APPENDICES

APPENDIX A
HPLC CHROMATOGRAMS OF FLAVONOIDS AND PHENOLIC ACIDS

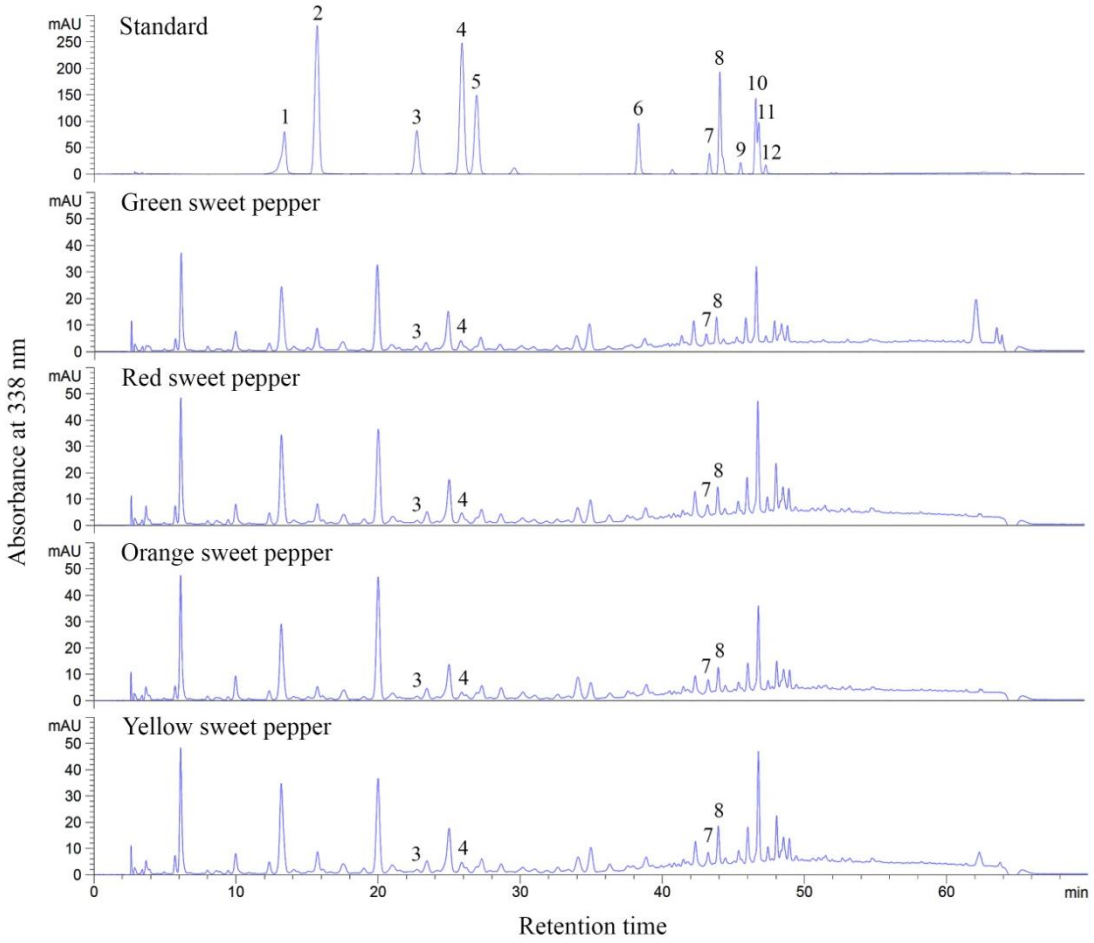


Figure A1 HPLC chromatograms of flavonoids and phenolic acids. Flavonoids and phenolic acids of four color sweet peppers were determined using HPLC analysis at 338 nm. Peaks are labeled as follows: 1: chlorogenic acid, 2: caffeic acid, 3: *p*-coumaric acid, 4: ferulic acid, 5: sinapic acid, 6: myricetin, 7: quercetin, 8: luteolin, 9: hesperetin, 10: kaempferol, 11: apigenin and 12: isorhamnetin.

APPENDIX B
HPLC CHROMATOGRAMS OF CAROTENOIDS

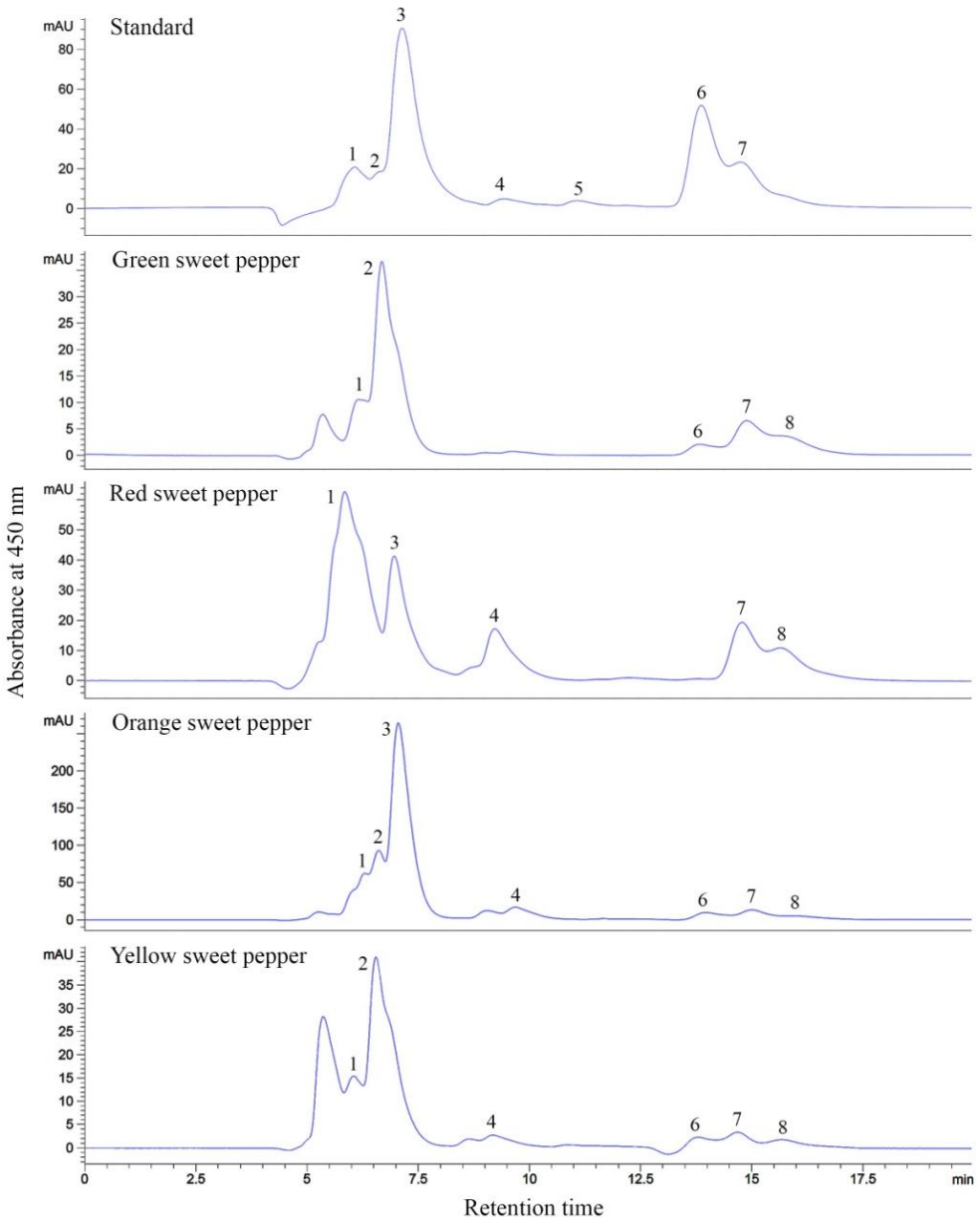


Figure B1 HPLC chromatograms of carotenoids. Carotenoids of four color sweet peppers were determined using HPLC analysis at 450 nm. Peaks are labeled as follows: 1: capsanthin, 2: lutein, 3: zeaxanthin, 4: β-cryptoxanthin, 5: lycopene, 6: α-carotene, 7: *trans*-β-carotene and 8: *cis*-β-carotene.

APPENDIX C
GC-MS CHROMATOGRAMS OF VOLATILE COMPOUNDS

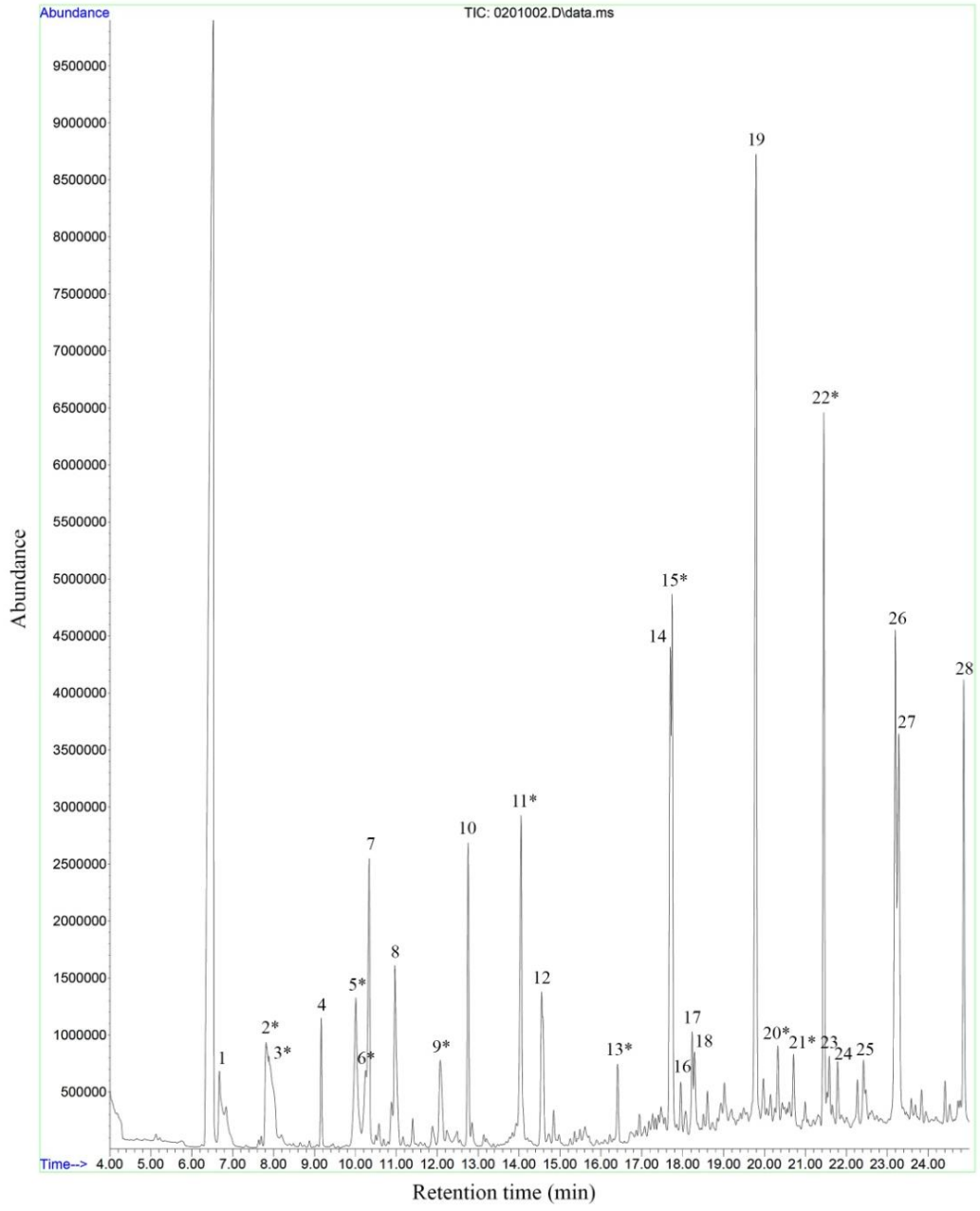


Figure C1 GC-MS chromatogram of volatile compounds from green sweet pepper. Peaks are labeled as Table C1. *Detected contaminants from using DVB/CAR/PDMS fiber and column

