CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Skin

The skin is one the larger organs in terms of surface area. For the average adult, the skin occupies a surface area of approximately 2 square meters (3,000 square inches) (11)

2.1.1 Functions of the skin

The numerous functions of the skin are as follows: (11-13)

A) Regulation of body temperature

Perspiration produced by sweat glands and changes in the flow of blood to the skin help regulate body temperature.

B) Protection

The skin provides a physical barrier against abrasion, microbial and fungal invasion, dehydration and damaging effects of ultraviolet (UV) radiation.

C) Reception of stimuli

Numerous nerve endings and receptors detect stimuli related to temperature, touch, pressure and pain.

D) Excretion

Small amounts of water, salts, and several organic compounds, components of perspiration, are excreted by sweat glands.

E) Synthesis of vitamin D

The skin is the site of Vitamin D production which results from the ultraviolet-mediated conversion of precursors. Vitamin D is a substance that aids in the absorption of calcium and phosphorus.

F) Immunity

Cells of the skin assume a role in bolstering immunity, your ability to fight disease by producing antibodies.

2.1.2 Structure of the skin

The skin consists of two principal parts (Figure 1). The outer, thinner portion, which is composed of epithelium, is called the epidermis. The inner, thicker, connective tissue portion is called the dermis. Beneath the dermis is a subcutaneous (SC) layer, which is layer of loose connective tissue consisting mainly of subcutaneous adipose tissue (12, 13).

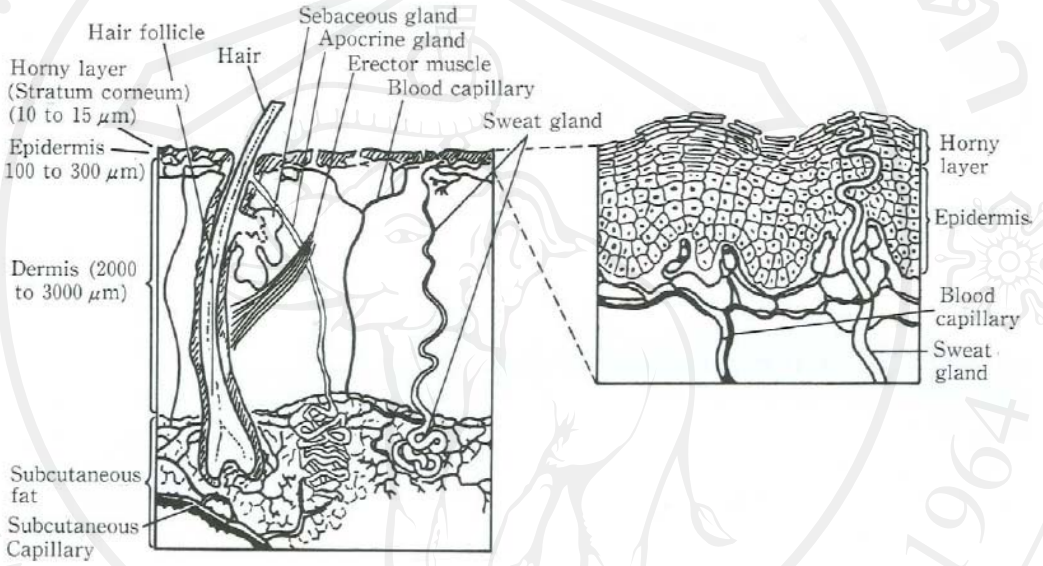


Figure 1 Basic structure of the skin (11)

2.1.2.1 Epidermis

The epidermis is composed of stratified squamous epithelium and contains four types of cells. The most numerous is known as a keratinocyte, a cell that undergoes keratinization. Keratinocyte produces keratin, which helps waterproof and protect the skin, and protect the skin, and they participate in immunity. This structure provides the major barrier function of the skin. The second type of cell is called a melanocyte, which can also be found in the dermis. It produces melanin, one of the pigments responsible for skin color, and absorbs ultraviolet (UV) radiation. The third and fourth types of cells are both called nonpigmented granular dendrocytes, but they are two distinct cell types, formerly known as Langerhans' cells and Granstein cells. These cells are involved with the immune response (12-14).

The name of the five layers of the epidermis (Figure 2) from the deepest to the most superficial are as follows: (12)

A) Stratum basale

This single layer of cells produces melanin and is capable of continued cell division. As the cell multiply, they push up toward the surface and become a part of the layers. Eventually the cells are shed from the top layer of the epidermis. The whole process takes about 2 weeks.

B) Stratum spinosum

This layer has eight to ten rows of cell with spine like projections that join the cells together. Melanin is also found in this layer.

C) Stratum granulosum

The third layer consists of three to five rows of flattened cells that contain keratohyanin, a substance involved in keratin formation.

D) Stratum lucidum

This is the layer found only in the palms and soles. It consists of several rows of clear, flat, dead cells that are translucent.

E) Stratum corneum

This layer consists of 25 to 30 rows of flat, dead cells completely filled with keratin. These cells are continuously shed and replaced.

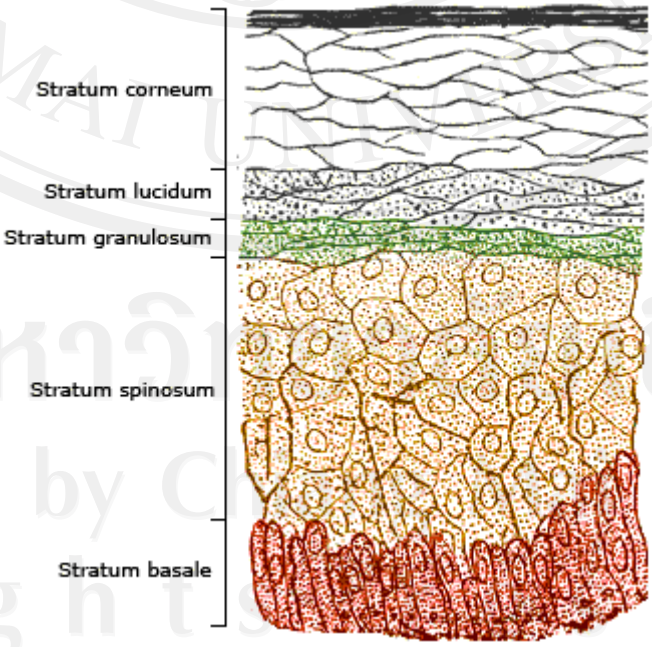


Figure 2 The five layers of the epidermis (12)

2.1.2.2 Dermis

The second part of the skin, the dermis, is very thick in the palms and soles and very thin in the eyelids, penis and scrotum.

