



Effects of Polishing on Proximate Composition, Physico-Chemical Characteristics, Mineral Composition and Antioxidant Properties of Pigmented Rice

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Abstract: The effects of polishing on proximate compositions, physico-chemical characteristics, mineral compositions and antioxidant properties of the rice flours obtained from three different pigmented rice varieties (Chak-hao Angangba, Chak-hao Amubi and Chak-hao Poireiton) were investigated. The rice varieties were significantly ($P < 0.05$) different in the contents of the test characteristics. Lipids, ash, minerals, phytochemicals (phenolic acids and flavonoids) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity of rice flours were decreased after polishing (9% degree of milling), while amylose content and lightness were increased. X-ray diffraction pattern of rice flours exhibited A-type crystalline pattern with reflections at 15.1°, 17.1°, 18.2° and 23.0°. Pasting properties and transition temperatures were decreased after polishing treatment. Polishing resulted in changes in the crystallinity, enthalpy and morphology of rice flours.

Key words: amylose; antioxidant property; crystallinity; gelatinization; mineral; pigmented rice; polishing; pasting property; bran

Rice is the primary cereal of regular diet and one of the highest cultivated food crops in the world. It is the main staple food for half of the world's population, predominantly in developing countries (Monks et al, 2013). As a result of the high consumption and main source of carbohydrates, minerals, vitamins and bioactive components, rice represents an appropriate vehicle for nutrient delivery to these populations (Sompong et al, 2011; Nile et al, 2016). In a nutritional point of view, rice flour is a chief ingredient in numerous traditional foods, which is possibly due to the absence of gluten, low amount of calcium and allergenic proteins (Gujral and Rosell, 2004). There are numerous factors which are known to affect the nutritional value of rice such as rice genotype, agronomic and cultivation condition, storage and processing (Singh et al, 2000; Falade and Christopher, 2015).

Rice is consumed as the whole kernel of the white rice which is obtained after milling of the rough rice. The principle of rice milling is the removal of the husk (husking) followed by the rice bran (polishing), which gives us the edible portion (endosperm) of rice grain (Savitha and Singh, 2011). Usually, rice polishing is carried out by the industries to improve the physical and sensory properties of the rice grain and the storage stability (Monks et al, 2013; Paiva et al, 2016). Further, removal of the germ and bran layers of the rice caryopsis is done by polishing process. Rice bran layer is rich in minerals, vitamins, fat and dietary fibers (Roy et al, 2008; Paiva et al, 2014), while proteins and fats are concentrated in the germ of the rice caryopsis (Itani et al, 2002). Typically, a high degree of milling enriches the sensorial and physical quality of rice, while most of the nutritional components

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of rice are removed during the polishing process (Itani et al, 2002; Paiva et al, 2016).

Recently, pigmented rice varieties have received great attention owing to their high content of polyphenols, minerals, vitamins and numerous biological activities. In general, rice bran contains numerous nutrients, including fiber, minerals and vitamins (Patil and Khan, 2011), as well as health-promoting bioactive phytochemicals such as phenolics, flavonoids, γ -oryzanol, tocopherols, ferulic acid, phytic acid and tocotrienols (Friedman, 2013). Phenolic compounds are secondary metabolites in plants, and can scavenge free radicals and further decrease the oxidative stress and protect the biological macromolecules from potential damage (Okarter et al, 2010; Ti et al, 2014). The anti-oxidant properties of phenolic compounds are responsible for prevention of chronic diseases such as obesity, diabetes, atherosclerosis, cancer and cardiovascular diseases (Kong and Lee, 2010; Okarter et al, 2010).

Due to increase in the awareness on nutrition and health, research has taken a turn to improve the functional food products with bioactive components. Pigmented rice with augmented bioactive components such as phenolic, tocopherol and flavonoid compounds shows a key role in the improvement of functional foods. Numerous studies are being conducted to examine the effect of polishing on nutritional, physico-chemical and functional properties of pigmented and non-pigmented rice varieties (Singh et al, 2005; Sompong et al, 2011; Min et al, 2014; Zhang et al, 2015; Paiva et al, 2016). However, few studies have been carried out on the nutrient compositions, quality characteristics, and antioxidant properties of the pigmented rice cultivated in North-east India (Saikia et al, 2012; Reddy et al, 2016). Moreover, the impacts of polishing on the crystallinity, chemical composition, mineral content, phytochemicals, physico-chemical and antioxidant characteristics of the traditional pigmented rice varieties cultivated in North-east India are still unclear till now. Therefore, the objective of this study was to investigate the effects of polishing on proximate composition, morphology, mineral content, phytochemical content (phenolic and flavonoids), antioxidants and physico-chemical characteristics of three pigmented rice varieties cultivated in North-east India.

MATERIALS AND METHODS

Materials

Three different pigmented rice varieties, Chak-hao

Angangba (CAng, brown rice), Chak-hao Poireiton (CP, purple rice) and Chak-hao Amubi (CA, black rice), were obtained from the Rice Research Centre, Central Agricultural University, Manipur Province, India. All the rice samples were from the recent harvest of December, 2015. All the paddy materials were dried, cleaned, further packed in screw-capped plastic containers and stored at 4 °C. Folin-Ciocalteu reagent, catechol, quercetin, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from HiMedia Co., Ltd. (Mumbai, India). All chemicals were of analytical grade unless specially mentioned.

Processing of rice

Rice grains were dehusked with a Rice Husker (THU-34A, Satake Engineering Co., Japan) to produce raw pigmented rice (RPR), which was further polished using the Rice Mill (TM05, Satake Manufacturing Co., Japan) to remove the bran with 9% degree of milling (DOM), resulting in the formation of the white rice or polished rice (PPR). Then, both RPR and PPR grains were milled to flour using a laboratory mill (A11B, IKA Inc, India). All the flour samples were sieved through 100 μ m sieve-size and stored in airtight plastic containers.

