‘ULAM’:
CONSUMPTION AMONGST KELANTANESE MALAY FROM SELECTED DISTRICTS AND THEIR ANTIOXIDANT PROPERTIES

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by

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ABBREVIATIONS

$ABTS^{\bullet+}$	2,2'- azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
BCB	ß-Carotene Bleaching
BHA	Butylated Hydroxyl Anisole
BHT	Butylated Hydroxyl Toluene
CAP-e	Cell-Based Antioxidant Protection In An Erythrocyte
CAT	Catalase
CDC	Centers for Disease Control and Prevention
$CoQH_2$	Coenzyme Q
DMSO	Dimethyl sulfoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
FDA	Food and Drug Administration
FRAP	Ferric Reducing Antioxidant Power
FTC	Ferric Thiocyanate
GPx	Gluthation Peroxidase
HCl	Hydrogen Chloride
HCN	Hydrogen Cyanide
HNO_2	Nitrous Acid
H_2O_2	Hydrogen Peroxide
$HO_2^{\bullet}$	Hydroperoxyl
HOCl	Hypochlorous acid
NO_2^+	Nitronium cation
N_2O_3	Dinitrogen trioxide
N_2O_4	Dinitrogen tetroxide
$ONOO^-$	Peroxynitrite

ONOOH	Peroxynitrous acid
$^1O_2$	Singlet oxygen
O_2	Superoxide
O_3	Oxone
OD	Optical Density
$OH^{\bullet}$	Hydroxyl
ORAC	Oxygen Radical Absorption Capacity
PBS	Phosphate Buffer Saline
PUFA	polyunsaturated fatty acid
RNS	Reactive Nitrogen Species
ROONO	Alkyl peroxynitrites
$RO^{\bullet}$	Alkoxyl
$RO_2^{\bullet}$	Peroxyl
ROOH	Lipid Hydroperoxide
ROS	Reactive Oxygen Species
TBARS	Thiobarbituric Acid-Reactive-Substances
TEAC	Trolox Equivalent Antioxidant Capacity
TRAP	Total Radical Trapping Antioxidant Parameter
SCN	Standing Committee on Nutrition
SOD	Superoxide Dismutase
USM	Universiti Sains Malaysia
UV	Ultraviolet
WHO	World Health Organization

ULAM: PENGAMBILAN OLEH ORANG MELAYU KELANTAN DARI DAERAH TERPILIH DAN KESAN ANTIOKSIDANNYA

ABSTRAK

Gaya pemakanan memberi impak yang besar kepada kehidupan berkualiti dan menggambarkan keadaan kesihatan seseorang. Pepatah mengatakan ‘kita adalah apa yang kita makan’. Kajian ini dijalankan bertujuan menilai pengambilan ulam-ulaman oleh orang Melayu Kelantan yang senonim dengan gaya masakan Kelantan yang terkenal seperti nasi kerabu dan nasi ulam. Seramai 168 orang peserta iaitu 67 orang lelaki dan 101 orang perempuan telah bersetuju mengambil bahagian dalam soal selidik ini. Mereka terdiri daripada penduduk di daerah Batu Mengkebang, Salor dan Tawang yang berumur di antara 18 tahun dan keatas. Hasil kajian mendapati 32.8% daripada peserta lelaki and 32.7% daripada peserta perempuan mengambil ulam setiap hari. Manakala peserta berumur 40-59 tahun adalah penyumbang terbesar bagi peratusan individu yang memakan ulam. Kajian mendapati terdapat kaitan di antara pendapatan dan pengambilan ulam bagi setiap daerah. Kajian ini juga menunjukkan 83.6% peserta lelaki dan 74.3% peserta perempuan menggabungkan pelbagai jenis ulam semasa pengambilannya iaitu kebanyakan mereka mengambil 2 jenis ulam semasa makan. Kebanyakan peserta bersetuju bahawa ulam boleh meningkatkan selera makan dan selamat di makan. Topografi kawasan dan kebolehperolehan setiap ulam berkemungkinan mempengaruhi pengambilan jenis ulam di setiap daerah. Kesimpulannya, terdapat persamaan di antara gender dalam pengambilan ulam. Kajian ini di harap dapat memberi pengetahuan mengenai pengambilan dan kebaikan pelbagai ulam yang di makan, terutamanya ulam yang terdiri daripada tumbuhan liar di Kelantan.

Ekstrak metanolik 10 jenis ulam telah dikaji dari aspek aktiviti antioksidannya. Aktiviti antioksidan ekstrak metanolik *Luffa acutangula* (Petola segi), *Oroxylum indicum* (Buah beko), Jenerih (tiada nama saintifik lagi), *Leucaena leucocephala* (Petai belalang), *Emilia Sonchifolia* (Bayam peraksi), *Acrostichum aureum* (Piai), *Garcinia xanthochymus* (Asam kandis), *Syzygium inophylla* (Gelam tikus), *Curcuma longa* (Bunga kunyit) dan *Moringa Oleifera* (Merungai) telah di saring dengan menggunakan kaedah pemerangkapan radikal DPPH (0.5mg/mL) dan kaedah ferric thiocyanate (1mg/mL). Daripada 10 jenis ulam, ulam seperti *Syzygium inophylla*, *Curcuma longa*, *Emilia Sonchifolia*, *Moringa Oleifera* and *Oroxylum indicum* telah menunjukkan tinggi aktiviti antioksidannya dalam kedua-dua kaedah tersebut (>90%). Kesimpulannya, hasil penyelidikan ini boleh membantu menyumbang kepada pembangunan diet kawalan atau pembikinan neutraceutikal untuk penyakit-penyakit kronik. Kajian ini telah membantu meningkatkan jumlah maklumat tentang ulaman yang digunakan oleh rakyat Malaysia dimana ia dapat membantu dalam pengenalan sumber antioksidan semulajadi yang efektif.

