# Integrated Biochemical Analysis of Red Rice (*Oryza sativa* L.): Unveiling Nutritional and Functional Attributes

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**Abstract**: The present study evaluates the physicochemical and nutritional characteristics of red rice varieties collected from different districts of Himachal Pradesh namely Shimla, Kullu, Chamba and Kangra, each representing distinct geographical locations. There were significant differences in the physicochemical and nutritional characteristics of varieties. Grain size classifications showed that Chhohartu, Matali, Jattoo, Karad, Jhinjhan and Kaluna were short, whereas Gocha, HPR-2720 and HPR-2795 were medium. Grain shapes were medium in Chhohartu, Karad, Jhinjhan, HPR-2720 and HPR-2795, while Matali, Jattoo, Kaluna, and Gocha were bold. Amylose content ranged from 18.27% (Karad) to 25.17% (Chhohartu). The proximate composition indicated that Jattoo (13.67%) had the highest moisture content, whereas Kaluna (3.56%) showed the highest ash content. Protein content was high across all the varieties ranging from 8.66% (Jhinjhan) to 13.89% (Gocha). Jhinjhan (402.2 mg/kg) showed the highest iron content, while Karad (83.6 mg/kg) had the lowest. The highest zinc content was observed in Kaluna (46.55 mg/kg) while Matali (32.35 mg/kg) had the lowest. The significant negative correlation was observed between carbohydrate and protein (r= -0.810**), energy and moisture (r = -0.877**), and a moderate negative correlation between energy and ash (r = -0.711*), indicating that higher moisture and ash content in red rice varieties correspond to lower energy content. The current research demonstrated that the variations observed in these characteristics are influenced by environmental factors across the different geographical regions.

**Keywords**: Red rice, Himachal Pradesh, Physicochemical characteristics, Nutritional parameters

Red rice (*Oryza sativa* L.), known for its unique red bran layer, is gaining significant attention for its remarkable nutritional and functional properties. A distinctive feature of this type of rice is that even after extensive milling, a slight red remains in the outermost layer. The bran colour, varying from light to dark red, is caused by anthocyanin and proanthocyanidin pigments in different bran layers. It is an Asian native that has long been grown in the Philippines, Japan, China, Sri Lanka, Korea, India, and other rice-growing countries. The northwest Himalayas regions, such as Uttarakhand, Jammu and Kashmir and Himachal Pradesh, exhibit significant diversity in indigenous rice cultivars. Several well-known red rice cultivars in India are Matta rice from Kerala, Patni from Maharashtra, Jattoo and Matali from Kullu Valley in Himachal Pradesh (Devraj et al., 2020). These red rice varieties are part of both indica and japonica subspecies, exhibiting a range of characteristics such as glutinous or non-glutinous, aromatic or non-aromatic, and early or late maturing. They also vary in grain length, from short to long, although the majority are coarse-grained. Himalayan red, a long-grained red rice variety has gained recognition in the export market (Ahuja et al., 2007).

The nutritional profile of red rice is notably remarkable, as it contains a high concentration of essential nutrients that support overall health and welfare. Shao and Bao (2015) documented that whole grain contains carbohydrates, protein, lipids, dietary fibres, minerals, vitamins (E and B complex) and phytochemicals like phenolic compounds. The primary components of whole grains are carbohydrates, mainly starch. Amylose is the main factor influencing the various physiochemical characteristics of starch (Wani et al., 2012). Moreover, whole grains are higher in dietary fibre, which can be widely divided into soluble and insoluble fibre (Slavin et al., 2003). Protein is a major constituent of red rice after starch, with four distinct fractions: globulin, albumin, glutelin (the predominant protein) and a minor protein prolamin (Amagliani et al., 2017). The third naturally occurring component in red rice is lipids, or fats, mainly located in the outer bran layer and exit in both starch and non-starch-associated forms. Saleh et al., (2019) observed that lipid content significantly influences the quality of rice throughout processing and storage. Micronutrients, including minerals and vitamins, are essential for good health while being required in small quantities. Red rice has a two to three times higher zinc and iron content than white rice. contains magnesium which lowers the risk of heart attacks and plays a critical function in blood pressure regulation. Manganese and calcium are present in abundance which helps in strengthening metabolism, and bones. Selenium is also a part of the nutritional package of red rice and helps the body fight against infection. Red rice's low glycemic index makes it suitable for several conditions, including diabetes. Red rice

may also be helpful for the prevention or cure of diseases caused by vitamin A and B deficiencies (Kristamtini et al., 2012).

