

## CHAPTER V

### RESULTS

#### 5.1 Phytochemicals of sweet peppers

All four colored sweet pepper extracts were investigated regarding their phytochemicals (flavonoids, phenolic acids, carotenoids and volatile compounds), which might be varied according to colored fruits. Flavonoids, phenolic acids and carotenoids were detected by HPLC analysis, while volatile compounds were detected by GC-MS analysis.

##### 5.1.1 Flavonoids and phenolic acids

Four colored sweet peppers contained different content of flavonoids, including quercetin (72-102  $\mu\text{g/g}$  dry weight) and luteolin (56-96  $\mu\text{g/g}$  dry weight) (Table 5.1). Among the four sweet peppers, yellow sweet pepper was found the significantly highest concentrations of quercetin (102.33  $\mu\text{g/g}$  dry weight), while the rest contained insignificant lower amount. Likewise, luteolin content (95.89  $\mu\text{g/g}$  dry weight) was found to be the highest in yellow sweet pepper, followed by those in red sweet pepper, green sweet pepper and orange sweet pepper, respectively. These data were led to the highest total flavonoids content detected in yellow sweet pepper, followed by red sweet pepper, orange sweet pepper and green sweet pepper, respectively.

Similarly, sweet peppers contained phenolic acids (*p*-coumaric acid (10-20  $\mu\text{g/g}$  dry weight) and ferulic acid (13-28  $\mu\text{g/g}$  dry weight)), depending on colored fruits of the sweet peppers (Table 5.1). Green sweet pepper was found to possess the highest content of *p*-coumaric acid (19.62  $\mu\text{g/g}$  dry weight), followed by yellow sweet pepper, red sweet pepper and orange sweet pepper, respectively. On the contrary, red sweet pepper was found to possess the highest quantity of ferulic acid (27.67  $\mu\text{g/g}$  dry weight), followed by yellow sweet pepper, green sweet pepper and orange sweet pepper, respectively. These results could partially lead to the quantity of total phenolic

acids being detected in these different colored sweet peppers. The results suggested that green sweet pepper possessed the highest total phenolic acids, followed by those in red sweet pepper, yellow sweet pepper and orange sweet pepper, respectively.

### 5.1.2 Carotenoids

Carotenoids consisting of capsanthin, lutein, zeaxanthin,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene, *trans*- $\beta$ -carotene and *cis*- $\beta$ -carotene were observed in four colored sweet peppers (Table 5.2). The present data showed that only capsanthin (16-178  $\mu\text{g/g}$  dry weight), *trans*- $\beta$ -carotene (8-42  $\mu\text{g/g}$  dry weight) and *cis*- $\beta$ -carotene (7-34  $\mu\text{g/g}$  dry weight) were found in all four sweet peppers. Lutein (45-115  $\mu\text{g/g}$  dry weight), zeaxanthin (71-192  $\mu\text{g/g}$  dry weight),  $\beta$ -cryptoxanthin (8-40  $\mu\text{g/g}$  dry weight) and  $\alpha$ -carotene (4-9  $\mu\text{g/g}$  dry weight) were only detected in particular sweet peppers.

Capsanthin, *trans*- $\beta$ -carotene and *cis*- $\beta$ -carotene contents in different colored sweet pepper were found in similar trend, in which red sweet pepper significantly contained the highest concentration of these carotenoids (178.20  $\mu\text{g/g}$  dry weight of capsanthin, 41.72  $\mu\text{g/g}$  dry weight of *trans*- $\beta$ -carotene and 34.28  $\mu\text{g/g}$  dry weight of *cis*- $\beta$ -carotene). Capsanthin was significantly decreased in orange sweet pepper, yellow sweet peppers and green sweet peppers, respectively, while *trans*- $\beta$ -carotene and *cis*- $\beta$ -carotene were lowest in yellow sweet pepper. Likewise, the highest quantity of  $\beta$ -cryptoxanthin (40.49  $\mu\text{g/g}$  dry weight) was detected in red sweet pepper, followed by orange sweet pepper and yellow sweet pepper, respectively. However, none was detected in green sweet pepper. On the contrary, yellow sweet pepper provided the significantly highest lutein (115.16  $\mu\text{g/g}$  dry weight), followed by green sweet pepper and orange sweet pepper, respectively, while none was detected in red sweet pepper. Zeaxanthin was only detected in orange sweet pepper (191.8  $\mu\text{g/g}$  dry weight) and red sweet pepper (70.7  $\mu\text{g/g}$  dry weight), while the rest were not measurable. Trace amount of  $\alpha$ -carotene (3.5-9.0  $\mu\text{g/g}$  dry weight) was detected in orange sweet pepper, yellow sweet pepper and green sweet pepper. None was detected in red sweet pepper. As for total carotenoids, it was suggested that red sweet pepper contained the highest content of total carotenoids (365.40  $\mu\text{g/g}$  dry weight), followed by those in orange sweet pepper, yellow sweet pepper and green sweet pepper, respectively.

