Analytical Monitoring of Triglyceride Ethanolysis Reaction

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Tomas Bata University in Zlín
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Theses guidelines

1. Review current literature aimed at analytical monitoring of alcoholysis reaction (transesterification) of triglycerides. Focus especially at methods suitable for on-line analysis.
2. Monitor triglyceride ethanolysis reaction with chosen method(s), preferably in an on-line manner.
3. Evaluate performance of tested method(s) and perform basic validation against standard reference method – gas chromatographic determination of reaction mixture composition.
4. Critically evaluate gained results, discuss them and summarize in a form of Master thesis.

Form processing of diploma thesis: **printed/electronic**
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Recommended resources:

[1] Zagonel GF, Peralta-Zamora P, Ramos LP. Multivariate monitoring of soybean oil ethanolysis by FTIR. Talanta 2004;63:1021–5. https://doi.org/10.1016/j.talanta.2004.01.008.
[2] Mahamuni NN, Adewuyi YG. Fourier transform infrared spectroscopy (FTIR) method to monitor soy biodiesel and soybean oil in transesterification reactions, petrodiesel-biodiesel blends, and blend adulteration with soy oil. Energy and Fuels 2009;23:3773–82. https://doi.org/10.1021/ef900130m.
[3] Mwenge P, Seodigeng T. In situ Real-Time Multivariate Analysis of Methanolysis Monitoring of Sunflower Oil Using FTIR. Int J Chem Mol Eng 2020;14:136–48.
[4] Husár J, Pecha J, Šánek L. Development and validation of a simple and reliable alternative method for process monitoring and final product quality control during fatty acid ethyl esters production. Talanta 2021;235:122752. https://doi.org/10.1016/J.TALANTA.2021.122752.
[5] Knothe G, Krahl J, Van Gerpen JH. *The Biodiesel Handbook*. 2nd ed. Urbana, Ill.: AOCS Press, c2010, online (xiii, 501 p.). ISBN 9781615835843.

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ABSTRACT

The current world energy crisis had led to the need for alternative energy source, one of these sources is the use of oil for the production of biodiesel using the transesterification process. This in turn requires the full process understanding in order to efficiently and easily monitor and control the transesterification reaction. In this work Transesterification reaction, using rapeseed oil and ethanol as an esterifying agent with Sodium Hydroxide as a catalyst, was monitored using Fourier Transform Infrared Spectroscopy (FTIR). This method was implemented as the analysis method in an on-line manner.2-Dimensional Correlation Spectroscopy was used on samples taken directly from the reaction mixture, in order to find regions of interest that directly correlate to the concentration or percentage of parameters of interest including : Triglycerides, Monoglycerides, Diglycerides, Fatty Acid Ethyl Esters, and Conversion. It was found that 2-Dimensional Correlation Spectroscopy can be easily implemented and leads to promising results, and Fourier Transform Infrared Spectroscopy was found to be reliable, with results within 5% error of the reference method used (Gas Chromatography). Fourier Transform Infrared (FT-IR) Spectroscopy with 2D-COS analysis was found to be quick, with the possibility of automation and would provide a jump forward for large-scale process control in the Ethanolysis On-Line monitoring when compared to classical Off-Line method.

Keywords:

Transesterification, Fourier Transform Infrared Spectroscopy, 2D-COS, FTIR, Ethanolysis, Fatty Acid Ethyl Esters.

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TABLE OF CONTENTS

I. INTRODUCTION

I.I. BACKGROUND

Fuel and energy security have been on the forefront of recent world developments; with Europe being the main player, as well as the most pressured to switch from conventional fossil fuels, and embrace all kinds of alternative renewable energy sources, like Wind, Solar, Hydro, Geothermal, Biofuels. As the energy blockade continues with Russia, Europe needs to find alternative, green, sustainable, and renewable energy sources in order to achieve energy security, meet European energy demand, as well as stopping the current trends in emissions of greenhouse gases in Europe, and to lead the way for other countries to implement such technologies. [1]

On the other hand, reports have been published by the U.S. Global Climate Change Research Program: Fourth National Climate Assessment and the UN IPCC Special Report on Global Warming. These reports had stated that the impact of climate change is already being felt in communities worldwide, and any further changes and increase in the average temperature of the planet would lead to devastating effects, on all seven continents, from ice melting in Antarctica, to uncontrollable floods in once arid and desert regions. [2]

Fossil fuel has been the main source of energy worldwide, and especially Europe with natural gas imported from Russia; burning fossil fuels emit large amounts of carbon dioxide, methane, nitrous oxide, and sulfur dioxide, as well as particulate matter (PM), which all of them have a significant and devastating effect on humans, with many kinds of effects ranging from respiratory problems to cancer risk. [3]

I.II. OVERVIEW OF BIOFUEL PRODUCTION

Biofuels are fuels derived from biomass (mostly organic sources) originating from plants and other living things, the goal of biofuel production is to be able to ideally replace petroleum or fossil fuels in engines, since the transportation sector is one of the biggest industries currently using petroleum or fossil fuels worldwide, the introduction of biofuels that have the compatibility to be used in currently operating engines could have a substantial impact on greenhouse gas (GHG) emissions worldwide, and could have the potential of significantly reducing the reliance on fossil fuels, or complete migration from fossil fuel sources with the combination of other renewable and sustainable sources such as solar, wind, hydroelectric, and other renewable energy sources. [4]

Biofuels can be categorized as biodiesel (mainly produced from vegetable oil, wax, or fats) or bioethanol (alcohol produced from starch and sugar containing plants e.g., corn) or biogas. One of the main advantages of using biofuels is the reduction of the amount of brown haze and significantly lower carbon monoxide percentage, and sulfur compounds. Biofuels used as an alternative to fossil fuels can reduce these emissions, as well as being sustainable, and being originating from renewable sources with less impact on the planet than fossil fuels is also considered an advantage to move on from classic fuels.[4]

Biofuels can be generally and broadly categorized into four main groups, 1st, 2nd, 3rd, and 4th generation biofuels. 1st generation biofuels are those biofuels made from plant oils, or plant sugars, this type of biofuels is one of the oldest, most used, and most developed of the four groups. 2nd generation biofuels are those made from waste organic matter from other industries, mainly lignocellulosic biomass sourced from agricultural activities and forestry, this generation of biofuels mitigates the argument that is raised with the 1st generation biofuels where it competes with food for landmass, where 2nd generation biofuels are mainly sourced from biomass that is considered waste matter and does not compete with land used for agriculture or to feed humans directly or indirectly. 3rd generation biofuels are produced by algae, which are provided with the required nutrients in order to grow and flourish, then is used for the production of biofuel in a process that is gaining a lot of momentum. Finally, 4th generation biofuels are the ones made from cyanobacteria which had been engineered to produce the feedstock required for the production of biofuels, this generation is the newest in the development of biofuel production and scientific research. [5]

In the years from 2021-2026 global demand for biofuels is projected to grow by 41 billion liters or 21% according to the International Energy Agency shown in Figure 1, one of the important reasons for this growth is the policies introduced by the United States and the European Union which helps increase the demand for biofuels in the near and far future. [6]

I.III. BIOFUEL

Biofuels from oils can be produced using a multitude of techniques that have been improved for decades, to name a few of these techniques: Pyrolysis, Micro-Emulsification, and Transesterification. Micro emulsion is one of the oldest techniques used for the production of biofuel from oils, which is mainly done by mixing the oil with a cosolvent to produce the biodiesel. Pyrolysis on the other hand involves using heat to decompose the long alkyl chains into smaller ones at high temperatures. [2]

Biofuels are defined by the EEA (European Environment Agency) as: A fuel produced from dry organic matter or combustible oils produced by plants. [7], biofuel made from plant oil by transesterification can also be called Fatty Acid Methyl Esters (FAME), or in case of using Ethanol in its production it would therefore be called Fatty Acid Ethyl Esters (FAEE); biodiesel can be mixed with petroleum diesel up to a certain percentage, biodiesel differs from petroleum diesel in its lubricity, sulfur content where biodiesel has no sulfur content, biodiesel is much less toxic that petroleum diesel, which would be beneficial in case of spills and mishandling. [8]
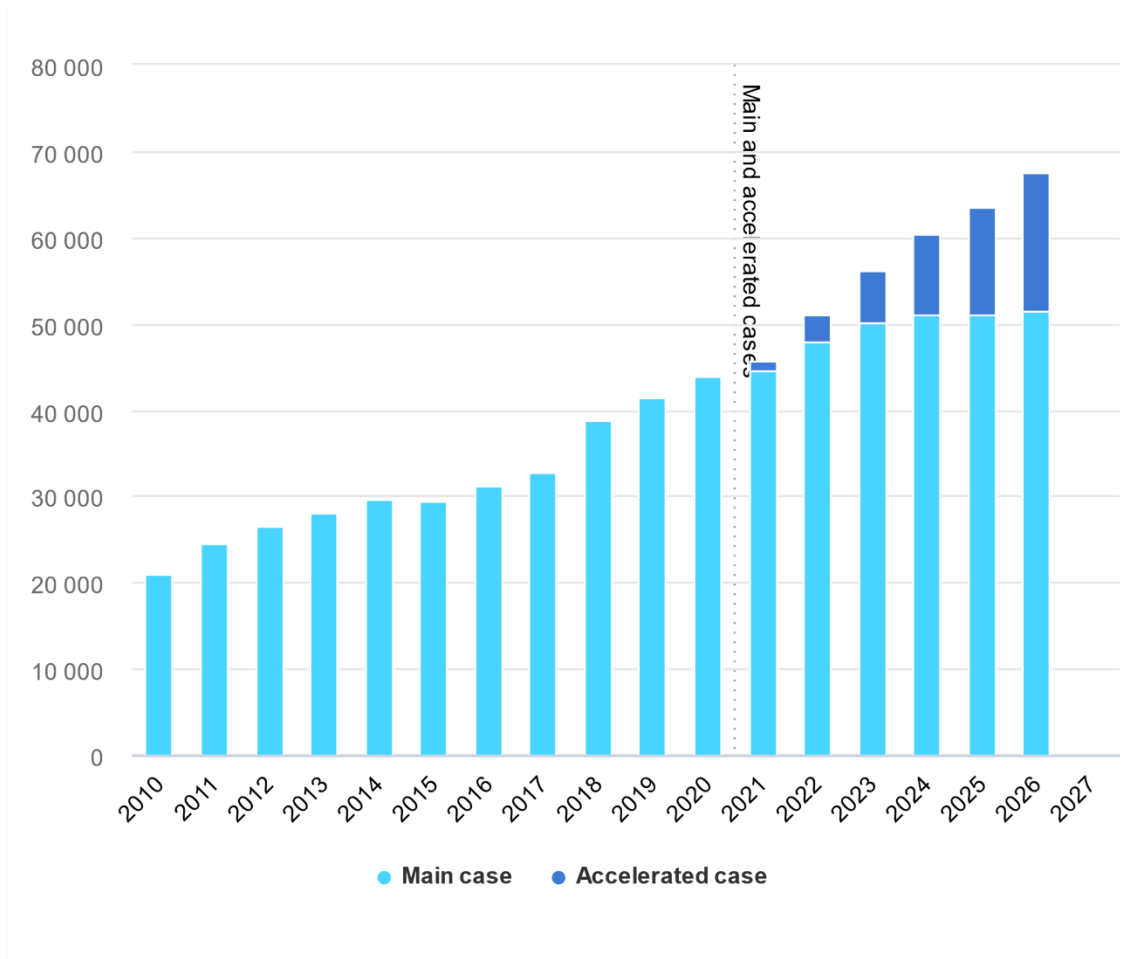
Figure 1 - Global Biodiesel Consumption, years 2021-2026, in Million Liters / Year. Source: IEA.org [6]

Predicted by the International Energy Agency, biodiesel consumption is increasing steadily through 2026.

I.IV.   TRANSESTERIFICATION PROCESS

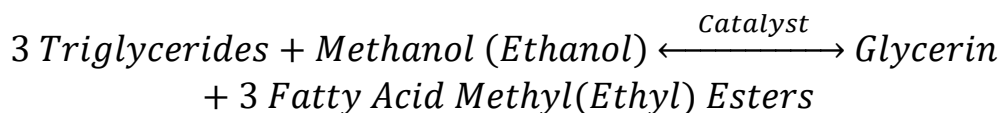### General description of the Transesterification Process.

Transesterification is a chemical process which is used commonly in the production of biodiesel. It entails the conversion of triglycerides which are the main component in plant oils or animal fats into a different fatty acid, whether Fatty Acid Methyl Esters (FAMEs) of Fatty Acid Ethyl Esters (FAEEs) depending on the kind of alcohol used in the production process. Transesterification is carried out by mixing some type of oil or animal fat with alcohol, in the presence of a certain catalyst to form biodiesel and glycerol. Once the reaction is complete, the mixture is separated into two layers, the top layer is a mixture of fatty acids, while the bottom layer consists mainly of glycerol which is considered as a byproduct of the process. [9]

Sources of Oil.

One of the most important considerations of biodiesel production is choosing the right feedstock depending on geographical availability for the production, sources of oil can be characterized into: Edible vegetable oil, non-edible vegetable oil, waste, and recycled oil, as well as animal fat, oils derived from algae, and microbial sources. [9]

The Transesterification process can be simplified as follows shown in Equation 1:

Equation 1 - Stoichiometric Equation for the General Transesterification Reaction. [10]

$$3\,Triglycerides + Methanol\,(Ethanol) \xrightarrow{\ Catalyst\ } Glycerin \\ + 3\,Fatty\,Acid\,Methyl(Ethyl)\,Esters$$

Catalysts and their Effect

For an effective and efficient transesterification process, the oil is first mixed with ethanol or methanol, then a catalyst is added to the system, whether it be a heterogeneous or homogenous catalyst. Heterogeneous catalysts are favored since they are much easier to remove from the reaction mixture after the reaction has taken place. [11]

Homogenous Catalysis: Is defined as the process of when the reactants are miscible, these homogenous catalysts can be acidic, alkaline, or enzymatic. Homogenous catalysts are used because of their relatively simplified usage, as well as their straightforward application, preparation, and availability [11]. The most used homogenous catalysts in the Transesterification process for the production of biodiesel are Sodium Hydroxide (NaOH), as well as Potassium Hydroxide (KOH), in addition to Sodium Methoxide (CH_3ONa) [12] , although Sodium Methoxide is considered to be the most expensive, and least popular, it is found to be the most applicable homogenous catalyst for the process because of its ability to prevent the formation of water and soap, which is considered one of the roadblocks in the process of biodiesel production. [13] Other types of homogenous catalysts have also been used in the process, such as enzymatic

catalysts, as well as organometallic catalysts, which will not be discussed in the current study. A disadvantage of using homogenous catalysts is that these catalysts are usually found to be corrosive, as well as introducing a new step where the resulting solution needs to be separated, and washed from the product which produces a substantial amount of wastewater, ultimately, these catalysts are rarely capable of being reused again. [14] [15]

Heterogeneous Catalysis: Is defined as the process of when the reactants and the catalyst are of different phases in the reaction system. Advantages for using heterogeneous catalysts are often associated with their ability to separate easily from the mixture when the reaction had taken place, in biodiesel production, they are advantageous because they produce higher glycerol purity when compared to using a homogenous catalyst [13]. Heterogeneous catalysts also eliminate the need to the washing process introduced when using homogenous catalysts, this also leads to significantly less waste produced in the process. Heterogeneous catalysts have sparked much interest in research, a few examples of heterogeneous catalysts are zeolites (mesoporous KIT-6, Zirconia ZrO_2, Li/NaY) [16][17] *Rice Husk Ash, Eggshells, Fly Ash, and Red Mud [18] [14]* . It is important to note that using recyclable, reusable, and readily available materials as catalysts would significantly reduce the cost of production of biodiesel, since the catalysts are one of the most important constituents in the reaction of production of biodiesel, in addition to the majority of the production cost which can be related to the cost of feedstock (oils or fats). [14]

Effect of Reaction Temperature

The Transesterification process can take place at certain different temperatures depending on the feedstock used, e.g., Methanolysis process of castor oil required a temperature of 20 – 35 C to produce a satisfactory result, while other vegetable oils like Soybean oil required a temperature of 45 – 50 C to produce similar results of satisfactory conversion rates after a specific duration (often 1 – 2 Hours) [19] [20]. Reaction temperature directly influences the rate of the reaction, controlling temperature's influence on the reaction comes to the requirements of the production process, feedstock, and energy requirements and cost cutting measures put in place, if any.

