisation (EI) mode at 70 eV. The mass range was *m/z* 40–350.

The GC × GC–TOF-MS system consisted of a HP 6890 (Agilent Technologies) gas chromatograph equipped with an Optic 2 programmable injector with a multicapillary liner (ATAS, Veldhoven, The Netherlands) and a Pegasus II time-of-flight mass spectrometer (LECO, St. Joseph, MI, USA). TOF-MS was operated at a storage rate of 50 Hz, using a mass range of *m/z* 40–400 and a multi-channel plate voltage of −1800 V.

For GC × GC–FID, a HP 6890 (Agilent Technologies) gas chromatograph equipped with a split/splitless injector and a FID system capable of producing a digital signal at a rate of 200 Hz was used.

For both GC × GC systems, a 30 m × 0.25 mm I.D., 0.25 μm BP21 (polyethylene glycol, TPA-treated) column (SGE Europe) was used as the first-dimension column. The second-dimension column was a 1 m × 0.1 mm I.D., 0.1 μm BPX-35 column (SGE Europe). The columns were connected with a press-fit connector (Varian universal quick seal; Varian–Chrompack, Middelburg, The Netherlands). The carrier gas, helium, was used at a constant flow of 1.3 ml/min. The temperature of the two GC columns, which were housed in the same oven, was programmed from 40 °C (2 min hold) to 240 °C (3 min hold) at 5 °C/min.

Thermal modulation was performed with a laboratory-made CO_2-cooled dual-jet modulator [7]. Cooling was effected through the Joule–Thompson effect of expanding liquid CO_2 (technical grade; HoekLoos). Briefly, two short sections of the second-dimension column are directly and alternately cooled in order to trap and focus each subsequent fraction which is, next, remobilized by the heat from the surrounding oven air. The modulation time was 3 s; the modulator temperature was kept about 100 °C below the oven temperature.