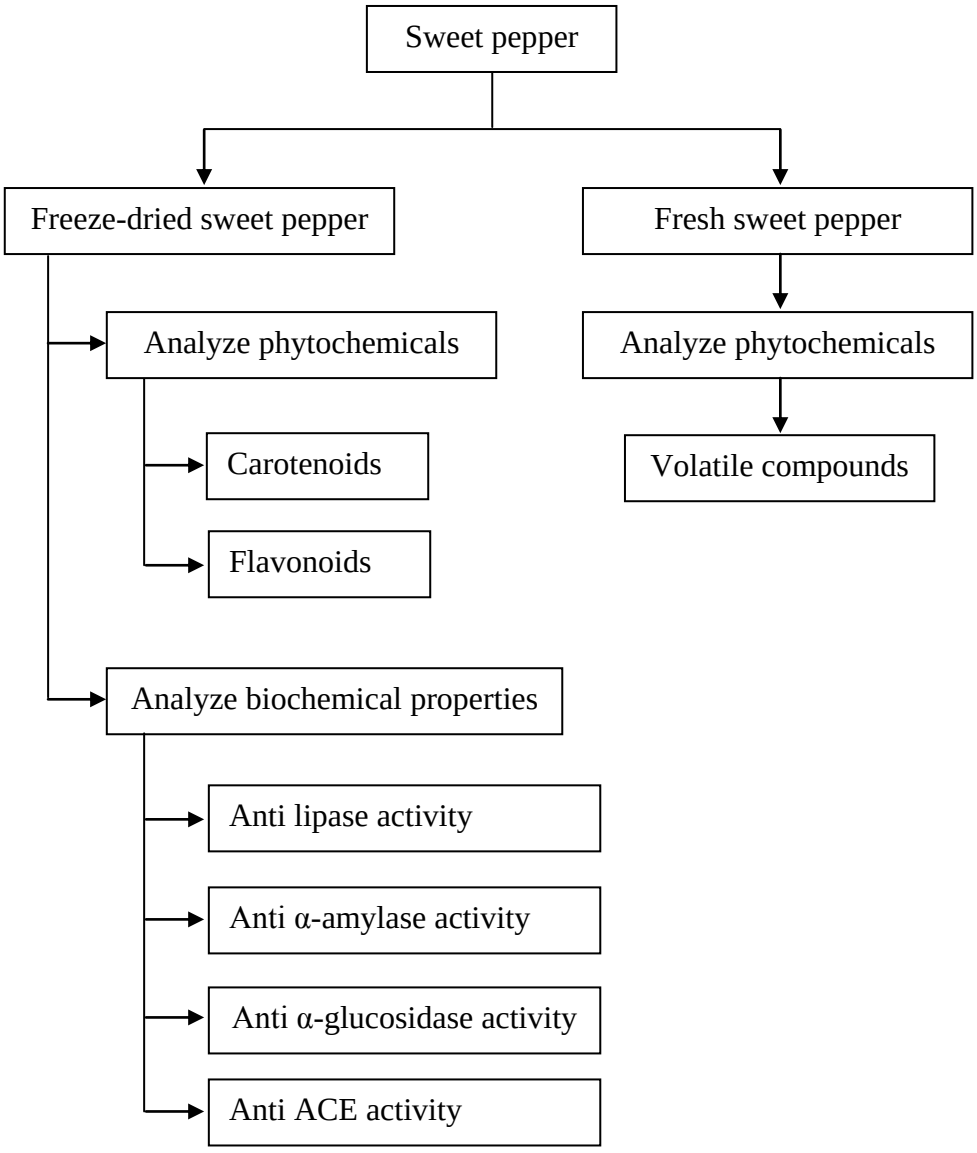
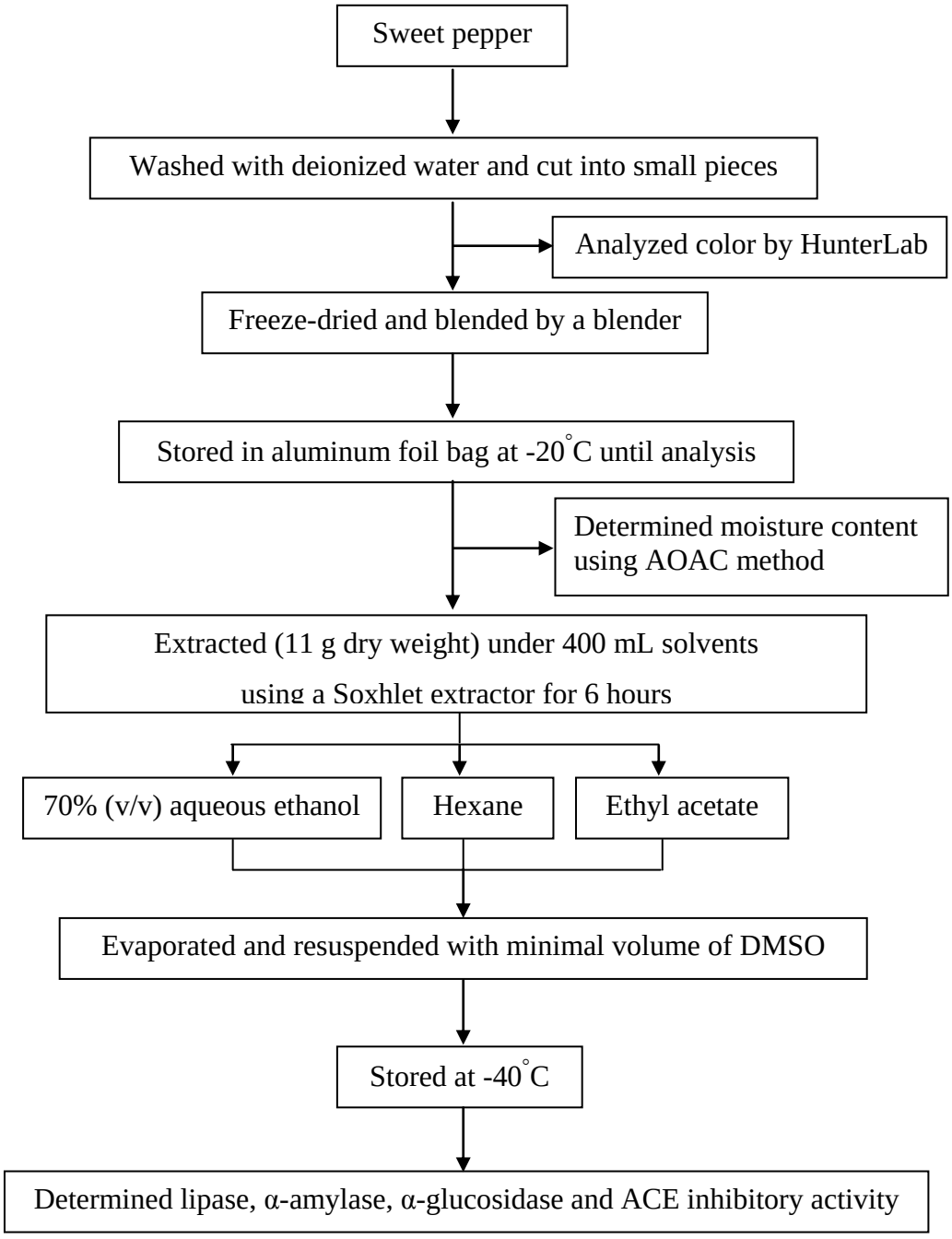
APPENDICES