**Table 5.1** Flavonoids and phenolic acids contents of four colored sweet peppers ( $\mu\text{g/g}$  dry weight)

Types of sweet pepper	Flavonoids		Total flavonoids	Phenolic acids		Total phenolic acids
	Quercetin	Luteolin		<i>p</i> -Coumaric acid	Ferulic acid	
Green	71.71 $\pm$ 1.57 <sup>c</sup>	62.31 $\pm$ 5.02 <sup>b</sup>	137.02 $\pm$ 6.59 <sup>d</sup>	19.62 $\pm$ 0.68 <sup>a</sup>	23.59 $\pm$ 0.01 <sup>c</sup>	43.21 $\pm$ 0.69 <sup>a</sup>
Red	91.98 $\pm$ 2.05 <sup>b</sup>	68.43 $\pm$ 0.98 <sup>b</sup>	160.41 $\pm$ 3.03 <sup>b</sup>	9.96 $\pm$ 0.08 <sup>bc</sup>	27.67 $\pm$ 0.13 <sup>a</sup>	37.63 $\pm$ 0.21 <sup>b</sup>
Orange	92.00 $\pm$ 0.64 <sup>b</sup>	56.34 $\pm$ 0.65 <sup>c</sup>	148.33 $\pm$ 1.29 <sup>c</sup>	9.53 $\pm$ 0.25 <sup>c</sup>	13.45 $\pm$ 0.05 <sup>d</sup>	22.98 $\pm$ 0.30 <sup>d</sup>
Yellow	102.33 $\pm$ 1.95 <sup>a</sup>	95.89 $\pm$ 2.19 <sup>a</sup>	198.22 $\pm$ 4.14 <sup>a</sup>	10.67 $\pm$ 0.20 <sup>b</sup>	24.75 $\pm$ 0.15 <sup>b</sup>	35.42 $\pm$ 0.35 <sup>c</sup>

All data were expressed by mean values  $\pm$  standard deviation.

The different letters <sup>a-d</sup> within same column showed the mean significantly difference at  $p$  value  $< 0.05$  using one-way ANOVA followed by Tukey's *b post hoc* test.

**Table 5.2** Carotenoid contents of four colored sweet peppers ( $\mu\text{g/g}$  dry weight)

Types of sweet pepper	Capsanthin	Lutein	Zeaxanthin	$\beta$ -Cryptoxanthin	$\alpha$ -Carotene	<i>trans</i> - $\beta$ -Carotene	<i>cis</i> - $\beta$ -Carotene	Total carotenoids
Green	16.13 $\pm$ 1.30 <sup>d</sup>	60.04 $\pm$ 5.63 <sup>b</sup>	ND	ND	3.56 $\pm$ 0.23 <sup>c</sup>	13.09 $\pm$ 1.27 <sup>c</sup>	9.64 $\pm$ 0.73 <sup>b</sup>	102.48 $\pm$ 9.17 <sup>d</sup>
Red	178.20 $\pm$ 5.25 <sup>a</sup>	ND	70.71 $\pm$ 0.94 <sup>b</sup>	40.49 $\pm$ 1.66 <sup>a</sup>	ND	41.72 $\pm$ 2.17 <sup>a</sup>	34.28 $\pm$ 1.77 <sup>a</sup>	365.40 $\pm$ 11.78 <sup>a</sup>
Orange	45.48 $\pm$ 0.01 <sup>b</sup>	45.16 $\pm$ 0.58 <sup>c</sup>	191.76 $\pm$ 1.24 <sup>a</sup>	19.45 $\pm$ 0.19 <sup>b</sup>	9.02 $\pm$ 0.07 <sup>a</sup>	17.74 $\pm$ 1.86 <sup>b</sup>	8.32 $\pm$ 0.03 <sup>bc</sup>	336.95 $\pm$ 0.98 <sup>b</sup>
Yellow	22.46 $\pm$ 1.04 <sup>c</sup>	115.16 $\pm$ 8.57 <sup>a</sup>	ND	7.55 $\pm$ 0.53 <sup>c</sup>	4.22 $\pm$ 0.23 <sup>b</sup>	8.32 $\pm$ 0.41 <sup>d</sup>	6.81 $\pm$ 0.28 <sup>c</sup>	164.53 $\pm$ 10.83 <sup>c</sup>

All data were expressed by mean values  $\pm$  standard deviation.

