IV RESULTS

4.1 General analysis of broccoli seeds

The data from the experimental section 3.2, as the results of the chemical analyses of the examined broccoli seeds are tabulated, calculated and presented in Table 4.1 (the raw data are in appendices C and D1-D9, page 135-144). The moisture content and dry matter percentages in broccoli seeds varied between 1.68 and 4.05, 95.95 and 98.32. Meanwhile the percentage of ash and fat varied between 1.07 and 3.13, 24.87 and 28.92, respectively. It can be reported that the highest amounts of moisture and ash were found in 'Green Queen' cultivar, whereas the highest amount of fat was found in 'Pak Ging' cultivar.

Table 4.1 Percentage contributions of moisture, dry matter, ash and fat of each broccoli seeds cultivars.[a]

Test	Cultivars				
	Green Queen	Packman	Pak Ging	Rod Fai	Top Green #067
%Moisture	3.96±0.09aA	2.09±0.09aA	1.82±0.02bA	2.41±0.08cA	1.72±0.05bA
%Dry matter	96.04±0.09aB	97.91±0.09aB	98.18±0.02bB	97.59±0.08bC	98.28±0.05bB
%Ash	3.05±0.13aA	1.79±0.12bA	1.86±0.87bA	1.78±0.27bA	1.46±0.11bA
%Fat, Crude	26.69±1.16C	27.47±1.32C	27.59±1.34C	25.90±0.95C	26.13±1.17C

[a] Data (% w/w) are expressed as mean ± SD in triplicate measurements. Small letters (a-c) compare means between cultivar in each row, capital letters (A-C) compare means between general chemical tests(the same genotype) at 5% level according to ANOVA test.

4.2 Glucosinolates analysis

4.2.1 Content of bioactive glucosinolates in broccoli seeds

The seven glucosinolates were detected in the seeds which were classified into three categories: (1) methylsulfinylalkyl glucosinolates (3-methylsulfinylpropyl-; glucoiberin, 4-methylsulfinylbutyl-; glucoraphanin and 5-methylsulfinylpentyl-; glucoalyssin); (2) methylthioalkyl glucosinolates (4-methylthiobutyl-; glucoerucin) and (3) indole glucosinolates (4-hydroxy-3-indolylmethyl-; 4-hydroxyglucobrassicin, 4-methoxy-3-indolymethyl-; 4-methoxyglucobrassicin and 1-methoxy-3-indolylmethyl-; neoglucobrassicin). Total and individual glucosinolates levels varied significantly among cultivars. The compound 4-methylsulfinylbutyl glucosinolate (glucoraphanin) was the predominant glucosinolate in broccoli seeds. Total glucosinolates were significantly higher in 'Top Green #067' cultivar (64.5 μmol/g DW). While other cultivars were reported in decending order 'Packman' (58.6 μmol/g DW), 'Green Queen' (51.2 μmol/g DW), 'Pak Ging' (25.5 μmol/g DW) were lower and 'Rod Fai' (20.3 μmol/g DW) was the lowest. All five cultivars were planted in the same environment and there was no special treatment to the soil bases. A typical glucosinolates chromatogram from broccoli seed is shown in Figure 4.1.

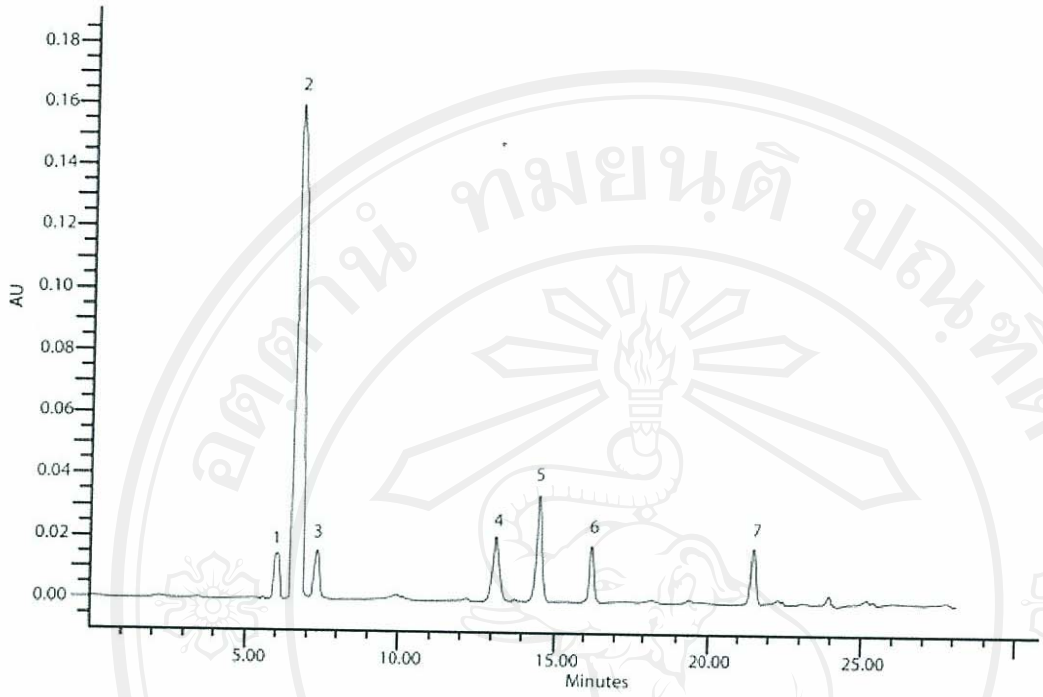

Figure 4.1 Typical glucosinolates chromatogram from broccoli seeds. Peaks; 1, 3-methylsulfinylpropyl-; 2, 4-methylsulfinylbutyl-; 3, 5-methylsulfinylpentyl-; 4, 4-hydroxy-3-indolylmethyl-; 5, 4-methylthiobutyl-; 6, 4-methoxy-3-indolymethyl-; 7, 1-methoxy-3-indolylmethyl.

