Kajian Keamanan Pangan Senyawa Ester 3-MCPD dalam Produk Minyak/ Lemak Pangan dan Produk Pangan Lainnya

Reviewon Food Safety of 3 – MCPD Esters in Edible Oils/ Fats and in Other Foods

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ABSTRAK: Dalam permintaan produk pangan untuk kesehatan dan keamanan pangan global sekarang ini, konsumen menghendaki dan sedang mencari produk pangan tanpa atau paling sedikit terkena kontaminasi produk. Senyawa ester 2- dan 3-monochloropropan-1,2-diol (MCPD) dan ester glisidol diketahui merupakan salah satu komponen kontaminan pada produk hasil olahan minyak makan yang telah banyak digunakan sebagai bahan pangan atau bahan ingredien pangan. Senyawa 3-monochloropropan-1,2-diol atau lebih dikenal dengan 3-MCPD merupakan kontaminan pangan yang diklasifikasikan sebagai bahan yang kemungkinan bersifat karsinogen;oleh karena itu, hanya boleh dikonsumsi dengan dosis/konsentrasi sebesar 2 µg/kg berat badan. Hasil studi terkini menunjukkan adanya senyawa ester 3-MCPD teridentifikasi dalam jumlah cukup tinggi pada produk minyak/lemak pangan, seperti margarin dan minyak goreng serta pangan yang mengandung lemak termasuk produk pangan *infant formula* dan susu manusia. Senyawa ester-ester lain seperti 2-MCPD dan ester glisidol pun diduga dapat terjadi. Namun, hingga saat ini hanya terdapat sedikit data informasi tentang toksikologi yang dapat diperoleh untuk senyawa ester 3-MCPD pada produk pangan. Tulisan ini akan membahas dan menjelaskan proses terjadinya senyawa 3-MCPD pada produk pangan, faktor-faktor yang memungkinkan menyebakan munculnya pembentukan senyawa ester 3-MCPD pada produk pangan, studi tentang efek beracun senyawa 3-MCPD atau toksikologi dan penentuan senyawa ester 3-MCPD dalam produk pangan.

Kata kunci: Kajian, ester 3-MCPD, minyak/lemak pangan, keamanan pangan

ABSTRACT: In today's global demand for healthy and safe foods, consumers are looking for foods without or with least contaminants. Esters of 2–and 3–mono-chloro-propane–1.2–diol(MCPD) and glycidol esters are important contaminants of processed edible oils used as foods or food ingredients. 3–mono-chloro-propane–1.2–diol (3–MCPD) esters is a food contaminants classified as a possible human carcinogen, so for a tolerable daily intake was established of 2 µ g/kg body weight.Recent studies have identified high levels of 3 – MCPD esters in refined edible fats, such as margarine and oils and in fat containing foods including infant formula and human milk. Other related esters compounds such as 2-MCPD esters and glycidol esters are also expected to occur. Only a little toxicological data are available in 3–MCPD esters. This review describes the occurrence of 3–MCPD esters in food products, possible factors that cause the formation of 3–MCPD esters, toxicological studies and determination of 3–MCPD esters in food products.

Keywords: review, 3 – MCPD esters, edible oils, food safety

1. Introduction

Food safety has emerged as an important global issue with international trade and health implications. Since food safety hazards can occur at any stage in food chain, it is essential that adequate control measures be put in place to avoid or minimize food safety hazards (Prati and Mc.Intyre, 2004). In response to the increasing number of foodborne illness, governments all over the world are intensifying their efforts to improve food safety (Sudershan *et.al.*, 2009).

Meanwhile, food as a basic need for all people, must be wholesome and safe. Therefore, in today's global demand for safe foods, consumers are looking for foods without or with least contaminants (Shahrim*etal.*, 2012). Consequently, convenient foods have led to continuous improvements of existing food-processing techniques; all designed to produce safe foods, while maintaining nutritional and sensory qualities. These developments require a more structured approach for the safety evaluation of foods and food ingredients. However, in the production of edible oils and fats from the crude oils; most oils are refined to remove free fatty acids, peroxides and other oxidative compounds which contribute to the aroma of the oils, also resulted in new contaminants, which called esters of 3-monochloropropane–1.2–diol (3–MCPD)(Weishaar, 2008). Even, recent studies have identified high levels of 3–MCPD esters in such as margarine and in fat - containing foods including infant formula (both starter and follow-on) and human milk (Larsen, 2009).

