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DETERMINATION OF 3-MONOCHLOROPROPANE-1,2-DIOL (3-MCPD) ESTERS IN EDIBLE PLANT OILS BY INDIRECT ACIDIC TRANSESTERIFICATION METHODS AND THE BPX-5 CAPILLARY COLUMN

(Penentuan Ester 3-Monokloroprapana-1,2-diol (3-MCPD) dalam Minyak Tumbuhan Boleh Dimakan dengan Kaedah Transesterifikasi Berasid Tidak Langsung dan Turus Kapilari BPX-5)

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Abstract

3-monochloropropane-1,2-diol (3-MCPD) and its ester are categorized as food toxicants that formed during high-temperature processing of fat-based matrices. It has been classified as a possible human carcinogen (Group 2B) due to its potential to induce cancer in humans. This study investigated the level of 3-MCPD esters in palm oil and other edible plant oils available in the Malaysian market. The samples were analyzed using indirect methods involving acidic transesterification, then quantified by gas chromatography-mass spectrometry (GC-MS) equipped with BPX-5 capillary column using selected ion monitoring (SIM) mode. The method performance showed good recovery between 92.80% to 105.22% and good reproducibility with RSD of 4.18% to 5.63% for three different concentrations of spiked 3-MCPD. The limits of detection (LOD) and quantification (LOQ) were 0.11 mg kg^{-1} and 0.14 mg kg^{-1}, respectively. The highest concentrations of 3-MCPD esters were detected in refined palm oil, ranging from 2.29 to 4.10 mg kg^{-1}, while 3-MCPD esters in other edible plant oils varied from <LOQ to 3.75 mg kg^{-1}. The validated results are presented to attract policymakers' attention and achieve the goal of ensuring consumer rights while monitoring food safety and quality.

Keywords: 3-monochloropropane-1,2-diol, gas chromatography-mass spectrometry, edible plant oils, food safety, food control

Abstrak

3-monokloroprapana-1,2-diol (3-MCPD) dan esternya dikategorikan sebagai bahan toksik makanan yang terbentuk semasa pemprosesan bersuhu tinggi matriks berasaskan lemak. Ia telah diklasifikasikan sebagai karsinogen manusia yang berkemungkinan (Kumpulan 2B) kerana potensinya untuk menyebabkan kanser pada manusia. Kajian ini dijalankan untuk menyiasat tahap ester 3-MCPD dalam minyak sawit dan minyak tumbuhan boleh dimakan lain yang terdapat di pasaran Malaysia. Sampel dianalisis

menggunakan kaedah tidak langsung yang melibatkan transesterifikasi berasid, yang kemudiannya dikuantifikasi oleh kromatografi gas-spektrometri jisim (GC-MS) yang dilengkapi dengan turus kapilari BPX-5 menggunakan mod pemantauan ion (SIM) terpilih. Prestasi kaedah menunjukkan perolehan semula yang baik antara 92.80% hingga 105.22% dan juga kebolehulangan yang baik dengan RSD sebanyak 4.18% hingga 5.63% untuk tiga kepekatan berbeza 3-MCPD terpaku. Had pengesanan (LOD) dan kuantifikasi (LOQ) didapati masing-masing pada 0.11 mg kg^{-1} dan 0.14 mg kg^{-1}. Kepekatan tertinggi ester 3-MCPD dikesan dalam minyak sawit ditapis, antara 2.29 hingga 4.10 mg kg^{-1}, manakala ester 3-MCPD dalam minyak tumbuhan boleh dimakan lain berbeza daripada <LOQ hingga 3.75 mg kg^{-1}. Keputusan yang disahkan dibentangkan dengan harapan dapat menarik perhatian penggubal dasar dan mencapai matlamat untuk memastikan hak pengguna sambil memantau keselamatan dan kualiti makanan.

Kata kunci: 3-monokloropropana-1,2-diol, kromatografi gas-spektrometri jisim, minyak tumbuhan boleh dimakan, keselamatan makanan, kawalan makanan

Introduction

Edible plant oils, also known as vegetable oils, are extracted from seeds, fruits, nuts, legumes, and pulps of plants. They are mainly used in cooking to change the flavor, color, and fragrance of foods and provide diversified flavor and enrich the sense of satiety during the cooking process [1]. In 2019, the global market of vegetable oils was almost 203 million tons [1]. With the increasing demand for edible plant oils globally, their quality and safety have gained significant attention as they play an essential role in human dietary nutrition. Hence, oils undergo a refining process to enhance their stability and quality. Although unwanted impurities are removed from the oils, refining often originates new contaminants such as chloropropanols and glycidols that can cause additional health hazards to consumers [2].

3-monochloropropane-1,2-diol (3-MCPD) and its esters are a class of food processing contaminant that belongs to chloropropanols, a group of alcohols comprised of a 3-carbon backbone substituted with one or two chlorine atoms [3]. The formation of such contaminants is mainly due to the high-temperature processing of fat-based matrices [4, 5]. 3-MCPD was first identified in its free form by Velisek et al. in acid-hydrolyzed vegetable proteins (acid-HVP) used in soy sauce production, while its esterified (bound) form was then discovered in 1980 [6, 7]. Their occurrence existed in soy sauces and other flavoring materials and foodstuffs such as edible oils, heat-processed foods, cereal, meat products, infant formula, and even human breast milk [8-12].

Previous studies have reported that high 3-MCPD esters were found in refined edible oils, especially palm oils [10]. They were formed during the refining process of vegetable oils in the deodorization step at high temperatures exceeding 200 °C to remove free fatty acid contents in oils while giving a good color and odor [13]. The potential risks of such contaminants have raised immediate concern among the public since 3-MCPD esters can be metabolized to release free 3-MCPD in the human gastrointestinal system [14]. In addition, 3-MCPD has been categorized as a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC) due to its potential to induce cancer in experimental animals [15]. Hence, it is vital to have a sensitive and robust method to determine the concentration of 3-MCPD in edible oils and fats.

