International Food Research Journal 23(2): 556-563 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

# Effects of cooking methods at different time durations on total phenolics and antioxidant activities of fresh and dried-stored fruits of *Sonneratia apetala* (Buch.-Ham.)

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**Article history**

*Received: 10 April 2015*
*Received in revised form: 7 August 2015*
*Accepted: 13 August 2015*

**Keywords**

*Antioxidant*
*Cooking*
*Mangrove fruit*
*Polyphenols*
*Sonneratia apetala*

**Abstract**

Cooking is the major method of consumption of the edible mangrove fruit of *Sonneratia apetala* (Buch.-Ham.). Effects of cooking methods-autoclaving, boiling, microwaving, pressure cooking and steaming on total polyphenol contents and antioxidant activities of both fresh and dried-stored seeds and pericarps of the fruits were studied. All the methods significantly ($p < 0.01$) enhanced total polyphenol contents, reducing power and total antioxidant capacity of both fresh and stored seeds on cooking for 20 min. Cooking for 20 min released two times higher amount of polyphenols from fresh seeds than from stored seeds. DPPH free radical scavenging activity of fresh seeds decreased at 2, 5 and 10 min cooking but increased the activity of stored seeds at 5, 10 and 20 min significantly. Cooking of both fresh and stored seeds significantly showed the higher contents of total polyphenol, DPPH scavenging, reducing power and total antioxidant capacity than pericarps did. In general, 20 min cooking is essential to obtain the highest amount of polyphenols and antioxidants from the fruit. No cooking method showed more significant advantages than the others. However, heat and pressure-tolerant polyphenols and antioxidants of *S. apetala* seeds might be used in food industries to produce functional foods and dietary supplements.



## Introduction

Mangrove fruits are grown in coastal tidal wetlands with saline habitats and low nutrients, in high temperature, solar radiation and diverse stresses. Coastal dwellers in many countries such as Bangladesh, India, Myanmar, Sri Lanka, Malaysia, Papua New Guinea, Philippines and some parts of Africa use various mangrove fruits and plant parts as foods and vegetables. Due to stressful coastal environment, mangrove fruits might have high content of antioxidant enzymes and components to mitigate stress mediated generation of reactive oxygen species (ROS). Fruits are not only rich in nutritional antioxidants such as vitamins C and E but also abundant in non-nutritional antioxidants such as carotenoids and phenolic compounds, especially flavonoids (Wach *et al*., 2007). Polyphenols as well as antioxidants show high redox potential, neutralize free radicals and reactive oxygen species (ROS), chelate metal ions and turn beneficial to physical and mental health. Consumption of fruits and vegetables is associated with reduced risk of several degenerative diseases, such as cataract (Christen *et al*., 2008), cardiovascular diseases and cancer (Kris-Etherton *et al*., 2002), Parkinson's and Alzheimer's (Di Matteo and Esposito, 2003), as well as inflammation and aging (Ames *et al*., 1993). These functional activities are often attributed to different antioxidant components, such as vitamin C, vitamin E, carotenoids, lycopenes, polyphenols and other phytochemicals. Moreover, polyphenols show antihyperglycemic (Hossain *et al*., 2002; Hanamura *et al*., 2006; Hossain *et al*., 2007), antidiabetic (Zunino *et al*., 2007), antiallergic (Matsuo *et al*., 2000), antimicrobial (Taguri *et al*., 2004) and anxiolytic (Vignes *et al*., 2006) activities. We reported polyphenol contents and antioxidant activities of common edible fruits (Hossain *et al*., 2008), fruity and leafy vegetables (Hossain *et al*., 2014; Hossain *et al*., 2015) and antidiabetic medicinal plants (Basar *et al*., 2013) in Bangladesh.