APPENDIX A

OVERVIEW OF THIS STUDY



APPENDIX B
SAMPLE PREPARATION AND EXTRACTION



APPENDIX C
REAGENT PREPARATIONS

C1. Reagent Preparations for Carotenoid Content Determination

Reagents

- Ascorbic acid
- Potassium hydroxide (KOH)
- Sodium Chloride (NaCl)
- Acetonitrile (CH_3CN)
- Methanol
- Dichloromethane (CH_2Cl_2)
- Triethylamine (TEA)
- Ammonium acetate

Reagent preparation

1. **1% (w/v) Ascorbic acid**

Weight 1 g of ascorbic acid into 100 mL volumetric flask. After that, adjust the volume to 100 mL with deionized water and mix well.

2. **2N KOH**

Weight 67.2 g of KOH into 1 L beaker. Then, add deionized water about 60 mL, mix well. Then, add 95% Ethanol about 540 mL and mix well.

3. **10% (w/v) NaCl**

Weight 50 g of NaCl into 500 mL volumetric flask. After that, adjust the volume to 500 mL with deionized water and mix well.

4. **Mobile phase**

Weight 1 g of ammonium acetate into 100 mL beaker. Then, add methanol about 50 mL, mix well. Then, adjust the volume to 100 mL with methanol. Take 800 mL of acetonitrile into 1000 mL cylinder and add the prepared ammonium acetate. Then, add 5 mL of methanol and adjust the volume to 1000 mL with

dichoromthane. After that, add 1 mL of TEA and mix well. Keep mobile phase in 1000 mL bottle.

C2. Reagent Preparations for Flavonoid Content Determination

Reagents

- Methanol
- Hydrochloric acid (HCl)
- Ascorbic acid
- Trifluoroacetic acid (TFA)
- Acetonitrile (CH_3CN)

Reagent preparation

1. 1% Ascorbic acid

Weight 1 g of ascorbic acid into 100 mL volumetric flask. After that, adjust the volume to 100 mL with deionized water and mix well.

2. 62.5% (v/v) Methanol

Take 625 mL of methanol into 1000 mL cylinder and add up to 1000 mL with deionized water and mix.

3. 6M HCl

Take 200 mL of deionized water into 500 mL volumetric flask. Add 26 mL of HCl, adjust the volume to 500 mL with deionized water and mix.

4. 5% (w/w) TFA in methanol

Add 210 µL of TFA in 1000mL of methanol and then, mix it.

5. 0.5% (w/w) TFA

Add 210 µL of TFA in 1000mL of water and then, mix it.

6. 0.5% (w/w) TFA in acetonitrile

Add 210 µL of TFA in 1000mL of acetonitrile and then, mix it.

C3. Reagent Preparations for Lipase Inhibitory Assays

Reagents

- Absolute methanol

- Tris(hydroxymethyl) aminomethane ($NH_2C(CH_2OH)_3$)
- Di–potassium hydrogen phosphate (K_2HPO_4)
- Potassium dihydrogen phosphate (KH_2PO_4)
- Potassium Chloride (KCl)
- Triton X–100
- Ethylenediaminetetraacetic acid (EDTA)
- 5,5'– dithiobis (2–nitro benzoic acid) (DTNB)
- 2,3–dimercapto–1–propanol tributyrate (DMPTB)
- Bovine serum albumin (BSA)
- *Candida rugosa* lipase (Type 8, ≥700 unit/mg)

Reagent preparation

1. 50 mM Tris, 10 mM KCl, 1 mM EDTA, pH 7.2 (Assay buffer)

Weight 6.057 g of $NH_2C(CH_2OH)_3$, 0.7455 g of KCL and 0.3722 g of EDTA into 1 L beaker. Then, add deionized water about 500 mL, mix well and adjust pH to 7.2. Then, adjust the volume to 1 L with deionized water and mix well. Keep the solution in a refrigerator.

2. 10% (v/v) Triton X–100 in 50 mM Tris, 10 mM KCl, 1 mM EDTA, pH 7.2 (Substrate buffer)

Take 90 mL of assay buffer into 150 mL beaker. Then, add 10 mL of Triton X–100 and mix well. Keep the solution in a refrigerator.

3. 50 mM Tris, 10 mM KCl, 1 mM EDTA with 0.1% BSA, pH 8.0 (Enzyme buffer)

Take 250 mL of assay buffer into 250 mL beaker. Then, add 0.5 g of BSA and mix well. Adjust pH to 8.0 with a few drops of a NaOH concentrated solution. Keep the solution in a refrigerator.

4. 50 mM potassium phosphate buffer (KPB), pH 7.4

Take 30.1 mL of 1 M K_2HPO_4 stock solution into 1 L volumetric flask and add 9.9 mL of 1 M KH_2PO_4 stock solution and then add deionized water about 500 mL and mix well. Then, adjust pH to 7.4 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.

5. 200 mM DTNB stock solution in Methanol

Weight 0.7927 g of DTNB into 10 mL volumetric flask and adjust the volume to 10 mL with absolute methanol.

6. 16 mM DTNB in 50 mM KPB

Take 920 µL of 50 mM KPB into 1.5 mL tube. Then, add 80 µL of 200 mM DTNB stock solution and mix well.

7. 10 mM DMPTB stock solution

Take 10 mL of substrate buffer into 15 mL tube and remove 33.45 µL. Then, add 33.45 µL of DMPTB solution and mix well. Keep the solution in a refrigerator.

8. 0.2 mM DMPTB

Take 10 mL of assay buffer into 15 mL tube and remove 200 µL. Then, add 200 µL of 10 mM DMPTB stock solution, mix well and store in an ice bath before used.

9. 1 mg/mL *Candida rugosa* lipase stock solution

Weight 1 mg of *Candida rugosa* lipase into 1.5 mL tube. Then, adjust the volume to 1 mL with enzyme buffer. Mix well and keep the solution in a refrigerator.

10.0.002 mg/mL *Candida rugosa* lipase

Take 10 mL of enzyme buffer into 15 mL tube and remove 20 µL. Then, add 20 µL of 1 mg/mL *Candida rugosa* lipase stock solution, mix well and store in an ice bath before used.

C4. Reagent Preparations for α-amylase Inhibitory Assays

Reagents

- Di–potassium hydrogen phosphate (K_2HPO_4)
- Potassium dihydrogen phosphate (KH_2PO_4)
- Potassium Chloride (KCl)
- *p*-nitrophenyl-α-D-maltopentaoside (PNPG-5)
- Porcine pancreatic α-amylase (Type 7, ≥10 unit/mg)

Reagent preparation

1. 50 mM potassium phosphate buffer KPB, pH 7

Take 30.1 mL of 1 M K_2HPO_4 stock solution into 1 L volumetric flask and add 9.9 mL of 1 M KH_2PO_4 stock solution and then add deionized water about 500 mL and mix well. Then, adjust pH to 7 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.