The European Food Safety Authority (EFSA) published a scientific opinion in May 2016 based on a survey done by the Panel on Contaminants in Food Chain (CONTAM) regarding the issue of contaminants found in processed vegetable oils and foods. They stated that the highest levels of 3-MCPD esters were found in palm oil [16]. Previous literature reports indicated that refined palm oil contained the highest levels of 3-MCPD esters [10, 17]. Due to the rising concern about the 3-MCPD potential health risk to humans, the European Commission (EC) Scientific Committee on Food and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have set a maximum tolerable daily intake (TDI) of 2 μg kg^{-1} of body weight of 3-MCPD levels [18, 19]. Such food safety issues from 3-MCPD cause implications for major vegetable oil producers, especially Malaysia, with high palm oil production [20]. Furthermore, about 85% of global palm oil produced was used in food applications [21]. Therefore, it is of utmost importance to identify the 3-MCPD levels in various edible plant oils so that the researchers and

manufacturers can control and mitigate the occurrence and intensity of 3-MCPD esters formation to ensure the refined oils are safe and of the best quality for consumers [20].

Several analytical methods have been developed and published over the years to determine 3-MCPD esters in edible oils. Generally, there are two main approaches for detecting 3-MCPD esters in edible oils: the direct and indirect analysis methods. Direct methods involve the direct determination of individual MCPD esters using liquid chromatography coupled with time-of-flight mass spectrometry (LC-TOFMS) [22, 23]. This method has the advantage of being simple, requiring a minor degree of sample preparation without any additional steps of analyte transformation, is not time-consuming, and gives detailed information on the chemical structure of the esters [24]. However, it has not been widely used in routine analysis due to its high cost and the possibility of contamination of complex matrices on the instrument [24]. In contrast, indirect methods involve quantifying the total content of free 3-MCPD released from their esterified form mainly by using gas chromatography-mass spectrometry (GC-MS) [25]. Due to the detection of only a single analyte (free 3-MCPD) and the need for just a single internal standard, this method provides higher sensitivity for the quantification of 3-MCPD. It is more suitable for routine analysis [4]. Indirect methods follow similar protocols, which are the addition of an internal standard to the sample (either free or esterified form of 3-MCPD-d5) for quantification purposes, acid or alkaline transesterification to release free 3-MCPD from its esterified form, followed by neutralization and salting out, derivatization of the extracted 3-MCPD, extraction of the derivative, and finally GC-MS analysis [5].

This research aims to determine the levels of 3-MCPD esters in edible plant oils. The validated results are presented to attract policymakers' attention and ensure consumer rights while monitoring food safety and quality. Palm oil and other edible plant oils such as peanut oil, corn oil, sunflower oil, and olive oil were analyzed using a GC-MS equipped with a BPX-5 capillary column and selected ion monitoring (SIM) mode determine and quantify the concentration of 3-MCPD esters. Because the performance of the BPX-5 column for detecting 3-MCPD esters has not been reported, its performance for the analysis of the target analyte was also a focus of this study. This research aimed to identify the concentration of 3-MCPD esters in two different local brands of unrefined and refined palm oil and two different brands of peanut oil, corn oil, sunflower oil, and olive oil available in the Malaysia market. The results obtained from the analysis were used to compare the levels of 3-MCPD esters among the edible plant oils and different brands for each type of oil sample.

Materials and Methods

Chemicals and samples

3-monochloropropane-1,2-diol (3-MCPD, 98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA), while the internal standard deuterated 3-monochloropropane-1,2-diol (3-MCPD-d5, 98.7%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Tetrahydrofuran (≥99.9%), sulfuric acid (H_2SO_4, 95-97%), methanol (≥99.8%), sodium sulfate (Na_2SO_4, ≥ 99.0%), sodium bicarbonate ($NaHCO_3$, ≥99.0%, and n-hexane (≥99.0%) were purchased from Merck (Selangor, Malaysia). Phenylboronic acid (PBA, ≥ 97.0%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of analytical and LC grades.

Twelve edible oil samples were used to analyze 3-MCPD esters by the indirect method, including two different local brands of unrefined and refined palm oil, two different local brands of peanut oil, corn oil, sunflower oil, and olive oil, respectively. An extra virgin olive oil was used as a blank sample for method performance and validation. All the edible oil samples were obtained from local supermarkets and were stored at room temperature (25 °C) before analysis.

Preparation of reagents and standards solutions

Methanolic sulfuric acid solution (1.8%, v/v) used in acidic transesterification was prepared by dissolving 1.8 mL of sulfuric acid in 100 mL of methanol. The saturated solution of sodium bicarbonate was prepared by mixing 4.8 g of NaHCO3 in 50 mL of ultra-pure water. In contrast, sodium sulfate solution (20%, w/v)

was prepared by dissolving 20 g of Na_2SO_4 in 100 mL of ultra-pure water. The derivatization agent, PBA solution (25%, w/v), was prepared by dissolving 1 g of PBA in 4 mL of acetone: water mixture (19:1, v/v).

The concentration of 1 mg mL^{-1} of 3-MCPD stock solution was prepared by weighing 10 mg (± 0.1 mg) of the 3-MCPD standard into a 10 mL volumetric flask and diluted to the mark with tetrahydrofuran. 10 µg mL^{-1} of 3-MCPD standard solution (S1) was prepared by pipetting 100 µL 3-MCPD stock solution into a 10 mL volumetric flask and filled up to the mark by diluting it with tetrahydrofuran. In comparison, 1 µg mL^{-1} of 3-MCPD standard solution (S2) was then prepared by pipetting 1 mL of 3-MCPD standard solution (S1) into a 10 mL volumetric flask and filled up to the mark by diluting it with tetrahydrofuran. Both S1 and S2 3-MCPD standard solutions were used for calibration purposes.