The upper region of the dermis consists of loose connective tissue containing fine elastic fibers and its surface area is greatly increased by small, fingerlike projections called dermal papillae. Dermal papillae cause ridges in the epidermis, which produce fingerprints and help us to grip objects.

The lower region of the dermis consists of dense, irregular connective tissue containing collagenous and elastic fibers, adipose tissue, hair follicles, nerves, oil glands and the duct of sweat glands. The combination of collagenous and elastic fibers gives the skin its strength, extensibility and elasticity (extensibility is the ability to stretch; elasticity is the ability to return to original shape after extension or contraction). Collagen provides strength to the skin structure and elastin provides the snap and resiliency. The ability of the skin to stretch can readily be seen during pregnancy, obesity and tissue swelling (edema). Small tears in the skin due to extensive stretching that remain visible as silvery white streaks are called striae. When the elastin fibers undergo changes that cause them to lose their resiliency or snap, the skin no longer is able to return to its original state. As a result sagging and crinkling occur in a pattern that is called wrinkles (12, 14).

2.1.2.3 Subcutaneous (SC) layer

Beneath the dermis, there is subcutaneous tissue that contains many adipose cells in between the connective tissue. The subcutaneous tissue protects skin from blunt and pressure-related trauma and serves as an insulator of heat loss. The loss of this protective padding results in an increase in problems of weight-bearing and pressure-prone surface, and other injuries, as well as the risk of hypothermia (12, 15).

2.1.3 Age-related changes in the skin

2.1.3.1 Physiological change in the skin (14, 16)

A) Epidermal change

The epidermis tends to become thinner with age, due to an increased scaling off its cells and a declining rate of cellular division that typically

accompanies aging. There is a decrease in the number of cells in the epidermis capable of producing the pigment melanin. However, the pigment cells (melanocytes) present tend to be larger and group together, forming dark pigment plaques calls aging spots that are typical of older persons.

B) Dermal changes

There is a general reduction in the number of fibroblasts of fibroblasts and fibers with aging. Thus, the dermis becomes thin and somewhat translucent. At the same time, there are an increasing amounts of insoluble collagen accumulate in the extracellular space, preventing the flow of nutrients and oxygen to cells and causing them to starve and die.

During aging, the elastic fibers of the dermis become less resilient due to structure changes resulting from the formation of cross-links. These changes in the elastic fibers cause the skin to be less able to smooth out. Thus, wrinkles and sags become common with age.

There is a reduction in the number of sweat glands, sebaceous glands, hair follicles and pigment in the hair with aging.

C) Subcutaneous changes

The subcutaneous tissue is essentially a layer of loose connective tissue in which much fat is stored. With aging there is a generalized loss of fat from the subcutaneous tissue. This loss is usually most obvious in the face and the limbs. The loss of subcutaneous fat is a major cause of the wrinkles that are common with age and is largely responsible for the emaciated appearance of many older persons.

2.1.3.2 Wrinkle (16, 17)

A) Classification of wrinkles

a) Crinkling-type wrinkle

There are fine wrinkles formed from folded skin. They usually are seen in persons from around 75 years old, and in sun-damaged individuals with post-inflammatory changes in the skin called elastosis.

b) The glyphic wrinkle

This wrinkle has a crisscross pattern and is frequently seen on the cheeks and the neck.

c) The deep wrinkle

This wrinkle forms a major line or deep groove that is long and straight. This is the most troublesome wrinkle because it is so visible and so difficult to eliminate.

B) Causes of wrinkle

a) Sun exposure

Top the list of causes of damaged skin. The skin must be protected from both UVA and UVB irradiation (that is 280-400 nm). Sun rays denature protein and enzymes, affect deoxyribonucleic acid (DNA) and the total cellular structure which results in profound abnormal structures.

Aging changes of skin protected from the sun are similar to, but less severe than, those in sun-exposed areas. The severity of the damage seems to depend on the amount of exposure and the specific regions involved.

b) Smoking cigarettes

There is growing evidence that free radicals are one of the major causes of abnormal proteins in the body. The resultant abnormal proteins produce the signs of aging in the various body tissues. These free radicals bind to our proteins and cause tissue damage.

c) Alcoholic beverages

A good healthy liver can burn up about an ounce of alcohol per hour depending on a number of variables. It is generally medically acceptable to advise a limit of one ounce of alcohol per day as not being likely to have any adverse effects on the skin. Above this amount alcohol can be quite deleterious and will obviate most efforts to treat or prevent wrinkles.

d) Soap-based products

The use of soap has some side effects on the skin. The most common emulsion systems used in these types of product are stearic and triethanolamine. The pH of these emulsions is often above neutrality, often higher than pH 8, which is one hundred times the pH of the skin (approximately 5.5).

Soap must remove oil and dirt to clean. Unfortunately it removes lipids, the natural skin oils, at the same time. In addition soap reacts with keratins of the skin, denatures them and causes them to lose function. This produces the dry, tight feeling associated with soap use.

