VOLATILE SULFUR COMPOUNDS AND COOKED HAM AROMA

C. THOMAS[1], J.L. VENDEUVRE[1], A.S. GUILLARD[1], J. SUDRON[2], F. MERCIER[2], P. TOURNAYRE[2], P. FUCHSMANN[3], K. BREME[3] AND J.-L. BERDAGUÉ[2]

[1] IFIP, 3/5 Rue Lespagnol, F-75020 Paris, France. [2] INRA, UR370 QuaPA, F-63122 Saint Genès Champanelle, France. [3] AGROSCOPE, ALP Research Station, Analytical Department, CH-3003 Bern, Switzerland.

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Economic Affairs, Education and Research EAER
Agroscope

INTRODUCTION

The aim of this study was to acquire knowledge about volatile sulfur compounds (VSCs) of cooked ham, which are poorly described in the literature - maybe because the analysis of these compounds is particularly difficult. Indeed, they are (i) present at trace levels (limited production during the cooking at low temperature : T < 70 °C) and are furthermore (ii) very difficult to extract and to detect due to a strong adsorption by the matrix and high chemical reactivity of thiols during analyses.

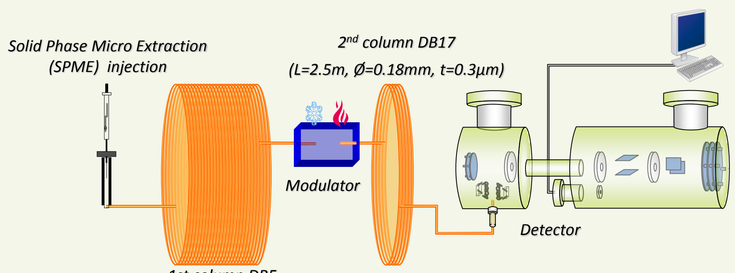
Here we report on the implementation of different methods of extraction and gas chromatographic analysis and detection to achieve an extensive identification of VSCs in industrial hams: Headspace-Solid Phase Microextraction coupled to the Comprehensive Gas Chromatography-time of flight Mass Spectrometry (HS-SPME-GCxGC-MStof), Dynamic Headspace coupled to the Gas Chromatography-Mass Spectrometry (D-HS-GC-MSquad), HS-SPME coupled to the Gas Chromatography/Pulsed Flame Photometric Detector and Mass Spectrometry (HS-SPME-GC/PFPD-MSquad) or a specific extraction of thiols with mercury salts (adapted from [1]) followed by a GCxGC-MStof analysis.

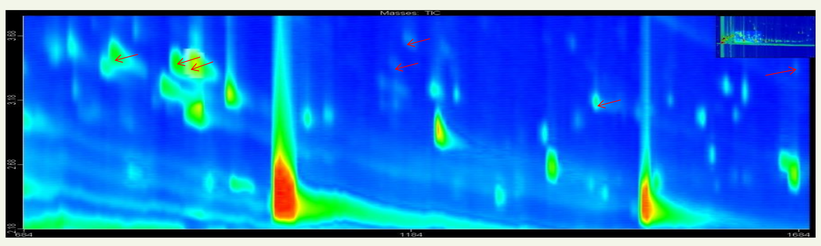
An exhaustive detection of odorants was performed by Dynamic Headspace coupled to the 8 ways Gas Chromatography-Olfactometry (D-HS-GC-8O) followed by Heartcut Gas Chromatography-Mass Spectrometry/Olfactometry (D-HS-GC-GC-MS/O) analyses.

MATERIALS and METHODS

Structures identification

Comprehensive Gas Chromatography -Time of Flight Mass Spectrometry

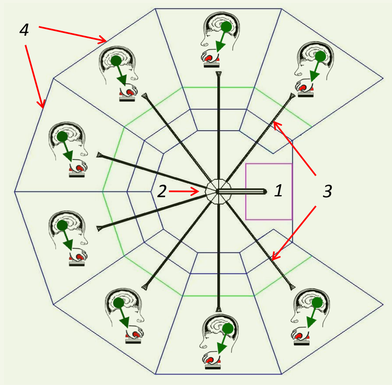




This method allows to clearly **identify** compounds co-eluted at trace levels like VSCs (Red arrows)

Odorants detection and identification

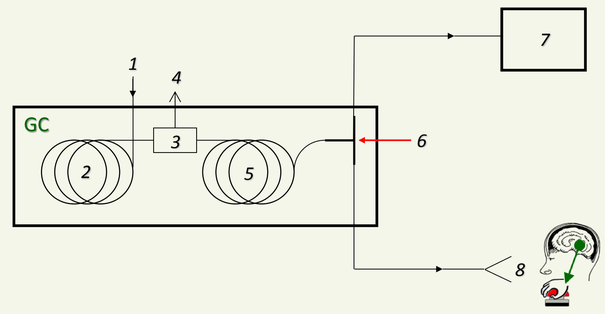
8 ways Gas Chromatography-Olfactometry device



1: GC-MS; 2: Effluents divider; 3: 8 transfer lines; 4: eight booths with terminals of olfaction for simultaneous olfactory detection by 8 judges in one single GC run.

