Analysis of neo-formed volatile heterocycles during meat cooking

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Abstract

For nutritional purposes, edible animal products are often enriched through supplementation of animal feed with unsaturated fatty acids. However, in the case of meat it is important to know the fate of these fatty acids undergone during cooking. Depending on the cooking conditions, a non-negligible proportion of these acids can be consumed by oxidative mechanisms and Maillard reactions leading to the production of neo-formed substances which can be detrimental for human health. This work aims at devising a set-up to study the volatile heterocyclic compounds produced during cooking. For this purpose, we developed a cooking cell allowing the control of thermodynamic variables and atmosphere. This cell was coupled to a system designed to collect volatile compounds by solid phase micro-extraction (SPME). The compounds were further analysed in details using comprehensive gas chromatography – time-of-flight mass spectrometry (GCxGC-MStof). More than 1000 compounds were identified including 112 heterocycles showing a broad structural and chemical diversity. The developed equipments are now on use to gain a better understanding of the impact of cooking conditions on the production of heterocyclic structures according to the degree of unsaturation of meat lipids.

Materials and methods

Meat samples: Charolais beef cattle was fed a standard diet either supplemented or not with linseed for 50 days prior to slaughter. After slaughter, a one-gram sample of aged meat was cut in the *Semi membranosus* muscle and placed on a stainless steel needle (Figure 1).

Cooking process: Each sample was cooked for 15 minutes. Cooking temperature was 40°C initially and then was increased to 200°C according to a 50°C.min^{-1} gradient. A 50 ml.min^{-1} air flow in the cooking cell directed the emanations to the SPME fibre.

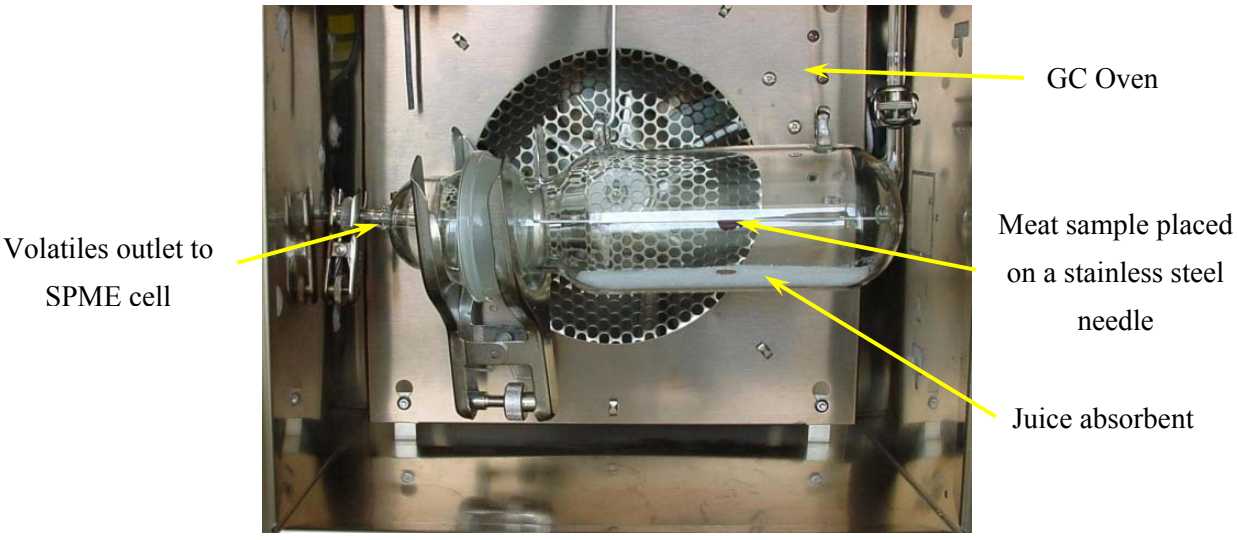

Figure 1: The cooking cell was installed into the oven of a gas chromatograph in order to take advantage of the temperature regulations.

Sampling of volatiles: An SPME 75 µm fibber (Carboxen/PDMS) (Supelco, Bellefonte, PA, USA), was used for the extraction of volatiles and introduced into a 250 ml expansion chamber (cooled at 5°C to trap the water vapour) directly coupled to the cooking cell. The SPME needle was exposed to the cooking vapours during the last ten minutes of cooking. After sampling, the fiber was thermally desorbed in the GC injection port for 2 min at 280°C.

Separation and identification of volatiles: The samples were analysed using a GCxGC-MStof instrument (LECO Pegasus IV, 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 dimension chromatographic column 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 at this temperature. The column 2 oven was constantly maintained at a temperature 15°C higher than that of column 1 oven. Each chromatographic run took 50 min. Chromatograms were processed using the ChromTOF™ software.

Results and discussion

Due to the great complexity of the extracts, the GCxGC-MStof analysis was essential to separate and to identify the volatile heterocycles produced during meat cooking. Actually, the reliable identification of cyclic structures that co-elute with a large number of carbonylated aliphatic compounds derived from meat fatty acids is made quite difficult without bidimensional chromatographic separation (Figure 2). Also the high sensitivity of time-of-flight mass spectrometers made possible by the high acquisition frequency provides high quality mass spectra even for trace compounds.