The compounds 4-methylsulfinylbutyl-, 4-methoxyindol-3-ylmethyl, 4-methylthiobutyl and 4- hydroxy-3-indolylmethyl glucosinolate were common to all cultivars. Only 1-methoxyindol-3-ylmethyl could not be detected in 'Rod Fai' cultivar. Glucoraphanin concentrations in broccoli seeds ranged from 11.4 to 48.9 μmol/g DW. While neoglucobrassicin ranged from 0.0 to 0.2 μmol/g DW. Glucoerucin concentration was lowest in 'Pak Ging' cultivar and highest in 'Top Green #067' cultivar which was no significant from 'Packman' cultivar. 4-methoxyglucobrassicin concentration was highest in 'Top Green #067' and the

lowest were in 'Pak Ging' and in 'Green Queen' cultivars. Table 4.2 shows the amount of the total and main individual glucosinolate expressed in term of micromol per gram of dry weight seeds. 'Top Green #067' cultivar contained the high level of total and individual glucosinolates, glucoraphanin 48.9 μmol/g DW (75.8%), glucoerucin (11.0 μmol/g DW), 4-methoxyglucobrassicin (1.8 μmol/g DW), 4-hydroxy-3-indolylmethyl (3.3 μmol/g DW) and neoglucobrassicin (0.1 μmol/g DW).

Table 4.2 Total glucosinolate levels (μmol/g DW) of the five cultivars of broccoli seeds cultivated in Thailand.[a]

Glucosinolates	Cultivars				
	Green Queen	Packman	Pak Ging	Rod Fai	Top Green #067
Total glucosinolates	51.2a	58.6a	25.5b	20.3b	65.5a
3-methylsulfinylpropyl-	0.2	0.05	0.1	0.05	0.2
4-methylsulfinylbutyl	37.5a	44.3a	15.8b	11.4b	48.9a
5-methylsulfinylpentyl-	0.1	0.05	0.1	0.05	0.1
4-hydroxy-3-indolylmethyl-	3.1a	2.4b	2.8b	1.6c	3.3a
4-methylthiobutyl-	8.9b	10.2a	5.2c	5.6c	11.0a
4-methoxy-3-indolymethyl-	1.3c	1.5b	1.3c	1.6a	1.8a
1-methoxy-3-indolylmethyl-	0.1	0.1	0.2	0.0	0.1

[a] Data are expressed as mean in triplicate measurements. Small letters (a-c) compare means between cultivar in each row at 5% level according to ANOVA test.

4.2.2 Method validation

4.2.2.1 Accuracy

The accuracy of the method was evaluated with the recovery test. The recovery data were determined by spiking glucosinolates standards with different levels in each cultivars. Recovery data ranged from 85 to 106% are considered to be accurate according to the current guidelines.[130] Considering the results of the recovery test, as shown in Table 4.3 below, the method is deemed to be accurate.

Table 4.3 Percentages of recovery of glucosinolates standards at different concentration levels in each broccoli seeds cultivars.

Concentration level of the standard represents (%)	Recovery, %
25	99.96
50	99.89
100	100.01
125	106.04

4.2.2.2 Precision of the chromatographic system

Intra- and inter-day analyses of the same solution were used to validate the precision of the chromatographic system. The %R.S.D. values of retention times and peak areas were shown as represented in Table 4.4 and 4.5. It was concluded

that there was no significant difference for the analyses tested within day and between days.

Table 4.4 The repeatablility parameters in the determination of intact glucosinolates from broccoli seeds (n=15) in intraday analysis.

Glucosinolates	Repeatablility			
	Mean retention times	RSD, %	Mean area ratio	RSD, %
3-methylsulfinylpropyl-	6.10	0.74	0.0469	0.73
4-methylsulfinylbutyl-	6.72	0.43	12.4160	0.21
5-methylsulfinylpentyl-	7.20	1.02	0.0313	1.19
4-hydroxy-3-indolylmethyl-	13.10	1.18	0.2723	0.71
4-methylthiobutyl-	14.52	0.86	3.0049	0.13
4-methoxy-3-indolymethyl-	16.21	1.07	0.1373	0.99
1-methoxy-3-indolylmethyl-	21.55	0.65	0.0073	0.51

Table 4.5 The reproducibility parameters in the determination of intact glucosinolates from broccoli seeds (n=45) in inter-days analysis.

Glucosinolates	Reproducibility			
	Mean retention times	RSD, %	Mean area ratio	RSD, %
3-methylsulfinylpropyl-	6.10	0.92	0.0469	1.04
4-methylsulfinylbutyl-	6.72	0.56	12.4150	0.19
5-methylsulfinylpentyl-	7.20	0.97	0.0314	1.02
4-hydroxy-3-indolylmethyl-	13.10	1.11	0.2720	0.85
4-methylthiobutyl-	14.52	0.68	3.0047	0.16
4-methoxy-3-indolymethyl-	16.21	0.95	0.1375	1.46
1-methoxy-3-indolylmethyl-	21.55	1.01	0.0073	0.60

4.2.2.3 Precision of the extraction procedure

The certified rapeseed reference standard material and five broccoli seeds cultivars prepared from the same procedure were checked by carrying out three replicate analyses. The relative standard deviation (RSD) of repeatability and reproducibility ranged from 0.9 to 1.7 %. These results suggest that the method represented good precision.

Table 4.6 Precision parameters for all glucosinolates analysis.

Precision	SD	RSD, %
Repeatablility	2.20	1.23
Reproducibility	3.42	1.50

4.2.2.4 Specificity

Determination of intact glucosinolates from broccoli seeds showed that no significant interferences with impurities substances from the chromatogram in Figure 4.1, page 66.

