Preliminary Large Scale Mitigation of 3-Monochloropropane-1, 2-diol (3-MCPD) Esters and Glycidyl Esters in Palm Oil

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Abstract: Approximately 900 tonne of crude palm oil (CPO) underwent washing using 5 to 10% hot water (90 to 95°C) at a palm oil mill. The aim of the CPO washing was to eliminate and/or reduce total chlorine content present in the conventional CPO, as it is known as the main precursor for the formation of 3-monochloropropane-1, 2-diol esters (3-MCPDE). By a simple hot water washing, more than 85% of the total chlorine was removed. However, washing did not have significant (p > 0.05) effect on other oil quality parameters such as the deterioration of bleachability index (DOBI), free fatty acid (FFA) content and diacylglycerol (DAG) content of the oil. The latter has been established as the main precursor for glycidyl esters (GE) formation. The treated CPO was then transported using tankers and further refined at a commercial refinery. Refining of washed CPO resulted in significantly (p < 0.05) lower formation of 3-MCPDE, but GE content remained slightly high. Post-treatment of refined oil significantly reduced the GE content (p < 0.05) to an acceptable level whilst almost maintaining the low 3-MCPDE level. The study has proven that water washing of CPO prior to refining and subsequent post-refining is so far the most effective way to produce good quality refined oil with considerably low 3-MCPDE and GE contents. Dry fractionation of refined palm oil showed these contaminants partitioned more into the liquid olein fraction compared to the stearin fraction.

Key words: 3-MCPDE and GE, water washing of crude palm oil, commercial refinery, dry fractionation, palm oil mill

1 Introduction

The presence of 3-monochloropropane-1, 2-diol esters (3-MCPDE) in vegetable oils and fats has been widely reported and discussed ever since it was first discovered in edible oils by Zelinková et al.7. Whilst the 3-MCPDE generally are not detected in most crude oils, it is prevalent in most refined vegetable oils and fats, including fish oils1,21. The esters are a result of high temperature processing where chlorinated compounds react with acylglycerols to form a mixture of 2- and 3-MCPDE3. Unlike seed oils such as soybean, sunflower, canola and corn oils, palm oil has been found to contain relatively higher amount of these contaminants due to the uptake of chlorinated compounds into the oil palm, possibly from soil and fertilisers4,5. Abd. Razak et al.6 in a first comprehensive survey on palm oil has documented 3-MCPDE ranging from below 0.25 mg kg\(^{-1}\) to as high as 5.8 mg kg\(^{-1}\) in refined palm oil.

Both organic and inorganic chlorines have been postulated to affect the formation of 3-MCPDE, and these chlorinated substances are inherent in the oil well before processing7,8. Mitigation of the formation of these esters should be carried out as early as at plantation stage. For palm oil, the production process starts from harvesting and transportation of fresh fruit bunch (FFB) to palm oil mill. In order to preserve the quality of crude oil produced, FFB must be transported as soonest possible to the mill so that the milling process can be completed on the same day. In the past few years, labour shortage caused delays in FFB harvesting and transportation of fresh fruit bunch (FFB) to palm oil mill. In order to preserve the quality of crude oil produced, FFB must be transported as soonest possible to the mill so that the milling process can be completed on the same day. In the past few years, labour shortage caused delays in FFB harvesting and delivery to the mill. Intervention at the plantations and estates is almost impossible at this stage. Hence, elimination of chlorinated compounds, especially the inorganic counterpart is more practical to be initiated at the palm oil mills. Treated water with lowest amount of chlorine should be used for CPO washing instead of alcohol.
due to practicality and economic viability.

Glycidyl esters (GE), which are formed from high temperature process particularly during deodorisation of oils and fats, has been another area of concern. Again, among all edible oils, palm oil has been singled out to yield higher levels of GE due to its inherent diacylglycerol (DAG) content. Unlike 3-MCPDE, the main precursor for GE is DAG. 3-MCPD is classified as a possible human carcinogen (group 2B) according to The International Agency for Research on Cancer (IARC), whereas glycidol is categorised as probably carcinogetic for humans (group 2A). Formation of GE from DAG is substantial at temperatures above 230°C and it has been suggested that deodorisation should be carried out at temperatures below 230°C to limit the GE formation. Furthermore, the GE formation accelerates when the DAG levels in oils exceed 3-4% of total lipids. Ideally, free fatty acid (FFA) content in crude oils, especially crude palm oil (CPO) must be kept around 1.9-2.5%, which corresponds to DAG levels of below 4%. However, it is well known that deodorisation of CPO at temperature below 230°C is not possible due to high carotene content in the crude oil. Lower deodorisation temperature will result in darker colour refined oil, which exceeds the 3 Lovibond red. In addition, only partial removal of FFA will be achieved with lower deodorisation temperature.

