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Glycidyl Fatty Acid Esters in Refined Edible Oils: A Review on Formation, Occurrence, Analysis, and Elimination Methods

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Abstract: Glycidyl fatty acid esters (GEs), one of the main contaminants in processed oils, are mainly formed during the deodorization step in the refining process of edible oils and therefore occur in almost all refined edible oils. GEs are potential carcinogens, due to the fact that they readily hydrolyze into the free form glycidol in the gastrointestinal tract, which has been found to induce tumors in various rat tissues. Furthermore, glycidol has already been identified as a "possible human carcinogen" (group 2A) by the Intl. Agency for Research on Cancer (IARC). Therefore, significant effort has been devoted to inhibit and eliminate the formation of GEs. The aim of this review is to provide a comprehensive summary on the following topics: (i) GE occurrence data for different edible oils and oil-based food products, (ii) precursors of GEs, (iii) factors influencing the formation of GEs, (iv) potential reaction mechanisms involving the leaving group and reaction intermediates, and (v) analytical methods, including the indirect and direct methods. More importantly, the various elimination methods for GEs in refined edible oils are being reviewed with focus on 3 aspects: (i) inhibition and removal of reactants, (ii) modification of reactive conditions, and (iii) elimination of GE products.

Keywords: elimination methods, formation mechanisms, glycidyl fatty acid esters, heat-induced food toxicants, refined edible oils

Introduction

Glycidyl fatty acid esters (GEs) have been identified as a new class of food-processing contaminant. These substances contain a common terminal epoxide group but exhibit different fatty acid compositions (Figure 1). For the 1st time, this class of compounds has been reported in edible oils after overestimation of 3-monochloropropane-1,2-diol (3-MCPD) fatty acid esters analyzed by an indirect method (Weisshaar and Perz 2010). Since 1980, 3-MCPD esters have been studied as food-processing contaminants and are found in various food types and food ingredients, particularly in refined edible oils (Velisek and others 1980; Kuśters and others 2011; Razak and others 2012; Becalski and others 2015). It has already been shown that, similar to 3-MCPD esters, GEs may also be detected in significant concentrations in refined edible oils (Zelinkova and others 2006; Masukawa and others 2010; Weisshaar and Perz 2010). Although harmful effects on humans and animals have not been demonstrated, the corresponding hydrolysates, 3-MCPD and glycidol, have been identified as rodent genotoxic carcinogens, ultimately resulting in the formation of kidney tumors (3-MCPD) and tumors at other tissue sites (glycidol). Therefore, 3-MCPD and glycidol have been categorized as "possible human carcinogens" (group 2B) and "probably carcinogenic to humans" (group 2A), respectively, by the Intl. Agency for Research on Cancer (IARC) (IARC 2000; Grosse and others 2011). In 2009, diacylglyceride (DAG)-based oils produced by Kao Corp. (Japan) were banned from the global market due to "high levels" of GEs (AOCS 2009).

Several reports have also suggested that a bidirectional transformation process may occur not only between glycidol and 3-MCPD but also their esterified forms in the presence of chloride ions (Figure 1) (Weisshaar and Perz 2010; Destaillats and others 2012a; Shimizu and others 2012a; Ermacora and Hrncirik 2014a). The transformation rate of glycidol to 3-MCPD was higher than that of 3-MCPD to glycidol under acidic conditions in the presence of chloride ion (Kaze and others 2011). Concurrently, the corresponding esters may exhibit similar conversion behavior. Furthermore, orally administered glycidol and the corresponding esters are metabolized to 3-MCPD in the gastrointestinal tract of F344 rats (Onami and others 2015). This finding indicates that the biological availability of 3-MCPD may be reduced by the removal of GEs. In recent years, increasing attention is therefore being paid to the formation mechanisms of GEs, together with

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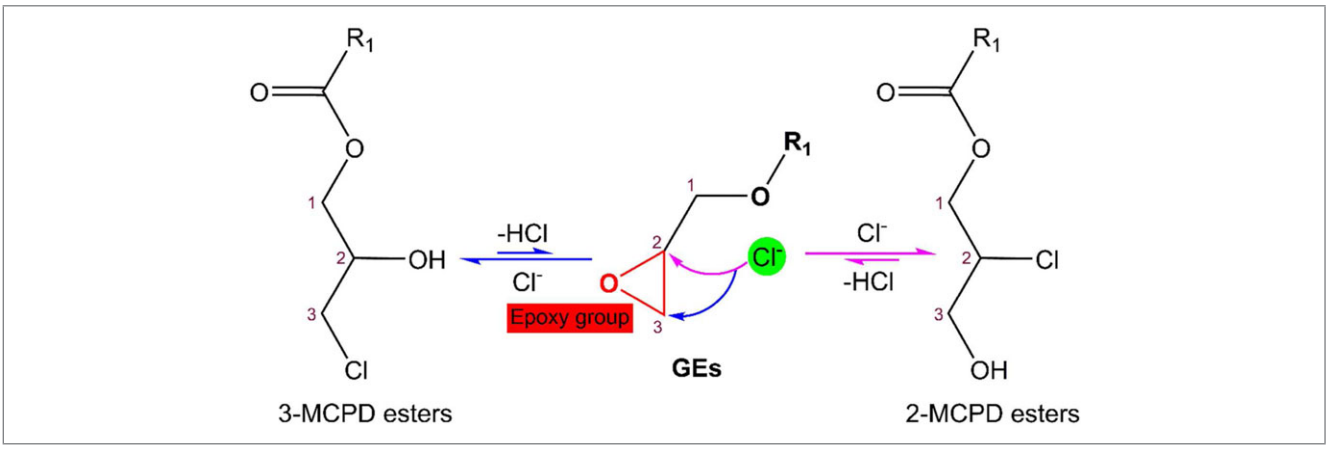

Figure 1–Potential conversion mechanism of GEs to 3-MCPD esters or 2-MCPD esters. R^1, fatty acyl group.

their determination and their elimination methods, specifically in edible oils and oil-based/fat-based food products.

Precursors of GEs in refined oils have been identified as partial acylglycerols, that is, DAGs and monoacylglycerides (MAGs); however, whether they also originate from triacylglycerides (TAGs) is still a topic of controversial debates. Several authors noted that pure TAGs were stable during heat treatment (such as 235 °C) for 3 h and were therefore not involved in the formation of GEs (Destaillats and others 2012a; Shimizu and others 2012a). However, our experimental results have shown that small amounts of GEs are present in a heat-treated oil model consisting of almost 100% TAGs. The results from Fourier-transform infrared (FTIR) studies indicate that the formation of GEs from TAGs can be attributed to the pyrolysis of TAGs to DAGs and MAGs (Cheng and others 2016). In contrast, 3-MCPD esters in refined oils can be obtained from TAG (Destaillats and others 2012b; Hori and others 2016). Presently, the mechanism for the formation of GE intermediates and the relationship between GEs and 3-MCPD esters are still unknown. At present, 2 methods have been developed for the determination of GEs in edible oils: a direct one involving converting GEs into the derivatives and analyzing with gas chromatography-mass spectrometry (GC-MS) as well as an indirect one quantifying each GE with liquid chromatography-mass spectrometry (LC-MS). Crews and others (2013) reviewed analytical methods of GEs in vegetable oils, some other food products, and various biological samples. Here, the advantages and disadvantages of the routine quantification methods were compared. In recent studies, however, GC-MS and nuclear magnetic resonance (NMR) have also been used for the direct analysis of GEs in edible oils (Steenbergen and others 2013; Song and others 2015). Therefore, we believe that an updated review about these determination methods is urgently needed. More importantly, efficient methods should be developed for the reduction of GE levels to ensure the safety of oils, while not negatively affecting food quality (such as taste, aroma, and color) as well as consumer acceptance. To the best of our knowledge, the only reviews on GE elimination methods available to date have been reported by Craft and others (2013) and Stadler (2015). However, these studies are based on limited research results and the methods used involve the modification of the refining process, renovation of deodorization equipment, as well as the inhibition of DAG and MAG formation by agricultural practices. Moreover, further studies on the formation mechanisms of GEs, together with additional elimination methods, need to be developed.

Our review aims to deliver a detailed summary of studies on the occurrence of GEs in different refined edible oils, in combination with mechanisms and factors responsible for the formation of GEs and proposed measures for reducing the GE levels in refined edible oils. Moreover, the identified gaps and future research prospects are also discussed in subsequent sections.

Occurrence of GEs

Available data on GE contents in food is mainly limited to refined edible oils and oil-based food products. However, it has already been established that GEs are formed during the deodorization step of oil-refining processes. As shown in Table 1, GEs can be detected in diverse refined oils and fats such as palm oil, rice oil, soybean oil, and corn oil. Among these, rice oil and palm oil prove to be most susceptible to the formation of GEs, both exceeding 30 mg/kg of oil. The precursor (DAG) contents of GEs are particularly high in oil, ranging from 4% to 12% with a mean of approximately 6.5% in palm oil (Long and others 2005). This fact also explains the relatively high concentrations of GEs in palm oil, while the actual reason for the high GE contents in rice oil is still unclear. Crude or unrefined oils and fats, such as extra virgin olive oil, do not contain GEs or merely trace amounts of GEs (MacMahon and others 2013a).

Generally, a high-temperature treatment, such as deodorization, is the most important factor for the formation of GEs. Apart from deodorization, frying, barbecuing, and baking at high temperature also seem to result in the potential formation of GEs in edible oils and oil-based food products. As reported previously, GEs are detected in edible meat patties cooked by both gas-fired and char-grilling cooking methods at approximately 200 °C or higher in concentrations ranging between 0.07 and 0.17 mg/kg and 0.67 and 1.11 mg/kg in meat samples, respectively (Inagaki and Hirai 2016). GEs are also detected in cookies containing different types of fat (MacMahon and others 2013a), presumably caused by high-temperature baking. In contrast, Dingel and Matissek (2015) have reported the absence of GEs formation in samples of potato crisps and the corresponding oils during deep frying of potato crisps. Subsequent reports confirmed that frying for a long time (>8 h) led to the degradation of GEs, instead of the formation and a higher GE degradation was found in the oil used to fry snacks (95%) than that used to fry potato chips (87%). The corresponding degradation rate largely depends on the type of oil (Aniołowska and Kita 2015, 2016b, 2016c). Similarly, 3-MCPD esters in commercial deep-fried food products containing fat were also found in

Table 1–Occurrence of GEs in refined edible oils and oil-based food products.

