



International Journal of Research in Agronomy

E-ISSN: 2618-0618
P-ISSN: 2618-060X
© Agronomy
NAAS Rating (2025): 5.20
www.agronomyjournals.com
2025; SP-8(8): 114-119
Received: 07-06-2025
Accepted: 11-07-2025

Chinmaya Swarup Pattanaik
Ph.D. Scholar, Department of
Plant Physiology, SOADU,
Bhubaneswar, Odisha, India

Dr. Nandita Jena
Associate Professor, Department of
Plant Physiology, Faculty of
Agricultural Sciences (FAS),
SOADU, Bhubaneswar, Odisha,
India

Corresponding Author:
Chinmaya Swarup Pattanaik
Ph.D. Scholar, Department of
Plant Physiology, SOADU,
Bhubaneswar, Odisha, India

Nutrient enriched black rice: A sustainable substitute to alleviate deficiency

Chinmaya Swarup Pattanaik and Nandita Jena

DOI: <https://www.doi.org/10.33545/2618060X.2025.v8.i8Sb.3503>

Abstract

Black rice (*Oryza sativa* L.) is a highly nutritious, good source of vitamins and bioactive compounds i.e., flavonoids and anthocyanin etc. A lab experiment was conducted at Institute of Agricultural Sciences, SOA on seven rice varieties i.e., Chakhao, Kalabati, Kala biroin, Gedi Ganjam, MTU-1061, Kalachampa and Jamuna by identifying their nutritional status and bioactive compounds. Black rice landraces Chakhao, Kala biroin and Kalabati showed most promising results in multiple parameters. Kalabati showed highest result in anthocyanin (40.25 mg/100 g) and flavonoid (23.87 mg/100g) content, followed by Chakhao and Kala biroin. In terms of protein and fat content Gedi Ganjam which is a white landrace showed effective result i.e., 10% and 0.29% respectively. In context with dietary fibre and energy content Chakhao gave highest result, 5.56% and 294.35 Kcal/100g respectively. Gedi Ganjam showed highest non reducing sugar followed by Kalachampa and MTU-1061 but in Chakhao, reducing sugar content showed best result followed by Kala biroin and Kalabati. Chakhao also recorded highest TSC content than other six varieties. Carotenoid content in black rice varieties was observed better than white rice varieties. From this experiment it was also revealed that Kalabati and Chakhao showed highest result in mineral content i.e., Fe (8.24 mg/100g) and Zn (3.02 mg/100g) respectively. As black rice has more fibre, antioxidant, vitamin and mineral content, its consumption offers numerous health benefits. Antioxidants and minerals like Fe & Zn, the components present in black rice are responsible for reducing chronic diseases such as heart disease, cancer and diabetes, hence its use is beneficial for dietary use, which alleviate malnutrition.

Key Words: Black rice, nutritious, antioxidants, landrace, anthocyanin

Introduction

Over 50% of people in the planet eat rice (*Oryza sativa* L.) as a staple diet. It is grown on 43.86 million hectares of land in India, producing 117.47 million tons of fruit (DAC&FW, 2019-20). It has a big impact on the economy of the country. There are various varieties of rice, distinguished by the size, texture, aroma, maturity period, growth conditions, and colour (polished, black, red, purple, and brown). The kinds of coloured rice are said to have many health advantages. The violet or dark purple hue of black rice (*Oryza sativa* L. indica) is due to the very high levels of anthocyanin pigments in the aleurone layer compared to white and red rice (Hou *et al.*, 2013) [14].

In India Black Rice is mostly cultivated in Manipur, West Bengal, Assam, Odisha, and Jharkhand. For a very long time, it has been integral to the socio-cultural activities of the indigenous Meitei group in Manipur (Asem, 2015) [31]. One variety of sticky black rice that is native to Manipur is called Chak-hao Amubi. "Ambui" means dark, while "Chakho" means tasty. It is typically offered on festival festivities and special occasions in Manipur. Its black colour turns purple when cooked, and its flavour becomes somewhat nutty. Manipur is now India's top producer of black rice.

Black rice is cultivated in the districts of Odisha such as Koraput, Malkangiri, Mayurbhanj, Kalahandi etc. Out of 30 landraces of black rice available in India, six black rice varieties available in Odisha are stored in the indigenous seed germplasm unit of Bargarh district of the state.