0.5 mg mL^{-1} of 3-MCPD-d5 stock solution was prepared by weighing 5 mg (± 0.1 mg) of 3-MCPD-d5 into a 10 mL volumetric flask and diluted to the mark with tetrahydrofuran. The working solution of 3-MCPD-d5 was used as the internal standard. It was prepared by pipetting 100 µL of its stock solution into a 10 mL volumetric flask and diluted to the mark with tetrahydrofuran to give a final concentration of 5 µg mL^{-1}. All the standard solutions prepared were stored at 4 °C for up to 3 months.

Determination of 3-MCPD esters using the indirect method

Analysis of 3-MCPD esters in edible oil samples was carried out using the indirect method of acidic transesterification by Zelinková et al. and Hrncirik et al. [5, 10]. 100 mg (± 5 mg) of oil sample was weighed in a clean glass tube, dissolved in 0.5 mL of tetrahydrofuran, and vortexed for 20 s. 80 µL of the internal standard solution, 3-MCPD-d5, was then added to the sample, followed by 1.8 mL of methanolic sulfuric acid solution (1.8%, v/v) vortexed for another 20 s. The tube was capped and incubated in a water bath at 40 °C for 16 hours. After the incubation period, 0.5 mL of a saturated aqueous sodium bicarbonate solution was added to the tube to stop the reaction and vortexed for 10 s. The organic solvents were evaporated to dryness at 55 °C by using a vacuum rotary evaporator.

Then, 2 mL of aqueous sodium sulfate solution (20%, v/v) was added to the mixture, followed by liquid-liquid extraction with n-hexane (2×2 mL) to remove the fatty acid methyl esters (FAMEs). FAMEs were removed by discarding the organic phase (upper layer) after phase separation. 250 µL of PBA derivatizing solution (25%, w/v, acetone/water, 19/1, v/v) was added to the aqueous extract. The tube was capped, sealed, vortexed for 15 s, and heated in a water bath at 80 °C for 20 min. After 20 min, the tube was allowed to cool to room temperature (25 °C). N-hexane (2×1 mL) was added, and the tube was vortexed for 30 s to extract the 3-MCPD-PBA derivative. The tube was allowed to stand for phase separation, and the supernatant (n-hexane) was transferred to a clean tube. A small amount of anhydrous sodium sulfate was added to the supernatant to remove any excess water. Finally, the supernatant was transferred to a sterile vial. 1 µL of the sample was injected and analyzed using GC-MS. The analysis was carried out in triplicate.

Gas chromatography-mass spectrometry analysis

Analyte detection was performed by a single quadrupole gas chromatography-mass spectrometer GCMS-QP2010 Plus equipped with an AOC-20i auto-injector from Shimadzu (Kyoto, Japan). 1 µL of the sample was introduced into the injector operating at 280 °C in splitless mode. The chromatographic separation was carried out on a bonded, poly(dimethylsiloxane) capillary column BPX-5 (SGE Analytical Science, 30 m length, 0.25 mm id, 0.25 µm film thickness). Helium (99.999%) was used as a carrier gas set at a constant flow of 1.2 mL min^{-1}. The column temperature was programmed at 60 °C for 1 min, which increased to 150 °C at the rate of 6 °C min^{-1} and held for another 2 min. The temperature was further increased to 280 °C at the rate of 10 °C min^{-1} and held constant for 5 min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV ionization energy. The MS transfer line temperature was maintained at 280 °C, while the ion source and quadrupole temperatures were at 250 °C and 150 °C, respectively. Qualitative analysis was carried out by running the mass spectrometer in a full-scan

mode over m/z 50 to 400 for peak confirmation. The quantitative analysis was performed by selected ion monitoring (SIM) mode to monitor characteristic ions at m/z 91, 147, and 196 for 3-MCPD derivatives, while for 3-MCPD-d5, the characteristic ions were at m/z 93, 150, and 201.

Method validation

A calibration curve was established by spiking a blank sample with eight different concentration levels of 3-MCPD. 100 mg (± 5 mg) of the blank sample (extra virgin olive oil), with no detection of the 3-MCPD esters, was spiked with different concentrations of the 3-MCPD standard. The concentrations of spiked 3-MCPD were 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mg kg^{-1} of 3-MCPD per 100 mg of sample, respectively. The known amount of 3-MCPD-d5 internal standard (4.0 mg kg^{-1} per 100 mg of the sample) was also spiked before extraction based on the sample preparation procedure.

Recovery and repeatability were evaluated by selecting three different concentrations of 3-MCPD (low, medium, and high: 0.25, 3.0, and 6.0 mg kg^{-1}), prepared for six replicates (n = 6), and subjected to 3-MCPD analysis. Besides, within-laboratory reproducibility was also conducted by measuring three different concentrations of 3-MCPD (low, medium, and high: 0.25, 3.0, and 6.0 mg kg^{-1}), with six replicates for each concentration level (n = 6) for three consecutive days. Limit of detection (LOD) and limit of quantification (LOQ) were performed by preparing one lowest concentration of 3-MCPD and subjected to 3-MCPD analysis. The same concentration was then repeated ten times. LOD and LOQ were calculated as three- and ten-fold standard deviations of ten independent samples.

Statistical evaluation

The oil samples were analyzed in triplicate. General statistical evaluation was carried out using Microsoft Office Excel 365. The average of triplicate analyses was reported together with the standard deviation (± SD). Significant differences between the results obtained were examined using Student's t-test with a 95% confidence interval ($p < 0.05$).

Results and Discussion

3-MCPD-PBA derivative chromatogram

This study was conducted using the BPX-5 capillary column, which directly replaces the GC columns used by previous literature, such as HP-5MS and DB-5MS [13, 26, 27]. These columns have similar phases of (5%-phenyl)-methylpolysiloxane (non-polar) with very low bleed characteristics, making them ideal for GC-MS applications. The BPX-5 capillary column is a multi-purpose column that can be used for a wide range of applications and is suitable for almost 80% of routine GC analyses, such as ultra-trace analyses, hydrocarbons, pesticides, herbicides, and triglycerides. Nonetheless, the BPX-5 column has not been applied and reported to detect 3-MCPD-PBA derivatives. Although the BPX-5 and HP/DB-5MS columns have similar phases in general, the retention times of the target analytes with similar characteristic ions may be slightly different. Likewise, retention times also vary depending on the type of columns employed and the analysis method. Several factors such as column length, column temperature, oven temperature, or gas flow rate may influence the retention time of a target analyte even if similar phases of GC columns are used. For example, the retention times for both 3-MCPD esters and their deuterated form varied between 14.50 min to 17.17 min [13, 26, 27].

