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Comparisons and Correlations of Phenolic Profiles and Anti-oxidant Activities of Seventeen Varieties of Pineapple

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Abstract Major phenolic, β-carotene, and ascorbic acid (AA) contents in 17 pineapple varieties were quantified and compared. Anti-oxidant activities were evaluated using 2,2-Diphenyl-l-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing anti-oxidant power (FRAP), and metal chelating capacity (MCC) assays. MD-2 exhibited the highest AA and total phenolic (TP) contents and DPPH and ABTS assay results, but was lower in β-carotene contents. Ripley had the highest total flavonoid (TF) content with a low AA content. Comte de Pairs exhibited the highest MCC and the lowest FRAP values. TP contents and both DPPH and ABTS activities, FRAP values and both AA contents and DPPH activities, and TF contents and ABTS activities were positively correlated. MD-2 exhibited the greatest diversity of phenolics and highest anti-oxidant activities in all assays. Information included herein can be useful for development of pineapple-based food products containing high levels of health promoting anti-oxidants.

Keywords: anti-oxidant, correlation, phenolic profile, pineapple

Introduction

Pineapple (*Ananas comous* L.) is a popular tropical fruit that is widely cultivated in Thailand, The Philippines, China, Brazil, and India (1). In addition to fresh-cut pineapple for direct consumption, pineapple is widely processed into juice and canned products. More than 100 volatile compounds have been identified in fresh-cut pineapple and juice (2,3). Hence, pineapple has been used as a flavor ingredient for baked foods, such as pies, pizzas, and cakes, to enhance flavor and taste (1).

Pineapple flesh is a good source of nutrients such as vitamins, minerals, and phenolic phytochemicals (4). Phenolics provide health promoting functions for prevention of inflammation, atherosclerosis, tumor formation, and other chronic diseases (5). Although phenolic profiles and anti-oxidant activities of pineapples and other tropical fruits, including mangos, bananas, and papayas have been reported, comprehensive information is still limited because few varieties were involved (4,6). Thus, in this study, 17 pineapple varieties were investigated for differences in phenolic profiles, β-carotene and ascorbic acid contents, and anti-oxidant activities. Results reflected relationships of bioactive compounds and anti-oxidant activities in pineapples.

In this study, anti-oxidant activities of different varieties of pineapple flesh were evaluated using 2,2-Diphenyl-l-picrylhydrazyl (DPPH) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), assays and reducing power (FRAP) and metal chelating capability (MCC) assays for evaluation of free radical destruction and inhibition of redox and transition metal oxidation. Correlations of total phenolic and flavonoid contents and anti-oxidant activities measured using different assays revealed contributions to anti-oxidant activities. In general, phenolic profiles and levels, β-carotene and AA contents, and anti-oxidant activities of 17 pineapple varieties reported in this study will be useful for selection of pineapple varieties for production of pineapple-based food products with high health promoting capabilities.

Materials and Methods

Chemicals NADH (nicotineamide adenine dinucleotide), PMS (phenazine mentosulphate), DPPH (2,2-diphenyl-l-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-s-triazine), Trolox, Folin-ciocalteau reagent, sodium fluorescein, and ascorbic, gallic, cinnamic, chlorogenic, coumaric, sinapic, ferulic, vanillic, and p-hydroxybenzoic acids, and

catechin, β-carotene, and quercetin standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile, acetic acid, acetone, and methanol were purchased from Fisher Chemicals (Fair Lawn, NJ, USA).

