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Determination of total phenolics and ascorbic acid related to an antioxidant activity and thermal stability of the Mao fruit juice

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Abstract

This study was aimed to determine total phenolics and ascorbic acid in association with their antioxidant activity and thermal stability. Both total phenolics and ascorbic acid contents of the mao juice were 337.52 mg GAE/100 mL and 175.34 mg/100 mL, respectively. Their antioxidant activity was comparatively assayed by four antioxidant methods, and found to be 590.81 mg BHT/100 mL, 95.41 mg Trolox/100 mL, 488.96 mg Trolox/100 mL and 15.77 mM Fe^{2+}/100 mL, according to each corresponding substrate of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2´-azinobis(3-ethylbenzothiazoline 6-sulphonate (ABTS), N,N-dimethyl-p-phenylenediamine dihydrochloride (DMPD) and ferric reducing antioxidant power (FRAP), respectively. The heating effect on total phenolics of the mao juice was also investigated. It was found that changes in the antioxidant activity related total phenolics of the juice sample were not significantly found, indicating high thermal stability.

Introduction

Nowadays, there is growing interest in the use of polyphenolic antioxidant-rich plant as dietary supplements (Alonso *et al*., 2002; Rababah *et al*., 2004). Current recommendation of health experts is to increase the consumption of fruit since there is convincing evidences linking a diet rich in fruit with reduced incidence of coronary heart disease, cancer and various age relating chronic disease (Margetts *et al.*, 2003). The protective effect of fruits has been attributed to their bioactive antioxidant constituents, including polyphenols and vitamins such as vitamin C (Shi *et al*., 2001). Polyphenols are secondary metabolites present in all vegetal tissues, as well as in flowers and fruits. They are important antioxidants of human diet (Gharras, 2009). Vitamin C or ascorbic acid is also one of the essential phytonutrients for the metabolism of living cells that occurs in different concentrations in natural foods, especially fruits and their products. Both polyphenols and ascorbic acid are major contributors to the total antioxidant activity in vegetable and fruit. It was reported that vitamin C accounted for 65-100% of antioxidant capacity of citrus juices (Gardner *et al*., 2000). Bangladesh fruit had high level of total phenolic content and reducing activity (Aklima *et al*., 2014).

In Thailand, the compiled fruits of the mao tree (*Antidesma thwaitesianum* Muell. Arg.) are commonly consumed as commercially available products of 100% juice and wine, particularly the fruit juice contains a very rich source of antioxidants. Mao fruit is favorable to be consumed and sold in the local market because of its good color and taste. Both mao juice and mao wine have become more popular as healthy nourishment (Puangpronpitag *et al*., 2008). There are about eighteen species of *Antidesma* plants mostly grown in Northeast, especially in Phu Phan district, Sakon Nakhon province. It is a shrub plant approximate 6-15 meters tall. Each of its compiled fruits is an oval shape with dark green fruit, turns to orange-red and dark-purple at a fully ripe stage and becomes sweet with slightly tart (Figure 1).

Figure 1. Picture showing green and ripe compiled fruits of *A. thwaitesianum* Müll. Arg or the so-called "Mao" fruit. (photo by T. Sripakdee)

Total phenolics, antioxidant activity and nutritive values of the mao fruits have previously been reported. Polyphenolic compounds and proanthocyanidins isolated from the mao extracts exhibited much higher antioxidant activities than that of standard Trolox and had similar antioxidant potential to grape seed

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proanthocyanidin extract (Puangpronpitag *et al*., 2008). Fifteen varieties of the Mao Luang fruits contained three different flavonoids, i.e. catechin, procyanidin B1 and procyanidin B2 (Butkhup *et al*., 2008). These organic compounds are the major flavonoids in all analyzed fruit samples. The skin contact the Mao Luang red wine had higher amounts of flavonoids, phenolic acids, anthocyanins, organic acids than the non-skin contact one (Samappito *et al*., 2008).

Since the great importance of antioxidant activity mainly attributed from polyphenols and ascorbic acid for human health and also because of the growth in commercial fruit juice production and consumption (Mahdavi *et al.*, 2010), the aim of this study was to evaluate the antioxidant activity related to total phenolics and ascorbic acid contents, and their thermal stability of the mao fruit juice.

