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A Predictive Model for Determination of the Iodine Value of Coconut Oil by GLC Analysis of the Component Fatty Acids

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Abstract

A study was conducted to develop a predictive model for determination of iodine value (IV) of coconut oil using gas liquid chromatographic (GLC) analysis of the component fatty acids (FA). Altogether twenty-six samples were selected to represent three sub-categories of coconut oil, namely ordinary coconut oil, virgin coconut oil, and coconut paring oil. Out of the twenty-six, fifteen samples were used as a calibration set while the remaining eleven samples were kept for validation purpose. All twenty-six samples were analyzed for iodine value using the AOCS method Cd Id-92 and for FA composition using GLC detection of fatty acid methyl esters (FAME). Pearson correlation analysis between IV and individual FA indicated that lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) were the five parameters having strong correlation with the iodine values. When these five parameters were used as independent variables in a stepwise regression procedure, a predictive model for iodine value was obtained with $C_{16:0}$ and $C_{18:1}$ as independent variables (coefficient of determination, R^2 = 0.9611 and standard error, SE=0.93). When the model was validated with an independent set of eleven samples, the coefficient of determination was 0.946 with an overall SE of 0.95. The study concludes that the iodine value measured by the GLC method was comparable to that obtained by AOCS method Cd Id- 92.

Keywords: Coconut oil, Fatty acid, GLC, Iodine value, Oil analysis, Quality control, VCO.

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Introduction

Iodine value is one of the most important and frequently used quality parameters in the oils and fats industry. Chemically, it quantifies the number of grams of iodine absorbed by 100 g of oil or fat and indicates the basic nature of oil or fat with regard to its degree of unsaturation (Rossell, 1987). Determination of the iodine value of an oil or fat can help to predict its stability during storage or in frying operations. Since iodine value is a basic characteristic of any oil or fat, it can be used to identify adulteration of oil or fat samples. In many natural oils and fats a significant deviation in iodine value could be taken as preliminary evidence to suspect the purity of the sample. The iodine value is also an important process control parameter to monitor fractionation and hydrogenation of oils and fats (Hoffman, 1989). For instance, it is often used to optimize operating conditions in fractionation to achieve the desired product quality.

There are several ways of determining the iodine value of oils and fats. Wij's method, Hanus and Hubl method, Rosenmund-kuhnhenn method (Paquot, 1979; Rossell, 1987) are some of them. Of the various methods and modifications proposed from time to time, Wij's method is widely considered as a standard method (AOCS, 1993). However, most of these methods are titrimetric in nature and involve the use of toxic, carcinogenic and environmentally unfriendly chemicals. Additionally, they are tedious and time consuming procedures and depend on the skills of the analyst. Therefore, developing alternative methods for iodine value determination has been the interest of many research groups. As such, there were many investigations employing various instrumental techniques for this purpose (Ng & Ping, 2001; Che Man *et al,* 1999; Haryati *et al,* 1997 & 1998). Gas liquid chromatography (GLC), for instance, is a technique which is widely used to determine the fatty acid profiles of oil and fat samples. Many previous reports have already highlighted the relationship between the fatty acid profile and the iodine value of oils and fats (Kyriakidis & Katsiloulis, 2000; Lynm & King, 1996). However, these reports did not give in an in-depth analysis into different kinds of coconut oil samples and their fatty acid distribution. Moreover, Nathanael (1966) pointed out that coconut kernel had some unique features in that the distribution of oil varied within the different layers. The layer closest to the water cavity was least rich in oil (56.3%) while the layer nearest to the brown testa was richest in oil (75.4%). Later investigations into this aspect showed that the oil characteristics of the brown testa were slightly different from those of the oil obtained from inner layers of the kernel. For these reasons, commercial coconut oil appears to have three subgroups namely; ordinary coconut oil (CNO), virgin coconut oil (VCO) and coconut paring oil (CPO). These subgroups may differ slightly in their fatty acid composition and iodine value (Marikkar *et al,* 2007; Yalegama *et al,* 1999). Therefore, investigations to determine the co-relationship of iodine value with the varying proportion of different fatty acids would be worthwhile for the coconut oil industry. With this objective, a study was conducted to develop a predictive model for the determination of iodine value of coconut oil using fatty acid data.

