World Congress on Oils and Fats
& 28th ISF Congress
27 - 30 September 2009 • Sydney Australia

# PAHs IN EDIBLE FATS AND OILS: AN OVERVIEW OF THEIR OCCURRENCE AND RELATED ANALYTICAL TECHNIQUES

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# PAHs and laws: a short introduction

## PAHs:

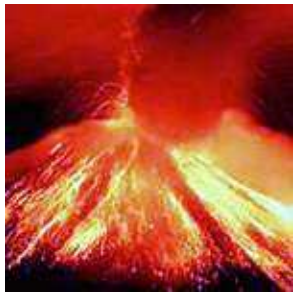
-ubiquitarious organic compounds

- Formed through incomplete combustion of organic matter (400-700℃).

- Cancerogenic according to l'EPA and IARC

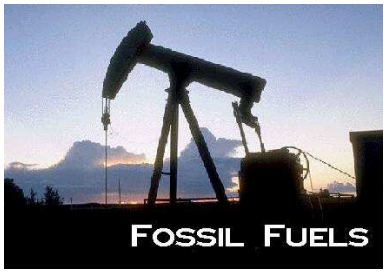
# PAHs in the atmosphere depending on combustion processes

## Natural sources



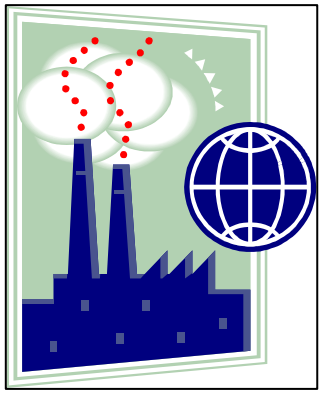
Volcanic eruption

Fire




Geochemical processing

## Anthropic sources



Industry

Home heating

Waste burning

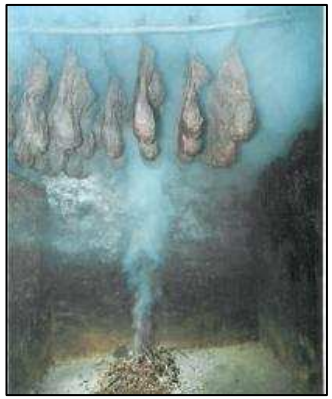
Car and trucks

# CONTAMINATION OF FOODS

## Atmospheric particulate fall down

## Poor technology applied

Grilling



Smoking

Mineral oil contamination

# LEGISLATION

## 2005: EEC limit harmonisation

BaP chosen as contamination marker

8.2.2005 EN Official Journal of the European Union L 34/3

COMMISSION REGULATION (EC) No 208/2005

of 4 February 2005

amending Regulation (EC) No 466/2001 as regards polycyclic aromatic hydrocarbons

(Text with EEA relevance)

In Annex I to Regulation (EC) No 466/2001 the following Section 7 is added:

'Section 7: Polycyclic aromatic hydrocarbons (PAH)

| | Product | Maximum level (µg/kg wet weight) | Performance criteria for sampling | Performance criteria for methods of analysis |
|---|---|---|---|---|
| 7.1. | Benzo(a)pyrene (1) | | | |
| 7.1.1. | Oils and fats intended for direct human consumption or use as an ingredient in foods (2) | 2,0 | Directive 2005/10/EC (4) | Directive 2005/10/EC |
| 7.1.2. | Foods for infants and young children | | | |
| 7.1.2.1. | Baby foods and processed cereal-based foods for infants and young children (3) | | | |
| 7.1.2.2. | Infant formulae and follow-on formulae, including infant milk and follow-on milk (4) | 1,0 | Directive 2005/10/EC | Directive 2005/10/EC |
| 7.1.2.3. | Dietary foods for special medical purposes (5) intended specifically for infants | | | |
| 7.1.3. | Smoked meats and smoked meat products | 5,0 | Directive 2005/10/EC | Directive 2005/10/EC |
| 7.1.4. | Muscle meat of smoked fish and smoked fishery products (6), excluding bivalve molluscs | 5,0 | Directive 2005/10/EC | Directive 2005/10/EC |
| 7.1.5. | Muscle meat of fish (7), other than smoked fish | 2,0 | Directive 2005/10/EC | Directive 2005/10/EC |
| 7.1.6. | Crustaceans, cephalopods, other than smoked | 5,0 | Directive 2005/10/EC | Directive 2005/10/EC |
| 7.1.7. | Bivalve molluscs | 10,0 | Directive 2005/10/EC | Directive 2005/10/EC |

(1) See page 15 of this Official Journal.
(2) Benzo(a)pyrene, for which maximum levels are listed, is used as a marker for the occurrence and effect of carcinogenic PAH. These measures therefore provide full harmonisation on PAH in the listed foods across the Member States. The Commission shall review the maximum levels for PAH in the listed food categories by 1 April 2007, taking into account the progress in scientific and technological knowledge on the occurrence of benzo(a)pyrene and other carcinogenic PAH in food.
(3) Cocoa butter is excluded from this category whilst investigations into the presence of benzo(a)pyrene in cocoa butter are made. This derogation will be reviewed by 1 April 2007.
(4) Baby foods and processed cereal-based foods for infants and young children as defined in Article 1 of Directive 96/5/EC. The maximum level refers to the product as sold.
(5) Infant formulae and follow-on formulae as defined in Article 1 of Directive 91/321/EEC. The maximum level refers to the product as sold.
(6) Dietary foods for special medical purposes as defined in Article 1(2) of Directive 1999/21/EC. The maximum level refers to the product as sold.
(7) Fish and fishery products as defined in the categories (b), (c), and (f) of the list in Article 1 of Regulation (EC) No 104/2000.
(8) Fish as defined in the category (a) of the list in Article 1 of Regulation (EC) No 104/2000.'

8.2.2005 EN Official Journal of the European Union L 34/43

COMMISSION RECOMMENDATION

of 4 February 2005

on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods

*(notified under document number C(2005) 256)*

8.2.2005 EN Official Journal of the European Union L 34/45

ANNEX

Polycyclic aromatic hydrocarbons (PAH) highlighted to be carcinogenic by the Scientific Committee on Food (1), for which further investigation of the relative levels in certain foods is required:

benzo(a)anthracene

benzo(b)fluoranthene

benzo(j)fluoranthene

benzo(k)fluoranthene

benzo(g,h,i)perylene

benzo(a)pyrene

chrysene

cyclopenta(c,d)pyrene

dibenzo(a,h)anthracene

dibenzo(a,e)pyrene

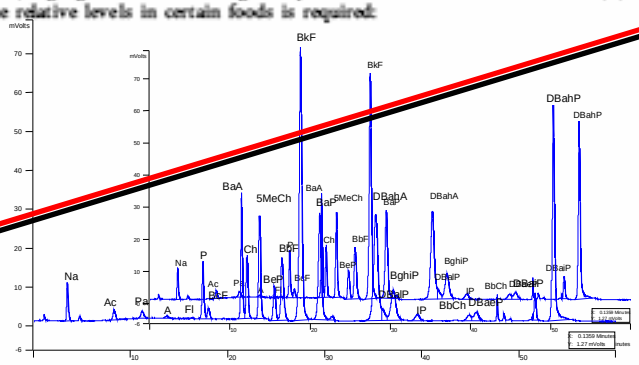
dibenzo(a,h)pyrene

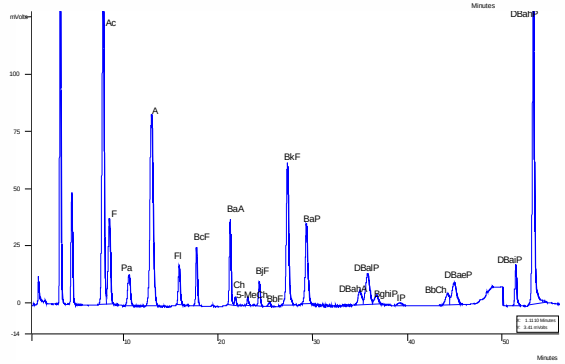
dibenzo(a,i)pyrene

dibenzo(a,l)pyrene

indeno(1,2,3-cd)pyrene

5-methylchrysene




**SFC (nowadays named EFSA) considered 33 PAHs and 15 are considered both carcinogenic and genotoxic.**

**+ BcF on proposal by JECFA (Joint FAO/WHO Expert Commitee on Food Additives)**

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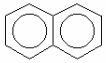
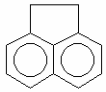
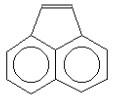
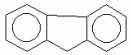
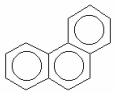
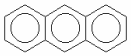
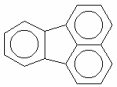
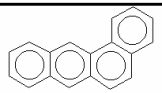
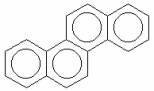
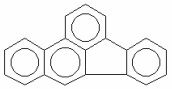
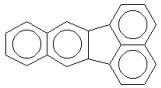
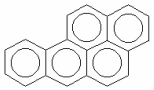
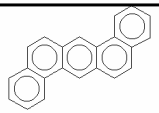
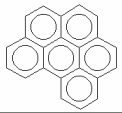
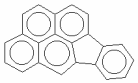
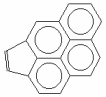
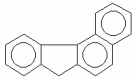
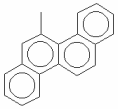
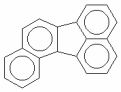
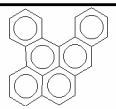
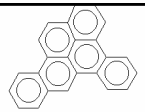
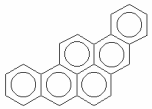
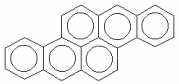
## IPA EPA

| Composto | Sigla | Formula |
|---|---|---|
| Naphthalene | Na |  |
| Acenaphtene | Ac |  |
| Achenaphthylene | Ap |  |
| Fluorene | F |  |
| Phenanthrene | Pa |  |
| Anthracene | A |  |
| Fluoranthene | Fl |  |
| Pyrene | P |  |

## IPA EPA e EU

| Composto | Sigla | Formula |
|---|---|---|
| Benzo(a)Anthracene | BaA |  |
| Chrysene | Ch |  |
| Benzo(b)Fluoranthene | BbF |  |
| Benzo(k)Fluoranthene | BkF |  |
| Benzo(a)Pyrerne | BaP |  |
| Dibenzo(a,h)anthracene | DBahA |  |
| Benzo(g,h,i)perilene | BghiP |  |
| IndenoPirene | IP |  |

## IPA EU

| Composto | Sigla | Formula |
|---|---|---|
| cyclopenta(c,d)pyrene | CPP |  |
| Benzo(c)fluorene | BcF |  |
| 5-methylchrysene | 5-MeCh |  |
| Benzo(j)fluoranthene | BjF |  |
| Dibenzo(a,l)pyrene | DBalP |  |
| Dibenzo(a,e)pyrene | DBaeP |  |
| Dibenzo(a,i)pyrene | DBaiP |  |
| Dibenzo(a,h)pyrene | DBahP |  |

DIRETTIVA 2005/10/CE DELLA COMMISSIONE

del 4 febbraio 2005

recante definizione dei metodi di campionamento e di analisi per il controllo ufficiale del tenore di benzo(a)pirene nelle derrate alimentari

## Method Performance

| *Parameter* | *Value/notes* |
|---|---|
| **Applicability** | **Foods as specified in EC Regulation n. 208/2005** |
| **LOD** | **< 0,3 μg/kg** |
| **LOQ** | **< 0,9 μg/kg** |
| **Precision** | **$HORRAT_r$ e $HORRAT_R$ < 1,5 as evaluated by collaborative study** |
| **Recovery** | **50-120%** |
| **Specificity** | **No inteferring by matrix, verify positive identification** |

# An European SCOOP Task to assess PAHs content in diet

**October 2004**

**Report on task for scientific cooperation.**
**http://ec.europa.eu/food/food/chemicalsafety/contaminants/scoop_3-2-12_final_report_pah_en.pdf**

## Partner countries:

- Austria
- Belgium
- Denmark
- Suomi/Finland
- France
- Germany
- Greek
- Ireland
- Italy
- Netherlands
- Norvey
- Portugal
- Spain
- UK

