SPECTROSCOPIC ANALYSIS OF PLASTIC ADULTERATED COOKING OIL- A PRELIMINARY TEST

by

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HDPE	High-density polyethylene
LDPE	Low- density polyethylene
LLDPE	Linear low-density polyethylene
ATR-FTIR	Attenuated total reflectance- Fourier Transform infrared
FTIR	Fourier Transform Infrared
FT-NIR	Fourier Transform-Near Infrared
FT-MIR	Fourier Transform-Mid Infrared
FT-RAMAN	Fourier Transform- Raman
VCO	Virgin Coconut Oil
EVOO	Extra Virgin Olive Oil
PO	Palm Oil
PLS	Partial Least Square
PCR	Principle Component Regression
PCA	Principle Component Analysis
HCA	Hierarchical Cluster Analysis
RMSEC	Root Mean Square Error of Calibration
UCO	Unused Cooking Oil
U	Used Cooking Oil
PLS	Plastic
RPLS	Raw plastic
STDEV	Standard Deviation
%RSD	Percentage Relative Standard Deviation

ABSTRAK

Minyak masakan bergred tinggi seperti minyak zaitun dara *(VOO)* dan minyak zaitun dara tambahan *(EVOO) sering dicampur bersama minyak masak bergred rendah seperti minyak kacang tanah dan minyak kelapa sawit.* Objektif utama bagi proses pencemaran ini adalah supaya keuntungan boleh dimaksimumkan. Walaupun pencemaran ini tidak mendatangkan kesan negatif secara langsung kepada kesihatan pengguna, tetapi hal ini akan menjejaskan usaha ekonomi. Selain itu, ia juga membahayakan kesihatan para pengguna apabila berlakunya pencemaran di antara minyak masakan dengan bahan atau sebatian yang tidak boleh dihadam oleh sistem tubuh manusia seperti plastik dan lemak haiwan yang berbahaya. Laporan menunjukkan bahawa segelintir peniaga yang tidak bertanggungjawab telah mencemari minyak masakan yang digunakan dengan memasukkan bahan yang tidak boleh dihadam sistem tubuh manusia , bagi tujuan untuk memastikan keranggupan barangan jualan perniagaan mereka bertahan bagi tempoh yang lebih lama. Objektif utama projek ini dijalankan adalah bagi mengesan kewujudan plastik yang tidak boleh dihadam di dalam minyak masakan, menggunakan *ATR-FTIR spectrometer* dan teknik kemomatrik iaitu Analisis Komponen Utama *(PCA)* dan analisis kluster hirarki *(HCA)*. Minyak masakan yang diperolehi di kawasan sekitar Kubang Kerian, Kelantan telah dicampurkan dengan plastik yang tidak boleh dihadam pada kadar suhu yang tinggi. Seterusnya minyak tersebut disejukkan pada suhu bilik, minyak masakan yang dicemari itu dianalisis melalui *ATR-FTIR* dan *FTIR* spectra (dalam lingkungan cap jari) yang tertakluk pada *PCA*.

ABSTRACT

Expensive edible oil for examples virgin olive oil (VOO) and extra virgin olive oil (EVOO) are commonly adulterated with cheap edible oil for examples peanut oil and palm oil. The main purpose of such an adulteration is for profiteering. Although such an adulteration does not have a direct impact on the consumer's health, it affects the order of the economy. More sinister and of potential hazard to the consumer's health is the adulteration of edible oil with inedible materials or compounds for example inedible plastic for instances low-density polyethylene (LDPE), high density polyethylene (HDPE) and polypropylene (Achilias *et al.*) use in carrier bag, plastic packaging and also plastic straws. It has been reported that some irresponsible street hawkers selling banana fritters, fish fritters and fried chicken, mixed their cooking oil with inedible plastic prior to cooking so as to make their fried foods produce crispier for a longer period of time. The primary objective of this project was to detect the presence of inedible plastic in edible cooking oil using ATR-FTIR spectrometer and chemometrics techniques of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Six bottles of Neptune® cooking oils purchased from a convenience store within the Kubang Kerian, Kelantan area were adulterated with transparent plastic bags at high temperature. Upon cooling to room temperature, the plastic adulterated cooking oils (PLS) together with their fresh counterparts i.e. unused cooking oils (UCO) were analysed using the ATR-FTIR and their FTIR spectra (within the fingerprint region) were subjected to PCA. Distinctive groupings of plastic adulterated cooking oil (PLS) and unused cooking oils (UCO) suggested that PLS can be differentiated with UCO using chemometrics techniques of PCA and HCA which also indicates the potential used of ATR-FTIR coupled with chemometrics techniques for monitoring edible oil adulterated with inedible plastic.

CHAPTER 1: INTRODUCTION

1.1 Frying Process

Frying is perhaps one of the most popular culinary techniques in both industrial and domestic food preparation processes. Fried products are usually better in taste, texture and are also attractive in terms of their appearances (Susana *et al.*, 2010). In essence, frying is the cooking of food in oil or fat in a receptacle. In general there are three types of frying techniques, which are dry frying, shallow frying and deep frying. These techniques differ in terms of the amount of cooking oil used and the time taken to fry the food.

