SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science in Industrial Chemistry.

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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ABSTRACT

Thermally oxidized oil such as recycled cooking oil and repeatedly used oil were reported to impose deleterious effect to health. In light of the presence of those oils in the market and food preparation process, this study was carried out to differentiate between fresh and thermally oxidized oil and propose parameter that can replace total polar compounds (TPC), the international standard in determining oil degradation status but it is time consuming. In this study, samples were fresh oil, oil subjected to controlled heating and frying in the laboratory at 180 °C to 200 °C for 6 hr and waste oils collected from various food outlets. The differences between fresh and thermally oxidized oil were evaluated based on several parameters; total polar compounds (TPC), fatty acids composition, short chain fatty acids, trans fatty acid, iodine value (IV), free fatty acids (FFA) content, adsorption at 233 and 269 nm under ultra violet (UV) spectrum and oil color. Results showed that fresh and thermally oxidized samples had significantly different level of total polar compound. Color index or absorption at 420 nm showed good correlation (r= 0.848) to TPC but depended on frying parameter especially the food medium. Thermally oxidized oil had decrease in unsaturated fatty acids and increase in saturated fatty acids content. No trans fatty acid was detected in all samples. Short chain fatty acid, the octanoic acid (C8:0) only present in thermally oxidized oil, with correlation of r= 0.750 to TPC. Free fatty acids level showed good correlation (r= 0.863) to TPC but depended on frying parameter especially the moisture content. Iodine value showed acceptable correlation (r = 0.5602) to TPC, however no significant difference between fresh and thermally treated oil. Absorption at 233 and 269 nm, showed correlation of r= 0.8469 and r= 0.8295 to TPC respectively. The presence of octanoic acid (C8:0) was proposed to be used as marker component to differentiate between fresh and thermally oxidized oil as it only present in the later, with simple analytical procedure to be applied as routine analysis and showed good correlation with total polar compounds (r= 0.750).

Keywords: Thermally oxidized oil; Total polar component; Coefficient correlation (r); Fatty acids composition; octanoic acid; Trans fatty acid; Iodine value; Free fatty acids; Adsorption under UV spectrum.

ABSTRAK

Minyak teroksida haba seperti minyak masak kitar semula dan minyak yang digunakan berulang kali dilaporkan memberi kesan buruk kepada kesihatan. Dengan kehadiran minyak tersebut di pasaran dan proses penghasilan makanan, kajian ini telah dijalankan untuk membezakan minyak masak yang belum digunakan dan minyak masak teroksida haba, dan juga untuk mencadangkan parameter yang boleh menggantikan amaun komponan polar, standard antarabangsa dalam menentukan degradasi kualiti minyak masak yang mana kaedah ini memerlukan banyak masa. Di dalam kajian ini, sampel adalah minyak yang belum digunakan, minyak yang dipanaskan dan digoreng di dalam makmal, dengan suhu terkawal antara 180 °C ke 200 °C selama 6 jam dan juga minyak masak terbuang yang dipungut dari beberapa tempat penghasilan makanan. Perbezaan antara minyak masak yang belum digunakan dan minyak masak teroksida haba dinilai berdasarkan beberapa parameter; amoun komponan polar (TPC), komposisi asid lemak, asid lemak rantai pendek, asid lemak trans, nilai iodine (IV), asid lemak bebas (FFA), penyerapan di bawah spectrum UV dan warna minyak. Minyak masak yang belum digunakan dan minyak masak teroksida haba menunjukkan perbezaan amoun komponen polar yang signifikan. Indeks warna atau penyerapan di 420 nm menunjukkan korelasi r= 0.848 kepada amaun komponan polar (TPC) tetapi dipengaruhi parameter sewaktu menggoreng terutamanya medium makanan. Minyak masak teroksida haba menunjukkan penurunan dalam jumlah asid lemak tak tepu dan peningkatan dalam jumlah asid lemak tepu. Tiada asid lemak trans dikesan dalam kesemua sample. Asid lemak berantai pendek iaitu asid ocranoik (C8:0) hanya hadir di dalm minyak masak teroksida haba dengan korelasi r= 0.750 kepada amaun komponan polar (TPC). Jumlah asid lemak bebas (FFA) menunjukkan korelasi r= 0.863 kepada amaun komponan polar (TPC), tetapi bergantung kepada parameter sewaktu proses menggoreng. Nilai iodine (IV), menunjukkan korelasi yang boleh diterima (r= 0.5602) kepada TPC tetapi tiada perbezaan signifikan antara minyak masak yang belum digunakan dan minyak masak teroksida haba. Penyerapan di 233 and 269 nm masing- masing korelasi r= 0.8469 dan r= 0.8295 kepada amaun komponan polar (TPC). Kehadiran asid octanoic (C8:0) dicadangkan sebagai penanda untuk membezakan minyak masak yang belum digunakan dan minyak masak teroksida haba kerana ia hanya hadir di dalam minyak yang terdegradasi, prosedurnya mudah, sesuai untuk diaplikasi dalam analisis rutin dan juga menunjukkan korelasi yang baik dengan amoun komponan polar

Kata kunci: Minyak teroksida oleh haba; Amoun komponan polar; Pekali korelasi (r); Komposisi asid lemak; Asid octanoic; Asid lemak trans; Nilai iodine; Asid lemak bebas; Penyerapan di bawah spectrum UV.

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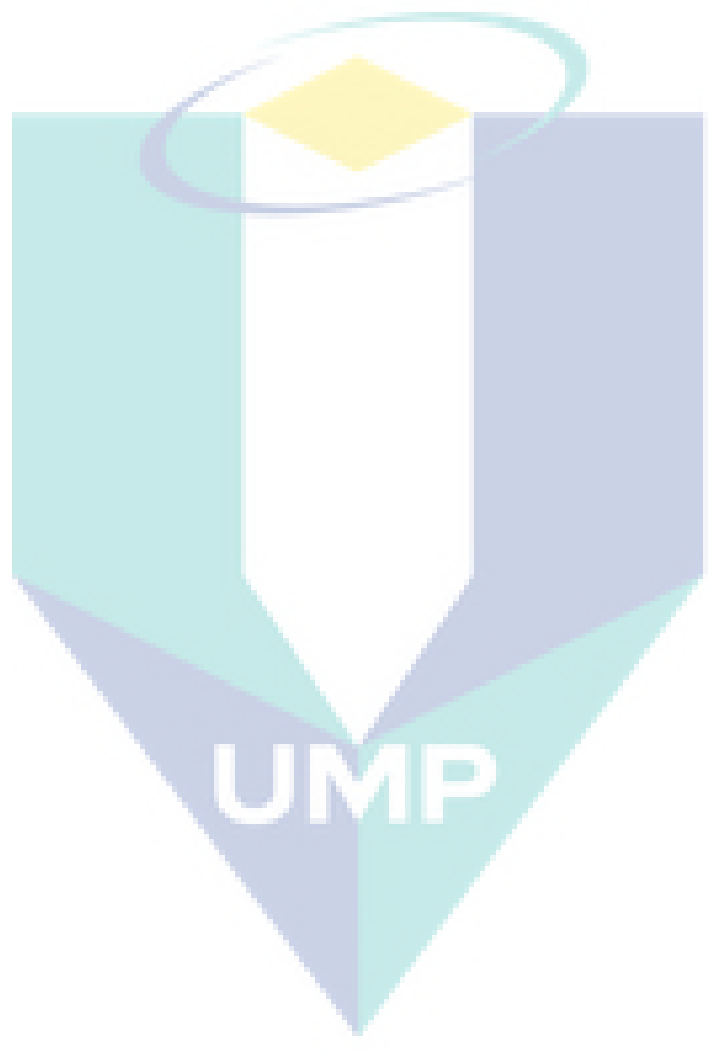

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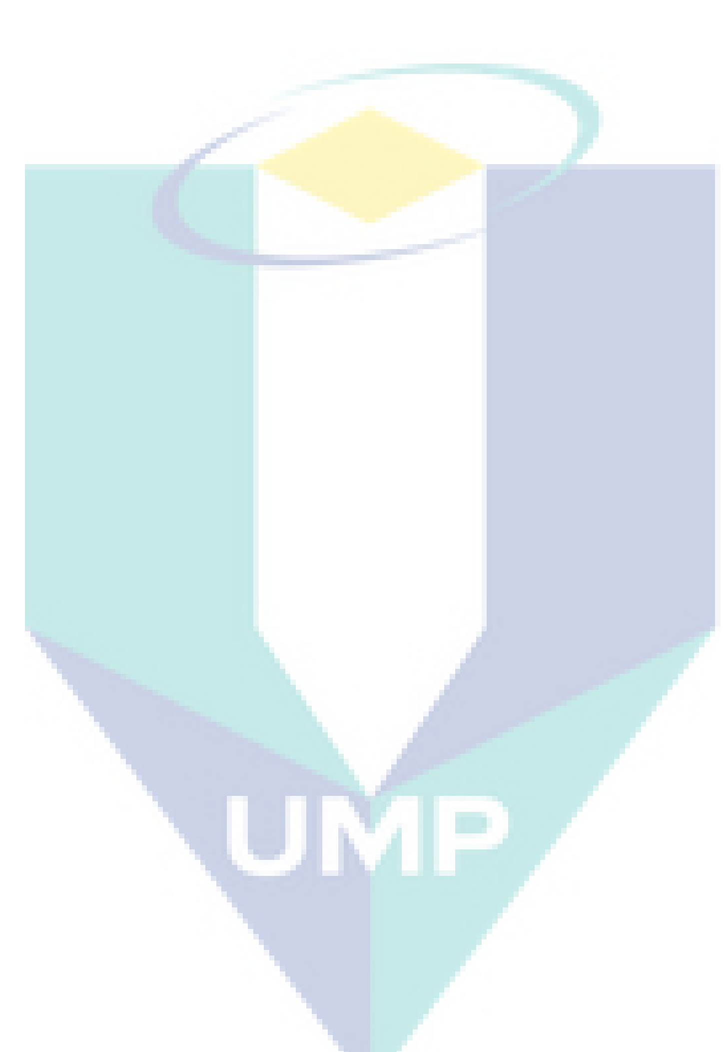

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LIST OF SYMBOLS

Symbol	Meaning
°C	degree Celsius
g	gram
kg	kilo gram
m	meter
nm	nano meter
mm	millimeter
µm	micro meter
µL	micro liter
ml	milliliter
%	percentage
hr	hour
min	minute
min^{-1}	per minutes
L	liter
ω	omega
mg	mili gram
cm	centimeter
v/v	volume/ volume
<	less than
r^2	coefficient of determination
r	correlation coefficient
σ	standard deviation
S	slope of calibration curve

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOCS	American Oil Chemists' Society
CVD	Cardio vascular disease
FA	Fatty acid
FAME	Fatty acid methyl ester
FFA	Free fatty acid
FID	Flame Ionization Detector
FTIR	Fourier transform infra red
GC	Gas chromatography
HDL	High density lipoprotein
IV	Iodine value
IUPAC	International union of pure and applied chemistry
LDL	Low density lipoprotein
LOD	Limit of detection
MSD	Mass spectrometry detector
MUFA	Mono unsaturated fatty acid
PAH	Poly aromatic hydrocarbon
PORIM	Palm Oil Research Institute of Malaysia
PUFA	Poly unsaturated fatty acid

RSD	Residual standard deviation
SFA	Saturated fatty acids
SPSS	Statistical Package for Social Sciences
TAG	Triacylglycerol
TFA	Trans fatty acid
TPC	Total polar compounds
UV	Ultra violet
UV Vis	Ultra violet visible

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Most Malaysians are exposed to unhealthy and unethical food servings whether dining out or at home. For instance, a food manufacturing factory was ordered to closedown for using recycled cooking oil in preparing its processed food products (*The Sun*. 2008. 30 January; *The Star*. 2008. 13 February). Recycled oil, or repeatedly heated and thermally oxidized oil have been well reported to cause deleterious effect to health such as genotoxic, mutagenic, increase of blood pressure, deleterious effect in the endothelial function and carcinogenic (Hageman et al., 1988; Williams et al., 1999; Rueda Clausen et al., 2007; Yildiz et al., 2007 and Leong et al., 2008).

That is the case where the misconduct of food manufacturer was reported. Probably there are many other cases of food manufacturing process that are unreported or proven to be unethical. The recycled cooking oil, besides used as food ingredient for lower cost purpose, might be repackaged and sold as brand new oil in the market. Though have not been proven yet, fast food outlets operators, banana fritters sellers, are notorious for selling their used or discarded oil to third party. It is alleged that the used oils are repackaged to be resold as brand new oil. Consumers who bought this allegedly recycled oil are exposed to anti nutritional properties and deleterious effect of the oil towards health.

A publication written by Riera et al. (2000) claimed, even if recycled oils are used as animal feed, the deleterious effect of the recycled oil can still enter human diet. This is because hazardous compounds in recycled oil such as dioxin, poly aromatic hydrocarbon (PAH) can accumulate over time and through food chain, thus still posing danger to human. This situation shows how consumption of recycled oil can pose deleterious effect to human.

A survey conducted by Norimah et al. (2008) revealed most urban dwellers depend more on food eateries and fast foods outlet for food consumption. The availability of fast food restaurant, 24 hours-eateries has been accounted for this trend. Foods served at these outlets normally are notorious for its processed, fried, and with high calorie and fat content. What more if the foods are prepared by using recycled cooking oil or repeatedly heated oil. Similar to recycled cooking oil, repeatedly heated, or thermally oxidized oil, have been extensively reported to cause adverse effect to health such as cardiovascular disease. It is a normal practice for food outlets operators to use frying oil repeatedly until the oil is fully degraded for cost cutting measure.

A case-controlled study conducted by Iqbal et al. (2008) involving subjects from 52 countries demonstrated that an unhealthy diet, mostly derived from fried food can increase the risk of cardio vascular disease (CVD). The incidence of CVD will become the leading cause of mortality in Malaysia, where 25% of medically certifiable deaths are due to CVD (Rahim, 2009). By 2010, it is projected to be the leading cause of death in Malaysia and other developing countries (*New Straits Times*. 2008. 4 April). This situation shows how dietary habit of Malaysians, whether under or out of their control, especially in terms of fried food, are exposing themselves to cardiovascular disease and other deleterious effects mentioned above. To prevent the hazardous impact of recycled, repeatedly heated cooking oil and increase in cardiovascular disease in Malaysia, changing the dietary habit of Malaysian such as avoiding fried or processed food is deemed to be impossible. Thus, one of the measures that can be taken is controlling the quality of the frying oil itself, which is the compulsory ingredient in fried or processed food. Quality and quantity of fat in foods served at food outlet or food manufacturing company should be monitored especially by health regulatory authorities.

Foods served by outlet operators and food manufacturer usually prepared by deep frying technique. The technique is preferred as it can give valuable sensory taste such as good aroma, crispy, crunchy texture, juicy taste, convenient and relatively low cost in large scale frying (Saguy and Dana, 2003). In practice, frying oil is usually replenished and reused several times prior discarded for lower cost purpose.

However, with the presence of air and moisture during the frying process especially at high temperature between 160°C to 180°C, reusing and recycling the oil will degrade the oil quality as several processes occur such hydrolysis, polymerization, (Bhattacharya et al., 2008; Ramadan, 2010), formation of conjugated dienes, decomposition of hydroperoxides (Yoon et al., 2000). The processes lead to the formation of decomposition product which consists of volatile and non volatile component. Most of the volatile compounds are lost during the frying process while non volatile are of concern as it can accumulate in the degraded oil, absorbed into food, ingested, and enter human diet, causing hazardous effect to health.

Due to the hazardous impact of thermoxidized oil to health, food production process by the food outlet operators and food manufacturer need to be monitored by regulatory authorities to avoid unethical food preparation process such as overused of heated oil, using degraded oil as food ingredient and recycling or selling degraded oxidized oil as a new product.

But the biggest question remain is what is the best, simplest, conclusive method that can be applied as routine analytical approach to determine quality of frying oil. Determination of total polar compounds is recognized as the most reliable method to measure oil degradation (Fritsch, 1981; Marquez Ruiz et al., 1995; Gil et al., 2004; Bansal et al., 2010) and this method has been a standard method to determine oil degradation in some countries (Akoh and Min, 2002).

However, determination of total polar components is time consuming for routine analytical purpose, and the use of large volume of solvents is considered potential environmental problem (Innawong et al. 2004; Ramadan, 2010). These disadvantages are the reason to search for a new generation of rapid methods for the analysis of deep-

frying oils. Other method that correlate well with total polar materials need to be determined to replace this time consuming method.

Rapid test kits to determine oil quality are commercially available. This should solve the problems of time consuming total polar compounds method. However, according to Bansal et al. (2010b), most of the test kits need to be calibrated, so it is really 'rapid' in a real sense. It also based on colorimetric reactions, which is subjective. The biggest setback for these kits is disagreement of results with acceptable conventional wet chemical method.

Physical evaluation such as the odor, color, formation of excessive foaming of the oil is normally conducted and they are the most obvious changes that can be observed even for the non-expert (Abdulkarim et al., 2007). The advantage of physical properties is they are relatively fast and easier to be measured. Moreover, physical properties evaluation usually does not involve hazardous chemicals (Bansal et al., 2010a). However this evaluation depends heavily on the perceptions, judgments of the evaluator and is not reliable between different analysts (Billek et al, 1978).

Chemical methods involve measuring fatty acids composition, free fatty acids value, iodine value, and content of conjugated double bounds (Al Harbi and Al Kahtani, 1993; Gil et al., 2004; Naz et al., 2005; Abdulkarim et al., 2007; Bansal et al., 2010a). All of the chemical methods above depend on the frying parameters such as type of food, oil and the temperature during frying operation. Other factors during frying such as frying temperature, emulsifiers, trace metals, food scraps, free fatty acids and alkaline-reacting materials (Bhattacharya et al., 2008) in the frying oil will cause different type of degradation. Oil consists of fatty acids with a variety in chain length and degree of saturation, so different oil will react different way towards degradation factors.

In addition, chemical method can only roughly determine the quality of oil (Hein et al., 1998), inconclusive and involve measuring non specific compounds. A specific method may be ideal for one operation but completely useless in another (Fritsch, 1981).

Hence, in a study dealing with oil oxidation and degradation, it is desirable to have simple method, independent of factors involve during frying process and will give conclusive results. Specific component need to be detected, regardless of its amount, and will give clear cut difference between unused oil and degraded oil, and can act as marker for oil degradation.

This present study was carried out to understand oil degradation level after thermally treated and oxidized. Several chemical parameters that have been used in literatures in determining oil degradation status will be tested. The assessment parameters were fatty acid composition and saturation level of the oil, presence of trans and short chain fatty acid, free fatty acid content, iodine value, level of conjugated fatty acid. These parameters were tested to determine which one correlates well with total polar compounds and can replace the time consuming method.

Statistical analysis was conducted to determine correlation between those chosen parameters and total polar compounds. The assessment parameters also were evaluated to determine which one is the simplest to be applied as routine analytical procedure, and can give conclusive, clear cut different between fresh and used oil regardless of oil type and frying parameters.

Palm oil was chosen as sample in this study owing to their common use as cooking medium by Malaysian. The samples in this study were fried oils collected from several food outlets. These fried oils were different in terms frying parameters and condition such as frying temperature, type of food, type of oil and fryer dimensions. This is to signify different frying variables and condition that can influence type and rate of oil degradation.

Oil samples also were subjected to frying and heating in laboratory under controlled condition. These samples acted as control sample in this study. Frying and heating were compared to understand the influence of food towards oil degradation. Corn oil also was included as sample control in this study because it is relatively has

high activity towards oxidation compared to palm oil, and comparison was made between these two oils.

1.2 PROBLEM STATEMENT

Quality of edible oil sold in the market and oil used in food manufacturing process and food outlets are questionable. The quality should be regulated by health regulatory bodies. But the question remains, what is the best method to determine oil degradation status. Several analytical approaches are available to determine oil deterioration level. However those methods are time consuming, not suitable for routine analysis, depends on oil type and frying condition, and some could not give conclusive results. Most published researches on degraded oil mostly conducted on corn, canola and soya bean oil which properties are different from palm oil, the main cooking oil consumed by Malaysian. Thus the results from those studies could not be applied on palm oil. Previous analysis normally conducted by severe heating and condition of oil in laboratory, which is not representative of real frying condition, and no sampling at food outlets were conducted.

This study was carried out to understand palm oil degradation level. Oil samples were collected from various food outlets that are usually patronized by Malaysian and notorious for using oil until fully degraded. It is also to determine simple, conclusive method that can be applied as routine analysis to evaluate palm oil degradation status and replace total polar compounds as standard method as this method is reliable but time consuming for routine analysis.

1.3 OBJECTIVE

- To determine properties of fresh and thermally oxidized palm oil

- To test available analytical methods in oil degradation evaluation to determine the best and simplest method that can be applied as routine analysis in determining palm oil degradation level.

- To determine component that is conclusive and can act as marker to degraded oil

1.4 HYPOTHESIS

- Fresh and thermally oxidized oil have different properties in terms of fatty acid composition, total polar compounds, iodine value, free fatty acid, color formation.

- Analysis using wet chemical methods such as total polar compounds, iodine value, free fatty acids are expected to be time consuming compared to analysis run by instrument such as gas chromatography and UV spectrophotometer. Color formation, free fatty acid, iodine value, total polar compounds, conjugated fatty acid, changes in fatty acid composition is expected to be influenced by oil and food composition

- Octanoic acid, a short chain fatty acid, can act as marker to differentiate between the fresh and degraded oil.

CHAPTER 2

LITERATURE REVIEW

2.1 FATS AND OIL

Fats and oil are major nutrient in the human diet and more than 90% of global oil production is used as food or as ingredient in food products (Moreno et al., 1999). Oils and fats consist mostly of more than 95% of triacylglycerol (Heldman and Lund, 2007; Othmer, 2008). One molecule of triacylglycerol (TAG) composed of three fatty acids esterified to a glycerol, hence its name. The hydroxyl group of the glycerol joins with carboxyl groups of the fatty acid to form ester bonds (Akoh and Min, 2002). The three fatty acids may or may not be identical, thus an edible fat or oil may contain more than 500 different TAG (Heldman and Lund, 2007). Figure 2.1 illustrates how fatty acids are connected to one molecule of glycerol to form TAG in oil.

Gilbert et al. (2009) and Othmer (2008) defined fats as triacylglycerol that composed primarily of saturated fatty acids and solid at room temperature, while oils are composed predominantly of unsaturated fatty acids and liquid at room temperature.In food preparation, oils and fats contribute to flavor and palatability (Akoh and Min, 2002) while during frying, oil acts as heat transfer medium (Al Harbi and Al Kahtani, 1993).

Oxidized oils and fats are responsible for rancidity, development of off flavor, loss of fat soluble vitamin and pigment in foods (Heldman and Lund, 2007), production of toxic compounds and can cause the oil and fats to become unacceptable for consumers for edible or other use (Othmer, 2008).

In biological aspect, fats and oil supply energy, support structural aspects of the body, and provide substances that regulate physiological process. However excess in fats in the body can produce harmful effect (Akoh and Min, 2002).

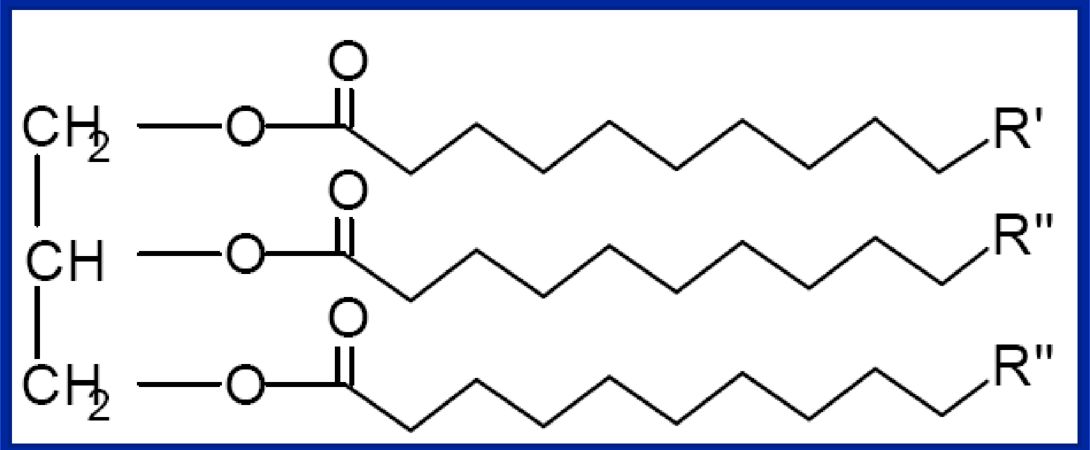


Figure 2.1: Structure of triacylglycerol in oil.
R', R'' and R''' indicate fatty acid chain length.

2.2 PALM OIL

Palm oil is widely used in various food products, such as margarines, shortenings, cooking oils, confectionery fats and vanaspati (Saad et al., 2007). Most Malaysian are consumers of palm oil. Corley (2009) stated that Malaysia has highest palm oil per capita consumption at 183 kg/head, compared to other region such as China with 10.6 kg, 17.7 kg in India and 39.3 kg in the USA. This high consumption might be due to its relatively low in price compared to other vegetable oil.

Survey in hypermarket chain also confirmed that the price is cheaper compared to other vegetable oil. This is in line with status of Malaysia as a leading producer of palm oil in the world (Basiron, 2002; Othmer, 2008; Abdullah et al. 2009).

Palm oil is semi solid at room temperature, and palm olein, which is fraction of palm oil, is widely used for industrial frying (Berger and Idris, 2005; Bansal et al.,

2010a). It is commonly used for preparation of fried foods due to its availability and better stability (Bhattacharya et al., 2008).

Palm oil occupies an intermediate position among natural fats as its saturated acid is 50% and the rest is unsaturated fatty acid. The fatty acid composition contributes to palm oil semi solid properties (Yadav, 2006), thus it is not required to undergo hydrogenation process, which is process of oil hardening, and can result in formation of trans fatty acids.

Trans fatty acid is the main issue in edible oil industry, as it is said to be deleterious to health. The move away from trans-fatty acids favors palm oil, as it can be used without hydrogenation (Muller et al., 1998; Tang, 2002; Berger and Idris, 2005; Moraes Mizurini et al., 2010). Palm oil contains high level of saturated fatty acids and it is the most important edible source of palmitic acid or hexadecanoic acid (C16:0), which is saturated fatty acid (Bautista et al., 2001). Its saturated and unsaturated fatty acid ratio is close to one and contains a considerable amount of carotene, an antioxidant (Noh et al., 2002).

Palm oil usually is consumed in the oxidized state due to heat exposure during cooking (Leong et al., 2008). In terms of susceptibility towards oxidation, palm olein and palm oil are low in sensitivity compared to oil with high unsaturated fatty acids contents (Al Harbi and Al Kahtani, 1993). Therefore it is widely used as a long-lived frying medium and gaining popularity in food industry (Berger and Idris, 2005; Leong et al. 2008).

Leong et al. (2008) observed that prolonged consumption of repeatedly heated palm oil may result in an increase in blood pressure level with necrosis of cardiac tissue. Al Harbi and Al Kahtani (1993) studied effect of feeding discarded palm oil sample to rats. They discovered oil palm did not give effect as deleterious as other oil with high polyunsaturated fatty acids contents, this is in contrast to research carried out by Rueda Clausen group in 2007. They discovered palm oil gave similar effect with oil with high unsaturated contents like sun flower and olive oil.

2.3 DEEP FRYING METABOLISM AND ITS EFFECT

Deep frying involves the process of immersing food item in large quantity of heated oil (Al Harbi and Al Kahtani, 1993). It is one of the most popular procedure for food preparation since it is rapid, develops desirable flavor, color and texture (Takeoka et al. 1997; Rossi et al. 2009). In cooking process, frying is favored over other methods. This is because, in roasting or oven cooking, the heat conductor is the air, but air is not a good heat conductor, so cooking with this method is a slow process (Berger, 2005) while cooking with oil will result in more rapid penetration of heat into the product being cook (Stevenson et al., 1984). For boiling process, the temperature of cooking is limited to the boiling point of water which is 100 °C, thus desirable crispy texture would not be achieved. Meanwhile, immersing food in hot oil can retain all the food flavors and juices within the crispy crust (Sanchez-Gimeno et al., 2008), thus frying can produce crispy texture at the outside and juicy texture at the inside.

Frying operation normally is conducted at 175–195 °C (Saguy and Dana, 2003; Alvis et al., 2009). Heat and mass transfer occur during frying and the amount depends on factors such as temperature, warm up time, oil type, food, oil rotation, its manipulation, and finally, the equipment used (Alvis et al. 2009; Rossi et al. 2009). Heat is transferred from the hot oil to the surface of the food material, while moisture is transferred from the food interior to the food surface (Yildiz et al., 2007). It is also considered food dehydration process and more exactly a procedure of water extraction by convection with change of state (Alvis et al., 2009).

