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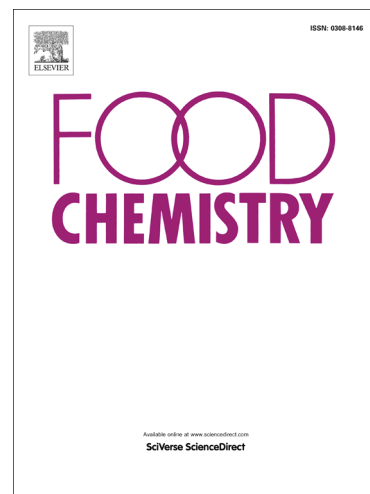
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Effects of pan cooking on micropollutants in meat

Christelle Planche^{a,b,c}, Jérémy Ratel^a, Patrick Blinet^a, Frédéric Mercier^a, Magaly Angénieux^a,
Claude Chafey^d, Julie Zinck^d, Nathalie Marchond^d, Sylvie Chevolleau^{b,c}, Philippe Marchand^e,
Gaud Dervilly-Pinel^e, Thierry Guérin^d, Laurent Debrauwer^{b,c}, Erwan Engel^{a*}

^a INRA, UR370 QuaPA, MASS group, F-63122 Saint-Genès-Champanelle, France

^b Toxalim, Université de Toulouse, INRA, INP-ENVT, INP-EI-Purpan, Université de
Toulouse 3 Paul Sabatier, F-31027 Toulouse, France

^c Axiom Platform, UMR 1331 Toxalim, MetaToul-MetaboHUB, National Infrastructure of
Metabolomics and Fluxomics, F-31027

^d Université Paris-Est, Anses, Laboratory for Food Safety, F-94701 Maisons-Alfort, France

^e LUNAM Université, Oniris, LABERCA, USC INRA 1329, F-44307 Nantes, France

* Corresponding Author:

Erwan Engel: Email: erwan.engel@inra.fr Tel: +33 4 73624589

Abstract

This work presents the effects of pan cooking on PCBs, PCDD/Fs, pesticides and trace elements in meat from a risk assessment perspective. Three different realistic cooking intensities were studied. A GC×GC-TOF/MS method was set up for the multiresidue analysis of 189 PCBs, 17 PCDD/Fs and 16 pesticides whereas Cd, As, Pb and Hg were assayed by ICP-MS. In terms of quantity, average PCB losses after cooking were $18 \pm 5\%$ for rare, $30 \pm 3\%$ for medium, and $48 \pm 2\%$ for well-done meat. In contrast, average PCDD/F losses were not significant. For pesticides, no loss occurred for aldrin, lindane, DDE or DDD, whereas losses exceeding 80% were found for dieldrin, sulfotep or phorate. Losses close to the margin of error were observed for trace elements. These results are discussed in light of the physicochemical properties of the micropollutants as well as of water and fat losses into cooking juice.

Keywords

Meat; Pan cooking; Polychlorinated biphenyls (PCBs); Polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs); Trace elements; Pesticides; Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOF/MS); Chemical risk assessment

1. Introduction

Food represents one of the main sources of human exposure to harmful pollutants, of which meat in particular can form a non-negligible source (Engel et al., 2015). For example, about 90–98% of the average exposure of humans to PCBs and PCDD/Fs results from dietary intake, with food of animal origin being the predominant source (Malisch *et al.*, 2014). Most

micropollutants, whether environmental or phytosanitary, can accumulate in animal tissues during stock raising and remain in meat end-products for human consumption (Takaki *et al.*, 2015). Among the toxic contaminants liable to being found in foods, and in particular meat products, polychlorinated biphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), pesticides and trace elements are important to monitor because these chemicals include many toxic congeners that are especially persistent in the environment (Darko *et al.*, 2007; Zheng *et al.*, 2013 ; Malisch *et al.*, 2014). PCBs were massively used in industry as insulators until the 1980s. PCDD/Fs are released in the environment by industrial and other human activities. Pesticides used as crop-control agents persist in cereals and other plants consumed by animals, and levels of trace elements naturally present in the environment can be increased by industrial activities or pollution.

Although these micropollutants are generally found in trace amounts in animal tissues, the human health risk they imply cannot be ignored. It is therefore important to assess precisely the risk incurred by consumers. To this end, the technological processes the food undergoes before consumption, in particular cooking (Hori *et al.*, 2005), have to be considered. Indeed, levels of contaminants can be affected by cooking, since contaminants can be released in cooking juices, evaporate, or be broken down by heat (Abou-Arab, 1999; Bayen *et al.*, 2005; Rawn *et al.*, 2013; Muresan *et al.*, 2015). A wide examination of literature data regarding the effects of cooking on food micropollutants show major discrepancies between laboratories (Stachiw *et al.*, 1988; Zabik *et al.*, 1995; Schechter *et al.*, 1998; Petroske *et al.*, 1998; Rose *et al.*, 2001). For instance, Hori *et al.* (2005) reported an approximately 40% cooking-induced decrease of PCBs and PCDD/Fs in beef (250-g chunks), whereas Perelló *et al.* (2010) found that cooking enhanced PCB concentration in veal steak, loin of pork, breast and thigh of chicken or even steak and rib of lamb. At least two factors may explain these inconsistencies. First, cooking conditions could vary a lot between studies but

the extent of these variations is difficult to assess because cooking protocols are generally poorly documented, with no or very little information regarding temperature monitoring and control. Second, most of these studies deal with naturally contaminated food matrices. Thus, these matrices differ significantly not only in micropollutant concentration but also in water and fat content, which may explain the variability of the cooking effects observed on micropollutants. Due to these discrepancies, no clear conclusion can be drawn regarding the effect of cooking on micropollutants. Thus, these former studies are not sufficient to feed risk assessment models, which require robust and reproducible data. To address this challenging issue, a realistic, standardized and reproducible cooking method must be set up. It is also necessary to use a homogeneous matrix intentionally and uniformly contaminated at a known and high enough concentration to be detected, even after cooking (Northcott *et al.*, 2000). Finally, since it is expected that micropollutants have different behaviors during cooking depending on their physicochemical properties, there is a need to widen the range of micropollutants investigated.

To enable a high-throughput assessment of the effects of cooking on a wide variety of harmful contaminants in food, multiresidue methods (MRMs) are particularly suitable (Tang *et al.*, 2013). Several studies demonstrated the relevance of comprehensive two-dimensional gas chromatography (GC×GC) in tandem with time-of-flight mass spectrometry (TOF-MS) to assay PCBs, PCDD/Fs (Focant *et al.*, 2004a; Focant *et al.*, 2005) and pesticides (Engel *et al.*, 2013). Some authors have focused on the combined assay of PCBs and PCDD/Fs (Planche *et al.*, 2015) or PCBs and pesticides (Van der Lee *et al.*, 2008), but to our knowledge, no GC×GC-TOF/MS method had yet been developed for the simultaneous assay of PCBs, toxic PCDD/Fs, and organochlorine and organophosphorus pesticides. For trace elements, a method was validated by the French National Reference Laboratory (Chevallier *et al.*, 2015) to

quantify 31 essential and non-essential trace elements in food by inductively coupled plasma mass spectrometry (ICP-MS).

