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Optimization of Palm Oil Physical Refining Process for Reduction of 3-Monochloropropane-1,2-diol (3-MCPD) Ester Formation

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Supporting Information

ABSTRACT: The reduction of 3-monochloropropane-1,2-diol (3-MCPD) ester formation in refined palm oil was achieved by incorporation of additional processing steps in the physical refining process to remove chloroester precursors prior to the deodorization step. The modified refining process was optimized for the least 3-MCPD ester formation and acceptable refined palm oil quality using response surface methodology (RSM) with five processing parameters: water dosage, phosphoric acid dosage, degumming temperature, activated clay dosage, and deodorization temperature. The removal of chloroester precursors was largely accomplished by increasing the water dosage, while the reduction of 3-MCPD esters was a compromise in oxidative stability and color of the refined palm oil because some factors such as acid dosage, degumming temperature, and deodorization temperature showed contradictory effects. The optimization resulted in 87.2% reduction of 3-MCPD esters from 2.9 mg/kg in the conventional refining process to 0.4 mg/kg, with color and oil stability index values of 2.4 R and 14.3 h, respectively.

KEYWORDS: *water degumming, magnesium silicate, response surface methodology (RSM), 3-MCPD ester reduction*

INTRODUCTION

3-Monochloropropane-1,2-diol (3-MCPD) ester contamination in refined oils has become a new safety issue worldwide (Figure 1). Formed at elevated levels in refined oils, these chloroesters are reported in various food products including infant formulas, follow-up formula, and baby foods.[1,2] Free 3-MCPD is carcinogenic in animal studies and induces infertility and malfunction of certain organs, but no toxicological data are available for 3-MCPD esters yet. A recent toxicological study showed that hydrolysis of 3-MCPD esters occurs in cells,[3] although 3-MCPD esters have been found in human breast milk fats[4] and raw goat milk fats,[5] which may suggest the possibility of direct absorption of the esters and their bioaccumulation in the mammalian body.

The formation of 3-MCPD esters is associated with high temperature during the deodorization step of oil refining. However, the mechanism of 3-MCPD ester formation in the refining process has not been clearly understood. Other steps of the refining process also contribute to the activation of 3-MCPD ester precursors. Recent research suggested that intrinsic components of the oil itself could be the precursors to 3-MCPD ester formation,[6,7] while high-temperature stripping steam liberates the source of protons and supplies sufficient energy for the reaction to take place.[8] Introduction of acid, whether from the acid degumming step or from the acidity of the bleaching clay, could lead to the formation of 3-MCPD ester precursors especially at high temperature.[9,10]

A mitigation process seems possible with removal of these precursors prior to the deodorization step. Washing crude palm oil with water or ethanol showed reduction of 3-MCPD ester formation.[7] Adsorbents such as calcinated zeolite, synthetic magnesium silicate,[11,12] and ion-exchange resin such as carboxymethyl cellulose[13] can impart sufficient removal of the precursors when in contact with palm oil during bleaching and reduce the level of 3-MCPD esters in the final refined oil. However, modification of the established physical refining process of palm oil for reduction of 3-MCPD esters may have an impact on the overall quality of the resulting refined palm oil.

Physical refining of palm oil requires crude palm oil to pass through three main processing steps, namely, degumming, bleaching in the pretreatment step, and also deodorization, for its purification into refined bleached and deodorized palm oil. The pretreatment step is most crucial in the physical refining process, which warrants sufficient removal of impurities and undesired components, which otherwise adversely affect the quality of the refined end product when subjected to deodorization.[14] Deodorization of palm oil has to be conducted at sufficiently high temperature to strip off free fatty acids and

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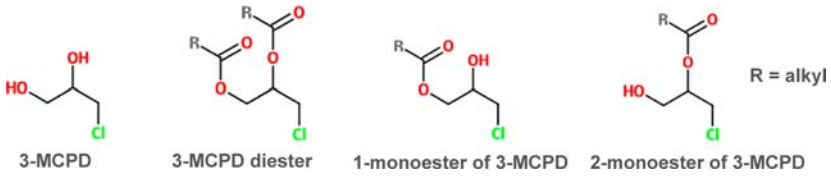

Figure 1. Structure of 3-monochloropropane-1,2-diol (3-MCPD) and 3-MCPD mono- and diesters.

Table 1. Seven Different Combinations of Bleaching Methods

bleaching method	adsorbent combination	3-MCPD ester (mg/kg)[b]	color (red)[b]	FFA (%)[b]
wet bleaching	1% R60 (20% slurry)	0.39 ± 0.06 A	4.2 ± 0.1 A	0.56 ± 0.02 A
	1% R60 (20% slurry)[a] + 0.5% Wac Supreme	0.29 ± 0.02 B	2.3 ± 0.0 D	0.12 ± 0.02 B
	0.5% Wac Supreme[a] + 1% R60 (20% slurry)	0.23 ± 0.03 C	2.5 ± 0.0 CD	0.10 ± 0.01 B
dry bleaching	1% R60	0.39 ± 0.02 A	2.6 ± 0.1 C	0.05 ± 0.01 C
	1% R60[a] + 0.5% Wac Supreme	0.42 ± 0.01 A	3.2 ± 0.0 B	0.04 ± 0.01 C
	0.5% Wac Supreme[a] + 1% R60	0.36 ± 0.02 A	2.7 ± 0.1 C	0.04 ± 0.01 C
	1% R60 + 0.5% Wac Supreme (mixture)	0.40 ± 0.01 A	2.3 ± 0.1 D	0.06 ± 0.01 C

[a]Bleaching was conducted with the first adsorbent then followed by the other. [b]Means in the same column followed by similar upper case letters do not differ significantly at $p \leq 0.05$ for $(n = 2)$ number of samples.

the majority of the volatile substances responsible for off-flavors. The final refined palm oil quality is largely influenced by the deodorization step, whereby a light color, bland tasting, and good oxidative stability oil is required.

