

Study on Functional Properties of Hakuwa Rojina Khichaju and Bhaskar Mani Adhikari*

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Abstract

This research is based on the study of functional property of Hakuwa. In the present work, normal rice and Hakuwa were comparatively analyzed for physiochemical attributes. The anthocyanin content, antioxidant property and phenolic content in Hakuwa were determined. The result showed Hakuwa is nutritionally rich food. Proximate composition of rice and Hakuwa showed that; fat content is increased from 0.67 ± 0.29 to $1.62 \pm 0.21\%$, fiber content from 1.10 ± 0.18 to $2.47 \pm 0.34\%$ and ash content 0.88 ± 0.34 to $1.08 \pm 0.14\%$, and protein decreased from 6.8 ± 0.77 to $6.40 \pm 0.53\%$ and carbohydrate from 91.05 ± 0.94 to $88.42 \pm 0.14\%$, Calcium and iron content in rice were found to be 22.57 ± 4.12 mg and 1.41 ± 0.21 mg, respectively, and that for Hakuwa were found to be 31.57 ± 0.98 , and 3.42 ± 0.14 mg respectively. Calcium and iron contents in Hakuwa were significantly increased. The reducing sugar and total sugar were increased whereas total starch was significantly decreased in Hakuwa. Protease and amylase activity were significantly increased. Milling yield of Hakuwa was found to be higher in Hakuwa. Decrease in length while increase in breadth was observed in Hakuwa. Total anthocyanin, IC₅₀ and phenolic contents in Hakuwa were obtained to be 114.14 ± 8.55 mg CGE/100g, 1428.95μ g/mL and 47.9 mg of GA/100g of extracts, respectively.

Keywords: Hakuwa; Anthocyanin content; Antioxidant activity; Total polyphenol content

Introduction

Rice is the staple food of almost half the World population and increasingly becoming popular because of its nutritional and health beneficial properties. Colored rice refers to purple- or red grained rice and this coloration is the result of accumulated anthocyanins in the pericarp. Anthocyanins have been recognized as health-enhancing substances due to their antioxidant activity, anti-inflammatory, anticancer and hypoglycemic activities [1]. The health benefits of coloured/pigmented rice are attributed in part to their phytochemicals, mainly phenolic compounds which has received the increasing attention because of its potent antioxidant properties. Phenolic compounds in dietary cereals possess potent antioxidant activity and provide health benefits associated with reduced risk of chronic diseases [2].

Prior to the consumption, food grains are subjected to different processes, which alter the bioavailability of both micro- and macronutrients as well as other phytochemicals of nutraceutical value [3]. Hakuwa is one of such processed food grains, which is indigenous to Newar community of Nepal. Hakuwa has its own traditional value and; it is believed that it has medicinal importance. Hakuwa is generally used in the preparation of traditional beverage known as Hyautho. Hyautho is a traditional beverage similar to jand but it has got its characteristic red color from Hakuwa. Hyautho is used as a medicinal healer of various kinds of diseases (Personal communication). The general objective of this work is to compare physiochemical attributes of Paddy and Hakuwa.

Materials and Methods

The methodology adapted to conduct this study was divided into two parts. At first a small survey was conducted in Bhaktapur district to understand about processing technology involved in Hakuwa preparation and sample preparation and laboratory analysis were done.

Sample collection and preparation

Hakuwa samples were collected from Bhaktapur district of Nepal. While collecting the samples a survey was conducted with the help of questionnaire to understand about traditional processing technology. Samples were sun dried and packed in LDPE bags and stored at room temperature $32 \pm 2^{\circ}$ C until use. The foreign, earthy matter and residual materials were removed from the rice and Hakuwa. It was then mechanically reduced to coarse powder form using electric grinder (Black and Decker, India) and passed through 60mm sieve and the powder was kept in sealed glass jars under refrigerated condition (4 ± 2°C) till use.