Effect of Reaction Time

Different vegetable oils had been studied in similar conditions to determine optimum reaction time and its effects on the transesterification process. It was found that during the first minute, the reaction rate is very slow due to the mixing and dispersion of oils with the used alcohol and catalyst, afterwards, the reaction rate was found to be significantly faster, up to a threshold of about 1 – 2 hours, when most feedstock of vegetable oils were found to yield a conversion of (93-98%) under identical conditions, this relationship can be shown in Figure 2. [20] [21]
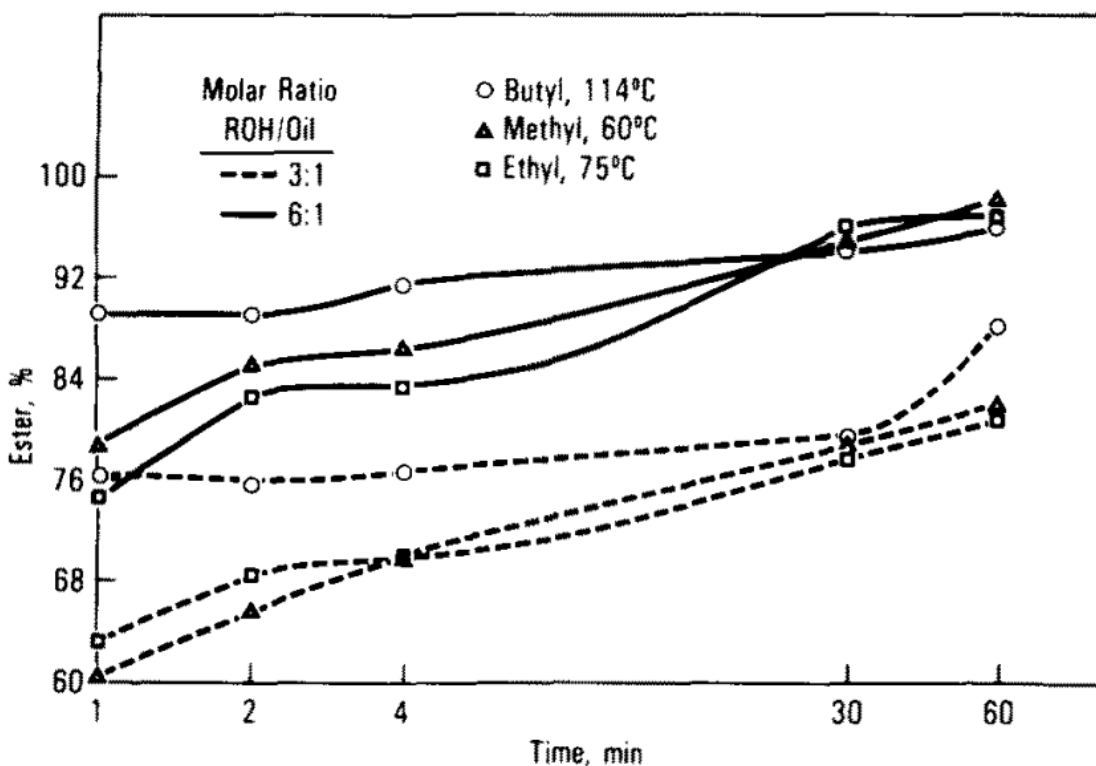
Figure 2 - Effect of Molar Ratio and Reaction Time on the Conversion rate in the Transesterification reaction using 0.5% Sodium Methoxide as a Catalyst. [22]

Effect of Oil to Alcohol Molar Ratio

One of the most important variables affecting the Transesterification process, and its yield, is the molar ratio of oil to alcohol used. Several studies had found that for the reaction to yield acceptable yields, it was required to have 6 moles of alcohol for 1 mole of glyceride in order to yield 3 moles of fatty esters and 1 mole of glycerol. This ratio changes depending on the type of catalyst used, in acidic homogenous catalysts, the ratio is significantly higher for the yield to be acceptable, while generally when using an alkaline catalyst, the ratio was found to be much lower compared to acidic catalysts. Generally, higher molar ratio of alcohol to oil tends to result in a greater conversion in less time, Figure 2 represents the relationship between the molar ratio and conversion. When using alkaline catalysts, it was found that a molar ratio of (3-6:1) to be sufficient with acceptable conversion in a reasonable time. [21]

I.V. ANALYTICAL METHODS FOR MONITORING BIOFUEL PRODUCTION

Determining the products of the Transesterification reaction is essential to the production of biofuel, since it provides a window into establishing a standardized method and requirements for the produced biodiesel, as well as providing the opportunity for adjustments and deeper knowledge of the actual products being formed after the reaction had taken place. A reliable analytical method is essential for the production and control of the reaction for industrial purposes. [23]

These methods provide the deeper knowledge required about the products which in turn deeply analyze the reaction components of interest and can give an idea of the reaction's progress. These components are mainly the Total Alkyl Esters, glycerol, and glycerides (monoglycerides, diglycerides, and triglycerides); triglycerides are the main components of oils and fats, which has three long chain fatty acids that are connected through an ester bond to a molecule of glycerol, while diglycerides, and mono glycerides are considered intermediaries in the general transesterification reaction. [24] [23] [25]
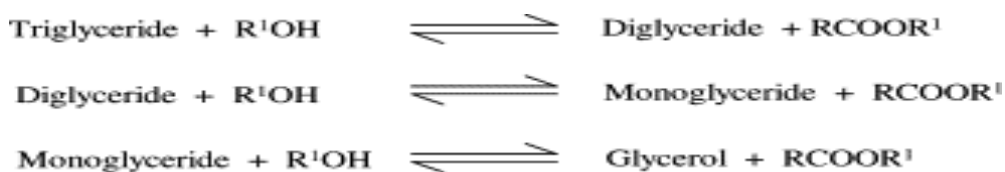
Figure 3 - Transesterification of Triglycerides Showcasing the Intermediary steps of the reaction. [24]

Chromatographic Methods
Chromatography is a technique which separates a mixture of contents based on the relative amount of each solute and its affinity to the stationary phase; multiple chromatographic methods have been implemented in the process of Transesterification and Biodiesel Production.

Gas Chromatography
Gas Chromatography (GC) is widely used in the Transesterification process; Gas Chromatography is mainly used in the analysis of the concentration, or amount of triglycerides, as well as the analysis and concentration of fatty acids and esters in the product. In GC, the amount of monoglycerides, diglycerides, as well as triglycerides, is determined and the biodiesel's composition is analyzed further to in order to determine the composition and concentration of any subsequent intermediary compounds during the course of the reaction. Gas Chromatography is used in the analysis of the transesterification process, e.g., standards EN14103[Reference Number] and 14105 [Reference Number] because of its robustness, its reproducibility, as well as its preciseness and accuracy in the determination of analytes; although very reliable, GC can sometimes be unfavored because of the amount of sample preparation, as well as the analysis time

required to obtain reproducible results, thus it could be used as a reliable analytical method to control and benchmark whichever technique has been used. [25]

High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is an analytical technique used to separate and quantify components in a liquid mixture, this separation and identification is based on their interaction with a stationary and mobile phase within the measuring instrument. The mixture is injected into a column packed with certain materials called the stationary phase and a mobile phase, a solvent that flows through the column. [26]

Alongside the Transesterification process for Biodiesel production, HPLC with an ultraviolet detector is often used for the monitoring of the production of biodiesel. Main advantages of using HPLC in monitoring the Transesterification process is that unlike Gas Chromatography, no derivatization is usually needed (In chromatography derivatization refers to the process in which a functional group is introduced to into an analyte to improve its detectability and in case of GC is to increase the analyte's volatility in the instrument.). In addition to that, in HPLC, glycerides and glycerol contents can be easily detected. With this said, in HPLC, samples should be processed for the glycerides analysis. [25]

Spectroscopic Methods

Spectroscopy can be defined as the study of materials depending on their ability to absorb parts of the electromagnetic spectrum. Spectroscopic techniques have been studied and expanded through the years, with the possibility of using these methods for quantitative as well as qualitative analyses for various samples.

Proton Nuclear Magnetic Resonance

(H-NMR) can be used to monitor the progression of the Transesterification process. This technique can measure and identify the fatty acid composition of the biodiesel, as well as having the capacity to measure the concentration of the biodiesel produced in the process, H-NMR can also determine the acidity of the biodiesel produced; disadvantages to using H-NMR is using High Field H-NMR is relatively expensive, as well as posing some difficulties in acquiring information on the composition of the mixtures analyzed. [25]

Infrared Spectroscopy

Infrared radiation was discovered by William Herschel in 1800 while investigating the energy levels associated with the wavelengths in the visible spectrum. Infrared radiation is not visible to the human eye, which responds to wavelengths between 390 to 750nm. The infrared spectrum starts at 0.75nm; the infrared spectrum can be divided into three regions: Near Infrared (NIR), Mid Infrared (MIR), and Far Infrared (FIR).

Infrared (IR) spectroscopy is an analytical technique that is widely used for the identification of chemical bonds and structures of substances. Infrared spectroscopy studies the interaction of substances with the infrared radiation when it travels through the substance medium; this infrared radiation causes rotational, and stretching interactions in the substances, depending on their energy

states, with distinct peaks for certain molecules that can help identify their presence in the analyte, as well as having the possibility to quantify their amount. Since infrared spectroscopy interacts mainly with the dipoles of molecules, having analytes with no dipole moment would lead to the analysis using infrared to be impossible and would not produce any infrared spectrum. [27]
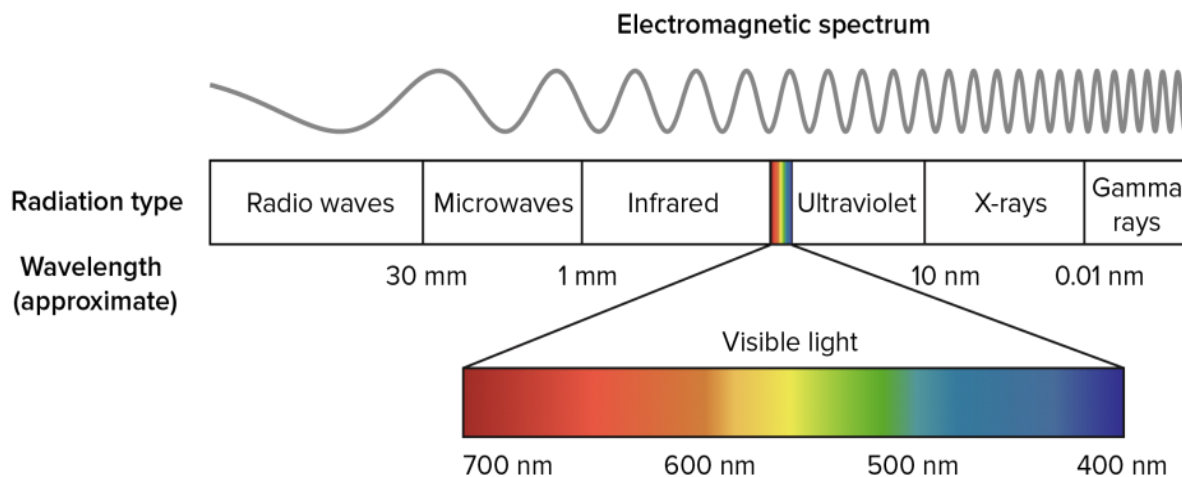

Figure 4 - Electromagnetic Spectrum with the Visible Region Highlighted [28]

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectrometry (FT-IR) was developed to overcome difficult aspects in traditional infrared spectroscopy, one of those difficulties was the slow scanning process, older design spectrometers used to be able to measure one wavelength at a time, so more advanced techniques needed to be developed and to have the ability to scan a multitude of wavelengths simultaneously; this is where the interferometer comes to play. An interferometer can produce a signal that has all the infrared frequencies encoded in one; this introduced the possibility of scanning a sample for the whole infrared frequency region in seconds rather than minutes. [27]

Fourier transform infrared spectroscopy introduced the ability for analysis that was unavailable before, being a non-destructive analysis technique, as well as requiring a small amount of analyte for it to be used were the game-changing characteristics of its popularity as an analytical method. It opened the door to the ability to identify functional groups involved in a certain reaction and to monitor the reaction progression.

Monitoring the Transesterification using FT-IR

Using FT-IR to monitor the transesterification process had been found to be effective, (Mahamuni et. al. 2009)[29] had found that using FT-IR to determine the biodiesel (Fatty Acid Methyl Esters) content in the reaction mixture and to monitor the transesterification reaction in soybean oil. In their work reference spectra of biodiesel and oil and mixtures, were then analyzed and the region that interested the authors most was between $1222 – 1184$ cm^{-1}, a software (ECFM) was used to automatically determine the concentration of biodiesel in the regions of interest using nonlinear Classical Least Square (CLS) method. Many processing steps were used before the actual analysis. Steps include distillation to remove the methanol, a washing step, and a separation step, these steps would in turn make using this method tedious for quick and reliable analysis even when using FT-IR, in the analysis the region of $1700 – 1800$ cm^{-1} was not taken into account because the peaks were difficult to distinguish in the software that was used. Being an off-line method decreases its possibility of being used and scaled-up efficiently for accurate process control and reaction management.

(Rabelo et. al. 2015)[30] found that conducting the transesterification reaction using soybean oil with methanol as the esterifying agent with sodium methoxide as the chosen catalyst. Off-line FT-IR reaction monitoring had been done. As promising as the results are, methods used needed many preparation steps, including a separation step, usage of adsorbents to remove excess water, and the removal of methanol, all before the analysis. Their region of interest was wide $600 – 1800$ cm^{-1} and the best model fit was selected based on statistical criteria (PCA and RMSEC).

(Rosset et. al.)[31] performed the Methanolysis reaction in different conditions using FT-IR and GC as analytical techniques. It was found that results obtained using FT-IR were comparable with the obtained results using Gas Chromatography with FT-IR analysis being quicker, simpler, in addition to being more economical. Some limitations apply to this method, the introduction of several preparation steps including filtration and separation, the model was created based on the area under the band between 1427 cm^{-1} and 1445 cm^{-1}.

(Mwenge et. al.)[32] The authors had shown in this research the feasibility of using FT-IR analysis in the Methanolysis reaction. Gas Chromatography was used as a reference method. Some disadvantages implementing this method are the required steps before analysis. The introduction of a washing step with 3% phosphoric acid, heating for moisture removal, and preprocessing steps e.g., mean-centering, and variance scaling. These steps are the main reason why this method cannot be implemented in an on-line manner. several spectral regions were selected for investigation, a full region from $701 – 1822$ cm^{-1} was investigated, and the best model fit was selected based on PLS analysis.

(Zagonel et. al.)[33] Had shown that FT-IR is very promising with the resulting calibration model, nevertheless, the reaction was treated with ammonium chloride solution, which would introduce extra steps and possibility of error, as well as sample centrifugation before the actual analysis. It was found that the region $1700 – 1800$ cm^{-1} was the most promising and a regression model was

built in that region using MATLAB to implement PCA on said region, then built a calibration model using PLS method for testing.

(Reyman et. al. 2013)[34] used sunflower oil as feedstock for the reaction, Methanolysis monitoring using FTIR. Many peak locations were considered, but only the peak at 1437cm^{-1} was considered for quantitative on-line Methanolysis monitoring of the production of methyl esters, spectral manipulation, and normalization at 1464 cm^{-1} peak, normalization might lead to information loss, information that might significantly alter the research results, some quantification limitations apply here as well, since validation was not implemented on actual data other than the created calibration blends.

While (Yuan et. al. 2013)[35] used an on-line Methanolysis process monitoring using a mid-FTIR spectrometer with a temperature controlled flow cell; canola oil had been used as the feedstock, then multiple regions of the spectrum were assessed to determine the reaction progress, it was found that using FT-IR for the monitoring of the reaction to be workable, as well as simple and accurate enough to be used to acquire data in real-time; Beer's Law and Least Squares calibration models were used to quantify the components of interest. Even though the results were promising, some disadvantages of this method are that the authors were using a specially made instrument, as well as proprietary software were used. Multiple regions were considered but only the peaks at 1198 cm^{-1} were considered for regression analysis, comparing the area under that wavenumber's peak.

Even though using FT-IR in the monitoring of the transesterification had been extensively researched and improved, most of the previous research focused on Methanolysis and the production of FAME, which in turn represents the need to further improve the analysis techniques implemented in the use of Ethanol and the production of FAEE. Previous research was mostly focused on building calibration models solely from standards and blends in an off-line manner, and the reaction mixture's actual contents and intermediaries were not taken into account. Processing steps were the biggest hurdle in the application of an on-line method. On-line monitoring is a gateway into an effective process control and production.

I.VI. TWO-DIMENSIONAL CORRELATION SPECTROSCOPY AND ITS BENEFITS IN REACTION MONITORING

FT-IR is a versatile and an important analytical technique used for the monitoring of the transesterification reaction, though in the first steps of the reaction and investigation of infrared spectra since FT-IR depends on the vibrational energy to produce the spectra. It is often times difficult to differentiate analytes in the matrix of compounds being analyzed; due to their similar compositions, or closely interrelated vibrational regions. Transesterification reaction is one of those cases where the feedstock and the resulting compounds are closely related to each other. This issue can be overcome by using a multitude of spectral manipulation techniques that could be used, such as spectral subtraction, peak intensity correlation, or a more complex computational method is needed. Generally, an additional technique is needed as a complementary method to mere investigation of the FT-IR spectra. In this case, the gathered spectra are considered complicated, therefore, more in-depth techniques were used to supplement the gathered spectra, and to assist with the FT-IR spectral regions analysis. [27]

Two-dimensional correlations spectroscopy (2D-COS) was introduced in 1989 and was developed through the decades to be one of the most essential analysis tools for spectroscopic analysis and investigation in multiple disciplines and applications, fields like pharmaceuticals, protein analysis, biomedical applications, as well as nanotechnology research. [36]

2D-COS investigates the dynamic changes in a sample that is responding to an external perturbation. 2D-COS then helps explore the correlations that could occur during said perturbation. 2D-COS is also especially useful in understanding the sequence of changes happening in the sample in response said perturbation; 2D-COS is dynamic, and adaptable to multiple spectral acquisition techniques, such as Raman spectroscopy, Fourier Transform Infrared Spectroscopy, Ultraviolet-Visible (UV-Vis) spectroscopy. Sources of perturbation applied to the sample being analyzed can range from changes in temperature, pH, pressure, concentration, or spatial coordinate changes in time. Implementing 2D-COS into any spectral analysis method is one of the important reasons it has been popular since its inception. [36]

The application of 2D-COS to any spectral analysis technique thus involves the use of the generalized 2D-COS method. The Generalized 2D-COS method was introduced by Isao Noda [36] which had made possible the use of 2D-COS in multiple spectral analysis techniques. The application of the Generalized 2D-COS method then produces a correlation map, which shows how the two signals are correlated with each other at different time delays or frequency shifts, this correlation map can reveal important information about the analyzed spectral data, how different regions correlate to each other in response to the perturbation, as well as the sequential relationship between the different aspects affected by the perturbation whatever it might be.