With the ultimate aim of making a better assessment of risks due to chemical contamination of food, we have studied the effects of cooking on the levels of PCBs, PCDD/Fs, pesticides and trace elements in meat. For this purpose the first part of this paper was focused on the set up of a GC×GC-TOF/MS multiresidue method for the simultaneous assay of PCBs, PCDD/Fs and pesticides in cooked ground beef. In a second part, this method, together with the ICP-MS method, was used to assess the effects of different realistic cooking intensities on micropollutant levels in contaminant-spiked meat. The validity of these results obtained with spiked meat will be discussed in the light of contaminant levels measured after cooking in naturally contaminated meat samples.

2. Materials and Methods

2.1. Chemicals and standards

Hexane, dichloromethane, acetone, methanol and toluene were organic trace analysis grade solvents (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Activated aluminum oxide (acidic, Brockmann I) and diatomaceous earth used for the preparation of accelerated solvent extraction (ASE) cells were obtained from Sigma-Aldrich. Micropollutant reference standards were obtained from AccuStandard Europe (Niederbipp, Switzerland) for the 209 PCBs, the organochlorine and organophosphorus pesticides, and from LGC Standards (Molsheim, France) for the 17 PCDD/Fs. The 209 PCBs were divided into nine $10 \text{ ng } \mu\text{L}^{-1}$ stock solutions. For PCDD/Fs, the concentration of the stock solution was different according to the congeners: tetra- $2.5 \text{ ng } \mu\text{L}^{-1}$, penta-, hexa- and hepta- $6.25 \text{ ng } \mu\text{L}^{-1}$ and octa- $12.5 \text{ ng } \mu\text{L}^{-1}$. Organochlorine and organophosphorus pesticide solution concentrations were $2000 \text{ ng } \mu\text{L}^{-1}$.

Internal standards were used for the accurate quantification of target compounds: 3'-F-PCB-28 (Chiron, Trondheim, Norway), 3'-F-PCB-81 (Chiron), ¹³C-labeled PCB-111 (Wellington laboratories, Guelph, ON, Canada), ¹³C-labeled PCB-194 (Wellington) and fenchlorphos (Sigma-Aldrich). For trace elements analysis, standard solutions containing 1000 mg L⁻¹ arsenic, cadmium, mercury and lead and internal standard solutions of 1000 mg L⁻¹ of scandium, rhenium, indium, yttrium and bismuth were purchased from Analytika (Prague, Czech Republic) or from Ultra Scientific (North Kingstown, RI). Suprapur HNO₃ (67% v/v) and Rectapur HNO₃ (54% v/v) was purchased from VWR (Fontenay-sous-Bois, France). Working standards were prepared daily in 6% HNO₃ (67%, v/v) and were used without further purification.

2.2. Meat samples

Two types of meat samples were used, corresponding to both intentionally and naturally contaminated meat. Intentionally contaminated meat was prepared with ground beef samples from a blend of muscles (11% fat) purchased from a French supplier. Aliquots of 125 g were stored at -80 °C before use. Matrix blanks of these samples were made before spiking. Naturally contaminated meat samples were obtained in the frame of the French project SOMEAT (Contract No. ANR-12-ALID-0004. Safety of Organic Meat; available at www.someat.fr) and were ground before use.

2.3. Spiking and cooking

2.3.1. Sample spiking

Ground beef was spiked according to Planche *et al.* (2015) combining micropollutant addition to ground meat with matrix homogenization. Briefly, ground beef (120 g) was immersed in dichloromethane (20 mL) containing the micropollutants (PCBs, PCDD/Fs, pesticides and trace elements) followed by evaporation under a hood and homogenization for 2 min in a blender. For PCBs, PCDD/Fs and pesticides, a spiking concentration of 8 ng g^{-1} of fresh meat was chosen to give concentrations in ready-to-run samples within the range of linearity of our GC×GC-TOF/MS method. For all trace elements, meat was spiked at a concentration of 0.1 ng g^{-1} fresh meat corresponding to the authorized limit for lead in meat and double the authorized limit for cadmium in meat according to EU Directive 466/2001 (there is no authorized limit in meat for arsenic and mercury).

2.3.2. Cooking method

To study the fate of micropollutants during cooking, circular small ground beef patties weighing 26 g (2.5 cm thickness) were shaped to resemble commercial ground beef patties. Ground beef patties were cooked in a stainless steel frying pan (17 cm diameter) on a controlled-temperature induction hob (Bosch Electroménager, Saint-Ouen, France). A sheet of 11 μm thick aluminum foil was laid on the bottom of the frying pan to recover juice released during meat cooking. Three different cooking conditions were used to simulate rare (core $50 \text{ }^{\circ}\text{C}$), medium (core $70 \text{ }^{\circ}\text{C}$, according to WHO recommendations for ground meats) and well-done (core $85 \text{ }^{\circ}\text{C}$) meat ($n = 3$ for each cooking condition). These cooking conditions corresponded to: 7 min heating (patty turned over once) at $160 \text{ }^{\circ}\text{C}$ at the bottom of the pan, 14 min heating (turned over three times) at $200 \text{ }^{\circ}\text{C}$ at the bottom of the pan and 14 min heating (turned over three times) at $250 \text{ }^{\circ}\text{C}$ at the bottom of the pan, respectively. Temperatures at the

core of the meat and at the bottom of the pan were monitored by thermocouples (RS Components, Beauvais, France).

2.3.3. Determination of fat content

The determination of fat content was realized according to Blanchet-Letrouvé *et al.* (2014) with slight modifications. Raw and cooked meat was freeze-dried; 1 g of the powder obtained was then extracted by accelerated solvent extraction (ASE) using a Dionex ASE 350 extractor (Sunnyvale, CA) fitted with 22 mL stainless-steel extraction cells. A toluene-acetone (70:30) mixture was used as extraction solvent at a temperature of 120 °C and a pressure of 1500 psi with three extraction cycles per sample. The extract obtained was then evaporated under a hood and weighed to determine fat content.

2.4. Sample extraction

2.4.1. Extraction of PCBs, PCDD/Fs and pesticides

Before extraction, the cooked ground beef patties were powdered with a liquid nitrogen grinder. Extraction, clean-up and concentration of the extract were then carried out according to Planche *et al.* (2015) with slight modifications. Briefly, 5 g of meat powder were extracted by accelerated solvent extraction (ASE) using a Dionex ASE 350 extractor (Sunnyvale, CA). Stainless-steel extraction cells (34 mL volume) were used, with 12 g of acidic alumina placed at the bottom of the cells. Paper filters were placed at the bottom and top of the alumina layer. The cells were then filled with 5 g of ground beef dispersed in diatomaceous earth. Hexane was used as extraction solvent at a temperature of 100 °C and pressure of 1500 psi. ASE extraction included heating (5 min), static time (5 min) and purging

(90 s) with two extraction cycles per sample. After filtration through a glass fiber prefilter and a 0.2 μm nylon filter (Phenomenex, Torrance, CA), the extract (ca. 40 mL) was evaporated (Rocket; Genevac Ltd, Ipswich, UK) using toluene as a keeper to minimize analyte losses during the evaporation step, and 4 mL of dichloromethane were then added. To clean up extracts, gel permeation chromatography (GPC) (Gilson, Middleton, WI) was carried out on an S-X3 Bio-Beads column (Bio-Rad, Philadelphia, PA) using dichloromethane as eluting solvent at a flow rate of 5 mL min^{-1} . The fraction obtained was evaporated to dryness (Rocket, Genevac Ltd), and 100 μL of hexane were then added prior to analysis. All the samples were spiked with internal standards (100 ng mL^{-1}) at different steps.