The different letters <sup>a-d</sup> within same column showed the mean significantly difference at  $p$  value  $< 0.05$  using samples independent t-test and one-way ANOVA followed by Tukey's-b *post hoc* test. ND: not detected

### 5.1.3 Volatile compounds

Sweet pepper showed various types of volatile compounds. However, only 15 main compounds (from ~20-40 peaks of all detected volatile compounds) with high peak area (%) were selected (Table 5.3). Green and orange sweet peppers possessed higher quality of volatile compounds (9 compounds) than those of red sweet pepper (7 compounds) and yellow sweet pepper (3 compounds), respectively. The compounds were found in all four sweet peppers including copaene (1-3%) and n-nonyl-cyclopropane or 1-dodecanol (1-8%). Hexadecane (~1%) was also found in all sweet peppers in exception of yellow sweet pepper. The compound that only found in red and orange sweet pepper was heptadecane (~1%). In addition, the volatile compounds that only presented in green and red sweet peppers included 2-isobutyl-3-methoxypyrazine (1-4%), 2-mercapto-4-phenylthiazole (~1%), and thiocyanic acid carbazol-3,6-diyl ester (2-3%). For alloaromadendrene, it was detected in orange and yellow sweet peppers (1-2%). Besides, the volatile compounds that found only in green sweet pepper was benzhydryl alcohol, benzophenone and  $\beta$ -*cis*-ocimene, and those of orange fruits were isolongifolene, 9,10-dehydro-,  $\beta$ -elemene,  $\beta$ -selinene and  $\gamma$ -selinene. Totally, the volatile compounds of sweet pepper that showed in this study were approximately 10-30% peak area of all detected compounds, while other compounds were mainly from contaminants by instruments [84]. Heptane, for an example, was used to test the ability of instrument in each sample analysis.

**Table 5.3** Selected volatile compounds of four colored sweet peppers (% peak area)

Volatile compounds	Green	Red	Orange	Yellow
<i>Selected volatile compounds from sweet peppers</i>				
9,10-Dehydro-isolongifolene	ND	ND	3.64	ND
2-Isobutyl-3-methoxypyrazine	4.28	0.81	ND	ND
2-Mercapto-4-phenylthiazole	1.48	0.71	ND	ND
Alloaromadendrene	ND	ND	0.89	1.87
Benzhydryl alcohol	2.21	ND	ND	ND
Benzophenone	2.40	ND	ND	ND
Copaene	3.43	2.55	2.42	0.70
Heptadecane	ND	0.76	0.71	ND
Hexadecane	0.23	1.15	1.02	ND
n-Nonyl-cyclopropane / 1-Dodecanol	8.09	1.32	0.75	6.68
Thiocyanic acid carbazol-3,6-diyl ester	3.36	1.66	ND	ND
$\beta$ - <i>cis</i> -Ocimene	0.37	ND	ND	ND
$\beta$ -Elemene	ND	ND	2.20	ND
$\beta$ -Selinene	ND	ND	3.27	ND
$\gamma$ -Selinene	ND	ND	3.99	ND
<i>Total</i>	26.06	8.96	18.89	9.25
<i>Detected contaminants from using DVB/CAR/PDMS fiber and column [84]</i>				
Hexamethylcyclotrisiloxane	3.44	1.88	1.45	3.04
Octamethylcyclotetrasiloxane	2.55	3.39	2.98	3.33
Decamethylcyclopentasiloxane	1.23	2.20	1.59	2.20
Dodecamethylcyclohexasiloxane	1.53	0.92	0.91	0.75
Tetradecamethylcycloheptasiloxane	1.23	2.20	1.59	2.20
Oxime-, methoxy-phenyl-	20.29	26.04	4.34	17.27
Butylated hydroxytoluene (BHT)	0.57	ND	ND	ND
<i>Total</i>	30.84	36.63	12.86	28.79
<i>Others</i>	43.1	54.41	68.25	61.96

ND: not detected

## **5.2 Anti-Alzheimer's disease of sweet pepper extracts**

Freeze-dried samples of four colored sweet peppers (green, red, orange and yellow) without seeds were extracted by solvents with different polarities including hexane, ethyl acetate and 70% (v/v) aqueous ethanol that represented non-polar solvent, semi-polar solvent and polar solvent, respectively. The extracts were evaporated and re-dissolved in minimum volume of DMSO. The extracts were then analyzed regarding the biochemical properties against oxidative stress and AChE, BChE and BACE1 activities.

