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Effect of parboiling on phytochemical content, antioxidant activity and physicochemical properties of germinated red rice

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ABSTRACT

In order to improve functional properties and palatability of germinated red rice, this study investigated differences in phytochemicals and physicochemical properties of germinated red rice at 2, 5, 10, 15 min of parboiling. Total free phenolic content and antioxidant activity of germinated red rice parboiled for 5 and 15 min was higher than that of germinated red rice. Free *p*-coumaric acid increased from 0.20 to 0.67 mg/100 g with parboiling time increasing from 5 to 15 min. Bound vanillic (0.17-0.27 mg/100 g) and *p*-coumaric acid (6.56-8.59 mg/100 g) had higher levels at 0, 2, or 5 min. During 15 min of parboiling, color difference (ΔE) increased from 0.58 to 9.09, heat enthalpy (ΔH) decreased from 4.69 to 1.94 J/g, and internal structure of rice was destroyed. Overall, parboiling time of less than 5 min was suitable to improve the quality of germinated red rice.

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1. Introduction

Rice, as one of the most important crops, feeds almost 1/3 of world's population. With the improvement of living standards, whole grains have become popular in western countries, and are being gradually accepted in developing countries. It is well known that germinated brown rice is considered to be a functional rice product which has high content of phytochemicals such as phenolics, flavonoids, and γ-aminobutyric acid (GABA). Compared with brown rice, germinated brown rice has shorter cooking time, softer texture and higher nutrition (Komatsuzaki et al., 2007). While, some undesirable odor could be generated by the corruption of microorganisms during germination processing (Chinma, Anuonye, Simon, Ohiare, & Danbaba, 2015), which might affect the flavor of germinated brown rice. Parboiling germinated rice, obtained from heat-moisture treatment of germinated rice, encompasses more nutraceuticals, better palatability and longer shelf life than germinated rice (Oli, Ward, Adhikari, & Torley, 2014). As one of the products of germinated rice, it is considered to be good to many chronic diseases, such as Alzheimer's disease, type-2 diabetes, hypercholesterolemia, colon cancer, cardiovascular diseases, and obesity (Patil & Khan, 2011).

Parboiling is a heat-moisture treatment consisted of three stages: 1) soaking the rough rice in cold water at room temperature to saturation moisture content; 2) gelatinization of rice starch through steaming or boiling; 3) drying the parboiling rice by sunshine or oven at low temperature until the water content is reduced to less than 14% (Ayamdoo, Demuyakor, Dogbe, Owusu, & Ofosu, 2013). In these processes, parboiling treatment result in the transformation of nutrition, pigments, vitamins and minerals from embryo and brown layer to endosperm which make parboiled polished rice more nutrition. It could also increase the hardness of rice so as to have fewer broken grain (Elbert, Tolaba, & Suárez, 2001), inactivate lipase for better storage and process, eliminate multiple microorganisms that occurred during soaking germination process (Bhattacharya, 2013), remove some undesirable odor, and change the physicochemical properties of whole rice grain (Cheevitsopon & Noomhorm, 2015). However, the effect of parboiling on the physicochemical properties of germinated brown rice has been rarely reported.

The phenolic acid, as one of secondary metabolites, mostly occurred in bran and embryo of rice grain accounting for 59.2% of total phenolic acids (Ti et al., 2014). It is an important source of antioxidant activity. Phenolic acids are present in two forms in rice: soluble form, including soluble free and soluble bound form; and insoluble bound form, which are esterified to cell walls (Shao, Xu, Sun, Bao, & Beta, 2015). Among the three phenolic constituents, bound phenolic acids are the most abundant, followed by soluble bound phenolic acids with free phenolic acids at least (Shao, Xu, Sun, Bao, & Beta, 2014). Both of ferulic and *p*-coumaric acid are

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observed to be the most abundant phenolic acids in pigment rice. Barley, which could be used in the brewing and food industries, contains many kinds of antioxidants such as flavanols (catechin dihexoside), flavonols (quercetin), and phenolic acids (ferulic, caffeic, coumaric acid) (Gangopadhyay, Rai, Brunton, Gallagher, & Hossain, 2016). They play an important role in promoting well-being and reducing the risk of diet-related diseases. Up to date, several studies have investigated total phenolic content, antioxidant activity, and phenolic acids in rough, brown, or germinated brown rice (Moongngarm & Saetung, 2010; Ti et al., 2014). However, little information has been reported about changes of phytochemical content, antioxidant activity and physicochemical properties of germinated brown rice at different parboiling time.

The novelty of this study was to improve functional properties and palatability of germinated red rice. It is greatly needed to find a suitable parboiling time. Parboiled rice with better quality would get good acceptability in the market. The aim of the work was to investigate the effect of different parboiling time on total phenolic, flavonoids, proanthocyanidins content, ABTS and DPPH antioxidant activity, phenolic acids, pasting, thermal properties, color differences and micro-structure. It would provide useful information on functional food in industrialized processes and commercial practices.

2. Materials and methods

2.1. Sample and chemistry reagents

Red rice (Anhui 02) was harvested from Anhui Academy of Agricultural Science in 2014. After sun drying to the moisture content less than 14%, the grain was put in a tight plastic bag at room temperature for about 3 months, and then stored at 4 °C in dark before analysis.