Determination of proximate composition

The proximate composition of rice flours was determined using the standard AOAC methods (AOAC, 1990). Moisture content was done by the gravimetric method in a hot air oven at 105 °C until constant weight, and the quantity of ash was analyzed using a muffle furnace at 550 °C. Crude protein content was measured by a standard Kjeldahl method using 5.95 as the conversion factor. Flour lipids were analysed by the solvent extraction procedure. The content of total carbohydrates was determined by difference from the analysis of moisture, ash, protein and lipids. Amylose content was assessed using the standard method described by Sowbhagya and Bhattacharya (1979).

Measurement of colour characteristics

Colour measurement of rice flours was performed using a Hunter Lab Colorimeter (D-25, Hunter Lab Associates Inc., Ruston, USA). The instrument was calibrated using the Hunter Lab colour standards, and lightness to darkness (L^*), redness to greenness (a^*) and blueness to yellowness (b^*) values of the rice flours were measured.

X-ray diffraction (XRD) investigation

X-ray diffraction (XRD-7000, Shimadzu, Japan) was used to investigate the degree of crystallinity using the procedure described by Reddy et al (2016). The sample was placed into a sample holder, and XRD patterns were observed in a reflection mode (2θ) ranged from 5° to 50° at room temperature using a voltage of 40 kV and filament current of 30 mA and scan speed of 1.5 $^\circ$ /min.

Analysis of pasting properties

The pasting parameters of rice flours were analysed using a rapid visco-analyzer (RVA) (RVA Starch Master 2, Newport Scientific Ltd, Australia) with the method by Reddy et al (2015). Rice flour (3.5 g, dry weight) was weighed into an RVA canister and then 25 mL distilled water was added. The dispersion was heated from 50°C to 95°C at a heating rate of $6^\circ\text{C}/\text{min}$ (after incubation at 50°C for 1 min), and maintained at 95°C for 5 min before cooling to 50°C (cooling rate $6^\circ\text{C}/\text{min}$) and held at 50°C for 2 min.

Determination of thermal properties

Gelatinization temperatures and enthalpy of the rice flours were carried out using a differential scanning calorimeter (DSC) (DSC-204, Netzsch, Germany). Accurately weighed samples (3 mg, dry weight) with added distilled water to make 70% rice flour slurry were packed in an aluminium DSC pan and allowed to stand for 2 h at 4°C before heating with an empty DSC pan used as reference. The scanning temperature and the heating rate were 35°C to 150°C and $10^\circ\text{C}/\text{min}$, respectively.

Mineral composition analysis

Mineral composition analysis of rice flours was performed using X-ray fluorescence spectrometer (WD-XRF, Bruker, Germany). Rice flour (2 g) was mixed with 0.5 g boric acid, and made into pellets by using a hydraulic press. Finally, the pellets were placed into the sample slots and the mineral composition of the rice flour was evaluated.

Determination of antioxidant activity

Extract preparation

The rice flours (50 g) were treated by stirring with 250 mL methanol for 3 h in an orbital shaker (Technico, India). Then, the supernatant was separated after centrifugation at 3 000 r/min for 10 min, and the

extraction process was performed twice. The obtained supernatants were combined and concentrated under vacuum. The extract was recovered by adding 10 mL methanol, and then stored at 4°C until use.

Total phenolic content (TPC)

TPC of each extract was estimated using the method described previously (Reddy et al, 2016). Briefly, the extract (200 μL) was mixed with 1 000 μL of 10% Na_2CO_3 solution and 1 000 μL Folin-Ciocalteu reagent. The resulting solution was stirred and diluted to a final volume of 5 mL with distilled water. The dispersion was left in dark for 2 h, and the absorbance at 765 nm was recorded using a spectrophotometer (UV-1800, Shimadzu, Japan). Catechol was used as the standard.

Total flavonoid content (TFC)

TFC of each extract was determined using the method described by Dewanto et al (2002). Briefly, 250 μL the extract and standard solution of quercetin were diluted with 1.25 mL distilled water. Then, 75 μL of 5% NaNO_2 solution was added and the mixture was kept at ambient temperature for 6 min. After incubation, 150 μL of 10% AlCl_3 solution was added and the mixture was incubated for 5 min followed by addition of 0.5 mL of 1 mol/L NaOH solution. Further, the mixture was diluted to 3 mL with distilled water and the absorbance was measured immediately at 510 nm using a spectrophotometer (UV-1800, Shimadzu, Japan).

DPPH radical scavenging activity

DPPH radical scavenging activity of each extract was examined using the procedure described previously (Cheng et al, 2006). Briefly, 100 μL the extracts was taken into 1.4 mL freshly prepared DPPH (0.2 mmol/L) methanolic solution. The mixture was shaken vigorously and left in dark for 30 min. The absorbance of the resulting solution was measured at 517 nm, compared with that of the control (100 μL methanol in 1.4 mL DPPH radical solution).

Observation of morphological characteristics

The powdered sample was mounted on metal stub with an adhesive tape and coated with carbon material to produce conductive samples. The samples were analysed and photographs were taken using a scanning electron microscope (S-3400N, Hitachi, Japan) with a potential acceleration of 15 kV.

Statistical analysis

The data were represented as means with standard

deviations of triplicate observations. An analysis of variance was performed with 5% significant level, and Duncan's multiple range method was performed to measure significant differences among mean values using SPSS20 software (SPSS Inc., Chicago, USA).

RESULTS

Proximate compositions of pigmented rice

Table 1 contains the proximate compositions such as moisture, protein, fat, ash and carbohydrate contents of the rice flours. As is evident from the results, the polishing treatment with 9% DOM resulted in decreases in the moisture, ash, protein and fat contents of pigmented rice, whereas increase in the carbohydrate content of pigmented rice. The moisture content significantly ($P < 0.05$) reduced in PPR when compared to their respective RPR and the values ranged from 11.12% to 12.05% (RPR) and 8.17% to 8.72% (PPR), respectively. Similar declining trend was also noted in the fat content (Table 1).

