Comparative Study of Nutritional Profile of Rice Varieties in Nepal

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Abstract

The purpose of this study was to evaluate and compare the nutritional quality of different rice varieties (*Taichung-176, Khumal-4* rice, and Black rice) with *Jumli Marsi* rice. The highest nutritional factors and phytochemical components were found in the *Marsi* rice (RR) and Black rice (BR). The highest amount of antioxidant property and phenolic content was found in Black rice which was $61.58 \pm 0.02\%$ and 22.75 ± 0.02GAE/100g respectively. The reducing sugar was found to be highest in the TR rice variety, which was $2.74 \pm 0.01\%$. The results also highlight the cooking and physicochemical properties of rice depending on the amylose content of rice varieties. The qualitative analysis of the phytochemical content in different rice varieties showed the presence of tannin, flavonoid, alkaloid, and terpenoid in *Marsi* and BR. While anthraquinone and saponin were negative for all the rice varieties, protein and glycoside were found to be present in all the rice varieties. Also, the pigmented rice varieties were found to have high nutritional components compared to the non-pigmented rice varieties. All the data observed in the study was found to be statistically significant ($p < 0.05$).

Keywords: Pigmented Rice**,** Antioxidant, phytochemicals, *Jumli Marsi*

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Introduction

Cereal grains are consumed worldwide and are considered an important dietary source of proteins, carbohydrates, vitamins, minerals, and fiber [1]. Rice is the cereal grain that is consumed as a major staple food around the world. Rice (*Oryza sativa* L) belongs to the family Poaceae. The contribution of rice as a percentage of total dietary energy supplied in developing countries, which is 715 kcal/day, 27% of total dietary energy supply, 20% of dietary protein, and 3% of dietary fat [2,3,4].

There are different varieties of rice available around the world. Among those, pigmented rice varieties such as red, black, purple, and brown rice are known to be consumed for a long time in Southeast Asian countries. They are also known to be originating in Southeast Asia [1]. In the case of Nepal, there are three different regions (Tarai, mountains, and hilly) classified according to their geographic location. Different varieties of rice are cultivated from Tarai (lowland plains) to high Hilly regions.

Jumla (2300 MSL) is the key site for growing cold-tolerant rice genotypes for the hilly regions of Nepal. The major area for the production of *Jumli Marsi* is Tila and Sinja valleys in Jumla, which are the world heritage sites present in Nepal [5]. *Marsi* (RR), sometimes spelled as *Marsee* is a variety of rice cultivated in the highest altitude in the world, Chhumchaur, Jumla [6]. It is commonly known as nutritionally rich in compounds such as protein, polyphenol, flavonoids, and antioxidants. The *Marsi* rice is classified by the farmers in different groups namely, *Kali Marsi, Raato Marsi* (can be seen in photograph 4)*, Seto Marsi, Daarime Marsi, Mehele Marsi,* etc. Marsi is also known to have medicinal value [7,8].

Black rice (BR) is a type of pigmented rice that has black bran covering the endosperm of rice grain. They are black (shown in **Photograph 1**) and turn purple when cooked. The color intensities of pigmented rice are known to be related to the value of lightness, redness, and yellowness. It takes a longer time to cook and has stickier consistency compared to white rice [9]. The supplementation of diets with black rice and the anthocyanin-rich extract of the black rice is found to have various health benefits [10].

The composition of rice variety is important for health-conscious consumers as well as useful to reduce fuel consumption during the process of cooking. Amylose content can be a factor that can influence the cooking time of rice. These are different in different varieties of rice [11]. Amylose is a branched carbohydrate mainly based on α (1–4) bonds. Amylose content is an important factor in determining the cooking properties of a rice variety [12,13]. The amylose content in different rice varieties is classified into waxy, low, intermediate, and high according to the percentage of amylose present in the rice, which is classified as waxy (0-2% amylose), low (10-20% amylose), intermediate (20-25% amylose), and high (>25% amylose) [14].

