Determination of PAHs in food matrices

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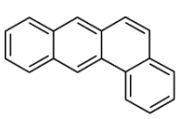
Abstract

When we talk about oil quality, we usually refer to what emerges from the chemical-physical analysis. For every olive grower, the first step to take, the most spontaneous and immediate among all, consists in immediately establishing the goodness of what is produced, bringing the oil just extracted to the nose. Sensations are immediately perceived. Then, there is a whole world behind that can not be overlooked. Sensory evaluation is not enough, it is also necessary to evaluate the oil analytically. It is done with instruments present in the mill, but they are not sufficient. It is the case to deepen analyzing the oil samples produced in a laboratory. Have you ever heard about contaminants? It is important to understand that the concept of quality is very complex and involves detailed studies, by those who produce and sell the oil, not only on the usual parameters, but also on non negligible details, related to the presence of any contaminants. In this paper the content of polycyclic aromatic hydrocarbons (PAHs) in various food matrices was analytically evaluate, in particular in different types of oil, cocoa and cereal-based product for children.

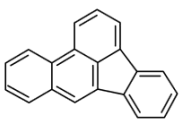
Introduction

Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that are composed of two or more fused aromatic rings with a pair of carbon atoms shared between rings in their molecules. PAHs containing up to six fused aromatic rings are often known as “small” PAHs, and those containing more than six aromatic rings are called “large” PAHs. The simplest PAHs, as defined by the International Agency for Research on Cancer (IARC) [1], are phenanthrene and anthracene, which both contain three fused aromatic rings. Naphthalene, which consists of two coplanar six-membered rings sharing an edge, is another aromatic hydrocarbon. The most extensively studied PAH is benzo(a)pyrene (BaP) and the most commonly analyzed PAHs are given in Figure 1. PAHs solely consist of carbon and hydrogen and do not contain heteroatoms. They are primarily formed by incomplete combustion or pyrolysis of organic matter and during various industrial processes. Consequently, the natural and anthropogenic sources in the environment are numerous. PAHs generally occur in complex mixtures which may consist of hundreds of compounds.



Benzo[a]anthracene



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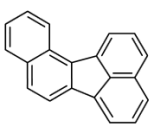
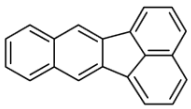
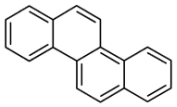


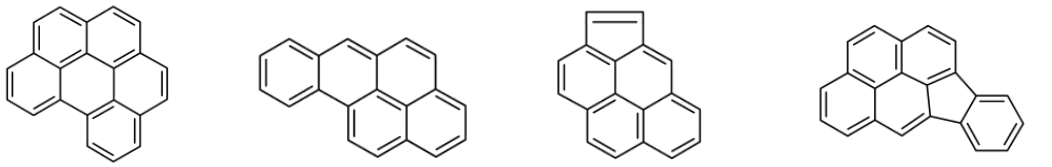
Benzo[j]Fluoranhene



Benzo[k]Fluoranthene



Chrysene



Benzo[ghi]Perylene Benzo[a]Pyrene Dibenzo[ah]anthracene Indeno[1,2,3-cd]Pyrene

Figure 1. Structure of polycyclic aromatic hydrocarbons.

Source of PAHs

PAHs derive from natural sources through three different processes: 1) pyrolysis of organic material at high-temperature (350°C – 1200°C), 2) creation of fossil fuel from organic material at low to medium temperature (100°C – 150°C) and 3) biosynthesis by microbes and plants [2]. The mode of PAHs formation can be either natural or anthropogenic. Examples of natural sources of PAHs formation include: forest and brush fires, volcanoes, bacterial and algal synthesis, petroleum seeps, erosion of sedimentary rocks containing petroleum hydrocarbons, and decomposition of vegetative liter fall. Moreover some types of food cooking can, for example, lead to the formation of PAHs, in particular heat treatments linked both to the preservation of the product (smoking), and to the preparation of the food (grilling, cooking, frying). An additional source of contamination, in addition to the aforementioned, is environmental one. In fact, PAHs can also be found in broad-leaved vegetables such as lettuce and spinach for the deposition of these substances carried with the air on the leaves during growth [3].

