CHAPTER I
INTRODUCTION

We are now increasingly aware of the fact that natural resources are limited. We are also convinced of the need to preserve the natural environment. Recycling by-products and minimizing wastes are crucial aspects of this project. The recovery of high value-added chemicals is of special interest.

Processing agricultural and forestal materials produces residues which disposal is often a problem for industries. These industrial wates, particularly of plant origin, contain a variety of biologically active species, which mostly go to waste. At the same time there is increasing consumer appreciation of natural products as alternatives to synthetic components in variety of goods going from food to personal care formulation. In any case, the rocovery of such bioactive components has to be economically viable. Research must be oriented towards the improvement of extraction technologies, the correct assessment of biological activities and proving the security of the products.

Recycling by-products is of interest from the environmental viewpoint (reduction of contaminant charge) and for health benefits derived from their biological activities. These products may be used as such or may be starting materials for preparation of novel compounds. Figure 1.1 summarizes the main bioproducts, their sources and their main field of applications. Some of polysaccharides such as pectins from fruit by-products and chitin derived chitosan from shrimp shells have different applications, mostly related to their film forming properties [1, 2]. Oligosaccharides from fruits and wood are used as prebiotic fibre components of functional food [3, 4]. Monosaccharides are starting materials for drug development [5]. Other sugars such as xylitol, derived from xylose, are used as alternative sweeteners [6, 7]. The fruit beverage and fruit canning industries generate fruit seeds, which are rich in oils of high content in unsaturated fatty acids and glycerides of possible direct application in cosmetics. Such oils may be also used as starting materials for the preparation of novel chemicals [8], such as biocompatible food preservative [9]. Protein rich meals are by-products from production of seed (e.g. soy, sunflower) oils. These protiens and their hydrolysates may be used in animal and poultry feed and/or human functional nutrition [10, 11]. Hydrolysates from keratinous parts of animals are used as their

moisturizing agents. A great variety of secondary metabolites with biological activities are present in plants. They have been given increasing significance as functionally active components of foods and drinks and are present in great amounts in by-products from food and forestry industries.

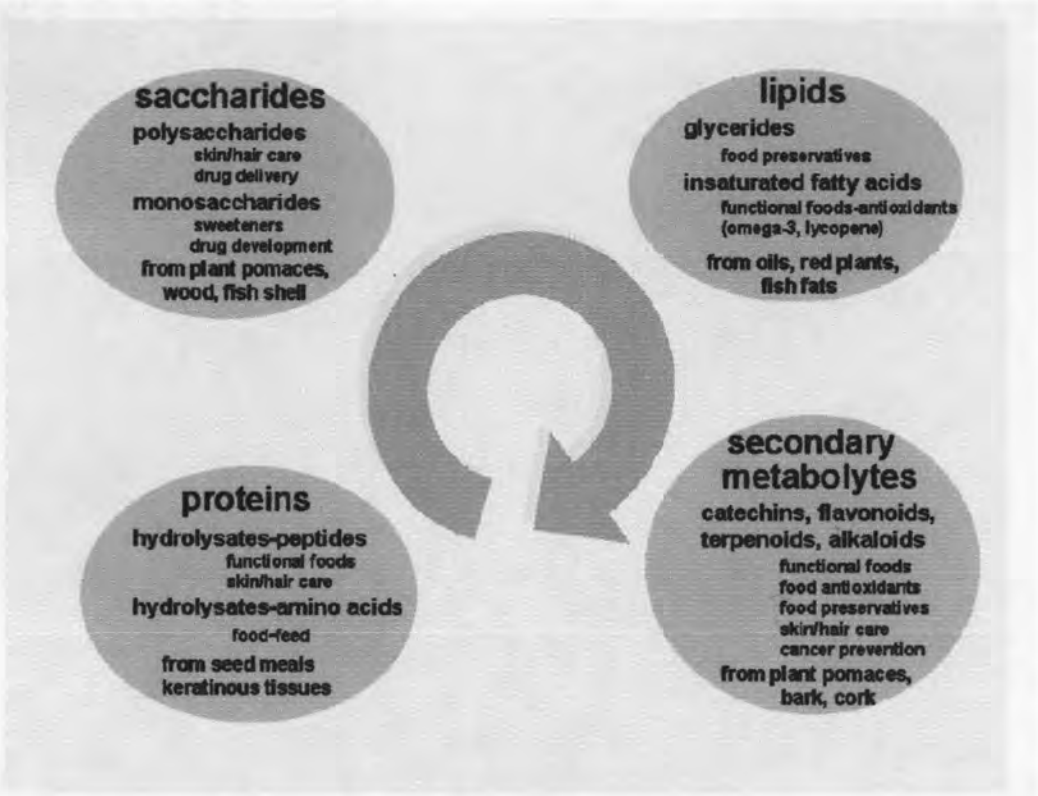


Figure 1.1 summarizes the main kinds of bioproducts, their sources and main field of applications (*Electro. J. Environ. Agric. Food Chem.* ISSN 1579-4377)

In Europe alone, hundreds of million tons of by-products from biological sourecs are generated every year. These by-products contain a myriad of bioactive compounds, which are there free for the taking. While the environmental benefits seem clear, the value of the products obtained must compensate for the costs of recovering them. In the sense it is important to both improve the extraction processes and substantiate the activity and safety claims.

The most agricultural countries, particularly of asian countries. Agricultural by-products are an important source of energy. Cellulosic biomass is an abundant renewable resource on earth and includes various agricultural residues. The degradation of cellusic material has gained increasing research attention due to its worldwide availibility and immense potential for its transformation into sugars, alternative fuels and chemical feedstock.

Thailand is an agricultural country. Each year the country produces not only agricultural products but also a lot of agricultural residues (Table 1.1). It is estimated that there are more than 50 million tons of agricultural residues per year [12]. Only a few residues are used, so most of them are considered useless wastes.

Table 1.1 Resources of agriculture and their residue in Thailand (1998)

Type	Production (1000 ton)	Residues	Residue to product ratios (RPR)	Residue generated (1000 ton)
1. Sugar cane	56,394	Bagasse	0.291	16,411
2. Paddy	22,332	Husk	0.230	5,136
		Straw	0.447	9,982
3. Oil palm	2,688	Empty bunches	0.428	1,150
		Fiber	0.147	395
		Shell	0.049	132
		Frond	2.604	7,000
		Male bunches	0.233	626
4. Coconut	1,419	Husk	0.362	514
		Shell	0.160	227
		Empty bunches	0.049	70
		Frond	0.225	319
5. Cassava	18,084	Stalk	0.088	1,591
6. Maize	4,533	Corn cob	0.273	1,133
7. Groundnut	147	Shell	0.323	47
8. Cotton	75	Stalk	3.232	242
9. Soybean	359	Stalk, leave, shell	2.663	956
10. Sorghum	225	Leaves and stem	1.252	282

Sources: 1. Center for Agriculture Information, agricultural statistics of Thailand crop year 1996/1997, Bangkok, Thailand, 1998

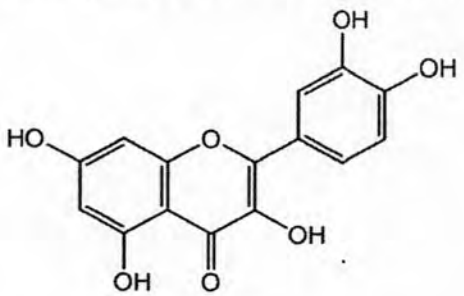
2. Black & Veatch (Thailand), Thailand biomass-based power generation and cogeneration within small rural industries (progress report), January 1999

Literature reviews

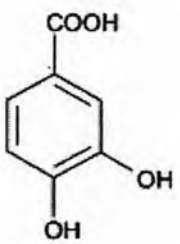
Recently, many research projects deal with agricultural by-products. Active compounds obtained from agricultural extraction processes were tested for biological activities such as antioxidant, antimicrobial, anticancer, anti-aging, anti-inflamatory, antimutagenic, anti-tyrosinase and UV absorption activities etc.

In 1994, Yen and coworker investigated the scavenging effect of methanolic extracts of peanut hulls (MEPH) on free radical and active-oxygen species. MEPH showed marked activity as a radical scavenging in the experiment using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), indicating that MEPH has effective activities as a hydrogen donor and as a primary antioxidant to react with lipid radicals. MEPH also possessed antioxidative activity toward hydrogenperoxide (H_2O_2) and superoxide ($O_2^{\bullet-}$), indicating that MEPH has scavenging activity on H_2O_2 and $O_2^{\bullet-}$ [13].