Methodology

Moisture content (Hot air oven method), crude protein (Macro Kjeldahl method), crude fiber were determined as per AOAC [4]. Fat content (Soxhlet extraction method), ash content, calcium content, iron content, reducing sugar, total sugar, total starch content were determined as per Rangana [5] and total carbohydrate was calculated by difference method. Protease and amylase activity were determined as per Manandhar and Sharma [6]. The length: bradth ratio of samples was determined as per Chakravorty and Gosh [7], milling yield as per Schramm [8] and moisture uptake ratio as per Sareepuang [9]. Anthocyanin in Hakuwa was determined by spectrophotometrically method as per Tsai [10]. The ability of the plant extracts to scavenge DPPH free radicals was assessed by the standard method Williams [11] adopted with some suitable modification. The corresponding % of inhibition was then calculated by using the formula: % inhibition = (Absorbance of control- Absorbance of extracts / Absorbance of control) ×100

The TPC of crude Hakuwa extracts was determined by spectrophotometric method using Folin-Ciocalteu's phenol reagent [12].

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Statistical analysis

All the experiments were performed seven times and the results were expressed as average of seven analysis \pm SD (standard deviation). The magnitude of correlation between variables was performed using t-test (Microsoft Excel 2007). The linear regression line for antioxidant and phenolic content was also determined using Microsoft Excel 2007.

Results and Discussion

Findings of survey

According to the survey, it was found that its production is decreasing day by day. Only few people are involved in its production depending on their need. Its use is limited only for the production of Hyautho, a traditional beverage used in Newar community which is use as medicinal healer of various kinds of diseases. Generally short grained Japonica varieties are used for the preparation of Hakuwa, as its best variety used for jand preparation.

Change in moisture and temperature during Hakuwa preparation

During Hakuwa preparation, after threshing paddy was kept in stack of straw. As shown in Figure 1 moisture content decreased and temperature increased. The initial moisture content and initial temperature of paddy were found to be $22.051 \pm 0.18\%$ and $35.56 \pm 0.68^{\circ}$ C and the final moisture content and temperature $20.14 \pm 0.39\%$ and $66.27 \pm 0.40^{\circ}$ C, respectively.

The obtained result agree with the finding of Stockli et al. [13] that rough rice stored at 26% moisture content reached a maximum temperature of 63°C in 6 days and 50°C in 17days when stored at 22% moisture content. Rough rice having moisture content 26% decreased to 22% after 15 days. Park et al. [14] also reported decrease in moisture content of grain when stored at 30 and 40°C.

Compositional analysis

The moisture content, fat, fiber, ash, protein and carbohydrate content in rice and Hakuwa was found to be 11.06 ± 0.23 , 0.67 ± 0.29 , 1.10 ± 0.18 , 0.88 ± 0.34 , 6.8 ± 0.77 , $91.05 \pm 0.94\%$, and 8.57 ± 0.11 , 1.62 ± 0.21 , 2.47 ± 0.34 , 1.08 ± 0.14 , 6.40 ± 0.53 , $88.42 \pm 0.14\%$, respectively. As shown in Table 1, there was significant difference in moisture, fat, fiber ash and carbohydrate content of Hakuwa and rice, and crude protein was not found significantly different for each other. Hakuwa has higher value in fat, fiber and ash content.

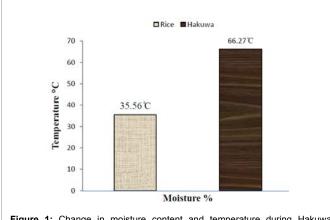


Figure 1: Change in moisture content and temperature during Hakuwa preparation.

Similar results were obtained for composition by various workers. Decrease in moisture content is due to heat generation during preparation of Hakuwa and higher sun drying. The result obtained from experiment is similar to Bhattacharya [15]; parboiled rice has higher nutritional value than its non-parboiled counter part due to migration of bran components (e.g., vitamins, minerals) into the endosperm during the hydrothermal treatment.

Similarly, acid-insoluble ash of rice and Hakuwa was found to be 0.16 \pm 0.05 and 0.32 \pm 0.06%, respectively. The calcium content in rice was 22.57 \pm 4.12 and 31.57 \pm 0.98 mg in Hakuwa. Likewise, iron content in rice and Hakuwa were found to be 1.41 \pm 0.21 mg and 3.42 \pm 0.14 mg respectively. From the analysis, rice and Hakuwa were found to be significantly different in terms of acid insoluble ash, calcium and iron.

