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Detection of lard contamination in five different edible oils by FT-IR spectroscopy using a partial least squares calibration model

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Abstract: Lard is defined as animal fat acquired from the adipose tissue of pigs and is not permitted for human consumption or external use by certain religions such as Islam and Judaism. Due to its low-cost availability for commercial use, it is often mixed with other vegetable oils mistakenly or deliberately and causes loss of consumer trust; hence, its detection in food products is essential. Consumers tend to know the authenticity of commercially available edible oils. However, edible oils are subjected to adulteration risks with lard, which breaches consumer rights. In the present study, we designed a transmission Fourier transform infrared spectroscopy (FT-IR)-based method for the rapid detection of lard in sunflower, canola, coconut, olive, and mustard oils. For this purpose, the selected oils were adulterated with lard in different concentration ratios (10:0, 9:1, 7:3, 6:4, 4:6, 3:7, 0:10). A single calibration model was developed for 35 standards (seven standards from each individual five oils) in the frequency range between 1078.01 and 1246.75 cm^{-1} to determine the relationship between actual adulterant concentration and FT-IR predicted concentrations using a partial least squares (PLS) method. The results of the present study indicated that FT-IR in combination with PLS has the potential to evaluate adulteration of edible oils with lard through single calibration as a rapid, nondestructive, and effective alternative method.

Key words: Edible oils, lard, detection, Fourier transform infrared spectroscopy

1. Introduction

The credibility and transparency of vegetable oils available in the market is an important topic of concern for consumers [1]. All vegetable oils contain triglycerides, diglycerides, and free fatty acids, beside other minor components such as phospholipids, terpenes, tocopherols, and sterols. Good quality oils and many phenolic compounds play important roles in lowering plasma cholesterol levels of consumers [2].

Generally, vegetable oils consist of monounsaturated and polyunsaturated fatty acids and fats along with plenty of saturated fatty acids. When one oil is mixed with any other oil in small concentration (<1%) it becomes very difficult to differentiate through a single technique [3]. The industrial use of lard along with vegetable oils is commonly practiced for producing shortenings, margarines with a specific solid content profile, for taste enhancements and introducing unique textural properties in several food products [4–6]. The food industry needs a quick, robust, reliable, and applicable method for the assurance of pure edible oils without any mixing of a contaminant, e.g., lard, to maintain consistent product quality [7]. For commercially available

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edible oils in local markets a systematic, economical, and consistent approach is required to ensure their purity [8]. Consequently, the development of new, facile, and efficient analytical methods is imperative due to commercial, ethical, religious, and aesthetic reasons. Several analytical methods including GC-MS, HPLC-MS-MS, NMR, and Fourier transform infrared (FT-IR) spectroscopy, and electronic nose and voltammetric analysis are available for the quality assurance of vegetable oils and fats [3,9]. Over the last 20 years, FT-IR spectroscopy has been widely used in research related to various fields due to substantial improvements in FT-IR spectroscopic instrumentation [10–14]. In the study of edible oils and fats, FT-IR has been recognized as an authoritative analytical tool. It is a nondestructive and rapid tool with no or minimal sample preparation. It permits the qualitative determination of organic compounds. Each organic molecule has the characteristic vibrational mode of each functional group. Therefore, each molecular group causes the appearance of bands in the infrared region at a specific frequency between 400 and 4000 cm^{-1} . Absorption frequency can be further influenced by the neighboring functional groups [14–18].