With repeated frying, the presence of moisture, heat, the incorporation of oxygen into triacylglycerol structure in the oil can cause occurrence of several processes. If oxygen is scarce, thermolytic reactions will take place. In the presence of air, both oxidative and nonoxidative reactions will occur simultaneously, while heat will accelerate the changes (Nawar, 1984).

During frying, water presence in food will vaporize and the steam that is formed will hydrolyze triacylglycerol, forming free fatty acids (Bhattacharya et al., 2008).

Besides hydrolysis of triacylglcerol molecule by water, free fatty acids can be formed as a result of oxidation of double bonds (Stevenson et al., 1984).

Presence of air can lead to oxidation. Oil oxidation initiates through a chain reaction and is referred as induction state while the time before a dramatic increase in the rate of oxidation is called induction period (Tan et al., 2002). During oxidation initial stage, hydroperoxides accumulate as primary oxidation product. Due to the instability of the hydroperoxide, it will further take part in few reactions, then decomposed and broken down into low molecular weight oxygenated constituents secondary oxidation products such as alcohols, aldehydes, free fatty acids, and ketones, volatile compounds, carboxylic acids, polymers, oligomers and monomers of modified triglycerides (Bester et al., 2008; Farhoosh et al., 2009). The structure and reaction of hydroperoxide can exert influence on degradation rates. Scission at the hydroperoxide molecule yield alkoxy radicals. If the alkoxy radicals gain hydrogen atom, hydroxy derivatives will be formed while losing hydrogen will lead to the formation of keto derivatives (Nawar, 1984). Fission reaction can result in the production of alcohol, aldehydes, acids and hydrocarbon while dehydration of hydroperoxide results in ketone formation (Stevenson et al., 1984; Gullen and Cabo, 2002).

The rate of oxidation is reported to be roughly proportional to the degree of unsaturation of the fatty acids present (Stevenson et al., 1984). It is generally established that the reaction of unsaturated fatty acids with molecular oxygen proceeds via typical free radical mechanisms. Since direct reaction of unsaturated linkages with oxygen is thermodynamically difficult (Nawar, 1984), it has been proposed that preformed hydroperoxides due to heat or exposure to light or by mechanisms where singlet O_2 as free radical is the active species involved in attacking double bond. The free radicals can propagate the abstraction of hydrogen atoms adjacent to double bonds of fatty acids, followed by O_2 attack at double bonds (Naz et al., 2005).

Besides heat, moisture and oxygen, oil alterations are influenced by several factors such as food emulsifiers, trace metals, free fatty acids and alkaline-reacting materials in the frying oil. Sodium and potassium ions that are transported to the frying oil can form alkaline soaps, which in turn stimulate foaming of the oil (Bhattacharya et

al., 2008). All non lipid material from the food such as proteins, carbohydrates, water, enzymes, salts, vitamins, antioxidant and pro oxidant can interact with the oil (Nawar, 1984). Protein will form complex with oxidized lipid and the degree of complex increase with the increase level of fatty acid unsaturation (Narayan and Kummerow, 1963). Complex chemical reactions take place such as gelatinization of starch, denaturation of protein and Mailard reaction (Berger, 2005). Mailard reaction occurs with the presence of sugar and protein, resulting in the browning of food being fried (Stevenson et al., 1984). Maillard browning products and their precursors are the major contributing substances to the discoloration of frying oil.

Oil that is used for deep frying normally is replenished and reused several times before being disposed. The presence of water, heat, moisture will cause oil changes and decomposition. Oil degradation products consist of volatile and non volatile products. In general, products with molecular weights more than 1800 daltons are non volatile, and those with molecular weights less than 1800 daltons are volatile. Volatile products usually are removed during volatilization process, while triacyglycerol, which are 99% of unused oil composition, weights in the range of 900-1000 daltons and are not very volatile at normal frying temperatures (Melton et al. 1994). All these decomposition products will affect the quality of an oil.

Bansal et al. (2010a) showed that oxidation progresses more rapidly in heating process where oil is heated without presence of food compared to frying process. It was the other way around for Bhattacharya et al. (2008). They found out fried oil deteriorated further than heated oil. As stated by Alvis et al. (2009), combination of frying parameters such as moisture content of the food, oil rotation and manipulation, warm up time, oil type and the equipment can lead to different rate of degradation between different frying and heating process.

Besides effecting nutritional value of the oil, food functional and sensory quality, thermoxidized oil can give deleterious effects on health. This is because the non volatile compound with high molecular weight and polarity that is formed during oil degradation can remain in the oil and subsequently enter human diet.

Leong et al. (2008) reported that feeding heated oil to rats resulted in increase of blood pressure and organ damage. Due to the decomposition products that are formed, oxidized oil also is said to be genotoxic (Hageman et al., 1988), mutagenic (Hagemen et al., 1988; Bautista et al., 1999), promote intestinal tumor (Aladedunye and Przybylski, 2009), organoleptic failure (Hein et al., 1998), carcinogenic (Yildiz et al., 2006), liver damage, promote deleterious effect in the endothelial function (Williams et al., 1999; Rueda Clausen et al., 2007) and growth retardation (Al Harbi and Al Kahtani, 1993).

Due to adverse effect of over heated oil, oil quality in food preparation process need to be monitored. Usually the physical and chemical properties of the oil are investigated. Physical properties such as smoke point, odor, color, formation of foam, viscosity are evaluated. Chemical properties are fatty acid composition, free fatty acid value, iodine value, peroxide value, and total polar compounds. The physical properties are relatively easier to measure as compared to the parameters involving chemical analysis, (Bansal et al., 2010a), however, assessment based on color, taste and odor can give some doubt in evaluation and depends on the perception of the evaluator (Billek et al., 1978).

For chemical evaluation, the determination of total polar materials in a frying fat provides the most reliable measure of the extent of deterioration in most cases (Fritsch 1981; Bansal et al., 2010a), but the methods are impractical, use a large amount of solvent, uneconomic and time consuming for large scale and routine operation (Billek et al., 1981; Paradis and Nawar, 1981; Innawong et al., 2004; Ramadan, 2010). Other chemical methods, such as fatty acid composition, free fatty acid value, involve measuring nonspecific compounds (Abalos et al., 2000). Iodine value depends heavily on the type of oil (Naz et al., 2005).

Since many factors involve in frying process, specific method might be impractical between different frying conditions (Abalos et al., 2000). For example, amount of free fatty acids has been an indicator of oil degradation. However, free fatty acids are the result of hydrolysis caused by presence of moisture in the food being fried in the oil. Oil that is used to fry dry food such as crackers and chips will produce amount of free fatty acids that are relatively low compared to oil that is used to fry food

that contain high moisture content such as fish and meats. Low free fatty acids level does not signify that the fried oil is not degraded. This is because, besides hydrolysis, there are other degradation processes such as oxidation and polymerization that can lead to oil degradation. So relying on the amount of free fatty acids can lead to underestimation of oil degradation (Al Harbi and Al Kahtani, 1993).

All of the methods above can be used to evaluate frying oil quality, however, some methods are non conclusive, can not give clear cut difference between fresh and heat abused oil. Some methods such as free fatty acid value, iodine value, depend on type of food and oil used during frying. Method that is conclusive such as total polar compounds are time consuming. Simple, objective method (Augustin et al., 1987), be independent of type of food and oil, insensitive to water content of food, (Ramadan, 2010) is needed for routine analysis.

2.4 FATTY ACIDS

Edible fats and oils consist almost entirely of fatty acids (Priego-Capote et al., 2007). In oil, three fatty acids are bound to one molecule of glycerol, forming triacylglycerol. Fatty acid has carboxyl group (COOH). The carbon chain is either saturated which is containing no carbon-carbon double bond or unsaturated which is containing one or more carbon-carbon double bond. Figure 2.2 shows general structure of fatty acid. Most fatty acid that occur in natural oil and fats are straight chain acids which contain an even number of carbon (Heldman and Lund, 2007; Othmer, 2008).

There are several ways in naming fatty acid. In IUPAC nomenclature, fatty acid is named after parent hydrocarbon, and double bond is counted from carboxyl acid group (IUPAC, 1978). For example, 18 carbon hydrocarbon chain, with double bond located between 9th and 10th carbon from carboxyl group is called 9-octadecenoic acid. Fatty acid also has trivial name. For example oleic acid is trivial name for 9-octadecenoic acid. Shorthand nomenclature is also common for fatty acid. The shorthand designation is the carbon number in the fatty acid chain followed by a colon, then the number of double bond and the position of the double bond closest to methyl side of the fatty acid molecule (Akoh and Min, 2002). For example, short hand

nomenclature for oleic acid is 18:1 ω9. Naming of fatty acids in this paper is based on three systems above, where appropriate and convenience.

Figure 2.2: Saturated and unsaturated fatty acid

2.4.1 Saturated fatty acids

Saturated fatty acids do not contain any double bonds or other functional groups along the chain. Carbons atoms apart from the carboxylic acid group contain maximum hydrogen atoms possible. Figure 2.2 shows example of saturated fatty acid. Besides naturally occurred, a process called hydrogenation will produce saturated fatty acid. This process will add hydrogen atoms to carbon molecule in fatty acids. Hydrogenation process is conducted to make the fats to become solid at room temperature and higher melting points, rendering stability towards the oil (Tang, 2002). Thus, oil with high saturated acid content is said to have lower sensitivity towards oxidation.

Palmitic acid (16:0) is one of the main saturated fatty acid in the human diet (Bautista et al., 2001). However several studies have shown that palmitic acid has a hypercholesterolemic effect (Khosla and Hayes, 1992; Kritchevsky et al. 2001). Leong et al. (2008) observed that diet containing high amount of saturated fatty acids can increase blood pressure. Moraes Mizurini et al. (2010) in their studies on diet rich in saturated fats discovered the diet can have a negative effect on the haemostatic

parameters that may influence the propensity for thrombosis, a marker of cardiovascular risk.

2.4.2 Monounsaturated fatty acids (MUFA)

Fatty acid is termed as monounsaturated fatty acid (MUFA) when it has one double bond along the carbon chain. The most common monounsaturated fatty acid is oleic acid (Akoh and Min., 2002). Figure 2.2 shows structure of monounsaturated fatty acid. These fatty acids are considered healthy fats because they tend to lower the Low Density Lipoprotein (LDL) or termed as bad cholesterol without lowering the good one, which is the High Density Lipoprotein or HDL (Karabulut et al., 2003; Abdulkarim et al., 2007; Molla et al., 2007; Priego-Capote et al., 2007; Kandhro et al., 2008).

Despites having good health effect compared to saturated fatty acid, MUFA has undesirable property in terms of frying stability. Priego-Capote et al. (2007) found out oil with high MUFA content turns rancid easily. Composition of oleic acid (18:1), one of MUFA, decreased after heat treatment (Cuesta et al., 1991). It shows that MUFA also is not stable when thermally treated. However, compared to polyunsaturated fatty acids, MUFA is relatively higher in stability towards oxidation (Abdulkarim et al., 2007).

2.4.3 Polyunsaturated fatty acids (PUFA)

This fatty acid has two or more double bond along the chain. The double bonds in poly unsaturated fatty acid (PUFA) can be methylene interrupted or conjugated. In methylene interrupted double bond, the double bonds are separated from each other by a single methylene group, while in conjugated double bonds, one of the double bond undergoes migration or shifted towards a structure where unsaturated centre are adjacent to each other (Leray, undated). Figure 2.3 shows example of methylene interrupted and conjugated PUFA.

Polyunsaturated fatty acids (PUFA) occur mostly in vegetable oil source (Priego-Capote et al., 2007). Fatty acids such as linoleic and linolenic acids are called

essential fatty acids as it cannot be synthesized in the body, only can be obtained from the diet (Molla et al., 2007).

HO O CH3
Methylene interrupted double bond

HO O CH3
Conjugated double bond

Figure 2.3: Polyunsaturated acid: Methylene interrupted and conjugated

Polyunsaturated fatty acids are not relatively stable as the structure is not packed together very well. During processing and storage, they are easily oxidized even at room temperature. This is because oxygen usually attacks the triglyceride close to the double bonds in the fat due to lowered activation energy in the initiation of free radical formation (Akoh and Min, 2002). Warner et al. (1997) discovered that content of linoleic acid which is one of PUFA, decreased after frying cycle. It also can influence the formation of polar compounds which is the compounds that is considered as marker for oil degradation.

Augustin et al. (1987) discovered strong linear correlation between linoleic acid content and the total polar compound content. Moreno et al. (1999) claimed decrease in unsaturation degree after an oil has been fried or heated is evidence of transformation towards PUFA and can indicate a decrease of oil nutritional value.

Despite instability towards thermal treatment and oxidation, the presence of PUFA in the diet is desirable. Oils that contain mainly unsaturated fatty acids are preferable compared to saturated animal fats like butter, bacon fat and also hydrogenated vegetable products (Molla et al., 2007). Ingestion of PUFA in diet is

associated with great influence to health. It has been reported that unsaturated fatty acid rich oils have a beneficial effect on the endothelial function (Rueda Clausen et al., 2007), protective against myocardial infarction (Baylin et al., 2007), regulation of lipid levels (Kandhro et al., 2008), necessary for the normal development and functioning of human tissues (Moreno et al., 1999), prevents high blood pressure (Ajayi et al., 2006), implicated in cancer prevention and treatment (Gleissman et al., 2010).

2.4.4 Linoleic acid/ palmitic acid (C18:2/C16:0) ratio

The linoleic acid/ palmitic acid (C18:2/ C16:0) ratio has been one of the parameter to determine oil degradation. This is because linoleic acid is more susceptible to oxidation, whereas palmitic acid is more stable toward oxidation (Bansal et al., 2010a).

Lower 18:2/16:0 value indicates that linoleic acid content has decreased, a signal of oil degradation. Al Harbi and Al Kahtani, (1993), Cuesta et al. (1991), Aladedunye and Przybylski (2009) and Bansal et al. (2010a) discovered lower value after frying cycles. Aladedunya and Przybylski (2009) and Rossi et al. (2009) found out oil with higher unsaturation degree will have higher degradation of linoleic acid content after thermally treated.

2.4.5 Trans fatty acids

In fatty acid, normally double bond occurs in cis configuration. However, geometrical isomerization or cis trans isomerization can occur. Cis and trans configuration differ in terms of hydrogen atom arrangement on the double bond. In cis configuration, the double bond contains hydrogen atm on the same side of each other while in trans configuration, the hydrogen atoms are opposite site of each other. This double bond can be located on different sides of the aliphatic chain, resulting in positional isomers (Ledoux et al., 2000). Figure 2.4 illustrates configuration of cis and trans isomer.

Trans fatty acid (TFA) has different physico-chemical, nutritional, biological and biochemical properties from cis isomer (Ledoux et al., 2000). TFA has higher melting points than the cis isomer due to its ability to pack themselves in systematic manner (Sherazi et al., 2009).

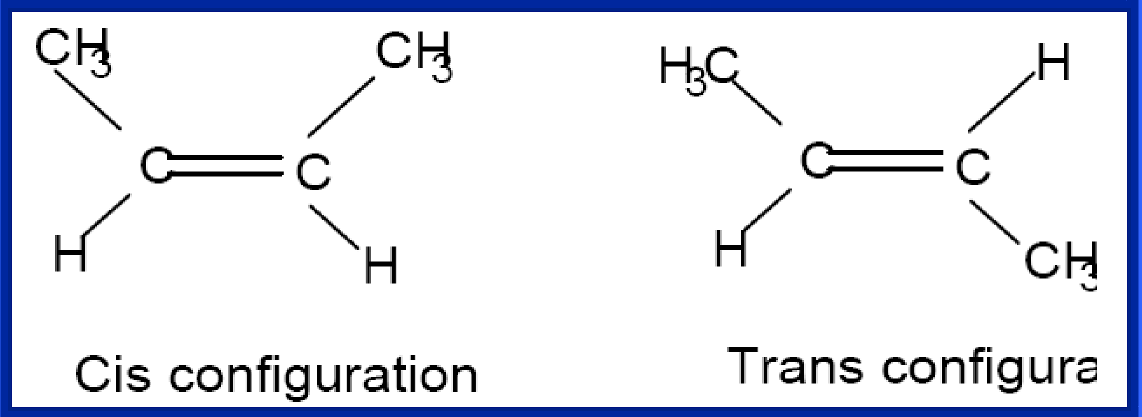


Figure 2.4: Cis and trans configuration of fatty acid

Figure 2.5 shows the structure of elaidic acid, which is the trans isomer of 18:1 fatty acid and the structure of oleic acid, which is the cis isomer. It can be seen from the structure that cis configuration has slight kink in its structure compared to trans where the structure is straight. Oleic acid and linoleic acid are illustrated as straight in figure 2.2 and 2.3, due to comparison purpose in terms of presence of double bond with saturated fatty acid counterpart. But in reality, cis unsaturated fatty acid is kink in its structure. Straight properties enable the trans fatty acid to pack themselves, thus having higher boiling points.

In nature, fatty acids normally occur in cis isomer. However, in herbivore especially the lactating cows, trans fatty acids are produced by anaerobic fermentation of polyunsaturated fatty acids in the rumen and the fermentation process is called biohydrogenation (Destaillats et al., 2007). Incomplete hydrogenation results in formation of trans fatty acids (Alves et al., 2008).

Trans fatty acids normally produced by industrial process such as deodorization (Aladedunye and Przybylski, 2009) and hydrogenation. Hydrogenation is the major source of trans fatty acids in the human diet (Tavella et al., 2000; Mjos, 2005; Huang et al., 2006; Baylin et al., 2007; Liu et al., 2007; Priego-Capote et al., 2007; Saunders et al., 2008; Bansal et al., 2009; Sherazi et al., 2009). Hydrogenation is the process where at elevated temperature, with nickel catalyst, in the absence of oxygen, hydrogen is bubbled through the fat.

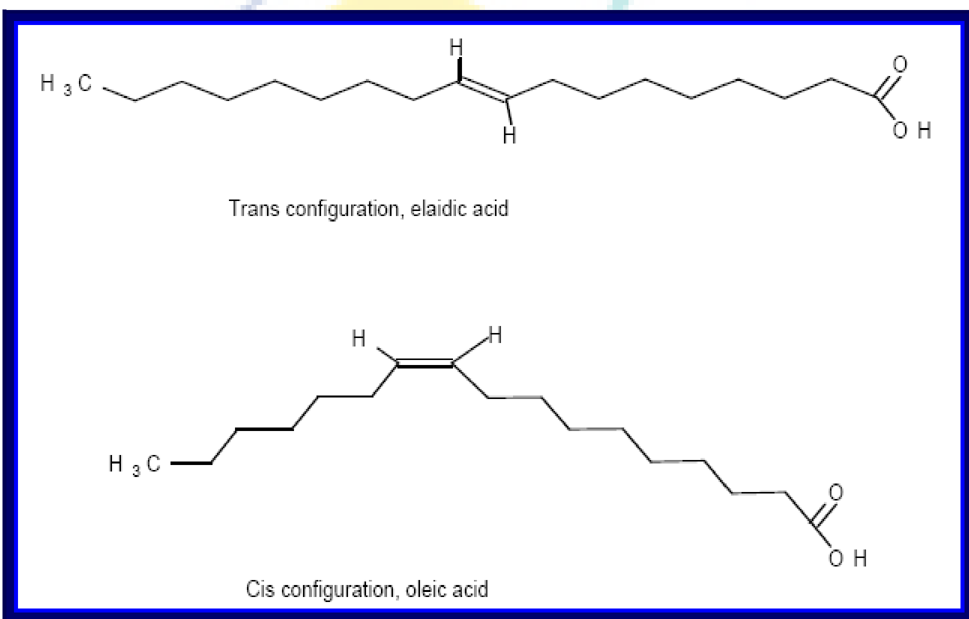


Figure 2.5: Trans fatty acid and cis fatty acid

Incomplete or partial hydrogenation rearranges the double bonds, converting some of them to the trans configuration and shifting the double bonds along the carbon chain (Priego-Capote et al., 2007). Vassenic acid (trans-11-18:1) and elaidic acid (trans-9-18:1) are the main product in biohydrogenation and industrial hydrogenation respectively (Destaillats et al., 2007). Besides bio and industrial hydrogenation, TFAs can also be produced during thermal treatment process such as deep frying (Romero et al., 2000; Bansal et al., 2009) and heating (Mjos, 2005; Tsuzuki et al., 2008). For hydrogenation, TFAs are mainly in one or two double bond (Sebedio et al. 1981). For thermal treatment, TFAs can occur in more than one and two double bonds. However

thermal isomerisation is almost exclusively geometrical isomerisation, not positional isomerization (Mjos, 2005).

The main concern of trans fatty acids is the intake of trans fatty acids is reported to be deleterious to health (Mjos, 2005; Baylin et al., 2007). It has been associated with coronary heart disease (Katz, 2002), produce adverse effects on blood lipids, including increasing LDL-cholestrol concentration and decreasing HDL-cholestrol concentration (Priego-Capote et al. 2000; Han et al. 2002; Mauger et al. 2003; Bansal et al. 2009), disturb development and growth of infants (Elias and Innis, 2001), influence vascular functions, cardiac arrhythmias (Kandhro et al., 2008), cause systemic inflammation in women, which may be involved in the pathogenesis of coronary artery disease (Mozaffarian et al., 2004), endothelial dysfunction (Baer and Judd, 2004), inhibit the metabolic conversion of linoleic acid to arachidonic acid and to other polyunsaturated fatty acids, a risk factor in the development of coronary heart disease (Kummerow et al., 2004). TFAs also can interfere with the metabolism of essential fatty acids, reducing the availability of fatty acid precursors for the synthesis of antihemostatic prostaglandins (Kinsella et al., 1981). Trans fatty acids is said to cause more unfavorable effect on postprandial tissue plasminogen activator activity than one rich in palmitic acid, which has been shown to correlate with a decrease in HDL cholesterol (Moraes Mizurini et al., 2010).

Due to health impact of trans fatty acids and requirement to include it in food labeling, the analysis of trans fatty acids require higher precision to avoid under or overestimation of its content. Effective January 1, 2006, the U.S. Food and Drug Administration requires the labeling of the amount of trans fatty acids per serving in packaged foods (Moss, 2006). Various methods available to analyze TFA, but the choice of method depend on the goal of studies. Normally food manufacturer wants to know the total level of trans fatty acids while biochemist needs detailed information of TFAs isomer (Ledoux et al., 2000).

Tang (2002) screened TFA content of edible oil available in Malaysian market. He discovered TFA is generally absent in crude palm oil. However in refined cooking palm oil, TFA present at 0.25%-0.67%. In non palm based cooking oil, especially

cooking oil with higher unsaturation degree showed higher content of TFAs, about 0.43% - 3.83%.

In thermally treated or deep fried oil, Aladedunye and Przybylski, (2009) and Bansal et al. (2009) discovered that the amount of trans fatty acids increased in correlation with frying temperature and time. They also discovered that cooking oil that contains more polyunsaturated fatty acids showed higher amounts of trans fatty acids after frying cycles. Palm oil gave lowest amount of trans fatty acids as this oil is highly saturated. In Bansal group research, isomers that are more prone to geometrical isomerization were linolenic acid, followed by linoleic and oleic. These finding is similar with research by Tsuzuki group in 2008. They suggested that triolein was most resistant to heat deterioration among the triolein, trilinolein and trilinolenin.

The exchange of TFA from food and frying oil has also been observed by some researchers. Bansal et al. (2009) discovered that fried oil contained more trans fatty acids compared to heated fatty acids, might be due to the transfer of trans fatty acids from food that is fried in the frying oil to the oil. The foods being fried might already contain trans fatty acids. Romero et al. (2000) in studying the interaction between food and frying medium discovered that frequent replenishment of fresh oil throughout the frying process minimized the fatty acid changes. Fried oil with non replenishment showed higher amount of TFAs compared to fried oil with frequent replenishment. According to them, this might be due to the dilution of altered compounds happen when frying medium is replenished with fresh oil.

Tsuzuki et al. (2008) studied the influence of lipid oxidation towards TFA formation. In their studies, they discovered when triolein was heated under a nitrogen stream, neither trans isomerisation nor polar compounds were detected. Addition of antioxidant considerably prevented geometrical isomerization. They found out TFA formation in heat treatment is accompanied or as a side chain of formation of polar compounds but the polar compound itself did not catalyze the cis trans isomerization.

2.4.6 SHORT CHAIN FATTY ACIDS

These fatty acids are of particular interest in this present study as several previous studies indicated that these fatty acids can differentiate between fresh and thermally oxidized oil. These fatty acids are saturated short-chain fatty acid, which are heptanoic acid (C7:0) and octanoic acid (C8:0), and saturated short chain aldehydic fatty acid, which are 8-oxo-octanoic acid (8-oxo-C8:0) and 9-oxononanoic (9-oxo-C9:0). These compounds are originated from the oxidation of oleic acid and linoleic acid as proposed by Berdeaux et al. (2002), Kamal Eldin et al. (1997) and Velasco et al. (2005) because these two fatty acids are the most representative of fatty acids undergoing oxidation in oil.

Oxidation causes formation of hydroperoxide, but hydroperoxide is unstable, further taking part in reaction such as homolytic β-scission of the alkoxy radicals of the hydroperoxides, result in the formation of secondary oxidation product, which are volatile and non volatile compounds. Figure 2.6, Table 2.1 and 2.2 show formation of these compounds. The volatile product will escape into the air while non volatile will remain in the oil (Berdeux et al., 1999; Velasco et al., 2005).

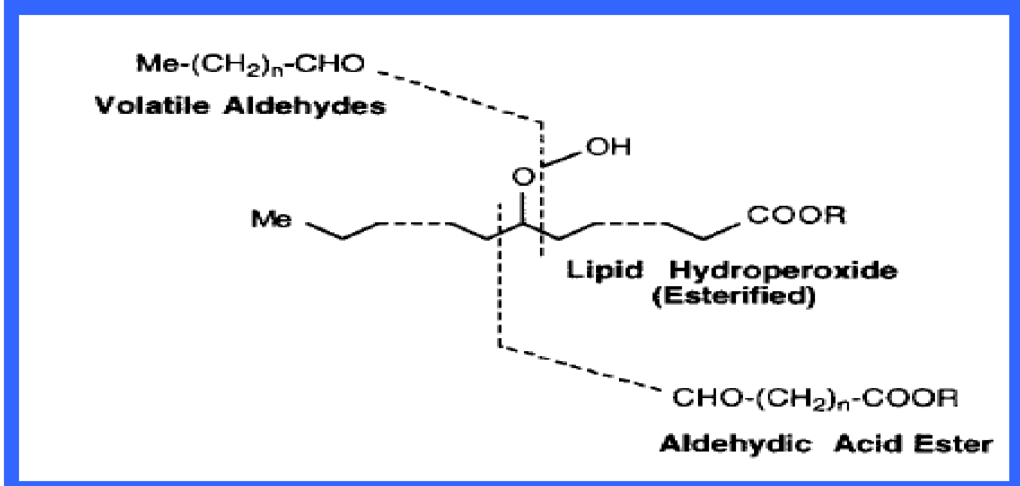


Figure 2.6: Break down of hydroperoxide to volatile and non volatile component

Source: Kamal Eldin et al. (1997)

Table 2.1: Volatile compounds formed by decomposition of hydroperoxides of oleic acid and linoleic acid

Fatty acyl group	Hydroperoxides	VOLATILE COMPOUNDS		
		Route A*	Route B**	
			+ $OH^{\cdot}$	+ $H^{\cdot}$
Oleate	8-ROOH	undec-2-enal	decanal	dec-1-ene
	9-ROOH	dec-2-enal	nonanal	non-1-ene
	10-ROOH	nonanal	octanol	octane
	11-ROOH	octanal	heptanol	heptane
Linoleate[1]	9-ROOH	deca-2,4-dienal	non-3-enal	nona-1,3-diene
	13-ROOH	hexanal	pentanol	pentane

Source: Dobargens, M.C. (2009)

Table 2.2 Short-chain glycerol-bound compounds formed by decomposition of hydroperoxides of oleic acid and linoleic acid

Fatty acyl group	Hydroperoxides	NON-VOLATILE COMPOUNDS		
		Route A*		Route B**
		+ $OH^{\cdot}$	+ $H^{\cdot}$	
Oleate	8-ROOH	7-hydroxyheptanoate	heptanoate	8-oxooctanoate
	9-ROOH	8-hydroxyoctanoate	octanoate	9-oxononanoate
	10-ROOH	9-oxononanoate	non-8-enoate	10-oxodec-8-enoate
	11-ROOH	10-oxodecanoate	dec-9-enoate	11-oxoundec-9-enoate
Linoleate[1]	9-ROOH	8-hydroxyoctanoate	octanoate	9-oxononanoate
	13-ROOH	12-oxododec-9-	dodeca-9,11-	13-oxotrideca-

	enoate	dienoate	9,11-dienoate

Source: Dobargens, M.C. (2009)

As shown by Table 2.2, among the four components, formation of C8:0 and 9-oxo-C9:0 are higher because they originated both from oleic acid and linoleic acid while C7:0 and 8-oxo-octanoic only originated from oleic acid (Kamal Eldin et al., 1997; Velasco et al., 2005; Dobargenes, 2009). Content of saturated fatty acid, C8:0 normally is higher than aldehydic fatty acid, 9-oxo-C9:0 because aldehydic compounds normally will participate in further reaction whereas the saturated short-chain acids accumulate due to higher stability (Velasco et al., 2005).