Research efforts to mitigate the formation of 3-MCPDE and GE have been initiated since 2009. The mitigation strategies include optimization of degumming conditions, neutralization of the degummed oils, selection of appropriate bleaching clays, optimisation of deodorisation conditions as well as post-refining. The potential applications of enzymes and adsorbents to remove both 3-MCPDE and GE in refined oils have also been discussed in the literature. Mitigation strategies for the reduction of 3-MCPDE and GE in vegetable oil processing have also been reviewed by Sergio et al.

The effect of water degumming in limiting the formation of 3-MCPDE and GE has been widely documented. Water degumming can also be regarded as a water washing step and is considered as the most effective way to mitigate the formation of 3-MCPDE as it removes hydratable inorganic chlorine. Water (1 to 5%) used for degumming gave a reduction of up to 80% of 3-MCPDE in the resulting refined (RBD) oil. However, most of the experiments were conducted in laboratories where removal of water is relatively easy. Water degumming/washing at commercial refineries may pose significant challenges with regards to the amount and treatment of the effluent. Most refineries, which are located near to ports, have very limited capacity of water treatment plant and also shortage of land area to expand the treatment plant.

Dual deodorisation strategy suggested by some researchers has been found to be effective in reducing the formation of 3-MCPDE and GE. In this step, deodorisation is carried out for a short period of time (5 min to 1 hr) at higher temperature (240 to 270°C) followed by longer period of time (2 to 4 hrs) at lower temperature (180 to 200°C) and vice versa with strong vacuum. This approach encountered little problem when applied under laboratory conditions. However, implementation at commercial refineries is not the most viable approach. Maintaining strong vacuum throughout the deodorisation process is not easy and generally requires new processing equipment.

Modification of oils and fats by means of hydrogenation, interesterification and fractionation has been documented to affect the levels of 3-MCPDE and GE. Palm oil is the most widely fractionated oil. Upon fractionation, 3-MCPDE has been reported to partition more into the liquid olein as compared to the solid stearin with partition coefficient, \( K_p \), of 2.8. A relationship equation predicting the amount of 3-MCPDE or GE in liquid olein has been developed and is based on the solid fat content of the slurry of the fractionating feed oil. The equation is given as:

\[
\text{3-MCPDE in liquid oil} = \frac{100}{(100 - \% \text{crystallised oil})^3}
\]

By applying the equation, partitioning of the esters in liquid and solid fractions can be predicted and calculated. In a wet fractionation of palm oil using acetone as reported by Kyselka et al., the resulted super olein (IV 64.2) was concentrated with 3-MCPDE in a factor of only 1.06 but subsequent wet fractionation of the super olein resulted in exceptionally high level of 3-MCPDE of almost 3.5 times in the top olein (IV 75.2). In the case of GE, its content in the super olein was almost similar with the original palm oil but increased rapidly in the top olein for almost 9 times higher.

While most publications in the literature with regards to the mitigations of 3-MCPDE and GE were generated from laboratory scale experiments, studies and findings from commercial plant are limited due to complexity of the work. Moreover, companies which have tried the mitigation and successfully reduced the esters formation are unlikely to publish their results, preferring to keep their secrets. This paper reported the first findings of a commercial/industrial mitigation of 3-MCPDE and GE. Water washing of CPO, physical refining of the washed CPO and dry fractionation of the resulted refined oil were carried out at a palm oil mill and refinery in Malaysia.

2 Materials and Methods
2.1 Raw materials and chemicals
A palm oil mill equipped with a water washing facility was identified for the commercial trial. The premise was located in the southern region of Peninsular Malaysia. CPO was sourced directly from the mill. The 120 tonne hr\(^{-1}\) of
capacity mill processes fresh fruit bunch (FFB) from its own plantation and independent smallholders. Processed/softened water with total chlorine content of 5 mg kg⁻¹ was used for the washing. For the refining trial, refining aids such as phosphoric acid (85% concentration, Merck, Darmstadt, Germany) and acid activated bleaching earth (Taiko Supreme 1B, Taiko Group, Malaysia) were commonly used at commercial refineries.

Two internal standards, i.e., 1, 2-dipalmitoyl-3-chloropropanediol (PP-3-MCPD-d5) and glycidyl palmitate (Gly-P-d5), were purchased from Toronto Research Chemical (North York, Canada), phenylboronic acid (PBA) was acquired from Merck (Darmstadt, Germany). Tert-butyl methyl ether (t-BME), sulphuric acid (H₂SO₄), sodium bicarbonate (NaHCO₃), iso-hexane, cyclohexane and iso-octane of analytical and GC grades were procured from Merck.

For survey studies, 41 CPO samples were collected from various palm oil mills in Malaysia and analysed for total chloride, and 2- and 3-MCPDE contents after heat treatment. In addition, refined, bleached and deodorised palm oil (RBDPO) from five refineries denoted as Refinery A to E were collected from southern region of Malaysia, where most refineries are located, and were subjected to quality and MCPDE and GE analyses. Besides that, another 141 RBDPO samples were also collected from various refineries in Malaysia and analysed for 3-MCPDE.

