QUALITATIVE AND QUANTITATIVE DETERMINATION OF *TRANS* FATTY ACIDS IN FOOD USING FOURIER TRANSFORMED INFRARED SPECTROSCOPY (FTIR)

By
Anchalee Arunrat

An Independent Study Submitted in Partial Fulfillment of the Requirements for the Degree
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Department of Food Technology
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การใช้ ฟูเรียทรานสฟอร์มอินฟาเรด สเปกโตรสโคปี ในการวิเคราะห์เชิงคุณภาพและปริมาณของไขมันชนิดทรานส์ ในผลิตภัณฑ์อาหาร

โดย
นางสาวอัญชลี อรุณรัตน์

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The Graduate School, Silpakorn University has approved and accredited an independent study title of "Qualitative and quantitative determination of *trans* fatty acids in food using fourier transformed infrared spectroscopy (FTIR)" submitted by Miss Anchalee Arunrat as a partial fulfillment of the requirements for the degree of Master of Science in Food Technology.

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The objective of this research attempted the determination and classification of *trans* fatty acid. A set of fatty acid samples were chosen for this study via mainly focusing on their structural differences; that allowed to determine the qualitative and quantitative of *trans* fatty acid via analytical instrument, fourier transformed infrared spectroscopy, FT-IR. Each compound exhibited unique its absorbance range, so call as its fingerprint; therefore. The varieties of absorbance bands could be further evaluated. The absorptivity of the spectrum were observed and analyzed with theoretical values of infrared absorbance regions from appendix in chemical reference book. These peaks of absorptivity were capable to relate to the quality and quantity of each fatty acid. As a result, *trans* fatty acids expressed the different wavenumber regions of FTIR absorbance the distinctive absorbance ranges in infrared spectrum from each sample. The range for absorbance for *trans* fatty acid showed at 1000 – 950 cm^{-1}, where *cis* configuration of fatty acid should be absorbed within the range of 780 – 680 cm^{-1}. As expected, *trans* fatty acids samples were feasibly classified the qualitative and quantitative analyses of *trans* fatty acids in food.

According to the study of margarine sampling by comparing the results between FTIR and GCMS, it finally concluded that these two equipments could determine both quality and quantity of *trans* fatty acid. However, GCMS can gave better data, but consumed more time. Therefore, it could be not suitable for using GCMS in the industry, which had time limitation and did not need the deep detail in analyzing. So, using FTIR would be the best for classification and determination of *trans* fatty acid in the industry.

Department of Food Technology Graduate School, Silpakorn University Academic Year 2009
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อัญชลี อรุณรัตน์ : การใช้ฟูเรียทรานสฟอร์มอินฟาเรด สเปกโตรสโคปี ในการวิเคราะห์เชิงคุณภาพและปริมาณของ ไขมันชนิดทรานส์ ในผลิตภัณฑ์อาหาร. อาจารย์ที่ปรึกษาการค้นคว้าอิสระ : ผศ.ดร.บัณฑิต อินณวงศ์. 67 หน้า.

งานวิจัยนี้ศึกษาการตรวจสอบในเชิงคุณภาพและปริมาณ ของไขมันชนิดทรานส์ ในผลิตภัณฑ์อาหาร โดยใช้เครื่อง Fourier Transformed Infrared Spectroscopy (FTIR) โดยจะเลือกสารที่ทราบโครงสร้างแน่นอน ทั้งโครงสร้างแบบ *cis* และโครงสร้างแบบ *trans* เป็นตัวแทนในการศึกษาแต่ละโครงสร้าง ซึ่งสารแต่ละโครงสร้างจะเกิดการดูดกลืนรังสีอินฟราเรดในระดับที่แตกต่างกัน ซึ่งความแตกต่างนี้จะเกิดขึ้นในช่วงที่เรียกว่า Fingerprint area จากข้อมูลอ้างอิงพบว่า การดูดกลืนรังสีอินฟราเรดของไขมันแบบ *trans* นี้จะพบในช่วง 1000-950 cm^{-1} และโครงสร้างแบบ *cis* พบการดูดกลืนรังสี อินฟราเรดในช่วง 780-680 cm^{-1} ซึ่งผลการศึกษาในงานวิจัยชิ้นนี้ก็ให้ผลที่สอดคล้องกันและมีความน่าเชื่อถือทางสถิติ นอกจากนี้ยังสามารถนำข้อมูลที่ได้ไปสร้างเป็น calibration graph โดยใช้โปรแกรม Spectrum Beer’s Law เพื่อนำไปใช้ในการศึกษาในเชิงปริมาณของไขมันชนิดทรานส์ในผลิตภัณฑ์อาหารได้อีกด้วย

จากการศึกษาในตัวอย่างมาร์การีน โดยเปรียบเทียบผลการทดลองที่ได้จาก FTIR และ GCMS พบว่า เครื่องมือทั้ง 2 ชนิดสามารถตรวจสอบทั้งในเชิงคุณภาพและปริมาณของไขมันชนิดทรานส์ได้ แต่ GCMS จะให้ประสิทธิภาพในการวัดที่ดีกว่า แต่ใช้ระยะเวลาในการวิเคราะห์นาน จึงไม่เหมาะกับการใช้งานในโรงงานอุตสาหกรรม ที่ต้องการความสะดวกรวดเร็ว และไม่ต้องการความละเอียดในการวิเคราะห์มากนัก ดังนั้น FTIR จึงเป็นทางเลือกที่ดีสำหรับการวิเคราะห์ไขมันชนิดทรานส์ในโรงงานอุตสาหกรรม

ภาควิชาเทคโนโลยีอาหาร บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร ปีการศึกษา 2552
ลายมือชื่อนักศึกษา ....
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CHAPTER 1

INTRODUCTION

Fatty acid can be classified as carboxylic acid with a presence of aliphatic chain attachment. Naturally occurring fatty acid can be varied in number of carbon, ranging from 4 – 24 carbon atom. The shortest carboxylic acid chain composed of 4 carbon atoms backbone, is known as butyric acid. Mostly, naturally occurring fatty acids are even numbered of carbon backbone and unbranced (Fennema, 1996). Consequently, the characteristic of fatty acid allows biosynthesis that involves acetyl-CoA, a coenzyme that caring two carbon atoms. Hydrolysis of ester linkage in fat or oil that contains no glycerol allows the production of fatty acid as well (Richard and O'Brien, 2004).

Fatty acid can be categorized into two classes; saturated and unsaturated fatty acids depending mainly on their number of double bonds and length. Saturated fatty acid may contain any double bond or any functional groups along the chain. Unsaturated fatty acid is the similar form but contain more of alkenyl functional group that can be observed along the chain, this observation may insist with the alkenes substituting single bond as a part of the chain as well as double bond (Mossoba et al., 2007). In the chain, the behavior of carbon atoms that are bond to either side the other carbon atom may lead to occurrence of *cis* or *trans* configuration (Figure 1.1) The *cis* conformation has adjacent hydrogen atom and double bond on the same side; therefore, the stiffness of double bond forces the chain to bend and consequently limiting the choice of conformation. The infrared spectrum of *cis* configuration should be observed in 780 – 680 cm^{-1} ranges. In contrast, *trans* configuration is a straight chain of saturated fatty acid; to be more precise, double bond and hydrogen are found on the opposite side. This does not restrict the conformation of the chain as the conformation of the chain is observed to be straight as mentioned (Devries and Prakash, 2009). The infrared spectrum of *trans* configuration should be observed in 1000 – 950 cm^{-1}.

cis configuration *trans* configuration

Figure 1 *Cis* and *trans* configuration

A *trans* fatty acid; so called *trans* fat is main interest for this research paper. For its molecular behavior of an alignment in the chain via double bond localization; these characteristic raised pros and cons which are stated in many past papers. *Trans* fat behaves like saturated fatty acid in way that it give rise to low density lipoprotein (LDL) or bad cholesterol as it is lowering high density lipoprotein (HDL) or good cholesterol (Booker and Mann, 2008). Some suggests that *trans* fat intake amount correlates with circulatory diseases such as artherosclerosis and coronary heart disease more than the same amount of *cis* fat intake at the same level. (Saunders, 2008) Other harmful risk to human health relating to *trans* fat intake such as increasing triglycerides and lipoprotein level, causing inflammation, and damaging cell of blood vessel (Lopez-Garcia et al., 2005).

From the past research about *trans* fatty acid, most researchers used GC to analyzed the data. However, there is another interesting method which can reduce cost and time in analyses. This method is to use FTIR, this method has been used to determined peroxide value, free fatty acid of frying oil content (Innawong et al., 2004). FTIR is used to analyzed quality and quantity of food composition at the different level of infrared absorptions. This depends on the vibrations of each type of bonds, where each type of bond has different vibration range. At the temperature above 0°c, every molecules would vibrate. When the frequency of vibration becomes equal to that of infrared radiation, the molecules would absorb the radiation. For *trans* fatty acid should be observed in 1000-950 cm^{-1} and for *cis* fatty acid should be observed at 780-680 cm^{-1} (Cross, 1960).

In this study the possibility of using the spectra from fourier transform infrared spectroscopy in transmission mode for qualitative and quantitative determination of *trans* fatty acid in food was investigated.

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CHAPTER 2

LITERATURE REVIEW

Fourier transformed infrared spectroscopy

Fourier transformed infrared spectroscopy is capable of determining compounds that has unique absorption ability. Since all of the bond type exhibits unique frequency, also known as chemical fingerprint; not single pair of molecule exhibits different structures that show exactly the same absorption pattern (or so called Infrared Spectrum) (Pavia et al., 2001). However, the absorption pattern may reveal similarities if the frequencies absorbed for two molecules are the same. Usually, each type of bond is found to be absorbed in a small portion of vibration of infrared region. When the absorption ranges are not matched with theoretical region, it is said to be some other type of bond. Stretching and bending characteristics of molecules in a compound are the simplest parameter that gives rise to absorption peaks. Simple applied this idea; we say that stretching vibration is found to occur at a high frequency than bending vibration (Firestone and Labouliere, 1965). In applying infrared theory to determination of *trans* fatty acid, one must be aware of the fact that double bond is stronger than single bond between the same atom and is adopted higher frequency absorption.

Fourier transformed infrared spectrometer is designed to produce interferogram; a complex signal containing all frequencies that make up an infrared spectrum. Interferogram is a plot of intensity versus time, called time domain spectrum (Christy et al., 2003). A fast and accurate result can be accounted from FTIR instrument for its known advantage to produce greater speed and sensitivity.

Trans fatty acid

Fat can be separated into two types, which are saturated fatty acid and unsaturated fatty acid. These two types of fat are different in their structure. The difference is that saturated fatty acid structure consist of only single bond, but unsaturated fatty acid structure consist of at least one double bond. The double bond structure can make the unsaturated fatty acid arranged into two types are *cis* configuration and *trans* configuration. The difference of these two configurations is the hydrogen (H) position. For *cis* configuration, H are at the same side. However, H are at the opposite side for *trans* configuration.

Trans fatty acid is rarely found in the nature. It's mostly found in cattle's stomach and dairy product (John et al., 2004). Beside that it can also be found in the cooking oil by hydrogenation process to change structure from *cis* configuration to *trans* configuration. This processed made it possible for vegetables oil to be converted into plastic fat forms. Hydrogenated edible fats and oils product can be prepared with creaming properties, frying stability, sharp melting properties, and the others functional characteristic desired for specific application (Richard and O'Brien, 2004).

However, *trans* fatty acid has two main cons which are increasing risk of heart attack in human, and it's also increase low density lipoprotein (LDL) that is dangerous to human body while it's decreasing high density lipoprotein (HDL) that is good for health (Booker and Mann, 2008).

According to these effects, it make the consumer think more to consuming fat. Also, it makes many countries concern about this effect by giving the law to control the quantity of Trans fat in food industry. However, this law has not been used in Thailand (national food institute, 2009).

Spectrum Beer's Law

The basis for quantitative analysis of absorption spectrometry is the Bouguer-Beer-Lambert Law commonly called Beer's Law. For a single compound in homogenous medium, the absorbance at any frequency is expressed as

$$A = abc$$

Where A is the measured sample absorbance at given frequency, a is the molecular absorptivity at the frequency, b is the pathlength of source beam in the sample, and c is the concentration of the samples. This law basically states that the intensities of absorption bands are linearly proportional to the concentration of each component in a homogenous mixture or solution. Resolution is closely related to the slit width in dispersive IR instruments or the optical path difference between two beam in the interferogram of FTIR spectrometer. The Beer's law deviation result in a nonlinear relationship for plots of absorbance (A) against concentration (c). As absorbance is defined as logarithm of transmittance; therefore, to achieve linear relationship exist only between the sample concentration and absorbance.

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CHAPTER 3

QUALITATIVE AND QUANTITATIVE DETERMINATION OF REFERENCE FATTY ACIDS COMPOUND USING FOURIER TRANSFORMED INFRARED SPECTROSCOPY (FTIR)

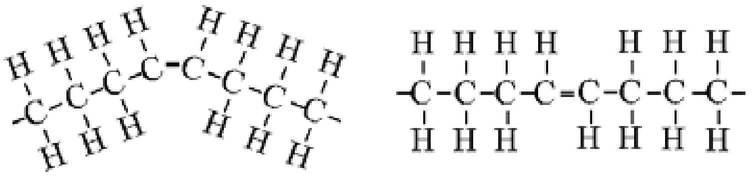
Abstract

The objective of this research attempted to determine and classify each type of *trans* fatty acid with respect to its FTIR absorbance. A set of fatty acid samples were chosen for this project based mainly on their structural differences; that allowed the comparison for qualitative and quantitative determination of *trans* fatty acid. This research was to study the determination of *trans* fatty acid sample via analytical instrument, fourier transformed infrared spectroscopy, FT-IR. Each compound exhibited unique absorbance range, so call as its fingerprint; therefore. The varieties of absorbance bands could be observed and then interpreted as compared to reference handbook. The major result of this research was distinctive absorbance ranges resulting in infrared spectrum from each sample. The range for absorbance for *trans* fatty acid showed in the regions of 1000 – 950 cm^{-1}, where *cis* configuration of fatty acid should be absorbed within the range of 780 – 680 cm^{-1}. As a result, samples were classified the qualitative and quantitative analyze of *trans* fatty acid composition in food.

Keywords : fourier transformed infrared spectroscopy, *trans* fatty acid

Introduction

Fatty acid is and organic compound consisting of a hydrocarbon chain and a terminal carboxyl group. Chain length ranges from 4 hydrogen atoms to nearly 24 carbon atoms. Naturally occurring fatty acid can be varied in number of carbon, ranging from 4 – 24 carbon atom. The shortest carboxylic acid chain composed of 4 carbon atoms backbone, and is said to butyric acid. Mostly, naturally occurring fatty acids are even numbered of carbon backbone and unbranced (Fennema, 1996). Long chain fatty acid most commonly occur as constituents of certain lipids, notably glycerides, phospholipids, sterols, and waxes, in which they are esterifiedwith alcohols. These long chain fatty acids generally have and even number of carbon atoms; unbranced chains predominate over brances chains. They may be saturated or unsaturated, with one double or more double bond. Saturated fatty acids are those in which every carbon atom carries its full quota of hydrogen atoms, and therefore there are only single bonds between adjacent carbon atom. Unsaturated fatty acid is the similar form but contain more of alkenyl functional group that can be observed along the chain, this observation may insist with the alkenes substituting single bond as a part of the chain as well as double bond (Mossoba et al., 2007). In the chain, the behavior of carbon atoms that are bond to either side the other carbon atom may lead to occurrence of *cis* or *trans* configuration (figure 2.) The *cis* conformation has adjacent hydrogen atom and double bond on the same side; therefore, the stiffness of double bond forces the chain to bend and consequently limiting the choice of conformation. The infrared spectrum of *cis* configuration should be observed in 780 – 680 cm^{-1} ranges. In contrast, *trans* configuration is a straight chain of saturated fatty acid; to be more precise, double bond and hydrogen are found on the opposite side. This does not restrict the conformation of the chain as the conformation of the chain is observed to be straight as mentioned (Devries and Prakash, 2006). The infrared spectrum of *trans* configuration should be observed in 1000 – 950 cm^{-1}.



cis configuration *trans* configuration

Figure 1 *Cis* and *trans* configuration

Trans fatty acids are produced from the naturally occurring *cis* unsaturated form by heating liquid vegetable oils in the presence of metal catalysts and hydrogen. Like hydrogenation (addition of hydrogen across a double bond to make it saturated), this process can also cause isomerisation of *cis* double bonds into the *trans* form. Such partially hydrogenated vegetable oils are attractive to the food industry because of their longer shelf life, oxidative stability and semi solidity at room temperature. *Trans* fatty acids raised pros and cons which are stated in many past papers. *Trans* fat behaves like saturated fatty acid in way that it give rise to low density lipoprotein (LDL) or bad cholesterol as it is lowering high density lipoprotein (HDL) or good cholesterol (Booker and Mann, 2008). Some suggests that *trans* fat intake amount correlates with circulatory diseases such as artherosclerosis and coronary heart disease more than the same amount of *cis* fat intake at the same level (Saunders, 2008). Other harmful risk to human health relating to *trans* fat intake such as increasing triglycerides and lipoprotein level, causing inflammation, and damaging cell of blood vessel (Lopez-Garcia et al., 2005). Associations with adverse health effects have led to efforts to decrease the intake of *trans* fatty acids by consumers, including labeling of food products for *trans* fatty acid content, most recently in the United states and Canada (Saunders, 2008).

From the past research about *trans* fatty acid, most researchers used GC to analyzed the data. However, there is another interesting method which can reduce cost and time in analyses. This method is to use FTIR, this method has been used to determined peroxide value, free fatty acid of frying oil content (Innawong et al., 2004). FTIR is used to analyzed quality and quantity of food composition at the different level of infrared absorptions. This depends on the vibrations of each type of bonds, where each type of bond has different vibration range. At the temperature above 0°c, every molecules would vibrate. When the frequency of vibration becomes equal to that of infrared radiation, the molecules would absorb the radiation. For *trans* fatty acid should be observed in 1000-950 cm^{-1} and for *cis* fatty acid should be observed at 780-680 cm^{-1} (Cross, 1960).

Materials and methods

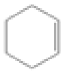
Preparation of Reference compounds

This experiment was conducted by using 8 known chemical substances to represent each fatty acids structure that purchased form Sigma-Aldrich. The first was single ene, it had 2 configuration, *cis* and *trans*. For *cis* configuration used *cis*-2-heptene, methyl oleate and cyclohexene are shown in figure 3. For *trans* configuration used *trans*-2-hexene, *trans*-2-pentenal and methyl palmitelaidate shown in figure 4. The second was conjugated diene consist of, conjugated *cis, cis* used 1,3-cyclohexadiene, conjugated *trans, trans* used *trans, trans*-2,4-hexadienyl acetate and conjugated *trans, cis* used *trans*-7, *cis*-9-dodecadienyl acetate were shown in figure 5. The last structure was nonconjugated diene consist of, nonconjugated *cis, cis* used methyl linoleate, nonconjugated *trans, trans* used methyl linolelaidate and nonconjugated *trans, cis* used *trans*-2, *cis*-6-nonadienal was shown in figure 6. All of chemicals were prepared by diluting with hexanes and the varied to 5 different concentration.

single ene

Cis-2-heptene

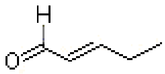
Methyl oleate
(Methyl *cis*-9-octadecanoate)



Cyclohexene

Figure 2 *Cis* configuration

Trans-2-hexene

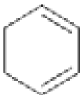


Trans – 2- pentenal

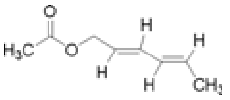
Methyl palmitelaidate

Figure 3 *Trans* configuration

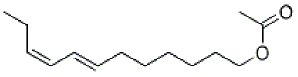
conjugated diene



1, 3-cyclohexadiene



Trans, trans-2,4-hexadienyl acetate



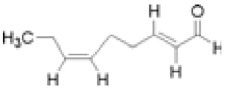
Trans-7, *cis*-9-dodecadienyl acetate

Figure 4 Conjugated diene cofiguration

nonconjugated diene

Methyl linoleate (methyl *cis, cis*-9, 12-octadecadienoate)

Methyl linolelaidate (methyl *trans, trans*-9, 12-octadecadienoate)



Trans-2, *cis*-6-nonadienal

Figure 5 Nonconjugated diene configuration

FTIR Spectrometer analysis

The FTIR spectrometer (spectrum100, Perkin Elmer) in transmission mode with a deuterated triglycine sulfate detector was to use in this experiment and use liquid KBr cell for measurements. The spectra were collected from 4000-450 cm^{-1} by using 16 scans/sample at 4 cm^{-1} resolution and fix pathlength at 0.1 mm.

Statistic analysis by PCA

Each sample was scanned for three times, after that, FTIR data would be analyzed using principal component analysis (PCA) technique from WIN-DAS software to differentiate among each concentration of fatty acids sample. PCA was an exploratory multivariate technique and involved a mathematical operation that determined the transformation of a set of predictable variables (possibly correlated) into a (smaller) set of new uncorrelated variables called principle components. The unique feature of these principle components was the first component accounting for the most variability in the data, the second component was known to use as the most remaining variability in the data. The remaining components obtained showed less variability in the data (Innawong et al., 2004).

Quantitative determination by spectrum Beer's law

Five different concentrations of chemical would be used as a sample for the study by scanning each samples for three times. After that the application of the spectrum Beer's law software was to figure out the calibration graph of each fatty acid chemical. The calibration graph would be used for quantitative determination in food.