Chemical reagents, such as Folin-Ciocalteu reagent, sodium hydroxide, DPPH (2,2-diphenyl-1-picrylhydrazyl), trolox (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid), $ABTS^{\cdot+}$ (2,2′-Azino-bis-(3-ethylbenzonthiazoline-6-sulfonic acid) diammonium salt) and gallic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The HPLC grade methanol and ethyl acetate, used in the extraction, purification and HPLC analysis, were purchased from Merck (Darmstadt, Germany) and Tedia (Fairfield, USA), respectively. The phenolic acid standards (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapic, isoferulic, and *o*-coumaric acid) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium hydroxide, sodium sulfate, and hydrochloric acid were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Preparation of germinated and parboiled germinated red rice

The preparation of germination red rice can separate into two processes: soaking and sprouting (Cáceres, Martínez-Villaluenga, Amigo, & Frias, 2014), which was performed by using the method reported by Moongngarm and Khomphiphatkul (2011) with slight modification. Red rough rice (200 g) was sterilized with 100 ml of 0.05% sodium hypochlorite for 30 min, and then washed by distilled water for three times, and soaked in 500 ml distilled water for 12 h to saturate moisture content. Then, the soaked red rough rice was placed on 25 cm × 30 cm iron tray with two layers of medical gauze laying on the bottom, and certain amount of water was added to keep medical gauze moist. The soaked red rough rice was sprouted for 48 h in the darkness at 30 °C with 90% relatively humidity in an incubator (MCR-450B, Hangzhou, China). Parboiling treatment was performed according the method reported by Lamberts, Rombouts, Brijs, Gebruers, and Delcour (2008) with minor modifications. Briefly, after germinating, the germinated red rough rice was immediately steamed with an automatic electric rice cooker for 2, 5, 10 and 15 min, respectively. All of the parboiled samples and germinated red rough rice were dried at 40 °C for 24 h until the moisture content reduced to less than 14%. The determination of moisture content of all samples was performed through drying at 105 °C to reach the constant weight. The red rough rice, germinated red rough rice and parboiled germinated red rough rice were de-husked and ground by a mill (Foss, Switzerland) and passed through an 80 mesh sieve. The red rice was marked as RR, the germinated red rice was marked as PGRR-0, and the germinated red rice parboiled for 2, 5, 10, 15 min were marked as PGRR-2, PGRR-5, PGRR-10, PGRR-15, respectively. All samples were performed in triplicate and stored at −4 °C prior to analysis.

2.3. Extraction of free and bound phenolics

Free phenolics were extracted by using the method reported by Shao et al. (2014) with minor modifications. Briefly, 1 g rice flour was extracted twice with 20 ml 80% methanol. Each time, the mixture was shaken on a shaker (HY-8 Speed control shaker, China) for 30 min at room temperature. The mixture was centrifuged (Himac CR21GII, Hitachi, Japan) at 10,000×*g* for 15 min at 4 °C. The supernatants were collected and combined together. After adjusting the pH to about 1.5–2.0 with concentrated HCl, the supernatants was concentrated using a rotary evaporator (IKA RV10, German) at 37 °C. The concentrated free fraction was defatted once with hexanes (10 ml) prior to extraction three times using ethyl acetate (70 ml). The ethyl acetate extracts were pooled and rotary evaporated at 35 °C. The dried extracts were dissolved in 5 ml 50% methanol as free phenolic extracts. The residue was washed with distilled water, and used to extract the bound phenolics. After digesting with 4 M NaOH at room temperature for 2 h, the mixture was adjusted to pH 1.5–2.0 with concentrated HCl. After centrifugation at 10,000×*g* for 30 min at 4 °C, the supernatant was defatted three times using 25 ml hexane and extracts thrice with ethyl acetate (70 ml). The ethyl acetate fractions were combined and evaporated at 35 °C. The dried extracts were dissolved in 5 ml 50% methanol and used as bound phenolic extracts. All analyses were performed in triplicate.

2.4. Determination of total phenolic content (TPC)

TPC was assayed by Folin-Ciocalteu method with minor modifications (Shen, Jin, Xiao, Lu, & Bao, 2009). Briefly, 200 μl of appropriate diluted free, bound extracts or standard solutions were mixed with 1.5 ml of 10-fold diluted Folin-Ciocalteu reagent, and then neutralization with 1.5 ml saturated sodium carbonate (75 g/L). The absorbance of the mixture was measured at 725 nm by a spectrophotometer (Unico Co, USA) after 2 h of reaction at room temperature in dark. Gallic acid solution (0, 25, 50, 75, 100, 125 mg/L) was used as standard to generate the straight line equation ($y = 124.86x - 3.7956$, $R^2 = 0.9992$). The results of TPC were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of dry rice flour (mg GAE/100 g). Duplicate determinations were carried out for each extract.

2.5. Determination of DPPH radical scavenging activity (DPPH)

The DPPH radical scavenging activity of extracts was determined according to the method reported by Brand-Williams, Cuvelier, and Berset (1995), and modified by Shao et al. (2014). Appropriately diluted free, bound extracts or standards (200 μl) were mixed with 100 μmol/L DPPH solution (3 ml). After incubating for 30 min in dark, the absorbance was measured at the

wavelength of 517 nm. The DPPH radical scavenging activity (%) was calculated by using the formula as follows:

$$\text{DPPH}\ (\%) = (1 - A_{sample}/A_{control}) \times 100\%$$

The straight line equation was generated from Trolox standard and the results were expressed as μmol of Trolox equivalent DPPH radical scavenging activity per 100 g of dry rice flour (μM TE/100 g). Duplicate determinations were carried out for each extract.