4.2.2.5 Detection and quantification limits

LOD and LOQ were established by the procedures described in the Section 2.12. Table 4.7 shows the LOD and the LOQ values of glucosinolates in broccoli seeds. The range of LOD and LOQ of benzylglucosinolates were 0.016-0.026 μg/mL and 0.125-0.251 μg/mL, respectively.

Table 4.7 Limits of detection (LOD) and Limits of quantification (LOQ).

Glucosinolates	LOD (μg/mL)	LOQ (μg/mL)
3-methylsulfinylpropyl-	0.026(±0.012)	0.112(±0.040)
4-methylsulfinylbutyl-	0.031(±0.011)	0.482(±0.020)
5-methylsulfinylpentyl-	0.025(±0.11)	0.119(±0.029)
4-hydroxy-3-indolylmethyl-	0.022(±0.012)	0.217(±0.041)
4-methylthiobutyl-	0.028(±0.012)	0.362(±0.026)
4-methoxy-3-indolymethyl-	0.021(±0.011)	0.209(±0.031)
1-methoxy-3-indolylmethyl-	0.022(±0.011)	0.101(±0.050)

Values expressed are mean ± S.D. of three experiments.

4.2.2.6 Linearity

The linearity of the photodiode array detector response was tested by analysing benzylglucosinolates standard containing each broccoli seeds cultivars. The validating parameters of each calibration curve (slope (a), intercept (b), correlation coefficient (R^2), standard deviation of the slope and standard deviation of the intercept) were obtained. Excellent linearity was observed for all these compounds between peak areas ratio and concentrations ($R^2 \geq 0.99$) over the range tested. All the data are summarized in table forms, staring from Table 4.8 through 4.14.

Table 4.8 The data used to construct calibration curve for 3-methylsulfinyl-propyl glucosinolate (raw data, appendix F1, page 146).

Concentration of 3-methylsulfinylpropyl glucosinolate (μmol/g)	Mean of area ratio of 3-methylsulfinylpropyl glucosinolate
0.026	0.06
0.057	0.09
0.11	0.17
0.15	0.24
0.17	0.27

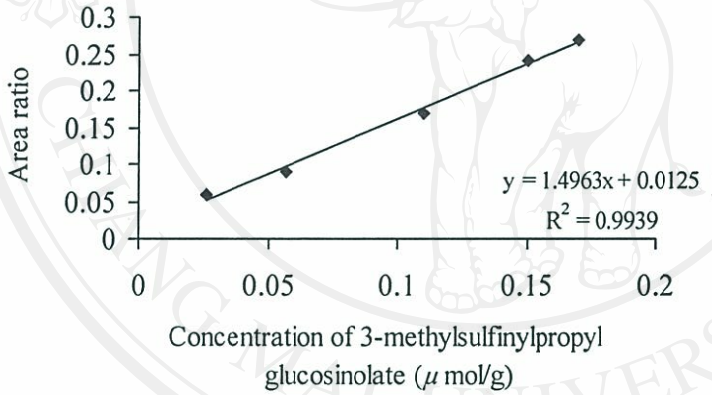

Figure 4.2 Calibration curve for 3-methylsulfinylpropyl glucosinolate.

Table 4.9 The data used to construct calibration curve for 4-methylsulfinyl-butyl glucosinolate (raw data, appendix F2, page 147).

Concentration of 4-methylsulfinylbutyl glucosinolate (μmol/g)	Mean of area ratio of 4-methylsulfinylbutyl glucosinolate
0.031	0.40
0.10	0.85
0.20	1.61
0.30	2.40
0.40	3.15

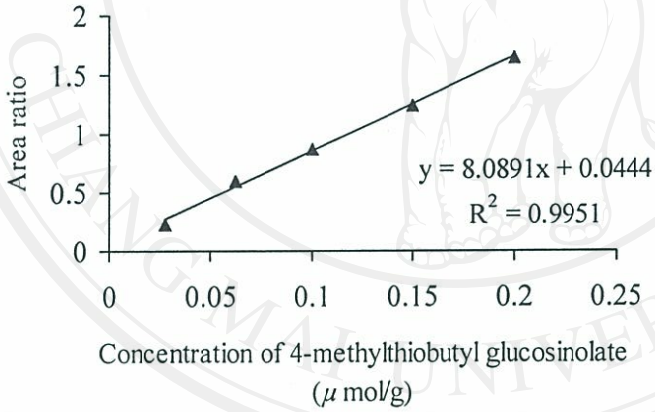

Figure 4.3 Calibration curve for 4-methylsulfinylbutyl glucosinolate.

Table 4.10 The data used to construct calibration curve for 5-methylsulfinyl-pentyl glucosinolate (raw data, appendix F3, page 148).

Concentration of 5-methylsulfinylpentyl glucosinolate (μmol/g)	Mean of area ratio of 5-methylsulfinylpentyl glucosinolate
0.025	0.05
0.05	0.08
0.10	0.15
0.15	0.23
0.18	0.29

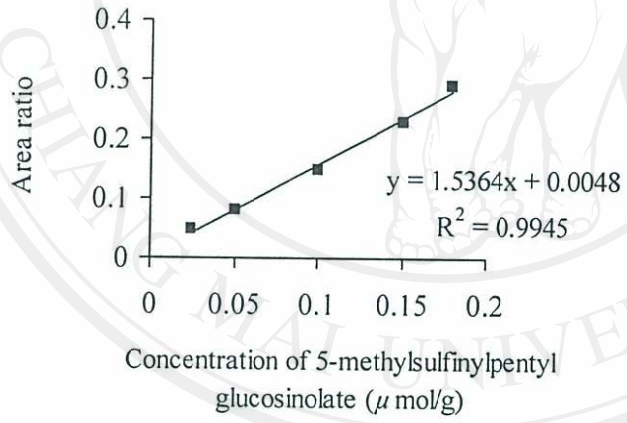

Figure 4.4 Calibration curve for 5-methylsulfinylpentyl glucosinolate.