The 3–Mono-chloro-propane–1.2–diol (3–MCPD) esters was reported has been detected in thermally treated foodstuff such as breadcrumbs and crusts (Dolezal*et al.*, 2009), coffee (Dolezal*etal.*, 2005), baked cereal products (Hamlet and Sadd, 2004), doughnuts and french–fries (Svejkovska*etal.*, 2004). These esters are part of larger group of chloropropanediols (CPD). Surprisingly, the compound has also been detectedin infant and baby food (Zelinkova*etal.*, 2009) which triggered further investigation since the compounds is suspected to be a non-genotoxic carcinogen (JECFA, 2002).

3-MPCD has been found to be genotoxic in most in vitro assays, although negative results were obtained in vivo assays (Bakhiya *et al.*, 2011). Evidence of carcinogenic activity in male rats and some evidence of carcinogenicity activity in female rats has been reported (Cho *et al.*, 2008).This concern is justified considering that 3–MCPD has shown nephrotoxic properties as well as having an ability to affect male fertility and induce cancer in experiments with animals. It has been classified as a possible human carcinogen group 2 B (IARC, 2012). For these reasons, this discovery has been considered a priority issue in relation to food safety (Arisseto*etal.*, 2013).

Preliminary studies and intake assessment, considering that the levels if 3–MCPD esters found in foods are hydrolized during digestion. This evidence showed that the exposure to free 3–MCPD may exceed the provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg body weight (bw) that currently established for this compound, so it become a potential health risk (B f R, 2007).Experiments recently conducted in rodents have shown that the relative concentration of 3–MCPD metabolites excreted in urineafter oral administration of equivalent molar doses of 3–MCPD. This indicatethat 3-MCPD ester may be hydrolyzed almost completely as suggested by its high bioavailability oral administration (Abraham *et al.*, 2013).Therefore, for risk assessment purposes, this evidence suggest that the complete hydrolysis of 3–MCPD di-esters should be considered and, thus the determination of the relationship between the concentrations of monoesters and di-esters is highly recommended (Arisseto*etal*., 2013). The present study was an attempt to review and case study on 3–MCPD esters in edible oils/fats and in other foods as food safety concern.A review on the occurrence of 3 – MCPD esters in food products, possible factors that cause the formation of 3–MCPD esters, toxicological studies and determination of 3–MCPD esters in food products especially in oils/fats will be illustrated.

2. The Occurence of 3-MCPD Esters In Food Products

3–Mono-chloro-propane–1.2–diol (3–MCPD) is well known as food processing contaminant since 1978 (Weishaar, 2008),in various food such as liquid seasoning or bakery goods (Larsen, 2009). 3–MCPD is formed when fat (glycerol or acyl-glycerol) and salt (Chloride ions) – containing foods are processed at high temperature during production (Larsen, 2009). Concentration of free 3 – MCPD in the low µg/kg range are present in many foodstuffs like acid–hydrolized vegetable protein, soy sauces, crackers, bread, toast and other bakery products, malt products and soups (Hamlet *etal*., 2002).

According to the European Union (EU) legislation in 2001 (EC Scientific Committee on Food/SCF) was considered that the *tolerable daily intake* (TDI) of 3–MCPD is 2 µg/kg body weight for hydrolised vegetable protein (HVP) and soya sauces (SCF, 2001). It was also used by Joint FAO/WHO Expert Committee on Food Additive (JECFA) in 2002 for established a provisional maximum tolerable daily intake for 3–MCPD at 2 µg/kg body weight (JECFA, 2002). Recently, free 3–MCPD has also been detected in foods like bread, toast, noodles and smoked products. Interestingly however, bread and noodles could be important contributors to the total daily intake especially for their strong consumption rather than for their content in 3–MCPD but the same TDI value seems to be applied (Shahrim*etal*., 2012).