2.2 Experimental design

2.2.1 Commercial trials

Experimental design of the commercial trials is as depicted in Fig. 1. The trials consisted of CPO washing at a palm oil mill, refining and dry fractionation at a commercial refinery.

2.2.1.1 Washing of CPO at palm oil mill

The washing plant has a capacity of 2.5 tonne hr⁻¹. The plant consists of a high shear dynamic mixer, reactor, centrifuge (separator) and vacuum dryer. 5-10% of hot (95°C) water was first mixed with hot (100°C) CPO in the mixer before pumped into a holding reactor for further mixing for up to 30 min. Subsequently, the mixture was fed into the separator to remove the washed water. The washed oil was then dried in the vacuum dryer. Moisture in washed CPO before and after the vacuum drying was 0.50 and 0.25%, respectively. Quality of the CPO before and after washing was monitored daily. Free fatty acid (FFA) content, deterioration of bleachability index (DOBI), and moisture and impurities (M&I) were within the trading CPO specification from the Palm Oil Refiners' Association of Malaysia (PORAM) (FFA of maximum 5%; DOBI of minimum 2.31; and M&I of maximum 0.25%).

Washing of freshly produced CPO was carried out for more than 30 days to obtain 900 tonne of washed oil. The washing plant was operated for up to 12 hrs daily. The washed CPO was then transported in multiple 25 tonne tankers to a nearby refinery for further processing.

Both feed and the washed CPO were sampled 3 times a day; i.e., early, middle and at the end of processing day. The samples were then mixed and reported as composite feed and washed CPO, respectively.

2.2.1.2 Refining at a commercial refinery

Refining of the washed CPO was conducted at a 500 tonne day⁻¹ capacity continuous physical refinery. Both washed and control (unwashed) oils were subjected to similar refining treatment that includes dry degumming with phosphoric acid, bleaching with acid activated clay, and

![Fig. 1 Process flow diagram of the commercial trials. It is assumed that oil losses during processing is negligible.](image-url)
filtration and deodorisation. The dosages of phosphoric acid and bleaching clay, operating conditions and residence time of both bleacher and deodoriser were not disclosed due to confidentiality requirements by the refinery. Overall, the refining operation was capable of producing an acceptable quality of refined palm oil in accordance to the trade specification of Palm Oil Refiners’ Association of Malaysia (PORAM); FFA of maximum 0.1%; colour of maximum 3R; and M&I of maximum 0.1%.

2.2.1.3 Post-refining

The refined, bleached and deodorised palm oil (RBDPO) was subjected to another cycle of mild physical refining. In the post-refining, the degumming step was omitted while the dosage of acid activated bleaching clay and deodorisation temperature were much lower as compared to first refining. However, the detail condition was undisclosed in accordance with the confidentiality requirements.

2.2.1.4 Dry fractionation

Crystallisation was carried out in two crystallisers. Refined oil was first heated to 70°C to eliminate all crystals. The liquid oil was then gradually cooled to pre-determined temperature with agitation. When the oil fractionation temperature was reached, the slurry was filtered using membrane filter press. Iodine values (IV) for olein and stearin were averaging at 57 and 34, respectively with an olein yield of 80%. Oil samples of feed (RBDPO) and the fractionated products (olein and stearin) were collected at the end of dry fractionation process.

2.2.2 Survey studies

Three survey studies with three different objectives were also conducted. The first survey involved 41 CPO samples collected from palm oil mills around Malaysia and the samples were subjected to heat test (see section 2.3). The objective of this study was to develop correlation between total chloride content in the CPO and formation of 2- and 3-MCPDE upon heat treatment. For the second survey, 5 RBDPO samples were collected from 5 refineries in the southern region of Malaysia and were analysed for refined oil quality and 2-, 3-MCPDE and GE content. The objective of this study was to compare the quality and 2-, 3-MCPDE and GE content of refined oil derived from the industrial trials and the commercial samples. For the third survey, a total of 141 RBDPO samples were collected from 42 refineries in Malaysia and analysed for 3-MCPDE. The objective of this survey was to obtain current scenario and establish a baseline on capability of refineries in Malaysia in producing refined palm oil containing low 3-MCPDE and GE without any mitigation measures.

2.3 Heat test

Crude oil samples (2 mL) were heated in a laboratory in a covered vial to 260°C under atmospheric pressure, the vial being held in a sand bath. The oil was maintained at 260°C for 1 hr, then cooled and analysed. This test was to provide the 3-MCPDE and GE values under laboratory conditions simulating the deodorisation process. Heat test is the simplest and fastest method to examine the effect of total chloride in CPO on the formation of 3-MCPDE. In the actual refining process, chloride from other resources including sparging steam, bleaching earth and phosphoric acid could also contribute to the formation of 3-MCPDE. As such, heat test is a more accurate procedure to study on the effect of total chloride in CPO on the 3-MCPDE formations while eliminating chloride precursor from other resources.