The gas chromatograms for both 3-MCPD esters and their deuterated form in the spiked blank samples are shown in Figure 1. The retention time of 3-MCPD-d5 was 17.08 min, while the retention time of 3-MCPD esters was 17.17 min. The EI mass spectra for the PBA derivative of 3-MCPD and 3-MCPD-d5 were also illustrated in Figure 2. SIM mode was used for GC-MS quantification, where m/z 147 and m/z 150 were served as the quantifier ions for 3-MCPD derivatives and 3-MCPD-d5, respectively. In addition, m/z 91 and 196 were used as the qualifier ions for 3-MCPD derivatives and m/z 93 and 201 for 3-MCPD-d5. Table 1 shows detailed information on the retention times and characteristic ions used for GC-MS analysis.

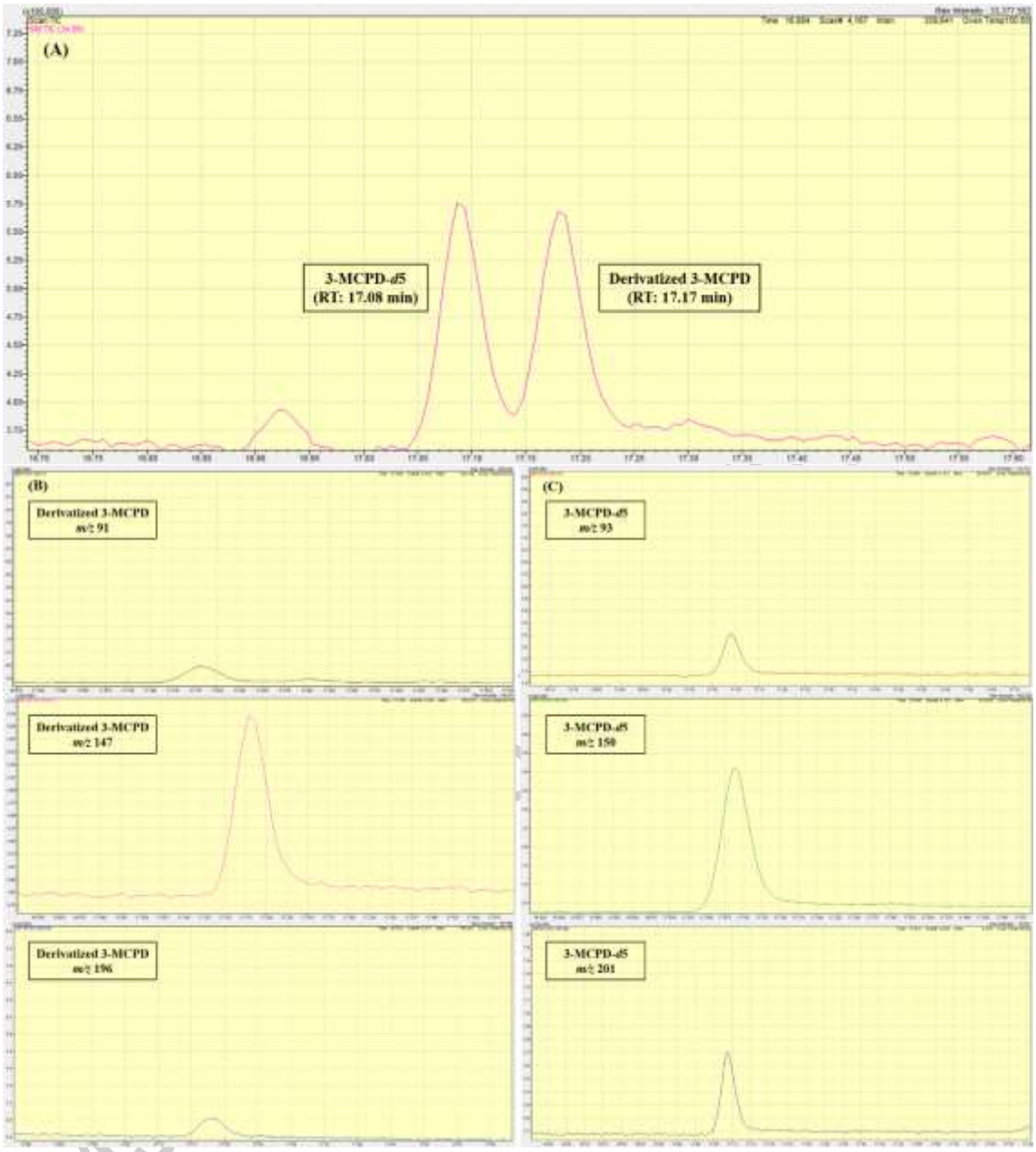

Figure 1. GC chromatograms of 3-MCPD esters and 3-MCPD-d5 in spiked blank samples (RT = retention time). (A) TIC of derivatized 3-MCPD, (B) SIM chromatogram of 3-MCPD esters, (C) SIM chromatogram of 3-MCPD-d5

