

Effect of freeze drying on antioxidant activity and phenolic contents of Mango (*Mangifera indica*)

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Abstract

This study was carried out to determine the antioxidant activity and the total phenolic content of fresh and freeze-dried mango. DPPH method was used to determine the antioxidant activity and Folin-Ciocalteu method was used to determine the phenolic contents of the samples. For measurement of antioxidant activity different concentration of the sample were taken. Methanol extract of fresh green mango at 50mg/ml has showed high scavenging activity ($98.72 \pm 0.88\%$) and low scavenging activity was showed by Green freeze dried mango ($97.26 \pm 1.8\%$) at 50mg/ml compared to the other samples. For total phenolic contents, absorbance at different concentration of samples were plotted against the standard Gallic acid curve and the value shows that fresh ripe mango contain higher amount of phenolic content. This study also shows a positive correlation between phenolic content and antioxidant activity where $r^2=0.916$. Significant ($p < 0.05$) differences were found between the fresh and freeze-dried fruit samples.

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Introduction

Mangifera indica, which is also known as mango, belongs to the family of Anacardiaceae. The most cultivated *mangifera indica* species is originated from India and Myanmar. Mango is one of the most popular consumed tropical fruits which is found in almost all the country of the world. The interior flesh part of the fruit is known as pulp, goes through several color changes from ripe (unripe) to yellow (ripe). Mango is an excellent source of phytochemicals (Ajila and Prasada, 2008) and also contains nutrients. Among all other tropical fruits, mangoes are valued as one of the potential sources of antioxidants. Antioxidants are believed to control and reduce the oxidative damage in foods and biomolecules by delaying or inhibiting the oxidation process caused by reactive oxygen species, thus enhancing the shelf-life and quality of the products as well as protecting the biological systems (Duthe *et al.*, 1996; Issara *et al.*, 2014). There is a strong relationship between the intake of these antioxidants and reduced mortality of many diseases (Halliwell *et al.*, 1992). Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage cell or kill cells. The use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Therefore antioxidants are widely used as ingredients in dietary supplements. Medicinal

plants play important roles in preventing various diseases and have taken much attention from many researchers over the last few decades. Studies on the antioxidant contents of fruits and vegetables are increasing day by day because high intake of fruits and vegetables has been shown to prevent certain chronic diseases, such as cardiovascular disease and some cancers (Zino *et al.*, 1997; Kalt *et al.*, 1999; Guo *et al.*, 2003). Processing and preservation of fruits and vegetables cause significant changes of their nutritional property and composition. Post harvest processes such as drying cutting, storage, packaging, fermentation, and cooking etc. may affect the phenolics composition and antioxidant activity of foods (Patthamakanokporn *et al.*, 2008; Rodrigues *et al.*, 2009; Robles- Sanchez *et al.*, 2009; Perez-Gregorio *et al.*, 2011). Freeze drying is mostly used for its best preservation and unchanged nutritional property. In some studies, losses of food vitamins and nutritional value due to freeze drying have been reported (Marcia *et al.*, 2000; Chang *et al.*, 2006). The aim of the present study was to investigate the freeze drying free effect on antioxidant activity and total phenolic content of mango.

Materials and Methods

Preparation of fruit extracts

Green and Ripen mango (*Mangifera indica*) were purchased from a local wholesale market of Sylhet,

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Bangladesh. All the fruits were washed under tap water and peeled. Mangoes were cut into (2 × 2) cm. Fresh fruits were analyzed immediately. Fresh green and ripen mango were cut and kept in the oven at 50°C for 24h to freeze-drying. For the freeze-drying experiment, the small pieces of mango were taken in a plate and frozen at -10 ± 1°C for 24 h. The frozen samples were put in the freeze-drier for 12 h until they were completely dried. Both fresh and freeze dried mango small pieces were ground with the help of blender. Extraction was carried out based on a modified method in the literature (Kosar, *et. al.*, 2007). The freeze-dried and fresh green and ripen ground 10g samples were extracted using pure methanol for 1 h using shaker. The residues, separated by filtering through Whatman filter paper, were re-extracted twice with the fresh solvent. The three extracts were pooled and then methanol was distilled off at 40°C using a rotary vacuum evaporator. The resulting crude concentrated extracts were used for analysis of total phenolic compounds and antioxidant activity. All analyses were carried out in triplicates.

Determination of moisture content

Determination of moisture content was measured based on the Association of Official Analytical Chemists method (1995). A known amount of fresh fruits were dried in the oven at 105 ± 1°C. Readings were taken hourly until constant weight was achieved.

