Pertanika J. Sci. & Technol. 27 (3): 2399 - 2408 (2019)

PERTANIKA JOURNALS

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Effect of Parboiling on *in vitro* Physiological Antioxidant Capacity of Brown Rice

Elaine Lee, Phene Neoh Pei Nee, Lee Huei Hong, Wong Sie Chuong, Wong Tze Jin, Koo Lee Feng and Yiu Pang Hung*

Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia, 97000 UPM, Bintulu, Sarawak, Malaysia

ABSTRACT

Parboiling process has been widely implemented in brown rice processing, but its effect on *in vitro* physiological antioxidant capacity of brown rice was not known. In this study, an *in vitro* method simulating the human physiological conditions was used to investigate the effect of parboiling on antioxidant capacity of brown rice in three Bario rice varieties. In this method, bacterial inocula were prepared from rat cecal contents. Results showed that parboiling process gave significant impacts on *in vitro* physiological antioxidant capacity of brown rice. The process improved total phenolic content at small intestine (Adan Halus), DPPH scavenging activity at both small and large intestines (Adan Halus and Bario Merah) and ferrous ion-chelating activity at large intestine (Bario Hitam). However, changes in antioxidant capacity were variety dependent, possibly due to different bran pigmentation. These suggested that parboiling process could improve physiological antioxidant capacity with *in vitro* simulation at small and large intestines by selecting a suitable rice variety and parboiled brown rice could offer good antioxidant properties to maintain physiological health.

Keywords: Bario rice, brown rice, DPPH scavenging activity, ferrous chelating activity, *in vitro* physiological antioxidant capacity, parboiled rice, total phenolic content

ARTICLE INFO

Article history:
Received: 27 August 2018
Accepted: 25 June 2019
Published: 21 October 2019

E-mail addresses:
ylang_329@hotmail.com (Elaine Lee)
phenеneoh@yahoo.com (Phene Neoh Pei Nee)
hueihong24@gmail.com (Lee Huei Hong)
wongsie@upm.edu.my (Wong Sie Chuong)
w.tzejin@upm.edu.my (Wong Tze Jin)
leefeng@upm.edu.my (Koo Lee Feng)
yiuph@upm.edu.my (Yiu Pang Hung)
*Corresponding author

ISSN: 0128-7680
e-ISSN: 2231-8526

INTRODUCTION

Whole grain rice or brown rice is a rich source of antioxidants for human diets. Significant scientific evidence indicates that consumption of whole grain products may reduce the risk for various types of chronic diseases (Liu et al., 2000). Brown rice is rich in phytochemicals such as derivatives of benzoic and cinnamic acids, anthocyanidins, flavonols and phenolic compounds. Some of these phytochemicals, such as ferulic acid, and diferulates are predominantly found in grains but are not present in significant quantities in fruits and vegetables (Bunzel et al., 2001). Due to the escalating health consciousness among the consumers, brown rice is gaining interest for its excellent health beneficial properties.

Nevertheless, the slow water absorption and rapid rancidity of brown rice lead to low acceptance among rice consumers. Parboiling process can strengthen the kernel integrity, prolong the shelf life of rice, shorten the cooking period, and prevent the loss of nutrients and the proliferation of fungus in rice (Bhattacharya, 2004). Besides, parboiling process also increases the nutritional values of the rice by induce nutrients from the bran into the kernel.

Several studies have reported on higher level of antioxidants in parboiled rice than non-parboiled rice (Bhattacharya, 2011; Byungrok et al., 2012). Antioxidant properties are commonly evaluated on solvent extracts of rice samples, which may not reflect the *in vivo* availability of the antioxidants (Serrano et al., 2007). Colonic microbial fermentation may also releases the additional antioxidants which are not possible to be evaluated using solvent extraction method alone (Serrano et al., 2007). Thus, it is important to evaluate the antioxidant release under physiological conditions to stimulate the human digestive system via gastric and intestinal digestion (Etcheverry et al., 2012).

Parboiling process could amend the drawbacks in brown rice and provide better nutritional quality. Brown rice could have different antioxidant capacity at small and large intestines after being parboiled. In this study, parboiling process was first applied on Bario brown rice to compare the *in vitro* physiological antioxidant capacity of non-parboiled and parboiled Bario brown rice at physiological conditions.

