

Research Article

Effects of germinating conditions on antioxidant properties, total polyphenol and phytate contents in quick-cooking husked *Hom Dam* Sukhothai 2 rice

Vareeporn Pitiwiwattanakul¹, SuthayaPhimphilai^{1*}, Surat Nuglor¹ and Chiyawat Chiyasut²

¹Faculty of Engineering and Agro-industry, Maejo University, Chiang Mai 50290, Thailand.

²Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.

*Email: suthaya@mju.ac.th

Abstract

Germinated husked-rice (brown rice) is an innovative food product as it contains an active substance for protecting against Alzheimer's symptoms in humans. However, longer soaking time in the germination process sometimes causes fermentation and unpleasant flavour. In combination, quick-cooking process of the rice facilitated a short cooking time but possibly affected the unique antioxidant properties. This study was aimed to investigate effects of germinating conditions and quick-cooking process on physical and chemical properties of husked coloured rice, *Hom Dam* Sukhothai 2, as well as antioxidant properties, polyphenol and phytate contents.

Husked coloured rice of *Hom Dam* Sukhothai 2 was germinated under three conditions; soaking in water (So), water soaking in combination with water spraying (SS) and water spraying (Sp) for a total time of 10-30 hrs. All conditions were controlled under 30±0.5°C and 35±0.5°C. Under assigned temperatures, the rice samples (SS and Sp) were germinated within 10 hrs. The best condition for a quick-cooking germinated rice product was when a heat treatment (121°C for 0.5 min) and drying process in a hot air oven (84°C for 130 min) were applied.

Soaking the rice under the germinating time period (So) caused lower pH in soaking water with unpleasant odour. However, qualities of germinated rice under a combination of water soaking and water spraying (SS) as well as water spraying (Sp) were qualified. Lower antioxidant properties, total polyphenol and phytate contents were found by comparing to the raw material (p<0.05). The rice germinated under 35±0.5°C showed higher antioxidant properties than that under 30±0.5°C (p<0.05). A quick-cooking process caused a reduction in antioxidant properties and polyphenol content.

The rice germination and a quick-cooking process caused a reduction in antioxidant properties and polyphenol content. However, water spraying is an alternative method for quality control of the rice during germination. The technique can be used in combination with or without an optimal traditional soaking time.

Keywords: germinated rice, phytate, polyphenol, antioxidant, soaking, *Oryza sativa*, Thailand.

Introduction

Brown rice (*Oryza sativa* L.) has higher nutritional components especially dietary fibre, vitamins, phenolic compounds and antioxidants than ordinary white rice grain [1, 2]. These bio-functional components exist mainly in the germ and bran layers. Brown rice, however, is not considered as a suitable table rice because of its dark appearance and hard texture. Many studies found that the germination of rice grain (Germinated brown rice; GBR), by soaking brown rice grain in warm water for 24 hr, increased the bio-functional components due to activated hydrolytic enzymes and softer texture [3], including the lowest phytate content [4].

γ -aminobutyric acid (GABA) is a free amino acid widely distributed in nature, and is a neurotransmitter in the brain and spinal cord of mammals [5]. Decarboxylation of L-glutamic acid catalyzed by the glutamate decarboxylase (GAD) enzyme is a major mechanism [6].

Phytic acids (PA) are an antinutritional factor as they inhibit availability of divalent cations such as Fe^{2+} , Ca^{2+} , Mg^{2+} and Zn^{2+} . The phosphate groups of phytic acid (inositol-hexakisphosphate; IP6) possibly form stable complexes with such cations, preventing bioavailability of minerals from food [7, 8, 9, 10].

Quick-cooking rice (QCR) is a pre-cooked and gelatinized product using water and steam to hydrate 60-75% moisture content to the rice. The cooked rice is partially dried to porous grains and open-structure. For rehydration, boiling water was added resulting in ready-to-eat rice in a short time period [11].

Techniques in rice germination as well as a quick-cooking process possibly affect properties of the brown rice. This study was aimed to verify germination techniques on the rice qualities and investigate effects of the quick-cooking process on physical and chemical properties, as well as antioxidant properties, polyphenol and phytate contents of germinated brown rice.

