

ANTIOXIDANT PROPERTIES OF GUAVA FRUIT: COMPARISON WITH SOME LOCAL FRUITS

LIM YAU YAN^a
LIM THENG TENG
TEE JING JHI
Monash University Malaysia

ABSTRACT

Two varieties of guava fruit were analyzed for total phenol contents, ascorbic acid contents and antioxidant activities. The antioxidant activities were assessed based on the ability of the fruit extracts in 50% ethanol to scavenge DPPH, reduce Fe(III) to Fe(II) and to bind to Fe(II) ion. The results were compared to several other local fruits as well as orange. It was found that the guava fruit contains relatively high amounts of antioxidant. It also has high primary, but low secondary antioxidant potential. Storage at 4°C has the effect of increasing ascorbic acid content, and the non-peeled fruit has higher total phenol and ascorbic acid contents compared to the peeled fruit. The length and width of the seedy guava were also monitored over a period of 17 weeks to define specific stages of fruit ripening.

Key words: Guava, antioxidant activities, total phenol and ascorbic acid contents, antioxidant assays.

INTRODUCTION

Guava (*Psidium guajava* L.), also known locally as jambu batu, is grown commercially and in many home gardens in Malaysia. The tree is very hardy and can grow to about 7-8 metres high with characteristic smooth, pale mottled bark that peels off in thin flakes. The fruits vary in size, shape and flavour depending on the variety. The better varieties are sweet while others may be astringent. On average, the fruit contains 74–87% moisture, 13–26% dry matter, 0.5–1% ash, 0.4–0.7% fat and 0.8–1.5% protein (Chin and Yong, 1980). It is rich in ascorbic acid (vitamin C), at levels far higher than most imported and local fruits. The fruit, in particular the pink flesh cultivar, has a fair amount of vitamin A (beta-carotene). Some vitamin B such as thiamin (B1), riboflavin (B2), niacin and pantothenic acid are also found in the fruit. In addition, it also contains a fair amount of phosphorous, calcium, iron, potassium and sodium (Lim and Khoo, 1990).

Guava, as in many other fruits and vegetables, is also rich in antioxidants that help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart disease, inflammation and brain dysfunction. In addition, antioxidants were reported to retard ageing (Feskanich et al., 2000; Gordon, 1996; Halliwell, 1996) besides preventing or delaying oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species. These include reactive free radicals such as superoxide, hydroxyl, peroxy, alkoxy, and non radicals such as hydrogen peroxide and hypochlorous acid. They scavenge radicals

E-mail: ^aLim.yau.yan@artsci.monash.edu.my.

by inhibiting initiation and breaking of chain reaction, suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Shi et al., 2001). Among the most abundant antioxidants in fruits are polyphenols and ascorbic acid. The polyphenols, most of which are flavonoids, are present mainly in ester and glycoside forms (Fleuriet and Macheix, 2003). In the case of guava, free elagic acid and glycosides of myricetin and apigenin are found to be present (Misra and Seshadri, 1968; Koo and Mohamed, 2001).

The purpose of this paper is to investigate the antioxidant content and activities in two varieties of locally grown guava. For comparison, similar analyses were carried out on banana, dragon fruit, star fruit, sugar apple, water apple and orange. Orange is the only imported fruit in the list.

MATERIALS AND METHODS

Samples

The two varieties of guava were a Kampuchean cultivar (Vietnam GU8) and a seedless variety from the wet market. The fruit of the Kampuchean variety is oval and seedy with an average length of 8–10 cm and width of 7–9 cm. The weight is 300–550 g. The fruit of the seedless variety is smaller, apple-shaped with length of 5.0–6.6 cm, width of 7.0–8.8 cm and weight of 145–260 g. The other fruits for comparison are orange (Valencia, a seedless variety from Australia), banana (*Musa sapientum*, mas cultivar), dragon fruit (*Hylocereus undatus*, grey pulp variety), star fruit (*Averrhoa carambola*), sugar apple (*Annona squamosa*, reddish-brown and green varieties) and water apple (*Syzygium aqueum*).

