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APPENDIX

Appendix A

MICRO-ALGAL GROWTH MEDIA

Bold's Basal Medium (BBM) (Stein, 1973)

1. KH_2PO_4	stock solution	8.75 g/500 ml	10 ml
2. $CaCl_2.2H_2O$		1.25 g/500 ml	10 ml
3. $MgSO_4.7H_2O$		3.75 g/500 ml	10 ml
4. $NaNO_3$		12.5 g/500 ml	10 ml
5. K_2HPO_4		3.75 g/500 ml	10 ml
6. NaCl		1.25 g/500 ml	10 ml
7. Na_2EDTA-KOH		10 g/L/ 6.2 g/L	1 ml
8. $FeSO_4.7H_2O$-H_2SO_4 (conc.)		4.98 g/L/ 1 ml/L	1 ml
9. Trace Metal Solution See below*			1 ml
10. H_3BO_3		5.75 g/500 ml	0.7 ml

***Trace Metal Solution:** (g/l)

1. H_3BO_3	2.86 g
2. $MnCl_2.4H_2O$	1.81 g
3. $ZnSO_4.7H_2O$	0.222 g
4. Na $MoO_4.5H_2O$	0.390 g
5. $CuSO_4.5H_2O$	0.079 g
6. $Co(NO_3)_2.6H_2O$	0.0494 g

Deionized water 1 liter

pH of medium was adjusted to 6.8 with 0.1 N NaOH. The medium was autoclaved at 121°C for 15 min.

Appendix B

CHEMICALS AND SOLUTIONS

1. Solutions for DNA extraction (Gibco BRL)

TE buffer (10 mM Tris-HCl, 1 mM EDTA. pH 7.5)

0.12 g Tris-HCl, 0.037 g EDTA were added to distilled water. The final volume was made to 100 ml. 0.1 N NaOH was used to adjust pH to 8.0 before autoclaving at 121°C for 15 min.

10% SDS

Dissolve 10 g SDS in 90 ml water with gentle stirring and bring to 100 ml with distilled water

Phenol:Chloroform:Isoamyl alcohol 25: 24: 1 (v/v/v)

Chloroform:Isoamyl alcohol 24: 1 (v/v/v)

3 M Sodium acetate

24.61 g Sodium acetate was added 100 ml distilled water.

Absolute ethanol

70% Ethanol

2. Solutions for β-carotene extraction

60% KOH

60 g KOH was added to distilled water. The final volumn was made to 100 ml.

95% Ethanol

9 % NaCl

9 g NaCl was added to distilled water. The final volumn was made to 100 ml.

Diethyl ether

Na_2SO_4

β-carotene (Merck)

Mobile phase

Acetonitrile: Dichloromethane: Methanol = 70: 20: 1

3. Solution for Quercetin extraction

0.2% Tert Butyl Hydro Quinone (Merck: 8.41424)

0.2 g was added to 100 ml Methanol.

0.1% L-Ascorbic acid (Sigma: A-5960)

0.1 g was added to 100 ml Milli-Q-Water.

10 M HCl

Milli-Q-Water

Quercetin standard (Sigma: Q-0125)

Quercetin-3- β-D-glycoside (Fluka: 17793)

Standard Matrix Solution (SMS)

1. Tert Butyl Hydro Quinone	25 ml
2. L-Asorbic acid	2 ml
3. Milli-Q-Water	19 ml
4. 10 M HCl	6 ml
5. Methanol	150 ml
Total Volumn	202 ml

Mobile phase

0.025 M KH_2PO_4 pH 2.4: Milli Q-water: Acetonitrile: = 1: 9: 3.6

Appendix C

CALCULATIONS FOR β-CAROTENE AND QUERCETIN CONTENTS

1. **Spectrophotometry for β-carotene contents**

MW = 536.85

A= εlc (Beer's Lambert Law)

A = Absorbance

ε = Extinction Coefficient ($cm^{-1}.mole^{-1}.l$) which $\varepsilon^{1\%}_{M}$ in Diethyl ether = 2500

l = length path cuvette (1 cm)

c = concentration (mole/ l)

A= εlc

c = A/ (εl)

c= A/ (2500x1) mole/ l

1000 ml contain A/2500 mole

If adjust to 25 ml contain $\frac{A \times 25}{2500 \times 1000}$ mole

g = mole x MW

$= \frac{A \times 25}{2500 \times 1000} \times 536.85$

Dry cell weight 50 mg contain β-carotene $= \frac{A \times 25}{2500 \times 1000} \times 536.85$ g

If 1 mg $= \frac{A \times 25}{2500 \times 1000 \times 50} \times 536.85 \times 10^{6}$ μg/mg cells

2. reversed-phase HPLC for β-carotene contents

Standard solution 1 mg/ml; 200, 400, 600 and 800 μg/ml.

