DETERMINATION OF PEROXIDE VALUE IN DEEP- FRYING COOKING OIL COLLECTED AT NIGHT MARKET IN KEPALA BATAS

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UNIVERSITI SAINS MALAYSIA

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BY

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DECLARATION

I hereby declare that I am the sole author of this thesis entitled “Determination of Peroxide Value in Deep-Frying Oil at Night Market in Kepala Batas”. I declare this thesis is being submitted to Universiti Sains Malaysia for the purpose of the award of Master Science in Health Toxicology. This dissertation is the result of my own research under supervision of Dr Hasni b Arsad except as cited in the references. This dissertation has been accepted for the study performed and is not concurrently submitted in candidature of any other degree. I authorize Universiti Sains Malaysia (USM) to publish to this dissertation to other institution and individuals for the purpose of scholarly publication.

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ABSTRACT

DETERMINATION OF PEROXIDE VALUE IN DEEP-FRYING COOKING OIL COLLECTED AT NIGHT MARKET IN KEPALA BATAS

Deep oil frying is one of the most procedures used for preparing food. The essential polyunsaturated fatty acid is important in human nutrition that obtained from vegetable oils. It is necessary in giving several health benefits to protect against heart disease, inflammatory disease, asthma, mood disorders and retinal diseases. However, lipid oxidation can lower the nutritional value and quality of oils, together with the formation of toxic compounds, off-flavors and off-odors. Fatty acids, especially polyunsaturated fatty acids are subject to rapid and extensive oxidation and other chemical changes upon exposure to air, light, transition metals, or heat during food processing. The oxidation process has resulted in the formation of hydroperoxide. Thus, the objective of this study is to quantify the peroxide compound in deep-frying cooking oil collected at night market in Kepala Batas. Eight samples of deep-frying cooking oil were collected at two selected night market at Kepala Batas. Standard methods for peroxide value determination in oil sample, which are based on volumetric titration by sodium thiosulphate. The results were showed a high value of peroxide in all eight samples with average from 3 to 27 meq/g.

ABSTRAK

PENENTUAN NILAI PEROKSIDA DI DALAM MINYAK MASAK BERGORENG YANG DI AMBIL DARI PASAR MALAM DI KEPALA BATAS

Minyak masak yang digunakan dengan kaedah menggoreng adalah salah satu teknik untuk menyediakan makanan. Asid lemak poli tak tepu yang terdapat dalam minyak sayuran adalah penting dalam diet pemakanan. Ia adalah sangat penting kerana dapat memberikan beberapa manfaat kesihatan bagi melindungi daripada penyakit jantung, penyakit radang, asma, gangguan emosi dan penyakit retina. Walaubagaimanapun, pengoksidaan lipid boleh menurunkan nilai khasiat makanan dan kualiti minyak, kerana ia mampu menghasilkan pembentukan sebatian toksik, rasa dan bau yang kurang enak. Asid lemak, terutamanya asid lemak poli tak tepu mampu meningkatkan kadar pengoksidaan dan perubahan kimia lain apabila ia terdedah kepada udara, cahaya, logam peralihan, atau haba semasa pemprosesan makanan. Proses pengoksidaan mengakibatkan pembentukan hydrperoksida. Oleh itu, objektif kajian ini adalah untuk mengukur sebatian peroksida dalam minyak masak bergoreng yang diambil di pasar malam Kepala Batas. Lapan sampel minyak masak bergoreng telah dikumpulkan dari dua pasar malam terpilih di Kepala Batas. Kaedah asas untuk penentuan nilai peroksida dalam sampel minyak, adalah dengan kaedah titratan isipadu natrium tiosulfat. Keputusan telah menunjukkan nilai peroksida yang tinggi dalam ke semua lapan sampel dengan purata 3-27 meq / g.

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LIST OF ABBREVIATIONS

Symbols

%	Percentage
●	Radical
g	Gram
L	Litre
meq	Mill equivalent
ppb	part per billion
ppm	part per million
h	hour
€	*trans*

Abbreviations

ACA	Active cutaneous anaphylasis
AV	Acid value
CH_3COOH	Acetic acid
$CHCl_3$	Chloroform
CHS	Contact hypersensitivity
EC	European Commision
FDA	Food and Drug Administration
FFA	Free Fatty Acid
IV	Iodine Value
KI	Potassium iodide
KOH	Potassium hydroxide
MRL	Maximum residue level
$Na_2S_2O_3$	Sodium thiosulphate
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychorinated Biphenyls
POM	Polymerized and Oxidized Matter
PUFA	Poly Unsaturated Fatty Acid
PV	Peroxide Value
ROS	Reactive Oxyggen
SON	Standard Organization of Nigeria
SV	Saponification Value
TAN	Total Acid Number
TBARS	Thiobarbituric Acid-reactive substance
t-BHP	*tert*-butylhydrperoxide
TGs	Triglycerides
UV	Ultra Violet

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Deep frying and the use of recycled oil for frying many times is a commonly practiced in commercial and sometimes in domestic cooking processes. The way of this cooking method may create a lipid peroxidation product which brings harm to human health. Most of the products are non-volatile, so they will be remained in the food or oils and give effect to the physical properties and form other radicals. This is due to the high temperatures in the existence of air and moisture, which make the occurrence of oxidative degradation of amino acids and the partial conversion of these lipids to volatile chain-scission products, non-volatile oxidized derivatives and dimeric, polymeric or cyclic substances leading to the formation of toxic or carcinogenic compounds (Zahir *et al*., 2014).