In this study, the palm oil physical refining process, particularly the pretreatment steps, was modified and therefore optimized for the reduction of 3-MCPD ester formation yet maintaining an acceptable quality of the refined palm oil. In this investigation, a mathematical model based on a small central composite rotatable design (CCRD) was proposed for modeling and analyzing the effects of the processing conditions on levels of 3-MCPD esters formed and important refined palm oil quality characteristics. Response surface methodology (RSM) is an important tool for developing, improving, and optimizing the process design, in which a response of interest is influenced by several variables.[15] It has become a popular tool for process optimization in lipid studies including the refining process of edible oils.[16,17] The mathematical model generated is used to describe the effects of process parameters and predict the optimum process conditions to achieve desirable responses.

MATERIALS AND METHODS

Materials. Crude palm oil was of Standard Quality I supplied by Sime Darby Golden Jomalina Sdn. Bhd. (Teluk Panglima Garang, Malaysia). The quality parameters of crude palm oil were deterioration of bleachability index (DOBI), 2.9 ± 0.08; free fatty acid (FFA), 3.5 ± 0.03%; peroxide value (PV), nil; β-carotene content, 597 ± 1.4 ppm; phosphorus content, 4.8 ± 0.3 ppm. 3-Monochloropropane-1,2-diol was purchased from Aldrich (Lyon, France), and isotope-labeled 3-MCPD-d_5 internal standard was purchased from Cambridge Isotope Laboratories (Andover, MA). Phenylboronic acid of 99.0% purity was purchased from Fluka (Shanghai, China). All other chemicals and solvents were of analytical grade. Synthetic magnesium silicate, Magnesol R60 (Dallas Group of America, Inc., Whitehouse, NJ), was provided by Global Specialty Ingredients (M) Sdn. Bhd. (Port Klang, Malaysia). Wac Supreme 1B activated clay (Taiko Group Sdn. Bhd., Pasir Gudang, Malaysia) and phosphoric acid, 85% (Merck, Darmstadt, Germany) were supplied by Sime Darby Golden Jomalina Sdn. Bhd. (Teluk Panglima Garang, Malaysia).

Process Conditions. Approximately 700 g of crude palm oil was refined in a five-step process that included water degumming, acid degumming, washing of the acid-degummed palm oil with 1% water, bleaching with activated clay and 1% Magnesol R60, filtering, and deodorizing to obtain the refined palm oil. The modified palm oil refining was conducted in a 2 L laboratory-scale refining flask with a stirrer and heating mantle that was connected to a vacuum pump (Shidae Electric Co., Ltd., Buchon City, Korea), and the oil was deodorized in a custom-made glass deodorizer consisting of a 1 L flask equipped with a built-in sparging steam and a thermocouple. The deodorizer was connected through three-stage water-jacketed traps and two empty flask traps before reaching a vacuum pump. Stripping steam was produced from distilled water and evaporated in a glass steam generator that was connected directly to the deodorizer steam inlet.

Bleaching Method. Both wet bleaching and dry bleaching methods were tested, with different combinations of bleaching adsorbents in two replicates, as shown in Table 1, to obtain the best bleaching method for the greatest removal of 3-MCPD esters and color. Wet bleaching was performed utilizing a 20% (w/w) slurry of Magnesol R60 synthetic magnesium silicate (of oil weight) alone and in combination with 0.5% Taiko Supreme 1B activated clay added before or after the Magnesol R60 slurry at 90 °C for 30 min. Dry bleaching was conducted at 90 °C for 30 min with four different bleaching adsorbent combinations, as shown in Table 1, namely, 1% Magnesol R60 alone, 1% Magnesol R60 followed by 0.5% Taiko Supreme 1B, 0.5% Taiko Supreme 1B followed by 1% Magnesol R60, and a mixture of 1% Magnesol R60 and 0.5% Taiko Supreme 1B. The refined palm oils obtained were analyzed to determine the 3-MCPD ester levels, free fatty acid contents, peroxide values, and color.

Experimental Design. A five-factor face-centered ($\alpha = 1$) small CCRD was employed to optimize the modified refining process for the production of refined palm oil with reduced 3-MCPD ester formation and acceptable oil quality, generating 26 experimental settings. The five factors and their levels were water degumming dosage (X_1, 0–5%), phosphoric acid dosage (X_2, 0–0.1%), degumming temperature (X_3, 40–80 °C), activated clay dosage (X_4, 0–1%), and deodorization temperature (X_5, 220–280 °C). The response variables were 3-MCPD ester levels and refined palm oil quality parameters, including the color and the oil stability index (OSI) from the Rancimat test. Other oil quality parameters, such as the FFA content and the PV, were also assessed for quality assurance.

