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ORIGINAL RESEARCH PAPER

Nutritional Assessment and Comparison between Brown and Milled Rice Landraces (*Anadi*, *Bhotange*, and *Kalo Nuniya*) of Nepal

Sakul Rai*[1], Dambar Bahadur Khadka[1], and Bimala Pokharel[2]

[1]*Central Campus of Technology, Tribhuvan University, Hattisar, Dharan, Nepal*

[2]*Ministry of Land Management, Agriculture & Cooperative, Koshi Province, Nepal*

Abstract

The main objective of this study was to assess and compare the nutritional quality of brown- and milled rice landraces (Kalo Nuniya, Anadi, and Bhotange) of Koshi Province. Paddy samples were cleaned to remove dockage and sun-dried until about 13-14% moisture to ensure maximum hulling- and milling recovery. Paddy samples were shelled and milled to obtain brown (unpolished) rice and milled (white or polished) rice, respectively and were stored in double-sealed re-closable low-density polyethylene (LDPE) zipper bags (62.5 μm) at ambient temperature (23-27°C) until analysis. As expected, brown rices had better nutritional profiles than their milled counterparts with significantly higher ($p<0.05$) crude fat (2.99 ± 0.10 – 3.99 ± 0.09%), crude fiber (0.12 ± 0.00 – 0.70 ± 0.02%), and total ash (1.53 ± 0.2 – 3.71 ± 0.30%) content. Among the brown rices, Anadi brown had better nutritional profile with the highest iron (1.56 ± 0.14 mg/ 100 g), calcium (86.4 ± 3.30 mg/100 g), total ash (3.71 ± 0.30%), crude fiber (0.70 ± 0.02%), and crude fat content (3.99 ± 0.09%). Bhotange and Kalo Nuniya had uniform distribution of iron and calcium, respectively, throughout the entire rice kernel.

Keywords:
Dockage
Nonstarch lipids
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*Corresponding author at:
Central Campus of Technology
Tribhuvan University, Nepal.
E-mail address: sakul.d.rai@gmail.com
Contact no.: +9779860867285

Introduction

Rice is not only the primary food source for a large portion of the world's population but also a crucial staple in regions where agriculture is the mainstay, and industrial development is limited. A significant portion of the global population with low incomes relies heavily on rice and rice-based diets for their nutritional needs (Bhattacharya, 2011). Cooked rice is a preferred food for almost the entire population in Nepal (Crop Development Directorate, 2015) and accounts for more than 50% of the total calorie contribution of Nepalese people (Kharel et al., 2018). From this perspective, rice is the most important global strategic crop for food and nutrition security.

Rice landrace, cultivated and consumed for centuries, plays a vital role in livelihoods and food security (Bhat & Riar, 2017). Traditional landraces are valued for their superior nutritional content compared to hybrids and they have also been a part of the Ayurveda system for ages, used to treat various ailments such as diarrhea, fever, vomiting, hemorrhage, burns, as well as to enhance eyesight, vocal clarity, fertility, and more. These health advantages have led to a growing demand among consumers (Hegde et al., 2013). However, information on the physicochemical qualities of Nepalese rice landraces is either not available or very limited (Pokharel et al., 2020) and no systematic initiatives appear to have been taken to identify, document, and promote Nepal's rich landraces diversity. Conversely, Nepalese farmers who continue to cultivate landraces are encountering challenges related to low crop yields and difficulties in achieving financial stability (LI-BIRD, 2020).

Since Nepalese rice germplasm possesses a great diversity both in qualitative and quantitative traits and all modern varieties can be traced back to landraces, it is necessary to identify and document those traits through collection, conservation, characterization, and utilization. Many rice landraces are vanishing from general cultivation and many of them are under threat. Rice landraces are rich in phenological and agro-morphological diversity. It is necessary to estimate the extent and magnitude of rice diversity and the useful traits that should be used in rice breeding. Conservation of these genetic resources is essential to plant breeders for current and future crop improvement and to farmers to meet their immediate livelihood needs (Upreti, 2017).