Many studies or survey have been carried out on free or bound 3 – MCPD esters in foods until recently, where 3 – MCPD esters are reported have been found in oils and fats. Various papers have documented the presence of free and 3 – MCPD esters in many foods products, such as cereal,

roasted coffee, malts, bread, etc (Hamlet and Sadd, 2004; Dolezal*etal*., 2005; Divinova*etal*., 2007). Reported values are between 0.2 and 6.6 mg/kg in most analysedfoodstuffs and the levels of bound 3 – MCPD are generally much higher than the free form. Meanwhile, salami and other meat products also recorded high values of up to 6.4 mg/kg (Reece, 2005; Svejkovska*etal*., 2004; Zelinkova*etal*., 2006). Further studies showed that in foodstuffs only a small percentage of 3 – MCPD is present as free 3 – MCPD, while the major part is ester – linked with fatty acids (Svejkovska*etal*., 2004) especially in vegetable oils (Larsen, 2009).

Further investigations have shown that 3 – MCPD is present in food not only in its free form but also in the form of mono – or di–esters with fatty acids; in many foodstuffs most of the 3 – MCPD is actually in ester – linked form (Weishaar, 2011; Crews *etal*., 2013). High levels of 3 – MCPD esters have been reported in edible refined plant oils and fats, especially palm oil, as well as in other products, such as crisp bread (Svejkovska*etal*., 2004; Weishaar, 2011). By the action of lipases, 3–MCPD can be released from the esters *invivo* (Hamlet andSadd, 2004).

Oils and fats are deemed to have a higher potential of forming 3 – MCPD esters upon on high thermal treatments, especially during odorisation where temperatures typically reach 240 ˚C and above (Shahrim*etal*., 2012).Some oils appear more receptive to the formation of these esters, as was discussed by Weishaar (2011). No 3–MCPD esters, or only traces, were detectable in native and unrefined fats and oils. Deodorisation was clearly identified as the crucial step for 3–MCPD ester formation in the refining process of fats and oils, with almost the total quantity of 3–MCPD esters being formed at the last step of the process (Larsen, 2009).

Weishaar (2011) classified refined vegetable oils and fats into three groups according to the levels of 3–MCPD found to be ester – bound, i.e.: (1) Low levels (0.5 – 1.5 mg/kg): rapeseed, soybean, coconut, sunflower oil; (2) Medium level (1.5 – 4 mg/kg): safflower, groundnut, corn, olive, cotton seed, rice bran oil; and (3) High levels (> 4 mg/kg): hydrogenated fats, palm oil and palm oil fractions, solid frying fats. Weishaaralso mentioned that levels of 0.5 – 10.5 mg/kg fat (median: 2.3 mg/kg) have been found in margarine; < 0.1 – 16.9 mg/kg fat (median: 1.5 mg/kg) in the fillings and toppings of cookies, crackers and bars; 2.3 – 10.3 mg/kg fat (median: 4.9 mg/kg) in sweet spreads (hazelnut nougat spreads) and 0.5 – 8.5 mg/kg fat (median: 2.5. mg/kg) in infant formula (powder). It was reported also by Weishaar (2011) that the highest concentrations of 3–MCPD esters (up to 2.7 mg/kg) were found in unused frying fats. In used frying fat, 3–MCPD levels decreased with increasing time of use. During the deep frying process nearly 240 ˚C no additional 3 –MCPD is formed. Therefore, the levels of 3 – MCPD esters in French fries and other frieds foodstuffs only depends on its concentration in the used frying fat.

Although there is a lack of data about 3–MCPD esters for many foodstuffs, it is obvious that thermally processed foods and refined fat and oils (as such or as a component of other foodstuffs) are the most significant sources of 3 – MCPD esters for consumers I particular, refined palm oil in different kinds of foodstuffs is responsible for a significant part of the exposure (Weishaar, 2011). Even so, although palm oils have shown higher values in comparison to other refined vegetable oils, the history and source of oils have to be checked against the methodology used (Shahrim*etal*., 2012).Raznim*etal*. (2012) provided details of 3–MCPD esters in refined palm oil, olein and stearin where using the B f R (Bundesinstitutfur Risikobewertung) 008 indirect method used. The highest recorded value was 5.7 mg/kg. Currently, the palm oil industry in Malaysia is taking measures to reduce the formation of 3 – MCPD esters based on research knowledge gained in recent years. The risk of exposure to 3 – MCPD esters has not been fully evaluated as potentially all vegetable oils in the presence of chlorides which are subjected to thermal treatments as in cooking, roasting, baking and frying, will have the probability of forming these components (Shahrim*etal*., 2012).

