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Assessing polycyclic aromatic hydrocarbons in cereal-based foodstuffs by using a continuous solid-phase extraction system and gas chromatography–mass spectrometry

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A B S T R A C T

Polycyclic aromatic hydrocarbons (PAHs) are a family of sixteen compounds of which are deemed priority pollutants by the European Commission and the US Environmental Protection Agency for their carcinogenic and mutagenic properties. A method for their determination in a variety of cereal-based foodstuffs including bread, cookies, flours and cereals is reported here. The method uses solid–liquid extraction with a semi-automated solid-phase extraction system to clean up sample extracts, and gas chromatography–mass spectrometry to determine and quantify the target analytes. The analytical figures of merit of the proposed method, which include high precision, sensitivity and selectivity for all 16 PAHs, testify to its robustness. Thus, the limits of detection for 1 g of sample span the range from 2 to 60 ng kg^{-1}, relative standard deviations are all lower than 7.5% and recoveries range from 88 to 103%. The method was successfully applied to several cereal-based foodstuffs samples. The European Commission has set a tolerated limit of 1 μg kg^{-1} for PAH4 [a combination of benzo(*a*)pyrene, benzo(*a*)anthracene, benzo(*b*)fluoranthene and chrysene] in cereal products for infants that was exceeded by three breakfast cereal foodstuffs (3.1–7.5 μg kg^{-1}). Also, home toasting bread was found to raise the concentrations of some PAHs above the allowable levels.

1. Introduction

Cereal products and their derivatives are staple foods throughout the world. According to FAO, the global cereal consumption is about 147.1 kg per person per year, of which 65.43 kg correspond to wheat, 53.92 kg to rice, 17.89 kg to maize and the remainder to other cereals such as oats or rye. In Europe, cereal consumption amounts to about 132 kg person^{-1} yr^{-1}, of which 109, 4.64 and 7.21 kg correspond to wheat, rice and maize (and their derivatives), respectively. In Africa, total cereal consumption is 151.2 kg person^{-1} yr^{-1}, with 47.67 kg for wheat and its derivatives, 44.48 kg for maize and 23.91 kg for rice. In America, total consumption is lower than the global average (118.6 kg person^{-1} yr^{-1}), with 61.35, 34.8 and 18.52 kg for wheat, maize and rice, respectively (FAO, 2107). About 90% of all cereal production comes from wheat, rice or maize, the geographic differences in consumption rate depending on their availability and the particular culture (Beta & Isaak, 2016). Although cereal products are typically scarcely contaminated, their heavy consumption makes them potential sources of polycyclic aromatic hydrocarbons (PAHs).

PAHs can reach cereal products by deposition of atmospheric pollutants and also from polluted water, by the effects of fire, etc. Also, food drying and cooking can result in thermal decomposition of organic matter to form PAHs, also the use of flavoring procedures by smoking. The amounts of PAHs present in cereal foodstuffs are influenced not only by the temperature reached in the food drying, processing or cooking process, but also by the way heat is applied and what type of energy source is used [3], and by whether semi-volatile gaseous chemicals are used in plants or oil fumes evolve during the handling and cooking of food (e.g., toasting bread) (Rey-Salgueiro, García-Falcón, Martínez-Carballo, & Simal-Gándara, 2008; Rey-Salgueiro, Martínez-Carballo, García-Falcón, & Simal-Gándara, 2008).

In 2011, the European Commission issued a regulation for PAHs in processed cereal-based foods, and foods for infants and young children, where it set a maximum allowed level of 1 μg kg^{-1} for benzo(*a*)pyrene (BaP) and an identical one for the combination of BaP, benzo(*a*)anthracene (BaA), benzo(*b*)fluoranthene (BbF) and

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chrysene (Chry) (European Commission, 2011). Also, the American Environmental Protection Agency (US EPA) has deemed the following 16 PAHs priority contaminants: Chry, BaA, BbF, BaP, naphthalene (Nap), acenaphthylene (Ap), acenaphthene (Ac), fluorene (F), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (BP), dibenzo[a,h]anthracene (DBahA) and indeno[1,2,3-*cd*]pyrene (IP) (USEPA, 2012).