2.4.2. Sample digestion for trace elements analysis

Samples were digested using a Multiwave 3000 microwave digestion system (Anton-Paar, Courtaboeuf, France), equipped with a rotor for eight 80-mL quartz vessels (operating pressure, 80 bar). The sample digestion procedure was performed according to the EN 13805 standard and had previously been optimized (Noël *et al.*, 2003). Before use, the quartz vessels were decontaminated with 6 mL of 50% HNO_3 (54%, *v/v*) in the microwave digestion system, then rinsed with ultrapure water (18 $\text{M}\Omega$ cm) and dried in a 40 $^\circ\text{C}$ oven. From 0.3 to 0.5 g (dry food) and from 0.5 to 2.0 g (fresh diet) of dietary samples were weighed precisely in the quartz digestion vessels and wet-oxidized with 3 mL ultrapure water and 3 mL Suprapur HNO_3 (67%) in the microwave digestion system. One randomly selected vessel was filled with reagents only and taken through the entire procedure as a blank. After cooling to room temperature, sample solutions were transferred into 50-mL polyethylene flasks. Then, 100 μL of the internal standard solution (1 mg L^{-1} internal standards and 10 mg L^{-1} gold chloride was added to the internal standard solutions to maintain Hg as Hg^{2+} in solution) were added to a

final concentration of $2 \mu\text{g L}^{-1}$ internal standards and $20 \mu\text{g L}^{-1}$ Au; the digested samples were filled with ultrapure water to the final volume before analysis by ICP-MS.

2.5. Micropollutant analysis

2.5.1. Multiresidue analysis of PCBs, PCDD/Fs and pesticides

Samples were analyzed on a time-of-flight mass spectrometer (Pegasus 4D, Leco, St Joseph, MI) coupled to a two-dimensional gas chromatograph (6890, Agilent Technologies) equipped with a dual stage jet cryogenic modulator (licensed from Zoex) according to Planche *et al.* (2015). An Rtx-Dioxin2 1D column ($60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$; Restek, Bellefonte, PA) was connected to a BPX-50 2D column ($2 \text{ m} \times 0.1 \text{ mm} \times 0.1 \mu\text{m}$) (SGE, Austin, TX) with a deactivated ultimate union (Agilent Technologies, Santa Clara, CA). A splitless injection of $1 \mu\text{L}$ of sample extract was performed through a CTC CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) with an inlet temperature set at $280 \text{ }^\circ\text{C}$. A split/splitless inert liner (Sky® 4.0 mm ID liner, Restek) was used. Ultra-pure grade helium (purity 99.9995%) was used as carrier gas with a constant flow rate of 1.5 mL min^{-1} . Purge time was set to 60 s with a flow rate of 50 mL min^{-1} . The primary oven temperature was initially set at $90 \text{ }^\circ\text{C}$ for 1 min, then ramped to $200 \text{ }^\circ\text{C}$ at $20 \text{ }^\circ\text{C min}^{-1}$, then to $308 \text{ }^\circ\text{C}$ at $2 \text{ }^\circ\text{C min}^{-1}$ and lastly to $330 \text{ }^\circ\text{C}$ at $5 \text{ }^\circ\text{C min}^{-1}$ for 10 min. The secondary oven temperature was set $5 \text{ }^\circ\text{C}$ higher than the primary oven temperature. The modulator temperature was set $15 \text{ }^\circ\text{C}$ higher than the primary oven temperature, and the modulation period was 5 s with 1.20 and 1.30 s for the hot and cold pulses, respectively. The transfer line temperature was set at $280 \text{ }^\circ\text{C}$. The mass spectrometer was operated with an electron ionization source (ionization energy: 70 eV), a detector voltage of 1800 V and a data acquisition rate of $100 \text{ spectra s}^{-1}$. The run time for each sample was 75 min. Analytical blank samples of pure solvent were run

to check for absence of targeted analytes. GC×GC data were processed using LECO ChromaTOF software (version 4.50.8.0).

In order not to be restricted by the sensitivity of the GC×GC-TOF/MS method, naturally contaminated samples, in which the concentration of contaminants is unknown, were analyzed according to Berge *et al.* (2011) by a French National Reference Laboratory (LABERCA, Nantes, France).

2.5.2. Analysis of trace elements

ICP-MS measurements were performed with a 7700x Series instrument (Agilent Technologies, Courtaboeuf, France) equipped with a third generation Octopole Reaction System (ORS³) using He gas. The sample solutions were pumped by a peristaltic pump from tubes arranged on a CETAC ASX-500 Series autosampler (CETAC Technologies, Omaha, NE). The torch position, ion lenses, gas output, resolution axis (10% of peak height, $m \pm 0.05$ amu) and background (<20 shots) were optimized daily with the tuning solution (1 mg L^{-1}) to carry out a short-term stability test of the instrument, to maximize ion signals and to minimize interference effects due to high oxide levels ($\text{CeO}^+/\text{Ce}^+ < 1.2\%$) and doubly charged ions ($\text{Ce}^{2+}/\text{Ce}^+ < 2\%$). Linearity response in the pulsed and analogue modes (P/A factor determination) was verified daily using PA tuning solutions. Further details of instrumental settings, optimization and validation process for the simultaneous determination of 31 elements are given in Chevallier *et al.* (2015).

2.6. Data processing

Micropollutant losses induced by cooking were determined according to Rawn *et al.* (2013):

$$\text{Loss} = 1 - \frac{([\text{Micropollutant cooked meat}] \times \text{mass cooked meat})}{([\text{Micropollutant raw meat}] \times \text{mass raw meat})}$$

where [Micropollutant raw meat] and [Micropollutant cooked meat] are the micropollutant concentrations in raw or cooked meat (ng g^{-1} of meat) and mass raw meat and mass cooked meat are the mass of meat (g) before and after cooking.

Data were processed using Statistica software version 10 (StatSoft, Maisons-Alfort, France). Principal component analysis (PCA) was performed on the level of micropollutants in meat before and after cooking to visualize the structure of the data. To determine whether the cooking process had an effect on the level of micropollutants in meat, a one-way analysis of variance (ANOVA; $p < 0.05$) was performed on data from GC×GC-TOF/MS and ICP-MS analyses. A Newman-Keuls mean comparison test was then performed on the resulting dataset, to determine which cooking intensity distinguished between the three ($p < 0.05$).

3. Results and Discussion

3.1. Multiresidue assay of PCBs, PCDD/Fs and pesticides in cooked meat

To study the effects of cooking on levels of PCBs, PCDD/Fs and pesticides in meat, we first designed a multiresidue method based on GC×GC-TOF/MS for the simultaneous monitoring of these three families of contaminants in cooked meat. Thus, we adapted the method recently developed by Planche *et al.* for the simultaneous monitoring of PCBs and PCDD/Fs to extend its scope to the assay of pesticides (Planche *et al.*, 2015).