3. Possible Factors That Cause The Formation of 3-MCPD Esters

The presence of esters in refined oil was first published by Gardner *etal.* (1983), who detected high amounts of the compound in adulterated Spanish rapeseed oil. Since then several authors have reported the presence of the compound in other types of refined vegetable oils (Zelinkova*etal*., 2006; Seefelder*etal*., 2008; Hrncirik, 2009; Weishaar, 2009). The level of 3–MCPD esters is either not detected or only present in trace amounts in crude or virgin oils, but the level is higher in refined oil (0.5 to 6.0 mg/kg) (Hoenicke, 2009).

The factors, which influence the formation of 3–MCPD esters are: the level of chloride, level of acyl-glycerols (tri, di – and mono-acyl-glycerols), pH, temperature and time. Heat has been identifiedas the major cause (Pudel*etal*., 2011; Zelinkova, 2006), whilst mono-acyl glycerol, di-acyl-glycerol and chloride are thought to be direct precursors for the formation of 3–MCPD esters (Larsen, 2009; Franke*etal*., 2009). Zelinkova*etal*. (2006) reported that heat treatment of oilseed during refining could influence the formation of 3 – MCPD.

The formation of 3–MCPD esters could only be effected in the presence of chlorides in any food matrix; presence of lipids and at high thermal processing conditions. In oils and fats, these components and factors are clearly available, although chlorides may not have been so evident until now. As for palm oil being more susceptible to the formation of chloro-propane–diol-esters, the reason has not been clearly elucidated, although it could be due to higher levels of di-acyl-glycerols (DAG) compare to other oils (Hamlet *etal*., 2011).

The mechanism for the formation of 3–MCPD esters, outlined by Svejkovska*etal*. (2006), shows that all lipids can undergo to nucleolipic substitution of the acyl group by chloride anion, thus forming chloro-propane–diol-esters. Cyclic acyl-oxomium intermediates may be formed in the process. In the presence of Lewis acids or under acidic conditions, cyclic acyl oxomium ions may be fromtri-acyl glycerol (TAG) and di-acyl glycerol (DAG) during refining. Although 3–MCPD esters are prevalent, 2–MCPD esters can also be expected. Hamlet *etal*. (2011) and Rahn*etal*. (2011) have elaborated comprehensively on the possible mechanism of the process.

Hrncirik (2009) has also tried to show the effect of Free Fatty Acids (FFA) and DAG on the formation of 3–MCPD esters. In this case, enzymatic hydrolysis was applied to increase the FFA and DAG contents in the oil samples. Despite the increase in the FFA and DAG content, there was slight drop in 3–MCPD esters content as observed in sample P2. Hrncirik (2009) concluded that DAG and FFA may play a role in the formation of the compound, but the results were inconclusive due to the poor correlation between the two factors (FFA and DAG) and 3–MCPD esters. A clear link between the precursor (DAG) could not be established by Stadler (2009) too. In a later study by Hrncirik and Ermacora(2010), the authors concluded that partial acyl-glycerols seem to be involved in the formation of 3–MCPD esters, but they are not the critical factors determiningthe final levels.

Actually, the temperature of refining, particularly that of deodorization has been suggested as the factors causing the formation of 3–MCPD esters in oils and fats (Franke, 2009; Hrncirik, 2009; Hrncirik and Van Duijin, 2011).Further studies by Ramli*etal*. (2011) have established that acid degumming and acid activated clays, especially of low pH can contributed to the formation of the esters. They concluded that besides the high deodorization temperature, the acidityof bleaching clays and the dosage of phosphoric acid are also contributing factors of the formation 3–MCPD esters.

4. Toxicology Studies

Toxicological animal studies have shown that the main target organ for 3 – MCPD toxicity is the kidney, with chronic oral exposure resulting in nepropathy and tubular hyperplasia and adenomas (as a reviewed by the joint FAO/WHO Expert Committee on Food Additives, JECFA (JECFA, 2002; in particular, Sunahara*etal*., 1993). 3–MCPD itself is an animal carcinogen producing tumours at various sites in male F344 rats (mammary tissue, testes and preputial gland) and renal tubular adenomas and carcinoma in both sexes of F344 rats (Sunahara*etal.*, 1993; Lynch, 1998). 3–MCPD has also been shown to reduce infertility in rats and suppression of the immune function (Lee*etal*., 2004; Lee *etal*., 2005). Evidence of carcinogenic activity in male rats and some evidence of carcinogenicity activity in female rats has been reported (Cho*etal*., 2008).