Model Verification. A validation experiment was conducted to confirm the validity and reproducibility of the model. The selected optimum solution was conducted using the same laboratory-scale physical refining process in two replicates. The 3-MCPD content and the oil quality parameters of refined palm oil were compared with the predicted values using a one-sample t test.

3-MCPD Ester Determination Using GC-MS/MS. The 3-MCPD ester contents of refined palm oil were determined using the modified BfR 8 method,[18] based on an indirect method using acid trans-

esterification, which involves the release of 3-MCPD from its esters, purification by ammonium sulfate extraction, phenylboronic acid derivatization, and quantitation using gas chromatography with tandem mass spectrometry detection (GC-MS/MS). A quantitative analysis of the derivatized 3-MCPD compound on a Thermo TSQ Quantum XLS triple quadruple GC-MS/MS system (Thermo Fisher Scientific Inc., Waltham, MA) was carried out by monitoring characteristic ions (quantifier) at m/z 147 of 3-MCPD and m/z 150 of isotopically labeled internal standard (3-MCPD-d_5). Ions at m/z 91 and 196 (3-MCPD) and m/z 93 and 201 (3-MCPD-d_5) were used as qualifiers. The column used was a 30 m × 0.25 mm i.d., 0.25 μm, HP-1MS capillary column (Agilent, Waldbronn, Germany). One-microliter volumes of the derivatized sample solutions were injected, and the programmable temperature vaporizer injector was programmed as follows: initial temperature of 60 °C for 0.05 min, ramped at 10 °C/s up to 250 °C, held for 1 min for evaporation and 1 min for analyte transfer onto the column. Finally, the temperature was ramped at 14.5 °C/s up to 300 °C and held for 1.5 min for postinjector cleaning with helium gas at a flow rate of 10 mL/min. The oven temperature program for the GC was set at an initial temperature of 50 °C (1 min hold), ramped at 10 °C/min up to 210 °C and then at 30 °C/min up to 300 °C, followed by holding for 20 min. Operating conditions for the mass spectrometer were as follows: ionization voltage of 70 eV, emission current of 50 μA, electron lens of 15, Q1 (first quadrupole) resolution of 0.4 Da, collision gas (argon) pressure of 1.5 mTorr, transfer line temperature of 250 °C, and MS source temperature of 230 °C.

Oil Quality Analysis. Free fatty acid, peroxide value, and color of crude palm oil and refined palm oil of each runs were determined according to AOCS official method (2009) Ca5a-40, Cd 8b-90, and *Cc* 13e-92, respectively.[19] Deterioration of bleachability index for crude palm oil was conducted according to MPOB test methods (2005).[20]

Oil Stability Index. The OSI of refined palm oil was determined according to the AOCS official method (2009) Cd 12b-92[19] with a Rancimat 743 apparatus (Metrohm Ltd., Herisau, Switzerland) by measuring the induction periods of the oils at 110 °C based on a conductometric method. The oxidation process was monitored in 3 g oil samples at an air velocity of 20 L/h.

Statistical Analysis. The data from the RSM were statistically analyzed using Design Expert version 6.0.10 (Stat-Ease, Inc., Minneapolis, MN). Each model was analyzed using ANOVA, regression analysis with hierarchical backward elimination at a 95% significance level ($p < 0.05$), and response surface plotting to establish an optimum condition for the reduction of 3-MCPD ester formation in refined palm oil. The insignificant ($p > 0.05$) factors and the interactions between them were removed. The data from each response were fitted with the factors by multiple regression to a second-order model polynomial equation, as follows:

$$Y_i = \beta_0 + \sum_{5}^{i=1} \beta_i X_i + \sum_{5}^{i=1} \beta_i X_i^2 + \sum_{4}^{i=1} \sum_{5}^{j=i+1} \beta_i X_i X_j$$

where Y_i is the response; β_0, β_i, β_{ii}, and β_{ij} are the constant coefficients of the intercept, linear, quadratic, and interaction terms, respectively; and X_i and X_j are independent variables. Subsequently, using numerical optimization, the responses of interest from the developed models were overlaid and optimized for the lowest 3-MCPD content and acceptable oil quality.

All of the experimental results were reported as the mean ± standard deviation of triplicate analyses. One-way ANOVA was applied to analyze statistical differences between group means established at $p < 0.05$ using the MINITAB version 14 statistical software (Minitab, Inc., State College, PA). Multiple comparisons of means were performed using Tukey's test.

RESULTS AND DISCUSSION

Modified Refining Process. The established physical refining process of palm oil was modified with addition of pretreatment steps to improve the removal of 3-MCPD ester precursors, prior to the deodorization step in which the 3-MCPD ester formation occurs. Water degumming was incorporated together with acid degumming to impart precursor elimination besides sufficient removal of gums and impurities. Most of the gums and impurities are removed during water degumming, but acid degumming resulted in further precipitation of residual gums, expected to be the nonhydratable phospholipid, which can be removed only by a strong acid wash.[21] This shows that acid degumming is crucial for palm oil physical refining because water degumming alone is not sufficient to get rid of the nonhydratable phospholipid, the presence of which could impart undesirable flavors and colors and shortens the shelf life of refined palm oil.[22] Subsequently, the acid-degummed oil was washed with 1% water because the acid-degumming step could lead to activation of 3-MCPD ester precursors.