FT-IR alone is used just to confirm the presence of different functional groups for qualitative analysis. However, for quantitative analysis, chemometric models such as partial least squares (PLS) and Beer's law are applied to determine the actual concentration of analyte present in the sample. FT-IR combined with chemometrics is an excellent tool for quantitative analysis since the intensities of the spectral bands are proportional to concentration. Chemometric techniques are extensively applied, especially when the numbers of independent variables are significantly greater than the number of data points. The analyte can be quantitatively determined by multivariate data analysis techniques such as principal component analysis (PCA), PLS regression, and Beer's law. Among the chemometric techniques, PLS has been frequently used for quantitative determinations. PLS regression is a statistical model that provides linear regression by projecting the predicted values and the actual values into a new space. The benefits of PLS are only applicable when the true correlation between the actual values and predicted values is linear or at least near to linear. The performance of PLS regression is considered to be poor if it is not linear [19–24]. FT-IR together with chemometric analysis has also been already used to detect the presence of lard in chocolates, ham sausages, meatballs, and animal fats with the combination of PLS, PCA, and discriminant analysis [4,5,25–27]. The method reported by Rohman et al. [28] is arduous and focused on analyzing different oils individually at various IR frequencies by referring to first and second derivatives to detect lard, which is a laborious and lengthy procedure. Previous reported FT-IR methods related to the determination of lard are based on a single oil and their developed calibrations could be used for just that specific oil. If the oil is changed then a new calibration will be required with changed parameters. For the first time in the present study a general calibration model is reported for all studied oils and only one calibration model is applicable using the same wavelength and the same parameters. The majority of consumers use industrial vegetable oils and the chances of adulteration in them are higher than in oil extracted from natural sources. Therefore, it was planned to establish a method based on the actual scenario. Furthermore, when oil is extracted from the seeds or fruits it is known as crude oil and after industrial processing (neutralization, bleaching, deodorization, etc.), it can be used for human consumption. Therefore, the authors selected industrial vegetable oils for the present study. To the best of our knowledge, to date no study has been reported in which FT-IR spectroscopy is used for detection of lard through common or single calibration for five different oils. Therefore, the aim of the present study was to use FT-IR spectroscopy for the detection of lard adulteration in commonly used edible oils and to develop a robust, rapid, green, and solvent-free FT-IR method for the detection of lard present in five different vegetable oils through a single PLS calibration model.

2. Materials and methods

2.1. Test samples, reagents, and standards

Five samples of pure edible oils, namely sunflower, canola, coconut, olive, and mustard oils, were collected from edible oil processing facilities in Karachi, Pakistan. Lard and IR grade potassium bromide for making IR pellets were purchased from Sigma Aldrich.

Thirty-five standards were prepared by mixing lard in sunflower, canola, coconut, olive, and mustard oils (w/w) in the ratios of 10:0, 9:1, 7:3, 6:4, 4:6, 3:7, and 0:10. Before mixing, the lard and edible oils were heated at 60 °C to make them homogenize. The prepared standards were transferred to 5-mL amber glass reagent bottles after passing through nitrogen gas. The ratio of prepared standards (edible oils:lard oil) and the actual concentrations of lard in the sunflower, canola, coconut, olive, and mustard oils are presented in Table 1, while the mixing ratio of lard in the edible oils for the preparation of KBr pellets and their codes are provided in Table 2. The same procedure was applied for the preparation of corn oil samples purchased from the local market to check the efficiency of the FT-IR method developed.

Table 1. Ratio and concentration of lard in the sunflower, canola, coconut, olive and mustard oils.

Ratio of prepared standards (Edible oil:lard)	Concentrations of lard in the sunflower, canola, coconut, olive, and mustard oils
10:0	0.500 mg lard + 0.000 mg pure edible oils
9:1	0.050 mg lard + 0.450 mg edible oils
7:3	0.150 mg lard + 0.350 mg edible oils
6:4	0.200 mg lard + 0.300 mg edible oils
4:6	0.300 mg lard + 0.200 mg edible oils
2:8	0.400 mg lard + 0.100 mg edible oils
0:10	0.000 mg of lard + 0.500 mg pure lard

Table 2. Mixing ratio of vegetable oils and their codes.