## BaP concentration in selected food groups in Europe

| Food groups | Number of samples |
|---|---|
| Additives | 83 |
| alcoholic beverages excluding beer & wine | 1 |
| beer & wine | 22 |
| Biscuits | 12 |
| **bread / rolls** | **103** |
| breakfast cereals | 12 |
| butter & animal fats | 6 |
| cereals (others) | 63 |
| Cheese | 41 |
| chocolate (chocolate & cocoa products) | 16 |
| coffee (green bean, filter, instant) | 32 |
| crisps & ready-to-eat savouries (salted snacks & seeds) | 11 |
| Crustaceans | 24 |
| dairy products | 1 |
| **dried fruits** | **158** |
| Eggs | 4 |
| **fish / fish products** | **1179** |
| fries / chips | 2 |
| fruit juices | 2 |
| Fruits | 95 |
| hot drinks (tea, infusion excluding cocoa powder) | 57 |
| Margarine | 34 |
| **meat** | **519** |
| Milk | 1 |
| mixed dishes | 20 |
| **Molluscs** | **300** |
| nuts & oleaginous grains | 30 |
| Offals | 2 |
| Others | 12 |
| pastas / rice | 16 |
| pastry goods | 8 |
| pizzas & quiches | 20 |
| Potatoes | 17 |
| poultry & game | 28 |
| products for special nutritional use (infant & dietetic formulae) | 22 |
| **sausages & ham** | **2358** |
| soft drinks excluding fruit juices | 2 |
| spices/sauces/condiments | 51 |
| sugar & by-products including honey | 16 |
| vegetables (canned) | 1 |
| vegetables (leaf) | 64 |
| vegetables (others) | 87 |
| vegetables (root excluding potatoes) | 24 |
| **vegetal oils** | **2110** |
| **waters (mineral, source, tap)** | **1195** |
| **Total** | **8861** |

## Nuts and Bearing Oil Seeds

*option: samples < LOD or < LOQ were assigned a value of LOD/2 or LOQ/2 (medium bound)*

| food group | Nb samples 0-0.5 ppb | Nb samples 0.5-1 ppb | Nb samples 1-2 ppb | Nb samples 2-5 ppb | Nb samples 5-10 ppb | Nb samples 20-50 ppb | Nb samples 50-100 ppb | Nb samples > 100 ppb |
|---|---|---|---|---|---|---|---|---|
| nuts & oleaginous grains | 28 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |

## Vegetable Oils

*option: samples < LOD or < LOQ were assigned a value of LOD/2 or LOQ/2 (medium bound)*

| food group | Nb samples 0-0.5 ppb | Nb samples 0.5-1 ppb | Nb samples 1-2 ppb | Nb samples 2-5 ppb | Nb samples 5-20 ppb | Nb samples 20-50 ppb | Nb samples 50-100 ppb | Nb samples > 100 ppb |
|---|---|---|---|---|---|---|---|---|
| virgin & extra virgin olive oil | 575 | 48 | 34 | 11 | 2 | 1 | 0 | 0 |
| olive oil | 188 | 57 | 18 | 7 | 4 | 4 | 2 | 0 |
| olive pomace oil | 60 | 21 | 17 | 170 | 9 | 65 | 9 | 9 |
| oil in canned food | 115 | 27 | 6 | 21 | 16 | 4 | 2 | 0 |
| Grape seed oil | 44 | 13 | 4 | 16 | 11 | 2 | 2 | 0 |
| sunflower oil | 107 | 20 | 15 | 30 | 28 | 0 | 0 | 1 |

**The only harmonysed method is the ISO 15302 method that is for BaP determination, only.**

**Then ISO standardysed another method (ISO/AWI 22959), that however requires a peculiar instrumentation based on a donor acceptor HPLC phase**

**A simpler approach was developed by means of SPE that Admit the evaluation of several PAHs.**
**Two methods were developed in Italy and underwent to ring test**

# Ring Test:a comparison between two SPE method developed in Italy for olive oils

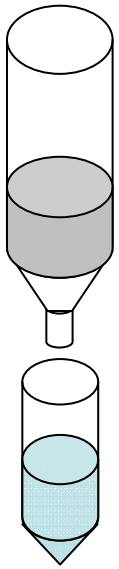
## Moret e Conte (2002)

**2 g oil in 10 mL C6**

**1 mL** | **8 mL C6/$CH_2Cl_2$**

**8 mL C6/$CH_2Cl_2$**

**SPE Si**



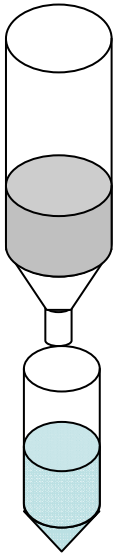
## Cortesi e Fusari (2005)

**2 g oil in 10 mL isoctane/CE**

**1 mL** | **25 mL isoctane/CE**

**6 mL $CH_2Cl_2$**

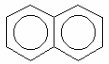
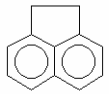
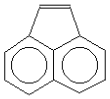
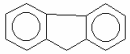
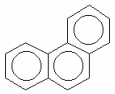
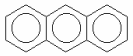
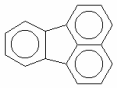
**SPE PPL**



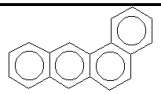
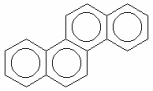
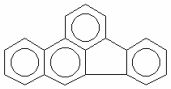
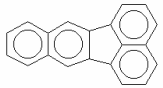
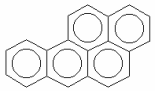
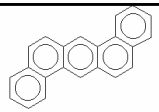
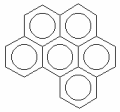
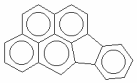
**Both methods characteristics lay inside thje limit of EEC Directive**

# IPA EPA – IPA EU

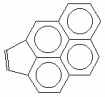
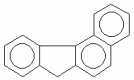
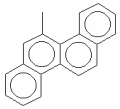
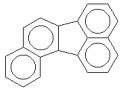
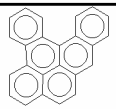
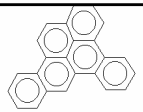
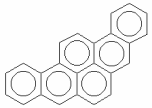
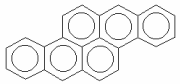
## IPA EPA

| Composto | Sigla | Formula |
|---|---|---|
| Naphthalene | Na |  |
| Acenaphtene | Ac |  |
| Achenaphthylene | Ap |  |
| Fluorene | F |  |
| Phenanthrene | Pa |  |
| Anthracene | A |  |
| Fluoranthene | Fl |  |
| Pyrene | P |  |

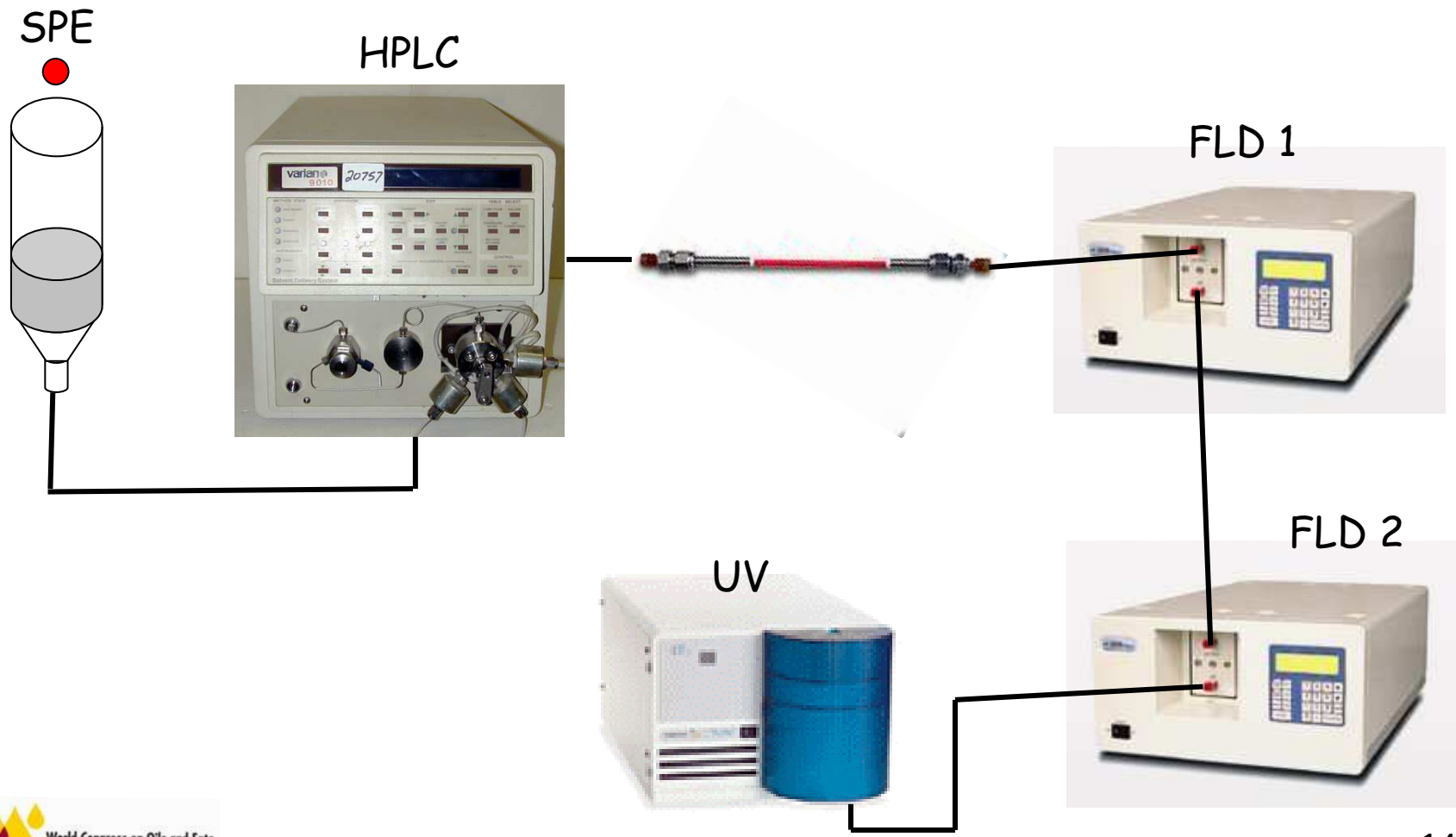
## IPA EPA e EU

| Composto | Sigla | Formula |
|---|---|---|
| Benzo(a)Anthracene | BaA |  |
| Chrysene | Ch |  |
| Benzo(b)Fluoranthene | BbF |  |
| Benzo(k)Fluoranthene | BkF |  |
| Benzo(a)Pyrerne | BaP |  |
| Dibenzo(a,h)anthracene | DBahA |  |
| Benzo(g,h,i)perilene | BghiP |  |
| IndenoPirene | IP |  |

## IPA EU

| Composto | Sigla | Formula |
|---|---|---|
| cyclopenta(c,d)pyrene | CPP |  |
| Benzo(c)fluorene | BcF |  |
| 5-methylchrysene | 5-MeCh |  |
| Benzo(j)fluoranthene | BjF |  |
| Dibenzo(a,l)pyrene | DBalP |  |
| Dibenzo(a,e)pyrene | DBaeP |  |
| Dibenzo(a,i)pyrene | DBaiP |  |
| Dibenzo(a,h)pyrene | DBahP |  |

