



## Simple Spectrophotometric Method for Determination of Iodine Value of Vegetable Oils

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### ABSTRACT

A spectrophotometric method has been developed for the determination of iodine value of vegetable oils. The method is based on the reaction of Hanus solution with oils and subsequently treated with potassium iodide solution producing triiodide ions. The absorbance at 350 nm of triiodide ions was used for analytical purposes. The calibration graph was constructed by plotting the absorbance at 350 nm versus the molar concentration of Hanus solution. Under the optimal conditions a linear calibration graph ranged from 0.02 to 0.10 mol/L of Hanus solution with  $r^2$  of 0.999. The proposed method was feasible to determine a wide range of iodine value of vegetable oil samples. The method was applied to commercially available vegetable oils and the results obtained by the proposed method agreed well with those obtained by the reference method. The standard deviation of iodine value ranged from 0.7 to 2.1 ( $n=3$ ). The analytical procedures were simple, required short analysis time, and used small amount of solvent and reagent.

**Keywords:** spectrophotometry, iodine value, vegetable oils

### 1. INTRODUCTION

Vegetable oils are commonly produced from seeds and nuts. It may contain different type and composition of fatty acids [1]. These chemical compositions relate to quality of vegetable oils. The fatty acids composition of oils could be determined by gas chromatography after their derivatization to methyl ester [2]. Iodine value is a chemical index which has been used to express the degree of unsaturation of fats and oils. The official methods for determination of

iodine value [3] involve the reaction of sample with halogenating reagent, Hanus or Wijs solution follows by iodometrically determine the unreacted reagent. The procedures are time consuming, and involve a large amount of solvent (10 mL per analysis), reagents (25 mL per analysis), and chemicals.

Several methods have been developed for determination of iodine value. The recently spectroscopic techniques[4-11], e.g.

FTIR, FT-NIR etc., have been proposed for fast and nondestructive analysis of oils for iodine value. However, the method involves much mathematic calculations, and uses sophisticated instrument which is not normally available in general laboratory. In addition to instrumental analysis, potentiometric titration was proposed as an alternative approach for analysis of biodiesel from palm oil [12].

Flow injection system coupled with potentiometric or spectrophotometric detector has been proposed by Lee *et al.* [13]. Spectrophotometric detection at 360 nm of unreacted reagent, i.e. Wijs solution (ICl in glacial acetic acid), allows the determination of iodine value of fatty acids in the range of 40 to 120. The standards which used for constructing a calibration graph were fatty acids with the known iodine value obtained by AOAC Wijs solution method. A spectrophotometric based analytical system was also proposed by Thomaidis *et al.* [14] for determination of olive oil iodine value. The method involves the absorbance measurement at 392 nm of unreacted Hanus solution, i.e. IBr in glacial acetic acid. The standardized olive oils with different iodine values range from 9 to 125 were used for constructing a calibration graph. The method is precise since the flow injection system is employed. However, the range of iodine value is limited by both the iodine value and weight of olive oils used as standards. Both two flow injection methods stated above involve the detection of unreacted reagent after mixing with samples. In addition, the methods required the standardization of oils or fatty acids used for construction of calibration graph by using the time consuming official methods.

In this paper, we propose a simple spectrophotometric method for determination of iodine value of edible oils.

The method is based on the spectrophotometric measurement of iodine, i.e. triiodide ions, liberated by the reaction of unreacted Hanus solution with aqueous KI solution. Neither fatty acids nor olive oils with known iodine value were used as standard for constructing calibration graph as used by previous reports. This is the first work that uses Hanus solution for both calibration graph construction and as a reagent.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

All chemicals used were reagent-grade or otherwise stated. Liquid bromine (Panreac, Spain), iodine (UNIVAR, Australia) and glacial acetic acid (RCI Lab-Scan, Thailand) were used for preparing Hanus solution. The Hanus solution was prepared freshly according to the procedures described in the AOAC method. Its concentration was iodometrically determined.

### 2.2 Instrumentation

The absorbance measurement was performed using the UV-Vis spectrophotometer (model UV-1800, Shimadzu, Japan) with 10 mm optical path of quartz cell.

### 2.3 Sample Preparation

Vegetable oil samples were bought from supermarkets. These included coconut, palm, olive, rice-bran, sesame, canola, corn, sunflower, and soybean oils. The 0.10 grams of accurately weighed sample was dissolved and then diluted with isooctane to 10 mL in volumetric flask. One-milliliter aliquot of this solution containing approximately 0.010 g of oil sample was subjected to analysis.

