https://doi.org/10.17221/225/2017-CJFS

Fractionation, Antioxidant and Inhibitory Activity of Thai Mango Seed Kernel Extracts

*Chaianun NAMNGAM[1], Supakorn BOONYUEN[2] and Praphan PINSIRODOM[1]**

[1]Department of Food Science and Technology Faculty of Agro-industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand [2]Department of Chemistry, Faculty of Science and Technology, Thammasat University, Bangkok, Thailand

**Corresponding author: kpprapha@kmitl.ac.th*

Abstract

Namngam Ch., Boonyuen S., Pinsirodom P. (2018): Fractionation, antioxidant and inhibitory activity of Thai mango seed kernel extracts. Czech J. Food Sci., 36: 8–15.

Mango seed kernel extracts (MSKE) from Kaew and Choke-Anan mango cultivars were fractionated using Sephadex LH-20 column chromatography. Antioxidant activity and the inhibitory effects on tyrosinase, 5-lipoxygenase, hyaluronidase and α-glucosidase of MSKE fractions were evaluated. The key components of polyphenol, found in the most active fraction, were identified using LC-ESI-MS. Three major isolates were obtained from both cultivars of the MSKE with the absorbance being higher for Choke-Anan than Kaew. Fraction 3 of MSKE from both cultivars showed significantly ($P \leq 0.05$) higher antioxidant activity and 5-lipoxygenase, hyaluronidase and α-glucosidase inhibitory activity with Choke-Anan more effective than Kaew. Six different polyphenols were found in fraction 3 of both cultivars. These were tri-*o*-galloyl-glucoside, tetra-*o*-galloyl-glucoside, maclurin tri-*o*-galloyl-glucoside, penta-*o*-galloyl-glucoside, hexa-*o*-galloyl-glucoside, and hepta-*o*-galloyl-glucoside. In the MSKE from Choke-Anan, hexa-*o*-galloyl-glucoside, and tetra-*o*-galloyl-glucoside were the two major components, whereas in the MSKE from Kaew tetra-*o*-galloyl-glucoside was the only major component. The results indicate that MSKE is a suitable by-product that could be utilised for adding value to the mango processing industry and could represent a valuable input into functional foods and pharmaceutical production.

Keywords: tyrosinase; 5-lipoxygenase; hyaluronidase; α-glucosidase galloyl-glucoside; *Mangifera indica* extract

Mango (*Mangifera indica* L.) belongs to the *Anacardiaceae* family and is one of the most important tropical fruits. It is consumed both fresh and processed. Thailand is the third largest producer of mango fruits in the world (Dube & Zunker 2004). Among Thai cultivars, Choke-Anan and Kaew are the two that are most commonly used for processing. Major by-products produced during mango processing are seeds and peels in the range 35–60% depending on the cultivar (Larrauri *et al.* 1996).

Mango seed kernels contain various polyphenols, including flavonols, xanthones and gallotannins, and can be a good source of natural antioxidants (Abdalla & Darwish 2007). The anti-tyrosinase, anti-inflammatory, hepatoprotective and anti-microbial activities of mango seed kernel extracts (MSKE) have previously been reported (Maisuthisakul & Gordon 2009; Nithitanakool *et al.* 2009; Khammuang & Sarnthima 2011). In our previous study (Namngam & Pinsirodom 2017), we found, using different methods (DPPH, FRAP, ABTS, and H_2O_2), that Thai MSKE from Kaew and Choke-Anan cultivars exhibited antioxidant activities and showed inhibitory effects on tyrosinase, 5-lipoxygenase, hyaluronidase and α-glucosidase activities.

In a previous study (Maisuthisakul 2011), the polyphenol compounds of MSKE were fractionated and it was found that fraction 3 of 5 fractions obtained using shaking extraction and fraction 2 of two obtained using acid hydrolysis extraction from Choke-Anan MSKEs exhibited the highest antioxidant efficiency. A previous report showed that MSKE

https://doi.org/10.17221/225/2017-CJFS

is a rich source of gallotannins. Five gallotannins, penta-*o*-galloyl-glucoside, hexa-*o*-galloyl-glucoside, hepta-*o*-galloyl-glucoside, octa-*o*-galloyl-glucoside and nona-*o*-galloyl-glucoside were identified in Chinese mango seed kernels using LC-ESI-MS/MS and NMR (Luo *et al.* 2014). Tri-*o*-galloyl-glucoside, tetra-*o*-galloyl-glucoside, penta-*o*-galloyl-glucoside, hexa-*o*-galloyl-glucoside, hepta-*o*-galloyl-glucoside and octa-*o*-galloyl-glucoside were also identified in Hiesy mango seed kernels (Resoanaivo *et al.* 2014) and 21 gallotannins were identified in Tommy Atkins mango seed kernels (Berardini *et al.* 2004).

The present studies were carried out to investigate the fractionation, antioxidant properties and selected enzyme inhibition activities of different fractions of MSKE and to identify key components using extracts from the mango cultivars Kaew and Choke-Anan. An additional objective was to identify by-products that could be obtained from the mango processing industry and which could add value and be used in functional foods and pharmaceutical products.

