# New methods for the identification of dry-cured ham odour active compounds

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**Abstract**

The aim of this work is to present reliable methods for the identification of odour-active substances in meat products using powerful up-to-date tools for the analysis of the volatile fraction. Most often, the identification of odorous volatile compounds is delicate and requires caution due to sensory limitations arising from variability in responses with small number of sniffers and to instrumental limitations in the identification of the odorous substances. These substances often co-elute and they are present in trace amounts. They cannot therefore be readily identified using ordinary routine analysis by Gas Chromatography Mass Spectrometry. To overcome these difficulties, we used a Dynamic Headspace – Gas Chromatography – eight-way Olfactometry set-up with a panel of eight sniffers. This provided a stabilised sensory response and generally avoided individual non-detection. To identify the odorous substances we used a Dynamic Headspace – Gas Chromatography – Mass Spectrometry / Olfactometry combination, together with a Solid Phase Micro-Extraction – Comprehensive Gas Chromatography – time-of-flight Mass Spectrometry set-up. Analyses were carried out on dry cured ham and more than 600 compounds were identified. They covered a wide diversity of structures, chemical functions and origins. Only 29 of them proved to be odour-active.

## Materials and methods

*Origin of hams and sampling*

The hams were produced according to the criteria of the French quality certification scheme "Indication Géographique Protégée Jambon de Bayonne".

*Dynamic Headspace Gas Chromatography – Mass Spectrometry*

The volatile components were extracted by dynamic headspace gas chromatography (Tekmar, Cincinnati, OH 45234, USA). For each analysis, 6 g of mixed ham was placed on deactivated glass wool at the bottom of a Pyrex extractor. The extractor was maintained at 30°C and purged for 60 min with a helium stream at a flow rate of 60 $ml.min^{-1}$. The Tenax® trap TA 60/80 mesh operated at 30°C. The volatile components were then desorbed from the trap at 180°C using high purity helium and sent into the cryo-focalisation area (-150°C). Volatiles were separated and detected using a GC-MS set-up (GC 6890 Agilent Technologies; capillary column RTX-5, length 60 m, Ø: 0.32 mm, film thickness: 1 µm and a quadrupole mass detector MSD 5973 Inert, Agilent Technologies).

*Solid Phase Micro-Extraction – Comprehensive Gas Chromatography – time-of-flight Mass Spectrometry*

An SPME 75 µm fibber (Carboxen/PDMS) (Supelco, Bellefonte, PA, USA), was used for the extraction of volatiles. For each sample, 4 g of mixed ham was placed in a sealed 2 ml vial and pre-incubated for 60 min at 35°C. After the headspace equilibrium procedure, the SPME needle was exposed to the ham headspace for 30 min at 21°C. After sampling, the fibber was thermally desorbed in the GC injection port for 2 min at 280°C. The samples were analysed using a LECO Pegasus IV GCxGC-*tof*MS instrument (LECO Corporation, St. Joseph, MI, USA) equipped with a cryogenic modulator (LECO Quad Jet Modulator). The first dimension chromatographic column was an SPB5 capillary column (length 30 m, Ø: 0.32 mm, film thickness: 1 µm) and the second was a DB17 capillary column (length 2.5 m, Ø: 0.178 mm, film thickness: 0.30µm). The columns and the modulator (cycle = 7 s) were placed in a gas chromatograph (6890N Agilent Technologies). The first column oven was held at 40°C for 5 min, ramped at 3°C $min^{-1}$ to 230°C and held for 10 min. The column 2 oven was a constant 15°C higher than the column 1 oven. Chromatographic runs took 78 min. Chromatograms were processed using ChromTOF™ software.