Table C1 Volatile compounds of green sweet pepper

Peak no.	Retention time (min)	Volatile compounds
1	6.679	1-Anthracenamine
2*	7.818	Octamethylcyclotetrasiloxane
3*	7.886	Octamethylcyclotetrasiloxane
4	9.168	β-*cis*-Ocimene
5*	10.009	Hexamethylcyclotrisiloxane
6*	10.250	Hexamethylcyclotrisiloxane
7	10.341	*p*-Phenylenebis(trimethylsilane)
8	10.971	2-(4-Nitrophenyl)-quinazolin-4(3H)-one / 5-Ethyl-3-(3-methyl-5-phenylpyrazol-1-yl)-1,2,4-triazol-4-amine
9*	12.075	Decamethylcyclopentasiloxane
10	12.756	2-Isobutyl-3-methoxypyrazine
11*	14.055	Octamethylcyclotetrasiloxane
12	14.553	6,7-Dimethoxy-2-(4-methoxyphenyl)-4-propyl-4H-3,1-benzoxazine / Octadecane-12-on-1-ol, TMS
13*	16.412	Dodecamethylcyclohexasiloxane
14	17.700	Copaene
15*	17.740	Decamethylcyclopentasiloxane
16	17.951	1-Cyclohexyldimethylsilyloxyoctade cane
17	18.232	Bis(pentamethylphenyl)-phosphine oxide / 1-Propylpentachlorotriphosphazene
18	18.289	1-Cyclohexyldimethylsilyloxyoctadecane
19	19.794	n-Nonyl-cyclopropane / 1-Dodecanol
20*	20.326	Tetradecamethylcycloheptasiloxane
21*	20.709	Butylated hydroxytoluene (BHT)
22*	21.448	Dodecamethylcyclohexasiloxane
23	21.585	7-Chloro-2,3-dihydro-3-(4-N,N-dimethylaminobenzylidene)-5-phenyl-1H-1,4-benzodiazepin-2-one
24	21.785	5α-Cholestan-2-one, oxime
25	22.420	Hexadecane
26	23.198	Benzophenone
27	23.284	Benzhydryl alcohol
28	24.869	Thiocyanic acid carbazol-3,6-diyl ester

*Detected contaminants from using DVB/CAR/PDMS fiber and column

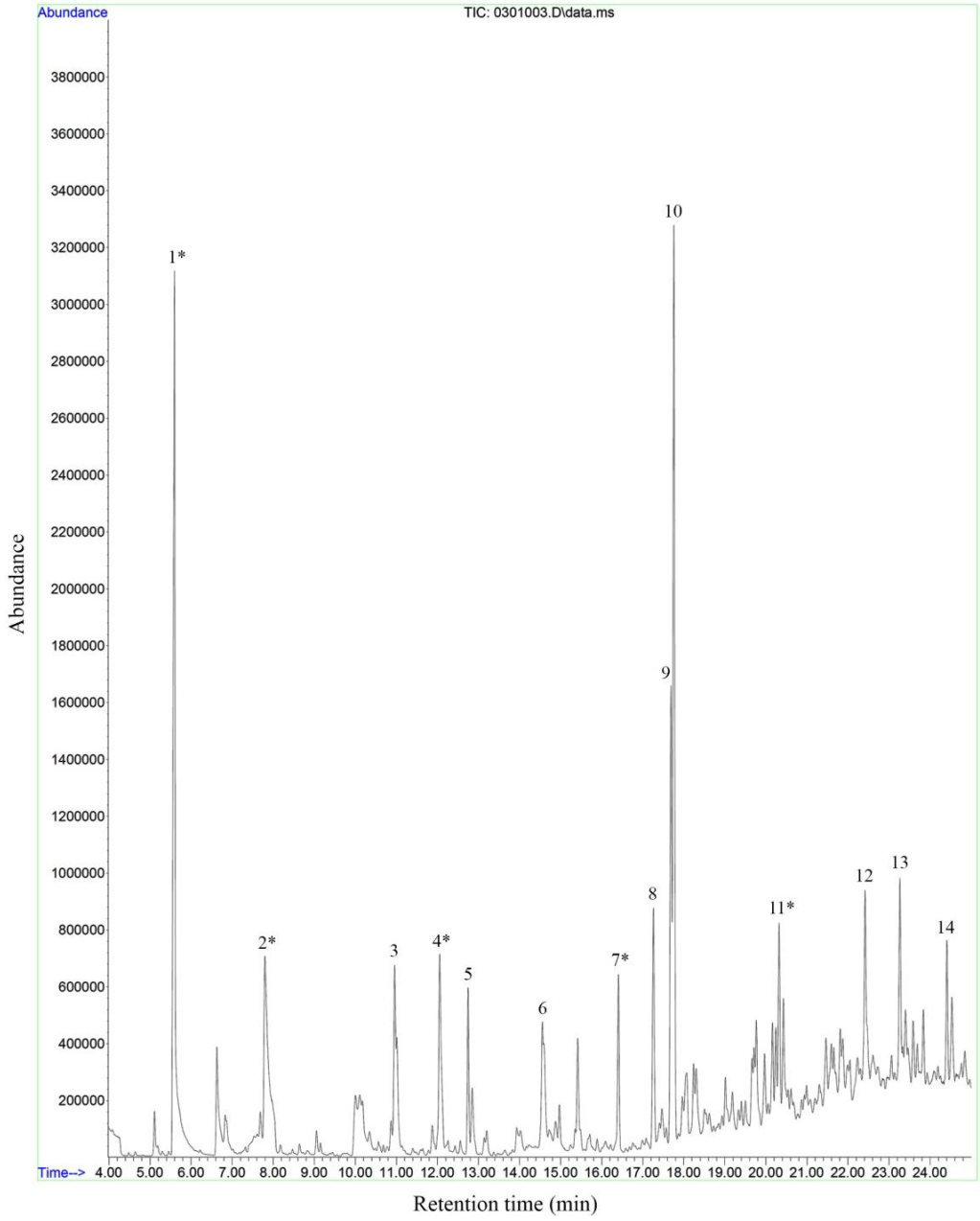


Figure C2 GC-MS chromatogram of volatile compounds from red sweet pepper. Peaks are labeled as Table C2. *Detected contaminants from using DVB/CAR/PDMS fiber and column

Table C2 Volatile compounds of red sweet pepper

Peak no.	Retention time (min)	Volatile compounds
1*	5.598	Oxime-, methoxy-phenyl-
2*	7.800	Octamethylcyclotetrasiloxane
3	10.959	Ethyl 2-trimethylsilyloxy-2-(3-trimethylsilyloxyphenyl)acetate
4*	12.058	Decamethylcyclopentasiloxane
5	12.750	2-Isobutyl-3-methoxypyrazine
6	14.558	2-Chloro-4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidine
7*	16.412	Dodecamethylcyclohexasiloxane
8	17.265	[(3,5,6-Trichloro-2-pyridinyl)oxy]acetic acid
9	17.688	Copaene
10	17.762	4-Bromo-2-chlorobenzenamine
11*	20.326	Tetradecamethylcycloheptasiloxane
12	22.420	Hexadecane
13	23.267	1,2,3,4,4a,5,8,9,12,12a-decahydro-1,4-methanobenzocyclodecene
14	24.411	Heptadecane

*Detected contaminants from using DVB/CAR/PDMS fiber and column

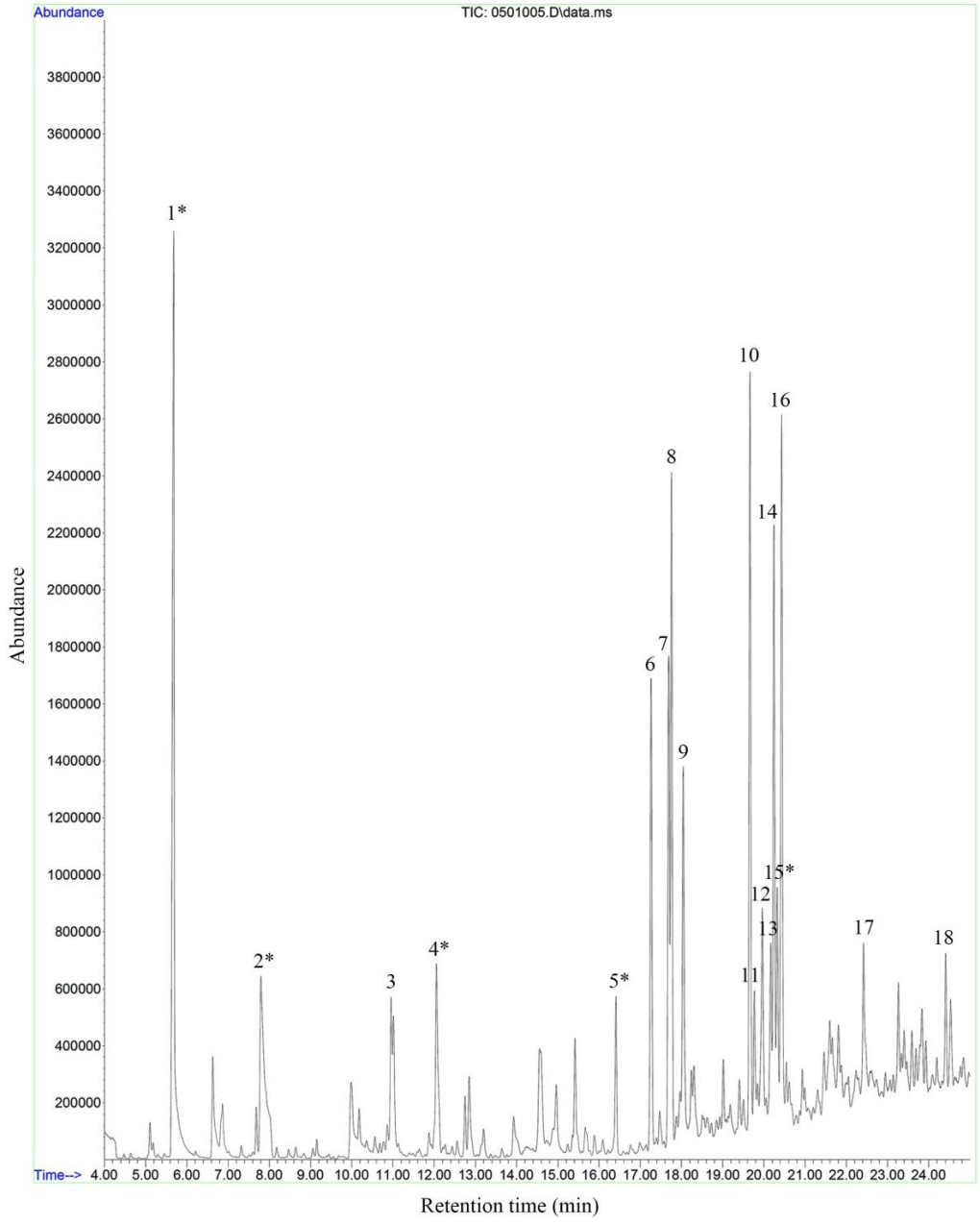


Figure C3 GC-MS chromatogram of volatile compounds from orange sweet pepper. Peaks are labeled as Table C3. *Detected contaminants from using DVB/CAR/PDMS fiber and column