Results and discussion

The infrared absorption represented the total number of *trans*-double bonds in the portion examined, regardless of the chain length, and it was only slightly affected by degree of saturation. Thus, the spectral interpretations should not be confined to one or two bands as the whole spectra should be examined. The simplest interpretation should be done first by examine *cis* or *trans* existence behavior (Mossoba et al., 2005). The results for infrared absorption region of reference compound were given in table 1.

From the result, it could be concluded that *cis* configuration absorbed infrared around 758–697 cm^{-1} but *trans* configuration absorbed infrared around 987-965 cm^{-1} (table 1.). The different infrared absorption depended on the different vibration of each bond. The different spectral of each bonds would be shown as fingerprint area (1000-600 cm^{-1}) in the bending mode out-of-plane of vibration. Moreover, the absorption at each fingerprint region depended on functional groups of compound. For *cis* and *trans* C-H group, that was the major functional group of fatty acids should be observed. According to many researches, *trans* configuration absorbed the infrared around 1000-950 cm^{-1} and *cis* configuration absorbed the infrared around 780-680 cm^{-1} (Cross, 1960). Even though, each compound had similar structure, the difference of each one was length arrangement which caused the vibration and infrared absroption at different levels (Pavia et al., 2001). In this study, each reference compound in 5 different concentrations were scanned. The result showed that FTIR responses could be clearly separated among the different concentrations.

Table 1 Analytical result for infrared absorption region of reference chemicals

sample	structure	wavelength (cm^{-1})	vibration	functional group
Cis-2-heptene	*cis*	697	bending	C-H
Methyl oleate	*cis*	758	bending	C-H
Cyclohexene	*cis*	718	bending	C-H
1,3-cyclohexadiene	Conjugated *cis, cis*	747	bending	C-H
Methyl linoleate	Nonconjugated *cis, cis*	724	bending	C-H
Trans-2-pentenal	*trans*	974	bending	C-H
Trans-2-hexene	*trans*	965	bending	C-H
Methyl palmitelaidate	*trans*	966	bending	C-H
Trans, trans-2,4-hexadienyl acetate	Conjugated *trans, trans*	987	bending	C-H
Methyl linolelaidate	Nonconjugated *trans, trans*	968	bending	C-H

Raw data obtained from FTIR method was further analyzed by PCA covariance technique from Win-Das software to differentiate each concentration of reference compound by plotting between PC score 1 and PC score 2, which the data was grouping in each concentration and could be determined by PC score, that showed each grouping efficiency. The high PC score 1 showed more grouping efficiency (Sivakesava and Irudayaraj, 2001). It found that the data scanned by FTIR could be used to represent the qualitative and quantitative determination in food. The result of PCA plot between PC score 1 and PC score 2 of each chemical were given in table 2.

The result could be further study by calibrating the FTIR data and formulating equations for relating to Beer's law, which this method could determine but quality and quantity of *trans* fatty acids in food.

Table 2 Analytical result of PCA plot between PC score 1 and PC score 2

Chemical	**PC score 1 (%)**	**PC score 2 (%)**
1,3-cyclohexadiene	94.04	98.41
Cis-2-heptene	98.03	99.99
Cyclohexene	88.04	99.98
Trans-2-pentenal	99.85	99.97
Trans-2-hexene	99.89	99.96
Trans, trans-2,4-hexadienyl acetate	99.72	99.94

Conclusions

This research had provided alternative prodedure for determining *trans* fatty acid presented in food products via FTIR. This was simple, fast, accurate, relatively inexpensive, and flexible method for application. Qualitative analysis had trustworthy statistic figures for having the acceptable and consistent predictions of conformation of fatty acid. Quantitative analysis had also been shown some prediction by Beer Lambert's law; however, the further experiment was crucial step in order to specify exactly what the percentage was or ratio of *trans* fatty acid was presented. There was factor verifying this research important; due to the need of studying some other aspects of *trans* fat behavior and characteristic in order to cover all areas that would work together for a beneficial development on human health. Data imported to this research paper by figures, tables, spectrum, and graph had made clear assumption of qualitative and quantitative aspects.

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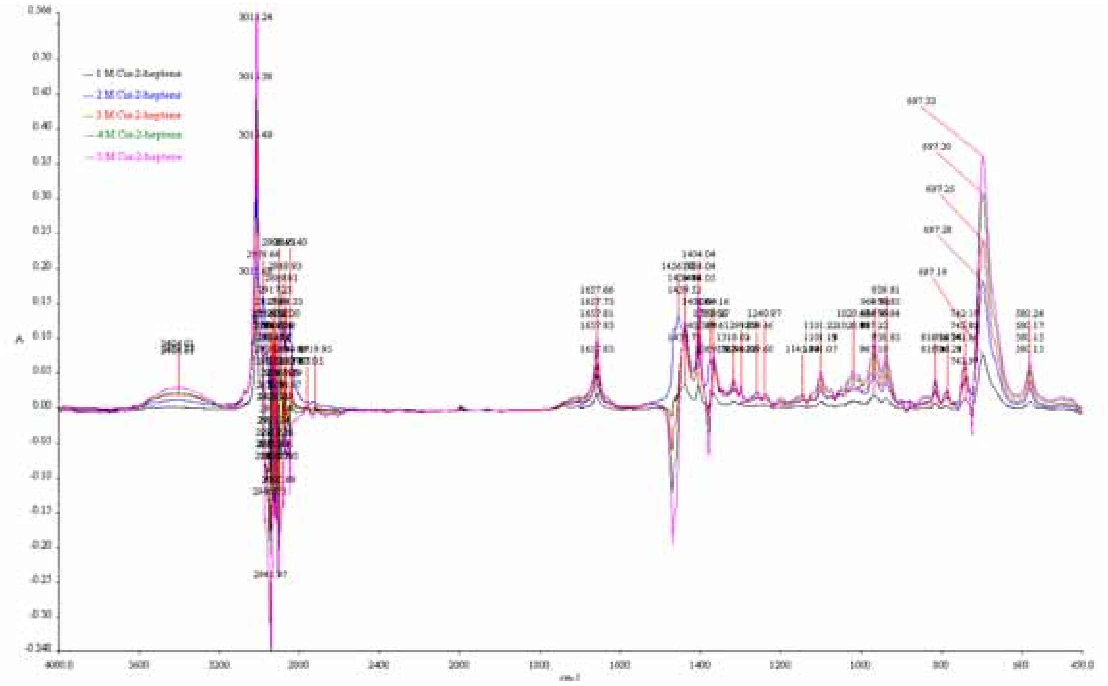

