CHAPTER 1

INTRODUCTION

1.0 Introduction

Edible oils such as palm oil, olive oil, sunflower oil and coconut oil are widely used in many food industries. Fats and oil, which contain fatty acids, are oxidized to hydroperoxides in the presence of oxygen. These hydroperoxides further decompose to form polymers, gummy materials, aldehydes, ketones or acids. The presence of food hydroperoxides and their decomposition products causes fats, oils and food containing fats and oils to develop off-flavors and malodors (Frankle, 1996). Antioxidants, for examples, the α-tocopherols, Butylated Hydrosyanysole (BHA), Butylated Hydroxytoluene (BHT), and citric acid can inhibit the oxidation of unsaturated fatty acids, but do not prevent the formation and decomposition of hydroperoxides.

The replacement of synthetic antioxidants by natural ones may have benefits due to health implications and functionality such as solubility in both oil and water, of interest for emulsions, in food systems (Moure et al., 2000). Vegetable materials contain many compounds with antioxidants activity. Several plants have been studied as sources of potentially safe natural antioxidants for the food industry. This antioxidant compounds may protect against lipid oxidation. In the food industry, the addition of antioxidants is required to preserve flavor and color and to avoid vitamin destruction (Pokorny et al., 2001).

Lipid peroxidation and the generation of free radicals are natural phenomena in the biological systems. Lipid peroxidation is associated with several types of biological damage example atherosclerosis, aging and carcinogenesis. Environmental

pollutant such as mercury especially methylmercury can promote lipid peroxidation. Free radical is highly unstable form of oxygen. It is due to loss of electron in its molecules.

Free radicals are both created in metabolic processes and also as a result of environment pollution. Free radical production is encouraged by several exogenous factors at least some are a prominent features of modern lifestyle.

In this case, antioxidant plays an important role to protect us from the unavoidable oxidative stresses. An oxidative stress is defined as the oxidative damage inflicted on biological system by reactive oxygen species (ROS) (Sen et al., 2000). This reactive oxygen species (ROS) then will act on oxygen to create free radicals thus, initiate the lipid peroxidation process. Edible oils may contain sources of antioxidants substances which can scavenge free radicals and play an important role in the prevention of free radical-induced diseases. By donating hydrogen radicals, the primary radicals are reduced to non-chemical compounds and are then converted to oxidize antioxidant radicals (Yamaguchi et al., 1998).

1.1 Edible oils

Fats and oils are water-insoluble, hydrophobic substances of vegetable, land animal or marine animal origin, which consist predominantly of glyceryl esters of fatty acids, so called triacylglycerides. Reversible changes in state owing to variation in temperature may obliterate the common conception that fats are solids and oils are liquids (Patterson, 1983). Edible oils are any oil substances that suitable and can be used as food. For example, palm oil is widely used as cooking oil. The unsaponifiable of palm oil is rich in carotenoids and tocopherols. In the other hand, sunflower oil is one type of edible oil but it cannot be used in frying of food because of the lack of autoxidative stability due to high linoleic acid content.

Olive is an edible fleshy elliptical drupe and contains a hard oval-shaped stone. Its fruit was used for edible purposes and for production of olive oil (Salunkhe et al., 1992). The high oleic acid content in olive oil plus some natural antioxidants combines to give an oil of acceptional stability even during deep frying (Quiles et al., 2001). Red palm oil was cholesterol-free, trans-free oil that is loaded with phytonutrients such as vitamin E (tocotrienols), α-carotene, β-carotene and other carotenoids (Bierenbaum, 1997). Its have tasty alternative of cooking, baking and food processing.

Listed are few more types of edible oil and usage of each type of oil:

I. Canola Oil: One of the healthiest cooking oils due to its low saturated fat content and high monounsaturated fat content. Good for frying at medium temperature.
II. Corn Oil: One of the cheapest and most widely used, corn oil is suitable for frying. It is also used in some margarine and for baking. It should not be used for salad dressings; it has a fairly strong flavor. Corn is rich in omego-6 fatty acids, which are believed to lower bad cholesterol.
III. Grapeseed Oil: Made from the oil pressed from grape seeds left over from wine-making. It has a delicate mild flavor and is good for cooking, frying and in salad dressings.
IV. Safflower Oil: Light all-purpose oil that comes from the seeds of the safflower. It can be used in place of sunflower and peanut oils. It is suitable for deep frying.
V. Sunflower Oil: Low in saturated fat and high in vitamin E, sunflower is the best all-purpose oil. It can be used to cook, fry and in salad dressings.
VI. Soy Oil: Neutral flavored oil which comes from soybeans. It is most widely used for frying because it has a high smoke point. Soy oil is rich in polyunsaturated and monounsaturated fats and low in saturates.

1.1.1 Saturated oils

When the carbon atoms in the hydrocarbon chain of a fatty acid hold their full complement of hydrogen, they are described as saturated (Patterson, 1989). Therefore, saturated oils mean that the molecule has all the hydrogen atoms it can hold. Saturated fatty acids are highly flexible molecules that can assume a wide range of conformations because there is relatively free rotation around each of their C—C bonds (Voet et al.,1998).

Saturated fats are important in the biological functions since they are needed for energy, hormone production, and cellular membranes and also needed for important signaling and stabilization processes in the body. The saturated fatty acids that play important roles in these processes are palmitic acid, myristic acid and lauric acid. These saturated fatty acids are found in certain food fats. Myristic acid is a minor constituent of the mixed fatty acids of most animal and vegetable fats which constitutes 15-30% of coconut oil (Formo et al., 1979).

Some examples of saturated oils are butyric acid, lauric acid, myristic acid, palmitic acid.

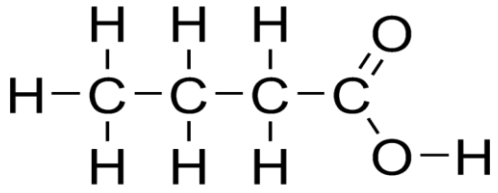


Figure 1: The structure of Butyric acid

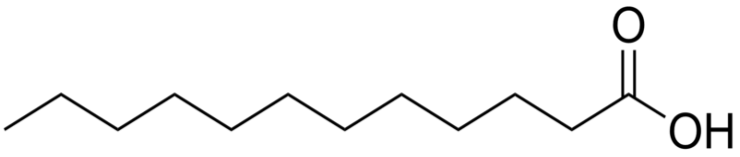


Figure 2: The structure of Lauric acid

1.1.2 Unsaturated Fatty acids

When two adjoining carbon atoms in the hydrocarbon chain of a fatty acid each lack a hydrogen atom, a double bond forms between them. The fatty acid was then said to be unsaturated (Patterson, 1989). Such double bonds are potential centers of chemical reaction and hence disruption. It is because when the hydrogen atoms have removed, this will opens the structure of the molecule in a way that makes it susceptible to attack by free radicals. Free radicals are reactive molecular fragments that occur even in healthy cells, and can damage the cell. When unsaturated oils were exposed to free radicals, they can create chain reactions of free radicals that spread the damage in the cell, and contribute to the cell's aging.

A large number of unsaturated fatty acids occur naturally; they are more difficult to isolate, purify, and characterize than the saturated fatty acids. Unsaturated oils, especially polyunsaturated oil, weaken the immune system's function in ways that are similar to the damage caused by radiation, hormone imbalance, cancer, aging or viral infections.

Some examples of unsaturated oils are oleic acid and palmitoleic acid.

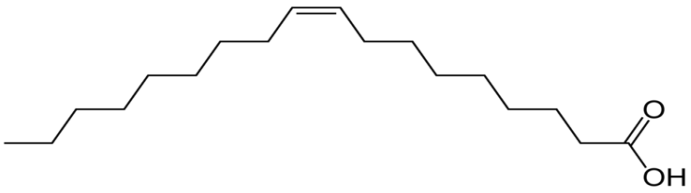


Figure 3: The structure of Oleic acid

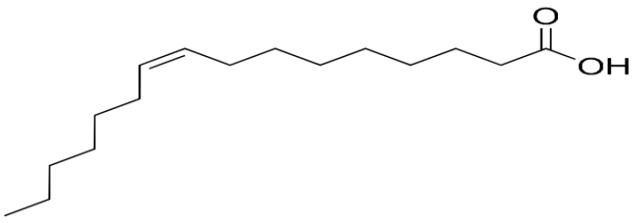


Figure 4: The structure of Palmitoleic acid

1.2 Antioxidant

1.2.1 Definition

Antioxidant is the substances that protect biological systems against the potentially harmful effects of oxidation; especially those caused by oxygen derived free radicals. Free radicals are responsible for aging and causing various human diseases. Antioxidants which scavenge free radical play an important role in the prevention of free radical-induced diseases. The primary radicals are reduced to non-radical chemical compounds by donating hydrogen radicals and are then converted to oxidize antioxidant radicals (Yamaguchi et al., 1998). This helps in protecting the body from degenerative diseases.

Biological antioxidants fall into two classes, the enzymatic antioxidant and non-enzymatic antioxidants (Krinsky.,1992b). The major enzymes directly involved in the scavenging of reactive oxygen species (ROS) are superoxide dismutase which scavenge $O_2^{\bullet-}$, catalyses which scavenge H_2O_2 and glutathione peroxidases which scavenge organic hydroperoxides.

One of the strategies is the control of the levels of transition metals such as iron or copper, which are capable of generating the extremely reactive hydroxyl radical. Human diet contains an array of different compounds that possess antioxidant activities or have been suggested to scavenge ROS. The most common dietary antioxidants are ascorbic acid (Vitamin C), tocopherols (vitamin E), carotenoids and flavonoids (Packer et al., 1999).

1.2.2 Classification of antioxidant

There are two sources of antioxidant which are synthetic antioxidant and natural antioxidant. The natural antioxidant is obtained from natural sources for examples α-tocopherol, β-carotene and flavonoid. The natural antioxidant can be divided into two groups due to their several of criteria which are enzymatic and non-enzymatic group (Yanishlieva-Maslarova., 2001). Since 1980, natural antioxidant has

appeared as an alternative to synthetic antioxidant. Synthetic antioxidants are not safe enough to use after a long period compared to natural antioxidant. The examples of popular synthetic antioxidant are phenolic compound such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), tertiary butyhydroquinone (TBHQ) and polyhydroxyphenols (eg; gallates).

Antioxidants are grouped mainly according to their mode of action. The classification of antioxidant is divided into primary antioxidant, secondary antioxidant and synergistic antioxidant.

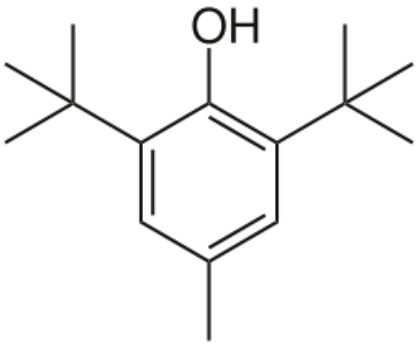


Figure 5: The structure of butylated hydroxytoluene (BHT)

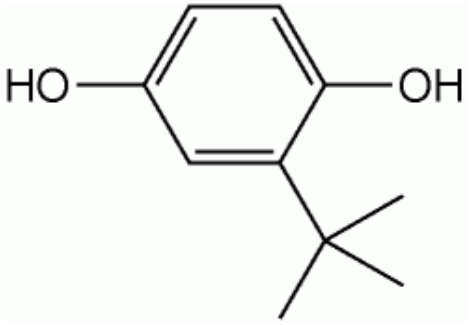


Figure 6: The structure of tertiary butylhydroquinone

1.2.2.1 Primary antioxidant.

Primary antioxidant is also known as chain-breaking antioxidant. They are compounds that suppress or prevent the formation of new free radical species. They are also able to donate hydrogen atom rapidly to a lipid radical, forming new radical that are more stable than the initial one. Primary antioxidant also known as prevention antioxidants and include all those enzymes such as superoxide dismutase, glutathione peroxidase, catalase and metal sequestering proteins such as transferrin and ferritin. Primary antioxidants are effective at very low concentration, and at higher levels, they may become pro-oxidants (Madhavi et al., 1996).