Table C3 Volatile compounds of orange sweet pepper

Peak no.	Retention time (min)	Volatile compounds
1*	5.683	Oxime-, methoxy-phenyl-
2*	7.801	Octamethylcyclotetrasiloxane
3	10.959	Ethyl 2-trimethylsilyloxy-2-(3-trimethylsilyloxyphenyl)acetate
4*	12.058	Decamethylcyclopentasiloxane
5*	16.412	Dodecamethylcyclohexasiloxane
6	17.265	2,3-Dihydro-4H-1-benzoselenin-4-one
7	17.688	Copaene
8	17.757	4-Bromo-2-chlorobenzenamine
9	18.043	β-Elemene
10	19.662	9,10-Dehydro-isolongifolene
11	19.771	Cyclododecane
12	19.960	γ-Selinene
13	20.166	Alloaromadendrene
14	20.246	β-Selinene
15*	20.326	Tetradecamethylcycloheptasiloxane
16	20.429	α-Selinene
17	22.420	Hexadecane
18	24.411	Heptadecane

*Detected contaminants from using DVB/CAR/PDMS fiber and column

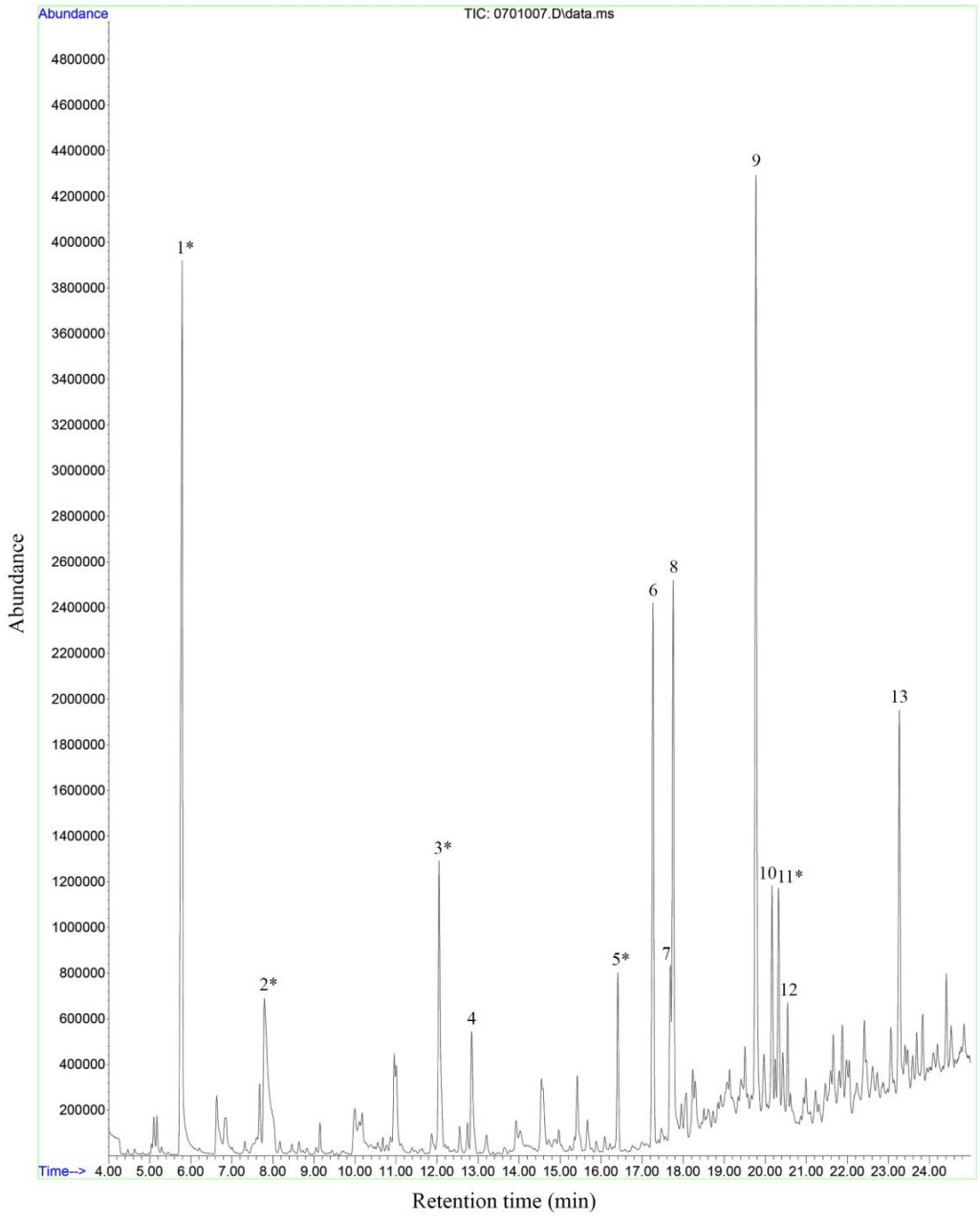


Figure C4 GC-MS chromatogram of volatile compounds from yellow sweet pepper. Peaks are labeled as Table C4. *Detected contaminants from using DVB/CAR/PDMS fiber and column

Table C4 Volatile compounds of yellow sweet pepper

Peak no.	**Retention time (min)**	**Volatile compounds**
1*	5.792	Oxime-, methoxy-phenyl-
2*	7.801	Octamethylcyclotetrasiloxane
3*	12.052	Decamethylcyclopentasiloxane
4	12.847	Naphthalene
5*	16.406	Dodecamethylcyclohexasiloxane
6	17.265	6-Chloro-7-hydroxy-4-methyl-2H-chromen-2-one / 2,4,5-Trichloro-pyrimidine
7	17.688	Copaene
8	17.757	4-Bromo-2-chlorobenzenamine
9	19.777	n-Nonyl-cyclopropane / 1-Dodecanol
10	20.166	Alloaromadendrene
11*	20.326	Tetradecamethylcycloheptasiloxane
12	20.549	α-Farnesene
13	23.267	1,2,3,4,4a,5,8,9,12,12a-Decahydro-1,4-methanobenzocyclodecene

*Detected contaminants from using DVB/CAR/PDMS fiber and column

APPENDIX D

PHYSICAL PROPERTIES OF FRESH SWEET PEPPER

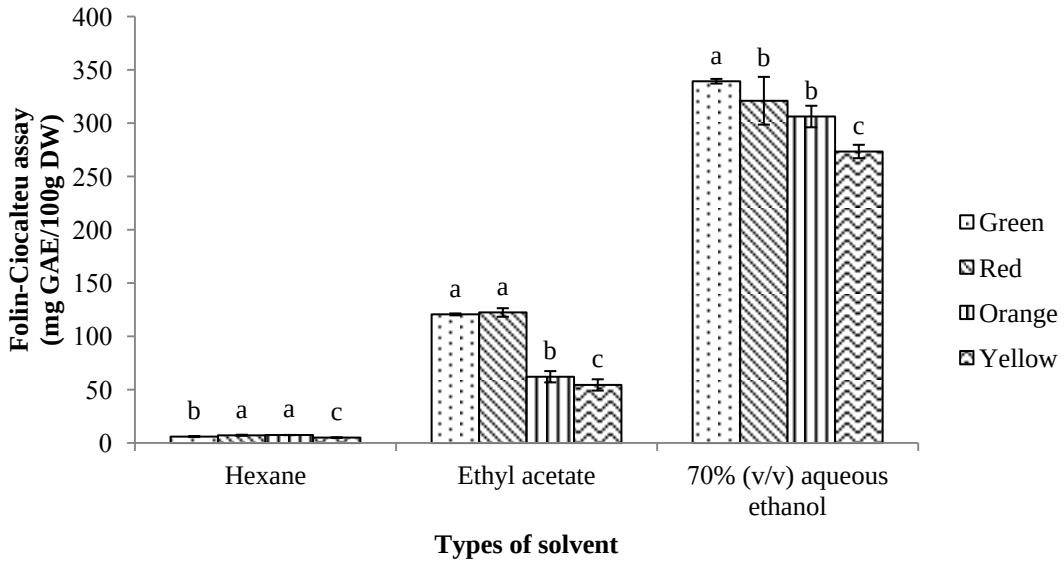
Table D1 Color characteristics and moisture contents of fresh sweet peppers

Types of sweet pepper	Color values			Moisture contents* (%)
	L*	a*	b*	
Green	+28.92	-8.56	+16.22	94.09 ± 0.45
Red	+27.52	+34.09	+19.45	92.55 ± 0.80
Orange	+46.49	+31.41	+50.19	92.52 ± 0.69
Yellow	+52.19	+15.74	+55.23	92.98 ± 0.46

*The data were expressed by mean values ± standard deviation.

APPENDIX E
BAR GRAPHS OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY

(A)



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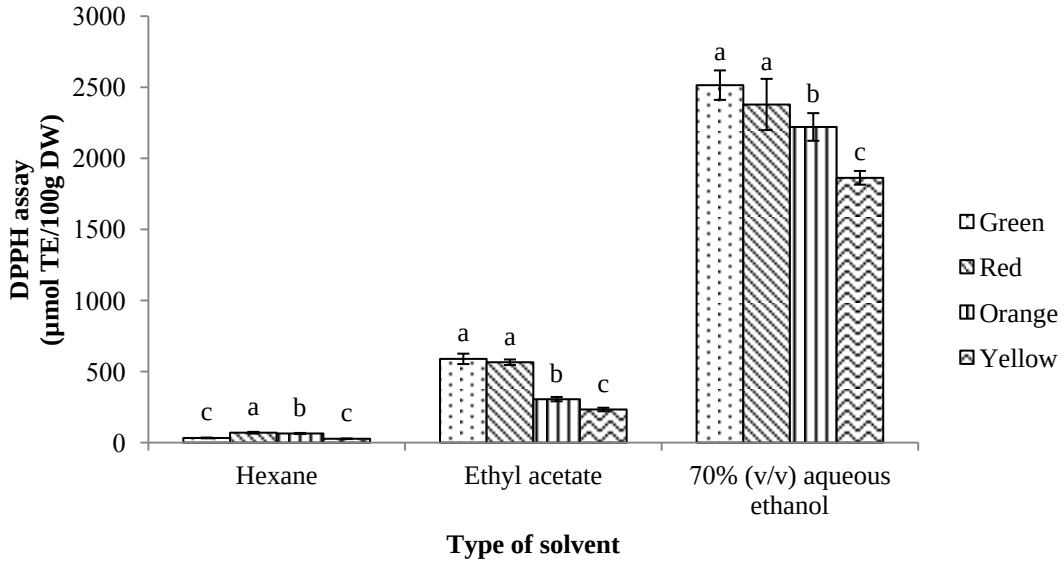