Figure 6 Whole spectra of *cis*-2-heptene from 4000-450 cm^{-1}

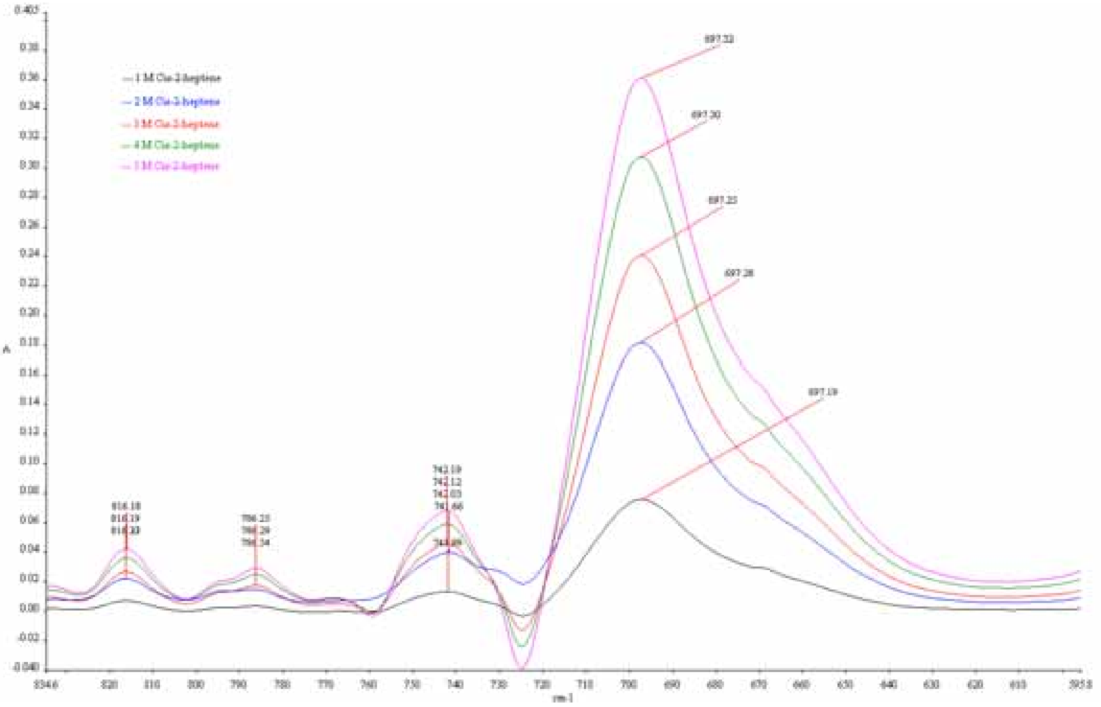

Figure 7 Spectra of *cis*-2-heptene at 697 cm^{-1} from 1M-5M

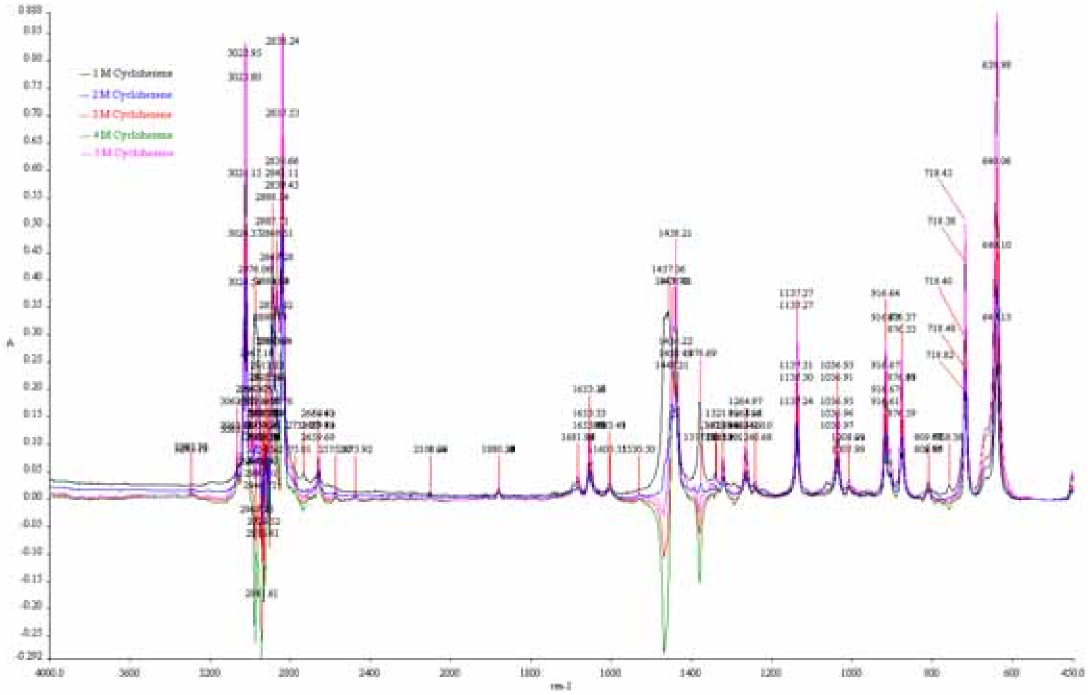

Figure 8 Whole spectra of cyclohexene from 4000-450 cm^{-1}

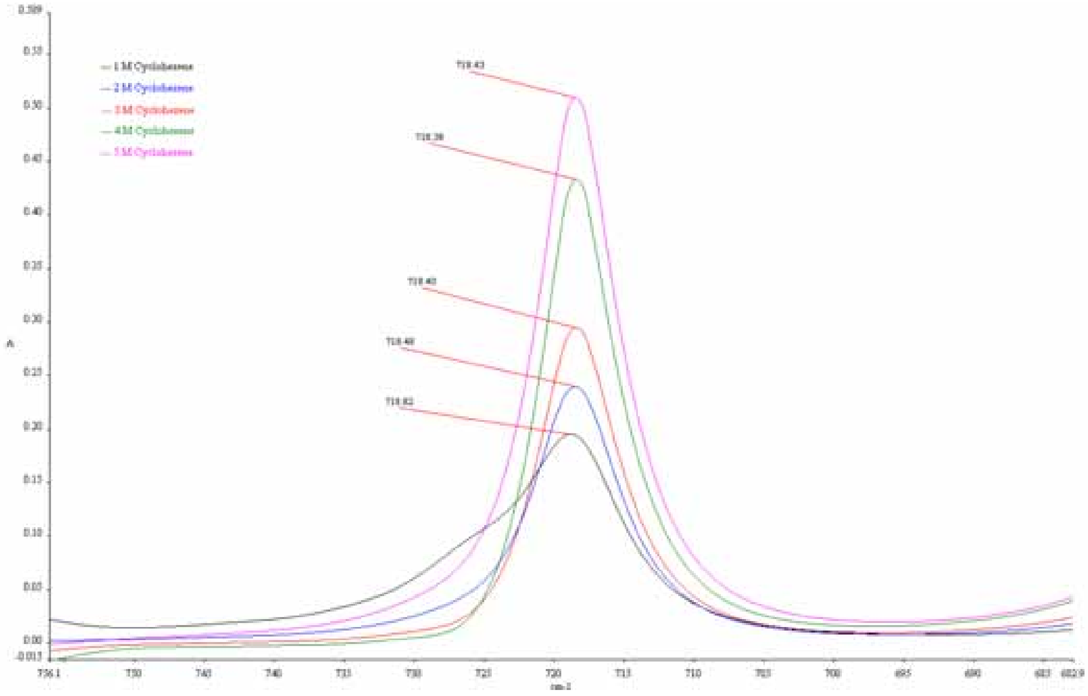

Figure 9 Spectra of cyclohexene at 718 cm^{-1} from 1M-5M

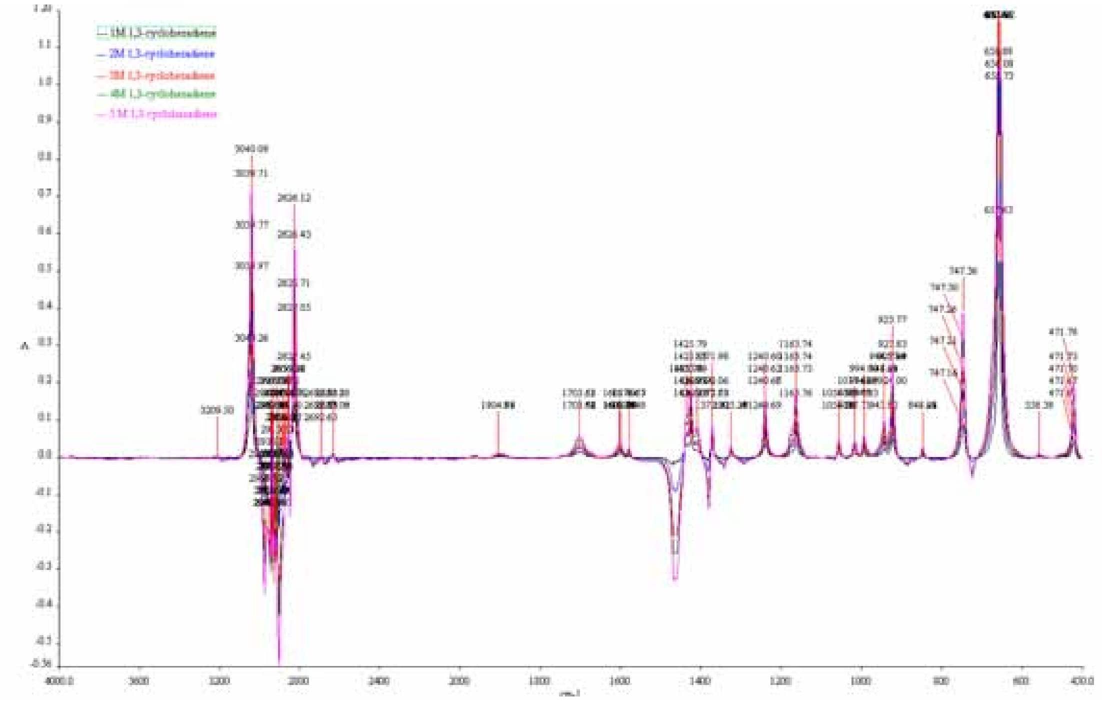

Figure 10 Whole spectra of 1, 3-cyclohexadiene from 4000-450 cm^{-1}

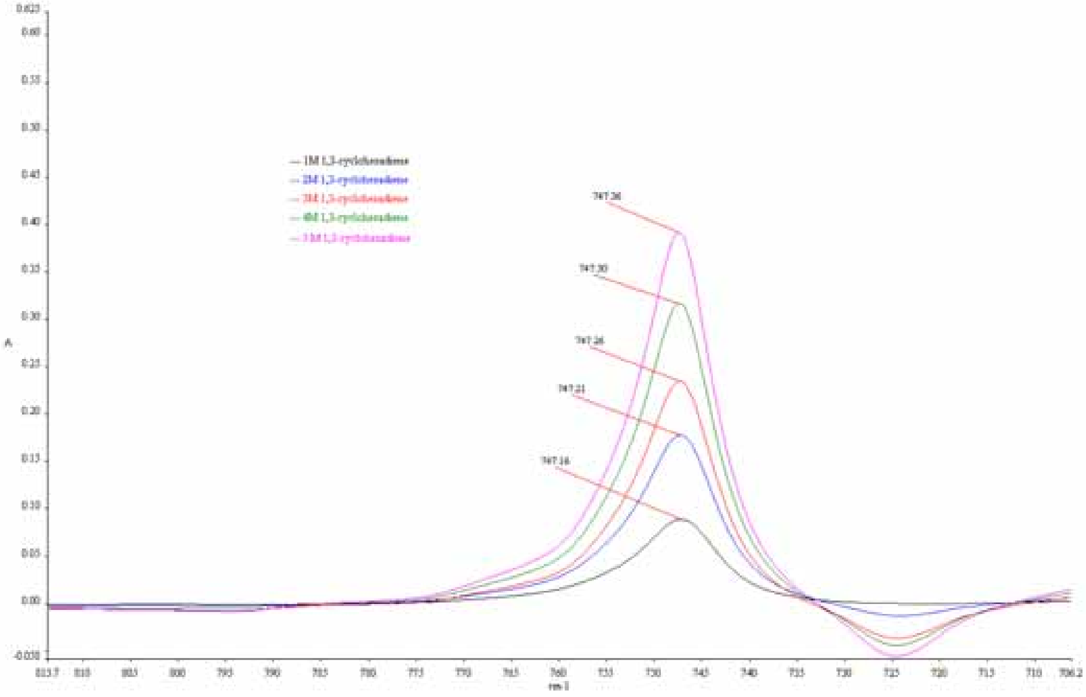

Figure 11 Spectra of 1, 3-cyclohexadiene at 747 cm^{-1} from 1M-5M

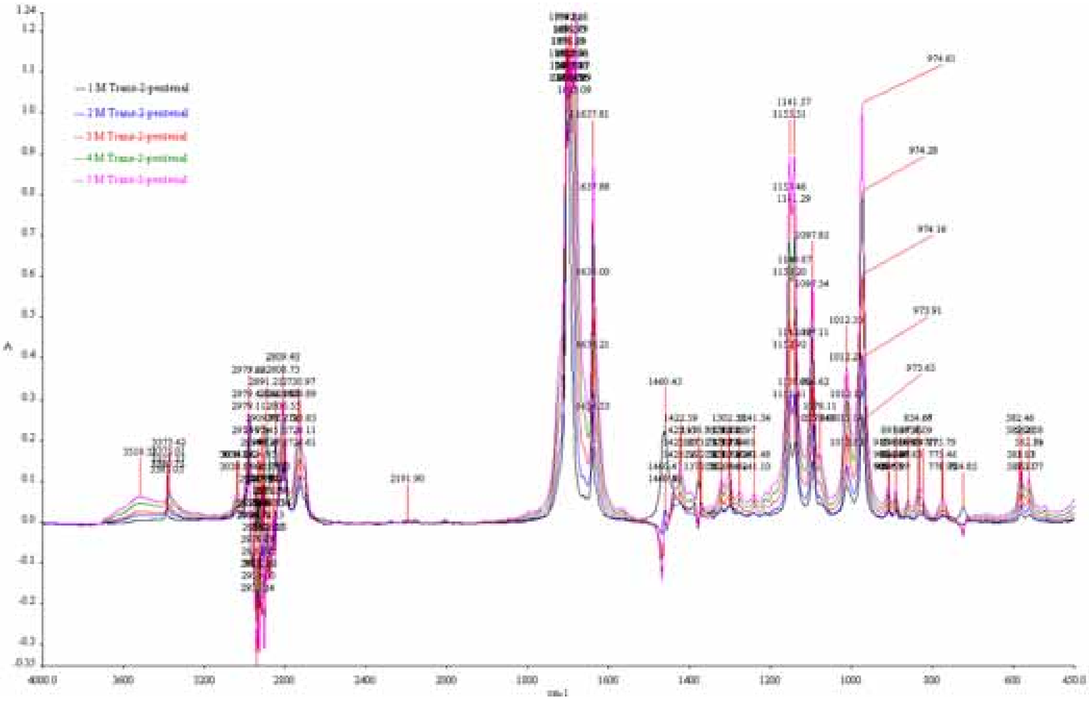

Figure 12 Whole spectra of *trans*-2-pentenal from 4000-450 cm^{-1}

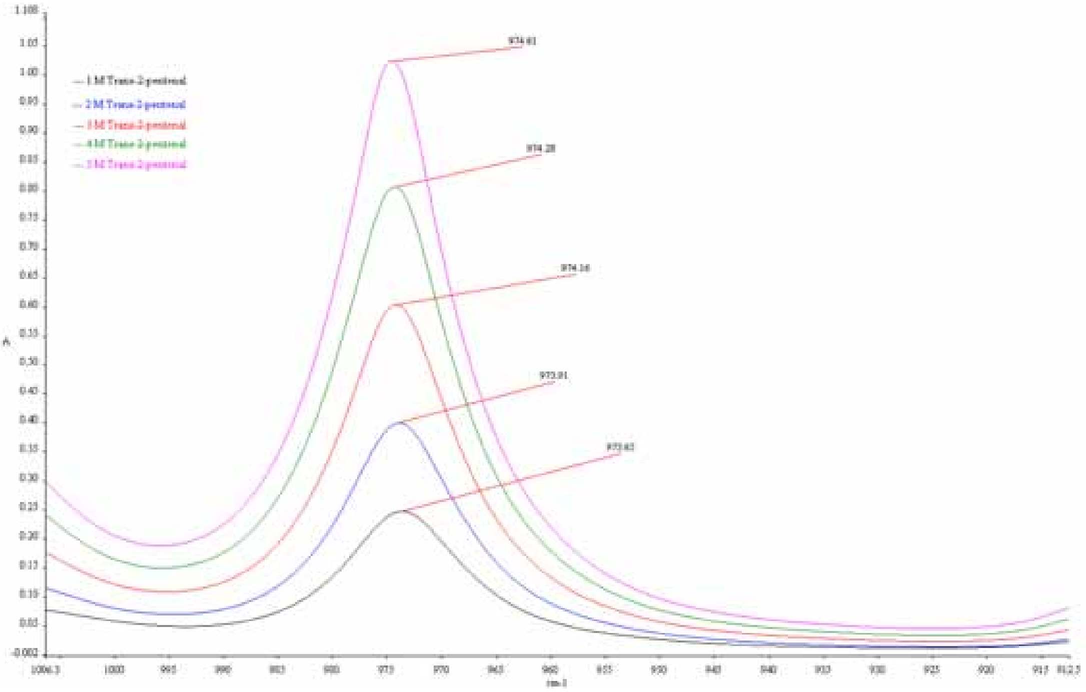

Figure 13 Spectra of *trans*-2-pentenal at 974 cm^{-1} from 1M-5M

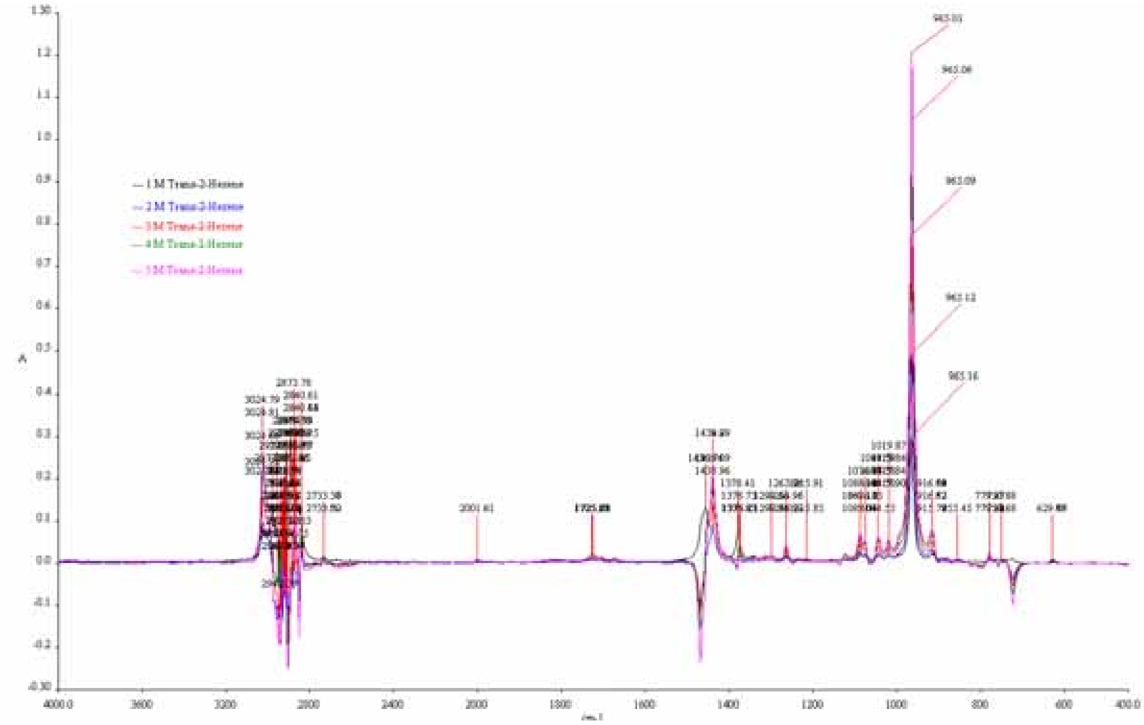

Figure 14 Whole spectra of *trans*-2-hexene from 4000-450 cm^{-1}

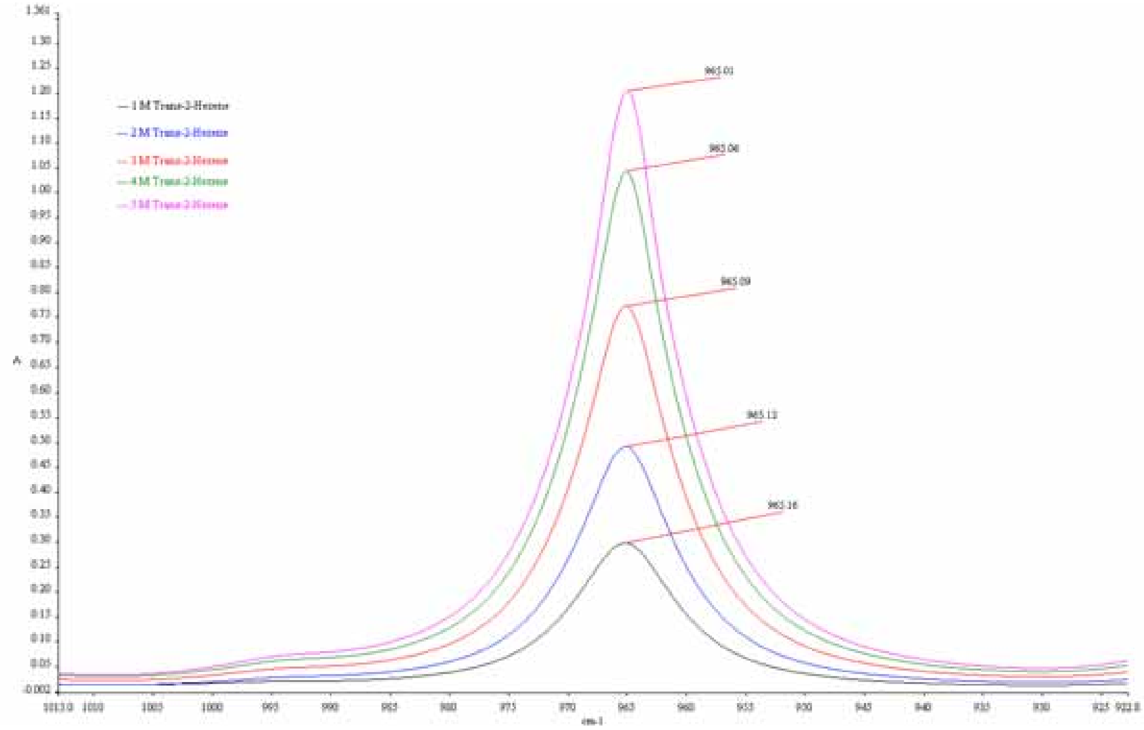

Figure 15 Spectra of *trans*-2-hexene at 965 cm^{-1} from 1M-5M

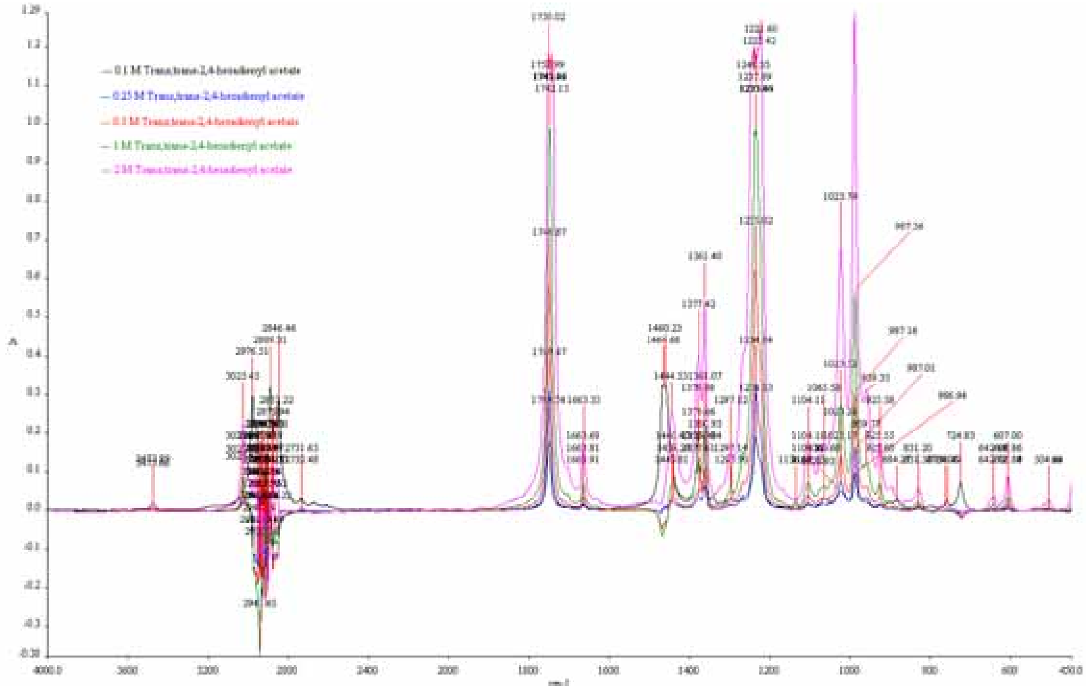

Figure 16 Whole spectra of *trans, trans*-2, 4-hexadienyl acetate from 4000-450 cm^{-1}

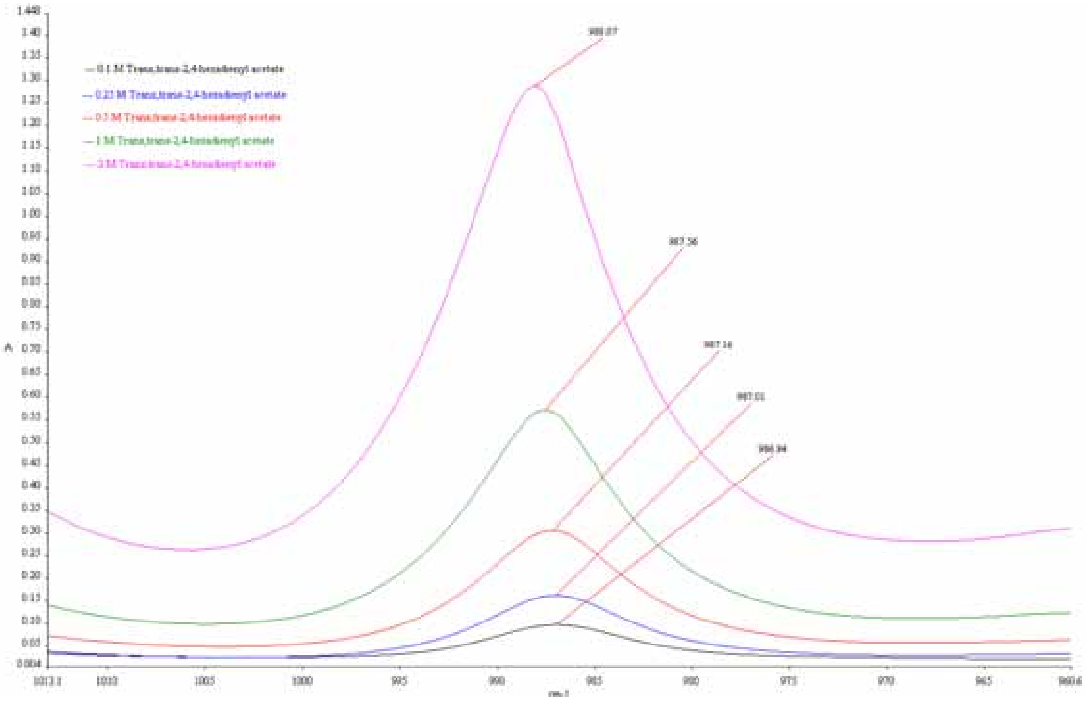

Figure 17 Spectra of *trans, trans*-2, 4-hexadienyl acetate at 987 cm^{-1} from 0.1M-2M

