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An investigation of antioxidant capacity of fruits in Singapore markets

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Abstract

The antioxidant capacity of a group of fruits obtained in the Singapore markets was investigated. A total of 27 fruit pulps were tested for their general antioxidant capacity based on their ability to scavenge 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical. The contribution of L-ascorbic acid (AA) to the total antioxidant activity of fruits was investigated by using RP-HPLC. The antioxidant capacity of the fruit pulp was measured by monitoring the change of absorbance of the free radical solution at 414 nm in the test reaction mixture following addition of the fruit extract, as compared with AA. The results were expressed as mg of AA equivalents per 100 g, i.e. the quantity of AA required to produce the same scavenging activity as the extract in 100 g of sample (L-ascorbic acid equivalent antioxidant capacity, AEAC). Total antioxidant capacities of AA acid, trolox, hydroquinone, pyrogallol and several fruits were also evaluated based on its ability to scavenge the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical. Results obtained were compared with those of ABTS assay. Every mol of AA, trolox or hydroquinone, was found to reduce about 2 mol of $ABTS^{\bullet+}$ or DPPH•. However, 4 mol of DPPH• or 7 mol of $ABTS^{\bullet+}$ were scavenged by every mol of pyrogallol. A good correlation of AEAC was observed between the two methods. Both methods have been recommended to be useful tools to evaluate antioxidant capacities of fruits. According to the AEAC value of binary extract solution of fruits in the ABTS model, ciku shows the highest antioxidant capacity, followed by strawberry, plum, star fruit, guava, seedless grape, salak, mangosteen, avocado, orange, solo papaya, mango, kiwi fruit, cempedak, pomelo, lemon, pineapple, apple, foot long papaya, rambutan, rambutan king, banana, coconut pulp, tomato, rockmelon, honeydew, watermelon and coconut water. The AA contribution to AEAC of fruits varied greatly among species, from 0.06% in ciku to 70.2% in rambutan.

Keywords: ABTS; DPPH; HPLC; Antioxidant capacity; L-Ascorbic acid; Fruits

1. Introduction

High consumption of fruits and vegetables has been associated with a lowered incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction and cataracts (Ames, 1983; Ames, Shiganaga, & Hagwn, 1993; Feskanich et al., 2000; Gordon, 1996; Haegele et al., 2000; Halliwell et al., 1996; Michels et al., 2000). These protective effects are considered, in large part, to be related to the various antioxidants contained in them. There is overwhelming evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Antioxidants, which can inhibit or delay the oxidation of an oxidisable substrate in a chain reaction, would therefore seem to be very important in the prevention of these diseases (Ames et al., 1993; Aruoma, 1998; Jacob & Burri, 1996; Steinberg et al., 1991; Maxwell & Lip, 1997; Pratico & Delanty, 2000; Wang et al., 1996). However, knowledge of the potential antioxidant compounds present in a food does not necessarily indicate its antioxidant capacity. Furthermore, the synergistic effect which could exist between different antioxidants (termed synergism) means that the total antioxidant effect may be greater than the sum of the individual antioxidant activities and the isolation of one compound will not exactly reflect the overall action (Jia, Zhou, Yang, Wu, & Liu, 1998; Poeggeler, Reiter, Hardeland, Sewerenek, Melchiorri, & Barlow-Warden, 1995; Wu, Sugiyama, Zeng, Mickle, & Wu, 1998). In addition, there are many different antioxidant

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components in animal and plant tissues, it is relatively difficult to measure each antioxidant component separately. Several analytical methods have been proposed for determining total antioxidant activity of biological extracts in order to evaluate the total antioxidant capacity of biological samples (Cano, Hernandez-Ruiz, Garcia-Canovas, Acosta, & Arnao, 1998; Cao, Alessio, & Culter 1993; Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993; Robert, Pellegrini, Poteggente, Pannala, Yang, & Rice-Evans 1999; Wayner, Burton, Ingold, & Locke, 1985; Whitehead, Thorpe, & Maxwell, 1992).

Fruits from the tropical and subtropical climates are known to be associated with many medicinal properties (Morton, 1987). Many of these fruits are taken as remedies for coughs, intestinal bleeding, diarrhoea and other medical conditions. Pineapple juice, for example, can be taken to alleviate sore throat and seasickness. Thus, it is likely that these fruits will also have protective effects towards degenerative disease.