# SPE-HPLC-FLD/UV

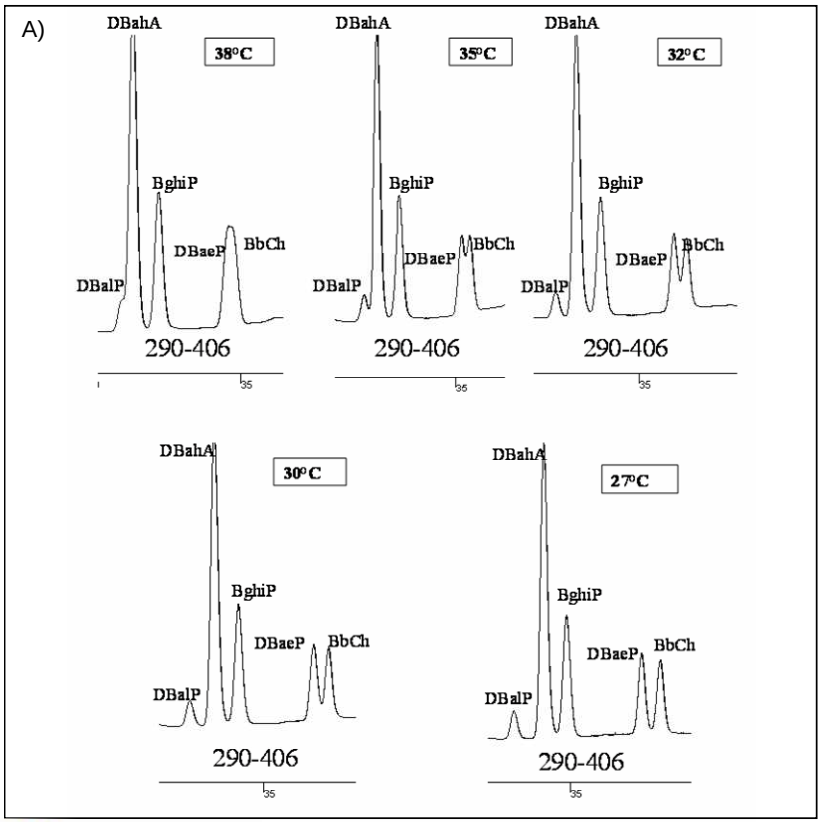


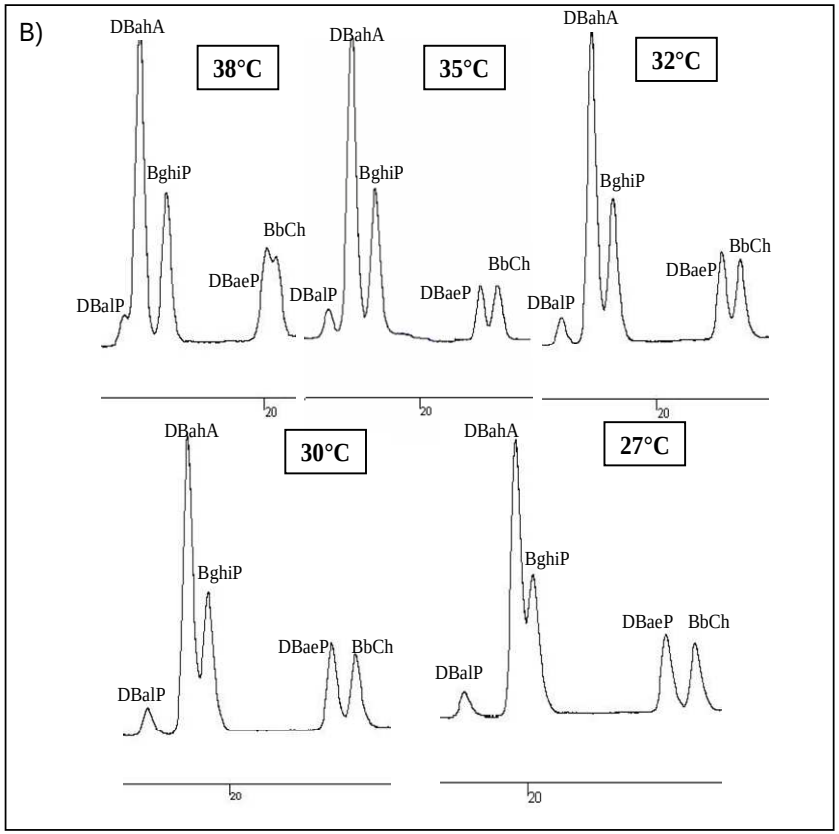
## “Fast gradient elution”

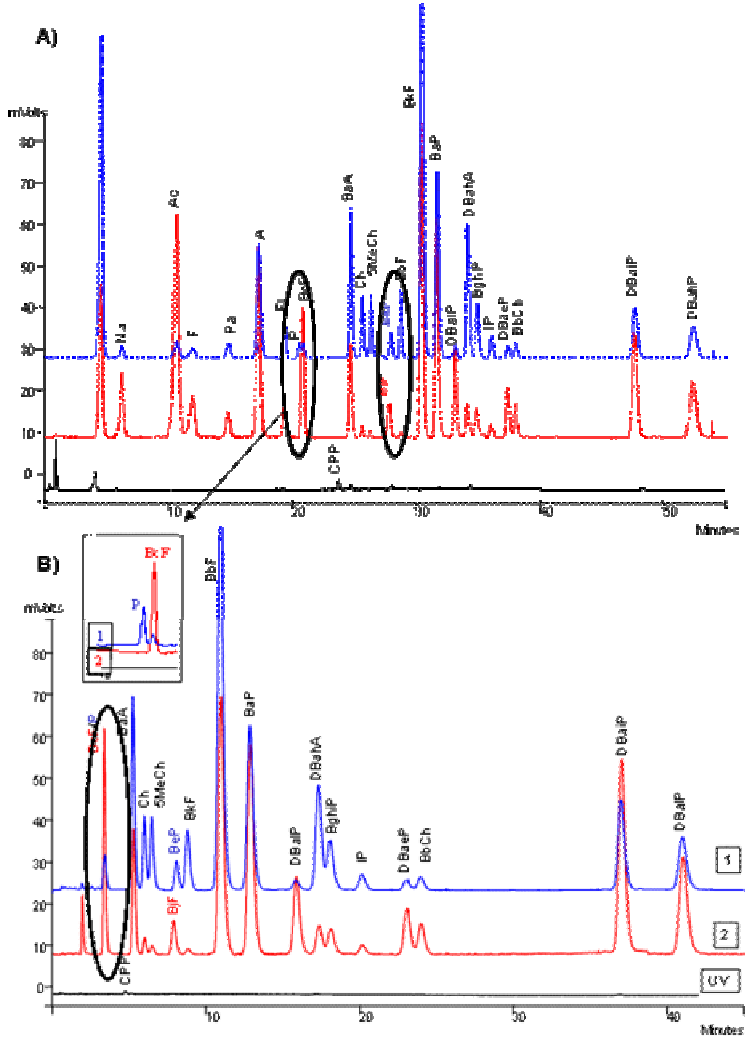
| time | $CH_3CN$ | $H_2O$ |
|---|---|---|
| 0 | 75 | 25 |
| 20 | 75 | 25 |
| 35 | 100 | 0 |

## “Long time gradient elution”

| time | $CH_3CN$ | $H_2O$ |
|---|---|---|
| 0 | 50 | 50 |
| 10 | 50 | 50 |
| 15 | 60 | 40 |
| 20 | 75 | 25 |
| 35 | 75 | 25 |
| 45 | 100 | 0 |






| Standard | λex- λem 1 | λex- λem 2 |
|---|---|---|
| Na<br>Ac<br>F | 270-390 | 276-330 |
| Pa | | 250-366 |
| A | | 250-402 |
| Fl | | 270-470 |
| CPP | | 280 (UV) |
| P<br>BcF | | 300-354 |
| BaA<br>Ch<br>5-MeCh | | 290-404 |
| BeP<br>BjF<br>BbF | 280-410 | 310-548 |
| BkF<br>BaP | 290-410 | 256-410 |
| DBahA<br>DBalP<br>BghiP | 290-406 | 270-446 |
| IP | 290-484 | |
| BbCh<br>DBaeP | 290-450 | 290-400 |
| DBaiP | | 290-434 |
| DBahP | | 300-452 |

# Method validation

| | Level 1 | | | | Level 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | µg/kg | Recovery (%) | intra-day repeatability (n=6) | inter-day repeatability (n=12) | µg/kg | Recovery (%) | intra-day repeatability (n=6) | inter-day repeatability (n=12) |
| **CPP** | 18.86 | 88.18 | 10.2 | 14.7 | 25.19 | 84.67 | 6.6 | 14.0 |
| **BcF** | 0.60 | 124.10 | 8.2 | 8.4 | 1.20 | 111.13 | 6.4 | 8.4 |
| **BaA** | 1.20 | 79.84 | 6.7 | 6.8 | 2.40 | 98.47 | 6.3 | 7.2 |
| **Ch** | 1.19 | 116.16 | 7.6 | 8.5 | 2.38 | 114.83 | 4.3 | 6.6 |
| **5MeCh** | 0.60 | 105.47 | 8.2 | 8.2 | 1.20 | 111.61 | 8.4 | 9.2 |
| **BeP** | 2.05 | 101.74 | 10.0 | 10.5 | 4.10 | 109.35 | 2.7 | 6.1 |
| **BjF** | 3.59 | 89.72 | 9.3 | 14.4 | 7.19 | 102.12 | 9.3 | 9.5 |
| **BbF** | 1.80 | 85.69 | 7.6 | 12.0 | 3.59 | 102.29 | 5.3 | 6.4 |
| **BkF** | 1.19 | 85.07 | 7.5 | 11.9 | 2.38 | 103.21 | 3.2 | 8.6 |
| **BaP** | 1.19 | 78.96 | 6.4 | 13.1 | 2.38 | 94.92 | 8.1 | 8.9 |
| **DBalP** | 1.78 | 59.65 | 6.8 | 7.1 | 3.58 | 86.68 | 7.9 | 10.9 |
| **DBahA** | 1.79 | 91.30 | 6.4 | 11.5 | 3.56 | 97.87 | 8.6 | 9.1 |
| **BghiP** | 1.78 | 92.09 | 6.2 | 9.4 | 3.57 | 108.45 | 1.8 | 4.4 |
| **IP** | 1.19 | 81.61 | 7.5 | 11.9 | 2.38 | 98.38 | 6.0 | 6.3 |
| **BbCh** | 0.12 | 63.25 | 9.4 | 10.4 | 0.24 | 100.97 | 7.2 | 8.3 |
| **DBaeP** | 0.59 | 71.22 | 9.6 | 11.5 | 1.19 | 95.21 | 9.3 | 11.0 |
| **DBaiP** | 1.20 | 70.70 | 11.0 | 12.1 | 2.39 | 97.62 | 3.2 | 8.3 |
| **DBahP** | 0.59 | 62.86 | 11.3 | 16.2 | 1.19 | 80.77 | 11.3 | 13.8 |

| Compounds | Linear range (µg/kg) | Slope (l=5) | Intercept (l=5) | $r^2$ | LOD (µg/kg) (n=6) | LOQ (µg/kg) (n=6) |
|---|---|---|---|---|---|---|
| **CPP** | 27.81-125.97 | 1844 ± 44 | 699 ± 167 | 0.994 | 18.86 | 27.81 |
| **BcF** | 0.60-5.99 | 2963103 ± 64438 | 5676 ± 8117 | 0.996 | 0.06 | 0.11 |
| **BaA** | 1.20-11.98 | 1609014 ± 28744 | 6501 ± 10422 | 0.997 | 0.003 | 0.04 |
| **Ch** | 1.19-11.89 | 669093 ± 9871 | 3912 ± 3522 | 0.998 | 0.43 | 0.67 |
| **5-MeCh** | 0.60-5.99 | 1420780 ± 37305 | 4130 ± 6258 | 0.993 | 0.19 | 0.30 |
| **BeP** | 2.05-20.52 | 193012 ± 5412 | 4353 ± 3332 | 0.992 | 0.43 | 0.70 |
| **BjF** | 3.59-35.94 | 130964± 5054 | 3989 ± 5500 | 0.984 | 0.17 | 0.63 |
| **BbF** | 1.80-17.96 | 485465 ± 13160 | 9245 ± 7091 | 0.993 | 0.003 | 0.22 |
| **BkF** | 1.19-11.91 | 6059566 ± 112129 | 15804 ± 40433 | 0.996 | 0.04 | 0.08 |
| **BaP** | 1.19-11.89 | 2807969 ± 63838 | 8851 ± 21266 | 0.995 | 0.01 | 0.05 |
| **DBalP** | 1.79-17.88 | 868664 ± 24195 | 16689 ± 13049 | 0.992 | 0.14 | 0.29 |
| **DBahA** | 1.78-17.81 | 1221445 ± 36272 | 2609 ± 13630 | 0.993 | 0.04 | 0.09 |
| **BghiP** | 1.78-17.84 | 598048 ± 12224 | 3831 ± 4180 | 0.997 | 0.14 | 0.33 |
| **IP** | 1.19-11.90 | 239921 ± 4566 | 2010 ± 1522 | 0.996 | 0.04 | 0.14 |
| **BbCh** | 0.12-1.19 | 5435687 ± 129764 | 4637 ± 4340 | 0.992 | 0.04 | 0.08 |
| **DBaeP** | 0.59-5.93 | 1616733 ± 34087 | 6849 ± 6118 | 0.995 | 0.07 | 0.17 |
| **DBaiP** | 1.20-11.96 | 3117311 ± 51940 | 39720 ± 18206 | 0.998 | 0.03 | 0.23 |
| | | 4369382 ± 102794 | 1990 ± 11702 | 0.996 | 0.11 | 0.21 |