MATERIAL AND METHODS

Material. Mushroom tyrosinase and soybean 5-lipoxygenase were purchased from Sigma-Aldrich (USA). Bovine hyaluronidase and yeast α-glucosidase were purchased from Merck (Germany). L-3-4-dihydroxyphenylalanine, arbutin, sodium linoleate, rutin, *p*-nitrophenyl-α-D-glucopyranoside, dimethylaminobenzaldehyde, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, and Sephadex LH-20 were purchased from Sigma-Aldrich (USA). Dimethyl sulfoxide (DMSO) was purchased from Fluka (Switzerland). The other chemicals and solvents used in this study were purchased from Sigma-Aldrich (USA).

The two cultivars of mango used in this study, Kaew and Choke-Anan, were obtained from a local orchard in the Nakornratchasima Province of Thailand between March and May, 2016. The samples were randomly selected based on size (4–5 mangoes/kg) and all were judged to be mature-green. Mango peel and pulp were removed from the fruits by hand and the seeds were washed and stored at –18°C for no longer than four months before being used. The kernels were then separated from their shells for analysis.

Preparation of mango seed kernel extracts. Preparation of crude MSKE using ultrasonic-assisted extraction was as described by Gharfoor & Choi (2009). Briefly, samples were ground and blended with 95% ethanol (100 ml) in a blender (Moulinex, Mexico) for 5 minutes. The samples were then incubated in a sonication water bath (JAC Ultrasonic 2010P; Jinwoo Engineering Co., Ltd., Korea), with the frequency fixed at 20 kHz, at a temperature of 25°C for 15–60 minutes. Samples were then further incubated in a water bath at 80°C for 1 hour. The mixtures were cooled at room temperature and the supernatant was passed through filter paper (Whatman No. 4; Merck, Germany). All filtrates were evaporated in a rotary evaporator (Büchi Rotavapor R II; USA) at 50°C under vacuum (to 70% solids) and the extracts were weighed to determine the yield of soluble components.

Fractionation of MSKE. The samples (1 g) were dissolved in 5 ml methanol and applied to a Sephadex LH-20 chromatography column (35 mm diameter × 450 mm height). The flow rate was 1 ml/min. Methanol fractions (10 ml) were collected in test tubes. Eluted fractions were then pooled based on their elution profiles. Their absorbance was measured at 280 nm using a spectrophotometer (UV-1601; Shimadzu, Japan). After evaporation of methanol, the samples were weighed to determine the yield of different fractions. Each fraction was used to determine the total polyphenol content (TPC), antioxidant activity and inhibitory action on tyrosinase, 5-lipoxygenase, hyaluronidase and α-glucosidase activity.

Determination of total polyphenol contents (TPC). The TPC were determined using Folin-Ciocalteu's phenol reagent and a method modified from Singleton and Lamuela-Raventos (1999). Briefly, each crude extract of 0.5 g was dissolved in 20 ml dimethyl sulfoxide (DMSO). Samples of 0.5 ml were mixed with 0.5 ml of Folin-Ciocalteu's phenol reagent and adjusted to 10 ml with distilled water. After mixing for 5 min, 0.8 ml of 10% (w/v) sodium carbonate was added. The mixtures were agitated and incubated for 10 min at room temperature in the dark. The absorbance was measured at 765 nm on a spectrophotometer. The total polyphenol contents of the samples were expressed as milligrams of gallic acid equivalent per gram of MSKE (mg GAE/g MSKE) using a linear equation.

DPPH radical scavenging assay. The radical scavenging activity of MSKE was measured by using the stable free radical, DPPH (2,2-diphenyl-2-picrylhydrazyl), as described by Nishaa *et al.* (2012). DPPH

https://doi.org/10.17221/225/2017-CJFS

(0.8 mM) in ethanol was prepared and 0.6 ml of this solution were added to 4.4 ml of sample solution in ethanol. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm with a spectrophotometer. The ability to scavenge DPPH was calculated as percent DPPH scavenging activity using Equation (1) and EC_{50} was calculated.

$$\% \text{ DPPH scavenging} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (1)$$

Ferric reducing antioxidative power assay (FRAP). FRAP assays on MSKE were carried out as described by Nishaa *et al.* (2012). FRAP reagent was freshly prepared by mixing 25 ml acetate buffer (3 mM, pH 3.6), 2.5 ml 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) solution (10 mM TPTZ in 40 mM HCl) and 2.5 ml $FeCl_3$ (20 mM) water solution. Each sample (150 µl, 0.5 mg/ml dissolved in ethanol) was added to 4.5 ml of freshly prepared FRAP reagent and stirred, and, after 5 min, the absorbance was measured at 593 nm using a spectrophotometer. A calibration curve of various concentrations of Trolox was used and results were expressed as milligrams of Trolox equivalent per gram of MSKE (mg Trolox/g MSKE).