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‘ULAMS’: CONSUMPTION AMONGST KELANTANESE MALAY FROM SELECTED DISTRICTS AND THEIR ANTIOXIDANT PROPERTIES

ABSTRACT

Eating styles have been shown to exert a major impact on the quality of life and this can be shown in the health status of the individuals. So the saying ‘We are what we eat’. The present study was carried out to evaluate the consumption of ‘ulam’ amongst Kelantanese Malay who were synonyms with the famous Kelantanese cuisine of nasi kerabu and nasi ulam. 168 participants, 67 male and 101 female had agreed to participate in this survey. They were residents from the districts of Batu Mengkebang, Salor and Tawang, aged 18 years old and above. The result showed 32.8% of male and 32.7% of female participants took ‘ulam’ daily. Meanwhile, participants who aged 40 – 59 years old contributed the major percent in practicing ‘ulam’ intake daily. The study shows the relationship between income and ‘ulam’ intake in each district. The result also revealed that 83.6% of male and 74.3% of female participants combined various type of ‘ulams’ at one sitting. Most of them took two type of ‘ulams’ during meal time. Majority of participants agreed that ‘ulams’ can increase appetite and are safe to consume. Topography of the area of study and availability of ‘ulams’ in the vicinity possibly influenced the type of ‘ulam’ consumed in each district. In conclusion, there was similarity between the genders in practice of ‘ulam’ intake. It is hoped that this study will provide relevant information pertaining to the consumption and properties of various ‘ulams’ that are being consume in society, especially those ‘ulams’ that are edible wild plants available in Kelantan.

Methanolic extract of 10 ‘ulams’ were investigated for their antioxidants properties. Antioxidant activity of the methanolic extracts of *Luffa acutangula* (Petola segi), *Oroxylum indicum* (Buah beko), Jenerih (no scientific name available as yet), *Leucaena leucocephala* (Petai belalang), *Emilia Sonchifolia* (Bayam peraksi), *Acrostichum aureum* (Piai), *Garcinia xanthochymus* (Asam kandis), *Syzygium inophylla* (Gelam tikus), *Curcuma longa* (Bunga kunyit) and *Moringa Oleifera* (Merungai) were screened by using DPPH radical Scavenging (0.5mg mL^{-1}) and Ferric thiocyanate assay (1mg mL^{-1}). Out of the ten ‘ulams’, ‘ulams’ viz of *Syzygium inophylla*, *Curcuma longa*, *Emilia Sonchifolia*, *Moringa Oleifera* and *Oroxylum indicum* showed significantly higher in antioxidant activity (> 90%) detected by both assay. In conclusion, the outcome of the study may help in contribution towards the development of dietary control or production of neutraceutical for chronic diseases. This study assisted in increasing the available information regarding ‘ulams’ that used among Malaysian and it can also help in identification of potent sources of natural antioxidant.

THE STUCTURE OF THESIS

This thesis is divided into two parts. Part one consist of contents of the questionnaire pertaining ‘ulam’ consumption among Kelantanese Malay. This thesis also provided demographic data of participants which influence the practice of ‘ulam’ intake in Kelantan. An attempt is also made to identify the practice of ‘ulam’ consumption amongst Kelantanese Malay and include the frequency score of ‘ulam’ intake amongst Kelantanese. The frequency of ‘ulams’ eaten was calculated according to popularity of those ‘ulams and the list of ‘ulam taken by the Kelantanese are also made. The result will be shared via publication of the information gathered.

Meanwhile, part two of the thesis involves an experimental study. 10 ‘ulams’ were selected for antioxidant assay. The basis of selection is really random with some elements of non-studied items being given priority. Two assays which were DPPH radical scavenging and Ferric Thiocyanate Assays were used. The comparison between the selected ‘ulams’ and standard antioxidants BHT and Ascorbic acid were also done to identify the strongest antioxidants among these ‘ulams’. The information gather in this thesis can help in the identification of natural antioxidants from plants which are comparable to the synthetic antioxidants.

CHAPTER ONE

INTRODUCTION

1.1 Plant as medicine

The use of natural products as sources of drugs is as ancient as the human civilization (De Pasquale, 1984; Rates, 2001). These natural products include materials such as mineral, plants, and animals that have been shown some therapeutic properties. Despite major scientific and technological progress in combinatorial chemistry and other modern analytical methodologies, drugs derived from natural product still make up an enormous contribution to drug discovery today (Rocha *et al.*, 2001). In fact, about 25% of drugs prescribed worldwide come from plants (Elliot, 1986; Rates, 2001). Furthermore, World Health Organization (WHO) estimates that about 80% of world's population still depends on medicinal plants as a source of health care (Malaysia Timber Council, 2004). Socioeconomic factors such as poverty and lack of access to modern medicine among developing countries are some of the factors which may also contribute to the above mentioned estimate (Calixto, 2005).

Modern approaches in the attempt for new drug discovery from medicinal plants utilize either preparation of plants or substances from plants (also known as natural product) that is believed to have potential value as pharmaceutical agent (Balunas and Kinghorn, 2005; McChesney *et al*., 2007). According to Li *et al.* (2008), medicinal plants have been used to treat human diseases in the East for centuries. In recent years, there has been a growing interest amongst the public

towards alternative therapies and the therapeutic use of natural products, especially those derived from plants (Rates, 2001).

Malaysia is one of the greatest bio-diverse countries with a high index of plants and animals believed to be of medicinal potentials (Mohsin, pers. Comm., 2005). According to Burkill, (1935) Malaysia has about 12,000 species of flowering plants of which about 1,300 plants are used in traditional medicine. In the past five decades, medicinal plant research in Malaysia has been carried out mainly by researchers from government- funded universities and research institutes with little involvement of industries and multinationals (Ibrahim, 2005).

There are some scientific evidences developed over the last couple of decades which show the bioactivities of various plants species in Malaysia. These include some of the natural resources which are used in the traditional medicine among the Malays. They contain unique organic moieties that have been shown to have therapeutic properties. However, the practice of using plants for medicinal purpose by the Malays is not based on knowledge about the therapeutic properties but rather on the facts that these plant materials were thought to be beneficial for their health based on information derived from and passed down from generation to generation (Muhamad and Mustafa Ali, 1992). Even though certain plants have now been shown not to have therapeutic properties and that the utilization of these plants was based on indigenous theories and beliefs among the Malays, they however show some benefit in the maintenance of health. This is probably because of the lack of scientific insight to explain and predict the curative action of the plants. Meanwhile in some countries the use of medicinal plants is often associated with witchcraft and

superstition. Sometimes it is based on the assumption that the appearance of plants may give clues to their medicinal properties (Gurib-Fakim, 2006). Generally, the specific plants to be used and the methods of application for particular ailments were passed down verbally from generations to generations (Balunas and Kinghorn, 2005) but enormous amount of knowledge about these plants has remained undocumented.

