CHAPTER VI
DISCUSSION

6.1 Phytochemicals in Sweet Peppers

6.1.1 Carotenoid contents

The different colored sweet peppers have been reported to contain particular contents of nutrients and phytochemical compounds such as vitamin C, flavonoids and carotenoids [3, 76, 77]. Carotenoids are pigments in fruits and vegetables, which can act against oxidative stress as antioxidants and immune enhancer [78-80]. Carotenoids can promote health benefits against various diseases such as cataracts, cancer and cardiovascular disease [5, 81]. Although carotenoid contents in sweet peppers have been studies, sweet pepper cultivars in Thailand have not yet been investigated. Therefore, this research provided the information about carotenoid contents of sweet pepper cultivars in Thailand.

Chilies were reported to contain various carotenoids such as capsanthin, β-carotene, zeaxanthin and lutein [4, 82, 83]. Some of these carotenoids in chili were studied for their health benefit. Previous research suggested that capsanthin in red paprika (*C. annuum*) showed potent anti-tumor-promoting activity [84]. Maturity stage affects the quantity of pigments in sweet pepper. The color of pepper fruits changes from green to red, yellow or orange during maturity stage, depending on cultivars [82]. The results from this study showed that total carotenoid contents of mature sweet peppers (red, orange and yellow) were higher than immature sweet peppers (green). This result was consistent with other studies, which reported that carotenoids were increase during maturation [4, 19, 82]. Besides, red sweet pepper was reported to contain the largest amount of total carotenoids, followed by orange, yellow and green sweet peppers, respectively [4]. The green color of pepper fruit is formed by chlorophyll and some carotenoids such as xanthophylls, violaxanthin, lutein and β-carotene [82]. The yellow and orange colors of pepper fruit are due to zeaxanthin,

lutein, β-cryptoxanthin, α- and β-carotene [85]. On the other hand, the red color of pepper fruit is formed by ketocarotenoids, capsanthin and capsorubin [85]. This information is corresponded to our studies, which showed that green, orange and yellow sweet peppers contained lutein, while it was not detected in red sweet pepper. Besides, our results indicated that capsanthin content of red sweet pepper was 4-10 folds greater than those of green, orange and yellow sweet peppers.

6.1.2 Flavonoid and phenolic acid contents

Flavonoids and phenolic acids are a group of phenolic compounds that commonly found in plants [86]. Flavonoids are one of the largest groups of phenolic compounds, which provide important colors and flavors of fruits and vegetables. Flavonoids and phenolic acids are effective antioxidants that can promote health benefits [86]. Flavonoids can reduce the risk of various diseases such as cancers, cardiovascular disease, obesity, diabetes and hypertension. [6, 53, 65, 87, 88]. Amount and type of flavonoids and phenolic acids in each plant are different. Catechins are a type of flavonoids that are mostly found in tea [89]. Caffeic acid was found in coffee, grapes, apples and olives, while anthocyanidin was found in grapes, cherries and strawberries [86].

Sweet peppers are also a source of flavonoids and phenolic acids (Figure 6.1 A-D). In this study, *p*-coumaric acid and ferulic acid are a group of phenolic acids that found in sweet pepper. The results showed that green sweet pepper exhibited the highest level of *p*-coumaric acid, while red sweet pepper exhibited the highest level of ferulic acid. Quercetin and luteolin are main flavonoids in sweet peppers [3, 90, 91]. Different colored sweet peppers had different amount of flavonoids [3, 90]. Likewise, this study exhibited the different content of flavonoids in each colored sweet pepper. Yellow sweet pepper exhibited the highest quercetin and luteolin levels, followed by red, orange and green. This result was different from the previous study, which indicated that red sweet pepper demonstrated the highest quercetin and luteolin levels [3]. Nevertheless, cultivar, climate and cultivation of sweet peppers might be the cause of this variation. However, green sweet pepper exhibited the lowest quercetin and luteolin levels in both studies. Besides, quercetin is a flavonoid that was found the most in peppers followed by luteolin [2, 91]. Our result was corresponded to the prior

study, which found that the level of quercetin was higher than luteolin [2]. Interestingly, it was found that these flavonoids could potentially inhibit lipase, α-amylase, α-glucosidase and ACE activities [53, 65, 87, 88]. Thus, these compounds could possibly control obesity, diabetes and hypertension through enzyme inhibition.

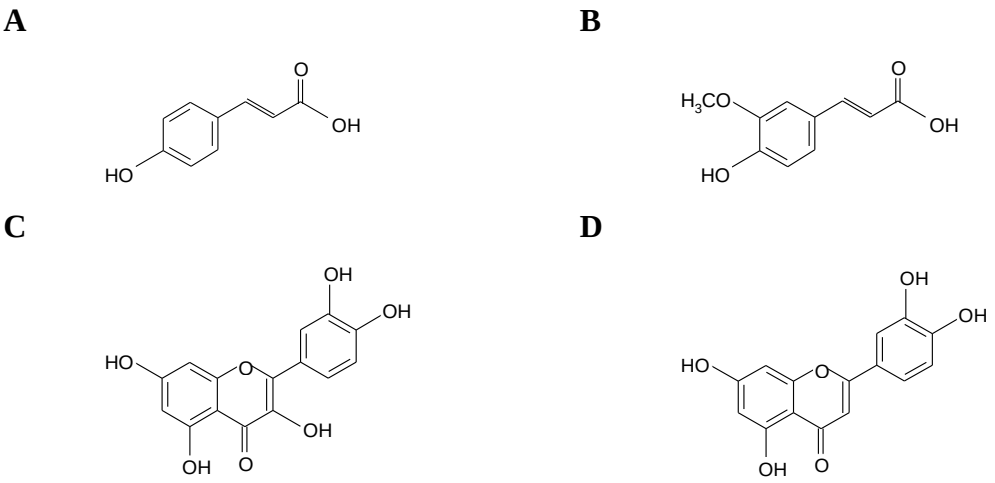

Figure 6.1 The chemical structures of (A) *p*-coumaric acid, (B) ferulic acid, (C) quercetin and (D) luteolin These compounds, A-B and C-D, are in a group of phenolic acids and flavonoids, respectively.