Dry frying is probably the healthiest way of frying compared to other frying technique as it uses small quantity of animal fat or vegetable fat. Shallow frying or sometimes known as stir frying uses small quantity of cooking oil at very high temperature. As it is relatively quick frying process, the food needs to be "supervised" at all time to prevent charring. Deep frying process normally uses large quantity of cooking oil or fat at temperature relatively lower than shallow frying. In this frying technique, food is submerged in the cooking oil for considerable length of time before being removed, drained and served. The last frying technique is the most popular technique among Malaysia's street hawkers selling banana fritters, fish fritters and fried chickens.

There are a numbers of chemical reactions occur in cooking oil during frying process. The reactions include hydrolysis, oxidation and thermal alteration (Li *et al.*, 2015). Hydrolysis occurs due to the presence of moisture in the food. This involves breaking of ester bonds hence releasing free fatty acids and diacylglycerols. Thermal alteration and oxidation occurs due to exposure to high temperature and the presence of air during frying. In both conditions, the unsaturated fatty acids are modified to diacylglycerols with at least one of three fatty acyl chains being altered.

1.2 Cooking Oils

There are various types of cooking oils available in the market nowadays for examples corn oil, olive oil, sunflower oil, soybean oil rapeseed oil and peanut oil. In our country, almost half of our food products are being prepared by frying using palm oil. Other than being abundance (due to the fact that Malaysia is one of the largest producer of palm oil), palm oil is relatively cheap. Furthermore, palm oil contains high saturated fats therefore it can withstand high heat and resistance towards oxidation (high oxidative stability) compared to unsaturated vegetables oil (Marcoa *et al.*, 2007).

Triglycerides are the major constituents of cooking oils. They constitute mainly of carbon and hydrogen atoms with six oxygen atoms per molecule (Sakhashir, 2008). The chemical reactions occur during frying process occur between the oxygen atom bonding for stabilization of molecules.

1.3 Adulterated Products

According to Nurrulhidayah *et al.* (2013), adulterated products refer to products that fail to meet the specific or set standards by the governing bodies. They may also be referred as products that have undergo or experienced intentional substitution, addition, and misrepresentation of food, food ingredients or food packaging. The products in which false or misleading statements have been made about them may also be considered as adulterated products (Spink J and Moyer, 2011). Adulterated products may be harmful to consumers' health. They can also undermine the integrity of the economy (Lohumi *et al.*, 2015). In the case of cooking oil, the most common adulteration involved mixing high priced cooking oil for example olive oil with low priced cooking oil for example coconut oil (Durme and Vandamme, 2016). The ultimate purpose of such an adulteration is for profiteering.

Though it is beyond the scope of this study, it is also worth mentioning that sometimes animal fats are being added to cooking oil. In the preparation of a British delicacy such as fish and chips, lard or beef dripping is added to enhance its taste. Addition of animal fats into food products is a serious concern to certain religious groups. In Islamic and Kosher dietary laws, foods containing porcine based substances are strictly forbidden and in Hinduism, the consumption of beef fats is strictly prohibited (Eliasi and Dweyer, 2002; Marikkrar et. al., 2005).

1.4 Plastics as Adulterant

Plastics are synthetic polymeric materials, which meant that they are being produced in factories. The building blocks for making plastics are small organic molecules that constitutes of hydrogen atom along with other elements for examples sulphur, nitrogen, hydrogen and oxygen, which originated from crude oil. The repeating units are known as ethylene, or ethane (Zhang *et al.*, 2013). When ethylene molecules are polymerized to form polyethylene, they form a long chain of carbon atoms in which each carbon is bonded two hydrogen atoms. Different forms of polyethylene can be made from the ethylene units.

Plastic bag is an example of polymerization product of ethylene units. It is typically categorized into three basic types, which are high-density polyethylene (HDPE), low-density polyethylene (LDPE) and linear low-density polyethylene (LLDPE). The thick and glossy shopping bags from the mall are usually made from LLDPE, while grocery bags are mainly made from HDPE and clear plastic bags, used for packaging of cooking oil are made from LDPE (Zhang *et al.*, 2013). The above mention plastics are classified as thermoplastic, which means that they are resistant towards high temperature. Thermoplastic has long, linear polymer chains that are only weakly chemically bonded and connected to each other however the bonds are easily broken which allow them to glide easily on each other like the strands of freshly cooked spaghetti. They are readily re-molded compounds and the weak bonds between molecules cause the molecules to be easily shaped (Hu *et al.*, 2016).

1.5 Problem Statement

In Malaysia's food industry setting, plastic bags including plastic straws have been reported to be added into cooking oil by irresponsible food operatives in the preparation of their fried products for examples banana fritters, fish fritters and fried chickens. Although the actual mechanism is unknown, the presence of plastic in cooking oil allows fried foods to stay fresh and crispier for a longer period of time. A prime concern regarding this issue is, it impact on consumers' health since plastic materials are not intended for consumption as they contain hazardous and toxic compounds for example bisphenol (BPA).