An assortment of criteria is being discussed to review on when the frying oils need to be discarded. Restaurants and other food services had monitored the changes in physical properties of frying oils which used as an indicator of oil quality. For example, sample of frying oil could be discarded if the oil presented in dark, too much smoke, strong odor, greased texture, or when a persistent foam layer of specified thickness is observed.

Nevertheless, before an operator found the physical effects, the oil has typically undergone considerable decomposition (Innawong *et al.,* 2004). Oxidation is a major deteriorating process that gives huge implication in the conditions of the quality and assessment such as developing the rancidity and off-flavor of fats and oils (Xiuzhu *et al*., 2007). Basically, frying oils undergo common degradation and complex chemical alteration when heated. Acceleration of the deterioration in frying oil and also producing a number of polar molecules as resulted by viscosity and density data of oils can be caused by the presence of air and water (Clark and Serbia, 1991). Hydroperoxide is from the formation of primary oxidation products that develop from triacylglycerol, which may later decompose in order to become lower molecular weight compounds like free fatty acids, alcohols, aldehydes, and ketones which ultimately leading to a rancid product (Zahir *et al*., 2014).

In addition, the oxidation process that occurs in oils, whether in storage or heating, condition may contribute in low nutritional quality, alter the flavor and may generate toxic compound (Niazmand *et al*., 2011). Indeed, Al-Khusaibi *et al*. (2012) claimed that, higher temperature use during frying can make the oil form a radical compound that give bad impact to health. Therefore, the peroxide compound in deep-frying cooking oil has been measured. The objectives of this study have been restricted to the following:

i) To quantify the peroxide compound in deep-frying cooking oil collected at night market in Kepala Batas

ii) To understand the quality of deep-frying cooking oil before and after collecting at night market in Kepala Batas

CHAPTER 2

LITERATURE REVIEW

2.1 Quality of Cooking Oil

Edible oil is one of the chosen ingredients of the diet that used for cooking purposes. Researchers had studied the effect of temperature on the stability, viscosity, peroxide value, and iodine value to assess the quality and functionality of the oil (Jinfeng *et al.,* 2011). It is necessary to observe the quality of oil in order to avoid the use of abused oil, bad health consequences due to high consuming foods fried in degraded oil, to keep the quality of fried foods and to minimize the production costs associated with early disposal of the frying medium (Vijayan *et al.,* 1996).

Oil that rich with unsaturated fatty acids may allowed in rapid degradation because of the oxidation activity (Bou *et al*., 2012), Atmospheric oxygen responds instantly with lipid and other organic compounds of the oil to cause structural degradation in the oil, which leads to loss of quality of food and is harmful to human health (Bhattacharya *et al.,* 2008). Besides, the occurrence of hydrolysis is generally caused by the present of water that came from the fried food (Chammen *et al.,* 2015). Then, increasing of free fatty acid value is mainly from the degradation of secondary oxidation products formed during heating and the presence of oxygen.

The existence of moisture that may be provided by air could have an influence on increasing acidity. However, the drop off in the acidity level after 24 h and 30 h may be caused by the evaporation of the volatile free fatty acids under the heating effect. The polymerization of fatty acids during the heating process may also block the acid functions that are no longer assayed (Chammem *et al.*, 2015).

A various type of physical and chemical parameters of edible oil was used to examine the compositional quality of oils (Mousavi *et al*., 2012). These physicochemical parameters include iodine value (IV), saponification value (SV), viscosity, density and peroxide value (PV). Osawa et al. (2007) clarified about hydrolytic rancidity that happen due to hydrolysis of fats and oils and it has potential in yielding free fatty acid (FFA). The level of acidity in 1g of sample can be determined by neutralize the FFA using milligrams of potassium hydroxide. A part from that, Europe is the only state in the world that has guidelines on frying oil safety (Sebastian *et al*., 2014).

Furthermore, the color change in oils is mainly caused by an accumulation of highly conjugated oxidation products. Color was closely correlated with all the assessed parameters. However, it needs to be considered with color measurement because they could be influenced by pigments extracted from the food (Sebastian *et al*., 2014).