Studies conducted by Berdeaux et al. (2002) and Velasco et al. (2005) discovered the amount of these short chain components increased with frying time. Velasco group also reported that the total content of these compounds gave linear correlation with total polar components which is an acceptable method in determining oil quality and suggested that the short chain component can provide a good indication of the total alteration level of oils heated at frying temperature.

2.5 ANALYSIS OF FATTY ACIDS BY GAS CHROMATOGRAPHY

Analysis of fatty acids profile in fats and oil by gas chromatography (GC) is widely applied (Ulbergh et al., 1999). It has been the method of choice due to its ability to provide reliable qualitative and quantitative analysis of fatty acid (Adam et al. 1999; Laakso et al. 2002). Besides that, it can offer accuracy, convenience at relatively lower cost (Huang et al., 2006; Bansal et al., 2009).

Gas chromatography technique is similar to any forms of chromatography which consists of mobile phase and stationary phase but in GC, the mobile phase is gas and the stationary phase is liquid. In gas chromatography, sample is injected in some form of inlet, volatilized by the high temperature at the inlet, and brought by carrier gas in passing through a column or stationary phase. Samples spend different time in the carrier gas and the stationary phase, depend on their relative affinity. Upon reaching the

end of the column, samples which are separated into individual component then detected by a detector.

Identification of components in gas chromatography analysis usually can be made by comparing the component retention time, which is the time the component travel from the beginning of the column until reaching injector against retention time of authentic standard. The weight of the component usually is expressed as the relative percentage in the composition (Christie, 1989).

GC conditions such as program of the temperature, split injection, carrier gas and detector are important determinant to obtain high accuracy during analysis. Carrier gas in GC normally is helium and hydrogen and the detector chosen to analyze fatty acids normally is Flame ionization Detector (FID) or Mass Spectrometry Detector (MSD). Programmed temperature is preferred in order to obtain the best resolution, compared to isothermal temperature program (Alves et al., 2008).

The main concern of most researchers in analyzing fatty acid is type of stationary phase or column**.** Different types of column available with non polar, polar and highly polar stationary phase to analyze fatty acid. Alves et al. (2008) tested the columns with the three different stationary phase polarity and discovered differences in resolution and elution order. Generally, on non-polar columns unsaturated FAME elutes in front of saturated ones and cis elutes before trans isomer. The elution order of these compound classes is reversed with polar phases. Analysis of fatty acid in GC normally is carried out using column with medium or high polarity. Yamamoto et al. (2008) tested weakly capillary polar in analyzing FAME. They found out the column can be used to separate FAME, but separation of geometrical and positional isomer was not afforded. For the separation of geometrical and positional isomer, columns with highly polar stationary phase of cyanoalkyl polysiloxane have been extensively used, and the CP-Sil 88 (100% cyanopropylsilicone) of 100m long is the most popular one (Alves et al., 2008). The problem with highly polar column is low thermal stability (Alves et al., 2008).

The analysis of trans fatty acids (TFA) using GC is challenging due to various positional isomers that can occur along the carbon chain that contain double bond (Huang et al., 2006), so TFA analysis need high efficiency even by present GC analysis. (Laakso et al., 2002).

To avoid underestimation of trans fatty acids profile, long, highly polar capillary column is the most acceptable method to obtain isomeric profile of trans fatty acids. (Kramer et al., 1997; Ledoux et al., 2000; Tang, 2002; Golay et al., 2006; Baylin et al., 2007; Destaillats et al., 2007; Bansal et al., 2009). Previous researchers have proven that using high polarity, long column with at least 50 m in length, has improved the resolution of trans and cis isomer. The CP-Sil 88 capillary column which coated with 100% cyanopropylpolysiloxane was used by Destaillats et al. (2007), Golay et al. (2006), Molkentin and Precht, (1995), Wolff and Bayard, (1995) to analyse TFAs in food. Other researchers use equivalent of the column such as SP2560 by Baylin et al. (2007) and BPX-70 by Mjos (2005) and Hartig (2008) used HP 88.

The main drawback of GC is, it does not allow direct analysis of fatty acids. Prior analyzed by GC, fatty acids need to be converted to ester derivatives, normally fatty acid methyl ester (FAME) to render volatility, thus analysis time considerably increased (Priego-Capote et al., 2007) especially for vast quantity of samples.

2.5.1 TRANSESTERIFICATION

To be analyzed by gas chromatography (GC), fatty acids need to be converted to fatty esters to render volatility (Yunusova et al., 1998). Process converting fatty acids to fatty ester is commonly referred as transesterification (Meier et al., 2006).

Transesterification is a process where exchange of organic group of an ester with organic group of alcohol occurs during transesterification. If methanol is used in this process, it is called methanolysis, producing fatty acids methyl ester (FAME) and glycerol (Meher et al., 2006). Figure 2.7 illustrates transesterification using methanol process.

Methyl ester is the simplest fatty acids derivatives that can be prepared, with the lowest molecular weight, thus they elute from GC columns at lower temperatures than

other derivatives. However, due to its volatility, analysis of short chain fatty acids is rather difficult (Christie, 1990).

For routine analysis of fatty acids by GC, the preparation of FAME should be rapid, simple, without structural changes and produce side reaction (Metcalfe and Schmitz, 1961), and quantitative which means the esters obtained representative of fatty acid composition of the original sample (Bannon et al., 1985). Other factors that influence the yield of esterification are purity of the reactant from water content and free fatty acids (Demirbas, 2008). Problems that occur during FAME preparation are failure of fatty acids to be methylated quantitatively, saponification of the esters when alkaline methylation media are used, loss of short chain esters in aqueous layer when aqueous extraction procedure is used and evaporation of the short chain esters during storage (Bannon et al., 1985).

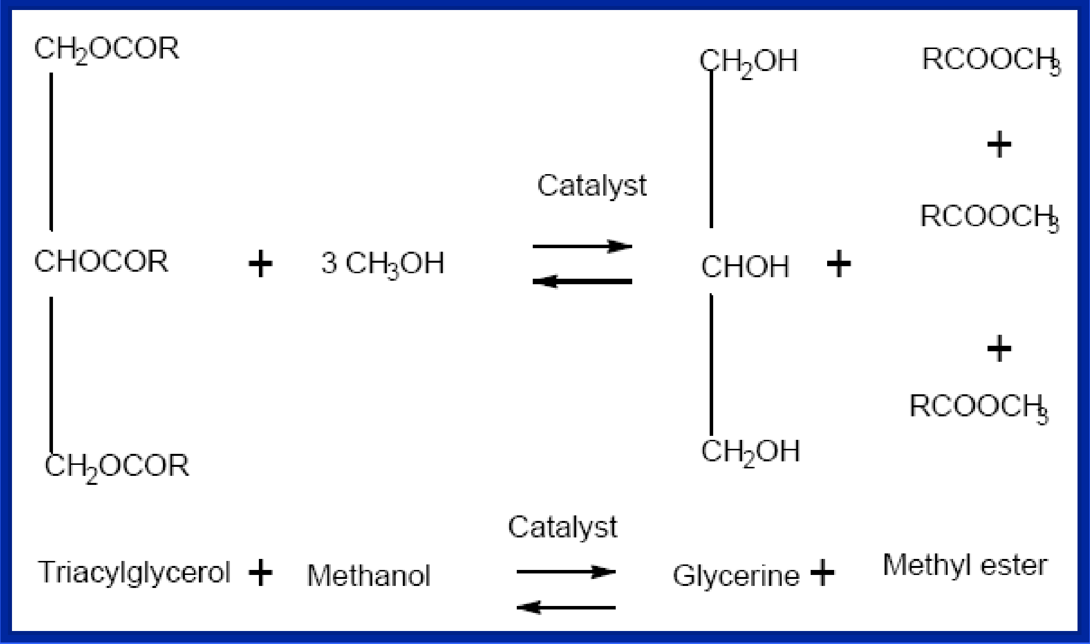


Figure 2.7: Transesterification process using methanol.

Source: Meher et al. (2006)

Catalyst is used to improve the reaction rate and yield of transesterification. The catalyst could be basic or acidic. Acidic catalyst reacts by donating a proton to carbonyl group of fatty acids while base catalyst reacts by removing proton from alcohol (Demirbas, 2008).

The advantage of acidic catalyst is it can be used to catalyze low grade fats or oil with high free fatty acid content (Freedman et al., 1984) and produce high yield of FAME (Demirbas, 2008). Problem with acidic catalyst is long reaction time (Demirbas, 2008). Kramer et al. (1997) reported that methylation of fatty acids using acid catalyst results in decreased of cis/trans isomer while increasing trans/trans isomer. Golay et al. (2006) observed degradation of conjugated linoleic acid during acidic catalyzed.

Reaction of acidic catalyst normally involves temperature up to 90 ◦C for 1 hr, oxidation of unsaturated fatty acids might occur (Sowa and Subbaiah, 2004). Boron trifluoride, an acidic catalyst, is popular for FAME preparation due to its fast and effective methylation, however it is expensive, and has a short shelf life (Antolin et al., 2008). Previous studies with almost similar objective and sample with present paper that used acidic catalyst are Billek et al. (1978), Kamal-Eldin et al. (1997), Takeoka et al. (1997), Ovesen et al. (1998), Romero et al. (2000), Tavella et al. (2000), Giebler et al. (2003), Karabulut et al. (2003), Glew et al. (2006), and Tsuzuki et al. (2008).

Common alkali catalyst are sodium or potassium hydroxide, and sodium or potassium methoxide. Alkaline catalyst is simpler and faster even at room temperature compared to acid catalyst (Demirbas, 2008; Freedman et al., 1984) and can produce higher yield (Predojevic, 2008). However base catalyst is sensitive to free fatty acid content of the oil that is to transesterified (Ackman, 1998). The free fatty acids are not converted to ester, but to side product as soap (Leung and Guo, 2006). Base catalyzed method is suitable for oil with acid value less than 2 (Bansal et al., 2010a).

Base catalyst method is also sensitive to water (Demirbas, 2008), thus it requires anhydrous condition. Prolonged contact with air will diminish the effectiveness of alkaline catalysts through interaction with moisture and carbon dioxide (Freedman et al., 1984). Other problem of alkaline catalyzed is saponification or formation of soap especially for short chain fatty acids (Ackman, 1998). The problem can be reduced by

using sodium methoxide as the catalyst and neutralizing the reaction mixture after a reaction period of six minutes. The six minutes time is the optimum time between a short reaction time in order to minimize saponification and a long reaction time in order to promote methanolysis of the long chain fatty acids.

Other benefit of neutralizing catalyst is to reduce appearance of artifacts in GC chromatogram and prolong GC column lifespan as base compounds are damaging towards GC column (Bannon et al., 1985). Predojevic (2008) recommended purification of FAME using silica gel or phosphoric acid. Previous studies that used base catalyst are Berdeaux et al. (1999), Golay et al. (2006), Priego-Capote et al. (2007), Destaillat et al. (2007) and Bansal et al. (2009, 2010a).

2.6 TOTAL POLAR COMPOUNDS

Total polar compounds (TPC) refer to all the degraded products other than the initial triglycerides present in the fresh oil (Bansal et al., 2010a). Determination of total polar compounds (TPC) in a frying fat provides the most reliable measure (Abdulkarim et al., 2007), best assessment parameter (Aladedunye and Przybylski, 2009) of oil degradation. It is an official method in Europe to determine oil degradation (Akoh and Min, 2002) with regulatory limits around 25 % (Takeoka et al., 1997). Almost all countries determine quality of oils and fats through total polar compounds amount (Hein et al., 1998).

TPC is measured using column chromatography. In the column chromatography method, oil will be divided into two fractions. The first fraction consists of unaltered triglycerides or non polar fraction, the second fraction consists of altered triglycerides which is the polar fraction. This is based on properties that oxidized triglycerides are highly polar and adsorbed more strongly on silica gel (Cuesta et al., 1991).

Besides oil quality issue, polar fraction gives big concern to health. Hagemen et al. (1988) studied mutagenic activity of polar fraction, and the polar fraction showed higher mutagenic activity compared to non polar fraction. Hagemen et al. (1988) discovered that differences of TPC level in unused and used fat for deep frying can be as big as 42%. In study conducted by Aladedunye and Przybylski in 2009, TPC content

increased linearly with frying time and temperature, but only achieved the 25% discard level 4 days of frying at 215 °C. Cuesta et al. (1991), Arroyo et al. (1992) and Abdulkarim et al. (2007) also found TPC to increase with frying time.

Lot of studies such as studies conducted by Cuesta et al. (1991), Takeoka et al. (1997), Warner et al. (1997) and Abdulkarim et al. (2007), indicated that oil with higher level of polyunsaturated acids showed higher amount of TPC after thermally treated compared to oils rich in monounsaturated acid and saturated acid. According to Al Harbi and Al Kahtani, (1993), TPC content more reliable to be adapted to polyunsaturated oils and might not be applicable to more saturated oils as saturated oils are relatively lower in sensitivity towards oxidation.

Although TPC is a standard method in determining oil oxidative state, this method is extremely laborious, time consuming, use hazardous organic solvent (Hein et al., 1998; Gil et al., 2004). Other assessment method that correlates well with column chromatography method may provide faster and simpler alternative (Aladedunye and Przybylski, 2009). Xu (2000) discovered strong correlation between total polar compounds content and spectrophotometric method, measuring absorption at 350 and 650 nm wavelength in determining oil degradation level. Aladedunye and Przybylski in 2009, discovered low correlation between acid value and TPC but high correlation with color index of the oil. Takeoka et al. (1997) also discovered that TPC was highly correlated with color index and significantly correlated with iodine value. However the absolute degree of unsaturation as determined by the iodine value was not directly proportional to the amount of polar compounds formed. Augustin et al. (1987) reported good correlation with iodine value and C18:2/C16:0 ratio.

Abdulkarim et al. (2007) found good correlation of TPC with oil viscosity. Billek et al. (1978) reported good correlation between TPC and petroleum ether insoluble oxidized fatty acids, which also one of method to determine oil degradation. Arroyo et al. (1992) found out polar compounds were not significantly correlated with free fatty acids level, and suggested that intensive thermoxidative rather than a hydrolytic process took place in experimental deep-fat frying.

2.7 IODINE VALUE

One of the methods to determine oxidative degradation of an oil is measuring iodine value. Iodine value (IV) is a measure of the unsaturation of fats and oils and it expresses the amount of iodine absorbed by double bonds of fatty acids (Haryati et al., 1998). Iodine value can indicate overall change in the degree of unsaturation.

Decrease in iodine value is attributed by destruction of double bonds caused by oxidation, polymerization and scission (Cowan, 1954; Cuesta et al., 1991; Al Harbi and Al Kahtani, 1993; Abdulkarim et al., 2007). That processes occur by reaction of double bonds with oxygen. However direct reaction is thermodynamically difficult. It has been proposed that preformed hydroperoxides due to heat or exposure to light or by mechanisms where singlet O_2 as free radical is the active species involved in attacking double bond. The free radicals can propagate the abstraction of hydrogen atoms adjacent to double bonds of fatty acids, followed by O_2 attack at double bonds (Naz et al., 2005). Decrease in unsaturation degree is directly related with the degradation of polyunsaturated fatty acids in oil (Moreno et al., 1999).

There are many methods to determine iodine value. Takeoka et al. (1997), Karabulut et al. (2003) determined iodine value according to AOCS Official methods while Mamat et al. (2005) referred to PORIM test method. Naz et al. (2005) applied IUPAC Standard Methods in measuring iodine value. Kyriakidis and Katsiloulis (2000) in their analysis of comparing iodine value measurement method, discovered that the iodine value is strongly dependent on the method by which it is determined.

Noh et al. (2002) screened iodine value in several palm oil samples. They discovered that the iodine value is positively correlated with oleic and linoleic acid content, the higher the level of oleic and linoleic acid composition in an oil, the higher the iodine value. Factors that can influence reduction in iodine value are presence of air, heat and lights. Naz et al. (2005) discovered deep frying influenced higher reduction rates of IV compared to exposure of oil to light and air. They also reported presence of antioxidant in oil helped in avoiding high reduction rate of iodine value.

Iodine value also depends on the type of sample. Knothe and Steidley (2009) discovered, after oxidized, different samples experienced various magnitude of increase in saturation, however independent sample obtained from the same source give little consistency in increase of saturation. Al Harbi and Al Kahtani (1993), Naz et al. (2005) and Abdulkarim et al. (2007), also gave similar finding. They discovered significant differences of IVs with respect to variety of oil.

Takeoka group in 1997 discovered different temperature treatments will give different level of reduction rate of IV. In measuring IV of thermally treated soy bean oil, corn oil, canola oil cottonseed oil, they discovered all of the oil experienced progressive decrease in unsaturation of all oil after heat treatment. However when treated at 190 °C, corn oil had the fastest loss of unsaturation followed by canola salad oil, cottonseed oil, soybean salad oil. When heated at 204 °C, soybean salad oil had the fastest loss of unsaturation. Corn oil which had the fastest loss of unsaturation at 190 °C had only the fourth fastest loss when heated at 204 °C. The most saturated oil had slower changes in unsaturation compared to other oil. Moreno at al. (1999) discovered significant increase in saturation after temperature reach 150 °C. Augustin et al. (1987) reported iodine value highly correlated with other oil measure of oil degradation method, which is the total polar compound (TPC).

2.8 FREE FATTY ACIDS (FFA) CONTENTS

Presence of moisture and air during frying can result in the hydrolysis of triacylglycerol molecule and oxidation of fatty acid double bonds, producing non esterified fatty acid or free fatty acids (FFA) (Abdulkarim et al., 2007). At high temperature, the moisture turn into steam, which will hydrolyze triacylglycerol, forming free fatty acids (Bhattacharya et al., 2008), while air will initiate a cycle of oxidation reactions involving the formation of hydroperoxides. The hydroperoxides subsequently result in free radical-mediated reactions, attacking double bond of fatty acid (Cuesta et al., 1991). Free fatty acids (FFA) are more prone to oxidation than intact triacylglycerol due to the presence of polar carboxyl groups (Colakoglu, 2007), thus oil that is rich in FFA has higher oxidation rate.

Even though hydrolysis product has little impact on nutritional quality of an oil (Fritsch, 1981), free fatty acid which is the result of hydrolysis is more prone to oxidation than corresponding methyl ester (Colakoglu, 2007), so hydrolysis product can trigger oxidation process which is said to be detrimental to oil quality. Free fatty acids, monoglycerol, diacylglycerol contain hydrophilic and hydrophobic groups in the same molecules, and then concentrate at the surface of oil. Oxygen dissolved more in the presence of these compounds hence oxidation is triggered (Colakoglu, 2007).

Free fatty acid can accumulate in the frying oil through repeated use (Innawong et al., 2004). It also can contribute to oil rancidity (Othmer, 2008). Thus, the amount of free fatty acid can indicate oil degradation level. Abdulkarim et al. (2007) analyzed frying stability of few types of cooking oils and they reported increase in FFA content after frying cycle. Al Harbi and Al Kahtani, (1993), Innawong et al, (2004) and Ozbay et al. (2008), also reported higher amount of FFA in oils that were fried in relatively longer period. Fritsch (1981) claimed initial amount of FFA in an oil also can influence the rate of FFA formation in degraded oil. The higher the initial FFA, the greater will be the amounts of FFA formed by hydrolysis.

Fritsch (1981) and Abdulkarim et al. (2007) found high correlation of FFA amount with total polar compounds (TPC). Since these FFAs are caused by presence of moisture, Bansal et al. (2010a) and Bhattacharya et al. (2008) reported high correlation of FFAs with moisture content of food being fried in the oil. When the moisture content of food was high, the oil quality was inferior in terms of TPC and FFA.

Fritsch (1981), claimed the determination the amount of FFA produced by hydrolysis is too small to effect frying oil quality because adverse effect of oil are due to oxidation of unsaturated fatty acids. Since the determination of FFA does not differentiate between acids formed by oxidation and those by hydrolysis, FFA amount is not a good indicator if it is used alone in analyzing oil oxidative stability. Al Harbi and Al Kahtani (1993) also claimed FFA value is not a recommended procedure for measuring fat deterioration.

Usually FFA content is determined by titration method, where oil is dissolved in alcohol, titrated with potassium hydroxide solution using phenolphthalein as an indicator to a pale-pink end point. The principle of this method is to measure the number of mg of KOH required in neutralizing the free fatty acids contained in 1 g of fat. Several studies that have used titration method are Abdulkarim et al. (2007), Al Harbi and Al Kahtani (1993) and Fritsch, (1981).

The subjectivity of judging the colorimetric end point means will lead to difficulty in getting consistent results for routine analysis among different analyst (Gerde et al., 2007). Innawong et al. in 2004 found strong correlation between FTIR absorbance at 3300 cm^{-1} and free fatty acid content that was determined by titration method.

2.9 CONJUGATED FATTY ACIDS

Besides chemical method to determine oil quality, physical method using ultra violet visible (UV/Vis) spectrophotometer can be used to assess degradation level of an oil. It also offers fast analysis and convenience analysis (Xu, 2000). UV/Vis spectrophotometer operates by determining the absorption or transmission of ultra violet light from 190 to 800 nm by a sample (Pavia et al., 2009). Except for a few unusual oil, double bond in naturally occurring fats and oil are interrupted by methylene group, not conjugated (Othmer, 2008). During oxidation, methylene interrupted double bond show a shift in their double bond position, due to isomerization and conjugation reaction. (Akoh and Min, 2002).

Methylene interrupted double bonds are transparent to most UV spectrum while the conjugated isomers exhibit intense absorption under UV spectrum and can be conveniently detected by UV spectroscopy (Akoh and Min, 2002). Bands at 233 nm, 270 nm, 305 nm are diagnostic for conjugated system in dienes which is two double bond separated by conjugate system, trienes with three double bond and tetraenes with four double bond respectively.

Al Harbi and Al Kahtani. (1993) measured absorption at both 232 nm and 268 nm in fresh and used oil. They reported stronger absorption by used oil for both wavelengths. Abdulkarim et al. (2007) discovered levels of conjugated dienes throughout frying period are lowest in palm oil compared to polyunsaturated oil. Augustin et al. (1987) reported higher absorption of oil heated at higher temperature compared to low temperature. Caution must be exercised in interpreting absorption because minor constituent in fats and oil may contain chromophore compounds absorbing in the same region (Othmer, 2008).

2.10 OIL COLOR

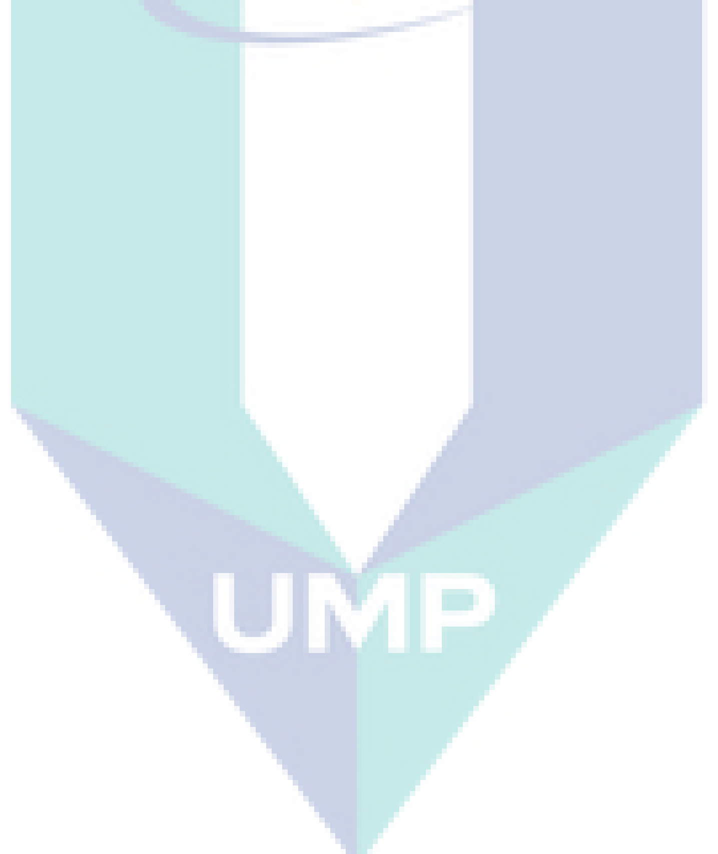
When oil is fried, color changes will happen. Coloring is due to the increase in high molecular weight compounds and viscosity of the oil (Melton et al., 1994; Tan et al., 1985). Color also is influenced by the type and amount of food being fried in the frying oil. Food interacts with oil to form color constituents such as Maillard browning product (Takeoka et al., 1997) and caramelization of food particles and then released into frying medium can result in the darkening of the oil (Baixauli et al., 2002). Although it is the result of oil deterioration, it can be a useful indicator of oil degradation for frying operators as it is the easiest to be observed (Tan et al., 1985).

Color can be a rapid indicator of oil degradation. However, it depends on the skill of the operator. Tan et al. (1985), Al Harbi and Al Khatani (1993), and Abdulkarim et al. 2007 stated that palm oil showed rapid darkening compared to other types of oil but it does not necessarily means reduction in quality (Al Harbi and Al Khatani, 1993) and it can lead to underestimation as the value of 25 percent polar compounds probably is far away to be reached (Dobarganes and Marquez-Ruiz, 1998). Moreover, color formation is the result from more than one chemical processes, so it is not a good indicator of extend of oil degradation (Takeoka et al., 1997).

For frying operators, oil quality is judged merely based on visual observation. The evaluation relies on the one’s experience and it might differ between another. Usually, oil that is darkened is considered bad and can be the discarding point. For oil

quality control in the laboratory, oil color is monitored using spectrophotometer, color standard, and tintometer (Takeoka et al., 1997).

In UV visible spectroscopy, absorbance at 420 nm is the color index or browning index that can reflect an overall chemical degradation and polymerization such as Mailard reaction product that occur during frying (Kim et al. 2002; Yaghmur et al. 2001; Budryn et al. 2009). It is also considered as browning index (Lee and Nagy, 1988). Yaghmur et al. (2001), Sanibal et al. (2004), Lee et al. (2004) and Farhoosh et al. (2009), measured absorption at 420 nm to study oxidative stability of frying oil.



CHAPTER 3

MATERIALS AND METHOD

3.1 SAMPLING PROCEDURE

Discarded oil samples were collected from four different food outlets in May 2010.All of the food outlets are located in Kuantan, Pahang. Sample D, E,F,G were collected from an eatery, food caterer, banana fritters stall and fast food outlet respectively. The outlets operators were asked to conduct their normal frying procedure and save 1 L of the oil prior discarded. Oil samples were collected at the end of their daily operation. The outlets operators were queried about frying condition such as frying time and temperature, type of fryer, cooking oil and food medium. Sample D, E, F, G were used to fry papads, chicken meat, banana fritters, French fries respectively. For sample D, E, F, the food outlets operators used open conventional gas-fried steel pan that is normally used in household frying. In contrast, for sample G, the operator used a well-designed electric deep fryer. All the frying processes were conducted with replenishment.However no information on the time and temperature of frying were revealed by the outlets operators.

Fresh counterparts of the oil were purchased from a local hypermarket in Kuantan. There was no fresh counterpart from sample G as the origin of the oil was not revealed by the fast food outlet operator.The collected samples were filtered through Whatman No. 4 filter paper to remove suspended food particles. All the samples were kept under 4 °C, in dark bottles to avoid further oxidation.

For control samples, two brands of palm oil, denoted as sample A and B were purchased from local hypermarket. Sample C was corn oil, bought from a local

Table 3.1: Oil sampling and treatment

Sample	Type/ Source	Treatment	Food medium
A	Control sample/ Palm oil	I) Fresh oil II) Heated, 180-200 °C, 6 hour in electric deep fryer III) Fried, 180-200 °C, 6 hour in electric deep fryer	I) - II) - III) Chicken nugget
B	Control sample/ Palm oil	I) Fresh oil II) Heated, 180-200 °C, 6 hour in electric deep fryer III) Fried, 180-200 °C, 6 hour in electric deep fryer	I) - II) - III) Chicken nugget
C	Control sample/ Corn oil	I) Fresh oil II) Heated, 180-200 °C, 6 hour in electric deep fryer III) Fried, 180-200 °C, 6 hour in electric deep fryer	I) - II) - III) Chicken nugget
D	Collected sample/ Food eatery	I) Fresh oil II) Waste oil, gas fried steel pan	I) - II) Papads
E	Collected sample/ Food caterer	I) Fresh oil II) Waste oil, gas fried steel pan	I) - II) Chicken meet
F	Collected sample/ Banana fritters stall	I) Fresh oil II) Waste oil, gas fried steel pan	I) - II) Banana fritters
G	Collected sample/ Fast food outlet	I) Waste oil, electric deep fryer	I) French fries

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hypermarket. Chicken nuggets as food medium in control samples were purchased from local hypermarket. Table 3.1 summarizes how all samples were collected and treated.