1.6 Objectives of the Study

1.6.1 General Objective

The general objective of this study was to analyse cooking oil preparations using spectroscopic technique of ATR-FTIR and chemometrics technique of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA).

1.6.2 Specific Objective

The specific objectives of the study were:

1. To analyse un-used, used and plastic adulterated cooking oils using ATR-FTIR spectroscopy.
2. To visually examine the ATR-FTIR spectra of the un-used, used and adulterated cooking oils for similarities and differences.
3. To differentiate cooking oil and plastic adulterated cooking oils using chemometrics techniques of Principle Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA).

1.7 Significance of the Study

This study will provide a mean for differentiation between plastic adulterated cooking oil with non-adulterated cooking oil, in other words, it will permit monitoring and detection of inedible plastic in cooking oils.

CHAPTER TWO: LITERATURE REVIEW

2.1 Production of Cooking Oil

Cooking oil mostly originated from plant base or animal resources. Cooking oil from plant base either from kernel or seeds. Many type of oil are available in the market todays and common oils in Malaysia are coconut oil, palm oil, sunflower oil and corn oil.

Coconut fruits are abundant in Asian countries such as India, Philipine, Indonesia and Malaysia. It has been used as edible oil for consumption. The coconut oil are either from kernel or meat of mature harvested coconut (Hamsi *et al.*, 2014). Coconut oil is extracted via two methods either dry or wet processing (Santos *et al.*, 2014). In dry process the coconut oil is extracted from the meat and dried using sunlight or fire to create copra, a dried kernel (Hamsi *et al.*, 2014).

Next, the copra will be mixed and pressed with substance such as hexane to produce the coconut oil. The coconut oil then undergo refining, bleaching and deoduorising which involve chemicals and heating process at high temperature (Xu, 2015). In wet processing method which is known as the process to extract virgin coconut oil (Hamsi *et al.*, 2014). Wet processing of coconut oil using a fresh coconut is used instead of dried copra. The wet process required a prolonged heating step.

Corn oil is also one of the most common oil among consumers because it is literally cheaper compared to other vegetable oils. The corn oil produced from the germ of corn (Alama and Alandis, 2014). First, the germs of corns undergo cleaning process before pressing process. Pressing process is a method to pass the seeds passing through extraction process. The corn oil extracted using solvent such as hexane and then the extracted corn undergo alkali treatment to remove phosphatides and finally the extracted corn will proceed to refining process and deodorize by stream distillation under high vacuum (Sievers, 1920).

The palm oil production is almost similar with the production of corn oils. The palm fruits will undergo cleaning and pressing process first before proceeds to the extraction process. After the extraction process, the extract will undergo alkali treatment with centrifuge process. The centrifuge process is a process to separate the oil with unwanted solutions. Lastly removal of odor and colour of palm oil are done. This step is known as deodorization and bleaching process. This study will focus more on palm cooking oil since it is low price edible oil (Muth *et al.*, 1998).

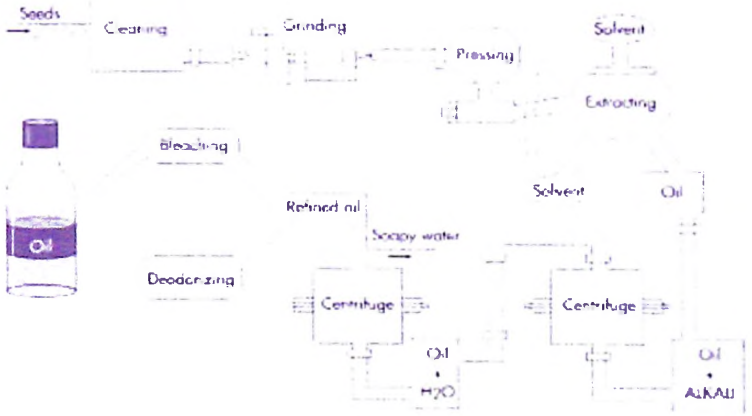


Figure 2.1: Basic manufacturing method of cooking oils (Muth *et al.*, 1998).

Chemically, cooking oil is categorized as hydrocarbon materials which also known to be a liquid fat. It has a general structure of carbon, hydrogen linked by single and double bond. Figure 2.2 showed the general structure of cooking oil compound.

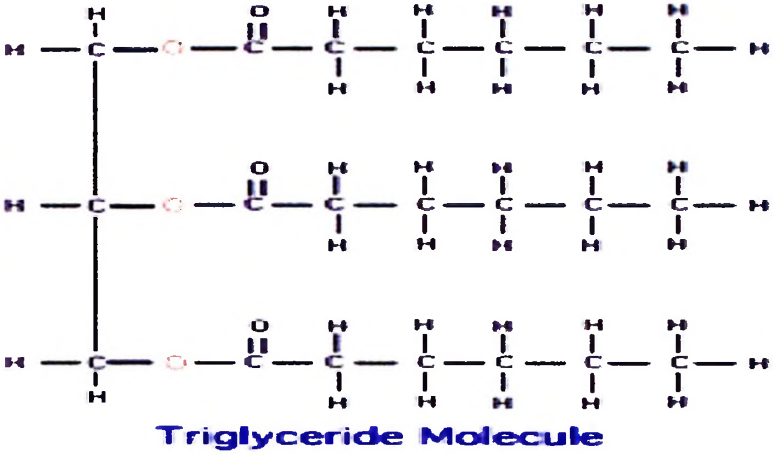


Figure 2.2: The general structure of cooking oil compound (Chang, 2010).