2.6. Determination of $ABTS^{\cdot+}$ radical scavenging activity

The radical scavenging activity was measured by using a spectrophotometer according to the method previously reported (Shen et al., 2009). Briefly, $ABTS^{\cdot+}$ solution was produced by mixing 7 mM ABTS stock solution and 2.45 mM potassium peroxydisulfate (v/v = 2:1) in dark at room temperature overnight. The $ABTS^{\cdot+}$ working solution was diluted from $ABTS^{\cdot+}$ solution by ethanol to adjust the absorbance to 0.700 ± 0.020 at the wavelength of 734 nm. Appropriated diluted free, bound extracts or standards (100 μl) were mixed with 3.9 ml $ABTS^{\cdot+}$ working solution thoroughly on a vortex. After reacting for about 6 min, the absorbance was tested at 734 nm. Trolox solution (0, 25, 50, 75, 100, 125 mg/L) was used as standard to generate the straight line equation (y = 124.5–177.03x, R^2 = 0.9997). The results were expressed as μmol of Trolox equivalent activity per 100 g of dry rice flour (μM TE/100 g). Duplicate determinations were carried out for each extract.

2.7. HPLC analysis of phenolic acids

The phenolic acid content was analyzed on a waters HPLC system (Waters Associates, Milford, Mass, USA) that consisted of a Waters 2707 autosampler, a Waters 2489 UV/Visible detector and a Waters 1525 binary pump. An XBridge 5 μm C18 column (250 mm × 4.6 mm) was used to separate the phenolic acids. Gradient elution was performed with a mobile phase consisted of A (0.1% acetic acid in water) and B (0.1% acetic acid in methanol). The flow rate was 1 ml/min, and the gradient was set according to the method reported by Shao et al. (2014) as follows: 0–11 min, 9–14% B; 11–14 min, 14–15% B; 14–17 min, 15% B; 17–24 min, 15–16.5% B; 24–28 min, 16.5–19% B; 28–30 min, 19–25% B; 30–36 min, 25–26% B; 36–38 min, 26–28% B; 38–41 min, 28–35% B; 41–46 min, 35–40% B; 46–48 min, 40–48% B; 48–53 min, 48–53% B; 53–70 min, 53–70% B; 70–72 min, 9% B; 72–75 min, 9% B. The injection volume was 10 μl. Free and bound phenolic extracts were filtered through 0.45 μm membrane filters before analysis. The column temperature was kept at 40 °C. The phenolic acids were detected at a wavelength of 280 nm, and quantified by using the external calibration curves according to the retention time of phenolic acid standards.

2.8. Determination of total flavonoid content (TFC)

Sample flour (0.5 g) was extracted three times with 10 ml of 80% methanol. After shaking for 45 min (HY-8 Speed control shaker, China), the mixture was centrifuged at 10,000×g for 15 min by using a high-speed centrifuge (Himac CR21GII, Hitachi, Japan). The supernatants of each extraction were collected and combined together. The extractions were temporary stored at −20 °C, and analyzed in 2–3 days. All analyses were performed in triplicate.

TFC was tested by using a colorimetric method with slight revisions (Jia, Tang, & Wu, 1999; Shen et al., 2009). Briefly, 150 μl of 5% $NaNO_2$ was mixed with 500 μl of the sample extracts or standard solutions in 15 ml tubes and then incubated for 5 min. After adding 150 μl of 10% $AlCl_3{\cdot}6H_2O$ and allowing 5 min of reaction, 1 ml NaOH (1 M) and 3 ml double-distilled water were added. The absorbance was measured at 510 nm after thoroughly mixing and reacted for about 10 min. TFC was expressed as milligrams of catechin equivalent per 100 g of dry rice flour (mg CE/100 g). Duplicate determinations were carried out for each extract.

2.9. Determination of total proanthocyanidin content (TPAC)

Sample flour (0.5 g) was extracted three times with 15 ml of methanol: 1 M HCl (85:15, v/v) by a shaker under dark condition. After centrifuging at 4, 100×g for 15 min at room temperature, the clear supernatants were collected, combined together, and stored at −20 °C before analysis. Triplicate extractions were carried out for each sample. TPAC was measured by using the method of vanillin assay with slight modifications (Sun, Ricardo-da-Silva, & Spranger, 1998). Briefly, appropriately diluted sample extracts or standard solutions (0.6 ml) were added to 1.5 ml of solution A (1% vanillin solution in methanol), and mixed thoroughly. Before reacting for 20 min in a 30 °C water bath, 1.5 ml of solution B (sulfuric acid/methanol solution (1:4, v/v) was added. The absorbance was measured at 500 nm. Each extracts treated with 100% methanol instead of solution A was used as blank in order to eliminate interferences (e.g., anthocyanin, which mainly exists in black rice). TPAC was expressed as mg catechin equivalent per 100 g of dry rice flour (mg CE/100 g). Duplicate determinations were carried out for each extract.

2.10. The determination of pasting properties

The pasting properties were determined using a Rapid Visco-Analyzer (RVA) (Newport Scientific model (+), Warriewood, Australia). The weight of sample and addition of water were calculated by software of RVA according to the moisture content of sample (14% moisture basis). The heating and cooling cycles were conducted following the general pasting method (STD 1). The paddle rotating was held at 50 °C for 1 min, heated to 95 °C within 3 min 42 s, and then held at 95 °C for 2 min 30 s. It was continuous cooled to 50 °C for 2 min, which the paddle rotating speed maintained at 160 rpm. The viscosity value was expressed in rapid viscos units (RVU).