Source of human exposure

There is a continuous concern regarding the exposure of the population to PAHs. This concern arises from the fact that PAHs are ubiquitous environmental pollutants that possess mutagenic, teratogenic and carcinogenic effects. Table 1 lists all PAHs quantified and their carcinogenic (IARC) classifications [4].

PAH	MW	No. aromatic rings	IARC Group
Benz[a]anthracene	228	4	2A
Chrysene	228	4	3
Benzo[b]fluoranthene	252	5	2B
Benzo[k]fluoranthene	252	5	2B
Benzo[j]fluoranthene	252	5	2B
Benzo[e]pyrene	252	5	3
Benzo[a]pyrene	252	5	1
Perylene	252	5	3
Benzo[ghi]perylene	276	6	3
Indeno[1,2,3-cd]pyrene	276	6	2B
Benzo[b]chrysene	278	6	3
Dibenzo[a,j]anthracene	278	6	
Dibenzo[a,h]anthracene	278	6	2A

Group 1: "carcinogenic to humans"
Group 2A: "probably carcinogenic to humans" and
Group 2B: "possibly carcinogenic to humans".

Table 1. Degree of evidence for carcinogenicity evaluated by IARC classifications.

- Legislative framework

Maximum limits have been set by Commission Regulation (EC) No 1881/2006 for PAHs in key foodstuffs, e.g. smoked meat and smoked meat products, smoked fish and smoked fishery products, oils and fats, infant formulae and follow-on formulae and processed cereal-based foods and baby foods for infants and young children [5]. The limits laid down in the Regulation are shown in Table 2.

FOODSTUFFS (1)	PAH	MAXIMUM LEVELS (µg/kg)
Polycyclic Aromatic Hydrocarbons		
Dietary foods for special medical purposes (9) (29) intended specifically for infants	Benzo(a)pyrene	1.0
	Sum of PAH4 (45)	1.0
Oils and fats (excluding cocoa butter and coconut oil) intended for direct human consumption or use as an ingredient in food	Benzo(a)pyrene	2.0
	Sum of PAH4 (45)	10.0
Cocoa beans and derived products	Benzo(a)pyrene	5.0 µg/kg fat
	Sum of PAH4 (45)	30.0 µg/kg fat

Table 2. Maximum levels in Regulation 1881/2006 for polycyclic aromatic hydrocarbons in foodstuffs.

Benzo[a]pyrene is the most known compound from the toxicological point of view as evidenced by IARC classification and more frequently determined in the various matrices, both environmental and food. For this reason it has been used as an indicator of the class of PAHs. Then, new maximum levels for the sum of four substances (PAH4) (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene) were introduced, whilst maintaining a separate maximum level for benzo[a]pyrene.

Materials

In this work, a total of 59 samples were analyzed: 37 samples of oil, 7 samples of cocoa and 15 samples of products for children. The analyzed oil samples can be divided into:

- 15 sunflower oils
- 15 extra virgin olive oils
- 4 pomace oils
- 2 exhausted olive oil

The commercial samples were taken from the territorial ASUR inspectors of the region Marche and from the command for the protection of public health (“Comando per la Tutela della Salute Pubblica, N.A.S di Ancona”).

Experimental Method

This analytical procedure was validated by the “Istituto Superiore di Sanità” (ISS) through an external quality control ring test organized and managed by the ISS itself. The outcome of the test was more than

satisfactory and the laboratory of Ascoli Piceno was enabled and encouraged to carry out the determination of PAHs in olive oil with this analytical procedure. This method consists of three phases:

1. **Extraction**

Ten grams of oil are weighed and dissolved in 25 ml of pentane. 5 µl of benzo[a]anthracene D12 solution at a concentration of 5 mg/l and 15 ml of DMSO are added to the pentane solution. Dimethylsulfoxide is a substance with electronic doublets and electrons π, so it is also very similar to PAHs. For this reason, DMSO is a specific solvent for PAHs and it allows the separation of aliphatic hydrocarbons from aromatic hydrocarbons and the latter from triglycerides found in vegetable oils and fats, resulting in a considerable advantage in subsequent instrumental determination. The extraction is performed by stirring in a 100 ml separating funnel for about 30 seconds. The clear DMSO solution, stratified in the lower part, drains into a 250 ml separating funnel with a ground necked flask. The extraction is repeated twice with 10 ml portions each of DMSO. To the portions of DMSO combined in a separating funnel, 75 ml of distilled water are added and three times extracted with 50 ml of cyclohexane each. The water complexes the DMSO and binds it more strongly than the PAHs. For this reason the solubility of PAHs in DMSO will decrease and will be moved to the cyclohexane phase. The cyclohexane solutions, which are also combined in a 250 ml separating funnel with a ground necked flask, are washed with 100 ml of distilled water and stirred to prevent the formation of emulsions. The aqueous solution below is eliminated, and the cyclohexane solution is filtered on anhydrous sodium sulfate placed in a funnel with glass wool and collected in a flask with a ground necked flask. Two washes of the anhydrous sodium sulfate were carried out with 10 ml each of cyclohexane and the cyclohexane solution obtained was evaporated through rotavapor at the temperature of 40°C. The solution is transferred to a vial, washing the sides of the flask twice and the solution obtained is evaporated at about 100 µl under the flow of nitrogen.

2. **Purification**

The sample is completely seeded on the starting line of a TLC plate. With a pencil we draw two parallel lines distant from the edge 1.5 cm (starting or sowing line of the sample) and 13.5 cm (solvent arrival line or end chromatographic runs). The vial washing liquids are deposited above the sample. The plate is placed in a chromatography tank containing toluene-hexane 50:50. The solvent is left to run until the distance of 12 cm is covered. After that the solvent is evaporated and the plate is quickly observed in the light of Wood to delimit the blue spot corresponding to the PAHs (Rf about 0.8). The silica portion corresponding to the PAHs is scraped with a flexible steel spatula on an aluminum sheet. The silica is transferred onto a tube with glass septum and eluted with 1 ml of dichloromethane portions until approximately 3 ml of eluate is obtained. It is evaporated to dryness under nitrogen flow, it is taken up with 100 µl of cyclohexane, 5 µl of perylene D12 solution at a concentration of 5 mg/l is added and the sample is ready for the gas chromatographic determination.

3. **Instrumental analysis**

The analysis is performed by a gas chromatograph (GC) Agilent Technologies 7890A. The gas chromatograph is combined with a Mass Selective Detector (MSD) that is 5975C (Agilent Technologies). An example of a chromatogram of a solution of PAHs at 250 µg/kg is shown below (Figure 2).

