Malaysian Journal of Analytical Sciences
(MJAS)
Published by Malaysian Analytical Sciences Society

GLYCIDYL ESTERS: AN UPDATED MINI-REVIEW OF ANALYTICAL METHODS (2015-2022)

(Glisidil Ester: Suatu Tinjauan Terkini Ulasan Mini bagi Kaedah Analisis (2015-2022))

Nor Syuhada Mat Shukri[1], Nur Syafiqah Subri[1], Siti Munirah Ishak[2], Nur Nadhirah Mohamad Zain[2], Mazidatulakmam Miskam[3], Wan Nazwanie Wan Abdullah[3], Sazlinda Kamaruzaman[4], Nor Suhaila Mohamad Hanapi[5], Wan Nazihah Wan Ibrahim[5], Muggundha Raoov[6], and Noorfatimah Yahaya[2*]

[1]Faculty of Industrial Science & Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak,
26300 Gambang, Kuantan, Pahang, Malaysia
[2]Department of Toxicology, Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia, 13200 Bertam Kepala Batas, Penang, Malaysia
[3]School of Chemical Sciences, Universiti Sains Malaysia, 11800, USM Pulau Pinang, Malaysia
[4]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
[5]Faculty of Applied Sciences, Universiti Teknologi MARA, 40450, Shah Alam, Selangor, Malaysia
[6]Department of Chemistry,Faculty of Science, Universiti Malaya, Kuala Lumpur 50603, Malaysia

[*]Corresponding author: noorfatimah@usm.my

Received: 2 September 2022; Accepted: 21 August 2023; Published: 30 October 2023

Abstract

Recent issues on the presence of glycidyl ester (GE), a potentially carcinogenic compound, in food have sparked consumer worry. Fatty acid esters of glycidol pose potential food safety concerns and have been classified by IARC as a “likely human carcinogen”. They are generated during the high-temperature deodorization step. Vegetable oils consist primarily of five fatty acids, palmitic acid, linolenic acid, linoleic acid, oleic acid, and stearic acid. Palm kernel and coconut oil also contain significant quantities of lauric acid and myristic acid. These fatty acids of edible oil undergo various reactions, producing different GE. Researchers have developed a GE quantitation method called “indirect” methodology, but this method uses hydrolysis to release glycidol from GE instead of directly targeting the intact fatty acid esters. It also requires a derivatization step before gas chromatography-mass spectrometry (GC–MS) analysis. While the early methods developed to target these contaminants exclusively utilized the ‘indirect’ approach, they were critical in bringing attention to the presence of GE in oils. The factors that are highly related to GE formations include process temperature, process duration, and the presence of precursors. This paper reviews previous studies on various methods and instruments that focused on detecting GE in different matrices from 2015 to 2022.

Keywords: glycidyl ester, deodorization, hydrolysis

Abstrak

Isu terkini mengenai kehadiran ester glisidil (GE), sebatian berpotensi karsinogenik, di dalam makanan telah mencetuskan kebimbangan dalam kalangan pengguna. Ester asid lemak glisidol menimbulkan potensi keselamatan makanan dan telah diklasifikasikan oleh IARC sebagai "kemungkinan karsinogen manusia". Ia dihasilkan semasa proses penyahbauan pada suhu tinggi. Minyak sayuran yang utama terdiri daripada lima asid lemak, asid palmitik, asid linolenat, asid linoleat, asid oleik, dan asid stearat. Biji kelapa sawit dan minyak kelapa juga mengandungi asid laurik dan asid miristik dalam jumlah yang banyak. Asid-asid lemak dalam minyak yang boleh dimakan tersebut melalui pelbagai tindak balas, menghasilkan GE yang berbeza. Para penyelidik telah membangunkan kaedah kuantitasi GE yang disebut metodologi "tidak langsung", tetapi kaedah ini menggunakan hidrolisis untuk membebaskan glisidol dari GE dan bukannya menyasarkan secara terus ester asid lemak yang utuh. Kaedah ini juga memerlukan langkah derivatisasi sebelum analisis kromatografi gas-spektrometri jisim (GC–MS). Meskipun kaedah-kaedah awal yang dibangunkan untuk mengesan bahan cemar ini menggunakan pendekatan tidak langsung secara eksklusif, kaedah-kaedah tersebut sangat penting dalam mengarah perhatian kepada kehadiran GE di dalam minyak. Antara factor-faktor yang berkait rapat dengan penghasilan GE termasuklah suhu proses, masa proses dan kehadiran pelopor. Makalah ini membentangkan resensi kajian-kajian terdahulu mengenai pelbagai kaedah dan instrumen yang tertumpu kepada pengesanan GE dalam pelbagai matriks dari tahun 2015 hingga 2022.

Kata kunci: glisidil ester, penyahbauan, hidrolisis

Introduction

Glycidyl fatty acid esters or glycidol (GE) are contaminants commonly found in most refined fats and oils, including edible oil such as palm oil and vegetable oil or any food made from these oils like margarine and fried products. Palm oil, corn oil, and coconut oil have been recorded with the highest presence of GE. The food industry uses palm oil because of its stability to high temperatures and long shelf life [1]. In some research, GE has been found in infant formula due to the use of vegetable oils [2]. It was also reported that the most abundant GE of fatty acids were esters of the palmitic and oleic acids [3]. Hence, the presence of GE has raised the concern of many people as GE is a carcinogenic compound. In general, a process called oil deodorization at a temperature within 230 ℃ to 270 ℃ could lead to the formation of GE as byproducts [4]. Specifically, this compound is formed from diacylglycerol (DAG) or monoacylglycerol (MAG) at high temperatures. The release of glycidol happens after GE is broken down during the digestion process. According to a report in European Food Safety Authority (EFSA) Journal in 2016, of all the available refined vegetable oils, palm-based fat and oils contain the highest amount of monochloropropanediol (MCPD) in the form of ester. Figure 1 and Figure 2 depict the general structure of GE and the chemical structures of several GE, respectively.

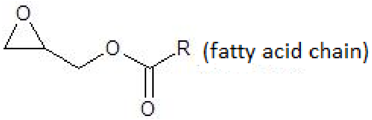

Figure 1. General structure of GE

Since gut lipases rapidly digest GE to free glycidol, GE intake should be considered a source of glycidol. Therefore, International Agency for Research on Cancer (IARC) suggested the application of the ALARA (As Low As Reasonably Achievable) principle on this genotoxic carcinogenic compound [3]. In 1990, the U.S. National Toxicology Program declared the multisite carcinogenicity of glycidol towards rodents. Later in 2010, an estimation of 1.3 mg/kg/day of glycidol was recorded for human cancer potency by the California Environmental Protection Agency [5].

As mentioned above, the presence of GE is due to the process of oil deodorization at high temperatures. The synergistic interaction between stripping stream rate and temperature is significant to the formation of GE. In the refining process of conventional edible oils, deodorization is the last procedure performed. The main purpose of this step is to remove undesirable substances. This is done to achieve the standard of oil production in taste, odor, color, and oxidative stability [6]. According to Stadler (2015), MCPD and GE's formation pathways in oil are different. Unlike MCPD, GE cannot be generated from triacylglycerols (TAG), but it is generated through intramolecular conversion and a free fatty acid chain loss. GE can also be generated via hydrolysis of MAG [4]. Arisseto et al. [8] proposed the formation of GE via either the internal nucleophilic attack of DAG or the nucleophilic attack of acyloxonium ion generated from the displacement of hydroxide group [7]. Even though GE could be formed in all edible oil during the deodorization process, they tend to occur at the highest concentration in palm oil [8].