Red rice pericarp contains a significant amount of phenolic substances (in soluble form) and antioxidants, in addition to its high functional food value (de Mira et al., 2009). Phenolic acids, flavonoids and tannins are considered the primary dietary phenolic compounds (King and Young 1999). Among red rice varieties, the most abundant bound phenolic acid is ferulic acid followed by *p*-coumaric and vanillic acid (Sompong et al., 2011). Cyanidin 3-O-glucoside is the primary anthocyanin found in red rice (Tomoyuki et al., 2002). The flavonoids identified in red rice include proanthocyanidins, which belong to the class of flavanols (Min et al., 2011). Proanthocyanidins, also known as condensed tannins, are the most abundant polyphenols in the plant kingdom and are made up of oligomers of flavan-3-ol and flavan-3,4-diol (Smerglio et al., 2017). The red rice of Himachal Pradesh is poorly understood, and only a few studies have been done on its quality characteristics. The study aims to determine the nutritional and functional attributes of red rice grown in different regions of Himachal Pradesh.

## MATERIAL AND METHODS

**Sample collection:** Seven landraces (Chhohartu from Shimla district, Matali and Jattoo from Kullu district, Karad and Jhinjhan from Chamba district and Gocha and Kaluna from Kangra district) and two released varieties (HPR-2720 and HPR-2795 from Kangra district) of red rice were collected from different regions of Himachal Pradesh during the years 2022 and 2023.

Details regarding the naming of the red rice varieties were obtained from the Wheat and Rice research station, Malan CSKHPKV Palampur (H.P.). The samples were cleaned manually to remove debris and attached soil, followed by shade-drying. After drying, the grains were dehusked using a de-husker (available at the Wheat and Rice research station, Malan), finely ground into a powder with a laboratory grinder and passed through a 60-mesh sieve. The powdered samples were kept in airtight containers to maintain their stability until further analysis was performed. All the experiments were done in triplicate.

**Physicochemical characteristics**: The size and shape classification were determined by the scale of (Cruz and Kush 2000). The shape of the kernel was assessed by calculating the length-to-width (L/W) ratio. The alkali spreading value (ASV) was assessed using the procedure by Little et al. (1958) with some alternations. The starchy endosperm of the rice kernels was visually rated using the 7-point numerical spreading scale. The amylose content (AC) was measured using the procedure by Juliano et al., (1971) with slight modifications. The amylose content was estimated using the standard curve of potato amylose.

**Proximate composition and food energy:** The moisture content of the sample was determined using the hot air oven method (AOAC 2000). Ash content was determined by the muffle-furnace method as per the procedure referred to by AOAC (2000). The samples in the crucibles were heated at 550 ℃ for 5-6 hrs in a muffle furnace. The fat content was determined using Soxhlet extraction following the method (AOAC 2005). The extraction was conducted 4-6 hours at 60-68 ℃ temperature. Crude fibre content of the sample was measured by the AOAC (2000) procedure. The crude protein content was analysed using the Kjeldahl method as per the procedure detailed in AOAC (1990), which encompassed protein digestion and distillation.

The total carbohydrate content in the rice sample was calculated (James 1995): =100- (% of Moisture + % of ash + % of fat + % of crude fibre + % of crude protein)**.** Food energy was calculated by the formula of (Verma and Srivastav 2017): Food energy (kJ/100g) = (%CP*4) + (%F*9) + (%CHO*4), Where CP = crude protein, F = fat and CHO = carbohydrate.