2.2 Parameter in Measuring The Oil Quality

2.2.1 Viscosity and Density Measurement

A quality of fried food is also corresponds with the using oil because as the oil degrades, it will be changing the quality of the food products. Now a day, there are many physical test and chemical test found in the food industry. One of the methods and instrument that available to check on the oil quality is by measuring the viscosity. The designed instrument was to measure the formation of polymerized and oxidized matter (POM) contained in used cooking oil (Stier, 2004).

The viscosity of oils refers to the present of complex triglyceride (TGs) that is naturally present in oils. The alteration of fatty acids arrangement on the glycerol backbone of the triglyceride molecule can change the viscosity of the oils. Consequently, the chain length with the chemical properties of saturated and unsaturated chain give affects to the viscosity of oils (Zahir *et al*., 2014). Moreover, the viscosity and density decreases with an increase of unsaturation chain (Kim *et al.,* 2010). Sheer stress and temperature are also related to viscosity.

However, sheer stress does not give much effect toward storage of oils which are used for edible purposes compared to the effect of temperature. When the temperature goes high the kinetic energy will be also increase to enhance the movement of the molecules and lowered the intermolecular forces. The layers of the liquid easily pass over one another and thus contribute to the reduction of viscosity.

This nature is also revealed by another study that discussed the viscosity is depends on the molecular structure and decreases with the unsaturation of fatty acids (Kim *et al*., 2010). The density of oils will be decreased when the temperature is arisen as well as when using the same oil for frying three times. The densities of oils were related to the standard range of 0.898–0.907 g/ ml approved by the Standard Organization of Nigeria (SON, 2000). Frying process could undergo thermo-oxidative or lipid oxidation and hydrolytic reactions which results in deterioration in quality of the oil (Romero *et al*., 2000).

2.2.2 Saponification Value Measurement

Saponification value (SV) is an index of an average molecular mass of fatty acid in the oil sample. The lower value of saponification represent that the mean molecular weight of fatty acids is lower or that the number of ester bonds is less. This might imply that the fat molecules did not interact with each other (Denniston *et al*., 2004).

2.2.3 Iodine Value Measurement

Iodine value (IV) is a measure the degree of unsaturation in a fat or vegetable oil. It verifying the oxidative stability of oils and can qualitatively determine the whole unsaturation of fats (Asuquo *et al.,* 2012). The result of low iodine values can contribute to its greater oxidative storage stability (Zahir *et al.,* 2014).

2.2.4 Free Fatty Acid Measurement

There are several methods to investigate the quality of oil is such as by measuring the Free fatty acid (FFA) and total polar compound level (Chen *et al*., 2013). FFA levels represent the mechanism of triacylglycerol elements had undergone hydrolytic degradation. Generally during the refining process, high level of FFAs compounds could be removed from the crude oils. Gunstone (2008) stated that, the refined oil have to be 0.1% less of FFA content. When the content of FFAs in frying oil is more than 1%, it is suggested to be discarded (Tseng *et al*., 1996). Furthermore, the oils could be discarded if the FFA content had reached to 2% as claimed by the United States Department of Agriculture. Other than that, the limit of FFA in frying oil that have been proposed by some of the European countries as their regulatory rule ranging from 1.0 to 2.5% (Sebastian *et al*., 2014).

2.2.5 Peroxide Value Measurement

Peroxide value (PV) is related in measuring rancidity reactions and can be used as an indication of the quality and stability of fats and oils (Ekwu and Nwagu, 2004). The peroxide value could increase corresponds with the storage time, contact with air of the oil samples and temperature (Zahir *et al.*, 2014).

2.3 Toxic Compound

The quality and the stability of cooking oil in frying are affected from rapid deterioration. This deterioration has gone through a series of reactions which lead to a qualitative and a nutritional change such as rancidity, discoloration and loss of essential fatty acids. Furthermore, the oil deterioration can promote the production of toxic compounds such as peroxides, aldehydes and epoxides (Zhang *et al*., 2012). Oxidation is one of the most general chemical reactions that bring to this problem (Chammen *et al.*, 2015).

Intake of vegetable oil both raw and thermally treated was being increased in the market and daily use. Stalls and fast food restaurants, mostly prepared a food by using edible oils treated with high temperature (Blaszczyk, 2014). Contamination of vegetable oil may come from technological processes in seed smoke-drying or indirectly by the vehicles released gases and atmospheric particle from combustion sources that deposited on crop (Wang and Guo, 2010).

Incomplete combustion of organic matter and the burned of fossil fuels or vegetation could generate polycyclic aromatic hydrocarbons (PAHs) compound. Other combines factors that contaminated the oil are during solvent extraction, packaging materials, residue of mineral oil and relocation of contaminated soil (Hossain and Salehuddin, 2012). The process of refining edible oil can help in minimizing the total of contaminants. However, deodorization process does not show significantly high effect on reducing high molecular PAHs but mainly it can get rid the light PAHs (Yusty, 2005).