Table 4.11 The data used to construct calibration curve for 4-hydroxy-3-indolylmethyl glucosinolate (raw data, appendix F4, page 149).

Concentration of 4-hydroxy-3-indolylmethyl glucosinolate (μmol/g)	Mean of area ratio of 4-hydroxy-3-indolylmethyl glucosinolate
0.022	0.28
0.031	0.49
0.062	0.99
0.093	1.30
0.12	1.79

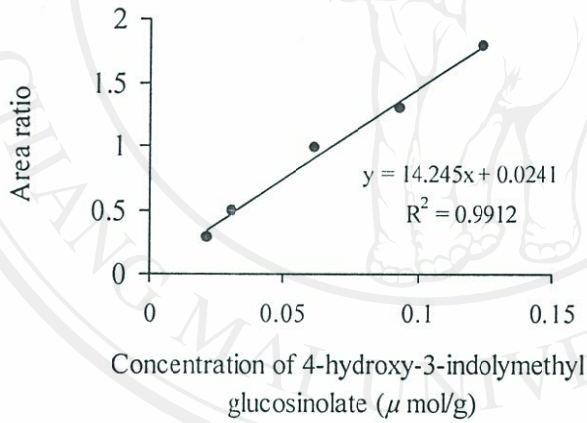

Figure 4.5 Calibration curve for 4-hydroxy-3-indolylmethyl glucosinolate.

Table 4.12 The data used to construct calibration curve for 4-methylthiobutyl glucosinolate (raw data, appendix F5, page 150).

Concentration of 4-methylthiobutyl glucosinolate (μmol/g)	Mean of area ratio of 4-methylthiobutyl glucosinolate
0.028	0.22
0.062	0.60
0.10	0.87
0.15	1.25
0.20	1.65

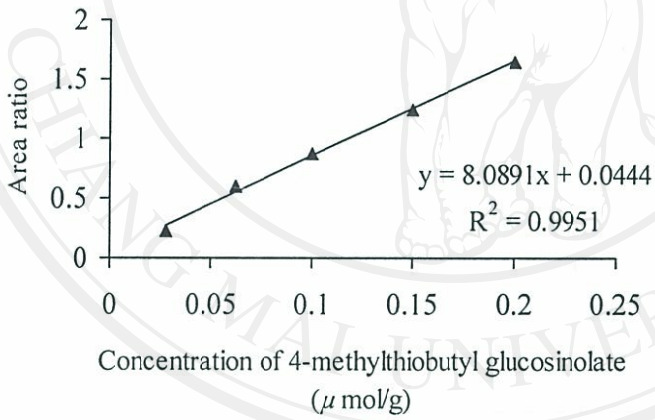

Figure 4.6 Calibration curve for 4-methylthiobutyl glucosinolate.

Table 4.13 The data used to construct calibration curve for 4-methoxy-3-indolylmethyl glucosinolate (raw data, appendix F6, page 151).

Concentration of 4-methoxy-3-indolylmethyl glucosinolate (μmol/g)	Mean of area ratio of 4-methoxy-3-indolylmethyl glucosinolate
0.021	0.11
0.04	0.27
0.08	0.66
0.10	0.78
0.12	0.97

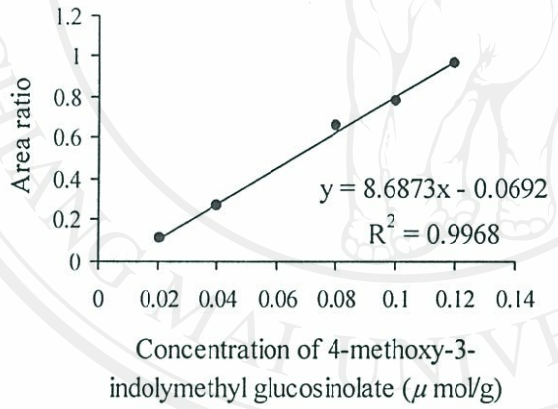

Figure 4.7 Calibration curve for 4-methoxy-3-indolylmethyl glucosinolate.

Table 4.14 The data used to construct calibration curve for 1-methoxy-3-indolylmethyl glucosinolate (raw data, appendix F7, page 152).

Concentration of 1-methoxy-3-indolylmethyl glucosinolate (μmol/g)	Mean of area ratio of 1-methoxy-3-indolylmethyl glucosinolate
0.022	0.13
0.030	0.24
0.056	0.50
0.098	0.91
0.14	1.27

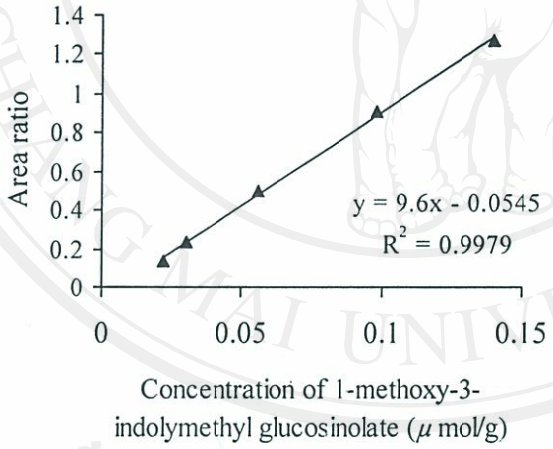

Figure 4.8 Calibration curve for 1-methoxy-3-indolylmethyl glucosinolate.

4.2.3.7 Range

To established the optimun conditions, the linerity plot was constructed for peak areas response versus 4-methylsulfinylbutyl glucosinolate concentration in range 0.10-500 mg/L. Table 4.15 represented all the data and Figure 4.9 is the linearity range plot.