Chemicals and Instruments

The following chemicals were used for the following analyses. *Total phenol determination*: Folin-Ciocalteu's phenol reagent (Fluka, 2N), gallic acid (Fluka, 98.0%), anhydrous sodium carbonate (Fluka, 99.0%). *Ascorbic acid determination*: potassium iodide (Hamburg, 99%), arsenic trioxide (Fluka, 99.5%), iodine (Fisher, 99.9%), sodium hydrogen carbonate (Fisher, 99.8%), sodium hydroxide (Hamburg, 98%), sulphuric and hydrochloric acids (Fisher). *1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity*: 1,1-diphenyl-2-picrylhydrazyl (Sigma, 90%), L(+)-ascorbic acid (Merck, 99.7%), methanol (Mallinckrodt, 100%). *Ferric reducing power*: iron(III) chloride 6-hydrate (Fisher, 100%), potassium ferricyanide (Unilab, 99%), trichloroacetic acid (Fisher, 99.8%), potassium dihydrogen phosphate (Bendosen, 99.5%), dipotassium hydrogen phosphate (Merck, 99%). *Ferrous ion chelating activity*: Iron(II) sulphate 7-hydrate (Hamburg, 99.5%), ferrozine iron reagent (Acros Organics, 98+%).

Water used was purified by Elga deionizer and ethanol for extraction was from HmbG Chemicals (95 V%).

Optical absorbance was measured with an Anthelie Advanced 5 Secoman or GBC Cintra 5 UV-Vis spectrophotometer. Acidity (pH) was measured with a Hanna pH211

meter. High pressure liquid chromatography (HPLC) determination of ascorbic acid was measured with a Waters Associate (2487) instrument using a C-18 column.

Sample Preparation

Depending on the type of fruits, 10 to 30 g of the edible portions were either blended for approximately one minute to a paste, or crushed and homogenized using a pestle and mortar. Guava, star fruit and water apple were homogenized without peeling the skin while the other four fruits were crushed after the skin was removed. The homogenized sample was transferred into a 100-mL volumetric flask and 50% ethanol was added up to the mark. The mixture was shaken manually or with a vibrator for 10–15 min and filtered under suction. In situations where the filtrate appeared to be very cloudy, the filtrate was centrifuged to obtain a clear supernatant liquid, which was subsequently used for the various analyses. All tests were performed in triplicates within a week and the extracts were stored at -20°C until used.

To study the effect of storage on total phenol content (TPC) and ascorbic acid content (AAC), the freshly cut fruit was sealed in a plastic bag and stored at 4°C for 4–5 days after which it was treated with 50% ethanol in the same way as before.

Chemical Analysis

Antioxidant Contents

a. Ascorbic acid content (AAC)

The AAC was determined by the iodine titration method (Suntornsuk et al., 2002) or the reversed phase-HPLC method: Waters C-18 column (3.9x150 mm, 5 μm particle size), mobile phase 5% acetic acid, flow-rate 0.5 mL/min and 254 nm detection wavelength. Both methods gave similar results to within 5%.

b. Total phenol content (TPC)

TPC was determined using the Folin-Ciocalteu's reagent (Singleton and Rossi, 1965). Samples (triplicate) of 0.3 mL were introduced into test tubes followed by 1.5 mL of Folin-Ciocalteu's reagent (diluted 10 times with water) and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min. Absorption at 765 nm was measured. If the sample absorbance exceeded 1, the sample was appropriately diluted to give a reading of less than 1. Total phenol contents were expressed in gallic acid equivalents (mg per 100 gram fresh fruit). The gallic acid standard line has the equation $y = 0.0111x - 0.0148$ ($R^2 = 0.9998$), where y is absorbance at 765 nm and x is concentration of gallic acid in mg/L.

Since ascorbic acid also contributes to the formation of the blue molybdenum-tungsten complex, it is important to correct for the absorbance originating from it. An ascorbic acid calibration curve was therefore prepared. The TPCs reported in this paper have been

corrected for ascorbic acid. The calibration equation for ascorbic acid was determined to be $y = 0.0072x - 0.0176$ ($R^2 = 0.9900$), where x is the concentration of ascorbic acid in mg/L.

