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Levels, fingerprint and daily intake of polycyclic aromatic hydrocarbons (PAHs) in bread baked using wood as fuel

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ABSTRACT

Concentrations, fingerprint and daily intake of 16 PAHs in 15 bread samples baked using wood as fuel are examined in this work. Analysis was performed by GC/MS after saponification of the samples and clean up of the extract. The total concentration of the 16 analytes varies from 6 to 230 μg/kg on dry weight (d.w.). The better extraction procedure was estimated by analyzing test-samples and using different extraction methods. Additionally, for every analyzed sample, the extraction yield has been determined by the use of surrogate standards. Extraction yields were never less than 77% and in most cases almost 100%. The profiles of PAHs (percentage) are similar for all the analyzed samples but are different from those reported when other types of fuels are taken in consideration. The daily intake of PAHs was found to range between 1.6 and 68 μg day^{-1}, while the intake of B[*a*]P ranges from 0.33 to 8.0 μg day^{-1}. These results are considerably lower than the slope factor for 14 of the 15 analyzed samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds containing two or more aromatic rings [1] and are of special apprehension because of their widespread distribution throughout the environment [1,2] and their often toxic carcinogenic and mutagenic properties, which are particularly effective for 4–6 rings PAHs [3–9]. Natural and anthropogenic processes produce PAHs. Among these sources, pyrogenic (incomplete burning of coal, oil, gas, wood, garbage or other organic substances) and petrogenic inputs are the two main sources of PAHs [2,10]. A maximum amount of PAHs is formed when materials burn at temperatures in the range 500–550 °C, as in wood fires or in cigarettes [11]. In addition to the many domestic and industrial combustion processes, coal tar containing coating systems is also a major source of PAHs in aquatic systems and in the atmosphere [12,13]. To these we may add offshore activities, oil spills, offshore installations and shipping exhausts [2]. Under anaerobic conditions, some PAHs can also be derived from biogenic precursors such as natural substances (pigments, steroids, etc.) [14,15].

Non-smokers are mainly exposed to PAHs through food and air [9,16,17]. Food of plant origin can be contaminated by an accumulation of PAHs if the edible part of the vegetable is exposed to an anthropyzed area [18,19].

PAHs are significantly present in food due to heat processes such as smoking, grilling and smoke-drying, although environmental contamination is also an issue [20]. This holds especially true for vegetable organisms that do not take up significant PAHs from the soil but other sources of contamination are available, such as particles from the air [18,21,22]. Some types of edible oils (e.g., olive oil extracted by means of organic solvents) may be contaminated with PAHs through artificial drying and heating during processing, if preventive measures are not taken [23].

Bread is an essential food in human nutrition. It is a good source of energy, contains vitamins, proteins, lipids and minerals, which are essential in human diet. In Italy, bread is a major component of people's diet and the per capita consumption is among the highest in the world [24]. Local bread is produced in a number of different types of bakeries.

Italian bread manufacturers typically utilize wheat flours with high protein contents (about 12%). Commercial yeasts, in some cases, are used, but true artisanal bakers make use of natural yeast present in the environment to create a sourdough starter. The colony is periodically refreshed by adding more nutrients in preparation for the next batch. The sponge obtained mixing the flour and water with the yeast is then fermented for 3–5 h. Typical bread manufacture involves a mechanical dough-handling process-kneading, shaping and cutting - that is too rough for lower-strength, higher-hydration artisan doughs. Artisan bread is baked on the hearth of a deck oven (T = 300–400 °C)

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with some type of stone decking using wood as fuel. Refractory bricking, which absorbs and reflects heat very well, is commonly used.

In cities, most of the bakeries are either fully or partially automated and electricity and natural gas are the main sources of energy used to operate. In contrast, bakeries in the countryside are less well equipped and are fueled with wood, although a considerable number of bakeries are using waste wood [25]. Despite the high magnitude of bread consumption in Italy, few attempts, in the last years, were conducted to map out the levels of these contaminants in bread [26,27].

In the present work, considering that in Italy many people (self-made naturalists and/or ecologists) prefer to eat bread produced in countryside bakeries in which firewood (or waste wood) is burned and, known that wood smoke contains a large number of PAHs [2,28], we investigated the levels, distribution of 16 PAHs on bread baked using wood as fuel.

The work is focused on EPA-priority PAHs among which only 8 are recognized as both genotoxic and carcinogenic. In addition eight 4–6 rings PAH have been recently introduced in the EU-priority PAH list. The PAHs highlighted to be carcinogenic by the Scientific Committee on Food (SCF), for which further investigation of the relative levels in certain foods is required are: benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g,h,i*]perylene, benzo[*a*]pyrene chrysene, cyclopenta[*c,d*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]-pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene, 5-benzo[*j*]fluoranthene, cyclopenta[*c,d*]pyrene, 5-methylchrysene [29].