Table 4.15 The data used to construct linearity of 4-methylsulfinylbutyl glucosinolate.

Concentration (mg/L)	Peak area of 4-methylsulfinylbutyl glucosinolate (AU)
0.1	540
0.5	1020
1	2106
5	12389
10	25530
50	120620
100	240239
150	361860
200	482490
250	608156
300	730720
350	872645
400	1010900
450	1148978
500	1280823

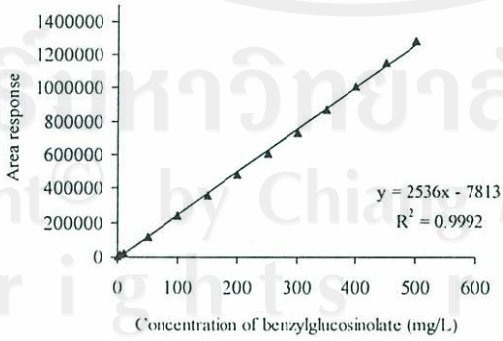

Figure 4.9 Linearity range of 4-methylsulfinylbutyl glucosinolate at the optimum conditions.

4.2.3.8 Robustness

According to resolution test (Table 4.16), it showed the reliability of an analysis with respect to deliberate variations. The data indicated that the method is robust.

4.2.3.9 System suitability testing

The chromatographic parameters (resolution, selectivity and tailing factor) were satisfactory for these compounds (Table 4.16). The calculated resolution values between each peak-pair were not less than 2.70 and the selectivity values were not less than 1.07.

Table 4.16 Column performance for the separation of glucosinolates compounds.

Glucosinolates	Retention time, t_R (min)	Number of theoretical plates, N	Resolution, R_s	Selectivity, α	Tailing factor, T
3-methylsulfinylpropyl-	6.10	12,413	–	–	1.02
4-methylsulfinylbutyl-	6.72	12,863	2.70	1.10	1.06
5-methylsulfinylpentyl-	7.20	17,137	5.11	1.07	0.97
4-hydroxy-3-indolylmethyl-	13.10	19,216	23.45	1.82	1.07
4-methylthiobutyl-	14.52	28,177	29.80	1.11	1.03
4-methoxy-3-indolymethyl-	16.21	22,950	31.25	1.12	1.01
1-methoxy-3-indolylmethyl-	21.55	35,268	45.58	1.33	1.05

4.3 Antioxidant assay

4.3.1 Total antioxidant activity

4.3.1.1 ABTS radical scavenging assay

4.3.1.1.1 Calibration curves

A plot of the calibration curves of the three standard antioxidants included ascorbic acid, quercetin and Trolox were also shown in Figure 4.10. Data are shown in Table 4.17, 4.18 and 4.19.

Table 4.17 The data used to construct calibration curve for ascorbic acid standard.

Concentration of ascorbic acid (mM)	ABTS scavenging activity, %
0.5	12.13
1.0	23.81
1.5	36.25
2.0	50.25
2.5	63.91

Table 4.18 The data used to construct calibration curve for quercetin standard.

Concentration of quercetin (mM)	ABTS scavenging activity, %
0.05	9.28
0.10	17.03
0.20	30.51
0.25	38.72
0.50	78.37

Table 4.19 The data used to construct calibration curve for Trolox standard.

Concentration of Trolox (mM)	ABTS scavenging activity, %
0.1	5.30
0.5	19.19
1.0	36.56
1.5	60.74
2.0	82.35

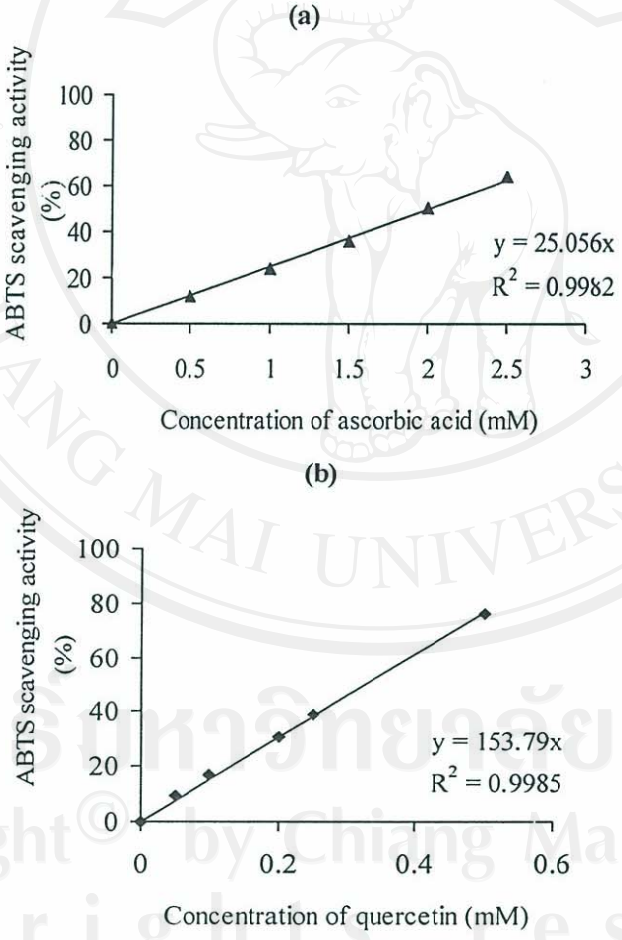

Figure 4.10 Concentration-response curves for the absorbance at 734 nm for $ABTS^{+\circ}$ as a function of concentration of standard ascorbic acid (a), quercetin (b) and Trolox (c) solutions.