The fruit of *Sonneratia apetala* (Buch.-Ham.) is abundantly grown in the world largest mangrove forest, the Sundarbans along with the coastal areas of Bangladesh, India, Myanmar, Malaysia, New Guinea, China etc. People adjacent to the coastal Bangladesh, Myanmar and India extensively consume this fruit by cooking and, sometimes, through other preparations. In many regions including some parts of Africa, the fruits are also processed to produce sour sauce

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which is marketed. Ripe fruits are also used to expel intestinal parasites (Malay) and half-ripe fruits are good for coughs. Fermented juice of the fruit is useful in arresting haemorrhage. In previous studies, we reported polyphenols, flavonoids, vitamin C contents and antioxidant, antidiabetic and antibacterial activities of *S. apetala* fruit (Hossain *et al*., 2013; Hossain *et al*., 2016). However, cooking is the major method of consumption of the fruit. Cooking such as simple boiling, microwaving, pressure cooking, autoclaving etc. would lead to a number of changes in physicochemical characteristics of the fruit. Reduction of phenolics and loss of antioxidant capacity have been reported for some vegetables after cooking (Ismail *et al*., 2004; Zhang and Hamauzu, 2004). It has otherwise been reported that total antioxidant activity of vegetables on cooking remained unchanged or got enhanced (Gahler *et al*., 2003; Turkmen *et al*., 2005). Oboh (2005) reported that blanching of vegetables resulted in the decrease of antioxidant activity. Sasipriya *et al.* (2014) showed that pressure cooking enhanced phenolics and antioxidant activity of unripe banana fruit and flower. Cortez-Garcia *et al.* (2015) reported steaming and microwaving had no effects on phenolics and antioxidant activity of xoconostle *(Opuntia joconostle)* fruit whereas boiling and grilling reduced them significantly. Therefore, till today, effects of cooking and comparison of cooking methods on polyphenols content and antioxidant activity of fruits and vegetables have remained inconclusive. No report described the effects of different cooking methods on polyphenols content and antioxidant activity of fresh and stored fruit of *S. apetala*, and, thus, the present research was undertaken.

## Materials and Methods

*Chemicals and reagents*

Ascorbic acid, Folin-Ciocalteu's phenol reagent and gallic acid were purchased from Sigma-Aldrich Co (St. Louis, MO). DPPH (1,1-diphenyl-2 picrylhydrazyl) was purchased from Wako Pure Chemical Industry, Ltd., Osaka, Japan. Ammonium molybdate, ethanol, methanol, potassium ferricyanide, sodium carbonate and trichloroacetic acid were purchased from Merck, Germany.

*Collection and processing of the fruit*

The fruits of *Sonneratia apetala* (Buch.-Ham.) were collected from the world's largest mangrove forest, the Sundarbans. Collected fruits were washed by distilled water to remove undesirable materials. After removing excess water, the pericarps were peeled off from the seeds. For storage, seeds and pericarps were shed dried and stored separately in air tight containers for 06 (six) months at room temperature.

*Sample preparation*

Forty gram (40 g) of fresh/stored seeds or pericarps was taken in 100 mL distilled water and kept in room temperature for 24 h. Then uncooked and cooked extracts were prepared following the methods as stated below:

Uncooked: The preparations were used as if for extractions.

Autoclaving: The preparations were capped with aluminum foil and placed in an autoclave at 121$^{o}$C for 2, 5, 10 or 20 min before performing extractions.

Boiling: The preparations were capped with aluminum foil and boiled for 2, 5, 10 or 20 min before performing extractions.

Microwaving: The preparations were cooked in a 1000 W domestic microwave oven (Whirlpool) for 2, 5, 10 or 20 min before performing extractions.

Pressure cooking: The preparations were capped with aluminum foil and cooked in a domestic pressure cooker for 2, 5, 10 or 20 min before performing extractions.

Steaming: Water was taken in a tea kettle and placed on a gas burner. When the water was boiled, the lid of the kettle was opened and a net was kept on it. Seeds or pericarps containing beaker was placed on the net to perform steaming for 2, 5, 10 or 20 min before extractions.

*Extraction*

Uncooked/cooked seeds or pericarps were homogenized with a homogenizer at 500 rpm for 3-5 min. The homogenate was filtered through Whatman no. 1 filter paper. The filtrate was finally adjusted to 1000 mL with distilled water and stored in a refrigerator at 4$^{o}$C no longer than 3 days for conducting the experiments.

*Determination of total phenolic content*

The total concentration of phenolics (TPH) in the extracts was determined according to the Folin-Ciocalteu method (Ough and Amerine, 1988) with gallic acid (GA) as the standard and expressed as mg gallic acid equivalents (GAE)/g of fresh (FW) or dried weight (DW). One milliliter (1 mL) of diluted extract was mixed with 1 mL of Folin-Ciocalteu's reagent and vortexed for 5 s. Then, 1 mL of a 10% (w/v) sodium carbonate aqueous solution was added to the mixture. The mixture was incubated at room temperature for 1 h and thereafter colorimetric

measurement was made at 700 nm. Each experiment was conducted three times.

