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Antioxidant activity and phenolic content of selected fruit seeds

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Abstract

The total antioxidant capacity and phenolic content of edible portions and seeds of avocado, jackfruit, longan, mango and tamarind were studied. In addition, the relationship between antioxidant activity, phenolic content and the different degrees of heating of mango seed kernel was investigated. The seeds showed a much higher antioxidant activity and phenolic content than the edible portions. The contribution of all the fruit seed fractions to the total antioxidant activity and phenolic content was always >70%. ABTS cation radical-scavenging and FRAP assays were employed for the determination of antioxidant activity; FCR assay was used to measure the total phenolic content. The AEAC and FRAP of ethanolic extracts of MSKP products increased to a maximum after heating to 160 °C. The total phenolic content in extracts of MSKP products increased from 50.3 to 160 mg/g GAE with an increase in heating temperature to 160 °C.

Keywords: Antioxidant capacity; Avocado; Jackfruit; Longan; Mango; Phenolic content; Tamarind

1. Introduction

Epidemiological studies show that many phytonutrients of fruits and vegetables may be beneficial in protecting the human body against damage by reactive oxygen and nitrogen species (Diplock et al., 1998; Halliwell, 1997). Thus, it is considered important to increase the antioxidant intake in the human diet and one way of achieving this is by enriching food with antioxidants. As some synthetic antioxidants may exhibit toxicity and require high manufacturing costs but show lower efficiency than natural antioxidants, there is a need to identify natural and possibly more economic and effective antioxidants with potential to be incorporated into foods.

Several natural antioxidants have already been isolated from different kinds of plant materials, such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs (Ramarathnam, Osawa, Ochi, & Kawakishi, 1995). Antioxidant compounds have been identified in the seeds of citrus (Alessandra, Marie-Elisabeth, Hubert, & Claudette, 1998), grape (Jayaprakasha, Singh, & Sakariah, 2001), mango (Puravankara, Boghra, & Sharma, 2000), canola (Krygier, Sosulski, & Hogge, 1982; Naczk, Amarovicz, Sullivan, & Shahidi, 1998; Wanasundara, Amarovicz, & Shahidi, 1994), sunflower (Kubicka, Jedrychowski, & Amarowicz, 1999), primrose (Balasinska & Troszynska, 1998; Wettasinghe & Shahidi, 1999), sesame (Shahidi, Amarovicz, Abou-Gharbia, & Shehata, 1997), flaxseeds (Oomah, Kenaschuk, & Mazza, 1995) and lupin (Tsaliki, Lagouri, & Doxastakis, 1999); yet, studies relating to the antioxidant activity of tropical and subtropical fruit seeds have been sparsely reported. Fruit seeds have not generally received much attention as antioxidant sources and this could be due to their lack of popularity and lack of commercial applications (unlike oil seeds). However, there are considerably higher ratios of by-products arising from fruit-processing plants as

Abbreviations: AEAC, L-ascorbic acid-equivalent antioxidant capacity; ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); FCR, Folin–Ciocalteu reagent; FRAP, ferric-reducing antioxidant power; GAE, gallic acid equivalents; MSKP, mango seed kernel powder; TPTZ, 2,4,6-tripyridyl-*s*-triazine.

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fruit juices and derived products have experienced growing worldwide popularity. For example, there are about 3×10^5 ton of dry mango seed kernels available annually in India after consumption or industrial processing of mango fruits (Narasimha Char, Reddy, & Thirumala Roa, 1977; Narasimha Char & Azeemoddin, 1989). It would be beneficial, in improving the complete utilization of the seeds, if they could be used as a source of natural food additives and ingredients.

