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Effects of toasting procedures on the levels of polycyclic aromatic hydrocarbons in toasted bread

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Abstract

Some polycyclic aromatic hydrocarbons (PAHs), particularly those with a high molecular weight, have been classified as probably carcinogens to humans by the International Agency for Research on Cancer (IARC). The significance of the determination of PAHs is reflected by the special attention of the European Union, which is paying to regulate the maximum allowed levels of PAHs in foodstuffs such as smoked foods. Like other thermally processed foodstuffs, toasted bread can contain these carcinogenic chemicals, not only due to a contamination at source but also during toasting. In order to check PAHs generated from toasting in sandwich bread, several treatment conditions were evaluated: direct toasting (flame-toasting, coal-grilling or gas oven-toasting) or indirect toasting (electric oven-toasting). PAHs were extracted by solid–liquid extraction (SLE) and determined by liquid chromatography with fluorescence detection (LC-FD). Based on the results, the used toasted technique would strongly affect in PAH levels in the final product. No samples obtained by electric oven and toaster were polluted; otherwise the samples toasted by charcoal and flame grilling presented very important levels. Up to 350 μg/kg of total PAHs were detected in toasted samples by wood flame. Differences between different ways of toasting could be ascribed to deposition of PAHs from smoke. Finally, several commercial toasted samples of bread were tested to determine PAHs. Overall, the PAH levels were very low. Benzo[*a*]pyrene ranged from no detectable to 0.23 μg/kg.

Keywords: PAHs; Toasted bread; Activated carbon; Indirect and direct toasting; Liquid chromatography; Fluorescence spectrophotometry

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals that are formed during the incomplete burning of coal, oil, gas, wood, garbage, or other organic substances, such as tobacco and charbroiled meat. PAHs have been included in the priority pollutant lists of the Agency of Toxic Substances and Disease Register (ATSDR), the International Agency for Research on Cancer (IARC), in the European Union (EU) and in the Environmental Protection Agency (EPA), due to their mutagenic and carcinogenic properties. In 1984 the United States Environmental Protection Agency (USEPA) designed 16 PAHs as compounds of interest under a suggested procedure for reporting test measurement results (USEPA, 1984). In the International Programme on Chemical Safety (IPCS, 1998), other 17 PAHs were added to the 16 PAHs listed by USEPA. Of all the 33 risk assessed PAHs, 15 PAHs (benzo[*a*]anthracene, cyclopenta[*c*,*d*]pyrene, chrysene, 5-methylchrysene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno [1,2,3-*c*,*d*]pyrene, dibenzo[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene, dibenzo[*a*,*l*]pyrene, dibenzo[*a*,*e*]pyrene, dibenzo[*a*,*i*]-pyrene, dibenzo[*a*,*h*]pyrene) were recognized as clearly mutagenic and carcinogenic (Commission Regulation,

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2006). Bay and fjord regions in their structure make them highly reactive (Codex Alimentarius Commission, 2004).

Contamination of foodstuffs by PAHs can occur at source (e.g. by atmospheric deposition on crops), during smoking and, particularly, intense thermal processing (toasting, roasting, frying, etc.). Intense thermal processes can be applied to foods in an indirect or direct way. When the thermal agent or smoke does not enter in direct contact with food an indirect thermal processing is used. Electric oven toasting could be an example of this process. A direct contact between the thermal agent or smoke and food takes place in direct thermal processes like in barbecues and gas oven toasting. Contamination of PAHs by intense thermal processing occur due to generation by direct pyrolysis of food nutrients and also, due to direct deposition of PAHs from smoke produced through incomplete combustion of different thermal agents (Fazio & Howard, 1983). Food, water and air are the main exposition routes to PAHs (OPS, 1985). Food accounts for the 99%, being smoked foods and those submitted to severe thermal treatments the foodstuffs providing the highest levels through intake. According to dietary intake studies in Italy, United Kingdom and Spain, BaP daily intakes range from 0.04 to 0.42 μg/day (Dennis, Massey, McWeeny, Knowles, & Watson, 1983; Ibáñez et al., 2005; Lodovicci, Dolara, Casalini, Ciappellano, & Testolin, 1995). In Spain, the daily intake for BaP and total PAHs was 0.14 and 8.6 μg/day, respectively (Ibáñez et al., 2005), being the group of cereals (bread, cookies, cakes, rice, pasta, etc.) the one accounting for a 20% and 40%, respectively, of BaP and total PAHs to daily intake. Bread consumption is very high in Spain; while the consumption of fresh bread was reduced from 82 to 56 kg by person and year in the last 20 years, the consumption of packaged bread was increased from 3.3 to 7.6 kg by person and year (CEOPAN, 2007). No EU maxima levels for PAHs are regulated in bread. Nevertheless the Scientific Committee on Food (SCF) demands of EU members a detailed analysis of relative ratios of these compounds in foods during technological procedures.

