Limitation of Indirect Quantification for Glycidol Fatty Acid Ester in Edible Oils

Kao Corporation
Global RD, Healthcare Food Labs.
Indirect Quantification for GE (DGF Method)

Combination of two different pretreatment

- **Option A**
  - Test Oil
  - Ester Cleavage
  - Derivatization (phenylboronic ester)
  - GC/MS
  - 3-MCPD forming substance (MCPD-FS)
    - 3-MCPD ester (MCPD-E)
    - Glycidol ester (GE)

- **Option B**
  - Test Oil
  - Acid Treatment ➔ GE Elimination
  - Test Oil
  - Ester Cleavage
  - Derivatization (phenylboronic ester)
  - GC/MS
  - 3-MCPD ester (MCPD-E)

GE is calculated as the difference in two options.
Keys for Indirect Determination

Indirect determination is based on the assumption that

- MCPD-FS consist of GE and MCPD-E
- Complete elimination of only the GE by acid treatment

Acid treatment condition

\[
\text{(Test oils + sulfuric acid in 1-propanol)} \\
\downarrow \\
\text{Sonication at 45 deg-C for 15min}
\]

Acid treatment in option B is the critical step.
**cf. Direct Quantification for GE**

Masukawa *et al.* (2010)

Test Oil

↓

Double Solid-phase Extraction

(Reverse $\rightarrow$ Normal phase)

→ Removal of Acylglycerols

↓

LC/MS

↓

Glycidol esters

(Palmitate, Stearate, Oleate, Linolate, Linoleate)

**Five species of GE are directly detected.**
Objectives

In preliminary tests, the indirect method gave lower GE levels than the direct method in some oil compositions.

Verification of scope and limitation of the indirect method from the aspect of oil composition.

Focusing on the acid treatment in option B.

- Dose the GE eliminate completely?
- Any influences of partial acylglycerols (PGs)?
Materials and Methods

- Test oils: “MCPD-FS free oils + GE”
  → Oils contain only GE as MCPD-FS
- Method: Monitoring MCPD-FS levels during acid treatment
  → treatment time: 0 to 90min

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Test oils</th>
<th>Objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>“TAG+GE”</td>
<td>- GE decomposition</td>
</tr>
<tr>
<td>#2</td>
<td>“TAG+PG”</td>
<td>- Impact of PG</td>
</tr>
<tr>
<td>#3</td>
<td>“TAG+PG+GE”</td>
<td>- Interaction</td>
</tr>
</tbody>
</table>
Preparation for Test Oils

- MCPD-FS free oils were prepared from rapeseed oil with enzymatic and chromatographic technique.
- Oils containing GE (25 to 810 µmol/kg) → Glycidyl stearate (GS) was spiked to the oils.
- TAG+DAG (10 to 100 wt% DAG) → Mixture of TAG- and DAG-rich oil.
- TAG+MAG (5 and 10 wt% MAG) → Mixture of TAG- and MAG-rich oil.

<table>
<thead>
<tr>
<th>MCPD-FS free oils</th>
<th>MAG-rich oil</th>
<th>DAG-rich oil</th>
<th>TAG-rich oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAG (wt%)</td>
<td>99.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DAG (wt%)</td>
<td>0.9</td>
<td>98.1</td>
<td>0.0</td>
</tr>
<tr>
<td>TAG (wt%)</td>
<td>0.0</td>
<td>1.9</td>
<td>100</td>
</tr>
<tr>
<td>MCPD-FS (µmol/kg)*</td>
<td>&lt; 1.8</td>
<td>&lt; 1.8</td>
<td>&lt; 1.8</td>
</tr>
</tbody>
</table>

* LOQ = 1.8 µmol/kg
GE decomposition during acid treatment

Test oils: TAG + GS

Changes in GS levels as MCPD-FS

Spiked GS and residual MCPD-FS levels

Complete elimination of GE was not achieved

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18th May, 2010, AOCS meeting in Phoenix AZ
Influence of PG on MCPD-FS level in op.B

- **TAG-DAG** test oils without GE

- **TAG-MAG** test oils without GE

MCPD-FS were generated in proportional to PG

18th May, 2010, AOCS meeting in Phoenix AZ
Residual MCPD-FS and underestimation of GE

Presence of PG and GE in test oils caused residual MCPD-FS not originated from MCPD-E in option B.

\[
GE = \text{option A} - \left( \text{option B} \right)
\]

\[
= \text{option A} - \left( \text{MCPD-E} + \text{GE} \right) - \left( \text{MCPD-E} + \text{MCPD-FS} \right)
\]

Residual MCPD-FS leads to underestimation of GE
**GE in the Market Oils with Different Method**

<table>
<thead>
<tr>
<th></th>
<th>Cooking oil A (Palm blend)</th>
<th>Cooking oil B (Rice bran)</th>
<th>Spread A (Oil phase)</th>
<th>Spread B (Oil phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Partial acylglycerols, wt-%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>0.0</td>
<td>0.0</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>4.0</td>
<td>6.8</td>
<td>2.5</td>
<td>1.1</td>
</tr>
</tbody>
</table>

**“Indirect” determination, mg as 3-MCPD/kg**

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<tbody>
<tr>
<td>MCPD-FS (Level A)</td>
<td>3.9 ± 0.1</td>
<td>9.6 ± 0.1</td>
<td>1.1 ± 0.0</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>MCPD-E (Level B)</td>
<td>2.3 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>GE (A - B)</td>
<td>1.7</td>
<td>7.6</td>
<td>-0.2</td>
<td>-0.5</td>
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**“Direct” determination, mg as 3-MCPD/kg**

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<tr>
<td>GE</td>
<td>2.3</td>
<td>9.6</td>
<td>1.2</td>
<td>0.7</td>
</tr>
</tbody>
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Difference in both method

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<th>Spread B (Oil phase)</th>
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<tr>
<td>Difference</td>
<td>-0.6</td>
<td>-2.0</td>
<td>-1.4</td>
<td>-1.2</td>
</tr>
</tbody>
</table>

**Indirect method gave lower GE levels**

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Conclusion

Applying the indirect method for GE quantification,

- Oils containing higher GE or PG brought about underestimation of GE, due to their incomplete decomposition and/or MCPD-FS generation.

- Thus, PG and GE levels of test oils should be taken into consideration.