However, in a 2008 statement from the European Food Safety Authority Panel in Contaminants in Food Chain (EFSA CONTAM) concluded that: there was no comprehensive toxicological and bioavailability data on 3–MCPD esters available. Nevertheles, the German Federal for Risk Assessment (B f R) based its recent risk assessment on toxicological data on free 3 – MCPD; under the assumption that 100 % of 3 – MCPD are release from the esters. Subsequently, Seefelder and co-workers in 2007, using the intestinal model lipase studies have shown that the release of 3 – MCPD from 3 – MCPD esterswas much shown than from the monoester. Furthermore, it was found that in vegetable oils, only 15 % of 3 – MCPD are in mono-ester forms, while the majority are di-esters.

Recently, a 90-days rat study was carried out by the University of Parma (Italy) in response to the European Food Safety Authority (EFSA) call, to evaluate the toxicological profile of 3 – chloro-propane – 1.2 – diol (3 – MCPD) esters (mono – and di-ester) compared to that of free (or unesterified) 3–MCPD. This study aimed to compare the toxicity of 3 – MCPD di-palmitate and free 3–MCPD, performed on male and female rats (Barocelli*etal*., 2011). Considering that only a small part (< 15 %) of the 3–MCPD bound in esters is in fact bound in monoesters (Seefelder*etal*., 2008), the studies were performed using only di-esters form. Palmitic-acid and fatty acid was proposed due to the fact that highest levels of 3 – MCPD were found in palm oil and is the most commonly used di-estertostudy the formation and the composition of 3 – MCPD esters in vitro. This report covers the whole 90 – days study with either 3–MCPD (respectively 29.5, 7.57, and 1.84 mg/kg of body weight per day) or 3 – MCPD di-palmitate (respectively 156.75, 30.9 and 9.78 mg/kg per day). In male rats, Bench Mark Dose $(BMD)_{10}$ for severe renal and testicular damage

induced by 3 – MCPD di-palmitate were 41 and 64.4 mg/kg per day, respectively. The corresponding BMD_{10} were 17.4 and 44.3 mg/kg per day. The values for damage induced by the free 3 – MCPD were much lower, indicating a higher toxicity level.

Research carried out by the MPOB (Malaysian Palm Oil Board) on 3–MCPD esters in palm oil by looking at all stages of the refining process to identify the cause of its formation and conducting collaborative toxicological studies with other research institutions was reported by Shahrim*etal*. (2012). An acute oral toxicity study was conducted on the effect of 3 – MCPD palmitate – oliate using animal model. This study was undertaker to assess the health hazard potential of 3–MCPD esters by determining adverse effects following an oral administration in rats.

In the study, a single dose of acute oral toxicity was performed using Sprague Dowley rats by oral gastric intubation. It was demonstrated that there were no ill effects nor did any death occurred in any of the groups of male and female rats fed with the 3–MCPD palmitate – oliate at 50, 200 and 400 mg/kg of body weight. The 3–MCPD esters which is known to be the most abundant 3 – MCPD esters found in palm oil, show no pattern and unlikely toxicity at every does tested in terms of body weight changes and pathology (Shahrim*etal*., 2012). However, the International Agency for Research on Cancer (IARC) has classified 3–MCPD as a "possible human carcinogen (group 2 B)" (IARC, 2012).Do to its mutagenic potential practice in the United Kingdom (UK) had previously been to reduce exposure to 3–MCPD to as low a level as practicable (Robjohns*etal*., 2003).

5. Determination of 3 – MCPD Esters In Food Products Especially in Oils/Fats

The proposed analytical approaches for determining 3–MCPD esters involved both indirect analysis – in which the total concentration of the compounds is measured as free 3 – MCPD obtained after a hydrolysis/methanolysisprocedure; and direct analysis, in which the different spesies of 3–MCPD esters are identified individually.Indirect methods have shown good application for routine tests due to the high sensitivity and the need of a reduced number of analytical standards (Arisseto*etal*., 2013). These methods are based on indirect determination of bound 3 – MCPD via trans–esterification in acid (B f R Method 008) or in alkali (B f R Method 009 and 010) (DGF, 2009; Weishaar, 2008). The principle of the indirect method involves the conversion of the esters of free 3–MCPD, and then the 3 – MCPD is quantified using Gas Chromatography – Mass Selective Detector (GC-MSD).