2.4 Analytical methods for quality parameters

2.4.1 Free fatty acid (FFA)

Free fatty acids (FFA) were analysed according to AOCS Official Method Ca 5a-40. The oil sample (5-20 g) was dissolved in iso-propanol with phenolphthalein as indicator. The mixture was then titrated with potassium hydroxide until permanent faint pink appeared and persisted for at least 1 minute. The free fatty acid was calculated as a percentage of palmitic acid.

2.4.2 Deterioration of bleachability index (DOBI)

Deterioration of bleachability index (DOBI) is a test to predict the refiningability of palm oil as it is well correlated with the colour of refined oil. It was analysed according to Malaysian Palm Oil Board (MPOB) Test Method p2.9:2004. Crude palm oil was measured in a UV visible spectrophotometer, Lambda 35, Perkin Elmer (Connecticut, USA) using a 10 mm cell. The solvent was iso-octane (Merck, Darmstadt, Germany). The oil sample of 0.1 g was weighed into a 25 mL volumetric flask and the solvent was added. Measurement at 269 nm and 446 nm was taken. The DOBI was reported as the ratio of absorbance at 446 nm to 269 nm.

2.4.3 Diacylglycerol (DAG)

Analysis of diacylglycerol (DAG) was performed by Ultra High Performance Liquid Chromatography (U-HPLC) (ACQUITY UPLC H-Class System, Waters Corp., Milford, Massachusetts, USA) with accordance to AOCS Official Methods Ce 5b-89 and Ce 5c-93. Sample was dissolved in acetone to form a 5% w/v solution. The prepared sample was filtered through a 0.2 µm syringe filter (GH, Waters). About 1 µL of the solution was injected through an ACQUITY UPLC® BEH C18 column (particle size of 1.7 µm, id. 2.1 mm × 150 mm length) and maintained at 30°C. The elution solvent consisted of a mixture of acetone (TEDA Company, Inc., Fairfield, Ohio, USA) and acetonitrile (Merck, Darmstadt, Germany) in a volume to volume ratio of 63.5:36.5 and fixed at a flow rate of 0.25 mL/min. The sample was maintained at 40°C in the auto sampler prior to sample injection. DAGs were detected using a refractive index detector (Waters Corp.,) set at 35°C. DAGs were quantified using peak area normalisation method.
4.4 Colour

Colour was identified using a Lovibond Tintometer Model F (The Tintometer Ltd., Salisbury, England) apparatus, in reference to the AOCS Official Method Cc 13e-92. A glass cell of 5/4 in (133.4 mm) was filled with the sample and placed inside a lighting cabinet. The colour of the sample can be immediately determined using the colour ranks in the ratio of 10 yellow (Y) to 1 red (R) as the base line. The intensity of the glass was assigned through matching the colour ranks with the colour of the sample.

2.5 Analysis of 3-MCPDE and GE

Analysis of 2- and 3-MCPDE and GE was carried out in accordance to AOCS Official Method Cd 29a-13: 2- and 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils and fats by acid transesterification. The method determined the sum of derivatized 2-MCPD, 3-MCPD and 3-MBPD (converted from glycidyl ester) compounds using gas chromatography with mass spectrometry (GC-MS) technique.

Determination and quantification of the analyte were achieved by means of GC-MSD from Agilent Technologies (Santa Clara, CA, USA); equipped with a Series 5975C quadrupole detector and controlled by a programmable GC 7890A. Chromatographic separation was performed on a bonded poly(dimethylsiloxane) capillary column, HP-5MS inert (30 m length, 0.25 mm id, 0.25 µm film thickness). Helium (purity 99.999%) was used as a carrier gas at a constant flow of 0.8 mL min⁻¹. The column temperature was programmed at 80°C (1 min) to 170°C (1 min) at the rate of 10°C min⁻¹ to 200°C at 3°C min⁻¹ and was then accelerated to 300°C at the rate of 15°C min⁻¹ (15 min). 1 µL of the sample was injected in a pulsed split-less mode. The quantitative analysis was carried out by monitoring the characteristic ions at m/z 147, 196 and 198 for derivatized 2-MCPD; m/z 196 and 198 for derivatized 2-MCPD while for 3-MCPD-d₄, characteristic ions were at m/z 150, 201 and 203. For derivatized 3-MBPD and 3-MBPD-d₄, the ions were monitored at m/z 147 and 240; and m/z 150 and 245, respectively.

2.6 Analysis of total chlorine content

Analysis of total chlorine content was carried out using ASTM D 4929 – 04 method B for crude oil, with slight modification using Total Charge Analyser (TCA) model NSX 2100 (Mitsubishi, Japan). The principle of TCA analysis is based on combustion followed by micro-coulometric titration. Oil sample (50 mg) was either injected or placed into a combustion boat where the temperature was set at 1000°C. Argon and oxygen were passed through the boat and any chlorine containing components were completely combusted. The resulting combustion products were swept into titration cell. The electrolyte solution containing silver ion was immediately combined with any chloride ions. The amount of chloride was calculated from the quantity of electricity required for titration.

