Update on Analytical Methodology for 3-MCPD Esters and Glycidyl Esters in Oils and Fats

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BLL
Bund für Lebensmittelrecht und Lebensmittelkunde e.V.

OVID
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Intro: 3-MCPD/glycidyl ester analytics

- extremely vivid field
- very relevant for other areas
  (mitigation, mechanism, toxicity, oil supply chain, …)
- new (modified) methods presented (+)
- number of methods used is increasing (-)
- quality of results: ??? - which methods are correct?

Today's objective:

- to summarize current progress
- to review data available that allow the evaluation of individual methods

Outline

- **evolution of 3-MCPD/glycidyl esters methodology**
- analytical protocol of indirect methods
- results of recent ring-tests
- several methods in detail
- summary of current status

Evolution of 3-MCPD esters methodology

2004: **acid-based transesterification** (Velisek, ICT)[1]
2007-2010: several modifications, e.g.:
- use of HFBI (Seefelder, Nestlé)[2]
- replacing NaCl (Hrncirik, Unilever)[3]
- further modified, ring-tested as BfR 008

2008: **alkaline-based transesterification** (Weisshaar, CVUA)[4]
- method adopted by DGF (DGF C-III 18)
- Nov 2008: the method shown to be unspecific (Kuhlmann, SGS)
- DGF method modified (C-III 18 option B)
- another method developed by BfR, ring-tested as BfR 009

2009: first direct method presented (Collison, ADM)

2010: several groups working on direct methods (e.g. Pinkston, P&G; Granvogl, DFA; …)

References:
1. Divinova et al., *Cz. J. Food Sci.* **22**, 182–189 (2004)
2. Seefelder et al., *Food Addit. Contam.* **25**, 391–400 (2008)
3. Hrncirik et al., *Eur. J. Lipid Sci. Technol.* **113** (2011)
4. Weißhaar, *Eur. J. Lipid Sci. Technol.* **110**, 183–186 (2008)

Evolution of glycidyl esters methodology

2009: differential methods, e.g. DGF C-III 18 option A and B

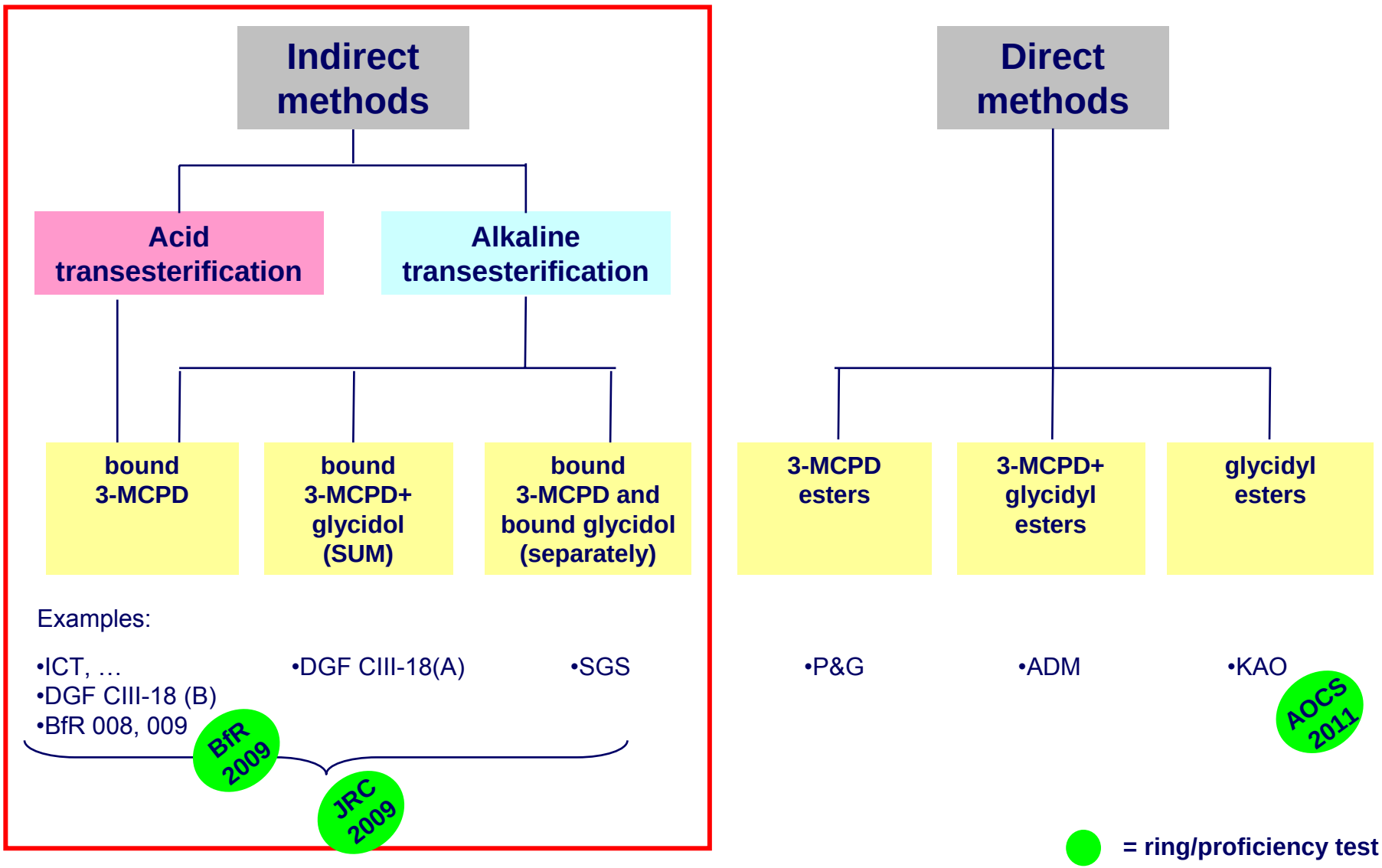
2009: salting-out with bromide salt (SGS, Kuhlmann)