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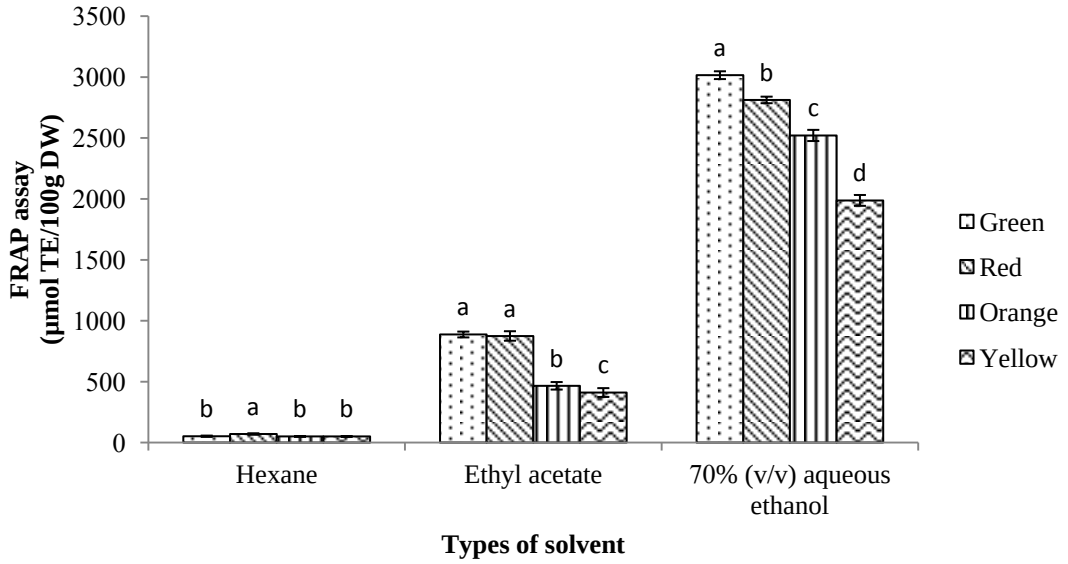


(D)

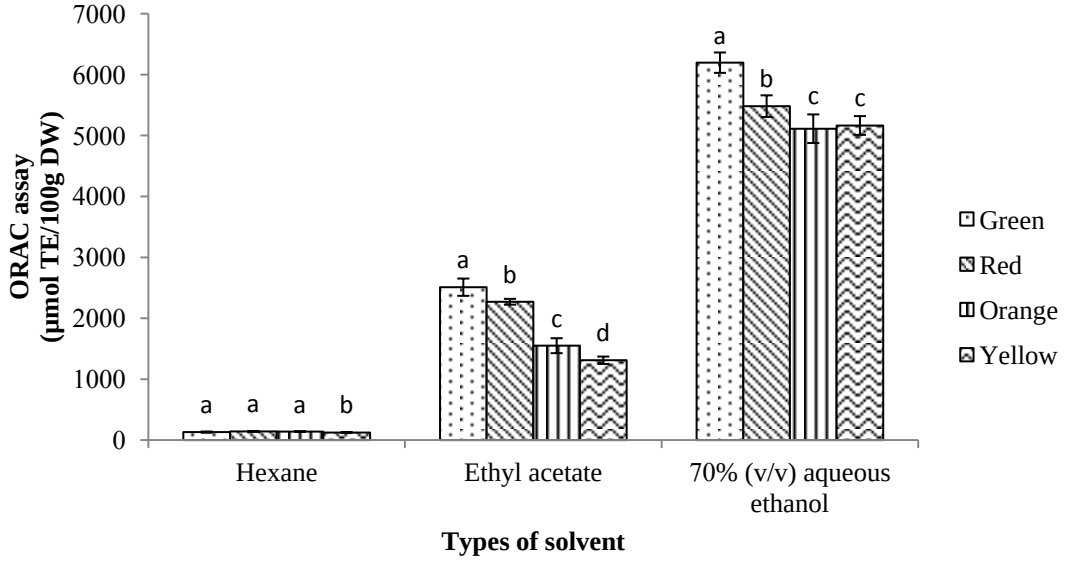
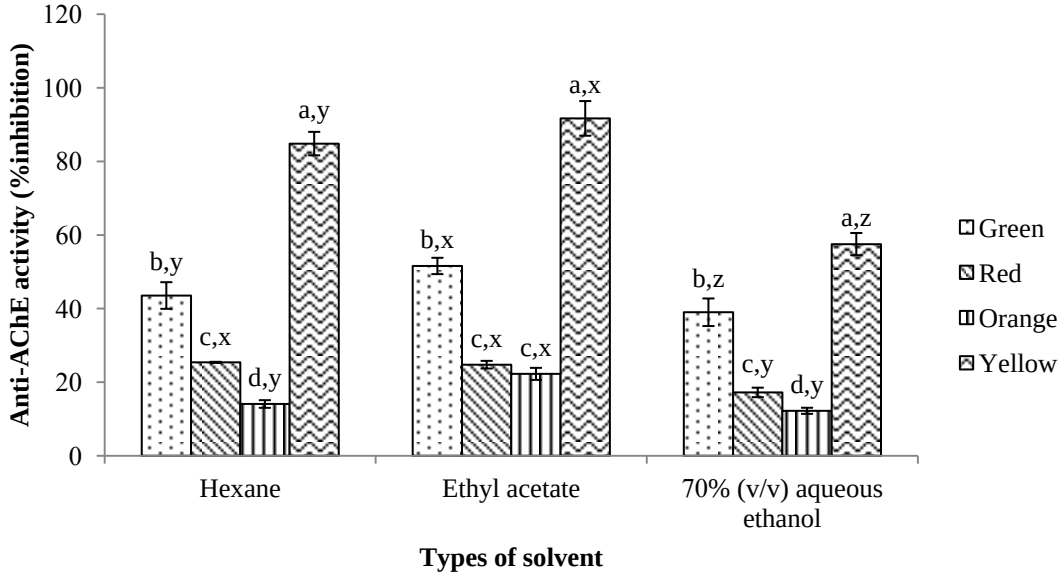


Figure E1 TPC and antioxidant activity of sweet pepper extracts. The results of (A) Folin–Ciocalteu, (B) DPPH radical scavenging, (C) FRAP and (D) ORAC assays of four colored sweet peppers extracted with hexane, ethyl acetate and 70% (v/v) aqueous ethanol were showed in bar graphs. All data were expressed by mean values ± standard deviation. The different letters $^{a\text{-}d}$ 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, DW: dry weight.

APPENDIX F

BAR GRAPHS OF ENZYME INHIBITORY ACTIVITIES

(A)



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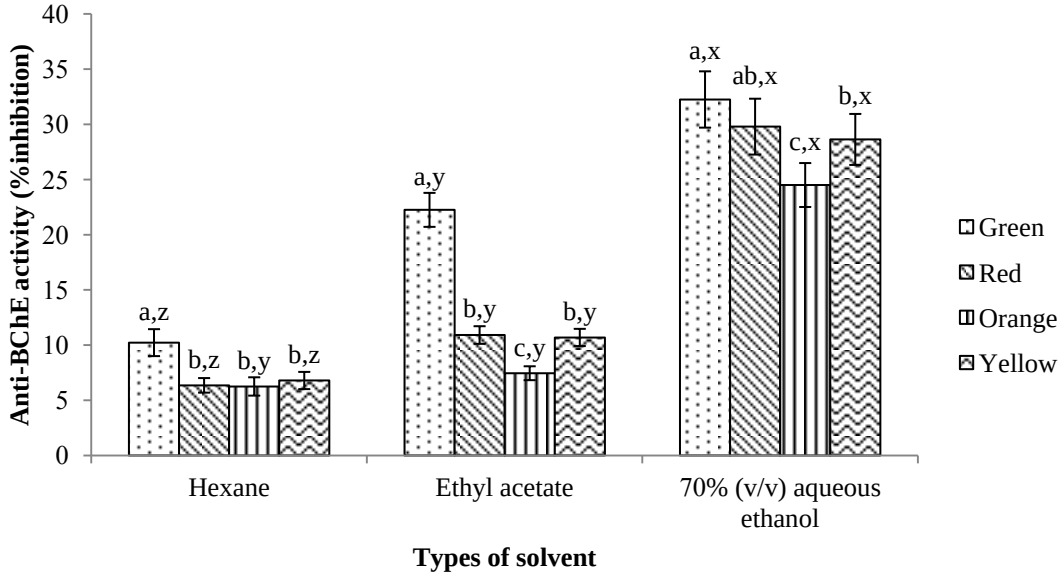


(C)

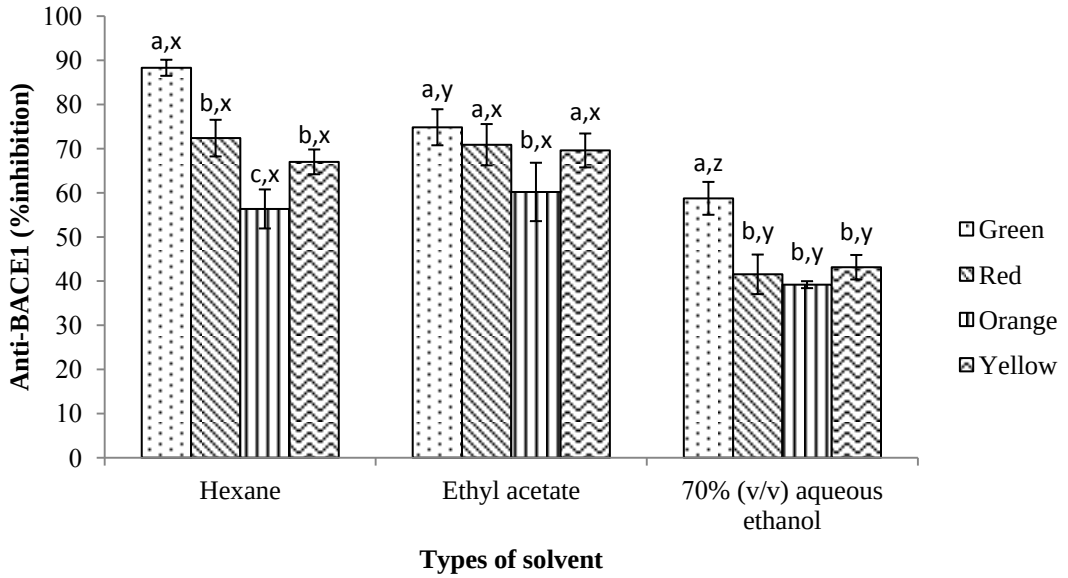


Figure F1 Cholinesterase and β-secretase inhibitory activities of sweet pepper extracts. (A) AChE, (B) BChE and (C) BACE1 inhibitory activities of four colored sweet peppers extracted with hexane, ethyl acetate and 70% (v/v) aqueous ethanol were exhibited in bar graphs. All data were expressed by mean values ± standard deviation. The different letters including $^{a\text{-}d}$ and $^{x\text{-}z}$ 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.