2.2 Free radicals

A free radical is an atom or molecule that has one or more unpaired electrons and is capable of independent existence. It can react actively with other nearby molecules to alter or destroy them (18). If a series of electrons is added to oxygen, four oxygen products with the formation of water as the fourth one are produced. That can be described the oxygen tetravalent reduction as: (17, 18)

Oxygen + one electron = Superoxide ($^1O_2^{\bullet-}$)

Superoxide + $2H^+$ = Hydrogen peroxide (H_2O_2)

Hydrogen peroxide + e^- + H^+ = Hydroxyl radical ($OH^\bullet$) + H_2O

Hydroxyl radical + one more electron + hydrogen = Water (H_2O)

Three reactive oxygen species (ROS) are seen, but only two are free radicals. These are the superoxide and the hydroxyl radical. Hydrogen peroxide is an ROS, but not a free radical (19).

2.2.1 Superoxide radical ($O_2^{\bullet-}$)

Superoxide is known as attack enzymes and cell membrane. It tends to attack unsaturated fatty acid in the cell membrane causing them to break down. The process is cell lipid peroxidation. The three major steps in lipid peroxidation can be diagrammed as follows:

Polyunsaturated fatty acid (PUFA) + superoxide ⟶ Lipid free radical (LFR)

LFR + oxygen ⟶ Peroxyl lipid radical

Peroxyl lipid radical + PUFA (new) ⟶ Lipid hydroperoxide + LFR (new)

The most important aspect of superoxide is its ability to react with hydrogen peroxide and form the more reactive hydroxyl which attacks DNA. Superoxide can be destroyed by the enzyme superoxide dismutase (SOD) before it can be converted to hydroxyl radical. We can describe this reaction as follows:

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

2.2.2 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is dangerous because it diffuses into cells, particularly into the nucleus of the cells to react with DNA. It also reacts with proteins to cross-link and to denature them, making these compounds either nonfunctional or no longer able to function in normal way.

2.2.3 Hydroxyl radical ($OH^{\bullet}$)

The hydroxyl radical is one of the most potent oxidants. Hydroxyl radical can react with almost every compound in the body such as enzymes, proteins, carbohydrates and lipid. It can attack lipids and produce lipid peroxides, and cross link all kinds of proteins, but the attack of DNA is probably the most injurious to the body overall. Many scientists believe it to be one of the major causes of the changes we see in aging.

These consequences of oxidative stress construct the molecular basis in the development of many diseases. The role of free radicals in various diseases is highlighted in Table 1.

Table 1 Role of free radical in various diseases (3)

Diseases	Role of free radicals in pathophysiology
Atherosclerosis	- Superoxide-mediated endothelial dysfunction, activation of macrophages
Myocardial infarction	- ROS driven ischemic reperfusion injury and myocyte necrosis and/or apoptosis
Hypertension	- ROS-mediated vascular smooth muscle cell proliferation, oxidant production viaNADH/NADPH oxides and endothelial dysfunction

Table 1 (continued)

Diseases	Role of free radicals in pathophysiology
Diabetes	- ROS accelerated formation of advanced glycation end products (AGEs) - Superoxide-mediated endothelial dysfunction
Aging	- Cell damage and metabolic abnormalities
Cancer	- ROS-mediated gene mutations (modification of pyridine and purine bases) and post-translational modifications leading disruption of cellular processes
Parkinson's disease	- ROS-mediated mitochondrial dysfunction
Alzheimer's disease	- Amyloid peptide and advanced glycation end Products - ROS-mediated neurotoxicity to hippocampal cells and the synaptosomal membranes
Huntington's disease	- ROS-mediated transcriptional dysregulation and mitochondrial impairment
Autoimmune disorders	- ROS-mediated inflammation and tissue Destruction
Age-related macular degeneration	- Photochemical reactions in the oxygen-rich environment of the outer retina lead to the liberation of cytotoxic (ROS)
Acute lung injury, acute respiratory distress syndrome, inflammation and hyperoxia	- ROS-mediated inflammation and endothelial dysfunction

2.3 Antioxidant

An antioxidant is a molecule which stops an oxidative reaction chain through various mechanisms and prevents the damage caused by them. Thus it is believed to be in health maintenance through the modulation of oxidative process in the body. Oxidative damage with the unregulated production of ROS has been implicated in a growing list of clinical disorders such as Table 1. Antioxidants can be classified into two major groups, i.e., enzymatic and non-enzymatic antioxidants. Some of these antioxidants

are endogenously produced which include enzymes, low molecular weight molecules and enzyme cofactors. Among non-enzymatic antioxidants, many are obtained from dietary sources (3, 18, 20). Dietary antioxidants can be classified into various classes, of which polyphenols is the largest class. Polyphenols consist of phenolic acids and flavonoids. The other classes of dietary antioxidants include vitamins, carotenoids, organosulfural compounds and minerals. The antioxidants that were tested and evaluated for the treatment of oxidative stress are presented in Table 2.

Table 2 Some select antioxidants and their mechanism of action (3)

Enzymatic antioxidants	**Properties**
Superoxide dismutase	•located in both mitochondria and cytosol •dismutates superoxide radicals
Glutathione peroxidase	•located in mitochondria, cytosol and cell membrane •removes hydrogen peroxide and organic hydroperoxides
Catalase	•located primarily in peroxisomes •removes hydrogen peroxide
Non-enzymatic antioxidants	**Properties**
Vitamin E	•lipid soluble phenolic compound; major chain breaking antioxidant found in cell membranes,
Vitamin C	•major classes: tocopherols and tocotrienols
Vitamin A	•located in aqueous phase of cell; acts of radical scavenger and recycles vitamin E •derived from cleavage of carotene in intestine •has biological activity of retinol •unsaturated lipid •inhibits lipid peroxidation

Table 2 (continued)

Non-enzymatic antioxidants	**Properties**
Uric acid	•by-product of purine metabolism in humans and higher apes; may be an important physiological antioxidant; scavenges hydroxyl radicals
Glutathione	•nonprotein thiol in cells; serves multiple roles in the cellular antioxidant defense
a-Lipoic acid	•effective as an antioxidant and in recycling vitamin C •potent pro-glutathione agent
Carotenoids	•lipid soluble antioxidants located primarily in membranes of tissues •major component of "phytochemicals" •lipid soluble radical savenger and metal chelators
Flavonoids and polyphenols	•by-product of heme metabolism; may serve as an extracellular antioxidant
Bilirubin	•lipid soluble quinone derivatives; reduced forms are efficient antioxidants
Ubiquinones	•pineal hormone •thought to be localized in nucleus of cell
Melatonin	•lipid soluble radical scavenger

