Validated vs. Not validated – Challenges in Analytical Measurements of MCPD Esters and Glycidyl Esters in Different Food Matrices

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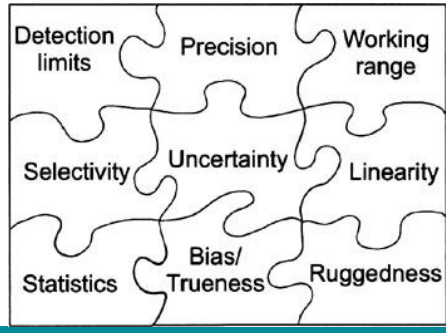
DGF Symposium on MCPD Esters and Glycidyl Esters
Analytics, Toxicology, Risk Assessment, Mitigation – Where we are today?
20-21 June 2017, Rocket Tower Conference Center, Berlin

Several levels of method validation

- In-house validation
  - a very first step, a must for each new published method; validation protocol not always (extensively) described
  - one may assume all users (sufficiently) validated the method prior to reporting/publishing results (reality?)

- Validation through a simple cross-laboratory method comparison
  - next step, beyond in-house
  - typically one or two methods in two (or more) laboratories

- Validation through official programs (ISO, AOAC, AOCS, …)
  - an ultimate level
  - fixed list of strict requirements (method performance)
  - typically the same method in multiple laboratories



MCPDE/GE analysis in oils and fats

2011-2012

Several methods published:

Ermacora & Hrncirik, JAOCS (2013)

Kuhlmann, EJLST (2011)

Fiebig, EJLST (2011)

2013

Collaborative Study

AOCS

2014

AOCS Official methods issued:

Cd 29a-13
Cd 29b-13
Cd 29c-13

2015-2017

ISO 18363 (in progress)

ISO

3-MCPDE/GE occurrence in food

refined vegetable oils/fats

bottled oils
(for consumption/cooking)

fried food
(snacks)

spreads

Baked goods,
confectionary

infant
formulas

- an issue across entire food-industry (many sectors affected)
- levels in food needed for an exposure assessment, a product comparison, …

From the analysis of oils/fats to foodstuffs

When developing methods for different food matrices, attention to be paid to:

- Sufficient/complete extraction of oil/fat from foodstuff (representative sample?)
- Avoiding degradation/inter-conversion of the analytes during sample preparation
- Matrix effect (interfering compounds, e.g. other ingredients)
- Assessing method accuracy

EFSA report – November 2013

EFSA Journal 2013;11(9):3381

SCIENTIFIC REPORT OF EFSA

Analysis of occurrence of 3-monochloropropane-1,2-diol (3-MCPD) in food in Europe in the years 2009-2011 and preliminary exposure assessment [1]

European Food Safety Authority[2, 3]

- increasing interest in monitoring MCPD/glycidyl esters in foodstuffs
- however, established methods validated solely for oils and fats
- EFSA report (Nov 2013); quality of reported results – a cause of concern

EFSA report – May 2016

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 3 March 2016

doi: 10.2903/j.efsa.2016.4426

Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food

EFSA Panel on Contaminants in the Food Chain (CONTAM)

Abstract

EFSA was asked to deliver a scientific opinion on free and esterified 3- and 2-monochloropropane-1, 2-diol (MCPD) and glycidyl esters in food. Esters of 3- and 2-MCPD and glycidol are contaminants of processed vegetable oils; free MCPDs are formed in some processed foods. The Panel on

- EFSA report (May 2016) – an improvement of the situation, yet many questions remain as no officially validated methods are available

Analyses of processed food…
a number of methods limited so far

Unilever combined method (AOCS Cd 29a-13) applied to various foodstuff
(in combination with an isolation/purification procedure):

- **spreads, dressings, mayonnaises** (Ermacora & Hrncirik, 2014)

- **bakery goods, fish/meat, potato/cereal based snacks** (Wenzl et al. 2015)

- **infant formulae** (Wohrlin et al, 2015)

… work in progress, more are expected to come

MCPDE/GE analysis in spreads/dressings

2013

Method development

Ermacora & Hrncirik,
Fd Addit. Contam. A (2014)

2014

Collaborative Study
(preparation)

AOCS

Jan 2015

Collaborative Study
(Training phase)