Hydrogen peroxide scavenging assay. The hydrogen peroxide scavenging ability of MSKE was measured using the method of Yen and Chen (1995). H_2O_2 solution (4 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). Samples of 4 ml were mixed with 0.6 ml of H_2O_2 solution. The mixture was incubated for 10 min. The absorbance of the solution was determined at 230 nm using a spectrophotometer against a blank solution containing the plant extract in phosphate buffer without H_2O_2. The H_2O_2 scavenging activity was expressed as milligrams of Trolox equivalent per gram of MSKE (mg Trolox/g MSKE) using a linear equation.

ABTS radical scavenging assay. The ABTS 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity of MSKE was measured as described by Re *et al.* (1999). The ABTS radical cation was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate solution (1 : 1) and the mixture was allowed to stand in the dark at room temperature for 12 h before use. The mixture was diluted with deionised water and 95% ethanol (1 : 1) to ensure an absorbance of 0.7 ± 0.02 at 734 nm in spectrophotometric analysis. Extracts of 20 µl were mixed with 6 ml of diluted ABTS radical cation solution. The decrease in absorbance was recorded at 6 min after mixing. Trolox was used as a standard. The ABTS scavenging activity of the samples was expressed as milligrams of Trolox equivalent per gram of MSKE (mg Trolox/g MSKE) using a linear equation. All determinations were performed in triplicate.

Determination of tyrosinase inhibition activity. The inhibition activity of MSKE was measured using the modified dopachrome method with mushroom tyrosinase and L-3,4-dihydroxyphenylalanine (L-DOPA) as substrates (Masuda *et al.* 2005) and their effect on the enzyme was determined based on dopachrome formation at 450 nm using a 96-well reader. Samples were dissolved in DMSO at various concentrations. Each well contained 40 µl of sample with 80 µl of phosphate buffer (0.1 M, pH 6.8), 40 µl of tyrosinase (100 units/ml) and 40 µl of L-DOPA (2.5 mM). Each sample was accompanied by a blank that had all the components except L-DOPA. Results were compared to arbutin as a reference standard. The percent inhibition of tyrosinase was calculated using Equation (2). The inhibitory effect (%) of the samples was expressed as an inhibitor concentration causing a 50% loss of enzyme activity (IC_{50}).

$$\% \text{ inhibition activity} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (2)$$

Determination of 5-lipoxygenase inhibition activity. The inhibition activity of MSKE was measured using linoleic acid as a substrate as described by Shinde *et al.* (1999). The reaction was initiated by the addition of aliquots (50 µl) of a soybean lipoxygenase (1000 units/ml) solution (prepared daily in 1 M potassium phosphate buffer, pH 9.0) and 2.0 ml of sodium linoleate (100 µM) in phosphate buffer. The samples (30 µl) were dissolved in DMSO at various concentrations and then added. The mixture was incubated for 5 min. The absorbance of the solution was taken at 234 nm on a spectrophotometer. Results were compared to rutin as reference standard. The percentage inhibition of 5-lipoxygenase was calculated according to Equation (2). The inhibitory effect (%) of the samples was expressed as an inhibitor concentration causing a 50% loss of enzyme activity (IC_{50}).

Determination of hyaluronidase inhibition activity. Inhibition of hyaluronidase was determined by measuring the amount of N-acetyl glucosamine that was split from sodium hyaluronate as described by Lee *et al.* (2001). The assay medium consisted of 50 µl of hyaluronidase (1000 units/ml prepared in 0.1 M acetate buffer, pH 3.5), 50 µl of various concentrations of MSKE dissolved in 5% DMSO and 12.5 mM

https://doi.org/10.17221/225/2017-CJFS

calcium chloride. The medium was incubated for 10 min at 37°C. The reaction was then initiated by the addition of 250 µl of sodium hyaluronate as substrate (1.2 mg/ml dissolved in 0.1 M acetate buffer, pH 3.5) and then 1.5 ml of *p*-dimethylaminobenzaldehyde (4 g PDMAB dissolved in 350 ml of glacial acetic acid and 50 ml of 10 N HCl) was added to the reaction mixture followed by incubation for 20 min at 37°C. The absorbance was measured at 585 nm using a spectrophotometer. Results were compared to vitamin C as reference standard. The percent inhibition of hyaluronidase was calculated according to Equation (2). The inhibitory effect (%) of the samples was expressed as the inhibitor concentration causing a 50% loss of enzyme activity (IC_{50}).

Determination of α-glucosidase inhibition activity. The inhibitory effects of MSKE on α-glucosidase was measured according to Apostolidis *et al.* (2011). The reactions contained dilutions of the MSKE (0–200 µl) and 100 µl of α-glucosidase (1000 units/ml 0.5 mg/ml) in phosphate buffer (0.1 M, pH 6.9) solution and were incubated at 25°C for 10 min. Then, 50 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside in phosphate buffer (0.1 M, pH 6.9) solution was added. The mixtures were incubated at 25°C for 5 min, before reading the absorbance at 405 nm on a spectrophotometer. Acarbose was used as reference standard. The percent inhibition of α-glucosidase was calculated according to Equation (2). The inhibitory effect (%) of the samples was expressed as an inhibitor concentration causing a 50% loss of enzyme activity (IC_{50}).