2009: first direct methods developed (Musukawa, KAO; Collison, ADM)[1,2]

2010: "mild" alkaline-based transesterification, followed by the conversion with bromide salt (Kuhlmann, SGS)[3]

References:
1. Masukawa et al., *J. Oleo Sci.* **59**, 81-88 (2010)
2. Haines et al., *J. Am. Oil Chem. Soc.* (2011)
3. Kuhlmann, *Eur. J. Lipid Sci. Technol.* **113** (2011)

Method classification



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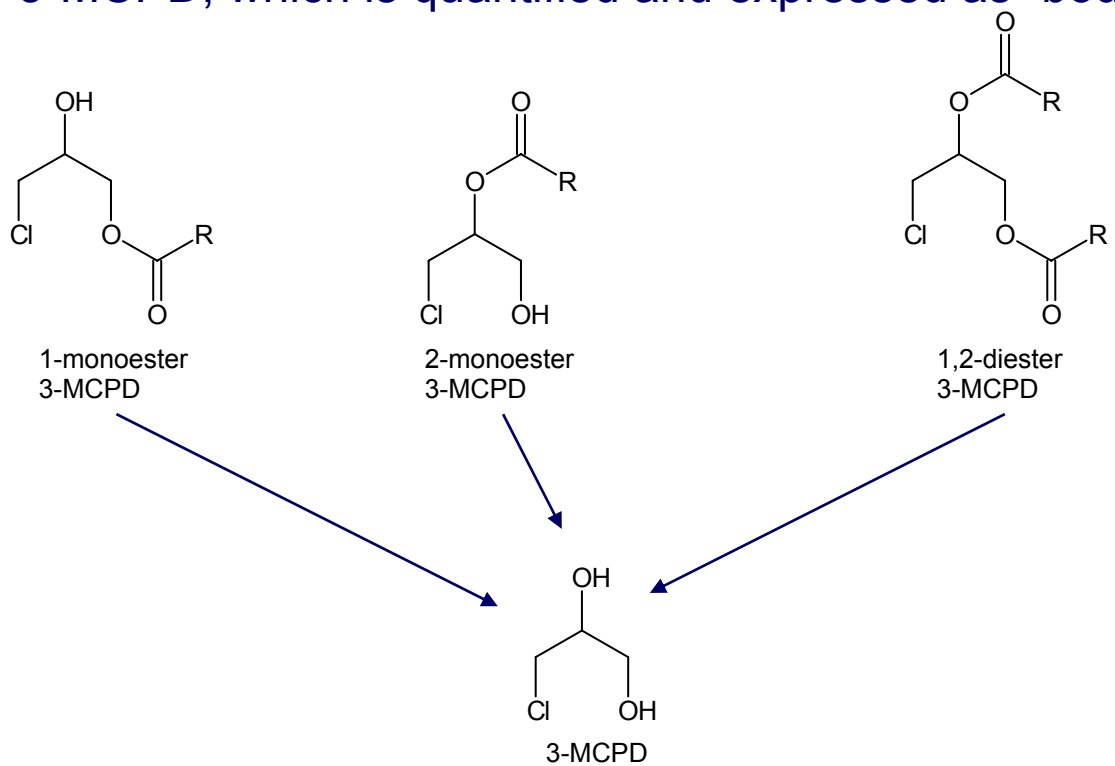
Indirect v. direct method

