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

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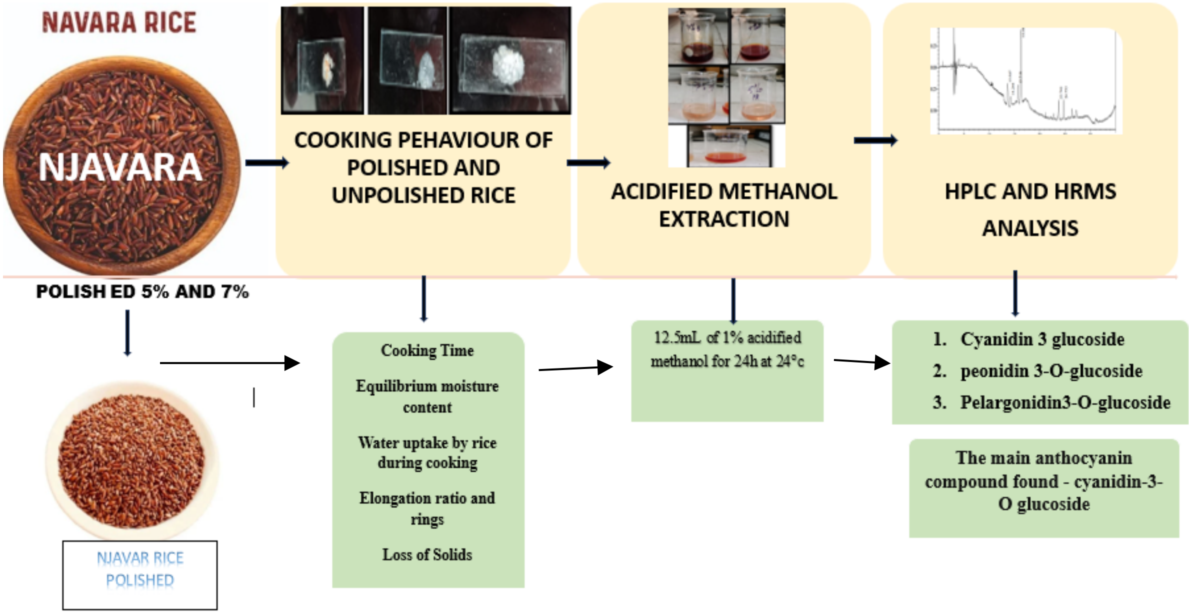
Abstract

The aim of this study was to assess how rice polishing properties affect the nutritional value and anthocyanin composition and cooking performance of Njavara rice which grows as a traditional red rice variety in Kerala's Indian region. Red rice exists as unpolished rice which was examined together with 5% polished rice and 7% polished rice including their bran components. Red rice contains the highest nutritional value because it holds 8.565 mg GAE/g of total phenolic content which exceeds the 8.180 mg GAE/g found in 5% polished rice and 7.537 mg GAE/g found in 7% polished rice. Total anthocyanin content was highest in the 7% bran fraction (8.28 ± 0.06 mg/100 g), followed by red rice (6.81 ± 0.05 mg/100 g), 5% bran fraction (4.56 ± 0.08 mg/100 g), 5% polished rice (3.51 ± 0.02 mg/100 g), and 7% polished rice (2.53 ± 0.03 mg/100 g). The cooking properties showed red rice needed the longest cooking duration of 45 minutes while 5% polished rice needed 35 minutes and 7% polished rice needed 30 minutes with respective elongation ratios of 1.11, 1.18, and 1.21. The protein content was higher in red rice (9.42 ± 0.15%) as compared 9.23 ± 0.56% found in 5% polished rice and 8.12 ± 0.48% present in 7% polished rice. The research shows that polishing reduced bioactive compounds and antioxidant potential while decreasing cooking time and increasing solid loss during cooking.

Keywords: *Njavara*, Anthocyanin, Cooking Properties, Polishing, Nutritional Composition.



1. Introduction

Rice (Oryza sativa L.) is an essential crop for more than half of the world's population and accounts for over 20% of the total calorie intake in the world with 90% of the total global production from Asia [1]. White rice is the most consumed rice variety throughout the planet. Some Asian countries prefer the colored varieties such as red, browns, black, reddish-brown, and purple-black rice.

Pigmented rice, especially the Njavara variety, has great significance in tropical areas like Kerala, India, which is famous for its varied cultivars and the healing properties it has in Ayurveda. The Njavara rice, which is characterized by its white or black husks, has been the medicine of choice by Indian folk healers for ages. The dehusked Njavara rice is made up of 73% carbohydrates, 9.5% protein, 2.5% fat, 1.4% ash, and provides 1628 kJ per 100 g of energy [2]. On the other hand, milling can take away much of the rice's nutrients, as the different nutrients (protein, fat, fiber, minerals, oryzanol, thiamine, and phenolic compounds) which the embryo and bran layer that are removed are concentrated in, play a crucial role in controlling rice quality characteristics [3].