Plants have excellent antioxidant properties and these effects are mainly attributed to their phenolic constituents. The phenolic constituents of mango seed kernel are reported to be mainly gallic and ellagic acids, as well as gallates (Puravankara et al., 2000). Gallotannins and condensed tannin-related polyphenols are also reported to be present (Arogba, 2000). A phytochemical investigation of mango stem bark extract has led to the isolation of gallic acid, 3,4-dihydroxy benzoic acid, gallic acid methyl ester, gallic acid propyl ester, mangiferin, (+)-catechin, (−)-epicatechin, and benzoic acid and benzoic acid propyl ester (Alberto et al., 2002). Antioxidative activity of tamarind seed coat is due to the presence of 2-hydroxy-3′,4′-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate and epicatechin (Tsuda et al., 1994). Avocado seeds are claimed to be rich in a complex mixture of polyphenolic compounds, ranging from (+)-catechin and (−)-epicatechin to highly polymeric substances; a proanthocyanidin has also been identified (Geissman & Dittmar, 1965). The objectives of the present study were to determine the total antioxidant capacities and phenolic contents of avocado, jackfruit, longan, mango and tamarind seeds, to compare the antioxidative capacity with their edible portions, and to investigate the relationships between antioxidant activity, phenolic content and the effects of different degrees of heating of mango seed kernel.

2. Materials and methods

2.1. Materials

2.1.1. Fruits

Avocado (*Persea Americana* Mill.), jackfruit (*Artocarpus heterophyllus* Lam.), longan (*Dimocarpus longan* Lour.), mango (*Mangifera indica* L.), and tamarind (*Tamarindus indica* L.) were purchased on several separate occasions from local markets in Singapore.

2.1.2. Chemicals

ABTS, L-Ascorbic acid, potassium persulfate were purchased from Sigma (MO, USA). Folin–Ciocalteu reagent, iron (III) chloride hexahydrate, anhydrous sodium acetate, acetic acid, ethanol and hydrochloric acid were purchased from Merck (Darmstadt, Germany). TPTZ, iron (II) sulfate heptahydrate and gallic acid were purchased from Acros Organics (NJ, USA). Anhydrous sodium carbonate was purchased from J.T. Baker (NJ, USA).

2.2. Methods

2.2.1. Sample preparation

The edible portions and seeds of the five selected fruits were freeze-dried (−50 °C, 24 h). Freeze-dried samples were separately ground using a stainless-steel grinder. Portions of the freeze-dried ground mango seed kernels were defatted with petroleum ether by the Soxhlet method. The defatted meals were air-dried for 12 h to remove all traces of solvent. All the samples were stored in vacuum-packaged polyethylene pouches at −20 °C until required for analysis. For the analysis of fresh fruits, the edible portions and seeds were ground just before use.

Approximately 1 g and 200 mg of each of the edible portions or seeds were accurately weighed and refluxed with a total volume of 20 or 50 ml of ethanol:water (50:50, v/v), respectively, in a water bath at 70 °C for one hour. The extracts were then passed through a Whatman no. 4 filter paper.

Portions of the fresh mango seed kernels were dried in a conventional air-oven (35, 105 °C) until a constant weight was attained. Those that had been oven-dried at 105 °C were then re-heated at 140 ± 2, or 160 ± 2, or 180 ± 2 or 200 ± 2 °C for 20 min in the oven. Weighed amounts (200 mg) of each oven-dried and heated sample were refluxed with 50 ml of ethanol:water (50:50, v/v) in a water bath at 70 °C for an hour. The extracts were then filtered as above.

All filtrates were directly measured in the ABTS, FRAP and FCR assays (see below). Triplicate extractions were prepared from each sample and all extracts were measured five times in each assay. The mean values of antioxidant capacity and phenolic content were calculated.

2.2.2. ABTS cation radical-scavenging assay

The ABTS cation radical-scavenging assay was carried out using an UV/visible spectrophotometer (Shidmazu UV-1601) with a Shidmazu CPS-240A temperature controller. The procedure was adapted from a previous report by Robert et al. (1999). Briefly, stock solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate to generate the ABTS cation chromophore. The mixture was diluted with absolute ethanol to give an absorbance of 1.5–2 at 414 nm. An aliquot of the samples (10–40 μl) was added to 3 ml of ABTS reagent and the absorbance reading was taken after initial mixing and up to 90 min until it reached a plateau. Total antioxidant capacity was calculated relative to the reactivity of ascorbic acid as a standard under the same

conditions and the results were expressed as μmol/g AEAC.