• a great number of individual compounds (esters) converted to a single substance: 3-MCPD, which is quantified and expressed as “bound 3-MCPD“



• avoids synthesis of a series of standards
• indirect method performance (specificity, truness, ... ?)

3-MCPD esters analysis (indirect methods)

Basic analytical protocol:

Oil sample + I.S.

↓

Transesterification (methanolysis)

↓

Neutralisation & salting out

↓

Derivatisation

↓

GC/MS analysis

The most common variations:

Internal standard:
- free form: 3-MCPD-d5
- bound form: e.g. dipalmitoyl-3-MCPD-d5

Transesterification:
- acid: H_2SO_4/MeOH (16 h, 40°C)

- slow; mild conditions
- alkaline: $NaOCH_3$/MeOH (1-10 min., RT)

- fast; decomposition of IS/analyte

Salting out:
- NaCl (specificity! - reaction with glycidol → additional 3-MCPD)
- SO_4^{2-}
- NaBr

Derivatisating agents:
- phenylboronic acid (PBA)
- heptafluorobutyrylimidazole (HFBI)

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BfR: 2nd Collaborative Study for the Determination of 3-MCPD Fatty Acid Esters in Edible Fats and Oils (2009)

BfR
Risiken erkennen – Gesundheit schützen

Setup:

- participation: 36 laboratories
- samples: 5 samples (no spiked sample) in two series
- three indirect methods tested, incl.:
  - **BfR 008** (**acid transesterification**): 6 datasets
  - **BfR 009** (**alkaline transesterification**): 27 datasets

Comments:

- methods: without using NaCl for salting out (+)
- methods: free 3-MCPD is prescribed as internal standard (-)
- samples: no spiked sample, trueness not evaluated (-)

Outcome:

- both methods gave reproducible results (different level of uncertainty due to # of labs)
- more than 90 % of labs provided satisfactory results ($|z| \leq 2$)

Further details in the report (available to the participants of the test); publication in preparation

JRC: Proficiency test on the determination of 3-MCPD esters in edible oil (2009-2010)

Setup:

- participation: 34 laboratories
- samples: 2 samples
  - refined palm oil
  - virgin olive oil spiked by dioleoyl-3-MCPD
- variety of in-house methods used, incl.:
  - acid transesterification based methods
  - alkaline transesterification based methods (different modifications: DGF A/B, Weisshaar 2008, …)
  - unspecified (8x)

Comments:

- methods: no restrictions
- samples: evaluation of the trueness included (+)

Outcome:

- … (see next slides)

JRC: Proficiency test on the determination of 3-MCPD esters in edible oil (2009-2010)