1.2 Phytochemicals in Plants

The term ‘phyto’ of the word phytochemicals is derived from the Greek root word meaning plant. Therefore, phytochemicals are referred to plant chemicals (Oliveri, 2003; Liu, 2004). These phytochemicals are defined as bioactive nonnutrients plant compounds in fruits, vegetables, grains, and other plant parts that are linked to reducing the risk of major chronic diseases (Liu, 2004). It is estimated that more than 4000 of these compounds have been discovered to date and it is expected that scientists will discover many more (The Caribbean Food and Nutrition Institute, 2005). The above report further mentioned that any one serving of vegetables could provide as many as 100 different phytochemicals.

Plants show biological and chemical diversity as they synthesize various chemicals as defense agents against pests, diseases, and predators (Ibrahim, 2005; Rocha *et al.*, 2001). In order to survive, plants have developed sophisticated mechanisms including an elaborate chemical arsenal of toxic substances, such as terpenoids and alkaloids that inhibit the growth of other organisms or make them unattractive to predators (Rocha *et al.*, 2001). Plants also produced various antioxidant compounds like terpenoids, steroids and phenolic compounds such as

tannins, coumarins and flavonoids (Srinivasan *et al.*, 2007) as part of their natural defense mechanism in order to survive and to counteract the oxidative damage caused by Reactive Oxygen Species (ROS) (Shon *et al*., 2003; Kumaran and Karunakaran, 2007; Ganesan *et al.*, 2007). In the tropic, plants are exposed to long periods of intense sunlight, thus they absorb the sun's radiation and generate high levels of oxygen as secondary product of photosynthesis. This oxygen is a free radical agent and is easily initiated by Ultraviolet and heat from sunlight to produce toxic ROS (Vimala *et al*., 2003).

Wang *et al*., (1999) and Huda-Faujan *et al*., (2007) elucidated that the antioxidant properties of plants are due to presence of low molecular weight of phenolic compounds, which are secondary metabolites derived from pentose phosphate, shikimate and phenylpropanoid pathways in plants (Randhir *et al*., 2004; Aberoumand and Deokule, 2008). Phenolic acid compounds are thought of as powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory action (Aberoumand and Deokule, 2008) as well as platelet aggregation inhibition activity (Asami, *et al*, 2003).

The chemical structure of these phenolic acid compounds comprise an aromatic ring, bearing one or more hydroxyl substituents and range from simple phenolic molecules to highly polymerased compounds or 'polyphenols' (Aberoumand and Deokule, 2008). Several polyphenols have been demonstrated to have clear antioxidant properties *in vitro* as they can act as chain breakers or radical scavengers (Masella *et al*., 2005). This property is also thought to help in reducing

the risk of chronic diseases such as cancers, cardiovascular diseases and age related-neuronal degeneration (Ames *et al*., 1993; Teow *et al*., 2007). These claims have also been supported by some studies. Extracts from natural products, such as fruits, vegetables and medicinal herbs all contain polyphenols. They have been shown to have positive effects against cancer, comparable to chemotherapy or recent hormonal treatments (Lee *et al.*, 2004). However, the quantity and quality of polyphenols present in plant foods can vary significantly due to different factors such as plant genetics and cultivar, soil composition and growing conditions, maturity state, and post harvest conditions (Faller and Fialho, 2009). Figure 1.2 (a) shows classification of dietary phytochemicals.

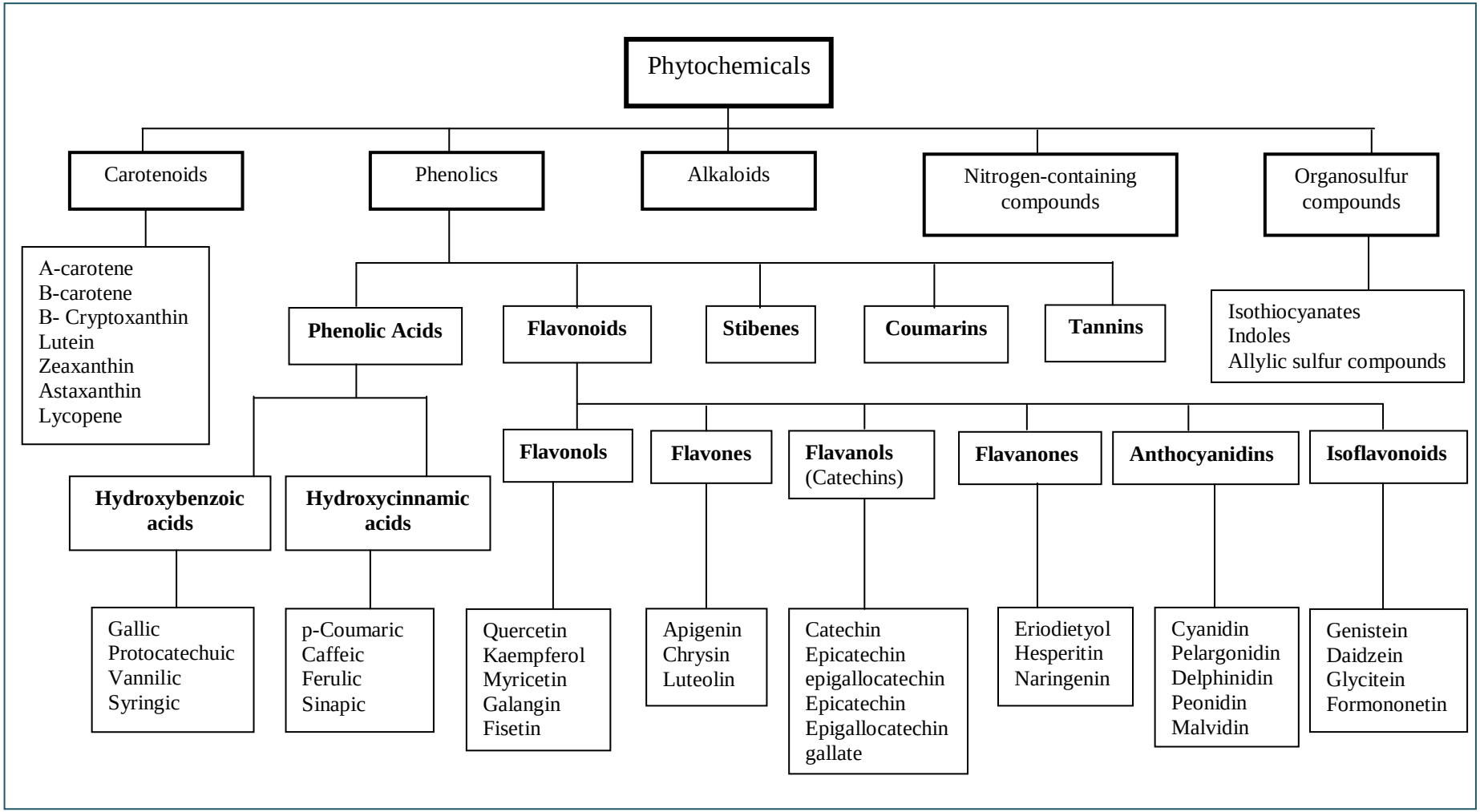


Figure 1.2(a): Classification of dietary phytochemicals (Liu, 2004).