Pineapple sample preparation All 17 pineapple varieties were cultivated in experimental fields of the Pineapple Germplasm Repository of South Subtropical Crops Research Institute (SSCRI) in Zhanjiang, China and harvested in July of 2013. Endowed with a typical tropical to subtropical marine climate, Zhangjiang has an average annual temperature of 23°C and an average annual precipitation of 1,500 mL. Five ripened pineapple fruits of each variety were randomly selected from experimental fields. After peeling, flesh of pineapples was cut into 1 cm×1 cm pieces. Then, 500 g of fresh-cut pieces was homogenized in a blender (MBB518; Waring, East Windsor, NJ, USA). Twenty g of homogenized pineapple pulp was mixed with 60 mL of methanol and incubated at 60°C for 30 min with agitation (Helmer PC900i I series Platelet Incubator W/ PF48i; Platelet Agitator, Helmer Scientific, Noblesville, IN, USA). After centrifugation (Thermo Scientific, Waltham, MA, USA) at 5,000×*g* for 10 min, the methanol layer was collected. The residue was mixed with 60 mL of methanol to repeat the extraction procedure. All methanol layers were combined and evaporated to dryness at 40°C using a vacuum rotary evaporator (P/N Hei-VAP Precision ML/G3 564-01300-00; Hei-VAP Precision, Heidolph, Schwabach, Germany). Three independent batches of dried extract were prepared, and each dried extract was separately applied to a stock methanol solution at 100 mg/mL.

Determination of total phenolic, total flavonoid, and ascorbic acid contents The total phenolic (TP) content was determined following the method of Jang and Xu (7). Folin-Ciocalteau reagent (0.75 mL) was mixed with 0.1 mL of a 1 mg/mL diluted extract stock solution and allowed to react for 5 min. The mixture was mixed with 0.75 mL of sodium bicarbonate (60 g/L) at 25°C for 90 min in the dark. Then, the absorbance of the reaction solution was measured using a 1600 UV-Vis spectrometer (Shimadzu, Kyoto, Japan) at 750 nm. The TP content was calculated and expressed as mg of gallic acid equivalents (GAE)/100 g of fresh weight (FW) based on a standard curve prepared using gallic acid.

The total flavonoid (TF) content was determined as described by Kim *et al*. (8). One mL of a 1 mg/mL diluted stock solution was mixed with 0.3 mL of 5% $NaNO_2$ and 4 mL of distilled water. A 0.3 mL aliquot of 10% $AlCl_3$ was transferred to the mixture, followed by addition of 2 mL of 1 M NaOH. After the reaction solution (200 μL) was diluted with 1.8 mL distilled water, the absorbance of the diluted solution was measured at 506 nm by a UV-Vis spectrometer (1600; Shimadzu). The TF content was calculated using a curve obtained from a quercetin standard and expressed as mg of quercetin equivalents (QE)/100 g (FW).

The ascorbic acid (AA) content was measured following the method of Kampfenkel *et al*. (9). Homogenized pineapple flesh was mixed with 8 mL of 6% trichloroacetic acid and cooled in an ice bath for 10 min before centrifugation at 12,000×*g* for 20 min. The supernatant was collected for measurement of the absorbance by a UV-Vis spectrometer (1600; Shimadzu) at 525 nm expressed as mg/100 g (FW) using the standard curve of ascorbic acid which was obtained using ascorbic acid standard.

Determination of anti-oxidant activities DPPH assays were performed following the method of Liyana-Pathirana and Shahidi (10) with minor modification. One mL of 0.135 mM DPPH was mixed with 1 mL of a 1 mg/mL diluted stock solution. The mixture was vortexed by a Vortex Mixer (Cole-Parmer, Vernon Hills, IL, USA) for 1 min and incubated for 30 min at 25°C in the dark. The absorbance of the mixture was measured by a UV-Vis spectrometer (1600; Shimadzu) at 517 nm. The scavenging DPPH free radical activity was expressed as μM Trolox equivalents (TE)/100 g (FW) and calculated using a standard curve of Trolox which was obtained by Trolox standard.

The ABTS assay method was similar to the method of Re *et al.* (11). A mixture consisting of 7 mM of ABTS and 2.4 mM of potassium persulfate (1:1, v/v) was incubated in the dark for 12 h at 40°C, then diluted with methanol to obtain an absorbance of 0.70±0.02 at 734 nm by a UV-Vis spectrometer (1600; Shimadzu). Subsequently, 1 mL of the prepared mixture was mixed and reacted with 1 mL of a 1 mg/mL diluted stock solution for 7 min, or with methanol as a blank. The absorbance of the reaction solution was measured at 734 nm by a UV-Vis spectrometer (1600; Shimadzu). The ABTS free radical scavenging activity was expressed as μM Trolox equivalents (TE) /100 g (FW) using the standard curve of Trolox.