Some PAHs have been demonstrated to be carcinogenic and mutagenic, however, those PAHs that have not been found to be carcinogenic may be active as synergists increasing the carcinogenicity of other PAHs [8,9]. Exposure of humans to single PAHs does not occur because they are always encountered as complex mixtures. The fact that exposure to PAHs is always due to a mixture, which is not always of constant composition, renders health consequences difficult to assess.

According to the Scientific Committee on Food [30], 15 PAHs, in experimental animals *in vivo*, demonstrate evidence of mutagenicity and/or genotoxicity in somatic cells. They may be regarded as potentially genotoxic and carcinogenic to humans; their carcinogenicity depends on their structure (bay region) [31–33]. Toxicological investigations showed different carcinogenic potency for various PAHs-mixtures [34–36]. Very mutagenic and carcinogenic is B[*a*]P and it has been accepted as a marker of carcinogenic PAHs in food and environmental samples [37,38]. Since the ratio of the contents of B[*a*]P and the other carcinogenic PAHs is rather constant, the use of B[*a*]P as a marker for the contamination of food by PAHs may be justified. B[*a*]P is also regarded and recommended as a marker in Air Quality Standards [39]. However, it is necessary to underline that in a recent report [40], the Commission of European Food Safety Authority (ESFA) concluded that there are some doubt about this possibility.

Through several different studies it became evident that it is possible to specify the carcinogenic effect of individual PAH compared with B[*a*]P and to weight it by means of a conversion factor. These factors are called toxicity equivalence factors (TEF) (Table 1) [34–37].

Latest European legislation concerning PAHs in foods was introduced in 2006 [38] in response to food-contamination problems, based on data collected by the European Member States and assessed by SCF. According to regulations, the content of B[*a*]P in processed cereal-based foods and baby foods for infants and young children should not exceed 1 μg/kg [39].

Table 1
Toxic equivalency factors (TEF) according to the Environmental Protection Agency, Office of Pollution Prevention and Toxicity (US EPA OPPTS) [37]

Compound	TEF	Compound	TEF
Acenaphthylene	0.01	Chrysene	0.01
Acenaphthene	0	Benzo[*b*]fluoranthene	1
Fluorene	0	Benzo[*k*]fluoranthene	0.1
Phenanthrene	0	Benzo[*a*]pyrene	1
Anthracene	0.01	Perylene	0
Fluoranthene	0.01	Indeno[1,2,3-*cd*]pyrene	0.1
Pyrene	0	Dibenz[*a,h*]anthracene	1
Benz[*a*]anthracene	0.1	Benzo[*g,h,i*]perylene	0.01

2. Materials and method

The methods for PAHs determination, used in research laboratories and for routine monitoring of these specimens in foods, have undergone marked improvements during the past years [41–45]. There is still no official procedure accepted by all concerned that would solve the difficulties associated with quantitative analysis of PAHs from the food material [46].

2.1. Sampling

Samples of bread were collected on winter 2005, in 15 different bakeries, using wood as fuel, in the countryside near the city of Palermo. Bread samples collected for this study were baked from the same type of *duro* wheat.

Samples collected from a minimum of three pieces of 250 g, wrapped in aluminum foil, were immediately stored avoiding exposure to light and then rapidly transferred to the laboratory where they were frozen (−20 °C) less than 24 h later prior to analysis. Samples were taken directly after being baked not to let them exposed to other source of contamination. The bread samples of each bakery were separately cut into small pieces and mixed thoroughly, before three sub-samples, each of 25 g, were taken from each bakery for analysis.

In addition, as reference, we have analyzed three samples of flour and three samples of bread industrially produced purchased in supermarkets of Palermo.

2.2. Laboratory equipment

All glassware and sample containers were thoroughly washed with hot detergent solution followed by rinsing with purified water and acetone (analytical grade) respectively. These were finally baked in the oven at 110 °C overnight. To avoid contaminations of samples, different glassware and syringes were used for standards and for solutions extracted from samples.

2.3. Chemicals

All the solvents were of HPLC grade, and water was purified by a Milli-Q system. The compounds used as internal and surrogate standard were perdeuterated PAHs (Supelco, Milano). The standard solution containing 16 compounds at different concentrations (100–2000 mg/l) was the so-called calibration mix B 31455 (Supelco 610). Fluka chemicals supplied the standard solution of perylene (no. 48079 lot LB 20731) and KOH for trace analysis. Alumina (150 basic, type T particle size 0.063–0.2 mm) and silica (Silica gel, particle size 0.063–0.2 mm; Merck, Darmstadt, Germany) were washed with CH_2Cl_2 and activated for 14 h at 150 °C.