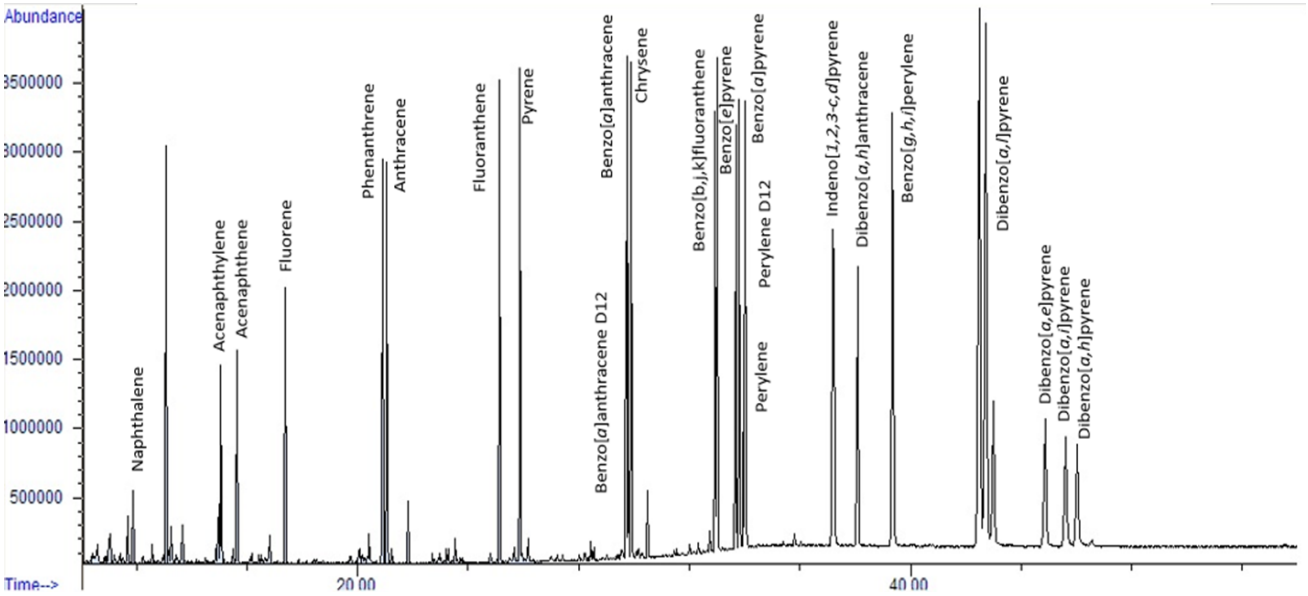


Figure 2. Chromatogram of a solution of PAHs at 250 µg/kg.

This work also concerned the validation of the test method for the research of PAHs in oil. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. In this regard, the laboratory must use test methods and procedures defined by rules, techniques or official methods in force. The current legislation on foodstuffs requires control units to use accredited testing methods. The ARPAM laboratory in Ascoli Piceno is accredited by Accredia (Ente Italiano di Accreditamento). The method was validated by determining the following parameters:

- Selectivity
- Linearity
- Recovery
- Repeatibility
- Limits of quantification (LOQ)
- Uncertainty of measurement by Horwitz

Results and Discussion

PAHs content in sunflower oil

Commission regulation has set a maximum level of 2 µg/kg for benzo[a]pyrene in oils and fats intended for direct consumption or for use as an ingredient in foods and has set a maximum level of 10 µg/kg for the sum of 4 PAHs (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene). In the graph below (Figure 3) the maximum levels are indicated with a red line that allows to see if in the analyzed sample there will be a surplus of benzo[a]pyrene or the sum of 4 PAHs.

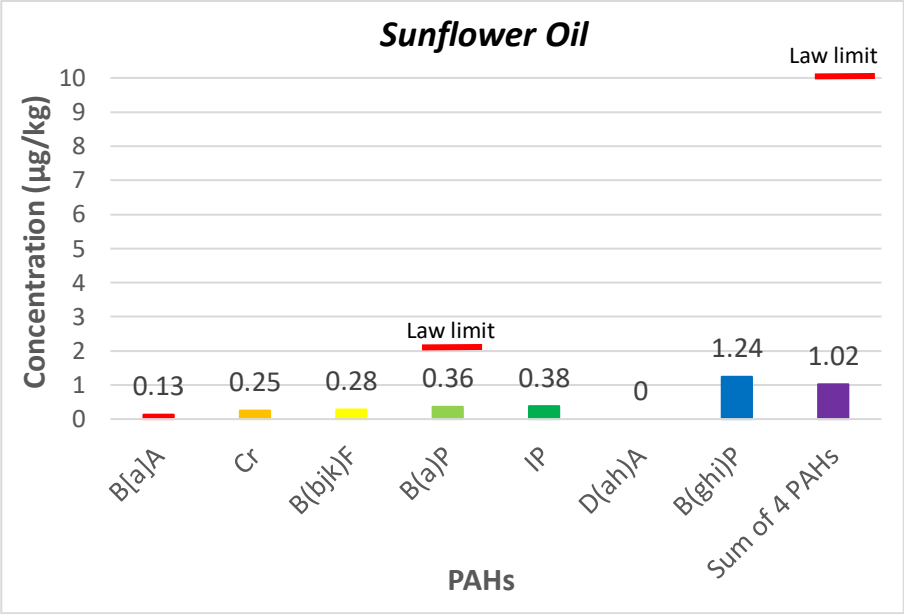


Figure 3. Sample of sunflower oil.

In seed oils, low concentration values of PAH are generally found both because the seeds are protected from atmospheric pollution (the sunflower seed from the shell), and also because seed oils are subjected to grinding. Indeed under no circumstances is the threshold limit of 10 µg/kg exceeded for the sum of the four components. The limit of 2 µg/kg for benzo[a]pyrene was also not exceeded.

- PAHs content in extra virgin olive oil

Also in this case, as well as for sunflower oils, the maximum level of benzo[a]pyrene is 2 µg/kg while the maximum level for the sum of 4 PAHs is 10 µg/kg. The levels are indicated with a red line in the graph below (Figure 4).