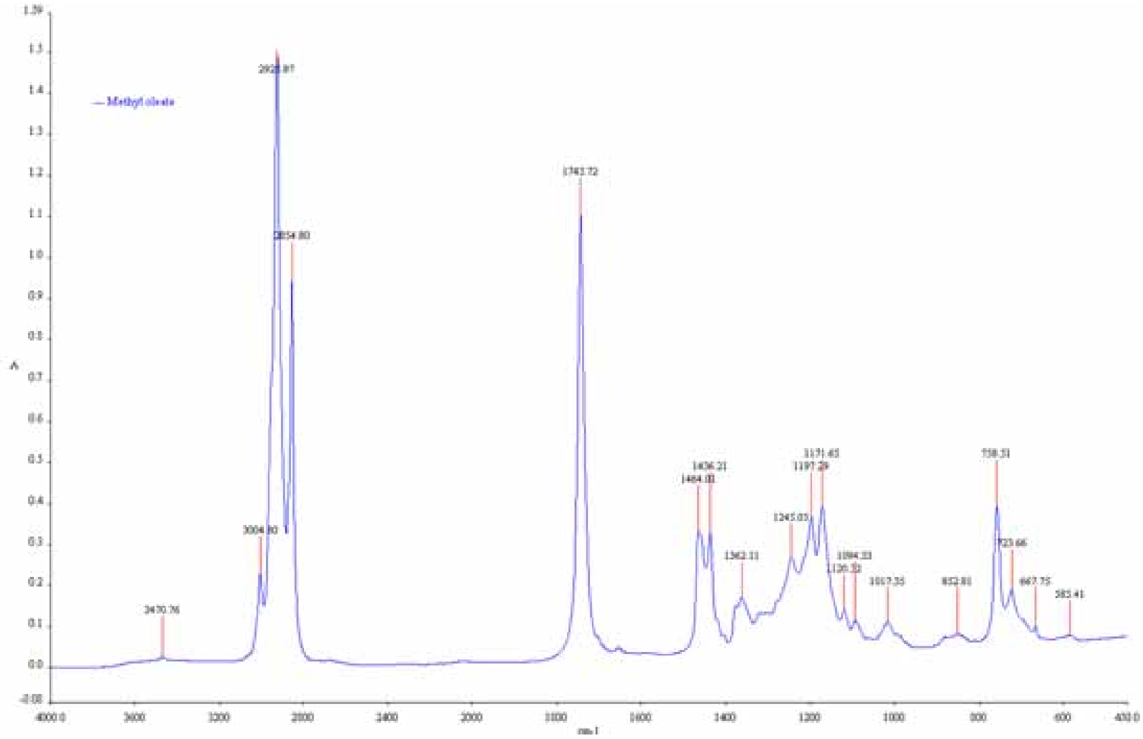

Figure 18 Whole spectra of methyl oleate from 4000-450 cm^{-1}

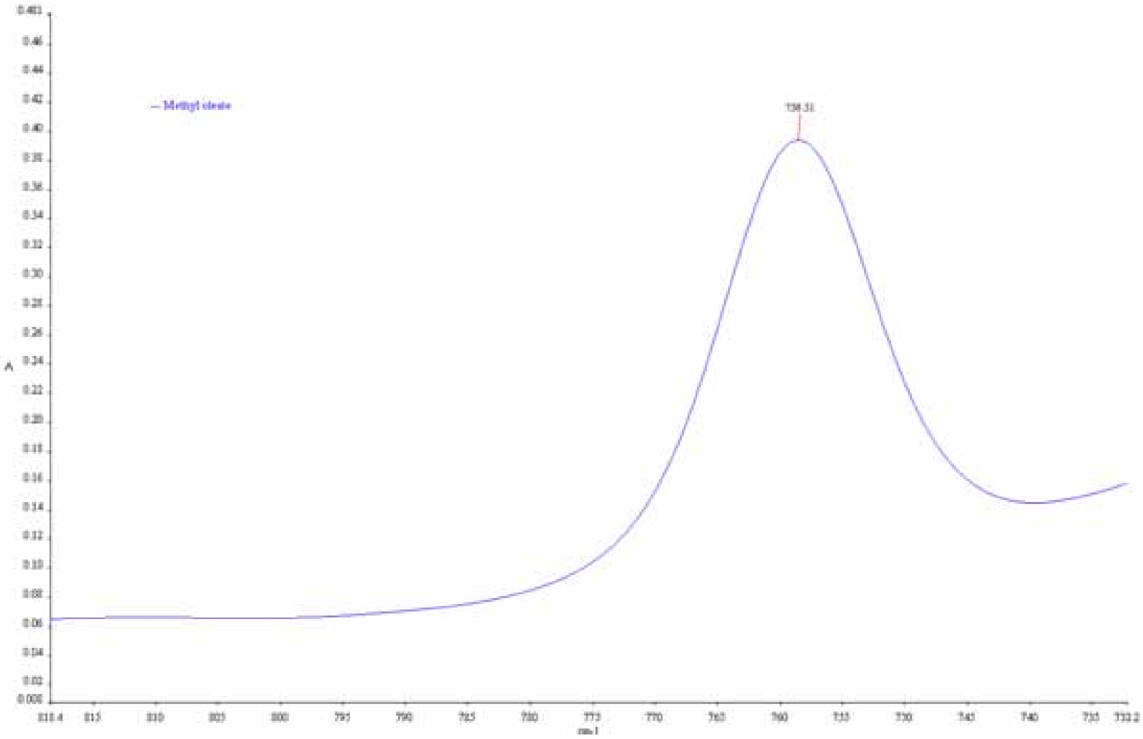

Figure 19 Spectra of methyl oleate at 758 cm^{-1}

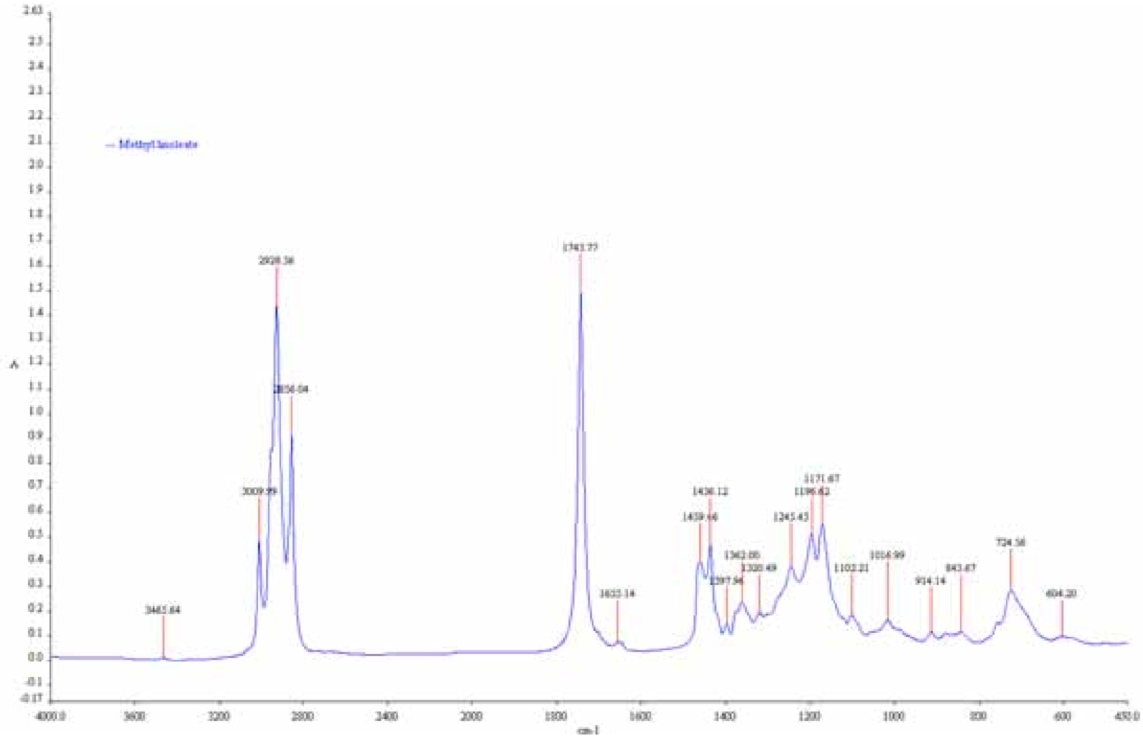

Figure 20 Whole spectra of methyl linoleate from 4000-450 cm^{-1}

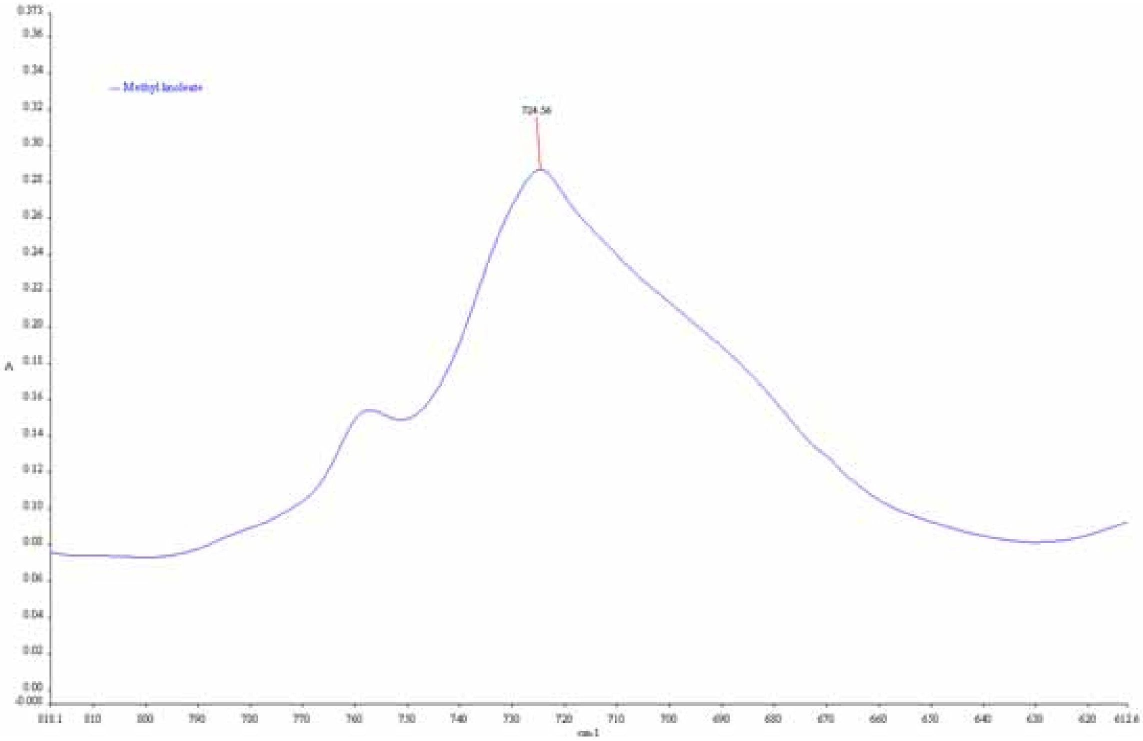

Figure 21 Spectra of methyl linoleate at 724 cm^{-1}

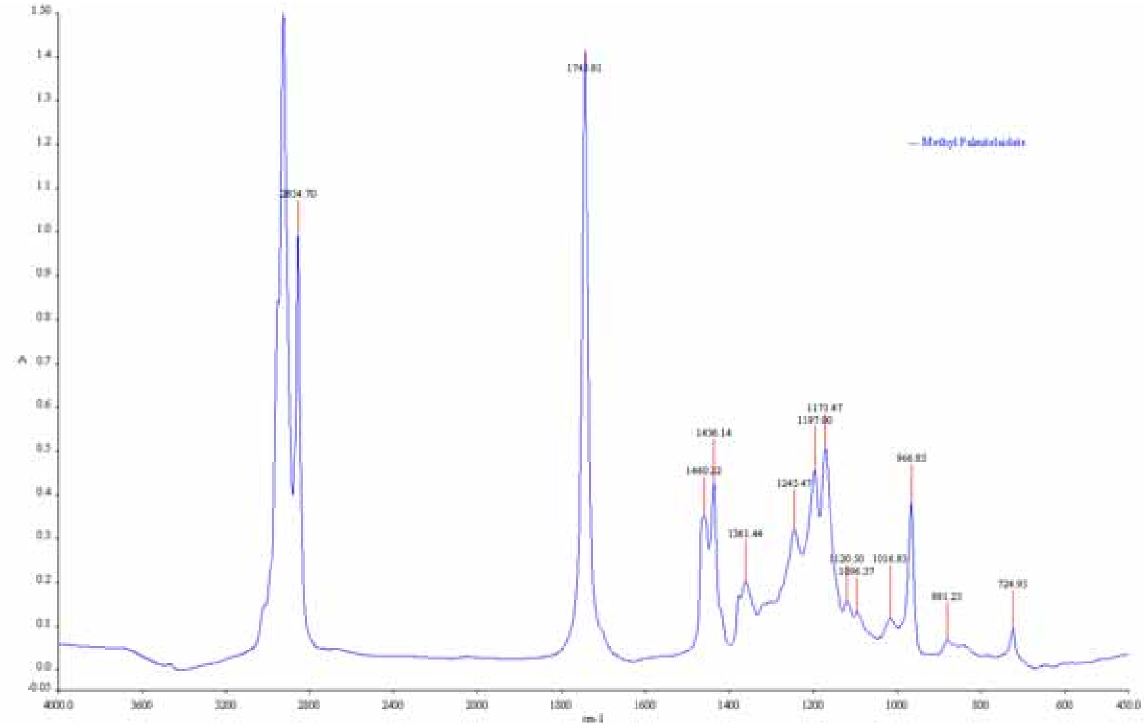

Figure 22 Whole spectra of methyl palmitelaidate from 4000-450 cm^{-1}

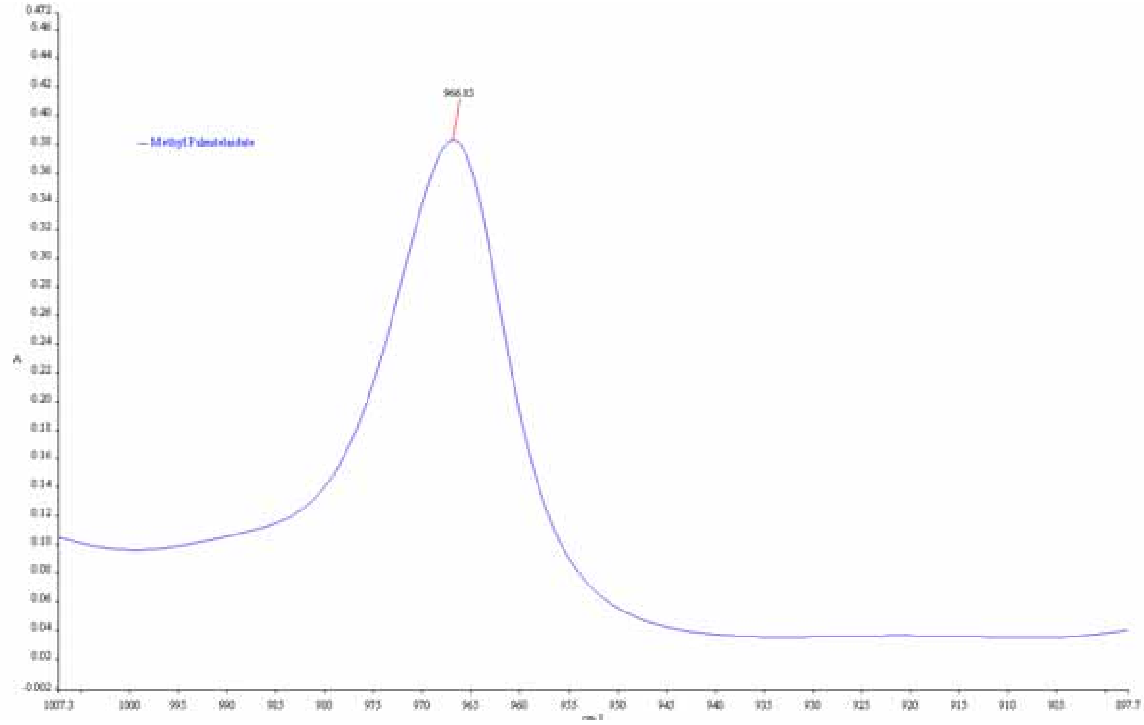

Figure 23 Spectra of methyl palmitelaidate at 966 cm^{-1}

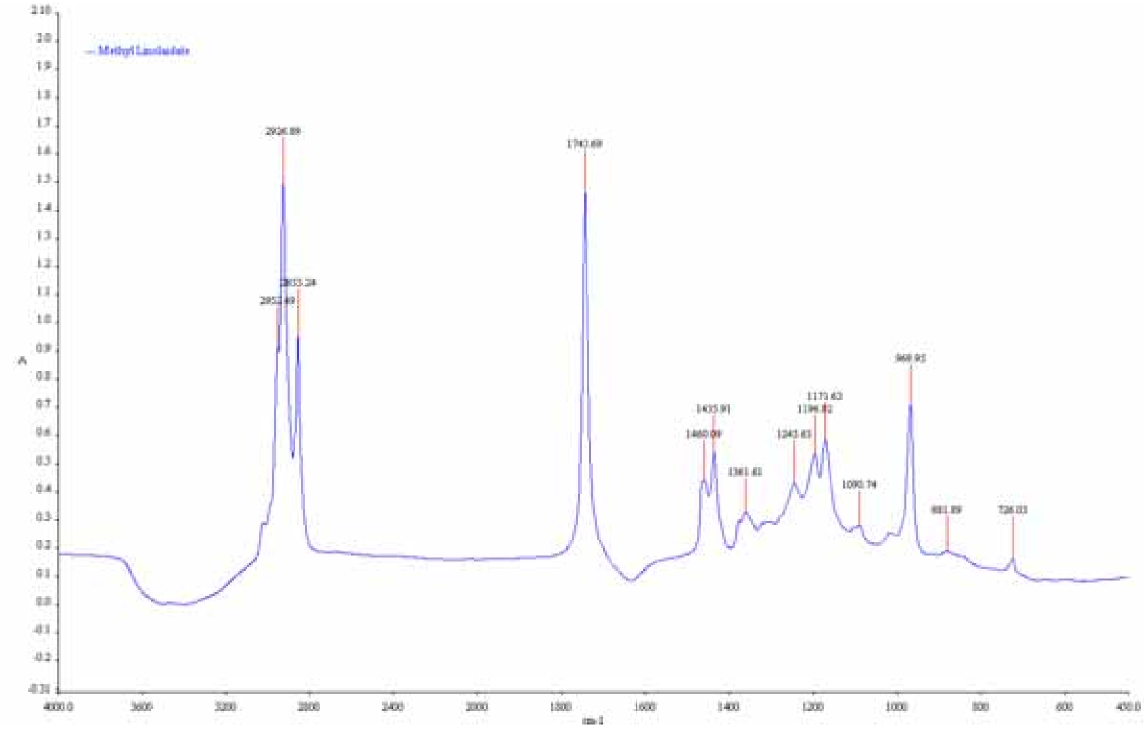

Figure 24 Whole spectra of methyl linolelaidate from 4000-450 cm^{-1}

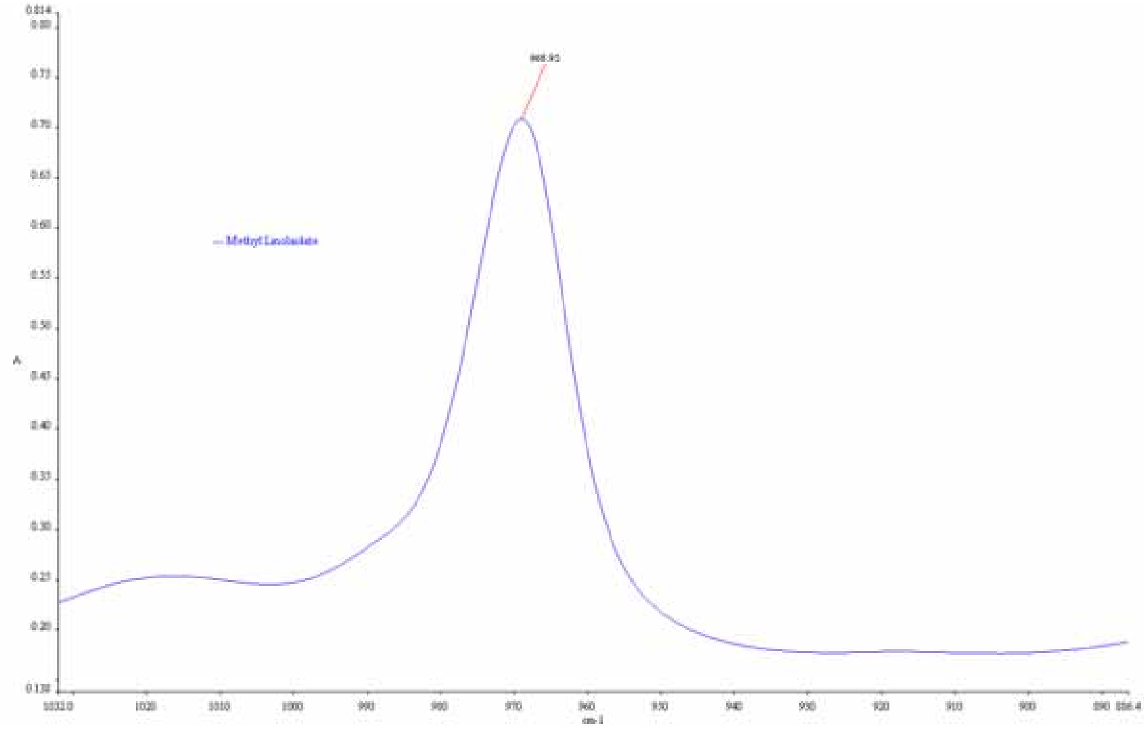

Figure 25 Spectra of methyl linolelaidate at 968 cm^{-1}

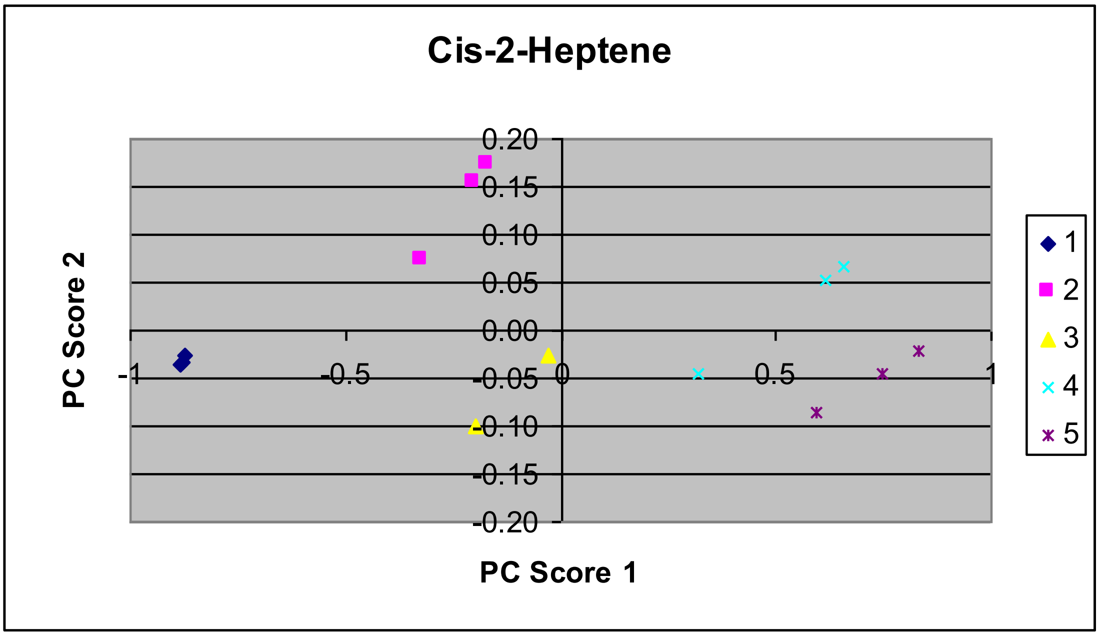

Figure 26 PCA plot between PC score 1 and PC score 2 of *cis*-2-heptene

Table 3 PC score of *cis*-2-heptene

PCScore:	1	2	3
Variance:	3.64E-01	7.27E-03	2.76E-05
%Variance:	98.03	1.96	0.01
Cumulative%Var:	98.03	99.99	100

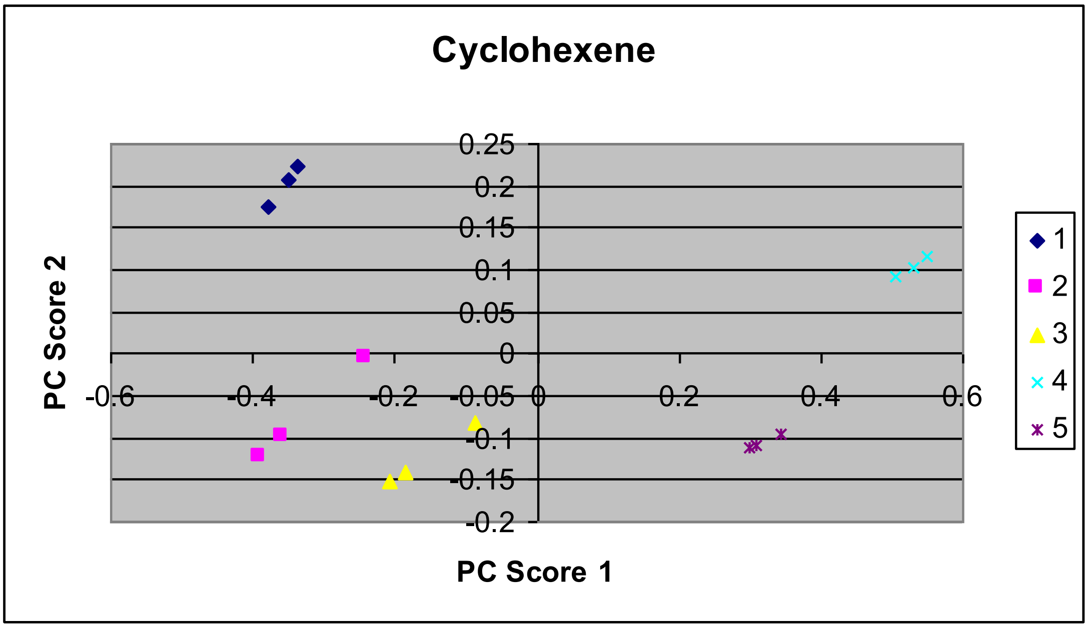

Figure 27 PCA plot between PC score 1 and PC score 2 of cyclohexene

Table 4 PC score of cyclohexene

PCScore:	1	2	3
Variance:	1.39E-01	1.89E-02	3.64E-05
%Variance:	88.04	11.94	0.02
Cumulative%Var:	88.04	99.98	100

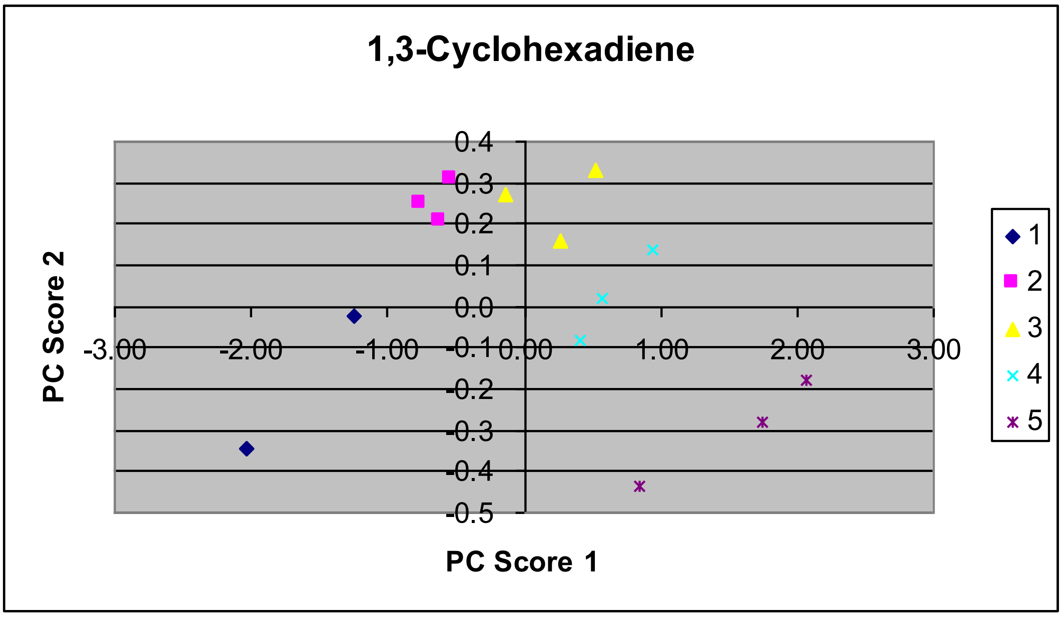

Figure 29 PCA plot between PC score 1 and PC score of 1, 3-cyclohexadiene

Table 5 PC score of 1, 3-cyclohexadiene

PCScore:	1	2	3	4
Variance:	1.49E+00	6.94E-02	1.29E-02	7.03E-03
%Variance:	94.04	4.38	1.50	0.09
Cumulative%Var:	94.04	98.41	99.91	100