The obtained result agreed with the findings of Juliano [16]; calcium and iron content in milled rice is 10-30 mg and 10-50 mg and in brown rice is 0.2-2.8 mg and 0.2-5.2 mg. Yodmanee [17] reported iron content tends to be higher in aromatic and colored (red and black) rice varieties than in colorless varieties.

Changes in reducing sugar, total sugar and total starch

Reducing sugar, total sugar and total starch in rice and Hakuwa was found to be 0.225 ± 0.005 , 0.36 ± 0.07 , 73.05 ± 7.43 and 0.234 ± 0.014 , 0.41 ± 0.012 , $70.78 \pm 6.17\%$, respectively. Statistical analysis in Table 2 showed that there is no significant difference in reducing sugar, total sugar and total starch of rice and Hakuwa. There is only a small increase in reducing sugar and total sugar in Hakuwa, while total starch was decreased. Increase in reducing sugar up to 12.44% in the 9th day of germination has been reported by Ayernor and Ocloo [18]. Veluppillai et al. [19] also described the increase in reducing sugar content is due to the hydrolysis of starch by the endogenous amylase produced during germination. Ali and Bhattacharya [20] have explained that increasing level of reducing sugar during parboiling is due to sucrose conversion during soaking and degradation of starch during steaming. The obtained result for total starch is similar to the result observed by Moongngram [21] during germination.

Changes in amylase and protease activity

The pattern of increment of amylase and protease during Hakuwa

Parameters (%)	Rice	Hakuwa	T _{stat}	T _{tab}
Moisture	11.06 ± 0.23ª	8.57 ± 0.11 ^₅	31.233	2.447
Crude Protein(db)	6.8 ± 0.77 ^a	6.40 ± 0.53ª	0.048	2.447
Crude Fat(db)	0.67 ± 0.29ª	1.62 ± 0.21 [♭]	6.507	2.447
Crude Fiber(db)	1.10 ± 0.18ª	2.47 ± 0.34 ^b	9.534	2.447
Ash(db)	0.88 ± 0.34^{a}	1.08 ± 0.14 ^b	1.333	2.447
Carbohydrate(db)	91.05 ± 0.94ª	88.42 ± 0.14 ^b	7.404	2.447
Acid Insoluble Ash	0.16 ± 0.05ª	0.32 ± 0.06 ^b	5.318	2.447
Calcium (mg)	22.57 ± 4.12ª	31.57 ± 0.98 [♭]	5.511	2.447
Iron (mg)	1.41 ± 0.21ª	3.42 ± 0.14 ^b	46.523	2.447

Note: the values are expressed as mean \pm S.D % of seven times for samples determination

 $t_{\rm cal}$ = Calculated value of t at 5% level of significance $t_{\rm tab}$ = Tabulated Value of $t_{\rm db}$ = dry basis

Table 1: Compositional comparison of rice and Hakuwa.

Parameter	Rice%	Hakuwa%	T _{stat}	T _{tab}
Reducing sugar	0.234 ± 0.014ª	0.234 ± 0.014^{a}	1.729	2.447
Total sugar	0.36 ± 0.07 ^a	0.41 ± 0.012^{a}	2.084	2.447
Total sugar	70.78 ± 6.17 ^a	70.78 ± 6.17^{a}	0.819	2.447

 Table 2: Change in reducing sugar, total sugar and total starch during Hakuwa preparation.

preparation is shown in Figure 2. Low activity of protease was found as compared to amylase activity during Hakuwa preparation. Moongngram [21] reported increase in amylase activity in germinated rice varieties. Veluppillai et al. [19] also reported increase in amylase and protease activities in germinated rice varieties.

Change in length:breadth (l/b) ratio

The length:breadth ratio in rice and Hakuwa was found to be 3.42 ± 0.39 and 2.77 ± 0.51 respectively. From the analysis, rice and Hakuwa were found to be significantly different in terms of l/b ratio. From the result obtained it is seen that length is decreased and breadth is increased in Hakuwa than that of rice. Similar result is obtained by Chaudhary [22] milled parboiled rice is slightly shorter and broader than milled raw rice.