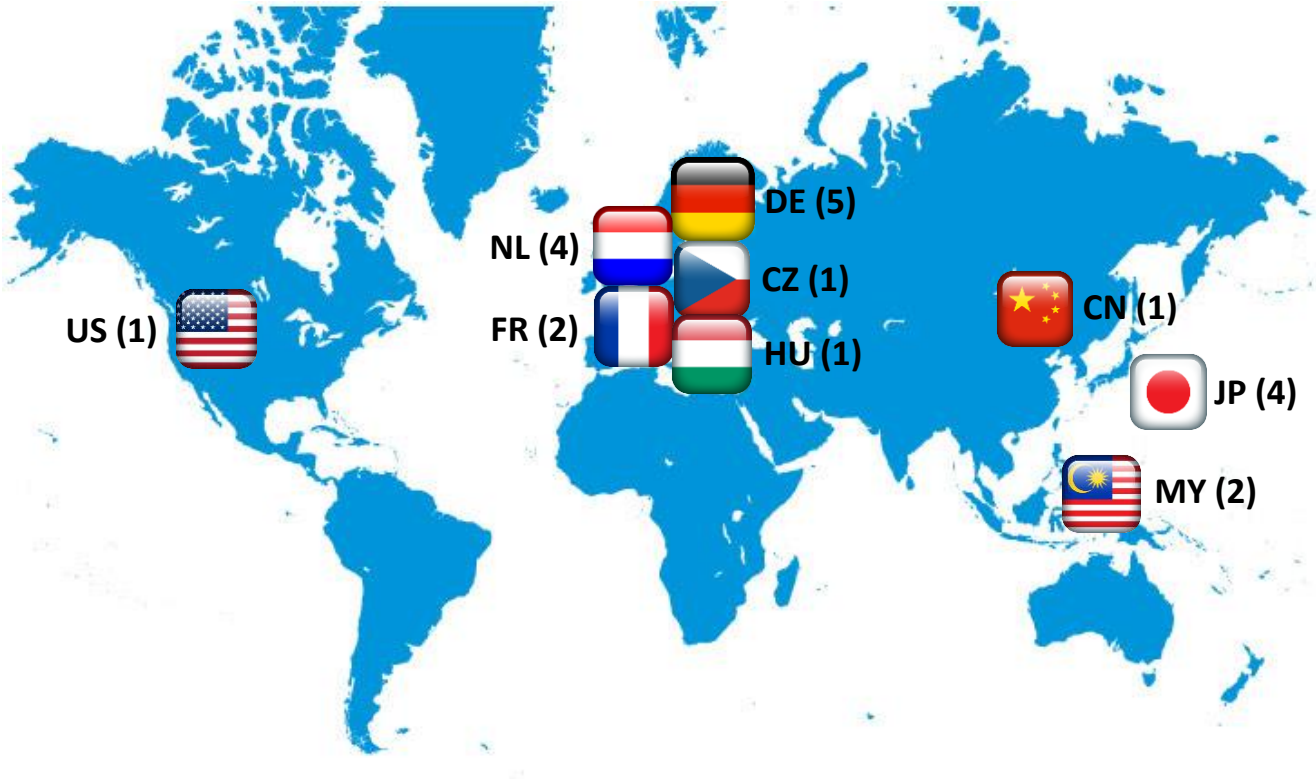
Jun 2015

Collaborative Study
(Official phase)

