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Article

Simple Methods via Mid-IR or ^{1}H NMR Spectroscopy for the Determination of the Iodine Value of Vegetable Oils

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Two methods for determining the iodine value in vegetable oils are described. One employs mid-infrared (mid-IR) spectroscopy and the other uses hydrogen nuclear magnetic resonance (^{1}H NMR). The determination of the iodine value is based on either the transmittance intensity of mid-IR signals or on the ^{1}H NMR signal integration and multivariate calibration. Both of the methods showed adequate coefficients of determination (r^2 = 0.9974 and 0.9978, respectively) when compared to Wijs method, which is recommended by the norm EN 14111. A statistical comparison between the results from the proposed methods and from Wijs method shows that both instrumental methods offer equivalent results and greater precisions compared to Wijs method. The regressions obtained from the constructed models were considered statistically significant and useful for making predictions. The proposed methods present several advantages compared to Wijs method because they significantly reduce analysis time, reagent consumption and waste generation. Furthermore, an analyst can choose between the mid-IR or ^{1}H NMR to determine the iodine value.

Keywords: iodine value, vegetable oils, infrared, nuclear magnetic resonance, green methods

Introduction

Oils and fats have always been associated with their nutritional characteristics and considered as raw material for industrial processes. Thus, the characterization and quality control of these products have always been important. Currently, in addition to their use as food, oils represent renewable energy sources for the production of biodiesel, which further reinforces the importance of characterizing oils and assessing their quality.[1]

Oils are essential reagents for biodiesel production. They are composed of triglycerides, and their transesterification with short-chain alcohols produces a mixture of long-chain monoesters (biodiesel) and glycerin as a byproduct.[1-3]

Characterizing oils and biodiesel is relevant for identifying fraud, contamination or adulteration, in addition to evaluating their quality.[4] The iodine value, a parameter that characterizes oils, fats and biodiesel, indicates the degree of unsaturation of these products.[3-6]

It is expected that the composition of different vegetable oils derived from the same vegetable source may vary by geographic, climatic and other factors.[7,8] However, vegetable oils are associated with average characteristic compositions. Therefore, it is possible to estimate a mean range for some characterizing parameters, such as the iodine value.[9-11]

The iodine value is a measure of the number of double bonds in a sample. It specifies the mass of iodine (I_2) consumed *per* 100 g of sample.[6,12] The iodine value of oils depends on several factors, mainly on the quantity of carbon-to-carbon double bonds present in the sample. Additional factors that influence the iodine value are the storage conditions and the age of the oil, especially if the sample has undergone oxidation processes.[12]

Considering that oxidation reactions also influence the iodine value, this index is directly related to another very important parameter in oils and in biodiesel, i.e., the oxidative stability.[6,13] Oils and biodiesels with high degrees of unsaturation, and therefore with high iodine values, are more susceptible to oxidative degradation.[5,13-16] Factors, such as high temperature and exposure to light, air and moisture, can promote the degradation of oils and biodiesels.[14-16] One of the proposed reaction mechanisms of oxidation considers the removal of an allylic hydrogen.[14-16] Based on that hypothesis, it can be readily understood that a molecule with a greater number of unsaturations would have a greater number of allylic hydrogens available to initiate oxidation reactions.

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One of the most used procedures to determine the iodine value is known as Wijs method.[12] This method is described in the American Oil Chemists' Society Cd 1-25 method,[17] and its use is determined by the norm EN 14111.[18] It is based on the reagent ICl dissolved in glacial acetic acid.[12,18] This mixture is known as Wijs reagent or Wijs solution. Wijs method yields analytical quality, however, the use of the pertinent reagent requires careful handling because of its toxicity.[3] Moreover, this procedure is relatively expensive, slow, and it consumes a significant amount of reagents, producing wastes that require a specific treatment before they can be discarded. These facts become important when large numbers of determinations are to be performed. Therefore, a safer, faster, greener and lower-cost procedure to determine iodine value (or other important parameters, such as the acid number)[2,19,20] is desirable.[21]

In this work, two instrumental analytical methods are proposed to determine the iodine value; one uses mid-infrared (mid-IR) spectroscopy, and the other uses hydrogen nuclear magnetic resonance (^{1}H NMR). These two techniques are widely used for the identification and characterization of organic compounds. From mid-IR spectroscopy, information is obtained about bond vibrations, while from ^{1}H NMR, it is possible to extract data about the molecular, structural and geometric formulas of organic compounds.[22]

It is known that oils can be formed by different types of esters. Based on this principle, Gopinath, Puhan and Nagarajan[23] built a theoretical model that used a multiple linear regression method to predict the iodine value of different biodiesels from their fatty acid methyl ester composition. Because the composition of the different esters attached to glycerol in oils, including their unsaturations, can generate different responses in both mid-IR and ^{1}H NMR techniques, the objective of the present work is to correlate these responses with the iodine value to develop two different methods for determining the iodine value.