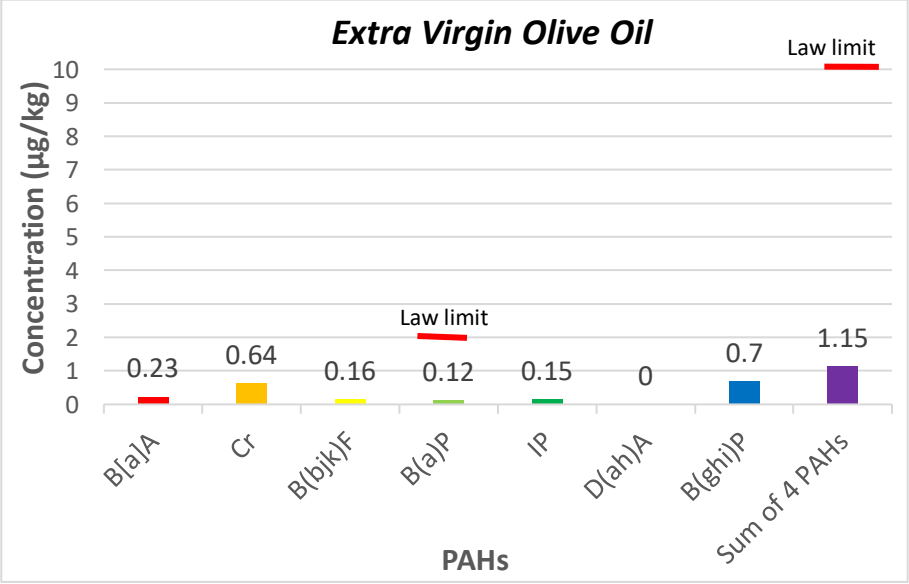


Figure 4. Sample of extra virgin olive oil.

In light of the new legal limits imposed for PAHs in oils, it was checked whether the thresholds of 10 µg/kg for the sum of the four components and of 2 µg/kg for benzo[a]pyrene are exceeded. In no case is the threshold limit exceeded.

- PAHs content in exhausted extra virgin olive oil

Some trials were carried out to investigate the fate of PAHs when oil was heated to frying temperatures. Aliquots of extra virgin olive oil previously analyzed (blue line in Figure 5) and without PAHs were heated with a domestic electrical fryer at 180°C for various times. In particular the red line shows PAH concentrations in the extra virgin olive oil after the first frying (heating time of 30 min), and the green line shows PAH concentrations in the extra virgin olive oil after the fourth frying (heating time of 4 h).

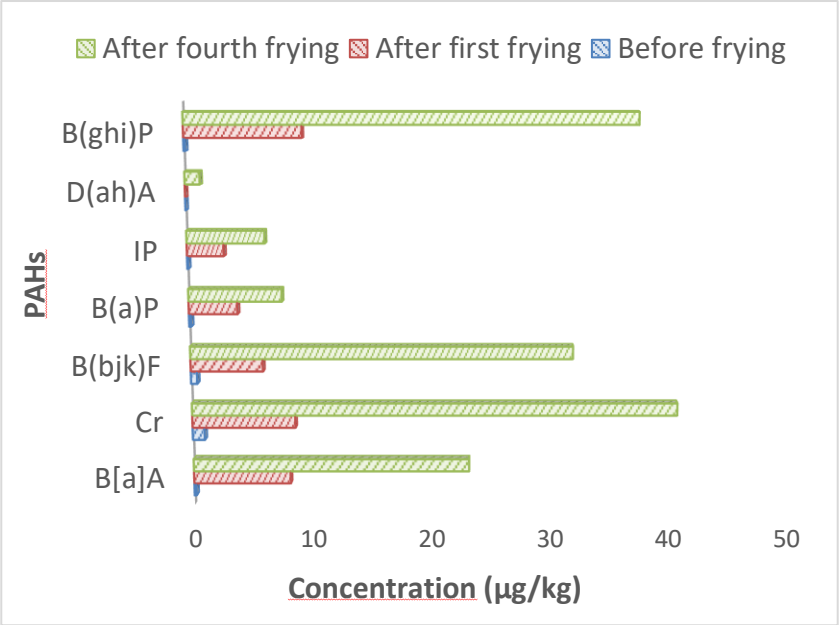


Figure 5. Sample of exhausted extra virgin olive oil.

