

Greener Analytical Method for Determination of Iodine Number of Edible Oils

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Abstract

A greener analytical method for determination of iodine number (IN) of oils is presented. As per the AOAC standard method, a large amount of solvent and reagent was used, and long incubation time was required. This research is aimed at using less amount of solvent and reagent, less sample weight, and shorten the analysis time by using the modified titrimetric AOAC standard method. The study showed that by reducing the sample size, the amount of reagent could be decreased to 1.00 mL and the reaction time of 1 min is enough for completion of the reaction. The amount of reagent used was at least 25 times less than that of the classical method. There was no significant difference at 95% confidence level between the results obtained by the proposed method and the standard method, and both results correlated well. The present method can be applied to edible oils commonly found in the market (iodine number range of 6.0 to 130).

Keywords: iodine number, edible oil, green analytical method, modified titrimetric method

1. Introduction

Nowadays, iodine number (IN) is one of the great interest parameters for measuring the degree of unsaturation of oil or fat and quickly indicating the group to which the oil belongs to. It is defined as the number of halogen in centigram, expressed as iodine, which is absorbed by one gram of oil sample (percent iodine absorption). The higher iodine number, the more unsaturated fatty acid parts are present in oil. The interesting application of iodine number is its use as a parameter in process control as well as a quality parameter in traded palm oil product. It is used as a guidance tool for the purchasing of raw materials and controlling hydrogenation reaction (Weiss, 1970). It is also used to investigate the properties of vegetable oil that used to produce biodiesel that will affect some properties of biodiesel, such as viscosity, cloud point, and storage stability which are changed due to the oxidation and polymerization reactions of double bonds in the oil. Moreover, since oil of high iodine number will contain high content of unsaturated bonds in fatty acids, it indicates the nutritional value (Knothe, 2002).

Several analytical techniques for determination of iodine number of edible oils have been reported. Some studies are undertaken by measuring the amount of remaining reagent from the reaction by using ion selective electrode (ISE) in potentiometric method (Honda & Kashimoto, 1978; Mahapatra, 2011) or measuring the absorbance of the remaining reagent (Kamson, 1986; Lee & Pollard, 1984), which is simple but still using large amount of reagent and solvent. Some papers also introduced flow injection techniques (FI) to make a more comfortable and faster analysis (Thomaidis & Georgiou, 2000). However, though the method provided automatic measurement the continuous flowing of reagent still generated large amounts of waste solution. Furthermore, nuclear magnetic resonance (NMR), near infrared (NIR), and Fourier transform infrared (FT-IR) spectroscopic techniques have been proposed (Baeten & Aparicio, 2000; Che Man & Setiowaty, 1999; Foca et al., 2016; Hendl, 2001; Yang et al., 2005). The gas chromatographic method is an alternative method for determination of IN as per AOCS Cd1c-85 (Firestone, 1989). By this method, IN is calculated from the fatty acid composition in the oil sample. With this technique, quantification of different free fatty acids can be made simply. However, it involves

a complicated mathematical calculation, high-cost instrument, and well operation skill.

The determination of iodine number of edible oils according to the AOAC standard methods (Helrich, 1990) is carried out by using the titration method. An oil sample is analyzed by reacting with Hanus solution (iodine monobromide in glacial acetic acid) or Wij's solution (iodine monochloride), and then the amount of the remaining reagent is determined. This titrimetric method is simple and requires only general laboratory skills and apparatus. However, as per the AOAC titrimetric method, 25 mL of IBr reagent and 10 mL of chlorinated solvents per analysis is required with 30 to 60 min incubation time.

For many years, the green chemistry movement has been promoting ways to reduce the risks of chemical use to humans and the environment. The main concept of green chemistry is the application of chemistry skills and knowledge to reduce or eliminate the use and/or generation of hazardous substances (Anastas, 1999; De Marco et al., 2019).

In this research, it is aimed to modify the titrimetric AOAC standard method for the determination of iodine number of edible oils by using less amounts of sample and reagent, and shorter incubation time. The experimental conditions were studied for reducing the amount of reagent and solvent, using short analysis time, and avoiding the usage of chlorinated solvent.

2. Method

2.1 Materials and Reagents

All chemicals, i.e., glacial acetic acid, potassium iodide, starch soluble, dichloromethane (as per AOAC titrimetric method), n-propanol, iso-octane, and oleic acid were analytical reagent grade. Standard solutions of 0.1 M and 0.005 M sodium thiosulfate were prepared by dissolving in deionized (DI) water and standardized against potassium iodate. Hanus solution (iodine monobromide, IBr) concentration of 0.10 M was prepared with glacial acetic acid according to the AOAC method (Helrich, 1990). The Hanus solution is stable for at least 1 month.

2.2 Sample Preparation

Nine samples of commercially available edible oil were purchased from local stores in Chiang Mai, Thailand, with IN range from 5 to 130. These included coconut, palm, olive, rice bran, sesame, canola, corn, sunflower, and soybean oils. Weighed 0.10 g of sample and dissolved it with iso-octane to 10 mL. One-mL aliquot of this sample solution (0.010 g sample) is used for analysis.