Participants

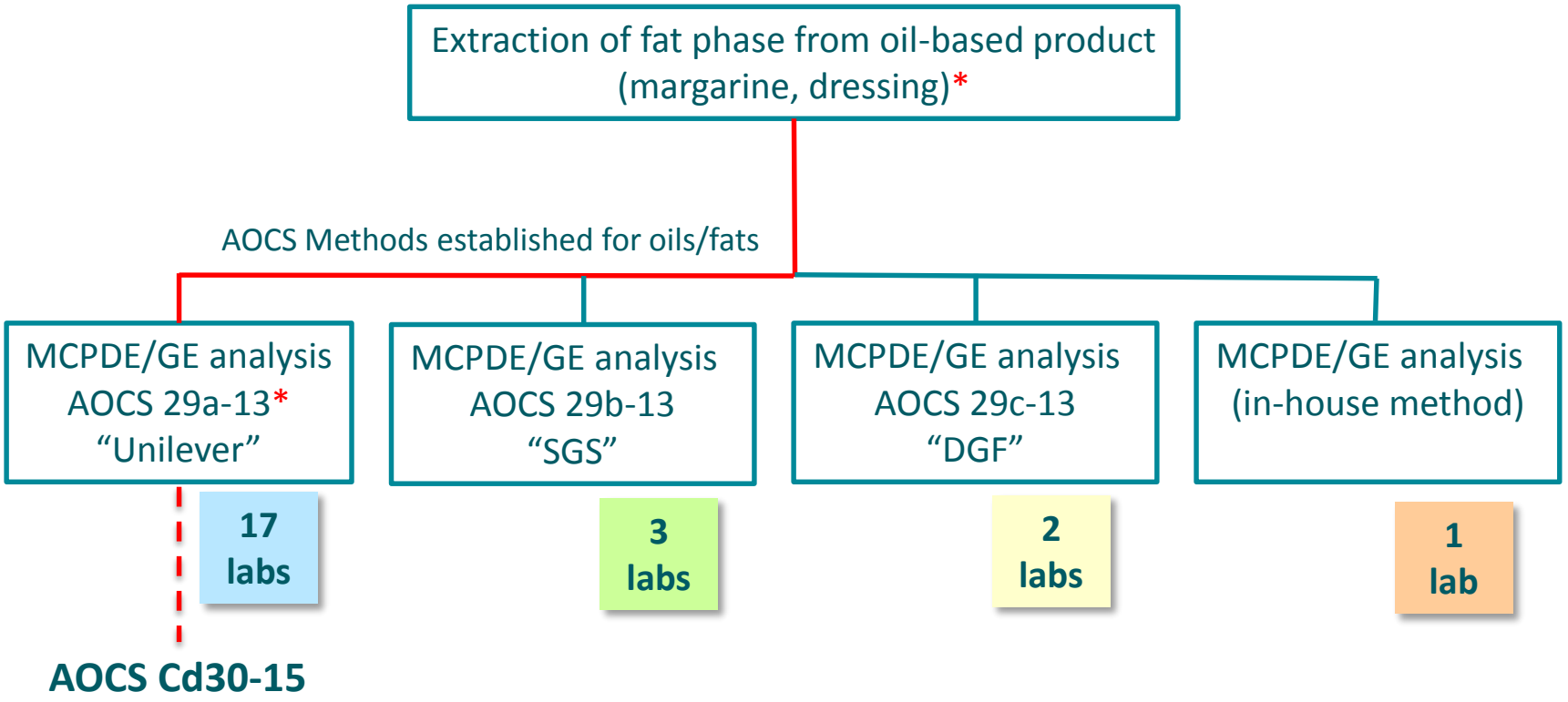
AOCS Collaborative study Cd30-15

- 21 laboratories affiliated to Industry, Research Institutes, commercial laboratories, Authorities, Academia (from nine countries)



Scope

AOCS Collaborative study Cd30-15



* Based on: Ermacora & Hrncirik, Food Additives & Contaminants: Part A, Vol. 31, No. 6, 985–994
Free download at: http://www.tandfonline.com/eprint/TBrjc2Ch8TiQEzrhA2JE/full

From the analysis of oils/fats to foodstuffs

When developing methods for different food matrices, attention to be paid to:

- Sufficient/complete extraction of oil/fat from foodstuff (representative sample?)
- Avoiding degradation/inter-conversion of the analytes during sample preparation
- Matrix effect (interfering compounds, e.g. other ingredients)
- Assessing method accuracy

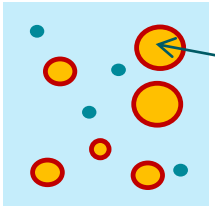
Major challenge

Assessing method accuracy

- Certified reference material?
- Comparison with the established method?
- Spiked samples?