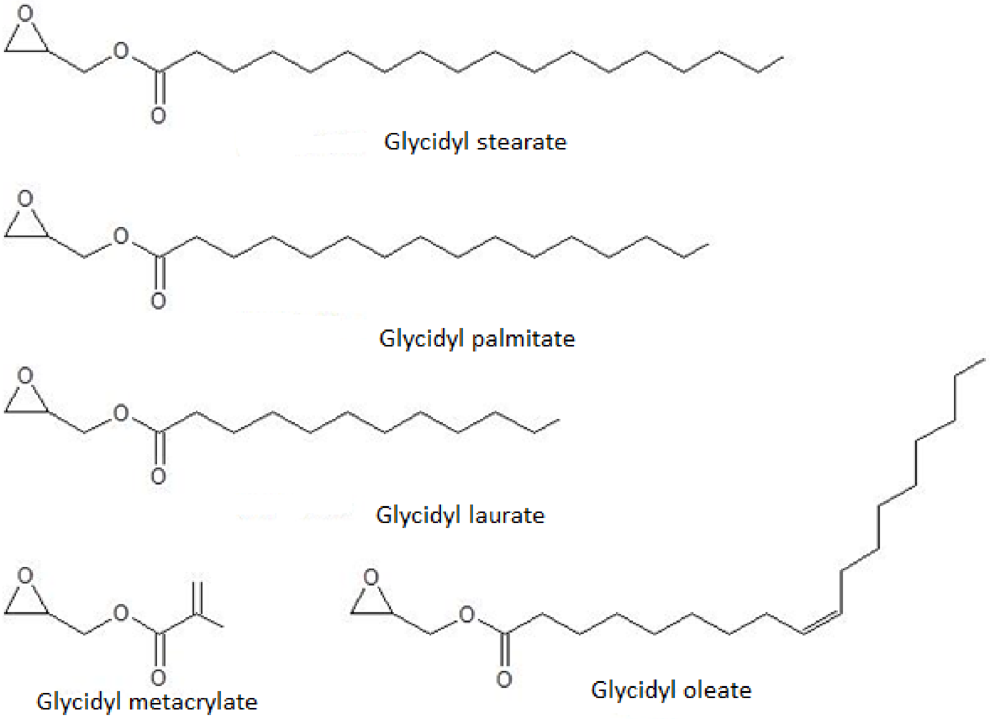

Figure 2. Chemical structures of several GE

Responding to such concern, much research is currently focusing on determining the presence of GE in edible oil. Edible oil such as palm oil was typically chosen as it provides the primary source of GE. Zelinkova et al. [10] studied the direct analysis of glycidyl ester laurate, myristate, palmitate, stearate, oleate, linoleate, and linolenate, and this method was successfully applied for the quantitation of GE in edible oils. Some research focused on determining the effect of frying temperature on GE formation since frying is one of the most convenient and common food preparation processes. According to Aniolowska et al. [3], by subjecting fat to high temperatures in the presence of the water and air during the deep-frying process, high number of new compounds that may be formed during the various hydrolytic, thermal, and oxidative reactions could be detected. In the frying process, the product of oil degradation, DAG, would react at high temperatures to form GE. GE also increased in concentration with increasing frying duration, frying temperature, and NaCl concentration [9]. Heating meat at high temperatures using an open fire charcoal grill can also generate a significant level of GE [10]. In 2017, the European Commission recommended compiling analytical data on the occurrence of GE in a broader range of food than only for oils, fats, and margarine [11]. At around the same time, the American Oil Chemists' Society (AOCS) publicized three methods suitable for analyzing GE, namely Cd 29a-13, Cd 29b-13, and Cd 29c-13 [4].

Due to the major advancement in analytical detection technologies, it is possible to analytically detect processing-derived food contaminants like GE at the level of parts per billion (ppb) [12] by using either direct or indirect approaches [7]. The direct methods allow the use of instruments like liquid chromatography-mass spectrometry (LC-MS) for quantitative and qualitative analysis of MCPD esters and GE, exactly as they are found in food. On the other hand, the indirect determination is based on the conversion of GE to glycidol. Glycidol will then be separated, derivatized, chromatographically isolated, and quantified. Several analytical methods have been customized to accommodate the indirect method for measuring GE and MCPD ester in oils and fats [11]. Solid-phase extraction (SPE) or gel permeation chromatography clean-up is adequate to achieve acceptable separation of GE and MCPD from the oil matrix [11]. Wang et al. reported the involvement of cation-mediated and free radical-mediated intermediates in the formation mechanisms of GE [13]. Elimination of fatty acid radical or hydroxyl radical would form a free radical intermediate with five or six-membered cyclic acyloxionium structure. The presence and reactivity of chlorine compounds play vital roles in the formation of MCPD esters and, to a certain degree, GE.

In recent years, the amount of GE found in processed food and edible oils has significantly increased. Their presence is concerning to public health due to their ability to release potentially toxic glycidol via hydrolysis in the digestive tract [14]. The high level of chlorinated metabolites, the use of fertilizers that contain chloride salt, and the use of HCl-activated clays during the bleaching step of the refining process of palm oil contributed to its high GE concentration compared to other edible oils. Owing to the potential health risks and the levels of these contaminants in edible oils and foodstuff, the European regulatory has set limits of 1 mg/kg and 0.5 mg/kg of bound glycidol in processed food and infant formulas, respectively. One of the main strategies to contain the formation of GE is by removing the chlorinated precursor before deodorization [14], other than blending oils, refining methods improvement and acid and enzymatic degumming.

To produce refined oil of high quality in terms of organoleptic quality, stability, and nutritional quality, undesirable compounds are removed for safe human consumption [15]. The amount of GE contents in refined palm oil (RPO) range from 0.30 to 18.00 mg/kg, much higher than the level of GE in other oils like maize oil, olive oil, etc., which is in the range of 15 to 650 μg/kg. As shown in Figure 1 and Figure 2, GE contain an epoxide group and an ester group in addition to the long chain of fatty acid compositions. Both GE and its free form after hydrolysis in gastrointestinal tract, glycidol, are carcinogenic due to the presence of epoxide group that can react with DNA to form DNA adducts. Glycidol is known as genotoxic non-threshold carcinogen with evidences of toxicity in animal even at a very small dose [15], suggesting neurotoxicity, mutagenicity, and reproductive toxicity [16]. Detection of GE using indirect quantification based on gas chromatography-mass spectrometry (GC-MS) requires extensive sample preparation including transesterification, neutralization and derivatization steps and thus, more time-consuming. Albeit needing a high number of internal standards to detect a wide range of GE, direct methods based on LC-MS are much simpler and more commonly applied for the analysis of GE present in oily matrices due to their high sensitivity and specificity [17].

In this review, analytical method development including the sample preparation and detection of different types of GE are summarized in Table 1. The present article will address the development of sample preparation methods and analytical detection system of GE in various matrices, from the year 2015 to 2022.

Analytical Determination of GE

AOCS has published three indirect methods, namely Cd 29a-13, Cd 29b-13 and Cd 29c-13 to determine the amount of MCPD esters and glycidol in edible oils such as palm oil, canola oil and sunflower oil [4]. In these methods, the sum of MCPD esters and GE from various sources were expressed as free 3-MCPD and free glycidol. For instance, in the AOCS Official Method Cd 29c-13, the addition of dilute sodium hydroxide solution or sodium methoxide in methanol frees up glycidol and 3-MCPD via base catalysis, while the addition of excess acidic chloride-containing salt solution stops the reaction [18]. The free glycidol reacts with inorganic chloride to generate additional 3-MCPD and 2-MCPD. Then phenylboronic acid (PBA) is used for derivatization process, followed by the determination by GC-MS [4]. On the other hand, having the reaction stopped with an acidic chloride-free salt solution in excess does not generate additional 3-MCPD from free glycidol. As a result, this method allows for the indirect detection of GE (bound glycidol) under the assumption that no other compounds present are reactive towards inorganic chloride at ambient temperature to produce 3-MCPD [18].

In 2015, there were research developed to study the effect of the type of oil and degree of degradation on GE content during the frying process of French Fries. The study with the main purpose to determine the content of GE in oil that used for frying French fries were developed by Anilowska et al. By using LC-MS the presence of GE can be determined and the sample were analyzed for the acid and anisidine values, colors, refractive index (RI), composition of fatty acid and also content and composition of the polar fraction [19]. As in this study it was observed that the higher of time frying the content of GE were decreased. It is recorded with losses from 47% in the rapeseed oil and 78% in palm oil. This data was recorded after the frying process finished.

