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Identification and origin of odorous sulfur compounds in cooked ham

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ABSTRACT

The aim of this work was to identify and gain further knowledge on the origin of sulfur compounds present in the volatile fraction of cooked ham, and on their role in the aroma of this product. To this end, we performed analyses by one- and two-dimensional gas chromatography coupled with mass spectrometry, and olfactometry. Among the odorant sulfur compounds identified, three furans present in trace amounts proved to have very intense odours responsible for the "meaty, cooked ham" notes of this pork product. They were 2-methyl-3-furanthiol, 2-methyl-3-(methyldithio)furan and bis(2-methyl-3-furyl) disulphide. Addition of thiamine or cysteine also enabled us to study the effect of these odour precursors on the formation of odorant furans during the cooking of ham. The results revealed a direct link between the thermal degradation of thiamine and the formation of these compounds. By contrast, addition of cysteine in the presence of fructose or xylose did not appreciably increase their production.

1. Introduction

The volatile fractions of foods contain a large number of compounds of which only some contribute to their aroma. In the case of cooked meats, the role of sulphur compounds is well-known (Werkhoff et al., 1990). Their importance can be partly explained by their very low detection thresholds. Their presence in trace amounts is enough to impart the pleasant "meaty, roasty, cooked ham, etc." notes (Mottram, 1998) that help give these products their typical odour profile. Among these compounds, furan and thiophene rings possess a thiol group in position 3 that brings a "meat savoury" note (Buttery, Haddon, Seifert, & Turnbaugh, 1984; Evers et al., 1976). In the case of cooked ham, Thomas, Mercier, Tournayre, Martin, and Berdagué (2013) have confirmed the important role of sulphur compounds in its aroma.

Studies during the cooking of meats confirm the formation of many volatile sulphur compounds. At high temperatures, they can originate from the advanced stages of the Maillard reaction (Bailey, 1994; Mottram, 1994), hydrolysis or thermal degradation of amino acids (Belitz & Grosch, 1999). Several studies in model media have been conducted to elucidate the reaction mechanisms involved. These studies, combining varying physicochemical conditions and often high temperatures, showed that addition of thiamine or cysteine in the presence of certain sugars increased the production of odour-active sulphur compounds (Cerny & Davidek, 2003; Tai & Ho, 1997; Wang, Yang, & Song, 2012). However, in the conditions of low-temperature slow cooking of cooked ham (Hennel, 1966), the effect of these precursors on the formation of odour-active sulphur compounds has never been assessed.

The aim of this study was accordingly (i) to identify as fully as possible the sulphur compounds in the volatile fraction of cooked ham, including those that have an olfactive impact, and (ii) to assess the effects of adding sulphur precursors on the production of these compounds during the cooking of model hams.

2. Materials and methods

2.1. Materials and sample origins

The analyses were performed on commercially available cooked ham (to identify sulphur compounds, both odorant and non-odorant), and model cooked hams (to assess the effects of aroma precursors). The commercially available ham, selected for its intense cooked ham aroma, came from the Cellier Company (Aubière, France) and was made in the traditional way (Hennel, 1966). The model hams (weight of 300 g) were produced in the laboratory from a specific pig muscle (*M. semimembranosus*). We mixed 1 kg of chopped raw meat with 100 g of brine (18 g of curing salt containing 0.6% nitrite in solution in 82 g of water). This mix was then distributed in closed glass jars under vacuum. The cooking programme was 40–69 °C at 0.1 °C/min^{-1} and 120 min hold at 69 °C. In our experiment, a broad range of aroma precursors was added to test their effects on the formation of odorant sulphur

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compounds detected by olfactometry. Some brines contained thiamine (0, 8, 80, 800 and 8000 mg added to 100 g of brine), cysteine plus fructose (0, 100, 1000, 3000 and 10,000 mg of cysteine and 3 g of fructose added to 100 g of brine) or cysteine plus xylose (0, 100, 1000, 3000 and 10,000 mg of cysteine and 3 g of xylose added to 100 g of brine).

2.2. Additives and reference compounds

2.2.1. Additives

Thiamine (purity 99%, Ref.: T4625), xylose (purity 99%, Ref.: X1500), fructose (purity 99%, Ref.: F0127), cysteine (purity 97%, Ref.: W326305) were purchased from Sigma–Aldrich (St. Louis, MO). Sodium nitrite (0.6% w/w) was added in mixture with sodium chloride 99.4% w/w (Supplier: Coopérative des Charcutiers, 43 bis rue Anatole France, 63000 Clermont-Ferrand, France).

2.2.2. Reference compounds

3-Methylthiopropanal (purity 97%, Ref.: W274704), 2-methyl-3-furanthiol (purity 95%, Ref.: W318809), 2-methyl-3-(methyldithio)furan (purity 98%, Ref.: W357308), bis(2-methyl-3-furyl disulphide (purity 98%, Ref.: W325900), 2-methylthiophene (purity 98%, Ref.: M84208), dimethyltrisulphide (purity 98%, Ref.: W327506) were purchased from Sigma–Aldrich.