2.3 Determination of IN by AOAC Titrimetric Method (Helrich, 1990)

The AOAC titrimetric method was slightly modified. An aliquot of 0.25 g oil sample was dissolved in 10 mL of dichloromethane (instead of chloroform), follows by adding 25 mL of IBr solution. Shook it and incubated in the dark for 30 min. Then, added 15% w/v potassium iodide solution 10 mL. Titrated with 0.1 M sodium thiosulfate to pale yellow. Added few drops of starch indicator and continued titrating until the blue color entirely disappears. Blank determination was conducted similarly but without oil. The iodine number was then calculated using equation (1).

$$IN = [(V_B - V_S) \times M \times 12.69] / W \quad (1)$$

Where V_B = mL $\text{Na}_2\text{S}_2\text{O}_3$ used for blank determination, V_S = mL $\text{Na}_2\text{S}_2\text{O}_3$ used for sample determination, M = molarity of $\text{Na}_2\text{S}_2\text{O}_3$, W = weight of sample (g)

2.4 Greener Titrimetric Method for Determining Iodine Number

This method was modified from the AOAC titrimetric method to be a small scale titration. Weighed 0.10 g sample and dissolved it with iso-octane to 10 mL. One-mL aliquot of this solution (0.010 g sample) was mixed with IBr solution 1 mL. Shook it and incubated in the dark for 1 min. Then, added 6 % w/v potassium iodide solution 1 mL. Titrated with 0.005 M sodium thiosulfate to pale yellow. Added few drops of starch indicator and continued titrating until blue entirely disappears. Conducted blank determination. The iodine number was calculated using equation (1).

3. Results and Discussion

3.1 Optimization Studies

The parameters which affected analytical performance and the optimum condition are presented in Table 1.

Table 1. Optimization of the conditions of downscaled titrimetry

Parameters	Condition
Sample weight	0.010 ± 0.001 g
I ₂ solution	
- Concentration	0.10 M
- Volume	1.00 mL
Incubation time	1 min
Solvent	Iso-octane 1.0 mL

3.1.1 Effect of Type and Amount of Solvent

Chloroform has long been used for dissolving oil sample and its use has been banned in some countries. n-Propanol was used for an online spectrophotometric approach (Thomaidis & Georgiou, 2000) since it readily mixed with I₂ solution. However, when this mixture is mixed with an aqueous solution of potassium iodide, the emulsion is formed. In this work, dichloromethane, n-propanol, and iso-octane (Takeshita et al., 1994) were compared for use in the determination of IN. Although dichloromethane is a chlorinated solvent, it was used for comparison purpose. The results as shown in Table 2 indicated that there was no significant difference in the iodine number by using these solvents. Iso-octane was readily mixed with I₂ solution, did not form an emulsion with oil and aqueous solution. To avoid emulsion forming and to eliminate the usage of chlorinated solvent, iso-octane was chosen for dissolving oil samples.

Table 2. The determination of IN using different solvents (n=3)

Type of solvent	Palm oil		Soybean oil	
	IN	%RSD	IN	%RSD
Dichloromethane	53.6	2.6	131.7	2.2
n-Propanol	58.5	0.7	136.5	1.1
iso-Octane	55.4	0.3	125.6	0.9

It was also attempted to reduce the volume of solvent used for dissolving oil samples. By utilizing the procedures described in section 2.4 but using the different volumes of solvent, iso-octane, i.e., 1.00, 5.00, and 10.00 mL, the resulting iodine numbers of oil samples are shown in Table 3. It was found that there was no significant difference observed in the IN of these oil samples by using 1.00, 5.00, and 10.00 mL of solvent. Therefore, 1.00 mL of iso-octane was selected for dissolving 0.01 g of oil.

Table 3. Effect of the amount of solvent for IN determination of palm and soybean oils. (n=3)

Volume of solvent (mL)	Palm oil		Soybean oil	
	IN	%RSD	IN	%RSD
1.00	55.6	0.5	127.7	0.9
5.00	56.6	1.0	129.6	1.2
10.00	56.2	1.1	128.2	1.1

3.1.2 Effect of Incubation Time

The rate and extent of the halogenation reaction depend on incubation or reaction time (Earle & Milner, 1939; Markley, 1947). As suggested in the AOAC standard method, the higher iodine number of oil has required the longer the incubation time to complete the reaction. The effect of incubation time ranges from 1 to 30 min was investigated as shown in Table 4. The results were shown that there was no significant difference in iodine number of both palm oil and soybean oil for the analysis employing the incubation time of 1, 5, 15, and 30 min (one-way ANOVA test; F-cal = 0.074, F-crit = 6.59 for palm oil, and F-cal = 0.013, F-crit = 6.59 for soybean oil). Therefore, the incubation time of 1 min was chosen. It should be noted that this incubation time is appropriate for 0.01 g of oil having iodine number equal or less than that of soybean oil.