### **5.2.1 Total phenolic content and antioxidant activity**

This hypothesis was emphasized on TPC and antioxidant activity of different colored sweet peppers. The TPC of extracts were determined using Folin-Ciocalteu method, while antioxidant activity were measured using DPPH radical scavenging, FRAP and ORAC assays. As results, it was found that 70% (v/v) aqueous ethanolic extracts significantly provided the highest TPC and antioxidant activity among all solvents (TPC of 274-339 mg GAE/g dry weight, DPPH radical scavenging values of 1,863-2,515  $\mu\text{mol TE}/100\text{g dry weight}$ , FRAP values of 1,987-3,015  $\mu\text{mol TE}/100\text{g dry weight}$  and ORAC values of 5,112-6,197  $\mu\text{mol TE}/100\text{g dry weight}$ ). Ethyl acetate extracts (TPC of 55-123 mg GAE/g dry weight, DPPH radical scavenging values of 233-589  $\mu\text{mol TE}/100\text{g dry weight}$ , FRAP values of 410-887  $\mu\text{mol TE}/100\text{g dry weight}$  and ORAC values of 1,311-2,510  $\mu\text{mol TE}/100\text{g dry weight}$ ) and hexane extracts (TPC of 5-8 mg GAE/g dry weight, DPPH radical scavenging values of 33-70  $\mu\text{mol TE}/100\text{g dry weight}$ , FRAP values of 50-70  $\mu\text{mol TE}/100\text{g dry weight}$  and ORAC values of 125-142  $\mu\text{mol TE}/100\text{g dry weight}$ ) provided lower TPC and antioxidant activity (Table 5.4 and Figure E1).

Fruits colors of sweet pepper also significantly influenced on TPC and antioxidant activity (Table 5.4 and Figure E1). Red and orange sweet peppers extracted by hexane exhibited the highest quantity of TPC (7.3 and 7.5 mg GAE/g dry weight, respectively), followed by those of green sweet pepper and yellow sweet pepper, respectively. The level of antioxidant activity as being detected by DPPH radical scavenging and FRAP assays were also similar to TPC, in which red sweet pepper exhibited the highest activity among all colored sweet peppers (DPPH radical

scavenging value of 69.5  $\mu\text{mol TE}/100\text{g dry weight}$  and FRAP value of 69.8  $\mu\text{mol TE}/100\text{g dry weight}$ ). However, the results of ORAC assay suggested that antioxidant activity of red, orange and green sweet pepper extracts not significantly different (132-142  $\mu\text{mol TE}/100\text{g dry weight}$ ), while those of yellow sweet pepper extract provided significantly the lowest.

Under extraction with ethyl acetate, green and red sweet pepper extracts significantly exhibited the highest TPC and antioxidant activity as being detected by Folin-Ciocalteu (120.7 and 122.5 mg GAE/g dry weight, respectively), DPPH radical scavenging (589.4 and 565.5  $\mu\text{mol TE}/100\text{g dry weight}$ , respectively), and FRAP assays (886.6 and 874.6  $\mu\text{mol TE}/100\text{g dry weight}$ , respectively), followed by those of orange extract and yellow extract, respectively. ORAC assay suggested that green sweet pepper extract could significantly provide higher prevention of oxidative stress induction (2,510.4  $\mu\text{mol TE}/100\text{g dry weight}$ ) than red, orange and yellow sweet pepper extracts.

The results of TPC and antioxidant activity of ethanolic extracts were followed a similar trend as those extracted under ethyl acetate extracts. Green sweet pepper significantly possessed the highest TPC (339.4 mg GAE/g dry weight), followed by those of red sweet pepper, orange sweet pepper and yellow sweet pepper, respectively. The results of antioxidant activity were consistent to TPC, in which green sweet pepper extract significantly showed the highest antioxidant activity among all colored sweet peppers (FRAP value of 3,015.3  $\mu\text{mol TE}/100\text{g dry weight}$  and ORAC value of 6,196.5  $\mu\text{mol TE}/100\text{g dry weight}$ ).

