CHAPTER III
LITERATURE REVIEW

3.1 Alzheimer's disease

Currently, elderly population (≥ 60 years old) is continuingly increased because of reducing death rates [10] resulting from development in medicinal treatment and improvement of lifestyle. In 2005-2010, Thai people have lifespan of about 74.1 years old; however, Thais in 2025-2030 will have life expectancy at birth with average lifespan of 79.3 years old [11]. As lifespan increases, elevated occurrence of Alzheimer's disease (AD) is also reported. In 2006, about 26.6 million of AD patients worldwide are under diagnostic [12]. The number of AD patients is predicted to be continuingly increased in 2050, in which every one person in 85 people will have AD [12]. Therefore, AD is the important disease to study regarding its prevention and treatment.

AD, a neurodegenerative disorder, is the most recognized type of dementia in elderly people [13]. The disease is often found in individual with age of over 65 years old [14]. Among these, the prevalence in age groups of 65-74 years is 2.5%, of 75-79 years is 4%, of 80-84 years is 11% and of 85-93 years is 24% [15]. AD occurrence is a result of destruction in brain cells and nerves, especially in the part of storing memories in hippocampus. Nerves cell commands to different parts of the body, which receives information and forwards to neighboring cells by neurotransmitter. When nerve cell is destroyed, data transmission is interrupted, resulting in non-responding organ. The early stage of AD symptoms can be observed as loss of memory in recent events. Confusion, irritability, mood swings, trouble with language and long-term memory loss are detected symptoms in the advanced stage. Finally, body functions are lost, leading to death in average 8-10 years after founding symptom [16].

3.1.1 Causes of Alzheimer's disease

Nowadays, the causes of AD are still not well understood. However, four recent pathways including oxidative stress induction, termination of physiological role of cholinergic synapses, β-amyloid formation and abnormality of tau protein are hypothesized for AD occurrences.

Oxidative stress induction

Oxidative stress is caused by free radical and reactive oxygen species (ROS), a highly unstable and reactive atom (or molecule) due to an unpaired electron in an outer orbit. ROS is the free radicals in body generated during normal physiological and biochemical processes by organisms. On the other hand, the imbalance of radicals' overproduction could lead to destroys major components of cells such as nucleus, mitochondrial DNA, membranes and cytoplasmic proteins and cause many oxidative stress related diseases including AD [17, 18]. As results, these damages could lead to cell apoptosis [19], especially in brain, due to requirement of high energy and oxygen consumption rate [20]. In addition, oxidative stress of ROS is related to the formation and accumulation of β-amyloid in the brain of AD patients through formation of amyloid precursor protein (APP) oxidation [21]. Accumulation of ROS is also found to be increased in proportion to ageing [19]. This matter is highly related to the pathogen of the neurodegenerative disorders in elderly, leading to AD occurrence. Therefore, the oxidative stress reduction by antioxidant may decrease the risk of AD. It was previously suggested that the intake of antioxidant rich-food can reduce the risk of AD occurrence in elderly subjects with cognitive impairment or relieve the symptoms in AD patients [18].

Cholinergic hypothesis

The cholinergic hypothesis of AD is caused by decreasing forebrain cholinergic neurons and level of acetylcholine (ACh) [22]. The brain of AD patient shows the decreased production of ACh, a neurotransmitter carrying messages in the brain. This neurotransmitter is located in hippocampus, the brain responsible for memory, and cerebral cortex, the brain area for thinking and making decision. Activity of ACh can be terminated by cholinesterases, the enzymes that hydrolyze ACh into

inactive metabolites, choline and acetyl-CoA [22] (Figure 3.1). These cholinesterase enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are the major causes of reduced ACh in AD brain. Besides, increased AChE and BChE are detected in the neuritic plaques in β-amyloid formation and neurofibrillary tangles in hyperphosphorylated tau proteins in the early stages of AD, suggesting the relation among these hypotheses [16, 17, 23]. AChE is the specific cholinesterase, hydrolyzing predominantly choline esters in the central nervous system, nerve and red blood cells (RBCs). BChE, on the other hand, is the non-specific or pseudo-cholinesterase, hydrolyzing choline esters as well as other esters in glial cells, endothelial cells, pancreas, liver and neurons [22, 24]. Moreover, BChE activity was increased in AD brain in the range of 40-90% [23]. Therefore, AD treatment in this hypothesis could be mainly focused on AChE, while inhibition of BChE could support the increased ACh activity in brain.

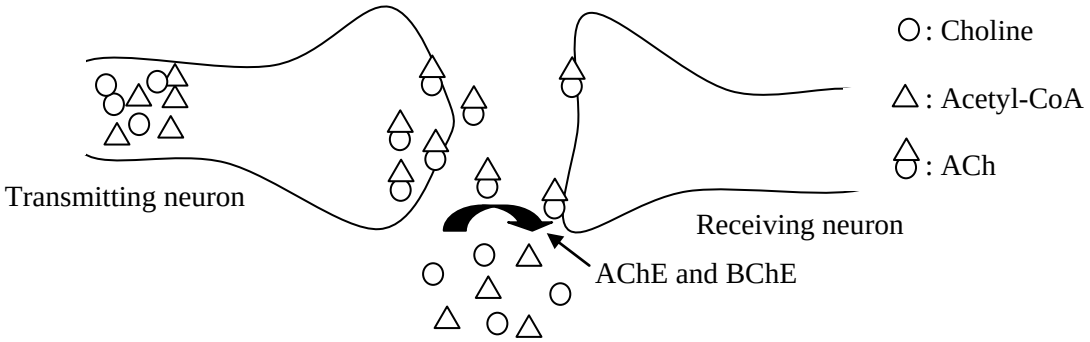


Figure 3.1 Degradation of acetylcholine (ACh) [22]. The scheme shows degradation of neurotransmitter, ACh, by cholinesterase enzymes, AChE and BChE, to produce inactive molecules, choline and acetyl-CoA, at the synaptic cleft of neurons.

β-amyloid formation hypothesis

Accumulation of overproduced β-amyloid in hippocampus and other areas of the cerebral cortex are hypothesized as one cause of AD occurrence. The β-amyloid is toxic on dementia brain due to disruption on cell-to-cell communication, leading to death of neurons in the brain [13]. The β-amyloid is the peptide with 36-43 amino acids, which is generated from long chain protein called amyloid precursor protein (APP, 695-770 amino acids) by proteolytic enzymes such as β-secretase (also known

as β-site amyloid precursor protein cleaving enzyme1 or BACE1) [25]. This phenomenon was supported by the previous study [23], which indicated that inhibition of BACE1 activity in mice could lead to less β-amyloid production. APP is important for growth and repair of neurons. In normal brain, APP can be hydrolyzed into two pathways, a non-amyloidogenic and an amyloidogenic routes (Figure 3.2) [26]. The former is suggested as APP being mainly digested by α-secretase at the α-site within the β-amyloid peptide region, thus preventing the toxic β-peptide formation. The later is occurred as APP being digested by β-secretase at the β-site, thus producing the C-terminal fragment and β-amyloid containing peptide [13, 25]. This step is followed by γ-secretase digestion at γ-site in the β-amyloid containing peptide, thus generating the isoforms of toxic β-amyloid peptide, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ [27]. The $A\beta_{1–42}$ isoform is interesting for AD study, because its concentration in cerebrospinal fluid (CSF) of AD's patients is reduced, while $A\beta_{1–40}$ level is unchanged. The reduction of $A\beta_{1–42}$ may be due to self-interaction of $A\beta_{1–42}$ to form β-amyloid plaques [15]. This matter could promote β-amyloid formation, which self-associated into β-amyloid oligomers and eventually insoluble senile plaques in brain (Figure 3.2 and 3.3). These toxic β-amyloid plaques could lead to neurodegenerative changes. Thus, β-secretase inhibitor is also the important agent for reducing the progress of AD. In addition, mutation of presenilin-1 and 2 genes (coding for γ-secretase protein) causes high activity in γ-secretase, leading to elevated β-amyloid formation [27]. The evidence that supports this hypothesis is indicated as a relationship between abnormal chromosome 21 (causing Down syndrome) and AD. Individual with an extra gene copy of chromosome 21 (trisomy 21), which is the same chromosome for translating APP, is always found senile plaques and neurofibrillary tangles, leading to AD at 40 years old of age [28].