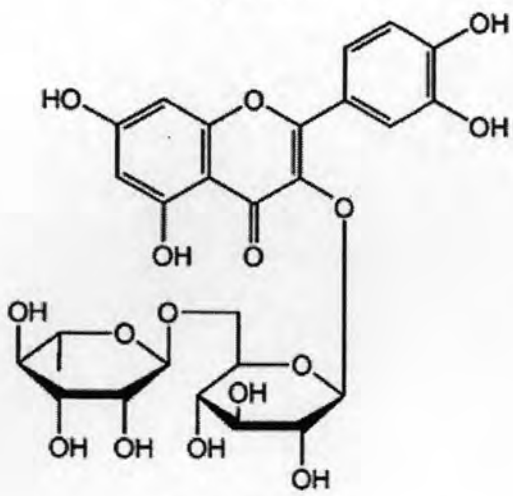
In 1997, Watanabe and coworker seperated antioxidant compounds from Buckwheat *(Fagopyrum esculentum)* hulls by using Sephadex LH-20 column chromatography. Five antioxidant compounds were isolated and identified as quercetin, hyperin, rutin, protocatechuic acid and 3,4-dihydroxybenzaldehyde (Figure 1.2) [14].



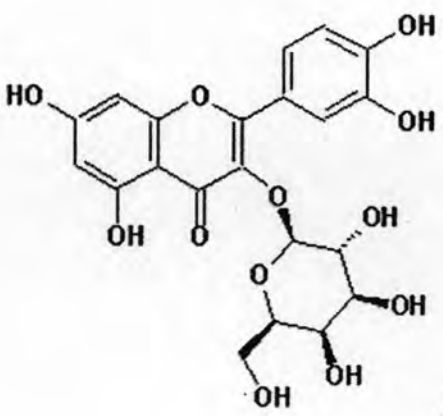
quercetin



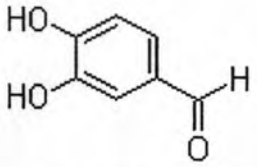
protocatechuic acid



hyperin



rutin

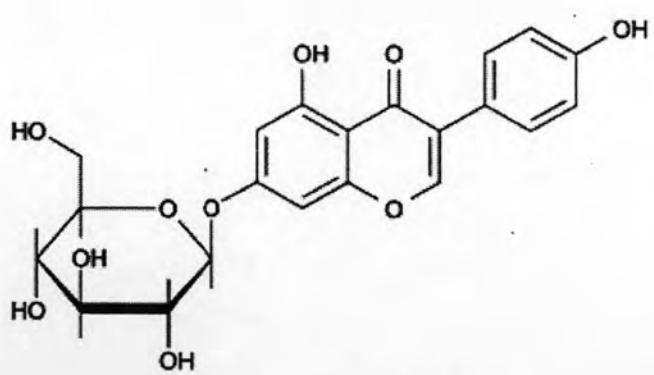


3,4-dihydroxybenzaldehyde

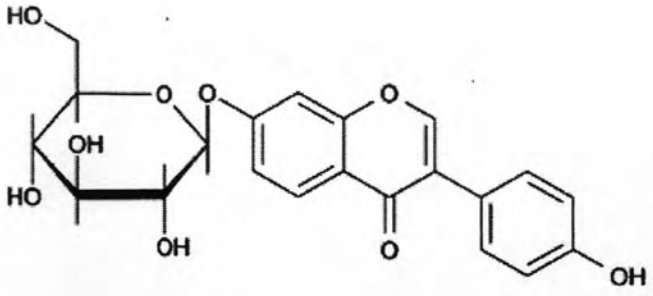
Figure 1.2 The molecular structures of quercetin, protocatechuic acid, hyperin, rutin and 3, 4-dihydroxybenzaldehyde

In 1998, Bocco and coworker extracted citrus peels and seeds, which are byproducts of the juice extraction industry and test their antioxidant activity. The results indicated that seed possessed greater antioxidant activity than peels. The composition of all tested samples was studied by HPLC, methanol extract are rich in flavones and glycosylated flavanones, wheras hydrolyzed extracts contain mainly phenolic acid and flevonols [15].

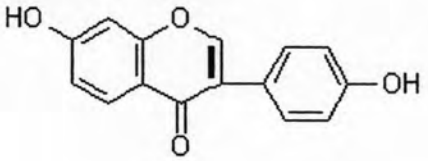
In 2001, Plewa and coworker isolated antigenotoxic components and cancer cell growth suppressors from soy hull and corn distillated solid (CDS). The isolated isoflavone, daidzein expressed antigenotoxic activity, however, genistein and genistin enhanced DNA damage. In microplate assay, genistin, daidzein and daidzin (Figure 1.3) expressed a wide range of growth supression on HT-29 human colon cancer cells [16].



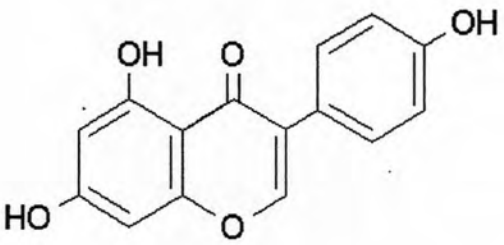
Daidzein



Daidzin



Genistin



Genistein

Figure 1.3 The molecular structures of daidzein, daidzin, genistin, and genistein

In 2003, Miyazawa and coworker investigated the inhibitor of tyrosinase activity in black rice bran. The ethyl acetate extract had the most potent inhibition against tyrosinase activity. Inhibitory compound in the ethyl acetate extract was further isolated by silica column chromatography and identifed by GC-MS, IR, and ^{1}H and ^{13}C NMR spectroscopy. The inhibitory compound is protocatechuic acid methyl ester (Figure 1.4) [17].

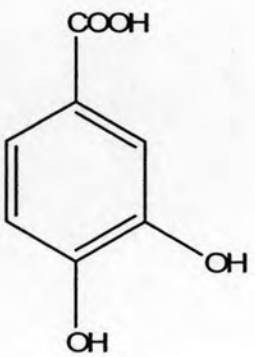
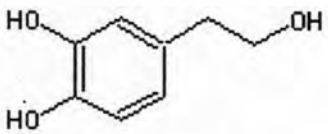


Figure 1.4 The molecular structure of protocatechuic acid methyl ester

In 2004, Louli and coworker recoverd the phenolic compounds contain in the wine industry by-products and examine their antioxidant activity. This work employed a combined process of liquid and supercritical solvent extraction. The extract of a higher antioxidant activity was obtained by using ethyl acetate as solvent and then, these extracts were further treated with supercritical carbondioxide (SC-CO_2) at various extract pressure, which resulted in their significant enrichment in phenolic compounds and the improvement of their antioxidant and organoleptic properties [18].

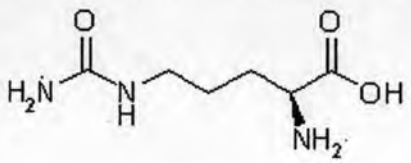
In 2005, Bouzid and coworker used fungal enzyme, cinnamoyl esterase, as a tool to release simple phenolic compounds from olive oil by-product. Free simple phenolic compounds including hydroxytyrosol (Figure 1.5) were released. The evluation of antioxidant activity of hydroxytyrosol indicated that the compound possess higher antioxidant activity than ascorbic acid and BHT. Its good solubility in oily and aqueous media makes natural hydroxytyrosol vary useful for application in multi-component foods [19].



Hydroxytyrosol

Figure 1.5 The molecular structure of hydroxytyrosol

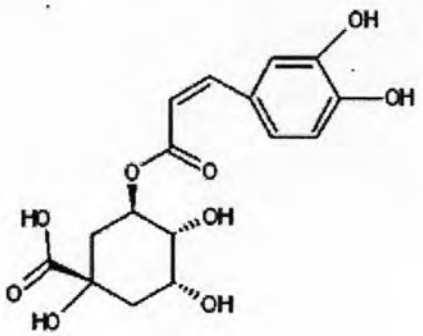
In 2005, Rimando and Perkins-Veazie determined the range of citrulline in watermelon rind. Citrullin (Figure 1.6) is a non-essential amino acid first identified from watermelon juice and is used in the nitric oxide system in human and has potential antioxidant and vasodilation roles. A method using gas chromatography-mass spectrometry (GC-MS) is separation citrullin from glutamic acid and determination the citrullin content. These results indicated that watermelon rind, an unutilized agricultural waste, offers a source of natural citrullin [20].



