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ORIGINAL PAPER

Determination of Free Fatty Acids in Edible Oils with the Use of a Variable Filter Array IR Spectrometer

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Abstract The feasibility of employing a portable variable filter array (VFA) IR spectrometer equipped with a transmission flow cell to quantitatively analyze edible oils or biodiesel feedstocks for free fatty acids (FFA) was evaluated. The approach to FFA determination employed was based on a previously reported FTIR method that involves the extraction of FFAs into methanol containing the base NaHNCN, which converts the FFAs to their salts, followed by measurement of the carboxylate absorbance at $\sim$1,573 cm^{-1} in the spectrum of the methanol phase. When this methodology was implemented on the low-resolution VFA-IR spectrometer, the analytical performance was comparable to that of conventional FTIR instrumentation at FFA concentrations of <1%. However, at higher FFA levels, the relatively weak pulsed IR source of the VFA-IR spectrometer was found to provide insufficient energy for accurate measurement of the carboxylate absorption superimposed on the strong methanol absorption at $\sim$1,450 cm^{-1}. By changing the extraction solvent to ethanol (EtOH), good spectra and calibrations could be obtained over an FFA range of 0–5%, having an overall SD of ±0.07% FFA. Based on this assessment, a VFA-IR spectrometer provides an economical instrumental means for at-line monitoring of FFA levels in crude and refined edible oils and biodiesel feedstocks, capable of analyzing $\sim$20–30 prepared samples per hour.

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Introduction

Extensive work in our laboratory directed toward the development of Fourier transform infrared (FTIR) spectroscopy as a quantitative tool for the analysis of fats and oils as well as lubricants has resulted in a variety of IR analytical methods for these products [1, 2]. Although this work has focused on FTIR analysis, for certain applications the powerful capabilities provided by FTIR spectroscopy may not be required once an analytical method has been developed and is to be implemented. Thus, IR instruments with more limited resolution or wavelength range may suffice for some dedicated applications, as exemplified by milk analysis [3, 4], where simple, fixed-wavelength filter instruments are more than adequate. In this context, the recent development of miniaturized, low-resolution IR spectrometers with no moving parts through the combined use of variable filter array (VFA) technology and electronically modulated (pulsed) sources affords the possibility of designing simple, low-cost portable analyzers. These spectrometers are constructed by interposing a transmission cell or ATR accessory between a pulsed IR source and a linear variable filter (LVF), mounted on a pyroelectric infrared detector array [5]. LVFs are wedge-shaped interference filters that pass a range of wavelengths across the detector array, with the nominal spectral resolution attained being dependent on both the type of LVF and the size of the detector array. At the present stage of development of this technology, the nominal spectral resolution is >10 cm^{-1}, as compared to ≤2 cm^{-1} for most

FTIR spectrometers, and the resolution is further reduced by both optical and thermal "bleed" between adjacent pixels of the detector array [5]. This low spectral resolution and the limited wavelength range of VFA-IR spectrometers (by comparison with FTIR spectrometers) clearly restrict their applicability but are sufficient for many practical applications.

Among the various IR methods that have been developed for fats and oil analysis, we deemed the determination of free fatty acid (FFA) content to be the most suitable candidate for implementation on a VFA-IR spectrometer. FFA content is an important quality or process control variable since it strongly affects the utility and value of an oil when used as an edible oil or as feedstock for biodiesel methyl ester production and is also a key determinant of oil quality in frying operations. The FFA content of oils is traditionally determined by titrating an alcoholic solution of the oil with a strong base to a phenolphthalein end point [6]. Although standardized and relatively simple, such titrimetric methods are tedious, consume substantial amounts of solvent and use environmentally problematic reagents, as well as being of limited reliability when dark, crude oils are analyzed. FTIR spectroscopy provides an instrumental means of determining FFA content, and numerous FTIR methods for this purpose based on a wide variety of approaches have been described in the literature [7–11], among which the most sensitive and versatile is that developed by Al-Alawi et al. [10]. This paper describes the implementation of the latter method on a VFA-IR spectrometer, including modifications made to both the instrument and the method to optimize analytical performance, and evaluates its capability to quantitatively determine FFA content of edible oils relative to that of a conventional FTIR spectrometer.