### 2.4 Spectrophotometric Procedures for Iodine Value Determination

### 2.4.1 Construction of Calibration Graph

A series of standardized Hanus solutions to use for constructing a calibration graph was prepared by appropriate diluting the 0.10 mol/L Hanus solution with glacial acetic acid. A 1.00 mL of various concentrations of Hanus solutions were added with 1.00 mL isooctane and kept in the dark place for one minute followed by adding with 1.00 mL of 15% w/v KI solution. One milliliter of the resulting solution was mixed with 1.00 mL of 15% w/v KI solution and then diluted to 100 mL with deionized water in volumetric flask before measuring absorbance. All procedures were performed at 25°C. The plot of absorbance versus concentration of Hanus solution was used for determining the concentration of unreacted Hanus solution.

### 2.4.2 Sample Analysis

One milliliter of sample solution was treated with 1.00 mL of 0.10 mol/L Hanus solution for one minute in the dark place and followed by the remaining procedures as described in section 2.4.1. The reagent blank was also performed by using isooctane instead of sample solution. The concentration of unreacted Hanus solution was determined via calibration graph.

### 2.5 Reference Method

We have investigated the downscaled titrimetric method for determination of iodine value of edible oils. The method was modified and based on the Hanus AOAC method. Method validation showed that the iodine value obtained by this proposed titrimetric method were not significant difference with those by standard conditions of AOAC method. In addition, the reaction time was dramatically reduced to 1.0 min. and the amount of reagent, i.e. Hanus solution, was 1.00 mL compared to conventional

procedures which required ca. 30 min incubating sample with 25.00 mL of Hanus solution. Thereby we used this method for comparing the analytical results in this work. The briefly procedures were as follows. One-milliliter aliquot of 0.010 g/mL of oil sample in isooctane was reacted with 1.00 mL of Hanus solution in the dark place for 1.0 min. Then, to this solution 1 mL of 6% w/v KI solution was added. This liberated iodine which can be quantified by titration with sodium thiosulfate solution and using starch solution as indicator.

## 3. RESULTS AND DISCUSSION

### 3.1 Basis of the Method

The basis of the official method of AOAC involves simple determining the iodine absorbed by the sample via the blank and sample determinations. This was also the basis of this research. Rather titrating, we determined the unreacted IBr reagent by spectrophotometric measurement of the triiodide ions. The absorbance measured at a specific wavelength of IBr reagent was used to calculate the concentration of IBr remained in the solution. The amount of iodine absorbed by the sample was calculated by subtraction of blank with sample determinations. The equation (1) used for calculation of iodine value was formulated as follows.

$$\text{Iodine value} = \frac{(M_b - M_s) \times 100 \times 126.9 \times 2}{1000 \times W_s} \quad (1)$$

Where  $M_b$  and  $M_s$ , were molar concentration of IBr obtained from blank and sample determinations, respectively, and  $W_s$  was weight in gram of sample.

### 3.2 The Detection Wavelength

The Hanus solution was mixed with KI solution and diluted with deionized water before recording the absorption spectrum using the spectrophotometer. The absorption

spectrum of the mixed solution of Hanus solution and KI solution, and this solution with starch solution showed the maximum absorption at 350 and 610 nm, respectively.

It is possible to determine the concentrations of Hanus solution via triiodide or blue iodine-starch complex. The characteristic of calibration graphs obtained by different detection species was investigated. The detection at 350 nm gave better results. The graph was linear in the range of 0.02 - 0.10 mol/L Hanus solution with good coefficient of determination ( $r^2$ , 0.9954). The detection at 610 nm gave a curve line for the range of concentration of Hanus solution tested. Therefore, the absorbance measurement at 350 nm was chosen.

### 3.3 KI Concentrations

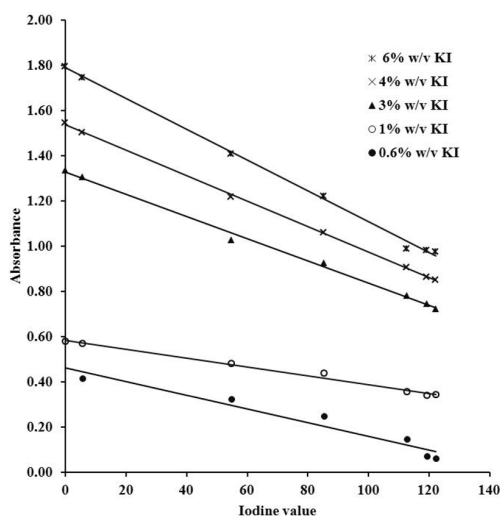
Iodides undergo the oxidation reaction with IBr to form triiodides, therefore its concentration will affect the sensitivity, i.e. slope of calibration graph, of the method. The concentration of KI was studied. Sample solutions prepared as described in section 2.3 were treated with 1.00 mL of 0.10 mol/L Hanus solution for one minute in the dark, to this solution 10.00 mL of various concentrations of KI (0.6 - 6% w/v) was added. The resulting solutions were diluted 100 folds with deionized water before measuring the absorbance at 350 nm. The data shown in Figure 1 were the plots between the absorbance and iodine value of oil samples (determined by Hanus AOAC method). The results showed that using the higher concentration of KI the higher the slope of the graphs was resulted. It should be noted that the absorbance at "0" iodine value was the absorbance of blank solution. In addition, the high absorbance values, i.e. more than 1.50 unit, were resulted when using 4.0 and 6.0% w/v KI, and this high