2.11. Color characteristics of sample

The color measurement of red rice, germination red rice and germination red rice parboiled for 2, 5, 10, 15 min were carried out with optical sensor (Konica Minolta CM-5, Japan) on the basis of CIE L*, a*, b* color system (Jung et al., 2015). The total color difference (ΔE) was calculated by applying the following equation:

$$\Delta E = ((L^* - L^*_{RR})^2 + (a^* - a^*_{RR})^2 + (b^* - b^*_{RR})^2)^{0.5}$$

2.12. The differential scanning calorimetry (DSC) analysis

The DSC measurements were performed using a differential scanning calorimeter (TA Instrument DSC Q200, USA) under an ultrahigh-purity nitrogen atmosphere. The instrument was calibrated using indium as a standard and an empty aluminum pan was used as a reference. The DSC analysis of samples was prepared according to the method reported by Samutsri and Suphantharika (2012) with little variations. Briefly, approximately 5.0 mg sample flour was weighted into an aluminum crucible, mixed with 8 μL of distilled water, and hermetically sealed in an aluminum pan immediately. After equilibrating at room temperature for 1 h, sealed samples were analyzed by the DSC instrument.

Table 1
Total phenolic content and antioxidant activity of free and bound fraction of samples at different parboiling time.*

	TPC		DPPH		ABTS	
	FREE	BOUND	FREE	BOUND	FREE	BOUND
RR	15.3 ± 1.9^{a}	46.8 ± 0.4^{bc}	44.3 ± 0.5^{a}	124.5 ± 3.3^{ab}	74.9 ± 6.6^{a}	210.1 ± 9.1^{bc}
PGRR-0	7.1 ± 0.4^{d}	55.5 ± 1.7^{a}	26.7 ± 0.8^{c}	131.1 ± 1.4^{a}	30.6 ± 1.7^{c}	220.8 ± 1.3^{ab}
PGRR-2	8.9 ± 1.5^{cd}	52.6 ± 6.5^{ab}	32.4 ± 0.3^{bc}	131.2 ± 1.9^{a}	24.9 ± 5.8^{c}	233.4 ± 16.0^{a}
PGRR-5	11.6 ± 0.8^{bc}	51.4 ± 3.1^{ab}	37.1 ± 2.8^{b}	133.1 ± 4.4^{a}	56.6 ± 6.1^{b}	212.9 ± 2.4^{ab}
PGRR-10	10.1 ± 0.5^{bcd}	44.4 ± 1.2^{bc}	29.5 ± 1.2^{c}	110.6 ± 5.3^{c}	47.9 ± 0.1^{b}	190.7 ± 6.0^{cd}
PGRR-15	12.6 ± 2.2^{ab}	42. 9 ± 2.3^{c}	31.6 ± 4.8^{bc}	111.5 ± 10.6^{bc}	55.5 ± 2.3^{b}	177.5 ± 3.5^{d}

* The results are present as means ± SD (n = 3). Values in each column with different letters are significantly different ($P < 0.05$); TPC is expressed as mg GAE/100 g dry rice flour (mg GAE/100 g), DPPH and ABTS are expressed as μM TE/100 g dry rice flour (μM TE/100 g). TPC, total phenolic content; DPPH, antioxidant capacity tested by DPPH radical scavenging; ABTS, antioxidant capacity tested by $ABTS^{\cdot+}$ radical scavenging; FREE, free phenolic fraction; BOUND, bound phenolic fraction; RR, red rice; PGRR-0, germinated red rice parboiled for 0 min; PGRR-2, germinated red rice parboiled for 2 min; PGRR-5, germinated red rice parboiled for 5 min; PGRR-10, germinated red rice parboiled for 10 min; PGRR-15, germinated red rice parboiled for 15 min.

Scans procedure was performed from 25 to 100 °C at a constant rate of 10 °C/min. The transition temperature, concluding the onset temperature (T_0), peak temperature (T_p), conclusion temperature (T_c) and ΔT, and gelatinization enthalpy (ΔH) were determined according to the DSC heating curves. Melting enthalpy (ΔH) was calculated from the area of the main endothermic peak and expressed in terms of J/g of dry starch using the equipment software. Measurements were performed in triplicate.

2.13. Scanning electron microscopy (SEM)

The micro-structure of the rice endosperm fragment was observed by scanning electron microscopy (TM3000, Hitachi, Japan) by using the method reported by Liu and Ng (2015). Final moisture content of all the samples was controlled to less than 8%. The samples were coated with gold-palladium, and examined on the scanning electron microscopy.

2.14. Statistical analysis

All the parameters were measured in triplicate, and presented on a dry weight basis as means ± standard deviation (SD). Data analysis was performed with SAS program version 8 (SAS Institute Inc., Cary, NC, U.S.A). Differences among different parboiling time were found using ANOVA, followed by Duncan multiple comparison tests. Statistical significance was defined at level of $P < 0.05$.

3. Results and discussion

3.1. Analysis of total phenolic content (TPC) and antioxidant activity

TPC and antioxidant activity (DPPH and $ABTS^{\cdot+}$ radical scavenging capacity) of free and bound phenolic fractions of all samples are shown in Table 1. Free TPC of RR (15.3), PGRR-5 (11.6) and PGRR-15 (12.6) were significantly higher than that of PGRR-0 (7.1 mg GAE/100 g). It was consistent with those found in wheat (Fares, Platani, Baiano, & Menga, 2010). Bound TPC of PGRR-0 (55.5) was significantly higher than that of RR (46.8 mg GAE/100 g). PGRR parboiled for 0–5 min had similar bound TPC, and they were significantly ($P < 0.05$) higher than that parboiled for 15 min (42.9 mg GAE/100 g). It was attributed to the heat treatment which could partially break down the ester bond between phenolics and cell walls, and some bound phenolics were released as free phenolics.

In order to reduce the interferences of other substances in the tests of antioxidant activity, two methods were used to measure the total antioxidant activity, DPPH radical and ABTS cationic scavenging activity. The trends of antioxidant activity were generally similar to that of TPC, which might be due to the high correlation between antioxidant activity and TPC (Adom & Liu, 2002; Shen et al., 2009).

