



Impact of Polishing on Physicochemical Properties, Cooking Behavior, and Anthocyanin Content in *Njavara* Red Rice

Anju Augustine^{✉,1} Naomi Vincent^{✉,2} Shruti Joshi^{✉,2,*}

¹ Independent Researcher, Mysore 570005, India

² Department of Grain Science Technology, CSIR–Central Food Technological Research Institute (CFTRI), University of Mysore, Mysore 570005, Karnataka, India

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Abstract

The aim of this study was to assess the effect of rice polishing on the nutritional value, anthocyanin composition, and cooking performance of *Njavara* rice, a traditional red rice variety cultivated in the Kerala region of India. Red rice in its unpolished form was examined alongside 5% polished rice and 7% polished rice, including their corresponding bran fractions. Unpolished red rice retained the highest total phenolic content, with 8.565 mg GAE/g, compared with 8.180 mg GAE/g in 5% polished rice and 7.537 mg GAE/g in 7% polished rice. The highest total anthocyanin content was observed in the 7% bran fraction (8.28 ± 0.06 mg/100 g), followed by red rice (6.81 ± 0.05 mg/100 g), the 5% bran fraction (4.56 ± 0.08 mg/100 g), the 5% polished rice (3.51 ± 0.02 mg/100 g), and the 7% polished rice (2.53 ± 0.03 mg/100 g). The cooking properties showed that red rice required the longest cooking duration of 45 min, while 5% polished rice required 35 min and 7% polished rice required 30 min, with respective elongation ratios of 1.11, 1.18, and 1.21. The protein content was higher in red rice ($9.42 \pm 0.15\%$) as compared to $9.23 \pm 0.56\%$ found in 5% polished rice and $8.12 \pm 0.48\%$ present in 7% polished rice. The results demonstrated that polishing reduced the levels of bioactive compounds and antioxidant capacity while decreasing cooking time and increasing solid loss during cooking.

Keywords:

Njavara; anthocyanin; total phenolic content; pigmented rice varieties; differential scanning calorimetry

1. Introduction

Rice (*Oryza sativa* L.) is a staple crop for more than half of the world's population and accounts for over 20% of the global caloric intake, with Asia contributing approximately 90% of total worldwide rice production [1]. White rice is the most widely consumed rice variety worldwide. However, several Asian countries preferentially consume colored rice varieties, including red, brown, black, reddish-brown, and purple-black rice.

Among pigmented rice varieties, *Njavara* is particularly important in Kerala, India, owing to its traditional therapeutic applications in Ayurveda. *Njavara* rice, characterized by its white or black husks, has been widely used in traditional Indian folk medicine for centuries. De-

husked *Njavara* rice consists of approximately 73% carbohydrates, 9.5% protein, 2.5% fat, and 1.4% ash, and provides an energy value of 1628 kJ per 100 g [2]. However, milling removes a substantial proportion of rice nutrients because the embryo and bran layers removed during processing are rich in protein, fat, fiber, minerals, oryzanol, thiamine, and phenolic compounds. These nutrients play a crucial role in determining the nutritional and quality characteristics of rice [3].

Pigmented rice, including varieties with an intact bran layer and colored pericarps, such as black, purple, and red rice, possesses greater nutritional value than white rice [4]. The coloration is attributed to the presence of acetylated procyanidins, anthocyanins, and other phenolic compounds, which exhibit considerable free radical

* Corresponding Author:

Shruti Joshi, Department of Grain Science Technology, CSIR–Central Food Technological Research Institute (CFTRI), University of Mysore, Mysore 570005, Karnataka, India, shruti.cftri@csir.res.in



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scavenging activity [5,6]. Although pigmented rice has traditionally been used primarily in desserts and specialty foods prepared for festivals and religious offerings, it is increasingly being recognized as a delicacy and a valuable source of nutrients with medicinal and pharmacological potential [7]. Different rice varieties exhibit distinct grain colors and anthocyanin profiles, with both the composition and concentration of anthocyanins varying according to the rice variety. As rice processing progresses, both the intensity of rice coloration and the anthocyanin content decrease, as evidenced by the significant loss of color during the milling process [8–10]. Kato & Horibata [11] reported that the antioxidant activity of polished rice is lower than that of red rice. A lower milling degree, particularly around 4%, preserved higher levels of total anthocyanins while maintaining acceptable sensory quality. However, total anthocyanin loss ranged from 53% to 91% as the milling degree increased from 4% to 7% [12]. Additionally, at a milling degree of 9%, nearly 100% loss of anthocyanins was observed [8]. These findings indicate that increasing the milling degree not only results in greater nutrient loss but also demonstrates that anthocyanins are predominantly concentrated in the bran layer of colored rice. Polishing preferentially removes the aleurone and bran layers, leading to substantial anthocyanin losses of 53–91% at 4–7% milling and nearly complete loss at higher milling degrees. This process may also alter anthocyanin profiles, reducing the diversity of glycosidic compounds present in red rice. *Njavara* specifically loses red pigmentation and antioxidant capacity during processing, whereas the bran fractions retain the highest levels, with antioxidant capacity reported to be approximately 4–8 times greater than that of the grains [13].

Furthermore, Lu et al. [14] reported that changes in the water absorption capacity of rice during cooking may be associated with interactions between glutenin and starch [14]. Although *Njavara* has long been used in traditional medicine and as a functional food, limited scientific literature is available on how processing methods affect its nutritional and functional properties. Previous HPLC-HRMS studies have primarily focused on common black and red rice varieties, whereas the anthocyanin profile of *Njavara* and the effects of low-degree polishing (5–7%) on its cooking properties and nutrient composition remain largely unexplored. This investigation uniquely quantified the effects of polishing (0%, 5%, and 7%) on the nutrient composition, detailed anthocyanin profile determined by HPLC-HRMS, and cooking characteristics (cooking time and elongation) of *Njavara* rice. This study addresses the existing gap in varietal-specific data for minimally processed *Njavara* rice. Accordingly, different polishing degrees were evaluated to determine their influence on the

nutritional composition and cooking properties of *Njavara* rice. The present investigation further evaluates the effect of polishing on the anthocyanin profile of *Njavara* rice through high-performance liquid chromatography (HPLC) and high-resolution mass spectrometry (HRMS).

