Kasetsart J. (Nat. Sci.) 41 : 561 - 569 (2007)

Antioxidant Capacity and Phenolic Content of Chilies

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ABSTRACT

The antioxidant capacity of red and green Prik Khee Nu (*Capsicum frutescens* Linn.), and red and green Prik Chee Fah (*Capsicum annuum* Linn. var. acuminatum Fingerh.) which have been normally used in Thailand as a food ingredient was estimated by three different methods; Ferric reducing antioxidant power (FRAP) assay, Improved ABTS radical cation decolorization assay, and DPPH free radical scavenging activity; together with their total phenolic content. Percentages of 95% ethanol in extraction solvents were also studied. Solvents with 60 and 80%(v/v) of 95% ethanol gave higher results for all samples and methods. FRAP values of all samples calculated on the basis of mg vitamin C per gram of fresh weight were 0.62 ± 0.15 to 2.40 ± 0.13 (red Prik Khee Nu > green Prik Khee Nu and red Prik Chee Fah > green Prik Chee Fah). Values of ABTS calculated on the basis of mg vitamin C per gram of fresh weight were 1.32 ± 0.05 to 6.68 ± 0.84 (red and green Prik Khee Nu > red Prik Chee Fah > green Prik Chee Fah). Values of DPPH calculated on the basis of mg vitamin C per gram of fresh weight were 0.36 ± 0.04 to 1.23 ± 0.31 (red Prik Chee Fah > red and green Prik Khee Nu > green Prik Chee Fah). Total phenolic content calculated on the basis of mg gallic acid per gram of fresh weight was 0.85 ± 0.20 to 3.48 ± 0.58 (red Prik Khee Nu > green Prik Khee Nu > red Prik Chee Fah > green Prik Chee Fah). Correlations of all antioxidant capacity results and total phenolic content (r = 0.310 - 0.909) were found highly significant ($p \leq 0.01$).

Key words: antioxidant capacity**,** phenolic content, chilies

INTRODUCTION

Chili is an erect, branched, shrub-like herb with fruits used as garnishing and flavoring in culinary purposes. There are many different species and varieties but two of them are normally used in Thailand. The first one is called Prik Khee Nu or bird chili (*Capsicum frutescens* Linn.), which is a small type (tiny cone) and only 3/4 to $1\frac{1}{2}$ inch in length, but it is very hot (50,000 - 100,000 scoville units). The second is called Prik Chee Fah or chili spur pepper (*Capsicum annuum* Linn. var. acuminatum Fingerh.), a finger shape with 4 to 6 inches in length, with a heat of 30,000 - 60,000 scoville units. Chilies contain 0.2 to 2% capsaicinoids, (vanillylamides of monocarboxyl acids) which are responsible for the pungency or bite in capsicums. Capsaicin accounts for about 50 to 70% of total capsaicinoids. It gives the bite but has no odour. Other bite contributing components are 20 to 25% dihydrocapsaicin which together with capsaicin provides fiery notes from mid-palate to throat, 7% nondihydro-capsaicin which is fruity and sweet and has the least burning

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Received date : 20/04/06 Accepted date : 04/09/06

sensation, and 1% homocapsaicin and 1% homodihydrocapsaicin which give a numbing and prolonged burn (Uhl, 2000).

Chilies have been recognized by many cultures around the world for their medicinal qualities. When chilies are eaten, capsaisin stimulates the release of endorphins, which give a pleasurable feeling. Moreover, chilies are believed to increase circulation, relieve rheumatic pain, treat mouth sores and infected wounds, reduce blood clots, and aid digestion by stimulating saliva and gastic juice flow (Uhl, 2000). Capsaicin has been tested by many investigators for its effects on experimental carcinogenesis and mutagenesis. There is no solid evidence showing that chili and capsaicin are carcinogenic in humans. In contract, many studies reveal substantial antioxidant, antigenotoxic and anticarcinogenic effects of chili extracts and capsaicin (Surh *et al*., 1998; Prasad *et al*., 2004). Therefore, capsaicin is suggested as an important dietary phytochemical with antioxidant and chemopreventive activities.