Class	Food products	Number	Method	Range (mg/kg)	Average (mg/kg)	References
Oils and fats	Palm oil	3	Two-step SPE/LC-MS	9.26 to 9.40	–	(Blumhorst and others 2013)
	Palm oil	3	Two-step SPE/LC-MS	25.60 to 28.00	–	(Shiro and others 2011)
	Palm oil	2	NPLC/GC-MS	–	30.20	(Steenbergen and others 2013)
	Palm oil	20	Alkaline/Br^- GC-MS	0.30 to 18.00	3.70	(Kuhlmann 2011)
	Palm oil	4	Two-step -SPE/LC-MS	–	31.24	(Aniołowska and Kita 2015c)
	Palm oil and palm Oil-based fats	12	GPC/GC-MS	0.32 to 6.30	2.38	(Weisshaar and Perz 2010)
	Palm kernel oil	2	Alkaline/Br^- GC-MS	0.30 to 2.50	–	(Kuhlmann 2011)
	Palm olein	3	LC-TOF-MS	–	15.60	(Haines and others 2011)
	Soybean oil	3	NPLC/GC-MS	–	1.56	(Steenbergen and others 2013)
	Soybean oil	3	UPLC: ultra performance liquid chromatography-TOF-MS	–	0.12	(Hori and others 2012)
	Rice oil	3	Two-step SPE/HPLC-MS	27.22 to 28.76	–	(Shiro and others 2011)
	Rice oil	3	LC-TOF-MS	–	33.70	(Haines and others 2011)
	Olive oil	2	NPLC/GC-MS	–	4.31	(Steenbergen and others 2013)
	Corn oil	3	UPLC-TOF-MS	–	2.09	(Hori and others 2012)
	Sesame oil	3	LC-TOF-MS	1.30 to 3.70	–	(Haines and others 2011)
	Rapeseed oil	5	GC-MS	0.01 to 1.10	0.51	(Kuhlmann 2016)
	Peanut oil	4	Alkaline/Br^- GC-MS	0.40 to 1.10	–	(Kuhlmann 2011)
	Peanut oil	3	LC-MS/MS	0.44 to 0.57	0.49	(MacMahon and others 2013a)
	Sunflower oil	11	GC-MS-HS	0.02 to 0.90	0.36	(Kuhlmann 2016)
	Walnut oil	5	Acidic/Br^- GC-MS	–	5.83	(Ermacora and Hrncirik 2013)
	Walnut oil	5	Alkaline/Br^- GC-MS	0.70 to 1.40	–	(Kuhlmann 2011)
	Coconut oil	2	Alkaline/Br^- GC-MS	0.50 to 3.00	–	(Kuhlmann 2011)
	Almond oil	1	LC-MS/MS	–	0.03	(MacMahon and others 2013a)
	Grapeseed oil	3	LC-MS/MS	0.14 to 3.02	1.14	(MacMahon and others 2013a)
Infant and baby food	Infant formula (fat fraction)	70	Acidic/Br^- GC-MS	0.16 to 0.87	0.36	(Wöhrlin and others 2015)
Meat sample	Pork, beef, chicken cooked by gas-fired frying pan and charcoal grill	6	Two-step SPE/LC-MS	0.07 to 0.17 for gas-fired frying pan, 0.67 to 1.11 for charcoal grill	–	(Inagaki and Hirai 2016)
Conventional samples	Oxtail soup (powder)	3	Acidic/Br^- GC-MS	–	0.31	(Kūsters and others 2011)
	Gravy (powder)	3	Acidic/Br^- GC-MS	–	0.07	(Kūsters and others 2011)
	Vegetable soup (powder)	3	Acidic/Br^- GC-MS	–	0.14	(Kūsters and others 2011)
	Margarine	3	Acidic/Br^- GC-MS	–	3.63	(Kūsters and others 2011)
	Cookies	3	Acidic/Br^- GC-MS	–	0.94	(Shiro and others 2011)
	Sauce for chicken (powder)	3	Acidic/Br^- GC-MS	–	0.07	(Kūsters and others 2011)
	Chocolate—hazelnut bar	3	Acidic/Br^- GC-MS	–	0.07	(Kūsters and others 2011)

"–" Represents not available/analyzed data.

small amounts, ranging from 0 to 0.99 mg/kg, expressed by the 3-MCPD equivalent (Arisseto and others 2015). This finding most likely indicates that the digestion of GEs and 3-MCPD esters from fried food exhibits a lower risk to human health. Moreover, some food products, prepared using contaminated vegetable oils and fats as the main ingredients, such as infant formula, margarine, creams, and mayonnaise, may contain certain amounts of GEs derived from the raw oil, without high temperature exposure (Kūsters and others 2011; Ermacora and Hrncirik 2014b). In recent years, with increasing social awareness of oil safety, several industrial standards have been established for limiting the GE contents in oils and fats: below 0.60 mg/kg of oil set by China's health care and food industry, below 0.50 mg/kg of oil set by Nestlé, and below 0.10 mg/kg of oil set by Yili Company. However, no universally and globally accepted standard has been set so far.

A significant discrepancy has also been observed among the GE levels determined by different methods in the same type of oils. For example, 30.2 mg/kg of GE levels in palm oil were detected by GC-MS with normal phase LC (NPLC) separation, but only 9.26 to 9.40 mg/kg were detected by LC-MS with 2 solid phase extractions (SPEs) (Blumhorst and others 2013; Steenbergen and others 2013). Several likely reasons have been proposed for this phenomenon: First, the crude oils, such as palm oil, were collected from different geographic locations with varying climatic conditions. This may have caused a distinct hydrolysis of TAGs by lipase during the maturity and harvesting of oil plants for the formation of DAGs and MAGs (Siew and Ng 1995). Second, the difference in refining process parameters, particularly the deodorization step, likely caused discrepancies in the found GE levels. Third, no unified standard method is available for analysis of GEs, and the GE levels in the same type of oil determined by these methods with different sensitivity, specificity, and accuracy cannot be compared. This general limitation will be further discussed in detail below. Finally, the overall reliability of some data in certain cases may also be questionable.

Except for refined edible oils and fats, the data of GE occurrence available for oil-based food products can be found listed in Table 1. The GE levels in infant formula ($n = 70$) purchased from local markets are reported by Wöhrlin and others (2015). The highest concentration of GEs found was 0.87 mg/kg, expressed on a raw fat basis. Ideally, the report motivated government officials and industry to improve the safety of infant formula as an artificial

substitute for human breast milk, commonly used for feeding infants in their 1st year of life. To ensure the accuracy of GE content in infant formula, a more recent report proposed a microwave extraction method of fat with the limit of detection (LOD) and limit of quantitation (LOQ) equaling 0.0008 and 0.0028 mg/L, respectively (Marc and others 2016). In conventional oil-based food sources, relatively high contents of GEs can be found in margarine and cookies, with a mean of 3.63 and 0.94 mg/kg, respectively. The amounts of GEs in oil-based food products are caused by the addition of contaminated oils and fats as well as high temperatures in the manufacturing processes.

Formation of GEs

The occurrence of GEs in refined edible oils has attracted significant attention with respect to the formation mechanism, including the precursors of GEs and factors influencing the formation of GEs. The deodorization step in the oil refining process significantly affects the formation of GEs (Weisshaar and Perz 2010; Destaillats and others 2012a). In initial studies, GEs were regarded as a pathway of 3-MCPD ester formation or their degradation (Hamlet and others 2002; Svejkovska and others 2006). Subsequently, it was proposed that GEs, as well as 3-MCPD esters, form an acyloxonium ion intermediate 1st and then rearrange through charge migration, finally forming GEs (Weisshaar and Perz 2010; Rahn and Yaylayan 2011a). However, it was also considered that GEs and 3-MCPD esters could be formed following a different pathway, depending on the applied temperature and reaction time (Haines and others 2011; Hrncirik and Duijn 2011). In this review, we focus on 3 aspects of the formation of GEs from macroscopic and microscopic perspectives, that is, precursors and factors influencing the formation of GEs for the macroscopic section and reactive mechanisms for the microscopic section.

Precursors

DAGs and MAGs, the minor components in edible oils, may be formed not only through lipase hydrolysis of TAGs during maturing, harvesting, and transportation of oil fruits/seeds, but also through pyrolysis of TAG at high temperatures, including conventional heating and deodorization (Shimizu and others 2012a; Lucas-Torres and others 2014). Thus, different distributions of DAGs and MAGs may be observed in the different varieties of oils or the same type of oil from different locations, with MAGs being present in much smaller amounts than DAGs. For example, the total DAG contents, including 1,2-DAGs and 1,3-DAGs, in corn oil, palm kernel oil, soybean oil, coconut oil, and sunflower oil are all different, that is, 4.1%, 2.8%, 2.6%, 2.3%, and 2.2%, respectively (Hamlet and others 2011). Moreover, due to the specificity of TAG lipase at Sn-1 or Sn-3 position, the 1,2-DAG contents are found to be higher than the 1,3-DAG contents in freshly extracted oils. However, 1,3-DAGs exhibit a higher stability, which explains the relative decrease in 1,2-DAG levels compared to 1,3-DAGs and total DAG levels during storage (Hamlet and others 2011). Because of a longer water exposure compared to seed oil, fruit oils are more susceptible to potential hydrolysis reactions. DAG levels are therefore particularly high in fruit oils, such as palm oil and olive oils. For example, palm oil features the highest amount of DAG, ranging from 4% to 12%, with a mean of 6.5%. Furthermore, high contents of GEs have been reported in refined palm oil (Pudel and others 2011; Aniołowska and Kita 2016a). The GE levels in DAG-rich oil are 12 to 43 times higher than those in the normal edible oil consisting chiefly of TAGs (Masukawa and others 2010). Haines and others (2011) reported that only 5 mg/kg or less of GEs were detected in oil consisting of a DAG content of <2%. However, high DAG contents (> 6%) in oil were found to correspond to the prominent GE levels. This notion seems to indicate that DAGs are likely to be involved in the formation of GEs.

Furthermore, Destaillats and others (2012a) as well as Shimizu and others (2012a) conducted a series of model reactions simulating oil deodorization with pure TAG, DAG, and MAG. GEs were detected in heated DAG and MAG, but only in trace amounts in TAG. Concurrently, Freudenstein and others (2013) also demonstrated the contribution of DAGs and MAGs to the formation of GEs in refined oil. In another approach, increasing amounts of pure DAG and MAG were added into the model oil without polar fractions and the mixtures were heated at 240 °C for 2 h. In both tests, the formation of GE increased in a linear fashion, with the increasing amounts of DAGs and MAGs. However, other minor components, such as the phospholipids, chloride, and free fatty acids (FFAs), did not affect or merely slightly affected the formation of GEs. Therefore, it can be concluded that DAGs and MAGs are the precursors of GEs. Our experiments also prove further that MAGs exhibit a higher formation capacity than DAGs. This could be achieved by comparing real edible oils and chemical models (Cheng and others 2016). However, due to the low molecular weight of MAGs that are readily stripped into the deodorizer distillate or due to possible interesterification reactions from 2 MAGs to DAG, MAGs can be found in refined edible oils only in trace amounts (< 0.5%) (Goh and Timms 1985; Shimizu and others 2012a), accounting for its low contribution to the formation of GEs. Previously, glycerol had been shown to function as a precursor of GEs (Freudenstein and others 2013), but it could be completely removed by distillation off due to its low molecular weight.