Materials and Methods

Reagents

Two extraction solvents were used in this work: reagent grade MeOH and absolute EtOH, both dried over molecular sieves for 24 h prior to use. Reagent-grade oleic acid, used as the fatty acid standard, and sodium hydrogen cyanamide (NaHNCN; also known as sodium carbodiimide) were obtained from Sigma-Aldrich (St Louis, MO). NaHNCN was dissolved in the extraction solvents at concentrations of $\sim$2 g/L of absolute EtOH or $\sim$9 g/L of MeOH; dissolution of the salt was facilitated by mild heating in a microwave oven, and these concentrations correspond to the maximum amounts that could be readily dissolved under these conditions. The MeOH solution was "aged" for a minimum of 4 days prior to use to allow for completion of the cyanamide to carbodiimide conversion (NaHN-C$\equiv$N $\rightarrow$ NaN=C=NH) that occurs in MeOH, as indicated by the complete disappearance of the ν(C$\equiv$N) band at 2,100 cm^{-1} [10]. Aging was not required for the EtOH solution owing to the reasonable stability of the cyanamide form, which underwent slow transformation to the carbodiimide form over a period of 60 days. Refined, bleached and deodorized (RBD) oils of various types were purchased from local retail outlets and were used for the preparation of calibration standards.

Calibration

For the preparation of calibration standards, locally purchased RBD oils were run through an activated silica gel column to remove any residual FFAs that may have been present and were then gravimetrically spiked ($\pm$0.0001 g) with varying amounts of oleic acid to obtain two sets of calibration standards spanning FFA ranges of 0–1 and 0–5% (expressed as percent oleic acid), respectively. These standards were prepared for FTIR analysis in accordance with the sample preparation protocol of the NaHNCN-based FTIR method for FFA determination described previously [10]. For the calibration range of 0–1% FFA, 6 g ($\pm$0.0001 g) of oil was weighed into a tared 15 mL clinical centrifuge tube, and 6 mL of the NaHNCN/MeOH extraction solvent was then added to the tube with a calibrated propipette. In the case of the 0–5% FFA calibration, 10 mL of the NaHNCN/EtOH extraction solvent was added to 2 g of oil. Following addition of the NaHNCN–containing extraction solvent, samples were mixed on a vortex mixer for 30 s and then centrifuged for 2 min at 5,000 rpm to separate the oil and alcohol layers. The alcohol layer in each tube was then aspirated by vacuum into a 100 μm CaF_2 transmission cell and its spectrum recorded as described below. Calibration equations relating FFA concentration to the carboxylate absorption in the spectrum of the alcohol layer were derived from duplicate spectra of each standard. Spectral data processing and statistical analysis were carried out using TQ Analyst 7.2 (Thermo Electron Inc.) and Origin.

Instrumentation

An InfraSpec VFA-IR spectrometer (Wilks Enterprise, South Norwalk, CT) controlled by proprietary software running on a Windows platform (Windows 98 or higher) was employed for this study; key instrument specifications are presented in Table 1. This instrument collects a spectrum every second (i.e., with each pulse of the electronically modulated source), and a pre-set number of spectra can be collected consecutively and co-added automatically to increase the S/N ratio; for the present

Table 1 Specifications of the Wilks InfraSpec VFA-IR spectrometer employed in this study

Characteristic	Specification
Dimensions	5″ × 5″ × 1.5″; 12.7 × 12.7 × 3.8 cm^3
Weight	3.5 lbs, 1.5 kg
PC interface	RS 232
Power requirements	9 V DC, 2.0 amps
Power supply	Universal AC/DC converter type
Suggested operating range	15–60 °C
Detector array	64-pixel linear pyroelectric array
Spectral range	5.5–11 μm (1,818–910 cm^{-1})
Nominal spectral resolution[a]	14 cm^{-1}

[a] Effective spectral resolution is ~30 cm^{-1}

study, the optimal number of co-added scans was empirically determined to be 32. The InfraSpec spectrometer was equipped with a 100 μm CaF_2 flow cell (International Crystal Laboratories, Garfield, NJ) with Luer-Lok fittings. The EtOH or MeOH extracts were loaded into the cell by aspiration using a stainless steel Luer-Lok needle (Fig. 1). A two way valve controlled the loading and emptying of the cell, and a solvent trap was located in the outlet line.

For comparative purposes, the InfraSpec was run side-by-side with a Bomem WorkIR FTIR spectrometer (ABB Bomem, Quebec City, QC, Canada), sharing the same cell and cell loading assembly. The FTIR spectra were collected at a resolution of 4 cm^{-1} by co-adding 16 scans and were ratioed against a background spectrum collected with the methanolic reagent solution in the cell to eliminate its contributions from the spectra of the samples.

