Contaminants in oils and fats: analysis and regulations

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Presentation of ITERG

- Guillaume CHANTRE
- ISO 9001
- ISO 17025
- 6 M€ (turnover)
- 80 Staff
- Technical Industrial Center
- French Ministry of Industry

AOCS-CCOA Joint Symposium on Functional Lipids, Shanghai, Nov 2014
Contents

Polycyclic aromatic hydrocarbons
Mineral oil
Phthalates
3-MCPD esters & glycidol esters

Origins ?
Regulation ?
Recognized analytical methods ?
Risks ?
Polycyclic aromatic hydrocarbons

- Environmental contamination
- Production process
- Regulation

➔ (EC) n°1881/2006 – contaminants in foodstuffs
PAHs origins in vegetable oils

**Oilseeds**

- Extraction
- Crude oil + HAP
- Deodorisation
- Active carbon
- Light PAHs
- Heavy PAHs
- Refined oil (+ HAP)

* Grapeseed oil
* Coconut oil
* Pomace olive oil
* Sunflower oil

**Steps:**
1. Extraction of Crude oil from Oilseeds
2. Deodorisation of Crude oil
3. Active carbon treatment
4. Refined oil production

**Oils:**
- Grapeseed oil
- Coconut oil
- Pomace olive oil
- Sunflower oil
Structure of PAHs

**HEAVY PAHs**
- dibenz(ah)anthracene
- benzo(ghi)perylene

**LIGHT PAHs**
- fluoranthene
- pyrene
- benz(a)anthracene
- chrysene

**GENOTOXIC**

GT : genotoxic

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PAHs regulation: (EC) n°1881/2006 contaminants in foodstuffs

<table>
<thead>
<tr>
<th>PAHs</th>
<th>Maximum levels oils and fats (µg/kg)</th>
<th>Maximum levels coconut oil (µg/kg)</th>
<th>Maximum levels cocoa butter (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sum of 4 PAHs:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- benzo[a]pyrene</td>
<td></td>
<td></td>
<td>35,0</td>
</tr>
<tr>
<td>- benz[a]anthracene</td>
<td></td>
<td></td>
<td>from 1.4.2013</td>
</tr>
<tr>
<td>- benzo[b]fluoranthene</td>
<td></td>
<td></td>
<td>until 31.3.2015</td>
</tr>
<tr>
<td>- chrysene</td>
<td></td>
<td></td>
<td>30,0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>from 1.4.2015</td>
</tr>
<tr>
<td><strong>benzo[a]pyrene</strong></td>
<td>2,0</td>
<td>2,0</td>
<td>5,0</td>
</tr>
<tr>
<td></td>
<td>from 1.4.2013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PAHs: determination methods

Principle

✓ **PAH isolation**: liquid chromatography (alumina, silica gel, C18-silica gel) or HPLC (donor-acceptor complex chromatography, size-exclusion chromatography)

✓ **Analysis**: HPLC/fluorescence or GC/MS

<table>
<thead>
<tr>
<th>Method</th>
<th>ISO 15302</th>
<th>ISO 15753</th>
<th>ISO 22959</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaP LOQ (µg/kg)</td>
<td>0,1</td>
<td>0,2</td>
<td>0,1</td>
</tr>
<tr>
<td>Reproducibility (CVR%)</td>
<td>27 % (2,1µg/kg)</td>
<td>41% (3,21µg/kg)</td>
<td>10 % (2,6µg/kg)</td>
</tr>
</tbody>
</table>

ISO 15302 : benzo[a]pyrene in oils (LC + HPLC/FLD)
ISO 15753 : 12 PAHs in oils (2 SPE + HPLC/FLD)
ISO 22959 : 17 PAHs in oils (DACC on-line + HPLC/FLD)
EN 16619 : 4 PAHs in foodstuffs (SEC + SPE + GC/MS)
BaP : occurrence in vegetable oils

- Virgin olive oil
- Olive oil
- Pomace olive oil
- Grapeseed oil
- Sunflower oil

**EU limit** 2 µg/kg

*EU Scoop Task (Oct 2004)*
Edible oils: PAH4 Levels

Screening 2012: 21 vegetable oils

EU limit PAH4: 10 µg/kg

ITERG data (2012)

benzo(a)pyrene
chrysene
benz(a)anthracene
benzo(b)fluoranthene
Mineral oil

- Environmental contamination (air, soil)
- Crop protection
- Transport & storage
- Production process
- Regulation

→ (EC) n°1151/2009 - import of sunflower oil from Ukraine
→ EFSA Scientific Opinion on Mineral Oil Hydrocarbons in food (2012)
Mineral oil composition

**EFSA Scientific Opinion, 2012**

Mineral oil: complex mixture of hydrocarbons

→ MOSH: straight or branched alkanes & alkylated cycloalkanes

→ MOAH: aromatic hydrocarbons including alkyl-substituted

Hydrocarbon compound number in mineral oil > 100 000 for those with less than 20 carbon atoms!