The quality of edible oils can be determined by their freshness, storability and toxicity. It can also be evaluated by the present of several trace metals. Example of trace metals such as copper, iron or manganese that is able to increase the speed of oxidation. A part from that, elements like cadmium, chromium, mercury, silver and lead are very significant on measure of their toxicity and metabolic role (Anthemidis *et al.*, 2005). Every of the toxic compounds can act with other food compounds like a pigments and proteins and extend to showed pathological effects on the digestive system and increase carcinogenic effect. It has been designed for each type of edible oil with different legislated level of trace element. For an example, a maximum residue level of arsenic, copper and iron in olive oils and olive-pomace oil had been established by the International Olive Council (2009). Whereas for the other vegetable oils, the maximum level of copper and iron is ranging from 0.1 to 5ppb as legislated by Codex Alimentarius (2009) (Llorent-Martinez *et al*., 2011).

In addition, the vegetable oil like extra virgin oils which not undergoes refining process during the manufacturing is able have high amount of trace metals. In fact, metal content is one of the most main quality criteria of extra virgin olive oil. It is important to keep on monitoring the existence of these elements in order to preserve the high nutritional value and the high organoleptic quality of product. However, the precise measure of trace elements in vegetable oils is still in analytical test because of their low concentration level and the difficulties that occur due to the features and interference contained in the matrix (Cabrera Vique et al., 2012).

There are some factors on the present of constituent of metals in edible oils. The source may come from the soil, environment, genotype of the plant, fertilisers and metal-

containing pesticides, initiate during the production process such as bleaching, hardening, refining and deodorisation or by contamination from the metal processing equipments (Lloren-Martinez *et al*,, 2011).

Toxic substances produced from degraded frying oils may cause harm to health. The dielectric constant is increased directly with an amount of producing polar molecule which occurs from the oil thermally and oxidatively break down (Fritsch et al., 1979). In frying conditions, oils are hydrolyzed to form FFA and mono- and diglycerides and these compounds could build up in the repeated use of frying oil. In addition, the oxidation of oil could form compounds of conjugated dienoic acid, hydroperoxide, epoxide, hydroxides, ketone and aldehyde. Cleavage is able in converting the compounds into smaller fragments or go to the triglyceride molecule to cross-link each other. This reaction will lead to higher polymeric triglycerides and form dimeric. Thus, the value of peroxide and FFA may increase in oils due to the increasing of volatile compounds. The volatile rancidity marker can give effect on rancidity of oils which can be analyzed by GC-MS methods (Cabrera-Vique *et al*., 2012).

2.3.1 Hydroperoxide

The oil may oxidised with existence of some trace elements and also support the generation of aldehyde, ketone, peroxide, acid, epoxide and other compounds (Lloren-Martinez *et al.,* 2011). Hydroperoxide is one of the major oxidation products of vegetable oil (Morales *et al*., 2014).

The present of copper could speed up the decomposition rate of hydrogen peroxide until 50 to 100 times faster compared to iron (Choe & Min, 2006). Thus, the finding and analyze of copper become one of the common practice in performing the determination of oil quality (FAO–WHO, 2003; COI, 2006; Commission of the European Communities, 2003). Besides, the generating of ketone and aldehyde could alter the taste of oils due to the present of copper which acts as prooxidant in the catalytic oxidation of hydroperoxide with the existence of oxygen. Catalytic oxidation will also keep the new forming of radicals and continue the occurring of oxidation process (Pinto *et al.,* 2006). A few studies had demonstrated that copper was given greater catalytic effect compared to iron. At any of experimental condition an analysis done on soybean oil showed are kinetic oxidation and the catalytic effect of copper was higher than iron. Copper autooxidative capacity was at low level than 30ppb (Marfil *et al.,* 2008).

Moreover, trace element may come from natural environment and technology in oil processing. While the natural present of chromium in raw material could increase from the interaction with elements in apparatus, ceramic utensil, metal packages in the industry (stainless steels). The roles of iron as catalyst to speed up the decomposition of lipid peroxide. Furthermore, Iron is able to disturb the oxidative stability of virgin olive oil by decomposing the phenolic compounds such as caffeic acid (Choe & Min, 2006). It is also may come from the source of processing equipment and contamination in environment (Mendil *et al.,* 2009).

2.3.2 Adverse Health Effect

Nowadays, the consumption of dietary fats and oils has increased in industrialized countries and this has mainly been attributed to an increase in the intake of fast food, which include heated and processed dietary fats such as frying oil. The most often used cooking oils include olive oil, corn oil, and sesame oil, all of which contain the glycerol triesters of oleic acid and linoleic acid as their unsaturated fatty acids and palmitic acid and stearic acid as their saturated fatty acids (Surh and Yun, 2012). Component of fatty acids leads to the oxidation of unsaturated fatty acids is easier with heating and radicals are more likely to be generated at the carbon–carbon double bond-neighboring methylene groups (Min and Boff, 2002). The radicals are very reactive and counter easily with other lipid molecules.

