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Comparison on the antioxidant properties of fresh and convection oven-dried guava (*Psidium guajava* L.)

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Abstract

Antioxidant properties of both fresh and convection oven-dried guavas (*Psidium guajava* L.) were determined. Guava slices of 1.0 cm wide, 3.0 cm long and 0.5cm thick (20 g) were subjected to convection drying at 40°C for 9, 12 and 14 hours, respectively, and their water activity, total phenolic content (TPC) and antioxidant activities were measured. Guavas that had been subjected to drying for 9, 12 and 14 hours were shown to achieve the water activity of 0.36-0.49. Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) of guava was found to decrease for all the drying durations. Convection oven-drying of guava for 12 and 14 hours showed a significant decrease in TPC ($p < 0.01$) and Ferric Reducing Power Assay (FRP) ($p < 0.01$). Nine hours of convection oven-drying was shown to retain most of the TPC, AEAC and FRP of guava.

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Introduction

Guava (*Psidium guajava* L.) fruit is generally ovoid or pear shaped and depending on cultivar, their sizes vary from 2.5 to 10 cm in diameter and weight 50 to 500 g (Yusof, 2003). The flesh may be pink, white or yellow, either with seed or seedless (Yusof, 2003). Guava is a native to Mexico and it is also available throughout South America, Europe, Africa and Asia as it is able to grow in all subtropical areas (Gutierrez *et al*., 2008). Guava is characterized by low carbohydrate content (13.2%), fat (0.53%), proteins (0.88%) and high water content (84.9%) (Gutierrez *et al*., 2008). Antioxidant properties of fresh guava have been previously reported (Leong and Shui, 2002; Lim *et al*., 2007). Lim *et al*. (2007) found that seeded guava has a higher ascorbic acid equivalent antioxidant capacity (AEAC) (218 ± 79 mg/100 g) compared to that of seedless guava (176 ± 54 mg /100 g). In a study by Leong and Shui (2002), AEAC of fresh guava has been reported at 270 ± 18.8 mg/100 g. Guava fruit has been used as a traditional medicine to treat ulcers, wound and diarrhea in Philippines and anorexia, diarrhea, digestive problems, inflammation and ulcers in Brazil (Gutierrez *et al*., 2008).

Like many other fruits, guava is highly perishable. Drying is one of the methods used to prolong the shelf life of guava and prevent surplus of guava especially guava that is not satisfactory for other types of processing such as canneries (Yusof, 2003). Various drying methods including osmotic dehydration (Vieira *et al*., 2007; Duangmal and Khachonsakmetee, 2009), hot air drying and lyophilisation (Osorio *et al*., 2011) have been studied on guava. In general, dehydration often causes loss of qualities such as colour, appearance, texture, flavor and nutritional value.

Dried fruits such as apricots, cranberries, dates, figs, raisins and plums have been shown to have a lower phenolic content compared to their fresh counterparts based on dry weight basis (Vinson *et al*., 2005). In another study, semi-dried tomatoes using forced-air drier at 42°C have been shown to decrease in the total phenolic contents (Toor and Savage, 2006). Since no work has been conducted on the effect of drying on the antioxidant properties of guava, this study aims to compare the antioxidant properties of fresh and convection oven-dried guava.

Materials and Methods

Fruits

Guava (*Psidium guajava* L.) with similar shape, colour, size, firmness and with no apparent damage were purchased from local markets in Taman Sri Muda, Shah Alam, Selangor. The flesh of each

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fruit was observed to be white and the central pulp contains yellowish seeds. Only fruits ranging from 6-8°Bx and in the pH range of 4.0-4.5 were selected in this study.

Chemicals and equipment

The following chemicals were used in this study. Extraction: methanol (Merck), ethanol (HmbG® Chemicals). Total phenolic determination: Folin-Ciocalteu's phenol reagent (Fluka, 2N), gallic Acid (Sigma, 98%), sodium carbonate anhydrous (Fluka, 99%). Free radical scavenging assay: 2,2-diphenyl-1-picryhydrzyl (DPPH) (Sigma, 90%), ascorbic acid (Kollin chemicals, 99.7%). Ferric ion chelating activity (FIC): ferrozine (Acros organics, 98%), iron (II) sulfate (Merck, 99%). Ferric reducing power (FRP): Iron (III) chloride-6-hydrate (Fisher Scientific, 99.8%), di-potassium hydrogen phosphate (Merck), potassium dihydrogen phosphate (Fisher chemicals), potassium ferricyanide (Unilab laboratory Reagent, 99%), trichloroacetic acid (Fisher Scientific).