Spiking oil? ✓

Bulk oil

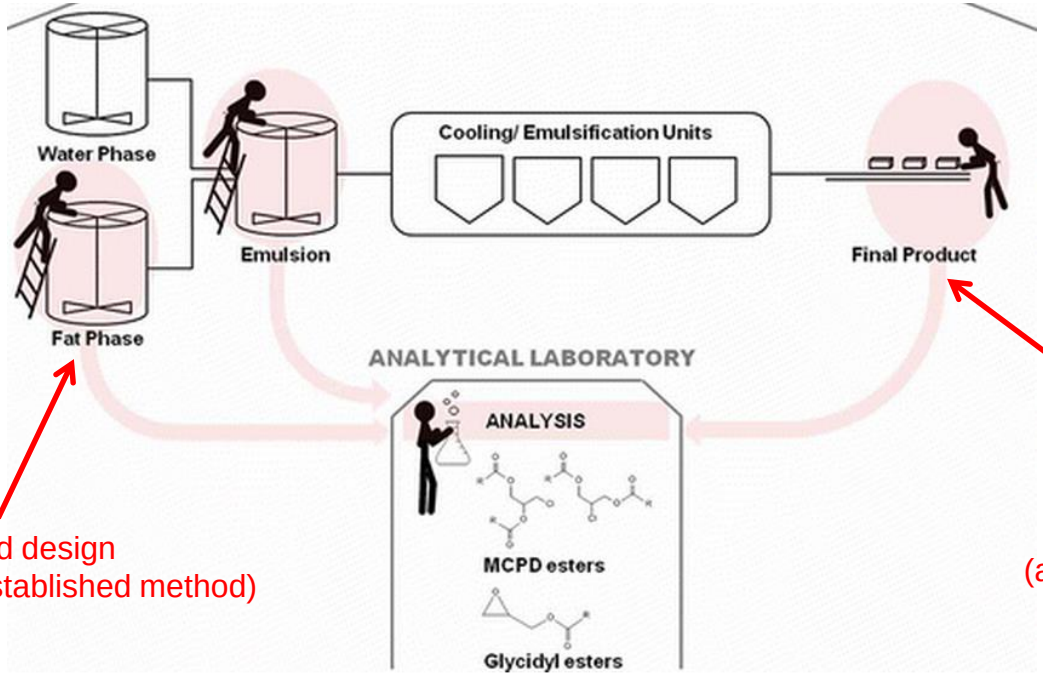


Food product (example)

Spiking food products? ✗
(→ different recovery of native/spiked compounds!)

Tackling major challenge

Manufacturing of a reference material with known levels of MCPDE/GE
(known contamination level in raw material and final product)



Fat blend design
(analysis by the established method)

Final product
(analysis by the tested method)

Scheme from: Alessia Ermacora, Karel Hrnčiřík (2014). Food Additives & Contaminants: Part A, Vol. 31, No. 6, 985–994.

Testing material

AOCS Collaborative study Cd30-15

- samples produced at pilot-plant scale by Unilever
- different matrix (o/w- & w/o- emulsions), fat content and formulations
- desired level of contamination achieved by blending oils/fats with known levels of MCPDE/GE

Product	Sample ID	Fat level (%)
A. Brick margarine	AOCS 2015 - 07 / 10	75
B. Soft margarine	AOCS 2015 - 06	75
C. Soft margarine	AOCS 2015 - 11	40
D. Mayonnaise	AOCS 2015 - 09	75
E. Mayonnaise	AOCS 2015 - 05 / 08	30

Testing material

AOCS Collaborative study Cd30-15

Homogeneity check

- according ISO/IEC 17043:2010; ISO 13528:2005; IUPAC → passed ✔

Stability study

- for various storage/transport temperatures (-18°C, 4°C, ~20°C) / 6 wk
- no degradation observed ✔

Assigned values [1,2]

Sample ID	Bound 3-MCPD (mg/kg)	Bound 2-MCPD (mg/kg)	Bound glycidol (mg/kg)
AOCS 2015 - 07 / 10	1.06 ± 0.06	0.53 ± 0.02	3.03 ± 0.20
AOCS 2015 - 06	0.45 ± 0.05	0.23 ± 0.03	0.37 ± 0.04
AOCS 2015 - 11	0.23 ± 0.04	0.11 ± 0.02	0.13 ± 0.02
AOCS 2015 - 09	0.43 ± 0.05	0.21 ± 0.03	0.19 ± 0.03
AOCS 2015 - 05 / 08	0.11 ± 0.02	*(0.06 ± 0.01)* [3]	*(< 0.03)* [4]

1) assigned values determined by applying the extraction method coupled with Cd29a-13 (isotope dilution mass spectrometry with bracketing calibration)
2) ± expanded uncertainty of the assigned value (k=2)
3) concentration of the analyte was below the limit of quantification
4) concentration of the analyte was below the limit of detection