Factors

As mentioned above, GEs, as well as 3-MCPD esters, are formed predominantly during the deodorization step in the oil refining process. Deodorization temperature and time have been shown to represent the most crucial factors for the formation of GEs. Several research groups have reported that GE levels increase upon increasing the incubation temperature ranging from 140 to 280 °C (Hrncirik and Duijn 2011; Destaillats and others 2012a). Thus, the finding explains why no correlation between the GE levels and DAG contents were observed in in the production of commercial oil samples by different suppliers using different deodorization temperatures (Craft and others 2012). However, topic of controversial debates is the issue whether GEs can degrade via thermal degradation reactions at certain temperatures and reaction time. Pudel and others (2011) reported that the GE content increased with time below 250 °C, favoring the formation of GEs. However, at temperatures up to 290 °C, the GE levels decreased within 2 h, which was considered as a consequence of GE degradation or distillation. GEs have been detected in deordorizer distillates (Craft and others 2012), and recently, it has been found that GE levels decreased rapidly with time after 2 h at 200 °C in DAG-model reaction occurring in sealed ampoule bottle (Cheng and others 2016). If the deodorization temperature was set at 230 °C, GE levels increased with time in the range of 1 to 5 h (Hrncirik and Duijn 2011). Therefore, the accumulation of GEs in refined oil depends on the formation rate of GEs from DAGs and MAGs as well as their degradation and distillation rate during deodorization in oil refining process. It seems likely that the higher

the temperature that is implemented, the higher the degradation rate of GEs that is observed in the deodorization step. This finding may further explain why the GE content in frying oil decreases during frying for a long period of time (>24 h), regardless of the applied frying temperature (Aniołowska and Kita 2016b).

In the oil deodorization step, apart from deodorization temperature and time, the stripping steam rate can also influence the formation of GEs. Özdikicierler and others (2015) investigated the effects of process parameters (temperature, pressure, and stripping steam) on the formation of GEs during steam distillation of olive oil and olive pomace oil by response surface methodology. It was found that the interaction between the stripping steam rate and temperature was statistically significant for the formation of GEs. When the water flow rate was higher than 2.0 mL/min, the increasing mass of distillation vapor from oil to cooler, which may contain MAGs, DAGs, and even GEs, likely explained why the GE contents decreased despite of the high temperature.

Additionally, due to an uneven heat distribution in oil fruits/seeds during the roasting or drying processes, at the edge of the roaster the temperature may increase beyond 200 °C (Sánchez Moral and Ruiz Méndez 2006), high enough to form GEs. In some cases, small amounts of GEs could also be found in crude oils (Matthäus and others 2011; MacMahon and others 2013a; Qi and others 2015). Although GE levels remain constant during the refining process prior to deodorization, DAG and MAG may be reduced to a certain degree (Bailey 2005), which indirectly influences the formation of GEs. Additionally, it is well known that GEs are unstable substances due to the presence of an epoxide group in the chemical structures. It has been proposed that the epoxy structure of GEs may be destructed under acidic conditions, removing different amounts of GEs that depend on the concentration of acid in refined oil (Matthäus and others 2011). However, Freudenstein and others (2013) have suggested that the presence of FFAs could support the formation of GEs. Although it is believed there is little effect on the formation of GEs in comparison with DAGs, certain amounts of FFAs exist in bleached oil during physical refining. This notion seems to indicate that a relatively low level of acid favors the formation of GEs, but higher concentration of acid seems also to reduce the occurrence of GEs in refined oil. However, further studies and follow-up work must be conducted in order to confirm this hypothesis.

Reactive mechanisms

It has already been well documented that DAGs and MAGs represent the most important reactants for the formation of GEs formed during high temperature exposure and depending on both temperature and temperature exposure time. Unlike the chlorine-containing 3-MCPD esters, GEs are formed without chloride. Presently, there are 4 proposed mechanisms of GE formation derived from DAGs and MAGs (Figure 2A), all involving an intramolecular rearrangement through charge migration and differing from each other depending on either the nature of the intermediate or the leaving group. Two of the proposed mechanisms involve a common reactive intermediate formed by either deacidification of 1,2-DAGs (Figure 2A, pathway d) or dehydration of MAGs (either 1-MAGs or 2-MAGs) (Figure 2A, pathways c and c′). The other 2 pathways consider a direct intramolecular rearrangement followed by elimination of fatty acid for DAGs (either 1,2-DAGs or 1,3-DAGs) (Figure 2A, pathways b and b′) or of water for 1-MAGs (Figure 2A, pathway a). The next subsection will review each mechanism, providing evidence supporting these mechanisms in refined edible oils and oil-based food products.

For a long time, the cyclic acyloxonium ion, a well-known reactive intermediate in organic chemistry (Paulsen 1971), has been proposed as a possible reactive intermediate in the formation of 3-MCPD esters and 2-MCPD esters (Collier and others 1991; Hamlet and others 2002; Velíšek and others 2002). It may be readily formed by the elimination of hydroxyl groups from either DAGs or MAGs. Rahn and Yaylayan (2011a) confirmed the actual formation of cyclic acyloxonium ions through real-time monitoring of FTIR spectra of pure acylglycerols with a chloride species treated at 100 °C. Considering the similarity in molecular structure, formation condition, and widespread distribution between 3-MCPD esters and GEs (MacMahon and others 2013a), it has been concluded that cyclic acyloxonium ions may also represent a reactive intermediate in the formation of GEs (Weisshaar and Perz 2010). However, the hypothesis has not yet been experimentally confirmed. In a recent study conducted by our group, the cyclic acyloxonium ion could be identified through FTIR spectra of pure 1,2-dipalmitin and 1-monopalmitin during high-temperature treatment (Cheng and others 2016). Therefore, we can confirm that a cyclic acyloxonium ion I is formed through the transformation of an electron along with the elimination of fatty acid for 1,2-DAGs (Figure 2A, pathway d) and water for MAG (Figure 2A, pathways c and c′). The cyclic structure may then be opened through an intramolecular rearrangement at high temperature, ultimately generating GEs. Theoretically, due to the steric hindrance at the Sn-2 position, the cyclic acyloxonium ion cannot be formed by the deacidification of 1,3-DAGs. Furthermore, in comparison with DAGs, the reactivity of MAGs is expected to proceed more rapidly in an acidic medium, such as partially hydrolyzed oils, not only due to more similar molecular structures of MAGs and GEs, but also due to the superior leaving group water compared to a fatty acid chain (Hamlet and others 2004). However, Hrncirik and Duijn (2011) as well as Shimizu and others (2012a) have concluded that the formation reactions of 3-MCPD esters in the presence of chloride are completed within a relatively short period of time. However, steady levels may be reached and GEs form continuously throughout the heating period, without chloride influencing the formation of GEs. The nonsynchronous and independent occurrence lead to a possible conclusion that 3-MCPD esters and GEs feature no common intermediates. The authors also reported that the levels of GEs and 3-MCPD esters reached an equilibrium after 1 to 2 h of heating and depending on the temperature level (Shimizu and others 2013a). The biggest difference between the 2 formation reactions is the absence of chloride, which may be responsible for the different formation rates. It has also been reported that, due to the stability of 3-MCPD esters, the plateau levels of 3-MCPD esters in the model test are the result of completed reactions resulting from the lack of an available chloride source. Hence, the formation of 3-MCPD esters is limited to not only the quantity, but also the availability of chloride, such as the molecular species. Conversely, GEs represent unstable substances at high temperatures. Therefore, the GE formation and transformation both take place in a simultaneous fashion, which may partially explain why GE levels reach an equilibrium in a longer period of time than 3-MCPD esters.

To the best of our knowledge, Destaillats and others (2012a) were the 1st to investigate the formation mechanisms of GEs in refined palm oil. The authors conducted a series of model reactions mimicking palm oil deodorization with pure TAG,

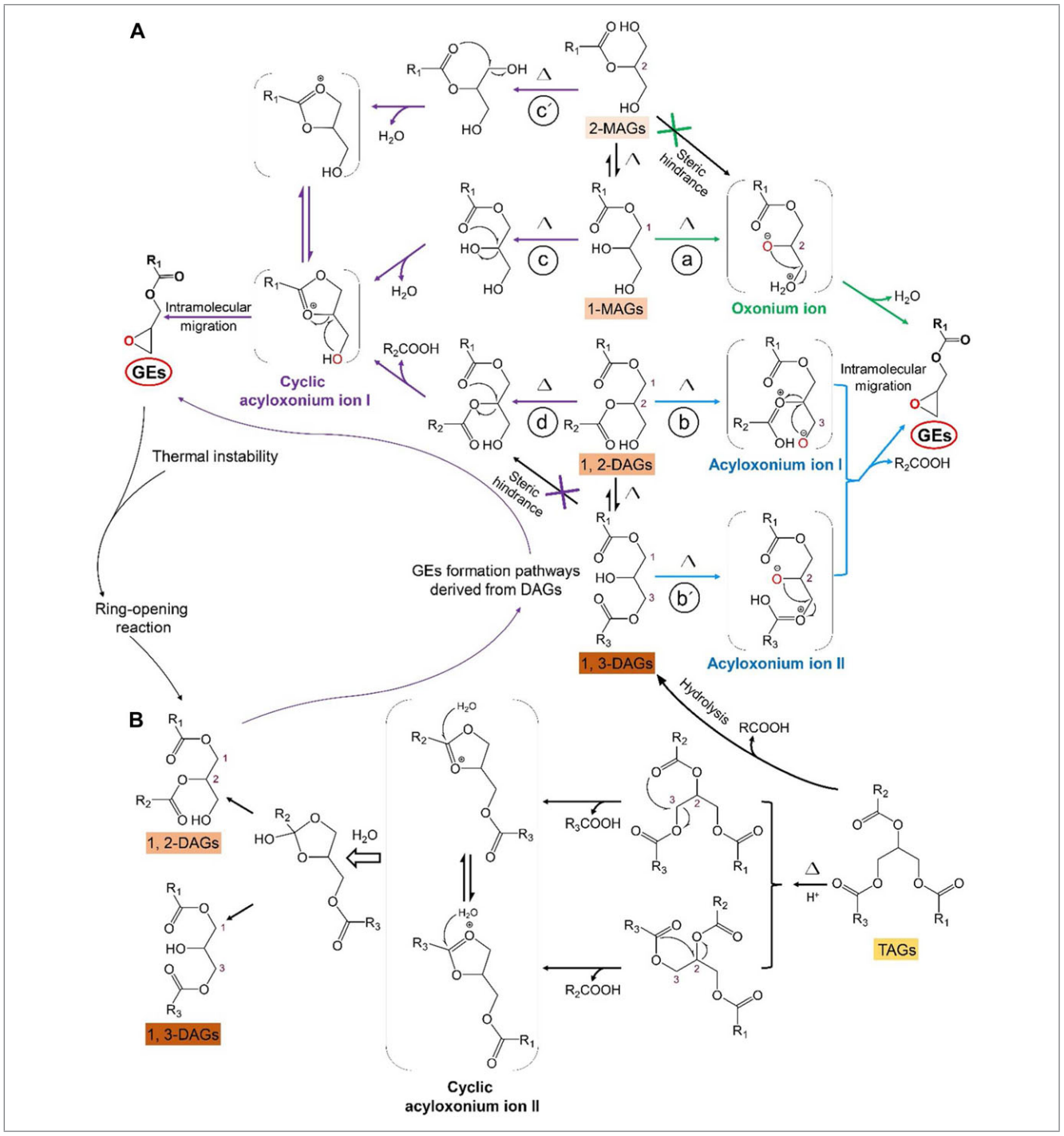

Figure 2–Summary of the proposed pathways of GE formation: (A) reaction mechanisms derived from DAGs and MAGs and (B) mechanisms of formation of DAGs and MAGs derived from TAGs at high temperature (Rahn and Yaylayan 2011a, 2011b; Destaillats and others 2012a). R_1, R_2, and R_3, fatty acyl groups (either "same" or "different").

