



Nutritional Composition, Total Polyphenol Content, and Antioxidant Activity of Traditionally Processed Parole (*Vigna unguiculata*) Seed—A Wild Cowpea of Bangladesh

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ABSTRACT

This study evaluated the effects of boiling and pressure cooking on the nutritional composition, total polyphenol content (TPC), and antioxidant activity of unconventional cowpea ("parole") from the Chittagong region of Bangladesh. Parole seeds subjected to pressure cooking exhibited a higher proximate composition than those cooked by boiling. Raw seeds contained higher protein (25.47%), fat (3.87%), and ash (4.47%) than processed samples, with boiling causing the greatest reduction (17.46%, 2.84%, and 3.22%, respectively). Pressure cooking better retained nutrients (20.22% protein, 2.98% fat, 3.71% ash). Carbohydrate content increased in boiled samples (61.45%) compared to raw (51.38%) and pressure-cooked (58.58%) parole seeds. Thermal processing reduced calcium and magnesium levels but did not significantly affect moisture or energy content. TPC decreased most markedly in boiled samples ($1.06 \pm 0.02 \mu\text{g}/\text{mg}$), followed by pressure-cooked ($1.51 \pm 0.10 \mu\text{g}/\text{mg}$) and raw samples ($2.76 \pm 0.08 \mu\text{g}/\text{mg}$), representing reductions of 61.59% and 45.28%, respectively. In contrast, antioxidant activity remained relatively constant across groups, with values of 71.03% for boiled samples, 72.76% for pressure-cooked samples, and 72.83% for raw seeds. The findings suggest that boiling and pressure cooking enhanced antioxidant activity despite reducing polyphenols and certain nutrients. Further research should optimize processing methods to improve nutrient retention and explore value-added product development.



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1. Introduction

Legumes are a vital and cost-effective source of dietary protein and fiber, second only to cereals in global human nutrition, serving as an affordable alternative to animal protein for millions in developing nations by providing high-quality protein (Zhang et al. 2024), essential carbohydrates, minerals, and trace elements (Duke 1981; Maphosa and Jideani 2017).

Cowpea or parole (*Vigna unguiculata* (L.) Walp) serves as a key protein source in tropical Africa (Abebe and Alemayehu 2022) and other regions, despite its classification as a minor pulse. Its seeds are nutritionally dense, offering high-quality protein (~25%), dietary fiber,

and a rich profile of essential amino acids, particularly lysine (Ferreira et al. 2022). This makes cowpea an ideal complementary food for cereal-based diets, improving overall protein quality.

Cowpea contains various bioactive compounds, including phenolic acids and flavonoids, which contribute to its antioxidant properties and associated health benefits, such as reduced risk of chronic diseases (Apea-Bah et al. 2017, Sombié et al. 2018). However, these compounds can also act as anti-nutritional factors, impairing the bioavailability of essential nutrients (Villavicencio et al. 2000).

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Traditional processing methods, such as boiling and pressure cooking enhance digestibility, inactivate anti-nutritional factors, and improve the overall nutritional profile by increasing insoluble fiber (Deol and Bains 2010; Veena et al. 1995). While these methods can reduce levels of specific bioactive compounds and may degrade some heat-sensitive nutrients, the net effect is a significant improvement in the food's nutritional value and safety.

Despite extensive research on legume processing, critical gaps remain in understanding how specific cooking methods (e.g., boiling vs. pressure cooking) influence the antioxidant activity, phenolic content, and nutrient retention of understudied cowpea cultivars like parole. This study aimed to analyze the proximate composition (moisture, protein, fat, fiber, ash, carbohydrates, energy) of parole seeds subjected to boiling and pressure cooking, quantify mineral content (Ca, Mg) in processed flour, and evaluate total phenolic content and antioxidant activity to determine the impact of these processing techniques. By addressing these objectives, the study seeks to optimize parole's nutritional utility, offering actionable insights to enhance its role in combating protein-energy malnutrition, a persistent challenge in Bangladesh and similar regions where affordable, nutrient-dense foods are urgently needed.