As the fat were extracted from the French fries, the GE analysis were conducted on the frying oil samples [19]. This lab work developed a double SPE procedure, chloroform and acetone were used as the medium for the oil to be dissolved. Spike extract was applied for the simultaneous determination of GE, it was used as the internal standard. The percent of recovery for each of 5 standard spiked GE which is 500 ng g^{-1},1000 ng g^{-1}, 2500 ng g^{-1} and 12, 500 ng g^{-1} were 99.5-103%, 98.5-1025%, 98.0-101.5% and 89-97.5% respectively. In the fresh oil that ranged from 0.80 to 25.34 kg^{-1} the total amount of GE can be detected. The GE content for all of the samples decreased during the frying process that was carried out. In within 24 hours (1 day), GE content in palm oil and palm olein recorded about 44% and 38% losses respectively. Within 40 hours after the frying process the losses for both of these oils, which is palm oil and olein were 78% and 67% respectively. From this observation it was stated that the main parameter of the losses of GE was dependent on the type of oil used. The time range was considered as the minor effect to the losses of GE in this oil. The GE degradation was stated to undergo fast degradation in the palm oil compared to the rapeseed oil.

The data obtained from the result showed that GE tend to undergo the stabilization while in the frying condition [19]. There was thing that can influence the content of GE in the ready product, it was the composition of the oil used for the pre-frying process. This phenomenon arises due to the fat exchange that occurs between the frying medium and the products being fried during the frying process [19]. The initial absorption of fat quality during the frying process can impact the subsequent increase in GE content within the fat. The duration of frying also plays a role in the reduction of GE levels within the extracted fat from fried French fries, potentially influencing the conversion and composition of GEs in this context. Moreover, the GE content can be significantly related to the aid and refractive index value. From this study, the GE content with the highest amount can be found in the fresh oil which is in palm olein (25 mg/kg) and the lowest content of GE was found in the rapeseed oil (0.8 mg/kg) [19].

Becalski et al. [5] published data on the presence of 2 and 3-MCPD ester and glycidol ester (MCPD and GE) in over 100 edible fats, oils, and products comprising fats and oils. In this study, GE and MCPD esters were determined using stable isotope dilution analysis with a glycidol-d5 labelled standard by LC-MS/MS in the mode atmospheric pressure chemical ionization (APCI), and GC-MS in the mode SIM after derivatization. All of the food samples were purchased in the local market in Ottawa, Canada. The sample was homogenized first before proceeding with the next step. LC-MS/MS was used for the determination of target glycidol fatty ester. The target of GE which was lauric and mystric revealed the recovery from the spiked cookies was 102% and 109% respectively. Meanwhile, the recovery of glycidyl laurate 103% and myristate 101% from the spiked of virgin olive oil. From the observation, the unprocessed oil does not contain any detectable levels of GE or MCPD ESTERS except for palm oil that recorded 100-550 ng/g for MCPD ESTERS. The GE and MCPD ESTERS concentration in processed oil or fats were found to be high, at 10.6 and 17.1 g/g, respectively.

Table 1. Extraction and determination of glycidyl esters (2015–2022)

Analytes	**Extraction Techniques**	**Quantitation Instruments**	**Sample Matrices**	**Linearity (mg/kg)**	**LOD (mg/kg)**	**Precision, RSD%**	**Relative Recovery/ Recovery, %**	**Concentration Found (mg/kg)**	**References**
2-MCPD 3-MCPD GE	SPE	LC-MS/MS	Edible oil	N/A	N/A		101 - 109	N/A	[5]
2-MCPD 3-MCPD GE	Pressurized liquid extraction: Oven temperature: 40 ◦C, pressure: 103 bar, pre-heating time 1 min, static extraction time 5 min, heat	GC-MS Column: DB5-MS Temperature was programmed as follows: 60 ◦C for 1.0 min, from 60 ◦C to 150 ◦C at 6 ◦C min^{-1}, 2.0 min at 150 ◦C and from 150 ◦C to 300 ◦C at 10 ◦C min^{-1}	Food matrices	20-1850	7-17	<17	82-114	The highest levels of both 3-MCPDEs and GEs were found in margarines followed by a potato crisps sample from sliced potatoes and fine bakery wares such as cookies, fatty cake products and puff pastries	[23]
3-MCPD GE	Petroleum ether extraction	GC-MS Column: Restek Rxi-5 ms Temperature was programmed as follows: 60 °C for 1 min, 6 °C min^{-1} to 190 °C, 30 °C min^{-1} to 280 °C, isothermal 10 min Sample volume:1 µL with pulse pressure of 25 psi	Sunflower oil	N/A	N/A	N/A	98.8-102.0 98.7-100.2	N/A	[21]
2-MCPD 3-MCPD GE	Accelerated solvent extraction (ASE) Extraction solvent: A mixture of isohexane and acetone (80/20, v/v)	GC-MS Column: DB5-MS GC oven temperature programme was 60°C (held for 1 min), ramped at 6°C min−1 to 180°C, followed by 30°C min−1 to 300°C (held for 10 min)	Infant formula	0.5-2.0	0.04-0.05	<12	94.7-109	0.011-0.070	[24]

	ASE conditions: pressure, 1500 psi; temperature, 100°C; heating period 6 min; extraction (static) time 5 min, with three static cycles per sample. flush volume 100%; and purge time, 60 s								
Glycidol	Double SPE Extraction solvent: chloroform diluted with acetone to the mark	LC-MS consists of ternary pump and autosampler equipped with APCI interface. Column: Luna 3 µm PFP(2) 100 Å column equipped with precolumn. Separation temperature: 25°C. Flow rate: 0.2 mL min−1, sample injection volume: 10 µL	Palm oil	N/A	N/A	N/A	N/A	5.04-38.95	[3]
GE	SPE	LC-MS equipped with APCI interface (positive mode)	Refined oil	5-1000 ng/mL^{-1}	27.7-45 ng g^{-1}	<5.4	89 - 103	0.33-12.7	[22]

	Extraction solvent: chloroform that is diluted with acetone to the mark. Reverse phase: Cartridge conditioned with methanol, eluted with SPE manifold under gravity (1 drop per 2-3 seconds), solvent evaporated (at 40 °C in a nitrogen stream) Normal phase: Cartridge conditioned with n-hexane/ethyl acetate (95:5 v/v)	APCI parameters applied: corona discharge current, 2 µA; shield voltage, 600 V; capillary voltage, 59 V; vaporizing gas temperature, 500 °C, drying gas temperature, 250°C; dwell time, 200 ms each Column: Luna 3 µm PFP(2) 100 Å column equipped with a precolumn Separation temperature: 25 °C. Flow rate: 0.2 mL min−1, sample injection volume: 10 µL							
Glycidol 3-MCPDE	LLE Extraction solvent: mixture of solvent consists of hexane: MTBE (1:2)	GC-MS by SIM Injection temperature: 180 °C. Column: capillary column VF-1ms Flow rate: 1.2 mL/min. The following temperature program was used in the oven: 60°C (held for 1 min), 6°C/min to 190°C, 20°C/min to 280°C (held for 30 min)	Infant formula	0-5.97 0-2.60	0.10 E: 0.08	<15	82-97 93-108	Not detected (ND) to 0.75	[2]
G-laurate G-palmitate G-Stearate G-oleate G-linoleate G-Linolenate G-palmitate	LLE Extraction solvent: 20% Na_2SO_4 (w/v); 2 × 2 mL n-heptane SPE Extraction solvent: 500 mL n-hexane: ethyl acetate (85:15, v/v). Cartridge conditioned with elution solvent (n-	GC-MS with SIM mode. Column: J&W DB-5 MS GC Injection (pulse pressure 200 kPa for 0.30 min) at a Temperature of 250°C Flow rate: 1.2 mL/min. The transfer line temperature was set to 300 °C. The oven temperature program was as follows: initial temperature 60°C held for 1 min, 6°C/min till	Food matrices	N/A	N/A		92.3-103.3	N/A	[11]