For this study, 3 important landraces of Nepal (*Anadi*, *Kalo Nuniya/Kalo Nunia*, *Bhotange*), each of unique quality were chosen for the study. Anadi, the only glutinous (sticky) variety native to the middle hills of Nepal was selected for its special food value, and supposed nutritional and medicinal value (Sthapit et al., 2005). *Kalo Nuniya/Kalo Nunia* was chosen for its aromatic property (CDD, 2015; Joshi et al., 2021), popularity, high cultural-, social- (Joshi et al., 2020) and market-value (Adhikari et al., 2017). *Bhotange* rice landrace was selected to properly document, promote and assess the relevant quality parameters, thereby providing further scope for quality breeding and its conservation as the literature regarding this landrace remains very scanty.

Figure 1
Sample collection site (province and districts)

Whole grain rice is the chemical reservoir storing mostly starch (80%, with varied amounts of amylose and amylopectin) and 6-8% proteins (Butardo & Sreenivasulu, 2016). Gross composition and individual components of rice milling fractions have been studied by Champagne et al. (2004). The distribution of protein in rice has also been discussed by many authors, including Cagampang et al. (1966), Juliano et al. (1973), Resurreccion et al. (1979), and Shih (2006). Compensation for an increase in protein content lowers starch percentage value (Juliano, 1993). Lipids, dietary fiber, minerals, and secondary metabolites are in trace amounts (located mostly in rice bran) but are lost upon milling (Butardo & Sreenivasulu, 2016). Nonstarch lipids are distributed in various layers of rice (Choudhury & Juliano, 1980). Grain types, planting environments, and extraction methods used affect its value (Amissah et al., 2003; Kitta et al., 2005; Wang et al., 2006). Crude fiber concentrated in the bran fraction is lost during milling (Juliano & Tuaño, 2018). Reducing sugar content of milled- and brown rice has been studied by Ali & Bhattacharya (1980). Champagne et al. (2004) reported higher free sugar content in brown rice than in milled rice. Mineral content in different rice landraces and cultivars (brown and white rices) varies due to genotypic differences (Jiang et al., 2008; Zeng et al., 2010; Huang et al., 2016). This wide variation in the chemical composition of rice landraces provides further scope for improving its nutrition and grain quality through breeding (Pokharel et al., 2020).

Materials and Methods

Collection of paddy (rough rice) samples

About 10 kg each of *Kalo Nuniya* (black-, fine grain-, aromatic rice), *Anadi* (bold-, glutinous-, late maturing rice), and *Bhotange*, was obtained from the Ministry of Industry, Agriculture & Cooperative, Koshi Province (see Figure 1 for sample collection site). Paddy samples were cleaned to remove dockage (foreign particles) and sun-dried until about 13-14% moisture to ensure maximum hulling- and milling recovery (International Rice Research Institute, n.d.) Half the amount of paddy was shelled (INDOSAW) to obtain brown (unpolished) rice. The remaining paddy was milled (INDOSAW) to obtain milled (white or polished) rice. Each rice type was kept in double-sealed re-closable low-density polyethylene (LDPE) zipper bags (62.5 μm) at ambient temperature (23-27°C) until analysis.

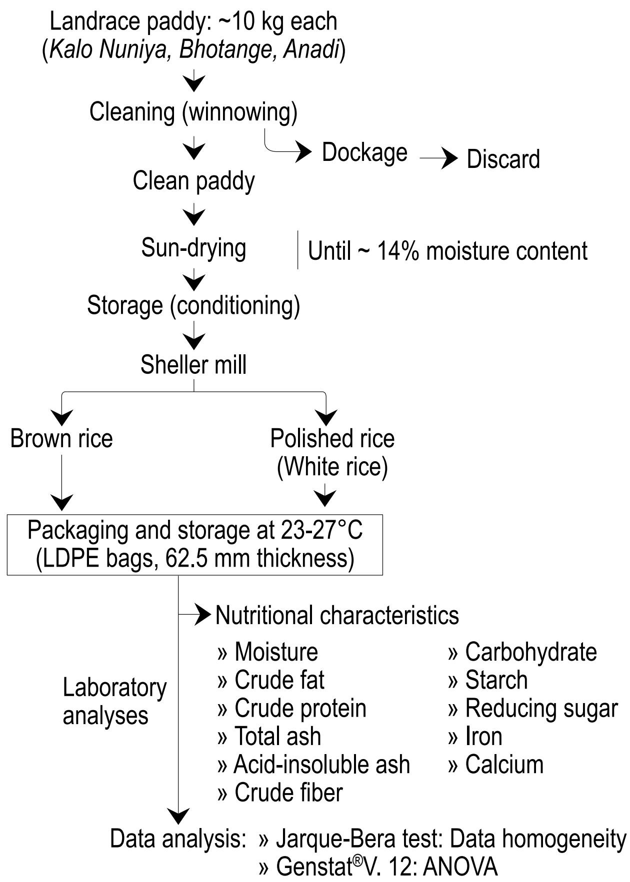

Figure 2
An overview of workflow

Nutritional profile (proximate and ultimate constituents)