2.4. Analysis and quality assurance

Before to analyze bread samples, a quality assurance study was carried out in terms of recovery of PAHs and precision. In order to verify the accuracy (which corresponds to the percentage of recovery) and the precision (reproducibility) of the analytical procedure, some experiments (Table 2) were carried out (at least three-fold replicates) by using samples, previously dried at 105 °C, containing known quantities of PAHs, prepared by us, because a reference certified standard of bread containing the analytes taken into account in this work is not commercially available. The samples, containing known quantities of PAHs, were obtained by performing several extraction steps in 72 h on three bread samples (1 g), using Soxhlet apparatus, after the complete PAHs extraction (controlled by GC–MS analysis), a known amount (100 μl) of EPA 610 mixture of 15 analytes (Mix Supelco 458743) diluted 1:5000 (Table 2) was added to each purified blank samples. After 2 days, the test-samples obtained in this way, after having been carefully homogenized, were extracted by using three different methods. The extraction tests, together with the average recoveries, calculated for the compounds spiked, and the relative mean deviations are reported in Table 2. The results favor the saponification method because it allowed recovering the highest amount of PAHs. Therefore, from this moment onwards, for the analysis of samples of breads the saponification method described below will be used.

In addition, for every analyzed sample, the extraction yield has been determined utilizing a mix of two surrogate standards containing known concentrations of anthracene-d_{10} and benz[*a*]anthracene-d_{12}, added to samples prior of analysis. Considering the analysis of all the samples, extraction yields were never less than 77% and in most cases almost 100% and are in good agreement with literature data.

To increase the quality of results, every five to seven analyses, a blank experiment using purified water and following all procedures of extraction and clean up was carried out which gave a clean background.

The detection limit (LOD), estimated as 3σ (three times the background noise) (IUPAC criterion), was similar for all analyzed compounds and results were less than 0.015 μg/kg d.w. for all analytes. The blank values of analytical procedure remained always below the quantification limit (LOQ): 0.05 μg/kg d.w., estimated as 10 times σ.

2.5. Extraction, and clean up of bread samples

A known amount (500 μl) of anthracene-d_{10} and benz[*a*]anthracene-d_{12} solution (10 μg/l each) was added to 1 g (previously dried at 105 °C) of bread at least an hour before the analysis and the sample was digested for 4 h under reflux after addition of 50 ml of an ethanolic solution of KOH (2 mol/l). After cooling, decantation and addition of 20 ml of water, the digest was liquid–liquid extracted three times with 15 ml of pentane. The extracts were reduced to a small volume using a rotary evaporator ($T = 35 \pm 1$ °C).

The extract from such complex matrices as bread, contains not only PAHs, but also numerous other hydrophobic and slightly no polar compounds. These components must be removed in further steps of analysis in order to facilitate the separation and quantification of single PAHs. From among a number of clean up procedures, the most efficient and selective is column chromatography on Al_2O_3. An eluent of low solvent strength extracts all no polar aliphatic and aromatic hydrocarbons, polychlorinated biphenyls, some pesticides, and PAHs from such a column, while the components that make up the major mass of the dissolved material, such as (saponified) triacylglycerols and more polar lipids, remain adsorbed in the column.

The purification of the extract was performed by chromatography after dilution with dichloromethane/pentane (35:65 v/v) on an alumina micro-column (1.4 g of Al_2O_3, length ≈7 cm). The hydrocarbons were eluted with 8 ml of dichloromethane/pentane (35:65 v/v). This procedure, often treated as preliminary clean up, has been suggested as a method of choice for the actual ISO standard [42].

The solution was taken up to dryness, diluted with 1 ml of pentane and then transferred to a silica micro-column (0.8 g of SiO_2). The stationary phase was saturated with pentane, the alkanes were eluted with 2 ml of pentane and the aromatic fraction was eluted with 5 ml of the $C_2H_2Cl_2/C_5H_5$ mixture.

The last stage in the procedure involved drying the solution containing PAHs under a weak nitrogen flow at room temperature. The dry residue was dissolved in 500 μl solution containing the following perdeuterated internal standards in cyclohexane (250 μg/l each): acenaphthene d_{10}; phenanthrene d_{10}; chrysene d_{12} and perylene d_{12}. This solution is suitable for analysis directly by GC–MS.

2.6. PAHs analysis

In this research, analyses of all samples were carried out using a gas-chromatograph since, preliminary analysis by us and literature data [46] had determined that not all peaks are well separated using HPLC technique, because the extracted solutions from bread samples contain many more components than the standards of 16 PAH thus a HPLC column has an insufficient number of theoretical plate to separate, for example, benzo[*b*]fluoranthene from benzo[*k*]fluoranthene. The attempts at separation of analytes were carried out with a liquid chromatograph operating at high pressure (Jasco PU-980 Gulliver) equipped with column C:18 (Supelco 15 cm × 4.6 mm). The elution mixture was made up with acetonitrile (HPLC solvent, Carlo Erba, Milano) – water (bidistilled on alkaline $KMnO_4$ and filtered on 0.45 μm membranes). The signal from the UV/vis detector regulated at 254 nm, and that from the fluorimetric detector (Jasco FP 6200) regulated at 290 nm for excitation and at 430 nm for emission was used for the identification of the components.