A set of 16 pesticides (12 organochlorine and 4 organophosphorus) likely to be found in meat were targeted in this work. The separative capacity of the column set used (Rtx-Dioxin2/BPX50) was determined by calculating resolution factors (R_s) according to Zapadlo

et al. with $R_s = \Delta t_R/w_b$, where t_R is the retention time and w_b the average peak width at the base (Zapadlo *et al.*, 2011). Two successive peaks were considered resolved when $R_{s,1D} \geq 0.6$ in 1D or $R_{s,2D} \geq 0.4$ in 2D. The average resolution factors for pesticides were 2.44 in the first dimension and 0.45 in the second one. These values are of the same order of magnitude as those obtained by Planche *et al.* (2015) for PCBs and PCDD/Fs (on average, $R_{s,1D} = 2.05$ and $R_{s,2D} = 0.51$). The GC×GC-TOF/MS method optimized for the analysis of PCBs and PCDD/Fs was thus also satisfactory for the analysis of pesticides. Figure 1 presents an expanded region of the contour plot obtained from the analysis of an extract of cooked meat spiked beforehand with PCBs, PCDD/Fs and pesticides, showing the separation of PCBs 105, 141 and 127 (Nos. 6, 7 and 8 in Figure 1), and of PCB 126 (No. 28) and 2,3,7,8-TCDD (No. 29) using the second chromatographic dimension of the GC×GC-TOF/MS. In addition, compounds that were not chromatographically resolved could be individually monitored provided they displayed specific ions in their respective mass spectra: this was the case for DDT (No. 19) and PCB 160 (No. 18). The GC×GC-TOF/MS method used thus allowed the simultaneous assay of 222 micropollutants (189 PCBs, 17 PCDD/Fs and 16 pesticides), in 75 min (Table S1. Supplementary material). These results further support GC×GC-TOF/MS as an effective method for multiresidue analysis.

In order to further use this method for PCB, PCDD/F and pesticide quantification after cooking, recovery rates of these micropollutants were determined after spiking, extraction and analysis of cooked meat using the protocol developed by Planche *et al.* on raw meat (Planche *et al.*, 2015). Table 1 shows the recovery rates obtained from medium cooked meat for a set of 21 PCBs (including 15 of the 18 PCBs considered as the most relevant due to their occurrence in meat), the 16 pesticides listed in Table S1. (Supplementary material), and a set of 7 PCDD/Fs including 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, the most toxic dioxins with WHO-TEF = 1 (Sirot *et al.*, 2012). If the expanded uncertainty is taken as twice the RSD, we can

note that many of the component recoveries shown for 2 degrees of freedom are not significantly different from 100%. Indeed, most of the recovery rates lay between 70% and 130%, a classically acceptable interval (EPA Method 8000C, 2003), with $RSD \leq 10\%$. Hexachlorodibenzo-dioxins/furans, PCBs 156, 167 and 209, heptachlor, DDT and *O,O,O*-triethylphosphorothioate had recovery rates and/or RSD values outside the classical acceptable limits. For these compounds, the results subsequently obtained in this study must therefore be interpreted with caution. Note that for 16 of the 21 PCBs monitored, cooking lowered recovery rates relative to the values obtained in raw meat by Planche *et al.* (2015). This decrease was especially marked for PCBs 105 and 156, with respective recovery rates of 72% and 59% after extraction and analysis of cooked meat, against 115% and 108% from raw meat. Sporning *et al.* (2004) also showed for an ASE extraction method that PCB recovery rates varied widely according to the matrix, for example averaging 113% ($RSD = 1.8\%$) when extracting from a vegetable feedstuff, against 46% ($RSD = 2.2\%$) when extracting from pork fat. According to Ishiwatari *et al.* (2013), the different matrix structures could explain these differences. In our study conditions, the protein denaturation that occurs during cooking causes a marked change in the structure of the meat matrix that may account for the decreased recovery rates obtained from cooked relative to raw meat.

The recovery rates of the micropollutants monitored were determined in the same way from rare-cooked and well-done meat. Contrary to what one might expect, the results were of the same order of magnitude as those obtained after medium cooking (data not shown). Indeed, although high cooking intensity raised the lipid content of the meat (13%, 15% and 16% of lipids respectively in rare, medium and well-done cooked meat) and lowered its water content (61%, 52% and 50% of water respectively in rare, medium and well-done cooked meat), the recovery rates obtained were not significantly affected.

3.2. Effects of cooking on contaminant loads in meat

3.2.1. PCBs and PCDD/Fs

As shown in the first map of the PCA (Figure 2), clear differences in meat PCB concentrations in ng g^{-1} of fat (classical expression of PCB concentration in food for risk assessment according to Reg 1259/2011/EC) can be observed according to the cooking intensity. Figure S1 (Supplementary material) points out the major loadings for the first factorial plane. A similar separation was obtained starting with the quantities of PCBs in raw or cooked meat (Figure S2. Supplementary material). The examination of the effect of the different cooking intensities on each PCB (Table 2 and Table S2. Supplementary material) confirms these observations. The average total PCB concentration decreases ($p < 0.05$) from 79 ng g^{-1} of fat in raw meat ($n = 3$) to 69, 63 and 43 ng g^{-1} of fat after rare ($n = 3$), medium ($n = 3$), and intense ($n = 3$) cooking. This corresponds to a loss of respectively 18%, 30% and 48% of total PCBs (ng) in the meat. Higher cooking intensity thus causes significantly greater PCB losses ($p < 0.05$), which induce a decrease in PCB concentration in meat. Although both the matrix studied and the cooking intensities were different, these results are in line with those of Bayen *et al.* (2005) for pan-fried fish (5 min at $180 \text{ }^\circ\text{C}$) containing 8.3–18.3% lipids, in which average losses of PCBs were $36 \pm 11\%$ (Bayen *et al.*, 2005). The loss observed for intense cooking is also consistent with the average decrease of 44% in PCDD/F and dioxin-like PCB concentration reported by Hori *et al.* (2005) after a 10-minute broiling of ground beef ($15.7 \pm 4.6\%$ fat). In contrast, Rawn *et al.* (2013) reported narrow variations in PCB concentrations when cooking fish, these variations being partly attributed to the uncertainty in the quantification method used. Considering the different PCB congeners (Table S2. Supplementary material), cooking effect varied with the congener assayed, with for example non-significant losses of PCBs 8, 19 and 52, but losses greater than 50% of PCBs 77, 153,

169, 206 and 209 after medium cooking. Note that the losses observed for PCBs 126 and 169, which had the highest WHO-TEF values of all the PCB congeners studied (0.1 and 0.03), were respectively 16% and 57%, showing the importance of taking into account the mitigating effects of cooking when assessing risks ascribed to this family of contaminants. These losses cannot be explained by a volatilization of PCBs during cooking because PCBs are resistant to temperatures up to 300 °C whereas, even under the most intense cooking condition, the temperature at the bottom of the pan is 250 °C and only 85 °C at the core of the meat. In our study, the greatest losses (>55% for medium cooking and >80% for intense cooking) were observed for PCBs 206 and 209, the most highly chlorinated PCB congeners (9 and 10 chlorine atoms, respectively). Bayen *et al.* also found greater losses of more highly chlorinated PCBs after cooking: they explained this effect by the expulsion of these particularly lipophilic compounds into cooking juices along with lipids contained in the food matrix (Bayen *et al.*, 2005). This explanation also fits our results, where PCBs 206 and 209 were the most lipophilic, owing to their high chlorination level (highest log K_{ow} values of 7.9 and 8.3, respectively). Also, Aaslyng *et al.* (2003) showed that the higher the core temperature in the meat during cooking, the greater were the lipid losses in the cooking juice. Extraction of PCBs in the expelled lipids thus explains why losses caused by intense cooking (core 85 °C) were greater than those observed with gentle cooking (core 50 °C). The matrix studied, and in particular its initial lipid content and the method of cooking chosen thus seem to strongly influence variations in PCB loads observed during cooking.