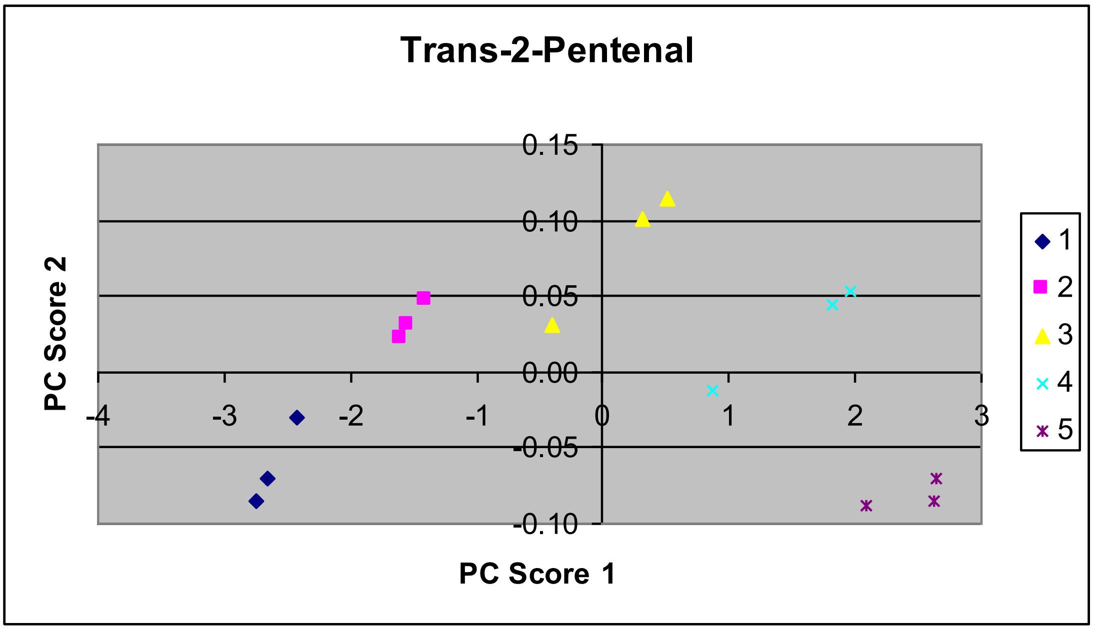

Figure 30 PCA plot between PC score 1 and PC score 2 of *trans*-2-pentenal

Table 6 PC score of *trans*-2-pentenal

PCScore:	1	2	3	4
Variance:	3.86E+00	4.69E-03	9.52E-04	1.23E-04
%Variance:	99.85	0.12	0.02	0.01
Cumulative%Var:	99.85	99.97	99.99	100

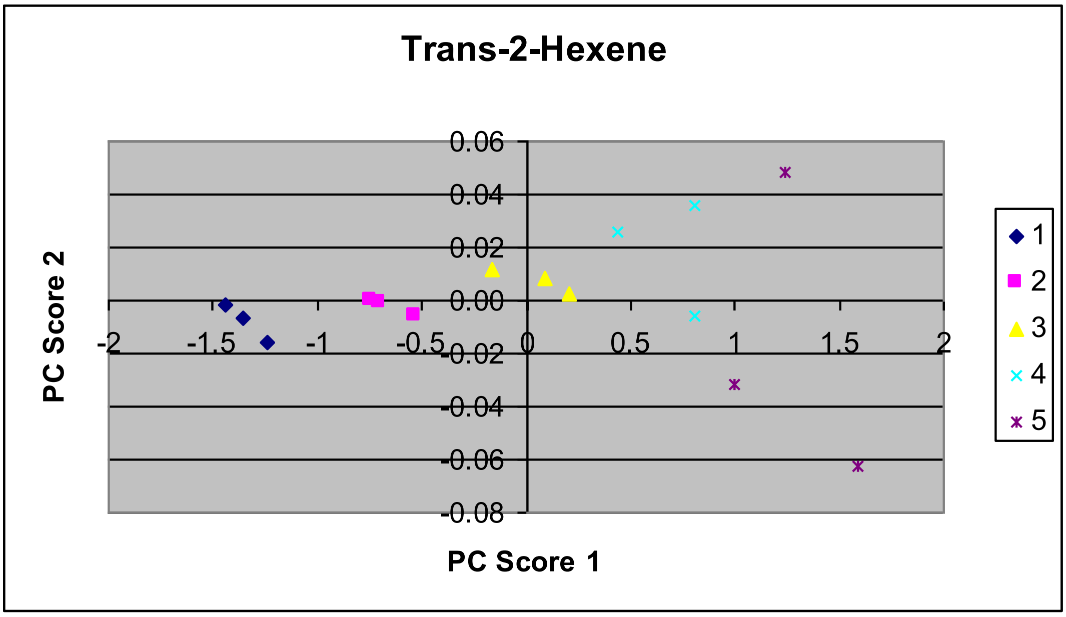

Figure 31 PCA plot between PC score 1 and PC score 2 of *trans*-2-hexene

Table 7 PC score of *trans*-2-hexene

PCScore:	1	2	3	4	5
Variance:	9.57E-01	6.93E-04	1.93E-04	1.21E-04	5.79E-05
%Variance:	99.89	0.07	0.02	0.01	0.01
Cumulative%Var:	99.89	99.96	99.98	99.99	100

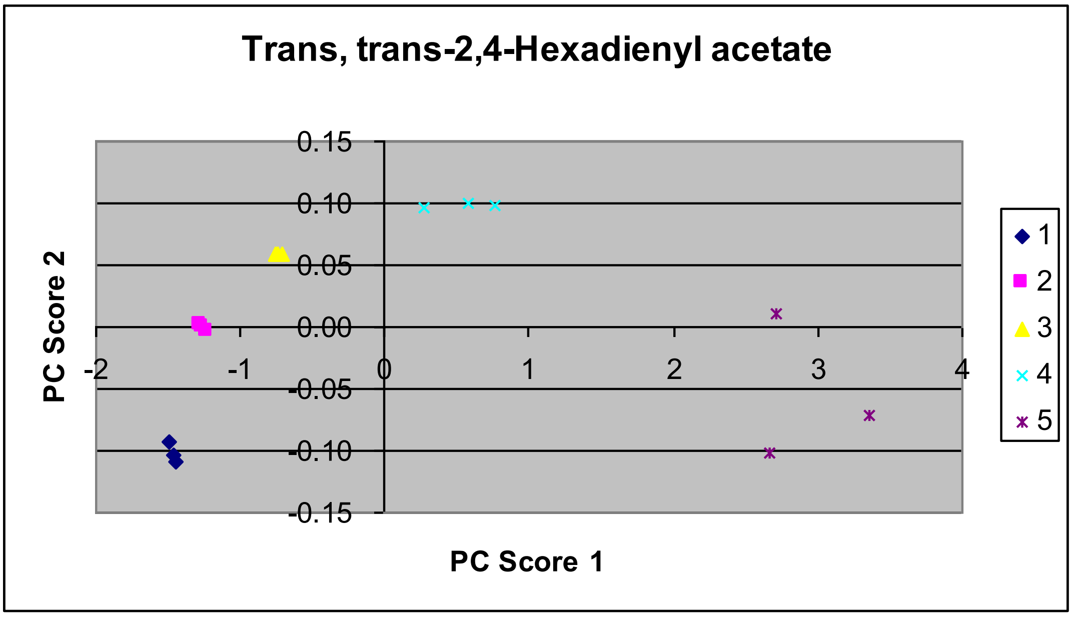

Figure 32 PCA plot between PC score 1 and PC score 2 of *trans, trans*-2, 4-hexadienyl acetate

Table 8 PC score of *trans, trans*-2, 4-hexadienyl acetate

PCScore:	1	2	3	4	5
Variance:	2.82E+00	6.19E-03	1.04E-03	4.63E-04	1.38E-04
%Variance:	99.72	0.22	0.04	0.02	0
Cumulative%Var:	99.72	99.94	99.98	99.99	100

<u>*Cis*-2-heptene</u>

Calibration Line:

Slope: 12.9114
Intercept: -0.0964
Correlation: 0.9980
Standard error: 0.1140
StdErr of Prediction: 0.1805

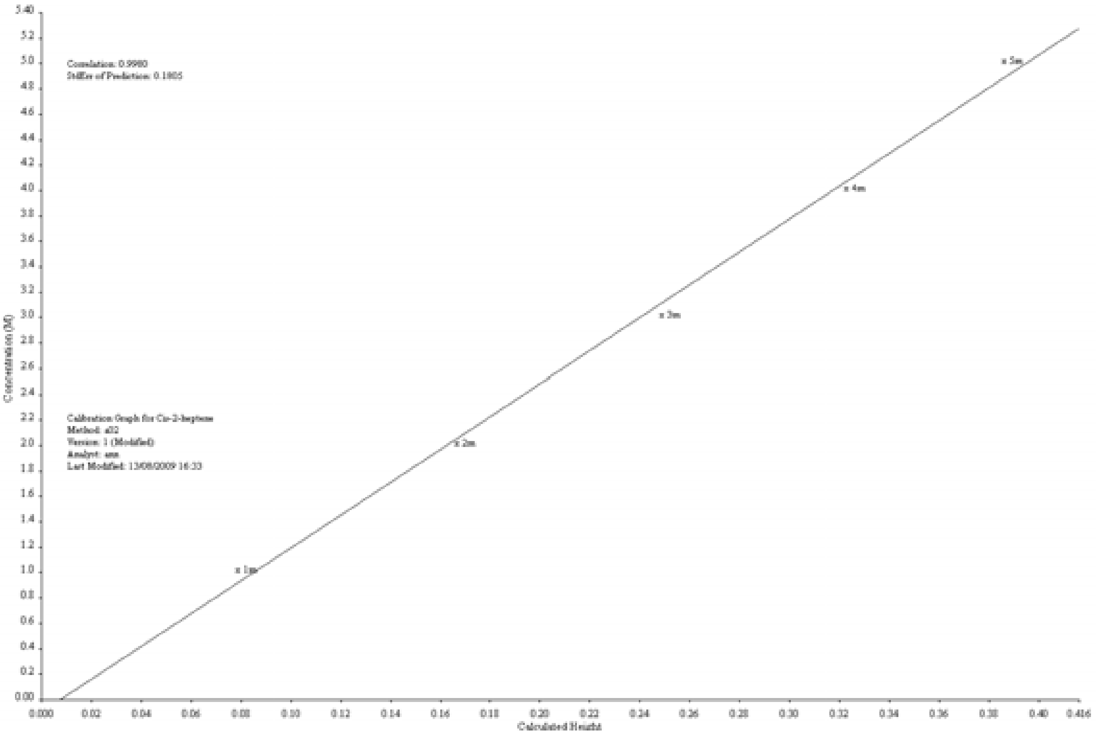

Figure 33 Calibration graph of *cis*-2-heptene

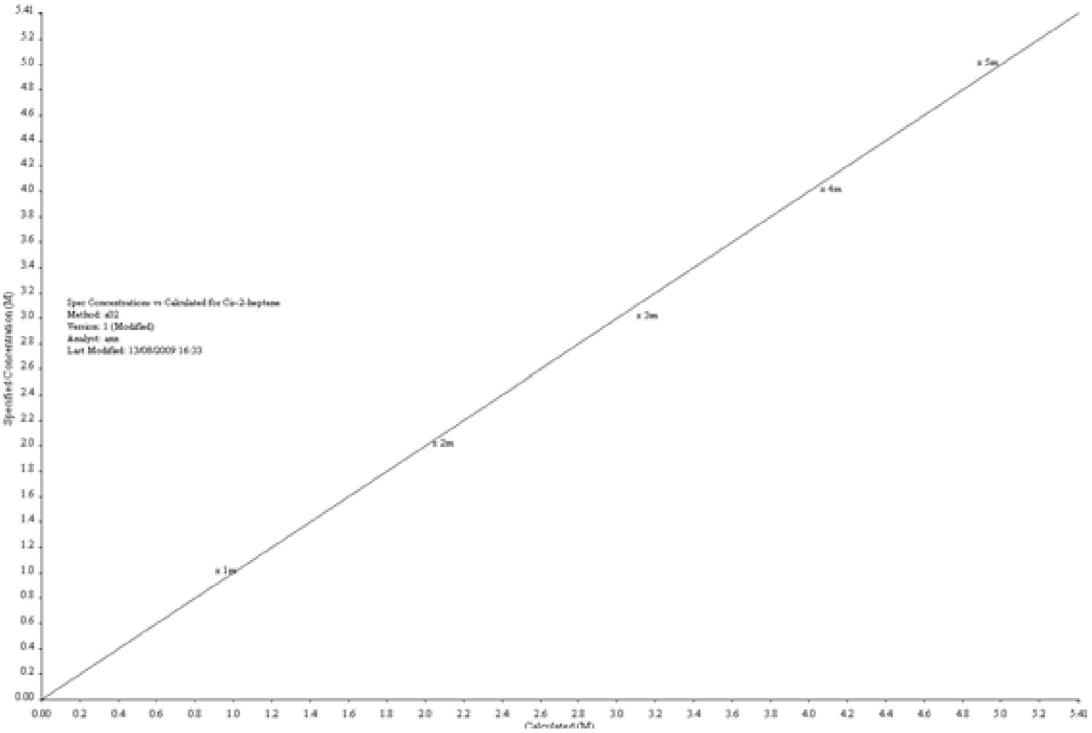

Figure 34 Spec concentration vs. calculated of *cis*-2-heptene

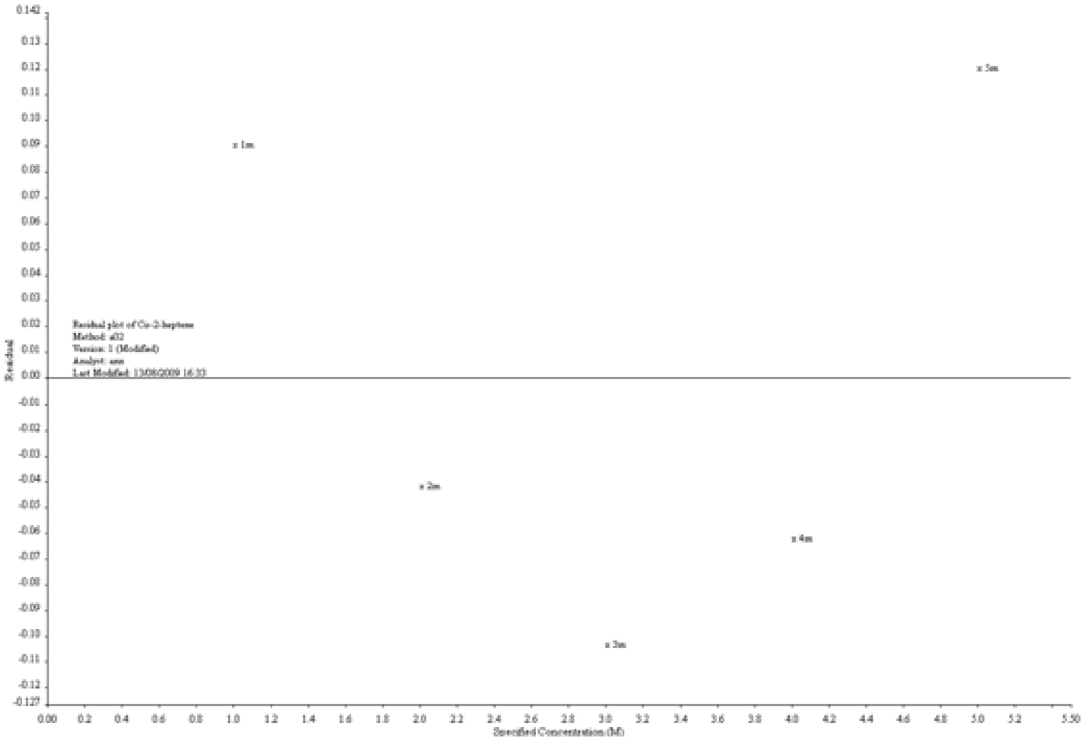

Figure 35 Residual plot of *cis*-2-heptene

Cyclohexene

Calibration Line:

Slope: 10.9837
Intercept: -0.5395
Correlation: 0.8946
Standard error: 0.8159
StdErr of Prediction: 1.2957

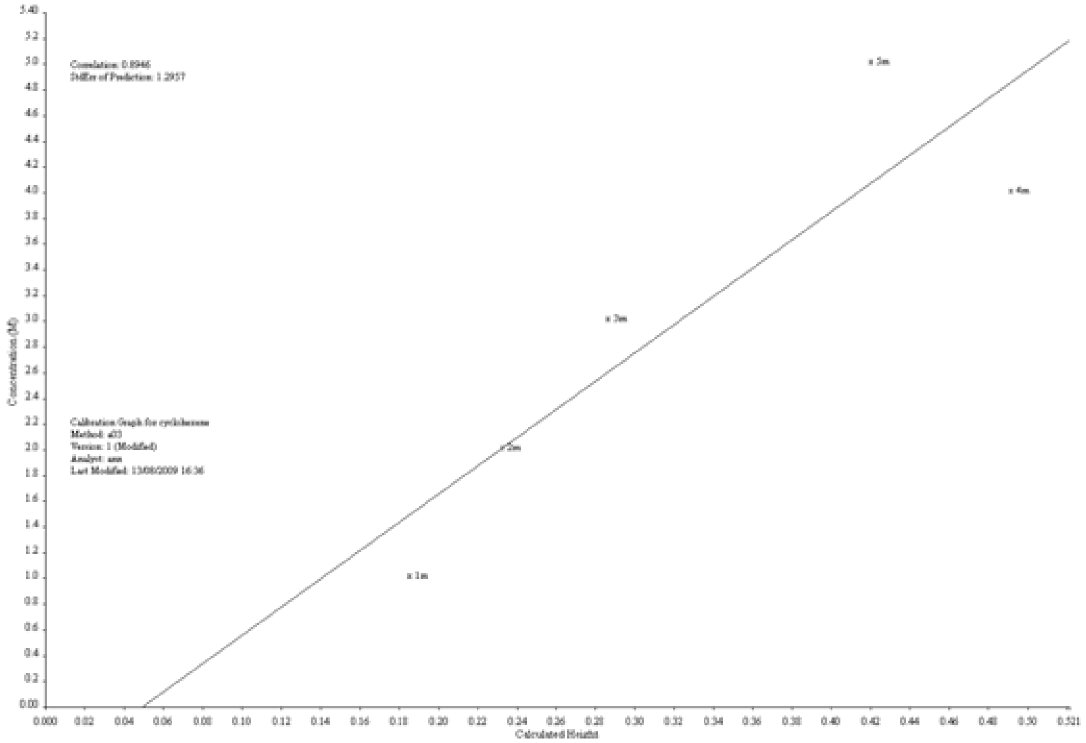

Figure 36 Calibration graph of cyclohexene

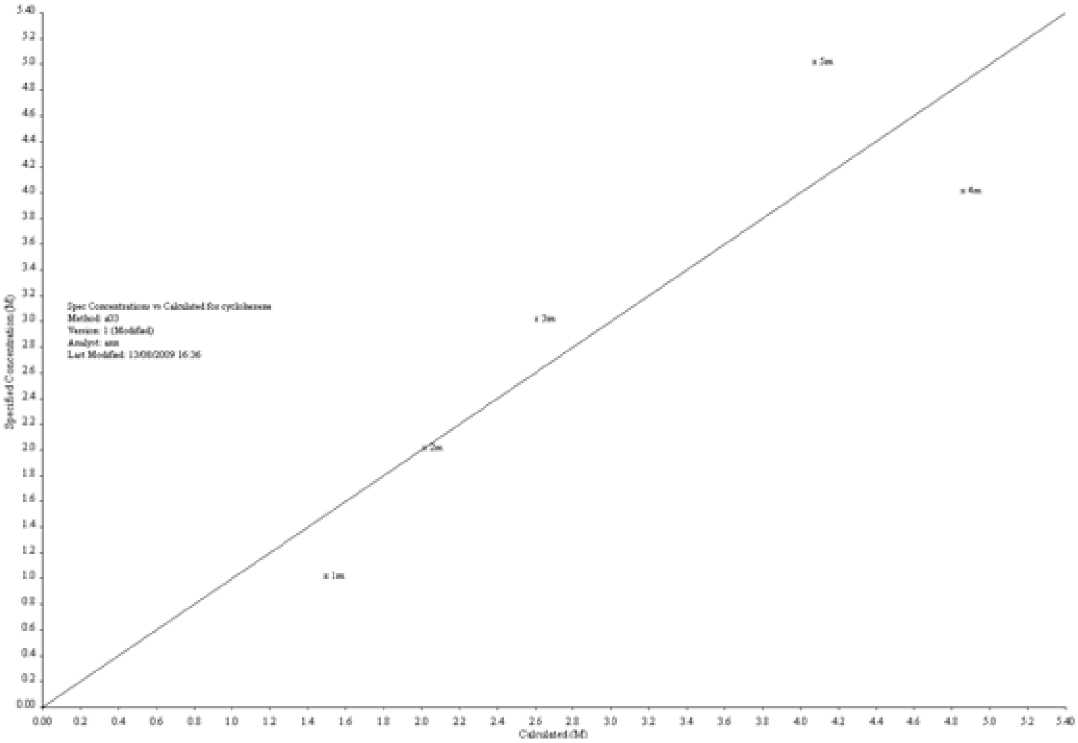

Figure 37 Spec concentration vs. calculated of cyclohexene

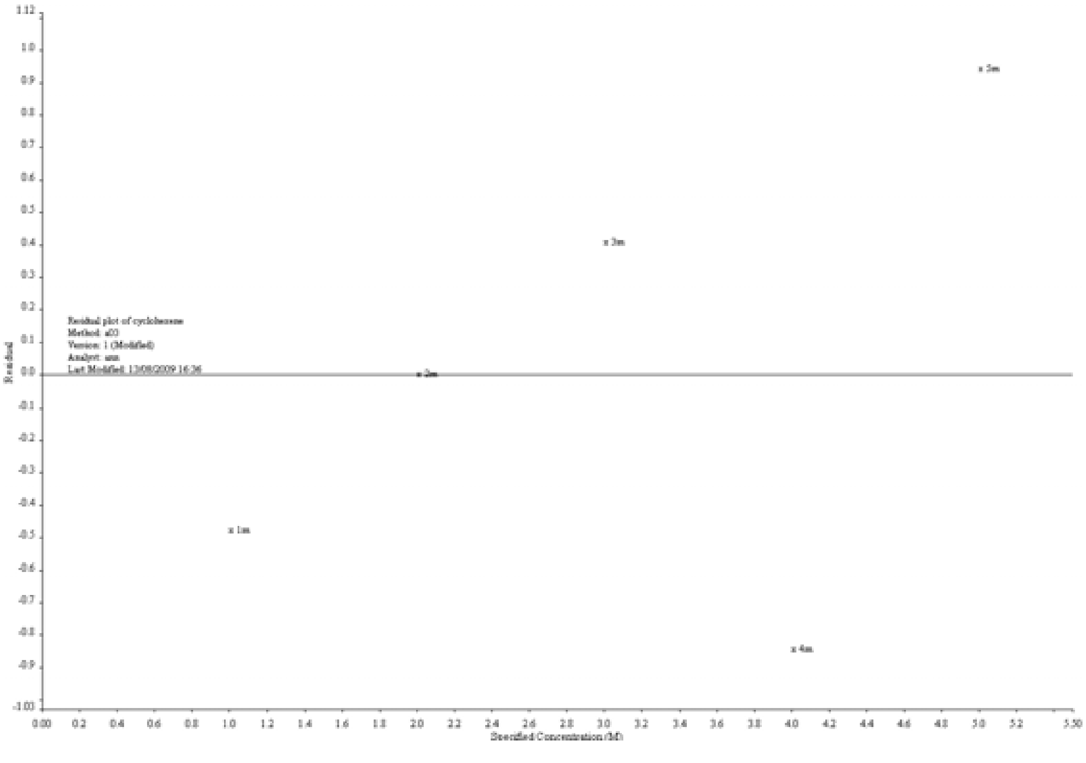

Figure 38 Residual plot of cyclohexene

<u>1,3-cyclohexadiene</u>

Calibration Line:
Slope: 11.6434
Intercept: -0.0972
Correlation: 0.9993
Standard error: 0.0681
StdErr of Prediction: 0.0973

