

Research Paper

Antiradical scavenging activity and polyphenolic compounds extracted from Thai mango seed kernels

Pitchaon Maisuthisakul

School of Science, University of the Thai Chamber of Commerce, Bangkok 10400, Thailand

Author to whom correspondence should be addressed, email: pitchaon@yahoo.com

Paper originally presented at Food Innovation Asia 2007

Abstract: Thai mango (*Mangifera indica* Linn.) seeds were collected from a local market and kernels were separated and dried. This study was carried out to determine antioxidant activities using the stable radicals 1-diphenyl-2-picrylhydrazyl (DPPH•) and phenolic compounds of the ethanolic seed kernel extracts of eleven Thai mango cultivars. These are commonly known as *Kaew* (KW), *Nam Dokmai* (NM), *Khiew Sawoey* (KS), *Pimsaen* (PS), *Chok-Anan* (CA), *Rad* (RD), *Phalun* (PL), *Hua Chang* (HC), *Mun Duan Kao* (MK), *Okrong* (OR) and *Maha Chanok* (MN). The results showed that all the mango seed kernels demonstrated antioxidant activities comparable to α -tocopherol. Extracts of cultivars KW and CA showed the highest antioxidant efficiency, resulting in 110-113% amplification in antioxidant activity compared to the reference. The phenolic acids of mango seed kernels were in form of free phenolic acid (42-56%), more than esterified phenolic acid (10-19%) and insoluble bound phenolic acid (15-20%). The cultivar KW exhibited the highest values of phenolic acid, 74.99 ± 0.65 milligrams of gallic acid equivalent per 100 grams of fresh weight (mg of GAE/100 g fresh), whereas CA gave the highest flavonoid content (30.16 ± 0.21 milligrams of rutin equivalent per 100 grams of fresh weight (mg of RUE/ 100 g fresh)). Most of the phenolic compounds of KS, PS, PL and MK were comprised of phenolic acids and flavonoids, whereas other phenolic constituents were found in KW, ND, CA, RD, HC, OR and MN extracts. The antioxidant activities in the mango seed kernel extracts did not correlate with the yield of the extracts, or the yield of seed kernels from mango seeds, however the activities were related with phenolic, flavonoid and phenolic acid content. Mango seed kernels show promise as a potential material for extracting natural antioxidants.

Keywords: mangifera indica, antioxidant, phenolic compounds, flavonoid, phenolic acid

Introduction

Mango (*Mangifera indica* Linn.) is one of the most important tropical fruits in the world and currently ranked 5th in total world production among the major fruit crops (FAO, 2004). As mango is a seasonal fruit, about 20% is processed for products such as puree, nectar, leather, pickles, canned slices and chutney and these products experience worldwide popularity and have also gained increased importance in the US and European markets (Abdalla *et al.*, 2007). During processing of mango, by-products such as peel and kernel are generated. Kernels take up about 17-22% of the fruit (Soong *et al.*, 2006). As seed kernel is not currently utilized for any commercial purposes, it is discarded as a waste and becomes a source of pollution. This waste should be treated as a specialized residue due to the high levels of phenolic compounds and stable fat rich in saturated fatty acids. Recently, industry has been increasing investment in waste treatment to utilize these kernels as a good source of natural antioxidants and lipids (Arogba, 2000).

Mango seed kernel is by-product obtained during processing and it has been shown to enhance oxidative stability of fresh type cheese and ghee. Its extract extended their shelf life (Parmar and Sharmar, 1990). This could be attributed to the phospholipids and phenolic compounds in mango seed extract. In addition, mango kernel has also been shown to be a good source of phytosterols, such as campesterol, β -sitosterols, stigmasterol and to also contain tocopherols (Soong and Barlow, 2004). The antioxidant effect of mango seed kernel is due to their high content of polyphenols, sesquiterpenoids, phytosterols and microelements like selenium, copper and zinc (Schiber *et al.*, 2003).