APPENDIX G
SUMMARY OF RESULTS

Table G1 Summary of results from this thesis

Types of analyses	Green	Red	Orange	Yellow
Flavonoids				
Quercetin	C	B	B	A
Luteolin	B	B	C	A
Total flavonoids	D	B	C	A
Phenolic acids				
p-Coumaric acid	A	BC	C	B
Ferulic acid	C	A	D	B
Total phenolic acids	A	B	D	C
Carotenoids				
Capsanthin	D	A	B	C
Lutein	B	ND	C	A
Zeaxanthin	ND	B	A	ND
β-Cryptoxanthin	ND	A	B	C
α-Carotene	C	ND	A	B
trans-β-Carotene	C	A	B	D
cis-β-Carotene	B	A	BC	C
Total carotenoids	D	A	B	C
TPC				
Hexane extracts	B, Z	A, Z	A, Z	C, Z
Ethyl acetate extracts	A, Y	A, Y	B, Y	C, Y
70% (v/v) aqueous ethanol extracts	A, X	B, X	B, X	C, X
DPPH assay				
Hexane extracts	C, Z	A, Z	B, Z	D, Z
Ethyl acetate extracts	A, Y	A, Y	B, Y	C, Y
70% (v/v) aqueous ethanol extracts	A, X	A, X	B, X	C, X
FRAP assay				
Hexane extracts	B, Z	A, Z	B, Z	B, Z
Ethyl acetate extracts	A, Y	A, Y	B, Y	C, Y
70% (v/v) aqueous ethanol extracts	A, X	B, X	C, X	D, X
ORAC assay				
Hexane extracts	A, Z	A, Z	A, Z	B, Z
Ethyl acetate extracts	A, Y	B,Y	C, Y	D, Y
70% (v/v) aqueous ethanol extracts	A, X	B, X	C, X	C, X
AChE				
Hexane extracts	B, Y	C, X	D, Y	A, Y
Ethyl acetate extracts	B, X	C, X	C, X	A, X
70% (v/v) aqueous ethanol extracts	B, Z	C, Y	D, Y	A, Z
BChE				
Hexane extracts	A, Z	B, Z	B, Y	B, Z
Ethyl acetate extracts	A, Y	B, Y	C, Y	B, Y
70% (v/v) aqueous ethanol extracts	A, X	AB, X	C, X	B, X
BACE1				
Hexane extracts	A, X	B, X	C, X	B, X
Ethyl acetate extracts	A, Y	A, X	B, X	A, X
70% (v/v) aqueous ethanol extracts	A, Z	B, Y	B, Y	B, Y

The different letters including A-D and X-Z within same analysis or 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. ND: not detected.

APPENDIX H
OVERVIEW OF THIS THESIS

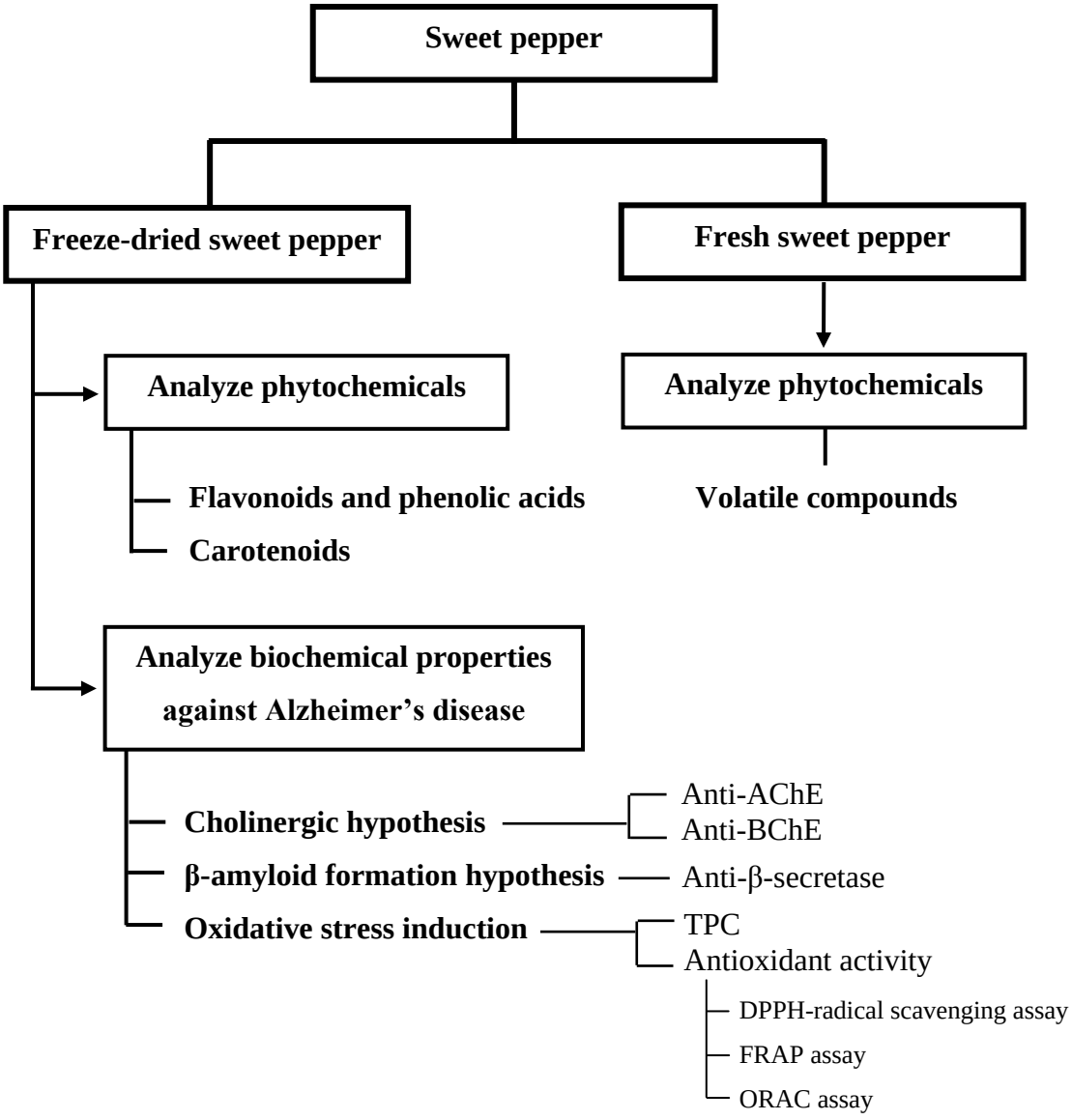


Figure H1 Overview of this study. This thesis focused on the analyses of phytochemicals and biochemical properties against Alzheimer's disease of four colored sweet peppers.

APPENDIX I

SAMPLE PREPARATION AND EXTRACTION FOR ANTI-ALZHEIMER'S DISEASE ANALYSES

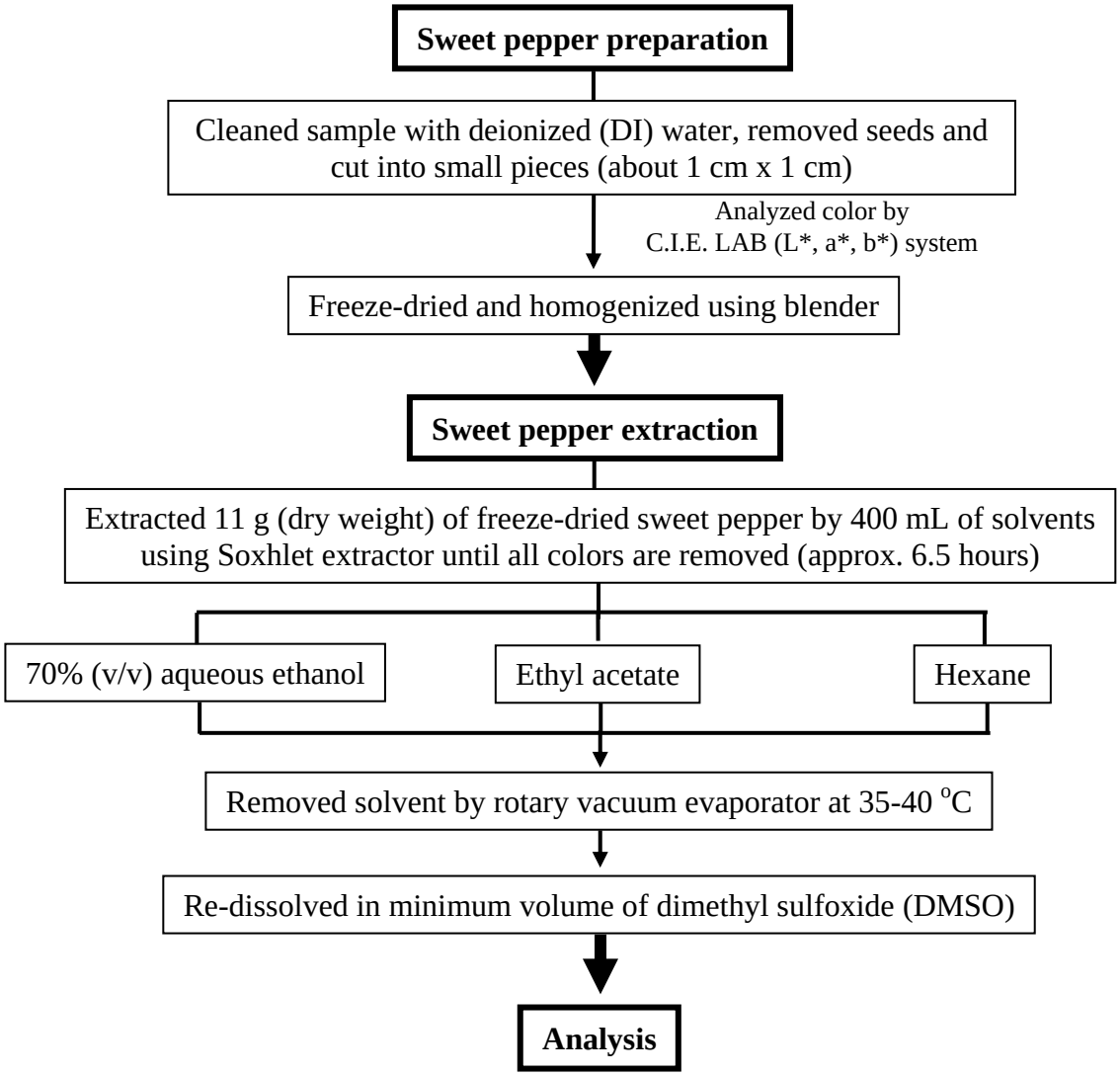


Figure I1 Sample preparation and extraction for anti-AD analyses. Freeze-dried sweet peppers were extracted by 70% (v/v) aqueous ethanol, ethyl acetate and hexane using Soxhlet extractor.

APPENDIX J
REAGENT PREPARATIONS

J1. Reagent preparations for flavonoid and phenolic acid analyses

Reagents

1. Methanol, pa
2. Hydrochloric acid (HCl), pa
3. Ascorbic acid, pa
4. Water, HPLC grade
5. Trifluoroacetic acid (TFA)
6. Acetronitrile, HPLC grade
7. Methanol, HPLC grade

Reagent preparations

1. 62.5% (v/v) methanol

Dilute 625 mL of methanol to 1000 mL with deionized water. Keep the solution at ambient temperature.