*DPPH free radicals scavenging activity*

The reaction mixture (total volume, 3 mL), consisting of 0.5 mL of 0.5 M acetic acid buffer solution at pH 5.5, 1 mL of 0.2 mM DPPH in ethanol, and 1.5 mL of diluted extract was shaken vigorously (Blois, 1958). After incubation at room temperature for 30 min, the amount of DPPH remaining was determined by measuring absorbance at 517 nm. Mean values were obtained from triplicate experiments.

*Reducing power activity*

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Briefly, 1 mL of extract was mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6 and 2.5 mL of 1% potassioum ferricyanide solution. After incubation at 50°C for 20 min, the mixtures were mixed with 2.5 mL of 10% trichloroacetic acid followed by centrifugation at 650 g for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of this solution was measured at 700 nm. Ascorbic acid served as positive control.

*Determination of total antioxidant capacity*

The assay was done according to the method described by Prieto *et al.* (1999). The tubes containing the extracts and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 90°C for 90 min. After cooling at room temperature, the absorbance was taken at 695 nm against a blank. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

*Statistical analysis*

All data were presented as means ± SD (standard deviation) of at least three replicates. Differences between variables were tested for significance by using ANOVA. Differences between means were considered to be significantly different at $p < 0.05$ using the Statistical Package for Social Sciences (SPSS), version 20.0.

## Results

*Total phenolic content (TPC)*

Total phenolic content in uncooked and cooked extracts of both seeds and pericarps of *S. apetala* was determined in fresh and stored states that were expressed as mg gallic acid equivalent (GAE).

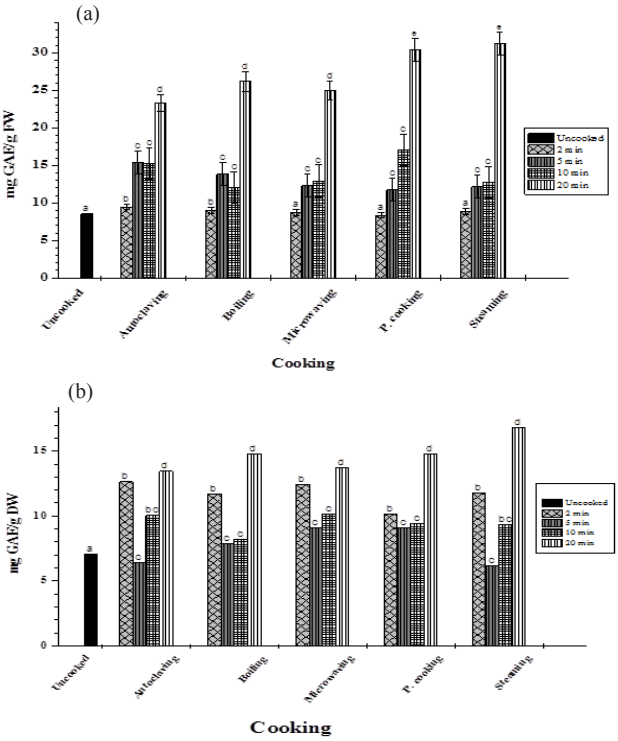


Figure 1. Total polyphenol content of (a) fresh (mg GAE/g FW) and (b) stored (mg GAE/g DW) seeds of *S. apetala.* GAE: gallic acid equivalent, FW: fresh weight, DW: dry weight. Values are means ± SDs (bars), n=3. Different alphabet indicates significant difference at $p < 0.05$