The cooking quality of rice depends on the components of the rice variety such as proteins and amylopectin [15]. The antioxidant activities of black and red rice and their crude extracts have given results that show the addition of the pigmented rice could increase antioxidant property in daily meals. The pigment in rice is the indicator of bioactive compounds present in the rice [9]. These varieties are studied and found to have high anticancer, anti-inflammatory properties as well as antioxidant content. Some phytochemicals such as phenolic compounds, bioflavonoids, terpenoids, and alkaloids are also found in pigmented rice [8,16]. The grains with red and black (darker) colored pericarp grains have higher molecular weight compared to the light brown pericarp color [16].

Carotenoids, vitamin E, terpenoids, and phenolics are some important groups of phytochemicals found in whole grains [17]. Phenolics are produced by the process of secondary metabolism in plants, which are also found to have a positive impact on human health [18]. The anthocyanins and pro-anthocyanidins/condensed tannins are some of the commonly found pigments in black rice and red rice, respectively. The flavonoids like luteolin, apigenin, quercetin, isorhamnetin, kaempferol, and myricetin are found in rice. Black rice has high phenolic and antioxidant compounds, which contribute to multiple biological activities leading to an increase in today's market demand [19,20].

The *Taichung-176* (TR) rice variety (shown in Photograph 3) is one of the commonly used rice varieties in Nepal and it is used to prepare traditional fermented food varieties such as *Chyang, selroti,* etc. *Marsi* and Black rice varieties are expensive compared to the other two rice varieties. *Khumal-4* (KR) (shown in **Photograph 2**) rice is affordable and is consumed by the locals for a daily meal [21]. There have been various research studies performed to study different rice varieties, however, no researches have been performed to compare Red rice (RR), also known as *Marsi,* which is mainly found in the altitudes of Nepal, with other rice varieties. Therefore, in this study, the main objective was to comparatively study the nutritional characteristics of *Marsi* rice with other rice varieties available in Nepal.

Materials and Methods
Sample Collection and identification
Four different varieties of rice (*Taichung-176* rice, *Khumal-4* rice, Black rice, and Red rice/ *Jumli Marsi* rice) were collected from the local market of Khumaltar and Kathmandu Organics (as suggested by Food Research Division, NARC, Khumaltar, Nepal).

Determination of physical properties of rice
Determination of length, breadth was done by randomly selecting 10 grains from each sample using a slide caliper [22].

Length = Main scale reading + Vernier scale reading × Vernier constant

Breadth = Main scale reading + Vernier scale reading × Vernier constant

Weight of kernels
Kernel grains (1000 grains counted manually) from each variety were randomly selected and weight was recorded in grams [23].

Determination of cooking characteristics of rice varieties
Swelling property/ Water uptake
For the determination of swelling property, finely ground rice was mixed in distilled water (0.35 g in 12.5 ml). The mixture was then heated in the water bath for 30 minutes at a temperature of 60°C. It was then centrifuged at 3500 rpm for 20 minutes. The supernatant was decanted in a pre-weighed dish. The temperature was maintained at 100 °C for drying (20 minutes) [24]. The residue was weighed. The swelling power was calculated by:

Swelling Property = Weight of the Residue /Original weight

Cooking time
Rice (2 g) of each variety was cooked in distilled water (20 ml) separately, and the temperature was maintained at 90°C in a water bath. Two glass slides were used to press cooked rice samples in between (to find out the minimum cooking time) [23,24].

Proximate Analysis of rice varieties
Determination of moisture and ash was done based on the standard method. The reducing sugar content was determined by the anthrone method. The carbohydrate content was determined using the anthrone method for carbohydrate estimation. Spectrophotometric analysis was done at 630 nm [24,25,26].

Calculation:
Amount of carbohydrate present in 100mg of the sample = Glucose (in mg)/ Volume of test sample × 100

% carbohydrates = (concentration sample * dilution factor)/ mass sample X 100

Amylose content
The rice flour was mixed with ethanol (95%) and 1 N NaOH. A mixture was prepared by thoroughly mixing it

in a volumetric flask. The mixture was heated in a boiling water bath for 10 minutes. It was then cooled to room temperature, to gelatinize the starch present in the rice sample. Five milliliters of the solution was then transferred to another volumetric flask. One ml of 1N acetic acid and iodine solution was added to the solution. The distilled water was used to adjust the volume to 100 ml. The vortex mixing method was used to mix it again. It was then allowed to stand for the time duration of 20 minutes. The absorbance of the sample solution was measured at 620 nm using a UV Spectrophotometer. Finally, the determination of amylose content in samples was done by using the amylase standard curve [23].