From all the Rice varieties Black Rice is most nutritious variety. It is also called as superfood due to its high nutritional composition. Black Rice has more protein, iron and fibre than Brown Rice. Due to its dark purple colour caused by anthocyanins, which are also provide antioxidants.

Anthocyanins are regulated by the usage of flavonoid groups (anthocyanins, proanthocyanidins, flavonoids, flavones, flavonones, and flavan-3-ols), which are most notably linked to the prevention of hypertension. After evaluating the anthocyanin and tocopherol extracts from black rice bran, it was discovered that the anthocyanin extract had a significant impact on cholesterol maintenance but a less significant effect on fatty acid oxidation inhibition. The high fibre content of black rice aids in the removal of waste and toxins from the digestive system and promotes regular bowel movements, can help people lose weight because it fills them up. The fibre in rice helps to avoid diverticulitis, haemorrhoids, bloating, constipation, irritable bowel syndrome, and duodenal ulcers. Hence, considering the health protection due to potential antioxidants, the objective of the study was based on to compare the biochemical components present in between black rice and white rice varieties.

Materials and Methods

The research materials are seven rice varieties from which three are black rice landraces and four are white rice varieties. The three black rice landraces are Chakhao, Kala biroin and Kalabati. Chakhao and Kala biroin were collected from college Research Farm and Kalabati from local farmers of Koraput district, Odisha. Four white rice varieties are Kalachampa, Gedi Ganjam, MTU 1061 and Jamuna from which Gedi Ganjam and Jamuna are grown in coastal areas of Odisha. MTU 1061 which is a high yielding variety and tolerant to pest like Brown Plant Hopper (BPH) cultivated in alluvial soils. Kalachampa is an indigenous rice variety, mostly cultivated in Keonjhar district of Odisha and it is particularly suitable for organic and sustainable farming. All four white rice varieties are collected from farmers from various districts of Odisha.

Determination of Total Flavonoid and Anthocyanin: Total flavonoid and anthocyanin content in rice varieties were determined by spectrophotometric method. The total flavonoid and anthocyanin were measured at 374 and 535 nm respectively. The total anthocyanin content was represented as cyanidin-3-glucoside equivalents (mg g⁻¹), whereas total flavonoid content was represented as quercetin equivalents (mg g⁻¹) using the following formulas.

$$\text{Total Flavonoid (TF)} = \frac{A_{374\text{nm}} \times \text{dilution factor}}{76.6}$$

$$\text{Total Anthocyanin (TA)} = \frac{A_{535\text{nm}} \times \text{dilution factor}}{76.6}$$

Estimation of Fat: 100g sample was put into soxhlet thimble and n-hexane solvent was added. The solvent is extracted for 8 hours. Fat has been separated using rotary evaporator. Fat% was calculated using the following formula

$$\% \text{ Fat} = \left(\frac{W_3 - W_2}{W_1} \right) \times 100\%$$

Where, W₁= Sample weight taken in g

W₂= Weight of the plate with sample after heating

W₃= Weight of plate after desiccating

- **Determination of Total Carbohydrate:** Total Soluble Carbohydrate was determined by using Anthrone method explained by Scott and Melvin 1953. Total soluble sugar estimated by measuring colour intensity at OD 630nm.
- **Estimation of Protein:** Protein was estimated using Lowry's method (1951) [17]. About 200 mg samples were taken and powdered with 10 ml of 10% TCA solution. Then the sample was centrifuged and 10 ml of 1N-NaOH was added and again centrifuged for 10 minutes. After centrifugation different reagents were added and OD was taken at 660 nm using spectrophotometer.
- **Estimation of Reducing Sugars and Non-Reducing Sugars:** Reducing Sugars was determined by using Ferricyanide standard method given by Gremli, 1970 [29]. Non reducing sugars were calculated by subtracting reducing sugars from total soluble sugars.
- **Estimation of Dietary Fibre:** The Dietary Fibre was estimated by using FOSS Fiberteesystem, model 1020 hot extractor and crucible-fitted glass.
- **Determination of Energy:** The energy was calculated by using the following formula

$$\text{Energy (Cal/100g)} = (\text{Protein} \times 4.27) + (\text{Lipid} \times 9.05) + (\text{Carbohydrate} \times 3.85)$$

- **Determination of Iron (Fe) and Zinc (Zn):** The samples were digested and transferred to 100ml volumetric flask and volume was made with distilled water and then filtered. Samples were analysed by Atomic Absorption spectrophotometer method given by AOAC, 1990.
- **Determination of Total Carotenoid:** Carotenoid content were determined by using the method stated by Jensen A, 1978. Carotenoid was measured at 470 nm OD and calculated using the following formula

$$\text{Carotenoid (mg/g)} = (1000 \text{ OD } 470 - 2.27 \text{ Chl a} - 81.4 \text{ Chl b}) / 2270$$

Determination of Moisture: Total Moisture percentage was estimated by using Moisture meter.