The sample, which initially didn't contain PAH or contained in reduced quantities, has undergone a strong increase in these substances during the cooking process. In particular, it was possible to notice that there is a directly proportional relationship between frying time and the amount of PAHs that are formed. PAHs concentration increased as heating time increased. The results indicated that the cooking method significantly affected PAH emissions and frying increases the amounts of PAHs and B[a]P.

- PAHs content in olive pomace oil

Also in this case, as well as for sunflower oils and extra virgin olive oils, the maximum level of benzo[a]pyrene is 2 µg/kg while the maximum level for the sum of 4 PAHs is 10 µg/kg. Below is shown the graph with the concentrations of PAHs found in the sample of olive pomace oil analyzed.

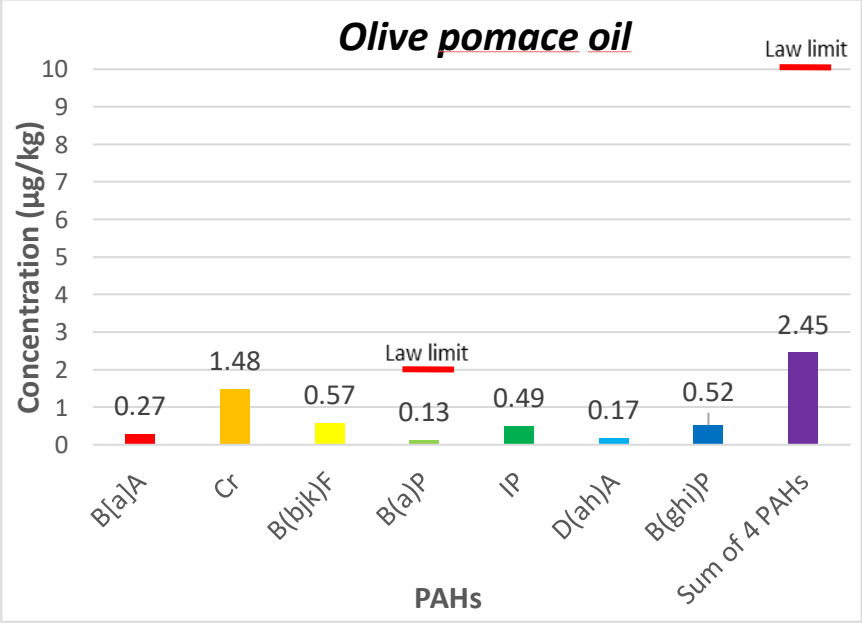


Figure 6. Sample of olive pomace oil.

Along olive pomace oil production, contamination can occur during pomace the drying and solvent extraction steps. Sometimes, the olive pomace is dried by direct contact with combustion fumes. PAH content depends highly on the conditions used prior to the oil extraction process, and can be relatively high especially if harsh conditions are used. Nevertheless, PAH content is generally reduced during refining.

Anyway, under no circumstances is the threshold limit of 10 µg/kg exceeded for the sum of the four components and the limit of 2 µg/kg for benzo[a]pyrene was also not exceeded.

- PAHs content in cocoa

It should be recalled that Commission regulation (EC) 835/2011 has set a maximum level of 5 µg/kg fat for benzo[a]pyrene in cocoa beans and derived products and has set a maximum level of 30 µg/kg fat for the sum of 4 PAHs. The limits are marked with a red line in the graph below.

