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Determination of PAH profiles by GC-MS/MS in salmon muscle meat processed with four cold smoking techniques

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1 **Determination of PAH profiles by GC-MS/MS in salmon muscle**
2 **meat processed with four cold smoking techniques**

3
4 **ABSTRACT:**

5
6 An analytical method based on gas chromatography / tandem mass spectrometry (GC-
7 MS/MS) (triple quadrupole device) has been developed for quantification of polycyclic
8 aromatic hydrocarbons (PAHs) in smoked salmon. This method was applied to determine
9 PAH concentrations in smoked fish and to assess the impact of four industrial smoking
10 processes on their profiles. Two smokehouse temperatures and three times of smoke
11 exposure were applied. All the smoking techniques used lead to acceptable PAH levels:
12 the quantities recovered are one hundred times lower than the legal limit ($5 \mu\text{g.kg}^{-1}$)
13 concerning the principal PAH of concern, *i.e.* benzo(a)pyrene. In order to compare
14 different smoking processes, the TEQ (Toxic Equivalent Quantity) approach was chosen.
15 Smouldering leads to the highest TEQ while liquid smoke leads to the lowest TEQ.

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17
18 **KEYWORDS:** PAH, smoked salmon, smoking process, TEQ, purification, tandem mass
19 spectrometry

1 INTRODUCTION

2
3 Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic food and environmental
4 contaminants (Baird et al., 2005; Mottier et al., 2000). Environmental exposure can be
5 through inhalation (Easton et al., 2002; Storelli et al., 2003; Grova et al., 2005) and
6 deposition from the air can lead to contamination of cereals and vegetables, resulting in
7 human exposure from uncooked foodstuffs (SCF, 2002). However, home-cooking and
8 industrial food processes represent the major source of human exposure from the diet
9 (Zabik et al., 1996; Kannappan et al., 2000; Stolyhwo et al., 2005). Smoked foods (Chen
10 et al., 1996; Jira, 2004) have been known for several decades, to be a source of PAHs
11 especially benzo(a)pyrene (Šimko, 1991; Kazerouni et al., 2001)

12
13 In the smoking process, PAHs are generated during smoke production by wood pyrolysis.
14 Because of their lipophilic properties (log Kow range between 4 and 7), PAHs can
15 accumulate in the lipid fraction of food products and are not easily extracted from food
16 with a high fat content, such as smoked salmon. Moreover, many analytical strategies
17 have been developed to detect and measure these compounds (Rivera et al., 1996; Moret
18 et al., 2000) such as de-fatting, which is especially used during the extraction step
19 (Nyman et al., 1993; Yeakub Ali et al., 2001; Dugay et al., 2002). Accelerated Solvent
20 Extraction (ASE), Solid-Phase Extraction (SPE) clean-up on selected cartridges, and
21 saponification have been developed in order to avoid lipids suspected of disrupting the
22 analysis (Wang et al., 1999; Kishikawa et al., 2003; Marcé et al., 2000). All the studies
23 dealing with PAH analysis in smoked food have been carried out with a single detector
24 and GC/MS (Šimko, 2002; Yurchenko et al., 2005) and HPLC/FD (Chen et al., 1996; Koffi
25 Houessou et al., 2005) are the two main analytical methods for their measurement at low
26 levels. However, co-elution which could be attributed to co-extracted lipids as in the case
27 of smoked fish (Wang et al., 1999) cannot be totally removed (Chiu et al., 1997 ; Moret et
28 al., 1999). Indeed, the lipidic substances of food matrices can disrupt the extraction step
29 but they are also involved in chromatographic coelutions, disturbing the detection of PAHs
30 by creating interferences with the analytes or by increasing the global noise. Gas
31 chromatography coupled to tandem mass spectrometry appears to be a candidate method
32 to lower the limit of detection (LOD) of PAHs even in oily matrices (Munoz et al., 2001 ;
33 Veyrand et al., 2006) and leads to low limits of detection (e.g. 0.07 $\mu\text{g.kg}^{-1}$ for
34 benzo(g,h,i)perylene in oil) (Ballesteros et al., 2006). Nevertheless, this detection system
35 has not been used for PAHs monitoring in smoked food and especially in smoked salmon.

1 The presence of PAHs - especially benzo(a)pyrene - in smoked fish has previously been
2 reported (Šimko et al., 2002) but little information is available concerning the influence of
3 the smoking processes. Most work on PAHs and smoked fish has focused on methods of
4 extraction and determination (Järvenpää et al., 1996; Wang et al., 1999; De Boer et al.,
5 2003). Some studies compare modern and traditional kilns (Karl, 1996 and 1997) but, to
6 our knowledge, no study is available that compares modern industrial smoking processes
7 for fish with respect to the twenty PAHs suspected to be carcinogens (EC, 2005b). This is
8 one of the reasons that justifies this study.

9
10 Moreover, in order to reduce PAH levels in smoked fish, a liquid smoke atomization
11 smoking process has been developed in recent decades. It consists of the vaporization of
12 liquid smoke obtained from a condensation process of wood smoke, onto the fish.
13 However, legal maximum residue limit for PAHs have not been set for this technique.
14 Studies concerning PAHs in liquid smoke have only focused on the PAH composition of
15 liquid smoke itself or the PAH composition of food smoked with this technique (Guillén et
16 al., 2000a,b; Šimko, 2005), but no comparison of liquid smoke with traditional smoking
17 techniques applying wood pyrolysis are available.

18
19 The aims of this work were, firstly, to assess a GC-MS/MS (SRM acquisition)
20 quantification method for PAHs in smoked salmon by comparison with GC-MS (SIM
21 acquisition). To carry out PAH quantification, an extraction method for smoked salmon
22 was developed. It consists of a liquid-solid extraction followed by an optimized SPE
23 purification step. Secondly, we have applied this method to carry out a determination of
24 PAHs in salmon flesh, smoked according to the four smoking processes most used in
25 industry. The effect of time of smoke exposure and smokehouse temperatures on PAH
26 content has also been assessed.

27 28 **MATERIALS AND METHODS**

29 ***Materials and reagents***

30 All solvents for PAH analysis were analytical or HPLC grade and purchased from SDS
31 (Peypin, France) except toluene and tetrahydrofurane from Fluka (Buchs, Switzerland).
32 ENVI Chrom P cartridges of 6 mL, 0.50 g bonded phase were obtained from Supelco
33 (Bellefonte, USA). All PAH standards (fluorene, phenanthrene, anthracene,
34 cyclopenta(c,d)pyrene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene,
35 indeno(1,2,3-c,d)pyrene, dibenzo(a,e)pyrene, dibenzo(a,i)pyrene, benzo(g,h,i)perylene,
36 dibenzo(a,h,)anthracene, dibenzo(a,l)pyrene and dibenzo(a,h)pyrene) were from LGC
37 Promochem (Wessel, Germany) except benzo(a)anthracene, chrysene and

1 benzo(a)pyrene, which were obtained from Chiron (Trondheim, Norway). All ¹³C-labelled
2 PAHs were from CIL (Cambridge Isotope Laboratories, USA) except for
3 cyclopenta(c,d)pyrene, dibenzo(a,h)pyrene and dibenzo(a,l)pyrene for which ¹³C-labelled
4 standards were not available. Ultrapure water was obtained from a MilliQ[®] system
5 (resistivity < 18 MΩ.cm).
6

7 ***Fish processing***

8 Salmon (*Salmo salar*) reared in Norway were purchased from a seafood wholesaler
9 (Nantes, France). The time between their capture and their filleting was not more than one
10 week. Nine gutted fish of 3 ~ 4 kg of the same batch were received in a box in ice. They
11 were directly filleted, trimmed and put on grids in a cold chamber at + 3°C for 2 h. All the
12 fillets were about 1 kg. Next, they were hand-salted with refined salt (Salins du Midi,
13 France) and left for 3 h at +12°C before being rinsed on grids with water (15°C) and
14 stored at 3°C for 18 h until smoking.
15

16 Before smoking, a drying step was carried out by putting the fillets in the smokehouse at
17 18°C for 15 min. The aim of this step is to dry the product surface for a better smoke
18 penetration according to industrial procedures. Secondly, this step allows the
19 standardization of the internal temperature at 8°C for all the samples which were
20 previously stored in cold room at + 2°C. Then, at the beginning of the smoking process,
21 smoke was introduced into the cell on fillets that had the same internal temperature
22 whatever the smokehouse temperature. The smoked fillets were stored for less than one
23 week at + 2°C prior to sensorial analysis. The medium parts of smoked fillets (about 200
24 g) were put at – 80°C and freeze-dried for PAH analysis.
25

26 Raw salmon coming from the same batch of salmon were separated in order to prepare
27 blank matrices to measure the environmental contamination in PAHs in salmon.
28

29 ***Smoking equipment and procedures***

30 The smokehouse was an HMI Thirode (PC90 Model) device (Thirode, France), 1500 ×
31 1300 ×2250 mm with a capacity of 380 kg, mounted on a trolley with 28 grids on which the
32 fillets were deposited. For each smoking technique, the fillets were placed at the same
33 level (grid numbers 10, 12 and 14) 20 cm from the door of the smokehouse. The
34 air/smoke circulation was horizontal. To study the effect of time of smoke exposure and
35 smokehouse temperatures, salmon fillets were swept by the smoke for 1, 2 or 3 hours at a
36 temperature of 22 or 32°C.
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3 1 The exhaust valve opening was 1/3 but was closed for liquid smoke vaporization and the
4 2 relative hygrometry was set at 60 %. For each process, except liquid smoke, the smoke
5 3 was introduced into the smokehouse at a flow-rate of 90 m³/h.
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10 5 For the production of wood smoke, four processes are used: smouldering, thermostated
11 6 plates and friction, which generate smoke from wood sawdust, chips and logs,
12 7 respectively, and atomization of liquid smoke (Varlet et al., 2006).
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16 9 **Smouldering parameters**

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20 11 A generator (Thirode, France) produced smoke by pyrolysis (between 400 and 450 °C)
21 12 from beech sawdust using the smouldering method. The sawdust was poured onto an
22 13 electrically heated ring and pyrolyzed. The pyrolysis temperature was determined with a
23 14 probe placed onto the heated ring. The ring was heated only for the ignition period and
24 15 was treated further only by electric pulses. The pyrolysis was also maintained through an
25 16 air intake producing a continuous flow around the heated ring by a fan. The sawdust fell
26 17 on the heated ring by gravity from a hopper. Introduction of sawdust was programmed
27 18 every 6 min. The sawdust was before moistened and homogenized in order to obtain a
28 19 moisture rate of 20 %.
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36 21 **Thermostated plates parameters**

