Influence of Microwave Cooking on Proximate, Mineral and Radical Scavenging Activities of Tree Bean Seeds and Pods

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A B S T R A C T

The experiment has been carried out to investigate the proximate composition, mineral contents and radical scavenging activities (RSA) of tree bean (Parkiaroxburghii G. Don) seeds and pods owing to microwave cooking and compared with that of traditional cooking. Eventually, proximate, mineral and RSA decreased significantly under both the cooking methods in comparison to the uncooked seeds and pods. However, the rate of reduction in microwave cooking was lower while compared with traditional cooking. Microwaved tree bean apparently showed better DPPH (2,2-diphenyl-2-picryl hydrazyl; 43.96-67.31%), ABTS[2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; 57.72-65.79%] and MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; 1.19-1.53 Abs] activities than that of conventional cooking. Thus, microwave cooking was proven worthy in turn of retaining the nutritional properties in tree bean seeds and pods which was severely sacrificed under traditional cooking methods. The result of this study would be helpful in ensuring food and nutritional security in a sustainable way.

Keywords
Parkiaroxburghii, Traditional cooking, Microwave, Radical scavenging activities, Nutritional properties

Introduction

Tree bean (Parkiaroxburghii G. Don) is one of the most nutritive legume grown and consumed in abundance by the people of north eastern region of India. Tender pods and seeds are rich sources of protein, carbohydrate, fat, vitamins, minerals and antioxidants compared to other realm of legumes (Singh et al., 2009; Seal, 2011). The high-quality protein content in pods and seeds may be a substitute of animal protein among the low-income people. These legumes are consumed fresh or cooked traditionally by boiling and incorporating in different curries. Various cooking methods viz., boiling, roasting, steaming, microwave and pressure-cooking reportedly diminish nutritional properties, bioactive compounds and neutraceutical properties. In addition, cooked vegetables exhibit poor color and quality in comparison to the fresh ones (Turkmen et al., 2006). Reports showed that an appropriate process condition influences the morphological and nutritional
characteristics of vegetable species (Bernhardt and Schlich, 2006; Podsedek, 2007). Similar studies indicated that the method of preparation and cooking can even improve the nutrition quality of food (Adriana and Guy, 2016). However, there is no information available on effect of cooking on nutritional properties of tree bean. Hence, the present experiment was conducted in order to study the effect of conventional and microwave cooking on proximate, mineral and radical scavenging activities of tree bean (*Parkiaroxburghii* G. Don).

**Materials and Methods**

**Crop material and treatment conditions**

Tree bean pods were collected from the local market of Imphal, Manipur, India. Uniform, fresh and matured green pods free from mechanical injury were selected for the experiment and cleaned properly before cooking treatments. Matured pods were scrapped and cut into pieces. Seeds were also collected from the pods and studies for the nutritional changes owing to cooking.

For conventional cooking, a set of pod pieces and seeds (100 gm) were boiled in 200 ml water for 5 min and another set for 10 min. In case of microwave cooking, two sets of bean pieces (100 gm each) were microwaved at 600 W and 1000 W for 3 min each. Excess moisture of the cooked bean was decanted and soaked in blotting paper. Proximate composition, mineral composition and free radical scavenging activities were analyzed in three replicates with duplicate determinations.

**Analyses**

**Proximate analysis**

Moisture, ash, crude fat, crude fibre, crude protein, nitrogen free extracts and energy of the cooked and uncooked tree bean were determined according to the AOAC (2012) methods.

**Mineral content**

Nitrogen content was determined by Kjeldahl’s method. Other minerals were analysed after wet ashing by concentrated nitric acid and perchloric acid (1:1, v/v). Mineral contents *viz.*, iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), copper (Cu) and manganese (Mn) were analyzed by atomic absorption spectrometry whereas; sodium (Na) and potassium (K) were estimated by flame photometry and phosphorus (P) using spectrophotometry, according to the methods of AOAC (2012).

