We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Advances in the Application of Spectroscopic Techniques in the Biofuel Area over the Last Few Decades

João Cajaiba Da Silva, Alex Queiroz,
Alline Oliveira and Vinícius Kartnaller

Additional information is available at the end of the chapter

Abstract

Guided by the instability of the oil market, as well as limited availability of and, especially, the environmental impacts of fossil fuels, the needs of the market for environmental-friendly energy sources have increased. However, as with any other product that is intended to place on the market, it is essential to ensure the quality of the fuel for successful marketing and acceptance by consumers. Spectroscopic techniques have been widely used for different purposes in the literature for the past decades, from biological applications to the measurement of the elemental composition of planets. From studies focused on biodiesel, bioethanol, biomass and biofuel in general, different spectroscopic techniques have also been applied in the area. The focus of this chapter is to elucidate what has been published in the last few decades over the subject, detailing the basic concepts of the main spectroscopic techniques applied and showing the results and developments over biofuel. The aim of the chapter is to achieve a set of information that can be used as a bigger compile of information of the state of the art regarding the theme.

Keywords: biofuel, spectroscopy, infrared, Raman, NMR, UV/Vis, image analysis, ICP-OES

1. Introduction

Spectroscopy is a general term for the science that deals with the interaction between the various types of radiation and matter [1]. Therefore, these types of measures are only possible if the interaction between photons and matter causes some kind of change in one or more properties

of the sample. The electromagnetic spectrum can be divided into regions according to the wavelength of the radiation, as shown in **Figure 1**.
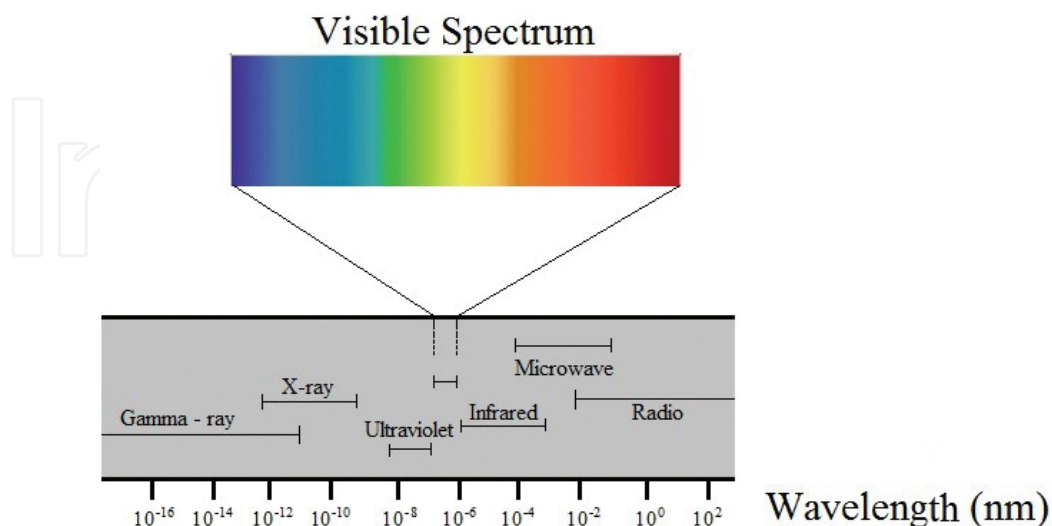
Figure 1. The electromagnetic spectrum showing the wavelength limits corresponding to each type of electromagnetic wave. The colored area shows the visible region.

According to the region of the electromagnetic spectrum, different types of spectroscopic techniques can be used, and the phenomena explored by them are of totally a different nature. This fact explains the applicability of each technique for specific cases. **Table 1** shows the spectroscopic methods according to the spectral region and the transition type.

Because of its versatility, the spectroscopic methods have been widely used for different purposes in literature for the past few decades, from biological applications [3, 4] to the measurement of elemental abundance in astronomical objects [5, 6]. Since the beginning of the century, the number of publications involving such techniques has grown exponentially.

At the same time, guided by the instability of the oil market, as well as limited availability and especially the environmental impacts of fossil fuels [7], the needs of the market for environ-mental-friendly energy sources have increased. However, as with any other product that is intended to place on the market, it is essential to ensure the quality of the fuel for successful marketing and acceptance by consumers [8]. For this reason, many countries have specific legislation that establishes desirable characteristics to minimize problems arising from the use of these compounds. Quality control, not only for biofuels but also for their increasingly used blends, is of paramount importance. The type (the chain length, degree of unsaturation, and presence of other chemical functions) and the concentration of the fatty acids used in the synthesis of biodiesel, for example, can influence the properties of the final product and its prerequisites in terms of storage and their tendency to oxidize [9]. The raw materials used and the process of obtaining biofuel can be decisive in terms of the introduction of some harmful contaminants in the final product [7]. Therefore, the monitoring of reactions is critical for quality control [10], and the monitoring of minor components, such as glycerol, water, residual alcohol, and heavy metals, is very relevant [11].

Type of energy transfer	Region of electromagnetic spectrum	Spectroscopic technique	Quantic transition type
Absorption	ɣ-ray	Mossbauer spectroscopy	Nuclear
	X-ray	X-ray absorption spectroscopy	Inner electrons
	Ultraviolet/Visible	UV/Vis spectroscopy	Bounding electrons
		Atomic absorption spectroscopy	Bounding electrons
	Infrared	Infrared spectroscopy	Rotation/vibration of molecules
		Raman spectroscopy	Rotation/vibration of molecules
	Microwave	Microwave spectroscopy	Rotation of molecules
	Radio wave	Electron spin resonance spectroscopy	Spin of electrons in a magnetic field
		Nuclear magnetic resonance spectroscopy	Spin of nuclei in a magnetic field
Emission (thermal excitation)	Ultraviolet/Visible	Atomic emission spectroscopy	Bounding electrons
Photoluminescence	X-ray	X-ray fluorescence	Inner electrons
	Ultraviolet/Visible	Fluorescence spectroscopy	Bounding electrons
		Phosphorescence spectroscopy	Bounding electrons
		Atomic fluorescence spectroscopy	Bounding electrons
Chemiluminescence	Ultraviolet/Visible	Chemiluminescence spectroscopy	Bounding electrons

[a] Adapted from Ref. [2].

Table 1. Spectroscopic methods based on electromagnetic radiation[*].

Chromatographic and spectroscopic methods are the most popular ways for biofuel analysis [11]. Spectroscopic techniques, however, have attributes that make them ideal for use in these kinds of analytical measurements. These attributes include the following [12]:

• Fast and simple analysis, either qualitative or quantitative, is possible;

• Little or no sample preparation is usually required, and they do not use polluting solvents;

• It is easy to find appropriate calibration standards and methods of analysis that are validated by recognized institutes;

• Direct and non-invasive analyses are possible;

• Normally, non-destructive methods can be employed, with a few exceptions, such as atomic spectrometry methods;

- The observed response is usually directly proportional to the concentration of the species in the system, except for ^{13}C NMR spectroscopy.

- In-situ analysis can be conducted, for example, in the case of real-time monitoring of biofuel production reactions;

- They are relatively inexpensive with respect to analysis time and reagents.

For the above reasons, it is relevant to examine the recent advances provided by the introduction of spectroscopic methods for biofuel analysis. This chapter aims to provide the theoretical basis of the most widely used techniques for this purpose and to analyze the impact of their use on this area.

2. Vibrational spectroscopy: infrared and Raman spectroscopy for biofuel evaluations

The notion that systems comprised by molecules can undergo vibrational motions, in which their atoms are in constant movement around their equilibrium position, can be explained by vibrational spectroscopy [13]. Every molecule contains a ground state energy (including vibrational energy), which can be described as the sum of different components. This energy defines the minimum energy with which atoms in a molecule can move in a periodic motion, described mainly by the six movements shown in **Figure 2**.
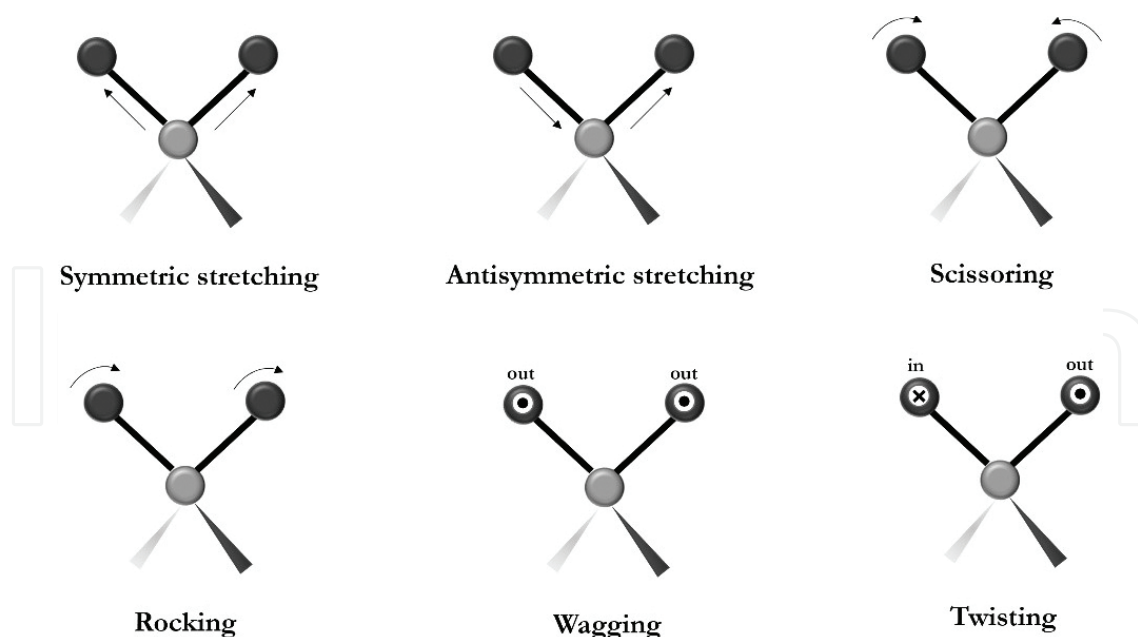
Figure 2. Main vibrational modes for molecules.

The principle of this spectroscopy type is that electromagnetic radiation can interact with molecules, leading them from their ground state energy to a vibrational excited state energy

in which the atoms' movements have higher energy. The state transition follows quantum mechanical rules and obeys quantized energy levels; for a harmonic oscillator view of the vibration, the energy that takes a molecule from a vibrational state to an immediately higher state is ($\frac{1}{2}hv$), where h is the Planck constant and v is the frequency of the vibration. Hence, the photon that can interact with a molecule must have an energy that equals this value in order to change its vibrational state; otherwise, the transition is not achieved. The portion of the electromagnetic spectrum that can lead to these transitions is the infrared region. This region can be divided into three different parts, with different applications for spectroscopic study: near infrared (NIR), 14,000–4000 cm^{-1}, allows the study of overtones and harmonic or combination vibrations; mid infrared (MIR), 4000–400 cm^{-1}, allows the study of the fundamental vibrations and the rotation-vibration structure of small molecules; and far infrared (FIR), 400–10 cm^{-1}, allows the study of low-heavy atom vibrations [14]. A polyatomic molecule can undergo many types of vibration, depending on their number of atoms and their degree of freedom. For nonlinear molecules, the number of possible vibrations can be defined by the $3N - 6$ rule, where N is the total number of atoms. Hence, different molecules undergo different types of vibration, leading to different possible transitions by absorbing infrared radiation. This is the premise of this type of spectroscopy, since a spectrum achieved by such technique can be seen as the fingerprint of a molecule and can be used specially for functional group analysis [15].

The two major techniques to study these vibrations are infrared and Raman spectroscopy, and they have been studied to a greater degree than the other techniques for applications since the middle of the twentieth century. Although the theoretical knowledge was developed during the prior century, it was not until World War II that infrared spectroscopy had a breakthrough, with the number of instruments going from less than 20 prior to the war to 700 by 1947 [16, 17]. In fact, several UK/US programs during the war prompted research and development of the early commercially available infrared instruments, such as those used in petroleum analysis (e.g., to trace the origin of gasoline used by the German air force), in quality control for the production of synthetic rubber, and in resolution of the structure of penicillin [16–18]. Examples of early publications range from industrial applications to more academic studies For example, Downing et al. [19] used infrared spectroscopy to differentiate isomers of the molecule dichlorodiphenyltrichloroethane and to qualitatively detect the presence of impurities in its samples, and Pfann et al. [20] used infrared spectroscopy to evaluate the presence of molecular fragments of the initiators used to catalyze a polymerization reaction in the structure of the final polymer. Regarding the biofuels area, since it is a more recent subject, the first described applications of infrared spectroscopy started to appear only by the end of the 1990s. In 1996, Adjaye et al. [21] used it to characterize different mixtures of silica-alumina and HZSM-5 as catalysts for the catalytic conversion of a biofuel from the thermal processing of maple wood to liquid hydrocarbons; in the same year, Sanderson et al. [22] used the technique for the compositional analysis of biomass feedstocks. Raman spectroscopy, on the other hand, was applied for biofuel studies almost a decade later than infrared spectroscopy, as in the work of Oliveira et al. [23]. They used the technique to determine the adulteration of diesel/biodiesel blends by the addition of vegetable oil. In this study, both

Raman and near-infrared spectroscopy were applied for the determination, and both gave a final similar accuracy result for the quantification of the adulteration, dependent on the algorithm used for the calculation.