The proximate components (moisture content, crude fat, crude protein, crude fiber, total ash, and total carbohydrate) of rice varieties were determined as per the methods described in KC & Rai (2007). Iron was determined colorimetrically and calcium was determined by the volumetric method as described in KC & Rai (2007).

Reducing sugar was determined using 3,5-Dinitrosalicylic Acid (DNSA Method) as described by KC & Rai (2007) and Jain et al. (2020). The absorbance was read at 540 nm against the blank in a UV-spectrophotometer (Agilent Cary 60 UV-Vis). The trendline was computed from the slope of standard glucose curve and reducing sugar in the aliquot was calculated from the trendline equation and back calculated to % of reducing sugar as glucose anhydrous.

Total starch was determined using the Lane and Eynon method with slight modifications described in KC & Rai (2007). A 2 g ground rice sample was mixed with water and heated to 60°C for 0.5 h to create a starch solution. Next, 25 ml of 95% alcohol was added, and the supernatant was removed after centrifugation. Again, 15 ml (50%) of alcohol was added, stirred, and centrifuged until the filtrate tested negative for the presence of sugar using α-naphthol. The residue was transferred to a 250 ml conical flask with about 50 ml water. 20 ml of concentrated HCl was added and placed in a boiling water bath for 2.5 h.

After cooling, the final volume was made up to 100 ml with water and filtered using filter paper. The solution was neutralized with NaOH solution. For clarification, 2 ml each of 0.3N $Ba(OH)_2$ and 5% $ZnSO_4$ was added to 10 ml of extract, and volume was made up to 100 ml with distilled water. The solution was filtered to get a clear extract. The starch content was then determined on the hydrolyzed extract by Lane and Eynon method.

Statistical analysis

The data generated were tested for homogeneity using the Jarque-Bera method followed by ANOVA (using Genstat® version 12.1.0.3338) for comparing the values.

Results and Discussion

Proximate components

Table 1 shows the proximate constituents of the 3 rice landraces taken for the study. Table 2 shows the ultimate components of the 3 rice landraces taken under this study. The moisture content among rices showed no significant difference ($p>0.05$) and was within the acceptable limit (<16%) in the mandatory standard set by the Department of Food Technology and Quality Control (2020). However, it must be noted that a vast difference in the degree of milling (DOM) and between varieties was obtained in this study with Anadi showing 19.61% DOM, *Bhotange* showing 23.19% DOM and *Kalo Nuniya* showing 35.05% DOM. So, all the parameters studied should be associated with their respective DOM.

There was no significant difference ($p>0.05$) in crude protein among all the rices (milled and brown). Since the greatest part of the total proteins is located in the endosperm (Shih, 2006), during analysis, in milled rice, the endosperm compensates for the reduced weight from bran removal, making the net protein percentage unchanged. Also, protein distribution becomes more even as protein content increases in the grain (Cagampang et al., 1966; Juliano et al., 1973; Resurreccion et al., 1979). The assessed rice landraces' (both brown and milled) crude protein was found to be within the range reported by Champagne et al. (2004) for brown- and milled rice. It must be noted that Champagne et al. (2004) used 5.95 as a conversion factor for Kjeldahl nitrogen.

All the rices in this study had higher crude fat contents than those reported by Champagne et al. (2004) for brown- and milled rice. This could be due to different grain types, planting environments, and extraction methods used between studies (Amissah et al., 2003; Kitta et al., 2005; Wang et al., 2006). A significant difference ($p\leq0.05$) in crude fat content was also seen among the rices under study which also could be due to difference in grain types and planting environments. Choudhury & Juliano (1980) mentioned the distribution of nonstarch lipids (major lipid in rice) to be 14%-18% in embryo, 39%-41% in bran, 15%-21% in polish, and 25%-33% in sub-aleurone layer and inner endosperm. The significant reduction ($p\leq00.05$) in crude fat content in milled rices from brown rices of the same landrace can be attributed to the milling process which results in the loss of the layers mentioned above to various extents.