1.2.2.2 Secondary antioxidant.

Secondary antioxidant is also known as radical scavenging antioxidants. They function as decomposer of lipid peroxides into stable end products (Hudson, 1990). They also retard the rate of autoxidation of lipid by processes other than that of interrupting the autoxidation chain by converting free radicals to more stable species. Secondary antioxidant plays two main roles, which are suppressing chain reaction initiation and breaking chain reaction propagation. They remove newly formed free radicals before chain reaction is initiated, which can lead to cell damage and also further free radical formation. They are often used in combination with primary antioxidants to yield synergistic stabilization effects.

1.2.2.3 Synergistic antioxidant.

This class of antioxidant can be broadly classified as oxygen scavengers and chelators. The cooperatives effect of the inhibitors during oxidation which results by their reinforcing each other is known as synergism. Synergists function by various mechanisms. They may act as hydrogen donors to the phenoxyl radical thereby regenerating the primary antioxidant (Madhavi et al., 1996).

1.2.3 Antioxidant activity

Oxygen is very important in our lives as it is involved in the biological processes, but at the same time, it can be harmful as oxygen can chemically transformed into free radicals. Free radicals are natural by-products of organic processes within our cells and by exposure to various environmental stresses. The over-oxidation of free radicals can cause stress at a cellular level that in turn can lead to disease (Mcdonald et al., 1993). In this case, antioxidant plays their roles to prevent them from oxidizing.

Therefore, antioxidant activity can be defined as an ability of an antioxidant compounds to inhibit lipid peroxidation process and to trap the free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. This free radical may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease.

Antioxidant compounds like phenolic acids, polyphenols, Vitamin E, Vitamin A and flavonoids, which usually obtained from plant sources can scavenge free radical such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Champe and Harvey, 1994). These free radicals also form because of oxidative stresses in biological organisms.

1.3 Free Radicals

1.3.1 Definition

Free radical defined as a chemical species that posses an unpaired electron in the outer (valence) shell of the molecules. This is the reason why they are highly reactive and because of that, it was trying to gain stability by capturing needed electron quickly from their surroundings. Hence, free radicals attack the nearest stable molecule, "stealing" its electron. When the attacked molecule loses its electron, it become a free radical itself and begins the chain reaction. Once the process started, it can cascade, finally resulting in the disruption of a living cell.

1.3.2 Types of free radicals

The hydroxyl radical is an extremely reactive oxidizing radical that will react to most biomolecules at diffusion-controlled rates, which means that reactions will occur immediately with biomolecules. The hydroxyl free radical is important in biological damage and it is more reactive towards cellular constituents than superoxide radicals are. The other example was nitric oxide; it is a common gaseous free radical. It is now recognized to play a role in vascular physiology and is also known as endothelium derived relaxing factor. Vascular endothelium produces nitric oxide, as do neutrophils and macrophages from arginine using the enzyme nitric oxide synthetase. This event can be stimulated by cytokines, tumor necrosis factor, or interleukins (Beckman and Ames, 1993). Inhibition of production is known to reduce microbicidal and tumouricidal activities of macrophages.

Free radicals and reactive oxygen species are either synthesized endogenously example in energy metabolism and by the antimicrobial defense system of the body, or produced as reactions to exogenous exposures such as cigarette smoke, imbalanced diet, exhaustive exercise, environmental pollutants and food contaminants (Reavley, 1998). ROS are either radicals (molecules that contain at least one unpaired electron)

or reactive non-radicals, both of which are capable of oxidizing biomolecules. ROS are produced as normal consequence of biochemical processes in the body (Champe and Harvey, 1994).

This ROS sometimes is non-radical but they will readily extract electrons from other molecules, converting them to free radical and thereby initiate the chain reaction (Voet et al., 1999). Therefore, ROS was known as molecules that initiate the formation of free radical either naturally or accidentally. The random nature of free radical attacks makes it difficult to characterize their reaction products, but all classes of biological molecules are susceptible to oxidative damage caused by free radicals. Several degenerative diseases, including Parkinson's, Alzheimer's and Hungtington's diseases, are associated with oxidative damage to mitochondria (Voet et al., 1999). The examples of ROS are shown below;

Table 1: Biologically significant free radicals and reactive oxygen species with their chemical structure (Champe and Harvey, 1994).

Superoxide radical	$O_2^{\bullet -}$
Hydroxyl radical	•OH
Peroxyl radical	ROO•
Hydrogen peroxide	H_2O_2
Singlet oxygen	$^1O_2$
Nitric oxide	NO•
Hypochlorous acid	HOCl

1.4 Antioxidant in edible oil

Table below shown the content of edible oil:

Table 2: The content and function of antioxidants in edible oil (Salunkhe et al., 1992).

Type of oil	Content of antioxidant	Function
Palm oil	Vitamin A • α-carotene • β-carotene	Protect against oxidative damage Protect against certain form of cancer and stimulates the body's immune defence.
	Vitamin E • α-tocopherols • tocotrienols	Potent peroxy radical scavenger Inhibit the formation of free radicals and peroxides
	Phenolic compound • Flavonoids • Polyphenols	Help to prevent certain formation of cancer Protect against lipid peroxidation
Sunflower oil	Vitamin E • α-tocopherol	Potent peroxy radical scavenger

	Phenolic compound • Polyphenols	Protect against lipid peroxidation
Olive oil	Vitamin E • α-tocopherol • tocotrienols	Potent peroxy radical scavenger Inhibit the formation of free radicals and peroxides
	Phenolic compound • Polyphenols	Protect against lipid peroxidation
Red palm oil	Vitamin A • α-carotene • β-carotene	Protect against oxidative damage Protect against certain form of cancer and stimulates the body's immune defence.
	Vitamin E • tocotrienols	Inhibit the formation of free radicals and peroxides
Coconut oil	Vitamin E • α-tocopherol	Potent peroxy radical scavenger

Edible oil may contain different antioxidant compounds or their active ingredients. For example palm oil, contains high amount of tocotrienols and α-tocopherols. Some of the antioxidant presents in the plant or animals are lipid-soluble whereas, some are water-soluble antioxidant. For example, carotene is a lipid soluble orange substance present in fruit and roots of plants (Birtigh et al., 1994). There are some examples of antioxidant compound exist in edible oils including polyphenols, vitamin E and vitamin A.

1.4.1 Polyphenols

Phenolic compounds have a basic chemical structure; all have an aromatic ring bearing one or more hydroxyl groups. They arise from 2 main of biosynthetic pathway that is 1) Shikimic acid pathway and 2) Acetic acid pathway. They occur primarily in glycosylated form, with one or more sugar residues linked to hydroxyl groups or directly to an aromatic carbon atom. A large range of low and high molecular weight plant polyphenolics presenting antioxidant properties has been studied and proposed for protection against lipid oxidation.

Polyphenols have other undesirable effects in food systems such as the formation of strong complexes with dietary proteins. Currently, interest has focused on polyphenols as a natural source of antioxidants and most studies centre of flavonoids. Among the flavonoids, the most common compounds are flavanols, flavonols, flavonones, flavones, isoflavones, anthocyanidins and proanthocyanidins.

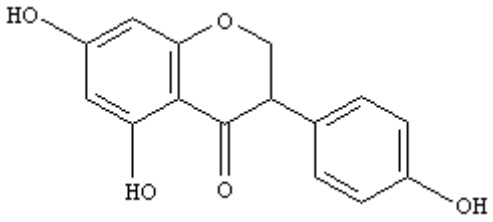


Figure 7: The structure of flavanols

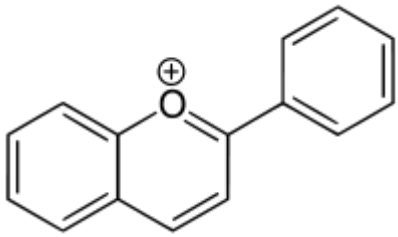


Figure 8: The structure of anthocyanidins

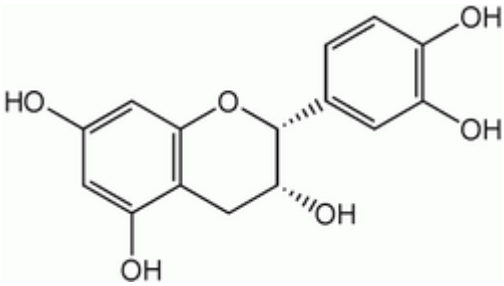


Figure 9: The structure of proanthocyanidins

1.4.2 Vitamin E

Vitamin E is an essential nutrient because the body cannot synthesize its own vitamin E, which must be provided by foods and supplement. Vitamin E possesses natural antioxidant capabilities. It functions as an antioxidant to protect fat in membranes around cells such as nerves, heart, muscles and red blood cells from damage by oxygen.

There are four isomers of vitamin E; α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol but only the α-tocopherol is usually mentioned, since it accounts for about 80% of total activity of the vitamin.

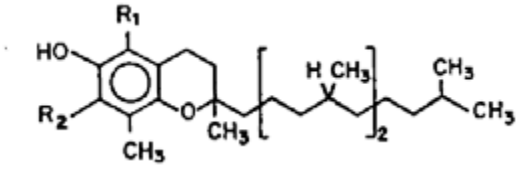


Figure 10: The structure of tocopherols (Ball, 2004)

Tocotrienols are minor plant constituents found abundantly in rice bran and in palm oil, which can provide a significant source of vitamin E animal feeds. Tocotrienols also able to inhibit the formation of free radicals and peroxides produced during linoleic acid oxidation in food and biological system.

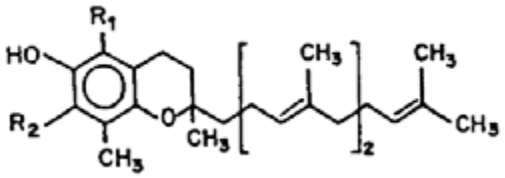


Figure 11: The structure of tocotrienols (Ball, 2004).

1.4.3 Vitamin A

Vitamin A is water-insoluble and soluble in fats, oils and in most organic solvents example ethanol, methanol (Rucker et al., 2001). Vitamin A in crystalline form or in oil, if kept under a dry nitrogen atmosphere in a dark cool place, is stable for long periods. On the other hand, vitamin A and its analog are particularly sensitive to oxidation by air in the presence of light, particularly when spread in thin surface film.

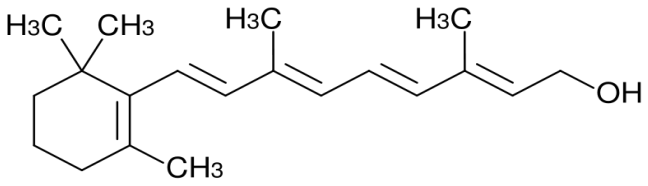


Figure 12: The structure of vitamin A

Common dietary sources of preformed vitamin A are various dairy products, such as milk, cheese, egg, liver; other internal organ such as kidney and heart; and many fishes and also sources from plants, example carrots (carotenoids) and green leafy vegetables; spinach. The richest sources of carotenoids are red palm oil, which contain about 0.5 mg of mixed α-carotene and β-carotene per milimiters (Rucker et al., 2001). Carotene is used in the food processing industry for cloning purposes. β-carotene have many function such as may protect against certain form of cancer and stimulates the body's immune defense mechanism by way of an increased capacity of the macrophages to kill the tumor cells and increases the production of the tumor necrosis factor. β-carotene also widely regarded as an anticancer agent (Salunkhe, 1992).