2.2 Production of Plastic

Plastic is scientifically known as low-density polyethylene (LDPE) and commonly known as poly-bags, which originated from crude oil. Polyethylene is the chemical compounds presence in LDPE plastic bag which ethylene is its monomer. LDPE plastic bags are biodegradable, light and highly available in market. LDPE are the major type of thermoplastic, recently used throughout the world such as housewares, electrical components, gas pipe and others (Achilias *et al.*, 2007
).

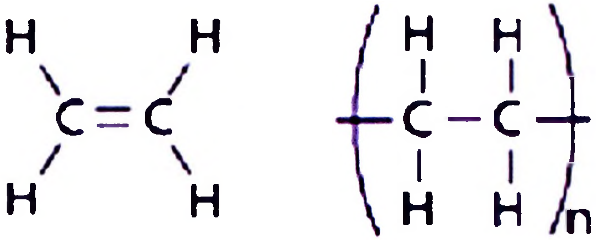


Figure 2.3: Chemical structure of ethylene (left) and polyethylene (right) (Chang, 2010).

Different method are used in the production of LDPE plastic bag is conventional extrusion, injection molding and film blowing technique. The most common production method of LDPE bag is by blown film extrusion or also known as tubular film process (Matzinos *et al.*, 2002). As mention above, LDPE produced from polyethylene might be treated with an addictive such as anti-block and ultraviolet inhibitor. It was done by insert the palletized polyethylene into hopper and the force applied on the materials causes it to move into the barrel where in a high temperature.

The plastic soon starts to melt completely. After travelling through the channel, as shown in figure 2.4, the melting materials travel through the screen pack for filtering of foreign matter before proceed with supporting beaker and adapter for dying process. Next the melted plastic is then forced to the flow channel for shaping purpose. The plastic thickness are made from 20 µm -200 µm (McWilliams, 2006).

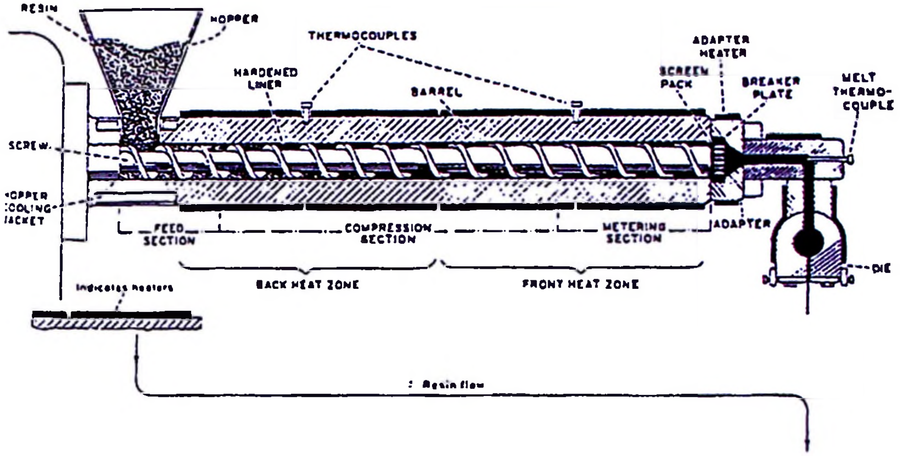


Figure 2.4: Flow chart of plastic production using blown film extrusion method.

2.3 Study on Cooking Oil

The first extensive investigation on cooking oil was done by Zeddelmen and Wurzinger (1973). , In their study, they used soybean oil, groundnut oil, hardened groundnut oil and coconut oil and compared them against palm oil. The results of the experiment had shown that palm oil and hardened groundnut oil had the highest oxidative stability at high temperature compared to animal fats and highly unsaturated oil.

In a study conducted by Man et al. (2005) where bleached, refined and deoudorised palm olein and coconut oil were compared, it was found that palm olein was superior in term of free fatty acids content, iodine value (Susana *et al.*), foaming tendency and smoke point.

Xu *et al.* (2015) In their study on detection of virgin coconut oil adulterated with animal fats had used GC X GC-TOF/MS to detect the presence of the animal fats in the virgin coconut oil (VCO). The level of sterol trimetylsilyl ethers, which was derived from cholesterol and cholestenol was used to differentiate between the VCO, animal fats and VCO adulterated with the animal fats. (Xu *et al.*, 2015).

Li *et al.* (2015) had studied the effect of frying condition on fatty acid profiles and other polar materials in cooking oil. The study was conducted for quality control purpose, which was to observe the relationship between the frying conditions and the chemicals composition of the oil. The study showed that the viscosity of the cooking oil increased with temperature. A model that predicts the oil degradation was successfully developed using the process parameters.

