Determination of Iodine Value of Palm Oil Based on Triglyceride Composition

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ABSTRACT: The triglyceride (TG) composition of palm oil is normally determined by high-performance liquid chromatography (HPLC). The HPLC chromatograms indicated a good separation of most of the TG components in the oil. The TG can be classified based on either the TG groups, i.e., triunsaturated, monosaturated, disaturated, or trisaturated, or the number of double bonds, i.e., zero, one, two, three, or four double bonds. The more unsaturated the fatty acid, the greater the iodine value (IV). Therefore, it is hypothesized that the IV of an oil can be determined based upon the TG composition of the oil. Based on the TG groups, stepwise regression analysis showed that the areas of the disaturated, trisaturated, and triunsaturated TG peaks could predict the IV with a coefficient of determination (R^2) of 0.990. The regression based on the number of double bonds yielded a good regression equation with $R^2 = 0.992$. The important variables were the peak area of the fatty acids that contained zero, one, two, and three double bonds. This study concludes that the TG composition can be used to predict the IV of palm oil. The best prediction model is obtained by using the number of double bonds in the TG as the independent variable.

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KEY WORDS: HPLC, instrumental analysis, iodine value, palm oil, triglyceride.

Iodine value (IV) is a measure of the unsaturation of fats and oils and it expresses the amount of absorbed iodine. It is an important parameter in the palm oil industry and can be used as a guide in palm oil processing, such as in hydrogenation (1).

In the palm oil industry, IV is usually determined by a titration method that uses toxic solvents. Many different methods are available to determine IV of fats and oils, such as the Wijs, Hanus, and Hubl (2), Hofmann and Green (3), and Rosenmund-Kuhnhenn (4) methods. Although many methods have been developed, the Wijs method is considered to be the standard, and it is the most widely used. The methods require mostly toxic chemicals that are hazardous to the analyst as well as to the environment. To reduce the use of particularly toxic solvents, cyclohexane has been substituted for carbon tetrachloride (5–7). Numerous efforts have been made to find an appropriate substitute for the IV determination by instrumental methods to further reduce the use of chemicals. Near infrared (NIR), Fourier transform (FT) nuclear magnetic resonance, and FT-infrared methods have been used as instrumental analyses to determine IV (8,9). Haryati *et al.* (10) examined the correlation of refractive index to IV of palm oil. Further, Haryati *et al*. (11) developed a technique to determine IV of palm oil based on differential scanning calorimetry. The fatty acid composition from gas chromatography could also be used for calculating IV according to an AOCS method (5).

High-performance liquid chromatography (HPLC) is a technique that is capable of separating triglyceride (TG) components for determining the composition of an oil or fat. Each TG component has a specific number and position of double bonds. IV can be expressed as the average degree of unsaturation of the fatty acids present in the TG molecules of an oil. The more unsaturated the fatty acids, the greater the IV of the oil. Therefore, it is hypothesized that the IV of an oil can be determined based on its TG composition. This study is intended to present an instrumental method for IV determination by HPLC.

MATERIALS AND METHODS

Malaysian refined, bleached, deodorized palm oil (RBDPO) with an IV of 51, RBD-palm olein with an IV of 56, RBD-palm stearin with an IV of 29, and super olein with an IV of 60 were used in this study. From these oils, 16 blends were prepared to obtain an IV range of 29–60. All chemicals used were of either analytical or HPLC grade. TG standards of myristic-myristic-myristic (MMM), myristic-myristic-palmitic (MMP), palmitic-palmitic-myristic (PPM), oleic-oleic-oleic (OOO), oleic-oleic-palmitic (OOP), palmitic-palmitic-oleic (PPO), palmitic-palmitic-palmitic (PPP), oleic-oleic-stearic (OOS), palmitic-oleic-stearic (POS), and stearic-oleic-stearic (SOS) were purchased from Sigma Chemical Co. (St. Louis, MO).

Each blend was analyzed for its IV by a standard AOCS method (12) and for its TG composition by HPLC. The HPLC system used was composed of a Shimadzu LC-10 AD liquid chromatograph, Shimadzu SIL-10A auto injector, Shimadzu system controller SCL-10A, and RID-6A Shimadzu refrac-

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tive index detector (Kyoto, Japan). The column was Nova-Pak C_{18} (3.9 × 300 mm; Waters, Milford, MA) packed with a particle size of 5 µm. The mobile phase was a mixture of acetone/acetonitrile (63.5:36.5), and the flow rate was 1 mL/min. The injection volume was 10 µL of 5% (wt/vol) oil in chloroform. Sensitivity was adjusted to 16×10^4 refractive index units full-scale deflection. TG peaks were identified based on TG standard and earlier results (13–16).