The daily intake of BaP in Italy is 0.04–0.42 μg day^{-1} (Lodovici, Dolara, Casalini, Ciappellano, & Testolin, 1995). On the other hand, Spain's daily consumption of BaP and total PAHs in bread, cookies, cakes, rice and related products are 0.14 and 8.6 μg day^{-1}, respectively (Ibáñez et al., 2005). In France, daily exposure to BaP, BaA, BaF and Chry in combination has been estimated at 1.48 μg kg bw^{-1} day^{-1} in adults and 2.26 μg kg bw^{-1} day^{-1} in children (Veyrand et al., 2013). Studies in Kuwait have shown daily intakes of BaP, based on consumption of 300 g of bread per day, range from 0.85 to 4.96 μg person^{-1} day^{-1} (Al-Rashdan, Helaleh, Nisar, Ibtisam & Al-Ballam, 2010). These values are lower than those reported by Ahmed, Abdel, El Samahy & Youssof (2000), and Orecchio and Papuzza (2009). A number of studies have confirmed the presence of PAHs in different types of food. In cereal grain-based foodstuffs, PAHs have been found at 350 μg kg^{-1} in bread toasted by wood flame, and BaP, in particular, at concentrations from 0 to 0.23 μg kg^{-1} (Rey-Salgueiro, García-Falcón et al., 2008). In corn, PAHs have been encountered at levels from 5.9 to 127 μg kg^{-1} (De Lima et al., 2017). Some processed foodstuffs have been found to contain similar PAH levels (e.g., 9.9–90 μg kg^{-1} in youtiao, a wheat-based fried food) (Li, Wu, Wang, & Akoh, 2016). In a recent study, bread and cereals were shown to contain four priority PAHs at joint levels of 0.22–1.62 μg kg^{-1} (Rozentale, Zacs, Perkons, & Bartkevics, 2017).

Although the presence of PAHs in some cereal grain-based foodstuffs is well-known, a fast, selective semi-automated method for their determination capable of detecting the analytes at very low levels is needed. Thus, the European Commission PAHs factsheet mentions several methods but none for cereal-based products (Lerda, 2011). Some authors have determined PAHs by using liquid chromatography in combination with fluorimetric (HPLC-FLD) (García-Falcón, Cancho-Grande, & Simal-Gándara, 2005; Germán-Hernández, Pino, Anderson, & Afonso, 2011; Rey-Salgueiro, Martínez-Carballo et al., 2008; Rey-Salgueiro, García-Falcón et al., 2008) or UV detection (Germán-Hernández et al., 2011). However, some PAHs are not fluorescent; also, positive identifications must be confirmed with other chromatographic techniques. One alternative is using gas chromatography coupled to mass spectrometry (GC–MS) (Al-Rashdan et al., 2010; De Lima et al., 2017; Escarrone et al., 2014; Li, Wu, Zeng, Wang, & Yu, 2016; Li et al., 2016; Liu & Korenaga, 2001; Martorell et al., 2012; Rozentale et al., 2017), a tandem of mass spectrometers (GC-MS/MS) (Rozentale et al., 2017) or flame ionization detection (Singh, Vashishth, & Vishal, 2011).