2.4 Carotenoids

Food carotenoids are usually C40 tetraterpenoids built from eight C5 isoprenoid units, joined so that the sequence is reversed at the center. The basic linear and symmetrical skeleton, which can be cyclized at one or both ends, has lateral methyl groups separated by six C atoms at the center and five C atoms elsewhere. A distinctive characteristic is an extensive conjugated double-bond system, which serves as the light-absorbing chromophore responsible for the yellow, orange, or red color that these compounds impart to many foods. Hydrocarbon carotenoids (i.e., carotenoids made up of only carbon and hydrogen) are collectively called carotenes; those

containing oxygen are termed xanthophylls. In nature, they exist primarily in the more stable all-*trans* isomeric form, but *cis* isomers do occur. Because plants are able to synthesize carotenoids de novo, the carotenoids composition of plant foods is enriched by the presence of small or trace amounts of biosynthetic precursors, along with derivatives of the main components. Although commonly thought of as plant pigments, carotenoids are also encountered in some animal foods. Animals are incapable of carotenoid biosynthesis, thus their carotenoids are diet derived, selectively or unselectively absorbed, and accumulated unchanged or modified slightly into typical animal carotenoids.

In the early stages of carotenoids biosynthesis, the C5 primer for chain elongation undergoes successive additions of C5 units, yielding in sequence C10, C15, and C20 compounds. Dimerization of the latter produces phytoene, the first C40 carotenoid. The succeeding transformations are schematically shown in Figure 3.

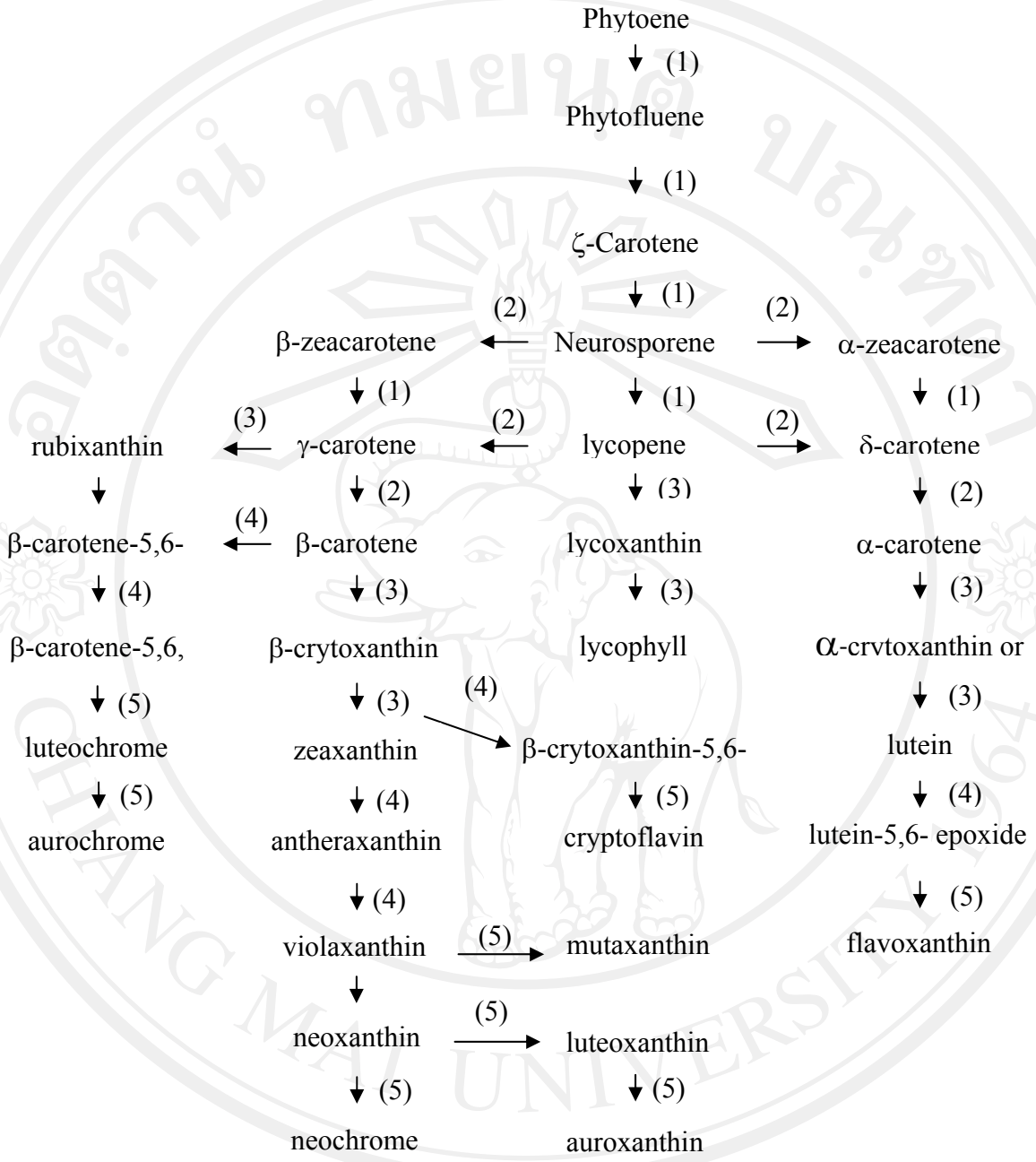


Figure 3 Later stages of carotenoids biosynthesis and possible transformations of carotenoids: (1) desaturation, (2) cyclization, (3) hydroxylation, (4) epoxidation, and (5) epoxidefuranoxide rearrangement (7)

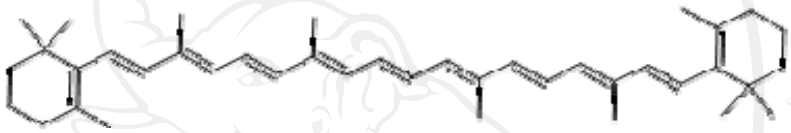
2.4.1 Structure and common food of carotenoids

Of the acyclic carotenes (Figure 4), lycopene is the most common. Lycopene is the principal pigment of many red-fleshed fruits and fruit vegetables, such as tomato, watermelon, red-fleshed papaya and guava, and red or pink grapefruit (21).