FT-IR had been used to analyze adulteration in milk, in Figure 5, 2D-COS was used to find, analyze, and determine a band in the spectra of milk powder to quantify melamine adulteration.
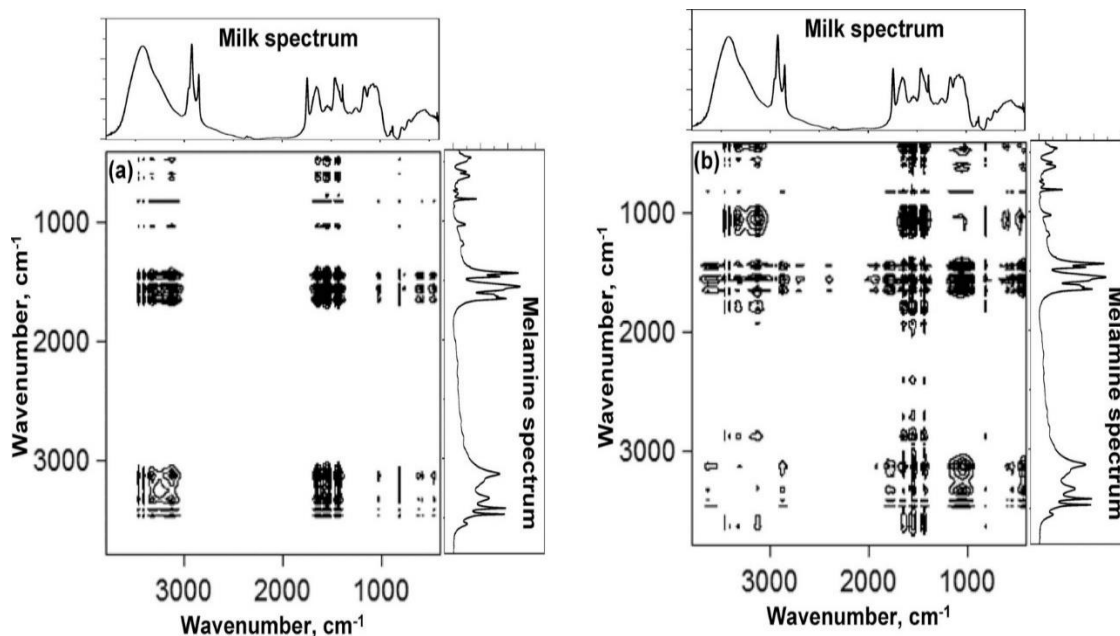
Figure 5 - Example of a Two-Dimensional Correlation Map used in the Analysis of Milk Powder Adulteration, in this paper 2D-COS had proved essential to determine the sequential change as well as the determination of which bands hold the possibility for quantification of melamine. [37]

While in figure 6, FT-IR was used to obtain 2D-COS maps in order to locate bands that correlate with the crystallization of ion-irradiated poly(ether-ether ketone), 2D-COS assisted in the determination of such band and then it was found that the area under that specific band changed linearly with the degree of crystallization.
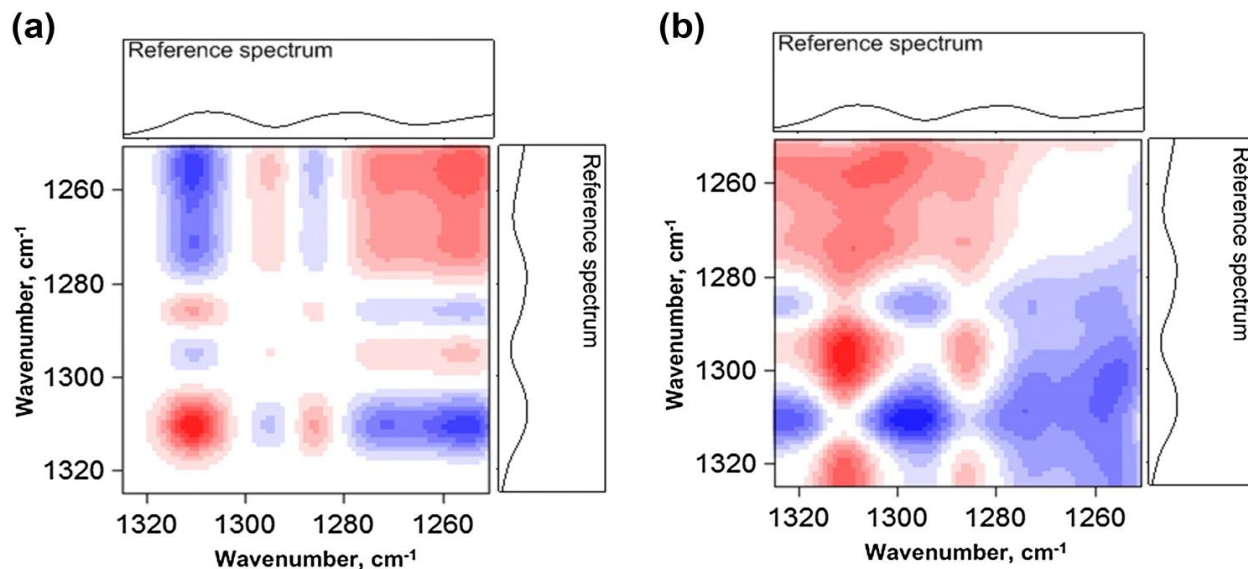
Figure 6 - Example of a Two-Dimensional Correlation Map used in the Analysis Crystallization in Cross-Linked Poly (Ether-Ether Ketone), in this article 2D-COS was used also to determine where to locate crystallization sensitive bands in order to perform quantifications and to build a linear model. [38]

Interpreting 2D-Correlation Maps

In order to understand the computed 2D-Correlation maps, one has to implement what is known as the Noda Rules, which are:

1. If the sign of a cross peak at a spectral coordinate pair $(\nu 1, \nu 2)$ of a synchronous 2D correlation spectrum is positive, i.e., $\Phi (\nu 1, \nu 2) > 0$, the spectral intensities measured at $\nu 1$ and $\nu 2$ are changing in the same direction, i.e., both intensities are either increasing or decreasing simultaneously.
2. On the other hand, if the sign of a synchronous cross peak is negative, i.e., $\Phi (\nu 1, \nu 2) < 0$, the spectral intensities measured at $\nu 1$ and $\nu 2$ are changing in the different directions, i.e., one is increasing while the other is decreasing.
3. If the sign of a cross peak at a spectral coordinate pair $(\nu 1, \nu 2)$ of an asynchronous 2D correlation spectrum is positive, i.e., $\Psi (\nu 1, \nu 2) > 0$, the spectral intensity measured at $\nu 1$ varies before that measured at $\nu 2$ with respect to the perturbation.
4. If the sign of an asynchronous cross peak is negative, i.e., $\Psi (\nu 1, \nu 2) < 0$, the spectral intensity measured at $\nu 1$ varies after that measured at $\nu 2$ with respect to the perturbation.
5. However, if the sign of a synchronous cross peak located at the same spectral coordinate $(\nu 1, \nu 2)$ is negative, i.e., $\Phi (\nu 1, \nu 2) < 0$, the above two rules are reversed. [39] [40]

These rules were implemented to understand and interpret the 2D-Correlation Maps acquired from experimental spectral data and will be discussed in further detail in later parts of the paper.

Using R Programming Language to Acquire 2D-Correlation Maps

R is a programming language similar to S, often used use for statistical computing and graphical interpretation, R has a multitude of linear and nonlinear statistical modeling, classification, and graphical methods that are highly adaptable, modifiable, and extensible with packages hosted on CRAN (the Comprehensive R Archive Network). One of R's strengths is its ease of use, and the providing the capability to produce publication ready plots including mathematical formulas and symbols, default settings are implemented with research papers in mind, but also being user-friendly and adjustable. R is available as free and open-source software under the GNU General Public License. [41]

Corr2D Package for R

Corr2D is a package created by Robert Geitner, Robby Fritzsch, Thomas Bocklitz, and Juergen Popp. It is an implementation of the two-dimensional correlation analysis described by Isao Noda, while also being adjustable in its properties and how to handle the input matrices and how to plot the resulting 2D Correlation Maps. [42]

II.   MATERIALS AND METHODOLOGIES

II.I.   MATERIALS

Gustona® rapeseed oil was acquired from a local vendor. Ethanol (99.8%) was procured from BC-Chemeservis s.r.o. (99.7%) analytical grade Sodium Hydroxide (pellets) acquired from IPL Labs.

II.II.   EXPERIMENTAL SETUP AND PROCEDURE

Rapeseed oil was mixed with Ethanol in a flat-bottomed glass reactor mixed at 2000rpm, this mixture was heated to temperatures of (40, 50, or 60 °C) using a thermostatic water bath. Sodium Hydroxide pellets (0.25%, 0.5%, or 1% wt./wt. of Sodium Hydroxide to Oil was used in multiple experiments) was mixed with Ethanol (amount required for dissolution of NaOH) in a different flask to dissolve it into the alcohol, this catalyst solution was then added to the Oil and Ethanol in the glass reactor being mixed for the start of the Transesterification reaction. The molar ratio of oil to ethanol was 1:6 for all experiments. Samples were taken at the specified time intervals starting from the moment of adding the catalyst into the reactor as follows (0, 2, 5, 10, 15, 20, 30, 60, 120 min). These samples were instantly analyzed in an FT-IR instrument and the spectra were recorded and saved for future interpretation.

II.III.   ANALYTICAL METHODS

A continuous flow injection cell with a total volume of (0.2 mL) was used, in conjunction with Attenuated Total Reflectance (ATR)-FT-IR analysis using Nicolet® FT-IR iS50 from Thermo Scientific equipped with an ATR (Attenuated Total Reflectance) sampling accessory. All spectra were collected at lab temperature of 20°C, with number of scans set to 10, resolution set to 4cm^{-1}, and background data were collected at the start of the experiment. Approximately 5mL of sample were withdrawn directly from the reaction vessel, and analyzed in a way where the solution flows through the cell as the analysis is ongoing. The cell was cleaned with 5mL of ethanol, and 20mL of air after each sample spectra collection, The spectra were collected using the OMNIC ® software provided with the instrument by Nicolet ®.

RI measurements were done according to the method implemented by (Husar et. al) [23], and GC sample preparation, where approximately 2mL of the reaction mixture were withdrawn and mixed with a n-butanolic acid solution of adipic acid (0.1mol/L, 3mL), and vigorously shaken to stop the reaction. The analysis was done according to (Šánek et. al) [43]

R programming language[41] was used to construct 2D-COS maps, with Corr2D Package acquired from CRAN.

III. RESULTS AND DISCUSSION

III.I.   FTIR SPECTRAL ACQUISITION

Acquiring the FTIR spectra for the Transesterification reaction, it was noted that the spectra for oil and the produced biodiesel are highly similar and interconnected through the process, this similarity is due to the fact that the chemical composition of triglycerides and ethyl esters which form the dominant components in the transesterification reaction are remarkably similar. This can be observed in figure 7 and figure 8:
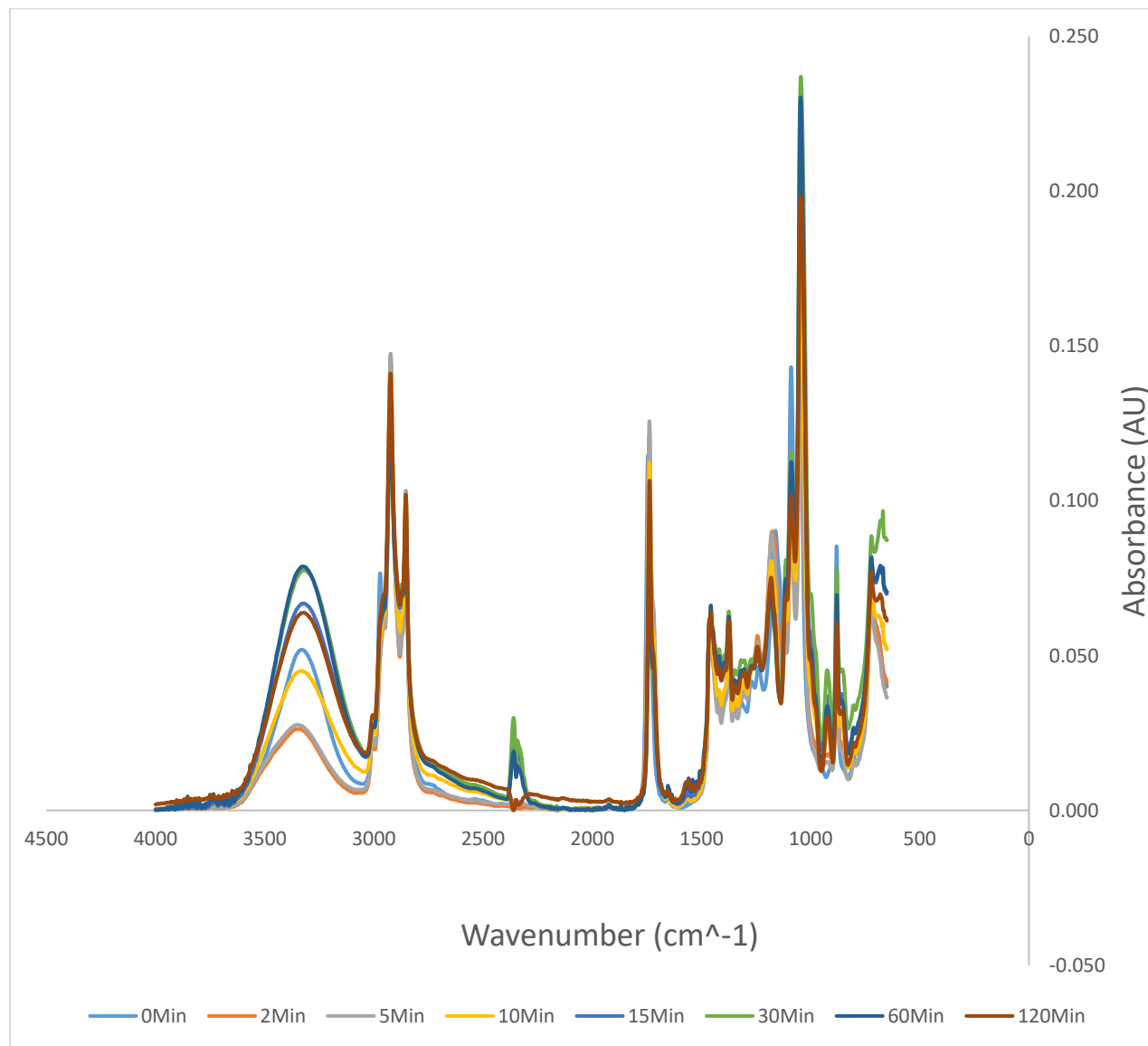
Figure 7- FT-IR Spectra throughout the Reaction from 650 – 4000 cm^-1. Temperature: 40 °C, 0.25% NaOH : Oil, 1 : 6 Ethanol : Oil ratio.
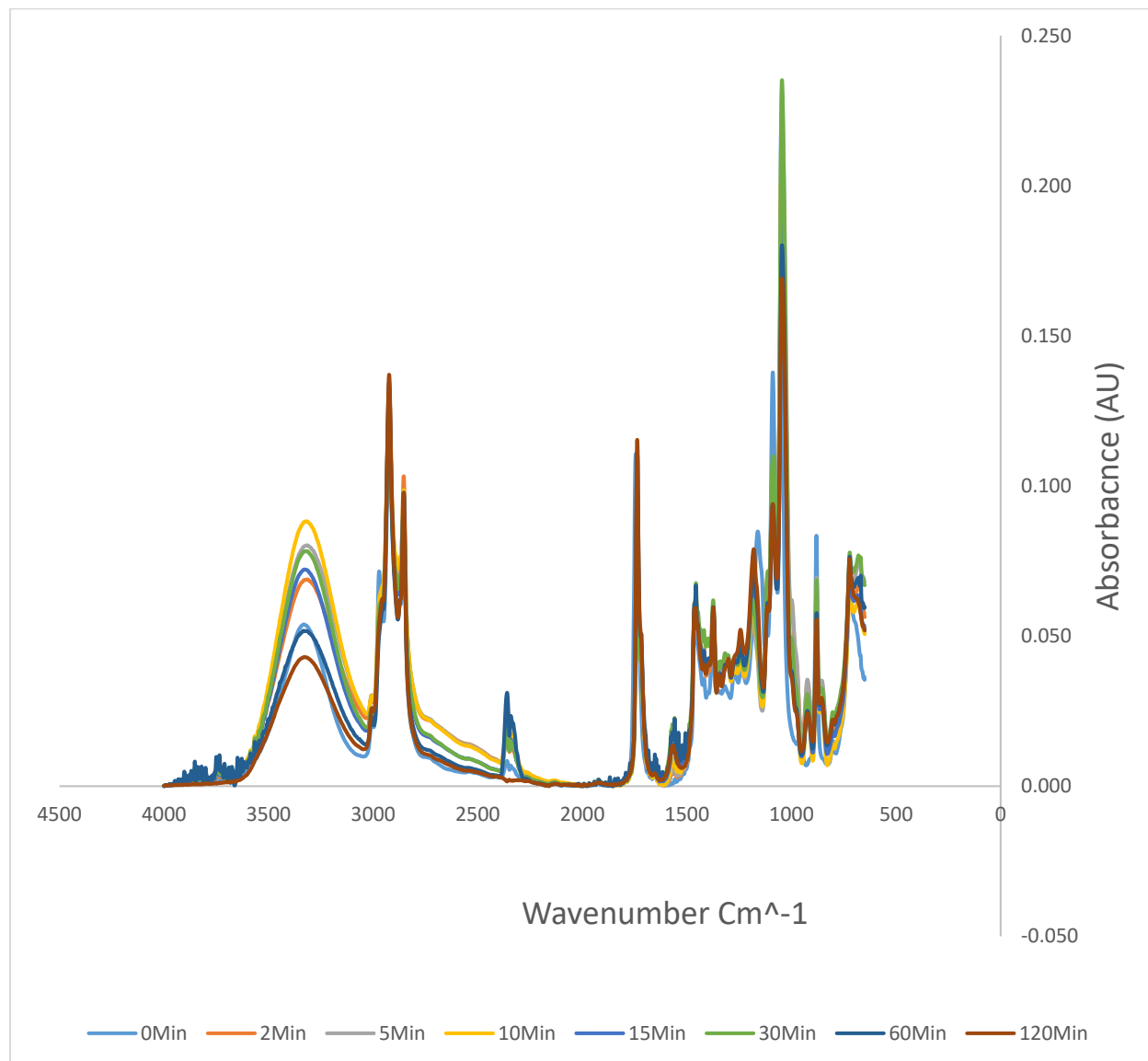
Figure 8 - FT-IR Spectra throughout the reaction from 640 – 4000 cm^-1. Temperature: 50 ℃, 0.54% NaOH : Oil, 1 : 6 Ethanol: Oil ratio.