The Ferric reducing anti-oxidant power (FRAP) method used herein was similar to the method developed by Benzie and Strain (12). The FRAP reagent contained 25 mL of 300 mM sodium acetate in acetic acid at pH 3.6, 2.5 mL of a 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 2.5 mL of a 20 mM $FeCl_3 \cdot 6H_2O$ solution. Ten μL of a 100 μg/mL diluted stock solution, or 1 mM $FeSO_4$ as a reference was mixed with 1 mL of distilled water and 1.8 mL of the FRAP solution. Then, the mixture was allowed to react at 37°C for 10 min. Absorbance values of the reaction and $FeSO_4$ reference solutions were determined by a UV-Vis spectrometer (1600; Shimadzu) at 593 nm and they were compared to obtain FRAP activity values and converted to μM Trolox equivalents (TE)/100 g (FW) using the Trolox standard curve.

The metal chelating capacity (MCC) was determined following the method of Dinis *et al*. (13). One mL of a 100 μg/mL diluted stock solution was mixed with 2.8 mL of distilled water, 50 μL of 2 mM $FeCl_2 \cdot 4H_2O$, and 150 μL of 5 mM ferrozine. After reaction for 10 min, formation of a ferrous ion-ferrozine complex was monitored by a UV-Vis spectrometer (1600; Shimadzu) at 562 nm to determine the MCC value as:

$$\text{MCC (Chelating capacity \%)} = (Abs_{blank} - Abs_{sample})/Abs_{blank} \times 100$$

where Abs_{blank} and Abs_{sample} are absorbance values of blank and pineapple sample mixtures, respectively.

Determination of phenolic and β-carotene contents Individual phenolic and β-carotene contents were determined using a Shimadzu LC-20A HPLC apparatus coupled with a UV-Vis multi-wavelength detector and a C18 reversed phase column (i.d. 250×4.6 mm, 4 μm) (Hypersil ODS). The mobile phase conditions and HPLC methods for determination of phenolic and β-carotene contents followed the methods of Shen *et al.* (14).

Statistical analysis TP, TF, AA, phenolics, and β-carotene measurement data, and DPPH, ABTS, FRAP, and MCC assay results for all 17 pineapple varieties from 3 independent experiments were analyzed, and results were expressed as mean±standard deviation (SD). A one-way analysis of variance (ANOVA) was used for evaluation of overall significant differences, followed by a post-hoc Tukey's studentized range test at $p<0.05$ using SAS software, v. 9.1.3 (SAS Institute, Cary, NC, USA). The correlations between TP, TF, and AA contents and different anti-oxidant activities among the 17 pineapple varieties were carried out at $p<0.05$ (*) and $p<0.01$ (**).

Results and Discussion

Phenolic profiles and total phenolic, flavonoid, ascorbic acid, and β-carotene contents Phenolic profiles in the flesh of 17 pineapple varieties are listed in Table 1. Comte de Paris and MD-2 showed the greatest phenolic profile diversity consisting of 11 phenolics, and catechin, epicatechin, myricetin, gallic acid (GA), *p*-hydroxybenzoic acid (pHA), chlorogenic acid (ChA), vanillic acid (VA), coumaric acid (CmA), ferulic acid (FA), sinapic acid (SA), and cinnamic acid (CiA) (Fig. 1). In contrast, DN5 only had 6 phenolics and showed the lowest phenolic profile diversity among varieties (Table 1). Catechin was the most abundant phenolic in Red Pineapple at 285.15 μg/g (FW), followed by 152.38 μg/g (FW) in Fresh Premium and 122.24 μg/g (FW) in Ripley, while Shenwan had the lowest level at 24.00 μg/g (FW) (Table 1). SA was present in most varieties with a level in Jaspine of 84.89 μg/g (FW) and 82.77 μg/g (FW) in Comte de Paris. Catechin and SA are effective anti-oxidants for scavenging free radicals in the human body and prevention of cell inflammation (5). The richness of catechin and SA in pineapples could play a major role in health promotion functions of this tropical fruit.