Pigmented rice, which comprises the varieties with a bran layer that has not been peeled off and also colored pericarps such as black/purple and red, gives a rice of better nutritional value than white one [4]. The colors are influenced by the presence of acetylated procyanidin, anthocyanins, and other phenolics that have considerable free radical scavenging activity [5], [6]. While pigmented rice has been used mainly in desserts and special foods for festivals and religious offerings, it is now being considered more and more as a delicacy and a source of nutrients with medicinal and pharmacological potential[7]. Different types of rice have different colors with different anthocyanins, and the types and amounts of anthocyanins depend on the rice variety. As rice processing goes on, the color of the rice and the amount of anthocyanins decrease, which has been confirmed by the significant reduction of the rice color during the milling process [8], [9], [10]. Kato & Horibata, (2021) [11] proved that the antioxidant activity of polished rice is lower than that of red rice. Low milling degree (>4%) preserved total anthocyanins in high amounts and the sensory quality of the sample was found to be good, but total anthocyanins loss was in the range of 53-91% while the milling degree increased from 4% to 7% [12]. Additionally, at the milling degree of 9% almost 100% of anthocyanins loss occurred[8]. This showed that not only different rice milling degrees resulted in loss of nutrients but also that the anthocyanins content was mainly confined in the bran of colored rice. Polishing preferentially removes aleurone/bran layers, slashing total anthocyanins by 53-91% from 4-7% degree and nearly 100% at 9-12%; profiles shift from diverse glycosides in red rice to trace monomers in polished forms. Njavara specifically loses red pigmentation and antioxidant capacity, with bran fractions retaining highest levels (e.g., 4-8x more than grains) [13].

Furthermore, Lu et al. (2021) reported that rice's water absorption ability declined during cooking, which might be due to the glutenin and starch interaction[14]. For a long time, people have been using it in traditional medicine and as a functional food, but not much scientific literature has been written about its response to different processes like these, which are important to the preservation of its nutritional and functional qualities. Prior HPLC-HRMS work focused on generic black/red rice (2-30 compounds), overlooking Njavara's unique Ayurvedic profile and precise low-degree polishing (5-7%) impacts on cooking-nutrient synergies. This investigation uniquely quantifies polishing effects (0%, 5%, 7%) on Njavara rice's nutrients, detailed anthocyanin profiles via HPLC-HRMS, and cooking metrics (time, elongation). It addresses gaps in varietal-specific data for minimally processed forms. Hence, this study proposal is to unveil the masked facts by involving the degrees of milling in the discussion and analyzing in terms of their nutritional composition and cooking properties. 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).

Materials and methods

Njavara rice for the sample preparation were obtained from the local market of 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 had undergone cleaning, dehusking through dehusker and polishing with 5-7% moisture content in a Satake rice polisher. The polished fractions were (5% and 7%) and dried in pulverizer. Then it was sieved through 60 mesh. The sieved sample along with bran samples (5% and 7%) were collected in a zip lock bag. The samples were then stored at 4 degrees for further studies.

2.2 Extraction of anthocyanin using acidified methanol

Anthocyanins were extracted following the method described by Constantin (2022) [15] with slight modifications. Finely ground rice bran and rice flour samples were used for extraction. The solvent employed was acidified methanol, prepared as 1% (v/v) hydrochloric acid in methanol. A solid-to-solvent ratio of 1 : 12.5 (w/v) was maintained, wherein 1 g of sample was mixed 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 carefully collected. To ensure maximum recovery of anthocyanins, the extraction process was repeated three times on the residual pellet under identical conditions. The supernatants from all extractions 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 AOAC method (2016) [16], including determination of moisture content through two-hour drying at 130°C, fat extraction using a Soxhlet apparatus. Ash was determined using the method of incinerating the sample in a furnace at high temperatures (around 550°C). Crude protein, calculated by multiplying total nitrogen by a factor of 6.25, were measured 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 for milled rice was followed by using the Ranghino test [18]. 5 grams of the sample was taken out and boiled in 20mL of preheated distilled water in a water bath at 90°C. Minimum cooking time was determined by pressing the sample 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)

In a 100 ml beaker a proper amount of rice (5-15 grams) was taken. The water was given the rice enough time to absorb and reach the moisture content it can hold with the passing of time. After the soaking period, the rice was taken out of the water and excess surface moisture was removed with a paper towel. The rice was weighed one more time and the last weight was noted.