2. 50 mM KPB, 200 mM KCl (Buffer)

Weight 1.491 g of KCl into 100 mL beaker. Then, add potassium phosphate buffer (KPB) about 100 mL, mix well. Keep the solution in a refrigerator.

3. 25 mM PNPG-5 stock solution

Take 1.053 mL of buffer into stock PNPG-5 bottle, mix well and keep the solution in a refrigerator.

4. 5 mM PNPG-5

Take 4 mL of buffer into 15 mL tube. Then, add 1 mL of PNPG-5 stock solution, mix well and store in an ice bath before used.

5. 40 mg/mL porcine pancreatic α-amylase

Weight 0.4 g of porcine pancreatic α-amylase into 50 mL tube and dissolve enzyme overnight. Then, centrifuge at 4,500 rpm for 10 minites, filtrate and store in an ice bath before used.

C5. Reagent Preparations for α-glucosidase Inhibitory Assays

Reagents

- Di–potassium hydrogen phosphate (K_2HPO_4)
- Potassium dihydrogen phosphate (KH_2PO_4)
- *p*-nitrophenyl-α-D-glucopyranaide (PNPG)
- *Saccharomyces cerevisiae* α-glucosidase (Type 1, ≥10 unit/mg)

Reagent preparation

1. 50 mM potassium phosphate buffer KPB, pH 7

Take 30.1 mL of 1 M K_2HPO_4 stock solution into 1 L volumetric flask and add 9.9 mL of 1 M KH_2PO_4 stock solution and then add deionized water

about 500 mL and mix well. Then, adjust pH to 7 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.

2. 2 mM PNPG

Weight 0.006 g of PNPG into 15 mL tube. Then, add 10 mL of potassium phosphate buffer (KPB) mix well and store in an ice bath before used.

3. 100 U/mL *Saccharomyces cerevisiae* α-glucosidase stock solution

Take 1 mL of KPB, pH 7 into stock enzyme bottle mix well. Keep the solution in a refrigerator (-20°C)

4. 0.1 U/mL *Saccharomyces cerevisiae* α-glucosidase

Take 9.99 mL of enzyme buffer into 15 mL tube. Then, add 0.01 mL of 0.01 U/mL *Saccharomyces cerevisiae* α-glucosidase stock solution, mix well and store in an ice bath before used.

C6. Reagent Preparations for ACE Inhibitory Assays

Reagents

- Di–potassium hydrogen phosphate (K_2HPO_4)
- Potassium dihydrogen phosphate (KH_2PO_4)
- Sodium hydroxide (NaOH)
- Sodium Chloride (NaCl)
- Hydrochloric acid (HCL)
- *o*-phthaldialdehyde
- Hippuryl-histidyl-leucine (HHL)
- Rabbit lung angiotensin-converting enzyme (≥2 unit/mg)

Reagent preparation

1. 100 mM potassium phosphate buffer KPB, pH 8.3

Take 47 mL of 1 M K_2HPO_4 stock solution and 3 mL of 1 M KH_2PO_4 stock solution into 500 mL beaker Then, add deionized water about 400 mL and mix well. Then, adjust pH to 8.3 with a few drops of a NaOH concentrated solution. After

that, adjust the volume to 500 mL with deionized water and mix well. Keep the solution in a refrigerator.

2. 0.025 M NaOH

Weight 0.1 g of NaOH into 100 mL beaker and adjust volume to 100 mL with deionized water.

3. 0.28 M NaOH

Weight 5.6 g of NaOH into 500 mL beaker and adjust volume to 500 mL with deionized water.

4. 3 M NaCl

Weight 17.53 g of NaCl into 100 mL beaker and adjust volume to 100 mL with deionized water.

5. 3 M HCl

Take 24.87 mL of 12.06 M HCl into 100 volumetric flask and adjust volume to 100 mL with deionized water.

6. 20 mg/mL *o*-phthaldialdehyde

Weight 40 mg of *o*-phthaldialdehyde into 2 mL tube. Then, add 2 mL of absolute methanol and mix well.

7. 3 mM Hippuryl-histidyl-leucine (HHL)

Weight 25.77 mg of HHL (429.27 g/mol) into 50 mL tube and add 4.165 mL of 0.025 NaOH. Then, 13.835 mL of 100 mM KPB, pH 8.3 and add 2 mL of 3 mM NaCl. Mix well and keep the solution in a refrigerator.

8. 1 U/mL ACE stock solution

Take 1 mL of KPB into stock enzyme bottle mix well. Keep the solution in a refrigerator (-20°C)

9. 0.5 U/mL ACE

Take 0.5 mL of KPB, pH 8.3 into 1 mL tube. Then, add 0.5 mL of 1 U/mL ACE stock solution, mix well and store in an ice bath before used.