3.2 FRYING PROCEDURE

One kg of sample A, B, and C were fried in 3L stainless steel domestic deep fryer (Anvil Deep Fryer, USA, dimensions370 X 455 X 350 mm) at 180 °C – 200 °C with total frying time of 6 hr. The frying temperature from 180 °C to 200 °C was chosen because it is normal frying practice (Ni and Datta, 1999; Saguy and Dana, 2003; Alvis et al., 2009).Frying time of 6 hours can generally signify frying time in daily operation of food outlets where 8 to 12 hours is considered as normal daily operation of food outlets in Malaysia.Chicken nuggets which acted as food medium were fried in 200 g batches, 10 min apart for 6 hr.

To understand the influence of food in oil deteriorative changes, heating and frying condition was conducted. Heating conditions was applied by keeping all the process variables the same as those in frying except there was no food in the oil. No replenishment of the oil during frying and heating to avoid dilution of decomposed and oxidized product.

3.3 FATTY ACID TRANSESTERIFICATION

3.3.1 Materials

2M KOH in methanol was prepared by dissolving 5.6g KOH (Merck, Germany) in 50ml methanol (Merck, Germany, analytical grade); Hexane (Merck, Germany, analytical grade); anhydrous sodium sulphate (Merck, Germany).

3.3.2 Method

Esterification procedure was done according to Berdeux et al. (1999), with slight modification. 100 mg of oil sample were dissolved in 10 ml hexane in test tube, then 1ml of 2M KOH in methanol was added to the tube. Then the tube was vortex occasionally. After 15 min, the hexane phase was collected and washed twice with 4ml

water, twice to get rid of remaining catalyst and methanol. The solution then was dried over sodium sulfate and filtered using filter paper.

3.4 GAS CHROMATOGRAPHY ANALYSIS

3.4.1 Materials and equipment

HP Innowax capillary column (30 m, 0.32 mm, 0.25 µm, Agilent, Germany), HP 88 capillary column (100 m, 0.32 mm, 0.25 µm, Agilent, Germany), Agilent 7890 GC equipped with flame ionization detector (FID);Fatty acid authentic standard (C4-C24, 37 component FAME Mix, Sigma Aldrich, Supelco, USA); Hexanoic acid methyl ester (C6:0), Octanoic acid methyl ester (C8:0), Decanoic acid methyl ester (C10:0), Undecanoic acid methyl ester (C11:0), Dodecanoic acid methyl ester (C12:0), Tridecanoic acid methyl ester (C13:0), Tetradecanoic acid methyl ester (C14:0), Tetradecenoic acid methyl ester (C14:1), Pentadecanoic acid methyl ester (C15:0), Cis-10- Pentadecenoic acid methyl ester (C15:1), Hexadecanoic acid methyl ester (C16:0), Cis-9-Hexadecenoic acid methyl ester (C16:1), Heptadecanoic acid methyl ester (C17:0), Cis-10-Heptadecenoic acid methyl ester (C17:1), Octadecanoic acid methyl ester (C18:0), Trans-9-Octadecenoic acid methyl ester (C18:1n9t), Cis-9-Octadecenoic acid methyl ester (C18:1n9c), Trans-9, 12-Octadecadienoic acid methyl ester (C18:2n6t), Cis-9, 12-Octadecadienoic acid methyl ester (C18:2n6c), Eicosanoic acid methyl ester (C20:0), Cis-6, 9, 12-Octadecatrienoic acid methyl ester (C18:3), Cis-11-Eicosenoic acid methyl ester (C20:1), Cis-9,12,15-Octadecatrienoic acid methyl ester (C18:3n3), Heneicosanoic acid methyl ester (C21:0), Cis-11,14-Eicosadienoic acid methyl ester (C20:2), Docosanoic acid methyl ester (C22:0), Cis-8,11,14-Eicosatrienoic acid methyl ester (C20:3n6), Cis-13-Docosenoic acid methyl ester (C22:1n9), Cis-11,14, 17-Eicosatrienoic acid methyl ester (C20:3n3), Cis-5,8,11,14-Eicosatetraenoicacid methyl ester (C20:4n6), Tricosanoic acid methyl ester (C23:0), Cis-11,16-Docosadienoic acid methyl ester (C22:2), Tetracosanoic acid methyl ester (C24:0), Cis-5,6,11,14,17-Eicosapentaenoic acid methyl ester (C20:5n3), Nervonic acid methyl ester (C24:1), Cis-4,7,10,13,16, 19-Docosadienoic acid methyl ester (C22:6n3)).

3.4.2 Method

1 μL of fatty acid methyl ester solution was injected into an Agilent 7890 GC equipped with flame ionization detector (FID) with split ratio of 50. Helium was used as carrier gas. Both injection port and detector temperatures were 250 °C.

For analysis of common fatty acids, HP Innowax, fused silica capillary column was used.Helium gas flow was 1.5 ml/min.Different temperature programs were tried. At the end of the investigation, the best temperature program was selected for a good resolution of fatty acid mixture standard.The column temperature was programmed from 50 °C for 1 min, increased to 70°C at 5 °C min^{-1}, held for 9 min, and further programmed to 200 °C at 15 °C min^{-1}, held for 10 min. Final temperature was 230 °C, held for 4 min.

For detection of trans fatty acid, HP 88 fused silica capillary column that is specialized in detecting trans fatty acid was used.Helium gas flow was 2.0 ml/min.The column temperature was programmed from 120 °C for 1 min, increased to 175 °C at 10 °C min^{-1}, held for 10 min, increased to 210 °C at 5 °C min^{-1}, held for 5 min and further programmed to 230 °C at 5 °C min^{-1}, held for 5 min. Fatty acids were identified by comparison of retention time with authentic standards (C4-C24, Supelco 37 component FAME Mix). Results of fatty acids are given as relative percentage area of the sum of all identified peaks.

3.4.3 Limit of Detection

The octanoic acid limit of detection (LOD) was evaluated by serial dilutions of octanoic acid standard (Fluka, Sigma Aldrich) stock solutions in hexane (Merck). A calibration graph was constructed in the range of1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.001563 and 0.000782 % (v/v).

Based on ICH Harmonized Tripartite Guideline (2005), Limit of Detection (LOD) was expressed as:

$$LOD = \frac{3.3\sigma}{S}$$

where σ isthe residual standard deviation (RSD) of the response and S isthe slope of the calibration curve.

3.5 DETERMINATION OF TOTAL POLAR COMPOUNDS

3.5.1 Materials

Silica gel (Merck, Germany, particle size 0.063-0.200 mm); Petroleum ether (Merck, Germany, analytical grade); Diethyl ether (Merck, Germany, analytical grade); Calcined sea sand (Merck, Germany,); Glass column (Favorit, USA, 2.1 cm internal diameter and 45 cm in length dimension); Glass wool.

3.5.2 Method

Silica gel was brought to activity of 5% water content by drying at 160 °C for 4 hr. After cooling to ambient temperature in a desiccator, the silica gel then was put into a flask. Water was added to make a 5% (w/w) mixture, and the flask was shaken. 261 ml petroleum ether was added to 39 ml diethyl ether to create 87:13 (v/v) mixture of petroleum ether and diethyl ether. This solvent was denoted as solvent 1.

Column chromatography procedure was done according to Gil et al. (2004). Glass wool was packed tightly over the outlet of the glass column, and about 4 g of calsined sea sand was added over the glass wool. Then 30 ml of solvent 1 was added into the column. 2.5 g deactivated silica gel, slurried in solvent 1 then added into the column through a funnel. The silica gel layer in the column was leveled by tapping with rubber pipe. 4g of calsined sea sand was added over the silica gel to preserve the silica

layer from disturbance. Solvent in the glass column was drained off until the level of the sand. 2.5g oil sample, dissolved in 5 ml of solvent 1 then introduced into the column.

Solvent 1 was kept on added into the column, while fraction was collected in a beaker, through the outlet of the column. Fraction collecting was stopped after the volume had reached 150 ml and this fraction was denoted as fraction 1. Second fraction, denoted as fraction 2 was collected by eluting 150 ml diethyl ether through the column.

Separately, both fractions was put into pre weight round bottom flask and the solvent in the fraction was distilled off using rotary evaporator, at 55 °C. Then the flask was weighed again. Total polar compounds were calculated as followed:

$$total\ polar\ compounds\ (\%) = \frac{m - m1}{m} \times 100$$

where m_1 is the weight (g) of the non polar fraction and m is the weight (g) of the sample added to the column.

3.6 DETERMINATION OF FREE FATTY ACIDS CONTENT

3.6.1 Materials

0.1 M KOH solution was prepared by dissolving 5.6g KOH (Merck) was dissolved in 1L deionized water. 1% phenolphthalein indicator was prepared by dissolving 1g phenolphthalein (Sigma Aldrich, USA) in 100ml ethanol (Merck, Germany, analytical grade).

3.6.2 Method

Free fatty acid determination was based on AOAC Official Method Ca5a-40 (1989). 1L of ethanol was neutralized by titrating with 0.1 M KOH solution, with phenolphthalein indicator to pale pink end point. With three replications, 10g of oil was

dissolved in 50ml neutralized ethanol. The solution turned yellow in color. Then, the solution was titrated against 0.1 M KOH solution, with phenolphthalein indicator, until the solution turn pink in color that persisted about 30 seconds. The volume of 0.1 M KOH solution needed to titrate the sample to a pale pink end point was recorded. The free fatty acid value was calculated using the equation;

$$\text{Free fatty acids (\%)(as oleic acid)} = \frac{(V)(M)(MW)}{10\,(w)}$$

where V is volume in ml of KOH needed to titrate the sample, M is concentration of KOH, MW is molecular weight of fatty acid, expressed as molecular weight of oleic acid, which is 282 and w is sample amount, in g.

3.7 DETERMINATION OF IODINE VALUE

3.7.1 Materials

Wijs reagent (Fluka, Sigma Aldrich, USA); In preparing 2.5% mercuric acetate in glacial acetic acid (Merck, Germany), 2.5g mercuric acetate was dissolved in 100 ml glacial acetic acid (Fisher, USA, analytical grade); 150g potassium iodide (Merck, Germany) was dissolved in 1L deionized water to prepare 15% KI solution; Starch indicator solution was prepared by dissolving 10g soluble starch (Sigma Aldrich, USA) in 1L water; To prepare 0.1mole/L sodium thiosulphate solution, 24.8181g of $Na_2S_2O_3.5H_20$ (Merck, Germany) were dissolved in water, completed to 1000 mL in volumetric flask; Cyclohexane (Merck, Germany, analytical grade); Glacial acetic acid (Fisher, USA, analytical grade).

3.7.2 Method

Method to determine iodine value was according to AOCS Official methods Cd 1-25 (1989) and Abdul Karim et al, (2007). Iodine value determination was done in three replications. 0.5g oil sample was weight into two 500ml glass-stoppered iodine

flasks. The flasks were labeled as Sample 1, Sample 2. Third flask was labeled as Blank and was left without oil sample. 20mL cyclohexane: acetic acid (1/1) (v/v) was added, and then 20ml Wijs reagent was pipetted into the flasks. 10ml of 2.5% mercury (II) acetate in glacial acetic acid solution was added. The flasks then left in the dark for 30 min. After reaction period, 100ml distilled water and 20mL 15% KI solution were added. The solution then titrated against 0.1mole/L sodium thiosulphate solution, to a pale yellow end point. Several drops of starch indicator were added and titration continued until disappearance of purple color. Iodine value is calculated based on the following equation;

$$iodine\ value = \frac{blank\ titer\ (ml) - sample\ titer\ (ml)(0.01269)(100)}{sample\ (g)}$$

3.8 UV/VIS SPECTROPHOTOMETER ANALYSIS OF CONJUGATED FATTY ACID

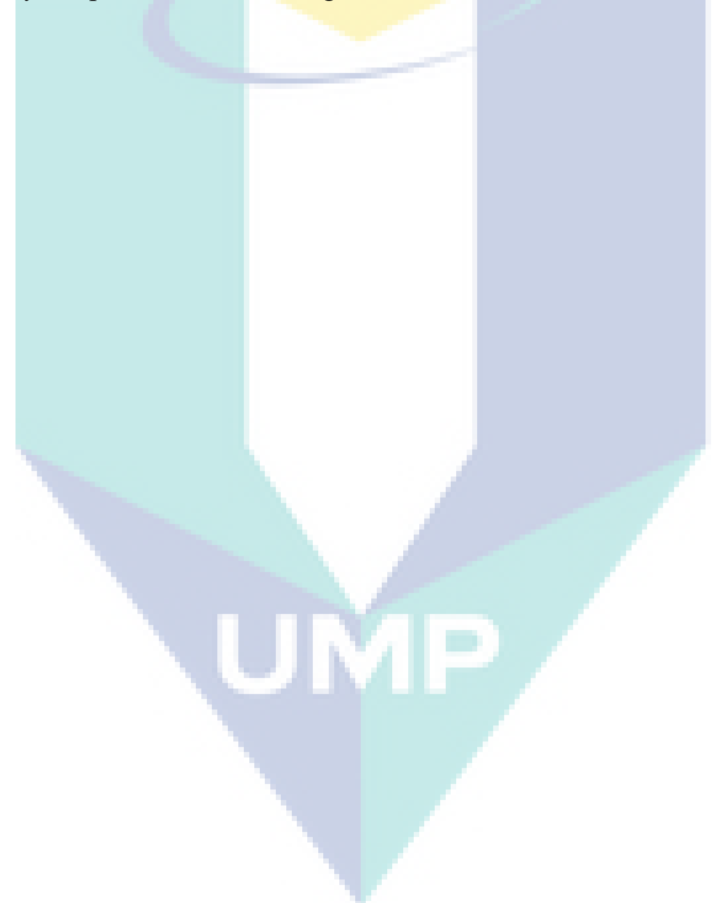
2% of oil solution in hexane (Merck, Germany) was placed in a quartz cuvette (1 cm optical path). Spectrophotometric absorbance was measured using a Hitachi U-1800 Spectrophotometer (Tokyo, Japan). The oil samples were scanned at 233 nm and 269 nm wavelengths. Analyses were done in triplicate and values reported are means.

3.9 OIL COLOR

Absorption spectra of 2% of oil solution in hexane (Merck, Germany) were obtained in the range 190–1100 nm using UV- Visible spectrophotometer Thermo Scientific, USA. The radiation source is a combination of a deuterium-discharge lamp for the ultraviolet wavelength range and a tungsten lamp for the visible wavelength range. The cells are rectangular cells in quartz glass with a path length of 1cm.Analysis were done in triplicate and values reported are means.

3.10 STATISTICAL ANALYSIS

All data for the oil samples are the means and standard deviations for three replications. Data were analyzed by one way analysis of variance (ANOVA). Specific statistical differences between means were compared by Tukey's honestly significant difference test and significance was accepted at 5% level ($P < 0.05$). All statistical analyses were performed using the Statistical Package for Social Sciences version 16.0 software (SPSS, Inc., Chicago). The relationships between the parameters were determined by simple correlation using Microsoft Excel 2003.



CHAPTER 4

RESULTS AND DISCUSSION

4.1 OIL SAMPLING AND THERMAL TREATMENT JUSTIFICATION

Present study consisted of waste oil collected from various food outlets and oil subjected to heating and frying in laboratory. Waste oil collected from food outlets will be denoted as collected samples afterwards and oils subjected to heating and frying in laboratory acted as control samples. Figure 4.1 and 4.2 show oil sampling and treatment for control and collected samples respectively.

Two commercial brands of palm oil were included as control samples, and denoted as sample A and B. Enquiries to the hypermarket operator revealed sample A has been the most sought after brand by consumers while sample B is the hypermarket in-house brand. Sample B is relatively cheaper in price than sample A and it is perceived to be low in quality by consumers due to its packaging and appearance. Sample B appeared cloudy compared to sample A which was more transparent and translucent in color.

Palm oil was chosen as sample in this study owing to their common use as main cooking medium by Malaysian (Corley, 2009). Corn oil, denoted as sample C, was included in control group because it has been extensively reported in literatures to be more susceptible to oxidation than palm oil. Moreover, survey in several hypermarkets revealed that there are more brands of corn oil in the market compared to other polyunsaturated oils such as soybean, sunflower, and peanut oil. So it can be generally accepted that corn oil is polyunsaturated oil that is consumed the most by Malaysian.

Sample	Source	Treatment		
Sample A	Palm Oil	Fresh Oil	Heated Oil 180-200 °C, 6 hr	Fried Oil 180-200 °C, 6 hr
Sample B	Palm Oil	Fresh Oil	Heated Oil 180-200 °C, 6 hr	Fried Oil 180-200 °C, 6 hr
Sample C	Corn Oil	Fresh Oil	Heated Oil 180-200 °C, 6 hr	Fried oil 180-200 °C, 6 hr

UMP

Figure 4.1: Oil samples and treatment in control samples

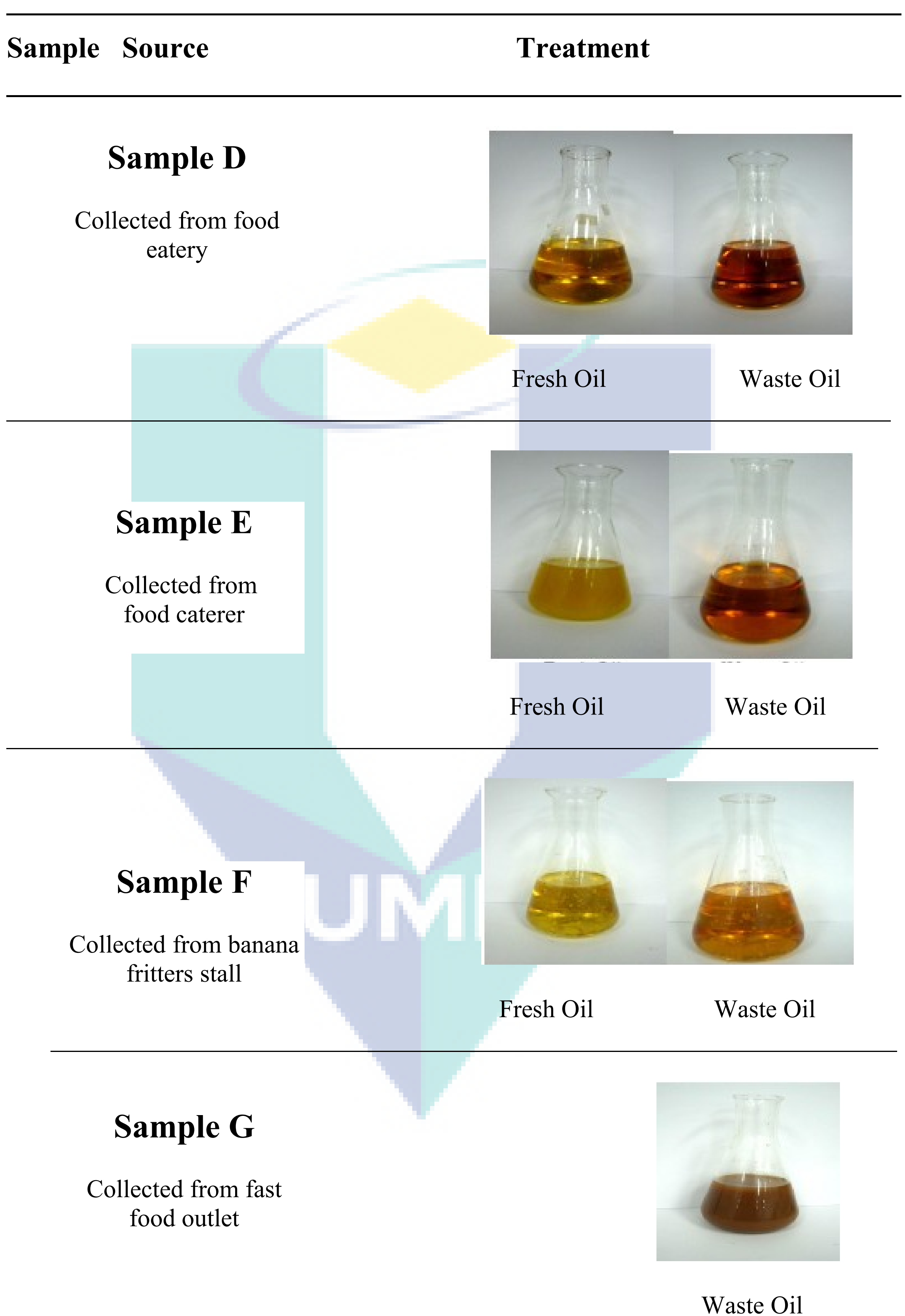


Figure 4.2: Oil sampling and treatment in collected samples

Therefore degradation level of palm and corn oil was compared to determine how stable palm oil towards degradation relatives to corn oil.

For the control samples; sample A, B, and C, the oils were subjected to thermal treatment in the laboratory, under controlled temperature from 180 °C to 200 °C for 6 hr. The treatment involved frying the oil, in stainless steel deep fryer, with the presence of food medium, which was chicken nugget. Heating the oil was conducted with the same condition applied to frying process, but minus the presence of food medium.

The temperature from 180 °C to 200 °C was chosen because it is normal frying practice (Ni and Datta, 1999; Saguy and Dana, 2003; Alvis et al., 2009). Frying time of 6 hours can generally signify frying time in daily operation of food outlets where 8 to 12 hours is considered as normal daily operation of food outlets in Malaysia. The frying time and temperature are also without the exaggeration in thermal conditions that sometimes occur in laboratory oil-heating or frying experiments (Al Harbi and Al Kahtani, 1993).

Heating and frying were compared to understand the influence of food in oil deteriorative changes. This is because few studies reported that food will influence type of degradation. It is also because very few reports could be found in the literature to explore the stability of oils under both frying and controlled heating conditions (Aladedunye and Przybylsky, 2009; Bansal et al., 2010a).

For collected samples, waste oils were sampled from outlets that are commonly patronized by Malaysian. The outlets were food eatery, food caterer, banana fritters stall, and fast food outlet. Sample from those outlets are denoted as sample D, E, F, and G respectively afterwards. Samples were collected from different food outlets with different food medium to find out whether differences in frying parameters such as food medium, frying utensils, time and temperature of frying can lead to different rate of degradation.

More samples from food outlets especially from fast food outlet were intended to be collected as they are notorious in using oil until fully degraded. Due to poor

cooperation from food outlets operators, only one sample for every outlet was managed in giving oil sample. For fast food outlet, the origin of the oil was not revealed by the fast food outlet operator due to corporate secrecy policy hence there was no fresh counterpart for sample G in this study.

The samples were collected from various food outlets to signify different frying parameters such as type of oil, food medium and temperature applied during frying process. This is because different frying parameters will contribute to different type and level of oil degradation (Augustin et al., 1987). According to Alvis et al. (2009), frying variables can be the temperature, warm up time, oil type, oil rotation, its manipulation, and the frying utensil used. Food medium can cause variety of chemical reactions occurring such as gelatinization of starch, Maillard reaction, denaturation of protein and decrease of moisture.

For sample from food eatery (D), food caterer (E), banana fritters stall (F), the food outlets operators used open conventional gas-fried steel pan that is normally used in household frying. In contrast, for sample from fast food outlet (G), the operator used a well-designed electric deep fryer. According to respective food outlets operators in this study, sample D was used to fry papads, sample E for chicken meat, sample F for banana fritters and sample G for French fries. These differences in frying parameters are expected to give different type of degradation, and will be discussed further in the next sections.

4.2 ESTERIFICATION OF FATTY ACIDS

Fatty acids of interest in this study are short chain fatty acids and trans fatty acids. Before fatty acid is analyzed by gas chromatography, it is required to be converted to fatty acid methyl ester (FAME) to render volatility. Since those fatty acids are labile and heat sensitive (Kramer et al. 1997; Berdeaux et al. 1999), the method of esterification of fatty acids is discussed in detail.

Various methods are available in converting fatty acid to fatty acid methyl ester (FAME) and normally the methods involve transesterification using basic or acidic catalyst. In this study, transesterification using basic catalyst was the method of choice. This is because, for the preparation of FAME, the method should be rapid, simple, without structural changes and produce side reaction (Metcalfe and Schmitz, 1961). These reaction conditions can be offered by basic catalyst. Basic catalyst requires short amount of reaction time, can be carried out at room temperature and does not lead to artifact formation while acidic catalyst is generally harmful, compared to basic catalyst that is generally milder (Kramer et al. 1997).

Berdeaux et al. (1999) claimed transesterification using potassium hydroxide (KOH) is the best method in transesterification of short chain fatty acids. Kramer et al. (1997) reported that methylation of fatty acids using acid catalyst results in decreased of cis/trans isomer while increasing trans/trans isomer. Golay et al. (2006) observed degradation of conjugated linoleic acid during acidic catalyzed. These claims led to the choosing of basic catalyst instead of acidic one in this study, as both trans and conjugated fatty acids are also the component of interest.

The setback of basic catalyst is saponification or formation of soap can occur during transesterification process (Ackman, 1998). However, for routine analysis of cooking oil from food outlets, basic catalyst is recommended. Some precautionary steps were taken such as using anhydrous condition in all chemicals to prevent saponification (Demirbas, 2008). The catalyst that was used was catalyst that was freshly prepared as prolonged contact with air will diminish the effectiveness of alkaline catalysts through interaction with moisture and carbon dioxide (Freedman et al., 1984).

4.3 GAS CHROMATOGRAPHY ANALYSIS OF FATTY ACIDS

In this present work, two fused silica capillary columns were used in GC analysis of fatty acid methyl ester (FAME). The columns were moderately polar, 30 m, HP Innowax and highly polar, 100 m HP 88. HP Innowax was chosen because it is generally used in GC analysis to analyze various compounds while HP 88 is specific

column for trans fatty acid detection. Trans fatty acid is expected to be discovered in degraded oil, hence the HP Innowax column was included in this study.

Figure 4.3 and 4.4 represent chromatograms of FAME standard mixture from HP Innowax column and HP 88 column respectively. The compounds that eluted in the chromatograms are listed in Table 4.1 for HP Innowax column and in Table 4.2 for HP 88 column. Elution order for both columns was according to fatty acid chain length. As the capillary column used was cyanopolysiloxane stationary phase, unsaturated fatty acid eluted later than saturated fatty acid with the same chain length and trans fats eluted earlier than their corresponding cis isomers. For fatty acid identification in samples, retention times were compared with those of standard methyl esters.

Several temperature programs were tested to achieve the best separation of the fatty acid standard mixture. The temperature program that was applied in this study was the optimized temperature program that achieved good separation in running analysis of fatty acid methyl ester authentic standard mixture. The chromatograms for the fatty acid authentic standard are shown in figure 4.3 and 4.4 for HP Innowax column and HP 88 column respectively.

Though HP Innowax column is not meant to achieve separation of cis and trans fatty acid, it could separate trans-9, 12-octadecadienoic acid methyl ester (C18:2n6t) and cis-9, 12-octadecadienoic acid methyl ester (C18:2n6c). However, it could not resolve trans-9-octadecenoic acid methyl ester (elaidic acid methyl ester) and cis-9-octadecenoic acid methyl ester (oleic acid methyl ester) as only oleic acid was detected in analysis using HP Innowax column while the presence of elaidic acid was not detected. HP 88 column is specific column for trans fatty acid, thus it could achieved good resolution of all trans isomers that were available in the standard mixture.

It can be concluded here, for general analysis of fatty acid methyl ester, the use of HP Innowax column is satisfactory as it can resolve the common fatty acids that are normally present in palm oil. The separation obtained was almost equal as to those produced by highly polar HP 88 column. However, for detailed analysis of trans fatty acid isomers in oil, the use of HP 88 column is a must.