Antioxidant Activities

a. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The free radical scavenging activity of the fruit extracts was measured by the decrease in absorbance of methanolic DPPH solution at 517 nm in the presence of the extract (Krings and Berger, 2001). Different dilutions of the extract amounting to 1.0 mL were added to 2.0 mL of DPPH. The initial concentration of DPPH was 0.1 mM and the reading was taken after allowing the solution to stand for 30 min. The antioxidant activity was expressed as:

$$\% \text{ disappearance} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100\% \quad \text{where A is absorbance.}$$

IC_{50} , the amount of sample extracted into 1 mL solution necessary to decrease by 50% the initial DPPH concentration was derived from the % disappearance versus concentration plot (at this point concentration means mg of fruit extracted into 1.0 mL solution). The results were also expressed as ascorbic acid equivalent antioxidant capacity (AEAC) (Leong and Shui, 2002) using either one of the following equations:

$$\text{AEAC (mg AA/100g)} = (A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}} - \text{AAA}) \times \text{conc. AA (mg/mL)} \times \text{vol extract (mL)} \times (100/\text{g sample})$$

where AA is ascorbic acid, and AAA is absorbance of AA, or

$$\text{AEAC} = (IC_{50(\text{AA})}/IC_{50(\text{sample})}) \times 10^5$$

$IC_{50(\text{AA})}$ value used was determined to be $(3.72 \pm 0.24) \times 10^{-3}$ mg/mL based on 18 repeated measurements.

b. Ferric reducing antioxidant power (FRAP)

The ferric reducing power of the fruit extracts was determined by using the potassium ferricyanide–ferric chloride method (Oyaizu, 1986). Different dilutions of extracts amounting to 1 mL were added to 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 min, after which 2.5 mL trichloroacetic acid (10%) was added. An aliquot of the mixture (2.5 mL) was taken and mixed with 2.5 mL water and 0.5 mL 1% $FeCl_3$. The absorbance at 700 nm was measured after allowing the solution to stand for 30 min. FRAP of a sample is estimated in terms of gallic acid equivalents (GAE) in mg GAE/g of fresh sample.

c. Ferrous ion chelating activity

The ferrous ion chelating (FIC) activity was measured by the decrease in absorbance at 562 nm of the iron(II)-ferrozine complex (Carter, 1971; Dinis et al., 1994). One milliliter of 0.125 mM FeSO₄, was added to 1.0 mL sample (with different dilutions), followed by 1.0 mL of 0.3125 mM ferrozine. The mixture was allowed to equilibrate for 10 min before measuring the absorbance. Sample solutions with appropriate dilutions were used as blanks as the fruit extracts may also absorb at this wavelength. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and ferrozine only) using the formula:

$$\text{Chelating effect \%} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

RESULTS AND DISCUSSION

Guava Growth

The change in dimension (length and width) of the fruit was measured over a period of 17 weeks as a means of monitoring its growth pattern, beginning from the formation of fruit bud. A total of 10 fruits (Kampuchea variety) from two trees were measured. The results for two of the fruits (labeled 1 and 2) are shown in Figure 1. Both length and width were