The ash content in RPR was found to be 3 to 4 times higher than that in PPR (Table 1), and the percentage of ash content in RPR ranged from 0.83% to 1.79%. From the results, we observed that the ash content of PPR was affected significantly by polishing and the values ranged from 0.31% to 0.57%.

The protein content of RPR ranged from 5.57% to 8.75% (Table 1). The polishing process resulted in a reduction of 3.09%, 4.12%, and 5.03% in the protein content of CA, CP and CAng, respectively. The findings suggested that the loss of protein content was lower in pigmented rice (3.09% to 5.03%) due to polishing, when compared to the loss of fat (87.69% to 93.11%) and ash content (60.24% to 77.54%). The amount of the total carbohydrates of selected

pigmented rice varieties as affected by polishing treatment was analysed by the variance in the respective moisture, ash, fat and protein contents of the samples.

Amylose content

Significant differences ($P < 0.05$) were observed among the tested pigmented rice, of which CP and CAng exhibited lower amylose content whereas CA exhibited the highest amylose content (Table 1). Amylose content of RPR ranged from 1.93% to 3.16%, and the amylose content of PPR ranged from 3.98% to 6.11%. On the basis of amylose content, CAng (1.93%) and CP (1.98%) were categorised as waxy rice varieties and CA (3.16%) as non-waxy rice variety.

Colour of rice flours

There were significant differences observed in the colour characteristics of rice flours among selected pigmented rice varieties (Table 1). Among RPR, the flour obtained from CAng showed the highest lightness (74.41), in comparison with the flour from CP (64.52) and CA (61.53). a^* and b^* of rice flours showed significant difference ($P < 0.05$) among RPR, whereas CAng showed the highest values (7.68 and 11.29, respectively).

X-ray diffraction

X-ray diffraction patterns of the pigmented rice varieties are presented in Fig. 1. RPR and PPR flours both represented an A-type X-ray diffraction pattern, with reflections (2θ) appearing at 15.1° ; a doublet at 17.1° and 18.2° ; and one very broad peak at 23.0° . RPR flours showed slight but significant ($P < 0.05$) differences in the crystallinity in the following order: CAng (10.75%) > CP (10.32%) > CA (9.56%) (Table 1).

Table 1. Proximate compositions, amylose content, colour parameters, and crystallinity of raw and polished pigmented rice.

Parameter	Chak-hao Amubi		Chak-hao Poireiton		Chak-hao Angangba	
	Raw	Polished	Raw	Polished	Raw	Polished
Moisture content (%)	11.40 \pm 0.30 b	8.72 \pm 0.60 c	12.05 \pm 0.20 a	8.54 \pm 0.22 cd	11.12 \pm 0.70 b	8.17 \pm 0.10 d
Ash content (%)	0.83 \pm 0.10 c	0.33 \pm 0.03 e	1.79 \pm 0.20 a	0.57 \pm 0.09 d	1.38 \pm 0.20 b	0.31 \pm 0.03 e
Protein content (%)	8.75 \pm 1.20 a	8.48 \pm 0.17 a	7.77 \pm 0.05 b	7.45 \pm 0.04 c	5.57 \pm 0.10 d	5.29 \pm 0.07 e
Fat content (%)	3.33 \pm 0.20 b	0.41 \pm 0.07 d	3.73 \pm 0.20 a	0.34 \pm 0.05 d	3.05 \pm 0.10 c	0.21 \pm 0.03 e
Carbohydrate content (%)	74.67 \pm 1.40 d	82.13 \pm 0.61 b	74.38 \pm 1.50 d	83.27 \pm 0.30 b	78.24 \pm 2.60 c	85.57 \pm 0.47 a
Amylose content (%)	3.16 \pm 0.30 c	6.11 \pm 0.39 a	1.98 \pm 0.10 d	4.08 \pm 0.09 b	1.93 \pm 0.10 d	3.98 \pm 0.15 b
L^*	61.53 \pm 1.10 e	84.34 \pm 0.72 b	64.52 \pm 0.90 d	75.36 \pm 0.58 c	74.41 \pm 1.20 c	88.64 \pm 0.43 a
a^*	2.21 \pm 0.19 d	1.58 \pm 0.07 e	3.45 \pm 0.09 b	2.68 \pm 0.18 c	7.68 \pm 0.07 a	1.46 \pm 0.26 e
b^*	2.86 \pm 0.12 c	1.72 \pm 0.07 d	3.49 \pm 0.16 b	0.24 \pm 0.06 e	11.29 \pm 0.11 a	3.25 \pm 0.15 bc
Crystallinity (%)	9.56 \pm 0.12 c	9.18 \pm 0.15 d	10.32 \pm 0.22 b	10.08 \pm 0.15 b	10.75 \pm 0.21 a	10.34 \pm 0.18 b

L^* , Lightness to darkness; a^* , Redness to greenness; b^* , Blueness to yellowness.

Values (Mean \pm SD, $n = 3$) with the same lowercase letters in a row did not differ significantly ($P < 0.05$) by the Duncan's multiple range method.