Epicatechin ranged from a level of 11.15 μg/g (FW) in DL3 to undetectable in DN5, Shenwan, and Chenhuang, while VA was only detected in DN2, MD-2, Shenwan, Comte de Paris, and Jaspine at a level below 0.13 μg/g (FW) (Table 1). In the previous studies, chlorogenic acid, luteolin-7-O-glucoside, ferulic acid, and proto-catechuic, catechin, quercetin, and cinamic acids were identified in several pineapple varieties (6,15). Differences in phenolic profiles in pineapple varieties may have been due to genetically controlled biosynthesis of phenolic compounds in pineapples.

Among the 17 pineapple varieties, MD-2 had the highest TP content of 72.57 mg GAE/100 g (FW), while Fresh Premium and Smooth Cayenne had TO levels above 40 mg GAE/100 g (FW) (Table 2). The TP content was in a range of 25.51 to 38.69 mg GAE/100 g

Table 1. Phenolic profiles of 17 pineapple varieties

Variety	Phenolics (μg/g, FW)										
	GA[1)]	Catechin	*p*HA	ChA	VA	Epicatechin	CmA	FA	SA	Myricetin	CiA
N1	0.26*f*[2)]	38.51*h*	0.45 *d*	0.01*h*	-[3)]	6.76*c*	0.02*h*	-	5.06*h*	0.24*f*	-
DL1	0.70*a*	52.48*g*	0.47*d*	0.05*e*	-	4.38*e*	0.01*i*	-	26.66*e*	-	-
DL2	0.26*f*	53.70*g*	0.26 *g*	0.01*h*	-	5.27*d*	0.13*d*	-	3.47*i*	-	-
DL3	0.24*f*	78.50*e*	0.34 *f*	0.04*f*	-	11.15*a*	0.03*g*	-	20.00*f*	-	-
DN2	.0.17*g*	58.16*g*	0.52*c*	0.10*c*	0.05*c*	4.94*d*	0.04*g*	-	12.55*g*	-	-
DN5	0.06*i*	41.10*h*	0.14*i*	0.03*f*	-	-	0.07*f*	-	-	0.40*d*	-
Fresh Premium	0.30*e*	152.38*b*	0.48*d*	0.01*h*	-	4.41*e*	0.16*c*	-	59.10*c*	-	-
Ripley	0.48*c*	122.24*c*	0.31*f*	0.03*f*	-	4.40*e*	0.22*b*	0.14*b*	-	-	0.03 *c*
MD-2	0.24*f*	80.94*e*	0.68*a*	0.06*d*	0.13*a*	2.14*f*	0.11*e*	0.20*a*	1.36*k*	0.71*b*	0.05*b*
Tainung 17	0.14*h*	43.57*h*	0.21*h*	0.03*f*	-	10.15*a*	-	-	2.48*j*	0.38*d*	0.07*a*
Red Pineapple	0.31*e*	285.15*a*	0.16*i*	0.03*f*	-	0.88*g*	-	0.12*c*	70.57*b*	0.30*e*	-
Shenwan	0.30*e*	24.00*i*	0.16*i*	0.19*a*	0.08*b*	-	-	0.10*c*	26.63*e*	0.33*e*	-
Comte de Paris	0.28*e*	77.48*e*	0.52*c*	0.12*b*	0.07*b*	6.65*c*	0.25*a*	0.11*c*	82.77*a*	1.02*a*	0.01 *e*
Smooth Cayenne	0.54*b*	63.33*f*	0.57 *b*	0.04*e*	-	3.70*e*	-	-	38.20*d*	0.08*g*	0.08 *a*
Chenhuang	0.40*d*	53.25*g*	0.20*h*	0.06*d*	-	-	-	-	0.84*l*	0.54*c*	0.03 *c*
Jaspine	0.43*c*	91.70*d*	0.48 *d*	0.01*h*	0.13*a*	6.90*c*	0.26*a*	0.07*d*	84.89*a*	-	0.02*d*
New Phuket	0.76*a*	80.68*e*	0.39 *e*	0.02*g*	-	7.89*b*	-	0.05*e*	35.44*d*	0.10*g*	0.01 *e*

[1)]FW (fresh weight), GA (Gallic acid), ChA (chlorogenic acid), CiA (cinnamic acid), CmA (coumaric acid), FA (ferulic acid), *p*HA (*p*-hydroxybenzoic acid), SA (sinapic acid), and VA (vanillic acid)

[2)]Values with different letters in the same column are significantly different at $p<0.05$.