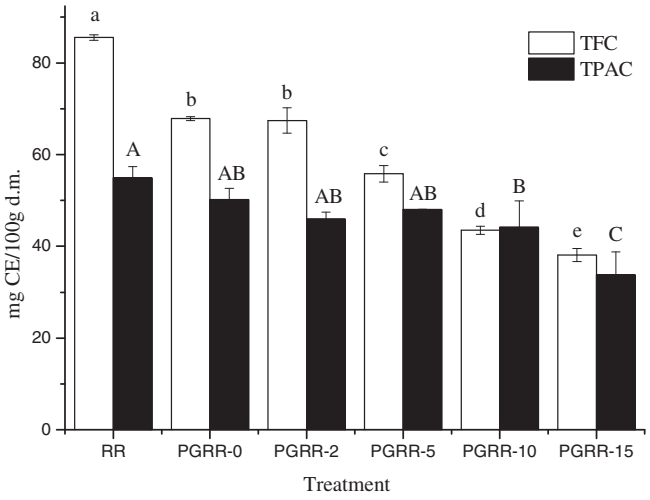

Fig. 1. Total flavonid content and total proanthocyanidin content of germinated red rice at different parboiling time. The results are present as means ± SD (n = 3). TFC and TPAC are expressed as mg CE/100 g dry rice flour (mg CE/100 g). Different lowercase and capital letters above bars are significantly different, respectively ($P < 0.05$). TFC, total flavonid content; TPAC, total proanthocyanidins content; RR, red rice; PGRR-0, germinated red rice parboiled for 0 min; PGRR-2, germinated red rice parboiled for 2 min; PGRR-5, germinated red rice parboiled for 5 min; PGRR-10, germinated red rice parboiled for 10 min; PGRR-15, germinated red rice parboiled for 15 min.

As shown in Table 1, RR had higher free DPPH and ABTS than PGRR-0, but had similar values of bound DPPH and ABTS as PGRR-0. Free fraction of PGRR-5 (37.1) had higher DPPH radical scavenging capacity than that of PGRR-0 (26.7) and PGRR-10 (29.5 μM TE/100 g). Bound fractions of PGRR parboiled for 0–5 min (131.1–133.1) had higher DPPH radical scavenging capacity than that parboiled for 10–15 min (110.6–111.5 μM TE/100 g). Free fractions of PGRR-0 (30.6) and PGRR-2 (24.9) had similar $ABTS^{\cdot+}$ radical scavenging capacity, both of which were significantly ($P < 0.05$) lower than that of PGRR parboiled for 5–15 min (47.9–56.6 μM TE/100 g). Bound $ABTS^{\cdot+}$ radical scavenging capacity of PGRR parboiled for 0–5 min (212.9–233.4) were significantly ($P < 0.05$) higher than that parboiled for 10–15 min (177.5–190.7 μM TE/100 g).

3.2. Total flavonoid content (TFC) and total proanthocyanidin content (TPAC)

The effects of parboiling on TFC and TPAC are shown in Fig. 1. Germination caused significantly decrease of TFC ($P < 0.05$). During parboiling processing, PGRR-0 and PGRR-2 had similar TFC, and

Table 2
Changes of free and bound phenolic acids in germinated red rice during different parboiling time.*

	RR	PGRR-0	PGRR-2	PGRR-5	PGRR-10	PGRR-15
FREE						
GA	0.30 ± 0.15^{a}	0.28 ± 0.06^{a}	nd	nd	nd	nd
PA	0.35 ± 0.01^{a}	0.11 ± 0.01^{d}	0.16 ± 0.00^{c}	0.16 ± 0.00^{c}	0.16 ± 0.00^{c}	0.21 ± 0.01^{b}
p-HA	tr	tr	tr	tr	tr	tr
VA	tr	tr	tr	tr	tr	tr
SA	nd	nd	nd	nd	nd	nd
p-CA	0.18 ± 0.02^{c}	0.09 ± 0.01^{d}	0.19 ± 0.04^{c}	0.20 ± 0.00^{c}	0.48 ± 0.01^{b}	0.67 ± 0.02^{a}
FA	0.20 ± 0.20^{a}	0.15 ± 0.00^{cd}	0.12 ± 0.01^{d}	0.13 ± 0.02^{cd}	0.16 ± 0.00^{bc}	0.18 ± 0.00^{ab}
BOUND						
GA	0.12 ± 0.01^{b}	0.16 ± 0.01^{a}	nd	nd	nd	nd
PA	1.27 ± 0.03^{bc}	1.62 ± 0.06^{a}	1.46 ± 0.18^{abc}	1.51 ± 0.10^{ab}	1.33 ± 0.01^{bc}	1.22 ± 0.09^{c}
p-HA	0.50 ± 0.06^{b}	0.71 ± 0.06^{ab}	0.76 ± 0.05^{ab}	0.85 ± 0.10^{a}	0.62 ± 0.21^{ab}	0.67 ± 0.09^{ab}
VA	0.20 ± 0.00^{cd}	0.22 ± 0.00^{bc}	0.27 ± 0.01^{a}	0.25 ± 0.04^{ab}	0.19 ± 0.01^{cd}	0.17 ± 0.01^{d}
SA	0.18 ± 0.00^{ab}	0.23 ± 0.01^{a}	0.21 ± 0.01^{ab}	0.17 ± 0.05^{b}	0.16 ± 0.03^{b}	0.19 ± 0.01^{ab}
p-CA	7.44 ± 0.31^{b}	8.59 ± 0.14^{a}	7.65 ± 0.26^{b}	7.15 ± 0.16^{b}	6.70 ± 0.16^{c}	6.56 ± 0.23^{c}
FA	13.32 ± 0.72^{ab}	14.32 ± 0.10^{a}	13.43 ± 0.89^{ab}	12.76 ± 0.23^{b}	12.63 ± 0.22^{b}	12.28 ± 0.21^{b}

