CHAPTER 2

LITERATURE REVIEWS

2.1 Noni Fruit

Noni (*Morinda citrifolia Linn.*) comes from a plant family Rubiaceae (comprised of 80 plant species) is found mainly in South Pacific (Tahiti), but also in Malaysia, Indonesia, Taiwan, Philippines, Vietnam, India, Africa, Fiji, Guam and the Hawaiian Island. It has large leaves and small white flowers that bloom all year-round. Noni flowers become a bumpy, pitted fruit several inches long. Upon ripening, noni's yellow skin becomes thinner translucent and an offensive odor will be developed. Noni contains numerous brown seeds that can float and can be transported in water thus the plant is found all over the world (Heinicke, 2003).

M. citrifolia grows in many different parts of the world, mainly tropical environments and produces a strong-tasting fruit that has innumerable health benefits. In the South pacific and Southeast Asia, knowledge of noni's healthful benefits has been passed from parents to children for countless generations. Families used the noni fruit both internally and externally (Heinicke, 2003). The common name of noni in Indian is "Indian mulberry" and in Thailand is called as "Yoh" (Sabiletou, 2002).

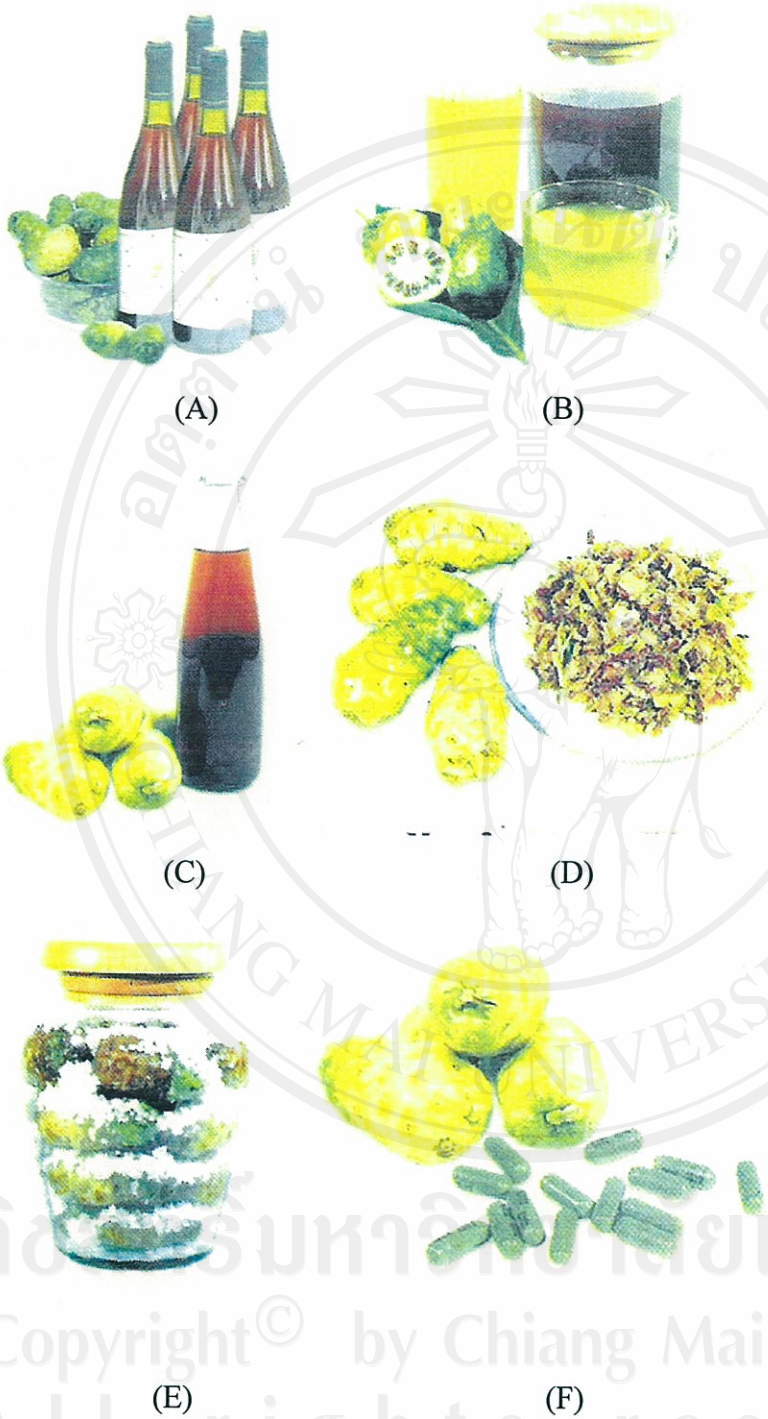
Noni fruits have a distinctive and not altogether pleasant aroma. They were traditionally eaten by native cultures in Samoa, Fiji, Southeast Asia, Polynesia, India, South Pacific and the Caribbean. Noni was a feminine food and also fed to livestock. The root and bark of the noni tree were sources of fabric dyes in Polynesia, Asia and Europe until 1950's (Heinicke, 2003). The nutritional values of the fresh leaf noni and raw noni fruit can be seen in Table 2.1.

Table 2.1 Nutritional values of fresh leaf and raw noni fruit

Nutritent	Fresh leaf	Raw fruit	Unit
Carbohydrate	11.1	7.5	gram
Protein	3.8	0.5	gram
Fat	0.8	0.0	gram
Fiber	1.9	1.1	gram
Calcium	350	39	milligram
Phosphorus	86	17	milligram
Iron	4.9	0.4	milligram
Vitamin A	9146	-	IU
Vitamin B1	0.3	0.06	milligram
Vitamin B2	0.14	0.04	milligram
Vitamin C	78	208	milligram

Source : Malisuwan (2003)

Several Thai conventional processing of noni fruit include noni wine, noni fresh juice, boiled noni juice, fermented noni juice, noni salad, noni capsules, preserved noni fruit and "boui Yoh" are shown in Figure 2.1.



(A) (B)

(C) (D)

(E) (F)

Figure 2.1 Noni products. A: noni wine, B: fresh noni juice and boiled noni juice, C: fermented noni juice, D: noni salad, E: "boui Yoh" and F: noni capsules.

Source: Subcharoen (2002)

2.2 Antioxidant components

Antioxidants in food may be defined as any substance which is capable of delaying, retarding or preventing the development of rancidity or other flavour deterioration in food due to oxidation. Antioxidants delay the development of off-flavours by extending the induction period. Addition of antioxidants after the end of this period tends to be ineffective in retarding rancidity development (Pokorny *et al.*, 2001).

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Zheng and Wang, 2001).

Antioxidants can inhibit or retard oxidation in two ways: either by scavenging free radicals, in which case the compound is described as a *primary antioxidant,* or by a mechanism that does not involve direct scavenging of free radicals, in which case the compound is a *secondary antioxidant.* Primary antioxidants include phenolic compounds such as vitamin E (α-tocopherol). These components are consumed during the induction period. Secondary antioxidants operate by a variety of mechanisms including binding of metal ions, scavenging oxygen, converting hydroperoxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen. Normally, secondary antioxidants only show antioxidant activity when a second minor component is present. This can be seen in the presence of metal ions. Reducing agents such as ascorbic acid are effective in the presence of tocopherols or other primary antioxidants (Pokorny *et al.*, 2001).