MATERIALS AND METHODS

Chemicals

The enzymes used for the digestive enzymatic treatment were pepsin (Acros), pancreatin (Sigma-Aldrich, from porcine pancreas), lipase (Sigma-Aldrich, from porcine pancreas), bile extract (Sigma-Aldrich, bile extract from porcine), α- amylase (Sigma-Aldrich, from *Aspergillus oryzae*), and amyloglucosidase (Fluka, from *Aspergillus niger*). Reagents used for preparing the buffer for digestive treatment were: hydrochloric acid (37%, Merck), potassium chloride (Merck), dipotassium phosphate (Scharlau), potassium phosphate monobasic (Riedel-de Haen), Tris (Vivantis), and maleic acid.

Chemicals for the colonic fermentation medium were ammonium bicarbonate (Merck), sodium bicarbonate (Fluka), disodium phosphate, anhydrous (Merck), monopotassium phosphate, anhydrous (Merck), magnesium sulfate heptahydrate, calcium chloride dehydrate (Merck), manganese (II) chloride tetrahydrate (Merck), cobalt (II) chloride hexahydrate (Merck), iron (III) chloride hexahydrate (Merck), resazurin sodium salt powder (Sigma-Aldrich), dithiothreitol (Merck), sodium sulfide monohydrate (Fisher), lactulose (Sigma-Aldrich), and trypticase peptone (pancreatic digest of casein).

Rice Samples

Three popular rice cultivars (*Oryza sativa* L.) of Sarawak, Malaysia namely Bario rice, Adan Halus, Bario Merah, and Bario Hitam were selected. The paddy (3 – 5 kg) of Bario rice varieties were collected from Bario Highland, Miri, Malaysia, de-husked and stored at -20°C until use (Lee et al., 2015).

Parboiling Treatment

Parboiling treatment at laboratory-scale was conducted according to modified parboiling method from Patindol and colleagues (2008). Full grains of brown rice (300 mg) were soaked in 120 mL water at 60°C for 4 hours. The soaked rice grains were then immersed in water bath at the same temperature for another one hour after the excess water was drained off. Later, the rice grains were autoclaved at 121 °C for 20 min at 15 psi. Finally, the rice grains were dried at 60°C for 45 minutes in an oven. The parboiling treatment was conducted with factorial arrangement in completely randomized design. Brown rice untreated with parboiling process was assigned as a control for comparison.

***In vitro* simulation of physiological conditions**

In vitro physiological digestion and colonic fermentation were conducted according to Serrano et al. (2007).

Digestive Enzymatic Treatment. Parboiled and non-parboiled rice of three rice varieties were first incubated with digestive enzymes in three replications. Briefly, 300 mg of rice grains were crunched and incubated with pepsin (0.2mL of a 300mg / mL solution in HCl-KCl 0.2 M buffer, *pH 1.5, 40* °C, 1 h), pancreatin (1mL of a 5mg /mL solution in phosphate buffer 0.1 M, pH 7.5, 37 °C, 6 h), lipase (2mL of a 7 mg/mL solution in phosphate buffer 0.1 M, pH 7.5, 37 °C, 6 h), bile extract porcine (2mL of a 17.5 mg/mL solution in phosphate buffer 0.1 M, pH 7.5, 37 °C, 6 h) and α-amylase (1mL of a 120mg/mL solution in tris-maleate buffer 0.1 M, pH 6.9, 37 °C, 16 h). After the incubation period, the samples were centrifuged for 15 minutes at 25 °C (3000 g). Upon removal of the supernatants, pellet residues for each sample were washed twice with 5 mL of distilled water and all the

supernatants were combined. The collected supernatants were incubated with 100 µL of amyloglucosidase for 45 minutes at 60 °C. The supernatants were then stored at -20 °C for antioxidant capacity analysis. Blanks containing no substrate and lactulose were included in the experiment for negative and positive control respectively.