APPENDIX D

ENZYME REACTION OF LIPASE ASSAY

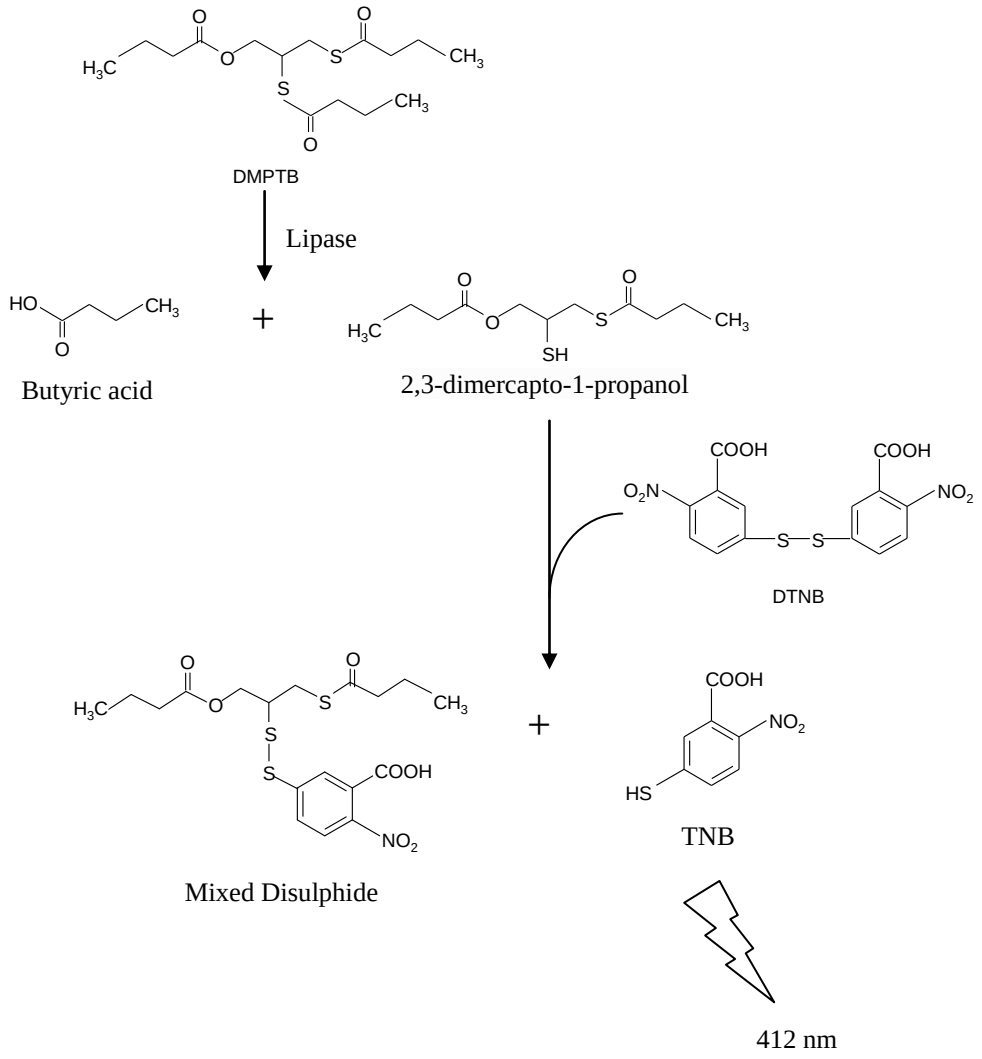


Figure D. Lipase hydrolyzes 2,3–dimercapto–1–propanol tributyrate (DMPTB) into butyric acid and 2,3-dimercapto-1-propanol. Then, 2,3-dimercapto-1-propanol reacts with 5,5'– dithiobis (2–nitro benzoic acid) (DTNB) releasing mixed disulphide and 2-nitro-5-mercaptobenzoic acid (TNB). TNB has yellow color and absorbs light at 412 nm.

APPENDIX E
ENZYME REACTION OF α-AMYLASE ASSAY

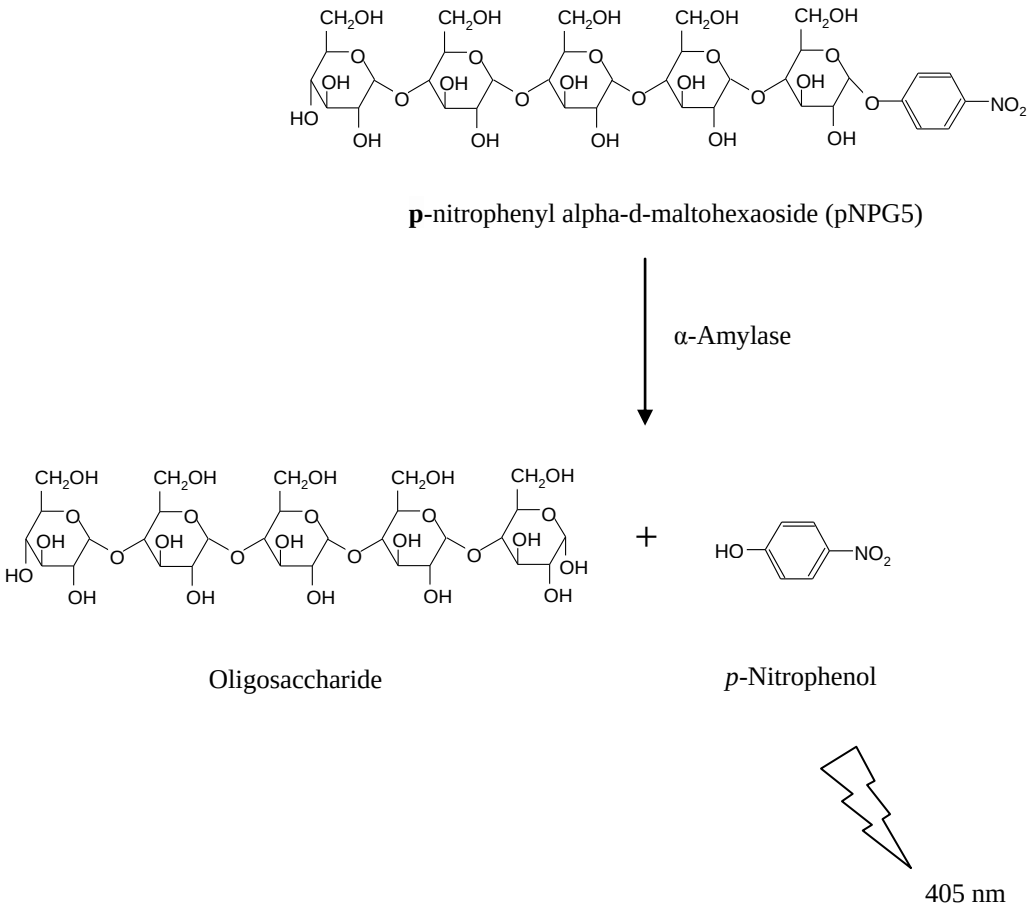


Figure E. The α-amylase hydrolyzes *p*-nitrophenyl alpha-d-maltohexaoside (pNPG5) into oligosaccharide and *p*-nitrophenol. The *p*-nitrophenol absorbs light at 405 nm.

APPENDIX F
ENZYME REACTION OF α-GLUCOSIDASE ASSAY

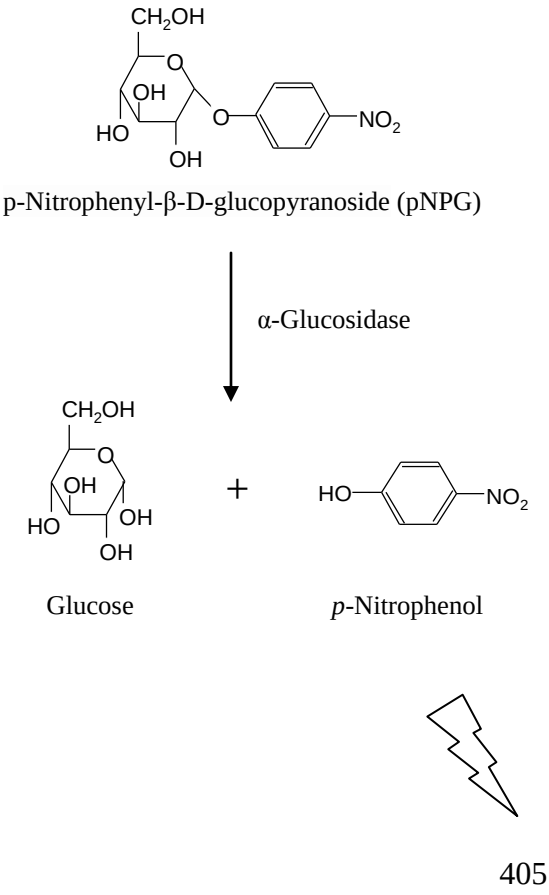


Figure F. The α-glucosidase hydrolyzes *p*-Nitrophenyl-β-D-glucopyranoside (pNPG) into glucose and p-nitrophenol. The *p*-nitrophenol absorbs light at 405 nm.