## Performance Criteria EU Dir. 2005/10

**LOD** 0.3 µg/kg

**LOQ** 0.9 µg/kg

## A case study – 1: A survey on frying oils

**Applied Temperatures:**

**180 - 200 °C.**

**Some epidemiologycal survey carried out in Taiwan and China (Wu-Williams et al., 1990; Ko et al., 1997; Gao et al., 1987) highlighted a relationship between lung cancer and frying in women exposed to frying smokes**

- **Aromatic etherocyclic amines**
- **1,3-butandiene**
- **acroleine**
- **formaldheyde**
- **benzene**
- ***PAHs* (Chen et al., 2001; Chiang et al., 1997; Vainiotalo et al., 1993)**

# Sampling protocol

| Source of samples | Code | Type of oil[a] | Type of products fried |
|---|---|---|---|
| Continuous process- Large-scale Spanish producers | plan 1 | po/so (60/40) | chips |
| | plan 2 | po | corn extruded snack |
| | plan 3 | so | chips |
| | plan 4 | sbo | chips |
| | plan 5 | sbo | potato extruded snack |
| | plan 6 | po/sbo (50/50) | chips |
| Discontinuos process Sample from University's canteen | C1a | pno | french fries |
| | C1b | pno | french fries, fish |
| | C2 | pno | french fries, fish |
| Snacks from Italian market | S1 | so | chips |
| | S2 | so | chips |
| | S3 | so | chips |
| | S4 | so | chips |
| | S5 | pno | chips |
| | S6 | pno | chips |
| | S7 | pno | chips |
| | S8 | pno | chips |
| | S9 | pno | chips |
| | S10 | pno | chips |
| | S11 | pno | chips |
| | S12 | pno | corn extruded |
| | S13 | pno | wheat extruded |
| | S14 | pno | cereal mix extruded |

[a] **po**: palm olei; **so**: sunflower oil; **sbo**: soybean oil; **pno**: peanut oil;

# PAHs in fried oils and products

| Code | Frying oil[a] | Sample[b] | Na | Ac | F | Pa | A | Fl | P | *L-PAH* | BaA | Ch | BeP | BbF | BkF | BaP | DBahA | BghiP | IP | *H-PAH* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| plan 1 | po/so (60/40) | fresh oil | 6.1 | < LOQ | 0.7 | 2.2 | 0.1 | 0.2 | 1.1 | *4.3* | 0.3 | 0.5 | < LOQ | 0.2 | 0.1 | 0.2 | < LOQ | 0.2 | 0.1 | *1.5* |
| | | fried oil | 3.5 | < LOQ | 0.6 | 1.7 | 0.1 | 0.7 | 0.9 | *4.0* | 0.3 | 0.3 | < LOQ | 0.2 | 0.1 | 0.2 | < LOQ | 0.3 | 0.1 | *1.4* |
| | | chips | 13.5 | 0.2 | 2.0 | 12.2 | 0.3 | 1.9 | 3.2 | *19.5* | 0.5 | < LOQ | < LOQ | 0.3 | 0.1 | 0.2 | < LOQ | < LOQ | 0.1 | *1.2* |
| plan 2 | po | fresh oil | 7.8 | 0.3 | 1.0 | 4.5 | 0.6 | 2.7 | 3.6 | *12.4* | 1.1 | 0.1 | < LOQ | 0.4 | 0.3 | 0.7 | < LOQ | 0.4 | 0.3 | *3.4* |
| | | fried oil | 4.2 | 0.1 | 1.0 | 3.9 | 0.5 | 2.6 | 4.2 | *12.3* | 1.3 | 0.2 | 0.1 | 0.6 | 0.2 | 0.7 | < LOQ | 0.4 | 0.2 | *3.6* |
| | | corn ext. | 9.3 | < LOQ | 1.2 | 4.8 | 0.4 | 2.3 | 4.4 | *13.2* | 1.0 | 0.3 | < LOQ | 0.5 | 0.2 | 0.6 | < LOQ | 0.5 | 0.3 | *3.5* |
| plan 3 | so | oil fresh | 7.2 | < LOQ | 1.5 | 8.4 | 1.0 | 3.9 | 5.3 | *20.1* | 0.8 | 0.2 | 0.1 | nq | 0.2 | 0.3 | < LOQ | < LOQ | 0.1 | *1.8* |
| | | oil fried | 4.3 | < LOQ | 0.4 | 5.0 | 0.5 | 3.1 | 4.7 | *13.6* | 0.7 | 0.6 | < LOQ | 0.7 | 0.2 | 0.3 | < LOQ | < LOQ | 0.2 | *2.6* |
| | | chips | nq | 0.1 | 1.5 | 5.7 | 0.4 | 2.6 | 5.0 | *15.3* | 1.2 | 0.7 | < LOQ | 0.8 | 0.3 | 0.5 | < LOQ | < LOQ | 0.4 | *3.9* |
| plan 4 | sbo | fresh oil | 5.9 | < LOQ | 0.6 | 2.3 | 0.2 | 2.0 | 3.7 | *8.7* | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | < LOQ | < LOQ | 0.1 | *0.8* |
| | | fried oil | 4.7 | < LOQ | 0.5 | 1.8 | 0.1 | 1.5 | 2.6 | *6.5* | 0.2 | 0.1 | < LOQ | < LOQ | 0.1 | < LOQ | < LOQ | < LOQ | < LOQ | *0.4* |
| | | chips | nq | 0.1 | 4.2 | 7.8 | 0.3 | 2.1 | 4.5 | *19.0* | 0.3 | 0.3 | < LOQ | 0.4 | 0.1 | 0.1 | 0.1 | 0.1 | < LOQ | *1.4* |
| plan 5 | sbo | fresh oil | 4.2 | < LOQ | 0.9 | 1.8 | 0.1 | 2.1 | 2.6 | *7.5* | 0.2 | 0.1 | 0.1 | 0.1 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | *0.5* |
| | | fried oil | 4.3 | < LOQ | 1.7 | 0.9 | 0.1 | 2.2 | 4.3 | *9.3* | 0.2 | 0.1 | nq | 0.2 | 0.1 | nq | 0.2 | < LOQ | < LOQ | *0.7* |
| | | patato ext. | 20.1 | < LOQ | 2.4 | 5.7 | 0.3 | 2.8 | 5.8 | *17.1* | 0.4 | 0.2 | nq | 0.4 | 0.2 | 0.2 | 0.1 | < LOQ | < LOQ | *1.4* |
| plan 6 | po/sbo (50/50) | fresh oil | 5.4 | < LOQ | 0.6 | 2.3 | 0.1 | 0.8 | 1.9 | *5.7* | 0.2 | 0.1 | < LOQ | 0.1 | 0.1 | 0.1 | < LOQ | < LOQ | 0.1 | *0.6* |
| | | fried oil | 5.8 | < LOQ | 1.3 | 3.0 | < LOQ | 0.8 | 2.2 | *7.3* | 0.4 | < LOQ | < LOQ | 0.2 | 0.1 | 0.1 | < LOQ | < LOQ | < LOQ | *0.9* |
| | | chips | 16.9 | < LOQ | 1.8 | 5.8 | 0.3 | 1.1 | 2.3 | *11.3* | 0.2 | 0.1 | < LOQ | 0.1 | 0.1 | 0.1 | 0.1 | < LOQ | < LOQ | *0.6* |
| C1a | pno | fresh oil | 2.3 | < LOQ | 0.2 | 0.6 | < LOQ | 0.5 | 0.5 | *1.8* | 0.2 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | *0.2* |
| | pno | fried oil (p) | 1.7 | < LOQ | 0.1 | 0.9 | < LOQ | 0.3 | 0.1 | *1.4* | 0.2 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | *0.2* |
| C1b | pno | fresh oil | < LOQ | < LOQ | < LOQ | 0.6 | 0.1 | 1.1 | 1.1 | *2.9* | 0.3 | 0.3 | 0.1 | 0.3 | 0.2 | 0.2 | < LOQ | 0.3 | 0.2 | *1.9* |
| | pno | fried oil (p) | 0.8 | < LOQ | 0.5 | 2.1 | 0.1 | 1.0 | 1.7 | *5.4* | 0.3 | 0.1 | 0.1 | 0.4 | 0.1 | 0.2 | 0.1 | 0.4 | 0.3 | *2.1* |
| | pno | fried oil (f) | 2.2 | < LOQ | 0.4 | 0.8 | 0.1 | 0.9 | 1.5 | *3.6* | 0.3 | 0.1 | 0.0 | 0.4 | 0.1 | 0.2 | < LOQ | 0.2 | 0.3 | *1.7* |
| C2 | pno | fresh oil | 4.4 | < LOQ | 0.6 | 1.8 | 0.1 | 0.9 | 1.3 | *4.7* | 0.4 | < LOQ | 0.1 | 0.2 | 0.1 | 0.1 | < LOQ | < LOQ | 0.1 | *1.0* |
| | pno | fried oil (p) | 3.6 | < LOQ | 0.7 | 3.2 | 0.1 | 0.9 | 1.0 | *5.8* | 0.3 | < LOQ | < LOQ | 0.2 | 0.3 | 0.2 | < LOQ | < LOQ | 0.7 | *1.7* |
| | pno | fried oil (f) | 7.2 | 0.3 | 2.0 | 4.5 | 0.2 | 1.1 | 1.1 | *8.9* | 0.1 | 0.2 | 0.1 | 0.3 | 0.1 | 0.1 | < LOQ | < LOQ | 0.2 | *1.1* |

[a] **po**: palm olei; **so**: sunflower oil; **sbo**: soybean oil; **pno**: peanut oil;

[b] ext.: extruded; p : potatoes; f : fish

***L-PAH***: sum of light PAHs, excluded Na and Ac due to the high variability; ***H-PAH***: sum of heavy PAHs

**Purcaro G., Navas J.A., Guardiola F., Conte L.S., Moret S., *Polyciclic aromatic hydrocarbons in frying oils and snacks*, J.Food Prot., 69(1), 2006**