2.7 Trace metal and phosphorus analyses

Trace metal and phosphorus analyses were performed according to the AOAC Official Method 984.27. About 0.5 g oil sample was mixed with 20 mL of nitric acid and soaked thoroughly. Then the sample was added with 5 mL hydrogen peroxide and heated slowly until it evaporated completely. The resulting solution was subsequently analysed for trace metal and phosphorus using Inductive Coupled Plasma Optical Emission Spectrometry (Agilent 5110 ICP-OES) (Agilent Technologies, Santa Clara, CA, USA).

2.8 Statistical analysis

Data obtained from the analyses were subjected to one-way analysis of variance (One-way ANOVA) with Fisher’s Multiple Comparison to determine the significant differences among the samples defined at 95% confidence interval (p < 0.05). All analyses were conducted in triplicate and reported as mean ± standard deviation (SD) of independent trials. The statistical analyses were performed using the Minitab software Version 16 Statistical Package (Minitab Inc., State College, PA, USA).

3 Results and Discussion

3.1 CPO washing at palm oil mill

The effect of water washing on total chlorine content and other quality parameters is tabulated in Table 1. Removal of total chlorine content as high as 85% was achieved upon CPO washing. From the literatures, the removed chlorine would be mostly the inorganic chlorinated compounds although this has not been quantified and validated. The remaining chlorinated compounds (15%) were likely the organochlorines. Other quality parameters of the washed CPO, namely as FFA, DOBI and DAG were remain unchanged. As expected, 3-MCPDE was not detected in either untreated or washed CPO. It is also noted that water washing is not a good option to reduce the DAG in CPO, which is the main precursor for GE.

Iron and phosphorus were slightly reduced in the washed CPO as illustrated in Table 1. Based on the typical phosphorus content in CPO between 10 and 20 mg kg⁻¹, the result obtained (13.08 mg kg⁻¹) is lower than the mean value of 15 mg kg⁻¹. There are literatures reported the phosphorus content in CPO exceeding 30 mg kg⁻¹. In this case, a marginal but insignificant reduction was observed in the phosphorus level with water washing, presumably because most of the hydratable phosphorus had been removed in the earlier milling process, and only non-hydratable phosphorus remained in the oil. To ascertain
the result, another set of CPO samples was collected from a different palm oil mill that has a washing facility recently installed. The samples were subjected to phosphorus analysis. The result showed that phosphorus content in that particular sample dropped from 21.4 mg kg$^{-1}$ in the untreated sample to 14.2 mg kg$^{-1}$ in the washed CPO. The reduction of phosphorus in the washed CPO was likely due to the removal of hydratable phosphatides (HP). Zulkurnain et al.\textsuperscript{19} highlighted that water degumming effectively removed hydratable phosphatides from 9.78 to 1.55 mg kg$^{-1}$.

The efficiency of phosphorus removal using water washing therefore depends on amount and type of hydratable phosphorus in the oil. Simple heat treatment to the washed CPO showed lower tendency of the oil to form 3-MCPDE. However, effect of CPO washing on the GE formation was less evident.

A further heat test was conducted on a total of 41 CPO samples and the results showed a weak correlation ($R^2 = 0.3152$) between total chlorine content and the formation of 3-MCPDE (Fig. 2), even though a better correlation ($R^2 = 0.7303$) between total chlorine content in CPO and 3-MCPDE formation in the refined oil has been reported\textsuperscript{8}.

Heat test is a simple procedure to demonstrate the capability of the oil to form MCPDE under heat treatment. The oil is heated to and held at 260°C for 1 hr to simulate the deodorisation process. It is important to note that the heat test performed was not fully simulates the deodorisation conditions at refinery due to the absence of vacuum. In the presence of precursors, 3-MCPDE can only be formed when the oil is subjected to high deodorisation temperature (260°C).

| Quality Parameter | CPO | Washed CPO | Heated CPO | Heated Washed CPO |
|-------------------|-----|------------|------------|------------------|
| Free fatty acid (%) | 4.65 ± 0.02$^a$ | 4.61 ± 0.01$^b$ | 4.61 ± 0.01$^b$ | 4.61 ± 0.01$^b$ |
| DOBI (%) | 2.46 ± 0.00$^a$ | 2.45 ± 0.00$^b$ | 2.45 ± 0.00$^b$ | 2.45 ± 0.00$^b$ |
| DAG (%) | 5.69 ± 0.06$^a$ | 5.65 ± 0.04$^a$ | 5.65 ± 0.04$^a$ | 5.65 ± 0.04$^a$ |
| Phosphorus, P (mg kg$^{-1}$) | 13.03 ± 0.42$^a$ | 12.73 ± 0.55$^a$ | 12.73 ± 0.55$^a$ | 12.73 ± 0.55$^a$ |
| Iron, Fe (mg kg$^{-1}$) | 3.57 ± 0.14$^a$ | 2.80 ± 0.08$^b$ | 2.80 ± 0.08$^b$ | 2.80 ± 0.08$^b$ |
| Copper, Cu (mg kg$^{-1}$) | N.D. | N.D. | N.D. | N.D. |
| Total chlorine (mg kg$^{-1}$) | 8.11 ± 0.06$^a$ | 0.93 ± 0.06$^a$ | 0.93 ± 0.06$^a$ | 0.93 ± 0.06$^a$ |
| 3-MCPDE (mg kg$^{-1}$) | N.D. | N.D. | 5.23 ± 0.48$^a$ | 5.23 ± 0.48$^a$ |
| 2-MCPDE (mg kg$^{-1}$) | N.D. | N.D. | 1.98 ± 0.24$^a$ | 1.98 ± 0.24$^a$ |
| GE (mg kg$^{-1}$) | 0.28 ± 0.01$^b$ | 0.31 ± 0.01$^b$ | 0.33 ± 0.06$^b$ | 0.40 ± 0.04$^c$ |