Materials and methods

Materials:

All the chemicals used in this study were of analytical grade, unless otherwise specified. Eight samples of CNO and seven samples of CPO were collected from reliable oil millers who use screw press oil expellers to extract oil from copra and dried coconut parings. Eight samples of VCO were extracted through a low-pressure oil expeller using eight batches of dried-pulverized samples of coconut. An additional set of three samples were prepared by mixing CNO with CPO. These samples were pre-analyzed for iodine value using the AOCS method Cd 1c-92. The iodine value of the samples ranged from 7.6-8.3 for CNO, 15.5-16.9 for CPO, and 4.7-5.6 for VCO. In

the composite pool of 26 samples, 15 samples were used as calibration standards while the remaining 11 samples were kept separately for validation purpose. The validation samples were chosen such that their iodine values fell within the iodine value range of the calibration set. Rather than calibrating individual oil types separately, a combined calibration has the advantage that it can be applied over a wider range of iodine values with the possibility of extrapolation for adulterated blends which may have an iodine value outside the ranges of the individual oil type. An additional set of three commercial VCO samples was employed for cross-validation.

Determination of fatty acid composition of oil samples:

Samples for the detection of fatty acid methyl esters (FAME) were prepared by dissolving aliquots of oil (50 mg) with petroleum ether (0.8 ml) and sodium methoxide (1M, 0.2 ml) (PORIM Test Method, 1995) and analyzed (in duplicate) on an Agillent 4890D gas chromatograph (Agillent Technologies, Singapore) equipped with a Flame Ionization Detector (FID). A non-polar capillary column HP-5 MS (0.25mm internal diameter, 30m length and 0.25μm film thickness, Hewlett Packard Company, Singapore) was used at a column pressure of 10 bars. The initial temperature of the column was at 100°C and was programmed to increase to 220°C at 4°C/ min and then remain at 220°C for 15min. The temperatures of the injector and detector were maintained at 250°C and 275°C, respectively (Marikkar *et al.*, 2006).

Statistical analysis:

The relationships between each GLC parameter and IV (Wij's) were determined by Pearson's correlation analysis. The iodine value and fatty acids compositional data of the calibration set were analyzed using a stepwise procedure in SAS to develop a calibration model. The significance level of an independent variable to enter and stay in the calibration mode was set to 0.15 during execution of the stepwise variable selection in SAS procedure "REG". A separate validation set with IV (Wij's) and fatty acid data was used to validate the calibration model.

Results and discussion

Analyses of oil samples according to the AOCS method showed that the iodine value of samples in each oil category fell within narrow ranges (Table 01). In a composite pool, the iodine value ranged from 4.7 to 16.9 showing that the sample pool represented almost all possible coconut oil products. GLC analyses of fatty acids as summarized in Table 02, indicated that the changes in iodine value of the samples had a direct influence on the relative proportion of fatty acids such as $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, and $C_{18:1}$. For instance, the proportion of $C_{12:0}$ in VCO was in the higher range while the proportion of the same fatty acid in CPO was in the lower range. On the other hand, the reverse order could be seen between these two categories of oils with regard to the proportion of the $C_{18:1}$ fatty acid. Since there is a linear variation between fatty acid data and the iodine value of the samples, they could be statistically analyzed to establish correlation between them. The Pearson correlation coefficients between IV (Wij's) and individual GLC parameters showed that only five out of the nine GLC parameters had good correlation with the IV (Wij's) of the oils (Table 03). While $C_{12:0}$ and $C_{14:0}$ had strong negative correlation, $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ had strong positive correlation. In addition, the correlations for each comparison were highly significant ($p<0.01$). Therefore, these five parameters could be chosen to run as a stepwise procedure in the development of a predictive model for iodine value.

Development of a calibration model for iodine value:

The outcome of the stepwise regression analysis is given in Table 04. It shows that out of the five independent variables entered to the

Table 1. Variation of Iodine Value among the Calibration Standards[1]

Oil Series	Range	Mean IV (Wij's)	SD
VCO	4.7 - 5.6	5.2	0.34
CNO	7.6 - 8.3	7.9	0.38
CPO	15.5 - 16.9	16.4	0.60

[1]Abbreviations: VCO, virgin coconut oil; CNO, coconut oil; CPO, coconut paring oil; IV, iodine value; SD, standard deviation.

