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International Journal of Agricultural Sciences

ISSN: 2598 – 1145 (online)

Research article

# Effect of Rice Milling on Their Polyphenol, Protein Content and Antioxidant Activity

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ARTICLE INFORMATION

*Article history*:

Received 10 February 2019
Revised: 70 March 2019
Available online: 02 April 2019

Keywords:
Rice milling, **P**olyphenol, protein, antioxidant activity

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**A B S T R A C T**

Phenolic compound is the main source of antioxidant that reduces the incidence of chronic diseases such as heart disease, blood cholesterol, blood pressure, diabetic and so no. The aim of this experiment was to investigate the effect of three milling process (dehusking, auto and polishing) of rice on polyphenol content with their antioxidant activity and nutritional profile. The polyphenol content determined according to the Folin Ciocalteu method. Polishing rice contain maximum phenolic compound (36.28 ± 0.73 mg/g of extract) that was statistically different from other milling rice ($p<0.05$). Polishing rice contained highest amount of protein (262.69 ± 0.38 mg/g extract) whereas auto rice contained highest amount of free amino acid content (14.06 ± 0.46 mg/g extract) and total sugar content (1312.29 ± 2.28 mg/g extract). Reducing sugar content was statistically similar to all milling rice. Antioxidant activity was comparatively assessed by ABTS (2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) free radical decolorization assay method. It was investigated that polishing rice showed strong free radical scavenging capacity by ABTS radical. It is concluded that polishing rice can be used as functional ingredient with high polyphenol and protein content with strong antioxidant activity.

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## INTRODUCTION

Rice is one of the world's most important food crops and major sources of staple food throughout the world. More than half of the world's population depends on rice as a primary source of food, and approximately 20% of the world's dietary energy supply is exclusively from rice-based nutrition. Rice is a primary staple food in most Asian countries, where nearly 90% of the world's rice is consumed. At present, China is the world's leading rice producer, with nearly 30% of global rice volume (FAO, 2021). The top 10 rice-producing countries, in the order of highest to lowest annual production, are China, India, Indonesia, Bangladesh, Vietnam, Thailand, Burma, Philippines, Japan and Brazil (FAO, 2021).

Cereals are the most important sources of food (FAO, 2002) that possesses great nutritional and bioactive properties such as phenolic acids-antioxidants, carbohydrate, protein, free amino acid, fibers, vitamin and minerals. It is the major source of energy for the world population. Cereal grain comprises unique health-promoting bioactive components as potential sources for functional food and ingredients. Based on the World Health Organization report for 2012–2016 (WHO, 2018), consumption of cereal grains may decrease the risk of

DOI : 10.25077/ijasc.5.2.60-65.2021

non-communicable diseases (e.g., type 2 diabetes, cardiovascular disease, hypertension and obesity). Instead, they comprise most of the micronutrients, fiber, and phytochemicals of the grain that could significantly impact on the nutritional quality of human food if integrated in flours or used as food ingredients (Brouns *et al*, 2012).

A large portion of the paddy harvested in the Asian region is retained and milled in the farm. Milling is usually done when paddy is dry (about l4 percent moisture content) (Lantin, 1999). The milling of rice involves at least two basic operations. i.e., removing the outer covering called the husk (Dehusking), or hull and removing the seed coat called the bran (polishing) to produce the edible portion (endosperm) for consumption. The former called dehusking or dehulling while the latter, is polishing or whitening process (Lantin, 1999).. This process has to be accomplished with care to prevent excessive breakage of the kernel and improve the recovery of the paddy. Actual milling process, however, removes also the germ and a portion of the endosperm as broken or powdery materials reducing the quantity of grains recovered in the process.

The large capacity multiple machine rice mill uses a different machine for each processing step: cleaning, dehusking, separating, bran removal and grading. These processes are integrated into one system by bucket elevators linking machine to machine to accomplish each stage of processing to the end of the output polished rice (Lantin, 1999).. This former named as auto rice mill. The modern multiple machine rice mill is more efficient than the traditional steel huller and consumes about one-half to two-thirds the power of the steel huller operating at the same capacity. The rice recovery rate is considerably higher in terms of total rice and head yields. The extent of losses on the edible portion of the grain during milling depends on so many factors as variety of paddy, condition of paddy during milling, degree of milling required, the kind of rice mill used, the operators and others. In this study, it was investigated that the change of nutritional value of rice by three different rice milling such as dehusking, polishing and auto rice milling (Lantin, 1999).

The main objective of this study was to investigate the change of polyphenol and nutritional component as well as anti-oxidant capacity of rice at different rice milling technique.