Kalo Nuniya white (K_w) and *Kalo Nuniya* brown (K_b) had lower crude fiber than those reported by Champagne et al. (2004). White rices had significantly different ($p\leq00.05$) crude fiber content among themselves and so was the case among brown rices. The difference in crude fiber content could be due to varietal difference and difference in the DOM. Significantly lower ($p\leq00.05$) crude fiber content in milled rices than their unpolished counterparts are because the crude fiber concentrated in the bran fraction is lost during milling (Juliano & Tuaño, 2018).

No significant ($p>0.05$) varietal difference in terms of ash (mineral) content was found among white rices. However, significantly ($p\leq0.05$) different ash content among brown rices could be the result of differences in bran's ash content. *Anadi* brown (A_b) and *Bhotange* brown (B_b) had more than 2-times higher ash content than the upper-range value reported by Champagne et al. (2004) for brown rice. The ash content of the white rices in this study also slightly exceeded the upper-range value reported by Champagne et al. (2004). This difference in ash content could be the result of different rice varieties taken between the studies. All brown rices had significantly higher ($p\leq0.05$) total ash and acid insoluble content than their milled counterparts. This is because minerals (total ash), in trace amounts (located mostly in rice bran) are lost upon milling

(Butardo & Sreenivasulu, 2016). Significant difference ($p \leq 0.05$) in acid insoluble ash content was seen among brown rices but not among white rices.

Carbohydrate content among white rices and among brown rices were similar ($p>0.05$). Significantly lower ($p \leq 0.05$) carbohydrate percentage observed in A_b and K_b than their milled counterparts are because the bran layer contributes to the rice weight, thereby apparently lowering the carbohydrate percentage value. However, no significant increase ($p>0.05$) in carbohydrate percentage in *Bhotange* white (B_w) compared to B_b might suggest a lesser contribution of bran to the total weight of the rice kernel.

Table 1
Proximate constituents of the rice landraces

Parameter	A_w	A_b	B_w	B_b	K_w	K_b	*LSD (5%)*
Moisture (%)	14.0 ± 0.55^a	12.45 ± 1.50^a	14.19 ± 0.47^a	12.25 ± 0.70^a	14.01 ± 0.73^a	12.80 ± 0.60^a	1.563
Crude protein (% db)	8.20 ± 0.34^a	9.00 ± 1.72^a	9.44 ± 1.47^a	9.00 ± 1.13^a	9.22 ± 0.08^a	10.5 ± 0.06^a	2.074
Crude fat (% db)	1.17 ± 0.12^b	3.99 ± 0.09^e	2.30 ± 0.05^c	3.83 ± 0.23^e	0.80 ± 0.01^a	2.99 ± 0.10^d	0.203
Crude fiber (% db)	0.50 ± 0.02^e	0.70 ± 0.02^f	0.15 ± 0.01^c	0.25 ± 0.01^d	0.08 ± 0.00^a	0.12 ± 0.00^b	0.032
Total ash (% db)	0.88 ± 0.08^a	3.71 ± 0.30^d	1.04 ± 0.11^a	3.37 ± 0.36^c	0.92 ± 0.04^a	1.53 ± 0.21^b	0.602
Acid insoluble ash (% db)	0.11 ± 0.01^a	0.46 ± 0.09^d	0.15 ± 0.01^a	0.37 ± 0.01^c	0.11 ± 0.03^a	0.24 ± 0.00^b	0.066
Carbohydrate (% db)*	75.02 ± 0.19^b	70.75 ± 2.65^a	73.02 ± 1.74^{ab}	72.30 ± 0.28^a	75.20 ± 0.98^b	72.12 ± 0.94^a	2.661

Note. A_w: *Anadi* white; A_b: *Anadi* brown; B_w: *Bhotange* white; B_b: *Bhotange* brown; K_w: *Kalo Nuniya* white; K_b: *Kalo Nuniya* brown; *Carbohydrate calculated by difference. Values are means ± standard deviation of triplicate samples. Means in the same row bearing the same superscript letter(s) are not significantly different ($p>0.05$).