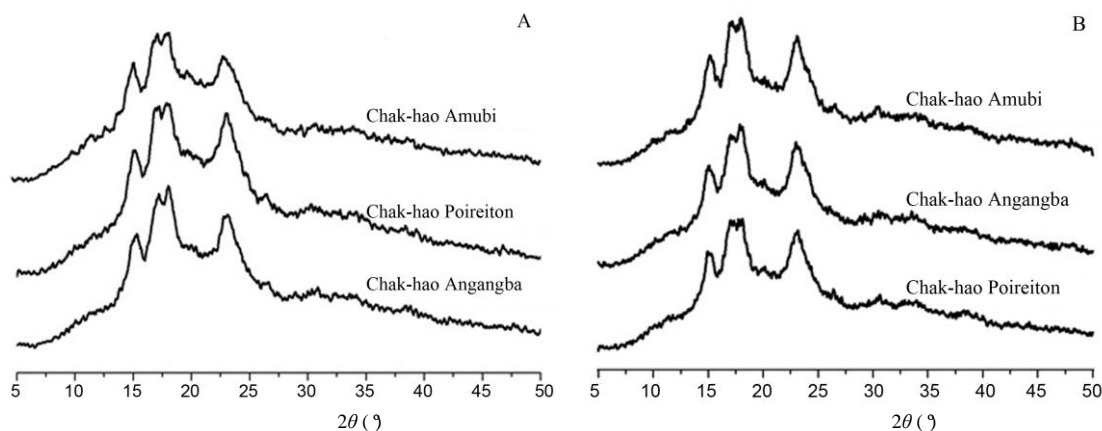


Fig. 1. X-ray diffraction of raw (A) and polished (B) pigmented rice varieties.

Polishing reduced the relative crystallinity in PPR compared to that of RPR, and the crystallinity in PPR was in the following order: CAng (10.34%) > CP (10.08%) > CA (9.18%).

Pasting properties

The typical RVA pasting profiles of pigmented flours are shown in Fig. 2, and the data are given in Table 2. Various pasting parameters including pasting temperature, peak viscosity, hold viscosity, final viscosity, break down viscosity and setback viscosity were found to be significantly different for the selected pigmented rice varieties (RPR) ($P < 0.05$). The peak viscosity, hold viscosity, final viscosity and setback viscosity were found to be the lowest in CAng whereas CA exhibited the highest peak viscosity, hold viscosity, final viscosity and breakdown viscosity followed by CP.

However, after the polishing, significantly decreased peak viscosity, hold viscosity, final viscosity, breakdown viscosity and setback viscosity were observed with enhanced pasting temperature for CA and CP ($P <$

0.05) (Fig. 2-B). However, for CAng, increased pasting temperature, hold viscosity, final viscosity and setback viscosity were noted with reduced peak viscosity and break down viscosity due to polishing.

Thermal properties

As shown in Table 2, the gelatinization temperatures showed no significant ($P < 0.05$) differences for the selected pigmented rice (RPR). The gelatinization occurred between 70.79 °C to 75.26 °C (onset temperature), 81.70 °C to 82.10 °C (peak temperature), and 91.98 °C to 93.11 °C (conclusion temperature), respectively. Gelatinization enthalpy of RPR have also shown significant difference, whereas CA (12.19 J/g) had the highest enthalpy followed by CAng (11.09 J/g) and CP (9.73 J/g).

However, after polishing, significant reduction in gelatinization temperatures and enthalpy values were observed in PPR as compared to RPR, and the thermal properties of PPR ranged from 53.86 °C to 56.31 °C (onset temperature), 73.56 °C to 77.13 °C (peak

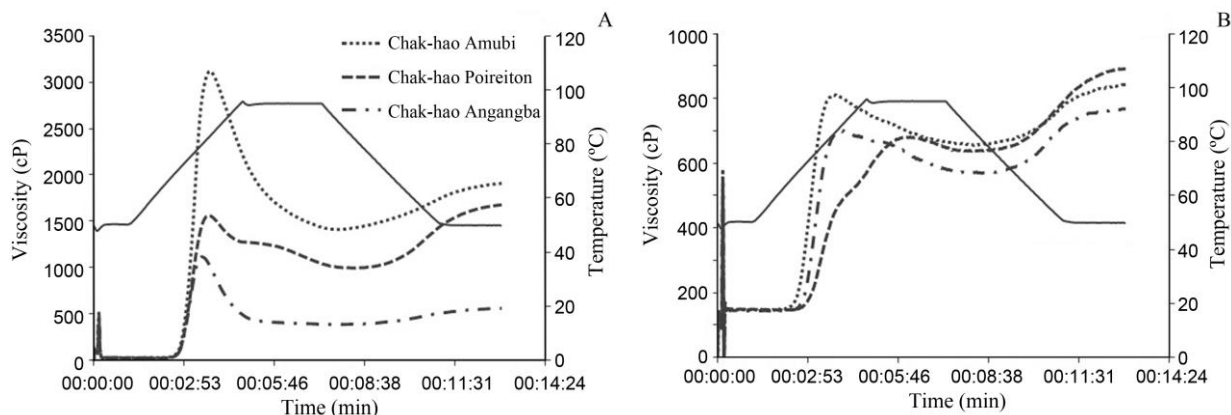


Fig. 2. Pasting properties of raw (A) and polished (B) pigmented rice varieties.

Table 2. Pasting and thermal properties of raw and polished pigmented rice.