For total PCDD/Fs in meat, the average losses were not significant, irrespective of the cooking intensity (Table S2. Supplementary material). These results are consistent with those obtained by Rose *et al.*, who also found no significant losses of PCDD/Fs after beef cooking, whatever the method used (Rose *et al.*, 2001). Table 2 shows that meat concentration in 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF (in ng g⁻¹ of fat, which is the classical expression

of PCDD/F concentration in food for risk assessment according to Reg 1259/2011/EC) increases after rare or medium cooking whereas no significant increase could be observed after intense cooking. However, these results must be interpreted with caution for these two compounds, their recovery rates being respectively 63.8% and 63.9% after intense cooking, with RSD $\geq 10\%$. For the other targeted PCDD/Fs, no significant difference in concentration during cooking could be observed, whatever the cooking intensity. These results contrast with those obtained by Hori *et al.* (2005), who showed an average decrease in concentration (pg TEQ g⁻¹) of 54% for PCDD/Fs when ground beef ($15.7 \pm 4.6\%$ fat) was broiled (approximately 10 min) (Hori *et al.*, 2005). As already reported by Perelló *et al.* (2010), we thus observed different behavior during cooking between key PCBs and PCDD/Fs, even though both families of compounds are lipophilic.

The immersion of meat in dichloromethane during the spiking step may modify the properties of the meat sample and the effects of cooking on micropollutants. Thus, in order to determine whether a difference may exist in the fate of contaminants during cooking, between spiked contaminants and contaminants bioaccumulated in muscles during animal breeding, beef ($n = 3$), pork ($n = 3$) and chicken ($n = 3$) samples naturally contaminated with PCBs and PCDD/Fs were studied. Table S3. (Supplementary material) presents concentrations of PCBs and PCDD/Fs in these raw or medium-cooked samples. Except for one beef sample (No. 3), an increase was observed in the concentration of PCBs and PCDD/Fs in meat after cooking. In terms of quantity, no significant losses were observed for these compounds during cooking (data not shown), which is consistent with the results observed for PCDD/Fs in spiked meat but differs from those obtained for PCBs in spiked meat (30% loss). These differences could be partly explained by the fact that meat fat content may vary significantly between samples and that these variations could affect the fate of micropollutants during cooking (Watkins *et al.*, 2010). Moreover, it is also important to note that the lipid content of naturally

contaminated samples (from 1.0% to 5.3% fat) is much lower than in spiked samples (11% fat). As PCBs are expelled in the cooking juice with fat, it can be hypothesized that for lean meat, there are, according to Oroszvári *et al.* (2006), small lipid losses in the cooking juice, which thus induce no significant loss of PCBs during cooking. To confirm this hypothesis, additional analyses were carried out on chicken meat samples (mean fat level: 1.5%) intentionally spiked with PCBs before cooking. Consistent with the results observed with naturally contaminated samples, no significant losses of PCBs were observed during cooking for these low-fat spiked samples (data not shown). Thus, differences that were observed between 11% fat spiked samples and naturally contaminated samples seems to be due to the different lipid level between these samples and not to the spiking protocol.

3.2.2. Pesticides

Table 3 gives the concentrations in ng g^{-1} of meat of the 16 pesticides (12 organochlorine and 4 organophosphorus) for the different cooking intensities, these concentration units being classically used in risk assessment according to Reg 396/2005/EC. Losses in the total load of pesticides in meat during cooking are reported in Table S2. (Supplementary material).

Different behaviors can be observed in Table 3. First, an increase in concentration during cooking could be observed for 6 pesticides (aldrin, DDE, DDD, lindane, methoxychlor, and beta-BHC). This increase may be explained by the mass loss of meat during cooking ($22.6 \pm 1.5\%$, $34.0 \pm 1.5\%$ and $35.6 \pm 1.8\%$ of mass loss after respectively rare, medium and well-done cooking) that exceed pesticide losses reported in Table S2. (Supplementary material), resulting in a concentration effect. We can note that most of these pesticides are not decreased according to the cooking intensity. Secondly, three pesticides

(alpha-BHC, heptachlor epoxide and disulfoton) showed no significant variation in concentration during cooking. Indeed the losses reported in Table S2 (Supplementary material) were of the same order of magnitude as mass loss of meat during cooking. These losses can be explained by an elimination of these compounds with juice during cooking or by their volatilization, in particular for disulfoton which is more volatile than the others. Note that the high variability of the results obtained for disulfoton ($RSD \geq 10\%$) may also explain that its small concentration increase after cooking observed in Table 3 was not significant. Lastly, a decrease in concentration could be observed for 7 pesticides (DDT, heptachlor, dieldrin, sulfotep, phorate, *O,O,O*-triethylphosphorothioate and delta-BHC), with losses of contaminants, that can exceed 80% after medium cooking (Table S2. Supplementary material), greater than meat mass losses. These 7 pesticides included 3 organophosphorus pesticides (sulfotep, phorate and *O,O,O*-triethylphosphorothioate), which are known to be thermosensitive even at current cooking temperature (Abou-Arab, 1999). Their breakdown during cooking could thus explain their lowered concentration in cooked meat. For heptachlor, dieldrin and DDT, Muresan *et al.* (2015) reported lower losses than in our study after baking pork (1 h at 110 °C): loss of 44.1%, 46.3% and 51.1% for respectively heptachlor, dieldrin and DDT (loss of 61.6%, 81.9% and 74.7% in our study for medium cooking). In our study, the conversion of DDT into DDD by reductive dechlorination or into DDE by dehydrochlorination under the effect of heat can explain the marked losses of DDT during cooking (Bayarri *et al.*, 1994; Muresan *et al.*, 2015). There seems to be an equilibrium state for DDD and DDE between losses in cooking juice and formation from DDT, which could explain why their quantities in meat remained almost constant during cooking (except for DDE during intense cooking with losses $\geq 40\%$), as shown in Table S2 (Supplementary material). According to Muresan *et al.* (2015), the oxidation of heptachlor into heptachlor epoxide could also explain the marked losses and lowered concentration of heptachlor during

cooking. This is consistent with the smaller losses of heptachlor epoxide during intense cooking than during medium cooking (Table S2. Supplementary material), possibly due to the formation of heptachlor epoxide by oxidation of heptachlor. In contrast, neither the physical and chemical properties of dieldrin and delta-BHC, nor their high temperature stability data available in the literature can explain the decrease in concentration of these two pesticides during cooking.