1.5 Lipid peroxidation

Lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds. As Lipid peroxidation involves complex reactions and great variety of substrates, understanding of these processes has come from studies of simple model substrate such as fatty acids of increasing unsaturation degree (the main fraction of membrane lipid) or cholesterol (abundant in blood plasma and cell membranes).

As a free radical reaction, lipid peroxidation proceeds in three distinct steps. Those are initiation, propagation and lastly termination step. In the first step, that is initiation, the polyunsaturated lipid will form lipid radical in the presence of metal and

hydroperoxides. Abstraction of a hydrogen atom by a reactive species such as hydroxyl radical may lead to initiation of lipid oxidation.

In aerobic condition, alkyl radical will react rapidly with oxygen to form peroxyl radical and after that react with polyunsaturated lipid to form hydroperoxides (ROOH). Finally, the reaction will finalize with the termination step, which free radical combine to form molecules with a full complement of electrons. On the other hand, the hydroperoxides will be decomposing in the presence of metal to form alkoxyl radical (ROO•).This alkoxyl radical will cleave into complex mixture of aldehyde that due to cause damage in the biological tissue. Lipid peroxidation involves 3 stages;

Initiation

$$X\bullet + RH \longrightarrow R\bullet + XH$$

Propagation

$$R\bullet + O_2 \longrightarrow ROO\bullet$$

$$ROO\bullet + R'H \longrightarrow ROOH + R\bullet$$

Termination

$$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2$$

$$ROO\bullet + R\bullet \longrightarrow ROOR$$

$$R\bullet + R\bullet \longrightarrow RR$$

Figure 13: The stages involves in lipid peroxidation process (Frankel,1999).

1.6 Heat and antioxidant activity

Antioxidants are substances that may protect your cells against the effects of free radicals. Free radicals are molecules produced when your body breaks down food, or by environmental exposures like tobacco smoke and radiation. Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases. Antioxidant substances include:

- Vitamin A
- Vitamin C
- Vitamin E

The stability of vitamin A in fortified wheat and corn flour is excellent. Studies show that wheat flour and yellow corn flour, stored under normal conditions, retain over 95% of their vitamin A after six months at room temperature. However, the stability of vitamin A under high storage temperatures is not as good. Wheat flour stored for three months at 45 ^{o}C retained only 72% of vitamin A. Baking fortified bread causes only limited losses of vitamin A, while frying has an adverse effect on the stability of the vitamin. After an initial frying of vitamin A-fortified soybean oil, about 65% of the original vitamin A remained; however, after four repeated frying, less than 40% of the original level of vitamin A was retained (Favaro et al., 1991).

The stability of vitamin E depends on its form, dl-α-tocopherylacetate being the most stable. Vitamin E, occurring naturally in foods in the form of α-tocopherol, oxidizes slowly when exposed to air. Losses of vitamin E occur only during prolonged heating such as in boiling and frying (Cort et al., 1975).

Ascorbic acid (vitamin C) is easily destroyed during processing and storage through the action of metals such as copper and iron. Both exposure to oxygen and prolonged heating in the presence of oxygen destroy ascorbic acid; thus, the stability

of vitamin C in fortified foods depends on the product, processing method, and type of packaging used.

It is important to assess the oxidative degradation of fats and oils in the food industry, because free radicalinitiated oxidation is one of the main causes of rancidity. Free radicals are known to be responsible for the oxidation of food components, resulting in alterations of the major quality-control parameters, such as colour, flavour, aroma and nutritional value of foodstuffs (Donelli et al., 1995). Excessive free radical formation, contributing to the onset of certain pathologies, may demand a high dietary intake of fruits, which are rich in antioxidant vitamins and phenolics (Owen et al., 2000). That is a good reason to assess the amounts of these compounds in dietary oils and how different technological processes, such as frying, affect their availability. There are numerous studies that report changes in fats and oils after heating or frying procedures (Che Man et al., 2000). Most of them conclude that such changes depend on the temperature, the heating cycles, the surface/ volume and food/oil ratios, the fatty acid and the antioxidant composition of the oils (Melton et a., 1994). On the other hand, the methods developed to assess the effects of frying on oils are not always consistent, making interpretation of the phenomena difficult. Thus, studies of thermal oxidation of oils are far from completed. All chemical changes of fats and oils and their natural contaminants at elevated temperatures originate in oxidation, hydrolysis, polymerisation, isomerisation or cyclisation reactions. All these reactions may be promoted by oxygen, moisture, traces of metal and free radicals (Gertz, 1996). These processes may reduce the amount of antioxidants in the oil, decrease its stability and produce new products which are responsible for loss of the nutritional value and quality of the oil (odour, flavour, absorption).

1.7 Method for determination of antioxidant activity

1.7.1 Measurement of antioxidant activity

Antioxidant activity cannot be measured directly but rather by the effects of the antioxidant in controlling the extent of oxidation. Methods show extreme diversity. Some methods involve a distinct oxidation step followed by measurement of the outcome as, for example, oxidation of linoleic acid followed by determination of diene conjugation.

1.7.2 2,2-diphenyl-1-picrylhydrazyl (DPPH) method

The antioxidant activity of various foods can be determined accurately, conveniently, and rapidly using DPPH testing. This method can be used successfully for solid samples without prior extraction and concentration, which saves time. Antioxidant activity measured using DPPH accounts partially for the bound and insoluble antioxidants. Relative antioxidant content provides an indication of importance of each of the foods. Antioxidant activity and nutritional labeling data including vitamins, fibers, and minerals will aid in the interpretation of clinical results obtained as various food products are tested in biological models for chronic disease. It is reasonable to expect that high antioxidant foods have greater potential to reduce free radicals in the body than do low antioxidant foods. Thus it is important to know the antioxidant content of foods, in addition to knowing the basic nutritional information such as the protein, fiber, fat, mineral and vitamin contents (Aruna et al., 2000).

The model of scavenging the stable DPPH• radical is widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidant on DPPH• radical scavenging is thought to be due to their hydrogen donating ability (Brand et al., 1995). In the DPPH test, the scavenging of the DPPH radicals was followed by monitoring the decrease in absorbance at 515 nm, which occurs due to reduction by the antioxidant or a reaction by the radical species.

CHAPTER 2

EXPERIMENTAL

2.1 Reagent

The following chemicals used in the analysis of antioxidant content in edible oils:

a. 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

b. Spectrometry grade methanol (99.9%)

c. Butylated Hydroxytoluene (BHT)

d. The oil sample as listed below were used in this analysis:

- Tiga Udang Palm Oil
- Giant Palm Oil
- Alif Palm Oil
- Knife Palm Oil
- Cesar Olive Oil
- Borges Olive Oil
- Family Choice Canola Oil
- Naturel Canola Oil
- Everest Sunflower Oil

- Naturel Sunflower Oil
- Olife Soy Bean Oil
- Family Choice Soy Bean Oil
- Daisy Corn Oil
- Mazola Corn Oil
- MP Lingams & Sons Gingerly Oil
- Baba's Gingerly Oil
- Green Love Rice Bran
- Smart Balance (Soy, Palm and Canola) Mixed Oil
- Naturel (canola & sunflower) Mixed Oil
- Ayurvedic Oil

2.2 Apparatus

The following apparatus were used in the analysis:

- Serum bottles
- Aluminium foil
- Clonical flask 100 ml
- Clonical flask 1000 ml
- Micropipette
- Oven

2.3 Sample Collection

Samples were purchased at supermarkets and grocery shop around Kajang area.

2.4 Preparation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Solution

39.43 milligrams of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is dissolved in spectrometry grade methanol (99.9%) in a 1000 ml conical flask. Methanol is added to 1000 ml mark. This produces 0.1 mM of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution.

2.5 Preparation of Butylated Hydroxytoluene (BHT) Standard Solution

100 mg of Butylated Hydroxytoluene (BHT) is dissolved in 100 ml of spectrometry grade methanol to produce a solution with 1000 µg/ml concentration. 1 ml of 1000 µg/ml solution is added into a new conical flask and topped up with spectrometry grade methanol up to the 100 ml mark. This step produces a solution with 1×10^{-5} g/ml concentration. Various concentrations of standard solutions were produced based on the table below.

Table 3: Volume of 1 x 10^{-5} g/ml BHT solution (ml) required to produce standard solution

Concentration of BHT (mM)	Volume of 1 x 10^{-5} g/ml solution (ml)	Volume of methanol added (ml)
0	0	10
0.1	0.1	10
0.5	0.5	10
1	1	10
2	2	10
3	3	10
4	4	10
5	5	10

2.6 Instrumentation

The absorbance of the standard and samples were obtained by using Shimadzu UV 1700 spectrophotometer equipped with 1.0 cm^3 quartz cell.

2.7 Analytical Procedure

2.7.1 Preparation of Calibration Graph

BHT was dissolved in spectrometry grade methanol to produce 0mM, 0.5mM, 1.0mM, 2.0mM, 3.0mM, 4.0mM and 5.0 mM. 5 mL of 0.10 mM DPPH in methanol were added in seven serum bottles which have been covered with aluminium foil. Then, 56 µL of 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mM BHT is added into each bottle respectively. The mixtures were allowed to stand at room temperature, 25^{o}C for 120 minutes and absorbance of DPPH was determined. A calibration graph absorbance of DPPH against BHT concentration is plotted.

2.7.2 Sample Preparation and Analysis

5 mL of 0.1 mM DPPH in spectrometry grade methanol (99.9 %) were mixed with 56 µL oil samples in a 30 mL serum bottle covered with aluminum foil. The samples prepared in triplicated were left in 25 ^{o}C for 120 minutes. Absorbance of the sample mixture was measured at 517 nm using UV/Vis-spectrometry. The absorbances were recorded in the table. The entire process was repeated at temperature of 80 ^{o}C, 120 ^{o}C and 180 ^{o}C.