[3)]Not detectable.

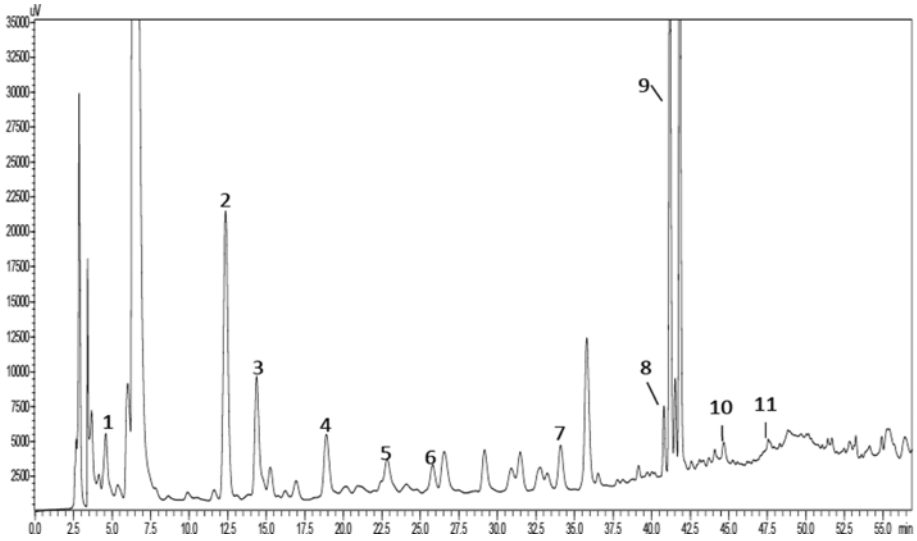

Fig. 1. Typical chromatogram of phenolic profile in pineapple flesh: 1. gallic acid; 2. catechin; 3. *p*-hydroxybenzoic acid; 4. chlorogenic acid; 5. vanillic acid; 6. epicatechin; 7. coumaric acid; 8. ferulic acid; 9. sinapic acid; 10. myricetin; 11. cinnamic acid

(FW) in other varieties. TP contents were related to the reducing power of phenolics and relied not only on quantities but also chemical structures of phenolics, which may have different numbers and positions of hydroxyl group (15,16). The TF content in pineapples varied significantly (p<0.05) from 10.27 mg of QE/100 g (FW) for Smooth Cayenne to 50.57 mg QE/100 g (FW) for Ripley (Table 2). DN2, DN5, Shenwan, Comte de Paris, Chenhuang, and Jaspine had TF contents of approximately 20 mg of QE/100 g (FW) (Table 2). However, TF contents in most pineapple varieties in this study, especially Ripley and MD-2, were higher than for other tropical fruits, such as mangos and loquats, for which TF contents were between 11.2 and 16.9 QE/100 g (FW) (16,17).

The attractive yellowish color and palatable sour taste of pineapples are mainly due to β-carotene and AA, respectively. MD-2 had the highest AA content of 26.19 mg/100 g (FW), which was approximately 2x higher than for DL1 at 13.87, Shenwan at 12.98, Tainung 17 at 12.08, and New Phuket at 11.74 mg/100 g (FW), while the AA content was below 9 mg/100 g (FW) in other pineapple varieties (Table 2). AA is recognized as an essential vitamin and is closely associated with immune system function. Therefore, consumption of pineapple would be a direct and efficient intake of AA for health promotion purposes. β-Carotene has a provitamin A activity and can