The bleaching treatment was employed with synthetic magnesium silicate as the auxiliary adsorbent, which has the ability to remove chloroester precursors from the oil,[11,12] in addition to the activated bleaching clay. The magnesium silicate alone is not sufficient to remove carotene and resulted in darker color oil, as reported in a previous study.[6] In this study, the magnesium silicate was adopted for bleaching at 1% and its synergistic effect with activated clay was studied in the bleaching methods study.

Bleaching Methods. A preliminary study was conducted on wet and dry bleaching of the synthetic magnesium silicate in combination with activated clay for the greatest removal of 3-MCPD ester precursors, oil impurities, and color, as the incorporation of synthetic magnesium silicate is novel to the palm oil refining process. The synthetic magnesium silicate was fixed at a 1% dosage because it is costly. Wet bleaching resulted in high FFA content due to triglyceride degradation in the presence of water, although the combination of magnesium silicate with activated clay imparted significantly higher ($p < 0.05$) removal of 3-MCPD esters and comparable color removal compared with dry beaching combinations, as shown in Table 1. This effect manifested as poor finished oil quality, as the FFA contents were higher than the acceptable standard specified for refined palm oil by Palm Oil Refiners Association of Malaysia (PORAM), which is less than 0.1%.[23]

There were no significant differences in the levels of 3-MCPD esters and contents of FFA among the four different dry-bleaching methods, but the color removal obtained from the mixture of magnesium silicate and activated bleaching clay was significantly ($p < 0.05$) enhanced compared with bleaching using magnesium silicate alone or using both adsorbents in sequential bleaching steps. This result might be due to the synergistic effects of both adsorbents when used in combination. Therefore, dry bleaching using a mixture of magnesium silicate and acid-activated bleaching clay was the preferred method and was used in the subsequent optimization of the refining process.

Optimization of the 3-MCPD Ester Reduction. *Model Evaluation.* Fitting experimental values of 3-MCPD esters, color, and OSI value to various models showed that the responses were most suitably described with a reduced quadratic model, except for the OSI, which showed a reduced cubic model, with insignificant factors and interactions removed from the model by backward elimination. The adequacy and fitness of these final reduced models were sufficiently explained by the coefficient of determination, R^2, and lack-of-fit test. The overall predictive capabilities of the models are expressed by the