	hexane: ethyl acetate, 85:15, v/v)	150°C (held for 2 min), 30°C/min till 300°C held for 10 min HPLC-MS Mobile phase A: (methanol:H2O, 92:8,v/v) and mobile phase B (2-propanol) were programmed as follows: isocratic elution 100% mobile phase A till 18.0 min, linear gradient 100% B till 18.1 min, isocratic elution 100% B till 25.0 min, linear gradient 100% A till 25.1 min, isocratic elution 100% A till 35.0 min. The flow rate was 1 mL/min, Column temperature: 40°C APCI at gas pressure of 30 psi, vaporization temperature of 500 °C							
2-MCPD	Microwave extraction The septa used was 22 mm EPA Septa Silicone/PTFE, 3.2mm thick. Extraction solvent: Ethyl acetate Time: 10 min	GC-MS Column: HP 5 MS capillary column Injector temperature: 250°C Flow rate: constant of 1.7 mL/min, the transfer line was at 250°C, the ion source at 230°C and the quadrupole at 150°C. The GC column oven was programmed from an initial temperature of 80°C held for 1 min, increased at a rate of 10°C/min up to 200°C, and then ramped up again at 15°C/min up to 250°C, which was held for 15 min	Food matrices	0-5	0.0008-0.03	N/A	N/A	N/A	[25]
3-MCPD				0-5					
GE				0-10					

3-MCPD GE	LLE Extraction solvent: ethyl acetate and LC/MS grade water. Incubation temperature: 35°C and 500 rpm for 1.5 hours. Centrifuged for 20 minutes.	LC-MS/MS equipped with ESI source Column: Pursuit XRs C18 column Column temperature: constant at 30°C Initial flow rate: 200 µL/min , 100% mobile phase A (2 mM ammonium formate/0.05% formic acid, 92:8 MeOH/H2O) for 2 min Injection volume: 5 µL	Infant formula	N/A	N/A	1.0-9.5	88.7-107.5	0.090 -5.92 µg/g 0.89 -8.19 µg/g	[26]
GE	Solvent extraction Extracting solvent: n-hexane Steamed over boiling water for 5 min.	GC-MS Capillary column: Agilent TG-5MS Constant flow rate of 1.18 mL/min GC oven temperature program followed by: 80°C with an increase 171 of 5°C/min to obtain 155°C, followed by increase by 60°C/min to obtain 300°C that 172 was held for 5 min	Edible oil	N/A	0.01	N/A	N/A	N/A	[27]
3-MCPD GE	SPE Extraction solvent: diethyl ether	GC-MS/MS Column: two HP-5MS Injector Temperature: 250°C Injection volume: 2 µL Flow rate: constant 1 mL min^{-1} Transfer line Temperature: 280°C Backflush time: 5 min Backflush pressure: 50 psi	Fish oil	N/A	0.05 0.02	0.2-6.1 4.6-14.4	93.4 98.9	0.37	[28]
2-MCPD 3-MCPD Glycidol	SPE Extraction solvent: Ethyl acetate	GC-MS	Food matrices	N/A	N/A	N/A	82-109	N/A	[8]

3-MCPD GE	**N/A**	**GC-MS** **LC-MS/MS**	**Food and Oil**	**N/A**	**0.05**	**N/A**	**N/A**	**N/A**	**[7]**
3-MCPD Glycidyl ester	Extraction with methyl tert-butyl ether and diethyl ether	GC-MS Column: Rxi-17Sil Ms Temperature program: 85°C(hold 0.5 min) to 150 °C at 6°C/min to 180°C at 12°C/min to 280°C at 25°C/min (hold 7 min)	Edible oil	N/A	0.13-0.14	N/A	N/A	N/A	[31]
Glycidyl palmitate Glycidyl palmitate D5	FTIR wavelength range between 4000 and 600cm^{-1} with 40 interferograms at a resolution of 4 cm^{-1} NMR Frequency of 499.887 MHz followed by parameters: temperature of the machine set at 26 °C, number of scans was 64, pulse width (90° pulse angle) was 8.6 ms, and relaxation delay time was 2.0 s to complete the total acquisition time of 3.53 min. The spectral width adjusted from − 1 to 14 ppm	GC-MS Mode: SIM	Palm based cooking oil	>0.997	0.24	N/A	N/A	0.6-21.3	[30]
3-Chloro-1,2-propanediol and 2-Chloro-1,3-propanediol, glycidyl stearate, rac 1,2-Bis-palmitoyl-3-chloropropanediol, 1,3-Dipalmitoyl-2-chloropropanediol	Agilent Intuvo 9000 GC system coupled to triple Quadrupole mass spectrometer Working in splitless mode in MMI conditions (initial conditions 60°C	Gas chromatography tandem mass spectrometry (GC–MS/MS) Column: DB-17-MS Flow of 1.2 ml/min	Extra virgin olive oil	N/A	0.01	>11	81-115	25 µg/mL	[32]

	Rate 300°C/min to 280°C, hold time 10 min). Oven temperature: 60°C hold time 1 min, rate 20°C/min to 160°C; rate 10°C/min to 220 °C; rate 25°C/min to 320°C hold time 4 min, post run 325°C, total run time 20 min								
3-MCPD esters 2-MCPD esters Glycidyl esters	HS–SPME Derivation temperature: 100°C Extraction time: 80 min	GC-MS Column: HP-5 Oven temperature: initial temperature 50°C hold 1 min, increase of 2°C/min to 90°C then by 40°C/min to 325°C hold 4 min and post-run at 325°C for 5 min	100–500 mg of oil sample or 100 µL of PP-MCPD ESTERSs (500 µg/mL)	N/A	3.9 µg/L	0.51-5.04	91.1-102.1	N/A	[29]
3-MCPD esters Glycidyl esters	Reax 2 rotatory shaker from Heidolph Centrifuged at 5000 rpm for 10 min	Ultra-high performance liquid chromatography tandem mass spectrometry with triple quadrupole analyzer (UHPLC-MS/MS-QqQ) Mobile phase: Solvent A methanol:H2O (90:10, v/v) with 2 mM ammonium formate and 0.05% (v/v) of formic acid Solvent B isopropanol:H2O (98:2, v/v) with 2 mM ammonium formate and 0.05% (v/v) of formic acid at flow rate of 0.2 mL min^{-1} Injection volume: 5 µL Column temperature: kept at 25 °C	Oil samples	0.9932-0.9995	1.72	< 20	N/A	10 mg/L	[17]
3-MCPD esters 2-MCPD esters Glycidyl esters	A capillary column HP-5MS (30 m, id 0.25 mm, film thickness 0.25 µm, 5%-Phenyl methylpolysiloxane	GC-MS Column: HP5-MS	Frying oils	0.06-5 0.03-3 0.06-2	0.0015 0.0019 0.013	1.5 5.17 2.79	94.90 95.15 91.03	N/A	[9]

2-MCPD esters 3-MCPD esters Glycidyl esters	A high-speed vertical shaker Extracted at 1500 rpm for 5 min , added 10 g of sodium sulfate anhydrous, extracted at 1500 rpm for 3 min , centrifuged at 4800 rpm	GC-MS/MS 70 °C for 0.02 min increased to 280 °C at a rate of 8.5 °C/s for 8 min then increased to 330 °C at a rate of 10 °C/s for 14.5 min	60 samples from different manufacturers	N/A	0.0003-0.0032	< 15	75 - 110	0.0023-0.0011	[10]

Wenzl et al. [20] in 2015, has reported that the EFSA and Joint Research Centre (JRC) were cooperate in order to obtain the data regarding the analysis of 3-monochloropropane-1,2-diol (3MCPD) and 2-monochloropropane-1,3-diol (2MCPD) and glycidyl ester (GE). They had developed a suitable method for analysis of these compounds. The tests were run under the different types of food such as meat products, potato based-snacks, cereal-based snack and margarines. In this study, the method developed will determine the ester-bound analytes, and the other analysis will determine the free 2-MCPD and 3-MCPD. The GE will be converted to the monobromopropandeiol ester and well known as MBPD. Both MCPD and MBPD will undergo the process transesterification before the derivatization process takes place. During the derivatization process the analytes will be treated with phenylboronic acid (PBA) in organic solvent. Gas chromatography mass spectrometry (GC-MS) was used to measure the PBA derivatives. The quantifications used the stable isotope labelled analogues. The sample extraction used was pressurized liquid extraction (PLE) with presence of tert-Butyl methyl ether 100% solvent and with temperature of 40 ℃. The fat from the food matrices were extracted and were determined by gravimetry. Both MCPD esters and GEs showed limits of quantification (LOQs) of 100 μg/kg or lower. In this study, the MCPD ester will be expressed as free MCPD meanwhile GEs will be expressed as glycidol. The estimated amount of LOD and LOQs for GEs were 17 μg/kg and 31 μg/kg respectively. For all of the targeted analyte, the gas chromatography mass spectrometry (GC-MS) with the presence of electron ionization with the selected ion monitoring mode.