The consumption of cooking oils may exacerbate some allergic diseases. In the research study, the effects of naturally oxidized olive oil on immediate and delayed-type allergic reactions were investigated in mice. Naturally oxidized olive oil had a high peroxide value and exacerbated contact hypersensitivity (CHS), active cuteneous anaphylaxis (ACA) and DNFB-induced hypersensitivity correspond with peroxide level. Ultraviolet (UV)-irradiated olive oil, corn oil, sesame oil and triolein had high peroxides, and same goes with acid value (AV) and thiobarbituric acid-reactive substance (TBARS) level. However, fresh olive oil and the representative oxidation product with a high AV or TBARS level had not represent effect on CHS, whereas all UV-irradiated oils and naturally oxidized olive oil exacerbated it. This result indicated that the exacerbation of CHS by oxidized olive oil may depend on the total amount of peroxides contained in the oxidized oil (Ogino *et al.*, 2015).

Apart from that, the ingestion of highly-oxidized dietary oil may be responsible for an increased risk of allergic diseases even though the incidence is low. The exacerbation of allergic diseases such as allergic asthma has been correlated with an increase in oxidative stress, as indicated by the high levels of oxidative products in asthma patients. Oxidative stress and altered antioxidant defenses are involved in the pathophysiology of acute exacerbations in atopic dermatitis. Polyunsaturated fatty acids also mediate degranulation from rat basophilic leukemia. Recently, the saturated fatty acids with 7–12 carbons have been shown to induce expression of thymic stromal lymphopoietin which suggests exacerbation of allergic inflammation. However, oleic acid or the malondialdehyde precursor did not affect CHS in the present study. Therefore hydroperoxides generated from unsaturated fatty acids are principally involved in the exacerbation of allergic reactions (Ogino *et al.*, 2015).

Liver disease and alcoholic liver injury had been correlated with reactive oxygen species (ROS). During the occurrence of oxidative stress and elimination of liver toxins and xenobiotics, there is an amount of oxygen free radical formed due to ROS (Comelli *et al.*, 2007). At the liver tissue, there are rich of endogenous antioxidant which then scavenged with ROS. Nevertheless, at high acute dose or chronic exposure of toxic substance may hit the hepatic system and overcome the defence system which then leads to liver damage (Fruehauf and Meyskens, 2007). The structure of cellular macromolecules could trigger nuclease and protease, the porous of membrane and alter the gene expression after the cell being reacted with ROS and become the important factors why toxin attracted to liver function (Wu *et al.*, 2006 and Fruehauf and Meyskens, 2007).

Besides, the occurrence of oxidative stress and cell injury are also caused by the pro-oxidants like tert-butyl hydroperoxide (t-BHP) (Williams and Jeffrey, 2000). The mechanism of cell damage that was the cause of acute oxidative stress has been studied with the use of analog model of short chain t-BHP. An observation towards hepatocyte cultures and liver showed cytochrome P450 is able to metabolize t-BHP to free radical intermediates. Then the radical could harm the cell and initiate lipid peroxidation (Lee *et al*., 2008).

Excessive event of lipid peroxidation will lead to cytotoxic effect and even at low concentration of ROS may activate the cell signalling. Cell culture and in vivo study had conducted on the role of small concentrations of lipid hydroperoxide may inhibit the lung cell DNA (Luo *et al*., 1999). It is well known that lipid hydroperoxide is a significant mediator of lung growth. Thus, there was study investigated on the present of diphenyl phenyl diamine to inhibit the lipid peroxidation. The mechanism will occur with the presence of ferric ion and the scavenger of the radical from lipid peroxidation. Lipid hydroperoxide can initiate both alveologenesis and physiological apoptosis (Jamal *et al*., 2012).

Oxidative stress occurs when hydrogen peroxide and tBHP is operated in many others tissue in the body with the existence of transition metals. Iron and copper react with peroxide to produce free radicals with highly reactive hydroxyl radical. In vivo study had done on rat liver and hepatoma cell line with inducing a concentration which able to generate oxidative stress and observe the pathological process at middle to high micromolar and also at low millimolar range (Sabaretnam *et al.*, 2010).

Aging is also found with increase vulnerability to oxidative stress. They observed in the hepatocytes of 4-6 month and 24-26 months, rats which induced with hydrogen peroxide and tert-butylhydrperoxide. Aging may occur due to the liver function that are important in regulating the systemic metabolism, immunity and detoxification (Schmucker, 2005). In addition, there was prognosis on liver diseases relate with oxidative stress like fibrosis, cirrhosis and ischemia (Jansen, 2002). The oxidant tBHP had lowered the mitochondrial function in isolated hepatocytes and its also correlated with reducing hepatic antioxidant enzymes (Drahota *et al.*, 2005).