Frying and heating process of edible oil lead to the formation of trans-fatty acids in the oil. The study on the formation and eventually the intake of such acids during frying and heating process in fried food had been conducted by Tsuzuki *et al.* (2010). In their study, they had fried sliced potatoes using canola oil at various temperatures. The presence of the trans-fatty acids was examined in both the sliced potatoes and the canola oil using gas chromatography.

Marcoa *et al.* (2007) had conducted a study on the frying performances of a sunflower and palm oil blend in comparison to pure palm oil. The study focused on the analytical parameters for examples free acidity, spectrophotometric indices, total polar composition, short-chain fatty acids, tocopherol and tocotrienol composition, colour and also the flavour of the oils (Ta€hka€pa€a€ *et al.*, 2014). The study had shown that all the analytical parameters were relatively higher in the blended cooking oil compared to pure palm oil.

A study conducted by Susana *et al.* (2010) on the stability of olive oil under deep frying conditions proved that the oil is resistant towards degradation under domestic frying condition which was around 170°C. Extra virgin olive oil (EVOO) was the most resistance compared to other olive oil categories, which meant that EVOO undergo less oxidation and reduction reactions. The samples were evaluated for free acidity, peroxide value, β-carotene and total phenols up until the total polar compounds achieved the maximum legal values (Susana *et al.*, 2010).

2.4 Principle of Attenuated Total Reflectance-Fourier Transform Infrared ATR-FTIR Spectroscopy

Infrared spectroscopy is a technique often use in organic chemistry. It is an analytical technique that can identify the presence of certain functional group in a molecule. It is also being used to evaluate the purity of a compound.

ATR-FTIR spectroscopy is the interactions between matter and the electromagnetic radiation in the infrared region. The electromagnetic waves usually couple with the molecular vibrations where the molecule will be excited to a high vibrational state after absorbing the infrared radiation. The probability of particular infrared frequency being absorbed depends on the actual interaction between frequency and the molecules. Infrared spectroscopy is a very powerful technique which can provide fingerprint information on a chemical composition of a sample (Qu *et al.*, 2016).

In ATR-FTIR analysis the light i.e. the IR radiation passes through the beam-splitter and sends the light into two different directions at right angles. One beam travels to a stationary mirror then back to the beam-splitter while the other goes to a moving mirror (Hsu, 1997). The motion of the mirror makes the total path length variable versus that taken by the stationary mirror beam. When both beams meet up again at the beam-splitter, it recombines and creates constructive and destructive interference which is known as interferogram. Figure 2.1 shows a typical layout of an FTIR spectrometer and its basic signal transformation process i.e. from interferogram to IR spectrum.

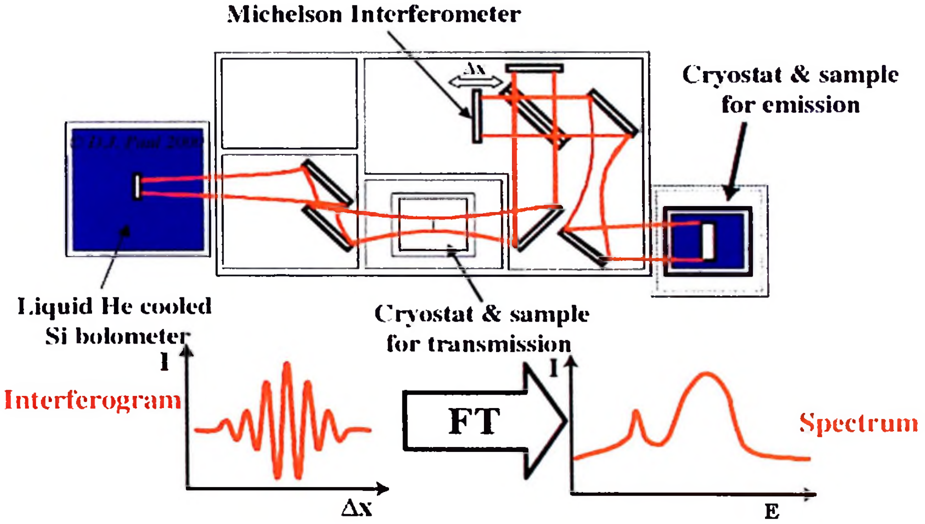


Figure 2.5: Typical layout of a FTIR spectrometer and its basic signal transformation process.

The recombined beam passes through the sample and the sample absorbs all the different wavelengths characteristic of spectrum and subtracts specific wavelength from the interferogram. The detector interprets variation in energy versus time for all the wavelengths simultaneously. The output or result of the process is in the form of spectrum that represents the wavenumber of specific functional group in a compound (Hsu, 1997). Figure 2.2 illustrates a typical IR spectrum showing the absorption bands and also the functional groups. The ATR-FTIR is the most widely adopted method in for food quality and authenticity analysis. It is based on the total internal reflectance of the infrared beam by an internal reflection crystal with reflective index (Lohumi *et al.*, 2015).