Materials and Methods

Materials

Brown rice grain (not exceeding 2 weeks after milling process) cultivars of *Khao Hom Dam* Sukhothai 2, were obtained from Natural Rice Co., Ltd., Sukhothai Province.

Methods

Optimal moisture content for brown rice germination

Brown rice grain was thoroughly washed in drinking water for 5 min and drained for an extra 5 min. The rice sample was divided into two portions then subjected to different water uptake techniques; soaking in water and water spraying, under $30\pm 0.5^\circ\text{C}$ for 18 hrs. The sample was collected during the process for moisture content analysis [12].

Germination of brown rice

Brown rice grain was thoroughly washed in drinking water for 5 min and drained for an extra 5 min. The rice sample was divided into three portions then subjected to different water uptake techniques for 30 hrs; soaking in water (So), soaking in water and water spraying (SS) and water spraying (Sp). The chamber temperature was controlled at $30\pm 0.5^\circ\text{C}$ and $35\pm 0.5^\circ\text{C}$. The rice samples were collected in hermetically sealed containers and stored at -20°C before analysis.

Determination of antioxidant activity (AOA)

Germinated rice samples (5 g) were extracted with 50 ml of 95% ethanol, in a continuous shaker for 12 hrs at room temperature. Samples extract were filtered and stored at -20°C before analysis.

ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) assay

Radical scavenging activity was determined using the method of Re *et al.* [13] with some modifications. In brief, the ABTS^{•+} radical cation was generated by the interaction of ABTS (7 mM, 5 ml) and K₂H₂O₈ (2.45 mM, 7.5 ml). The mixture was incubated at room temperature for 12 hours to give a dark green solution. Before analysis, the solution was diluted with distilled water to reach the absorbance of 0.7-0.9 at 730 nm using a UV-VIS Spectrophotometer (Perkin Elmer, Germany). For measurement, 2 ml of ABTS^{•+} solution was mixed with 20 µl of the extracts and incubated at room temperature for 3 min then measured an absorbance at 730 nm. Trolox solution was used to generate a standard line and activity was reported as trolox equivalent antioxidant capacity in mg/1g dry matter (DM) (mg TEAC/1g DM)

FRAP (Ferric reducing ability power) assay

The ferric reducing property of the extracts was determined using the method of Benzie and Strain [14], with some modifications. In brief, 1.2 ml of FRAP reagent (300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, 20 mM FeCl₃•6 H₂O, ratio 10:1:1) was mixed with 40 µl of the extracts and incubated at room temperature for 4 min then measured for absorbance at 593 nm. FeSO₄ solution was used to generate a standard line. Results were expressed as FeSO₄ equivalent in mg /1 g DM.

Determination of total polyphenol contents (TPC)

Fresh and dried rice samples, 1 g DM, were refluxed with 95% ethanol (50 ml) at 80°C for 1 hour. Each extracted sample was filtered through a filter paper then adjusted to 50 ml before storage under -20°C until analysis.

The TPC was determined using the method of Pinsirodom and Changnoi [15], with some modifications. In brief, 500 µl of the extracts, 9.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent were thoroughly mixed in a test tube and incubated at room temperature for 5 min. Two millilitres of 10% (w/v) sodium bicarbonate was added and incubated for 10 min before an absorbance measurement at 734 nm. Gallic acid solution was used to generate a standard line. Results were reported as gallic acid equivalent in mg/1 g DM (mg GAE/1g DM).

Phytate content (PC)

The PC was determined using the method of Reddy, *et al.* [16], with some modifications. The rice samples, 0.3-0.5 g DM, were extracted in 6 ml HCl (0.5 M) in a shaker bath at room temperature for 12 hrs. Sample extracts were centrifuged for 15 min. Supernatant was digested with Ceric solution (5% (w/v), 2 ml) and 0.8 ml conc. H₂SO₄ then centrifuged for 15 min. The pellet was digested with 3 ml conc. H₂SO₄ at 330°C for 150 min. The extracts were adjusted to a volume of 50 ml. Each sample extract was mixed with reagent solution and incubated at room temperature before mixing with Malachite green solution. Optical density at 610 nm was recorded. Phosphorus solution was used to generate the standard line. Results were reported as potassium dihydrogen phosphate (KH₂PO₄) equivalent in mg/100 g DM (mg KDP/100g DM).

