Effects of pickling steps on antioxidant activity of guava

N S Ramli* and N R Mohamad Saadon
Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*E-mail: shazini@upm.edu.my

Abstract. Pickling has been used for centuries to increase the shelf life of foods. Pickling of fruit involves several steps including washing and salting. This processing may affect the retention of antioxidant compounds. The consumption of antioxidant-rich fruits has been associated with reduce the risk of oxidative stress-related diseases. However, there is limited information available on the antioxidant activity of fruits during the pickling steps. Therefore, the study is conducted to determine the ascorbic acid content (AA), total phenolic content (TPC), total flavonoid content (TFC), and antioxidant properties using ferric reducing power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH) assays during the pickling process (salting, washing, and pickling) of guava. The results showed that guava pickle showed a higher amount of TPC and TFC, but lower AA (p<0.05) in comparison to the fresh guava. There were no differences in the antioxidant activities of pickled and fresh guava. Similarly, salting and washing did not significantly change the AA, TPC, TFC, and antioxidant properties of the samples (p>0.05). Findings from the present study revealed that guava pickle could be a good source of antioxidant polyphenols. Further study is needed to identify the phenolic compounds responsible for antioxidant activity.

1. Introduction
Fruits contain abundant nutrients and antioxidant compounds such as phenolics and flavonoids that confer many benefits to human health. Antioxidants compounds neutralize the free radicals by accepting or donating electrons to eliminate the unpaired radicals which are active and dangerous [1]. Therefore, the consumption of antioxidant-rich foods has been associated with reduce the risk of oxidative stress-related diseases such as cancer, diabetes, and cardiovascular diseases [2]. However, fruits are highly perishable after being harvested. Many processing methods have been applied to increase their shelf life and preserve the nutrients and antioxidant contents. Drying methods including oven- and freeze-drying have been shown to reduce the antioxidant content while microwave drying did not affect the antioxidant capacity of fruit samples [3]. Besides, a study on the effects of fruit jam processing on the active compounds showed that the antioxidant compounds were reduced during the peeling and filtration steps [4].

Pickling has been used for centuries to preserve foods and it is still being used today [5]. The pickling process involves soaking in vinegar or anaerobic fermentation in brine. According to the Food and Agriculture Organization [6], the savory flavor of pickled fruits or vegetables is due to the combinations of ingredients during pickling which will reduce the pH and moisture contents and eventually prevent the microorganism growth. It has been reported that the antioxidant properties of pickled papaya was lower than fresh papaya [7]. On the other hand, Fang et al. [8] observed an increase in the total free phenolic acids of potherb mustard after 5 weeks of pickling, and the antioxidant capacities of potherb mustard pickles were retained above 65%. Similarly, Karat et al. [9]
found that Indian pickles contained a high amount of ascorbic acid could prevent cell damage. Fruit pickles are widely sold and consumed by Asian people however, to the best of our knowledge, limited information is available on the antioxidant properties changes during the pickling process. The present study is important to provide information to consumers on the health benefits of fruit pickles. Besides, the data obtained from the study will assist the food industries to improve and optimize the pickling process for the production of high antioxidant guava pickles. Therefore, the study is conducted to investigate the ascorbic acid, polyphenols contents, and antioxidant capacity during the pickling process (salting, washing, and pickling) of guava. Guava is analyzed in the present study since it is considered as an excellent source of ascorbic acid and antioxidant content.

2. Methodology

2.1. Sample preparation and extraction
Fresh guava was bought from the fresh market in Serdang, Selangor, Malaysia. The fruits were cleaned and subjected to the pickling process based on the procedures outlined by Food and Agriculture Organization [6]. In brief, the fruits were immersed in 15% salt solution for two weeks and washed thoroughly before added into a solution containing sugar, honey, and apple vinegar. Samples were collected at each pickling process, freeze-dried, and ground into a fine powder. The sample extraction was conducted according to the method described by Falleh et al. [10] using 70% (v/v) ethanol with the sample to solvent ratio is 1:10. The extracts later were used for further analysis.

2.2. Total ascorbic acid content
The ascorbic acid content was conducted using AOAC [11]. The samples at each processing step were collected and cut into small pieces. Then, an aliquot of sample extract (10 mL) was mixed with oxalic acid (50 mL). The mixture was stirred before 10 mL of the filtrate was taken to titrate with 2,6-dichlorophenolindophenol (dye solution) until a distinctive rose-pink endpoint was appeared and last more than five seconds.

2.3. Total phenolic content (TPC)
The TPC was measured using Folin-Ciocalteau’s reagent according to Mediani et al. [12]. The reagent was prepared by diluting it with distilled water at the ratio of 10:1. An aliquot of sample extract (0.3 mL) was mixed with Folin-Ciocalteu reagent (1.5 mL). Then, 1.2 mL of 7.5% sodium carbonate was added to the mixture. The tubes were covered with aluminum foil and incubated in a dark place for 30 minutes. The absorbance was read at 765 nm using a spectrophotometer. A gallic acid (Sigma Aldrich, Germany) standard curve was constructed (0.02 – 0.16 mg/mL) and calculated as gallic acid equivalents (GAE) /100 g of dried weight.

2.4. Total flavonoid content (TFC)
The TFC was determined spectrophotometrically based on the procedures outlined by Ahmed et al. [13]. The sample extract (1 mL) was added into a test tube containing the same volume of 2% aluminum chloride hexahydrate (AlCl₃H₂O₆). The absorbance was taken at 415 nm after 15 minutes against a blank (70% ethanol). The results were expressed as Quercetin equivalents/100 g of dried weight (Sigma Aldrich, Germany).