(c)

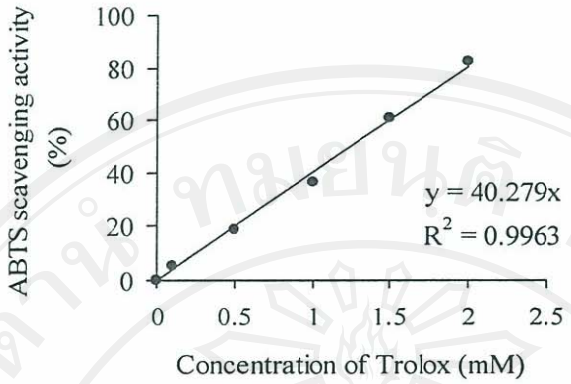

Figure 4.10 *continued.*

4.3.1.1.2 Antioxidative activity of the broccoli seeds extracts

Antioxidant activity of the three standard antioxidants for each cultivar were provided in Figure 4.11-4.13. Our laboratory results showed that the methanolic extract of broccoli seeds gave the highest ABTS radical scavening activity followed by water, ethanol (70%), ethyl acetate and chloroform extracts. The chloroform extracts showed the least scavenging activity (<25%). This activity was increased by increasing the concentration of broccoli seeds samples. The TEAC value of ascorbic acid is highest in 'Pak Ging' (33.2%) cultivar followed by 'Packman' (30%), 'Top Green #067' (29.8%), 'Green Queen' (27.2%) and 'Rod Fai' (23%) cultivars. The TEAC value of quercetin is highest in 'Green Queen' (71.2%) cultivar followed by 'Top Green #067' (67.1%), 'Packman' (64.5%), 'Pak Ging' (60.3%) and 'Rod Fai' (53.2%) cultivars. The TEAC value of Trolox is highest in 'Packman' (39.2%) cultivar followed by 'Pak Ging' (38.3%), 'Top Green #067'

(36.4%), 'Green Queen' (25.9%) and 'Rod Fai' (19.2%) cultivars. The highest overall antioxidant activity was 'Top Green #067' cultivar.

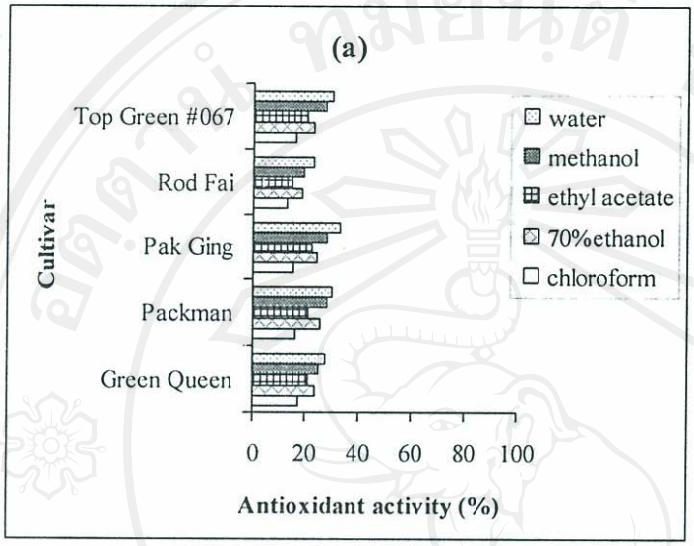

Figure 4.11 Antioxidant activity of the standard antioxidant ascorbic acid (a) with different broccoli seeds cultivars in different solvent extracts in ABTS assay.

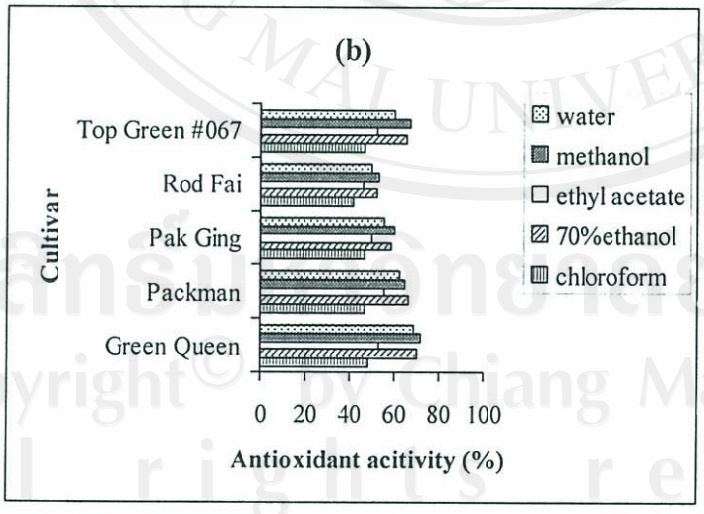

Figure 4.12 Antioxidant activity of the standard antioxidants quercetin (b) with different broccoli seeds cultivars in different solvent extracts in ABTS assay.

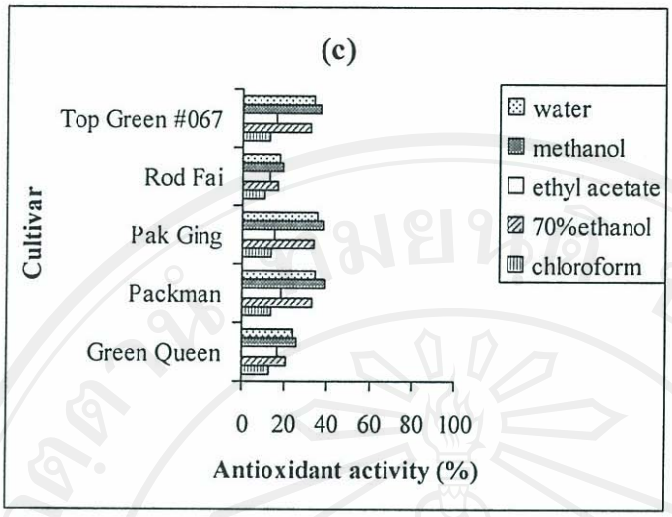

Figure 4.13 Antioxidant activity of the standard antioxidants Trolox (c) with different broccoli seeds cultivars in different solvent extracts in ABTS assay.