The relationship between peak areas of the HPLC chromatograms and IV was determined by stepwise regression in SAS (Statistical Analysis System) release 6.08 (SAS Institute, Cary, NC). In this study, the TG were classified based either on the TG composition (i.e., triunsaturated, monosaturated, disaturated, or trisaturated) or on the number of double bonds (i.e., no double bond, one double bond, two double bonds, three double bonds, or four double bonds) that were present in the TG molecules. The area (A) of each peak was measured. It is hypothesized that, if IV can be predicted from these characteristics, the HPLC method could be used to determine the IV of palm oil.

RESULTS AND DISCUSSION

The standard analysis showed that the IV of the samples ranged from 29 to 60. Palm Oil Refiners Association of Malaysia standard specifications for IV-processed palm oil are as follows: RBDPO is 50–55, RBD palm olein minimum is 56, and RBD palm stearin maximum is 48, whereas super olein minimum is 60 (17). Thus, this range covered almost all palm oil products.

In this study, 10 standard TG were analyzed, and the results showed that the order of retention times was MMM, MMP, PPM, OOO, OOP, PPO, PPP, OOS, POS, and SOS (Fig. 1). HPLC analysis of each blend showed a different elution pattern chromatogram. A sample chromatogram is presented in Figure 2. There were 15 peaks in the chromatogram. These peaks were identified based on the retention times of TG standards and on the results of Swe *et al.* (13,15), Ghazali *et al.* (14), and Wong (16). The peaks were identified as MMM, MPL, OOL, MMP, PLO, PPL, PPM, OOO, OOP, PPO, PPP, OOS, POS, PPS, and SOS where M stands for myristic, P for palmitic, O for oleic, L for linoleic, and S for stearic. The 15 distinct peaks were calculated for their areas. These peaks were then classified as monosaturated (a) [PLO, OOP, OOS], disaturated (b) [MPL, PPL, PPO, POS, SOS], trisaturated (c) [MMM, PPM, PPP, PPS], and triunsaturated (d) [OOL, OOO]. Therefore, from each sample, four variables were observed, namely peak area of monosaturated (A_a), of disaturated (A_b), of trisaturated (A_c), and of triunsaturated (A_d). These variables were then used as independent variables in the stepwise regression analysis with IV as the dependent variable. The summary of the stepwise regression is presented in Table 1.

The stepwise regression analysis showed that three variables were adequate to provide a good prediction of the IV with $R^2 = 0.990$. The variables were the areas of A_b, A_c, and A_d. Therefore, the regression model of step 3 is used to predict IV and rewritten as:

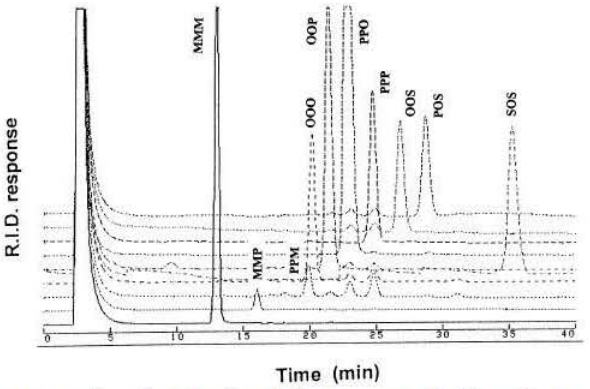

FIG. 1. High-performance liquid chromatogram of triglyceride standards: MMM, myristic-myristic-myristic; MMP, myristic-myristic-palmitic; PPM, palmitic-palmitic-myristic; OOO, oleic-oleic-oleic; OOP, oleic-oleic-palmitic; PPO, palmitic-palmitic-oleic; PPP, palmitic-palmitic-palmitic; OOS, oleic-oleic-stearic; POS, palmitic-oleic-stearic; SOS, stearic-oleic-stearic.

$$\text{IV} = 56.487 - 0.135 \times 10^{-5} A_b - 0.347 \times 10^{-5} A_c + 1.018 \times 10^{-5} A_d \quad [1]$$

(1.96) (1.6×10^{-7}) (2.0×10^{-7}) (1.9×10^{-6})

Numbers in parentheses are the standard deviations of the respective coefficients.