Table 2. TP, TF, AA, and β-carotene contents of 17 pineapples varieties

Varieties	TP (mg GAE/100 g, FW[1)])	TF (mg QE/100 g, FW)	AA (mg/100 g, FW)	β-Carotene (μg/g, FW)
N1	38.22±3.54*bc*[2)]	14.76±2.56*c-e*	4.25±0.19*h*	2.16±0.01*b*
DL1	32.44±3.43*bc*	14.63±2.24*c-f*	13.87±0.19*b*	1.91±0.01*c*
DL2	36.53±4.48*bc*	19.24±2.71*cd*	6.04±0.00*f*	1.19±0.01*f*
DL3	31.66±3.34*bc*	13.90±2.70*d-f*	8.84±0.39*d*	3.06±0.02*a*
DN2	30.75±1.27*cd*	20.82±0.78*cd*	5.56±0.00*g*	2.08±0.01*b*
DN5	35.04±0.37*bc*	22.83±0.22*c*	0.40±0.00*k*	1.77±0.01*d*
Fresh Premium	43.76±1.67*b*	17.28±0.32*c-e*	6.04±0.00*f*	1.46±0.01*e*
Ripley	36.66±1.63*bc*	50.57±2.63*a*	0.93±0.23*j*	1.90±0.01*c*
MD-2	72.57±8.99*a*	33.60±2.94*b*	26.19±0.00*a*	0.40±0.00*h*
Tainung 17	31.39±2.10*cd*	10.40±0.78*g*	12.08±0.34*c*	2.44±0.02*b*
Red Pineapple	25.51±1.14*e*	12.28±1.72*ef*	8.47±0.23*d*	0.37±0.00*h*
Shenwan	36.12±2.87*bc*	21.12±0.96*cd*	12.98±0.34*b*	0.31±0.00*i*
Comte de Paris	34.94±5.10*bc*	20.23±3.40*c*	6.94±0.20*e*	0.52±0.00*g*
Smooth Cayenne	40.42±3.22*b*	10.27±2.40*ef*	6.71±0.00*e*	1.15±0.00*f*
Chenhuang	29.26±2.26*cd*	20.40±0.38*cd*	4.23±0.23*h*	0.30±0.00*i*
Jaspine	32.91±0.63*cd*	21.72±0.27*cd*	2.91±0.23*i*	2.08±0.01*b*
New Phuket	38.69±4.41*b*	14.50±1.73*ef*	11.74±0.58*c*	1.72±0.01*d*

[1)]FW, fresh weight

[2)]Values with different letters in the same column are significantly different at p<0.05.

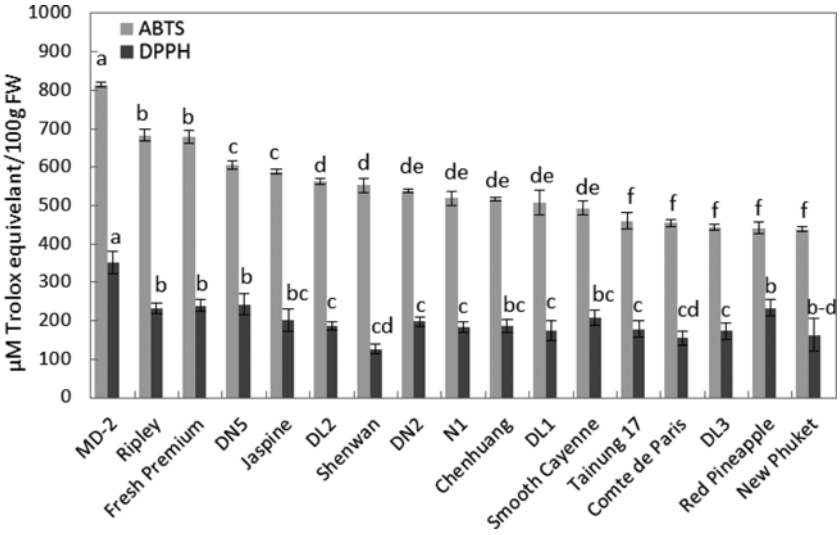

Fig. 2. Scavenging DPPH and ABTS free radical activities of the seventeen pineapple varieties

reduce risks of cardiovascular diseases (5) with a reported range of 1.39 to 3.47 μg/g (FW) in Indonesian pineapples (18). Levels of β-carotene in the 17 varieties were between 0.30 in Chenhuang and 3.06 μg/g (FW) in DL3 (Table 2). Except Chenhuang, all varieties exhibited higher than average levels of β-carotene at 0.30 μg/g (FW) reported in the U.S. Department of Agriculture (USDA) database (19).