Lycopene

Figure 4 Chemical structure of lycopene

The bicyclic β-carotene (Figure 5) is the most widespread of all carotenoids in foods e.g., red palm oil, carrot, tomato, apricot, mango, orange and leafy vegetables (22).



β-carotene

Figure 5 Chemical structure of β-carotene

Structurally, vitamin A (retinol) is essentially one half of the molecule of β-carotene with an added molecule of water at the end of the lateral polyene chain. Thus, β-carotene (Figure 5) is a potent provitamin A to which 100% activity is assigned. An unsubstituted β ring with a C11 polyene chain is the minimum requirement for vitamin A activity (23). γ- Carotene, α-carotene, β-cryptoxanthin, α-cryptoxanthin and β-carotene-5,6-epoxide, all of which have one unsubstituted ring, would have about half the bioactivity of β-carotene. The acyclic carotenoids, which are devoid of β rings, and the xanthophylls other those mentioned above, in which the β rings have hydroxy, epoxy, and carbonyl substituents, are not provitamins A (7, 23).

2.4.2 Factors influencing carotenoids composition

The carotenoids composition of foods are affected by factors such as cultivar or variety; part of the plant consumed; stage of maturity; climate or geographic site of production; harvesting and postharvest handling; processing and storage. In carotenogenic fruits and vegetables, ripening is usually accompanied by enhanced

carotenogenesis as chlorophylls decompose and the chloroplasts are transformed into chromoplasts. The one factor that decisively affects the carotenoids content is the maturity of the plant food when harvested and offered for consumption. This variability was attributed to the wide differences in maturity stage, because these fruit vegetables can be harvested over a long period and have a long shelf life during which carotenoids biosynthesis continues. Carotenogenesis may continue even after harvest as long as the fruit or vegetable remains intact (24, 25).

Carotenoids losing during postharvest storage were reported in some vegetables, especially under conditions favorable to wilting, high temperature, and light exposure. Geographic effects were shown that greater exposure to sunlight and elevated temperature heighten carotenoids biosynthesis in fruits (7).

Carotenoids are susceptible to isomerization and oxidation during processing and storage, the practical consequences being loss of color and biologic activity and the formation of volatile compounds that impart desirable or undesirable flavor in some foods. The occurrence of oxidation depends on the presence of oxygen, metals, enzymes, unsaturated lipids, prooxidants, or antioxidants; exposure to light; type and physical state of carotenoid present; severity of the treatment (i.e., destruction of the ultrastructure that protects the carotenoids, increase of surface area, and duration and temperature of heat treatment); packaging material; and storage conditions. Heating promotes *trans-cis* isomerization (25-27).

2.4.3 Physicochemical properties of carotenoids

2.4.3.1 Solubility

Carotenoids are lipophilic. They are insoluble in water and soluble in organic solvents, such as acetone, alcohol, ethyl ether, chloroform, and ethyl acetate. Carotenes are readily soluble in petroleum ether, hexane, and toluene. Crystalline carotenoids may be difficult to dissolve in the above solvents but do dissolve in benzene and dichloromethane. Solubility of β-carotene in tetrahydrofuran was shown to be excellent (27).

2.4.3.2 Light Absorption

The conjugated double-bond system constitutes the light-absorbing chromophore that gives carotenoids their attractive color and provides the visible absorption spectrum that serves as a basis for their identification and quantification. The color enables analysts to monitor the different steps of carotenoids analysis. Loss or change of color at any time during the analysis gives an immediate indication of degradation or structural modification. The ultraviolet and visible spectrum is the first diagnostic tool for the identification of carotenoids. The wavelength of maximum absorption (λmax) and the shape of the spectrum (spectral fine structure) are characteristic of the chromophore. Most carotenoids absorb maximally at three wavelengths, resulting in three-peak spectra. The greater the number of conjugated double bonds, the higher the λmax values. Thus, the most unsaturated acyclic carotenoid lycopene, with 11 conjugated double bonds, is red and absorbs at the longest wavelengths (λmax at 444-505 nm in hexane). The bicyclic β-carotene, although possessing the same number of conjugated double bonds as lycopene, is yellow orange and has λmax at 450-478 nm and a inflection (shoulder) at 425 nm in hexane (7, 27).

2.4.3.3 Isomerization and oxidation

The highly unsaturated carotenoid is prone to isomerization and oxidation. Heat, light, acids, and adsorption on an active surface (e.g., alumina) promote isomerization of *trans*-carotenoids, their usual configuration, to the *cis* forms. Oxidative degradation, the principal cause of extensive losses of carotenoids, depends on the availability of oxygen and is stimulated by light, enzymes, metals, and co-oxidation with lipid hydroperoxides. Thus, total loss of color and biologic activities are the final consequences (27, 28). Conditions necessary for isomerization and oxidation of carotenoids exist during preparation, processing, and storage of food (29). Thus, retention of naturally occurring or added carotenoids in prepared, processed, and stored foods is an important consideration. Carotenoids are also subject to isomerization and oxidation during analysis (25).