There are many cultivars of Thai mangoes such as Nam-Dokmai, Kaew and Rad. We thus examined and report on the antioxidant activity of eleven cultivars of Thai mango seed kernels. DPPH activity, total phenolic content, total flavonoid content, free phenolic acid, esterified phenolic acid, insoluble bound phenolic acid and yield were determined with the aim of exploiting the high potential source from mango kernel cultivars as natural antioxidants.

Materials and Methods

Materials

Eleven cultivars of ripe mangoes which are commonly known as Kaew (KW), Nam Dokmai (NM), Khiew Sawoey (KS), Pimsaen (PS), Chok-Anan (CA), Rad (RD), Falun (FL), Hua Chang (HC), Mun Duan Kao (MK), Okrong (OR) and Maha Chanok (MN) were purchased from a local market during March to April 2007. The seeds were washed and the kernels were obtained by manually removing shells, and were then sun dried for 24 h. The dried material was kept in a freezer at -20°C for no longer than two months.

Folin Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, hexamethyltetramine, aluminium chloride, rutin were purchased from Sigma Chemical Co., Ltd (St. Louis, USA). Gallic acid and Ellagic acid were purchased from Acros Organics (New

Jersey, USA). The other chemicals and solvents used in this experiment were analytical grade purchased from Sigma-Aldrich Co., Ltd (Steinheim, Germany).

Extraction of mango seed kernel antioxidants

Frozen kernel (80g) were blended for 1 min with ethanol at -20°C and the containers were then flushed with nitrogen and shaken for 4.5 hours in the dark at 30°C (Maisuthisakul *et al.*, 2007). The supernatant, after filtration through cheesecloth and Whatman No 4 filter paper, was evaporated under vacuum. The sample was dried in a freeze dryer and stored in aluminum foil after flushing with nitrogen at -20°C until analysis to determine their yield, radical DPPH scavenging activity, total phenolic content, total flavonoid content, free phenolic acid, esterified phenolic acid and insoluble bound phenolic acid.

Determination of the yield of extract

The dried extracted sample was weighed to calculate the yield by the following equation;

$$\text{Yield (\%, wet weight basis)} = (W_1 \times 100) / W_2$$

Where W_1 is the weight of extracts after freeze drying and W_2 is the weight of fresh samples.

Determination of radical DPPH scavenging activity

Free radical scavenging capacity of betel leaf extract was evaluated according to the previous reported procedure using the stable 2,2-diphenyl -1-picrylhydrazyl radical (DPPH●) (Masuda *et al.*, 2007). The final concentration was 100 μM for DPPH●. The absorbance at 517 nm was measured against a blank of pure methanol at 30 min of reaction used to estimate the remaining radical levels. The DPPH activity was expressed in terms of EC_{50} ($\mu\text{g}\cdot\text{mL}^{-1}$). Antiradical activity was reported in the form of $1/\text{EC}_{50}$.

Determination of total phenolic content

The volume of total phenolic content in 11 mango seed kernel extracts was determined using Folin-Ciocalteu reagent (Kähkönen *et al.*, 2007). Each extracted sample (200 μl) was introduced into test tubes and then 1 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5% w/v) were added. The tubes were mixed with a vortex and allowed to stand for 30 min in the dark, then centrifuged at 3300g for 5 min. Absorption was measured at 765 nm (UV/Vis 1601 spectrophotometer, Shimadzu, Japan). Results of total phenolic content were expressed as milligrams of gallic acid equivalent per 100 gram of fresh weight (mg GAE/100 g fresh).

Determination of total flavonoid content

Total flavonoid content of 11 samples from various cultivars of mango seed kernels was measured by a colorimetric assay (Bonvehí, Torrento & Lorente, 2001). 1 ml of 0.5% hexamethyl tetramine (w/v), 20 ml of acetone and 2 ml of 0.1 mol/l HCl were added to the finely ground frozen sample (5 g) and set to boil with reflux for 30 min. The resulting solution was filtered, and the residue was washed with 20 ml of acetone, the volume being leveled to 100 ml with acetone. 10 ml of the extract was put into a separation funnel, along with 20 ml of H_2O and 25 ml of ethyl acetate. Extraction with ethyl acetate was carried out three times. The extract was washed twice; using 50 ml of H_2O each time, and was subsequently made up to 100 ml with ethyl acetate. The total flavonoid content was determined in 10 ml of the extract using 1 ml of 2% AlCl_3 in methanol solution (5% acetic acid in methanol). Absorbance was read at 425 nm (UV/Vis 1601 spectrophotometer, Shimadzu, Japan) and flavonoid percentage was estimated using calibration curves and

expressed as rutin equivalents in milligrams per 100 gram fresh weight (mg RUE/100 g fresh).