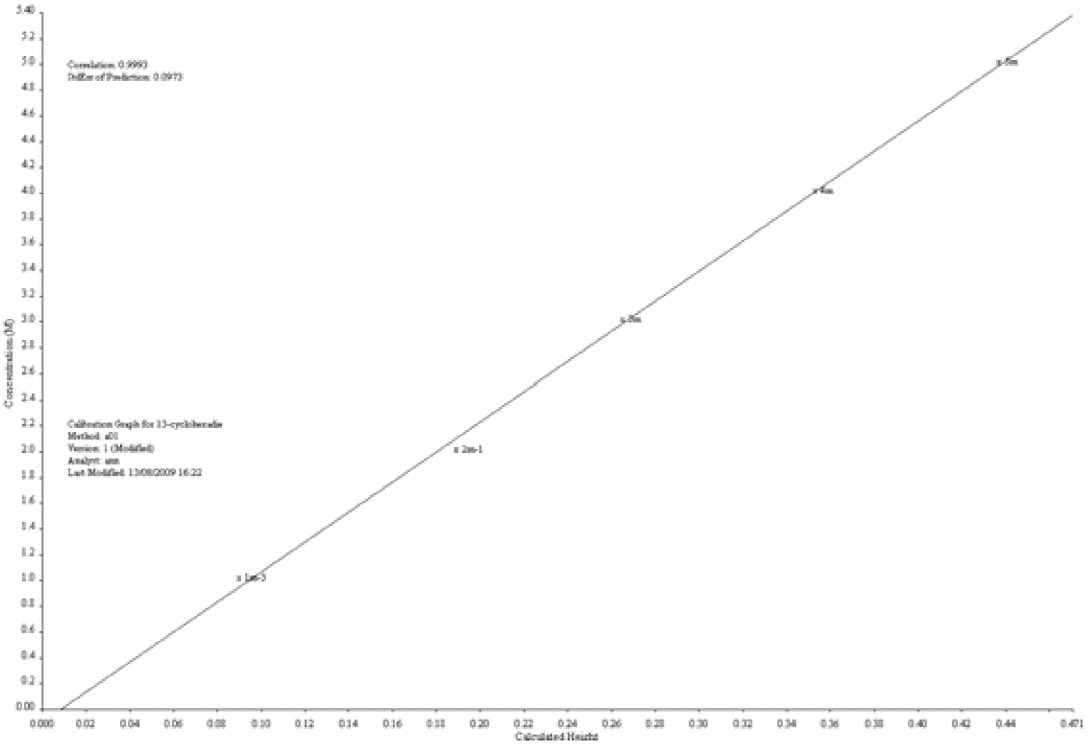

Figure 39 Calibration graph of 1, 3-cyclohexadiene

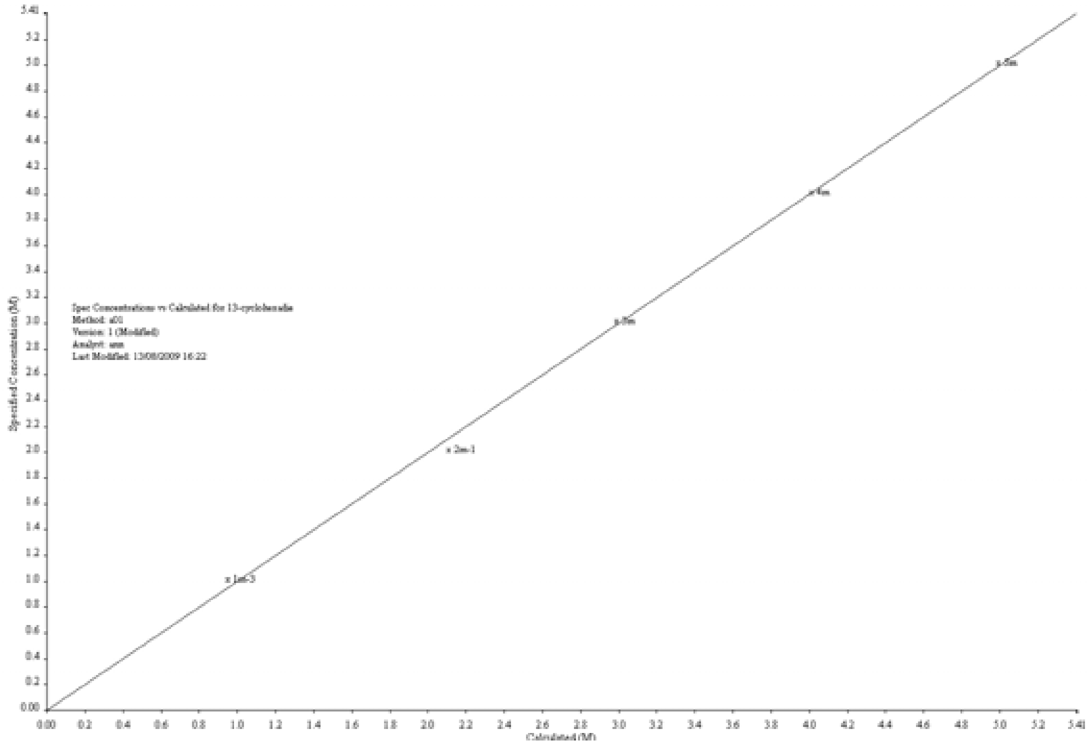

Figure 40 Spec concentration vs. calculated of 1, 3-cyclohexadiene

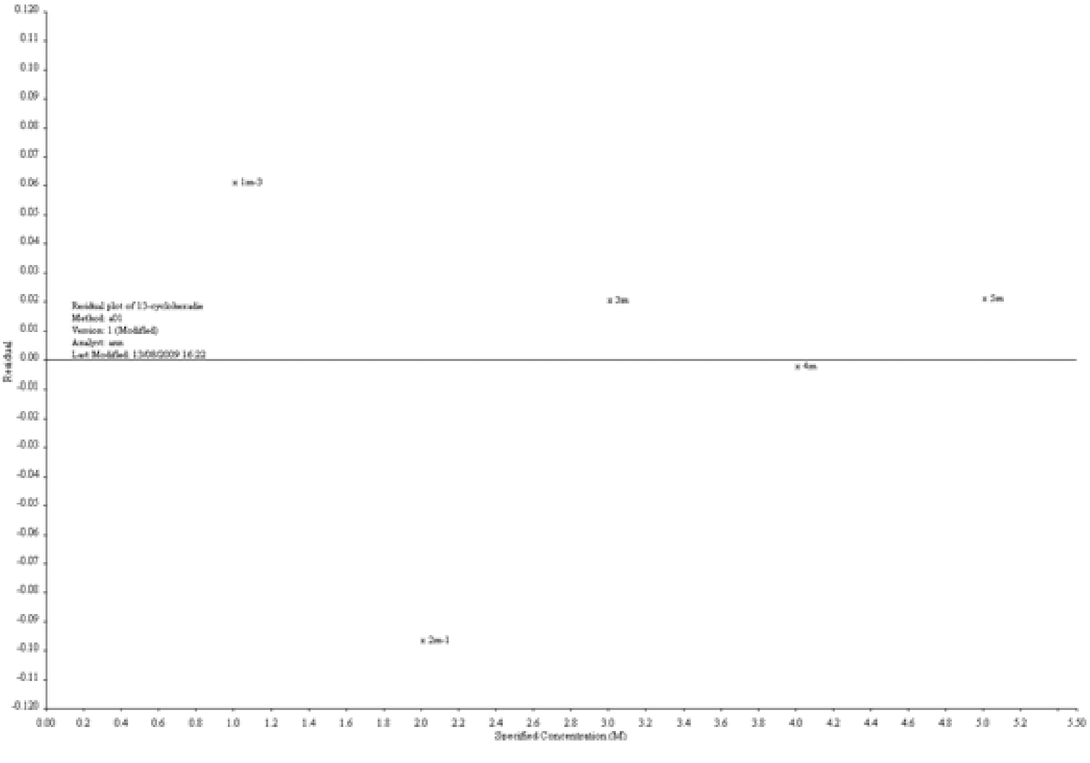

Figure 41 Residual plot of 1, 3-cyclohexadiene

<u>*Trans*-2-pentenal</u>

Calibration Line:
Slope: 5.9829
Intercept: -0.1569
Correlation: 0.9990
Standard error: 0.0814
StdErr of Prediction: 0.1269

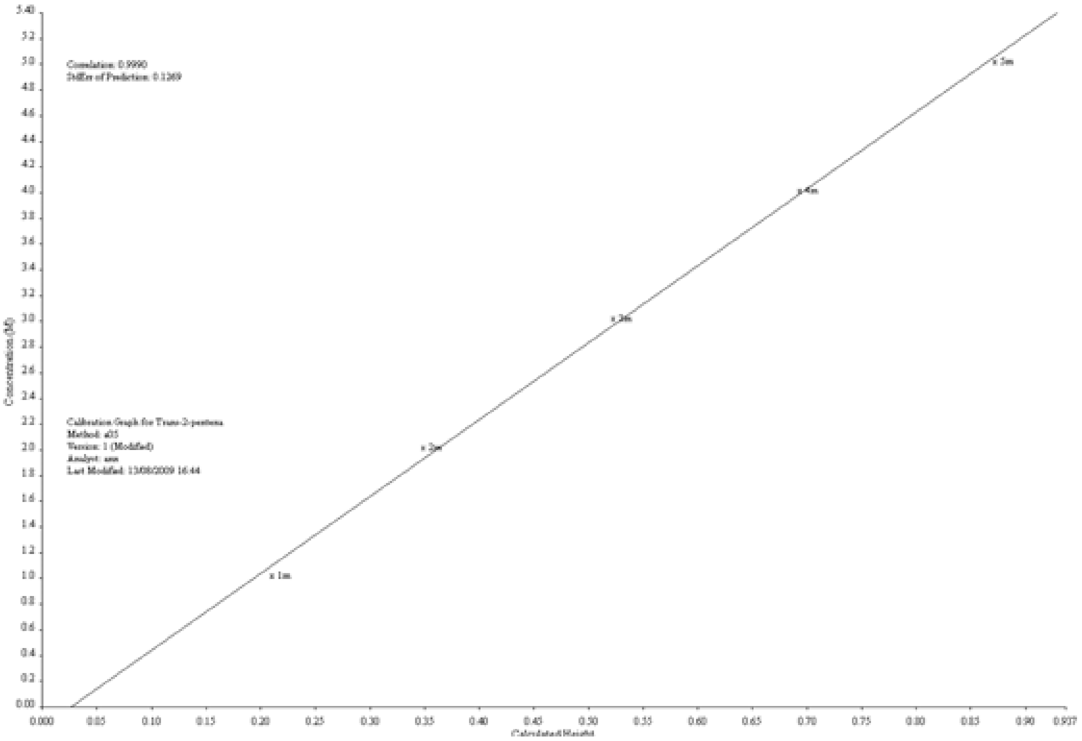

Figure 42 Calibration graph of *trans*-2-pentenal

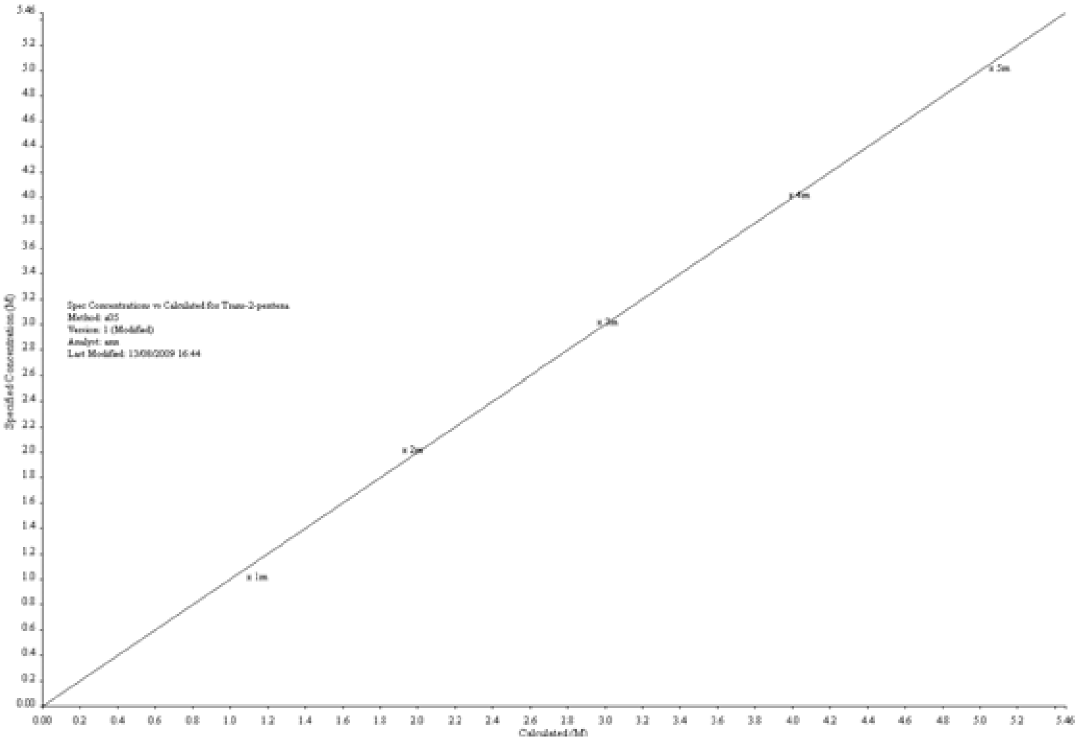

Figure 43 Spec concentration vs. calculated of *trans*-2-pentenal

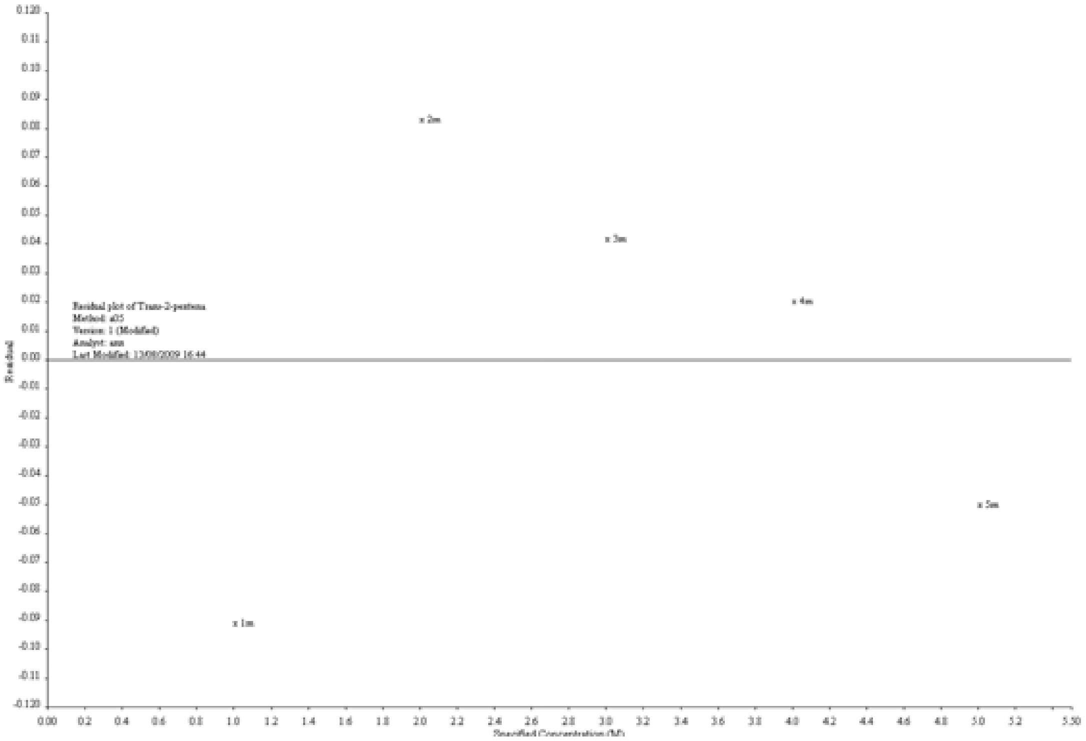

Figure 44 Residual plot of *trans*-2-pentenal

<u>*Trans*-2-Hexene</u>

Calibration line
Slope: 4.3456
Intercept: -0.2013
Correlation: 0.9962
Standard error: 0.1588
StdErr of Prediction: 0.2161

