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Original Research Article

The application of multistep extraction and liquid chromatography with fluorescence detection for analysis of azaarenes in edible oil samples

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ABSTRACT

A method was adapted for the analysis of 6 azaarenes: benzo[h]quinoline, benzo[a]acridine, benzo[c]acridine, dibenzo[a, c]acridine, dibenzo[a, j]acridine and dibenzo[a, h]acridine, in raw ("recently opened") and thermally treated edible oils (cold-pressed rapeseed and olive). The purification procedure used was based on alkaline hydrolysis, tandem solid-phase extraction on columns filled with Extrelut – a diatomaceous earth and cation exchanger (propylsulphonic acid). The procedure enabled the selective isolation of potentially carcinogenic compounds belonging to benzoacridines and dibenzoacridines from oil samples. The eluted fractions of azaarenes were analyzed by high-performance liquid chromatography with programmed fluorescence detection. The detection limits for the azaarenes were between 0.0003 and 0.01 ng/g of oil. The recoveries for the analyzed compounds were from 59 to 79%. The concentrations of the individual azaarenes found in the investigated samples were between 0.08 and 2.55 ng/g. The total concentrations of the B[h]Q, benzoacridines and dibenzoacridines (expressed in ng/g of oil) fall within the range of 3.18–3.82 in the "recently opened" sample and from 2.65 to 4.68 in the thermally treated oil samples.

1. Introduction

Consumption of vegetable oils, both raw and thermally treated, continues to grow. This increase is the result of a change in eating habits, i.e. animal fats have been substituted with vegetable fats. Fast foods prepared with edible oils treated at high temperatures are widely consumed. Numerous investigations have shown that there are compounds in edible oils from the group of polycyclic aromatic hydrocarbons (PAHs) classified by the International Agency for Research on Cancer (IARC) as carcinogenic, most likely or possibly carcinogenic to humans (Alomiraha et al., 2010; Błaszczyk et al., 2008; Bogusz et al., 2004; Lage Yusty and Cortizo Daviña, 2005; IARC Monographs, 2010, 2012; Rojo Camargo et al., 2011; Węgrzyn et al., 2006). Benzo[a]pirene has been classified as human carcinogen (Group 1A) and selected to serve as a marker of PAH intake and its mutagenic activity in the diet. However, according to the Report of European Food Safety Authority (EFSA) (2008), in order to evaluate human exposure to PAHs through the diet, it is better to measure the total concentration of several PAHs rather than only the B[a]P concentration (IARC Monographs, 2010, 2012; EFSA, 2008; European Commission, 2005, 2006). PAHs can be present in edible oils because of the wide distribution of PAHs in the environment. However, the commercial preparation of edible oil, such as direct drying of the oil raw material with smoke, has been recognized as more important source of food contamination (Moret and Conte, 2000).

In many environmental and food samples, PAHs and their nitrogen analogues, azaarenes (aza-PAHs) were detected (Bleeker et al., 2002; Koči et al., 2007; Machala et al., 2001; Rivera et al., 1996; Švabenský et al., 2007; Warzecha, 1993; Wilhelm et al., 2000). These compounds may be formed during the same processes as polycyclic aromatic hydrocarbons, i.e. as a result of pyrolysis or the incomplete combustion of organic matter, especially coal and crude oil products (Nito and Ishizaki, 1997). The structures of 6 azaarenes are presented in Table 1. Due to the

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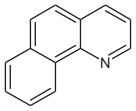
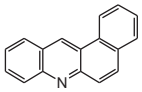
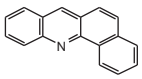
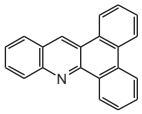
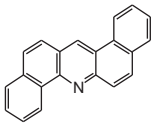
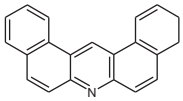
Table 1
Structures and abbreviations of the investigated azaarenes.

Azaarene	IARC class[a]	Abbreviation	Structure
Benzo[h]quinoline	No data[b]	B[h]Q	
Benzo[a]acridine	3	B[a]Ac	
Benzo[c]acridine	3	B[c]Ac	
Dibenzo[a, c]acridine	No data[b]	DB[a, c]Ac	
Dibenzo[a, h]acridine	2B	DB[a, h]Ac	
Dibenzo[a, j]acridine	2A	DB[a, j]Ac	

[a] IARC group: 2A – most likely carcinogenic to humans; 2B – possibly carcinogenic; 3 – not classifiable as to carcinogenicity to humans (IARC, 1993; IARC Monographs, 2010, 2012).
[b] No data in the IARC monographs.

presence of the nitrogen atom in the molecule, azaarenes express higher mutagenic and carcinogenic activity than do the corresponding PAHs (Bleeker et al., 1999, 2002; Sovadinova et al., 2006). Most metabolites of azaarenes show strong carcinogenic activity in experimental animals (Kumar et al., 2001). The International Agency for Research on Cancer (IARC) classified dibenzo[a, h]acridine (DB[a, h]Ac) as possibly carcinogenic to humans (group 2B) and, in 2010, upgraded dibenzo[a, j]acridine (DB[a, j]Ac) to probable carcinogenic compounds (group 2A) (IARC, 1993; IARC Monographs, 2010, 2012) (Table 1).