Figure 7 and Figure 8 represent the FT-IR spectra acquired from two different experiments done at separate times; it is apparent that the spectra are interlocked and remarkably similar to begin with, even though there are some differences apparent in the region from $1000 – 1500 cm^{-1}$, this region is known as the fingerprint region. Peaks that appear in the fingerprint region are often useful to identify certain molecular vibrations since the pattern of peaks is unique for each molecule, this region is most often useful for qualitative analysis and identification of unknown compounds. In this case, the interconnected spectra of the complete reaction mixture which predominantly contains triglycerides, oil, ethanol, and glycerol, are complex, similar, and require extra analysis techniques to recognize the sequence, and relationship between the peaks, and components taking part in the reaction mixture simultaneously. Most often than not, in similar

cases where the analytes have similar spectra and are not easily distinguishable for interpretation, researchers have implemented in-situ multivariate methods to analyze the acquired spectra and to be able to understand the flow of the transesterification reaction; in order to use such methods, it is first required to perform exploratory studies using statistical methods using different mixtures of the analytes in question, one of the statistical methods often used is PCA [44] (Principal Component Analysis) which is a multivariate statistical technique used to analyze large datasets and to identify patterns in the analyzed data. [23] [45]

PCA does not give a straightforward linear relationship on spectral data acquired directly from the Ethanolysis reaction mixture. Therefore a robust correlation visualization technique needs to be used in order to directly analyze the complex components taking part in the Ethanolysis reaction.

III.II.   DATA PREPROCESSING & A VISUAL TECHNIQUE TO IDENTIFY SPECTRA IN R

Raw spectral data acquired immediately when performing the reaction were studied, and a spectral manipulation technique was picked to preprocess the data before analysis; it was found that the most reliable spectral manipulation technique to implement was automatic baseline correction provided with the OMNIC® software. Baseline correction is a method that should be used with care because it has the potential to affect the actual peaks gathered in the process and could lead to the spectra to hide important information and in turn would make it difficult to perform quantitative analysis when needed. This said, the automatic baseline corrections packaged with most FT-IR spectral acquisition software including OMNIC® are the safest and most reliable, the reason being is that they implement statistical methods to determine the correction, rather than depending on the person analyzing the spectra and his/her biases towards the visible peaks and what they represent. [46] Automatic Baseline Correction using the OMNIC® Software package bundled with the instrument had been used on all collected spectra during each individual experiment. [46]

Figure 9 and Figure 10 represent the acquired spectral data from certain experiments at the beginning and end of the experiment, highlighting the difference between the raw acquired data with no manipulation and the same data series where Automatic Baseline Correction technique had been used as a preprocessing method.

The usage of spectral preprocessing (Automatic baseline correction in OMINC®) was further tested against non-manipulated data and the results were compared to determine the best fit model. See section : Statistical Modelling and Validation.
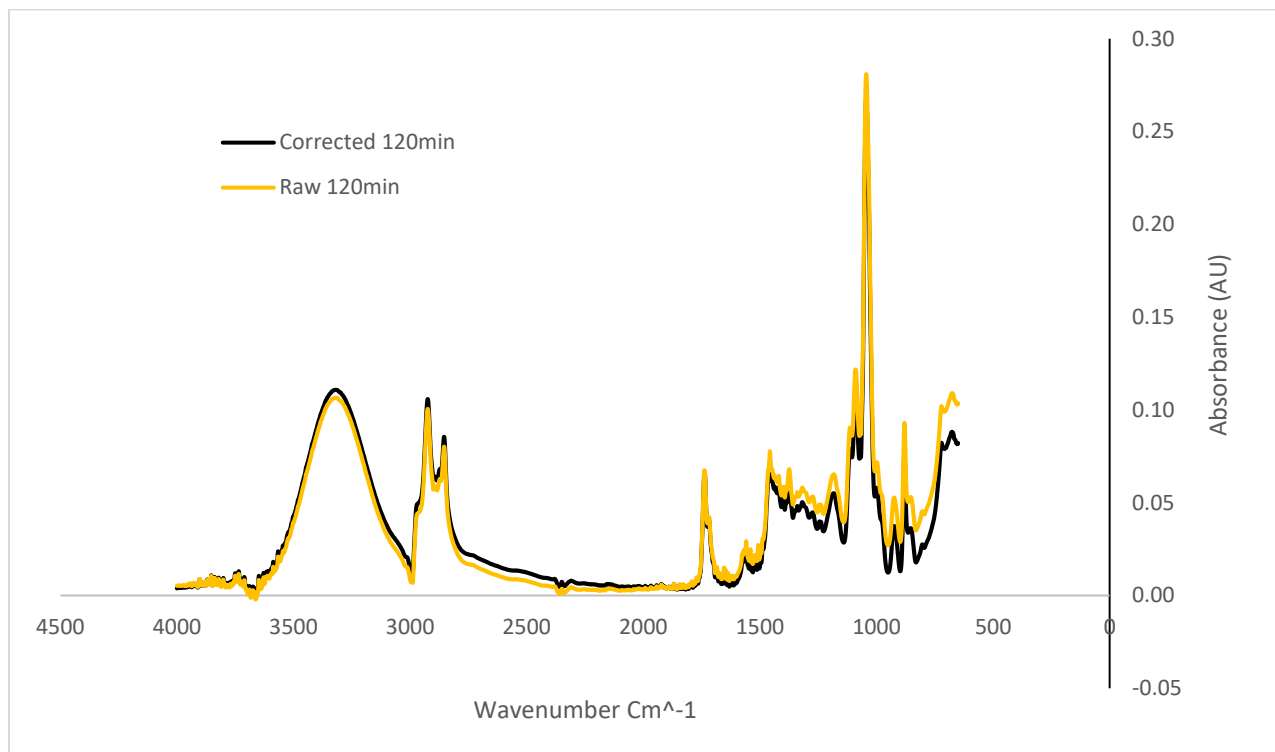
Figure 9 – A comparison between the raw data with no manipulation and the Effect of using Automatic Baseline Correction on the Acquired Spectra at the end of the experiment.
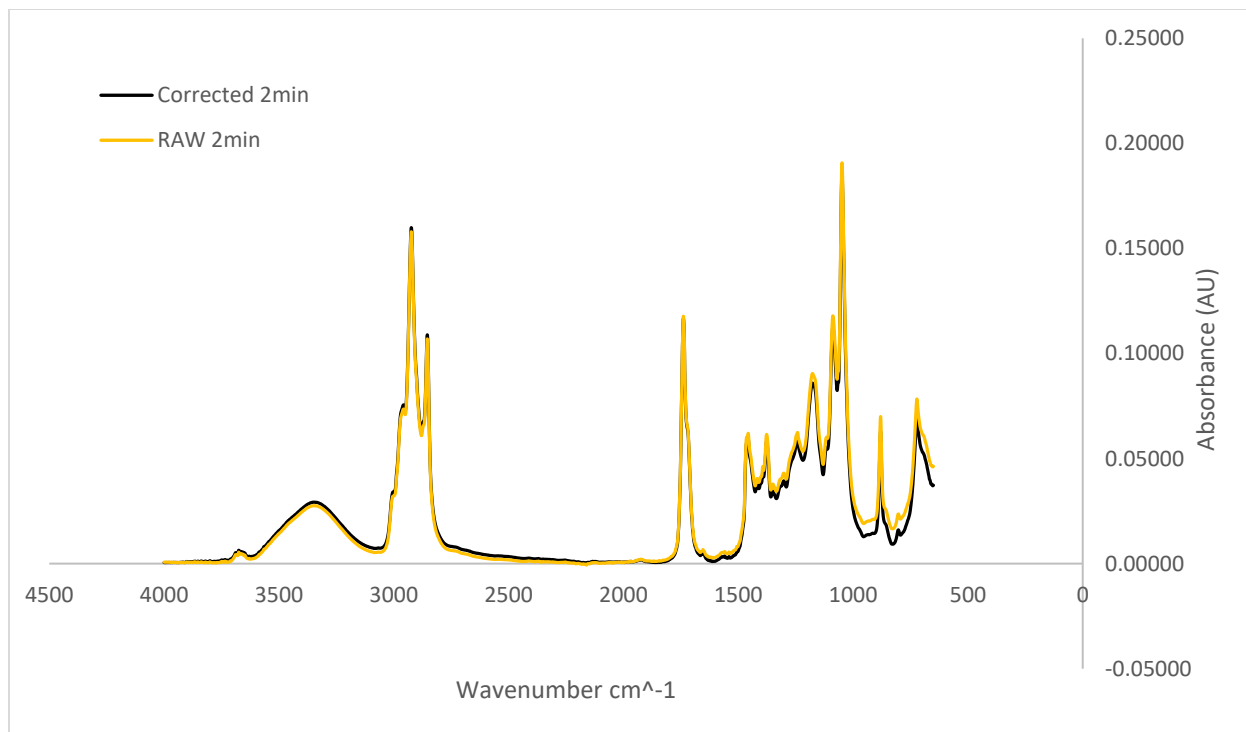
Figure 10 - A comparison between the raw data with no manipulation and the Effect of using Automatic Baseline Correction on the Acquired Spectra at the beginning of the experiment.

III.III. 2-D COS MAPS & ITS INTERPRETATIONS

2D COS analysis hinges on the acquisition of the 2D Synchronous and Asynchronous maps, and the subsequent analysis of said maps. The first step is to take a broad, eagle-eye look on all regions of the reaction, this approach will provide an idea of the regions and the correlation between them as the reaction progresses; Figure 11, and Figure 12, represent such global maps acquired from different experiments to aid in the decision-making of further close-up investigation of the regions and their interconnectivity.
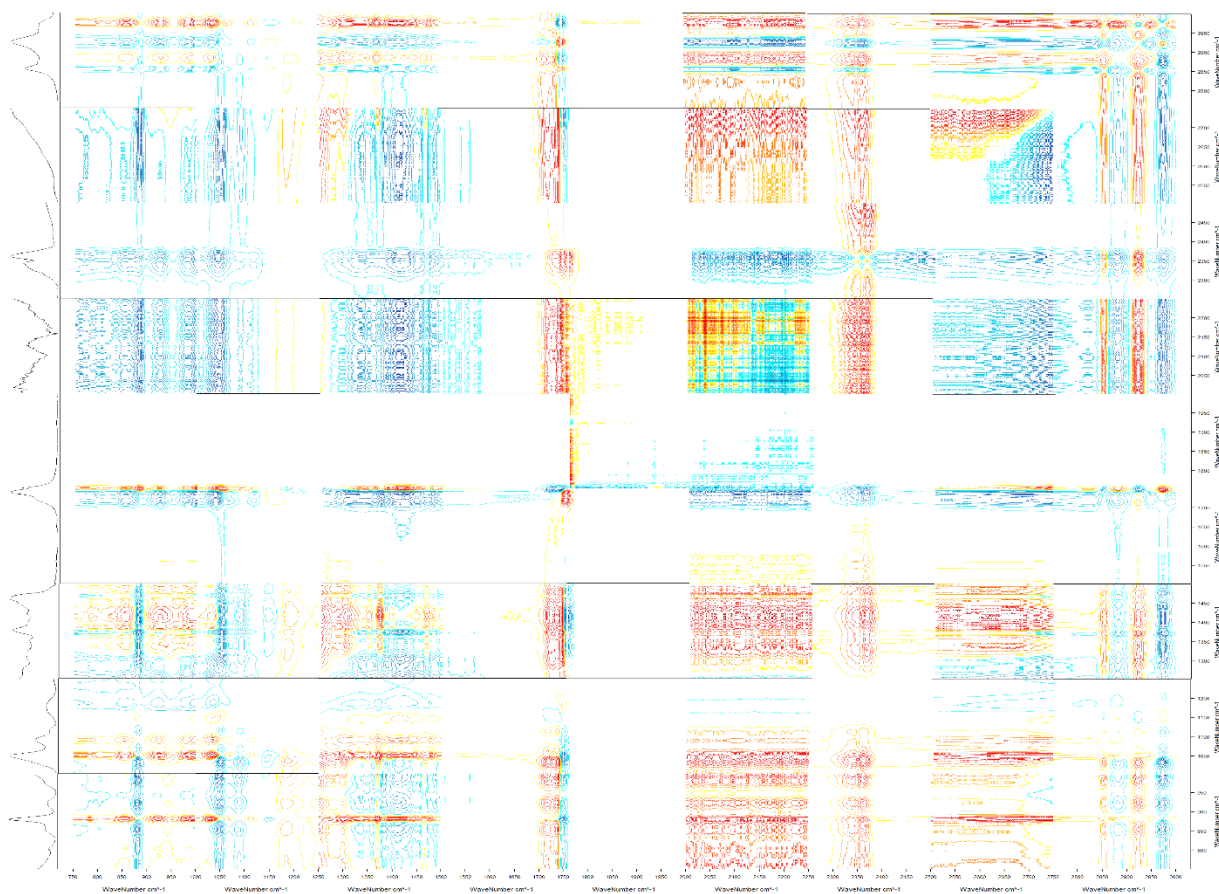
Figure 11 - A Full 2D Asynchronous Correlation Map Representing the Sequence of Changes as the Reaction Continues. Reaction Conditions : Temperature: 40 °C, 0.25% NaOH : Oil, 1 : 6 Ethanol : Oil ratio. For a higher quality image see Appendix .
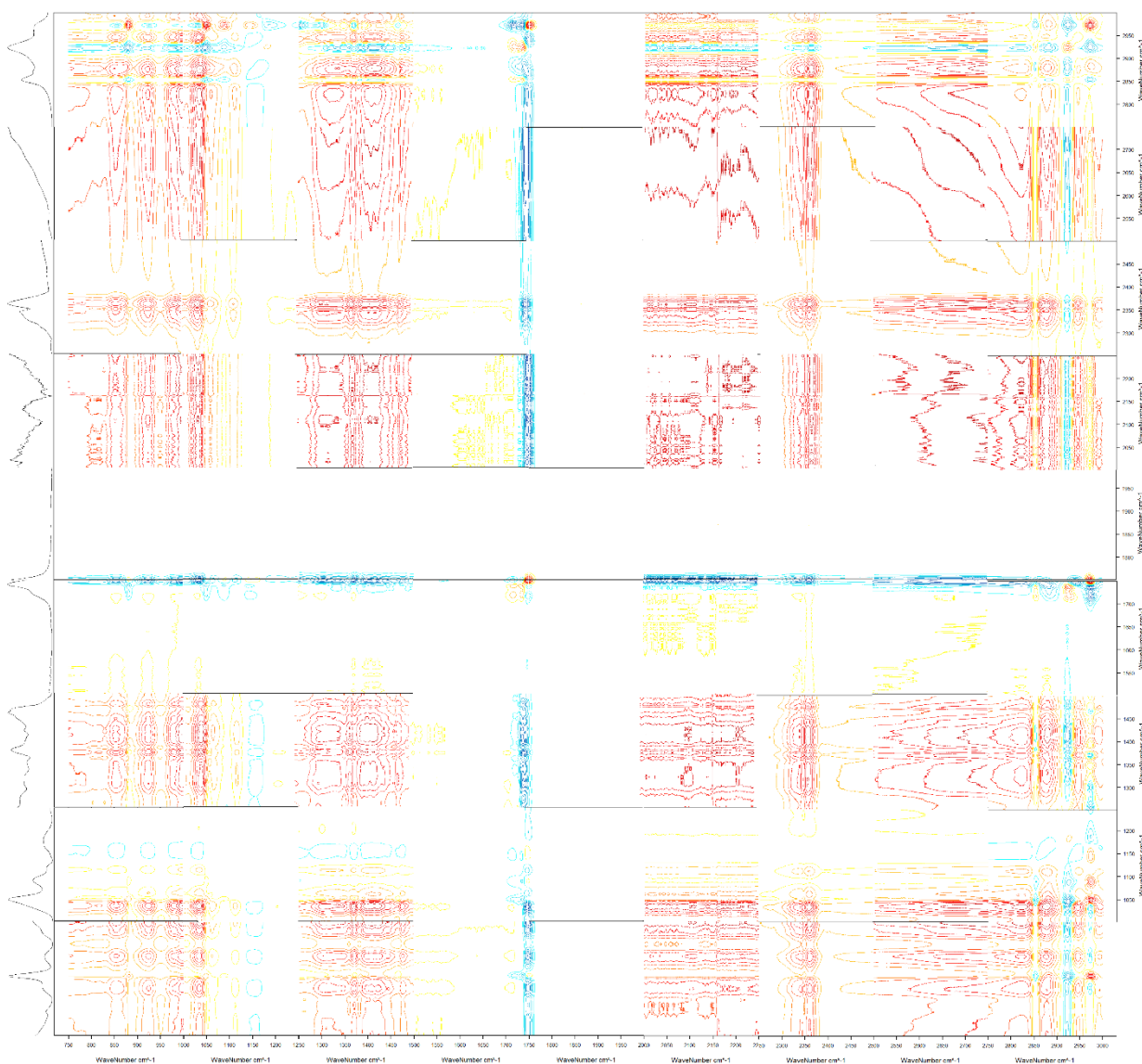
Figure 12 - A Full 2D Synchronous Correlation Map Representing the Sequence of Changes as the Reaction Continues. Reaction Conditions: Temperature: 40 °C, 0.25% NaOH : Oil, 1 : 6 Ethanol : Oil ratio. For a higher quality image, see Appendix.