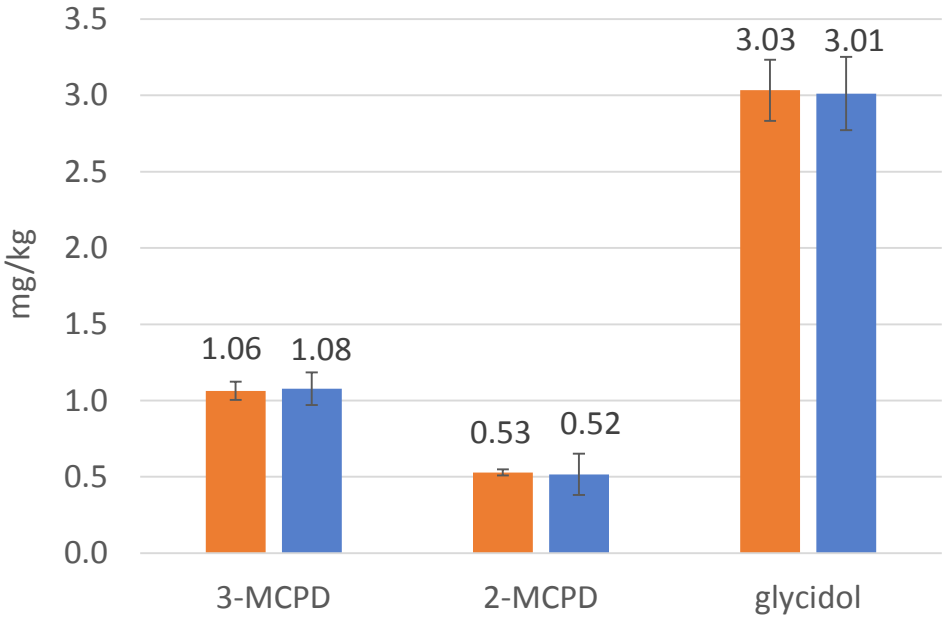
Data sets collected

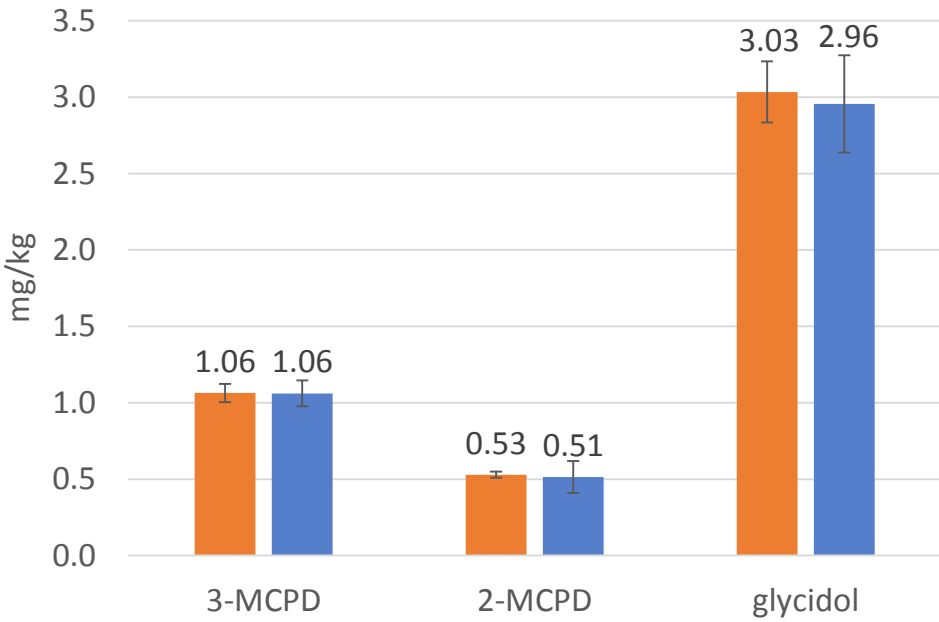
	AOCS Cd29a-13	AOCS Cd29b-13	AOCS Cd29c-13	Other methods
Bound 3-MCPD	17	3	2	1
Bound 2-MCPD	16	3	1	1
Bound glycidol	17	3	2	1