2.2.3. Ferric-reducing antioxidant power assay

The FRAP assay was carried out by using a modified method of Benzie and Szeto (1999). An aliquot of the samples (10–40 μl) was mixed with 3 ml of ferric-TPTZ reagent. The change in absorbance was measured at 593 nm after initial mixing and up to 90 min until it reached a plateau. Aqueous solutions of known Fe(II) concentration ($FeSO_4 \cdot 7H_2O$) were used for calibration of the FRAP assay and antioxidant power was expressed as μmol/g FRAP.

As the antioxidant activity is strongly dependent on the model system in which it is evaluated, a single analytical assay may be inadequate. Therefore, both ABTS assay and FRAP assay were used for the measurement of total antioxidant capacity.

2.2.4. Folin–Ciocalteu reagent assay

The Folin–Ciocalteu reagent assay was used to determine the total phenolics content (Singleton & Rossi, 1965). An aliquot of the samples (10–40 μl) was mixed with 1.8 ml of Folin–Ciocalteu reagent previously diluted with distilled water (1:10). The solution was allowed to stand at 25 °C for 5 min before adding 1.2 ml of 15% sodium carbonate solution in distilled water. The absorbance at 765 nm was read after initial mixing and up to 90 min until it reached a plateau. Gallic acid was used as a standard for the calibration curve. The total amount of phenolic compounds was calculated and expressed as GAE (mg/g).

3. Results and discussion

3.1. Determination of total antioxidant capacity and phenolic content of edible portions and seeds of fruits

Solvent extraction is frequently used for isolation of antioxidants and both extraction yield and antioxidant activity of extracts are strongly dependent on the solvent, due to the different antioxidant potentials of compounds with different polarity (Julkunen-Tiito, 1985; Marinova & Yanishlieva, 1997). The ethanol–water systems were used as extraction solutions in the present studies as they are the most widely employed solvents for hygiene and abundance reasons; also, the solvent is compatible with food.

The total antioxidant capacity and polyphenol content vary considerably from one kind of fruit to another. In addition, they are found to be different for different parts of the fruits. Antioxidant activity and phenolic content of both edible portions and seeds of avocado, jackfruit, longan, mango and tamarind were measured by the use of ABTS, FRAP and FCR assays, which were carried out on three different independent occasions, using fruit purchased on three separate occasions. The AEAC, FRAP and GAE of fruit seeds and edible portions are presented as means of three determinations ± SD (standard deviation). On the basis of the wet weight, mango seed kernel had the highest antioxidant activity, followed by the seeds of tamarind, longan, avocado, and jackfruit. For the edible portion (wet weight), tamarind showed the highest antioxidant activity, followed by the mango, avocado, longan and jackfruit. On the basis of dry weight, mango seed kernel also had the highest antioxidant activity and phenolic content, followed by the seeds of tamarind, avocado, longan, and jackfruit. For the edible portion (dry weight), tamarind also showed the highest antioxidant activity and phenolic content, followed by mango, longan, avocado and jackfruit. Overall, seeds showed a much higher antioxidant capacity and phenolic content than the edible portions in the fruits tested. Their phenolic contents are correlated with the antioxidant activity, as shown in Table 1. In most fruits, the contribution of the fruit seed fraction to the total antioxidant activity and phenolic content was more than 95%, except for jackfruit, which was about 70%. This suggests that the fruit seeds should be further utilized rather than just discarded as waste.

3.2. Scavenging effect of extracts from heated MSKP products on ABTS cation radical

As mango seed kernel showed the highest antioxidant activity and phenolic content among the samples tested, it was subjected to heating in order to evaluate the effect of different degrees of heating on the antioxidant capacity and phenolic content. In India, mango seed kernel is traditionally roasted and eaten by the tribal people, so it is assumed to be suitable for human consumption. However, some further work on possible toxicity of the product is currently underway. Mango is one of the most important tropical/subtropical fruits (Ramteke, Vijayalakshmi, & Eipeson, 1999). World mango production reached 23,800,000 metric ton in 1999, which is 1.2 million metric ton higher than the 1995 production based on FAO statistics. India, China, Mexico, Thailand, Philippines, Pakistan, Nigeria, Indonesia, Brazil and Egypt are the top ten mango-producing countries in the world and production is heavily concentrated in Asia.

