CHAPTER III
LITERATURE REVIEW

3.1 Types of Tea

Tea is one of the most widely consumed beverages in the world and has become an important agricultural product. Tea could be produced from the leaves of conventional *C. sinensis* or from other parts of herbal plants, including leaves, flowers, seeds, fruits, bark and roots. Tea does not only posses inspiring aroma, it also provides health benefits. As healthy drinks, conventional tea and herbal tea become more famous among new generations and anyone who wants a substitute beverage other than coffee.

3.1.1 Conventional tea (*Camellia sinensis*)

Tea is made from the leaves of *C. sinensis*, an evergreen shrub of the Theaceae family, which grows mainly in many parts of Asia, Middle East and Africa. Tea plants in Thailand are originated from China and have been well–known for centuries. Tea is mostly grown in mountain villages of Chiang Rai and Chiang Mai provinces, especially the regions of Doi Mae Salong, Doi Wawee, Doi Tung and Doi Chang [16]. Nowadays, the areas of northern Thailand have gained the popularity as famous tea–growing areas, contributing to one of the highest tea quality providers in the world.

The tea plants can reach a height of 9–12 meters, but cultivated tea plants are usually pruned to around 1–1.5 meters for convenient maintenance and harvest proposes. This sub–tropical plant grows best in warm/rainy condition and mostly in high altitude areas, where the slope of the land does not exceed 45 degrees. The ideal temperature is 18–30 °C with 75–90% relative humidity. Two main varieties (subspecies) of *C. sinensis*, Assam variety (*C. sinensis* var. *assamica*, also known as

C. assamica) and Chinese variety (*C. sinensis* var. *sinensis*), are used for tea production. Most commercial tea production in the world is comes from Assam variety, including most of black teas, while Chinese variety is used to produce green tea and Chinese–type black tea [17].

Types of conventional tea and chemical compositions

According to fermentation process, tea can be divided into three main types, including unfermented, semi–fermented and fully fermented tea. Three most common types of tea are green, oolong and black teas. There are also some less common types such as white tea and also compressed teas (e.g., Pu–erh). All of these teas are made from the same species of *C. sinensis*, but they are processed in different protocols [17]. The tea processing method defines different categories of tea as well as their chemical compositions. The main chemical compositions in tea are polyphenols (flavan–3–ols, or commonly known as catechins, and flavonoides), alkaloids (caffeine, theophylline and theobromine), volatile oils, polysaccharides, amino acids, lipids, vitamins and inorganic elements such as potassium and manganese [1].

Tea processing method (Figure 3.1) is first prepared by softening up tea leaves that are harvested and placed on the racks known as “withering”. This step is followed by the “rolling” process, which breaks down plant cells, releases and exposes plant enzymes to environmental oxygen. Thus, this process initiates the oxidation reaction and is commonly referred as fermentation, which occurs when the enzymes in tea leaves interact with oxygen. This step lasts for several hours, leading to a change in color and chemical compositions of tea. Normally, shorter oxidation process provides teas with richer flavor and aroma, while longer process emphasizes on giving more color (Figure 3.2). After fermentation, the leaves are taken to the “firing” or drying ovens to reduce moisture content by exposure to hot air at high temperature [1].

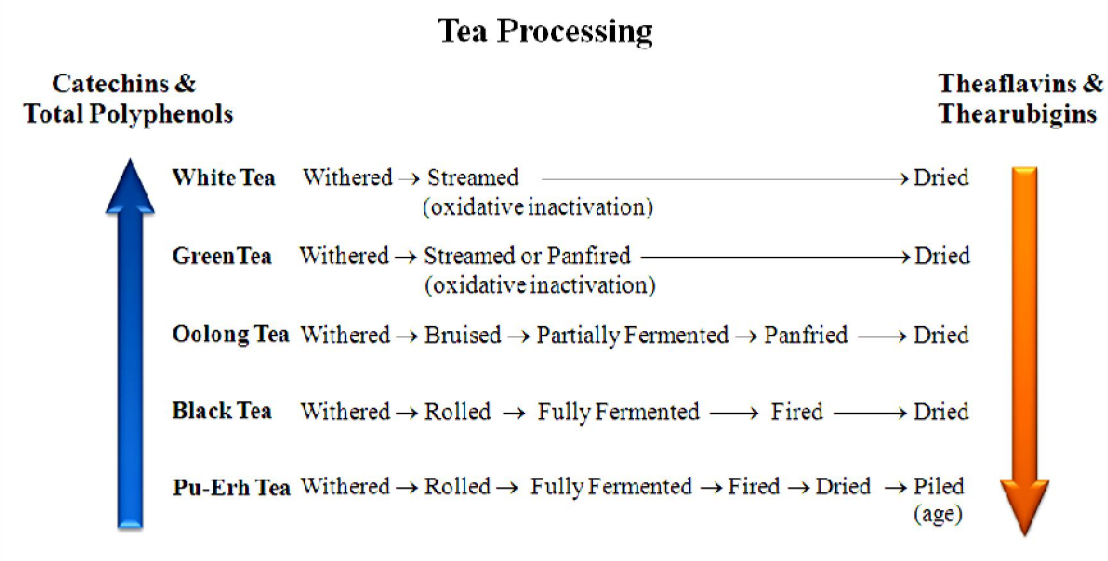


Figure 3.1 Tea processing [18]. Tea leaves are picked and withered with warm air to reduce moisture and to make the leaves pliable for other processes. These procedures will determine the types of tea. White and green teas are both unfermented tea, but white tea is steamed directly after harvest. Oolong tea is a partially fermented tea, while black teas are fully fermented tea leaves. Pu–erh tea is not only fully fermented but is also aged.

Figure 3.2 Five main types of conventional tea [18]. Each type of tea results from various steps of tea processing and different level of oxidation due to fermentation process. More oxidation produces darker–colors in tea. Left to right, white tea, green tea, oolong tea, black tea and pu–erh tea.

Five main types of conventional tea are as follow:

White tea is essentially type of unfermented tea and is the minimally processed type of tea. Buds and few young tea leaves are collected shortly before the buds have fully opened. Then, the leaves are steamed, dried and slightly oxidized. The brewed liquor of white tea is very pale yellow in color and mild tasting. White tea contains theobromine and gallic acid along with high levels of catechins and caffeine [1]. Tea catechins have been found to be better antioxidants than vitamins C and E and carotene [1].

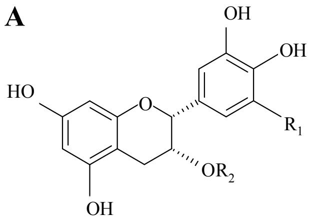
Green tea is an unfermented tea. This type of tea is steamed directly after harvest and withering. The steaming process immediately kills some bacteria and enzymes in the leaves, thus preventing fermentation. This step also helps to soften up the leaves for rolling process. Tea leaves are then fired to prevent further fermentation. The liquor of this tea is typically green or yellow in color. Its flavor ranges from smooth, nutty, toasty, to sweet. The chemical compositions of green tea highly resemble that of fresh tea leaves. It contains polyphenolic compounds, which account for up to 30% of dry weight. Most of green tea polyphenols are catechins (6–16% of dry green leaves). The four main catechin derivatives present in green tea are (−)–epicatechin (EC), (−)–epigallocatechin (EGC), (−)–epicatechingallate (ECG) and (−)–epigallocatechingallate (EGCG). Other than catechin, green tea also contains alkaloids such as caffeine, theobromine, theophylline and phenolic acids (i.e., gallic acids) [1] (Figure 3.3A and C).

Oolong tea is a partially fermented tea. This type of tea undergoes all stages of tea processing, including partially withered, fermented, fired, rolled and partially re–fermented steps. Oolong teas undergo only 10–80% fermentation comparing to black tea, measured by amount of brown or red colors on the leaf during tea processing. The brewed liquor of oolong tea is clear and orange–brown in colors. This tea contains majority of monomeric catechin, theaflavins and thearubigins with marginal amount of epigallocatechin ester, theasinenins, dimeric catechin and dimeric proanthocyanidins [17, 19].

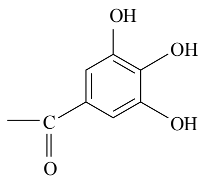
Black tea is a fully fermented tea. It utilizes all of tea processing and completely made within a day. The brewed liquor of black tea ranges from dark brown to deep red in colors. During the fermentation process, the monomeric flavan–3–ols undergo polyphenol oxidation, resulting in conversion to theflavins, therarubigins and other oligomers [20] (Figure 3.3B). The theflavin (about 3–5% of dry weight), a benzotropolone–linked heterodimeric catechin, is responsible for the characteristics of black tea (color and taste). Therarubigin (20% of dry weight) with a wide molecular weight range depending on different oligomeric and polymeric polyphenolic structures, on the other hand, is less well characterized [21]. Even though approximately 77% of the polyphenols in green tea are reported as catechins, only 3–4% catechins from total polyphenols in black tea are discovered [22]. The decrease in level of this bioactive compound is expected from the results of tea processing as well as fermentation process.

Pu–erh tea is another fully fermented tea. This tea is steamed either as loose–leaf tea or pressed into dense cakes with decorative shapes. Tea is stored in special temperature– and humidity–controlled conditions during which time the tea is gradually oxidized and fermented for a number of years. This process results in lower concentrations of tea polyphenols and tea catechins than other types of tea [17].

