

**DETERMINATION OF ANTIOXIDANT ACTIVITIES, TOTAL PHENOLIC
CONTENT, TOTAL FLAVONOID CONTENT AND ASCORBIC ACID IN
POMEGRANATE (*Punica granatum*) AND PASSION (*Passiflora edulis*)
FRUIT PEEL, SEED AND JUICE**



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This Final Year Project Report entitled “**Determination of antioxidant activities, total phenolic content, total flavonoid content and ascorbic acid in pomegranate (*Punica granatum*) and passion (*Passiflora edulis*) peel, seed and juice**” was submitted by Fara Syazana binti Ahmad Murshid, in partial fulfillment of the requirements for the Degree of Bachelor of Science (Hons.) Food Science and Technology, in the Faculty of Applied Sciences, and was approved by

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LIST OF ABBREVIATIONS

BHA	:	Butylated hydroxyanisole
BHT	:	Butylated hydroxianisole
DPPH	:	1,1-diphenyl-2-picrylhydrazyl
ET	:	Electron transfer
FCR	:	Folin-Ciocalteu reagent
FRAP	:	Ferric Reducing Antioxidant Power
HAT	:	Hydrogen atom transfer
LDL	:	Low Density Lipoprotein
OG	:	Octyl gallate
ORAC	:	Oxygen Radical Absorbance Capacity
PG	:	Propyl gallate
ROS	:	Reactive Oxygen Species
TBHQ	:	Tertiary butyl hydroquinone
TEAC	:	Trolox Equivalent antioxidant capacity
TRAP	:	Total radical trapping antioxidant parameter
UV	:	Ultra-violet
UV-VIS	:	Ultra Violet- Visible
mg	:	milligram
min	:	minutes
Mm	:	milliMol
rpm	:	revolution per minutes
%	:	percentage
°C	:	Degree Celcius

ABSTRACT

DETERMINATION OF ANTIOXIDANT ACTIVITIES, TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT, AND ASCORBIC ACID IN POMEGRANATE (*Punicagranatum*) AND PASSION (*Passifloraedulis*) PEEL, SEED AND JUICE.

The aim of this study is to determine the total phenolic content, total flavonoid content, ascorbic acid content and their antioxidant activities by DPPH and FRAP assays on six samples which are the pomegranate fruit peel, seed, juice, passion fruit peel, seed, and juice. The samples are all extracted by using ethanol to obtain the extracts before the determination of antioxidant content. For total phenolic content, it was determined using Folin-Cioclatau's reagent with gallic acid as standard and measured at 750 nm. The highest amount of total phenolic content was in pomegranate peel with 182.71 ± 0.57 mg GAE/100 g sample and the lowest was in passion juice with 16.05 ± 0.00 mg GAE/100 g sample. The total flavonoid content was determined at 415 nm with rutin as standard and expressed as mg RE/100 g sample. The highest flavonoid content was in passion peel with 100.94 ± 0.00 mg RE/100 g sample and the lowest was in pomegranate seed with $5.00 \pm 0/00$ mg RE/100 g sample. Ascorbic acid content was determined according to AOAC method. The highest amount of ascorbic acid was in pomegranate peel with 62.84 ± 10.88 mg/100 g sample and the lowest was in passion seed with 15.71 ± 5.44 mg/100 g. Each sample was treated with DPPH and FRAP assay for the determination of antioxidant activities in each components and all the samples exhibited good antioxidant activities indicates the presence of antioxidant in the samples. All the samples values have significant difference at ($p < 0.05$). Among the three components in pomegranate fruit, the peels shows the highest antioxidant capacity and activity, while for passion fruit, the peel also shows the highest antioxidant capacity and activities compared to its seed and juice.