The term 'food antioxidants' is generally applied to those compounds that interrupt the free-radical chain reactions involved in lipid oxidation. However, the term should not be used in such as narrow sense because of the complexity of food systems (Baskin and Salem, 1997). Antioxidants are classified into five types:

(1) Primary antioxidants are compounds, mainly phenolic substances, that terminate the free radical chains in lipid oxidation. Natural and synthetic tocopherols, alkyl gallates, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), etc., are belong to this group, and their function as electron donoirs.

(2) Oxygen scavengers, for example, ascorbic acid (vitamin C), ascorbyl palmitate, erythorbic acid (D-isomer of ascorbic acid), and its sodium salt, etc., which react with oxygen, and can thus remove it in a closed system. The regeneration of phenolic antioxidants, an entirely different mechanism, by ascorbic acid (present in many fruits and vegetables) has also been proposed to explain the synergistic action of mixed antioxidants.

(3) Secondary antioxidants, such as dilauryl thiopropionate and thiodipropionic acid, function by decomposing the lipid hydroperoxides into stable end products.

(4) Enzymic antioxidants, for example, glucose oxidase, superoxide dismutase, catalase, glutathione peroxidase, etc, function either by removing dissolved/headspace oxygen, e.g. with glucose oxidase, or by removing highly oxidative species (from food system), e.g. with superoxide dismutase. This group of antioxidants appears to function by reducing the general rate of radical formation and acts by destroying/deactivating active species and/or their precursors. For example, catalase decomposes hydrogen peroxide without the formation of radicals.

(5) Chelating agents or sequestrants, for example, citric acid, amino acid, ethylenediaminetetra-acetic acid (EDTA), etc., chelate metallic ions such as copper and iron that promote lipid oxidation through a catalytic action. The chelates are sometimes referred to as synergists since they greatly enhance the action of phenolic antioxidants. Most of these synergists exhibit little or no antioxidant activity when used alone, except amino acids which can show antioxidant or pro-oxidant activity. Phospholipids such as cephalin act as antioxidant synergists in some systems, perhaps also due to their chelating effect (Hudson, 1990).

In the past few years, there has considerable interest in natural products endowed with antioxidant properties. A lot of traditionally used medicinal plants have been proposed for their interesting antioxidant activities and recommended for people's normal diets. In fact, it is widely accepted that diets rich in fruits and vegetables are protective against several human diseases. The protective effects have been attributed to the antioxidant nutrients present such as vitamin C and vitamin E

but also to the minor polyphenolic component of plants. These components contain a number of phenolic hydroxyl groups attached to ring structures responsible for antioxidant activity and often occur in the glycosidic form (Cervellati *et al.*, 2002).

Epidemiological studies have shown that dietary patterns were significantly associated with the prevention of chronic diseases such as heart disease, cancer, diabetes and Alzheimer's disease. Consumption of fruits and vegetables has been highly associated with reducing the risk of cancer (Sun *et al.*, 2002).

In the status of normal metabolism, the levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions. Overproduction of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and protein. More and more evidence suggests that this potentially cancer-inducing oxidative damage might be prevented or limited by dietary antioxidants found in fruits and vegetables (Sun *et al.*, 2002). Oxidative damage increases the risk of degenerative diseases such as cancer and cardiovascular diseases. Antioxidants reduce oxidative damage to biomolecules by modulating the effects of reactive oxidants. Therefore, an increase in the consumption of fruits and vegetables containing high levels of antioxidants has been recommended (Adom and Liu, 2002).

Several epidemiological studies indicated a beneficial effect of tomato consumption in the prevention of some major chronic diseases, such as some types of cancer and cardiovascular diseases. It has been postulated that the protective role of the tomato is due to tomato antioxidants that could contribute to the inhibition of abnormal oxidative processes. Tomato contains different classes of antioxidants such as carotenoids, ascorbic acid, phenolic compounds and α-tocopherol, and due to its high consumption rates, it can provide significantly to the total intake of these components. The antioxidant content of fresh tomatoes can be affected by many pre- and postharvest factors. The influence of cultivar, cultural practice, ripening stage at harvest and storage conditions on antioxidants accumulation has been studied during the past decade. Moreover, it is well-known that the positive effect on health associated with the consumption of fresh fruits and vegetables is exerted by the pool of antioxidants, with noticeable synergistic effects (Raffo *et al.*, 2002).

Free radical

Radicals are chemical species with one or more unpaired electrons, and free radicals are radicals that have moved out of the immediate molecular environment of their generation. A free radical is any chemical species that has an odd number of electrons, because it contains one or more unpaired electron(s), that is, an electron that occupies an atomic or molecular orbital by itself. The adjective *free* has been used to underline the fact that such radicals are capable of independent a structural entity in a molecular formula. A radical will thus be indicated, as recommended, by a superscripted dot at the right side of the formula in the parentheses so as to show that no judgment as to the location of the unpaired electrons is made (Roberfroid and Calderon, 1995).

Free radicals are chemical species, which have unpaired electrons. Molecules are composed of atoms and electrons. Electrons are present generally in pairs. However, under certain conditions, molecules have unpaired electrons and as such they are called free radicals. Unpaired electrons usually seek other electrons to become paired. Thus, free radicals are in general reactive and attack other molecules, although some radicals are not reactive but stable enough to have long life. Free radicals and oxidants can trigger lipid peroxidation, as well as the oxidation of proteins and DNA, causing extensive damage to body cells (Papas, 1998).

Examples of reactive free radicals are the hydroxyl ($HO^{\cdot}$) and alkoxyl ($LO^{\cdot}$) radicals, while vitamin E (tocopheroxyl) and vitamin C (dehydroascorbate) radicals are examples of stable radicals (Papas, 1998).

2.2.1 Ascorbic acid (vitamin C)

L-ascorbic acid (AA) is a carbohydrate-like compound whose acidic and reducing properties are contributed by the 2,3-enediol moiety. Two electron oxidation and hydrogen dissociation convert L-ascorbic acid to L-dehydroascorbic acid (DHAA). DHAA exhibits approximately the same vitamin activity as AA because it is almost completely reduced to AA in the body. AA occurs naturally in fruits and vegetables. In addition to its function as an essential nutrient, AA is widely used as a food ingredient/additive because of its reducing and antioxidative properties. AA effectively inhibits enzymatic browning by reducing *ortho*-quinone products. Other

functions include (a) reductive action in dough conditions, (b) protection of certain oxidizable compounds (e.g. folates) by reductive effects, free radical scavenging and oxygen scavenging, (c) inhibition of nitrosamine formation in cured meats and (d) reduction of metal ions. The antioxidative role of ascorbic acid is multifunctional, with ascorbate inhibiting lipid autoxidation by several mechanisms. These include (a) scavenging singlet oxygen, (b) reduction of oxygen- and carbon-centered radicals, with formation of a less reactive semidehydroascorbate radical or DHAA, (c) preferential oxidation of ascorbate, with concurrent depletion of oxygen and (d) regeneration of other antioxidants, such as through reduction of the tocopherol radical. AA is surprisingly effective as an antioxidant when dispersed in oils as well as in emulsions. Combinations of ascorbic acid and tocopherol are especially effective in oil-based systems, while the combination of α-tocopherol and the lipophilic ascorbyl palmitate is more effective in oil-in-water emulsions. Similarly, ascorbyl palmitate has been shown to act synergistically with α-tocopherol and other phenolic antioxidants (Gregory, 1996).