37 22 A generator 720 × 1120 × 1730 mm (Thirode, France) produced smoke by pyrolysis (500
38 23 °C) of beech chips. The pyrolysis temperature was determined with a probe placed on the
39 24 plates. A system spreads the chips on thermostated plates and the plates were cleaned
40 25 after 3 minutes of combustion. The smoke was pulsed by a ventilator in order to obtain the
41 26 same flow rate of smoke in the smokehouse than smouldering and friction.
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48 28 **Friction parameters**

49 29 A generator type FR 1002 (Muvero, The Netherlands) produced smoke by friction (380
50 30 °C) by pressing a beech log (8 × 8 × 10 cm) against a rotating friction wheel during 10
51 31 seconds and every 30 seconds. The pyrolysis temperature was determined with a probe
52 32 placed into the log. The beech log is pressed pneumatically by means of a wood gripper
53 33 with a pressure of 3,5 bars.
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59 35 **Liquid smoke parameters**

60 36 Liquid smoke was purchased from a smoke flavouring manufacturer (France). It was a
37 37 purified condensate of beech smoke. Liquid smoke is utilised by atomising by pressurized

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3 1 air in the smokehouse at ambient temperature. The vaporization device (Lutetia, France)
4 2 allows the setting of air and liquid smoke pressures in order to obtain a consumption of
5 3 liquid smoke of 1 L/h as in industrial procedures. Liquid smoke was injected in the
6 4 smokehouse for 2 min every 3 min. For this type of smoking process, the hygrometry of
7 5 the smokehouse was set at 70 %.
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13 7 **Solid-Liquid extraction of PAH**

14 8 2 g of freeze-dried fillet spiked with a mixture of 20 ¹³C-PAHs at 1 µg.kg⁻¹, considered as
15 9 internal standards, was homogenized in 40 mL of cyclohexane / ethyl acetate (50:50;v/v)
16 10 and shaken for 30 min, then centrifuged at 5000 g for 30 min at 0°C. The liquid part was
17 11 carefully isolated and evaporated to dryness under a gentle stream of nitrogen. The
18 12 residue was dissolved in 6 mL of cyclohexane. Each PAH quantification was the result of
19 13 the mean of triplicate measurements carried out on three individual fillets which have been
20 14 smoked.
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28 16 **SPE Clean-up procedure**

29 17 Solid-phase extraction cartridges placed on a Vac Elut system were conditioned with 5 mL
30 18 of water, then 5 mL of methanol and finally with 5 mL of cyclohexane. 6 mL of sample in
31 19 cyclohexane was introduced into the cartridge and washed with 3 mL of cyclohexane in
32 20 order to remove the fat. The PAHs were eluted with 12 mL of a mixture of cyclohexane
33 21 and ethyl acetate (50:50; v/v) then evaporated to dryness under a nitrogen stream. Finally,
34 22 the residue was dissolved in 40 µL of toluene.
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42 24 **GC/MS/MS analysis**

43 25 For GC/MS/MS experiments, an HP-6890 gas chromatograph (Agilent Technologies,
44 26 Palo-Alto, USA) was coupled to a triple stage quadrupole Quattro Micro mass
45 27 spectrometer (Waters Milford, USA). A liner, 4 mm i.d., loosely filled with silanized glass
46 28 wool, was used in splitless mode GC injector (280°C). The GC column Zebron was a ZB-
47 29 5MS 30 m × 0.25 mm i.d., film thickness 0.25 µm (Phenomenex, Torrance, USA). The GC
48 30 oven temperature was maintained at 110°C for 1 min after injection then programmed at
49 31 20°C.min⁻¹ to 240°C, then at 5°C.min⁻¹ to 340°C. Helium was used as carrier gas. Electron
50 32 ionization was used and SRM (Selected Reaction Monitoring) was selected as acquisition
51 33 technique. The monitored ions were those listed amongst the recommended ones by the
52 34 European Commission (European Commission, 2005b) with, in addition, fluorene,
53 35 phenanthrene, anthracene, fluoranthene, pyrene from the US EPA 16 priority pollutants
54 36 (INERIS, 2003). The range of mass to charge ratio (m/z) was 166 (fluorene), 178
55 37 (phenanthrene, anthracene), 202 (fluoranthene, pyrene), 226 (cyclopenta(c,d)pyrene),
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3 1 228 (benzo(a)anthracene, chrysene), 242 (5-methylchrysene), 252 (benzo(b), (j) and
4 (k)fluoranthene, benzo(a)pyrene), 276 (benzo(g,h,i)perylene, indeno(1,2,3-c,d)pyrene),
5 2
6 3 278 (dibenzo(a,h)anthracene) and 302 (dibenzopyrenes).
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10 5 Analytes were identified on the basis of their retention time and at least two transitions;
11 6 ¹³C-labelled internal standards corresponding to each PAHs studied were used for
12 7 quantification excepted for cyclopenta(c,d)pyrene, reported to ¹³C benzo(a)anthracene,
13 8 dibenzo(a,h)pyrene reported to ¹³C dibenzo(a,i)pyrene and dibenzo(a,l)pyrene reported to
14 9 ¹³C dibenzo(a,e)pyrene.
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19 11 To express the concentrations in µg.kg⁻¹ of wet tissue, the concentrations expressed in
20 12 µg.kg⁻¹ of freeze-dried tissue have been multiplied by individual freeze-drying factor for
21 13 each PAH. These factors have taken into account the loss of weight due to water
22 14 removing and the loss of analytes signal during freeze-drying. They were determined by
23 15 addition of internal standards before and after this step.
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17 **GC-MS analysis**

18 18 For GC-MS experiments, an HP-6890 gas chromatograph was coupled to an HP-5973
19 19 (Agilent Technologies, Palo-Alto, USA) quadrupole mass spectrometer (low-resolution
20 20 single MS). Injector and transfer-line temperatures were 250°C and 305°C, respectively,
21 21 and source and analyzer temperatures were 230°C and 150°C, respectively. A glass
22 22 insert, 4 mm i.d., loosely filled with silanized glass wool was used in the split/splitless GC
23 23 injector (250°C, purge splitless 1,5 min). The GC column was 30 m × 0,25 mm i.d., film
24 24 thickness 0,25 µm, OV-1 (Ohio Valley). The GC oven temperature was maintained at
25 25 110°C for 10 min after injection then programmed at 20°C.min⁻¹ to 160°C and then at 15
26 26 °C.min⁻¹ to 300°C which was maintained for 10 min. Helium (N55) was used as carrier gas
27 27 at 1,1 mL.min⁻¹. The acquisition mode was SIM (Single Ion Monitoring). Electron ionization
28 28 was used with energy of 70 eV.
29
30

31 **Statistical analysis**

32 31 Analysis of variance (ANOVA) was performed on PAH concentrations data using
33 32 Statgraphics Plus 5.1 software (Statistical Graphics Corp., USA). The significant statistical
34 33 level was set at $p < 0.05$ (Cardinal et al. 2006). Multivariate data processing was
35 34 performed with Uniwin Plus 5.1 software (Sigma Plus, France). A correspondence
36 35 factorial analysis (CFA) was carried out on the PAH content according to the smoking
37 36 parameters (3hrs of smoke exposure, 32°C) and the type of smoking process.
37

RESULTS AND DISCUSSION

Efficiency of the analytical method

Extraction method and recovery yields

The main critical point for PAH analysis in food remains their lipophilic properties. Indeed, PAHs are often co-extracted with lipids. In order to decrease the fat content in the final sample, the volumes of washing and elution have been precisely chosen in order to avoid lipid residues after the concentration with nitrogen and to prevent fast contamination of the mass spectrometer. The potential loss caused by the cyclohexane washing step during SPE is compensated by repeatable recovery yields (Table 1) for each monitored PAH. The estimation of recovery yields were performed with three replicates on spiked unsmoked freeze-dried salmon (1 ng/g of each PAH). When the number of rings increases in the PAH molecule, the polarity decreases and the PAH concerned has a high affinity for the lipid fraction and is less extracted by the solvent mixture (cyclohexane / ethyl acetate). As required for benzo(a)pyrene (EC 2005/10, 2005c), the recovery yields of all PAHs are between are between 50 and 120 %, from 43 % (for dibenzo(a,e)pyrene and dibenzo(a,l)pyrene) to 99 % (for phenanthrene). These results are results are acceptable for fatty matrices such as salmon in which we have previously measured a fat content of 13 % before smoking.

Good reproducibility of extraction can be observed with coefficients of variation less than 10 % for all the molecules studied except for dibenzopyrenes. Nevertheless, in all cases, the coefficient of variation is under the 15 % required for other structure-like contaminant analysis (EC 2002/69, 2002).

GC-MS/MS approach and comparison with GC-MS (SIM)

GC-MS/MS analysis provides several advantages in PAH detection and quantification in food. The first benefit is that it allows treatment of the food matrix under gentler conditions of extraction than those developed until now, which remove lipids under strong conditions such as saponification. The extraction must lead to samples which can be injected after removing lipids and the detection is improved because of further specific monitored transitions, which enables co-extracted lipid substances to be minimized and separated from the PAHs.

The second advantage is that the specificity of GC-MS/MS allows a focus on the transitions of PAHs. It enables the extraction to be optimised to improve the signal, especially in the chromatographic zone of the peaks of analytes. Consequently, the levels

1 of detection are decreased (Monteau et al., 2005 ; Veyrand et al., 2006). Comparing GC-
2 MS with SIM mode acquisition and GC-MS/MS with SRM acquisition (Figure 1), especially
3 for high molecular weight PAHs like benzo(g,h,i)perylene, the signal for GC-MS/MS
4 appears with fewer interferences and a more stable baseline. Therefore, detection limits
5 were significantly improved. For lipophilic PAHs like benzo(g,h,i)perylene, suspected of
6 being co-eluted with lipid substances, a level of detection is reached at $0.5 \mu\text{g}\cdot\text{kg}^{-1}$ with
7 GC-MS (SIM) and less than $0.1 \mu\text{g}\cdot\text{kg}^{-1}$ with GC-MS/MS. Therefore, gas chromatography
8 coupled to MS/MS detection increases by more than five times the sensitivity in this type
9 of food matrix, noticeably for the main heavy PAHs. These results are in accordance with
10 other studies applying tandem mass spectrometry to the recovery of PAHs in food oils
11 (Ballesteros et al., 2006).