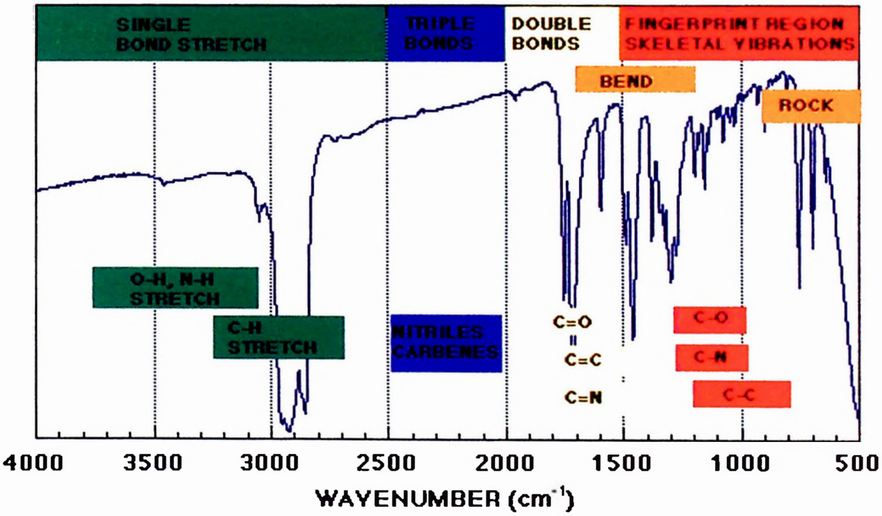


Figure2.6: A typical IR spectrum

The mid infrared region is the most common region used in IR spectroscopy. This region is divided into two regions namely the functional group region, which is from 4000 to 1500 cm^{-1} and the fingerprint region that covers from 1500 to 400 cm^{-1}. The advantages of FTIR is fast spectrum acquisition, easy to operate, and does not need complex sample preparation therefore it was chosen for this study.

2.5 Study on Adulteration using FTIR Spectroscopy

In a study conducted by Zhang Q. et al. (2012) on authentication of edible vegetable oil adulterated with used frying oil, FTIR spectroscopy had been found to be effective for detecting such an adulteration.

FTIR spectroscopy had also been used to authenticate hazelnut oil mixed with different type of oils. The spectral data obtained were analyzed by using discriminant analysis and partial least-squares techniques (Ozen and Mauer, 2002). Other than that FTIR had been used to investigate the thermal stabilities of various types of edible oils (Moros et al., 2009; Pinto et al., 2010).

Rohman A. and Che Man Y.B. (2009) had shown that pure extra virgin olive oil (EVOO) can be discriminated with EVOO adulterated with palm oil using FTIR spectroscopy combined with chemometrics techniques of Partial Least Square (PLS), Principal Component Regression (PCR) and discriminant analysis (DA). In the study, PLS and PCR had successfully quantified the level of EVOO contents while the DA had enabled discrimination of EVOO with its potential adulterant i.e. the palm oil.

Yang et al. (2005) had used FTIR, Fourier Transform near Infrared (FT-NIR) and FT-Raman spectroscopy to discriminate edible oils and fats. They had found that FTIR was the most superior technique over the FT-Raman and FT-NIR for discrimination of edible oils and fats.

A study conducted by Lohumi *et al.* (2013) showed the effectiveness of FTIR to discriminate between viable and nonviable watermelon seeds. Other spectroscopic techniques for examples-MIR and Raman spectroscopy are widely and successfully used for authentication and quality analysis of a variety of agro-food products (Lohumi *et al.*, 2015).

2.6 Chemometrics Techniques

2.6.1 Principle Component Analysis (PCA)

PCA is the fundamental of the unsupervised method in chemometrics. PCA is commonly used to find hidden structures in a large dataset and used to discover natural grouping within the data. The PCA performs well when dealing with highly collinear variables such as those generated from spectroscopic techniques including IR spectroscopy.

PCA is used to identify patterns in data set in such way as to highlight their similarities and differences (Everitt, 2001). In PCA the given dataset is described using new set of variables known as principle components, which derived from linear combination of the original variables of given dataset with its specific principle component loadings. The power of PCA lies in its ability for dimensionality reduction. After principle components transformation, a given dataset can be described using only the first few principal components that describe most of the variability in a given dataset rather than the whole set of variables before the transformation. Graphical representation known as score plot is normally constructed from the first few principal components selected. The score plot displays the arrangement of the samples or objects of a given dataset across their sample space when their similarities and differences are deduced according to their principal component scores.