The GC instrument (Shimadzu mod.GC-17A) was coupled with a mass spectrometer (Shimadzu, quadrupole detector mod. GCMS-QP5000) equipped with an acquisition data system (Shimadzu, CLASS 5000). The MS detector can operate in two different modes: scanning and selected ion monitoring (SIM).

The data were acquired operating in SIM mode that allows quick identification and quantification of the preselected ion peaks. On the other hand, non-preselected peaks, e.g., substituted PAHs and other interferences are not quantified. SIM is more sensitive technique for trace quantitative analysis and can result in as much as a 500-fold increase in sensitivity.

The used column is an Equity-5 (30 m 0.25 i.d., 0.5 μm) fused-silica capillary column from Supelco (Milano, Italy) that has a high efficiency, thus the components of environmental and/or food extracts can be much better separated than by using HPLC. Ultra pure (99.999%) helium is used as a carrier gas (20.6 ml/min). The 1 μl solutions of the extracted are injected in the splitless mode at 0.61 min split delay. The injection of both extracts from samples and standard solutions was performed by hand. The use of an injector equipped with electronic pressure control allows maintaining a constant flow rate during the entire separation. The injector temperature was maintained at 280 °C. The GC temperature program was: from 40 °C (2 min) to 100 °C at 40 °C/min, to 200 °C at 10 °C/min, to 325 °C (8 min) at 30 °C/min.

Table 2
Concentrations of the solutions added to purified blank samples to value the accuracy of analytical methods and results* of recovery tests carried out on samples of bread (previously dried) containing known quantity of 15 compounds

Compound	Concentration of solution used for spiking (μg/l)	Mathematical concentration in bread (μg/kg)	Recovery (μg/kg d.w.)*			
			Soxhlet	Saponification	Ultrasound (C_5H_5/CH_2Cl_2 1:1)	Ultrasound (CH_2Cl_2)
Acenaphthylene	400	40	14	25	10	11
Acenaphthene	200	20	2.9	12	11	13
Fluorene	40	4	2	2.8	1.7	2
Phenanthrene	20	2	1.8	1.5	1.5	1.4
Anthracene	20	2	1.1	1.5	1.2	1.1
Fluoranthene	40	4	3.9	3	3.1	2.7
Pyrene	20	2	1.6	1.5	1.4	1.2
Benz[*a*]anthracene	20	2	1.5	1.6	1.2	1.4
Chrysene	20	2	1.8	1.7	1.6	1.6
Benzo[*b*]fluoranthene	40	4	3.5	2.9	3.3	3.2
Benzo[*k*]fluoranthene	20	2	1.9	1.6	1.9	2.1
Benzo[*a*]pyrene	20	2	1.9	1.8	1.1	1.5
Indeno[1,2,3-*cd*]pyrene	20	2	1.8	1.5	1.5	1
Dibenz[*a,h*]anthracene	40	4	2.7	2.8	2	3
Benzo[*g,h,i*]perylene	40	4	3.5	2.9	1.5	1.6
Total PAHs*		96	46 ± 5	64 ± 5	44 ± 10	48 ± 11

* Mean of three analysis.

Identification of the components of the standard mixture was carried out by comparing retention times for each component of the mixture with those of pure components analyzed under the same experimental conditions. Identification was confirmed by comparing the spectra of the single components with those stored in the acquisition system library (NIST). The identification of PAHs in the solutions extracted from samples was carried out on the basis of previously determined retention times and confirmed by using mass spectra previously acquired.

The content of each single analyte in the sample was quantified relatively to the perdeuterated PAHs added to the dry residue. The complete calibration for all the analytes was measured by injecting, every 2–3 days, five solutions containing 15 standard compounds at known concentration, prepared for dilution of a concentrated mix (Mix Supelco 458743) and having the same concentration of perdeuterated PAHs as that used for spiking the sample. Most of the analytes have response factor of the same order of magnitude. The response of the GC–MS instrument was checked every morning using a solution containing only four compounds.

The list of analyzed compounds, the perdeuterated standards employed, the quantification ion and the confirmation ion for each analita are shown in Table 3.

2.7. Water content analysis

About 2 g of homogenized sample was dried at 105 °C for one night. The water content was determined by weight loss and was utilized to correlate all the results with dry weight (d.w.).

3. Results and discussion

3.1. Levels and distribution of PAHs

The concentrations of single PAHs measured in the reference samples (wheat and bread prepared industrially) are very similar to the quantification limits.