Apart from that, there are also a few productions of oxidation compounds like epoxide, ketone, aldehyde, alcohol, hydrocarbon, cyclic polymers and peroxide which have tended to make oxidized oil become cytotoxic to the body and cause bad effect on physiochemical regulation of the lipid profile (Cao *et al.*, 2013; Osaka *et al*., 2002). The mechanism of peroxidation, cross-linkage and other reaction of cell membrane fatty acids that cause by oxidative stress of lipid derived oxygen. The compound of lipid derived oxygen is also resulting in dislodging fatty acids from triacylglycerol anchorage (North *et al*., 1994). In other views, cytotoxicity could happen to rise up of free fatty acids contain in frying oil and accumulate in tissues (Ng *et al.,* 2014).

CHAPTER 3

METHODOLOGY

3.1 Chemicals and Reagents

Chemicals like acetic acid (CH_3COOH), chloroform ($CHCl_3$), potassium iodide (KI), starch and sodium thiosulphate ($Na_2S_2O_3$) were purchased from Fisher Scientific.

3.2 Collection of Sample

Used cooking oil was collected at two selected night market at Kepala Batas which is located at Bertam Perdana and Bertam Putra night market. A total of eight samples was focussed on the usage of deep-frying oil. Each of a sample is going to have two times of sampling in a single day. The first sampling was the unused cooking oil and next sampling will be after the oil been using in frying.

3.3 Peroxide Value Test

Peroxide value (PV) is a measure of peroxides contained in the oil. PV is determined by measuring iodine released from potassium iodide. The American Oil Chemists' Society (AOCS) Official Method Cd 8-53 is often present in determining peroxide value of fats and oils (Moigradean *et al*., 2012). Firstly, 2.5g of each sample was weighted into 250

ml Erlenmeyer flask and 15 ml mixture of acetic acid and chloroform solution was added together in a proportion of 3:2. The solution was shaken before 0.5 ml of KI saturated was filled with it. KI solution was made by adding KI solute drop by drop to distilled water until it became saturated. Next, 15ml of distilled water and 0.5 ml of starch indicator was mixed with the solution. A starch indicator was prepared by pouring 100 ml of hot distilled water to 2 g of starch. Saturated KI mixture is added to the sample and the measure of iodine liberated from KI by the oxidative action of peroxides present in the oil is determined by titration with standard 0.1 N sodium thiosulphate using the starch solution as an indicator (Zahir et al., 2014). Titration was also performed for blanks. The solution was titrated with 0.1 N sodium thiosulphate until the solution turn from oily yellow to colorless. All the samples were repeated for three replicates. Peroxide value was computed utilizing the rule below:

$$\text{Peroxide Value} = \frac{(S-B) \text{ x } N \text{ x } 1000}{W\,(g)}$$

Where, S = ml of sodium thiosulphate use for sample titration

B = ml of sodium thiosulphate use for blank titration

N = Normality of sodium thiosulphate

W = Gram of oil sample

CHAPTER 4

RESULT

4.1 Peroxide Value Test

A test on Peroxide Value (PV) was handed out to investigate the level of peroxide that occurred due to the lipid oxidation of used cooking oils. A method by The American Oil Chemists' Society (AOCS) Official Method Cd 8-53 is a common mode that has been used as an indicator of the initial stage of oxidative change. Figure 4.1 had shown the peroxide value by compared with 8 samples of cooking oil before and after use in frying that collected from both Bertam Putra and Bertam Perdana night market. Table 4.1 tabulated the data on the amount of milliequivalents of peroxide per gram of oil.

Table 4.1: Data on milliequivalents of peroxide per gram of oil.

Peroxide Value (meq/g)		
Sample Of Used Cooking Oil	Before Frying	After Frying
1	14.618	22.581
2	3.976	11.982
3	9.268	19.975
4	11.945	25.286
5	9.319	27.939
6	3.997	22.639
7	9.329	17.321
8	3.993	14.662