## METHOD

### *Raw materials*

Rice sample was collected from 3 rice mill (Dehusking, polishing and auto rice mill) in the region of Ishurdi, Pabna district, Bangladesh.

### *Chemicals and other materials*

Tannic acid, Folin &Ciocalteu's phenol, 2,2'-azinobis-(3-ethylbenzothiazoline-6- sulfonic acid) (ABTS), DPPH (1,1-diphenyl-2-picrylhydrazyl) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Quercetin, sulfuric acid, Sodium carbonate ($Na_2CO_3$), Sodium hydroxide, Rochelle salt, Sodium sulfate, Phenol, 3,5 Dinitro salicylic acid, Bovine serum albumin fraction V, $CuSO_4 \cdot 5H_2O$, Potassium sodium tartrate tetrahydrate, sucrose, L-tyrosine, glucose (dextrose) were aslo from Sigma Aldrich (St. Louis, MO, USA).

### *Preparation of extraction*

Milling rice grain were ground with a laboratory grinder to make flour and sieved with a 100-mesh sieve. One gram of sample was extracted by 10 mL water for boiling at 95ºC temperature for 3 to 10 hours and then centrifuged 10 min on 4000 rpm. Extract sample filtered by syringe filter (0.45 µm) and preserved in refrigerator at -20ºC for chemical analysis.

### *Total polyphenol content*

Total phenolic content (TPC) of rice sample extract was determined by the reported method (Singleton *et al.*, 1999) with slight modification. Mixed 100 µL of each sample and 100 µL of Folin-Denis reagent firstly, after 3 minutes, added 1 mL of 0.7 M Sodium carbonate in the mixture and then reacted at room temperature for 1 h. Absorbance of sample was measured at 750 nm. Total phenolic compounds in the cereal grain extracts were determined using an equation obtained from a standard curve of tannic acid (0–500 µg/mL, $Y = 0.0028x - 0.0351$, $R^2 = 0.9968$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis. The results are expressed as mg Tannic acid equivalents (TA eq) per g of extract of cereal grain sample. All determinations were carried out in triplicate.

### *Determination of protein contents*

The protein content was measured by Lowry Assay (Lowry *et al.*, 1951) with minor modification. For protein content determination, Bovine serum albumin (BSA) was used to make the standard calibration curve. Stock Bovine serum albumin (BSA) solution was prepared by dissolving 10 mg BSA powder in 1.0 mL distilled water, then the standard solutions of BSA were prepared by serial dilutions using distilled water (0–500 $\mu$g/mL). An amount of 100 µL diluted standard BSA solutions and all sample extracts was separately mixed with 1 mL Lowry reagent. Lowry reagent was prepared by mixing 0.5 mL of 1% cupric sulfate with 0.5 mL of 2% sodium potassium tartrate, followed by the addition of 50 mL of 2% sodium carbonate in 0.1 N NaOH. Each sample reacted with Lowry reagent for 10 minutes then react with Folin's reagent. Color was allowed to develop for 30

minutes at room temperature and the absorbance measured at 750 nm and blanked on the water only control. Protein content in the cereal grain sample extracts were determined using an equation obtained from a standard curve of BSA (0–500 µg/mL, $Y = 0.0004x + 0.0135$, $R^2 = 0.999$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis. The results are expressed as mg BSA equivalents per g of extract or per 100 g of sample. All determinations were carried out in triplicate.

***Detection of free amino acid contents***

Free amino acid content of cereal grain was detected by the method described by Setsuro *et al.*, 1966 with minor modification. For free amino acid content determination, L-tyrosine was used to make the standard calibration curve. Stock L-tyrosine solutions was prepared by dissolving 10 mg L-tyrosine powder in 400 µL, 1N HCl then add 600 µL distilled water. Standard solutions of L-tyrosine were prepared by serial dilutions using distilled water (0–500 $\mu$g/mL). An amount of 100 µL diluted standard L-tyrosine solutions and all sample extracts was separately mixed with 1 mL of 0.55 M $Na_2CO_3$ for 5 minutes then react with 100 µL of 2N Folin & Ciocalteu's phenol reagent. Color was allowed to develop for 30 minutes at room temperature and the absorbance measured at 750 nm and blanked on the water only control. Free amino acid content in the cereal grain sample extracts were determined using an equation obtained from a standard curve of L-tyrosine (0–500 µg/mL, $Y = 0.0022x + 0.0068$, $R^2 = 0.9956$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis. The results are expressed as mg L-tyrosine equivalents per g of extract or per 100 g of sample. All determinations were carried out in triplicate.