2. 6M HCl

Add 200 mL of deionized water into a 500-mL volumetric flask and add 26 mL of HCL. Then, adjust the volume to 500 mL with deionized water and mix it. Keep the solution in ambient temperature.

3. 5% (w/w) TFA in methanol

Mix 210 µL of TFA and 1000 mL of methanol.

4. 0.5% (w/w) TFA

Mix 210 µL of TFA and 1000 mL of water.

5. 0.5% (w/w) TFA in acetronitrile

Mix 210 µL of TFA and 1000 mL of acetronitrile.

J2. Reagent preparations for carotenoid analysis

Reagents

1. Ascorbic acid
2. Potassium hydroxide (KOH)
3. Ethanol (EtOH)
4. Sodium chloride (NaCl)
5. Acetonitrile (CH_3CN)
6. Methanol (MeOH)
7. Dichloromethane (CH_2Cl_2)
8. Triethylamine (TEA)
9. Ammonium acetate

Reagent preparations

1. 10% ascorbic acid solution

Weight 1 g of ascorbic acid into a 100-mL volumetric flask. Then, adjust the volume to 100 mL with reverse osmosis (RO) water and mix well.

2. 2N KOH solution

Weight 67.2 g of KOH into a flask, add 60 mL of RO water and mix well. Then, add 540 mL of 95% (v/v) aqueous ethanol

3. 10% NaCl solution

Weight 50 g of NaCl into a 500-mL volumetric flask. Then, adjust the volume to 500 mL with RO water and mix well.

4. Mobile phase (prepare in hood)

Weight 1 g of ammonium acetate and add 50 mL of methanol into 100-mL beaker and mix well. Then, adjust the volume to 100 mL with methanol. After that, add 800 mL of acetonitrile, the ammonium acetate solution and 10 mL of methanol into 1000-mL cylinder. Then, adjust the volume to 1000 mL with dichloromethane and add 1 mL of triethylamine. Finally, filtrate the mobile phase with filter paper Nylon before using.

J3. Reagent preparations for Folin-Ciocalteu assay

Reagents

1. Folin-Ciocalteu reagent
2. Sodium bicarbonate (Na_2CO_3)
3. Gallic acid monohydrate

Reagent preparations

1. 10% (v/v) Folin-Ciocalteu reagent

Dilute 500 µL of Folin-Ciocalteu reagent (stock solution) to 5 mL with deionized water. Keep the reagent at ambient temperature.

2. 7.5% (w/v) Na_2CO_3 solution

Weight 7.5 g of Na_2CO_3 in a 100-mL volumetric flask. Then, adjust the volume to 100 mL with deionized water and mix well. Keep the solution at ambient temperature.

3. 1000 µg/ml gallic acid solution (stock solution)

Weight 100 mg of gallic acid monohydrate in a 100-mL volumetric flask. Then, adjust the volume to 100 mL with deionized water. Keep the solution in a freezer.

4. 200 µg/ml gallic acid solution (working solution for serial dilution)

Dilute 80 µL of 1000 µg/mL gallic acid solution (stock solution) to 400 µL with deionized water. After that, dilute 200 µg/ml gallic acid solution by serial dilution to be 100, 80, 60, 40, 20 and 10 µg/mL, respectively. Keep the solution in an ice bath before using.

J4. Reagent preparations for DPPH radical scavenging assay

Reagents

1. Absolute ethanol
2. 2,2-diphenyl-1-picrylhydrazyl (DPPH)
3. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox)

Reagent preparations

1. 95% (v/v) aqueous ethanol

Dilute 950 mL of absolute ethanol to 1000 *m*L with deionized water. Keep the solution at ambient temperature.

2. 150 μM DPPH in 95% (v/v) aqueous ethanol

Weight 0.00148 g of DPPH into a 25-mL volumetric flask. Then, adjust the volume to 25 mL with 95% (v/v) aqueous ethanol and mix well**.** Keep the solution in an ice bath before using.

3. 8 mM trolox solution (stock solution)

Weigh 0.20 g of trolox into a 100-mL volumetric flask. Then, adjust the volume to 100 mL with 95% (v/v) aqueous ethanol and mix well. Keep the solution in a freezer.

4. 0.64 mM trolox solution (working solution for serial dilution)

Dilute 20 μL of 8 mM trolox solution (stock solution) to 250 μL with 95% (v/v) aqueous ethanol. After that, dilute 0.64 mM trolox solution by serial dilution to be 0.32, 0.16, 0.08, 0.04, 0.02 and 0.01 mM, respectively. Keep the solution in an ice bath before using.

J5. Reagent preparations for FRAP assay

Reagents

1. Glacial acetic acid
2. Hydrochloric acid (HCl)
3. 2,4,6-tripyridyl-*s*-triazine (TPTZ)
4. Sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$)
5. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$)
6. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox)

Reagent preparations

1. 300 mM acetate buffer (pH 3.6)

Weight 3.1 g of sodium acetate trihydrate into a 1-L volumetric flask and add 16 mL of glacial acetic acid. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

2. 10 mM TPTZ solution in 40 mM HCl

Weight 0.312 g of TPTZ into 100-mL volumetric flask. Then, adjust the volume to 100 mL with 40 mM HCl (0.4 mL of conc. HCL + 99.6 mL of deionized water) and mix well. Keep the solution in a refrigerator.

3. 20 mM $FeCl_3 \cdot 6H_2O$ solution

Weight 0.5406 g of $FeCl_3 \cdot 6H_2O$ into 100-mL volumetric flask. Then, adjust the volume to 100 mL with deionized water and mix well. Keep the solution in a refrigerator.

4. FRAP reagent

Mixing the reagent of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM $FeCl_3 \cdot 6H_2O$ solution in ratio of 10:1:1 respectively and warm in water bath at 37 °C before using.

5. 250 µM trolox solution (working solution for serial dilution)

Dilute 60 µL of 1000 µM trolox solution (stock solution, from ORAC assay) to 240 µL with deionized water. After that, dilute 250 µM trolox solution by serial dilution to be 125, 62.5 31.25, 15.625 and 7.8125 µM, respectively. Keep the solution in an ice bath before using.

J6. Reagent preparations for ORAC assay

Reagents

1. Di-potassium hydrogen phosphate (K_2HPO_4)
2. Potassium dihydrogen phosphate (KH_2PO_4)
3. 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)
4. Fluorescein sodium salt
5. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox)

Reagent preparations

1. ORAC buffer (stock solution)
   - Prepare 0.75 M KH_2PO_4 solution (stock solution)

Weight 102.07 g of KH_2PO_4 into a 1-L volumetric flask. Then, adjust the volume to 1 L with deionized water and mix well**.** Keep the solution in a refrigerator.

- Prepare 0.75 M K_2HPO_4 solution (stock solution)

Weight 130.64 g of K_2HPO_4 into a 1-L volumetric flask. Then, adjust the volume to 1 L with deionized water and mix well**.** Keep the solution in a refrigerator.

For ORAC buffer (stock solution) preparation, mix 351 mL of 0.75 M KH_2PO_4 solution (stock solution) and 603 mL of 0.75 M K_2HPO_4 solution (stock solution), this yields 954 mL of the ORAC buffer (stock solution). Keep the solution in a refrigerator.

2. ORAC buffer (working solution)

Take 100 mL of ORAC buffer (stock solution) into a 1-L volumetric flask. Then, adjust the volume to 1 L with deionized water and mix well**.** Adjusted pH to 7.2 with a few drops of a NaOH concentrated solution. Keep the solution in a refrigerator.

3. 153 mM AAPH

Weigh 0.414 g of AAPH into a 10-mL volumetric flask. Then, adjust the volume to 10 mL with ORAC buffer (working solution) and mix well. Keep the solution in an ice bath before using.

4. 8.37×10^{-4} mM fluorescein solution (concentrated solution)

Weight 0.045 g of fluorescein sodium salt into a-100 mL volumetric flask. Then, adjust the volume to 100 mL with ORAC buffer (working solution). Keep the solution in a freezer.

5. 4.19 μM fluorescein solution (stock solution)

Dilute 100 μL of 8.37×10^{-4} mM fluorescein solution (concentrated solution) to 20 mL with ORAC buffer (working solution) and mix well. Keep the solution in a freezer.

6. Fluorescein solution (working solution)

Dilute 244 μL of 4.19 μM fluorescein solution (stock solution) to 12.5 mL with ORAC buffer (working solution) and mix well. Warm the solution in water bath at 37 °C before using.

7. 1000 μM trolox solution (stock solution)

Weigh 0.025 g of trolox into a 100-mL volumetric flask. Then, adjust the volume to 100 mL with ORAC buffer (working solution) and mix well. Keep the solution in a freezer.

8. 100 μM trolox solution (working solution for serial dilution)

Dilute 20 μL of 1000 μM trolox solution (stock solution) to 200 μL with ORAC buffer (working solution). After that, dilute 100 μM trolox solution by serial dilution to be 50, 25, 12.5, 6.25 and 3.125 μM, respectively. Keep the solution in an ice bath before using.

J7. Reagent preparations for AChE inhibitory assay

Reagents

1. Absolute methanol
2. Di-potassium hydrogen phosphate (K_2HPO_4)
3. Potassium dihydrogen phosphate (KH_2PO_4)
4. 5-5'-dithiobis(2-nitrobenzoic acid) (DTNB)
5. Acetylthiocholine (ATCh)
6. *Electrophorus electricus* acetylcholinesterase (AChE)

Reagent preparations

1. 1 M K_2HPO_4 solution (stock solution)

Weight 228.23 g of K_2HPO_4 into 1-L beaker. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

2. 1 M KH_2PO_4 solution (stock solution)

Weight 136.09 g of K_2HPO_4 into 1-L beaker. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

3. 50 mM potassium phosphate buffer (KPB), pH 7.0

Take 30.7 mL of 1 M K_2HPO_4 solution (stock solution) into 1-L volumetric flask and add 19.2 mL of 1 M KH_2PO_4 solution (stock solution) and then add deionized water about 500 mL and mix well. Then, adjust pH to 7.0 with a few drops of a NaOH concentrated solution. After that, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

4. 200 mM DTNB solution in methanol (stock solution)

Weight 0.7927 g of DTNB into 10-mL volumetric flask and adjust the volume to 10 mL with absolute methanol. Keep the solution at ambient temperature.

5. 16 mM DTNB solution in 50 mM KPB pH 7.0 (working solution)

Take 966 µL of 50 mM KPB pH7.0 into 1.5-mL tube. Then, add 84 µL of 200 mM DTNB solution (stock solution) and mix well.

6. 10 mM ACTh solution (stock solution)

Weight 28.918 mg of ACTh into 10-mL volumetric flask. Then, adjust the volume to 10 mL with 50 mM KPB pH 7.0 and mix well. Keep the solution at -20^{o}C. (Molecular weight of ACTh = 289.18 g/mol)

7. 0.32 mM ACTh solution (working solution)

Dilute 184 µL of 10 mM ACTh solution (stock solution) to 5750 µL with 50 mM KPB pH 7.0. Keep the solution in an ice bath before using.

8. 0.01 mg/mL AChE solution (stock solution)

Weight 0.1 mg of AChE into 10-mL volumetric flask. Then, adjust the volume to 10 mL with 50 mM KPB pH 7.0 and mix well. Keep the solution at -20^{o}C.