Results and Discussion

The present study revealed significant genotypic variation among seven black rice varieties across a wide range of nutritional and biochemical parameters. Detailed analysis of the black rice landraces indicated substantial differences in their nutritional composition, bioactive compound content, and micronutrient profiles. Understanding varietal differences in nutrient and phytochemical composition is crucial for nutritional health benefit and functional food development; this study identified notable diversity among the evaluated black rice landraces.

Table 1: Protein (%), Fat (%), Dietary fibre (%) and Energy (kcal/100g) content in different rice varieties

Varieties	Protein%	fat%	Dietary fibre%	Energy kcal/100g
Kalabati	5.31	0.11	4.76	212.88
Chakhao	7.81	0.20	5.56	294.35
Kala biroin	6.75	0.17	5.08	251.24
Gedi Ganjam	10.0	0.29	3.01	286.53
Kalachampa	6.03	0.20	2.67	212.71
MTU-1061	6.04	0.19	2.89	213.83
Jamuna	5.22	0.09	2.02	186.24
SE(m) ±	0.12	0.01	0.06	5.01
CD(P=0.05)	0.36	0.03	0.17	15.20

*Each value is an average of three observations. There are some significant differences among rice varieties at $P \leq 0.05$.

The proximate analysis of three black rice landraces and four white rice varieties revealed significant inter-varietal differences (Table-1). The protein concentration in Gedi Ganjam (10%) was found comparatively remarkable among the seven rice varieties. Chakhao (7.81%) and Kala biroin (6.75%) also analyzed more protein levels compared to other varieties. On the other hand, Jamuna and Kalabati showed the lowest protein content. Statistically significant variation was observed in protein content across varieties at $P=5\%$. High protein rice is especially critical in protein-deficient populations, providing a plant-based protein source that supports growth and development (Juliano, 1993). Fat content was uniformly low, consistent with typical rice composition. The values ranged from 0.09% (Jamuna) to 0.29% (Gedi Ganjam), confirming rice as a low-fat staple grain. Dietary fibre content, a key determinant of glycemic response and gastrointestinal health, varied notably among the varieties. Chakhao (5.56%) exhibited the highest fibre content, followed closely by Kala biroin (5.08%) and Kalabati (4.76%), suggesting their superior value for functional and health-promoting rice-based products. Fibre-rich diets have been shown to reduce the

risk of type 2 diabetes and cardiovascular diseases (Slavin, 2005). The energy content followed a pattern generally reflective of both macronutrient composition and varietal biomass allocation. Chakhao recorded the highest energy value of 294.35 kcal/100g, aligning with its encouraging protein and fibre content. In contrast, Jamuna had the lowest energy density (186.24 kcal/100g). The standard error and critical difference confirm the statistical significance of these energy variations at $P \leq 0.05$. These findings highlight Chakhao, Kala biroin, and Gedi Ganjam as varieties with notable nutritional advantages. These results are consistent with earlier findings supported by Sompong *et al.* (2011) [26] and Shao *et al.* (2018) [24], who reported notable differences in protein, fiber, and anthocyanin levels among pigmented rice accessions.

The carbohydrate profile, particularly the sugar composition, plays a crucial role in influencing both the nutritional quality and the organoleptic properties of rice. The black rice varieties exhibited statistically significant variations ($P \leq 0.05$) in their non-reducing sugar, reducing sugar and total soluble carbohydrate (TSC) contents (Table-2).

Table 2: Non Reducing Sugar (mg/g), Reducing Sugar (mg/g) and TSC (mg/g) content in different rice varieties

Varieties	Non Reducing Sugar mg/g	Reducing Sugar mg/g	TSC mg/g
Kalabati	2.03	2.88	4.92
Chakhao	2.82	3.91	6.73
Kala biroin	2.05	3.69	5.74
Gedi Ganjam	5.34	0.92	6.26
Kalachampa	3.29	1.52	4.81
MTU-1061	3.30	1.54	4.84
Jamuna	2.20	2.04	4.24
SE(m) ±	0.03	0.06	0.09
CD(P=0.05)	0.10	0.18	0.28

*Each value is an average of three observations. There are some significant differences among rice varieties at $P \leq 0.05$.