2. Materials and Methods

2.1. Collection and preparation of parole seed samples

Raw parole seeds were collected from the Chittagong region. The seeds were stored at 4°C until processing. Raw samples were prepared by manually dehulling 100 g of raw parole seeds. Boiled samples were subject to boiling for 45 min in tap water at 100°C in the ratio 1:10 (w/v) on a gas burner until it became soft when touched with finger, and then dehulled. Pressure-cooked samples were subject to cooking in tap water (1:10, w/v) at 15 psi for 35 minutes on a gas burner until it became soft when touched with finger, and then dehulled. All samples were then dried at 60°C for 92 hours, cooled in a desiccator, and ground into a fine powder.

2.2. Proximate analysis

2.2.1. Moisture content determination

Moisture content was determined following AOAC (2012) guidelines, where 5 g of powdered sample was oven-dried at 100°C for 24 hours and reweighed. The moisture percentage was calculated using the standard gravimetric formula:

$$\text{Moisture (\%)} = \left[\frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \right] \times 100$$

2.2.2. Ash content determination

The ash content was determined following the AOAC (2012) guidelines, where a 2 g oven-dried sample was ignited using a gas burner until white smoke ceased, then transferred to a muffle furnace and burned at 550°C until a light gray ash was obtained. After cooling in a desiccator,

the sample was weighed, and the ash content (%) was calculated using the formula:

$$\text{Ash (\%)} = \left(\frac{\text{Weight of residue}}{\text{Weight of sample}} \right) \times 100$$

2.2.3. Protein content determination

The protein content was determined by using the Kjeldahl method, as outlined in Rangana (2005). The following formula was used for the calculation:

$$\text{Protein (\%)} = [\text{Nitrogen Content (\%)} \times 5.55]$$

2.2.4. Crude fat content determination

The crude fat content was determined using the AOAC (2005) method with a Soxhlet extraction apparatus. The following formula was used to calculate the crude fat content:

$$\text{Crude Fat (\%)} = \left(\frac{\text{Weight of Extracted Lipids}}{\text{Weight of Sample}} \right) \times 100$$

2.2.5. Crude fiber content determination

The crude fiber content was determined by (AOAC, 2012) boiling 2 g of the sample successively in 1.25% sulfuric acid and 1.25% sodium hydroxide to remove soluble components, followed by drying, ashing in a muffle furnace (550°C), and weighing. The crude fiber content (%) was calculated using the formula:

$$\text{Crude Fiber (\%)} = \left(\frac{\text{Weight of Residue}}{\text{Weight of Sample}} \right) \times 100$$

2.2.6. Total carbohydrate determination

The total carbohydrate content was determined using the method as described by Srivastava and Sanjeev (Srivastava and Sanjeev 2002). The following formula was used to calculate the carbohydrate content:

$$\begin{aligned} \text{Total Carbohydrate (\%)} &= 100 - [\text{Moisture (\%)} \\ &+ \text{Crude Protein (\%)} + \text{Crude Lipid (\%)} \\ &+ \text{Crude Fiber (\%)}] \end{aligned}$$

2.2.7. Energy content determination

The total energy content was calculated using the following formula (Souci et al. 2000):

$$\text{Energy} = [9 \times (\text{g fat}) + 4 \times (\text{g protein}) + 4 \times (\text{g carbohydrate})]$$

2.3. Calcium and magnesium content determination

The calcium and magnesium contents of the sample were determined using the method described by AOAC (1995).