Antioxidant assay

The scavenging effects of samples for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were monitored according to the method of the previous report by Yen and Chen (1995). Briefly, 2.0 ml aliquot of test sample (in methanol) was added to 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark, and its absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1 - (A_{\text{sample}} - A_{\text{sample blank}}) / A_{\text{control}}] \times 100$$

Where, A_{control} is the absorbance of the control (DPPH solution without sample); A_{sample} is the absorbance of the test sample (DPPH solution plus test sample) and $A_{\text{sample blank}}$ is the absorbance of the sample only (sample without DPPH solution). Synthetic antioxidants: BHT, gallic acid and ascorbic acid were used as positive controls.

Determination of total phenolic content

Total phenolic compounds were determined according to Folin-Ciocalteu method (Velioglu *et al.*, 1998; Zzaman *et al.*, 2013). A 1.0 ml aliquot of sample was added to 1.5 ml of deionized water and 0.5 ml of 0.1 M Folin-Ciocalteu reagent, and the contents were mixed thoroughly. After 1 min, 1.0 ml of 20% sodium carbonate solution was added, and the mixture was again mixed thoroughly. The control contained all reaction reagents except the sample. After 30 min of incubation at 37°C, the absorbance was measured at 750 nm, and compared to gallic acid calibration curve. Total phenolics were estimated as gallic acid equivalent (GAE).

Statistical analysis

Data obtained from experiments were analyzed using the Statistical Package for the Social Sciences (Version 21; IBM corporation, 1989). Analysis of Variance was used to determine significant difference between fresh and freeze-dried fruits for antioxidant compounds and activity. Significant difference was determined at $p < 0.05$.

Results and Discussion

Moisture content

The results in Table 1 show the moisture content as it is expected. Ripe mango contains high amount of moisture (85.63%) whereas Green mango contains less moisture which is 55.44% on fresh weight (FW) basis. In a previous study we found that fresh mango contains 88.67 % moisture (Norshahida *et al.*, 2011).

Table 1. Moisture content of mango samples

Samples	Moisture (%)
Green mango	55.44 ± 1.67 ^a
Ripe mango	85.63 ± 2.45 ^b

*Data given are mean ± standard deviation for three different value of each sample. Values denoted with the different letter within the same column are significantly ($p \leq 0.05$) different

Yield of methanolic extract

Due to crude extract we used methanol as organic solvent. Methanol acted as good extractor where it extracted highest amount (0.707 g/g DW sample) of crude from fresh ripe mango sample followed by raw freeze dried (0.646 g/g DW sample), fresh green (0.644 g/g DW sample), ripe freeze dried (0.581 g/g DW sample) is shown in Table 2.

DPPH (1,1-diphenyl-2-picrylhydrozyl) radical scavenging activity

DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. In

Table 2. Yield of methanolic extract of mango samples

Samples	Methanolic extract
Fresh Green	0.644 ±0.30 ^b
Green Freeze dried	0.646±0.15 ^b
Fresh Ripe	0.707±0.17 ^c
Ripe Freeze dried	0.581± 0.29 ^a

*Data given are mean ± standard deviation for three different value of each sample. Values denoted with the same letter within the same column are not significantly (p > 0.05) different

the DPPH test, the extracts were able to reduce the stable radical DPPH to the yellow-colored diphenyl hydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction (Shon *et al.*, 2003).

Table 4 shows the antioxidant activity of the methanolic extract of four different mango samples were evaluated by free radical scavenging assay. Fresh Green Mango extracts (concentration 50 mg/mL) exhibited excellent scavenging effects on DPPH radicals in the range of 98–<99%. Fresh Ripen Mango (concentration 50 mg/mL) also showed good scavenging effect like Fresh Green mango in the range of 98-<99%. There were no significant (p > 0.05) differences observed for free radical scavenging activity between fresh and freeze-dried mango samples. Freeze-dried mango samples (concentration 50 mg/mL) showed antioxidant activity around 97%. But it was found that freeze-dried sample showed less antioxidant activity than that of the fresh sample.

Table 4. Antioxidant activity of mango methanolic extracts at different concentration

Samples	Scavenging effect (%)				
	50 (mg/ml)	10 (mg/ml)	2 (mg/ml)	0.4 (mg/ml)	0.08 (mg/ml)
Gallic acid	98.64±0.23 ^d	94.55±0.54 ^{bc}	93.20±0.87 ^b	90.21±1.57 ^a	88.22±1.23 ^a
Ascorbic acid	97.00±0.82 ^d	94.93±0.37 ^{bc}	93.82±0.25 ^b	92.27±0.18 ^a	90.02±1.02 ^a
Fresh Green	98.72±0.88 ^d	97.42±0.29 ^d	94.77±0.87 ^c	92.34±0.64 ^b	91.69±2.02 ^a
Green Freeze dried	97.26±1.8 ^d	94.53±0.26 ^c	94.04±0.77 ^{bc}	91.38±1.94 ^b	87.38±2.06 ^a
Fresh Ripe	98.53±0.51 ^d	95.85±0.30 ^c	94.73±1.24 ^b	94.78±1.37 ^b	92.00±1.79 ^a
Ripe Freeze dried	97.67±1.75 ^c	96.13±0.09 ^{bc}	94.71±0.43 ^b	94.02±1.53 ^b	91.03±1.39 ^a

