

Evaluation of antioxidant properties in fresh and pickled papaya

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Abstract : Preservative fruits have gained popularity in recent years as part of food consumption, but their benefits towards human health are not known. This study compared total phenolic (TPC), total flavonoid (TFC), β -carotene, lycopene, ascorbic acid (AA) contents and antioxidant properties between fresh and pickled papaya. The results indicated that mean TPC (mg gallic acid equivalent/100 g dry samples), TFC (mg rutin equivalent/100 g dry samples), β -carotene ($\mu\text{g}/100$ g edible portions), lycopene ($\mu\text{g}/100$ g edible portions) and AA content (mg/100 g edible portions) were higher in fresh papaya (141.66 ± 11.71 ; 57.80 ± 2.11 ; 793.83 ± 5.47 ; 779.69 ± 5.55 ; 70.37 ± 0.65) as compared to pickled form. Antioxidant activity (%) measured by DPPH and β -Carotene-Linoleate bleaching method was higher in fresh papaya (56.83 ± 4.68 ; 77.56 ± 1.40). Total phenolic, total flavonoid, ascorbic acid, beta carotene and lycopene were strongly correlated with antioxidant activity and scavenging activity ($0.905 \leq r \leq 1.00$) indicating that were important contributors to antioxidant properties in papaya extracts. The pickling process of papaya caused a significant decrease in their antioxidant component and activity.

Keywords: Pickled papaya, antioxidant activity, total phenolic content, total flavonoid, carotenoid

Introduction

Free radicals are naturally produced in the body through normal metabolism of carbohydrates, amino acids and fats. Other factors known to increase free radicals in our body include chronic diseases, smoking, environmental poisons, alcohol and ionizing radiation. Overproduction of free radicals can result in oxidative stress, a deleterious process that damages the cell structure. Antioxidants are chemical compounds that can bind to free oxygen radicals preventing these radicals from damaging healthy cells where as pro-antioxidant act indirectly either by modulation of direct agents or by regulation of the biosynthesis of antioxidant proteins (Halliwell, 2011).

Plants (fruits, vegetables, medicinal herbs, etc.) may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, and some other endogenous metabolites, which are rich in antioxidant activity. Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anti-carcinogenic, antibacterial, or antiviral activities to a

greater or lesser extent (Pe' rez-mature *et al.*, 2009).

Papaya is originated in Southern Mexico and Costa Rica; subsequently it was introduced as a plantation crop in Australia, Philippines, Hawaii, South Afrika, Sri Lanka, India and in all tropical regions (Krishna *et al.*, 2008). Papaya when consumed regularly will ensure a good supply of vitamin A and C which is essential for good health especially for eyesight. A study by Krishna *et al.* (2008) indicated that the consumption of guava and papaya fruits reduced oxidative stress and altered lipid profiles. Thus, it could reduce the risk of diseases caused by free radical activities such as cancer, and cardiovascular disease. Pickled papaya is widely sold and consumed among Malaysians. The fermentation process of pickles is very simple and there is no need for specific equipment. Salt, sugar, vinegar and water are the essential or basic ingredients in making pickles. Pickles also contain acetic acid which acts as preservative in order to keep the product quality for a longer time.

To our knowledge, no large-scale systematical surveys of the antioxidant activity and phenolic compounds of tropical fruits and their pickled products have been researched. Findings from this study will increase the scientific data available and

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such data will be beneficial in constructing nutrition database in the future. Thus, this study was initiated to compare total phenolic, total flavonoid, ascorbic acid, beta carotene, lycopene contents and antioxidant activity in papaya (*Carica papaya*) between fresh and pickled form.

Materials and Methods

Food sampling

Convenience sampling method was used to obtain the samples. One kilogram of fresh papaya (*Carica papaya*) was purchased from Pasar Tani Masjid Jamek, Serdang while five hundred grams of pickled papaya was purchased from Pulau Pinang. All the samples were immediately transported to nutritional laboratory, Universiti Putra Malaysia for analysis.

Samples preparation and extraction

Five hundred grams of papaya samples were cleaned and washed with tap water. They were chopped into small pieces and homogenized using a blender for 2 min. The homogenized samples were kept in the freezer maintained at -800°C for three days. Later, all the samples were grounded into fine powder using a dry grinder and stored in a freezer at -200°C before extraction. Ten grams of samples were homogenized in 250 ml 80% (v/v) methanol at room temperature. The mixture was shaken using shaking incubator at 200 rpm for 120 min at 500°C . The mixture was then centrifuged at 3000 rpm for 15 min at room temperature and the supernatant was taken. This supernatant was stored at -200°C until further analysis.

Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu's reagent as described by Singleton and Rossi (1965). The sample extract (200 μL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (previously diluted tenfold with distilled water) and allowed to stand at room temperature for 5 min. A 1.5-mL sodium bicarbonate solution (60 g/L) was added to the mixture. The tubes were vortexed, covered and allowed to stand for 90 min. Triplicate measurements were carried out and the absorbance was measured at 750 nm against a blank containing all the reagents without the sample. Results were calculated as gallic acid equivalent (GAE)/100g dry weight of sample.

Determination of total flavonoid content

Flavonoid content was estimated by using Aluminium trichloride method (AlCl_3) (Lamaison and Carnat, 1990): 1 ml of methanolic extract solution was added to 1 ml of 2% methanolic AlCl_3 , 6

H_2O . The absorbance was measured 10 minutes later at 430 nm. Distilled water was used as blank. The result was expressed in mg rutin/100g dry matter by comparison with standard routine treated in the same condition.

Determination of β -carotene, lycopene and ascorbic acid using High Performance Liquid chromatography (HPLC)

β -carotene and lycopene

Beta-carotene and lycopene contents were analysed according to the method of Charoensiri *et al.* (2009) with slight modification. Five grams of homogeneous sample were placed in conical flask. The sample was mixed with 50 ml of 5% (w/v) ethanolic potassium hydroxide (KOH) solution. The resulting mixture was mixed by continuous shaking for 2 min before transferring to the separating funnel and 50 ml of hexane was added. The mixture was shaken vigorously and the upper layer was transferred to a conical flask. The sample was twice extracted with 50 ml of hexane. The combined hexane extract was washed with 100 ml aliquots of water until alkali-free. An aliquot was collected and evaporated in a rotary evaporator under vacuum in a 450°C water bath. The residue was dissolved in 1 ml of chloroform and 1 ml of methanol. Analyses were performed using Waters 515 HPLC instrument equipped with Hewlett Packard series 1050 UV-Vis detector. Separation of beta carotene and lycopene in each sample was performed using a C18 (for carotenoids, C 30 column was used) (Vydac 20i TP 54, 25 x 0.46 cm) and a mobile phase mixture consisting of acetonitrile (CH_3CN):tetrahydrofuran (THF):methanol (CH_3OH):triethylamine (TEA), of 80:14:6:0.1 (v/ v/v/v) containing 0.2% ammonium acetate at a flow rate of 1.0 ml/min, and monitoring at 450 nm. The results of beta-carotene and lycopene were evaluated from triplicate analyses and were expressed as mean values \pm SD.

Ascorbic acid

Ascorbic acid (vitamin C) was extracted according to the modified method of Abdunabi *et al.* (1997). The sample (10 g) was homogenised with an extracting solution containing meta-phosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask (wrapped with aluminum foil) and it was then filtered through a Whatman No. 4 filter paper to obtain a clear extract. The ratio of the sample to extraction solution was 1:1. All samples were extracted in triplicates. Ascorbic acid was determined by a reverse-phase HPLC technique. A Hewlett Packard HPLC Series 1100, USA equipped

with degasser, quaternary pump, autosampler and diode array detector was used. An Ultrasphere octadecylsilyl (ODS) Hypersil C18, 5 mm particle size, in a 250 mm length x 4.0 mm I.D stainless steel column (Hewlett Packard) was used to determine the vitamins. The mobile phase used was a mixture of acetonitrile-water (50:50) at a flow rate of 1.5 ml/min, and monitoring at 254 nm. Ascorbic acid standard was prepared by dissolving 100 mg of L-ascorbic acid in a metaphosphoric acid (0.3 M) – acetic acid (1.4 M) solution at the final concentration of 1 mg/ml.