A



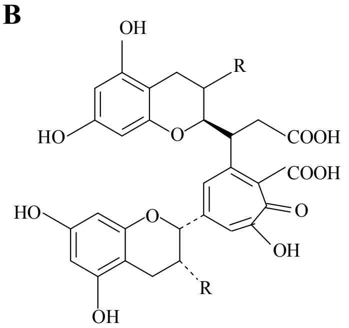
Catechins



Gallate Group

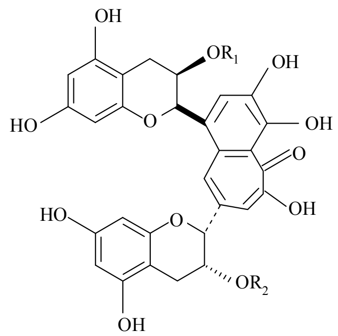
Tea catechins	R1	R2
(-)-Epicatechin (EC)	H	H
(-)-Epigallocatechin (EGC)	H	OH
(-)-Epicatechin gallate (ECG)	H	gallate
(-)-Epigallocatechin gallate (EGCG)	OH	gallate

B



Thearubigins

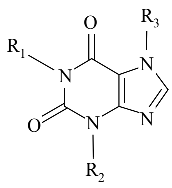
(R = gallate or other group)



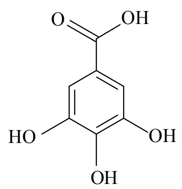
Theaflavins

(R = gallate or H)

C



Tea alkaloids	R1	R2	R3
Caffeine	CH_3	CH_3	CH_3
Theophylline	CH_3	CH_3	H
Theobromine	H	CH_3	CH_3



Gallic acid

Figure 3.3 Chemical structures of tea constituents [23, 24]. The main chemical compositions of tea are polyphenols known as catechins. Four main types of catechins, including (A) (−)–epicatechin (EC), (−)–epigallocatechin (EGC), (−)–epicatechingallate (ECG) and (−)–epigallocatechingallate (EGCG), are mostly present in green tea. Likewise, major polyphenols in black tea are (B) theaflavins and thearubigins, being formed by the oxidation and polymerization of catechins during fermentation process. Beside polyphenols, tea also contains alkaloids such as (C) caffeine, theobromine and theophylline, as well as phenolic acids such as gallic acids.

3.1.2 Herbal Teas

Herb is a plant or plant part used for therapeutic properties and also used as functional food, food coloring and cosmetics as well as beverages. Thai traditional herbal beverage and herbal tea have played an important role, not only for quenching the thirst, but also provides health benefit. Consumers have increased interest in drinking herbal teas due to the belief of health benefit and less side effects comparing to synthetic medicine. Herbal teas are prepared from various parts of plants such as leaves, flowers, seeds, fruits, stems, roots and barks. Teas are carefully dried and stored in containers in order to retain and preserve the herbal properties until use.

Herbal teas can be brewed through the process of decoction or infusion. The decoction is made by boiling herbal with water and usually prepared into extract fluids from hard plant materials such as roots and barks. This process is normally accomplished in the preparation for medicinal purposes as in Chinese herbal remedies and in brewed teas for refreshments as well. On the other hand, the infusion is made by pouring boiling water over dried or powdered herbs in a cup or a teapot. Normally, the herbs are ground into small granules or powder so that it dissolves immediately in water.

Each herb contains different health effects and limit of consumption. It can divide herbal tea into three groups according to purposes of drinking. First is herbal tea that focuses on good taste and nice aroma with some health benefits. Thai herbal teas in this group include *Chrysanthemum indicum* (Kek–huay), *Zingiber officinale* (Khing), *Centella asiatica* (Bai–bua–bok), *Hibiscus sabdariffa* (Kra–jiab) and *Aegle marmelos* (Ma–toom). These herbal teas usually possess no side effect and thus not harmful. Second is herbal tea with minor medicative properties such as *Orthosiphon aristatus* (Ya–nuad–maew), *Thunbergia laurifolia* (Rang–jued), *Carthamus tinctorius* (Dok–kum–phoi) and *Vernonia cinerea* (Ya–dok–khao). These herbal teas could be taken twice a day for several days without any side effect. The last group is aimed for medicative action such as laxative *Senna alata* (Chum–hed–ted) and *Senna alexandrina* (Ma–kham–kaek). These herbal teas are required limited consumption and should be taken into consideration as prolonged consumption is more harmful than good.

Thai Food and Drug Administration (FDA) defines additional plants and their parts to be used as raw materials for herbal tea as in accordance with the Notification of Ministry of Public Health (MOPH) No. 280 B.E. 2547 (2004). The plants and parts thereof that have been allowed as raw materials for herbal tea are reported (Table 3.1) [25].

Table 3.1: List of plants or their parts used as raw materials for herbal tea, approved by Thai FDA [25]

Scientific name	***Thai common name***	***Part used***
Aegle marmelos (L.) Corr.	Ma–toom	Fruit
Phyllanthus emblica L.	Ma–kham–pom	Fruit
Momordica grosvenori Swingle	Luo–han–gua	Fruit
Hibiscus sabdariffa L.	Kra–jiab	Flower
Carthamus tinctorius L.	Dok–kum–phoi	Flower
Chrysanthemum indicum L.	Gek–huay	Flower
Ganoderma lucidum (Fr.) Karst.	Hed–lim–jue	Flower
Cynara scolymus L.	Ar–ti–choke	Flower and leaf
Morus alba L.	Bai–mon	Leaf
Centella asiatica (L.) Urban	Bai–bua–bok	Leaf
Pandanus amaryllifolius Roxb.	Bai–toey	Leaf
Gynostemma pentaphyllum (Thunb.) Mak.	Jiao–gu–lan	Leaf
Stevia rebaudiana Bertoni	Ya–wan	Leaf
Cymbopogon citratus (DC.) Stapf	Ta–khrai	Stem and leaf
Derris scandens Benth.	Thao–wan–priang	Stem and leaf
Zingiber officinale Roscoe	Khing	Root
Alpinia galanga (L.) Willd	Kha	Root
Glycyrrhiza glabra L.	Cha–ame –ted	Root

Currently, herbal teas have been gaining popularity for Thai people as healthy beverages due to their fragrances and results of promoting health on antioxidant properties and therapeutic applications by media. In this study, fifteen popular Thai herbal teas will be focused and investigated in term of their biological functions against selected key enzymes that control some major chronic diseases. These herbal teas are divided into five main groups based on part of plants including fruit, flower, leave, stem/leave and root parts. The characteristics of herbs and their therapeutic properties could be summarized as follow:

1) Fruits

Bael fruit (Ma–toom)

Scientific name: *Aegle marmelos* (L.) Corr.

Family: Rutaceae

Characteristic: Bael fruits possess oval shape with smooth and woody skin that ranges from green to brown color. The shell is thick and very hard. Numerous seeds are surrounded by slimy mucilage (Figure 3.4A).

Therapeutic uses: Antiseptic, astringent, carminative, expectorant, stimulant, stomachic

Indian gooseberry (Ma–kham–pom)

Scientific name: *Phyllanthus emblica* L.

Family: Phyllanthaceae

Characteristic: Indian gooseberry fruits are small in size with spherical shape that lies with six vertical stripes. Its color is light greenish yellow with a quite smooth and hard on appearance (Figure 3.4B).

Therapeutic uses: Antioxidant, antipyretic, antitussive, aphrodisiac, astringent, blood tonic, ditretic, expectorant, hemostatic, laxative, nutritive tonic, refrigerant, stomachic

Bitter cucumber (Ma–ra–kee–nok)

Scientific name: *Momordica charantia* Linn.

Family: Cucurbitaceae

Characteristic: Bitter cucumber fruits are bright green in color, small oval shape with roughly and knobby skin. Inside the fruit, a large hollow contains a thin layer surrounding the seed (Figure 3.4C).

Therapeutic uses: Antioxidant, antiviral, antidiabetic, immunomodulating

2) Flowers

Rosella (Kra–jieb)

Scientific name: *Hibiscus sabdariffa* L.

Family: Malvaceae

Characteristic: The rosella flowers are white to pale yellow that have a deep red spot at the base of each petal. There are 8–12 petals of the pointed petals underneath the flower. The sepals are spreading out together to cover the seeds (Figure 3.4D).

Therapeutic uses: Antitussive, carminative, diuretic, expectorant, refrigerant, tonic

Safflower (Kum–phoi)

Scientific name: *Carthamus tinctorius* L.

Family: Asteraceae

Characteristic: The safflower flowers are usually yellow or orange in color. Each flower produces 15–30 seeds with seed oil content usually being between 30–45% (Figure 3.4E).

Therapeutic uses: Alterative, antirheumatic, carminative, diaphoretic, diuretic, emmenagogue, laxative, male tonic, tonic, stimulant

Chrysanthemum (Kek–huay)

Scientific name: *Chrysanthemum indicum* L.

Family: Asteraceae

Characteristic: The chrysanthemum flower comes in several varieties of shape and color, but originally it was just a small yellow flower. Yellow or white chrysanthemum flowers are used for tea (Figure 3.4F).

Therapeutic uses: Alterative, antipyretic, antispasmodic, bitter tonic, carminative, diaphoretic, emmenagogue, nervine, sedative, stimulant

3) Leaf

White mulberry (Mone)

Scientific name: *Morus alba* L.

Family: Moraceae

Characteristic: White mulberry leaves are usually bright green, alternate, simple and ovate. The margins usually contain larger, more rounded teeth (Figure 3.4G).

Therapeutic uses: Anthelmintic, antipyretic, antitumor, antitussive, aphrodisiac, carminative, laxative, purgative, sedative

Asiatic pennywort (Bua–bok)

Scientific name: *Centella asiatica* (L.) Urban

Family: Apiaceae

Characteristic: The leaves are in a single form with long stems and a kidney shape. There is a deep curve at the base of each leaves. The leaves are jagged around in a triangle form (Figure 3.4H).

Therapeutic uses: Alterative, antioxidant, antipyretic, antirheumatic, astringent, bitter tonic, blood tonic, diuretic, emmolient, expectorant, nervine, vulnerary

Pandanus (Toey–hawm)

Scientific name: *Pandanus amaryllifolius* Roxb.

Family: Pandanaceae

Characteristic: The leaves are green oblong shape, pointed tip and smooth edges. The leaves grow on the stalk and around, which abundantly arrange themselves in order (Figure 3.4I).

Therapeutic uses: Antipyretic, carminative, digestive, diuretic, expectorant

Jiaogulan (Pan–ja–khan)

Scientific name: *Gynostemma pentaphyllum* (Thunb.) Mak.

