Antioxidant Potential and Phenolic Constituents of Mango Seed Kernel from Various Extraction Methods

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ABSTRACT

The antioxidant properties of Mango (*Mangifera indica* cultivar Chok-Anan) seed kernel (MSK) extracted by various extraction (shaking, refluxing, acid hydrolysis) methods were examined by applying 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS+) radical-scavenging assays and antioxidant activity using the ferric thiocyanate test (FTC). All three methods proved that extraction methods affected the antioxidant potential of MSK extracts. The antioxidant capacity of the acid hydrolysis extract had the highest value and was significantly ($P < 0.05$) higher than that of α-tocopherol, which is the commercially used natural antioxidant. Their phenolic composition (saponin, flavonoids, anthraquinones and tannins) and total phenolic content were also determined. The total phenolic content of MSK from different extraction methods varied between 90.03 and 285.70 mg of tannic acid equivalents per gram dry weight of product. Both flavonoids and tannins were major contributors to the phenolics in MSK. This research suggests that the extract has potential as a natural antioxidant.

Key words: mango, seed kernel, antioxidant, phenolic

INTRODUCTION

Mangoes (*Mangifera indica* L.) are tropical fruit-bearing plants of Africa and Asia. As mangoes are a seasonal fruit, about 20% of the fruit is processed for products in Thailand. During mango processing, by-products such as peel and kernel are generated. The mango seed kernel (MSK) contributes about 17-22% of the fruit (Soong and Barlow, 2004) and is discarded as residue by manufacturers. MSK was shown to enhance the oxidative stability of fresh cheese and ghee (Parmar and Sharmar, 1990). This could be attributed to the phospholipids and phenolic compounds in the MSK extract. Gallotannins and condensed tannin-related polyphenols detected by thin-layer chromatography were reported to be present in MSK (Arogba, 1997). In addition, phenolics from dry MSK meal were reported to contain tannic acid, gallic acid, and epicatechin in the ratio 17: 10: 1, respectively (Arogba, 2000). Abdalla *et al*. (2007) recently characterized the phenolic compounds in Egyptian MSK as tannins, flavonoids, and unknown compounds. These data showed that MSK contains various phenolic compounds so it can be a good source of natural antioxidants (Puravankara *et al*., 2000; Abdalla *et al*., 2007).

The present study was undertaken to evaluate the antioxidant activity of mango seed kernel from several extraction methods using Folin Ciocalteu's phenol reagent, 2,2-diphenyl-1-

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picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), including the ferric thiocyanate method. Moreover, the qualitative determinations of some classes of phenolics and the total phenolic content were also determined.

MATERIALS AND METHODS

Materials

Three batches of sun-dried seeds from ripened mango (*M. indica* cultivar Chok-Anan) were donated by Woraporn Co., Ltd., a mango-processing manufacturer in Thailand from March to June in 2007 as by-products. The seeds were washed and sun dried in the greenhouse for 3 d and the kernels were removed manually from the seeds for further extraction.

Chemicals

Folin Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Chemical Co., Ltd (St. Louise, USA). Sodium bicarbonate and the other chemicals and solvents used in the experiment were analytical grade purchased from Sigma-Aldrich Co., Ltd (Steinheim, Germany).

Preparation of MSK extracts

Samples of kernels were divided into three sets. Each set weighed about 200 g. The first set of samples was ground and blended with 95% ethanol (600 mL) in a blender for 1 min and shaken for 4.5 h (method 1). The other two sets of samples were refluxed with ethanol for 3 h (method 2) or with 1.2 M hydrochloric acid in ethanol for 3 h (method 3). The supernatant was passed through Whatman filter paper no. 4. All filtrates were evaporated under reduced pressure and the remaining water was removed by freeze drying and weighed to determine the yield of soluble

components. The moisture content of sample materials was measured according to AOAC (1990). The color of the MSK extract was observed. The dried extracts and reference samples (α-tocopherol, ascorbic acid and tannic acid) at the same concentration (100 $\mu g \cdot L^{-1}$) were used to estimate the antioxidant properties through DPPH, ABTS and the thiocyanate methods. The MSK extracts were also used to evaluate the total phenolic content and phenolic components.

Determination of antioxidant potential

The total free radical-scavenging capacity of the MSK or reference samples was determined using the DPPH and ABTS methods. The antioxidant activity in a linoleic acid emulsion system was also determined.

The free radical scavenging activity of the MSK or reference samples was evaluated using the stable radical DPPH according to the method of Masuda *et al*. (1999). The radical scavenging activity (%) was plotted against the plant extract concentration (μg/mL) to determine the concentration of extract that reduced activity by 50% (EC_{50}). These values were changed to antiradical activity (A_{AR}) defined as $1/EC_{50}$, since this parameter increases with antioxidant activity. All determinations were performed in triplicate.