Closer inspection of Figure 11, and Figure 12, looking for determinate peaks it was found that the regions that undergo changes during the reaction progress are easily distinguishable between 1000-1100cm^{-1}, 1500-1800 cm^{-1}, in addition to peaks found in the region between 2800-3000 cm^{-1}; these regions were picked for further inspection and analysis because they contain the best peaks and the most reliable Asynchronous and Synchronous peaks to determine the flow and the inner-workings of the interacting regions seen through FT-IR spectra.

III.IV. UNDERSTANDING THE 2D-COS PEAKS IN SELECTED REGIONS

Figures 13 to 16 showcase the 2D-COS synchronous and asynchronous maps. Which in turn show the relationship and correlation in the regions where changes occur in response to the perturbation affecting the Ethanolysis reaction.
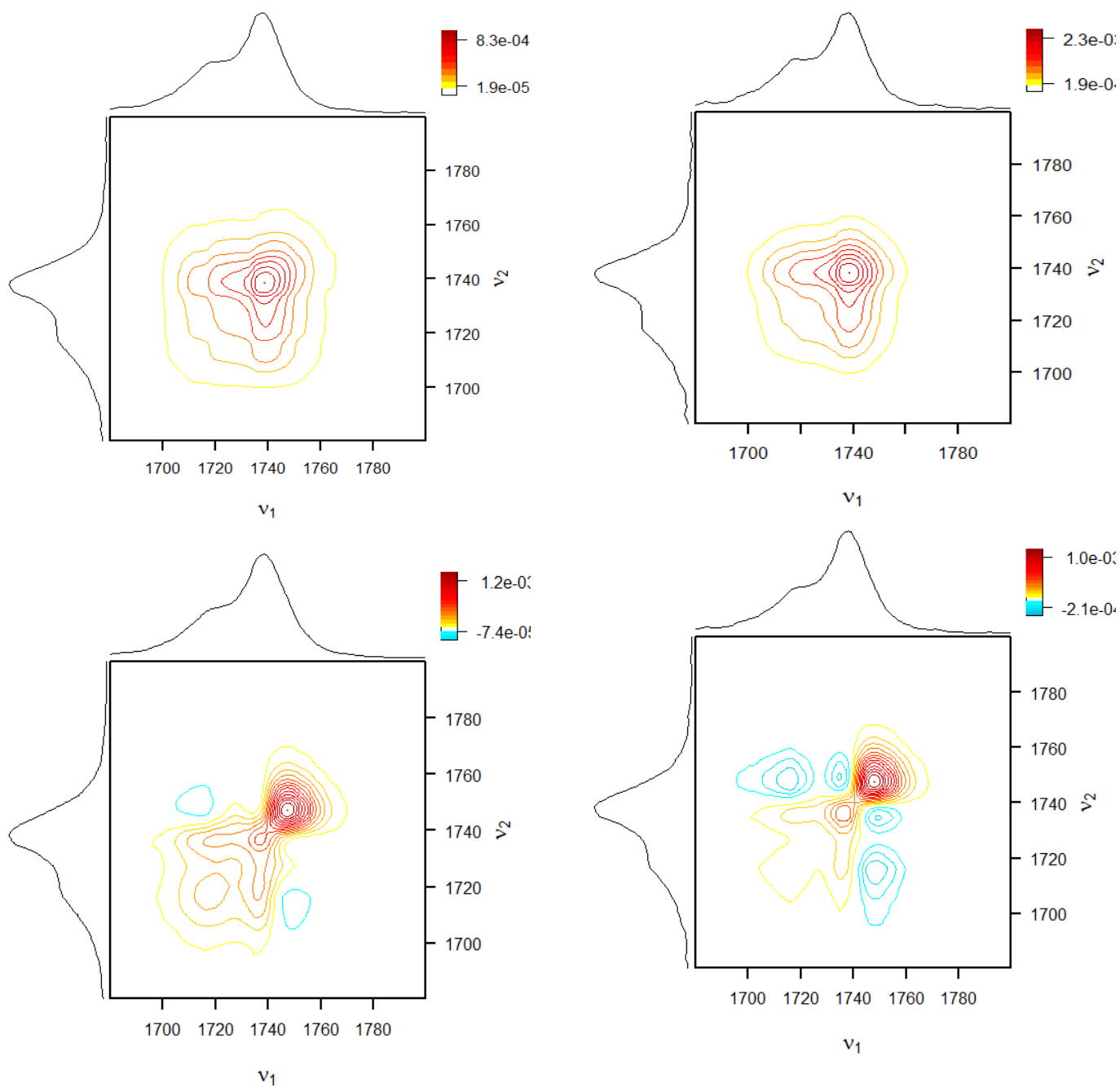
Figure 13 - Synchronous 2D Maps of the Region Between 1680 – 1800 cm⁻¹ acquired from four different experiments conducted with varying conditions.
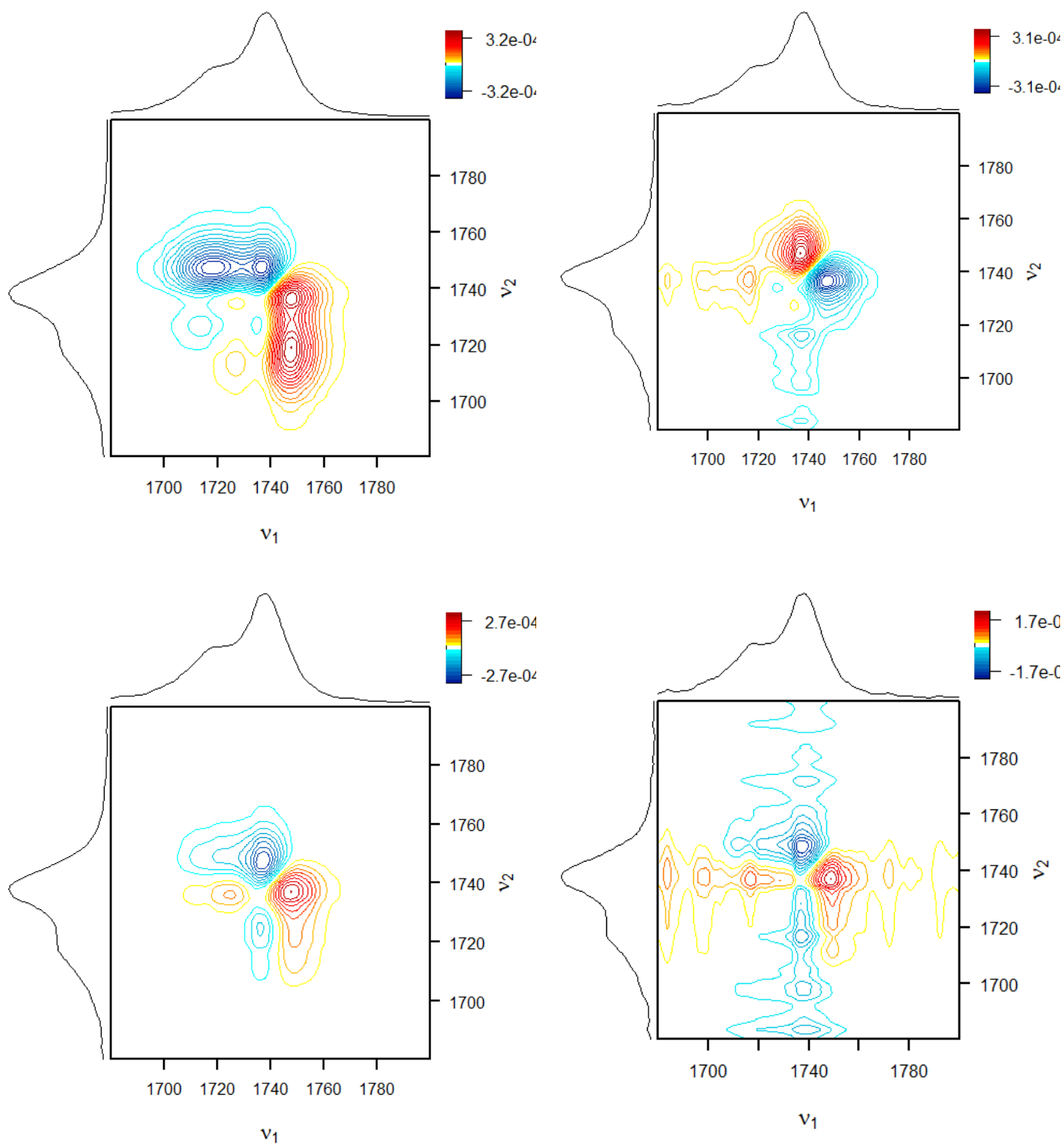
Figure 14 - Asynchronous 2D Maps of the Region Between 1680 – 1800 cm⁻¹ acquired from four different experiments conducted with varying conditions.
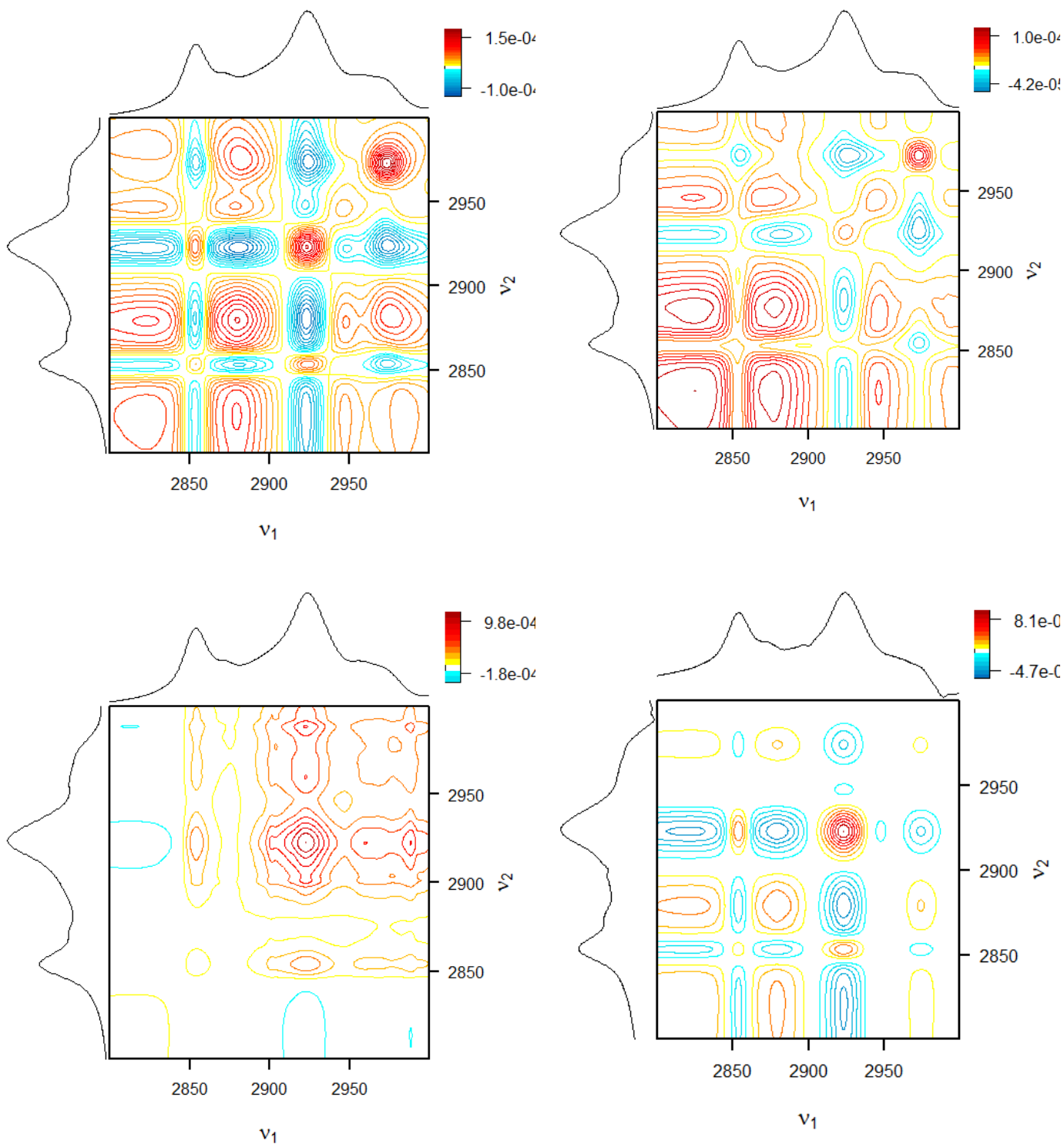
Figure 15 - Synchronous 2D Maps of the Region Between 2800 – 3000 cm^{-1} acquired from four different experiments conducted with varying conditions.
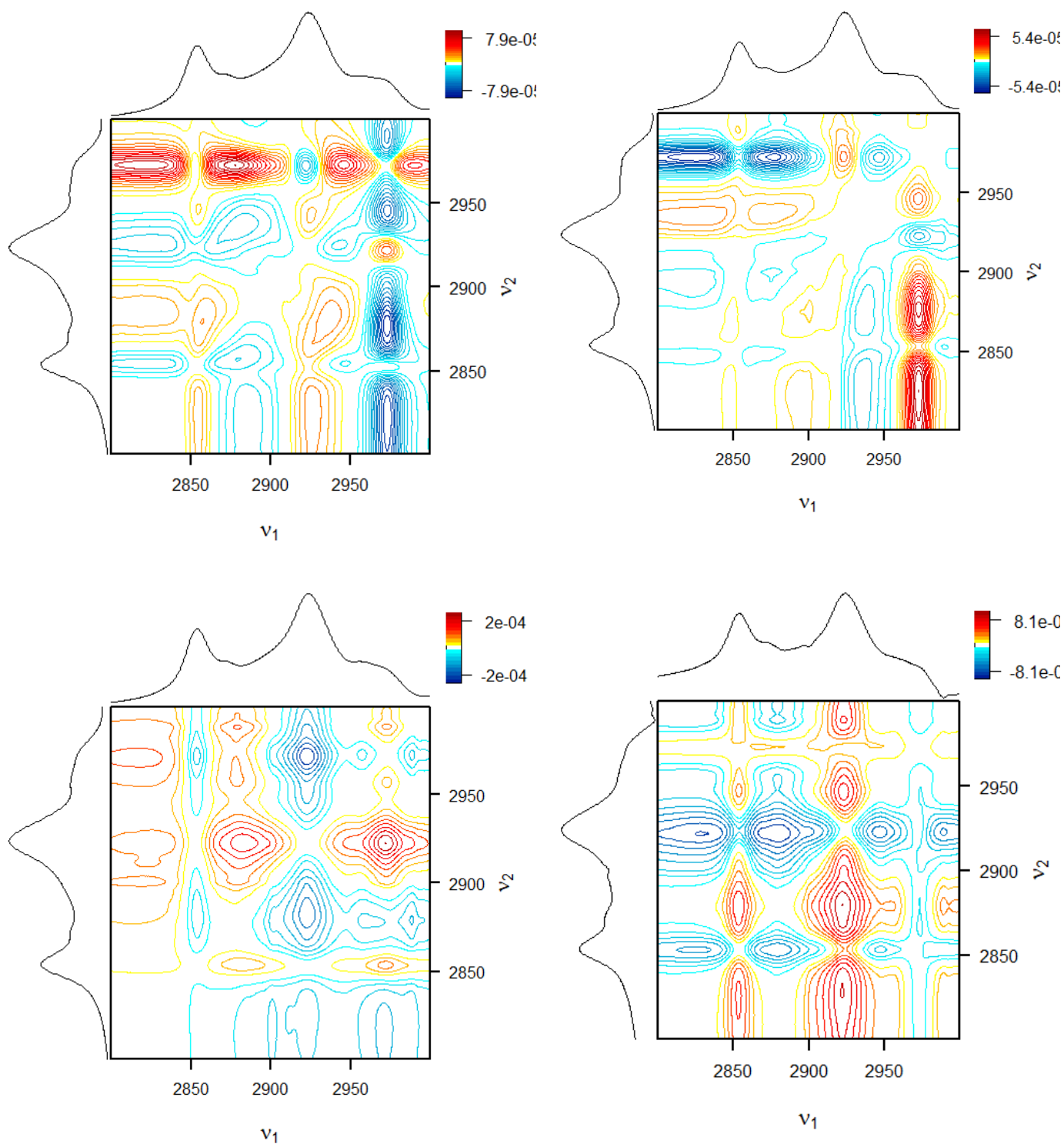
Figure 16 - Asynchronous 2D Maps of the Region Between 2800 – 3000 cm^{-1} acquired from four different experiments conducted with varying conditions.

