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Trace amount determination of monocyclic and polycyclic aromatic hydrocarbons in fruits: Extraction and analytical approaches



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ABSTRACT

Two reliable and complementary approaches have been established for the determination of volatile and semi-volatile aromatic hydrocarbons at $\mu\text{g}/\text{kg}$ level in fruits. Headspace solid-phase microextraction was used for extraction of monocyclic and light polycyclic aromatic hydrocarbons ($\text{MW} \leq 178 \text{ g/mol}$). Method based on ultrasound-assisted extraction followed by solid-phase extraction allowed extraction of heavy polycyclic aromatic hydrocarbons ($\text{MW} \geq 202 \text{ g/mol}$). Extraction techniques coupled with analysis by gas chromatography-mass spectrometry were successfully applied to 18 aromatic compounds in different fruit matrices (apple, pear, plum, and grape). Applicability of methods to these several fruits had been statistically verified through ANOVA test, by comparison of linear regression responses (slopes) of calibrations curves. Developed methods showed good linearity ($r^2 = 0.919\text{--}0.999$) within a range of concentrations from 0 to $10 \mu\text{g}/\text{kg}$ wet weight and acceptable values were obtained for repeatability ($\text{RSD} = 1\text{--}22\%$) and intermediate precision ($\text{RSD} 3\text{--}30\%$). Limits of detection and quantification did not exceed 0.2 and $0.6 \mu\text{g}/\text{kg}$, respectively, except for fluorene in grape (0.5 and $1.8 \mu\text{g}/\text{kg}$, respectively). Extraction and analytical methodologies implemented in the present study allow sensitive determination of trace levels of monocyclic and polycyclic aromatic hydrocarbons in fruit.

1. Introduction

Aromatic hydrocarbons, which are hydrophobic organic pollutants containing one (or more) benzene ring(s), are ubiquitous. The most commonly investigated compounds are the 16 polycyclic aromatic hydrocarbons (PAHs) from the US-EPA list of priority pollutants. Benzene, toluene, and ethylbenzene, from the group of monocyclic aromatic hydrocarbons (BTEX: benzene, toluene, ethylbenzene, and xylenes), are also considered as priority pollutants (US-EPA priority pollutant list, 2014).

Benzo[a]pyrene (BaP) and benzene have been classified by the International Agency for Research on Cancer (IARC) as carcinogenic compounds. Moreover, some PAHs (BaP, dibenz[a,h]anthracene, benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene) show enough evidence of mutagenicity/genotoxicity in animal somatic cells *in vivo* to be considered as potentially genotoxic and carcinogenic to humans through the diet (EFSA, 2008).

Food is known as the main source of contamination by aromatic hydrocarbons for non-smokers (Menzie and Potocki, 1992). Contamination of raw products (animals and agricultural products) can occur during growth, transport, storage, and transformation (drying,

grilling, roasting, smoking, and frying). Fruits and vegetables have usually a smaller aromatic hydrocarbon level than meat, probably due to their lower lipid content and their possible consumption as raw product. PAH contents (wet weight; w.w.) are respectively lower than $1 \mu\text{g}/\text{kg}$ and $4.1 \mu\text{g}/\text{kg}$ for fruits and vegetables from Catalonia, while their levels reach $365 \mu\text{g}/\text{kg}$ in meat (salami) (Martorell et al., 2010).

However, fruits and vegetables are consumed in large quantities and remain a considerable source of aromatic hydrocarbons. Fruits and vegetables consumption represents about 31% of the total food intake and is responsible for between 5 and 10% absorption of PAHs (Martorell et al., 2010). In the Czech Republic, PAH intake (sum of 12 PAHs) is estimated at $449 \text{ ng}/\text{day}$ for apple (average annual consumption of 23.8 kg) (Jánská et al., 2006). Thus, contamination of fruits and vegetables by potentially carcinogenic aromatic hydrocarbons is a significant concern, and involves development of techniques to monitor these pollutants in such complex solid food matrices.