* The results are present as means ± SD (n = 3) and expressed as mg/100 g dry rice flour (mg/100 g). Values in each row with different letters are significantly different ($P < 0.05$). GA, gallic acid; PA, protocatechuic acid; *p*-HA, *p*-hydroxybenzoic acid; VA, vanillic acid; SA, sinapic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; tr, trace amount; nd, not detected. FREE, free phenolic fraction; BOUND, bound phenolic fraction; RR, red rice; PGRR-0, germinated red rice parboiled for 0 min; PGRR-2, germinated red rice parboiled for 2 min; PGRR-5, germinated red rice parboiled for 5 min; PGRR-10, germinated red rice parboiled for 10 min; PGRR-15, germinated red rice parboiled for 15 min.

then it gradually decreased from the parboiling time of 5–15 min. For TPAC, red rice and germinated red rice parboiled for 0–10 min had similar contents, but significant decrease was observed by parboiling for 15 min ($P < 0.05$). The varying TFC and TPAC at different parboiling time could be attributed to rice husk which was seemed as a barrier and hindered the loss of functional compound, such as flavonoids, proanthocyanidins and polyphenols (Moongngarm & Saetung, 2010).

3.3. Analysis of phenolic acids

The high performance liquid chromatography (HPLC) analysis was used to quantify phenolic acids. The results are presented in Table 2. A total of seven phenolic acids were found in free and bound fractions, such as gallic acid (GA), protocatechuic (PA), *p*-hydroxybenzoic (*p*-HA), vanillic (VA), sinapic (SA), *p*-coumaric (*p*-CA) and ferulic acid (FA). FA and *p*-CA were the most dominant bound phenolic acid among the seven phenolic acids.

For free phenolic acids (Table 2), GA was only detected in red and germinated red rice with similar contents, which might be due to the heat treatment lead to the decomposition of GA. Germination caused lower levels of PA, *p*-CA, and FA, which might be due to the discard of some water-soluble components in soaking treatment (Liang, Han, Nout, & Hamer, 2008). PA of PGRR-15 was significantly higher than that of PGRR-0, PGRR-2, PGRR-5, PGRR-10 with the latter three significantly higher than the former one. *p*-HA and VA were detected in trace amounts, and SA was not detectable in free fractions of all the detected samples. For *p*-CA, parboiling caused an increase from 0.09 (PGRR-0) to 0.67 mg/100 g (PGRR-15). It might be because that heat treatment broke down the ester bond, so the bound *p*-CA was released from the cell walls and became as free phenolic acid. FA contents of PGRR parboiled for 0–15 min was seemed to be at similar levels.

For bound phenolic acids, germination caused higher levels of GA, PA, and *p*-CA, which might be due to the division and multiplication of cells in germinated process (Ti et al., 2014). As shown in Table 2, only red and germinated red rice had detectable GA with the latter (0.16) significantly higher than the former (0.12 mg/100 g). PA of PGRR-0 (1.62) and PGRR-5 (1.51) were significantly higher than that of PGRR-15 (1.22 mg/100 g). Red rice and PGRR parboiled for 0–15 min had similar contents of *p*-HA. For VA, PGRR parboiled for 2–5 min (0.25–0.27) had higher contents than that parboiled for 10–15 min (0.17–0.19 mg/100 g). SA, which was observed only in bound fractions, had higher content in PGRR-0 (0.23) than in PGRR-5 (0.17) and PGRR-10 (0.16 mg/100 g). *p*-CA content of PGRR-0 (8.59) was significantly higher than that of PGRR-2 (7.65), PGRR-5 (7.15), PGRR-10 (6.70), and PGRR-15 (6.56 mg/100 g), with the former two significantly higher than the latter two. FA did not differ significantly at most parboiling time with higher levels in PGRR-0 (14.32 mg/100 g).

3.4. Pasting properties

The pasting characteristics of RR and PGRR are shown in Table 3. It showed that germination could cause decrease of viscosity profiles of red rice, which was similar as previous study (Chinma, Adewuyi, & Abu, 2015). Moreover, it found that parboiling processing could greatly improve the pasting profile of germinated red

Table 3
Pasting properties of germinated red rice at different parboiling time.*

Treatment	Peak viscosity (RVU)	Trough viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)	Peak time (min)	Pasting temperature (°C)
RR	110.4 ± 3.0^{a}	84.6 ± 2.8^{a}	25.8 ± 0.2^{a}	172.7 ± 0.5^{a}	62.3 ± 2.5^{a}	5.8 ± 0.0^{b}	86.3 ± 0.0^{e}
PGRR-0	13.5 ± 0.1^{f}	4.9 ± 0.0^{e}	8.6 ± 0.1^{b}	23.4 ± 0.2^{f}	9.9 ± 0.2^{e}	4.9 ± 0.0^{c}	88.6 ± 0.1^{d}
PGRR-2	73.0 ± 1.0^{b}	71.9 ± 1.0^{b}	1.0 ± 0.0^{c}	128.9 ± 0.3^{b}	55.9 ± 1.2^{b}	7.0 ± 0.0^{a}	92.5 ± 0.1^{c}
PGRR-5	67.4 ± 0.2^{c}	67.4 ± 0.2^{c}	0.0 ± 0.0^{d}	121.3 ± 0.5^{c}	54.0 ± 0.3^{b}	7.0 ± 0.0^{a}	93.3 ± 0.0^{b}
PGRR-10	63.2 ± 1.4^{d}	63.4 ± 1.4^{d}	−0.1 ± 0.0^{d}	111.6 ± 0.1^{d}	48.4 ± 1.5^{c}	7.0 ± 0.0^{a}	93.4 ± 0.0^{b}
PGRR-15	59.1 ± 1.3^{e}	60.6 ± 1.3^{d}	−1.5 ± 0.0^{e}	101.6 ± 0.6^{e}	42.5 ± 1.9^{d}	7.0 ± 0.0^{a}	94.9 ± 0.0^{a}

RR, red rice; PGRR-0, germinated red rice parboiled for 0 min; PGRR-2, germinated red rice parboiled for 2 min; PGRR-5, germinated red rice parboiled for 5 min; PGRR-10, germinated red rice parboiled for 10 min; PGRR-15, germinated red rice parboiled for 15 min.
* The results are present as means ± SD (n = 3). Values in each column with different letters are significantly different (P < 0.05).