Ascorbic acid is moderately strong reducing agent, hence, it is readily oxidized during food processing and storage. Copper and iron are effective oxidation catalysts at level as low as 0.5 ppm. At food concentrations stability is superior at pH 3 – 4.5 than at pH 6 – 7. Ascorbic acid is more stable in concentrated solutions under anaerobic conditions, and the pH optimum is closer to neutrality. It reacts readily with antocyanin pigments (Harris and Karmas, 1975).

The vitamin C content of fruits increases until just before ripening, and then decreases. Whether the increase in weight on ripening offsets the slight loss is not certain. Usually the outer portions of fruits contain the most vitamin C for a given weight. When fruits are cooked, most of the ascorbic acid is dissolved out of the tissue into the liquid, oxidation occurring more easily in iron copper or badly tinned vessels. Enamel, Pyrex glass and stainless steel are the best materials. Rapidly bringing the material to the boil facilitates speedy inactivation of the ascorbic acid oxidase and minimizes loss due to oxidation. Fruits show losses in vitamin C content when stored for long periods. The losses are minimized by keeping at a low temperature, storing out of contact with air and in the dark. Canning fulfils two of these conditions, and losses in canned fruit pulps over a period of 12 months are about

10%. Addition of sulphite has a preserving effect on the vitamin C in fruit products, especially juices with little sugar (Woollen, 1996).

Ascorbic acid is an effective scavenger *in vivo* because it can react with the O_2^-, and $HO_2^\cdot$, $HO^\cdot$, water soluble peroxyl radical ($RO_2^\cdot$), singlet oxygen, and hypochlorous acid. Ascorbic acid is an antioxidant because the semidehydroascorbate radical (formed from ascorbic acid and a free radical) is much less reactive than most of the radicals scavenged by ascorbate. The ascorbyl radical is reduced back to ascorbic acid by enzymatic systems using either NADH or reduced glutathione as the cofactor (Baskin and Salem, 1997).

Ascorbic acid acts in the plasma to scavenge free radicals, dissipating these reactive species before they can react with biological membranes and lipoproteins. Ascorbic acid also has the ability to regenerate the activity of lipid-soluble antioxidants, such as γ-tocopherol and β-carotene by interacting with biological membranes at the aqueous-lipid interphase. Ascorbate may have a dual antioxidant function in biological systems: to inactivate damaging radicals in the plasma and to preserve the activity of lipophilic antioxidants (Basu *et al.*, 1999).

The substantially high cellular levels of vitamin C provide antioxidant protection to the eye against photosynthetically generated free radicals and against plasma and low-density lipoprotein oxidation. Vitamin C also functions as a reducing agent for mixed-function oxidases involved in drug metabolism by inactivating a wide variety of xenobiotic substances and hormones (Wang *et al.*, 2002).

Pasteurization of yellow passion fruit juices resulted in a 25% loss in L-ascorbic acid, which was completely destroyed after 14 days of storage; losses coincided with increased juices browning and formation of 5-hydroxymethylfurfural. The time and temperature sensitivity of ascorbic acid, brown color formation, and the development of off-orders during storage are issues that affect product quality (Talcott *et al.*, 2003).

Raffo *et al.* (2002) reported that no significant difference in ascorbic acid content was observed at different ripening stages of cherry tomatoes.

Ascorbic acid also indirectly contributes to several key oxidative and reductive enzyme system and has the ability to regenerate other biologically important antioxidants, such as glutathione and vitamin E, into their reduced state. Furthermore,

vitamin E can play an improtant role in reducing oxygen toxicity and is also an excellent nitrite-trapping agent for preventing gastric cancer (Wang *et al.*, 2002).

2.2.2 Carotenoid

Carotenoids and related compounds are the colours of nature. They consist of a group of over 600 naturally occurring coloured pigments that are widespread in plants, but only about 24 commonly occur in human foodstuffs. In the plant, they serve two essential functions: as accessory pigments in photosynthesis and in photoprotection. These functions are achieved through the polyene structure of carotenoids, which allows the molecules to absorb light and to quench, or inactivate, singlet oxygen and free radicals (Pokorny *et al.*, 2001).

Carotenoids are lipid soluble, and are therefore carried within lipoprotein particles. Normally, 1 mol of Low density lipoprotein (LDL) contains only about 0.29 mol of β-carotene, 0.12 mol of α-carotene and 0.16 mol of lycopene. Carotenoids are thought to be highly efficient quenchers of singlet oxygen, $^1O_2$. At a low oxygen pressure, normally present in the physiological situation, carotenoids are also an excellent substrate for free radical attack. β-carotene has an added advantage of being able to trap more lipid free-radicals than α-tocopherol, the latter being able to trap a maximum of two. This is because of β-carotene has ability to form multiple resonance stabilized molecules as subsequent binding of free radicals occurs (Gregory, 1996).

A singlet β-carotene molecule is believed to eliminate up to 1,000 singlet oxygens by physical mechanisms involving energy transfer, before it is oxidized and losed its antioxidant properties. The rate of oxidation of β-carotene is dependent on the oxygen partial pressure. The carbon-centred radicals are resonance-stabilized when the oxygen pressure is lowered. The reactivity of β-carotene towards peroxyl radicals and the stability of the resulting carbon-centred radicals are two important features that give the carotene molecule antioxidant capabilities (Basu *et al.*, 1999).

The hypothesis, based on epidemiological evidence, that health benefits arising from the consumption of coloured fresh fruit and vegetables are at least in part due to their carotenoid content has led to interest in the non-provitamin, as well as the provitamin A carotenoids. At present, there are no quantifiable biochemical or physiological markers of carotenoid 'status' (other than in relation to the vitamin A

activity). Neither carotenoid 'deficiency' nor 'toxicity' is recognised. However, low plasma carotenoid concentration is used as an indicator of those 'at risk' of chronic disease based on the direct association between the intake of carotenoid containing vegetables and fruit, plasma and tissue concentrations of carotenoids and the development of chronic disease states, particularly cardiovascular disease and cancer of various organs (Pokorny *et al.*, 2001).

During processing there are a number of physical and chemical changes that need to be considered for their possible impact on bioavailability of carotenoids. Thermal processing is normally undertaken to render the product edible, to eliminate any spoilage/pathogenic organisms and to activate enzymes. Cooking therefore softens the cell walls so that they are easily separated or broken mechanically, all cellular membranes are destroyed and proteins denatured. The carotenoid, normally stable within the original structure, are then exposed to the external environment where they may be subject to light, atmospheric oxygen and oxidised or reactive products of other components. Lycopene (tomatoes) and lutein and β-carotene (green leaves) appear to be quite stable in the fresh tomato and green leaf even when exposed to intense sunlight. During processing the protection of the native environment is lost and the carotenoids are readily oxidised and photobleached. This is particularly true if the product is dried and exposed to the air and light (Pokorny *et al.*, 2001).

Losses of carotenoid that occur after thermal processing and storage in anaerobic and light-free conditions (e.g. canning) are slight and may be as a result of oxidation by compounds formed enzymically or thermally during processing. Processing, however, increases the availability of lycopene for absorption, particularly if processed in the presence of lipid. It is also recognised that dietary fat itself improves carotenoid bioavailability (Pokorny *et al.*, 2001).