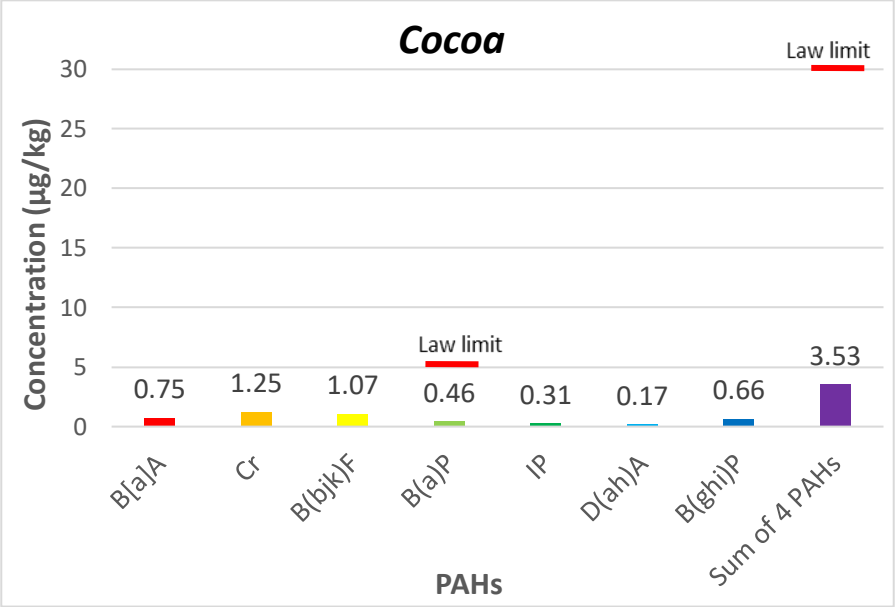


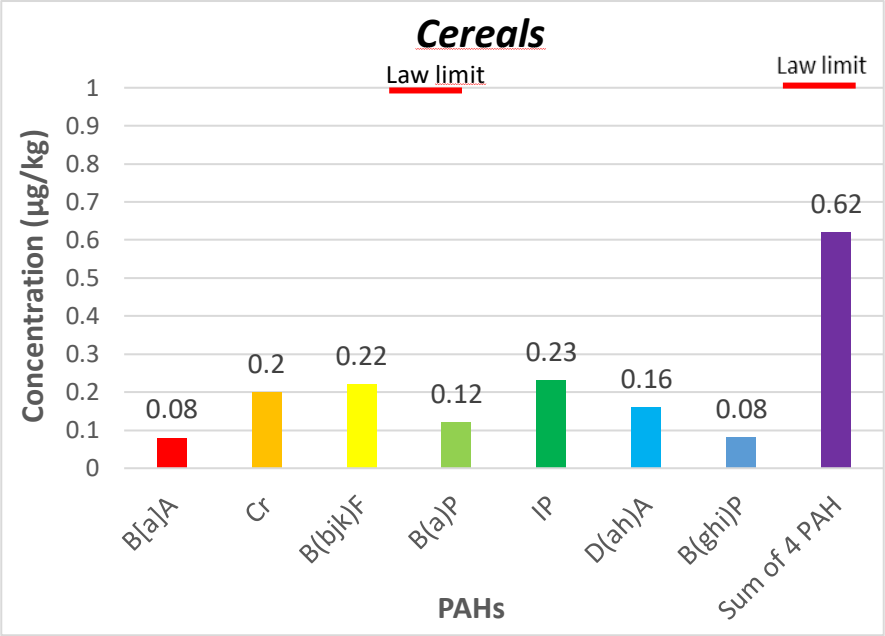
Figure 7. Sample of cocoa.

Most PAHs content in cocoa bean originated from outside penetrate into cocoa cotyledon. The PAHs were supposed to come from smoke produced from wood and or/ fuel burning. Cocoa bean shell effectively absorbs the PAHs passing through and contacting with it. These evidences showed a risk for the PAH contamination in cocoa bean cotyledon from the contaminant in their shell.

There is the possibility that the PAH compounds may be formed during cocoa beans drying and fermentation. From the results obtained it should be noted that the assigned analyte contents for B[a]P and the sum of four PAHs, expressed on fat basis did not exceed the above mentioned maximum levels.

- PAHs content in early childhood products

For the protection of the health of infants and children, which is a vulnerable group, it is appropriate to define maximum levels at the lowest levels obtainable. For this reason Regulation 2005/108/EC specifies a maximum level of 1.0 µg/kg for BaP and also for the sum of 4 PAHs in processed cerealbased foods and baby foods for infants and young children, in infant formulae and follow-on formulae, including infant milk and follow-on milk, and in dietary foods for special medical purposes intended specifically for infants.



Cereals are important constituents of the human diet across the world both in terms of quantities consumed and nutritional value. Although the reported levels are overall rather low compared with those in seeds and edible oils the frequent occurrence of PAHs contamination and the large cereal consumption can also make them a significant source to human exposure.

Figure 8. Sample of cereal-based products for children.

