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# Evaluation of polycyclic aromatic hydrocarbons in Circassian cheese by high-performance liquid chromatography with fluorescence detection

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAH) in traditional/industrial smoked and unsmoked Circassian cheeses available in Turkish markets were analysed using solid-phase extraction, followed by high-performance liquid chromatography (HPLC) with fluorescence detection. Mean levels of a total of 9 PAHs in smoked traditional and industrial Circassian cheeses were 19.6 and 6.73 μg kg$^{-1}$, while levels in unsmoked traditional and industrial cheeses were 0.77 and 0.49 μg kg$^{-1}$, respectively. The dominant individual PAHs found were naphthalene and acenaphthene. Benzo[*a*]pyrene, a marker compound representing carcinogenic PAHs, was found in 90% and 30% of traditional smoked and unsmoked Circassian cheeses, 52% and 24% of industrial smoked and unsmoked cheeses, respectively. Correlation statistical analysis showed that benzo[*a*]pyrene was a good marker for total 9 PAHs in Circassian cheese samples ($r_{B[a]P/sum\ of\ 9\ \ PAHs} = 0.816$, $p < 0.01$) as well as the best marker for 5 carcinogenic PAHs ($r_{B[a]P/carcinogenic\ PAHs} = 0.904$, $p < 0.01$). Risk assessment conducted using daily intakes of sum of 9 PAH levels found in both traditional and industrial smoked Circassian cheese samples showed high risk compared with unsmoked cheeses.



## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 200 different chemicals that are widespread environmental contaminants resulting from the incomplete combustion or pyrolysis of organic materials, during industrial processes and other human activities (Aygun and Kabadayi, 2005; Ciecierska and Obiedzinski, 2010; Ishizaki et al., 2010). They are characterized by two or more condensed aromatic rings (Anastasio et al., 2004; Lorenzo et al., 2010). These contaminants in environmental matrices including air, water, and soil, are important because of their mutagenic and carcinogenic properties (Titato and Lancas, 2006). Recent studies indicate that skin, lungs, bladder, breast, and colon cancers in humans have been associated with PAHs (Boffetta et al., 1997; Marti-Cid et al., 2008). Seven individual PAHs (benzo[*a*]anthracene (B[*a*]A), benzo[*a*]pyrene (B[*a*]P), benzo[*b*]-fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), chrysene (Chr), dibenzo[*a,h*]anthracene(DB[*ah*]A) and indeno[1,2,3-*c,d*]pyrene (Ip)) have been classified as probable human carcinogens in group B2 by the US Environmental Protection Agency (EPA) (Environmental Protection Agency, 2002). However, various epidemiological studies have showed that dietary exposure to PAHs is associated with an increased risk of some human cancers (Brody et al., 2007; Lee and Shim, 2007; Vineis and Husgafvel-Pursiainen, 2005; Yoon et al., 2007). Therefore B[*a*]P has been recently classified as a human carcinogen (Group 1) by the International

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Agency for Research on Cancer (IARC). DB[*ah*]A is classified as probably carcinogenic (Group 2A), whereas others are classified as possible human carcinogens (Group 2B) (IARC, 2012).

Due to their hydrophobic properties and chemically inert nature, they accumulate in lipids at the end of the food chain in both plants and animals (Grova et al., 2002; Ishizaki et al., 2010). In animal-based foodstuffs, PAHs can originate not only from environmental deposition but also from thermal processing (Ciecierska and Obiedzinski, 2010; Guillen and Sopelana, 2003; Perello et al., 2009). Baking, frying, drying, grilling, roasting, toasting and smoking can contribute to PAH formation (Pagliuca et al., 2003; White et al., 2008). Traditional smoking under uncontrolled conditions can cause dangerous PAH contamination because the food is directly in contact with the smoke (Pagliuca et al., 2003).

Currently, there is no limit for B[*a*]P and sum of PAHs in smoked cheese in Turkey. However, the Turkish Food Codex has established limit values for B[*a*]P and the sum of four PAHs (B[*a*]P, B[*a*]A, B[*b*]F and Chr) of 5 and 30 $\mu g\ kg^{-1}$, for smoked meat and smoked meat products, respectively (Turkish Food Codex, 2011). In addition, some countries (such as Germany, Austria and Czech Republic) have established a legal limit of 1 $\mu g\ kg^{-1}$ for B[a]P in smoked foods (Conde et al., 2005; Lorenzo et al., 2010).

The traditional Circassian cheese, originally named *Adyghe Koaye*, is produced from goats', sheep' and cows' milk or a mixture of them by acid coagulation (Guneser and Yuceer, 2011; Uysal et al., 2006). For the production of this cheese, milk is boiled and then cooled for fermentation. Sour whey or sour yoghurt is added as coagulating agent at a ratio of 3% and stirred. Formed curd is transferred into special cheese baskets (30 cm in diameter and around 5–10 cm in height). Whey is drained for 5–10 min and then the cheese is molded in the same basket. The upper surface of the cheese is salted and the next day the cheese is turned upside down and salted on the other side. The cheese is offered for consumption in fresh (unsmoked), dried (hung in the sun in the same basket and turned upside down frequently), and smoked form (smoked with the smoke of beech and oak trees in stoves for 3–4 days, after wrapping with a small cloth) (Guneser and Yuceer, 2011; Kamber, 2008; Uysal et al., 2006).