Determination of free phenolic acid, esterified phenolic acid and insoluble bound phenolic acid

The free, esterified and insoluble-bound phenolic acids of mango seed kernel extract were isolated using the procedure of Krygier *et al.* (1982) with slight modification. The extract (2 g) was extracted six times with 40 ml of water-methanol-acetone (6:7:7, v/v/v) at room temperature, using a vortex for 2 min. After each extraction, samples were centrifuged for 15 min at 5000 × g and supernatants were collected (residue kept for further analysis). Combined supernatants were evaporated at 30°C under vacuum to 40 ml and the pH of the mixture was adjusted to 2 using 6 M HCl. The samples were centrifuged at 5000 × g and supernatants were extracted 6 times with diethyl ether-ethyl acetate mixture (1:1, v/v) at a supernatant to solvent ratio of 1:1 (v/v). The ether extracts were combined and evaporated to dry at 30°C under vacuum. The extracted phenolic acids contained free phenolic acids (FPA).

The aqueous layer remaining after extraction of free phenolic acids and the residue after centrifugation were mixed together and then treated with 30 ml of a 4 M NaOH solution under nitrogen for 4 h at room temperature to release esterified phenolic acids. The resultant hydrolysate was acidified to pH 2 using 6 M HCl and extracted into diethyl ether-ethyl acetate mixtures, as before, to yield esterified phenolic acids (EPA).

The residue, kept for further analysis after extraction of free and esterified phenolic acids, was treated with 20 mL of 4M NaOH under nitrogen at room temperature, then acidified with 6 M HCl to pH 2 and centrifuged at 5000 × g for 15 min. The supernatants were extracted six times with diethyl ether-ethyl acetate mixtures, as before, and extracts were evaporated to dry under vacuum to obtain insoluble-bound phenolic acids (IBPA). The dried phenolic acids (FPA, EPA, and IBPA) were dissolved in methanol, separately, and contents of phenolic acids were determined colorimetrically, using Folin-Ciocalteu reagent for total phenolic content determination. The contents of free, esterified and insoluble-bound phenolic acids were expressed as mg of gallic acid equivalents per 100 gram of fresh weight (mg GAE/100 g fresh).

Statistical analysis

Results are presented as mean value ± standard deviation (at least three replicate experiments). Statistic analysis among treatments were determined at the significance level of $P < 0.05$.

Results and Discussion

Determination of yields

Taking into consideration the industrial requirements for extraction, both yields and economic parameters were emphasized before detailed study of their antioxidant potential. The high moisture content of the fresh products investigated here required the use of a sun drying process which would add slightly to the overall cost. Solvent extraction is frequently used for isolation of antioxidants. The antioxidant activities of the extracts are strongly dependent on

the solvent, due to the different antioxidant potentials of compounds with different polarity (Soong *et al.*, 2004). The ethanol was used as extraction solution in the present study as it is the most widely used solvent for hygiene and abundance reasons; also, the solvent is compatible with food.

Yields of the kernels from several cultivars of mango seeds are shown in Figure 1. CA seed had the largest size compared to the other mango seeds, hence there was no doubt that the yield of kernel from the CA cultivar would have the highest value. In addition, the CA seed also gave the highest yield of extract (Table 1). A multiple mixture of different phenolic classes in a wide range of polarities can be assumed for mango kernel extract yields.