Identification of polyphenols using LC-ESI-MS. The fractions with the highest bioactivities were identified to harbour the main polyphenol constituents using LC-ESI-MS. Electrospray ionisation mass spectrometry analysis of polyphenol compounds in fractions of MSKE was performed for identification using an applied TSQ Quantum Ultra-LCMS (Thermo Fisher, USA) based on the method described by Abdullah *et al.* (2015). The mass spectra were acquired in positive electrospray ionization (ESI) modes with high resolutions of up to 3000 Daltons. The spray voltage used was 3500 V. The sheath/auxiliary/sweep gas was 99% nitrogen and sheath gas pressure was 30 psi with 5 psi for auxiliary gas pressure. The capillary temperature was 270°C. The injection volume was 10 µl and the flush speed was 100 µl/s. In the MS analysis (full scan), data were collected over masses of 50 to 2000 *m/z*.

Statistical analysis. The results are expressed as the mean ± standard deviation. All determinations were performed in triplicate ($n = 3$). Statistical analyses were carried out using one-way ANOVA in SPSS version 16.0. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Fractionation of mango seed kernel extracts. The crude ethanol extracts of MSKE from the two cultivars were fractionated on Sephadex LH-20 chromatography columns using methanol as the mobile phase. The elution profiles measured by the spectrophotometer at 280 nm are shown in Figure 1. Three major isolates were obtained for the MSKE from both cultivars with the absorbance generally higher for the Choke-Anan MSKE fractions than for those of Kaew.

Recovery yield, total polyphenols and antioxidant activities. The recovery yield and TPC of all the obtained fractions from Kaew and Choke-Anan MSKE are shown in Table 1. The recovery yields were in the range of 6.41–17.11 and 9.49–27.90%, respectively. Crude MSKE from Kaew and Choke-Anan exhibited TPC values of 132.78 ± 0.77 and 175.06 ± 0.61 mg GAE/g, respectively. Fraction 3 of MSKE from Kaew and Choke-Anan had approximately 1.75 and 1.50-fold higher levels of TPC than crude MSKE, respectively, which were significant differences ($P \leq 0.05$). Table 1 shows the antioxidant activities of crude fractions MSKE from Kaew and Choke-Anan and gallic acid as a reference standard using four different methods. Fraction 3 of both cultivars exhibited significantly ($P \leq 0.05$) increased antioxidant activities in all the methods tested compared to crude extracts, with approximately 1.54, 1.54, 1.48, and 1.12-times higher antioxidant activities for Kaew and 1.51, 1.49,

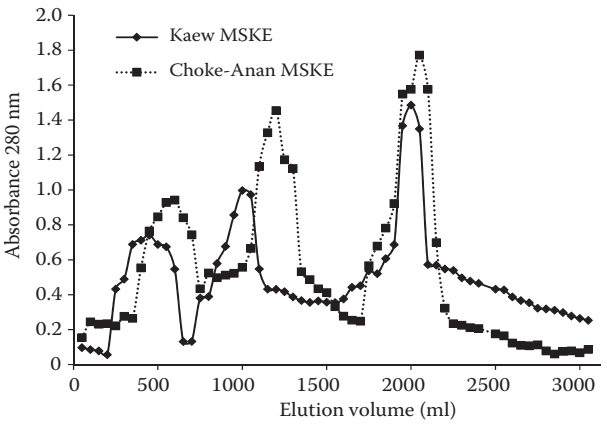

Figure 1. Column chromatography profile of the fractions from mango seed kernel extracts (MSKE)

https://doi.org/10.17221/225/2017-CJFS

Table 1. Results of the Sephadex LH-20 column chromatography

Fractions		Yield (%)	TPC (mg GAE/g)	Antioxidant properties			
				DPPH EC_{50} (μg/ml)	FRAP	H_2O_2	ABTS
					(mg Trolox/g)		
Kaew	crude	nd	132.78 ± 0.77^{g}	10.22 ± 0.05^{a}	209.35 ± 1.52^{f}	95.58 ± 0.25^{h}	163.14 ± 0.89^{g}
	F1	6.41 ± 0.02^{e}	130.30 ± 0.87^{g}	10.37 ± 0.07^{a}	204.45 ± 1.15^{g}	103.13 ± 1.58^{g}	155.59 ± 0.72^{h}
	F2	9.44 ± 0.01^{d}	184.72 ± 0.59^{d}	8.66 ± 0.32^{b}	225.06 ± 0.91^{e}	121.15 ± 0.63^{e}	171.59 ± 1.09^{f}
	F3	17.11 ± 0.03^{b}	232.00 ± 1.33^{b}	6.63 ± 0.08^{d}	322.70 ± 0.91^{b}	141.61 ± 1.21^{c}	184.54 ± 1.76^{c}
Choke-Anan	crude	nd	175.06 ± 0.61^{e}	7.17 ± 0.03^{c}	229.18 ± 0.54^{d}	112.69 ± 0.60^{f}	177.52 ± 0.70^{d}
	F1	9.49 ± 0.16^{d}	150.95 ± 6.79^{f}	6.13 ± 0.15^{e}	232.26 ± 1.23^{d}	113.57 ± 0.81^{f}	175.73 ± 0.34^{e}
	F2	16.30 ± 0.03^{c}	206.01 ± 0.74^{c}	5.46 ± 0.20^{f}	254.41 ± 2.31^{c}	125.29 ± 0.31^{d}	184.56 ± 0.93^{c}
	F3	27.90 ± 0.02^{a}	263.28 ± 1.50^{a}	4.74 ± 0.14^{g}	342.10 ± 1.00^{a}	158.50 ± 0.74^{b}	191.45 ± 1.00^{b}
Gallic acid		nd	nd	4.67 ± 0.24^{g}	344.29 ± 3.04^{a}	180.55 ± 0.74^{a}	200.22 ± 1.40^{a}