Although the antioxidant activity of chilies has been widely accepted, the information on antioxidant capacity of chilies is still limited. This activity is varied by many factors, such as varieties, origins, growing areas and conditions. Pellegrini *et al.* (2003) reported that the antioxidant capacity of chilies was equal to 23.54 mmol Fe^{2+} / kg fresh weight by ferric reducing antioxidant power (FRAP) assay, 6.42 mmol Trolox / kg fresh weight and 7.62 mmol Trolox / kg fresh weight for total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) methods, respectively. Other related works reported in the terms of antioxidative compounds in chilies such as flavonoid content (Nair *et al*., 1998; Miean and Mohamed, 2001), alpha-tocopherol content (Ching and Mohamed, 2001), carotenoid ester content (Breithaupt and Bamedi, 2001), and beta-carotene content (Siripongvutikorn *et al*., 2005).

Since various methods should be used to monitor and compare the antioxidant activity of foods, and results could be affected by extraction solvents (Iwatsuki *et al*. 1994; Zhou and Yu, 2004; Leelarungrayub *et al*., 2006). This study aimed to estimate the antioxidant capacity of Prik Khee Nu (*Capsicum frutescens* Linn.) and Prik Chee Fah (*Capsicum annuum* Linn. var. acuminatum Fingerh.) by three different methods including Ferric reducing antioxidant power (FRAP) assay, Improved ABTS radical cation decolorization assay, and DPPH free radical scavenging activity. Effects of extraction solvents (95% ethanol percentages) were studied, and total phenolic content of extracts was also estimated.

MATERIALS AND METHODS

1. Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) [Aldrich], TPTZ (2,4,6-tripyridyl-s-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl) [Sigma], ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), Folin-Ciocalteu phenol reagent, ferric chloride, ferrous sulphate, gallic acid, glacial acetic acid, hydrochloric acid, sodium acetate, potassium persulphate, sodium carbonate, vitamin C [Fluka] were analytical grade.

2. Sample extraction

Fresh samples (green and red Prik Khee Nu and Prik Chee Fah) were purchased from fresh markets. Their moisture content was analyzed by AOAC (1997). Sample extraction method of Leong and Shui (2002) was modified for sample preparation. Edible portion of fresh samples were homogenized using a blender. Two grams of each homogenized sample were weighted into a 25 cm × 150 cm tube, and 10 ml of a solvent were added. Deionized water was used as a primitive solvent and percentages of 95% ethanol were varied to prepare six different extraction solvents (0, 20, 40, 60, 80, and 100%(v/v)). The extraction was done

by using a vortex mixer for 60 s. The mixture was filtered by Whatman No 1 and the filtrate was used for FRAP, ABTS, DPPH, and total phenolic content assays. Spectronic 20D+ spectrophotometer of Milton Roy was used for all assays.

3. Ferric reducing antioxidant power (FRAP) assay

The FRAP was assessed according to Benzie and Strain (1999). Briefly, 6 ml of working FRAP reagent prepared daily was mixed with 20 µl of extract sample. The absorbance at 593 nm was recorded after a 30-min incubation at 37°C. Absorbance increases were calculated as FRAP values by comparing with standard curves created by ferrous sulphate, vitamin C, and Trolox, and reported as mg Fe^{2+}, Trolox, and vitamin C per gram of fresh weight.

4. ABTS radical cation decolorization assay

The ABTS method of Re *et al*. (1999) was modified. ABTS radical cation ($ABTS^{+}$) was produced by reacting 7 mM ABTS stock solution with 2.45 mM postassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 - 16 hours before use. The $ABTS^{+}$ solution was diluted with deionized water and 95% ethanol (1:1) to an absorbance of 0.70 ($\pm$ 0.02) at 734 nm. Twenty to one hundred microliters of extract sample was mixed with 6 ml of diluted $ABTS^{+}$ solution. The decrease of absorbance was recorded at 1, 5, 10, and 30 min after mixing. Absorbance decreases were calculated as ABTS values by comparing with standard curves created by Trolox and vitamin C, and results were reported as mg Trolox, and vitamin C per gram of fresh weight.

5. DPPH free radical scavenging activity

The method of Brand-Williams *et al.* (1995) was used with some modifications. 0.8 mM DPPH radical solution in 95% ethanol was prepared. One hundred to four hundred microliters of extract sample was diluted to 5.4 ml using deionized water and 95% ethanol (1:1) before 0.6 ml DPPH solution was added and mixed. The decrease of absorbance was recorded at 1, 5, 10, and 30 min after mixing. Absorbance decreases were calculated as DPPH values by comparing with standard curves created by Trolox and vitamin C, and results were reported as mg Trolox, and vitamin C per gram of fresh weight.