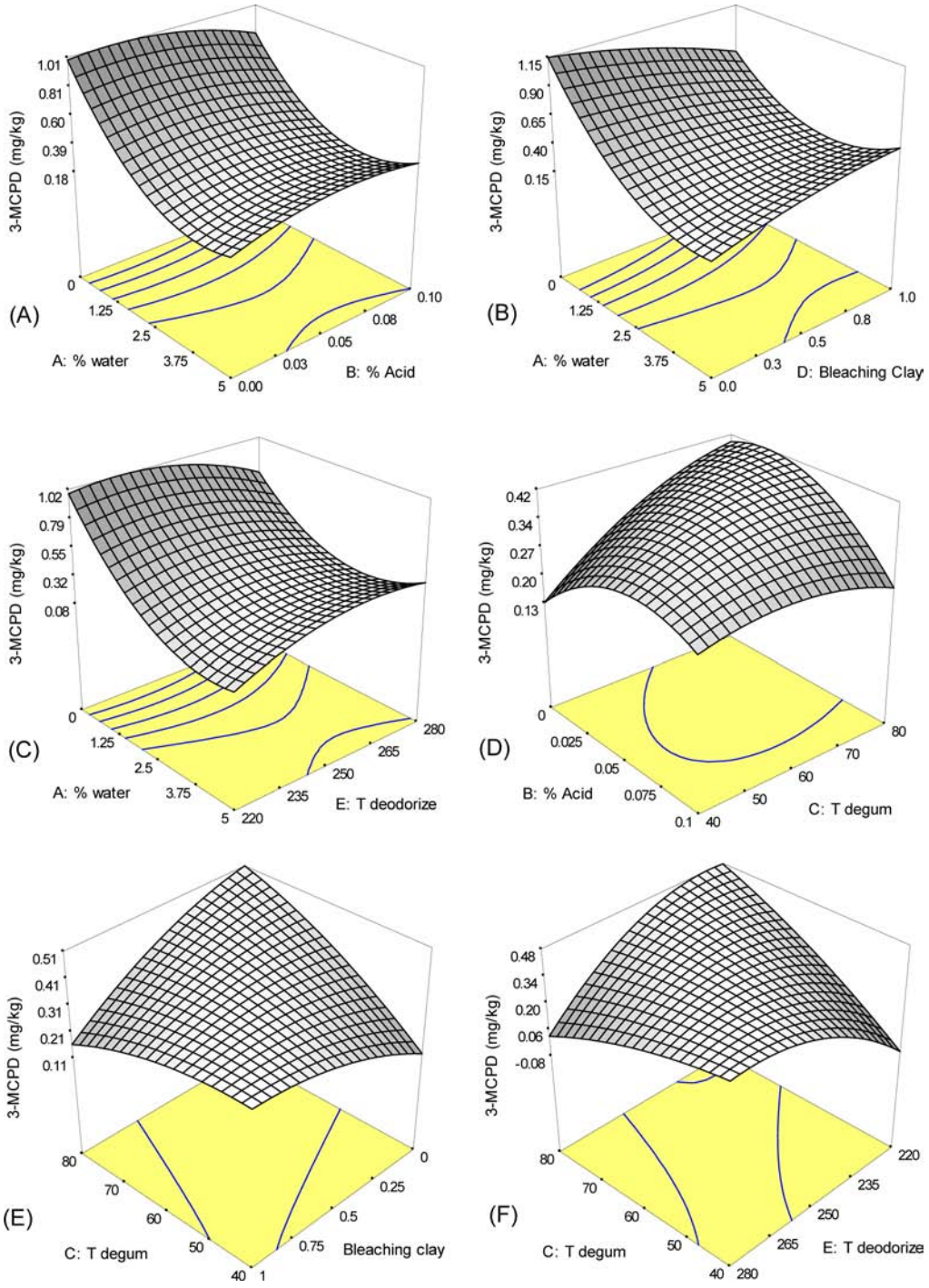

Figure 2. Responses surface plots of 3-MCPD levels showing significant interactions between (A) water and acid dosage; (B) water and clay dosage; (C) water dosage and deodorization temperature; (D) acid dosage and degumming temperature; (E) degumming temperature and clay dosage; and (F) degumming and deodorization temperature, with other factors held at center points.

coefficients of determination, R^2, which were close to 1, indicating that more than 90% of the variations in the models were adequately explained by the regression models and no significant ($p > 0.005$) lack of fit for all of the responses, indicating that the models accurately represent the data in the experimental regions.

Effects of Processing Parameters on the Formation of 3-MCPD Esters. The modified refining process resulted in 3-MCPD ester levels ranging from 0.2 to 1.0 mg/kg, representing a 89.7−42.4% reduction from the conventional process, which has 3-MCPD ester levels of 1.7 mg/kg. The greatest 3-MCPD ester reduction was significantly ($p < 0.05$) contributed by the increment of the water dosage (X_1), while increasing the activated clay dosage (X_4) and decreasing the degumming temperature (X_3) also showed a significant reduction but to a much lesser extent. The reduction of 3-MCPD esters could be primarily based on the elimination of precursors, when employing water degumming and greater amounts of activated clay during the pretreatment steps. Reduction of temperature during acid degumming may cause less activation of the

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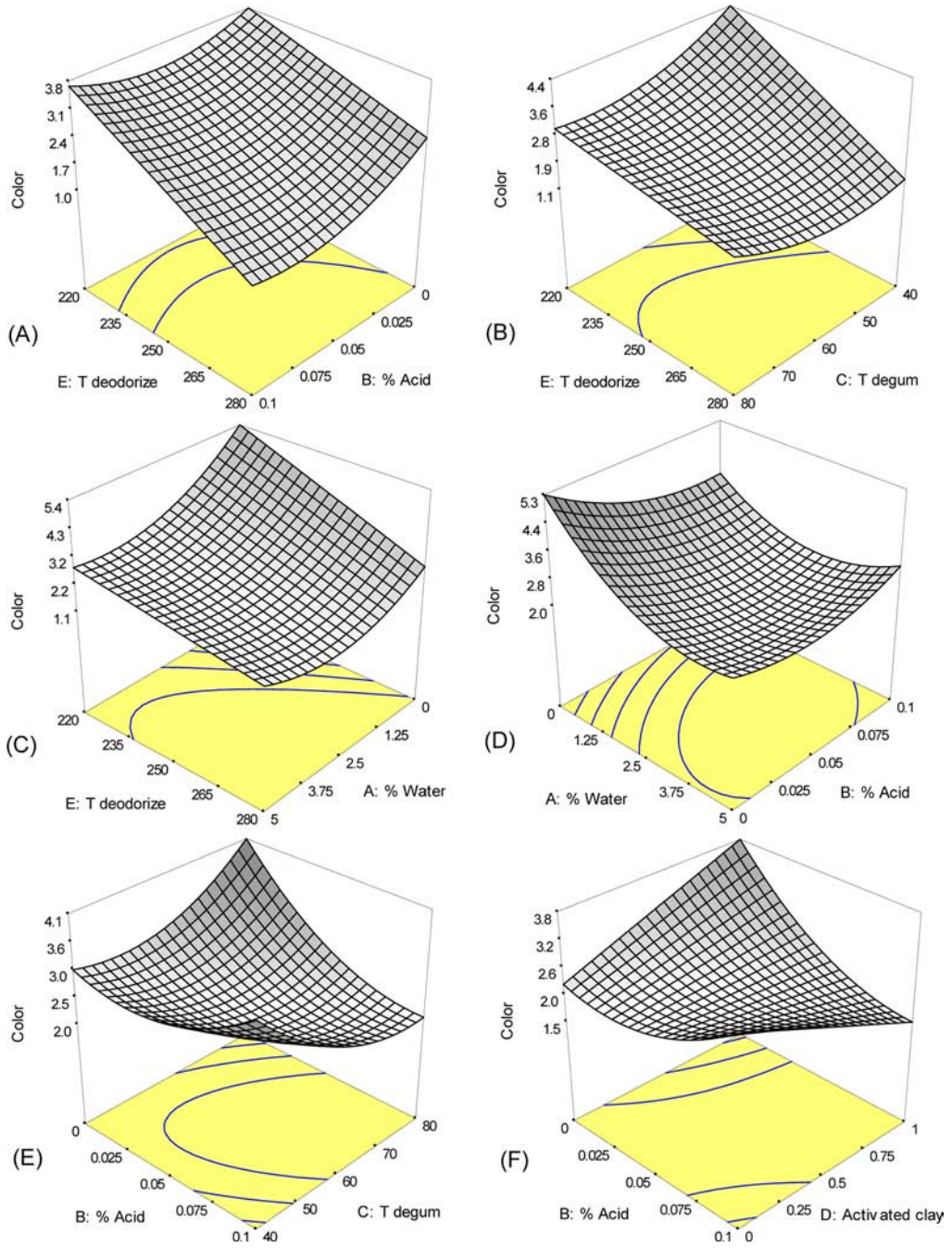