Table 1. Yield and Antioxidant activity of mango seed kernel extracts and reference compounds determined by the DPPH method [§]

Mango cultivars	Yield (%) of extracts	EC ₅₀ (µg/ml)	Antiradical activity (1/EC ₅₀)
α- tocopherol	-	14.95 ± 0.23 ^c	2.68 ± 0.04 ^e
Kaew	3.25 ± 0.08 ^{bc}	13.06 ± 0.86 ^a	3.04 ± 0.20 ^h
Namdokmai	3.09 ± 0.03 ^{ab}	14.49 ± 0.22 ^{bc}	2.73 ± 0.04 ^f
Keawsawoi	3.07 ± 0.04 ^{ab}	16.48 ± 0.43 ^{de}	2.40 ± 0.06 ^c
Pimsaen	3.41 ± 0.06 ^d	18.72 ± 0.21 ^f	2.11 ± 0.02 ^b
Chokeanan	3.33 ± 0.07 ^{cd}	13.67 ± 0.44 ^{ab}	2.90 ± 0.09 ^g
Rad	3.17 ± 0.05 ^b	15.65 ± 0.30 ^{cd}	2.53 ± 0.05 ^d
Phalun	3.27 ± 0.04 ^c	19.69 ± 0.16 ^{fg}	2.01 ± 0.02 ^b
Huachang	3.25 ± 0.04 ^{bc}	14.11 ± 0.07 ^b	2.80 ± 0.01 ^f
Munduankao	3.11 ± 0.03 ^b	20.54 ± 0.14 ^g	1.93 ± 0.01 ^a
Okrong	3.09 ± 0.03 ^{ab}	16.94 ± 0.14 ^e	2.33 ± 0.02 ^c
Mahachanok	3.01 ± 0.03 ^a	14.41 ± 0.22 ^b	2.75 ± 0.04 ^{ef}

Note: [§] Data followed by different letters within each column are significantly different according to Duncan's multiple range test at $P < 0.05$. Data obtained from at least three replicates.

Determination of radical DPPH scavenging activity and phenolic compounds of mango seed kernel extracts

Screening of 11 mango cultivars demonstrated the kernel to be a rich source of phenolic acid. The antiradical activity, total phenolic, flavonoid and phenolic acid content varied considerably from one cultivar of mango seed kernel to another. On a fresh weight basis, KW, CA, MN, NM showed high antiradical activity compared to the α-tocopherol (Table 1).

According to the results presented in Figure 2, we note that extracts from the KW cultivar showed the highest total phenolic content (116.98 ± 0.37 mg GAE/100 g fresh). Conversely, extracts obtained from the MK cultivar showed the least total phenolic content. It was observed that most cultivars of mangoes having flesh exhibiting a sour taste, showed the highest phenolic content. Most of the phenolic constituents of KW, PS, PA and MK cultivars were phenolic acids and flavonoids (Figure 2). The main phenolic compound of mango seed

kernel of 11 cultivars was phenolic acid. It can be seen that extracts obtained using ethanol solvent composed high polarity phenolic compounds. When only phenolic acid contents were considered, it was found that the amount of free phenolic acids of the studied kernel samples were higher than esterified and insoluble bound phenolic acids (Figure 3). The results also indicated that, for the given raw materials, the antiradical activities (with some minor exception) correlated with their total phenolic contents.