The total concentration of the 16 compounds investigated, expressed as the sum of concentrations ($\sum$PAH), in bread baked using wood as fuel, varies from 6 to 230 μg/kg of dry weight (d.w.) (Table 4), also, considering the mean value of 15 samples, we report the percentages of single compounds found in the bread prepared in different bakeries, using wood as fuel. To evaluate the precision of the analysis, three replicates of all samples were analyzed. The R.S.D. of the replicas on the concentrations of individual compounds ranged from 7 to 25%.

Concentration difference found in the bread samples is related more to exposure and time of backing while heterogeneous levels (sources) of contamination is usually indicated by a variation of PAHs spectrum. In most of the samples analyzed in this study, the profiles of PAHs (percentage) (Fig. 1) distribution are similar among themselves but are different from those reported in a research that takes in to consideration other types of fuels (heavy oil, light oil, solid waste and electricity) [25].

The differences in distribution of the single compounds in the analyzed bread samples were probably a consequence of the specific methods of each artisan baker.

Considering the average of the results of all the studied samples, fluorene, phenanthrene and indeno[1,2,3-*cd*]pyrene are the three

Table 3
List of 16 PAHs, the deuterated standards employed, the quantification ion and confirmation ion for SIM GC–MS mode (Underlined compounds are the internal standards utilized in the analysis)

Group	Chemical	Quantification ion	Confirmation ions
1	Acenaphthylene	152	76, 151
	Acenaphthene	154	152, 76
	Fluorene	166	164, 165
	Acenaphthene d_{10}	164	
2	Phenanthrene	178	188, 89
	Anthracene	178	188, 89
	2 Methyl anthracene	192	96, 82
	9 Methyl anthracene	192	96, 82
	Fluoranthene	202	101, 200
	Pyrene	202	101, 200
	1 Methyl pyrene	216	108, 94
	Benzo(a)anthracene	228	114, 226
	Phenanthrene d_{10}	188	
3	Chrysene	228	114, 226
	Benzo(b)fluoranthene	252	126, 250
	Benzo(k)fluoranthene	252	126, 250
	Benzo(a)pyrene	252	126, 250
	Chrysene d_{12}	240	
4	Perylene	252	126, 250
	Indeno(1,2,3-*cd*)pyrene	276	277, 138
	Dibenzo(*a,h*)anthracene	278	279, 139
	Benzo(*g,h,i*)perylene	276	277, 138
	Perylene d_{12}	264	

Table 4
Individual, total and B[*a*]P equivalent PAHs concentrations (averages of three replicas)* of PAHs (μg/kg d.w.) detected in bread samples baked with wood fuel and % of single PAHs (mean of 15 samples)

Compound/sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	% Mean
Acenaphthylene	1.05	0.73	0.32	0.57	2.6	2.6	0.38	6.7	6.9	1.6	6.9	16	11	5.0	7.6	4.7
Acenaphthene	0.95	0.23	0.29	0.64	10	5.0	0.31	7.8	11	6.1	13	7.5	3.8	6.1	11	5.6
Fluorene	0.95	0.32	0.32	1.8	14	8.3	0.27	16	24	11.	23	19	8.4	8.8	21	10
Phenanthrene	1.5	0.93	0.50	6.0	24	13	0.76	27	35	20	44	67	30	18	36	22
Anthracene	0.09	0.09	0.05	0.27	1.7	1.0	0.07	1.7	1.9	1.4	2.6	10	2.8	1.8	2.9	1.9
Fluoranthene	0.51	0.27	0.21	0.74	3.8	1.7	0.23	3.8	4.2	3.4	8.4	24	7.3	3.9	12	5.0
Pyrene	0.52	0.21	0.23	0.90	0.07	1.5	0.33	4.7	3.1	2.3	7.3	21	5.0	2.9	11	4.1
Benz[*a*]anthracene	0.32	0.05	0.15	0.22	0.22	0.33	0.18	0.40	0.29	0.22	3.5	4.4	0.27	0.31	4.8	1.0
Chrysene	0.54	0.14	0.38	0.85	1.60	0.84	0.43	1.01	0.79	0.61	9.1	11	1.3	0.91	7.5	2.5
Benzo[*b*]fluoranthene	1.04	0.28	0.36	0.54	0.83	0.81	0.30	0.59	0.87	0.14	5.7	8.1	0.48	1.6	7.1	1.9
Benzo[*k*]fluoranthene	0.61	0.23	0.26	0.36	0.77	1.9	0.16	1.1	0.68	0.18	8.6	9.3	0.24	1.3	11	2.4
Benzo[*a*]pyrene	0.72	0.37	0.28	0.54	0.73	1.5	0.36	1.2	0.13	0.16	4.4	9.4	0.25	0.16	8.4	1.9
Perylene	0.65	0.091	<0.015	0.22	0.73	0.32	<0.015	<0.015	<0.015	0.032	0.32	0.19	<0.015	0.15	0.17	0.19
Indeno[1,2,3-*cd*]pyrene	2.1	5.0	1.6	9.1	15	4.4	1.0	16	7.1	9.1	18	10	2.9	0.34	16	7.9
Dibenz[*a,h*]anthracene	1.5	0.70	0.23	1.3	4.6	1.2	0.89	3.6	1.2	1.6	1.6	5.6	0.18	0.50	5.7	2.0
Benz[*g,h,i*]perylene	1.4	0.49	0.40	0.83	5.2	0.54	1.0	2.6	1.3	4.2	13	6.6	0.66	0.46	7.6	3.1
Total PAHs	15	10	5.5	25	86	45	6.8	94	98	62	169	230	74	52	171	76
B[*a*]P equivalent PAHs	3.6	1.9	1.1	3.4	7.9	4.2	1.7	7.3	3.2	3.0	15	26	1.5	2.6	25	7.2