Figure 3. Response surface plots of refined palm oil color showing significant interactions between (A) deodorization temperature and acid dosage; (B) deodorization and degumming temperature; (C) deodorization temperature and water dosage; (D) water and acid dosage; (E) acid dosage and degumming temperature; and (F) acid and clay dosage, with other factors held at center points.

precursors during this step. In contrast, the acid dosage (X_2) and the deodorization temperature (X_5) demonstrated significant effects ($p < 0.05$) only in the quadratic pattern and in the interaction effects.

The water-degumming step imparted a reduction of up to 80% from 1.0 mg/kg to 0.2 mg/kg with increasing the water dosage from 0% to 5%. Other factors caused only a small variation of 3-MCPD ester level as increasing factor levels. At a water dosage of 5%, the 3-MCPD esters ranged from 0.2 to 0.4 mg/kg, while without water degumming the 3-MCPD ester level increased to the range of 0.7 to 1.5 mg/kg. The modified refining process affected 3-MCPD ester formation in a particular way that is contrary to expectations. The mechanisms that removed 3-MCPD ester precursors were the factors that exerted a great influence on 3-MCPD ester reduction. In contrast, the effects of factors that were expected to influence 3-MCPD ester formation, such as the deodorization temperature and the acid dosage, were not significant. Perhaps, when the precursors have been washed out in the first step of water degumming, the other steps are guaranteed a low chance of 3-MCPD ester formation.

The interaction effects of different factors were examined using the generated response surface plots of 3-MCPD ester level (Figure 2), based on the following second-order equation:

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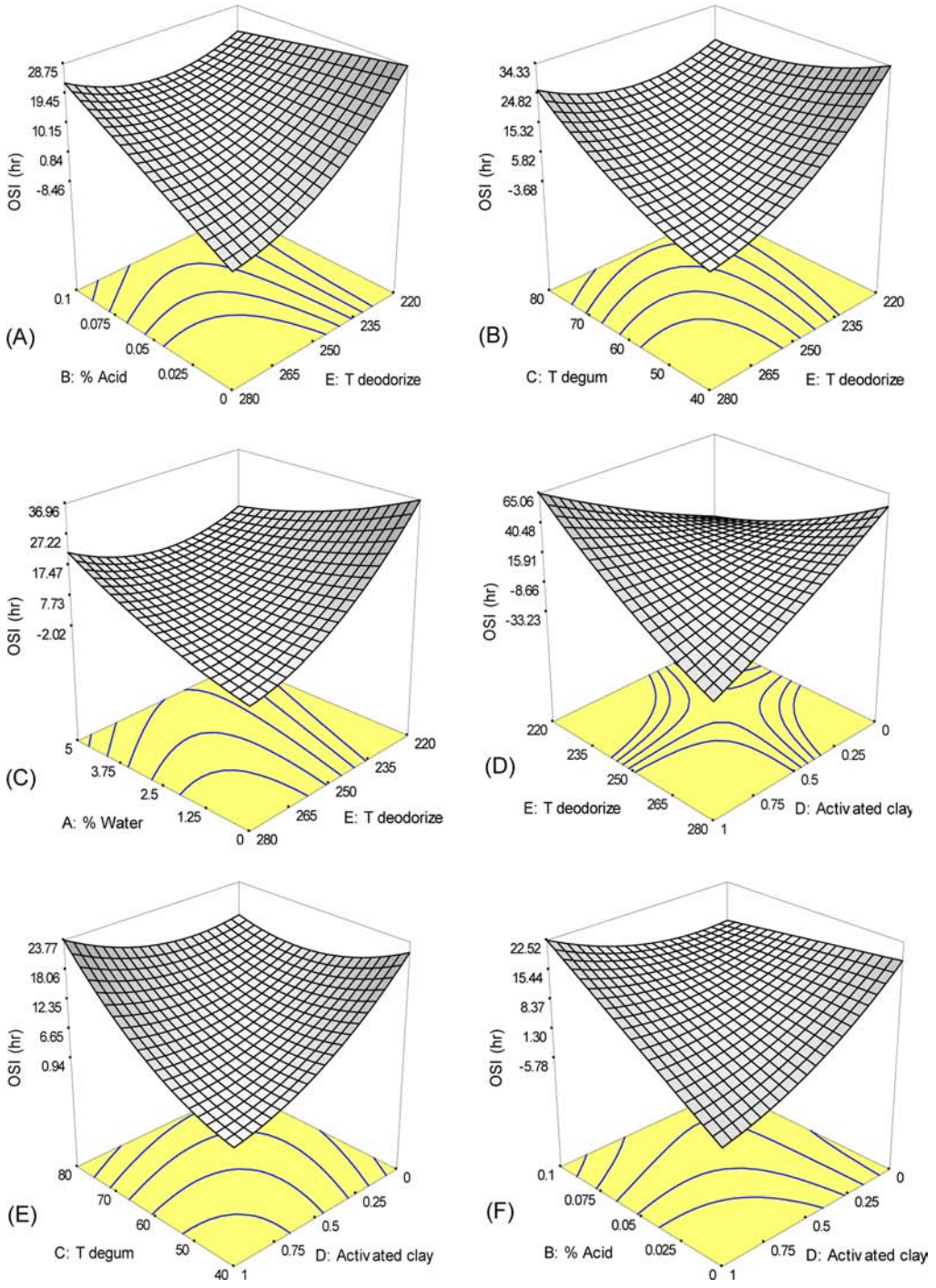