**Table 5.4** Total phenolic content (TPC) and antioxidant activity of four colored sweet peppers extracted with hexane, ethyl acetate and 70% (v/v) aqueous ethanol were analyzed by Folin-Ciocalteu, DPPH radical scavenging, FRAP and ORAC assays

Types of sweet pepper extract	Folin-Ciocalteu assay (mg GAE/100g dry weight)		Antioxidant activity ( $\mu\text{mol TE}/100\text{g dry weight}$ )			
			DPPH assay	FRAP assay	ORAC assay	
<b>Hexane extracts</b>						
Green	6.1 $\pm$ 0.5 <sup>b</sup>		32.9 $\pm$ 2.7 <sup>c</sup>	51.6 $\pm$ 5.3 <sup>b</sup>	131.5 $\pm$ 10.1 <sup>a</sup>	
Red	7.3 $\pm$ 0.5 <sup>a</sup>		69.5 $\pm$ 6.1 <sup>a</sup>	69.8 $\pm$ 6.1 <sup>a</sup>	142.4 $\pm$ 7.4 <sup>a</sup>	
Orange	7.5 $\pm$ 0.1 <sup>a</sup>		64.2 $\pm$ 3.8 <sup>b</sup>	49.8 $\pm$ 1.1 <sup>b</sup>	140.2 $\pm$ 6.1 <sup>a</sup>	
Yellow	5.1 $\pm$ 0.3 <sup>c</sup>		27.4 $\pm$ 2.1 <sup>d</sup>	49.6 $\pm$ 3.3 <sup>b</sup>	124.7 $\pm$ 7.5 <sup>b</sup>	
<b>Ethyl acetate extracts</b>						
Green	120.7 $\pm$ 0.9 <sup>a</sup>		589.4 $\pm$ 36.5 <sup>a</sup>	886.6 $\pm$ 23.9 <sup>a</sup>	2,510.4 $\pm$ 142.0 <sup>a</sup>	
Red	122.5 $\pm$ 4.0 <sup>a</sup>		565.5 $\pm$ 19.5 <sup>a</sup>	874.6 $\pm$ 38.9 <sup>a</sup>	2,271.5 $\pm$ 47.6 <sup>b</sup>	
Orange	62.2 $\pm$ 5.3 <sup>b</sup>		305.9 $\pm$ 15.3 <sup>b</sup>	465.6 $\pm$ 30.9 <sup>b</sup>	1,549.9 $\pm$ 122.5 <sup>c</sup>	
Yellow	54.5 $\pm$ 5.3 <sup>c</sup>		232.9 $\pm$ 12.8 <sup>c</sup>	410.3 $\pm$ 36.3 <sup>c</sup>	1,310.9 $\pm$ 60.7 <sup>d</sup>	
<b>70% (v/v) aqueous ethanol extracts</b>						
Green	339.4 $\pm$ 2.2 <sup>a</sup>		2,515.1 $\pm$ 103.7 <sup>a</sup>	3,015.3 $\pm$ 32.0 <sup>a</sup>	6,196.5 $\pm$ 167.2 <sup>a</sup>	
Red	321.1 $\pm$ 22.4 <sup>b</sup>		2,379.1 $\pm$ 180.3 <sup>a</sup>	2,812.2 $\pm$ 26.9 <sup>b</sup>	5,482.1 $\pm$ 178.0 <sup>b</sup>	
Orange	306.3 $\pm$ 10.1 <sup>b</sup>		2,220.9 $\pm$ 97.3 <sup>b</sup>	2,520.6 $\pm$ 45.8 <sup>c</sup>	5,112.3 $\pm$ 254.6 <sup>c</sup>	
Yellow	273.5 $\pm$ 6.3 <sup>c</sup>		1,863.2 $\pm$ 48.2 <sup>c</sup>	1,987.3 $\pm$ 44.6 <sup>d</sup>	5,165.1 $\pm$ 154.1 <sup>c</sup>	

All data were expressed by mean values  $\pm$  standard deviation.

The different letters including <sup>a-d</sup> within same solvent extraction condition showed the mean significantly difference at  $p$  value  $< 0.05$  using one-way ANOVA followed by Tukey's-b *post hoc* test.  
TE: trolox equivalent, GAE: gallic acid equivalent

### 5.2.2 Cholinesterase inhibitory activities

The results suggested that four colored sweet pepper extracted with all three solvents (hexane, ethyl acetate and 70% (v/v) aqueous ethanol) at final concentration of 30.56 g dry weight/L or 305.6 g fresh weight/L (moisture content of fresh sweet pepper of ~90%) could inhibit AChE activity (Table 5.5 and Figure F1A). Among all three solvent extracts, ethyl acetate extracts significantly showed the highest anti-AChE activity (22-92% inhibition) in exception of red sweet pepper. It was found that anti-AChE activity of red sweet pepper extracted with hexane did not significantly differ from that of ethyl acetate. The extracts of 70% (v/v) aqueous ethanol significantly possessed the lowest AChE inhibitory activity (12-58% inhibition) in exception of orange sweet pepper. The extracts of orange sweet pepper with 70% (v/v) aqueous ethanol and hexane were not significantly different.