Colonic Fermentation. The pellet residues collected were stored in at -20 °C prior to *in vitro* colonic fermentation. Male rats (body weight of 100 ± 5 g) were killed in a carbon dioxide chamber. Fresh rat cecum was collected through the abdominal midline incisions of rat's body (AUP-R049/2013). The rat cecal contents was scraped, weighed and added to a flask containing sterile anaerobic medium to give a 100 g/L inoculum. The anaerobic medium was prepared according to Goering and Van (1970) which contained trypticase, micromineral and macromineral solutions and also the resazurin as anaerobic redox indicator. The indigestible fractions (pellet residues) collected from the digestive enzymatic treatment were mixed with fermentation medium (8 mL, 4 °C, 16 h) followed by addition of 2 mL inoculums. The headspace of test tubes were rinsed with carbon dioxide before sealed with parafilm. The colonic fermentation was conducted at 37°C for 24 hours. Blanks containing no substrate and lactulose were included in the experiment to function as zero and completely fermentable substrate, respectively. After the incubation period, pH values of the samples were measured and neutralized with 1 M NaOH. The test tubes with fermentation mixtures were centrifuged (2500g, 10 min, 25°C). The supernatants were collected and stored at -20°C for antioxidant capacity analysis.

In vitro Physiological Antioxidant Capacity

Antioxidant capacity was measured on the supernatant collected from digestive enzymatic treatment and colonic fermentation. The supernatant collected contains the physiological available fractions of the chemical compounds, which contributes to antioxidant capacity physiologically. Three antioxidant capacity, namely phenolic content, DPPH scavenging activity and ferrous ion chelating activity were measured.

Phenolic Content. Phenolic content was determined from supernatants collected according to Butsat and Siriamornpun (2010). An aliquot of 80 µL supernatant was mixed with 400 µL of freshly prepared Folin-Ciocalteu reagent (0.2 N). Then, 320 µL of sodium carbonate (Na_2CO_3) and 600 µL of ultrapure water were added. The mixtures were then topped up to 1 mL and left to stand for 2 hours at room temperature. Absorbance at 760 nm was measured by using a UV-vis spectrophotometer. Phenolic content was expressed as mg gallic acid equivalent per gram sample.

DPPH Radical Scavenging Activity. DPPH radical scavenging activity of the supernatants was determined according to Bran-Williams et al. (1995). Supernatant (0.5 mL) was

mixed with 2.5 mL of a 0.5 mM methanolic DPPH solution. The mixture was then shaken vigorously and incubated for 30 minutes in the dark at room temperature. The absorbance at 517 nm was measured by using a UV-vis spectrophometer. DPPH free radical-scavenging ability was calculated by using the formula: DPPH scavenging activity (%) = [Absorbance of control-Absorbance of sample / Absorbance of control] ×100.

Ferrous Ion Chelating Activity. Ferrous ion-chelating activity of the supernatants was measured according to Zhao et al. (2008). Supernatant (50 µL) was mixed with 50 µL of ferrous chloride solution. Then, 1.6 mL of 80% methanol was added to the mixture and incubated for 5 minutes at room temperature. Ferrozine (100 µL) was then added and further incubated for 10 minutes at room temperature. The absorbance at 562 nm was measured by using a UV-vis spectrophometer. Ferrous ion-chelating ability was calculated by using the formula: (%) = [1-Absorbance of sample / Absorbance of control] ×100. Disodium EDTA had been used as a standard.

Data Analysis

Data was analyzed statistically using independent t-test to detect the differences of antioxidant capacity between parboiled and non-parboiled rice, and between small and large intestines ($p<0.05$). The statistical software used was Statistical Analysis System (SAS) version 9.3.

RESULTS AND DISCUSSION

Total Phenolic Content

Brown rice or non-parboiled rice of "Adan Halus" and "Bario Hitam" showed higher bioaccessibility of phenolic compounds at large intestine (Table 1). After parboiling treatment, these rice samples showed higher phenolic content at small intestine than the large intestine. The phenolic content of parboiled "Adan Halus" at small intestine increased up to 69.79 % (8.30 ±1.53 to 14.09 ± 0.87 µg gallic acid equivalents/g rice) compared to non-parboiled "Adan Halus". The phenolic content of "Bario Hitam" at small intestine increased 22.05 % compared to non-parboiled rice. Parboiled "Bario Merah" showed 24.83 % loss in phenolic content compared to non-parboiled "Bario Merah". The loss of phenolic content was also accompanied by the change in bran color after parboiling (Figure 1). "Bario Merah" could contained high level of readily soluble or unbound antioxidants which then contributed to the higher extractability of phenolic compounds at small intestine.