Citrullin

Figure 1.6 The molecular structure of Citrullin

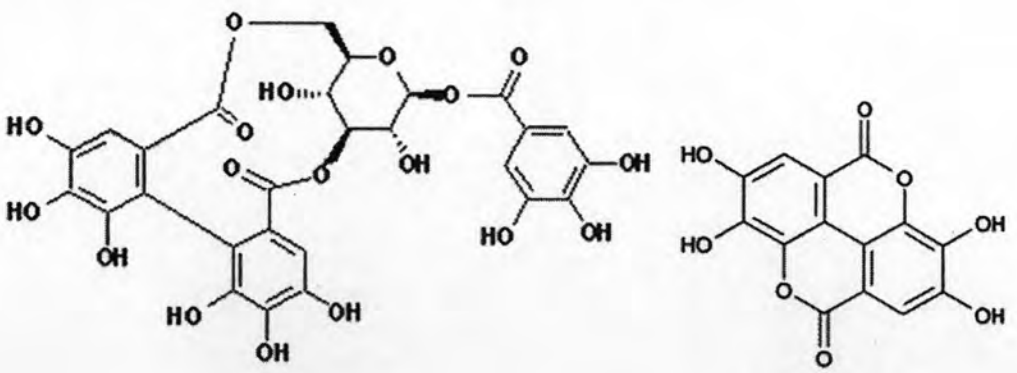
In 2005, Kanatt and coworker studied the effective utilization of potato peel, a waste generated in large quantities by the food industry, as antioxidant. Potato peel extract (PPE) exhibited high phenolic content. Chlorogenic acid (Figure 1.7) was the major component and showed excellent antioxidant activity as determined by β-carotene bleaching and radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) [21].



Chlorogenic acid

Figure 1.7 The molecular structure of chlorogenic acid

In 2005, Rangkadilok and coworker studied the phytochemicals extract from peel, pulp, and seed tissue of longan fruit (*Euphoria longana* Lam.) and identified the major component as galic acid, corilagin (an ellagitannin), and ellagic acid (Figure 1.8) by using reverse phase HPLC method. From quantification, seed contained the highest levels of the three phenolics and pulp contained the lowest [22].



Corilagin Ellagic acid

Figure 1.8 The molecular structures of corilagin and ellagic acid

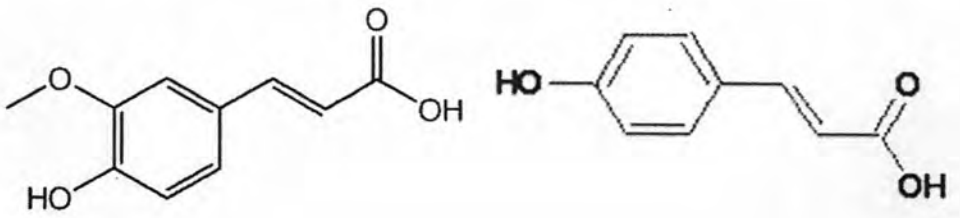
In 2005, Iqbal and coworker evaluated antioxidant activity by measurement of total phenolic content antioxidant activity in linoleic acid system, reducing power, metal chelating ability, scavenging capacity by DPPH radical and ABTS cation radical

and conjugated diene in five indegenous rice bran varieties, i.e. Rice bran-super kernel (RB-kr), Rice bran-Super 2000 (RB-s2), Rice bran-Super Basmati (RB-bm), Rice bran-Super-386 (RB-86) and Rice bran-Super fine (RB-sf). The overall order of antioxidant activity was RB-kr > RB-s2 > RB-bm > RB-86 > RB-sf [23].

In 2005, Esposito and coworker studied antioxidant activity of wheat bran by-products, which were obtained by an industrial milling process. The antioxidant activity is higher for the internal bran fraction. The antioxidant activity of some durum wheat by-product fraction is comparable to that of widespread fruits and fresh vegetables, likely due to the presence of fibre-bound phenol compounds. The high fibre content and antioxidant activity of durum wheat bran by-products can be of particular interest for their use in cereal-based products [24].

In 2005, Lapornik and coworker investigated the effect of solvent and extraction time on the yield of extracted antioxidants from grape, black, and red currant by-products (marc). Ethanol and methanol extracts of red and black currant contain twice more anthocyanins and polyphenols than water extract. Extracts made from grape marc had seven times higher values than water extracts. The highest antioxidant activity was in extracts made from black currant marc while the lowest was in red currant marc extracts. Grape extracts had high antioxidant activity due to its high content of polyphenols [25].

In 2005, Xu and coworker determined ferulic (FA) and *p*-coumaric acid (*p*-CA) (Figure 1.9) and related phenolic compounds in the cell wall of sugarcane bagasse (SCB) by using mild alkaline hydrolysis of the alkali-soluble lignin preparations and acid hydrolysis of the 90% acidic dioxane-soluble lignin fraction. The hydrolysis by alkaline released 48.8% of the total ester-linked *p*-CA and 43.8% of the total esterified FA while the hydrolysis by 90% acidic dioxane released 38.8% of the total ester-linked *p*-CA and 43.8% of the total esterified FA [26].



Ferulic acid *p*-coumaric acid

Figure 1.9 The molecular structure of ferulic acid and *p*-coumaric acid

In 2006, Colombo and coworker used high performance liquid chromatograhphy (HPLC) with photo-diode array to separate and quantify flavonoids in sugarcane leaves and bagasses. The result showed sugarcane flavonoids consist of a complex mixture of aglycones and glycosides (including flavonolignan glycosides) [27].

In 2006, Kim and coworker evaluated the effect of heating and physical conditions of grape seeds, whole and powdered forms, on the antioxidant activity of their extracts. After heating, grape seeds were extracted with 70% ethanol, and total phenol contents (TPC), radical scavenging activity (RSA) and reducing power of the extracts were determined. The antioxidant activity of grape seed extract was increased after thermal treatment. The extract was analyzed by GC-MS and found several low-molecular-weight phenolic compounds, such as azelaic acid, 3,4-dihydroxy benzoic acid, and *o*-cinnamic acid (Figure 1.10). These results indicated that antioxidant activity of grape seed extract was affected by heating condition (temperature and time) and physical condition of grape seeds at time of heat treatments [28].

Azelaic acid

Figure 1.10 The molecular structure of azelaicacid

In 2006, Anagnostopoulou and coworker evaluated radical scavenging activity and determined the total phenolic content in seven different extracts of Navel sweet orange (*Citrus sinensis*) peel. High phenolic content and radical scavenging activities were found for the ethyl acetate fraction. The antioxidant activity was compared with reference compounds, Trolox, ascorbic acid and quercetin, which are already known for their good antioxidant activity. The radical scavenging activity of the ethyl acetate fraction approached the activity of the standards [29].

In 2007, Farhoosh and coworker evaluated the antioxidant activity of various extracts of old tea leaves (OTL) and black tea waste (BTW) in comparison with that of green tea leaves (GTL). The highest extraction yield and antioxidant activity were found in hot water extracts of GTL. Hot water extracts of OTL an BTW showed the same statically significant antioxidant activity ($P<0.05$). BTW extracts had antioxidant activities comparable with or even better than those of OTL extracts. Hence, OTL and BTW, which are often considered as agricultural wastes, can be used as potent natural antioxidant sources [30].

Biological activities in cosmetic and personal care products

1. Antioxidant activity

The word "free radicals" and "antioxidants" have become well known for health-conscious consumers [31]. Free radical and reactive oxygen species, collectively known as ROS are generated continuously via normal physiological processes, more so in pathological conditions. Reactive oxygen intermediates (ROIs) are partially reduced forms of atmospheric oxygen (O_2). They typically result from excitation of O_2 to form singlet oxygen (O_2^1) or from the transfer of one, two or three electron to form a superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide or a hydroxyl radical ($HO^{\bullet}$), respectively. The role active oxygen and free radicals in tissue damage, in different diseases of humans are becoming increasingly recognized [32] and various degenerative disorders of human such as cardiovascular disease, aging, cancer and neuro-degenerative disease like Alzheimer's disease [33-36].

Active oxygen and related species play an important physiological role and, at the same time, they may exert toxic effect as well. The active oxygen species are essential for production of energy, synthesis of biological essential compounds, and phagocytosis, a critical process of our immune system. They also play a vital role in

signal transduction, which is important for cell communication and function. However, there is now increasing evidence which shows that these active oxygen species may play a causative role in a variety of diseases as mention before.

Antioxidant is defined as any substances that, when present at low concentrations compared with those of an oxidative substrates, significantly delay or prevent oxidation of the substrate [37]. Consequently, the roles of antioxidants, which suppress such oxidative damage are important in aerobic organism [38]. Humans have evolved with antioxidat systems to protect against free radicals. These systems include some antioxidants producted in the body (endogenous). The (a) enzymatic defense, such as glutathione peroxidase, catalase, and superoxide dismutase, which metabolize peroxide, hydrogen peroxide, and lipid peroxide, and (b) non-enzymatic defense, such as glutathioe, histidine-peptide, the iron-binding proteins transferin, ferritin, dihydrolipoic acid, reduced CoQ_{10}, metalonin, and others obtained from the diet (exogenous) [39] such as Vitamin A, E, C, phytochemical, food antioxidant (BHA, BHT) [40]. However, natural defenses are overwhelmed by an excessive generation of free radicas, which can be dealt with external factors (environmental insults, smoking). Hence, dietary intake of antioxidant compounds becomes important to maintain adequate antioxidant status [41].