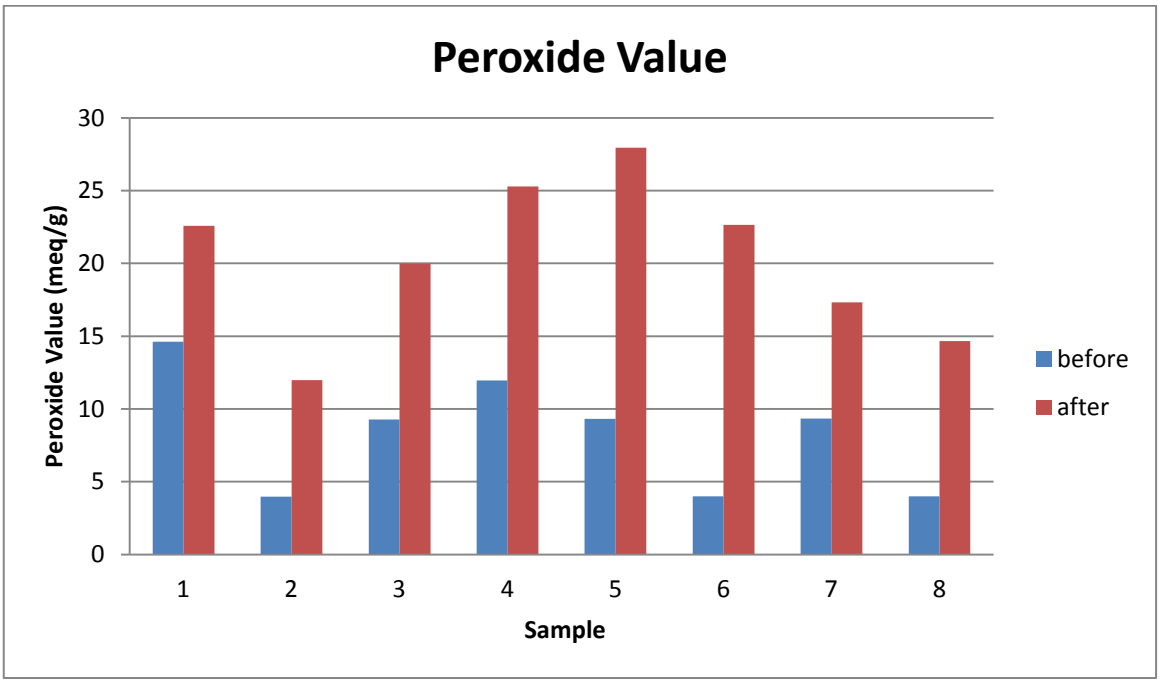

Figure 4.1: Comparison of peroxide value of oils before and after frying

All of the eight samples either before or after frying had shown a high value of peroxide. The level of peroxide was in the range of 3.976 to11. 945 meq/g for the oil before frying. While the range of peroxide value for after frying sample was from 27.939 to 11.982. Based on the result, the sample can be classified into moderate oxidation and high oxidation state based on the calculation of value of peroxide. A product with peroxide value between 5 and 10 meq/g is at moderate oxidation and above10 meq/g is classified as a high oxidation state (Moigradean *et al*., 2012).

CHAPTER 5

DISCUSSION

5.1 Vegetable oil

Oil and fats are needed in our body for a purpose of energy source, as a structural component and to make powerful biological regulators (Gunstone, 2008). They also act as vital role in metabolic reactions in the human organism. Basically, vegetable oils are beneficial and well accepted due to their cholesterol-lowering effect. However, by comparing with animal fats, that are mostly saturated and don't usually react with other chemicals like oxygen while the unsaturated vegetable oils are more reactive (Mendil *et al.,* 2009). Vegetable oils are become necessary needs in worldwide nutrition and come out with different varieties and different qualities, by referring on the country and regional state (Lloren-Martinez *et al.,* 2011).

Vegetable oil can be contaminated in many ways, such as by the technological processes on smoke-drying oil seeds, indirectly exposed to exhaust gases from vehicles and other combustion derived atmospheric particles deposited on the crops during growing (Wang and Guo, 2010). Other combines factors that contaminated the oil are during solvent extraction, packaging materials, residue of mineral oil and relocation of contaminated soil (Hossain and Salehuddin, 2012). The process of refining vegetable oil can help in minimizing the total of contaminants (Yusty, 2005).

Refining operation includes the stages of defaming, neutralization, bleaching and deodorization. The last step of deodorization, required high temperature and oil is put under steam-distillation to remove malodorous compound. Thus, the temperature condition is able to promote the generation of undesirable product (Messeguer, 2011). Furthermore, forming of radical or a smaller unstable fragment after being cracked by the higher temperature (Dost & Ideli, 2012).

Vegetable oils is a complex mixture of triglyceride and contain different chain-length, point of the double bond attaches to the carbon chain, degree of unsaturation and the isomer of the double bond (*cis* and *trans* isomers) (Abdulkarim *et al*., 2007). In a real sample of vegetable oils, there are different content of oleic acid, linoleic and linolenic acids (Morales *et al.,* 2014). In all the common edible oil, most of it contents an abundant of monounsaturated oleic acid (Gunstone, 2000). The condition of oleic acid is more stable compared to polyunsaturated fatty acid because it is more resistant to oxidation whether at storage temperature or at high temperature during frying. Hence, oil with the highest total of oleic acid will undergo slow rate in developing oxidative rancidity during shelf life or while the occurrence of oxidative decomposition during frying compare to those oils that have high amounts of polyunsaturated fatty acids (Abdulkarim *et al*., 2007).