2. Materials and Methods

Njavara rice used for sample preparation was obtained from a local market in Mysore, India. Rice was stored at 4 °C in a cold storage room and packed in airtight bags to prevent any insect or pest contamination. All chemicals used for analysis were of analytical grade unless otherwise specified and were mostly sourced from Sigma.

2.1. Sample Preparation

Njavara rice was cleaned, dehusked using a dehusser, and polished to obtain 5% and 7% polishing degrees using a Satake rice polisher. The 5% and 7% polished rice samples were dried, pulverized, and passed through a 60-mesh sieve. The sieved samples, together with the bran fractions (5% and 7%), were collected in zip-lock bags and stored at 4 °C until further analysis.

2.2. Extraction of Anthocyanin Using Acidified Methanol

Anthocyanins were extracted according to the method described by Constantin (2022) [15], with slight modifications. Finely ground rice bran and rice flour samples were used for extraction. Acidified methanol, prepared by adding 1% (v/v) hydrochloric acid to methanol, was used as the extraction solvent. A solid-to-solvent ratio of 1:12.5 (w/v) was maintained by mixing 1 g of sample with 12.5 mL of the extraction solvent. The mixture was kept under static extraction conditions at 24 °C for 24 h to facilitate efficient solubilization of anthocyanins. After extraction, the mixture was centrifuged using an Eppendorf centrifuge (model 5430) at 2000 × g for 15 min. The clear supernatant was then carefully collected. To ensure maximum anthocyanin recovery, the extraction process was repeated three times on the residual pellet under identical conditions. The supernatants obtained from all extraction cycles were pooled and stored at 4 °C until further analysis.

2.3. Proximate Analysis

The proximate composition of the *Njavara* rice variety was analyzed according to the AOAC method (2016) [16], including the determination of moisture content by drying at 130 °C for 2 h and fat content by Soxhlet extraction. Ash content was determined by incinerating the sample in a muf-

fle furnace at high temperature (around 550 °C). Crude protein content, calculated by multiplying the total nitrogen content by a factor of 6.25, was determined using the Dumas method [17]. Total carbohydrates in *Njavara* rice were determined by difference, calculated as 100 minus the sum of moisture, crude protein, total fat, and ash percentages.

2.4. Cooking Properties

2.4.1. Cooking Time

The minimum cooking time of the milled rice was determined using the Ranghino test [18]. A 5 g rice sample was cooked in 20 mL of distilled water maintained at 90 °C in a water bath. The minimum cooking time was determined by pressing the cooked grains between two glass plates until no white core remained.

2.4.2. Equilibrium Moisture Content Attained by Rice Upon Soaking in Water at Ambient Temperature (EMC-S)

A measured quantity of rice, ranging from 5 to 15 g, was placed in a 100 mL beaker. Water was added, and the rice was soaked until equilibrium moisture content was reached. After the soaking period, the rice was removed from the water, and excess surface moisture was gently blotted with a paper towel. The soaked rice was then weighed, and the final weight was recorded to calculate the equilibrium moisture content.

2.4.3. Water Uptake and Loss of Solids

The rice samples were weighed and rinsed with cold water to remove surface starch before cooking. The rice was carefully drained using a mesh sieve. A measured volume of water was added for cooking at a defined rice-to-water ratio. After cooking, the rice and cooking water were separated, and the cooked grains were dried to determine water uptake and solid loss.

2.4.4. Elongation Ratio and Rings

The length of the cooked rice grains was measured using a Vernier caliper.

2.5. Bioactive Compound Analysis

2.5.1. Total Phenolic Content

The total phenolic content of red and polished rice samples was determined using the Folin–Ciocalteu (F–C) colorimetric method, following the procedure originally described by Martins et al. (2021) [19], with appropriate modifications.

Briefly, the sample extract was diluted five-fold with distilled water. An aliquot (0.2 mL) of the diluted extract was mixed with 6.0 mL of distilled water, followed by the addition of 0.5 mL of Folin–Ciocalteu reagent. After 3 min of reaction, 1.5 mL of 20% (w/v) sodium carbonate (Na₂CO₃) solution was added, and the final volume was adjusted to 10 mL with distilled water. The reaction mixture was incubated in the dark at room temperature for 15 min, after which the absorbance was measured at 765 nm against a reagent blank using a UV–Visible spectrophotometer. Gallic acid was used as the reference standard, and a calibration curve was constructed using a series of gallic acid standard solutions at different concentrations. The absorbance values were plotted against the corresponding gallic acid concentrations using Microsoft Excel (Figure 1). A linear regression equation and the corresponding coefficient of determination (R²) were subsequently obtained [20].

The total phenolic content was calculated from the calibration curve and expressed as mg gallic acid equivalents per g of sample (mg GAE/g) using the corrected formula:

$$\text{TPC (mg GAE/g)} = \frac{C \times V}{M}$$

where C is the concentration of phenolics obtained from the calibration curve (mg/mL), V is the volume of extract (mL), and M is the mass of the sample (g).

2.5.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

40 µL of sample extracts were mixed with 2.9 mL of DPPH solution (4 mg DPPH in 100 mL methanol), incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm [21].

Inhibition calculated as % = [(A_{control} – A_{sample})/A_{control}] × 100

2.5.3. Determination of Total Anthocyanin Content

The total anthocyanin content was determined as described by Anggraeni et al. (2019) [22]. Briefly, the sample extract was mixed with two buffer solutions, maintaining a maximum test portion-to-buffer ratio of 1:4. The absorbance of the diluted test portion was then measured at 520 nm and 700 nm using buffers at pH 1.0 and 4.5. Absorbance measurements were performed against the appropriate reagent blank. To ensure accurate determination of total anthocyanin content, absorbance was measured within 20–50 min after sample preparation.