Determination of antioxidant activity *β*-carotene-linoleate bleaching assay

Determination of antioxidant activity using *β*-Carotene-linoleate bleaching method was done according to the method by Velioglu *et al.* (1998). One millilitre of *β*-carotene solution (0.2 mg/ml chloroform) was pipetted into a round-bottom flask (50ml) containing 0.02 ml of linoleic acid and 0.2 ml of 100 % Tween 20. The mixture was then evaporated at 40°C for 10 min by means of a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 ml of distilled water. The distilled water was added slowly to the mixture with vigorous agitation to form an emulsion. Five ml aliquots of the emulsion were transferred into different test tubes containing 0.2 ml of samples in 80% methanol at 1 mg/ml. The tubes were then gently mixed and placed at 45°C in a water bath for 2 hours. Absorbance of the samples was measured at 470 nm using a spectrophotometer at initial time (t=0) against a blank, consisting of an emulsion without *β*-carotene. Standards BHT at the same concentration with samples were used as comparison. An amount of 0.2 ml of 80% methanol in 5 ml of the above emulsion was used as the control. The measurement was carried out at 15 min intervals. All determinations were performed in triplicate.

The antioxidant activity (AA) was calculated according to the following equation:

$$AA = [1 - (A_o - A_t) / (A^o_o - A^o_t)] \times 100$$

where,

A_o and A^o_o are the absorbance values measured at initial time of the incubation for samples and control respectively, while A_t and A^o_t are the absorbance values measured in the samples or standards and control at $t = 120$ min.

2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method

The free radical scavenging activity of the papaya extracts and the ascorbic acid (standard) were determined according to the DPPH free radical scavenging assay as described by Lee *et al.* (2007). Two hundreds microliters of sample extract (1-8 mg/ml in 80% (v/v) methanol) or ascorbic acid (standard) (0.005-1.28 mg/ml) were mixed with 1 ml of 0.05 mM DPPH in 80% methanol. The mixture was shaken vigorously and leave to stand for 30 minutes at room temperature in a dark room. The absorbance was read using a UV-Vis spectrophotometer at 517 nm with 200 μ l of 80% methanol and 1 ml DPPH served as a blank. The scavenging effect on the DPPH radical is calculated using the following equation:

$$\text{Scavenging effect (\%)} = 1 - \left[\frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}} \right] \times 100$$

EC_{50} is determined from the plotted graph of scavenging activity against the concentration of sample extracts, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out, and their scavenging effect was calculated based on the percentage of DPPH scavenged.

Statistical analysis

All data were presented as means \pm standard deviations. Data were analyzed using SPSS for windows version 17.0. One-way ANOVA and Pearson Correlation Coefficient were used. One way ANOVA was used to test whether there is significant differences in total phenolic, total flavonoid, *β*-carotene, lycopene and vitamin C content and antioxidant activity of papaya between fresh and pickles form. Pearson Correlation Coefficient was used to determine the correlation between the parameters studied in fresh and pickled papaya. Statistical significance were set at $p < 0.05$.

Results and Discussion

Sample preparation and extraction

The present study used freeze-dried sample as it has been shown that it minimized the loss of antioxidant components in the sample. Freeze-drying of foods can preserve the labile analytes and at the same time rupture the cell compartments. This rupturing by lyophilization could result in better extraction efficiency compared to extracting fresh materials. In this present study, a mixture of water

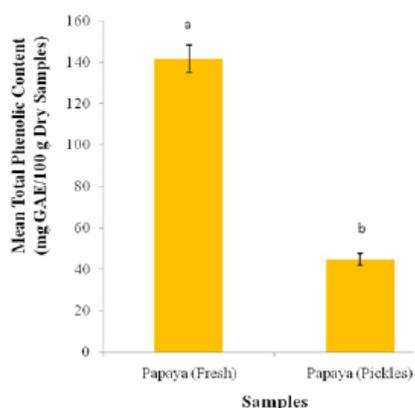


Figure 1. Mean total phenolic content of samples assayed by Folin-Ciocalteu method. Means with different letters were significantly different at the level of $p < 0.05$.