APPENDIX G
ENZYME REACTION OF ACE ASSAY

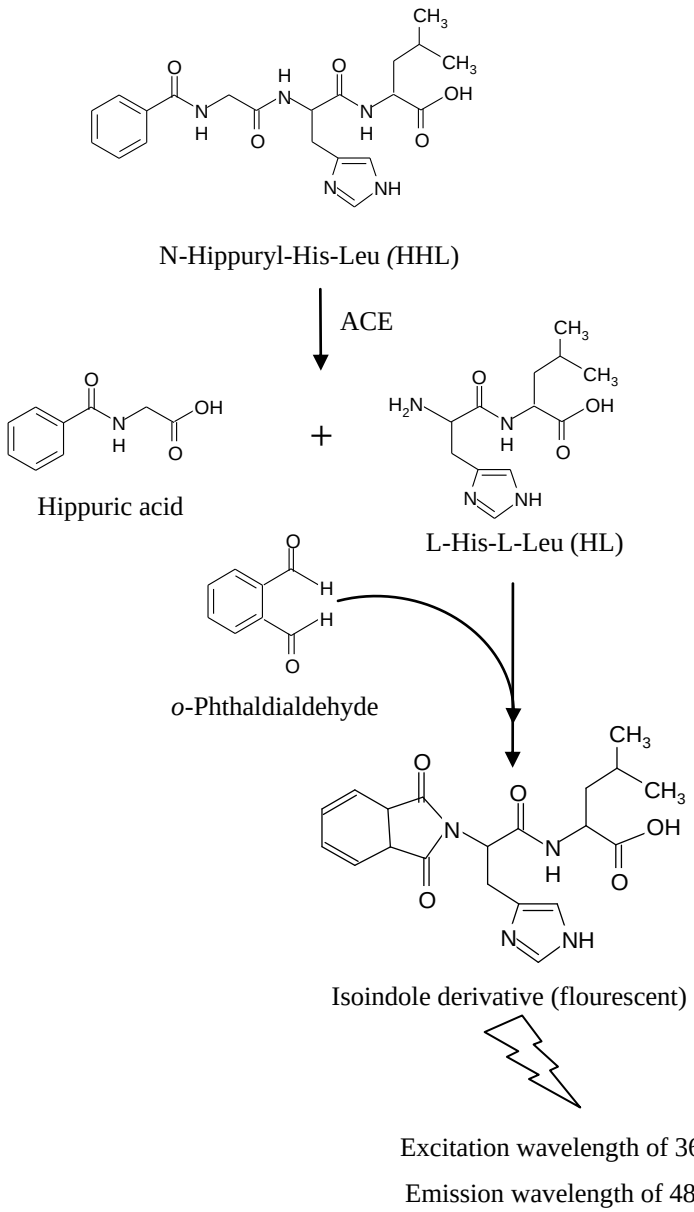


Figure G. The ACE hydrolyzes *N*-Hippuryl-His-Leu *(*HHL) into hippuric acid and *L*-His-*L*-Leu (HL). Then, HL reacts with *o*-phthaldialdehyde releasing isoindole derivative, a fluorescent product. The assay is monitored by measuring excitation wavelength of 360 nm and emission wavelength of 485 nm.

APPENDIX H
HPLC CHROMATOGRAM OF CAROTENOIDS

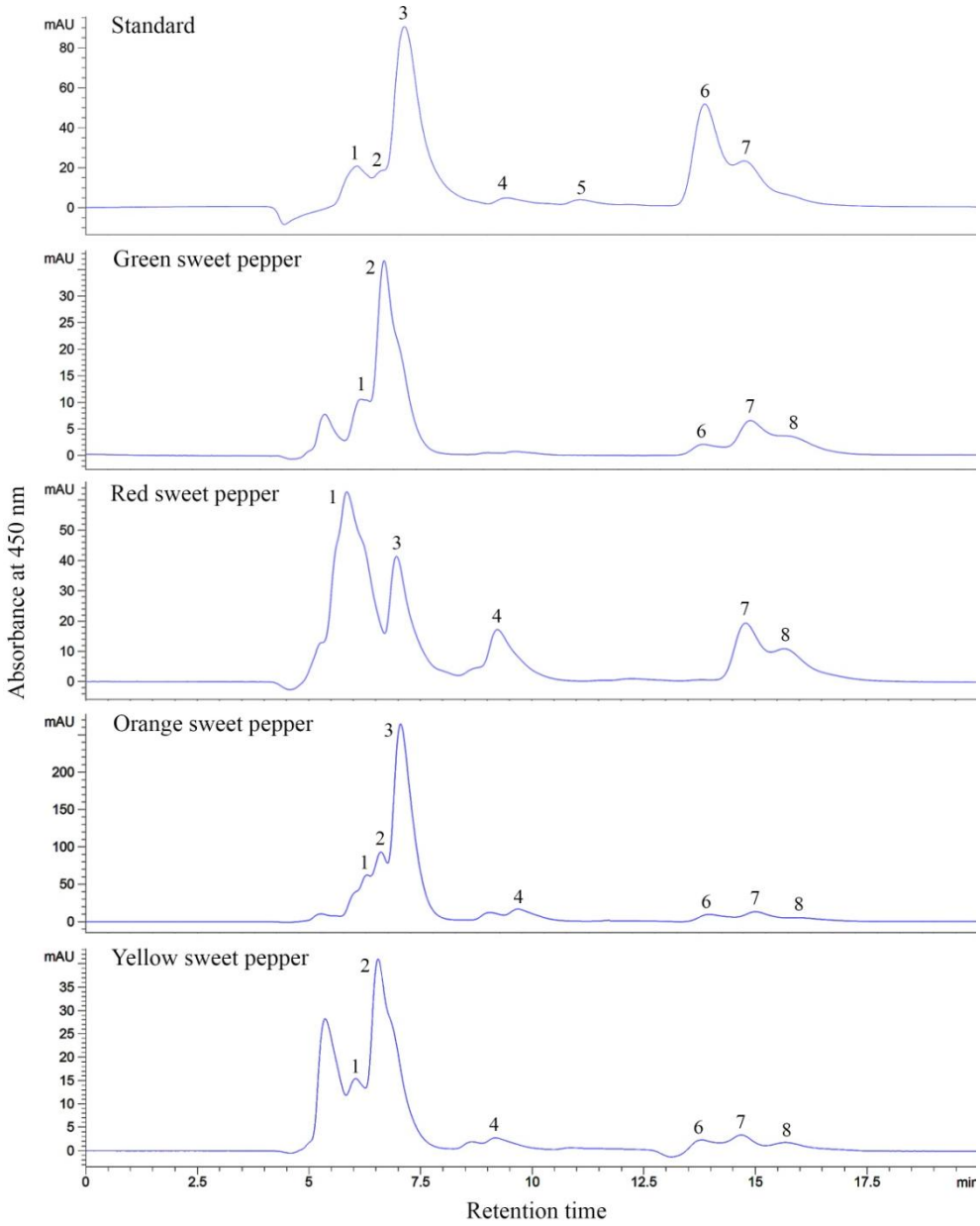


Figure H1. The carotenoids chromatogram of standard, red and yellow sweet peppers (a: Capsanthin, b: Lutein, c: Zeaxanthin, d: β-cryptoxanthins, e: Lycopene, f: α-carotene, g: trans-β-carotene and h: cis-β-carotene)