The two techniques, although related to the vibrational modes of the molecules, have different principles and are complementary. Infrared spectroscopy is based on radiation absorption, while Raman spectroscopy is based on the interaction via inelastic collisions. In infrared spectroscopy, an infrared photon of certain energy (with a frequency v), can be absorbed by a molecule if that energy is exactly the same as the energy difference between a vibrational ground state and a vibrational excited state; using a simplified harmonic model, the energy difference between the two states is ($\frac{1}{2}hv$), as mentioned before in the text. Hence, the photon energy (E_p) needed so that a transition is possible is defined by:

$$E_p = \Delta E = \frac{1}{2}hv. \tag{1}$$

However, even if a photon has such energy, this state transition may not exist due to the main selection rule of infrared spectroscopy: for absorption to occur (hence, transition), the vibration needs to lead to a change in the dipole moment. If no change occurs, then that transition is considered "infrared forbidden" [13]. The intensity in which the transitions occur also depends on the molecular bonds related to the vibration, since it is proportional to the square of this dipole moment [24].

For Raman spectroscopy, the dipole moment change by the vibration is not the cutting rule, and vibration transitions that are not allowed in infrared spectroscopy may be allowed in Raman spectroscopy. This is due to the different phenomena taking place in this spectroscopy; as said, the technique takes advantage of an inelastic collision between the photon and the molecule, leading to the scattering of the electromagnetic radiation with different wavelength than the irradiated. The electric field in the electromagnetic radiation can interact with the molecule, creating an additional induced dipole moment as a response of the electrons and nuclei moving in opposite directions of the field, in accordance with Coulomb's law [25]. The dependency in this case relates to the ability of the molecule to be polarized, measured by its polarizability, that is, the deformability of the electron cloud around the molecule by an external electric field. In fact, the selection rule for a vibration transition in Raman spectroscopy is that it causes a change in the polarizability of the molecule. The incident photon is then momentarily absorbed by the molecule, leading to a transition from the ground state into a virtual state; a new photon is created and scattered by a transition from this virtual state to a lower energy vibrational state, as shown in **Figure 3**. Most of the scattered light is the same frequency as the initial light, as described by Rayleigh scattering, which does not contain any information regarding the molecule. The information comes from the Stokes and anti-Stokes scattering, in which the molecule goes from a ground state to a virtual state or from an excited state to a virtual state; in the first case, the scattered light has a frequency smaller than the initial

light, while for the second, the scattered light has a frequency higher than the initial light. Hence, the Raman spectra can be divided by both types of scattering spectra, and the frequency difference is equivalent to the one in infrared spectroscopy.
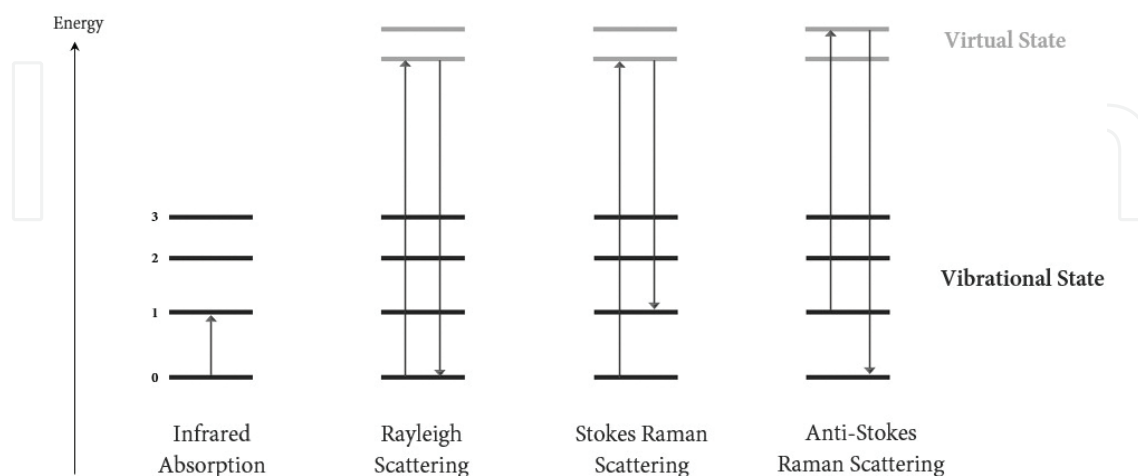
Figure 3. Energy-level diagram for the possible transitions in infrared and Raman spectroscopy.

For both techniques, characterization is one of the main possibilities, since the frequencies of the molecular vibrations depend on the masses of the atoms, their geometric arrangement, and the strength of their chemical bonds. The spectra provide information on the molecular structure, dynamics, and environment [24].

In terms of application, both techniques have different apparatus schemes, which will not be dealt by the authors here, but can be easily found elsewhere. Regarding the difference between the techniques for comparison purposes, Peter Larkin has described it in his book "*Infrared and Raman Spectroscopy: Principles and Spectral Interpretation*," as shown in **Table 2**.

	Raman	Infrared	Near-IR
Ease of Sample Preparation	Very simple	Variable	Simple
Liquids	Very simple	Very simple	Very simple
Powders	Very simple	Simple	Simple
Gases	Simple	Very simple	Simple
Fingerprinting	Excellent	Excellent	Very good
Best vibrations	Symmetric	Assymetric	Comb/Overtone
Group frequencies	Excellent	Excellent	Fair
Aqueous solutions	Very good	Very difficult	Fair
Quantitative analysis	Good	Good	Excellent
Low-frequency modes	Excellent	Difficult	No

Table 2. Comparison of Raman, mid-IR and near-IR spectroscopy, taken from [24].

The difference between the spectra of samples achieved by infrared spectroscopy and Raman spectroscopy is illustrated by Corsetti et al. [26], in which both techniques were used to quantitative measurements in systematically varied blends of ethanol and a gasoline, a subject of interest due to the production of bioethanol. **Figure 4** shows a comparison between the two techniques for the mixture and for pure ethanol and gasoline.
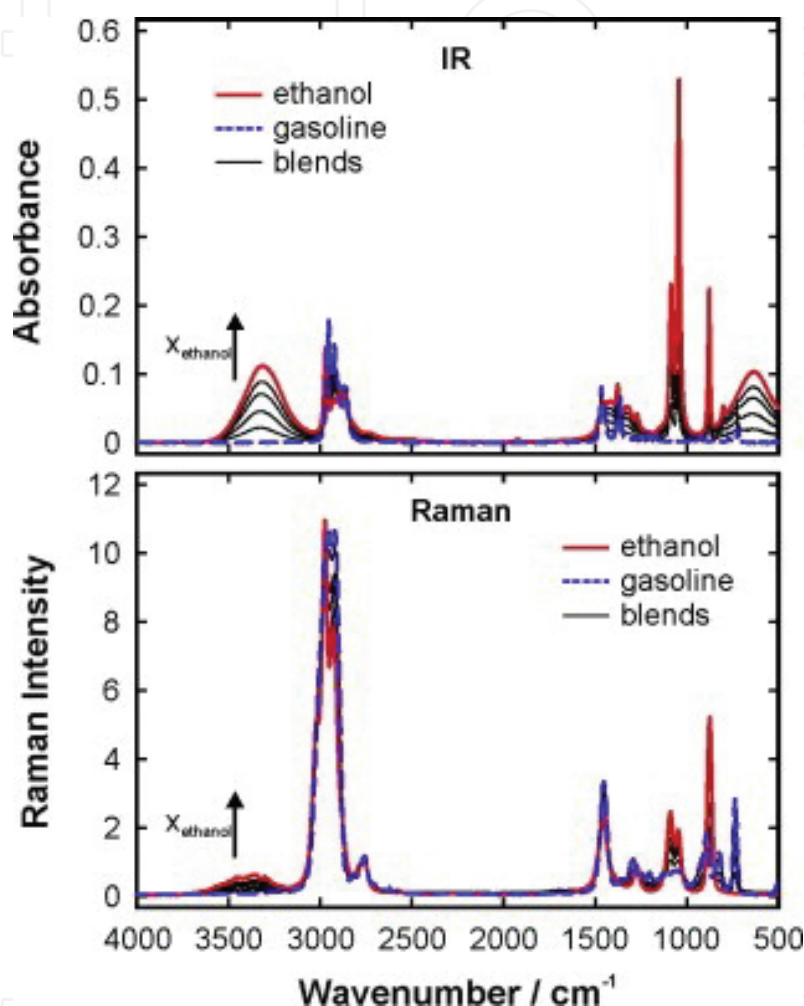

Figure 4. IR and Raman spectra of pure ethanol (red), pure gasoline (dashed blue), and blends (black), presented in [26].

A comparison between the spectra can be very direct, in which bands that are very intense for IR spectroscopy have low intensity in Raman spectroscopy, and vice versa. The vibrational bands related to alcohol molecules, which are the stretches of the O—H (3600–3000 cm^{-1}) and the C—O (1000–1100 cm^{-1}) bands, are intense in the IR spectra due to the electronegativity that causes great change in the dipole moment; however, these vibrations are not very polarizable, so they are shown with low intensity in the Raman spectra. On the other hand, the bands related to the stretches and bending of the C—H bands (3000–2800 cm^{-1} and 1600–1200 cm^{-1}) do not cause much change in the dipole moment and are not very intense in the IR spectra, while they tend to be more polarizable and have greater intensity in the Raman spectra.

As mentioned previously, the first applications of the two techniques in the biofuel area were for the characterization and quantification of components/properties of biofuels. These types of applications are very common, and a great amount of work from the past two decades can be found in the literature regarding it. Characterization is the principal feature of these techniques, which have been applied in different situations. One of them is the evaluation of catalysts, as in the study of heterogeneous biocatalysts from sucrose, saw dust, and chicken egg shells for biodiesel production by Wembabazi et al. [27]. Another study investigated the production of hydrocarbon biofuels through hydroprocessing of carinata oil [28], while other looked at the production of bioadditive fuels from bioglycerol under eco-friendly conditions [29]. Many other works can be found that deal with this theme [21, 30–32] Characterization has been applied not only to the catalysts used for biofuel processes but also to the biofuel itself, such as the structural analysis of bio-oils from the subcritical and supercritical hydro-thermal liquefaction of a plant from Turkey in a study by Durak and Aysu [33], or the characterization of insoluble material isolated from Colombian palm oil biodiesel in Plata et al. [34]. Also, Nanda et al. evaluated horse manure through catalytic supercritical water gasification as a possible next generation feedstock for biofuel production [35]. Many others have used this approach [36–42].

Another great feature of these techniques is their concentration dependency. The intensity of the signals is related to the characteristic of the band vibration and to the number of molecules needed to make the state transition (i.e., molecular concentration). This dependence can be defined by the Lambert-Beer law, which states that there is a linear relationship between signal and concentration. Hence, this can be used for the estimation of the concentration of components present in mixtures or for other properties in different systems. As commented earlier, one of the first articles published using infrared spectroscopy aimed to achieve a compositional analysis of biomass feedstock, where they determined the chemical composition of several woody and herbaceous feedstocks (121 samples in total) using NIR spectroscopy. Samples were analyzed for ethanol extractives, ash, lignin, uronic acids, arabinose, xylose, mannose, galactose, glucose, and C, H, N, and O, and all those responses were quantified using spectroscopy. The results showed great correlations for most responses, as depicted in **Figure 5**, suggesting that infrared spectroscopy was able to quantify components related to biomass feedstocks with high accuracy responses.