**Mineral Analysis:** The minerals present in the samples were estimated using atomic absorption spectroscopy (AAS). The

**Table 1.** Collection sites and GPS coordinates of red rice varieties

| Varieties | Collection sites | Latitude | Longitude |
|-----------|------------------|----------|-----------|
| Chhohartu | Peja, Shimla district | 31° 22'72"N | 77° 86'25"E |
| Matali | Prini, Kullu district | 32° 21'50"N | 77° 19'58"E |
| Jattoo | Prini, Kullu district | 32° 22'34"N | 77° 19'70"E |
| Karad | Kari Chaonri, Chamba district | 32° 48'02"N | 76° 01'46"E |
| Jhinjhan | Nagori, Chamba district | 32° 56'68"N | 76° 11'03"E |
| Kaluna | Dungla, Kangra district | 32° 21'60"N | 76° 30'68"E |
| Gocha | Supara, Kangra district | 32° 31'38"N | 76° 18'37"E |
| HPR-2795 | Tarindi, Kangra district | 32° 10'31"N | 76° 35'87"E |
| HPR-2720 | Tarindi, Kangra district | 32° 10'31"N | 76° 35'87"E |

**Table 2.** Analysis of proximate composition of red rice

| Parameters | Methods | References |
|------------|---------|------------|
| Moisture | Hot air oven | AOAC 2000 |
| Ash | Muffle furnace | AOAC 2000 |
| Fat | Soxhlet extraction | AOAC 2005 |
| Crude fibre | Acid and alkali digestion | AOAC 2000 |
| Crude protein | Micro Kjeldhal | AOAC 1990 |
| Total carbohydrate | By difference: 100- (Moisture + ash + fat + crude fibre + crude protein) | James 1995 |

diacid mixture method was used to prepare the samples (Renuka et al., 2016).

**Statistical analysis:** The data were statistically analysed using Duncan's multiple range tests at the 0.05 significance level and Pearson's correlation coefficients at 0.05 and 0.01 significance levels with SPSS software [Version 29.0.2.0 (20)].

## RESULTS AND DISCUSSION

**Physicochemical characteristics:** Grain size is characterized by its maximum dimension, while grain shape is based on the length-to-breadth ratio. The average grain length ranged from 4.86 (HPR-2795) to 6.30 mm (Kaluna). The average grain width varied between 2.09 (HPR-2720) to 2.81 mm (Matali). The length-to-width (L/W) ratio of red rice varieties varied from 1.88 (Kaluna) to 2.89 (HPR-2720). Chhohartu, Matali, Jatto, Karad, Jhinjhan and Kaluna were short in size, while Gocha, HPR-2720 and HPR-2795 were medium in size. L/W ratio Chhohartu, Karad, Jhinjhan, HPR-2720 and HPR-2795 were medium, while Matali, Jattoo, Kaluna and Gocha were bold in shape. Abeysekera et al., (2017) documented that the grain length of varieties ranged from 4.12 to 5.98 mm and length to width ratio ranged from 1.81 to 3.03.

The alkali spreading value (ASV) of the various red rice varieties ranged from 1.66 (HPR-2720 and HPR-2795) to 5.16 (Karad). The majority of red rice varieties exhibited an intermediate gelatinization temperature (GT). The high GT with low ASV was observed in Kaluna, HPR-2720 and HPR-2795. The intermediate GT and ASV were in Chhohartu, Matali, Jattoo, Karad, Jhinjhan and Gocha. Lahkar et al. (2020) observed a similar range of ASV in aromatic Joha rice, ranging from 1 to 5 most of which exhibited intermediate scores. Amylose content (AC) varied significantly ranged from 18.27 (Karad) to 25.17% (Chhohartu). A study by Thongbam et al. (2010), reported amylose content in rice cultivars from Manipur ranging between 14.33 to 29.47%, most of the cultivars classified as having intermediate amylose content (20-25%).