DAG, and MAG. The results revealed that thermally treated DAG with high amounts of GEs induced 5 times more FFAs than thermally treated TAG with trace amounts of GEs did. However, FFAs could be detected at a lower amount in thermally treated MAG, also with high amounts of GEs. Based on the above results and a previous report (Velíšek and others 2002), the acyloxonium ion intermediates have been suggested to be generated through the initial elimination of FFAs by abstraction of the proton in the hydroxyl group (at the Sn-2 position for 1.3-DAGs and at the Sn-3 position for 1,2-DAGs) and the vicinal carboxyl group. The rearrangement through charge migration eventually results in the formation of GEs (Figure 2A, pathways b and b′). In comparison with the cyclic acyloxonium ion I, acyloxonium ion intermediates exhibit a higher potential energy, suggesting a lower stability due to the fact that these species do not feature a resonance-stabilized structure (Hamlet and others 2011). This notion seems to suggest the cyclic acyloxonium ion I in pathways c and d features a greater potential for the formation of GEs than acyloxonium ions in

pathways b and b′ (Figure 2A) do. Moreover, the latter species are difficult to be detected due to their short time occurrence. Therefore, the species have never been identified *in vitro*. For MAGs, the elimination of water could be initiated by abstraction of the proton in the hydroxyl group as well as the hydroxyl group in the vicinal diol following the formation of GEs (Figure 2A, pathway a). This mechanism has been proposed in a similar fashion before in the literature (Hamlet and others 2011). The reaction does not occur in 2-MAGs, unless it is transferred to 1(3)-MAGs. The latter is most notably due to the steric hindrance at the Sn-2 position.

The trace amounts of GEs detected in pure TAG suggest that TAGs do not represent the precursors of GEs. However, DAGs and MAGs can be formed from the direct hydrolysis of TAGs through the elimination of fatty acids under high-temperature conditions. Unlike GEs, the formation of 3-MCPD esters has been reported to be predominantly derived from TAGs that involves the cyclic acyloxonium ion II reaction intermediate (Destaillats and others 2012b). The cyclic acyloxonium ion II may be attacked by the chloride ion to form 3-MCPD esters and may also decompose to form DAGs through the hydrolysis reactions (Figure 2B) (Rahn and Yaylayan 2011a). DAGs and MAGs formed from TAGs through the 2 routes described above may contribute to the minor formation of GEs in pure TAG.

Except for the 4 possible pathways mentioned above, GEs may also be formed through the decomposition of MCPD monoesters involving 3-MCPD monoesters, and 2-MCPD monoesters rather than their diesters. Velíšek and others (2002) have discussed the decomposition of 3-MCPD, 2-MCPD, and their enantiomers in alkaline media from a glycidol intermediate, which was found to then hydrolyze to form glycerol. The latter was also observed as the decay pathway of 3-MCPD and 2-MCPD in model dough systems, as well as a side reaction in the production of epichlorohydrin via dehydrochlorination of dichloropropanols (Hamlet and others 2003; Milchert and others 2012). Based on a classical organic chemistry theory, the combination of a hydroxy group and a chlorine atom on neighboring carbon atoms is responsible for the most common elimination reaction of vicinal chlorohydrins, such as by a dehydrochlorination to form substituted oxiranes (epoxides) (Ketola and others 1978). It has also been shown that 3-MCPD is almost 10 times more stable than 2-MCPD that decomposes readily under alkaline conditions (Dolezal and Velisek 1994). More importantly, their formation mechanisms involving the SN2 reaction are susceptible to steric effects (Collier and others 1991). As expected, the levels of 3-MCPD esters in almost all oils are significantly higher than the levels of 2-MCPD esters (Koyama and others 2015; Jędrkiewicz and others 2016). Therefore, it seems that the potential of 2-MCPD to form glycidol is much greater than 3-MCPD. Similarly, the basic reactions of an esterified form, such as the formation and decomposition, are considered to be analogous to the reactions of the free form in the presence of a vicinal chlorohydrin structure, such as 2-MCPD monesters and 3-MCPD-1-esters. The possible formation pathways of GEs derived from both MCPD monoesters are shown in Figure 3. In slightly acidic as well as neutral environments, the dehydrochlorination of vicinal chlorohydrin groups in MCPD esters involves the elimination of a chloride anion, resulting in the formation of a carbocation intermediate. Then, the hydroxyl group in MCPD monoesters may act as a nucleophile, leading to the formation of a protonated epoxide and the formation of GE epoxides via proton elimination (Figure 3). Conversely, the decomposition reaction of MCPD esters takes place rather rapidly in an alkaline environment. As outlined in Figure 3, the exogenetic hydroxyl anions react with hydrogen in the hydroxyl group of chlorohydrin to reach an equilibrium as the corresponding alcoholate anion. The alcoholate oxygen then attacks the carbon bearing the leaving chloride atom to form GEs as the rate-determining step in this dehydrochlorination reaction. Due to the steric hindrance at the Sn-2 position, the 3-MCPD-2-ester, the isomer of 3-MCPD-1-ester, cannot directly form GEs through the 2 routes described above at pH $\leq$7 and pH >7 until transformation into 3-MCPD-1-ester bearing a vicinal chlorohydrin structure takes place (Figure 3). Also, the high intrinsic hydrolysis tendency of oils exposed to alkali media restricts the presence of alkali conditions in edible oils, and leads to reduce contribution for the total GE levels compared with those exposed to acidic and neutral media. Although these predictions seem to be feasible in theory, 3-MCPD monoesters or 2-MCPD monoesters in refined oils are only found in trace amounts, and, in part, are derived from the hydrolysis of their corresponding diesters, the most general form of 3-MCPD esters (Zelinkova and others 2006; Ermacora and Hrncirik 2014a). Furthermore, it is possible that 3-MCPD esters may be formed through the dehydrochlorination of dichloropropanol esters, which can be either found not at all or merely in small amounts in some oils, such as sunflower and tea seed oil (Milchert and others 2012; Yang and others 2015; Kuhlmann 2016). It is thus believed that the contribution of 3-MCPD or 2-MCPD esters to the formation of GEs in refined edible oils is most likely negligible.

As mentioned before, the GE concentrations in refined oils can be obtained as a result of competition reactions between the formation and transformation, due to the fact that the epoxy group is not stable upon heating. In comparison, the transformation of GEs can be observed at lower temperatures, suggesting the transformation reaction exhibits a lower activation energy than the formation reaction (Shimizu and others 2013a). To date, the transformation mechanism of GEs in refined edible oils under high temperature conditions still remains unclear. The ring-opening reaction of GEs has been demonstrated to take place under acidic conditions, leading to the formation of DAGs and MAGs (Shimizu and others 2012b). Accordingly, it seems possible that GEs may transform back to MAGs and DAGs during heating in the presence of FFAs. However, the hypothesis for this action still remains unexplored. It had been proposed before that one of the formation pathways of 3-MCPD esters takes place via GEs, meaning that GEs may transform into 3-MCPD esters in the presence of a chlorine source (Figure 1). Rahn and Yaylayan (2011b) as well as Shimizu and others (2013b) have confirmed that GEs may represent precursors for 3-MCPD monoesters which exhibit merely a very low concentration in edible oils (Zelinkova and others 2006). However, the transformation rate between these species has been shown to be very low (Shimizu and others 2013b), most notably due to the fact that no significant difference between GE levels in model reactions with and without a chloride source (Shimizu and others 2012a). The thermal degradation test of 3-MCPD diesters carried out in a model system mimics the deodorization process of vegetable oils and also demonstrates that the continuous formation of 3-MCPD monoesters is not due to the degradation of the corresponding diesters, but instead is likely due to the transformation of GEs in the presence of chloride ion (Ermacora and Hrncirik 2014a). Additionally, degradation reactions of the ester are thought to be possible to take place at high temperature, for example, thermal degradation of TAGs during heating (Lucas-Torres and others 2014). Therefore, it is believed that free glycidol detected in the deodorizer distillate may be formed from GEs. However, in a sealed heating system, the species is not formed

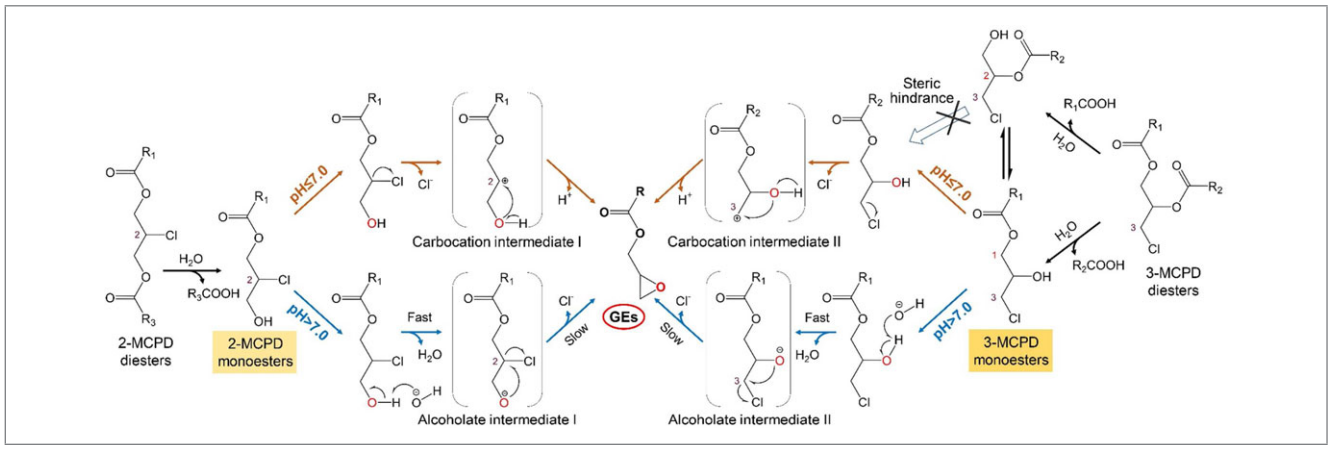

Figure 3–Proposed pathway of GE formation derived from 2-MCPD esters and 3-MCPD esters in neutral, acidic, and alkaline media (Hamlet and others 2011). R, R_1, and R_2, fatty acyl groups (either "same" or "different").

in diolein, whereas GEs are formed at identical concentrations (Shimizu and others 2012a), indicating that the deacidification of GEs via thermal degradation reactions is rather impracticable.

Analysis of GEs

Because of structural similarities of GEs and 3-MCPD esters, initial methods for the detection of GEs provide *the analytical protocol based on that of* 3-MCPD esters, involving the transesterification of esters to release the free forms (Divinova and others 2004; Seefelder and others 2008; Weisshaar and Perz 2010). It has already been shown that some technological drawbacks exist, ultimately limiting the efficient detection of GEs as well as 3-MCPD esters. For example, the absence of a proper chromophore renders high-performance liquid chromatography (HPLC) with ultraviolet (UV) or fluorescence detectors unsuitable. Furthermore, a high boiling point limits the use and development of a direct GC method. To the best of our knowledge, only 1 report describes the GC analysis of GEs with silylation in silicon oil. However, this method is not applicable for edible oils consisting of high amounts of acylglycerols (Engbersen and Van Stijn 1976). Therefore, as shown in Table 2 and 3, therefore, the present analysis methods to determine GEs in edible oils may be grouped into 2 categories: indirect and direct methods. In the following subsections, we will provide a comprehensive summary of the 2 methods.