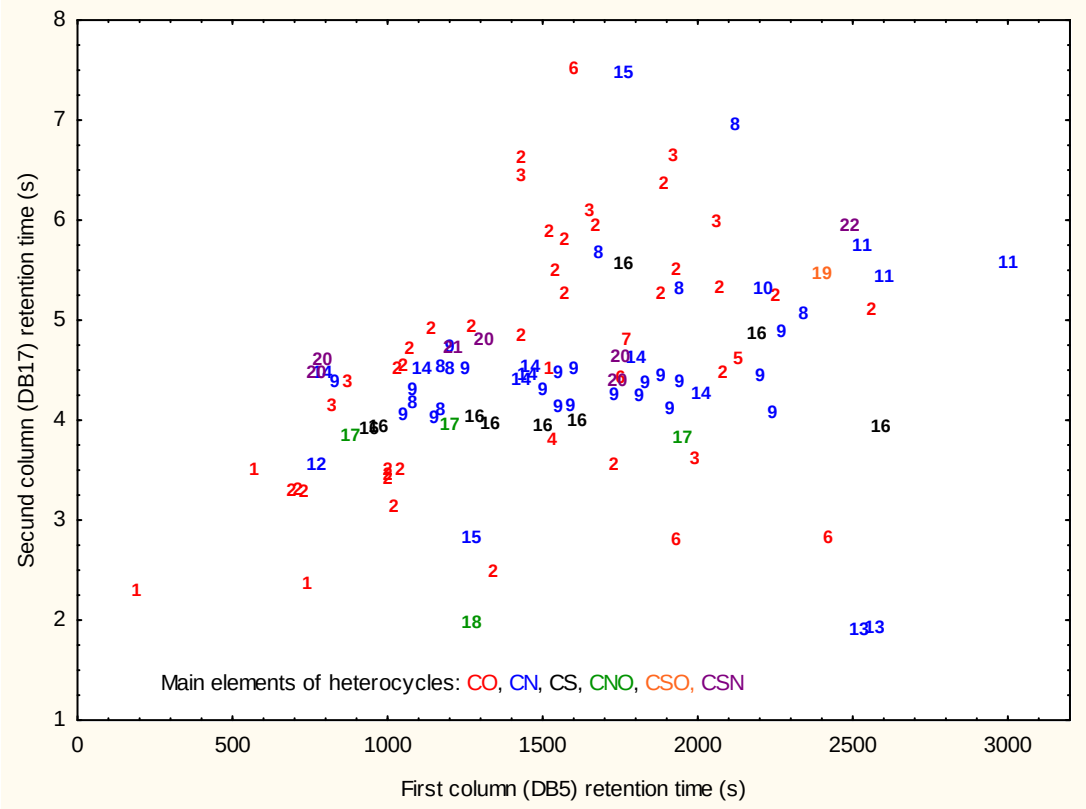

Figure 2: Zoom of the GCxGC (LECO Pegasus IV) Signal. Among the large number of volatile compounds identified, only the apex of heterocycles identified on the basis of their spectral similarities and their chromatographic retention indices are presented. The numbers correspond to the cyclical structures identified: **1** = Oxyrane, **2** = Furane, **3** = Pyrane, **4** = Oxepine, **5** = Benzofurane, **6** = Dioxolane, **7** = Benzodiazole, **8** = Pyrrole, **9** = Pyridine, **10** = Pyridine, **11** = Indolizine, **12** = Pyridazine, **13** = Pyrimidine, **14** = Pyrazine, **15** = Tetrazole, **16** = Thiophene, **17** = Oxazole, **18** = Oxazine, **19** = Cyclopentathiapyrane, **20** = Thiazole, **21** = Isothiazole, **22** = Benzothiazole.

In the present analytical conditions, GCxGC-MStof analyses allowed to identify more than 2000 compounds including 112 heterocycles showing broad structural and chemical diversities (Figure 2 and 3). These heterocycles originate mainly from lipid oxidation and Maillard reactions. In our cooking conditions, CO and CN were the heterocycles present in highest quantities. Among these compounds furans and pyridines were the best represented both in number and in quantity in the chromatographic profiles. From a safety perspective, a large number the heterocyclic compounds identified can be considered as potentially toxic or mutagenic according to the TOXNET® database (Wexler, 2001). To date many studies have confirmed the toxicity of heterocyclic amines produced during the cooking of meat (Machiels et al., 2003). However little work has been undertaken to evaluate the risk associated with neoformed heterocyclic compounds of lower molecular weight contrary to the work undertaken to demonstrate their aromatic

properties (Mottram, 1998).

The first results of the present work show that cooked meats containing higher unsaturated fatty acids also contain higher quantities of neo-formed volatiles heterocycles.

Conclusion and prospects

The developed equipments are now on use to gain a better understanding of the impact of cooking conditions on the production of heterocyclic structures according to the degree of lipid unsaturation of meats. Our current research aims at evaluating if the production of undesirable heterocyclic compounds during the cooking of meats does not counteract the beneficial nutritional effect expected from an increase in their polyunsaturated fatty acids content.

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