12
13 The improvement of the chromatographic signals is also due to the chemical structures of
14 PAHs. Indeed, PAHs are highly condensed and stable so the $M^{+} > M^{+}$ transition can be
15 monitored. Their stability increases with the number of rings in their chemical structure.
16 For this reason, the energy in the collision cell is increased from 25 to 35 eV for
17 dibenzo(a,h)anthracene, indeno(1,2,3-c,d)pyrene, benzo(g,h,i)perylene and all the
18 dibenzopyrenes in order to cause a loss of two protons and to generate the ion $M-2$ (Table
19 2). This second ionization causes a decrease in the noise by fragmenting the co-extracted
20 substances co-eluted with PAHs whereas PAHs are weakly affected. With GC-MS/MS
21 analysis, thanks to the energy of collision and the monitoring of specific transitions, a
22 mass clean-up is carried out that reduces the noise due to lipidic substances thus
23 increasing simultaneously the signal-to-noise ratio of PAHs.

24
25 The method reported here provides a suitable analysis of PAHs in investigative studies. It
26 is faster in extraction time and takes place under gentle conditions, which avoid the
27 generation of potential interferences or matrix effects. The benefits brought by the
28 extraction step are only noticeable because the GC-MS/MS analysis has been optimized
29 for the monitoring of PAHs.

30 ***PAH content in smoked salmon according to the smoking process***

31 The analytical method presented above was employed to investigate PAHs in salmon
32 smoked by four different industrial smoking processes: smouldering, thermostated plates,
33 friction and liquid smoke. In parallel, PAH quantification in unsmoked salmon are compiled
34 as comparison points in the tables of each smoking process in order to differentiate PAHs
35 coming from the environment and salmon feed and those generated during the smoking
36 process (Tables 4 to 7). The environmental contamination due to the bioaccumulated
37

1 PAHs is in agreement with the literature (Easton et al., 2002). PAHs of low molecular
2 weight are predominant but in weak concentrations (between 0.06 for fluorene to 0.19
3 $\mu\text{g}\cdot\text{kg}^{-1}$ for pyrene) and all PAHs of heavy molecular weight are all below 0.02 $\mu\text{g}\cdot\text{kg}^{-1}$.

4 Concerning the four processes studied, it can be noted that 5-methylchrysene,
5 indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene and all the dibenzopyrenes were not
6 found in all the samples whatever the time of smoke exposure and the smokehouse
7 temperature. These PAHs are considered as more toxic than low molecular weight PAHs
8 like fluorene.

9
10 Because the PAHs do not all have the same level of toxicity, a TEF (Toxic Equivalent
11 Factor) expressed in comparison to benzo(a)pyrene, the leading substance for PAH, was
12 defined for each PAH (Table 3). The concentration of each PAH is multiplied by its
13 corresponding TEF and then added in order to obtain a single value that illustrates the
14 toxicity of the foodstuff studied. This value corresponds to the TEQ (AFSSA, 2003). TEQ
15 approach was chosen to express the total PAH contamination of a smoked or unsmoked
16 product. Even if this presentation of PAH content is empirical because the effects of PAHs
17 in a mixture are not sufficiently known, we have used this approach for its possibility to
18 express PAH contamination of food by a single value.

19
20 The contamination of the samples for each experimental point (one smokehouse
21 temperature and one time of exposure) is summarized in the form of the TEQ in each
22 table from Table 4 to Table 7. Except for thermostated plates, the analysis of the variance
23 of the TEQs does not show a significant influence of time of smoking or smokehouse
24 temperature. The low level of PAHs at 1 hour in fish fillets smoked with thermostated
25 plates could be due to the inertia of the device.

26
27 Whatever the settings of the parameters, all the smoking processes lead to high levels of
28 PAHs of low molecular weight, which seem to be generated mainly during smoking
29 (Figure 2). The concentrations of PAH from fluorene to fluoranthene are comprised
30 between 1 and 5 $\mu\text{g}\cdot\text{kg}^{-1}$. Smouldering leads to the highest concentrations of PAHs of low
31 molecular weight and liquid smoke to the lowest concentrations of the same PAHs.
32 Friction and thermostated plates lead to intermediary similar levels of contamination,
33 especially for fluoranthene and pyrene (from 0.10 to 0.40 $\mu\text{g}\cdot\text{kg}^{-1}$). These PAHs and
34 especially phenanthrene whose bioavailability in water is significant, can form the
35 fingerprint of the initial contamination of marine organisms (Pointet et al., 2000; Marsili et
36 al., 2001, Easton et al., 2002). It is interesting to note that this predominance of PAHs of
37 low molecular weight is also remarkable after the smoking of the fish.

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5 2 ***Effects of the time of smoke exposure and smokehouse temperature on the***
6
7 3 ***generation of PAHs according to the process used***

8 4 Smouldering

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10 5 All the fillets smoked according to this technique are acceptable because none of the
11 6 samples exceeded the legal limit (benzo(a)pyrene concentration $< 5 \mu\text{g.kg}^{-1}$) (EC
12 7 208/2005, 2005a). Under the more extreme conditions (3 h, 32°C), benzo(a)pyrene
13 8 reached only $0.05 \mu\text{g.kg}^{-1}$. No effect of time of smoke exposure or smokehouse
14 9 temperature was noticeable for this PAH in smouldering mode (results compiled in Table
15 10 4). In fish fillets smoked by smouldering, there are more low molecular weight PAHs than
16 11 high molecular weight PAHs. An analysis of variance showed a significant influence of the
17 12 parameters studied (smokehouse temperature and time of smoke exposure) on the
18 13 quantity of PAHs recovered in salmon fillets, especially phenanthrene and chrysene (p -
19 14 values ≤ 0.05). Increasing the smokehouse temperature and the time of smoke exposure
20 15 raises the concentration of phenanthrene in salmon fillets. For chrysene, only an effect of
21 16 increasing smokehouse temperature is noticeable. This effect could be explained by a
22 17 higher amount of salmon lipids in liquid state at 32°C than at 22°C. Thus, PAHs that are
23 18 lipophilic could be better adsorbed in the fillet (Moret et al., 1999). Measurements on a
24 19 greater number of fillets could confirm this trend.
25 20

26 21 Thermostated plates

27 22 The results presented in Table 5 show that this mode generates low levels of PAHs
28 23 (benzo(a)pyrene concentration $< 0.04 \mu\text{g.kg}^{-1}$ whatever the setting of parameters)
29 24 whereas it is the technique that reaches the highest temperature of pyrolysis (500°C) and
30 25 it was reported that PAH occurrence was greater with a high temperature of pyrolysis
31 26 (Clifford et al., 1980). Thus, the generation of PAHs at high pyrolysis temperature can be
32 27 compensated by the device used in the smoke production. Below 400°C, the smoke
33 28 contains very low amounts of PAHs but also fewer odour-active compounds and
34 29 molecules that influence the required organoleptic characteristics of the final product
35 30 (Hamm, 1977). Clearly, pyrolysis must be optimized in order to obtain a flavoured smoke
36 31 while minimizing PAH generation. For anthracene, benzo(b) and benzo(j)fluoranthene and
37 32 benzo(a)pyrene, the results of ANOVA show a significant effect of time of smoke
38 33 exposure with a 5 % risk. A significant effect of smokehouse temperature is also
39 34 noticeable for anthracene.
40 35

41 36 An increase in the smoking parameters (especially time of smoke exposure) generated
42 37 PAHs, nevertheless all the PAH quantities were below the European legal limit for smoked

1 fish. The highest concentration of benzo(a)pyrene was found after three hours of smoke
2 exposure and reaches $0.04 \mu\text{g.kg}^{-1}$, still clearly below $5 \mu\text{g.kg}^{-1}$. In general, there is an
3 important difference between the benzo(a)pyrene concentrations found in smoked salmon
4 whatever the smoking processes and parameters and the maximum legal value. The
5 heterogeneity in the PAH contamination of food in the different European countries and
6 the different diets can explain this high legal limit.

7 8 Friction

9 Like with the thermostated plates process, there are half as many low molecular weight
10 PAHs as for the smouldering process (Table 6.). All the PAH quantities are generally
11 below those of thermostated plates. However, the benzo(a)pyrene content is higher than
12 in thermostated plates ($0.06 \mu\text{g.kg}^{-1}$ and $0.04 \mu\text{g.kg}^{-1}$ respectively) and at 32°C , 3 hours of
13 smoke exposure, benzo(g,h,i,)perylene concentration is double that in the thermostated
14 plates method. That is why this process could be more hazardous than thermostated
15 plates due to a higher occurrence of certain high molecular weight PAHs, even if the PAH
16 content is lower than with the smouldering process.

17
18 Benzo(a)pyrene reaches a content close to $0.06 \mu\text{g.kg}^{-1}$ after three hours of smoking. It is
19 the highest concentration found for all the smoking processes studied but this quantity is
20 much lower than the legal limit. A concentration of benzo(a)pyrene of $0.10 \mu\text{g.kg}^{-1}$ has
21 already been reported in commercial smoked fish (Kazerouni et al., 2001), which
22 strengthens our results and shows that the conditions of the smoking process used are
23 similar to those found in industry. However, greater amounts of benzo(a)pyrene have also
24 been reported in commercial smoked herring with $0.5 \mu\text{g.kg}^{-1}$ and eel with $0.3 \mu\text{g.kg}^{-1}$
25 (Storelli et al., 2003).

26
27 It is not surprising to find very low quantities of PAHs because friction is the smoking
28 technique that leads to the lowest wood pyrolysis temperature (380°C). It has already
29 been reported that when the thermal degradation of the wood does not exceed 425°C ,
30 PAHs are nearly absent in wood smoke (Sainclivier, 1985). Friction is the only modern
31 smoking process that produces wood smoke under 400°C , which is the natural
32 temperature of wood ignition.