Indirect analysis

The indirect analysis of GEs is based on the transformation of glycidol into a halogenated derivative involving a derivatization reaction with derivatizing agents such as phenylboronic acid (PBA), heptafluorobutyrylimidazole (HFBI), bis(trimethylsiyl)trifluoroacetamide (BSTFA), and heptafluorobutyric anhydride (HFBA). In indirect analyses, intact 3-MCPD esters and GEs are 1st hydrolyzed into the corresponding free 3-MCPD and glycidol under either acidic or alkaline conditions, followed by a purification process (liquid-liquid extraction), derivatization, and quantification by GC-MS. The latter has been regarded an official method by the German Society for Fat Science (DGF) (DGF Standard Methods C III 18 (09) 2009). This method consists of 2 pretreatments (option A and option B) with different analytical mechanisms (Figure 4A). In option A, the released glycidol from GEs by sodium methoxide, together with 3-MCPD, can be converted to a 3-MCPD-PBA derivative in the presence of chloride and PBA, thus determining the sum of GEs and 3-MCPD esters. Option B only determines the level of 3-MCPD esters resulting from the elimination of GEs by acid treatment; the total GE amounts are calculated as the difference between both measurements with a modification by multiplication with a stoichiometric factor of 0.67. The development of the method is based on the assumption that glycidol can be completely converted to 3-MCPD in option A, and completely eliminated by acid treatment in option B, whereas no other substance will react with inorganic chloride to form 3-MCPD. As a reference, a modified approach has been approved as a standard method by the American Oil Chemists' Society (AOCS) (Joint AOCS/JOCS Official Method Cd 29c-13 2013).

Weisshaar (2008) showed that the acidic transesterification procedure incorrectly produced higher levels of 3-MCPD esters than the transesterification procedure with sodium methoxide\methanol. This finding is most likely due to the additional formation of 3-MCPD under acidic conditions. Nevertheless, the previously published findings determined that, when free 3-MCPD is released by the alkaline-catalyzed transesterification, the presence of GEs and chloride can lead to an overestimation of 3-MCPD ester levels in the tested oil (Kuhlmann 2008; Hrncirik and others 2011). Conversely, Hrncirik and others (2011) demonstrated that, due to the irreversible degradation of GEs during acidic transesterification, the subsequent conversion to 3-MCPD proved to be impossible. The latter seemed to be the reason for a better robustness and selectivity of this acid transesterification. Meanwhile, NMR studies indicated that a bidirectional conversion takes place between GEs and 3-MCPD esters in option A with the alkaline transesterification as depicted above (Kaze and others 2011). Furthermore, it was found that 37% of the 3-MCPD mass was converted to glycidol during the derivatization step in option A, while >70% of the glycidol mass was converted to 3-MCPD. Additionally, the incomplete epoxide ring-opening reaction of glycidol and its esters (about 90%) could also be observed by acid treatment in option B. Furthermore, 3-MCPD and its esters were determined to be formed from partial acylglycerols and related chloride-containing substances in the tested oil during acidic pretreatment, which interfered with the accurate determination of the 3-MCPD esters. The results obtained led to an underestimation of the GE contents in edible oils when this indirect method was used. This, in turn, explains why lower GE levels were obtained through the indirect method, compared to the direct method, using the same sample (Shimizu and others 2010). Furthermore, acid

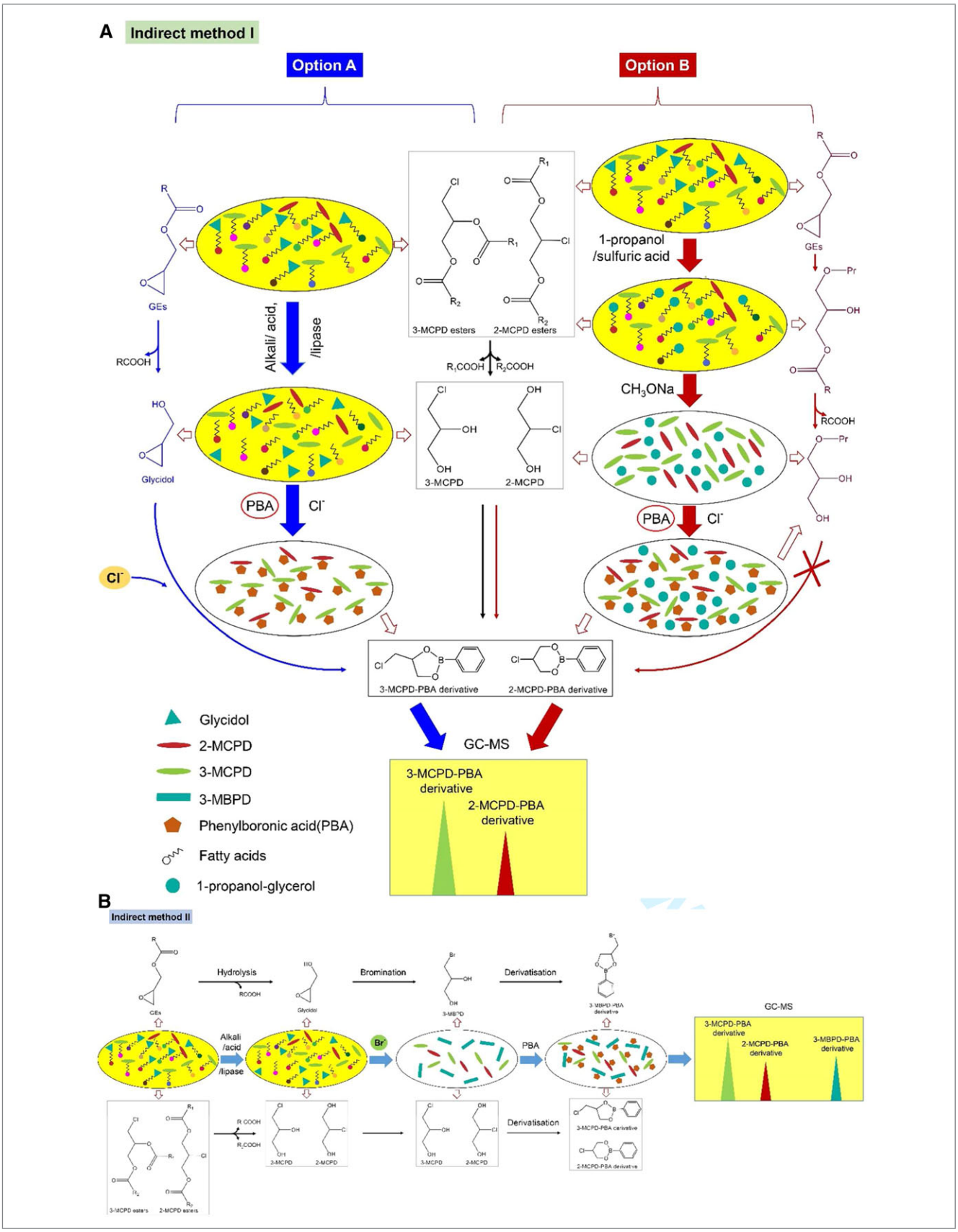

Figure 4–Reaction scheme of the indirect determination of GEs by GC-MS: (A) chlorination and (B) bromination of GEs followed by PBA derivation. R, R_1, and R_2, fatty acyl groups (either "same" or "different").

Table 2–Indirect analysis methods for GEs in edible oils.

Method denomination	Analyte	Internal standard	Transesterification (time)	Derivatization agent	Comments	References
Alkaline (DGF standard method C-III 18 (09))	GEs	3-MCPD-d_5	Sodium methoxide /methanol (10 min)	PBA	Bidirectional conversion between glycidol and 3-MCPD leads to inaccuracy of method.	(DGF Standard Methods C III 18 (09) 2009; Kaze and others 2011)
Alkaline	GEs and 3-MCPD esters	1,2-Dipalmitoyl-3-chloropropane-d_5	Methanol/sulfuric acid (1 min)	PBA	It is the 1st to determine simultaneously GEs and 3-MCPD esters in different food products	(Küsters and others 2011)
Alkaline or acidic, mild	GEs and 3-, 2-MCPD esters,	1,2-Dipalmitoyl-3-chloropropane-d_5, 3-MBPD-d_5	2.5 mg/mL methanolic sodium hydroxide solution (16 h); 1.8% by volume of sulfuric acid–methanol solution (16 h)	PBA	GEs were quantified based on the 3-MBPD/3-MBPD-d_5 ratio due to the transformation of glycidol to MBPD.	(Kuhlmann 2011; Ermacora and Hrncirik 2013, 2014b)
Enzymatic (*Candida rugosa*)	GEs and 3-MCPD esters	3-MCPD-d_5, 3-MBPD-d_5,	Lipase (30 min)	PBA	On the basis of (Kuhlmann 2011) alkaline, methanolysis is displaced by lipase hydrolysis to shorten the testing time, shortening the detection time	(Miyazaki and others 2012; Koyama and others 2015; Miyazaki and Koyama 2016)

transesterification generally required a much longer reaction time (16 h), based on the developed determination methods of 3-MCPD esters reported previously (Zelinkova and others 2006; Ermacora and Hrncirik 2012). To improve the accuracy and time requirements of the indirect GE determination, a further modification of sample pretreatment was made to avoid using the acid cleavage of the esters in the determination of GEs. Several researchers also implemented the lipase-catalyzed hydrolysis of GEs using a lipase from *Candida rugosa* (30-min incubation at room temperature), which greatly shortens detection time (Miyazaki and others 2012; Koyama and others 2015).

Different from the dependence of GE determinations on 3-MCPD esters in the initial indirect methods described above, Kuhlmann (2011) developed a new type of indirect determination method for GEs which allowed for the direct detection of glycidol derivatives as well as 3-MCPD derivatives, independently of one another (Figure 4B). The analytical protocol involves a series of distinct steps: transesterification under mildly alkaline or lipase-catalyzed conditions, transformation of glycidol into monobromopropanediol (MBPD), derivatization of MBPD, and GC-MS analysis. Two of 3 published methods by AOCS are based on this protocol (Joint AOCS/JOCS Official Method Cd 29a-13 2013; Joint AOCS/JOCS Official Method Cd 29b-13 2013). Although this approach enables GEs to be determined in a simultaneous fashion with 3- and 2-MCPD esters, and lipase has been employed for the hydrolysis of esters, avoiding the formation of additional GEs from MCPD esters or partial acylglycerols (Miyazaki and others 2012; Koyama and others 2015), the incomplete bromination may still result in an underestimation of GE levels in the oils tested. Therefore, it becomes obvious why a direct determination method, requiring no transesterification and derivatization, is needed.

