International Food Research Journal 23(4): 1827-1831 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Short Communication

The effect of storage conditions on antioxidant activities and total phenolic contents of parboiled germinated brown rice (Khao Dok Mali 105)

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Article history

Received: 1 July 2015
Received in revised form: 2 February 2016
Accepted: 9 February 2016

Keywords

Antioxidant activities
Parboiled germinated brown rice
Storage conditions
Total phenolic contents

Abstract

Rice *(Oryza sativa)* is a staple crop that has supplied over half of the world's population. Our preliminary data suggested that parboiled germinated brown rice exhibited higher antioxidant activities and total phenolic contents than germinated brown rice, brown rice and white rice, respectively. Nevertheless, the information on the stability of the antioxidant capacities and total phenolic contents in parboiled germinated brown rice is unavailable. Therefore, this research aimed to investigate the effects of storage conditions of parboiled germinated brown Thai Jasmine rice (Khao Dok Mali 105) regarding its antioxidant capacities and total phenolic contents. The determination of antioxidant capacities was employed using ferric reducing antioxidant power (FRAP), oxygen radical antioxidant (ORAC) assays and DPPH (1,1–diphenyl–2–picrylhydrazyl)–radical scavenging assays, while total phenolic content was determined using Folin-Ciocalteu reagent. The results found that the antioxidant activities (FRAP values of 348-371 µmol TE/100 g and ORAC values of 2475-2812 µmol TE/100 g, DPPH values of 62-66 %) and total phenolic contents (62-64 mg GAE/g) had no statistically significant difference under the storage temperatures of 30 and 40°C for 6 months. Since these temperatures are average temperatures in Thailand (30-40°C), rice can be kept for consumption for half a year without any significant changes in anti-oxidative agents and total phenolic contents.

Introduction

Rice *(Oryza sativa)*, a plant developed as food that has become a staple crop, has supplied over half of the world's population in a form of milled rice. Bran layer of rice is a source of nutrients and bioactive compounds; however, it is removed during milling process. To enhance or retain nutrients and other compounds, various rice processing steps were introduced to promote high quality of rice production. Partial milling process in brown rice is able to retain bran and germ layer, thus can maintain more nutrients than milling white rice. Besides, rice germination can initiate chemical and physical modifications with enzyme being activated to create hydrolyzed bioactive compounds that may possess bio–functional activities (Hossain *et al.*, 2004; Patil and Khan, 2011). Moreover, heating and soaking processes with husk of parboiled rice production cause the nutrients to diffuse into rice endosperm (Oli *et al*., 2014). Increase in nutrient compositions and content of bioactive compounds are possibly resulted in enhancement of various potential biological properties.

Rice processing methods such as germination and milling processes have an impact on rice compositions, which may affect on antioxidant content. It was previously found that different types or techniques of rice processing can change amount of antioxidant and also induce physic-chemical change, which contribute to enhance antioxidant activity. During the germination of rice grain, endosperm reserves are rapidly hydrolyzed and degraded by numerous enzymes (i.e., protease, amylase, RNase and R–enzyme). This matter results in an increase of peptides, free amino acids and reducing sugar as well as contribution of new bioactive compounds (Juliano and Palmiano, 1972). The potential antioxidants can be found in form of phenolics such as ferulic acids and p-coumaric acids, which are predominantly found in bran layer of rice (Adom *et al*., 2005). Non-phenolics antioxidant is also found in rice such as vitamin E and oryzanol. Therefore, rice with retained bran or germ layer exhibits higher antioxidant activity than white rice. Besides, different quantity of bioactive compounds was reported in particular type of rice (Tian *et al*., 2006). Antioxidant activity of whole brown rice is similar to those being detected in

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wheat and oat (Tian *et al*., 2006). Interestingly, these antioxidant potentials are even higher than those of some fruits and vegetables such as pear, banana, orange, grapefruit, broccoli and carrot (Liu, 2007).

Unlike germination, little information of parboiling process of rice has been reported. Interestingly, our preliminary results suggested that parboiled germinated brown rice (PGBR) exhibited higher antioxidant activity and total phenolic content (TPC) than those of germinated brown rice (GBR), brown rice (BR) and white rice (WR). These results suggested that bioactive compounds and phenolic contents could be lost due to rice polishing process, while parboiling process is a considerable factor that contributed antioxidants retaining in rice. Migration of bioactive compounds into rice core, heat denaturation of rice cell wall and heat modification of some bioactive compounds might increase amount of antioxidants and TPCs in PGBR.