33 34 Liquid smoke

35 This process leads to the lowest amounts of PAHs (Table 7). As it is reported in
36 Regulation 2065/2003 (EC 2065/2003, 2003), the use of smoke flavourings is generally
37 considered to be of less concern than traditional smoking process. The low PAH

1 quantities found in salmon treated with liquid smoke confirm the legislation view and the
2 improvements during the process of producing liquid smoke. Nevertheless, liquid smokes
3 can be very different in their composition according to the process of their production and
4 the nature of the wood used not only from an organoleptic point of view but also from a
5 PAH content point of view (Hattula et al., 2001). The ANOVA results do not show any
6 effects of time of smoke exposure or smokehouse temperature. Thus, the maximum
7 amount of PAHs that can be adsorbed from this liquid smoke by the product could be
8 considered as reached. The highest benzo(a)pyrene concentration is $0.05 \mu\text{g.kg}^{-1}$, found
9 for one hour of smoke exposure and 32°C . If liquid smoke atomization is considered as a
10 flavouring process, this value is one and a half times the legal limit ($0.03 \mu\text{g.kg}^{-1}$) but, if it
11 is considered as a smoking process, this value is well below the legal limit of $5 \mu\text{g.kg}^{-1}$.
12 Actually, the use of liquid smoking should be ruled by EC 88/388. Thus, it leads to non
13 compliant products (benzo(a)pyrene concentration of $0.05 \mu\text{g.kg}^{-1}$ in the product versus
14 the legal limit of $0.03 \mu\text{g.kg}^{-1}$). However, this contamination is below these brought by
15 traditional smoking process whose legal limit has been set at $5 \mu\text{g.kg}^{-1}$ of benzo(a)pyrene
16 (EC 208/2005, 2005a). Clearly, the liquid smoke process must be further investigated.
17 Indeed, for the same product, different liquid smokes exist that lead to different limit
18 values of contamination. Much work has already been done on the volatile composition of
19 liquid smokes (Guillén et al., 1996a and b; 1999) but not enough on their PAH
20 composition. Today, the same kind of investigation must be led to assess the PAH content
21 of smoked products treated by liquid smokes and to understand the kinetic of deposition
22 and penetration of PAHs brought by smoke flavourings in the smoked products.

23 24 Comparison of the four processes

25 Smouldering seems to be the smoke generation process that leads to smoked fish with
26 the highest TEQs. This difference is caused by higher amounts of low molecular weight
27 PAHs than in the fish smoked by other techniques. Fish smoked by liquid smoke
28 vaporization present the lowest TEQs. When friction is used, the smoked fish fillets have
29 the lowest PAH content but the benzo(a)pyrene concentration is high. Therefore, as the
30 TEF of this PAH is 1, the TEQ is significantly increased. Conversely, when the
31 thermostated plates process is used, individual PAH concentrations are higher while the
32 benzo(a)pyrene concentration is lower than with the friction technique. Consequently, the
33 TEQs of the thermostated plates mode are always lower than for the friction mode. Thus,
34 smoke generation by thermostated plates is the technique applying wood pyrolysis that
35 leads to the lowest TEQs and the least contaminated fillets.

36

1 A correspondence factorial analysis (CFA) (Figure 3) performed on the PAH content of
2 fish smoked for 3 h at 32°C shows the main effects of the four smoking processes studied
3 according to PAH contamination. The three processes applying wood pyrolysis seem to
4 form a homogeneous group against liquid smoke according to their effect on the PAH
5 composition of smoked fish. In fact, the PAH composition of the four groups of smoked
6 fish is very similar but the fish fillets smoked by processes applying wood pyrolysis
7 present higher quantities of fluorene, phenanthrene cyclopenta(c,d)pyrene and
8 benzofluoranthenes. For 3 hrs of smoke exposure and a temperature of smokehouse of
9 32°C, the concentration of benzo(a)pyrene is 0.03 $\mu\text{g.kg}^{-1}$ in fillets treated by liquid smoke,
10 0.04 $\mu\text{g.kg}^{-1}$ in fillets smoked by thermostated plates and 0.05 $\mu\text{g.kg}^{-1}$ in fillets smoked by
11 friction and smouldering. After fluorene, phenanthrene presents the highest content in all
12 the smoking processes studied. This result has been reported in other studies (Karl, 1996;
13 Wang et al., 1999; Storelli et al., 2003). This PAH could deserve special interest but its
14 toxicity is not very high. However, the determination of phenanthrene content could be an
15 indicator of the intensity of the smoking process or be used to discriminate products
16 smoked by processes applying wood pyrolysis and products treated with liquid smoke,
17 especially with long times of smoke exposure. Indeed, phenanthrene concentration is the
18 second highest PAH concentration by comparison with the other PAH concentrations in
19 salmon smoked by liquid smoke but phenanthrene concentration with this technique is the
20 smallest concentration of phenanthrene (1.53 $\mu\text{g.kg}^{-1}$) by comparison with those found
21 with the other smoking techniques (from 2.73 $\mu\text{g.kg}^{-1}$ with friction to 5.20 $\mu\text{g.kg}^{-1}$ with
22 smouldering). This difference seems to increase with the time of smoke exposure.

23 CONCLUSIONS

24 The method reported here provides a suitable analysis of PAHs in investigative studies.
25 However, this kind of analysis is only possible thanks to technologies like GC-MS/MS and
26 the use of ^{13}C -labelled internal standards, which demonstrates their suitability and
27 accuracy for the determination and quantification of contaminants in food. It offers
28 reduced extraction times and takes place under gentle conditions, which avoid the
29 generation of potential interferences or matrix effects. The benefits brought by the
30 extraction step are only noticeable thanks to GC-MS/MS analysis optimized for the
31 monitoring of PAHs.

32
33
34 This study assesses the occurrence of PAHs in smoked salmons following the four
35 smoking processes most used in industry. The potential effects of two essential
36 parameters (time of smoke exposure and smokehouse temperature) have been
37 evaluated. Among the three techniques applying wood pyrolysis, smouldering, which is

1 the most used, leads to the more contaminated products. Salmons smoked by the friction
2 mode have the lowest quantities of PAHs except for benzo(a)pyrene, which mainly
3 contributes to the increase in TEQ of smoked products. The thermostated plates mode
4 generates higher PAH amounts but the benzo(a)pyrene concentration is lower than in the
5 friction mode, which leads to the lowest overall contamination. More research should be
6 carried out about the conditions of generation of PAHs in modern smoking processes to
7 better understand these TEQs. Variation of the parameters settings of smoking such as
8 moisture of sawdust, others time of smoke exposure and smokehouse temperature, wood
9 nature could bring other important information of PAH generation. This study focused on
10 the impact of various process parameters on PAH profiles. More investigations should be
11 led in order to study the influence of smoking parameters on the deposition kinetics of
12 PAHs (adsorption, migration in the product) during the process. Liquid smoke is a
13 particular case because, when considered as a smoking process, the lowest individual
14 quantities of PAHs and TEQ are then found. However, when considered as a flavouring
15 process, the benzo(a)pyrene value is higher compared to its legal value thus the
16 legislation about liquid smoke in the fish smoking process should be clarified. Finally, the
17 TEQ approach was chosen to express the total PAH contamination of a smoked or
18 unsmoked product. This presentation of PAH content is empirical because the effects of
19 PAHs in a mixture are not sufficiently known. Nevertheless, we have shown that the TEQ
20 approach can compare and discriminate products smoked by various smoking processes.
21 However, this kind of analysis is only possible thanks to technologies like GC-MS/MS and
22 the use of ¹³C-labelled internal standards, which demonstrates their suitability and
23 accuracy for the determination and quantification of contaminants in food.

24 25 **REFERENCES**

26
27 AFSSA (2003). Avis de l'AFSSA relatif à une demande d'avis sur l'évaluation des risques
28 présentés par le benzo[a]pyrene B[a]P et par d'autres hydrocarbures aromatiques
29 polycycliques (HAP), présents dans diverses denrées ou dans certaines huiles végétales,
30 ainsi que sur les niveaux de concentration en HAP dans les denrées au-delà desquels
31 des problèmes de santé risquent de se poser. Agence Française de Sécurité Sanitaire
32 des Aliments. Saisine n°2000-SA-0005.

33
34 Baird, WM, Hooven, LA, Mahadevan, B. 2005. Carcinogenic polycyclic aromatic
35 hydrocarbon-DNA adducts and mechanism of action. Environmental and Molecular
36 Mutagenesis 45:106-114.

- 1
2
3 1 Ballesteros, E, García Sánchez, A, Ramos Martos, N. 2006. Simultaneous
4 multidetermination of residues of pesticides and polycyclic aromatic hydrocarbons in olive
5 and olive-pomace oils by gas chromatography/tandem mass spectrometry. *Journal of*
6 *Chromatography A* 1111:89-96.
7
8
9
10
11 6 Chen, BH, Wang, CY, Chiu, CP. 1996. Evaluation of analysis of polycyclic aromatic
12 hydrocarbons in meat products by liquid chromatography. *Journal of Agriculture and*
13 *Food Chemistry* 44:2244-2251.
14
15
16
17
18 10 Chiu, CP, Lin, YS, Chen, BH. 1997. Comparison of GC-MS and HPLC for overcoming
19 matrix interferences in the analysis of PAHs in smoked food. *Chromatographia*, 44(9):497-
20 504.
21
22
23
24 14 Clifford, MN, Tang, SL, Eyo, AA. 1980. Smoking of foods. *Process Biochemistry*
25 June/July:8-11.
26
27
28
29 17 Collins, JF, Brown, JP, Alexeeff, GV, Salmon, AG. 1998. Potency equivalency factors for
30 some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbons derivatives.
31 *Regulatory Toxicology and Pharmacology* 28:45-54.
32
33
34
35
36 21 De Boer, J, Law, RJ. 2003. Development in the use of chromatographic techniques in
37 marine laboratories for the determination of halogenated contaminants and polycyclic
38 aromatic hydrocarbons. *Journal of Chromatography A* 1000:223-251.
39
40
41
42 25 Easton, MDL, Luszniak, D, Von der Geest, E. 2002. Preliminary examination of
43 contaminant loadings in farmed salmon, wild salmon and commercial salmon feed.
44 *Chemosphere*, 46:1053-1074.
45
46
47
48
49 29 EC 88/388. 1988. Official Journal of the European Union, L 184.
50
51
52 31 EC 2002/69. 2002. Official Journal of the European Union, L 209:5-14.
53
54
55 33 EC 2065/2003. 2003. Official Journal of the European union, L 309 :1-8.
56
57
58
59 35 EC 208/2005. 2005a. Official Journal of the European Union, L 34:3-5.
60
36
37 EC 2005/108. 2005b. Official Journal of the European Union, L 34:43-45.