Direct analysis

Compared to the indirect determination of GEs, a direct method generally quantifies the levels of every GE species bearing different fatty acyl chains by LC-MS without any chemical transformation. Therefore, the method provides full information on the composition of the sample without any side reactions. An overview of the methods from the recent literature can be found listed in Table 3. The development of a direct method, instead of an indirect method, has become of great interest in the last few years. A major challenge of the method is that the presence of large amounts of acylglycerols, especially TAGs in tested edible oil, will negatively influence the precision, accuracy, and susceptibility of the direct method, and therefore they must be removed before analysis. The distribution pattern of GEs is directly related to the fatty acid profiles of the tested oil (Aniołowska and Kita 2016a). It has already been proposed that the GE contents in most edible oils may be adequately represented by the analysis of 7 GEs, that is, GEs of lauric, myristic, palmitic, stearic, oleic, linoleic, and linolenic acids (Dubois and others 2011). Compared with the indirect method, a further separation and purification, as well as a larger initial investment in analytical standards, are required for the composition complexity of the corresponding analyte. Several purification techniques, such as gel permeation chromatography (GPC) (Dubois and others 2011) and 2-step SPE, have been designed to remove a large amount of tri- and partial acyl glycerides prior to LC-MS analysis. From an inspection of Table 3, the 2-step SPE purification, the 1st reversed phase SPE to remove nonpolar fractions (such as TAG) and the following normal phase SPE removal of partial acylglycerols, seem to be the superior pretreatment process and are applied in a variety of developed direct methods (Table 3). Furthermore, it has already been demonstrated that the implementation of the SPE procedure in the order listed above provides better analytical results than a SPE procedure in reversed order (Masukawa and others 2010). If the diluted oil is injected directly into the LC-MS system, a fast deterioration of system performance can be observed. Haines and others (2011) described the determination of 7 GEs and 20 3-MCPD esters in edible oils using LC-time-of-flight-mass spectrometry (LC-TOF-MS) in the positive-ion mode with the simplest implementation of sample preparation. The oil samples were only diluted by methanol–sodium acetate solution/methylene chloride/ acetonitrile (0.26 mM, 1:8:1

Table 3–Direct analysis methods for GEs in edible oils.

Method denomination	Analyte	Calibration	Sample preparation	Chromatographic column	Mobile phase composition	Recovery and LOD/LOQ	References
LC-MS, SIM: selected ion monitoring, APCI, in positive-ion mode	Five GEs	External standard method	Liquid/liquid partitioning of sample in acetonitrile; 2-step SPE cleanup: reversed phase SPE using Sep-Pak Vac RC C18 cartridge 500 mg (Waters) followed by the normal phase SPE using a Sep-Pak Vac RC Silica cartridge 500 mg (Waters); evaporation of eluent and reconstitution of residue in methanol/2-propanol 1:1 by volume	UPLC-MS: Acquity UPLC BEH: ethylene bridged hybrid C18 column 100 × 2.1 mm i.d., 1.7-μm particle size (Waters); HPLC-MS: L-column ODS: octadecasilyl-silica 150 × 4.6 mm i.d., 5-μm particle size	A: acetonitrile /methanol/water 17:17:6 by volume; B: 2-propanol	71.3% to 94.6% (average 79.4%) for TAG-rich oils; 90.8% to 105.1% (average 97.2%) for DAG-rich oil, LOQ: 0.0045 to 0.012 mg/mL	(Masukawa and others 2010; Masukawa and others 2011)
LC-MS, SIM, APCI: atmospheric pressure chemical ionization, in positive-ion mode	Five GEs	Internal standard method with $C_{17:0}$-GE	Liquid/liquid partitioning of sample in tert-butyl methyl ether (MTBE)/ethyl acetate 4:1 by volume; 2-step SPE cleanup as depicted by Masukawa and others (2010); evaporation of eluent and reconstitution of residue in methanol/2-propanol 1:1 by volume	L-column ODS 150 × 4.6 mm i.d., 5-μm particle size	A: methanol; B: 2-propanol	Close to 100%, LOQ: 82 to 110 mg/kg	(Shiro and others 2011; Blumhorst and others 2013)
LC-TOF-MS for method development, SIM, ESI, in positive-ion mode LC-MS/MS for routine analysis, SIM, ESI, in positive-ion mode	Seven GEs	Standard addition	Liquid/liquid partitioning of sample in cyclohexane/ethyl acetate (1:1 by volume); GPC extraction on Bio-Beads S-X3 column 280 × 25 mm i.d.; additional cleanup for oils containing high DAG and MAG content by SPE: 500 mg silica (eluent dichloromethane)	LC-TOF-MS: Acquity UPLC 50 × 2.1 mm i.d., 1.8-μm particle size LC-MS/MS: Luna C18 column 50 × 3 mm i.d., 3-μm particle size	LC-TOF-MS: A: methanol/water/formic acid 75:25:0.1 by volume; B: 2-propanol: formic acid 99.9:0.1 by volume LC-MS/MS: A: methanol/water/formic acid 75:25:0.5 by volume; B: 2-propanol: formic acid 99.5:0.5 by volume	68% to 111% (average 93%), LOQ: 0.05 to 0.10 μg/kg	(Dubois and others 2011)
LC-TOF-MS, SIM of sodiated adducts, ESI, in positive-ion mode	Seven GEs and 20 3-MCPD mono- and diesters	Internal standard method with MCPD-d_5 dioleic acid ester and palmitic acid-d_{31} GEs	Dilution of sample in mobile phase B with 0.1% by volume of internal standard stock solution	Luna C18 column 50 × 3 mm, i.d., 3-μm particle size	A: 0.26 mM methanol-sodium acetate solution (MSA)/methanol/acetonitrile 1:8:1 by volume; B: MSA/methylene chloride/acetonitrile 1:8:1 by volume	LOD: 0.07 to 0.29 mg/kg for GEs, 0.21 to 1.69 mg/kg for 3-MCPD monoesters, and 0.10 to 0.40 mg/kg for 3-MCPD diesters	(Haines and others 2011)

(*Continued*)

Table 3–Continued.

Method denomination	Analyte	Calibration	Sample preparation	Chromatographic column	Mobile phase composition	Recovery and LOD/LOQ	References
LC-MS/MS, MRM, APCI, in positive-ion mode	Five GEs	Stable isotope dilution analysis with d_{31}-glycidyl palmitate and d_{35}-glycidyl stearate	Liquid/liquid partitioning of sample in acetone; 2-step SPE cleanup: reversed phase SPE using Sep-Pak C18 cartridge followed by normal phase SPE using silica cartridge (300 × 10 mm i.d.); evaporation of eluent and reconstitution of residue in diethyl ether	Gemini C18, 250 × 2.0 mm, i.d., 5-μm particle size	100% methanol	84% to 108%, LOD: 0.07 to 0.15 mg/kg using 10 mg sample and 0.001 to 0.003 mg/kg using 0.5 g sample of oil	(Becalski and others 2012)
LC-TOF-MS, SIM of sodiated adducts, ESI, in positive-ion mode	Five GEs, 9 3-MCPD mono- and diesters	Internal standard method with 1-palmitoyl-3-MCPD-d_5, 1,2-bis-linoleoyl-3-MCPD-d_5, 1,2-bis-palmitoyl-3-MCPD-d_5	Liquid/liquid partitioning of sample in *n*-hexane; 2-step SPE cleanup: normal phase SPE using Sep-Pak Plus SI cartridges, 500 mg (Waters) followed by reversed phase SPE using SPE Sep-Pak Plus C18 cartridge, 500 mg (Waters); evaporation of eluent and reconstitution of residue in acetonitrile	AQUITY UPLC BEH C18 column 50 × 2.1 mm i.d., 1.7-μm particle size	A: methanol /water 15:85 by volume; B: methanol/phase 97.5:2.5 by volume	62.6% to 108.8%, LOD: 0.16 ng/mL for GEs, 0.86 ng/mL for 3-MCPD monoesters, and 0.22 ng/mL for 3-MCPD diesters	(Hori and others 2012)
LC-MS/MS, MRM, ESI, in positive-ion mode	Six GEs, 12 sn-1 and sn-2 3-MCPD monoesters	Internal standard method with 6 deuterated GEs and 1-oleoyl-3-MCPD-d_5, 1-palmitoyl-3-MCPD-d_5	Dissolution of sample in 20% by volume ethyl acetate/MTBE; 2-step SPE cleanup: reversed-phase SPE using 1000 mg/6 mL C18 cartridge followed by normal phase SPE using 500 mg/3 mL Si cartridge; evaporation of eluent and reconstitution of residue in isopropanol	Pursuit XRs C18 column 150 × 2.0 mm i.d., 3.0-μm particle size	A: 2 mM ammonium formate /0.05% formic acid in 92:8 by volume methanol/water; B: 2 mM ammonium formate /0.05% formic acid in 98:2 by volume isopropanol/water	84% to 115% for GEs, 95% to 113% for sn-1 3-MCPD monoesters, 76.8% to 103% for sn-2 3-MCPD monoesters, LOD: below 0.03, 0.06, and 0.18 mg/kg, respectively	(MacMahon and others 2013b)
GC-MS, SIM, ESI, in positive-ion mode	Seven GEs	Internal standard method with glycidyl palmitate-d_5	Liquid/liquid extraction of sample in acetonitrile and heptane; 2-step SPE cleanup as depicted by Masukawa and others (2010); NPLC separation: Lichrosorb 5 Diol columns 250 mm × 4.6 mm i.d.	DB-5 ms column 15 m × 0.25 mm i.d., 0.10-μm particle size	Helium gas	85% to 115%, LOD: 0.01 mg/kg	(Steenbergen and others 2013)
^{1}H NMR	Intact GEs	Internal standard method with benzene	Dissolution of sample in hexane; silica gel column (particle size 75 to 150 μm) partitioning; evaporation of eluent; reconstitution of residue in deuterated chloroform		–	93.20% for palm olein, 100.65% for DAG-rich oil, LOD: 0.0511 mmol/L, LOQ: 0.170 mmol/L	(Song and others 2015)

by volume) prior to analysis. Nonetheless, the LOD of GEs in refined palm oil was estimated to be about 0.1 mg/kg, with 1 exception of 0.29 mg/kg for glycidyl myristate. The LODs were approximately 3 times higher than that reported by Shiro and others (2011). Unfortunately, the analyte recoveries were not available and likely varied significantly as a consequence of matrix effects. In addition, the developed method by Hori and others (2012), involving the addition of sodium salts to the mobile phase for the formation of sodiated adducts, caused significant negative effects on the MS instrument. The latter finding demonstrates that frequent instrument vvcleaning is critical, thereby avoiding a premature corrosion of the electrospray ionization (ESI) components, such as the nebulizer needle.