**Radical scavenging activities and MTT assay**

**Extraction**

Samples (10 g) were extracted with 100 ml of aqueous methanol (80%, v/v) at ambient temperature with agitation for 18-24 hr.

The extracts were filtered using Whatman No. 1 filter paper and the filtrates were separately concentrated to dryness by using a rotary evaporator (Buchi, Switzerland) and aliquots (10 mg/ml) were analysed for free radical scavenging activity and MTT activity.

**DPPH (2,2-diphenyl-2-picryl hydrazyl)**

DPPH activity was examined as according to Blois (1958).

Solution mixtures of 100 µl of sample extracts and 3.9 ml of 0.5 mM DPPH were incubated for 30 min at 25°C in dark. The discolorisation of the purple colour was measured at 518 nm and percent scavenging activity was calculated.
ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)]

ABTS was determined by using the method of Re et al., (1999). ABTS radical cations (ABTS+) were produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium per sulphate (kept in the dark for 12-16 hrs before use). The absorbance was read at 745 nm after 10 min after adding 20 μl extract to 980 μl of ABTS solution and the per cent inhibition was calculated.

MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]

MTT assay was carried out using the protocol derived by Lui and Nair (2010) with some modifications. Stock solutions of extracts were prepared in DMSO at 40 mg/ml and MTT was dissolved in water at 0.5 mg/ml. The two solutions were added in a glass vial at 1:1 v/v and mixed thoroughly for 1 min. The reaction mixture was then incubated at 37°C for 6 hr. The absorbance was measured at 570 nm. Ascorbic acid, Gallic acid and Trolox were used as positive control.

Statistical analyses

The experiment was conducted in completely randomized design (CRD). The data were analysed statistically following the analysis of variance (ANOVA) and significance was tested at probability level P ≤ 0.01. All data represented are mean of three replications with duplicate determinations.

Results and Discussion

Effect of microwave cooking on proximate composition of tree bean

Table 1 showed that moisture content in tree bean pods and seeds under conventional and microwave cooking was significantly higher than uncooked control, which is in accordance to the findings of Lewu et al., (2009). However, ash, crude fat, crude fibre, crude protein and energy showed a decreasing trend under cooking either ways. Microwave cooked tree bean seed at 600W appeared to be with higher ash content (3.43% in seeds and 4.20% in pods) among the cooking treatments with lower decrease over uncooked control (Table 1). Similarly, crude fibre (9.74% in seeds and 5.53% in pods) and crude protein (4.96% in seeds and 2.51% in pods) was less affected under microwave cooking at 600W while compared with control (Table 1). On the contrary, fat content (1.13-1.37% in seeds and 0.35-0.88% in pods) was highly decreased under microwave cooking in comparison to conventional cooking (Table 1). The decrease in proximate content could be due to diffusion of the contents into cooking water (Alajaji and El-Adawy, 2006). However, nitrogen free extracts and energy showed non-significant differences among the cooking treatments in this study although there was a significant variation between the cooked and uncooked tree bean samples. Similar observations were reported in Irish and Sweet potato (Ikanone and Oyeka, 2014). Proximate content was found to be significantly affected by cooking procedure depending on the individual compounds (Hwang et al., 2012). Overall results indicated that microwave cooking at 600W exhibits comparatively higher proximate compositions in tree bean among all the cooking treatments.