L-Ascorbic acid (AA) has numerous biological functions, which include the synthesis of collagen, hormones and neurotransmitters (Block, 1993). It is believed that the role of AA acid in disease prevention is due to its ability to scavenge free radical in the biological systems. Cancer, which is due to uncontrolled cell proliferation, may be initiated by oxidative and free radical damage to DNA and cell. Since AA may act as an effective antioxidant, it is able to slow down or prevent such damage (Block, 1993). AA is abundant in many fruits and is more prevalent in most fruits than is vitamin E. Therefore, it is believed that the AA contributes largely to the antioxidant activity in these extracts. The objective of this study was to measure the antioxidant properties of some common fruits available in the Singapore market by using an improved 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical decolourisation assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay. RP- HPLC was used to determine the AA (Vitamin C) content, thus the contribution of total antioxidant capacity in these fruits can be calculated.

2. Materials and methods

2.1. Materials

2.1.1. Fruits

Twenty-seven varieties of fruits were purchased on several separate occasions from local supermarkets and the wholesale centre. The 27 fruits comprised ciku (*Manilkara zapota*), strawberry (*Fragaria virginiana*), 'Flame seedless' grape (*Vitis vinifera*), guava (*Psidium guajava*), plum (*Prunus domestics*), star fruit (*Averrhoa carambola* L.), kiwi fruit (*Actinidia chinensis*), mango (*Mangifera indica* L.), lemon (*Citrus limon*), papaya *var.* solo (*Carica papaya* L.), mangosteen (*Garcinia mangostana* L.), salak (*Salacca edulis*), avocado (*Persea Americana*), foot long papaya (*Carica papaya* L.), pomelo (*Citrus grandis*), orange (*Citrus aurantium*), cempedak (*Artocarpus integer* Merr.), rambutan king (*Nephelium mutabile*), rambutan (*Nephelium lappaceum* L.), apple (*Malus pumila*), pineapple (*Ananas comosus* Merr.), tomato (*Lycopersicon esculentum*), banana (*Musa paridasiaca*), rockmelon (*Cucumis melo* var. *cantalupensis*), honeydew (*Cucumis melo* var. *inodorus*), watermelon (*Citrullus vulgaris*) and coconut (*Cocos nucifera*) .

2.1.2. Chemicals

ABTS, DPPH, AA, pyrogallol, hydroquinone, potassium persulfate and glycine were purchased from Sigma (MO, USA); trolox from Acros Organics (NJ, USA); ethanol, acetic acid, methanol and hydrochloric acid were purchased from Merck (Darmstadt, Germany); acetonitrile from EM Science (NJ, USA).

2.2. Sample preparation

Coconut water was weighed, diluted and filtered for analysis. All other fruits, except seedless red grapes, strawberries, and tomatoes, were peeled. Ciku was tested before natural ripening. The edible portion of the fruit was homogenized using a blender and weighed into a 50 ml centrifuge tube, and 25 ml of 50% aqueous ethanol (HPLC grade of pure water, the solution was sonicated by a Bransonics cleaner) was added (1:10 w/v) and mixed in a vortex mixer for 60 s. The extract was centrifuged at 2000 ***g*** for 5 min at room temperature. The supernatant was filtered and used directly for the ABTS, DPPH and AA assay.

2.3. ABTS free radical decolorization assay

The total antioxidant capacity assay was carried out on the Ultraspec 3000 UV/Visible Spectrophotometer (Pharmacia Biotech Ltd., Cambridge CB4 4FJ, England). The procedure was based on a method developed by Robert et al. (1999) with some modification. $ABTS^{\bullet+}$ was generated by reacting ABTS (7.4 mM) with potassium persulphate (2.6 mM). The solution was diluted to obtain an absorbance of 1.4 units at 414 nm (molar extinction coefficient $\epsilon = 3.6 \times 10^4$ mol^{-1} l cm^{-1}, Forni, Morla-Arellano, Packer, & Willison 1986) with 50 mM glycine-HCl buffer (pH 4.5) before use. Three millilitres of the solution were added to 20–80 μl of AA, trolox, hydroquinone, pyrogallol and fruit extracts separately. The changes in absorbance at 414 nm were recorded at 1, 3, 6, 10, 20, 30, 40, 60 and 90 min after mixing and until the absorbance reached a plateau. The antioxidant capacities, obtained by comparing the absorbance change at 414 nm in a test reaction mixture containing extract of fruit with that containing AA,

were expressed as mg of AA equivalents per 100 g of homogenate (AEAC). The AEAC was calculated using the following equation:

$$\text{AEAC} = \frac{\Delta A}{\Delta A_{\text{AA}}} \times C_{\text{AA}} \times V \times \frac{100}{W}$$

where, ΔA is the change of absorbance after addition of fruit extract, C_{AA} is the concentration of AA standard solution (mg/ml), ΔA_{AA} is the change of absorbance obtained from a calibration curve when the same volume of AA standard solution as that of fruit extract was added, V is the volume of filtrate (ml) and W is the weight of homogenate used for extraction (g).