Mean of triplicate analysis ± SD
Values within a row with different superscript letters are significantly different ($p < 0.05$)
DOBI, Deterioration of bleachability index
N.D., Not detected
Level of detection (LOD) for 3-MCPDE = 0.02 mg kg$^{-1}$
Level of detection (LOD) for GE = 0.05 mg kg$^{-1}$
Level of detection (LOD) for Cu = 0.03 mg kg$^{-1}$

![Figure 2](image_url)

**Fig. 2** Correlation between total chlorine content and formation of (A) 3-MCPDE and (B) 2-MCPDE in heated CPO samples ($n = 41$).
As such, the heat test is considered as the direct and fastest method to predict the formation of MCPDE without going through the actual refining condition. Although chlorine donors affect the levels of both 3- and 2-MCPDE, some samples showed lower formation with higher total chlorine content. This finding demonstrated that some chlorinated compounds present in CPO may not be reactive and do not contribute to the formation of MCPDE.

Chlorinated compounds present in palm oil have been comprehensively examined by Nagy et al.\(^7\). The mass-destillate filtering method applied to quantify the chlorine compounds yielded approximately 300 substances. The chlorine compounds can be categorised as organic and inorganic. The most abundant organochlorine in CPO is mainly phytosphingosines\(^8\), which is found to be the most active chlorine precursor in the formation of 3-MCPDE\(^9\). Sphingolipid organochlorine compounds increased as oil content per dry matter of oil palm mesocarp. On the other hand, the inorganic chlorines are found mostly in the form of iron (II), iron (III), magnesium and calcium chloride. These inorganic salts, which are higher in polarity, could be extracted using polar solvent. Laboratory experiments of removing inorganic chlorine using water and water-alcohol mixtures in crude oils have been able to reduce the formation of 3-MCPDE but not GE in refined oils\(^7,10\).

Water washing of CPO could also neutralise the acidity of the oil. It has been demonstrated that phosphoric acid used for degumming\(^7\) and acidity of CPO\(^20\) imparted higher formation of the 3-MCPDE in refined palm oil. Neutralisation of degummed palm oil with potassium hydroxide (KOH), sodium hydroxide (NaOH) and calcium oxide (CaO) has also proven to retard the 3-MCPDE formation\(^4,20\).

### Table 2  Normal conditions for CPO refining.

| Process       | Condition                        | Aid                              |
|---------------|----------------------------------|----------------------------------|
| Degumming     | 85°C, atmospheric pressure, 15 min | 0.06-0.08% phosphoric acid       |
| Bleaching     | 110°C, 50 mbar vacuum, 30 min    | 0.8-1.0% acid activated clay     |
| Deodourisation| 260°C, 5-3 mbar vacuum, 2 hrs    | –                                |

### Table 3  Properties of refined oils produced from control and washed CPO in comparison to commercial RBDPO samples (\(n=7\)).