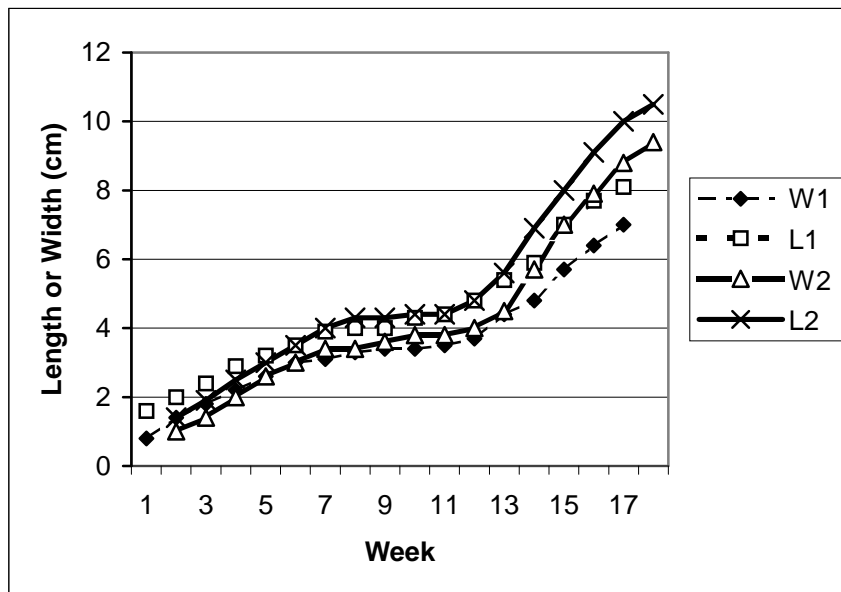


Figure 1. Changes in length(L) and width(W) of two guava fruits during their growth

observed to increase in a similar manner. From week 1 to week 6, the increase was fast. Between week 6 and 11, the increase was slower. From week 11 onwards, both length and width increased sharply until week 17 when the fruits turned greenish yellow and were considered as ripe. The other eight fruits studied also showed similar pattern of growth, although the exact week when the fruit underwent a rapid expansion may differ by approximately one week. The pattern of increase was similar to the changes in weight and diameter during guava fruit development reported by Yusof and Mohamed (1987). Based on these results, guava fruits used here were considered to be matured but unripe between week 11–14 and ripe after week 17. This will be used as the basis for classifying ripe and unripe guava fruits in the next study.

Factors Affecting Antioxidant Contents (TPC and AAC) of Guava Fruit

Effects of Ripeness

The TPC and AAC of the ripe and unripe guava fruits (Kampuchean variety) are summarised in Table 1. As the fruit ripens, TPC decreases but AAC increases. According to Taylor (1993), the decrease in polyphenol content of guava fruit causes a loss in astringency during ripening of the fruit. The increase in AAC as the fruit matures is due to the breakdown of starch to glucose which is used in the biosynthesis of ascorbic acid. The DPPH scavenging activity of the unripe guava as measured by the AEAC value is primarily due to its higher TPC relative to AAC. Decrease of AEAC during ripening suggests that the antioxidant activities of guava fruit declined during fruit ripening.

Table 1. Total Phenolic Content (TPC) and Ascorbic Acid Content (AAC) of Ripe (> 17 weeks old) and Unripe (11–14 weeks old) Guava Fruits (Kampuchea Variety)

	TPC (mg GAE/100g)	AAC (mg/100g)	AEAC (mg AA/100g)	% contribution of AEAC by AA
Unripe (n=4)	270 ± 100	32 ± 28	310 ± 170	10
Ripe (n=8)	138 ± 30	144 ± 60	218 ± 79	66

Effects of Storage

Increase in AAC was observed during storage of ripe and unripe guava (Table 2). A similar increase was also observed in dragon fruit and water apple. The trend for TPC appeared to increase in unripe samples but decrease in ripe samples. However due to the small changes observed and the limited number of samples, a definite conclusion on TPC trend during storage cannot be reached and more studies are required.

The increase in AAC after storage was due to several reasons. After harvest, ascorbic acid is still being synthesized in the living fruit tissues. It is known that fruit ripening continues after harvest and this process leads to significant changes in the contents of the antioxidants. Tudela et al. (2002) observed similar ascorbic acid increase in freshly cut

Table 2. Effects of Storage on Total Phenolic Content (TPC) and Ascorbic Acid Content (AAC) of Guava Fruits

Sample	TPC (mg GAE/100g)		AAC (mg/100g)	
	Before Storage	After Storage	Before Storage	After Storage
1(Unripe)	235 ± 4	253 ± 4	29.8 ± 0.1	47.2 ± 0.3
2(Unripe)	220 ± 10	239 ± 3	63.6 ± 0.7	92.4 ± 0.7
3(Ripe)	169 ± 7	161 ± 5	92.2 ± 0.5	96.7 ± 0.8
4(Ripe)	155 ± 15	128 ± 3	192 ± 0.5	208 ± 1

potatoes under the same conditions. It was explained that when stored in low temperature and/or wounded, the respiration rates of freshly cut potatoes increased resulting in the accumulation of glucose which is required for ascorbic acid biosynthesis.