6.1.3 Volatile compounds

Sweet peppers have unique color, taste, pungency and aroma [92]. It can be consumed in fresh or processed forms. Fresh sweet pepper fruits have different flavor from other fruits and vegetables. Flavor has been determined as the overall sensation during consumption and provided by odor and taste [93]. Odor is caused by volatile compounds, in which human recognize by the sense. Volatile compounds of fruits and vegetables are different. Therefore, this study analyzed the volatile compounds in four colored sweet pepper including green, red, orange and yellow by GC-MS method. The results suggested that sweet pepper contained various particular volatile compounds. Some volatile compounds in sweet pepper that were found in this study including pyrazine,2-methoxy-3-(2-methylpropyl), hexadecane, heptadecane and copaene were also found in chili peppers (*Capsicum annuum*) from previous researches [94, 95]. Base on our results, each colored sweet pepper had different

quality and quantity of volatile compounds. Green sweet pepper contained the highest level of 2-methoxy-3-(2-methylpropyl)pyrazine. Nevertheless, red sweet pepper contained this compound only in trace amount, while it was not detected in orange and yellow sweet peppers. These results were corresponded to the previous studies, in which 2-methoxy-3-(2-methylpropyl)pyrazine was found to possess green aroma [96]. Therefore, 2-methoxy-3-(2-methylpropyl)pyrazine can be found mostly in green pepper as being reported in our results. Besides, other volatile compounds that could be found in sweet pepper were alloaromadendrene, 2-mercapto-4-phenylthiazole, benzhydryl alcohol, benzophenone, n-nonyl-cyclopropane / 1-dodecanol, thiocyanic acid carbazol-3,6-diyl ester, β-cis-ocimene, β-elemene, β-selinene, γ-selinene and 9,10-dehydro-isolongifolene. Interestingly, previous research suggested that alloaromadendrene had an antioxidant property [97]. Thus, alloaromadendrene in sweet pepper might possess an antioxidant property. Nevertheless, some volatile compounds that were found in our study were not compounds from sweet pepper. These compounds were hexamethylcyclotrisiloxane, octamethylcyclotetrasiloxane, methoxy-phenyl-oxime, butylated hydroxytoluene, decamethylcyclopentasiloxane and tetradecamethylcycloheptasiloxane. These compounds were reported to be contaminants from DVB/CAR/PDMS fiber and column coating [98].

Volatile compounds have been studied for their benefit. Essential oil contains volatile compounds, which extracted from plant. Nowadays, essential oil is used in industry such as perfume, soap and cosmetic. In addition, their health benefit was studied. Prior research investigated the anti-diabetic properties of turmeric essential oil through enzyme inhibition [99]. The results suggested that turmeric essential oil could inhibit α-amylase and α-glucosidase at IC_{50} of 64.7 and 1.32 μg/mL, respectively. Turmeric essential oil contained various volatile compounds such as α-phellandrene, β-elemene, β-caryophyllene β-ocimene and α-selinene [100]. The β-elemene and β-ocimene were also found in sweet peppers. Thus, it was possible that these compounds might possess α-amylase and α-glucosidase inhibitory activities. Nevertheless, the volatile compounds of sweet pepper have not been directly studied for their health benefits. Thus, the information received from this research would lead to further investigation on health benefit from sweet pepper's volatile compounds.

6.2 Sweet Peppers and Health Benefits

6.2.1 Anti-lipase activity

Sweet pepper contains significant quantity of bioactive compounds such as flavonoids, carotenoids and phenolic acids [3]. Flavonoids have been suggested to possess anti-obesity thought lipase inhibition. Quercetin, kaempferol and luteolin (Figure 6.1 C-D and Figure 6.2) were reported to inhibit lipase activities [7, 26, 87]. Bioactive compounds in sweet pepper have various chemical structures, so the extraction solvents will effect on the extracted compounds. This study suggested that sweet peppers extracted with 70% (v/v) aqueous ethanol exhibited higher lipase inhibitory activity than those extracted with ethyl acetate and hexane. The result related to total phenolic compounds in our laboratory study, in which sweet pepper extracted with 70% (v/v) aqueous ethanol exhibited the highest total phenolic compounds. Phenolic compounds that were reported to inhibit lipase activity were flavonoids and phenolic acids [26, 87, 101]. Flavonoids and phenolic acids are polar compounds, so it can be extracted with polar solvent. Hence, this reason is possibly causes sweet pepper extracted with 70% (v/v) aqueous ethanol exhibited the highest lipase inhibitory activity. In addition, prior study that evaluated bioactive compounds in different solvent extractions from hot pepper (*Capsicum* spp.) [20] was in good agreement with the results from our studies. The results from previous study showed that the highest amount of flavonoids was found in methanol extracts (polar solvent), followed by ethyl acetate and acetone, while none was detected in hexane [20]. In nature, carotenoids are lipophilic compounds [102], thus non-polar solvent is needed to quantify carotenoids. Therefore, it can be concluded that compounds in hexane extracts are mainly carotenoids, while flavonoids and phenolic acids were rarely found in hexane. Since some flavonoids and phenolic acids can act as anti-lipase agents [7, 26, 87], sweet peppers extracted with hexane exhibited the lowest anti-lipase activity.