Family: Asteraceae

Characteristic: The leaves are green, serrated and commonly grow in group of five (Figure 3.4J).

Therapeutic uses: Anti–inflammatory, antioxidant, antiproliferative

Stevia (Ya–wan)

Scientific name: *Stevia rebaudiana* Bertoni

Family: Cucurbitaceae

Characteristic: The leaves are small, oblanceolate shape and serrated above the middle. The leaves of stevia contain sweetening compounds that has a pleasantly sweet and refreshing taste (Figure 3.4K).

Therapeutic uses: Antioxidant, adjuvant

Cat's whisker (Ya–nuat–maeo)

Scientific name: *Orthosiphon aristatus* (Blume) Miq

Family: Lamiaceae

Characteristic: The leaves are oval–shaped, bright to dark green and coarsely toothed (Figure 3.4L).

Therapeutic uses: Diuretic

4) Stem and leaf

Lemon grass (Ta–krai)

Scientific name: *Cymbopogon citratus* (DC.) Stapf

Family: Poaceae

Characteristic: The plants are tall striped leaves, jagged edges and a sharp (Figure 3.4M).

Therapeutic uses: Antiemetic, anti–inflammatory, antitussive, diuretic, carminative, diaphoretic, digestive, expectorant, refrigerant, stomachic, tonic

Jewel vine (Thao–wan–priang)

Scientific name: *Derris scandens* Benth.

Family: Leguminosae

Characteristic: *Derris scandens* is a woody vine, an evergreen climbing branch shrub with rich green leaves. The vines are often twisted and the leaves are compound and alternate pinnate (Figure 3.4N).

Therapeutic uses: Analgesic, antispasmodic, diuretic, purgative

5) Roots

Ginger (Khing)

Scientific name: *Zingiber officinale* Roscoe

Family: Zingiberaceae

Characteristic: Ginger root possesses a distinctive thickened with a swollen hand–like appearance. The rhizome exhibits a brown outer layer and a pale yellow centre with a lemon like smell and spicy flavor (Figure 3.4O).

Therapeutic uses: Adjuvant, analgesic, antiemetic, anti–inflammatory, antirheumatic, antiseptic, antitussive, aphrodisiac, carminative, diaphoretic, digestive, emmenagogue, expectorant, galactogogue, stimulant, stomachic, tonic

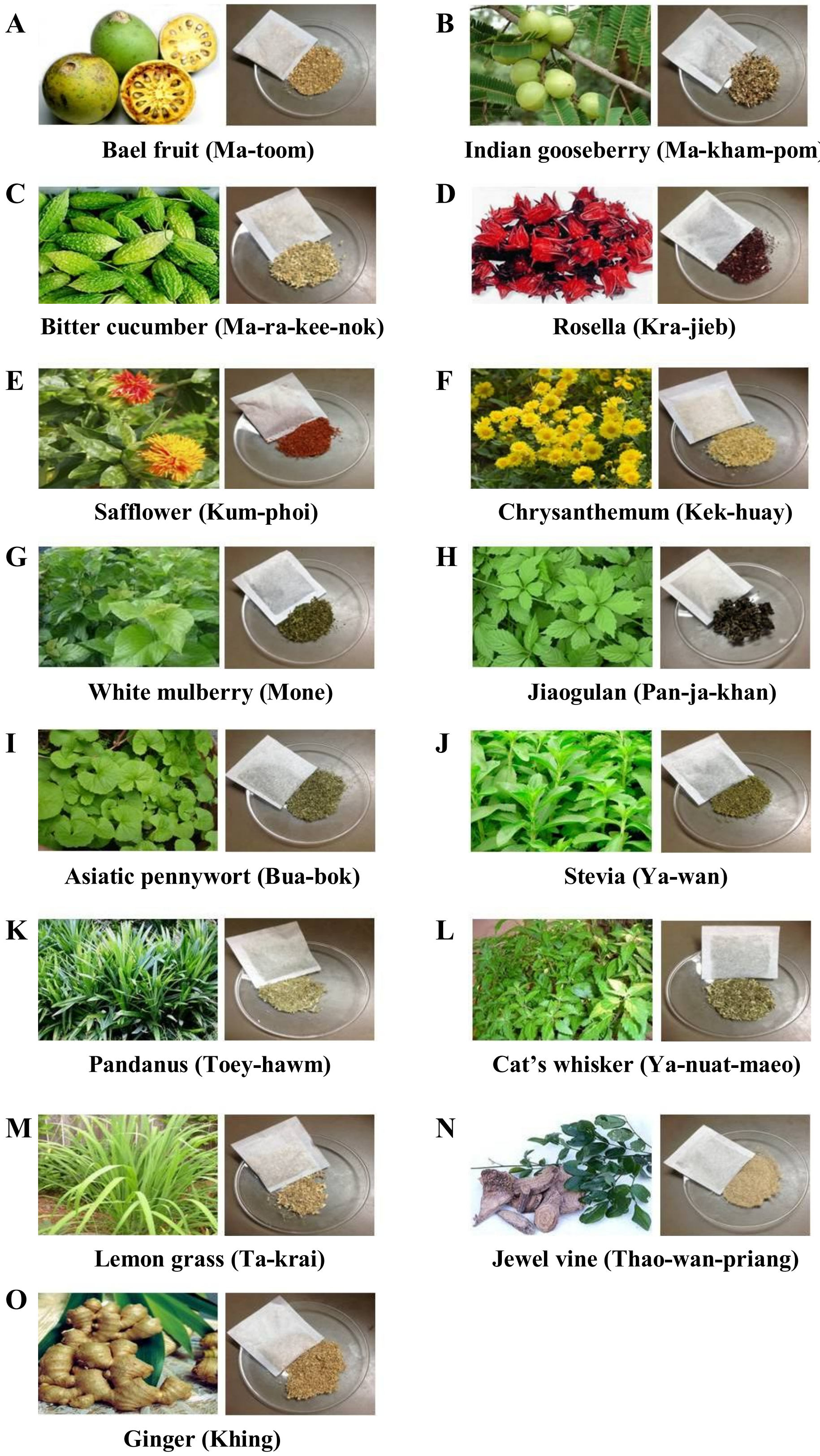


Figure 3.4 Fifteen Thai herbal teas from each part of plants. Fruit tea categories consist of (A) beal fruit (Kra–jieb), (B) Indian gooseberry (Ma–kham–pom) and (C)

bitter cucumber (Ma–ra–kee–nok). Flower tea categories consist of (D) rosella (Kra–jieb), (E) safflower (Kum–phoi) and (F) chrysanthemum (Kek–huay). Leave tea categories consist of (G) white mulberry (Mone), (H) asiatic pennywort (Bua–bok), (I) pandanus (Toey–hawm), (J) jiaogulan (Pan–ja–khan), (K) stevia (Ya–wan) and (L) cat's whisker (Ya–nuat–maeo). Stem and leave tea categories consist of (M) lemon grass (Ta–krai) and (N) jewel vine (Thao–wan–priang). Root tea categories consist of (O) ginger (Khing). The pictures showed fresh herbs on the left and dry herbs that are used in this experiment on the right.

3.2 Tea and Health Promotion

Medicinal plants or green medicines are an important therapeutic alternative pathway for treatment and prevention of various ailments. Tea is a most popular beverage that is associated with lifestyle and nutraceuticals. Tea provides a good source of bioactive compounds with vast biological functions to human body. Traditionally, consumption of teas suggested an important role in treatment and protective effects for a wide variety of health conditions such as cardiovascular disease, neurodegenerative disease, diabetes, hypertension and obesity. In this study, the potential efficacy of tea as preventive agents of oxidative stress, glycation, obesity and hypertension will be focused.

Since fat represents a major source of unwanted calories, the inhibition of fat digestion is an interesting approach for reducing fat absorption. Pancreatic lipase inhibition is one of the most widely studied mechanisms for the determination of potential efficacy of natural product as anti–obesity agents. Interestingly, sodium retention plays a central role in the development of obesity–related hypertension. Renin–angiotensin–aldosterone system (RAAS) is one of the most important mechanisms in the body concerning the regulation of blood pressure. Angiotensin–converting enzyme (ACE) is a key enzyme in RAAS, which associated to angiotensin II production, thereby further increasing renal sodium and water reabsorption in kidneys, leading to increased blood pressure. Therefore, the inhibition of ACE is considered as a targeted approach for anti–hypertension.

3.2.1 Oxidative stress

Oxidative stress is a disturbance in the systemic balance between the oxidant and antioxidant. It results from the overproduction of free radicals, including reactive oxygen species (ROS). Free radicals are generated in the human body from endogenous (e.g. respiratory chain and oxidative enzymes) and exogenous (e.g. air pollution, smoking, drug, X–ray and ozone) sources [26]. Free radicals play an essential role in enzyme–catalyzed reaction, activation of nuclear transcription factors and gene expression. Although free radicals are essential to the body, excess amount may lead to cytotoxicity and oxidative damage to healthy tissue, leading to development of some diseases. Free radicals interact with other molecules within the cells, which results in damage of biomolecules such as lipid, protein, amino acids, DNA and RNA. Several clinical conditions suggested scientific evidence of free radical that causes damage to major organs and the brain by the chronic diseases and by neurological diseases such as obesity, cancers, diabetes, cardiovascular diseases, Parkinson's disease and Alzheimer's diseases [27, 28]. However, the normal biological system can be maintained by exclusion of excessive free radicals by antioxidants.

Antioxidants and antioxidant capacity assays

Antioxidants have been defined as any substances that inactivate free radicals or prevent free radical–initiated chemical reactions through either 1) directly interactions of antioxidants and free radicals or 2) indirectly effect by changing the redox balance. Antioxidants can suppress free radicals and protect cells against oxidative stress, thus preventing cell damages and some severe diseases. Based on their functions, antioxidants are classified as primary (or chain breaking) and secondary (or synergists) compounds. Primary antioxidants are able to inhibit chain reactions by acting as hydrogen donors or free radical acceptors. On the other hand, the action mechanism of the secondary antioxidants is based on chelating property of prooxidant and free metals.