- 1
2
3 1
4
5 2 EC 2005/10. 2005c. Official Journal of the European Union, L 34:15-20.
6
7 3
8 4 Grova, N, Monteau, F, Le Bizec, B, Feidt, C, André, F, Rychen, G. 2005. Determination of
9
10 5 Phenanthrene and Hydroxyphenanthrenes in Various Biological Matrices at Trace Levels
11 6 using Gas Chromatography Coupled to Mass Spectrometry. Journal of Analytical
12 7 Toxicology 29:175-181.
13
14 8
15 9 Guillén, MD, Manzanos, MJ. 1996a. Study of the components of a solid smoke flavouring
16 10 preparation. Food Chemistry 55:251-257.
17 11
18 12 Guillén, MD, Ibargoitia, ML. 1996b. Volatile components of aqueous liquid smokes from
19 13 *Vitis vinifera* L shoots and *Fagus sylvatica* L wood. Journal of Science and Food
20 14 Agriculture 72:104-110.
21 15
22 16 Guillén, MD, Manzanos, MJ. 1999. Extractable components of the aerial parts of *Salvia*
23 17 *lavandulifolia* and composition of the liquid smoke flavoring obtained from them. Journal of
24 18 Agriculture and Food Chemistry 47:3016-3027.
25 19
26 20 Guillén, MD, Sopelana, P, Partearroyo, MA. 2000a. Determination of polycyclic aromatic
27 21 hydrocarbons in commercial liquid smoke flavorings of different compositions by gas
28 22 chromatography-mass spectrometry. Journal of Agriculture and Food Chemistry 48:126-
29 23 131.
30 24
31 25 Guillén, MD, Sopelana, P, Partearroyo, MA. 2000b. Polycyclic aromatic hydrocarbons in
32 26 liquid smoke flavorings obtained from different types of wood. Effect of storage in
33 27 polyethylene flasks on their concentrations. Journal of Agriculture and Food Chemistry
34 28 48:5083-5087.
35 29
36 30 Hamm, R. 1977. Analysis of smoke and wood smoke. Pure and Applied Chemistry
37 31 49:1655-1666.
38 32
39 33 Hattula, T, Elfving, K, Mroueh, UM, Luoma, T. 2001. Use of liquid smoke flavouring as an
40 34 alternative to traditional flue gas smoking of rainbow trout fillets (*Oncorhynchus mykiss*).
41 35 Lebensmittel-Wissenschaft Und Technologie 34:521-525.
42 36
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 1 INERIS. Doornaert, B, Pichard, A, Gillet, C. 2003. Hydrocarbures aromatiques
4 polycycliques (HAPs). Evaluation de la relation dose-réponse pour des effets
5 cancérigènes : Approche substance par substance (facteurs d'équivalence toxique – FET)
6 et approche par mélanges. Evaluation de la relation dose-réponse pour des effets non
7 cancérigènes : Valeurs Toxicologiques de Référence (VTR). INERIS-DRC-03-47026-
8 ETSC-BDo-N°03DR177.
9
10
11
12
13
14 8 Järvenpää, E, Huopalahti, R, Tapanainen, P. 1996. Use of supercritical fluid extraction-
15 high performance chromatography in the determination of polynuclear aromatic
16 hydrocarbons from smoked and broiled fish. *Journal of Liquid Chromatography and*
17 *Related Technologies* 19(9):1473-1482.
18
19
20
21
22
23 13 Jira, W. 2004. A GC/MS method for the determination of carcinogenic polycyclic aromatic
24 hydrocarbons (PAH) in smoked meat products and liquid smokes. *European Food*
25 *Research and Technology* 218:208-212.
26
27
28
29 17 Kannappan, S, Indra Jasmine, G, Jeyachandran, P, Tamil Selvi, A. 2000. Polyaromatic
30 hydrocarbons in processed fishery products. *Journal of Food Science and Technology*
31 37:596-601.
32
33
34
35
36 21 Karl, H, Leinemann, M. 1996. Determination of PAH in smoked fishery products from
37 different smoking kilns. *Zeitschrift fur Lebensmittel Untersuchung und Forschung*, 202:459-
38 464.
39
40
41
42 25 Karl, H. 1997. Influence of the smoking technology on the quality of smoked fish regarding
43 undesirable compounds. In: *Seafood from Producer to Consumer, Integrated Approach to*
44 *Quality*. Luten JB, editor. Amsterdam: Elsevier Science Publishing. p:633-639.
45
46
47
48
49 29 Kazerouni, N, Sinha, R, Hsu, CH, Greenberg, A, Rothman, N. 2001. Analysis of 200 food
50 items for benzo(a)pyrene and estimation of its intake in an epidemiologic study. *Food*
51 *Chemistry and Toxicology* 39:423-436.
52
53
54
55 33 Koffi Houessou, J, Benac, C, Delteil, C, Camel, V. 2005. Determination of polycyclic
56 aromatic hydrocarbons in coffee brew using solid-phase extraction. *Journal of Agriculture*
57 *and Food Chemistry* 53:871-879.
58
59
60 36

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
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40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 Larsen, JC, Larsen, PB. 1998. Chemical carcinogens. In : Air Pollution and Health.
2 Hester, RE, Harrison, RM, editors. Cambridge (UK), p:33-56.

3
4 Marsili, L, Caruso, A, Fossi, MC, Zanardelli, M, Politi, E, Focardi, S. 2001. Polycyclic
5 aromatic hydrocarbons (PAHs) in subcutaneous biopsies of Mediterranean cetaceans.
6 *Chemosphere* 44 :147-154.

7
8 Monteau, F, Sarcher, L, Varlet, V, Maume, D, André, F, Le Bizec, B. 2005. Compared
9 mass spectrometry approaches for PAH determination in food. Work presented at the
10 Dioxin 2005 / ISPAC 20 congress, Toronto, Canada.

11
12 Moret, S, Conte, L, Dean, D. 1999. Assessment of polycyclic aromatic hydrocarbon
13 content of smoked fish by means of a fast HPLC/HPLC method. *Journal of Agriculture and*
14 *Food Chemistry* 47:1367-1371.

15
16 Mottier, P, Parisod, V, Turesky, RJ. 2000. Quantitative determination of polycyclic
17 aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to
18 mass spectrometry. *Journal of Agriculture and Food Chemistry* 48:1160-1166.

19
20 Munoz, D, Doumenq, P, Guiliano, M, Jacquot, F, Scherrer, P, Mille, G. 1997. New
21 approach to study of spilled crude oils using high resolution GC-MS (SIM) and metastable
22 reaction monitoring GC-MS-MS. *Talanta* 45:1-12.

23
24 Nyman, PJ, Perfetti, GA, Joe Jr, FL, Diachenko, GW. 1993. Comparison of two clean-up
25 methodologies for the gas chromatographic / mass spectrometric determination of low
26 nanogram / gram levels of polynuclear aromatic hydrocarbons in seafood. *Food Additives*
27 *and Contaminants* 10:489-501.

28
29 Pointet, K, Milliet, A. 2000. PAHs analysis of fish whole gall bladders and livers from the
30 Natural Reserve of Camargue by GC/MS. *Chemosphere* 40:293-299.

31
32 Sainclivier, M. 1985. L'industrie alimentaire halieutique. Des techniques ancestrales à
33 leurs réalisations contemporaines : salage, séchage, fumage, marinage, hydrolysats.
34 *Bulletin scientifique et technique de l'Ecole Nationale Supérieure Agronomique Centre de*
35 *Recherches de Rennes*. p.219-285. (In French).

- 1
2
3 1 Scientific committee on Food (2002). Opinion of the Scientific Committee on Food on the
4 risks to human health of Polycyclic Aromatic Hydrocarbons in food (expressed on 4
5 december 2002). SCF/CS/CNTM/PAH/29 final.
6
7
8 4
9
10 5 Šimko, P. 1991. Changes of benzo(a)pyrene contents in smoked fish during storage.
11 Food Chemistry 40:293-300.
12
13 7
14 8 Šimko, P. 2002. Determination of polycyclic aromatic hydrocarbons in smoked meat
15 products and smoke flavouring food additives. Journal of Chromatography B 770:3-18.
16
17 9 Šimko, P. 2005. Factors affecting elimination of polycyclic aromatic hydrocarbons from
18 smoked meat foods and liquid smoke flavorings. Molecular Nutrition and Food Research
19 49:637-647.
20
21 12
22
23 13
24 14 Stołyhwo, A, Sikorski, ZE. 2005. Polycyclic aromatic hydrocarbons in smoked fish – a
25 critical review. Food Chemistry 91:303-311.
26
27 15
28 16
29 17 Storelli, MM, Stuffer, RG, Marcotrigiano, GO. 2003. Polycyclic aromatic hydrocarbons,
30 polychlorinated biphenyls, chlorinated pesticides (DDTs), hexachlorocyclohexane, and
31 hexachlorobenzene residues in smoked seafood. Journal of Food Protection 66:1095-
32 1099.
33
34 20
35
36 21
37 22 Varlet, V., Serot, T., Knockaert, C., Cornet, J., Cardinal, M., Monteau, F., Le Bizec, B.,
38 Prost, C. (2006). Organoleptic characterization and PAH content of salmon (*Salmo salar*)
39 smoked according to four industrial smoking techniques. J. Sci. Food Agric., accepted.
40
41 24
42 25
43 26 Veyrand, B, Brosseaud, A, Sarcher, L, Varlet, V, Monteau, F, Marchand, P, André, F, Le
44 Bizec, B. 2006. Innovative method for determination of 20 polycyclic aromatic
45 hydrocarbons in food and oil samples using GC-MS/MS based on an isotope dilution
46 approach. Journal of Chromatography A, submitted.
47
48 28
49 29
50 30
51 31 Wang, G, Lee, AS, Lewis, M, Kamath, B, Archer, RK. 1999. Accelerated solvent extraction
52 and gas chromatography/mass spectrometry for determination of polycyclic aromatic
53 hydrocarbons in smoked food samples. Journal of Agriculture and Food Chemistry
54 47:10626-1066.
55
56 33
57 34
58 35
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
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40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 Yeakub Ali, Md, Cole, RB. 2001. SFE-plus-C₁₈ cleanup and selective extraction method
2 for GC/MS quantitation of polycyclic aromatic hydrocarbons in smoked meat. Journal of
3 Agriculture and Food Chemistry 49:4192-4198.

