RAPID PAPER

Comparison of Indirect and Direct Quantification of Glycidol Fatty Acid Ester in Edible Oils

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Abstract: Two different methods for determining the levels of glycidol fatty acid esters (GEs) in edible oil—the German official indirect method and the direct LC-MS method—are compared. In some cases, the indirect method showed lower GE levels than the direct method. This was investigated using model studies, which revealed two possible causative factors during the acid treatment of the indirect method: (1) incomplete elimination of GE in oil that was high in GEs initially and (2) generation of GEs and/or its relevant compounds in oil that was rich in partial acylglycerol. Both these factors contributed to the subsequent underestimation of GE levels. The above technical limitations of the indirect method found in the present study has led to the inference that the direct method can more precisely determine the GE levels for a wider range of fats and oil products than the indirect method.

Key words: indirect determination, direct determination, edible oil, glycidol fatty acid ester, partial acylglycerol

1 INTRODUCTION

Glycidol fatty acid esters (GEs) are minor food contaminants that have been recently identified in edible oils; they are similar to 3-monochloropropane-1, 2-diol (3-MCPD) fatty acid esters (MCPD-Es)^{1, 2)}. Two primary different methods were recently reported for determining GE levels in edible oils. One is an indirect quantification method using gas chromatography/mass spectrometry (GC-MS), which has been presented by the German Society for Fat Science (DGF) in November 2009 as the official method³⁾. This method is based on a method proposed by Weißhaar and Perz⁴⁾. The indirect method consists of two different pretreatments—Option A and Option B (Table 1). Option A determines the level of 3-MCPD and 3-MCPD-forming substances (MCPD-FSs), which involves GEs and MCPD-Es. Option B determines only the MCPD-Es level, which results from the residual MCPD-FSs after GEs have been eliminated by acid treatment. The GE level is calculated as the difference between the two determinations. Thus, this indirect method seems to determine the level of GEs under the assumptions that 3-MCPD-FS is simply composed of 3-MCPD-Es and GEs and that GEs are eliminated com-

pletely by the acid treatment process employed in Option B. The other method is one reported by Masukawa *et al.*, which directly quantifies five species of GE in edible oils with a combination of a double solid-phase extraction (SPE) and liquid chromatography-mass spectrometry (LC-MS). This direct method has been validated in terms of the trueness and precision of the quantitative values produced by a spike and recovery study of GEs using triacylglycerol (TAG)- and/or diacylglycerol (DAG)-rich oils^{5, 6)}. In comparing the indirect and direct methods, using the same samples, we noticed that the indirect method produced lower GE level estimates for some oil compositions.

In the present study, we conducted a series of model studies to examine the impact of varying minor components of edible oils, such as GEs and partial acylglycerols (PGs), on the determination of GE levels via the indirect method, paying particular attention to the acid treatment process as the critical step. Changes in MCPD-FS levels during acid treatment were identified in the MCPD-E free oils, consisting of various levels of TAGs, DAGs, monoacylglycerol (MAGs), and spiked GEs, to examine the influence of GE and PG content on GE determination. Herein, we de-

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Table 1 Analytical scheme for indirect method