Among the tested varieties, Gedi Ganjam showed the highest concentration of non-reducing sugars (5.34 mg/g). Kalachampa (3.29 mg/g) and MTU-1061 (3.30 mg/g) also analysed relatively high values, whereas Kala biroin registered the lowest content (1.05 mg/g). The high non-reducing sugar level in Gedi Ganjam may also be linked to a trait favourable for diabetic-friendly rice varieties. Where, Kala biroin grains exhibited the highest reducing sugar content of 4.69 mg/g, followed by Chakhao with 3.91 mg/g. These sugars, primarily glucose and fructose, are metabolically active. Their abundance in Kala biroin may contribute to enhanced aroma and flavor complexity during thermal preparation (Buttery *et al.*, 1983) [5]. Chakhao recorded TSC with 6.73 mg/g, followed by Gedi Ganjam (6.26 mg/g) and Kala biroin (5.74 mg/g). These values are statistically significant as per the $P \leq 0.05$ value showing a

crucial role for rapid energy release. Varieties such as Jamuna and Kalachampa, with lower TSC values, may offer reduced glycemic impact and could be appropriate in therapeutic diets targeting metabolic syndrome (Tian *et al.*, 2004) [27].

More reducing sugar level in Kala biroin and Chakhao corroborate the findings of Deepa *et al.* (2007) [9], noted that aromatic pigmented rice tends to accumulate higher levels of reducing sugars, likely due to the interplay between sugar metabolism and anthocyanin biosynthesis.

Anthocyanins and flavonoids are key secondary metabolites contributing to the antioxidant potential and pigmentation of black rice. These bioactive compounds have been widely associated with anti-inflammatory, anti-carcinogenic, and cardiovascular protective properties, making them critical targets in functional food development (Wu *et al.*, 2006) [28].

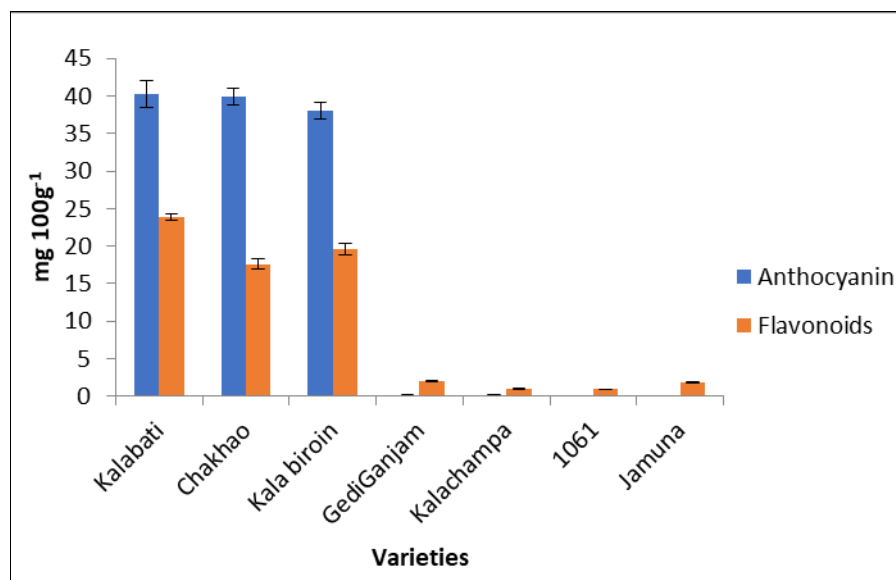


Fig 1: Total Anthocyanin (mg/100g) and Total Flavonoid content (mg/100g) content in all seven rice varieties, with error bars representing \pm standard error of the mean (n=3)

The Anthocyanin content in black rice varieties like Kalabati (40.25 mg/100g), Chakhao (39.91 mg/100g), and Kala biroin (38.06 mg/100g), contained over 100-fold more anthocyanins than the pale or white-hulled varieties (Fig-1). In contrast, the varieties of white rice as Gedi Ganjam, Kalachampa, MTU-1061, and Jamuna had negligible anthocyanin levels (<0.25 mg/100g), indicating a lack of pigmentation in their pericarp or aleurone layers. All varieties are showing a significant difference at $P \leq 0.05$.