The most prominent role of carotenoid pigments in the diet of humans and other animals is their ability to serve as precursors of vitamin A. Although the carotenoid β-carotene possesses the greatest provitamin A activity because of its two β-ionone rings, other commonly consumed carotenoids, such as α-carotene and β-cyptozanthin, also possess provitamin A activity. Provitamin A carotenoids present in fruits and vegetables are estimated to provide 30-100% of the vitamin A requirement in human populations. A prerequisite to vitamin A activity is the existence of the

retinoid structure (with β-ionone rings) in the carotenoid. Thus, only a few carotenoids possess vitamin activity (Gregory, 1996).

Edible plant tissues contain a wide variety of carotenoids. Red, yellow, and orange fruits, root crops and vegetables are rich in carotenoids. All green leafy vegetables contain carotenoids but their color is masked by the green chlorophyll (Gregory, 1996).

Many factors influence the carotenoid content in plants. In some fruits, ripening may bring about dramatic changes in carotenoids. For example, in tomatoes, the carotenoid content, especially lycopene, increases significantly during the ripening process. Thus, concentrations differ depending on the stage of plant maturity. Even after harvest, tomato carotenoids continue to be synthesized. Since light stimulates biosynthesis of carotenoids, the extent of light exposure is known to affect their concentration. Other factors that alter carotenoid occurrence or amount include growing climate, pesticide and fertilizer use, and soil type (Gregory, 1996).

Because carotenoids can be readily oxidized, it is not surprising that they have antioxidant properties. In addition to cellular and *in vitro* protection against singlet oxygen, carotenoids, at low oxygen partial pressures, inhibit lipid peroxidation. At high oxygen partial pressure, β-carotene has pro-oxidation properties. In the presence of molecular oxygen, photosensitizers (i.e., chlorophyll) and light, singlet oxygen may be produced, which is highly reactive active oxygen species. Carotenoids are known to quench singlet oxygen and thereby protect against cellular oxidative damage. Not all carotenoids are equally effective as photochemical protectors. It has been proposed that the antioxidant functions of carotenoids play a role in limiting cancer, cataracts, atherosclerosis, and the processes of aging. For the stability during processing, carotenoids are relatively stable during typical storage and handling of most fruits (Gregory, 1996).

2.2.3 Flavonoid

There is a growing interest in food compounds which possess a possible health-protecting capacity and which were previously regarded as non-nutrients. Those compounds, if derived from plants, are described as phytochemicals. They hardly contribute to the nutritional value of the product but might play an important

role in maintaining human health. Flavonoids are an example of these compounds, and in epidemiological studies inverse relations with aging diseases such as coronary heart diseases and cancer have been described. This is ascribed to their function as antioxidants, or in modulation enzyme activity (Van der Sluis *et al.*, 2002).

Flavonoids are a group of polyphenolic compounds ubiquitously found in fruits and vegetables. The increasing interest in flavonoids is due to the appreciation of their broad pharmacological activity. Beneficial effects of flavonoids have been described for diabetes mellitus, allergies, cancer, viral infections, headache, stomach and doudenal ulcers, parodentosis and inflammatory diseases. Different flavonoids have different antioxidant capacities. The antioxidant potential of flavonoids is dependent on the number and arrangement of hydroxyl groups across the structure, as well as the presence of electron-donating and electron-withdrawing substituents in the ring structure. The hydroxyl radical scavenging activities of flavonoids increase with the number of hydroxyl groups substituted on the B-ring, especially at position 3'. A single hydroxyl substituent generates little antioxidant activity. A great variation in the flavonoid contents of different fruit crops and different growing conditions has also been reported (Wang *et al.*, 2002).

The three most important groups of flavonoids present in apple and apple products are flavonols or catechins, flavonols, and anthocyanins, with the main representatives are epicatechin, queretin glycosides, and cyanin galactoside, respectively. All of these compounds belong to the "polyphenolics" group, together with procyanidins, which consist of oligomeric acid and phenolic acids, which are also present in apple. Besides their contribution to potential health benefits, flavonoids contribute to the color and taste of apples. After processing apples to an apple juice, a low flavonoid concentration is found in the juice (Van der Sluis *et al.*, 2002).

Flavonoids are diphenylpropanes that commonly occur in plants and are frequently components of human diet. They are consumed in relatively high quantities in our daily food. The main source of flavonoids is vegetables, fruits and beverages. For example, the content of quercetin glycoside in outer leaves of lettuce could be as high as 237 mg/kg fresh weight, and the content of kaemferol glycoside in kale could be 250 mg/kg fresh weight (Hertog *et al.*, 1992).

Flavonoids are distributed widely in plant foods such as vegetables and fruits. They possess a unique C6-C3-C6 structure with phenolic OH groups, and more than 4000 different varieties has been identified as natural flavonoids. Quercetin, a typical flavonol present in a wide variety of vegetables such as broccoli, onions and lettuce, contains phenolic OH groups at the 5 and 7 position in the A ring and at the 3' and 4' position in the B ring (Figure 2.2). Tea catechins are flavanol-type flavonoids in which phenolic OH groups are bound to the 5 and 7 position (Figure 2.3). The hydroxyl group at the 3 position is esterified frequently by gallic acid (epicatechin gallate and epigallocatechin gallate) (Packer *et al.*, 1999).

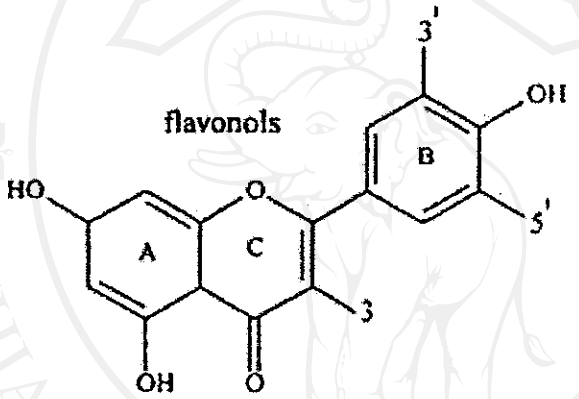


Figure 2.2 Structure of flavonols.

Source: Wang and Mazza (2002)

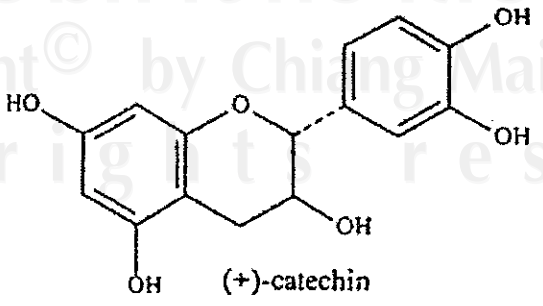


Figure 2.3 Structure of catechin.

Source: Wang and Mazza (2002)

Flavonoids have important effects in plant biochemistry, acting as antioxidants, enzyme regulators, precursors of toxic substances, pigments and light screens. Selected flavonoids have been shown in numerous *in vitro* and *in vivo* experiments to have antiallergic, anti-inflammatory, antiviral and antioxidant activities. Polyphenolic flavonoids can scavenge reactive oxygen radicals, such as the hydroxyl radical and superoxide anion radical, by donating a hydrogen atom or electron. Their antioxidant activity seems to be substantially dependent on pH because phenolic OH groups are dissociated with the elevation of pH, and the electron-donating capacity of phenolic OH group in flavonoids increases with its dissociation (Packer *et al.*, 1999).