Considering the high cost and complex data analysis of LC-MS, Steenbergen and others (2013) have suggested a novel direct detection method for intact GEs in edible oils based on GC-MS. To reduce any interferences of acylglycerols, the preparation of oil samples involves the extraction of analytes with acetonitrile and heptane. The purification employs a 2-step SPE, as depicted above, together with a final isolation of the GEs by NPLC. The authors also considered that a potential formation of GEs might occur from the precursors in the injection port of the GC with a high temperature. Therefore, a cold on-column injection was adopted to avoid potential thermal degradation of GEs with the formation of artifacts. The LODs of GEs were determined to be about 0.01 mg/kg for the individual GE, which proves to be significantly lower than the developed LC-MS-based methods (Table 3). The recovery values were in the range of 85% to 115%, largely depending on the chain identity and level of GEs. However, both direct approaches based on GC-MS and LC-MS require a variety of expensive reference materials and a rather complicated sample preparation process. This ultimately limits their applicability in routine analyses. A more recent report describes another direct method for the determination of GEs based on ^{1}H NMR spectroscopy, an important analytical technique now widely used in the study of lipids, such as the determination of fatty acid composition, real-time monitoring of transesterification, and adulteration identification of vegetable oils (Anderson and Franz 2012; Zhang and others 2013a; Vicente and others 2015). In this method, a quantification formula was deduced from the diagnostic signals of epoxy methylene protons at chemical shifts of 2.56 and 2.76 ppm. By introducing a weighted average factor, the individual GE was calculated as the stoichiometric ratio of the glycidol-to-esterified lipid component followed by conversion of molar to weight percentage. The recovery values in palm olein and DAG-rich oil were determined to be 93.20% and 100.65%, respectively, and the LOD and LOQ were found to be 0.0511 and 0.170 mmol/L, respectively, which was far higher than LC-MS-based method. These results confirm the potential of the analytical model to for GEs determination. Unfortunately, no information was provided regarding GE levels of various oils, which might have been below the LOD due to the absence of a further purification process. Accordingly, further work is still required to evaluate the availability of NMR method used as an alternative routine analysis of GEs in refined oil.

From the above description of direct and indirect methods, the major advantages of the indirect method are the very simple sample pretreatment saving detection time, no need for intact GEs standards, which compensates for the lack of LC-MS-based and GC-MS-based direct methods. After further development of enrichment techniques, it is expected that the direct method will progress as an alternative for the routine analysis of GEs.

Elimination Methods of GEs

As mentioned above, GEs represent potential carcinogens widely found in refined edible oils and oil-based food products, with possible adverse effects on the human body and health upon ingestion. Due to the lack of limit standards yet worldwide, therefore, there is an urgent need to mitigate GE levels to ensure the quality and safety of edible oils and oil-based food products. Some elimination methods have been shown to be promising on a research laboratory or pilot plant scales and have therefore also been adopted in the oil production industry. Most studies on the elimination of GEs to date have been performed using palm oil, most notably due to its extraordinarily high amounts of GEs and a generally high consumption rate around the world. The following subsections will summarize the currently developed elimination methods, based on the formation mechanisms of GEs, including inhibition and removal of precursors, modification of formation conditions, and elimination of formed GEs (Figure 5).

Inhibition and removal of precursors

Unlike 3-MCPD esters, the precursors of GEs are comprised of only DAGs and MAGs rather than chloride ions which prove to be water soluble. Therefore, we believe that the levels of 3-MCPD esters can be reduced through the removal of chloride ions by water wash even though this process does not change the GE concentrations (Matthäus and others 2011). It has already been established that initial DAG and MAG contents in crude oils exhibit a pronounced impact on the formation of GEs. The removal of DAGs and MAGs seems to be the most straightforward way to reduce the formation of GEs. As mentioned above, DAGs and MAGs mainly form through hydrolysis of TAGs, resulting from the activity of endogenous lipase after maturation of oil plants before inactivation. It has also been reported that bruised oil seeds/fruits display more lipolytic activity than undamaged oil seeds/fruits. Moreover, postmature fruits, processing delay, as well as rough handling of oil plant bunches may all contribute to the presence of high DAG and MAG concentrations in crude oils (Kopas and Kopas 2009; Matthäus and Pudel 2014). Chilling the oil fruit can also enhance lipase hydrolysis, which has been found to induce up to 70% of FFAs in palm fruits when subjected to 5 °C chilling (Sambanthamurthi and others 1995; Cadena and others 2013). Taken in concert, these factors explain why the GE contents are different in palm oils from different locations as reported by Matthäus and others (2011). Only 1.3 mg/kg of GEs was detected in palm oil from Ghana, but up to 14 mg/kg was found in palm oil from Malaysia. For oil producers, the elimination of GEs should begin with the selection of plants from different locations and cultivation conditions. Taking some agricultural practices between harvest and processing of oil fruits/seeds are essential to diminish the activity of lipase. These practices include the modification of harvesting conditions, minimizing cracking of oil fruits when transforming to factories, avoiding bruised fruit, reducing the time between harvest to milling, and so on. All these practices taken in concert aim to inhibit the formation of DAGs and MAGs (Figure 5, stage A: 1 to 7). For this reason, the sterilization step during milling of oil seeds or fruits, intended to inactivate enzymes such as lipases and to soften the fruit for speedy removal from the bunches, should be kept at or below 120 °C (Figure 5, stage B: 8) (Craft and Nagy 2012; Stadler 2015).

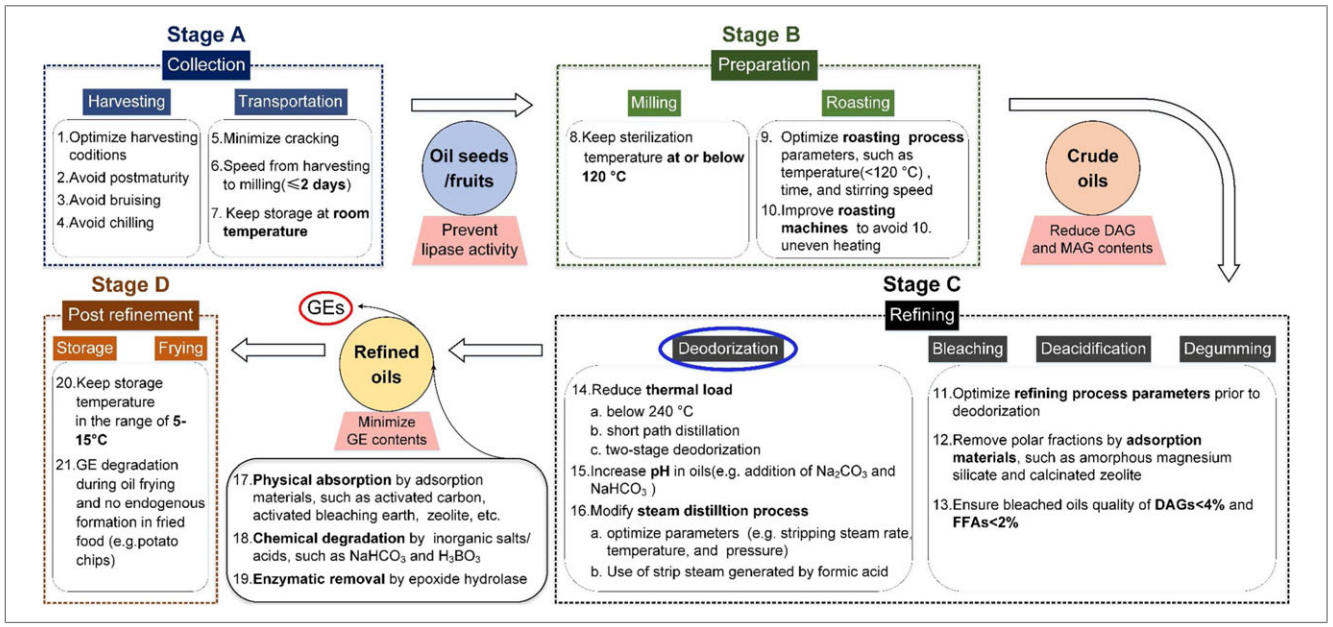

Figure 5—Summary of the elimination methods of GEs in the oil seeds/fruits collection (Stage A), preparation (Stage B), refining (Stage C), and application (Stage D) of edible oils. The provided information was obtained from different literature sources.

As mentioned above, the refining steps prior to deodorization, that is, degumming, neutralization, and bleaching, may remove some of the DAGs and MAGs. In part, this explains the concentration reduction of 3-MCPD esters and GEs at different refining stages (Pudel and others 2011). DAGs and MAGs can also be removed by addition of adsorption materials (Figure 5, stage C: 12), a process that has been well established for the removal of polar components from frying oil (Yates and Caldwell 1993; Lin and others 2001). Strijowski and others (2011) reported that amorphous magnesium silicate and calcined zeolite may reduce polar components as well as DAGs and MAGs by approximately 25%. Therefore, an improved bleaching procedure with additional removal of polar components is needed in the future to ensure a reduced formation of GEs as well as 3-MCPD esters during oil deodorization. Based on a report by Craft and others (2012), it seems feasible that the levels of DAGs and FFAs in crude palm oil below 4% and 2.5%, respectively, should be kept to reduce the formation of GEs during deodorization (Figure 5, stage C: 13).

Modification of formation conditions

From a chemistry point of view, it is well known that high temperature represents the most important reaction condition for the formation of GEs from DAGs and MAGs from a chemistry point of view. In the production of edible oil, the general deodorization process of bleached oils involves steam distillation at temperatures between 250 and 260 °C for 0.5 to 3.0 h. This process represents the last, but indispensable step for the removal of FFAs and any toxic polycyclic aromatic hydrocarbons. Furthermore, this step is crucial for the decomposition of pesticide residues and the inactivation of pigments. However, the deodorization step during oil refining has also been demonstrated to contribute to the widespread production of GEs in refined edible oils (Weisshaar and Perz 2010; Destaillats and others 2012a). Proper modification of deodorization temperature and time are crucial parameters for inhibiting the formation of GEs. Pudel and others (2011) have reported that the formation of GEs during deodorization at a temperature of <240 °C is negligible (≤5 mg/kg). However, at a temperature of 250 °C, the GE concentrations increased significantly with time. To further understand this phenomenon, Craft and others (2012) carried out a laboratory-scale deodorization experiment with pure DAG, thereby confirming the significant formation of GEs at temperatures above 230 to 240 °C. Hence, it may be feasible to reduce the formation of GEs by keeping the deodorization temperature below 240 °C (Figure 5, stage C: 14a). Instead of conventional deodorization, a short-path distillation (Figure 5, stage C: 14b) with a condenser temperature of 60 °C, an evaporator temperature of 170 °C, a stirrer speed of 100 r/min, and a pump frequency of 20 Hz were used by Pudel and others (2016). The authors found that the refined palm oil produced by this mild deodorization process contained a low amount of GEs and 3-MCPD esters, whereas the sensory quality, in terms of taste and odor could be improved. Additionally, several studies involving a 2-stage deodorization (Figure 5, stage C: 15c), that is, 1 short step at high temperature (250 to 270 °C), and a 2nd longer step at a lower temperature (200 °C), have been shown to significantly reduce the GE concentrations, irrespective of the processing sequence (Pudel and others 2012; Stadler 2015).

In addition, the roasting step during oil pressing also involves a heat treatment, with a local temperature of over 200 °C, which seems to be the proper temperature range for the formation of GEs. It has already been reported that the roasting process leads to the formation of 3-MCPD ester, as well as benzo(a)pyrene (Cheng and others 2015; Li and others 2016). However, it is still questionable whether GEs are formed in this process. Recently, some findings, obtained through studies carried out in our laboratory, have demonstrated that the levels of GE in hot-pressed crude oils are significantly higher than the levels in cold-pressed and solvent-extracted crude oils. Furthermore, owing to the pyrolysis of TAG during roasting, DAG and MAG contents were found to be slightly higher in hot-pressed crude oil compared with the crude oils from the other 2 methods. The latter finding may actually lead to an increased difference of GE levels among the 3 refined oils. Therefore, further studies on the optimization

of roasting parameters, such as roasting temperature, time, and stirring speed, should be carried out in an effort to lower the GE, DAG, and MAG contents in crude oils (Figure 5, stage B: 9, 10).