4
5 Yurchenko, S, Mölder, U. 2005. The determination of polycyclic aromatic hydrocarbons in
6 smoked fish by gas chromatography mass spectrometry with positive-ion chemical
7 ionization. Journal of Food Composition and Analysis 18:857-869.

8
9 Zabik, ME, Booren, A, Zabik, MJ, Welch, R, Humphrey, H. 1996. Pesticides residues,
10 PCBs and PAHs in baked, charbroiled, salt boiled and smoked Great Lakes lake trout.
11 Food Chemistry 55:231-239.

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Table 1. Recovery yields carried out on unsmoked salmon of the 20 studied PAHs and coefficient of variation of the extraction method

Compounds	Recovery yield (mean of six extractions)	Coefficient of variation
Fluorene	95 %	5%
Phenanthrene	99 %	6%
Anthracene	52 %	6%
Fluoranthene	83 %	2%
Pyrene	85 %	4%
Benzo(a)anthracene	57 %	5%
Cyclopenta(c,d)pyrene	60 %	7%
Chrysene	65 %	6%
5-methylchrysene	53 %	7%
Benzo(b)fluoranthene + Benzo(j)fluoranthene	53 %	6%
Benzo(k)fluoranthene	59 %	5%
Benzo(a)pyrene	49 %	6%
Indeno(1,2,3-c,d)pyrene	47 %	5%
Dibenzo(a,h)anthracene	48 %	9%
Benzo(g,h,i)perylene	61 %	10%
Dibenzo(a,l)pyrene	43 %	11%
Dibenzo(a,e)pyrene	43 %	12%
Dibenzo(a,i)pyrene	44 %	11%
Dibenzo(a,h)pyrene	40 %	15%

Each recovery yield is the mean of three measurements.

Table 2. Molecular weights, log Kow, energy of collision used and mass transitions monitored for the 20 studied PAHs

Compounds	Mw	Log Kow	Number of rings	Collision cell energy (eV)	Monitored transition
Fluorene	166	4.18	3	20 25	166 > 166 166 > 164
Phenanthrene	178	4.57	3	20 25	178 > 178 178 > 176
Anthracene	178	4.45	3	20 25	178 > 178 178 > 176
Fluoranthene	202	5.10	4	20 25	202 > 202 202 > 200
Pyrene	202	5.32	4	20 25	202 > 202 202 > 200
Cyclopenta(c,d)pyrene	226		5	20 25	226 > 226 226 > 224
Benzo(a)anthracene	228	5.63	4	20 25	228 > 228 228 > 226
Chrysene	228	5.81	4	20 25	228 > 228 228 > 226
5-methyl-chrysene	242		4	20 25	242 > 242 242 > 240
Benzo(b)fluoranthene	252	6.57	5	20 25	252 > 252 252 > 250
Benzo(j)fluoranthene	252	6.11	5	20 25	252 > 252 252 > 250
Benzo(k)fluoranthene	252	6.84	5	20 25	252 > 252 252 > 250
Benzo(a)pyrene	252	6.00	5	20 25	252 > 252 252 > 250
Benzo(g,h,i)perylene	276	6.63	6	20 35	276 > 276 276 > 274
Indeno(1,2,3-cd)pyrene	276	6.60	6	20 35	276 > 276 276 > 274
Dibenzo(a,h)anthracene	278	6.70	5	20 35	278 > 278 278 > 276
Dibenzo(a,l)pyrene	302		6	20 35	302 > 302 302 > 300
Dibenzo(a,e)pyrene	302		6	20 35	302 > 302 302 > 300
Dibenzo(a,h)pyrene	302	7.28	6	20 35	302 > 302 302 > 300
Dibenzo(a,i)pyrene	302	7.28	6	20 35	302 > 302 302 > 300

Table 3. Toxic Equivalent Factor for the studied PAHs

List of PAHs	TEF (INERIS)	TEF (Larsen et al., 1998)
Fluorene	0.001	0.05
Phenanthrene	0.001	0.0005
Anthracene	0.01	0.0005
Fluoranthene	0.001	0.05
Pyrene	0.001	0.001
Benzo(a)anthracene	0.1	0.005
Cyclopenta(c,d)pyrene	0.1	0.02
Chrysene	0.01	0.03
5-methyl-chrysene		
Benzo(b)fluoranthene	0.01	0.1
Benzo(j)fluoranthene		0.05
Benzo(k)fluoranthene	0.01	0.05
Benzo(a)pyrene	1	1
Benzo(g,h,i)perylene	0.01	0.02
Indeno(1,2,3-cd)pyrene	0.1	0.1
Dibenzo(a,h)anthracene	1	1.1
Dibenzo(a,l)pyrene		1
Dibenzo(a,e)pyrene		0.2
Dibenzo(a,h)pyrene		1
Dibenzo(a,i)pyrene		0.1

TEF of 5-methylchrysene was only assessed for an aerial contamination and sat at 1 (Collins et al., 1998).

Table 4. Toxic Equivalent Factor (INERIS) and PAH content in salmon muscle smoked by smouldering at three different times of smoke exposure at two smokehouse temperatures (in $\mu\text{g}\cdot\text{kg}^{-1} \pm$ standard deviation wet tissue).

	Initial contamination	Parameters of smoking					
		1 h, 22°C	1 h, 32°C	2 h, 22°C	2 h, 32°C	3 h, 22°C	3 h, 32°C
Fluorene	0.06 ± 0.041	1.29 ± 0.215	2.38 ± 0.430	2.42 ± 0.331	4.09 ± 0.739	2.84 ± 0.325	5.74 ± 1.511
Phenanthrene	0.04 ± 0.018	1.36 ± 0.353	2.43 ± 0.563	2.28 ± 0.764	3.94 ± 0.735	3.42 ± 0.318	5.20 ± 0.979
Anthracene	0.01 ± 0.005	0.33 ± 0.038	0.50 ± 0.069	0.50 ± 0.076	0.71 ± 0.078	0.54 ± 0.101	0.90 ± 0.147
Fluoranthene	0.075 ± 0.100	0.27 ± 0.043	0.05 ± 0.008	0.37 ± 0.072	0.55 ± 0.113	0.62 ± 0.137	0.63 ± 0.197
Pyrene	0.19 ± 0.015	0.16 ± 0.268	0.14 ± 0.123	0.26 ± 0.266	0.48 ± 0.223	1.00 ± 0.763	0.35 ± 0.092
Benzo(a)anthracene	< 0.02	0.02 ± 0.005	0.02 ± 0.001	0.02 ± 0.003	0.02 ± 0.004	0.02 ± 0.003	0.03 ± 0.001
Cyclopenta(c,d)pyrene	< 0.01	0.01 ± 0.011	0.02 ± 0.004	0.05 ± 0.002	0.05 ± 0.005	0.02 ± 0.002	0.03 ± 0.003
Chrysene	0.01 ± 0.03	0.08 ± 0.010	0.10 ± 0.019	0.08 ± 0.007	0.09 ± 0.002	0.07 ± 0.002	0.09 ± 0.007
Benzo(b)fluoranthene + Benzo(j)fluoranthene	< 0.02	0.03 ± 0.003	0.03 ± 0.003	0.03 ± 0.002	0.03 ± 0.001	0.02 ± 0.006	0.03 ± 0.003
Benzo(k)fluoranthene	< 0.01	0.01 ± 0.001	0.01 ± 0.003	0.01 ± 0.001	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.002
Benzo(a)pyrene	< 0.02	0.04 ± 0.003	0.04 ± 0.005	0.04 ± 0.003	0.04 ± 0.009	0.04 ± 0.005	0.05 ± 0.004
Benzo(g,h,i)perylene	< 0.01	0.07 ± 0.008	0.10 ± 0.001	0.05 ± 0.011	0.07 ± 0.017	0.07 ± 0.022	0.06 ± 0.014
TEQ ($\mu\text{g}/\text{kg}$)	5.65.10⁻⁴	0.055	0.065	0.059	0.065	0.059	0.079

TEQ are calculated with TEFs from INERIS table.

TEQ are lower-bound TEQs (PAH contents below the limit of quantitation were not been taken into account).

For 1 h, 32°C, fluoranthene concentration was temptatively quantified because of its very weak quantities.

Table 5. Toxic Equivalent Factor and PAH content in salmon muscle smoked by thermostated plates at three different times of smoke exposure at two smokehouse temperatures (in $\mu\text{g}\cdot\text{kg}^{-1}$ \pm standard deviation of wet tissue).