And make a new standard curve from β-carotene standard extraction

Direct Injection 20 μl; serial dilution 0.2, 0.4, 0.6 and 0.8 μg/μl

Construct equation from standard curve Y= mX

	1 μl	contain β-carotene	X μg
If dilute	500 μl	contain β-carotene	X x 500 = Y μg
Dry cell weight	50 mg	contain β-carotene	Y μg
	1 mg	contain β-carotene	Y/50 = A μg/mg dry cell weight

3. reversed-phase HPLC for Quercetin contents

Standard solution 0.5 mg/ml; 0.5, 1.0, 1.5, 4.0 and 20.0 μg/ml

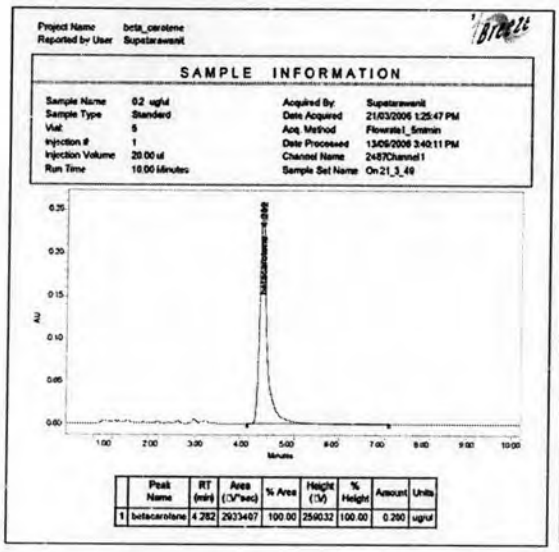
Construct equation from standard curve Y= mX

Inject	20 μl	contain Quercetin	X μg
Adjust volumn to	5000 μl	contain Quercetin	$\frac{X \times 5000}{20}$ = Y μg
Dry cell weight	200 mg	contain Quercetin	Y μg
	1 mg	contain Quercetin	Y/200 = A μg/mg dry cell weight

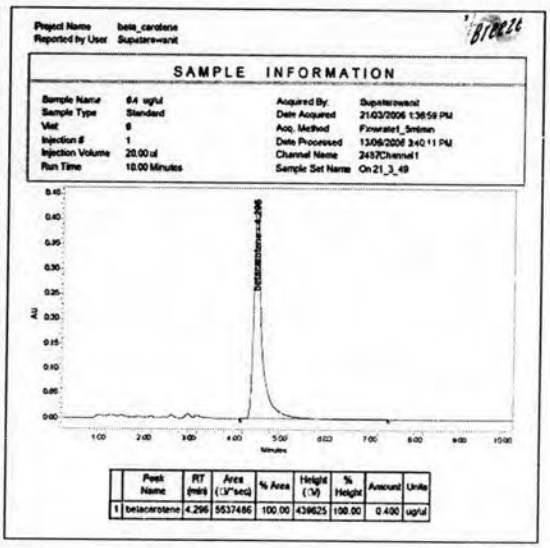
Appendix D

REPRESENTATIVE CHROMATOGRAMS FOR DETERMINATIONS OF β-CAROTENE AND QUERCETIN CONTENTS

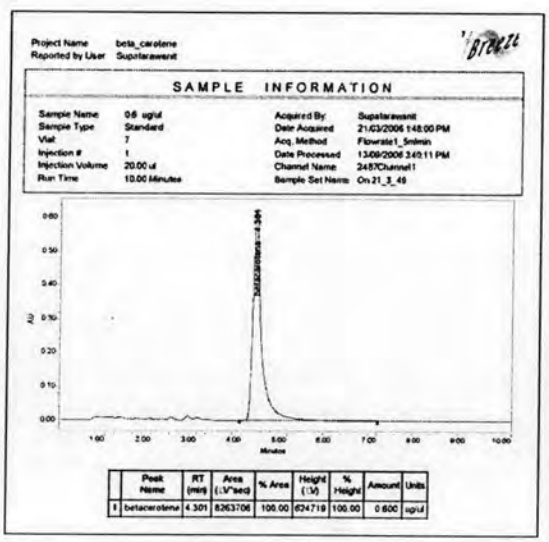
Representative chromatograms of β-carotene standards as determined by reversed phase HPLC were shown in Figure D.1 The retention time of β-carotene standard was 4.2 minutes.