**Proximate composition and food energy:** Statistically significant differences were observed in the proximate composition among red rice varieties. The moisture content ranged from 11.56 (Chhohartu) to 13.67% (Jattoo). Saikia et al. (2012) showed similar outcomes in pigmented rice varieties from Manipur ranging from 11.6 (Chak-haoamubi) to 13.7% (Bakuljoha). Ash content varied from 2.27 (Jhinjhan) to 3.56% (Kaluna). Lahkar et al. (2020) reported ash content in traditional aromatic (Joha) rice ranged from 1.0 (Tulsi Joha and Rampal Joha) to 7.47 % (Boga Kunkuni Joha). The fat content ranged from 1.02 (Matali) to 3.46% (Jattoo). Sompong et al., (2011), reported fat content in rice varieties (nine red and three black) varied from 1.15 (Sri Lanka Red rice 1) to 3.72% (Niaw Dam Pleuak Khao). Crude
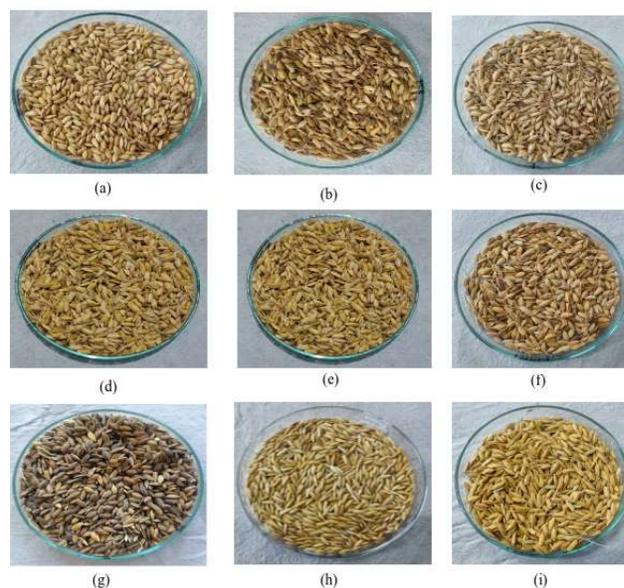


**Fig. 1.** Red rice varieties (a) Chhohartu (b) Matali (c) Jattoo (d) Karad (e) Jhinjhan (f) Gocha (g) Kaluna (h) HPR-2795 (i) HPR-2720

**Table 3.** Physical characteristics of red rice (Mean±SE, n=30)

| Varieties | Grain length (mm) | Grain breadth (mm) | L/B ratio | Grain size | Grain shape |
|---|---|---|---|---|---|
| Chhohartu | 5.49±0.05 | 2.61±0.03 | 2.11±0.02 | Short | Medium |
| Matali | 5.41±0.04 | 2.81±0.02 | 1.93±0.02 | Short | Bold |
| Jattoo | 5.17±0.04 | 2.52±0.02 | 2.05±0.02 | Short | Bold |
| Karad | 5.34±0.06 | 2.41±0.04 | 2.23±0.05 | Short | Medium |
| Jhinjhan | 5.23±0.03 | 2.12±0.02 | 2.46±0.04 | Short | Medium |
| Kaluna | 4.86±0.03 | 2.57±0.01 | 1.88±0.01 | Short | Bold |
| Gocha | 5.56±0.04 | 2.67±0.03 | 2.09±0.03 | Medium | Bold |
| HPR-2720 | 5.97±0.05 | 2.09±0.04 | 2.89±0.05 | Medium | Medium |
| HPR-2795 | 6.30±0.05 | 2.48±0.01 | 2.54±0.02 | Medium | Medium |

fibre content ranged from 0.73 (HPR-2720) to 1.95% (Jattoo). Dasgupta and Handique (2018) studied the crude fibre content in twenty Indigenous landraces of paddy, varied from 0.90 (Lalkartisali) to 1.67% (Kura binni) in pigmented red rice landraces.

Crude protein content ranged from 8.66 (Jhinjhan) to 13.89% (Gocha). The findings obtained in this study are similar to Lahkar et al., (2020), who reported protein content in traditional aromatic Joha rice varied from 6.4 (Tulsi Joha) to 17.3% (Krishna Joha). The carbohydrate content ranged from 66.30 (Jattoo) to 72.05% (Matali). Subudhi et al., (2013) also observed that carbohydrate content in aromatic rice varied from 64.6 (Pusa Sugandh-2) to 89.15% (Kala Namak). Food energy values varied significantly ranged from 334.36 (Kaluna) to 345.65kJ/100g (Gocha). Verma and Srivastav (2017) also reported food energy values for Indian rice cultivars varied from 348.79 (Govind Bhog) to 361.07 kCal/100g (Gopal Bhog).