Convection oven (Memmert, loading model 100-800) was used for drying. Absorbance was measured using UV-vis spectrophotometer (Hitachi model U-1800). Orbital shaker (Protech model 719), pH meter (Mettler Toledo SevenGo), analytical balance (Sartorius CPA224S), water activity meter (Aqualab, Decagon) and sonicator (Ultrasonic LC130H) were used in this study.

Sample preparation

Six individual fruits were used for each drying duration. Guava was washed, halved, pitted and cut into slices of 1.0 cm wide, 3.0 cm long and 0.5 cm thick. An approximately 20 g of guava slices was weighed and subjected to drying in a convection oven. The convection oven was operated together with a fan at the highest speed. Convection drying was carried out at 40°C for 9, 12 and 14 hours, respectively. Fresh guava (control) and guava that has been subjected to convection oven-drying were from a same fruit. Both control and dried guavas were then subjected to extraction.

For sample extraction, samples were ground into thick paste using a mortar and pestle. The paste was then extracted with 50 ml of methanol, and transferred into a 100 ml conical flask. The flask was then placed on an orbital shaker for 1 hour. Similar procedures were repeated for the dried samples. Liquid nitrogen was used to assist in grounding the dried samples by dipping the samples into liquid nitrogen to achieve glassy state. At glassy state, the dried samples became brittle and this would ease pounding. Extracts were then filtered under reduced pressure and stored at 4°C for no more than 14 days for further analysis.

Measurement of moisture loss and water activity (a_w)

Samples were weighed prior to and after each drying treatment. The percentage of moisture loss was determined based on the difference in the weight of guava slices before and after drying. Samples were kept in a desiccator after each drying interval to ensure the samples were cooled to room temperature before weighing the samples. The percentage of moisture loss was calculated based on the following equation:

Moisture loss (%)
= [(Initial weight (g) - weight after drying (g))/initial weight (g)] × 100

Water activity (a_w) was measured using Aqualab water activity meter calibrated using a standard salt solution (8.5 M LiCl) at a water activity of 0.5. Samples were first closely arranged in sample cups to ensure that the base of the cup was fully covered. The cups were then covered and sealed with parafilm to avoid moisture absorption from the atmosphere before taking the water activity reading.

Determination of total phenolic content (TPC)

Total phenolic content of the extracts was determined by the Folin-Ciocalteu method described by Kahkonen *et al*. (1999) with slight modification. Triplicates of sample extracts, which consisted of 300 µl each, were transferred into a test tube and 1.5 ml of Folin-Ciocalteu's reagent (10 %v/v) together with 1.2 ml of sodium carbonate was added. The test tubes were wrapped in aluminum foil to prevent light exposure and left to stand for 30 minutes before measuring the absorbance at 765 nm. Distilled water was used as a blank instead of sample extract. Total phenolic count, was expressed as g of gallic acid equivalents (GAE) per 100g of material (g GAE/100g).

2,2-diphenyl-1-picryhydrzyl (DPPH) free radical scavenging assay

2,2-diphenyl-1-picryhydrzyl (DPPH) free radical scavenging assay was carried out according to the method described in literature (Miliauskas *et al*., 2004) with slight modifications. Various dilutions of sample extract amounting to 1 ml were transferred into test tubes followed by the addition of 2 ml of DPPH (0.15 mM). The solution mixture was then left to stand for 30 minutes before the measurement of absorbance at 517 nm. Methanol was used to replace the sample extracts in blank. Samples were prepared in triplicates. The antioxidant activity can be expressed as:

% Inhibition = $[(A_{control} - A_{sample})/A_{control}] \times 100\%$

where A is absorbance

The inhibitory concentration IC_{50} was determined by plotting the DPPH scavenging activity (%) against the amount of extract (mg). Results were also expressed as ascorbic acid equivalence antioxidant capacity (AEAC) using the following equation:

$$AEAC\ (g\ AA/100g) = [IC_{50}\ (ascorbate)/IC_{50}\ (sample)] \times 100$$

Ferric reducing power assay (FRP)