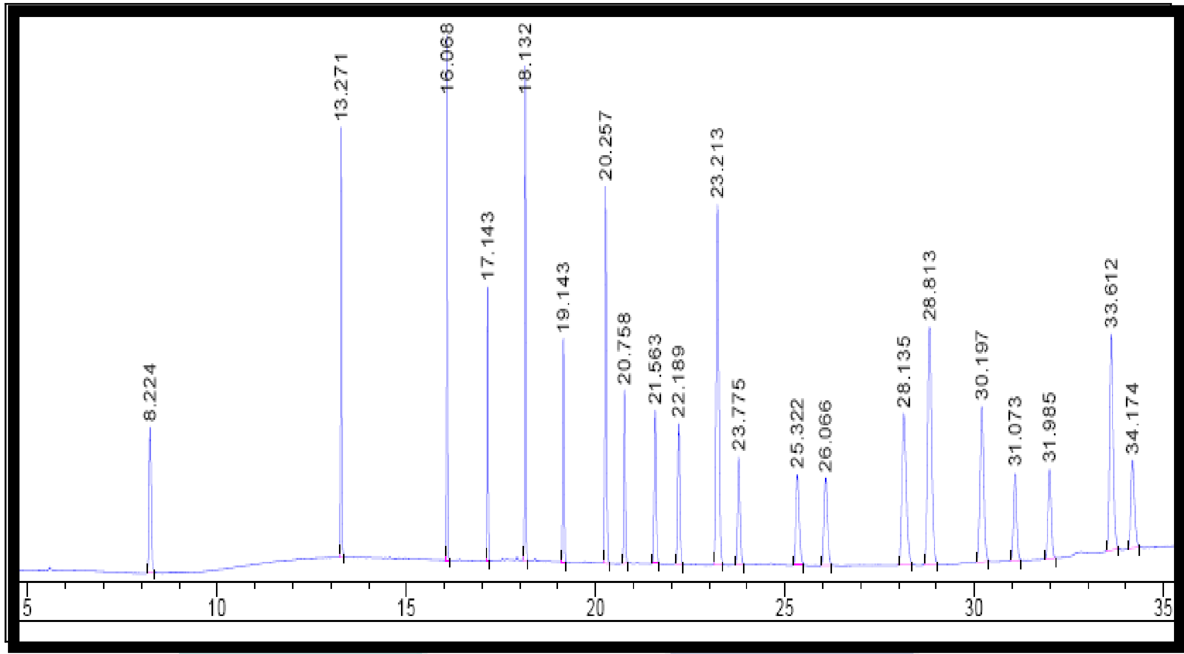


Figure 4.3: Chromatogram of fatty acid methyl esters standard mixture in HP Innowax column. Numbers above each peak represent retention time of the peak.

Table 4.1: List of fatty acid methyl esters and respective retention times that eluted in HP Innowax column

Peak	Retention time	Fatty Acid Methyl Ester
1	8.224	Hexanoic acid methyl ester (C6:0)
2	13.271	Octanoic acid methyl ester (C8:0)
3	16.068	Decanoic acid methyl ester (C10:0)
4	17.143	Undecanoic acid methyl ester (C11:0)
5	18.132	Dodecanoic acid methyl ester (C12:0)
6	19.143	Tridecanoic acid methyl ester (C13:0)
7	20.257	Tetradecanoic acid methyl ester (C14:0)
8	20.758	Tetradecenoic acid methyl ester (C14:1)
9	21.563	Pentadecanoic acid methyl ester (C15:0)
10	22.189	Cis-10- Pentadecenoic acid methyl ester (C15:1)
11	23.213	Hexadecanoic acid methyl ester (C16:0)
12	23.775	Cis-9-Hexadecenoic acid methyl ester (C16:1)
13	25.322	Heptadecanoic acid methyl ester (C17:0)
14	26.066	Cis-10-Heptadecenoic acid methyl ester (C17:1)
15	28.135	Octadecanoic acid methyl ester (C18:0)
16	28.813	Cis-9-Octadecenoic acid methyl ester (C18:1n9c)
17	30.197	Trans-9, 12-Octadecadienoic acid methyl ester (C18:2n6t)
18	31.073	Cis-9, 12-Octadecadienoic acid methyl ester (C18:2n6c)
19	31.985	Eicosanoic acid methyl ester (C20:0)
20	33.612	Cis-6, 9, 12-Octadecatrienoic acid methyl ester (C18:3n6)
21	34.174	Cis-11-Eicosenoic acid methyl ester (C20:1)

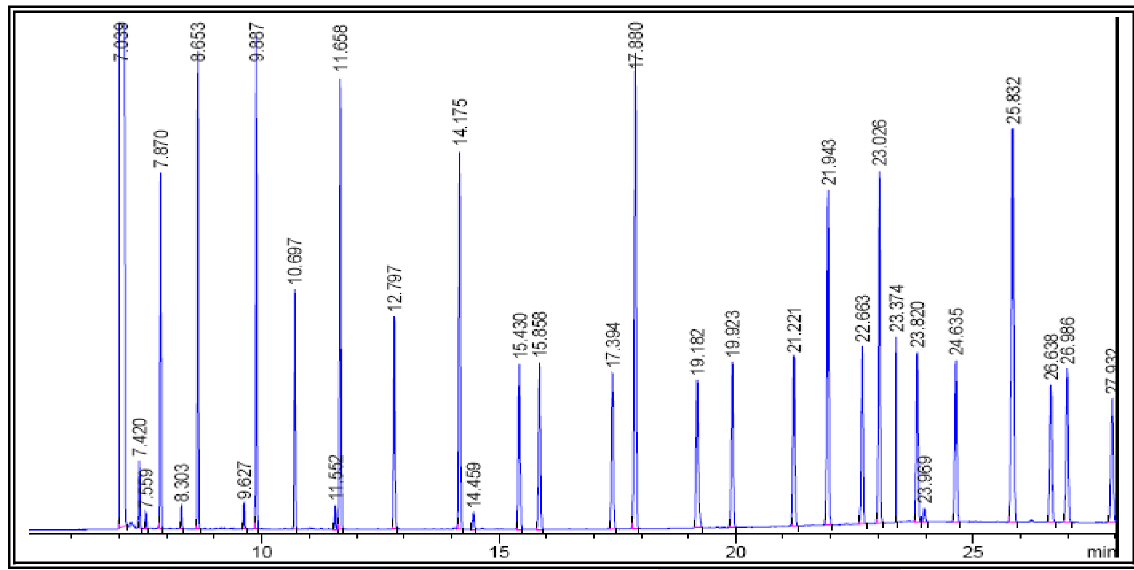


Figure 4.4: Chromatogram of fatty acid methyl esters standard mixture in HP 88 column. Numbers above each peak represent retention time of the peak

Table 4.2: List of fatty acid methyl esters and respective retention times that eluted in HP 88 column

Peak	Retention time	Fatty Acid Methyl Ester
1	7.870	Hexanoic acid methyl ester (C6:0)
2	8.653	Octanoic acid methyl ester (C8:0)
3	9.887	Decanoic acid methyl ester (C10:0)
4	10.697	Undecanoic acid methyl ester (C11:0)
5	11.658	Dodecanoic acid methyl ester (C12:0)
6	12.797	Tridecanoic acid methyl ester (C13:0)
7	14.175	Tetradecanoic acid methyl ester (C14:0)
8	15.430	Tetradecenoic acid methyl ester (C14:1)
9	15.858	Pentadecanoic acid methyl ester (C15:0)
10	17.394	Cis-10- Pentadecenoic acid methyl ester (C15:1)
11	17.880	Hexadecanoic acid methyl ester (C16:0)
12	19.182	Cis-9-Hexadecenoic acid methyl ester (C16:1)
13	19.923	Heptadecanoic acid methyl ester (C17:0)
14	21.221	Cis-10-Heptadecenoic acid methyl ester (C17:1)
15	21.943	Octadecanoic acid methyl ester (C18:0)
16	22.663	Trans-9-Octadecenoic acid methyl ester (C18:1n9t)
17	23.026	Cis-9-Octadecenoic acid methyl ester (C18:1n9c)
18	23.820	Trans-9, 12-Octadecadienoic acid methyl ester (C18:2n6t)
19	24.635	Cis-9, 12-Octadecadienoic acid methyl ester (C18:2n6c)
20	25.832	Eicosanoic acid methyl ester (C20:0)
21	26.638	Cis-6, 9, 12-Octadecatrienoic acid methyl ester (C18:3n6)
22	26.986	Cis-11-Eicosenoic acid methyl ester (C20:1)
23	26.638	Cis-9,12,15-Octadecatrienoic acid methyl ester (C18:3n3)

4.4 FATTY ACIDS COMPOSITION

Changes in fatty acids composition have been the parameter to determine oil degradation level. This is because, with repeated frying, the presence of moisture, heat, oxygen (Innawong et al., 2004) in the oil can cause occurrence of several processes that lead to oxidation, decomposition and isomerization of polyunsaturated fatty acids. Polyunsaturated fatty acids are generally sensitive to oxidation. The decomposition and isomerization of polyunsaturated fatty acid (PUFA) will change overall fatty acid composition.

Oil that is rich in PUFA is expected to have drastic changes in fatty acid composition. Palm oil is relatively more stable as it contains high amount of saturated fatty acid (Al Harbi and Al Kahtani, 1993; Bhattacharya et al., 2008). So the changes in fatty acid composition are expected to be not prominent compared to oil with high degree of PUFA. The present study was carried out to determine whether the changes in composition of fatty acids in palm oil after it is thermally treated is significant and whether it can be used as indicator for the degradation status indicator for palm oil.

The fatty acids composition of control samples and samples collected at food outlets are presented in Table 4.3 and 4.4 respectively. Results are produced based on the average of three replications with standard deviation. Sample A and B, which were the control samples of palm oil, produced fatty acids composition that is characteristic of palm oil. Generally in palm oil, palmitic acid and oleic acid are abundant and the amount of saturated and monounsaturated fatty acids composition is almost equal (Noh et al. 2002). This is in agreement with those found by Al Harbi and Al Kahtani (1993), and Abdulkarim et al. (2007) in their studies on palm oil fatty acids composition.

Corn oil sample, namely Sample C, produced fatty acids composition that is characteristic of corn oil where generally, content of linoleic acid is prominent (Guillen and Goicoechea 2009). As expected, collected samples from food eatery (Sample D), food caterer (Sample E) and banana fritters stall (Sample F) showed characteristic of palm oil where amount of palmitic acid is high. For sample collected from fast food outlet (Sample G), which the origin of the oil was not revealed, also showed

characteristic of palm oil. It is noticed that palm oil are the frying oil of choice for all food outlet operators that involved in this studies.

A total of 10 fatty acids were detected in fresh oil and 11 in heated, fried and waste oil. The fatty acids carbon ranges for fresh oil was from C10 to C20 and C8 to C20 in thermally oxidized oil. The number of double bonds in unsaturated fatty acids was from one to three double bonds. Generally for all samples, saturated fatty acids consisted of octanoic acid (C8:0), decanoic acid (C10:0), dodecanoic acid or lauric acid (C12:0), tetradecanoic acid (C14:0), hexadecanoic acid or palmitic acid (C16:0), octadecanoic acid or stearic acid (C18:0) and eicosanoic acid (C20:0). Monounsaturated fatty acids consisted of hexadecenoic acid (C16:1) and octadecenoic acid or oleic acid (C18:1). Polyunsaturated fatty acids consisted of octadecadienoic acid or linoleic acid (18:2) and octadecatrienoic acid or linolenic acid (18:3). Major fatty acids in all samples were palmitic acid, stearic acid, oleic acid and linoleic acids.

Regardless of the oxidative state, the major fatty acids in palm oil samples, namely sample A, B, D, E, F, G, were palmitic acid which is the saturated fatty acid and oleic acid which is the monounsaturated fatty acid. The high amount of saturated fatty acids contributed to oxidative stability towards palm oil while high amount of monounsaturated fatty acids is desirable due to its nutritional benefits. Amount of polyunsaturated fatty acids is not prominent in palm oil.

Corn oil sample (sample C) is highly consisted of polyunsaturated fatty acid which is the linoleic acid and linolenic acid (Guillen and Goicoechea 2009). Except in fried oil, the portions of those fatty acids were more than 50% of all fatty acid composition.

As can be seen from Table 4.3 and 4.4, all samples had increase in saturation degree and decrease in unsaturation degree. Decrease in unsaturation is the results of degradation, isomerization and decomposition of polyunsaturated fatty acids. Overall fatty acids composition will change after the content of unsaturated fatty acid is altered.

Table 4.3: Heating and cooking effect on individual fatty acid amount (% of total fatty acids) of control samples

Fatty Acids	Sample A			Sample B			Sample C		
	Fresh	**Heated**	**Fried**	**Fresh**	**Heated**	**Fried**	**Fresh**	**Heated**	**Fried**
Saturated FA									
C8:0	nd	0.02^{*a}	0.09^{*b}	nd	0.09^{*b}	0.09^{*b}	nd	0.19^{*c}	0.14^{*d}
C10:0	0.015^{*a}	0.016^{*a}	0.020^{*b}	0.02^{*c}	0.08^{*d}	0.02^{*c}	nd	nd	nd
C12:0	0.16^{*b}	0.18^{*b}	0.21^{*c}	0.19^{*b}	0.47^{*d}	0.21^{*c}	nd	nd	nd
C14:0	1.04^{*a}	1.06^{*a}	1.07^{*a}	0.98^{*b}	1.10±0.07^{a}	1.05±0.04^{a}	0.04^{*c}	0.05^{*c}	0.18^{*d}
C16:0	37.89±0.02^{a}	36.61±0.02^{a}	39.24±0.02^{a}	39.17^{*a}	42.67±3.02^{b}	42.04±3.02^{b}	12.5^{*d}	15.9±0.02^{d}	17.5±0.04^{d}
C18:0	4.23^{*a}	4.17^{*a}	4.31^{*b}	4.14^{*a}	4.46±0.27^{c}	4.48±0.11^{c}	2.01^{*d}	1.62^{*f}	2.63^{*e}
C20:0	0.36^{*a}	0.32^{*a}	0.13^{*b}	0.20^{*c}	0.20^{*c}	0.14^{*b}	0.75^{*d}	0.49^{*e}	0.49^{*d}
Total	**43.70**	**42.38**	**45.07**	**44.70**	**49.07**	**48.03**	**15.3**	**18.25**	**20.8**
Monounsaturated FA									
C16:1, n-9c	0.18^{*a}	0.31^{*b}	0.38^{*b}	0.17^{*a}	0.17^{*a}	0.33±0.14^{b}	nd	nd	nd
C18:1, n-9c	43.60±0.03^{a}	44.33±0.01^{a}	45.14±0.05^{a}	44.17^{*a}	43.28 ±0.76^{a}	43.72±0.75^{a}	29.43^{*b}	27.91±0.06^{b}	33.72±0.07
Total	**43.63**	**44.64**	**45.52**	**44.34**	**44.04**	**44.05**	**29.43**	**27.91**	**33.72**
Polyunsaturated FA									
C18:2, n-6c	12.31±0.02^{a}	12.40±0.02^{a}	8.60±0.06^{b}	10.61^{*c}	7.25±2.91^{d}	7.35±1.55^{d}	54.61^{*e}	53.03±0.03^{e}	44.03±0.11^{f}
C18:3, n-6c	0.36^{*a}	0.32^{*a}	0.38^{*a}	0.38^{*a}	0.38^{*a}	0.38^{*a}	0.42^{*b}	0.51^{*c}	0.46^{*b}
Total	**12.67**	**12.72**	**8.98**	**10.99**	**7.63**	**7.73**	**55.03**	**53.54**	**44.49**
C18.2/C16:0	0.33^{a}	0.33^{a}	0.21^{b}	0.27^{a}	0.16^{b}	0.17^{b}	4.37^{a}	3.22^{b}	2.52^{c}

n.d., not detected; * standard deviation < 0.01; data with different letter, eg ^{a,b,c} are statistically different within samples

Sample A, Palm oil Brand A; Sample B, Palm oil brand B; Sample C, corn oil

Table 4.4: Heating and cooking effect on individual fatty acid amount (% of total fatty acids) of oil samples collected from food outlets

Fatty Acids	Sample D		Sample E		Sample F		Sample G
	Fresh	Waste	Fresh	Waste	Fresh	Waste	Waste
Saturated FA							
C8:0	nd	0.03^{*a}	nd	0.02^{*a}	nd	0.02^{*a}	0.06^{*b}
C10:0	0.02^{*a}	0.02^{*a}	0.02^{*a}	0.02^{*a}	nd	0.02^{*a}	0.02^{*a}
C12:0	0.23^{*a}	0.20^{*a}	0.18^{*a}	0.23^{*b}	0.18^{*a}	0.18^{*a}	0.28^{*c}
C14:0	0.96^{*a}	0.96^{*a}	0.94^{*a}	0.96^{*a}	1.00^{*b}	1.07^{*b}	1.00^{*a}
C16:0	37.38^{*a}	38.39^{*a}	38.52^{*b}	38.82±0.09^{b}	36.94^{*b}	36.67^{*b}	39.82^{*b}
C18:0	4.14^{*a}	4.28^{*b}	4.19^{*a}	4.13^{*a}	4.05^{*c}	4.19^{*a}	4.71^{*d}
C20:0	0.15^{*a}	0.14^{*a}	0.15^{*a}	0.19^{*b}	0.21^{*b}	0.31^{*c}	0.32^{*c}
Total	**42.88**	**44.02**	**44.00**	**44.37**	**42.38**	**42.46**	**46.21**
Monounsaturated FA							
C16:1, n-9c	0.18^{*a}	0.18^{*a}	0.16^{*a}	0.18^{*a}	0.18^{*a}	0.31^{*b}	0.23^{*b}
C18:1, n-9c	45.23^{*b}	44.60±0.02^{a}	44.48±0.02^{a}	44.23 ±0.04^{a}	45.57^{*b}	44.30 ±0.04^{b}	43.08 ±0.04^{c}
Total	**45.41**	**44.78**	**44.64**	**44.41**	**45.75**	**44.61**	**43.31**
Polyunsaturated FA							
C18:2, n-6c	11.10^{*c}	9.96^{*b}	10.62^{*a}	10.60 ±0.13^{a}	11.51^{*c}	12.35±0.02^{d}	9.93^{*b}
C18:3, n-6c	0.38^{*a}	0.38^{*a}	0.37^{*a}	0.37^{*a}	0.36^{*a}	0.36^{*a}	0.38^{*c}
Total	**11.48**	**10.34**	**10.99**	**10.97**	**11.87**	**12.71**	**10.31**
C18.2/C16:0	0.30^{a}	0.26^{b}	0.27^{b}	0.27^{b}	0.31^{a}	0.33^{a}	0.24^{b}

n.d., not detected; * standard deviation < 0.01; data with different letter, eg ^{a,b,c} are statistically different within samples

Sample D, from food eatery, papads as food medium; Sample E, from food caterer, chicken meat as food medium; Sample F, from banana fritters stall, banana fritters as food medium; Sample G, from fast food outlets, French fries as food medium

4.4.1 Fatty acids saturation degree

After intense heating, the double bond of unsaturated fatty acids can be disrupted by oxidation, scission, and polymerization (Cowan, 1954). Unsaturated fatty acids then will undergo transformation into decomposition product. Saturated fatty acids do not contain any double bond or other functional groups along the chain. Thus, saturated fatty acid is said to have lower sensitivity towards oxidation.

The decomposition of unsaturated fatty acid will increase the saturation level of an oil. Increase in saturation degree is attributed by the increase in saturated fatty acids relative to monounsaturated fatty acids and polyunsaturated fatty acids. Fatty acids saturation degree was investigated in this study to find out whether the changes in saturation degree in palm oil is significance and can be one of parameter to determine oil degradation level.

As shown by Table 4.3 and 4.4, saturated fatty acids amount increased while amount of polyunsaturated acids decreased after the oil was thermally treated. Figure 4.5, 4.6 and 4.7 show fatty acids saturation degree in the fresh and thermally treated oil samples. Sample A, B which were control samples of palm oil, were measured as a batch, Sample D, E, F and G which were palm oil collected samples were measured as a batch.

In palm oil control samples (A and B), the amount of saturated fatty acids in fresh state was 44.2%. After heated, the amount increased to 45.73%, while in fried state, the amount was higher, 46.65 %. The same tendency also applied to monounsaturated fatty acids. The amount was higher after thermally treated. It was the other way around for polyunsaturated fatty acids where the amount underwent decreases in thermally treated oil. The decreases were more prominent in fried oil where the amount was 8.45 % compared to 11.83% in fresh state.

From Figure 4.6, it was observed that a big decrease of polyunsaturated fatty acid amount in fried corn oil of sample C. The amount declined to 44.49 %, where the content was originally 55.03 % in fresh state. The amount decreased to 53.54 % in

heated sample. Berdeaux et al. (2002) claimed that linoleic is the most representative of fatty acid undergoes degradation in oil. The claims can explain the great loss of polyunsaturated fatty acids in sample C as this sample highly contained linoleic acid.

As can be observed from Figure 4.5 and 4.6, fried oil showed sharper decline in polyunsaturated fatty acids amount compared to heated oil. It shows here that heating and frying medium underwent different degradation process and food medium may play its part in influencing oil deterioration. Compounds from the food such as moisture, trace metal, may trigger more deterioration steps.

From Figure 4.7 that represents fatty acids saturation degree in sample from food outlets, namely D, E, F and G, it is observed that the saturated fatty acids amount increased and polyunsaturated fatty acids amount decreased in waste oil. However, monounsaturated fatty acids experienced slight decline in its amount, from 45.26 % fresh oil to 44.33 % in waste oil.

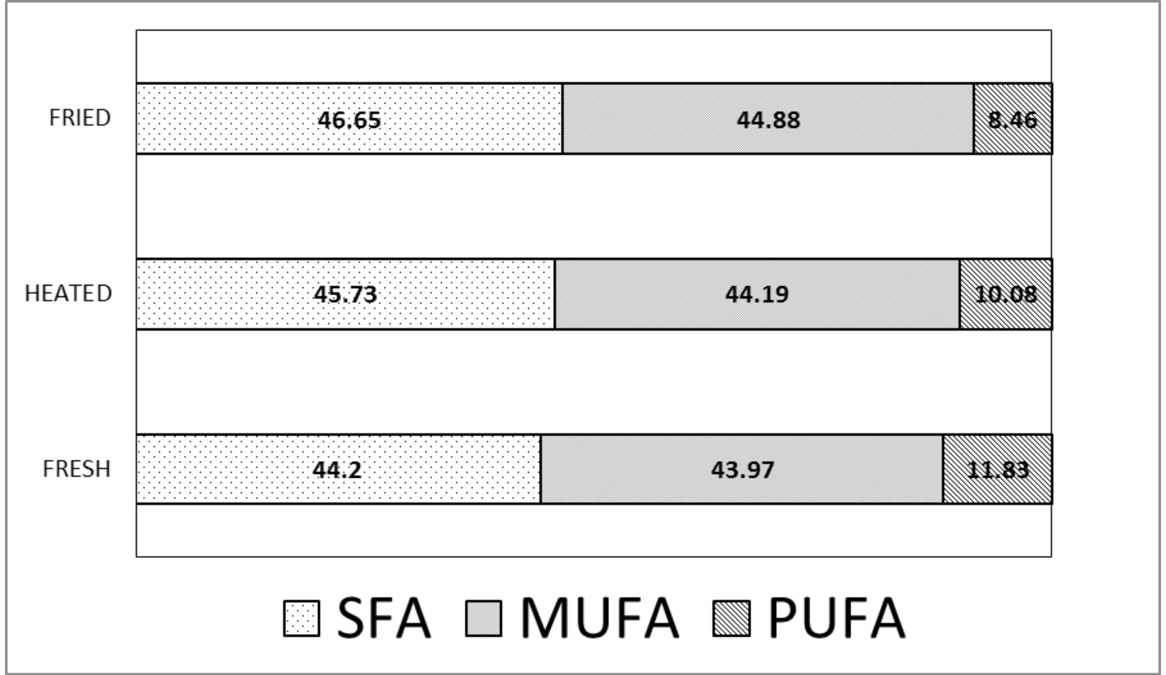


Figure 4.5: Fatty acids saturation (%) in fresh, heated and fried oil of sample A and B. SFA: saturated fatty acid. MUFA: Monounsaturated fatty acid. PUFA: Polyunsaturated fatty acid.

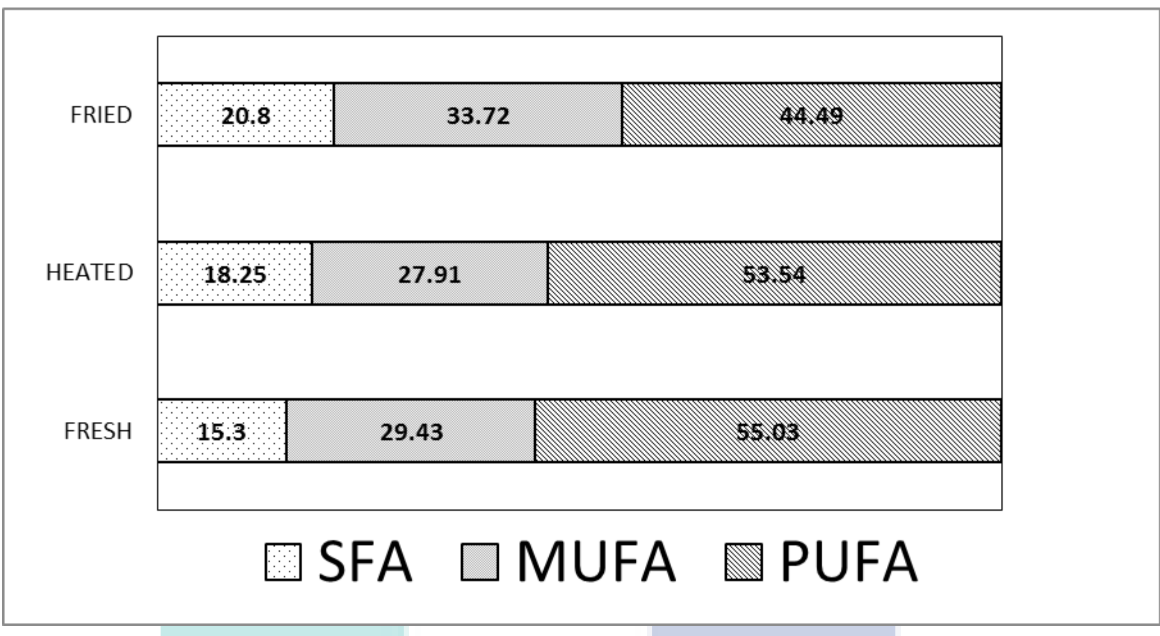


Figure 4.6: Fatty acids saturation (%) in fresh, heated and fried oil of sample C.
SFA: saturated fatty acid. MUFA: Monounsaturated fatty acid. PUFA: Polyunsaturated fatty acid.

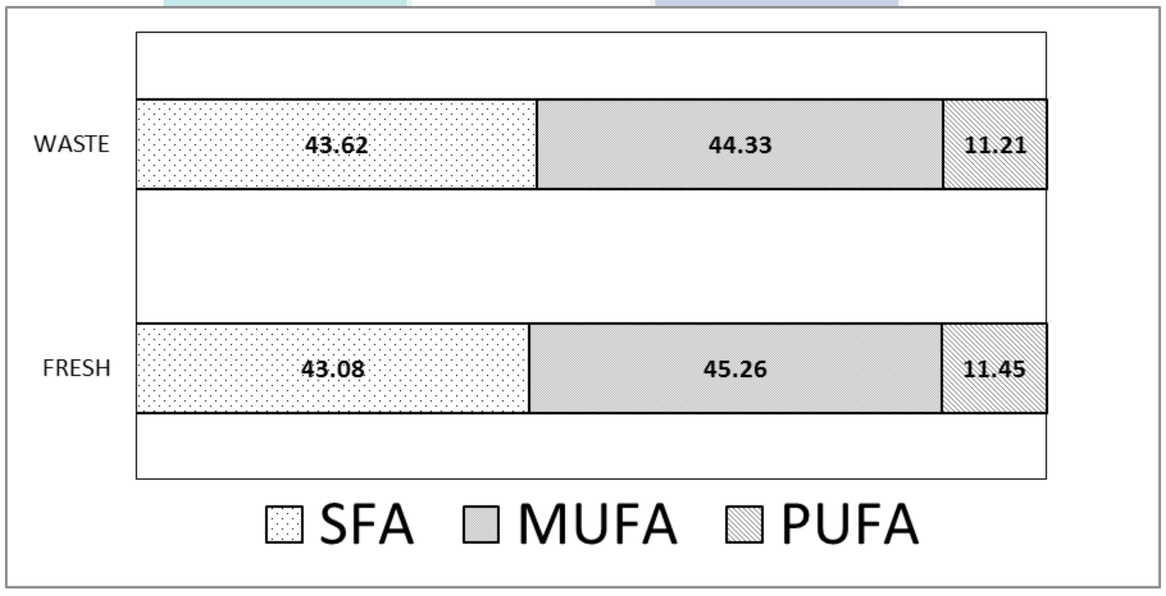


Figure 4.7: Fatty acids saturation (%) in fresh and waste oil of sample D,E ,F, G.
SFA: saturated fatty acid. MUFA: Monounsaturated fatty acid.
PUFA: Polyunsaturated fatty acid.