*a-dEach value is presented as mean ± SD (n = 3). Means within the same raw with different letters differ significantly (p ≤ 0.05)

Total phenolic content (TPC)

Standard curve was used for the measurement of total Phenolic content. Standard curve was made by using Gallic acid at different concentration (0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm, 1 ppm).The results from the study (Table 3) showed that TPC of fresh and freeze-dried mango fruits tested varied significantly (p < 0.05) ranging from 14 to 17 mg GAE/100 g dry sample. Fresh green mango (17 mg GAE/100 g

Table 3. Total phenolic content of fresh and freeze dried mango samples

Samples	Phenolic content
Fresh Green	17.22±0.22 ^c
Green Freeze dried	14.22±0.35 ^a
Fresh Ripe	15.95±0.45 ^b
Ripe Freeze dried	14.75±0.54 ^a

*Data expressed in mg GAE/100 g are in dry weight basis. Values denoted with the same letter within the same column are not significantly (p > 0.05) different. Data given are mean ± standard deviation for three different value of each sample

DW) was found to have the highest TPC, followed by fresh ripen mango (15 mg GAE/100 g DW), then ripen freeze dried mango (14.75 mg GAE/100 g DW) and green freeze dried mango (14.22 mg GAE/100 g DW). TPC values reported by other researchers for selected tropical fruits, total phenolics content of mango (56.0 mg GAE/100 g FW) (Luximon-Ramma *et al.*, 2003) and TPC was reported in mango (113 mg GAE/100 g FW) which were different from our present analysis (Patthamakanokporn *et al.*, 2008). In a study of some tropical fruits including mango subjected to freeze drying reported reduced levels of phenolic content but increased amount of ascorbic acid (Norshahida *et al.*, 2011).

Correlation between antioxidant compounds antioxidant activity of fruits

Coefficient of correlation between the antioxidant compounds (phenolic compound) and antioxidant activity of the mango fruits was studied, as shown in Figure 1.

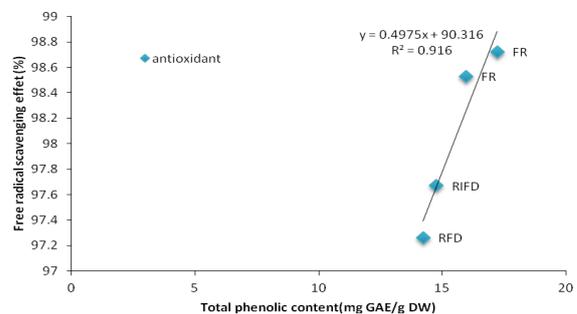


Fig. 1. Correlation between free radical scavenging activity and total phenolic compounds of selected samples (FR- Fresh Green; RFD- Green Freeze Dried; FRI- Fresh Ripe; RIFD- Ripe Freeze Dried)

Antioxidant activity (free radical scavenging activity) was significantly correlated (p < 0.05) with TPC with r² = 0.916 as shown in Figures. Significant positive correlation indicated that the free radical scavenging

activities are mainly attributed to the TPC involved. TPC are more likely to be responsible for scavenging most of the free radicals in the studied. This results were similar to a previous study which found that the content of phenolics in the medicinal and aromatic plant extracts (Miliauskas, 2004). Another study showed that the phenolic compounds contributed greatly towards antioxidant activity than that of ascorbic acid or carotenoids (Lim *et. al.*, 2007). Therefore, it could be expected that phenolic compounds might have been the major contributor of antioxidant activity in the presently tested mango fruits.

Conclusion

The results of the present study showed that mango is a good source of anti-oxidants and phenolic compounds. It revealed that freeze-drying may be a good method for processing and preservation of mango fruits but this technique can noticeably affect the composition of some antioxidant components and antioxidant activity of the fruits. Besides this, the results of the study showed that both fresh green and ripe mango are good sources of antioxidants, compared to green freeze dried and ripe freeze dried mango. Further research on the structural elucidation of the mango fruits' individual phenolic compounds and evaluation of their mechanisms of action and biological principles using some in-vivo models is recommended.

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