The occurrence of polycyclic aromatic hydrocarbons in cereal-based food is the result of different food preparation methods (e.g. roasting or drying) and the bioaccumulation in the oily seeds. Nevertheless, no sample result had a value above 1 µg/kg. Despite the low levels found, cereals and cereal products were identified as a major contributor to the intake of PAHs, owing to their high consumption. Therefore, PAH levels in these two product groups should be further monitored.

Conclusions

PAHs are present, in widely varying quantities, almost in all foods. This presence may be due to environmental contamination (mainly by deposition of atmospheric particulate material and by absorption from contaminated matrices, such as soil and river or sea water), or to formation during certain processing processes (especially drying through combustion fumes and smoking with traditional methods) and some heat treatments (in particular, grilling or frying). For this reason a study was carried out to assess the PAHs content in different food matrices such as oils, cocoa and products for children. The objective of this work was to determine the content of PAHs in food to verify if the limits imposed by the European Commission for benzo[a]pyrene and the sum of the four PAHs were respected. This has also allowed us to define the environmental quality status of the territory about the presence of substances considered dangerous and the extent of their return to humans through food. As for oils, three types of oil were analyzed: extra virgin olive oil, sunflower oil and pomace oil. When the olives are subjected to polluting sources containing PAHs, the resulting vegetable oil is contaminated by both light PAHs and heavy PAHs. The former are an expression of background pollution and concern extensive man-made areas (heavily trafficked areas). The latter are an expression of direct pollution by car-vehicle exhausts or discharges of equipment with an internal combustion engine used in the field for mechanized harvesting of olives (shaking or suction) and tree pruning, as well as mechanical handling of olives once collected. From the analysis of extra virgin olive oil, based on the results obtained, it is possible to have an indication about the location of the olive grove, whether it is in a pollution-free area or in an area affected by polluting sources. In the samples analyzed for heavy PAHs, in no case was found a higher value for benzo[a]pyrene and the sum of four PAHs than that imposed by the European Commission. On the other hand, very high values were found for light PAHs. From this we can deduce

that our territory is polluted mainly by vehicular traffic and this is reflected in a significant amount of light PAHs in the oil. As far as sunflower oil is concerned, low concentrations of PAH are generally found both because the seeds are protected from atmospheric pollution (sunflower seeds from the shell) and because seed oils are subjected to grinding. In our case, however, the samples show an appreciable concentration of PAHs. This finds an explanation in the fact that sunflower oil is subjected to a similar treatment to that of pomace oil in which the seeds are dried by direct contact with hot fumes, rich in PAHs. However, in no case was the threshold limit of 10 μg/kg exceeded for the sum of the four components. The limit of 2 μg/kg for benzo[a]pyrene was also not exceeded. As regards pomace oils, higher PAH values were found than those found in extra virgin olive oils and sunflower seed oils. Knowing the production cycle of this oil it is easy to identify the source and the mechanism of pollution from olive pomace oil; the smoke coming from the combustion of the exhausted pomace is in close contact with the raw material to which part of the combustion products is released, including PAHs. However, in no case was the threshold limit of 10 μg/kg exceeded for the sum of the four components and also the limit of 2 μg/kg for benzo[a]pyrene was not exceeded. On the other hand, it was interesting to evaluate the effect of cooking in a sample of extra virgin olive oil. The latter was analyzed as such and was subsequently analyzed after a single frying at 180°C for 30 minutes and after four frying at 180°C for 4 hours. The data obtained showed that the cooking process has significantly influenced PAH emissions and frying increases the amount of PAH and B[a]P. The sample of extra virgin olive oil, which initially did not contain PAH or contained in reduced quantities, has undergone a strong increase in these substances during the cooking process. In particular, it was possible to notice that there is a directly proportional relationship between frying time and the amount of IPA that are formed. In this case the two fried oils exceeded the maximum allowed limit (10 μg/kg) set by the European Union for the sum of four PAHs and also exceeded the maximum allowed limit of 2 μg / kg for B[a]P. With regard to cocoa, positive PAH concentration can be found due to inappropriate cocoa drying practices. The same goes for cereals used for baby products. In both cases the limits imposed by the law have been respected. In conclusion, keeping in mind the data obtained regarding the contamination of food which is an important indicator of the quality of life, no particularly critical situations have been identified that could compromise the natural process of sustainable development.

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