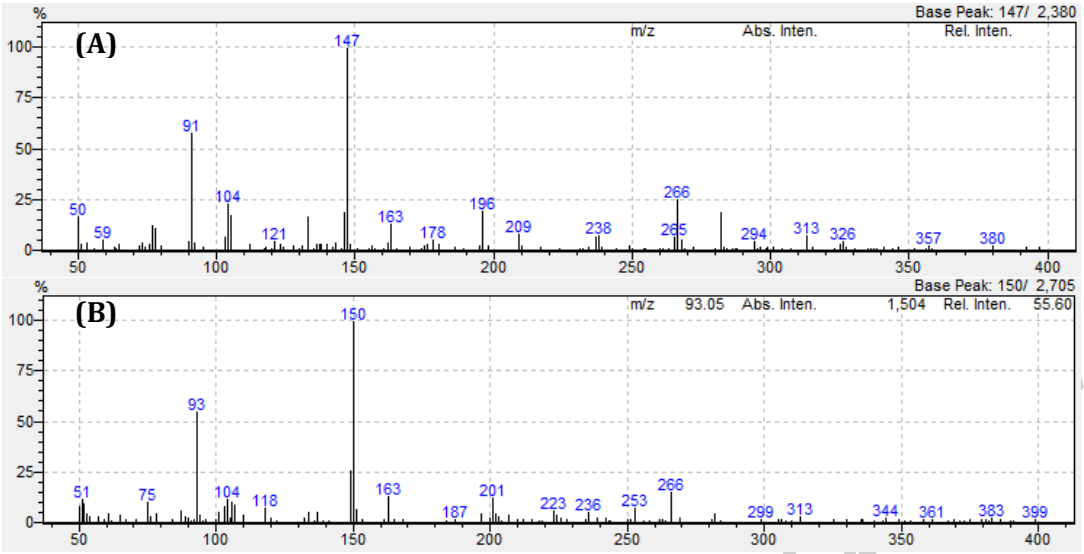

Figure 2. EI mass spectra for PBA derivatives of (A) 3-MCPD and (B) 3-MCPD-d5

Table 1. Retention times and characteristic ions used for SIM mode in GC-MS analysis

Analyte	Retention Time (min)	Quantifier Ion (m/z)	Qualifier Ion (m/z)
3-MCPD-d5	17.08	150	93 & 201
3-MCPD	17.17	147	91 & 196

Method validation

Method validation for the analysis of 3-MCPD esters using GC-MS was conducted about linearity, recovery, precision (repeatability and within-laboratory reproducibility), LOD, and LOQ. Linearity was evaluated using eight calibration points from 0.25 mg kg^{-1} to 6.0 mg kg^{-1} of the 3-MCPD standard. The calibration curve was constructed by plotting the response ratio of the 3-MCPD standard and 3-MCPD-d5 internal standard against the concentration of the 3-MCPD standard, as illustrated in Figure 3. The results showed a linear relationship between the concentration of 3-MCPD standards and the area ratio of 3-MCPD/3-MCPD-d5, with the linearity was verified at $r^2 \geqslant 0.99$. The calibration curve obtained shows that the area ratio of 3-MCPD/3-MCPD-d5 is directly proportional to the concentration of spiked 3-MCPD standards, which obeys Beer's law. The linear range is broad enough to cover the total concentration of 3-MCPD esters found in most edible oils.

In addition, method validation was also conducted based on the recovery test. The result in Table 2 shows the percentage recovery for three different concentrations of 3-MCPD, with the lowest recovery of 92.80% was obtained for the lowest concentration of 3-MCPD (0.25 mg kg^{-1}), while the highest concentration of 3-MCPD (6.0 mg kg^{-1}) recorded the highest recovery of 105.22%. Apart from the recovery determination, the intra-day and inter-day (intermediate) precision were also performed in this study to determine the repeatability and within-laboratory reproducibility of the method, respectively. The RSD percentage obtained for repeatability was 4.30%, 6.38%, and 5.01% for 3-MCPD levels of 0.25, 3.0, and 6.0 mg kg^{-1}, respectively (Table 2). On the other hand, the percentage of RSD

obtained for within-laboratory reproducibility was 5.63% for the lowest concentration (0.25 mg kg^{-1}), 5.32% for the intermediate concentration (3.0 mg kg^{-1}), and 4.18% for the highest concentration of 3-MCPD (6.0 mg kg^{-1}) (Table 2). The low RSD of the replicates showed the reliability of the data acquired by an indirect method and the usage of the BPX-5 column, which attest to satisfactory repeatability and reproducibility. The high accuracy, good repeatability, and reproducibility are crucial in method validation as these parameters build a degree of confidence in obtaining the results. For instance, the RSD values must show the degree of variation expected when the method was repeated several times under standard situations [28]. Any tedious repeats can be eliminated, resulting in better time management for subsequent analyses using the same procedure and settings.

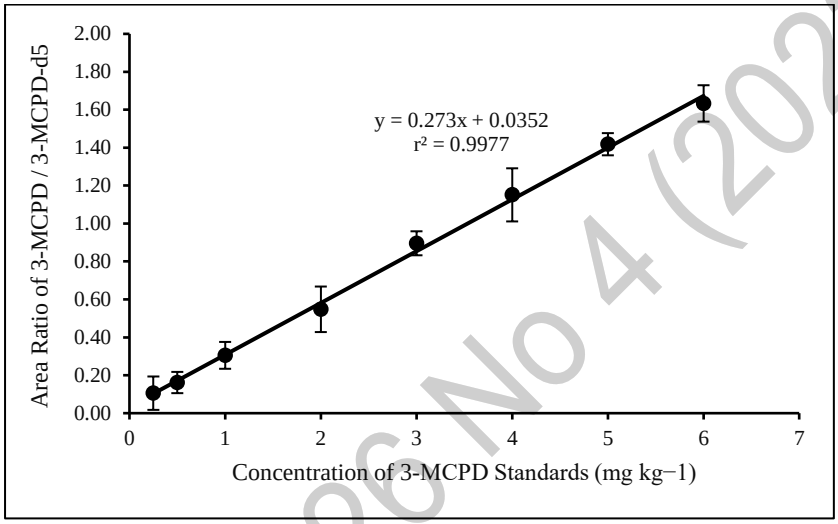

Figure 3. Calibration curve of 3-MCPD esters. Error bars are standard deviations obtained from the triplicate analysis

Table 2. Recovery and precision (repeatability and within-laboratory reproducibility) for 3-MCPD esters (n = number of replicates for each spike level)