The 8 ways Gas Chromatography-Olfactometry allows an exhaustive olfactory **detection** of odorants [2,3].

Heartcut Gas Chromatography-Mass Spectrometry/Olfactometry device



1: ham headspace; 2: DB5 Capillary column; 3: Switching / cryofocusing module; 4: vent; 5: DBWax Capillary column; 6: Effluent divider; 7: Mass spectrometer; 8: terminal of olfaction.

Heartcut Gas Chromatography-Mass Spectrometry/Olfactometry (GC-GC-MS/O) is an efficient tool to **identify** odorants.

RESULTS

Identified sulfur compounds

Sulfides

Dimethylsulfide [a, b, d] — Allyl methyl sulfide [b] — 3-(methylthio)propanal [d]
Dimethyldisulfide [a, b, d] — Hydrogen sulfide [a, b, d] — 1-(methylthio)prop-1-ene [a]
Dimethyl trisulfide [a, b, d] — carbon disulfide [a, b, d] — (methylthio)acetate [a, b]
Dimethyl tetrasulfide [a] — Carbonyl sulfide [b, d] — 2-methyl-3-(methylthio)-butane [b]

Thiophenes

Thiophene [a, b, d] — 3-(methylthio)tiophene [b] — 2,5-dimethylthiophene [a]
3-acetyl-thiophene [a, c] — 2/3-methylthiophene [a, b, d] — 2-acetyl-thiophene [a, c]
2-methyl-3-thiophenethiol [a] — Thieno[3,2-b]thiophene [a]
2-butylthiophene [a] — 2-ethylthiophene [a] — 2-pentylthiophene [a]

Identifications

a = HS-SPME-GCxGC-M*stof* (identification of 32 VSCs)
b = HS-SPME-GC-PFPD/MS*quad* (21 VSCs)
c = Specific thiols extraction [1], GCxGC-M*stof* (5 VSCs)
d = DH-GC-MS/*O and* = DH-GC-GC-MS*quad* (20 VSCs)

Similarity Indices of Mass spectra ≥ 900 and Retention Indices coherent with databases values.

Thiazoles

5-methylthiazole [a] — Thiazole/isothioazole [a, b, d] — Benzothiazole [c]

Furans

2-methyl-3-furanthiol [a, b, c, d] — 2-methyl-5-(methylthio)furan [a, b]
methyl 2-methyl-3-furyl disulfide [a, b, d] — bis(2-methyl-3-furyl)disulfide [a, c, d]