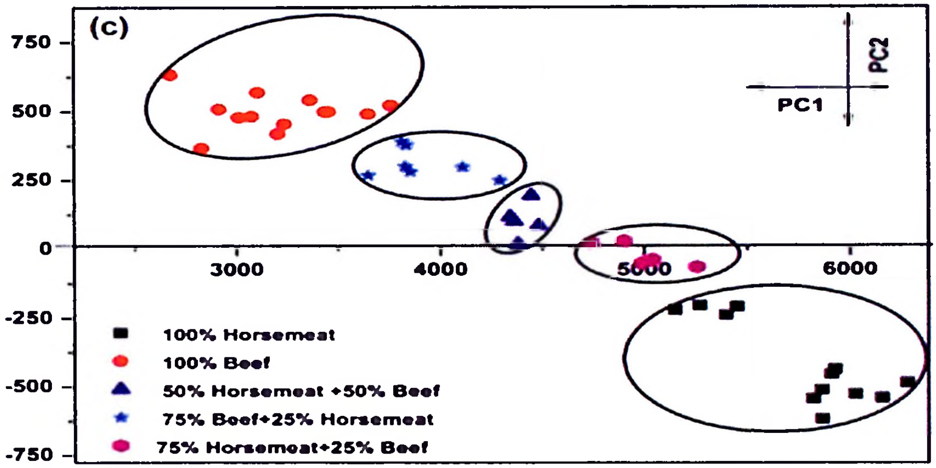
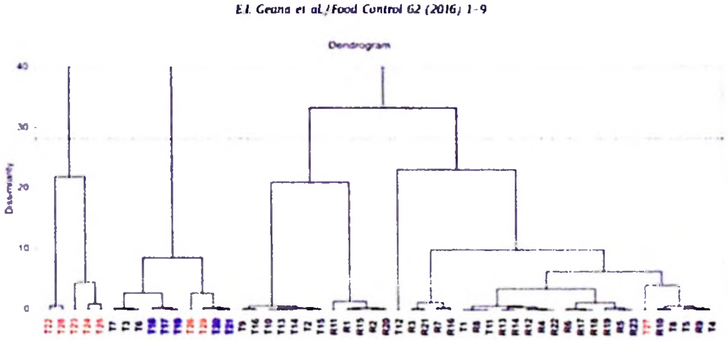


Figure 2.7: The example of PCA results based on previous study by Boyacı, 2014, on a novel method for discrimination beef and horsemeat using Raman spectroscopy.

2.6.2 Hierarchical Cluster Analysis (HCA)

HCA is a clustering technique that produces dendrogram (similar to tree diagram) as its final outcomes. The dendrogram shows the linkage between an individual sample with group or cluster of samples that exist within the dataset. HCA uses either agglomerative or divisive methods to identify clusters in a given dataset (Everitt, 1993). The former is the most common approach where clustering start with all samples being separate and join to form cluster in series of steps until all cluster exist within a given dataset are joined together to form a one large group. The divisive method, works the opposite way of agglomerative technique. The closeness between samples of a given dataset are computed most commonly using the Euclidean distance function whilst single, average and complete linkage are amongst the linkages functions commonly used to link between clusters that arise from a given dataset.



Class				
C1	C2		C3	C4
R1	R3	R18	T3	T22
R2	R4	R19	T6	T23
R11	R5	R21	T7	T24
R15	R6	R22	T17	T25
R20	R7	R23	T18	[illegible]
T2	R8	T1	T19	
T9	R9	T5	T20	
T10	R10	T5	T21	
T13	R12	T8	T26	
T14	R13	T11	[illegible]	
T15	R14	T12		
T16	R16	[illegible]		
	R17			

Figure 2.8: The example of dendrogram from study of verifying the red wines adulteration through isotopic and chromatographic investigations coupled with multivariate statistic interpretation of the data by Geana, 2016.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Sample Collection and Preparation

Six bottles of 1 kg Neptune® cooking oil (Neptune Sdn. Bhd., Malaysia) as shown in Figure 3.1 were purchased from a convenience store in the Kubang Kerian, Kelantan area. According to the information available on the label, the cooking oil was blended oil comprising of palm oil and selected groundnuts and sesame oil. The plastic packaging or bags used to adulterate the cooking oils were also purchased from the same convenience store. The sample was prepared in the food preparation laboratory, Universiti Sains Malaysia. The samples were heat on the stove, Zanussi available in the food preparation laboratory.

Figure 3.1: Neptune® cooking oils purchased from the convenience store.

3.1.1 Preparation of Fresh or Unused Cooking Oil (UCO)

Fresh or unused cooking oil samples (UCO) were prepared by drawing 1 mL oil from each of the purchased Neptune® oil into six different glass vials. The samples were labeled as UCO1, UCO2, UCO3, UCO4, UCO5 and UCO6 respectively.

3.1.2 Preparation of Used Cooking Oil (U)

Used cooking oil (U) samples were prepared by heating approximately 1 L of the Neptune® oil in a pan at four different cycles using four different temperatures i.e. 150°C, 180°C, 200°C and 250°C. At each cycle, approximately 1 mL of oil was drawn from the pan and the samples were heat up for approximately 15 minutes until it reached the appropriate temperature. Next, the samples transferred into glass vial using a glass pipette after cooling at room temperature for approximately 10 min. The heating temperatures were monitored using a thermocouple. The vials were labeled as U150, U180, U200 and U250 respectively.