Ferric ion reducing power of the extracts was determined according to the method described in literature (Chu *et al*., 2000) with slight modifications. Triplicates of samples (1 ml) were transferred into test tubes followed by the addition of 2.5 ml of 0.2M potassium phosphate buffer and 2.5 ml potassium ferricyanide (1 %w/v). The test tubes were incubated at 50 °C for 20 minutes. Trichloroacetic acid solution (2.5 ml) was added to stop the reaction in the tubes. Samples (2.5 ml) were added to 2.5 ml of water and 500 µl of ferric chloride (0.1% w/v). Test tubes were incubated at room temperature for 30 minutes and the absorbance was measured at 700nm. Results were expressed as mg of gallic acid equivalents (GAE) per g of material (mg GAE/g).

Data analysis

Data was reported as means ± standard error of means, where n = 6. Independent sample t-test was used and the least significant difference (LSD) at $p < 0.05$ was calculated using R-2.8.1 program to determine significant differences between the fresh and dried guavas.

Results and Discussion

Moisture loss and water activity of convection oven-dried guava

Moisture loss of guava was constant after 9 hours of drying (result not shown). Therefore dehydration periods of 9, 12 and 14 hours were selected for subsequent antioxidant study. Water activity of the convection oven-dried guava was within the range of 0.3-0.5 (result not shown).

Extraction efficiency

Ethanol and methanol have been used to extract phenolic compounds from plant material (Lim and Murtijaya, 2007). In the current study, it was found that sample extracted with 50 %(w/v) methanol exhibited the highest total phenolic content (TPC) compared to other concentrations whilst sample extracted with 50 %(w/v) ethanol showed the highest TPC compared to other concentrations (Table 1). There was no difference between TPC extracted in 50 %(w/v) methanol or ethanol (p = 0.44), similar to a previous study (Alothman *et al*., 2009). The 50 %(w/v) methanol was used in subsequent study as methanol could degrade cell wall within plant materials, thus enabling better extraction of intracellular materials. Methanol could also inactivate polyphenol oxidase within fruits, which alters phenolic compounds and consequently changes the antioxidant activity (Robards, 2003).

Table 1. TPC of guava extracted using various solvents at different concentrations

Solvent (w/v)	TPC (mg GAE/100g)
Methanol 90%	113.11 ± 4.57 [d]
Methanol 70%	121.67 ± 1.30 [c]
Methanol 50%	138.32 ± 9.13 [a]
Ethanol 90%	140.44 ± 5.05 [a]
Ethanol 70%	130.05 ±3 .54 [b]
Ethanol 50%	143.63 ± 2.90 [a]

All results were presented in mean ± SD where n = 6, $p < 0.05$; different letters beside values indicates significant difference between the samples

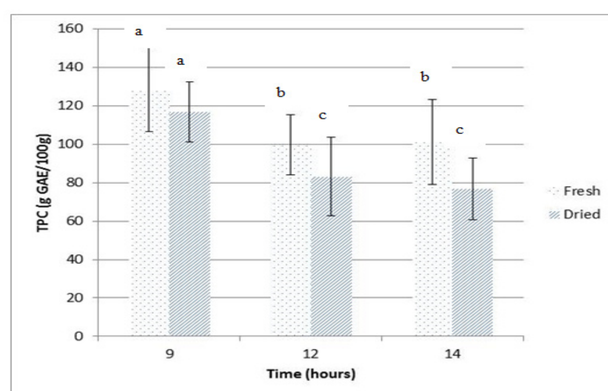

Figure 1. TPC of fresh and dried guavas under various convection oven-drying durations at 40°C. Superscript indicates significant difference between drying times

Total phenolic content (TPC)