***Determination of total sugar***

Total sugar content was determined by the phenol–sulfuric acid method described by DuBois *et al.*, 1956 with minor modification. For total sugar content determination, Sucrose was used to make the standard calibration curve. Stock sucrose solution was prepared by dissolving 10 mg sucrose powder in 1mL distilled water. Standard solutions of sucrose were prepared by serial dilutions using distilled water (0–500 $\mu$g/mL). An amount of 100µL diluted standard sucrose solutions and all sample extracts was separately mixed with 100 µL of 5% phenol solution. Then 500 µL of sulfuric acid (highly concentrated) was added in this mixture. This mixture needed 10 minutes for reaction in boiling water and then cooled in ice bath for another 15 minutes. Finally, the absorbance of each sample was measured at 490 nm and blanked on the water only control. Total sugar content in the cereal grain sample extracts were determined using an equation obtained from a standard curve of Sucrose (0–500 µg/mL, $Y = 0.0027x + 0.0585$, $R^2 = 0.9998$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis. The results are expressed as mg Sucrose equivalents per g of extract or per 100 g of sample. All determinations were carried out in triplicate.

***Determination of reducing sugar***

Reducing sugars were measured by the method of Somogyi and Nelson (Somogyi, 1952; Nelson, 1944) with minor modification. For reducing sugar content determination, Glucose (Dextrose) was used to make the standard calibration curve. Stock glucose solution was prepared by dissolving 10 mg glucose in 1mL distilled water. Standard solutions of glucose were prepared by serial dilutions using distilled water (0–500 $\mu$g/mL). An amount of 100 µL diluted standard glucose solutions and all sample extracts was separately mixed with 300 µL of DNS reagent. DNS reagent was prepared by mixing 1% Sodium hydroxide, 20% Rochelle salt, 0.05% Sodium sulfate, 1% 3,5 Dinitro salicylic acid with 0.2 mL Phenol. DNS reagent mixing all sample put in water bath at 95°C for 5 minutes for reaction and then cooled in ice bath for another 5 minutes. Finally, the absorbance of each sample was measured at 540 nm and blanked on the water only control. Reducing sugar content in the cereal grain sample extracts were determined using an equation obtained from a standard curve of glucose (0–500 µg/mL, $Y = 0.0021x + 0.0605$, $R^2 = 0.9920$) where concentrations on the X-axis and their corresponding absorbance values on the Y-axis. The results are expressed as mg Glucose equivalents per g of extract or per 100 g of sample. All determinations were carried out in triplicate.

***Determination of antioxidant activity***

Antioxidant activity was determined by the ABTS (2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) free radical decolorization assay method developed by Re *et al.*, 1999. The ABTS. Positive (+) radical cation was progenerated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and incubating for 12–16 h in the dark at room temperature until the reaction was complete and the absorbance was stable. The absorbance of the ABTS. + solution was equilibrated to 0.70 (± 0.02) by diluting with water at room temperature, then 100µl was mixed with 50 µl of the test sample and the absorbance was measured at 734 nm after 6 min. All experiments were repeated three times. The radical scavenging activities of cereal grain sample were calculated by the following equation:

 DOI : 10.25077/ijasc.5.2.60-65.2021

$$ABTS\ radical\ scavenging\ activity\ (\%) = \left(1 - \frac{Sample\ absorbance}{Control\ absorbance}\right) \times 100$$

Then, curves were constructed by plotting percentage of inhibition against concentration in µg/mL. The equation of this curve allowed to calculate the $IC_{50}$ corresponding to the sample concentration that reduced the initial ABTS absorbance of 50 %. A smaller $IC_{50}$ value corresponds to a higher antioxidant activity. All test analyses were realized in triplicate.

***Statistical Analysis***

All assay data for continuous variables were conducted in triplicates. The values were expressed as the mean ± standard deviation (SD) calculated using Microsoft Excel 2010 and Sigma plot 12.5. All assay data were subjected to one-way analysis of variance (ANOVA) using PROC GLM in SAS program (SAS Institute, 1989). Mean values were compared with Duncan's Multiple Range Test at 0.05 level of Type I error.

## RESULTS AND DISCUSSION

***Extraction yield and polyphenol content* of different milling rice**

The health benefits of polyphenols on the human body are mainly due to their oxidation resistance. Polyphenols in rice have a stronger antioxidant effect in the body through the synergistic effect of multiple bioactive compounds than the single active ingredient and can eliminate too many oxidation free radicals in the body as anti-oxidants or after the intestinal digestion. They are known to have antioxidant activity and it is likely that the activity of these extracts is due to this phenolic compound (Tepe *et al.,* 2006). The result of phenolic content of different milling rice sample were presented in Table 1. The results showed that maximum polyphenol content (36.28 ± 0.73 mg/g extract) in polishing rice though it was lower extraction yield 0.90%. Minimum polyphenol content was observed in auto milling rice followed by dehusking rice.