For quantification or classification purposes, many chemometric algorithms can be applied to vibrational data to transform the data to more relevant quantitative information. In the case of the Sanderson's work presented above, partial least squares regression (PLSR) was used to convert the spectra data to the responses. Several other works in the biofuel area have used different algorithms for the quantitative evaluation of different responses or for quality control and classification, such as multivariate linear regression (MLR), principal component regression (PCR), support vector machines (SVM), artificial neural network (ANN), partial least squares-discriminant analysis (PLS-DA), principal component analysis (PCA), K-nearest neighbors (KNN), linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), regularized discriminant analysis (RDA), amongst others [39, 43–60] (see **Figure 6**).
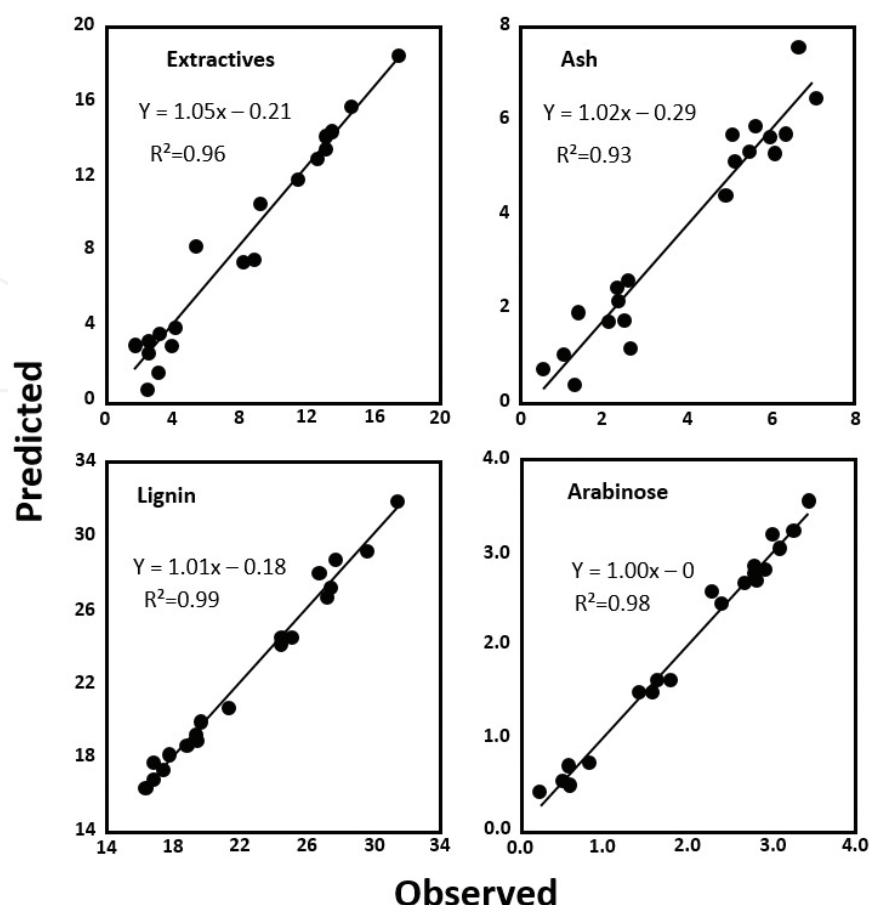
Figure 5. Graph of predicted vs. observed values of the responses studied in [22] for the quantification of components present in biomass feedstock.
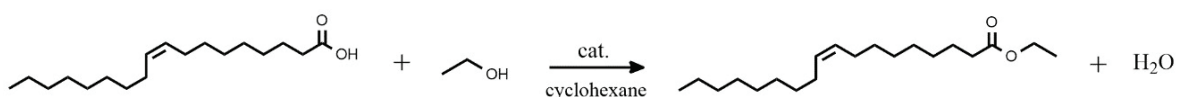
Figure 6. Scheme of the biodiesel production reaction made by [79].

Other applications in the area using the vibrational techniques are the evaluation of biofuel degradation [61–64] and process evaluation [65–70]. Regarding the latter subject, a new strategy for process optimization and control emerged in the last decade, namely, the process analytical technology (PAT) initiative [71]. PAT can be defined as the optimal application of technologies in feedback process-control strategies, information management tools, and/or product-process optimization strategies, as described by Chadwick et al. [72]. To meet its objectives, real-time sensing techniques are employed to control and monitor parameters throughout the processing chain, achieving a great amount of data. In the literature, PAT has been applied to follow reaction paths, monitor conversion over time, and even quantitatively evaluate catalyst efficiencies for biodiesel production [73–79]. As an example, from our research group, Kartnaller et al. have used infrared spectroscopy and chemometrics to follow

the esterification reaction of a free fatty acid to biodiesel, quantifying each component in the mixture and evaluating different enzymatic catalysts [79]. **Figure 7a** shows the change in the spectra of the reaction mixture in which the bands related to different components change as the reaction progress. As discussed, the spectroscopic signal is concentration dependent per the Lambert-Beer law; hence, if a product in a mixture changes in its concentration, so will the intensity of its bands. With the advance of technology, the infrared equipment has changed in the past few decades and new types have been shown in the market. This has allowed the equipment to acquire more information in less time and in an automated manner, which may generate a great deal of information and allow easy monitoring of reaction paths, as shown in **Figure 7b**.
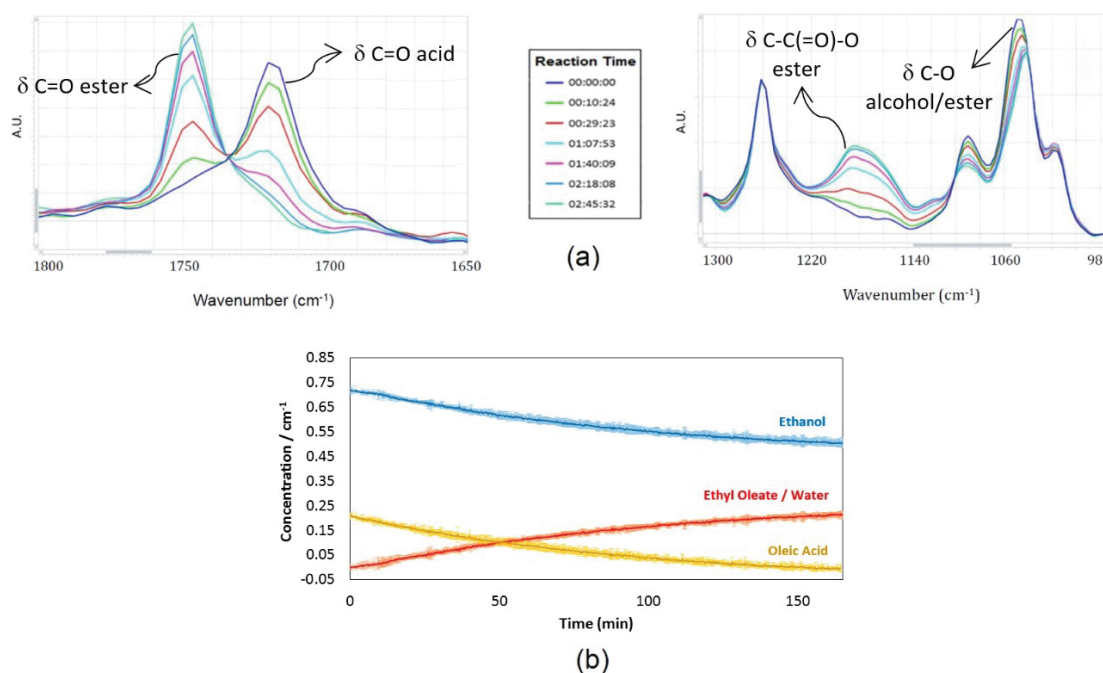
Figure 7. (a) Change in the intensity of infrared bands from different components in a mixture due to reaction and (b) quantitative monitoring of the different components of the reaction over time, calculated by the Lambert-Beer law, from [79].

Hence, it is easy to conclude that vibrational spectroscopy has a large range of applications in general, and the biofuel area has used it for many types of goals in the past years. With the increase of technological advances, both from the technical perspective and the biofuel research, the use of the technique is bound to increase even more.

# 3. Nuclear magnetic resonance and biofuel applications

The theory of nuclear magnetic resonance (NMR) spectroscopy was proposed by Pauli in 1924. He suggested that when certain atomic nuclei have spin and a nuclear magnetic moment, in

the presence of an applied magnetic field (Bo), they could be induced to absorb energy and change their spin orientation with respect to this applied field [1, 15].

The independent works of Felix Block and Eduard Purcell in 1946 experimentally showed that by absorbing electromagnetic radiation in the presence of an intense magnetic field, the nucleus experiences an unfolding of its spin state energy levels, as shown in **Figure 8**, which is dependent on the spin itself. In addition, the energy absorbed must equal the energy difference between the two states involved; in this case, the energy absorption is a quantized process [15]. The stronger the applied magnetic field, the greater the energy difference between the two inverse spin states, as shown in **Figure 9**.
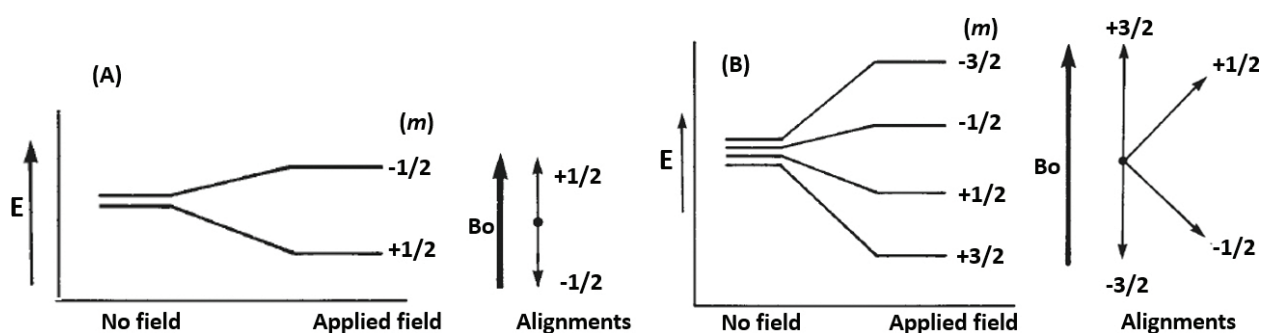
Figure 8. Energy levels for a nucleus with spin quantum number; (a) $I = 1/2$ and (b) $I = 3/2$ in the absence and in the presence of an applied magnetic field (Bo).
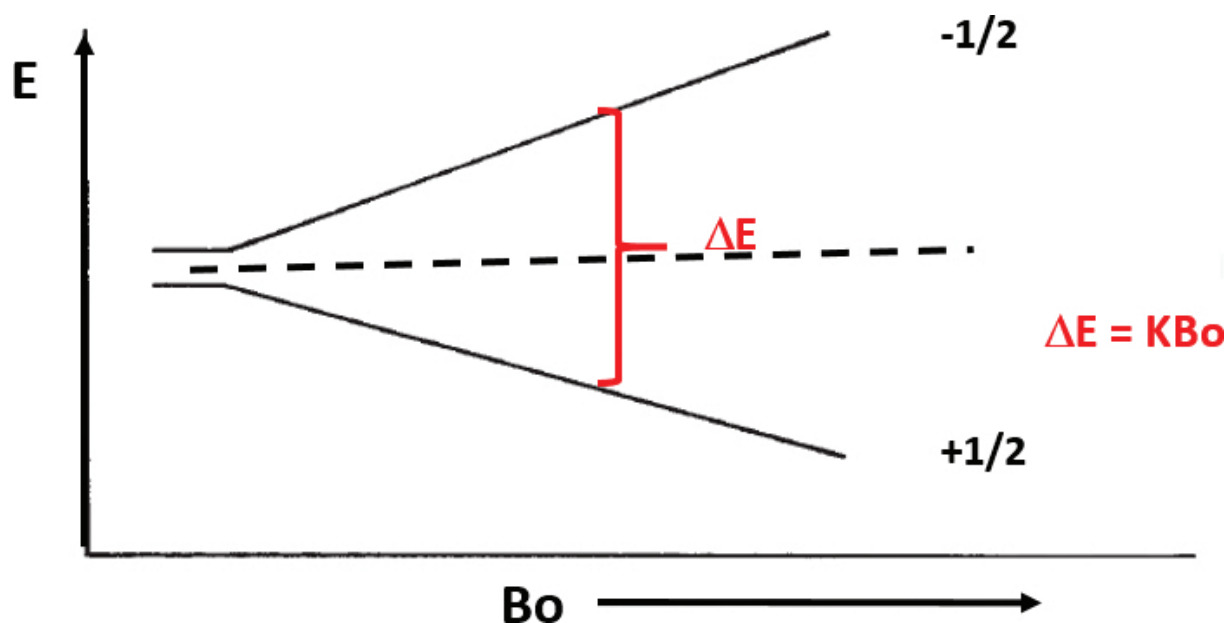
Figure 9. Spin state energy separation as a function of the increase in the magnetic field.

NMR spectroscopy is based on the measurement of the absorption of electromagnetic radiation in the radiofrequency region (4–900 MHz). The applied magnetic field differentiates the energy

of the two inverse spins of the nuclei that was previously degenerated, allowing the state transition to be achieved by the absorption of radiation and generating the signal. Hence, in this technique, the nuclei of the atoms are the main parts involved in the absorption process, while there is a great dependence on the applied magnetic field.