Comparing all the control samples, TPC of fresh guava used as a control for 9 hours of drying was higher than that of controls for 12 and 14 hours of drying (Figure 1). Variation in TPC of the fresh guavas indicated the variation in fruits despite the fact that the ripeness in terms of total soluble solids of 6-8°Bx and pH range of 4.0-4.5 were selected. Variation between controls could also be due to unspecific reduction of the Folin Ciocalteu's reagent as different phenolic compounds respond differently with the reagent (Kahkonen *et al*., 1999). Adding to phenolic compounds, non-phenolic compounds such as proteins, ascorbic acid and sugar are also able to reduce the Folin Ciocalteu's reagent, thus resulting in a higher reading of TPC (MacDonald *et al*., 2006). In

Table 2. AEAC of fresh and dried guava under different drying durations

Treatment	AEAC (mg/100g)	Reduction
Fresh	120.65 ± 0.07[a]	~26.96%
9 hours drying	88.12 ± 1.14[b]	
Fresh	60.29 ± 0.81[a]	~28.87%
12 hours drying	42.88 ± 0.32[b]	
Fresh	84.97 ± 0.25[a]	~44.43%
14 hours drying	47.21 ± 2.02[b]	

All results are presented in mean ± SD where n=6, p<0.05. Different uppercase letters beside the values indicates significant difference between values within the same row

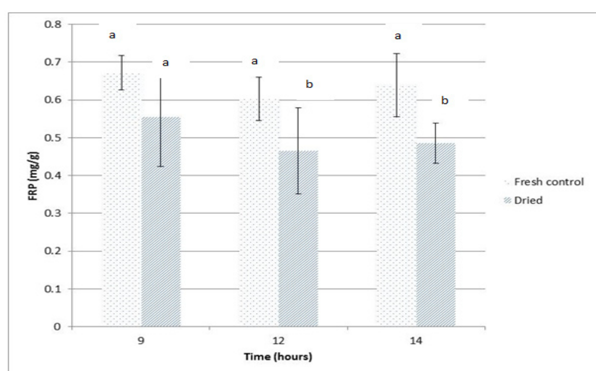

Figure 2. FRP of fresh and dried guava under various convection oven-drying durations at 40°C. Superscript indicates significant difference between drying times

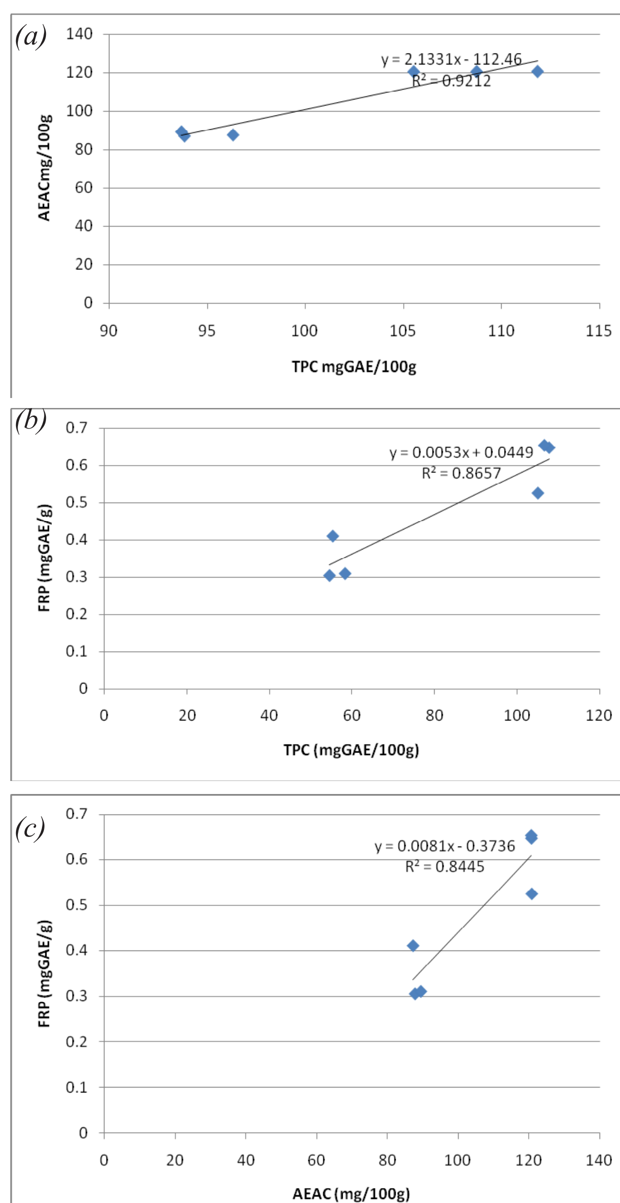

Figure 3. Correlations between (a) TPC and AEAC; (b) TPC and FRP; (c) AEAC and FRP of fresh and convection oven-dried guava

the current study, proteins and ascorbic acid content of the fruits were not determined. This could contribute to the variation in TPC between control samples.