**Food energy and relationships with proximate composition:** The significant negative correlation was observed between carbohydrate and protein (r = - 0.810**) at the 0.01 level, indicating an inverse relationship between carbohydrate and protein.

Additionally, a highly significant negative relationship was revealed between energy and moisture (r = - 0.877**) at the 0.01 level as well as a moderate but significant negative correlation between energy and ash (r = - 0.711*) was shown at the 0.05 level, which indicates that as the moisture and ash content of red rice varieties increases, their energy content tends to decrease. The significant negative correlation between energy and both moisture and ash were reported in several previous studies by (Verma and Srivastav 2017).

**Mineral analysis**: The concentrations of manganese (Mn), copper (Cu), zinc (Zn), iron (Fe), magnesium (Mg), phosphorus (P) and potassium (K) in the nine red rice varieties ranged from 24.7-40.45 mg/kg, 2.2-17.5 mg/kg 32.35-46.55 mg/kg, 83.6-402.2 mg/kg, 0.1-0.17%, 0.05-0.13% and 0.07-0.2% respectively.

Iron (Fe) was the highest micro-nutrient among all the red rice varieties followed by zinc (Zn), manganese (Mn), and copper (Cu), whereas magnesium (Mg) was the highest macro-nutrient across all the varieties, except Karad and HPR-2795, in both of these varieties, Potassium (K) was the highest macro-nutrient. The mineral content in this research is similar to or near Verma and Srivastav (2017) in six aromatic rice cultivars.

**Table 4.** Chemical characteristics and proximate composition of red rice

| Varieties | Alkali spreading value | Gelatinization temperature | Amylose content (%) | Moisture (%) | Ash (%) | Fat (%) |
|---|---|---|---|---|---|---|
| Chhohartu | 4.5[d] | I (70-74° C) | 25.17[f] | 11.56[a] | 2.69[bc] | 3.13[g] |
| Matali | 3.33[c] | I (70-74° C) | 24.94[f] | 12.77[b] | 2.63[abc] | 1.02[a] |
| Jattoo | 4.66[d] | I (70-74° C) | 20.55[cd] | 13.67[d] | 2.82[c] | 3.46[h] |
| Karad | 5.16[d] | I (70-74° C) | 18.27[a] | 12.91[bc] | 2.46[abc] | 2.76[e] |
| Jhinjhan | 3.5[c] | I (70-74° C) | 20.07[ab] | 13.35[bcd] | 2.27[a] | 2.62[d] |
| Kaluna | 2.5[b] | H (75-79° C) | 22.36[e] | 13.03[bcd] | 3.56[d] | 2.81[b] |
| Gocha | 4.83[d] | I (70-74° C) | 20.38[abc] | 11.95[a] | 2.35[ab] | 1.43[f] |
| HPR-2720 | 1.66[a] | H (75-79° C) | 20.71[d] | 13.55[cd] | 3.25[d] | 2.73[de] |
| HPR-2795 | 1.66[a] | H (75-79° C) | 20.31[bcd] | 12.98[bcd] | 2.74[c] | 2.43[c] |

H: High; I: Intermediate; L: Low
Different letters within the same column represent significant differences (*P*< 0.05).

**Table 5.** Proximate composition and food energy of red rice

| Varieties | Crude fibre (%) | Crude protein (%) | Total carbohydrate (%) | Food energy (kJ/100g) |
|---|---|---|---|---|
| Chhohartu | 1.17[d] | 13.08[f] | 68.33[c] | 345.00[d] |
| Matali | 1.12[cd] | 10.39[b] | 72.05[e] | 339.13[bc] |
| Jattoo | 1.95[f] | 11.78[c] | 66.30[a] | 336.19[ab] |
| Karad | 1.11[cd] | 13.18[g] | 67.55[bc] | 339.81[c] |
| Jhinjhan | 0.99[b] | 8.66[a] | 72.08[e] | 339.22[bc] |
| Kaluna | 1.07[c] | 13.13[fg] | 67.76[bc] | 334.36[a] |
| Gocha | 1.61[e] | 13.89[h] | 67.31[b] | 345.65[d] |
| HPR-2720 | 0.73[a] | 12.16[e] | 67.64[bc] | 334.37[a] |
| HPR-2795 | 0.79[a] | 11.90[d] | 69.13[d] | 338.82[bc] |

Different letters within the same column represent significant differences (*P* < 0.05).