The ABTS radical scavenging activity was determined according to Re *et al*. (1999). The activity of each antioxidant was determined at three concentrations, within the range of the dose-response curve of Trolox, and the radical-scavenging activity was expressed as the Trolox equivalent antioxidant capacity (TEAC), defined as mMol of Trolox per gram of sample. All determinations were performed in triplicate.

The antioxidant activity in a linoleic acid emulsion system of the MSK or reference samples was determined using the thiocyanate method (Hu *et al*., 2004), with some modifications. A sample of 0.5 mL in absolute ethanol was mixed with 0.5 mL of 5.21% linoleic acid, 1 mL of 0.05 M

phosphate buffer (pH 7), and 0.5 mL of distilled water and placed in a screw capped tube. The reaction mixture was incubated in the dark at 40 °C in an oven. Aliquots of 0.1 mL were removed every 24 h during incubation and the degree of oxidation was measured by sequentially adding ethanol (9.7 mL, 75%), ammonium thiocyanate (0.1 mL, 30%) and ferrous chloride (0.1 mL, 0.02 M in 3.5% HCl). After the mixture had rested for 3 min, the peroxide value was determined by monitoring absorbance at 500 nm until the absorbance of the control reached the maximum. The degree of linoleic acid peroxidation was calculated using Equation 1:

$$\text{Antioxidant activity} = [(A_{control} - A_{sample})/A_{control}] \times 100 \tag{1}$$

The antioxidant activity was plotted against the sample concentration in order to determine the concentration required to achieve a 50% inhibition of linoleic acid oxidation [AA_{50}]. All tests and analyses were carried out in triplicate and averaged.

Determination of total phenolic content

The total phenolic content of extracts was determined using the Folin-Ciocalteu's phenol reagent (modified from Kähkonen *et al.*, 1999). The concentration of total phenolic compounds in all plant extracts was expressed as mg of tannic acid equivalent per g dry weight of MSK using a linear equation. All determinations were performed in triplicate.

Determination of phenolic constituents

A qualitative chemical screening of MSK was performed in order to determine the occurrence of some classes of active phenolic constituents: saponin, flavonoids, anthraquinones and tannins. The UV-visible spectra (200-400 nm) of the MSK extracts and tannic acid were also measured.

A saponin test was determined using foam formation (Babayemi *et al.*, 2006). Extract powder was diluted 10 times in boiling water and the mixture was transferred to a 100-mL-graduated cylinder. After cooling and covering with parafilm, the cylinder was shaken vigorously for 30 seconds and set for 15 minutes. The saponin content was evaluated from the height of the foam layer as negative (<5 mm), low (5-9 mm), medium (10-14 mm) and high (>15 mm).

A flavonoid test of MSK was determined using alkali reaction and the Shinoda test (Olabiyi *et al.*, 2008). An aliquot of 4 mL of ammonia solution was added to 2 mL of each ethanolic extract. If a yellow precipitate was observed, it indicated the presence of flavone, flavonol and xanthone. If the precipitate showed other colors; browning-orange, reddish-orange, and reddish purple, it indicated the presence of flavonol, flavonone and chalcone, respectively. For the Shinoda test, 4 mL of ethanolic extract, a piece of magnesium ribbon and 1 mL of concentrated hydrochloric acid were added. If the solution showed a pink red or red color, it indicated the presence of flavonoids. An amount of 6 mL of distilled water and 1 mL of octanol were added to the mixture. After covering, the tube was shaken vigorously for 30 seconds and set for 15 minutes. If a reddish-purple color was observed, it indicated the presence of flavonol and flavonone. An orange color indicated the existence of flavone, xanthone and chalcone.

Bornträger's test (Kumar *et al.*, 2007) was used for anthraquinone evaluation. Firstly, 50 mg of ethanolic extract was heated with 10 mL of 10% ferric chloride solution and 1 mL of concentrated hydrochloric acid. The extract was cooled, filtered and the filtrate was shaken with 10 mL of diethyl ether. The ether extract was further extracted with 5 mL of ammonium hydroxide. A pink or deep red color of the alkali layer indicated the presence of anthraquinones.

Braemer's test (Karumi *et al.*, 2004) was used for tannin determination. To 1 mL of ethanolic extract, 10 mL of 10% alcoholic ferric chloride

solution was added. A blue-black or greenish grey color of the solution indicated the presence of tannins in the extract.

Statistical analysis

Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were analyzed by the SPSS software (SPSS Inc., Chicago, IL, USA). The general linear model procedure was applied and Duncan's multiple range tests was used to compare the mean values at $p < 0.05$. Mean values and pooled standard error of the mean (SEM) were then estimated.