The freeze-dried and oven-dried MSKP products showed similar antioxidant capacities (Tables 1 and 2), which illustrated the relative heat stability of the antioxidant substances. Surprisingly, the ethanolic extracts of heated MSKP products showed higher antioxidant activities in the ABTS cation radical-scavenging assay than did the oven-dried MSKP products. A decrease in

Table 1
The total antioxidant activity and phenolic contents of fresh and freeze-dried edible portion and seed of mango, tamarind, longan, avocado and jackfruit by ABTS, FRAP and FCR assays

Item	Fresh sample	Freeze-dried sample		
	Antioxidant activity	Antioxidant activity		Phenolic content
	AEAC[a] (μmol g^{-1})	AEAC[a] (μmol g^{-1})	FRAP[a] (μmol g^{-1})	GAE[a] (mg g^{-1})
Mango				
Kernel	762 ± 72.9	1397 ± 59.3	2572 ± 129.7	117 ± 13.5
Flesh	7.2 ± 1.6	27.1 ± 2.7	36.6 ± 5.0	2.4 ± 0.3
Defatted mango				
Kernel	ND	986 ± 39.3	1976.2 ± 205.6	78.0 ± 3.8
Tamarind				
Seed	698 ± 30.3	1160 ± 140.0	2486 ± 311.4	94.5 ± 4.9
Flesh	17.0 ± 2.3	30.0 ± 1.0	49.9 ± 4.4	3.9 ± 0.9
Longan				
Seed	488 ± 82.5	692 ± 24.2	1388 ± 136.8	62.6 ± 3.2
Flesh	3.7 ± 1.6	14.8 ± 1.4	41.5 ± 16.1	1.6 ± 0.1
Avocado				
Seed	236.1 ± 45.1	725 ± 39.4	1484 ± 15.7	88.2 ± 2.2
Flesh	4.9 ± 1.1	13.1 ± 2.4	9.6 ± 1.4	1.3 ± 0.0
Jackfruit				
Seed	7.4 ± 2.0	25.4 ± 2.6	2.8 ± 0.3	27.7 ± 3.4
Flesh	3.0 ± 0.4	11.0 ± 0.3	6.8 ± 0.5	0.9 ± 0

[a] Means of three determinations ± SD (standard deviation).

Table 2
The total antioxidant activity and phenolic contents of extracts from heated MSKP products by ABTS, FRAP and FCR assays

Item	Antioxidant activity		Phenolic content
	AEAC[a] (μmol g^{-1})	FRAP[a] (μmol g^{-1})	GAE[a] (mg g^{-1})
MSKP products			
Oven-dried at 35 °C	1571 ± 178	2787 ± 550	118 ± 7.4
Oven-dried at 105 °C	1888 ± 426	3078 ± 737	127 ± 15.6
Heated at 140 °C	2464 ± 210	3235 ± 820	159 ± 22.8
Heated at 160 °C	2568 ± 170	3822 ± 768	160 ± 10.7
Heated at 180 °C	2275 ± 244	3679 ± 36.6	149 ± 17.8
Heated at 200 °C	1353 ± 124	2337 ± 231	103 ± 9.1

[a] Means of three determinations ± SD (standard deviation).

total antioxidant capacity was only observed when heating temperature was increased above 160 °C.