In this context, this work proposes classical multivariate calibration with mid-IR and ^{1}H NMR signals for determining the iodine value. Once the multivariate model is built and updated regularly, it determines the iodine value in vegetable oils using safe and reliable procedures, while minimizing excessive experimental steps, analysis time, consumption of reagents and waste generation.

Experimental

Samples

All vegetable oils were purchased in a local market or donated by the Laboratory of Extraction, Applied Thermodynamics and Equilibrium (Faculty of Food Engineering, Unicamp). Eleven different sources of oils were used in this study: sunflower (*Helianthus annuus*), canola (*Brassica napus L. var. oleifera Moench*), soybean (*Glycine max*), corn (*Zea mays*), Brazil nut (*Bertholletia excelsia*, *Nobilis*, *Myrtaceae*), cottonseed (*Gossypium* spp.), rice (*Oryza sativa*), golden flaxseed and brown flaxseed (*Linum usitatissimum*), sesame (*Sesamum indicum*) and a mixed oil of sesame and toasted sesame.

Mid-IR spectra

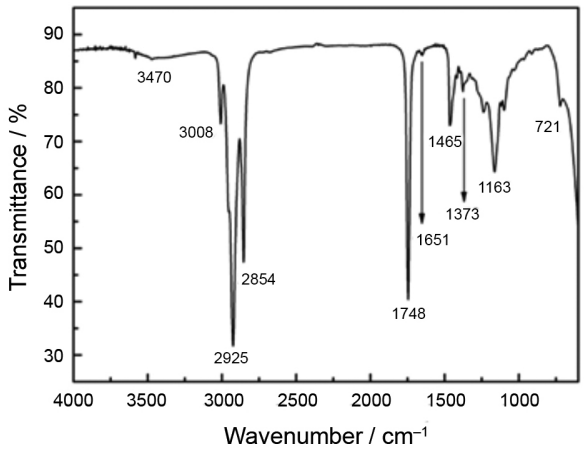
To acquire absorption spectra, each oil sample was applied as a film between two NaCl plates. The spectra were obtained in an MB102 Bomem Fourier tranform infrared (FTIR) spectrometer using the following experimental conditions: spectral width, 4000-600 cm^{-1}; spectral resolution, 0.4 cm^{-1}; number of scans, 16. Each spectrum was normalized from 0 to 1, dividing all points of the spectrum by the highest value, to mitigate the influence of the film thickness in signal intensities.

^{1}H NMR spectra

All of the ^{1}H NMR spectra were recorded in a Bruker Avance III 500 MHz NMR spectrometer. To obtain the spectra, 20 μL of each oil were dissolved in 600 μL of deuterated chloroform ($CDCl_3$), containing tetramethylsilane (TMS) as an internal reference, using the following experimental conditions: spectral width, –4.00-16.00 ppm; spectral size, 32768 points; 90º pulse, 11.75 μs; delay, 5 s and number of scans, 16.

Iodine value determination by the Wijs method (EN-14111-2003)[18]

An aliquot of the oil sample (0.13-0.15 g) was weighed to the nearest 0.001 g and dissolved in a 500 mL Erlenmeyer flask using 20 mL of solvent (prepared by mixing equal volumes of cyclohexane and glacial acetic acid) and 25 mL of Wijs solution. This solution was allowed to rest for 1 h in the dark with a blank prepared in the same manner except that it did not contain any sample. Then, 20 mL of potassium iodide solution (100 g L^{-1}) and 150 mL of distilled water were added. Titration with standardized 0.1 mol L^{-1} sodium thiosulfate solution using a 50 mL digital manual burette was carried out until the solution was a pale yellow color. Then, 3 mL of a starch solution was added. The titration was continued until the blue color disappeared. The iodine value (g of iodine *per* 100 g of oil) is given by equation 1:



Band / cm^{-1}	Assignment
3470	νO–H
3008	νC–H$_{olefinic}$
2925	$\nu_{as}CH_2$
2854	$\nu_{s}CH_2$
1748	ν_{as}C=O
1651	νC=C
1465	$\delta_{s}CH_2$
1373	$\delta_{s}CH_3$
1163	ν_{as}C–O
721	ρCH_2

Figure 1. Assignment of observed absorption peaks in mid-IR spectra, illustrated from a spectrum of sunflower oil.

$$\text{Iodine value} = \frac{12.69\ C\ (V_1 - V_2)}{m} \quad (1)$$

where C is the exact concentration (mol L^{-1}) of the standard sodium thiosulfate solution; V_1 is the volume (mL) of standard sodium thiosulfate solution used for blank test; V_2 is the volume (mL) of standard sodium thiosulfate solution used for sample titration; and m is the mass (g) of the oil sample.

Results and Discussion

The iodine values obtained by Wijs method for eleven oil samples ranged from 97.0 to 187.1 g *per* 100 g of oil. The Brazil nut oil sample presented the lowest iodine value, and the golden flaxseed oil the largest. The range between these two samples was the working range used to build the multivariable calibration model.

All of the iodine values obtained in this work are in agreement with the data given in the literature.[9] In other words, all of the iodine values are within the expected average range for each source. For example, according to the literature[9] it is expected for soybean oil an iodine value between 118 and 139 g *per* 100 g, and the iodine value for the sample analyzed in this work by Wijs method was 130.4 g *per* 100 g.

Figures 1 and 2 show the assignments of mid-IR and ^{1}H NMR signals, respectively.

To develop the methods using mid-IR and ^{1}H NMR techniques, six mid-IR signals and five ^{1}H NMR signals were chosen to provide further differentiation between the structures of the samples. These signals were mainly related to the double bonds, the positions of the double bonds, and the size of the carbon chains of the esters.

The wavenumbers of the selected mid-IR signals were 721, 1465, 1651, 2854, 2925 and 3008 cm^{-1}. The transmittance intensity values (T) of these signals were considered as variables to build the multivariate model.

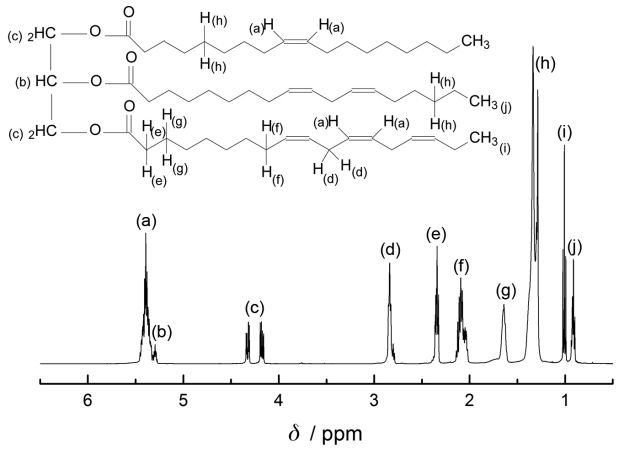


Figure 2. Assignment of hydrogens in the ^{1}H NMR spectra, exemplified from a ^{1}H NMR spectrum of golden flaxseed.

For the ^{1}H NMR spectra, the chemical shifts of the five selected signals were 0.99, 1.30, 2.00, 2.80 and 5.40 ppm. All of the signals in the spectra were integrated, and the integral of the signals at around 4.25 ppm was calibrated to 4.00, as those signals represent H-1 and H-3 of the glycerol, totaling four hydrogens. The calibrated values of the integral (I) of the five selected signals were used to perform the multivariate calibration. For the signal at 5.40 ppm, the resulting integral value was decreased by one unit because this signal contains the methylene hydrogen of H-2 of glycerol together with the olefinic hydrogen signals. Thus, only the olefinic hydrogens are taken into account at 5.40 ppm because the methylenic hydrogen H-2 of glycerol does not vary among samples.

For classical multivariate calibration, the data are organized in matrices. The **y** matrix is a column matrix that has the property of interest that is desired to be calibrated (in this case, the iodine value obtained by Wijs method), where each row represents one sample. As eleven oil samples were used, there are 11 rows in this matrix.

In the **X** matrix, the obtained experimental data are organized for all samples (rows) for each variable (column). Six mid-IR signals or five ^{1}H NMR were used as variables. The iodine value was calibrated separately using mid-IR or ^{1}H NMR data, which generated two methods for determining the iodine value, each using one of the mentioned spectroscopic techniques.

