Evaluation of Antioxidant Capacity of Non-Edible Parts of Some Selected Tropical Fruits

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Total polyphenol content and antioxidant activities of non-edible parts (seed and peel) of eight tropical fruits were analyzed and compared with those of their edible parts. The antioxidant activity was evaluated based on the ability of the fruit extracts (seed, peel and pulp) to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radicals, and the ferrous ion-chelating capacity. Total polyphenol content in seed, peel and pulp ranged from 0.2 to 153, 5.0 to 124, and 1.0 to 12 mg/g DW, respectively. Non-edible parts of the tropical fruits were found to have significant antioxidant activities. Among them, mango seed, mango peel, starfruit peel and avocado peel showed higher antioxidant potential by the DPPH and ABTS radical scavenging assays. The DPPH and ABTS radical scavenging abilities were highly correlated with total polyphenol content. Kiwano and papaya peels showed strong ferrous ion-chelating capacity, although they did not have high polyphenol content and DPPH and ABTS radical scavenging activities.

Keywords: tropical fruits; non-edible parts; phenolic content; antioxidant capacity

Introduction

Epidemiological studies have consistently shown an association between the consumption of fruits and vegetables and a reduced risk of human diseases, such as cardiovascular disease and cancer (Steinmetz and Potter, 1996; Ellingsen et al., 2008). The protective effects of such natural products are related to their antioxidants: phenolic compounds. Phenolics are products of secondary metabolism in plants, providing essential functions in the reproduction and growth of the plants. Phenolic compounds in our diet may provide health benefits. Fruits and vegetables are excellent sources of phenolic compounds (Wang et al., 1996; Vinson et al., 2001; Chu et al., 2002; Sun et al., 2002). Consumption of these compounds from dietary plant sources may increase protective antioxidants in the body.

Many tropical fruits are now available to consumers in Japan. The antioxidant properties of a number of these tropical fruits have been investigated using different analytical methods (Jimenez-Escrig et al., 2001; Leong and Shui, 2002; Someya et al., 2002; Lim et al., 2007). However, studies related to the antioxidant activity of non-edible parts of tropical fruits have been sparsely reported (Soong and Barlow, 2004). Non-edible parts of fruits have not generally received much attention as antioxidant sources. Thus the objectives of the present study were to determine the phenolic content and the total antioxidant capacity of non-edible parts of tropical fruits, to compare with those in edible parts, and to investigate the relationship between phenolic content and antioxidant capacity.

Materials and Methods

Fruits  Avocado (Persea americana Mill., Lauraceae), mango (Mangifera indica L., Anacardiaceae), canistel (Pouteria campechiana Baehni., Sapotaceae), passionfruit (Passiflora edulis Sims., Passifloraceae), papaya (Carica papaya L., Caricaceae), kiwano (Cucumis metuliferus E. Mey. ex Naudin, Cucurbitaceae), kiwifruit (Actinidia chinensis Planch.,
Actinidiaceae), and starfruit (*Averrhoa carambola* L., Oxalidaceae) were purchased from a local market in Sapporo, Japan.

**Chemicals**

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical was purchased from Wako Pure Chemicals Industries Ltd. (Japan). Ferrozine (3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine) and ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), diammonium salt) were obtained from Sigma-Aldrich Co. (USA), and FeCl$_2$$\cdot$4H$_2$O and Folin-Ciocalteu reagent were purchased from Merck, Darmstadt (Germany). All other chemicals and reagents used were of analytical grade, and were purchased from Wako Pure Chemicals Industries Ltd.

**Sample preparation**

Edible (pulp) and non-edible (seed and peel) parts of eight selected fruits were separately ground using a stainless-steel grinder. Separated samples were freeze-dried (−50°C, 72 h), and all samples were stored in vacuum-packaged polyethylene pouches at −20°C until analysis. Approximately 100 mg each of freeze-dried powders of the edible (pulp) and non-edible (seed and peel) parts was precisely weighed and extracted with 20 or 50 mL of 50% aqueous ethanol (v/v), respectively, in a water bath at 30°C for 24 h. The extracts were then filtered through Whatman no.4 filter paper. All filtrates were adjusted to a total volume of 20 or 50 mL with the same solvent, respectively, and used as a stock solution for further analyses.

**Total phenol content**

Total phenol content of fruit extracts was determined by Folin-Ciocalteu reagent (Kumazawa et al., 2002). Stock solution of each sample (1.0 mL, in triplicate) was introduced into test tubes followed by 1.0 mL Folin-Ciocalteu reagent (diluted 10 times with water) and 1.0 mL sodium carbonate (10%, w/v). Tubes were vortexed, covered with parafilm and allowed to stand for 60 min at room temperature. Absorption at 760 nm was then measured. If the sample absorbance exceeded 1, the sample was appropriately diluted to give a reading less than 1. Total phenol contents are expressed in gallic acid equivalents (mg/g dry fruit).

**DPPH radical scavenging assay**

The DPPH radical scavenging activity of the fruit extracts was measured by the decrease in absorbance of ethanolic DPPH radical solution at 734 nm (Re et al., 1999; Pellegrini et al., 2003). ABTS was dissolved in water to a 7 mM concentration. The ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration). The mixture was allowed to stand in the dark at room temperature for 12-16 h before use. The resultant ABTS radical solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. The stock solution of each sample (0.5 mL, in triplicate) was introduced into test tubes followed by 1.0 mL of ABTS radical solution. Tubes were vortexed, covered with parafilm and allowed to stand for 10 min at room temperature. Absorption at 734 nm was then measured. The antioxidant activity is expressed as TEAC (Pellegrini et al., 2003).