Bread toasting includes an intense thermal process, which can be applied by direct (flame-toasting, coal-grilling or gas oven-toasting) or indirect (electric oven-toasting) way. Although commercial bread toasting is performed at 220–250 °C in an electric oven, higher temperatures could be easily reached by consumers at home. As a result of intense thermal processes, partial carbonizations could take place in bread. The determination of PAHs in carbonized food samples is a very complex task because activated carbon is an ideal source for PAHs adsorption/retention, making their extraction more difficult. The determination requires the technique with both high sensitivity and selectivity. So far, a number of methodologies have been developed for the extraction of PAHs in different foodstuffs (Chen & Lin, 1997; Chen & Chen, 2001, 2003; Duedahl-Olesen, White, & Binderup, 2006; Jamoszka, Warecha, Blaszczk, & Bodzek, 2004; Kayali-Sayadi, Rubio-Barroso, García-Iranzo, & Polo-Díez, 2000; Larsson, Sahlberg, Eriksson, & Busk, 1983; Lintas, De Matthaeis, & MERLI, 1979; Mottier, Parisod, & Turesky, 2000; Rohrlich & Suckow, 1970; Wu, Wong, Lee, Shi, & Ong, 1997) and to our knowledge there is no publication about the difficulty of PAH extraction in food when activated carbon has been generated during intense thermal processes.

Owing to the gaps in knowledge on the PAH levels in toasted bread, an experimental design was elaborated to investigate 11 of the 15 mutagenic and carcinogenic PAHs listed on Commission Regulation 1881/2006 of 19 December 2006 (Commission Regulation, 2006) in toasted bread under different conditions. Cyclopenta[*c*,*d*]pyrene was not determined because its lack of fluorescence, and dibenzo[*a*,*l*]pyrene was selected from the group of the four isomers dibenzo[*a*,*l*]pyrene, dibenzo[*a*,*e*]pyrene, dibenzo[*a*,*i*]pyrene and dibenzo[*a*,*h*]pyrene because it is the most carcinogen (Devanesan et al., 1999). In this way, the obtained results can be used not only to select those contributing to minimize their presence in toasted bread, but also to establish maxima limits for PAHs in bread. Finally, several commercial toasted samples of bread were analyzed.

2. Materials and methods

2.1. Chemicals, solutions and materials

The 11 PAHs studied (benzo[*b*]fluoranthene (B[*b*]F, 98%), benzo[*k*]fluoranthene (B[*k*]F, 98%), benzo[*a*]pyrene (B[*a*]P, 97%), benzo[*ghi*]perylene (B[*ghi*]P, 98%), indeno [1,2,3-*c*,*d*]pyrene (I[1,2,3-*c*,*d*]P, 98%), benzo[*a*]anthracene (B[*a*]A, 98%), dibenzo[*a*,*h*]anthracene (DB[*ah*]A, 97%), chrysene (Chr, 99%), 5-methylchrysene (5-Mch, 99%), dibenzo[*a*,*l*]pyrene (DB[*a*,*l*]P, 99%) and benzo[*j*]fluoranthene (B[*j*]FA, 100%)) were purchased from Aldrich, Supelco or Isostandards (Madrid, Spain). HPLC grade acetonitrile and water, and analytical grade *n*-hexane and toluene were all supplied by Panreac. Individual 100 mg/L stock solutions of PAHs were prepared by dissolving about 0.01 g of product in a small amount of acetonitrile, hexane or acetonitrile:toluene (2:3) and diluting to 100 mL with the same solvent, which was selected depending on the solubility of the PAH. From these solutions, solutions containing 10 and 0.1 mg/L concentrations of PAHs in *n*-hexane were prepared separately. From these diluted individual solutions, mixed solution with PAHs ranging from 10 to 700 μg/L were prepared in acetonitrile following evaporation of the hexane. Working standard solutions used to construct the calibration line were prepared in acetonitrile by dilution to reach concentrations between 0.02 and 40 μg/L. These solutions were stored in amber flasks at 4 °C, where they were stable for at least 6 months. Waters Sep-Pak silica (690 mg) cartridges (Santiago de Compostela, Spain) were used as solid-phase extraction (SPE) minicolumns for purification and concentration. Analytical grade C-45 nitrogen was supplied by Carburos Metálicos (Vigo, Spain). Additional equipment included a rotary

evaporator (Heidolph, Barcelona, Spain), an ultrasonic bath (P-Selecta, Barcelona, Spain), an oven (P-Selecta, Barcelona, Spain), an analytical precision scale (Sartorius, Madrid, Spain) and a vortex shaker (Heidolph, Barcelona, Spain). Disposables used were nylon filters (0.45 μm), micropipettes (200–1000 μL) and injection vials (2 mL) furnished with screw caps and PTFE-lined butyl rubber septa and inserts (0.35 mL).