In 2015, Dingel et al. [21] has reported the study on the non-formation of the 3-MCPD ESTERS and GE during the deep frying of the potatoes crisps in the large scale. In this study, high oleic-sunflower oils (HOSO) were used for deep frying. GC-MS was used to analyze the sample of potato crisps. There were 9 samples that were used for this analysis. All of the samples were collected directly from the German production line. The potato crisps sample weighed approximately 35 g. The sample preparation process began with the use of a typical laboratory mill for finely ground samples. The petroleum ether was then used to extract the compounds. The HOSO frying oils were utilized immediately for the analysis without any pre-treatment needed. The sample aliquots were dissolved in tert-butyl methyl ether before being spiked with the internal standard 3-MCPD-1, 2-bs-palmitoylester-d5. In this experiment, ester cleavage occurs, and it will halt once the acidified sodium chloride or sodium bromide solution is introduced to both aliquots. Each sample was extracted using a diethyl or ethyl acetate combination. Before the assays were evaporated, phenylboronic acid (PBA) was employed to mix the organic extracts of each experiment. As a result, GE's recovery was estimated to be between 98.7% and 100.2%. MCPD ESTERS and GE were both varied in terms of measurement uncertainty as a result of the experiment. Furthermore, it did not show any increases when compared to the oil utilized at the start of this investigation. The MCPD ESTERS and GE levels in this study were also low, ranging between 0.10 and 0.85mg/kg fat and 0.09 (LOQ) and 0.91 mg/kg, respectively. From the result obtained there were no significant changes in level of MCPD ESTERS and Ge while frying process occurred.

Research study regarding the monitoring of the glycidyl ester (GE) in the refined vegetables oils by using liquid chromatography-single-quadrapole mass spectrometry (LC-MS) was performed by Aniolowska et al. in 2015. Basically, refined vegetable oils from the retail outlet in Poland was used in order to analyze the GE [22]. For the analysis, 12 rapeseed oil samples, 4 sunflower oil samples, and 4 palm oil samples were employed. This study's analyses were carried out in duplicate. The high-performance liquid chromatography (HPLC) was used to extract GE-rich extracts from oils. Except for water, all of the solvents utilized in the LC-MS study were under HPLC grade. The extraction method used was double solid phase extraction (SPE). Methanol was used to condition the reverse phase of the SPE cartridge, whereas n-hexane or ethyl acetate was used to condition the normal phase of the SPE cartridge. In this study, the composition of the fatty acid can be determined by using gas chromatography (GC). From the result obtained, different analytes possess different concentrations, and the concentration of the analytes were range within 0.17 and 44.33 mg/kg. The LODs were determined within the range 1.1 to 1.8 ng/mL while the LOQs were in range of 3.8 to 6 ng/mL. From the calibration curves the correlation was good with R^2 within 0.9981 and 0.9990. The percentage of recovery values were recorded from 89% to 103% and the standard deviation was less 5.4%. In 2016, Samaras et al. presented an indirect analytical method for simultaneous determination of 3-MCPD and GE in the various food stuff, including breads and roll, bakery wine, smoked fish products, fried and roasted meat, potato based snack, margarines and extra virgin olive oil blank sample, by using pressurized liquid extraction (PLE) and GC-MS [23]. The sample was immersed in liquid nitrogen, grinded, and then homogenized to produce a fine homogenous powder in order to increase the extraction efficiency. Based on the outcomes of literature and experimental studies, *tert*-butyl methyl ether (t-BME) was proposed as the best extractant for the determination of the MCPD esters and GE for this PLE technique. In order to overcome the overwhelming effect of fat content on the sample, for samples exceeding the 5% total fat contents (w/w), stable isotope-labelled internal standards would be added directly into 100 mg extract after PLE. After acid transesterification, the derivatization procedure was performed by using a low volume of derivatization reagent, phenylboronic acid (PBA), which provides lower background level and decreased limits of detection (LODs) compared to the AOCS methods [5]. Based on this study, the concentrations of 3-MCPD esters and GE were found to be the highest in margarines, followed by potato crisp snacks and bakery products. The targeted compounds were found only at trace levels in smoked fish, smoked meat and fried roasted meat. They were almost undetected in cereal-based products due to the products' low-fat content. This study suggested that food products prepared using palm oil are more likely to contain higher concentrations of MCPD esters and GE, implicating the threat of exposure of particularly European citizen toward these carcinogens [23].

Owing to the adverse health effects and widespread occurrence of bound MCPD and bound glycidol in foodstuff, Wohrlin et al. found it was necessary to study and monitor their concentrations in infant formula. In this study, accelerated solvent extraction (ASE) was performed to extract MCPD and GE from 14 samples of infant formula of Deutsche products [24]. After converting GE into monobromopropanediol (3-MBPD) esters, both MCPD esters and 3-MBPD esters were hydrolyzed and derivatized for detection by GC-MS. From the result, the recovery rates of the analytes can be determined by the fortification of the infant formula. It was stated that the recovery rates for 3-MCPD was between 93% to 103% while for 2-MCPD, it was between 91% to 96%. For GE it was determined that the rate of recovery values was between 92% to 109%. It was recorded that GE has the highest precision of the whole method, which is 12%. Meanwhile 3-MCP and 2-MCPD were 5% and 3% respectively [24].

Marc et al. [25] has reported the research study regarding the determination of MCPD ester and glycidyl ester (GE) by using microwave extraction in the different food stuffs in 2016. There were two types of sample preparation in this study which is sample preparation for oils and samples preparation for the infant formula. This research study used gas chromatography mass spectrometry (GC-MS) for the analysis of the sample. A new method of extraction for this study was developed which was using the microwave. Usually, the extraction method by using the microwave was used to extract the contaminants that come from non- food matrices such as soil or river water. However, this method was used by many laboratories before in order to determine the polycyclic aromatic hydrocarbons (PAH) in food matrices. In this study, the linearity of the method was studied starting from 0-10 mg/kg for the glycidol ester while the linearity for 2 and 3-MCPD ester were studied from 0-5 mg/kg. This method LOQs for both MCPD and glycidyl ester at 0.1 mg/kg, 0.02 mg/kg and 0.0025 mg/L respectively for oils, chips and crisps and infant formula. For LOD in oils was 0.03 mg/kg, 0.007 mg/kg for crisps and chips and 0.0008 mg/kg for infant formula for both MCPD and glycidyl ester.