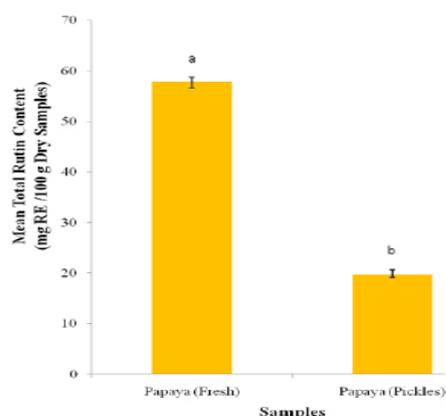


Figure 2. Mean total flavonoid content of samples assayed by aluminium trichloride method. Means with different letters were significantly different at the level of $p < 0.05$.

and methanol (80%) has been used in the extraction method. Antioxidants compounds in plants have different polarities which require the use of different solvent for efficient extraction. Ethanol is widely used for consumption and is very environmental friendly. In contrast, methanol is highly toxic and hazardous. However, methanol water system was the most universal and widely employed solvents for antioxidant extractions (Lee *et al.*, 2007).

Total phenolic content

Figure 1 shows mean total phenolic content of papaya in fresh and pickled form. Fresh papaya had total phenolic content of 141.66 ± 11.71 mg GAE/100 g dry weight while papaya in pickled form had 44.76 ± 4.73 mg GAE/100 g dry samples. This indicated that mean total phenolic content was higher in fresh papaya as compared to pickled papaya. In addition, analysis of variance revealed a significant different ($p < 0.05$) in mean total phenolic content between fresh and pickled papaya.

The treatment used for the production of pickles may cause the loss of phenolic compound in the sample. Therefore, this finding does not support the fact that high sugar content in pickled papaya may cause falsely elevated phenolic concentrations (Prior *et al.*, 2005). However, total phenolic content in the pickled form (44.76 ± 4.73 mg GAE /100 g dry samples) is still considered high as compared to total phenolic content of other fruits in fresh form, obtained from the previous research (Lako *et al.*, 2007; Lim *et al.*, 2007). The range of total phenolic content (mg GAE /100 g fresh samples) was 14 in banana, 15 in pineapple, 32 in malay apple, 26 in fresh papaya (Lako *et al.*, 2007), 33 in hog plum, and 28 in fresh papaya (Lim *et al.*, 2007). It was reported that fresh papaya cultivated in Thailand had phenolic content of 54 ± 2.6 mg gallic GAE /100 g fresh samples (Patthamakanokporn *et al.*, 2008) indicating that local fresh papaya used in our study (141.66 ± 11.71 mg GAE/100 g dry weight) has higher phenolic content than that from Thailand.

The different results obtained from the previous studies (Lim *et al.*, 2007; Lako *et al.*, 2007; Patthamakanokporn *et al.*, 2008) may be attributed to different cultivars, growing conditions, stages of ripening at harvest, or the storage conditions and time elapsed before the fruits were analyzed. Sample preparation method may also influence the results.

Total flavonoid content

Figure 2 presents mean total flavonoid content of papaya in fresh and pickled form. Papaya in fresh form had 57.80 ± 2.11 mg rutin /100g dry samples while papaya in pickled form had 19.71 ± 0.98 mg rutin /100 g dry samples. Statistical analysis showed there was a significant difference in mean total flavonoid content between these samples. Flavonoid compounds are considered to be the largest group of naturally occurring phenols (Vijayakumar *et al.*, 2008). Thus, it is not surprising to see that total flavonoid content accounts for almost half of the phenolic compounds in both samples. Total flavonoid content of both fresh and pickled papaya in this present study was higher than other widely consumed tropical fruits. Alothman *et al.* (2009) reported that total flavonoid content (mg /100 g fresh samples) of banana was 1.24; pineapple was 5.24 and 18.2 in guava. In a study it was shown that Thai fruits hog plum, pear and apples were in the range of 12.6 – 44.3 mg /100 g fresh samples of flavonoid contents (Maisuthisakul *et al.*, 2008). Therefore, it can be seen that fresh and pickled papaya had high total flavonoid content as compared to other tropical fruits.