4.3.1.2 Ferric reducing antioxidant power (FRAP assay)

4.3.1.2.1 Calibration curve

The calibration curve for the antioxidant determination of a FRAP assay is represented in Figure 4.12, and Table 4.20 below showed the data.

Table 4.20 The data used to construct calibration curve for FRAP assay.

Concentration of ferrous standard solution (mM)	Ferrous ion chelating ability, %
0.05	11.02
0.10	16.41
0.25	27.10
0.5	58.39
1.0	99.65

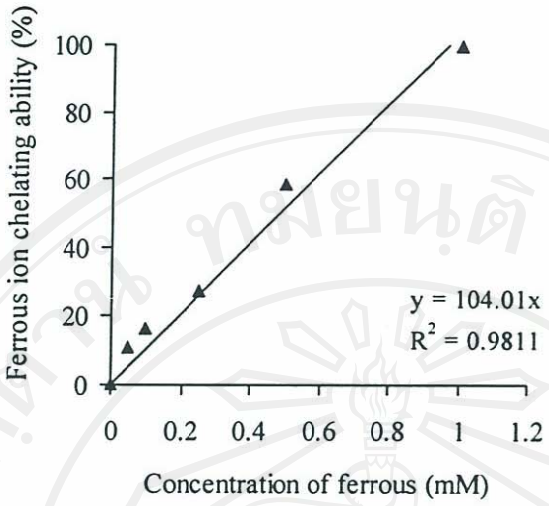

Figure 4.14 Calibration curve for FRAP assay in this study.

4.3.1.2.2 Antioxidative activity of the broccoli seeds extracts

Figure 4.15 demonstrates the ferrous ion chelating ability of the broccoli seeds extracts in various solvents. The methanolic seeds extract performed the best ferrous ion chelating ability followed by water, ethanol (70%), ethyl acetate and chloroform. 'Packman' cultivar showed the highest ferrous ion chelating ability (46.67%), 'Top Green#067' (44.26%), 'Green Queen' (39.21%) 'Pak Ging' (37.65%) and 'Rod Fai' (16.46%) cultivars.

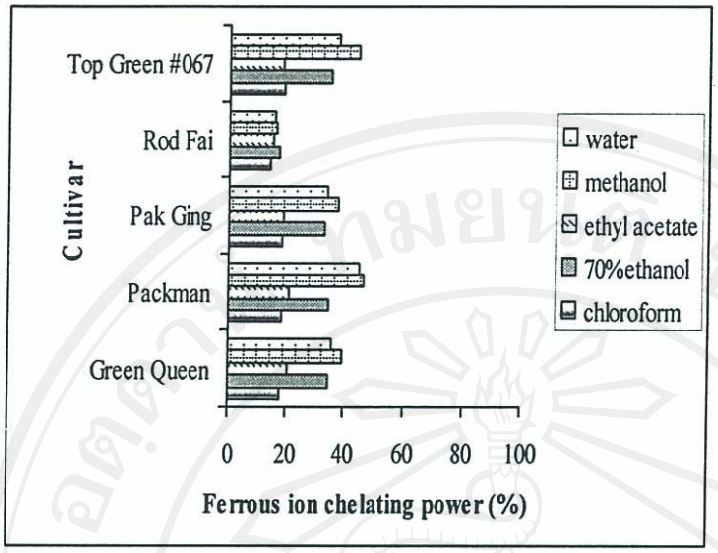

Figure 4.15 Ferrous ion chelating power of the extracts from broccoli seeds cultivar in various solvents.

4.3.2 Total Phenolics, flavonoids and flavonols compounds assay

The total antioxidants values presented in Table 4.21 is the sum of all these antioxidants detected in each cultivar. The content of phenolic compounds (mg/100g DW) in methanolic extracts, determined from regression equation of calibration curve (y = 9.6581x + 0.0796, R^2=0.99) and expressed in gallic acid equivalents (GAE), varied between 0.88 and 1.58. The concentration of flavonoids and flavonols, expressed in rutin equivalents (regression equation of calibration curve: y = 5.042x + 0.30, R^2=0.99) in mg/100 g of seed extract. The content of flavonoids (mg/100g DW) varied from 0.860 to 1.508. The concentration of flavonols varied in a same wide range as compared with total phenolics and

flavonoids, from 0.957 to 1.131. Results of the statistical analysis of the total amount of phenolics, flavonoids and flavonols in broccoli seeds data are reported in appendices G1-G11, page 153-168.

Table 4.21 Total amount of phenolics , flavonoids and flavonols in broccoli seeds cultivars avaiable in Thailand.[a]

Compounds	Cultivars				
	Green Queen	Packman	Pak Ging	Rod Fai	Top Green #067
Total phenolics (in GAE)	1.226aA	1.170bA	1.166bA	0.896cA	1.573dA
Total flavonoids (in RE)	1.027aB	1.017aB	1.063aB	0.898bA	1.467cB
Total flavonols (in RE)	1.024aB	1.070bC	1.060cB	0.962dB	1.128eC

[a] Data are means of triplicate experiments. All values are expressed in mg/100g of DW. Small letters (a-e) compare means between cultivar in each row, capital letters (A-C) compare means between antioxidant capacity (the same genotype) at 5% level according to ANOVA test.

4.3.3 Individual antioxidant activity

4.3.3.1 Phenolic compounds analysis

Using this analytical method, catechin, epicatechin, epigallocatechin gallate, gallic acid, quercetin and rutin could be determined simultaneously, and the validity of the method was also verified (Table 4.22 and Figure 4.16). There was no detectable phenolic compounds in broccoli seeds in all extracts from this study.