2.4.3 Water Uptake and Loss of solids

The rice was weighed and then rinsed with cold water to remove excess starch which would otherwise cause stickiness during the cooking process. A careful drainage was done through a mesh. Then the measured amount of water required for cooking, which usually follows the ratio of 1 part rice to 1.5 to 2 parts water, according to the desired texture and the specific rice type, was added. The rice was cooked then dried to get rid of the absorbed water and solid loss can be calculated along with water gain.

2.4.4 Elongation ratio and rings

For measuring the length of a sample of cooked rice grains, a vernier caliper was applied.

2.5 Bioactive compound analysis

2.5.1 Total Phenolic Content:

The total phenolic content of redand 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 of 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 (Na_2CO_3) solution was added, and the final volume was adjusted to 10 mL with distilled water. The reaction mixture was incubated at room temperature in the dark 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. A standard calibration curve was prepared using different concentrations of gallic acid . The absorbance values were plotted against gallic acid concentrations using Microsoft Excel (figure 1), and a linear regression equation along with the coefficient of determination (R^2) was obtained [20].

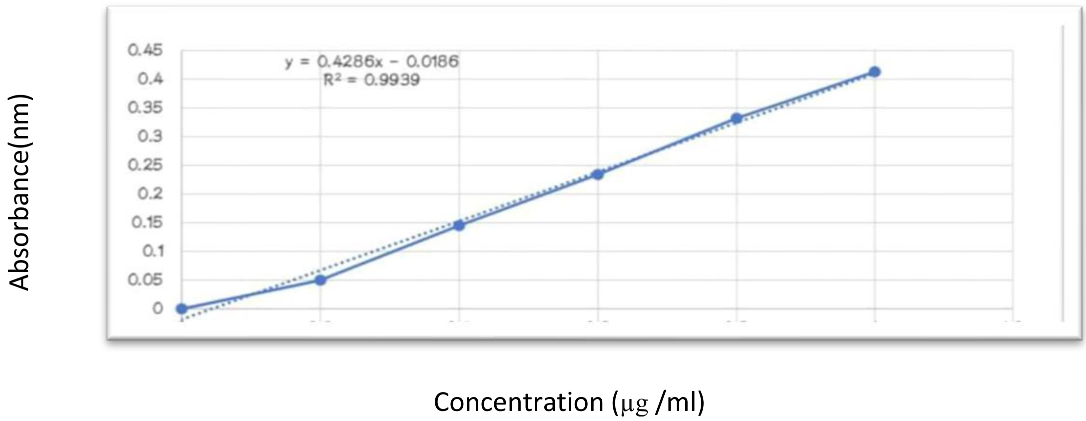


Figure 1. Standard graph of Gallic acid

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-picrylhydrazy (DPPH) Assay:

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

Inhibition calculated as % = [(A_control - A_sample)/A_control] × 100

2.5.3 Determination of total Anthocyanin content

The determination of total anthocyanin content, as described by Anggraeni et al., 2019 [22] involved the following steps: The sample extract was mixed with two buffer solutions, maintaining a maximum ratio of test portion to buffer at 1:4. The absorbance of the diluted test portion was then measured at both 520 nm and 700 nm using buffers with pH 1.0 and pH 4.5. These measurements was taken against a blank cell filled with distilled water. The absorbance measurements must be taken within 20-50 minutes of preparation to ensure accuracy in determining the total anthocyanin content.

2.6 Differential Scanning Calorimetry (DSC)

The instrument used was a PerkinElmer DSC8000 which was manufactured by PerkinElmer in U.S. The rice sample (usually a few milligrams) was taken in a small aluminium 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 done with the EVO® LS 15, a German built device controlled by SmartSEM™ software. It was necessary to subject the sample to high-voltage electron beam with energy from 100 to 30,000 electron volts. The image was created pixel by pixel as the electron beam, which was directed by scan coils, moved in straight lines. The electron detector recorded signals from the sample, such as secondary electrons (SE) and backscattered electrons (BSE). The SEM delivered a partly 3D image which was influenced by the topography, i.e. the shape, size, and surface texture, of the sample. The inclination of the sample surface not only influenced the topographic contrast but also the number of BSE and SE signals, with an inclination of more than 50^o to 70^o boosting the signals [23] .

2.8 Quantification profile

2.8.1 HPLC- Instrumentation and chromatographic conditions.

Anthocyanin content in 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 achieved using a Kromasil C18 reversed-phase column (particle size 5 µm, pore size 100 Å, dimensions 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):

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

Detection of anthocyanins was carried out using a UV–Vis detector at 525 nm, which corresponds to the maximum absorbance 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 carried out on 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 separation occurred based on their mass-to-charge (m/z) ratios. 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.