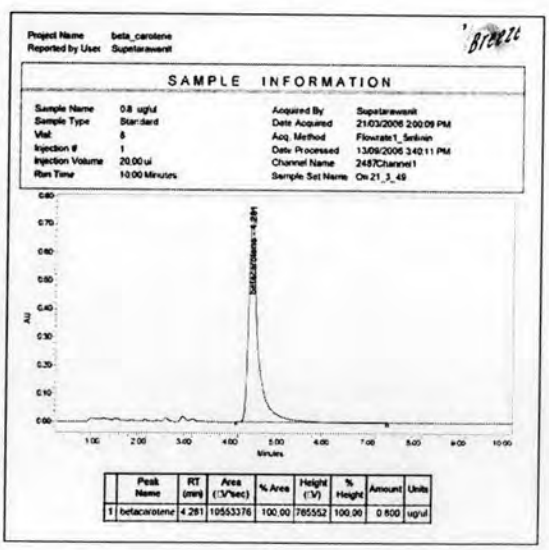
(A)



(B)



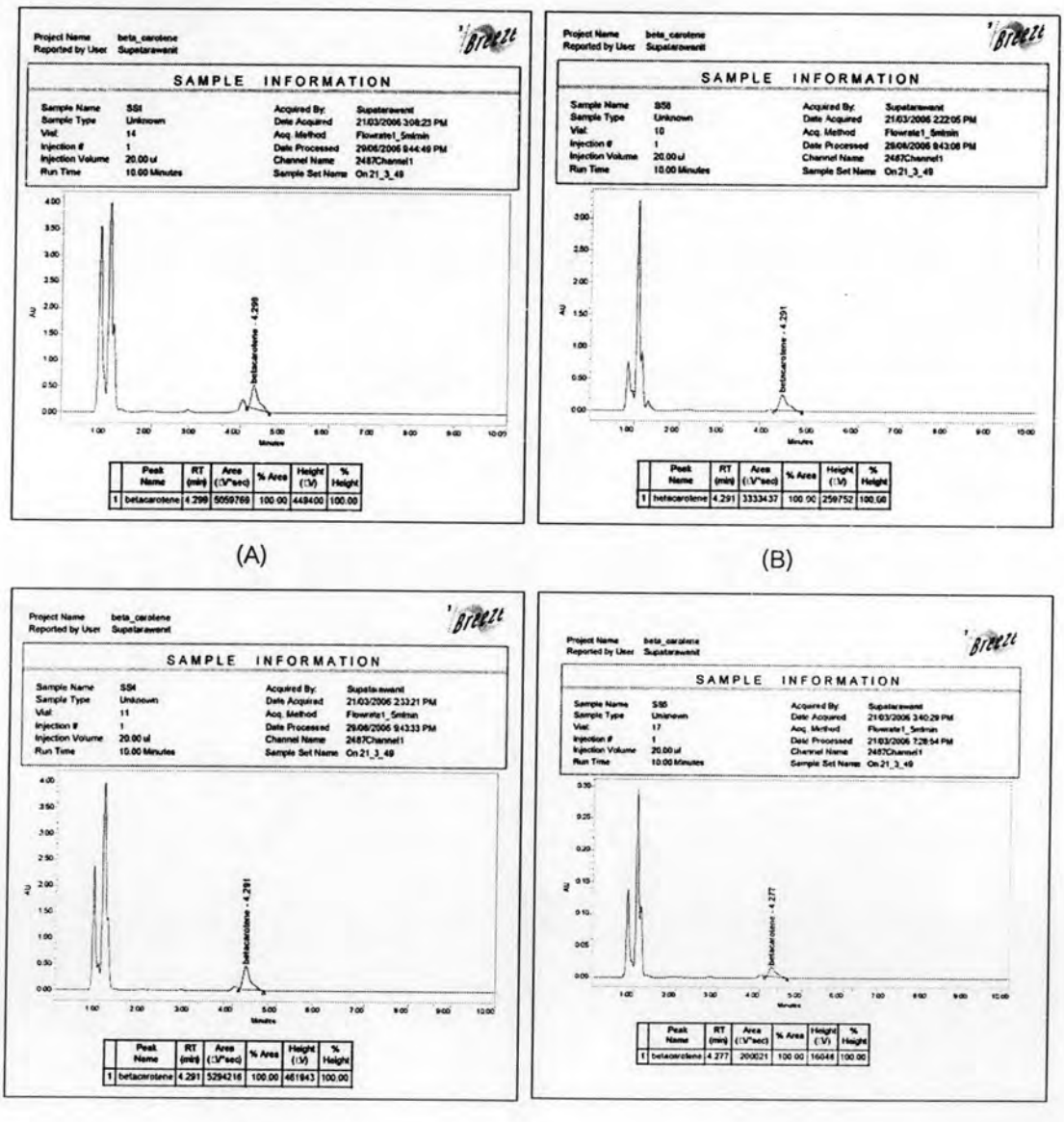
(C)