2.2. Chromatographic conditions

The liquid chromatographic system used was a Thermo Separation Products (TSP) P2000 binary pump equipped with a TSP AS1000 autosampler, a TSP SCM1000 vacuum membrane degasser and a Jasco FP-1520 fluorescence detector. Separations were performed with a 25 cm × 4.6 mm (length × i.d.), 5 μm particle, Supelcosil LC-PAH obtained from Supelco. The temperature of the HPLC column was kept constant at 33 °C to obtain reproducible PAHs retention times, what is of paramount importance when using a defined wavelength programme, which allows the optimization of sensitivity for each PAH. The used mobile phases were acetonitrile (ACN) and water. The gradient was: 80:20 ACN/H_2O change to 95:5 ACN/H_2O in 40 min change again to 80:20 ACN/H_2O in 1 min and hold for 10 min giving an analysis time of 51 min. The injection volume was set to 50 μL by LC flow rate of 1 mL/min. Detection was performed at selected excitation and emission wavelengths (Rey-Salgueiro, Martínez-Carballo, García-Falcón, & Simal-Gándara, 2008).

2.3. Samples

Laboratory toasted bread samples of white sandwich bread (28 g; 10 × 10 × 1.2 cm) were toasted under the conditions recorded in Table 1. Indirect and direct toasting was selected in order to verify the factor affecting PAHs generation.

2.3.1. Oven toasting

Electric and gas oven were tested. Samples were toasted during 20 min at 200 °C, obtaining medium toasted bread. To probe the PAHs generation when loss of temperature control takes place, bread slides were roasted in a muffle during 15 min at 300 and 500 °C.

2.3.2. Toaster toasting

An electric traditional 840 W toaster was used. Toasted conditions were 270 °C during 4 min.

2.3.3. Coal grilling

Charcoal and hot wood grills are used indistinctly because they produce the same heat intensity. Charcoal and hot oak wood were used in this work as primary means of delivering heat. First of all, charcoal was heated in a muffle at 300 °C before it was used for cooking bread. Oak wood was crushed and burned with an electric hot plate at 450 °C, avoiding flames, to obtain hot coal. Bread was positioned at 6 cm above coals till medium toasted degree was obtained. Periods of time of 5 min for charcoal and 9 min for oak wood were necessary. In both cases a grill, which allows direct contact between bread and combustion products, was used.

Table 1
Bread toasting experiments by an indirect (a) and direct (b) way

Toasting conditions	Indirect way			
	Electric oven	Muffle		Toaster
	200 °C	300 °C	500 °C	
(a)				
Source temperature (°C)	200	300	500	250–270
Bread temperature	200	300	500	250–270
Distance between source and bread (cm)	–	–	–	2
Toasting time (min)	20	15	15	4

	Direct way				
	Gas oven	Charcoal grilling		Flame grilling	
		Charcoal	Oak	Oak	Gas
(b)					
Source temperature (°C)	200	300–350	450	700–800	480–500
Bread temperature	200	190–200	140–150	100–110	300–330
Distance between source and bread (cm)	–	6	6	20	6
Toasting time (min)	15	5	9	10	10

2.3.4. Flame grilling

Bread was grilled in the flame of an oak log fire and gas fire. To obtain medium toasted bread was grilled at 20 cm above the oak log fire during 15 min and at 6 cm above the gas fire during 10 min. A grill, which allows direct contact between bread and combustion products, was used.

2.3.5. Commercial toasted bread

Twenty four samples of commercial toasted bread were collected in a market survey from three different trade names with a broad range of products (Table 2).

2.4. Sample treatment

The pre-analytical treatment used in this work was based on a procedure for the determination of PAHs in smoked foods or instant coffee previously reported by the present authors (García-Falcón, López de Alda Villaizán, González Amigo, Simal Lozano, & Lage Yusty, 1996; García-Falcón, Cancho-Grande, & Simal-Gándara, 2005a; García-Falcón & Simal-Gándara, 2005; García-Falcón, Cancho-Grande, & Simal-Gándara, 2005b). One gram toasted bread (or 0.12 g ash) was subjected to ultrasound-assisted solvent extraction with 3 × 10 mL *n*-hexane for 10 min each. The extract obtained was centrifuged (1000 U/min) during 5 min to facilitate separation of the