Fig. 1 The InfraSpec VFA-IR spectrometer with transmission flow cell and Luer-Lok needle used to aspirate the sample into the flow cell

Results and Discussion

The fats and oils sector requires simple, rugged at-line instrumentation capable of analyzing key quality and process control parameters such as FFA content, whether in relation to processing of edible oils or screening of biodiesel feedstocks. Such requirements generally cannot be met by conventional FTIR spectrometers, and ruggedization of these instruments to permit their use (and survival) outside a controlled laboratory environment, while possible for some suitably designed models, is extremely costly. In contrast, because VFA-IR spectrometers are highly compact and have no moving parts, these low-cost instruments are rugged and portable. However, these characteristics come at a price, in terms of analytical capabilities relative to FTIR spectrometers, and the implementation of a quantitative IR method on a VFA-IR spectrometer requires careful assessment on a method-by-method basis and, even if feasible, may require modifications of the original methodology.

In the present study, the feasibility of employing a VFA-IR spectrometer for the determination of FFA content by adapting methodology previously developed for FTIR spectrometers by Al-Alawi et al. [10] was investigated. As illustrated in Fig. 2, this FTIR method involves mixing oil with an immiscible solvent (MeOH) containing the weak base sodium hydrogen cyanamide in equilibrium with its tautomeric form sodium carbodiimide, thereby extracting the FFAs and stoichiometrically converting them to their respective sodium salts. The absorbance at the carboxylate band in the FTIR spectrum of the MeOH phase, recorded in a 100-μm transmission cell, is then measured, and the FFA content is predicted from a calibration devised from standards prepared by gravimetric addition of a pure fatty acid

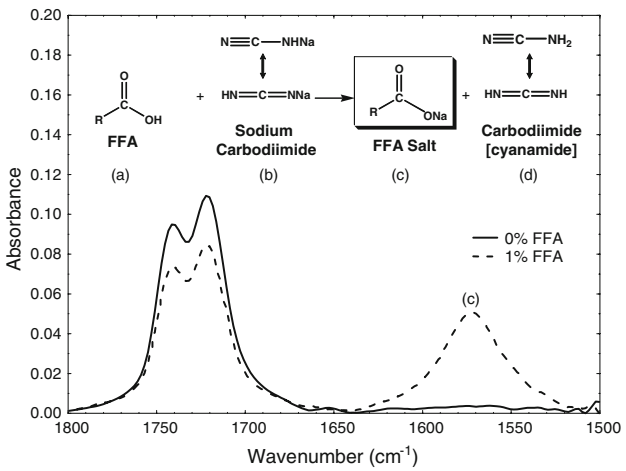

Fig. 2 The stoichiometric reaction associated with IR analysis for FFA and the corresponding spectral changes taking place when carboxylic acids extracted into methanol react with sodium hydrogen cyanamide

to an FFA-free oil. However, when the spectra of MeOH extracts of calibration standards were recorded on a VFA-IR spectrometer, "bad pixel" warnings were issued by the software, indicating that insufficient energy was reaching the detector in some portion of the spectrum collected. This was not an issue for the FTIR spectrometer, which uses a hot Globar with substantially higher energy output than the cool pulsed source of the VFA-IR spectrometer. These warnings were eliminated when the cell pathlength was reduced from 100 to 50 μm, but this resulted in an unacceptable reduction in sensitivity and restricted sample flow. It was found that the energy could be increased sufficiently to allow for use of a 100 μm cell simply by moving the source from its original location, ~5 cm from the cell window, to ~1 cm from the cell window. With the source re-located in this manner, the increased energy throughput was sufficient to eliminate "bad pixel" warnings. The close proximity of the source to the cell did not result in undue heating of the sample because the source is pulsed and hence cool. A standard curve for the determination of FFA content by measurement of the absorption of the FFA salts in the spectrum of the MeOH phase was then generated in the same manner as in the FTIR method previously reported [10]. Thus, FFA-free Canola oil was spiked with known amounts of oleic acid to obtain calibration standards covering a range of 0–1% FFA, considered appropriate for the analysis of process samples, in which FFA contents are generally in the range of <0.1–1%. These standards were then mixed in a 1:1 (w/w) ratio with the methanolic NaHNCN solution, and the spectra of the methanol extracts were recorded on both the VFA-IR and the FTIR spectrometer and compared (Fig. 3). In the FTIR spectra, recorded at 4 cm^{-1} resolution (Fig. 3a), the absorption band of the carboxylate salt produced by the reaction of oleic acid with NaHNCN is clearly observed at 1,573 cm^{-1}. This band is not resolved in the VFA-IR spectra but instead is completely overlapped by a strong MeOH absorption band centered at ~1,450 cm^{-1} (Fig. 3b) as a consequence of the low resolution (~30 cm^{-1}) of the VFA-IR spectrometer. Relating FFA concentration to the spectral response measured at ~1,573 cm^{-1} (relative to a baseline at 2,000 cm^{-1}) in the FTIR spectra produced an excellent standard curve (Fig. 4a). Despite its lower resolution, the VFA-IR spectrometer yielded similar quantitative information when the carboxylate response was measured at 1,598 cm^{-1} relative to a baseline at 1,687 cm^{-1} (Fig. 4b). The calibration equations obtained for the FTIR and VFA-IR spectrometers are presented below:

$$\mathrm{FFA_{FTIR}}\% = 0.008 + 5.167 \times \mathrm{Abs}_{1571/2000\,cm^{-1}} \quad R = 0.998 \quad \mathrm{SD} = 0.015\% \tag{1}$$

$$\mathrm{FFA_{VFA-IR}}\% = 0.002 + 6.024 \times \mathrm{Abs}_{1598/1687\,cm^{-1}} \quad R = 0.997 \quad \mathrm{SD} = 0.029\% \tag{2}$$

Thus, the VFA-IR spectrometer was capable of tracking low levels of added oleic acid quite well, with the SD being about double that for the FTIR spectrometer (0.029 vs. 0.015% FFA).

Subsequent work involved assessing the performance of both instruments over a range of FFA concentrations of 0–5%, this broader range considered appropriate for the analysis of crude oils and biodiesel feedstock, where high FFA levels are often encountered. In the case of the FTIR spectrometer, Fig. 5a illustrates the same clear-cut progression in absorbance at 1,573 cm^{-1} as the concentration of FFA increases, while in the case of the VFA-IR spectrometer, the trend previously observed over the 0–1% range is perturbed, with the higher concentration samples in the calibration series producing anomalous spectra and "bad pixel" warnings. The calibration equations derived in the same manner as those for the 0–1% FFA calibration (except for a change in the FTIR baseline point) produced the following regression equations:

$$\mathrm{FFA_{FTIR}}\% = -0.013 + 6.204 \times \mathrm{Abs}_{1571/1799\,cm^{-1}} \quad R = 0.999 \quad \mathrm{SD} = 0.045\% \tag{3}$$

$$\mathrm{FFA_{VFA-IR}}\% = 0.267 + 4.305 \times \mathrm{Abs}_{1598/1687\,cm^{-1}} \quad R = 0.977 \quad \mathrm{SD} = 0.402\% \tag{4}$$

As can be seen, there is a clear deterioration of the calibration SD over the 0–5% FFA range for the VFA-IR

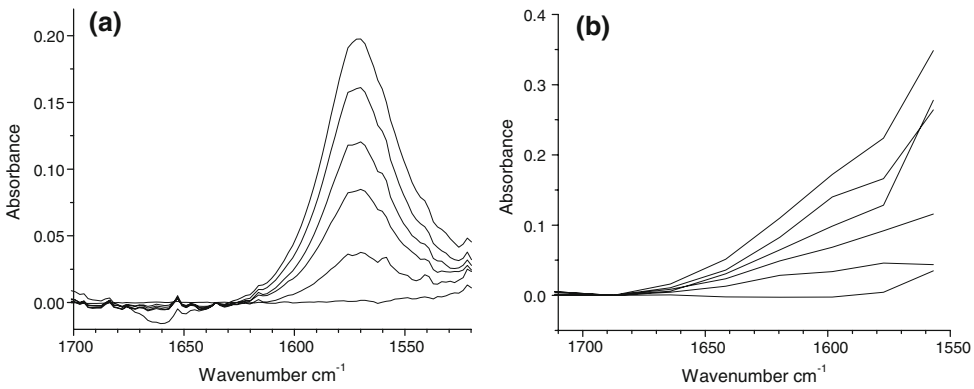

Fig. 3 **a** Calibration spectra for oleic acid standards (0–1%) in Canola oil obtained using an FTIR spectrometer and **b** VFA-IR spectrometer