JRC EUROPEAN COMMISSION

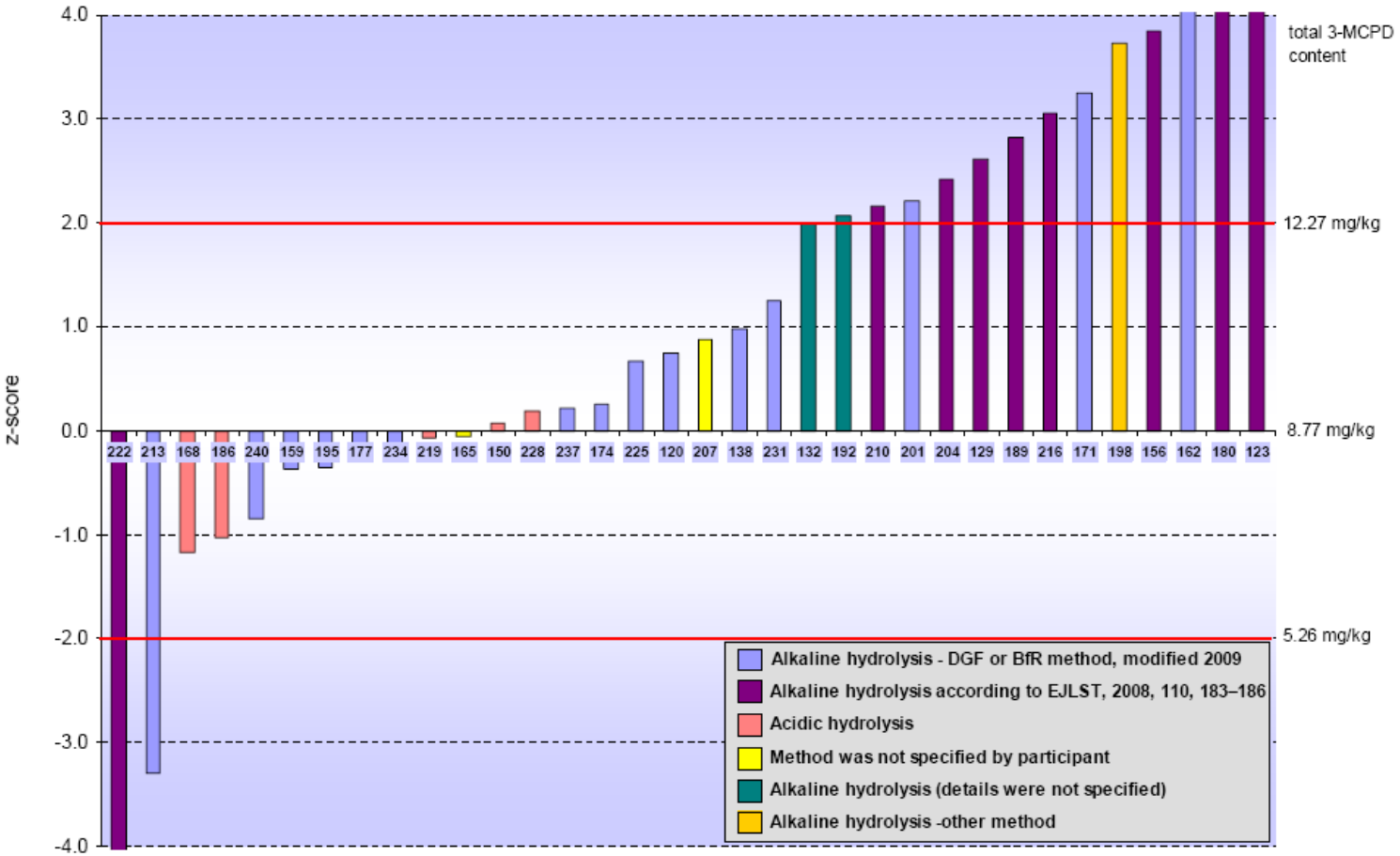
irmm Institute for Reference Materials and Measurements

	Spiked virgin olive oil	Refined palm oil
Assigned value (ppm)	**4.58**	**8.77**
Results (ppm)	**2.4-14.8**	**0.6-18.8**
Satisfactory results, $\|z\| \leq 2$ (%)	**85**	**56**
Comment - results	**results NOT affected by the method used**	**results affected by the method used (due to glycidyl esters?)**

Final report available at: www.jrc.ec.europa.eu

JRC: Proficiency test on the determination of 3-MCPD esters in edible oil (2009-2010)

Figure 4. 1: Plot of participants' z-scores for the contaminated palm oil test sample. The different analysis procedures applied are colour coded (from the Final report)



JRC: Proficiency test on the determination of 3-MCPD esters in edible oil (2009-2010)

Refined palm oil sample (assigned value 8.8 mg/kg)	Range (mg/kg)	Compliance (|z|≤2, i.e. range 5.3-12.3 mg/kg)
34 data sets in total, of which – 27 obtained by alkaline methods – 5 obtained by acid methods – 1 unspecified		 11/27 5/5 1/1
27 dataset obtained by **alkaline methods** – 15 specific methods (BfR or DGF B) – 9 unspecific methods ("Weisshaar") – 3 methods with details unknown	**3.0 – 16.5 mg/kg**	 11/15 0/9 0/3
5 dataset obtained by **acid methods**	**6.7-9.1 mg/kg**	5/5

- poor performance of alkaline methods partly caused by usage of unspecific methods
- several labs clearly struggled with the method (some probably not ready at that time)
- results for spiked crude oil (no glycidyl esters) much better (85% satisfactory)
  → interference with glycidyl esters is critical!