Other sulfur compounds

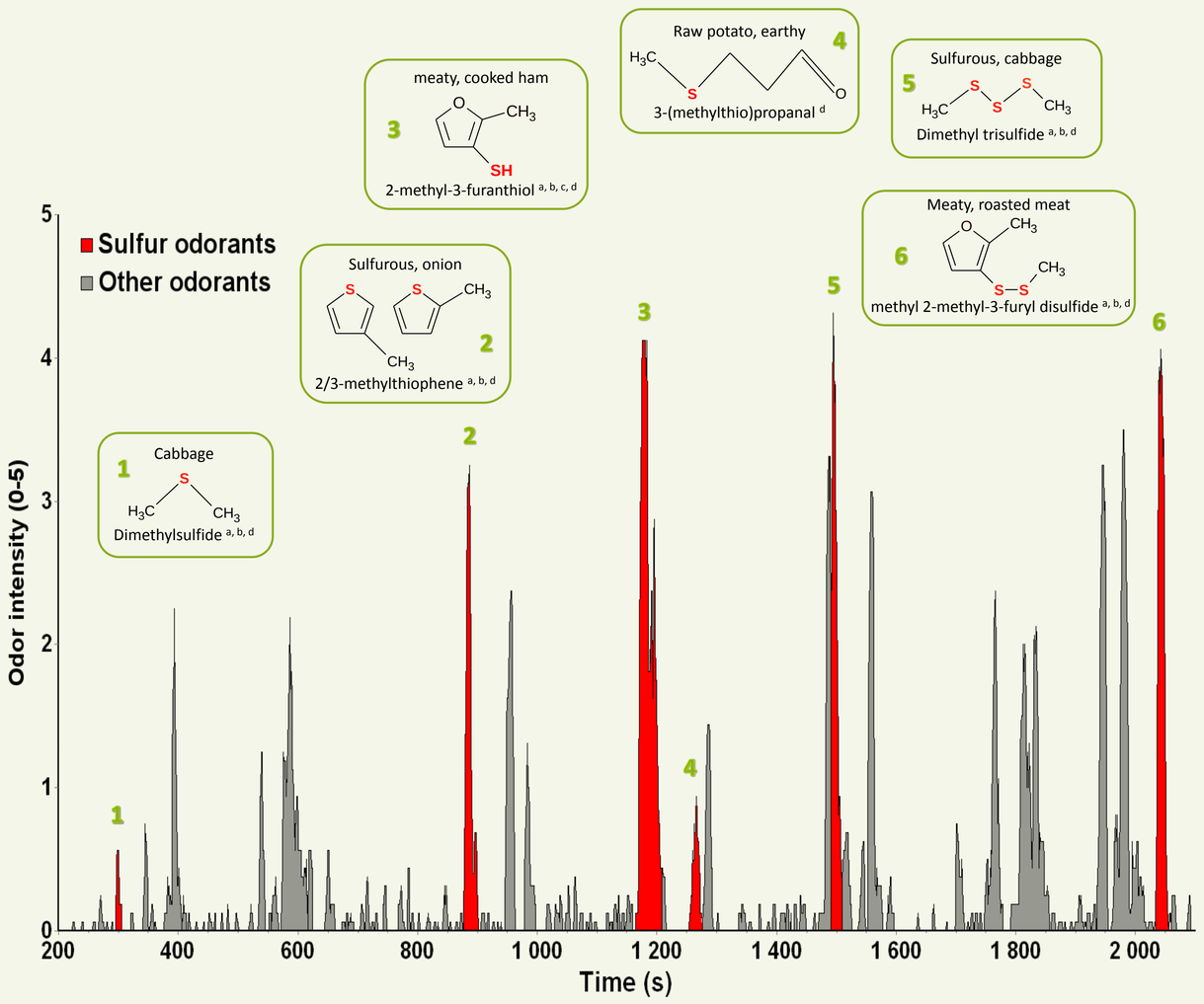
Thiirane [a] — *p*-Dithiane-2,5-diol [a] — Sulfur dioxide [a, b, d] — Dimethyl sulfone [a, b, d]

Aliphatic thiols

Methanethiol [a, b, d] — 1,2-ethanedithiol [a]

- Taking into account all employed techniques, a total number of 38 sulfur compounds were identified in the volatile fraction of cooked ham.
- According to the literature [4], most of these compounds originate from a Maillard reaction between sulfur containing amino acids (cystein or methionine) and sugars or from thermal degradation of thiamine. Aliphatic sulfur compounds and furans are mainly produced from Strecker degradation. Thiophene and thiazole are products of Amadori and Heyns rearrangements.
- The most efficient method of detection and identification is HS-SPME-GCxGC-MStof followed by HS-SPME-GC/PFPD-MSquad. The specific extraction of thiols with mercury salts allows to extract important quantities of 2-methyl-3-furanthiol but also induce a dimerisation of this compound into *bis*(2-methyl-3-furyl) disulfide during chromatography.

8 ways GC-O aromagram of a cooked ham



- This aromagram demonstrates the important role of sulfur odorants in cooked ham and reveals that only 7/38 compounds identified by mass spectrometry are perceived by olfactometry.
- Among these sulfur odorants; sulfides, methional, 2-methyl-3-furanthiol and methyl 2-methyl-3-furyl disulfide have very low detection thresholds (0.001-0.0025ng/L in air, [5])
- Olfactometry analyses indicate that pure 2-methyl-3-furanthiol and pure methyl 2-methyl-3-furyl disulfide have "meaty" odors very similar to the odor of cooked ham.

CONCLUSION

- The different analytical approaches have lead to the identification of 38 volatile sulfur compounds.
- Despite a low temperature of cooking , a large variety of cyclic or aliphatic sulfur compounds are produced in ham during cooking.
- Because of their intense meaty odors and important odor impact revealed by GC-O, 2-methyl-3-furanthiol and methyl-2-methyl-3-furyl disulfide appear to be the essential sulfur compounds for cooked ham aroma.

REFERENCES

[1] Tominaga, T.; Dubourdieu D. 2006. *J. Agricultural and Food Chemistry*, ***54***, 29-33.
[2] Berdagué, J.L.; Tournayre, P.; Cambou, S. 2007. *J. of Chromatography A*, **1146** (1), 85-92.
[3] AcquiSniff® Software Distributed by INRA Transfert, F-75015 Paris, France. AcquiSniff@clermont.inra.fr
[4] Mottram, D.S. 1998. *Food Chemistry*, **62**, (4), 415-424.
[5] Filippi, J.-J.; Fernandez, X.; Duñach, E. 2007. Sciences des Aliments, **27**, 23-46.

EUROFOODCHEM XVII - May 07-10, 2013 Istanbul, TURKEY

Centre de Clermont-Theix-Lyon
63122 Saint Genès Champanelle – France
www.clermont.inra.fr