Each colored sweet pepper provided different levels of AChE inhibitory activity. Yellow sweet pepper extracted with all three solvents significantly exhibited the most effective toward AChE inhibitory activity (58-92% inhibition) and the highest inhibitory activity was found in ethyl acetate extract (91.69% inhibition). Besides, green sweet pepper significantly exhibited less anti-AChE activity (40-52% inhibition) than those of yellow sweet pepper, followed by red sweet pepper (17-25% inhibition) and orange sweet pepper (12-22% inhibition), respectively. However, anti-AChE activity levels of red and orange sweet pepper extracted with ethyl acetate were not significantly different.

The  $IC_{50}$  values for inhibiting AChE activity from the sweet peppers extracted in all three solvents in a range of 5-118 g dry weight/L, which their trends exhibited similar as those of percentage of inhibition. The significantly highest activity was found in yellow sweet pepper, in which the lowest  $IC_{50}$  values were detected in the range of 5-13 g dry weight/L. Yellow sweet pepper extracted under hexane and ethyl acetate possessed the highest inhibitory activity (5.17 and 5.86 g dry weight/L, respectively). Nevertheless, the  $IC_{50}$  values of red and orange sweet peppers extracted with 70% (v/v) aqueous ethanol could not be determined, since these sweet peppers possessed trace inhibitory activity (17.21 and 12.21 % inhibition, respectively). Too high concentration of extract could interfere with the colorimetric analysis due to its rich color pigments.

Cholinesterase enzymes in cholinergic hypothesis were not only included AChE but also BChE. As results, sweet peppers extracted with three solvents at the concentration of 30.56 g dry weight/L could inhibit BChE activity (Table 5.5 and Figure F1B). The sweet peppers extracted under 70% (v/v) aqueous ethanol significantly possessed the highest anti-BChE activity (25-32% inhibition), the different inhibition trend than those of anti-AChE results. However, hexane and ethyl acetate extracts in this case exhibited only trace activity (6-10% inhibition and 7-22% inhibition, respectively).

Green sweet pepper extracted in all three solvents provided the highest inhibitory activity (10-32% inhibition). In 70% (v/v) aqueous ethanolic extraction, green sweet pepper exhibited slightly higher anti-BChE activity than that of red sweet pepper (30-32% inhibition), followed by those of yellow sweet pepper and orange sweet pepper, respectively. In hexane extraction, it was found that green sweet pepper exhibited the highest anti-BChE activity (10.22% inhibition), while the rest could only inhibit trace BChE activity with no significant difference. Similar results were observed in ethyl acetate extraction, in which green sweet pepper provided the highest BChE inhibitory activity (22.25% inhibition), while orange sweet pepper exhibited the lowest activity.

The  $IC_{50}$  values were in same trend as percentage of inhibition, in which 70% (v/v) aqueous ethanol could provide higher extraction of anti-BChE agents from sweet peppers among all three solvents ( $IC_{50}$  values of 63-82 g dry weight/L). It was found that green sweet pepper extracted with 70% (v/v) aqueous ethanol provided the lowest  $IC_{50}$  value at 62.64 g dry weight/L, followed by red sweet pepper, yellow sweet pepper and orange sweet pepper, respectively. When comparing to the only available  $IC_{50}$  of green sweet pepper, it was found that green sweet pepper extracted with aqueous ethanol could inhibit BChE more efficiently (lower  $IC_{50}$ ) than ethyl acetate extract. Nevertheless, ethyl acetate extraction from other sweet peppers and hexane extraction of all sweet peppers were incapable to analyze  $IC_{50}$  values, due to low inhibitory activity (6-11% inhibition).