Possibly the most important group of phenols in food is flavonoids, which consist of catechins, proanthocyanins, anthocyanidins and the flavones, and flavonol and their glycosides. The flavonoids are thought to possess a range of medicinal properties and act as antibacterial, antitumor and antiviral agents. Furthermore flavonoids also possess antiradical and antioxidant properties. However, not all flavonoids are equally potent. Hydroxylation of the B ring in positions 2', 3' and 4' promotes radical scavenging capability and inhibition of peroxidation of lipids, while hydroxylation of the 7- position increase the potency in inhibition of superoxide dismutase. The ability of a flavonoid to protect against lipid peroxidation is dependent not only upon its structure but also on its ability to interact with and penetrate the lipid bilayers (Baskin and Salem, 1997).

It has been found that flavonoids and other polyphenols possess antitumoral, anti-allergic, and anti-inflammatory activities. Epidemiological evidence has shown the importance of flavonoids in reducing mortality from coronary heart disease. Flavonoid and other dietary compounds have been mentioned as statistically beneficial and protective against carcinogenesis. Most of these biological effects are believed to come from their antioxidant properties. Flavonoids can exert their antioxidant activity by inhibiting the activities of enzymes including xanthine oxidase, myeloperoxidase, lipoxygenase and cyclooxygenase, by chelating metal ions, by interacting with other antioxidant such as ascorbate, and most importantly, scavenging free radicals (Pokorny *et al.*, 2001).

The hydroxyl radical is more reactive than the superoxide anion and is therefore more harmful to biological samples. Most flavonoids possess a high reactivity with the hydroxyl radical. For instance, (+)-catechin, (-)-epicatechin, 7,8-dihydroxy flavone, and rutin scavenge the hydroxyl radical at 100 – 300 times more than mannitol, a typical hydroxyl radical scavenger. The reactivity of flavonoids toward hydroxyl radical is generally much higher than that toward superoxide. Flavonoids inhibit the production of reactive oxygen species in cells. Flavonoids also react with singlet oxygen ($^1O_2$). Flavonoids in green tea such as (-)epigallocatechin gallate, (-)-epicallocatechin and (-)-epicatechin have been shown to scavenge $^1O_2$ (Packer *et al.*, 1999).

Flavonoids are an important part of the diet because they can modulate lipid peroxidation involved in atherogenesis, therombosis, and carcinogenesis, Known properties of flavonoids include free radical scavenging, strong antioxidant activities in preventing the oxidative enzymes, and anti-inflammatory actions. Two important properties of flavonoids include their antioxidant activity and their metal chelation properties. They behave as antioxidants by donating electrons to radicals and breaking the radical chain. Several antioxidant mechanisms may be considered, including scavenging radicals ($ROO^{\cdot}$, $RO^{\cdot}$) and activated oxygen species ($HO^{\cdot}$, $O_2^{\cdot}$, $^1O_2$), inactivating metal ions by chelation, complexing with proteins (enzymes, apoprotein-B of LDL-Low density lipoprotein) and metal-binding sites of enzymes, exhibiting synergism by reducing radicals from oxidized α-tocopherol and ascorbic acid, and partitioning acording to their polarity to become distributed at different oxidation sites. Flavonoids can also bind one or two copper atoms by chelation (Packer *et al.*, 1999). Oxidation of LDL plays a key role in the initial phases of atherosclerosis. When polyunsaturated lipids (LH) in LDL or tissue membranes become oxidized in the presence of metals and hydroperoxides, an alkyl radical $L^{\cdot}$ is formed. The alkyl radical reacts with oxygen to form peroxyl radicals ($LOO^{\cdot}$), which react again with LH to produce hydroperoxides (LOOH). These hydroperoxides decompose readily in the presence of metals to produce aldehydes. These aldehydes react with the apoprotein-B of LDL to produce oxidized LDL. Phenolic antioxidants can inhibit two important steps in the free radical chain reaction by reacting with (1) peroxyl radicals and inhibiting hydroperoxide formation and (2) alkoxyl radical and inhibiting

aldehyde formation. Phenolic compounds can participate in several antioxidant defenses, including preventing oxidant formation, scavenging activated oxidation, reducing reactive intermediates, and including repair system (Packer *et al.*, 1999).

The ability of flavonoids to inhibit lipid oxidation is well documented, both for natural lipid products and for model lipids. Flavonoids may act as antioxidants by scavenging radicals that include superoxide anions, lipid peroxyl radicals and hydroxyl radical. Other mechanisms of action of selected flavonoids include singlet oxygen quenching, metal chelation, as well as lipoxygenases inhibition. The glycosides are less effective as antioxidants than are the aglycones (Pokorny *et al.*, 2001). It is concluded that for maximal radical scavenging activity a flavonoid molecule needs to meet the following criteria (Figure 2.4) :

(a) 3', 4' – dihydroxy struture in the B-ring

(b) 2,3-double bond in conjunction with a 4-oxo group in the C-ring

(c) presence of a 3-hydroxyl group in the C-ring and 5-hydroxyl group in the A-ring (Pokorny *et al.*, 2001).

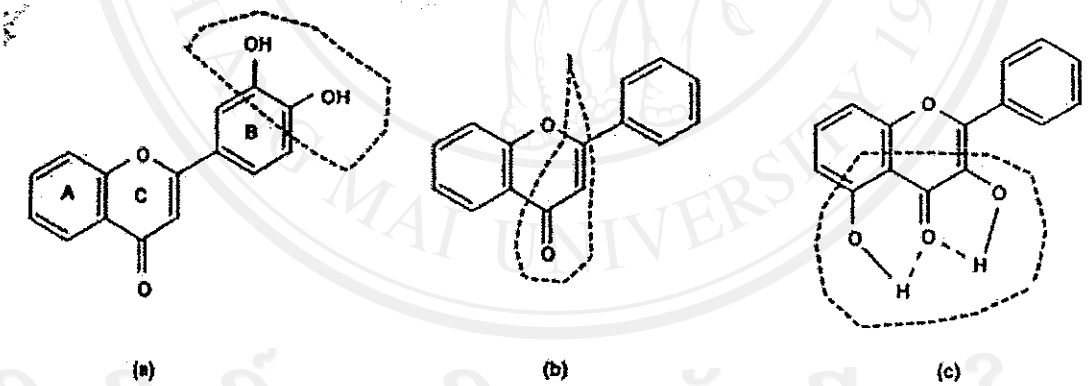


Figure 2.4 Structures of flavonoid molecules that have radical scavenging activities

(a) 3', 4' – dihydroxy struture in the B-ring

(b) 2,3-double bond in conjunction with a 4-oxo group in the C-ring

(c) presence of a 3-hydroxyl group in the C-ring and 5-hydroxyl group in the A-ring.

Source: Pokorny *et al.* (2001)

Flavonoids with free hydroxyl groups act as free-radical scavengers, and multiple hydroxyl group, especially in the B-ring, enhance their antioxidant acitivity. The hydroxyls in ring B are the primary active sites in interrupting the oxidation chain (Pokorny *et al.*, 2001).

2.2.4 Phenolic compounds

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. In general, there are two basic categories of antioxidants, natural and synthetic. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in food or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity. Herbs have been used for a large range of purposes including medicine, nutrition, flavorings, beverages, dyeing, repellents, fragrances, cosmetics, charms, smoking, and industrial uses. Since prehistoric times, herbs were the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century. Even though a variety of herbs are known to be sources of phenolic compounds, their compositional data are insufficient. Moreover, various herbs along with vegetables and fruits contain numerous phytochemicals in addition to phenolic compounds, such as nitrogen compounds, carotenoids, and ascorbic acid. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower incidence and lower mortality rates of cancer in several human cohort (Zheng and Wang, 2001).