After the studies performed by Block and Purcell, other scientists discovered that the frequency of absorbed radiation by certain nuclei is strongly affected by their chemical environment, which is influenced by their electrons and nuclei neighbors. Hence, this phenomenon can associate the absorption spectra with the molecular structure. This discovery showed the great utility of NMR, since it is based on spectrum information, it is possible to obtain the structural elucidation of a molecule.

Figure 10 shows the predicted ^{1}H NMR spectra for ethanol, in which the nucleus evaluated is of hydrogen-1. Since the hydrogen nucleus is composed solely on a proton, the spectroscopy is commonly referred to as proton NMR. As was said before, not all nuclei in a molecule have a transition resonance at the same frequency, since the nuclei in a molecule are surrounded by electrons and exist in slightly different electronic environments. For the molecule of ethanol, there are three different chemical environments, which generate small differences in the absorption frequency of the nuclei. This is defined by the chemical shift (δ). The chemical shift can be used to identify functional groups and can help in the elucidation of the structure arrangements of groups. These applications are based on empiric correlations between structure and shift. Several tables have been published with values of the chemical shifts related to functional groups and molecules [15, 80]. In addition, the exact values of chemical shift depend on the solvent type and solution concentration. For the proton NMR spectra, the chemical shift values are defined in relation to an internal standard molecule, tetramethylsilane (TMS), and they vary between 0 at 20 ppm, while TMS is defined as zero.
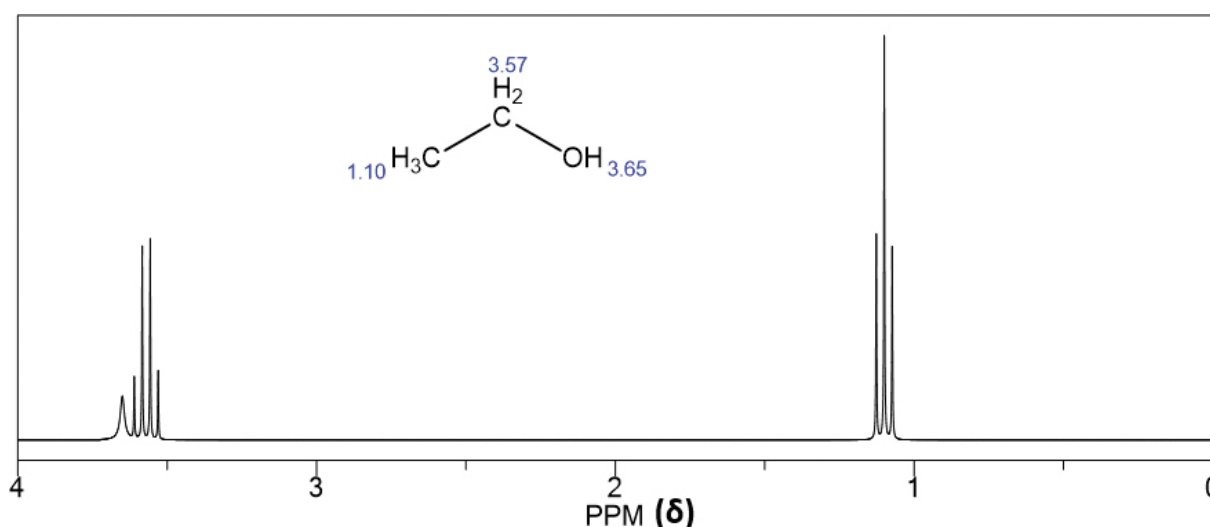

Figure 10. Predicted ^{1}H NMR spectra of the ethanol molecule.

Nowadays, two types of spectrometers are commercially available: the continuous-wave (CW) and the pulsed or Fourier transform spectrometer (FT-NMR). Historically, CW instruments

were mainly used for spectroscopic studies. Today, FT-NMR instruments dominate the market. For the CW instruments, the absorption sign is monitored while the frequency source is varied slowly. In some instruments, the frequency source stays constant, and the intensity of the magnetic field is varied. For the pulsed instruments, the sample is irradiated with a periodic energy pulse of radiofrequency, which leads to a signal in the time domain that decays in the interval between the pulses. This decay is known as the free induction decay (FID), and the signal is converted to a sign frequency domain using the Fourier transform (a mathematical operation), resulting in a spectrum, as shown in **Figure 11**.
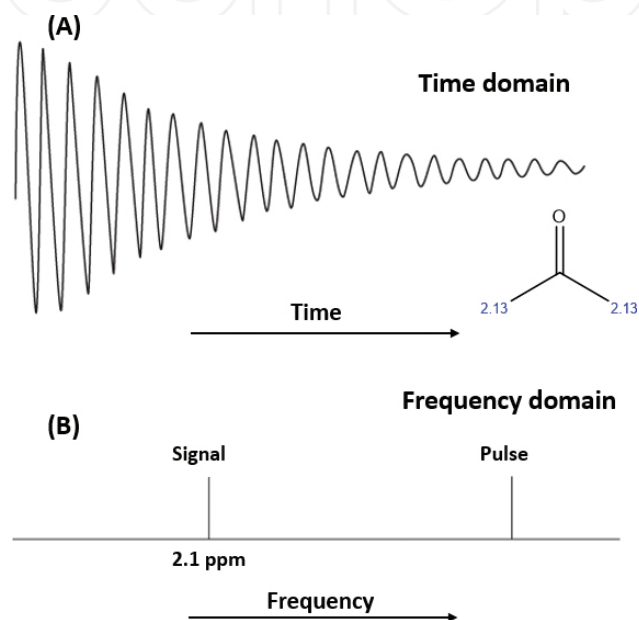
Figure 11. (a) Time domain. FID Curve for ^{1}H NMR for acetone; (b) spectra created using the Fourier transformation to convert the time domain to a frequency domain.

The integrated peak areas of ^{1}H NMR are proportional to the number of nuclei present in the molecule; that means, for the molecule of ethanol shown in **Figure 10**, the three different types of hydrogen have signals in which the areas are proportional to 3:2:1. However, for ^{13}C NMR spectra (another very common NMR technique in which the nuclei analyzed are from the carbon-13), the signals are not proportional to the number of nuclei present, and they cannot be used for this type of proportional characterization. Other applications use proton NMR for the determination of functional groups within the molecules, such as hydroxyl in alcohols and phenols, aldehydes, carboxylic acids, olefins, amines, and amides [1]. NMR can also be correlated to the concentration of the molecule itself, not only to the amount of nuclei within it. The technique can be used for the quantification of compounds in mixtures, especially when there are different types of functional groups [81, 82].

^{13}C NMR, as with ^{1}H NMR, can be used for the determination of organic and biochemical structures. These determinations are based on the chemical shifts and, as for the ^{1}H spectra, these values are relative to tetramethylsilane, with values between 0 and 200 ppm. In general, the effects of the environment are analogous to the ones observed for the ^{1}H NMR. The

applications of Fourier transform methodology and broadband decoupling of protons have accelerated the development of, and enhanced the interest in, ^{13}C NMR spectroscopy. Techniques such as distortionless enhancement by polarization transfer (DEPT) and ^{1}H-^{13}C COSY (HETCOR) have further endeared ^{13}C NMR spectroscopy to organic chemists. In general, ^{13}C NMR spectroscopy allows chemists to directly observe the carbon framework of organic moieties and to make (collaborative) inferences about active nuclei attached to the carbon backbone. The technique is particularly valuable in research on natural products, pharmaceutical drugs, and biochemical processes, where complex cyclic species are common and often difficult to identify with only ^{1}H NMR [82].

This section illustrates the utility and application of NMR spectroscopy as a probative tool in the field of biomass, bio-oils and biofuels. Examples of its application include describing the characterization of the pyrolysis of biomass to achieve bio-oils, analyzing oil quality using quantification methodologies, and real-time monitoring of reaction paths for biofuel production [83–87].

Many reports in the literature describe the techniques used to obtain liquid fuels from biomass, including fractionation, liquefaction, pyrolysis, hydrolysis, fermentation, and gasification. Biomass pyrolysis is the cheapest way to obtain these products [88], and therefore it is the most studied methodology for such conversion [89], either in terms of physical properties or characterization of the resulting components [84, 90–92]. Compounds such as acids, esters, alcohols, ketones, aldehydes, anhydrosugars, furans, and phenols are usually found in bio-oil [93, 94], and its exact composition is strongly dependent on a number of factors, including the biomass origin and composition, the reaction temperature and heating rates to which the biomass is submitted, and the residence time on reactors [95]. During the pyrolysis process, the oil fractions are condensed, collected in a sample tube, and posteriorly analyzed. NMR spectroscopy is a suitable method for biomass pyrolysis studies. Different from other spectroscopic techniques, which can be limited for only the qualitative analysis of bio-oil samples, ^{1}H and ^{13}C NMR can be used as a way to obtain approximate ratios of the chemical environments of protons and carbon atoms. Besides, it can be useful to determine the approximate aromatic/aliphatic ratios. Another advantage is that NMR is a nondestructive technique, and the sample fractions can normally be recovered after the procedure.

Mullen et al., for example, described a method using ^{1}H and ^{13}C NMR to characterize bio-oils fast pyrolysis from numerous types of biomass [95]. Based on the proton NMR spectra of six different bio-oils and the integral values of selected regions, the ^{1}H and ^{13}C NMR spectra were used to determine the functional groups present in each oil. Using the DEPT spectra that were enabled, it was possible to evaluate the extent of branching in the molecules. These procedures provided important structural data about the analyzed samples. The work of Ben and Ragauskas [96] described an in-situ investigation of the relationship between the structures of pyrolysis oil at various time points during the accelerated aging process at 80°C, using ^{1}H and ^{13}C NMR. It was possible to detect a reduction in the percentage of aldehyde, carboxylic acid, and ether compounds, while the level of aromatic, alkene, and alkane compounds increased. In other words, it was possible to use NMR spectroscopy in the investigation of structural changes of pyrolysis oil compounds before and after an accelerated aging process.

The NMR technique has an advantage with respect to other common methods, such as infrared spectroscopy or chromatographic techniques, as it has high resolution compared to the others, and it also offers the possibility of obtaining valuable structural information. Besides that, the accelerated aging process of pyrolysis oil is carried out inside the NMR equipment, so the analysis is made in situ and in real time [96].

NMR spectroscopy can also be used for quality control either of the vegetable oils used as raw material for biofuel production or the final obtained product [38]. The quality of the vegetable oil influences many properties in the produced biodiesel, such as viscosity, lubricity oxidative stability, cold flow, ignition quality, and the heat of combustion. The type of fatty acid also influences the final product. Monounsaturated and saturated fatty acids are more stable than highly unsaturated ones, although they show higher viscosity and a bigger tendency to solidify in cold weather.

Analytical techniques for fatty acid determination, such as gas chromatography and near-infrared spectroscopy, need calibration models using some standards with similar chemical composition related to the analyzed sample [38]. High-resolution NMR has an advantage above the others because calibration models are not necessary. It is possible to quantify compounds in the sample through observing the integrated area of peaks with the chemical shift corresponding to a specific type of fatty acid [97]. Usually, the acquisition time for this type of analysis is quite long. However, Prestes et al. developed a method that is useful in determining the fatty acid profile in intact seeds in a fast and automated way using low-resolution NMR. It was possible to analyze more than a thousand samples per hour, thus working as a powerful tool to speed up the selection of oilseeds that are suitable for biodiesel applications [98]. Garro Linck et al. successfully analyzed biodiesel obtained via the transesterification of different raw materials using low-field spectrometers [99].

Another NMR application is as the PAT tool (as described for infrared spectroscopy), which can be used for the *in-situ* monitoring of the biodiesel production process, where sampling is not necessary [85]. As was discussed, ^{1}H NMR spectroscopy can be a robust, rapid, and quantitative method that can be applied for determining the presence of multiple components due to specific chemical shifts in the spectrum, and reaction monitoring can be applied over time, based on the integration of individual proton signals. An example of this application is the work of Anderson and Franz, where they used high-resolution NMR equipment for the monitoring of the biodiesel production reaction, evaluating the transesterification of triacylglycerol (TAG) and the resulting products, including diacylglycerol (DAG), monoacylglycerol (MAG), glycerol, and fatty acid methyl esters (FAME) [100]. Due to different molecular structures and different environments and different forms of hydrogen, it is possible to differentiate the signals from each molecule and evaluate them over time, as shown in **Figure 12**.