For calcium content, the following equation was used:

$$\begin{aligned} \text{Calcium Content (mg/100g)} &= \left(\frac{\text{mg of Calcium Obtained}}{\text{Weight of Sample}} \right) \times 100 \end{aligned}$$

For magnesium content, the following equation was used:

$$\text{Magnesium Content (mg/100g)} = \frac{\text{(mg of Magnesium Obtained)}}{\text{/Weight of Sample}} \times 100$$

2.4. Total phenolic content (TPC) determination

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Slinkard and Singleton 1977). A primary stock solution of gallic acid was prepared at 1000 µg/ml, while the sample extract was obtained by mixing 1 g of cowpea with 10 ml of methanol overnight, followed by filtration and drying. Calibration was performed by diluting the stock to concentrations of 50, 100, 150, 250, and 500 µg/ml, with absorbance measured at 725 nm against a reagent blank to construct the standard calibration curve. 1 mL of sample (methanolic extract) was mixed with 9 mL distilled water in a 25 mL volumetric flask, followed by the addition of 1 mL Folin-Ciocalteu reagent. After 5 minutes, 10 mL of 7% sodium carbonate solution was added, and the volume was adjusted to 25 mL with distilled water. The mixture was incubated for 90 minutes at room temperature, and the absorbance was measured at 765 nm against a reagent blank. The phenolic content was quantified using a gallic acid standard calibration curve, and results were expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract (µg GAE/mg extract).

2.5. Total antioxidant activity determination

The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (Brand-Williams et al. 1995), with slight modifications. A 0.004% (w/v) solution of DPPH in methanol was prepared, and absorbance was measured at 517 nm. The solution was stored covered under cool conditions. Serial dilutions of the crude extract were prepared in Falcon tubes by mixing 1, 2, 3, 4, and 5 mL of the sample extract with 9, 8, 7, 6, and 5 mL of methanol, respectively. 1 mL from each dilution was transferred to separate tubes, mixed with 3 mL of the DPPH solution, and the volume in each tube was adjusted to 10 mL with 99% methanol. The mixtures were vortexed and incubated in the dark for 30 minutes. After incubation, absorbance was measured at 517 nm. All measurements were performed in triplicate. The percentage inhibition of DPPH by extracts was calculated by using following formula:

$$\text{Inhibition} = (A - B) \times \left(\frac{100}{A}\right)$$

Where A is the absorbance of pure DPPH in oxidized form and B is the absorbance of sample taken after 30 minutes of reaction with DPPH

2.6. Statistical analysis

The study followed a completely randomized design with triplicate measurements. Statistical analysis was performed using one-way analysis of variance (ANOVA). Where significant differences were found, means were compared using Tukey's HSD post-hoc test at a 95% confidence level (*p* < 0.05).

3. Results

3.1. Proximate composition of parole samples

The proximate composition (moisture, protein, ash, fat, fiber, total carbohydrates, and energy content) of parole samples was analyzed, and the results are presented in Table 1. The application of high pressure and temperature did not significantly ($p > 0.05$) affect moisture content, with values of $13.74 \pm 0.33\%$ (raw), $13.61 \pm 0.16\%$ (pressure-cooked), and $14.04 \pm 0.18\%$ (boiled), where the boiled sample exhibited the highest moisture. Significant ($p < 0.05$) reduction in protein content occurred. Raw ($25.47 \pm 0.86\%$), pressure-cooked ($20.22 \pm 0.25\%$), and boiled ($17.46 \pm 0.49\%$) samples was showing decreasing retention. Reduction of protein is due to heat treatment. Because, heat cause protein to denature and make some protein more soluble that are leaches out into cooking water (Belitz et. al., 2009). Similarly, fat content significantly ($p < 0.05$) decreased from $3.87 \pm 0.09\%$ (raw) to $2.98 \pm 0.25\%$ (pressure-cooked) and $2.84 \pm 0.07\%$ (boiled). Ash content also declined significantly ($p < 0.05$), with raw ($4.47 \pm 0.19\%$), pressure-cooked ($3.71 \pm 0.10\%$), and boiled ($3.22 \pm 0.11\%$) samples, while fiber content remained unaffected ($p > 0.05$) across treatments (raw: $1.07 \pm 0.05\%$, pressure-cooked: $0.91 \pm 0.07\%$, boiled: $0.99 \pm 0.11\%$). Carbohydrate content increased significantly ($p < 0.05$) from $51.38 \pm 1.11\%$ (raw) to $58.58 \pm 0.54\%$ (pressure-cooked) and $61.45 \pm 0.16\%$ (boiled), likely due to the reduction in other macronutrients. Despite these changes, energy content showed no significant ($p > 0.05$) differences, with raw (342.23 ± 0.58 kcal), pressure-cooked (342.02 ± 0.00 kcal), and boiled (341.2 ± 1.15 kcal) samples maintaining consistent energy values.