ABSTRAK

PENENTUAN AKTIVITI ANTIOKSIDAN, KANDUNGAN FENOLIK, JUMLAH FLAVONOID DAN ASID ASKORBİK PADA KULIT, BIJI DAN JUS BUAH DELIMA (*Punica granatum*) DAN MARKISA (*Passiflora edulis*)

Tujuan kajian ini adalah untuk menentukan jumlah kandungan fenolik, jumlah kandungan flavonoid, kandungan asid askorbik dan aktiviti antioksidan menggunakan DPPH dan FRAP di dalam enam sampel iaitu kulit, biji dan jus buah delima serta kulit, biji dan jus buah markisa. Semua sampel diekstrak dengan menggunakan etanol untuk mendapatkan ekstrak sebelum penentuan kandungan antioksidan. Untuk kandungan jumlah fenol, ia telah ditentukan menggunakan reagen Folin-Ciocalteu dengan asid galik sebagai piawaian yang diukur pada 750 nm. Jumlah tertinggi kandungan jumlah fenol adalah dalam kulit buah delima dengan 182.71 ± 0.57 mg GAE/100 g sampel dan yang terendah adalah jus markisa dengan 16.05 ± 0.00 mg GAE/100 g sampel. Jumlah kandungan flavonoid telah ditentukan pada 415 nm dengan rutin sebagai piawaian dan dinyatakan sebagai RE mg /100 g sampel. Kandungan flavonoid yang tertinggi ialah dalam kulit buah markisa dengan 100.94 ± 0.00 mg RE /100 g sampel dan kandungan flavonoid yang terendah adalah di dalam biji buah delima dengan 5.00 ± 0.00 mg RE /100 g sampel. Kandungan asid askorbik telah ditentukan mengikut kaedah AOAC. Jumlah tertinggi asid askorbik adalah dalam kulit buah delima dengan 62.84 ± 10.88 mg/100 g sampel dan terendah adalah di dalam biji buah markisa dengan 15.71 ± 5.43 mg/100 g. Setiap sampel telah diuji dengan DPPH dan FRAP bagi penentuan aktiviti antioksi dan dalam setiap komponen dan semua sampel menunjukkan aktiviti antioksidan yang baik dan membuktikan kehadiran antioksidan dalam sampel. Nilai semua sampel mempunyai perbezaan yang signifikan ($p < 0.05$). Antara tiga komponen dalam buah delima, kulit menunjukkan kapasiti dan aktiviti antioksidan tertinggi, manakala bagi buah markisa, kulitnya juga menunjukkan kapasiti dan aktiviti antioksi dan tertinggi berbanding dengan benih dan jusnya.

CHAPTER 1

INTRODUCTION

1.1 Background and problem statement

Antioxidant is one of the minor foods constituent that have a big role in inhibiting the oxidation of other molecules and also protect our cells against the effects of free radicals. Antioxidant is also defined as the substance which at low concentrations than of the oxidisable substrate, delays or prevents oxidation of that substrate (Li *et al.*, 2006). Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources (Prakash *et al.*, 2001). The transfer of electron or hydrogen from a substance to an oxidising agent is called the oxidation process and leads to the formation of free radicals. Free radicals can start the chain reactions and can cause damage or death to the cell. There is strong evidence that free radicals are responsible for the damage to the lipids, proteins and nucleic acids in cell that may leads to various physiological and pathological abnormalities, such as inflammation, cardiovascular diseases and ageing (Conteras-Calderón *et al.*, 2011). Antioxidant compounds like phenolic acids, polyphenols and flavonoids acts by scavenging free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Prakash *et al.*, 2001). Antioxidant reacts by terminating these chain reactions, remove the free radical intermediates, and inhibit other oxidation reactions. The antioxidants are the one that being oxidised and act as reducing agent.

Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress. Oxidative stress plays a role in heart diseases,

neurodegenerative diseases, cancer and in the aging process (Ramamoorthy and Bono, 2007). This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases. The dietary antioxidants oppose this and lower risk of disease (Atoui *et al.*, 2005).

Natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community (Hue *et al.*, 2012). Antioxidant present mainly in fruits, vegetables, nut, grains, some meats, poultry and fish. Antioxidant substances include phenolic compound, beta-carotene, lutein, lycopene, selenium, vitamin A, vitamin B, vitamin C (ascorbic acid) and more. Epidemiological studies show that many phytonutrients of fruits and vegetables may be beneficial in protecting the human body against damage by reactive oxygen and nitrogen species (Soong and Barlow, 2004) and another studies shown that consumptions of fruits and vegetables is negatively associated with morbidity and mortality of cardio and cerebro-vascular diseases, certain types of cancer and the antioxidant contents in fruits and vegetables including the ascorbic acid, carotenoids, flavanoids, hydrolyzable tannin, are supposed to play important role in the prevention of these diseases (Li *et al.*, 2006). The defensive effects of natural antioxidants in fruits and vegetables are related to three major groups: vitamins, phenolics, and carotenoids. Ascorbic acid and phenolics are known as hydrophilic antioxidants. It is important to increase the antioxidant intake in our daily diets by eating food that rich in antioxidants. Recent epidemiological studies indicate that consumption of fruits is associated with a lower risk of chronic diseases. The antioxidants capacities of fruits vary depending in their content of vitamin C, vitamin E, carotenoids, flavonoids, and other polyphenols (Conteras-Calderón *et al.*, 2011).

The uses of synthetic antioxidants are increasing nowadays. Some of the synthetic antioxidants are the butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), propyl gallate (PG), octylgallate (OG) and many more that had been used in foods, pharmaceuticals and cosmetics. However, although they are

powerful in protecting the product quality in food distribution, excess antioxidants added to food might produce toxicities or mutagenicities and thus endanger the health of people (Xiu-Qin *et al.*, 2009). Some other researches stated that some synthetic antioxidants may exhibit toxicity and require high manufacturing costs but show lower efficiency than natural antioxidants (Soong and Barlow, 2004).

1.2 Significance of study

This study is proposed to determine the antioxidant content specifically the total phenolic contents, total flavonoids content, and ascorbic acid in pomegranate and passion fruits by products such as their peel and seeds as compared to the juice. It would increase the usage of waste product into more useful products by exploiting them as a source of natural antioxidants that are beneficial. It can replace the usage of synthetic antioxidant in processed foods which are known to be unhealthy compared to the natural antioxidant.

Besides of the delicious taste, refreshing flavour and pleasant aroma, fruits also provide us with important vitamins, minerals, and other bioactive compound to the human diet. Epidemiological studies shown that the consumption of fruits showed positive correlation with the risk of chronic diseases and the combination of the vitamins, minerals, phenolic antioxidants and fibre seem to be responsible for these effects (Vasco *et al.*, 2008). However in some fruits by-products such as peel and seed, it contains higher amount of antioxidant and vitamins compared to its juice. According to many researchers, the content of total phenolic compounds and the antioxidant activity is particularly high in the peel of some fruits more than the whole fruit (Conteras-Calderón *et al.*, 2011).

Generally, Malaysian consumed vegetables that contain relatively abundant sources of antioxidant components with strong potential antioxidants activities (Ikram *et al.*, 2009). Malaysia is a country that rich in tropical fruits that has a unique taste of their own and different compositions of vitamins and antioxidants. These tropical fruits composition have been widely commercialised and many

studies have been made. However there are several other fruits which are not originated from Malaysia, but quite easy to obtain being ignored instead of their richness of flavour and nutritional values. The potential of these fruits and its by-products as natural antioxidants are wasted due to the lack of studies.

Nowadays, people are more aware of the importance of antioxidants for a better health. Food manufacturers prefer to use synthetic antioxidants and among the synthetic types of antioxidants, the most frequently used to preserve food are the butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) (Moure *et al.*, 2001). This is because both natural and synthetic antioxidants play the same role in retarding the oxidation process in foods product which can lead to the formation of undesirable compounds, off-flavour and off-odour and at the same time increase the shelf life of the products. However, the used of the synthetic antioxidant has been related to some health risks. Studies showed that BHA (butylated hydroxyanisole) can caused lesion formation in the rat forestomach while BHT (butylated hydroxitoluene) may cause internal and external haemorrhaging at high doses that is severe enough to cause death in some strains of mice and guinea pig (McCarthy *et al.*, 2001). Therefore, the search for alternative sources of natural antioxidant, which is probably safer than synthetic antioxidant are becoming increasingly important and more attractive (Oliveira *et al.*, 2009).