# PAHs contamination in fried snacks

| Code | Frying oil[a] | Sample[b] | Na | Ac | F | Pa | A | Fl | P | *L-PAH* | BaA | Ch | BeP | BbF | BkF | BaP | DBahA | BghiP | IP | *H-PAH* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S1** | **so** | **chips** | 7.9 | 0.2 | 1.0 | 1.3 | < LOQ | 0.6 | 0.8 | *3.7* | < LOQ | 0.1 | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 | < LOQ | 0.1 | *1.0* |
| **S2** | **so** | **chips** | 9.2 | < LOQ | 0.4 | 1.5 | 0.1 | 2.9 | 2.2 | *7.1* | 0.8 | 1.0 | < LOQ | 1.2 | 0.4 | 0.6 | < LOQ | 0.5 | 0.3 | *4.8* |
| **S3** | **so** | **chips** | 3.7 | < LOQ | 0.1 | 1.2 | < LOQ | 0.3 | 0.1 | *1.7* | < LOQ | 0.3 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | *0.3* |
| **S4** | **so** | **chips** | 9.6 | 0.1 | 0.6 | 2.1 | 0.1 | 0.9 | 1.4 | *5.0* | < LOQ | < LOQ | < LOQ | 0.1 | < LOQ | < LOQ | 0.1 | < LOQ | 0.1 | *0.3* |
| **S5** | **pno** | **chips** | 5.6 | < LOQ | 0.6 | 4.5 | 0.2 | < LOQ | 5.9 | *11.2* | 0.3 | < LOQ | < LOQ | 0.3 | < LOQ | 0.2 | < LOQ | < LOQ | 0.4 | *1.2* |
| **S6** | **pno** | **chips** | 5.9 | 0.1 | 1.1 | 5.2 | 0.1 | 0.8 | 1.5 | *8.8* | 0.1 | 0.1 | 0.2 | 0.2 | 0.1 | 0.4 | < LOQ | 0.1 | 0.2 | *1.3* |
| **S7** | **pno** | **chips** | 6.1 | < LOQ | 0.3 | 1.6 | 0.1 | 1.0 | 3.9 | *6.9* | 0.2 | < LOQ | < LOQ | < LOQ | < LOQ | 0.1 | < LOQ | < LOQ | < LOQ | *0.4* |
| **S8** | **pno** | **chips** | 3.1 | < LOQ | 0.5 | 4.2 | 0.1 | 1.7 | 4.9 | *11.4* | 0.7 | 0.4 | < LOQ | 0.5 | < LOQ | 0.5 | < LOQ | < LOQ | 0.4 | *2.4* |
| **S9** | **pno** | **chips** | 9.2 | < LOQ | 0.1 | 1.6 | 0.1 | < LOQ | 1.8 | *3.6* | 0.2 | 0.1 | 0.4 | 0.8 | 0.3 | 0.3 | 0.6 | 0.3 | 0.2 | *3.1* |
| **S10** | **pno** | **chips** | 24.9 | < LOQ | 1.3 | 10.4 | 0.4 | 1.9 | nq | *14.0* | 0.4 | 1.2 | < LOQ | 0.1 | < LOQ | 0.3 | < LOQ | < LOQ | 0.1 | *2.0* |
| **S11** | **pno** | **chips** | 20.4 | < LOQ | 1.0 | 4.4 | 0.3 | < LOQ | 5.1 | *10.7* | 0.8 | 0.8 | < LOQ | 0.7 | 0.3 | 1.2 | < LOQ | 1.0 | 1.3 | *6.1* |
| **S12** | **pno** | **corn ext.** | 14.3 | 0.1 | 1.3 | 3.3 | 0.1 | 0.8 | 1.3 | *6.8* | 0.5 | 0.3 | 0.1 | 0.7 | 0.2 | 0.3 | < LOQ | 0.1 | 0.8 | *3.1* |
| **S13** | **pno** | **wheat ext.** | 24.5 | < LOQ | 2.4 | 9.5 | 0.9 | 1.6 | nq | *14.3* | 0.4 | 0.5 | < LOQ | 0.5 | 0.1 | 0.5 | < LOQ | 0.3 | 0.2 | *2.4* |
| **S14** | **pno** | **cereal mix ext.** | 4.7 | 0.3 | 0.4 | 1.0 | 0.1 | 0.5 | < LOQ | *2.0* | 0.2 | 0.1 | < LOQ | 0.2 | 0.1 | 0.1 | 0.4 | < LOQ | 0.2 | *1.3* |

[a] **so**: sunflower oil; **sbo**: soybean oil; **pno**: peanut oil.

[b] ext.: extruded; p : potatoes; f : fish

**Purcaro G., Navas J.A., Guardiola F., Conte L.S., Moret S., *Polyciclic aromatic hydrocarbons in frying oils and snacks*, J.Food Prot., 69(1), 2006**

# A case study – 2: Olive pomace oil and PAHs contamination

# Contamination sources

## Storage

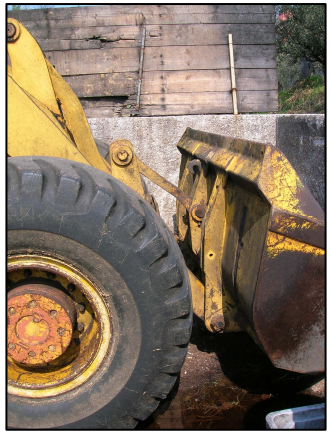
- Open yards → 
  - Atmospheric particulate
  - Vehicles smokes
  - Asphalt powder

Lubricating oils

Mineral oils

## MOVING:

- Old loaders → Hydraulic oil
- Moving systems → Lubricating oil



## Olive pomace

**Applied technologies:**

- Drying
- Extraction

# BaP and sum of heavy PAHs in pomace (µg/Kg)

**Sampled at crushing :**

**Sampled at extraction factory :**

| | | | | |
|---|---|---|---|---|
| **RA** | fresh pomace | *4,3* | 0,5 | 5,3 |
| | pomace 1-2 days old | *4,6* | 0,4 | 3,2 |
| | pomace 10-20 days old | *5,7* | 5,5 | 99,4 |
| **TS** | fresh pomace | *14,9* | 0,5 | 5,9 |
| | pomace 10-20 days old | *5,0* | 1,8 | 13,8 |
| **IM1** | pomace 2.-3 days old | *3,5* | 0,5 | 3,4 |
| | pomace 7-10 days old | *4,3* | 0,5 | 3,8 |
| **IM2** | fresh pomace | *3,9* | 1,5 | 7,0 |
| | pomace 15 days old | *2,5* | 24,4 | 206,5 |
| **BA** | pomace 7 days old | *6,9* | 0,8 | 4,2 |
| | pomace 7 days old | *5,4* | 0,5 | 2,4 |
| | pomace 15-20 days old | *3,6* | 67,6 | 437,3 |

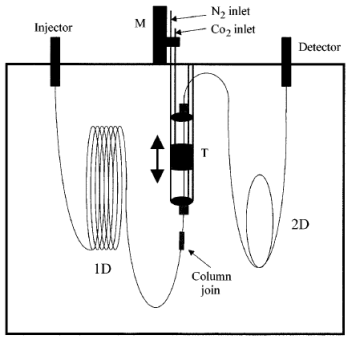
**Sampled at the different steps at extraction plant**

| | | | | |
|---|---|---|---|---|
| **BA** | not dried pomace | *4,5* | 0,8 | 2,1 |
| | dried pomace | *5,1* | 34,1 | 215,3 |
| | oil/hexane miscella | | 23,1 | 650,2 |
| | raw oil | | 349,8 | 2068,7 |
| | Virgin hexane (ng/mL) | | 0,2 | 2,3 |
| | Recycled hexane (ng/mL) | | 0,0 | 0,1 |
| **IM** | dried pomace | *5,6* | 58,2 | 908,7 |
| | oli/hexane miscella | | 1064,1 | 6432,5 |
| | raw oil | | 3081,2 | 13945,7 |

**Pomace presents a number of interferring substances that make the “normal” way of determination somewhat inadequate an improvement of separation was mandatory, so a multidimensional technique was applied, in cooperation with Royal Melbourne Institute of Technology**

GC × GC: Agilent 6890 con LMCS

Detection: TOF-MS Pegasus II




Lee retention index were used instead of Kovats retention index

for PAHs identification

$$RI = 100 * \frac{t_{r(x)} - t_{r(z)}}{t_{r(z+1)} - t_{r(z)}} + 100z$$

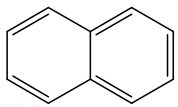
$t_{r(x)}$ : retention time analyte

$t_{r(z)}$ e $t_{r(z+1)}$: retention time of the standard

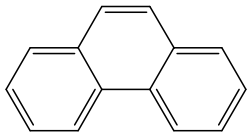
Compounds that elutes before and after the analyte

Reference standards for Lee retention index calculation:

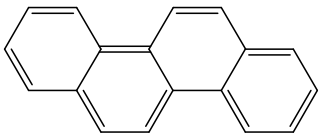
Na (200);



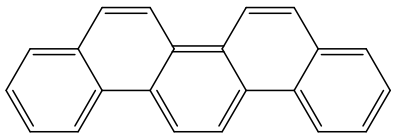
Pa (300);



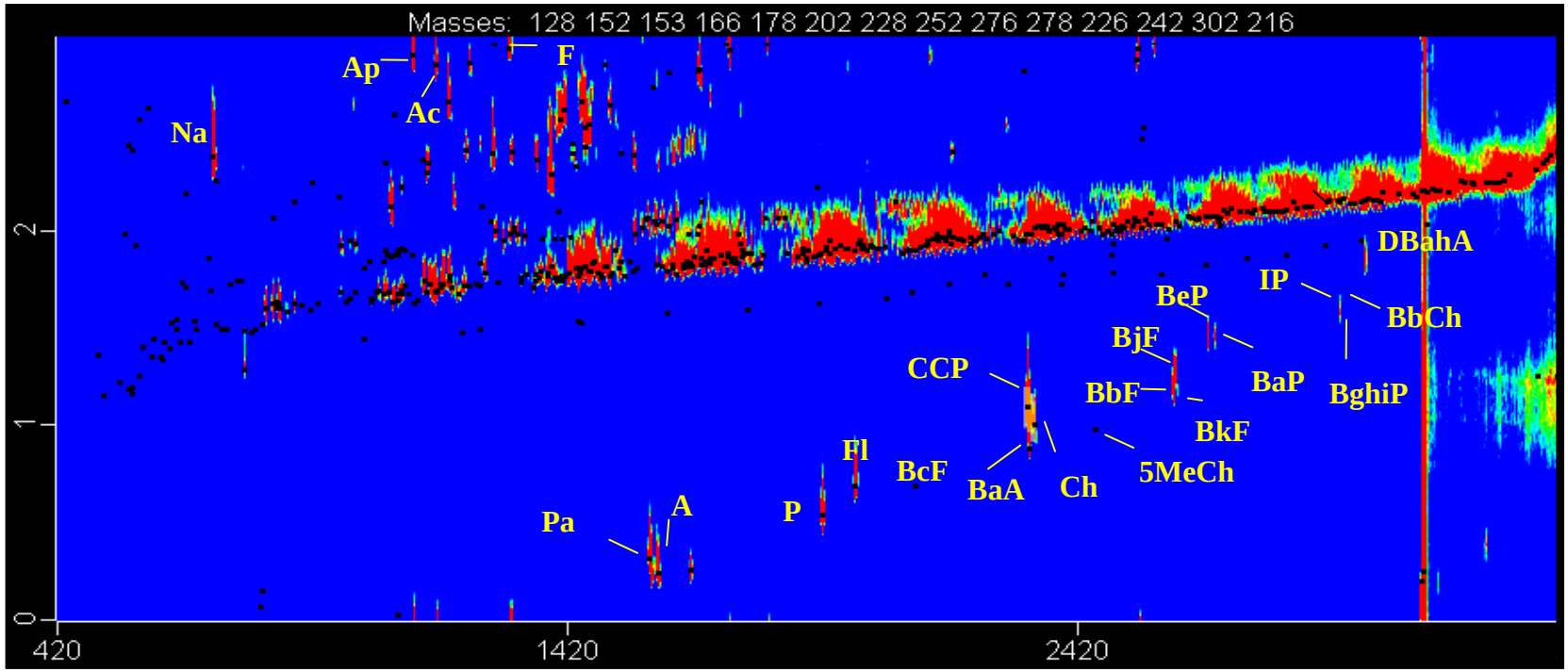
Ch (400);