In addition, sweet pepper extracts could inhibit AChE and BChE activities in a dose dependent manner. For example, yellow sweet pepper extracted with hexane at concentration of 2-23 g/L exhibited AChE inhibitory activity in a range of 35-91%

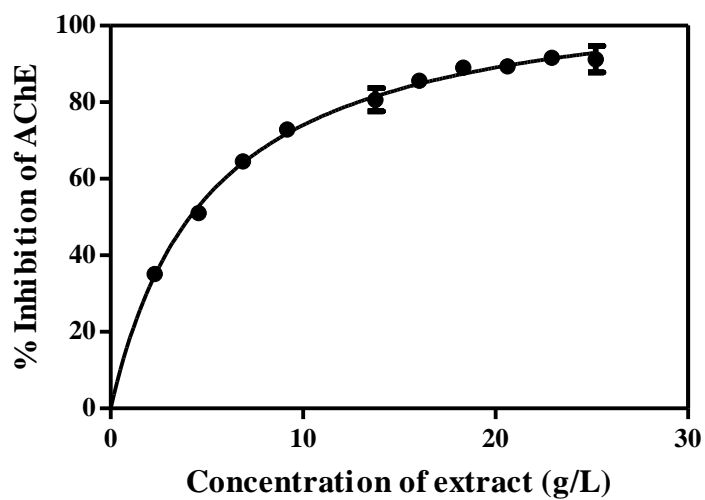
inhibition, respectively (Figure 5.1A). As well, the increasing concentration at 9-61 g/L of green sweet pepper extracted with 70% (v/v) aqueous ethanol led to increase BChE inhibitory activity in a range of 14-53%, respectively (Figure 5.1B). It indicated that if concentration of sweet pepper extract was increased, the ability of inhibitory activity also increased. However, the linear relationship between the concentration of extract and the inhibition was only observed at low concentration of the extract. However, the increase in inhibition was continuously decreased with higher concentration of the extract.

**Table 5.5** AChE and BChE inhibitory activities of four colored sweet peppers extracted with hexane, ethyl acetate and 70% (v/v) aqueous ethanol

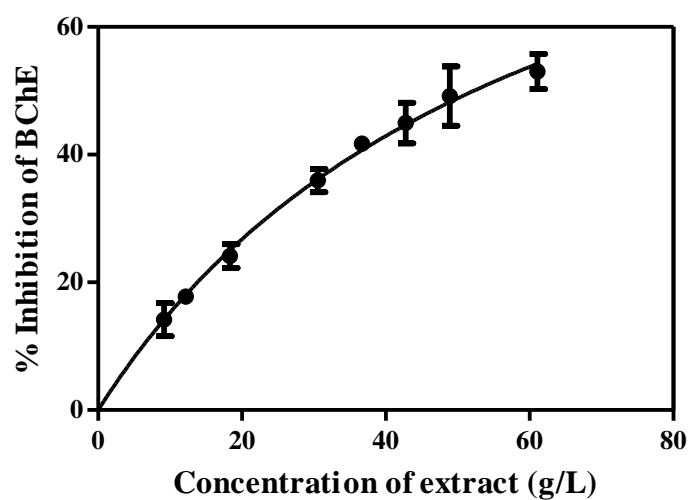
Types of sweet pepper extract	AChE		BChE	
	% Inhibition	IC <sub>50</sub> (g dry weight/L)	% Inhibition	IC <sub>50</sub> (g dry weight/L)
<i>Hexane extracts</i>				
Green	43.55 ± 3.62 <sup>b,y</sup>	52.90 ± 5.07 <sup>b,y</sup>	10.22 ± 1.22 <sup>a,z</sup>	NA
Red	25.38 ± 0.15 <sup>c,x</sup>	106.40 ± 5.09 <sup>c,y</sup>	6.35 ± 0.66 <sup>b,z</sup>	NA
Orange	14.06 ± 1.04 <sup>d,y</sup>	116.70 ± 3.69 <sup>d,x</sup>	6.25 ± 0.83 <sup>b,y</sup>	NA
Yellow	84.84 ± 3.19 <sup>a,y</sup>	5.17 ± 0.26 <sup>a,x</sup>	6.79 ± 0.78 <sup>b,z</sup>	NA
<i>Ethyl acetate extracts</i>				
Green	51.58 ± 2.22 <sup>b,x</sup>	37.69 ± 2.62 <sup>b,x</sup>	22.25 ± 1.54 <sup>a,y</sup>	85.34 ± 1.17 <sup>y</sup>
Red	24.75 ± 1.04 <sup>c,x</sup>	77.63 ± 0.99 <sup>c,x</sup>	10.91 ± 0.80 <sup>b,y</sup>	NA
Orange	22.25 ± 1.64 <sup>c,x</sup>	117.95 ± 0.64 <sup>d,x</sup>	7.45 ± 0.63 <sup>c,y</sup>	NA
Yellow	91.69 ± 4.71 <sup>a,x</sup>	5.86 ± 0.27 <sup>a,y</sup>	10.69 ± 0.78 <sup>b,y</sup>	NA
<i>70% (v/v) aqueous ethanol extracts</i>				
Green	39.00 ± 3.75 <sup>b,z</sup>	59.35 ± 2.62 <sup>b,z</sup>	32.25 ± 2.55 <sup>a,x</sup>	62.64 ± 2.13 <sup>a,x</sup>
Red	17.21 ± 1.29 <sup>c,y</sup>	NA	29.79 ± 2.53 <sup>ab,x</sup>	69.95 ± 3.90 <sup>b</sup>
Orange	12.21 ± 0.83 <sup>d,y</sup>	NA	24.50 ± 1.99 <sup>c,x</sup>	81.53 ± 3.20 <sup>c</sup>
Yellow	57.52 ± 3.02 <sup>a,z</sup>	13.38 ± 0.42 <sup>a,z</sup>	28.63 ± 2.31 <sup>b,x</sup>	79.48 ± 2.03 <sup>c</sup>