2.5. Antioxidant activity

2.5.1. Feric reducing antioxidant power (FRAP) assay
The assay was determined based on the method from Rabeta and Nur Faraniza [14]. Briefly, an aliquot of sample extract (0.2 mL) was added into 3 mL of FRAP reagent. The tubes were vortexed and incubated at 37 °C for 30 minutes before reading the absorbance at 593 nm. The samples were determined against blank. A standard solution of Tolox with the concentration between 0.05 to 0.55
mM were used to construct a calibration curve. The results were expressed as Trolox equivalents/100 g of dried weight.

2.5.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay
The DPPH free radical scavenging activity was determined according to Mediani et al. [12]. An aliquot of sample extract (100 μL) was mixed with 200 μL of DPPH solution (5.9 mg/100 mL) and incubated for 30 minutes kept at room temperature (24 ℃). Methanol The absorbance was recorded at 515 nm using a microplate reader (BioTek ELx800 Absorbance Microplate Readers) with the mixture of DPPH reagent and solvent as positive control and deionized water as blank. The IC₅₀ values were derived from scavenging activity. The following equation was used to calculate the scavenging effect:

\[
\text{Scavenging effect (\%)} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100
\]

2.6. Statistical analysis
Minitab 18 software (Minitab Statistical Software, Pennsylvania, USA) was used for data analysis. All analyses were reported in means ± standard deviation and were conducted triplicate measurement. The differences between the pickling steps were analyzed using a one-way analysis of variance (ANOVA). A significant value was set at a 95% confidence interval.

3. Results and Discussion
The ascorbic acid content of guava at different pickling steps is shown in Figure 1. The ascorbic acid content increased during salting and washing and reduced during pickling. The increase in ascorbic acid content during salting could be due to reduce moisture content which concentrated the ascorbic acid. Salt is added during pickling process to control the type and rate of fermentation. Besides, the addition of salt has been shown to deactivate the peroxidase enzymes that causes loss of ascorbic acid in fruits [6]. Vitamin C is very sensitive and unstable, the preparation steps to freeze-dry the samples before the extraction process also affect the reduction of vitamin C content. As suggested by Lee and Kade [15], vitamin C is lost during the cutting of vegetables. It can be observed that the values of ascorbic acid content for all types of fruits and vegetables in pickled form were decreased. In the commercial pickling process, sodium benzoate was added as a preservative towards mould. Sodium benzoate would react with ascorbic acid through a decarboxylation process to produce benzene [16]. Hence, this could be the underlying reason for the decrease in ascorbic acid content in pickle fruit.

![Figure 1. Ascorbic acid content of guava at different pickling steps. Small letters above the column indicate significant different (p < 0.05).](image-url)
content of the pickle was increased. Kebe et al. [17] explained that the reduction of total phenolic content may indicate leaching of the compounds into the brine following osmotic reaction. The increased value of total phenolic content in pickled form may due to the addition of sugar in the pickling step. Based on the study conducted by Shalaby et al. [18], the antioxidant activity of green tea increased after the addition of sucrose which may due to oxidized phenolic compound and sucrose molecules, resulted in the formation of reduced phenolic compounds.

![Figure 2. Total phenolic content of guava at different pickling steps. Small letters above the column indicate significant different (p < 0.05).](image)

The TFC of guava at different pickling steps is shown in Figure 3. The decreased value of total flavonoid content in the salting step could be due to the oxidation reaction during salting resulting in the leaching of the compounds [19]. According to Hwang and Thi [20], the addition of salt is thought to prevent the extraction of water-soluble compounds and also decrease the efficiency of extracting flavonoid compounds.

![Figure 3. Total flavonoid content of guava at different pickling steps. Different small letters above the column are significantly different (p < 0.05).](image)

Table 1 shows the antioxidant activities of guava at different pickling steps. The results indicated that the FRAP and IC50 value of guava pickle are comparable to the fresh guava. Almeida et al. [21] studied the effect of sucrose addition in a banana pickle and found that the presence of sucrose solution prevents the leakage of antioxidant compounds from the banana tissue. Therefore, it can be postulated that the sugar solution gives a protective effect on the antioxidant components of guava pickle.
Table 1. Antioxidant activities of guava at different pickling steps.

| Pickling step | FRAP value (mM Trolox Equivalent/100g) | IC₅₀ Value (DPPH) (mg/ml) |
|---------------|---------------------------------------|--------------------------|
| Fresh         | 1008.30±50.90ᵃ                      | 0.78±0.07ᵇ               |
| Salting       | 406.31±3.89ᵇ                        | 6.13±0.01ᵃ               |
| Washing       | 429.82±2.56ᵇ                        | 6.74±0.43ᵃ               |
| Pickle        | 1025.40±48.00ᵃ                       | 0.68±0.23ᵇ               |

Different superscript letters in the same column indicate significant different (p < 0.05).

4. Conclusion

In brief, pickle guava contains a higher amount of phenolic compounds including flavonoids compared to fresh guava, hence it can be a good source of polyphenols in the diet. The salting step during the pickling process reduced the antioxidant activities, TPC, TFC, and ascorbic acid content of guava. However, there were no changes observed in all the parameters measured during washing. The variations in the ascorbic acid content, TPC, and TFC with no change in the antioxidant activities during the pickling process suggesting a possible interaction between the interfering substances and reagents used. Further study could focus on the identification of polyphenols and other compounds responsible for the antioxidant activity in guava pickles. It is important to highlight the variability of the samples including fruit species and variety, geometrical factor, climatic conditions, and ripeness index as well as different pickling methods available across the country when evaluating the changes in antioxidant properties in future research.

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