Data used for the statistical evaluation

A. Brick margarine (75%)

AOCS 2015 - 07 / 10



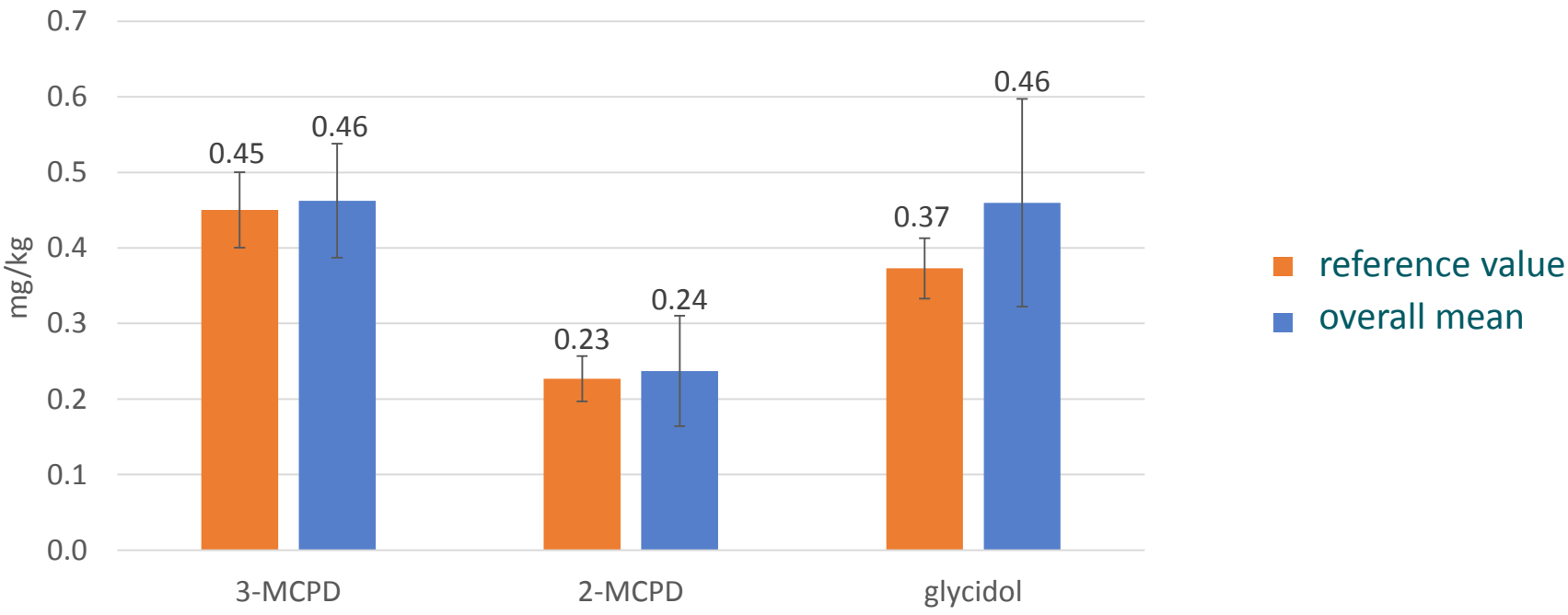


reference value

overall mean

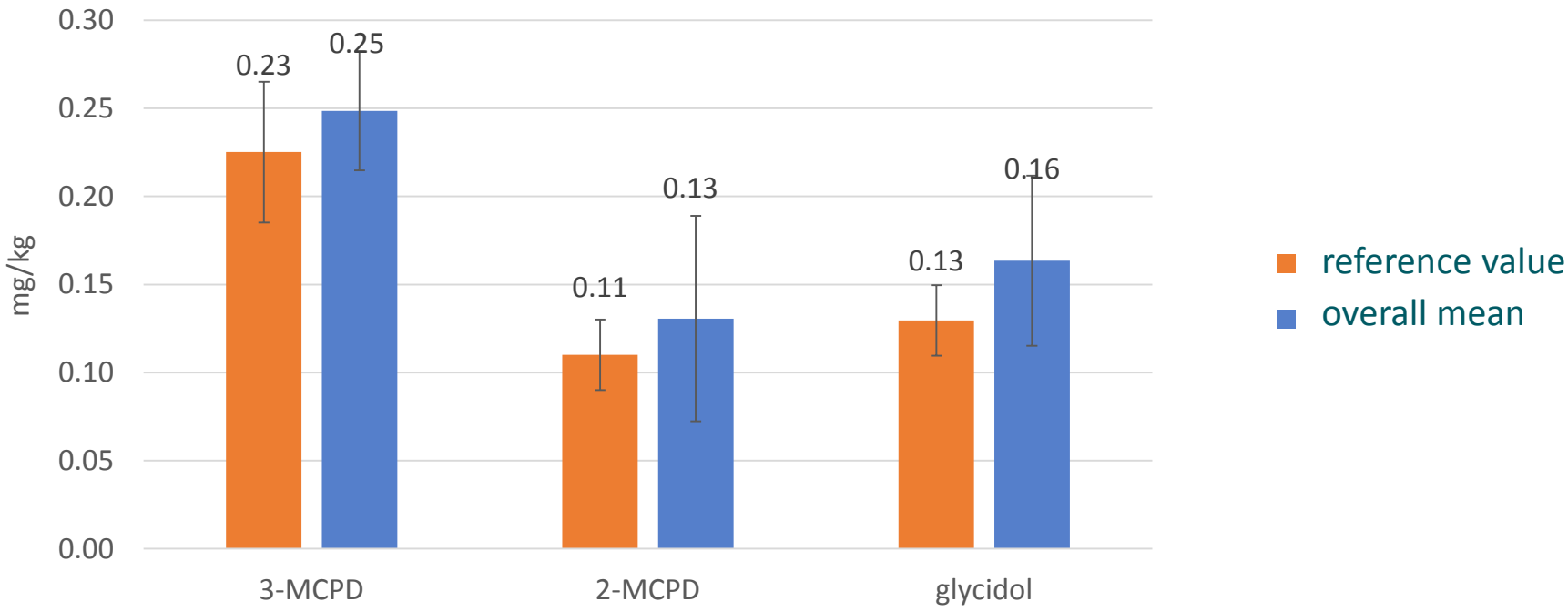
B. Soft margarine (75%)