1.3 Free Radical and Lipid Peroxidation

Free radical is defined as any species capable of independent existence that contains one or more unpaired electrons (Haliwell, 1994). There are two sources of free radicals that could cause cell damage in the body, the endogenous and exogenous sources. The endogenous sources are produced as a result of free radicals being produced inside the body via toxic byproducts of normal functions such as metabolism, biochemical reactions in cells, detoxification in the liver and energy generation by the mitochondria (Vimala *et al*., 2003). These free radicals are generally produced in biological systems in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Haliwell, 1996; Lee, 2004; Valko *et al*, 2007). Table 1 shows the list of radical and non-radical species produced in human body that can easily be reactive with other molecules.

Whereas, the exogenous sources are derived from smoking, dietary style, stressful lifestyle and radiation. These entities enhance the production of free radical in the body (Vimala *et al*., 2003). Meanwhile, plants also generate ROS by certain biochemical pathways such as the energetic electron transport reactions of photosynthesis in chloroplasts and of respiration in mitochondria. These organelles become major contributors of ROS in plants. Excited chrorophyl can yield singlet oxygen species when there are inadequate electron acceptors available (Close and Hagerman, 2006).

Table 1.3: The list of radical and non-radical species produced in human body

	Radical	***Non-Radical***
ROS	Superoxide $O_2^{\bullet -}$	Hydrogen Peroxide H_2O_2
	Hydroxyl $OH^{\bullet}$	Hypochlorous acid HOCl
	Peroxyl $RO_2^{\bullet}$	Oxone O_3
	Alkoxyl $RO^{\bullet}$	Singlet oxygen $^1O_2$
	Hydroperoxyl $HO_2^{\bullet}$	
RNS	Nitric Oxide $NO^{\bullet}$	Nitrous Acid HNO_2
	Nitrogen dioxide $NO_2^{\bullet}$	Dinitrogen tetroxide N_2O_4
		Dinitrogen trioxide N_2O_3
		Peroxynitrite, $ONOO^-$
		Peroxynitrous acid ONOOH
		Nitronium cation, NO_2^+
		Alkyl peroxynitrites, ROONO

(Halliwel, 1996).

As mentioned earlier, reactive oxygen species (ROS) are continuously being produced in our body because of oxygen consumption in some metabolic processes such as respiration and some cell-mediated immune functions (Ak & Gülçin, 2008). The major sites of oxygen consumption in the cell are peroxisomes that lead to the production of H_2O_2. ROS and RNS are well known to play important role in the body as deleterious as well as beneficial species, since they can be either harmful or beneficial to living systems (Valko, *et al*., 2006). Beneficial effects of ROS occur at low or moderate concentration which involve in cellular responses to noxia such as in defence against infectious agent and in the function of a number of cellular signaling systems. Whereas, the harmful effect of free radical include biological damage due to overproduction of free radicals in the body (Valko *et al*., 2007).

The overproduction of ROS and RNS can lead to a variety of diseases and conditions such as ageing, atherosclerosis, ischemic heart injury, cancer and other diseases by altering the protein, DNA, unsaturated fatty acid (lipids or fats) and cell membrane through lipid peroxidation process (Figure 1.3 shows the major step involved in lipid peroxidation). Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids (Senthil and Manoharan, 2004). Lipid peroxidation of membrane fatty acid leads to altered membrane fluidity, increased permeability, and altered membrane function due to damage to membrane components (Quindry and Powers, 2006). The condition of overproduction of ROS/RNS is called oxidative/nitrosative stress, whereby the body cannot neutralize the free radicals or repair the damage with endogenous antioxidant. This condition occurs because of the imbalance between antioxidant systems and the product of oxidants (Laquerre, *et al*., 2007).