The type of antioxidant are classified into two categories; synthetic and natural antioxidants. In general, the more popular synthetic antioxidants (Figure 1.11) used are phenolic compounds which are always substituted by alkyl to improve their solubility in fats and oils, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and esters of gallic acid, e.g. propyl gallate (PG) [42]. After a long period use of synthetic antioxidants, a new toxicological behaviors data impose some caution in their use [43]. In this context, natural products appear as more healthy and safer than synthethic antioxidants [44]. Natural antioxidants are found in almost all plants, microorganism, fungi, and even in animal tissue [45]. The natural antioxidants can be phenolic compounds (tocopherol, flavonoids, and phenolic acid), nitrogen compounds (alkaloids, chlorophyl derivatives, amino acids, and amines), or catotenoids as well as ascorbic acid [46].

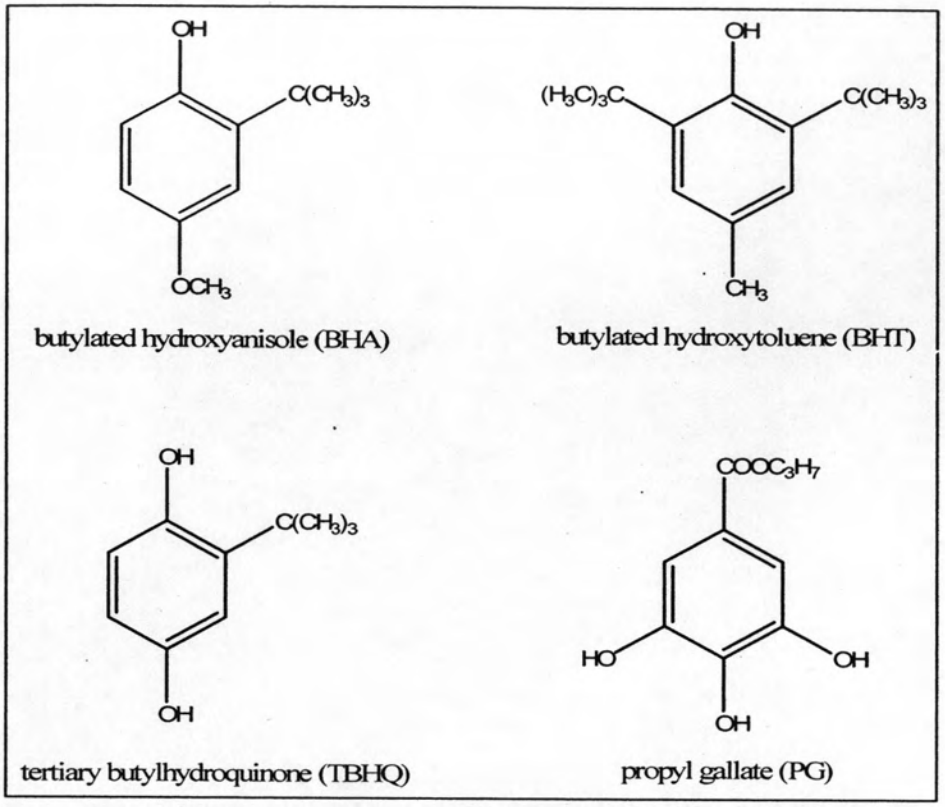


Figure 1.11 The synthetic antioxidants

The antioxidative plant constituents revealed various types of anti-oxidative phytochemicals (Figure 1.12)

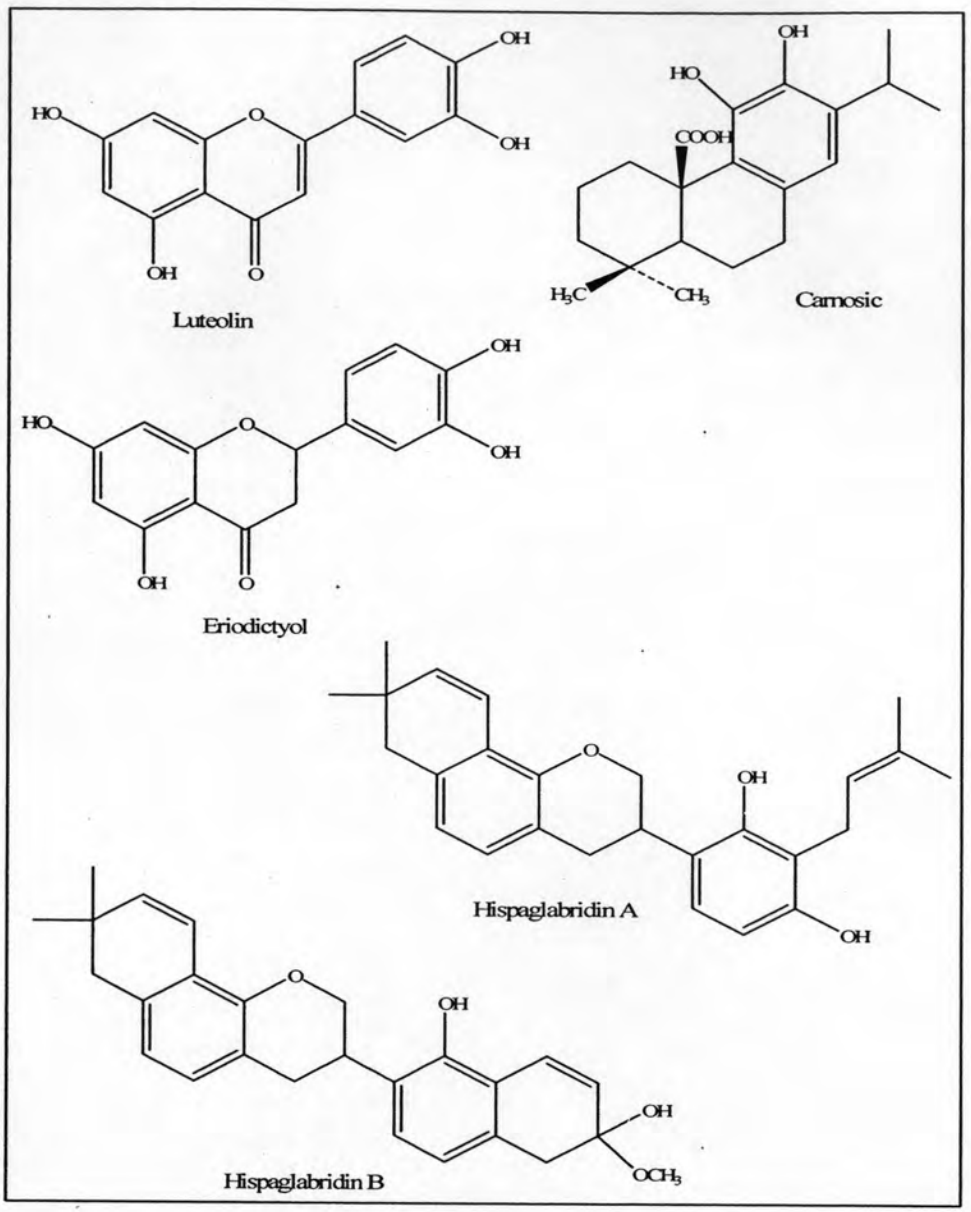
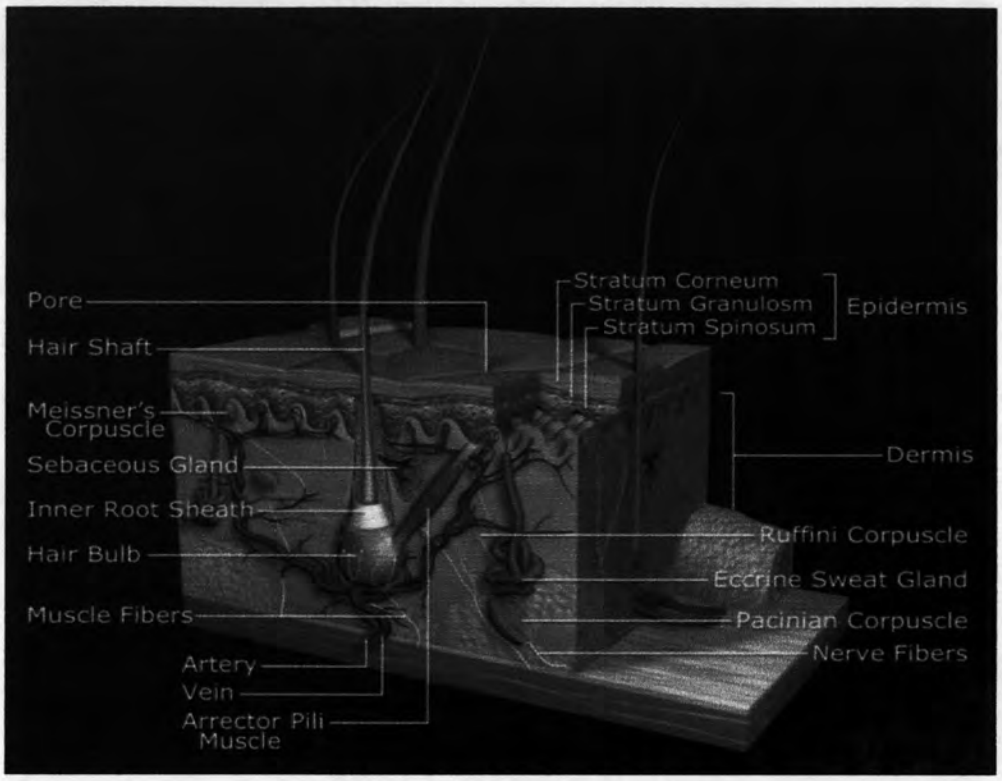