The high anthocyanin concentrations in Kalabati and Chakhao underscore their nutraceutical potential and commercial viability in health-oriented rice products.

Flavonoid content followed a similar trend among the rice varieties. Kalabati observed again highest value of flavonoid with 23.87 mg/100g followed by Kala biroin (19.54 mg/100g) and Chakhao (17.61 mg/100g). These three varieties were statistically superior to the rest ($P = 0.67$ mg/100g), suggesting a

strong correlation between dark pericarp pigmentation and total flavonoid concentration. In contrast, non-pigmented varieties such as MTU-1061 (0.98 mg/100g), Kalachampa (1.03 mg/100g), and Jamuna (1.86 mg/100g) showed minimal flavonoid content. Since flavonoids contribute to the total antioxidant activity, their co-occurrence with anthocyanins reinforces the value of pigmented rice in oxidative stress management and chronic disease prevention (He & Giusti, 2010) [12].

Black rice landraces could target markets for diabetic-friendly, anti-aging, or anti-inflammatory food products (Ling *et al.*, 2001) [19]. Similarly, Kim *et al.* (2008) [16] documented high anthocyanin concentrations (35-50 mg/100g) in black rice varieties, particularly those with dark pericarps, closely aligning with the values observed in Chakhao, Kalabati, and Kala biroin in the present study.

Table 3: Total Carotenoid (mg/g) and Moisture% in different rice varieties

Varieties	Total Carotenoid mg/g	Moisture%
Kalabati	27.23	8.68
Chakhao	59.47	12.55
Kala biroin	39.21	11.73
Gedi Ganjam	12.18	11.6
Kalachampa	8.64	10.89
MTU-1061	4.96	9.13
Jamuna	5.01	8.35
SE(m) \pm	0.40	0.17
CD(p=0.05)	1.22	0.52

*Each value is an average of three observations. There are some significant differences among rice varieties at $P \leq 0.05$.

Carotenoids are another important phytochemicals with provitamin an activity and strong antioxidant properties, contributing to eye health, immune function, and oxidative stress mitigation (Rodriguez-Amaya, 2001) [21]. The present study revealed a highly significant variation in total carotenoid content among the evaluated black rice varieties at $P \leq 0.05$.

The highest carotenoid concentration was observed in Chakhao (59.47 mg/g), followed by Kala biroin (39.21 mg/g) and Kalabati (27.23 mg/g), confirming their enhanced nutritional value for lipophilic antioxidants (Table-3). On the other end, Jamuna (5.01 mg/g) and 1061 (4.96 mg/g) registered the lowest

carotenoid levels, suggesting their limited contribution to vitamin A intake. These results highlighted Chakhao and Kala biroin along with Kalabati as promising landraces especially targeting populations vulnerable to vitamin A deficiency. Saini *et al.* (2017) [23] reported the potentiality of certain black rice varieties as sources of provitamin A and antioxidants.

Moisture content also significantly influenced the shelf life, storage stability, and processing behavior, most importantly the integrity of components of rice grains. In comparison, Chakhao with higher moisture content of 12.55%, may maintained hygroscopic balance without significant deterioration and post-

harvest microbial stability where as in Jamuna and Kalabati with their lower moisture percentages of <9%, may offer advantages in dry storage conditions. High-moisture grains (>17%) are more susceptible to fungal growth, spoilage, and carotenoid degradation during storage (Borah *et al.*, 2018) ^[4].

Micronutrients such as iron (Fe) and zinc (Zn) play pivotal roles in human health, particularly in combating iron-deficiency anemia and promoting immune defense function, respectively. The current study revealed significant variation among rice varieties for both Fe and Zn concentrations (Fig-2).