9. 0.0001 mg/mL AChE solution (working solution)

Dilute 82 µL of 0.01 mg/mL AChE solution (stock solution) to 8200 µL with 50 mM KPB pH 7.0 (working solution). Keep the solution in an ice bath before using.

J8. Reagent preparations for BChE inhibitory assay

Reagents

1. Absolute methanol
2. Di-potassium hydrogen phosphate (K_2HPO_4)
3. Potassium dihydrogen phosphate (KH_2PO_4)
4. 5-5'-dithiobis(2-nitrobenzoic acid) (DTNB)
5. Magnesium chloride ($MgCl_2$)
6. Butyrylthiocholine chloride (BTCh)
7. Equine serum butyrylcholinesterase (BChE)

Reagent preparations

1. 50 mM potassium phosphate buffer (KPB) pH 7.0 with 1mM $MgCl_2$

Weight 4.7605 mg of $MgCl_2$ and adjust the volume to 50 mL with 50 mM KPB pH 7.0 and mix well. Keep the solution in a refrigerator. (Molecular weight of $MgCl_2$ = 95.21 g/mol)

2. 16 mM DTNB solution in 50 mM KPB pH 7.0 (working solution)

Take 966 µL of 50 mM KPB pH 7.0 into 1.5-mL tube. Then, add 84 µL of 200 mM DTNB solution (stock solution) and mix well.

3. 10 mM BCTh solution (stock solution)

Weight 22.578 mg of BCTh into 10-mL volumetric flask. Then, adjust the volume to 10 mL with 50 mM KPB pH 7.0 and mix well. Keep the solution at -20^{o}C. (Molecular weight = 225.78 g/mol)

4. 0.4 mM BCTh solution (working solution)

Dilute 232 µL of 10 mM BCTh solution (stock solution) to 5800 µL with 50 mM KPB pH 7.0. Keep the solution in an ice bath before using.

5. 0.1 mg/mL BChE solution (stock solution)

Weight 1 mg of BChE into 10-mL volumetric flask. Then, adjust the volume to 10 mL with 50 mM KPB pH 7.0 with 1mM $MgCl_2$. Mix well and keep the solution at -20^{o}C.

6. 0.0005 mg/mL BChE solution (working solution)

Dilute 41 µL of 0.1 mg/mL BChE solution (stock solution) to 8200 µL with 50 mM KPB pH 7.0 with 1mM $MgCl_2$. Keep the solution in an ice bath before using.

J9. Reagent preparations for BACE1 inhibitory assay

Reagents

Using β-Secretase (BACE1) Activity Detection Kit (Fluorescent)

1. 0.5 mg BACE1 substrate
2. Dimethyl sulfoxide (DMSO)
3. 3 units/µL BACE1 enzyme)
4. Fluorescent assay buffer

Reagent preparations

1. 500 µM BACE1 substrate solution (stock solution)

Add 500 µL of DMSO into 0.5 mg BACE1 substrate. Keep the solution at -20^{o}C.

2. 50 µM BACE1 substrate solution (working solution)

Dilute 200 µL of 500 µM BACE1 substrate solution (stock solution) to 2000 µL with fluorescent assay buffer. Keep the solution in an ice bath before using.

3. 0.3 units/µL BACE1 enzyme

Dilute 20 µL of 3 units/µL BACE1 enzyme to 200 µL with fluorescent assay buffer. Keep the solution in an ice bath before using.

APPENDIX K
REACTIONS OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY ASSAYS

K1. Principle for determination of TPC by Folin-Ciocalteu assay

The phenol is ionized in alkaline condition to become ionized phenolic acid, which is subsequencely oxidized by Folin-Ciocalteu reagent. This reagent is a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$), thus changing yellow color of Mo(VI) to a blue complex of MoO(V) in a complex of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) under reduction process (Figure K1.1). The change of color can be measured in a spectrophotometer at 765 nm. Gallic acid is used as a control standard (Figure K1.2), and the concentration of TPC is usually reported as gallic acid equivalents (GAE) per gram dry weight.

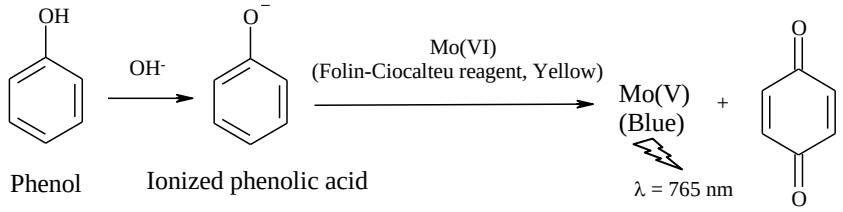
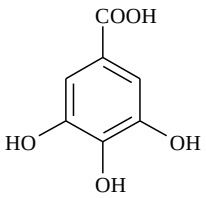


Figure K1.1 Reaction for determination of TPC by Folin-Ciocalteu assay. The yellow Mo(VI) in phosphomolybdic–phosphotungstic acid reagent is reduced to blue Mo(V) complex by ionized phenolic group. The changed color is measured in a spectrophotometer at 765 nm.



Gallic acid

Figure K1.2 Chemical structure of gallic acid. It is a type of phenolic acid, which is a standard used in Folin-Ciocalteu assay.

K2. Principle for determination of DPPH - radical scavenging assay

DPPH assay is based on both hydrogen transfer (HAT) reactions and single electron transfer (SET) reactions [112]. DPPH is a stable free radical, consisting of a reactive organic nitrogen atom that acts as a radical scavenger or hydrogen donor [113]. The DPPH$^{\bullet}$ radical contains an unpaired nitrogen with a deep purple color. If DPPH$^{\bullet}$ radical reacts with an antioxidant (AH) in a reduction reaction, the stable DPPH-H is generated to produce yellow product. Thus, DPPH assay is measured by a loss of deep purple color of DPPH at 520 nm after reacting with antioxidant (Figure K2). The data are presented as % radical scavenging activity or trolox equivalence (TE) per gram dry weight.

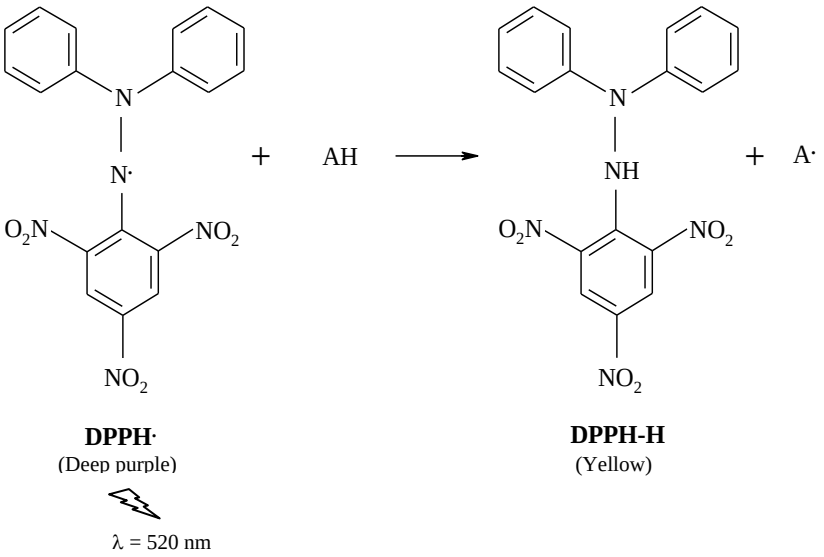


Figure K2 Reaction for determination of antioxidant activity by DPPH assay. The deep purple DPPH$^{\bullet}$ radical reacts with antioxidants (AH) to produce a yellow DPPH-H compound. Antioxidant activity is measured with the loss of deep purple by absorbance at 520 nm.

K3. Principle for determination of FRAP assay

FRAP assay based on single electron transfer (SET) reactions [112] is from the reaction between ferric 2,4,6-tripyridyl-*s*-triazine (TPTZ) and antioxidant. Ferric-TPTZ (Fe^{3+}-TPTZ) is reduced to ferrous complex (Fe^{2+}-TPTZ) by electron-

donated antioxidant, which changes the color from brown to indigo. This color change can be measured at a wavelength of 595 nm as an indicator of product formation (Figure K3). The FRAP value is reported as TE per gram dry weight using trolox as a standard.

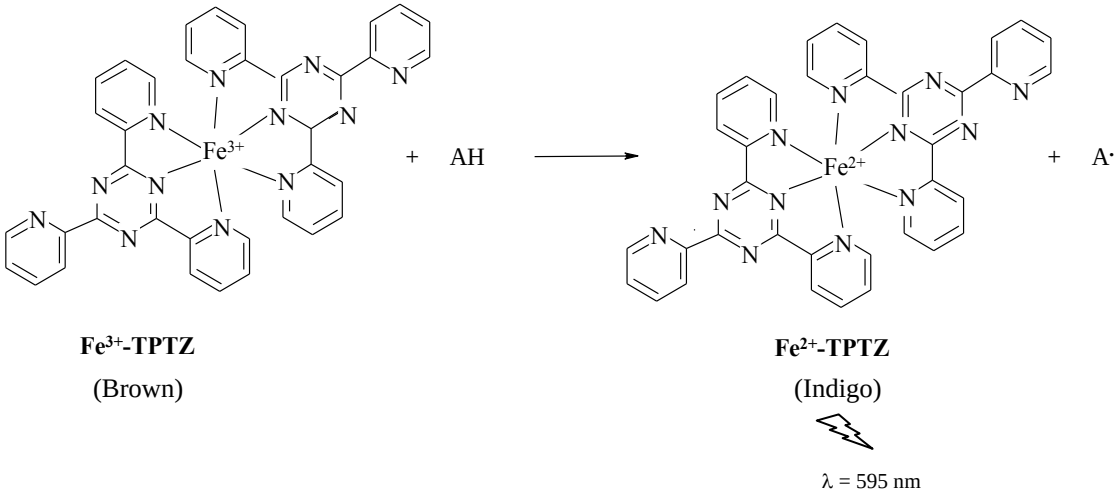


Figure K3 Reaction for determination of antioxidant activity by FRAP assay. The assay is measured by the reduction of brown Fe^{3+}-TPTZ to indigo Fe^{2+}-TPTZ by antioxidant (AH). The indigo color as indicator of product formation can be measured at a wavelength of 595 nm.

K4. Principle for determination of ORAC assay

ORAC assay based on hydrogen transfer (HAT) reactions [112] includes the reaction between peroxyl radical of AAPH and fluorescein by oxidative damage. AAPH is oxidized to produces peroxyl radical. Then, the peroxyl radical reacts with the fluorescein to form fluoresceinyl radical, a non–fluorescent product (Figure K4.1). Antioxidant activity in ORAC assay is determined by a kinetic measuring of fluorescence intensity loss and protection of the fluorescence loss by antioxidant. The fluorescence intensity will be monitored at 37 ºC with an excitation wavelength of 485 nm and emission wavelength of 528 nm. Trolox is commonly used as a control standard (Figure K4.2), and ORAC values are usually reported as Trolox equivalents (TE) per gram dry weight.