All data were expressed by mean values ± standard deviation. The different letters including <sup>a-d</sup> and <sup>x-z</sup> within same solvent extraction condition and same sweet pepper color, respectively, showed the mean significantly difference at *p* value < 0.05 using samples independent t-test and one-way ANOVA followed by Tukey's *post hoc* test. Final concentrations of all sweet pepper extracts were 30.56 g dry weight/L.

(A)



(B)



**Figure 5.1 Cholinesterase inhibitory activities of sweet pepper extracts in dose dependent manner.** (A) AChE and (B) BChE inhibitory activities of yellow sweet pepper extracted with hexane and green sweet pepper extracted with 70% (v/v) aqueous ethanol, respectively.

### 5.2.3 $\beta$ -secretase inhibitory activity

The final analysis for anti-AD property in this study is anti- $\beta$ -secretase1 (anti-BACE1) analysis, which involved  $\beta$ -amyloid formation. The activity against BACE1 of different colored sweet peppers extracted under different solvents (Table 5.6 and Figure F1C) suggested that sweet pepper with the concentration of 30.56 g dry weight/L could highly inhibit BACE1 up to ~90% inhibition. Sweet peppers extracted under hexane provided the highest BACE1 inhibitory activity (56-88% inhibition), followed by those under ethyl acetate (60-75% inhibition) and 70% (v/v) aqueous ethanol (42-59% inhibition), respectively.

For hexane extraction, green sweet pepper significantly provided the highest anti-BACE1 activity (88.30% inhibition), followed by red and yellow sweet pepper extracts. Orange sweet pepper, on the other hand, exhibited the lowest BACE1 inhibitory activity. Likewise, green sweet pepper extracted under 70% (v/v) aqueous ethanol exhibited the highest BACE1 inhibitory activity among all colored sweet peppers. Similar trend was also observed in sweet pepper extracted under ethyl acetate, in which orange sweet pepper significantly showed less inhibitions than green, red and yellow sweet pepper extracts.

**Table 5.6** BACE1 inhibitory activity of four colored sweet peppers extracted with hexane, ethyl acetate and 70% (v/v) aqueous ethanol

Types of sweet pepper extract	% Inhibition of BACE1
<i>Hexane extracts</i>	
Green	88.30 ± 1.82 <sup>a,x</sup>
Red	72.37 ± 4.15 <sup>b,x</sup>
Orange	56.33 ± 4.42 <sup>c,x</sup>
Yellow	66.99 ± 2.81 <sup>b,x</sup>
<i>Ethyl acetate extracts</i>	
Green	74.83 ± 4.08 <sup>a,y</sup>
Red	70.88 ± 4.68 <sup>a,x</sup>
Orange	60.18 ± 6.61 <sup>b,x</sup>
Yellow	69.58 ± 3.84 <sup>a,x</sup>
<i>70% (v/v) aqueous ethanol extracts</i>	
Green	58.73 ± 3.72 <sup>a,z</sup>
Red	41.52 ± 4.48 <sup>b,y</sup>
Orange	39.18 ± 0.79 <sup>b,y</sup>
Yellow	43.13 ± 2.77 <sup>b,y</sup>

All data were expressed by mean values ± standard deviation.

The different letters including <sup>a-d</sup> and <sup>x-z</sup> within same solvent extraction condition and same sweet pepper color, respectively, showed the mean significantly difference at *p* value < 0.05 using one-way ANOVA followed by Tukey's-b *post hoc* test.

Final concentrations of all sweet pepper extracts were 30.56 g dry weight/L.