Values are mean ± SD (n = 3); values in the same column that are followed by different superscript letters are significantly different each other ($P \leq 0.05$); nd – not determined

1.41, and 1.07-times higher antioxidant activities for the Choke-Anan cultivar. Fraction 3 of Choke-Anan MSKE did not have a significantly ($P > 0.05$) different antioxidant activity compared with gallic acid using the DPPH and FRAP methods. Gallic acid clearly exhibited the highest ($P \leq 0.05$) antioxidant activity using the H_2O_2 and ABTS methods. The recovery yields for both Kaew and Choke-Anan MSKE were highest in fraction 3. In addition, the fractions with the highest TPC in both cultivars corresponded to those with the highest antioxidant activities in all the methods tested. Maisuthisakul (2011) also reported that fraction 3 from Choke-Anan, obtained from MSKE using shaking extraction and fraction 2, obtained from MSKE using acid hydrolysis extraction, did not differ significantly ($P > 0.05$) in their antioxidant efficiencies when compared to methyl gallate.

Selected enzyme inhibitory activities. In this investigation, crude MSKE and fractions from Kaew and Choke-Anan were used to determine the inhibition of tyrosinase, 5-lipoxygenase, hyaluronidase and α-glucosidase activities compared to the reference standard. The values are presented as the inhibitor concentration causing a 50% loss of enzyme activity (IC_{50}). A smaller IC_{50} value means a higher enzyme inhibitory activity. Table 2 shows the IC_{50} values of enzyme inhibition activities of crude MSKE, fractions and the standard reference.

Tyrosinase is the main enzyme in the melanin synthetic pathway in melanocytes and Park *et al.* (2004) reported that inhibition of tyrosinase could be an important strategy for blocking melanogenesis. Tyrosinase inhibitors have been widely used as ingredients in some skin whitening cosmetics. Fraction 3 of MSKE from Kaew and Choke-Anan showed significant differences ($P < 0.05$) in tyrosinase inhibitory activity compared to all other fractions and crude MSKE; the IC_{50} values were 17.10 ± 0.15 and 15.35 ± 0.63 μg/ml, respectively. The results also showed that fraction 3 of both cultivars was more effective at inhibiting tyrosinase activity than the standard reference, arbutin. Therefore, MSKE is a promising functional food and natural cosmetic for whitening products.

Alitonou *et al.* (2006) showed that 5-lipoxygenase can catalyse the conversion of arachidonic, linoleic and other polyunsaturated fatty acids into biologically active metabolites, which are active mediators in a variety of inflammatory processes. The inhibitory activity of 5-lipoxygenase from crude MSKE and fractions of MSKE from Kaew and Choke-Anan, compared to rutin, showed a significantly ($P \leq 0.05$) higher 5-lipoxygenase inhibition activity than the other fractions and crude MSKE. The IC_{50} values ranged from 21.35 ± 0.65 μg/ml to 41.63 ± 0.52 μg/ml. The reference standard, rutin, had a lower IC_{50} value (10.96 ± 0.69 μg/ml). It is therefore clear that the inhibitory activity of 5-lipoxygenase from MSKE may hold potential for the potential development of new anti-inflammatory drugs.

Sahasrabudhe and Deodhar (2010) showed that hyaluronidase is a naturally occurring enzyme that catalyses the degradation of hyaluronic acid, whose action can lead to diminishing amounts of hyaluronic acid in the skin with the result that the skin becomes dried and wrinkled. Fraction 3 of Kaew MSKE and fractions 1, 2, and 3 of Choke-Anan MSKE showed

https://doi.org/10.17221/225/2017-CJFS

Table 2. Enzyme inhibition activities of crude extracts and different MSKE fractions