Table 2. Variation of Fatty Acid Composition among the Calibration Samples[1]

Fatty Acid	Range	Mean Value	SD
Capric ($C_{6:0}$)	0.09 - 0.43	0.26	0.13
Caprylic ($C_{8:0}$)	3.82 - 7.51	5.65	1.17
Caproic ($C_{10:0}$)	5.07 - 6.24	5.72	0.37
Lauric ($C_{12:0}$)	50.71 - 37.54	45.57	5.19
Myristic ($C_{14:0}$)	22.17 - 19.22	20.64	0.91
Palmitic ($C_{16:0}$)	7.78 - 13.12	9.79	1.94
Stearic ($C_{18:0}$)	0.0 - 0.13	0.03	0.03
Oleic ($C_{18:1}$)	5.63 - 17.60	10.09	4.39
Linoleic ($C_{18:2}$)	1.42 - 3.99	2.78	0.74

[1]For abbreviations see Table 1.

Table 3. Pearson Correlation Coefficient between IV (Wij's) and each of the GLC Fatty Acid parameters

Fatty Acid	Correlation Coefficient
Capric ($C_{6:0}$)	+ 0.033 ($p<0.401$)
Caprylic ($C_{8:0}$)	- 0.0685 ($p<0.178$)
Caproic ($C_{10:0}$)	+ 0.0005 ($p<0.935$)
Lauric ($C_{12:0}$)	- 0.9290 ($p<0.0001$)
Myristic ($C_{14:0}$)	+ 0.329 ($p< 0.0155$)
Palmitic ($C_{16:0}$)	+ 0.8985 ($p< 0.0001$)
Stearic ($C_{18:0}$)	+ 0.0003 ($p<0.7905$)
Oleic ($C_{18:1}$)	+0.9485 ($p<0.0001$)
Linoleic ($C_{18:2}$)	+0.7520 ($p<0.0001$)

Table 4. Summary of Stepwise Regression Analysis with GLC Fatty Acid Parameters versus Iodine Values[1]

Step	Regression equation	R^2	SE
1	IV = -1.13 + 1.08 $C_{18:1}$	0.9523 ($p<0.0001$)	1.55
2	IV = 7.52 - 1.6 $C_{16:0}$ + 1.79 $C_{18:1}$	0.9611 ($p<0.0001$)	0.95

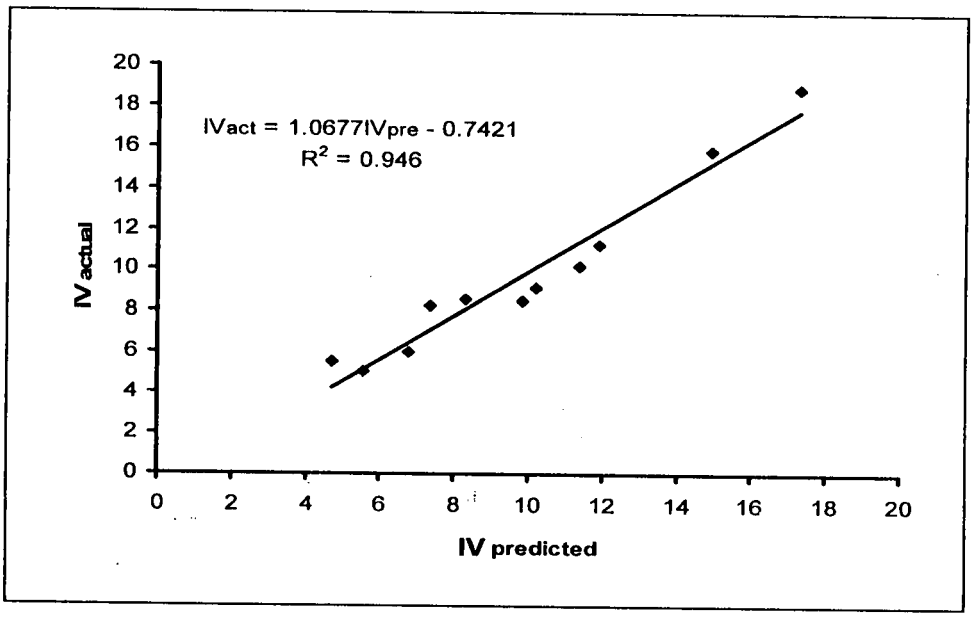
[1]Abbreviations: IV, iodine value; $C_{18:1}$, oleic acid proportion; $C_{16:0}$, palmitic acid proportion; R^2, coefficient of determination; SE, standard error.