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 1. Moisture content ranged from 10.8 ± 0.68% in 5% polished rice to 11.8 ± 0.46% in red rice, indicating a gradual increase in moisture retention with reduced polishing intensity. Fat content was highest in red rice (2.30 ± 0.22%), followed by 5% polished rice (1.30 ± 0.26%) and 7% polished rice (1.12 ± 0.24%), highlighting the contribution of bran layers to lipid retention. Similarly, protein content was higher in red rice (9.42 ± 0.15%) and 5% polished rice (9.23 ± 0.56%) compared to 7% polished rice (8.12 ± 0.48%). Ash content, which reflects mineral concentration, also increased with lower polishing intensity, ranging from 1.09 ± 0.58% in 7% polished rice to 1.74 ± 0.72% in red rice. These findings indicate that red rice retained superior nutritional quality, while increased polishing resulted in progressive nutrient loss.

3.2 Cooking Properties of Rice

Cooking behavior varied significantly 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 ± 1.23% to 11.2 ± 1.42%, and under soaked conditions from 11.2 ± 1.52% to 12.1 ± 1.65%, with red rice showing comparatively higher moisture absorption. Water uptake was highest in 7% polished rice (249.2 ± 1.48%), followed by 5% polished rice (241.7 ± 1.56%), while red rice exhibited the lowest value (211.4 ± 1.79%). In contrast, solid loss during cooking was highest in 7% polished rice (7.2 ± 1.56%) and lowest in red rice (2.5 ± 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 measured by DPPH and ABTS assays differed more prominently among the treatments. Red rice exhibited the highest antioxidant activity, with DPPH and ABTS scavenging activities of 95.2 ± 1.26% and 97.6 ± 0.52%, respectively. In comparison, 5% polished rice showed values of 90.5 ± 1.23% (DPPH) and 92.3 ± 1.53% (ABTS), while 7% polished rice recorded 89.7 ± 1.52% and 91.5 ± 1.24%, respectively. These findings indicate that reduced polishing helped preserve phenolic compounds and antioxidant potential, particularly in red rice.

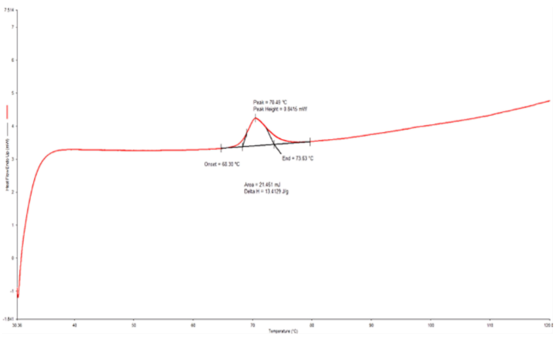
3.4 Differential Scanning Calorimetry

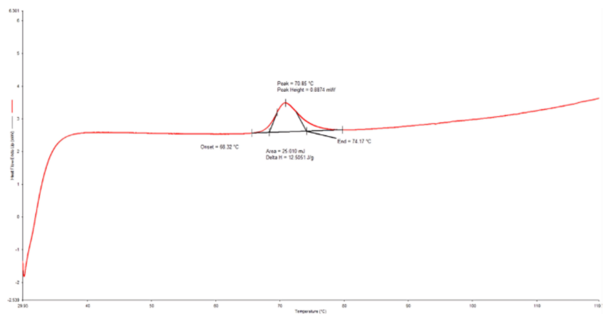
The differential scanning calorimetry (DSC) results presented in Table 1 and Figure 2a–c demonstrated variations in starch gelatinization behavior among the rice samples. Red rice exhibited the lowest onset temperature (62.81 °C) and peak temperature (66.53 °C), whereas 7% polished rice showed the highest 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 polished rice samples may be attributed to the removal of bran layers and pigments, which exposed the crystalline starch structure and increased the thermal energy required for starch gelatinization.

Table 2: Physico-chemical composition, thermal and cooking 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			
Normal	10.7±1.19 [a]	10.2±1.23 [a]	11.2±1.42 [a]
Soaked	11.5± 1.25 [a]	11.2±1.52 [a]	12.1±1.65 [a]
Water Uptake	241.7±1.56 [b]	249.2±1.48 [a]	211.4±1.79 [c]
Loss of solid (%)	6.2±1.43[b]	7.2±1.56[a]	2.5±1.26[c]
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]