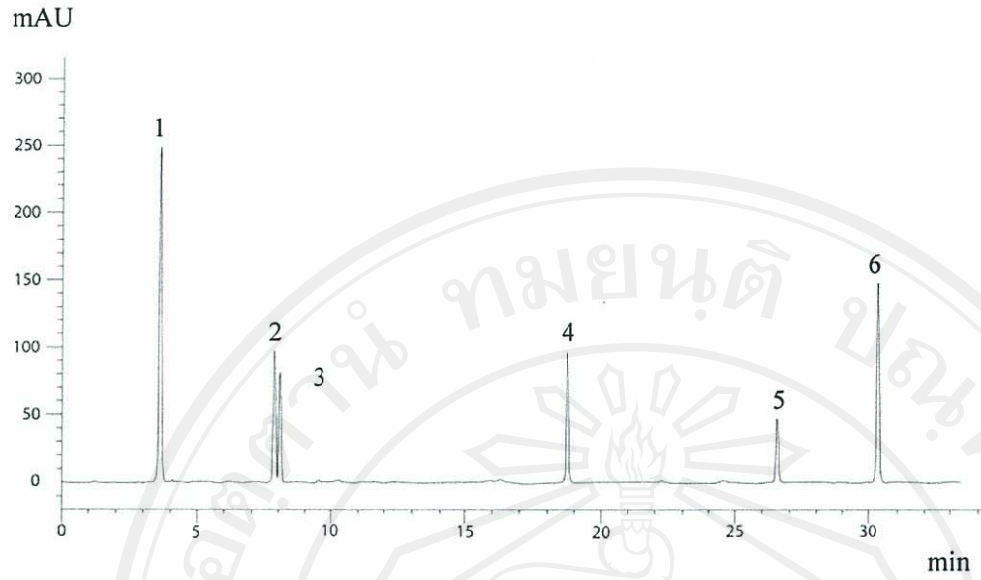

Figure 4.16 HPLC chromatogram of phenolic standard: 1, gallic acid (3.63 min); 2, catechin (7.92 min); 3, epicatechin (8.01 min); 4, (-)-epigallocatechin gallate (18.79 min); 5, quercetin (26.54 min) and 6, rutin (30.57 min).

Table 4.22 Parameters of calibration graphs for the phenolic standards in this study.

Peak no.	Phenolic compounds	Linearity[a]	LOD (μg/mL)[b]	LOQ (μg/mL)[b]	Recovery (%)[c]
1	Gallic acid	1.000	0.15	0.42	99.5 ± 2.0
2	(+)-Catechin	0.996	0.44	2.14	98.6 ± 3.4
3	(-)-Epicatechin	0.997	0.39	1.87	97.5 ± 3.6
4	(-)-EGCG	0.995	0.37	1.11	97.8 ± 2.6
5	Quercetin	0.997	0.46	2.47	100.5 ± 5.0
6	Rutin	0.998	0.21	0.18	104.6 ± 2.8

[a] Linearity was expressed as the correlation coefficient of each calibration curve, which was determined by five calibration points.
[b] Data were expressed as mean of triplicate measurements.
[c] Recovery are expressed as mean ± standard deviation carried out in broccoli seeds samples.

4.3.3.2 Ascorbic acid, β-carotene and tocopherols assay

The total concentration of ascorbic acid, β-carotene and tocopherols in each individual group was the sum of the individual concentrations for each group. The linear range, calibration formula, and the detection limit of the standards for each group are extracted from ANOVA analysis and listed in Table 4.23. The detection limit was defined as the concentration at which the signal to noise ratio (*S/N*) was equal to or greater than three. In the calibration formula, x stands for the concentration of the analyte, and y is the peak area. All samples were prepared and analyzed in duplicate.

Table 4.23 Calibration curves, detection limits and method validation data used For determination of ascorbic acid, β-carotene and tocopherols.

Parameters	Compounds		
	Ascorbic acid	β-carotene	Tocopherols
Linear range (μg/mL)	0.2-100	0.5-100	0.5-100
Formulae	$y = 25.74x - 10.51$	$y = 18.18x + 6.51$	$y = 20.34x + 8.33$
R^2	0.9991	0.9988	0.9994
LOD (μg/mL)	0.05	0.1	0.1
R.S.D.% (Rt)	0.78	0.95	1.20
R.S.D.% (Area)	2.0	1.8	1.3
Recovery, %	101	99.97	106

To investigate the amount of these vitamins, analysis of variance (ANOVA) were generated using means of individual compounds among the broccoli cultivar accessions (Table 4.24). Ascorbic acid concentrations in broccoli seeds ranged from 0.020 to 0.047 mg/100g DW. These results, however, indicated that β-carotene and tocopherol levels significant differences were found in 'Pak Ging', 'Rod Fai' and 'Top Green #067' cultivars surveyed here. The β-carotene concentrations ranged from 0.490 to 0.640 mg/100g DW, although tocopherols concentrations ranged from 0.013 to 0.023 mg/100g DW. The raw data were reported in appendices G9, G10 and G11.

Table 4.24 Total amount of ascorbic acid, β-carotene and tocopherols in broccoli seeds cultivars avaiable in Thailand.[a]

Compounds	Cultivars				
	Green Queen	Packman	Pak Ging	Rod Fai	Top Green #067
Ascorbic acid	0.034aA	0.038bA	0.032aA	0.021cA	0.045dA
β-carotene	0.526aB	0.566bB	0.596cB	0.502dB	0.636eB
Tocopherols	0.018aC	0.015bA	0.017cC	0.013dA	0.022eA

[a] Data are means of triplicate experiments. All values are expressed in mg/100g of DW. Small letters (a-e) compare means between cultivar in each row, capital letters (A-C) compare means between antioxidant capacity (the same genotype) at 5% level according to ANOVA test.