Table 4. The effect of reaction time for IN determination of palm and soybean oils. (n=3)

Incubation time (min)	Palm oil		Soybean oil	
	IN	%RSD	IN	%RSD
1	57.6	0.5	127.9	1.2
5	57.5	0.2	128.4	0.8
15	57.4	0.2	128.9	1.0
30*	56.8	1.3	129.7	0.9

* as per AOAC method

3.1.3 Effect of the Weight of Sample

As per AOAC method, 25.00 mL of IBr solution is recommended for 5.00 g of oil sample, therefore, for 1.00 mL of this reagent should be reacted quantitatively with 0.100 g of sample. The weight of soybean oil ca. 0.01, 0.02, and 0.05 g were studied for its effect on IN. The IN (mean \pm S.D., n=3) of 131.8 ± 3.3 , 102.1 ± 0.2 , and 45.9 ± 0.1 were obtained with the sample weight of 0.01, 0.02, and 0.05 g, respectively. The reaction of IBr with sample requires 50-60% excess of this reagent as mentioned in AOAC method (Helrich, 1990). Therefore, sample weight of 0.010 g of soybean oil and other oils with IN less than that of the soybean oil is appropriate for reacting with 1.00 mL of 0.10 M IBr solution.

3.1.4 Effect of Concentration and Volume of IBr Solution

Though the concentration of IBr solution of 0.10 M is stated in AOAC method, it was investigated whether or not the lower concentration of this reagent can be used. The effect of concentration of IBr solution was investigated by analysis of soybean oil with different concentrations of IBr solution ranged from 0.025 to 0.100 M, IN (mean \pm SD, n=3) obtained were 44.5 ± 1.1 , 82.5 ± 0.8 , 109.5 ± 1.5 , and 127.3 ± 1.3 , respectively. This results are also in consistency with the analysis of palm oil and sunflower oil at IBr concentration of 0.050 and 0.10 M. The volume of IBr solution was also investigated and the results are shown in Table 5.

Table 5. The effect of volume of IBr solution for determination of IN (n=3)

Sample	Volume of Hanus solution (mL)			IN by AOAC method
	1.00	1.50	2.00	
Coconut	5.6 ± 1.4	6.6 ± 1.3	3.4 ± 0.7	6.5 ± 0.7
Oleic acid	90.2 ± 1.3	91.1 ± 2.4	92.8 ± 4.5	90.3 ± 1.1
Linolenic acid	143.1 ± 1.0	153.3 ± 3.5	157.8 ± 6.0	154.9 ± 1.0
Soybean	121.7 ± 4.2	132.1 ± 3.5	135.0 ± 3.7	124.6 ± 1.5
Ricebran	97.7 ± 3.2	98.5 ± 3.6	104.8 ± 4.2	100.1 ± 1.2
Sunflower	116.5 ± 1.6	119.5 ± 2.3	135.2 ± 1.7	126.2 ± 1.1
Palm	59.1 ± 2.0	58.7 ± 2.6	61.3 ± 2.4	58.8 ± 1.0

It is shown in Table 5 that the volume of reagent had affected on the IN of linoleic acid, soybean oil, rice bran oil, and sunflower oil. For oil samples having IN less than 100, 1.00 mL of reagent is appropriate. However, for oil having IN more than 100, 2.00 mL of reagent should be used.

3.2 Real Sample Analysis

The proposed greener titrimetric method was applied for determination of IN of real oil samples. Oleic acid and nine vegetable oil samples were subjected to the analysis. The analytical results are shown in Table 6. To test whether or not the results obtained by both methods were different, the paired t-test was performed. The observed t-value, $t_{cal} = 0.011$, was less than the critical t-value, $t_{crit} = 2.10$, therefore, there was no significant difference between the results obtained by the proposed method and the AOAC standard method. The developed method has superior advantages on at least 25 folds reducing the amounts of reagent and analysis time, and consequently producing very small amounts of waste solution.

Table 6. Analytical results for IN determination of edible oils by greener titrimetric method (n=3)

Oil	AOAC standard method		Proposed method		% Difference
	IN	%RSD	IN	%RSD	
Coconut	7.5	17.1	6.5	11.1	14
Palm	59.0	0.3	58.8	1.7	1
Olive	82.1	0.6	84.4	0.9	3
Rice bran	100.1	0.5	101.9	2.0	2
Sesame	108.8	0.4	106.2	1.3	2
Canola	109.9	0.6	106.5	2.3	3
Corn	112.3	0.7	110.2	2.1	2
Sunflower	126.2	0.5	127.3	0.4	1
Soybean	126.8	0.8	124.6	1.2	2
Oleic acid	85.9	0.5	90.3	1.3	5

4. Conclusions

The greener titrimetric method for determination of iodine number of edible oils provided various advantages such as short analysis time, low reagent and solvent consumption. By reducing the sample size, reagent, solvent, and reaction time could be dramatically reduced. The amount of solvent and reagent used were at least 25 times less than that of the classical standard method. By using simple titration method, there is no significant difference between the results obtained by the proposed method and the standard method for the IN in the range of 6.0-130.

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