Effects of Skin Peeling

Table 3 shows that the antioxidant contents are always higher in guava fruit with intact skin compared to fruit with the skin peeled off. This result is consistent with the results reported earlier for guava (Bashir and Abu-Goukh, 2003; Jimenez-Escrig et al., 2001) and for other fruits such as pear (Sanchez et al., 2003). It suggests that the skin of guava contains relatively high amounts of antioxidants such as phenols and ascorbic acids.

Table 3. Antioxidant Contents of Peeled and Non-peeled Guava Fruits

Sample	TPC (mg GAE/100 g)		AAC (mg/100 g)	
	Non-peeled	Peeled	Non-peeled	Peeled
1	169	129	92	82
2	140	119	193	177
3	161	152	217	180
4	227	176	109	103
5	134	118	95	69

Antioxidant Properties of Guava Compared with Other Tropical Fruits

The TPC, AAC and antioxidant activity of guava are compared with a few local fruits in Table 4. Both varieties of guava fruit contain relatively high quantity of antioxidants as

shown by the high amount of TPC and AAC recorded. In the case of AAC, guava can contain as much as ten times that of other fruits such as banana, dragon fruit, starfruit and sugar apple. In the case of TPC, only starfruit and sugar apple have comparable quantity of the antioxidant. On the whole, the results suggest that guava is a healthy fruit to consume from the antioxidant viewpoint, and is better than the temperate fruit orange.

Table 4. The Antioxidant Content and Activities of Extracts from Tropical Fruits
(Orange is included for comparison)

Fruits	TPC (mg GAE/100g)	AAC (mg/100g)	IC ₅₀ (mg/mL)	AEAC (mg AA/100g)	FRAP (mg GAE/g)
Guava (seeded) ⁸	138 ± 31 _a	144 ± 60	1.71 ± 0.61 _f	218 ± 79	2.09 ± 0.18
Guava(seedless) ⁶	179 ± 44 _a	132 ± 46	2.11±0.63 _{f,g}	176 ± 54	1.65 ± 0.06
Banana (mas) ⁶	51 ± 7 _c	4.9 ± 0.6	13.4± 2.5 _i	27.8 ± 5.5	0.18 ± 0.06
Dragon fruit ³	21 ± 6 _d	8.0 ± 1.6	27.5 ± 3.9 _j	13.5 ± 2.1	0.07 ± 0.01
Star fruit ⁷	131 ± 54 _a	5.2 ± 1.9	3.8 ± 2.1 _{g,h}	98 ± 55	0.83 ± 0.02
Sugar apple(brown) ³	175 ± 36 _a	21.3 ± 2.1	3.9 ± 0.4 _{g,h}	82.1 ± 6.9	0.62 ± 0.10
Sugar apple(green) ³	165 ± 18 _a	6.8 ± 0.8	4.6 ± 0.8 _h	71.4 ± 11.8	0.58 ± 0.04
Water apple ⁴	35 ± 4 _e	4.1 ± 2.1	12.0 ± 3.8 _i	31 ± 10	0.30 ± 0.02
Orange ⁵	75 ± 10 _b	67 ± 9	5.4 ± 1.3 _h	70 ± 17	0.61 ± 0.05

The superscript numerals in the fruits column are the number of samples studied.

Values having the same superscript within the same column (TPC, IC₅₀) are not significantly different (p=0.05).