(D)

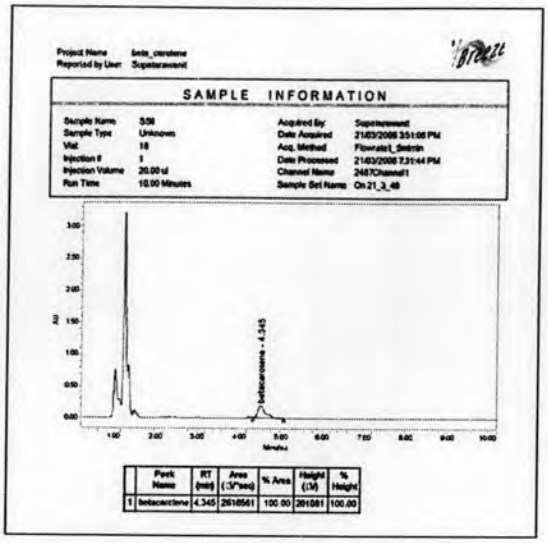
Figure D.1 Representative chromatograms of β-carotene standards (A) 0.2 µg/µl,(B) 0.4 µg/µl, (C) 0.6 µg/µl and (D) 0.8 µg/µl as determined by reversed phase HPLC. Procedures are described in Materials and Methods.

Chromatograms of β-carotene contents in mid-log phase cells of 5 strains of *Chlorella* spp. strains SS1, and SS8 and *Scenedesmus* spp. strains SS4, SS5, and SS9 as determined by reversed-phase HPLC were shown in Figure D.2. The retention time of β-carotene was at 4.2 minutes.



(A) (B)

(C) (D)

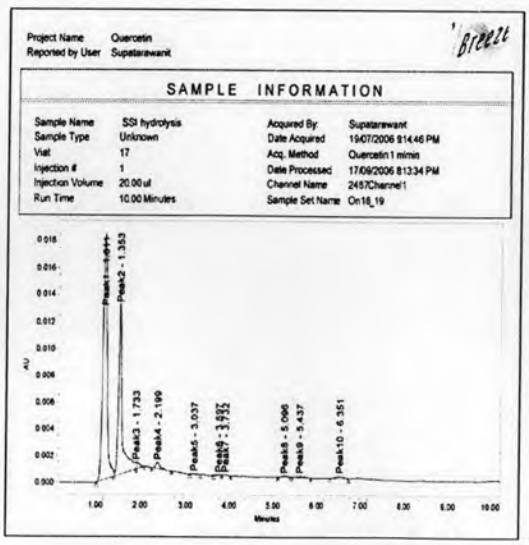


(E)

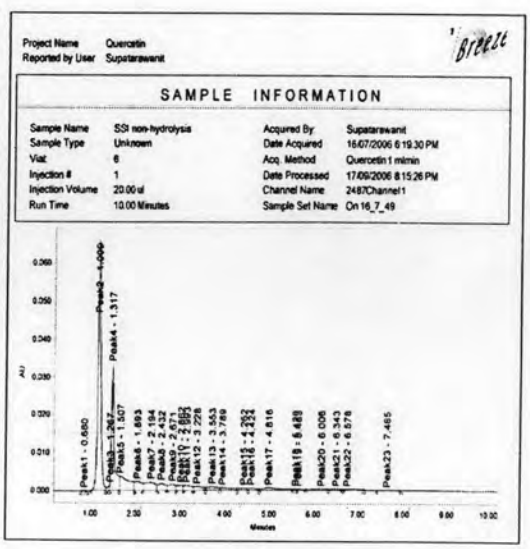
Figure D.2 Chromatograms of β-carotene contents in mid-log phase cells of (A) *Chlorella* sp. strain SS1, (B) *Chlorella* sp. strain SS8, (C) *Scenedesmus* sp. strain SS4, (D) *Scenedesmus* sp. strain SS5, and (E) *Scenedesmus* sp. strain SS9 as determined by reversed-phase HPLC. Procedures are described in Materials and Methods.