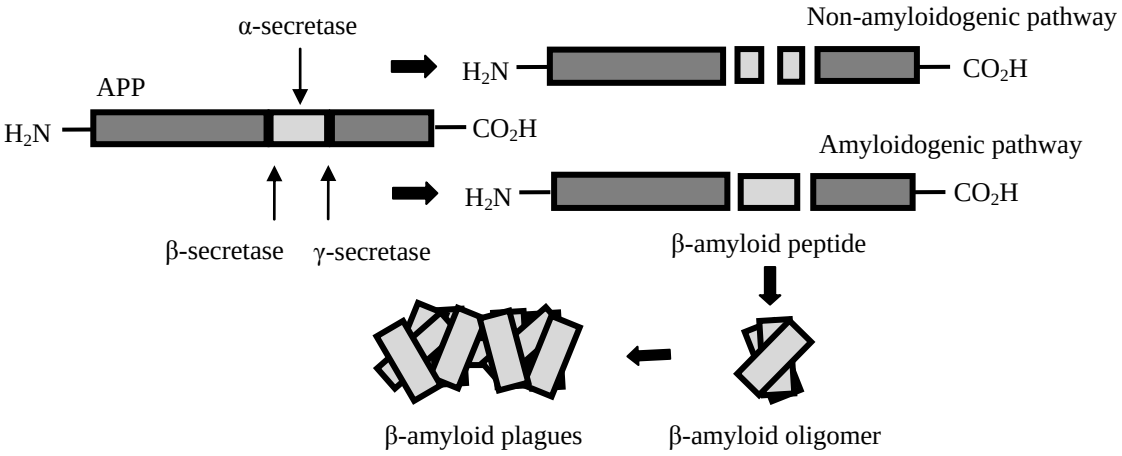


Figure 3.2 Amyloid plagues formation. It is originally caused by degradation of APP. Normally, APP digestions are divided into two pathways, non-amyloidogenic and amyloidogenic pathways. In non-amyloidogenic pathways, APP is hydrolyzed by α-secretase within the β-amyloid peptide region to prevent β-amyloid production. As for amyloidogenic pathways, APP is digested by β-secretase and γ-secretase to form β-amyloid peptides. Then, these β-amyloid peptides interact to each other, forming β-amyloid oligomer and β-amyloid plagues that lead to neuron cell death.

Tau protein hypothesis

Abnormal insoluble tau protein is also significant cause of AD. Tau protein is a cytoskeletal protein that binds and stabilizes microtubules. Thus, this protein is essential for axonal transport in neurons and maintenance of neuron's structure [23]. These microtubules carry nutrients and molecules between cell body and the ends of axon. For AD patient, tau protein is hyperphosphorylated and self-aggregated to form neurofibrillary tangle inside hippocampus neurons (Figure 3.3 and 3.4) [26]. As results, microtubules are disintegrated, leading to loss of the neuron's transport system [16].

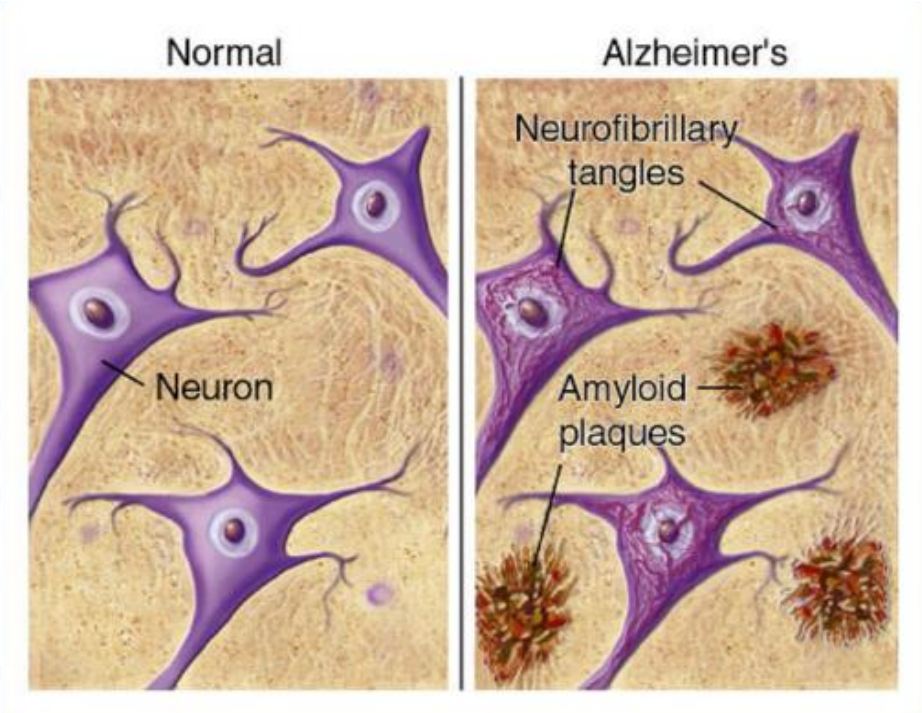


Figure 3.3 Neurofibrillary tangles and amyloid plagues [16]. The scheme shows normal neuron (left) and Alzheimer's neuron including neurofibrillary tangles and amyloid plagues (right). In the brain of patients with AD, the transportation of nutrient and neuromolecules as well as communication system of neuron are interrupted by neurofibrillary tangles and amyloid plagues formation, thus leading to neurological symptoms.

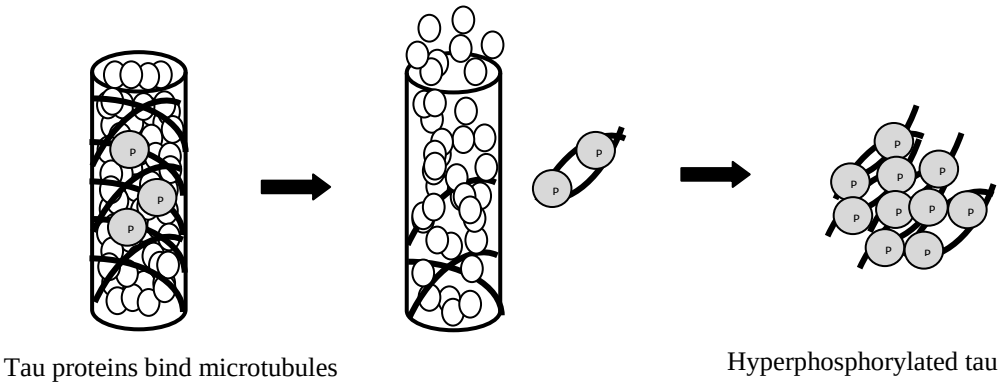


Figure 3.4 Hyperphosphorylation of tau protein. Protein hyperphosphorylation can cause aggregation of neurofibrillary tangle, leading to disintegrated microtubules in brain cells and then loss of the neuron's transport system.