3.2.3. Trace elements

Table 4 presents concentrations in mg kg^{-1} of meat (classical expression of trace element concentration in food for risk assessment according to Reg 466/2001/EC) of the four trace elements studied (arsenic, cadmium, mercury and lead). A significant increase ($p < 0.05$) was observed in the concentration of trace elements in the meat during cooking for all four elements monitored. This increase was of the same order of magnitude for cadmium, mercury and lead, with an average concentration for each metal before cooking of 0.116 mg kg^{-1} of meat (RSD = 1.83%) and an average concentration after cooking of 0.151 mg kg^{-1} of meat (RSD = 0.66%). This increase may be explained by the mass losses of meat during cooking that exceed trace element losses reported in Table S2 (Supplementary material), resulting in a concentration effect. A more marked concentration effect was observed for arsenic (from 0.133 mg kg^{-1} of meat before cooking (RSD = 5.60%) to 0.215 mg kg^{-1} of meat after cooking (RSD = 3.67%)) because its quantity in meat stays constant during cooking while meat mass decreases. This increase in the arsenic concentration in meat during cooking was already reported in earlier studies (Devesa *et al.*, 2001; Ersoy *et al.*, 2006; Perelló *et al.*, 2008). Note that losses of trace elements observed during medium cooking (Table S2 Supplementary material) are close to the margin of error of the method used to assay the trace elements,

making their critical reading difficult (Chevallier *et al.*, 2015). Thus, given the weak or nil effect of cooking on the trace element levels in meat, we did not collect data for rare or intense cooking in addition to medium cooking.

In order to assess the impact of spiking on the fate of trace elements during cooking, beef ($n = 9$), pork ($n = 7$) and chicken ($n = 6$) samples naturally contaminated with arsenic were studied. Table S4 (Supplementary material) presents concentrations (in mg kg^{-1} of meat) of arsenic in these raw or medium-cooked samples. Except one beef sample (No. 6) and one pork sample (No. 15), an increase was observed in the concentration of arsenic in meat after cooking, whereas its quantity in meat stays constant (data not shown). These results are consistent with those observed with spiked meat. Thus, spiking does not seem to affect the fate of trace elements during cooking.

4. Conclusion

The GC×GC-TOF/MS multiresidue method set up enabled the study of the effects of cooking on a broad range of PCBs, PCDD/Fs and pesticides in spiked ground beef meat. The fate of some trace elements in meat was assessed by ICP-MS analysis. Results show that cooking could induce a significant decrease in levels of PCBs and pesticides in meat, this decrease being more marked as cooking conditions became more intense. In contrast, cooking only slightly affected levels of PCDD/Fs and trace elements in meat. Our results indicate that meat lipid levels, hydrophobicity of the contaminants studied and their sensitivity to heat represent the major factors explaining the variations observed. Results obtained on spiked meat proved to be relevant for future modeling/prediction of micropollutant fate in naturally contaminated meat during cooking. The ultimate goal of the project will be to improve chemical risk

assessment procedures, taking into account the changes induced by cooking on micropollutants in meat.

Conflict of interest

The authors declare that there is no conflict of interest.

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Figure captions

Fig. 1. GC×GC-TOF/MS contour plot of a set of PCBs, PCDD/Fs and pesticides in medium-cooked meat. The zoom shows how DDT (No. 19) and PCB160 (No. 18) can be resolved by means of their specific ions.

Fig. 2. Discrimination of concentration (ng g^{-1} of fat) of PCBs in meat according to intensity of cooking of samples.

Table 1. Recovery rates obtained for a set of PCDD/Fs, PCBs and pesticides, both organochlorine (OC) and organophosphorus (OP), after spiking, extraction and analysis of medium cooked meat ($n = 3$). *: significantly lower at $p < 0.05$ than recovery rates obtained in raw meat by Planche et al. (2015).

Family	Compound	Recovery rate (%)	RSD (%)
PCDD/F	2,3,7,8-TCDD	86	8.3
PCDD/F	2,3,7,8-TCDF	94	11
PCDD/F	1,2,3,7,8-PeCDD	74	6.3
PCDD/F	2,3,4,7,8-PeCDF	72	5.4
PCDD/F	1,2,3,4,7,8-HxCDD	67	12
PCDD/F	1,2,3,4,7,8-HxCDF	69	12
PCDD/F	1,2,3,6,7,8-HxCDF	62	14
PCB (1)	2	91	6.7
PCB (8)	2,4'	102*	5.9
PCB (19)	2,2',6	91	6.8
PCB (28)	2,4,4'	103*	6.4
PCB (52)	2,2',5,5'	94*	8.1
PCB (77)	3,3',4,4'	107*	4.1
PCB (81)	3,4,4',5	83*	6.5
PCB (105)	2,3,3',4,4'	72*	7.3
PCB (114)	2,3,4,4',5	71*	6.5
PCB (118)	2,3',4,4',5	70*	6.4
PCB (123)	2',3,4,4',5	80*	3.2
PCB (126)	3,3',4,4',5	71*	3.2
PCB (153)	2,2',4,4',5,5'	80*	0.6
PCB (156)	2,3,3',4,4',5	59*	9.0
PCB (157)	2,3,3',4,4',5'	106	4.0
PCB (167)	2,3',4,4',5,5'	65*	7.5
PCB (169)	3,3',4,4',5,5'	113	5.0
PCB (172)	2,2',3,3',4,5,5'	107*	4.0
PCB (189)	2,3,3',4,4',5,5'	94	2.3
PCB (206)	2,2',3,3',4,4',5,5',6	83*	3.0
PCB (209)	2,2',3,3',4,4',5,5',6,6'	95*	21
Pesticide (OC)	aldrin	107	6.8
Pesticide (OC)	dieldrin	117	9.7
Pesticide (OC)	4,4'-DDT	136	10
Pesticide (OC)	4,4'-DDD	90	7.8
Pesticide (OC)	4,4'-DDE	92	3.3
Pesticide (OC)	lindane	88	9.0

Pesticide (OC)	alpha BHC	94	5.4
Pesticide (OC)	béta BHC	87	5.9
Pesticide (OC)	delta BHC	77	9.2
Pesticide (OC)	heptachlor	138	4.1
Pesticide (OC)	heptachlor epoxide	76	5.7
Pesticide (OC)	methoxychlor	96	11
Pesticide (OP)	sulfotep	101	7.6
Pesticide (OP)	<i>O,O,O</i> - triethylphosphorothioate	68	7.3
Pesticide (OP)	phorate	112	7.3
Pesticide (OP)	disulfoton	72	7.4

Table 2. Concentrations ($n = 3$) of PCBs and PCDD/Fs in spiked raw meat and in spiked meat cooked at different intensities (rare, medium and well-done). All results are corrected for individual recovery rates.^{a,b,c,d}: The letters represent the groups given by the Newman-Keuls mean comparison test when compared for each compound, based on the concentration obtained for the different cooking conditions. Two groups with distinct letters can be considered as significantly different ($p < 0.05$).