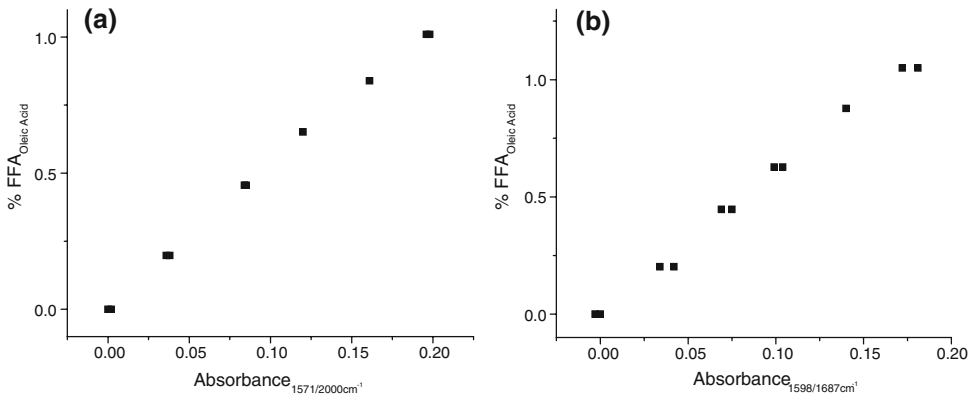

Fig. 4 **a** Calibration plot of % FFA versus absorbance for the FTIR spectrometer and **b** for the VFA-IR spectrometer

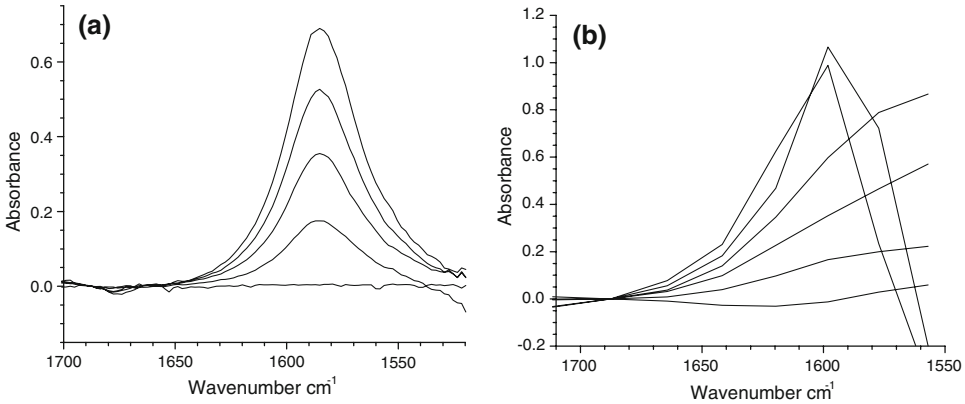

Fig. 5 **a** Calibration spectra for oleic acid standards (0–5%) in Canola oil obtained using an FTIR spectrometer and **b** a VFA-IR analyzer

spectrometer relative to the FTIR spectrometer. However, by moving the VFA-IR measurement one data point over to 1,620 cm^{-1}, the calibration equation SD improved to a more reasonable value of ±0.107%:

$$\mathrm{FFA_{VFA-IR}}\% = 0.21 + 7.93 \times \mathrm{Abs_{1620/1687\,cm^{-1}}} \quad R = 0.998 \quad \mathrm{SD} = 0.107\% \tag{5}$$

It was concluded that the VFA-IR spectrometer was becoming energy limited at higher FFA levels (>3%), owing to the combined absorption of MeOH and FFAs. Thus, while there was enough residual energy over an FFA range of 0–1% to obtain good quantitative results, at higher FFA concentrations the absorption at 1,598 cm^{-1} exceeded the available source energy, taking the absorbance off-scale. Moving the measurement to higher frequency compensated somewhat, but with a loss in sensitivity. Since no further energy could be gained by reconfiguring the instrument, other than by shortening the cell pathlength and thereby losing sensitivity, a modification of the method was considered. Specifically, the possibility of changing the extraction solvent from MeOH to EtOH was examined owing to the lower absorption of the latter in the spectral region of interest (Fig. 6), providing approximately twice as much residual infrared energy to work with to measure the FFA salts.