Determination of Reducing Sugar by using 3, 5- dinitrosalicylic acid / DNS method

For the rice sample, 100 mg of the sample was weighed and centrifuged with the hot 80% ethanol twice (5 ml each time). The supernatant was collected and evaporated by keeping it in a water bath at 80°C. 10 ml water was added to dissolve the sugars. 0.5 to 3 ml was pipette out of the extract in test tubes and volume was equalized to 3 ml with water in all the tubes. The standard sugar solution was prepared in the range of 0 to 3 ml in different test tubes. The final volume was made up to 3 ml with distilled water, which made the concentrations ranging from 0 to 750 mg. 3 ml of DNS reagent was added. The contents were heated in a boiling water bath for 5 minutes. It was then cooled and the intensity of dark color was read at 540nm. Reducing sugar in rice varieties was determined by using the 3, 5- dinitrosalicylic acid / DNS method by spectrophotometric analysis at 540 nm [27].

● Antioxidant and phenolic content of rice varieties

The antioxidant content was determined by using the DPPH assay method. The phenolic content was determined by the Folin-Ciocalteu method using spectrophotometric analysis (gallic acid as the standard).

Antioxidant activity (DPPH Assay method)

The antioxidant activity of rice samples was evaluated spectrophotometrically, as a measure of radical scavenging activity by using DPPH [24,25].
The capability to scavenge the DPPH radical (% inhibition) was calculated using the following equation:

Scavenging ability % =

[Absorbance of control (515 nm) - Absorbance of sample (515 nm)/Absorbance 515 nm of control] x 100

Phenolic content (Folin-Ciocalteu method)

Total phenolic content was determined by the standard graph of gallic acid, which was prepared by dissolving 1 mg of dry gallic acid, and absorbance was measured at 760 nm [26].

Phytochemical properties of rice varieties

The qualitative analysis of different rice varieties was done for the phytochemicals: terpenoid, flavonoid, tannin, anthraquinone, saponin, and glycoside [9].

Extraction

Each variety of rice was milled into flour and then the rice flour was kept in 20 ml of 80% methanol for 24 hrs at room temperature and then centrifuged at 4000 g for 15 minutes.

Terpenoid:

Rice extract (5 ml) was mixed with 2 ml of chloroform. The concentrated H_2SO_4 (3 ml) was added to the solution. Reddish-brown coloration formed in between two liquid phases indicated the presence of terpenoids.

Flavonoid:

Crude rice sample (2 g) was mixed with 10 ml of ethyl acetate heated in a water bath (5 minutes). The Whatman paper no.1 was used to filter the solution. Then, the filtrate (4 ml) and the dilute ammonia solution (10%) were mixed well and observed for color change (yellowish coloration), which indicated the presence of flavonoids.

Tannin:

A few drops of 0.1% $FeCl_3$ solution were mixed with the (10 ml) rice extract filtrate. The formation of bluish-black precipitation indicated the presence of tannin.

Phlobatannin:

The concentrated HCl was added to the rice extract filtrate (10 ml) in a test tube, and boiled for 1 minute. It was then observed for the formation of a red precipitate, which indicated the presence of phlobatannins.

Protein:

Rice extract (2 ml) was taken in a test tube was taken. One ml of 40% NaOH solution was added to it. The solution was then mixed properly. One to two drops of $CuSO_4$ solution were added to the solution. The change of color to violet indicated the presence of proteins.

Table 1. Bioactive compounds in different rice varieties

S.N.	Type of rice	Antioxidant (%inhibition)	Phenolic Content (GAE/100G)
1.	*Taichung-176* (TR)	8.13±0.01	9.35±0.01
2.	*Khumal-4* (KR)	16.38±0.00	11.74±0.01
3.	*Marsi*/ Red rice (RR)	53.06±0.01	15.47±0.03
4.	Black rice (BR)	61.58±0.02	22.75±0.02

Note: Phenolic content was expressed in the unit of mg GAE/100g. All the values are mean ± standard deviation of triplicate analysis. The data presented were found to be significant ($p < 0.05$).