APPENDIX I

HPLC CHROMATOGRAM OF FLAVONOIDS AND PHENOLICS

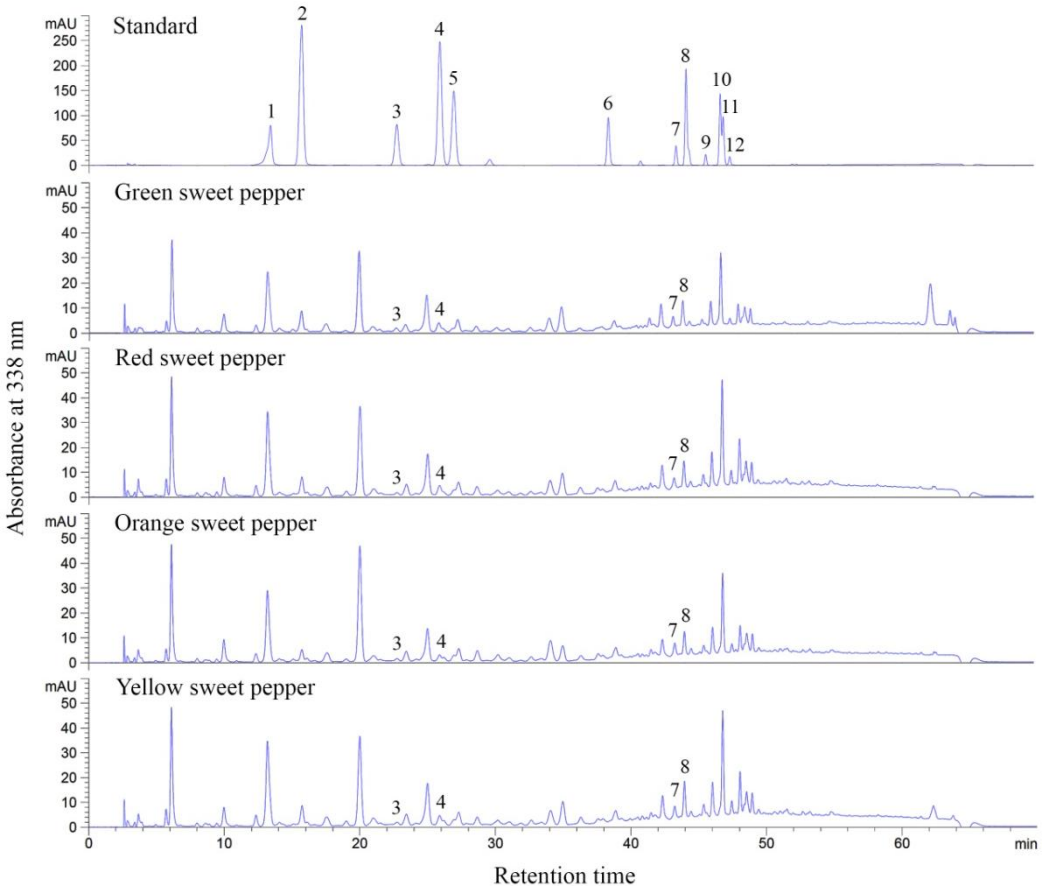
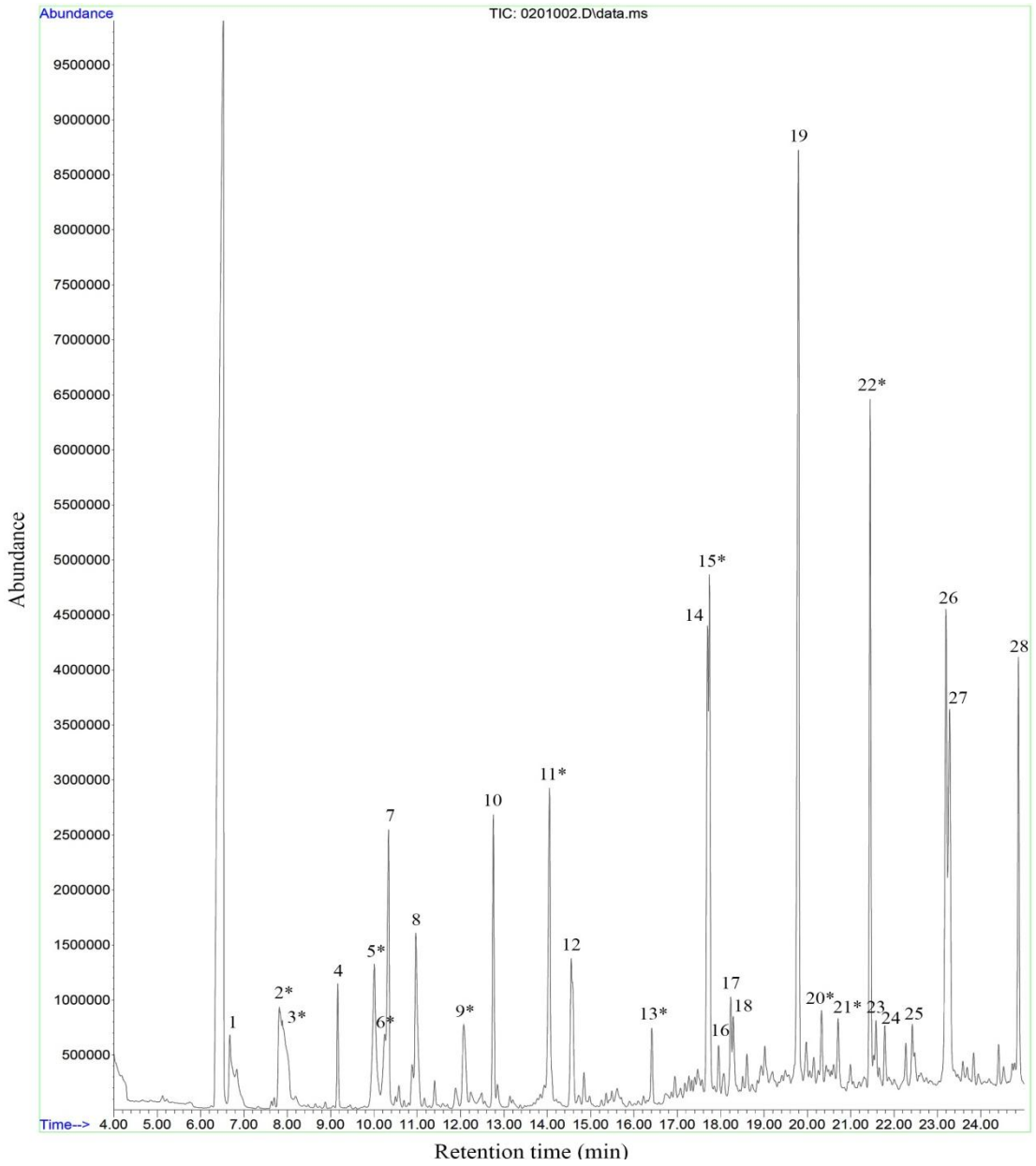