6. Total phenolic content

The Folin-ciocalteau micro method of Waterhouse (n.d.) was used to estimate total phenolic content. Sixty microliters of extract sample was diluted with deionized water to 4.8 µl, and 300 ml Folin-ciocalteau reagent was added and shaken. After 8 min, 900 µl of a 20% Sodium carbonate was added with mixing. The solution was left at 40°C for 30 min before reading the absorbance at 765 nm. Gallic acid was used as a standard, and results were reported as mg gallic per gram of fresh weight.

7. Statistical analysis

The experiment was repeated three times and was conducted on separate marketing purchases (triple measurements for each marketing purchase). A randomized complete block design (RCBD) was used. Extraction solvents and marketing purchases were served as treatment and block variables, respectively, in order to study the extraction solvent effects for each chili. Chili types and marketing purchases were served as treatment and block variables, respectively, in order to compare the antioxidant capacity of chilies. Mean comparisons was performed by Duncan's new multiple range test (DMRT). The bivariate correlations between all antioxidant capacity assays and total phenolic content were analyzed.

RESULTS AND DISCUSSION

1. Moisture content of samples

Moisture content of all samples from 3 marketing purchases was analyzed and shown in Table 1. Prik Chee Fah was higher in moisture content than Prik Khee Nu, and the green ones contained higher moisture content than the red ones.

2. Ferric reducing antioxidant power (FRAP) assay

The significant difference between extraction solvents was found for both red and green Prik Khee Nu, and green Prik Chee Fah as shown in Table 2. The extracts from 60 and 80%(v/v) of 95% ethanol produced higher FRAP values for red and green Prik Khee Nu, while the extracts from 40%(v/v) and more of 95% ethanol gave higher FRAP values for green Prik Chee Fah. When four chilies were compared, FRAP values of red Prik Khee Nu was highest, followed by green Prik Khee Nu and red Prik Chee Fah which their values were almost equal, and the lowest one was green Prik Chee Fah. Compared to the work of Pellegrini *et al.* (2003) which showed that FRAP value of chili was 23.54 mmol Fe^{2+} / kg fresh weight (or 1.31 mg Fe^{2+}/ g fresh weight) by using water and acetone as extraction solvents, however, their value was between values of green Prik Khee Nu or red Prik Chee Fah, and green Prik Chee Fah in this study.

3. ABTS radical cation decolorization assay

The reaction rate of ABTS assay (absorbance decrease / time) was highest in the first minute and then was slow down as shown in Figure 1. The significant difference between extraction solvents was found for all samples. Extracts from 40, 60, and 80%(v/v) of 95% ethanol yielded higher ABTS values for both red and green Prik Khee Nu. All mixed solvents (mixture of water and 95% ethanol) gave higher ABTS values than water or 95% ethanol for red Prik Chee Fah, but only the extract from 60%(v/v) of 95% ethanol produced the significant highest ABTS value for green Prik Chee Fah (Figure 1). Highest ABTS values at 10 min of extracts from 60%(v/v) of 95% ethanol for all samples were shown in Table 3 (red and green Prik Khee Nu > red Prik Chee Fah > green Prik Chee Fah). Values at 30 min were not used for calculating highest values because some of them reached zero before the measuring time. Pellegrini *et al.* (2003) reported that ABTS value of chili was 7.62 mmol Trolox / kg fresh weight or 1.91 mg Trolox / g fresh weight by using water and acetone as extraction solvents, however, their value was very close to the one of green Prik Chee Fah in this study.

4. DPPH free radical scavenging activity

In this study, the reaction rate of DPPH assay was almost completed within the first minute as shown in Figure 2. The significant differences between extraction solvents was also found for all samples. Their results were almost similar to ABTS assay, but extract from 60 and 80%(v/v) of 95% ethanol produced higher ABTS values for green Prik Chee Fah. Highest DPPH values at 10 min of extracts from 60% (v/v) of 95% ethanol of

Table 1 Moisture content of Prik Khee Nu and Prik Chee Fah.

Sample	Moisture content (wet basis)
Prik Khee Nu, red	67.09 ± 1.58^{d}
Prik Khee Nu, green	78.46 ± 1.27^{c}
Prik Chee Fah, red	84.28 ± 2.32^{b}
Prik Chee Fah, green	90.92 ± 1.15^{a}

1 Each value represents the mean and standard deviation from 3 marketing purchases.

2 Means with different superscripts are significantly different ($p \leq 0.05$).

Table 2 Ferric reducing antioxidant power (FRAP) assay of Prik Khee Nu and Prik Chee Fah when extraction solvents were varied[1].

