Advantages and disadvantages of different analytical techniques used to determine chloropropanols in food lipid matrices

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Poland in the World
Poland in numbers

- area: 312 679 km²
- population: 38.5 mln
- capital: Warszawa
- time zone: GMT +1

The Baltic Sea coast

The Tatra Mountains
Gdańsk

- over 1 000 year-old city,
- an important part of Baltic Europe,
- represents the traditions of the Hanseatic League, a medieval association for the promotion and protection of trade,
- world capital of amber,
- **fall of communism** – road to freedom,
- city of **famous people**: Farenheit, Heweliusz, Schopenhauer, Gunter Grass, Lech Wałęsa
Short agenda

• theoretical background,
• analytical aspects,
• application of the SPME technique,
• results.
Theoretical background
What’s are chloropropanols?

- chloropropanols = chlorohydrins,
- heat-induced food contaminants,
- appears in free and esterified (bound) form,
- the most popular: 3-MCPD, 2-MCPD, 1,3-DCP
- toxicity:
  - genotoxic in vivo studies,
  - carcinogenic in vivo studies (nephrotoxicity, antiferility effects).
Chloropropanols precursors

**CHEMICALS**
- chloride ions,
- glycerol,
- tri-, di- and monoacylglycerols

**PROCESSING**
- temperature,
- pH,
- hydrolytic enzymes
History

- Discovery of 3-MCPD: 1978
- Discovery of 3-MCPD-E: 1980
- Setting TDI (EU Scientific Committee on Food): 2001
- Development of new analytical methods: 2001-2015

- GC-MS
- 2 µg/kg bw
- Intensive toxicological research

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Chloropropanols in food lipids

- edible vegetable oils,
- margarines,
- infant milk formulas and human breast milk,
- fish oils, fish liver oils and encapsulated oils,
- thermally treated food rich in fats.
Analytical aspects
ANALYTICAL PROBLEMS

- LACK OF CHROMOPHORE
- HIGH POLARITY
- LOW MOLECULAR WEIGHT
- COMPLEX MATRIX
- HIGH BOILING POINT

CHALLENGE FOR ANALYTICAL CHEMIST
Analytical approach

DIRECT

- Sample preparation
- LC-MS analysis
- Remove of interfering matrix components

INDIRECT

- Sample preparation
- Hydrolysis
- I Extraction
- II Extraction
- Derivatisation
- GC-MS analysis
- Addition of deuterated internal standards
- Addition of derivatisation reagent
- Remove of interfering lipid components

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### Direct vs Indirect Determination

<table>
<thead>
<tr>
<th>DIRECT</th>
<th>INDIRECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>no side reactions,</td>
<td>possible analytes conversion,</td>
</tr>
<tr>
<td>precise information,</td>
<td></td>
</tr>
<tr>
<td>determination of single esters –</td>
<td>few analytical standards,</td>
</tr>
<tr>
<td>lots of analytical standards,</td>
<td>simple matrix removal,</td>
</tr>
<tr>
<td>high cost of single analysis,</td>
<td>easy separation,</td>
</tr>
<tr>
<td>matrix removal needed,</td>
<td>multi-step sample preparation,</td>
</tr>
<tr>
<td>nearly no sample preparation step,</td>
<td>time consuming and laborious,</td>
</tr>
<tr>
<td>problematic – equipment damage, ion source contamination,</td>
<td>derivatization reaction required,</td>
</tr>
<tr>
<td>LC-MS</td>
<td>GC-MS</td>
</tr>
</tbody>
</table>

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## Most popular indirect methods

<table>
<thead>
<tr>
<th>Method name</th>
<th>“improved Unilever”</th>
<th>DGFC-VI 18 (10)</th>
<th>SGS 3 in 1</th>
<th>“enzymatic”</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Author</strong></td>
<td>Ermacora &amp; Hrncirik</td>
<td>Kuhlmann</td>
<td>Kuhlmann</td>
<td>Miyazaki et al.</td>
</tr>
<tr>
<td><strong>Year</strong></td>
<td>2013</td>
<td>2010</td>
<td>2011</td>
<td>2012</td>
</tr>
<tr>
<td><strong>Hydrolysis</strong></td>
<td>Slow acidic</td>
<td>Fast alkaline</td>
<td>Slow alkaline</td>
<td>enzymatic</td>
</tr>
<tr>
<td><strong>Hydrolysis agent</strong></td>
<td>H$_2$SO$_4$ in MetOH</td>
<td>NaOH in MetOH</td>
<td>Triacylglycerol lipase from Candida rugosa</td>
<td></td>
</tr>
<tr>
<td><strong>Time and temp. of hydrolysis</strong></td>
<td>16 h, 40 °C</td>
<td>3-5 min, RT</td>
<td>16 h, -25 °C</td>
<td>30 min, RT</td>
</tr>
<tr>
<td><strong>Hydrolysis stopping agent</strong></td>
<td>NaHCO$_3$</td>
<td>NaBr acid solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Derivatisation</strong></td>
<td>PBA, 15 min, RT, ultrasound bath</td>
<td></td>
<td>PBA, 20 min, 85 °C</td>
<td></td>
</tr>
<tr>
<td><strong>Organic solvents</strong></td>
<td>3 mL THF</td>
<td>100 µL t-BME</td>
<td>600 µL diethyl ether</td>
<td>200 µL octane</td>
</tr>
<tr>
<td></td>
<td>8.5 mL heptane</td>
<td>1.2 mL hexane</td>
<td>6 mL hexane</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.8 mL diethyl ether/ethyl octane</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 µL octane</td>
<td>3 mL hexane</td>
<td></td>
</tr>
<tr>
<td><strong>Total sum of the organic solvents</strong></td>
<td>11.5 mL</td>
<td>3.6 mL</td>
<td>4.1 mL</td>
<td>11 mL</td>
</tr>
</tbody>
</table>
SGS „3 in 1”