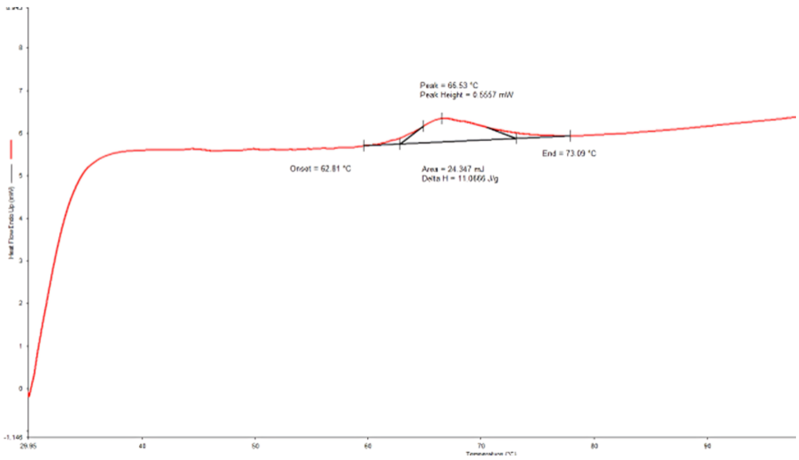


Figure 2. DSC thermogram showing starch gelatinization characteristics of red rice a) 5% Polished rice b) 7% Polished rice c) Red rice

3.5 Total Anthocyanin Content

Significantly lower amounts of anthocyanins were detected upon increasingly polished rice samples (Table 3). The maximum total anthocyanin content (TAC) was characterized 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 samples were a lot lower than this, with the 5% polished rice containing 3.51 ± 0.02 mg C3G/100 g and the 7% polished rice having only 2.53 ± 0.03 mg C3G/100 g.

Table 3: Total anthocyanin Content

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

3.6 Surface morphology

Red rice, due to its morphological properties, has a rough and grainy surface, and this uneven texture can be regarded as a sign of the original bran and germ layers. The intricate composition of the bran is not only revealed but also reflected in the variety of particle shapes and sizes on its surface, which is the result of the existence of compounds rich in anthocyanin. The keeping of the surface structure in red rice is very important for the retention of anthocyanins, which in turn helps the grain acquire its antioxidant properties and the health benefits associated with it, as indicated in Figure 3a . Smooth and uniform surfaces with the least irregularities show the characteristics of polished rice. The process of polishing, in which the outer bran and germ layers are removed, effectively destroys the main reservoirs of anthocyanins in the grain. Moreover, the presence of pores, voids, and structural defects like cracks and fissures is related to the mechanical stress that the polishing process imposed. 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 the smoother appearance of polished rice has become the most common choice in cooking, the nutritional advantages of red rice, especially those coming from its anthocyanin content, are likely to draw the attention of health-conscious consumers.

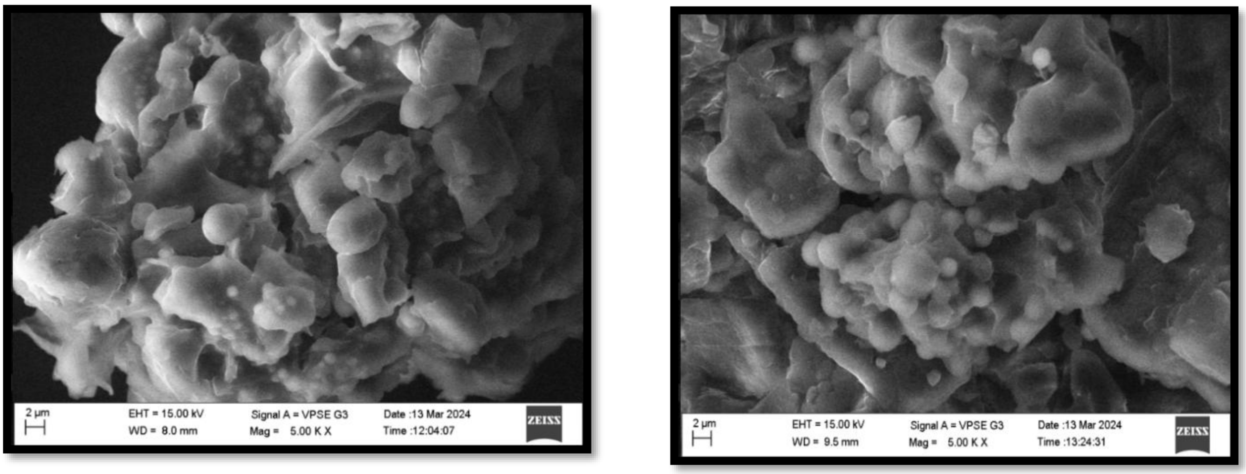


Figure 3: Scanning Electron microscopy of Rice a) Whole Rice Grain Surface b) Polished rice