CHAPTER 3

RESULT AND DISCUSSION

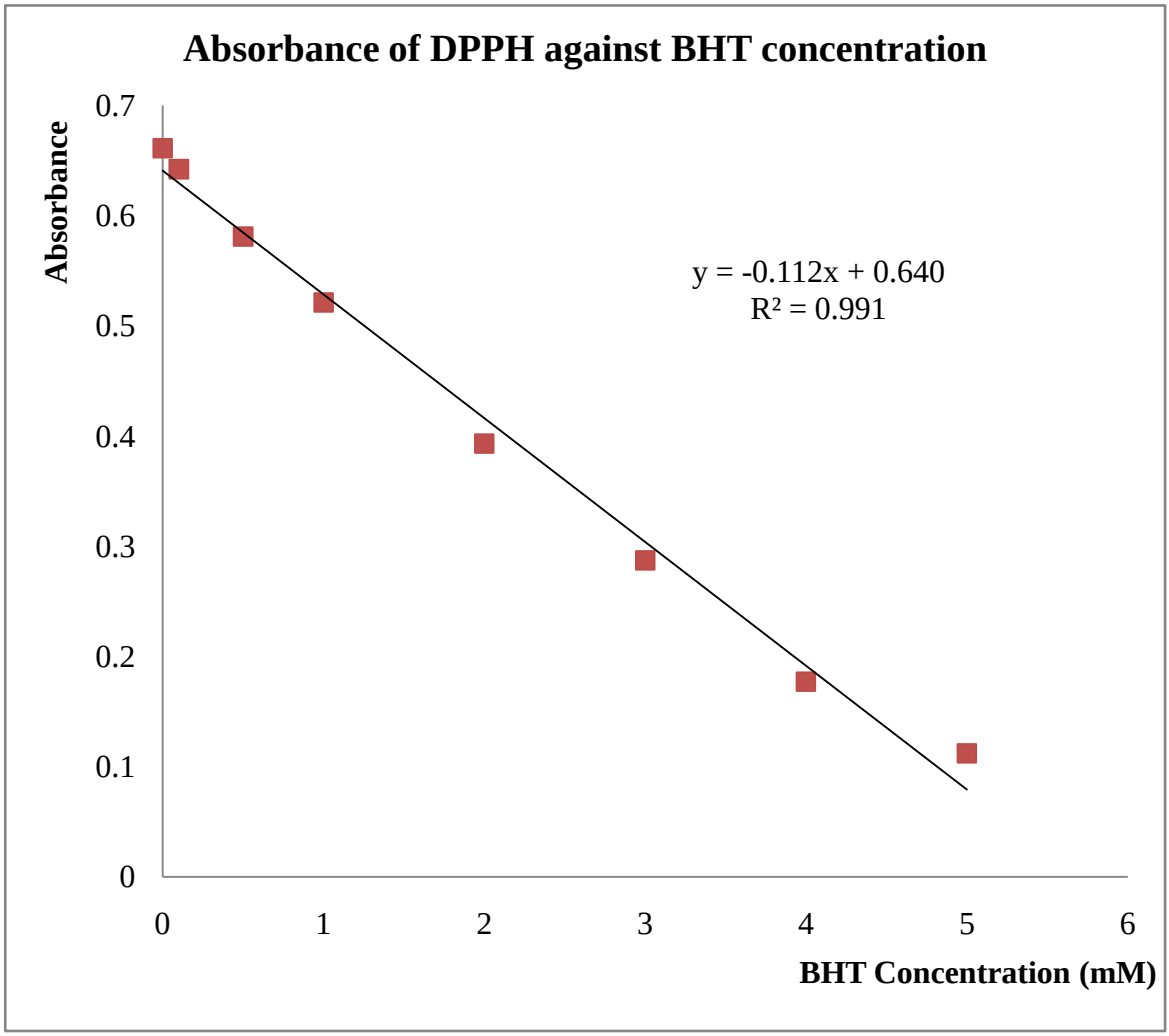
3.1 Calibration curve

A calibration graph for absorbance of 2,2-diphenyl-1-picrylhydrazyl (DPPH) against concentration of BHT were obtained based on a series of 8 standard solution containing known amount of BHT. The concentration of BHT standard sample was subsequently plotted against the absorbance of DPPH to obtain a calibration as shown in Graph 1. Table 1 shows the absorbance and concentration of standard samples.

Table 4: Absorbance of DPPH against BHT Concentration

BHT Concentration (mM)	0	0.1	0.5	1	2	3	4	5
Absorbance (A)	0.661	0.642	0.581	0.521	0.393	0.287	0.177	0.112

Graph 1: Calibration Graph of Absorbance of DPPH against BHT Standard Samples



3.2 Sample Analysis

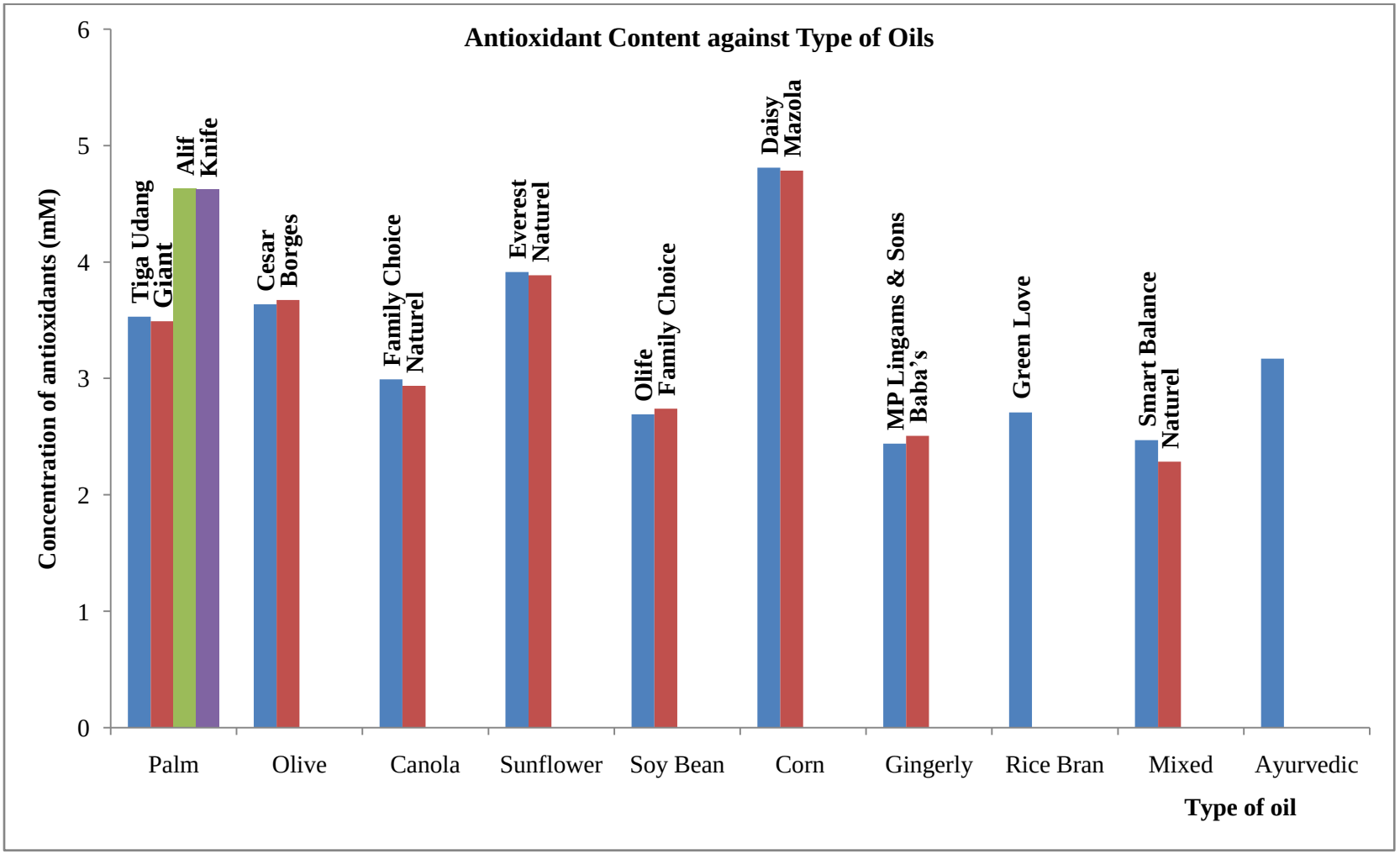
The samples were analyzed at four different temperatures to accomplish the objective of the research. Accordingly the temperatures chosen were 25 ^{o}C, 80 ^{o}C, 120 ^{o}C and 180 ^{o}C. Then readings were tabulated in tables and illustrated in graphs.

Table 5: Antioxidant Content of Oils at 25^{o}C

No	Type of oil	Brand	Absorbance at 517 nm	Concentration of Antioxidants (mM)	Average Concentration of Antioxidants (mM)
1	Palm Oil	Tiga udang	0.242	3.5536	3.5298 ± 0.0184
			0.247	3.5089	
			0.245	3.5268	
		Giant	0.249	3.4911	3.4911 ± 0.0073
			0.250	3.4821	
			0.248	3.5000	
		Alif	0.123	4.6161	4.6310 ± 0.0111
			0.120	4.6429	
			0.121	4.6339	
		Knife	0.123	4.6161	4.6220 ± 0.0084
			0.121	4.6339	
			0.123	4.6161	
2	Olive Oil	Cesar	0.232	3.6429	3.6369 ± 0.0042
			0.233	3.6339	
			0.233	3.6339	
		Borges	0.228	3.6786	3.6726 ± 0.0152
			0.231	3.6518	
			0.227	3.6875	
3	Canola Oil	Family Choice	0.303	3.0089	2.9911 ± 0.0146
			0.307	2.9732	
			0.305	2.9911	
		Naturel	0.311	2.9375	2.9375 ± 0.0000
			0.311	2.9375	
			0.311	2.9375	

4	Sunflower Oil	Everest	0.201	3.9196	3.9137 ± 0.0042
			0.202	3.9107	
			0.202	3.9107	
		Naturel	0.205	3.8839	3.8869 ± 0.0042
			0.205	3.8839	
			0.204	3.8929	
5	Soy Bean Oil	Olife	0.338	2.6964	2.6905 ± 0.0042
			0.339	2.6875	
			0.339	2.6875	
		Family Choice	0.333	2.7411	2.7411 ± 0.0073
			0.332	2.7500	
			0.334	2.7321	
6	Corn Oil	Daisy	0.101	4.8125	4.8095 ± 0.0042
			0.102	4.8036	
			0.101	4.8125	
		Mazola	0.101	4.8125	4.7857 ± 0.0193
			0.105	4.7768	
			0.106	4.7679	
7	Gingerly Oil	MP Lingams & Sons	0.368	2.4286	2.4405 ± 0.0111
			0.365	2.4554	
			0.367	2.4375	
		Baba's	0.359	2.5089	2.5060 ± 0.0111
			0.361	2.4911	
			0.358	2.5179	
8	Rice Bran	Green Love	0.335	2.7232	2.7083 ± 0.0111
			0.338	2.6964	
			0.337	2.7054	
9	Mixed Oil	Smart Balance (Soy, Palm and Canola)	0.362	2.4821	2.4702 ± 0.0111
			0.363	2.4732	
			0.365	2.4554	
		Naturel (canola & sunflower)	0.382	2.3036	2.2857 ± 0.0126
			0.385	2.2768	
			0.385	2.2768	
10	Ayurvedic Oil		0.289	3.1339	3.1696 ± 0.0292
			0.281	3.2054	
			0.285	3.1696	

Graph 2: Antioxidant Content of Oils at 25 ^{o}C



Above table 5 and graph 2 signify the antioxidant content of edible oils at the temperature of 25 ^{o}C which is room temperature. The preparation of the calibration curve also made at 25 ^{o}C.

Table 5 shows the samples prepared in triplicate and the average concentrations obtained from calibration curve. The bar chart indicates the antioxidant content of oils based on the type of oil and the brand of oil.

Subsequently the graph 2 shows Daisy and Mazola corn oil has the highest antioxidant content with 4.8095 ± 0.0042 mM and 4.7857 ± 0.0193 mM respectively followed by 2 types of palm oil that is Alif and Knife with the value of 4.6310 ± 0.0111 mM and 4.6220 ± 0.0084 mM.

Palm oil (Tiga Udang & Giant), Olive oil (Cesar & Borges), Canola oil (Family Choice & Naturel), Sunflower oil (Everest & Naturel), Soy Bean oil (Olife & Family Choice), Gingerly oil (MP Lingams & Baba's), Rice Bran oil (Green Love), Mixed oil (Smart Balance) and Ayurvedic oil are having antioxidant content between 2.4405 ± 0.0111 mM and 3.9137 ± 0.0042 mM.