Table 2.Physical characteristics of different rice varieties

S. N.	Rice Type	Kernel weight (g)	Length (mm)	Breadth (mm)	Cooking time (Min)	Amylose Content (%)	Amylose Type	Amylo-pectin (%)	Swelling property
1.	TR	19.21±0.23	4±0.01	3±0.2	23±2.0	27.62	High	72.40	3.83±0.25
2.	KR	17.12±0.05	5±0.20	3±0.1	20±0.5	24.12	Medium	75.88	3.25±0.02
3.	RR	18.04±0.03	6±0.25	4±0.25	35±1.0	20.67	Medium	79.33	2.32±0.18
4.	BR	17.03±0.02	6±0.25	3±0.5	40±2.0	5.28	Low	94.72	1.80±0.15

Note: All the tests (except length and breadth of rice) were performed in triplicate and the data listed are the mean ± standard deviation of triplicate readings. The data presented were found to be significant ($p < 0.05$).

Table 3. Proximate analysis of different rice varieties

S. N.	Tests performed	Types of Rice			
		TR	KR	RR	BR
1.	Ash content (%)	1.62±0.01	0.74±0.01	1.56±0.01	1.70±0.01
2.	Moisture (%)	9.05±0.01	7.91± 0.00	10.2± 0.03	12.15± 0.01
3.	Carbohydrate (%)	82.5±0.02	65.3± 0.01	74.5± 0.01	73.2±0.01
4.	Reducing Sugar (%)	2.74±0.01	1.42± 0.01	1.12± 0.02	1.87± 0.03

NOTE: X±Y= Mean ± S.D of triplicate values. All the experimental values were generated three times and the mean was then calculated. The data presented were found to be statistically significant ($p < 0.05$).

Saponin:

The crude sample (0.5 g) was mixed in distilled water, and boiled by using a water bath. After that, it was shaken well. The sample was then observed for the formation of froth, which indicated the presence of saponin.

Anthraquinone:

The crude sample and benzene were mixed in a conical flask. A magnetic stirrer was used to mix it properly for 4 hrs. The filtrate (10 ml) was taken and mixed with 0.5 ml ammonia solution (10%). The change in color of the solution indicated the presence of anthraquinone.

Glycoside:

The rice extract (5 ml) was mixed with a mixture of glacial acetic acid (2ml), 2% $FeCl_3$ solution and 1 ml concentrated H_2SO_4 (added slowly when mixed). The mixture was observed for the formation of a brown ring, which indicated the presence of glycoside.

Data Analysis

All the tests (except length, breadth of rice, and amylose content) were performed on triplicates (n =3). The length and breadth of rice were measured in n=10 by randomly selecting grains from each sample. The determination of the level of significance of various parameters of different rice varieties was performed by using Minitab Version 18.

One factor analysis of variance was performed for a completely randomized design to check the level of significance.