Together with the formation of 3-MCPD esters, it has been reported that the formation of GEs is strongly dependent on the pH value in oils (Šmidrkal and others 2011). Acidity has been shown to have a strong influence on the formation of 3-MCPD esters in crude oils heated at high temperature (Ramli and others 2015). Šmidrkal and others (2011, 2016) reported that the addition of alkali potassium or sodium bicarbonate to neutralize FFAs in sunflower oil can significantly prevent the formation of 3-MCPD esters. A decrease in pH value may also induce a higher potential to form GEs. Therefore, the neutralization of FFAs by the addition of alkaline substances such as Na_2CO_3 and $NaHCO_3$ can be expected to reduce the formation of GEs during oil deodorization (Figure 5, stage C: 15). This, in turn, means that the GE levels in chemically refined oils with preremoval of FFAs in the neutralization step should be lower than the GE levels in physically refined oils. Furthermore, compared with the latter process, chemical refining requires the deodorization temperature at a lower level (230 to 240 °C). Conversely, an increase in pH has also been shown to reduce the GE levels, as well as 3-MCPD and its esters, in refined oils (Sim and others 2004; Freudenstein and others 2013). However, the underlying mechanisms of this reduction in glycidol/3-MDPD (+esters) are still the topic of controversial debates. In order to answer the question whether a high pH value inhibits GE formation reactions or promotes their degradation, further experimental data are required. More recently, it has also been confirmed that the formation of 3-MCPD esters is related to oil oxidation due to the participation of the free radicals in the process of their formation (Zhang and others 2013b; Zhang and others 2015). Indeed, the addition of antioxidants, intended to inhibit oxidation or free radicals, to model systems involving real oil models and chemical models, reduces the formation of 3-MCPD esters in both systems treated at 230 °C for 30 min, compared with the corresponding control samples without addition of antioxidants (Li and others 2015). Zhang and others (2016) also demonstrated that the formation of 3-MCPD esters was mitigated by scavenging free radicals using antioxidants at the molecular level. Unfortunately, whether GE levels are also reduced through this treatment has never been the topic of investigations. Therefore, further studies are needed to provide definite answers to these questions.

Elimination of formed GEs

As discussed above, GEs have been proven to exist in almost all refined oils. For all formed GEs, the elimination methods are classified as physical adsorption and chemical degradation in the literature. Physical adsorption to eliminate GEs involves a process that does not destroy the molecular structure of GEs but adsorbs the compounds in adsorption materials, such as activated carbon, magnesium silicate, zeolite, activated bleaching earth, and so on (Figure 5, method 17). Previously, these adsorption materials were applied for the removal of polar components (Lin and others 2001), polycyclic aromatic hydrocarbons (PAHs) (Leon-Camacho and others 2003), and pesticide residues (Mendez and others 2005) during oil refining. Recently, Strijowski and others (2011) have investigated the possibilities of removing 3-MCPD esters and GEs from palm oil using different adsorption materials, such as amorphous magnesium silicate, zeolite, silicon oxide, sodium aluminum silicate, calcium silicate, and magnesium silicate. The results obtained show that calcined zeolite and synthetic magnesium silicate could remove, by up to 40%, the 3-MCPD esters and GEs. The treatment did not affect the oxidative stability and sensory properties of palm oil. Other studies have shown that mainly GEs could be eliminated by addition of adsorption materials. However, the actual elimination mechanisms for the both compound types still remain unclear. A study carried out thereafter not only demonstrated the elimination of GEs by activated bleaching earth in both TAG- and DAG-rich oils, but also investigated the elimination process in a model system (Shimizu and others 2012b). The authors also found that the elimination of GEs was not due to adsorption of activated bleaching earth but rather through the chemical transformation of GEs involving a ring-opening reaction. However, it seems likely that GEs are 1st adsorbed, and then a ring-opening reaction occurs in the adsorbed GEs on activated bleaching earth pretreated by acid. Therefore, follow-up studies should also focus on the evaluation of suitable adsorption materials for effectively reducing the GEs contents in edible oils.

Due to the relatively low molecular weight of GEs and MAGs, the compounds may be distilled off, to accumulate in distillates during the deodorization/steam distillation process. For the 1st time, Craft and others (2012) reported high amounts of GEs (>100 mg/kg) in distillate samples, and proposed that GE levels in refined palm oil can also be determined by the removal rate of GEs into deodorization distillates. Therefore, the modification of a steam distillation process may likely decrease the GE contents in refined oils (Figure 5, stage C: 16a). Özdikicierler and others (2015) investigated the effects of steam distillation process parameters (stripping steam rate, temperature, and pressure) on 3-MCPD esters and GE formation in olive oil and olive pomace oil. The results obtained showed that the interaction between stripping steam temperature and rate was statistically significant for the formation of GEs. However, when the steam rate was sufficiently high, the promoting effect of steam temperature on the formation of GE was not significant. Under optimum conditions, determined by response surface methodology, that is, steam distillation temperature of 230 °C, water flow rate of 1.2 mL/min, and pressure of 4 mbar for olive oil, as well as steam distillation temperature of 230 °C, water flow rate of 1.0 mL/min, and pressure of 2 mbar for olive pomace oil, both ester contents in the oils could be reduced. This proves to be particularly true for GEs which could be reduced to below 0.1 mg/kg. This elimination method may be further examined for other edible oils exhibiting high levels of GEs, such as palm oil and rice oil.

Additionally, as pointed out above, the formed GEs are not stable under acidic conditions. Therefore, in an effort to reduce the contents of GEs and 3-MCPD esters, Matthäus and others (2011) implemented the replacement of water by formic acid in the formation of the strip steam during oil deodorization. A higher concentration of formic acid led to lower amounts of the esters, particularly GEs, indicating that the use of acid solutions instead of water for the generation of strip steam during deodorization seemed to be a feasible method for the reduction of GE levels (about 35%) in refined edible oils (Figure 5, stage C: 16b). Different from the physical adsorption discussed above, the elimination mechanisms of this method may involve a structural degradation of GEs involving some chemical reactions. From this study, we assume that the addition of certain nontoxic substances to refined oils enables GEs to degrade or to transform into harmless compounds, such as glycerol, DAGs, MAGs, and so on (Figure 5, method 18). It was shown previously that both glutathione and cysteine could significantly lower the concentrations of formed 3-MCPD in the model system (Velisek and others 2003).

However, further investigations on the use of these specific substances should be required in order to decrease GE levels in refined edible oils even more efficiently.

Oil storage plays an important part in the production and distribution of edible oil. In 2008, during an academic conference, Matthäus and others (2008) reported for the 1st time that the concentrations of GEs and 3-MCPD esters decreased during oil storage at low temperature. More recently, Matthäus and others (2015) further verified the notion that GEs in refined palm oil stored at a temperature ranging between 5 and 15 °C degraded significantly. However, at room temperature (20 °C) and at –20 °C no degradation of GEs occurred. Interestingly, the levels of 3-MCPD esters remained constant at all tested temperature ranges. Presumably, upon transportation or use, edible oils should be stored or placed in the refrigerator (5 to 15 °C) to reduce the concentrations of GEs (Figure 5, stage D: 20). Another high-temperature application, such as oil frying, has recently been shown to degrade GEs in frying oils due to the high temperatures (<190 °C) and the long time exposure (Figure 5, stage D: 21). Here, the degradation rate largely depends on oil types, fried raw materials, and frying parameters (Aniołowska and Kita 2015, 2016a, 2016c). Furthermore, when potato crisps are deep fried using high-oleic sunflower oil on an industrial scale, no endogenous formation of both GEs and 3-MCPD esters in frying oil can be observed and the concentrations, particularly for GEs, are very low (about 0.09 mg/kg) (Dingel and Matissek 2015). Taken in concert, it can be determined that only little attention needs to be paid to GEs in frying oils, whereas the initial GE levels in refined oils require more attention.

Apart from the general elimination methods described above, the enzymatic removal of 3-MCPD and its esters has been reported by Bornscheuer and Hesseler (2010). Herein, 3 kinds of enzymes, halohydrin dehalogenase from *Arthrobacter* sp. AD2, epoxide hydrolase from *Agrobacterium* radiobacter AD1, and lipase A from *Candida antarctica*, were used in this experiment. These enzymes ultimately led to the conversion of 3-MCPD and its esters to the harmless product glycerol. One of the enzymes, epoxide hydrolase, may efficiently catalyze the hydrolysis of epoxides into the corresponding vicinal diol. Therefore, the enzyme can also be applied for the removal of GEs in refined edible oils (Figure 5, method 20). Here, GEs may be hydrolyzed into nontoxic MAGs. Moreover, the method does not require addition of organic solvents. Unfortunately, no reports can be found in the literature highlighting the enzymatic removal of GEs as of yet.

Conclusions

Oil deodorization represents a final and essential step in the refining process of edible oil for the removal of odoriferous materials and other undesirable components. These include pesticide residues, pigments, FFAs, and so on. Nevertheless, harmful heat-processing contaminants, such as GEs and 3-MCPD esters, are formed through pyrolytic reactions during high-temperature deodorization. The common occurrence of GEs in edible oils and oil-based food products, particularly infant formulas, has attracted increasing attention in the oil processing industry in the past 10 y. However, currently, no universal, global regulations restricting the maximum allowable GE concentrations in edible oils exist. Every aspect of GE studies, including analysis methods, formation mechanisms, and elimination methods, is evaluated in detail. For the analytical methods of GEs, and due to the uncertainty of the indirect analysis methods, the current research mainly focuses on direct methods, not only based on LC-MS. The precursors of GEs, DAGs, and MAGs, have been well identified in model systems, but the reactive pathways from DAGs or MAGs to GEs still remain controversial due to a lack of experimental data in the literature supporting any proposed mechanisms. This phenomenon ultimately limits the development of effective elimination methods, potentially resulting in the large amounts of GEs in commercially available edible oils. From the published reports, perhaps the most effective and most pragmatic method to extensively eliminate GEs in refined edible oils that exists to date is an upstream intervention in edible oil production. Oil manufacturers and oil plant growers need to work closely together in the future to reduce GE contents in edible oils as much as possible. Additionally, owing to the strong connection of formation mechanisms including precursors, formation conditions, and reaction process between GEs and 3-MCPD esters, increased research efforts should focus on the structural relationship of both species in formation mechanisms. We believe that both GEs and 3-MCPD esters may be eliminated by using effective, simple, and inexpensive techniques.

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Author Contributions

W. Cheng was responsible for the study design, searching and interpreting the literature, and preparing the manuscript; G. Liu and L. Wang were responsible for the study design and drafting the final manuscript. Z. Liu gave valuable assistance in the major revision of the manuscript. All authors reviewed the final manuscript before submission.

Conflicts of Interest

The authors declare no conflicts of interest.

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