The antioxidant defense systems includes 1) endogenous (enzymatic and non–enzymatic) antioxidants such as superoxide dismutase, catalase, thioredoxin, glutathione peroxidase, the peroxiredoxin family of proteins and glutathione and 2) exogenous antioxidants such as vitamin C, vitamin E, carotenoids and polyphenols, which can be obtained from the diet [29]. Polyphenols are the most abundant antioxidants, which found in varieties of plants, fruits and vegetables as well as tea from *C. sinenis* [30].

Various methods have been employed to evaluate the antioxidant capacity *in vitro*. The assays can be classified into two categories based on the reaction mechanism, including 1) hydrogen atom transfer (HAT) reaction and 2) a single electron transfer (SET) reaction (Table 3.2).

Table 3.2 *In vitro* antioxidant capacity assay (Adapt from Ndhlala *et al.*, 2010) [31]

Principle	***Antioxidant capacity assays***
Hydrogen atom transfer reaction (HAT–based assays)	- Oxygen radical absorbance capacity (ORAC) - *β*–Carotene/linoleic acid model system - Inhibition of phospholipid peroxidation
Single electron transfer reaction (SET–based assays)	- Total phenols by Folin–Ciocalteu (Folin–C) - 1,1–Diphenyl–2–picrylhydrazine (DPPH) radical scavenging - Trolox equivalent antioxidant capacity (TEAC) - Ferric reducing antioxidant power(FRAP)

The HAT–based assays measure the ability of an antioxidant to quench free radicals by hydrogen–atom donation. The mechanisms of HAT reaction can be explained as the hydrogen atom (H) of antioxidant (A) being transferred to an $ROO^{\bullet}$ radical [32] as follows.

$$ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet}$$

Antioxidant capacity measurements are based on competitive inhibition of chemical kinetics, and the quantity of antioxidant is derived from the kinetic curves. These assays generally are composed of a synthetic free radical generator, an oxidizable molecular probe and an antioxidant compound [31].

On the contrary, SET–based assays measure the ability of a potential antioxidant to transfer one electron from antioxidant to any potential electron acceptors (such as metals, carbonyls and free radicals). The mechanisms can be summarized by the reaction (1–3) [32]:

$$ROO^{\bullet} + AH \longrightarrow ROO^{-} + AH^{\bullet+} \quad (1)$$

$$AH^{\bullet+} + H_2O \rightleftharpoons A^{\bullet} + H_3O^{+} \quad (2)$$

$$ROO^{-} + H_3O^{+} \rightleftharpoons ROOH + H_2O \quad (3)$$

Antioxidant capacity was demonstrated at the end point of the redox reaction (equation (3)) by measuring an absorbance of probed oxidants (as indicated by color change). The color change occurs when an electron is removed from the antioxidant with the degree of color change being proportional to the concentration of antioxidants in the reaction mixture [31]. The reaction endpoint is then terminated when the color change stops.

However, due to the complex nature of biological systems and the multiple reaction characteristic, no individual assay for measuring antioxidant capacity will accurately reflect all radical sources and antioxidants [32]. Thus, in this study four difference methods, including ORAC assay as HAT mechanism as well as FRAP, DPPH radical scavenging and Folin–C assays as SET mechanism were employed to investigate antioxidant capacity (more detailed mechanisms in Appendix A).

Tea and anti–oxidant properties

Tea is the major source of antioxidants called polyphenols, the same compound that is found in fruits and vegetables. White tea and green tea have been reported as an effective antioxidant provider as it contains the highest quantity of polyphenols. Most of tea polyphenols are catechins [33]. Upon the oxidation step of tea leaves to make black tea, catechins are converted to complex components called theaflavins and thearubigins. These compounds can also act as antioxidants; however, they exhibit lesser activity than catechins from white tea and green tea. Thus, it can be concluded that the radical scavenging activity of white tea and green tea are usually higher than that of black tea due to the loss and change of antioxidants during tea processing. Thus, the cultivar type is critical in determining the antioxidant potency of tea product. Black teas that processed from suitable cultivars could be potent in antioxidant activity comparing to green teas [34]. In addition, tea catechins also enhance the activity of glutathione peroxidase, glutathione reductase, glutathione–*S*–transferase, catalase and quinine reductase, which act as detoxifying enzymes that serve as cellular antioxidants.

Tea catechins are better antioxidants than vitamins C and E. Interestingly, epigallocatechingallate (EGCG) provides the most potent antioxidant activity among all catechins both *in vitro* and *in vivo*. Due to the structure of catechins that consists of phenolic hydroxyl groups (Figure 3.3A), it can chelate metal ions and prevent a generation of free radicals [35]. Thus, it can be concluded that the most potent antioxidant activity of EGCG is due to several phenolic hydroxyl groups.

Likewise, antioxidant activities of plants are widely varied and depend on types of herb. Plants or herbs with higher phenolics content possess stronger primary antioxidant activities. Several herbs provide antioxidant–rich sources, which are capable of terminating free radical–initiated reactions and preventing oxidative damage. Generally, herbal teas exhibit lower antioxidant activity than tea of *C. sinensis*. However, recent studies have shown that antioxidants activities of *S. rebaudiana* and *Lagerstroemia speciosa* teas were comparable or even superior to those of *C. sinensis* [36]. Vast variety of Thai herbal teas are found to be the potent sources of antioxidants such as *P. emblica*, *S. rebaudiana, M. alba*, *H. sabdariffa*, *C. indicum*, *A. marmelos*, *C. asiatica*, *A. galanga* and *Z. officinale* [9-12]. Many chronic diseases including cardiovascular disease, renal disease, cancer as well as diseases associated with aging and inflammatory conditions are results of oxidative stress and subsequent generation of free radicals. Thus, tea may possess high potential nutraceutical properties against the progression of these diseases.

3.2.2 Glycation

Glycation is an oxidative stress related glycosylation process. Glycation is a non–enzymatic reaction between amino group (–NH_2) of protein or lipid molecules with reducing agents (such as sugar), resulting in the formation of advanced glycation end products (AGEs). Reducing sugars initiate a reversible reaction by forming a Schiff–base with the free amino group on protein. This Schiff–base then thermodynamically converts into Amadori product, the intermediate glycation product, wherein the rearrangement of hydrogen atom from the hydroxyl group adjacent to the carbon–nitrogen double bond moves to the amide carbon, leaving a ketone moiety. Over a period of weeks, these products undergo further rearrangement reactions to form AGEs. Finally, the reaction is driven by oxidative and non–oxidative pathway, forming specific AGEs such as pentosidine and Nε–(carboxymethyl)–lysine, and pyrraline, respectively (Figure 3.5) [37].

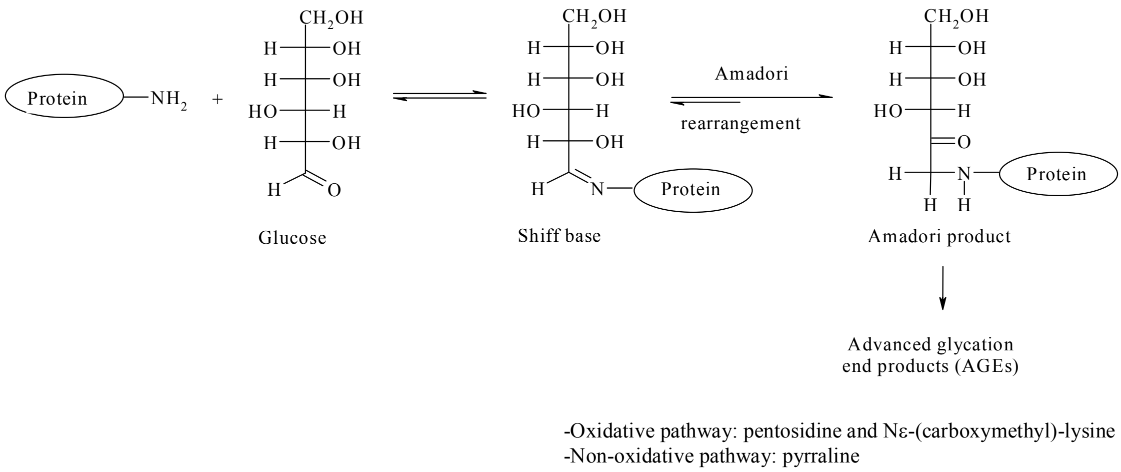


Figure 3.5 Glucose and AGEs formation pathway. Glucose reacts with the amino group of protein though a series of reactions, forming Schiff bases and Amadori product, and eventually AGEs.

Alternatively, the Schiff–bases may undergo spontaneous degradation to form reactive α, β–dicarbonyl species such as glyoxal and methylglyoxal (Figure 3.6). Both are cytotoxic compounds produced within body and can directly modify amino groups of amino acids to form AGEs [38]. Nε–(carboxymethyl)–lysine and Nε–(carboxyethyl)–lysine are the major AGEs adducts formed from glyoxal and methyglyoxal, respectively [39]. Beside, glyoxal and methylglyoxal can be generated by glucose breakdown (*via* the Wolff pathway), triose phosphate fragmentation, acetone/threonine fragmentation, lipid peroxidation and fructose–3–phosphate decomposition (Figure 3.7).

AGEs has been shown to cause oxidative stress in various cell types and also implicated in the progression of many age–related diseases such as cardiovascular disease, diabetes and Alzheimer's disease [40-42]. Although, the endogenous AGEs are formed in the cell of human body, they arise from exogenous sources such as smoke and diet with food processing products [42]. The accumulation of AGEs has been associated with the progression of many diseases, and oxidative stress also promotes AGEs formation. Therefore, the development of natural products with both anti–oxidative stress and anti–AGEs formation properties may have a potential to prevent such illness.