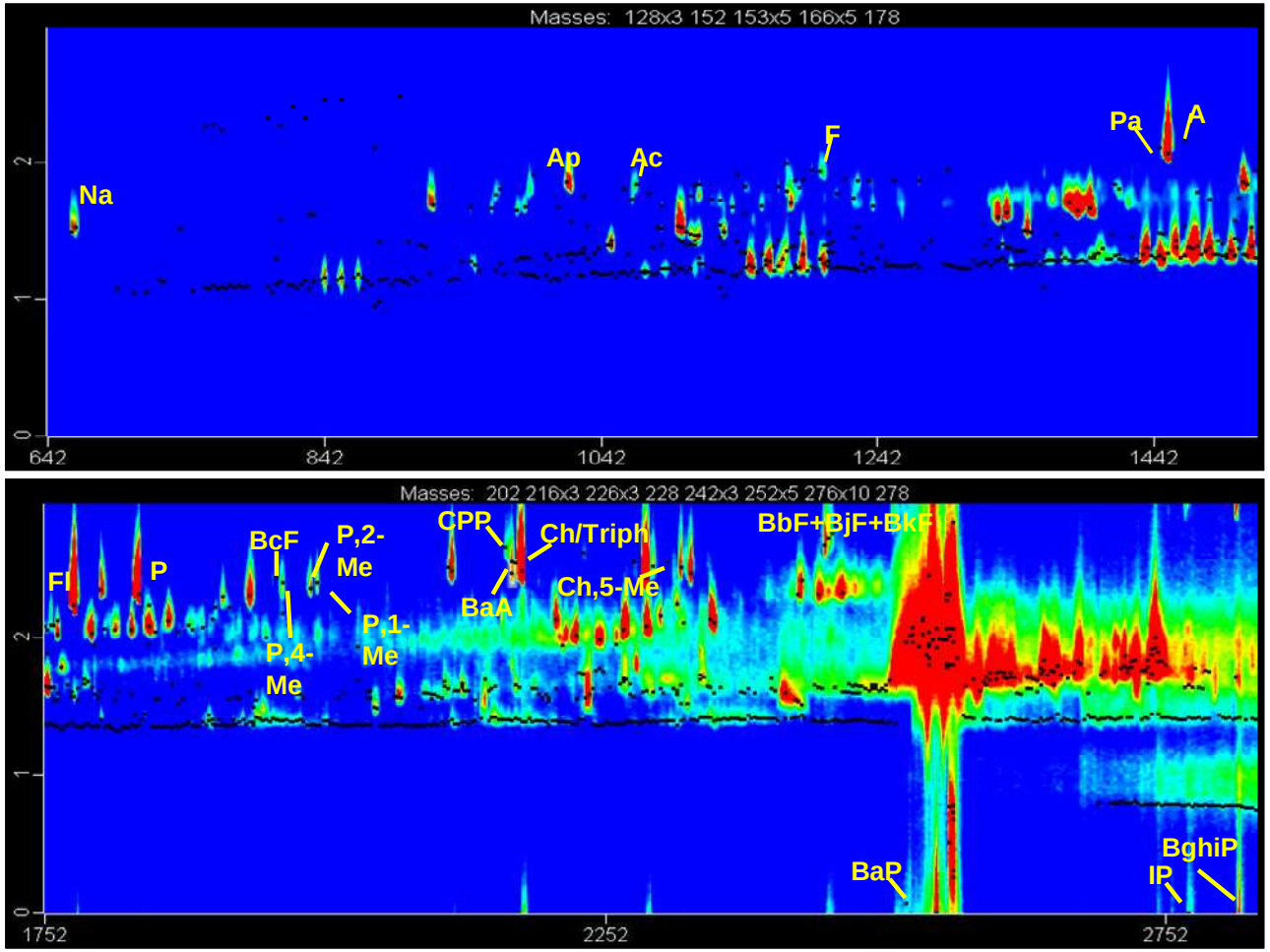
picene (500)



## GC x GC plot of a sample of olive oil spiked with a standard mix of PAHS



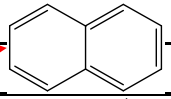
# GC x GC plot of a sample of olive pomace oil spiked with a standard mix of PAHS

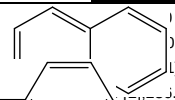


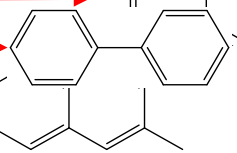

Pyrolysis inside the injector → Second dimension column saturated by polar compounds

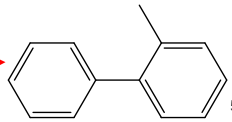
**More volatile compounds flad the second dimension column, so changing the polarity**

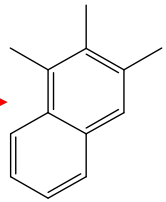
# Identification (tentative, too) of separed compounds-1

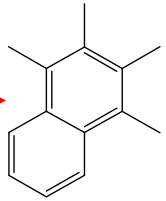












| Compounds | Lee RI | RI std | Lee RI from literature | presence of compounds in different oils | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | poo 1 | poo 2 | poo 3 | jdp | dp | sp | cpo 1 | cpo 2 |
| Naphthalene[a] | 200 | 200 | [1,2,3,4,5,6,7,8,9] | x | x | x | x | x | x | x | x |
| Naphthalene, 2-methyl-[a] | 218.32 | 217.82 | .73 [2]; 220.47 [3]; 216.32 [5] | x | | | | | | | |
| Naphthalene, 1-methyl-[a] | 220.99 | 220.83 | ]; 223.78 [2]; 223.42 [3] | x | | | | | | | |
| Biphenyl[a] | 232.70 | | .39 [3]; 236.44 [4]; 229.21 [5]; 231.10 [7] | x | | x | | x | x | x | x |
| Naphthalene, 2-ethyl- or isomer[a] | 236.12 | 2 | 236.08 [1]; 238.61 [2] | x | | x | | x | x | x | |
| Naphthalene, 2,7-dimethyl- or isomer[a] | 237.79 | 2 | 237.71 [1] | x | | x | | x | x | x | |
| Naphthalene, 1,3-dimethyl- or isomer[b] | 240.08 | | 240.25 [1] | x | | x | x | x | x | x | x |
| Naphthalene, 1,6-dimethyl- or isomer[b] | 240.84 | | 240.72 [1]; 243.57 [2] | x | | x | | | | x | |
| Naphthalene, 2,3-dimethyl- or isomer[b] | 243.89 | | 243.55 [1]; 246.42 [2] | | | | | x | x | | |
| Acenaphthylene[a] | 244.66 | 244.86 | 244.63 [1]; 240.52 [5]; 245.40 [7] | x | | x | | x | | | |
| Naphthalene, 1,2-dimethyl- or isomer[b] | 246.39 | | 246.49 [1]; 249.02 [2] | | | x | | x | | x | |
| Acenaphthene[a] | 251.33 | 251.01 | 251.29 [1]; 245.85 [5] | x | | x | | x | | | |
| 1,1'-Biphenyl, 2-ethyl- or isomer[b] | 251.89 | | 250.85 [1] | | | x | | | | | |
| 1,1'-Biphenyl, 4-methyl-[a] | 252.29 | 251.73 | | x | | x | | x | | x | |
| Naphthalene, trimethyl-[b] | 253.61 | | 252.95 [2] | | | | | x | | x | x |
| 1,1'-Biphenyl, methyl-[b] | 254.55 | | 54.71 [1]; 254.66 [2]; 254.70 [9] | | | x | | x | | | |
| Naphthalene, trimethyl-[b] | 256.11 | | 256.40 [9] | | | | | x | x | x | x |
| 1,1'-Biphenyl, methyl-[b] | 257.03 | | 256.80 [8] | | | | | x | | | x |
| Dibenzofuran[a] | 257.03 | | 257.17 [1]; 259.07 [3]; 259.74 [5] | | | | | x | | x | x |
| Naphthalene, 2-(1-methylethyl)-[b] | 258.17 | | 257.80 [9] | | | | | | x | x | |
| Naphthalene, trimethyl-[b] | 258.94 | | 257.48 [2]; 257.93 [5]; 257.80 [8]; 258.60 [9] | | | x | x | x | x | x | |
| Naphthalene, trimethyl-[b] | 260.08 | | 262.40 [8]; 261.30 [9] | | | x | | x | x | x | x |
| 1,1'-Biphenyl, methyl-[b] | 261.22 | | 262.70 [2] | | | | | x | | x | x |
| Naphthalene, trimethyl-[b] | 262.60 | | 263.31 [1]; 262.90 [8]; 262.30 [9] | | | | x | x | x | x | |
| Naphthalene, trimethyl-[b] | 263.50 | | 264.40 [8] ;264.70 [9] | | | | | x | x | | |
| Naphthalene, trimethyl-[b] | 265.02 | | 264.60 [8] ; 265.10 [9] | | | | | | x | x | |
| Naphthalene, trimethyl-[b] | 265.40 | | 265.90 [1] | | | | x | x | | | x |
| 1H-Phenalene[b] | 267.30 | | 266.90 [2] | | | | | x | | x | x |
| Fluorene[a] | 267.94 | 268.13 | 268.17 [1]; 270.01 [2]; 269.94 [3]: 269.73 [4]; 262.04 [5]; 267.70 [6]; 268.20 [7]; 270.10 [8] | x | x | x | x | x | x | x | x |
| ethyl biphenyl[b] | 269.08 | | 268.47 [5]; 270.50 [7]; 267.00 [9] | | | | | x | x | | |
| Naphthalene, trimethyl-[b] | 269.47 | | 268.50 [8]; 269.30 [9] | | | x | | x | x | x | x |
| 3,3'-Dimethylbiphenyl[b] | 271.21 | | 271.87 [1]; 271.00 [9] | x | | x | | x | x | x | x |
| Naphthalene, tetramethyl[b] | 273.00 | | 273.50 [9] | x | | | | x | | | |
| 4,4' dimethyl biphenyl[b] | 274.90 | | [1]; 277.49 [2]; 274.50 [7]; 276.30 [8]; 274.50 [9] | x | | | | | | x | x |
| Tetramethylnaphthalene[b] | 275.29 | | 276.80 [8]; 276.30 [9] | | | | | x | | | |
| Dibenzofuran, 4-methyl-[b] | 277.19 | | 274.89 [5]; 276.40 [9] | | | | | x | | x | x |
| Naphthalene, tetramethyl[b] | 279.09 | | 281.40 [8]; 279.30 [9] | | | | | x | | | |
| Xanthene[b] | 279.09 | | 280.48 [1]; 279.10 [7] | | | | | x | | | |

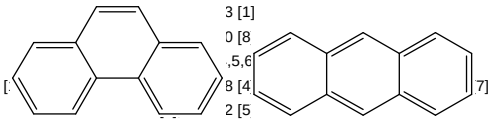
[a] identify on the basis of the mass spectrum and the retention index of the standard and reported in the literature; [b] tentatively identify on the base of the mass spectrum and the retention index reported in the literature

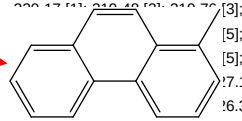
***[1]*** Lee et al., 1979; ***[2]*** Pio et al., 1999; ***[3]*** Wise et al., 1988; ***[4]*** Vassilaros et al., 1982; ***[5]*** Re-Poppi et al., 2002; ***[6]*** Zamperlini et al., 1997; ***[7]*** Durlak et al., 1998; ***[8]*** Paschke et al., 1992; ***[9]*** Bundt et al., 1991.

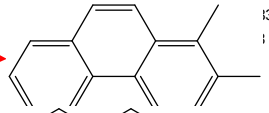
***poo*** : pomace olive oil from the market; ***dp*** : dried pomace extracted in laboratory; ***sp*** : stored pomace, extracted in laboratory; ***jdp*** : just delivered pomace, extracted in laboratory; ***cpo*** : crude pomace oil

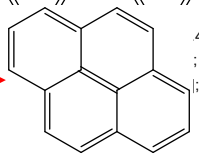
# Identification (tentative, too) of separed compounds-2