Figure 4. Response surface plots of oil stability index (OSI) of refined palm oil showing significant interactions between (A) acid dosage and deodorization temperature; (B) degumming and deodorization temperature; (C) water dosage and deodorization temperature; (D) deodorization temperature and clay dosage; (E) degumming temperature and clay dosage; and (F) acid and clay dosage, with other factors held at center points.

$$Y_1 = 0.38 - 0.31X_1 - 0.02X_2 + 0.06X_3 - 0.03X_4 - 0.007X_5 + 0.3X_1^2 - 0.090X_2^2 - 0.05X_3^2 - 0.053X_4^2 - 0.14X_5^2 + 0.08X_1X_2 + 0.18X_1X_4 + 0.13X_1X_5 - 0.08X_2X_3 - 0.05X_2X_4 - 0.13X_2X_5 - 0.14X_3X_4 - 0.22X_3X_5 - 0.09X_4X_5$$

Because water dosage showed the most significant effects, all of the interactions involving this factor (Figure 2A–C) showed considerable reduction in 3-MCPD esters with increasing water dosages, regardless of their factor levels. A greater amount of water allowed for more precursors to be washed out in the first step and reduced the chances of 3-MCPD ester formation in the later steps. The influence of the degumming temperature was also clearly seen in all of the interaction effects involving this factor, as shown in Figure 2D–F. When the degumming temperature was reduced from 80 °C to 40 °C, the 3-MCPD ester reduction was approximately 70%. This effect was rendered most significant by decreasing the acid dosage,

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increasing the activated clay dosage, and decreasing the deodorization temperature. Increasing activated clay dosages and reduction in the deodorization temperature demonstrated reduction in 3-MCPD ester levels, as shown in Figure 2B, E and Figure 2C, F, respectively.

Effects of the Processing Parameters on Refined Palm Oil Quality. *Refined Palm Oil Color.* Color is one of the important quality criteria in grading the final refined palm oil with the standard specification requirement to be below 3 Lovibond Red (R) using a 5.25″ Lovibond cell.[23] The experiments resulted in refined palm oil colors in the range of 1.2 to 6.6 R, in which color removal was significantly ($p < 0.05$) influenced by increasing deodorization temperature (X_5), the water dosage (X_1), and both the acid dosage (X_2) and the temperature (X_3) of the acid-degumming step. The activated clay dosage (X_4) significantly ($p < 0.05$) influenced several of the interaction effects.

The deodorization temperature exhibited the greatest effect in color reduction because heat bleaching removes most of the carotene, which leads to a light-colored oil (Figure 3A–C). High acid dosages assist in degradation of carotene compounds during the pretreatment steps, which leads to further color removal below 2.0 R with increasing degumming temperature (Figure 3E) and activated clay dosage (Figure 3F).[24] However, without acid degumming, a high degumming temperature and activated clay dosage are not able to remove color and imparted an unusual dark color. Color fixation occurred due to the presence of nonhydratable phospholipid during deodorization as a result of insufficient removal of gums.[22] This result shows that acid degumming is crucial for sufficient color removal (to less than 2.5 R) because the acid has the ability to sequester trace metals, in addition to the efficient removal of sludge or gums.[21] Water degumming also significantly ($p < 0.05$) assists in the color removal when acid degumming was not performed (Figure 3D). As the water dosage increased to 5%, a substantial reduction of color (close to 2.0 R) was observed.

Oil Stability Index. The oil stability index determines the relative resistance of the oil sample to isothermal oxidation at elevated temperatures that accelerates the development of oxidative rancidity and predicts the oil oxidative stability. The OSI, or the oxidation induction period (measured in hours), is a predictive value for the shelf life of an oil. For refined palm oil, the OSI at 110 °C usually falls in the range of 20 to 25 h.[25] The OSI value of the experiments was in the range of 0.8 to 25.9 h, and the oil oxidative stability was affected significantly ($p < 0.05$) by increasing deodorization temperature (X_5) and activated clay dosage (X_4) and decreasing both dosage (X_2) and temperature (X_3) of acid degumming. Interestingly, the oil oxidative stability was not significantly ($p < 0.05$) affected by the water degumming.

Increasing the deodorization temperature reduced the OSI value drastically (Figures 4A–D) due to the higher loss of tocopherols and carotenes at high temperature, as tocopherols are volatile and both types of molecules are heat-labile. Tocopherol and carotene are the natural antioxidants in palm oil that protect it from oxidation. Most crude oils have OSI values that are double those of their refined oil counterparts because of higher total tocopherol content.[26−28] High-temperature stripping may remove 30% to 50% of the tocopherols, which gives rise to a marked decrease in their protective power against future autoxidation of the refined oil.[29] For this reason, citric acid may be added as a preservative at 100 mg/kg (as a 30% solution) during the cooling-down period after deodorization, and synthetic tocopherol (vitamin E) may also be added to commercial refined palm oil to compensate for the loss of the natural antioxidants.