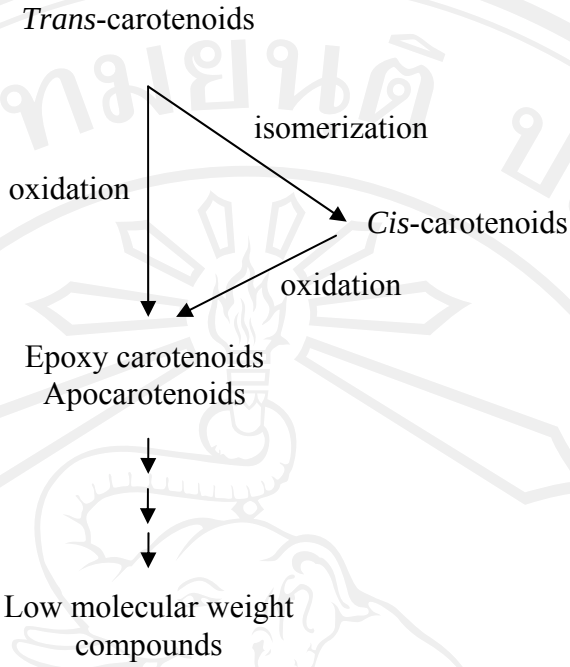


Figure 6 Possible scheme for the degradation of carotenoids (7)

2.4.4 Carotenoids and human health

Humans cannot synthesize carotenoids and, therefore, must rely on dietary sources to provide sufficient levels. Fruits and vegetables are primary sources of carotenoids in the human diet and their consumption has been associated with numerous health benefits (30). One of the most important physiological functions of carotenoids on human nutrition is to act as vitamin A precursors and non-pro-vitamin A carotenoids (lutein, zeaxanthin and lycopene) function as free-radical scavengers, enhance the immune response, suppress cancer development, and protect eye tissue (31). Other biologic functions or actions attributed to carotenoids (e.g., prevention of certain types of cancer, cardiovascular disease, and macular degeneration) are independent of the provitamin A activity and have been attributed to an antioxidant property of carotenoids through singlet oxygen quenching and deactivation of free radicals (32). Carotenoids can reduce the risk of cardiovascular disease through reductions in low-density lipoprotein oxidation and oxidative stress at locations of plaque formations (33). However, a possible mechanism which can explain the dual role of carotenoids as both beneficial and harmful agents in cancer is that their excess or deficiency may bring about changes in molecular pathways involved with apoptotic

signalling. Carotenoid ability in inhibiting or in enhancing apoptosis depends on several factors: carotenoid concentration, concerted action of multiple micronutrients, cell type, and redox status (34).

2.5 *Momordica cochinchinensis* (Lour.) Spreng

Figure 7 The characterization of *Momordica cochinchinensis* (Lour.) Spreng

2.5.1 Botanical aspect and distribution (35, 36)

Binomial name	:	*Momordica cochinchinensis* (Lour.) Spreng
Common name	:	Spring bitter gourd
Thai name	:	Fak-kao (ฟักข้าว)
Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Violales
Family	:	Cucurbitaceae
Genus	:	*Momordica*

2.5.2 Characteristics

This rampagenous perennial vine was given the name *Muricia cochinchin*ensis by Loureiro, a Portuguese missionary-priest who published Flora Cochinchinensis in 1790. Later, Sprengel concluded that the plant belonged in the Linnean genus *Momordica* and changed the name in 1826. *Momordica Cochinchinnensis* is also indigenous to China, Moluccas (Burma), Japan, India, Thailand, Laos, Cambodia, Philippines, Malaysia, and Bangladesh (37, 38). Other common names of the plant are listed in Table 3.

The plant can be cultivated either from seeds or root tubers. Leaves are alternate and deeply three-to-five-lobed with toothed margins. The leaf stalk is glandular. The plant is dioecious, that is, the male and female plants are separate. The flowers are pale-yellow and solitary in the axils of the leaves. The production of parthenocarpic fruits, which is of economic importance, can be accomplished using growth regulators in the female plant in the absence of male plants. However induced parthenocarpic fruits have no seed, whereas hand pollinated fruits contain 18 seeds per fruit on average (39).

The plant starts flowering about 2 months after root tubers have been planted. Flowering usually occurs in April and continues to July/ August and sometimes until September. On average, it takes about 18-20 days for a fruit to mature from emergence of the bud of the female flower. A plant produces 30 to 60 fruits on average in one season. The ripe fruit is picked from August to February (40).

Table 3 Names of *Momordica cochinchinnensis* (Lour.) Spreng in different languages (4)

Language	**Name**
Latin	*Momordica Cochinchinensis* Spreng
	Muricia cochinchinensis Lour.
	Muricia mixta Roxb.
Indian	Bhat kerala
Chinese	Moc Niet Tu
English	Spiny bitter gourd
	Sweet gourd
	Cochinchin gourd
Japanese	Kushika
	Mokubetsushi
Hindu	Hakur
	Kakrol
	Kakur
Laos	Mak-kao
Malais	Teruah
Thai	Fak-kao
Vietnamese	Gac

Fruits are large, densely aculeate, and green, turning to dark orange or red when ripe. Unlike that of the bitter gourd (*Momordica charantia*), the exocarp (rind) of the fruit is hard and is covered with conical points one-eighth-inch high. The fruit comes in oblong and almost round shapes. There are no differences in the ways the fruits are used or consumed. There are also variations among different fruits with respect to their spine and fruit tips. In some fruits, the spines are smooth and dense, whereas in some, they are hard and widely spaced. The fruits are picked at maturity when the fruit is bright red and seeds are hardened.

The mesocarp of the fruit is one-half-inch thick, spongy and orange. The core is divided into cartilaginous chambers containing bright red fleshy seed pods. Each fruit has on average between 15 to 20 rounds, compressed and sculptured

seeds. The seed membrane and kernels contain oil. There is no record of any uses of the mesocarp. The aril of a ripe fruit is bright red in color and has a palatable bland to nutty taste (39).