To date, only one study has been identified that studied azaarenes in edible oils (Szterk et al., 2012). Azaarenes have not often been analyzed in food. Several studies confirm the presence of these compounds in smoked, grilled or fried meat (Grimmer and Naujack, 1986; Janoszka et al., 2004; Janoszka, 2007, 2010; Rivera et al., 1996). For the isolation of the aza-PAH fraction from the complex matrix of food, methods based on multiple-step extraction were applied (Błaszczyk and Janoszka, 2008; Janoszka, 2010; Rivera et al., 1996; Szterk et al., 2012). During these procedures, food samples need first to be hydrolyzed to degrade the fat matrix before extraction (Janoszka et al., 2004; Błaszczyk and Janoszka, 2008; Janoszka, 2010). For oil samples, Szterk et al. (2012) used the classic liquid–liquid extraction connected with solid phase extraction.

Several methods have been introduced to measure the concentration of azaarenes, such as gas chromatography (GC) with flame ionization detection (FID), GC with mass spectrometry (MS) in selected ion monitoring (SIM) mode (Grimmer and Naujack, 1986; Nito and Ishizaki, 1997), high performance liquid chromatography with ultraviolet detection (HPLC-UV), HPLC with fluorescence detection (HPLC-FLD) (Błaszczyk and Janoszka, 2008; Janoszka, 2010) and liquid chromatography/tandem mass spectrometry (LC–MS/MS) (Janoszka et al., 2004; Rivera et al., 1996; Švabenský et al., 2007; Szterk et al., 2012).

In this context and due to lack of information concerning azaarenes in edible oils, the aim of this study was to adapt a method to determine 6 aza-PAHs in rapeseed and olive oil normally consumed in Poland, evaluating the possible influence of temperature (cooking) on the concentration of these contaminants in the oil samples. For these purposes the purification procedure based on a tandem-solid phase extraction, which was used for the isolation of azaarenes from a meat matrix (Janoszka et al., 2004; Janoszka, 2007; Błaszczyk and Janoszka, 2008), was adapted for the isolation of these compounds from the oil samples. Although GC-MS was found to be more efficient for the separation of different azaarenes, HPLC with fluorescence detection was shown to be the most sensitive method.

2. Materials and methods

2.1. Materials

2.1.1. Standards

6 standards were used for the study: benzo[h]quinoline (purity 95%) was from Ultra Scientific (North Kingstown, RI, USA), benzo[a]acridine (99.5%), benzo[c]acridine (99.7%), dibenzo[a, c]acridine (99.8%), dibenzo[a, j]acridine (99.6%) and dibenzo[a, h]acridine (99.3%) were purchased from Promochem (Wessel, Germany). The names, abbreviations and structures of the investigated compounds are given in Table 1. A standard mixture of 1 mg/L in acetonitrile was prepared from the standard stock solutions, each with a concentration of 0.2 g/L in acetonitrile.

2.1.2. HPLC-grade organic solvents

Dichloromethane, *n*-hexane, methanol, acetonitrile, water (POCH, Gliwice, Poland) were used as the components of the mobile phases and as extraction solvents. Sodium hydroxide, hydrochloric acid, and ammonium hydroxide (analytical-reagent grade) were purchased from POCH (Gliwice, Poland). Diatomaceous earth extraction columns (Extrelut, 20 mL) and the refill material were obtained from Merck (Darmstadt, Germany). The solid phase-extraction columns, filled with propylsulphonic acid (PRS, 500 mg, 3 mL), were purchased from J.T. Baker (Deventer, The Netherlands). The PRS columns were preconditioned with dichloromethane (4 mL).

2.2. Oil samples

2.2.1. Cold pressed oils being investigated (in quantities of 1 L)

Rapeseed oil (Polish production) and olive oil extra virgin (Italian production) were purchased in a local shop. Two bottles with the same batch number of each oil were purchased. Samples taken from the first bottle of each oil served as spiked and unspiked samples and were named "recently opened". In these samples, isolation of azaarenes fraction and analysis were performed. Every test was performed four times. Samples taken from the second bottle of each oil were named "thermally treated". Before later analysis, all of these samples were heated using the Petri plates according to the procedure given by the Polish Standard PN-60/A-86910. The oil samples were placed in identical Petri plates (7 cm in diameter and 4 cm high) and were heated in a thermostat heater made by Venticell (BMT, Slovakia) at a temperature of 180 °C ± 0.5 three times for 20 min, i.e. for 1 h altogether. Between succeeding heating cycles, the samples were cooled to room temperature. Using the temperature of 180 °C is similar to frying in deep oil (range from 150 to 200 °C). However, a cycle of heating and cooling is similar to frying three times in the same oil. One of four heated samples was taken for the procedure of isolation and qualitative–quantitative analysis.

2.3. Sample preparation

Samples of 2 g of oil (mass exact to 10^{-4} g) were dissolved in 8 mL of n-hexane, alkaline hydrolyzed using 30 mL of 1 mol/L