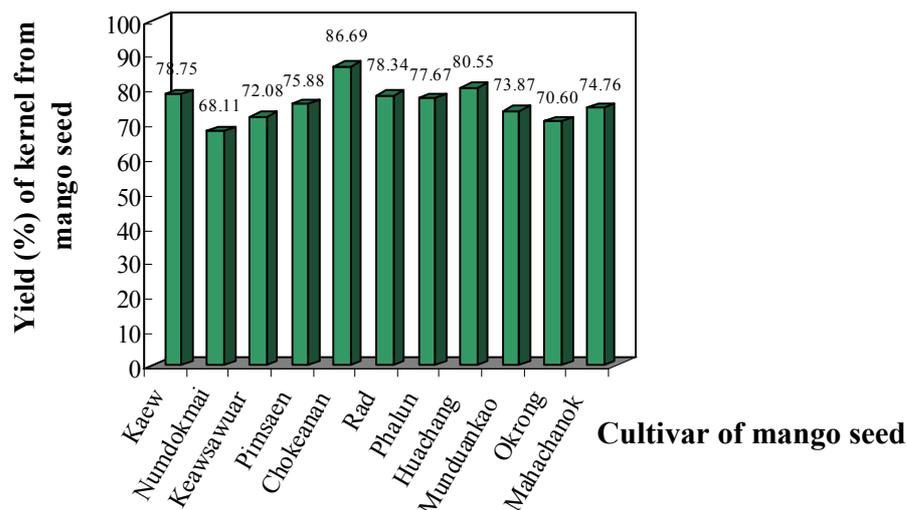


Figure 1. The yields (%) of mango seed kernel from various mango cultivars.

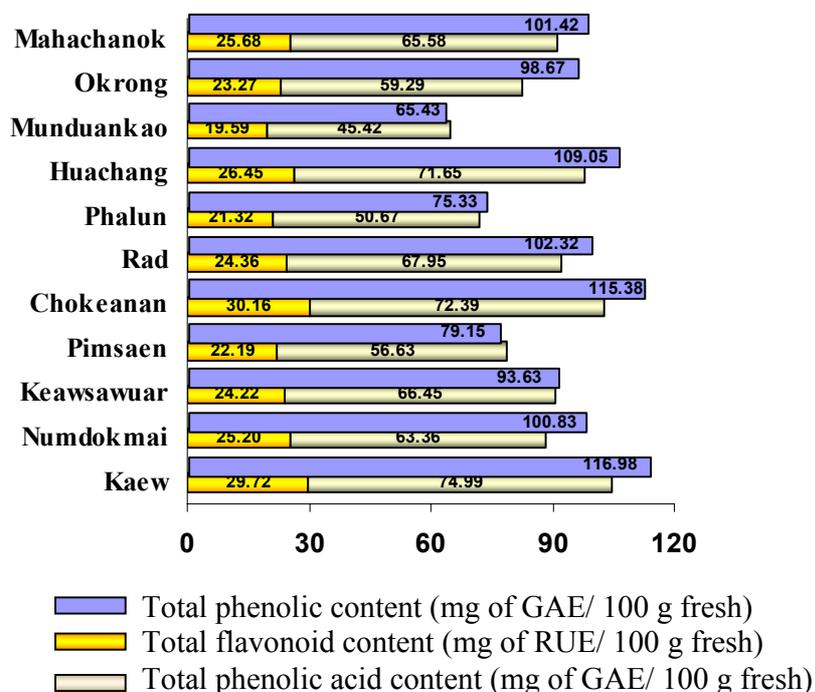


Figure 2. The total phenolic, flavonoid and phenolic acid contents of mango seed kernel from various mango cultivars.