Effect of microwave cooking on Mineral composition of tree bean

Mineral contents (N, P, K, Na, Ca, Mg, Fe, Zn, Cu and Mn) in cooked tree bean were significantly decreased while compared with uncooked samples (Table 2). The rate of decrease in mineral contents was comparatively lower at microwave cooking at 600W followed by 1000W.
Table 1 Proximate composition of tree bean seeds and pods as influenced by traditional and microwave cooking

| Treatments                      | Moisture content (%) | Ash content (%) | Crude Fat (%) | Crude Fibre (%) | Crude Protein (%) | NFE (%) | Energy (Kcal/g FW) |
|---------------------------------|----------------------|-----------------|---------------|-----------------|-------------------|---------|-------------------|
| Uncooked seeds                  | 62.59±3.57           | 3.80±0.44       | 5.82±0.18     | 5.63±1.36       | 8.23±0.35         | 13.93±0.35 | 140.98±18.00     |
| Traditional cooked seeds (10 min) | 79.05±1.75           | 2.50±0.46       | 1.62±0.68     | 8.73±1.01       | 4.67±0.27         | 3.43±2.87 | 46.99±8.05       |
| Traditional cooked seeds (20 min) | 81.33±4.28           | 2.33±0.29       | 1.82±0.56     | 4.47±0.42       | 3.97±0.27         | 6.09±3.98 | 56.58±20.50      |
| MW cooked(600W) seeds           | 76.33±1.75           | 3.43±0.45       | 1.37±0.71     | 9.74±4.05       | 4.96±0.27         | 4.17±5.47 | 48.83±26.33      |
| MW cooked(1000W) seeds          | 78.61±4.28           | 3.37±0.83       | 1.13±0.32     | 6.26±3.16       | 4.67±0.36         | 5.97±5.73 | 52.73±18.68      |
| Uncooked pods                   | 58.55±1.22           | 4.70±0.53       | 2.08±1.13     | 13.73±0.45      | 4.67±0.44         | 16.26±0.80 | 102.47±8.49      |
| Traditional cooked pods (10 min) | 76.43±3.15           | 4.13±0.91       | 0.73±0.28     | 7.57±4.60       | 2.16±0.20         | 8.98±5.83 | 51.13±24.56      |
| Traditional cooked pods (20 min) | 71.15±10.55          | 2.93±0.85       | 0.73±0.33     | 6.50±0.75       | 2.10±0.17         | 16.58±11.09 | 81.32±45.48    |
| MW cooked(600W) pods            | 73.71±3.15           | 4.20±0.52       | 0.88±0.53     | 5.53±1.63       | 2.51±0.36         | 13.16±4.31 | 70.63±17.67      |
| MW cooked(1000W) pods           | 68.43±10.55          | 3.83±0.93       | 0.35±0.35     | 5.93±2.55       | 2.39±0.20         | 19.06±13.77 | 88.95±53.37     |

SEm
- Moisture content: 3.16, Ash content: 0.38, Crude Fat: 0.33, Crude Fibre: 1.42, Crude Protein: 0.17, NFE: 3.97, Energy: 16.08
- CD (0.05): Moisture content: 9.32, Ash content: 1.12, Crude Fat: 0.97, Crude Fibre: 4.19, Crude Protein: 0.51, NFE: NS, Energy: 47.42
- CD (0.01): Moisture content: 12.71, Ash content: 1.53, Crude Fat: 1.33, Crude Fibre: 5.71, Crude Protein: 0.70, NFE: NS, Energy: NS

All data is mean of three replicates with duplicate determinations. Different letters represent significant difference (P ≤ 0.01) among the treatments according to Duncan’s multiple range test (DMRT).
Table 2: Mineral contents of tree bean seeds and pods as influenced by traditional and microwave cooking