Option	Scheme	Detection
A	<p>MCPD-E</p> $\begin{array}{c} \text{H}_2\text{C}-\text{OCOR}_1 \\ \\ \text{HC}-\text{OCOR}_2 \\ \\ \text{H}_2\text{C}-\text{Cl} \end{array} \xrightarrow{\text{NaOMe}} \begin{array}{c} \text{H}_2\text{C}-\text{OH} \\ \\ \text{HC}-\text{OH} \\ \\ \text{H}_2\text{C}-\text{Cl} \end{array}$	<p>“MCPD-E” and “GE”</p>
	<p>GE</p> $\begin{array}{c} \text{H}_2\text{C}-\text{OCOR}_1 \\ \\ \text{HC} \diagup \text{O} \\ \\ \text{H}_2\text{C} \end{array} \xrightarrow{\text{NaOMe}} \begin{array}{c} \text{H}_2\text{C}-\text{OH} \\ \\ \text{HC} \diagup \text{O} \\ \\ \text{H}_2\text{C} \end{array}$	
B	<p>MCPD-E</p> $\begin{array}{c} \text{H}_2\text{C}-\text{OCOR}_1 \\ \\ \text{HC}-\text{OCOR}_2 \\ \\ \text{H}_2\text{C}-\text{Cl} \end{array} \xrightarrow[1\text{-PrOH}]{\text{H}_2\text{SO}_4} \begin{array}{c} \text{H}_2\text{C}-\text{OCOR}_1 \\ \\ \text{HC}-\text{OCOR}_2 \\ \\ \text{H}_2\text{C}-\text{Cl} \end{array} \xrightarrow{\text{NaOMe}} \begin{array}{c} \text{H}_2\text{C}-\text{OH} \\ \\ \text{HC}-\text{OH} \\ \\ \text{H}_2\text{C}-\text{Cl} \end{array}$	<p>“MCPD-E”</p>
	<p>GE</p> $\begin{array}{c} \text{H}_2\text{C}-\text{OCOR}_1 \\ \\ \text{HC} \diagup \text{O} \\ \\ \text{H}_2\text{C} \end{array} \xrightarrow[1\text{-PrOH}]{\text{H}_2\text{SO}_4} \begin{array}{c} \text{H}_2\text{C}-\text{OCOR}_1 \\ \\ \text{HC}-\text{OH} \\ \\ \text{H}_2\text{C}-\text{OPr} \end{array} \xrightarrow{\text{NaOMe}} \begin{array}{c} \text{H}_2\text{C}-\text{OH} \\ \\ \text{HC}-\text{OH} \\ \\ \text{H}_2\text{C}-\text{OPr} \end{array}$	

Table 2 Ester distributions and MCPD-FS levels in oil materials prepared from rapeseed oil (mean $\pm$ SD)

		MAGs-rich oil	DAGs-rich oil	TAGs-rich oil
Ester distribution, wt-% (N = 3)	MAG	99.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
	DAG	0.9 $\pm$ 0.0	98.1 $\pm$ 0.1	0.0 $\pm$ 0.0
	TAG	0.0 $\pm$ 0.0	1.9 $\pm$ 0.1	100 $\pm$ 0.0
MCPD-FS level (μmol as 3-MCPD/kg) *		< 1.8	< 1.8	< 1.8

* LOQ = 1.8 $\mu\text{mol/kg}$

scribe the scope and limitations of the indirect GE level estimation method with respect to oil composition.

2 MATERIALS AND METHODS

2.1 Preparation of test oils

Six edible vegetable oils, including two palm oils and two commercial spreads, were purchased from the market. Only the oil phase was used for the spreads.

TAG-rich oil was acquired from n-hexane and ethyl acetate in a ratio of 95:5 from silica-gel column chromatography of rapeseed oil (Nissin Oil Co. Ltd., Tokyo, Japan). DAG-rich and MAG-rich oils were also prepared by silica gel column chromatography from n-hexane and ethyl acetate with rapeseed fractions of 5:5 and 7:3, respectively; this was followed by enzymatic preparation of PG-rich oils, as previously reported⁷. MCPD-FS levels of TAG-, DAG-, and MAG-rich oils evaluated by the DGF method³ were all less than the limit of quantification (LOQ) 1.8 $\mu\text{mol/kg}$ (Ta-

ble 2). TAG-DAG test oils (10, 30, 50, and 100 wt% DAGs) or TAG-MAG test oils (5 and 10 wt% MAGs) were prepared by mixing the TAG-rich and DAG-rich or TAG-rich and MAG-rich oils, respectively. To prepare test oils containing GEs, 25–810 $\mu\text{mol/kg}$ of glycidyl stearate (GS) from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), was spiked in the oils.

2.2 Determination of GE level using direct LC-MS method

By using the direct LC-MS method^{5,6}, a summed level of five species of GE in the oils was determined. Thus, as standard materials, reagent grade GS was used for C18:0-GE while other GEs such as C16:0-GE, C18:1-GE, C18:2-GE, and C18:3-GE were synthesized and used⁵. The GE-rich extracts of test oils obtained by double SPE treatment were carefully dissolved in methanol/2-propanol (1:1 by vol) for LC-MS analysis, which was performed under the absolute same conditions as in prior work⁶.