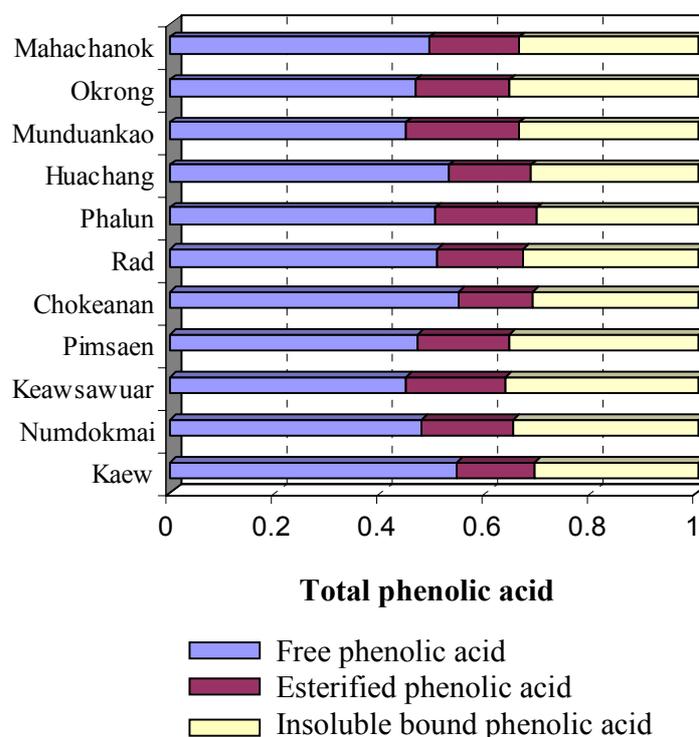


Figure 3. The fraction of free phenolic acid, esterified phenolic acid and insoluble bound phenolic acid in mango seed kernel. The total phenolic acid is calculated in terms of mg/ 100 g of fresh sample.

Relationship of antiradical activity with yields and phenolic compounds

The antiradical activity in all extracts did not correlate with yields of kernels from mango seeds or yields of the extracts (Figure 4). Furthermore, content of phenolic compounds correlated strongly with their antiradical activity ($R^2 = 0.92$) (Figure 5a). This confirms that the phenolics are likely to contribute to the radical-scavenging activity of kernel extracts. Similar results were reported for different plants by various studies (Soong and Barlow, 2004). The correlation between flavonoids and antiradical activity ($R^2 = 0.90$) (Figure 5b) of all extracts was slightly higher than that between phenolic acids and antiradical activity ($R^2 = 0.86$) (Figure 5c). These results suggest that the antioxidant capacity of extracts is due to the contribution of flavonoids (90%) and phenolic acids (86%).

To evaluate the procurement of raw material, economic characteristics such as availability (sufficient amount, season-dependence), pre-treatment (low influence of treatment on active compounds, possible standardization according to good agricultural practice guidelines), quality (homogeneity), market opportunities and cost (price, transport, additional pretreatment required) were assessed. Seed from the cultivar KW, exhibiting a high level of phenolic compounds, is only available in small amounts from factories. After ranking of both the phenolic yield and practical issues, the cultivar CA was selected for further investigation.

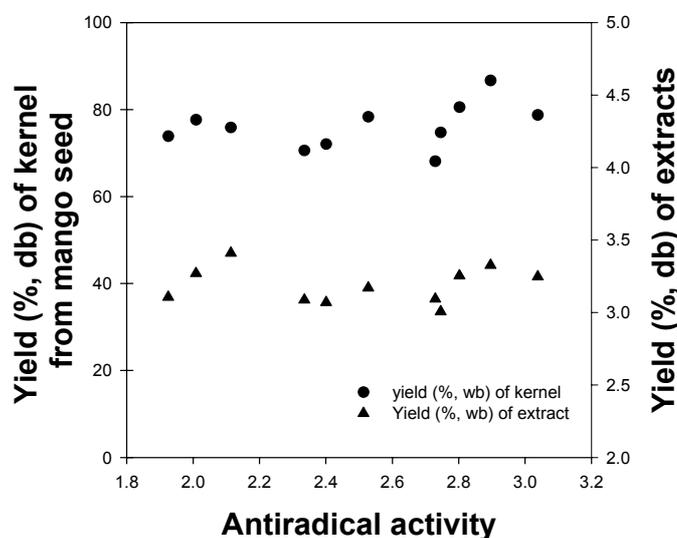


Figure 4. The relationship between yield of kernel from mango seed, yield of extracts and antiradical activity of mango seed kernel extracts.