For fresh seeds, the TPC ranged from 8.5 to 31.2 mg GAE/g fresh weight (FW) and the order being uncooked ≤ 2 < 5 ≈ 10 < 20 min cooking (Figure 1a). Total phenolic contents increased more significantly ($p < 0.05$) in cooked fresh seeds at 5, 10 and 20 min than in the uncooked, and the level at 20 min cooking, the order being, streaming ≈ pressure cooking > boiling ≈ microwaving ≈ autoclaving was 3 to 4 times as large as that of the uncooked. However, for dried stored seeds, the TPC ranged from 7.1 to 16.8 mg GAE/g dry weight (DW) and the order being, uncooked ≤ 5 ≤ 10 < 2 < 20 min cooking (Figure 1b). Uncooked fresh seeds had polyphenols content of 8.5 mg GAE/g FW, which was higher than stored seeds, 7.1 mg GAE/g DW. Though dried-stored seeds had higher amount of dry matter, cooking for 20 min showed about 2 times higher polyphenols in fresh seeds than in stored seeds. Polyphenols contents in both fresh and stored pericarps were very smaller than those of seeds. For fresh pericarps, uncooked showed 2.8 mg GAE/g FW which was near to the same at 2 min cooking, but the amount decreased significantly at 5 and 20 min cooking, though more surprising to note that 10 min cooking significantly increased the content in all cooking methods, where pressure cooking showed the highest content of polyphenols (5.1 mg GAE/g FW) in fresh pericarps followed by steaming (4 mg GAE/g FW). Stored

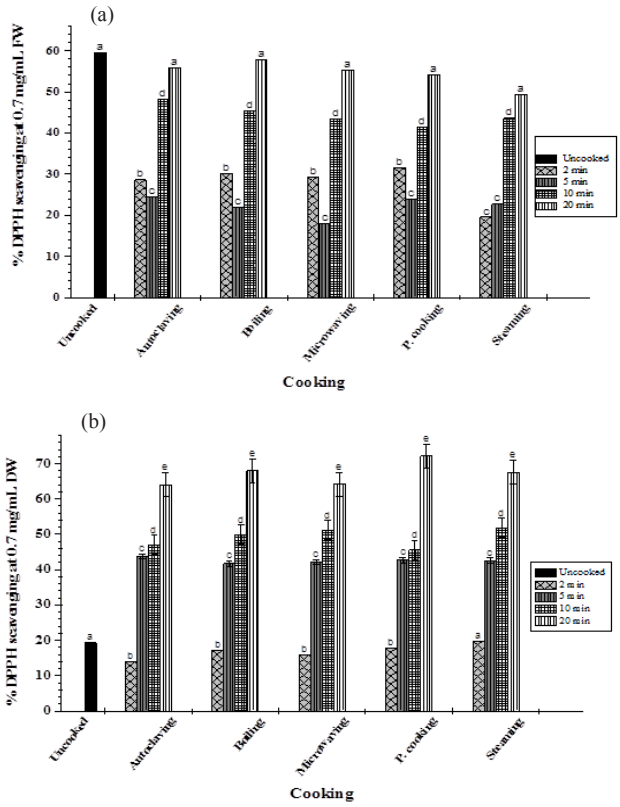


Figure 2. DPPH free radical scavenging activity of (a) fresh and (b) stored seeds at 0.7 mg/mL. FW: fresh weight, DW: dry weight. The data are presented as means ± SDs (bars), n=3. Different alphabet indicates significant difference at $p < 0.05$

uncooked pericarps showed polyphenols content of 4.1 mg GAE/g DW. Similarly 10 min cooking of stored pericarps showed significantly ($p < 0.05$) the higher content of polyphenols where pressure cooking showed the highest (9.9 mg GAE/g DW) followed by autoclaving (9 mg GAE/g DW).

*DPPH free radical scavenging*

The DPPH free radical scavenging ability of uncooked and cooked fresh seeds of S. apetala was shown in Figure 2a. Cooking decreased the DPPH free radical scavenging activity of fresh seeds. At the concentration of 0.7 mg/mL FW, uncooked seeds scavenged 59.6% DPPH free radical. In fact, cooking of fresh seeds at 2, 5 and 10 min significantly ($p < 0.05$) decreased the DPPH radical scavenging ability when compared with uncooked whereas 20 min cooking showed insignificant results and the order being, uncooked ≈ 20 > 10 > 2 > 5 min cooking. For stored seeds, cooking at 20 min showed the highest activity of DPPH free radical scavenging followed by 10 min cooking (Figure 2b). The DPPH radical scavenging activity of both fresh and stored pericarps decreased more significantly at 2, 5 and 10 min cooking than in the uncooked but it remained insignificant at 20 min cooking and the order being,