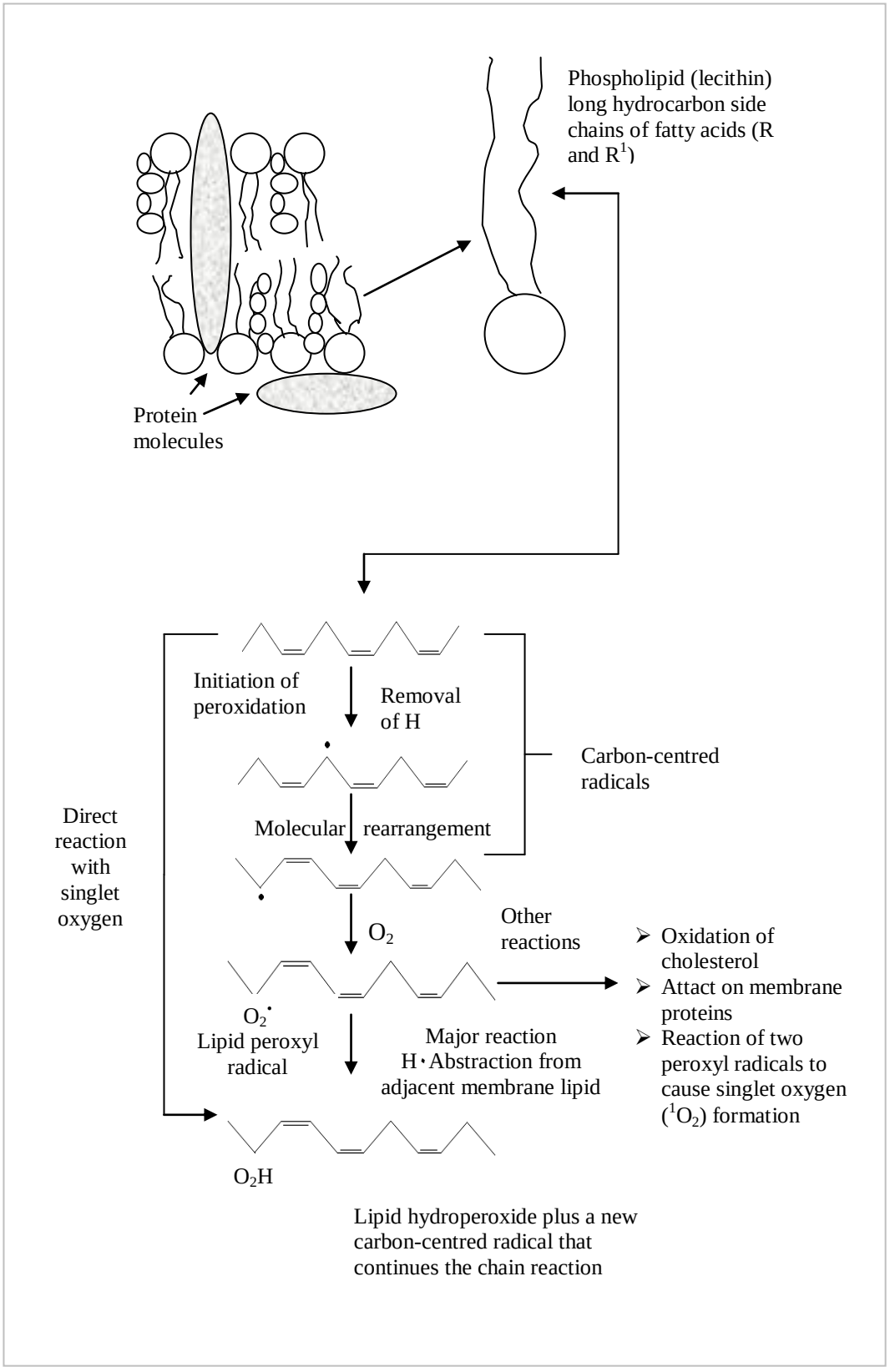


Figure 1.3: Lipid Peroxidation in cell membrane. Peroxidation of fatty-acid side chain with three double bonds is shown. Removal of an atom of hydrogen, the iniating event, can occur at several different places in the side chain. (Gutteridge and Halliwel, 1994).

Figure 1.3 shows the alteration of membrane cell lipid in the presence of free radical. Free radical reacts with non radical species to generate other free radical species. The lipid peroxidation is referred to as oxidation of lipid, whereby free radical steals electron from the lipid membrane in the cell. This process is a free radical chain reaction mechanism which involves three steps: initiation, propagation and termination (Valko, *et al*., 2006). The step below shows the principle of lipid peroxidation that can occur in cells.

Initiation

The initiator of lipid peroxidation process might be transition metal iron or copper, or oxidative intracellular enzyme such as lypoxygenase and myeloperoxidase or even free radical itself.

$$A\text{-}B \xrightarrow{\text{Initiator}} A\bullet + \bullet B$$

This initiation process is occurring when radical species (ROS or free radical) combines with an unsaturated fatty acid and abstracting hydrogen atom from fatty acid side chain (RH) of polyunsaturated fatty acid (PUFA) so generating carbon-centered radical (R•). PUFA is an unsaturated fatty acid that has more than one double bond in their C chain. The oxidation rate of fatty acid is increase in relative to degree of unsaturation. As an example, linolenic acid with 3 double bonds is three times more susceptible to oxidation compared to linoleic acid that has two double bonds.

$$A\bullet + RH \longrightarrow AH + R\bullet$$

Propagation

The propagation phase proceeds when carbon-centered (R•) radical from the iniation process react with O_2:

$$R\bullet + \bullet OO\bullet \longrightarrow ROO\bullet$$

The peroxyl radical (ROO•) formed are highly reactive, it can attack adjacent fatty acid side chains and lead to the formation of lipid hydroperoxide (ROOH). This reaction is repeated and called free radical chain reaction.

$$ROO\bullet + RH \longrightarrow ROOH + R\bullet$$

Termination

In term of termination process, antioxidant is needed to donate hydrogen atom to the system or excess radical is quenched by them to produce non radical species.

$$R\bullet + R\bullet \longrightarrow R\text{-}R$$

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

1.4 Antioxidant

According to Halliwel and Gutteridge (1990), antioxidant was defined as "a substance that when present at low concentrations, compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate". Many researchers reported that plants and vegetables have high antioxidant activity. This statement is also supported by Shon *et al*., (2003) and Ganesan *et al*., (2007), where they have found different types of antioxidants in various kinds of higher plants. The principle function of antioxidant is in delaying of the oxidation of other molecules by inhibiting the initiation or propagation of oxidation chain reaction by free radicals (as discussed in the previous page) and may reduce the oxidative damage to the human body (Namiki, 1990; Ismail *et al*., 2004).

Antioxidants are also added to a variety of foods as a preservative to prevent or deter free radical-induced lipid oxidation, which is responsible for the development of off-flavors and the undesirable chemical compounds in food (Angelo 1996; Lee *et al*., 2004). Unfortunately, the synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) which are used in industrial processes may possess some side effects and show toxic properties to the human (Anagnostopoulou *et al*., 2006; Manian *et al*., 2008; Pokorny, 2007; Orhan, *et al*., 2009). There are growing body of knowledge among the mass society about the health promoting impact of antioxidants in daily foods and the assumption that a number of common synthetic preservatives may have hazardous effects (Krishnakumar & Gordon, 1996; Peschel *et al*., 2006). Therefore, people are looking for natural antioxidants that are believed to be able to prevent degenerative diseases

and protect food lipids from oxidations (Hu and Kitts, 2005; Sharififar *et al*, 2009) as well as increase the shelf-life of foods (Schwarz *et al*., 2001; Ganesan, *et al.*, 2007).

1.4.1 Endogenous and Exogenous Antioxidants

In order to control the level of oxidative stress and maintain redox homeostasis, the human body needs to develop defense systems. The antioxidants defence systems produced by the human body are endogenous antioxidants (Masella *et al*., 2005). The human body has endogenous antioxidants which comprise the enzymatic and nonenzymatic antioxidant systems to defend and protect the body from reactive oxygen species (ROS) induced damage (Anderson, 1999 cited Manian *et al*., 2008). Enzymatic antioxidant systems especially superoxide dismutase (SOD), catalase (CAT), gluthation peroxidase (GPx) and thioredoxin systems are recognised as being highly efficient in ROS detoxification. Whereas, the main nonenzymatic antioxidants present in the human organism are glutathion, bilirubin, estrogenic sex hormones, uric acid, coenzyme Q, melanin, melatonin, α-tocopherol and lipoic acid (Laguarre *et al*., 2007). Table 1.4.1 shows endogenous and exogenous antioxidants present in human plasma.