Sample	FRAP values (mg / g FW[2]) calculated on the basis of		
	Fe^{2+}	Trolox	Vitamin C
Prik Khee Nu, red			
Overall	2.48 ^{x} ± 0.48	4.94 ^{x} ± 0.96	1.95 ^{x} ± 0.38
Percentage of 95% ethanol			
0%	1.75 ^{d} ± 0.08	3.49 ^{d} ± 0.16	1.38 ^{d} ± 0.06
20%	2.11 ^{c} ± 0.14	4.20 ^{c} ± 0.28	1.66 ^{c} ± 0.11
40%	2.48 ^{b} ± 0.12	4.94 ^{b} ± 0.24	1.95 ^{b} ± 0.09
60%	2.97 ^{a} ± 0.20	5.93 ^{a} ± 0.40	2.34 ^{a} ± 0.16
80%	3.05 ^{a} ± 0.16	6.09 ^{a} ± 0.32	2.40 ^{a} ± 0.13
100%	2.50 ^{b} ± 0.13	4.99 ^{b} ± 0.27	1.97 ^{b} ± 0.11
Prik Khee Nu, green			
Overall	1.90 ^{y} ± 0.36	3.78 ^{y} ± 0.72	1.49 ^{y} ± 0.28
Percentage of 95% ethanol			
0%	1.35 ^{e} ± 0.10	2.70 ^{e} ± 0.21	1.06 ^{e} ± 0.08
20%	1.60 ^{d} ± 0.12	3.19 ^{d} ± 0.23	1.26 ^{d} ± 0.09
40%	1.86 ^{c} ± 0.19	3.72 ^{c} ± 0.38	1.47 ^{c} ± 0.15
60%	2.14 ^{ab} ± 0.05	4.27 ^{ab} ± 0.10	1.68 ^{ab} ± 0.04
80%	2.35 ^{a} ± 0.09	4.67 ^{a} ± 0.19	1.85 ^{a} ± 0.07
100%	2.09 ^{bc} ± 0.10	4.16 ^{bc} ± 0.20	1.64 ^{bc} ± 0.08
Prik Chee Fah, red			
Overall	1.93 ^{y} ± 0.25	3.85 ^{y} ± 0.50	1.52 ^{y} ± 0.20
Percentage of 95% ethanol			
0%	1.85 ± 0.37	3.70 ± 0.74	1.46 ± 0.29
20%	1.77 ± 0.29	3.53 ± 0.58	1.40 ± 0.23
40%	1.92 ± 0.24	3.83 ± 0.48	1.51 ± 0.19
60%	1.99 ± 0.26	3.96 ± 0.51	1.56 ± 0.20
80%	2.18 ± 0.15	4.33 ± 0.30	1.71 ± 0.12
100%	1.88 ± 0.11	3.74 ± 0.21	1.48 ± 0.08
Prik Chee Fah, green			
Overall	0.66 ^{z} ± 0.21	1.31 ^{z} ± 0.42	0.52 ^{z} ± 0.16
Percentage of 95% ethanol			
0%	0.42 ^{c} ± 0.08	0.84 ^{c} ± 0.15	0.33 ^{c} ± 0.06
20%	0.52 ^{bc} ± 0.16	1.04 ^{bc} ± 0.31	0.41 ^{bc} ± 0.12
40%	0.68 ^{ab} ± 0.17	1.35 ^{ab} ± 0.34	0.53 ^{ab} ± 0.13
60%	0.78 ^{a} ± 0.24	1.54 ^{a} ± 0.48	0.61 ^{a} ± 0.19
80%	0.78 ^{a} ± 0.19	1.56 ^{a} ± 0.38	0.62 ^{a} ± 0.15
100%	0.78 ^{a} ± 0.19	1.55 ^{a} ± 0.38	0.61 ^{a} ± 0.15

[1] Each value represents the mean and standard deviation from three lots;
a, b, … Means with different superscripts for each sample are significantly different ($p \leq 0.05$) between extraction solvents for that sample;
x, y,… Means with different superscripts are significantly different ($p \leq 0.05$) between samples.

[2] FW = Fresh weight.

all samples were shown in Table 4. Values at 30 min were not used for calculating highest values because absorbance values were quite stable after 10 min. Red Prik Chee Fah had the highest value, followed by red and green Prik Khee Nu, but values of green Prik Khee Nu and green Prik Chee Fah were not significantly different ($p>0.05$).