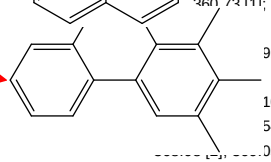
| Compounds | Lee RI | RI std | Lee RI from literature | presence of compounds in different oils | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | poo 1 | poo 2 | poo 3 | jdp | dp | sp | cpo 1 | cpo 2 |
| Naphthalene, tetramethyl[b] | 283.65 | | 283.40 [8]; 281.40 [9] | | | | | x | | | |
| Anthracene, 9,10-dihydro-[a] | 285.88 | 282.63 | 284.89 [1]; 285.60 [7] | x | | | | | | | |
| Naphthalene, tetramethyl[b] | 286.31 | | 287.30 [8] | | | | | x | | | |
| Phenanthrene, 9,10-dihydro-[a] | 286.31 | 285.27 | 287.09 [1]; 286.78 [2]; 287.86 [5]; 286.80 [7] | | | | | x | | | |
| Naphthalene, tetramethyl[b] | 287.07 | | 288.40 [8] | | | | | x | | | |
| 9H-Fluorene, 2-methyl-[b] | 287.83 | | 288.21 [1]; 288.78 [2]; 288.29 [3]; 288.42 [4] | | | | | x | | | |
| 9H-Fluorene, 1-methyl-[a] | 288.59 | 287.53 | 289.03 [1]; 290.45 [2]; 289.14 [3]; 289.20 [4] | | | | | x | | | x |
| 1,2,3,4,5,6,7,8-octahydrophenanthrene[b] | 291.57 | | 3 [1] | | | | x | | x | | |
| Naphthalene, tetramethyl[b] | 293.92 | | 0 [8 | | | | | x | | | |
| Phenanthrene[a] | 300 | 300 | ,5,6 | x | x | x | x | x | x | x | x |
| Anthracene[a] | 301.65 | 301.71 | 301.69 [ 8 [4 7] | x | x | x | x | x | x | x | x |
| Naphthalene, 1-phenyl-[b] | 312.40 | | 2 [5 | x | | | x | x | x | x | x |
| o-Terphenyl[a] | 317.36 | 319.43 | 321.70 [7] | x | x | x | x | x | x | x | |
| Phenanthrene, 3-methyl- or isomer[b] | 319.42 | | 319.46 [1]; 318.61 [2]; 318.93 [3]; 318.11 [4]; 318.22 [5] | x | | | | x | | x | x |
| Phenanthrene, 2-methyl- or isomer[a] | 320.25 | 319.43 | [3]; 319.53 [4]; 319.13 [5] | x | | | | x | | x | x |
| Phenanthrene, 4-methyl- or isomer[b] | 323.14 | | [5]; 321.82 [6] | x | | | | | | | |
| Anthracene, 1-methyl- or isomer[a] | 323.97 | 323.36 | [5]; 322.61 [6] | x | | | | | | x | x |
| Pyrene, 4,5-dihydro-[b] | 328.10 | | 7.10 [7] | x | | | | x | | | |
| 2-Phenylnaphthalene[b] | 330.58 | | 6.39 [5]; 333.90 [7] | x | x | x | x | x | x | | x |
| Phenanthrene, 9-ethyl-[b] | 334.30 | | 337.05 [1]; 336.76 [2]; 335.08 [5] | | | | | x | | | |
| Triphenylmethane[a] | 335.68 | 334.76 | | | | x | | | | | |
| Phenanthrene, 2,6-dimethyl- or isomer[b] | 337.19 | | 36.69 [5]; 336.89 [6] | x | | | | x | | x | x |
| Phenanthrene,2,7-dimethyl- or isomer[b] | 338.43 | | [1]; 337.68 [6]; 339.10 [7] | x | x | | | x | | x | x |
| Phenanthrene, 1,7-dimethyl- or isomer[b] | 341.32 | | 341.41 [6] | x | x | | | x | x | x | |
| Phenanthrene, 2,3-dimethyl- or isomer[b] | 342.15 | | 342.46 [6] | x | x | | | x | | x | x |
| Phenanthrene, 9,10-dimethyl- or isomer[b] | 344.63 | | 342.99 [6] | x | | | | | | | |
| Fluoranthene[a] | 345.04 | 345.08 | 49 [3]; 344.51 [4]; 340.35 [5]; 343.25 [6]; 344.80 [7] | x | x | x | | x | | x | x |
| Acepenanthrylene[b] | 348.35 | | ; 347.82 [3]; 347.67 [4]; 346.74 [5]; 347.12 [6] | x | x | x | | x | | x | x |
| Pyrene[a] | 352.89 | 352.92 | ; 351.51 [4]; 348.43 [5]; 350.52 [6]; 346.60 [7]; 353.20 [8] | x | x | x | x | x | x | x | x |
| Phenanthrene, trimethyl-[b] | 355.19 | | 356.30 [8] | | x | x | | x | | x | x |
| m-Terphenyl[a] | 357.02 | 355.89 | 360.73 [1]; 358.30 [2]; 352.60 [7] | x | x | x | x | x | x | x | x |
| Phenanthrene, trimethyl-[b] | 357.85 | | 356.30 [8] | x | | | | x | x | x | x |
| Phenanthrene, trimethyl-[b] | 359.09 | | 91 [1]; 360.10 [8] | x | | | | x | x | x | x |
| Phenanthrene, trimethyl-[b] | 361.57 | | 361.5 [8] | x | | x | | x | | x | x |
| p-Terphenyl[b] | 362.40 | | 10 [1]; 363.98 [2] | | | | x | | x | | |
| 11H-Benzo[a]fluorene[b] | 363.22 | | 54 [3]; 366.72 [4]; 366.37 [5]; 359.80 [7] | x | x | x | x | x | x | x | x |
| 11H-Benzo[b]fluorene[b] | 366.53 | | 05 [3]; 369.40 [4]; 369.33 [5] | x | x | x | x | x | x | x | x |
| Phenanthrene, tetramethyl-[b] | 368.18 | | 368.40 [8] | x | | x | x | x | x | x | x |











[a] identify on the basis of the mass spectrum and the retention index of the standard and reported in the literature; [b] tentatively identify on the base of the mass spectrum and the retention index reported in the literature

***[1]*** Lee et al., 1979; ***[2]*** Pio et al., 1999; ***[3]*** Wise et al., 1988; ***[4]*** Vassilaros et al., 1982; ***[5]*** Re-Poppi et al., 2002; ***[6]*** Zamperlini et al., 1997; ***[7]*** Durlak et al., 1998; ***[8]*** Paschke et al., 1992; ***[9]*** Bundt et al., 1991.

***poo*** : pomace olive oil from the market; ***dp*** : dried pomace extracted in laboratory; ***sp*** : stored pomace, extracted in laboratory; ***jdp*** : just delivered pomace, extracted in laboratory; ***cpo*** : crude pomace oil

# Identification (tentative, too) of separed compounds-3

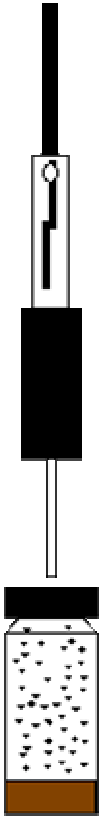
| Compounds | Lee RI | RI std | Lee RI from literature | presence of compounds in different oils | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | poo 1 | poo 2 | poo 3 | jdp | dp | sp | cpo 1 | cpo 2 |
| Benzo[c]fluorene[a] | 369.42 | 369.66 | | x | x | x | x | x | x | x | x |
| Phenanthrene, trimethyl-[b] | 370.66 | | 69.90 [8] | | x | x | | x | | x | x |
| Pyrene, 4-methyl- or isomer[b] | 370.66 | | 69.26 [2]; 370.10 [5] | x | x | x | x | x | x | x | x |
| Pyrene, 1-methyl- or isomer[b] | 373.97 | | 73.05 [2]; 371.86 [5] | x | x | x | x | x | x | x | x |
| Pyrene, methyl[b] | 374.79 | | 74.03 [2] | x | x | x | x | x | x | x | x |
| Benzo[ghi]fluoranthene[b] | 390.91 | | [3]; 389.92 [4]; 389.67 [5]; 390.96 [6] | | x | | | x | | x | |
| Benzo[c]phenanthrene[b] | 391.32 | | 391.39 [1]; 391.12 [2]; 391.07 [3]; 391.24 [4] | x | x | x | x | x | x | x | x |
| Cyclopenta[cd]pyrene[a] | 397.93 | 398.24 | 96.55 [4]; 397.63 [5]; 396.68 [6] | x | x | | | x | | | |
| Benz[a]anthracene[a] | 398.76 | 398.86 | [3]; 398.76 [4]; 398.36 [5] | x | x | x | x | x | x | x | x |
| Chrysene + thryphenylene[a] | 400 | 400 | 5,6,7,8,9] | x | x | x | x | x | x | x | x |
| Benz[a]anthracene, methyl or isomer[b] | 408.49 | | [5] | | | x | | x | | x | x |
| 1-methyl-triphenylenen or isomer[b] | 416.11 | | [2]; 415.14 [5] | x | x | x | | x | x | x | x |
| Chrysene, methyl- or isomer[b] | 417.21 | | 416.63 [1] | x | x | | | | | | |
| Chrysene, methyl-or isomer[b] | 417.45 | | 417.56 [1]; 418.21 [2] | | | | x | x | | x | x |
| Benz[a]anthracene, 5-methyl-or isomer[b] | 418.31 | | 2 [1] | x | | x | | | x | x | x |
| 5-Methylchrysene[a] | 419.25 | 419.81 | 8 [1] | x | x | x | x | x | x | x | x |
| Chrysene, 6-methyl or isomer[b] | 419.34 | | 418.21 [2] | | | x | | | | x | |
| Chrysene, 4-methyl or isomer[b] | 421.60 | | 420.91 [5] | x | | | | | | x | |
| Chrysene, 1-methyl- or isomer[b] | 422.07 | | 422.87 [1];422.97 [5] | x | x | x | | x | x | x | x |
| 2,2'-Binaphthalene[a] | 423.47 | 423.25 | 423.91 [1]; 426.90 [2]; 424.00 [5] | x | x | x | x | x | x | x | x |
| Benz[a]anthracene, 1,12-dimethyl- or isomer[b] | 439.07 | | 436.82 [1] | | | x | | x | | x | |
| Benzo[b]fluoranthene[a] | 442.65 | 442.75 | 441.74 [1]; 441.76 [2]; 443.11 [3]; 443.13 [4]; 440.37 [5]; 443.58 [6] | x | x | x | x | x | x | x | x |
| Benzo[j]fluoranthene[a] | 442.79 | 442.87 | 443.58 [6]; 432.90 [7] | x | x | x | x | x | x | x | x |
| Benzo[k]fluoranthene[a] | 443.13 | 443.22 | 442.56 [1]; 442.28 [2]; 444.06 [3]; 444.02 [4]; 441.09 [5]; 444.60 [6] | x | x | x | x | x | x | x | x |
| Benzo[e]pyrene[a] | 452.13 | 452.20 | 450.73 [1]; 451.28 [2];452.70 [3];452.29 [4]; 450.12 [5]; 452.86 [6] | | | x | | x | | x | x |
| Benzo[a]pyrene[a] | 454.50 | 454.52 | 453.44 [1]; 452.99 [2]; 454.57 [3]; 454.02 [4]; 451.92 [5]; 454.75 [6] | | | x | | x | | x | x |
| Indeno[1,2,3-cd]pyrene[a] | 492.89 | 492.47 | 481.87 [1]; 491.35 [2]; 493.88 [3]; 493.24 [4]; 490.80 [5] | | | | | x | | x | |
| Benzo[ghi]perylene[a] | 500.29 | 500.21 | 501.32 [1]; 500.00 [2]; 501.38 [3]; 501.32 [4]; 500.20 [5]; 501.88 [6] | | | x | | x | | x | x |

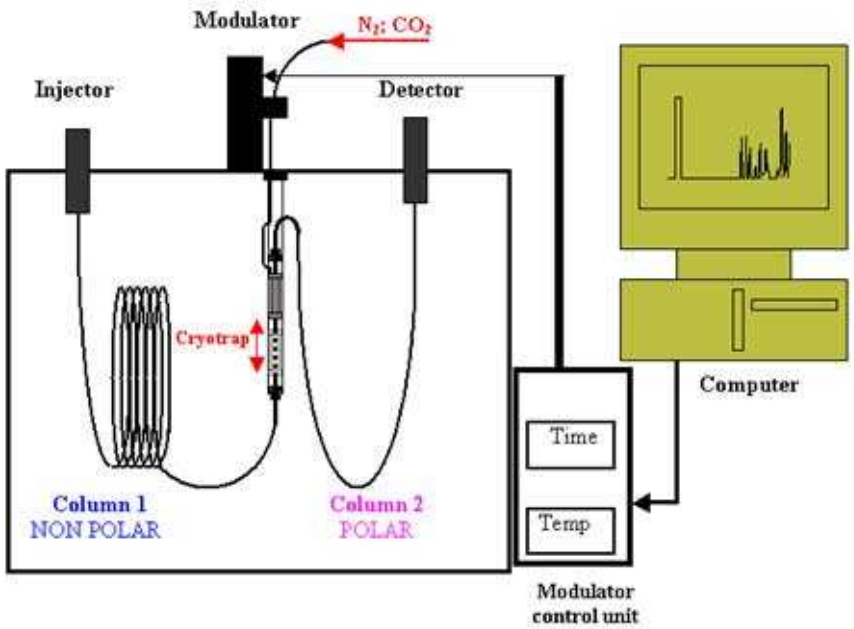
[a] identify on the basis of the mass spectrum and the retention index of the standard and reported in the literature; [b] tentatively identify on the base of the mass spectrum and the retention index reported in the literature

***[1]*** Lee et al., 1979; ***[2]*** Pio et al., 1999; ***[3]*** Wise et al., 1988; ***[4]*** Vassilaros et al., 1982; ***[5]*** Re-Poppi et al., 2002; ***[6]*** Zamperlini et al., 1997; ***[7]*** Durlak et al., 1998; ***[8]*** Paschke et al., 1992; ***[9]*** Bundt et al., 1991.