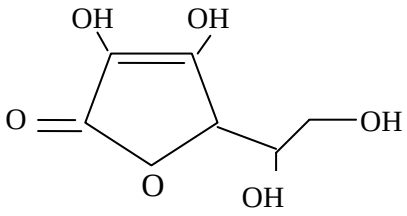
Table 1.4.1: Antioxidants in Human Blood Plasma

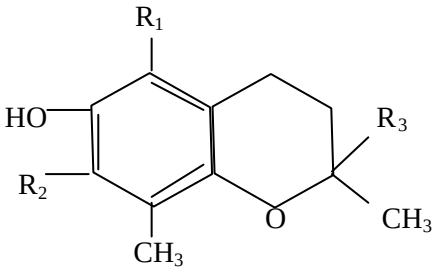
Antioxidant	***Plasma or serum concentrations***
Protein antioxidants	
Enzymatic	
• *Cu, Zn-superoxide dismutase (endothelium-derived)*	• **5-20 IU/mL**
• *Catalase*	• **Not detectable**
• *Glutathione peroxidise*	• **0.4 U/mL**
Non-enzymatic (binding of metalions or metal ion complexes)	
• *Albumin*	• **50 g/liter**
• *Haptoglobin*	• **0.5-3.6 g/liter**
• *Transferrin*	• **1.8-3.3 g/liter**
• *Hemopexin*	• **0.6-1.0 g/liter**
• *Ceruloplasmin*	• **0.18-0.4 g/liter**
• *Lactoferrin*	• **0.0002 g/liter**
Low- molecular-weight antioxidants	
Water soluble	
• *Ascorbic acid*	• **30 – 150 µM**
• *Glutathione*	• **1 – 2 µM**
• *Urate*	• **160 – 450 µM**
• *Bilirubin*	• **5 – 20 µM**
• *Thiol (nonalbumin)*	• **50 – 100 µM**
Lipid soluble (lipoprotein associated)	
• *α-Tocopherol*	• **15 – 40 µM**
• *γ-Tocopherol*	• **3 – 5 µM**
• *α-carotene*	• **0.05 - 0.1 µM**
• *β- carotene*	• **0.3 - 0.6 µM**
• *Lycopene*	• **0.5 - 0.1 µM**
• *Lutein*	• **0.1 - 0.3 µM**
• *Zeaxanthin*	• **0.1 - 0.2 µM**
• *Ubiquinol-10*	• **0.4 - 1.0 µM**

*adopted from Keaney and Frei, 1994

Overall, endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), thioredoxin reductase, peroxiredoxin and glutathione peroxidase (GPx), work as first line of defense system against superoxide and hydrogen peroxides (Masella *et al*., 2005). However, they are not able to be 100% effective because of certain compounds generated by the interactions of ROS with biological micromolecules. One of these compounds is carbon-centered radical (R•) that is

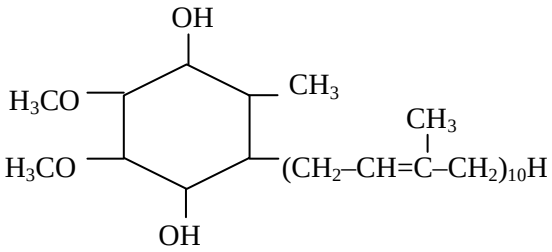
highly unstable being short-lived intermediates that stabilizes by abstracting a hydrogen from another chemical species (Laguerre *et al*., 2007). The second lines of defense system against ROS involve GPx, glutathione S-tranferase (GST), aldo-keto reductase and aldehyde dehydrogenase. These enzymes contribute in the detoxification of secondary products, so as to prevent further intracellular damage, degradation of cell components and eventual cell death (Masella *et al*., 2005). In many tissues, hydrogen peroxide is inactivated by catalase to produce water and oxygen (Close and Hagerman, 2006). The organelle that contains catalase is peroxisome (Valko *et al*., 2007). Figure 1.4.1(a) shows the chemical structure of non-enzymatic antioxidant present in human body.



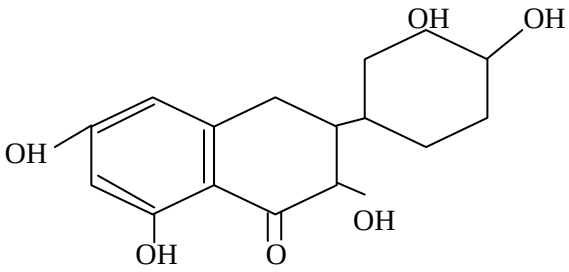


VITAMIN C

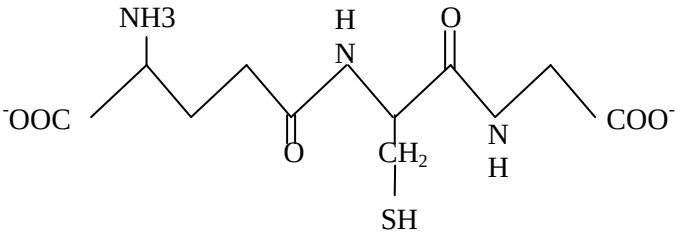
VITAMIN E



UBIQUINOL-10



QUERCETIN



GLUTATHIONE

	R_1	R_2	R_3
α- Tocopherol	CH_3	CH_3	$C_{16}H_{33}$
β- Tocopherol	CH_3	H	$C_{16}H_{33}$
γ- Tocopherol	H	CH_3	$C_{16}H_{33}$
δ- Tocopherol	H	H	$C_{16}H_{33}$

Figure 1.4.1(a): Chemical structure of non-enzymatic antioxidants (Briviba and Sies, 1994).

On the other hand, the production of antioxidant enzymes in the body declined as age increased. Therefore, the antioxidant enzymes are insufficient to scavenge and eliminate excess free radicals efficiently (Vimala *et al*., 2003). Antioxidants supplies from diet are essentially needed and these antioxidants are called exogenous antioxidants. Many studies have showed that exogenic antioxidant, especially those supplied by natural product such as plants in the form of phenolic compounds, ascorbic acid and carotenoids are essential for counteracting oxidative stress (Laguerre *et al*., 2007).