3.2. Mineral composition of parole samples upon boiling and pressure cooking

The calcium content (mg/100 g) in raw, pressure-cooked, and boiled samples is presented in Table 2. The raw sample contained the highest calcium content (1.06 ± 0.01 mg/100 g), followed by pressure-cooked (1.05 ± 0.01 mg/100 g) and boiled (1.03 ± 0.01 mg/100 g) samples. A significant ($p < 0.05$) reduction was observed due to heat treatment and cooking duration.

Magnesium levels (mg/100 g) varied significantly ($p < 0.05$) among samples. The raw sample exhibited the highest magnesium content (2.30 ± 0.10 mg/100 g), while pressure-cooked and boiled samples contained 1.96 ± 0.02 mg/100 g and 1.64 ± 0.02 mg/100 g, respectively. The decrease in magnesium was attributed to heat-induced nutrient loss.

3.3. Total phenolic content (TPC)

The total phenolic content (TPC, µg/mg) in raw, pressure-cooked, and boiled parole samples (Table 3) revealed that thermal treatments significantly ($p < 0.05$) reduced TPC levels, with the raw sample exhibiting the highest content (2.76 ± 0.08 µg/mg), followed by pressure-cooked (1.51 ± 0.10 µg/mg) and boiled (1.06 ± 0.02 µg/mg) samples, representing reductions of 45.28% and 61.59%, respectively.

Table 1. Proximate composition of raw and processed parole seeds

Proximate composition	Raw sample	Pressure cooked sample	Boiled sample
Moisture (%)	13.74± 0.33 ^a	13.61±0.16 ^a	14.04±0.18 ^a
Protein (%)	25.47± 0.86 ^a	20.22±0.25 ^b	17.46±0.49 ^c
Fat (%)	3.87± 0.09 ^a	2.98 ± 0.25 ^b	2.84±0.07 ^c
Ash (%)	4.47± 0.19 ^a	3.71 ± 0.10 ^b	3.22±0.11 ^c
Fiber (%)	1.07±0.05 ^a	0.91± 0.07 ^a	0.99±0.11 ^a
Total carbohydrate (% by difference)	51.38± 1.11 ^c	58.58± 0.54 ^b	61.45±0.16 ^a
Energy (kcal/100g)	342.23±0.58 ^a	342.02±.00 ^a	341.2±1.15 ^a

Data are the means ± standard deviations. Different superscript letters in rows indicate statistical differences.

Table 2. Mineral composition (mg/100g) of raw and processed parole seeds

Component	Raw sample	Pressure cooked seeds	Boiled seeds
Calcium	1.06±0.01 ^a	1.05±0.01 ^b	1.03±0.01 ^c
Magnesium	2.30±0.10 ^a	1.96±0.02 ^b	1.64±0.02 ^c

Data are the means ± standard deviations. Different superscript letters in rows indicate statistical differences.

Table 3. TPC (µg/mg) content of raw and processed parole seeds

Component	Raw sample	Pressure cooked seeds	Boiled seeds
TPC	2.76±.08a	1.51±.01b	1.06±.02c
% Difference		45.28%	61.59%

Data are the means ± standard deviations. Different superscript letters in rows indicate statistical differences.