The A_d group showed a positive coefficient, whereas the less unsaturated groups, i.e., A_b and A_c, showed negative coefficients. The increase of unsaturated TG will be complemented by the decrease in the more saturated groups and vice versa as shown by their correlations (Table 2). The less unsaturated TG have a lower IV. Consequently, an increase in saturated TG will decrease IV.

In the second grouping system, the TG compositions were classified based on the number of double bonds, i.e., no dou-

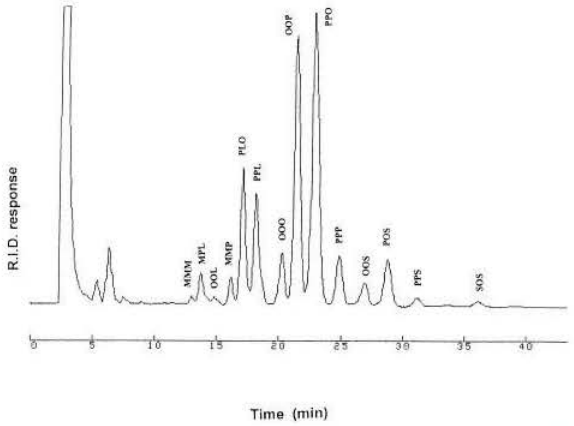

FIG. 2. High-performance liquid chromatogram of palm oil: MPL, myristic-palmitic-linoleic; OOL, oleic-oleic-linoleic; PLO, palmitic-linoleic-oleic; PPL, palmitic-palmitic-linoleic; PPS, palmitic-palmitic-stearic. For other abbreviations see Figure 1.

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TABLE 1
Stepwise Regression with Triglycerides as the Independent Variables[a]

Step	Regression equation	R^2
1	$IV = 55.715 - 0.465 \times 10^{-5} A_c$	0.919
2	$IV = 64.582 - 0.123 \times 10^{-5} A_b - 0.419 \times 10 A_c$	0.967
3	$IV = 56.487 - 0.135 \times 10^{-5} A_b - 0.347 \times 10 A_c + 1.018 \times 10^{-5} A_d$	0.990

[a]Abbreviations: IV, iodine value; A_b, area of disaturated triglyceride; A_c, area of trisaturated triglyceride; A_d, area of triunsaturated triglyceride.

ble bonds (MMM, PPM, PPP, PPS), one double bond (PPO, POS, SOS), two double bonds (MPL, PPL, OOP, OOS), three double bonds (PLO, OOO), and four double bonds (OOL). Therefore, for each sample, five variables were observed, namely peak area of no double bonds (A_{0D}), of one double bond (A_{1D}), of two double bonds (A_{2D}), of three double bonds (A_{3D}), and of four double bonds (A_{4D}).

The stepwise regression allowed only the areas of no double bond, one, two, and three double bonds to be in the model as expressed in Equation 2. This regression performs as well as Equation 1, with $R^2 = 0.992$. The summary of stepwise regression is presented in Table 3.

$$IV = 60.026 - 0.336\times 10^{-5} A_{0D} - 0.193 \times {}^{-5} A_{1D} + 0.054 \times 10^{-5} A_{2D} + 0.080 \times 10^{-5} A_{3D}$$
$$(1.5) \quad (2.1 \times 10^{-7}) \quad (1.9 \times 10^{-7}) \quad (1.6 \times 10^{-7}) \quad (2.9 \times 10^{-7}) \quad [2]$$

Numbers in parentheses are the standard deviations of the respective coefficients.

In this model, the coefficients of the less unsaturated variables [no double bonds (A_{0D}) and single double bond (A_{1D})] are negative. IV is measured based on a fixed weight; therefore, an increase in the value of the variable with fewer double bonds will be complemented by a decrease of the variable with a greater number of double bonds. This fact is supported by the negative correlation of A_{3D} with A_{0D} as well as with A_{1D} (Table 4). Because a greater double-bond number increases the amount of iodine that can be absorbed, an increase of the parameter with a low number of double bonds will reduce IV. This would explain the negative coefficients of A_{0D} and A_{1D}.