| Source of RBDPO | Free fatty acid (%) | Colour (R) | DAG (%) | Total chlorine (mg kg\(^{-1}\)) | 3-MCPDE (mg kg\(^{-1}\)) | 2-MCPDE (mg kg\(^{-1}\)) | GE (mg kg\(^{-1}\)) |
|-----------------|---------------------|------------|---------|-------------------------------|--------------------------|--------------------------|---------------------|
| Control CPO     | 0.11 ± 0.01\(^b\)  | 2.4 ± 0.1\(^d\) | 5.57 ± 0.02\(^a\) | 2.11 ± 0.06\(^d\) | 2.15 ± 0.01\(^d\) | 0.89 ± 0.03\(^c\) | 4.13 ± 0.06\(^c\) |
| Washed CPO      | 0.06 ± 0.00\(^b\)  | 2.6 ± 0.1\(^cs\) | 6.32 ± 0.08\(^c\) | 0.60 ± 0.07\(^b\) | 1.37 ± 0.33\(^c\) | 0.49 ± 0.12\(^d\) | 3.84 ± 0.42\(^d\) |
| Commercial oil A| 0.17 ± 0.00\(^e\)  | 2.2 ± 0.1\(^a\) | 5.80 ± 0.04\(^e\) | 4.56 ± 0.06\(^a\) | 5.14 ± 0.10\(^a\) | 2.20 ± 0.06\(^b\) | 2.20 ± 0.06\(^b\) |
| Commercial oil B| 0.41 ± 0.00\(^e\)  | 3.0 ± 0.0\(^e\) | 6.71 ± 0.00\(^c\) | 2.49 ± 0.09\(^b\) | 3.24 ± 0.11\(^b\) | 1.31 ± 0.09\(^b\) | 2.26 ± 0.07\(^b\) |
| Commercial oil C| 0.09 ± 0.01\(^e\)  | 3.0 ± 0.0\(^c\) | 5.41 ± 0.02\(^d\) | 1.16 ± 0.01\(^e\) | 2.00 ± 0.15\(^d\) | 0.97 ± 0.18\(^b\) | 2.65 ± 0.06\(^b\) |
| Commercial oil D| 0.06 ± 0.00\(^e\)  | 3.0 ± 0.0\(^c\) | 5.83 ± 0.05\(^c\) | 2.66 ± 0.31\(^a\) | 2.59 ± 0.18\(^a\) | 1.20 ± 0.10\(^b\) | 0.76 ± 0.03\(^d\) |
| Commercial oil E| 0.20 ± 0.00\(^e\)  | 2.3 ± 0.0\(^e\) | 5.82 ± 0.06\(^c\) | 3.30 ± 0.13\(^b\) | 0.84 ± 0.02\(^c\) | 0.38 ± 0.00\(^c\) | 2.85 ± 0.02\(^b\) |

Mean of triplicate analysis ± SD
Values within a column with different superscript letters are significantly different (\(p<0.05\))

### 3.2 Refining of washed CPO at commercial refinery

Under normal processing condition as outlined in Table 2, refining of washed CPO resulted in less 3-MCPDE content in the refined oil. Other quality parameters for the refined palm oil conformed to the specifications outlined by Palm Oil Refiners’ Association of Malaysia (PORAM) (Table 3). The refined oil from control unwashed CPO contained slightly higher 3-MCPDE content of 2.15 mg kg\(^{-1}\). Analysis of commercial refined oil samples collected from various refineries in Malaysia showed that Refinery E managed to produce an even lower 3-MCPDE. Refining condition and pre-treatment to the CPO were however undisclosed. This refinery produces refined oil for infant formula and hence their refining condition and oil pre-treatment must have already been established.

GE formation was nevertheless unaffected by CPO washing. As shown in Table 3, DAG content was significantly increased in the washed CPO. During washing, the CPO slightly hydrolysed and hence resulted in higher DAG content. The increase is however insignificant in term of quality as palm oil contains around 5.0 to 7.0 \(\%\) DAG. GE level in refined oils from untreated and washed CPO were not significantly different. Refined palm oil collected from Refinery D exhibited low GE content of 0.76 mg kg\(^{-1}\). The oil has most probably been through post refining at mild processing condition.

A limit of 1.0 mg kg\(^{-1}\) of GE in oils and fats has already been imposed by the European Commission\(^29\) while two limits of 1.25 mg kg\(^{-1}\) for soft oils including palm kernel oil.
and 2.50 mg kg\(^{-1}\) for other oils including fish oil and mixture of these oils have been proposed for 3-MCPDE. If limits of 1.0 mg kg\(^{-1}\) for GE and 2.50 mg kg\(^{-1}\) for 3-MCPDE are taken into consideration, none of the refineries in Table 2 are able to meet the specifications. However, post refining by Refineries C and E could be able to reduce the GE level and comply with the regulation.

### 3.3 Post-refining for reduction of GE

3-MCPDE and GE content in refined oil upon post-refining is shown in Fig. 3. Reduction in 3-MCPDE level in post-bleached oil was not significant as compared to GE. A depletion of more than 86% GE was achieved when the refined oil is bleached with acid activated clay. The result is in accordance with laboratory condition findings reported in the literature, where acid activated clays are able to eliminate 99% of GE in different types of oils, including palm and rice bran oils\(^{31}\). The acidic condition of acid activated bleaching clay plays a key role in initiating an interesterification reaction of ring-opening to convert glycidyl palmitate into monopalmitate and glycerol dioleate\(^{31}\). Even though the GE content increased after post-deodorisation, its level was just slightly above 1.2 mg kg\(^{-1}\) (Fig. 3). Optimization of the deodorisation process would ensure GE content of below 1.0 mg kg\(^{-1}\) in the final product.

### 3.4 Dry fractionation

Partitioning of 3-MCPDE and GE upon dry fractionation of RBDPO derived from CPO washing is shown in Table 4. Batch crystallisation was carried out in two crystallisers with capacity of 47 tonne each. The oil was cooled by means of internal cooling coils. Fractionation was carried out at temperature of 24.5°C with crystallisation period of 5 hrs. At the end of crystallisation, slurry was filtered by a membrane filter press. Palm olein and stearin with iodine value (IV) of 57 and 34 were produced. Olein yield of 80% was achieved. In both crystallisers, the same phenomenon was observed where 3-MCPDE and GE were partitioned more in the palm oleins. A significant \((p<0.05)\) increment of 10 to 20% of 3-MCPDE in the oleins was observed. In the case of GE, 8 to 30% increase in the oleins was recorded. The results presented in Table 4 provide useful information for refineries in predicting the level of these contaminants in their fractionated products.