The different colored sweet peppers have been reported to exhibit different level of bioactive compounds such as carotenoids and flavonoids [3]. In this study, amongst four colored sweet peppers extracted with 70% (v/v) aqueous ethanol, red sweet pepper showed the highest lipase inhibitory activities. The result was also consistent with flavonoid and phenolic acid contents, in which red sweet pepper

contained high contents of quercetin and luteolin. In addition, phenolic acids (salicylic, *p*-hydroxybenzoic, gentisic, protocatechuic, vanillic, syringic, *o*-coumaric, *p*-coumaric, caffeic, ferulic, sinapic) were reported to inhibit lipase activity [101]. Caffeic, ferulic and benzoic acids are the strongest lipase inhibitors among phenolic acids. Ferulic acid was also found in the largest amounts in red sweet pepper. Thus, it is corresponded to the result, which showed that red sweet peppers had the highest lipase inhibitory activities. Under ethyl acetate extraction, green and yellow sweet peppers exhibited the highest anti-lipase activities. Similarly, under hexane extraction, yellow sweet peppers exhibited the highest anti-lipase activity. These results related to flavonoid and phenolic acid contents. From our studies, yellow sweet pepper contained the greatest levels of flavonoids, while green sweet pepper had the highest amounts of *p*-coumaric acid. Orange sweet pepper exhibited the lowest anti-lipase activity in all solvent extractions. The result was supported by flavonoid and phenolic acid contents, which orange sweet pepper had the lowest amount of quercetin, luteolin, *p*-coumaric and ferulic acids.

Catalytic pocket of lipase consists of Ser, His and Asp [103]. Ser residue attacks the ester of triglyceride to form an acyl enzyme intermediate (Figure 6.3) [103]. His and Asp help stabilized Ser and substrate. Then, water enters the active site to release enzyme and free fatty acid. Orlistat, an anti-lipase drug, can form covalently bond with catalytic Ser in the active site of gastric and pancreatic lipases (Figure 6.4) [104]. Thus, the active sites of these enzymes are unavailable to interact with TGs, leading to a reduction in lipid digestion and absorption [105].

From our study, sweet pepper could inhibit lipase activity. The IC_{50} values of sweet peppers were in range of 10.68 to 61.96 mg/mL (143.36 to 828.34 mg/mL fresh weight). Sweet peppers extracted with 70% (v/v) aqueous ethanol possessed the most potent IC_{50} value of 10.68 to 24.68 mg/mL (143.36 to 329.95 mg/mL fresh weight). Previous research suggested that the IC_{50} values of bioactive compounds, including quercetin and ferulic acid were 0.0072 and 0.0241 mg/mL, respectively [87]. These bioactive compounds could be found in sweet pepper. Comparing to orlistat, the competitive inhibitor with the IC_{50} values of 0.0159 mg/mL [87], quercetin is a better lipase inhibitor than orlistat. Nevertheless, quercetin and ferulic acid were non-competitive inhibitors [101, 106]. These compounds and substrate could combine with

lipase to form enzyme-substrate-inhibitor (ESI), leading to a lower lipase activity. The inhibitor can bind to the enzyme whether substrate has been bound the enzyme or not. However, the mechanism on lipase inhibition of these compounds was required futher investigation.

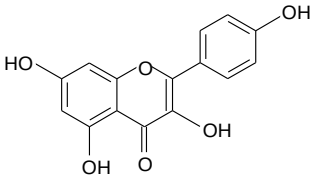

Figure 6.2 The chemical structures of kaempferol. This compound is flavonoid, which is found in chili peppers and sweet peppers.

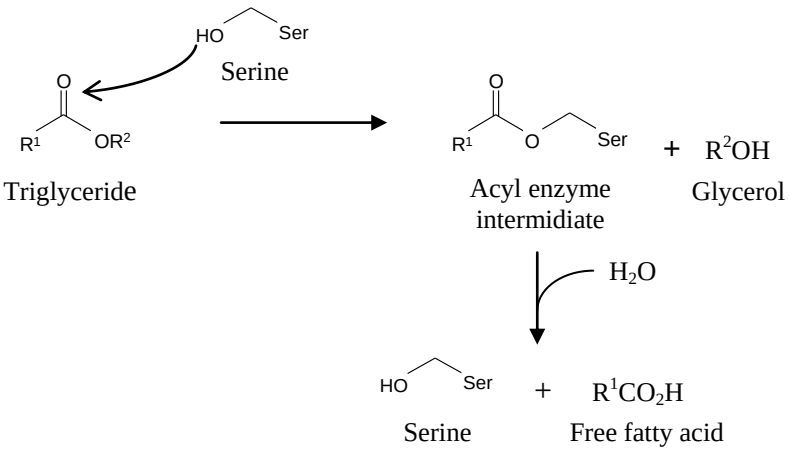

Figure 6.3 The mechanism of lipase. Serine residue in the active site of lipase attacks the ester on triglyceride to release acyl enzyme intermidate and glycerol. Then, water hydrolyses acyl enzyme intermidate into serine residue and free fatty acid.

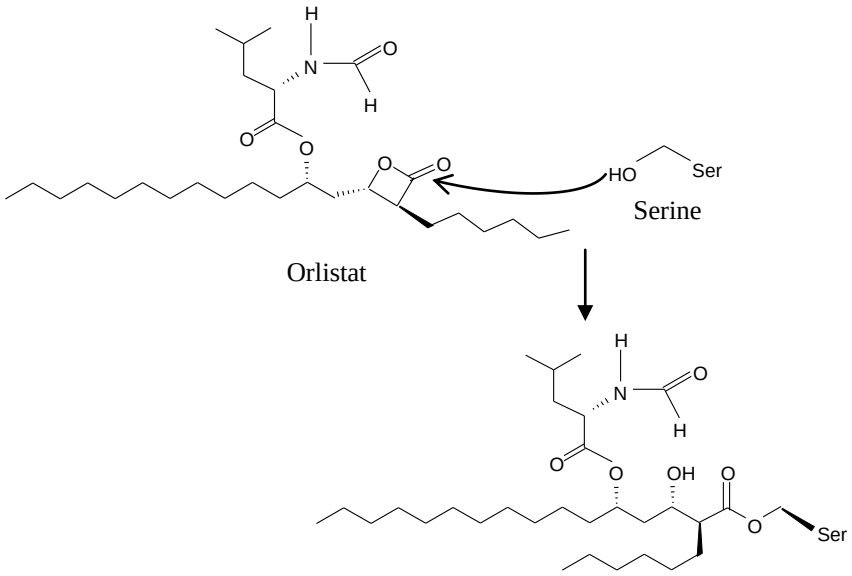

Figure 6.4 The mechanism of orlistat. Orlistat form bond with serine residue in the active site of lipase. Thus, lipase is unavailable to interact with triglceride, leading to a reduction in lipid digestion and absorption.