Due to these properties of PGBR (regarding higher antioxidant activity and TPC than GBR, BR and WR), it is of interest to investigate its storage conditions, including time and temperature, to improve and promote an appropriate post-harvest management. It was previously reported that most antioxidants and phenolic compounds are likely degraded under particular conditions such as extreme pH, time, thermal oxidation and UV exposure (Racine, 1981; Sanhueza *et al*., 2000). Thus, this research was focused on the investigation of storage conditions of parboil germinated brown Thai jasmine rice (Khao Dok Mali 105) in order to examine the time period and temperature to store rice sample regarding its antioxidant capacities and TPCs. This research was conducted by imitating the general conditions of rice packaging in a supermarket and rice storage for consumption. Therefore, the results could be applied in daily life. The information received from this study would provide supportive evidence of post-harvest management to effectively storage PGBR under suitable conditions to prolong its health property.

Materials and Methods

Sample preparation

Thai Jasmine parboiled germinated brown rice (Khao Dok Mali 105) was obtained from RCK Agri Marketing Co., Ltd. (Thailand). The sample was kept in separated clear vacuumed bags under 30 and 40°C incubator for 6 months. The sample was collected every month for analyses. The collected sample was grounded into fine powder by a cyclotex sample mill (series 1903 with 200–240V and 50/60 Hz from FOSS, Höganäs, Sweden). The moisture content was determined using Association of Official Analytical Chemists (AOAC) (930.15, AOAC International, 2005), which was found to be in range of 4–6%. All samples were kept in vacuum bag and stored at –20°C.

Extraction of rice sample

According to our preliminary data for optimized extraction conditions, rice powder (4 g) was extracted with 40% (v/v) aqueous ethanol (20 mL) in a water bath sonicator (model B1510, 40 KHz; Bransonic Bransonic® Ultrasonic, Danbury, CT) for 10 minutes before being shaken in a shaker (Memmert GmbH, Wisconsin, USA) at 100 rpm at 50°C for 2 hours. The mixture was centrifuged at 3000 rpm for 5 minutes. The supernatant was collected and filtered through Whatman No. 1 filter paper (GE Healthcare, Bangkok, Thailand). The filtrate was then kept at 4°C for analysis.

Determination of antioxidant capacity

Antioxidant activity was determined using ferric reducing antioxidant power (FRAP) assays, 1,1–diphenyl–2–picrylhydrazyl–radical scavenging (DPPH) and oxygen radical antioxidant capacity (ORAC). The FRAP assay was determined according to the method of Benzie and Strain, 1996 with some modifications. The FRAP reagent containing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl) and $FeCl_3 \cdot 6H_2O$ solution (20 mM) in a ratio of 10:1:1 was warmed at 37°C before use. The sample (20 µL) was mixed with FRAP reagent (150 µL) and incubated at room temperature (25°C) for 8 minutes. The reaction was monitored at a wavelength of 600 nm using a microplate reader (Synergy HT multi–detection microplate reader, BioTek Instruments, Inc., Winooski, VT) with Gen5 data analysis software. The FRAP values were determined using a standard curve of trolox, a water–soluble analogue of vitamin E, solution (6.25, 12.5, 25, 50, 100, 250, 500 and 1000 µM) and expressed as trolox equivalence (TE) per 1 g dry weight of sample.

The DPPH assay was performed according to the method of Fukumoto and Mazza, 2000 with some modifications as follows. The sample was mixed with DPPH (150 µM) in 95% (v/v) aqueous ethanol and incubated in dark at room temperature (25°C) for 30 minutes. The reaction was determined by measuring the absorbance at 520 nm using the microplate reader. The radical scavenging activity was calculated as a percentage of DPPH discoloration using the equation:

$$\% \text{ Radical scavenging activity} = 100 \times (1-(Abs_{sample} / Abs_{control})),$$

where Abs_{sample} is the absorbance of the sample and DPPH reagent, and $Abs_{control}$ is the absorbance of 95% (v/v) aqueous ethanol and DPPH reagent. Trolox solution (0.08, 0.16, 0.32, 0.64 or 1.28 mM) was used as the standard. The results were expressed as TE per 1 g dry weight of sample.