Anderson and Franz were able to see, then, the decrease of the TAG bands due to the biodiesel production and also the isomers of intermediates, such as DAG, throughout the reaction. This is a statement of the sensibility and sensitivity of the NMR spectroscopy technique in achieving information from the molecules, which could not be achieved by other techniques.
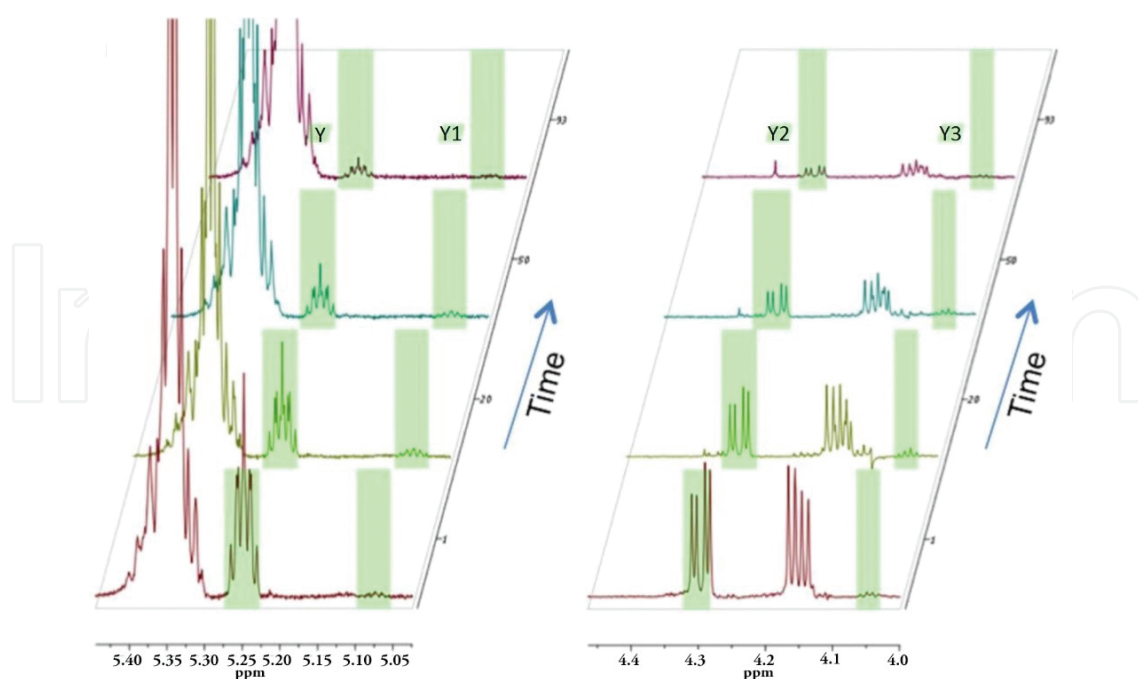
Figure 12. ^{1}H NMR stacked spectra over time of transesterification for biodiesel production, where Y indicates the TAG glyceryl methine at 5.27 ppm, Y1 indicates 1,2-DAG glyceryl methine at 5.08 ppm, Y2 indicates TAG glyceryl methylene at 4.29 ppm and Y3 indicates 1,3-DAG glyceryl methine at 4.09 ppm [100].

4. UV/Vis spectroscopy and image analysis for the quantification and classification of biofuels

The absorption of radiation in the ultraviolet/visible (UV/Vis; 200–700 nm) region results from the excitation of bounding electrons. The UV/Vis radiation has enough energy to promote electronic transitions, and this is the main principle investigated by UV/Vis absorption spectroscopy. This technique is very useful in identifying functional groups in a molecule because a correlation between the absorption bands and the functional group can be done. While inorganic compounds normally absorb light in the visible part of the spectrum, organic molecules usually present some functional groups capable of absorbing radiation from UV light sources. These functional groups must be unsaturated or have a heteroatom with non-bonding electrons, such as oxygen, sulfur, or halogens. **Table 3** shows some functional groups and the radiation wavelength absorbed [1].

According to the Lambert-Beer law, the intensity of the absorption of radiation by the species present in the sample is directly proportional to its concentration in the system. Thus, quantitative determination of compounds containing absorbing groups can be easily made. UV/Vis spectroscopy is widely used for many applications, including in the biofuel area, since it is low cost and allows the analyst to perform qualitative and quantitative analysis in a fast and reliable way.

Functional groups	Example	λ_{max} (nm)
Alkene	$C_6H_{13}CH{=}CH_2$	177
Alkyne	$C_5H_{11}{-}C{\equiv}C{-}CH_3$	178
		196
		225
Carbonyl	$\begin{array}{c} O \\ \| \\ CH_3CCH_3 \end{array}$	186
		280
Carboxyl	CH_3COOH	204
Aromatic	C_6H_6	255
Alcohol	CH_3OH	167
Esther	$C_6H_{10}O_3$	250
Halogen	CH_3I	258
Sulfur	$(CH_3)_2S$	229

Table 3. Ultraviolet absorption of some organic molecules.

UV/Vis spectroscopy is very useful, for example, for the evaluation and quantification of the blend level in biodiesel/diesel mixtures. The utilization of blends between biodiesel and conventional diesel is very common, and the blend level can vary. Usually, pure biodiesel is referred to as B100, while some blends are classified according to its percentage of biodiesel, such as B20, B5, and B2 (respectively presenting 20, 5, and 2% of biodiesel in conventional diesel). The blend level directly influences some important characteristics of the fuel, such as its lubricity and tail pipe emissions [101]. Thus, it is very important to develop adequate methods for such applications. For example, Zawadzki et al. studied different types of biodiesel blends, employing an UV-Vis spectrometer as a low-cost detector to identify the range of UV frequencies suitable for detecting the biodiesel blend level [102]. The proposed method is robust, even with changes in the biodiesel feedstock and the fuel diesel origin.

UV/Vis detectors are also widely employed in high-performance liquid chromatography (HPLC) [103–105]. Many papers describing the use of HPLC-UV/Vis for biofuel analysis can be found in the literature. An interesting example is a statistical study performed by Foglia et al. [106], in which the precision and accuracy of HPLC-UV/Vis were evaluated with good results. This system was employed to determinate the level of simple alkyl esters of fats and oils (biodiesel) blended in petroleum diesel and to validate this method for this kind of analysis using different biodiesel feedstocks.

Another interesting application involving biodiesel/diesel blends is the detection of adulterations using vegetable oil. Commonly, near/mid infrared spectroscopy and spectrofluorimetry are used for this kind of analysis together with chemometric tools for multivariate classification/calibration [104]. These are expensive methods, so it would be interesting to evaluate the possibility of detecting adulterated blends using UV/Vis spectroscopy, which is simpler and

cheaper. A study performed by Fernandes et al. employed UV/Vis and chemometric tools to detect soybean oil in biodiesel/diesel blends, focusing on biodiesel/diesel blend (B5) adulterations with soybean oil percentages from 0.5 to 2.5% (v/v) [105].

Another application that is indirectly correlated to the concept of visible light spectroscopy and that has been applied throughout the past few years is image analysis. The simplest definition for the word *image* can be understood as an optical replica of a luminous or illuminated object formed by a mirror of lens. Therefore, the object that gives rise to an image does so through an interaction with electromagnetic radiation, either by emission or absorption processes. Over the years, a wide variety of analytical methods involving image analysis as the main tool have been introduced, due to the ease with which they are carried out in a fast, noninvasive, and inexpensive way compared to more advanced spectroscopic techniques. New fields were opened with the introduction of chemometric methods to analyze image analysis results, such as exploratory image analysis, multivariate statistical process monitoring, multivariate regression, and image resolution [107]. The versatility of this technique has been explored in several areas, from flame determination of elements [108] to biochemistry analysis [109], providing fast and cheap results in a simple way.

In the field of renewable fuels, image analysis has been also explored for applications in various stages of production. For example, for the application of microalgae for biofuel production, an area that has attracted increasing attention recently, digital image processing has been successfully used as a way for monitoring and quantifying the amount of biomass present on photobioreactors, using the RGB color system [110], and also to build the light distribution profile in microalgae cultivation [111], a key factor for mobile productivity. Another interesting application related to microorganisms with potential use in biodiesel production is the determination of the intracellular accumulation level of lipids in yeast cells, which can be done in a dynamic and nondestructive manner via high-content images [112].

Because biodiesel quality control is becoming increasingly important, given the high market demand for renewable energy sources, methods such as image analysis have emerged as fast and reliable alternatives for the evaluation of some parameters. RGB/gray scale histograms obtained from digital images have been used together with statistical methods in biodiesel classification as an efficient way to determine the feedstock used in its production, since this influences the properties of the final product [113] without, however, employing time-consuming techniques, such as chromatographic methods. Another example is the application of image analysis together with thin layer chromatography, a very simple method, for glycerol detection in biodiesel [114]. This organic compound is a byproduct of the manufacturing process and is considered a contaminant in the final product. Similarly to fossil fuels, the burning of biodiesel, for example, causes the emission of pollutants, such as NOx species. Image analysis has proved useful in the prediction of the level of these emissions by the use of flame radical imaging to monitor the biomass combustion process [115].

In the coming years, researchers expect that image analysis will be explored in other stages of the production process because of its potential for easily obtaining relevant results for the biofuel area.

5. Atomic absorption and emission: trace element determination in biofuel samples

The determination of trace elements in biofuels is a key parameter for their use, and the importance of conducting such analyses reliably and quickly grows as the global demand for renewable energy sources becomes greater. The main spectroscopic techniques currently used for trace elements determination in biofuels are based on the phenomena of emission and absorption of electromagnetic radiation by atoms or elementary ions. In the case of the atomic absorption principle, electronic excitation occurs in atoms from the ground state to more energetic electronic levels because of energy absorption at specific wavelengths that are characteristic of each element. The excited electrons tend to quickly return to the ground state, releasing energy at wavelengths also characteristic of each element. This is the concept explored by atomic emission methods. Both of the concepts are shown in **Figure 13**.
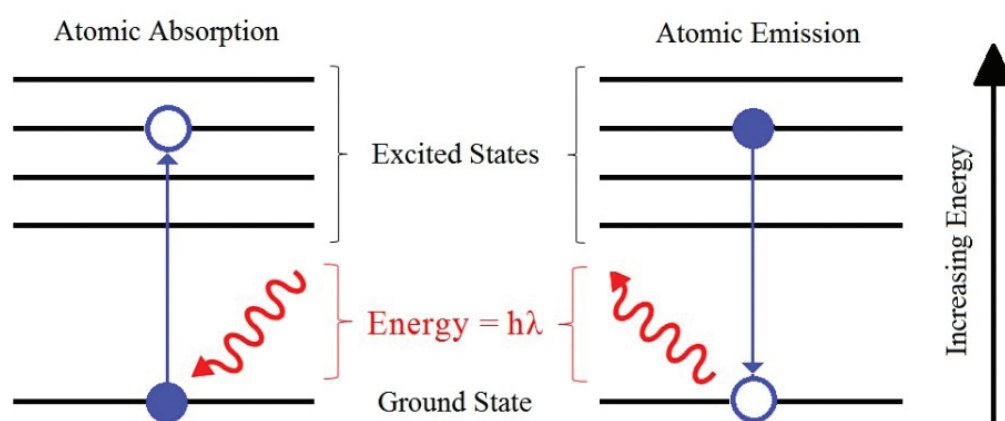
Figure 13. Atomic energy absorption/emission. The blue circles represent electrons, and the blue vacancies represent the final electron state after the absorption/emission occurs; h is the Planck constant and λ represents the wavelength in which the transition takes place.

The most common techniques are inductively coupled plasma-optical emission spectrometry (ICP-OES) and atomic absorption spectrometry (AAS) [116]. ICP-OES employs a highly energetic plasma, formed by an electrically neutral gas, usually argon, converted to positive ions and electrons. Such plasma has enough energy to atomize, ionize, and virtually excite all the elements of the periodic table to more energetic electronic states [117]. These species tend to return rapidly to the ground state, releasing energy at characteristic wavelengths depending on the elements present in the analyzed sample, and the radiation intensity is directly proportional to the concentration of the element in the sample [1]. AAS also employs atomization methods as well as emission techniques. Commonly, this process is carried out by means of a flame, in which desolvation, evaporation, and dissociation of the molecules into atoms take place [1]. Another common atomizing method is electrothermal atomization, in which the energy for volatilization and atomization is provided by means of an electric current applied to a graphite furnace [118]. Two more specific atomization techniques are hydride atomization by heating a quartz tube, a very common method for the analysis of some metals, such as

arsenic, and the cold vapor technique, which is widely used for mercury determination. After atomization, the analytes are submitted to radiation from a source, whether a single emission line source (e.g., hollow cathode lamps, multielement lamps or electrodeless discharge lamps) or a continuous source (e.g., halogen or deuterium lamps) that needs an auxiliary monochromator system to select the desired lines [119]. The analytes in the ground state absorb energy at specific wavelengths corresponding to their more favorable electronic transitions, thus generating an absorption spectrum whose intensity depends on the population of the atoms in the ground state, directly proportional to their concentration in the sample.