6.2.2 Anti-α-amylase activity

Previous research found that solvent extractions affect the amount of bioactive compounds in chili extracts [20]. Chili has various compounds, such as flavonoids, capsaicinoids and carotenoids, which have various polarities. Flavonoids were reported to inhibit α-amylase, such as luteolin, quercetin, myricetin, kaempferol, cyanidin, genistein and fisetin [56]. According to previous study, luteolin showed the strong inhibition against α-amylase [60, 107]. Additionally, capsaicinoids (capsaicin and dihydrocpsaicin) in peppers also inhibited α-amylase with IC_{50} of 83.00 and 92.00 μg/ml, respectively [60]. Capsiate is a large capsaicinoids in sweet pepper, which has a structure similar to capsaicin (Figure 6.3 G-H) [108]. It is possible that both compounds may have similar functions. The result from our study showed that sweet peppers extracted with ethyl acetate exhibited the highest α-amylase inhibitory activity. Ethyl acetate is a semi-polar solvent, so it may extract polar and non-polar compounds. Previous study suggested that capsaicin and dihydrocpsaicin were extracted to the maximum content in hexane (non-polar), followed by ethyl acetate, acetone and methanol, respectively [20]. In contrast, the highest amount of flavonoids was found in methanol (polar) extract, followed by ethyl acetate and acetone, while it

was not detected by hexane [20]. From previous research, both capsaicinoids and flavonoids that were reported to inhibit α-amylase were extracted well in ethyl acetate (semi-polar). Therefore, ethyl acetate is an optimal solvent for sweet pepper extraction in order to obtain the highest α-amylase inhibitory activity.

Sweet pepper has various colors including green, red, orange and yellow. Green is in immature stage of sweet pepper. Red, orange and yellow are in mature stage. Previous research suggested that the amount of phytochemicals in chilies is change during maturity [17, 19]. Additionally, other studies suggested that antioxidant activity, total phenolics and carotenoids of mature chilies are higher than immature ones [3, 18], in which some of these compounds were reported to inhibit α-amylase activity [56, 57]. Based on our results, red sweet peppers extracted with 70% (v/v) aqueous ethanol exhibited the highest anti-α-amylase activity, followed by yellow, green and orange sweet peppers, respectively. These results were consistent with the prior study, which suggested that red sweet pepper had higher α-amylase inhibitory activity than yellow sweet pepper [10]. Base on our study, red sweet pepper contained high content of flavonoids and phenolic acids. In addition, capsiate was also found in red sweet pepper [109]. Thus, these compounds might cause red sweet pepper extracted with 70% (v/v) aqueous ethanol to exhibit higher α-amylase inhibitory activity than those three colored sweet peppers. Under ethyl acetate extraction, all colored sweet pepper extracts exhibited insignificantly different anti-α-amylase activity. From this result, bioactive compounds from ethyl acetate extraction that could inhibit α-amylase might have same contents in four colored sweet peppers, such as quercetin, luteolin, capsaicin, dihydrocapsaicin, capsiate. Under hexane extraction, yellow sweet pepper exhibited the highest α-amylase inhibitory. The result was corresponded to total flavonoids, which were found the most in yellow sweet pepper.

The reaction of α-amylase with its natural substrate, polysaccharide (starch), could be initiated by the hydrolysis of α-1-4-glycosidic linkage on polysaccharide, thus producing smaller sugar units such as disaccharide (maltose) and glucose. The active site of enzyme is build by the number of subsites (Figure 6.6) [110]. The hydrolysis occurs between subsites -1 and +1. Catalytic triads of α-amylase consist of one Glu and two Asp. The mechanism between enzyme and substrate suggested that catalytic Glu could serve the proton to glycosidic oxygen (Figure 6.7)

[111]. Then, glucose is released. The catalytic Asp that acts as a nucleophilic forms interaction with C1 of sugar residue. Then, the water molecule donates the proton to Glu and hydrolyses the bond between the C1 of sugar residue and the nucleophile oxygen. Thus, the reaction mechanism is completed.

Acarbose, the commercial medicine, is used to treat diabetes through α-amylase and α-glucosidase inhibition. Acarbose is a competitive inhibitor, which exhibited the IC_{50} value of 0.08 mg/mL against α-amylase [45]. Acarbose can directly interact with catalytic Glu and Asp at the active site of α-amylase (Figure 6.8) [112-114]. The hydrolysis occurs between subsites -1 and +1. Nitrogen bond of secondary acarbose, which cannot be cleaved by α-amylase, also occurs between these subsites. Thus, acarbose can block substrate to bind with α-amylase at the active side.

Flavonoids were reported to act against α-amylase [56]. Flavonoids consist of benzopyran (A and C rings) and phenyl (B ring) groups. The α-amylase inhibitory activity of six groups of flavonoids including anthocyanidin, flavonol, isoflavone, flavanone, flavan-3-ol and flavone were studied (Figure 6.5 A-F) [56]. Each group contains different benzopyran (C ring) moieties and the linkage between benzopyran and phenyl groups. The results suggested that luteolin, quercetin and myricetin exhibited high α-amylase inhibitory activities with the IC_{50} of 0.10, 0.11 and 0.16 mg/mL, respectively. Moreover, the inhibitory activity increased with the increase of hydroxyl group on the phenyl (B ring) group. These flavonoids were found to be the competitive inhibitors [57]. The hydroxyl groups on A and B rings are important for α-amylase inhibition. The hydroxyl groups on B ring could form hydrogen bonds with the carboxylate groups of catalytic Asp and Glu at the enzyme active site. When inhibitor bounds to enzyme at the active site, enzyme was unavailable to form the complex with substrate. Thus, α-amylase inhibitor could against diabetes by delaying glucose absorption.