Quick-cooking process for germinated rice

The germinated rice was subjected to a quick-cooking process as reported in a study by Raweewat [17]. Heat treatment was conducted under 121°C 15 psi for 0.5 min before drying in a hot air oven at 84°C for 130 min. The quick-cooking germinated rice was stored at -20°C until analysis.

Statistical analysis

The data were analyzed using ANOVA (SPSS version 16.0 for Windows) with Duncan's new multiple range test to differentiate the means at $\alpha=0.05$.

Results and Discussion

Optimal moisture content for brown rice germination

Moisture content (MC) of soaked brown rice was as shown in Figure 1. The MC in rice samples rapidly increased to 35-40% after 5-7 hrs in water soaking and spraying under $30\pm 0.5^\circ\text{C}$. The MC level was adequate for germination of the rice. No significant difference was found between soaking and spraying at the same temperature ($p>0.05$). Spraying is an optional technique for moisture uptake in germinated rice process. Diffusivity of water in rice increased with temperature [18]. Higher temperature also possibly leads to higher water diffusivity into rice.

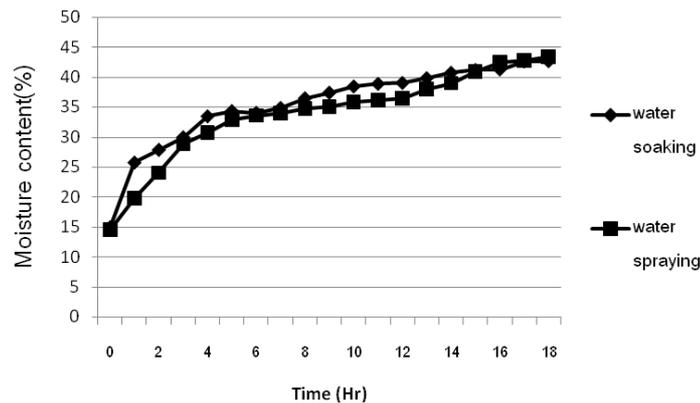


Figure 1. Moisture content of *Hom Dam Sukhothai 2* brown rice during water soaking and spraying under $30\pm 0.5^\circ\text{C}$.

Effect of germination on AOA, TPC, PC and quality of brown rice

AOA (TEAC, FRAP) in raw material were 73.10mg TEAC/100 g DM, 51.37mg FeSO₄/100 g DM, respectively, with TPC of 242mg GAE/100 g DM (Table 1). During the germination process, AOA and TPC in the rice samples decreased compared to raw material (Table 2). However, condition factors (time, temperature and water uptake technique) affected the AOA and TPC. The maximum AOA were found in the rice samples processed under the spraying technique at $35\pm 0.5^\circ\text{C}$ 20 hrs. Process temperature under $30\pm 0.5^\circ\text{C}$ tended to maintain higher TPC in the rice than that under $35\pm 0.5^\circ\text{C}$. The decrease of AOA and TPC is possibly due to germination conditions and type of phenolic compounds [19]. Tian *et al* [20], reported that major phenolic compounds in rice grain were mainly in a water soluble form. Spraying technique could dissolve more compounds into excess drained water than soaking. Germination of the brown rice at $35\pm 0.5^\circ\text{C}$ for 20 hrs resulted in 0.5-2 mm rice sprouts. Suwananon and Jaimyangyeun [21], reported that antioxidant properties in rice were significantly increased within 18 hrs of germination under $30-40^\circ\text{C}$.

PC in raw material of the studied brown rice was 2031mg KDP/100 g DM (Table 1). This particular component decreased during germination (Table 2). The lowest PC in the brown rice was found at 20 hrs germination under $35\pm 0.5^\circ\text{C}$ with the spraying technique. The results supported a study of Jianfen *et al* [22] as a reduction of PC progressed 4-60% with increased germination period at $30-35^\circ\text{C}$.

The rice water uptake technique was an important factor affecting quality of the germinated brown rice. Traditional germinated brown rice processed under a long soaking period was easily contaminated by microorganisms. Starch leakage from the rice kernel could be a crucial source for fermentation resulting in off-flavour and unpleasant products.