The higher concentration of phenolic content in the small intestine shown by "Adan Halus" and "Bario Hitam" indicated the release of bounded compounds from the originally complexes through the parboiling process. Higher concentration of phenolic content at

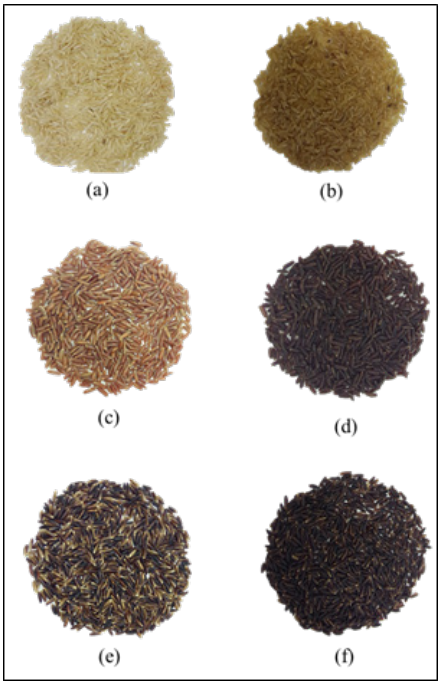

Figure 1. The diagram showed parboiled (right) and non-parboiled (left) rice samples for three rice varieties: "Adan Halus" (a&b), "Bario Merah" (c&d) and "Bario Hitam" (e&f).

small intestine was an important turn up as most of the polyphenols get absorbed in this site (Pandey & Rizvi 2009). Although the high concentration of antioxidants did not guarantee greater absorption of antioxidants at the small intestine, it was possible that the cellular uptake of metabolites is proportional to their unbound concentration. Hence, the possible uptake might enhance the function of antioxidants in human's body in preventing oxidative stress related diseases.

The effect of parboiling on overall phenolic content from small intestine until large intestine was not consistent among rice varieties. Overall phenolic content of "Adan Halus" and "Bario Hitam" increased after parboiling but not in "Bario Merah". The increment in overall phenolic content of parboiled rice was in congruence to previous study which reported on the release of phenolic compounds which were once bounded to the cell wall (Min 2006). However, different phenolics profiles were expected in "Adan Halus", "Bario Hitam" and "Bario Merah". Thermal energy in the presence of oxygen and moisture would accelerate the oxidative degradation of phenolic compounds and led to destruction of some phenolic compounds specifically present in Bario Merah. This could explained the lower overall phenolic content of "Bario Merah", which may due to the possible loss of proanthocyanidins with low degree polymerization (Min, 2006; Awika et al., 2003).

DPPH Scavenging Activity

DPPH scavenging activity of brown rice or non-parboiled rice of three rice varieties was higher in small intestine than large intestine (Table 2). After parboiling, DPPH scavenging activity was increased at both small and large intestine following the same trend of higher DPPH scavenging activity in small intestine. The increment of DPPH radical scavenging activity in all rice varieties was probably caused by detachment of the bounded antioxidants other than phenolic compounds from the cell wall after the thermal activity in parboiling process. These could be tocopherols, tocotrienols and γ-oryzanol (Iqbal et al., 2005). The increment of DPPH scavenging activity of brown rice after parboiled in

Table 1

Total phenolic content of parboiled and non-parboiled Bario rice varieties.

	Adan Halus		Bario Merah		Bario Hitam	
	Non-parboiled	Parboiled	Non-parboiled	Parboiled	Non-parboiled	Parboiled
Small intestine	8.30 ± 1.53y,b	14.09 ± 0.87x,a	18.02 ± 0.64x,a	13.54 ± 1.63x,b	13.00 ± 0.80y,a	16.74 ± 1.20x,a
Large intestine	5.06 ± 0.20x,a	4.56 ± 0.39y,b	4.96 ± 0.26y,a	4.31 ± 0.21y,b	5.28 ± 0.24x,a	4.47 ± 0.19y,a
Overall	13.35± 1.37	18.65 ± 0.54	22.97±0.40	17.85±1.50	18.28±0.85	21.22±1.20

Note.

1. Mean value ± standard deviation, n=3
2. Mean values followed by different letters of x and y within column show significant difference between small intestine and large intestine at $p<0.05$
3. Mean values followed by different letters of a and b within row show significant difference between non-parboiled and parboiled treatments of same variety at $p<0.05$

Table 2

DPPH scavenging activity of parboiled and non-parboiled Bario rice varieties.