	Initial contamination	Parameters of smoking					
		1 h, 22°C	1 h, 32°C	2 h, 22°C	2 h, 32°C	3 h, 22°C	3 h, 32°C
Fluorene	0.06 \pm 0.041	1.43 \pm 0.225	2.18 \pm 0.615	2.10 \pm 0.648	2.63 \pm 0.763	2.37 \pm 0.621	4.28 \pm 0.710
Phenanthrene	0.04 \pm 0.018	1.61 \pm 0.413	2.16 \pm 0.845	1.33 \pm 0.353	1.88 \pm 0.164	1.54 \pm 0.636	3.04 \pm 0.748
Anthracene	0.01 \pm 0.005	0.29 \pm 0.043	0.36 \pm 0.011	0.33 \pm 0.053	0.41 \pm 0.010	0.41 \pm 0.122	0.45 \pm 0.097
Fluoranthene	0.075 \pm 0.100	0.27 \pm 0.077	0.37 \pm 0.100	0.32 \pm 0.069	0.33 \pm 0.082	0.27 \pm 0.161	0.46 \pm 0.107
Pyrene	0.19 \pm 0.015	0.19 \pm 0.198	0.08 \pm 0.019	0.26 \pm 0.216	0.18 \pm 0.026	0.20 \pm 0.262	0.20 \pm 0.090
Benzo(a)anthracene	< 0.02	0.01 \pm 0.005	0.01 \pm 0.001	0.01 \pm 0.005	0.01 \pm 0.003	0.01 \pm 0.001	0.01 \pm 0.004
Cyclopenta(c,d)pyrene	< 0.01	< 0.01	< 0.01	0.02 \pm 0.007	0.02 \pm 0.002	0.02 \pm 0.005	0.01 \pm 0.008
Chrysene	0.01 \pm 0.03	0.06 \pm 0.006	0.05 \pm 0.005	0.07 \pm 0.004	0.07 \pm 0.003	0.05 \pm 0.008	0.06 \pm 0.016
Benzo(b)fluoranthene + Benzo(j)fluoranthene	< 0.02	< 0.02	< 0.02	0.02 \pm 0.005	0.02 \pm 0.004	0.02 \pm 0.004	0.03 \pm 0.005
Benzo(k)fluoranthene	< 0.01	< 0.01	< 0.01	0.01 \pm 0.003	0.02 \pm 0.004	0.01 \pm 0.001	0.0 \pm 0.0011
Benzo(a)pyrene	< 0.02	< 0.02	< 0.02	0.04 \pm 0.012	0.03 \pm 0.002	0.04 \pm 0.009	0.04 \pm 0.012
Benzo(g,h,i)perylene	< 0.01	0.07 \pm 0.064	0.08 \pm 0.019	0.08 \pm 0.026	0.11 \pm 0.064	0.08 \pm 0.004	0.08 \pm 0.007
TEQ ($\mu\text{g}/\text{kg}$)	5.65.10⁻⁴	0.009	0.011	0.052	0.044	0.053	0.056

TEQ are calculated with TEFs from INERIS table.

TEQ are lower-bound TEQs (PAH contents below the limit of quantitation were not been taken into account).

Table 6. Toxic Equivalent Factor and PAH content in salmon muscle smoked by friction at three different times of smoke exposure at two smokehouse temperatures (in $\mu\text{g}\cdot\text{kg}^{-1} \pm$ standard deviation of wet tissue).

	Initial contamination	Parameters of smoking					
		1 h, 22°C	1 h, 32°C	2h, 22°C	2 h, 32°C	3 h, 22°C	3 h, 32°C
Fluorene	0.06 ± 0.041	1.39 ± 0.521	1.60 ± 0.312	1.46 ± 0.652	2.57 ± 0.416	1.99 ± 0.345	3.16 ± 0.656
Phenanthrene	0.04 ± 0.018	1.33 ± 0.202	1.37 ± 0.172	1.82 ± 0.457	2.32 ± 0.282	1.34 ± 0.219	2.73 ± 0.262
Anthracene	0.01 ± 0.005	0.29 ± 0.015	0.35 ± 0.011	0.38 ± 0.035	0.50 ± 0.019	0.33 ± 0.058	0.49 ± 0.083
Fluoranthene	0.075 ± 0.100	0.28 ± 0.045	0.24 ± 0.042	0.30 ± 0.066	0.33 ± 0.040	0.23 ± 0.010	0.33 ± 0.080
Pyrene	0.19 ± 0.015	0.04 ± 0.044	< 0.01	0.07 ± 0.056	0.10 ± 0.048	0.05 ± 0.051	0.15 ± 0.099
Benzo(a)anthracene	< 0.02	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.005	0.01 ± 0.002	0.02 ± 0.006	0.01 ± 0.002
Cyclopenta(c,d)pyrene	< 0.01	0.02 ± 0.004	0.01 ± 0.003	0.02 ± 0.002	0.02 ± 0.001	0.03 ± 0.006	0.02 ± 0.002
Chrysene	0.01 ± 0.003	0.06 ± 0.009	0.05 ± 0.005	0.05 ± 0.002	0.07 ± 0.012	0.06 ± 0.007	0.06 ± 0.003
Benzo(b)fluoranthene + Benzo(j)fluoranthene	< 0.02	0.01 ± 0.001	0.01 ± 0.002	0.02 ± 0.010	0.01 ± 0.002	0.02 ± 0.003	0.01 ± 0.003
Benzo(k)fluoranthene	< 0.01	0.01 ± 0.003	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.003	0.01 ± 0.004
Benzo(a)pyrene	< 0.02	0.04 ± 0.004	0.04 ± 0.010	0.05 ± 0.006	0.04 ± 0.011	0.06 ± 0.015	0.05 ± 0.007
Benzo(g,h,i)perylene	< 0.01	0.07 ± 0.020	0.07 ± 0.023	0.10 ± 0.001	0.09 ± 0.039	0.16 ± 0.012	0.08 ± 0.020
TEQ ($\mu\text{g}/\text{kg}$)	$5.65 \cdot 10^{-4}$	0.050	0.050	0.062	0.055	0.074	0.066

TEQ are calculated with TEFs from INERIS table.

TEQ are lower-bound TEQs (PAH contents below the limit of quantitation were not been taken into account).

Table 7. Toxic Equivalent Factor and PAH content in salmon muscle smoked by liquid smoke at three different times of smoke exposure at two smokehouse temperatures (in $\mu\text{g}\cdot\text{kg}^{-1} \pm$ standard deviation of wet tissue).

	Initial contamination	Parameters of smoking					
		1 h, 22°C	1 h, 32°C	2 h, 22°C	2 h, 32°C	3 h, 22°C	3 h, 32°C
Fluorene	0.06 ± 0.041	0.67 ± 0.372	0.97 ± 0.212	1.48 ± 0.200	1.48 ± 0.249	1.00 ± 0.274	1.93 ± 0.785
Phenanthrene	0.04 ± 0.018	1.25 ± 0.469	1.53 ± 0.279	1.51 ± 0.344	1.05 ± 0.429	1.13 ± 0.455	1.53 ± 0.564
Anthracene	0.01 ± 0.005	0.22 ± 0.076	0.38 ± 0.059	0.36 ± 0.002	0.33 ± 0.089	0.29 ± 0.095	0.37 ± 0.048
Fluoranthene	0.075 ± 0.100	0.31 ± 0.050	0.23 ± 0.041	0.37 ± 0.104	0.25 ± 0.024	0.34 ± 0.094	0.29 ± 0.118
Pyrene	0.19 ± 0.015	0.08 ± 0.110	0.07 ± 0.072	0.45 ± 0.118	0.06 ± 0.023	0.39 ± 0.207	0.20 ± 0.250
Benzo(a)anthracene	< 0.02	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.002	0.01 ± 0.006	0.01 ± 0.003	0.02 ± 0.008
Cyclopenta(c,d)pyrene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Chrysene	0.01 ± 0.03	0.05 ± 0.004	0.05 ± 0.004	0.07 ± 0.007	0.07 ± 0.004	0.06 ± 0.005	0.07 ± 0.004
Benzo(b)fluoranthene + Benzo(j)fluoranthene	< 0.02	0.01 ± 0.001	0.01 ± 0.001	< 0.02	< 0.02	< 0.02	< 0.02
Benzo(k)fluoranthene	< 0.01	0.01 ± 0.001	0.01 ± 0.001	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(a)pyrene	< 0.02	0.03 ± 0.001	0.05 ± 0.012	0.03 ± 0.007	0.04 ± 0.004	0.03 ± 0.003	0.03 ± 0.027
Benzo(g,h,i)perylene	< 0.01	0.08 ± 0.064	0.12 ± 0.028	0.03 ± 0.016	0.05 ± 0.025	0.09 ± 0.19	0.09 ± 0.049
TEQ ($\mu\text{g}/\text{kg}$)	$5.65 \cdot 10^{-4}$	0.037	0.060	0.039	0.048	0.038	0.041

TEQ are calculated with TEFs from INERIS table.

TEQ are lower-bound TEQs (PAH contents below the limit of quantitation were not been taken into account).

Figure 1. **A** : GC-MS, SIM acquisition. Ion chromatograms corresponding to benzo(a)pyrene, indeno(1,2,3-c,d)pyrene and benzo(g,h,i)perylene in commercial smoked salmon extracts (masses monitored: m/z 252 and 276). **B** : GC-MS/MS, SRM acquisition. Ion chromatograms corresponding to benzo(g,h,i)perylene in smoked salmon extract (transition $276 > 274$). F1A: Friction technique, 1 hour of smoke exposure, 22°C in the smokehouse. F3A: Friction technique, 3 hours of smoke exposure, 22°C in the smokehouse.