Table 1. Amount of beta carotene, lycopene and ascorbic acid in 100 g edible portion of fresh and pickled papaya expressed as beta carotene, lycopene and ascorbic acid equivalent

Samples	Beta-carotene	Lycopene	Ascorbic acid
	($\mu\text{g}/100\text{ g edible portion}$)	($\mu\text{g}/100\text{ g edible portion}$)	($\text{mg}/100\text{ edible portions}$)
	Mean \pm SD	Mean \pm SD	Mean \pm SD
Papaya (Fresh)	793.83 \pm 5.47 ^a	779.69 \pm 5.55 ^c	70.37 \pm 0.65 ^a
Papaya (Pickles)	439.76 \pm 2.89 ^b	72.15 \pm 0.54 ^d	5.20 \pm 0.27 ^b

Ascorbic acid content

Table 1 presents mean ascorbic acid content in 100 g edible portion of fresh fruit extract. It showed that fresh papaya had ascorbic acid as much as 70.37 \pm 0.65 mg /100 g edible portions where as the pickled papaya had 5.20 \pm 0.27 mg /100 g edible portions. Fresh papaya had higher ascorbic acid content as compared to the pickled papaya. Analysis of variance also revealed that there was a significant difference in mean ascorbic acid content between fresh and pickled papaya ($p < 0.05$). Ascorbic acid content in pickled papaya was significantly lower as compared to the fresh papaya. This result was in line with the expectation that ascorbic acid as the water-soluble vitamin was easily lost during the process of soaking and marinating of the papaya. The pickled papaya had longer storage time than the fresh form. Longer storage time prior to analysis had been known to contribute substantially to ascorbate loss and retention (Franke *et al.*, 2004).

This finding was in agreement with Franke *et al.* (2004) who found that ascorbic acid content in fresh papaya was 73.7 mg /100 g edible portions. On the other hand, ascorbic acid content reported by Lim *et al.* (2007) was 108 mg /100 g edible portions and was consistent with Tee *et al.* in Malaysian Food Composition Database, 1997 (112.8 mg/100 g edible portions of ascorbic acid content). Variability that may influence ascorbic acid content includes species variety (genetic conditions), harvesting time, growing location, environmental conditions, storage and processing conditions (time, temperature, humidity) (Franke *et al.*, 2004; Wall, 2006). These determinants of ascorbic acid content were in part considered in some reports on ascorbic acid concentrations. For instance, longer day lengths and higher light intensities in summer months can increase the concentrations of

ascorbic acid and glucose, the precursor to ascorbic acid in fruits (Lee & Kader, 2000).

β -carotene

Table 1 presents mean β -carotene content in 100 g edible portion of fruit extracts. It showed that fresh papaya had 793.83 \pm 5.47 $\mu\text{g}/100\text{ g edible portions}$ where as the pickled papaya had 439.76 \pm 2.89 $\mu\text{g}/100\text{ g edible portions}$. Analysis of variance also revealed that there was a significant difference ($p < 0.05$) in mean beta carotene content between fresh and pickled papaya. This present study provides an interesting fact that papaya contains an excellent amount of beta carotene. The mean beta carotene content determined in this present study showed higher values than those previously reported. Charoensiri *et al.* (2009) reported only 471 $\mu\text{g}/100\text{ g edible portions}$. Tee and Lim (1991) in the study on carotenoid composition and content of Malaysian vegetables and fruits reported 228 $\mu\text{g}/100\text{ g edible portions}$ of beta carotene in ripe papaya.

Malaysian fresh papaya (Table 1) has almost equivalent beta carotene value to that of Thai Food (1043 $\mu\text{g}/100\text{ g edible portions}$) (Charoensiri *et al.*, 2009). As previously mentioned, there were a number of factors such as natural variation of fruits and geographical origin that may influence the nutrition composition of fruits. Also, post-harvest conditions such as time of picking to market (transportation), shelf time prior to purchase, may also influence the amounts of beta carotene (Bouderies *et al.*, 2007). Precaution steps that were taken into consideration during the analysis had minimized the lost of beta carotene which eventually contributed to high beta carotene content in the studied samples which included performing analysis under dim light. Performing the work at room temperature with exposure to dim or weak light can decrease the concentration of individual carotenoid that changes from one form to another.

Lycopene

Table 1 shows mean lycopene content in 100 g edible portions of fresh fruit extract. The lycopene content in fresh papaya was 779.69 \pm 5.55 $\mu\text{g}/100\text{ g edible portions}$ whereas papaya in pickled form was 72.15 \pm 0.54 $\mu\text{g}/100\text{ g edible portions}$. Analysis of variance revealed that there was a significant difference in mean lycopene content between papaya in fresh and pickled form ($p < 0.05$).