Once the **y** and **X** matrices are organized, the classical multivariable calibration is achieved by equation 2, where **β** matrix represents the coefficients that define the location of the line:[24,25]

$$\mathbf{y} = \mathbf{X}\,\boldsymbol{\beta} \quad (2)$$

$$\text{where } \mathbf{y} = \begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ \dots \\ y_{11} \end{bmatrix}, \mathbf{X} = \begin{bmatrix} 1 & x_{11} & x_{12} & x_{13} & x_{14} & x_{15} \\ 1 & x_{21} & x_{22} & x_{23} & x_{24} & x_{25} \\ 1 & x_{31} & x_{32} & x_{33} & x_{34} & x_{35} \\ \dots & \dots & \dots & \dots & \dots & \dots \\ 1 & x_{111} & x_{112} & x_{113} & x_{114} & x_{115} \end{bmatrix} \text{ and } \boldsymbol{\beta} = \begin{bmatrix} 0 \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{bmatrix}.$$

In this context, the objective is to find the β constant values, and for this, it is necessary to isolate the **β** matrix from the matrix calculations (equation 3):[24,25]

$$\boldsymbol{\beta} = (\mathbf{X}^t\,\mathbf{X})^{-1}\,\mathbf{X}^t\,\mathbf{y} \quad (3)$$

After obtaining the constants contained in this matrix, it is possible to express the iodine value based on the selected variables. Equations 4 and 5 show the general expressions for the iodine value (IV) from the mid-IR and ^{1}H NMR data, respectively.

$$IV = \beta_0 + \beta_1(T_{721\ cm^{-1}}) + \beta_2(T_{1465\ cm^{-1}}) + \beta_3(T_{1651\ cm^{-1}}) + \beta_4(T_{2854\ cm^{-1}}) + \beta_5(T_{2925\ cm^{-1}}) + \beta_6(T_{3008\ cm^{-1}}) \quad (4)$$

$$IV = \beta_0 + \beta_1(I_{5.40\ ppm} - 1) + \beta_2(I_{2.80\ ppm}) + \beta_3(I_{2.00\ ppm}) + \beta_4(I_{1.30\ ppm}) + \beta_5(I_{0.99\ ppm}) \quad (5)$$

where IV is the iodine value; β is a constant value related to the variable indicated in the subindex; T is the transmittance intensity value at the wavenumber indicated in the subindex; and I is the integration of the signal at the chemical shift indicated in the subindex.

Table 1 presents the β constants values with the related variables for the models built with the mid-IR and ^{1}H NMR spectra.

As the number of unsaturations in the oil increased, the transmittance values at 1651 and 3008 cm^{-1} decreased (i.e., an increase of the absorbance). This results in a higher iodine value and it is reflected in the negative values of β_3 and β_6, as noted in Table 1. Moreover, the low transmittance intensity related to the asymmetric stretching of $-CH_2-$ groups (2925 cm^{-1}) is also related to a higher iodine value, as indicated by the negative β_5.

The data in Table 1 show also that an increased number of olefinic hydrogens (5.40 ppm) implied an increase of the iodine value, indicated by the positive value of β_1. The other signals contributed to a lower iodine value, especially the hydrogens from long-chain monoesters (1.30 ppm).

After constructing the models, the samples were tested. Table 2 shows comparisons of the results obtained by multivariate calibrations versus the results obtained by Wijs method. Table 2 lists the iodine values predicted by mid-IR and by ^{1}H NMR of eleven different vegetable oils, and agreement can be observed with the results obtained by Wijs method.

The accuracy of the two proposed methods was verified by calculating the relative error of each sample, considering the results obtained by the Wijs method as the true values. The mid-IR method showed results with differences in relation to the Wijs method between zero and 2.6% and the ^{1}H NMR method between 0.2 and 2.1%. In terms of precision, the Wijs method generated results with relative standard deviation (RSD) ranging from 0.7 to 4.8% (mean RSD = 3.0%), while the range for the mid-IR and ^{1}H NMR methods were 0.1 to 3.3% (mean RSD = 1.7%) and 0.4 to 6.9% (mean RSD = 1.5%), respectively.