Although phenolic compounds are generally heat-resistant, prolonged thermal processing caused significant ($p < 0.05$) degradation, demonstrating that boiling and pressure cooking markedly decrease TPC in parole seeds.

3.4. Antioxidant activity

The DPPH scavenging activity of parole samples showed that the boiled sample had the slight inhibition (71.03%), followed by pressure-cooked (72.76%) and raw (72.83%) samples, with thermal processing not significantly affecting antioxidant properties; pressure cooking (10 min) and boiling (30 min) showed constant scavenging activity compared to raw sample, indicating that processing methods distinctly alter antioxidant capacity, where pressure cooking samples still exhibited superior activity despite the overall decline compared to boiling (Figure 1).

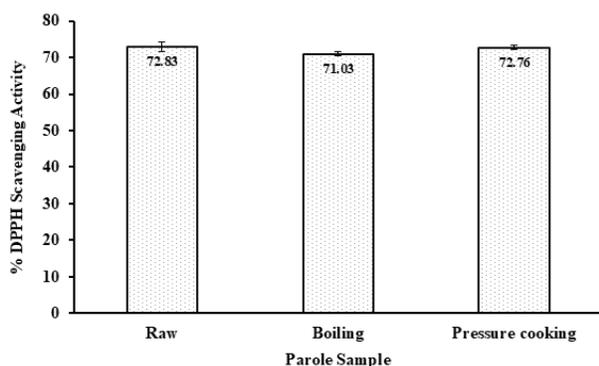


Figure 1. DPPH scavenging activity of parole seed samples

4. Discussion

4.1. Nutritional composition

Proximate analysis revealed significant variations in parole seed composition across treatments (Table 1). Raw samples contained the highest protein (25.47%), fat (3.87%), and ash (4.47%) content, while boiled samples showed the greatest nutrient reduction (protein 17.46%, fat 2.84%, ash 3.22%). Pressure-cooked samples exhibited intermediate values (protein 20.22%, fat 2.98%, ash 3.71%). Carbohydrate content followed an inverse pattern, with boiled samples containing the highest levels (61.45%) versus pressure-cooked (58.58%) and raw samples. Moisture content remained consistent (~14.04±0.18%) across treatments, exceeding the optimal 9-12% range for cowpea storage (Awosanmi et al. 2020), potentially affecting product stability through increased microbial risk and reduced compression strength.

The protein reduction (25.47% to 17.46%) due to thermal denaturation effects while maintaining peptide bonds (Fennema 1996). Fat content decreased (raw 3.81% to boiled 3.56%) progressively with processing intensity (Omenna et al. 2016). Ash content variation before and after cooking (3.34-4.47%) and (2.26 - 3.05%) respectively, reflected mineral loss during processing though remaining nutritionally significant. Fiber content showed no significant treatment differences ($p > 0.05$) (Soaud et al. 2021), possibly due to methodological or varietal factors. Energy content remained stable despite processing, with carbohydrate content (58.58-61.45%) within typical cowpea ranges (53-66%). These results confirm parole's nutritional robustness, with protein levels (17.46-25.47%) addressing protein-energy malnutrition while maintaining favorable fat profiles for cardiovascular health.

Processing methods differentially affected components, with boiling maximizing carbohydrates but reducing heat-sensitive nutrients most significantly. These results confirm cowpea's nutritional robustness, with protein levels (17.46-25.47%) addressing protein-energy malnutrition while maintaining favorable fat profiles for cardiovascular health. Processing methods differentially affected components, with boiling maximizing carbohydrates but reducing heat-sensitive nutrients most significantly.