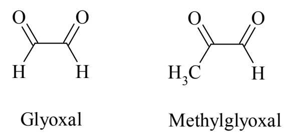


Figure 3.6 Chemical structures of glyoxal and methylglyoxal. Both compounds are cytotoxic and can cause AGEs through interactions with free amino group in protein.

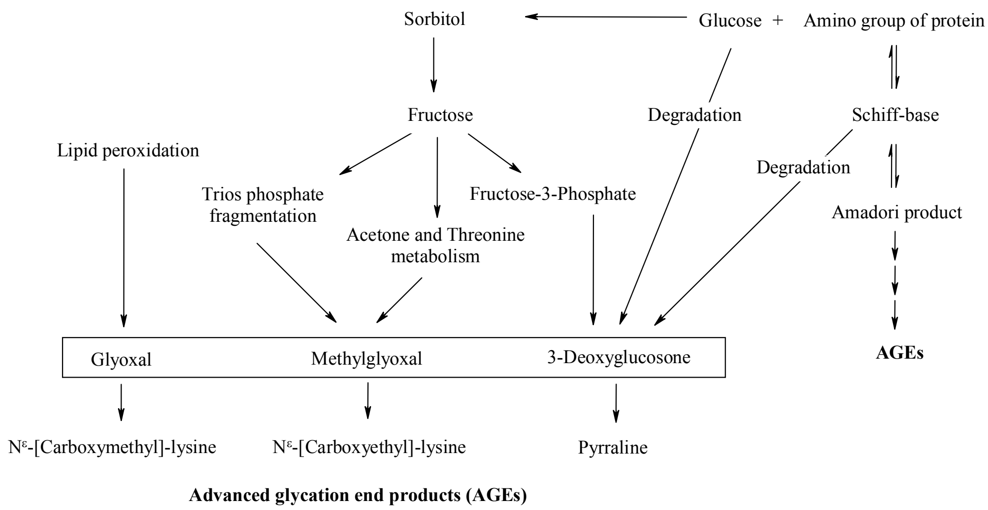


Figure 3.7 Formation of AGEs by glyoxal and methylglyoxal. These dicarbonyls can be generated by glucose degradation, triose phosphate fragmentation, acetone and threonine fragmentation, lipid peroxidation and fructose–3–phosphate decomposition. Nε–(carboxymethyl)–lysine and Nε–(carboxyethyl) lysine are the major AGEs adducts formed from glyoxal and methylglyoxal, respectively.

Tea and anti–glycation properties

Dietary plants with antioxidant phenolics possess a potential function against glycation process. Since tea from *C. sinensis* is an important dietary source of phenolic compounds, tea likely exhibits anti–glycation activity. Currently, little information regarding anti–glycation activity of teas is available. Previous studies reported that green tea, black tea and oolong tea extracts initiated anti–AGEs formation in glucose and methylglyoxal induced bovine serum albumin (BSA) systems *in vitro* [43]. Among these, green tea showed the highest anti–AGEs activity [10].

For herbal teas, several studies suggested that some plants not only exhibited antioxidant activity but also anti–glycation activity. The later was attributed to amounts of phenolics and flavonoids. Numerous herbal teas including *C. citratus*, *O. aristatus*, *M. alba*, *H. sabdariffa*, *Melissa officinali* and *C. tinctorius* exhibited potent anti–glycation activity [2, 10]. However, herbal teas generally possessed lower anti–glycation activity than those of *C. sinensis*. In contrast, recent studies suggested that anti–glycation activity of *S. rebaudiana* and *Caesalpinia sappan* teas were comparable to those of *C. sinensis* [10]. In addition, *M. officinalis* exhibited higher anti–glycation activity than green tea, both in BSA/glucose and BSA/methylglyoxal systems [2]. Thus, these herbal teas could be excellent choices of interest for healthy beverages.

3.2.3 Obesity

Overweight and obesity are defined by World Health Organization (WHO) as abnormal or excessive fat accumulation that presents a risk to human health [44]. A crude population measurement of obesity is body mass index (BMI), which can be calculated by weight (in kilograms) and height (in meters) of a person and has a unit of kg/m^2. For Asians, the limited BMI of overweight and obesity are ≥ 23.0 and ≥ 25.0 kg/m^2 [45]. In addition, waist circumference is another important measurement used to evaluate central adiposity and the risk of chronic disease on the consequences of being overweight and obese. The suggested waist circumference cut–offs are 90 cm for men and 80 cm for women [46]. The fundamental motive for overweight and obesity is an energy imbalance between calories consumption and calories expenditure. The risk factors contributing to obesity include genetics, environmental factors, social lifestyle, illnesses, and medications. Overweight and obesity are related to several chronic diseases such as hypertensions cardiovascular diseases, diabetes mellitus and certain types of cancer. The prevalence of such obesity–related diseases is likely increased in proportion of obesity itself [47].

Currently, overweight and obesity are the fifth leading risks of global deaths and are major public health problems that have become more common among adults and rapidly increased among children and adolescents. Nowadays, more than 1.1 billion people are undergone overweight worldwide, and 312 million are classified as obese. Among these, at least 2.8 million people die each year from these diseases [48]. In addition, 44% of the diabetes burden, 23% of the ischaemic heart disease burden and 7–41% of certain cancer burdens are attributable to overweight and obesity [44]. As in Thailand, overweight and obesity are also considered as serious problems for public health. The fourth national health examination survey in 2008 (NHES 4) found that the prevalence of overweight and obesity has significantly increased from the first survey in 1991 by three–fold in adults [49, 50]. Therefore, it is important to establish fundamental guidelines for the prevention and treatment of overweight and obesity.

Obesity and high fat diet

Obesity is resulted by an energy imbalance, which causes the focus on dietary fat intake to be over–emphasized at the expense of total energy intake. Many studies have shown a direct relatedness between the incidence of obesity and dietary fat consumption. Several mechanisms have been proposed to explain how high fat intake might lead to increased body fat [51, 52]. Fats are potent energy source in the diet providing 9 kcal per gram, whereas carbohydrate or protein provides 4 kcal per gram. Thus, fat enriched foods are high in energy density, and fat intake is an important contributor to energy balance. Obesity from high fat diet is then a result of positive energy balance. In addition, fats are often considered to yield greater food flavor and palatability, which are the possible causes of high fat consumption [53]. Furthermore, under metabolic conditions of macronutrients, fat produces a lower thermogenesis effect than protein and carbohydrate, suggesting that fats may be accumulated as body fat [54]. Therefore, high fat diets may play an important role in the increased prevalence of obesity and obesity–related diseases. Low fat diets are often prescribed as the prevention and treatment of overweight and obesity.

The digestive process of dietary lipid consists of several sequential steps, including emulsification, hydrolysis, micellization and lastly absorbance into the enterocytes (Figure 3.8). The major human lipases include the pre–duodenal (lingual and gastric) and the extra–duodenal (pancreatic, hepatic, lipoprotein and the endothelial) lipases [4]. Among these, pancreatic lipase produced by the pancreatic acinar cells is responsible for hydrolysis of 50–70% of total dietary fat, while gastric lipase exhibits approximately 10–30% digestion and lingual lipase only occupies minimal activity. The digestion of fats starts in oral cavity through exposure to lingual lipases and continues to the stomach through exposure to both lingual and gastric enzymes. The fats are emulsified by bile acids and then mixed with pancreatic enzyme for hydrolysis process. Most dietary fat is ingested as triglycerides (90%) comprising of a unit glycerol and three chain of fatty acids (FAs).These triglycerides (TGs) are hydrolyzed to free fatty acid and monoglycerides (MGs) by pancreatic lipases in conjunction with colipase (lipase coenzyme) to accelerate the enzyme reaction. The resulting products form mixed micelles that mainly contain cholesterol, bile salts and lysophosphatidic acid (LPA) before being absorbed from small intestine into enterocytes. This process eventually leads to the resynthesis of TGs, which are then stored in adipocytes as the main energy source.

Pancreatic lipase, the main enzyme in fat digestion, possesses a molecular weight of approximately 50 kDa from 449 amino acids. The encoded enzyme shows 86% and 68% sequence homology with porcine and canine pancreatic lipase, respectively. The overall structure of the enzyme consists of two domains, the large N–terminal domain (residues 1–336) and the smaller C–terminal domain (residues 337–449) (Figure 3.9). The large N–terminal domain contains the α/β–hydrolase fold, which acts as an active site with a catalytic triad formed by active residues, Ser^{152}, Asp^{176}, His^{263}. This catalytic triad is chemically analogous to serine proteases such as chymotrypsin but with structural distinction. A lid domain (Cys^{237}–Cys^{261}) acts as a surface loop that covers the active site from outside environment once the enzyme binds to its substrate or when the enzyme is in its resting state (closed conformation).

This closed conformation of the enzyme is stabilized by the two loop regions, β5 (residues 75–84) and β9 (residues 203–223). In the presence of bile salts micelles, the lid domain and the β5 loop adopt a new conformation to open the lid (opened conformation), which allows substrate to bind the enzyme's active site. The C–terminal domain is important for binding of colipase. This coenzyme exposes the hydrophobic tips of its fingers at the opposite site of its lipase–binding domain. These hydrophobic tips help to bring the catalytic N–terminal domain into close proximity with the interface area where the opening of the lid domain occurs, thus supporting the interactions of lipid–water interface [4, 55].