**Table 6.** Pearson's correlation coefficient among proximate composition and food energy values in red rice

| Parameters | Moisture | Ash | Fat | Fibre | Protein | Carbohydrate |
|---|---|---|---|---|---|---|
| Ash | 0.316 | -0.309 | 0.401 | 0.223 | -0.810** | 0.077 |
| | (0.408) | (0.419) | (0.284) | (0.565) | (0.008) | (0.845) |
| Fat | -0.045 | -0.241 | 0.221 | -0.430 | 0.259 | |
| | (0.909) | (0.532) | (0.568) | (0.248) | (0.500) | |
| Fibre | -0.105 | 0.294 | -0.552 | 0.258 | | |
| | (0.788) | (0.443) | (0.124) | (0.503) | | |
| Protein | -0.486 | -0.379 | 0.295 | | | |
| | (0.185) | (0.314) | (0.441) | | | |
| Carbohydrate | 0.017 | -0.711* | | | | |
| | (0.965) | (0.032) | | | | |
| Energy | -0.877** | | | | | |
| | (0.002) | | | | | |

**$p < 0.01$ level (2-tailed); * $p < 0.05$ level (2-tailed)

**Table 7.** Mineral content of red rice (n=3)

| Varieties | Micro-nutrients (mg/kg) | | | | Macro-nutrients (%) | | |
|---|---|---|---|---|---|---|---|
| | Mn | Cu | Zn | Fe | Mg | P | K |
| Chhohartu | 40.45 | 13.7 | 33.8 | 259 | 0.15 | 0.11 | 0.13 |
| Matali | 34.65 | 14.2 | 32.35 | 275.95 | 0.1 | 0.05 | 0.08 |
| Jattoo | 34.1 | 14.35 | 42.95 | 247.5 | 0.17 | 0.07 | 0.14 |
| Karad | 24.7 | 12.55 | 44.4 | 83.6 | 0.11 | 0.05 | 0.2 |
| Jhinjhan | 29.8 | 15.55 | 32.35 | 402.2 | 0.13 | 0.12 | 0.07 |
| Kaluna | 30.85 | 14.6 | 46.55 | 201.75 | 0.14 | 0.07 | 0.12 |
| Gocha | 28.9 | 14.1 | 36.75 | 140.3 | 0.13 | 0.08 | 0.12 |
| HPR-2720 | 33.2 | 12.2 | 33.8 | 345 | 0.15 | 0.13 | 0.09 |
| HPR-2795 | 30.5 | 17.5 | 46.4 | 147.1 | 0.12 | 0.07 | 0.19 |

## CONCLUSIONS

The current research provides a comprehensive assessment of the nutritional and functional attributes of red rice varieties collected from different regions of Himachal Pradesh. Red rice showed high levels of protein, ash, fat and crude fibre contributing to superior nutritional value compared to regular rice. The mineral content, particularly iron and zinc, is significantly high in varieties, this indicates the potential of red rice as a functional food that can address mineral deficiencies, especially in developing regions. The research concluded that the nutritional and functional characteristics of red rice are affected by the distinct geographical regions of Himachal Pradesh, making these varieties highly suitable for both nutritional and functional food applications. The red rice varieties of Himachal Pradesh show great potential for enhancing dietary nutrition and providing health benefits due to their rich nutrient content and bioactive compounds.

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## AUTHOR'S CONTRIBUTION

Pardeep Kumar – Conceptualization, Maneesha Devi – Original draft preparation, Leena Thakur, Pushpa Guleria, Neha Guleri, Purnima Sharma – reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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