Table 1. TEAC, FRAP, TPC and PC in raw material of *Hom Dam Sukhothai 2* brown rice.

	Antioxidant activity (AOA)		Total polyphenol content, TPC (GAE/100 g DM)	Phytate content, PC (KDP/100 g DM)
	ABTS ⁺ (mg TEAC/100 g DM)	FRAP (mg FeSO ₄ /100 g DM)		
<i>Hom Dam Sukhothai 2</i>	73.10±2.30	51.37±14.00	242.58±63.00	2031±151

Values are mean±SD (n=3).

Table 2. TEAC, FRAP, TPC and PC in germinated brown rice at different germinating conditions.

Time (hrs)	Temperature (°C)	Water uptake technique	Antioxidant activity (AOA)		Total polyphenol content, TPC(GAE/100 g DM)	Phytate content, PC (KDP/100 g DM)
			ABTS ⁺ (mg TEAC/100 g DM)	FRAP (mg FeSO ₄ /100 g DM)		
10	30	1	18.90±0.02 ^f	30.45±0.01 ^b	138.45±0.05 ^m	2000±51 ^j
		2	25.89±0.02 ^g	30.13±0.02 ^b	101.85±0.03 ^h	2007±47 ^j
		3	29.42±0.02 ^h	44.20±0.02 ^f	137.01±0.01 ^{lm}	939±65 ^g
	35	1	54.19±0.03 ^j	60.52±0.01 ^l	70.47±0.05 ^d	428±50 ^d
		2	62.10±0.02 ^k	58.27±0.02 ^{jk}	81.26±0.05 ^{ef}	430±65 ^d
		3	69.90±0.04 ^l	58.35±0.03 ^{jk}	57.11±0.03 ^a	137±113 ^b
15	30	1	59.90±0.04 ^k	56.30±0.01 ⁱ	97.73±0.04 ^h	1961±172 ^j
		2	52.84±0.02 ^j	52.12±0.02 ^h	94.35±0.02 ^g	1853±273 ^j
		3	51.95±0.02 ^j	51.74±0.02 ^h	100.65±0.01 ^h	1963±132 ^j
	35	1	59.55±0.02 ^k	51.73±0.03 ^h	102.13±0.03 ^h	2051±181 ^j
		2	61.37±0.02 ^k	48.14±0.02 ^g	101.06±0.05 ^h	1715±224 ^j
		3	50.07±0.02 ^j	55.46±0.02 ^{ji}	113.28±0.04 ^{hi}	1841±128 ^j
20	30	1	17.71±0.02 ^{ef}	39.98±0.02 ^e	161.76±0.15 ⁿ	2769±85 ^l
		2	23.81±0.05 ^g	40.64±0.02 ^e	163.63±0.04 ⁿ	2646±70 ^k
		3	38.45±0.02 ⁱ	53.52±0.03 ^h	120.89±0.05 ^{ij}	938±119 ^g
	35	1	61.56±0.02 ^k	66.14±0.04 ⁿ	136.31±0.03 ^{kim}	294±84 ^c
		2	68.39±0.03 ^l	76.21±0.03 ^o	131.48±0.04 ^k	244±59 ^c
		3	89.67±0.03 ^m	84.37±0.02 ^p	116.66±0.02 ⁱ	43±40 ^a
25	30	1	14.18±0.02 ^{cd}	31.57±0.01 ^{bc}	135.38±0.02 ^{kim}	2777±128 ^l
		2	24.28±0.05 ^g	35.71±0.01 ^d	138.35±0.04 ^m	2696±77 ^k
		3	29.18±0.03 ^h	53.06±0.01 ^h	95.53±0.02 ^g	786±119 ^f
	35	1	13.77±0.03 ^{cd}	63.84±0.03 ^m	85.45±0.03 ^f	990±82 ^g
		2	11.98±0.03 ^c	62.86±0.02 ^m	84.13±0.04 ^{ef}	853±81 ^f
		3	87.15±0.03 ^m	55.86±0.01 ⁱ	73.05±0.05 ^d	676±58 ^e
30	30	1	15.53±0.03 ^{de}	29.78±0.02 ^b	132.04±0.05 ^{kim}	2830±63 ^l
		2	15.06±0.03 ^{de}	27.77±0.02 ^a	123.87±0.02 ^j	2777±84 ^l
		3	23.89±0.03 ^g	32.65±0.02 ^c	116.66±0.03 ⁱ	805±135 ^f
	35	1	4.14±0.02 ^a	59.88±0.01 ^{kl}	64.27±0.04 ^{bc}	1119±53 ^h
		2	4.60±0.03 ^a	56.80±0.02 ^{ij}	68.49±0.04 ^c	1248±70 ^j
		3	8.70±0.02 ^b	47.29±0.03 ^g	61.04±0.05 ^{ab}	953±112 ^g