5.2 Deep-Frying Oil

Deep frying is one of the most common processes for the preparation of food that give sensory properties that a favored by consumer like typical flavor, taste, color and crisp texture. Product of new compound could be formed of oils and may also affect the fried

food because in several cases, lipid from frying oil can be absorbed into the food. Thus, most of the nutritional value of fried food depends on the type of oil used (Gertz and Matthäus, 2008). The repeated frying could cause several oxidative and thermal reactions that result in a change of the physicochemical, nutritional and sensory properties of the oil (Che Man and Jasvir, 2000). The mechanisms of hydrolysis, oxidation and polymerization processes which occur during frying have altered the composition of oil and make changes on the flavor and stability of its compounds (Li *et al*., 2008). Besides, different reactions depend on some factors such as replenishment of fresh oil, frying condition, original quality of frying oil and decrease in their oxidative stability (Choe and Min, 2007). Other than that, during frying condition, Vitamin E contained in oil will be degraded and able to inexistent after 3-6 h of frying (Casal *et al*., 2010).

Frying operation, which continuously and repeatedly used under high temperature with constant expose to oxygen, the existence of water from food will lead on occurring of chemical reaction and degradation of frying oil (Aladedunye and Przybylski, 2013). However, all of oil samples may result in lower signal response of oxidation by use of low temperature. This has happened because of less volatile compounds released from the hot oil at low temperature (Innawong *et al*., 2004).

5.3 Peroxide Value

All samples that collected before frying showed a high value of peroxide which is started from 3.976, 3.997, 3.993 and up till 9.268, 9.319, 9.329, 11.945 and 14.618 for sample 1 to 8 respectively. The measured of peroxide in oil was kept increasing after it's

used in frying until the value were reached from 11.982, 14.662, 17.321, 19.975, 22.581, 22.639, 25.286 and 27.939 in sample 1 to sample 8 respectively. The maximum level suggested by FAO of United Nation (2013) is up to 10 milliequivalents of active oxygen/kg oil in refined oil. The increase in peroxide value was also suggested that the peroxide form during the storage condition. Furthermore, the lipid hydroperoxide radical is formed when the heated oil is kept at room temperature and reused again in frying (Lamboni *et al*., 1999). They are parameter of standards for oil quality determination such as content of moisture, content of free fatty acids, trace heavy metals and peroxide value (Bell and Gillatt, 2013).

Peroxide value (PV), along with free fatty acids, is one of the most frequently determined quality parameters during oil production, storage and marketing. PV, often expressed as milliequivalents of hydroperoxide (ROOH) per kilogram of oil, is a measure of the hydroperoxides present in the oil as a product of primary oil oxidation. The first stage of standard method includes the reaction of the KI oxidation (at KI excess) by hydroperoxides in oils. The next stage is the volumetric titration which I_3 was iberated by $Na_2S_2O_3$ with the present of starch indicator: The iodine released is complexed with soluble starch, which acts as an indicator, and the iodine is quantitated by titration with sodium thiosulfate. Based on the stoichiometry of the two reactions, the hydroperoxide concentration can be calculated.

Freshly refined oils regularly have a PV, lower than 1 meq/kg oil and oil is considered to be rancid at a peroxide value above 10 meq/kg oil (Gunstone, 2008). Sulieman *et al.* (2006) reported that a good quality frying vegetable oil should have a PV of less than 2 meq/kg. Studied done in-use frying oils collected during frying operations showed that

PV ranges from 3.3 to 48.1 meq/kg. Then, in discarded samples the PV ranged from 4.7 to 247.5 meq/kg (Sebastian *et al*., 2014). This can prove that a large proportion of the frying oils used in these commercial establishments were highly oxidized.

5.4 Factors Affecting Formation of Peroxide

5.4.1 Heat

Intake of vegetable oil both raw and thermally treated was being increased in the market and daily use. Stalls and fast food restaurants, mostly prepared a food by using edible oils treated with high temperature (Blaszczyk, 2014). Marmesat (2010) stated that when oxidation is happening at high temperature of food processes, the formation of new compounds occur rapidly, oxygen pressure is reduced and hydroperoxide decomposed at a fast rate. According to Berdeaux et al. (2012) hydroperoxide is a primary oxidation compound which limited in high temperature because of the low stability. It decomposes rapidly into the high number of volatile and nonvolatile secondary oxidation product like triacyglycerol and volatile compounds of low molecular weight that produce from a breakdown of hydroperoxide. Shahidi and Ying (2005) justified that oxidation of lipid form of various volatile and non volatile secondary products from a decomposition of a continuous amount of hydroperoxide as a primary oxidation product.