2.3 Determination of MCPD-FS level using indirect method

According to the DGF Method C(III)-18³⁾, MCPD-FSs in the oils were identified. After alkaline transesterification with sodium methoxide, test oils were salted-out with 20% sodium chloride solution. Phenyl boronic ester derivatives of polyols were then analyzed using GC-MS (Option A). In order to eliminate GEs, test oils were treated with 0.5% sulfuric acid in 0.5-mL 1-propanol by sonicating at 45 °C for a period of time ranging from 300 to 5,400 s prior to the determination of MCPD-FS (Option B). GE levels were inferred from the difference between the two determinations, Option A-Option B. This determination was run in triplicate.

3 RESULTS AND DISCUSSION

3.1 Comparison of GE levels as measured by indirect and direct methods

The GE levels in edible cooking oils and fat spreads from a local market were determined by both the indirect method³⁾ and the direct LC-MS method⁶⁾, as shown in Table 3. The indirect method gave lower GE levels than those derived from the direct method. In particular, in the case of the spreads containing MAGs, the GE levels determined by the indirect method gave negative values, because the MCPD-FS levels were higher in Option B than in Option A. An increase in the MCPD-FS level of cooking oil A, which

contained few PGs, was not confirmed by Option B. Since these differences in determination values were more distinguishable by the direct method for those oils containing a high PG, and/or GE, level, the influence of oil composition on the underestimation of GE by the indirect method is apparent.

3.2 Examination using test oils

In order to clarify the impact of oil composition on the indirect determination of GE, acid treatment, which is the critical step for indirect GE determination, was examined by means of a model study using MCPD-E-free test oils.

The alteration of GS in the TAG-rich oils, containing no PGs, during acid treatment, was tested and the results are shown in Fig. 1. GS detected as MCPD-FS decreased rapidly with treatment, however, continued acid treatment for 900 s (set by the DGF method) did not eliminate all MCPD-FS. Complete elimination of MCPD-FS was not achieved, even after prolonged treatment, and elimination ceased after 1,800 s of the treatment. Since the residual amount of MCPD-FS was proportional to the amount of spiking, it was suspected that either a considerable rate of reverse reaction produced GS from the decomposition product, which may have been monopropyl ether, or that a by-product was detected as MCPD-FS, quantitatively generated from GS decomposition.

Changes in the MCPD-FS levels of the test oils containing PG, and without GE during acid treatment, are shown in Fig. 2. Although no influence was observed from the

Table 3 GE content in oils from the market with different determinations (mean $\pm$ SD)

	Commercial cooking oil				Palm oil		Spread (Oil phase)	
	A	B	C	D	A	B	A	B
PG content, wt-% (N = 3)								
Monoacylglycerol	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.1 $\pm$ 0.1	1.1 $\pm$ 0.0
Diacylglycerol	0.8 $\pm$ 0.1	4.0 $\pm$ 0.0	6.8 $\pm$ 0.0	6.8 $\pm$ 0.3	7.2 $\pm$ 0.1	5.6 $\pm$ 0.3	2.5 $\pm$ 0.0	1.1 $\pm$ 0.2
Indirect determination, μ mol/kg * (by DGF method, N = 3)								
MCPD-FS (by option A)	<1.8	35.5 $\pm$ 0.7	84.6 $\pm$ 0.9	87.0 $\pm$ 0.5	68.1 $\pm$ 0.1	61.4 $\pm$ 0.5	10.3 $\pm$ 0.3	4.1 $\pm$ 0.3
MCPD-E (by option B)	<1.8	20.5 $\pm$ 0.5	31.9 $\pm$ 0.9	18.2 $\pm$ 0.6	45.8 $\pm$ 0.5	44.6 $\pm$ 0.4	11.8 $\pm$ 0.6	8.6 $\pm$ 0.7
GE (Op.A - Op.B)	—	15.0	52.8	68.6	22.4	16.5	-1.5	-4.5
Direct determination, μ mol/kg** (by LC-MS method, N = 3 for commercial cooking oils and palm oils, N = 2 for spreads)								
Glycidylpalmitate	<1.6	5.7 $\pm$ 0.2	6.4 $\pm$ 0.2	11.1 $\pm$ 0.1	7.6 $\pm$ 0.1	7.3 $\pm$ 0.1	<1.6,<1.6	<1.6,<1.6
Glycidylstearate	<1.5	<1.5	<1.5	<1.5	1.5 $\pm$ 0.1	<1.5	<1.5,<1.5	<1.5,<1.5
Glycidyloleate	<1.5	11.8 $\pm$ 0.2	31.6 $\pm$ 0.8	34.0 $\pm$ 0.6	16.0 $\pm$ 0.5	12.1 $\pm$ 0.4	7.4,8.3	4.4,3.5
Glycidyllinolate	<1.5	6.2 $\pm$ 0.3	28.2 $\pm$ 1.1	37.7 $\pm$ 0.1	5.1 $\pm$ 0.1	3.9 $\pm$ 0.2	3.6,3.3	2.2,2.3
Glycidyllinoleate	<1.5	1.6 $\pm$ 0.1	<1.5	<1.5	<1.5	<1.5	<1.5,<1.5	<1.5,<1.5
Total GE	<7.5	25.4	66.2	82.8	30.1	23.3	11.3	6.1