Parameter	Chak-hao Amubi		Chak-hao Poireiton		Chak-hao Angangba	
	Raw	Polished	Raw	Polished	Raw	Polished
Pasting temperature (°C)	69.61 ± 0.37 d	76.40 ± 0.52 a	69.10 ± 0.25 d	73.27 ± 0.25 b	69.24 ± 0.50 d	70.13 ± 0.06 c
Peak viscosity (cP)	3 171 ± 47 a	813 ± 15 d	1 536 ± 33 b	679 ± 10 e	1 114 ± 12 c	697 ± 15 e
Hold viscosity (cP)	1 410 ± 28 a	658 ± 18 d	991 ± 22 b	810 ± 13 c	386 ± 11 f	572 ± 19 e
Final viscosity (cP)	1 903 ± 26 a	839 ± 16 d	1 662 ± 10 b	891 ± 15 c	571 ± 11 f	765 ± 21 e
Break down viscosity (cP)	1 699 ± 17 a	153 ± 8 d	562 ± 11 c	43 ± 4 f	738 ± 19 b	129 ± 7 e
Setback viscosity (cP)	492 ± 7 b	188 ± 5 d	683 ± 18 a	251 ± 6 c	175 ± 11 d	191 ± 6 d
T ₀ (°C)	70.79 ± 0.23 b	56.30 ± 1.03 c	75.26 ± 0.25 a	56.50 ± 1.00 c	74.94 ± 0.32 a	53.80 ± 0.64 d
T _P (°C)	81.70 ± 0.69 a	77.13 ± 1.10 b	82.10 ± 0.62 a	73.56 ± 2.10 c	81.80 ± 0.45 a	74.07 ± 1.50 c
T _C (°C)	93.11 ± 2.31 a	89.02 ± 1.52 b	91.98 ± 2.12 ab	85.98 ± 1.20 c	92.94 ± 2.32 a	85.57 ± 0.60 c
ΔH (J/g)	12.19 ± 0.34 a	10.05 ± 0.33 c	9.73 ± 0.15 c	9.84 ± 0.17 c	11.09 ± 0.75 b	10.17 ± 0.11 c

T₀, Onset temperature; T_P, Peak temperature; T_C, Conclusion temperature; ΔH, Gelatinization enthalpy.

Values (Mean ± SD, *n* = 3) with the same lowercase letters in a row did not differ significantly (*P* < 0.05) by the Duncan's multiple range method.

temperature), 85.57 °C to 89.02 °C (conclusion temperature), 9.84 to 10.17 J/g (enthalpy), respectively.

Mineral compositions

The mineral contents of various RPR and PPR flours were evaluated and the corresponding data are given in Table 3. The mineral content of rice flour was significantly different (*P* < 0.05) among all the selected rice varieties. Among all the minerals, phosphorus content (2 062.1 to 2 529.7 mg/kg) observed in RPR was the highest, followed by potassium (1 546.8 to 1 843.6 mg/kg), sulphur (743.5 to 966.4 mg/kg), magnesium (377.2 to 387.6 mg/kg), calcium (77.6 to 136.2 mg/kg), iron (47.2 to 88.8 mg/kg), and zinc (34.9 to 53.9 mg/kg), while copper content (27.5 to 33.4 mg/kg) was the lowest.

However, polishing led to significant declines in the mineral contents of the pigmented rice. The mineral content of RPR was found to be approximately 0.75 to 2.50 times more than the mineral content of PPR (Table 3). These mineral contents for various varieties of RPR were found to be significantly different (*P* < 0.05). After milling, high phosphorus content (456.7 to 1 401.6 mg/kg) was noted in PPR, followed by

potassium content (449.2 to 966.4 mg/kg), sulphur content (657.7 to 847.4 mg/kg), whereas zinc content (20.2 to 24.6 mg/kg) was found to be the lowest.

Total phenolic content

TPC of pigmented rice varieties (RPR and PPR) is represented in Fig. 3-A. Variations in TPC were observed within the RPR varieties. CAng (10.55 mg/g) showed the highest TPC, whereas the lowest value was noted for CP (7.75 mg/g). In addition, reduction (85.54% to 89.97%) in TPC in pigmented rice by polishing treatment was occurred. After polishing process, CA exhibited the lowest TPC (1.00 mg/g), and CP showed a relatively lower TPC (1.12 mg/g), while the highest TPC (1.45 mg/g) was observed for CAng.

Total flavonoid content

TFC in RPR ranged from 3.25 to 3.90 mg/g, which may be considered as abundant (Fig. 3-B). The highest TFC was noted in CAng rice flour (3.90 mg/g), followed by CA (3.49 mg/g) while the lowest TFC was observed for CP (3.25 mg/g). After polishing, CAng (0.18 mg/g) showed the lowest TFC while CA

Table 3. Mineral compositions of raw and polished pigmented rice.

mg/kg

Parameter	Chak-hao Amubi		Chak-hao Poireiton		Chak-hao Angangba	
	Raw	Polished	Raw	Polished	Raw	Polished
Calcium	136.2 ± 1.2 a	53.6 ± 1.5 e	114.6 ± 3.1 b	63.2 ± 1.1 d	77.6 ± 1.5 c	42.5 ± 1.1 f
Chlorine	63.4 ± 2.1 a	39.6 ± 1.2 d	60.6 ± 0.7 b	44.3 ± 0.9 c	35.7 ± 0.9 e	28.6 ± 1.9 e
Copper	33.4 ± 0.5 a	26.2 ± 1.1 c	27.5 ± 1.7 c	20.1 ± 0.7 e	30.6 ± 0.6 b	24.1 ± 1.8 d
Iron	88.8 ± 0.9 a	26.2 ± 0.7 e	47.2 ± 0.6 c	30.6 ± 0.4 d	57.1 ± 0.7 b	24.5 ± 0.5 f
Potassium	1 606.6 ± 43.7 b	566.1 ± 13.7 e	1 843.6 ± 51.8 a	966.4 ± 26.2 d	1 546.8 ± 21.7 c	449.2 ± 7.3 f
Magnesium	377.2 ± 14.6 a	106.6 ± 6.1 c	387.6 ± 8.6 a	215.8 ± 8.9 b	379.1 ± 6.8 a	56.8 ± 2.1 d
Manganese	38.8 ± 1.2 b	20.1 ± 1.2 d	42.7 ± 1.9 a	21.8 ± 1.5 d	23.6 ± 2.5 c	14.5 ± 1.1 e
Phosphorus	2 062.1 ± 106.2 c	718.5 ± 15.7 e	2 529.7 ± 41.7 a	1 401.6 ± 21.9 d	2 248.1 ± 40.4 b	456.7 ± 32.1 f
Sulphur	976.1 ± 62.4 a	847.4 ± 39.1 c	916.5 ± 10.9 b	795.8 ± 12.5 cd	743.5 ± 7.6 d	657.7 ± 27.8 e
Zinc	53.9 ± 1.1 a	24.6 ± 2.3 d	42.4 ± 0.6 b	24.7 ± 1.2 d	34.9 ± 3.3 c	20.2 ± 0.5 e

Values (Mean ± SD, *n* = 3) with the same lowercase letters in a row did not differ significantly (*P* < 0.05) by the Duncan's multiple range method.