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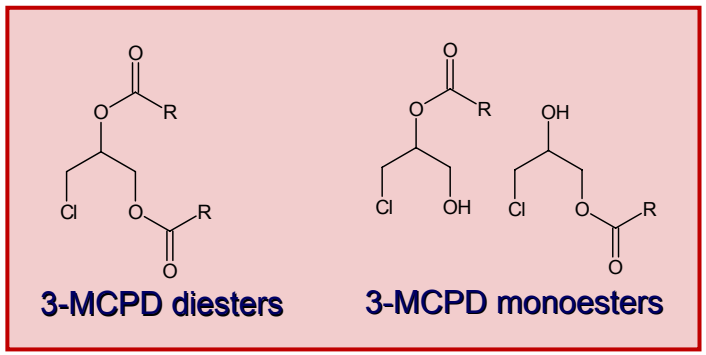
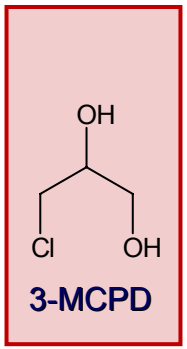
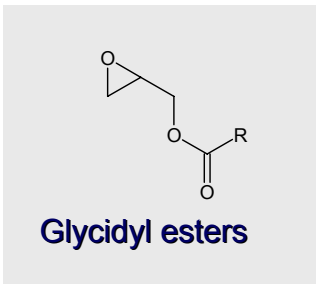
Method in details: BfR 009



trans-esterification

CH_3ONa/methanol

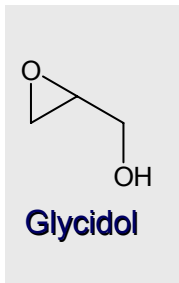




DAGs
MAGs
phospholipids
…

occurs but not interfering

probably occurs but not interfering



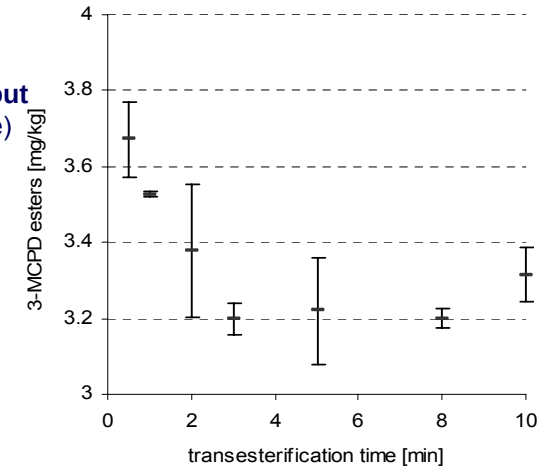
spontaneous conversion in alkaline soln

salting out (sulphate)

Comments [1]:

1. Degradation of 3-MCPD in alkaline medium during transesterification.
Recovery of 3-MCPD after:
1 min= 83-95%; 10 min=40-41% (decrease in sensitivity!)