Materials and Methods

Chemicals and instruments

2,2-Diphenyl-1-picrylhydrazyl, gallic acid, ascorbic acid were purchased from Sigma-Aldrich (USA). 2,4,6-Tri(2-pyridyl)-s-triazine was purchased from Sigma (Switzerland). 2,2'-Azino-bis(3-ethylbenzothiazolin-6-sulfonic acid and 2,6-dichlorophenolindophenol sodium salt were purchased from Fluka (Germany). N,N–Dimethyl-p-phenylenediamine dihydrochloride was purchased from Fluka (Switzerland). Potassium persulfate and iron chloride hexahydrate were purchased from QRëC® (New Zealand). Trolox was purchased from Aldrich (Russia). Ferrous sulfate heptahydrates, metaphosphoric acid and sodium acetate were purchased from Carlo Erba (Italy). Butylated hydroxytoluene and Folin-Ciocalteu reagent were purchased from Merck Chemical Supplies (Germany). Sodium carbonate was purchased from Univar (Australia). All chemicals and solvents used such as methanol, dichloromethane, acetic acid and hydrochloric acid were of analytical grade.

Ultraviolet and visible spectrophotometer (Agilent 8453, USA) was used to determine total phenolics, ascorbic acid and antioxidant activity. pH meter (Model Proline B 210, China) was used to measure the pH solution of the fruit juice.

Sample preparation

The sealed bottles of 100% mao juice were purchased from Phu Phan district, Sakon Nakhon province, Thailand. The mao juice was kept storing in refrigerator at 4°C before use. The juice sample was extracted with an acidified methanol. Briefly, 5 mL of the juice was mixed with 20 mL of 1%(v/v) HCl methanol and kept at room temperature overnight. The extract was centrifuged at 4000 rpm for 10 min. The supernatant was used for determination of antioxidant activity, total phenolics and ascorbic acid.

Chemistry background of the mao juice

The chemistry background of the mao juice including pH value, total acidity, volatile acidity, fixed acidity, total solids, specific gravity and buffering capacity was determined following the standard methods of AOAC (1995). Total acidity of the fruit juice was determined by titration method. The solution of dilute juice was titrated to pH 8.1 with 0.1 M NaOH standard solution using phenolphthalein as an indicator. Total acidity was calculated in terms of citric acid using its chemical formula as *Acidity (g/100 mL) = (Normality of the juice sample) x (Equivalent weight of citric acid)*. The pH value of 10% diluted juice was also determined by using pH meter.

Volatile acidity was also determined by titration method. The solution of the residual juice sample after heat treatment was titrated with 0.1 M NaOH standard solution until the end point reached at pH 8.1 as measured by pH meter. Fixed acidity is simply the difference between total (titratable) acidity and its volatile acidity. It refers to the amount of acidity that is not volatile under normal conditions. The fixed acidity was calculated according to the equation: *Fixed acidity (as citric acid) = (Total acidity (g/L) as citric acid) – (Volatile acidity, g/100 mL as citric acid x 10 x 1.17)*.

Buffering capacity is the ability of typical buffer solution to resist changes in pH. The initial pH was measured with pH meter and the buffering capacity was measured by adding 1 M NaOH in the increments of 0.2 mL until the pH value reached at pH 9.0. Buffering capacity is expressed as the molarity of sodium hydroxide required to increase pH value by 1.0 unit (Beynon *et al*., 1996).

Total solids are a measure of the amount of materials remaining after all the water has been evaporated. It is needed to clarify all dissolved materials including both inorganic (salts) and organic compounds presence in the juice sample. Five mL of the fruit juice was dried in hot oven at 70°C for 24 h. The residual weight was accurately obtained and then calculated as percentage of the sample used.

DPPH radical scavenging activity assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability of antioxidant solution was determined according to the method of Yang *et al.*

(2008). Briefly, 1 mL of sample solution was added to 3 mL of 0.1 mM DPPH solution. The mixed solution was kept for 30 min at ambient temperature (29°C) in dark and the absorbance of the solution was measured at 517 nm. The percentage inhibition was calculated according to the equation: Inhibition (%) = $(A_c - A_s / A_c)$ x 100. Where A_c is the absorbance of control (containing DPPH solution), A_s is the absorbance of sample. Antioxidant activity was expressed as mg BHT equivalent/ 100 mL of sample. All determinations were performed in triplicate.

