PROCESS-INDUCED TOXICANTS AND ODORANTS GENERATED BY MEAT COOKING

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Abstract – Cooking conditions are the key factor for organoleptic and sanitary qualities of meat products. This study focused on these bivalent properties by determining the odour-active compounds and polycyclic aromatic hydrocarbons (PAHs) in cooked meat with high resolution GC-olfactometry and GC×GC-TOF/MS, respectively. PCA permitted to stress that to achieve a balance between sanitary and organoleptic properties, a compromise in the cooking conditions has to be reached as both key odorants and toxicants are more produced when heating intensity is increasing.

Key Words – food acceptance, odour-active compounds, polycyclic aromatic hydrocarbons

I. INTRODUCTION

Cooking has drastic and contrasted influences on meat properties [1]. While it produces the compounds responsible for cooked meat odour/aroma, it generates heat-induced toxicants such as PAHs. The formation pattern of these molecular determinants of meat quality strongly depends on cooking conditions. The present paper investigates the consequences of the main cooking processes on beef meat profile in odour-active compounds and in 17 PAHs.

II. MATERIALS AND METHODS

Cooking processes. All beef samples were prepared in the same conditions. Different cooking modes were studied: pan frying at 170°C for 8 min, oven cooking for 20 min at 150, 200 or 250°C under different atmospheric gas conditions (air, nitrogen and oxygen), microwave heating at 600W for 15s, and grilling using proper electrical appliances. These cooking methods were selected objectively to represent several heat transfer techniques including conduction (pan cooking), convection (oven and grilling), and radiation (microwave). For each of the 12 cooking conditions, three restructured beef steaks were cooked and temperatures were monitored for meat core, surface and cooking chamber.

Comprehensive GC×GC-TOF/MS for PAH analysis. PAHs were first extracted from cooked meat by accelerated solvent extraction (ASE). After concentration of the ASE-extract by centrifugal evaporation, the corresponding sample was analysed by GC×GC-TOF/MS [2] on BPX-5 (30x0.25x0.25) × BPX-50 (1x0.1x0.1) column set. The identification and quantification of the compounds by TOF/MS were supported by a customised pesticide library. The precision of the method was evaluated in terms of repeatability, limit of quantification (LOQ), as well as recovery rates.

High resolution olfactometry for odour-active compound determination. A GC-MS analysis of the 36 samples was performed in order to differentiate four groups of cooking modes according to the cooked meat content in volatile compounds. Customized GC-MS-8O was run on one sample of each group with a panel of eight trained sniffers for the identification of well resolved odour-active compounds [3, 4]; a customized two-dimensional "heartcut" GC coupled to MS and olfactometry (GC-GC-MS-O) was further used to resolve coeluting substances in the aromagram and to identify the corresponding odour-active compounds. The nature of identified compounds was validated by using customized odour databases, olfactory comparisons with pure compounds as well as retention index and mass spectra databases.

Data treatments. Data were processed with the Statistica Software release 8.0 package (Statsoft, Maisons-Alfort, France) and the R software

release 2.14.0. One-way-ANOVAs were performed on PAH profiles and aromagrams in order to select the compounds whose level was affected by the mode of cooking. The data were normalized by Systematic Ratio Normalization [5] and PCAs were processed on the resulting datasets to discriminate meat cooking conditions based on PAH content or odour active compounds.

III. RESULTS AND DISCUSSION

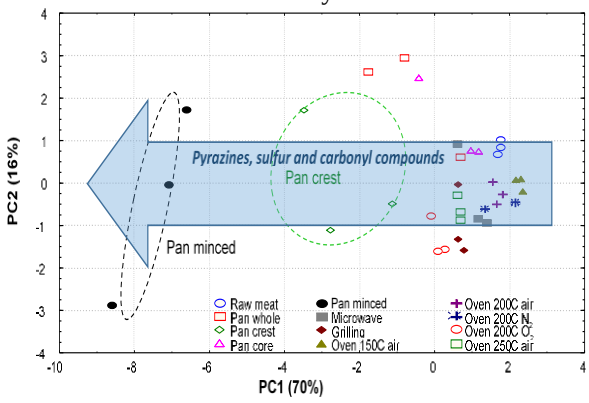
Process-induced odorants. The content in volatile compounds of the 36 samples was determined by GC-MS after solid-phase microextraction (SPME). A PCA enabled to differentiate four clusters of cooking modes. The use of the GC-8O/MS system on one sample of each group enabled to detect 53 significant odour zones and to identify by MS the corresponding odour-active compounds. Certain aroma zones were unresolved probably due to the coelution of aroma compounds leading to a possible masking of trace-level odour-active compounds by important interferences of olfactive impressions and resulting in unreliable olfactive characterization. To resolve these coeluting odour zones, a GC-GC-O-MS was run on the samples and 15 additional odour-active compounds were pointed out [6]. Finally, a total of 68 odour-active compounds were shown to contribute to the odour/aroma of cooked meat. Table 1 gives the major odour-active compounds as determined by the odour-activity value.