Most common methods for PAHs extraction in food are solvent extraction techniques. Ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE) have been notably used for PAH extraction from fish and meat products (Purcaro et al., 2009; Wang et al., 1999). These extraction procedures are most often followed by solid-phase extraction (SPE) as a clean-up

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step. Concerning fruits and vegetables, Soxhlet seems to remain a widely used technique for PAH extraction (Abou-Arab et al., 2014; Ashraf and Salam, 2012; Soceanu et al., 2014).

Less solvent consuming techniques, such as stir-bar sorptive extraction (SBSE) and solid-phase microextraction (SPME) by direct immersion or in the headspace (HS-SPME), are prevalent for extraction of the most volatile compounds in food matrices. HS-SPME and SBSE notably allow the assessment of benzene and toluene in cooked pork samples (Benet et al., 2015). HS-SPME technique provides concurrent monitoring of BTEX and PAHs up to four aromatic rings in olive oil (Vichi et al., 2005). SPME and HS-SPME techniques have also been used for the simultaneous recovery of BTEX and PAHs in liquid matrices. Direct immersion SPME, followed by an HS-SPME, step allows the determination of BTEX and heavy PAHs, such as dibenz[a,h]anthracene and benzo[ghi]perylene in Brazilian water (bottled, tap, and river water) (Bianchin et al., 2012). Nevertheless, until now, the simultaneous extraction of BTEX and total PAHs in complex matrices such as food remains challenging due to the difference in volatility between light and heavy aromatic hydrocarbons. The aim of this work, which is a development of existing protocols (Pointot et al., 2014), was to optimize and validate methodologies for aromatic hydrocarbons determination at trace level ($\mu\text{g}/\text{kg}$) in four different fruits (apple, pear, plum, and grape).

2. Material and methods

2.1. Chemical compounds evaluated

Acenaphthene (PubChem CID: 6734), acenaphthene-d10 (PubChem CID: 177559), acenaphthylene (PubChem CID: 9161), benz[a]anthracene (PubChem CID: 5954), benz[a]anthracene-d12 (PubChem CID: 12279531), benzene (PubChem CID: 241), benzo[a]pyrene (PubChem CID: 2336), benzo[b]fluoranthene (PubChem CID: 9153), benzo[ghi]perylene (PubChem CID: 9117), chrysene (PubChem CID: 9171), dibenz[a,h]anthracene (PubChem CID: 5889), ethylbenzene (PubChem CID: 7500), ethylbenzene-d10 (PubChem CID: 117648), fluoranthene (PubChem CID: 9154), fluorene (PubChem CID: 6853), indeno[1,2,3-cd]pyrene (PubChem CID: 9131), *m*-xylene (PubChem CID: 7929), naphthalene (PubChem CID: 931), *o*-xylene (PubChem CID: 7237), *p*-xylene (PubChem CID: 7809), pyrene (PubChem CID: 31423), and toluene (PubChem CID: 1140) were studied.

2.2. Reagents and standards

The solvents used were of GC grade. Methylene chloride (99.8%), ethyl acetate (99.8%), cyclohexane (99.5%), and ethanol (> 99.9%) were purchased from VWR Prolabo (Fontenay-sous-Bois, France). The standard mixture of PAHs (2 mg/mL certified in methylene chloride) and the BTEX stock solution (2 mg/mL in methanol, certified reference material 47505, ISO/IEC 17025 and ISO Guide 34) were obtained from Supelco (Bellefonte, PA). Working BTEX and PAH solution (0.1 mg/L for ultrasound-assisted extraction followed by solid-phase extraction (UAE/SPE) and 2 mg/L for HS-SPME) was prepared by mixing equivalent volumes of stock solutions in methylene chloride and then stored in a refrigerator (4–6 °C, for 2 weeks).