RESULTS AND DISCUSSION

The extract yield and color as a function of the extraction method are shown in Table 1. Statistical analysis indicated that the extraction method influenced the yield for all extracts

(P<0.05). The yield from acid hydrolysis was the highest, which was consistent with Troszynska and Ciska (2002). They found that acid hydrolysis released more phenolic compounds, such as protocatechuic acid from the plant seed. The color and moisture content of the extracts varied depending on the extraction conditions (Table 1). The color of the MSK extracts from acid hydrolysis (method 3) was darker than that of both other methods (shaking (1) and refluxing (2)), while the moisture content of the extract from shaking was higher than that of the acid hydrolysis method.

In the present study, the investigation of total antioxidant capacity was measured as the cumulative capacity of the compounds present in the sample to scavenge free radicals, using DPPH• and ABTS+• reaction. The scavenging of DPPH• and ABTS•+ of MSK were determined and compared to reference compounds (Table 2). The increasing order of antiradical activity was method

Table 1 Yield, color and moisture content of MSK extracts from different extraction methods [A].

Conditions	Yield (%)	Color	Moisture content (%, dry basis)
Shaking (method 1)	3.31±0.19 [a]	Yellow	4.12±0.06 [b]
Refluxing (method 2)	11.90±0.04 [c]	Yellow	4.07±0.05 [ab]
Acid hydrolysis (method 3)	10.75±0.07 [b]	Black	4.03±0.06 [a]

A = dry weight basis of mango seed kernel; mean of three replications ± SD (standard deviation).
Different superscript letters indicate a significant difference (P<0.05) between conditions in each column.

Table 2 Antioxidant capacities and total phenolic content of MSK obtained from different extraction methods [A].

Conditions	Antiradical activity $(A_{AR}, 1/EC_{50})$	ABTS activity (mMol of Trolox/g)	Antioxidant efficiency $(1/AA_{50})$	Total phenolic content (mg of TAE/ g)
Shaking (method 1)	1.75±0.15 [c]	1.03±0.02 [a]	0.014±0.000 [a]	90.03±0.06 [a]
Refluxing (method 2)	2.60±0.24 [d]	1.14±0.02 [b]	0.016±0.000 [b]	212.13±0.07 [b]
Acid hydrolysis (method 3)	4.16±0.54 [e]	1.41±0.01 [d]	0.019±0.000 [d]	285.70±0.28 [c]
α-tocopherol	2.68±0.04 [d]	1.30±0.03 [c]	0.017±0.000 [c]	ND
Ascorbic acid	0.70±0.00 [a]	1.12±0.00 [b]	0.048±0.000 [e]	ND
Tannic acid	0.95±0.01 [b]	3.10±0.01 [e]	0.054±0.000 [f]	ND

A = dry weight basis of mango seed kernel; means of three replications ± SD (standard deviation).
Different superscript letters indicate significant differences (P<0.05) between conditions in each column.
ND = not determined.

1 < method 2 < method 3 for the plant extracts, which was the same order as the total phenolics content of the MSK extract (Table 2). The antiradical activities of the MSK extract from acid hydrolysis were higher than from α-tocopherol and ascorbic acid. This work showed that the extraction and hydrolysis procedure have a substantial effect on the detection of phenolic compounds present in MSK. Acid hydrolysis did release and degrade phenolic compounds, which was in agreement with the work of Krygier *et al*. (1982) and Chiang *et al*. (2001). Most phenolic compounds in plant seeds occur primarily in the bound form as conjugates with sugars, fatty acids or proteins (Krygier *et al*., 1982). Therefore, it is important that a hydrolysis process is adopted in order to obtain the maximum yield of the phenolic content of MSK. Moreover, during acid hydrolysis at elevated heating temperatures, high molecular weight phenolics can degrade to low molecular weight phenolics (Chiang *et al*., 2001). Structurally different phenolics show different rates of hydrolysis (Nuutila *et al*., 2002). Degradation of some flavonoids, such as quercetin and myricetin, due to a reaction temperature of more than 95°C, has also been reported by Hertog *et al*. (1992). These reasons could explain the higher amount of total phenolic content and the loss of flavonoid content (Table 3) in the refluxed conditions with acid hydrolysis of MSK.