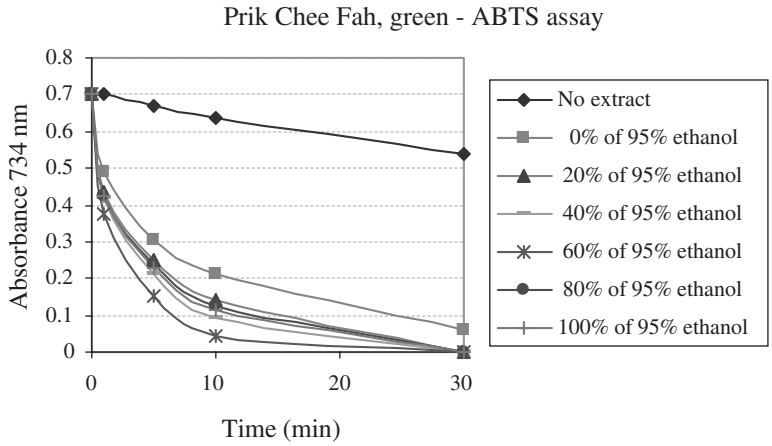

Figure 1 ABTS assay of green Prik Chee Fah when extraction solvents were varied.

Table 3 Highest ABTS values at 10 min of extracts from 60%(v/v) of 95% ethanol for Prik Khee Nu and Prik Chee Fah[1].

Sample	ABTS values (mg / g FW[2]) calculated on the basis of	
	Trolox	Vitamin C
Prik Khee Nu, red	10.13 [a] ± 1.51	6.68 [a] ± 0.84
Prik Khee Nu, green	9.82 [a] ± 1.33	6.48 [a] ± 0.88
Prik Chee Fah, red	5.80 [b] ± 0.56	3.82 [b] ± 0.37
Prik Chee Fah, green	2.00 [c] ± 0.07	1.32 [c] ± 0.05

[1] Means with different superscripts are significantly different ($p\leq0.05$) between samples.

[2] FW = Fresh weight.

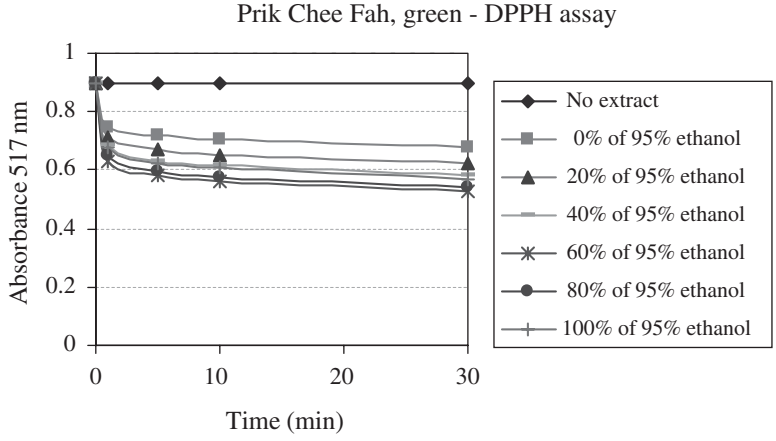

Figure 2 DPPH assay of green Prik Chee Fah when extraction solvents were varied.

Results showed that DPPH assay were lower than the ones of ABTS could also be found in previous works. Wang *et al.* (1998) who showed that some compounds, which have $ABTS^+$ scavenging activity may not show DPPH scavenging activity, and Arts *et al.* (2004) who found that some products of $ABTS^+$ scavenging reaction may have a higher antioxidant capacity and can react with $ABTS^+$. Moreover, in this study the extract of red Prik Chee Fah showed more DPPH scavenging activity than those of other samples. It was different from results of FRAP and ABTS assays which showed the higher antioxidant capacity of red Prik Khee Nu. Each method has its own reaction which may produce a different result, and natural samples compose of many compounds with various antioxidant capacity and mechanisms. Therefore, various methods should be used to monitor and compare for the antioxidant capacity study.

5. Total phenolic content

Total phenolic content of 4 chilies was estimated and shown in Figure 3. The significant difference of total phenolic content between extraction solvents was found for all samples, but they seemed to affect to Prik Khee Nu rather than Prik Chee Fah. Extracts from 60 and 80% of 95% ethanol provided higher values, and red Prik Khee Nu contained a higher phenolic content (3.48 ± 0.58 mg gallic acid / g fresh weight), followed by green Prik Khee Nu (2.83 ± 0.31 mg gallic acid / g fresh weight), red Prik Chee Fah (1.81 ± 0.23 mg gallic acid / g fresh weight), and green Prik Chee Fah (0.85 ± 0.20 mg gallic acid / g fresh weight), respectively.

