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Relevance of two-dimensional gas chromatography and high resolution olfactometry for the parallel determination of heat-induced toxicants and odorants in cooked food☆

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ABSTRACT

The assessment of the dual impact of heating treatments on food safety and aroma is a major issue for the food sector. The aim of the present study was to demonstrate the relevance of multidimensional GC techniques, olfactometry and mass spectrometry for the parallel determination of process-induced toxicants and odorants in food starting with cooked meat as a food model. PAHs were analyzed by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry after extraction by accelerated solvent extraction (ASE-GC × GC–TOF/MS). Odour-active compounds were determined by dynamic headspace–GC hyphenated with eightbooth olfactometry and mass spectrometry (DH–GC–MS/8O) and DH-heart-cutting multidimensional GC hyphenated with olfactometry and mass spectrometry (DH–GC–GC–MS/O). For PAH determination, the GC × GC conditions consisted of a combination of a primary non-polar BPX-5 column and a secondary polar BPX-50 column, and a modulation period of 5 s. In terms of linearity (R^2 ranging from 0.985 to 0.997), recovery rate (84–111%) and limit of detection (5–65 ng/kg of cooked meat), the ASE-GC × GC–TOF/MS method was found consistent with the multiresidue determination of 16 PAHs including benzo[*a*]pyrene in cooked meat. For aroma compounds, DH–GC–MS/8O and DH–GC–MS/O revealed 53 major meat odour-active compounds. A customized heart-cutting GC–GC–MS/O enabled the coeluting odour zones with high odour-activity to be resolved and revealed 15 additional odour-active compounds. Finally, these developments of multidimensional approaches were used to investigate the balance between 16 PAHs and 68 odour-active compounds generated with different cooking techniques.

1. Introduction

Cooking has both positive and negative impacts on food quality. While cooking is necessary to develop the desirable flavours in food, besides making it digestible as well as destroying harmful organisms, the oxidation and pyrolysis of fats, especially at frying temperatures, can give rise to compounds that decompose creating undesirable flavours and various process-induced toxicants, including polycyclic aromatic hydrocarbons (PAHs). Nowadays, there is an increasing demand to limit the negative influence of cooking, with a specific emphasis on heat-induced toxicants such as PAHs, while preserving sensory properties of processed food, focusing on odour-active compounds. It is therefore necessary to develop analytical tools for the parallel investigation of process-induced toxicants and odorants generated during food cooking.

More than 100 PAHs have been characterized in nature as genotoxic carcinogens and biological mutagens [1]. PAH-induced carcinogenesis may result when a PAH-DNA adduct is formed on a critical site for the regulation of cell differentiation or growth. Interactions of various PAHs have been shown to produce both synergistic and antagonistic effects in mutagenicity tests *in vitro* [2]. For non-smokers and non-occupationally exposed populations, dietary intake has been identified as the principal route of human exposure to PAHs [3]. Efforts have thus been made to limit their formation in food through understanding the influence of various food processes including cooking. Therefore, food control authorities including the United States Environmental Protection Agency (US EPA) [4] and the European Food Safety Authority (EFSA) [5]

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have started monitoring the priority PAHs and seeking advanced methodology related to their detection in food [6]. Recently, methods using comprehensive two-dimensional gas chromatography (GC × GC) have been reported to improve the chromatographic resolution for PAH determination in food [7–9]. The high resolution of GC × GC allows for separation of the target compounds from matrix interferences and may enable the resolution of most coelutions reputed to persist in one-dimensional GC [10], even if the recent development of congener-specific GC-columns for PAH analysis might also offer promising alternatives [11,12]. The time-of-flight mass spectrometry (TOF/MS) detector has been largely used to detect narrow peaks obtained from fast separation in the second chromatographic dimension making it easier to identify additional relevant compounds [10,13].

Cooking is also responsible for the development of food aroma [14,15]. For the screening of key odorants in food products, gas chromatography olfactometry (GC-O) coupled with mass spectrometry (MS) is commonly applied involving one or more human assessors or sniffers [16]. However, most of the reported techniques are unable to provide precise quantitative evaluation due to the wide variability of individual responses, variability between experimental sequences, as well as coelutions making it difficult to identify odour-active compounds. Pollien et al. [17] have demonstrated that a panel of at least eight sniffers was required to obtain a robust and reproducible aromagram. To overcome this drawback, a computerized multibooth GC–MS/8O system was developed to obtain an aromagram from a panel of eight sniffers for simultaneous detection of compounds using a single run [18]. Despite using high resolution GC, the 1D-separation achieved with the GC–MS/8O system is not always sufficient to resolve all the coeluted odour zones. One of the most popular options to elucidate these complex odour zones is to re-separate them with a second GC column hyphenated to olfactometry. The importance of multidimensional separation (MDGC) techniques, including heart-cutting GC–GC–MS/O providing an improved capacity to resolve the constituents of a sample, was recently reviewed by Marriott et al. [19].