3.1.2 Potential treatments

Up until now, there is no certain pathway for AD treatment. However, the treatments for patients in the early and middle stages of AD to prolong daily maintaining functions of body and nervous system involve improving the symptoms and delaying the progression of the disease [16]. The medicinal treatment has been focused on six major groups of anti-AD drugs including AChE inhibitors (AChE-I), *N*-methyl-D-aspartate (NMDA), receptor antagonists, monoamine oxidase (MAO) inhibitors, antioxidants, metal chelators and anti-inflammatory drugs [16]. Originally, anti-AD drug was discovered as AChE inhibitors, which cause an increase in ACh levels, leading to reduced AD symptoms such as memory loss, thinking disorientation and language abnormality. These drugs are approved by Food and Drug Administration (FDA) including donepezil, galantamine, rivastigmine and tacrine. Donepezil and tacrine are the reversible non-competitive AChE inhibitors [29]. On the other hand, galantamine is a reversible competitive AChE inhibitor with low BChE inhibitory activity [30]. Rivastigmine is a pseudo-irreversible AChE and BChE cholinesterase inhibitor [30]. In addition, physostigmine (or eserine), a tertiary amine containing natural compound isolated from the seeds of *Physostigma venenosum*, can inhibit both cholinesterase enzymes [16]. However, these synthetic drugs have side effects such as diarrhea, tiredness, dizziness, confusion, headache, vomiting, nausea, fatigue, insomnia, heart attack and stroke [31]. Tacrine can also cause hepato toxicity effects [16]. Thus, prevention and treatment of AD from natural products such as fruits and vegetables that can be consumed daily are of interest due to no/less side effect and cost effectiveness. As well, these natural products are not concerned regarding safety limitation as they can be consumed in daily diet [17].

3.2 Sweet pepper

Sweet peppers or bell peppers with a known scientific name of *Capsicum annuum* is belong to the Solanaceae family. The plant can be grown in a variety of climates, both tropical and sub-tropical regions of the world [32]. Unlike other peppers, the sweet peppers have non-pungent flavor, which is due to the non-pungent pericarp. However, its placenta tissue is still pungent [33]. The primary substance that

controls hotness in peppers is called capsaicin, which is found in very small amounts in sweet peppers [34]. Sweet peppers are plump, bell-shaped vegetables, consisting of either three or four fruit lobes. The fruit has thick wall with several forms, sizes, and colors [33]. Most unripe sweet pepper fruits are green; however, the color changes to red in the ripe stage. Other than ripe red pepper, other colors of unusual sweet pepper cultivars are yellow, orange, purple, brown or black, depending on the genotype or the seasonal period of breeding [33]. Different fruit colors can influence the taste and flavor of each particular pepper. For examples, the colored peppers such as red, yellow, and orange peppers, are sweeter than green peppers because of higher glucose content in ripening stage [1].

Because of the varieties of colors and tastes, sweet peppers are popular vegetable in many national cuisines worldwide to increase consumer attraction. Besides, sweet peppers are the second most important vegetable of the Solanaceae family in the world after tomato [35]. Traditionally, red pepper (*C. annuum*) in the form of paprika (ground powder) has been used in food ingredient as food colorant to give more food appearance [36]. Sweet peppers are commonly consumed as raw vegetables or cooked without seed because of their non-pungent flavor. Raw red or green sweet pepper is usual added to salad. They are also cooked as pickles, stuffing, and soups [35]. Thus, these peppers are of interest regarding their health benefits. They contain many bioactive compounds [2, 37-43], which provide several health benefits such as antioxidant [17, 39, 44-47] and anti-AD [8, 9, 17, 48, 49] properties. As well, volatile compounds found in this sweet pepper were previously reported to possess antioxidant property [50]. Sweet peppers were reported to possess biological function against hypoglycemic properties through inhibition of α-amylase and α-glucosidase [51]. However, little researches on relationship between sweet peppers and other health benefits are reported, especially AD.

3.2.1 Chemical compositions

Several previous studies reported that sweet peppers contain many significant bioactive compounds, such as phenolic compounds, carotenoids, ascorbic acid and tocopherols and capsaicin [2, 37-43, 51, 52]. Type and quantity of bioactive compounds are differed among colored fruits of sweet pepper. For example, the level

of chlorophyll in unripe green pepper was found to be decreased in ripe orange, yellow and red peppers (Table 3.1). It is possible that this chlorophyll might be structurally modificational changed into new pigments such as red or yellow carotenoids, related xanthophylls and anthocyanins [53]. In addition, the unique aroma of sweet pepper may also results from characterization of its volatile compounds. These phytochemicals were reported to provide many potential health benefits, especially phenolics (phenolic acids and flavonoids). These phenolics mainly act as antioxidants that can prevent the occurrence of some oxidative stress related diseases such as cancer, cardiovascular disease and neurodegenerative disorders [39]. Therefore, the health benefits of colored sweet peppers might be varied with colors.

Table 3.1 Main pigment contents of sweet peppers (μg/g dry weight) [37]

Pigments	Green[1]	Red[2]	Orange[3]	Yellow[4]
Total carotenoids	1,513.5[a]	7,137.0[d]	5,292.7[c]	2,236.3[b]
Zeaxanthin	ND	8.8	ND	ND
Lutein	70.2	ND	ND	ND
β-carotene	12.2[a]	43.9[c]	56.6[d]	15.9[b]
Chlorophyll-a	409.1[d]	284.7[c]	198.3[b]	34.5[a]
Chlorophyll-b	72.1[c]	12.2[a]	31.2[b]	11.5[a]

[a-d] Significant difference (p<0.05) is expressed by different letters in the same row.

ND: not detected.

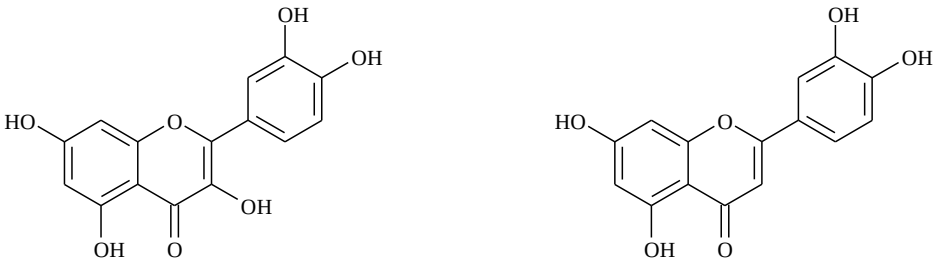
Detection limit for lutein and zeaxanthin: 1.1 μg/mL and 0.019 μg/mL, respectively.

[1] Orion, [2] Mazurca, [3] Simpaty and [4] Taranto

Flavonoids

Bioactive compounds in plants are generally flavonoids which are a group of secondary metabolites. These compounds are synthesized from adaptation of plant to biotic and abiotic stress conditions (i.e., infection, wounding, water stress, cold stress and high visible light) [45]. These stress-related pathways of biosynthesis lead them to act as antioxidants [17, 44, 45], which may prevent/treat oxidative stress related diseases such as neurodegenerative disorders [8, 9, 17, 48, 49]. Flavonoids are the antioxidants with particular hydroxyl group(s) attaching to specific locations in the

structure [54]. In addition to antioxidant function, flavonoids are reported to have pharmacological and medicinal properties, including vasodilatory, anticarcinogenic, immune-stimulating, antiallergenic, antiviral, coronary heart disease preventing, and estrogenic effects. Besides, flavonoids are reported to be capable of inhibiting various enzymes involving in carcinogenesis [55]. Sweet peppers contain both quercetin (a flavonol) and luteolin (a flavone). Quercetin has a hydroxyl group at C-3 in the aromatic ring, while luteolin does not (Figure 3.5). The structural difference between quercetin and luteolin influences on antioxidant activity, in which the presence of the hydroxyl group at C-3 in quercetin results in greater free radical-scavenging efficiency than luteolin [54].



Quercetin Luteolin

Figure 3.5 Chemical structures of quercetin and luteolin. The chemical structures of flavonoids in sweet peppers including quercetin (left) and luteolin (right). Quercetin tends to exhibit higher antioxidant activity than luteolin because of the presence of a hydroxyl group at C-3 in the aromatic ring.

Flavonoid levels are varied in sweet pepper cultivars, ranging from 22 to 245 mg/kg fresh weight (Table 3.2). The wide range of flavonoid content may be resulted from differences in genetics and growth environmental conditions. The stress environments of plant growth stimulate the phenylpropanoid pathway and production of various phenolic compounds [1]. Total flavonoid contents of pepper are generally reduced during ripening and color changing stages. Flavonoid loss during maturation may be due to the metabolic conversion of flavonoids to secondary phenolic compounds by polyphenol oxidase and peroxidase [1].