Table 2
Commercial toasted bread

Sample	Definition for sale, Characteristics	Nutritional information (100 g)			Principal ingredients
		Proteins	Carbohydrates	Fatty	
1	Toasted bread, Traditional	12	72	3.4	Wheat flour, vegetable fat, extract of malt, yeast, sugar
2	Toasted bread, Normal with milk and Ca	10	72	6.0	Wheat flour, yeast, vegetable fat, sugar, glucose and fructose syrup, fat free milk powder, flour of malted barley
3	Toasted bread roll, Normal	11	70	9.1	Wheat flour, yeast, vegetable fat, sugar, yeast
4	Toasted bread roll, Classics	11	69	6.4	Wheat flour, yeast, vegetable fat, sugar, yeast
5	*Grilletines*, Traditional	11	66	9.0	Wheat flour, yeast, vegetable fat (palm), sugar, wheat gluten, extract of malt, fat free milk powder
6	Toasted bread, Without salt and sugar	12	70	6.2	Wheat flour, yeast, vegetable fat, sugar, wheat gluten
7	Toasted bread, Traditional without salt	11	74	2.9	Wheat flour, yeast, vegetable fat, sugar, extract of malt
8	Toasted bread, Without fat and salt	13	75	1.1	Wheat flour, yeast, inulin (4%), wheat gluten, sugar, glucose and fructose syrup
9	Toasted bread, With milk	13	72	5.7	Wheat flour, sugar, milk powder (4%), yeast, vegetable fat
10	Toasted bread, Integral	13	64	6.6	Integral wheat flour, yeast, vegetable fat, glucose and fructose syrup, wheat gluten
11	Toasted bread, Integral	12	65	5.2	Integral wheat flour, yeast, vegetable fat , sugar, malt extract
12	Toasted bread, Integral	13	67	0.6	Integral wheat flour, sugar, vegetal soluble fibre, yeast, salt, vegetable oil
13	Toasted bread roll, Integral	12	62	10	Integral wheat flour, sugar, integral flour of crushed wheat (30%), vegetal oil, yeast
14	Toasted bread roll, Integral	11	61	8.6	Integral wheat flour, vegetal oil, sugar, yeast, malt extract
15	Toasted bread, With integral flour	12	71	5.4	Wheat flour, integral wheat flour (31%), sesame seed, yeast, palm oil, flour of malt cereals (wheat and barley)
16	*Grilletines*, Integral	13	58	12	Wheat flour, integral wheat flour (12%), vegetal oil (palm), sugar, yeast, wheat gluten, fat free milk powder, extract of barley malt
17	Toasted bread, Integral	14	62	7.0	Integral wheat flour, vegetal oil, yeast, wheat gluten
18	Toasted bread, Integral without salt and sugar	14	62	7.0	Integral wheat flour, vegetal oil, yeast, wheat gluten, salt, flour of malted barley
19	Toasted bread, Integral without salt and sugar	14	62	5.3	Integral wheat flour, vegetal oil, yeast, wheat gluten, flour of malted barley
20	Toasted bread, Integral without salt	14	68	0.6	Integral wheat flour, sugar, yeast, vegetal oil
21	Toasted bread, 10 cereals and fibre	12	68	6.1	Wheat flour, cereal flour (21%), yeast, glucose and fructose syrup, vegetal oil
22	Toasted bread, Multi cereals	14	65	2.6	Integral wheat flour, sugar, yeast, vegetal oil, salt, malted barley flour, cereals, linen, sunflower and sesame seeds, gold linen
23	Toasted bread roll, Multi cereals	12	63	4.8	Wheat flour, yeast, oat flour, rye, barley, sugar, vegetal oil, linen seeds and gold linen, sesame and sunflower seeds
24	Toasted bread, Integral con 8 cereals 97% fat free	14	64	2.0	Wheat flour, sugar, vegetal oil, malted barley flour, oat, triticale, rye, yeast, salt, rice flour, buckwheat and millet

liquid fraction. The residue was cleaned up directly with sep-pack silica plus cartridges (Waters, Spain), adding 10 mL *n*-hexane to avoid losses. Finally, the extract was evaporated till dryness under a stream of nitrogen in a TurboVap LV Concentration Workstation (Caliper Life Sciences, Spain) and filled up to a final volume of 0.5 mL or 1 mL with ACN.

3. Results and discussion

3.1. HPLC procedure performance

Chromatographic conditions were based on the method developed by García-Falcón and Simal-Gándara (2005) and García-Falcón et al. (2005b), where 7 of 11 selected PAHs in this work were detected in different foodstuffs. In order to select the most appropriate detection wavelengths for the news selected analytes such as chrysene, 5-methylchryse, benzo[*j*]fluoranthene and dibenzo[*a*,*l*]pyrene, excitation and emission fluorescence spectra were recorded. Though different gradient elution programs were applied in order to obtain a good resolution of chromatographic peaks, retention times of dibenzo[*a*,*l*]pyrene and dibenzo[*a*,*h*]anthracene were very similar. To solve the close coelution problem that pose these two PAHs, an alternative λ_{ex} and λ_{em} were selected at their elution retention time window between 23 and 33 min that are selective for dibenzo[*a*,*l*]pyrene 393/453 nm (Rey-Salgueiro et al., 2008). No selective excitation and emission wavelengths for dibenzo[*a*,*h*]anthracene was found and λ_{ex} at 296 nm and λ_{em} at 406 nm were used to its quantification.