*Gas Chromatography – Olfactometry*

Analyses were performed using: - (i) an eight-way gas chromatography-olfactometry device (GC-8WO). This apparatus consists of a chromatograph (Hewlett Packard 4890D) coupled to a divider that distributes the volatile component outflow synchronously to eight transfer lines, the outlets from which are connected to eight sniffing ports located in separate booths (Tournayre and Berdagué, 2005). The overall architecture of the system is depicted in Figure 1. The GC-8WO analytical conditions are described in detail elsewhere (Berdagué et al.,2007).

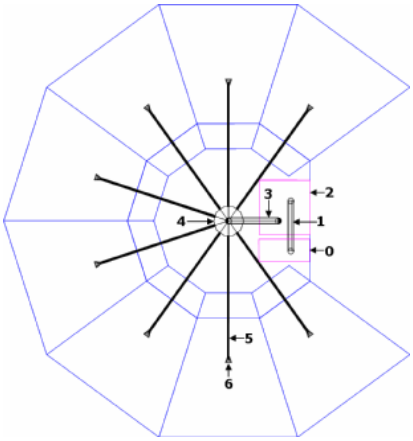


**Figure 1.** Architecture and overview of the GC-8WO device. The configuration presented was coupled to a system of extraction-concentration of volatile components of the purge-and-trap type. The set-up consists of eight individual booths each equipped with its own lighting, soundproofing and laminar flow conditioned air (22°C and deodorised by filtering through active carbon). The diameter of the whole system was 4.5 m and comprised the following: 0 = Purge-and-trap system; 1 = First transfer line; 2 = Gas chromatograph equipped with a silica capillary column; 3 = Second transfer line; 4 = Effluent splitter; 5 = Third transfer lines; 6 = Sniffing ports.

- (ii) an independent Gas Chromatography–quadrupole Mass Spectrometry / one-way Olfactometry (GC-MS/O) set-up composed of a chromatograph (GC 6890, Agilent Technologies), a mass detector (MSD 5973 Inert, Agilent Technologies) and a one-way home-made sniffing port. GC-MS/O analyses were performed by three of the eight most efficient sniffers selected after the GC-8WO analyses. The GC-8WO and the GC-MS/O were each coupled to an identical Purge and Trap device (Tekmar, Cincinnati, OH 45234, USA) device. The GC signals obtained with the GC-MS/O chromatogram and the GC-8WO aromagram were processed and aligned with the AcquiSniff® software (Berdagué and Tournayre, 2002) and analysed by the VIDEO-Sniff® method *(20)*. This method makes full use of the sensory information comprising the number of detections, their intensities, their durations and the vocabulary used to describe the odours perceived by the sniffers. The identification of odour-active compounds was achieved by crossing GC-MS/O, SPME-GCxGC-*tof*MS data, retentions indices, odour databases and olfactory comparisons with pure compounds.

**Results and discussion**

Overall, 612 compounds were identified by GCxGC-*tof*MS (data not presented!). The use of the 8W-GC/O device allowed a rapid and efficient selection of the odour active parts of the chromatograms. The aromagram obtained revealed thirty-four odorous areas (Figure 2). The main biochemical origins of the odour-active compounds are identified: **1)** The lipolysis of triglycerides and phospholipids is responsible for the release of carboxylic acids, which can be more easily oxidised to odorous compounds like aldehydes, ketones, alcohols and shorter-chain carboxylic acids produced throughout the refining stage. Aldehydes ('vegetable, green, rancid or fruity' notes) and 1-Octen-3-ol, 1-octen-3-one and 1-nonen-3-ol ('fungus-like' odours) arise from the oxidation of unsaturated fatty acids. Heptanoic and octanoic acids ('animal, pungent, rancid, meaty' odours) may come directly from the lipolysis or from the oxidation of fatty acids. **2)** The catabolism of amino acids such as valine, leucine and isoleucine is responsible for the formation of short-chain branched aldehydes, alcohols or carboxylic acids such as propanal, 2-methyl and butanal, 3-methyl ('empyreumatic' odours). These compounds are formed by amino acid oxidation mechanisms *via* Strecker degradation which may be favoured in dry-cured ham by a high free amino acid content (intense proteolysis), low water activity (effect of salting) and by the long ripening period. 1-butanol, 3-methyl ('cheesy, malty, burnt, grilled' odour) comes from the reduction of butanal, 3-methyl and 3-methyl butanoic acid ('cheesy, feet, sweaty' odour) in the oxidative catabolism of leucine and isoleucine. The sulfur-containing compounds arise from the breakdown of sulfur-containing amino acids cysteine and methionine. Dimethyl trisulfide ('cabbage, gas' odours) comes from the condensation of methanethiol, and methional ('potato' odour) is formed by the oxidative catabolism of methionine *via* Strecker degradation. **3)** The catabolism of glucids (hydrolysis and subsequent oxidative degradation of glycogen) is responsible for the formation of 2,3-butanedione ('butter' odour). **4)** Among the compounds derived from condensation reactions, we have identified esters and pyrazines. Esterification reactions are relatively active in dry-cured ham. We identified