Table 4
Color analysis and thermal properties of germinated red rice at different parboiling time.*

	L*	a*	b*	ΔE	T_0 (°C)	T_p (°C)	T_c (°C)	ΔT (T_c–T_0) (°C)	ΔH (J/g)
RR	76.2 ± 0.0^{b}	5.2 ± 0.0^{a}	9.5 ± 0.0^{a}	–	66.6 ± 0.9^{e}	74.9 ± 0.1^{f}	86.3 ± 0.0^{e}	19.7 ± 0.9^{a}	4.7 ± 0.1^{b}
PGRR-0	76.5 ± 0.1^{a}	4.9 ± 0.0^{b}	8.5 ± 0.0^{b}	0.6 ± 0.0^{e}	68.4 ± 0.2^{d}	75.7 ± 0.1^{e}	87.3 ± 0.1^{d}	18.9 ± 0.3^{a}	5.8 ± 0.0^{a}
PGRR-2	75.5 ± 0.1^{c}	4.5 ± 0.1^{d}	8.2 ± 0.1^{d}	1.3 ± 0.1^{d}	72.0 ± 0.5^{c}	78.1 ± 0.2^{d}	88.3 ± 0.2^{c}	16.3 ± 0.8^{b}	3.4 ± 0.0^{c}
PGRR-5	73.9 ± 0.2^{d}	4.7 ± 0.0^{c}	8.1 ± 0.0^{e}	3.7 ± 0.6^{c}	73.0 ± 0.0^{c}	79.5 ± 0.2^{c}	89.2 ± 0.4^{b}	16.3 ± 0.3^{b}	3.3 ± 0.0^{c}
PGRR-10	73.6 ± 0.1^{e}	4.5 ± 0.0^{d}	8.1 ± 0.0^{f}	4.5 ± 0.2^{b}	74.3 ± 0.2^{b}	80.5 ± 0.4^{b}	89.6 ± 0.1^{b}	15.3 ± 0.1^{bc}	2.6 ± 0.3^{d}
PGRR-15	72.1 ± 0.1^{f}	4.7 ± 0.0^{c}	8.3 ± 0.0^{c}	9.1 ± 0.2^{a}	75.9 ± 0.0^{a}	82.3 ± 0.1^{a}	90.6 ± 0.3^{a}	14.8 ± 0.3^{c}	1.9 ± 0.1^{e}

RR, red rice; PGRR-0, germinated red rice parboiled for 0 min; PGRR-2, germinated red rice parboiled for 2 min; PGRR-5, germinated red rice parboiled for 5 min; PGRR-10, germinated red rice parboiled for 10 min; PGRR-15, germinated red rice parboiled for 15 min.

* The results are present as means ± SD (n = 3). Values in each column with different lowercase letters are significantly different ($P < 0.05$).

rice. All of the viscosity parameters increased significantly from 0 to 2 min of parboiling except break down value, and then gradually decreased or remained at similar levels with the increase of parboiling time.

During parboiling process, the gelatinisation and recrystallisation occurred in a starch-water mixture where dispersed amylose chains form double-helical associations of 40–70 glucose units through hydrogen bonding (Jane & Robyt, 1984; Leloup, Colonna, Ring, Roberts, & Wells, 1992). Therefore, the rearrangement and association of the outermost short branches of rice starch granules could result in the increase of pasting profiles of parboiling germinated red rice. With the increase of parboiling time, the viscosity of parboiling germinated red rice gradual decreased. It might be due to the disruption of hydrogen bounds and/or the breaking of covalent linkages of double helices (da Rosa Zavareze & Dias, 2011). Overall, short time of parboiling can improve the viscosity characteristics of germinated red rice with better palatability.

3.5. Color difference, thermal properties and scanning electron microscopy (SEM)

The color parameters of L*, a*, b*, and Δ*E* value, and the thermal properties of T_0, T_p, T_c, Δ*T*, and Δ*H* of all the samples are listed in