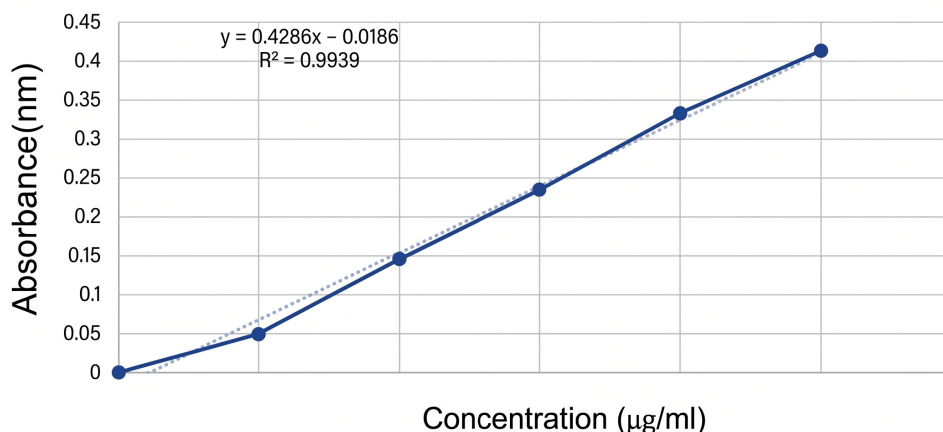


Figure 1: Standard graph of gallic acid.

2.6. Differential Scanning Calorimetry (DSC)

The analysis was performed using a PerkinElmer DSC 8000 (PerkinElmer, USA). A small amount of the rice sample (typically a few milligrams) was placed in an aluminum pan. The sample pan and the reference pan were placed in the DSC instrument. The experiment was started by heating or cooling the pans at a controlled rate (usually constant) while recording the heat flow between the sample and reference pans as a function of temperature.

2.7. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was performed using an EVO® LS 15 microscope (Germany) operated with SmartSEM™ software. During analysis, the sample was exposed to a high-voltage electron beam with an accelerating voltage ranging from 100 to 30,000 eV. SEM images were generated by scanning the electron beam across the sample surface. Secondary electron and/or backscattered electron signals were collected to obtain surface morphology images. SEM was used to observe the surface morphology, shape, size, and texture of the rice samples. The inclination of the sample surface affected not only the topographic contrast but also the intensity of the backscattered electron (BSE) and secondary electron (SE) signals, with surface inclinations between 50° and 70° resulting in enhanced signal intensity [23].

2.8. Quantification Profile

2.8.1. HPLC- Instrumentation and Chromatographic Conditions

The anthocyanin content of the rice bran and rice flour extracts was analyzed using a high-performance liquid

chromatography (HPLC) system equipped with a gradient pump (Shimadzu LC-10AVp). The chromatographic system was manufactured by Waters (Alliance series) and fitted with a UV-Visible detector. Prior to HPLC analysis, the pooled methanolic extracts were filtered through Whatman No. 2 filter paper, transferred into amber vials, and stored at 4 °C until analysis. Chromatographic separation was performed using a Kromasil C18 reversed-phase column (5 µm particle size, 100 Å pore size, 4.6 mm × 250 mm). The column temperature was maintained at 60 °C. The injection volume was 10 µL, and the flow rate was set at 1.0 mL min⁻¹. The mobile phase consisted of:

Solvent A: 0.1% (v/v) formic acid in water

Solvent B: 100% acetonitrile (HPLC grade)

A linear gradient elution program was employed as follows (Table 1):

Anthocyanins were detected using a UV-Vis detector set at 525 nm, corresponding to the maximum absorbance wavelength of anthocyanin pigments.

2.8.2. High-Resolution Mass Spectrometry (HRMS)

Further characterization and confirmation of anthocyanin compounds were performed using High-Resolution Mass Spectrometry (HRMS). The analysis was conducted using a Sciex QTRAP 6500/TripleTOF 5600 mass spectrometer (Sciex, USA). Samples were ionized using both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources. The instrument was operated in both positive and negative ionization modes. The generated ions were introduced into a hybrid quadrupole time-of-flight (Q-TOF) mass analyzer, where they were separated by mass-to-charge (m/z) ratio. Data acquisition and processing were conducted using Analyst TF software (Version 1.6). Mass spectra recorded predominantly in positive

ion mode provided detailed information on ion abundance and molecular mass, facilitating accurate identification of anthocyanin compounds present in the samples.

Table 1: Gradient elution program for HPLC analysis using solvent A and solvent B.

Time (min)	Solvent A (%)	Solvent B (%)
0	95	5
10	85	15
20	70	30
30	50	50
35	95	5
40	95	5

3. Results

3.1. Proximate Analysis of Njavara Rice

The proximate composition of *Njavara* rice and its polished variants (5% polished, 7% polished, and red rice) showed noticeable differences in moisture, fat, ash, and protein contents due to varying degrees of polishing. The results are presented in [Table 2](#). The moisture content ranged from $10.8 \pm 0.68\%$ in 5% polished rice to $11.8 \pm 0.46\%$ in red rice, indicating a gradual increase in moisture retention as polishing intensity decreased. Fat content was highest in red rice ($2.30 \pm 0.22\%$), followed by 5% polished rice ($1.30 \pm 0.26\%$) and 7% polished rice ($1.12 \pm 0.24\%$), highlighting the contribution of bran layers to lipid retention. Similarly, protein content was higher in red rice ($9.42 \pm 0.15\%$) and 5% polished rice ($9.23 \pm 0.56\%$) compared to 7% polished rice ($8.12 \pm 0.48\%$). Ash content, which reflects mineral concentration, also increased with lower polishing intensity, ranging from $1.09 \pm 0.58\%$ in 7% polished rice to $1.74 \pm 0.72\%$ in red rice. These findings indicate that red rice retained superior nutritional quality, whereas increased polishing resulted in progressive nutrient loss.