Concentration of 3-MCPD (mg kg^{-1})	**% Recovery (n = 6)**	**RSD (%)**	
		Repeatability (n = 6)	**Within-Laboratory Reproducibility**
0.25	92.80 ± 4.21	4.30	5.63
3.0	104.65 ± 6.70	6.38	5.32
6.0	105.22 ± 5.28	5.01	4.18

According to the guidelines in EURACHEM, a blank sample containing no detectable or too low level of analyte might need to be spiked for specific analytical techniques such as chromatography to obtain a non-zero standard deviation [29]. In this study, the LOD and LOQ were estimated by calculating the mean and standard deviation of ten spiked blank samples (extra virgin olive oil) corresponding to the lowest point of the calibration curve. The calculated mean and standard deviation values were fitted into the following equations for the estimation of LOD and LOQ,

$$LOD \cong \bar{x}_{blank} + 3\ SD_{blank} \quad (1)$$

$$LOQ \cong \bar{x}_{blank} + 10\ SD_{blank} \quad (2)$$

where $\bar{x}_{blank}$ is the mean of the spiked blank sample and SD_{blank} is the standard deviation of the spiked blank sample. Hence, the LOD and LOQ of the method obtained in this study were 0.11 mg kg^{-1} and 0.14 mg kg^{-1}, respectively.

Table 3 compares the analytical performances of this study (validated method) to those of other indirect methods previously reported. Except for the slightly lower recovery reported by Abd. Razak et al., who employed the AOCS official method, all the procedures mentioned here exhibited good recovery (> 85%) and repeatability (RSD <10%). The linear range in this study was significantly more extensive than the AOCS official method and the method reported by Arisseto et al., which can determine a wide range of target analyte concentrations without excessive dilution. However, the LOQ was established at a minimum of 0.10 mg kg^{-1}, implying that none of the methods given are sensitive enough to detect 3-MCPD esters within the maximum tolerable limit of 0.002 mg kg^{-1} set by the Scientific Committee on Food (SCF) of the European Commission [18]. This suggests that all of the reported indirect methods (including direct HPLC methods) cannot reliably detect edible oil samples with low concentrations of 3-MCPD esters (below the maximum tolerable limit of 0.002 mg kg^{-1}). In other words, all indirect methods face the same challenges and have the same limitation. The detectable 3-MCPD esters in most edible oils are much greater than the maximum tolerable limit, even though the indirect method's LOD and LOQ are higher than the maximum tolerable limit of 0.002 mg kg^{-1}. In summary, this indirect method, combined with the BPX-5 capillary column, is suitable for routine analysis in practice.

Table 3. Comparison of the analytical performance of the current method with other indirect methods that have been reported

Methods	BfR Method 008 (Column: HP-5MS)	BfR Method 008 (Column: DB-5MS)	AOCS Official Method Cd 29a-13 (Column: HP-5MS)	Acid Cleavage	Acid Cleavage	Special Remark
Linear Range (mg kg^{-1})	0.25–6.00	0.25–6.00	0.08–2.39	0–4.00	0.25–6.00	Compared to the official AOCS method, this method has a wider linear range.
Recovery (%)	97.60–107.90	85.40–110.50	80.30–85.80	101.00–110.00	92.80–105.22	The recovery % range is narrower and closer to 100%.
Repeatability (RSD, %)	0.02–0.12 (n = 6)	4.18–4.79 (n = 8)	7.00–10.20 (n = 7)	4.40–8.40 (n = 6)	4.30–6.38 (n = 6)	This method outperforms the standard AOCS method in terms of precision.

Table 3 (cont'd). Comparison of the analytical performance of the current method with other indirect methods that have been reported

Methods	BfR Method 008 (Column: HP-5MS)	BfR Method 008 (Column: DB-5MS)	AOCS Official Method Cd 29a-13 (Column: HP-5MS)	Acid Cleavage	Acid Cleavage	Special Remark
Reproducibility (RSD, %)	NA	NA	NA	3.90–6.10 (n = 6)	4.18–5.63 (n = 6)	In real-world applications, this approach has proven to be reliable.
LOD (mg kg^{-1})	0.25	0.09	0.02	0.05	0.11	Despite having a greater LOD than the standard AOCS method, this method is reliable for quantifying the concentration of 3-MCPD esters in all edible oils examined.
LOQ (mg kg^{-1})	0.50	0.23	NA	0.10	0.14	In comparison to other studies, this approach has a decent LOQ.
Reference	[13]	[27]	[30]	[31]	This study	

[a]Column: VF-1MS; Temperature program: 60 °C held for 1 min, 6 °C min^{-1} to 190 °C, 20 °C min^{-1} to 280 °C, 280 °C held for 30 min
[b]Column: BPX-5; Temperature program: 60 °C held for 1 min, 6 °C min^{-1} to 150 °C, 150 °C held for 2 min, 10 °C min^{-1} to 280 °C, 280 °C held for 5 min
NA means not available

3-MCPD esters in edible plant oils

The indirect method with PBA as the derivatizing agent was applied in this study as it readily reacts with 3-MCPD to form a stable derivative 4-chloromethyl-2-phenyl-1,3,2-dioxaborolane. Due to its high selectivity and ability to extract with non-polar solvents such as n-hexane, PBA was suitable for the choice of derivatization agent for GC-MS analysis. In this study, a total of 12 edible plant oils available in local supermarkets were analyzed to detect their 3-MCPD esters content, with two different brands for each type of oil. The concentrations of 3-MCPD esters in these samples ranged from <LOQ to 4.10 mg kg^{-1}, as shown in Figure 4.