3.7 Analysis and Quantification of Anthocyanin by HPLC

The results from HPLC analysis conducted on Njavara rice samples indicated that cyanidin-3-glucoside was the major anthocyanin compound as seen in Table 4. The highest concentration in red rice (418.6 µg/100 g, Peak 6, retention time 13.885 min, peak area 36,965) was revealed by the quantification. The 5% bran fraction had 364.4 µg/100 g (Peak 10, retention time 13.199 min, peak area 32,183), while the 7% bran fraction was reduced to 191.7 µg/100 g (Peak 3, retention time 13.786 min, peak area 16,930). The content of cyanidin-3-glucoside in white rice was very low with the 5% white rice having 34.9 µg/100 g (Peak 5, retention time 13.647 min, peak area 3,082) and the 7% white rice containing 3.5 µg/100 g which was the lowest (Peak 13, retention time 13.656 min, peak area 305). The results of the study show that cyanidin-3-glucoside gets lost progressively and in large amounts when polishing is done, thus it means that most of this anthocyanin is present in the outer bran layers which get removed during the polishing process. They are represented in supplementary material as figures (Fig. S1-Fig S5).

Table 4: HPLC Peaks

Sample	**Peak**	**Ret. Time**	**Area**	**Compound Name**	**Concentration µg/100g**
5% Bran fraction	Peak 10	13.199	32183	Cyanidin 3 glucoside	364.4

7% Bran fraction	Peak 3	13.786	16930	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	36965	Cyanidin 3 glucoside	418.6

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

The High-Resolution Mass Spectrometry (HRMS) analysis of *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 exactly to its theoretical m/z of 287 and molecular formula $C_{21}H_{21}ClO_{11}$. Pelargonidin 3-O-glucoside appeared with an observed m/z of 271.05, matching the theoretical m/z of 271 ($C_{21}H_{21}ClO_{10}$). Peonidin 3-O-glucoside was detected at an observed m/z of 301, in agreement with the theoretical m/z of 301 and molecular formula $C_{22}H_{23}O_{11}Cl$ (Table 5). These spectral data confirm that cyanidin, pelargonidin, and peonidin derivatives constitute the major anthocyanins in *Njavara* rice, supporting its characteristic pigmentation and antioxidant properties reported in the literature. The precise mass accuracy and characteristic molecular ions validate the molecular identity of these bioactive flavonoid compounds contributing to the rice's functional and therapeutic attributes. They are represented in supplementary material as figures (Fig. S6-Fig S8).

Table 5: Spectral Data

Compound	Observed m/z	Theoretical m/z	Formula
Cyanidin 3 glucoside	287.05	**287**	$C_{21}H_{21}ClO_{11}$
Pelargonidin 3-O-glucoside	271.05	**271**	$C_{21}H_{21}ClO_{10}$
peonidin 3-O-glucoside	301.07	**301**	$C_{22}H_{23}O_{11}Cl$

3.9 Correlation analysis

The correlation analysis of Njavara rice properties has shown some significant interrelationships between the thermal, cooking, nutritional, and bioactive parameters. The Pearson correlation matrix revealed that there were high positive correlations among thermal transition parameters (end temperature, onset temperature, peak temperature), elongation ratio, water uptake, solid loss, protein content, equilibrium moisture, fat, ash, cooking time, and soaked moisture, with most correlation coefficients above 0.90. This points to a close relationship between these characteristics and their tendency to either increase or decrease together, thus demonstrating their interdependence in the grain matrix.. For instance, higher gelatinization temperatures correlated with improved grain expansion during cooking and prolonged cooking times, indicating that starch properties, protein content, and moisture uptake all play a substantial role in determining functional rice quality (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). This result demonstrates that increasing the degree of polishing leads to significant depletion of antioxidant phenolics and anthocyanin pigments, which are vital for the rice's nutraceutical value. Furthermore, TPC and anthocyanin content showed a strong positive correlation (r = 0.90), indicating that anthocyanins make a major contribution to the overall phenolic profile and antioxidant activity of *Njavara* rice. Collectively, these results emphasize the crucial importance of minimal polishing for preserving nutritional, functional, and health-promoting properties of this traditional rice variety (Figure 5).