Benz[a]anthracene-d12 (2 mg/mL in methylene chloride), ethylbenzene-d10 (2 mg/mL in methanol), and acenaphthene-d10 (2 mg/mL in methylene chloride) used as internal standards respectively for heavy PAHs, BTEX, and light PAHs, were obtained from Supelco (Bellefonte, PA). Working internal standard solutions (0.1 mg/L for UAE/SPE and 2 mg/L for HS-SPME) were prepared using dilution of the stock solutions in methylene chloride and stored at 4–6 °C, for 2 weeks.

Helium Alphagaz was purchased from Air Liquide (Paris, France).

2.3. Samples

Varieties of fruits used in this study were Boskoop and Gala apples (based on seasonal availability), William pears, Black Amber red plums, and Italia white grapes. Uniform fruits without skin damage or imperfections were purchased in a French local supermarket. Fruits were neither washed nor peeled only freed from the core and seeds or kernel. The whole fruit (skin and pulp) was crushed in a blender before extraction. Measurements were all performed in triplicate and each sampling of crushed fruits was spiked with working solutions to attain final concentrations of 10 $\mu\text{g}/\text{kg}$ for internal standards and between 0 and 10 $\mu\text{g}/\text{kg}$ for BTEX (excepted for *m/p*-xylenes with concentrations between 0 and 20 $\mu\text{g}/\text{kg}$) and PAHs. Total volume introduced in samples was systematically adjusted to 200 μL by addition of methylene chloride.

Fruits were exposed to exhaust gas from a diesel engine vehicle firstly driven on the road in 2005. The car, equipped with a diesel particulate filter, did a 30-min journey on the road. Then, apple, pear, plum, and grape (about 200 g) were introduced independently in a duct directly connected to the exhaust pipe for 30 min. For each fruit variety, skin and pulp were crushed in a blender. Internal standards (10 $\mu\text{g}/\text{kg}$) were added to crushed samples and the volume introduced was adjusted to 200 μL with methylene chloride. HS-SPME and UAE techniques were performed in triplicate immediately after exposure. HS-SPME extracts were analyzed immediately after extraction. UAE extracts were stored at 4–6 °C for 1 week and their clean-up by SPE was carried out prior to their analysis.

2.4. Instrumentation

Chromatographic separations were carried out with a Shimadzu (Kyoto, Japan) gas chromatograph equipped with a DB-5 ms capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness) from J&W Scientific (Folsom, CA). Helium (99,999 %) was used as the carrier gas. Mass spectrometric detection was performed with a QP 2010 mass spectrometer (Shimadzu) using an electron ionization source (EI) followed by a simple quadrupole (qMS) analyzer (different from Pointot et al., 2014). Ion source temperature was maintained at 250 °C and interface temperature was 300 °C. Improvement in selectivity was provided by selective detection achieved using the selected ion monitoring (SIM) mode of the mass analyzer. Ions used for analyte detection with their corresponding retention time are presented in Table 1. Retention indices were calculated for all compounds using a DB-5 ms column (Table 1).

2.5. Extraction and analysis procedures

HS-SPME was used for extraction of BTEX and light PAHs and UAE/SPE allowed the extraction of heavy PAHs.

2.5.1. Headspace solid-phase microextraction

2.5.1.1. Extraction. Crushed fruit (20 g) was weighed and placed in a 125-mL glass flask containing a magnetic stir bar. The flask, which was sealed with a silicone septum, was half-immersed in a water bath at 60 °C. Constant agitation (750 rpm) allowed reproducible and homogeneous mixing throughout extraction. After 15 min equilibrium, a 75 μm CAR/PDMS (Carboxen/polydimethylsiloxane) fiber was exposed to the headspace above the sample for an extraction time of 45 min.

2.5.1.2. Analysis. Carrier gas (helium) flow was at 1 mL/min. Injector temperature was 200 °C and injections were carried out in splitless mode with optimized desorption time of 1 min. After this time, split ratio was fixed at 200:1. Initial oven temperature was 40 °C and was held for 2 min. Then temperature was increased by 2 °C/min to 55 °C, held for 5 min, increased by 4 °C/min to 100 °C, held for 5 min,

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