	Adan Halus		Bario Merah		Bario Hitam	
	Non-parboiled	Parboiled	Non-parboiled	Parboiled	Non-parboiled	Parboiled
Small intestine	24.11 ± 0.72x,b	26.96 ± 1.76x,a	20.76 ± 3.58x,b	24.89 ± 2.34x,a	34.88 ± 2.68x,a	40.78 ± 1.80x,a
Large intestine	14.03 ± 2.15y,b	16.63 ± 1.45y,a	10.21 ± 0.62y,b	24.10 ± 1.84y,a	10.43 ± 2.68y,a	9.27 ± 0.43y,a
Overall	19.07±1.12	21.80±1.54	15.49±1.93	24.50±1.88	22.65±1.98	25.02±1.08

Note.

1. Mean value ± standard deviation, n=3
2. Mean values followed by different letters of x and y within column show significant difference between small intestine and large intestine at $p<0.05$
3. Mean values followed by different letters of a and b within row show significant difference between non-parboiled and parboiled treatments of same variety at $p<0.05$

Table 3

Ferrous chelating activity of parboiled and non-parboiled Bario rice varieties.

	Adan Halus		Bario Merah		Bario Hitam	
	Non-parboiled	Parboiled	Non-parboiled	Parboiled	Non-parboiled	Parboiled
Small intestine	19.79 ± 7.96y,a	N.D y,a	N.D. y,a	N.D. y,a	16.82 ± 4.13x,b	19.54 ± 5.51y,a
Large intestine	79.61 ± 38.94x,a	92.85 ± 12.39x,a	94.40 ± 3.74x,a	97.11 ± 3.40x,a	15.77 ± 11.39x,b	96.05 ± 6.85x,a
Overall	56.31±5.28	46.42±6.20	47.20±1.87	48.55±1.70	16.29±7.22	57.79±4.63

Note.

1. Mean value ± standard deviation, n=3
2. Mean values followed by different letters of x and y within column show significant difference between small intestine and large intestine at $p<0.05$
3. Mean values followed by different letters of a and b within row show significant difference between non-parboiled and parboiled treatments of same variety at $p<0.05$
4. N.D. indicates non detectable

small and large intestines possess benefits for optimal antioxidant absorption and also maintenance of colon health. The free antioxidant compounds might counteract the pro-oxidant or toxic species produced during colonic bacterial metabolism (Serrano et al., 2007).

In general, parboiling treatment gave similar increment trend of DPPH scavenging activity on all rice varieties but at different magnitude. DPPH radical-scavenging activity of "Adan Halus" (5.45 %) and "Bario Merah" (18.02 %) increased significantly after parboiling, except "Bario Hitam". The increases in DPPH scavenging activity could be explained by release of antioxidant compounds from parboiling treatment (Brighente et al., 2007). However, there's no changes in "Bario Hitam" possibly due to the loss of anthocyanin during parboiling with observed discoloration of the grains. The loss of DPPH scavenging activity due to loss of anthocyanin might have been compensated with the release of other antioxidant compounds after parboiling, and resulted in no significant difference between parboiled and non-parboiled rice for "Bario Hitam".

Ferrous Chelating Activity

Ferrous chelating activity of brown rice or non-parboiled rice of three rice varieties was higher in large intestine than small intestine (Table 3). The ferrous chelating activity of parboiled rice at the large intestine was 92.85 ± 12.39 %, 97.11 ± 3.40 % and 96.05 ± 6.85 % for "Adan

Halus", "Bario Merah" and "Bario Hitam", respectively. This was most probably caused by the low stability of chelating agents in the low pH conditions before enter small intestine (Moon & Shibamoto, 2009). Ferrous ion-chelating activity detected in the small intestine is therefore lesser than the activity in large intestine.

After parboiling treatment, ferrous chelating activity was increased at both small and large intestine only in "Bario Hitam". The chelating activity of parboiled "Bario Hitam" showed significantly higher values than the non-parboiled "Bario Hitam" in the large intestine with the increment of approximately 80.28%. Besides, "Bario Hitam" could contain bounded antioxidants with catechol and galloyl groups (Perron & Brumaghim, 2009). Parboiling process successfully released these antioxidants and contribute to higher ferrous chelating activity in parboiled "Bario Hitam".

CONCLUSIONS

Parboiling treatment significantly affected the *in vivo* antioxidant capacity of Bario rice varieties base on the current simulation study. The effect of parboiling was variety dependent due to the differences in antioxidants composition. Parboiled rice showed improved phenolic content at small intestine ("Adan Halus"), DPPH scavenging activity at both small and large intestines ("Adan Halus" and "Bario Merah") and ferrous ion-chelating activity at large intestine ("Bario Hitam"). The study indicated that antioxidant capacity from parboiling process is varied across rice varieties.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the research fund given by Universiti Putra Malaysia, Malaysia (9589200) and generous assistance from the Sarawak Department of Agriculture.