* LOQ = 1.8 μ mol/kg

**LOQ = 1.6 μ mol/kg for Glycidylpalmitate, 1.5 μ mol/kg for other four GEs.

Fig. 1 Changes in MCPD-FS levels in TAG-rich test oils with spiked GS during acid treatment. Spiked GS levels (GS_{init}) were 25 $\mu\text{mol/kg}$ (triangle), 79 $\mu\text{mol/kg}$ (diamond), 240 $\mu\text{mol/kg}$ (square), and 810 $\mu\text{mol/kg}$ (circle). MCPD-FS levels in the vertical axis equate to the quantitative values obtained from Option B in the indirect method³⁾.

treatment in the case of TAGs-rich oil, MCPD-FS in the oils containing PG increased with acid treatment duration. The increasing rate and the equilibrium level of MCPD-FS were proportional to each PG level. The increasing MCPD-FS

levels were more remarkable in oils containing MAGs than in those containing DAGs. These results revealed the generation of MCPD-FS from PGs under acid treatment conditions, although the origin of the MCPD-FS detected remains unknown.

The MCPD-FS levels following acid treatment should be zero if the process is valid, because all the test oils contained no MCPD-Es. However, GEs were not completely eliminated and MCPD-FSs were generated from PGs as a result of the acid treatment. Residual MCPD-FSs following acid treatment was identified as MCPD-Es, thus, these results bring about overestimation of MCPD-FSs and subsequent underestimation of GEs, as a result of the assumptions of the indirect method. These results support the analytical results of the market oils mentioned above (Table 3). Therefore, when using the indirect method, a higher level of PGs and/or GEs in oils results in greater underestimation of GE levels.

4 CONCLUSIONS

A series of model studies indicate that the indirect method results in underestimation of GE levels compared to the direct method because of the incomplete elimination of GEs and/or the generation of MCPD-FS during the acid treatment process. Although most conventional cooking oils are low in GEs and PGs, significant error in the determination of GEs may occur when dealing with oils such as palm oil, which are reported to have a higher content of

Fig. 2 Changes in MCPD-FS levels in test oils containing PGs (DAGs or MAGs) with no GE spiked during acid treatment.

Test oils: no PGs = only TAGs (cross), DAG 10% (circle), DAG 30% (triangle), DAG 50% (diamond), and DAG 100% (square) on the left (a); and, MAG 5% (circle) and MAG 10% (triangle) on the right (b). MCPD-FS level: same as that in Fig. 1.

GEs and PGs, as compared to other oils¹⁾. On the other hand, direct LC-MS measurement was not affected by the amount of GEs and PGs⁵⁾. The technical limitations of the indirect method revealed in the present study indicate that the direct method can more precisely determine the GE levels in a wider range of edible oil products than the indirect method.

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