Figure 1.12 The natural antioxidants

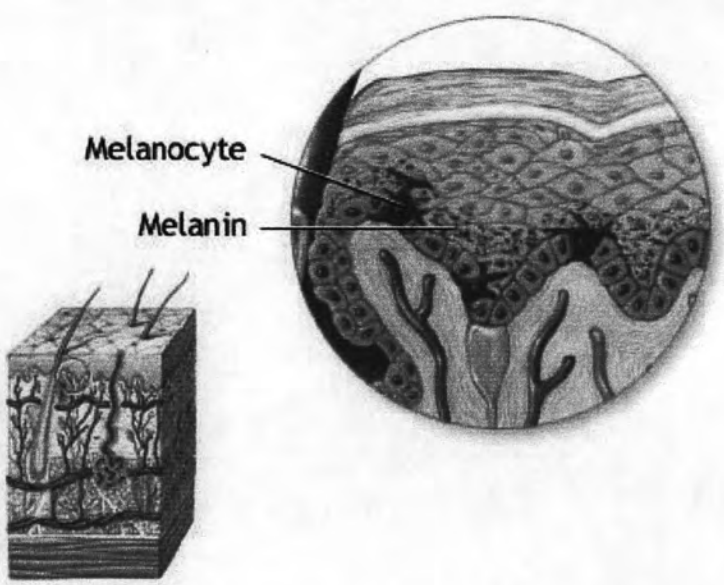
2. Anti-tyrosinase activity

The color in human hair, skin and iris is produced by a pigment melanin, which is produced by dermal melanocyte cells. The melanocyte cells (Figure 1.13) transform tyrosine and phenylalanine into two different forms of melain, which then spread throughout the dermal cells and the keratinocytes via melanosomes to darken tissue. Chemical metabolism occurs intra-cellulary to produce melanin from the precursors phenylalanine and tyrosine (Figure 1.14). There are two forms of melanin, Eumelanin and Pheomalanin. Eumelanin is metabolized from 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and produces a brown color in hair in its intact form and

pheomalanin is metabolized from 5,6-indolequinone, which produces a red color in hair in its intact form. Various degrees of these two slightly different forms produce different shades of hair [47]. In addition to coloration, melanin pigmentation in the skin also provides photoprotection from UV radiation to the skin [48].



(A)



(B)

Figure 1.13 (A) Diagram layer of human skin, (B) Melanin in melanocyte of skin [48]

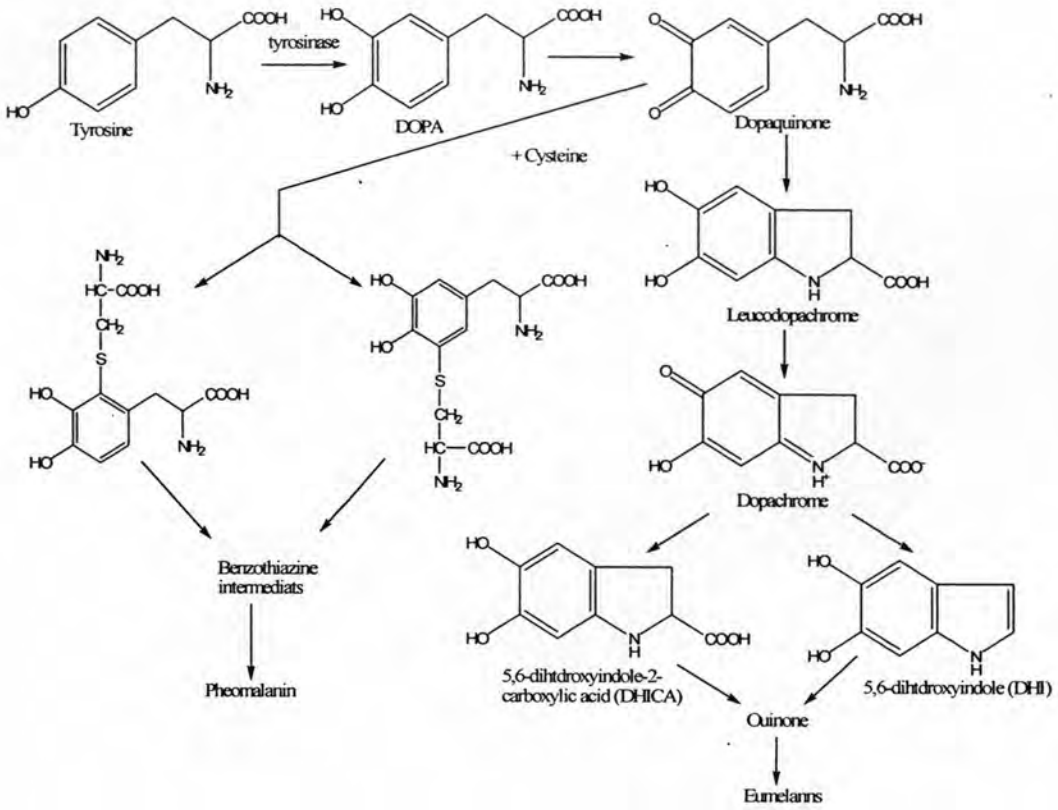


Figure 1.14 Melanin synthesis pathways

Tyrosinase

Tyrosinase (EC 1.14.18.1) is a copper-containing monooxygenase catalysing the o-hydroxylation of monophenols to the corresponding catechols (monophenolase activity), and the oxidation of catechols to the corresponding o-quinone (diphenolase or catecholase activity) (Figure 1.15) [49]. Tyrosinases are widespread in the living world, where they are often referred to as phenolases, phenol oxidases, polyphenol oxidases, catechol oxidases, depending on the particular source or also on the Authors who have described any particular enzyme.

In higher plants, the enzyme protects the plant against insects and microorganisms by catalyzing the formation of an impervious scab of malanin against further attack. This defensive role is well documented for the tyrosinase from the trichomes of *Solanaceae* species. Tyrosinase is upregulated in wounded tissue in apple [50], potato [51], tomato leaf, broad bean, grapes, spanish, carrot and pokeweed [52-56]. Such finding suggests a defense-related role of tyrosinase in higher plants, however, no tyrosinase-induction occurred upon infection of *Nicotiana* hybrids with various microorganisms [57] or upon challenge of watermalon *(Citrullus hatus)* with

Fusarium [58]. In insects, tyrosinase is involved in sclerotization of the exoskelaton and in the protection against other organisms by encapsulating them in melanin [59]. Tyrosinase is also found in mammals and it is responsible for skin pigmentation [60]. In most fruits and vegetables, tyrosinase is responsible for enzymatic browning, following bruising, cutting or other damage to the cell. For example, mushroom tyrosinase is responsible for the undesired browning of mushrooms that take place during senescence or damage during post harvest handling.