Based on the results of this study, we concluded that IV of palm oil can be determined or predicted based on the TG composition of the oil. The best prediction model was obtained by using the double-bond numbers in the TG as the independent variables, as expressed in Equation 2. To predict IV of palm oil, the TG composition of the oil sample should be analyzed by HPLC to obtain the area of the peak with no double bonds, one double bond, two double bonds, and three double bonds and substitute these values in Equation 2.

The results of the IV analysis based on the standard AOCS method (12) and HPLC methods developed in this study are shown in Table 5. Statistically, all predicted IV are not signif-

TABLE 2
Correlation Among Triglyceride Groups[a]

	A_b	A_c	A_d
A_b	1	0.4002	–0.1693
A_c		1	–0.6678
A_d			1

[a]See Table 1 for abbreviations.

TABLE 3
Summary of the Stepwise Regression with Double-Bond Numbers as the Independent Variables[a]

Step	Regression equation	R^2
1	$IV = 55.715 - 0.465 \times 10^{-5} A_{0D}$	0.919
2	$IV = 64.204 - 0.390 \times 10^{-5} A_{0D} - 0.1654 \times 10^{-5} A_{1D}$	0.983
3	$IV = 62.397 - 0.366 \times 10^{-5} A_{0D} - 0.176 \times 10^{-5} A_{1D} + 0.0364 \times 10^{-5} A_{2D}$	0.987
4	$IV = 60.026 - 0.336 \times 10^{-5} A_{0D} - 0.193 \times 10^{-5} A_{1D} + 0.054 \times 10^{-5} A_{2D} + 0.080 \times 10^{-5} A_{3D}$	0.992

[a]Abbreviations: A_{0D}, area of triglyceride with no double bonds; A_{1D}, area of triglyceride with one double bond; A_{2D}, area of triglyceride with two double bonds; A_{3D}, area of triglyceride with three double bonds. For other abbreviations see Table 1.

TABLE 4
Correlation Among Independent Variables[a]

	A_{0D}	A_{1D}	A_{2D}	A_{3D}
A_{0D}	1	0.52074	–0.51298	–0.27049
A_{1D}		1	0.07825	0.02721
A_{2D}			1	–0.10073
A_{3D}				1

[a]See Table 3 for abbreviations.

TABLE 5
Comparison of IV Determined by Standard AOCS and HPLC Methods[a]

Blends	IV by AOCS method[b]	IV by HPLC method[b] TG groups	IV by HPLC method[b] Number of double bonds
1	59.02	58.52 ± 0.91	58.28 ± 0.89
2	56.82	56.51 ± 0.48	56.92 ± 0.50
3	55.81	54.49 ± 0.60	55.76 ± 0.62
4	53.55	53.77 ± 0.37	53.74 ± 0.35
5	52.89	53.72 ± 0.38	53.51 ± 0.35
6	51.21	50.93 ± 0.35	50.46 ± 0.31
7	48.15	48.89 ± 0.42	47.72 ± 0.31
8	49.09	47.77 ± 0.53	48.40 ± 0.46
9	43.78	44.47 ± 0.35	45.36 ± 0.40
10	40.75	42.14 ± 0.37	40.82 ± 0.95
11	44.31	45.83 ± 0.59	45.67 ± 0.57
12	39.39	39.86 ± 0.32	39.74 ± 0.30
13	39.33	37.86 ± 0.52	38.33 ± 0.44
14	34.16	34.24 ± 0.45	33.54 ± 0.42
15	32.05	32.04 ± 0.59	33.30 ± 0.46
16	28.62	27.89 ± 0.69	27.99 ± 0.64

[a]Abbreviations: HPLC, high-performance liquid chromatography; IV, iodine value; TG, triglyceride.
[b]Average of two replications. Figures following ± sign are the standard deviations for the respective predicted IV. All IV calculated by standard AOCS method are not significantly different from those predicted at a confidence level of 95%.

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icantly different from the IV obtained by the standard AOCS method at the confidence level of 95% ($P > 0.05$). Due to easier sample preparation, HPLC can be used to determine TG composition of oil more rapidly than the analysis of fatty acid composition of oil by GC. Thus, the IV prediction by HPLC will also be faster than by GC.

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