As shown in Table 1, the AEAC of the ethanolic extracts of defatted MSKP products was reduced by 30% with respect to those samples freeze-dried. Even though there is some loss of phenolics (extracted by petroleum ether), the difference indicated the presence of non-phenolic type antioxidants. Several natural lipophilic antioxidants, such as phospholipids, tocopherols and carotenoids, are reported to occur in mango seed kernel, but most of these would be expected to be degraded at elevated temperature (Azizah, Nik Ruslawati, & Swee Tee, 1999). However, a higher antioxidant capacity was observed in the ethanolic extracts of heated MSKP products than in those freeze-dried. The most likely explanation for the higher antioxidant activity, at least in part, is an increased antioxidative principle due to the generation and accumulation of Maillard-type antioxidants (MRP) during the heating process. This suggestion is further confirmed in a recent paper by Antonio, Alessandra, and Giampaola (2003) who showed an increase in hydroxymethylfural, an intermediate in MRP production, in plums dried at 60 and 85 °C, respectively. MRPs are obtained by reactions between sugars and amino acids, peptides, or enzymic protein hydrolysates and they are suggested to have varying degrees of antioxidant activity, depending on their origin (Kim, Hayase, & Kato, 1986; Yamaguchi, 1986). Nevertheless, MRPs are also expected to be degraded at elevated temperature, which may explain the reduction in the antioxidant activity of extracts from MSKP heated at above 160 °C (Azizah et al., 1999).

3.3. Reducing power of extracts from heated MSKP products

Although ABTS and FRAP assays were carried out in different solutions, namely, ethanol and aqueous buffer, respectively, and they work by different mechanisms, i.e., scavenging of ABTS cation radicals in the ABTS assay and reduction of ferric ion in the FRAP assay, the results from these two assays were significantly correlated in all examined samples (Fig. 1).

Since the optimum antioxidant activity of mango seed kernels was attained after mild heating from 140 to 180 °C for 20 min, functional food(s) might be developed based on such products or the purified extracts being incorporated into food products to give therapeutic effects. The under-utilization of the mango seed kernel could be partly due to the limited knowledge of its toxicological properties. According to Berger, Saharty, and Krings (1999), the small amounts of toxic compounds generated under mild roasting conditions are highly unlikely to have an adverse effect on human consumers. In Nigeria, mango seed kernel is processed into a powder, e.g., processed mango kernel flour, which can be substituted for wheat flour in biscuits (Arogba, 1999). The lipid compositions of various mango kernel varieties have recently attracted increased research interest because of their potential application in the confectionery industry as a source of a cocoa–butter substitute (Ali, Gafur, Rahman, & Ahmed, 1985; Gaydou & Bouchet, 1984; Hemavathy, Prabhakar, & Sen, 1987; Lakahminarayana, Rao, Ramalingaswamy, & Chandrasekhara Rao, 1983; Rukmini & Vijayaraghavan, 1984). Rukmini and Vijayaraghavan (1984) reported that toxicity was not evident in rats fed with diets containing 100 g/kg of mango kernel crude fat. By extrapolation, consumption of 0.4 kg of the kernel or 0.8 kg of the processed flour per 70 kg body weight is deemed safe. Based on the toxicological evaluations conducted on the mango seed kernels in the past and the availability of huge amounts of kernels, they seem promising as a possible source of safe antioxidants.

3.4. Total polyphenols in extracts from MSKP products with different degrees of heating

The temperature during the drying and heating process affects compound stability due to chemical and enzymatic decomposition, losses by volatization or thermal decomposition; these latter have been suggested to be the main mechanisms causing the reduction of polyphenol contents. However, the total polyphenols in extracts of MSKP products increased from 117 to 160 mg/g of GAE after heating to 160 °C. This is most likely attributed to the formation of phenolic substances under milder heating temperature whereas polyphenols are degraded at elevated heating temperatures. As shown in Table 1, the total polyphenol contents of MSKP products heated at 200 °C were considerably reduced. Yen and Chuang (2000) reported that the decrease in antioxidant activity of extracts of roasted *Cassia tora* was related to the degradation of polyphenols during roasting at elevated temperature. Nicoli, Anese, Manzocco, and Ferici (1997) also reported that the increase in antioxidant activity of coffee brewed under minimum roasting was due to the formation of MRP and its phenolic content whereas the phenolics were degraded after over-roasting. The increase of polyphenol contents of MSKP products after heating is correlated with the increase in antioxidant activity as illustrated in Figs. 2 and 3. Therefore, it may be suggested that the increases in antioxidant activity of extracts of heated MSKP products are related to the increase in polyphenols rather than the formation of MRPs. The formation of phenolic compounds during the heating process might be due to the availability of precursors of phenolic molecules, by non-enzymatic interconversion between phenolic molecules subjected to the effects of external factors, such as temperature. Thus, the plant composition and the degree of heating could be important factors contributing to high total polyphenol content. However, further investigation is needed to truly explain this phenomenon.