Over the past three decades, the free radical theory has greatly stimulated interest in the role of dietary antioxidants in preventing many human diseases including cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, and diabetes (Yun, *et al*., 2002). Therefore, it is important to consume a diet high in antioxidant, such as fruits and vegetable to reduce the harmful effects of oxidative stress (Teow *et al*., 2007). Furthermore, fruits and vegetables are rich sources of phytochemicals such as carotenoids, flavonoids and other phenolic compounds (Teow *et al*., 2007). Besides, fruits and vegetables are also the main sources of vitamins and provitamin such as tocopherols, ascorbic acids and carotenoids (Ismail, *et al*., 2004; Weisburger, 1999). These kinds of vitamins are also thought to have antioxidant properties (Weisburger, 1999).

In addition to antioxidant vitamins, carotenoids, and polyphenols, vegetables provide a large group of glucosinolates, which according to Plumb *et al*., (1996) possess rather low antioxidant activity, but the products of their hydrolysis can protect against cancer (Nautiyal, *et al*., 2008). Glucosionalates, which are present in

cruciferous vegetables, are activators of liver detoxification enzymes. Consumption of cruciferous vegetables offers a phytochemical strategy for providing protection against carcinogenesis, mutagenesis and other forms of toxicity due to electrophiles and reactive forms of oxygen (Fahey *et al*., 1997; Dillard and German, 2000). Furthermore, dietary antioxidants have the potential to reduce the genetic instability of cancer cells and thus may be useful in treatment (Reddy *et al*., 2001). Recently, many plants have been examined to seek new and effective antioxidant and anticancer compounds, as well as to elucidate the mechanism of cancer prevention and apoptosis (Swamy and Tan, 2000; Lee *et al*., 2004).

The mechanism by which plants dietary substances may inhibit carcinogenic process is shown in figure 1.4.1(b). The role of diet in affecting carcinogenic process is complicated, whereby these diets may possiblely be involved in different mechanism of actions. The anticarcinogenic action of dietary compounds can be classified into two groups – those that block and those that suppress depending on the site of action (Wattenberg *et al*., 1992). Some compounds in diets can both block and suppress synergetically. The main action of blocking agents is to stimulate the carcinogen detoxifying enzymes and to inhibit enzymes which have potential to activate precarcinogens into carcinogens. The suppressing agents include compounds that inhibit the apprearance of tumour, even after the administration of carcinogen. Suppressing agents may act by modifiying intracellular signaling, inhibition of oncogene expression, or modification of polyamine or oestrogen metabolism (Williamson *et al*., 1999). The failures of maintenance and repair pathways effectively determine the course of aging, the origin of age-related diseases and eventual death (Rattan and Clark, 2005; Rattan, 2006; Rajawat, *et al*., 2009).

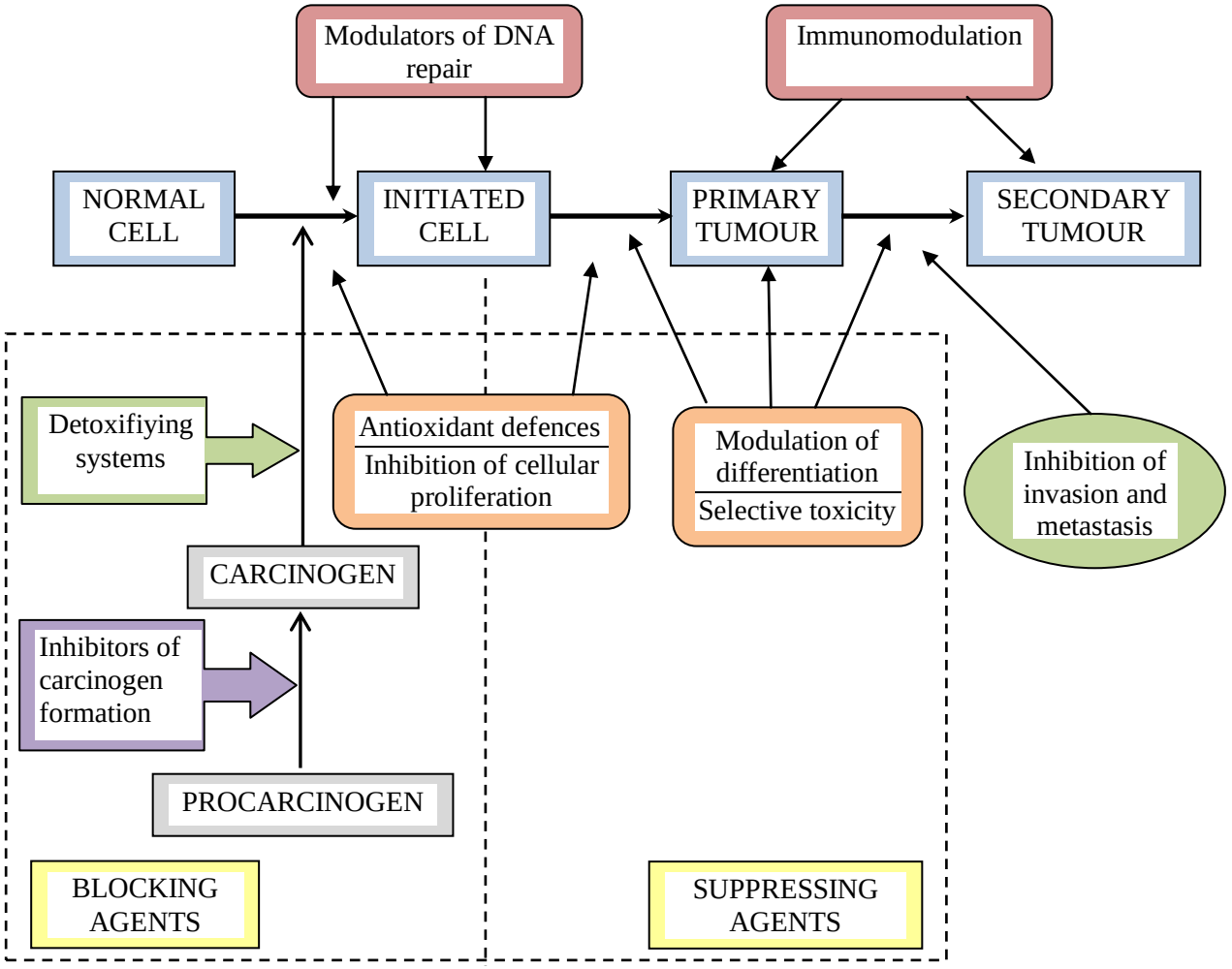


FIGURE 1.4.1(b): Mechanisms and sites of interaction whereby plant dietary substances may inhibit the carcinogenic process (Johnson, *et al*., 1994)

1.4.2 Mechanism of Actions of Antioxidant.

The mechanisms of action of antioxidants involve two different ways of donating H atom to free radical or scavenge species that are responsible in initiating the oxidation. This is called preventive antioxidants whereas the secondary antioxidants work as chain-breaker by termination of the propagation step in lipid peroxidation (Laguarre *et al*., 2007). According to Saha *et al*, (2004) antioxidants may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing the first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds and chain-breaking to prevent continued hydrogen abstraction from substrates. The principle described below shows that the antioxidants may act through different mechanisms.