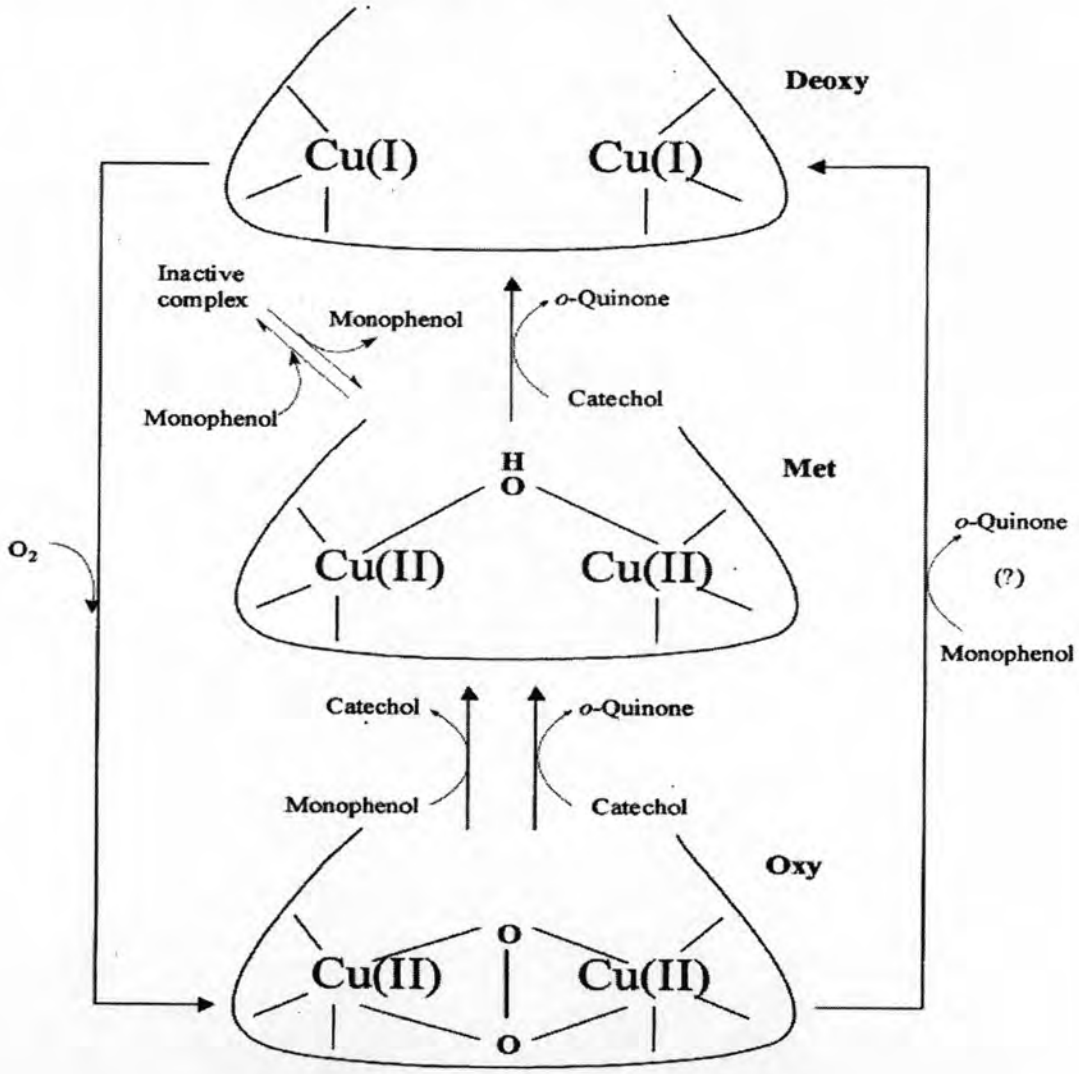


Figure 1.15 The three physiological states of the active site of tyrosinase: from top to bottom, the cuprous, *deoxy* form, and the cupric forms, respectively *met* and *oxy*

Tyrosinase inhibitors

Tyrosinase, the enzyme that controls the synthesis of melanin, is a unique product of melanocytes. It is considered to be the rate-limiting step enzyme for the biosynthesis of melanin in epidermal melanocytes. Therefore, tyrosinase activity is thought to be a major regulatory step in melanogenesis.

Among inhibitors, a distinction could be made between copper chelators (competitive with respect to oxygen) (Figure 1.16) and substrate analogues (competitive towards phenol and/or diphenol substrates) (Figure 1.17). However, such a classification is purely indicative, as many inhibitors cannot be ascribed to a particular group and many of them behave as mixed-type inhibitors (competitive/noncompetitive) [49].

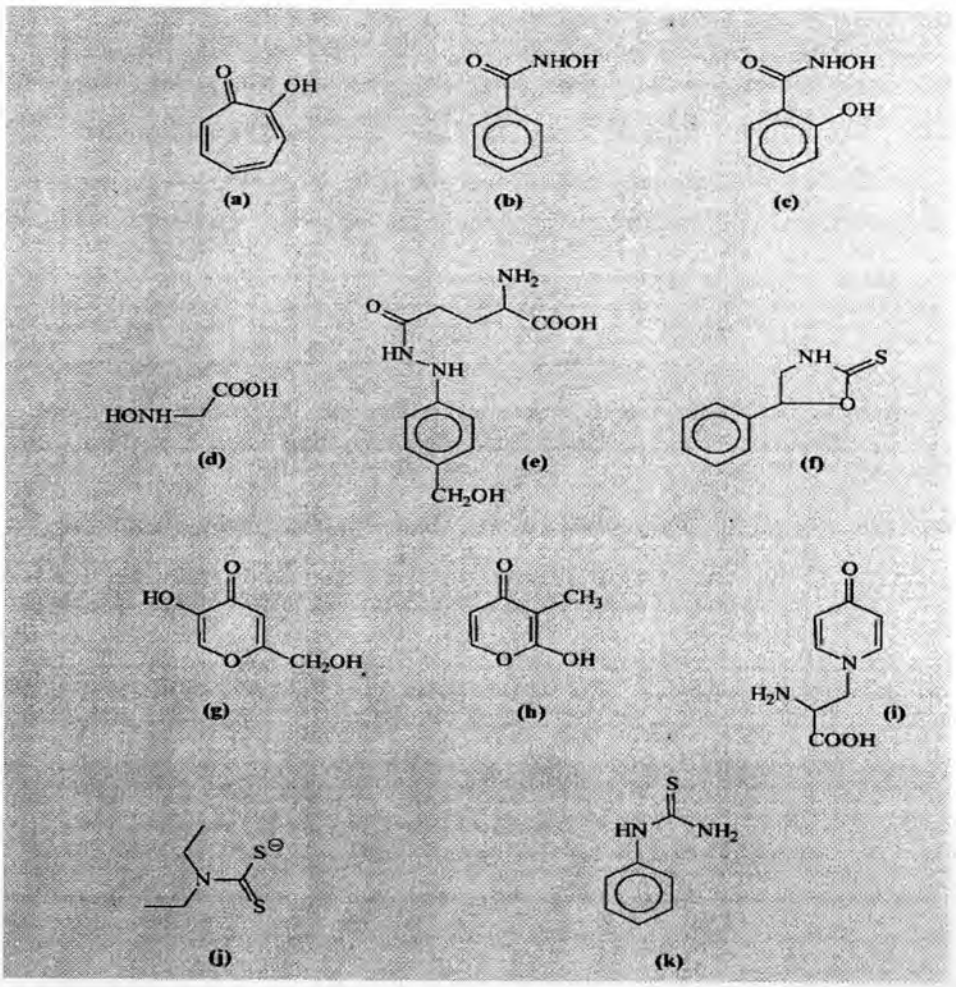


Figure 1.16 The structure of some tyrosinase inhibitors, that presumably act as copper chelators: (**a**) tropolone; (**b**) benzhydroxamic acid; (**c**) salicylhydroxamic acid; (**d**) *N*-hydroxyglycine; (**E**) agaritine; (**F**) barbarine; (**g**) kojic acid; (**h**) maltol; (**i**) minosine; (**j**) diethyldithiocarbamate; (**k**) phenylthiourea