Comparision of milling yield

The milling yield in rice and Hakuwa was found to be $94.36 \pm 0.45\%$ and $97.20 \pm 0.285\%$ respectively. From the analysis, rice and Hakuwa were found to be significantly different in terms of milling yield. Similar result is obtained by Chaudhary (1990) [22] head rice recovery is improved due to parboiling.

Change in water uptake ratio

Water uptake ratios in rice and Hakuwa were found to be 2.18 ± 0.06 and 1.75 ± 0.08 , respectively. The water uptake ratio in rice and Hakuwa is higher than that reported in Sareepuang [9]. Chaudhary (1990) [22] reported the water uptake in parboiled rice is slower than raw rice and parboiled rice can absorb more water resulting greater volume.

Anthocyanin content

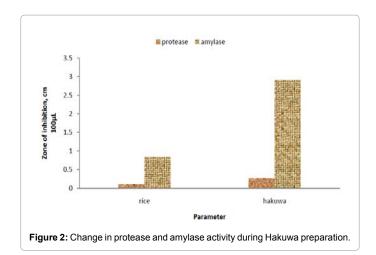
Anthocyanin content in Hakuwa was found to be 114.14 ± 8.55 mg CGE (cyanidin 3-O-glucoside equivalents)/100g. The obtained result is less than the finding from Park [23]; total anthocyanin content in the black rice was 1214.85 mg CGE/kg of black rice. According to the findings of Maisuthisakul and Changchub [24], mean anthocyanin contents among the white, red and black rice were found to be 123.7, 75.6 and 199.2 mg/L of cyanidin-3-glucoside, respectively. From the experiment it was found that the anthocyanin content in Hakuwa was found lesser than that in black rice and white rice, but higher than red rice.

DPPH scavenging activity

The IC₅₀ value for Hakuwa was found to be 1428.95µg/mL. The IC₅₀ value for standard Gallic Acid (GA) was found to be 604.295µg/mL. By comparing the IC₅₀ value of Hakuwa with that of an authenticated antioxidant, GA, we found antioxidant activity of Hakuwa was less than Gallic acid. The result obtained from the experiment is in accordance with the result obtained from Seo et al. [25] which reported IC₅₀ value of 12 varieties of rice ranges from >1000 to 246.94 mg/ml (Figure 3). The result obtained from the experiment is lower than that obtained by Rao et al. [26] where IC₅₀ for rice bran ranges from 48.88-87.72 µg/ml.

Total Phenolic content

The total phenolic contents in the examined Hakuwa extracts using the Folin-Ciocalteu's reagent is expressed in terms of Gallic acid equivalent. The total phenolic content in Hakuwa was found to be 47.9 mg of GA/100g of extracts. Similar results had been reported by Goffman and Bergman [27] and Shen et al. [28]. According to Yodmanee et al. [17], total phenolic content in colored rice ranges from 58- 329 mg of GA/100g.



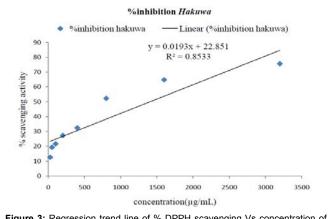


Figure 3: Regression trend line of % DPPH scavenging Vs concentration of Hakuwa IC_{50} value of Hakuwa= 1428.95µg/ml.

Conclusions

From the survey conducted, it was found that Hakuwa is rarely prepared these days and is on the verge of extinction. From the proximate analysis, Hakuwa was found to be nutritionally rich in comparison to the normal rice. The total sugar and reducing sugar were found to be higher; and starch content was found to be lower in Hakuwa than in normal rice. Hakuwa showed both protease and amylase activity; however, protease activity was quite lower. Milling yield of Hakuwa was comparatively higher than that of normal one, with less brokens. Anthocyanin content and total phenolic content in Hakuwa was found to be 114.14 \pm 8.55 mg CGE/100g and 47.9 mg of GA/100g of extract respectively, which shows that Hakuwa has antioxidant property. This research leads to an indication that Hakuwa is nutritionally rich and possess several functional properties.

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