		raw meat		rare-cooked meat		medium-cooked meat		well-done meat	
<i>lipid content</i>		11%		13%		15%		16%	
<i>cooking mass loss</i>				23%		34%		36%	
Compound	Toxic toxic equivalency factor (TEF)	Concentration concentration (ng g ⁻¹ of fat)	RSD (%)	Concentration concentration (ng g ⁻¹ of fat)	RSD (%)	Concentration concentration (ng g ⁻¹ of fat)	RSD (%)	Concentration concentration (ng g ⁻¹ of fat)	RSD (%)
PCB 1		64 ^b	4.6	49 ^a	8.2	51 ^a	12	47 ^a	11
PCB 8		77 ^a	5.6	75 ^a	14	79 ^a	13	60 ^a	23
PCB 19		68 ^a	8.6	55 ^a	11	58 ^a	32	53 ^a	15
PCB 28		99 ^d	0.60	81 ^c	1.3	73 ^b	2.9	63 ^a	7.5
PCB 52		78 ^{bc}	2.2	92 ^c	8.2	73 ^b	11	56 ^a	20
PCB 77	0.0001	84 ^d	5.3	49 ^c	5.1	44 ^b	3.1	38 ^a	5.3
PCB 81	0.0003	76 ^b	6.4	70 ^b	13	66 ^{ab}	3.1	55 ^a	12
PCB 105	0.00003	87 ^c	0.62	68 ^b	11	69 ^b	3.5	47 ^a	7.7
PCB 114	0.00003	76 ^b	1.6	79 ^b	11	68 ^b	8.5	48 ^a	6.6
PCB 118	0.00003	78 ^b	3.1	71 ^b	11	70 ^b	2.2	49 ^a	8.0
PCB 123	0.00003	81 ^c	4.1	68 ^b	11	71 ^b	2.7	30 ^a	6.1
PCB 126	0.1	72 ^c	2.3	62 ^b	11	67 ^{bc}	4.5	54 ^a	2.8
PCB 153		97 ^c	3.9	47 ^b	8.6	50 ^b	15	32 ^a	27

PCB 156	0.00003	80 ^b	2.7	76 ^b	4.5	80 ^b	4.8	38 ^a	7.3
PCB 157		67 ^b	4.6	105 ^c	7.8	73 ^b	2.1	55 ^a	3.0
PCB 167	0.00003	77 ^b	0.90	102 ^c	7.8	71 ^b	0.43	51 ^a	4.4
PCB 169	0.03	74 ^c	1.3	80 ^d	4.1	36 ^b	9.5	26 ^a	15
PCB 172		88 ^c	7.6	56 ^b	11	61 ^b	1.1	40 ^a	8.9
PCB 189	0.00003	72 ^c	3.7	60 ^b	6.5	72 ^c	1.4	32 ^a	8.7
PCB 206		89 ^c	9.9	50 ^b	8.9	41 ^b	4.4	15 ^a	8.3
PCB 209		82 ^d	8.1	49 ^c	10	39 ^b	5.3	13 ^a	3.3
2,3,7,8-TCDD	1	74 ^a	12	70 ^a	17	71 ^a	15	67 ^a	16
2,3,7,8-TCDF	0.1	63 ^a	9.4	72 ^a	15	77 ^a	1.8	68 ^a	16
1,2,3,7,8-PeCDD	1	66 ^a	12	77 ^a	5.0	65 ^a	30	49 ^a	6.4
2,3,4,7,8-PeCDF	0.3	55 ^a	11	66 ^a	25	74 ^a	8.3	58 ^a	3.7
1,2,3,4,7,8-HxCDD	0.1	62 ^a	18	90 ^a	15	97 ^a	7.2	74 ^a	35
1,2,3,4,7,8-HxCDF	0.1	51 ^a	9.6	85 ^b	11	81 ^b	1.4	64 ^a	16
1,2,3,6,7,8-HxCDF	0.1	45 ^a	7.9	72 ^b	10	75 ^b	18	49 ^a	14

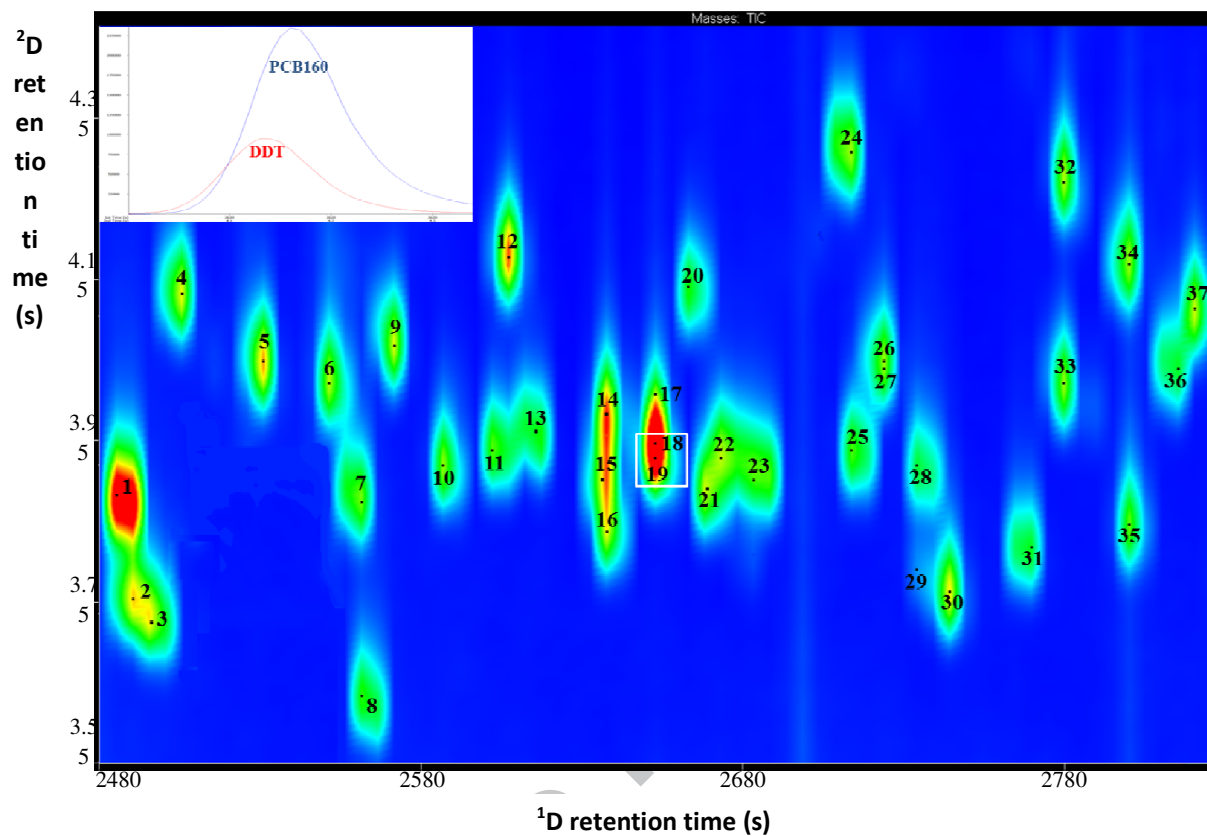
Table 3. Concentrations ($n = 3$) of organochlorine (OC) and organophosphorus (OP) pesticides in spiked raw meat and in spiked meat cooked at different intensities (rare, medium and well-done). All results are corrected for individual recovery rates. ^{a,b,c,d}: The letters represent the groups given by the Newman-Keuls mean comparison test when compared for each compound, based on the concentration obtained for the different cooking conditions. Two groups with distinct letters can be considered as significantly different ($p < 0.05$).