Figure I. The flavonoids and phenolic acids chromatogram of standard, green, red, orange and yellow sweet peppers (1: chlorogenic acid, 2: caffeic acid acid, 3: *p*-courmaric acid, 4: Ferulic acid, 5: sinapic acid, 6: myricetin, 7: quercetin, 8: luteolin, 9: hesperitin, 10: kamepferol, 11: apigenin and 12: isorhamnetin)

APPENDIX J
GC-MS CHROMATOGRAM OF VOLATILE COMPOUNDS

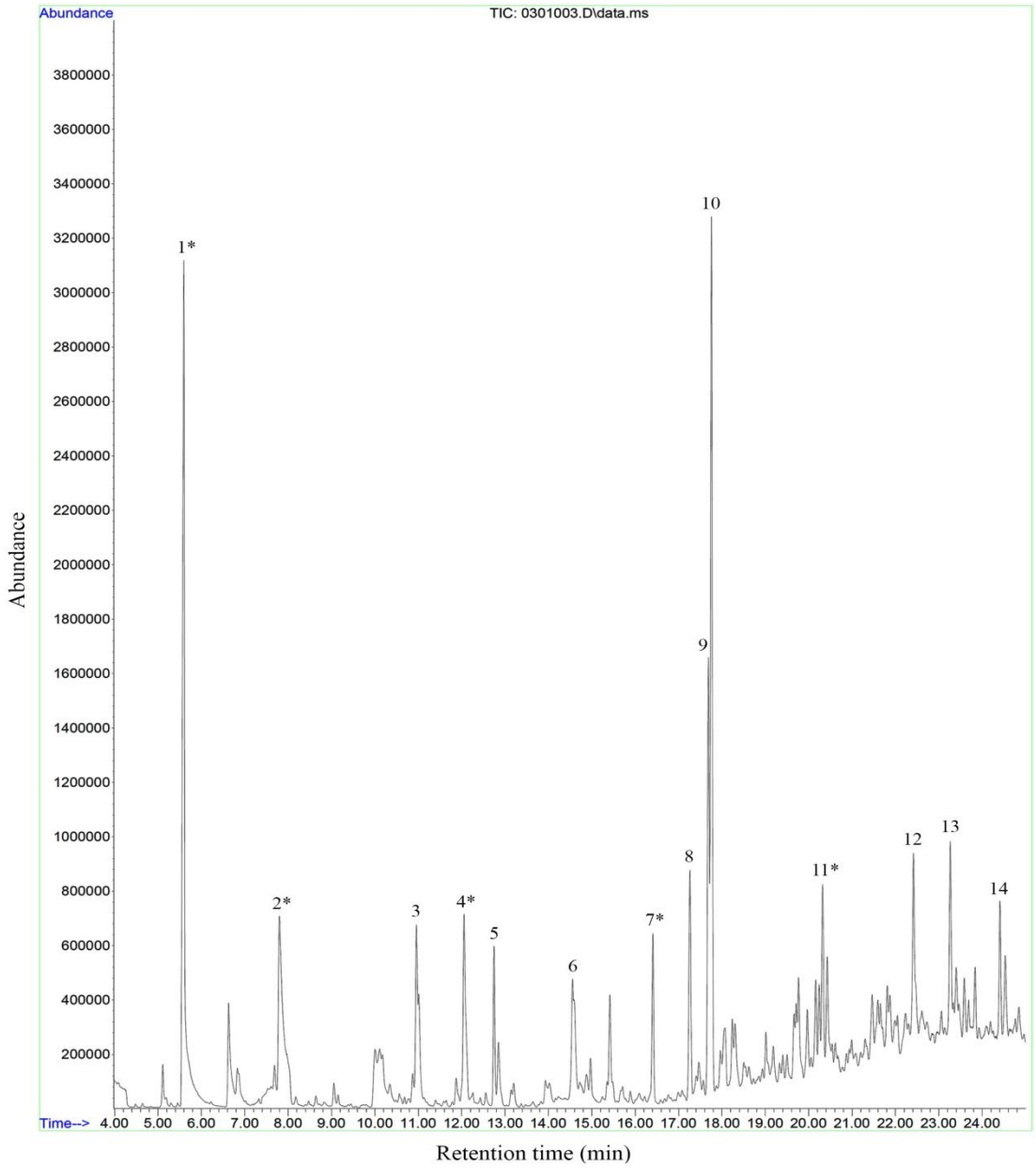
J1. Green sweet pepper



Peak	RT	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 or 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 or 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 or 1-Propylpentachlorotriphosphazene
18	18.289	1-Cyclohexyldimethylsilyloxyoctadecane
19	19.794	n-Nonyl-cyclopropane or 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