The statistical paired Student's *t*-test at a 95% confidence level was performed, and the results (Table 3) indicate complete agreement between both of the two

Table 1. β constant values, with the respective related variables, for multivariate calibration obtained from mid-IR spectra and from ^{1}H NMR spectra

Mid-IR							
Variable	1	$T_{721\ cm^{-1}}$	$T_{1465\ cm^{-1}}$	$T_{1651\ cm^{-1}}$	$T_{2854\ cm^{-1}}$	$T_{2925\ cm^{-1}}$	$T_{3008\ cm^{-1}}$
β	$\beta_0 = 334.7$	$\beta_1 = 27.18$	$\beta_2 = 278.8$	$\beta_3 = -261.6$	$\beta_4 = 136.2$	$\beta_5 = -6303$	$\beta_6 = -226.5$
^{1}H NMR							
Variable	1	$I_{5.40\ ppm} - 1$	$I_{2.80\ ppm}$	$I_{2.00\ ppm}$	$I_{1.30\ ppm}$	$I_{0.99\ ppm}$	
β	$\beta_0 = 270.4$	$\beta_1 = 6.178$	$\beta_2 = -1.415$	$\beta_3 = -2.380$	$\beta_4 = -3.109$	$\beta_5 = -0.4335$	

T: Transmittance intensity value at the wavenumber indicated in the subindex; I: integration of the signal at the chemical shift indicated in the subindex; β: a constant value related to variable indicated in the subindex.

Table 2. Iodine value predicted by mid-IR and by ^{1}H NMR, and iodine value by Wijs method

Oil	Wijs method / (g 100 g^{-1})	mid-IR / (g 100 g^{-1})	^{1}H NMR / (g 100 g^{-1})
Brazil nut	97.0 ± 3.9	96.7 ± 1.0	96.1 ± 6.6
Brow flaxseed	176.9 ± 1.3	178.8 ± 0.1	176.0 ± 0.7
Canola	112.7 ± 3.6	113.2 ± 2.2	111.6 ± 0.5
Corn	118.1 ± 3.5	118.9 ± 1.4	118.6 ± 1.3
Cottonseed	121.0 ± 4.3	120.1 ± 1.4	120.6 ± 1.8
Golden flaxseed	187.1 ± 2.7	185.9 ± 0.9	187.7 ± 3.2
Rice	104.1 ± 2.3	105.5 ± 0.8	103.9 ± 1.3
Sesame	114.0 ± 2.2	114.0 ± 3.8	115.8 ± 1.6
Sesame + toasted sesame	110.5 ± 4.8	111.3 ± 2.1	112.1 ± 0.4
Soybean	130.4 ± 6.2	130.9 ± 4.0	131.5 ± 0.7
Sunflower	130.0 ± 4.7	126.6 ± 4.0	127.3 ± 0.5

Table 3. Results from Snedecor's F-test and Student's t-test, with n = 3 and confidence level $(1 - \alpha) = 0.95$

Oil	Wijs method × mid-IR		Wijs method × ^{1}H NMR	
	Calculated		Calculated	
	$F = s_a^2 / s_b^2$	t	$F = s_a^2 / s_b^2$	t
Brazil nut	15	0.11	2.9	0.17
Brow flaxseed	169	2.03	3.4	0.85
Canola	2.7	0.17	52	0.43
Corn	6.3	0.30	7.2	0.19
Cottonseed	9.4	0.28	5.7	0.12
Golden flaxseed	9.0	0.60	1.4	0.20
Rice	8.3	0.82	3.1	0.11
Sesame	3.0	0.00	1.9	0.94
Sesame + toasted sesame	5.2	0.22	144	0.47
Soybean	2.4	0.10	78	0.25
Sunflower	1.4	0.78	88	0.81

F critical value = 19.00 ($\alpha = 0.05$); t critical value = 2.78 ($\alpha = 0.05$).[24-26]

proposed methods and Wijs method. Snedecor's F-test (Table 3) shows that, considering the mean of the relative standard deviation, the proposed methods tended to be more precise than the Wijs procedure.

The t calculated values that are shown in Table 3 clearly indicate that, at the confidence level of 95%, all results obtained with the two proposed methods were statistically equivalent to the results obtained with Wijs method. With respect to the precision, some high F values were observed: brow flaxseed for the comparison with mid-IR; and canola, sesame + toasted sesame, soybean and sunflower for the comparison with ^{1}H NMR. In all of those cases, the high F values were consequences of the results obtained with Wijs method having higher standard deviations, meaning that the proposed methods were more precise than Wijs method.