* The R.S.D. of the three replicas on the concentrations of individual compounds ranged from 7 to 25%.

most abundant components. Phenanthrene is always much more abundant than the isomeric anthracene.

Of all PAH analyzed in this context some of them, especially the slightly volatile ones, are not regarded as carcinogenic. Within the remaining PAHs, there are substantial differences of potency in the size of several orders of magnitude. Concentrations of carcinogenic PAHs were calculated by:

$$\mathrm{TEQ} = \Sigma(\mathrm{PAH}_i \times \mathrm{TEF}_i)$$

where TEQ = toxic equivalents of reference compound; PAH_i = concentration of PAH congener *i*; TEF_i = toxic equivalent factor for PAH congener *i* (Table 1) [37].

Carcinogenic PAHs, expressed as B[*a*]P equivalent [37] ranged from 1.1 to 26 μg/kg d.w. (Table 4).

In samples of bread baked using wood as fuel, concentrations of B[*a*]P ranged from 0.13 to 9.4 μg/kg d.w. Five samples exceeded the legal limits (1 μg/kg) proposed by the Official Journal of the European Union for processed cereal-based foods [38].

Dennis et al. [42] reported that the level of benzo[*a*]pyrene was lower than 0.1 μg/kg in English white flour and similar amount was found in bread, meanwhile they reported that higher concentrations up to 2.2 μg/kg were detected in cereal-derived products containing higher levels of edible oils.