3.2. Cooking Properties of Rice

Cooking behavior varied among the rice samples ([Table 2](#)). Red rice required the longest cooking time (45 ± 1.25 min), whereas 7% polished rice exhibited the shortest cooking time (30 ± 1.23 min), followed by 5% polished rice (35 ± 1.26 min). The elongation ratio ranged from 1.11 ± 0.43 in red rice to 1.21 ± 0.25 in 7% polished rice, while 5% polished rice recorded an intermediate value of 1.18 ± 0.74 . Equilibrium moisture content under normal conditions ranged from $10.2 \pm 1.23\%$ to $11.2 \pm 1.42\%$, and under soaked conditions from $11.2 \pm 1.52\%$ to $12.1 \pm$

1.65% , with red rice showing comparatively higher moisture absorption. Water uptake was highest in 7% polished rice ($249.2 \pm 1.48\%$), followed by 5% polished rice ($241.7 \pm 1.56\%$), while red rice exhibited the lowest value ($211.4 \pm 1.79\%$). In contrast, solid loss during cooking was highest in 7% polished rice ($7.2 \pm 1.56\%$) and lowest in red rice ($2.5 \pm 1.26\%$). These findings suggest that increased polishing reduced cooking time and enhanced water absorption and elongation, but also increased solid loss during cooking.

3.3. Bioactive Compound Analysis

The bioactive compound analysis presented in [Table 2](#) revealed only slight variations in total phenolic content (TPC) among the samples. Red rice showed the highest TPC (8.565 ± 1.62 mg GAE/g), followed by 5% polished rice (8.180 ± 0.42 mg GAE/g) and 7% polished rice (7.537 ± 1.80 mg GAE/g). However, antioxidant activity, as determined by the DPPH and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, differed more markedly among the treatments. Red rice exhibited the highest antioxidant activity, with DPPH and ABTS radical scavenging activities of $95.2 \pm 1.26\%$ and $97.6 \pm 0.52\%$, respectively. In comparison, 5% polished rice showed values of $90.5 \pm 1.23\%$ (DPPH) and $92.3 \pm 1.53\%$ (ABTS), while 7% polished rice recorded $89.7 \pm 1.52\%$ and $91.5 \pm 1.24\%$, respectively. These findings indicate that reduced polishing helped preserve phenolic compounds and antioxidant potential, particularly in red rice.

3.4. Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) results presented in [Table 2](#) and [Figure 2a–c](#) demonstrated differences in the starch gelatinization behavior among the rice samples. Red rice exhibited the lowest onset temperature

(62.81 °C) and peak temperature (66.53 °C), whereas 7% polished rice showed the highest peak temperature (70.85 °C) and end temperature (74.17 °C). The onset temperature of 5% polished rice was 68.30 °C, with a peak temperature of 70.49 °C and an end temperature of 73.63 °C.

The higher gelatinization temperatures observed in the polished rice samples may be attributed to the removal of the bran layers and pigments, which exposed the crystalline starch structure and increased the thermal energy required for starch gelatinization.

Table 2: Physicochemical, thermal, cooking, and antioxidant properties of *Njavara* rice.

Analysis	5% Polished Rice	7% Polished Rice	Red Rice
Differential scanning calorimetry			
Onset temperature (°C)	68.30	68.32	62.81
Peak temperature (°C)	70.49	70.85	66.53
End temperature (°C)	73.63	74.17	73.09
Proximate Analysis			
Moisture (%)	10.8 ± 0.68 ^a	11.2 ± 0.35 ^a	11.8 ± 0.46 ^a
Ash (%)	1.30 ± 0.25 ^a	1.09 ± 0.58 ^a	1.74 ± 0.72 ^a
Fat (%)	1.3 ± 0.26 ^a	1.12 ± 0.24 ^a	2.3 ± 0.22 ^b
Protein (%)	9.23 ± 0.56 ^a	8.12 ± 0.48 ^b	9.42 ± 0.15 ^a
Cooking Properties			
Cooking time(min)	35 ± 1.26 ^b	30 ± 1.23 ^a	45 ± 1.25 ^c
Elongation ratio	1.18 ± 0.74 ^a	1.21 ± 0.25 ^a	1.11 ± 0.43 ^a
Equilibrium moisture			
Antioxidant profile			
Total Phenolic Content (mg GAE/g)	8.180 ± 0.42 ^a	7.537 ± 1.80 ^a	8.565 ± 1.62 ^a
DPPH (%)	90.5 ± 1.23 ^b	89.7 ± 1.52 ^b	95.2 ± 1.26 ^a
ABTS (%)	92.3 ± 1.53 ^b	91.5 ± 1.24 ^b	97.6 ± 0.52 ^a

3.5. Total Anthocyanin Content

Significantly lower anthocyanin contents were detected with increasing degrees of rice polishing (Table 3). The highest total anthocyanin content (TAC) was observed in the 7% bran fraction (8.28 ± 0.06 mg C3G/100 g), then red rice (6.81 ± 0.05 mg C3G/100 g) and 5% bran fraction (4.56 ± 0.08 mg C3G/100 g) followed. The polished rice samples exhibited substantially lower anthocyanin contents, with the 5% polished rice containing 3.51 ± 0.02 mg C3G/100 g and the 7% polished rice containing only 2.53 ± 0.03 mg C3G/100 g.

3.6. Surface Morphology

Red rice showed a rough and grainy surface, which may be attributed to the presence of intact bran and germ layers. The heterogeneous surface structure may reflect the presence of bran-associated particles and surface irregularities. The preservation of the bran layer may contribute to anthocyanin retention, thereby contributing to the grain's antioxidant properties and associated health benefits, as shown in Figure 3a. Smooth and uniform surfaces with minimal irregularities were characteristic of the polished rice samples. The polishing process removes the outer bran and germ

layers, which are major reservoirs of anthocyanins in pigmented rice. Moreover, the presence of pores, voids, and structural defects, such as cracks and fissures, was associated with the mechanical stress imposed during the polishing process. The particles of bran and germ that sometimes can be seen on the polished rice's surface are the traces of the anthocyanin-rich layers that were lost during the milling process, as shown in Figure 3b. Although polished rice is often preferred for culinary applications because of its smoother appearance, the nutritional advantages of red rice, particularly those associated with its anthocyanin content, are likely to appeal to health-conscious consumers.