Representative chromatograms of extraction Quercetin of mid-log phase cells of 5 strains of *Chlorella* spp. strains SS1, and SS8 and *Scenedesmus* spp. strains SS4, SS5, and SS9 as determined by reversed-phase HPLC were shown in Figure D.3-D.7.

The results showed that Quercetin was not detected in all the algal cells. More cells(up to 2 g) were used with no Quercetin detection.

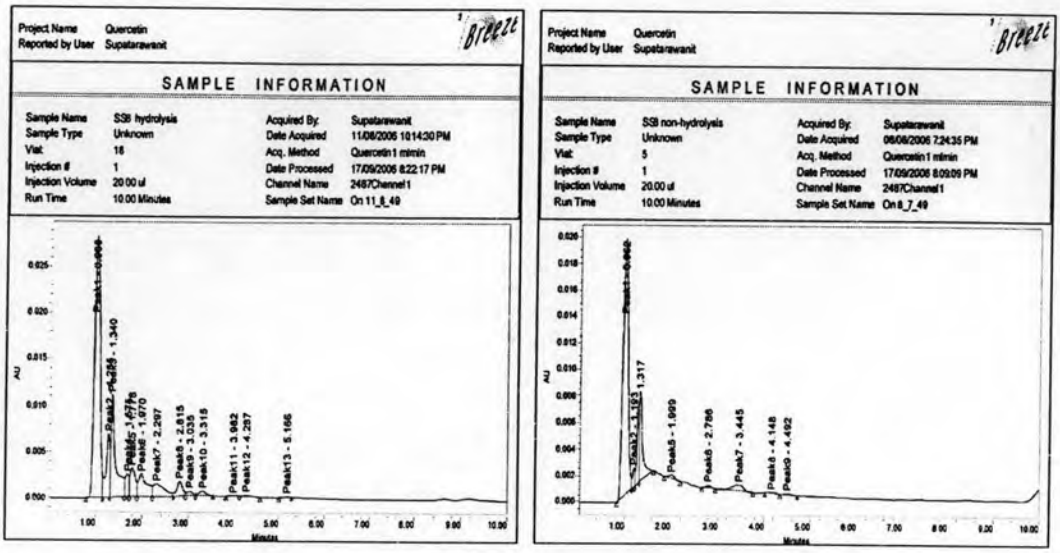


(A)



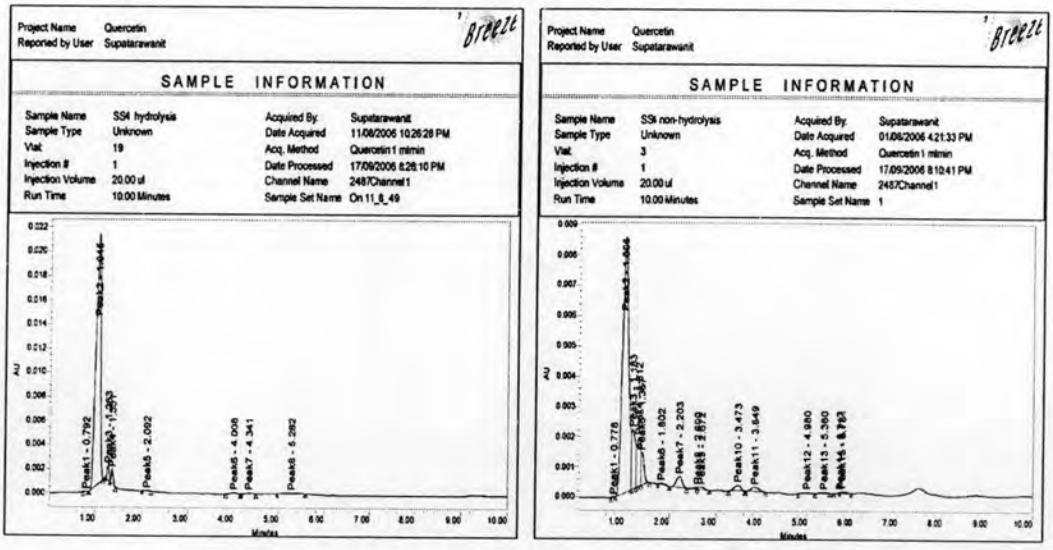
(B)

Figure D.3 Representative chromatograms of *Chlorella* sp. strain SS1: (A) with hydrolysis and (B) without-hydrolysis.