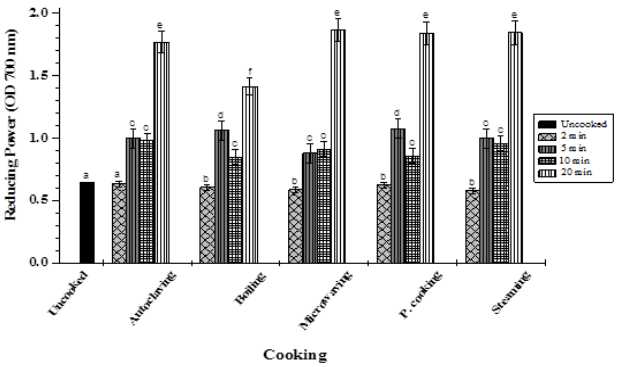


Figure 3. Reducing power of seed at 0.75 mg FW/mL; FW: fresh weight. The data are presented as means ± SDs (bars), n=3. Different alphabet indicates significant difference at $p < 0.05$

uncooked ≥ 20 > 2 > 5 > 10 min. Uncooked stored pericarps scavenged 58.3% DPPH free radical at the concentration of 0.7 mg/mL. Cooking at 20 min of stored pericarps showed the following results of DPPH scavenging: autoclaving 59.8 ± 1.4%, boiling 64.2 ± 3.5%, microweaving 62.4 ± 1.3%, pressure cooking 65.5 ± 3.3% and steaming 66.1% ± 3.1. DPPH radical scavenging activity of fresh and stored seeds or pericarps showed almost similar results after 20 min cooking.

*Reducing power*

The more antioxidant compounds reduce more oxidized form of ferric iron ($Fe^{+3}$) to ferrous iron ($Fe^{+2}$). In this study, reducing powers of both seeds and pericarps in cooked and uncooked were measured at 700 nm OD using the potassium ferricyanide reduction method, where ascorbic acid was used as positive control. The reducing power (OD) of uncooked fresh seeds was 0.65 at 0.75 mg/mL FW, which decreased a little at 2 min cooking in all methods. The highest reducing power of fresh seeds was observed at 20 min cooking in all methods and the order being 2 ≤ uncooked < 10 ≤ 5 < 20 min. Microwaving, pressure cooking, steaming and autoclaving showed the highest and similar reducing powers at 20 min cooking of fresh seeds (Figure 3). Stored uncooked seeds showed reducing power (OD) of 0.94 at 0.75 mg/mL DW. Cooking increased reducing power of stored seeds in all cooking methods at different time durations where 20 min cooking showed the highest results and OD of autoclaving, boiling, microwaving, pressure cooking and steaming was 1.2, 0.95, 1.2, 1.1 and 1.3 respectively, at 0.75 mg/mL DW. Cooking of fresh seeds for 20 min showed significantly the higher ($p < 0.05$) reducing power than that of stored seeds. Uncooked fresh pericarps showed small reducing power, OD 0.37 at 0.75 mg/mL FW whereas stored

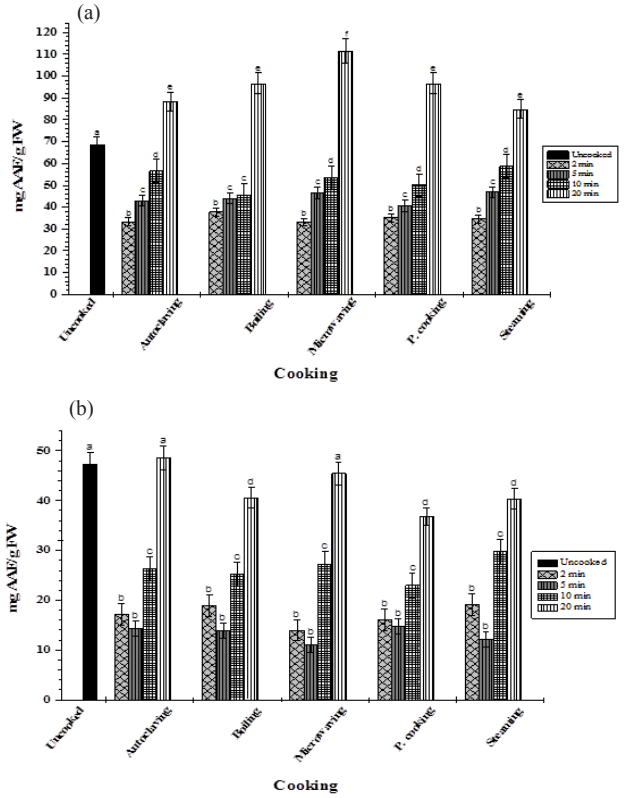


Figure 4. Total antioxidant capacity (mg AAE/g FW) of (a) seed and (b) pericarp of *S. apetala*. AAE: ascorbic acid equivalents, FW: fresh weight. The data represent the means ± SDs (bars), n=3. Different alphabet indicates significant difference at $p < 0.05$