3.2. PAH extraction procedure performance

As it was previously commented, commercial bread toasting is performed between 220 and 250 °C but higher temperatures could be reached easily. PAH generation has been evaluated under temperature conditions between 300 and 700 °C. At this point, a new matrix appears because of activated carbon generation in toasted bread samples. This fact was already verified by our previous studies (García-Falcón, Soto-González, & Simal-Gándara, 2006; Rey-Salgueiro, García-Falcón, Soto-González, & Simal-Gándara, 2004) where PAHs were measured in wood ash from different sources. We observed activated carbon generation when wood is burnt at high temperatures. There is no information as regards the recovery, precision, or quantification limits of methods for PAHs in such an activated-carbon-rich matrix. Therefore, in the present work PAH recoveries were determined by spiking materials such as commercial toasted bread and carbonized bread samples obtained at 300 °C and 500 °C. After spiking, samples were stored at refrigeration in the dark for 24 h to facilitate equilibration with the sample matrix. The set of samples analyzed was processed together with a blank to test for the background PAH levels in the material. Spiking levels were selected in accordance with the PAH levels found previously but a bit higher in the case of the higher molecular weight PAHs (Table 3). As shown in Table 3, recoveries obtained were between 82% and 103% in commercial toasted bread with RSD (%) below 7%, but between 86% and 116% in bread toasted at 300 °C with RSD (%) below 9% whereas in a range from 43% to 81% with RSD (%) lower than 5% for bread toasted at 500 °C. Production of activated carbon causes an important decrease in the efficiency of the selected extraction procedure, especially in samples toasted at temperatures higher than 500 °C. Nevertheless, as it can be seen in Table 1, laboratory bread toasting temperature was between 200 and 330 °C; only in the muffle 500 °C were reached. Therefore, the selected method was robust enough to quantify PAHs in toasted bread samples.

Table 3
PAH recoveries % (±RSD %) in commercial toasted bread and laboratory toasted bread at 300 °C and 500 °C ($n = 4$)

PAH	Commercial toasted bread	Laboratory toasted bread		Spike (μg PAH/kg)
		300 °C	500 °C	
B[*a*]A	82 ± 3	86 ± 6.0	81 ± 5.0	0.80
Chr	85 ± 2	112 ± 2.7	79 ± 0.50	5.0
5-Mch	102 ± 2	113 ± 3.0	75 ± 2.5	5.0
B[*j*]F	95 ± 4	116 ± 4.6	68 ± 3.4	14
B[*b*]F	92 ± 6	114 ± 1.4	69 ± 0.30	1.5
B[*k*]F	102 ± 7	101 ± 9.1	77 ± 3.0	0.20
B[*a*]P	103 ± 5	105 ± 5.3	70 ± 2.0	0.52
D[*a*,*l*]P	90 ± 3	91 ± 4.6	51 ± 2.0	6.0
D[*ah*]A	97 ± 4	98 ± 3.3	47 ± 3.0	1.8
B[*ghi*]P	98 ± 3	101 ± 2.3	44 ± 2.1	14
I[1,2,3-*cd*]P	89 ± 3	103 ± 6.5	43 ± 4.0	7.0

Detection and quantification limits (LODs and LOQs) were evaluated on the basis of the noise obtained with the analysis of unfortified bread samples ($n = 6$). LOD and LOQ were defined as the concentration of the analyte the produced a signal-to-noise ratio of 3 and 10, respectively (ACS, 1980) and were then tested experimentally by spiking blank samples at such levels (Table 4). External standard calibration was used to quantify the samples by LC-FD technique using multicomponent standards (Table 4). Good precision of the curves (RSD $\leqslant$ 6%) was obtained. Linear calibration plots—verified by the Mandel fitting test ($P = 99\%$) (Mandel, 1964)—were obtained over a concentration range of two or three orders of magnitude, depending on the compound.

3.3. Determination of PAHs

The method was applied to laboratory direct/indirect and commercial toasted samples.