The amount of phenolic compounds present in the hydrolysate of samples related to antioxidant activity. The results suggested that some redundant conjugated phenolics were released by acid hydrolysis and the free form might have provided more potent antioxidant activity. The higher the amount of phenolic compounds, the greater the antioxidant activity observed. Interestingly, the order of antiradical activity of pure compounds from the DPPH and ABTS methods were different (Table 2). The increasing order of DPPH activity was ascorbic acid < tannic acid < α-tocopherol, whereas the order of ABTS activity was ascorbic acid < α-tocopherol < tannic acid. These differences may be explained because of their solubility in the solvent used for each method.

The antioxidant capacity of MSK was determined using the ferric thiocyanate method (FTC), which measures increases in peroxide formation in the emulsion during incubation. All MSK samples obtained from methods 1, 2 and 3, exhibited antioxidant activity similar to DPPH and ABTS assays (Table 2). This implied that the phenolic compounds found in MSK were capable of exhibiting both antiradical activity and lipid oxidation inhibition. However, the antioxidant capacity of MSK extract from acid hydrolysis was only higher than α-tocopherol. From the current study, the antiradical activities of MSK extract from acid hydrolysis were higher than those from either α-tocopherol or ascorbic acid. Interestingly, the order of antioxidant activity of the references was quite different from that determined by the

Table 3 Qualitative contents of saponin and flavonoids of MSK extracts from different extraction methods.

Conditions	Saponin		Flavonoids			
	Foam (mm)	Results	Alkali reaction	Results	Shinoda test	Results
Shaking (method 1)	1	Negative	Brown-orange	Flavanone	Reddish purple	Flavonol, flavonone
Refluxing (method 2)	1	Negative	Brown-orange	Flavanone	Reddish purple	Flavonol, flavonone
Acid hydrolysis (method 3)	1	Negative	Black	Negative	Black	Negative

DPPH and ABTS tests seen in Table 2. The antioxidant order for pure antioxidants may be explained based on their hydrophobicity and thus their solubility in linoleic acid emulsions in accordance with the observation that polar antioxidants are more active in bulk oil systems, whereas non-polar antioxidants (hydrophobic) are more active in lipid suspended in an aqueous system, as reported by Frankel and Meyer (2000).

Both MSK from shaking and refluxing methods showed the presence of flavanone, flavonol and flavonone (Table 3). The qualitative analysis for tannins in all MSK extracts was judged positive (Table 4). Although analysis of the extracts from acid hydrolysis did not find any flavonoid, the total phenolic content of the extract was the highest (Table 2), suggesting that acid hydrolysis at high temperature was able to degrade flavonoid to other forms of phenolic compounds. These results are consistent with Arogba (2000) and Abdalla *et al*. (2007) Interestingly, the UV-vis spectrum of MSK extracts from method 3 was similar to that of tannic acid (Figure 1).

CONCLUSIONS

The extraction technique significantly affected extraction yield, the level of phenolic compounds and antioxidant activities determined by the DPPH and ABTS methods, including activity in a linoleic acid emulsion system of several extracts from MSK. The optimal extraction conditions for mango seed kernel were refluxing with 1.2 M hydrochloric acid in ethanol for 3 h. The acid hydrolysis conditions allowed higher yields, a greater total phenolic content and greater antioxidant activity. The MSK extract was capable of exhibiting both antiradical activity and lipid oxidation inhibition. The antioxidant activities of MSK extract were higher than those of a-tocopherol. The phenolic compounds from MSK contained flavonoids and tannins. The flavonoids were affected during hydrolysis treatment with hydrochloric acid. Furthermore, the present study showed that the extracts from seed kernels of mango (*Mangifera indica* L. cultivar Chok-Anan) have potential as an economically interesting phytochemical source for the nutraceutical and functional food market.
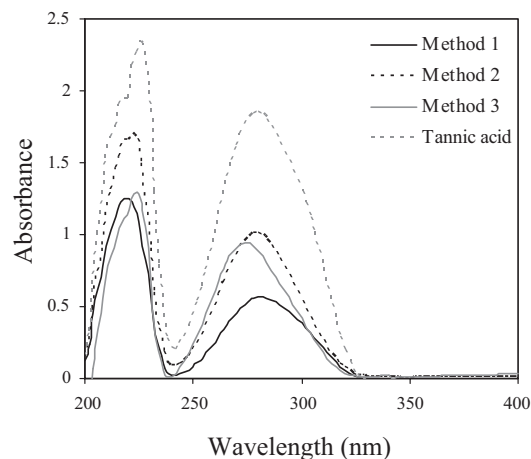
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Figure 1 Spectra of MSK from different extraction methods and tannic acid.

Table 4 Qualitative contents of anthraquinones and tannins of MSK extracts from different extraction methods.

Conditions	Anthraquinones	Tannins
Shaking (method 1)	Negative	Positive
Refluxing (method 2)	Negative	Positive
Acid hydrolysis (method 3)	Negative	Positive

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