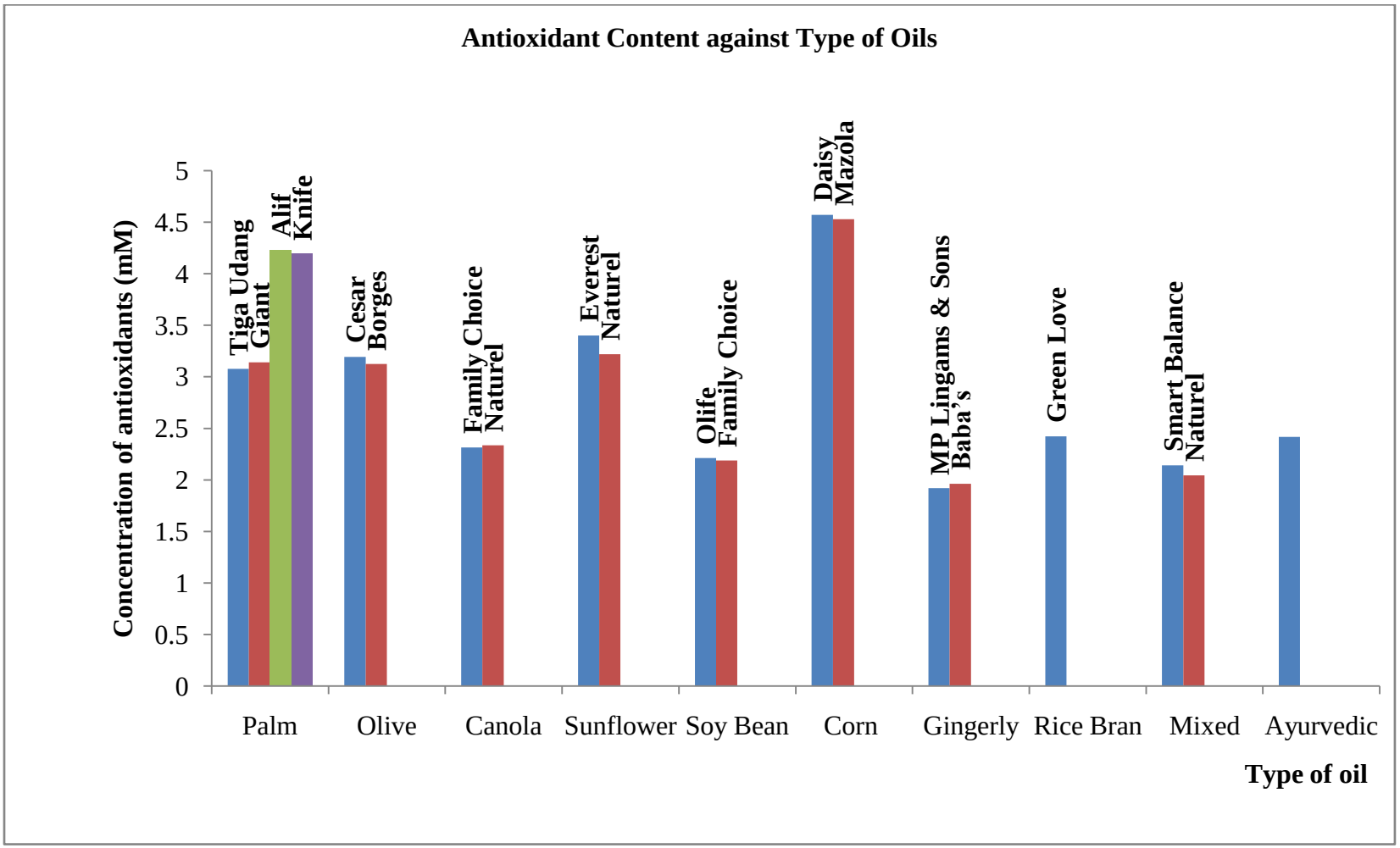
The lowest antioxidant content 2.2857 ± 0.0126 mM is found in Naturel brand mixed oil along with it contains mixture of canola and sunflower oil.

Table 6: Antioxidant Content of Oils at 80 ^{o}C

No	Type of oil	Brand	Absorbance at 517 nm	Concentration of Antioxidants (mM)	Average Concentration of Antioxidants (mM)
1	Palm Oil	Tiga udang	0.293	3.0982	3.0774 ± 0.0183
			0.295	3.0804	
			0.298	3.0536	
		Giant	0.289	3.1339	3.1399 ± 0.0084
			0.287	3.1518	
			0.289	3.1339	
		Alif	0.165	4.2411	4.2262 ± 0.0111
			0.167	4.2232	
			0.168	4.2143	
		Knife	0.171	4.1875	4.1934 ± 0.0042
			0.170	4.1964	
			0.170	4.1964	
2	Olive Oil	Cesar	0.282	3.1964	3.1934 ± 0.0112
			0.281	3.2054	
			0.284	3.1785	
		Borges	0.289	3.1339	3.1250 ± 0.0073
			0.290	3.1250	
			0.291	3.1161	
3	Canola Oil	Family Choice	0.379	2.3304	2.3155 ± 0.0111
			0.381	2.3125	
			0.382	2.3036	
		Naturel	0.379	2.3304	2.3363 ± 0.0042
			0.378	2.3393	
			0.378	2.3393	
4	Sunflower Oil	Everest	0.258	3.4107	3.4018 ± 0.0073
			0.260	3.3929	
			0.259	3.4018	
		Naturel	0.278	3.2321	3.2202 ± 0.0111
			0.281	3.2054	
			0.279	3.2232	
5	Soy Bean Oil	Olife	0.392	2.2143	2.2113 ± 0.0042
			0.393	2.2054	
			0.392	2.2143	

		Family Choice	0.395	2.1875	2.1875 ± 0.0000
			0.395	2.1875	
			0.395	2.1875	
6	Corn Oil	Daisy	0.129	4.5625	4.5714 ± 0,0073
			0.128	4.5714	
			0.127	4.5804	
		Mazola	0.132	4.5357	4.5298 ± 0.0042
			0.133	4.5268	
			0.133	4.5268	
7	Gingerly Oil	MP Lingams & Sons	0.425	1.9196	1.9196 ± 0.0073
			0.426	1.9107	
			0.424	1.9286	
		Baba's	0.418	1.9821	1.9613 ± 0.0152
			0.421	1.9554	
			0.422	1.9464	
8	Rice Bran	Green Love	0.371	2.4018	2.4226 ± 0.0152
			0.368	2.4286	
			0.367	2.4375	
9	Mixed Oil	Smart Balance (Soy, Palm and Canola)	0.402	2.1250	2.1399 ± 0.0152
			0.401	2.1339	
			0.398	2.1607	
		Naturel (canola & sunflower)	0.412	2.0357	2.0446 ± 0.0073
			0.411	2.0446	
			0.410	2.0536	
10	Ayurvedic Oil		0.371	2.4018	2.4167 ±0.0111
			0.368	2.4286	
			0.369	2.4196	

Graph 3: Antioxidant Content of Oils at 80 ^{o}C



The table 6 and graph 3 indicates the antioxidant content of edible oils in the temperature of 80 ^{o}C.

The table 6 shows the samples prepared in triplicate and the average concentrations obtained from calibration curve. The bar chart indicates the antioxidant content of oils based on the types of oil and the brand of oil.

Subsequently the graph 3 shows Daisy and Mazola corn oil has the highest antioxidant content with 4.5714 ± 0.0073 mM and 4.5298 ± 0.0042 mM respectively followed by 2 types of palm oil that is Alif and Knife with the value of 4.2263 ± 0.0111 mM and 4.1934 ± 0.0042 mM.

Palm oil (Tiga Udang & Giant), Olive oil (Cesar & Borges), Canola oil (Family Choice & Naturel), Sunflower oil (Everest & Naturel), Soy Bean oil (Olife & Family Choice), Gingerly oil (Baba's), Rice Bran oil (Green Love), Mixed oil (Smart Balance & Naturel) and Ayurvedic oil are having antioxidant content between 1.9613 ± 0.0152 mM and 3.4018 ± 0.0073 mM.

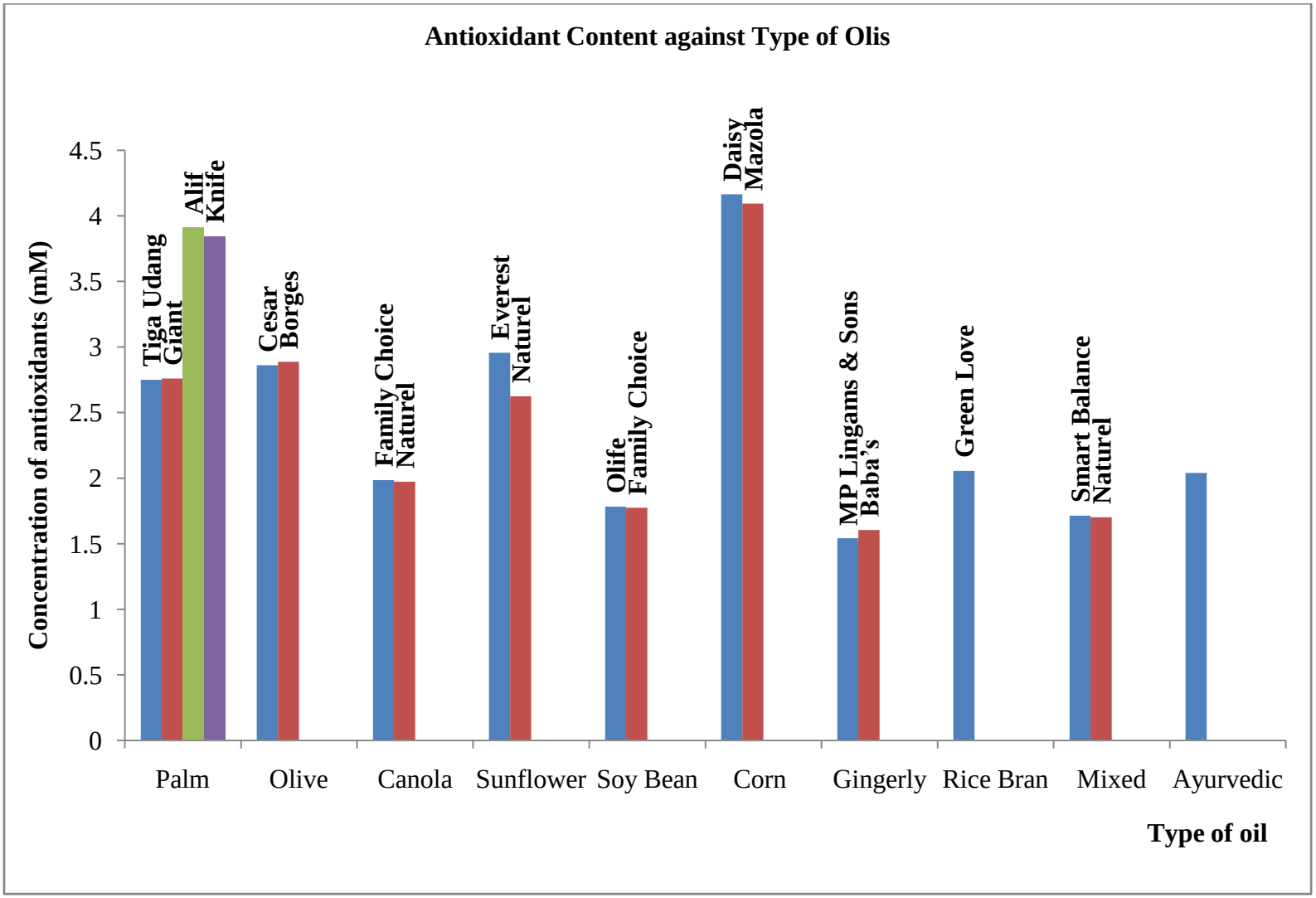
The lowest antioxidant content 1.9196 ± 0.0073 mM is found in MP Lingams & Sons brand gingerly oil.