- developed by Jan Kuhlmann in 2011,
- first published methods which allows to simultaneous determination of 3-MCPD, 2-MCPD and glycidol,
- mild condition of transesterification 16h at -25°C,
- derivatisation with PBA,
- detection and identification with GC-MS

Oil sample
- Add diethyl ether
- Add internal standard

Ester cleavage
- Freezing for 30 min at -22 to -25°C
- Add methanolic sodium hydroxide
- Freezing for 16 h at -22 to -25°C
- Add acidified sodium bromide solution
- Concentration under gentle stream of nitrogen
- Washing with iso-hexane

Organic layer
- Extraction with a mixture of diethyl ether/ethyl acetate
- Drying with anhydrous sodium sulphate

Derivatization
- Add PBA
- Ultrasound 15 min at RT
- Concentration under gentle stream of nitrogen

GC-MS Analysis

Reference:

Research Article

Determination of bound 2,3-epoxy-1-propanol (glycidol) and bound monochloropropanediol (MCPD) in refined oils

Jan Kuhlmann

Ju: SGS Germany GmbH, Hamburg, Germany
Enzymatic 3 in 1

- method developed by Miyazaki and co-workers in 2012,
- first apply to determine 3-MCPD,
- collaborative study within 13 laboratories of Japan Oil Chemists’ Society,
- simultaneous enzymatic hydrolysis and bromination step,
- derivatisation with PBA,
- detection and identification with GC-MS
Sample preparation

SPME
Alternative methods of isolation

- **HS (headspace analysis):**
  - isolation of 1,3-DCP and glycidol in soya sauces,

- **SPME:**
  - isolation of 1,3-DCP (PA fiber) and than derivatization and isolation of 3-MCPD (PDMS fiber),
  - simultaneously isolation of 1,3-DCP and 3-MCPD (PA fiber) with on fiber derivatization mode.
SPME in on-fiber derivatisation mode

Advantages:
• simplification
• shortened time
• reduced matrix interference

Loses of analytes  ➔ enrichment of analytes

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Derivatisation agents

\[
\begin{align*}
\text{Cl-} & \quad \text{OH} \\
\text{Cl-} & \quad \text{OH} \\
\text{F}_3\text{C} & \quad \text{N} & \quad \text{Si(CH}_3\text{)}_3 \\
\text{F}_7\text{C}_3 & \quad \text{N} & \quad \text{Si(CH}_3\text{)}_3 \\
\text{O} & \quad \text{Si(CH}_3\text{)}_3
\end{align*}
\]
Different fiber coatings

DVB/CAR/PDMS

PEG

PA
SPME with ionic liquids phase

1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C4MIM][TFSI])

Problems to be solved: increase fiber lifetime in derivatisation conditions
Results
Margarines

The graph shows the concentration of 2-MCPD and 3-MCPD in five different samples of margarines (M_1 to M_5). The concentration is measured in mg/kg. Sample M_5 has the highest concentration of both 2-MCPD and 3-MCPD.
Infant formulas

<table>
<thead>
<tr>
<th>Concentration [mg/kg]</th>
<th>IF_1</th>
<th>IF_2</th>
<th>IF_3</th>
<th>IF_4</th>
<th>IF_5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-MCPD</td>
<td>1.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>3-MCPD</td>
<td>2.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

2-MCPD

3-MCPD

Poster session
Analytics, Quality and Safety
Poster No CFP 092R

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Determination of MCPD fatty acid esters
in lipid fractions of retailed infant formulas
Renata Jedzkiewicz, Agnieszka Glowacz, Justyna Gromadzka, Jacek Namieśnik
Fish oils

2-MCPD 3-MCPD

Poster session
Analytics, Quality and Safety
Poster No CFP 089R

Lipid Oxidation and antioxidants
Poster No CFP 090R
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Vegetable oils

Poster session
Analytics, Quality and Safety
Posters No CFP 070R
and CFP 091R

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Chloropropanols in different lipids

- Refined edible oils
- Margarines
- Fish oils
- Infant formulas

Concentration [mg/kg]

- 2-MCPD
- 3-MCPD
DGF C-III 17 vs SGS „3 in 1” method

3-MCPD concentration [mg/kg]

Margarine 1  Margarine 2  Margarine 3  Margarine 4  Margarine 5  Rapeseed oil

3 in 1 method
DGF method

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Take home message

CHLOROPROPANOLS

Chaos in toxicological data

New promising analytical techniques

Presents in food lipids
Research TEAM

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Renata Jędrkiewicz, MSc Eng

Justyna Gromadzka, PhD Eng

Prof. Jacek Namieśnik
Acknowledgements

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Thank You

for Your kind attention