pericarps in uncooked showed reducing power of OD 0.58. Reducing power of fresh and stored pericarps significantly ($p < 0.05$) decreased at 2 and 20 min cooking whereas increased significantly at 5 and 10 min cooking. Ten (10) min cooking of stored pericarps showed the highest reducing power, OD of autoclaving 0.95, boiling 0.94, microwaving 0.84, pressure cooking 0.92 and steaming 0.81 at 0.75 mg/mL DW.

*Total antioxidant capacity*

Total antioxidant capacity (TAC) of cooked and uncooked seeds or pericarps was expressed as ascorbic acid equivalent (AAE). Fresh seeds cooked at 2, 5 and 10 min showed more decreased amount of TAC ($p < 0.05$) than the uncooked did (68.7 mg AAE/g FW); whereas 20 min cooking significantly enhanced TAC in all methods used and the order being, $2 < 5 \leq 10 <$ uncooked $< 20$ min. Microwaving showed the highest TAC (111.5 mg AAE/g FW) followed by pressure cooking and boiling (Figure 4a). Stored uncooked seeds showed TAC of 40.3 mg AAE/g DW which significantly increased at 10 and 20 min cooking where 20 min cooking showed highest TAC (autoclaving 72.5, boiling 69.8, microwaving 66.6, pressure cooking 70.7 and steaming 73.4 mg AAE/g DW). Uncooked fresh pericarps had total antioxidant capacity of 47.2 mg AAE/g FW, which significantly decreased in all cooking methods at different time durations except for cooking at 20 min by autoclaving and microwaving, that showed TAC near to uncooked pericarps (Figure 4b). Stored pericarps showed TAC of 13.4 mg AAE/g DW, which significantly increased at 2, 5, 10 and 20 min cooking in all methods but 20 min cooking showed the highest TAC content of autoclaving 44.3, boiling 48.7, microwaving 42.5, pressure cooking 46.4 and steaming 45.6 mg AAE/g DW.

## Discussion

All cooking methods significantly increased phenolic content of the extracts of fresh and stored seeds (Figure 1a,b) of which the largest amount was at 20 min cooking whereas the highest polyphenols was found at 10 min cooking of pericarps. It may be due to cooking which softens or disrupts the plant cell walls and destructs the complex phenolics (Bernhardt and Schlich, 2005). Since seed tissues were more compact and hardy than pericarp, they took longer time to become soft and release more phenolics. Reportedly, total phenolics are usually stored in pectin or cellulose networks and can be released during thermal processing. Individual phenolics may, sometimes, increase because heat can break supramolecular structures and release the phenolic sugar glycosidic bounds, which react with the Folin-Ciocalteau's reagent (Bunea *et al.*, 2008). Ewald *et al.* (1999) reported that boiling, microwaving, frying or warm holding had not affected the level of polyphenols, quercetin and kaempferol in onions, green beans and peas. On the contrary, Crozier *et al.* (1997) showed that microwaving caused a loss of quarcetin in tomatoes and onions. We have already reported polyphenols-caffeic acid, (+)-catechin, (-)-epicatechin, ellagic acid, gallic acid and quarcetin in methanol fraction of *S. apetala* seeds (Hossain *et al*., 2015). In this study, we found that both stored seeds and pericarps at room temperature reduced total polyphenol contents significantly when cooked at 20 or 10 min respectively. This may be due to detrimental effects of processing, drying and storage on phenolics of seeds and pericarps. Losses of polyphenols upon cooking or blanching were reported also (Ismail *et al*., 2004; Zhang and Hamauzu, 2004). On an average, cooking of fresh seeds at 2 or 5 min declined near about 55% DPPH free radical scavenging ability compared with uncooked fresh seeds (Figure 2a) whereas the amount for fresh pericarps was 40%. It may be due to the higher content of heat liable antioxidants such as vitamin C in