Phytochemicals in fruits and vegetables can have complementary and overlapping mechanisms of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects. Recent studies showed that the phytochemicals, especially phenolics, in fruits and vegetables are the major bioactive compounds for human health benefits. There was a direct relationship between the total phenolic contents and the antioxidant activities in fruit and vegetables (Wang and Mazza, 2002).

Eberhardt *et al.* (2002) demonstrated that the vitamin C in apples only contributed to less than 0.4% of the total antioxidant activity, suggesting that a complex mixture of phytochemicals in fruits and vegetables provided protective health benefits mainly through a combination of additive and/or synergistic effects. In the human gastrointestinal system, food is digested in the stomach (an acid environment with enzymes), small intestine (a mild base environment with enzymes), and colon (a neutral pH environment with intestinal microflora). Phenolics in fruits are in both soluble free and bound forms. Bound phenolics mainly in the form of β-glycosides, may survive the human stomach and small intestine digestion and reach the colon intact, where they are released to exhibit their bioactivity with health benefits (Sun *et al.*, 2002).

Phenolic compounds are widely distributed in the higher plants, being found mostly in fruits, vegetables, seeds, herbs, and medicinal plants. Phenolic contents vary among different cultivars of fruits and vegetables, and within different tissues. Most phenolic compounds in food are naturally present in conjugated forms; in higher plants, low molecular weight phenols occur as glycosides or esters with sugars or related compounds. Phenol in the free state are normally found only in dead or dying tissues. It is, hence, of metabolic significance that flavonols are widely distributed in plant vegetative tissues in unconjugated forms, while most of the other groups of flavonoids occur as glycosides (Imeh and Khokhar, 2002).

Vison and co-workers (2001) reported that the conjugated fraction of phenolic compounds varied widely among commonly consumed fruits, from 8.7% in cranberry to as much as 90% in watermelon. Phenolic compounds are closely associated with the sensory and nutritional quality of food, contributing directly or indirectly to desirable or undesirable aroma and taste. In low concentrations, phenolics may protect food from oxidative deterioration; however, at high concentrations, they (or their oxidation products) may participate in discoloration of food, and interact with proteins, carbohydrates and mineral. Phenolic compounds are thus good antioxidants and substrates for oxidative browning (although, if uncontrolled, the latter one may be detrimental) (Imeh and Khokhar, 2002).

The action of phenolics as antioxidants is beneficial in food and in biological systems, where they are preferentially oxidized, thereby sparing nutrients (such as

vitamin E), body cells and tissues. Recently, phenols in foods have gained much attention, owing to their antioxidant activities and their possible beneficial implications in human health, a consequence of their demonstrated biological activity in prevention of cancer and cardiovascular diseases. Reliable composition data on phenolics and assessment of their activity are essential for calculating dietary intakes for epidemiological intervention and for planing clinical studies to elucidate health protective aspects of fruits (Imeh and Khokhar, 2002).

Polyphenols in vegetables, fruits, and teas can prevent degenerative diseases including cancers through an antioxidative action and/or the modulation of several protein functions. For example, the intake of antioxidative polyphenols reduces coronary heart disease mortality by suppressing the oxidation of low-density lipoprotein. Polyphenols exhibit agonism and/or antagonism of carcinogenesis-related receptors such as epidermal growth factor. The number of natural polyphenols has estimated to be over one million, because they are generally occurred as glycosides, and the sugar species and binding forms show great variety (Sakakibara *et al.*, 2003).

Increased consumption of fruit and vegetables can increase the plasma antioxidant capacity in humans and has been associated with protection against various diseases, including cancers and cardio- and cerebrovascular disease. It is not known what dietary constituents are responsible for this association, but it is often assumed that antioxidants contribute to the protection. However, the effects of increased consumption of fruit and vegetables on the overall antioxidant status is not known, and the results from intervention trials have not been conclusive regarding the protection of supplementation with pure antioxidants. It is therefore plausible that the putative beneficial effects of a high intake of fruit and vegetables on the risk of diseases may not result exclusively from the action of antioxidants, such as the well-characterized vitamins E and C or β-carotene. Rather, they may result from the action of lesser known compounds or from a concerted action of a combination of different antioxidants present in food (Cao *et al.*, 1998).

2.2.5 Antioxidant functionality during food processing

Types of changes in antioxidants during food processing and storage

The most importance processing technologies which affect antioxidants and oxidative stability of foods are listed in Table 2.2.

Table 2.2 Types of food processing which affect antioxidants and oxidative stability of foods

Temperature	Type of process	Examples
Elevated temperature	Water as heat transfer medium	Pasteurisation
		Sterilisation
		Blanching
		Evaporation
		Extrusion
	Air as heat transfer medium	Drying
		Roasting, Baking
	Oil as heat transfer medium	Frying
	Waves giving energy	Microwave
		Infrared heating
Ambient temperature	Effect of enzymes	Fermentation
	Effect of chemicals	Curing, smoking
	Effect of time	Storage

Source: Pokorny *et al.* (2001)

Antioxidants present in foods change during the processing, in a similar way to other food components. Although many studies deal with estimated losses of food nutrients, including antioxidants, through different operations of food processing, only the residual concentration of antioxidants has been determined in most cases, rather than total antioxidant capacity of foods. Very different, and sometimes even opposite, effects on the intrinsic antioxidant properties of foods can occur during processing and storage, as evident from Table 2.3 (Pokorny *et al.*, 2001).

Table 2.3 Changes in antioxidant properties of foods during processing and storage

Resulting resistance against oxidation	Examples of changes in foods, affecting the antioxidant activity
No effect	In case of moderately intensive processes positive and negative influences are counterbalanced
Increased resistance	Transformation of antioxidants into more active Compounds, such as glycosides into aglycones,
	formation of novel compounds, such as Maillard products,
	destruction of pro-oxidants, especially photosensitizers or
	heavy metals
	Inhibition of oxygen access, e.g. encapsulation
Decreased resistance against oxidation	Destruction of antioxidants by oxidation or interactions with other food components
	Losses of antioxidants by evaporation
	Improved access of oxygen, e.g. caused by drying
	Formaton of pro-oxidants or their liberation from inactive complex

Source: Pokorny *et al.* (2001)

The most important losses of antioxidant activity occur as a result of chemical changes in antioxidants present in food materials.

Naturally, the most pronounced changes result from oxidation reactions occurring rapidly on heating or slowly in storage (Table 2.4). Antioxidants are oxidised either by lipid oxidation products (mainly hydroperoxides) or directly by oxygen, either dissolved in lipidic and aqueous phases or absorbed from the air. Other changes are mostly neglected even when they affect food resistance more than oxidation processes, such as removal of water or evaporation of volatile antioxidants or pro-oxidants (Pokorny *et al.*, 2001).