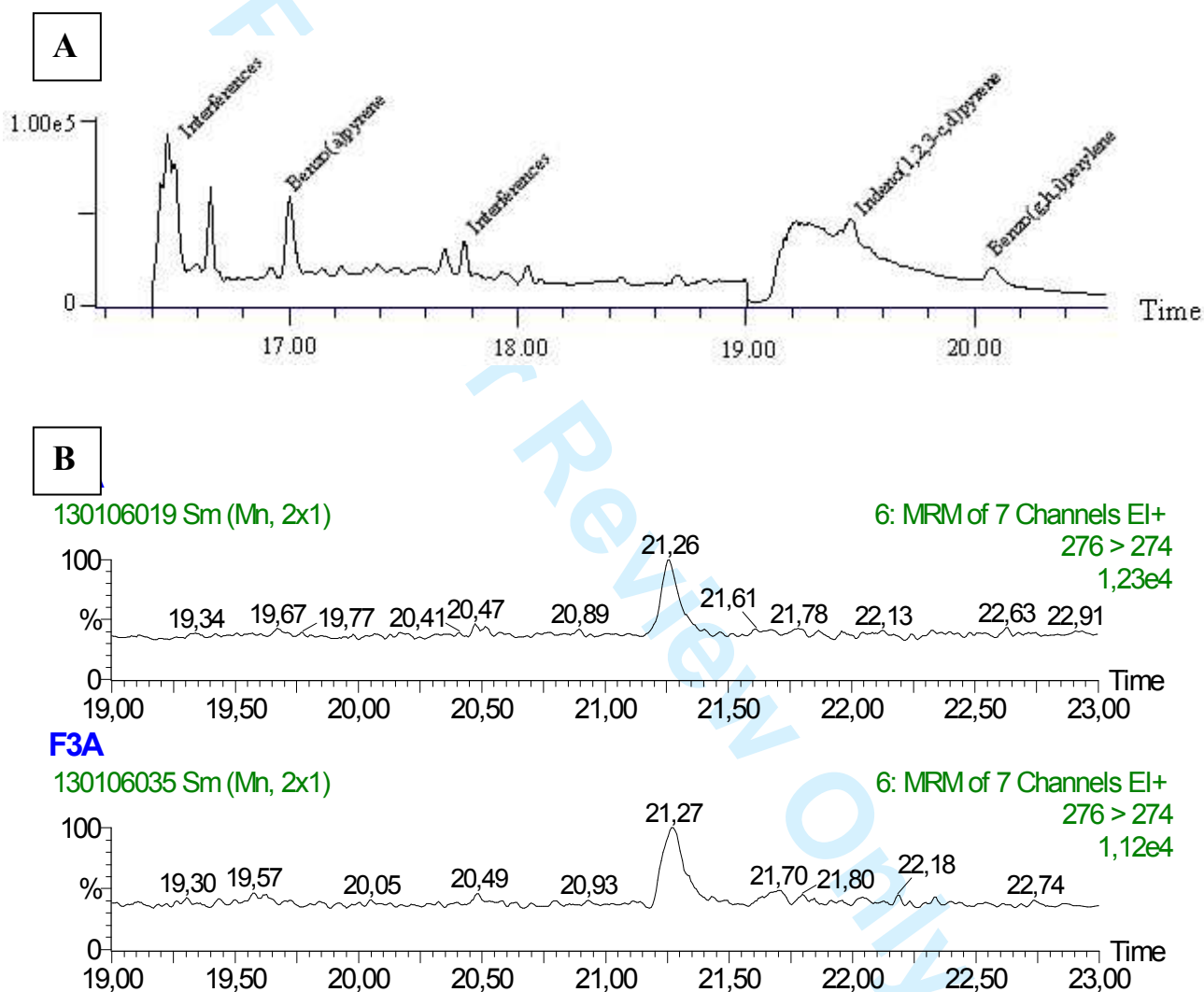
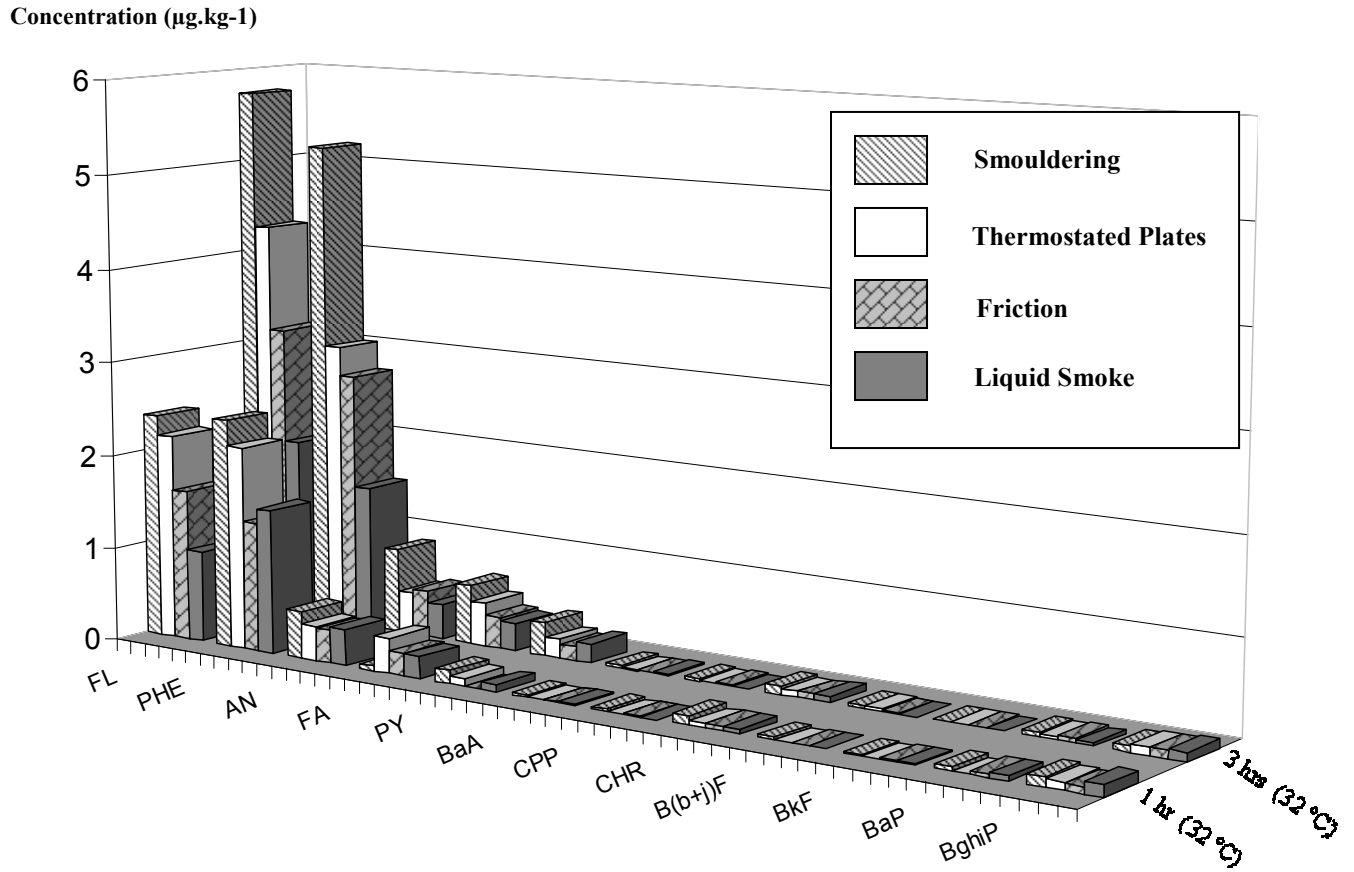
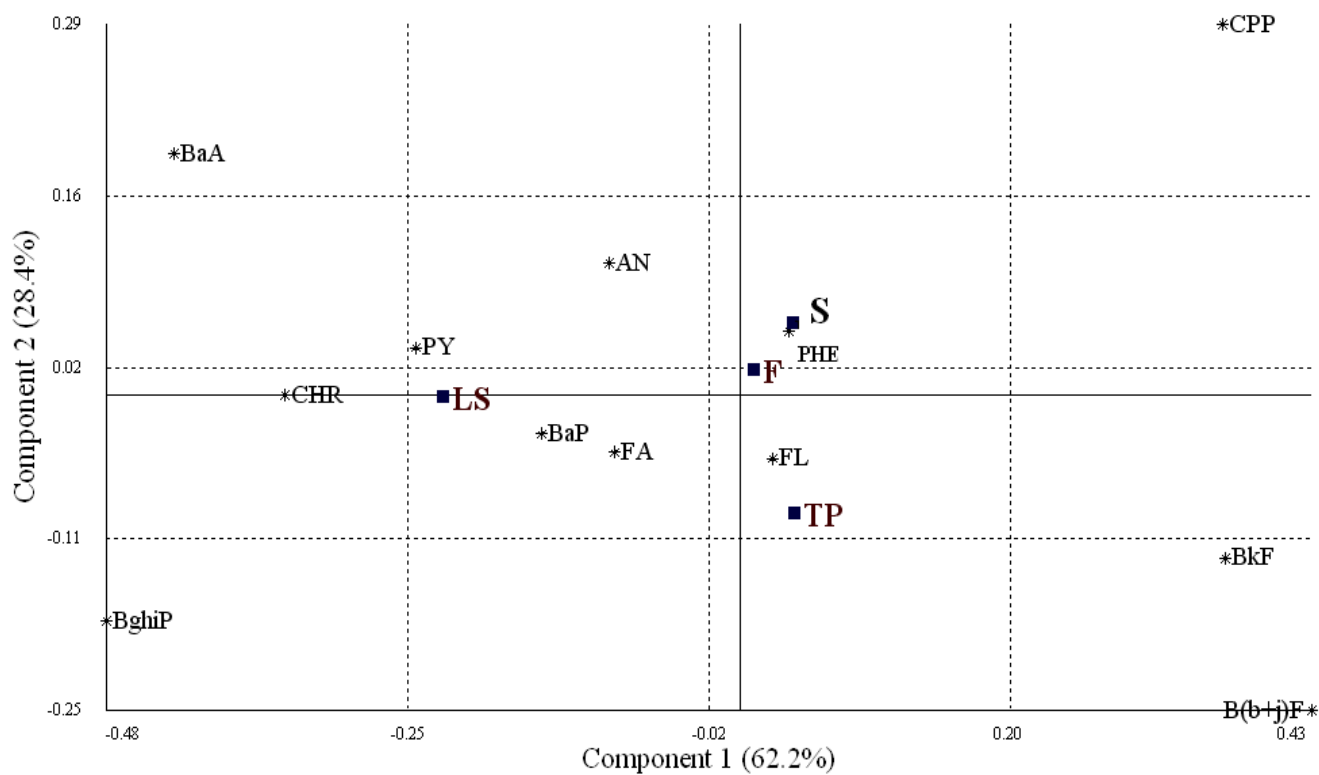


Figure 2. Predominance of PAHs of low molecular weight according to the smoking process (1 or 3 hours of smoke exposure at 32°C).



FL: Fluorene, PHE: Phenanthrene, AN: Anthracene, FA: Fluoranthene, PY: Pyrene, BA: Benzo(a)anthracene, CPP: Cyclopenta(c,d)pyrene, CHR: Chrysene, B(b+j)F: Benzo(b)fluoranthene + Benzo(j)fluoranthene, BkF: Benzo(k)fluoranthene, BaP: Benzo(a)pyrene and BghiP: Benzo(g,h,i)perylene.

Figure 3. Projection of processes and PAH contents in the plane 1-2 of Factorial Analysis of Correspondences.



For the smoking processes (in bold), S: Smouldering, TP: Thermostated Plates, F: Friction and LS: Liquid Smoke and for PAHs, FL: Fluorene, PHE: Phenanthrene, AN: Anthracene, FA: Fluoranthene, PY: Pyrene, BA: Benzo(a)anthracene, CPP: Cyclopenta(c,d)pyrene, CHR: Chrysene, B(b+j)F: Benzo(b)fluoranthene + Benzo(j)fluoranthene, BkF: Benzo(k)fluoranthene, BaP: Benzo(a)pyrene and BghiP: Benzo(g,h,i)perylene.