3.7. Analysis and Quantification of Anthocyanin by HPLC

The HPLC analysis of *Njavara* rice samples identified cyanidin-3-glucoside as the predominant anthocyanin compound, as shown in Table 4. Quantitative analysis revealed the highest concentration in red rice (418.6 µg/100 g; Peak 6; retention time, 13.885 min; peak area, 36,965). The 5% bran fraction contained 364.4 µg/100 g (Peak 10, retention time, 13.199 min; peak area, 32,183), whereas the 7% bran fraction contained 191.7 µg/100 g (Peak 3, retention time, 13.786 min; peak area, 16,930). Cyanidin-3-

glucoside content was markedly lower in the polished rice samples, with 5% polished rice containing 34.9 $\mu\text{g}/100\text{ g}$ and 7% polished rice containing the lowest concentration, 3.5 $\mu\text{g}/100\text{ g}$ (Peak 13; retention time, 13.656 min; peak area, 305). The results of the study demonstrate that cyanidin-3-glucoside is progressively depleted during polishing, indicating that this anthocyanin is predominantly localized in the outer bran layers, which are removed during polishing. The corresponding HPLC chromatograms are presented in the [Supplementary Material](#) as [Figures S1–S5](#).

3.8. Identification and Characterization by High-Resolution Mass Spectrometry (HRMS)

High-resolution mass spectrometry (HRMS) analysis of the *Njavara* rice samples confirmed the presence of key anthocyanin compounds based on their observed mass-to-charge ratios (m/z), which closely matched theoretical values. Cyanidin 3-glucoside was identified with an observed m/z of 287.05, corresponding closely to its theoretical m/z of 287 and molecular formula $\text{C}_{21}\text{H}_{21}\text{ClO}_{11}$. Pelargonidin 3-O-glucoside was identified with an observed m/z of 271.05, matching the theoretical m/z of 271 ($\text{C}_{21}\text{H}_{21}\text{ClO}_{10}$). Peonidin 3-O-glucoside was detected with an observed m/z of 301.00, consistent with its theoretical m/z of 301 and molecular formula $\text{C}_{22}\text{H}_{23}\text{O}_{11}\text{Cl}$ ([Table 5](#)). These spectral data confirm that cyanidin, pelargonidin, and peonidin derivatives are the predominant anthocyanins present in *Njavara* rice, supporting its characteristic pigmentation and antioxidant properties, as previously reported in the literature. The high mass accuracy and characteristic molecular ions confirmed the molecular identities of these bioactive flavonoid com-

pounds, which contribute to the functional and therapeutic properties of the rice. The corresponding HRMS spectra are presented in the [Supplementary Material](#) as [Figures S6–S8](#).

3.9. Correlation Analysis

Correlation analysis of the properties of *Njavara* rice revealed interrelationships among the thermal, cooking, nutritional, and bioactive parameters. The Pearson correlation matrix showed strong positive correlations among several thermal, cooking, and nutritional parameters, with most correlation coefficients exceeding 0.90. This suggests that these characteristics may vary together within the grain matrix, thus demonstrating their interdependence in the grain matrix. For instance, higher gelatinization temperatures were positively correlated with grain expansion during cooking and cooking time, suggesting that starch properties, protein content, and moisture uptake may influence the functional quality of rice ([Figure 4](#)).

In contrast, the simplified matrix focusing on polishing, total phenolic content (TPC), and anthocyanin levels revealed strong negative correlations between polishing and both TPC ($r = -0.93$) and anthocyanin ($r = -1.00$). These results demonstrate that increasing the degree of polishing significantly depletes antioxidant phenolics and anthocyanin pigments, which are essential contributors to the nutraceutical value of rice. Furthermore, TPC exhibited a strong positive correlation with anthocyanin content ($r = 0.90$), indicating that anthocyanins contribute substantially to the overall phenolic profile and antioxidant activity of *Njavara* rice. Collectively, these findings emphasize the critical importance of minimal polishing in preserving the nutritional, functional, and health-promoting properties of this traditional rice variety ([Figure 5](#)).

Table 3: Total Anthocyanin Content (TAC) polished rice and bran.

Total Anthocyanin Content (TAC) (mg/100 g)	5% bran fraction	5% polished rice	7% bran fraction	7% polished rice	Red rice
	4.56 ± 0.08^c	3.51 ± 0.02^b	8.28 ± 0.06^c	2.53 ± 0.03^a	6.81 ± 0.05^d

Table 4: HPLC peaks.

Sample	Peak	Ret. Time	Area	Compound Name	Concentration $\mu\text{g}/100\text{ g}$
5% Bran fraction	Peak 10	13.199	32,183	Cyanidin 3 glucoside	364.4

Table 4: *Cont.*

Sample	Peak	Ret. Time	Area	Compound Name	Concentration $\mu\text{g}/100\text{ g}$
7% Bran fraction	Peak 3	13.786	16,930	Cyanidin 3 glucoside	191.7
5% Polished Rice	Peak 5	13.647	3082	Cyanidin 3 glucoside	34.9
7% Polished rice	Peak 13	13.656	305	Cyanidin 3 Glucoside	3.5
Red rice	Peak 6	13.885	36,965	Cyanidin 3 glucoside	418.6