Anti-oxidant activities of pineapples obtained using DPPH, ABTS, FRAP, and MCC assays DPPH, ABTS, FRAP, and MCC assays are traditional methods for assessment of anti-oxidant activities, especially for fruits and vegetables. Comparison and combination of assay measurements allowed comprehensive investigation of anti-oxidant properties of different pineapple varieties and relationships with phenolic and ascorbic acid contents. DPPH and ABTS assays are mainly used for measuring anti-oxidant activities of organic nitrogen scavenging DPPH• and cation ABTS+ radicals, respectively, based on hydrogen donation capabilities of anti-oxidants (20). Due to high TP, TF, and AA contents, MD-2 was the leading variety for free radical scavenging activities in both DPPH at 351.62 μM of TE/100 g (FW) and ABTS at 814.63 μM of TE/100 g (FW) assays (Fig. 2). DN5, Fresh Premium, Ripley, and Red Pineapple also had DPPH scavenging activities of 241.95, 240.00, 233.03, and 233.03 μM of TE/100 g (FW), respectively (Fig. 2) related to the diversity and high levels of phenolics in Red Pineapple and Fresh Premium, and high TF contents in Ripley (Table 2). Fresh Premium and Ripley were the 2 of the top 5 varieties in ABTS assays, exhibiting good performance in scavenging of ABTS free radicals (Fig. 2). Additionally, diversity in the phenolic profile of Jaspine and a high TF content in DN5 may also have been responsible for effective capabilities in scavenging of ABTS free radicals (Table 2 and Fig. 2).

FRAP assays are used for determination of reducing powers of compounds with active hydroxyl groups (21). Previous studies have suggested that lipid peroxidation or protein modification caused by increased formation of reactive oxygen species (ROS) can be alleviated and inhibited by anti-oxidants with strong reducing powers (22). Thus, FRAP results obtained in this study can be important indicators for evaluation of activities of pineapple varieties for inhibition of oxidation involving reactive oxygen and metal ion oxidants. FRAP results for pineapples varied by variety. Shenwan at 609.50 and MD-2 at 519.50 μM of TE/100 g (FW) were 2 typical varieties with strong FRAP values (Fig. 3). High FRAP value may have been mainly due to relatively high TP, TF, and AA contents. FRAP values of other pineapple varieties ranged from 254.50 for Jaspine to 429.50 μM of TE/100 g (FW) for Chenhuang (Fig. 3). Average FRAP values of 211.93 for mangos and 350 μM of TE/100 g (FW) for figs were reported by Pande and Akoh (23) and Palafox-Carlosa *et al*. (24), respectively, which were lower than for half of the 17 pineapple varieties in this study.

Additionally, transition metals likely undergo redox cycling reactions and lead to a series of harmful oxidation reactions in biological systems that induce many chronic diseases, such as atherosclerosis, neurological disorders, Alzheimer and Parkinson diseases, and inflammation symptoms (22). Thus, MCC assays were used for examination of the effectiveness of each pineapple variety for reducing transition metals for prevention of related chronic diseases. DL2, Tiannung17, Comte de Paris, N1, DL1, Red Pineapple, and Smooth Cayenne all exhibited similar levels of ferrous ion chelation power (Fig. 3). However, MCC values of DL3, DN2, DN5, Ripley, MD-2, Chenhuang, Jaspine, and New Phuket were all below 9% (Fig. 3).