Table 5. Cross-validation with Selected Commercial Samples of Virgin Coconut Oil[1]

Sample	Relative Proportion of $C_{16:0}$	Relative Proportion of $C_{18:1}$	IV (Wij's)	IV $_{Pre}$	IV $_{Act}$
VCO-a	9.6	7.94	5.2	6.37	6.05
VCO-b	7.9	6.1	5.8	5.80	5.45
VCO-c	8.3	6.4	5.3	5.7	5.3

[1]Abbreviations: IV_{pre} , predicted IV; IV_{Act}, actual IV. For other abbreviations see Table 1.

Figure 1. Scatter plot showing validation data of actual vs. predicted IV. Each value represents the mean of two independent determinations. R^2, coefficient of determination



program, only two were necessary to predict the iodine value of coconut oil samples. The predictive models for the iodine value of coconut oil using $C_{18:1}$ and $C_{16:0}$ as independent variables are as given below:

$IV_{Pre} = -1.13 + 1.08\ C_{18:1}$; $R^2 = 0.9523$ ($p<0.0001$) and SE= 1.55 [1]

$IV_{Pre} = 7.52 - 1.6\ C_{16:0} + 1.79\ C_{18:1}$; $R^2 = 0.9611$ ($p<0.0001$) and SE= 0.93 [2]

where $C_{18:1}$, is the oleic acid proportion and $C_{16:0}$, the palmitic acid proportion

Both the above models show a higher coefficient of determination with good confidence limits. However, based on the highest coefficient of determination (R^2 value) and smallest standard error (SE), the second model would be more accurate for the prediction of the iodine value of coconut oil. The effectiveness of this regression model for future prediction could be checked by means of a separate validation data set.

Validation analysis:

The validation set contained eleven samples which were not represented in the calibration data set. Based on the outcome of the validation test (Figure 1), the relationship between actual IV (IV_{Act}) and the predicted IV (IV_{Pre}) could be given by a regression equation as shown below:

$IV_{Act} = 1.07\ IV_{Pre} - 0.74$; $R^2 = 0.946$ and SE= 0.95

According to the validation analysis, the iodine value of coconut oil samples can be predicted within a standard deviation of 0.95 IV units. Although there is a fair comparison between the predicted and actual iodine values, a cross-validation with commercial samples of coconut oil could provide an added degree of confidence for this method. So the fatty acid data for three commercial VCO samples were substituted in the prediction model and the values obtained for IV_{Pre} and IV_{Act} are compared with IV(Wij's) of these samples as shown in Table 05. Obviously, IV_{Act} for VCO-

a and VCO-b did not tally with IV(Wij's) but the deviations were found to be within the limit of the standard error calculated for the predictive model (Table 04). Of course, this kind of discrepancy could arise due to experimental errors associated with the analysis instruments. As noted before, the method described here is dependent on the estimation of the relative proportions of the C16:0 and C18:1 fatty acids. In general, accuracy of the estimation of these two fatty acids might be affected by the baseline drift in the GLC chromatograms. Baseline drifts in GLC chromatograms are sometimes observed when the sample analysis reaches the higher region of the temperature program. Therefore, this has to be taken into consideration when developing an in-house calibration for GLC analysis.

Conclusions

This study examined the potential application of GLC method for iodine value determination. It concluded that the iodine value of coconut oil as measured by the GLC method was comparable to that obtained by AOCS method Cd Id-92. The method uses reduced amount of reagents which are usually irritant or toxic to the analyst. Although this GLC method is faster and more convenient, adequate attention should be given to precautionary measures that are essential for better accuracy.

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