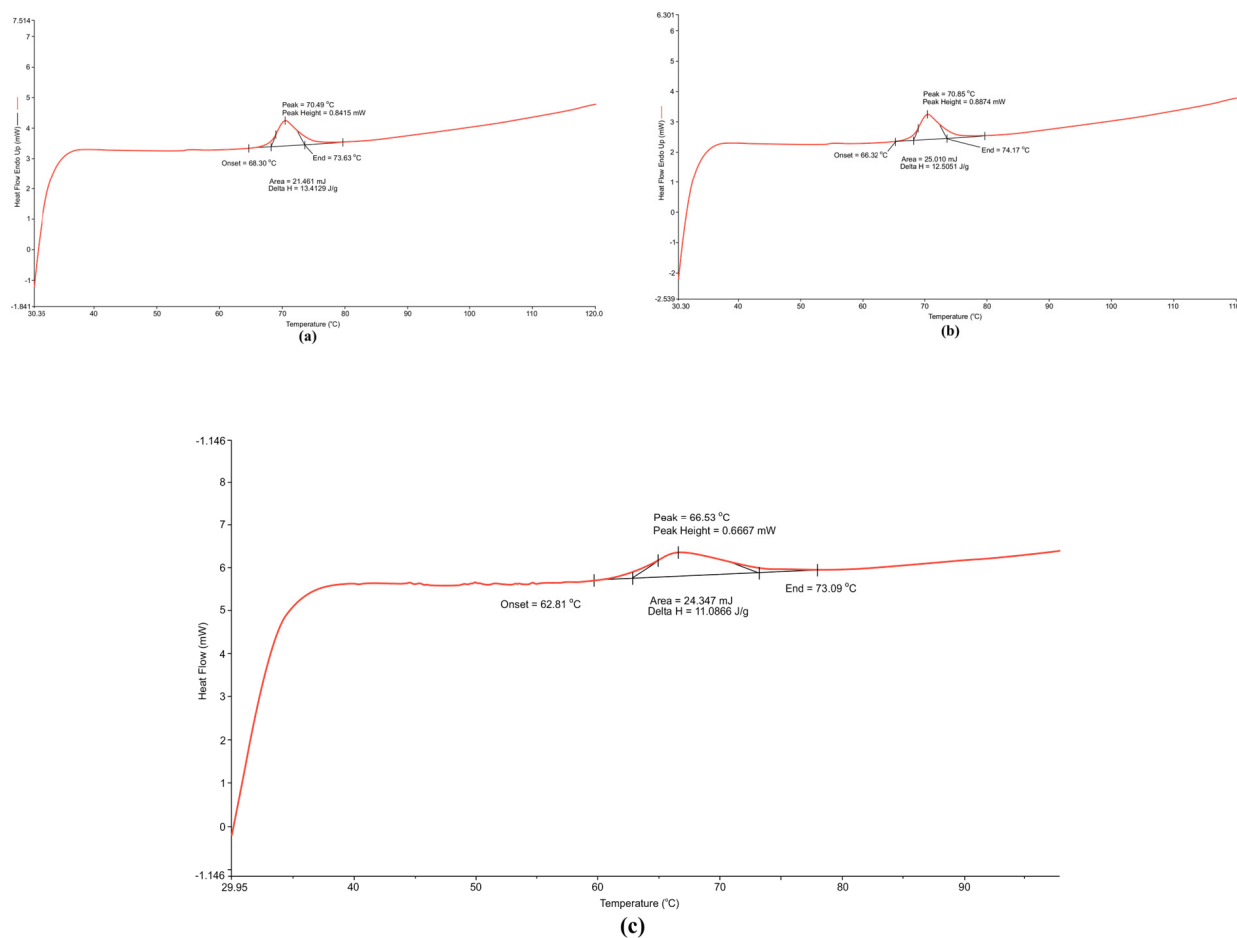


Figure 2: DSC thermograms showing starch gelatinization characteristics of (a) 5% polished rice, (b) 7% polished rice, and (c) red rice.

Table 5: Spectral data of the anthocyanin compound identified in Njavara red rice.

Compound	Observed m/z	Theoretical m/z	Formula
Cyanidin 3 glucoside	287.05	287	$\text{C}_{21}\text{H}_{21}\text{ClO}_{11}$
Pelargonidin 3-O-glucoside	271.05	271	$\text{C}_{21}\text{H}_{21}\text{ClO}_{10}$
peonidin 3-O-glucoside	301.07	301	$\text{C}_{22}\text{H}_{23}\text{O}_{11}\text{Cl}$

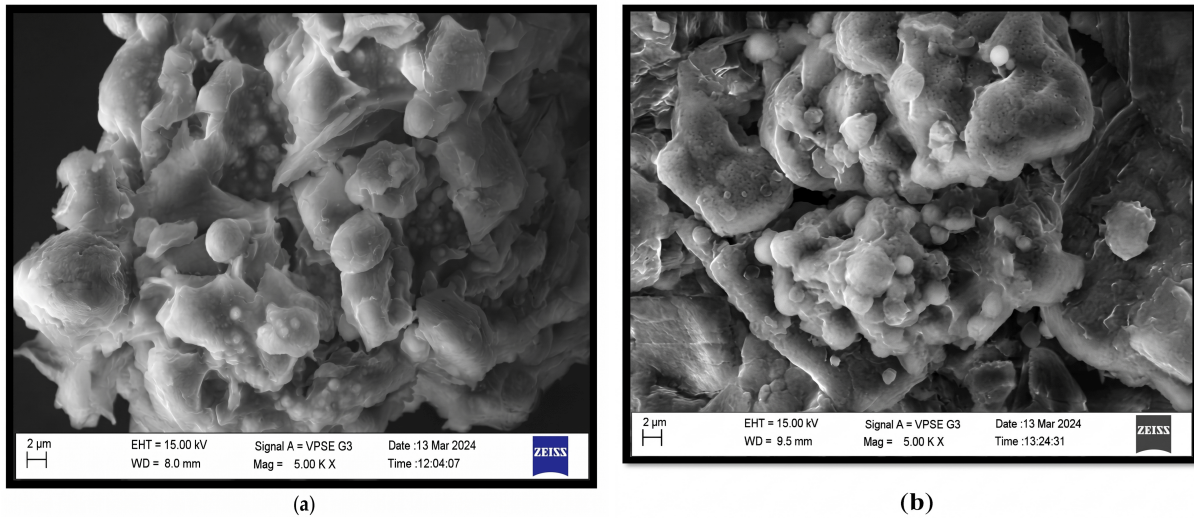


Figure 3: Scanning electron microscopy of rice (a) whole rice grain surface (b) polished rice.