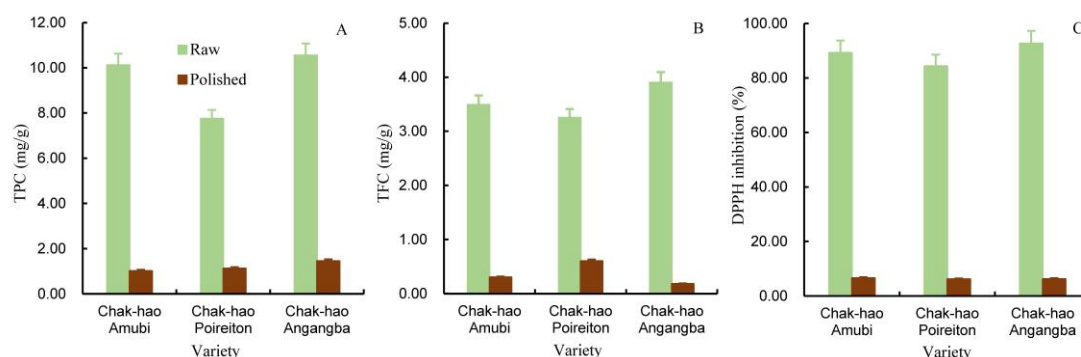


Fig. 3. Total phenolic content (TPC, A), total flavonoid content (TFC, B) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition (C) of raw and polished pigmented rice varieties.

(0.30 mg/g) had a relatively low TFC. The highest TFC was observed for CP (0.60 mg/g).

Antioxidant properties

The antioxidant activities of various types of pigmented rice (RPR and PPR) were analyzed by DPPH assay and the results presents in Fig. 3-C. All the pigmented rice varieties showed significant scavenging activity towards the free radical. Variations in the scavenging activity were observed within the different RPR varieties. DPPH activity ranged from 84.77% to 92.67%. CAng exhibited the highest activity (92.67%), followed by CA (89.28%), while the lowest was observed for CP (84.77%).

After polishing, CP (6.11%) showed the lowest DPPH activity which was relatively lower for CAng (6.17%). The highest DPPH activity was observed for CA (6.55%).

Morphological characteristics

The morphology and arrangement of the rice flours obtained from RPR were analysed (Fig. 4-A). It was evident that there were no momentous changes in the morphology of the rice flours obtained from selected pigmented varieties (RPR). It could be seen that after polishing, starch granules were released from clump in the matrices of plant cell wall and the shape of starch granules was changed from closely spherical to polygonal (Fig. 4-B).

DISCUSSION

Proximate composition

Reduced moisture content of PPR was observed in this study due to the generation of heat during milling

of rice grains. Decline in fat content was also observed after polishing, which could be due to the removal of bran layers of pigmented rice. Monks et al (2013) and Roy et al (2008) also reported that fat content decreased in brown rice due to polishing (up to 12% DOM). Differences in the quantity and composition of bran can occur as a function of genotypes, environmental conditions and agronomic practices (Monks et al, 2013). Later, Paiva et al (2016) reported a continuous decrease in fat content of black and red rice varieties during milling (up to 12% DOM) and parboiling.

Typically, the bran layers of the rice caryopsis contain high levels of ash content. Reduction in ash content in rice as an effect of polishing (up to 12% DOM) was also observed in different rice varieties (Lamberts et al, 2007; Paiva et al, 2014). These findings indicated that ash content was decreased in pigmented rice due to polishing, which could be due to the removal of outer bran layers of RPR. Further, Lamberts et al (2007) and Singh et al (2000) reported that the highest mineral content (approximately 61%) presents in the bran fraction of pigmented rice. Later, Heinemann et al (2005) compared the chemical and nutritional compositions of brown, parboiled brown, parboiled milled and milled rice, reporting a decrease of 61.15% in ash content in milled rice compared to the brown rice.

The protein content of RPR ranged from 5.57% to 8.75% (Table 1). These observations are in comparable with the reports from other types of pigmented rice (Monks et al, 2013). The loss of protein content was lower in pigmented rice (3.08% to 5.02%) due to polishing (9% DOM), which is possible due to the fact that proteins in the endosperm of the rice grains is higher than that in the bran layers (Lamberts et al, 2007; Paiva et al, 2016). Similar results were observed by Sompong et al (2011), who informed loss of