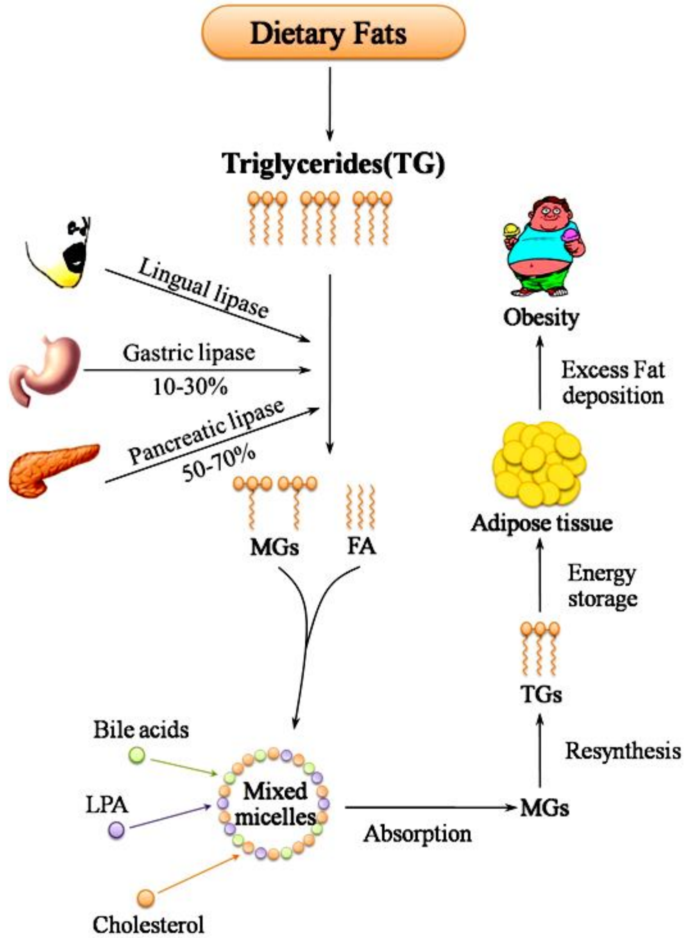
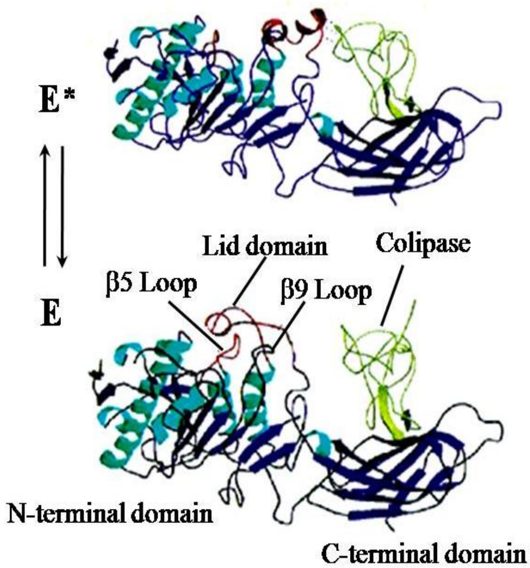


Figure 3.8 Processing of dietary lipid and lipid absorption [4]. Dietary lipids are mainly comprised mixed triglycerides (TGs), which required to be hydrolyzed by lipases from various tissues for absorption. Among these, pancreatic lipase is responsible for the hydrolysis of approximately 50–70% of total dietary lipid to fatty

acids (FA) and monoglycerides (MGs). The MGs and free FAs then form mixed micelles comprised of bile salts, cholesterol and lysophosphatidic acid (LPA), which consequently absorbed into enterocytes. These micelles act as sources of fat components for the resynthesis of TGs that are eventually stored in adipocytes as the main energy source.



$$E^* + S \rightleftharpoons E^*S \rightarrow E^* + P$$

E* = Enzyme in closed conformation

E = Enzyme in opened conformation

Figure 3.9 Three–dimensional structures of the human pancreatic lipase–colipase complex in the closed conformation (E) and in the open conformation (E*) [55]. The three main surface loops surrounding the active site are β5–loop, β9–loop and lid domain (lower part) and the conformational changes in the lid domain, the β5–loop and the colipase during interfacial activation (upper part).

Obesity and treatment

There are numerous options available for the treatment of obesity such as 1) lifestyle modification, which includes dietary interventions, physical activity and behavior therapy, 2) pharmacological interventions and 3) surgical interventions [56]. Dietary interventions for obesity are designed to create a negative energy balance by reducing daily energy intake to be lower than individual's energy requirements. These energy requirements vary by sex, weight and level of physical activity. There are several diet patterns for weight loss such as low fat diet, low calorie diet, very low calorie diet and low carbohydrate/high protein diet. Most weight loss diet pattern restricts energy intake in one way or another. However, weight maintenance requires long term changes in lifestyle. Physical activity is one of lifestyle modification and is defined as body movement that produced by skeletal muscle. This process requires energy expenditure, thus is suitable for the maintenance of weight loss [57]. The previous study has shown that people who exercise regularly are more successful in maintaining weight losses than those who do not [58]. In addition, behavior changes are important tasks for weight loss. This method can facilitate the adherence to the diet and activity goals. Common techniques include self–monitoring of food and activity, goal setting, stimulus (environment) control, cognitive strategies, social support and reinforcement [59].

Although reduction of caloric intake by diet and increased level of physical activity are recognizable approaches to support weight loss, but in certain cases pharmacological treatment may need to be considered. Potential anti–obesity drugs can be classified into four broad categories, including 1) agents that act through central nerve impulse to primarily decrease appetite, 2) agents that act through peripheral action to primarily increase metabolic rate, 3) agents that act on gastrointestinal tract and 4) agents that affect obesity and overall metabolic syndrome [60]. So far, only two drugs including sibutramine (commercial name as Meridia or Reductil), which affects central nerve action as a serotonin and noradrenalin reuptake inhibitor, and orlistat (commercial name as Xenical), which is a gastrointestinal lipase inhibitor, are currently available on market and approved by the Food and Drug Administration (FDA) in the United States of America, the Therapeutic Products Directorate (TPD) in Canada, and the European Medicines Agency (EMEA) for long–term use in obesity [61]. However, in October 2010 Abbott laboratories and FDA had withdrawn sibutramine from market, because experimental data from the Sibutramine Cardiovascular Outcomes Trial (SCOUT) suggested a 16% increased risk of serious heart events and even death [62]. Thus, currently orlistat is the only pharmacological treatment for obesity.

Orlistat is a hydrogenated derivative of a lipostatin isolated from *Streptomyces toxytincini*. It can act as a gastrointestinal lipase inhibitor that competes with dietary fats for the active sites on the lipase enzyme. Orlistat can form covalently bond with the active Ser residue on the catalytic triad of gastric and pancreatic lipases (Figure 3.10) [63]. The active sites on these enzymes are then unavailable to hydrolyze dietary TGs leading to a reduction in absorption of lipolysis products, monoglycerides and free fatty acids. Thus, orlistat is capable of preventing the absorption up to 30% of dietary fat at a therapeutic oral dose of 120 mg, three times a day [64]. This matter also results in caloric deficit and may yield a positive effect on weight control. However, several studies have reported on orlistat's side effects such as flatus with discharge, oily spotting, oily stool, faecal urgency, incontinence, abdominal pain and malabsorption of fat soluble vitamin [65]. Thus, this leads to the development of natural products for obesity treatment with low or no side effect, which may also initiate an alternative strategy for further investigation on an effective, safe anti–obesity drug.

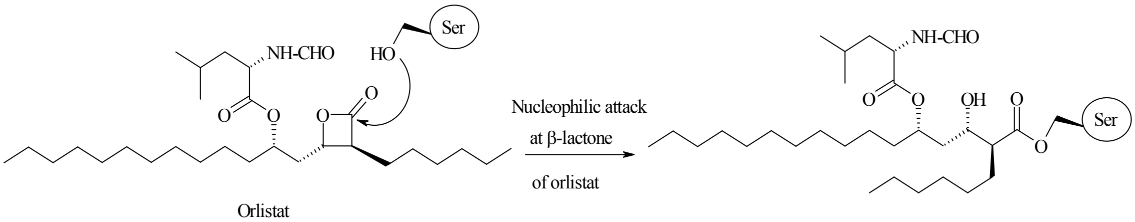


Figure 3.10 The inhibition of enzyme lipase by orlistat [63]. The inhibitory reaction of orlistat with pancreatic lipase is occurred through the covalently bond formation between the catalytic residue, Ser^{152}, of enzyme and the β–lactone ring of orlistat. This adduct formation thus prevents the interaction of the enzyme with its actual substrate (i.e., TGs).

Bariatric surgery is another procedure for the obesity treatment, but is only suitable for the severe case of obesity. This method is appropriate only for those individuals with a BMI ≥40 kg/m^2 or BMI ≥35 kg/m^2 in the presence of comorbidities. The surgery may reduce the size of the stomach by removing a portion of the stomach or resetting/rerouting small intestines to a small stomach pouch. After bariatric surgery, patients typically lose more than 50% of their excess weight. Besides, obesity–related diseases are also markedly improved after bariatric surgery i.e., reduced cardiovascular risk and improved life expectancy [66].

Obesity and trend of natural therapeutics

Even though lifestyle modifications including dietary interventions, physical activity and behavior therapy are well–known approaches to lose weight, these clinical approaches are not long term lasting, and weight regain is often seen in certain cases. Pharmaceutical treatment that prevents weight regain appears to become necessary among obese. However, the dissatisfactions of anti–obesity drugs including high costs and potentially hazardous side–effects are often doubtfully raised the questions of applying this treatment procedure. Thus, the development of natural products for the obesity treatment is a significant matter. Many medicinal plants in the form of crude extract or isolated compounds can induce body weight reduction and may also provide safety and cost–effective alternatives comparing to synthetic drugs. The bioactive compounds from natural sources are potentially useful in obesity treatments through reduction of fat absorption.

Pancreatic lipase inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of natural product as anti–obesity agents. At present, several bacterial, fungal and medicinal plants have been screened in hope to discover new compounds with pancreatic lipase inhibitory activity. So far, the bioactive compounds such as 1) saponins, polyphenols (i.e., apigenin, genistein and catechins) and terpenes from plants sources and 2) lipstatin, panclicins, valilactone, ebelactones, esterastin, caulerpenyne, vibralactone and percyquinin from microorganisms are capable of inhibit lipase activity [3, 4]. These compounds can function in a similar enzyme reaction as orlistat, thereby reducing the fat absorption into body. Other than these small compounds, lipase inhibitors from plant origin are found to be certain peptides and proteins such as protamine, ovoalbumin and β–lactoalbumin [67]. Besides, synthetic peptide (ε–polysine) and polysaccharides (chitosan oligosaccharide, water–soluble chitosan and polydrextrose) have the ability to inhibit the lipase activity as well [68, 69]. These larger molecules can strongly inhibit hydrolysis of TGs by interfering at the interface of lipid and the enzyme (change in interfacial quality), thus promoting desorption of lipase from its substrate.