People do not know that the by-product of pomegranate fruits and passion fruits mainly their peel and seeds can be exploited as natural antioxidants. Throwing them away would be such a waste. Because of the lack of information and studies on the benefits of these fruits seeds and peels, they are still being considered as waste products. Besides that, some studies reported that there are more antioxidants present in the peel or seed than the pulp itself. Other parts of plants such as bark, leaves, fruit peels and roots are also being exploited extensively for their antioxidant properties (Hue *et al.*, 2012). The amount of antioxidant specifically phenolic compound, flavonoids and ascorbic acid can be compared

among these fruits to identify which fruits contain the highest amount of these antioxidants.

1.3 Objectives

The objectives of this study are:

- i. to determine the total phenolic content, total flavonoids content, and ascorbic acid in pomegranate (*Punica granatum*) and passion fruit (*Passiflora edulis*) peel, seed and juice.
- ii. to compare the antioxidant activities of pomegranate (*Punica granatum*) and passion (*Passiflora edulis*) fruits peel, seed and juice by using DPPH and FRAP assays.

CHAPTER 2

LITERATURE REVIEW

2.1 Pomegranate (*Punica granatum*)

2.1.1 General Information

Pomegranate is an apple shaped fruit which is red or sometimes orange in colour outside or easily known as the peel. Inside the pomegranate fruit, there are many small seeds which are surrounded by water-laden pulp or the edible aril, may be varied in colour from white, deep purple or deep red. The seeds are also embedded in a white, spongy and astringent pulp. Most people like to eat the flesh raw but sometimes they used in cooking. Pomegranate (*Punica granatum* L.) belongs to Punicacea family adapted to the Mediterranean climate. It is a tropical and sub-tropical fruits (Ekşi and Özhamamcı, 2009). This fruit is native to the area of modern day Iran and Iraq. The pomegranate has been cultivated in Caucasus since ancient times and later spread to Asian areas such as the Caucasus as well as the Himalayas in Northern India.

Today, it is widely cultivated throughout Turkey, Iran, Syria, Azerbaijan, Armenia, Afghanistan, India, Pakistan, Bangladesh, Iraq, Lebanon, Egypt, China, Burma, Saudi Arabia, Israel, Jordan, the drier parts of Southeast Asia, the Mediterrenean region of Southern Europe, and tropical Africa. It was introduced into Latin America and California by Spanish in 1769. Pomegranate is also cultivated in parts of California and Arizona for juice production. It is also a seasonal fruits where in the northern hemisphere, the fruit season from September to February while in the southern hemisphere, it is from March to May.

The *Punica granatum* leaves are glossy, narrow oblong. The length is about 3–7 cm and 2 cm broad. The flowers are bright red, usually 3 cm in diameter, with four to five petals normally. The edible part of the fruit are berry like which is about 5–12 cm in diameter with a rounded hexagonal shape, and has thick reddish skin. The exact number of seeds in a pomegranate can vary from 200 to about 1400 seeds. Figure 2.1 shows the picture of pomegranate fruits.



Figure 2.1 Pomegranate fruits (*Punica granatum*)

Source: Jorgensen and Brennand, (2005)

2.1.2 Chemical composition

Pomegranate (*Punica granatum*) is native to the mediterranean region and has been used widely in the folk medicine of many countries. It has been used in the form of juice, concentrate, canned beverage, wine, jam, and jelly. The edible parts of pomegranate fruit represented 52 % of total weight, comprising 78 % juice and 22% seeds. Table 2.1 and Table 2.2 show nutritional composition in 100 ml of pomegranate juice and nutritional composition in seeds of pomegranate fruits.

Table 2.1 Nutritional composition in 100 ml of pomegranate juice

Nutrient	Contents (%)
Moisture	85.4
Total sugars	10.6
Pectin	1.4
Citric Acid	0.1
Ascorbic Acid	0.7
Free amino nitrogen	0.05

Source: Orak *et al.*, (2011)

Table 2.2 Nutritional composition in seeds of pomegranate fruit

Nutrient	Contents (%)
Total lipids	27.2
Protein	13.2
Crude fibers	35.3
Ash	2.0
Pectin	6.0
Total sugars	4.7

Source: Orak *et al.*, (2011)

2.1.3 Potential health benefits of pomegranate fruits (*Punica granatum*)

The juice of the pomegranate was effective in reducing the risk factors of heart disease, including low LDL (low density lipoprotein) oxidation, macrophage oxidative status, and foam cell formation based on laboratory research. Those are the steps leads to atherosclerosis and cardiovascular disease. Besides that, pomegranate juice has also been shown to reduce systolic blood pressure by inhibiting serum angiotensin-converting enzyme (Aviram *et al.*, 2004). Viral

infections may also be inhibited (Aviram and Dornfeld, 2001). It also may have antibacterial effects against dental plaque (Kaplan *et al.*, 2001).