Correlations of TP, TF, and AA contents and anti-oxidant activities obtained using DPPH, ABTS, FRAP, and MCC assays Correlations of anti-oxidant contents and anti-oxidant activities measured using 4 different assays were evaluated for identification of relationships between different types of anti-oxidants in pineapples and anti-oxidant activities (Table 3). Significant positive correlations between

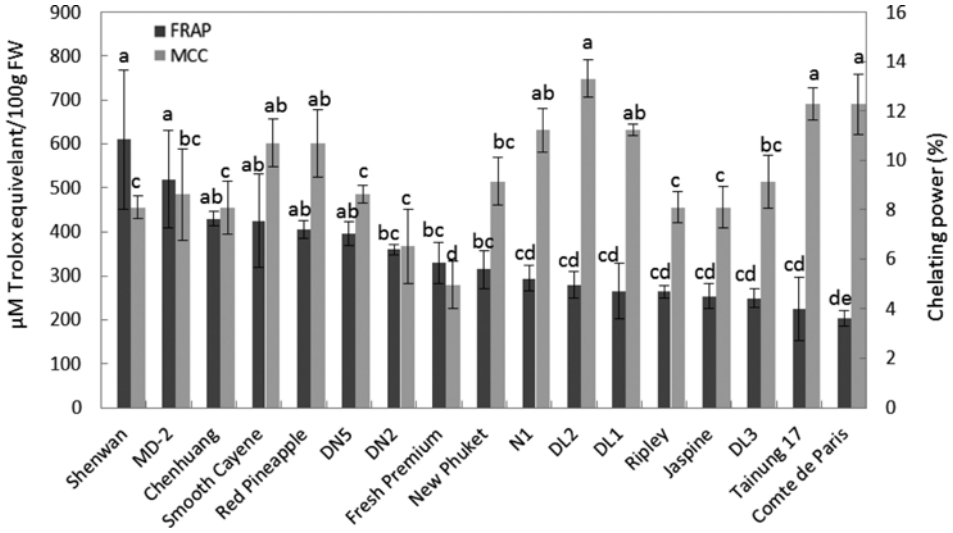

Fig. 3. Ferric reducing antioxidant powers (FRAP) and metal chelating capabilities (MCC) of the seventeen pineapple varieties

Table 3. Correlations between TP, TF, and AA contents and different anti-oxidant activities of 17 pineapple varieties

	TP	TF	AA	DPPH	ABTS	FRAP	MCC
TP	1.00						
TF	0.29	1.00					
AA	0.30	-0.33	1.00				
DPPH	0.81**	0.28	0.49*	1.00			
ABTS	0.81**	0.62**	0.06	0.79**	1.00		
FRAP	0.50*	-0.03	0.73**	0.66**	0.43	1.00	
MCC	0.03	-0.33	0.40	0.05	-0.27	0.07	1.00

*p<0.05
**p<0.01

TP content and DPPH scavenging (r=0.81) and ABTS (r=0.81) activity were observed. Thus, TP contents in pineapples were significantly (p<0.01) associated with activities of scavenging free radicals based on the post-hoc Tukey's studentized range test. Also, TF contents were correlated with ABTS scavenging activities with r=0.62, but not with DPPH scavenging activities (r=0.28). Flavonoids may be more specific in scavenging of ABTS+ cations than DPPH radicals (25,26). Poor correlations between TF and DPPH assay activities probably related to the ABTS assay being more sensitive to structural conformation and steric accessibility of anti-oxidant molecules (25). Different from TF contents, AA contents were significantly (p<0.01) correlated with FRAP results (r=0.73) but not with ABTS scavenging activities (r=0.06). Furthermore, scavenging of DPPH radicals and FRAP assay reducing powers were positively positive correlated (r=0.66). Correlation of AA contents with FRAP powers was reported for guava fruits (26). In this study, positive correlations between TP contents and FRAP powers in different pineapple varieties was identified (r=0.50). The correlation was also reported in mandarin (27) and pears (28).

MCC assay results were not correlated with other assay results or with anti-oxidant activities in this study (Table 3). Solubility values of the anti-oxidant catechin and other flavonoids were reportedly low in the reaction media of an MCC assay (29). Low solubility values of catechin and other flavonoids in pineapples may result in reduced reactions with metal ion oxidants. Therefore, compared with highly soluble anti-oxidants, contributions of flavonoids were relatively weak for metal chelating activities.

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