Fractions tyrosinase		Enzyme inhibition activities (IC_{50}, μg/ml)			
		tyrosinase	5-lipoxygenase	hyaluronidase	α-glucosidase
Kaew	crude	21.11 ± 0.50^{cd}	39.36 ± 0.61^{bc}	41.39 ± 0.85^{a}	154.42 ± 3.64^{b}
	F1	37.10 ± 0.22^{a}	41.63 ± 0.52^{a}	42.69 ± 0.82^{a}	131.75 ± 2.51^{d}
	F2	36.59 ± 0.21^{a}	35.32 ± 0.57^{d}	38.03 ± 0.90^{b}	118.99 ± 3.02^{e}
	F3	17.10 ± 0.15^{e}	29.79 ± 0.74^{f}	32.80 ± 0.84^{d}	110.45 ± 4.01^{g}
Choke-Anan	crude	21.29 ± 0.81^{c}	38.75 ± 0.41^{c}	39.19 ± 0.91^{b}	157.83 ± 3.32^{a}
	F1	27.48 ± 0.46^{b}	40.16 ± 0.51^{b}	35.42 ± 0.82^{c}	133.85 ± 5.24^{c}
	F2	27.18 ± 0.46^{b}	32.71 ± 0.69^{e}	35.80 ± 0.81^{c}	116.08 ± 5.15^{f}
	F3	15.35 ± 0.63^{f}	21.35 ± 0.65^{g}	29.56 ± 1.23^{e}	108.00 ± 2.51^{h}
Standard references	arbutin	20.64 ± 0.75^{d}	nd	nd	nd
	rutin	nd	10.96 ± 0.69^{h}	nd	nd
	vitamin C	nd	nd	38.67 ± 0.78^{b}	nd
	acarbose	nd	nd	nd	105.00 ± 0.51^{i}

Values are mean ± SD (n = 3); values in the same column that are followed by different superscript letters are significantly different from each other ($P \leq 0.05$); nd – not determined

significantly higher ($P \leq 0.05$) hyaluronidase inhibitory activities than the reference standard, which was vitamin C. Fraction 2 from Kaew and crude Choke-Anan MSKE were not significant different ($P > 0.05$) in their hyaluronidase inhibition activities. Moreover, MSKE from Choke-Anan showed significantly higher ($P \leq 0.05$) activity than that from Kaew. These results suggest that the hyaluronidase inhibitory activity of MSKE has potential as a new ingredient in functional foods and anti-wrinkle cosmetics.

Hydrolysed dietary carbohydrates are the major source of blood glucose, and are hydrolysed by pancreatic α-amylase with absorption by the small intestine aided by α-glucosidases (Elsenhans & Caspary 1987). Kumar *et al.* (2011) described the control of postprandial hyperglycaemia as a practical therapeutic approach for the management of diabetes. They suggested that the progression of diabetes mellitus can be stopped by inhibiting the absorption of dietary carbohydrates in the small intestine. The α-glucosidase inhibitory activities from crude extracts and fractions of MSKE from both Kaew and Choke-Anan compared to acarbose are shown in Table 2. In addition, fraction 3 of both cultivars exhibited significantly higher ($P \leq 0.05$) α-glucosidase inhibitory activity compared to crude extracts and other fractions with IC_{50} values of 110.45 ± 4.01 and 108.00 ± 2.51 μg/ml, respectively. However, acarbose, the reference standard, had significantly higher ($P \leq 0.05$) α-glucosidase inhibitory activity than all crude extracts and fractions of MSKE.

Fraction 3 of MSKE from both cultivars exhibited significantly higher ($P \leq 0.05$) tyrosinase, 5-lipoxygenase, hyaluronidase and α-glucosidase inhibitory activities than crude MSKE and other fractions. These results correspond with the TPC and antioxidant activity values shown in Table 1. Moreover, the IC_{50} values for tyrosinase and hyaluronidase inhibition activities of fraction 3 in both cultivars were lower than those of arbutin and vitamin C, respectively. Antioxidant activity mechanisms may also be one of the main reasons for tyrosinase inhibition activity (Kim *et al.* 2008). Some phenolic compounds such as ellagic acid, tannic acid (or gallotannins) and quercetin have been shown to inhibit tyrosinase activity (Shimogaki *et al.* 2000) and hyaluronidase activity (Girish & Kemparaju 2005). The experimental results indicate that fraction 3 of MSKE from both cultivars contained a key component that had stronger enzyme inhibitory activities.

Identification of key polyphenol constituents. The separation results for the polyphenols in fraction 3 of MSKE from Kaew and Choke-Anan using LC-ESI-MS mass spectrometry with a retention time of 0–8.3 min are shown in Table 3. Consistent with previous studies (Berardini *et al.* 2004; Luo *et al.* 2014; Resoanaivo *et al.* 2014), six polyphenols were detected in fraction 3 of MSKE from both cultivars and were the following:

Peak 1 showed an $(M\text{-}Na)^+$ ion of m/z 662.3 identified as tri-*o*-galloyl-glucoside.