The extracts of the fruit can inhibit the proliferation of human breast cancer cells. Pomegranates are also one of the fruit that is listed as high in fibre. Most of them are in the seeds as well as the unsaturated fat. Discarding the seed would be a great loss of fibre. Based on the clinical trial rationale and activity, it is found that metabolites of pomegranate juice ellagitannins localise specifically in the prostate gland, colon, and intestinal tissues of mice (Pedriali *et al.*, 2010) leading to clinical studies of pomegranate juice or fruit extracts for efficacy against several diseases.

Studies made by Aviram *et al.*, (2004) shows that pomegranate polyphenols can protect low density lipoprotein (LDL) against cell-mediated oxidation. The dietary supplementation of polyphenol-rich pomegranate juice to atherosclerotic mice significantly inhibited the development of atherosclerotic lesions, and this may be attributed to the protection of LDL against oxidation (Aviram *et al.*, 2004).

2.2 Passion Fruit (*Passifloraedulis*)

2.2.1 General information

Passion fruit is a species of passion flower and the fruit is about the size of an egg. There are two main varieties of the passion fruits; yellow passion fruit and purple passion fruit and another one is the giant granadilla. All are grown worldwide and edible. The fruit can be eaten raw or cooked in a variety of ways and most commonly used in baking.

Passion fruits are originated on the edges of South Amazon region of Brazil and possibly in Paraguay and North Argentina. The purple passion fruit is native from

southern Brazil through Paraguay to northern Argentina and usually adapted to the coolest subtropics or high altitudes in the tropics. The yellow passion fruit was originated in Australia as a sport from the purple passion fruit. However, it may have been introduced into Australia from tropical America (Akamine *et al.*, 1974)

The yellow passion fruit entered commercial cultivation more recently and is grown principally in Hawaii and Fiji. Other countries now undertaking to grow this yellow passion fruit are Brazil, Indonesia, Malaysia, Philippines, and Taiwan (Knight and Sauls, 1994). In Australia the purple passion fruit was flourishing and partially naturalised in coastal areas of Queensland before 1900. In Hawaii, seeds of the purple passion fruit, brought from Australia, were first planted in 1880 and the vine came to be popular in home gardens.

However, the species that will be used in this study is the *Passiflora edulis Sims.* or known as purple passion fruit. This species bears dark purple or nearly black, rounded or egg-shaped fruit about 5 cm (2 inches) in long, weighing 30-45 g (1-1.5 oz.). The fruits contain numerous small, black wedge-shaped seeds that are individually surrounded by deep orange-coloured sacs that contain the juice, the edible part of the fruit (Knight and Sauls, 1994)



Figure 2.2 Passion fruits (*Passiflora edulis*)

Source: Gerbaud, (2008)

2.2.2 Nutrient composition

Passion fruit is a good source of ascorbic acid (vitamin C), carotenoids (vitamin A), riboflavin and niacin with also a high mineral content. It is rich-flavoured and strongly, but pleasantly aromatic (Knight and Sauls, 1994). It has a unique flavour with °Brix value range of 12-20. It is also quite acidic. The undiluted juice is highly concentrated but is an excellent additive to other fruit juice. The juice makes an excellent jelly, pie filling or cake frosting. The seed with the surrounding juice sacs are often added to fruit salads. The purple passion fruit are usually sweeter and less acid than the yellow. The approximate composition in passion fruit cultivars per 100g edible portion are shown in the Table 2.3.