Ultimate components

Table 2
Ultimate components of the rice landraces

Parameter	A_w	A_b	B_w	B_b	K_w	K_b	*LSD (5%)*
Starch (% db)	68.35 ± 2.48^d	54.50 ± 2.96^a	67.69 ± 2.35^{cd}	62.90 ± 2.81^{bc}	64.48 ± 3.55^{cd}	58.90 ± 1.41^{ab}	4.967
Reducing sugar (% db)	0.08 ± 0.01^a	0.14 ± 0.03^a	0.08 ± 0.02^a	0.41 ± 0.14^b	0.08 ± 0.01^a	0.08 ± 0.02^a	0.115
Iron (mg/100 g)	0.55 ± 0.06^b	1.56 ± 0.14^d	0.69 ± 0.10^c	0.76 ± 0.04^c	0.13 ± 0.04^a	0.65 ± 0.05^{bc}	0.132
Calcium (mg/100 g)	19.64 ± 1.89^b	86.4 ± 3.30^d	13.98 ± 1.75^a	70.69 ± 4.06^c	19.63 ± 3.37^b	23.56 ± 2.98^b	5.579

Table 2 shows the ultimate components of the 3 rice landraces taken under this study. Starch content showed no significant difference ($p>0.05$) among white rices and among brown rices in the present study. The lower starch percentage in unpolished rices is due to the bran layer contributing to the rice weight, thereby apparently lowering the percentage value of starch. No significant increase ($p>0.05$) seen in the starch percentage of Bw than Bb could be due to less contribution of the bran layer to the total weight of rice. The total starch contents of the assessed landraces (both white and brown) were lower than those mentioned by Butardo and Sreenivasulu (2016). The lower starch content can be due to varietal differences. Also, compensation by an increase in protein content lowers starch percentage value (Juliano, 1993).

Reducing sugar content of milled rices in this study was similar ($p>0.05$) among each other and also to that reported by Ali and Bhattacharya (1980) for milled rice although the method of determination was different (Nelson-Somogyi) from the method used in this study (DNS). A_b and B_b had higher reducing sugar content than mentioned in Ali and Bhattacharya (1980) for brown rice whereas K_b had similar reducing sugar content.

This difference in reducing sugar content can be because of the difference in rice variety between the studies. A_b and K_b had similar ($p \leq 0.05$) reducing sugar content as their milled counterpart, indicating uniform distribution of reducing sugar content throughout the kernel rather than just being concentrated in the bran layer. A similar finding was also reported by Ali and Bhattacharya (1980) for milled and brown rice. Bb showed significantly higher ($p \leq 0.05$) reducing sugar content than its milled counterpart. Champagne et al. (2004) reported higher free sugar content in brown rice than in milled rice.

B_w and *Anadi* white (A_w) had iron content higher than the upper range value mentioned in Champagne et al. (2004) for milled rice whereas K_w had less than the reported lower range value. The difference in iron content among brown and white rices in these studies could be due to genotypic differences in various landraces and cultivars (Jiang et al., 2008; Zeng et al., 2010; Huang et al., 2016). Significantly higher iron content in A_b and K_b than their milled counterpart is because iron is reduced significantly in polished rice (Jiang et al., 2008). Even though B_b had significantly higher ($p \le 0.05$) total ash content than B_w, iron content did not show a significant difference between them, suggesting that iron was more uniformly distributed in the entire rice kernel of *Bhotange* than being just concentrated in the bran layer.

B_b and A_b had higher calcium content than mentioned in Champagne et al. (2004) for brown rice. The differences in the calcium content among white and among brown rices in this study might be due to genotypic differences in landraces and cultivars (Jiang et al., 2008; Zeng et al., 2010; Huang et al., 2016). Significantly higher calcium content of A_b and B_b ($p \le 0.05$) than their milled counterparts is because minerals are concentrated in the outer layers of brown rice or in the bran fraction (Juliano, 1993) which gets removed during milling (Butardo & Sreenivasulu, 2016). However, no significant difference in calcium content between K_b and K_w indicates uniform distribution of calcium throughout the entire kernel rather than being concentrated in bran only.

Conclusions

Brown rices of all the three landraces have a better nutritional profile than their milled counterparts with significantly higher crude fat, crude fiber, and ash content. Among brown rices, *Anadi* brown has a better nutritional profile with the highest iron, calcium, ash, crude fiber, and crude fat content. *Bhotange* and *Kalo Nuniya* had uniform distribution of iron and calcium, respectively, throughout the entire rice kernel.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare no conflict of interest.

Ethical approval

This work did not involve any animal study.

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