For antioxidant activities, this can be primary or secondary. Primary antioxidant properties are generally measured by DPPH assay (expressed as AEAC and IC₅₀) and FRAP. The DPPH assay measures the ability of the fruit extract to donate hydrogen to the DPPH radical resulting in bleaching of the DPPH solution. The greater the bleaching action, the higher the antioxidant activity (AEAC value), and this is reflected in a lower IC₅₀ value. FRAP on the other hand measures the ability of the extract to donate electron to Fe(III). The higher the FRAP value, the greater is the antioxidant activity. It is interesting to note that for the range of fruits considered here, there is a linear correlation between 1/IC₅₀ and FRAP (Figure 2; $y = 3.6408x - 0.1098$, $R^2 = 0.9804$). It suggests that the lower the IC₅₀ value, the higher the ferric reducing antioxidant power.

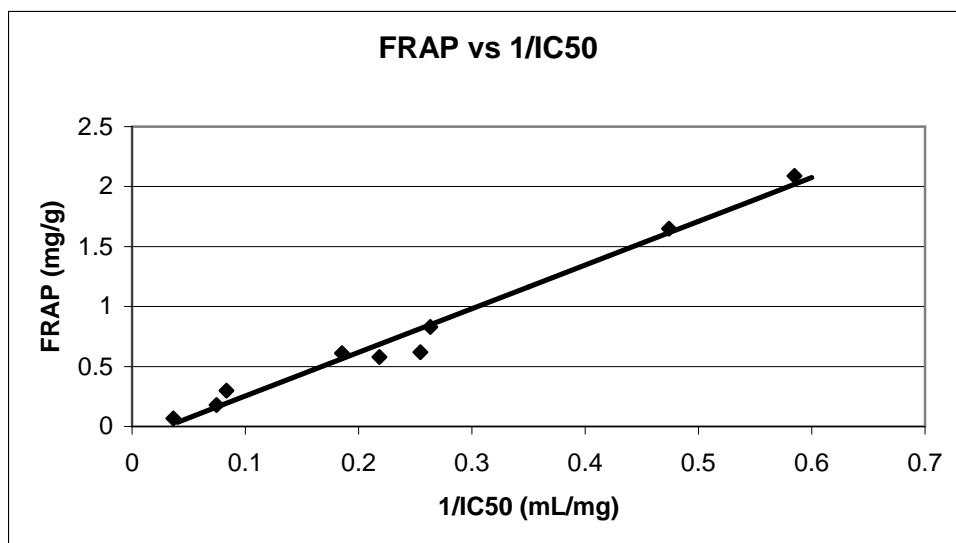


Figure 2. Correlation between FRAP and $1/IC_{50}$ of different fruits

In terms of the antioxidant activities of the fruits investigated, the two varieties of guava have the highest AEAC and FRAP values suggesting that guava has very high primary antioxidant activity compared with the others. This is also confirmed by the relatively low IC_{50} values recorded in guava fruits. The AEAC and FRAP values of guava fruit are especially distinctive as they can be at least a few times that of the other fruits.

Secondary antioxidant property is generally measured by the FIC activity which measures the chelating ability of the extract. The assay measures how effective the chemical compounds in the extract can compete with ferrozine for ferrous ion. The iron-ferrozine complex has maximum absorbance at 562 nm and a large decrease in absorbance indicates strong chelating power. By forming a stable iron(II) chelate, an extract with high chelating power reduces the free ferrous ion concentration thus decreasing the extent of the Fenton reaction which is implicated in many diseases (Halliwell and Gutteridge, 1990). The plot of percentage chelating power versus mg fruit extract/mL is presented in Figures 3a and 3b. Compared to other fruits, guava has very low chelating power. Banana has the highest, while starfruit and water apple are moderate. Other fruits such as sugar apple, dragon fruit and orange have relatively low chelating power.