Table 2.4 Oxidative destruction of antioxidants in foods

Reaction type	Examples
Oxidation with lipid oxidation products	Oxidation with lipidic free radicals ROO* or RO*
	Oxidation with lipid hydroperoxides ROOH
	Oxidation with lipid dioxolanes
Oxidation with singlet oxygen	In presence of chlorophyll pigments
Oxidation with triplet oxygen	Oxidation of antioxidant free radicals A*
	Formation of quinones from phenolics
Oxidation with heavy metals	Metal ions higher oxidation state

Source: Pokorny *et al.* (2001)

Changes under heating when water is the heat transfer medium

Exposure of food components to temperatures above ambient conditions (during heat processing) is a major cause of detectable changes, not only of nutrition quality, but also of antioxidant activity. Although some processes involving higher temperatures are used in order to produce positive changes, especially of the sensory value, they often result in loss of nutritional quality, and in some cases, in losses of their resistance against lipid oxidation (Pokorny *et al.*, 2001).

The application of moderate temperatures, up to 100°C, reduces the negative changes of nutritional quality. Various changes under these conditions are listed in Table 2.5. Food processing by application of such temperatures results in protein denaturation and aggregation reactions. Killing micro-organisms is the main reason for moderate heating. The denaturation of enzymes, which are also proteins, is often desirable. However, various changes occur in parallel, including changes in flavour, texture and colour as well as destruction of heat-sensitive nutrients. These factors have to be considered, and thermal processes must be carefully designed, to avoid overprocessing and unnecessary reductions in product quality (Pokorny *et al.*, 2001).

Table 2.5 Changes in antioxidants during treatment of food with hot water or steam

Type of process	Type of precursors	Type of products
Enzyme denaturation	Oxidoreductases	Inactive enzymes
Hydrolysis	Heteroglycosides	Aglycones
Pyrolysis	Ascorbic acid	Degradation products
Extraction	Vitamins, phenolics	Loss in cooking water

Source: Pokorny *et al.* (2001)

Pasteurisation and blanching are similar thermal processes utilising relatively mild thermal treatments to achieve the desired stability of food products during subsequent storage. The pasteurisation is most often associated with liquid foods, while the more complex blanching is associated with solid foods. It is generally recognised that application of a higher temperature for shorter time will lead to improve quality retention in pasteurisation and blanching. Boiling is often used for processing vegetables, fruit, meat and fish. Boiling temperatures are 100°C (or slightly higher or lower, depending on the atmospheric pressure) (Pokorny *et al.*, 2001).

Changes during pasteurisation

Losses of vitamins are a good marker of negative changes due to thermal destruction. Transformations of tocopherols (vitamin E) are the best known changes in antioxidants during thermal food processing. But they are only moderate during pasteurisation. Losses of ascorbic acid (an important inhibitor of oxidation) are used as an indicator of food quality and therefore the severity of pasteurisation, blanching, or the length of cooking. These changes are due mainly to thermal destruction, and to a lesser extent, to oxidation. In fruit juices the main cause of colour deterioration is enzymatic browning of polyphenolics, catalysed by polyphenoloxidases in the presence of dissolved oxygen. Polyphenoloxidases destroy phenolic antioxidants so their rapid inactivation is desirable when the preservation of phenolics is important. Losses of ascorbic acid and carotenes are minimised by de-aeration as well (Pokorny *et al.*, 2001).

Changes during blanching

Commercial methods of blanching involve passing solid foods through an atmosphere of saturated steam or a bath of hot water, so that only the water in both physical states is the carrier of heat. Rapid heating of the food material deactivates enzymes, such as lipoxygenases, which would otherwise catalyse lipid oxidation. The primary products of lipoxygenase-catalysed oxidation – lipid hydroperoxides – would partially destroy natural antioxidants. The deactivation of polyphenoloxidases is also very useful for the protection of phenolics against enzyme-catalysed oxidation into the respective quinones, the antioxidant activity of which is very low or non-existent (Pokorny *et al.*, 2001).

Changes during sterilisation

Another more severe thermal food process is referred to as a commercial sterilisation, which proceeds at higher temperatures than pasteurisation. The intensity of the commercial sterilisation process is such that is results in significant changes in the quality characteristics of the products. Excepting the elimination of micro-organisms, however, these changes are usually more detrimental than positive. In canned fruits and vegetables substantial vitamin losses may occur in all water-soluble vitamins, particularly ascorbic acid, which is the most important antioxidant in these foods. Therefore, the presence of residual oxygen in the medium has to be minimised. Lipoxygenases were deactivated during treatments of fruit juices at 70-90°C, depending on the type of juice, for instance, the lipoxygenase activity did not substantially change in apricot and apple juices, but decreased in carrot and green bean (Pokorny *et al.*, 2001).

Changes during boiling

Boiling is very common procedure for food preparation. In this case boiling water transfers heat. It is useful to add food to hot water to shorten the time for enzyme deactivation, especially the deactivation of oxidoreductases. During boiling, the antioxidant activity of proteins is affected because of their denaturation. The effect on antioxidants is similar to that occurring during sterilisation. The heat denaturation of haeme pigments in foods of animal origin could increase the pro-oxidative effect of

iron and thus reduce the activity of antioxidants. During boiling, antioxidants are partially extracted and remain in the boiling water. If the boiling water is not used but discarded these antioxidants are lost (Pokorny *et al.*, 2001).

Changes in antioxidants during fermentation processes

Fermentation processed are enzymatic reactions taking place for a relatively long time at temperatures close to the ambient temperature so that lipids are damaged by oxidation processes to a negligible degree, and thus, antioxidants are not damaged by oxidation as well. Oxygen is partially replaced by carbon dioxide (CO_2) in most fermentation processes (Pokorny *et al.*, 2001). Hydrolytic processes could cause cleavage of esters or glycosides of phenolic antioxidants into the respective acids or aglycones. They are usually more active as antioxidants than original compounds, such as quercetin and myricetin, which are more active than the respective glycosides. Polyvalent organic acids with a synergistic activity may be formed (Pokorny *et al.*, 2001).

Changes in antioxidant functionality during storage

The main reason for the application of antioxidants is to prolong the shelf life of food products by inhibiting the rancidification and other deterioration processes. Several methods exist for food storage. Their advantages and disadvantages from the standpoint of antioxidant preservation are compared in Table 2.6 (Pokorny *et al.*, 2001).

Table 2.6 Methods of food storage and their effect on antioxidant functionalities

Process	Advantages	Disadvantages
Simple storage with free Access of air	Low price, simplicity	Easy destruction of antioxidants
Vacuum packaging	Good protection, when low residual oxygen	High price
Packaging under inert gas	Good protection, when low residual oxygen	High price
Use of oxygen scavengers	Satisfactory, but no complete protection	High price, regulation should be observed
Addition of antioxidants	Satisfactory, but no complete protection	High price, regulation should be observed

Source: Pokorny *et al.* (2001)

Onion and garlic juices were added to ground lamb and after cooking the mixture was stored. Onion juice was found to be more efficient than garlic juice in inhibiting the warmed-over flavour, due to oxidation and rancidity of the product (Pokorny *et al.*, 2001).

The effects of packaging in a modified atmosphere and of cooking were evaluated in fresh-cut spinach, which contains both flavonoids and vitamin C. The content of total flavonoids remained fairly constant during storage both in air and in a modified atmosphere. A decrease in total antioxidant activity was observed during storage, particularly in spinach stored under modified atmosphere, which proves that antioxidants were gradually consumed by free radical scavenging. Boiling extracted 50% total flavonoids and 60% vitamin C in the cooking water, which could be used for further processing. Both ascorbic acid and total polyphenols were significantly

destroyed during cold storage at +5°C for 6 months. Wine flavonoids and anthocyanins are relatively stable in closed bottles but are easily oxidised once the bottle has been opened. The oxidation products easily polymerise. The flavour of wine, especially of red wine, is very much influenced by these processes so that wine should be consumed on the same day as the bottle is opened. Ascorbic acid added to extracts of elderberries and red grape skin caused higher degradation of anthocyanins during their storage for 6 months due to their reduction to colourless leucoanthocyanidins (Pokorny *et al.*, 2001).