DMPD radical scavenging activity assay

This method is based on *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride radical ($DMPD^{\bullet+}$) generated by reaction of Fe(III) in acidic medium with DMPD as described by Busuricu *et al.* (2008) with slightly modified. The stock solution of 100 mM DMPD was prepared by dissolving 209 mg of DMPD in 10 mL of deionized water; 1 mL of this solution was added to 100 mL of 0.1 M acetate buffer, pH 5.25, and the colored radical cation ($DMPD^{\bullet+}$) was obtained by adding 0.4 mL of 0.05 M ferric chloride. One mL of standard antioxidant or the juice sample (diluted in water with the ratio of 1:20) was added to 2 mL of the $DMPD^{\bullet+}$, and after 10 min at about 29°C under continuous stirring the absorbance at 505 nm was measured. The percentage inhibition was calculated according to the equation: *Inhibition (%) = (1- A_f / A_0) x 100.* Where A_0 is the absorbance of uninhibited radical cation and A_f is the absorbance measured after 10 min incubation followed by the addition of antioxidant sample. Antioxidant ability was expressed as TEAC (Trolox equivalent antioxidant capacity). All determinations were performed in triplicate.

ABTS radical cation decolorization assay

The 2,2'-azino-bis(3 - ethylbenzothiazolin - 6 - sulfonic acid (ABTS) assay is based on the ability of antioxidant molecule to scavenge the stable ABTS radical as slightly modified by Thaipong *et al.* (2006). The ABTS stock solution was prepared by an initiative reaction of 7 mM ABTS stock solution with 140 mM potassium persulfate and incubating overnight in the dark for 12-16 h. The $ABTS^{\bullet+}$ solution was diluted with absolute ethanol to adjust the absorbance at 734 nm to 0.70±0.02. One mL of antioxidant extract was mixed with 3 mL of the diluted $ABTS^{\bullet+}$ solution. After 5 min of the mixing, the decrease in absorbance was recorded. Trolox was used as a standard compound, and the result was also reported as mg Trolox equivalent antioxidant capacity (TEAC) per 100 mL sample.

FRAP (ferric reducing antioxidant power) assay

The FRAP assay was done according to Ayub Ali *et al*. (2010) with some modifications. The stock solutions included 300 mM acetate buffer pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3 \bullet 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \bullet 6H_2O$ solution and then warmed at 37°C before use. One mL of antioxidant extract (diluted with water 1:200) was allowed to react with 3 mL of the FRAP solution and the absorbance of the reaction mixture was measured at 593 nm after incubation at 37°C for 10 min. Standard solutions of $FeSO_4.7H_2O$ (50-300 mg/L) were used as a calibration curve. The results were expressed as mM Fe^{2+} per 100 mL sample.

Determination of total phenolics

The total polyphenol content was determined as slightly modified by Škerget *et al.* (2005). To 0.5 mL of diluted antioxidant extract, 2.5 mL of 10%(v/v) Folin–Ciocalteu reagent was added, followed by the addition of 2 mL of 7.5%(w/v) Na_2CO_3, then mixed well on a vortex vibrator for 5 min and incubated in the dark at ambient temperature (29°C) for 1 h prior to measuring the absorbance at 765 nm. Gallic acid was used as a calibration curve and the results were expressed as mg gallic acid equivalents per 100 mL sample.

Determination of ascorbic acid

Ascorbic acid content in the mao juice was determined according to the method of Klein *et al.* (1982) with slightly modified. The juice sample was extracted with 10 mL of 1% metaphosphoric acid for 45 min at room temperature (29°C) and filtered through Whatman No. 4 filter paper. One mL of the extract was mixed with 9 mL of 2,6-dichlorophenolindophenol and the absorbance was measured within 30 min at 515 nm against a blank solution. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid. The assays were carried out in triplicate, and the results were mean value ± standard deviation and expressed as mg ascorbic acid per 100 mL sample.

Thermal stability of total phenolics and antioxidant activity

Ten mL of the mao juice was heated in water bath at three levels of heating temperature (60, 80 and 100°C). At each heating temperature, three periods (30, 45 and 60 min) of an incubation time were undertaken, and then cooled down at room

temperature (29°C) before testing. The heated sample (100-fold dilution with deionized water) was used to determine both total phenolics and antioxidant activity following the procedures as mentioned above.

Statistical analysis

All assays were carried out in triplicate and results are expressed as mean ± SD.

Results and Discussion

Some physical and chemical properties as a general background of the mao juice determined in this study are shown in Table 1.