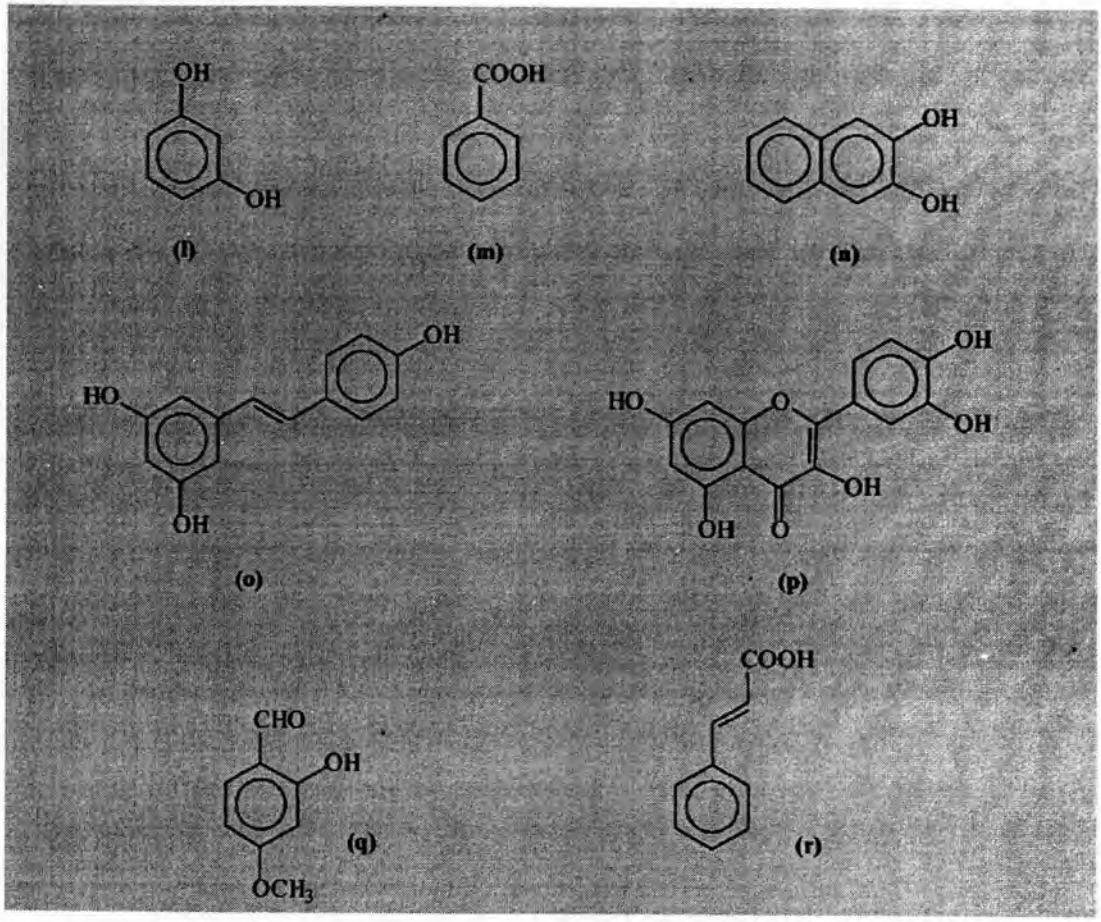


Figure 1.17 Some other tyrosinase inhibitors, that act as substrate analogues: (l) resorinol; (**m**) benzoic acid; (**n**) 2,3-dihydroxynaphthalene; (**o**) resveratro; (**q**) 2-hydroxy-4-methoxybenzaldehyde; (**F**) cinnamic acid

Several products on the market contain ingredients that inhibit tyrosinase and thus decrease melanin formation

Hydroquinone

Hydroquinone (Figure 1.18) was used in over-the-products and precription drugs as an ingradient in skin lighteners. It is also a natural ingredient in many plant-derived products, including vegetables, fruits, grain, coffee, tea, beer, and wine. For many years, hydroquinone had been the main treatment modality for postinflammatory hyperpigmentation and melasama. Hydroquinone exerts its depigmenting effect by inhibiting tyrosinase and by virtue of its cytotoxicity to melanocytes. It is known to cause reversible inhibition of cellular metabolism by affecting both DNA and RNA synthesis. Also, hydroquinone is an efficient blocker of tyrosinase and has been shown

to decrease its activities by 90% [61]. However, hydroquinone is now considered to be cytotoxic and hence potentially mitogenic [62].

Figure 1.18 Structure of hydroquinone

Arbutin

Arbutin ($C_{12}H_{16}O_7$) is naturally occuring β-D-glucopyranoside that consists of a molecule of hydroquinone bone to glucose (Figure 1.19). Traditionally used in Japan, arbutin is present in the leaves of pear trees and certain herbs. Its depigmenting mechanism involves a reversible inhibition of melanosomal tyrosinase activity rather than suppression of the expression and synthesis of tyrosinase. However, the utility of arbutin is a de-pigmenting agents in unclear. Nakajima et al. report that although tyrosinase activity was reduced in normal human melanocytes treated with arbutin, an increase of pigmentation occurred. These results have not been duplicated, and there are currently no published clinical studies evaluating the effects of arbutin on pigmentation disorder [61].

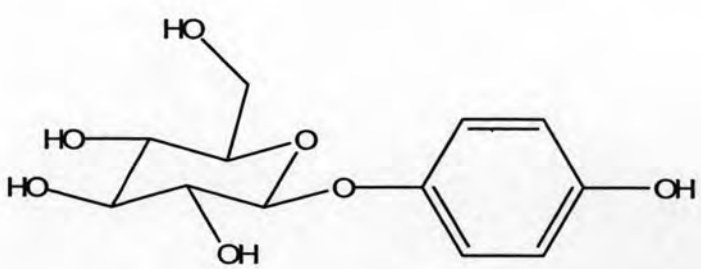


Figure 1.19 Structure of arbutin

Kojic acid

Kojic acid (5-hydroxy-2hydroxymethyl-γ-pyrone or $C_6H_6O_4$; Figure 1.20) is a fungal metabolite commonly produce by many species of Aspergillus, Acetobacter, and Penicillium. It is widely used as a food additive for preventing enzymatic browing and promoting reddening of unripe strawberries. Kojic acid suppresses tyrosinase activity. Mainly by chelating copper ions required for tyrosinase enzymatic activity [61].

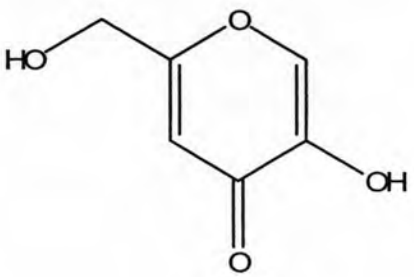


Figure 1.20 Structure of kojic acid

Licorice extract

Glabridin (*Glycyrrhiza glabra)* is the main ingredient of licorice extraction that affects skin. Glabridin (Figure 1.21) inhibiting tyrosinase activity in cell cultures without affecting DNA synthesis. Combined analysis of SDS-polyacrylamide gel electrophoresis and DOPA staining on the large granule fraction of these cells disclosed that glabridin specifically lowered the activities of some of the tyrosinase isozymes. Researchers have also shown that potential application of 0.5% glabridin inhibited ultraviolet B (UVB) induced pigmentation and erythema in the skin of guinea pig [61]

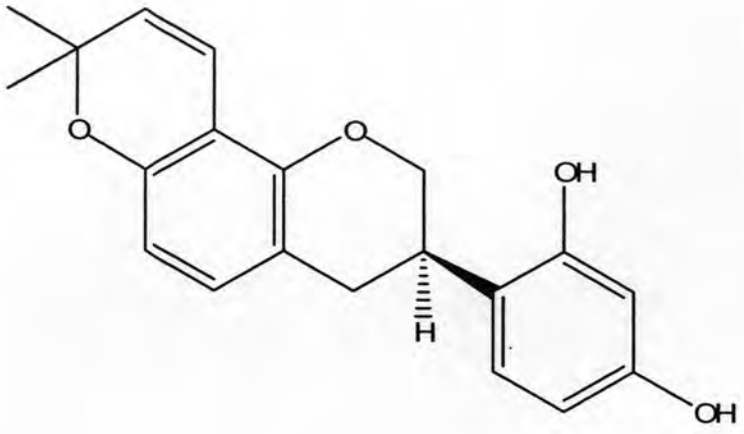


Figure 1.21 Structure of glabridin

3. UV-screening activity

Ultraviolet (UV) light is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than soft X-rays. When considering the effect of UV radiation on human health and the environment, the range of UV wavelengths is often subdivided into three major bands. UVC is radiation with wavelength in the region between 200 and 280 nm. UVC could not reach the surface of the present-day earth because of atmospheric Rayleigh scattering and ozone absorption, although these region are important parts of the extraterrestrial spectrum. UVB radiation is energetically less damageing than UVC. In the scientific literature, UVB radiation is often defined as 280-320 nm. However, the legal definition provided by the International Commission on Illumination sets the UVB radiation range as 280-315 nm. On earth, most of UVB radiation is attenuated by the ozone that absorbs strongly in the Hartley region (200-300 nm) and weakly in the Huggins Band (300-360 nm). Finally, UVA radiation (315-400 nm) reaches the surface of the earth relatively unattenuated and it is still less energetic than UVB radiation [62].

Safety aspect of UV

In human, prolonged exposure to solar UV radiation may result in acute and chronic health effects on the skin, eye and and immune system [63]. UVA, UVB and UVC can all damage collagen fibers and thereby accelerate aging of the skin. In general, UVA is the least harmful, but can contribute to the aging of skin, DNA damage and possibly skin cancer. It penetrates deeply and does not cause sunburn.

UVA light is also known as "black light" and, because of its longer wavelength, can penetrate many windows. It also penetrates deeper into the skin than UVB light and is thought to be a prime cause of wrinkles [64].

UVB light can cause skin cancer. The radiation excites DNA molecules in skin cells, causing covalent bonds to form between adjacent thymine bases, producing thymidine dimers (Figure 1.22). Thymidine dimers do not base pair normally, which can cause distortion of the DNA helix, stalled replication, gaps, and misincorporation. These can lead to mutations, which can result in cancerous growths. The mutagenicity of UV radiation can be easily observed in bacteria cultures [65]. This cancer connection is one reason for concern about ozone depletion and the ozone hole.