Changes in antioxidant properties of green and black tea infusions as a result of processing and storage were evaluated by measuring their chain breaking activity, oxygen-scavenging activity and redox potential. The results showed that pasteurisation, storage and forced oxygenation caused increased browning in both green and black tea extracts. These changes were accompanied by increasing colour intensity. Green tea catechins and residual catechins of fermented black tea were obviously oxidised into the respective quinones, which were then dimerised (Pokorny *et al.*, 2001).

2.2.6 Antioxidant activity

Antioxidant activity depends on many factors such as the lipid composition, antioxidant concentration, temperature, oxygen pressure, and the presence of other antioxidants and many common food components, e.g. proteins and water (Pokorny *et al.*, 2001).

Measuring Antioxidant activity

Method considerations

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods need special equipment and technical skills for the analysis. These types of methods published in the literature for the determinations of antioxidant activity of foods involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical-scavenging activity of antioxidants against

free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$) radical, the superoxide anion radical ($O_2^{\cdot-}$), the hydroxyl radical (OH$^{\cdot}$), or peroxyl radical (ROO$^{\cdot}$). The various methods use to measure antioxidant activity of food products can give varying results depending on the specificity of the free radical being used as a reactant. There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assays have been used extensively since the 1950's to estimate the peroxidation of lipids in membrane and biological system. These methods can be time consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix etc. and may not be practical when large numbers of samples are involved. Antioxidant activity methods using free radicals are fast, easy and simple. The ABTS [2,2'-azinobis(3-ehtylbenzothiazoline-6-sulfonic acid)] radical cation has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their properties as electron- or H-donating agents. Cao *et al.* (1998) have used the Oxygen Radical Absorbance Capacity (ORAC) procedure to determine antioxidant capacities of fruits and vegetables. In the ORAC method, a sample is added to the peroxyl radical generator, 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) and inhibition of the free radical action is measured using the fluorescent compound, B-phycoerythrin or R-phycoerythrin (Prakash, 2001).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of food. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity will helps to understand the functional properties of food (Prakash, 2001).

Radical-scavenging methods

Radical scavenging is the main mechanism by which antioxidants act in foods. Several methods have been developed in which the antioxidant activity is assessed by the scavenging of synthetic radicals in polar organic solvents, e.g. methanol, at room temperature. Those used include 2,2-dipheneyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzthiazoline-sulphonic acid) (ABTS) radicals (Pokorny *et al.*, 2001).

In the DPPH test, the scavenging of DPPH radicals is followed by monitoring the decrease in absorbance at 515 nm which occurs due to reduction by the antioxidant (AH) or reaction with a radical species ($R^{\cdot}$) (Pokorny *et al.*, 2001).

$$DPPH^{\cdot} + AH \longrightarrow DPPH - H + A^{\cdot}$$

$$DPPH^{\cdot} + R^{\cdot} \longrightarrow DPPH - R$$

Fast reaction of DPPH radicals occurs with some phenols e.g. α-tocopherol, but slow secondary reactions may cause a progressive decrease in absorbance, so that the steady state may not be reached for several hours. Most papers in which the DPPH method has been used report the scavenging after 15 or 30 min reaction time. The data is commonly reported as EC_{50}, which is the concentration of antioxidant required for 50% scavenging of DPPH radicals in the specified time period (Pokorny *et al.*, 2001).

The ABTS radical cation is more reactive than the DPPH radical, and reaction of the ABTS radical cation with an antioxidant is taken as complete within 1 min. The method of generation of the radical cation has changed several times since the method was first described. The most recent method describes the use of potassium persulphate to oxidise ABTS to the radical cation. The radical scavenging activity assessed by the ABTS method has been expressed as the TEAC (trolox equivalent antioxidant capacity) value in most papers employing this method (Pokorny *et al.*, 2001).

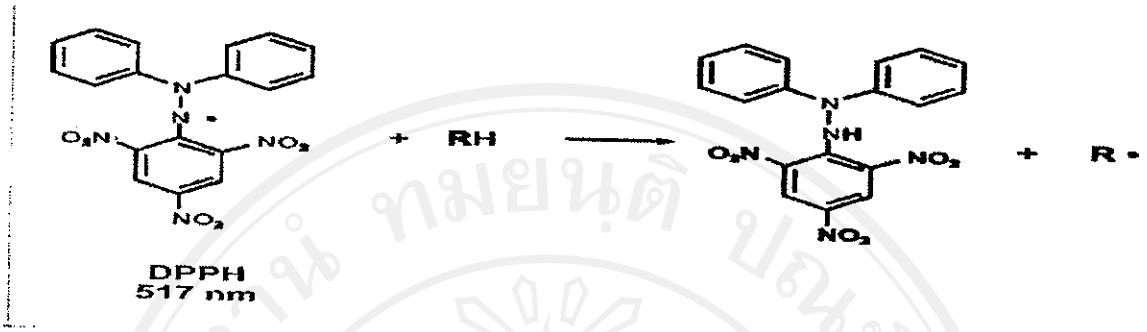


Figure 2.5 The structure of DPPH and its reduction by an antioxidant
Source: Prakash (2001)

The DPPH method

A simple method that has been developed to determine the antioxidant activity of foods utilizes a stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The antioxidant activity of various foods can be determined accurately, conveniently, and rapidly using DPPH. This method can be used successfully for solid samples without prior extraction and concentration, which saves time. The structure of DPPH and its reduction by an antioxidant are shown in Figure 2.5. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH redical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourisation is stoichiometric with respect to number of electrons captured. Antioxidant compounds may be water-soluble, lipid-soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in quantification of antioxidant activity of foods. 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, 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, mineral and vitamin content (Prakash, 2001).

2.3 Pasteurization

The definition of pasteurization is a partial sterilization of food at a temperature that destroys harmful microorganisms without major changes in the chemistry of the food. The main pasteurized foods that contribute significant nutrients are milk and some fruit juices. Pasteurization involves heating food for a short time to kill harmful micro-organisms that are present in the food. Not all micro-organisms are destroyed, and spoilage of the food may still occur on storage, but this can be delayed by refrigeration. Nutrient losses during pasteurization of milk and fruit juices are generally small, and in the case of fruit juices they must contain not less than a specified minimum amount of vitamin C. This generally means that vitamin C is added by the processor to make up for any losses that occurred during processing. To minimize further nutrient losses, milk and fruit juices should be stored away from light and in a cool place (Rattanapanon, 2002).

The main objective of the fruit juices pasteurization is to inhibit of pectinesterase and polygalacturonase. Example of the objective of the fruits juice pasteurization and the condition to do pasteurization are shown in Table 2.7 (Rattanapanon, 2002).

Table 2.7 Objective of fruit fuices pasteurization.

Food	Main Objective	Sub objective	Conditions
Fruit juices pH<4.5	Inactivate enzyme Pectinesterase and Polygalacturonase	Destroys food spoilage microorganisms such as yeast and mold	63^0C for 30 minute 71^0C for 1 minute 88^0C for 15 second* *then rapid reduce temperature to 3-7^0C

Source : Rattanapanon (2002)