2.4. DPPH free radical-scavenging assay

The free radical-scavenging activity of fruits was measured using the method described by Brand-Williams (Brand-Williams, Cuvelier, & Berset, 1995) with some modification. A 0.1 mM solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) in methanol was prepared. An aliquot of 20–80 μl of an antioxidant/fruit extract solution was added to 3 ml of this solution. The decrease in absorbance at 517 nm was measured at 0, 1, 5 and then every 10 min until the reaction reached a plateau. The decreased absorbance of DPPH remaining at the steady-state was calculated and expressed as mg of AA equivalents per 100 g of homogenate (AEAC).

2.5. HPLC assay

AA standard solution (1.36 g/ml) was prepared daily by accurately weighing 68 mg and dissolving it in 50 ml of 2% acetic acid. This was then diluted to give 0.068, 0.034, 0.017 and 0.0085 mg/ml working standard solutions. The HPLC system consisted of a Shimadzu HPLC (Model LC-10ATvp two Pumps and DGU-14A Degasser) equipped with a photo-diode array detector (Model SPD-M10A$_{\text{VP}}$; Shimadzu, Kyoto, Japan) interfaced with IBM Pentium-III personal computer. The separation was performed on a Shim-Pack VP-ODS column (250×4.6 mm i.d.; Shimadzu, Kyoto, Japan) with a guard column (GVP-ODS, 10×4.6 mm i.d.;

Table 1
AEAC of 27 selected fruits using ABTS$\bullet^+$ decolorization assay and their L-ascorbic acid content

Variety	AEAC[a] (mg/100 g)	AA content by HPLC[a] (mg/100 g)	Percentage contribution of AEAC by AA (%)[b]	Source	Classification by AEAC
Ciku	3396±387.9	2.0±0.7	0.06	Malaysia	Extremely high
Strawberry	472±92.9	53.9±11.2	11.4	Australia	
Plum	312±23.2	8.2±2.3	2.6	USA	
Star fruit	278±22.3	5.9±1.8	2.1	Malaysia	High
Guava	270±18.8	131±18.2	48.3	Thailand	
Grape seedless	264±83.6	0.5±0.2	0.2	USA	
Salak	260±32.5	2.4±1.5	0.9	Malaysia	
Mangosteen	150±23.3	4.1±1.2	2.7	Malaysia	
Avocado	143±16.5	9.0±2.1	6.3	Thailand	
Orange	142±22.6	36.1±15.9	25.5	Australia	
Solo papaya	141±26.7	67.8±12.6	48.0	Malaysia	
Mango	139±21.5	19.7±9.1	14.2	Philippines	
Kiwi fruit	136±18.2	52.8±22.5	38.7	New Zealand	
Cempedak	126±19.1	6.2±0.9	4.83	Malaysia	Medium
Pomelo	104±34.7	36.0±7.5	34.7	Malaysia	
Lemon	93.3±9.8	49.6±6.8	53.2	Australia	
Pineapple	85.6±21.3	54.0±7.9	63.0	Malaysia	
Apple	78.9±2.7	2.1±0.9	2.7	China	
Foot long papaya	72.5±2.6	45.2±10.3	62.3	Malaysia	
Rambutan	71.5±7.6	50.2±6.5	70.2	Malaysia	
Rambutan king	70.6±8.2	49.5±8.8	70.0	Malaysia	
Banana	48.3±1.2	2.1±0.8	4.4	Philippines	
Coconut pulp	45.8±6.5	0.9±0.3	19.7	Malaysia	
Tomato	38.0±1.7	11.0±2.6	29.1	Malaysia	
Rockmelon	26.2±3.5	2.7±0.6	10.3	Australia	Low
Honeydew	19.6±0.8	3.9±0.4	19.9	Malaysia	
Watermelon	11.9±0.1	3.7±0.2	31.0	Malaysia	
Coconut water	11.5±2.2	0.7±0.3	6.1	Malaysia	

[a] Mean of three determinations±S.D. (standard deviation).