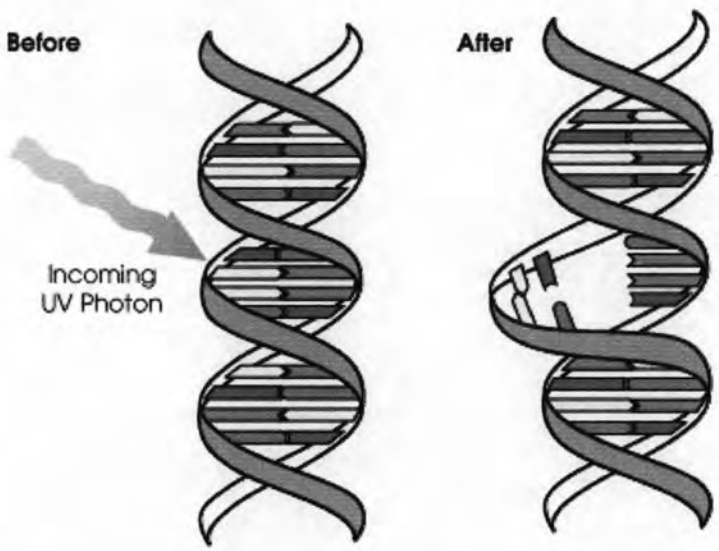


Figure 1.22 The ultraviolet photons harm the DNA molecules of living organisms in different ways

Protection against photo-degradation can be achieved in various ways as following:

- Screening UV absorption and its reduction by substrate, by using some UV absorber.
- Diminishing the initiation reaction rate by using quenchers for excited singlet/triplet states of complex groups.
- Decay of hydro-peroxides into non-radical products.
- Scavenger process of free radicals during their formation stage.

Sunscreen reduces the amount of damaging UV radiation reaching the skin. Suncreens works by filtering UV radiation with a chemical barrier that absorbs and/or reflects the UV radiations away from the skin. Many UV-screening activity have been found in natural product extract such as epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate from green tea extract [66], usinic acid from lichen extract [67] and silibinin from milk thistle *(Slyibrum marianum)* [68].

Rice Husk

Now, the effort using agricultural residues are interesting either in an energy aspect, an extraction technologies aspect and novel drug discovery aspect. Bioproducts from agricultural residues extraction will make an extra economically value to the residues. Because of rice is the Thailand's most important crop, a lot of agricultural residue from rice, including rice husk and rice straw are quit abundant.

In this research, therefore, the screening for antioxidant activity (DPPH radical scavenging assay), anti-tyrosinase activity (post TLC developing technique) and UV screening activity on rice husk have been investigated.

DPPH radical scavenging is measurement of discoloration of DPPH (2,2-diphenyl-1-picrylhydrazyl). DPPH (Figure 1.23) is a class of nitrogen-centered radical and stable with its resonance system and a radical generating substance to monitor the free radical scavenging abilities (the ability of a compound to donate an electron). The DPPH radical has a deep violet color due to its impaired electron, and radical scavenging can be followed spectrophotometrically by decrease of absorbance at 517 nm, as the pale yellow non-radical form is produced.

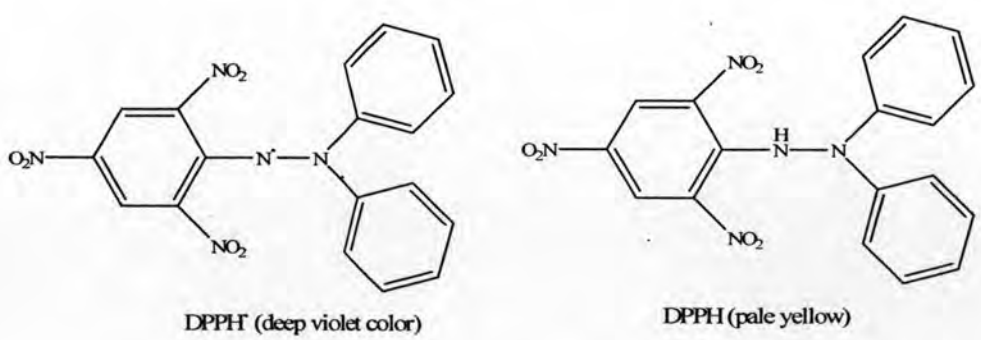


Figure 1.23 Structure of DPPH˙ and DPPH

The post TLC developing technique was used to detect substances which can inhibit tyrosinase activity. The method involved spraying the TLC plate or chromatographic paper containing sample spot(s) with tyrosinase and L-tyrosine solutions successively. The inhibitor molecule situated on the TLC plate can bind to the enzyme molecules around them. Tyrosinase molecules in the spot are, therefore, inactivated. Enzyme molecules in other area, however, are still active and, when in contact with L-tyrosine, will catalyse the formation of dark color product. This process will result in a browninsh-purple color all over the plate except the area with tyrosinase inhibitor.

In this research, the husks of five strains of rice cultivar, including Chi-nat 1 (CN), Look Daeng Pattani (LD), Lebnok Pattani (LN), Go Ko 1 (GK) and Jasmine (JM), were selected for screening their antioxidant activity, anti-tyrosinase activity and UV screening activity. The characteristics of rice in each strain are summarized in Table 1.2

Rice (*Oryza sativa)*

Rice have two species in the Poaceae ("true grass") family, Oryza sativa and Oryza glaberrima. These plants can be found in native to tropical and subtropical southern and southeastern Asia and Africa. Rice provides more than one fifth of the calories consumed by humans in their global diets [69]. Rice is a monocarpic annual plant, growing to 1–1.8 m tall depending on the variety and soil fertility. The grass has long, slender leaves 50–100 cm long and 2–2.5 cm broad. The small wind-pollinated flowers are produced in a branched arching to pendulous inflorescence 30–50 cm long. The seed is a grain (caryopsis) with 5–12 mm long and 2–3 mm thick. The structure of rice grain is illustrated in figure 1.24.

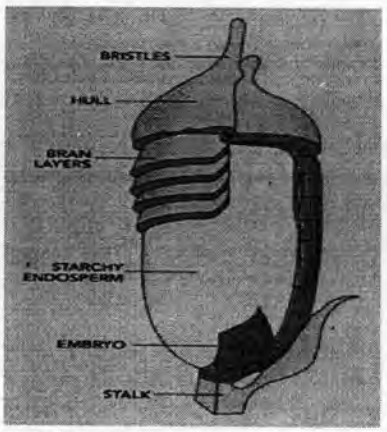


Figure 1.24 The structure of rice grain

Table 1.2 Characteristics and productivity of each rice strains [70]

Rice name	Characteristics	Productivity (kg/rai)
Chai-nat 1 (CN)	1. A height: 113 cm 2. Insensitive to photo period 3. Harvesting period: 120-130 days 4. Long ear and dense grain and green leaves 5. Unpolished rice: light brown 6. Seed dormancy period: 8 weeks 7. Amylose content: 26-27% 8. High yielding 9. Planting condition: area with water abundance	740
Look Daeng Pattani (LD)	1. A height: 160 cm 2. Sensitive to photo period 3. Harvesting period: January-February 4. Quite strong stem, light green leaves and long ear 5. Unpolished rice: dark brown 6. Seed dormancy period: 4 weeks 7. Amylose content: 25% 8. Planting condition: good resistant to saline and acid soil in Patthalung, Songkla, Satul, Pattani and Narathiwat	480

Table 1.2 continued

Rice name	Characteristics	Productivity (kg/rai)
Leb Nok Pattani (LN)	1. A height: 170 cm 2. Sensitive to photo period 3. Harvesting period: February 4. Long ear and dense. 5. Unpolished rice: light brown 6. Seed dormancy period: 3 weeks 7. Amylose content: 26% 8. Planting condition: good in lowland field	480
Go Ko I (GK)	1. A height: 115 cm 2. Sensitive to photo period 3. Harvesting period: 130 days 4. Light green stem and leaves and long and thin grain 5. Unpolished rice: light brown 6. Seed dormancy period: 3 weeks 7. Amylose content: 29-30% 8. Planting condition: area with water abundance	742
Jasmine (JM)	1. A height: 150 cm 2. Roots spread out at the horizontal surface 3. Unpolished rice: brown 4. Yellow-reddish husk 5. Harvesting period: mid-November 6. Planting condition: lowland field	600

Many researches on rice husk were published in the last decade.

In 2000, Khalid and coworker investigated the adsorption behavior of rice husks for antimony ions from aqueous solution. The studied showed that adsorption of antimony from aqueous solutions using rice husks can be achieved within a short contact time of 10 min [71].

In 2004, Adam and coworker synthesized silica-incorporated aluminum (RHA-Al) from rice husk ash (ash) using the sol-gel technique. The synthesized RHA-Al was used for an adsorption of palmitic acid [72].

In 2004, Mahvi and coworker studied the potential of using rice husk and rice husk ash for phenol adsorption from aqueous solution. A comparative study showed that rice husk ash is more effective than rice husk in phenol removal. The studied showed that the rice husk ash could be used as an efficient adsorbent material for a removal of phenolic from water and wastewater [73].

In 2005, Guo and coworker studied the adsorption of Rhodamine B (RB) by rice husk based porous carbons (RHCs) and commercial carbons from aqueous medium. The removing of RB from RHCs is larger than commercial carbons [74].

Objective of research

1. Screening for antioxidant activity, anti-tyrosinase activity and UV screening activity in organic extracts of rice husks.
2. Finding extraction processes to obtain extract with biological activities.
3. Isolating and characterizing the active components.