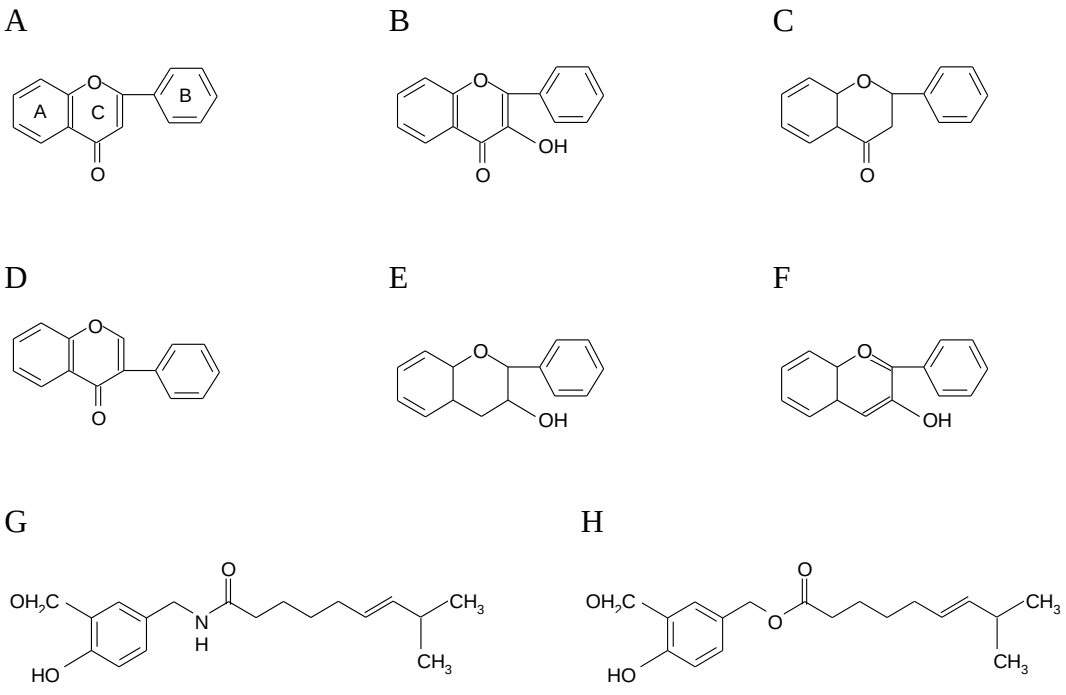

Figure 6.5 The chemical structures of (A) flavone, (B) flavonol, (C) flavanone, (D) isoflavone, (E) flavan-3-ol, (F) anthocyanidin and (G) capsaicin and (H) capsiate These compounds are a group of flavonoids, which include benzopyran (A and C rings) and phenyl (B ring) groups. G and H are the group of capsaicinoids, which are found in peppers.

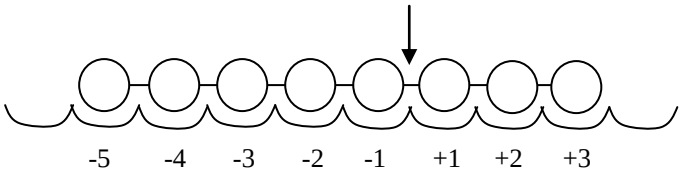

Figure 6.6 Arrangement of subsite with oligosaccharide Between subsides -1 and +1 is the cleavage area.