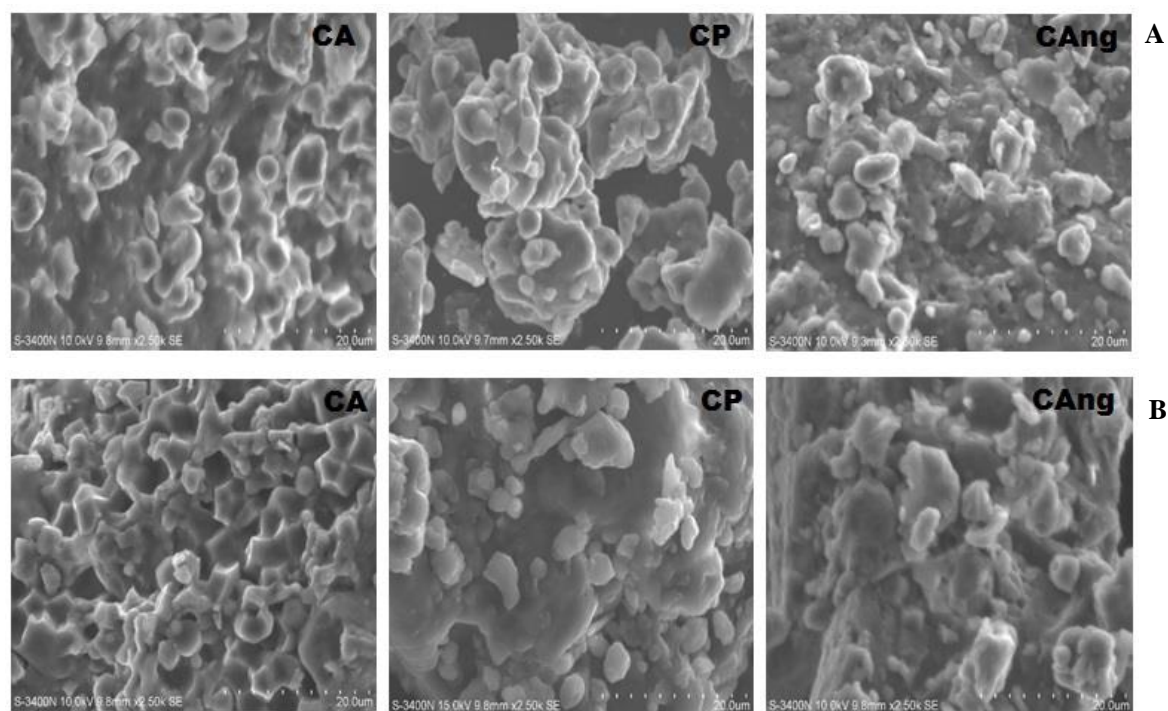


Fig. 4. Scanning electron micrographs (SEM) of raw (A) and polished (B) pigmented rice varieties.

CA, Chak-hao Amubi; CP, Chak-hao Poireiton; CAng, Chak-hao Angangba.

protein content between 2.5% to 10.0% for red and black rice varieties grown in Thailand, China and Sri Lanka during milling (up to 10% DOM).

PPR showed higher carbohydrate content than RPR, since the moisture, fat and ash contents were significantly affected by polishing (9% DOM). It could be due to the removal of bran, aleurone and germ portions leaving behind the starchy endosperm.

Amylose content

Generally, amylose content plays a crucial character in the cooking procedure, paste viscosity, rheological, thermal and texture of rice. The variance in amylose content of pigmented rice varieties are affected by rice genotype and agronomic or climatic conditions (Singh et al, 2000). From the results, increase in the amylose content was observed in case of PPR due to polishing (9% DOM) and the values were 1.93 to 2.00 times higher than that of RPR. This could be attributed to the removal of the bran layer components from rice grains, which results in the elevation of the proportion of starch in the grains (Monks et al, 2013).

Colour of rice flours

The amplification of the polishing treatment initiated an increase in the lightness of the grain. This increase

is associated with the removal of nutrients during the milling treatment, primarily the fat soluble and the mineral nutrients that are responsible for the yellowish colour of rice (Monks et al, 2013). Tian et al (2004) stated that colour of rice grain is mainly influenced by the content of phytochemicals, especially anthocyanins (black rice), ferulic acid and *p*-coumaric acids (brown rice). Results showed that the polishing process (9% DOM) increased the lightness (L^*) with reduced a^* and b^* values of pigmented rice. CAng exhibited the highest lightness, followed by CP, while the lowest value was observed for CA. This disclosed that the content of different pigments diminished from the surface of rice caryopsis, and also demonstrated that the rice bran contains more black, purple and red pigments (Lamberts et al, 2007). Saikia et al (2012) stated that the lightness of non-milled red rice was higher than that of black rice, since black rice has a high content of dark pigments in the outer bran layer of rice grains.

X-ray diffraction

Generally, the endosperm of rice caryopsis contains the starch granules with semi-crystalline structure, which can lead to the existence of reflections in X-ray diffraction pattern (Paiva et al, 2016). The types of

diffraction patterns in this study are comparable with the XRD patterns of the cereals (Yu et al, 2012; Paiva et al, 2016). Further, XRD offers data on the content of crystallites and amorphous regions which aids to the elucidation of crystal structures. It is known that starch crystallinity may depend upon amylopectin content, and also the crystalline arrangement of amylopectin is influenced by amylose molecules (Paiva et al, 2016; Zhang et al, 2016). Polishing (9% DOM) reduced the relative crystallinity in PPR compared to that in RPR, which could be due to the consequence of the enhanced amylose content in PPR (Paiva et al, 2016). However, the reduced crystallinity in PPR might also due to the reduction of other components, such as fat, ash, fiber and pigments during the polishing treatment (Monks et al, 2013).

Pasting properties

RVA pasting curve reflects the modifications in the viscosity of the sample upon heating and cooling in adequate water and helps to predict the texture of cooked rice (Zhang et al, 2016). The decreased breakdown viscosity of CP and CAng reflects the enhanced facility of the starch to tolerate heat and sheer stress upon cooling (Monks et al, 2013). It should be noted that CP exhibited the highest setback viscosity, followed by CA, while the lowest was observed for CAng. Variation in the pasting RVA curve of pigmented rice may occur due to the variances in structure of amylose and amylopectin, genotype, geographic and agronomic conditions, and other factors such as the presence of starch granule-associated proteins, protein and fat contents (Zhang et al, 2016). Increase in the pasting temperature of PPR due to polishing (9% DOM) may occur due to the dry milling of rice grains (Chen et al, 1999; Singh et al, 2005). Increase in pasting temperature and setback viscosity, and improved resistance to break down was observed in PPR when compared with RPR. It could be due to the increase in amylose content in pigmented rice due to polishing (9% DOM). Improvement in the amylose levels in PPR may lead to increase in the resistance to break down viscosity of rice flour (Chen et al, 1999).