Table 3.2 Flavonoid contents of sweet peppers (mg/kg fresh weight) [1]

Cultivars	Colors	Quercetin	Luteolin	Total flavonoids
Yellow Bell	Green[u]	22	11	33
	Orange[r]	13	9	22
Tam B-2	Green	44	9	53
Romanian Sweet	Yellow	219	26	245
YB 244	Yellow	81	10	91
YB 126	Yellow	112	15	127

[u]Unripe, [r]Ripe

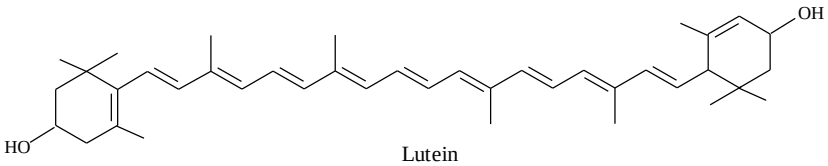
Carotenoids

Carotenoids are organic pigments in plants, which mainly found in chloroplasts and chromoplasts. These compounds can be divided into two groups, according to the presence of oxygen atom(s) in the structure. Carotenoid containing oxygen group(s) is known as xanthophyll such as lutein, zeaxanthin, capsanthin, capsorubin and β-cryptoxanthin. Purely hydrocarbons or oxygen-free carotenoid is known as carotene such as α-carotene, β-carotene, and lycopene (Figure 3.6). The carotenes including α-carotene and β-carotene as well as β-cryptoxanthin are provitamin A. Oxygenated carotenoids, except β-cryptoxanthin, on the other hand, do not possess provitamin A activity but can act as antioxidant [47]. Carotenoids can provide health benefits regarding prevention of oxidative stress environments, advanced age-related macular degeneration and cataracts [56]. In addition, capsanthin also exhibits anti-tumor (colon cancer) activities [57] and increases plasma HDL-cholesterol [58]. Lutein and zeaxanthin could absorb blue light that causes eye damage, thus leading to prevention of eye disease [59].

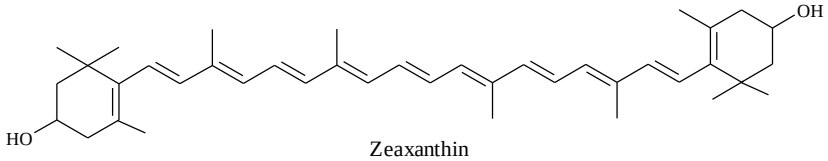
The important pigments in sweet peppers are carotenoids, especially in orange and red colors [2]. Therefore, various colored fruits of sweet peppers may come from different types and levels of carotenoids. Common carotenoids found in red sweet pepper are capsanthin and capsorubin, while yellow-orange peppers contain α-carotene, β-carotene, zeaxanthin, lutein and β-cryptoxanthin [1]. Besides, carotenoids possess antioxidant activity [1, 39, 46, 60], for example, α- and β-carotene as well as β-cryptoxanthin can act as provitamin A. Specifically, the quantity of

provitamin A in sweet peppers are ranged from 14 to 253 µg retinal equivalents (µg RE)/100 g fresh weight (Table 3.3). A wide range of provitamin A in sweet pepper is suggested to be the result of different colors in particular cultivar and maturity of sweet peppers [1]. Pepper cultivar (100g fresh weight) that contains more than 25% of Thai Recommended Daily Intakes (Thai RDI) for provitamin A required in males and females (Thai RDI = 800 µg RE) is Grande Rio 66 cultivar (red fruit color) [1]. Besides, lutein was suggested to be declined during ripening stage, while the opposite results were observed with the quantity of α-carotene, β-carotene, β-cryptoxanthin, capsanthin and zeaxanthin [1]. From this information, it can be suggested that fruit colors and maturity of sweet peppers plays a significant role in the determination of the quantity and quality of carotenoids.

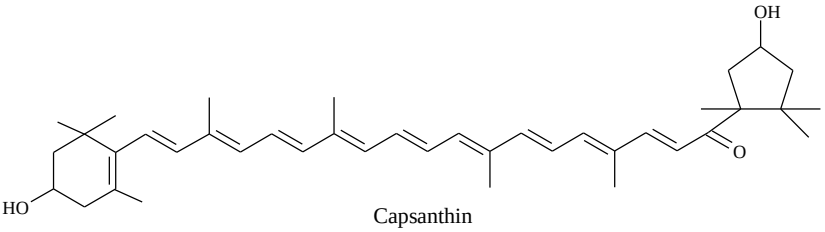
(A) Oxygenated carotenoids (xanthophyll)



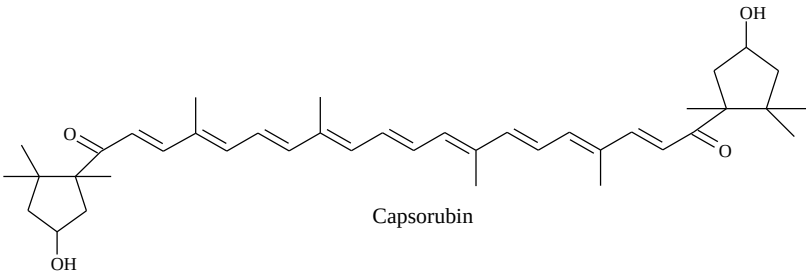
Lutein



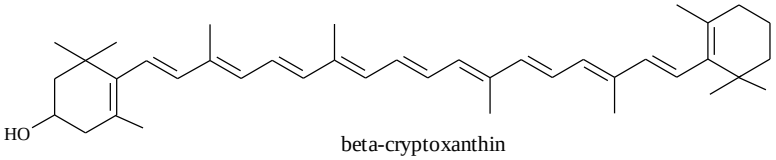
Zeaxanthin



Capsanthin



Capsorubin



beta-cryptoxanthin

(B) Free oxygenated carotenoids (carotene)

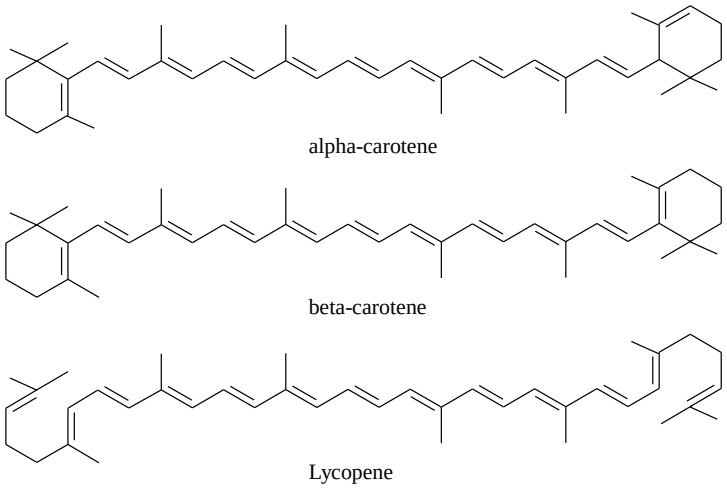


Figure 3.6 Chemical structures of major carotenoids in sweet pepper. Major carotenoids include (A) oxygenated carotenoids (xanthophyll) and (B) free oxygenated carotenoids (carotene). Carotenoids are pigments in various colored sweet peppers and possess health benefits such as provitamin A property and antioxidant activity.

Ascorbic acid (vitamin C)

Ascorbic acid (vitamin C) is an important nutrient for humans regarding its main health benefit of being antioxidants. Another form of vitamin C is dehydroascorbic acid, which occurred through degradation of ascorbic acid in the presence of oxygen (Figure 3.7). In addition to antioxidant property, ascorbic acid is used in collagen formation, prevention of scurvy and degenerative conditions including cancer, heart disease, cataracts, and stimulation of the immune system [1].