3.3.1. Laboratory toasted bread

Bread samples were processed by means of direct and indirect toasting. Results obtained by indirect toasting are shown in Table 5. PAH pollution from indirect toasting is mainly due to the pyrolysis of macronutrients (carbohydrates, lipids and proteins). Although commercial bread toasting is performed at 220–250 °C punctual temperature elevations could be reached. In order to prove the contribution of these effects in PAHs generation, bread was carbonized at 300 °C and 500 °C during 15 min. At 300 °C starts PAHs generation, detecting 2 μg/kg B[*a*]A, 10 μg/kg 5-Mch, 0.12 μg/kg B[*k*]F and 0.50 μg/kg B[*a*]P. It would be pointed out that at the beginning of chromatogram, where B[*a*]A, Chr and 5-Mch elute, some interferences could coelute with these PAHs. Similar results were obtained by Fazio and Howard (1983) where ashes from combustion of starch at different temperatures were tested. They verified that BaP levels did not exceed 0.7 μg/kg at

Table 4
Linear dynamic ranges ($r^2 > 0.999$), together with Limits of Detection (LOD) and Quantification (LOQ) in μg/kg ($n = 6$)

PAH	Standards concentration range (μg/kg)		LOD		LOQ	
	Bread sample	Bread sample	Bread sample	Bread ash	Bread sample	Bread ash
B[*a*]A	0.020–3.4	0.020–3.4	0.04	0.33	0.10	0.83
Chr	0.12–20	0.12–20	0.25	2.08	0.75	6.2
MCh	0.12–20	0.12–20	0.12	1.04	0.35	2.9
B[*j*]F	0.30–60	0.30–60	0.65	5.40	1.75	14
B[*b*]F	0.040–6.0	0.040–6.0	0.08	0.71	0.25	2.1
B[*k*]F	0.015–1.0	0.015–1.0	0.01	0.08	0.02	0.21
B[*a*]P	0.010–2.0	0.010–2.0	0.02	0.21	0.07	0.63
DB[*a*,*l*]P	0.15–25	0.15–25	0.16	1.30	0.50	4.1
DB[*ah*]A	0.040–7.0	0.040–7.0	0.08	0.71	0.25	2.1
B[*ghi*]P	0.30–60	0.30–60	0.70	5.80	1.75	14
I[1,2,3-*cd*]P	0.20–30	0.20–30	0.33	2.70	1.00	8.3

Table 5
PAH concentrations (mean ± standard deviation, $n = 3$) found in indirectly toasted bread samples

	Electric oven	Muffle ($t = 15$ min)		Toaster
	200 °C ($t = 20$ min)	300 °C	500 °C	250–270 °C ($t = 4$ min)
B[*a*]A	ND	2.1 ± 0.42	1.2 ± 0.16	ND
Chr	ND	ND	ND	ND
5-Mch	ND	10 ± 2.35	ND	ND
B[*j*]F	ND	ND	ND	ND
B[*b*]F	ND	ND	1.1 ± 0.19	ND
B[*k*]F	ND	0.12 ± 0.024	0.31 ± 0.020	ND
B[*a*]P	ND	0.50 ± 0.25	0.80 ± 0.13	ND
DB[*a*,*l*]P	ND	ND	ND	ND
DB[*ah*]P	ND	ND	ND	ND
B[*ghi*]P	ND	ND	ND	ND
I[1,2,3-*cd*]P	ND	ND	ND	ND
∑PAH (μg/kg)	–	12.7	3.4	–

ND, not detected.

temperatures between 370 and 390 °C but 17 μg/kg were detected at 650 °C. Evidences exist of lipid and cholesterol pyrolysis, which produces higher PAH levels.

With regard to toasting by direct way (Fig. 1A and B), of the three tested toasting sources, the grilling of bread in the flames of a log fire produces the higher PAH levels, followed by charcoal grilling and grilling in the flames of a gas source. When toasting is carried out by direct way, PAH pollution is generated by pyrolysis of macronutrients and also by PAH deposition from smoke of combustion. Because of pyrolysis of macronutrients does not contribute to increase PAH levels notably, as it has been previously shown, PAH deposition from smoke of combustion would be the most important source of PAHs in bread. To prove this affirmation, toasting by bread wrapped in aluminium foil and by a frying pan was carried out at the same conditions of the grilling in the flame of a log fire. As Fig. 1C shows, PAH levels decrease up to 99.5% and 97.5%, respectively. The levels of PAH in smoke depends on heat source (coal, wood, gas, etc.), temperature, flame intensity in flame combustion, particulate material generated during combustion, etc. (García-Falcón & Simal-Gándara, 2005; Muthumbi et al., 2003; Rey-Salgueiro et al., 2004). Of the selected heat procedures in this work (gas oven, charcoal and flame grilling) was flame of a log fire of wood the procedure that reached highest PAH levels as well as highest temperatures (Table 1). In our previous studies (García-Falcón & Simal-Gándara, 2005; Rey-Salgueiro et al., 2004) similar results were observed. In this way, the kind of combustion performed on the wood material affected the PAH levels in the smoke generated, being ignition