Thermal properties

Thermal characteristics of pigmented rice are similar with the gelatinization temperatures of rice varieties reported by Singh et al (2006), however, differed from the results of Chávez-Murillo et al (2011). Gelatinization temperatures of the pigmented rice are affected by the

content of starch granules available in the rice flours, existing interactions between the amylose and lipid contents (Shin et al, 2004). RPR showed a slight difference in gelatinization temperatures and enthalpy, which might be attributed to variances in the structure of amylose and amylopectin, rice genotype, geographic and agronomic conditions and other factors such as content of starch granule-associated proteins, protein and fat contents. Moreover, reduction in the gelatinization temperatures and enthalpy of pigmented rice is possibly due to the removal of bran and also depletion in lipid, ash and protein contents in pigmented rice (Chen et al, 1999). Such molecules interact with starch components in the rice, and increase the gelatinization temperature of starch and result in the increase of enthalpy.

Minerals

Minerals have an abundant nutritional importance in human health and are required to maintain a balanced diet, which is important for conserving all regular metabolic functions. All pigmented rice varieties are a rich source of minerals, such as iron, calcium, potassium, chlorine, copper, phosphorus, manganese, magnesium, sulphur and zinc. However, the results for the minerals were slightly different from the previous observations reported by Tsukada et al (2007) and Wang et al (2011). The variances in the mineral content of selected pigmented rice may happen due to various factors such as rice genotypes, agronomic and cultivation conditions. Reduction in mineral content in different varieties of pigmented rice was also observed due to polishing treatment (9% DOM) and this could be attributed to the removal of rice bran components during the polishing treatment (Itani et al, 2002; Heinemann et al, 2005). Moreover, Lamberts et al (2007) reported that the mineral content is the highest in bran (61.0%), followed by outer endosperm (23.7%), core endosperm (11.6%), and the lowest in the middle endosperm (3.7%).

Total phenolic content

CAng showed the highest TPC, whereas the lowest value was noted for CP. These findings considerably different from similar studies on pigmented rice varieties reported previously (Kong and Lee, 2010; Saikia et al, 2012). These observations reveal a promising vast discrepancy of TPC in pigmented rice, which might reflect the potential effects of genotype and agronomic conditions. Decreases in TPC of the

pigmented rice affected by polishing (9% DOM) were previously reported (Kong and Lee, 2010; Ti et al, 2014). The decrease in TPC could be attributed to the removal of rice bran, along with the pericarp, aleurone and embryo. All these components are known to contain a significant amount of phenolic compounds.

Total flavonoid content

The highest TFC was noted in CAng rice flour, followed by CA, while the lowest TFC was observed in CP. These results were similar to TFC of pigmented rice reported earlier (Saikia et al, 2012), which showed a significant difference from another report (Ti et al, 2014). Significant decline in TFC was observed in the pigmented rice by polishing (9% DOM) and TFC in PPR was 81.25% to 95.38% lower than that in RPR (Kong and Lee, 2010). This could be attributed to the removal of the rice bran affected by polishing treatment (9% DOM) as most of the flavonoid groups are distributed in rice bran.

Antioxidant properties

Increased antioxidant capacity of cereals was found due to its high content of phenolic compounds in the bran. These phenolic compounds exhibit decent antioxidant activity against free radicals. Generally, antioxidant capacity, which describes the ability of various food components in scavenging free radicals, has been recommended to evaluate the health effects of antioxidant-rich foods (Sompong et al, 2011). However, the results about the antioxidant properties in this study significantly differ from other pigmented rice studies reported previously (Sompong et al, 2011; Saikia et al, 2012).

The decrease in DPPH activity is predominantly attributed to the removal of rice bran during polishing treatment (9% DOM), which contains a significant quantity of phenolic compounds (Tuncel and Yilmaz, 2011). However, the DPPH activity of rice depends on not only the rice bran components, but also genetic diversity, and climatic or seasonal variations. These factors can also affect the secondary metabolites in rice bran. Further, the antioxidant capacity of rice grain is contributed not only by phenolic compounds, but also by other phytochemicals, such as carotenoids, tocopherols and γ -oryzanol (Choi et al, 2007).

Morphological characteristics

The granules in the flour samples were not uniform and regular in size, moreover, they formed a clump in

the matrices of plant cell wall (Reddy et al, 2016). As shown in Fig. 4-A, the starch granules in the rice flours were surrounded by non-starch components, such as proteins and fat bodies (Setyawati et al, 2016). After the polishing treatment (9% DOM), the scanning electron micrographs of PPR flour showed significant difference from those of RPR. This is possibly due to the destruction of the amyloplast and association of the starch complexes with lipids and proteins due to polishing and also it could lead to reduction of the protein and lipid contents in rice flour.

CONCLUSIONS

Milling is a traditional method which is adopted for processing of rice, and it helps to increase the stability and sensory quality of the rice upon storage. The work presented herein described a systematic evaluation of the process of polishing (9% DOM) on the nutrient compositions, physico-chemical properties, mineral contents, and antioxidant activities of various pigmented rice varieties (black, purple and brown rice). There was a notable loss in the phytochemical content (phenolic acids and flavonoids) of the rice varieties after polishing, which are otherwise beneficial to human health. The contents of various minerals, fats and proteins were also found to be decreased significantly after polishing (9% DOM). However, there were improvements in the lightness of the rice grains and the amylose content, and decreases in the pasting properties and the transition temperatures of the various rice varieties. The rice flours exhibited A-type X-ray pattern and remained unchanged even after polishing (9% DOM). This work may be considered as a development in the food situation because of the limited studies performed concerning pigmented rice varieties in India.

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