**Ferro ion-chelating capacity**

The method described by Hsu et al. (2003) was used to determine the ferrous ion-chelating activity of stock solution of the fruit extracts. Each sample (1.0 mL, in triplicate) was introduced into test tubes followed by 0.1 mL of 2 mM FeCl$_2$, 0.2 mL of 5 mM ferrozine and 3.7 mL ethanol. Tubes were vortexed, covered with parafilm and allowed to stand for 10 min at room temperature. Absorbance at 562 nm was then measured; a lower absorbance indicated a higher ferrous ion-chelating capacity, which was calculated as follows:

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\text{Ferro ion-chelating capacity} \times 100 = \left( 1 - \frac{A_{562 \text{ nm, sample}}}{A_{562 \text{ nm, control}}} \right) \times 100.
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**Results and Discussion**

**Polyphenol content**

The polyphenol content in eight tropical fruits (seed, peel and pulp) is shown in Fig. 1. For all fruits tested, the seeds showed a much higher phenolic content than the edible parts. Mango seed had the highest (153 mg/g DW) content of total phenolics, followed by canistel (35 mg/g DW) and avocado (35 mg/g DW) seeds. Soong et al. (2004) reported that the total phenolic content in freeze-dried mango seed was 117 mg gallic acid equivalent/g. The level of total phenols in this study was slightly higher than that reported by Soong, most likely due to the difference between the quality or harvest season. Peels also showed a much higher phenolic content than the edible parts. Mango, starfruit and avocado peels had the largest phenolic content (123, 80 and 75 mg/g DW, respectively). Ajila et al. (2007b) reported that the polyphenol content in mango peels ranged from 55 to 110 mg/g dry peel. In addition, Suda et al. (2005) reported that the phenolic contents in mango peel and seed were more than 5 times higher than that in mango pulp. On
the other hand, peels with relatively low phenolic content were found for kiwano, papaya and passionfruit (5.0, 13.2 and 12.2 mg/g DW, respectively), although studies on the non-edible parts of these fruits is limited. The range of values among the edible parts was as low as 1~12 mg/g DW.

**DPPH and ABTS radical scavenging activities** The TEAC of the fruits (seed, peel and pulp) was determined by DPPH and ABTS radical scavenging assay and is shown in Figs. 2 and 3, respectively. Most fruits with a high TEAC in the DPPH radical scavenging assay also had a high TEAC in the ABTS assay. As shown in Fig. 2, the seeds and peels showed a much higher radical scavenging activity than the edible parts. Mango seed had the strongest radical scavenging activity (4188 TEAC μmol/g DW), compared to other samples. Antioxidant activities of mango seed were in good agreement with reported data (Abdalla et al., 2007). Mango peel also had a strong radical scavenging activity (1846 TEAC μmol/g DW). A previous study has been reported that mango peel extract exhibits good antioxidant activity and thus may be used in nutraceuticals and functional foods (Ajira et al., 2007a). In our experiments, starfruit peel extract also exhibited good antioxidant activity (1000 TEAC μmol/g DW), followed by avocado peel (545 TEAC μmol/g DW) and avocado seed (462 TEAC μmol/g DW). On the other hand, peels with relatively low radical scavenging activity were found for kiwano (14.2 TEAC μmol/g DW), followed by papaya (58.4 TEAC μmol/g DW) and passionfruit (75.4 TEAC μmol/g DW). The range of values among the edible parts was as low as 7~153 TEAC μmol/g DW. As shown in Fig. 3, the seeds and peels showed a much higher ABTS radical scavenging activity than the edible parts.

**Correlation between DPPH radical scavenging activity and polyphenol content** Figure 4 shows a linear correlation of the DPPH radical scavenging activity and total polyphenol content. The correlation values of seed and peel were $R^2=0.9738$ and $R^2=0.9266$, respectively. Similar results were obtained with the ABTS radical scavenging activity and total polyphenol content (data not shown).

Thus, the non-edible parts of the eight tropical fruits showed a good correlation between the polyphenol content and DPPH and ABTS radical scavenging activities.

**Ferrous ion-chelating capacity** Polyphenols can chelate pro-oxidant metal ions, such as iron and copper, thus preventing free radical formation from these pro-oxidants (Kris-Etherton et al., 2002). The purpose of the evaluation of ferrous ion-chelating activity was to determine the capacity of non-edible parts of tropical fruits to bind ferrous ion catalyzing oxidation. The ferrous ion-chelating effect of selected fruit extracts is shown in Fig. 5. For all fruits tested, the peels showed a much higher ferrous ion-chelating capability compared to the edible parts. In particular, kiwano and papaya peels had the best chelating power, followed by canistel,
Fig. 2. DPPH radical scavenging activity of selected fruit extracts (mean±SD, n=3).

Fig. 3. ABTS radical scavenging activity of selected fruit extracts (mean±SD, n=3).
starfruit, passionfruit and avocado peels. Although kiwano and papaya peels did not show high polyphenol content and DPPH and ABTS radical scavenging activities, they could have potent secondary antioxidants which contain active components that bind to metal ions. As reports on the antioxidant capacity of non-edible parts of kiwano and papaya are very rare in the literature, our results could not be confirmed with those of previous studies. However, our results show kiwano and papaya peels have higher ferrous ion-chelating capacity.

In this study, non-edible parts (seed and peel) of mango, avocado, starfruit and canistel were found to have high radical scavenging capacity, while kiwano and papaya peels had weak radical scavenging capacity. However, kiwano and papaya peels have the best chelating power. Since little analysis of these samples has been performed, further study is required to characterize the active principles in the non-edible parts of tropical fruits.
References