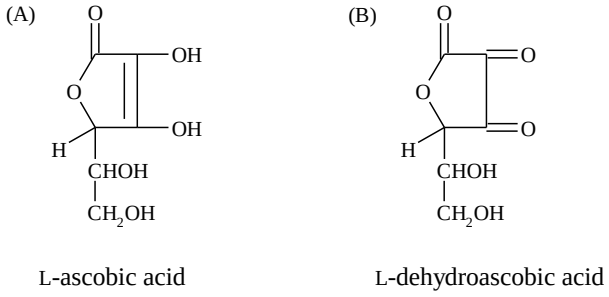
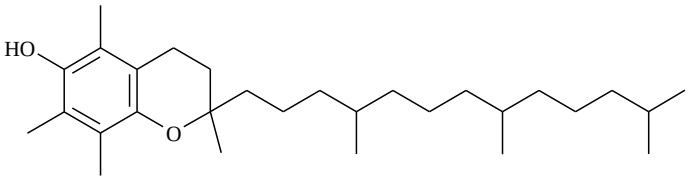


Figure 3.7 Chemical structures of different forms of vitamin C. Vitamin C can form (A) reduced L-ascorbic acid and (B) oxidized L-dehydroascorbic acid. Vitamin C is an important antioxidant, mostly found in fruits and vegetables.

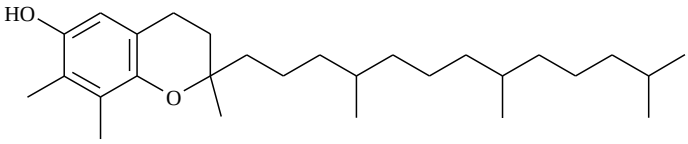
Sweet peppers are excellent sources of ascorbic acid, in which its amount per 100 g fresh weight is more than 100% of the Thai RDI (60 mg) required in both male and female (Table 3.3). Many ripe sweet peppers have been reported to exhibit higher level of ascorbic acid than the unripe ones. Higher ascorbic acid content in ripening stage may be due to the quantity of glucose, a precursor of ascorbic acid, which is increased during maturity of pepper fruit [1]. Other than maturity, different level of ascorbic acid in pepper is possibly the results of variations in types, cultivars, genetics or fertilization practices.

Tocopherols

Vitamin E, including tocopherols and tocotrienols (Figure 3.8), exhibits antioxidant activity against lipid oxidation in foods and biological systems. For example, tocopherols can neutralize free radical by donating hydrogen ions [61]. The health benefits of vitamin E are reported as to reduce the risk of coronary heart disease, some cancers, cataracts, diabetes, protection of cells oxidative damage and LDL oxidation, enhancement of the immune system, reduction of cholesterol synthesis and declined progression of neurological diseases [1].



alpha-tocopherol



gamma-tocopherol

Figure 3.8 Chemical structures of tocopherols. Tocopherols can be in the form of α-tocopherol (mainly found in pericarp of pepper), and γ-tocopherol (found in seeds). Tocopherols have antioxidant activity, which is good for health maintaining purpose.

Comparing to other investigated fruits, sweet peppers are excellent sources of vitamin E [62] (Figure 3.9). Most γ-tocopherol is found in pepper seeds, whereas α-

tocopherol is mainly found in pericarp tissue [1]. The α-tocopherol and γ-tocopherol contents of pepper fruit are affected by maturity stage, in which α-tocopherol contents in pericarp was found to be increased during ripening stage, while γ-tocopherol contents were decreased [63].

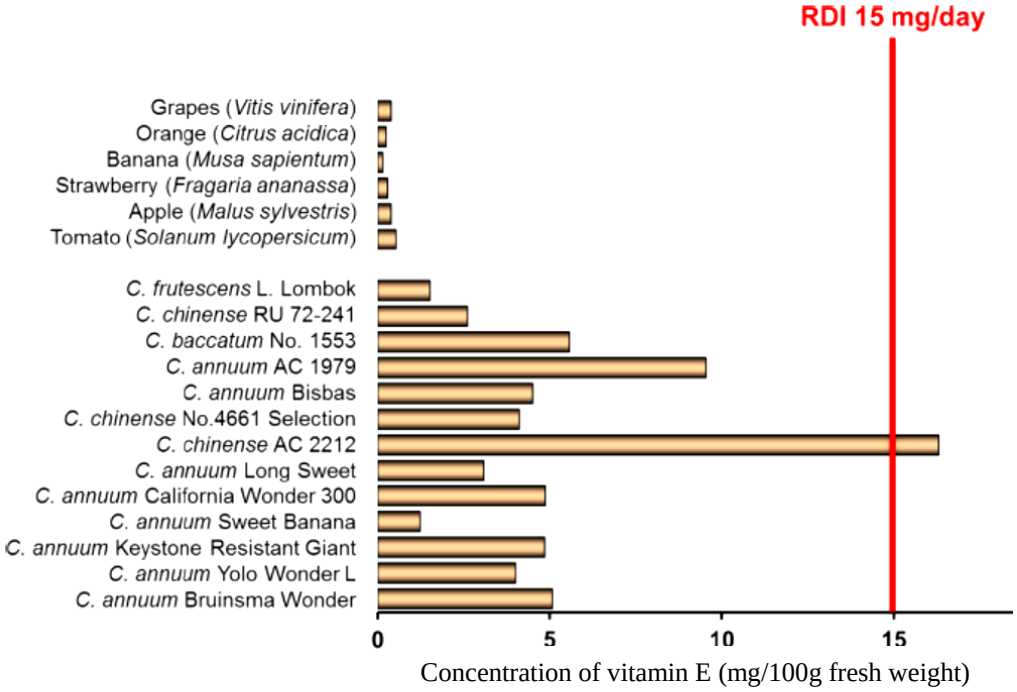


Figure 3.9 Vitamin E contents of ripe *Capsicum* fruits comparing to other fruits [62]. Sweet peppers include three cultivars, California Wonder, Bruinsma Wonder, and Yolo Wonder. The red line indicates the recommended daily intake (RDI) for adults, which is 15 mg/day.

Additionally, sweet peppers are excellent sources of three vitamins, including vitamin C (ascorbic acid), provitamin A (β-carotene), and vitamin E (α-tocopherol) (Table 3.4). Red pepper exhibited the highest levels of β-carotene and α-tocopherol, while green pepper possessed the lowest [37]. On the other hand, ascorbic acid was found to be the highest in green pepper. This matter may be due to variation of pigments and cultivars of different colored sweet peppers [37]. Besides, previous data suggested that the maturity stages were not correlated to the quantity of vitamins, which were differed from cultivar to cultivar (Table 3.3) [1]. Thus, this information suggested that the quantity of these vitamins might be varied according to the particular cultivars of sweet peppers.

Table 3.3 Provitamin A and ascorbic acid contents of sweet peppers [1]

Cultivars	Maturities	Provitamin A		Ascorbic acid	
		µg RE/100g FW	% Thai RDI *	mg/100g FW	% Thai RDI**
Cardinal	Green[u]	33	4.1	102	170
	Red[r]	110	13.8	124	207
King Arthur	Green[u]	33	4.1	84	140
	Red[r]	127	15.9	87	145
Var. 826R	Green[u]	38	4.8	88	147
	Red[r]	119	14.9	98	163
Red Bell G	Green[u]	44	5.5	95	158
	Red[r]	80	10.0	96	160
Red Bell C	Green[u]	35	4.4	72	120
	Red[r]	52	6.5	107	178
Tam Bell-2	Green[u]	33	4.1	109	182
	Red[r]	64	8.0	148	247
Grande Rio-66	Green[u]	81	10.1	98	163
	Red[r]	253	31.6	149	248
Klondike Bell	Green[u]	40	5.0	112	187
	Yellow[r]	31	3.9	109	182
Canary	Green[u]	31	3.9	112	187
	Yellow[r]	31	3.9	108	180
Orobelle	Green[u]	49	6.1	162	270
	Yellow[r]	36	4.5	95	158
Golden Bell	Green[u]	37	4.6	106	177
	Yellow[r]	32	4.0	90	150
Valencia	Green[u]	25	3.1	119	198
	Orange[r]	26	3.3	73	122
Oriole	Green[u]	37	4.6	91	152
	Orange[r]	99	12.4	86	143
Chocolate Beauty	Green[u]	38	4.8	62	103
	Brown[r]	108	13.5	100	167
Black Bird	Green[u]	32	4.0	66	110
	Black[r]	41	5.1	62	103
Ivory	White[u]	14	1.8	89	148
	Light yellow[r]	46	5.8	110	183
Dove	White[u]	16	2.0	77	128
	Light Orange[r]	29	3.6	103	172
Blue Jay	Purple[u]	22	2.8	95	158
	Orange[r]	59	7.4	123	205
Lilac	Purple[u]	17	2.1	67	112
	Orange[r]	86	10.8	104	173

[u]Unripe stage, [r]Ripe stage, RE: retinol equivalent, FW: fresh weight

*Thai RDI, male and female = 800 µg RE, where 1 µg RE = 1µg retinol = 6 µg β-carotene = 12 µg other provitamin A carotenoids.