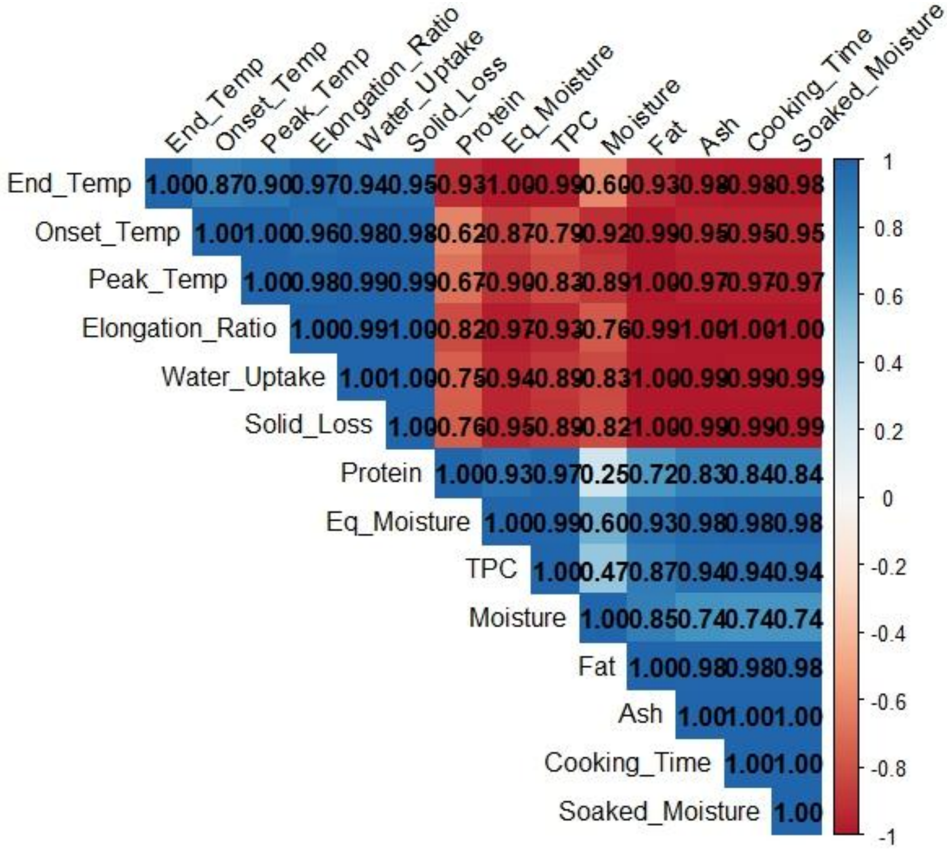


Figure 4: Correlation Matrix of Thermal, Cooking, and Nutritional Properties

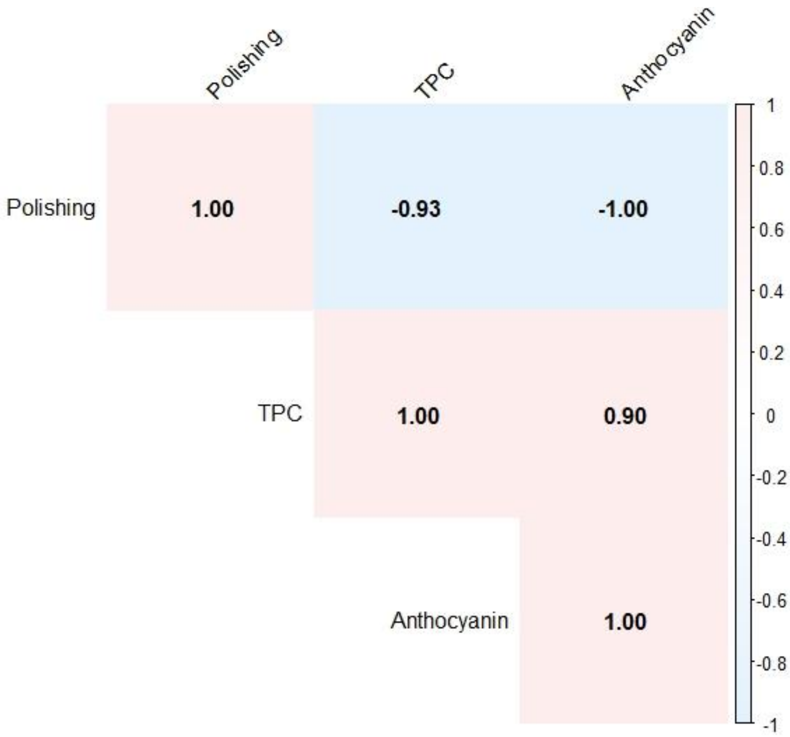


Figure 5: Correlation Matrix of Polishing Level with TPC and Anthocyanin Content

Discussion

The results of this research quite evidently indicate that the extent of milling has a great impact on the nutritional and functional properties of Njavara rice, which is in line with the previously observed trends for pigmented rice varieties. When all the grains were kept brown, they still managed to keep up to ten times their original amount of protein, fat, and mineral matter as well as moisture compared to 5% and 7% polished rice thus, confirming earlier studies [10], [24], [25]. These investigations had pointed out that the bran layers are the principal sources of the important nutrients and were confirmed by the current study which recorded fat and protein loss with the increase in the degree of polishing. The higher ash content, which is an indicator of the mineral richness, has backed the Deepa et al., 2008 [2] finding that the Njavara rice and pigmented genotypes, in general, are high in potassium, magnesium, iron, and phosphorus in the outer layers. As far as the cooking behavior is concerned, the decrease in cooking time with the rise of polishing is in line with the findings of Mardiah et al., (2017) [26], Nayeem et al., (2021) [27] who concluded that the reason for the faster cooking up was that the thinning of the husk allows the water to get in easily which in turn speeds up the gelatinization process. However, this is accompanied by a higher solid loss and a decrease in textural integrity that is in line with the increased leaching of more polished rice samples[10]. The acceptance of and improvement in texture after moderate (5%) polishing treatment, as seen in the present study, was similar to the findings of Mardiah et al., (2017) [26] who stated that a single mild polishing step not only conferred preferred sensory attributes on red rice without substantial sacrifices in nutritional quality but also led to developing consumer-preferred sensory attributes in red rice without major sacrifices in nutritional quality [26].