3.1.3 Preparation of Plastic Adulterated Cooking Oil (PLS)

Cooking oil was heat up to four cycles and plastic bag was added to the cooking oil during heating process. The commercial plastic bag will be added to the cooking oil once the oil was heated up. Plastic with size of 2 cm x 2 cm will be added to the cooking oil up to four pieces. The oil was used continuously up to four heating cycles without any addition or replacement. The oil was heated up until all plastics were soluble with no specific temperature. The plastic adulterated cooking oil then drawn into vial according to the number of plastic bags added. Each of the vials labeled as PLS1, PLS2, PLS3 and PLS4.

Table 3.1: Labeling for plastic adulterated cooking oil and the number of plastic dissolved in the cooking oil.

LABEL	NUMBER OF PLASTIC/ PCS
PLS1	1
PLS 2	2
PLS 3	3
PLS 4	4

3.2 Samples Analyses using ATR-FTIR Spectrometer

Determination of the spectroscopic profile of each of the sample was made using a Bruker Tensor 27 IR spectrometer (Bruker Optics, UK) fitted with an ATR attachment, as shown in Figure 3.2, which was linked to a personal computer by the Opus software. The samples were scanned from 600 - 4000 cm^{-1} range where 16 scans were collected at 4 cm^{-1} resolution each time. Background scan was performed prior to scanning each sample. The samples were analyzed directly without further sample preparation. Each sample was analyzed in triplicates.

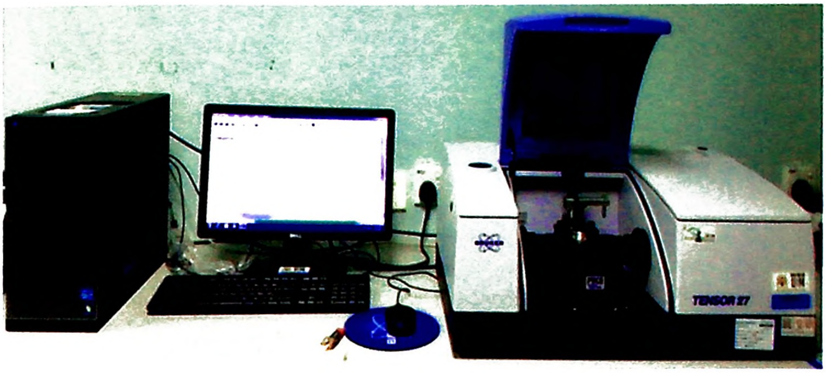


Figure 3.2: Bruker Tensor 27 IR spectrometer fitted with the ATR attachment.

3.3 Repeatability and Reproducibility Studies

Repeatability and reproducibility studies were performed to assess the variability of the analysis. For repeatability study, six consecutive measurements were made from one oil sample while for reproducibility study; one measurement was made from six different oil samples.

3.4 Statistical and Chemometrics Analyses.

The statistical analysis i.e. the calculation of mean, standard deviation and percentage relative standard deviation (%RSD) was performed using Microsoft Excel spreadsheet (Microsoft Office 2010, USA) while the chemometrics analyses were performed using Minitab version 16.2.0 software (Minitab Inc., USA). All data for chemometrics analyses was inputted first in Microsoft Excel spreadsheet prior to transferring to the Minitab environment.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 General Appearances of the Cooking Oils

The purchased cooking oils (each with net weight of 1 kg) were packed in a 1.5 mL transparent plastic bottle. The colour of the oil was yellowish brown and appeared to be consistent between bottles. The only different that can be detected between the oils was on the printed information available near the neck of each bottles. Imprint BLD17AS C1 as shown in Figure 4.1 can be seen on the UCO1 and UCO2 bottles while imprint BLD17AS C2 as shown in Figure 4.2 can be seen on the UCO3, UCO4, UCO5 and UCO6 bottles. These imprints could probably mean that the oils were from two different manufacturing batches or from two different manufacturing plants.

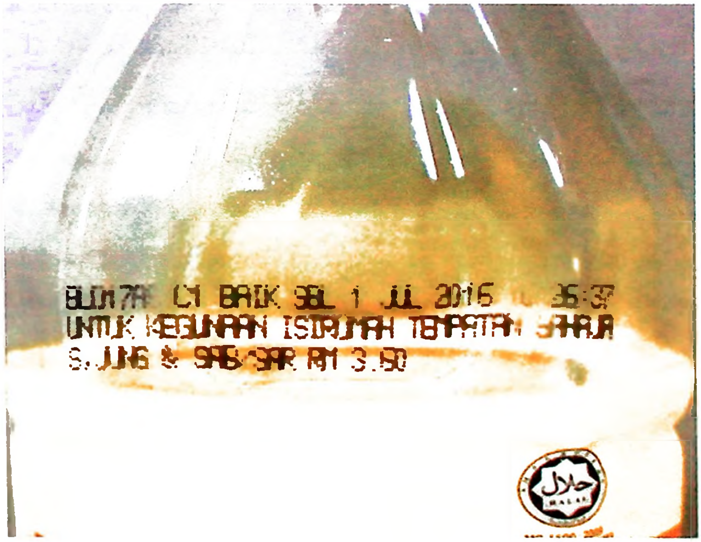


Figure 4.1: Imprint BLD17AS C1 appeared near the neck of the UCO1 and UCO2 bottles.