III.V. INTERPRETING THE 2D-COS SYNCHRONOUS MAPS IN SELECTED REGIONS

More in-depth analysis of spectral regions is needed to properly determine whether the method used could lead to statistically significant information and the possibility of modeling the reaction through this information; spectral regions where distinct peaks occur and are visible in the 2D-COS maps were taken into account.

Correlation peaks appear to be very clear in a correlation map between the region $1680 - 1800$ cm^{-1}, and the region $2800 - 3000$ cm^{-1} shown in Figure 17. As the reaction progresses, it was found that the peaks tend to be more concentrated around the 1740 cm^{-1} wavenumber, while being constant on the other axis; this phenomenon was found to be consistent with different experiments conducted at different times and conditions.
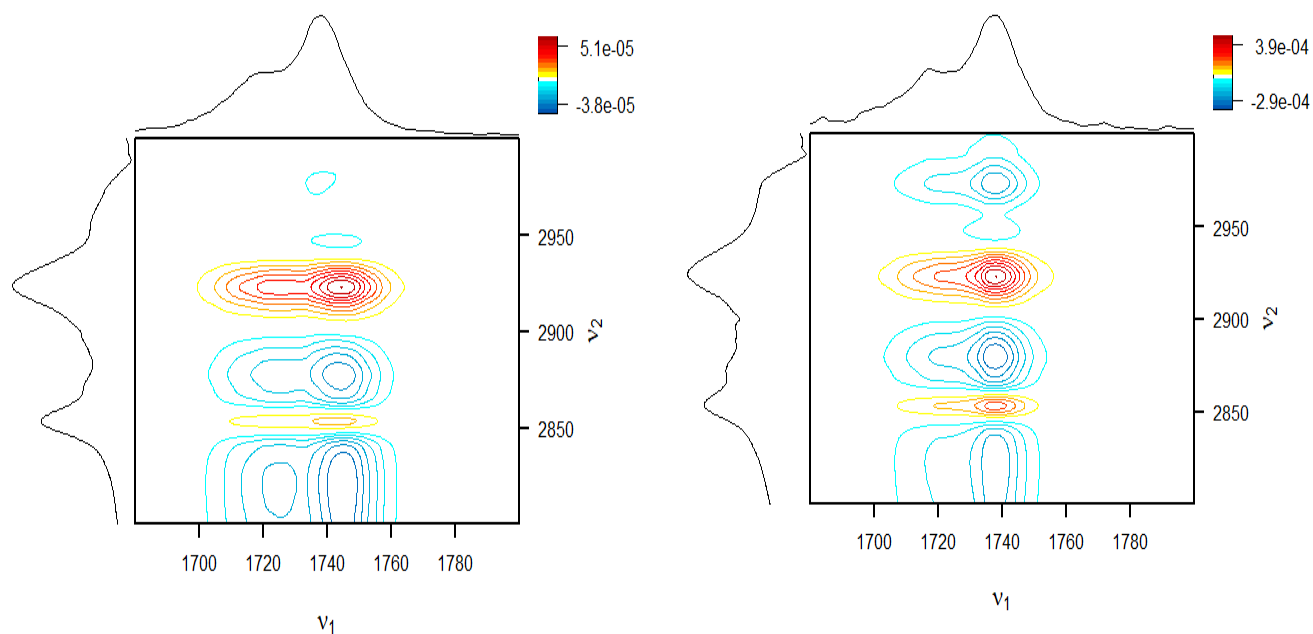
Figure 17 - (Left) Shows the Synchronous 2D-COS map in initial stages of the reaction with the main peaks easily discernible. (Right) Shows the 2D-COS Synchronous map in later stages of the reaction where the mostly the same peaks occur at similar y-axis locations, and a small shift in the x-axis.

Further investigation of the spectral region of $1680 - 1800$ cm^{-1} indicates that there is a shift in the position of the peak which can easily be shown in the 2D-COS maps in Figure 18. This shift most probably indicates a change in the vibrational intensity and properties of the functional group in that region, which is associated with the carbonyl group (C=O) that can evidently be found in esters. This shift in the peaks easily shows that the esters in triglycerides are being broken and new ester bonds are being formed between the alcohol and the fatty acid chains. The peak moving slightly lower indicates that a change in bond strength, or bond length resulting from the change in the molecular bonds in esters.
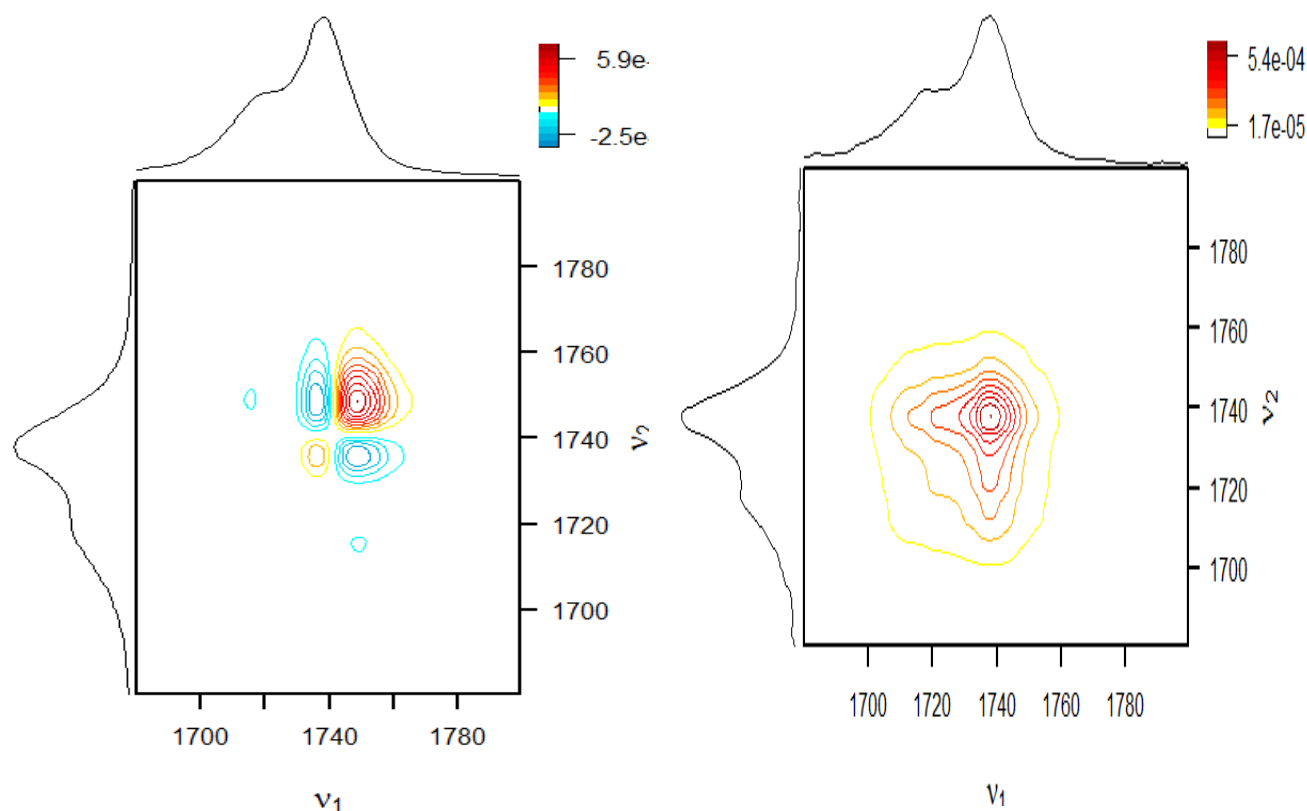
Figure 18 - (Left) Shows the Synchronous 2D-COS map in initial stages of the reaction with the main peak easily found around 1750 cm^{-1}. (Right) Shows the 2D-COS Synchronous map in later stages of the reaction where the shift had occurred, and the peak is visible around 1740 cm^{-1}.

This peak shift behavior was found to be consistent in different experiments performed at separate times and varying conditions, which can be used as an indication of the progression and optimum reaction time and conversion rate.

On the other hand, further investigation of the synchronous 2D-COS maps of the region between 2800 – 3500 cm^{-1} shown in Figure 19, represents the changes in the hydroxyl bonds as the reaction progresses, in the initial stages of the reaction, peaks are mostly visible around the 2950 cm^{-1} wavenumber, and as the reaction progresses these peaks start to fade and new peaks form around the 3300 cm^{-1} wavenumber; this switch and change in where the synchronous peaks are present could represent the rearrangement of the hydrogen bonds between C-H and O-H. [47]
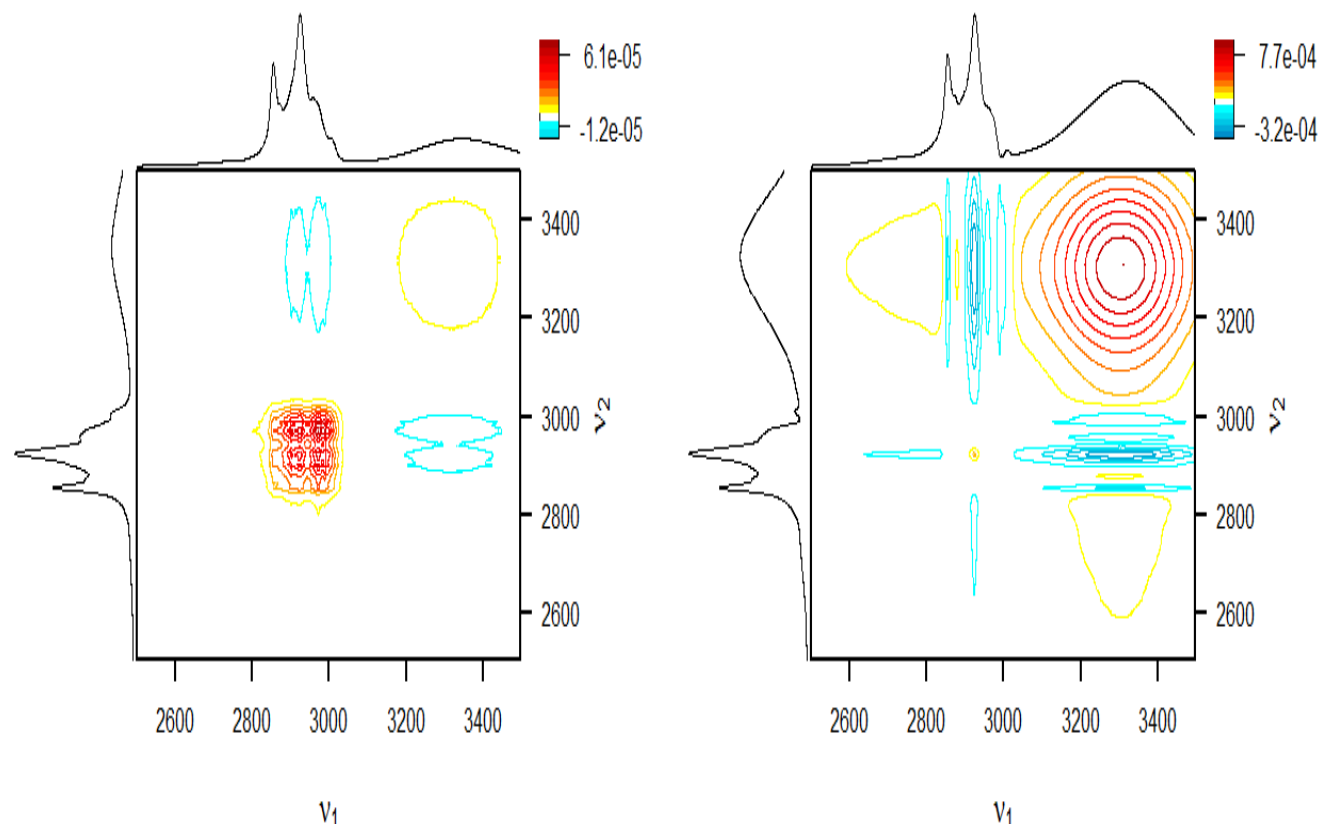
Figure 19- (Left) Shows the Synchronous 2D-COS map in initial stages of the reaction with the main peak easily found around 2950 cm⁻¹. (Right) Shows the 2D-COS Synchronous map in later stages of the reaction where the shift had occurred, and the peak is visible around 3300 cm^{-1.}

This switch in the peaks was also found to be consistent through different experiments with varying conditions, which provides another possibility for monitoring the progression of the reaction and optimum conversion.

2D characteristic changes and peak shifts were also found in the region between 1000 cm^{-1} and 1100 cm^{-1}, this peak shift from 1050 cm^{-1} into 1040 cm^{-1} was also found to be consistent, as shown in the following figure. This region could have the possibility of providing quantitative indications about the progress of the reaction.
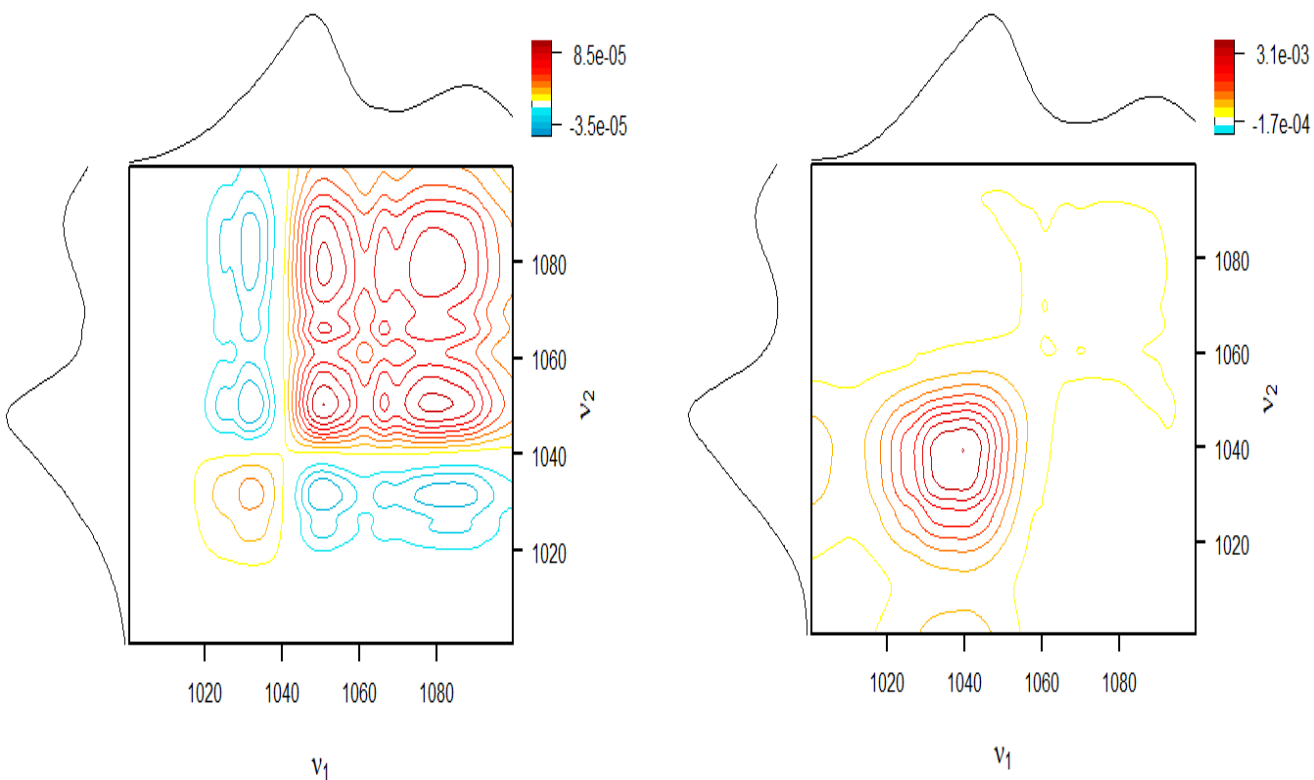
Figure 20 - (Left) Shows the Synchronous 2D-COS map in initial stages of the reaction with the main peak easily found around 1050 cm^{-1}. (Right) Shows the 2D-COS Synchronous map in later stages of the reaction where the shift had occurred, and the peak is visible around 1040 cm$^{-1.}$

All three 2D spectral regions were chosen to find the best region indicator of the progression and the actual quantification of the products and intermediaries.