Berdeaux et al. in (2002) stated that linoleic and oleic acids are representative of fatty acids undergo degradation in the oils. In this study, the decrease of linoleic acid

was accompanied with the decrease in unsaturation degree. However, in this study, there was no decrease in the amount of oleic acid. Probably this is because oleic acid, being a mono unsaturated fatty acid, is more resistant towards oxidation as it contains only one double bond compared to polyunsaturated fatty acids which have at least two double bonds. Bansal et al. (2010a) also discovered no decrease in oleic acid amount in their research of deep fried and heated palm oil. However, Cuesta et al. (1991) found that oleic acid showed a tendency to decrease in frying of olive oil. Rossi et al. (2009), observed higher degradation rate of sunflower oil, compared to other types of vegetable oil, due to the high content of linoleic acid. Aladedunye and Przybylski (2009) observed progressive decrease in linoleic acid of canola oil throughout frying period. All of the studies stated above claimed that oil with high polyunsaturated acids content showed more tendencies to deteriorate. These statements are in agreement with present study where corn oil sample showed more degradation of polyunsaturated fatty acids compared to palm oil samples.

Figure 4.5, and 4.6 show that palm oil control samples (A and B) and corn oil control sample (C) underwent increase of saturated fatty acid amount about 2.45 % and 5.50 % respectively. Corn oil, which amount of polyunsaturated fatty acids is high, showed more prominent increase in saturated fatty acids compared to palm oil. The increase was about two fold than the increase in palm oil. Unlike total polar compounds where the amount of 27 % is considered as the limit, no limit has been set yet for increase in fatty acids saturation degree. However this study can give estimation that palm oil samples that shows increase of 2.45 % in saturated fatty acids content relatives to its fresh counterpart has been thermally treated at 180 °C - 200 °C for about 6 hr. However, more samples should be tested to reach that conclusion.

4.4.2 Ratio of linoleic to palmitic acid (C18:2/ C16:0)

In a study dealing with oxidative stability of an oil, besides changes in fatty acids saturation degree, the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) has been used as an indicator of oil degradation. The ratio is represented by ratio of linolenic acid to palmitic acid (C18:2/ C16:0) (Aladedunye and Przybylski, 2009).

Linoleic acid (C18:2) and palmitic acid (C16:0) are major components in oil. While linoleic acid it is said to be representative of fatty acid undergoes degradation, palmitic acid is said to be highly stable, thus ratio of linoleic acid to palmitic acid (C18.2/C16:0), calculated by percentages of linoleic acids divided by the percentages of palmitic acid, has been suggested as a valid indicator of the level of oil deterioration. The lower the value of C18.2/C16:0 ratio, (decrease in linoleic acid and increase in palmitic acid), the higher the degradation level (Aladedunye and Przybylski, 2009).

Table 4.3 shows the value of C18.2/ C16:0 for control samples. As can be seen from the table, thermally treated oils show significantly lower value of C18.2/ C16:0 than the fresh counterparts. For control palm oil sample (A), only fried oil experienced a significant decline in value of C18.2/ C16:0, while heated oil value was not significantly different from fresh oil. For sample B, both heated and fried oil experienced significant decrease in the value of C18.2/ C16:0 the ratio. Even though sample A and B were both palm oils, they were different in terms of fatty acids composition and maybe in the amount of additives as they were different brands of oil. This might explain the differences in the magnitude of C18.2/ C16:0 reductions. Corn oil sample, namely Sample C, experienced significant difference in the value between fresh, heated and fried oil, with fried oil had higher decrease in the value compared to heated oil.

In agreement with the results of fatty acids saturation degree in section 4.5.1, it can be concluded that fried oil were more degraded than heated oil as all samples showed lower value of C18.2/ C16:0 in fried oil than heated oil. Bhattacharya et al. (2008) also found out fried palm oil deteriorated further than heated oil. Contrary with the results from Bansal et al. (2010a), they discovered oxidation process progressed more rapidly in the controlled heating process as compared to the frying process, indicated by lower value of C18:2/ C16:0 in their heated palm oil samples than fried oil samples. The differences in the result probably can be explained by frying variables that can influence degradation rate such as warm up time, oil rotation, manipulation, and the frying utensil (Alvis et al. 2009).

From Table 4.4, sample collected from food caterer (sample D) showed significant decrease in C18:2/ C16:0 values. All samples showed reduced value of

C18:2/ C16:0 except sample from banana fritters stall (sample F) where the value was higher in waste oil than fresh oil. There is no explanation can be given why waste oil of sample F produced higher value than the fresh counterpart. Probably, as claimed by Knothe and Steidley, (2009), the changes of C18:2/ C16:0 are more of a random nature. No comparison between fresh and waste oil can be made for sample G, however, sample G produced lowest value of C18:2/ C16:0 among waste oil samples.

Unlike total polar compound which maximum limit at 27% has been set by regulatory bodies, no maximum or minimum limit for C18:2/ C16:0 has been set yet. For example in this study, the C18:2/ C16:0 value of palm oil control samples can be the reference. C18:2/ C16:0 value of heated and fried oil increased by 0.10 unit to 0.12 unit. Thus, any discarded oil sample that shows increase of C18:2/ C16:0 value within the range can be proposed to has undergone treatment about 180 °C to 200 °C for 6 hr. However more samples is needed to reach this conclusion.

The results of C18:2/ C16:0 values did not show consistency, even within samples that received the same treatment. This study would like to agree with claims done by Knothe and Steidley, (2005), that the changes in C18:2/C16:0 is more of a random nature. Moreover, for routine analysis of oil degradation, the C18:2/ C16:0 values of discarded oil need to be compared with C18:2/ C16:0 value of fresh counterpart. This is to determine how much the C18:2/ C16:0 value has increased. Thus a mere value of C18:2/C16:0 in degraded oil means nothing if it is not compared with fresh oil to determine how far the value has changed from fresh to oxidative state.

4.4.3 Short chain fatty acid

Besides increase in saturation degree, oxidation of unsaturated fatty acids will produce other components. According to Kamal Eldin et al. (1997), Berdeaux et al. (2002), and Velasco et al. (2005), when oleic acid and linoleic acid are oxidized, hydroperoxide is formed. Due to its instability nature, the hydroperoxide will be further taking part in other reaction, forming secondary oxidation product consists of volatile and non volatile.

The non volatile compounds are in chemical and nutritional interest as it can remain in the frying oil, entered and ingested in human diet. The non volatile compounds usually consist of short chain fatty acid, which are heptanoic acid (C7:0) and octanoic acid (C8:0), and saturated short chain aldehydic fatty acid, which are 8-oxo-octanoic acid (8-oxo-C8:0) and 9-oxononanoic (9-oxo-C9:0). 9-oxononanoic, the major aldehydic acid in oxidized lipid, has adverse effect to health as it can induce hepatic lipid peroxidation and affect hepatic metabolisme (Berdeaux et al., 1999).

As indicated by fatty acid composition in Table 4.3 and 4.4, the most prominent about the fatty acid composition of all samples is octanoic acid (C8:0) was only detected in thermoxidized oils, which were the heated, fried and waste oil. Octanoic acid was not detected in fresh oil of all samples.

Gas chromatographic analysis using both columns, the HP Innowax and HP 88 has confirmed that C8:0 was only detected in degraded oil. Figure 4.9 and 4.10 present chromatograms of gas chromatographic analysis using HP Innowax and HP 88 respectively. In HP Innowax column, the presence of C8:0 was notified by a peak eluted at 13.256 minutes retention times. In HP 88 column, C8:0 eluted at 8.692 retention times. No peak eluting at both retention times, using both columns, was detected in fresh oil of all samples. Chromatograms for other samples are at the appendices.

In waste oil samples, amount of C8:0 ranging from 0.02 % to 0.06 %. Sample from fast food outlets, (Sample G), showed significantly higher amount of C8:0 than other waste samples followed by sample from food eatery (Sample D), sample from food caterer (Sample E) and sample from banana fritters stall (Sample F).

In control samples, the amount of C8:0 was much higher than collected samples, from 0.02 % to 0.19 %. Corn oil, namely sample C showed significantly highest amount of C8:0. In heated oil, 0.19 % was detected while 0.14 % was detected in fried oil. It was noticed that, in all control samples (A, B, and C), the amount of C8:0 in heated and fried oil did not show consistency in its pattern. In sample A, fried oil produced significantly higher amount of C8:0 than heated oil, in sample B, both heated and fried oil produced equal amount while in sample C, the amount of C8:0 was significantly

higher in heated oil than fried oil. Though all of the oil control samples received the same treatment, differences in terms of fatty acids composition, presence of additives, oil warm up time and rotation could led to the different level of degradation (Bhattacharya et al. 2008).

For the short chain fatty acid, no heptanoic acid was detected in this study. This is probably because, the formation of heptanoic acid (C7:0) is the result of oleic acid degradation singly, while formation of octanoic acid (C8:0) is the result of degradation of both oleic and linoleic acid (Velasco et al., 2005). Even though oleic acid is more abundant than linoleic acid in the oil samples, linoleic acid is relatively more reactive towards reaction with oxygen as it contains more double bond than oleic acid. Therefore, more chances for C8:0 formations than C7:0.

Generally aldehydic fatty acid present in lower amount in degraded oil, therefore, to be detected, it needs to be fractionated using column chromatography and they present in polar fraction (Berdeaux et al. 2002). However, in this study, even after the oil was fractionated using column chromatography, no aldehydic fatty acid was detected in gas chromatographic analysis. It could be that no aldehydic acid was formed during the oxidation process in this study. However, it is also possible that it was formed but it has degraded into other compound because aldehydic compounds normally will participate in further reaction due to its instability nature whereas the saturated short-chain fatty acids accumulate due to higher stability (Velasco et al., 2005).

Formation of C8:0 is the result of linoleic acid degradation as claimed by (Velasco et al., 2005). In this present study, the claim is likely to be true as strong correlation (r^2 =0.8806 and r= 0.938) was discovered between the changes in linoleic acid level to the amount of octanoic acid formed. Figure 4.10 shows the correlation plot between amount of octanoic acid formed and the decrease in linoleic acid.

Corn oil contains high amount of linoleic acid, as can be seen from Table 4.3 and it underwent a marked decrease in unsaturation level as shown by Figure 4.6. Thus the amount of C8:0 formed was much higher in Sample C compared to other samples.

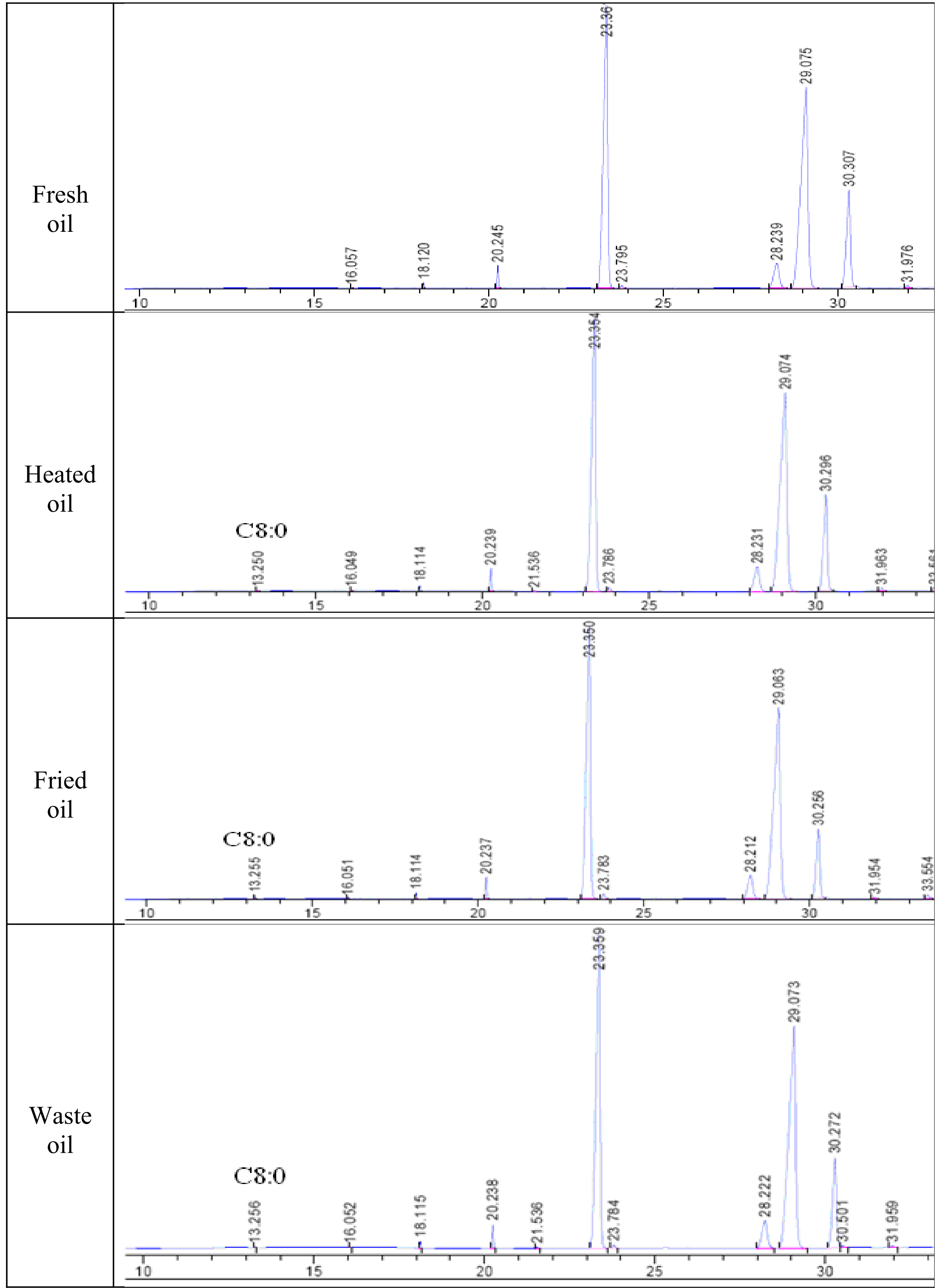


Figure 4.8: Chromatograms of fatty acids in fresh, heated, fried and waste oil using HP Innowax column. Octanoic acid (C8:0) presence is represented by a peak eluting at 13.256

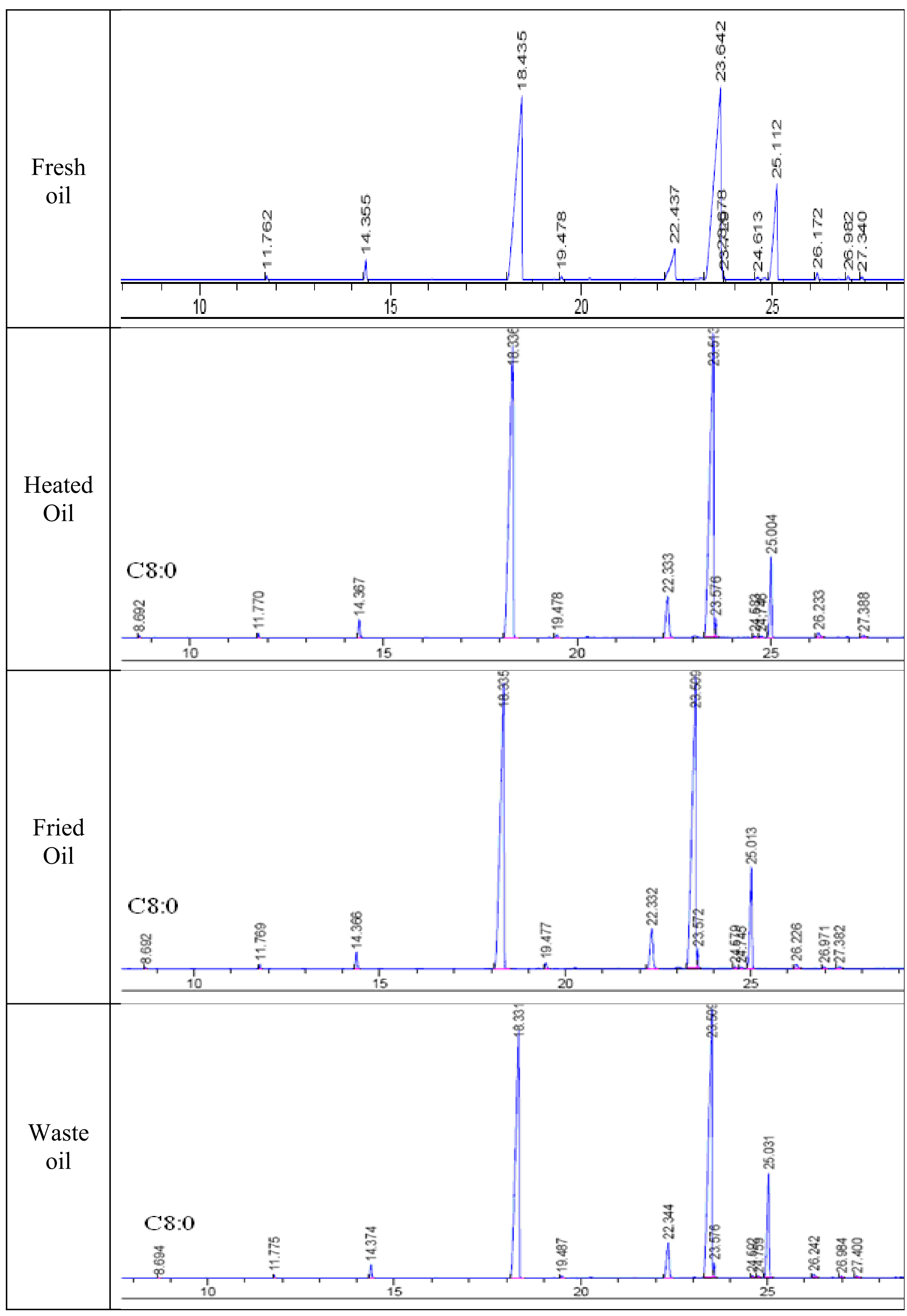


Figure 4.9: Chromatograms of fatty acids in fresh, heated, fried and waste oil using HP 88 column. Octanoic acid (C8:0) presence is represented by a peak eluting at 8.594.

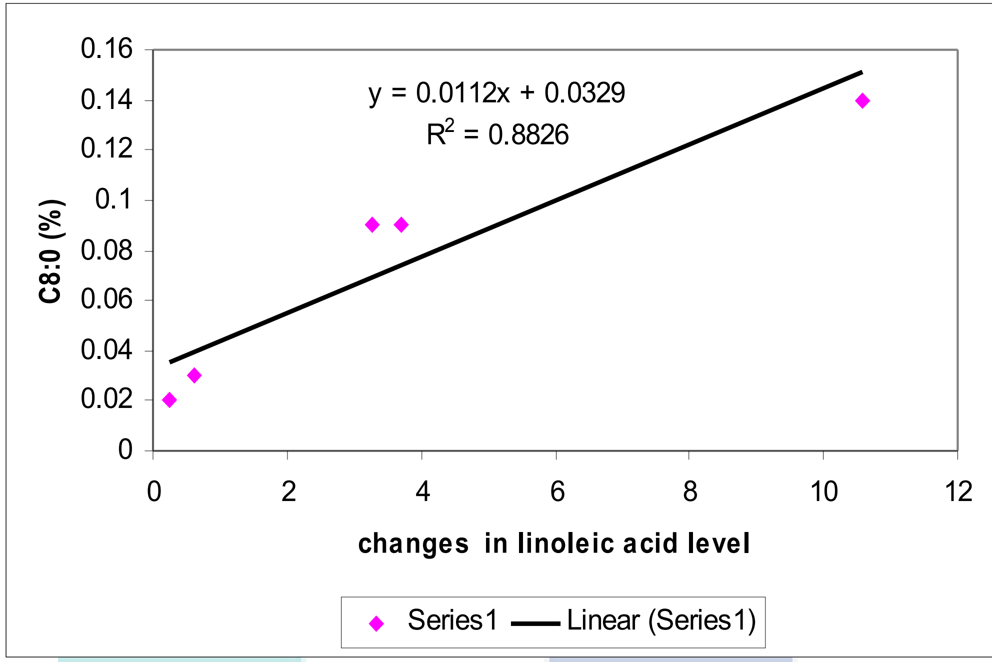


Figure 4.10: Correlation plot between changes of linoleic acids level and the amount of octanoic acids formed

Since the amount of octanoic acid detected by gas chromatography was too small, Limit of Detection (LOD) analysis was carried out to verify that the signal of octanoic acid detected by the GC does not come from GC noise. It is also to determine if the area detected by the gas chromatography is within the acceptable detection limit. From the calibration curve in Figure 4.11 and Table 4.5, the GC response was linear and the octanoic acid was detected down to 0.001 % area. The LOD value for octanoic acid was found to be 0.03 % (v/v) in concentration or 0.04 in area (%). From Table 4.3 and 4.4, the octanoic acids were detected at 0.02, 0.03, 0.06, 0.09, 0.14 and 0.19 area (%). From the calibration table of 4.5, it can be observed that the area (%) of 0.02 and 0.03 falls below the 0.03 % (v/v) in concentration or 0.04 in area (%). However, area (%) of 0.06, 0.09, 0.14 and 0.19 are above the minimum detection limit (0.03 % v/v) or 0.04 in area (%). However, it can be concluded that the signal of octanoic acid detected by the GC does not come from GC noise as most of the signal detected by the GC are above the minimum detection limit.

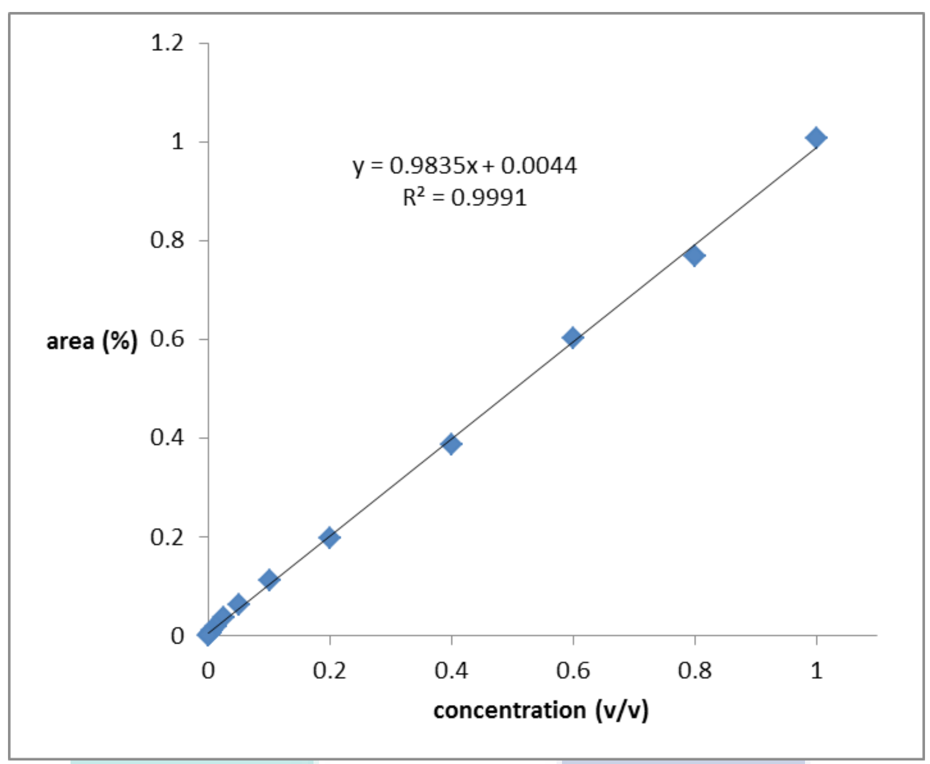


Figure 4.11: Calibration curve of octanoic acid standard

Table 4.5: Calibration table of octanoic acid

Concentration (% v/v)	area (%)
1.0	1.006
0.8	0.769
0.6	0.601
0.4	0.386
0.2	0.196
0.1	0.112
0.05	0.063
0.03 (LOD)	**0.04**
0.025	0.035
0.0125	0.018
0.00625	0.009
0.003125	0.005
0.001563	0.003
0.000782	0.001

UMP

In routine analysis, no comparison between fresh and oxidized is needed as this study has confirmed that fresh oil, regardless of oil type, is negative of C8:0. Therefore it can be proposed here that presence of C8:0 in oil can be conclusive indicator whether an oil has been thermally treated and oxidized. C8:0 amounts also correlated well with the decrease of linoleic acid, which can indicate the degradation of polyunsaturated fatty acids.

Moreover, the presence of C8:0 was independent of oil treatment, oil type and food medium. Even though the amount of C8:0 is too little to effect overall fatty acid composition, its presence can be a marker for thermally oxidized oil as it only present in oxidative state, not in fresh oil, regardless of frying conditions that were applied towards the samples. Bruhl and Matthaus (2008) also claimed production of octanoic acid was constant in thermally treated oil even though the amount was small.

4.4.4 Trans fatty acid

In nature, fatty acids in edible oil normally occur in cis isomer. During deep frying and heating (Mjos, 2005; Tsuzuki et al., 2008), double bonds in unsaturated fatty acids are reduced and some of them are converted from their natural cis to trans configuration (Bansal et al., 2009; Romero et al., 2000). Therefore, the presence of trans fatty acid in oil can indicate that the oil has undergone process such as deep frying.

This study investigated whether palm oil also susceptible to formation of trans fatty acid through heating and frying process. It was also to detect the presence of TFA in samples collected from food outlets. From this study, there was no trans fatty acid (TFA) detected in all samples. Trans fatty acid is well known for the difficulty to separate it from the cis fatty acid in gas chromatography analysis. To obtain good resolution from the cis counterpart, TFA need to be analyzed by highly polar column in gas chromatography analysis. In this study, even on high resolution HP 88 column, trans fatty acid was not detected and it was not due to poor gas chromatography parameter such as temperature program and gas carrier gas flow that was applied in this study. This is because, running analysis on mixture of fatty acid methyl ester authentic

standard containing trans fatty acid isomers using the HP 88 column with the applied gas chromatography parameters gave good resolution between trans and cis fatty acid.

Kramer et al. (1997) claimed that transesterification using acidic catalyst can result in decomposition of trans fatty acid. Hence, transesterification using mild catalyst, which was basic catalyst, was taken as precautionary step to avoid degradation of trans fatty acid. Still, there was no trans fatty acid detected in all samples.

This result is in agreement with study conducted by Liu et al. (2007) in their work on highly unsaturated soybean oil, that is said to be more susceptible for trans fatty acid formation than palm oil. No trans fatty acid formation was observed even after extensive heating at 160, 180 and 200 °C for 24 hr in the soybean oil sample. They suggested formation of TFA requires more severe condition. Aladedunye and Przybylski, (2009) obtained TFA in their fried oil probably because their canola oil sample contained high amount of polyunsaturated fatty acid which are more prone to isomerization at high temperature. The palm oil samples in this study were relatively more stable palm oil due to high saturation level.

Romero et al. (2000), though they obtained TFA in their fried palm oil, but the quantity of TFA that they obtained were so minor, even to the extent of non existence in certain sample. They suggested the TFA obtained in fried oil is not from the frying process itself, but more to transfer of TFA from food medium which already contain TFA to the frying medium. Food medium in this study was chicken nugget and the manufacturer revealed generally that all of their products contain zero level of trans fat. However, no fatty acid extraction and analysis was carried out towards the food medium to validate this claim. Bansal et al. (2009) discovered TFA in fried palm oil. However their fresh palm oil samples also contained trans fatty acid of linoleic acid isomers, whereas fresh palm oil in this study do not contain any trans fatty acid.

No trans fatty acid was discovered in degraded oil even after steps such as transesterification with basic catalyst and using high resolution column, the HP 88 column was taken. Romero et al (2000) suggested that trans fatty acid originally contain in the food medium can be transferred to frying medium. It is possible that no trans fatty

acid detected is because the food medium (chicken nugget) were zero in trans fat as revealed at the packaging, thus there was no transfer of trans fatty acid from the food medium to frying oil. So it can be concluded here that trans fatty acid is not a good indicator of oil degradation as it is independent on type of food. If trans fatty acid is made as an indicator in degraded oil, oil that is used to fry food without trans fatty acid will be considered superior compared to oil that is used to fry food with trans fatty acid where in reality, it does not mean necessarily so.

4.5 TOTAL POLAR COMPOUNDS

Total polar compounds (TPC) refer to all the degraded products other than the initial triacylglycerol or fatty acids present in fresh oil (Bansal et al., 2010a) and it has been the standard method to determine oil discarded level in some countries with regulatory limits of 27 % (Akoh and Min, 2002).

As can be seen from Table 4.6, the entire samples showed amount of polar compounds well below the limit. This can indicate that using palm oil for about 6 hr at high temperature is still acceptable in terms of production of polar components. Researches done by Abdulkarim et al. (1999) and Bansal et al. (2010a) showed that palm oil needed about 6 hr of frying time daily for five days to achieve discarded levels.