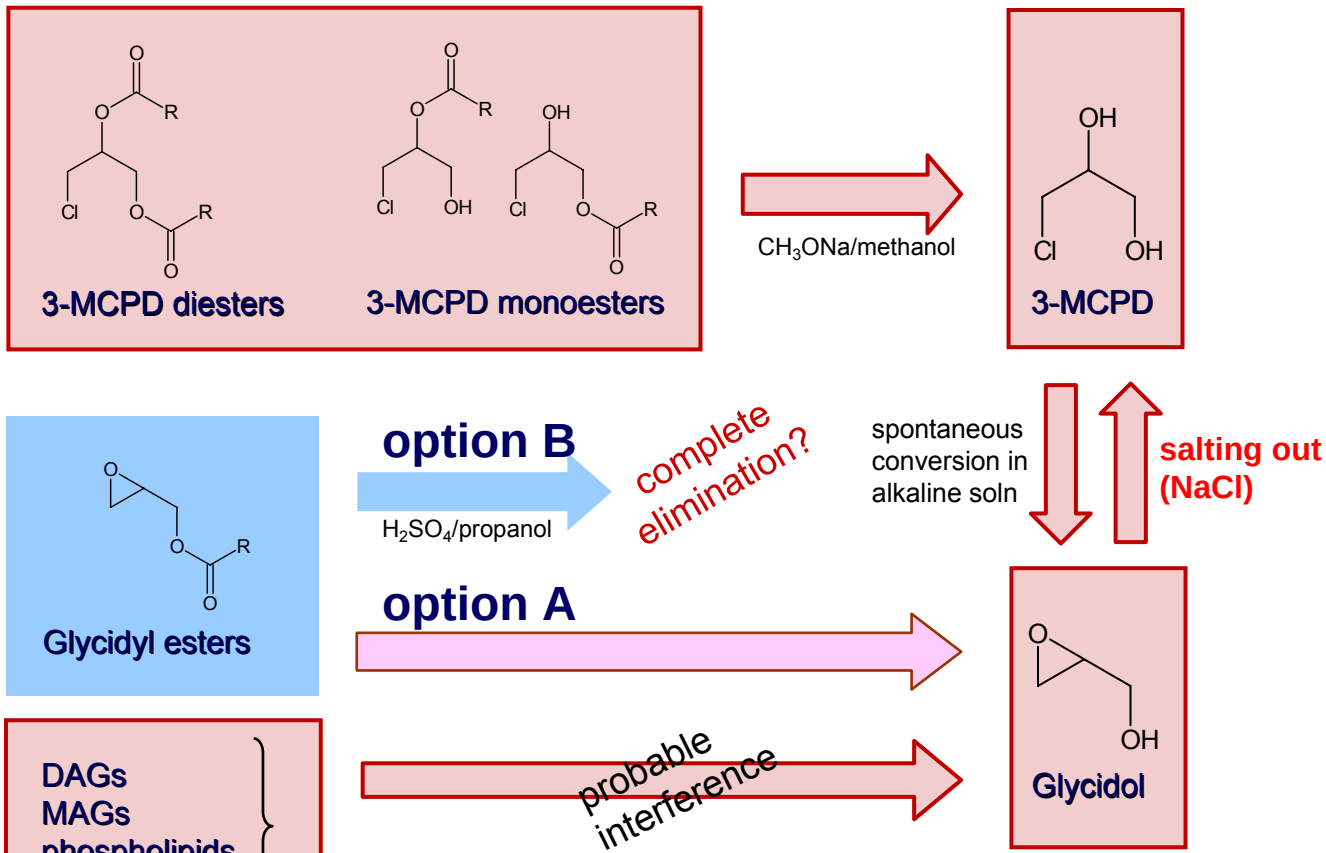
2. Results affected by transesterification time.



3. Results affected by the choice of I.S. (esterified v. free form of 3-MCPD-d5)

References:
1. Hrncirik et al., *Eur. J. Lipid Sci. Technol.* **113** (2011)

Method in details: DGF C-III 18 (option A and B)