Table 1. Extraction yield and polyphenol content of different milling rice

| Name of sample | Extraction yield (%) | Polyphenol content (mg/ g extract) |
|---|---|---|
| **Dehusking** | 1.42 | 29.97 ± 0.05$^{b}$ |
| **Auto** | 1.69 | 27.97 ± 0.12$^{c}$ |
| **Polishing** | 0.90 | 36.28 ± 0.73$^{a}$ |

Means having different letters are significantly different (DMRT, $p < 0.05$).

**Nutritional profile of different milling rice**

Nutritional profile was elucidated in Table 2. Maximum protein content was observed in polishing rice (262.69 ± 0.38 mg/g extract) that was statistically different from other milling rice. Low protein content was observed in dehusking milling rice that was 81.53 ± 0.85 mg/g extract. Maximum free amino acid (14.06 ± 0.46$^{a}$ mg/g extract) and total sugar (1312.29 ± 2.28 mg/g extract) content were determined in auto milling rice and both were statistically different from other milling rice. Reducing sugar content of three milling rice was statistically similar.

Table 2. Nutritional profile of different processing rice

| Name of sample | Nutritional profile (mg/ g extract) | | | |
|---|---|---|---|---|
| | Protein | Free amino acid | Reducing sugar content | Total Sugar |
| **Dehusking** | 81.53 ± 0.85$^{c}$ | 4.92 ± 1.92$^{b}$ | 62.79 ± 2.06$^{a}$ | 897.38 ± 2.44$^{c}$ |
| **Auto** | 158.87 ± 0.90$^{b}$ | 14.06 ± 0.46$^{a}$ | 61.02 ± 1.62$^{a}$ | 1312.29 ± 2.28$^{a}$ |
| **Polishing** | 262.69 ± 0.38$^{a}$ | 5.92 ± 0.08b$^{b}$ | 61.59 ± 1.28$^{a}$ | 1170.89 ± 2.24$^{b}$ |

Means having different letters are significantly different (DMRT, $p < 0.05$).

**Anti-oxidant activity of different milling rice**

The concentration of total polyphenol content in cereal grain has been positively associated with the antioxidant activity (Itani *et al.,* 2002, Goffman *et al.,* 2004, Zhang *et al.,* 2006), with potential beneficial effects on health, such as reduction of oxidative stress (Ling *et al.,* 2001 and Hu C. *et al.,* 2003), aid in the prevention of cancer (Hudson *et al.,* 2000; Hyun *et al.,* 2004), in the control of blood lipids and related diseases, which may help in the prevention of cardiovascular problems (Ling *et al.,* 2001), and in the prevention of the complications of diabetes (Morimitsu *et al.,* 2002, Yawadio *et al.,* 2007, Hu FB 2003, Orlich *et al.* 2014).

The antioxidant proprieties of extracts were measured in terms of their efficient $IC_{50}$ concentration corresponding to the sample concentration that reduced the initial ABTS radical absorbance of 50%. These $IC_{50}$ values for ABTS is given in table 3. From this Table 3 it was resulted that polishing rice had stronger radical scavenging activity compare to other milling rice. Low concentration of polishing rice had the ability to scavenge maximum ABTS radical.

Table 3: Anti-oxidant activity of different processing rice

| Name of sample | Radical scavenging activity ($RC_{50}$) (µg/mL) |
|---|---|
| | ABTS |
| **Dehusking** | 2188.40[a] |
| **Auto** | 1220.601[b] |
| **Polishing** | 740.62[c] |

Means having different letters are significantly different (DMRT, $p < 0.05$).

## CONCLUSIONS

Among three milling rice (Dehusking, Auto and polishing), polishing rice contains maximum polyphenol content with maximum protein content followed by auto rice in this experiment. These are responsible for strong antioxidant activity in polishing rice. On the basis of the results obtained in the present experiment, it is concluded that polishing rice has maximum capacity of antioxidant activity among three milling rice. Obtained results suggest that polishing rice can be used as functional ingredient with high polyphenol, protein content and antioxidant activity which is needed in order to be able to produce rice food products with maximum health benefits.

## ACKNOWLEDGMENT

We appreciate Hyunhhwa Lee for her over all cooperation to take this experiment. We thank Hwijae Jeong for ordering chemicals and test materials. We are grateful to Andong National University authority.

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