Since Malaysia is the second-largest producer of palm oil in the world, with approximately 85% of global palm oil produced was used in food applications, the levels of 3-MCPD esters in palm oil were studied in this research because once the validated results are reported in a scientific journal or a media article, it will draw the attention of policymakers. In short, it fulfills the objective of ensuring consumers' rights while monitoring food safety and quality. The concentration of 3-MCPD esters was compared among red palm oil and refined palm oil, in which refined palm oil comprised a higher concentration of 3-MCPD esters, ranging from 2.29 ± 0.09 to 4.10 ± 0.06 mg kg^{-1} when compared to

red palm oil (0.41 ± 0.03 to 3.40 ± 0.25 mg kg^{-1}). Red palm oil is often referred to as unrefined palm oil, and it is dark red due to the high content of carotenoids. Thus, it is vital to perform deodorization at low temperatures to avoid the thermal destruction of carotenoids [32]. Conversely, refined palm oil is light yellow and odorless as it undergoes deodorization at a high temperature of around 250 °C to remove any FFA and volatile components [32]. Such a process led to the formation of high 3-MCPD esters levels in refined palm oil. As shown in Figure 4, a low concentration of 3-MCPD esters with a value of 0.41 ± 0.03 mg kg^{-1} was identified in red palm oil (brand B). However, the other brand of red palm oil (brand A) detected a high concentration of 3-MCPD esters with a value of 3.40 ± 0.25 mg kg^{-1}. This suggested that the oil extraction conditions, for instance, the red palm oil sample (brand A), may consist of a mixture of unrefined and refined oils that eventually led to the occurrence of 3-MCPD esters.

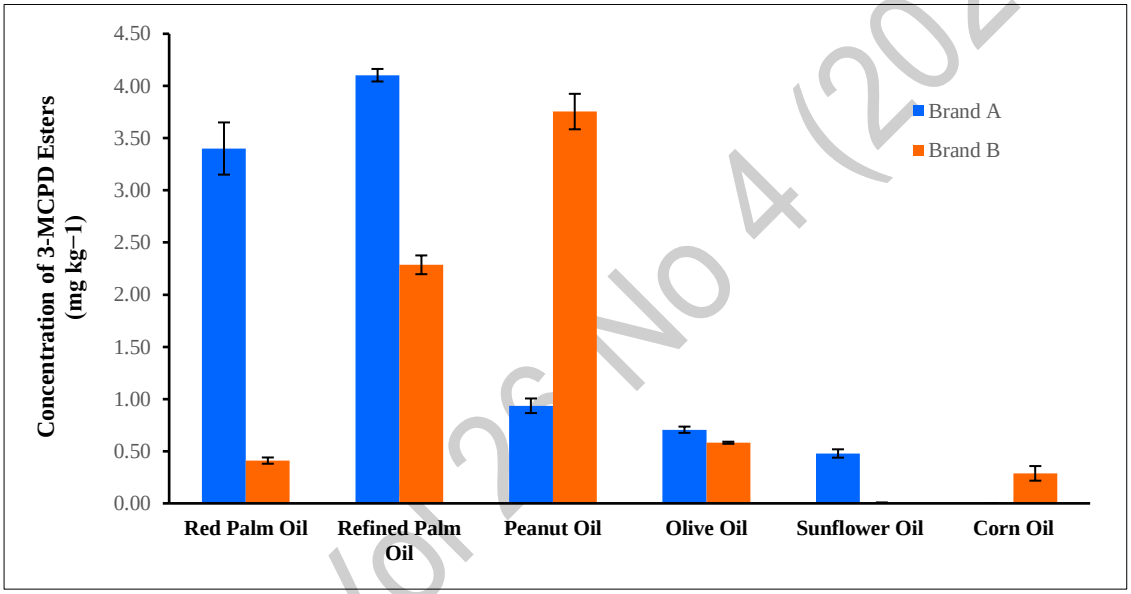

Figure 4. 3-MCPD esters levels determined in edible plant oils by indirect acidic transesterification methods and the BPX-5 capillary column, with two different brands for each type of oil

Besides palm oil, the other edible plant oils that consumers commonly used were also analyzed for the presence of 3-MCPD esters. Overall, olive oil, sunflower oil, and corn oil contained lower levels of 3-MCPD esters varied from <LOQ to 0.71 ± 0.03 mg kg^{-1}, a meanwhile higher concentration of 3-MCPD esters was found in refined palm oil and peanut oil, ranging from 0.94 ± 0.07 to 4.10 ± 0.06 mg kg^{-1} (Fig. 4). Sunflower oil and corn oil consisted of slightly lower concentrations of 3-MCPD esters (<LOQ to 0.48 ± 0.04 mg kg^{-1}) as compared to olive oil (0.58 ± 0.01 to 0.71 ± 0.03 mg kg^{-1}). Extra virgin olive oil was produced by physical pressing at low temperature (below 27 °C) without heating and therefore consisted of deficient levels of 3-MCPD esters [33]. In contrast, a higher temperature (150 °C) during deodorization involved in the production of refined olive oil resulted in higher content of 3-MCPD esters [33]. Since olive oil is a blend of both extra virgin olive oil and refined olive oil, thus it contains a higher concentration of 3-MCPD esters due to the combination of unrefined and refined olive oil in various ratios. Such a trend of results confirmed the previous literature by Zelinková et al. (2006) that reported a higher concentration of 3-MCPD esters was detected in refined olive oil than that of sunflower oil

and corn oil [10]. The highest concentration of 3-MCPD esters was found in refined palm oil (2.29 ± 0.09 to 4.10 ± 0.06 mg kg^{-1}), which was then followed by peanut oil (0.94 ± 0.07 to 3.75 ± 0.17 mg kg^{-1}). Peanut oil used in this study was a blended oil containing refined palm oil and peanut oil (as indicated on the product package and labeled). High deodorization temperature in the refining process further contributed to the formation of 3-MCPD esters in refined palm oil and peanut oil. Generally, refined fruits oil such as palm oil and olive oil generate higher levels of 3-MCPD esters than seed oils like sunflower oil and corn oil [10]. Palm oil derived from the mesocarp (pulp) of the fruit has a different composition from seed oils, which contain relatively high levels of potential precursors of 3-MCPD esters such as chloride ions and partial acylglycerols [34]. This eventually contributes to the high concentration of 3-MCPD esters in palm oil.