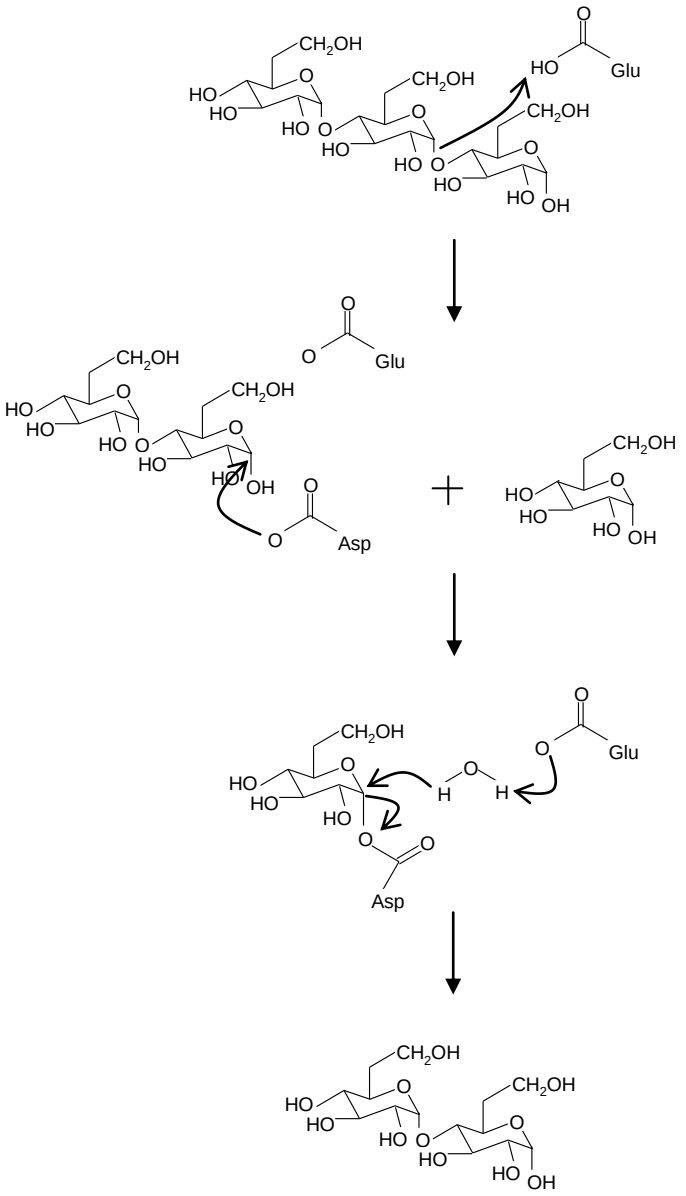

Figure 6.7 The mechanism of α-amylase The glutamate residue deprotonated the proton to glycosidic oxygen. The aspartate residue , a nucleophilic, attacks on the C1 of sugar residue. Then, the water molecule deprotonated the proton to glutamate and hydrolyses the bond between the C1 of sugar residue and the nucleophile oxygen. Then the glucose molecule is released.

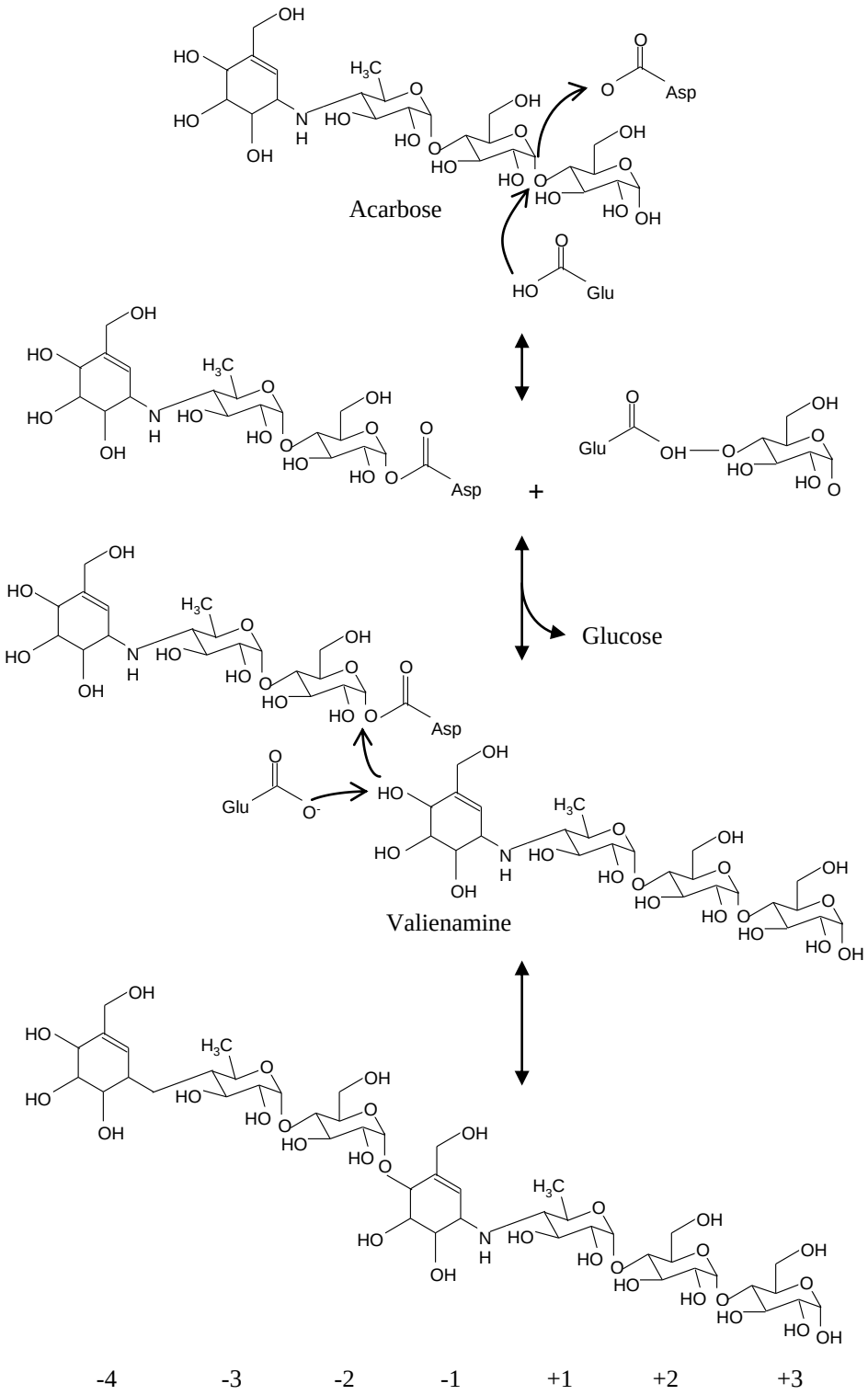

Figure 6.8 The reaction mechanism of acarbose. The aspartate and glutamate residues at the active site of enzyme form bond with the sugar residue on acarbose. Glucose molecule is released by the hydrolysis of bond between glutamate and sugar.

Then, the secondary acarbose bind with the fist acarbose structure and cause the acarbose stick on the active site of enzyme. The nitrogen bond occurs between subsites -1 and +1, which is the normal substrate hydrolysis area. The nitrogen bond cannot be cleaved by α-amylase. Thus, acarbose has an effective inhibitor to block the binding of enzyme and substrate.