Table 1. Some physical and chemical properties of the mao juice sample

Parameter	Value (Mean ± SD, n = 3)
pH value	3.30 ± 0.03
Total acidity	1.33 ± 0.07
Fixed acidity	0.43 ± 0.07
Volatile acidity	0.90 ± 0.13
Buffering capacity	0.11 ± 0.03
Total solids (%)	8.73 ± 0.04
Specific gravity (g/mL)	1.03 ± 0.01

Acidity is calculated in gram of citric acid per 100 mL sample

Commonly, the mao juice samples possess pH 3.30 with total acidity (1.33 g/100 mL) and total solids (8.73%). Previously, chemical properties of the ripe fruits of Mao Luang have also been reported. The mean values of pH, total soluble solid (°Brix) and total organic acids (mg/L) were 3.51, 16.50 and 49.36, respectively. And citric acid was ranged of 4.44-11.73 mg/g fresh weight (Samappito, 2008). In comparison with grape juices, the pH was ranged from 3.25 to 3.56. Total solids were found to be 33.87%, while total acidity was 8.2% (Mulero *et al*., 2010). For grape samples, pH was ranged from 3.41 to 3.70, and total acidity was 6.9% (Burin *et al*., 2010).

Total phenolics and antioxidant activity

Total phenolics of the mao juice was determined by Folin-Ciocalteu assay. The juice sample contained very high total phenolics (337.52 mg GAE/100 mL) (Table 2). The antioxidant activity was comparatively evaluated by four different spectrophotometric methods including DPPH, DMPD, ABTS and FRAP assays. These results are also shown in Table 2. It was demonstrated that the antioxidant activities of the mao juice were relatively high and variable according to their substrates of those antioxidant mechanisms.

Table 2. Total phenolics and antioxidant activity of the mao juice sample

Total phenolics (mg GAE/ 100 mL)[a]	Antioxidant activity			
	DPPH (mg BHT/ 100 mL)[b]	DMPD (mg Trolox/ 100 mL)[c]	ABTS (mg Trolox /100 mL)[c]	FRAP (mM Fe^{2+}/100mL)[d]
337.52 ±11.7	590.81 ±14.3	95.41 ±3.27	488.69 ±0.93	15.77 ±0.06

[a]GAE: gallic acid equivalents; [b]BHT: butylated hydroxytoluene equivalents; [c]Trolox: Trolox equivalents; [d]Fe^{2+}: Fe^{2+} equivalents

For such a case, the antioxidant activity of the juice sample determined by DPPH assay is found to be 590.81 mg BHT/100 mL or 26.81 mM BHT. Since specificity and sensitivity of each antioxidant method are different, it is somewhat difficult to compare the obtained results. However, the antioxidant activity depends on the chosen method, concentrations and physicochemical properties of the studied antioxidant (Kulisic *et al*., 2004).

As comparing total phenolics of the mao juice with those of other fruit juices (Lugasi *et al*., 2003), it is evident that the content of total phenolics of the mao juice is higher than those of other fruit juices (Table 3).

Table 3. Total phenolics and antioxidant activity of mao juice compared with other fruit juices

Sample	Total phenolics[e] (mg GAE/100 mL)	Antioxidant activity by DPPH assay
Mao	337.52 ± 11.7	26.81 mM BHT
Pomegranate[a]	381.73 ± 0.43	18-20 mM TEAC[f]
Red grape[b]	135.20 ± 0.36	2.51-11.05 mM TEAC
Apple[c]	42.81 ± 0.25	1.196 mM AEAC[g]
Aprico[c]	18.57 ± 0.41	0.974 mM AEAC
Orange[d]	42.85 ± 0.14	9.25 mM TEAC

[a]Gil *et al*., 2000; [b]Burin *et al*., 2010; [c]Pernicea *et al*., 2009; [d]Pisoschi *et al*., 2009; [e]Mahdavi *et al*., 2010; [f]TEAC: Trolox equivalent antioxidant capacity; gAEAC: ascorbic acid equivalent antioxidant capacity

However, the variation among the total phenolics of the commercial juices may be due to various factors such as different varieties of the fruit samples and/ or percentage of pure juice of their final products (Mahdavi *et al*., 2010). By comparing both antioxidant activity and total phenolics of the mao juice with other fruit juices, these values are found higher than those of other fruit juices. Therefore, these results indicate that the mao fruit juice posses a noticeable inhibition effect of free radicals.