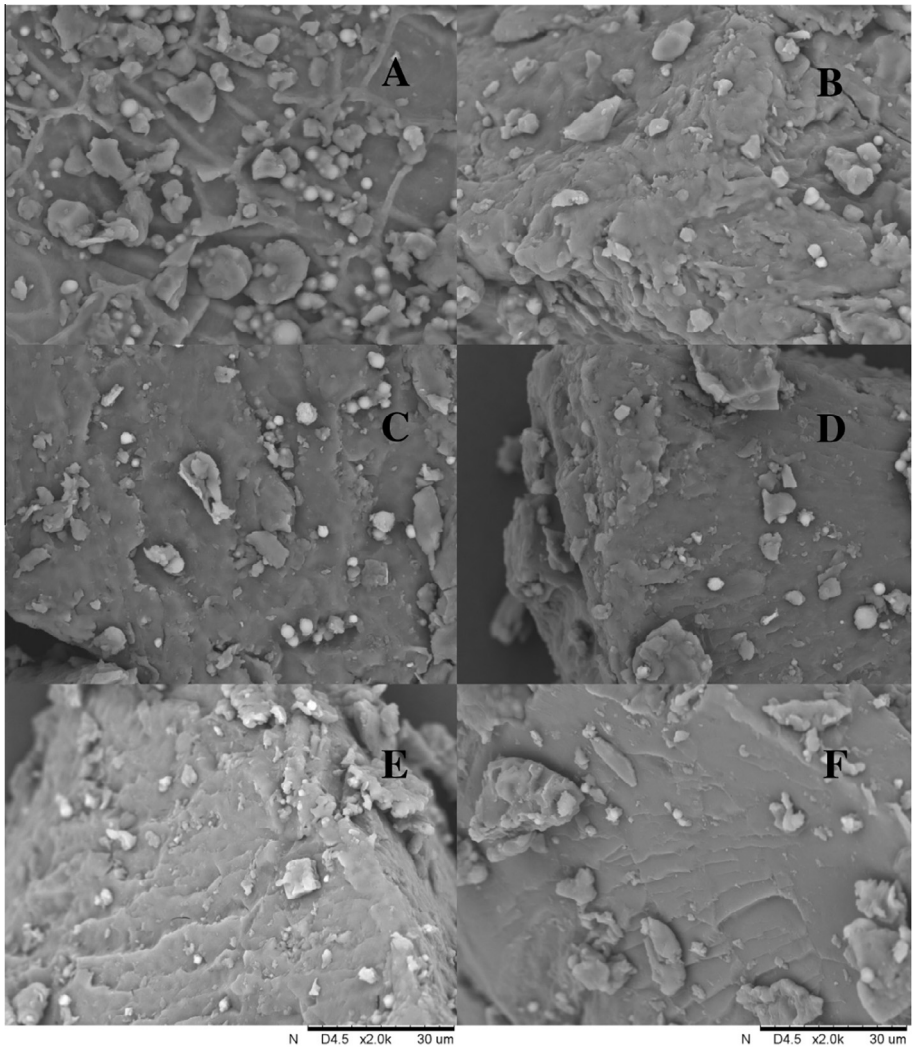

Fig. 2. Scanning electronic micrograph of germinated red rice at different parboiling time. A, red rice; B, germinated red rice parboiled for 0 min; C–F, germinated red rice parboiled for 2, 5, 10 and 15 min, respectively.

Table 4. It showed that PGRR-0 had higher L^*, but lower a^* and b^* value than RR. During different parboiling time, L^* significantly decreased from 76.5 to 72.1, and ΔE significantly increased from 0.6 to 9.1. It might be due to the transformation of pigments from embryo and brown layer to endosperm (Lamberts et al., 2008; Sirisoontaralak, Nakornpanom, Koakietdumrongkul, & Panumaswiwath, 2015).

For thermal properties analysis, germination caused the increase of all the thermal properties except ΔT value. With the increase of parboiling time, T_0, T_p, and T_c gradually increased from 68.4 to 75.9 °C, 75.7 to 82.3 °C and 87.3 to 90.6 °C, respectively, which was similar as the previous study reported by Sui et al. (2015). ΔH gradually decreased from 5.8 to 1.9 J/g indicating that the microstructure of rice starch granule could be destroyed easily after parboiling.

SEMs of germinated red rice at different parboiling time are shown in Fig. 2. In red rice, starch granules embedded in the network structure with a coarse surface. In germinated red rice, the network and the rough surface disappeared, and were replaced by a smooth exterior with a small number of regular starch granules. Parboiling treatment has great effect on microstructure of germinated red rice, as the starch granules become more and more irregular and the surface become smoother and compacter. It reflected that the net structure between protein and starch were destroyed and reconstructed, and internal texture of rice was changed greatly by parboiling process.

4. Conclusion

The above findings on phenolic content, total flavonoids, proanthocyanidins content, antioxidant activity, pasting, color, thermal properties, and scanning electronic micrograph of red rice at five different parboiling time provide some insight into how these parameters are changed in parboiling processing. In red rice, germination caused decreases of free TPC, antioxidant activity, and some phenolic compositions, but increases of bound TPC, antioxidant activity, and some phenolic compositions. Long time of parboiling (>5 min) caused increases of free TPC and ABTS antioxidant activity, but decreases of bound TPC and antioxidant activity. Seven phenolic acids were detected, where gallic acid was only detected in red and germinated red rice. *p*-Hydroxybenzoic and vanillic acid were detected in trace amount in free fraction of all the samples, and sinapic acid was only detected in the bound fractions of all the samples. Phenolic acids were mainly existed in bound form with ferulic and *p*-coumaric acids the most abundant. Germinated red rice parboiled for 2–5 min had higher levels of bound *p*-coumaric and vanillic acid than that parboiled for 10–15 min. TFC decreased significantly during germination and parboiling process, while TPAC kept at similar levels among all the samples except germinated red rice that parboiled for 15 min. Germination and parboiling treatment obviously increased the viscosity and thermal properties of germinated red rice, and decreased heat enthalpy. The increase of color parameter (ΔE) was observed during parboiling process. Scanning electron micrographs visually reflected that internal texture of rice was greatly changed, and the surface structure of rice fraction became more compact and tight. Therefore, germinated red rice parboiled for less than 5 min had higher amount of bound phenolics and better palatability. This study could help rice producers and food scientists to understand the complex changes of phytochemicals, antioxidants and physicochemical properties in germination and at different parboiling time. It would be useful to the food industry if new ways are to be explored in obtaining grains which are rich in health promoting phytochemicals with good palatability.

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