Peak 2 showed an $(M\text{-}Na)^+$ ion of m/z 813.5 identified as tetra-*o*-galloyl-glucoside.

https://doi.org/10.17221/225/2017-CJFS

Table 3. Characterisation of the main polyphenol constituents in fraction 3 of MSKE

Pea	Polyphenol	Relative abundance (%)		Retention time (min)		$(M\text{-}Na)^{\pm}$ (*m/z*)
		kaew	choke-anan	kaew	choke-anan	
1	tri-*o*-galloyl-glucoside	56	13	2.10	2.90	662.3
2	tetra-*o*-galloyl-glucoside	100	97	2.60	3.76	813.5
3	maclurintri-*o*-galloyl-glucoside	28	50	2.96	2.81	902.4
4	penta-*o*-galloyl-glucoside	34	88	3.48	3.99	961.5
5	hexa-*o*-galloyl-glucoside	24	100	4.16	3.21	1113.2
6	hepta-*o*-galloyl-glucoside	8	50	3.15	4.44	1265.0

Peak 3 showed an $(M\text{-}Na)^+$ ion of *m/z* 902.4 identified as maclurintri-*o*-galloyl-glucoside.

Peak 4 showed an $(M\text{-}Na)^+$ ion of *m/z* 961.5 identified as penta-*o*-galloyl-glucoside.

Peak 5 showed an $(M\text{-}Na)^+$ ion of *m/z* 1113.2 identified as hexa-*o*-galloyl-glucoside.

Peak 6 showed an $(M\text{-}Na)^+$ ion of *m/z* 1265 identified as hepta-*o*-galloyl-glucoside.

The retention time of the mass spectra of fraction 3 from both Kaew and Choke-Anan MSKE were in the range of 2.90–4.44 and 2.10–3.15, respectively, and exhibited ratios and a relative abundance of 5 > 2 > 4 > 3.6 > 1 and 2 > 1 > 4 > 3 > 5 > 6, respectively. Hexa-*o*-galloyl-glucoside and tetra-*o*-galloyl-glucoside were the two major components in fraction 3 of MSKE from Choke-Anan, whereas, in Kaew the only major component was tetra-*o*-galloyl-glucoside. The key components of fraction 3 of MSKE from Kaew and Choke-Anan contained the six main polyphenols: tri-*o*-galloyl-glucoside, tetra-*o*-galloyl-glucoside, maclurintri-*o*-galloyl-glucoside, penta-*o*-galloyl-glucoside, hexa-*o*-galloyl-glucoside and hepta-*o*-galloyl-glucoside. These results are similar to those described previously regarding the NMR spectra of ethanolic extracts of by-products from Hiesy mangoes in Madagascar. These authors identified ten gallotannins, which comprised glucose and three to nine gallic acids (Resoanaivo *et al.* 2014). Berardini *et al.* (2004) characterised polyphenols in MSKE Tommy Atkins mangoes and isolated 21 different gallotannins. A similar study identified the gallotannins in the seed kernels of three different Chinese mango cultivars (Maqiesu, Tainong-1, and Zihuamang) using LC-ESI-MS/MS, and found that the MSKE contained five major gallotannins, i.e., penta-*o*-galloyl-glucoside, hexa-*o*-galloyl-glucoside, hepta-*o*-galloyl-glucoside, octa-*o*-galloyl-glucoside and nona-*o*-galloyl glucoside (Luo *et al.* 2015).

CONCLUSIONS

The results of this study are consistent with previous studies on some other mango cultivars and show that fraction 3 of the MSKE from the mango cultivars Kaew and Choke-Anan exhibited the highest antioxidant activities as well as inhibitory effects against the selected enzymes. Moreover, hexa-*o*-galloyl-glucoside and tetra-*o*-galloyl-glucoside were identified as the two major components of fraction 3 from Choke-Anan, whereas tetra-*o*-galloyl-glucoside was found to be the only major component in Kaew. These findings demonstrate that Thai mango seed by-products can be a source of natural ingredients for functional foods, pharmaceutical and cosmetic products and that the type of antioxidant activity can vary between cultivars.

References

Abdullah A.H., Mohammed A.S., Abdullah R. (2015): Identification and quantification of phenolic compound in *Mangifera indica* waterlily kernel and their free radical scarvening activity. Journal of Advanced Agricultural Technology, 2: 1–7.

Abdalla E.M., Darwish S.M., Ayad E.H.E., El-Hamahmy R.M. (2007): Egyptian mango by-product 1. Compositional quality of mango seed kernel. Food Chemistry, 103: 1134–1140.

Alitonou G.A., Avlessi F., Sohounhloue D.K., Agnaniet H., Bessiere J.M., Menut C. (2006): Investigation on essential oil of cymbopogon giganteus from benin for it potential use as anti-inflammatory agent. International Journal Aromatherapy, 16: 113–119.

Apostolidis E., Li L., Lee C., Seeram N.P. (2011): *In vitro* evaluation of phenolic-enriched maple syrup extracts for inhibition of carbohydrate hydrolyzing enzymes relevant to type 2 diabetes management. Journal of Functional Foods, 3: 100–106.

https://doi.org/10.17221/225/2017-CJFS

Berardini N., Carle R., Schieber A. (2004): Characterization of gallotannins and benzophenone derivatives from mango (*Mangifera indica* L. cv. Tommy Atkins) peels, pulp and kernels by high-performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Communication in Mass spectrometry, 18: 2208–2216.