Tea and lipase inhibitory properties

The effects of tea on obesity have continuingly received increasing attention. The mechanisms of actions may be related to certain pathways, such as through the modulations of energy balance, appetite suppression and nutrients metabolism. Importantly, the inhibition of digestion and absorption of dietary fat has been used as target in obesity treatment, especially the inhibition of pancreatic lipase. Green tea and oolong tea are traditionally reported to possess anti–obesity and hypolipidemic actions, whereas black tea also contains many active ingredients [70]. Tea saponins in oolong tea have a good inhibitory effect on pancreatic lipase activity [71]. In previous study, it was suggested that saponin from oolong tea can prevent obesity through inhibiting pancreatic lipase activity and accelerating catecholamine–induced fat mobilization in rat induced by a high fat diet [6]. Besides, tea saponin was found to suppress an increase in body weight, parametrial adipose tissue weights and diameter in adipose cell size in rat induced by a high fat diet [72]. Under the same study, it also significantly increased triacylglycerol contents in feces. Furthermore, polyphenols identified from oolong tea such as (–)–epigallocatechin 3,5–di–O–gallate, prodelphinidin B–2 3,3'–di–O–gallate, assamicain A, theasinensin D, oolonghomobisflavan A and B, oolongtheanin 3'–O–gallate, theaflavin and theaflavin 3,3'–O–gallate showed the most potent pancreatic lipase inhibitory activities [4]. Similarly, catechin in green tea extract had direct inhibitory effect on gastric and pancreatic lipases and a stimulation of thermogenesis *in vitro* [5]. Under the same study, it is also found that after 3 months of 375 mg catechin consumption, body weight was decreased by 4.6% and waist circumference by 4.48%. These results suggested green tea extract to be the natural product for obesity treatment through its inhibitory activity toward lipases as well as stimulation of thermogenesis. Besides, white tea with substantial inhibitory activity of pancreatic lipase was even more effective than that of green tea (EC_{50} of 22 μg/mL for lipase inhibitory activity of white tea and of 35 μg/mL for green tea) [7]. Black tea, on the other hand, showed only little lipase inhibitory activity.

Thai herbal teas are potential dietary sources of bioactive components with medicinal properties such as anti–obesity agent. Additionally, there were several herbs that exhibited lipase inhibitory activity. Only some Thai medicinal plant have been shown to inhibit pancreatic lipases activity such as *S. rebaudiana* (IC_{50} of 0.53 mg/mL), *C. asiatica* (IC_{50} of 0.12 mg/mL), *C. tinctorius* (IC_{50} of 0.56 mg/mL), *O. aristatus* (IC_{50} of 0.13 mg/mL), *G. pentaphyllum*, (IC_{50} of 0.58 mg/mL) and *M. alba* (IC_{50} of 0.01 mg/mL) [13]. These results suggested that *M. alba* leave extract was the most potent effective pancreatic lipase inhibitor. In addition, *H. sabdariffa* has also been reported to negatively modulate obesity by inactivating porcine pancreatic amylase and inhibiting the adipocyte differentiation that is critical for adipogenesis [14, 73]. Therefore, it is suggested that a daily intake of these plants may be healthful for alternative prevention and treatment of obesity and hyperlipidemia.

3.2.4 Hypertension

Hypertension or high blood pressure is a chronic medical condition, in which the blood pressure in arteries is elevated. According to WHO criteria, the normal level for blood pressure is below 120/80 mmHg, where 120 represents the systolic blood pressure and 80 represents the diastolic blood pressure. Blood pressure between 120/80 and 139/89 is called prehypertension, while a blood pressure of 140/90 or above is considered hypertension [74]. High blood pressure also classified as primary (essential) or secondary hypertension. The primary hypertension (95% of cases) is diagnosed when no other cause for increased blood pressure is discovered. On the other hand, the secondary hypertension is high blood pressure that results from underlying causes such as pheochromocytoma, Conn's syndrome, Cushing's syndrome, renal diseases or drug induced [75].

Hypertension is an important public health problem worldwide and is an important risk factor for death such as cardiovascular disease, stroke and kidney diseases. The prevalence of hypertension in Thailand weighted to the national 2009 population was 21.4% (21.50% in men and 21.3% in women) that is rising rapidly and spread relatively across regions of Thailand [50, 76]. Furthermore, the levels of awareness of hypertension were low across the country [76]. Therefore, it is important to establish fundamental guidelines for screening, treatment and control of hypertension.

Hypertension and renin–angiotensin–aldosterone system

Obesity and in particular central obesity have been consistently associated with hypertension and increased cardiovascular risk. Sodium retention plays a central role in the development of obesity–related hypertension. This condition is largely attributable to increased activity of the sympathetic nervous system and insufficient suppression of the renin–angiotensin–aldosterone system (RAAS) (Figure 3.11) [77]. The RAAS is one of the most important mechanisms in the body concerning the regulation of blood pressure, fluid and electrolyte balance. When the blood pressure is decreased, the protease renin (angiotensinogenase) from granular cells of the juxtaglomerular apparatus in kidney will cleave an inactive angiotensinogen (kidney preprotein with a size of 342 amino acids) to yield the inactive angiotensin I (AngI, Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu). This decapeptide is capable of increasing marginal level of blood pressure and is then hydrolyzed to an active octapeptide angiotensin II (AngII, Asp–Arg–Val–Tyr–Ile–His–Pro–Phe) by angiotensin–converting enzyme (ACE), a key enzyme in the RAAS (Figure 3.12). AngII is the main hormone that functions as a potent vasoconstrictor. This hormone promotes increased renal sodium reabsorption and vascular hypertrophy, which eventually resulting in raised blood pressure. In addition, AngII promotes the release of aldosterone from the adrenal cortex and anti–diuretic hormone (ADH) from the posterior pituitary gland, thereby further increasing renal sodium and water reabsorption in kidneys (Figure 3.11). Interesting, the enzyme ACE is also able to inactivate the vasodilative peptide bradykinin in the kallikrein–kinin system, resulting in higher blood pressure. Therefore, ACE inhibitors can lower blood pressure *via* an action at the tissue level or through their effects on kinins, nitric oxide, or prostacyclin. ACE inhibition is likely considered to be the target for antihypertensive agents and also is the first line of treatment for patients with hypertension and heart failure [78].

There are two isoforms of ACE in human transcribed by the same gene, the somatic (sACE) and the testis (tACE) forms [79]. The sACE are synthesized by endothelial, epithelial and neuroepithelial cells, whereas the tACE are only found in sperm cell. The sACE with a molecular weight of 150–180 kDa (1,277 amino acids) consists of two homologous domains, the N– and C–terminal domains. These two domains are differed in term of their substrate specificities. The tACE with a molecular weight of 90–110 kDa (701 amino acids) consists of a single catalytic domain that resembles the C–terminal domain of sACE in exception of extra 36 amino acid residues at its N–terminus [80, 81]. The overall structures of sACE and tACE suggested homologous helical ellipsoids harboring three main substrate binding sites (S1, S1′ and S2′) and a narrow catalytic channel containing the catalytic zinc and the HExxH motif (Figure 3.13) [82, 83]. The zinc ion is located between subsites S1 and S1′ to participate in hydrolytic cleavage of the peptide bond of the substrate, resulting in release of the dipeptide product [84].

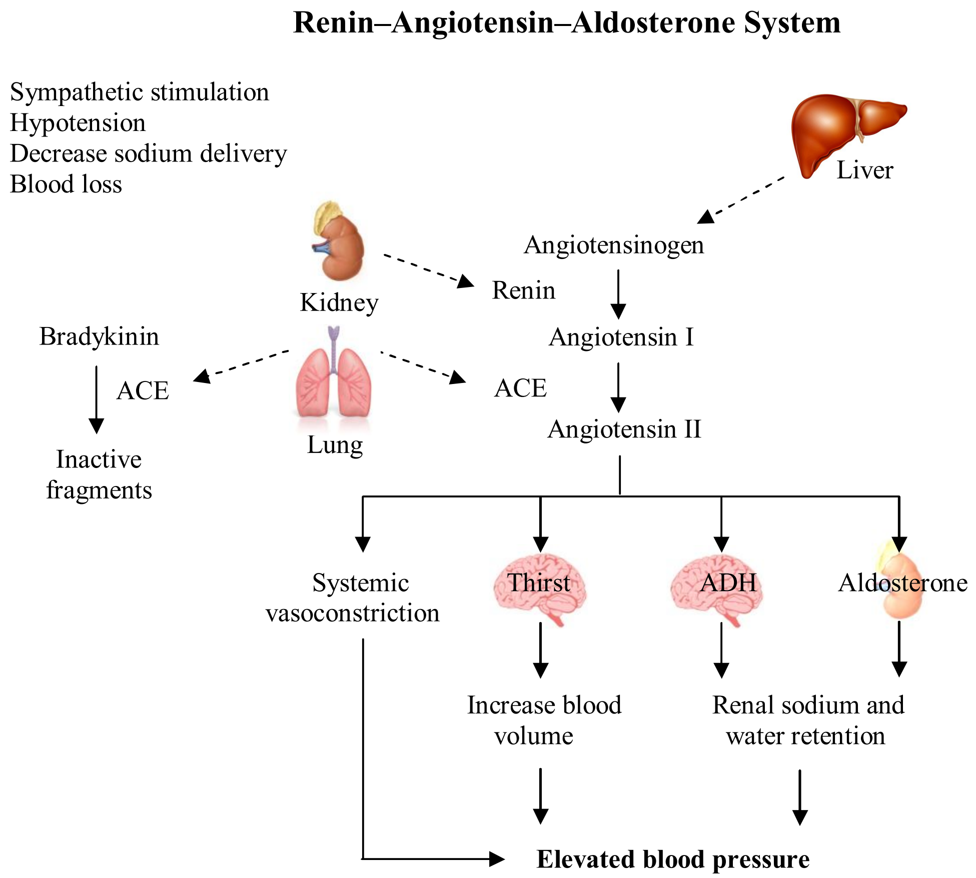


Figure 3.11 Rennin–Angiotensin–Aldosterone System (RAAS) [85]. RAAS can be activated with a loss of blood volume or a drop in blood pressure. Under normal circumstances, components of the RAAS interact to maintain salt and water homeostasis and blood pressure regulation. Dysfunction of the RAAS leads to aberrant fluid and electrolyte metabolism and alterations of blood pressure.