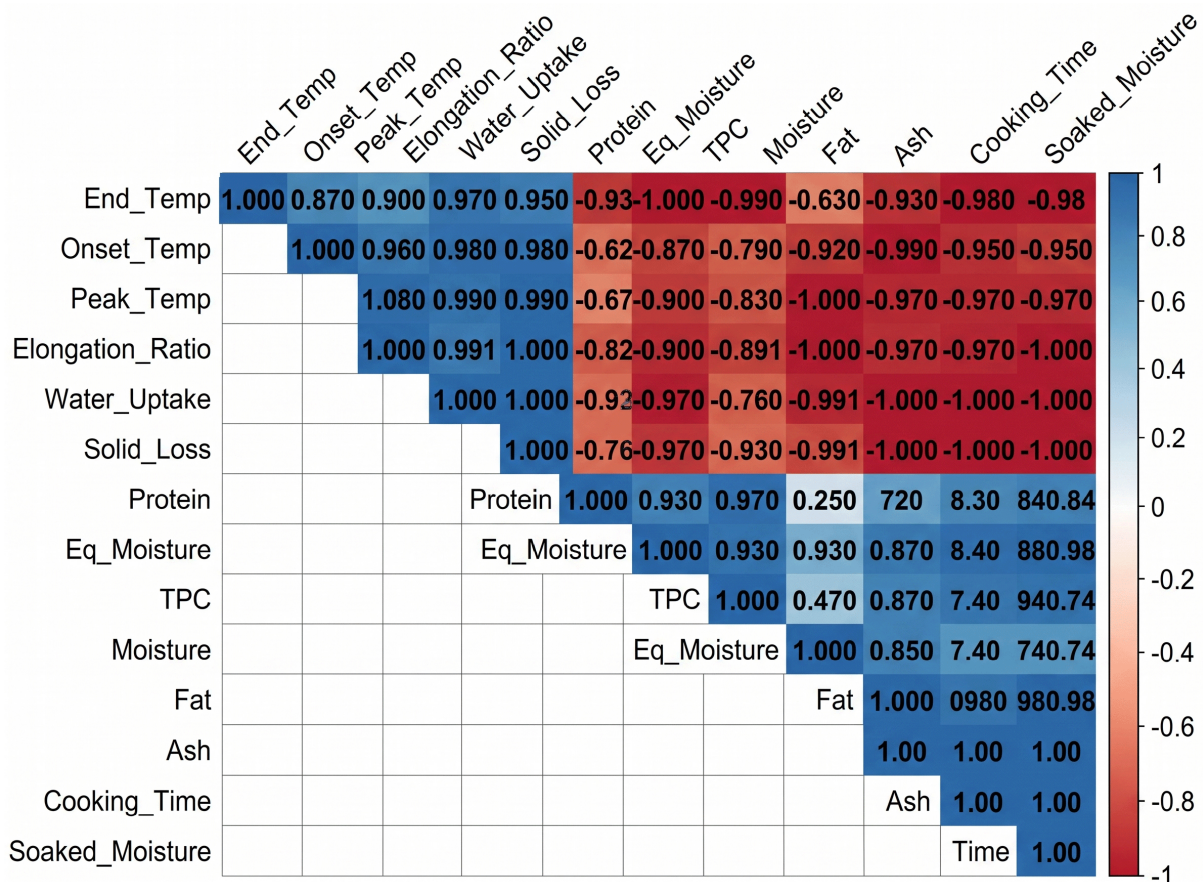


Figure 4: Correlation matrix of thermal, cooking, and nutritional properties.

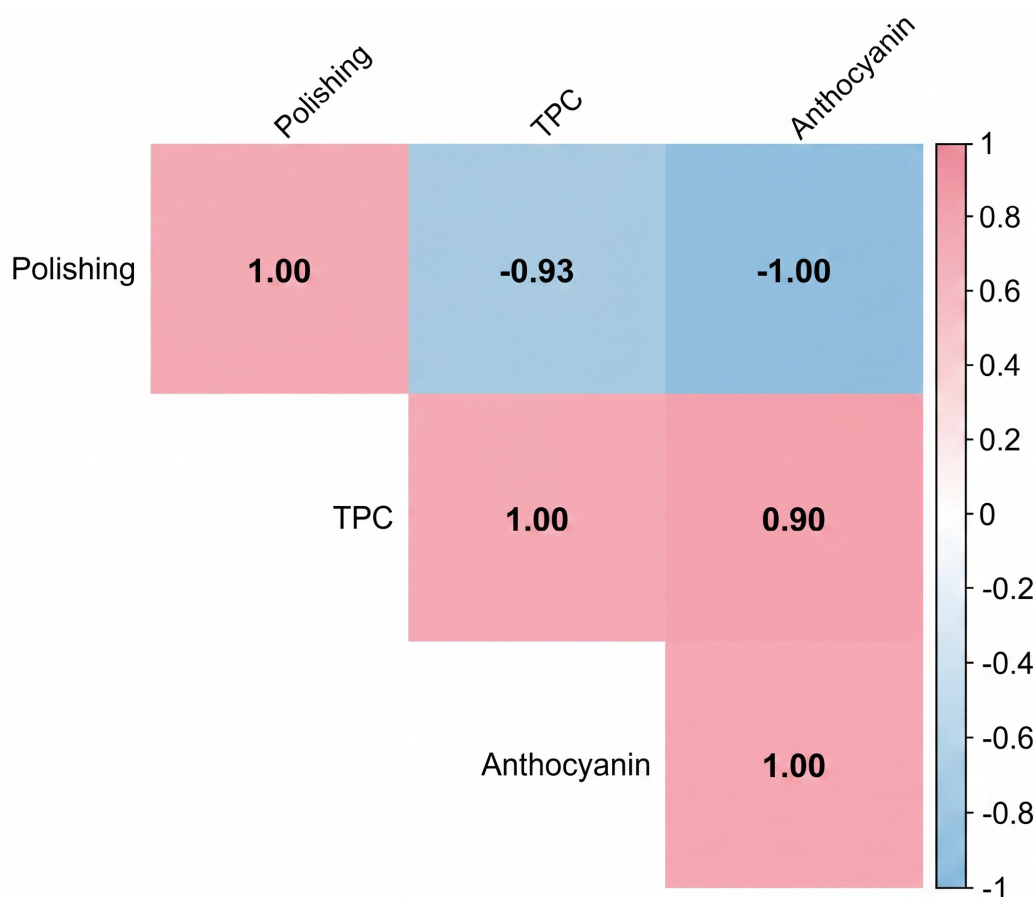


Figure 5: Correlation matrix of polishing level with TPC and anthocyanin content.