REFERENCES

Awika, J. M., Dykes, L., Gu, L., Rooney, L. W., & Prior, R. L. (2003). Processing of sorghum and sorghum products alters procyanidin oligomer and polymer distribution and content. *Journal of Agricultural and Food Chemistry, 51*(18), 5516-5521.

Bhattacharya, K. R. (2004). Parboiling of rice. In E. T. Champagne (Ed.), *Rice: chemistry and technology* (pp. 329-404). Louisiana, United States: Department of Agriculture Research Service, Southern Regional Research Center.

Bhattacharya, K. R. (2011). *Rice quality: A guide to rice properties and analysis*. Cambridge, United Kingdom: Woodhead Publishing Ltd.

Bran-William, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology, 28*, 25-30.

Brighente, I. M. C., Dias, M., Verdi, L. G., & Pizzolatti, M. G. (2007). Antioxidant activity and total phenolic content of some Brazilian species. *Pharmaceutical Biology, 45*(2), 156-61.

Bunzel, M., Ralph, J., Marita, J. M., Hatfield, R. D., & Steinhar, H. (2001). Diferulates as structural components in soluble and insoluble cereal dietary fibre. *Journal of the Science of Food and Agriculture, 81*, 653-660.

Butsat, S., & Siriamornpun, S. (2010). Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chemistry, 119*, 606-613.

Byungrok, M., Gu, L. W., Anna, M. M., Christine, J. B., & Chen, M. H. (2012). Free and bound total phenolic concentrations, antioxidant capacities, and profiles of proanthocyanidins and anthocyanins in whole grain rice (*Oryza sativa* L.) of different bran colours. *Food Chemistry, 133*, 715-722.

Etcheverry, P., Grusak, M. A., & Fleige, L. E. (2012). Application of *in vitro* bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Front Physiology, 3*, 1-21.

Goering, H. K., & Van, S. (1970). *Forage fiber analyses (Apparatus, reagents, procedures and some applications): Agriculture handbook No.379 Agricultural Research Service.* Washington DC, United States: Department of Agriculture.

Iqbal, M. J., Yaegashi, S., Ahsan, R., Shopinski, K. L., & Lightfoot, D. A. (2005). Root response to *Fusarium solani* f. sp. *glycines*: temporal accumulation of transcripts in partially resistant and susceptible soybean. *Theoretical and Applied Genetics, 110*, 1429-1438.

Lee, H. H., Loh, S. P., Bong, C. F. J., Sarbini, S. R., & Yiu, P. H. (2015). Impact of phytic acid on nutrient bioaccessibility and antioxidant properties of dehusked rice. *Journal of Food Science and Technology, 52*(12), 7806-7816.

Liu, S., Manson, J. E., Stampfer, M. J., Hu, F. B., Giovannucci, E., Colditz, G. A., ... & Willett, W. C. (2000). A prospective study of whole grain intake and risk of type 2 diabetes mellitus in U.S. women. *American Journal of Public Health, 90*, 1409-1415.

Min, B. (2006). Effects of hydrothermal processes on antioxidants in brown , purple and red bran whole grain rice *(Oryza sativa* L.). *Food Chemistry, 159*, 106-150.

Moon, J. K., & Shibamoto, T. (2009). Antioxidant assays for plant and food components. *Journal of Agricultural and Food Chemistry, 57*(5), 1655-1666.

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity, 2*(5), 270-278.

Patindol, J., Newton, J., & Wang, Y. J. (2008). Functional properties as affected by laboratory-scale parboiling of rough rice and brown rice. *Journal of Food Science, 73*, 370-377.

Perron, N. R., & Brumaghim, J. L. (2009). A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochemistry and Biophysics, 53*(2), 75-100.

Serrano, J., Goñi, I., & Saura-Calixto, F. (2007). Food antioxidant capacity determined by chemical methods may underestimate the physiological antioxidant capacity. *Food Research International, 40*(1), 15-21.

Zhao, H., Fan, W., Dong, J., Lu, J., Chen, J., & Shan, L. (2008). Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chemistry, 107*, 296-304.