| Treatments                        | N (%)   | P (ppm)  | K (ppm)  | Na (ppm) | Ca (ppm) | Mg (ppm) | Fe (ppm) | Zn (ppm) | Cu (ppm) | Mn (ppm) |
|----------------------------------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Uncooked seeds                   | 1.32±0.06 | 85.69±7.02 | 1568.0±121.24 | 303.33±2.89 | 1424.08±199.06 | 102.08±7.09 | 82.02±3.72 | 1.45±0.10 | 11.33±1.10 | 6.67±0.29 |
| Traditional cooked seeds (10 min)| 0.75±0.04 | 21.37±2.23 | 966.0±46.86 | 208.33±11.55 | 533.93±72.88 | 73.22±1.83 | 4.88±0.53 | 0.63±0.10 | 2.38±0.34 | 1.92±0.20 |
| Traditional cooked seeds (20 min)| 0.63±0.04 | 20.00±1.76 | 868.0±69.54 | 188.33±2.89 | 477.15±73.85 | 71.53±5.23 | 3.27±0.49 | 0.38±0.03 | 1.65±0.28 | 1.13±0.33 |
| MW cooked (600W) seeds           | 0.79±0.04 | 54.31±0.90 | 1514.0±336.50 | 258.33±11.55 | 798.97±58.11 | 88.22±1.83 | 6.77±2.82 | 1.02±0.13 | 2.72±1.05 | 3.18±0.87 |
| MW cooked (1000W) seeds          | 0.75±0.06 | 32.35±1.56 | 1102.0±141.01 | 238.33±2.89 | 688.80±174.01 | 86.53±5.23 | 5.62±2.94 | 0.78±0.03 | 2.32±1.34 | 2.17±0.75 |
| Uncooked pods                    | 0.75±0.07 | 90.39±10.30 | 2428.0±55.75 | 300.00±13.23 | 1405.80±82.49 | 94.77±1.18 | 36.87±7.79 | 1.40±0.30 | 6.95±0.25 | 4.85±0.49 |
| Traditional cooked pods (10 min) | 0.35±0.03 | 35.49±2.45 | 1644.0±156.92 | 175.00±10.00 | 539.50±42.72 | 68.73±4.33 | 6.17±1.91 | 0.48±0.10 | 1.35±0.39 | 1.55±0.35 |
| Traditional cooked pods (20 min) | 0.34±0.03 | 27.06±1.56 | 966.0±46.86 | 171.67±7.64 | 399.60±70.06 | 64.13±2.33 | 4.87±1.38 | 0.35±0.05 | 0.62±0.25 | 1.50±0.13 |
| MW cooked (600W) pods            | 0.40±0.06 | 44.51±1.48 | 2074.0±225.88 | 225.00±10.00 | 758.67±150.28 | 82.07±2.39 | 7.33±0.20 | 0.87±0.13 | 1.65±0.30 | 2.98±0.28 |
| MW cooked (1000W) pods           | 0.38±0.03 | 40.59±2.35 | 1890.0±421.03 | 223.33±7.64 | 510.95±21.87 | 75.80±3.88 | 4.68±1.10 | 0.77±0.20 | 1.53±0.73 | 1.83±0.19 |
| SEm                              | 0.03     | 2.47      | 117.34    | 5.11      | 63.42     | 2.29      | 1.81      | 0.08      | 0.42      | 0.26      |
| CD (0.05)                        | 0.08     | 7.28      | 346.11    | 15.07     | 187.05    | 6.77      | 5.34      | 0.24      | 1.23      | 0.77      |
| CD (0.01)                        | 0.11     | 9.93      | 472.04    | 20.56     | 255.11    | 9.23      | 7.29      | 0.33      | 1.67      | 1.05      |
Table 3: Effect of traditional and microwave cooking on radical scavenging activities and MTT assay of tree bean

| Treatments                        | DPPH (% activity) | ABTS (% activity) | MTT (Abs) |
|-----------------------------------|-------------------|-------------------|-----------|
| Uncooked seeds                    | 83.07±1.14        | 53.66±1.90        | 2.04±0.09 |
| Traditional cooked seeds (10 min) | 52.23±0.83        | 41.86±9.88        | 1.20±0.05 |
| Traditional cooked seeds (20 min)| 41.18±0.70        | 28.80±10.33       | 1.27±0.08 |
| MW cooked (600W) seeds            | 54.32±2.20        | 65.79±1.74        | 1.32±0.07 |
| MW cooked (1000W) seeds           | 43.96±1.04        | 57.72±0.97        | 1.53±0.12 |
| Uncooked pods                     | 89.51±0.50        | 68.56±2.44        | 2.67±0.28 |
| Traditional cooked pods (10 min)  | 52.76±0.87        | 63.73±8.05        | 1.49±0.11 |
| Traditional cooked pods (20 min)  | 30.59±1.59        | 54.39±2.58        | 0.91±0.06 |
| MW cooked (600W) pods             | 67.31±1.17        | 64.21±0.63        | 1.53±0.08 |
| MW cooked (1000W) pods            | 45.21±0.68        | 58.92±1.13        | 1.19±0.09 |