In this study, the concern was focuses on the detection of 3-MCPD and glycidyl ester in infant formula [26].The quantitative analysis was using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method provided for extracting MCPD and glycidyl esters from infant formula uses simple, liquid-liquid extraction processes that do not require complex instruments or expensive equipment. Ethyl acetate was used for extraction after the powdered infant formula was dissolved by using water. In this experiment all of the samples were purchased from the local market. The step of preparation of homemade infant formula for reference material were followed from the online recipe then followed by the preparation of the infant formula extracts. The infant formula extract solutions were prepared when the deuterated internal standard spiking solution of oil extract were pipetted for about 250µL of 5µg/mL into the glass centrifuge tube that contained oil extract. There were about 9 calibration curves that were used for the di-ester, monoester and glycidyl ester quantitation. The concentration prepared were 1, 2, 5, 10, 25, 50, 125, 250 and 400 ng/mL for each of the ester. The internal standard was 50 ng/mL. According to this study, infant formula was one of the food matrix components that presented a challenge due to their intricacy. It contained fats, carbs, proteins, vitamins, and minerals that had been properly homogenized and dried. During the development of this process, it was discovered that the quantity of fat extraction yields from various types of newborn formula available varied greatly from one type of infant formula to the next when the same set extraction was used. The percentage of recovery can be used to validate the method's performance. For the homemade powdered infant formula reference material, the ester recovery ranged from 84.9 percent to 109.0% on average. Meanwhile, the ester recovery rates for all concentrations and infant formulas ranged from 88.7 to 107.5 %.

The study in 2016 , Cheng et al. [27] has reported the concern to study and investigate the presence and the formation mechanism of glycidyl ester (GE) in the edible oils such as soybean oil, camellia oil and palm oil. This study focuses on the laboratory-scale preparation and refining during high temperature from 160 ℃ to 260 ℃ under nitrogen. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy was used in this experiment in order to monitor the formation of the GE in chemical model 1,2-dipalmitin (DPG) and 1-monopalmitin (MPG). All of the sample of crude oil such as palm oil, soybean oil and camellia oil were purchased from the local market in Guangzhou China. The extraction method used in the preparation of crude oil was solvent extraction and hot pressing and cold pressing methods were also used in the preparation of crude oil. After the preparation of crude oil, the preparation of model oil was continued by using column chromatography with solvent mixture of petroleum ether and diethyl ether and about 80 g of silica gel were used. For the laboratory scale refining procedure, all of the crude oil was processed at the industry standard state. Nitrogen was used in this procedure. The main purpose of using nitrogen is to avoid oil oxidation.

The total polar components (TPC) were employed as the gravimetric method to determine the total amounts of the polar fraction after eliminating the nonpolar component using column chromatography separation. TPC was then tested for composition using thin-layer chromatography (TLC). Following that, the TPC composition was examined using high performance size exclusion chromatography (HPSEC). The GE analysis was carried out in accordance with the AOCS Official procedure. The GEs content was estimated using an indirect method based on the detection of 3-MCPD ESTERSs levels. It entailed the ester's release of 3-MCPD ESTERS and glycidol, PBA derivatization, and quantitation by gas chromatography-mass spectrometry (GC-MS). According to the main findings, no statistical differences in the GE content of the tested oils with varying refining degrees before deodorization were identified. The results also revealed that the GE level in crude oil extracted by the solvent was lower than the detection limit of 10 µg/kg. The low quantity of GE was reported in the pressing method of extracted crude oils, whereas hot-pressed oil reported the low amount of GE corresponds to cold-pressed oil. GE levels were reported to be highest in palm oil followed by camellia oil and soybean oil. The GE level was monitored by GC-MS and the linear correlation between the DPG and GE was found at R^2 =0.9607, which is relatively poor.

Rubio et al. [28] has reported the study in a novel single –step method for the simultaneous determination of 3-MCPD and GE in the sample of winterized and non-winterized fish oil by using gas chromatography tandem-mass spectrometry (GC-MS/MS) in 2017. The process was based on the alkaline hydrolysis of ester at ambient temperature followed by a derivatization stage with phenylboronic acid (PBA) at 90 °C. The use of GC-MS/MS resulted in simpler sample treatment as well as improved limit quantification and precision of the analytical approach without the requirement for additional extract concentration. This approach verified both winterized and non-winterized fish oil by obtaining limits of quantification of 100 ng/g for 3-MCPD and 50 ng/g for glycidol. Furthermore, in order to validate the procedure, the laboratory results were compared to those obtained by an accredited reference laboratory (SGS Germany GmbH). The use of GC-MS/MS rather than GC-MS, which is often used to determine 3-MCPD and glycidyl derivatives, dramatically enhanced the signal-to-noise ratio while minimizing sample preparation and manipulation. The LOD and the LOQ for 3-MCPD were 0.05 mg/kg and 0.2 mg/kg respectively. While for glycidol, the LOD was 0.02 mg/kg and LOQ was 0.07 mg/kg. The recoveries obtained for both 3-MCPD and glycidol were almost near to 100 % which was 93.4% to 98.9% for 3-MCPD and glycidol respectively. Average value calculated were 99.8% for 3-MCPD and 99.0% for glycidol.

Previous studies on the analytical determination of the GE were fully validated only for oils, fats and margarine. This calls for a re-assessment of the critical steps in the AOCS Cd 29a-13 method, in 2017, for simultaneous determination of MCPD esters and GE in a broader range of different food, including meat, infant formula milk, fried fries, bakery product and cereal products [11]. Since overestimation of GE contents is more likely to occur with an indirect analysis of GE, the results can be confirmed by comparison of results achieved through an independent analytical method, such as the direct analysis of the GE by HPLC-MS/MS. In the direct method according to the AOCS standard method, internal standard is added to the pre-weighed fat and dissolved in cyclohexane: ethyl acetate (1:1, v/v) [11]. Before quantitative analysis, triacylglycerols (TAG) has to be removed from the extracted fats. The direct method proposed by Dubois et al. uses gel permeation chromatography (GPC) to separate the almost-similar analyte constituents. In this assay, six GE, namely glycidyl(G-) laurate, G-palmitate, G-stearate, G-oleate, G-linolate, and G-linolenate were determined, totaled up, and expressed in equimolar amounts of glycidol or glycidol equivalents for comparison of results with the indirect analysis method [11]. The results obtained from the two analysis methods were not significantly different. At 95% confidence level, only the results for three bakery products were significantly different. A linear correlation was found when plotting the difference of glycidol content measured by the direct and indirect method, against the content of MAG in the extracted fat sample. However, the results of the indirect method would be deduced as bias in the presence of higher than 1% MAG in the fat sample [11].

According to paper presented by Arisetto et al. [7], the analytical methods to determine these contaminant 3-MCPD and GE was using direct and indirect approaches. The indirect approaches were using gas chromatography coupled to mass spectrometry (GC-MS) for the analysis. This procedure necessitates the conversion of the compound into free form before the analysis can be carried out. The pros of using this approach were that it possesses a lowest number of analytical standards and was easier to comprehend the data. This method involves a few methods such as transesterification, neutralization, salting out and derivatization. Meanwhile, the direct method analysis was using high performance liquid chromatography-mass spectrometry (LC-MS/MS) for the identification and quantification of the different compounds. In direct method, a simple extraction procedure was required but it did not require transesterification and derivatization steps. In this study, samples such as fish oil, edible oil and several foodstuffs were used for the determination of 3-MCPD and GE. Generally, the LOD for both of these approaches was between 0.033 µg/kg to 50 µg/kg.