2.5.3 Growth

Momordica cochinchinensis (Lour.) Spreng grows on dioecious vines and is usually collected from fence climbers or from wild plants. The fruit itself becomes a dark orange color upon ripening. Its exterior skin is covered in small spines while its dark red interior consists of clusters of fleshy aril and seeds. The seeds should be planted immediately upon receipt. Long term storage (over 6 months) in the refrigerator (not freezer) is possible. The seeds should be placed half submerged in well draining soil mix. The seeds have one opening and this should be placed down in the soil. The soil should be kept wet. Warmth, air circulation, and bright light are required for germination. Seeds are easy to germinate and they will germinate in 7-10 days (4).

2.5.4 Chemical constituents from *Momordica cochinchinensis* (Lour.) Spreng

chondrillasterol ; cochinchinin ; columbin ; gypsogenin glycoside ; hemsloside MA-1 ; momorcochin ; momordic acid ; momordica saponin II ; momordin I-A ; momordin I-B ; momordin I-C ; momordin I-D ; momordin I-E ; momordin II ; momordin II-A; momordin II-B ; momordin II-C ; momordin II-D ; momordin II-E ; momordin III ; nardol oleanolic acid glycoside MG-1 ; oleanolic acid glycoside MG-2 (8, 41-45).

2.5.5 Chemical constituents from aril of *Momordica cochinchinensis* (Lour.) Spreng

Published values for carotenoids in aril of *Momordica cochinchinensis* (Lour.) Spreng vary widely, as shown in Table 4. Some studies previously, Vuong and King were reported that the oil extract from the aril contained 357 μg/ml α-tocopherol (46). The fatty acid content (of aril) was calculated to be 79–101 mg/g (5, 46, 47) implying an alpha-tocopherol concentration of about 36 μg/g if all the tocopherol activity is in the oil. The fatty acid composition of aril is given in Table 5, and the approximate nutrient composition of aril is shown in Table 6.

Table 4 Comparison of reported concentrations of carotenes in aril oil of *Momordica cochinchinensis* (Lour.) Spreng (mg/g of edible portion) (48)

Reported	β-carotene	Lycopene	Total carotenoids	Methods
West and Poortvliet (1993)	188	-	892	HPLC
Vien (1995)	458	-	-	-
Vuong *et al*.(2002)	175	-	-	HPLC/PDA
Aoki *et al*. (2002)	101	380	481	HPLC/PDA
Ishida *et al*. (2004)	641 (*trans*) 128 (*cis*)	1903 (*trans*) 170 (*cis*)	2926	HPLC/PDA
Voung *et al*. (2006)	83	408	497	HPLC/PDA

—: data not available; HPLC: high-pressure liquid chromatography; PDA: photodiode array detection.

Table 5 Fatty acid composition of aril of *Momordica cochinchinensis* (Lour.) Spreng (4)

Name	% Total fatty acids	Type of fatty acid
Arachidonic	0.10	Polyunsaturated
Tetracosanoic	0.14	Saturated
Gadoleic	0.15	Monounsaturated
Docasanoic	0.19	Saturated
Palmitoleic	0.26	Unsaturated
Eicosanoic	0.39	Saturated
Myristic	0.87	Saturated
Vaccenic	1.13	Monounsaturated
Alpha-linolenic	2.14	Polyunsaturated
Stearic	7.06	Saturated
Palmitic	22.04	Saturated
Linoleic	31.43	Polyunsaturated
Oleic	34.08	Monounsaturated
Total fatty acid	100	

Table 6 Nutrient compositions of aril oil of *Momordica cochinchinensis* (Lour.) Spreng (48)

Nutrients	100 g of edible portion
Energy (kJ)	523 (125 kcal)
Water (%)	77
Carbohydrates (g)	10.5
Lipids (g)	7.9
Proteins (g)	2.1
Cellulose (g)	1.8
Ash (g)	0.7
Calcium (mg)	56
Potasium (mg)	6.4

2.5.6 Traditional uses and potential health benefits of aril of *Momordica cochinchinensis* (Lour.) Spreng

In Viet Nam, this plant is called ‘‘gac’’, and the aril of the ripe fruit is widely used as a rice colorant due to its intense red color which is called “Xoi Gac”. Since Xoi Gac is served at festive occasions such as weddings, the lunar New Year, and other important celebrations, it is essential to mask the white color of rice, since white is considered the color of death (4). In a double-blind study with 185 Vietnamese preschool children, some were given Xoi Gac containing 3.5 mg/day β-carotene, while others were given an identical-looking dish containing 5 mg β-carotene powder, for 30 days. At the end, the former group had significantly greater plasma levels of β-carotene than the latter. Increases in plasma retinol, α-carotene, zeaxanthin, and lycopene levels were also significantly greater in children given gac (49). This is of great importance, because worldwide, vitamin A deficiency continues to be a major health problem and the leading cause of xerophthalmia and blindness in children (50). It is likely that the fatty acids in gac are what make its β-carotene more bioavailable than that of the synthetic form (49).

In Thailand, scientific studies have demonstrated that cream containing aril oil was statistically in improving skin wrinkles, hydration and viscoelasticity, compared with the cream base and the baseline from week eight onword. In addition,

no sign of skin irritation was observed (51). More recently, the fruit has begun to be marketed in the form of juice dietary supplements because of its high level of lycopene and β-carotene as a source of tomato (4).