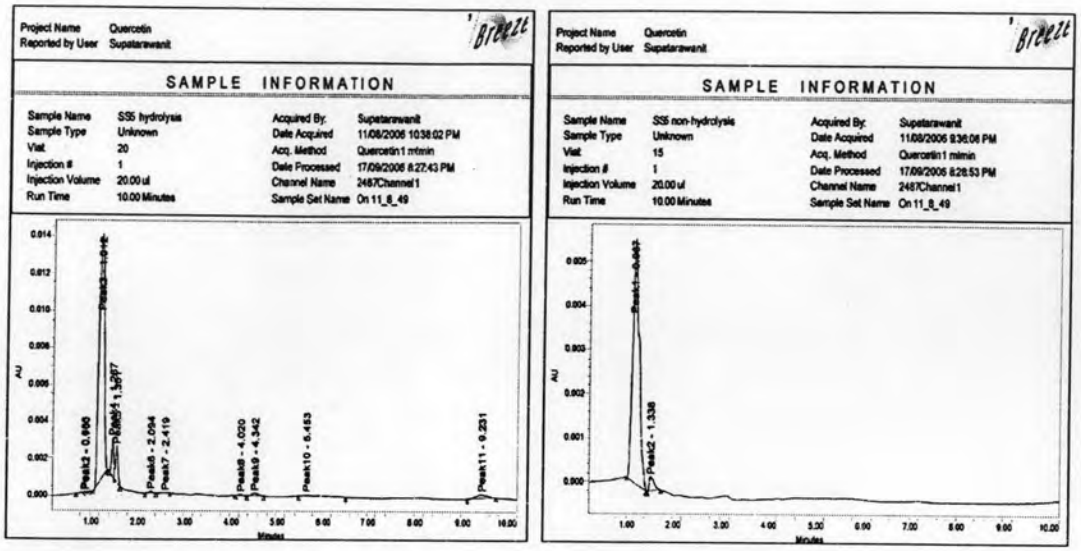
(A) (B)

Figure D.4 Representative chromatograms of *Chlorella* sp. strain SS8: (A) with hydrolysis and (B) without-hydrolysis.



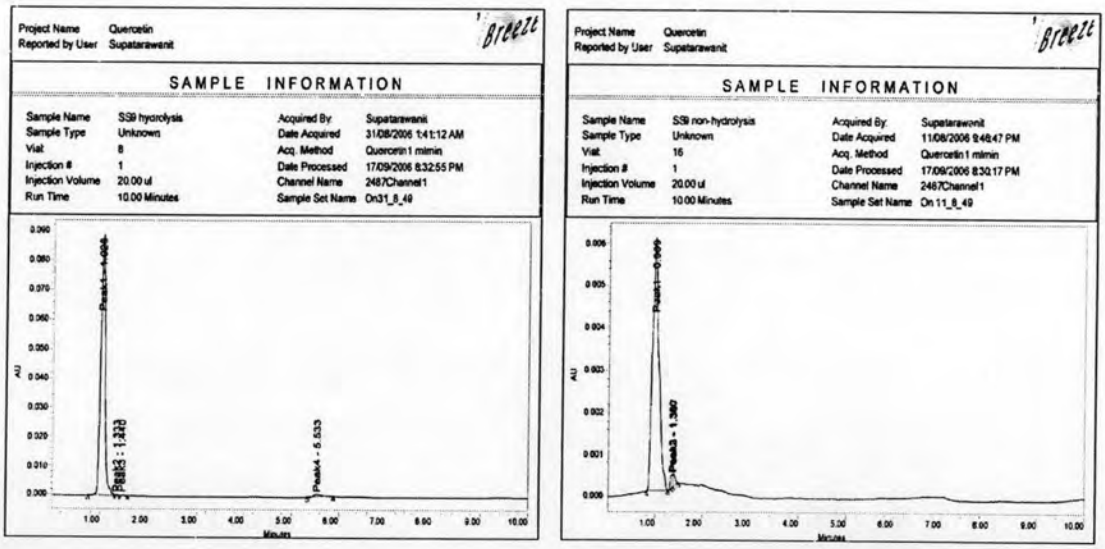
(A) (B)

Figure D.5 Representative chromatograms of *Scenedesmus* sp. strain SS4: (A) with hydrolysis and (B) without-hydrolysis.