The ORAC assay was determined according to the method of Ou *et al*. (2001) with some modifications as follows. The sample was mixed with fluorescein (40 nM) solution and incubated for 15 minutes at 37°C. After the incubation, AAPH (153 nM), a peroxyl radical generator, was added to the reaction mixture rapidly to start the reaction. The fluorescence intensity was monitored for 90 minutes in the microplate reader with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. The results were calculated based on the differences in areas under the sodium fluorescein decay curve (AUC) as follows:

$$AUC = 0.5 + f_1/f_0 + f_2/f_0 + f_3/f_0 + \ldots + (0.5)f_i/f_0,$$

where f_0 is the initial fluorescence reading at 0 min, and f_i is the fluorescence reading at time i minutes. Trolox (3.125, 6.25, 12.5, 25, 50 or 100 µM) was used as the standard. The results were expressed as TE per 1 g dry weight of sample.

Determination of total phenolic contents

The TPCs were determined according to the method of Folin–Ciocalteu method, which was adapted from Ainsworth and Gillespie (2007). The sample was mixed with 10% (v/v) Folin–Ciocalteu reagent (50 µL). After 5 minutes of incubation, saturated sodium bicarbonate (7.5% w/v, 200 µL) was added, and the reaction was mixed well. The mixture was then incubated at room temperature (25°C) in dark room for 2 hours. The reaction was measured at a wavelength of 765 nm using the microplate reader. Gallic acid (0–200 µg/mL) was used as a standard. The TPC was expressed in gallic acid equivalents per 1 g dry weight of sample (GAE mg/g DW sample).

Statistical analysis

All experiments were expressed as mean of triplicate assays ± standard deviation (SD). One way analysis of variance (ANOVA) and Tukey's multiple comparison tests were performed to determine the significant differences between values. Significance of difference was defined at $p<0.05$. All statistical analyses were carried out using SPSS statistics version 17 for Windows (SPSS Inc., Chicago, USA).

Results and Discussion

Rice sample was kept in a vacuum bag at 30 and 40°C for 6 months. The storage conditions were designed as to imitate how rice was packed (a clear vacuum bag) and bought by people from a local supermarket. Rice for consumption is normally stored at room temperature (30-40°C in Thailand) in a household for less than 6 months.

According to the results, PGBR exhibited antioxidant activities with FRAP values of 347.5±10 – 371.1±8 µmol TE/100 g, DPPH values of 62.2±1 – 66.3±1% and ORAC values of 2474.5±27 – 2864.3±94 µmol TE/100 g, while its TPCs were within the range of 61.8±1 – 64.9±5 mg GAE/g. It was also observed that there was no statistically significant difference in antioxidant activities and TPCs under the storage temperatures of 30 for 1-6 months (Table 1). Interestingly, no change in antioxidant activities and TPCs of PGBR was also observed at accelerated temperature (40°C), suggesting that PGBR could be kept at ambient temperature (approx. 30-40°C in Thailand) up to 6 months without any significant change in antioxidant activities and TPCs.

It was previously reported that the potential antioxidants in a form of phenolics in rice are ferulic acid, p-coumaric acids protocatechuic acid, hydroxyl benzoic acid, vanillic acid and caffeic acid, either in a free form or a bound form (Adom *et al*., 2005). Particularly, ferulic acid is the most abundant bioactive compound among phenolic acids found in bran layer of rice. Germinated brown rice was found to contain ferulic acid with a recovery yield of 16.67 mg/100 g, followed by p–coumaric acids with a recovery yield of 3.34 mg/100 g (Tian *et al*., 2006). Ferulic acid has been known as an effective antioxidant regarding protection against radiation–induced oxidative reactions and scavenging radicals (Shanthakumar *et al.*, 2012). As well, it functions toward ultraviolet A–mediated matrix metalloprotease–1 that acts as an enzyme responsible for collagen damage (Pluemsamran *et al.*, 2012) and ultraviolet radiation–induced skin damage (Saiji *et al.*, 2000). As for p–coumaric acid, this bioactive compound also possesses antioxidant activity (Zang *et al*., 2000), anti–inflammation (Luceri *et al.*, 2004) and inhibitory activity of platelet aggregation (Luceri *et al*., 2007).