Compound		raw meat		rare-cooked meat		medium-cooked meat		well-done meat	
		Concentration (ng g ⁻¹ of meat)	RSD (%)	Concentration (ng g ⁻¹ of meat)	RSD (%)	Concentration (ng g ⁻¹ of meat)	RSD (%)	Concentration (ng g ⁻¹ of meat)	RSD (%)
Aldrin	OC	7.8 ^a	3.9	10 ^b	9.6	13 ^b	12	12 ^b	12
Dieldrin	OC	8.4 ^c	8.5	1.3 ^a	5.9	2.4 ^b	4.7	0.87 ^a	16
4,4'-DDT	OC	8.5 ^c	5.9	6.5 ^b	9.9	3.4 ^a	13	4.3 ^a	4.6
4,4'-DDD	OC	7.5 ^a	12	9.4 ^b	12	11 ^b	3.3	12 ^c	8.4
4,4'-DDE	OC	6.9 ^{ab}	14	8.1 ^b	3.2	9.9 ^c	6.3	6.4 ^a	10
Lindane (Gamma BHC)	OC	7.4 ^a	6.6	12 ^b	9.8	10 ^b	4.3	10 ^b	12
Alpha alpha BHC	OC	6.9 ^a	7.3	7.0 ^a	10	8.5 ^a	7.0	8.1 ^a	12
Béta beta BHC	OC	6.5 ^a	5.4	9.8 ^c	4.5	10 ^c	4.6	8.3 ^b	11
Delta delta BHC	OC	5.9 ^b	4.9	3.6 ^a	15	3.2 ^a	4.6	4.2 ^a	20
Heptachlor	OC	8.2 ^c	1.8	4.5 ^{ab}	7.9	5.0 ^b	5.1	4.1 ^a	10
Heptachlor epoxide	OC	6.5 ^a	6.1	5.8 ^a	11	5.1 ^a	18	6.8 ^a	13
Methoxychlor	OC	7.3 ^a	4.5	10 ^b	6.0	10 ^b	4.7	7.5 ^a	12
Sulfotep	OP	7.5 ^b	6.8	1.2 ^a	17	1.5 ^a	29	1.2 ^a	6.9
<i>O,O,O</i> - triethylphosphorothioate	OP	6.7 ^b	12	1.6 ^a	17	1.9 ^a	10	1.5 ^a	11
Phorate	OP	8.4 ^b	6.4	2.1 ^a	15	2.3 ^a	20	1.7 ^a	28
Disulfoton	OP	6.0 ^a	4.6	7.8 ^a	26	8.8 ^a	11	8.0 ^a	14

Table 4. Concentrations ($n = 3$) of heavy metals in spiked raw meat and in spiked medium-cooked meat. All results are corrected for individual recovery rates. *: significantly different at $p < 0.05$.

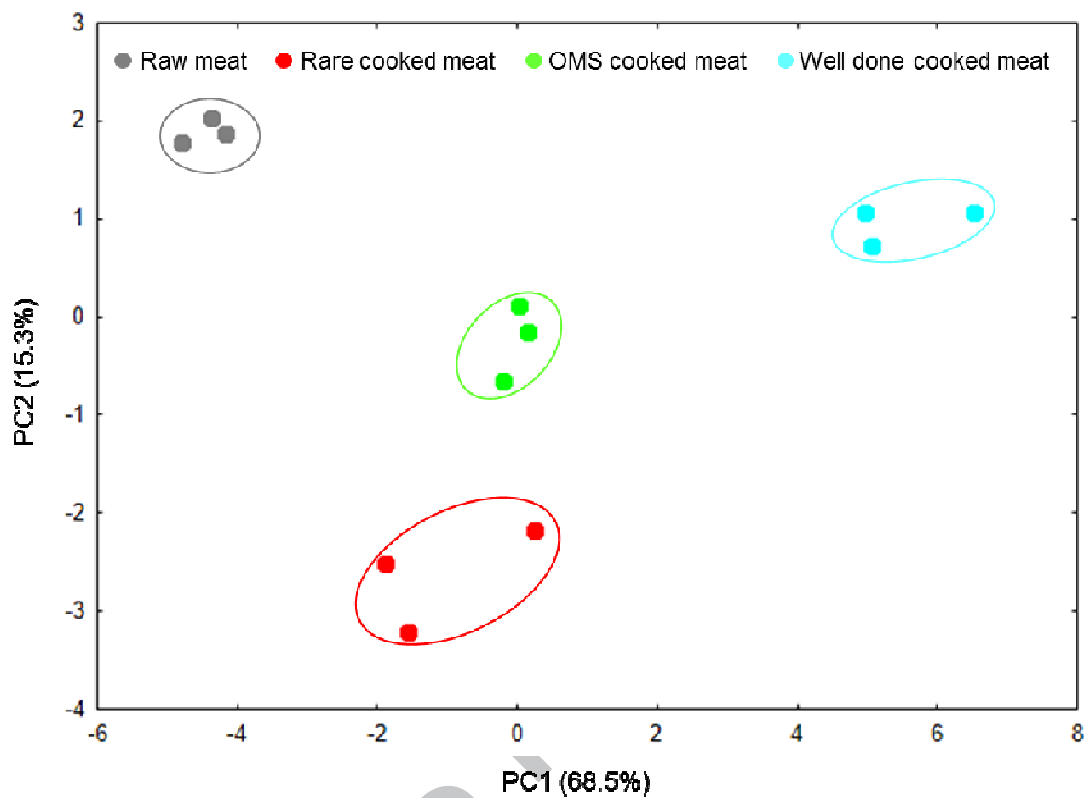
compound	raw meat		medium-cooked meat	
	concentration (mg kg^{-1} of meat)	RSD (%)	concentration (mg kg^{-1} of meat)	RSD (%)
arsenic	0.13	5.6	0.22*	3.7
cadmium	0.12	4.9	0.15*	4.8
mercury	0.11	6.0	0.15*	6.7
lead	0.12	3.6	0.15*	3.8

Fig. 1



1-DDD; 2-PCB168; 3-PCB153; 4-PCB132; 5-PCB179; 6-PCB105; 7-PCB141; 8-PCB127; 9-PCB176; 10-PCB137; 11-PCB130; 12-PCB186; 13-PCB164; 14-PCB163; 15-PCB138; 16-PCB178; 17-PCB158; 18-PCB160; 19-DDT; 20-PCB129; 21-PCB175; 22-PCB182; 23-2,3,7,8-TCDF; 24-Methoxychlor; 25-PCB187; 26-PCB166; 27-PCB183; 28-PCB126; 29-2,3,7,8-TCDD; 30-PCB159; 31-PCB162; 32-PCB128; 33-PCB185; 34-PCB174; 35-PCB167; 36-PCB202; 37-PCB181.

Fig. 2



Highlights

- GC×GC-TOF/MS successfully separated a mix of 189 PCBs, 17 PCDD/Fs and 16 pesticides
- The most common cooking practice induced an average loss of 30% of PCBs
- Cooking losses exceed 80% for some thermosensitive pesticides
- No cooking effect was observed for PCDD/Fs and trace elements

ACCEPTED MANUSCRIPT