Table 2.3 Nutritional composition of 100g of edible portion of passion fruits

Nutrient	Flesh	Arils and seeds
Moisture (%)	94.4	78.4
Protein (g)	0.112	0.209
Fat (g)	0.15	1.29
Crude fibre (g)	0.7	3.6
Ash (g)	0.41	0.8
Ca (mg)	13.8	9.2
P (mg)	12.5	24.6
Fe (mg)	0.8	2.93
Carotene (mg)	0.004	0.019
Thiamine (mg)	Trace	0.003
Riboflavine (mg)	0.033	0.12
Niacin (mg)	0.378	15.3
Ascorbic acid (mg)	14.3	Trace

Source: Morton, (1987)

2.2.3 Passion fruits benefits

Purple passion fruit peel extract has been reported to contain a mixture of bioflavonoids, phenolics acids, and anthocyanin and has been shown to reduce blood pressure in hypertensive rats and humans (Zibadi *et al.*, 2007). In addition, the purple passion fruits contain antioxidants, such as cyanidin, quercetin and edulilic acid. Some antioxidants, specifically pycnogenol, have been shown to have beneficial effects on hypertension and asthma. Also quercetin has been shown to have anti-inflammatory properties and is associated with the reduction of asthmatic symptoms. The significant antioxidant amount may serve as a natural antioxidants dietary source, helping prevent diseases, or as a food additive, increasing the stability and quality of food products (Malacrida and Jorge, 2012). It also has been used widely in folk medicine in South America to treat anxiety, insomnia, asthma, bronchitis and urinary infection (Sherma and Ronald, 2004). The giant granadilla fruit is valued in the tropics as antiscorbutic and stomachic. In Brazil, the flesh is prescribed as a sedative to relieve nervous headache, asthma, diarrhoea, dysentery, neurasthenia and insomnia. The seeds contain a cardiogenic principle, can be used as sedative (Morton, 1987).

2.3 Antioxidant

Antioxidant are the substance that when added to food products, especially lipids and lipid containing foods, can increase the shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage (Singh *et al.*, 2002). Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. Antioxidants are capable of stabilising or deactivating the free radicals before the latter attack cells and biological targets. They are crucial for maintaining cumulative and debilitating oxidative stress results (Atoui *et al.*, 2005).

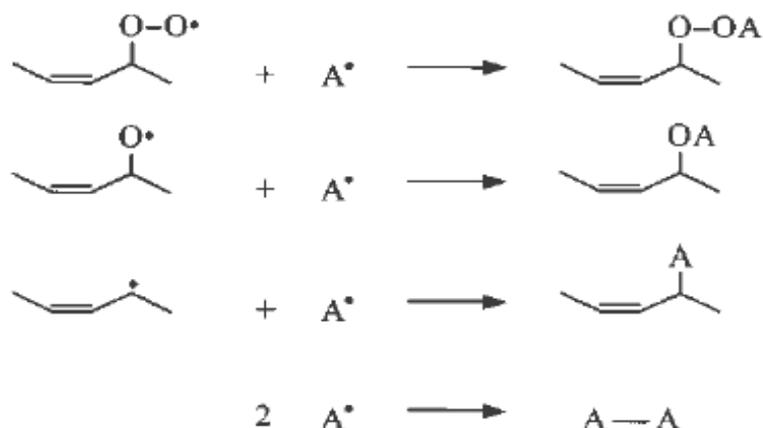


Figure 2.3 Termination reactions where antioxidants take place

Source: Dapkevicius, (2002)

Antioxidants can be divided into two types, the water soluble (hydrophilic) or lipid soluble (hydrophobic). Generally, the oxidants in the cell cytosol and the blood plasma will react with the soluble antioxidants while the lipid soluble antioxidants protect cell membranes from lipid oxidation (Seis, 1997). Variety of antioxidant defence to counter act with the auto-oxidation system can be non-enzymatic or enzymatic (Dapkevicius, 2002). Antioxidants can be grouped into the free radical inhibitors, propagation step of auto-oxidation inhibitors, singlet oxygen quencher, synergist antioxidants and also metal chelators.

Besides that, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in the food industry due to their abilities to prevent food deterioration and to extend the shelf life of foods. However, the usage of synthetic antioxidants was found to increase the risk of cancer occurrence and liver damage in human (Hue *et al.*, 2012). The synthetic antioxidant that commonly used are β -carotene, vitamin C and vitamin E are widely sold in the market and have been shown to increase the risk of mortality in adult who consumed them.