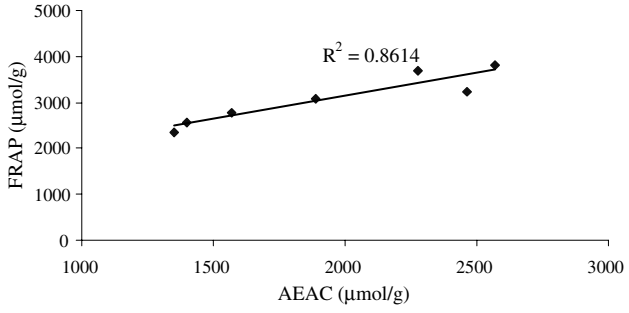

Fig. 1. Correlation of AEAC and FRAP of freeze-dried, oven-dried and oven heated MSKP products.

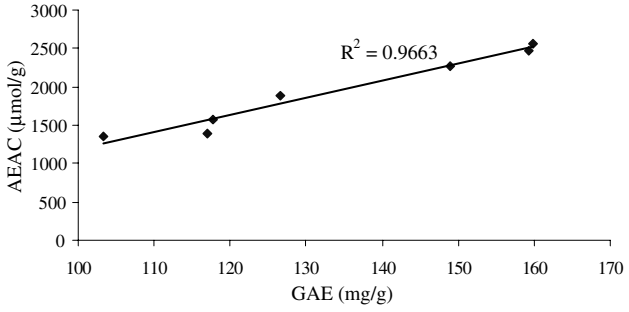

Fig. 2. Correlation of AEAC and total polyphenols content of freeze-dried, oven-dried and oven-heated MSKP products.

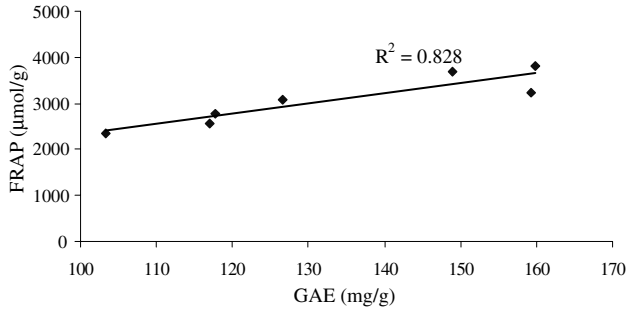

Fig. 3. Correlation of FRAP and total polyphenols content of freeze-dried, oven-dried and oven-heated MSKP.

4. Conclusion

The present study demonstrates a significantly higher total antioxidant capacity and phenolic content of fruit seeds than of the edible portions. The AEAC and FRAP of heated MSKP products were increased to considerably more than those of freeze-dried samples. This was most likely due to the production of MRPs or their intermediates with potent antioxidant activity, despite several other natural lipophilic antioxidants being degraded. Alternatively or in addition, it might also be due to the formation of phenolic compounds as the increase of polyphenol contents in MSKP products after heating is correlated with the increase in antioxidant activity. The total antioxidant activity and phenolic content were diminished when MSKP was heated above 160 °C. Therefore, it might be possible to use extracts of MSKP products heated between 105 and 160 °C as an additive in certain functional foods to boost their antioxidant capacity. Before final recommendations are made it is necessary to further confirm the lack of toxicity from such material and to investigate further dose/activity relationships.

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