2.6 Nanostructured lipid carriers

Lipid nanoparticles with solid particle matrix are derived from o/w emulsions by simply replacing the liquid lipid (oil) by a solid lipid, i.e. being solid at body temperature. The first generation of solid lipid nanoparticles (SLN) was developed at the beginning of the nineties. They were produced from a solid lipid only. In the second generation technology of the nanostructured lipid carriers (NLC), the particles are produced by using a blend of a solid lipid with a liquid lipid, this blend also being solid at body temperature. The production process is identical for both particles SLN and NLC. The solid lipid or lipid blend is melted, the pharmaceutical or cosmetic active dissolved in the melted lipid phase which is subsequently dispersed by high speed stirring in a hot aqueous surfactant/stabilizer solution of equivalent temperature. The obtained pre-emulsion is homogenized in a high pressure homogenizer yielding a hot o/w nanoemulsion. After cooling the emulsion droplets crystallize forming lipid nanoparticles with solid particle matrix, depending on the starting material either SLN or NLC. The advantage of the second generation technology is the increased loading with actives compared to SLN and firmer inclusion of the active inside the particle matrix during the shelf life. By preparing the particles from a solid lipid, especially highly purified solid lipids, the particle matrix tends to form a relatively perfect crystal lattice leaving limited space to accommodate the active. This limits the loading capacity and can lead to expulsion of active from the lipid matrix during storage. In contrast, the use of a lipid mixture with very differently structured (sized) molecules distorts the formation of a perfect crystal. The particle matrix contains many imperfections providing space to accommodate the active in molecular form or as amorphous clusters. One could state that “the perfectness” of the NLC system is its “imperfectness” in its crystalline structure. In the second half of the nineties, there was an increasing interest in investigating the SLN for dermal application, especially for cosmetic use (52, 53). Interesting cosmetic molecules were incorporated such as e.g. retinol and retinylpalmitate (54), vitamin E and vitamin E acetate (55) and coenzyme Q-10 (56).

At the turn of the millennium the NLC were developed (57) which - based on the controlled nanostructuring of the particle matrix – provide advantages with regard to loading capacity and long-term stability (firm inclusion of actives, physical stability of suspension). In previous reports, sensitive molecules such as retinols (58) and Ascorbyl palmitate (59) had been successfully incorporated into NLC and the chemical stability was enhanced.

2.6.1 Nanostructured lipid carriers preparation techniques

2.6.1.1 High shear homogenization

High shear homogenization is the dispersion technique which was initially used for the production of solid lipid nanodispersion. This technique is widespread and easy to handle. However, dispersion quality is often compromised by the presence of microparticles (60).

2.6.1.2 High pressure homogenization

High pressure homogenization (HPH) is a technology that has been applied for many years in various areas for the production of emulsion and suspensions. A distinct advantage of this technology is its ease for scale up, even very large volumes. Typical pressure for production of nanosuspension is 1000-1500 bars. Most of the homogenizers used are based on the piston-gap principle; an alternative is the jet-stream technology. In the piston-gap homogenizer, the macrosuspension coming from the sample container is forced to pass through a tiny gap (e.g., 10 μm); particle diminution is affected by shear force, cativation and impaction. In jet-stream homogenizer the collision of two high-velocity stream leads to particle diminution mainly by impact forces (61).

Very high shear stress and cultivation forces disrupt the particle down to the submicron range. Typical lipid contents are in the range 5-10% and represent no problem to the homogenizer (60). HPH leads to a product being relatively homogeneous in size, which is processing a higher physical stability of the aqueous dispersion. There are basically two different production methods (61).

2.6.2 Morphology and structure of nanostructured lipid carriers

The type of NLC depends on the chemical nature of the active ingredient and lipid, the solubility of actives in the melt lipid, nature and concentration of surfactants, type of production, and the production temperature. The three types of NLC can be summarized:

2.6.2.1 The imperfect type

The use of a lipid mixture with very differently structured (sized) molecules distorts the formation of a perfect crystal. The particle matrix contains many imperfections providing space to accommodate the active in molecular form or as amorphous clusters (Figure 8, left). One could state that "the perfectness" of the NLC system is its "imperfectness" in its crystalline structure.

2.6.2.2 The amorphous type

The lipid matrix is solid but not crystalline. It is in an amorphous state (Figure 8, middle).

2.6.2.1 The multiple type

It is an oil-in-solid lipid-in-water dispersion. The solid lipid matrix contains tiny liquid oil nanocompartments (Figure 8 right). This NLC type uses the fact that for a number of drugs, the solubility in oils is higher than their solubility in solid lipids.

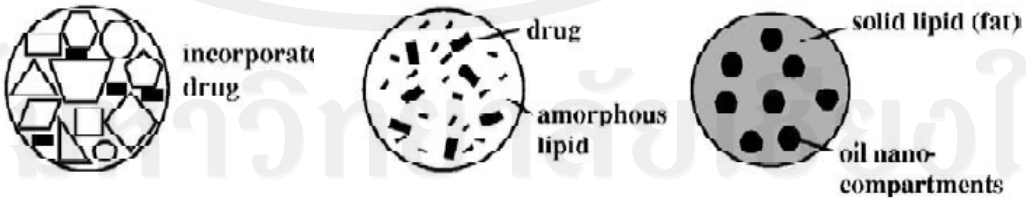


Figure 8 The three types of NLC: imperfect type concentrations of oil (left), amorphous type (middle), multiple type (right) (8)

2.6.3 Stability of nanostructured lipid carriers

Physical stability of the aqueous NLC is the absence of particles aggregation and creaming in the formulation of cosmetic and pharmaceutical products. NLC should be small size in the nanometer range. The particles are kept in suspension by the Brownian motion of the water molecules. NLC surface electrical charge, the particles are stabilized by electrostatics repulsion, and the physical stability is even further enhanced when sterictically stabilizing polymers. Han *et al*. (62) were studies the influence of surfactants on properties of NLC. The formulation in the study combined four types of additives including ionic surfactant (sodium deoxycholate), non-ionic emulsifier (Poloxamer 188 and Tween-80), and Lecithin to obtain favorably stable NLC drug delivery system, which could stabilize for more than 1 year without phase separation at 4°C. Teeranachaideekul *et al*. (10) studies on the formulation parameters affecting the stability of ascorbyl palmitate (AP) after incorporation into NLC were evaluated including types of lipids, types of surfactants, storage conditions. After storage for 90 days, the mean particle size analyzed by photon correlation spectroscopy (PCS) was lower than 350 nm. The zeta potential measured by the Zetasizer IV was higher than −30mV in all developed AP-loaded NLC formulations which varied according to the types of lipid and surfactant.