Sample Code	Sunflower:lard (%)	Sample code	Canola:lard (%)	Sample code	Coconut:lard (%)	Sample code	Olive:lard (%)	Sample code	Mustard:lard (%)
FM1A	10:0	FM2A	10:0	FM3A	10:0	FM4A	10:0	FM5A	10:0
FM1B	9:1	FM2B	9:1	FM3B	9:1	FM4B	9:1	FM5B	9:1
FM1C	7:3	FM2C	7:3	FM3C	7:3	FM4C	7:3	FM5C	7:3
FM1D	6:4	FM2D	6:4	FM3D	6:4	FM4D	6:4	FM5D	6:4
FM1E	4:6	FM2E	4:6	FM3E	4:6	FM4E	4:6	FM5E	4:6
FM1F	2:8	FM2F	2:8	FM3F	2:8	FM4F	2:8	FM5F	2:8
FM1G	0:10	FM2G	0:10	FM3G	0:10	FM4G	0:10	FM5G	0:10

2.2. FT-IR spectral measurements

A Bruker Vector 22 spectrometer was used to record the FT-IR spectra, and 0.50 mg of pure edible oils and standards (prepared by mixing of lard in five edible oils in the ratio shown in Figure 1) was added to 99.50 mg of KBr to make a pellet of 100 mg {Mallah, 2015 #28}. In order to get similar and reproducible results, homogeneous pellets were made with the same pressure. The FT-IR region of 4000–400 cm⁻¹ was selected to

record the spectra at 10 scans/min with the resolution of 2 cm^{-1} and deuterated triglycine sulfate was used as a detector. Three spectra were recorded for each sample and a fresh background was recorded prior to each sample collection.

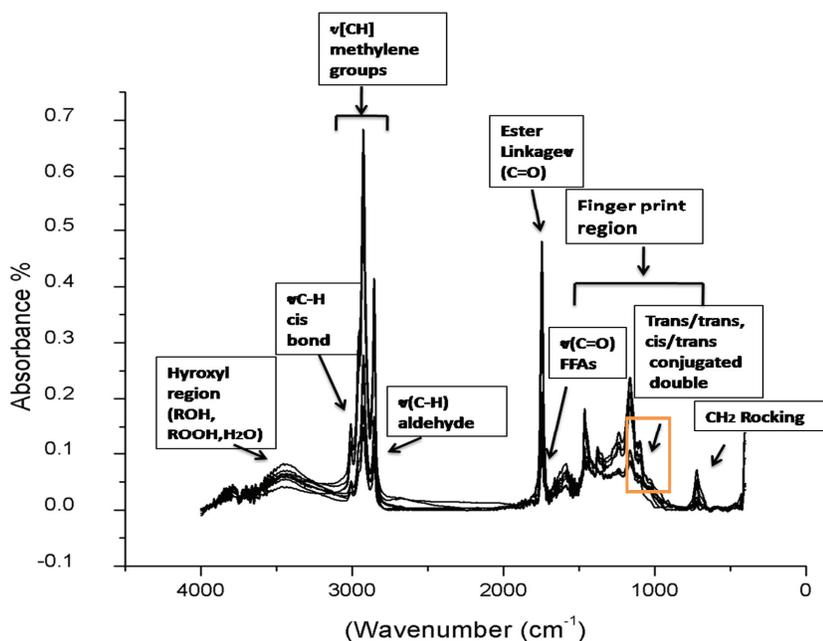


Figure 1. Group FT-IR spectra of the selected five oils and lard.

For validation, different samples of each vegetable oil containing lard were prepared other than the calibration set. All prepared calibration and validation sets were analyzed by FT-IR spectrometer.

2.3. Partial least squares chemometric analysis

Chemometric techniques coupled with FT-IR analysis have been used for a wide range of applications in food products due to their capability of assessing the fragmented range of infrared regions and extracted required information in the form of a calibration model [21]. This factorial multivariate model of calibration works on the assessment of scores and loadings along with process systems containing independent variables in relation to the data points [16].