By comparing different brands for each type of oil sample, there is a significant variation in the concentration of 3-MCPD esters in all the oil samples, except olive oil (Figure 4). A significant difference was observed at a 95% confidence interval using Student's t-test with a p-value less than 0.05 between two different brands of oil samples, with red palm oil and peanut oil specifically, as shown in Figure 4. The reason for the significant variation in the concentration of 3-MCPD esters found between two different brands of red palm oil was explained previously. The same goes for peanut oil. The refining process involving bleaching and deodorization may be one of the factors that cause the differences in the 3-MCPD esters levels between the two different brands. It may also link to the preliminary heat treatment of seeds, such as roasting peanuts, which results in a variation of 3-MCPD esters among different peanut oil brands. For other types of oil samples, it isn't easy to justify these variations because the main precursors in the reaction and the actual mechanism of 3-MCPD esters formation were not completely understood. The formation of 3-MCPD esters during the production of edible oils might be due to the presence of chlorine as a donor. Plants took up chloride ions via the soil either by the natural occurrence or through the fertilizers, which then further metabolized into hydrophilic chloride substances that may generate hydrochloric acid upon high-temperature treatment, thereby leading to the occurrence of 3-MCPD esters [3]. Table 4 compares the trend of 3-MCPD esters levels in different types of plant edible oil samples from this study to those from previous studies. It should be noted that this study and earlier literature found that among the six types of edible oils, refined palm oil had the most significant level of 3-MCPD esters. In contrast, corn oil had the lowest 3-MCPD esters, regardless of where the edible oil samples originated. Except for the high concentration of 3-MCPD esters identified in this study due to the type of red palm oil used for analysis, the concentration range of 3-MCPD esters for unrefined or red palm oil is essentially similar. This investigation suggests that the BPX-5 capillary column utilized has good separation performance, comparable to previously published analysis trends, indicating that this column could be useful in identifying 3-MCPD esters in a variety of edible oils.

Several factors such as thermal treatment, the presence of precursors, or soil conditions might trigger the formation of 3-MCPD esters beyond the maximum tolerable limit. Since 3-MCPD is a type of food-borne toxicant, it is vital to analyze and detect the content of 3-MCPD esters in edible oils as they have been widely used in our everyday life. Consumers have the right to choose the healthiest plant edible oils to be consumed. Consequently, continue researching on all possible pathways for the formation and mitigation of 3-MCPD esters should be done by researchers to ensure that the processed plant-based oils consumed by consumers are safe while maintaining the best nutritious quality of oils.

Table 4. 3-MCPD esters in edible oils found in this study compared to previously reported indirect methods using acidic transesterification

Methods	3-MCPD Esters Concentration Range (mg kg^{-1})						Reference
	Unrefined/Red Palm Oil	Refined Palm Oil	Peanut Oil	Olive Oil	Sunflower Oil	Corn Oil	
Bfr method 008	<0.25–0.90	<0.25–5.80	2.45	NA	0.60	<0.25–0.35	[13]
AOCS official method Cd 29a-13	<1.00	1.00–3.00	1.00–3.00	1.00–3.00	<1.00	<1.00	[30]
Acid cleavage (column: EquityTM-1)	NA	NA	<0.10	<0.30–2.46	<0.30	0.37	[10]
Acid cleavage (column: VF-1MS)	<0.05–0.33	1.05–2.95	0.13–0.29	0.14–0.33	0.10–0.27	<0.05–1.12	[31]
Acid cleavage (column: BPX-5)	0.41–3.40	2.29–4.10	0.94–3.75	0.58–0.71	<LOQ–0.48	<LOQ–0.29	This study

NA means not available

Conclusion

An indirect method involving acidic transesterification was used to detect and quantify 3-MCPD esters in various edible plant oils. PBA was chosen as the derivatizing agent since it reacts specifically with 3-MCPD to form a stable non-polar cyclic derivative (4-chloromethyl-2-phenyl-1,3,2-dioxaborolane) extractable with n-hexane. The use of the BPX-5 capillary column in GC-MS analysis was a direct replacement for other GC capillary columns that have been primarily used in previous literature. This provides researchers with a promising alternate choice/method for detecting 3-MCPD esters in various edible plant oil samples. The method validation verified that a linear calibration curve with extra virgin olive oil as a blank sample and spiked with eight different concentrations of 3-MCPD standards was established with $r^2 \geq 0.99$. Besides, the applied indirect method also showed the percentage recovery of three different concentration levels of 3-MCPD fell from 92.80% to 105.22%, together with RSD of 4.30% to 6.38% and 4.18% to 5.63% for both repeatability and within-laboratory reproducibility, respectively. The LOD and LOQ of the method were identified at 0.11 mg kg^{-1} and 0.14 mg kg^{-1}, respectively. As a result, this indirect method showed good accuracy and repeatability for the analysis of 3-MCPD esters in all studied edible plant oils.

From the results obtained for a total of 12 edible plant oils, 3-MCPD esters were found to be the lowest in corn oil (<LOQ to 0.29 mg kg^{-1}) and highest in refined palm oil (2.29 to 4.10 mg kg^{-1}). Refined palm oil contained a higher concentration of 3-MCPD esters than red palm oil (unrefined palm oil) due to high temperature during the deodorization step, contributing to the formation of 3-MCPD esters. Different brands for each type of edible plant oil samples also showed significant variation in 3-MCPD esters levels. Several factors, such as the refining process and the crude oil's quality, may contribute to forming 3-MCPD esters in various plant oils. Since 3-MCPD is a type of process contaminant that induces possibly carcinogenic to humans, it is significant to have potential mitigation of 3-MCPD in oils for the safety of consumers. Improvement can be made by upgrading the current technology of the refining process, for example, by implementing a more flexible deodorizing technology that could be carried out at low temperature (<250 °C) so that the occurrence of 3-MCPD in edible plant oils can be permanently reduced or eliminated in the future, without tarnishing the premium quality of the edible plant oils.

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