seeds (Hossain *et al*., 2013) which was very sensitive to heat in a short period of cooking. Prolongation of cooking time to 10 min decreased almost 25% of DPPH scavenging ability, but further prolongation to 20 min showed no larger differences in the cases of either cooked and uncooked fresh seeds or pericarps. Hence it showed resistance to deterioration in its radical scavenging activity. Yamaguchi *et al.* (2001) reported suppression of oxidation by antioxidants due to thermal inactivation of oxidative enzymes and the release of potent radical scavenging antioxidants from the destruction of cell walls and subcellular compartments of vegetables. However, our findings showed, cooking increased polyphenols contents though it did not correlate with the scavenging of DPPH radicals. Nguelefack *et al.* (2011) reported that only phenolics with a certain structure and with hydroxyl groups at specific positions can show radical scavenging activity.

The greater reducing power indicates greater antioxidant activity. Reducing capacity is enhanced by ortho or para orientation of the phenolic hydroxyl bond (Mcdonald *et al.*, 2001). Reportedly, primary and secondary antioxidants are electron donor compounds and they can reduce oxidized intermediates of lipid peroxidation as a reducing agent. All cooking methods lead to an increase in the reducing power of both fresh and dried-stored seeds. Twenty (20) min cooking of fresh seeds showed the highest reducing power, which was almost three times as high as that of uncooked fresh seeds (Figure 3). The gradual increase in reducing power of *S. apetala* seeds could be attributed to the possible breakdown of the tannins. Larger amount of released polyphenols probably compensated the loss of vitamin C and other heat liable antioxidants in seeds. Therefore, for seeds, strong correlation ($r = > 0.9$) was observed between polyphenols content and reducing power for each cooking method at different durations of time. Pericarp tissue had small amount of polyphenols (Hossain *et al.*, 2013) and was softer than that of seed, therefore, released polyphenol was the highest at 5 and 10 min cooking, which resulted in high reducing power at that time. But further prolongation of cooking time to 20 min, probably destructed some of the released polyphenols as well as decreased reducing power. Destruction of heat liable antioxidants, therefore, showed the lowest reducing power of pericarp at 2 min cooking. Oboh (2005) reported loss of ascorbic acid after blanching of some tropical green leafy vegetables. It has been reported that TAC is a combination of different antioxidant mechanisms, including free radical scavenging ability, reducing power and Fe(II) chelating ability. Total antioxidant capacity of fresh uncooked seeds or pericarps was higher than that of cooked at 2, 5, and 10 min (Figure 4a,b). It was interesting that 20 min cooking of fresh seeds significantly ($p < 0.05$) increased TAC from uncooked whereas for pericarps, a small reduction was observed. For stored seeds and pericarps, total antioxidant capacity gradually increased with increase of cooking time. However, loss of antioxidant capacity after boiling for several vegetables was reported (Ismail *et al*., 2004; Zhang and Hamauzu, 2004). In contrast, after cooking, TAC increased for tomato (Halvorsen et al., 2002; Wu *et al*., 2004) due to increased release of phytochemicals, such as lycopene, from the matrix (Gahler *et al*., 2003). Turkmen *et al.* (2005) reported significant increase of total antioxidant activity after boiling, microwave cooking and steaming of pepper, green beans, broccoli and spinach. Reports showed that cooking method affects the TAC differentially depending on vegetables (Natella *et al*., 2010). Cooking at 20 min significantly increased the TAC of *S. apetala* seeds, which might be attributed to the significant increase of total polyphenols (Figure 1), flavonoids and other phytochemicals released from the compact matrix of the seeds.

## Conclusion

Both fresh and stored seeds showed polyphenol contents, DPPH scavenging, reducing power and total antioxidant capacity higher than pericarps did in uncooked and cooked states at different cooking methods at different time durations. Fresh seeds and pericarps showed higher amount of polyphenols and antioxidant activities than the same amount of stored seeds and pericarps did, respectively. Cooking of seeds for 20 min and pericarps for 5 or 10 min showed the highest contents of polyphenol and antioxidant activities. All the cooking methods in this regard showed nearly the same results. However, heat and pressure-tolerant polyphenols and antioxidants of *S. apetala* fruit, especially from its seeds could be used in food and pharmaceutical industries to produce dietary supplements and functional foods.

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