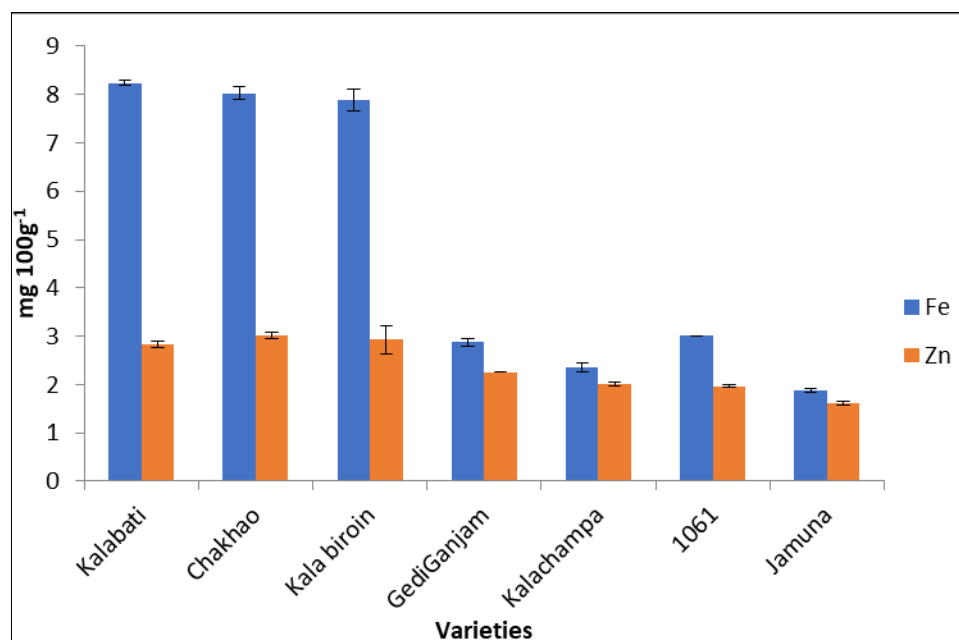


Fig 2: Iron (Fe) and Zinc (Zn) content (mg/100g) in different rice varieties, with error bars representing \pm standard error of the mean (n=3)

Iron content ranged from 1.88 mg/100g in Jamuna to 8.24 mg/100g in Kalabati, exceeding the critical difference (at $P \leq 0.05$). Kalabati (8.24 mg/100g), Chakhao (8.03 mg/100g), and Kala biroin (7.89 mg/100g) explained the importance of Indian landraces in terms of Fe concentration. These values are substantially higher than typical values reported for polished white rice, which often contains less than 2 mg/100g (Gregorio *et al.*, 2000) ^[10]. Zinc content followed a similar trend, though the concentration range was narrower from 1.62 mg/100g (Jamuna) to 3.02 mg/100g (Chakhao) than Fe content. Chakhao (3.02 mg/100g), Kala biroin (2.93 mg/100g), and Kalabati (2.84 mg/100g) again observed highest value and significantly above the P value (0.09 mg/100g). Zn deficiency is widespread in developing countries (Hotz & Brown, 2004) ^[13] and the results confirm that black rice varieties with dark pericarps tend to accumulate higher micronutrient levels, consistent with the reports on mineral accumulation in pigmented traditional landrace rice varieties compared to conventional white rice (Chen *et al.*, 2012) ^[6].

The superior Fe and Zn concentrations in Kalabati, Chakhao, and Kala biroin position them as core genetic resources for micronutrient-rich rice breeding programs, addressing the dual burden of energy sufficiency and hidden hunger (Bouis & Saltzman, 2017) ^[30]. These varieties could be deployed either directly as nutrient-dense staples or as donor parents in conventional and molecular breeding.

Conclusion

Rice is a major food crop in India and also in Odisha. The comparative analysis of seven rice varieties revealed especially in the context of nutrition and health-promoting characters. Chakhao, Kalabati, and Kala biroin emerged more promising profile in a wide spectrum of nutritional and biochemical traits across multiple parameters. These black rice landraces exhibited high value of protein, dietary fibre, energy, anthocyanins,

flavonoids and carotenoids. Furthermore, the considerable levels of iron and zinc observed in several varieties underscore their potentiality for micronutrient biofortification, a critical strategy in addressing global malnutrition and "hidden hunger".

Conversely, non-pigmented varieties such as Jamuna and MTU-1061, though less impressive nutritionally, an interesting metabolic divergence was noted in Gedi Ganjam, which had the highest protein and non-reducing sugar content, but minimal anthocyanin and flavonoid accumulation, highlighting the complex genotype-dependent between primary and secondary metabolism in rice.

Among different rice varieties, the landraces are highly nutritious, rich in antioxidants and also having higher micronutrient content. Black rice which are pigmented rice highly rich in anthocyanin and flavonoid content that contribute significantly to its numerous health benefits like potentially reducing the risk of cancer, heart disease and diabetes. Gedi Ganjam which is a white landrace, that are cultivated in coastal areas of Odisha gave a promising result in terms of protein and total soluble carbohydrate content and also have at par result in other observed parameters. More cultivation of black rice varieties and local landraces can be emerged as highly nutritious staple foods which have also numerous nutraceutical benefits.

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