The analytical protocol of these methods comprises a uniform series of steps: addition of an internal standard (either free or esterified form of isotopically labeled 3 – MCPD) to the sample: trans-esterification (commonly performed either in acid or alkaline medium), neutralization of the reaction mixture and salting out (using different neutralizing reagents and salts), derivatisation of the cleaved 3–MCPD/2–MCPD and GC-MS analysis (Crew *etal*., 2012).

Crew *etal*. (2012) has also reported that there has been enormous progress in the development of indirect methods for the determination of MCPD esters in the last decade, and in particular in last four years. Advances in the methodology have led to the improvement of the performance (e.g. sensitivity) of methods used and to better understanding of the limitations of these methods. Substantial effort on the development of methods based on different principles, and their further modifications, has resulted in a large number of different method differ in their scope and their performance is a cause of concerns. It would be highly desirable to harmonize current analytical methodology for MCPD and glycidyl esters and to identify those methods that meet certain performance criteria. Such harmonization will not improve the quality of the results, but also simplify their communication. To achieve this objective, it is seems inevitable that selected methods will be validated within international collaborative studies and ultimately adopted as official methods.

Table 1. shows the method of classification of two indirect methods: (a) acidic trans-esterification, and (b) alkaline trans-esterification. Generally, only two reagents are used for derivatisation, which are phenyl-bromic acid (PBA) and hepta – fluoro – butyrili-midazole (HFBI). The salting out reagents is either sodium chloride (NaCl) or sodium sulphate (Na_2SO_4) (Razak*etal*., 2012).

Table 1
Method classification for indirect methods (*)

Transesteri-ficatin	**Acidic**		**Alkaline**		
Derivatisation	**PBA**	**HFBI**	**PBA**		**HFBI**
Salting out	NaCl/ Na_2SO_4	-	NaCl	Na_2SO_4	Na_2SO_4
Methods	B f R 82.FC. 008	-	DGF C III. 18 (09)	B f R 82.FC. (009)	B f R 82.FC. (010)

(*) Source : Razak et al., (2012).

The direct quantification of 3–MCPD esters has also been developed using Liquid Chromatography – Time of Fight/Mass Spectrometry (LC-TOF/MS) (Haines *etal*., 2011). The direct method involves quantification based on the direct determination of

individual esters. Direct methods were initially based on GC-MS analysis of fraction isolated by Thin Layer Chromatography (Reece, 2005; Zelinkova*etal*., 2007), but the used of liquid chromatography–mass spectrometry (LC-MS) has proved more popular and convenient. Procedures have ranged from direct injection of oils solutions to the incorporation of solid – solid phase extraction clean-up. Glycidyl esters and MCPD esters can be determined simultaneously (Haines *etal*., 2011). For the independent determination of glycidyl esters, LC-MS is the method of choice, typically with gel permeation chromatography clean-up (Dubois *etal*., 2011; GranVogl and Schieberle, 2011b).

Ultra High Performance Liquid Chromatography (UHPLC) with high resolution time of flight mass spectrometry (UHPLC – TOF MS) analysis with the internal standardization with one isotope – labeled glycidyl ester and two isotope–labeled 3–MCPD esters was performed by Hori *etal*. (2012) for simultaneous determination of five glycidyl esters, three 3–MCPD mono-esters and six 3–MCPD di-esters in edible oils.Their sample preparation encompassed liquid-liquid portioning of the edible oil in n-hexane and acetonitrile, and subsequent solid-phase extraction on silica and C_{18} cartridges. In contrast to the method described above, the two cartridges were used in parallel. The n-hexane phase was loaded onto the silica cartridges, whereas the acetonitrile phase was applied to the C_{18} cartridges. The collected eluents were combined, evaporated and reconstituted in acetonitrile prior to the measurement (Crew *etal*., 2012).