6.2.3 Anti-α-glucosidase activity

At present, natural products that can act as nutraceutical have been focused to promote health benefits. Sweet peppers are potential sources of bioactive compounds such as quercetin, luteolin and myricetin that could function against α-glucosidase activity [3, 53, 55]. Polarity of solvent extraction is a cause that could affect the types of bioactive compounds in chili extracts. Based on our experimental results, it was showed that sweet peppers extracted with 70% (v/v) aqueous ethanol exhibited the highest α-glucosidase inhibitory activity, followed by ethyl acetate and hexane, respectively. This result was corresponded to the previous research, which suggested that methanol (hydrophilic solvent) extracts had the highest content of flavonoids [20]. In addition, previous study also used methanol to extract flavonoids from fresh pepper (*Capsicum annuum*) [2]. Therefore, flavonoids, which were reported to act as anti-α-glucosidase inhibitors, were extracted well with polar solvent. Among three solvent extractions, 70% (v/v) aqueous ethanol has the highest polarity, followed by ethyl acetate (semi polar) and hexane (non polar). Moreover, the result of our study was corresponded to total phenolic compounds, which suggested that sweet pepper extracted with 70% (v/v) aqueous ethanol had the highest total phenolic compounds. Thus, it could be suggested that 70% (v/v) aqueous ethanol is the most suitable solvent for sweet pepper extracts to get the maximum α-glucosidase inhibitors. Under ethyl acetate extraction, the extracts might be the polar and non polar compounds such as quercetin, luteolin, capsanthin, β-carotene. Under hexane extraction, the extracts might be the non polar compounds such as capsanthin, β-carotene, zeaxanthin, lutein. Nevertheless, the anti-α-glucosidase property of carotenoids has not been reported. Thus, it might be the cause that these solvent extractions exhibited lower anti-α-glucosidase activity than those extracted with 70% (v/v) aqueous ethanol.

The content of bioactive compounds in pepper is influenced by various factors such as cultivar, climate and ripening stages of fruits [3, 19, 115]. The different colors of sweet peppers may be due to different levels of bioactive compounds. The result showed that red and orange sweet pepper extracted by 70% (v/v) aqueous ethanol exhibited the most potent anti-α-glucosidase activity. Similarly, under ethyl acetate and hexane extraction, red sweet pepper exhibited the highest anti-α-glucosidase activity. This result was consistent to prior research, which studied α-glucosidase inhibitory activity of various peppers in *Capsicum annuum* species [10]. Red sweet pepper had the highest α-glucosidase inhibitory activity. Additionally, hypoglycemic activities of *Capsicum annuum* during fruits growth, which extracted by ethanol, were examined [60]. Mature fruits exhibited higher α-glucosidase inhibitory activity than immature ones. Flavonoids were reported to be able to inactivate α-glucosidase [53, 56]. Myricetin, quercetin, kaempferol and luteolin could inhibit α-glucosidase [56]. Additionally, ferulic acid could also inhibit α-glucosidase at IC_{50} of 0.45 mM [116]. Base on our results, red sweet pepper contained high amounts of quercetin, luteolin and ferulic acid. Thus, this information might be the reason that red sweet pepper exhibited the highest α-glucosidase inhibitory activity.

The mechanism of α-glucosidase is similar to α-amylase activity (Figure 6.7), but α-glucosidase hydrolyses polysaccharide into glucose at terminal non-reducing α-1-4-glycosidic linkage [117]. Glu and Asp are catalytic residues in the active site of α-glucosidase. The interaction between enzyme and substrate has three steps. First, catalytic Glu gives the proton to glycosidic oxygen [117]. The glucose residue is release in this step. Second, the catalytic Asp forms bond with C1 of sugar residue. Third, the water molecule gives the proton to Glu and hydrolyses the bond between the sugar residue and the Asp residue.

Acarbose is a competitive inhibitor on α-glucosidase [118]. This inhibitor is coordinated with α-glucosidase at the active site. The mechanism of acarbose on α-glucosidase is same as α-amylase (Figure 6.8) [119]. Catalytic Glu and Asp form bond with the non reducing sugar moiety on acarbose. Glucose molecule is released by the hydrolysis of bond between Glu and sugar. Then, the transglycosylation occurs to form a longer acarbose. The nitrogen bond of acarbose structure that is in the

hydrolysis area cannot be cleaved by α-glucosidase. Thus, acarbose has an effective inhibitor to block the binding of enzyme and substrate.

The anti-α-glucosidase of sweet peppers extracted with 70% (v/v) aqueous ethanol possessed the potent IC_{50} value of 6.46 to 13.71 mg/mL (86.71 to 231.98 mg/mL fresh weight). Quercetin and luteolin that could be found in sweet pepper exhibited the IC_{50} value of 0.0021 and 0.0060 mg/mL, respectively [56]. Comparing to acarbose with the IC_{50} of 0.036 mg/mL [46], these compounds could be potentially used for diabetic control through α-glucosidase inhibition. Luteolin could act as non-competitive inhihitor on α-glucosidase [58]. The hydroxyl groups on A ring (Figure 6.5 A) formed hydrogen bound with Thr residue on α-glucosidase. The binding site between luteolin and α-glucosidase was close to the active site [58]. Thus, luteolin indirectly blocks the entrance of substrate. This matter could lead to delayed glucose absorption into blood system, therefore, control of diabetes.