Kalkan et al. [9] evaluated the formation of 3-MCPD, 2-MCPD and GE in sunflower oil frying processes by using central composite design (CCD) methodology. Chemicals used were 3-MCPD, 3-chloro-1, 2 propanediol-d5 (3-MCPD-d5) and 2-MCPD, 2-chloro-1,3-propanediol-d5 (2-MCPD-d5), glycidyl stearate-d5 (Gly-Pd5) and glycidyl stearate (Gly-S-). Toluene, tertbutyl methyl ether, methanol, sodium hydroxide, diethyl ether, ethyl acetate, isohexane, n-heptane, isooctane, sodium bromide, anhydrous sodium sulfate, sulfuric acid, and PBA. Sample used was frying oil that were capped into brown-stoppered flasks and stored it in refrigerator at -18 °C because to protect for deterioration until the analysis. For the determination of GE, free and bound 3-MCPD esters were converted into free 3-MCPD form by adding dilute methanolic sodium hydroxide solution. Then, free and bound 3-MCPD were analyzed in GC/MS by derivatization with PBA. The amount of GE was obtained by multiplying the difference of total 3-MCPD and free and bound 3-MCPD by conversion factor. The conversion factor was the standard addition method which GE internal standard was added into oil sample. It was calculated by using slope of calibration curve. An indicator of the acceptability of method was based on the sensitivity of an analytical method. The LOD, LOQ, recovery and relative standard deviation tests were performed. It was observed that temperature, duration and salinity were the factors that had significant effects. MCPD ester in frying was transformed into GE species with the increasing of temperature, duration and salinity. The highest 3-MCPD (0.30 mg/kg), 2-MCPD (0.09 mg/kg) and GE (0.72 mg/kg) formations were observed in the hardest conditions with 40 min., 180 °C and 300 mg NaCl/ 100 mL for oil [10].

Nguyen et al. [10] reported that a GC/MS-MS method was developed, validated and applied for the determination of MCPD ESTERSs and GEs to analyzed 60 infant formula and baby food products. Different commercial food products such as powder infant formula, liquid infant formula, cereal based and vegetables-based baby foods which include smoothies, puffs, and biscuits. All the samples were homogenized and stored at −18 °C for analysis. Fat extraction was needed to extract contaminants out of food matrices because MCPD ESTERSs and GEs was highly lipophilic. The sample was spiked with 50 μL of an internal standard mixture (5 ng/μL) and placed on high-speed vertical shaker and extracted at 1500rpm for 5min. 10g of sodium sulfate anhydrous was added to get better phase separation and salting out effect. Extracted fat were obtained by evaporation of combined upper layers to dryness under nitrogen steam at 40 °C. Food items have been reported to contain a significant amount of partial glycerides especially MAGs that can cause artifact formation of GEs during bromination of GEs. Hence, a cleanup step by aminopropyl SPE cartridge was employed to remove MAGs. The results showed low levels of MCPD ESTERSs, and GEs were observed in most vegetable oil containing products while samples with no added oil showed < LOQs levels. The degree of MCPD ESTERSs and GEs contamination was in the following order: cereal based puree liquid formula cereal-based puree powder baby puffs < powder formula < baby biscuits, with the exception for GEs where baby puffs showed the highest concentrations.

A new method was developed based on the headspace solid-phase microextraction gas chromatography–mass spectrometry (HS–SPME–GC–MS) method for the quantification of 3-MCPD ESTERSs, 2-MCPD ESTERSs and GEs in edible oils [29]. Hydrolysis method was used in this research with the addition of NaBr and NaCl to convert glycidols into 3-MBPD or 3-MCPD. The authors also stated that a critical factor for the alkaline analysis was reaction time to indicate the sensitivity. Hence, to keep higher recovery, acidic hydrolysis was employed. The method of solid phase microextraction (SPME) involved fibre coated with porous stationary phase. Since the analyte have variable partition coefficient with different fibre coatings, then type of fibres play an important role in extraction efficacy [29]. A HS–SPME–GC–MS labor-friendly method demonstrates greater precision, accuracy and adequate LOD/LOQ as compared with liquid injection of GC–MS.

The analysis traces of 3-MCPD and GE has been carried out by using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry, using a triple quadrupole analyzer (UHPLC-MS/MS-QqQ) [17]. This method used a WX vortex from Velp Scientifica to homogenize the samples while a Reax 2 rotatory shaker from Heidolph extraction of the compounds. The MS conditions were optimized first then by applying full-scan MS, the characteristic ion of each compound was obtained. The target compounds were prepared at 10 mg/L in isopropanol to achieve a proper optimization. To reduce the analysis time and obtain the best peak shapes, chromatographic parameters need to be optimized. To guarantee the reliability of the results, an internal quality control has been carried out during the analysis which consisted of blank sample, a matrix calibration line from 0.01 to 0.5 mg/L and two samples which has been already spiked and analyzed at 0.01 and 0.2 mg/kg. Each of these quality control are to check the absence of interferences, to evaluate the sensitivity and performs quantification of samples also to evaluate the efficiency of the extraction process [17]. The results showed that most detected esters in analyzed oil samples were from oleic, linoleic and linolenic acid because it was the major fatty acids that are present in oils. It is normal for extra virgin and virgin olive oil to not show high concentrations of esters since both are not submitted to any refinement process. This method has a simple and fast extraction method based on the use of solvents. By using UHPLC-MS/MS-QqQ, it takes less time to analyze the samples and by using this approach, the most significant identification and quantification of 3-MCPS and GE that are present in the samples are available.

Lastly, Goh et al. [30] had studied the detection of glycidyl ester in edible palm-based cooking oil and a total result was acquired from the market in a different oil sample. The amount of GE content from 1.338-18.362 mg/kg. The result was not surprising because all the palm oils refinery in Malaysia uses high temperature so that it has been proven for the main factor that cause formation of GE. Mixed glycerides and high portions of oleic, linoleic and linolenic acid were existing in palm-based cooking oil. In this research, GC-MS and ATR-FTIR were used for identification and quantification of GE. However, for the confirmation of the presence of GE was further by using 1H NMR spectroscopy and the results was it showed some signals that known as a characteristic of fatty acids, polyunsaturated fatty acids (PUFA) and TAG. TAG has the most abundant fatty acids that present in edible palm-based cooking oil sample [30]. NMR technique has been proven as a useful technique in order to get data verification and have a potential for alternative spectrum input in modelling process. The results showed that contamination of GE in palm-based cooking oil sample ranged from 1.338 to 18.362 mg/kg which showed a non-normal distribution.

Conclusion

The first proposed analytical method for the detection of glycidyl esters was an indirect approach which is by hydrolyzing variety of fatty acid esters, releasing free glycidol and the analysis was greatly simplified. However, this method was shown to provide inaccurate results. Hence, different methods have been developed such as by using ultra-high performance liquid chromatography tandem mass spectrometry with triple quadrupole analyzer (UHPLC- MS/MS-QqQ), GC-MS, LC-MS, GC-MS/MS and LC-MS/MS. The newly developed methods offer a superior advantage simplified clean-up procedure that only required several hours to complete that much faster than hydrolysis step and it employs several isotopically labelled internal standards that avoided the need for time consuming. Great progress has been made on the analysis of glycidyl esters in oils and other matrices over the past few years, with a number of methods producing comparable and accurate results, the analysis of complex foods is still developing. The challenges of extracting the wide variety of fat-containing foods combined with the lack of commercial reference materials slow the progress of development. However, there are a number of published methods which have recently been validated in complex foods. Moving forward, comparing their performance to one another and to standard reference materials is necessary to ensure accurate data is being produced for exposure and risk assessment purposes. In addition, further studies related to the GE formation during food processing should be extended to other thermal methods.

Acknowledgement

This study was supported by the Ministry of Higher Education Malaysia through Fundamental Research Grant Scheme (FRGS) with reference code number: FRGS/1/2021/STG04/USM/02/4 and account code number: 203.CIPPT.6711973. The authors extend their gratitude to Siti Nurfazilah Awang and Nur ‘Ain Syaheeda Azizan for their invaluable contributions to the completion of this review.