Values are mean±SD (n=12).

^{a-p} Values within columns with different letters are significant difference (p<0.05).

So=Soaking in water, SS=soaking in water and water spraying, Sp=water spraying.

A combination of a short water soaking period and water spraying (SS) as well as water spraying (Sp) could be used as alternative methods for quality control of the rice during germination.

Effect of quick-cooking process on AOA, TPC and PC of germinated brown rice

AOA, TPC and PC of quick-cooking germinated brown rice are shown in Table 3. AOA and TPC in quick-cooking germinated brown rice were lower than that in raw material and traditional quick-cooked brown rice [19].

Table 3. TEAC, FRAP, TPC and PC in quick-cooking germinated brown rice at different germinating conditions and quick-cooking process.

Process	Water uptake technique	Antioxidant activity (AOA)		Total polyphenol content, TPC (GAE/100 g DM)	Phytate content, PC (KDP/100 g DM)
		ABTS ⁺ (mg TEAC/100 g DM)	FRAP (mg FeSO ₄ /100 g DM)		
After heat treatment	So	65.89±2.60 ^b	12.58±1.34 ^a	73.2±3.10 ^b	686±50 ^c
	SS	63.07±2.90 ^a	14.00±1.40 ^b	52.20±3.44 ^a	609±41 ^b
	Sp	69.22±3.05 ^c	19.25±1.00 ^c	50.43±4.20 ^a	485±51 ^a
After drying process	So	40.44±1.40 ^c	10.00±1.10 ^b	54.64±2.60 ^b	458±43 ^b
	SS	34.5±1.20 ^a	4.6±1.20 ^a	44.21±3.00 ^a	451±88 ^b
	Sp	36.03±1.40 ^b	4.7±1.30 ^a	62.75±4.50 ^c	273±52 ^a

Values are mean±SD (n=12).

^{a-c} Values within columns with difference letters are significant difference (p<0.05).

So=Soaking in water, SS=soaking in water and water spraying, Sp=water spraying.

Table 4. Reduction (% loss) of TEAC, FRAP, TPC and PC in germinated brown rice at different germinating conditions and quick-cooking processes compared to raw material.

Process	Water uptake technique	Antioxidant activity (AOA)		Total polyphenol content, TPC (GAE/100 g DM)	Phytate content, PC (KDP/100 g DM)
		ABTS ⁺ (mg TEAC/100 g DM)	FRAP (mg FeSO ₄ /100 g DM)		
After heat treatment	So	9.74±3.60 ^b	75.32±2.60 ^c	75.00±1.10 ^a	66.18±2.45 ^a
	SS	13.60±3.90 ^c	72.54±2.80 ^b	82.99±1.09 ^b	70.00±2.04 ^b
	Sp	6.25±3.80 ^a	62.24±2.00 ^a	84.43±1.30 ^c	76.10±2.54 ^c
After drying process	So	44.60±2.00 ^d	90.89±2.30 ^e	85.48±0.72 ^d	77.40±2.15 ^c
	SS	52.75±1.60 ^e	90.68±2.60 ^e	88.58±0.74 ^e	77.77±4.53 ^c
	Sp	50.63±2.00 ^e	80.37±2.10 ^d	84.79±1.03 ^{cd}	86.55±2.60 ^d

Values are mean± SD (n=12)

^{a-e} Values within columns with difference letters are significant difference (p<0.05).

So=Soaking in water, SS=soaking in water and water spraying, Sp=water spraying.