Higher degradation in mineral compositions was observed in traditional cooking for 20 minutes followed by cooking for 10 minutes. Cooking suppresses the mineral content in vegetables which may be minimized for ensuring better nutritive values in foods (Onyeike et al., 2003; Alajaji et al., 2006; Ikanone and Oyeka, 2014). Ash content represents nutritionally important minerals in food materials, hence, reduction of essential mineral in the food coupled with decrease in ash content under cooking (Onyeike and Oguike, 2003). Microwave cooking possessed greater retention of all minerals than that of boiling in various vegetables (Alajaji and El-Adawy, 2006), which is similar to the result of this experiment.

**Effect of microwave cooking on radical scavenging and MTT activity of tree bean**

DPPH activity in tree bean seeds and pulps was decreased in both traditional and microwave cooking while compared with uncooked samples. It is noteworthy that, microwave cooking at 600W had higher DPPH activities (54.32% in seeds and 67.31 in pods) among the cooking treatments (Table 3). Conventional cooking method by boiling led to detrimental effect in DPPH activity (Chuah et al., 2008). Ilyasoğlu and Burnaz (2015) reported microwave cooking as a better cooking process as compared to the traditional boiling treatment. Antioxidant capacities depend on the cooking procedures and vegetable structure (Ozyurt and Goc, 2013). Ng et al., 2011 reported that decreased in DPPH radical scavenging activity is correlated with the degradation of phenolic compounds owing to cooking.

Cooking for longer duration decreased radical scavenging activities such as DPPH and ABTS activities. ABTS activity was significantly higher in the seeds (65.79%) and pods (64.21%) while cooked in microwave at 600W which was observed to be lower in traditional boiling for 20 minutes (Table 3). Jiménez-Monreal et al., (2009) reported that microwave cooking led to lowest losses in radical scavenging assay and boiling for longer time lead to highest loss. According to
Hwang et al., (2012), cooking factors, including method, temperature, cooking time, and portion size, strongly affects the antioxidant activities in food.

MTT assay is a colorimetric assay for assessing cell metabolic activity which observed to be decreased both in traditional and microwave cooking processes. The activity appeared to be better in microwaved seeds (1.53 Abs at 1000W followed by 1.32 Abs at 600W) and pods (1.53 Abs at 600W) compared to that of uncooked pulp (Table 3). The least MTT activity was observed in conventional cooking (0.91-1.49 Abs). Previous studies indicated that antioxidant capacities of vegetables were decreased under thermal treatments (Ismail et al., 2004; Roy et al., 2007; Podsdek, 2007; Faller and Fialho, 2009). Inactivation of antioxidant compounds due thermal treatment could be the probable reason for decreased assays (Turkmen et al., 2005; Faller and Fialho, 2009).

Robinson et al., 2017 reported that antioxidant and free radical scavenging activity of the extract influences its anti-cancer properties which indicate that microwave cooking might be leading to have better antioxidant and cell metabolic activities as compared to other cooking methods.

In this study, microwave cooking exhibited comparatively better proximate composition, mineral contents and radical scavenging activities in tree bean seeds and pods over traditional cooking methods. Cooking by traditionally boiling techniques for longer duration resulted in maximum loss of nutrients. Hence, it could be concluded that microwave cooking not only saves time but also effectively retains the nutritive values in the food. The result of this study shed light on preventing nutritional loss in dietary supplements with appropriate cooking methods.

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