Turbo Quant (TQ) Analyst 9 software was used to assess the spectra and convert them into a PLS calibration model for detection in the five edible oils. It imports directly the group spectra file of all standards from OMNIC 9 software, in which all spectra were recorded. It generates the PLS calibration and provides the predicted value on the basis of actual values using a specific FT-IR region ($1246.75\text{--}1078.01\text{ cm}^{-1}$). A series of 35 calibration standards covering the edible oil to lard ratios of 10:0, 9:1, 7:3, 6:4, 4:6, 3:7, and 0:10 were analyzed by transmission FT-IR mode. Group spectra of the five pure oils (sunflower, canola, coconut, olive, and mustard) along with spectra of these oils spiked with known concentrations of lard were imported from OMNIC software into the TQ Analyst program to develop PLS calibrations. Different spectral regions were tried but the specific FT-IR region was selected on the basis of the maximum values of the coefficient of determination (R^2) and the minimum values of the root mean standard error of calibration (RMSEC). A suitable calibration region

was identified, which was used for the single calibration for the five selected oils. The PLS regression model used for calibration between the actual values and predicted values was in the frequency range of 1078.01–1246.75 cm^{-1} . The performance of the calibrations was assessed by the results of linear regression and evaluated by running the samples of known concentrations of lard in the selected oils. To evaluate the competence of the models to fit the calibration data and to calculate the deviation of the models, the RMSEC was used. The RMSEC is basically the calibration error related to the residuals of the calibration data. The value of RMSEC was provided by TQ Analyst 9 [16,17,21].

2.4. Efficacy of the FT-IR method

The efficacy was determined by recovery through the standard addition method [29]. The same ratios of lard and corn oil were mixed that were used for the calibration and then the developed FT-IR calibration model was applied to check the amount of lard added to the corn oil. Percent recovery was calculated by the following equation:

$$R(\%) = D \times 100/B,$$

where R is recovery (%) of lard, B is total concentration of lard added, and D is the total concentration of lard determined by FT-IR.

3. Results and discussion

3.1. FTIR spectra of oils

All vegetable oils showed weak infrared absorbance in the range 4000–3100 cm^{-1} . In the region of 3100–400 cm^{-1} , certain shoulders and peaks are responsible for the presence of specific functional groups and are dedicated to certain fatty acids [30]. Figure 1 shows group FT-IR spectra of the selected five oils and lard.

The wavelength of 3010 cm^{-1} is specified to trans=CH stretching vibration; 2954 cm^{-1} for asymmetrical stretching of $-\text{CH}_3$; 2922 cm^{-1} for symmetric stretching of $-\text{CH}_2$; 2853 cm^{-1} for asymmetric stretching of $-\text{CH}_2$; 1715 cm^{-1} for carbonyl stretching frequency; 1665 cm^{-1} for cis C=C stretching frequency; 1462 cm^{-1} for bending frequency of $-\text{CH}_2$; 1417 cm^{-1} for bending frequency of cis =CH; 1337 cm^{-1} for bending frequency of $-\text{CH}_2$; 1120 cm^{-1} , 1098 cm^{-1} , and 1032 cm^{-1} for carbonyl stretching frequency; 965 cm^{-1} for the out of plan bending frequency of trans $-\text{CH}=\text{CH}-$; 871 cm^{-1} for the wagging of CH_2 ; and 723 cm^{-1} for the out of plan bending of $-\text{CH}=\text{CH}-$ [31].

In Figure 2 the comparative spectra of the five edible oils and lard are represented. Visually the spectra seem to be equivalent except for some minor differences. The major peaks are the same in all of these spectra due to the presence of common functional groups. In the spectrum of coconut, a peak at 3010 cm^{-1} is not visible and at 1100 cm^{-1} only one peak is observable, which is shown in the circle [32]. Moreover, the peak at 750 cm^{-1} is also smaller than that for the other oils. The position of the peak at 1747 cm^{-1} and the shape of the peak at 1650 cm^{-1} in the FT-IR spectrum of lard is different than that of the other edible oils. A minor difference is noticeable in the height of peaks present in the FT-IR spectral region between 1130 and 1090 cm^{-1} [33].