**Thai RDI, male and female = 60 mg [64]

Table 3.4 β-Carotene, ascorbic acid, and α-tocopherol contents of sweet peppers (mg/g dry weight) [37]

Cultivars	Colors	β-Carotene	Ascorbic acid	α-Tocopherol
Orion	Green	0.012.2[a]	1.74[c]	0.98[a]
Mazurca	Red	0.043.9[c]	0.58[b]	3.65[d]
Simpaty	Orange	0.056.6[d]	0.49[a]	1.92[c]
Taranto	Yellow	0.015.9[b]	0.58[b]	1.23[b]

[a-d]Significant difference (p<0.05) is expressed by different letters in the same column.

Capsaicinoids

Capsaicinoids with alkaloid-like structures are unique pungent compounds in *Capsicum* species. These compounds include capsaicin, dihydrocapsaicin, homocapsaicin, nordihydrocapsaicin and homodihydrocapsaicin (Figure 3.10). The quantity of capsaicin and dihydrocapsaicin is approx. 90% of total pungency compounds in commonly consumed peppers [65]. Capsaicinoids exhibit antioxidant activity, in which 60 μg/mL of capsaicin could scavenge 91.9% of DPPH free radicals [66]. Capsaicin is used for treatment for pain and inflammation as well as prevention of many medical conditions including urticaria, diabetic neuropathy, arthritis, osteoarthritis, contact allergy, cluster headaches and urological disorders [1].

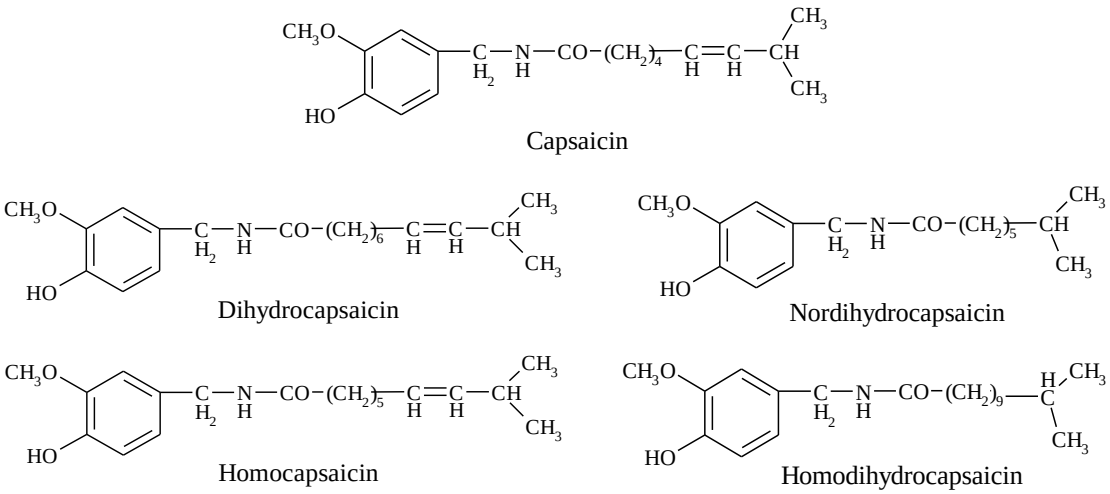
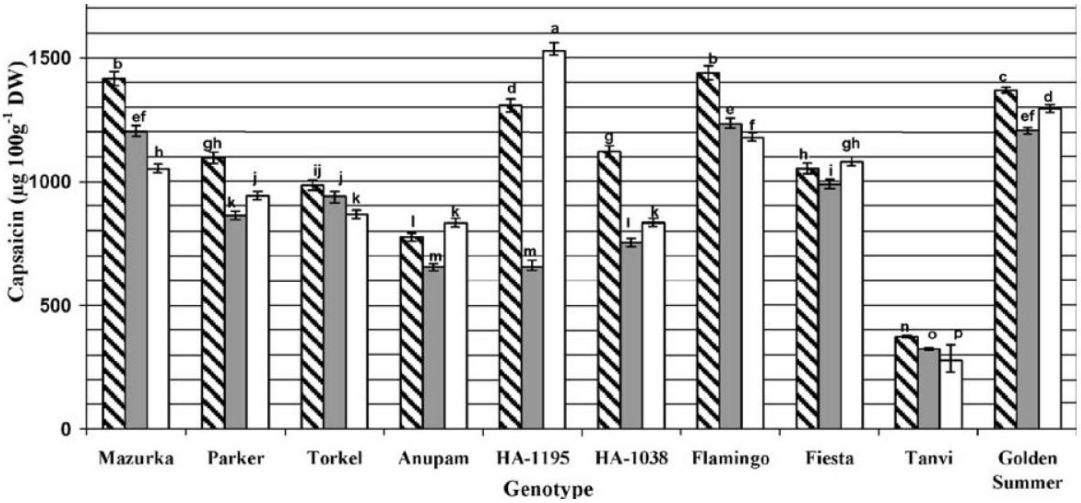


Figure 3.10 Chemical structures of various capsaicinoids. These capsaicinoids are the unique pungent compounds with antioxidant activity found in peppers.

The quantity of capsaicinoids in *Capsicum* sp. is varied, depending on genetics, environmental growth conditions and maturity (Figure 3.11) [52]. Comparing to other pungent red peppers, which contain high amounts of capsaicin ranging from 206 to 1,166 µg/g dry weight [67], sweet peppers are non-pungent peppers with only trace amount of capsaicin (less than 15 µg/100g dry weight) [52]. In addition, maturity seems to affect capsaicin content. Ripe sweet peppers possess similar or lower capsaicin level than the unripe ones [34]. Thus, under the same cultivar, unripe pepper may possess higher capsaicinoids and pungency levels than those of the ripe pepper. Besides, environmental stress can also induce synthesis of capsaicinoids. For example, warm weather can cause peppers to produce higher capsaicinoids than peppers growing in cool weather [1].



▨ : Green stage, ■ : Intermediate stage, □: Red/yellow stage

Figure 3.11 Capsaicin contents of 10 sweet pepper genotypes in different maturity stages [52]. In ten genotypes at the ripe stage, seven genotypes are red sweet peppers, including Mazurka, Parker, Torkel, Anupam, HA-1195, HA-1038 and Flamingo, and three were yellow peppers, including Fiesta, Tanvi, Golden and Summer. The green peppers (unripe) showed higher capsaicin contents than ripe ones. The ten genotypes at unripe green stage exhibited capsaicin content in range from 776–1,440 µg/100 g dry weight, while sweet pepper at the ripe red stage had 277–1,529 µg/100 g dry weight of capsaicin content. The green pepper showed higher capsaicin contents than ripe stage, except for two genotypes, HA-1195 and Anupam.