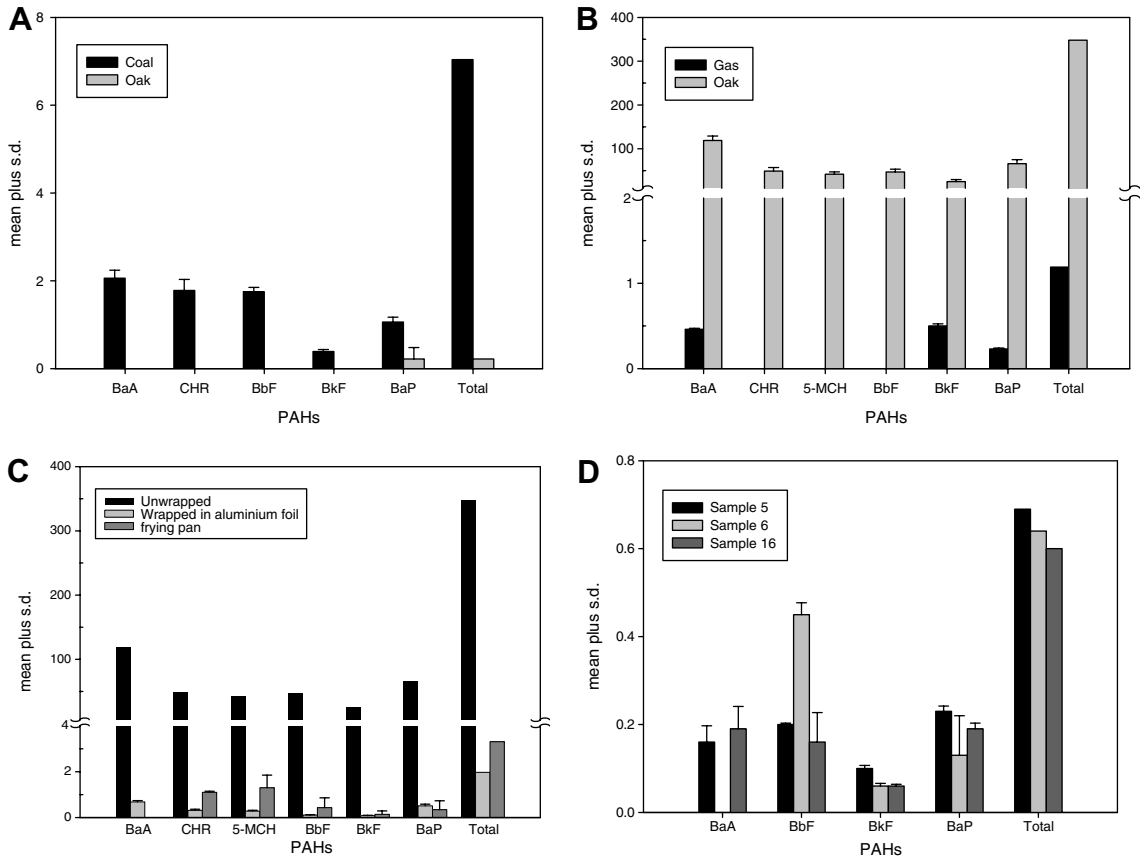

Fig. 1. Concentrations (μg/kg) of PAHs (mean ± standard deviation; $n = 3$) in (A) bread grilled over hot coal or oak, (B) bread toasted by flames from gas or oak, (C) unwrapped or wrapped bread toasted by flames from oak, and (D) commercial toasted bread samples (sample numbers are from Table 2).

and firing the material with flame what produces the highest PAH levels.

Several authors determined the effects of various processing methods, steaming, roasting, smoking, charcoal grilling, etc. on foods (Chen & Lin, 1997; Chen & Chen, 2001; Duedahl-Olesen et al., 2006; García-Falcón et al., 1996; Jamoszka et al., 2004; Larsson et al., 1983; Lintas et al., 1979; Mottier et al., 2000; Wu et al., 1997). All mentioned authors attribute the highest PAH generation during grilling or barbecue through pyrolysis during charbroiling of meat products and either deposition and penetration of smoke components into foods. Moreover, they found a link between fat foods and PAH levels. The hypothesis is that melted fat from the heated meat drips onto the hot coals and is pyrolyzed, giving rise to PAHs generation, which are then deposited on the meat surface as the smoke rises.