In Figure 3, some specific regions are displayed that show differences in the absorption frequency of the concerned oils. By zooming in on the individual spectra, one can distinguish the lard and canola, sunflower, olive, coconut, and mustard oils from each other. In the region of 3006 cm^{-1} the absorbance is due to the

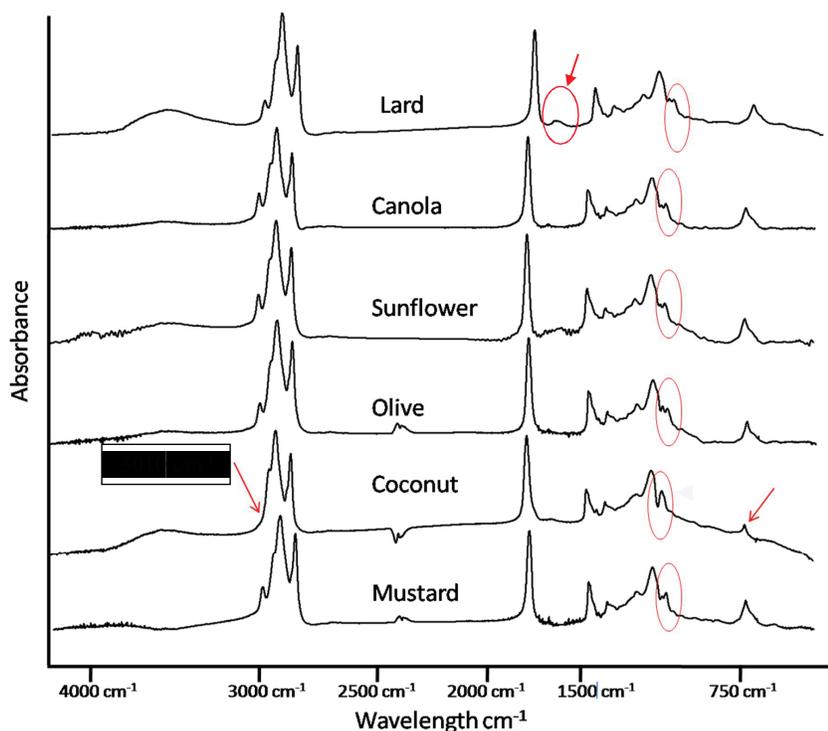


Figure 2. Stacked spectra of the edible oils and lard.

presence of methyl oleate (C18:1), which is in descending order as olive, canola, lard, sunflower, mustard, and coconut [34]. The absorbance at 3010 cm^{-1} is due to the degree of unsaturation attributed to methyl linoleate (C18:2), which is in ascending order as olive, lard, mustard, coconut, canola, and sunflower, as predicted by FT-IR spectra. The maximum absorbance in the region of 1753 cm^{-1} represents high content of the short carbohydrate chain and is in the descending order of coconut, lard, sunflower, mustard, canola, and olive. The region at 1665 cm^{-1} gives information about the C=C link and is representative of polyunsaturated fatty acid in the molecule and is in ascending order as coconut, lard, olive, mustard, canola, and sunflower. The region at $1440\text{--}1445\text{ cm}^{-1}$ represents vibration of deformation (CH) and total unsaturation, which is greatest in olive and least in coconut [35].

3.2. PLS calibration model

In order to build a calibration model, initially all the spectra were scanned in the full frequency region ($4000\text{--}400\text{ cm}^{-1}$) and afterwards two specific regions were selected for the development of a regression curve as predicted by the TQ Analyst software. The first range in this context was $713.53\text{--}732.82\text{ cm}^{-1}$, while the other range was $1246.75\text{--}1078.01\text{ cm}^{-1}$. The results obtained using the first range were not very useful; however, the second range revealed brilliant regression output. The results were obtained in normal mode without using first or second derivative FT-IR spectra.