Effect of extraction solvents

The type of solvent for extracting sweet peppers may affect on different level of bioactive compounds (Table 3.5). The quantity of capsaicin, quercetin and luteolin is differed, depending on extraction solvents. For examples, capsaicin in hot peppers was not detected in methanol extraction with the exception of Ixtapa cultivar [68]. However, it was detected in significant amount in hexane, ethyl acetate and acetone extraction [52, 68], suggesting that capsaicin is highly dissolved in lipophilic solvent. Similar results were observed with quercetin and luteolin in green and yellow peppers, in which both were not detected in methanol:water (70:30 v/v) extraction [37] but were present in methanol extraction [2]. Therefore, solvent system may influence the biological property of sweet peppers as a result of differed quantities of bioactive compounds. However, cultivars and growth conditions of sweet peppers including temperature, light, water, harvesting time and nutrient availability are greatly impacted on contents of bioactive compounds [37, 60]. As well, detecting methods are significant factors that are responsible for variation in quantity and quality of bioactive compounds.

Table 3.5 Amount of quercetin and luteolin in sweet peppers extracted using 70% (v/v) methanol [37] and methanol [2] (μg/g dry weight)

Colors	Quercetin		Luteolin	
	70% (v/v) methanol	**Methanol**	**70% (v/v) methanol**	**Methanol**
Green	ND	265[a]	ND	20[a]
Red	10.0	340[b]	ND	110[c]
Orange	ND	290[ab]	154.0	65[b]
Yellow	ND	296[a]	ND	70[b]

[a-d]Significant differences (p<0.05) are expressed by different letters in the same column. ND: not detect

3.2.2 Sweet peppers and Alzheimer's disease

Sweet peppers possess many bioactive compounds that promote health benefits. Being one of the major diseases in elderly, AD occurrence can cause personal effect and social disturbance regarding economic burden and health care delivery

systems. Therefore, the research on study of the biochemical properties of sweet peppers extracted in different solvent systems against AD is of interest.

Sweet peppers and impairments of learning and memory

In vivo study of red bell pepper (*Capsicum annuum* L.) on learning performance in the SAMP8, a typical senile-prone strain of senescence-accelerated mouse that being impairments in various learning and memory, was suggested that by feeding 20% (w/w) lyophilized powder of red bell pepper to the mice for 3 months could improve learning and memory impairment [4]. The mice that received continuous diet with red pepper exhibited better learning and memory performances (passive avoidance) than a control group that received a common diet. This research reported that the properties of red bell pepper on developing of learning and memory performances may be from capsanthin, β-carotene, α-tocopherol and ascorbic acid. Therefore, sweet peppers may be useful for preventing or treating the abnormality of brain with age-related learning in humans such as AD.

Sweet peppers and antioxidant

Oxidative stress induction is factor causing AD, thus antioxidants possess potential AD prevention and/or treatment. Sweet peppers were reported to be sources of antioxidants. Their extracts were reported to have higher level of total phenolic content (TPC) and antioxidant activity than other vegetables or fruits, such as tomatoes [69]. In addition, the investigation on methanolic extracted peppers (*Capsicum annuum* L.), including green, red, orange and yellow sweet peppers, were reported that green pepper exhibited the lowest TPC and antioxidant activity (Table 3.6) [2, 3]. Similar results were reported by another research on methanolic extracted sweet peppers, in which the orange and red sweet peppers were found to exhibit the highest TPC, but yellow and green peppers were found to exhibit the lowest TPC [3]. Antioxidant activity as being measured by DPPH assay were reported that the highest antioxidant activity was found in orange sweet pepper extract, followed by yellow, red and green peppers, respectively [3]. In addition, bioactive compounds that found in sweet peppers such as capsaicin, quercetin, luteolin, ascorbic acid, α-tocopherol, and β-carotene also exhibited high antioxidant activity (Table 3.6). The results suggested

that colors of sweet peppers could influence TPC and antioxidant activity, which may be come from their color pigments and flavonoids. Red, orange and yellow peppers contain more pigments than green pepper. These results may be due to high quantity of carotenoids in red, orange and yellow peppers, while green pepper has lower pigments. These data suggested that the antioxidant activity of different colored sweet peppers could lead to inhibition of oxidative stress and prevention of AD.

Table 3.6 Total phenolic content (TPC) and antioxidant activity of sweet pepper extracts and their bioactive compounds

Pepper species	Colors	TPC		DPPH radical scavenging activity			Ref
		µmol CE/g FW	mg GAE/g	µmol TE/g FW	µg/mL (IC_{50})	%Inhibition	
*C. annuum**	Green	2.4[a]	80.5[a]	2.1[a]	1,153.6[a]	-	[2, 3]
	Red	4.2[b]	121.9[c]	3.9[b]	882.3[b]	-	[2, 3]
	Orange	3.4[ab]	122.8[c]	3.5[b]	694.8[d]	-	[2, 3]
	Yellow	3.3[ab]	91.5[b]	3.2[b]	811.1[c]	-	[2, 3]
Bioactive compounds							
Capsaicin		-	-	-	-	91.9[#]	[66]
Quercetin		-	-	-	2.355	-	[44]
Luteolin		-	-	-	2.051	-	[44]
Ascorbic acid		-	-	-	5.851	96.6[#]	[44, 66]
α-Tocopherol		-	-	-	10.100	-	[44]
β-Carotene		-	-	-	-	85.2[$]	[46]

[a-d] Significant difference (p<0.05) is expressed by different letters in the same column.

The values are expressed as mean of triplicate.

CE: catechin equivalent, GAE: gallic acid equivalent, TE: trolox equivalent, FW: fresh weight, -: not available

*Using methanol for extraction

Sweet peppers and cholinesterase inhibitory activities

Cholinesterase inhibition is currently the major medicinal treatment for AD patient [16]. Since synthesized cholinesterase inhibitors possess several severe side effects [16], inhibitors from natural sources such as fruits and vegetables provide benefits regarding non/less toxicity, low cost and confidence to consume. Thus, anti-AChE and BChE agents from sweet peppers are of interest in this research, since sweet peppers are commonly consumed vegetables with good taste and low/no reported toxicity. The researches of cholinesterase inhibitory activities in sweet peppers are, however, unavailable. Nevertheless, this information is reported in other cultivars of pepper.

The investigation on the effect of ripening stage of methanolic extract of *Capsicum annuum* var. *acuminatum* regarding its AChE inhibitory activity was suggested that the half maximal inhibitory concentration (IC_{50}) of the premature green pepper extract exhibited the highest AChE inhibitory activity (IC_{50} of 84.30 µg/mL) (Table 3.7) [5]. This inhibitory activity was decreased during ripening stage of mature green and red peppers (IC_{50} of 96.69 and 130.03 µg/mL, respectively). However, when comparing to the IC_{50} of physostigmine (0.07 µg/mL), the commercial anti-cholinesterase drug, it was suggested that sweet peppers under this investigated extraction condition might be insufficient for treatment of AD. Nevertheless, when comparing to other fruits and vegetables such as ginkgo [16], pomegranate [70], mulberry [71], lemon juice [72], black chokeberry [72], turmeric [31], garlic [31], black pepper [31], ginger [31], and cinnamon [31], sweet peppers might be the potential food for prevention of AD. Besides, the colors of peppers, which are depended on the ripening stages (in this case, premature green pepper and mature green and red peppers), are the significant factor that might impact the quantity and quality of the bioactive compounds.

Additionally, *Capsicum chinense* Jacq. cv Habanero in two ripening stages, unripe (green) and ripe (red) peppers, that were extracted by ethanol and then hexane for lipophilic compounds were examined for their AChE and BChE inhibitory activities (Table 3.7) [6]. As results, ethanolic extract of green pepper exhibited higher anti-BChE activity than that of red pepper (IC_{50} values of 562 and 806 µg/mL, respectively). However, both exhibited trace activity of anti-AChE reaction

(IC_{50}>1000 µg/mL). On the other hand, the BChE inhibitory activity of lipophilic fractions were not observed in both green and red peppers (IC_{50}>1000 µg/mL). However, the AChE inhibitory activity was found in red pepper (IC_{50} of 733 µg/mL). Thus, polarity of solvents could affect AChE and BChE inhibitory activities, which may be due to different types of bioactive compounds extracted in particular solvents. In this study, bioactive compounds act as BChE inhibitors were likely dissolved in ethanol, while AChE inhibitors were likely dissolved in lipophilic solvent. Comparing to the IC_{50} of physostigmine toward AChE and BChE reactions (0.2 and 2.4 µg/mL, respectively), these results suggested that peppers exhibit cholinesterase inhibitory activities, which are much lower than the commercial drug.