AOCS 2015 - 06



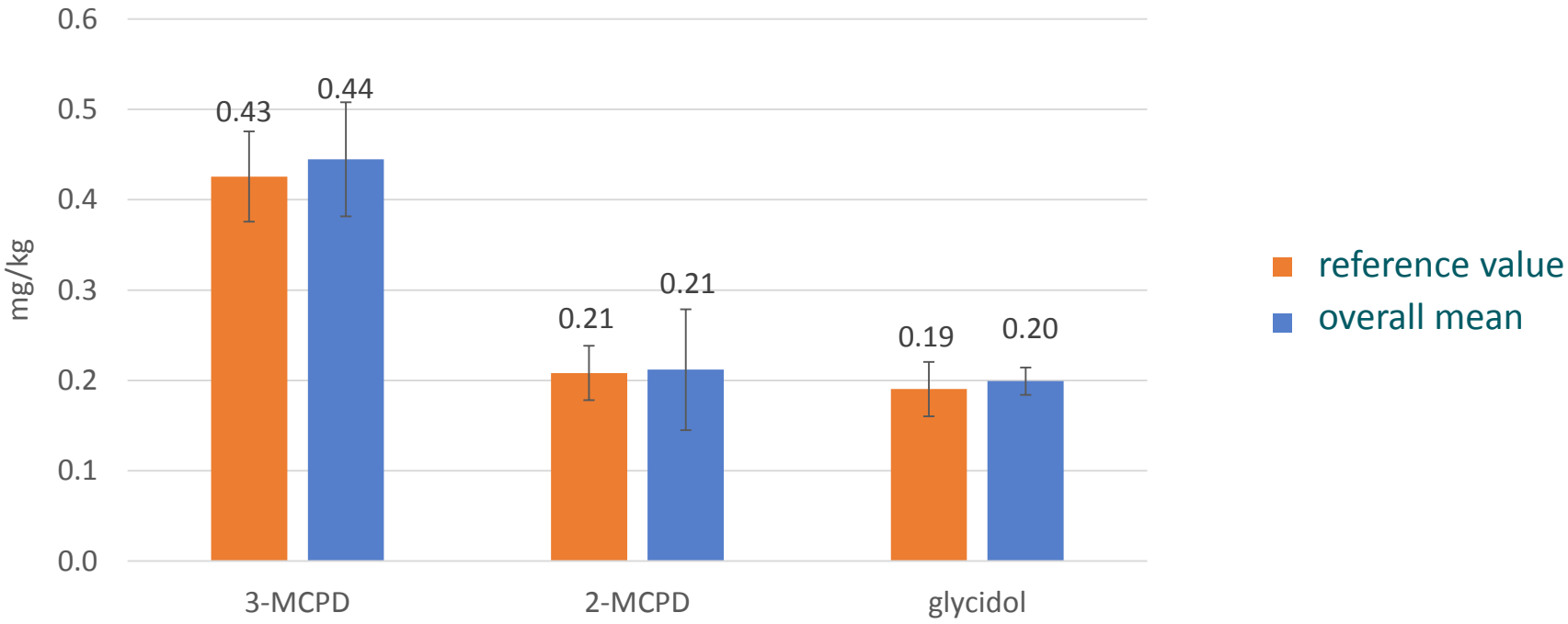
C. Soft margarine (40%)

AOCS 2015 - 11



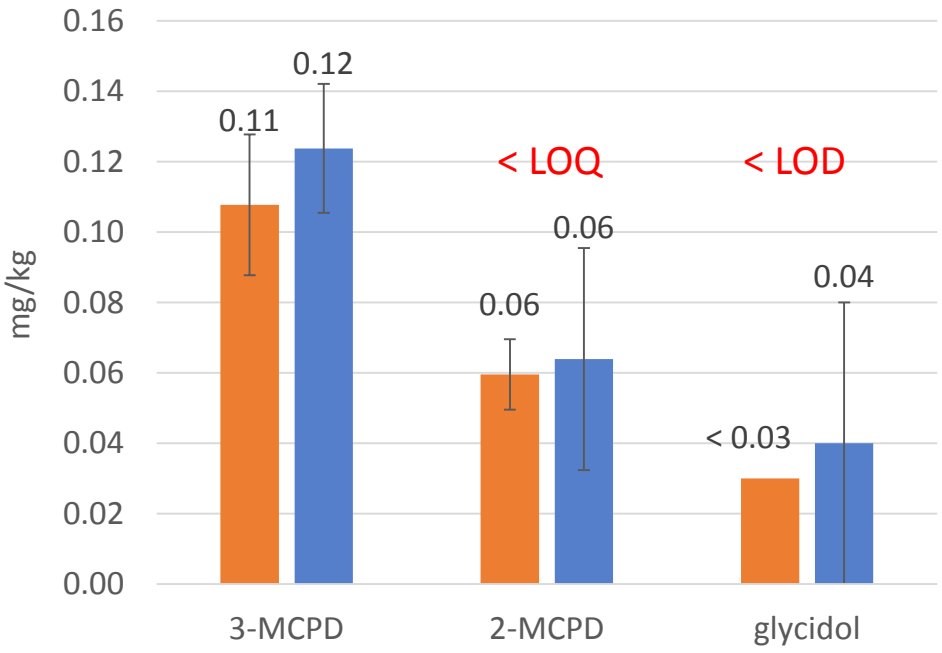
D. Mayonnaise (75%)