The results show the adequacy of the multivariate calibrations constructed from mid-IR and ^{1}H NMR data. In order to confirm the adequacy of the model, an analysis of the variance was performed according to Barros Neto, Scarminio and Bruns[24] and Box and Draper,[25] considering each regression as a whole.

Assuming that errors follow a normal distribution, the mean squares can be used to test whether the regression equation is statistically significant. When $\boldsymbol{\beta} = 0$, there is no correlation between **X** and **y**, and it has been demonstrated that the ratio of the mean squares follows an F distribution (equation 6):[24,25]

$$\frac{MS_R}{MS_r} \approx F_{(\nu_R, \nu_r)} \quad (6)$$

where MS_R is the mean square related to regression; MS_r is the mean square related to residual; ν_R is the degree of freedom related to regression; and ν_r is the degree of freedom related to residual.

Table 4. Analysis of variance for adjusting of the built linear models

Technique	Source	Sum of squares	Degrees of freedom (ν)	Mean square (MS)	r^2	Calculated $F = MS_R / MS_r$	F critical value (5%)
Mid-IR	Regression	8265	5	1653	0.9974	384	5.05
	Residual	21.3	5	4.3			
^{1}H NMR	Regression	8289	4	2072	0.9978	691	4.53
	Residual	17.9	6	3.0			

As equation 6 is only valid for $\boldsymbol{\beta} = 0$, this null hypothesis can be tested by using the calculated value of MS_R / MS_r and comparing it with tabled values at an appropriate confidence level. If it is verified that $MS_R / MS_r > F$, the hypothesis of $\boldsymbol{\beta} = 0$ must be discarded, showing that there is enough evidence that a linear correlation exists between the variables **X** and **y**.[24,25]

All of the analysis of variance results are presented in Table 4. The multivariable correlations provided as coefficient of determination (r^2) 0.9974 and 0.9978 for mid-IR and ^{1}H NMR, respectively.

Both of the constructed regressions have a higher value of MS_R / MS_r compared with the F critical value at a 95% confidence level, indicating that the regression equations are statistically significant. However, a correlation considered as significant by the F-test is not always useful for making predictions because the range of variation covered by the studied factors could be too small. The regression is useful for making predictions when MS_R / MS_r is at least ten times the value of the F distribution with the appropriate degrees of freedom at the selected confidence level.[24,25]

The calculated F for the regression by mid-IR was more than 76 times the F critical value, and for the regression by ^{1}H NMR, this number was even higher, 152 times larger than its respective F critical value. Thus, in addition to the regression equations being statistically significant, both of the regressions are considered useful for making predictions.

All these results demonstrate that the multivariate calibration models with mid-IR or ^{1}H NMR can be a safe and reliable alternative to Wijs method. A comparison among the three methods reveals that the two proposed methods are much faster, use a smaller amount of reagents/solvents and generate less waste compared with Wijs method. Therefore, the proposed methods can be considered to adhere to the principles of green chemistry.

There are other reports in the literature involving iodine value determination by ^{1}H NMR,[4,5,27] although they do not consider the experimental values obtained by Wijs method. The iodine value determination is based on a theoretical average molar mass derived from ^{1}H NMR.[4,5,27] While these methods do not require a calibration curve, they may predict different results compared with those obtained by Wijs method. This difference can occur because not every double bond is reactive to iodine (conjugated double bonds are not),[3] but all olefinic hydrogens are sensitive to ^{1}H NMR spectroscopy. The proposed method using ^{1}H NMR reported here does not encounter this problem because the experimental iodine value (obtained by Wijs method) is considered in the multivariable regression.

Other methods that use mid-IR or near-IR to determine iodine value are also reported in the literature; however, they employ more sophisticated calculations from chemometrics tools, which require appropriate software.[28-32]

Conclusions

This study demonstrated correlations between mid-IR and ^{1}H NMR data with iodine values. Once the model (with mid-IR or ^{1}H NMR) was built and implemented, the model demonstrated several advantages compared with Wijs method, such as the readiness of analysis with the possibility of automating the system, significant reduction in reagent consumption, and consequently low waste generation, all of which emphasize the green character of the methods.

Both multivariate calibrations have been shown to perform well and represent simple alternative methods of determining iodine value. Moreover, with mid-IR and ^{1}H NMR techniques, it is still possible to extract additional information on the character and quality of the oil samples. We highlight the results with mid-IR because this is a technique with a relatively low-cost spectrometer and low maintenance costs, and it is not necessary to solubilize the sample, unlike with ^{1}H NMR.

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