These AChE and BChE inhibitory activities may be from the main bioactive compounds in sweet peppers such as capsaicin, myricetin, quercetin and luteolin [1, 2, 37]. These compounds were reported to exhibit cholinesterase inhibitory activities (Table 3.7) [7, 8]. Capsaicin (1 mg/mL) was significantly inhibited AChE and BChE reactions with 62.7 and 75.3% inhibition, respectively [7]. In addition, myricetin, quercetin and luteolin also exhibited anti-human recombinant AChE (K_i of 37.8, 38.3 and 65.8 µM, respectively) and anti-human plasma BChE (K_i of 71.0, 68.0 and 166.1 µM, respectively) [8]. Besides, commercial ascorbic acid (60 mg/kg intraperitoneal injection), which was found to be in high levels in sweet peppers [1], significantly inhibited AChE activity with 17.13% inhibition in brains of mice [73]. Since these previous researches suggested that the sweet pepper extracts and the bioactive compounds found in sweet peppers exhibited cholinesterase inhibitory activities, Thai local market available sweet peppers extracted under different extraction conditions are of interest for optimizing the extraction of anti-cholinesterase agents.

Table 3.7 Summarized data on cholinesterase and BACE1 inhibitory activities of pepper extracts and their bioactive compounds

Pepper species	Pepper types	Solvents	% Inhibition		IC_{50} (ug/mL)			K_i (µmol/L)		Ref
			AChE	BChE	AChE	BChE	BACE1[$]	AChE	BChE	
C. annuum	Premature green	Methanol	-	-	84.3	-	-	-	-	[5]
	Mature green	Methanol	-	-	96.7	-	-	-	-	[5]
	Mature red	Methanol	-	-	130.0	-	-	-	-	[5]
C. chinense	Green	Ethanol	-	-	>1000	562	-	-	-	[6]
	Red	Ethanol	-	-	>1000	806	-	-	-	[6]
	Green	hexane fraction	-	-	>1000	>1000	-	-	-	[6]
	Red	hexane fraction	-	-	733	>1000	-	-	-	[6]
Bioactive compounds										
Capsaicin*			62.7	75.3	-	-	-	-	-	[7]
Myricetin			-	-	-	-	2.8	37.8	71.0	[8, 9]
Quercetin			-	-	-	-	5.4	38.3	68.0	[8, 9]
Luteolin			-	-	-	-	-	65.8	166.1	[8]
Ascorbic acid[#]			17.1	-	-	-	-	-	-	[73]
Medicine										
Physostigmine			-	-	0.2	2.4	-	-	-	[6]

*Concentration of **c**apsaicin = 1 mg/mL, [#]Concentration of ascorbic acid = 60 mg/kg i.p. (intraperitoneal injection)
[$]µmol/L, -: not available

Sweet peppers and BACE1 inhibitory activity

BACE1 is the key enzyme that controls AD in the β-amyloid formation hypothesis. Thus, inhibition of BACE1 may decrease AD occurrence. The main bioactive compounds in sweet peppers that reported to possess anti-BACE1 activity and reduce β-amyloid formation are myricetin and quercetin [9]. This study showed that myricetin and quercetin inhibited BACE1 enzyme activity with the IC_{50} of 2.8 and 5.4 μM, respectively (Table 3.7) [9]. In addition, both compounds exhibited BACE1 inhibitory activity in primary neuronal culture [9]. The neuronal cells with myricetin or quercetin were significantly exhibited decreased BACE1 activity (Figure 3.12). Besides, BACE1 inhibitory activity, myricetin (20 μM) and quercetin (20 μM) significantly reduced extracellular β-amyloid level (Figure 3.12). Myricetin decreased the level of $A\beta_{1\text{-}40}$ by 74.4% and the level of $A\beta_{1\text{-}42}$ by 84.4%, comparing to the control (0.1% DMSO without myricetin and quercetin). Similarly, quercetin reduced the level of $A\beta_{1\text{-}40}$ by 66.3% and the level of $A\beta_{1\text{-}42}$ by 80.1%.

In addition, ascorbic acid, β-carotene and α-tocopherol, which were reported in high amount in sweet peppers [1, 62], can act as antioxidants, thus preventing AD occurrence by reducing or preventing oxidation of APP [21]. It was previously suggested that APP oxidation could lead to production of β-amyloid in the brain of AD patients. Besides, vitamin C was reported to prevent AD *via* reduction of Aβ oligomer formation in transgenic AD mice by feeding a diet with vitamin C solution for 6 months [74]. Therefore, Thai local market available sweet peppers may also inhibit BACE1 activity due to these bioactive compounds.

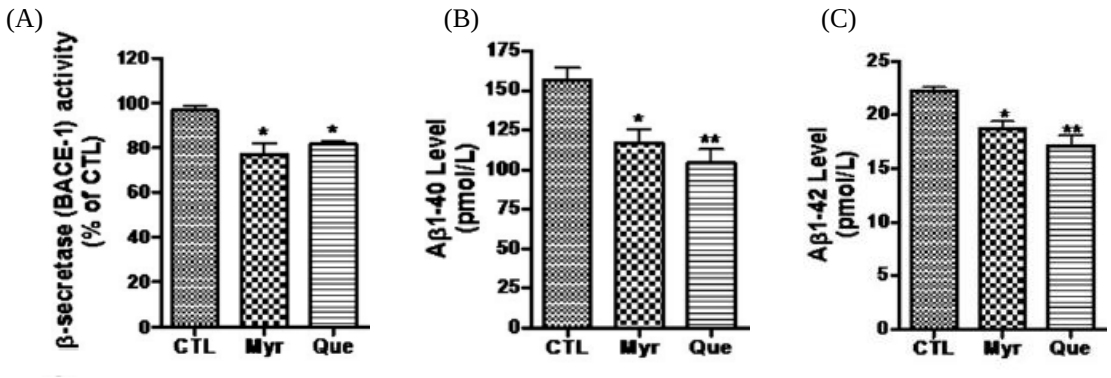


Figure 3.12 Effects of myricetin and quercetin on rat cortical neurons [9]. (A) BACE1 inhibitory activity, in which the data were expressed as percentage of CTL (control), (B) and (C) amyloid beta (Aβ) inhibitory activity using ELISA assay, in which the data were expressed as pmol/L.

Sweet peppers and tau protein abnormality

Tau protein abnormality is another cause of AD, which β-amyloid leads to induce hyperphosphorylation of tau protein [23]. Therefore, β-amyloid or BACE1 inhibitors from bioactive compounds that found in sweet pepper, such as myricetin and quercetin [9], may reduce hyperphosphorylated tau protein. In addition, enzyme that cause phosphorylation of tau protein is kinases (tau kinase), in which glycogen synthase kinase-3β (GSK-3β) is the main enzyme to transfer phosphate group to tau protein [23, 26]. Previous research reported that luteolin could decrease tau hyperphosphorylation and GSK activation elicited by traumatic brain injury (TBI) in AD mice model [75]. Ascorbic acid in a mixture of antioxidant drugs, including ascorbic acid, trolox and glutathione, possessed ability to prevent tau hyperphosphorylation [76]. Moreover, myricetin could also inhibit heparin-induced tau aggregation [26, 77]. Although little information of hyperphosphorylation of tau protein from sweet pepper extract is available, the bioactive compounds found in sweet pepper may lead to inhibitory activity of tau protein from sweet pepper extract.