value will exceed the output of spectrophotometer, i.e. Spectronic 21 (Milton&Roy).

The high slope of this graph is desired since it is the change of absorbance per iodine value unit. Therefore we have further examined the higher concentration of KI ranged to 20% w/v. In this case the resulting solutions after treated with KI were diluted 100 and/or 200 folds before subjecting to absorbance measurement. The results in Table 1 showed that the iodine value obtained by spectrophotometric method using 10% w/v, 15% w/v, and 20% w/v KI were not significantly difference with the reference method. Therefore, we selected, for safe, a 15% w/v KI solution with further 200 folds dilution. The final concentration of KI in diluted solution was 0.07% w/v.

### 3.4. Reduction the Use of KI

In section 3.3, the dilution step is necessary to obtain an appropriate absorbance value. Since high degree of dilution was involved, lot of KI was waste. Therefore, it was aimed to utilize KI effectively. The analytical procedures were slightly modified. After incubating the sample solutions with Hanus solution and followed by addition of KI solution, 9.00 mL of deionized water was added. One milliliter of the resulting solutions was mixed with various volumes of 15% w/v KI to give the final concentrations of KI of 0.01%, 0.06%, 0.10% and 0.16% w/v. The concentration of KI more than 0.16% w/v, i.e. 0.32%, was not further investigated since it gave an absorbance value of blank solution higher than 1.50 even 100 folds dilution was employed. Further dilution would make the absorbance below 1.50 but a tedious procedure is involved and a longer analysis time is resulted. The results showed that the slope of calibration graph increased dramatically when the final concentration of KI increased from 0.01 to



**Figure 1.** The absorbance-iodine value plots at various concentrations of KI.

**Table 1.** Iodine value obtained by using different concentration of KI.

Oil samples	Iodine value (mean $\pm$ SD, n=3)						Reference Method
	10% KI		15% KI		20% KI		
	200F*	100F	200F	100F	200F	100F	
Coconut	8.1 $\pm$ 1.0	6.0 $\pm$ 1.5	5.24 $\pm$ 0.9	6.7 $\pm$ 2.5	3.7 $\pm$ 0.4	3.8 $\pm$ 1.7	7.6 $\pm$ 1.0
Oleic acid**	82.4 $\pm$ 1.3	80.5 $\pm$ 1.0	80.4 $\pm$ 1.6	79.0 $\pm$ 1.2	79.0 $\pm$ 0.4	80.2 $\pm$ 0.2	83.3 $\pm$ 0.4
Sesame	105.7 $\pm$	103.6 $\pm$	108.2 $\pm$ 0.7	106.4 $\pm$ 0.4	104.6 $\pm$ 0.5	104.9 $\pm$ 0.5	107.9 $\pm$ 0.7
Sunflower	1.6	0.6	117.4 $\pm$ 0.7	116.7 $\pm$ 1.2	115.5 $\pm$ 0.8	114.5 $\pm$ 0.8	119.1 $\pm$ 1.2
Soybean	116.2 $\pm$	116.0 $\pm$	119.2 $\pm$ 1.5	118.0 $\pm$ 0.5	119.9 $\pm$ 0.6	120.7 $\pm$ 0.7	119.9 $\pm$ 1.1

\* F = dilution factor

\*\* Fatty acid.

0.06% w/v as shown in Figure 2. Increased the concentration of KI to 0.16% w/v resulted in slightly increased in slope of calibration graph and this concentration was therefore selected.

In addition, the results from the analysis of samples in Table 2 showed that the iodine value obtained were not significantly different from those by reference method when the final concentration of KI was 0.16% w/v. These may involve an ineffective conversion of iodide to triiodide at low concentration of KI.