III.VI.   REGRESSION TESTING ON REGIONS OF INTEREST

The mentioned regions were tested using linear regression to better have an idea of which region could have the possibility to reliably quantify the products and the progression of the reaction. Fatty Acid Ethyl Esters , Conversion , Monoglycerides, Diglycerides, Triglycerides, and Glycerin were tested in all three regions of interest with results shown in Figures 21 to 26.
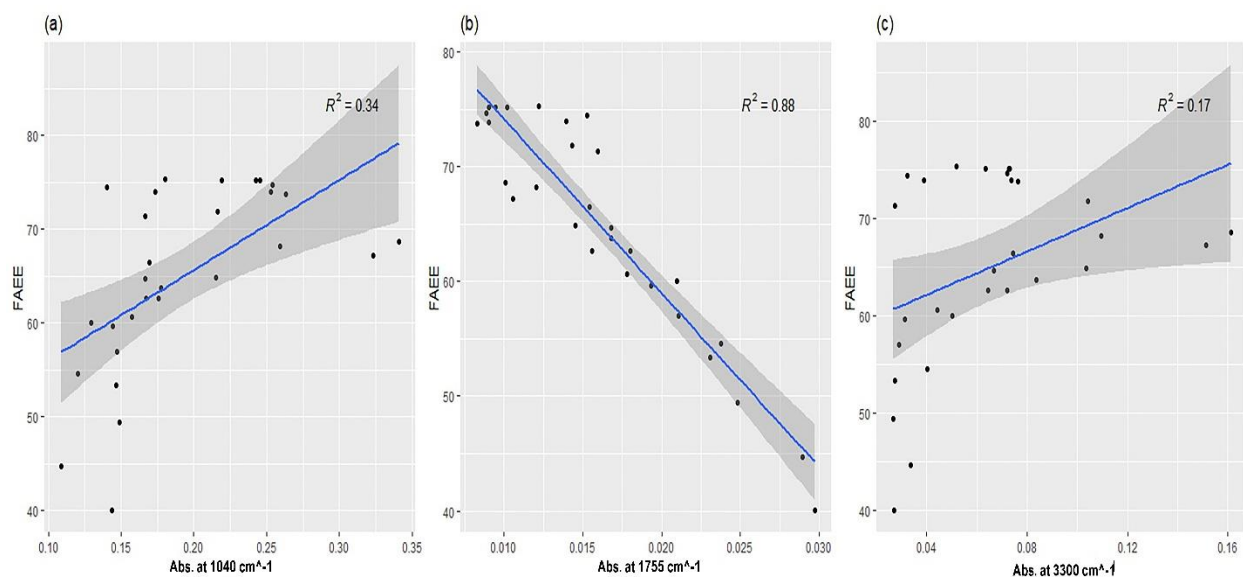
Figure 21 – (a) Linear regression model between Fatty Acid Ethyl Esters and Absorbance at 1040 cm^{-1} with R^2 = 0.34. (b) Linear regression model between Fatty Acid Ethyl Esters and Absorbance at 1755 cm^{-1} with R^2 = 0.88. (c) Linear regression model between Fatty Acid Ethyl Esters and Absorbance at 3300 cm^{-1} with R^2 = 0.17.
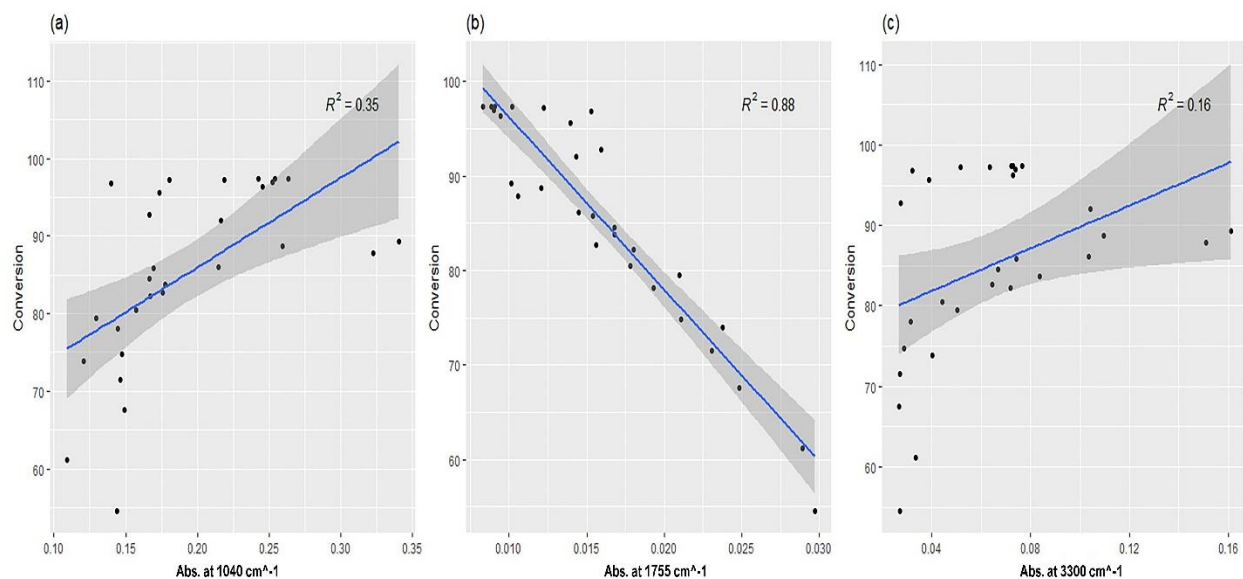
Figure 22 - (a) Linear regression model between Conversion % and Absorbance at 1040 cm^{-1} with R^2 = 0.35. (b) Linear regression model between Conversion % and Absorbance at 1755 cm^{-1} with R^2 = 0.88. (c) Linear regression model between Conversion % and Absorbance at 3300 cm^{-1} with R^2 = 0.16.
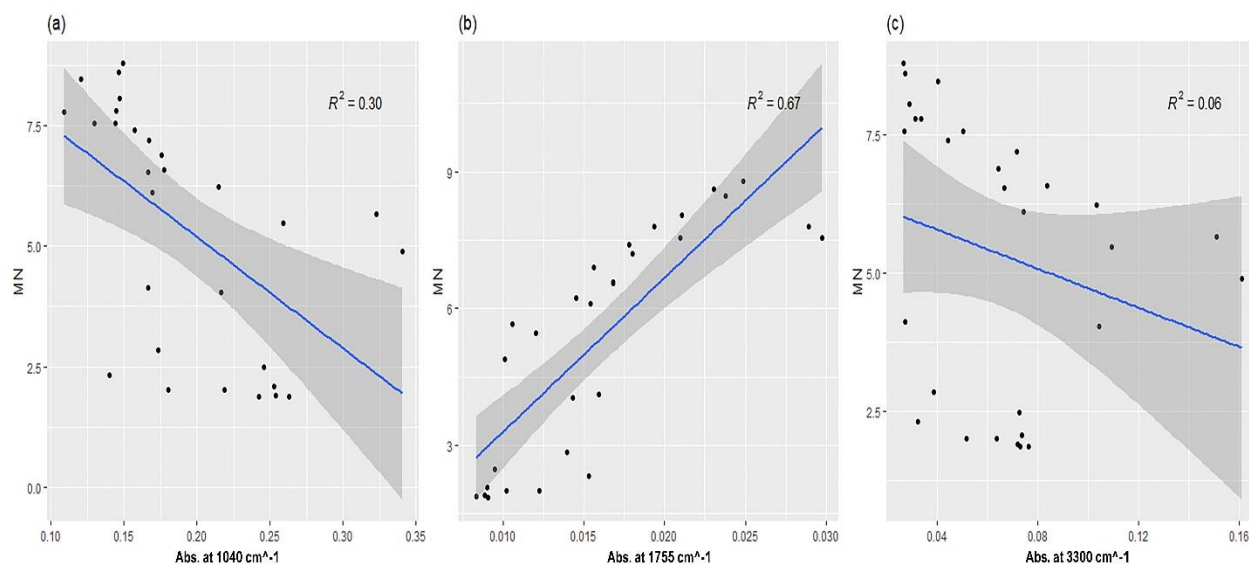
Figure 23 - (a) Linear regression model between Monoglycerides % and Absorbance at 1040 cm^{-1} with $R^2 = 0.30$. (b) Linear regression model between Monoglycerides % and Absorbance at 1755 cm^{-1} with $R^2 = 0.87$. (c) Linear regression model between Fatty Acid Ethyl Esters and Absorbance at 3300 cm^{-1} with $R^2 = 0.06$.
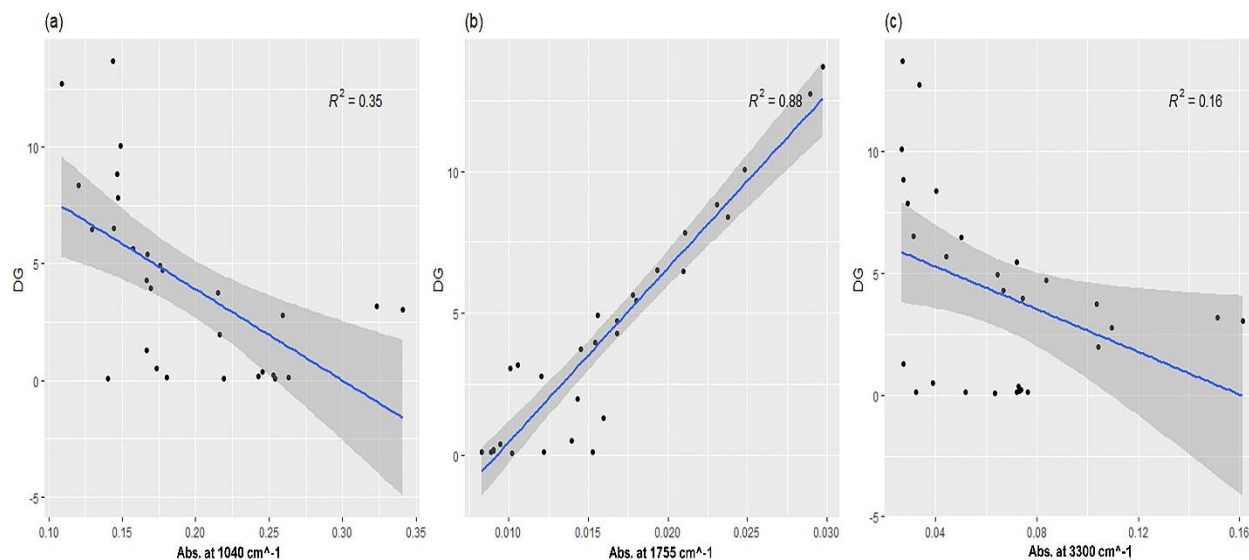
Figure 24 - (a) Linear regression model between Diglycerides % and Absorbance at 1040 cm^{-1} with $R^2 = 0.35$. (b) Linear regression model between Diglycerides % and Absorbance at 1755 cm^{-1} with $R^2 = 0.88$. (c) Linear regression model between Diglycerides % and Absorbance at 3300 cm^{-1} with $R^2 = 0.16$.
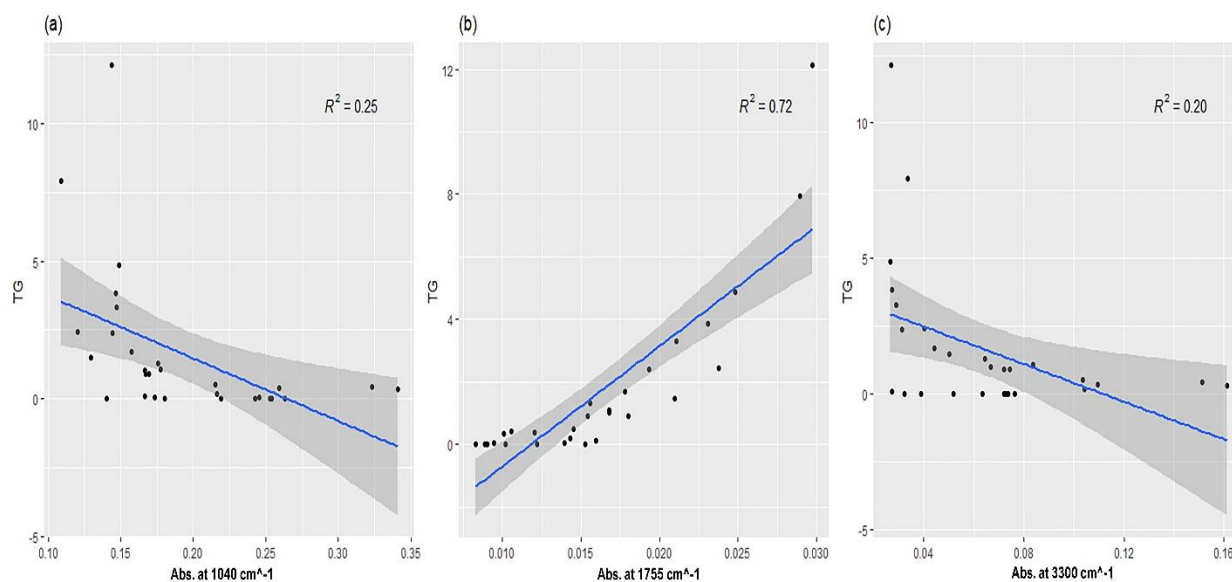
Figure 25 - (a) Linear regression model between Triglycerides % and Absorbance at 1040 cm^{-1} with R^2 = 0.25. (b) Linear regression model between Triglycerides and Absorbance at 1755 cm^{-1} with R^2 = 0.72. (c) Linear regression model between Triglycerides % and Absorbance at 3300 cm^{-1} with R^2 = 0.2.
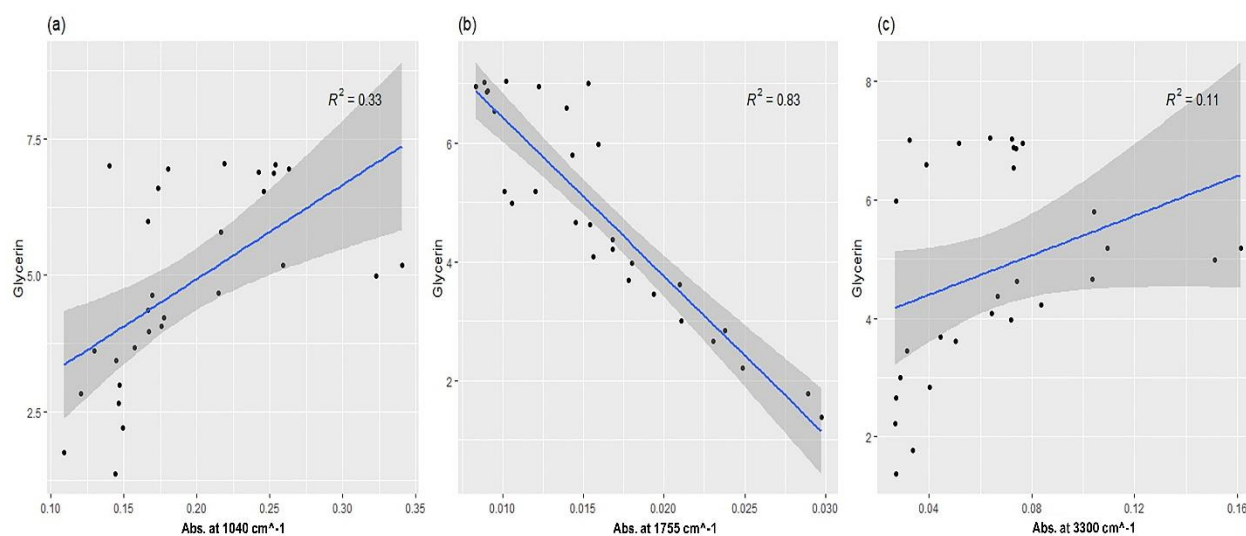
Figure 26 - (a) Linear regression model between Glycerin and Absorbance at 1040 cm^{-1} with R^2 = 0.33. (b) Linear regression model between Glycerin and Absorbance at 1755 cm^{-1} with R^2 = 0.83. (c) Linear regression model between Glycerin and Absorbance at 3300 cm^{-1} with R^2 = 0.11.

The previous charts (Figure 21 – Figure 26) indicate the possible relationships between the products and the regions of interest. What can be understood from those figures is that the regions around 1040 cm^{-1} and 3300cm^{-1} do not have the capacity to describe the changes to the reactants and products. While the region around 1755 cm^{-1} has the indicative results of describing the changes happening with Fatty Acid Ethyl Esters, Conversion , Diglycerides , Monoglycerides , as

well as Glycerin . This region was investigated further for more detailed interpretation and quantification.

III.VII. STATISTICAL MODELING, AND VALIDATION

Testing all regions of interest had led to a most promising region which lies around the 1755 cm^{-1} 2D peak. To fully test this region, the experimental data acquired with reference to the Gas Chromatography results were merged, and a random sample of 70% of the experimental data points were chosen for calibration to build a model, and the other 30% were tested against the model for validation. Two data sets were tested, the first is where automatic baseline correction was applied, the second was raw unprocessed spectral data. It was clear that in this case, using automatic baseline correction in OMNIC® software had led to omission or unwanted manipulation of the spectral data at regions of interest, therefore the models further taken into account are models built on raw spectral data with no baseline correction.
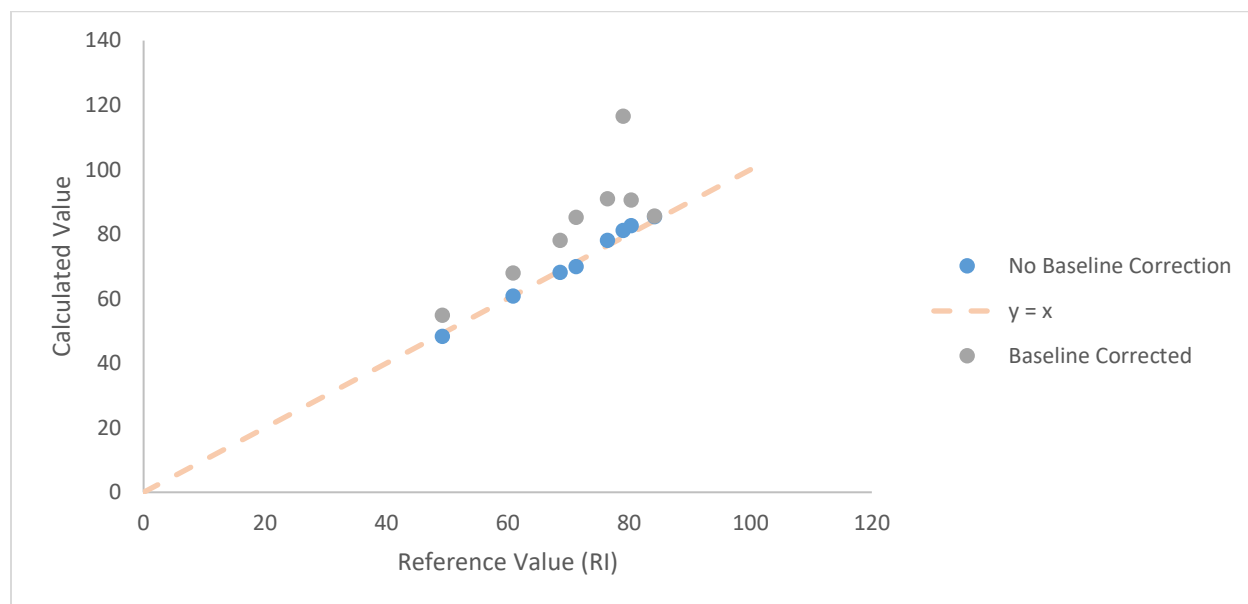
Figure 27 - Validation model of new values unseen by the model, Refractive Index method was used for reference values.