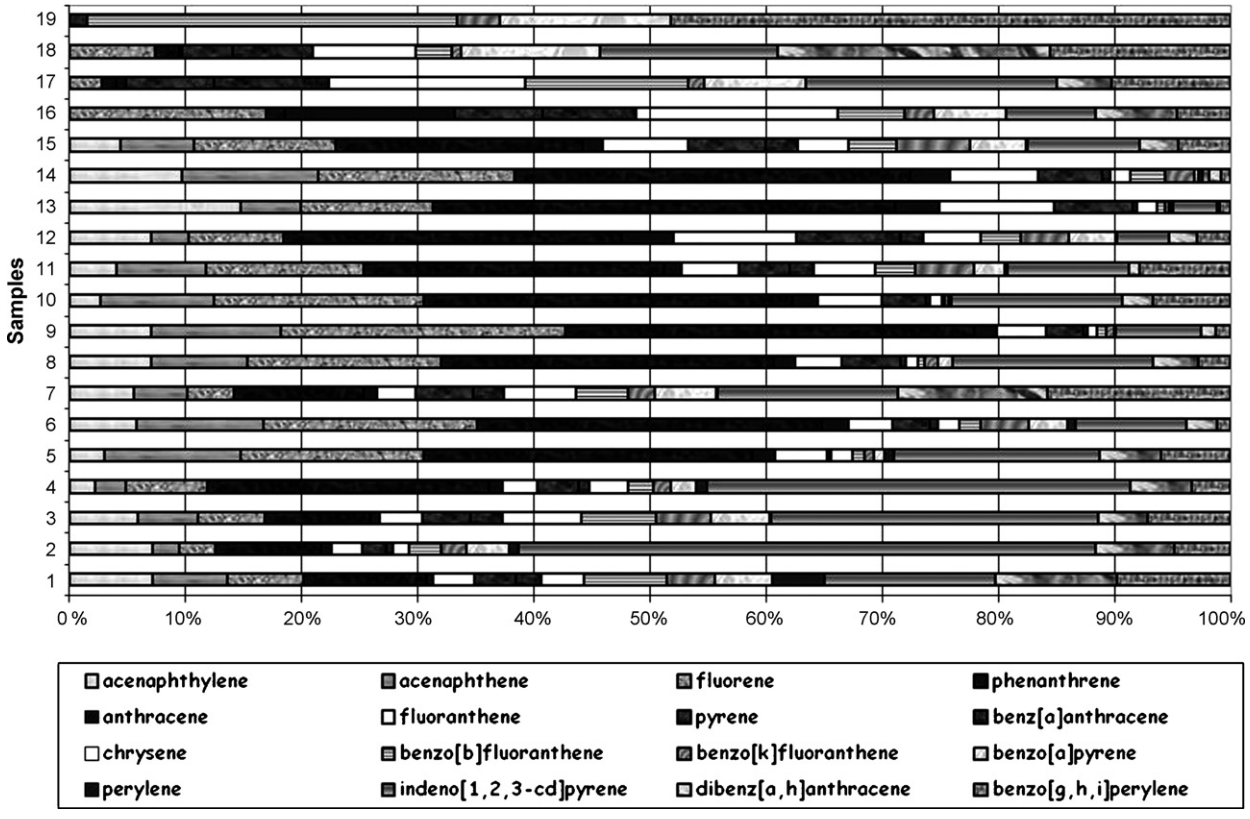

Fig. 1. Distributions of single compounds detected in bread baked with wood (1–15) and with other fuels (16–19).

Usually, in environmental matrices, the molecular patterns generated by diverse sources are like fingerprints, which makes it possible to hypothesize which processes generate PAHs by studying their distribution in samples [47–50].

Each sample was analyzed for its content of the 16 PAHs and the sum of five low molecular weight LPAHs (i.e., Aceph, Ace, Fl, Phen and Ant) and 11 high molecular weight HPAHs (i.e., Flu, Pyr, BaA, Chr, BbF, BkF, BaP, Per, DBA, BgP and Inp).

Attention has been paid to the distribution of low and high molecular weight PAHs (LPAHs and HPAHs, respectively) as a reliable tool for discriminating the petrogenic/pyrolytic origin of PAHs [51,52]. The lower the LPAHs/HPAHs ratio, the higher the prevalence of pyrolytic on petrogenesis origin of PAHs is. The LPAHs/HPAHs for our samples ranged from 0.24 to 0.88 and average ratio is ca. 0.62.

In analyzed samples the values of ratios anthracene to anthracene plus phenanthrene An/(An + Ph) ranged from 0.15 to 0.38 (Table 4), with an average of 0.27 and R.S.D. = 23%. Ratio <0.10 usually is an indication of low temperature sources (petroleum) while, a ratio >0.10 indicates a dominance of combustion [47–50].

For the bread baked using wood as fuel, ratios Fl/(Fl + Py) ranged from 0.41 to 0.98 with an average of 0.52 (Table 5), the R.S.D. (non-considering the values of ratio of sample no. 5) are very low (11%). Generally, for environmental matrices (sediments, organism, air, etc.), the Fl/(Fl + Py) ratio is below 0.50 for most petroleum samples and for gasoline, diesel and fuel oil combustion and above 0.50 in grass, most coal and wood combustion samples. The petroleum limit ratio appears closer to 0.40 than 0.50 for Fl/(Fl + Py) and ratios between 0.40 and 0.50 are more characteristic of liquid fossil fuel combustion whereas ratios >0.50 are characteristic of grass, wood or coal combustion [46–49].

For our samples, ratios B[*a*]A/(B[*a*]A + Chr) ranged from 0.12 to 0.39 with an average value of 0.27. The literature data [48] suggest that B[*a*]A/(B[*a*]A + Chr) ratios <0.20 involve petroleum, from 0.20 to 0.35 indicate either petroleum or combustion and >0.35 imply combustion. Lower value was obtained for sample no. 5.

IP/(IP + B[*g,h,i*]P) ratios for our samples ranged from 0.42 to 0.92. Accordingly to literature data, IP/(IP + B[*g,h,i*]P) ratios higher 0.50 imply combustion [45–47].

The results (Table 5) confirm that all the PAHs identified in the bread samples originate from combustion processes.

3.2. Daily intake of PAHs

The concentrations of PAHs in food have been studied in different parts of the world because of the fundamental importance of these matrices and the heavy reliance of some societies on them [53–56].

Mean level of PAHs in the analyzed samples is higher than that reported from Tawfic et al. for bread baked utilizing electricity but are lower compared to those obtained using other fuels; the total concentration of PAHs detected in bread prepared using mazot, solar and solid waste fuel had respectively an average of 321, 158 and 317 μg/kg [25].

Several studies have determined the level of PAHs intake associated with a normal human diet [9,57–60].