Table 1 Major aroma active volatile compounds in cooked beef under different cooking techniques

Compounds detected	Odour (from olfactometry experiments)
Sulfur dioxide	Sulfurous
2,3-butanedione	Butter, caramel, yogurt
Toluene	Chemical, rubber, glue
Butanoic acid	Rancid, cheese
Hexanal	Grass, green apple
3-methylthiopropanal	Mashed potatoes, cabbage
Ethylpyrazine	Toast, bread, cold coffee
Sulfonylbismethane	Cooked cabbage
γ-crotonolactone	Cream, butter, hot milk
2,3-dimethylpyrazine	Dry ham, cooked rice, peanut
4-methylpentanoic acid	Dirty socks, sweat, foot
1-octen-3-ol	Mushroom
1-octen-3-one	Cooked mushroom
Dimethyl trisulfide	Cabbage, salsify, cooked cauliflower
2-octanone	Cheesy, old cheese
Octanal	Citrus fruit, lemon, orange
2,3,5-trimethyl-6-ethylpyrazine	Bread, hot, dust
2,5-dimethyl-3-furanthiol	Cooked meat, roasted meat

The SPME-GC-MS dataset was restricted to the volatile compounds found odour-active in the GC-olfactometry experiment. A PCA was performed on the resulting dataset. Its first map, presented on Figure 1, confirms that the more intense the cooking conditions are, the more odour-active pyrazines, sulfur and carbonyl compounds are formed, which is consistent with Mottram's conclusions [7]. The generation of these key compounds mainly results from Maillard reactions associated with Strecker degradations [8, 9] and thermal degradation of lipids contained in meat matrix.

Figure 1. First map of a PCA processed on cooked meat odour-active compound data pointing out the correlation between the level of some pyrazines, sulfur and carbonyl compounds and heat treatment intensity



Process-induced toxicants. A GC×GC-TOF/MS based-method was developed in order to achieve a multiresidue separation of 17 PAHs not only in neat solvent but also in complex meat matrix [6]. The use of two-dimensional GC in adapted analytical conditions permitted to solve common coelution problems stressed in several studies associated with cyclopenta[*c,d*]pyrene, benzo[*a*]anthracene and chrysene as well as benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and

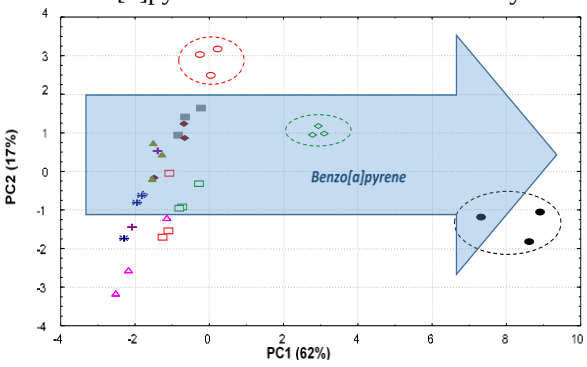
benzo[*k*]fluoranthene [10, 11]. Unfortunately a coelution between triphenylene and chrysene could not be solved [10, 12], inducing a probable overestimation of chrysene in our samples. The use of a chiral column in the second dimension could be a good option to solve this problem. Recovery data found were generally good, except for naphthalene, acenaphtalene and acenaphtene but the different PAHs were quantified with a good sensitivity (table 2) as the limit of quantification (LOQ) was determined around 0.016 µg/kg. The existing performance criteria for benzo[*a*]pyrene (BaP) are generally used as point of comparison for the other PAHs. The present analytical conditions for BaP in terms of LOQ were found to be compatible with the concentration range potentially met in food [13].

Table 2 Identification of PAHs, limits of quantification (LOQ) and toxic equivalency factors (TEF) of PAHs

PAH	Recovery %	LOQ (µg/kg)	TEF compared to Benzo[*a*]pyrene
Naphthalene	30	0.076	0.0001
Acenaphthylene	57	0.063	0.0001
Acenaphthene	41	0.057	0.0001
Fluorene	84	0.023	0.0001
Phenanthrene	109	0.036	0.0001
Anthracene	84	0.043	0.28
Fluoranthene	101	0.02	0.001
Pyrene	111	0.04	0.001
Cyclopenta[*c,d*]pyrene	86	0.03	0.012
Benzo[*a*]anthracene	85	0.036	0.014
Chrysene	104	0.016	0.026
5-methylchrysene	87	0.023	0.45
Benzo[*b*]fluoranthene			0.035
Benzo[*j*]fluoranthene	96	0.216	0.035
Benzo[*k*]fluoranthene			0.035
Benzo[*a*]pyrene	89	0.063	1

The 36 cooked meat samples (12 modes of cooking in triplicate) were run with the GC×GC-TOF/MS method in order to determine the PAH profile of each sample. A PCA was run on the corresponding dataset in order to assess a potential clustering of the cooking mode according to their ability to generate toxic PAHs. The first map of the PCA (figure 2) confirms that intense cooking modes promote the generation of benzo[*a*]pyrene which is by far the most toxic PAH congener.

Figure 2. First map of a PCA processed on PAH data pointing out the correlation between the level of benzo[*a*]pyrene and heat treatment intensity



IV. CONCLUSION

The present paper first demonstrated that high resolution olfactometry (GC-MS/8O combined with GC-GC-MS/O) was a relevant technique to determine odour-active compounds in meat matrix by resolving coeluting odour-active compounds. As demonstrated by its adding-value in terms of odour-active compounds detected (15 out of 68), this approach should be applied more systematically to processed food with complex aroma. In the same way, GC×GC-TOF/MS was confirmed to be a relevant technique for the multiresidue determination of toxicants such as PAHs. Finally, the parallel profiling of odour-active compounds and PAHs demonstrated that severely cooked meats are characterized by the important presence of the most carcinogenic PAH, benzo[*a*]pyrene, and aromatic pyrazines and carbonyl compounds [6]. Those results could be used for multi-objective optimization of the cooking process applied to meat in order to find a proper balance between flavour acceptability and food safety.

ACKNOWLEDGEMENTS

The team MASS thanks the conseil régional d'Auvergne and the FEDER in the project "territoire, agriculture, alimentation en Auvergne" of the CPER 2007-2013 for their financial help.

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