This paper is aimed at assessing the relevance of multidimensional GC techniques hyphenated to MS and olfactometry for the parallel investigation of process-induced toxicants and odorants generated during food cooking starting with meat as a food model. In a first step, a multiresidue method based on ASE-GC × GC–TOF/MS was developed in order to investigate 16 targeted PAHs in cooked meat matrices. In a second step, the main odour-active compounds of the same matrices were determined by high resolution olfactometry. This approach consists in refining the detailed aromagrams provided by GC–MS/8O with the high resolution of a customized heart-cutting GC–GC–MS/O. Finally, these analytical developments were used to investigate the parallel generation of PAHs and odour-active compounds for various domestic cooking conditions.

2. Materials and methods

2.1. Chemicals

PAH reference standards were obtained from Sigma–Aldrich (Steinheim, Germany). The concentrations of the 16 PAHs in the stock solution were 4 μg mL^{-1}. Three D-labelled PAHs (acenaphthylene-D8, *p*-terphenyl-D14 and benzo[*e*]pyrene-D12) were used as internal standards for the accurate quantification of target compounds (Wellington Laboratories Inc., ON, Canada). Solvents (analytical grade) and anhydrous sodium sulfate were purchased from Sigma–Aldrich. All glassware was deactivated by soaking in a 5% solution of dichlorodimethylsilane in heptane for 30 min, then rinsed three times with heptane and immediately washed with methanol.

2.2. Food and cooking processes

One beef semimembranosus muscle was ground in a meat mincer. The ground meat was restructured into beef steaks of 15 g, frozen by dipping in liquid N_2, then wrapped in aluminium foil, vacuum packed and stored at −80 °C. Before cooking, the frozen restructured beef steaks were thawed under running water for 20 min at 25 °C. For each cooking experiment, three restructured beef steaks were cooked and meat core, surface and cooking chamber temperatures were monitored. After cooking, the meat samples were immediately frozen in liquid nitrogen then wrapped in aluminium foil, vacuum packed and stored at −80 °C. Before analysis, the frozen meat samples were ground in liquid nitrogen to obtain a fine and homogeneous powder according to Berge et al. [20]. For the cooking processes, several methods were tested to represent different heat transfer techniques including conduction (pan frying), convection (oven and grilling), and radiation (microwave). The pan-frying process was carried out with a Teflon-coated pan (internal diameter: 30 cm). The steak samples were cooked for a total time of 8 min (4 min each side) after a preheating step of the pan for 10 min at 170 °C. For the oven cooking, the steak samples were put into a customized glass chamber placed in a temperature-controlled oven and roasted for 20 min at different temperatures (150, 200 and 250 °C), under several atmospheric gases (air, nitrogen and oxygen). The flow rate of the gas in the chamber was maintained at 100 mL min^{-1}. Microwave cooking was conducted for the beef steak samples at 600 W for 15 s, and grilling was carried out using electrical appliances to obtain more reproducible results than traditional charcoal/flame grilling.

2.3. PAH determination by GC × GC-TOF/MS

The PAHs were extracted from meat samples by pressurized liquid extraction (ASE 350, Dionex, Sunnyvale, CA, USA). 5 g of meat powder dispersed with diatomaceous earth were loaded into a 34 mL ASE extraction cell along with the three D-labelled internal standards. Cellulose filters were placed at the bottom of the cell. ASE extraction was performed using dichloromethane and isohexane (1:5) under the following conditions: extraction temperature: 100 °C; heating time: 5 min; static extraction time: 5 min; flush volume: 60% of cell volume; nitrogen purge: 120 s and 2 static extraction cycles. The extract was evaporated to dryness (Rocket, Genevac Ltd., France), then re-dissolved with 20 mL of acetonitrile. After 3 h at −20 °C, the cold extract was dried over 5 g of Na_2SO_4 and filtered in order to remove the frozen lipids and water. After a second evaporation step, the dried residue was re-dissolved with 1.5 mL of dichloromethane then evaporated with a gentle N_2 stream until the disappearance of the last drop of solution. Finally, the residue was dissolved in 100 μL of hexane before being injected into the GC × GC–TOF/MS system. The sample preparation procedure was designed to target a concentration of analytes a hundred times higher in the final extract.

The extracts were analyzed on a time-of-flight mass spectrometer (Pegasus 4D, Leco, St. Joseph, MI, USA) coupled to a two-dimensional gas chromatograph (6890, Agilent Technologies, Santa Clara, CA, USA) equipped with a dual stage jet cryogenic modulator (licensed from Zoex). The 1D column BPX-5 (30 m × 0.25 mm × 0.25 μmdf; SGE, Austin, TX, USA) was connected by a deactivated ultimate union (Agilent) to a BPX-50 2D column (1 m × 0.1 mm × 0.1 μmdf; SGE). A splitless injection of 1 μL of sample extract was performed through a CTC CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) with an injector temperature set at 280 °C. Helium (purity 99.9995%) was used as carrier gas