Shimadzu, Kyoto, Japan), using 2% (v/v) acetic acid/acetonitrile (95:5, v/v) as the mobile phase at a flow-rate of 0.8 ml/min at 40 °C oven temperature. Ten microlitres of the extract, prepared earlier, were injected into the HPLC. The measured AA content was expressed as mg AA/100 g edible portion.

3. Results and discussion

3.1. Total antioxidant capacity of fruits by $ABTS^{\bullet+}$ decolorization assay

This method measures the relative antioxidant ability of fruits to scavenge the radical $ABTS^{\bullet+}$ in the aqueous phase, as compared with a standard amount of AA. The $ABTS^{\bullet+}$, generated by potassium persulfate, is presented as an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain-breaking antioxidants (scavengers of lipid peroxyl radicals). Rice-Evans and co-workers (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995; Rice-Evans, Miller, & Paganga, 1996) and Roberta Re (Roberta et al., 1999) have demonstrated that $ABTS^{\bullet+}$ assay can be used to measure the antioxidant activity of a broad diversity of substances.

The antioxidant defence system of the body is composed of a mixture of antioxidants. Fruits are good sources of antioxidants that may be more effective and economical than supplements in protecting the body against oxidative damage under different conditions. Fruit antioxidants, which include AA, tocopherol, carotenoids and phenolics, vary greatly in their contents and profile among various fruits. As a result, the antioxidant capacity of one fruit differs considerably from another. By using the ABTS decolourisation assay, we have measured, for the first time, the antioxidant capacity of a variety of fruits available in the Singapore markets.

As shown in Table 1, total antioxidant capacity, in AEAC of fruits tested, was found to vary over 300-fold from that at the lowest value. Based on these values, the activity of the fruit extract to scavenge free radicals is classified into four categories. The fruit with AEAC of over 600 mg AA_{eq}/100 g is classified as containing extremely high antioxidant capacity. On the other hand, fruits with AEAC from 200 to 600 mg AA_{eq}/100 g, 70 to 200 AA_{eq}/100 g and less than 70 mg AA_{eq}/100 g are classified as containing high, medium and low antioxidant capacities respectively. On the basis of the wet weight of fruit (edible portion), ciku shows the highest antioxidant capacity, followed by strawberry, plum, star fruit, guava, seedless grape, salak, mangosteen, avocado, orange, solo papaya, mango, kiwi fruit, cempedak, pomelo, lemon, pineapple, apple, foot long papaya, rambutan, rambutan king, banana, coconut pulp, tomato, rockmelon, honeydew, watermelon and coconut water. The total antioxidant capacity of ciku was found to be extremely high (compared with the other fruits examined). We are currently looking into the compounds in ciku contributing such a high antioxidant capacity. Several tropical fruits from Southeast Asia, such as guava, star fruit and salak, are very good sources of antioxidants. Other fruits, such as avocado, papaya, mangosteen, pomelo, pineapple, cempedak, lemon and rambutan, also contain considerable amount of antioxidants. Wang et al. (1996) had previously measured the total antioxidant activity of 12 fruits using automated oxygen radical absorbance capacity (ORAC) assay. Of the eight fruits that were mutually tested, the ranking orders of total antioxidant capacity were as follows: strawberry > plum > orange > kiwi > banana > apple > tomato > honeydew melon. The ranking order was found to be the same here, except that apple was found to have a higher AEAC than banana. The difference could be related to seasonal variations or variety.

3.2. Content of AA in fruits

AA is one of the most effective antioxidants in fruits and vegetables. People with high intakes of dietary AA acid or citrus fruits have repeatedly been associated with lowered risk of developing cancer. Because of different criteria of adequacy and different interpretations of experimental evidence, the recommended dietary allowance (RDA) for AA varies between 30 and 80 mg/day. The RDA of AA for adult men and women is 30, 60 and 80 mg/day in Singapore, United States and Netherlands, respectively (Bender, 1993; Department of Nutrition, 1999). However, many scientific reports indicate that an increased intake of AA is associated with a reduced risk of chronic diseases such as cancer, cardiovascular disease, and cataract. One possible mode of action of AA is through its antioxidant capability. It is likely that the current RDA for AA to prevent deficiency disease, is not sufficient for optimum protection against the chronic diseases mentioned above, therefore, Carr and Frei (1999) suggested a new RDA of 120 mg AA/day.