An increase in both dosage and temperature of acid degumming increased the OSI value especially at high bleaching clay dosage (Figure 4E, F). Increasing the acid dosage improved the oil oxidative stability with the removal of impurities such as bound metal ions that associated with the nonhydratable phospholipids by insufficient acid treatments. Although present in small amounts, the inorganic phosphatides can cause oxidative instability in palm oil due to their strong emulsifying properties, which facilitate the dispersion of microparticulate impurities including iron and other undesirable materials.[30] Increases in the acid-degumming temperature also led to improvements in the oil oxidative stability because efficient acid degumming can be accomplished at high degumming temperatures.

Other Refined Palm Oil Quality Characteristics. Other important quality characteristics of refined palm oils such as FFA and PV were also tested. The FFA content ranging from 0.01% to 0.02% falls in the acceptable level of <0.1%, which shows sufficient removal of FFA was achieved with the deodorization process. The PV is an indication of the amount of hydroperoxides present in the oil that arises from lipid oxidation. The absence of PV from all of the refining treatments indicates that the modified process removed oxidized compounds from the crude oil sufficiently. Both the FFA and PV do not fit any significant model because they were not significantly affected by the modified process.

Numerical Optimization and Model Verification. The modified refining process was optimized for the highest reduction of 3-MCPD esters, lowest color formation, and highest OSI. Numerical optimization using the software generated a number of optimized processing conditions. The selected optimization conditions were a 3.5% water dosage, a 0.08% acid dosage, a 60 °C degumming temperature, a 0.3% bleaching clay dosage, and a 260 °C deodorization temperature, giving an 87.2% reduction in 3-MCPD esters, a color at 2.4 R, and an OSI value of 14.3 h when conducting the refining (Table 2). Graphical optimization as shown in Figure 5 demonstrates an overlay plot highlighting the optimum area with the responses at desired directions (3-MCPD ester reduction >85%, color <2.5 R, and OSI > 14 h) with respect to water and acid dosages at constant degumming temperature, clay dosage, and deodorization temperature. The model validation conducted in duplicate showed that there were no significant differences ($p > 0.05$) in observed and predicted values, as shown in Table 2, implying that the experimental values agreed with the predicted values at a 0.05 level of

Table 2. Observed and Predicted Values of Three Responses from Optimized Refining Conditions

	3-MCPD ester (mg/kg)	percent reduction (%)[c]	color (red)	OSI[d] (h)
observed[a]	0.37 ± 0.01	87.2 ± 0.2	2.4 ± 0.1	14.3 ± 0.3
predicted		87.5	2.2	17.34
p-value[b]		0.051	0.13	0.051

[a]All observed values are results of two replicates. [b]*p*-Value at 95% significant level. [c]Percent reduction based on 3-MCPD ester level in refined palm oil from conventional refining at 2.95 mg/kg. [d]OSI = oil stability index.

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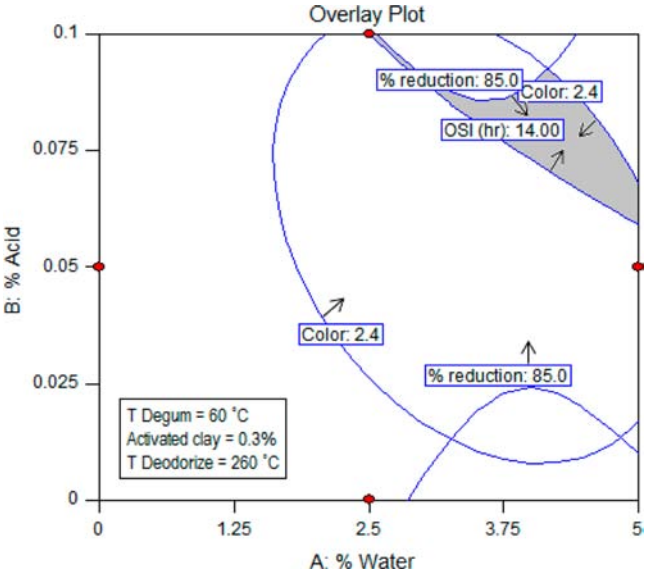

Figure 5. Graphical optimization of the modified refining process for the desired values of 3-MCPD ester reduction >85%, color <2.5, and OSI > 14 h (shaded gray).

significance. Thus, the models were sufficient to predict the 3-MCPD ester reduction, color, and OSI value of the refined palm oil.

In conclusion, the modifications of the palm oil refining process particularly at the pretreatment step and subsequent optimization successfully reduced 3-MCPD ester formation without compromising the final refined palm oil quality and stability. Water degumming helped both in 3-MCPD ester and color removal greatly and together with acid degumming enhanced oil oxidative stability. Bleaching with addition of synthetic magnesium silicate is advantageous in 3-MCPD ester reduction. The mathematical model generated using RSM can be used as a tool to identify optimum palm oil refining process conditions within the chosen constraints of minimum 3-MCPD esters, minimum color, and maximum OSI.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

3-MCPD, 3-monochloropropane-1,2-diol; OSI, oil stability index; FFA, free fatty acid; DOBI, deterioration of bleachability index; RSM, response surface methodology; CCRD, central composite rotatable design

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