4. Discussion

The findings of this study clearly demonstrate that the degree of milling substantially affects the nutritional and functional properties of *Njavara* rice, consistent with previously reported trends in pigmented rice varieties. When the grains were retained in their unpolished (brown) form, they preserved substantially higher levels of protein, fat, minerals, and moisture than the 5% and 7% polished rice samples, thereby confirming the findings of previous studies [10,24,25]. These studies identified the bran layers as the primary reservoirs of essential nutrients, a finding that was further supported by the present study, which demonstrated progressive losses of fat and protein with increasing degrees of polishing. The higher ash content, an indicator of mineral richness, supports the findings of Deepa et al. (2008) [2], who demonstrated that *Njavara* rice and pigmented rice genotypes generally contain higher concentrations of potassium, magnesium, iron, and phosphorus in their outer layers. The observed reduction in cooking time with increasing degrees of polishing is consistent

with the findings of Mardiah et al. (2017) [26] and Nayeem et al. (2021) [27], who attributed the shorter cooking time to the removal of the outer layers, which facilitates water penetration into the grain and accelerates the starch gelatinization process. However, this is accompanied by greater solid loss and reduced textural integrity, consistent with the increased leaching observed in the more extensively polished rice samples [10]. The improved cooking characteristics observed following moderate 5% polishing in the present study are consistent with the findings of Mardiah et al. (2017) [26], who reported that a single mild polishing step enhanced the sensory attributes of red rice while preserving most of its nutritional quality [26].

The findings of the present study further support the premise that *Njavara* rice is naturally rich in bioactive compounds. The total phenolic content (TPC) decreased only slightly from red rice to the 5% polished rice, whereas a marked reduction was observed in the 7% polished rice. This trend is consistent with the findings of Priyanthi and Sivakanesan (2021) and Avinash et al. (2024) [28,29], who reported that phenolic compounds are

predominantly localized in the bran layers and are among the first bioactive constituents to be lost during the milling process. The high antioxidant activities of the red rice samples, as determined by the DPPH and ABTS assays, are consistent with the findings of Rai et al. (2025) [30] and Ed Nignpense et al. (2024) [31], who reported that colored and traditional rice varieties exhibit greater antioxidant capacity than white or fully polished rice due to their higher phenolic and flavonoid contents. A very remarkable result was the significant decrease of anthocyanins with polishing [30,32,33]. These studies further suggest that anthocyanins, predominantly cyanidin-3-O-glucoside in red rice, are localized in the outer pericarp and aleurone layers, making them highly susceptible to removal even with minimal polishing. The present findings, which showed a substantial reduction in anthocyanin content following 7% polishing, further support the observations of Priyanthi and Sivakanesan (2021) [28], who reported that unpolished grains may contain up to 80% more anthocyanins than polished grains. The correlation analysis revealed a strong inverse relationship between polishing degree and anthocyanin content ($r = -1.00$), supporting the trend reported in the literature. Furthermore, the thermal and microstructural changes associated with polishing, as demonstrated by differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) in the present study, are consistent with those widely reported in previous studies.

Thermal and textural alterations following polishing, as demonstrated through DSC and microscopy in this study, are similarly well documented in the literature. The observed increase in gelatinization temperatures with increasing degrees of polishing can be attributed to the removal of phenolic–lipid–amylose complexes, as reported in [34], and is consistent with the reduction in lipid content described by Bani et al. (2024) [10]. In addition, studies by Abdel-Aal et al. (2006) [35] and Pereira-Caro et al. (2013) [36] have shown that anthocyanins and other phenolic compounds not only function as antioxidants but also influence starch behavior during cooking through interactions with amylose and amylopectin chains. Consequently, their removal during polishing may increase gelatinization temperatures and alter the textural properties of rice.

The present study is consistent with previous reports indicating that post-milling processing and storage conditions may influence anthocyanin stability, thereby compromising the nutritional value of rice. A comparison with the published literature on Indian pigmented rice further supports these observations [37]. The findings of Ed Nignpense et al. (2024) [31] and Deepa et al. (2008) [2] identified *Njavara* rice as a superior source of phenolic com-

pounds and antioxidant activity, providing a strong rationale for recommending minimal processing to preserve its nutritional and functional properties.

Overall, these findings provide strong scientific evidence supporting the traditional use of red and lightly polished *Njavara* rice. Minimal polishing preserves the grain's nutritional quality and functional properties, whereas more extensive polishing, despite improving cooking characteristics, leads to substantial losses of the bioactive compounds responsible for its medicinal and health-promoting benefits. These results underscore the importance of optimizing post-harvest processing and cooking methods to ensure that pigmented rice varieties deliver both sensory appeal and greater nutraceutical value in modern diets, as also advocated by multiple recent studies in the field.

5. Conclusions

The present study demonstrates that polishing influences the nutritional, functional, bioactive, thermal, and structural attributes of *Njavara* rice, a traditionally valued pigmented rice variety. Minimal processing (5% polishing) preserved much of the intrinsic nutritional value of red rice while significantly improving its cooking and textural properties, suggesting a favorable balance between consumer acceptability and health benefits. Future research should explore the bioavailability, long-term health effects, and clinical relevance of preserved bioactive compounds to better understand *Njavara* rice's nutraceutical potential.

List of Abbreviations

ABTS	2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
AOAC	Association of Official Analytical Chemists
APCI	Atmospheric Pressure Chemical Ionization
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DSC	Differential Scanning Calorimetry
EMC-S	Equilibrium Moisture Content (Sorption)
HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
TAC	Total Anthocyanin Content
TPC	Total Phenolic Content

Author Contributions

Investigation, resources, data curation: A.A.; Writing—original draft preparation, writing—review and editing, visualization: N.V.; Conceptualization, supervision: S.J. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

Data supporting the results of this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

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AI Declaration

The authors confirm that no AI tools were used to generate any content of this manuscript. The authors take full responsibility for the accuracy and integrity of the content.

Supplementary Materials

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