TPC of guava subjected to 9 hours of drying has no difference compared to its control fresh sample (p = 0.07) (Figure 1). Guavas dehydrated for 12 (p = 0.01) and 14 hours (p = 0.0006), however, showed a significant difference in TPC when comparing to its fresh counterparts (Figure 1). This could indicate that as oxidation progresses with drying time, there was an increasingly loss of phenolic compounds. The oxidation of fruits and vegetables is generally caused by oxidation of phenolic substrates by an enzyme known as polyphenoloxidases (PPO). PPO in guava can be deactivated by heating at 50°C for 10 minutes (Augustin *et al*., 1985). In the current study, drying temperature of 40°C may still able to allow the survival of polyphenoloxidases (PPO), thereby resulting in the loss of polyphenols as oxidation progresses with time. The loss of antioxidants could also be due to other factors such as thermal degradation, oxidation or polymerization of phenolic compounds, loss of antioxidant enzyme activities and Maillard browning (Kaur and Kapoor, 2001; Ling *et al*., 2005). TPC of guava dried for 12 and 14 hours showed no significant difference (p = 0.318) (Figure 1), indicating that prolonged drying of 12 hours resulted in a plateau of TPC.

Ascorbic acid equivalent antioxidant capacity (AEAC)

AEAC of guavas dried for 9, 12 and 14 hours was significantly different compared to their respective fresh counterparts (Table 2). This result indicates that drying at 40°C using a convection oven lowers the antioxidant ability (AOA) of guava particularly the radical scavenging ability. Flavonoids such as quercetin and quercetin glycosides showed good radical scavenging ability (Lu and Foo, 2000). These polyphenols could be more heat labile compared to

other polyphenols, hence resulting in the significant reduction of radical scavenging activity. In the current study, since 9 hours of convection oven-drying showed the lowest reduction in AEAC, the 9 hours of drying could be regarded as the least detrimental to the antioxidant activity (AOA) of guavas. Current AEAC result was lower than literatures (Lim *et al*., 2007; Leong *et al*., 2002) probably because of methanol instead of ethanol was used for sample extraction.

Ferric reducing power assay (FRP)

FRP of fresh guava was determined ranging between 0.6-0.65 mg/g (Figure 2). Phenolic compounds such as catechin are known to contribute to FRP but guavas have not been reported to contain catechin (Gutierrez *et al*., 2008). In this study, guavas that have been subjected to 9 hours of drying showed no significant difference in FRP compared to the fresh control ($p = 0.08$) whilst drying of 12 ($p = 0.03$) and 14 hours ($p = 0.004$) showed a significant reduction in FRP compared to its fresh controls (Figure 2).

Correlation between TPC, AEAC and FRP

A high positive correlation was found between TPC and AEAC ($R^2 = 0.92$) (Figure 3(a)). A high positive correlation was also shown between TPC and FRP ($R^2 = 0.87$) (Figure 3(b)). This could indicate that the phenolic compounds contribute to antioxidant activities such as radical scavenging and reducing ability of guavas. There was also a high positive correlation between AEAC and FRP ($R^2 = 0.84$) (Figure 3(c)). This could be due to the fact that DPPH and FRP assays rely on electron transfer in its reduction (Katsube *et al*., 2004; Park *et al*., 2006).

Conclusion

The present study shows that convection oven-drying of 9 hours is sufficient to achieve a desirable water activity of 0.486 for guava of 1.0 cm wide, 3.0 cm long and 0.5 cm thick, thus preventing microbial and fungal growth. Convection oven-drying of 9, 12 and 14 hours resulted in a significant decrease in the AEAC of guava. Unlike 12 and 14 hours of drying, there was no significant reduction in TPC and FRP of guava after the guava was subjected for 9 hours of convection oven-drying.

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