Table 7: Antioxidant Content of Oils at 120 ^{o}C

No	Type of oil	Brand	Absorbance at 517 nm	Concentration of Antioxidants (mM)	Average Concentration of Antioxidants (mM)
1	Palm Oil	Tiga udang	0.332	2.7500	2.7500 ± 0.0000
			0.332	2.7500	
			0.332	2.7500	
		Giant	0.330	2.7679	2.7589 ± 0.0073
			0.332	2.7500	
			0.331	2.7589	
		Alif	0.203	3.9018	3.9107 ± 0.0073
			0.202	3.9107	
			0.201	3.9196	
		Knife	0.211	3.8304	3.8452 ± 0.0111
			0.208	3.8571	
			0.209	3.8482	
2	Olive Oil	Cesar	0.323	2.8304	2.8620 ± 0.0281
			0.320	2.8571	
			0.316	2.8986	
		Borges	0.314	2.9107	2.8869 ± 0.0183
			0.317	2.8839	
			0.319	2.8661	
3	Canola Oil	Family Choice	0.419	1.9732	1.9851 ± 0.0084
			0.417	1.9911	
			0.417	1.9911	
		Naturel	0.421	1.9554	1.9732 ± 0.0146
			0.419	1.9732	
			0.417	1.9911	
4	Sunflower Oil	Everest	0.309	2.9554	2.9554 ± 0.000
			0.309	2.9554	
			0.309	2.9554	
		Naturel	0.311	2.9375	2.6250 ± 0.4674
			0.307	2.9732	
			0.308	1.9643	
5	Soy Bean Oil	Olife	0.442	1.7679	1.7827 ± 0.0111
			0.439	1.7946	
			0.440	1.7857	

		Family Choice	0.442	1.7679	1.7738 ± 0.0223
			0.444	1.7500	
			0.438	1.8036	
6	Corn Oil	Daisy	0.174	4.1607	4.1637 ± 0.0042
			0.174	4.1607	
			0.173	4.1696	
		Mazola	0.181	4.0982	4.0923 ± 0.0042
			0.182	4.0893	
			0.182	4.0893	
7	Gingerly Oil	MP Lingams & Sons	0.468	1.5357	1.5416 ± 0.0042
			0.467	1.5446	
			0.467	1.5446	
		Baba's	0.459	1.6161	1.6042 ± 0.0084
			0.461	1.5982	
			0.461	1.5982	
8	Rice Bran	Green Love	0.411	2.0446	2.0565 ± 0.0084
			0.409	2.0625	
			0.409	2.0625	
9	Mixed Oil	Smart Balance (Soy, Palm and Canola)	0.448	1.7143	1.7143 ± 0.0000
			0.448	1.7143	
			0.448	1.7143	
		Naturel (canola & sunflower)	0.451	1.6875	1.7024 ± 0.0111
			0.449	1.7054	
			0.448	1.7143	
10	Ayurvedic Oil		0.413	2.0268	2.0387 ± 0.0084
			0.411	2.0446	
			0.411	2.0446	

Graph 4: Antioxidant Content of Oils at 120 ^{o}C



The table 7 and graph 4 indicates antioxidant content of edible oils at the temperature of 120 ^{o}C. The table 4 shows the samples prepared in triplicate and the average concentrations obtained from calibration curve. The bar chart indicates the oil antioxidant content based on types of oil and the brand of oil.

Subsequently the graph 4 shows Daisy and Mazola corn oil has the highest antioxidant content with 4.1637 ± 0.0042 mM and 4.0923 ± 0.0042 mM respectively followed by 2 types of palm oil that is Alif and Knife with the value of 3.9107 ± 0.0073 mM and 3.8452 ± 0.0111 mM.

Palm oil (Tiga Udang & Giant), Olive oil (Cesar & Borges), Canola oil (Family Choice & Naturel), Sunflower oil (Everest & Naturel), Soy Bean oil (Olife & Family Choice), Gingerly oil (Baba's), Rice Bran oil (Green Love), Mixed oil (Smart Balance & Naturel) and Ayurvedic oil are having antioxidant content between 2.9554 ± 0.0000 mM and 1.6042 ± 0.0084 mM.

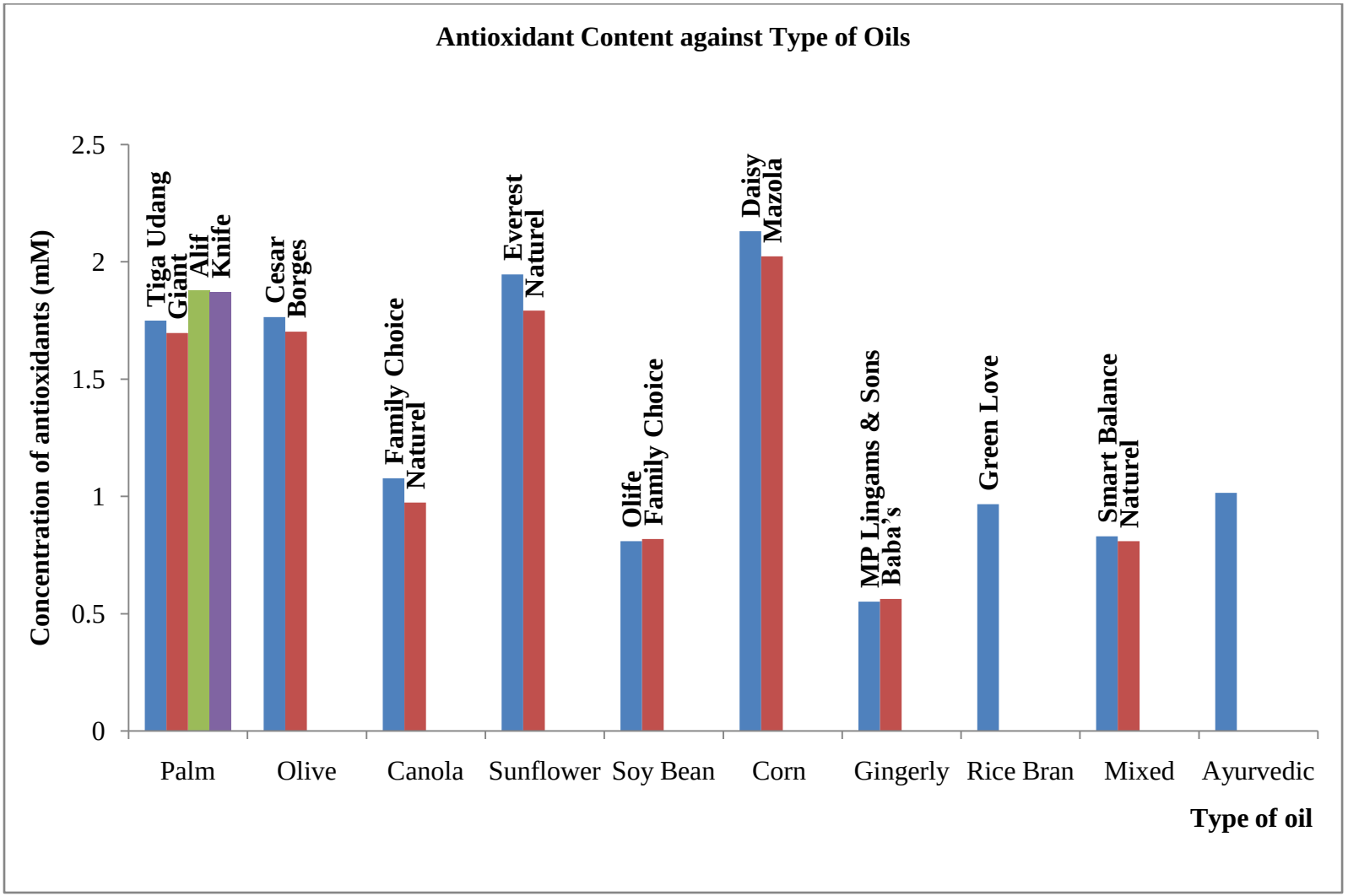
Thus, MP Lingams & Sons gingerly oil content lowest antioxidant with the value of 1.5416 ± 0.0042 mM.

Table 8: Antioxidant Content of Oils at 180 ^{o}C

No	Type of oil	Brand	Absorbance at 517 nm	Concentration of Antioxidants (mM)	Average Concentration of Antioxidants (mM)
1	Palm Oil	Tiga udang	0.442	1.7679	1.7500 ± 0.0146
			0.444	1.7500	
			0.446	1.7321	
		Giant	0.451	1.6875	1.6964 ± 0.0073
			0.449	1.7054	
			0.450	1.6964	
		Alif	0.428	1.8929	1.8780 ± 0.0111
			0.431	1.8661	
			0.430	1.8750	
		Knife	0.430	1.8750	1.8720 ± 0.0042
			0.431	1.8661	
			0.430	1.8750	
2	Olive Oil	Cesar	0.441	1.7768	1.7649 ± 0.0111
			0.442	1.7679	
			0.444	1.7500	
		Borges	0.451	1.6875	1.7024 ± 0.0111
			0.448	1.7143	
			0.449	1.7054	
3	Canola Oil	Family Choice	0.521	1.0625	1.0774 ± 0.0111
			0.519	1.0804	
			0.518	1.0893	
		Naturel	0.532	0.9643	0.9732 ± 0.0073
			0.531	0.9732	
			0.530	0.9821	
4	Sunflower Oil	Everest	0.423	1.9375	1.9464 ± 0.0073
			0.421	1.9554	
			0.422	1.9464	
		Naturel	0.438	1.8036	1.7917 ± 0.0111
			0.439	1.7946	
			0.441	1.7768	
5	Soy Bean Oil	Olife	0.551	0.7946	0.8095 ± 0.0111
			0.549	0.8125	
			0.548	0.8214	

		Family Choice	0.548	0.8214	0.8184 ± 0.0042
			0.548	0.8214	
			0.549	0.8125	
6	Corn Oil	Daisy	0.401	2.1339	2.1309 ± 0.0042
			0.401	2.1339	
			0.402	2.1250	
		Mazola	0.411	2.0446	2.0238 ± 0.0152
			0.415	2.0089	
			0.414	2.0179	
7	Gingerly Oil	MP Lingams & Sons	0.578	0.5536	0.5517 ± 0.0052
			0.581	0.5568	
			0.579	0.5446	
		Baba's	0.579	0.5446	0.5625 ± 0.0146
			0.577	0.5625	
			0.575	0.5804	
8	Rice Bran	Green Love	0.532	0.9643	0.9673 ± 0.0042
			0.531	0.9732	
			0.532	0.9643	
9	Mixed Oil	Smart Balance (Soy, Palm and Canola)	0.548	0.8214	0.8304 ± 0.0073
			0.547	0.8304	
			0.546	0.8393	
		Naturel (canola & sunflower)	0.551	0.7946	0.8095 ± 0.0111
			0.549	0.8125	
			0.548	0.8214	
10	Ayurvedic Oil		0.528	1.0000	1.0148 ± 0.0112
			0.526	1.0176	
			0.525	1.0269	

Graph 5: Antioxidant Content of Oils at 180 ^{o}C



The table 8 and graph 5 indicates the antioxidant content of edible oils at the temperature of 180 ^{o}C. Accordingly the table 5 shows the samples prepared in triplicate and the average concentrations were obtained from calibration curve.

The graph 5 indicates the antioxidant content of oils based on the types of oil and the brand of oil. In addition the bar chart also shows Daisy and Mazola corn oil has the highest antioxidant content with 2.1309 ± 0.0042 mM and 2.0238 ± 0.0152 mM respectively followed by 2 types of palm oil that is Alif and Knife with the value of 1.8780 ± 0.0111 mM and 1.8720 ± 0.0042 mM.