In the production of industrial Circassian cheese, milk is cooled to 30–32 °C at the end of the pasteurization and sour whey is then added for coagulation. After the completion of curdling at about 40–45 min, the curd is cut into small pieces (the size of peas) with a special knife or wires. The curd is transferred into a cheesecloth for whey drainage and then it is cut into blocks (around 15–20 cm length) with a knife. After the cutting step, blocks are placed into the fermentation room for about 10–16 h at room temperature. Then the blocks are broken into small pieces and heated at 70 °C for 2–2.5 min. After cooling, the curd is transferred to a clean surface and kneaded by hand. The cheese is cut into blocks and held for 6–10 h at room temperature. Blocks of cheese are then placed on racks at 17–20 °C and turned and salted every day. Industrial Circassian cheese may be consumed in either unsmoked (fresh) or smoked form. Smoking of the cheese is carried out with not only traditional smoke (previously described) but also with liquid smoke (0.01–0.1% liquid smoke, pH 2–4) for 15–60 min (Uçar, 2006).

There is no information about the levels of PAHs in Circassian cheese. Therefore, in this study we attempted to analyse the levels of 9 PAHs in industrial and traditional smoked and unsmoked Circassian cheese samples.

## 2. Material and methods

### 2.1. Sampling

For this study, 50 industrial (25 smoked and 25 unsmoked) and 20 traditional (10 smoked and 10 unsmoked) Circassian cheese samples were analysed. Industrial and traditional packaged smoked and unsmoked cheese samples were randomly acquired in local markets, supermarkets, and grocery stores from 7 representative cities (Antalya, Düzce, Izmir, Istanbul, Sakarya, Samsun and Sinop) of Turkey in 2011–2012. Traditional Circassian cheese is produced by Circassian families in regions of Anatolia, such as Düzce, Sinop, Balıkesir and Sakarya (Kamber,2008). Nowadays, it is produced on an industrial scale in commercial dairies in the cities of Düzce, Bolu, Adapazarı, Kayseri and Antalya and sold in places where the population is dense.

### 2.2. Chemicals and reagents

The standards of acenaphthene (Ace, 99%), anthracene (Ant, 99%), benzo[*a*]anthracene (B[*a*]A, 99%), benzo[*k*]fluoranthene (B[*k*]F, 98%), benzo[*ghi*]perylene (B[*ghi*]P, 98%), benzo[*a*]pyrene (B[*a*]P, 98%), dibenzo[*a,h*]anthracene (DB[*a,h*]A, 97%), naphthalene (Naph, 99%), pyrene (Pyr, >99%) were purchased from Sigma (Sigma–Aldrich, Steinheim, Germany) and the standard mixture of 16 PAHs (PAH mix 9) obtained from Dr. Ehrenstorfer GMBH (Augsburg, Germany). Water was purified with a Milli-Q ultra-pure water system (Millipore, Bedford, MA, USA) throughout the experiments. Acetonitrile, cyclohexane, dichloromethane, ethanol and methanol (all of HPLC grade, 99–99.9%) were supplied by Merck (Darmstadt, Germany). Solid-phase extraction (SPE) was performed with Isolate (silica 500 mg/3 mL) cartridges obtained from Supelco (Bellefonte, PA, USA).

### 2.3. Extraction and clean-up

The procedure described by Anastasio et al. (2004) with some modifications was applied for the extraction of PAHs from cheese samples. Essentially, 1 g cheese sample after homogenization was weighed into 30-mL Teflon centrifuge tubes and 5 mL of 1 M KOH ethanolic solution were added and then placed for 3 h in a water bath at 80 °C. After cooling to room temperature, 5 mL of distilled water and 10 mL of cyclohexane were added and the mixture was vortexed for 5 min. After centrifugation at 4000 × *g* for 15 min (Sigma, Model 3K30; Osterode am Harz, Germany), the supernatant layer was re-extracted with 10 mL of cyclohexane as previously described. The two cyclohexane phases were collected in a volumetric flask and concentrated by a rotary vacuum evaporator at 40 °C (Rotavapor R-200; Buchi, Flawil, Switzerland) followed by drying of the extract under a nitrogen stream. The residue was dissolved in 2 mL of acetonitrile, which was applied to an SPE cartridge.

The method described by Węgrzyn et al. (2006) was used for the cleanup. The SPE cartridge was previously activated by the passage of 10 mL of ultrapure water and 10 mL of methanol. The cartridge was then dried. After loading the 2 mL of eluate, the cartridge was left to dry by air for 1 min and PAHs were eluted with 10 mL of dichloromethane. Prior to HPLC analysis, the collected eluate was concentrated to near dryness in a water bath (40 °C) under a nitrogen stream. Finally, the PAH fraction was diluted with 500 μL acetonitrile.

### 2.4. Chromatographic analysis

The extracts of PAHs were analysed using an HPLC system from Shimadzu (Kyoto, Japan), consisted of a fluorescence detector (FLD) RF-10XL, quaternary pump LC-20AT, degassing device DGU-20A5, column oven CTO-10ASVP, auto-injector SIL-10A and system controller SCL-10AVP (data station LC-20AT). An Envirosep-PP column (125 mm × 4.6 mm, 4.6 μm; Phenomenex, Torrance, CA, USA) was used for chromatographic analyses. The HPLC-FLD was carried out under the following conditions; gradient elution