Figure 27 represents a comparison between two models where baseline correction had been used, against the other where no spectral manipulation was done. The test was performed on data not used for the model's calibration nor validation. It clearly shows that the model where no baseline correction was used can better predict the values in reference to the Refractive Index Method performed by (Husar et. al.)[23]. It was also found that using raw unprocessed data led to a better fitting model when different data points were used for calibration as well.
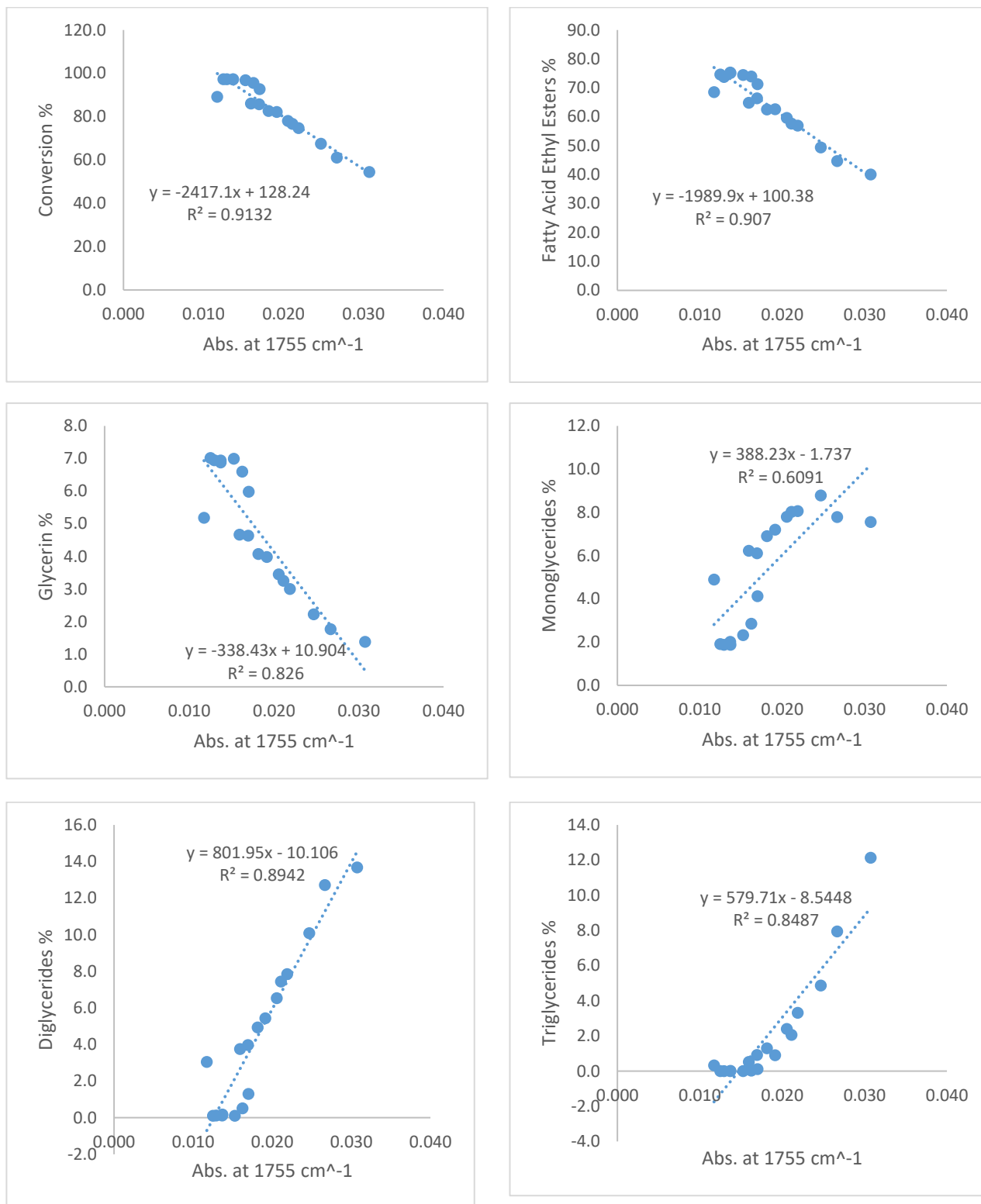
Figure 28 – Linear Regression models built from random calibration data between the absorbance at 1755cm⁻¹ without baseline correction and parameters of interest.

In Figure 28 where the linear regression models were built, it can be seen that some models including the Triglycerides, and Diglycerides models had a better fit when their percentages are higher than 2%, in that region the linear model could better predict Triglycerides, and Diglycerides in relatively higher concentrations rather than small quantities.

For validation, the created models were tested against unseen random data with results as follows:
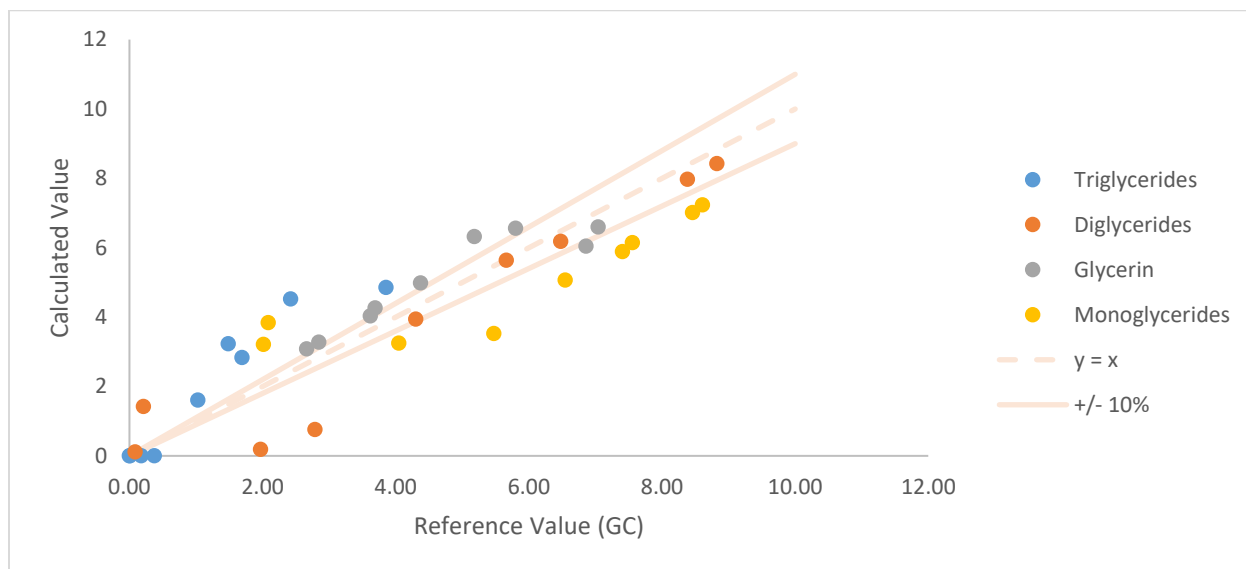
Figure 29 - Validation model for Monoglycerides, Diglycerides, and Glycerin % with a line showing a perfect fit. [Validation Data].

Figure 29 that the same case shown in Figure 28, it can be seen that the built model could better predict and fit data with higher confidence when the values are greater than 4%.
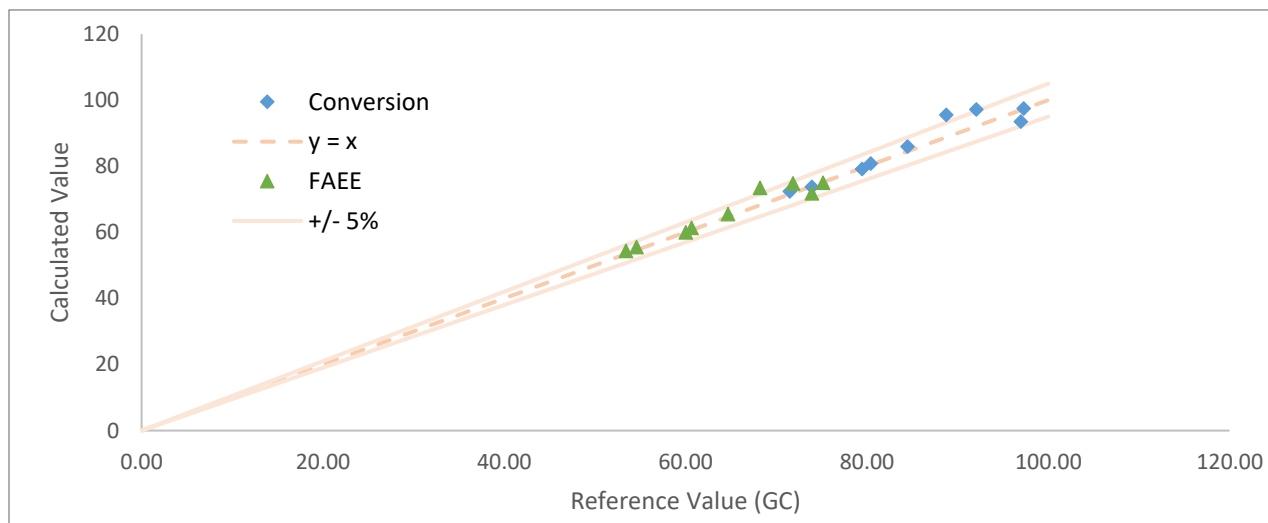
Figure 30 – Validation for FAEE and Conversion % with a line showing a perfect fit. [Validation Data]

Figure 30 showcases the model's prediction of FAEE and Conversion in comparison with Gas Chromatography as a reference method. Models built on unprocessed data were tried on different sets of randomized data points and compared to models that were built with data manipulated with automatic baseline correction, and it was found that the models trained on raw data better fit the Ethanolysis reaction in an on-line manner.

Using this method to monitor the Ethanolysis reaction progression and conversion rate was found to be reliable and comparable to reference methods such as Gas Chromatography, Figure 31 represents the progression of the reaction using FT-IR method with comparison to GC:
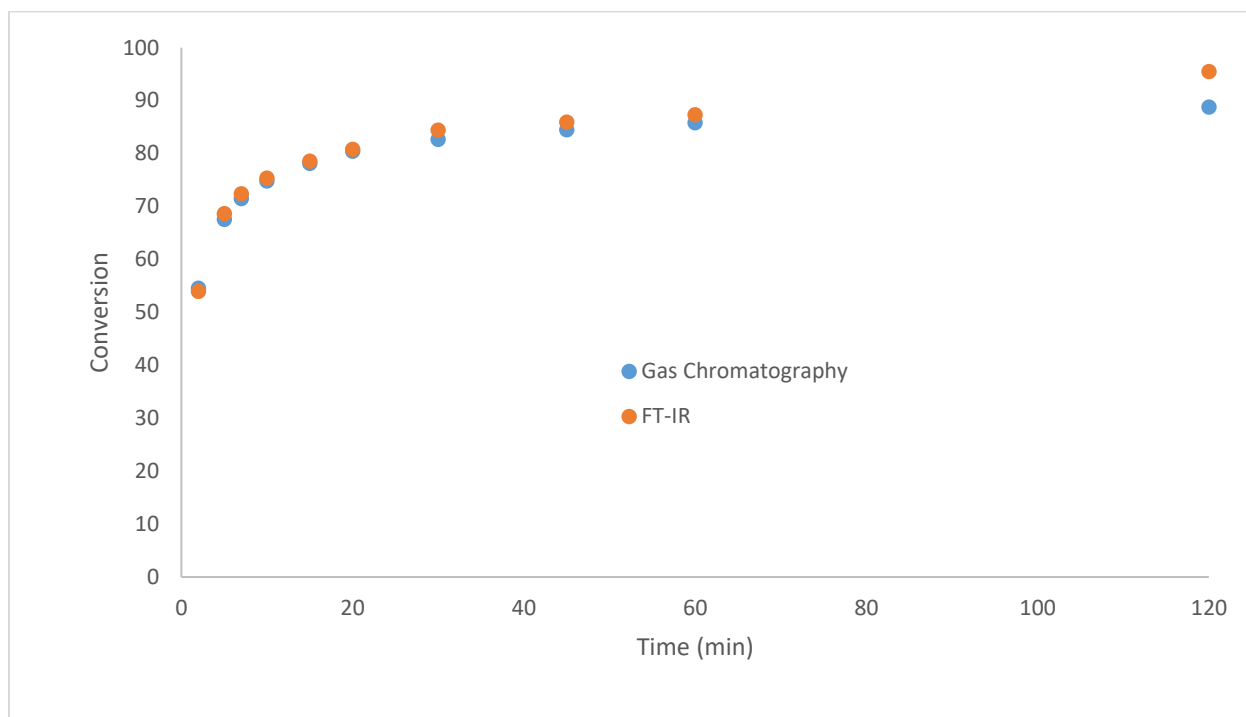
Figure 31 - Conversion of Oil to Fatty Acid Ethyl Esters Monitored with Gas Chromatography and FT-IR with the Help of 2D-COS. Reaction Conditions: Temperature 40C, 0.25% NaOH : Oil.1 : 6 Ethanol : Oil.

IV. CONCLUSIONS

This work focused on the development and validation of a quick, and reliable FT-IR analytical method, with the capability of quantitative on-line monitoring and assessment of the Ethanolysis reaction.

Samples of the reaction mixture were taken directly from the reaction vessel with neither processing nor preparation. These samples taken in specific time intervals were then analyzed using FT-IR directly. Acquired FT-IR spectra were preprocessed using only Automatic Baseline Correction in the OMNIC® software, subsequently Two-Dimensional Correlation maps were generated in R programming language using the Corr2D package. 2D-COS maps analysis had led to the detection of certain shifts in the peaks where it was determined that this shift indicated that the reaction had reached a certain stage, further analysis into these shifts in areas of interest had the potential to determine the percentage of Fatty Acid Ethyl Esters as well as Conversion percentage. This analysis can easily be applied in an online and automated manner and would provide the possibility of online monitoring of the Ethanolysis reaction, proving to be relatively reliable and significantly quicker than using conventional analytical methods like Gas Chromatography. These findings were found to be consistent throughout multiple experiments and differing reaction conditions. 2D-COS method can be easily implemented for the online monitoring of the Ethanolysis reaction with reliable and up-to-date results.

It was found that 2D-COS maps are not affected by automatic baseline correction, where synchronous and asynchronous maps were identical. The reason for this is that 2D-COS depends on the relationships and changes caused by the perturbation and not affected by shear values of the response from the machine. While using automatic baseline correction could be useful for the spectral data in different analysis methods, it was found to have the tendency to hide or manipulate actual spectral data that can be used for quantitative analysis of the transesterification reaction mixture.

Models built on the developed online FT-IR method can accurately predict the amount of Fatty Acid Ethyl Esters, Conversion percentage, as well as intermediaries in the reaction with a model fit of up to 92% (R^2) or better depending on the amount of training data and its accuracy. The Validation of the developed method based on random data points from multiple datasets revealed that the method's accuracy is comparable to those of Gas Chromatography and falls within 5% error from GC results in the monitoring of the progression of the Ethanolysis reaction (FAEE, Conversion). However, unlike GC, this method can easily be implemented in an on-line, and quick manner with no need for any spectral baseline correction or sample preparation or manipulation.

Using 2D-COS to monitor the transesterification reaction can be easily implemented, to determine the location of peaks of interest that have the possibility to describe the reaction, as well as forming a meaningful relationship between the signal at that location and products of interest. Automation is also a possibility, this method can be further improved, and implemented in conjunction with other new technologies like computer vision in order to acquire results interpreted instantaneously without the need to look into the FT-IR spectral data nor the use of any reference method.

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VI.   APPENDICES

VI.I.   SPECTROSCOPIC DATA

For a full resolution image, click on this link: FullPicture [https://utbcz-my.sharepoint.com/:f:/g/personal/t_jumaah_utb_cz/En6dZ8HqK0BMgxj090AbyoQBZP5iN8oU HZXo4U1LGoJr4A?e=BS7Hnl]

VI.II.   TABLE OF FIGURES