2.3. Solid-phase microextraction–comprehensive gas chromatography–time-of-flight mass spectrometry (SPME–GC×GC–MStof)

A slice of 100 g of industrial ham was minced and 2 g were placed at ambient temperature in a sealed 20-mL vial and incubated for 10 min. The extraction of volatiles was performed using a 75 mm solid-phase microextraction (SPME) Carboxen/PDMS fibre (Supelco Bellefonte, PA). The fibre was exposed to the ham headspace for 1 h at ambient temperature. After extraction, a splitless injection (at 220 °C for 2 min) of volatiles was carried out with an SPME Combipal autosampler (CTC Analytics AG, Zwingen, Switzerland). Separation and detection was performed using a GC×GC-MStof LECO Pegasus IV system (LECO Corporation, St. Joseph, MI). Volatiles were first separated on an RTX-5 capillary column (length 30 m, internal diameter 0.32 mm, film thickness 1 μm; Supelco, Saint-Germain-en-Laye, France) and secondly on a DB-17 capillary column (length 2.50 m, internal diameter 0.178 mm, film thickness 0.30 μm; J&W Agilent, Santa Clara, CA). Volatiles were detected by electron ionisation at 70 eV, and data were acquired over the mass range $18 < m/z < 220$ at 200 scan·s^{-1}. More details on the analytical settings are given in Théron et al. (2010).

2.4. Dynamic headspace sampling-gas chromatography–mass spectrometry (DHS-GC–MS)

For the industrial ham and for the model hams, 100 g were minced and 7 g were placed at ambient temperature in a Pyrex® extraction cartridge (Ref.: M3, Maillères, Aubière, France). The volatile compounds were then extracted by DHS (Tekmar, Cincinnati, OH), at 30 °C for 60 min with a helium stream (Messer, He/U purity: 99.995%) at a flow rate of 40 ml min^{-1}. The trap used contained Tenax® TA (60/80 mesh adsorbent, Supelco Bellefonte PA); working length 180 mm and inside diameter 1/8″. The dry purge step was set at 30 min. The volatile components were then desorbed from the trap at 180 °C for 10 min using helium (He/N55 purity: 99.9995%) and sent into the cryofocusing area (cooled at −150 °C with liquid nitrogen). After injection, by heating the trap at 180 °C for 2 min., volatiles were separated and detected using a GC–MS set-up composed of a chromatograph (GC 6890, Agilent Technologies; apolar capillary column RTX5-MS, length 60 m, internal diameter 0.32 mm, film thickness 1 μm) linked to a quadrupole mass detector (MSD 5973 Inert, Agilent Technologies). Volatiles were detected by electronic ionisation at 70 eV, and ions were scanned over the range $18 < m/z < 220$ a.m.u. For the trials with precursors, the sulphur odourants of interest were semi-quantified by measuring their peak areas from specific ions acquired in single ion monitoring mode. Analyses of model hams were performed in triplicate.

2.5. Gas chromatograph–mass spectrometry/olfactometry

Two complementary olfactometry instruments were used to detect and identify odorous compounds. They were both coupled to identical dynamic headspace devices (Tekmar) and the extraction conditions of the volatiles were similar to the conditions described in Section 2.4.

The first instrument made an exhaustive inventory of odorous compounds by eight-way olfactometry coupled to mass spectrometry (DHS-GC–MS/8O) (Berdagué & Tournayre, 2005; Berdagué, Tournayre, & Cambou, 2007). This system could detect a very large number of olfactory zones by accumulating the performances of eight sniffers. The selected sniffers were non-smokers with no known health disorders, less than 40 years old, and with tested sensitivity and ability to detect and consistently describe a wide range of odours. The nature of the samples analysed was communicated to the sniffers, who were asked to focus on the "meat, cooked ham" odour. To measure the intensity of the odours, a five-level scale (1, very weak; 2, weak; 3, moderate; 4, strong, and 5, very strong) was used. Olfactometric analyses lasted 40 min. Olfactometric data were acquired and processed with AcquiSniff® Software (Berdagué & Tournayre, 2002; Tournayre & Berdagué, 2003). Two sniffing sessions (with eight sniffers per session) of the industrial cooked ham were run with the DHS-GC–MS/8O device. The aromagram of the industrial cooked ham was obtained from the mean intensities of the individual aromagrams (Berdagué & Tournayre, 2002).

The second instrument was a chromatography–mass spectrometry–olfactometry system with a single olfaction port. This instrument worked either in gas chromatography–mass spectrometry–olfactometry mode (DHS-GC–MS/O) to fit DHS-GC–MS/8O analysis, or in two-dimensional chromatography mode (DHS-GC-GC–MS/O, also termed "heart-cut mode") to make a detailed olfactory exploration of all the odorous zones observed by DHS-GC–MS/8O, which is essential for reliable identification. The DHS-GC–MS/O instrument was composed of a chromatograph (GC 6890, Agilent Technologies; capillary column RTX5-MS, length 60 m, internal diameter 0.32 mm, film thickness:1 μm) hyphenated to a quadrupole mass detector (MSD 5973 Inert, Agilent Technologies). The capillary column was connected to the mass spectrometer *via* a deactivated capillary column (length 0.5 m, internal diameter 0.10 mm; SGE, Ringwood, Australia) and to the sniffing port *via* a deactivated capillary column (SGE, length 1.7 m, internal diameter 0.32 mm) using a zero dead volume T-connector (inside tubing diameter 1/16″; ZTIM Valco® Instruments Co. Inc., Houston, TX). The ratio of effluent between sniffing port and mass detector was 1/1. For the DHS-GC-GC–MS/O mode, the volatile compounds from the first separation on the non-polar (RTX5-MS) column were cryofocused on a second polar column (DB-WAXETR, J&W Scientific, Agilent Technologies: length 30 m, internal diameter: 0.32 mm, film thickness: 1 μm), and then sent by heating (180 °C, 2 min) to the sniffing port and to the mass spectrometer. Olfactometric data obtained by DH-GC–MS/O or by DH-GC-GC–MS/O were both acquired by 2 assessors with the AcquiSniff® Software in similar conditions (vocabulary, odour intensity (1–5) and odour duration) to the DH-GC–MS/8O data. The odour of the candidate structures identified by mass spectrometry was compared with their odour as described