Results

Four different varieties of rice TR, KR, RR, and BR were collected from four different places. **Table 1** shows that the antioxidant was highest for BR (61.58±0.02%), lowest for TR (8.13%) and the phenolic content was highest for BR (22.75 ±0.02GAE/100g), lowest for TR (9.35±0.01GAE/100g).
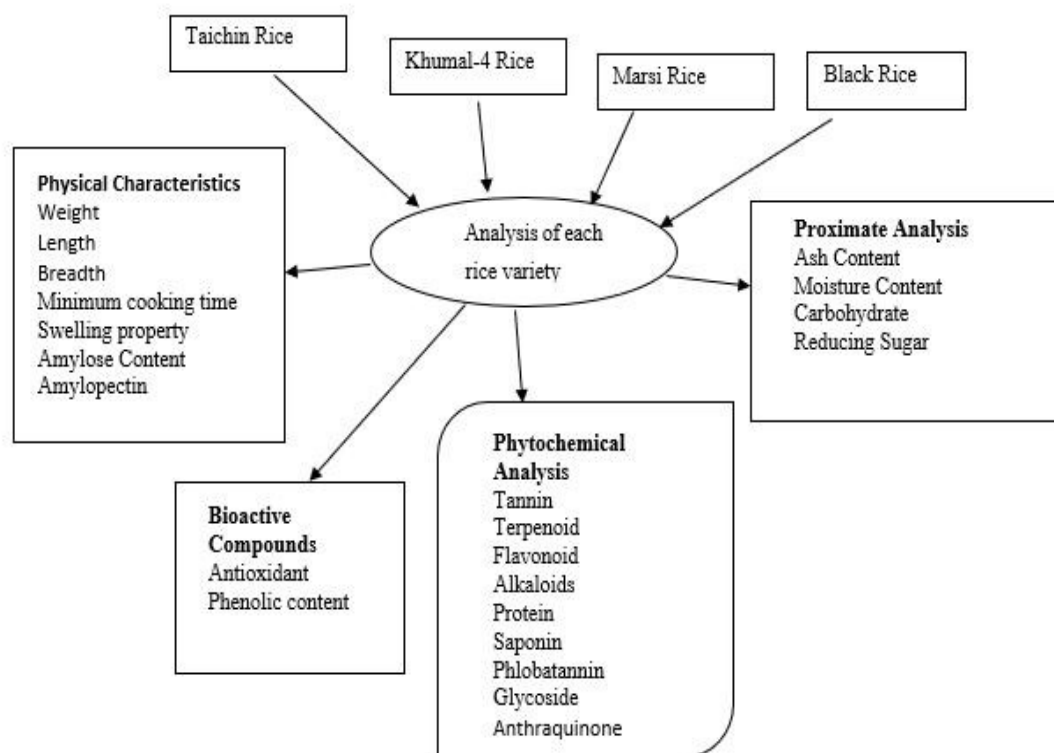
The physical characteristics and cooking characteristics of different rice varieties were studied. The rice kernel weight (g) (in **Table 2**) was 19.21±0.23, 17.12±0.05, 18.04±0.03, and 17.03±0.02 for TR, KR, RR, and BR respectively.

The results showed that the length and breadth of rice varieties ranged between 4 mm to 6 mm and 2 mm to 4 mm. The swelling capacity ranged from 1.80 w/w to 3.83 w/w. The cooking time ranged minimum for KR (20 minutes) and maximum for BR (40 minutes). The moisture content, ash content, and amylose content, as well as amylopectin, which was determined for each variety of rice (in **Table 3**). The moisture content of TR, KR, RR, and BR 9.05± 0.01%, 7.9± 0.00%, 10.2± 0.03%, and 12.15± 0.01% respectively.

Table 4. Qualitative analysis of phytochemical properties in rice sample

S. N.	Tests performed	Types of Rice			
		Taichung-176 **(TR)**	*Khumal-4* (KR)	Red rice/ *Marsi* **(RR)**	**Black rice (BR)**
1.	Tannin	-	-	+	+
2.	Flavonoid	-	-	+	+
3.	Alkaloids	-	-	+	+
4.	Terpenoid	-	-	+	+
5.	Protein	+	+	+	+
6.	Glycoside	+	+	+	+
7.	Anthraquinone	-	-	-	-
8.	Saponin	-	-	-	-

Note: (+) denotes the presence of the phytochemical and (–) denotes the absence of phytochemical

Flowchart 1. Schematic representation of study of different rice varieties

The ash was highest in BR 1.70±0.01%. Amylose content was highest in TR (27.62±0.01%) and lowest in BR (5.28±0.01%). The reducing sugar was found highest in TR (2.74±0.01%), and lowest in RR (1.12±0.03%). The phytochemical properties of different varieties of rice samples (**Table 4**). Tannin, alkaloids, flavonoids, and terpenoids were present only in red rice and black rice. Anthraquinone and saponin were absent in all the samples. Glycoside and protein were present in all the rice samples.

Discussion

The antioxidant activity was determined by the DPPH assay method by determining the percentage inhibition for every rice variety. The antioxidant activity of *Marsi* was compared with TR, KR, and BR. BR had the highest antioxidant property and the phenolic content which was 61.58± 0.02 % and 22.75± 0.02 GAE/100 g respectively. The lowest antioxidant activity was found in TR, which

was 8.13± 0.01%. The DPPH radical scavenging activity was found higher in BR variety (59.02 to 75.52%) with the highest observed in aromatic black rice *Poireiton chakhao* (75.52%) [26]. Another study reported that the DPPH scavenging activity of red rice, parboiled rice, and *Sona Masuri* were 57.06%, 9.13%, and 16.38%, respectively [18,29].