RP-HPLC was used to measure the content of AA due to its high sensitivity, high reproducibility, easy operation and short analysis time. Table 1 shows AA contents of the 27 selected fruits analysed. The AA contribution to scavenge $ABTS^{\bullet+}$ varies extensively up to 200 times the lowest value. AA accounts for a high percentage contribution to $ABTS^{\bullet+}$ scavenging activity in rambutan and rambutan king (70%), pineapple (63%), guava (48.3%), lemon (53.2%) and solo papaya (48%), foot long papaya (62.3%), kiwi fruit (38.7%), pomelo (34.7%), watermelon (31.7%), tomato (29.1%) and orange (25.5%). Wang et al. (1996) suggested that

the contribution of AA to ORAC activity of a fruit was usually less than 15%, except for kiwi fruit and honeydew melon. However, in this study, it was found that almost half of the fruits tested showed high contributions of AA to their total antioxidant capacity. Since Wang et al. (1996) did not test the antioxidant capacity of fruits having a high AA contribution in this study, the real L-ascorbic acid contribution to ORAC activity among these fruits needs to be confirmed. The contribution of AA to AEAC among other fruits was low, especially for ciku, plum, star fruit, salak, seedless grape, mangosteen, apple and cempedak. It seems fruits with a high AEAC value are more likely to have a low percentage contribution from AA to AEAC.

As discussed above, this set of data shows that the contribution of AA to the AEAC of fruits can differ extensively from one fruit to another. Therefore, contributions of other compounds to AEAC of these fruits must not be neglected. Among the most important compounds that contribute to the high AEAC value of these fruits are the polyphenolic compounds. Future studies are underway to measure the total phenolic content in these fruit extracts.

3.3. Scavenging DPPH free radicals

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Baumann, Wurn, & Bruchlausen 1979). AEAC of some fruits by DPPH and ABTS assay and their AA content are shown in Fig. 1.

Most fruits tested with a high AEAC in the ABTS model also have a high AEAC in the DPPH model. This high correlation may partly result from a similar mechanism and, also, both the antioxidants are soluble in aqueous/ethanol systems. Fig. 2 shows that a good correlation exits between two assay methods: $AEAC_{DPPH} = 0.9203 AEAC_{ABTS}$ and $R^2 = 0.9045$ (not including ciku, $R^2 = 0.9984$ if ciku was included). This

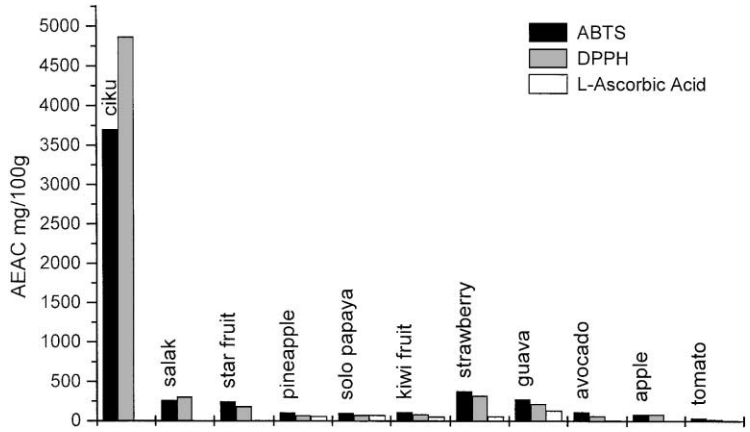

Fig. 1. AEAC of 12 fruits by ABTS- and DPPH-scavenging assay.

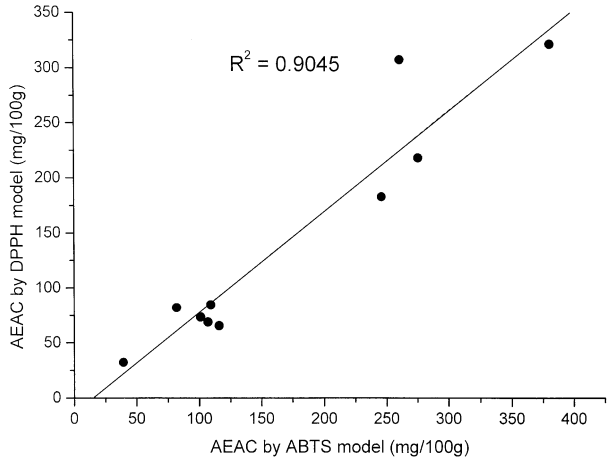

Fig. 2. Comparison of AEAC of fruits in ABTS and DPPH models.