Thus, Palm oil (Tiga Udang & Giant), Olive oil (Cesar & Borges), Canola oil (Family Choice & Naturel), Sunflower oil (Everest & Naturel), Soy Bean oil (Olife & Family Choice), Gingerly oil (Baba's), Rice Bran oil (Green Love), Mixed oil (Smart Balance & Naturel) and Ayurvedic oil contain antioxidant between 0.5625 ± 0.0146 mM and 1.7917 ± 0.0111 mM.

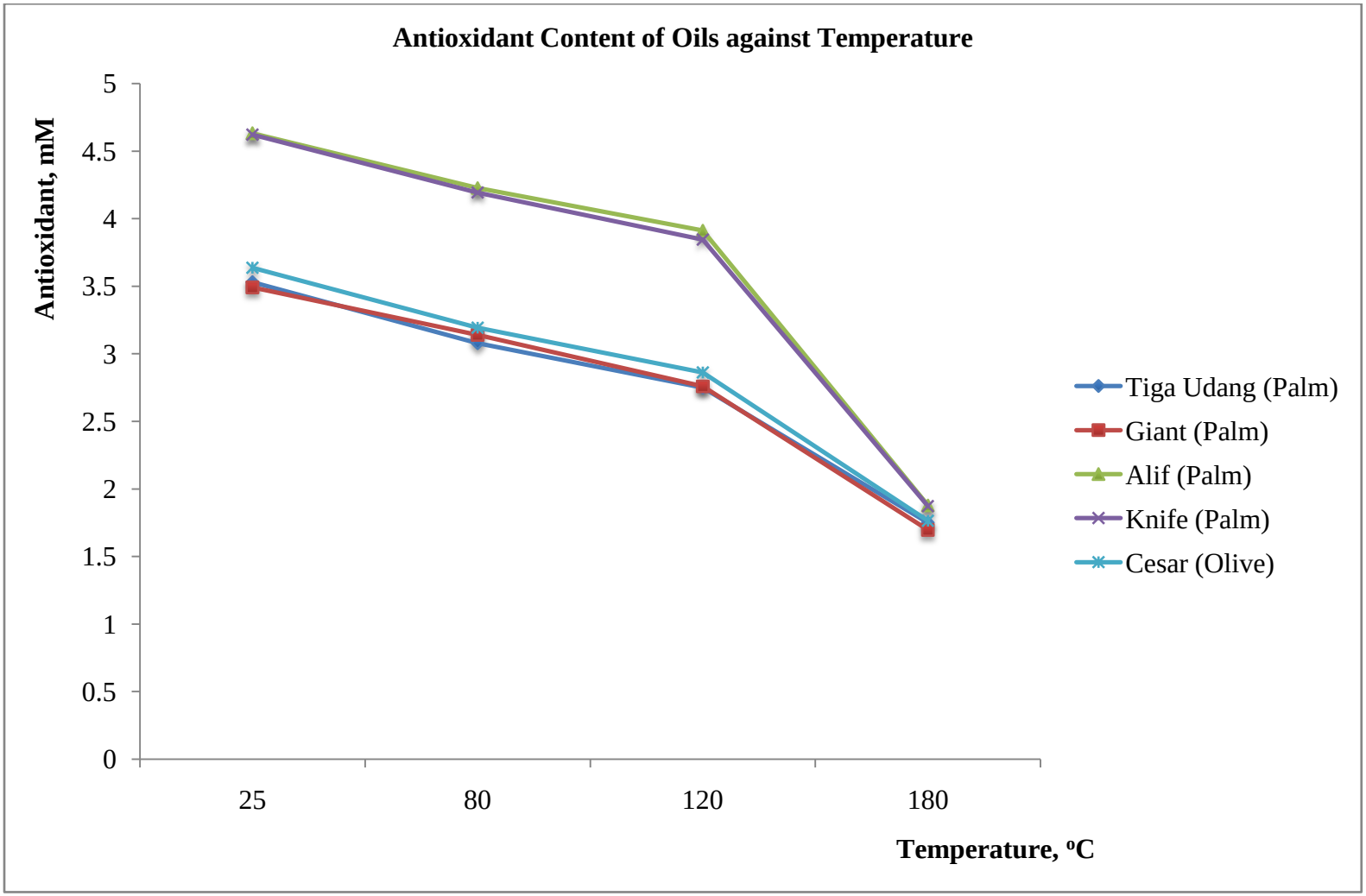
In additional the lowest antioxidant content found in MP Lingams & Sons gingerly oil with the value of 0.5517 ± 0.0052 mM.

Table 9: Comparison of Antioxidant Content of Oils at 25 ^{o}C, 80 ^{o}C, 120 ^{o}C and 180 ^{o}C

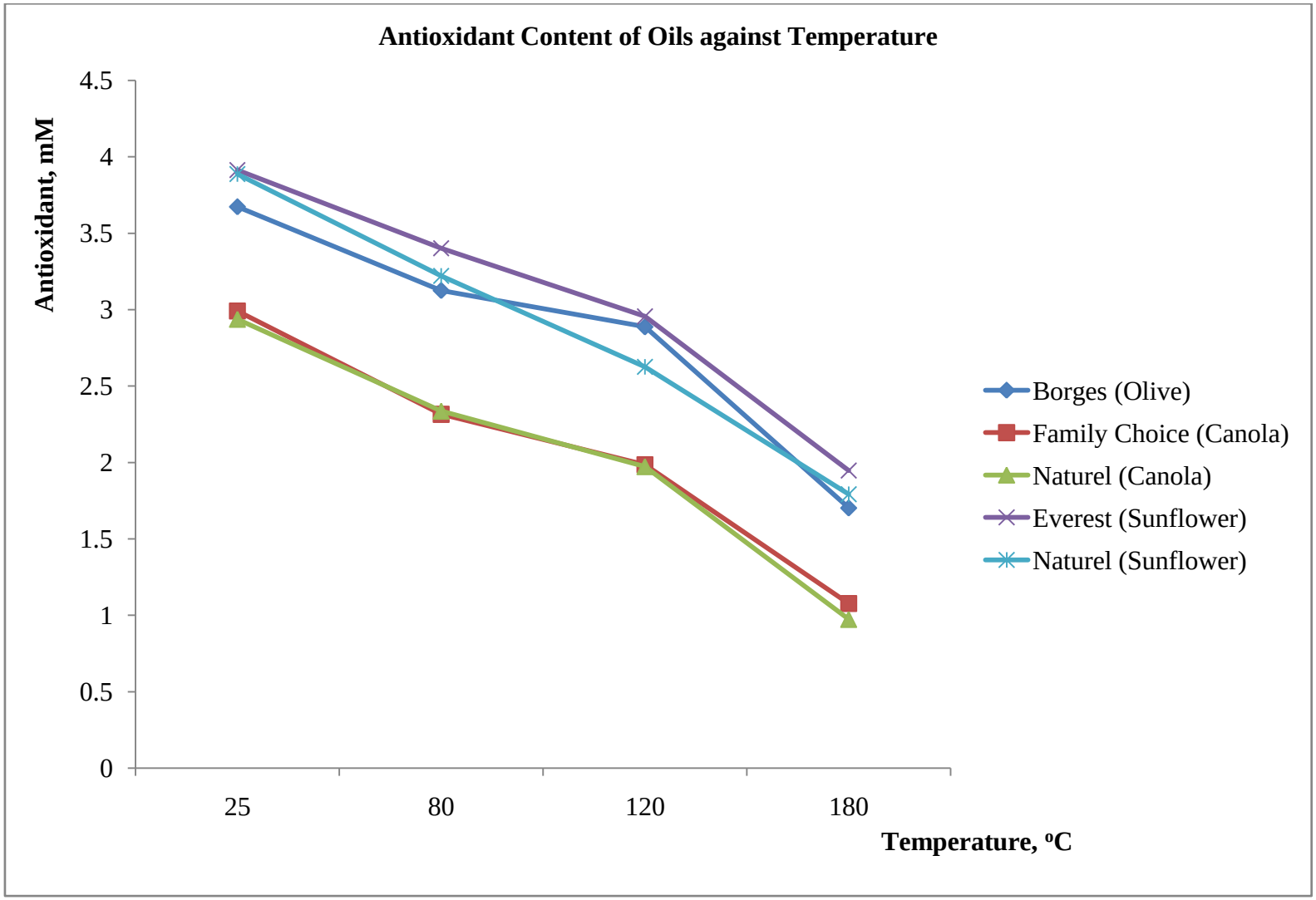
No	Type of oil	Brand	Antioxidant content at 25 ^{o}C	Antioxidant content at 80 ^{o}C	Antioxidant content at 120 ^{o}C	Antioxidant content at 180 ^{o}C
1	Palm Oil	Tiga udang	3.5298 ± 0.0184	3.0774 ± 0.0183	2.7500 ± 0.0000	1.7500 ± 0.0146
		Giant	3.4911 ± 0.0073	3.1399 ± 0.0084	2.7589 ± 0.0073	1.6964 ± 0.0073
		Alif	4.631 ± 0.0111	4.2262 ± 0.0111	3.9107 ± 0.0073	1.8780 ± 0.0111
		Knife	4.622 ± 0.0084	4.1934 ± 0.0042	3.8452 ± 0.0111	1.8720 ± 0.0042
2	Olive Oil	Cesar	3.6369 ± 0.0042	3.1934 ± 0.0112	2.8620 ± 0.0281	1.7649 ± 0.0111
		Borges	3.6726 ± 0.0152	3.1250 ± 0.0073	2.8869 ± 0.0183	1.7024 ± 0.0111
3	Canola Oil	Family Choise	2.9911 ± 0.0146	2.3155 ± 0.0111	1.9851 ± 0.0084	1.0774 ± 0.0111
		Naturel	2.9375 ± 0.0000	2.3363 ± 0.0042	1.9732 ± 0.0146	0.9732 ± 0.0073
4	Sunflower Oil	Everest	3.9137 ± 0.0042	3.4018 ± 0.0073	2.9554 ± 0.0000	1.9464 ± 0.0073
		Naturel	3.8869 ± 0.0042	3.2202 ± 0.0111	2.6250 ± 0.4674	1.7917 ± 0.0111

5	Soy Bean Oil	Olife	2.6905 ± 0.0042	2.2113 ± 0.0042	1.7827 ± 0.0111	0.8095 ± 0.0111
		Family Choise	2.7411 ± 0.0073	2.1875 ± 0.0000	1.7738 ± 0.0223	0.8184 ± 0.0042
6	Corn Oil	Daisy	4.8095 ± 0.0042	4.5714 ± 0.0073	4.1637 ± 0.0042	2.1309 ± 0.0042
		Mazola	4.7857 ± 0.0193	4.5298 ± 0.0042	4.0923 ± 0.0042	2.0238 ± 0.0152
7	Gingerly Oil	MP Lingams & Sons	2.4405 ± 0.0111	1.9196 ± 0.0073	1.5416 ± 0.0042	0.5517 ± 0.0152
		Baba's	2.5060 ± 0.0111	1.9613 ± 0.0152	1.6042 ± 0.0084	0.5625 ± 0.0146
8	Rice Bran	Green Love	2.7083 ± 0.0111	2.4226 ± 0.0152	2.0565 ± 0.0084	0.9673 ± 0.0042
9	Mixed Oil	Smart Balance (Soy, Palm and Canola)	2.4702 ± 0.0111	2.1399 ± 0.0152	1.7143 ± 0.0000	0.8304 ± 0.0073
		Naturel (canola & sunflower)	2.2857 ± 0.0126	2.0446 ± 0.0073	1.7024 ± 0.0111	0.8095 ± 0.0111
10	Ayurvedic Oil		3.1696 ± 0.0292	2.4167 ± 0.0111	2.0387 ± 0.0084	1.0148 ± 0.0112