Trace elements may cause significant problems with serious implications for the use of biofuels, such as biodiesel and bioethanol. Such elements can be present in original vegetable oils subjected to transesterification processes due to absorption from the soil by the plant used as a feedstock, or they may even be incorporated in the vegetable matrix by means of the catalysts used in the biofuel synthesis or extraction/refining procedures [120, 121]. Sodium and potassium, for example, are used in the form of hydroxides in the synthesis of biodiesel, and together with Al, Ca, Fe, Mg, and Ti, they form a group of elements that tend to form a large amount of ash (metal oxides) after the fuel is burned [122, 123]. It causes difficulties for the operation of the gears, reducing the longevity of the engines. Furthermore, these elements are also involved in corrosion processes [121]. Some transition metals, such as Cu, Pb, Cd, and Zn, can cause biodiesel oxidation [124], resulting in residues that may be deposited in the engines. Moreover, they can contribute to air pollution and cause environmental damage due to their toxicity [116]. The sulfur level in the produced biofuels also must be carefully monitored because of emission legislation. Sulfur has been related to the depletion of the Earth's ozone layer, acid rain incidence, and chronic respiratory diseases. Low sulfur levels are also needed for good performance in modern engines [125, 126]. Iron and vanadium can act as catalyst poisoners and may reduce the efficiency of advanced catalysts that are commonly used in gears [127]. Some additives that are added to biofuels to improve physical or burning characteristics also contain metals, and their levels must be monitored. Because of these problems, strict laws have been adopted for the maximum level of metal contaminants in commercial biodiesel. **Table 4** presents the tolerated level of some metals established by ASTM standards [128].

Biofuel	Element	Maximum level	ASTM standard (year)
Biodiesel	Na+K	5 ppm	D6571 (2012)
	Ca+Mg	5 ppm	D6571 (2012)
	S	Biodiesel S15: 0.0015% Biodiesel S500: 0.05%	D6571 (2012)
	P	0.001%	D6571 (2012)
Fuel ethanol	S	30 ppm	D4806 (2014)
Bioethanol	Cu	0.1 ppm	D1688 (2012)
	P	0.5 ppm	D3231 (2013)
	S	10 ppm	D3231 (2013)

Table 4. Maximum concentrations allowed for some trace elements in biofuels (ASTM standard).

It has become essential to develop precise, accurate, and sensitive methods that are applicable for monitoring metal contaminants to qualify biofuels according to the established standards for their use. The quantification of metals in bioethanol and biodiesel, for example, is associated with a number of difficulties, such as their low concentration in the organic matrix (in the range of μg/L), the limited number of certified standards, the sample complexity, and the dependence of the final product on the raw material used [128].

Despite ICP-OES and AAS being the most widely used techniques, the details involved in the determination of metals in biofuel samples must be observed. In general, organic matter decomposition procedures (often microwave assisted) can be applied to convert the matter to a simple aqueous matrix, therefore reducing the chances of spectral interference with the measurements and avoiding difficulties in the injection of these samples [129–134]. This process is also necessary because of the difficulties in introducing the samples in atomizers and in selecting appropriating calibration standards for the analyzed system [135].

In some cases, however, direct injection of samples of biodiesel, bioethanol or fuel ethanol may be advantageous in order to reduce the number of steps of the analysis procedure. This operation brings some drastic consequences. The injection, for example, can be greatly hindered due to the physical-chemical characteristics of the sample, such as viscosity and surface tension, thus modifying the ease of suction of the components. Both in the case of the ICP-OES technique (in which a nebulizer is used for generating an aerosol) [128] and in the case of the sample introduction systems used in flame atomic absorption equipment, problems in the injection of organic samples affect the efficiency and reproducibility of the analytical methods. Burning organic compounds normally generates instability in the plasma with ICP-OES [136], and the generated pyrolysis products cause some spectral interference. Another problem may be the deposition of material originating in the pyrolysis processes on the torch or other spectrometer facilities [128, 137]. When using a graphite furnace as an atomization method for AAS, organic samples usually cause excessive material spreading during volatilization [116].

Due to the importance of determining trace elements in biofuels, and taking into account the difficulties mentioned above, several research groups have been working on the development of new methods for this purpose. Some interesting approaches have been described in the past few years. Dilution with an organic solvent or water can be an interesting way to minimize the errors observed in the analysis of biodiesel samples [127, 138–142]. Methods involving pre-emulsification of the sample using surfactants have also achieved good reproducibility [121, 123, 143–148]. Furthermore, the extraction of analytes from a sample for further analysis provides good results in some cases [149–151].

Surely, the spectroscopic methods, whether based on atomic emission or absorption principles, are the most popular for elemental determination in biofuel samples due to their high sensibility and reliability, despite the specific characteristics of the analytical methods involving organic samples.

6. Summary

To conclude, biofuels have been presented in the last few decades as alternatives or substitutes for fossil fuels to decrease the amount of fossil fuels used. With the increase in awareness of the need to develop more sustainable ways to support the earth's energy system in the future, much study is still needed, mainly in the areas of production, characterization, and quality control. This chapter described several methods that involve spectroscopic techniques, including infrared, Raman, NMR, UV/Vis absorption, image analysis, ICP-OES, and AAS. With these techniques, applications can be performed to characterize organic components in biofuels, to study the development of methods for controlling quality (e.g., the validation of biodiesel blends or detection of adulterations), to develop methods for discerning trace elements in biofuel, and even to monitor the production of biofuels in real time.

The referenced works in this chapter represent only a brief summary of the uses of spectroscopic techniques, depicting their importance in terms of fostering new developments in the biofuels area.

Author details

João Cajaiba Da Silva, Alex Queiroz, Alline Oliveira and Vinícius Kartnaller[*]

*Address all correspondence to: vkartnaller@yahoo.com.br

Federal University of Rio de Janeiro, Chemistry Institute, Cidade Universitária, Rio de Janeiro, RJ, Brazil

References

[1] Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. Thomson Brooks/Cole; Belmonte, CA, USA; 2007.

[2] Harvey D. Modern Analytical Chemistry. McGraw-Hill Higher Education; USA; 2000.

[3] Beauvoit B, Chance B. Time-resolved spectroscopy of mitochondria, cells and tissues under normal and pathological conditions. Molecular and Cellular Biochemistry 1998;184:445–55.

[4] Diamandis EP. Immunoassays with time-resolved fluorescence spectroscopy: Principles and applications. Clinical Biochemistry 1988;21:139–50.

[5] Jura M. The elemental compositions of extrasolar planetesimals. Proceedings of the International Astronomical Union 2012;8:219–28.

[6] Orton GS, Moses JI, Fletcher LN, Mainzer AK, Hines D, Hammel HB, et al. Mid-infrared spectroscopy of Uranus from the Spitzer infrared spectrometer: 2. Determination of the mean composition of the upper troposphere and stratosphere. Icarus 2014;243:471–93.

[7] Stauffer E, Byron D. Alternative fuels in fire debris analysis: biodiesel basics. Journal of Forensic Sciences 2007;52:371–9.

[8] Knothe G. Analyzing biodiesel: standards and other methods. Journal of the American Oil Chemists' Society 2006;83:823–33.

[9] Pinto AC, Guarieiro LLN, Rezende MJC, Ribeiro NM, Torres EA, Lopes WA, et al. Biodiesel: an overview. Journal of the Brazilian Chemical Society 2005;16:1313–30.

[10] Fernando S, Karra P, Hernandez R, Jha S. Effect of incompletely converted soybean oil on biodiesel quality. Energy 2007;32:844–51.

[11] Knothe G. Analytical methods used in the production and fuel quality assessment of biodiesel. Transactions of the ASAE 2001;44:193–200.

[12] Baeten V, Dardenne P. Spectroscopy: Developments in Instrumentation and Analysis. Grasas y Aceites 2002;53:45–63.

[13] Diem M. Modern Vibrational Spectroscopy and Micro-Spectroscopy: Theory, Instrumentation and Biomedical Applications. John Wiley & Sons; Chichester, UK; 2015.

[14] Theophanides T. Infrared Spectroscopy – Materials Science, Engineering and Technology. Croatia: Intech; 2012.

[15] Pavia DL, Lampman GM, Kriz GS, Vyvyan JR. Introduction to Spectroscopy: Cengage Learning; USA; 2013.

[16] Rabkin YM. Technological innovation in science: the adoption of infrared spectroscopy by chemists. Isis 1987;78:31–54.

[17] Barth A, Haris PI. Biological and Biomedical Infrared Spectroscopy. Amsterdam: IOS Press BV; 2009.

[18] Miller FA. Reminiscences of Pioneers and Early Commercial IR Instruments. Analytical Chemistry 1992;64:824A–31A.

[19] Downing JR, Walker IF, Patterson GD, Freed WV. Applications of infrared spectroscopy to DDT. Industrial & Engineering Chemistry Analytical Edition 1946;18:461–7.

[20] Pfann HF, Williams VZ, Mark H. Mechanism of peroxide-initiated styrene polymerization. Infrared absorption of some polymer samples. Journal of Polymer Science 1946;1:14–21.

[21] Adjaye JD, Katikaneni SPR, Bakhshi NN. Catalytic conversion of a biofuel to hydrocarbons: effect of mixtures of HZSM-5 and silica-alumina catalysts on product distribution. Fuel Processing Technology 1996;48:115–43.

[22] Sanderson MA, Agblevor F, Collins M, Johnson DK. Compositional analysis of biomass feedstocks by near infrared reflectance spectroscopy. Biomass and Bioenergy 1996;11:365–70.

[23] Oliveira FCC, Brandão CRR, Ramalho HF, da Costa LAF, Suarez PAZ, Rubim JC. Adulteration of diesel/biodiesel blends by vegetable oil as determined by Fourier transform (FT) near infrared spectrometry and FT-Raman spectroscopy. Analytica Chimica Acta 2007;587:194–9.

[24] Larkin P. Infrared and Raman Spectroscopy: Principles and Spectral Interpretation. USA: Elsevier; 2011.

[25] Bernath PF. Spectra of Atoms and Molecules: Oxford University Press; New York, USA; 2005.

[26] Corsetti S, McGloin D, Kiefer J. Comparison of Raman and IR spectroscopy for quantitative analysis of gasoline/ethanol blends. Fuel 2016;166:488–94.

[27] Wembabazi E, Mugisha PJ, Ratibu A, Wendiro D, Kyambadde J, Vuzi PC. Spectroscopic analysis of heterogeneous biocatalysts for biodiesel production from expired sunflower cooking oil. Journal of Spectroscopy 2015;2015:1-8.

[28] Zhao X, Wei L, Cheng S, Kadis E, Cao Y, Boakye E, et al. Hydroprocessing of carinata oil for hydrocarbon biofuel over Mo-Zn/Al2O3. Applied Catalysis B: Environmental 2016;196:41–9.

[29] Mallesham B, Sudarsanam P, Reddy BM. Production of biofuel additives from esterification and acetalization of bioglycerol over SnO2-based solid acids. Industrial & Engineering Chemistry Research 2014;53:18775–85.

[30] Kumar P, Srivastava VC, Mishra IM. Dimethyl carbonate synthesis from propylene carbonate with methanol using Cu–Zn–Al catalyst. Energ Fuel 2015;29:2664–75.

[31] Cheng S, Wei L, Zhao X, Kadis E, Julson J. Conversion of Prairie Cordgrass to hydrocarbon biofuel over Co-Mo/HZSM-5 using a two-stage reactor system. Energy Technology 2016;4:706–13.

[32] Mandey R, Wijaya K, Triyono, Syoufian A. Chrome impregnated on Bentonite (Cr-Bentonite) as hydrocracking catalyst of used cooking oil. Asian Journal of Chemistry 2016;28:282–6.

[33] Durak H, Aysu T. Structural analysis of bio-oils from subcritical and supercritical hydrothermal liquefaction of Datura stramonium L. The Journal of Supercritical Fluids 2016;108:123–35.

[34] Plata V, Gauthier-Maradei P, Romero-Bohórquez AR, Kafarov V, Castillo E. Characterization of insoluble material isolated from Colombian palm oil biodiesel. Biomass and Bioenergy 2015;74:6–14.

[35] Nanda S, Dalai AK, Gokalp I, Kozinski JA. Valorization of horse manure through catalytic supercritical water gasification. Waste management 2016;52:147–58.

[36] Zou S, Wu Y, Yang M, Li C, Tong J. Thermochemical catalytic liquefaction of the marine Microalgae Dunaliella tertiolecta and characterization of bio-oils. Energ Fuel 2009;23:3753–8.