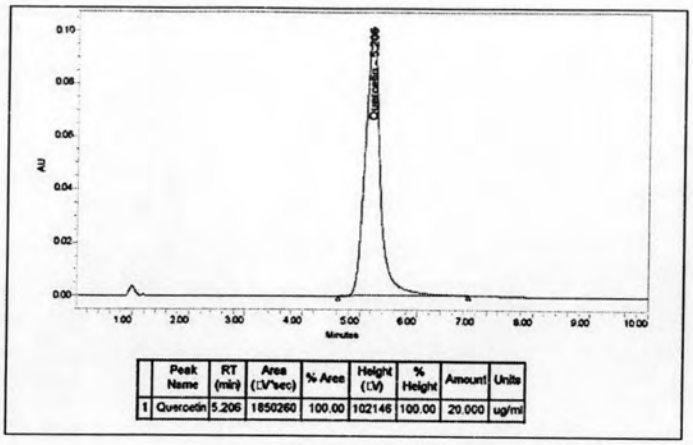
(A) (B)

Figure D.6 Representative chromatograms of *Scenedesmus* sp. strain SS5: (A) with hydrolysis and (B) without-hydrolysis.



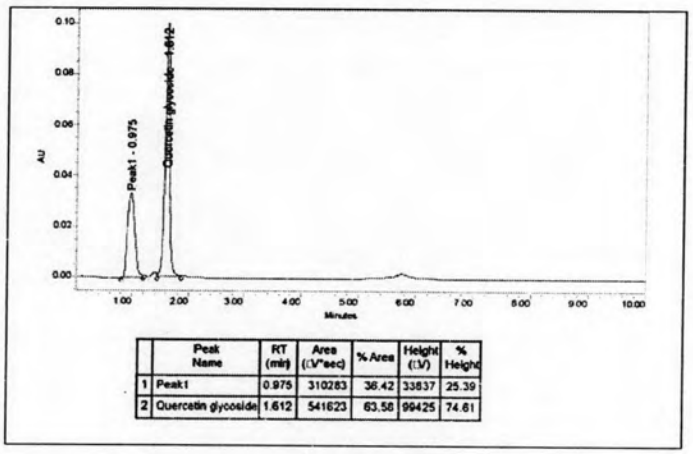
(A) (B)

Figure D.7 Representative chromatograms of *Scenedesmus* sp. strain SS9: (A) with hydrolysis and (B) without-hydrolysis.



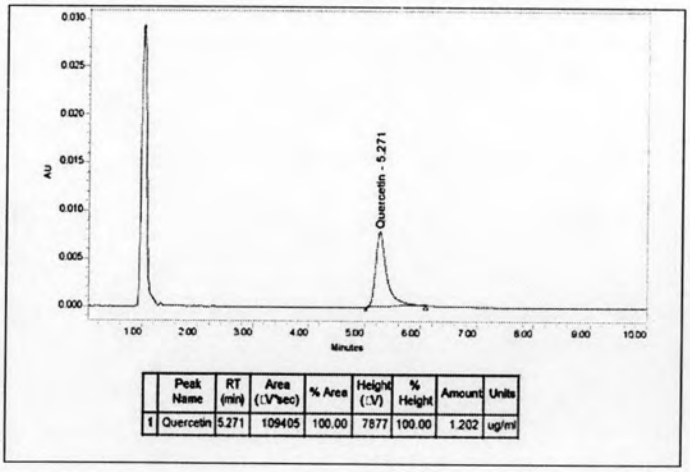
	Peak Name	RT (min)	Area (uV*sec)	% Area	Height (uV)	% Height	Amount	Units
1	Quercetin	5.206	1850260	100.00	102146	100.00	20.000	ug/ml

(A)



	Peak Name	RT (min)	Area (uV*sec)	% Area	Height (uV)	% Height
1	Peak1	0.975	310283	36.42	33837	25.39
2	Quercetin glycoside	1.612	541623	63.58	99425	74.61

(B)