In Italy, dietary exposure to PAHs (3 μg day^{-1}) was estimated to be significantly higher that respiratory intake of PAHs from polluted urban air (0.37 μg day^{-1}) [54]. A research estimates that the amount of weekly exposure to benzo[*a*]pyrene derived from food is commonly about 70% in the homes of non-smokers [55]. It is estimated that the median dietary intake of 3 μg day^{-1} of total PAHs represents around 96% of the total daily exposure for non-smokers [57]. Lifestyle is a potential factor to exposure, for example, tobacco smoke increases the intake of PAHs. The health risks for adults and children are different since the contact pathway with each exposure

Table 5
Ratios between isomer compounds

Isomer ratios	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Heavy oil[a]	Light oil[a]	Solid waste[a]	Electricity[a]
An/(An + Ph)	0.15	0.25	0.18	0.27	0.31	0.38	0.24	0.31	0.31	0.29	0.24	0.29	0.27	0.31	0.19	0.00	0.00	0.00	n.c.
Fl/(Fl + Py)	0.49	0.56	0.48	0.45	0.98	0.54	0.41	0.44	0.57	0.59	0.53	0.54	0.59	0.57	0.52	1.00	1.00	1.00	n.c.
BaA/(B[*a*]A + Chr)	0.37	0.27	0.29	0.21	0.12	0.28	0.30	0.28	0.27	0.27	0.28	0.28	0.17	0.26	0.39	0.32	0.37	0.44	1.00
In/(In + B[*g,h,i*]P)	0.60	0.91	0.80	0.92	0.75	0.89	0.50	0.86	0.85	0.69	0.57	0.61	0.81	0.42	0.68	0.63	0.68	0.50	0.0

n.c. = Not calculated because of the lack of data.
[a] Calculated from data [25].

medium (e.g., food) changes with age. Therefore, there would be a certain amount of discrepancy in health risks between age groups and the locality of the inhabitants [60–62].

In the present study, the estimated daily intake of total PAHs, based only on consumption of bread (300 g/person/day) [24], baked with wood as fuel range from 1.7 to 69 $\mu g\,day^{-1}$ per person.

Estimated daily intake of PAHs based on consumption of bread produced in bakeries using mazot, solar, solid waste and electricity, respectively were 48, 28, 80, and 5 μg/person/day. Meanwhile, the respective estimated daily intakes of benzo[*a*]pyrene were 3.7, 2.7, 8.1, and 0.81 μg/person/day [25].

Considering our data, the intake, of B[*a*]P, based on consumption of 300 g of bread per day, range from 0.04 to 2.8 $\mu g\,day^{-1}$ per person and result considerably lower than its slope factor (7.3 $\mu g\,day^{-1}$) [63] for all analyzed samples.

Reports of daily intake of B[*a*]P in other countries, based on all types of food consumption vary between 0.36 μg/person/day in Austria, 0.25 μg/person/day in the UK, 0.5 μg/person/day in the Netherlands and 0.1–0.3 μg/person/day in Italy [25]. The present results indicate the comparatively similar level of the daily intake of B[*a*]P in the study area, though it is only based on bread baked using wood as fuel consumption.

In accordance with the standard EPA methods, the risk of PAHs effects is expressed as the ratio of the dose resulting from exposure compared to a dose that is believed to be without risk of effects, even in sensitive individuals. This ratio is called the Hazard Quotient (HQ). If the HQ exceeds one, then there is a chance that non-carcinogenic effects may occur, with a probability which tends to increase as the value of HQ increases [64,65].

The World Health Organization (WHO) established a provisional tolerable intake (PTWI) of 7.3 $\mu g\,day^{-1}$ for B[*a*]P [63].

Considering our data, only a sample shows HQ values (calculated as reported in literature [61,62,65]) higher of 1 through consumption of bread baked using wood as fuel. This indicates that health risks associated with B[*a*]P exposure is insignificant.

Our main remarks are as follows:

(a) Bread baked with wood fuel constitutes a significant part of the human diet, important because of their desirable sensory properties, high nutritional value and abundance in carbohydrates and proteins.
(b) The present study made it possible to optimize extraction and analytical conditions for the determination of PAHs in bread samples. Under these conditions, recoveries are very high: never less than 77% and in most cases almost 100%. Reproducibility is also satisfactory (R.S.D. ranged from 7 to 25%).
(c) The wood smoke generated in baking of bread contains, depending predominantly on the condition of oven, a large variety of PAHs, including the most carcinogenic ones. The contents of B[*a*]P in the bread is, on average, lower than the limit set by National and European regulations.
(d) The greater presence of PAHs with high molecular weights in all samples and the isomeric ratios values used as PAHs distribution indexes confirm that most samples owe their PAHs to a combustion origin.
(e) Total PAHs content in nearly all samples are correlated with the concentrations of many of the single compounds. This evidence indicates that during the process of baking of the bread in the firewood furnace a characteristic mixture of PAHs is produced and consequently for routine analyses only a minor number of compounds could be analyzed.
(f) The daily intake of PAHs was found to range between 1.6 and 68 $\mu g\,day^{-1}$, while the intake of B[*a*]P ranges from 0.33 to 8.0 $\mu g\,day^{-1}$ and results in a considerably lower scale than its slope factor for 14 of the 15 analyzed samples.

The present work has indicated the comparatively high level of daily intake of B[*a*]P in comparison to levels reported from many other countries.

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