[37] Shuping Z, Yulong W, Mingde Y, Kaleem I, Chun L, Tong J. Production and characterization of bio-oil from hydrothermal liquefaction of microalgae Dunaliella tertiolecta cake. Energy 2010;35:5406–11.

[38] Ahmad M, Ullah K, Khan MA, Ali S, Zafar M, Sultana S. Quantitative and qualitative analysis of sesame oil biodiesel. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 2011;33:1239–49.

[39] Corsetti S, Zehentbauer FM, McGloin D, Kiefer J. Characterization of gasoline/ethanol blends by infrared and excess infrared spectroscopy. Fuel 2015;141:136–42.

[40] Rambo MKD, Alexandre GP, Rambo MCD, Alves AR, Garcia WT, Baruque E. Characterization of biomasses from the north and northeast regions of Brazil for processes in biorefineries. Food Science and Technology (Campinas) 2015;35:605–11.

[41] Grilc M, Likozar B, Levec J. Simultaneous liquefaction and hydrodeoxygenation of lignocellulosic biomass over NiMo/Al$_2$O$_3$, Pd/Al$_2$O$_3$, and zeolite Y catalysts in hydrogen donor solvents. ChemCatChem 2016;8:180–91.

[42] Xu Y, Hu X, Yu H, Wang K, Cui Z. Hydrothermal catalytic liquefaction mechanisms of agal biomass to bio-oil. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 2016;38:1478–84.

[43] Bertschi IT, Yokelson RJ, Ward DE, Christian TJ, Hao WM. Trace gas emissions from the production and use of domestic biofuels in Zambia measured by open-path Fourier transform infrared spectroscopy. Journal of Geophysical Research: Atmospheres 2003;108.

[44] Lestander TA, Rhen C. Multivariate NIR spectroscopy models for moisture, ash and calorific content in biofuels using bi-orthogonal partial least squares regression. The Analyst 2005;130:1182–9.

[45] Oliveira JS, Montalvão R, Daher L, Suarez PAZ, Rubim JC. Determination of methyl ester contents in biodiesel blends by FTIR-ATR and FTNIR spectroscopies. Talanta 2006;69:1278–84.

[46] Baptista P, Felizardo P, Menezes JC, Correia MJ. Multivariate near infrared spectroscopy models for predicting the methyl esters content in biodiesel. Anal Chim Acta 2008;607:153–9.

[47] Balabin RM, Safieva RZ. Biodiesel classification by base stock type (vegetable oil) using near infrared spectroscopy data. Anal Chim Acta 2011;689:190–7.

[48] Balabin RM, Safieva RZ, Lomakina EI. Gasoline classification using near infrared (NIR) spectroscopy data: comparison of multivariate techniques. Anal Chim Acta 2010;671:27–35.

[49] Satyarthi JK, Srinivas D. Fourier Transform infrared spectroscopic method for monitoring hydroprocessing of vegetable oils to produce hydrocarbon-based biofuel. Energy Fuel 2011;25:3318–22.

[50] Ault AP, Pomeroy R. Quantitative investigations of biodiesel fuel using infrared spectroscopy: an instrumental analysis experiment for undergraduate chemistry students. Journal of Chemical Education 2012;89:243–7.

[51] Pinzi S, Alonso F, García Olmo J, Dorado MP. Near infrared reflectance spectroscopy and multivariate analysis to monitor reaction products during biodiesel production. Fuel 2012;92:354–9.

[52] Baum A, Hansen PW, Meyer AS, Mikkelsen JD. Simultaneous measurement of two enzyme activities using infrared spectroscopy: a comparative evaluation of PARAFAC, TUCKER and N-PLS modeling. Anal Chim Acta 2013;790:14–23.

[53] Corgozinho CNC, Barbeira PJS. Quantification of biodiesel in biodiesel-diesel blends using spectrofluorimetry and multivariate calibration. Journal of Analytical Chemistry 2015;70:936–42.

[54] Mata Sánchez J, Pérez Jiménez JA, Díaz Villanueva MJ, Serrano A, Núñez N, López Giménez J. Assessment of near infrared spectroscopy for energetic characterization of olive byproducts. Renewable Energy 2015;74:599–605.

[55] Mazivila SJ, de Santana FB, Mitsutake H, Gontijo LC, Santos DQ, Neto WB. Discrimination of the type of biodiesel/diesel blend (B5) using mid-infrared spectroscopy and PLS-DA. Fuel 2015;142:222–6.

[56] Mitsutake H, Guimarães E, Freitas HC, Gontijo LC, Santos DQ, Borges Neto W. Multivariate control charts based on NAS and mid-infrared spectroscopy for quality control of B5 blends of methyl soybean biodiesel in diesel. Journal of Chemometrics 2015;29:411–9.

[57] Paiva EM, Rohwedder JJR, Pasquini C, Pimentel MF, Pereira CF. Quantification of biodiesel and adulteration with vegetable oils in diesel/biodiesel blends using portable near-infrared spectrometer. Fuel 2015;160:57–63.

[58] Alves JCL, Poppi RJ. Quantification of conventional and advanced biofuels contents in diesel fuel blends using near-infrared spectroscopy and multivariate calibration. Fuel 2016;165:379–88.

[59] Duca D, Pizzi A, Mancini M, Rossini G, Mengarelli C, Ilari A, et al. Fast measurement by infrared spectroscopy as support to woody biofuels quality determination. Journal of Agricultural Engineering 2016;47:17.

[60] Sitoe BV, Mitsutake H, Guimarães E, Gontijo LC, Santos DQ, Borges Neto W. Quality control of biodiesel content of B7 blends of Methyl Jatropha and methyl crambe biodiesels using mid-infrared spectroscopy and multivariate control charts based on net analyte signal. Energy & Fuel 2016;30:1062–1070.

[61] Neyestanaki AK, Klingstedt F, Lindfors L-E. Catalytic removal of NOx from gas mixtures simulating emissions from the combustion of biofuels. Reaction Kinetics and Catalysis Letters 2000;70:17–25.

[62] Yang H, Yan R, Chin T, Liang DT, Chen H, Zheng C. Thermogravimetric analysis: Fourier transform infrared analysis of palm oil waste pyrolysis. Energy & Fuel 2004;18:1814–21.

[63] Das B, Mandal TK, Patra S. A comprehensive study on Chlorella pyrenoidosa for phenol degradation and its potential applicability as biodiesel feedstock and animal feed. Applied Biochemistry and Biotechnology 2015;176:1382-401.

[64] Li H, Niu S, Lu C, Wang Y. Comprehensive investigation of the thermal degradation characteristics of biodiesel and its feedstock oil through TGA–FTIR. Energy & Fuel 2015;29:5145-53.

[65] Zhang T, Zhou Y, Liu D, Petrus L. Qualitative analysis of products formed during the acid catalyzed liquefaction of bagasse in ethylene glycol. Bioresource Technology 2007;98:1454-9.

[66] Gillespie GD, Everard CD, McDonnell KP. Prediction of biomass pellet quality indices using near infrared spectroscopy. Energy 2015;80:582-8.

[67] Kiran B, Pathak K, Kumar R, Deshmukh D, Rani N. Influence of varying nitrogen levels on lipid accumulation in Chlorella sp. International Journal of Environmental Science and Technology 2016;13:1823-32.

[68] Mishra S, Gupta S, Raghuvanshi S, Pal P. Energetic assessment of fixation of CO_2 and subsequent biofuel production using B. cereus SM1 isolated from sewage treatment plant. Bioprocess and biosystems engineering 2016;39:1247-58.

[69] Ortiz EG, Quintero I, Arévalo K. Biodiesel production from three mixes of oils with high free fatty content: quality evaluation and variable analysis. International Journal of Environmental Science and Technology 2016;13:1367-76.

[70] Ruan T, Zeng R, Yin X-Y, Zhang S-X, Yang Z-H. Water Hyacinth (Eichhornia crassipes) Biomass as a Biofuel Feedstock by Enzymatic Hydrolysis. BioResources 2016.

[71] Chen Z, Lovett D, Morris J. Process analytical technologies and real time process control a review of some spectroscopic issues and challenges. Journal of Process Control 2011;21:1467-82.

[72] Chadwick DT, McDonnell KP, Brennan LP, Fagan CC, Everard CD. Evaluation of infrared techniques for the assessment of biomass and biofuel quality parameters and conversion technology processes: a review. Renewable and Sustainable Energy Reviews 2014;30:672-81.

[73] Killner MHM, Rohwedder JJR, Pasquini C. A PLS regression model using NIR spectroscopy for on-line monitoring of the biodiesel production reaction. Fuel 2011;90: 3268–73.

[74] de Souza AVA, Cajaiba da Silva JF. Biodiesel Synthesis Evaluated by Using Real-Time ATR-FTIR. Organic Process Research & Development 2012;17:127–32.

[75] Natalello A, Sasso F, Secundo F. Enzymatic transesterification monitored by an easy-to-use Fourier transform infrared spectroscopy method. Biotechnology Journal 2013;8:133–138.

[76] Richard R, Dubreuil B, Thiebaud-Roux S, Prat L. On-line monitoring of the transester-ification reaction carried out in microreactors using near infrared spectroscopy. Fuel 2013;104:318–25.

[77] de Lima SM, Silva BFA, Pontes DV, Pereira CF, Stragevitch L, Pimentel MF. In-line monitoring of the transesterification reactions for biodiesel production using NIR spectroscopy. Fuel 2014;115:46–53.

[78] Reyman D, Saiz Bermejo A, Ramirez Uceda I, Rodriguez Gamero M. A new FTIR method to monitor transesterification in biodiesel production by ultrasonication. Environmental Chemistry Letters 2014;12:235–40.

[79] Kartnaller V, Junior II, de Souza AVA, Costa ICR, Rezende MJC, da Silva JFC, et al. Evaluating the kinetics of the esterification of oleic acid with homo and heterogeneous catalysts using in-line real-time infrared spectroscopy and partial least squares calibration. Journal of Molecular Catalysis B: Enzymatic 2016;123:41–6.

[80] Silverstein RM, Webster FX, Kiemle DJ. Spectrometric Identification of Organic compounds Wiley; USA; 2005.

[81] Evilia R. Quantitative NMR spectroscopy. Analytical Letters 2001;34:2227.

[82] Darbeau RW. Nuclear magnetic resonance (NMR) spectroscopy: a review and a look at its use as a probative tool in deamination chemistry. Applied Spectroscopy Reviews 2006;41:401–25.

[83] Monteiro MR, Ambrozin ARP, Lião LM, Ferreira AG. Determination of biodiesel blend levels in different diesel samples by 1H NMR. Fuel 2009;88:691–6.

[84] Vispute TP, Zhang HY, Sanna A, Xiao R, Huber GW. Renewable chemical commodity feedstocks from integrated catalytic processing of pyrolysis oils. Science 2010;330: 1222–7.

[85] Andrade DF, Mazzei JL, Kaiser CR, d'Avila LA. Assessment of different measurement methods using 1H-NMR data for the analysis of the transesterification of vegetable oils. Journal of the American Oil Chemists' Society 2012;89:619–30.

[86] Flores IS, Godinho MS, de Oliveira AE, Alcantara GB, Monteiro MR, Menezes SMC, et al. Discrimination of biodiesel blends with 1H NMR spectroscopy and principal component analyses. Fuel 2012;99:40–4.

[87] Killner MHM, Garro Linck Y, Danieli E, Rohwedder JJR, Blümich B. Compact NMR spectroscopy for real-time monitoring of a biodiesel production. Fuel 2015;139:240–7.

[88] Sharifzadeh M, Wang L, Shah N. Decarbonisation of olefin processes using biomass pyrolysis oil. Applied Energy 2015;149:404–14.

[89] Sharifzadeh M, Richard CJ, Liu K, Hellgardt K, Chadwick D, Shah N. An integrated process for biomass pyrolysis oil upgrading: a synergistic approach. Biomass and Bioenergy 2015;76:108–17.

[90] Venderbosch RH, Prins W. Fast pyrolysis technology development. Biofuels, Bioproducts and Biorefining 2010;4:178–208.

[91] Akhtar J, Saidina Amin N. A review on operating parameters for optimum liquid oil yield in biomass pyrolysis. Renewable and Sustainable Energy Reviews 2012;16: 5101–9.

[92] Pradhan D, Singh RK, Bendu H, Mund R. Pyrolysis of Mahua seed (Madhuca indica): Production of biofuel and its characterization. Energy Conversion and Management 2016;108:529–38.

[93] Junming X, Jianchun J, Yanju L, Jie C. Liquid hydrocarbon fuels obtained by the pyrolysis of soybean oils. Bioresource Technology 2009;100:4867–70.

[94] Fagernäs L, Kuoppala E, Tiilikkala K, Oasmaa A. Chemical composition of Birch Wood slow pyrolysis products. Energy & Fuel 2012;26:1275–83.