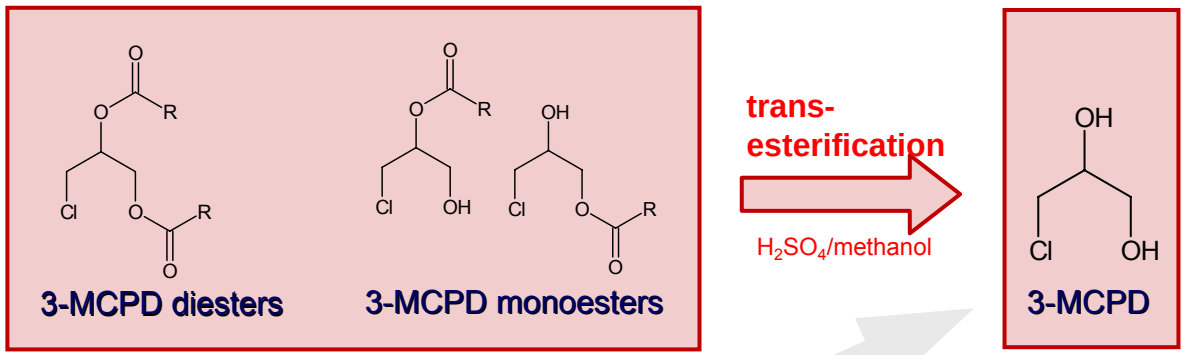


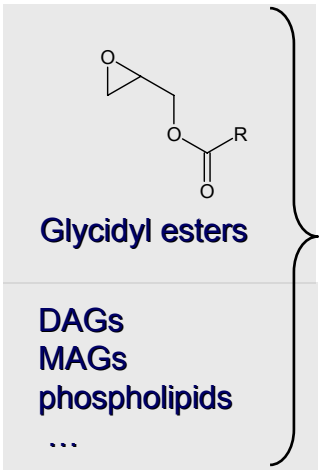
Comments:

- "DGF method does not accurately predict the amount of 3-MCPD and glycidyl esters in oil samples" [1]
- "Results of DGF method dependent on the oil composition (underestimation of glycidyl esters in some samples)" [2]
- "Conversion rate of glycidol to 3-MCPD is pH dependant, thus may be incomplete" [3]

References:
1. Collison 2010; details in: Haines et al., *J. Am. Oil Chem. Soc.* (2011)
2. Shimizu et al., *J. Oleo Sci.* **59**, 535-539 (2010)
3. Hrncirik et al., *Eur. J. Lipid Sci. Technol.* **113** (2011)

Method in details: Acid-transesterification method





potential conversion (if Cl^- present)

glycidol irreversibly degraded

Comments:

- transesterification proceeds under mild conditions (no harsh chemistry)
- no degradation of 3-MCPD occurs (recovery 100-102 %) [1]
- risk o conversion of glycidol to 3-MCPD by the reaction with chlorides, if present [2]
- good specificity (no conversion of glycidol) in regular oil samples [3]

References:
1. Hrncirik et al., *Eur. J. Lipid Sci. Technol.* **113** (2011)
2. Weißhaar, *Eur. J. Lipid Sci. Technol.* **110**, 183–186 (2008)
3. Ermacora et al., manuscript in preparation (2011)

Rough evaluation of analytical methods for 3-MCPD esters

	Sample prep. (time / labor)	Specificity	Robustness	Reproducibility (ring-tests)	Glycidyl esters simultaneously?
DGF C-III 18 (option A+B)	+/+	– [1]	± [2]	? [4]	Possible (quantification not precise)
BfR 009	+/+	+	± [3]	+ [4,5]	Not possible
Acid method	-/+	+	+	+ [5,6]	Not possible
SGS	-/?	+	?	?	Possible (comparison needed)

Symbols used:
(+) satisfactory
(±) doubtful
(−) unsatisfactory
(?) further evaluation needed

Notes:
1. interference with glycidyl esters, results dependent on sample composition
2. results may be dependent on the internal standard used and other factors (for details see slide #17-18)
3. results may be dependent on the internal standard used (for details see slide #17)
4. not possible to draw conclusions from JRC proficiency test (results of DGF and BfR009 methods combined)
5. based on the results of BfR ring test 2009
6. based on the results of JRC proficiency test 2010

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Summary of current status

	3-MCPD esters	Glycidyl esters
Indirect methods	▫ simple to perform, but… ▫ poor accuracy (results are dependant on sample composition) ▫ limited value/meaning (3-MCPD/glycidyl esters not related, formed differently!)	
	▫ number of methods available ▫ new BfR ring-test ongoing (fat-based matrices) ▫ good sensitivity, but risk of interference; reliable? ▫ provide just "total 3-MCPD", suitable for routine analyses	▫ differential DGF method just a rough "estimation" ▫ novel SGS method? (further evaluation needed) ▫ high uncertainty due to complex chemistry, reliable?
Direct methods	▫ few methods presented (other under development) ▫ substantial limitations (standards, instrumentation) ▫ provide a full profile, but remains difficult for routine analyses	▫ great progress in 2010, future trend ▫ AOCS/JOCS collaborative study ongoing (results expected May 2011) ▫ no conversion, reliable ▫ feasible for routine analyses