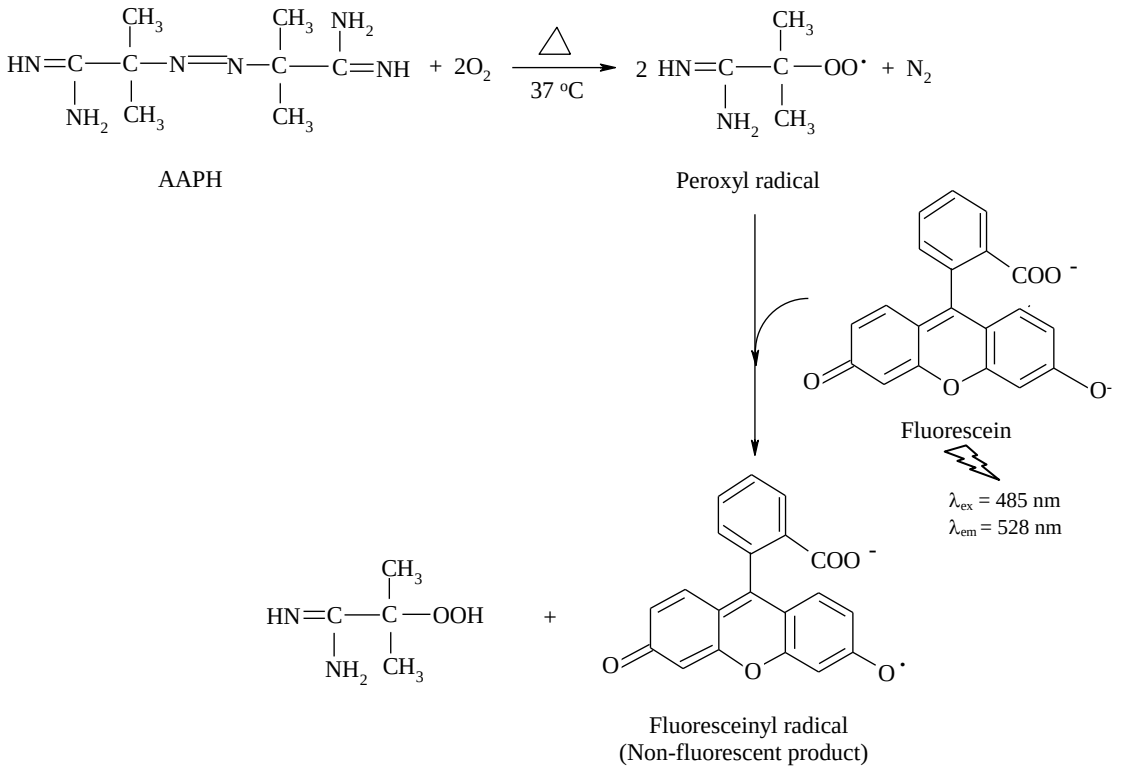


Figure K4.1 Reaction for determination of antioxidant activity by ORAC assay. AAPH is oxidized to generate nitrogen gas (N_2) and two peroxyl radicals. The peroxyl radical then reacts with the fluorescein to form fluoresceinyl radical, a non-fluorescent product. The assay measures the loss of fluorescein with an excitation wavelength of 485 nm and emission wavelength of 528 nm.

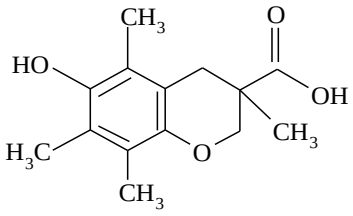


Figure K4.2 Chemical structure of trolox. It is a water soluble vitamin E analogue, which is a standard used in antioxidant assay.

APPENDIX L
REACTIONS OF ENZYME INHIBITORY ACTIVITY ASSAYS

L1. Principle of AChE inhibitory assay

The substrate, ATCh, is hydrolyzed by enzyme AChE to produce thiocholine, which is subsequently reacted with Ellman's reagent, DTNB, to form a yellow 2-nitro-5-mercapto-benzoic acid. This final product possesses yellow color that can be measured at a wavelength of 412 nm (Figure L1). However, under the presence of inhibitor, the production of thiocholine is reduced as a result of interference by inhibitor.

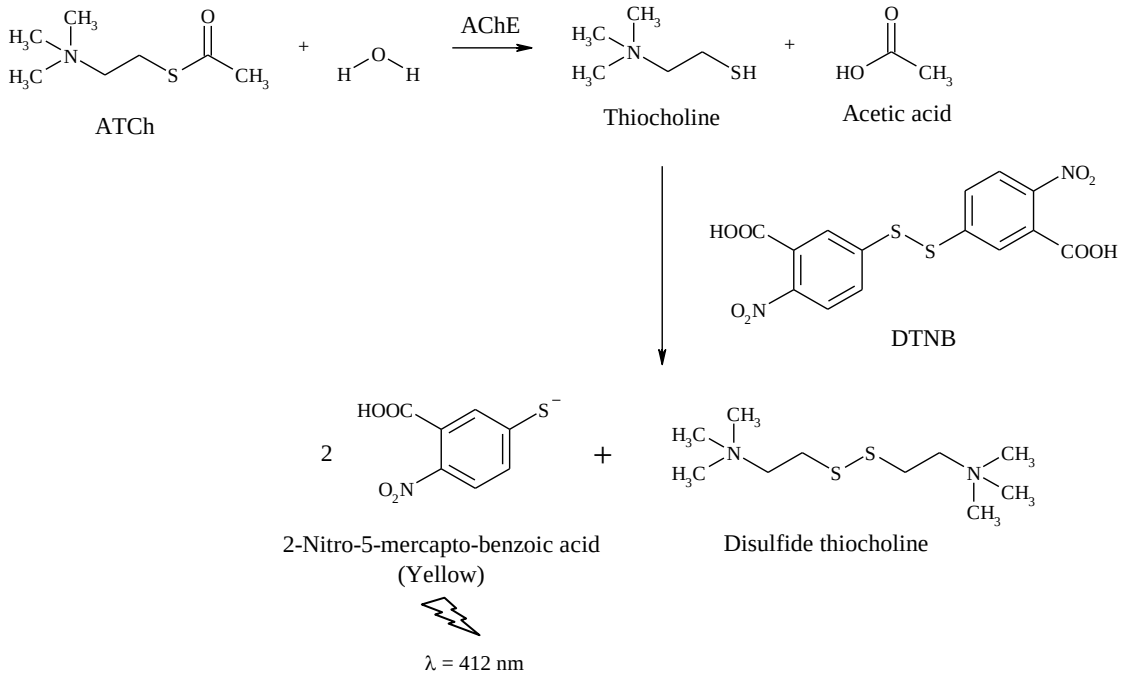


Figure L1 Reaction of AChE inhibitory assay. ATCh is hydrolyzed by AChE to form thiocholine and acetic acid. Thiocholine is reacted with DTNB to generate color product, which can be measured at a wavelength of 412nm.

L2. Principle of BChE inhibitory assay

The substrate, BTCh, is hydrolyzed by enzyme BChE to produce thiocholine, which is subsequently reacted with Ellman's reagent, DTNB, to form a yellow 2-nitro-5-mercapto-benzoic acid. This final product possesses yellow color that can be measured at a wavelength of 412 nm (Figure L2). However, under the presence of inhibitor, the production of thiocholine is reduced as a result of interference between inhibitor and enzyme.

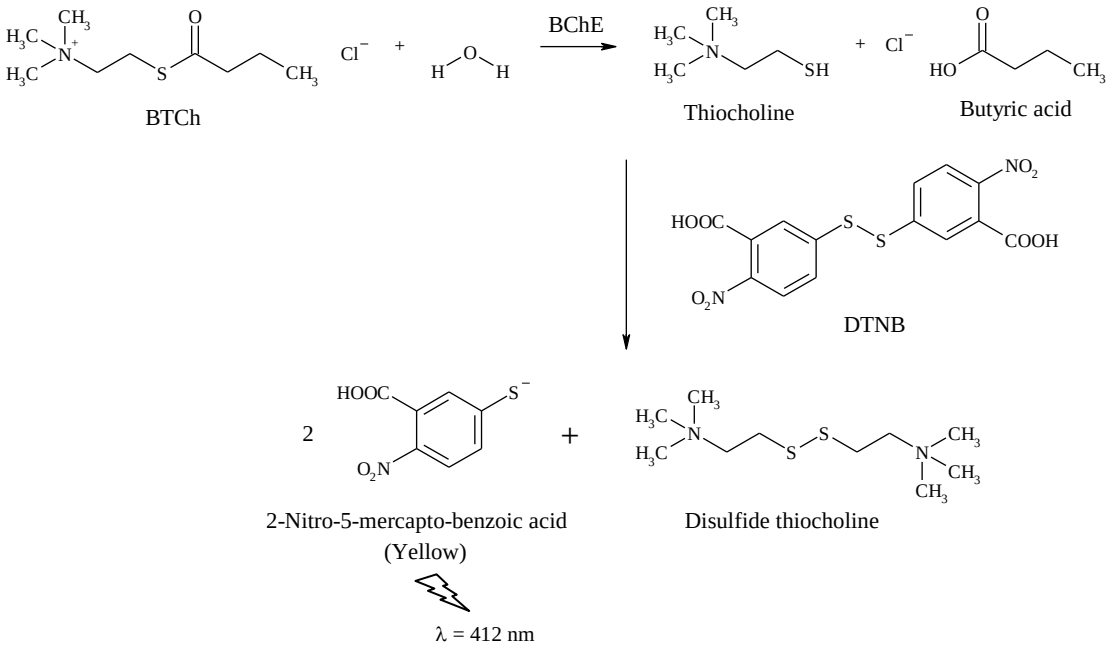


Figure L2 Reaction of BChE inhibitory assay. The reaction is similar to AChE inhibitory activity assay. BChE hydrolyzes BTCh to thiocholine and butyric acid. The BChE inhibitory activity is measured from the color of the product between thiocholine and DTNB at 412 nm.

L3. Principle of BACE1 inhibitory assay

The assay is based on a method of fluorescence resonance energy transfer (FRET). The FRET protease substrate, BACE1 substrate, consists of two fluorophores including a fluorescent donor (D) and a quenching acceptor (A). The energy of (D) is significantly quenched by (A) in resonance energy transfer (Figure L3), leading to dramatically reduced intrinsic fluorescence of the substrate. If BACE1 substrate is cleavage by BACE1, the fluorophore is separated from the quenching group, restoring the full fluorescence yield of the donor. Therefore, fluorescent intensity of peptide substrate becomes increased and can be measured at an excitation wavelength of 320 nm and an emission wavelength of 405 nm. However, if the inhibitor is present in system, the cleavage of BACE1 substrate will be reduced, leading to decreased fluorescence signal [114].

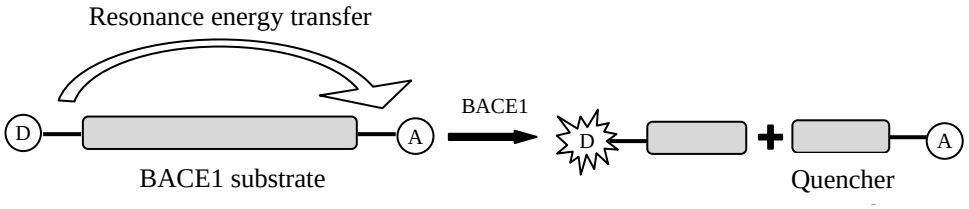


Figure L3 Reaction of BACE1 inhibitory assay. BACE1 substrate is cleavage from the quenching group (A) by BACE1 to increase fluorescence by fluorescent donor (D). The reaction can be measured at the excitation wavelength of 320 nm and the emission wavelength of 405 nm.