33 esters of which only 5 were detected by the panel of sniffers. Of these, 4 odorous esters were described as 'fruity'. These were ethyl esters, of which the acid part derives from the catabolism of branched amino acids (propanoic acid, 2-methyl-, ethyl ester; butanoic acid, 2-methyl-, ethyl ester; butanoic acid, 3-methyl-, ethyl ester) or fatty acids (hexanoic acid, ethyl ester). Vinyl acetate was perceived with a low intensity 'chemical, solvent' odour. Pyrazines, present in trace amounts, were the only odour-active heterocycles identified. Of the six pyrazines present in trace amounts and identified by SPME-GCxGC-*tof*MS, two were detected by olfactometry: 2,6-dimethylpyrazine and tetramethylpyrazine and described as having 'empyreumatic' odour by most of the sniffers. These compounds are formed by Maillard reactions, which are slow in ham, and often more active near the surface where the water content is lower. **5)** Some compounds such as alcohols and ketones or short-chain ketones or toluene have multiple biochemical origins. Toluene is also described as an environmental contaminant.

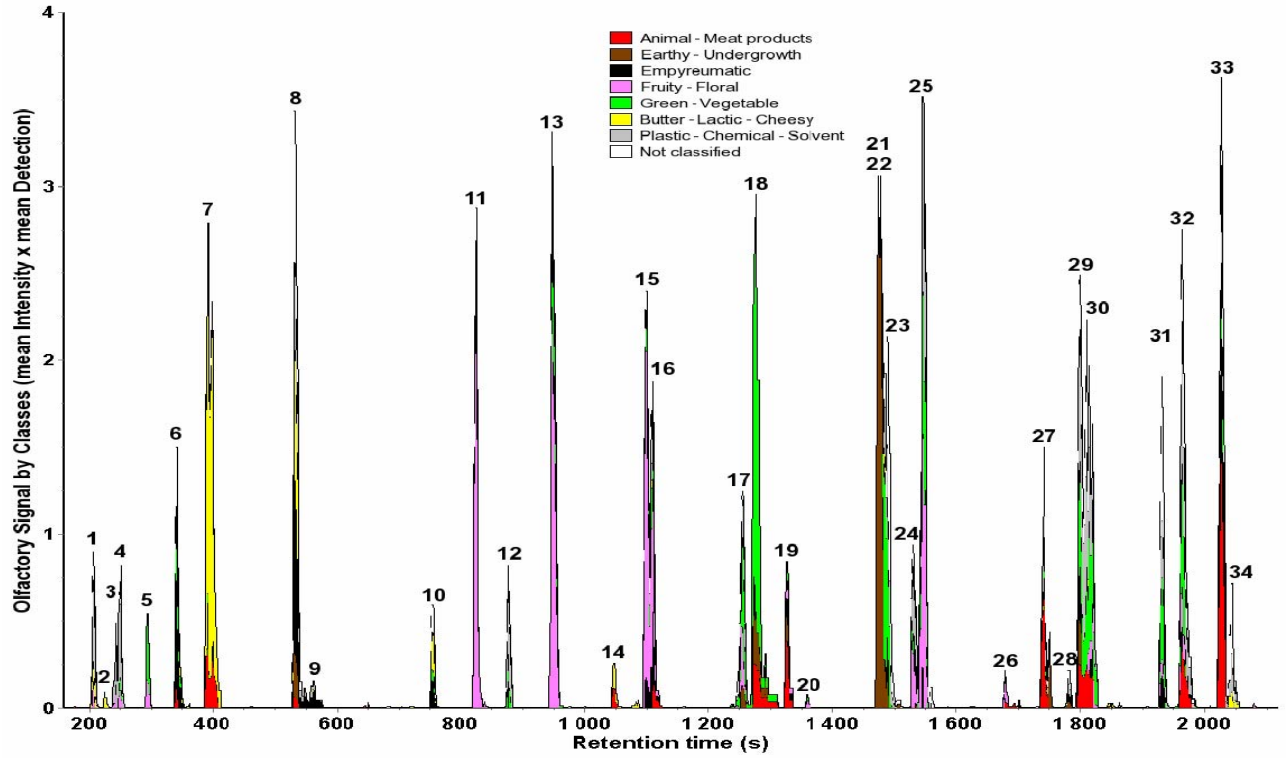


**Figure 2**. This aromagram corresponds to the mean of the 16 individual aromagrams obtained with the eight ways GC-O olfactometer. The breakdown of the olfactory signal into seven classes, using the AcquiSniff® software, clearly shows the aromagram zones belonging to a given olfactory class. **1-2** = ethanol (Alcohol), **3-4** = 2-propanone (solvent), **5** = 2-propanol (Fruity, alcohol), **6** = propanal, 2-methyl- (Empyreumatic), **7** = 2,3-butanedione (Butter, lactic), **8** = butanal, 3-methyl- (Chocolate, rancid cheese), **9** = vinyl acetate (Chemical, plastic), **10** = 1-butanol, 3-methyl-- (Empyreumatic, cheesy), **11** = propanoic acid, 2-methyl, ethyl ester (Fruity), **12** = toluene (Plastic, chemical), **13** = hexanal (Fruity, green apple, vegetable), **14** = butanoic acid, 3-methyl- (Vomit, cheesy), **15** = butanoic acid, 2-methyl-, ethyl ester (Fruity, citrus fruits), **16** = butanoic acid, 3-methyl-, ethyl ester (Fruity), **17** = heptanal (Fruity),. **18** = methional, 2-methyl- (Potatoes), **19** = 2, 6-dimethylpyrazine (Meaty, Roasted), **20** = phenol, 2-ethyl + unknown (Animal, mouldy, dry-cured product), **21** = 1-octen-3-one (Mushroom), **22** = 1-octen-3-ol (Mushroom), **23** = trisulfide, dimethyl- (Cabbage, gas), **24** = hexanoic acid, ethyl ester (Floral, fruity, solvent), **25** = octanal (Fruity, green, chemical), **26** = N.I. (Meaty), **27** = heptanoic acid (Animal, pungent, rancid), **28** = 1-nonen-3-ol (Mushroom), **29** = 2,3,5,6 tetramethylpyrazine (Caramel, chocolate), **30** = nonanal (Green, rancid, plastic), **31** = N.I. (Plastic, empyreumatic), **32** = 2-nonenal (Green), **33** = octanoic acid (Animal, meaty), **34** = N.I. (Detected but not described).

## Conclusions

The use of complementary methods of gas chromatography olfactometry combined with powerful separation and identification methods allows obtaining reliable information necessary for a better understanding of the chemical and biochemical origins of dry-cured ham and meat products aroma.

**References**

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