Initially, an FTIR-PLS calibration model was developed for each individual oil using the region of $1246.75\text{--}1078.01\text{ cm}^{-1}$ and excellent regression results were achieved. Then using the same parameters and the same FT-IR region, a single calibration model for canola, sunflower, coconut, olive, and mustard oils for the detection

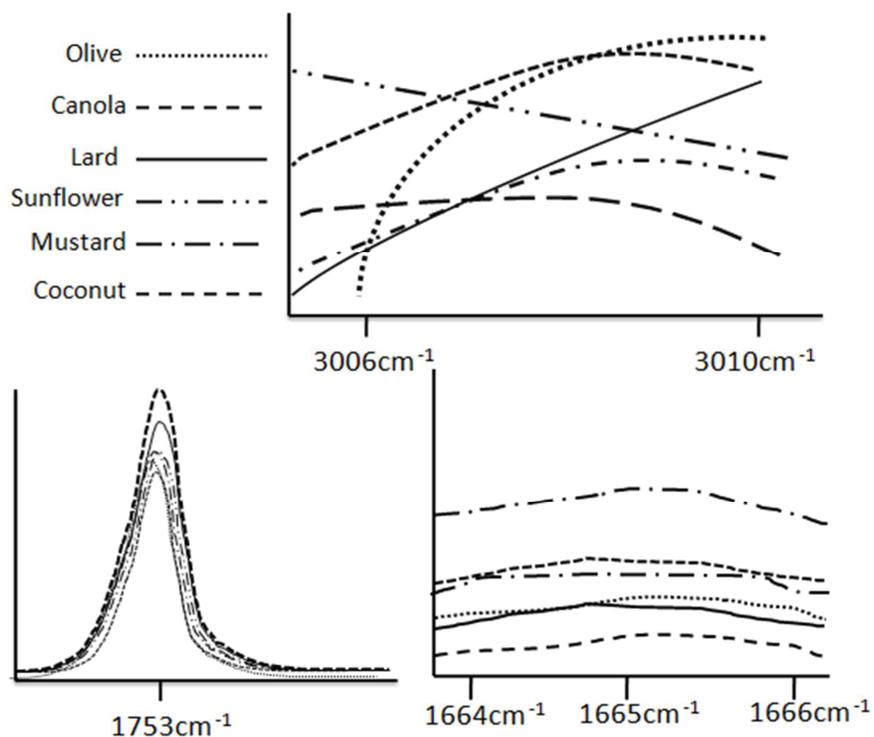


Figure 3. Representative spectra of the oils at different wavenumbers.

of lard adulteration was developed, which also provided encouraging results. PLS regression was performed and the relationship between the actual values of lard in the edible oils and the selected FT-IR region was developed as shown in Figure 4. Moreover, the details of the actual concentration of lard and the FT-IR predicted values obtained by TQ Analyst are shown in Table 3.

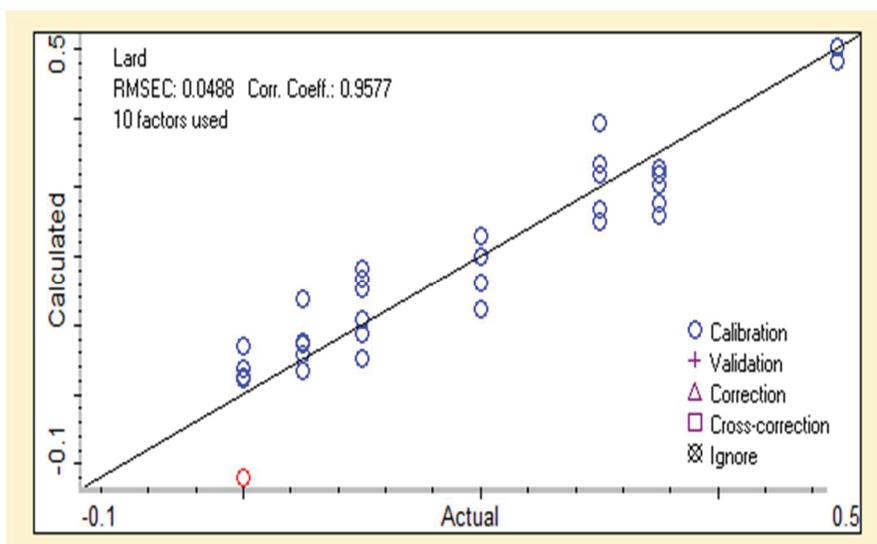


Figure 4. TQ Analyst calibration for lard mixing in the region of 1246.75–1078.01 cm^{-1} using PLS.