Sample C, which was corn oil sample, showed highest amount of polar compounds compared to other sample in all state, whether in fresh, heated or fried. The value was 3.9, 13.1 and 14.4 % respectively. Being an oil with high amount of unsaturated fatty acid, corn oil is more reactive towards oxidation. The differences in total polar compounds compared to palm oil are significant ($p< 0.05$). This finding is similar with works done by Takeoka et al. (1997) and Abdulkarim et al. (2007) where oil with high unsaturated fatty acids content produced relatively more polar compounds than oil with high saturated fatty acids content. According to Al Harbi and Al Kahtani, (1993), TPC contents is more reliable to be adapted to polyunsaturated oils and might not be applicable to more saturated oils as saturated oils are relatively lower in sensitivity towards oxidation.

Table 4.6: Total polar compounds (%) in control and collected samples

	Control Samples				Collected Samples			
	A	B	C		D	E	F	G
Fresh	3.2 ±0.2 $^{a, A}$	3.5 ±0.2 $^{a, A}$	3.9 ±0.5 $^{a, A}$	**Fresh**	3.3 ±0.5$^{a, A}$	3.9 ±0.3 $^{a, A}$	3.3 ±0.2 $^{a A}$	ND
Heated	10.1 ±0.5 $^{b, A}$	10.8 ±0.2 $^{b, A}$	13.1 ±0.6 $^{b, B}$	**Waste**	8.2 ±0.2$^{b A}$	7.5 ±0.5 $^{b A}$	7.8 ±0.3 $^{b A}$	14.1±0.4 ^{B}
Fried	11.8 ±0.6$^{c, A}$	12.0 ±0.5$^{c, A}$	14.4 ±0.3 $^{c, B}$					

Values are means of triplicates with standard deviation Values are means of three replicates with standard deviation. Mean values within each column followed by different letters (a, b, c, etc) are significantly ($P < 0.05$) different. Mean values within each row followed by different letters (A, B, C, etc) are significantly ($P < 0.05$) different. ND: not determined

Footnote:

Sample A, Palm oil Brand A;
Sample B, Palm oil brand B;
Sample C, corn oil
Sample D, from food eatery, papads as food medium;
Sample E, from food caterer, chicken meat as food medium;
Sample F, from banana fritters stall, banana fritters as food medium;
Sample G, from fast food outets, French fries as food medium

Sample A and B, both were palm oil sample, though perceived to be different in quality by consumers, were not significantly difference in terms of polar compound formation in fresh, heated and fried oil. After thermally treated, both samples showed significant differences between fresh, heated and fried. For sample A, the TPC amount evolved from 3.2 % in fresh oil to 10.1 % and 11.8 % in heated and fried oil respectively. In sample B, the value was 3.5, 10.8 and 12.0 % in fresh, heated and fried oil respectively.

The entire collected samples showed lower amount of polar compounds than palm oil control samples, except sample from fast food outlet (Sample G), where the value was 14.1 %. This can indicate that sample G was more deteriorated compared to palm oil control samples and could had been used for more than 6 hour at high temperature prior discarded. For waste oil, sample G with 14.1 % content produced the highest value, followed by sample D, F and E. Only TPC content of sample G differed significantly between samples. Total polar compounds content in waste oil of all samples were significantly ($p < 0.05$) higher than fresh oil.

Bansal et al. (2010a) found higher amount of polar compounds in heated oil compared to fried oil. They claimed food in the frying oil can act as protective barrier against oxidation. This is in contrast with result from present study where amount of polar compound was significantly higher in fried oil compared to heated oil. Argument for this situation is compounds that probably present in the food medium such as trace metals, moisture, alkaline reacting materials may initiate other reaction that can lead to higher formation of polar compounds. Bhattacharya et al (2008) also discovered that fried oil was more degraded than heated oil. He claimed that the presence of moisture from food initiate other chemical reactions that lead to higher formation of total polar compounds in fried oil.

Lots of previous researchers claimed that TPC amount is reliable in determining the quality of an oil. However, this method is extremely laborious, time consuming, use hazardous organic solvent and large volume of solvents is considered as potential environmental problem. In this study, about 30 minutes was required to analyze one sample. Prior analysis, silica gel in column chromatography need to be heated for about

4 hours and rehydrated to achieve correct activity and moisture content. Failure to do so will render separation of polar and non polar fractions to be inaccurate. Discrepancies also can occur between analyses if silica gel activation level is different. Silica gel activation also can render longer analysis time.

In this study, total polar compound is a good parameter to indicate oil degradation as the difference in TPC amount between fresh and thermally treated oil were significant. However, the method is time consuming, so parameter that correlates well with TPC amount is required to replace TPC as routine analysis in oil quality. Results of other parameters that will be presented in the next section will be compared with total polar compounds to determine which parameter correlate well with total polar compounds.

4.6 FREE FATTY ACIDS VALUE

Free fatty acids test measures the amount of detached, unbound or non esterified fatty acids to triaclyglycerol molecule. The free fatty acid is caused by presence of moisture and air at high temperature that can lead to hydrolytic and oxidative activity in fat. When triacylglycerol molecule is hydrolyzed, free fatty acid, monoacylglycerol and diacylglycerol will be formed (Bhattacharya et al., 2008). Thus, the amount of free fatty acids can indicate oil degradation level.

Table 4.7 and 4.8 present the amount of free fatty acids in the control and collected samples respectively. In fresh oil of sample A, B, C, the amount of free fatty acids was 0.11, 0.16, and 0.13 % respectively. The amount were not differed significantly, however sample B showed the highest amount. The appearance of sample B, as fresh oil also can indicate the quality in terms of free fatty acid (FFA) level. The oil was cloudy compared to other fresh oil. Cloudiness in oil can be attributed to the presence of monoacylglycerol and diacylglycerol, which are the results of hydrolysis of triaclyglycerol molecule.

In heated samples, no significant difference between sample A and B were discovered. However those samples differed significantly from heated corn oil sample,

the sample C. Fried oil also exhibited the same pattern where no significant difference between sample A and B, but both A and B differed significantly with sample C. This situation showed how corn oil which have received same treatment, can differ from palm oil in terms of free fatty acids formation. This is due to the presence of high content of polyunsaturated fatty acids in corn oil that are more prone to oxidation and hydrolysis.

For all control samples, the value of fried oil was significantly higher than heated oil. This is because presence of moisture in the food of fried oil can induce hydrolysis whereas in the heated oil, the free fatty acids formation only attributed by oxidation as heated oil is minus of food (Bhattacharya et al., 2008).

Fritsch (1981) claimed that initial value of FFA in an oil can influence the rate of formation of FFA after oxidation as the study discovered formation of FFA in degraded oil was relative to initial amount of FFA in fresh oil . It can be seen from table 4.6, oil with high FFA amount initially in fresh oil produced higher amount of FFA after thermally treated.

All waste oil samples gave FFA amount that was significantly difference from fresh oil. Waste oil sample of fast food outlet (sample G), which is fast food outlet discarded oil, gave highest amount of free fatty acid at 0.40 %. The value was even higher than the value of heated and fried palm oil control sample. The lowest FFA formation was in discarded oil from food eatery, which is the sample D at 0.17 %. The type of food that was fried in the oil might influence the rate of FFA formation in the oil. According to the food eatery operator, the oil was used to fry papads. Papads, being crispy food generally are low in moisture content. No measurement of moisture content of each food medium was conducted. However it can be generally concluded that papads, being a dry food, is lower in moisture content compared to other food medium (banana fritters, french fries, chicken meat), which are considered non- dry food. Bhattacharya et al. (2008) and Bansal et al. (2010a) also discovered that oil that was used to fry food with high moisture content produced more FFA compared to food with low moisture content.

Table 4.7: Free fatty acids value in control sample, expressed as % of oleic acid

	Control Sample		
	A	**B**	**C**
Fresh	0.11 ±0.02 $^{a, A}$	0.16 ±0.03 $^{a, A}$	0.13 ±0.03 $^{a, A}$
Heated	0.19 ±0.01 $^{b, A}$	0.21 ±0.04 $^{b, A}$	0.39 ±0.03 $^{b, B}$
Fried	0.25 ±0.03 $^{c, A}$	0.28 ±0.03 $^{c, A}$	0.48 ±0.04 $^{c, B}$

Values are means of triplicate with standard deviation. Values are means of three replicates with standard deviation. Mean values within each column followed by different letters (a, b, c, etc) are significantly (P < 0.05) different. Mean values within each row followed by different letters (A, B, C, etc) are significantly (P < 0.05) different.

Sample A, Palm oil Brand A; Sample B, Palm oil brand B; Sample C, corn oil

Table 4.8: Free fatty acids value in collected sample, expressed as % of oleic acid

	Collected Sample			
	D	**E**	**F**	**G**
Fresh	0.10 ±0.01 ^{a,A}	0.14 ±0.02 $^{a, A}$	0.13 ±0.05 $^{a, A}$	ND
Waste	0.17 ±0.02 ^{b,A}	0.22 ±0.01 $^{b, A}$	0.21 ±0.02 $^{b, A}$	0.40 ±0.01 $^{b, B}$

Values are means of triplicate with standard deviation. Values are means of three replicates with standard deviation. Mean values within each column followed by different letters (a, b, c, etc) are significantly (P < 0.05) different. Mean values within each row followed by different letters (A, B, C, etc) are significantly (P < 0.05) different. ND: not determined

Sample D, from food eatery, papads as food medium; Sample E, from food caterer, chicken meat as food medium; Sample F, from banana fritters stall, banana fritters as food medium; Sample G, from fast food outets, French fries as food medium

Unlike total polar compound which maximum limit at 27 % has been set by regulatory bodies, no maximum limit for FFA has been set yet. Thus for routine analysis of oil degradation, the FFA values of discarded oil need to be compared with FFA value of fresh counterpart. This is to determine how much the FFA value has increased. For

example in this study, the FFA value of palm oil control samples can be the reference. FFA value of heated and fried oil increased by 0.08 unit to 0.14 unit. Thus, any oil discarded sample that shows increase of FFA value within the range can be proposed to has undergone 180 °C to 200 °C for 6 hr. However, more samples need to be tested to reach that conclusion.

In conclusion, free fatty acid values of thermally treated oils were statistically different than the fresh oil. However, formation of FFA was independent on type of food that was fry in the oil. Foods that low in moisture content produced less FFA as shown by sample D. FFA formation also depended on initial amount of FFA in fresh oil as shown by sample A and B. Therefore, this study would like to recommend that FFA amount is not a good indicator in oil degradation as it is independent on type of food medium.

Generally, adverse effects of oil usually are caused by oxidation of unsaturated fatty acids (Abdulkarim et al., 2007). Free fatty acids test does not differentiate between acids formed by oxidation and those formed by hydrolysis (Fritsch, 1981). Thus FFA test is poor measure of oil degradation (Al Harbi and Al Kahtani, 1993). If FFA amount in fat is an ultimate criterion of oil degradation, it can lead to results where fully degraded oil due to other oxidation process but less in FFA amount is said to be better in quality compared to oil with high FFA amount but not so degraded in terms of oxidation process. It can be said that this method is only useful to determine degradation level of an oil that was used to fry food high in moisture. Moreover, in applying FFA test as routine analysis to determine oil degradation in food production and food serving industry, this test is quite tedious and the determination of colorimetric end point during titration may vary from one analyst to another and lead to inconsistent results.

4.7 IODINE VALUE

During frying and heating, double bonds of fatty acid are destructed by oxidation, polymerization and scission (Cowan, 1954; Cuesta et al., 1991; Al Harbi and Al Kahtani, 1993; Abdulkarim et al., 2007). This is because oxygen usually attacks the triglyceride close to the double bonds in the fat due to lowered activation energy in the

initiation of free radical formation (Akoh and Min, 2002). Besides fatty acids composition, C18:2/ C16:0, the evidence of destruction of double bonds can be indicated by the decrease in iodine value. Iodine value determines unsaturation degree of the oil by measuring the amount of iodine absorbed by double bond in a sample. Table 4.9 and 4.10 represent iodine value in control and collected samples respectively.

In control sample of palm oils (A and B), no significant ($p < 0.05$) difference in terms of iodine value in fresh, heated and fried oil. For sample A, the iodine value decreased by 1.04 % in heated oil and 1.59 % in fried oil. Though they were decrease in iodine value in thermally oxidized sample A, the iodine value of fresh, heated and fried oil were not statistically different at $p < 0.05$. For sample B, the iodine value decreased by 1.52 % in heated oil and 1.65 % in fried oil. Sample B showed the same pattern with sample A, though they were decrease in iodine value in thermally oxidized sample B, the iodine value of fresh, heated and fried oil were not statistically different at $p < 0.05$.

Though iodine value of sample A and B were not differed statistically, the magnitudes of reduction were different. Both received the same treatment. However, sample B showed higher increase in saturation compared to sample A. This finding is similar with previous researches such as Al Harbi and Al Kahtani (1993), Naz et al. (2005), Abdulkarim et al. (2007) and Knothe and Steidley, (2009), where they found different samples experienced different magnitude of decrease in unsaturation. Naz et al. (2005) discovered antioxidant in oil helped in avoiding high reduction rate of IV. Sample A was labeled as containing antioxidant by the manufacturer while sample B did not. That might explain the how the frying variable such as additives different magnitude in reduction rate of iodine value.

Table 4.9 indicates that corn oil was the only oil in control samples that showed statistically significant difference between fresh and thermally oxidized oil. The reduction rate was also bigger. In heated oil, the iodine value decreased by 3.32 % and fried oil decreased by 3.65 %. Takeoka et al. (1997) reported corn oil had the fastest loss of unsaturation of oils heated at 190 °C compared to other oil such as soybean and

canola oil. Abdulkarim et al. (2007) claimed palm oil were less susceptible to oxidation because they observed low changes of iodine value in palm oil, compared to other oil

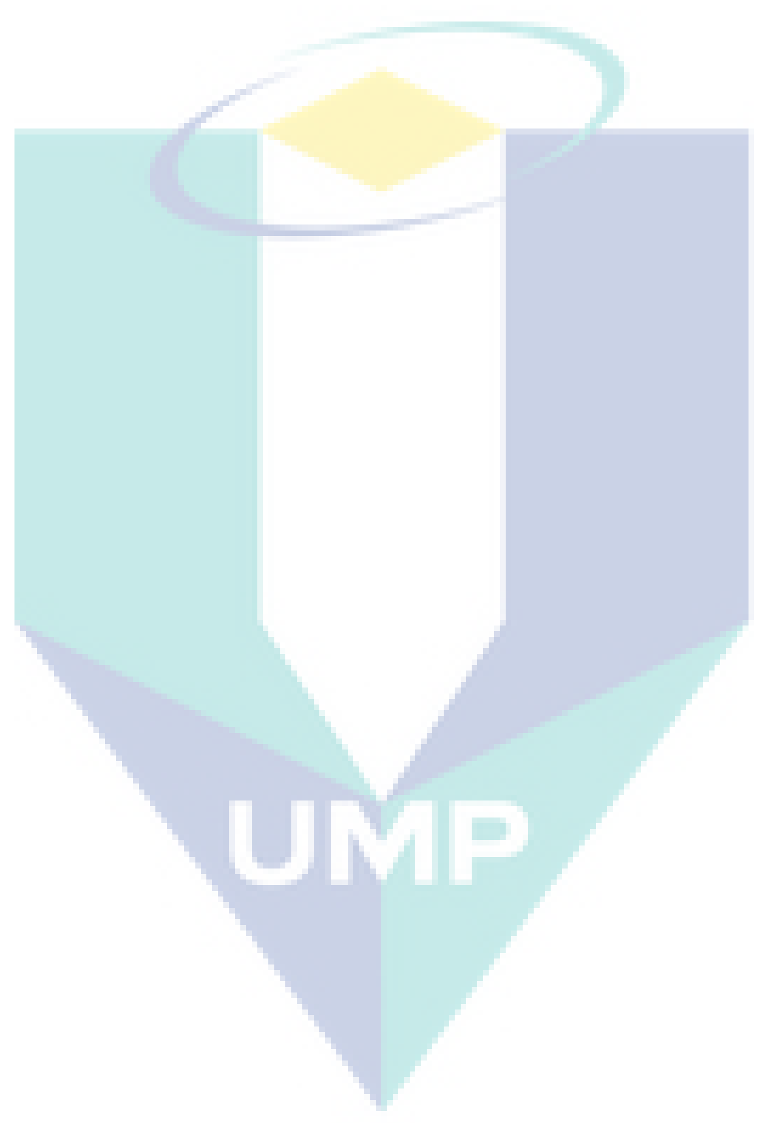


Table 4.9: Iodine value (g/100g) in control samples

Sample	Fresh	Heated	Decrease	Fresh	Fried	Decrease
A	55.6 ± 0.6 $^{a, A}$	55.0 ± 0.5 $^{a, A}$	**1.04 %**	55.6 ± 0.4	54.7 ± 0.3 $^{a, A}$	**1.59%**
B	55.1 ± 0.4 $^{a, A}$	54.3 ± 0.8 $^{a, A}$	**1.52 %**	55.1± 0.2	55.0 ± 0.2 $^{a, A}$	**1.65%**
C	125.9 ± 0.7 $^{a, B}$	121.7 ± 0.5 $^{a, B}$	**3.32 %**	125.9 ± 0.1	121.3 ± 0.4 $^{a, B}$	**3.65%**

Values are means of three replicates with standard deviation. Mean values within each column followed by different letters (a, b, c, etc) are significantly ($P < 0.05$) different. Mean values within each row followed by different letters (A, B, C, etc) are significantly ($P < 0.05$) different.

Sample A, Palm oil Brand A; Sample B, Palm oil brand B; Sample C, corn oil

Table 4.10: Iodine value (g/100g) in collected samples

Sample	Fresh	Waste	Decrease
D	55.2 ± 0.9 $^{a, A}$	54.5 ± 0.5 $^{a, A}$	**1.09 %**
E	55.6 ± 0.4 $^{a, A}$	55.0 ± 0.3 $^{a, B}$	**1.03 %**
F	56.1 ± 0.8 $^{a, A}$	55.5 ± 0.4 $^{a, B}$	**1.03 %**
G	ND	52.1 ± $^{a, C}$	**-**

Values are means of three replicates with standard deviation. Mean values within each column followed by different letters (a, b, c, etc) are significantly ($P < 0.05$) different. Mean values within each row followed by different letters (A, B, C, etc) are significantly different($P < 0.05$)

Sample D, from food eatery, papads as food medium; Sample E, from food caterer, chicken meat as food medium; Sample F, from banana fritters stall, banana fritters as food medium; Sample G, from fast food outets, French fries as food medium

with higher unsaturated fatty acids content. This can indicate that iodine value as assessment parameters are more suitable to be applied to oil with high unsaturation content.

For oil control samples (Sample A, B, C), fried oils appeared to have more reduction rate in iodine value than their heated counterparts. Possibly, the fatty acids that leached out from the foods in the frying medium altered the iodine value of the frying oil. Al Harbi and Al Kahtani, (1993) also observed fatty acid from the food can be transferred into the frying medium. Minus the food, heated oil still experienced reduction of iodine value due to destruction of double bond caused by oxidation.

From Table 4.10, sample from fast food outlets (sample G) showed lowest iodine value among the entire samples which means the sample was most saturated. No comparison could be made with its fresh counterpart to determine how much the iodine value has evolved after it had been thermally treated. Sample from food eatery (Sample D) has the biggest magnitude, with 1.09 % in iodine value reduction followed by sample from food eatery (E) and banana fritters stall (F), which had similar reduction magnitude at 1.03 %. It can be seen here that in collected samples, the reduction rate of iodine value is different between different samples. This is because all of the samples were treated differently. Frying variables such as frying time, temperature, food medium oil rotation, frying utensils might contribute to the differences.

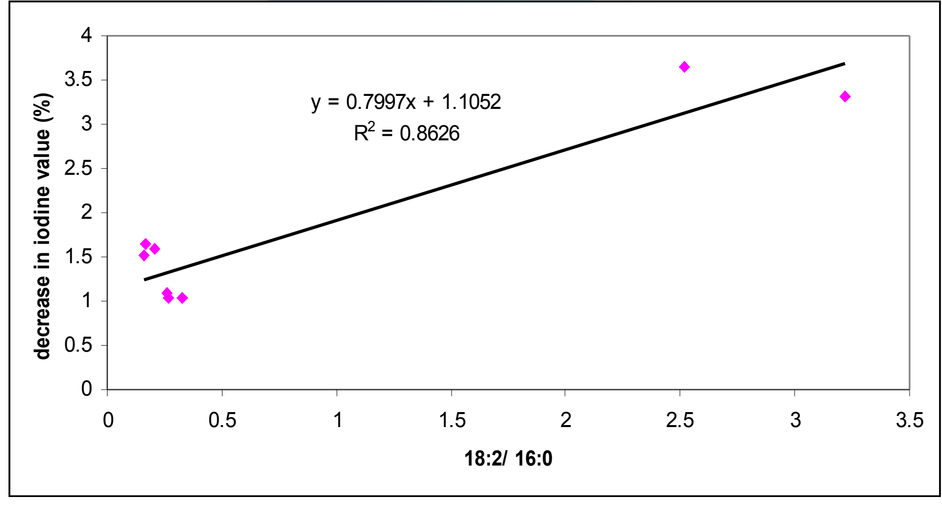


Figure 4.12: Correlation between 18:2/16:0 amount and the decrease in iodine value

Corn oil has the highest iodine value among the entire samples. The high value is attributed by high content of polyunsaturated fatty acid, the linoleic acid. Noh et al. (2002) discovered that iodine value was positively correlated with oleic and linoleic acid content, the higher the level of oleic and linoleic acid composition in an oil, the higher the iodine value. Moreno et al. (1999) claimed the decrease in unsaturation degree is directly related with the degradation of polyunsaturated fatty acids. The higher the polyunsaturated content in an oil, the higher the decrease in iodine value after it is thermally treated. Present study would like to agree with the claim made by the Moreno group. Figure 4.12 shows the decrease in iodine value correlates well (r^2 = 0.8626 and r= 0.9288) with the amount of polyunsaturated fatty acids (represented by ratio of linoleic acid to palmitic acid) present in the oil prior thermally oxidized.

Control samples showed oil thermally treated at 180 °C to 200 °C for 6 hour had iodine value reduction rate ranging from 1.59 % to 1.65 %. This study would like to propose that any palm oil sample that showed similar or more magnitude in reduction rate might have been treated in similar ways. However, reduction rate measurement means the iodine value of degraded oil need to be compared with its fresh counterpart, so more sample need to be analyzed, rendering analysis time.

Moreover, in this study, no significant difference was discovered between iodine value of thermally oxidized palm oil to its fresh counterpart. Only corn oil showed significant difference of iodine value between fresh and oxidized oil. It shows here that iodine value is more suitable to be degradation parameter for polyunsaturated fatty acids. Palm oil is highly saturated, thus it is more resistant to oxidation and reduction in iodine value.

4.8 CONJUGATED FATTY ACIDS

Double bond for polyunsaturated fatty acids in oil normally is in methylene interrupted form. When double bond is attacked by oxygen, a shift in double bond position, isomerization and conjugation reaction can occur (Akoh and Min, 2002), result in conjugated form. For polyunsaturated acid with two double bond and three double

bonds, a shift towards conjugated system will lead to conjugated diene and conjugated triene respectively.

Under UV spectrum, methylene interrupted system is transparent while conjugated system can absorb certain region. Triene absorb at 269 nm wavelength while conjugated diene absorb at 233 nm. In this study, absorption for heated and fried oil was compared to discover the influence of food towards conjugated fatty acid formation. The levels in waste oil were compared to find out the influence of different frying parameters towards the formation of conjugated fatty acid. Table 4.11 and 4.12 shows absorption at 233 nm and 269 nm respectively.

From Table 4.11, it is observed that there was no significant difference of adsorption at 233 nm in fresh state between two palm oils, the sample A and sample B. However, both samples differed significantly in thermally treated oil. In heated oil, sample B absorption was 1.45 while sample A adsorption was 1.33. In fried oil, sample B absorption was 1.48 while sample A adsorption was 1.35. Within sample, both sample A and sample B showed significant difference of adsorption between thermally treated and fresh oil. However, adsorption between heated and fried oil did not differed significantly for both sample A and sample B. This shows here that food medium did not influence conjugated fatty acid formation.

Sample C, which is corn oil showed higher adsorption than palm oils at the three states; fresh, heated and fried oil. Corn oil sample also showed the same tendency with sample A and B where adsorption for heated and fried oil did not differ significantly. Abdulkarim et al. (2007) also discovered lower levels of conjugated dienes in palm oil compared to polyunsaturated oil. Palm oil has lower levels of fatty acid with two double bond compared to corn oil, thus lower chances of forming conjugated diene compared to corn oil.

In collected samples, the highest adsorption at 233 nm was observed in sample from fast food outlet (Sample G), followed by sample from banana fritters stall (Sample F), sample from food eatery (sample D) and sample from food caterer (Sample E). The adsorptions were 2.09, 1.37, 1.34 and 1.22 respectively. These samples showed big

Table 4.11: Absorption at 233 nm in control and collected samples

	Control Sample				Collected Sample			
	A	**B**	**C**		**D**	**E**	**F**	**G**
Fresh	$0.31 \pm 0.02^{a, A}$	$0.33 \pm 0.03^{a, A}$	$0.45 \pm 0.01^{a, B}$	**Fresh**	$0.35 \pm 0.03^{a, A}$	$0.34 \pm 0.01^{a, A}$	$0.34 \pm 0.03^{a A}$	ND
Heated	$1.33 \pm 0.03^{b, A}$	$1.45 \pm 0.04^{b, B}$	$2.08 \pm 0.04^{b, C}$	**Waste**	$1.34 \pm 0.02^{b B}$	$1.22 \pm 0.02^{b A}$	$1.37 \pm 0.03^{b B}$	2.09 ± 0.04^{C}
Fried	$1.35 \pm 0.02^{b, A}$	$1.48 \pm 0.03^{b, B}$	$2.12 \pm 0.01^{b, C}$					

Values are means of three replicates with standard deviation. Mean values within each column followed by different letters (a, b, c, etc) are significantly ($P < 0.05$) different. Mean values within each row followed by different letters (A, B, C, etc) are significantly ($P < 0.05$) different. ND: not detected

Sample A, Palm oil Brand A; Sample B, Palm oil brand B; Sample C, corn oil
Sample D, from food eatery, papads as food medium; Sample E, from food caterer, chicken meat as food medium; Sample F, from banana fritters stall, banana fritters as food medium; Sample G, from fast food outets, French fries as food medium

Table 4.12: Absorption at 269 nm in control and collected samples

	Control Sample				Collected Sample			
	A	**B**	**C**		**D**	**E**	**F**	**G**
Fresh	0.11 ±0.04 $^{a, A}$	0.09 ±0.03 $^{a, A}$	0.89 ±0.03 $^{a, B}$	**Fresh**	0.12 ±0.03 $^{a, A}$	0.15 ±0.03 $^{a, A}$	0.16 ±0.02 $^{a A}$	ND
Heated	0.98 ±0.02 $^{b, A}$	0.81 ±0.03 $^{b, B}$	1.43 ±0.02 $^{b, C}$	**Waste**	0.65 ±0.02 $^{b A}$	0.61 ±0.03 $^{b A}$	0.72 ±0.01 $^{b B}$	1.01 ±0.03 ^{C}
Fried	0.96 ±0.02 $^{b, A}$	0.85 ±0.03 $^{b, B}$	1.41 ±0.03 $^{b, B}$					

Values are means of three replicates with standard deviation. Mean values within each column followed by different letters (a, b, c, etc) are significantly ($P < 0.05$) different. Mean values within each row followed by different letters (A, B, C, etc) are significantly ($P < 0.05$) different. ND. Not detected

Palm oil Brand A; Sample B, Palm oil brand B; Sample C, corn oil
Sample D, from food eatery, papads as food medium; Sample E, from food caterer, chicken meat as food medium; Sample F, from banana fritters stall, banana fritters as food medium; Sample G, from fast food outets, French fries as food medium

differences in adsorption between samples. It shows here how different in frying parameters such as food medium, frying utensils, time and temperature of frying can influence the formation of conjugated diene. All the collected samples; Sample D, E, and F showed significant different of adsorption in fresh and waste oil.

For adsorption at 269 nm, the pattern of adsorption was almost the same with adsorption at 233 nm, except the adsorption was much lower at 269 nm. Adsorption at 233 nm showed no significant difference between heated and fried oil, inferring that food medium did not influence conjugated triene formation. Sample C showed much higher adsorption at 269 nm than palm oils. This is because palm oil contains very low amount of fatty acid with three double bonds, thus the chances for formation of conjugated triene is very low.