Different products & composition: diesel fuel, white oil, lubricant ...

Technical grade mineral oil contain 15-35 % MOAH, which is minimised in food grade MOSH (white oils)
Mineral oil detected in vegetable oils

- 2008 → contamination of sunflower oil from Ukraine with a mineral oil from unknown origin
- 2009 → contamination of walnut oil with a food grade lubricant oil during refining process
- 2010 → identification of compounds eluted as mineral oil in grapeseed oils
- 2011 → contamination of milk fat with a food grade lubricant oil during production
MOSH determination: pr ISO17780

- Fractionation of the sample by liquid chromatography on silica gel impregnated with AgNO₃
- Quantification with an internal standard (C18 or C20)
- GC/FID analysis on an short apolar column

Elution with hexane
Solvent evaporation
GC analysis

1 g oil

18,5 g Silica gel

On-column injection (2 µl)
DB5 HT (15 m-0,25 mm-0,1 µm)
pr ISO 17780 validation

Sample 2: 50 mg/kg spiked olive oil

<table>
<thead>
<tr>
<th>Mineral oil – 50 mg/kg spiked olive oil</th>
<th>sample 2-2013</th>
<th>sample 4-2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiking level (mg/kg)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mean (m)</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Repeatability limit (r)</td>
<td>19,3</td>
<td>15,3</td>
</tr>
<tr>
<td>Reproducibility limit (R)</td>
<td>51,7</td>
<td>28,1</td>
</tr>
<tr>
<td>Horrat value</td>
<td>4,1</td>
<td>2,2</td>
</tr>
</tbody>
</table>

→ Method applicable from 50 to 1000 mg/kg
Phthalates

- Transport & storage
- Production process
- Regulation

→ EFSA Scientific Opinion on food additives, flavourings, processing aids and materials in contact with food (2005)
→ (EC) n°10/2011 - plastic materials and articles intended to come into contact with food
Chemical structures of phthalates

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>Abbreviation</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di-methyl PHT</td>
<td>DMP</td>
<td>R1=R2=methyl</td>
<td></td>
</tr>
<tr>
<td>Di-ethyl PHT</td>
<td>DEP</td>
<td>R1=R2=ethyl</td>
<td></td>
</tr>
<tr>
<td>Di-isobutyl PHT</td>
<td>DIBP</td>
<td>R1=R2=isobutyl</td>
<td></td>
</tr>
<tr>
<td>Di-butyl PHT</td>
<td>DBP</td>
<td>R1=R2=butyl</td>
<td></td>
</tr>
<tr>
<td>Di-hexyl PHT</td>
<td>DHexP</td>
<td>R1=R2=hexyl</td>
<td></td>
</tr>
<tr>
<td>Benzyl butyl PHT</td>
<td>BBP</td>
<td>R1=benzyl R1=butyl</td>
<td></td>
</tr>
<tr>
<td>Di-n-heptyl PHT</td>
<td>DHeP</td>
<td>R1=R2=heptyl</td>
<td></td>
</tr>
<tr>
<td><strong>Di-(2-ethyl hexyl) PHT</strong></td>
<td><strong>DEHP</strong></td>
<td>R1=R2=ethyl-2 hexyl</td>
<td></td>
</tr>
<tr>
<td>Di-n-octyl PHT</td>
<td>DNOP</td>
<td>R1=R2=octyl</td>
<td></td>
</tr>
<tr>
<td>Di-isononyl PHT</td>
<td>DINP</td>
<td>R1=R2=isononyl</td>
<td></td>
</tr>
<tr>
<td>Di-isodecyl PHT</td>
<td>DIDP</td>
<td>R1=R2=isodecyl</td>
<td></td>
</tr>
</tbody>
</table>

**DEHP**
- Oily viscous liquid
- MW: 390.6 g/mol
- BP: 385°C
- Water solubility: 3 µg/l
- High affinity for fat (log Kow : 7.5)
Phthalates are everywhere

Toys
Child-care articles

Flooring-Roofing- Wall covering
Adhesives-Sealant-Rubber
Paints-Shower curtains
Wires & cables-Fresheners

Shoes-Boots-Gloves
Out-door & rainwear

Cosmetics : Perfume
Hairspray-Deodorant
Skin emollient-nail polish
fingernail elongators

Car undercoating
Dashboard-Door panels-Safety glass

Pharmaceuticals
Medical devices : Catheters-Blood bag
(EC) No 10/2011 - Plastic materials & articles into contact with food