6.2.4 ACE inhibitory activity

Many researches are focused on ACE inhibitors from natural product [68, 120, 121]. Most researches showed that plant extracts are a rich source of phytochemicals, which were effective ACE inhibitors. Prior study was investigated the properties of peppers (*C. annuum*) on the inhibition of ACE [10]. It was suggested that peppers in *C. annuum* species exhibited high ACE inhibitory activities (73-84 %inhibition). However, the information on peppers regarding its anti-ACE activity is limited. In this study, the comparisons of different colored sweet peppers and solvent extractions were studied. The results suggested that sweet pepper extracted with 70% (v/v) aqueous ethanol exhibited the highest ACE inhibitory activities, followed by ethyl acetate and hexane, respectively. The result related to total phenolic contents in our laboratory study, which showed that sweet pepper extracted under 70% (v/v) aqueous ethanol had the highest total phenolic compounds. Besides, the result was consistent to previous study, which demonstrated that the ACE inhibitory activity had a relation with total phenolic contents [11]. Phenolic compounds were found to be an effective natural inhibitor for ACE [68]. Flavonoids were studied for their ability as ACE inhibitors [122]. The ACE inhibitory activities of 17 flavonoids were investigated [122]. The results suggested that flavonoids were a good source of anti-

hypertention through ACE inhibition. Luteolin had the highest ACE inhibitory activity. Base on our results, sweet peppers also consisted of quercetin and luteolin as main flavonoids. These compounds have polar structures (Figure 6.1 C-D). Thus, hydrophilic solvents are required to extract these compounds. Furthermore, previous study also suggested that the maximum contents of flavonoids were extracted in methanol, followed by ethyl acetate and not detected in hexane [20]. This matter could cause sweet peppers extracted with 70% (v/v) aqueous ethanol exhibited the highest ACE inhibitory activity.

In this study, four colored sweet peppers showed the different inhibitory activities. This result might be due to different contents of bioactive compounds in four colored sweet peppers. Some bioactive compounds that found in pepper were reported to inhibit ACE activities [122]. The result showed that red sweet pepper extracts with 70% (v/v) aqueous ethanol exhibited the highest ACE inhibitory activitiy. Similarly, under ethyl acetate red and green sweet peppers also exhibited the highest ACE inhibitory activities. In addition, our laboratory results suggested that total phenolic compounds of green and red sweet peppers extracts with 70% (v/v) aqueous ethanol and ethyl acetate were higher than orange and yellow sweet pepper. Therefore, ACE inhibitory activity was corresponded to total phenolic compounds. Besides, quercetin, luteolin, ferulic acid and *p*-coumaric acid could act against ACE activity [123, 124]. Thus, red and green sweet peppers exhibited the highest anti-ACE activity might be due to quercetin, luteolin and ferulic acid, which could act as ACE inhibitors.

Angiotensin-converting enzyme hydrolyzes angiotensin I into angiotensin II through the binding between angiotensin and the zinc atom in the active site of enzyme (Figure 6.9). Zinc ion is coordinated with two His, one Gln and water molecule [125]. In addition, the active site has several His residues. These residues can stabilize substrate and enzyme by hydrogen bounds. Water molecule serves the proton to Glu residue. This proton will move to the amine due to the cleavage of C-N bond. Then, angiotensin II is released.

Captopril is a drug that could act as the competitive inhibitor [126]. The sulfhydryl group of captopril binds directly with zinc atom at the active site of ACE. In addition, the carboxyl group on Pro moiety is bind with three residues, Gln, Lys and

Try, through hydrogen bond. This matter leads to the prevention of enzyme-substrate binding complex (Figure 6.10) [127].

Sweet peppers extracted with 70% (v/v) aqueous ethanol exhibited the most potent anti-ACE activity with the IC_{50} of 2.55 to 5.00 mg/mL (34.23 to 71.23 mg/mL fresh weight). Quercetin and luteolin exhibited the IC_{50} of 0.013 and 0.0065 mg/mL [122]. These flavonoids were the strong ACE inhibitors, which could be found in sweet peppers. Comparing to captopril, the anti-hypertensive drug with the IC_{50} of 0.000004 mg/mL [122], these compounds could be used for hypertensive treatment, and consumption of sweet pepper could possibly act against hypertension. Flavonoids were reported to be the competitive inhibitors, which generated the chelated complex within the active site of ACE [68, 128]. The hydroxyl groups of flavonoids probably bound with zinc ion [129]. This matter could restrain AngI transforming into AngII, leading to the decrease of aldosterone that causes blood vessels to shrink.

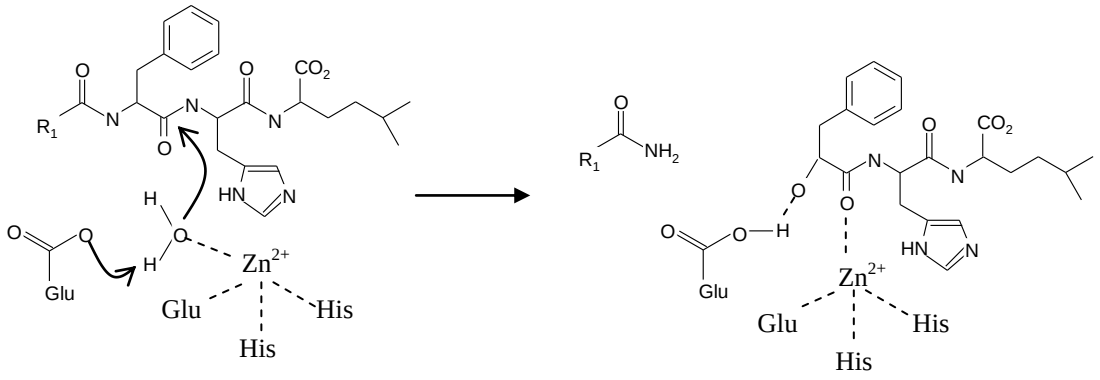

Figure 6.9 The mechanism of ACE Angiotensin I bind to zinc atom in the active site of ACE. Water molecule serves the proton to Glu residue. This proton will move to the amine due to the cleavage of C-N bond. Then, angiotensin II is released.

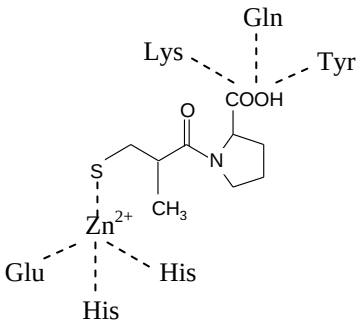

Figure 6.10 The mechanism of captopril The sulfhydryl group of captopril binds with zinc atom, leading to the prevention of enzyme-substrate binding complex.