A rapid method for the determination of the ratio of 3–MCPD mono-esters and di-esters in fats and oils using GC-MS and isotopically labeled 3–MCPD as internal standard has developed by Seefelder*etal*. (2008). They demonstrated that 3 – MCPD mono-esters and di-esters have been accepted by intestinal lipase as substrates in vivo using simple intestinal model. The paper also reported that 3–MCPD esters in human is unlikely to be completely hydrolisedinto 3–MCPD, as triglycerides and phospholipids are hydrolised in the intestine liberating 2 – mono-glycerides. From their study, it was found that a maximum of about 15 % of the total amount of 3–MCPD bound in esters is present in the mono-esterified form. In addition, it was also found that the release of 3–MCPD from 3–MCPD di-esters is slower then mono-esters, therefore suggesting that 3 – MCPD esters may contribute only marginally to the overall dietary exposure to 3–MCPD.

Reports in literature on the direct determination of 3–MCPD esters are very limited. This might be attributable to the lack of suitable commercial reference materials in the early days of the topic. Laboratories active in this field were until recently obliged to synthesize reference material themselves. Details of the synthesis of glycidyl esters and/or MCPD esters can be taken from general publication (Masukawa*etal*., 2011). However the commercial supply of reference materials has recently improved significantly. A broad range of both native and stable isotope – labeled glycidyl and MCPD esters is available from different suppliers. A non–exhaustive list of suppliers comprises (in alphabetical order) Chiron AS (Trondheim, Norway), Toronto Research Chemicals (Toronto, Canada) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan) (Crew *etal*., 2012).

Dubois (2011) recently presented the development of two direct analytical methods, the first targeting the direct analysis of intact MCPD mono-esters and the second one targeting direct analysis of MCPD di-esters. MCPD mono- and di-esters were isolated via double SPE/Solid Phase Extraction (C_{18} and silica) or silica gel columns respectively, and analysed by LC-TQFMS. Standard addition quantification was used, with labeled internal standards. The methods was compared with an indirect analytical method for 3– and 2–MCPD esters, which was based on the acid catalyzedtrans-esterification of the MCPD esters (Divinova*etal*., 2004). Table 2 shows a list of direct methods reported by various organization/research institutes.

A comparison of the indirect and direct method of analysis is described by Razak*etal*. (2012) and is shown in Table 3.

Table 2
Direct methods (*)

Method of Analysis	Reference
▪ LC-TOF/MS	▪ Collison and Haines (AOCS Meeting, 2010) ▪ Haines *etal*. (2010)
▪ LC-TOF/MS or LC-Q/TQF	▪ Mathew (AOCS Meeting, 2011)
▪ LC-MS/MS	▪ Pinkston and Stoffolana (AOCS Meeting, 2011) ▪ McMahon *etal*. (AOCS Meeting, 2011)
▪ LC-MS/MS with stable isotope dilution analysis	▪ Granvogl and Schieberle (AOCS Meeting, 2011).

(*) Source: Razak *etal*. (2012).

6. Conclusion

3–MCPD esters have been found in all refined vegetable oils. 3–MCPD esters are now also widespread in thermally processed foods like French fries, toasted bread, bread crust, donuts, salty crackers, roasted coffee, roasted barley, roasted dark malt and coffee creamer, and fermented foods.

Table 3
Summary of methods of analysis (*)

Indirect Method	Direct Method
▪ Sample to perform	▪ Several methods presented (other under development)
▪ High uncertainly (results are dependent on sample composition	▪ Provides a full profile, but it is difficult for routine analysis
▪ Number of methods available	▪ Substancial challenges (standards, instrumentation, sensitivity)
▪ Suitable for routine analysis (total bound 3–MCPD)	
▪ Good sensitivity	
▪ Trueness is questionable	

(*) Source: Razak *etal*. (2012).

The formation of 3–MCPD esters in oils and fats is a complex integration of multiple factors ranging, i.e. from chlorine donors, levels of free fatty acids, di-acyl-glycerides, acidic conditions of refining and high temperature of deodorization.

The indirect methods of analysis avoid the preparation of a series standards; the methods are also sensitive and easy to perform. Quantification is based only on the total content of 3–MCPD (free and bound form). On the other hand, the direct method offers as full profile quantification of the esters, but remains difficult for routine analysis.

Based on the observation and data generated and the findings suggest that this 3–MCPD di-ester tested was consider non-toxic. Nevertheless, other less known information regarding overall exposure to different foodstuffs, food preparation methods, bioavailability, metabolism, are in fact warranted before all dietary exposure can be fully determined.

Reference

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