<table>
<thead>
<tr>
<th>Specific migration limit in food</th>
<th>To be used as</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBP 30 mg/kg</td>
<td>Plasticizer in single-use material containing non-fatty foods except infant formulae</td>
</tr>
<tr>
<td>DINP ∑ = 9 mg/kg</td>
<td>Plasticizer in repeated use materials containing non-fatty foods</td>
</tr>
<tr>
<td>DIDP</td>
<td></td>
</tr>
<tr>
<td>DEHP 1,5 mg/kg</td>
<td></td>
</tr>
<tr>
<td>DBP 0,3 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

Material containing these phthalates cannot be used for oils & fats.
Phthalates: ITERG’s procedure

Advantages of the method:

- No contamination, no solvent
- Rapid analysis (45 min)
- Sensitive LOQ < 0.1 mg/kg (excepted DINP & DIDP)
DEHP in oils & fats

ITERG data, 2009

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3-MCPD esters & glycidol esters

- Production process (deodorisation)
- Regulation for free 3-MCPD
  \rightarrow  \text{(EC) n°1881/2006 – contaminants in foodstuffs (20 µg/kg in soya sauce)}
- Regulation for 3-MCPD esters
  \rightarrow  \text{EFSA statement – related to 3-MCPD esters (2008)}
  \rightarrow  \text{EFSA Scientific Report on 3MCPD occurrence in food in Europe (2013)}
3-MCPD esters & glycidol esters

Structure

« free » 3-MCPD

H₂C — OH

HC — OH

H₂C — Cl

7 fatty acids

C12 - C14
C16 - C18
C18:1 - C18:2
C18:3

H₂C

HC

H₂C — OH

Glycidol

« bound » 3-MCPD monoesters

H₂C — AG

HO — CH

H₂C — Cl

3-MCPD diesters

H₂C — AG

AG — CH

H₂C — Cl

« bound » 3-MCPD diesters

H₂C — AG

H₂C — Cl

3-MCPD monoesters « bound »

H₂C — AG

H₂C — Cl

Glycidol esters (GEs)

H₂C

HC

H₂C — AG

H₂C

HC

H₂C — OH

Hrncirik, OVID meeting, 2011

14

fatty acids

C12 - C14
C16 - C18
C18:1 - C18:2
C18:3

Hrncirik, OVID meeting, 2011

7

fatty acids

C12 - C14
C16 - C18
C18:1 - C18:2
C18:3

Hrncirik, OVID meeting, 2011
# 3-MCPD esters & glycidol esters

## Analysis strategy

<table>
<thead>
<tr>
<th>Pros</th>
<th>Indirect methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>No chemical reaction</td>
<td>Standards available</td>
</tr>
<tr>
<td>Individual quantification of species</td>
<td>Good sensitivity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cons</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards not available</td>
<td>Reactivity of the species</td>
</tr>
<tr>
<td>Expensive tools: HPLC/MS/MS</td>
<td>Global quantification of species</td>
</tr>
<tr>
<td>Purification step needed</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standards</th>
<th></th>
</tr>
</thead>
</table>
| AOCS-JOCS Cd 28-10 for GEs | AOCS Cd 29a-13 for 2- and 3-MCPD & GEs  
*From Ermacora & Hrnirick, JAOCS, 2012*  
*From Kuhlmann, EJLST, 2011*  
*AOCS Cd 29c-13 for 3-MCPD & GEs*  
*From DGF C-VI 18, 2010 & Weisshar, 2008* |
Esters de 3-MCPD & glycidol

2013 AOCS Collaborative Study

Sample 6

- Palm oil sample, not spiked.
- All indirect methods were acceptable

Sample 5

- RBD Canola sample, not spiked.
- Variable results between methods

- Comparable results for the 3 indirect methods
- In general, direct methods agreed with indirect methods
- Methods did not give reliable results for concentrations < 1 mg/kg
Occurrence of 3-MCPD & glycidol esters in vegetable oils

Screening ITERG 2013

Industrial specification 3MCPD = 2 mg/kg

Industrial specification glycidol = as low as possible
<table>
<thead>
<tr>
<th></th>
<th>Regulation</th>
<th>Methods</th>
<th>Risks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PAHs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mineral oil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Phthalates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>3-MCPD esters &amp; glycidyl esters</strong></td>
<td></td>
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</tr>
</tbody>
</table>
Conclusions

• Research of contaminants is part of multiple controls conducted by fat and oil industry to fulfill the EC regulation n°1881/2006.

• In the absence of regulation, the detection of contaminants must be addressed in partnership with authorities according to the toxicity of molecules.

• Risks are rather limited due to the efficient elimination during oil-refining steps.

• However some contaminants can be formed during the production process of vegetable oils such as 3-MCPD & glycidyl esters.
A special thanks to….

ITERG Analysis Department team