Testing indicated that the solubility of NaHNCN in EtOH was substantially lower than in MeOH (~2 g/L vs. ~9 g/L) and that the cyanamide to carbodiimide conversion was much slower (~60 d vs. ~4 d). Despite this difference, the reactivity of NaHNCN toward FFAs was similar in the two solvents. However, as a consequence of the lower solubility of NaHNCN in EtOH, the oil:solvent ratio had to be increased to ~1:5 to ensure sufficient reagent to convert up to 5% oleic acid to its salt form. Typical VFA-IR spectra obtained using the EtOH reagent solution for the calibration set covering the range of 0–5% FFA are presented in Fig. 7. As can be seen, there is a substantial improvement in the appearance of the calibration spectra, the energy gain manifesting itself in well-defined spectra, more in line with the FTIR spectra (Fig. 5a), albeit of lower resolution. These spectra yielded the following calibration equation:

$$\mathrm{FFA_{VFA-IR(EtOH-1:5)}}\% = 0.25 + 22.45 \times \mathrm{Abs_{1598/1687\,cm^{-1}}} \quad R = 0.999 \quad \mathrm{SD} = 0.075\% \tag{6}$$

Even though the EtOH method used a 1:5 oil:solvent ratio, resulting in the measurement of lower concentrations of FFA salt per unit volume (larger slope in Eq 6), the calibration SD improved (±0.075 vs. 0.107%) relative to the

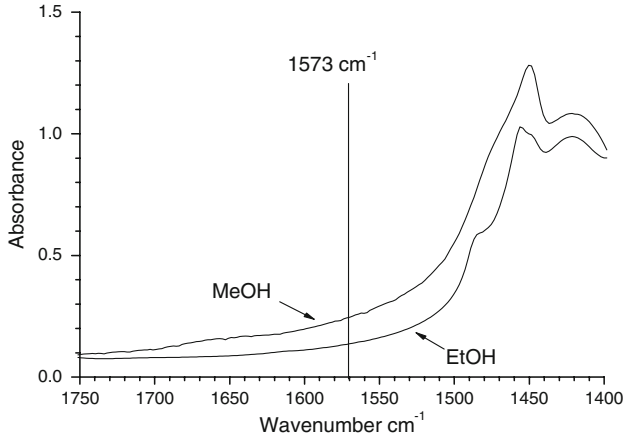

Fig. 6 Spectra of MeOH and EtOH in a 25 μm CaF_2 cell, illustrating their relative absorption profiles

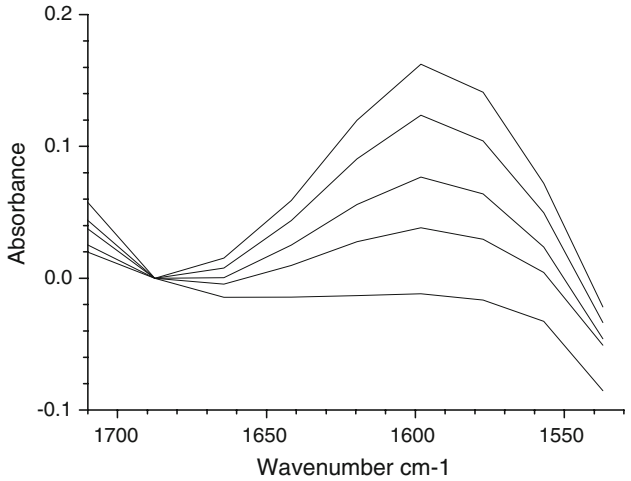

Fig. 7 Calibration spectra of FFA standards ranging from 0 to 5% FFA obtained using EtOH as extraction solvent in a 1:5 oil:solvent ratio

1:1 MeOH procedure, which was energy limited for higher FFA concentrations. Thus, the use of EtOH in place of MeOH allowed a wider range of FFA contents to be determined to within ±0.1%. However, if samples to be analyzed are routinely expected to have FFA contents below <1%, then the original MeOH procedure would be a better method in terms of sensitivity and accuracy as the extract is less dilute.

Based on the results of this work, a VFA-IR spectrometer is capable of replacing a conventional FTIR spectrometer to analyze for FFA content in oils using the method developed by Al-Alawi et al. [10]. If the method is only to be used to measure low levels of FFA (<1%), no modification of the original method is required; however, the use of EtOH as the extraction solvent in place of MeOH makes possible the accurate analysis of a wider range of FFA concentrations. Thus, a VFA-IR spectrometer can serve as a portable, dedicated FFA analyzer, providing a simple and economical instrumental means of determining FFA levels in crude and refined edible oils as well as biodiesel feedstocks and, as configured in this study, can readily analyze 20–30 samples/h once the samples have been prepared for analysis.

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