References

1. Albuquerque, T. G., Costa, H. S., Silva, M. A., and Oliveira, M. B. P. P. (2020). Are chloropropanols and glycidyl fatty acid esters a matter of concern in palm oil? *Trends in Food Science and Technology*, 105: 494-514.
2. Arisseto, A. P., Silva, W. C., Scaranelo, G. R., and Vicente, E. (2017). 3-MCPD and glycidyl esters in infant formulas from the Brazilian market: Occurrence and risk assessment. *Food Control,* 77: 76-81.
3. Aniołowska, M., and Kita, A. (2016). The effect of frying on glicydyl esters content in palm oil. *Food Chemistry,* 203: 95-103.
4. Stadler, R. H. (2015). Monochloropropane-1,2-diol esters (MCPDEs) and glycidyl esters (GEs): An update. *Current Opinion in Food Science,* 6: 12-18.
5. Becalski, A., Feng, S., Lau, B. P. Y., and Zhao, T. (2015). A pilot survey of 2- and 3-monochloropropanediol and glycidol fatty acid esters in foods on the Canadian market 2011-2013. *Journal of Food Composition and Analysis,* 37: 58-66.
6. Bognár, E., Hellner, G., Radnóti, A., Somogyi, L., and Kemény, Z. (2019). Formation of glycidyl esters during the deodorization of vegetable oils. *Hungarian Journal of Industry and Chemistry,* 46(2): 67-71.
7. Arisseto, A. P., Silva, W. C., Tivanello, R. G., Sampaio, K. A., and Vicente, E. (2018). Recent advances in toxicity and analytical methods of monochloropropanediols and glycidyl fatty acid esters in foods. *Current Opinion in Food Science,* 24: 36-42.
8. MacMahon, S. (2019). MCPD esters and glycidyl esters: A review of analytical methods. *In Encyclopedia of Food Chemistry*: pp. 569-577.
9. Kalkan, O., Topkafa, M., and Kara, H. (2021). Determination of effect of some parameters on formation of 2-monochloropropanediol, 3-monochloropropanediol and glycidyl esters in the frying process with sunflower oil, by using central composite design. *Journal of Food Composition and Analysis,* 96(7): 103681.
10. Nguyen, K. H., and Fromberg, A. (2020). Monochloropropanediol and glycidyl esters in infant formula and baby food products on the Danish market: Occurrence and preliminary risk assessment. *Food Control,* 110(11): 106980.
11. Zelinkova, Z., Giri, A., and Wenzl, T. (2017). Assessment of critical steps of a GC/MS based indirect analytical method for the determination of fatty acid esters of monochloropropanediols (MCPDEs) and of glycidol (GEs). *Food Control,* 77: 65-75.
12. Stadler, R. H., and Seefelder, W. (2016). An update on processing-derived food contaminants: acrylamide, monochloropropane-1,2-diol (MCPD) esters, and glycidyl esters. In Reference Module in Food Science. Elsevier.
13. Wang, S., Liu, G., and Cheng, W. (2021). Chloride-mediated co-formation of 3-monochloropropanediol esters and glycidyl esters in both model vegetable oils and chemical model systems. *Food Research International,* 140(381): 109879.
14. Santiago, J. K., Silva, W. C., Capristo, M. F., Ferreira, M. C., Ferrari, R. A., Vicente, E., Meirelles, A. J. A., Arisseto, A. P., and Sampaio, K. A. (2021). Organic, conventional and sustainable palm oil (RSPO): Formation of 2- and 3-MCPD esters and glycidyl esters and influence of aqueous washing on their reduction. Food Research International, 140(12): 109998.
15. Hew, K. S., Khor, Y. P., Tan, T. B., Yusoff, M. M., Lai, O. M., Asis, A. J., Alharthi, F. A., Nehdi, I. A., and Tan, C. P. (2021). Mitigation of 3-monochloropropane-1,2-diol esters and glycidyl esters in refined palm oil: A new and optimized approach. *LWT,* 139: 110612.
16. Wu, P. Y., Chen, H., Su, N. W., Chiou, T. Y., and Lee, W. J. (2021). First determination of glycidyl ester species in edible oils by reverse-phase ultra-performance liquid chromatography coupled with an evaporative light-scattering detector. *Molecules,* 26(9): 2702.
17. Hidalgo-Ruiz, J. L., Romero-González, R., Martínez Vidal, J. L., and Garrido Frenich, A. (2021). Determination of 3-monochloropropanediol esters and glycidyl esters in fatty matrices by ultra-high performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A,* 1639: 461940.
18. FEDIOL (2015). MCPD esters and glycidyl esters. Review of mitigation measures revision 2015. 15SAF108: pp. 1-17.
19. Aniołowska, M., and Kita, A. (2015). The effect of

type of oil and degree of degradation on glycidyl esters content during the frying of french fries. *Journal of the American Oil Chemists' Society,* 92(11–12): 1621-1631.

20. Wenzl, T., Samaras, V., Giri, A., Buttinger, G., Karasek, L., and Zelinkova, Z. (2015). Development and validation of analytical methods for the analysis of 3- MCPD (both in free and ester form) and glycidyl esters in various food matrices and performance of an ad-hoc survey on specific food groups. EFSA Supporting Publication 2015: EN-779, 12(3): 78.
21. Dingel, A., and Matissek, R. (2015). Esters of 3-monochloropropane-1,2-diol and glycidol: no formation by deep frying during large-scale production of potato crisps. *European Food Research and Technology,* 241(5): 719-723.
22. Aniołowska, M., and Kita, A. (2016a). Monitoring of glycidyl fatty acid esters in refined vegetable oils from retail outlets by LC–MS. *Journal of the Science of Food and Agriculture,* 96(12): 4056-4061.
23. Samaras, V. G., Giri, A., Zelinkova, Z., Karasek, L., Buttinger, G., and Wenzl, T. (2016). Analytical method for the trace determination of esterified 3- and 2-monochloropropanediol and glycidyl fatty acid esters in various food matrices. *Journal of Chromatography A,* 1466: 136-147.
24. Wöhrlin, F., Fry, H., Lahrssen-Wiederholt, M., and Preiß-Weigert, A. (2015). Occurrence of fatty acid esters of 3-MCPD, 2-MCPD and glycidol in infant formula. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment,* 32(11): 1810-1822.
25. Marc, C., Drouard-Pascarel, V., Retho, C., Janvion, P., and Saltron, F. (2016). Determination of MCDP esters and glycidyl esters by microwave extraction in different foodstuffs. *Journal of Agricultural and Food Chemistry,* 1(1): 1-15.
26. Leigh, J. K., and MacMahon, S. (2016). Extraction and liquid chromatography-tandem mass spectrometry detection of 3-monochloropropanediol esters and glycidyl esters in infant formula. *Journal of Agricultural and Food Chemistry,* 64(49): 9442-9451.
27. Cheng, W., Liu, G., and Liu, X. (2016). Formation of glycidyl fatty acid esters both in real edible oils during laboratory-scale refining and in chemical model during high temperature exposure. *Journal of Agricultural and Food Chemistry,* 64(29): 5919-5927.
28. Garballo-Rubio, A., Soto-Chinchilla, J., Moreno, A., and Zafra-Gómez, A. (2017). A novel method for the determination of glycidyl and 3-monochloropropanediol esters in fish oil by gas chromatography tandem mass spectrometry. *Talanta,* 165: 267-273.
29. Xu, M., Jin, Z., Yang, Z., Rao, J., and Chen, B. (2020). Optimization and validation of in-situ derivatization and headspace solid-phase microextraction for gas chromatography–mass spectrometry analysis of 3-MCPD esters, 2-MCPD esters and glycidyl esters in edible oils via central composite design. *Food Chemistry,* 307(5): 125542.
30. Goh, K. M., Maulidiani, M., Rudiyanto, R., Abas, F., Lai, O. M., Nyam, K. L., Alharthi, F. A., Nehdi, I. A., and Tan, C. P. (2021). The detection of glycidyl ester in edible palm-based cooking oil using FTIR-chemometrics and 1H NMR analysis. *Food Control,* 125(1): 108018.
31. Jana, B., Hepner, R., Sellers, K., Majer, H., and Konschnik, J. (n.d.). Optimizing GC-MS and GC-MS/MS analysis of 3-mcpd and glycidyl esters in edible oils. Technical Literature Library, *Restek Pure Chromatography*: pp 1-10.
32. Ioime, P., Piva, E., Pozzebon, M., and Pascali, J. P. (2021). Automated sample preparation and analysis by gas chromatography tandem mass spectrometry (GC–MS/MS) for the determination of 3- and 2-monochloropropanediol (MCPD) esters and glycidol esters in edible oils. *Journal of Chromatography A,* 1650: 462253.