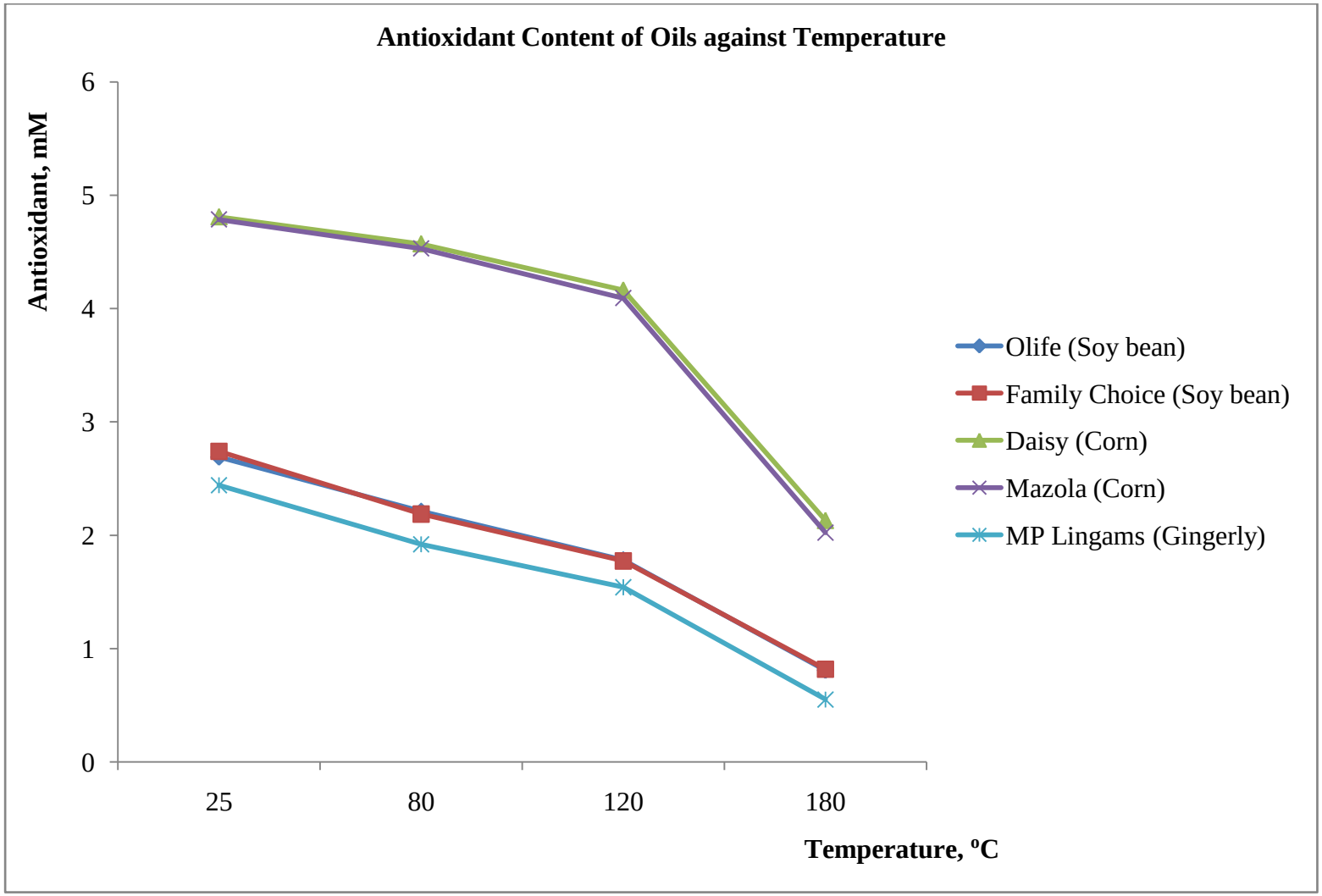
Graph 6: Antioxidant Content of Palm and Olive Oils at 25°C, 80°C, 120°C and 180°C



Graph 7: Antioxidant Content of Olive, Canola and Sunflower Oils at 25 ^{o}C, 80 ^{o}C, 120 ^{o}C and 180 ^{o}C.



Graph 8: Antioxidant Content of Soy Bean, Corn and Gingerly Oils at 25 ^{o}C, 80 ^{o}C, 120 ^{o}C and 180 ^{o}C.



Graph 9: Antioxidant Content of Gingerly, Rice Bran, Mixed and Ayurvedic Oils at 25°C, 80°C, 120°C and 180°C.

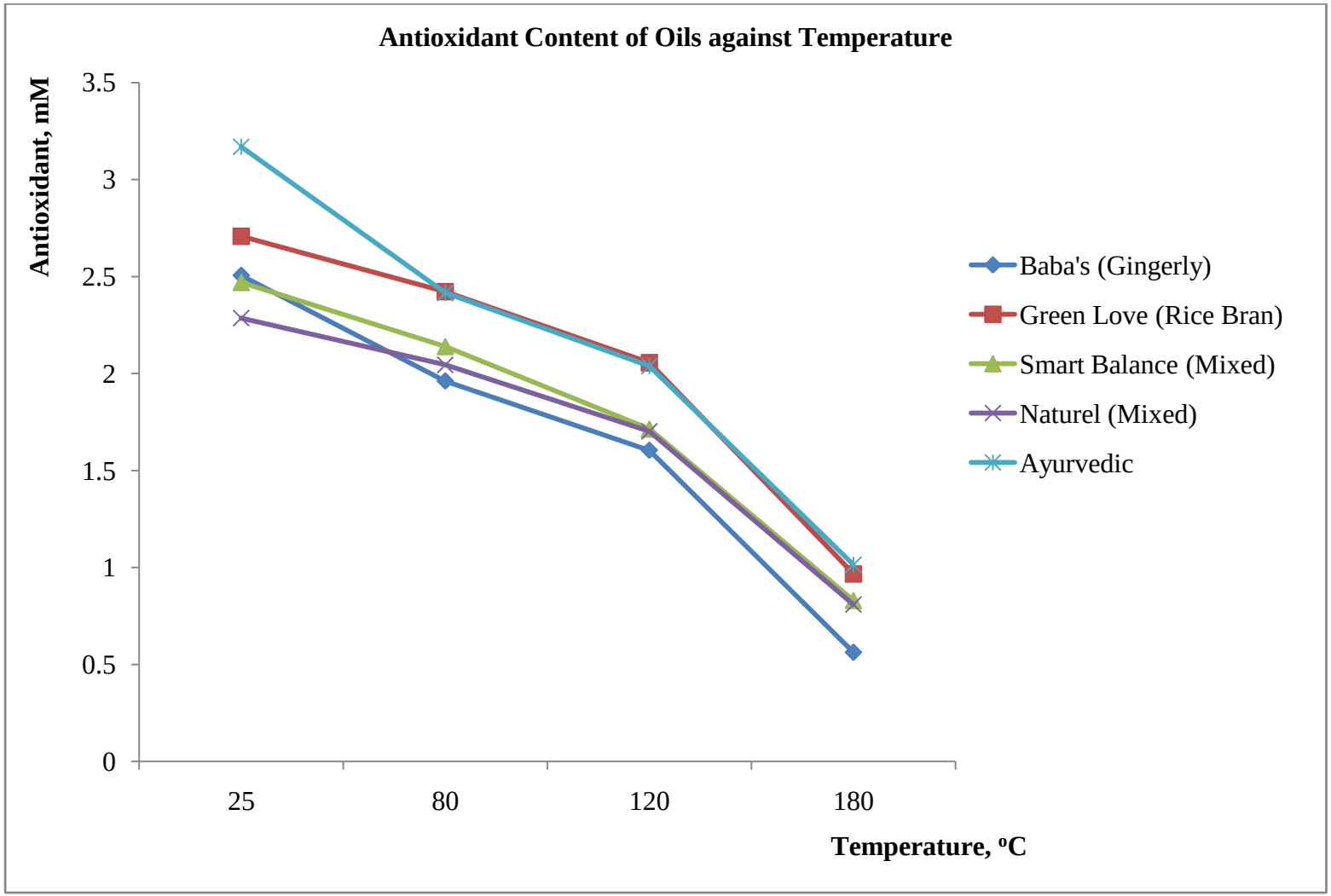


Table 7 shows the comparison of antioxidant content of edible oils at the temperature of 25 ^{o}C, 80 ^{o}C, 120 ^{o}C and 180 ^{o}C. Graph 6, 7, 8 and 9 indicate the trend of antioxidant content when the edible oils samples subjected to various temperature of incubation. The temperature of 25 ^{o}C was chosen in this experiment to identify the antioxidant content at the room temperature which is also the common storage temperature. The highest incubation temperature chosen is 180 ^{o}C because it is slightly below the smoke point of edible oils.

Table 7 shows the antioxidant content of all type of edible oils is at the highest at the temperature of 25 ^{o}C and the least at the temperature of 180 ^{o}C. Graph 6, 7, 8 and 9 show the decreasing trend of antioxidant content in the edible oils when the samples incubated at higher temperature. Mazola brand of corn oil show the most significant drop in antioxidant content up to 2.7619 mM. This is followed by Alif brand palm oil with decrease about 2.753 mM. Naturel brand Mixed oil show the least drop in the antioxidant content which is about 1.4762 mM.

The same trend has been observed in many other researches. Free radicals are known to be responsible for the oxidation of food components, resulting in alterations of the major quality-control parameters, such as color, flavor, aroma and nutritional value of foodstuffs (Donelli et al., 1995). Excessive free radical formation, contributing to the onset of certain pathologies, may demand a high dietary intake of fruits, which are rich in antioxidant vitamins and phenolics (Owen et al., 2000). That is a good reason to assess the amounts of these compounds in dietary oils and how different technological processes, such as frying, affect their availability. There are numerous studies that report changes in fats and oils after heating or frying procedures (Che Man et al., 2000). Most of them concluded that such changes depend on the temperature, the heating cycles, the surface/ volume and food/oil ratios, the fatty acid and the antioxidant composition of the oils (Melton et a., 1994). All chemical changes of fats and oils and their natural contaminants at elevated temperatures originate in

oxidation, hydrolysis, polymerisation, isomerisation or cyclisation reactions. All these reactions may be promoted by oxygen, moisture, traces of metal and free radicals (Gertz, 1996). These processes may reduce the amount of antioxidants in the oil, decrease its stability and produce new products which are responsible for loss of the nutritional value and quality of the oil (odour, flavour, absorption).

CHAPTER 4

CONCLUSION

It can be concluded from the results of the analysis of antioxidants content varied depending on the type of oils. Corn oil contains the highest amount of antioxidant followed by high grade palm oil, sunflower oil, olive oil, low grade palm oil, ayurvedic oil, canola oil, soy bean oil, rice bran oil, gingerly oil and mixed oil. Various brands of oil were chosen for each type of oil and the analysis indicates the antioxidant content of the samples depending only on the oil type not on the brand.

The characteristic of the DPPH methods are the determination of the initial concentration of inherent hydrogen donating antioxidants and the free radical formation rate from oxidized oils, which cannot be provided by other conventional methods. Small sampling size, relatively simple steps of sample preparation, and the easy quantitative comparisons among different sources are other features of DPPH method. However, there are some limitations in DPPH method. DPPH method is valid for the prediction of oxidative stability of fresh oils because highly oxidized oils cannot be differentiated from unoxidized samples.

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