PAHs present at low levels in cereal-based foodstuffs require preconcentration and extraction from the food matrix prior to their determination. So far, PAHs have been extracted with *n*-hexane (De Lima et al., 2017; Singh et al., 2011) or acetonitrile–acetone mixtures (Li, Wu, Wang et al, 2016), using ultrasound (Rey-Salgueiro, Martínez-Carballo et al., 2008; Rey-Salgueiro, García-Falcón et al., 2008) or microwave energy (Germán-Hernández et al., 2011) as auxiliary energy in some cases. Also, solid-phase extraction (SPE) has been used to purify *n*-hexane bread extracts obtained with the assistance of ultrasound (Li, Wu, Zeng et al, 2016). Rozentale et al. (2017) developed a method for PAHs in cereals based on solid-liquid extraction (SLE) under ultrasounds and subsequent clean-up of the extract by gel permeation chromatography. Pressurized liquid extraction (Martorell et al., 2012; Veyrand et al., 2013) and QuEChERS (Escarrone et al., 2014) have also been used to isolate PAHs from cereals and other frequently consumed foods. These methods typically use large amounts of organic solvents for small amounts of sample; also, analyte recoveries range widely (50–117%).

The aim of this work was to develop a sensitive method for the simultaneous determination of EPA's 16 PAH priority pollutants in a variety of cereal-based foodstuffs. To this end, samples were pretreated by solid–liquid extraction under ultrasound and then processed in an automated solid-phase extraction (SPE) system. After elution, the target analytes were detected by GC–MS in the electron impact mode (EI). The effect of the variables influencing the pretreatment and the SPE treatment were optimized. The ensuing method was assessed for analytical performance by application on field samples.

2. Materials and methods

2.1. Standards, reagents and samples

Standards for EPA's 16 PAH priority pollutants (Chry, BaA, BbF, BaP, Nap, Ap, Ac, F, Phe, Ant, Flu, Pyr, BkF, BP, DBahA and IP) were supplied by Dr. Erenstropher, Fluka or Acros in the highest available purity. Chromatographic-grade solvents (acetonitrile, methanol, *n*-hexane and acetone) were purchased from Merck (Darmstadt, Germany). The internal standard (IS), triphenyl phosphate, was supplied by Fluka (St. Louis, MO, USA). Reversed phase silica with octadecyl functional groups (RP-C18) was obtained from Supelco (Madrid, Spain). Finally, purified water was obtained in the laboratory by using a Milli-Q system (Mili-pore, Bedford, MA, USA).

All products were handled with care, using efficient fume hoods and wearing protective gloves.

A stock solution of each individual PAH at a 5 g L^{-1} concentration was prepared in acetone and stored at 4 °C. Working solutions were prepared on a daily basis by dilution of appropriate volumes of the stocks. The eluent used was acetonitrile containing 100 μg L^{-1} triphenyl phosphate, and also prepared daily.

All samples of cereal-based foodstuffs (cookies, flour, pasta, breakfast cereal, rice and bread) were collected in 0.5 L polypropylene containers that were precleaned with methanol. The samples were bought at different supermarkets in European countries, homogenized in a home mixer and stored at −20 °C prior to analysis. For recovery tests and method calibration, different volumes of a standard solution of PAHs were added to samples and left them into darkness for 12 h to facilitate the sorption in a similar way as how is carried out naturally. Finally, the samples were homogenized in an A320R1 grinder from Moulinex (Barcelona, Spain).

2.2. Equipment

Gas chromatography–mass spectrometry analyses were performed by using a Focus gas chromatograph coupled to a DSQ II quadrupole mass spectrometric detector (Thermo Electron, Madrid, Spain). The GC column used was a 30 m × 0.25 mm i.d. DB-5 MS capillary column (film thickness 0.25 μm) from J&W (Folson, CA, USA). Helium (purity 6.0) at constant flow rate of 1 mL min^{-1} was used as the carrier gas. Samples were injected in the splitless mode. The oven temperature was programmed as follows: 70 °C, held for 2 min, ramped to 240 °C at 10 °C min^{-1}, then to 290 °C at 15 °C min^{-1}, and held for 12 min (total run time, 34.3 min). The injector, transfer line and ion source temperature were 300, 280 and 200 °C, respectively, and the time for solvent delay was set at 6 min.

Ions were quantified in the selected-ion monitoring mode (ionization energy, 70 eV). The *m*/*z* values for each PAH are listed in Table 1.