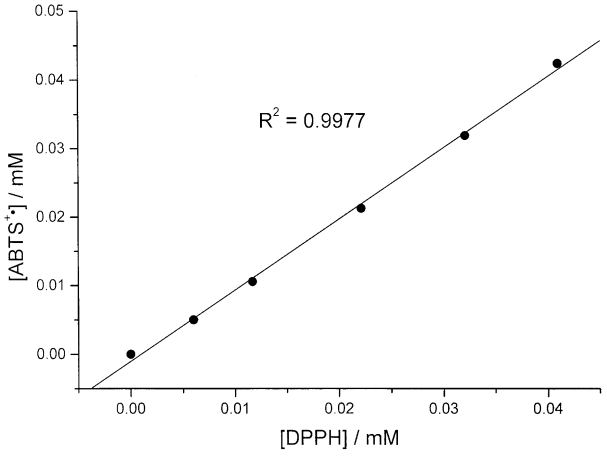

Fig. 3. Correlation of [ABTS•$^+$] and [DPPH•] reduced by AA [ABTS•$^+$] = 1.0088[DPPH•].

shows that the two methods are compatible when used to assess free radical-scavenging activity.

3.4. Correlation of ABTS assay and DPPH assay

Fig. 3 shows a linear correlation between reduced ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ after different concentrations of AA were added. Similar results were obtained when trolox, hydroquinone and pyrogallol were added, and their linear correlation could be described as $[ABTS^{\bullet+}] = 0.963[DPPH^{\bullet}]$ ($R^2 = 0.9993$), $[ABTS^{\bullet+}] = 0.9987\ [DPPH]$ ($R^2 = 0.9989$) and $[ABTS^{\bullet+}] = 1.688\ [DPPH^{\bullet}]$ ($R^2 = 0.9989$) respectively.

In this study, it is found that 1 mol of AA reacts with approximately 2 mol of ABTS radicals or DPPH radicals. The result is consistent with a previous report that 1 mol of AA reduces 2 mol of ABTS radicals (Cano et al., 1998). Similarly, 2 mols of ABTS or DPPH radicals are scavenged by 1 mol of trolox or hydroquinone. The stoichiometry of the reaction between pyrogallol and ABTS or DPPH radical is found to be 1:7 and 1:4, respectively.

For the antioxidants tested, the stoichiometry of reactions between antioxidant and ABTS$^{\cdot+}$ or DPPH$^{\cdot}$ is very close. However, when pyrogallol was used, the stoichiometry differs. Lissi, Modak, Torres, Escobar, and Urzua (1999) determined that total antioxidant potential of resinous exudates from Heliotropium species showed significant differences by employing ABTS and DPPH scavenging assay. Wang et al. (1998) found that some compounds, which have ABTS$^{\bullet+}$ scavenging activity may not show DPPH$^{\bullet}$ scavenging activity. Therefore, a linear correlation between two models may not be obvious among some other biological samples that may contain a variety of antioxidants.

However, the results obtained in this study, from 11 fruits show a good correlation between the two models. Among the eleven fruits tested in both models, six fruits have a relative AEAC difference of less than 20%, only two fruits more than 30% and none less than 50%. Since no significant difference of AEAC was observed among fruits by employing the two models, any one of the two models may be a useful tool for evaluating the total antioxidant capacity of fruits. Further research on the stoichiometry of reactions between pure fruit antioxidants and ABTS$^{\bullet+}$ or DPPH is currently under investigation.

4. Conclusion

The AEAC of 27 fruits in the ABTS model and the contributions of AA to the AEAC of fruits are measured. Among the 27 fruits tested, ciku had the highest AEAC value, followed by strawberry, plum, star fruit, guava, seedless grape, salak, mangosteen, avocado, orange, solo papaya, mango, kiwi fruit, cempedak, pomelo, lemon, pineapple, apple, foot long papaya, rambutan, rambutan king, banana, coconut pulp, tomato, rockmelon, honeydew, watermelon and coconut water. The contribution of AA to AEAC varies markedly from one fruit to another. A good correlation of AEAC, between ABTS model and DPPH model, was observed among 11 fruits. Both models are recommended as useful tools for evaluating the total antioxidant capacity of fruits.

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