A study done by Wall (2006) indicated that red-fleshed papaya had large amounts of lycopene. The study reported that lycopene content in red-flesh papaya was 1350.22 $\mu\text{g}/100\text{ g edible portions}$ while

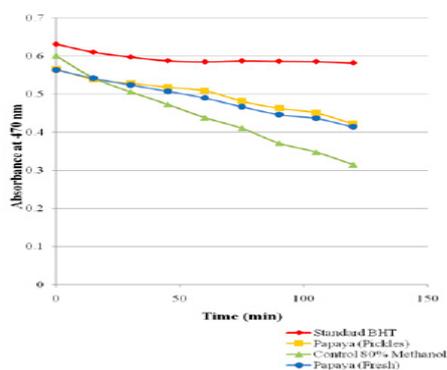


Figure 3. Degradation rate of samples assayed by β -carotene bleaching method

Table 2. Mean EC_{50} values of samples assayed by DPPH radical scavenging method

Samples	EC ₅₀ (DPPH) (mg/ml)
	Mean \pm SD
Papaya (Fresh)	4.28 \pm 0.30
Papaya (Pickles)	ND
Standard Ascorbic acid	0.027 \pm 0.00

Values were expressed as mean \pm SD (n=3)
ND = not detected

lycopene content in the yellow-fleshed cultivars was not detected. This is consistent with the earlier findings by Chandrika *et al.* (2003) that showed red-fleshed papaya had significantly higher beta carotene and lycopene than the yellow-fleshed variety. Similarly, Lako *et al.* (2007) found higher lycopene content in fresh papaya (1700 μ g/100 g edible portions) as compared to fresh papaya in our study. This was in agreement with previous reports by Setiawan *et al.* (2001) (5750 μ g /100 g edible portions) and Charoensiri *et al.* (2009) (2169 μ g /100 g edible portions) who showed high content of lycopene in fresh papaya.

Hence, it can be seen that the lycopene content obtained from our study especially in pickled papaya was lower than those previously reported. Difference varieties of papaya may also contribute to different lycopene content, thus it could be that the papaya in pickled form originated from yellow-fleshed variety used by manufacturers. It was difficult to control this factor since the pickled papaya were bought from the industry and we are not aware of the source of papaya.

Antioxidant activity

β -carotene bleaching method

Figure 3 shows the degradation rate of papaya in fresh and pickled form assayed by β -carotene bleaching method. BHT was used as a standard and

80% methanol was used as a control. Degradation rate of papaya in fresh form was slightly higher as compared to papaya in pickled form. As expected, the control had the highest degradation rate because it contains no antioxidant that can slow down the bleaching of beta carotene. A study done by Barros *et al.* (2007) confirmed that the absorbance decreased rapidly in the samples without antioxidant whereas, in the presence of antioxidant, they retained their color, and thus absorb light for a longer time. It can be seen that both papayas in fresh and pickled form had lower degradation rate than the control. It is probable that the antioxidant components in the papaya extracts can reduce the extent of beta carotene destruction by neutralizing the linoleate free radicals and other free radicals formed in the system. Overall, this present study showed a decreased in antioxidant activity in the order of BHT > fresh papaya > pickled papaya. BHT, the well known synthetic antioxidant, possessed the strongest antioxidant activity among the samples.

DPPH radical scavenging method

Table 2 shows mean EC_{50} values of papaya in fresh and pickled form. Mean EC_{50} value for fresh papaya was 4.28 \pm 0.30 mg/ml. The lower EC_{50} indicated the strongest ability of the extracts to act as DPPH scavengers. However, the difference in extraction methods used in previous studies should be taken into account in making the generalization from the results. A study by Pe'rez-Jime'nez *et al.* (2008) on updated methodology to determine antioxidant activity in plants foods had warrant that the solvent in which the reaction takes place was a key factor that caused the different results. This was due to the different polarity of the solvent that affect the mechanism of action.

As shown in table 2, EC_{50} values (extract concentration with 50% of maximum) for papaya in pickled form cannot be detected since it had mean scavenging effect below 50%. This finding indicated that papaya in pickled form was poor DPPH radical scavenger because even though concentration of samples was increased by 4 folds from the initial concentration of 1 to 8 mg/ml, scavenging effect was still below 50%.