For collected samples, the highest adsorption at 269 nm was in sample from fast food outlet (Sample G), followed by sample from banana fritters stall (Sample F), sample from food eatery (sample D) and sample from food caterer (Sample E). The adsorptions were 1.01, 0.72, 0.65 and 0.61 respectively. These samples showed big differences in adsorption between samples. It shows here how different in frying parameters can influence the formation of conjugated triene. All of the collected samples; Sample D, E, and F showed significant difference of adsorption between fresh and waste oil.

Results in this study showed that Sample A and B had significant difference of adsorption between thermally treated oil, though they had undergone the same treatment. This might be because generally palm oil contains carotene. Carotene also has conjugated system which is adsorptive under UV, thus disturbing measurement of diene and triene under UV spectrum (Othmer, 2008). Waste oil samples also indicated significant difference in their adsorption pattern, probably can be explained by frying parameters variables such as frying temperature, type of food and carotene content.

Adsorption showed significant difference between fresh and thermally treated oil, thus it can be proposed as alternatives to total polar compounds method. However,

caution must be exercised in interpreting absorption because minor constituent in fat and oil may contain chromophore compounds absorbing in the same region (Othmer, 2008), interfering with the reading of conjugated fatty acids.

4.9 OIL COLOR

Color evaluation is usually applied in determining discarded level of frying oil. This evaluation is normally applied during household frying as it is the most obvious changes that can be observed even for the non-expert (Abdulkarim et al., 2007). Oil that appears darker in color is usually considered as discarded level in household frying. Figure 4.1 and 4.2 show the appearance of all samples. Heated, fried and discarded oils were darker in color compared to fresh counterparts. Fresh oils were light yellow color in color, while heated, fresh and waste oils were amber and reddish brown in color.

In UV visible spectroscopy, absorbance at 420 nm is the color index or browning index that can reflect an overall chemical degradation and polymerization such as Mailard reaction product that occur during frying (Kim et al. 2002; Yaghmur et al. 2001; Budryn et al. 2009). Table 4.13 also indicates that absorbance at 420 nm were higher in thermally treated oil compared to fresh oil in all samples.

Fried oil appeared to be darker in color than heated oil. Table 4.13 also indicates that absorbance at 420 nm were significantly higher in fried oil than heated oil. This condition showed how food medium plays its role in determining oil quality. Substances from the food, such as carbonized compounds and browning pigments (Fritsch, 1981), might be diffused, released, dissolved in the frying medium therefore enhancing coloration and darkening of the oil (Al Harbi and Al Kahtani, 1993; Bansal et al., 2010a; Batthacharya et al., 2008).

Even without the presence of food, heated oils showed significantly higher absorption at 420 nm compared to the fresh counterparts. This is because, besides pigments from the foods that was released into the frying medium, accumulation of nonvolatile decomposition products such as oxidized triacylglycerols also can contribute in oil coloration (Abdulkarim et al., 2007). Change in oil colour also is the

Table 4.13: Absorption at 420 nm in control and collected samples

	Control Sample				Collected Sample			
	A	**B**	**C**		**D**	**E**	**F**	**G**
Fresh	0.54 ±0.01 $^{a, A}$	0.64 ±0.05 $^{a, B}$	0.50 ±0.03 $^{a, C}$	**Fresh**	0.68 ±0.02 $^{a, A}$	0.60 ±0.04 $^{a, B}$	0.50 ±0.04 $^{a C}$	ND
Heated	0.75 ±0.02 $^{b, A}$	0.72 ±0.01 $^{b, B}$	0.76 ±0.05 $^{b, A}$	**Waste**	0.90 ±0.04 $^{b A}$	0.80 ±0.03 $^{b B}$	0.73 ±0.03 $^{b C}$	1.03 ±0.02^{D}
Fried	0.94 ±0.01$^{c, A}$	0.83 ±0.05$^{c, B}$	0.98 ±0.04 $^{c, C}$					

Values are means of three replicates with standard deviation. Mean values within each column followed by different letters (a, b, c, etc) are significantly ($P < 0.05$) different. Mean values within each row followed by different letters (A, B, C, etc) are significantly ($P < 0.05$) different. ND: not detected

Sample A, Palm oil Brand A; Sample B, Palm oil brand B; Sample C, corn oil
Sample D, from food eatery, papads as food medium; Sample E, from food caterer, chicken meat as food medium; Sample F, from banana fritters stall, banana fritters as food medium; Sample G, from fast food outets, French fries as food medium

combined result of oxidation, polymerisation and other chemical changes (Bansal et al., 2010a).

Two palm oils in the control samples indicated significant difference in absorption at 420 nm though both were palm oil and received the same treatment. Sample B appeared more intense in color than sample A. This is probably because even though they were both originated from palm oil, their properties could not be 100% similar such as the level of antioxidant, pigment content, emulsifiers, trace metals, free fatty acids and alkaline-reacting materials. Those factors can influence oil alteration level (Bhattacharya et al. 2008) so they might influence the rate of color formation in oil.

As can be seen from Table 4.1, corn oil which received the same treatment with two palm oils in the control samples, was darker in color and showed higher absorption at 420 nm. This can indicate how type of oil also plays its role in determining the coloration of an oil.

Discarded oil samples also showed significant difference of absorption at 420 nm. Sample from banana fritters stall (Sample F) showed lowest absorption at 420 nm compared to other waste oils. Sample F color was yellowish, while sample from food eatery (Sample D) and sample from food caterer (Sample E) were reddish brown and sample from fast food outlet (sample G) was black in color. These oils were treated in different frying variables such as type of food and oil, thus explaining the differences in terms of color, influenced by various factors, as explained above. It is worth noting that waste oil of sample F was used to fry banana fritters. Generally, one of banana fritters main substance is turmeric and turmeric is meant to give yellow appearance in food. It is very likely that pigments from the turmeric has leaked into the oil, thus give yellowish appearance to waste oil of sample F. It can indicate here how type of food can influence the appearance of degraded oil.

Slight observation of waste sample of oil collected from fast food outlet, which was sample G, can indicate that the oil was really degraded as it appeared black in color. It gave the highest absorption at 420 nm among all samples.

4.10 CORRELATION OF EVALUATION PARAMETERS WITH TOTAL POLAR COMPOUNDS

Correlation of evaluation parameters with total polar compounds were determined to find out which parameter can replace total polar compounds in routine analysis to test frying oil quality. Table 4.14 lists the correlation coefficient of evaluation parameters towards total polar compounds.

Table 4.14: Relationship of evaluation parameter and total polar compounds

Evaluation Parameter	Changes during deep frying	Correlation with total polar compounds
Short chain fatty acids	Only detected in thermally treated oil	r = 0.750
Fatty acid saturation degree	Increase after frying process	-
18:2/16:0	Decrease after frying process	r = 0.526
Trans fatty acid	None detected	-
Free fatty acid	Increase after frying process	r = 0.863
Iodine value	Decrease after frying process	r = 0.5602
Conjugated diene	Increase after frying process	r = 0.8469
Conjugated triene	Increase after frying process	r = 0.8295
Color index	Increase after frying process	r = 0.8482

In this study, acceptable correlation (r^2= 0.563 and r= 0.750) was discovered between total polar component and the amount of C8:0 that was formed. Figure 4.13 shows the correlation plot between these two parameters. It is suggested that the measurement of octanoic acid can be a good indicator of frying quality as it gave acceptable correlation with total polar compounds.

As the plot in Figure 4.14 shows, relationship between C18:2/ C16:0 and total polar compound is discovered to be poor in this study, with coefficient determination (r^2) of 0.2768 and coefficient correlation (r) of 0.526. This is in contrast with studies done by Augustin et al. (1987) in oxidized palm oil. They discovered good linear relationship between these two parameters. This is probably because different frying parameters such as oil type, frying temperature, food composition led to different degradation rate. As claimed by Aladedunye and Przybylski (2009), and Bansal et al. (2010a), value of C18:2/C16:0 can be a reliable indicator for oil degradation. The claim could not be applied in this study because it gave poor correlation with total polar compounds, which have been the standard method in determining oil degradation.

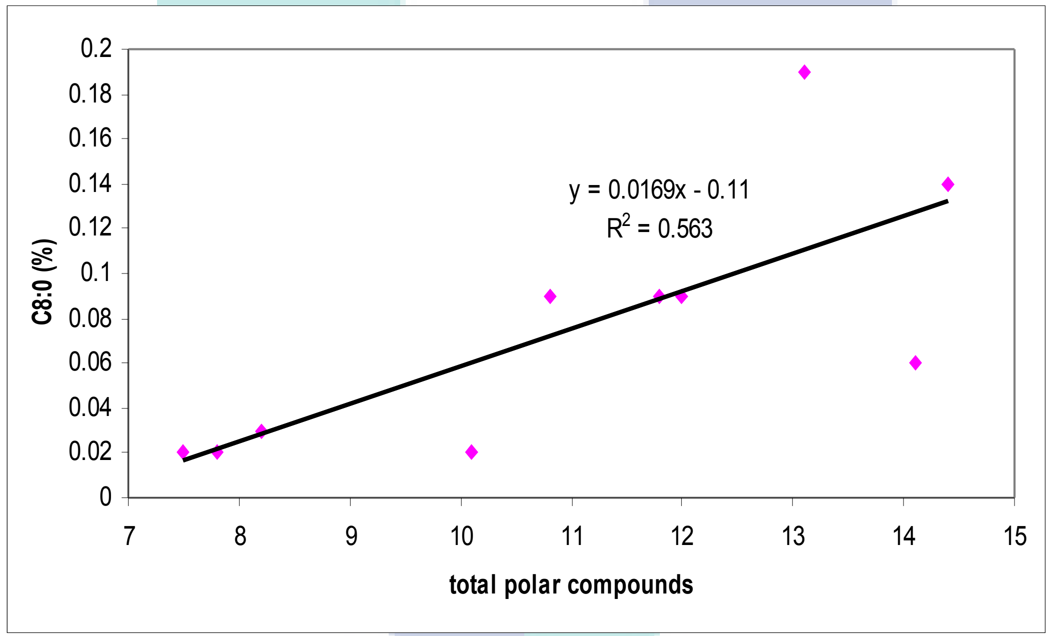


Figure 4.13: Correlation plot between changes of total polar compounds level and the amount of octanoic acid that was formed.

Free fatty acid content correlated well with total polar compounds with correlation determination (r^2) of 0.7449 and coefficient correlation (r) of 0.863. Figure 4.15 shows the correlation plot. It can be proposed that FFA test can replace the time consuming total polar compound determination.

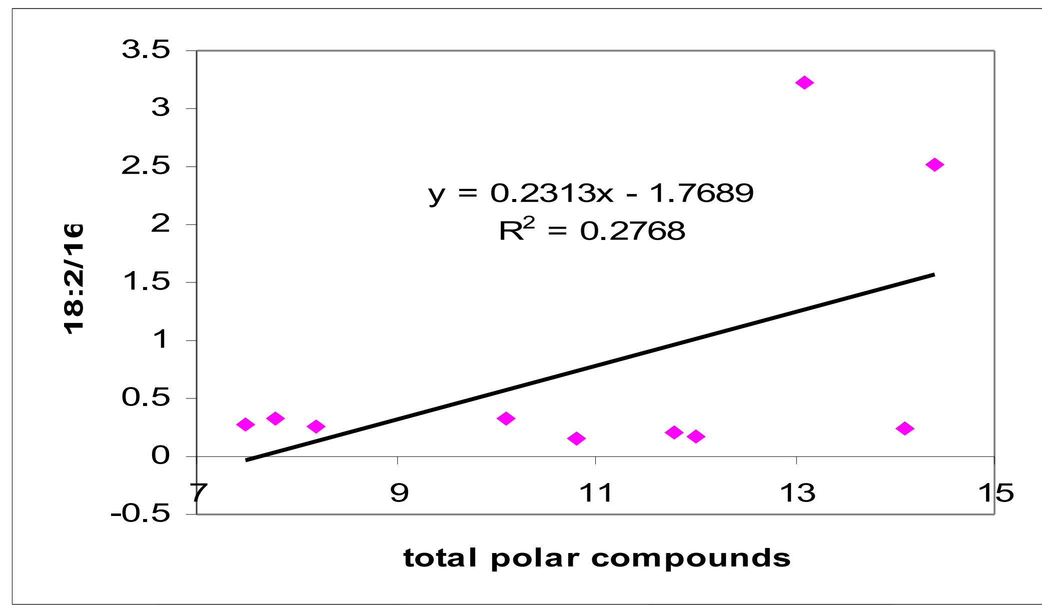


Figure 4.14: Correlation plot of total polar compounds and C18:2/ C16:0 value

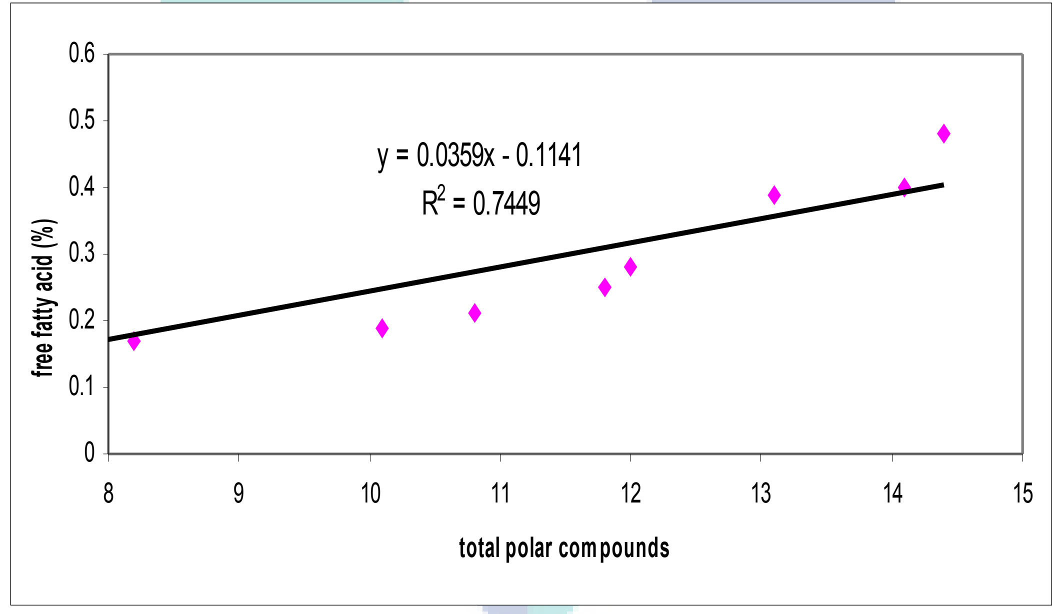


Figure 4.15: Relationship between amount of total polar compound and free fatty acids formation

Augustin et al. (1987) reported iodine value was highly correlated with total polar compound. Iodine value should have high correlation with total polar compounds. This is because, the more unsaturated an oil, the more degradation of fatty acid, thus higher polar compound will be formed. In this study, when total polar compound was

plotted against iodine value (Figure 4.16), acceptable correlation (r^2 = 0.3139, r= 0.5602) was obtained. When total polar compounds was plotted against decrease in iodine value (Figure 4.17), an acceptable correlation was also obtained (r^2 = 0.6043, r = 0.777). However, as stated in Section 4.7, no significant difference were discovered between iodine value of thermally oxidized palm oil to fresh palm oil, suggesting that iodine value is more suitable to be degradation parameter for polyunsaturated fatty acid.

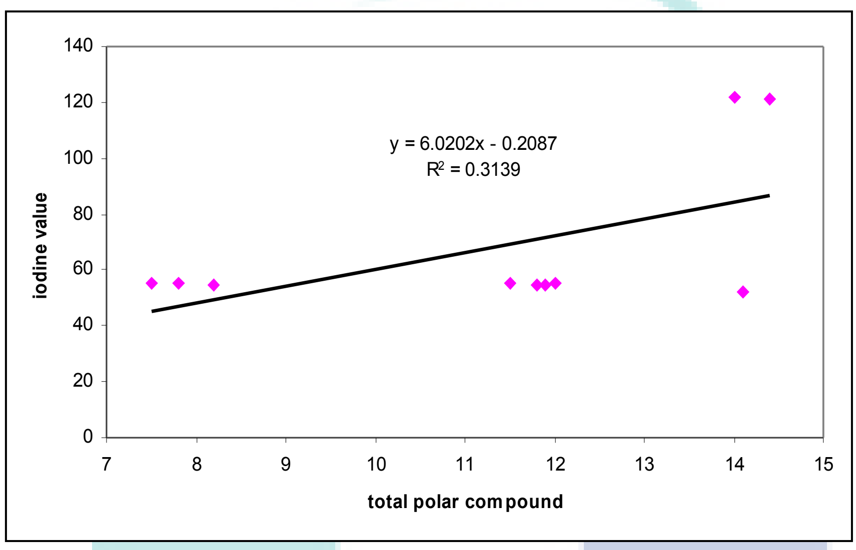


Figure 4.16: Correlation between total polar compounds content and iodine value.

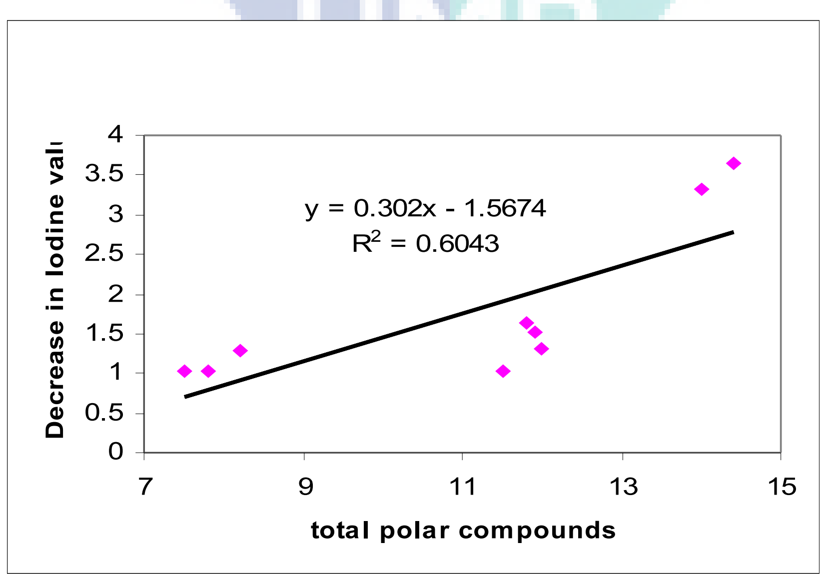


Figure 4.17: Correlation between total polar compounds content and the decrease in iodine value.

Figure 4.18 and 4.19 show correlation between total polar compounds and adsorption at 233 nm and 269 nm respectively. For adsorption at 233 nm, the coefficient determination (r2) was 0.7219 and coefficient correlation (r) was at 0.8496. For adsorption at 269 nm, the coefficient determination (r2) was 0.6881 and coefficient correlation (r) was at 0.8295.

Both adsorption at 233 nm and 269 nm correlated well with total polar compounds. Thus adsorption at 233 nm and 269 nm can be proposed as substitute or alternative to total polar compound method. However, there were significant difference of adsorption between two control samples of palm oil which received the same thermal treatment. This might be because generally palm oil contains carotene. Carotene also has conjugated system which is adsorptive under UV, thus disturbing measurement of diene and triene under UV spectrum (Othmer, 2008).

Figure 4.20 shows that absorption at 420 nm showed good correlation (r^2 = 0.7187, r= 0.8477) with total polar compounds. However, as discussed in section 4.9, oil color formation during frying could be influenced by the type of food medium. Pigments from the food can be solubilized in the oil, leading to different color formation between different foods medium.

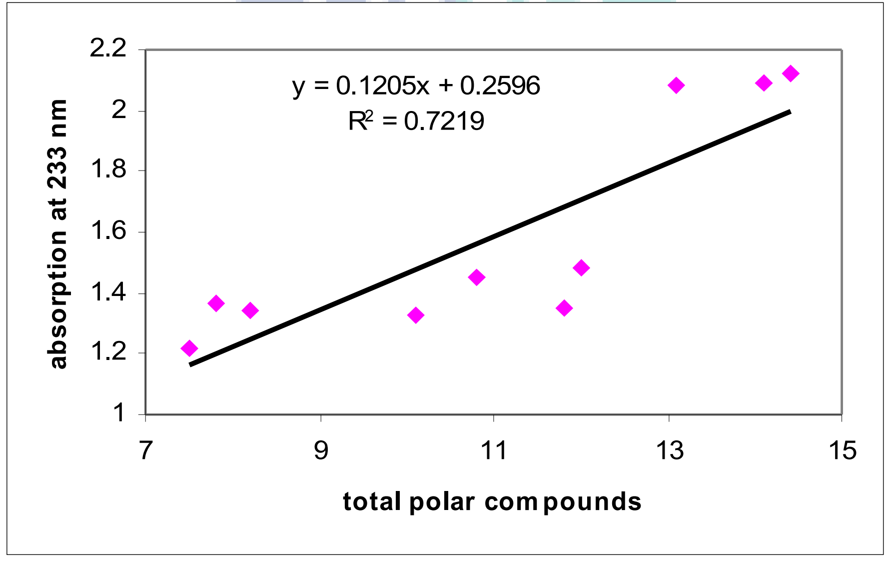


Figure 4.18: Correlation between total polar compounds and adsorption at 233 nm in oil sample.

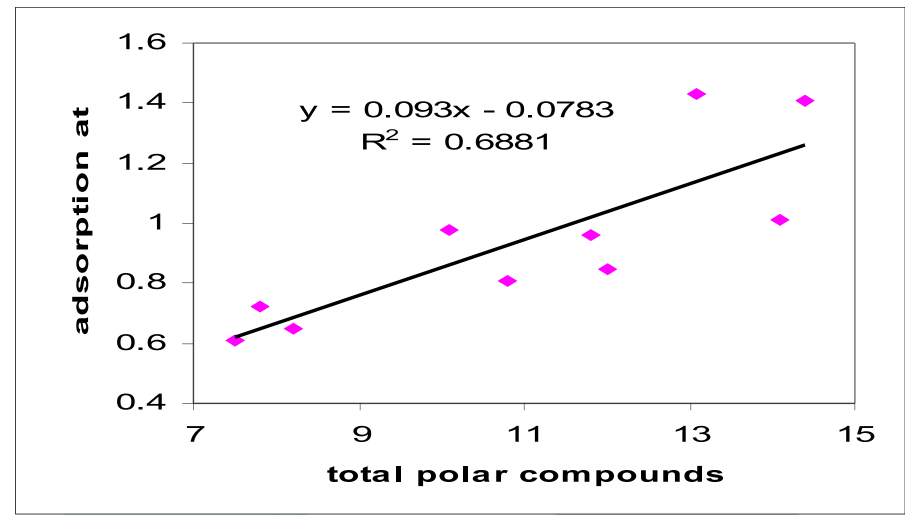


Figure 4.19: Correlation between total polar compounds and adsorption at 269 nm in oil sample.

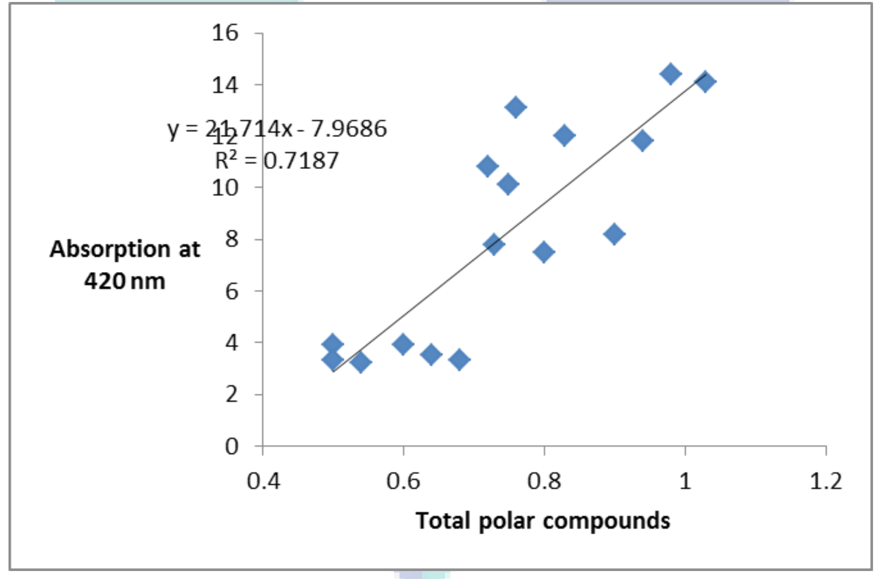


Figure 4.20: Relationship between amount of total polar compounds and absorption at 420 nm.

CHAPTER 5

CONCLUSION

5.1 Total polar compounds

Fresh oil and thermally oxidized oil gave significantly different amount of total polar compounds. However, as total polar compound method is time consuming, this evaluation is not suitable to be applied as routine analysis in determining oil quality.

5.2 Fatty acids composition, saturation degree, C18:2/C16:0

In all samples, the saturated fatty acids content increased while polyunsaturated fatty acids content decreased. The value of C18:2/C16:0 decreased after the oil was thermally treated. The value of C18:2/C16:0 gave coefficient correlation (r) of 0.526 towards total polar compounds. However, the value of C18:2/C16:0 differed significantly between samples that received the same treatment (A and B), thus the changes in C18:2/ C16:0 ratio only of a random nature and is not reliable to determine oil degradation status.

5.3 Short chain fatty acid

Short chain fatty acid (C8:0) only present in thermally degraded oil and was not detected in fresh oil regardless of sample treatment and frying variables. Even though the amount of C8:0 was not constant between samples that received the same treatment, the

presence of C8:0 was only detected in thermally treated oil. Therefore the presence of octanoic acid can be conclusive marker to indicate that the oil has been thermally treated. The contents of octanoic acid (C8:0) also produced good correlation (r= 0.750) with total polar compounds. Moreover, analytical procedure in detecting octanoic acid in oil is simple, involving converting oil sample to fatty acid methyl ester (FAME) and analyzed by gas chromatography (GC).

5.4 Trans fatty acid

In this study, there was no trans fatty acid detected in degraded oil samples even though analysis was carried out on a high resolution HP88 column that is specialized in detecting trans fatty acid. It can be concluded that palm oil was not susceptible towards trans fatty acid formation during normal frying temperature and time (180 ◦C to 200 ◦C, 6 hr). So trans fatty acid cannot be one of the parameter to determine fried oil oxidative status.

5.5 Free fatty acids

Free fatty acids value, which measure the unbound of non esterified fatty acids to glycerol molecule, gave good correlation (r= 0.863) to total polar compounds. However the amount of FFA was dependent on the type of food. Oil that was used to fry food high in moisture content (Sample D, E, and G) showed higher levels of FFA compared to oil that was used to fry food with low moisture content (Sample F) because one of the factors to cause FFA formation is moisture. Thus it cannot be a reliable parameter to determine oil degradation status as it depends on water content of food medium.

5.6 Iodine value

Iodine value, which measures the oil unsaturation degree gave acceptable correlation (r = 0.5602) with total polar compounds. However, in this study, no significant

difference was discovered between iodine value of thermally oxidized palm oil to its fresh counterpart, suggesting iodine value cannot be a reliable indicator of oil degradation.

5.7 Conjugated fatty acids

The adsorption at 233 nm and 269 nm showed good correlation (r=0.7219 for 233 nm r= 0.6881 for 269 nm for diene and trine respectively). Results in this study showed that Sample A and B had significant difference of adsorption between thermally treated oil, though they had undergone the same treatment. This is probably because, as cautioned by some literatures, the adsorption can be disrupted by the presence of carotene content in oil, so adsorption at 233 and 369 nm cannot be a good indicator of oil degradation.

5.8 Oil color

Absorption at 420 nm or color index showed good correlation (r= 0.848) to total polar compounds. However color formation was influenced by food medium as shown by samples. Therefore color index cannot be a reliable indicator in oil degradation.

5.9 Overall conclusion

Generally, corn oil was more degraded compared to palm oil based on evaluation parameters in this study. This is due to higher amount of polyunsaturated fatty acids in corn oil. Fried oil was more degraded than heated oil, showing the effects of food medium in oxidation and degradation process.

In determining frying palm oil status whether it is fresh or has been subjected to frying process, the presence of octanoic acid can be conclusive indicator compared to other parameters. This is because octanoic acid did not present in fresh oil. It is also dependent on type of food medium and oil. All samples regardless of type of oil and treatment received, showed the same pattern where C8:0 was only detected in oil that has been subjected to heating and frying process. Octanoic acid amount gave good correlation with total polar

compounds and its analytical procedure is simple to be applied as routine analysis. Other parameters in this studies gave inconclusive results, dependent on frying parameters such as food medium, gave poor correlation with total polar compounds, tedious analytical procedure and showed no significant difference between fresh and thermally treated oi

5.10 Future Study

Present study indicated that the presence of octanoic acid can differentiate between fresh and thermally treated oil. However, no study was conducted to find out the temperature and time of heating that can result in the formation of octanoic acid. For future study, it is recommended that the kinetics of octanoic acid formation to be conducted.

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