Interestingly, ferulic acid and p-coumaric acid were previously reported as stable antioxidants at ambient temperature (Sohn and Oh, 2003; Salameh *et al*., 2008). The decomposition data revealed that elapsed time to 90% of original ferulic acid powder remains at 25°C was 459 days (Sohn and Oh, 2003).

Table 1. The antioxidant activities and total phenolic contents of parboiled germinated brown rice that was incubated at 30 and 40°C under 0-6 months

Months	DPPH radical scavenging activity[1] (%)		Oxygen free radical scavenging activity [2] (µmol TE/100g)		Ferric reducing capacity activity [3] (µmol TE/100g)		TPCs (mg GAE/g)	
	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C
0	66.1 ± 1^{a}	66.1 ± 1^{a,A}	2812.0 ± 128^{a,A}	2812.0 ± 128^{a,A}	371.1 ± 8^{a,A}	371.1 ± 8^{a,A}	64.0 ± 5^{a,A}	64.0 ± 5^{a,A}
1	66.3 ± 1^{a,A}	64.8 ± 2^{ab,A}	2586.5± 236^{a,A}	2565.4 ± 168^{ab,A}	370.0 ± 19^{a,A}	360.8 ± 6^{a,A}	63.9 ± 4^{a,A}	64.9 ± 5^{a,A}
2	65.1 ± 2^{a,A}	64.3 ± 1^{ab,A}	2690.1 ± 333^{a,A}	2575.5 ± 27^{ab,A}	359.3 ± 11^{a,A}	355.3 ±15^{a,A}	64.9 ± 2^{a,A}	63.2 ± 1^{a,A}
3	64.7± 1^{a,A}	62.7 ± 1^{a,A}	2766.0 ± 89^{a,A}	2764.4 ± 200^{a,A}	358.0 ± 7^{a,A}	359.9 ±6^{a,A}	64.1 ± 3^{a,A}	62.7 ± 5^{a,A}
4	64.0 ± 3^{a,A}	62.9 ± 2^{a,A}	2730.0 ± 104^{a,A}	2864.3 ± 94^{a,A}	348.5 ± 1^{a,A}	347.5 ± 10^{a,A}	62.1 ± 2^{a,A}	62.8 ± 1^{a,A}
5	64.3 ± 1^{a,A}	62.2 ± 1^{a,A}	2703.2 ± 129^{a,A}	2721.7 ± 171^{a,A}	347.6 ± 10^{a,A}	350.5± 8^{a,A}	61.9 ± 4^{a,A}	62.2 ± 1^{a,A}
6	64.9 ± 3^{a,A}	63.7 ± 1^{ab,A}	2764.4 ± 200^{a,A}	2474.5 ± 27^{ab,A}	350.1 ± 8^{a,A}	353.6 ± 10^{a,A}	61.8 ± 1^{a,A}	62.6 ± 2^{a,A}

Each value was represent as mean ± SD (n = 3). Mean within a column in each tested conditions was shown with difference superscript letters, which was significantly different ($P < 0.05$). Small letter indicated statistical analysis within the same column (same incubating temperature but different time periods), and the capital letter indicated the statistical analysis between raw (same time period but different incubating temperatures) of each methods.

[1]DPPH radical scavenging activity was detected by using DPPH assay, [2] oxygen free radical scavenging activity was detected by using ORAC assay, [3]ferric ion chelating capacity activity was detected by using FRAP assay.

Similarly, p-coumaric acid was quite stable at ambient temperature, but it started to degrade once the temperature reached 75°C (Salameh *et al*., 2008). From these data, it was possible that the maintenance of antioxidant activities and TPCs found in PGBR over 1-6 months under 30 and 40°C might be a result of the stability of these bioactive compounds. Thus, PGBR could be appropriately kept at ambient temperature (approx. 30°C in Thailand) up to half a year without any significant change in anti-oxidative agents and TPCs.

Conclusion

Rice sample could be kept in a vacuum bag at ambient temperature in Thailand (30-40°C) for 6 months without any changes in antioxidant activities and total phenolic contents. The information received from this research could provide supportive evidence to promote suitable post-harvest handling regarding the storage temperature and time of Thai jasmine rice that passed through parboiled and germinated processes.

Acknowledgements

Financial support was provided by Agricultural Research Development Agency (ARDA) and National Research Council of Thailand (NRCT).

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