Correlation between total phenolic content, total flavonoid content, ascorbic acid, beta-carotene, lycopene and antioxidant activity and scavenging activity

Correlation between the assays was analyzed using Pearson Correlation Test. Our results showed that correlations between antioxidant activity and

Table 3. r-values of Pearson Correlation Test

	Antioxidant Activity	Scavenging Activity
Total Phenolic Content	0.905*	0.993**
Total Flavonoid Content	0.944**	0.995**
Ascorbic acid	0.958**	0.997**
Beta carotene	0.962*	0.999**
Lycopene	0.956*	1.00**

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

scavenging activity from all assays were positively very strong ($0.905 \leq r \leq 1.00$) (table 3) for all antioxidant assays tested. This correlation confirmed that the phenolic compounds were the main micro constituents contributing to the antioxidant activity of papaya. However, the ambiguous relationship between total phenolic and antioxidant activity from other studies (Azizah *et al.*, 2007; Reddy *et al.*, 2010) could be due to a high content of reducing agents such as ascorbic acid, minerals and carotenoids in the fruits (Deepa *et al.*, 2006), high protein content or genetic, agronomic and environmental influences (Jagdish *et al.*, 2007). A very strong correlation between total flavonoid content and antioxidant activity of papaya in fresh and pickled form ($r = 0.944$) was expected since flavonoids are a subset of phenolic compound. Previous research confirmed that there was a good correlation coefficient between total flavonoids and phenolic compound (Maisuthisakul *et al.* (2008). This indicated that the flavonoid was an important phenolic group representing the scavenging activity of the fruits studied. However, a recent research on non-traditional tropical fruits from Brazil revealed no correlation was observed between the beta carotene bleaching and flavonoid content of the fruits studied (Rufino *et al.*, 2010).

Ascorbic acid content in the fruit extract gave a significant contribution on the antioxidant activity of the fruit. A study by Rufino *et al.* (2010) found no correlation between beta carotene bleaching and any of the study variables including ascorbic acid content. It is possible that the high content of ascorbic acid in the fruits studied interfered in the system as a pro-oxidant factor. Lycopene content measured in tomato fruits exhibit a physical quenching rate constant with singlet oxygen almost twice as high as that of beta carotene (Chang *et al.*, 2006). This imply a correlation exist between lycopene content and antioxidant activity. In our study, lycopene showed a very strong correlation with antioxidant activity ($r = 0.956$).

Conclusions

Processing methods of the pickles may explain the low antioxidant components and antioxidant activity in pickles as compared to the fresh papaya. Although evaluation methods and results reported have not yet been sufficiently standardized, making comparisons difficult, the data add valuable information to current knowledge on the nutritional properties of tropical fruit and its pickled form. This research revealed that both fresh and pickled papaya contained considerable amount of antioxidant components which exhibited excellent antioxidant activity. However, it is important to highlight the limitation that warrant caution in the interpretation of the data from this study. Variability in the samples such as the fruits variety, origin or species as well as different processing method for making the pickles across country should be taken into consideration in evaluating and comparing the antioxidant components and their activity in future research. Hence, it is interesting to conduct more research on the comparison of the fresh tropical fruits and their pickled form so that consumers can make an informed choice.

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References

- Abdulnabi, A.A., Emhemed, A.H., Hussein, H.D. and Biacs, P.A. 1997. Determination of antioxidant vitamins in tomatoes. *Food Chemistry* (60): 207-212.
- Alothman, M., Bhat, R. and Karim, A. 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry* (115): 785-788.
- Azizah, O., Amin, I, Nawalyah, A. G. and Ilham, A. 2007. Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry* (100): 1523-1530.
- Boudries, H., Kefalas, P. and Hornero-Me'ndez, D. 2007. Carotenoid composition of Algerian date varieties (*Phoenix dactylifera*) at different edible maturation stages. *Food Chemistry* (101): 1372-1377.
- Chandrika, U.G., Jansz, E.R. and Wikramasinghe, S.M.D.N., Warnasuriya, N.D. 2003. Bioconversion of pro-vitamin A carotenoids and antioxidant activity of carica fruits. *Journal of Natural Science Foundation Sri Lanka* (31): 437-444.
- Chang, C.H., Lin, H.Y., Chang C.Y. ND Liu, Y.C. 2006. Comparisons on the antioxidant properties of fresh,