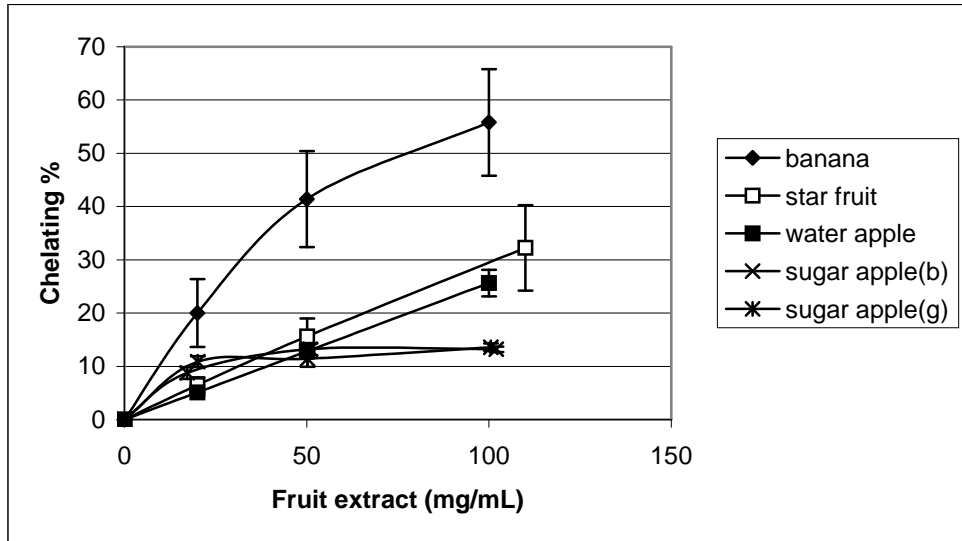


Figure 3a. Chelating power of fruit extracts of banana, star fruit, water apple and sugar apple
Note: Sugar apple (b =brown; g = green)

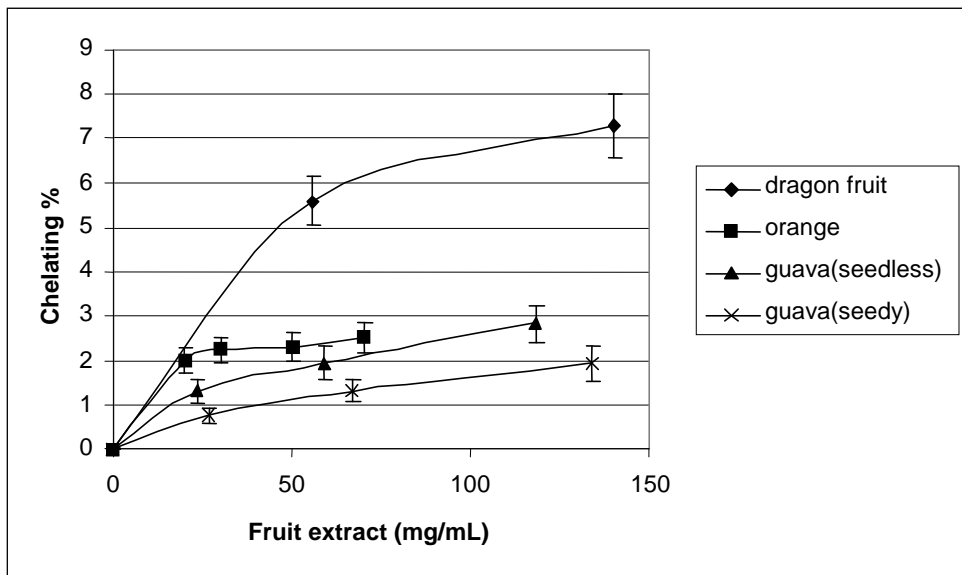


Figure 3b. Chelating power of fruit extracts of dragon fruit, orange and two varieties of guava

An interesting observation arising from the results is that guava has a potent primary antioxidant property (measured by IC_{50} and FRAP) but its function as a secondary or preventive antioxidant (measured by chelating power) is weak. Primary antioxidants

scavenge radicals to inhibit chain initiation and break chain propagation. Secondary antioxidants suppress the formation of radicals and protect against oxidative damage. On the other hand, though banana does not have high TPC and AAC values, it is a potent secondary antioxidant which contains active components that bind to metal ions strongly.