Ascorbic acid contents

The ascorbic acid content of the mao juice (175.34 mg/100 mL) was found higher than that of other commercial fruit juices (Mahdavi *et al*., 2010) as shown in Table 4. In literature, the ascorbic acid

Table 4. Ascorbic acid contents of mao juice compared with other commercial fruit juices[a]

Fruit juice	Ascorbic acid content (mg/100 mL)
Mao	175.34 ± 6.37
Orange	15.86 ± 0.62
Pomegranate	17.34 ± 0.23
Apple	13.40 ± 0.77
Sour cherry	16.44 ± 0.42
Red grape	15.18 ± 0.76
White grape	14.13 ± 1.31
Pineapple	13.60 ± 0.21
Mango	12.57 ± 0.02
Peach	15.63 ± 0.56
Apricot	16.20 ± 0.15

[a]Mahdavi *et al.* (2010)

contents of orange juice, lemon juice and grapefruit juice were found in the ranges of 14.61-33.97, 10.29-12.85 and 8.09-32.88 mg/100 mL, respectively (Klimczak *et al*., 2007; Pisoschi *et al*., 2008). Also, it was clearly shown that the ascorbic acid of pure orange juice immediately after production was found between 36.15 and 40.85 mg/100 mL.

Thermal stability of total phenolics and antioxidant activity

Thermal stability of total phenolics and antioxidant activity of the juice sample was also investigated by heating at various temperatures (60, 80 and100°C) and heating times (30, 45 and 60 min) prior to analysis. The results are shown in Figure 2 and Figure 3. It was found that losses of the total phenolic contents were found to be 14.34, 9.75 and 11.12% at 60, 80 and 100°C, respectively, after 60 min heating (Figure 2).

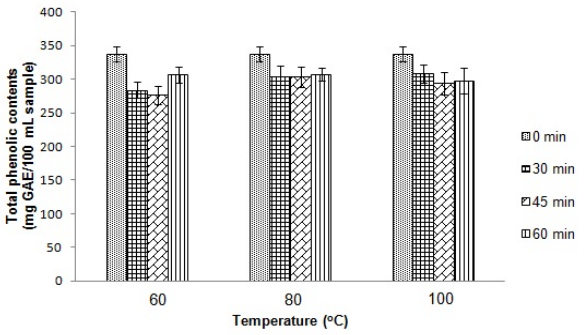

Figure 2. Effects of temperature and incubation time on total phenolics of mao juice

Accordingly, the juice sample still gave high thermal stability of the antioxidant activity with the reduction of 2.33, 10.97 and 14.84% at 60, 80 and 100°C, respectively, after 60 min heating (Figure 3).

It was demonstrated that both total phenolics and related antioxidant activity of the mao juice were not significantly decreased affected by various temperatures and incubation times, probably due to high stability of free and bound phenolic compounds in the fruit juice. Similar result was reported

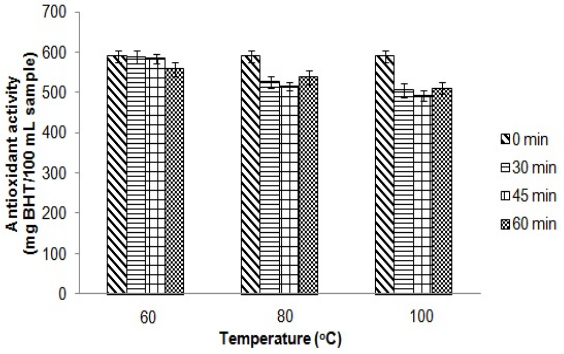

Figure 3. Effects of temperature and incubation time on the antioxidant activity of mao juice

concerning the thermal stability of anthocyanins in three pomegranate juices (Fischer *et al.*, 2013). This would be explained that simple heat treatment could not cleave covalently bound phenolic compounds from rice hull (Lee *et al*., 2003). This indicates that the phenolic compounds of plant materials should be present in different bound status depending on species. Therefore, in this study, it is indicating high thermal stability of the mao juice sample.

Conclusion

Total phenolics and ascorbic acid contents of the mao juice related to its antioxidant activity, and their thermal stability were investigated. The results indicated that the mao juice was rich in the phenolics and ascorbic acid contents and correspondingly exhibited high antioxidant activity when compared with other fruit juices. Moreover, the mao juice possessed high thermal stability. Therefore, it can be used as an important component of natural sources of antioxidants in fruit juices.

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