- freeze-dried and hot-air-dried tomatoes. *Journal of Food Engineering* (77): 478–485.
- Charoensiri, R., Kongkachuichai, R. & Sungpuag, P. 2009. Beta-carotene, lycopene, and alpha-tocopherol contents of selected Thai fruits. *Food Chemistry* (113): 202–207.
- Deepa, N., Kaur, C., Singh, B. AND Kapoor, H.C. 2006. Antioxidant capacity in some red sweet pepper cultivars. *Journal of Food Composition and Analysis* (19): 572–578.
- Franke, A.A., Custer, L.J., Arakaki, C. A. and Murphy, S.P. 2004. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. *Journal of Food Composition and Analysis* (17): 1–35.
- Halliwell, B. Free radicals and antioxidants – quo vadis? *Trends in Pharmacological Sciences* (32): 3
- Jagdish, S., Upadhyay, A.K., Kundan, P., Anant, B. and Mathura, R. 2007. Variability of vitamin C, E and phenolics in Brassica vegetables. *Journal of Food Composition and Analysis* (20): 106–112.
- Krishna, K.L., Paridhavi, M. and Patel, J.A. 2008. Review on nutritional, medicinal and pharmacological properties of Papaya (*Carica papaya*). *Natural Product Radiance* 7(4):364-373.
- Lako, J., Trenerry, C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S. and Premier, R. 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry* (101): 1727–1741
- Lamaison, J.L. and Carnat, A., 1990. Teneurs en acide rosmarinique, en dérivés hydroxycinnamiques totaux et activités antioxydantes chez les Apiacées, les Borraginacées et les Lamiacées médicinales. *Pharmaceutica Acta Helvetiae* (65): 315-320.
- Lee, S.K. and Kader, A.A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology* (20): 207-220.
- Lee, W.Y., Emmy Hainida, K.I., Abbe Maleyki, M.J. and Amin, I. 2007. Antioxidant capacity and phenolic content of selected commercially available cruciferous vegetables. *Malaysian Journal of Nutrition*. 13(1), 71-80.
- Lim, Y.Y., Lim, T.T. and Tee, J.J. 2007. Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry* (103): 1003-1008.
- Maisuthisakul, P., Pasuk, S. & Ritthiruangdej, P. 2008. Relationship between antioxidant properties and chemical composition of some Thai plants. *Journal of Food Composition and Analysis* (21): 229–240.
- Patthamakanokporn, O., Puwastien, P., Nitithamyong, A. and Sirichakwal, P.P. 2008. Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. *Journal of Food Composition and Analysis* 21(3): 241–248.
- Pe´rez-Jime´nez, J., Arranz, S., Taberner, M., Di´az-Rubio, M. E., Serrano, J. and Saura-Calixto, S. 2008. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International*. 41: 274–285.
- Pe´ rez-Matute, P., Zulet, M.A. & Marti´nez, J.A. 2009. Reactive species and diabetes: counteracting oxidative stress to improve health *Current Opinion in Pharmacology* (9): 771-779.
- Prior, R.L., Wu, X. and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agriculture and Food Chemistry* (53): 4290-4302.
- Reddy, C.V.K., Sreeramulu, D. and Raghunath, M. 2010. Antioxidant activity of fresh and dry fruits commonly consumed in India. *Food Research International* (4): 285–288.
- Rufino, M.S.M., Alves, R.E., Brito, E.S., Pérez-Jiménez, J., Saura-Calixto, F. and Mancini-Filho, J. 2010. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*. Article in Press.
- Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total of total phenolics with phosphomolybdemic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* (16): 144-158.
- Tee, E.S. and Lim, C.L. 1991. Carotenoid composition and content of Malaysian vegetables and fruits by the AOAC and HPLC methods. *Food Chemistry* (41): 309-339.
- Tee, E.S., Mohd Ismail, N., Mohd Nasir, A. and Khatijah, I. 1997. Malaysian Food Composition Database Programme.
- Velioglu, Y.S., Mazza, G., Goa, L. and Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agriculture and Food Chemistry* (46): 4113-4117.
- Vijayakumar, S., Presannakumar, G. and Vijayalakshmi, N.R. 2008. Antioxidant activity of banana flavonoids. *Fitoterapia* (79): 279–282.
- Wall, M.M. 2006. Ascorbic acid, vitamin A, and mineral composition of banana (*Musa sp.*) and papaya (*Carica papaya*) cultivars grown in Hawaii. *Journal of Food Composition and Analysis* (19): 434–445.