Table 3. Actual concentration of lard mixing in all vegetable oils and FT-IR predicted values of lard obtained by TQ Analyst.

Actual values of lard in each oil (mg)	FT-IR predicted values for the five edible oils (mg)				
	Sunflower	Canola	Coconut	Olive	Mustard
0.000	0.049	0.013	0.047	0.037	0.042
0.050	0.071	0.126	0.071	0.087	0.070
0.150	0.175	0.167	0.119	0.194	0.171
0.200	0.210	0.246	0.165	0.220	0.180
0.300	0.329	0.303	0.270	0.270	0.331
0.400	0.382	0.362	0.333	0.333	0.371
0.500	0.496	0.468	0.470	0.480	0.500

A quantity of lard is added gravimetrically (w/w) to the individual oil to develop the PLS model and obtain the relationship between actual and predicted values. In the PLS model 35 calibration standards were used to develop the regression curve. The difference between the calculated values and the predicted values is very small and the developed PLS model works as an accurate tool to measure lard in vegetable oils. A correlation coefficient of 0.9577 was achieved with the developed model. For the calibration of individual oils and common calibration, the regression coefficient was found to be excellent but combined PLS calibration is quick in determining the presence of lard in different types of edible oils. The RMSEC of the model is 0.0488. The major success of the developed model lies in its capacity to differentiate lard from other oils in a single calibration set.

3.3. Validation of the FT-IR method

Method validation was performed according to AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals [36]. A recovery test on the corn oil was carried out by the addition of a known amount of lard as used for the preparation of calibration standards. The recovery test (Table 4) and statistical figures of merit (Table 5) for the validation of the FT-IR method indicated satisfactory performance. These results clearly revealed that there is no substantial effect of the type of oil, which was different from the oils used in the calibration. Therefore, it could be assertively claimed that the proposed FT-IR method is an accurate, rapid, and green approach to evaluate the level of lard in edible oils.

4. Conclusion

FT-IR along with PLS multivariate calibration proved to be an accurate technique for detecting and quantifying the amount of lard mixed with sunflower, corn, coconut, olive, and mustard oil. The results are comparable to the concentration of lard in binary mixtures with other oil. The correlation coefficient of 0.9577 is produced in the region of 1078.01–1246.75 cm^{-1} and the RMSEC of the model is 0.0488. The best feature of this model is its robustness and accuracy. The proposed method is ecofriendly, economical, and solventless, and avoids the lengthy procedure of sample preparation compared to techniques such as gas chromatography. This method can effectively be adopted by food certifying agencies and food departments, which regularly check the authentication of vegetable oils for identification and quantification of lard in vegetable oils.

Table 4. Recovery test of neutral oil by standard addition method by FT-IR.

A (mg)	B (mg)	C (Total of A & B) (mg)	D Amount of lard by FT-IR (mg)	Difference between B and D (mg)	Relative difference (RD %)	Recovery (%)	Acceptable recovery [39] (%)
0.500	0.000	0.5 mg	0.003	-0.003	-	-	90-108
0.450	0.050		0.045	0.005	10	90	
0.350	0.150		0.16	-0.01	-6.666	105.3333	
0.300	0.200		0.213	-0.013	-6.500	106.5	
0.200	0.300		0.313	-0.013	-4.333	104.3333	
0.100	0.400		0.355	0.045	11.250	98.75	
0.000	0.500		0.53	-0.03	-6.000	106	

A = corn oil; B = actual amount of lard; C = total concentration of corn oil and lard; D = amount of lard determined by FT-IR.

Table 5. Statistical figures of merit for FT-IR method validation.

Parameters	Figures of merit
Mean recovery (%)	101.375
Correlation coefficient	0.9836
Limit of detection (LOD) (mg)	0.010
Limit of quantification (LOQ) (mg)	0.030

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