Dube M., Zunker K., Neidhart S. (2004): Effect of technological processing on the allergenicity of mangoes (*Mangifera indica* L.). Journal of Agricultural and Food Chemistry, 52: 3938–3945.

Elsenhans B., Caspary W.F. (1987): Absorption of carbohydrates. In: Capary W.F. (ed.): Structure and Function of the Small Intestine. Amsterdam Excerptia Medica, 139–159.

Ghafoor K., Choi Y.H. (2009): Optimization of ultrasound assisted extraction of phenolic compounds and antioxidants from grape peel through response surface methodology. Journal of the Korean Society for Applied Biological Chemistry, 52: 295–300.

Girish K., Kemparaju K. (2005): Inhibition of *Naja naja* venom hyaluronidase by plant derived bioactive components and polysaccharide. Biochemistry, 70: 1145–1150.

Khammuang S., Sarnthima K. (2011): Antioxidant and antibacterial activities of selected varieties of Thai mango seed extract. Pakistan Journal of Pharmaceutical Sciences, 24: 37–42.

Kim Y.J., Kang K.S., Yokozawa T. (2008): The anti-melanogenic effect of pycnogenol by its antioxidative actions. Food and Chemical Toxicology, 46: 2466–2471.

Kumar S., Narwal S., Kumar V., Prakash O. (2011): α-Glucosidase inhibitors from plants: a natural approach to treat diabetes. Pharmacognosy Review, 5: 19–29.

Larrauri J.A., Ruperez P., Borroto B., Saura-Calixto F. (1996): Mango peels as a new tropical fibre: Preparation and characterization. LWT-Food Science and Technology, 29: 729–733.

Lee K.K., Cho J.J., Park E.J., Choi J.D. (2001): Anti-elastase and anti-hyaluronidase activity of phenolic substance from *Areca catechu* as a new anti-ageing agent. International Journal of Cosmetic Science, 23: 341–346.

Luo F., Fu Y., Xing Y., Yan S., Hu G., Huang X., Huang G., Sun C., Li X., Chen K. (2014): Identification and quantification of gallotannins in mango (*Mangifera indica* L.) kernel and peel and their antiproliferative activities. Jounal of Functional Foods, 8: 282–291.

Maisuthisakul P., Gordon M.H. (2009): Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. Food Chemistry, 117: 332–341.

Maisuthisakul P. (2011): Antioxidant capacity of extracts and fractions from mango (*Mangifera indica* L.) seed kernels. International Food Research Journal, 18: 523–528.

Masuda T., Yamashita D., Takeda Y., Yonemori S. (2005): Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. Bioscience, Biotechnology, and Biochemistry, 69: 197–201.

Namngam C., Pinsirodom P. (2017): Antioxidant properties, selected enzyme inhibition capacities, and a cosmetic cream formulation of Thai mango seed kernel extracts. Tropical Journal of Pharmaceutical Research, 16: 9–16.

Nishaa S., Vishnupriya M., Sasikumar J.M., Hephazibah P.C., Gopalakrishnan V.K. (2012): Antioxidant activity of ethanolic extract of *Maranta arundinacea* L. tuberous rhizomes. Asian Journal of Pharmaceutical and Clinical Research, 5: 85–88.

Nithitanakool S., Pithayanukul P., Bavovada R. (2009): Antioxidant and hepatoprotective activities of Thai mango seed kernel extract. Planta Medica, 75: 1118–1123.

Park S.H., Kim D.S., Kim W.G., Ryoo I.J., Lee D.H., Huh C.H. (2004): Terrein: a new melanogenesis inhibitor and its mechanism. Cellular and Molecular Life Sciences, 61: 2878–2885.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Eran C. (1999): Antioxidant activity applying improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26: 1231–1237.

Resoanaivo L.H., Albrieux F., Lemaire M. (2014): Chemical constituents of peels, kernels and hulls of fruits of *Mangifera indica* Var. Hiesy and their potential valorization. Journal of Pharmacognosy and Phytochemistry, 3: 225–233.

Sahasrabudhe A., Deodhar M. (2010): Anti-hyaluronidase, anti-elastase activity of *Garcinia indica*. International Journal of Botany, 6: 299–303.

Shimogaki H., Tanaka Y., Tamai H., Masuda M. (2000): *In vitro* and *in vivo* evaluation of ellagic acid on melanogenesis inhibition. International Journal of Cosmetic science, 22: 291–303.

Shinde U.A., Phadke A.S., Nari A.M., Mungantiwar A.A., Dikshit V.J., Saraf M.N. (1999): Membrane stabilization activity a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia, 70: 225–257.

Singleton V.L., Lamuela-Raventos R.M. (1999): Analysis of total phenol and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, 299: 152–178.

Yen G.-C., Chen H.Y. (1995): Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry, 43: 27–32

Received: 2017–06–15
Accepted after corrections: 2018–01–22