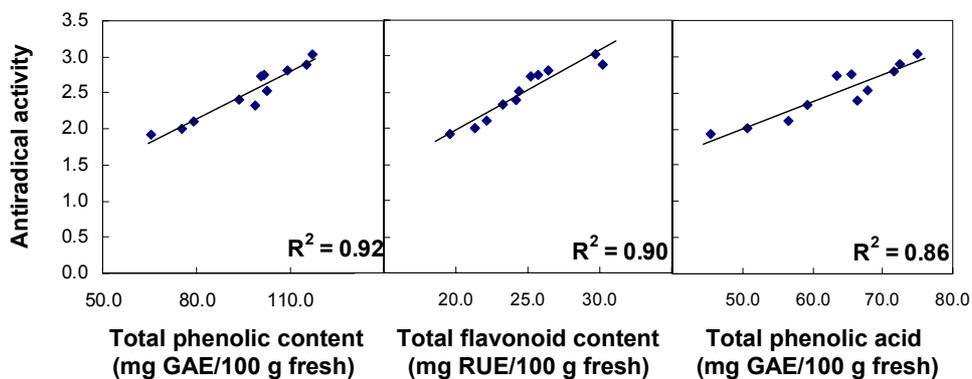


Figure 5. The relationship between antiradical activity and (a) total phenolic, (b) flavonoid and (c) phenolic acid content of mango seed kernel extracts.

Conclusion

The type of cultivar of mango seed kernel had significant affect on extraction yields, total phenolic, flavonoid and phenolic acid content. It was notable that extracts from *Mangifera indica* Linn. cultivars Kaew and Chok-Anan exhibited higher efficiency as antioxidant agents than did the other selected cultivars. However, since economic characteristics also need to be considered, Chok-Anan cultivar is regarded as being may be considered suitable for further investigation of their potential antioxidant agent.

Acknowledgement

This study was supported by a grant from University of the Thai Chamber of Commerce (UTCC). The authors would like to thank Dr. Leonard Fweje for his suggestions.

References

1. Abdalla, A.E.M., Darwish, S.M., Ayad, E.H.E. and El-Hamahmy, R.M. (2007). Egyptian mango by product 2: Antioxidant and antimicrobial activities of extract and oil from mango seed kernel. **Food Chemistry**, 103: 1141-1152.
2. Arogba, S.S. (2000). Mango (*Mangifera indica*) kernal: Chromatographic analysis of the tannin and stability study of the associated polyphenol oxidase activity. **Journal of Food Composition and Analysis**, 13: 149-156.
3. Bonvehí, J.S., Torrent, M.S. and Lorente, E.C. (2001). Evaluation of polyphenolic and flavonoid compounds in honey-bee collected pollen produced in Spain. **Journal of Agricultural and Food Chemistry**, 49, 1848-1853.
4. Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K. and Kujala, T.S. (1999). Antioxidant activity of plant extract containing phenolic compounds. **Journal of Agricultural and Food Chemistry**, 47, 3954–3962.
5. Krygier, K., Sosuiski, F.W. and Hogge, L. (1982). Free, esterified and insoluble bound phenolic acids 2. Composition of phenolic acids in rapeseed flour and hulls. **Journal of Agricultural and Food Chemistry**, 30, 334–336.
6. Maisuthisakul, P., Pongsawatmanit, R. and Gordon, M.H. (2007). Assessment of phenolic content and free-radical scavenging capacity of some Thai indigenous plants. **Food Chemistry**, 100, 1409-1418.
7. Masuda, T., Yonemori, S., Oyama, Y., Takeda, Y., Tanaka, T. and Andoh, T. (1999). Evaluation of the antioxidant activity of environmental plants: activity of leaf extracts from seashore plants. **Journal of Agricultural and Food Chemistry**, 47, 1749–1754.
8. Parmar, S.S. and Sharmar, R.S. (1990). Effect of mango (*Mangifera indica* L.) seed kernel pre-extract on the oxidative stability of buffalo ghee. **Food Chemistry**, 35: 99-107.
9. Schiber, A., Berardini, N. and Carle, R. (2003). Identification of flavonol and xanthol glycosides from mango peels by HPLC. **Journal of Agricultural and Food Chemistry**, 51: 5006-5011.
10. Soong, Y. and Barlow, P. (2004). Antioxidant activity and phenolic content of selected fruit seeds. **Food Chemistry**, 88: 411-417.

11. Soong, Y. and Barlow, P. J. (2006). Quantification of gallic acid and ellagic acid from Longan (*Dimocarpus longan* Lour) seed and mango (*Mangifera indica* L.) kernel and their effects on antioxidant activity. **Food Chemistry**, 97: 524-530.