CONCLUSION

The present study shows that a local fruit such as guava has a high quantity of antioxidant such as phenols and ascorbic acid. Guava also has high primary antioxidant potential when compared to other local fruits and an imported fruit such as orange. Banana though weaker than orange as a primary antioxidant is, however, a powerful secondary antioxidant.

ACKNOWLEDGEMENT

The authors thank Monash University Malaysia for financial support (Grant number ES-11-03), and Y. H. Kong, S. P. Pang and S. L. Goh for assistance in carrying out parts of the experimental work.

REFERENCES

- Bashir, H. A., & Abu-Goukh, A. A. (2003). Compositional changes during guava fruit ripening. *Food Chemistry*, 80, 557–563.
- Carter, P. (1971). Spectrophotometric determination of serum iron at the submicrogram level with a new reagent-ferrozine. *Anal. Biochem.*, 40, 450–458.
- Chin, H. F., & Yong, H. S. (1980). *Malaysian fruits in colour*. Kuala Lumpur: Tropical Press, Malaysia.
- Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, 315, 161–169.
- Feskanich, D., Ziegler, R. G., Michaud, D. S., Giovannucci, E. L., Speizer, F. E., Willett, W. C., & Colditz, G. A. (2000). Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *Journal of the National Cancer Institute*, 92, 1812–1823.
- Fleuriet, A., & Macheix, J. J. (2003). Phenolic acids in fruits and vegetables. In C. A. Rice-Evans & L. Packer, *Flavonoids in Health and Disease*. New York: Marcel Dekker Inc.
- Gordon, M. H. (1996). Dietary antioxidants in disease prevention. *Natural Product Reports*, 265–273.
- Halliwell, B. (1996). Antioxidants in human health and disease. *Ann. Rev. Nutr.*, 16, 33–50.
- Halliwell, B., & Gutteridge, J. M. C. (1990). Role of free radicals and catalytic metal ions in human disease: An overview. *Methods in Enzymology*, 186, 1–85.
- Jimenez-Escrig, A., Rincon, M., Pulido, R., & Saura-Calixto, F. (2001). Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *J. Agri. Food Chem.*, 49, 5489–5493.
- Koo, M. H., & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. *J. Agri. Food Chem.*, 49, 3106–3112.
- Krings, U., & Berger, R. G. (2001). Antioxidant activity of some roasted foods. *Food Chemistry*, 72, 223–229.

- Leong, L. P., & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, 76, 69–75.
- Lim, T. K., & Khoo, K. C. (1990). *Guava in Malaysia: Production, pests and diseases*. Kuala Lumpur: Tropical Press, Malaysia.
- Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *Japan J. Nutrition*, 44, 307–315.
- Sanchez, A. C. G., Gil-Izquierdo, A., & Gil, M. I. (2003). Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *Journal of the Science of Food and Agriculture*, 83, 995–1003.
- Shi, H. L., Noguchi, N., & Niki, E. (2001). Introducing natural antioxidants. In J. Pokorny, N. Yanishlieva & M.H. Gordon (Eds.), *Antioxidants in Food: Practical applications*. Cambridge: Woodhead Publishing Ltd and CRC Press.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Amer. J. Enol. Vitic.*, 16, 144–158.
- Suntornsuk, L., Kritsanapun, W., Nilkamhank, S., & Paochom, A. (2002). Quantitation of vitamin C content in herbal juice using direct titration. *Journal of Pharmaceutical and Biochemical Analysis*, 28, 849–855.
- Taylor, J. E. (1993). Exotics. In G. B. Seymour, J. E. Taylor, and G. A. Tucker (Eds.), *Biochemistry of fruit ripening* (pp. 163–165). Boca Raton: Chapman and Hall.
- Tudela, J. A., Espin, J. C., & Gil, M. I. (2002). Vitamin C retention in fresh-cut potatoes. *Postharvest Biology and Technology*, 26, 75–84.
- Yusof, S., & Mohamed, S. (1987). Physico-chemical changes in Guava (*Psidium guajava* L.) during development and maturation. *J. Sci. Food Agric.*, 38, 31–39.