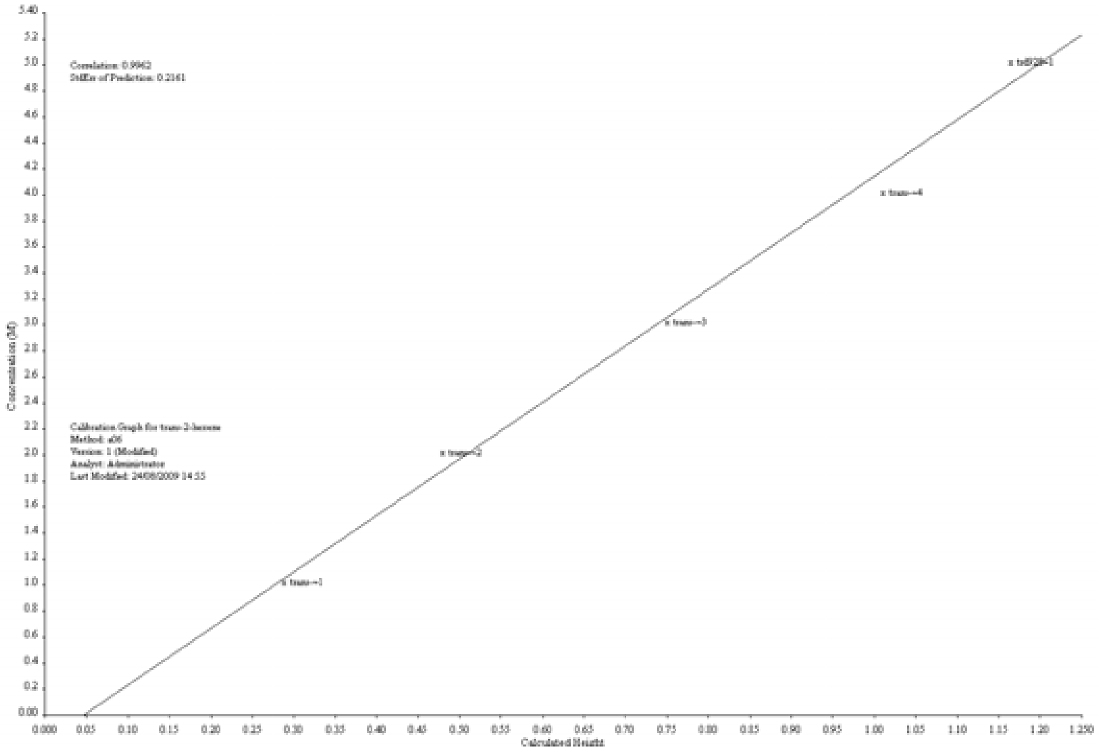

Figure 45 Calibration graph of *trans*-2-hexene

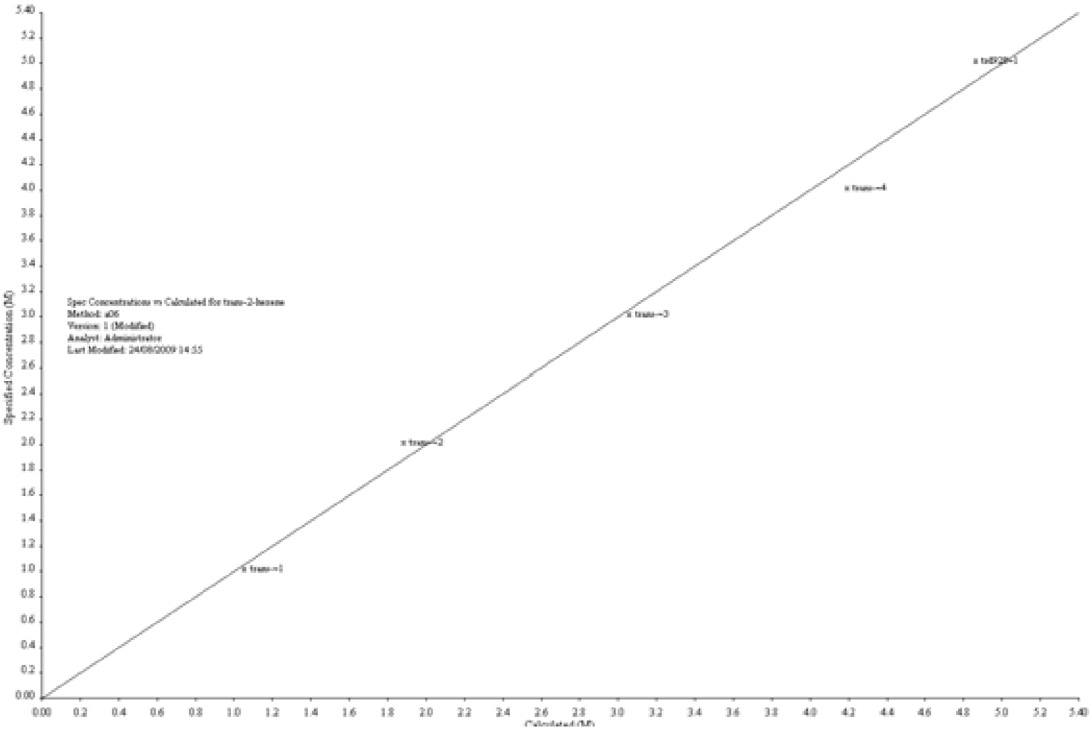

Figure 46 Spec concentration vs. calculated of *trans*-2-hexene

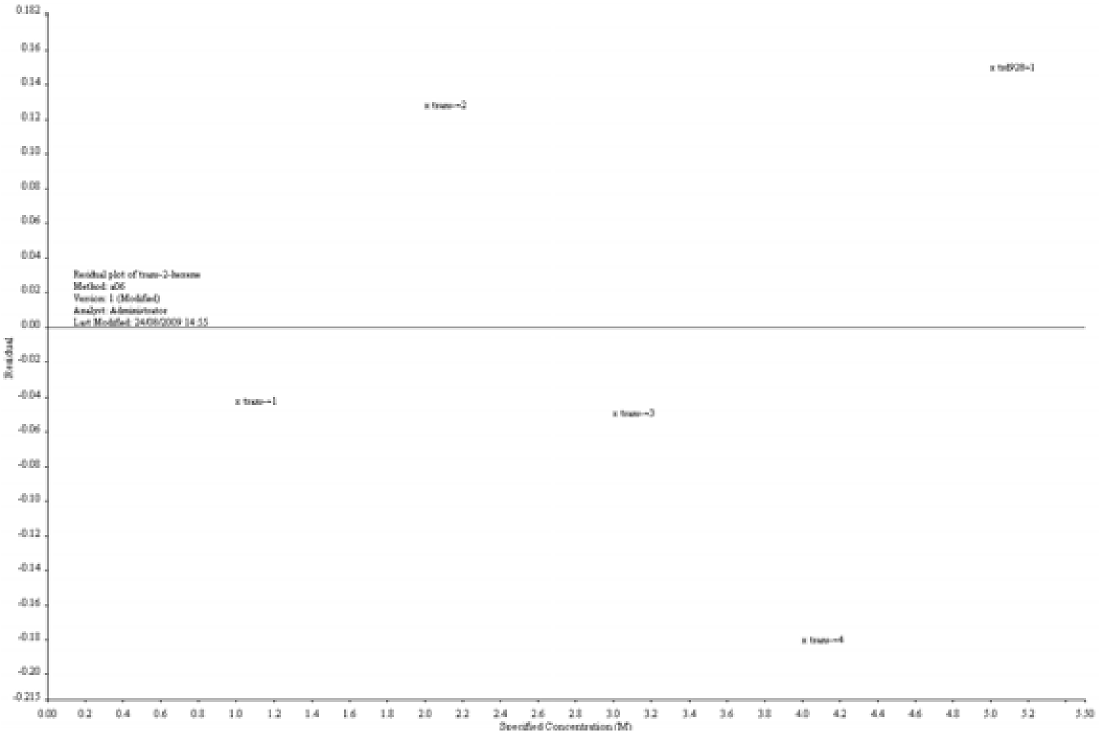

Figure 47 Residual plot of *trans*-2-hexene

<u>*Trans, trans*-2, 4-hexadienyl acetate</u>

Calibration Line:
Slope: 2.1177
Intercept: -0.0458
Correlation: 0.9995
Standard error: 0.0280
StdErr of Prediction: 0.0595

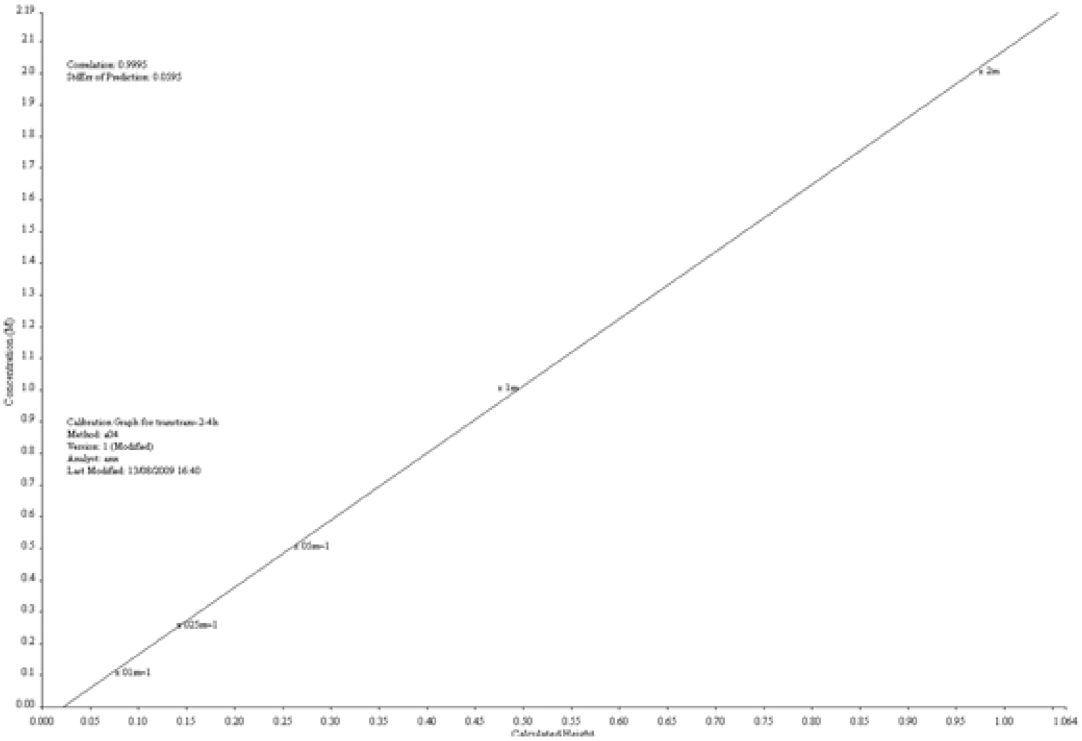

Figure 48 Calibration graph of *trans, trans*-2, 4-hexadienyl acetate

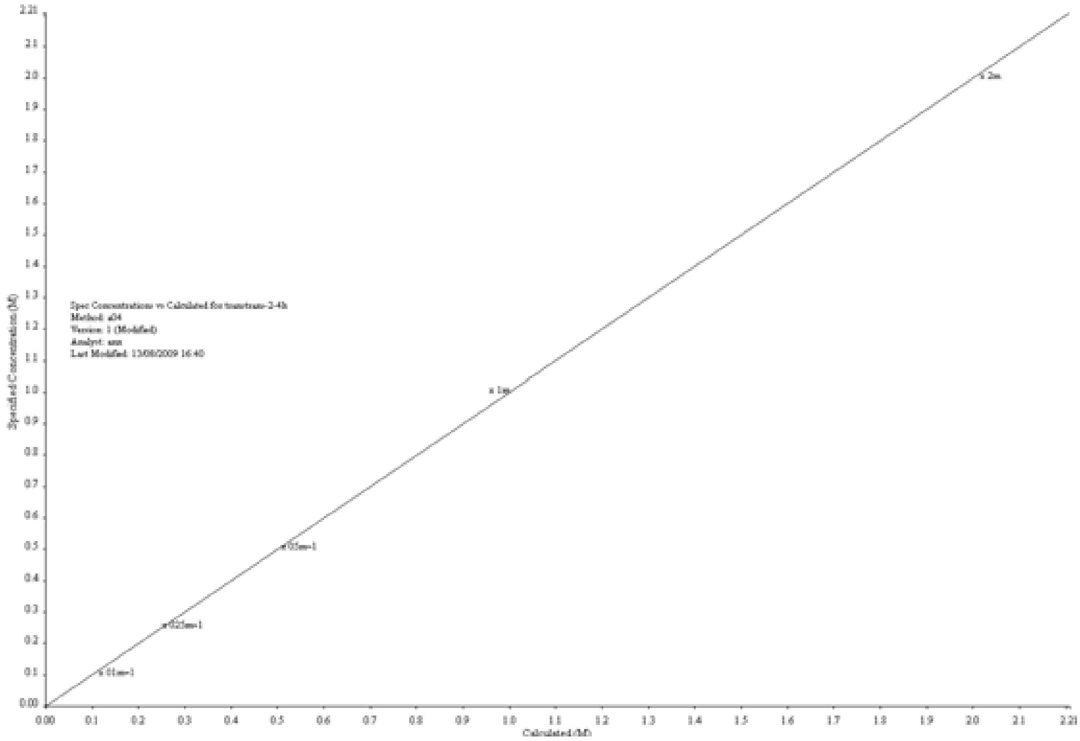

Figure 49 Spec concentration vs. calculated of *trans, trans*-2, 4-hexadienyl acetate

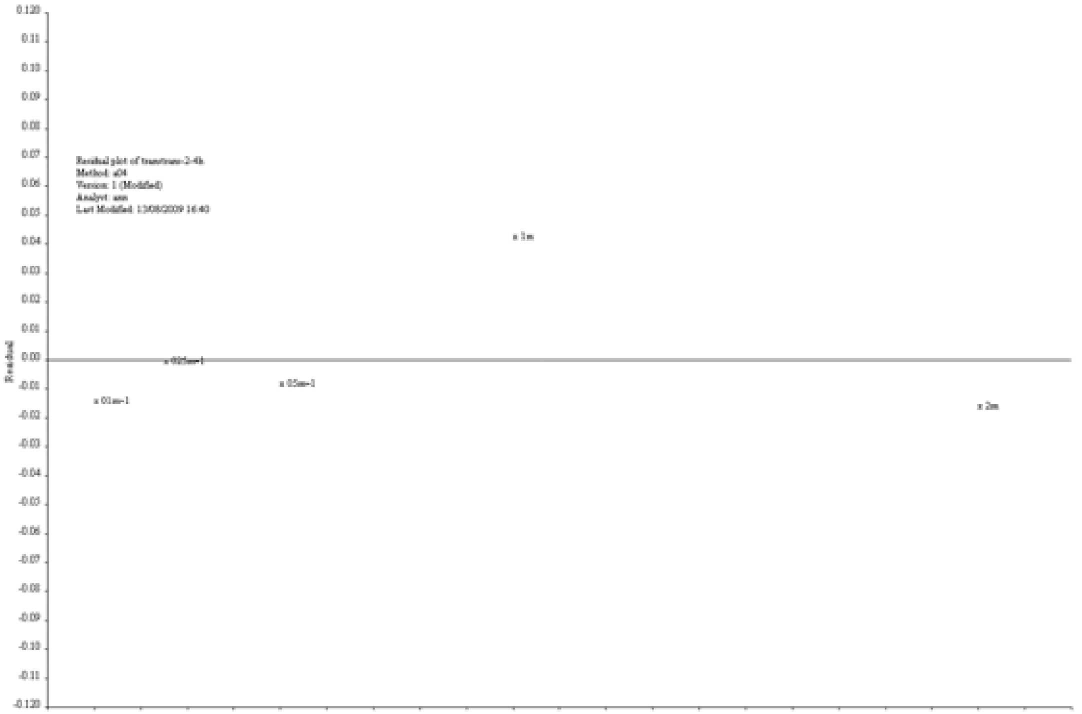

Figure 50 Residual plot of *trans, trans*-2, 4-hexadienyl acetate

CHAPTER 4

QUALITATIVE AND QUANTITATIVE DETERMINATION OF *TRANS* FATTY ACID IN MARGARINE USING FOURIER TRANSFORMED INFRARED SPECTROSCOPY (FTIR) COMPARING WITH GAS CHROMATOGRAPHY (GCMS)

Abstract

This research was mainly examined to the feasibility and compatibility of two different instruments (FTIR and GCMS) for determining both quality and quantity of *trans* fatty acids compound of margarine products. It could be concluded that two selected equipments were capable to determine both quality and quantity of *trans* fatty acid. However, GCMS could gave better result, but it took more time. Therefore, it was not suitable to use GCMS in the industry, which had time limitation and did not need the deep detail in analyzing. Thus, the use of FTIR would be alternative method for analyzing *trans* fatty acid in margarine products.

Keywords: fourier transformed infrared spectroscopy, gas chromatography, *trans* fatty acid, margarine

Introduction

Margarine is a flavored food product containing not less than 80% fat (Rader et al., 1996). That change the *cis* configuration to *trans* configuration by hydrogenation process. This processed made it possible for vegetables oil to be converted into plastic fat forms. Hydrogenated edible fats and oils product can be prepared with creaming properties, frying stability, sharp melting properties, and the others functional characteristic desired for specific application (Richard and O'Brien, 2004).

Concern has existed that the hydrogenation process may have adverse consequences because natural essential fatty acids are destroyed and the new artificial isomers are structurally similar to saturated fats. These isomers lack the essential metabolic activity of the parent compound, and inhibit the enzymatic desaturation of essential fatty acids. Metabolic studies have provided unequivocal evidence that *trans* fatty acids increase plasma concentrations of low density lipoprotein (LDL) cholesterol and reduce concentrations of high density lipoprotein (HDL) cholesterol relative to the parent natural fat (Booker and Mann, 2008). Some suggests that *trans* fat intake amount correlates with circulatory diseases such as atherosclerosis and coronary heart disease more than the same amount of *cis* fat intake at the same level. Other harmful risk to human health relating to *trans* fat intake such as increasing triglycerides and lipoprotein level, causing inflammation, and damaging cell of blood vessel (Lopez-Garcia et al., 2005).

From the past research about *trans* fatty acid, most researchers used GC to analyzed the data. However, there is another interesting method which can reduce cost and time. This method is to use FTIR, this method has been used to determined peroxide value, free fatty acid of frying oil content (Innawong et al., 2004). FTIR is used to analyzed qualitative and quantitative of food composition at the different level of infrared absorption. This depends on the vibrations of each type of bonds, where each type of bond has different vibration range. At the temperature above 0°c, every molecules would vibrate. When the frequency of vibration becomes equal to that of infrared radiation, the molecules would absorb the radiation. For *trans* fatty acid should be observed in 1000-950 cm^{-1} and for *cis* fatty acid should be observed at 780-680 cm^{-1} (Cross, 1960).

The GC method is the technique that separate the vaporized mix compound by mobile phase; use the inert gas that not react with mix compound such as Helium (He). The mobile phase is a carrier gas and the stationary phase is a column; that coated inside with microscopic layer such as silica gel. When the carrier gas and mix compound moving through the stationary phase, the mix compound would be separated depends on each compound polarity. So, each compound would be elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.

The GC method can be qualitative and quantitative determination of *trans* fatty acid in food. But the accuracy and reliability of method depends on the resolution and identification of *trans* fatty acid (Azizian, 2004).

Materials and Methods

Preparation of margarine

Margarine were purchased locally. It was prepared by soxhlet extraction with hexanes after that, evaporated the solvent by rotary evaporator (RII, Buchi) and then diluted with hexanes before use and finally analyzed using the same condition as the reference chemical.

Preparation of fatty acid methyl ester

The lipid extracts were then esterified according to the sodium methoxide method. Fat were approximately weighed 100 mg, transferred to a test tube, added 5 ml. of hexanes and vortex briefly to dissolve lipid, and add 250 µl of 0.5 M sodium methoxide reagent and vortex for 1 minutes, follow by 5 ml of saturated NaCl solution and shaked vigorously for 15 s. Let sit for 10 minutes. After that the fat would remove the hexane layer and transfer to a test tube containing a small amount of Na_2SO_4. Allow the hexane phase containing the methyl ester to sit in contact with Na_2SO_4 for at least 15 min prior to analysis.

FTIR Spectrometer analysis

The FTIR spectrometer (spectrum100, Perkin Elmer) in transmission mode with a deuterated triglycine sulfate detector was used for this experiment and liquid KBr cell was utilized for measurements. The spectra were collected from 4000-450 cm^{-1} by using 16 scans/sample at 4 cm^{-1} resolution and fix pathlength at 0.1 mm.

GCMS analysis

Analysis of fatty acid methyl ester was carried out on a agilent 7890A GC system and 5975C MSD (GCMS) with an automatic injector and a HP-5 capillary column (30 m. × 250 μm. × 0.25 μm). The injection volume was 1 μl with a split ratio of 10: 1. The injector temperature was 250°c. The carrier gas was high purity helium with a column flow rate 1 ml/min. The column temperature was programmed from 55°c for 2 min then 10°c/min to 265°c for 6 min.

Results and Discussion

In this study, 3 different commercial margarines were scanned by FTIR (table 9.) and analyzed by GCMS (table 10.)

Table 9 Analytical result of margarine scanned by FTIR

Sample	Wavenumber (cm^{-1})	Compound	Structure
Margarine 1	758	Methyl oleate	*Cis*
	724	Methyl linoleate	Nonconjugated *cis, cis*
Margarine 2	758	Methyl oleate	*Cis*
	724	Methyl linoleate	Nonconjugated *cis, cis*
Margarine 3	758	Methyl oleate	*Cis*
	724	Methyl linoleate	Nonconjugated *cis, cis*

In this study, only unsaturated fatty acid group was concentrated. The results of scanning 3 different commercial margarines by FTIR shown the same results of 2 different fatty acid compounds, because the raw materials of margarine production were the same, but the quantity of each compound were different, However, it depended on the process and the amount of each ingredient that was used. FTIR was usually detected functional group of compounds, so it shown the same result. Quantitative analysis could determined by fitting the data that scanned by FTIR with the calibration were obtained from reference compounds, which shown the quantitative result of each compound.

Table 10 Analytical result of margarine analyzed by GCMS

Sample	**Compound**	**Structure**
Margarine 1	Methyl linoleate	Nonconjugated *cis, cis*
	Methyl oleate, (*E*)	*Trans*
	Methyl Eicosanoate	*Cis*
	Methyl palmitoleate (*Z*)	*Cis*
Margarine 2	Methyl oleate	*Cis*
	Methyl linoleate	Nonconjugated *cis, cis*
	Methyl oleate, (*E*)	*Trans*
	Methyl Eicosanoate	*Cis*
	Methyl palmitoleate (*Z*)	*Cis*
Margarine 3	Methyl oleate	*Cis*
	Methyl linoleate	Nonconjugated *cis, cis*
	Methyl oleate, (*E*)	*Trans*
	Methyl Eicosanoate	*Cis*
	Methyl palmitoleate (*Z*)	*Cis*

From the result of margarine determination that compared between FTIR technique and GCMS technique shown that GCMS had given better result because of better sensitivity of equipment. It could be guaranteed from the result of 0.1 M cyclohexene that analyzed with both equipments. The result shown GCMS could detect cyclohexene at 0.1 M at retention time 2.235 (figure 58.) but FTIR could not determine at this concentration (figure 54.). Therefore, FTIR could determine at least 1 M of cyclohexene. So, it could be concluded that GCMS was sensitive and accurate for the analysis that the cause of different result.

On the others hand, analyzing fat and oil by the GC method was heated, oxidized or extensively processed by partial hydrogenation. Many *trans* fatty acid isomers formed under these conditions provided a challenge for the GC analyst. However, the FTIR method provided the *trans* fatty acid content irrespective of the location of the *trans* double bonds in the molecule. Complementary technique was required to analyze non-volatile fatty acid polymers or oxidized fatty acid containing *trans* double bond, that would be measured by IR but was missed if we measure only FAME that elute from GC column. (Azizian, 2004). Therefore, *trans* fatty acid determination in food industrial did not need the accuracy result as it is a routine job. Thus, FTIR would be alternative procedure to analyze because it reduced cost and time during analysis.