[95] Mullen CA, Strahan GD, Boateng AA. Characterization of various fast-pyrolysis bio-oils by NMR spectroscopy. Energy & Fuel 2009;23:2707–18.

[96] Ben HX, Ragauskas AJ. In situ NMR characterization of pyrolysis oil during accelerated aging. Chemsuschem 2012;5:1687–93.

[97] Joseph J, Baker C, Mukkamala S, Beis SH, Wheeler MC, DeSisto WJ, et al. Chemical shifts and lifetimes for nuclear magnetic resonance (NMR) analysis of biofuels. Energy & Fuel 2010;24:5153–62.

[98] Prestes RA, Colnago LA, Forato LA, Vizzotto L, Novotny EH, Carrilho E. A rapid and automated low resolution NMR method to analyze oil quality in intact oilseeds. Anal Chim Acta 2007;596:325–9.

[99] Garro Linck Y, Killner MHM, Danieli E, Blümich B. Mobile low-field 1H NMR spectroscopy desktop analysis of biodiesel production. Applied Magnetic Resonance 2013;44:41–53.

[100] Anderson LA, Franz AK. Real-time monitoring of transesterification by 1H NMR spectroscopy: catalyst comparison and improved calculation for biodiesel conversion. Energy & Fuel 2012;26:6404–10.

[101] Shahid EM, Jamal Y. A review of biodiesel as vehicular fuel. Renewable and Sustainable Energy Reviews 2008;12:2484–94.

[102] Zawadzki A, Shrestha DS, He B. Biodiesel blend level detection using ultraviolet absorption spectra. Transactions of the Asabe 2007;50:1349–53.

[103] Brandão LFP, Braga JWB, Suarez PAZ. Determination of vegetable oils and fats adulterants in diesel oil by high performance liquid chromatography and multivariate methods. Journal of Chromatography A 2012;1225:150–7.

[104] de Oliveira RR, de Lima KMG, Tauler R, de Juan A. Application of correlation constrained multivariate curve resolution alternating least-squares methods for determination of compounds of interest in biodiesel blends using NIR and UV–visible spectroscopic data. Talanta 2014;125:233–41.

[105] Fernandes DDS, Gomes AA, de Fontes MM, da Costa GB, de Almeida VE, de Araujo MCU, et al. UV-Vis spectrometric detection of biodiesel/diesel blend adulterations with soybean oil. Journal of the Brazilian Chemical Society 2014;25:169–75.

[106] Foglia TA, Jones KC, Phillips JG. Determination of biodiesel and triacylglycerols in diesel fuel by LC. Chromatographia 2005;62:115–9.

[107] Prats-Montalban JM, de Juan A, Ferrer A. Multivariate image analysis: a review with applications. Chemometr Intell Lab 2011;107:1–23.

[108] Silva Lyra W, dos Santos VB, Dionízio AGG, Martins VL, Almeida LF, Nóbrega Gaião E, et al. Digital image-based flame emission spectrometry. Talanta 2009;77:1584–9.

[109] Alexandre I, Hamels S, Dufour S, Collet J, Zammatteo N, De Longueville F, et al. Colorimetric silver detection of DNA microarrays. Analytical Biochemistry 2001;295:1–8.

[110] Sarrafzadeh MH, La H-J, Lee J-Y, Cho D-H, Shin S-Y, Kim W-J, et al. Microalgae biomass quantification by digital image processing and RGB color analysis. Journal of Applied Phycology 2014;27:205–9.

[111] Chen X, Goh QY, Tan W, Hossain I, Chen WN, Lau R. Lumostatic strategy for microalgae cultivation utilizing image analysis and chlorophyll a content as design parameters. Bioresource Technology 2011;102:6005–12.

[112] Capus A, Monnerat M, Ribeiro LC, de Souza W, Martins JL, Sant'Anna C. Application of high-content image analysis for quantitatively estimating lipid accumulation in oleaginous yeasts with potential for use in biodiesel production. Bioresource Technology 2016;203:309-17.

[113] Costa GB, Fernandes DD, Almeida VE, Araujo TS, Melo JP, Diniz PH, et al. Digital image-based classification of biodiesel. Talanta 2015;139:50–5.

[114] Bansal K, McCrady J, Hansen A, Bhalerao K. Thin layer chromatography and image analysis to detect glycerol in biodiesel. Fuel 2008;87:3369–72.

[115] Li N, Lu G, Li X, Yan Y. Prediction of NOx emissions from a biomass fired combustion process based on flame radical imaging and deep learning techniques. Combustion Science and Technology 2015;188:233–46.

[116] Lepri FG, Chaves ES, Vieira MA, Ribeiro AS, Curtius AJ, DeOliveira LCC, et al. Determination of trace elements in vegetable oils and biodiesel by atomic spectrometric techniques: a review. Applied Spectroscopy Reviews 2011;46:175–206.

[117] Taylor HE. Inductively Coupled Plasma-Mass Spectrometry: Practices and Techniques. Academic Press; USA; 2001.

[118] Schlemmer G, Radziuk B. Analytical Graphite Furnace Atomic Absorption Spectrometry: A Laboratory Guide. Birkhäuser Verlag; Basel, Switzerland; 1999.

[119] Welz B, Sperling M. Atomic Absorption Spectrometry. Wiley-VCH; Weinheim, Germany; 2005.

[120] Balat M. Production of biodiesel from vegetable oils: a survey. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 2007;29:895–913.

[121] Lisboa MT, Clasen CD, Vellar DCdS, Oreste EQ, Saint'Pierre TD, Ribeiro AS, et al. An easy and fast procedure for the determination of Ca, Cu, Fe, Mn, Mg, Na, K and Si in biodiesel by ICP OES using emulsification as sample preparation strategy. Journal of the Brazilian Chemical Society 2013;25:143–151.

[122] Baernthaler G, Zischka M, Haraldsson C, Obernberger I. Determination of major and minor ash-forming elements in solid biofuels. Biomass and Bioenergy 2006;30:983–97.

[123] de Jesus A, Silva MM, Vale MG. The use of microemulsion for determination of sodium and potassium in biodiesel by flame atomic absorption spectrometry. Talanta 2008;74:1378–84.

[124] Sarin A, Arora R, Singh NP, Sharma M, Malhotra RK. Influence of metal contaminants on oxidation stability of Jatropha biodiesel. Energy 2009;34:1271–5.

[125] Balcaen L, Woods G, Resano M, Vanhaecke F. Accurate determination of S in organic matrices using isotope dilution ICP-MS/MS. Journal of Analytical Atomic Spectrometry 2013;28:33–9.

[126] Amais RS, Long SE, Nobrega JA, Christopher SJ. Determination of trace sulfur in biodiesel and diesel standard reference materials by isotope dilution sector field inductively coupled plasma mass spectrometry. Analytica Chimica Acta 2014;806:91–6.

[127] Woods GD, Fryer FI. Direct elemental analysis of biodiesel by inductively coupled plasma-mass spectrometry. Analytical and Bioanalytical Chemistry 2007;389:753–61.

[128] Sánchez R, Sánchez C, Lienemann C-P, Todolí J-L. Metal and metalloid determination in biodiesel and bioethanol. Journal of Analytical Atomic Spectrometry 2015;30:64–101.

[129] Saleh MI, Murray RS, Chin CN. Ashing techniques in the determination of iron and copper in palm oil. Journal of the American Oil Chemists' Society 1988;65:1767–70.

[130] Carbonell V, Mauri AR, Salvador A, de la Guardia M. Direct determination of copper and iron in edible oils using flow injection flame atomic absorption spectrometry. Journal of Analytical Atomic Spectrometry 1991;6:581–4.

[131] Matos Reyes MN, Campos RC. Determination of copper and nickel in vegetable oils by direct sampling graphite furnace atomic absorption spectrometry. Talanta 2006;70:929–32.

[132] Cindric IJ, Zeiner M, Steffan I. Trace elemental characterization of edible oils by ICP–AES and GFAAS. Microchemical Journal 2007;85:136–9.

[133] de Oliveira AP, Villa RD, Antunes KCP, de Magalhães A, Silva ECe. Determination of sodium in biodiesel by flame atomic emission spectrometry using dry decomposition for the sample preparation. Fuel 2009;88:764–6.

[134] Gonzalvez A, Ghanjaoui ME, El Rhazi M, de la Guardia M. Inductively coupled plasma optical emission spectroscopy determination of trace element composition of argan oil. Food Science and Technology International 2010;16:65–71.

[135] Aucélio RQ, de Souza RM, de Campos RC, Miekeley N, da Silveira CLP. The determination of trace metals in lubricating oils by atomic spectrometry. Spectrochimica Acta Part B: Atomic Spectroscopy 2007;62:952–61.

[136] Blades MW, Caughlin BL. Excitation temperature and electron density in the inductively coupled plasma: aqueous vs organic solvent introduction. Spectrochimica Acta Part B: Atomic Spectroscopy 1985;40:579–91.

[137] Jai Kumar S, Gangadharan S. Determination of trace elements in naphtha by inductively coupled plasma mass spectrometry using water-in-oil emulsions. Journal of Analytical Atomic Spectrometry 1999;14:967–71.

[138] Karadjova I, Zachariadis G, Boskou G, Stratis J. Electrothermal atomic absorption spectrometric determination of aluminium, cadmium, chromium, copper, iron, manganese, nickel and lead in olive oil. Journal of Analytical Atomic Spectrometry 1998;13:201–4.

[139] Edlund M, Visser H, Heitland P. Analysis of biodiesel by argon-oxygen mixed-gas inductively coupled plasma optical emission spectrometry. Journal of Analytical Atomic Spectrometry 2002;17:232–5.

[140] Canário CM, Katskov DA. Direct determination of Cd and Pb in edible oils by atomic absorption spectrometry with transverse heated filter atomizer. Journal of Analytical Atomic Spectrometry 2005;20:1386.

[141] Saint'Pierre TD, Tormen L, Frescura VLA, Curtius AJ. The direct analysis of fuel ethanol by ICP-MS using a flow injection system coupled to an ultrasonic nebulizer for sample introduction. Journal of Analytical Atomic Spectrometry 2006;21:1340–4.

[142] dos Santos EJ, Herrmann AB, Chaves ES, Vechiatto WWD, Schoemberger AC, Frescura VLA, et al. Simultaneous determination of Ca, P, Mg, K and Na in biodiesel by axial view inductively coupled plasma optical emission spectrometry with internal stand-ardization after multivariate optimization. Journal of Analytical Atomic Spectrometry 2007;22:1300.

[143] Ibrahim H. Determination of lead in frying oils by direct current plasma atomic emission spectrometry. Journal of the American Oil Chemists Society 1991;68:678–9.

[144] Murillo M, Benzo Z, Marcano E, Gomez C, Garaboto A, Marin C. Determination of copper, iron and nickel in edible oils using emulsified solutions by ICP-AES. Journal of Analytical Atomic Spectrometry 1999;14:815-20.

[145] de Jesus A, Zmozinski AV, Barbará JnA, Vale MGR, Silva MrM. Determination of calcium and magnesium in biodiesel by flame atomic absorption spectrometry using microemulsions as sample preparation. Energy & Fuel 2010;24:2109–12.

[146] de Souza RM, Leocádio LG, da Silveira CLP. ICP OES Simultaneous Determination of Ca, Cu, Fe, Mg, Mn, Na, and P in biodiesel by axial and radial inductively coupled plasma-optical emission spectrometry. Analytical Letters 2008;41:1615–22.

[147] Silva JSA, Chaves ES, Santos ÉJd, Saint'Pierre TD, Frescura VLA, Curtius AJ. Calibra-tion techniques and modifiers for the determination of Cd, Pb and Tl in biodiesel as microemulsion by graphite furnace atomic absorption spectrometry. Journal of the Brazilian Chemical Society 2010;21:620–6.

[148] Lima AS, Silva DG, Teixeira LS. Determination of cadmium in biodiesel using micro-emulsion and electrothermal atomization atomic absorption spectrometry. Environ-mental monitoring and assessment 2015;187:4122.

[149] De Leonardis A, Macciola V, De Felice M. Copper and iron determination in edible vegetable oils by graphite furnace atomic absorption spectrometry after extraction with diluted nitric acid. International Journal of Food Science & Technology 2000;35:371–5.

[150] Kowalewska Z, Izgi B, Saracoglu S, Gucer S. Application of liquid-liquid extraction and adsorption on activated carbon of the determination of different forms of metals present in edible oils. Chem Anal (Warsaw) 2005;50.

[151] Tokay F, Bağdat S. Determination of iron and copper in edible oils by flame atomic absorption spectrometry after liquid–liquid extraction. Journal of the American Oil Chemists' Society 2015;92:317–22.