### 3.5 Precision Study

Oil samples having low and high iodine

value i.e. coconut and soybean oil, respectively, were subjected to analysis (n=5) by the proposed method. The iodine value obtained were  $7.3 \pm 1.2$  and  $125.3 \pm 1.3$  for coconut and soybean oil, respectively. A good precision was obtained. The absorbance measurement allows repeated measurement of treated sample which should improve precision and accuracy.

### 3.6 Application to Oil Samples

The proposed method was applied to real samples for determination of iodine value. The analytical results were shown in Table 3. To test whether or not the

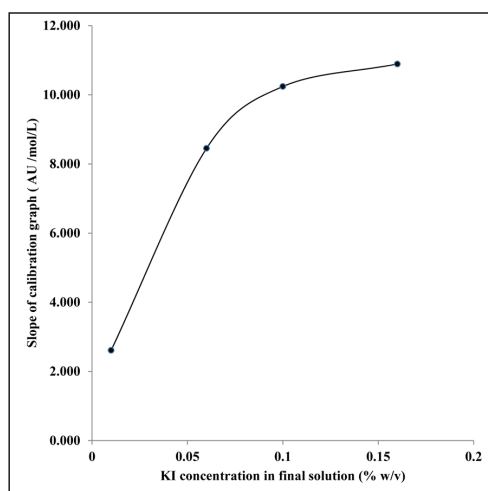


Figure 2. Plot of slope of calibration graph versus KI concentration.

Table 2. Effect of final concentration of KI on the iodine value.

Sample	Iodine value (mean $\pm$ SD, n=3)				
	% w/v KI in final solution				Reference method
	0.16	0.10	0.06	0.01	
Coconut oil	8.9 $\pm$ 0.6	5.9 $\pm$ 0.9	4.6 $\pm$ 1.1	4.0 $\pm$ 0.8	8.8 $\pm$ 0.7
Oleic acid	85.2 $\pm$ 1.2	83.1 $\pm$ 1.1	83.0 $\pm$ 0.8	67.0 $\pm$ 1.4	85.8 $\pm$ 1.2
Soybean oil	119.9 $\pm$ 0.9	117.9 $\pm$ 0.7	116.8 $\pm$ 0.4	102.7 $\pm$ 1.2	122.4 $\pm$ 0.7

Table 3. Iodine value of vegetable oils obtained by proposed spectrophotometric method.

Sample	Iodine Value (mean $\pm$ SD, n= 3)	
	Reference Method	Proposed Method
Coconut oil	7.8 $\pm$ 1.7	8.1 $\pm$ 1.6
Palm oil	57.0 $\pm$ 2.1	54.2 $\pm$ 2.0
Olive oil	84.1 $\pm$ 0.6	82.8 $\pm$ 0.7
Canola oil	91.0 $\pm$ 3.0	88.5 $\pm$ 1.7
Rice-bran oil	92.9 $\pm$ 0.8	90.3 $\pm$ 1.4
Sesame oil	106.6 $\pm$ 1.2	103.4 $\pm$ 2.1
Corn oil	107.1 $\pm$ 0.2	104.4 $\pm$ 1.3
Sunflower oil	116.6 $\pm$ 1.1	114.0 $\pm$ 2.0
Soybean oil	124.9 $\pm$ 0.6	124.8 $\pm$ 0.9
Oleic acid	85.8 $\pm$ 0.8	85.7 $\pm$ 2.7

results obtained by both methods were different, the t-test was performed. The observed t-value,  $t_{cal} = 1.88$ , was less than the critical value,  $t_{crit} = 2.45$  ( $p = 0.05$ ), therefore, there was no significant difference between the results obtained by the proposed and the reference methods. The correlation plot was also performed and it showed that the slope of the graph was close to 1.0 (Iodine value  $_{proposed\ method} = 0.98$  Iodine value  $_{reference\ method} + 0.52$ ,  $r^2 = 0.9987$ ). This indicated that both results correlated well.

#### 4. CONCLUSIONS

The proposed spectrophotometric method has several advantages. Iodine solution was used as a reagent and the standards for construction of calibration graph. This allows a wide range of iodine value could be determined. The analytical procedures were simple and required small volume of reagent and sample. The spectrophotometric measurement eliminates the time consuming involving in titration procedures, thus was suitable for analysis a large number of samples. The proposed procedures could reduce (i.e. potassium iodide) and eliminate (i.e. sodium thiosulfate) chemicals required in conventional titrimetric method of AOAC. Since a small volume was required for absorbance measurement, many aliquots of treated sample solution could be repeatedly analyzed which would result in accuracy and precision improvement. Although a dilution step is required for appropriate absorbance measurement, the pungent odor of glacial acetic acid contained in reagent is also dramatically reduced.

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