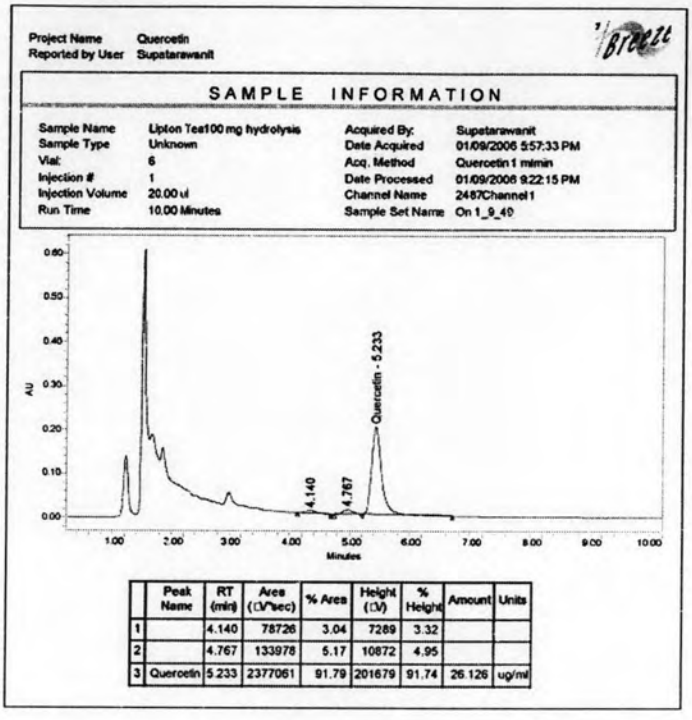


	Peak Name	RT (min)	Area (uV*sec)	% Area	Height (uV)	% Height	Amount	Units
1	Quercetin	5.271	109405	100.00	7877	100.00	1.202	ug/ml

(C)

Figure D.8 Representative chromatograms of Quercetin standards (A) Quercetin-3-β-D-glycoside (B) Quercetin-3-β-D-glycoside after hydrolysis(C)

Figure D.9 Showed a reprecentative chromatogram of Quercetin detection in Tea leave (Lipton Tea) as a positive control as determined by HPLC in a wavelength at 370 nm. Flow rate of mobile phase 1.0 ml.min^{-1}. The retention time of Quercetin was at 5.2 minutes as shown in Figure D.9.



	Peak Name	RT (min)	Area (uV*sec)	% Area	Height (uV)	% Height	Amount	Units
1		4.140	78726	3.04	7289	3.32		
2		4.767	133978	5.17	10872	4.95		
3	Quercetin	5.233	2377061	91.79	201679	91.74	26.126	ug/ml

Figure D.9 Representative chromatogram for Quercetin detection in tea leaves (Lipton Tea) as a positive control as determined by reversed-phase HPLC.

BIOGRAPHY

Miss Supatarawanit Sawangdee was born on August 16, 1980. She obtained a Bachelor of Science Degree in Microbiology from Khon Kaen University, Khon Kaen, Thailand, in 2002.

Publication

1. Chansa-ngavej, K., Chongfuengprinya, W., Ly Kim Pheng, Emampaiwong,D., Sulanchupakorn,S., and Sawangdee,S. 2006. Use of RAPD-PCR fingerprints to monitor changes in DNA and field distribution of soybean rhizobia biofertilizers. Proceedings of The 14th World Fertilizer Congress. Held at Lotus Kad Suan Kaew Hotel, January 23-27, 2006. Chiangmai, Thailand. 6 Pages (in press).

Presentation at Scientific Conferences

1) สุภัทรวนิช แสวงดี และ กาญจนา ชาญสง่าเวช. 2549. การวิเคราะห์ปริมาณบีตา-แคโรทีนในสาหร่ายสีเขียวขนาดเล็ก *Chlorella* spp. และ *Scenedesmus* spp. 5 สายพันธุ์. หนังสือรวมบทคัดย่อการประชุมวิชาการ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. ครั้งที่ 14 : หน้า 14.
2). Chansa-ngavej, K., Chongfuengprinya, W., Emampaiwong,D., Sulanchupakorn,S., and Sawangdee,S. 2006. Use of RAPD-PCR fingerprints to monitor changes in DNA and field distribution of soybean rhizobia biofertilizers. Abstract Book, The 14th World Fertilizer Congress. Lotus Kad Suan Kacu Hotel, January 23-27, 2006. Chiangmai, Thailand. Page 31.
3) Sawangdee, S. and Chansa-ngavej, K. 2005. Production of β-carotene and other anti-oxidants by cyanobacteria and micro-Algae. Abstract Book, The 2nd AgBiotech Graduate Conference II. May 16-17, 2005. Bangkok, Thailand. p. 113