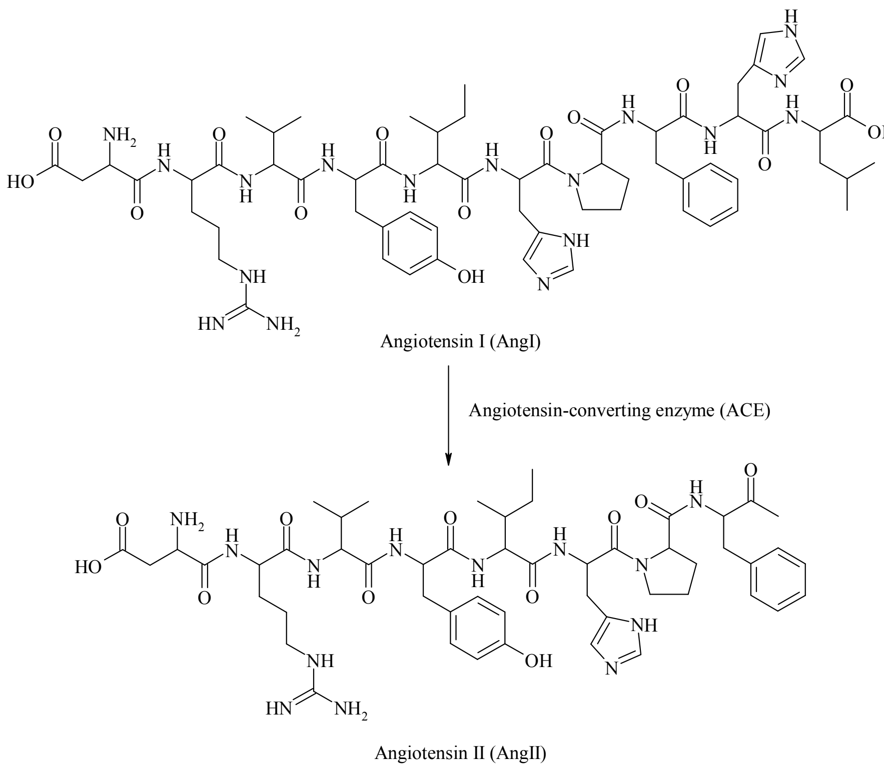


Figure 3.12 The convertion of angiotensin I into angiotensin II by the enzyme reaction of angiotensin–converting enzyme (ACE). ACE is a zinc protease enzyme, which can cleavage two C–terminal residues, histamine (His) and leucine (Leu) from inactive angiotensin I, thus creating active angiotensin II.

ACE acts as a peptidyl dipeptidase, removing the C–terminal dipeptide from substrates, His–Leu from AngI (Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu) and Phe–Arg from nonapeptide vasodilator bradykinin (Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg). The binding of AngI into the catalytic pocket of ACE is supported by the interactions between the side chains of Phe residue that fits into S1 cavity, His residue that fits into S1′ cavity and Leu residue that fits into S2′ cavity (Figure 3.13A). The amide bond between Phe and His residues are hydrolyzed by Zn^{2+}–dependent active site of ACE. In addition to AngI and bradykinin, ACE hydrolyzes numerous other peptides such as *N*–acetyl–seryl–aspartyl–lysyl–proline (Ac–SDKP), cholecystokinin, hemopressin and amyloid β–protein [86]. Indeed, the C–terminal domain of sACE is highly selective for angiotensin–converting site, whereas the N–terminal domain efficiently cleaves Ac–SDKP [87].

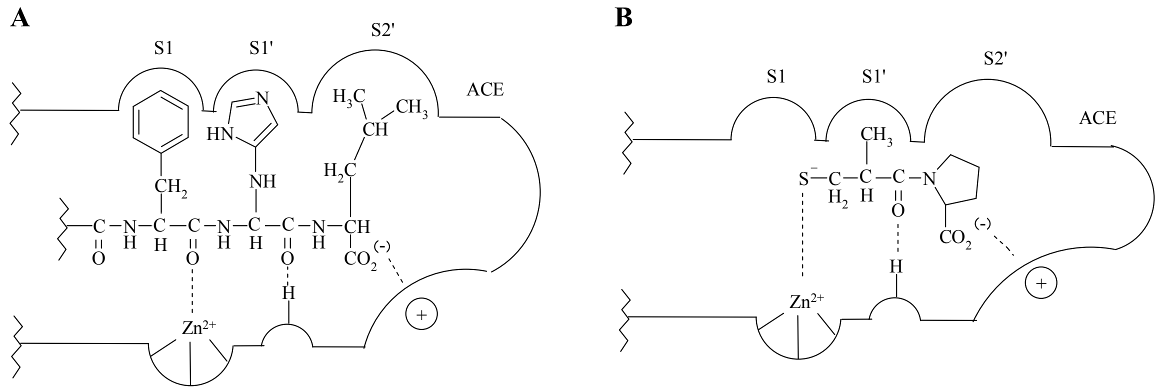


Figure 3.13 The catalytic pocket of ACE [84]. (A) The scheme showed the binding of AngI (Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu) into the catalytic pocket of ACE with side chains of Phe residue fitting into S1 cavity, His residue fitting into S1′ cavity and Leu residue fitting into S2′ cavity. The amide bond between Phe and His residues are hydrolyzed by Zn^{2+}–dependent active site of ACE. (B) The scheme showed the binding of ACE inhibitor, captopril, with the side chain $–S^{-}$ moiety forming a coordinating ligand to Zn^{2+} binding site.

Hypertension and treatment

Lifestyle changes are an important first step for treatment of hypertension especially exercise and control diet. However, in certain cases pharmacological treatment may need to be considered. Synthesized drugs such as captopril, enalapril and lisinopril are extensively used medicines in the treatment of hypertension. These drugs act as ACE inhibitor that is the first line of treatment for patients with hypertension and heart failure. The mechanism of action of these drugs is competitively inhibiting ACE by mimicking the structure of its substrate (Figure 3.13B). For example, the side chain $–S^{-}$ moiety forming a coordinating ligand to Zn^{2+} binding site, whereas the carbonyl group of molecule makes a hydrogen bond interaction with the HExxH motif [88]. The active sites on this enzyme are then unavailable to hydrolyze any substrate such as AngI and bradykinin, leading to a reduction of AngII and inactivation of bradykinin, which are resulting in lower blood pressure.

Nevertheless, these drugs have caused side effects such as hypotension, dry cough, hyperkalemia, rash, hepatotoxicity, dysgeusia, renal dysfunction and angioedema [89]. This matter leads to the development of natural products as nutraceuticals for alternative treatment of hypertension, especially for pre–hypertensive patients. Currently, ACE–inhibitory peptides have been isolated from diverse protein sources such as marine muscle protein, wheat, corn, soybean, mushrooms, garlic, egg, potatoes and even *C. sinenis* tea [90-93].

Tea and anti–hypertensive properties

Tea is believed to possess a positive effect on blood pressure relieving. Previous studies suggested that tea consumption is associated with an increase in urine volume and electrolyte elimination, especially sodium, along with a decreased blood pressure in hypertensive adenine–induced rats [94]. Similarly, tea polyphenols can also reduce blood pressure through their antioxidant properties in stroke–prone spontaneously hypertensive rats [95]. Besides, the study in human suggested that consumption of green or oolong tea (120 mL/day or more, for 1 year) significantly reduces the risk of developing hypertension [96]. These experiments led to the investigation in ACE inhibitory activity of tea extract, since generally the activation of RAAS causes an increase in blood pressure with ACE being the key enzyme. Therefore, the blockade of RAAS with ACE inhibition results in reduction of blood pressure.

Many studies had investigated the properties of tea on the inhibition of ACE both *in vivo* and *in vitro*. Flavanols in green tea and black tea can inhibit ACE activity in human endothelial cells [8]. Besides, oral intake of a single dose (400 mL) of green tea significantly inhibited ACE activity in healthy volunteers [97]. These studies suggested that tea may have a potential to prevent and protect against cardiovascular disease especially hypertension through inhibition of ACE activity.

At present, little information regarding anti–ACE of Thai herbal teas is available. Previous studies demonstrated that *H. sabdariffa* extracts can reduce blood pressure in humans and showed anti–ACE activities *in vitro* [98]. Thus, it has been claimed that the ACE inhibitory activity is probably due to flavones and anthocyanins in *H. sabdariffa* [98]. Recent study has shown that anthocyanins, delphinidin and cyanidin–3–*O*–sambubiosides, from *H. sabdariffa* can also inactivate ACE by competing with the substrate for the active site [99]. In addition, it was found that *C. asiatica* extracts could inhibit ACE activity *in vitro* [100]. Thus, consumption of some Thai herbal teas such as *H. sabdariffa* and *C. asiatica* may promote potential health benefits by reducing high blood pressure.

From all the above reasons, the investigation of medicinal properties of conventional and Thai herbal teas is an interesting and necessary topic that can provide an alternative source for the discovery of bioactive compounds as well as the development of nutraceuticals from natural food products for prevention and treatment of many chronic diseases.