Larsson et al. (1983) checked different methods of cooking meat products and the results revealed that the grilling of frankfurters in the flames of a log fire resulted in extremely high PAH levels, up to 212 μg/kg BaP, depending on the fat content of the food. Frying or electrical broiling does not lead to the production of PAHs. Chen and Lin (1997) detected that charcoal grilling of duck samples with skin contained the highest amount of total PAHs, followed by charcoal grilling of duck samples without skin, smoking, roasting, and steaming. For carcinogenic PAHs, smoking contained the highest amount, followed by charcoal grilling and roasting. Similar results were determined by Kazerouni, Sinha, Hsu, Greenberg, and Rothman (2001) in meat samples cooked by different techniques in controlled conditions, and by various restaurants and fat-food chains. The highest levels of BaP (up to about 4 μg/kg of cooked meat) were found in grilled/barbecued very well done steaks and hamburgers and in grilled/barbecued well done chicken with skin. Wu et al. (1997) detected fluorene, phenanthrene, anthracene, benzo[*a*]anthracene, chrysene and benzo[*k*]fluoranthene in a range between 14 and 54 μg/kg in *rougan*, a traditional Chinese barbecued pork dish. Mottier et al. (2000) analyzed PAHs in barbecued meat sausages, determining concentration levels below the quantification limit in all products.

3.3.2. Commercial toasted bread samples

Samples of commercial toasted bread were obtained from stores in Spain in order to determine their PAH contents. Fig. 1D shows the results obtained: B[*b*]F, B[*k*]F and B[*a*]P were detected in 3 of 24 selected samples in a range of 0.25–0.45, 0.059–0.098 and 0.13–0.23 μg/kg, respectively. Similar concentrations of 0.16 and 0.19 μg/kg B[*a*]A were detected in 2 of 24 selected samples. Two pollution sources could be attributed to PAH generation in commercial toasted bread, PAH contamination in raw materials (at source) or during thermal processing. Although 9 of selected samples contain in their packages claims like "slow fire toasted", these samples were PAHs free; therefore, PAHs detected in the other samples could be attributed to the contamination of the raw material. To our knowledge there are only few papers about PAH pollution in commercial toasted bread (Kayali-Sayadi et al., 2000; Nieva-Cano, Rubio-Barroso, & Santos-Delago, 2001). Kayali-Sayadi et al. (2000) detected naphthalene, acenaphthalene, phenanthrene and dibenzo[*a*,*h*]anthracene in all bread simples analyzed. PAH levels were detected in these samples within the range 0.32–9.4 μg/kg toasted bread. Nieva-Cano et al. (2001) determined 16 PAHs in commercial toasted bread. Only fluorene, phenanthrene, anthracene, fluoranthene and chrysene were analyzed in a range of concentrations between 7.4 and 18 μg/kg for anthracene and fluoranthene, respectively. Some authors have verified higher PAH levels in products, which contain granary flour (Dennis et al., 1991). Total PAHs at 5.6 μg/kg were obtained in products with granary flour and 1.5 μg/kg with white flour. These authors also proved that fats and oils contained the highest PAHs levels due to their lipophylic character. In the selected samples only one of the 3 contaminated samples contained granary flour but all had important fat levels between 6.0% and 12%. No EU maxima levels for PAHs are regulated in bread. Nevertheless B[*a*]P concentrations in the selected samples are lower than maximum levels regulated in other foods (Commission Regulation, 2006; Duedahl-Olesen et al., 2006), ranging between 1.0 μg/kg in baby foods and 10 μg/kg in bivalve molluscs.

4. Conclusions

As far as we know, there are no published methodologies to determine PAHs in different foodstuffs when activated carbon has been generated during thermal processes. The proposed method helps to cover the needs in this area of PAHs fate in toasting bread to asses the state of food pollution. The selected method is relatively rapid and reliable for determining PAHs in bread samples toasted at temperatures lower than 300 °C. At higher temperatures than 300 °C, generation of activated carbon takes place and therefore PAH adsorption increases complicating their extraction.

The results reveal that bread toasted by indirectly does not constitute a health risk, even when bread was toasted at temperatures higher than 300 °C. With regards to direct toasting, of the three tested toasting sources, the grilling of bread in the flames of a log fire produces the higher PAH levels, following by the charcoal grilling and grilling in the flames of a gas source. When toasting is carried out by direct way, PAH pollution is produced by their deposition from combustion smoke, diminishing considerably when this deposition is avoided (wrapped in aluminium foil or by a frying pan). Several commercial toasted bread samples were analyzed. B[*b*]F, B[*k*]F and B[*a*]P were detected in 3 of 24 selected samples in a range of 0.25–0.45, 0.059–0.098 and 0.13–0.23 μg/kg, respectively. Nevertheless B[*a*]P concentrations in the selected

samples are much lower than maxima levels regulated in other foods.

On the basis of the results of the present study and with the intention of decreasing the intake of carcinogenic substances, a recommendation concerning the toasting of bread at home could be issued. Toasting by indirect way should be used and, when grilling is selected, direct contact between food and combustion smoke should be avoided.

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