***** The contaminants from using DVB/CAR/PDMS fiber

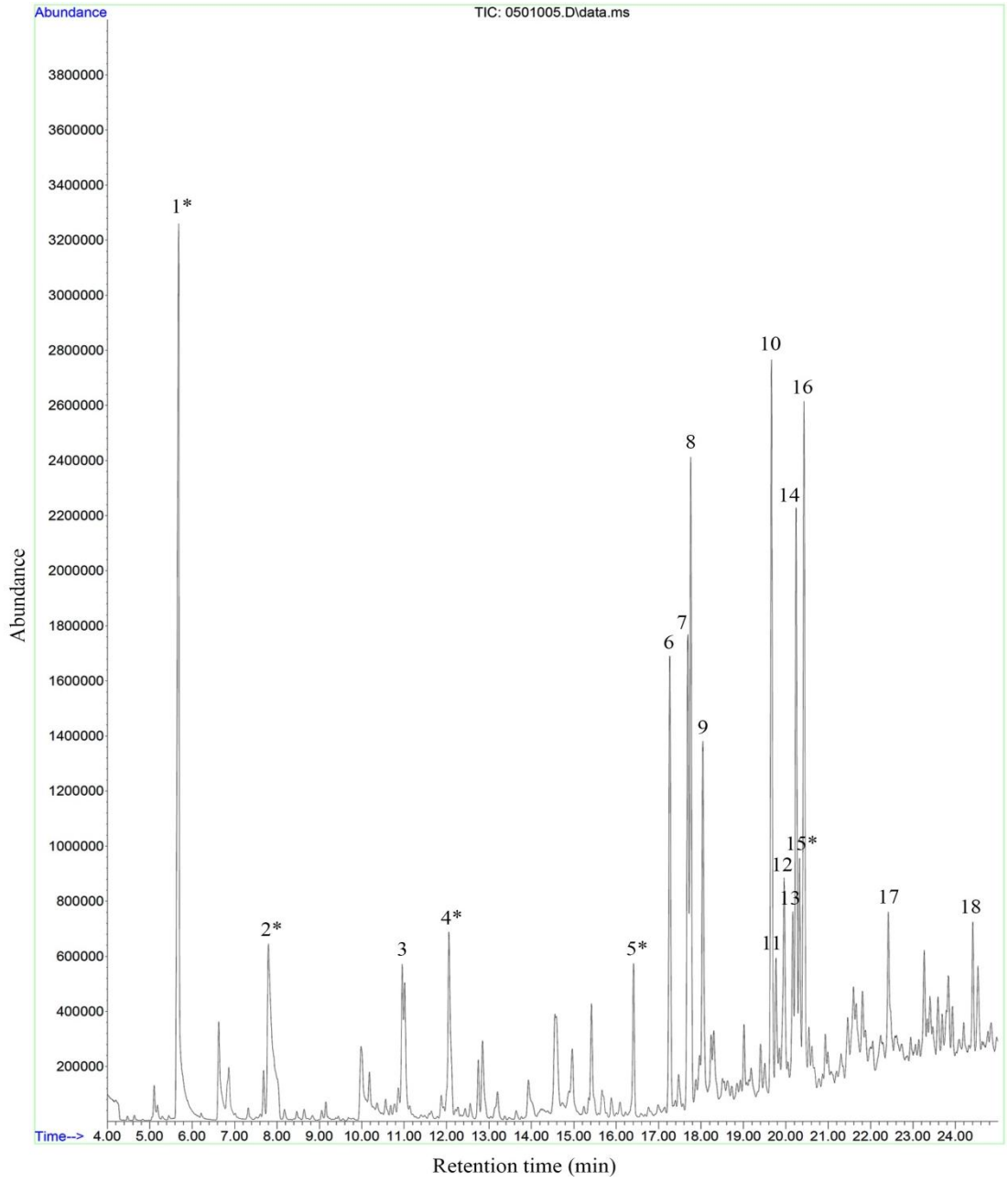
J2. Red sweet pepper



Peak	RT	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

***** The contaminants from using DVB/CAR/PDMS fiber

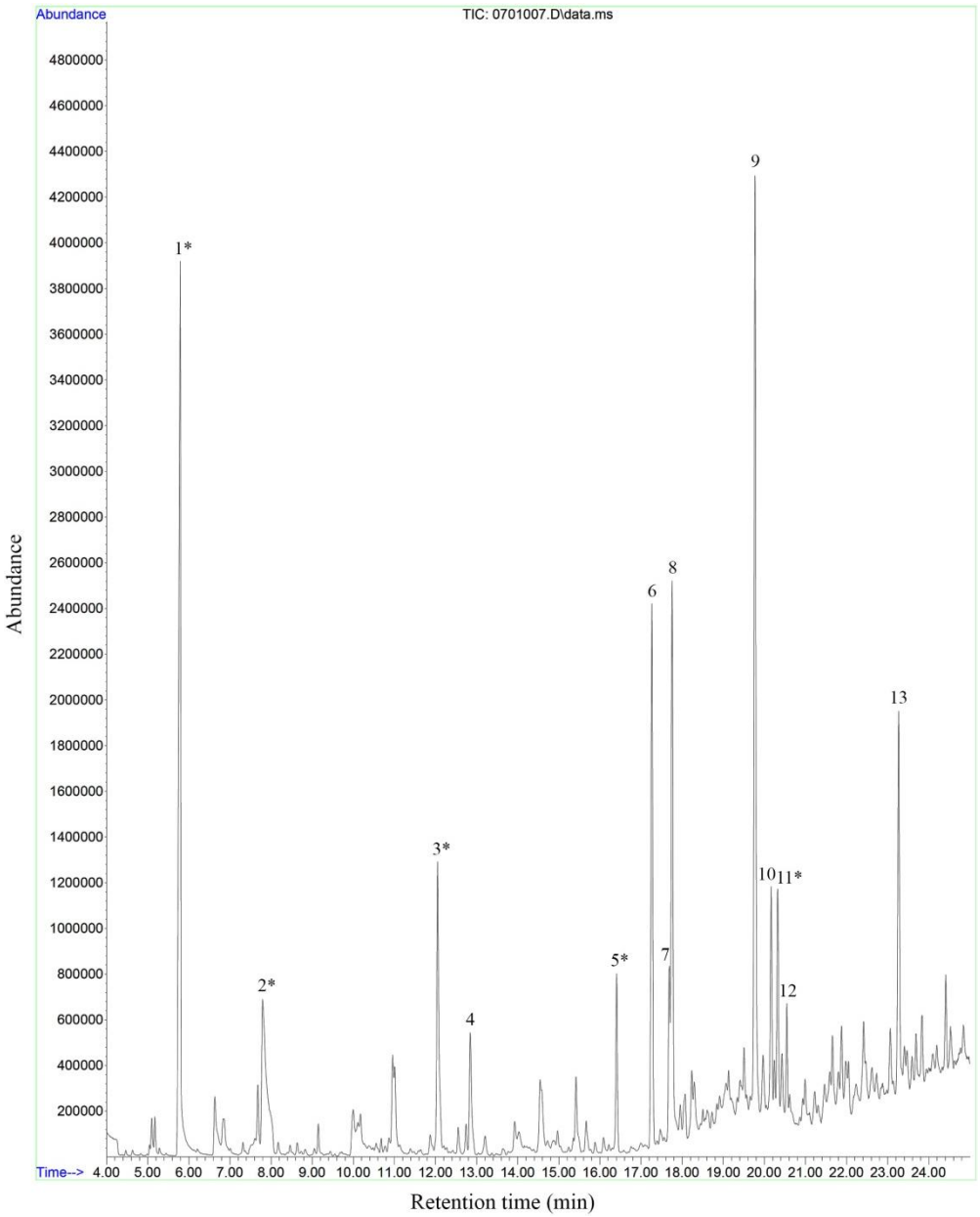
J3. Orange sweet pepper



Peak	RT	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

***** The contaminants from using DVB/CAR/PDMS fiber

J4. Yellow sweet pepper



Peak	RT	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 or 2,4,5-Trichloro-pyrimidine
7	17.688	Copaene
8	17.757	4-Bromo-2-chlorobenzenamine
9	19.777	n-Nonyl-cyclopropane or 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

***** The contaminants from using DVB/CAR/PDMS fiber

APPENDIX K
MOISTURE CONTENTS AND COLOR VALUES OF SWEET PEPPERS

Table K Moisture contents and color values (L*, a*, b*) of sweet peppers

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

L* = the value of brightness (0 = darkness, 100 = lightness)
a* = the value of red and green ((+) = redness, (-) = greenness)
b* = the value of yellow and blue ((+) = yellowness, (-) = blueness)