Bivariate correlations between all antioxidant capacity values and total phenolic

Table 4 Highest DPPH values at 10 min of extracts from 60%(v/v) of 95% ethanol for Prik Khee Nu and Prik Chee Fah.

Sample	DPPH values (mg / g FW[2]) calculated on the basis of	
	Trolox	Vitamin C
Prik Khee Nu, red	0.93 [b] ± 0.08	0.79 [b] ± 0.07
Prik Khee Nu, green	0.78 [bc] ± 0.11	0.66 [bc] ± 0.09
Prik Chee Fah, red	1.46 [a] ± 0.37	1.23 [a] ± 0.31
Prik Chee Fah, green	0.42 [c] ± 0.05	0.36 [c] ± 0.04

[1] Means with different superscripts are significantly different ($p \leq 0.05$) between samples.

[2] FW = Fresh weight.

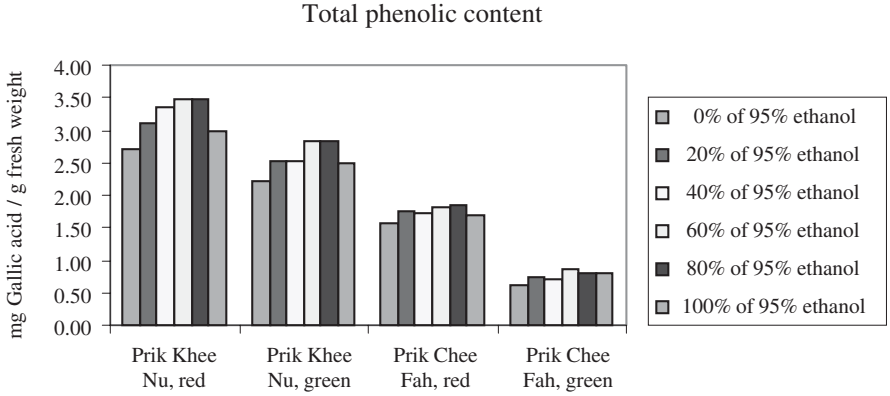

Figure 3 Total phenolic content estimation when extraction solvents were varied.

Table 5 Bivariate correlation results of three antioxidant capacity assays and total phenolic content from all extracts.

	Correlation coefficient	Sig. (2-tailed)
Total phenolic content	1.000	-
FRAP	0.866	0.000
ABTS (1 min)	0.561	0.000
ABTS (5 min)	0.478	0.000
ABTS (10 min)	0.423	0.000
ABTS (30 min)	0.310	0.008
DPPH (1 min)	0.811	0.000
DPPH (5 min)	0.906	0.000
DPPH (10 min)	0.909	0.000
DPPH (30 min)	0.896	0.000

content of all extracts were analyzed according to the fact that many phenolic compounds in plants are good sources of natural antioxidants (Ho, 1992; Amiot *et al.*, 1997). The results of bivariate correlations in Table 5 shows a good agreement between results of all antioxidant capacity assays and total phenolic content. Although correlation coefficients between all ABTS assays and total phenol content were not high, their correlations were still highly significant ($p \leq 0.01$). These lower coefficients might be caused by the different antioxidant activity of $ABTS^+$ scavenging reaction products (Arts *et al.*, 2004), and some reactions were completed before the measuring time at 30 min. Significance levels indicated that correlations were real, not due to chance in the form of random sampling error, even though the strength of a linear relationship was not high.

CONCLUSION

Both extraction solvents and analytical methods affected the estimation of antioxidant capacity and total phenolic content of samples. Therefore, extraction solvents should be considered and more than one method should be used for comparison. For chilies in this study, solvents containing 60 and 80 % (v/v) of 95% ethanol were more suitable. The results of FRAP and ABTS assays were much more agreeable than the ones of DPPH assays. However, the correlations of all three assays and total phenolic content were found. All results showed that chilies are good sources of natural antioxidants and phenolic compounds.

ACKNOWLEDGEMENTS

This work was a part of a research project supported by a grant from Office of Agricultural Research and Extension at Maejo University, Thailand.

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