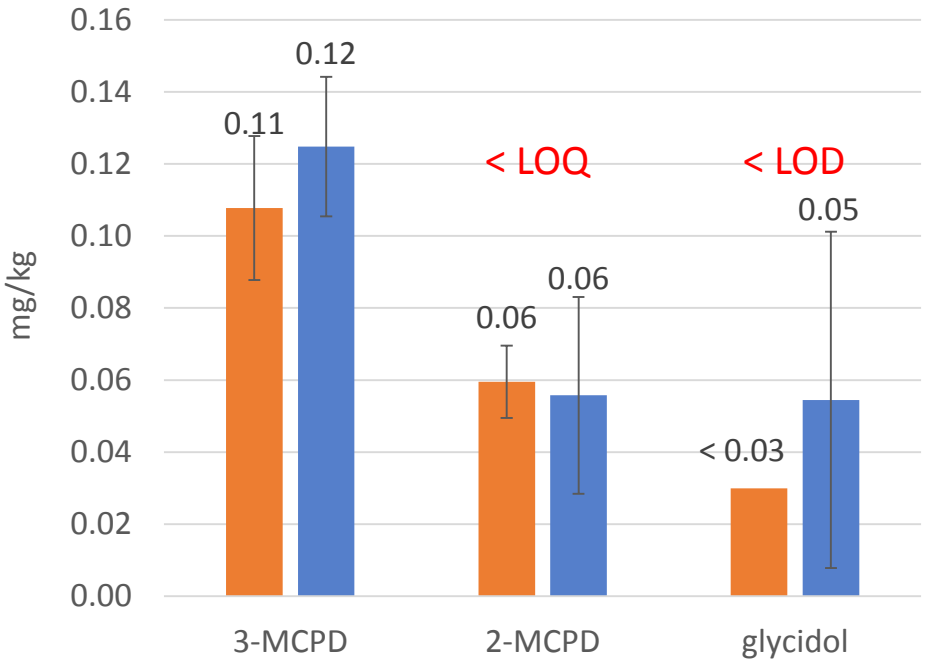
AOCS 2015 - 09



E. Mayonnaise (30%)

AOCS 2015 – 05 / 08





reference value

overall mean

Summary

AOCS Collaborative study Cd30-15

Current method extensively tested:

- on real samples (no spiking) with known levels of MCPDE/GE (accuracy)
- on different emulsified foodstuff varying in formulation (applicability)
- across a wide range of contamination level with the focus on low end (sensitivity of the method tested to its limits)

Results: “beyond expectations” for trace contaminant analysis

Method reasonably easy to adopt (a number of participants started from scratch)

Five new methods approved by the AOCS Uniform Methods Committee*:

- Ac 6-16 (Official Method) Extraction and Indirect Enzyme-Linked-Lectin-Assay (ELLA) Analysis of Soybean Agglutinin in Soybean Grain
- Cd 12c-16 (Standard Procedure) Accelerated Oxidation Test for the Determination of Oxidation Stability
- Cd 30-15 (Official Method) Analysis of 2- and 3-MCPD Fatty Acid Esters and Glycidyl Fatty Acid Esters in Oil-Based Emulsions
- Ce 12-16 (Official Method) Sterols and Stanols in Foods and Dietary Supplements Containing Added Phytosterols
- Ce 13-16 (Recommended Practice) Determination of Cyclopropenoic and Nutritional Fatty Acids in Cottonseed and Cottonseed Oil by Gas Chromatography

*Available for purchase through the AOCS website

Concluding remarks

Clear demand to establish reliable (official) methods for various food matrices

- methods for oil/fats can be potentially applied
- however, it must be only combined with an optimized fat isolation procedure

- each commodity (matrix) requires an individual approach

- extensive testing & validation are required by using reference material:
  - real samples (pref. avoiding spiking)
  - compositional variation (fat%, emulsifiers, …)
  - wide range of contamination levels (extra focus on low end)

Questions & inquiries:

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