1.4.2(a) Preventive Antioxidants:

The preventive antioxidation pathways are dependent on diverse range of available oxidation initiators. These pathways include transition metals chelators, singlet oxygen quenchers, ROS detoxification and others.

Transition metals chelators:

The concentration of free iron in healthy blood is very low. However, during stress conditions such as in septic shock, inflammation and tissue damage, iron can be released from its stores and will then be available to generate free radical (Williamson *et al*., 1999). Generally, iron and copper are almost always bound to

carrier proteins or locked away in storage proteins (Gutteridge and Haliwell, 1994). Chelators of transition metals such as copper and iron can prevent oxidation by forming complexes or coordination compounds with the metals. The chelators are proteins such as transferrin, ferritin, and lactalbumin that sequester iron, or ceruloplasmin and albumin that sequester copper (Laguerre *et al*., 2007).

Singlet oxygen quenchers

The antioxidant activity of carotenoids arises primarily as a consequence of the ability of the conjugated double-bonded structure to delocalise unpaired electrons (Mortensen *et al*., 2001 Valko *et al*., 2006). This is primarily responsible for the excellent ability of β-carotene to physically quench singlet oxygen ($^1O_2$) without degradation, and for the chemical reactivity of β-carotene with free radicals such as the peroxyl (ROO•), hydroxyl (•OH), and superoxide radicals ($O_2•^-$). This latter mechanism of action occurs through deactivation of $^1O_2$ into $^3O_2$.

$^1O_2$ + β-carotene $\longrightarrow$ $^3O_2$ + β-carotene •

Through the long conjugated polyenic system of these molecules, the excess energy generated in their excited state (β-carotene•) is dissipated via vibrational and rotational interactions with the solvent or the environment (Laguerre *et al*., 2007)

β-carotene• $\longrightarrow$ β-carotene + heat

Regenerated β-carotene can begin a new $^1O_2$ quenching cycle through this energy (heat) dissipation mechanism and thus become a nonstoichiometric quencher.

ROS detoxification

ROS detoxification is a crucial oxidation prevention pathway, mainly mediated by endogenous enzymatic antioxidant systems. Superoxide dismutase (SOD) catalyzes superoxide anion dismutation into hydrogen peroxide and oxygen (Laguerre *et al*., 2007).

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Glutathione peroxidase (GPx) provides an alternative route for distruction of hydrogen peroxide at the expense of the small molecule antioxidant glutathione (GSH) (Close and Hagerman, 2006).

$$2GSH + H_2O_2 \xrightarrow[GPx]{} GSSG + 2H_2O$$

The third enzyme is catalase and it's generally found in peroxisomes (Valko *et al*., 2007). This enzyme is very efficient in promoting the conversion of hydrogen peroxide into water and oxygen (Valko, *et al*., 2006)

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

1.4.2(b) Chain-breaking Antioxidants:

Vitamin E (a major lipid-soluble antioxidant) is the most effective chain-breaking antioxidant within the cell membrane which it protects membrane fatty acids from lipid peroxidation **(**Percival, 1998). It reacts with alkoxy radicals (LO•), lipid peroxyl radicals (LOO•) and alkyl radicals (L•) which are derived from PUFA oxidation (Blokhina, *et al*., 2003). These tocopherols are exhibit a very distinct lag phase (see figure 1.4.2). Chain-breaking antioxidants induce a lag phase during which substrate is not substantially oxidized. This phase continues until the antioxidant is completely consumed (Laguerre *et al*., 2007).

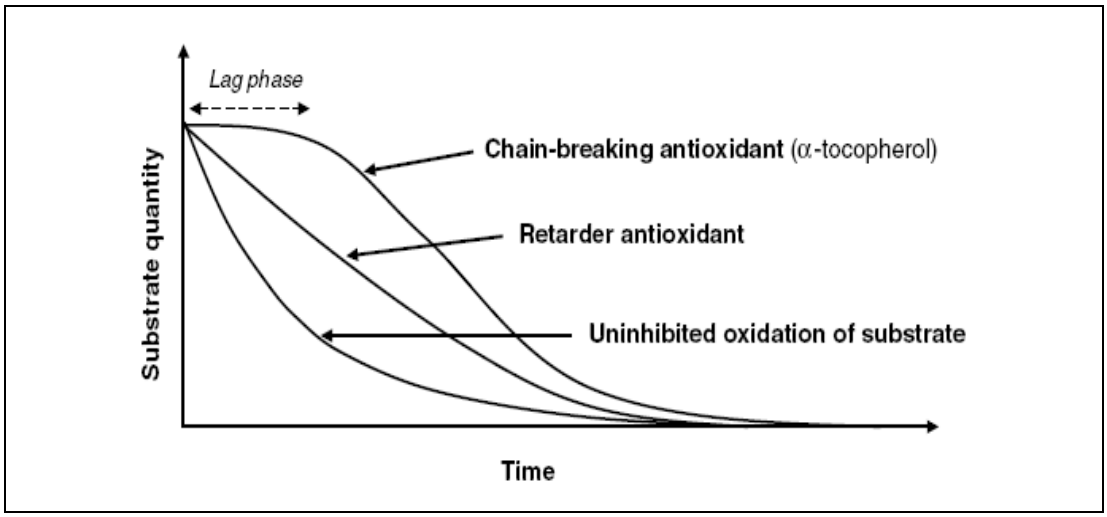


Figure 1.4.2: Theoretical time-course curves of antioxidation by chain breaker or retarder.

Another compound present in membranes that might sometimes act as a chain-breaking antioxidant is the reduced form of coenzyme Q, ubiquinol (Gutteridge and Haliwell, 1994). In lipid peroxidation, chain breaking antioxidants