Polarity of both 3-MCPDE and GE attributed to the higher concentration of these contaminants in liquid fractions upon fractionation. Palm olein has been documented to contain greater amounts of 3-MCPDE and GE when compared to palm stearin\(^{6, 22, 33}\). In a dry fractionation, Gibon et al.\(^{32}\) coined that the partitioning mechanism of 3-MCPDE and GE involves combination of selective co-crystallisation of the most saturated esters with higher melting triacylglycerols (TAGs) and preferred solubility of the most unsaturated ones with lower melting TAGs. In wet (acetone) fractionation, the partitioning could be explained as results of unsaturated fatty acyls solubility in acetone\(^{31}\).

Espinosa\(^{32}\) highlighted an enrichment of 13% of 3-MCPDE in palm olein from the dry fractionation of palm oil. Additionally, re-fractionation of the palm olein resulted in 32% increment of 3-MCPDE in the higher IV super olein. It is proposed that 3-MCPDE level in palm oil must be below 1.30 mg kg\(^{-1}\) to ensure that multiple fractionated super olein contains less than 2.0 mg kg\(^{-1}\) 3-MCPDE.

### Table 4 Levels of 3-MCPDE and GE in feed (post-refined RBDPO) and the dry fractionated products from two different crystallisers.

| Oil samples | 3-MCPDE (mg kg\(^{-1}\)) | 2-MCPDE (mg kg\(^{-1}\)) | GE (mg kg\(^{-1}\)) |
|-------------|--------------------------|--------------------------|---------------------|
| Feed        | 1.26 ± 0.00\(^{a}\)      | 0.70 ± 0.02\(^{a}\)      | 1.59 ± 0.01\(^{a}\) |
| Crystalliser 1 Olein | 1.53 ± 0.02\(^{a}\)      | 0.95 ± 0.00\(^{a}\)      | 2.08 ± 0.06\(^{a}\) |
| Stearin     | 0.80 ± 0.01\(^{d}\)      | 0.42 ± 0.01\(^{d}\)      | 1.30 ± 0.00\(^{d}\) |
| Crystalliser 2 Olein | 1.39 ± 0.03\(^{b}\)      | 0.84 ± 0.04\(^{b}\)      | 1.72 ± 0.00\(^{b}\) |
| Stearin     | 0.70 ± 0.02\(^{e}\)      | 0.31 ± 0.03\(^{e}\)      | 0.95 ± 0.00\(^{e}\) |

Mean of triplicate analysis ± SD

Values within a column with different superscript letters are significantly different \((p < 0.05)\)
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3.5 Current status of refined palm oil produced in Malaysia

A significant progress has been made with regards to the mitigation of 3-MCPDE and GE in refined palm oil produced in Malaysia. CPO washing plant has been installed in some of the mills to reduce the total chlorine content, while modification at several refineries to limit the formation of 3-MCPDE and GE is in progress. While GE level in refined palm oil could be reduced by post refining at mild temperature, mitigation of 3-MCPDE should take place as earliest as possible of the supply chain to eliminate the precursor. Latest surveys carried out in 2018-2019 revealed that 62% of the RBDPO samples (n = 141) collected from 42 local refineries contained 3-MCPDE below 2.5 mg kg⁻¹ (Fig. 4). Majority (40%) of the samples analysed exhibited 3-MCPDE level between 1.00-1.99 mg kg⁻¹. Nevertheless, countless effort is required to further reduce the 3-MCPDE formation in 38% of the RBDPO samples to an acceptable limit. With the right mitigation measures, it is expected that Malaysian palm oil could meet the 3-MCPDE limit of 2.50 mg kg⁻¹ by January 2021 as proposed by the European Commission.

4 Conclusions

Production of refined palm oil with low 3-MCPDE and GE on a commercial scale is possible with the implementation of mitigation measures generated from laboratory scale experiments. Water washing of CPO in a commercial palm oil mill is proven to be an effective way to reduce the presence of chlorinated substances in the oil, and hence, minimises the formation of 3-MCPDE. The treated CPO could be refined without any modification at the refinery to produce refined oil with lower 3-MCPDE. Although organochlorine substances could not be removed through washing, removal of water-soluble inorganic chlorine enables reduction of the 3-MCPDE formation by half compared to conventional refined oil. The GE content in refined palm oil could be reduced by post-refining.

Acknowledgements

The authors acknowledged the Malaysian Palm Oil Board (MPOB) for providing the fund for this research work and permission to publish the research outcomes. Cooperation by a mill and a refinery in the southern region of Malaysia to conduct the commercial trials is greatly appreciated.

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