***poo*** : pomace olive oil from the market; ***dp*** : dried pomace extracted in laboratory; ***sp*** : stored pomace, extracted in laboratory; ***jdp*** : just delivered pomace, extracted in laboratory; ***cpo*** : crude pomace oil

# AVOID SOLVENT EXTRACTION: SPME - GC × GC - TOFMS






**200 µL oil in 1.5 mL n- hexane.**
**Fibre dipped 30 min at room temp**
**Fiober washing 1 min**

G. Purcaro, P. Morrison, S. Moret, L.S. Conte, P.J.Marriott, *Determination of polycyclic aromatic hydrocarbons in vegetable oils using solid phase microextraction comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry*, **J. Chromatogr. A**, 116 (2007), 284-291

# METHOD VALIDATION

## External calibration

### Calibration curves

Linearity Range :1.5 ug/kg-30 ug/kg

### LOD&LOQ

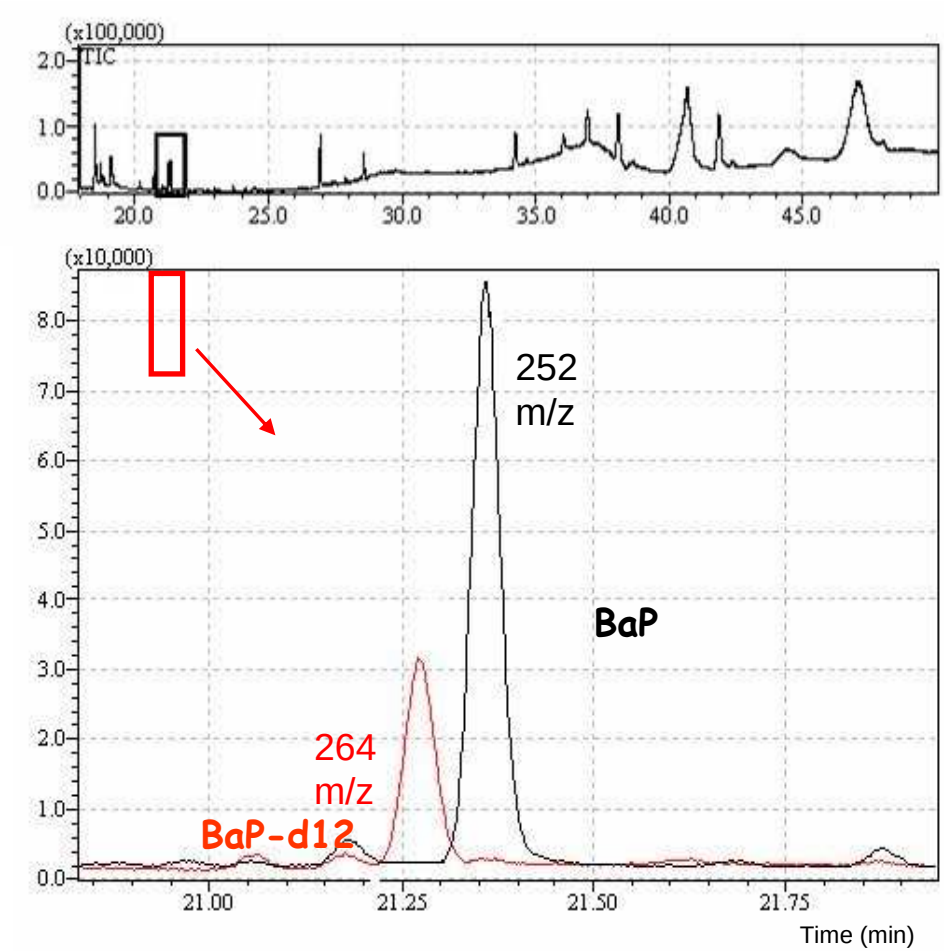
**As 3 and 10 times S/N**

| | range tested (µg/Kg) | | slope | intercept | r2 | LOD (µg/Kg) | LOQ (µg/Kg) |
|---|---|---|---|---|---|---|---|
| | min | max | | | | | |
| **BcF** | 1.00 | 24.64 | 1675.8 | 2406.8 | 0.957 | 0.76 | 2.54 |
| **BaA** | 1.50 | 36.83 | 5229.2 | -2.6 | 0.985 | 0.42 | 1.39 |
| **CPP** | 1.00 | 24.64 | 3047.1 | 332.9 | 0.988 | 1.38 | 4.62 |
| **Ch** | 1.49 | 36.71 | 4958.5 | 4200.3 | 0.992 | 0.40 | 1.33 |
| **5MeCh** | 1.00 | 24.64 | 1974.2 | 106.4 | 0.994 | 1.12 | 3.75 |
| **BkF+BjF+BbP** | 3.99 | 98.31 | 10166.8 | 18762.8 | 0.991 | 0.99 | 3.30 |
| **BaP** | 1.48 | 36.47 | 11071.7 | 6249.2 | 0.998 | 0.21 | 0.70 |
| **IP** | 1.49 | 36.71 | 23327.5 | 11477.7 | 0.980 | 0.12 | 0.40 |
| **DBahA** | 1.50 | 36.83 | 16767.2 | 4966.2 | 0.985 | 0.16 | 0.52 |
| **BghiP** | 1.49 | 36.71 | 18177.8 | 11005.0 | 0.991 | 0.13 | 0.44 |
| **DBalP** | 1.00 | 24.64 | 3705.5 | 1957.0 | 0.987 | 0.59 | 1.97 |
| **DBaeP** | 1.00 | 24.64 | 18723.2 | 5687.7 | 0.992 | 0.59 | 1.97 |
| **DBaiP** | 1.00 | 24.64 | 18704.2 | 2769.9 | 0.993 | 0.20 | 0.65 |
| **DBahP** | 1.00 | 24.64 | 16794.8 | 2910.6 | 0.988 | 0.23 | 0.78 |

Performance criteria
EU Dir. 2005/10

**0.3 µg/kg** **0.9 µg/kg**

# A simplify SPME method for “clean” samples: SPME - GC - MS



# Method validation

G. Purcaro, S. Moret, L.S. Conte, R*apid validated method for the analysis of benzo[a]pyrene in vegetable oils by using solid-phase microextraction-gas chromatography-mass spectrometry*., **J. Chromatogr. A**, 1176 (2007) 231-235.

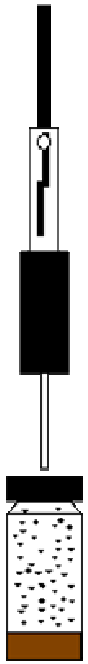


Table 1. Precision

| level tested (ng) | Intra-day repeatability (RSD %) (n=6) | inter-day repeatability (RSD%) (n=12) |
|---|---|---|
| 0.40 | 5.30% | 5.76% |
| 3.00 | 1.60% | 1.93% |

Table 2. LOD and LOQ.

| LOD | LOQ |
|---|---|
| 0.03 ng | 0.09 ng |
| **0.17 µg/kg *** | **0.46 µg/kg *** |
| **0.3 µg/kg** | **0.9 µg/kg** |

LOD: $y_d = \mu_b + 2t*\sigma_b$

LOQ: $y_q = \mu_b + 10*\sigma_b$

Criteri di Performance
EU Dir. 2005/10

Table 3. Trueness (as RSD)%.

| | ng added | ng observed | difference observed | Accuracy (relative error %) |
|---|---|---|---|---|
| **sample** | 0.00 | 0.24 ± 0.02 | - | - |
| **sample+0.4 ng** | 0.40 | 0.67 ± 0.01 | 0.42 | 6.19 |
| **sample+2.0ng** | 2.00 | 2.21 ± 0.01 | 1.96 | -1.81 |

# A case study – 3: Grapeseed oil and PAHs contamination

| Sample | storage (week) | BbF | | BkF | | BaP | | DBahA | | BghiP | | IP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Before Drying | After Drying | Before Drying | After Drying | Before Drying | After Drying | Before Drying | After Drying | Before Drying | After Drying | Before Drying | After Drying |
| 1 | 3 | 5,9 | 34,1 | 2,0 | 10,7 | 1,2 | 16,4 | < LOQ | < LOQ | 3,8 | 5,3 | 1,3 | 7,6 |
| 2 | 3 | 4,9 | 47,8 | 1,5 | 17,7 | 1,2 | 13,0 | 1,5 | < LOQ | 0,8 | 4,4 | 0,6 | 4,8 |
| 3 | 4 | 5,1 | 21,6 | 1,7 | 6,1 | 1,4 | 10,8 | 0,4 | < LOQ | 1,3 | 2,8 | 1,0 | 6,4 |
| 4 | 4 | 3,9 | 30,9 | 1,7 | 8,7 | 1,0 | 14,0 | 0,1 | < LOQ | 2,1 | 2,7 | 1,6 | 2,4 |
| 5 | 5 | 4,4 | 33,2 | 1,8 | 8,4 | 1,2 | 15,4 | < LOQ | < LOQ | 2,9 | 2,8 | 2,5 | < LOQ |
| 6 | 6 | 5,4 | 37,7 | 2,0 | 11,1 | 1,6 | 18,9 | < LOQ | < LOQ | 4,1 | 4,7 | 1,3 | 11,9 |
| 7 | 6 | 4,8 | 30,3 | 1,8 | 8,6 | 1,7 | 15,6 | < LOQ | < LOQ | 3,5 | 3,7 | 2,2 | 4,9 |
| 8 | 6 | 6,0 | 33,6 | 2,0 | 8,9 | 1,5 | 27,5 | 0,2 | < LOQ | 2,1 | < LOQ | 1,2 | < LOQ |
| 9 | 7 | 6,5 | 28,7 | 2,3 | 7,3 | 1,8 | 24,6 | < LOQ | < LOQ | 2,5 | < LOQ | 1,4 | < LOQ |
| 10 | 7 | 5,4 | 19,4 | 1,7 | 6,4 | 1,1 | 8,6 | < LOQ | < LOQ | 2,6 | 4,0 | 1,0 | 6,1 |
| 11 | 8 | 7,2 | 39,7 | 2,3 | 10,7 | 1,8 | 20,8 | 0,1 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| 12 | 8 | 4,2 | 18,8 | 1,5 | 4,4 | 1,2 | 11,8 | < LOQ | < LOQ | 2,7 | < LOQ | 1,1 | < LOQ |
| 13 | 9 | 4,2 | 35,6 | 1,6 | 9,2 | 1,2 | 21,5 | < LOQ | < LOQ | 2,7 | 2,8 | 1,1 | < LOQ |
| 14 | 9 | 6,5 | 18,5 | 2,3 | 5,2 | 1,8 | 11,4 | < LOQ | < LOQ | 1,8 | < LOQ | 1,3 | 4,2 |
| 15 | 11 | 4,2 | 24,5 | 1,6 | 7,0 | 1,1 | 23,0 | < LOQ | < LOQ | 2,4 | 4,6 | 1,0 | 6,0 |
| 16 | 12 | 6,3 | 29,0 | 2,3 | 13,9 | 1,5 | 32,9 | < LOQ | < LOQ | 1,4 | < LOQ | 2,4 | < LOQ |
| 17 | 12 | 6,1 | 24,6 | 1,9 | 7,2 | 2,2 | 22,2 | < LOQ | < LOQ | 2,5 | 1,7 | 1,7 | 9,8 |
| 18 | 13 | 2,6 | 52,5 | 0,9 | 14,4 | 0,9 | 40,8 | 1,0 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| 19 | 14 | 9,3 | 54,0 | 2,7 | 16,7 | 2,4 | 44,3 | 0,1 | < LOQ | 3,2 | < LOQ | 2,1 | < LOQ |
| 20 | 14 | 5,0 | 53,4 | 1,7 | 3,4 | 1,8 | 9,6 | 3,1 | < LOQ | 1,7 | < LOQ | 1,0 | 3,0 |

**S. Moret, A. Dudine, L.S.Conte Processing effects on the polyaromatic hydrocarbon content of grape seed oil JAOCS, 2000, 77, 12, 1289-1292**

# *Thanks for your kind attention*

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