Table 11 Analytical result of margarine analyzed by FTIR comparing with GCMS

Sample	**FTIR result**	**GCMS result**	**Structure**
Margarine 1	Methyl oleate	-	*Cis*
	Methyl linoleate	Methyl linoleate	Nonconjugated *cis, cis*
	-	Methyl oleate, (*E*)	*Trans*
	-	Methyl Eicosanoate	*Cis*
	-	Methyl palmitoleate (*Z*)	*Cis*
Margarine 2	Methyl oleate	Methyl oleate	*Cis*
	Methyl linoleate	Methyl linoleate	Nonconjugated *cis, cis*
	-	Methyl oleate, (*E*)	*Trans*
	-	Methyl Eicosanoate	*Cis*
	-	Methyl palmitoleate (*Z*)	*Cis*
Margarine 3	Methyl oleate	Methyl oleate	*Cis*
	Methyl linoleate	Methyl linoleate	Nonconjugated *cis, cis*
	-	Methyl oleate, (*E*)	*Trans*
	-	Methyl Eicosanoate	*Cis*
	-	Methyl palmitoleate (*Z*)	*Cis*

Beside that, the possible reason for different result was to the reference chemicals could not cover all of compounds that might be found in margarine. From the

FTIR spectra showed the other peak around 1000-650 cm^{-1} that could not be identified. It was possible for *cis* or *trans* fatty acids compound in margarine. So, the further study might pursued more reference chemicals in order to cover all of compounds in food sampling for analyzing and identifying all compounds in food and the result could be further used for quantitative determination.

Conclusions

Trans fatty acid determination in margarine by FTIR comparing with GCMS had the similar results, margarines consisted of *trans* fatty acid and others structure of fatty acids. It could determined the quality of fatty acid in margarines via both FTIR and GCMS. However GCMS procedure gave the better result than FTIR because of its superior sensitivity and accuracy. However, FTIR was more appropriate to determine *trans* fatty acid in food and could apply to be a routine work in food industry. So, using FTIR would be as option to quantified *trans* fatty acid in food products.

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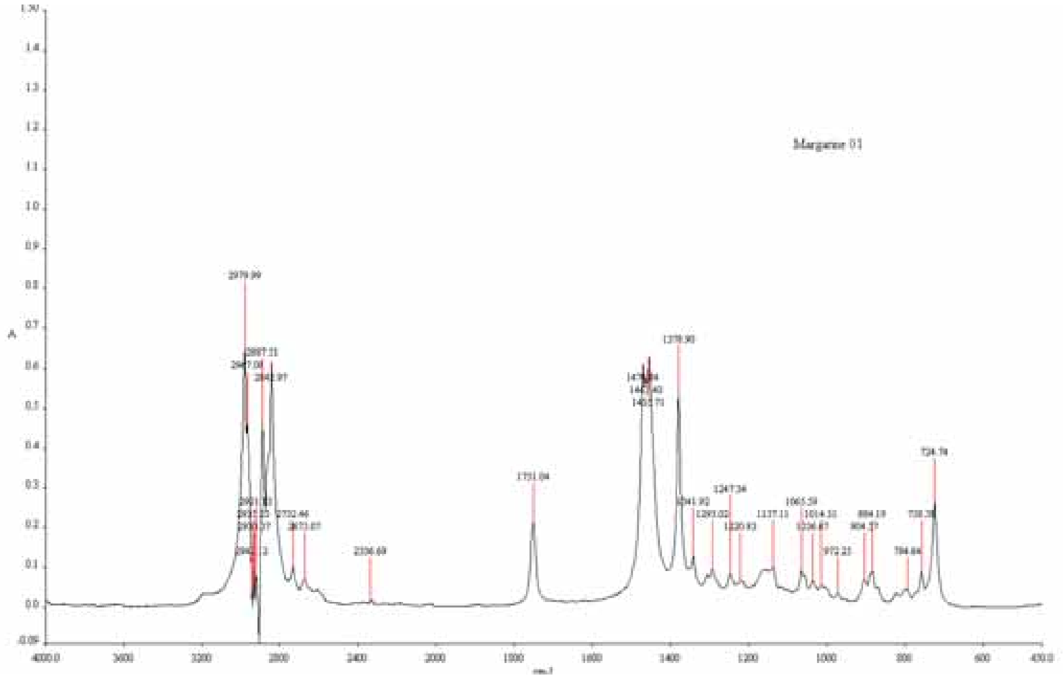

Figure 51 The FTIR spectra of margarine 1

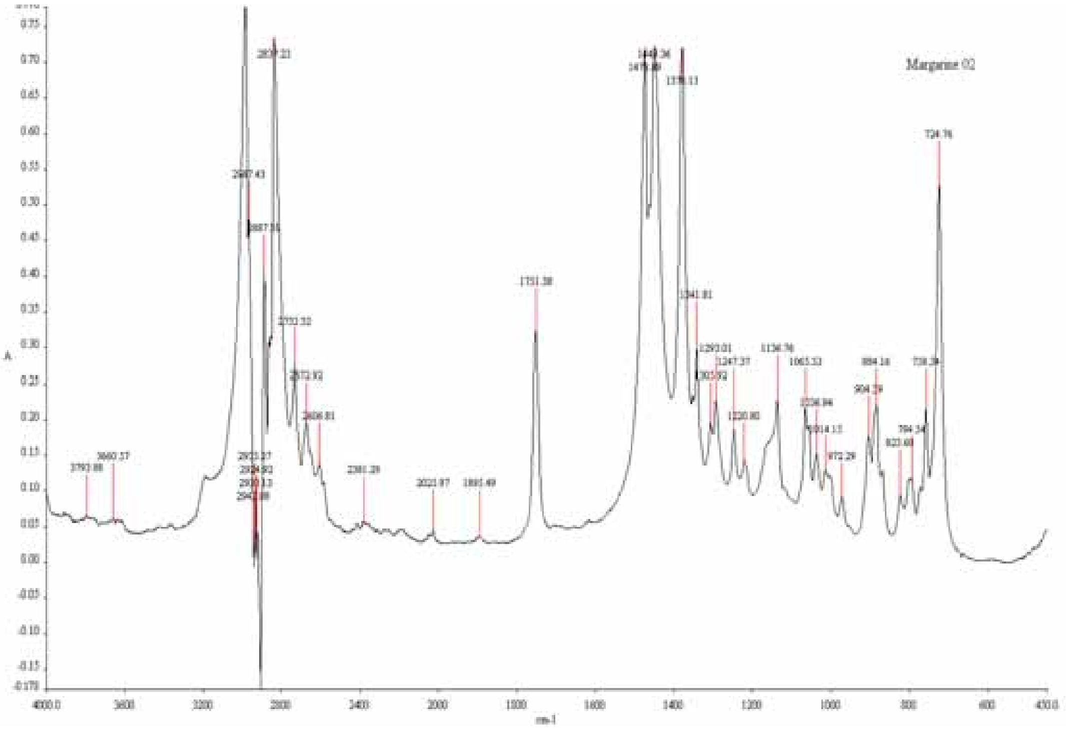

Figure 52 The FTIR spectra of margarine 2

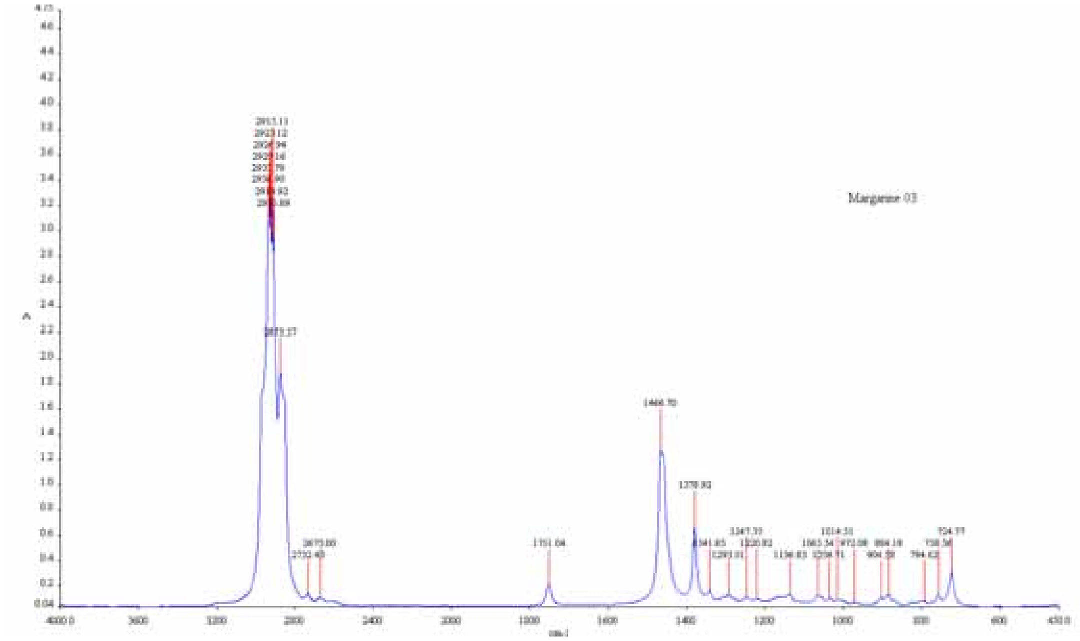

Figure 53 The FTIR spectra of margarine 3

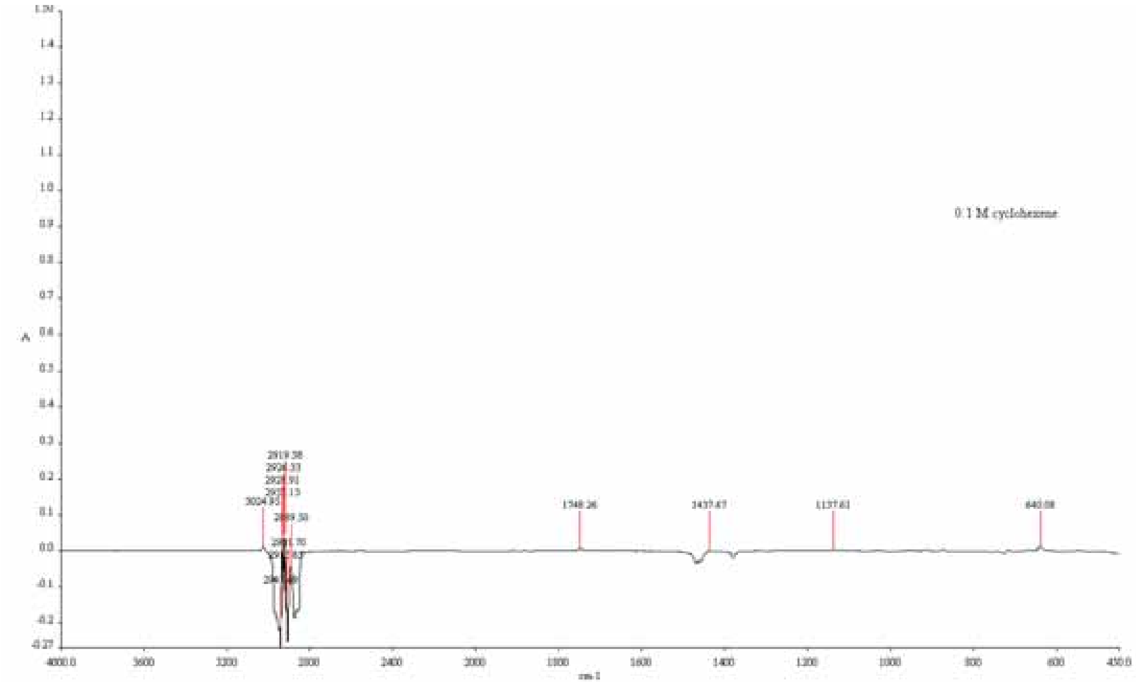

Figure 54 The FTIR spectra of 0.1M cyclohexene

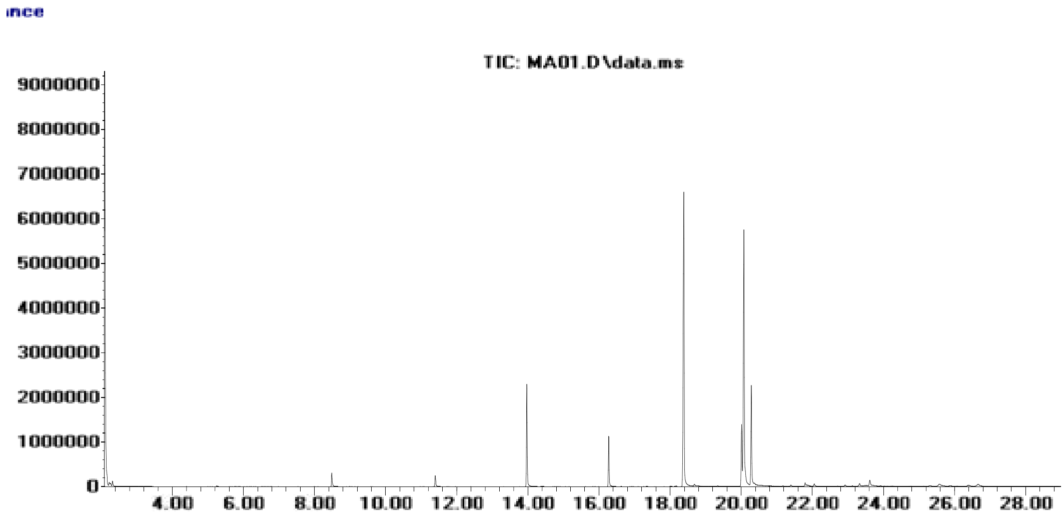

Figure 55 The GCMS chromatogram of margarine 1

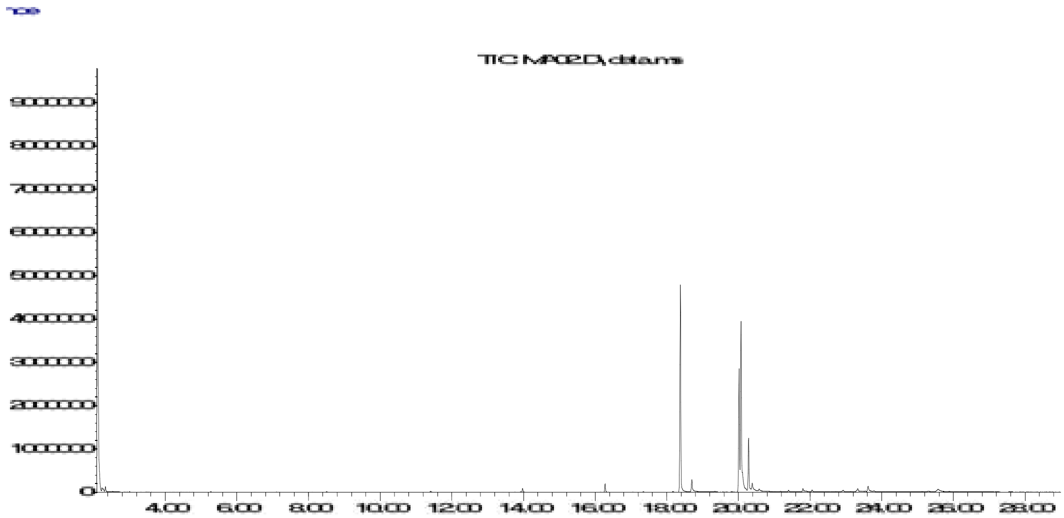

Figure 56 The GCMS chromatogram of margarine 2

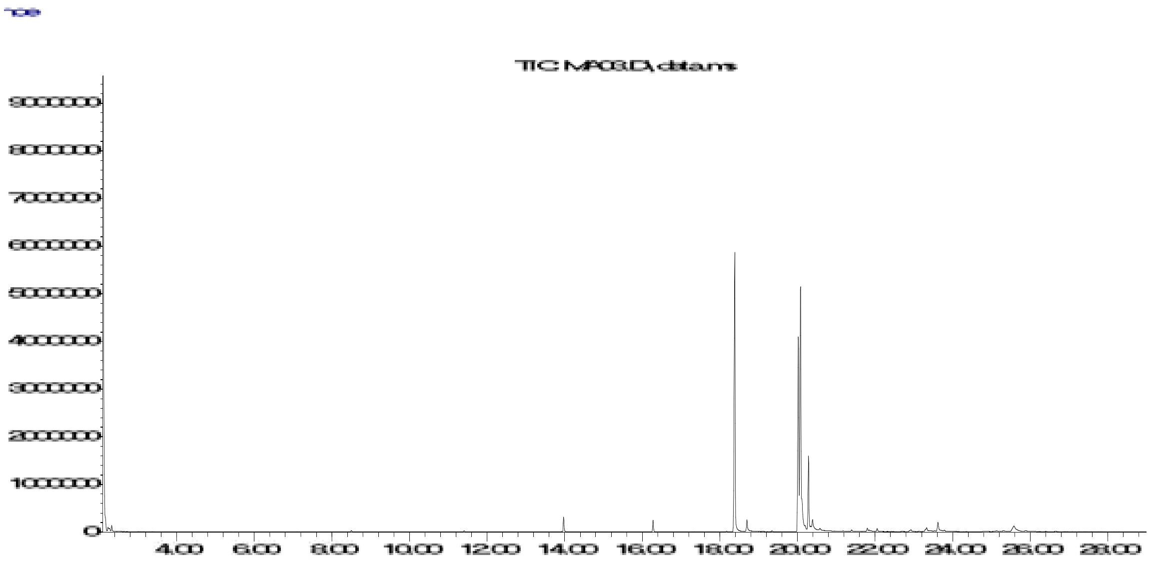

Figure 57 The GCMS chromatogram of margarine 3

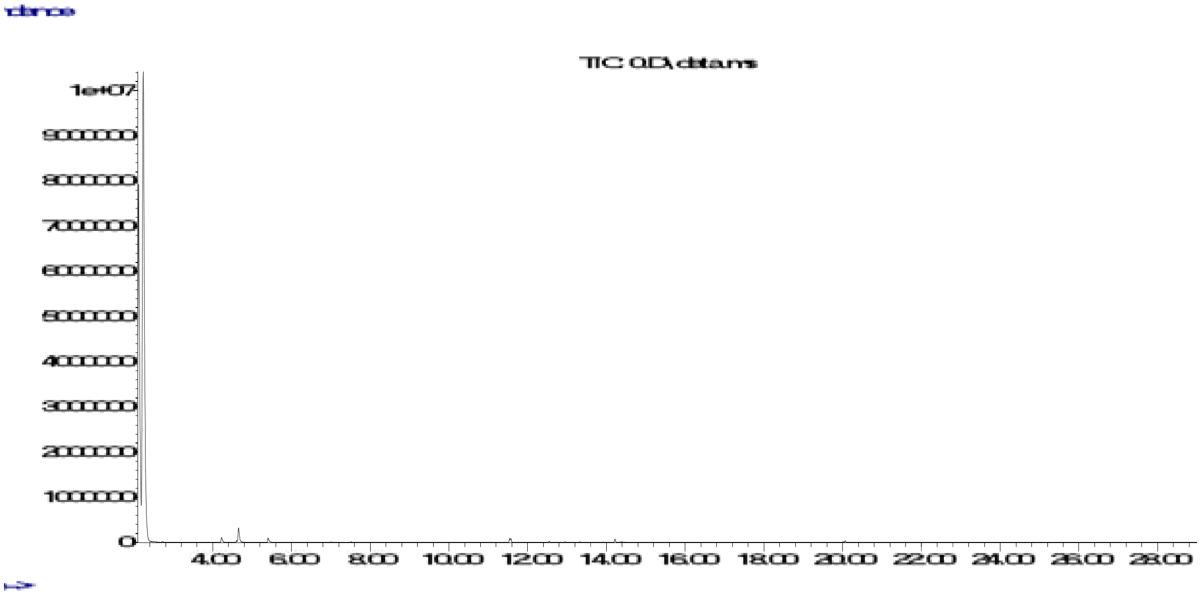

Figure 58 The GCMS chromatogram of 0.1M cyclohexene

CHAPTER 5

SUMMARY

The main aims of this study was to the qualitative and quantitative determination of *trans* fatty acid in food products. According to the result. It could concluded that fourier transformed infrared spectroscopy (FTIR) had ability to analyze quality of *trans* fatty acid in food. It found that *trans* configuration absorbed infrared at regions of 1000-950 cm^{-1}, but *cis* configuration exhibited around 780-680 cm^{-1}.

This study used the known reference compounds to represent each structure and determine by FTIR. The result showed in the range as above. Those data could use to calibrate the curves by Spectrum Beer's law for quantitative analysis. The FTIR responsed data would be fitted with the obtained calibration graph and then finally determined the quantity of each *trans* fatty acid structure.

For *trans* fatty acid determination in commercial margarines, the margarine samples composed of *trans* fatty acids and others structures of fatty acid. The next experiment would compare between data obtained from FTIR and GCMS. The both equipment could analyze *trans* fatty acids but GCMS could analyze more varieties data of fatty acids than FTIR, as concluded, the data from GCMS was more sensitive and accurate than FTIR.

This research could conclude that FTIR could measure quality of *trans* fatty acids because FTIR was able to specify and classify types of *cis* and *trans* configurations of fatty acid structure onsite respect to different regions of infrared absorption. However, FTIR could not identify the types of each compounds. The more reference compounds might be more collected before identification. Moreover, the result obtained from this research could further used to quantify quantitative by using calibration graph would each compound.

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