4.2. Mineral composition of parole seed

The mineral composition of paroles (Table 2) revealed that heat processing led to nutrient loss, likely due to leaching during thermal treatment, with the raw sample containing the highest amounts of calcium (Ca: 1.06 ± 0.01 mg/100 g) and magnesium (Mg: 2.30 ± 0.10 mg/100 g), while boiled and pressure-cooked samples showed the lowest levels of these minerals; notably, the boiled sample had even lower Ca and Mg content than the pressure-cooked sample, possibly due to prolonged heating, a trend consistent with findings in *Mucuna flagellipes*, where boiling significantly reduced mineral content (Udensi et al. 2008), and with previous studies demonstrating a 23% loss of Mg in mature cowpeas after pressure cooking (Hefnawy 2011).

Calcium is vital for bone formation, muscle function, and nervous system regulation, while magnesium supports nerve and muscle function, regulates blood pressure and sugar levels, and aids in protein, bone, and DNA synthesis. Based on these findings, parole seeds can be considered a valuable dietary source of essential minerals.

4.3. Total phenolic content (TPC)

Parole seeds are a rich source of bioactive compounds, particularly phenolic compounds, which exhibit redox properties that contribute to their antioxidant activity; however, total phenolic content (TPC), determined through acidic hydrolysis, often shows an inverse relationship with antioxidant capacity, as higher TPC may correspond to reduced antioxidant activity due to structural changes in phenolic compounds, with hydroxyl groups in plant extracts playing a key role in free radical scavenging. As shown in Table 3, boiling parole seeds for 30 minutes resulted in a significant ($p < 0.05$) reduction in TPC (61.29%) compared to pressure cooking for 5 minutes (45.28%), with this decrease attributed to cooking time and temperature, as hydrothermal processing leaches phenolic compounds into the water. The raw sample exhibited the highest TPC content (2.76 $\mu\text{g}/\text{mg}$), while pressure cooking and boiling reduced levels to 1.51 $\mu\text{g}/\text{mg}$ and 1.06 $\mu\text{g}/\text{mg}$, respectively, with the greater loss in boiled samples linked to prolonged heat exposure. Conventional thermal processing methods (boiling, pressure cooking, autoclaving, and soaking) have been shown to reduce TPC in cowpeas depending on treatment duration and temperature (Yadav et al. 2018), as extended heat exposure degrades phenolic compounds. Given that TPC measurement involves acidic hydrolysis, this study confirmed a gradual decline in phenolic content with increasing cooking temperature and time.

4.4. Antioxidant activity

The DPPH radical scavenging activity of parole seed extracts showed constant antioxidant potential with thermal processing. Despite expected phenolic leaching, suggesting thermal processing enhances bioactive compound availability. Antioxidant activity is consistent with the findings of Yadav et al. (2018), who demonstrated that time-temperature parameters during thermal processing modulate antioxidant capacity through mechanisms such as cell wall disruption (enhancing phenolic release) and Maillard reaction product formation (contributing additional antioxidant effects). The superior activity in boiled samples implies prolonged heating may generate additional antioxidants that compensate for phenolic losses, which is nutritionally significant as these compounds combat free radicals implicated in chronic diseases like cardiovascular disorders and cancer through prevention, scavenging, and catalytic decomposition pathways, making thermal processing potentially most beneficial for oxidative stress mitigation.

5. Conclusion

This study demonstrates that conventional processing techniques, such as boiling and pressure cooking, significantly affects the nutritional profiles of parole. Both techniques reduced several key nutrients, including protein, fat, and minerals, which is attributed to leaching into cooking water and the degradation of heat-sensitive compounds. Conversely, carbohydrate contents increased, and antioxidant activity was preserved despite a decline in phenolic content. The retention of antioxidant activity may be attributed to the liberation of bound compounds, during thermal processing, which enhances their bioavailability. Given the role of parole as an affordable and nutrient-dense staple, optimizing the processing methods is crucial for enhancing its nutritional value and mitigating malnutrition, particularly in developing regions. Further product development should focus on refining these thermal processing to better preserve the inherent nutritional benefits of parole.

A limitation of this study is its focus on a single cowpea variety and two common thermal processing methods. Future research should explore a wider genetic diversity and alternative processing techniques.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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