The current research outcomes also support the notion that Njavara rice is naturally packed with bioactive compounds. The measured total phenolic content (TPC) showed a very slight decrease from red to 5% polished rice, while in 7% polished samples the TPC was considerably lower, thus very close to the trends of the reports by

Priyanthi & Sivakanesan, 2021,Avinash et al., 2024) [28], [29] who claimed that phenolics localized in the bran were the first to go during milling. The high antioxidant activities measured in red rice samples, which were evaluated using DPPH and ABTS methods, are in agreement with the findings of Rai et al., (2025) [30] and Ed Nignpense et al., (2024) [31], who reported that colored and traditional rice varieties always win over white and fully polished rice in terms of antioxidant capacity thanks to their phenolic and flavonoid content. A very remarkable result was the significant decrease of anthocyanins with polishing [30], [32], [33]. These researchers contend that anthocyanins, mainly cyanidin-3-O-glucoside in red rice, are encompassed in the outer pericarp and aleurone layers and hence very much prone to removal by even little polishing. The current results showing that there was nearly total loss of anthocyanins after 7% polishing, strengthen the claim made by Priyanthi & Sivakanesan, (2021) [28] that the unpolished grains may have up to 80% more anthocyanins than the polished ones. The correlation data showing an inverse relationship between polishing and anthocyanin content ($r = -1.00$) very precisely supports these literature conclusions. The thermal and textural changes due to polishing, which were evidenced by DSC and microscopy in this research, are also highly cited in the literature.

Thermal and textural alterations following polishing, as demonstrated through DSC and microscopy in this study, are similarly well documented in the literature. The observed upward shift in gelatinization temperatures with increased polishing is explained by the removal of phenolic–lipid–amylose complexes, as discussed by [34], and matches the reduction in lipid content postulated by Bani et al., (2024) [10] In addition, studies by Abdel-Aal et al. (2006) [35] and Pereira-Caro et al., (2013) [36] reveal that anthocyanins and other phenolics not only serve as antioxidants but also modify starch behavior during cooking by interacting with amylose and amylopectin chains; their removal therefore increases gelatinization temperatures and changes textural outcomes.

The present study also aligns with reports that that post-milling handling—such as powdering and storage under suboptimal conditions increasing the loss of anthocyanins, further compromising nutritional value. In reviewing the comparative literature on Indian pigmented rice [37] . Ed Nignpense et al., (2024) [31] and Deepa et al., (2008) [2] positioned *Njavara* as a leader in phenolic content and antioxidant activity, providing a strong rationale for recommending minimal processing.

Overall, these findings establish that the traditional wisdom behind the use of red and lightly polished *Njavara* rice finds strong scientific support: minimal polishing preserves the grain's nutritional superiority and functional benefits, while more extensive polishing, though improving cooking characteristics, results in significant depletion of the compounds responsible for its medicinal and health-promoting properties. These results underscore the importance of optimizing post-harvest processing and cooking methods to ensure that pigmented rice varieties deliver both sensory appeal and maximum nutraceutical value in modern diets, as also advocated by multiple recent studies in the field**.**

Conclusion

The present study comprehensively demonstrates the profound influence of polishing on the nutritional, functional, bioactive, thermal, and structural attributes of *Njavara* rice—a traditionally valued, pigmented medicinal variety. Minimal processing (5% polishing) retained much of the intrinsic nutritional value of red rice while significantly improving cooking and textural properties, suggesting a favourable compromise between consumer acceptability and health benefits. Future research should explore long-term health impacts, clinical validations, and bioavailability studies of the preserved bioactive to fully harness *Njavara*'s nutraceutical potential.

Conflicts of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript."

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List of abbreviations

1. **HPLC** – High-Performance Liquid Chromatography
2. **HRMS** – High-Resolution Mass Spectrometry
3. **AOAC** – Association of Official Analytical Chemists
4. **EMC-S** – Equilibrium Moisture Content (Sorption)
5. **TPC** – Total Phenolic Content

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