Photograph 1. Black rice variety

Photograph 2. *Khumal-4* rice variety

Photograph 3. *Taichin* rice (*Taichung-176*)

Photograph 4. Red rice variety (*Jumli Rato Marsi*)

The phenolic content of *Marsi* was compared to that of other rice varieties. Only BR had a higher phenolic content than that of *Marsi* rice variety, which was 22.75± 0.02 GAE/100 g. The lowest phenolic content was found in TR, which was 9.36± 0.01 GAE/100 g. Similar findings of the phenolic content and antioxidant were found in another study on pigmented rice varieties [27] [29]. All the results from this study were found to be similar to the

result of previous experiments, and also were found to be statistically significant ($p < 0.05$).

The moisture content of *Marsi* rice was compared to that of TR, KR, and BR. The moisture content of *Marsi* was 10.2% and for TR, KR, and BR, it was 9.05%, 7.9%, and 12.15% respectively. Only BR was found to have more moisture than *Marsi*. Only BR was found to have the highest ash content compared to that of *Marsi*, which was found to be 1.70%. In a similar study, the ash value was higher in black rice varieties compared to the other varieties of rice, which is the result of a similar trend as in our study [28]. In another study, black rice was compared to white, brown, glutinous, and basmati rice, black rice was found to have higher ash content than other varieties [18][30]. Carbohydrate content was found to be 74.5± 0.01% in *Marsi* rice variety and only TR had carbohydrate content higher than that of *Marsi* which was 82.5±0.02%. Similar findings were reported in a previous study for the *Jumli Marsi* rice [7].

Amylose content plays an important role in determining the cooking and pasting properties of a rice variety [13,15]. The cooking quality of rice depends on the components of the rice variety such as proteins and amylopectin varieties [31][32]. Amylose content in TR, KR, *Marsi* rice, and BR were found to be 27.6± 0.01%, 24.12± 0.01 %, 20.67± 0.01%, and 5.28± 0.01% respectively. In this study, the relation was found to be positive as the rice varieties with a high amount of amylose had shorter cooking time duration **(Table 2)**. Similar trends in results were found in another study varieties [14,24,31].

In this study, a qualitative analysis of phytochemical properties was also performed in all the different varieties of rice. Tannin, alkaloids, flavonoids, and terpenoids were present only in red rice and black rice. Anthraquinone and saponin were absent in all the samples. Glycoside and protein were present in all the samples (pigmented and non-pigmented rice). Similar results were observed in a study performed on different rice varieties [9].

The purpose of the study was to compare different characteristics of varieties of rice available in Nepal. *Marsi* rice and BR are expensive when compared to the other two varieties, and they are mainly consumed for their nutritional benefits. The reason for choosing these four rice varieties is that they are of different price range and also have different colors as well as characteristics. The pigmented and non-pigmented range of rice varieties were taken for the study because of how they are promoted in the Food market these days. Pigmented rice varieties were found to be more nutritious as they

were found to have more amount of antioxidant content as well as phenolic content. This could be because the pigments of the rice varieties are directly linked to the amount of nutrition. *Marsi* and black rice get their pigment from anthocyanin, which is known to have antioxidant properties.

Conclusion

In conclusion, *Jumli Marsi* rice variety is highly valued in Nepal for its unique color and high nutritional content. From the result of this study, *Marsi* rice was found to be rich in antioxidants, phenolics when compared to the other two non-pigmented rice varieties but the black rice variety was found to be richest in the bioactive compounds. For a detailed comparison of these rice varieties, the determination of anthocyanin, tannin content, and flavonoid could be done. Since methanol is used as an extracting solvent for most of the polar components, there may be the presence of non-polar components which may not have been measured by using this method. Different methods could be used to quantify the non-polar content present in different rice varieties.

Author's Contribution

The study was performed by Ms. Evance Pakuwal under the supervision of Mr. Prakash Manandhar.

Competing Interest

There are no competing interests involved in this study.

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Ethical Approval and Consent

Not Applicable.

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