Compared to the raw material, heat treatment during starch gelatinization caused a reduction of AOA in a range of 6-13% (ABTS⁺) and 60-75% (FRAP) and drying further reduced the AOA for 44-52% (ABTS⁺) and 80-90% (FRAP). TPC and PC were reduced for 75-82% and 66-76%, respectively, during gelatinization. Consequently, the drying process further reduced the TPC and PC for 84-88% and 77-86%, respectively (Table 4). The Sp technique showed higher reduction effects than SS and So, respectively. The results supported a previous study of Raweewat [19], that AOA, TPC and PC were intolerant to thermal conditions during the quick-cooking process in brown rice.

Conclusion

The quick-cooking process for germinated brown rice had three major steps. Water uptake into the rice is an initial part wherein spraying technique under $35\pm 0.5^{\circ}\text{C}$ for 20 hrs could be applied. Starch gelatinization mainly used heat treatment. Drying process is a final step for a stable shelf-life in ambient temperature. Combination of all steps caused reductions of 52%, 90%, 89% and 86% in AOA (ABTS⁺⁺), AOA (FRAP), TPC and PC, respectively, in the final product compared to raw material.

Acknowledgements

The authors would like to acknowledge the Thailand Research Fund (TRF), MAG Window I for financial support and Natural Rice Co., Ltd., Sukhothai Province for supplying the raw material used in this study.

References

1. Graf, E. and Empson, K.L. (1987). Phytic acid: a natural antioxidant. *The Journal of Biological Chemistry*, 262:11647-11650.
2. Hunt, J. R., Johnson, K.L. and Juliano, B.O. (2002). Bioavailability of zinc from cooked Philippine milled, under-milled and brown rice, as assessed in rats by using growth, bone zinc and zinc-65 retention. *Journal of Agricultural and Food Chemistry*, 50:5229–5235.
3. Ohtsubo, K., Keitaro, S., Yuji, Y. and Takafumi, K. (2005). Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. *Journal of Food Composition and Analysis*, 18: 303-316.
4. Jianfen, L., Bei, Z.H., Nout, M.J.R. and Robert, J.H. (2008). Effects of soaking, germination and fermentation on phytic acid, total and *in vitro* soluble zinc in brown rice. *Food Chemistry*, 110:821-828.
5. Manyam, B.V., Katz, L., Hare, T., Kanifefski, A. and Tremblay, R.D. (1981). Isoniazid-induced elevation of cerebrospinal fluid (CSF) GABA levels and effects on chorea in Huntington's disease. *Annals of Neurology*, 10:35-37.
6. Mayer, R, Cherry, J. and Rhodes, D. (1990). Effects of heat shock on amino acid metabolism of cowpea cells. *Plant Physiology*, 94:796-810.
7. Larsson, M., Minekus, M. and Havenaar, R. (1997). Estimation of the bioavailability of iron and phosphorus in cereals using a dynamic *in vitro* gastrointestinal model. *Journal of the Science of Food and Agriculture*, 74: 99-106.
8. Lestienne, I., Caporiccio, B., Besançon, P., Rochette, I. and Trèche, S. (2005). Relative contribution of phytates, fibres, and tannins to low iron and zinc *in vitro* solubility in pearl millet (*Pennisetum glaucum*) flour and grain fractions. *Journal of Agricultural and Food Chemistry*, 53(21): 8342-8348.

9. Lestienne, I., Mouquet, C., Vernière, I., Rochette, C. and Trèche, S. (2005). The effects of soaking of whole, dehulled and ground millet and soybean seeds on phytate degradation and Phy/Fe and Phy/Zn molar ratios. *International Journal of Food Science and Technology*, 40: 391-399.
10. Lestienne, I., Vernière, I., Mouquet, C., Picq, C. and Trèche, S. (2005). Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chemistry*, 89:421-425.
11. Alexander, W.P. (1954). Process of Preparing Quick-cooking Rice. November 9, 1954. U.S. Patent No. 507,242. Referred by Naivikul, A. 2007. Rice: Science and Technology. 2nd ed. Kasetsart University Publishing. Bangkok. p.366.
12. AOAC International (1995). Official Method of Analysis. 16th, Ed. Arlington: The Association of Analytical Chemists: Gaithersburg, MD.
13. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26:1231-1237.
14. Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" the FRAP assay. *Analytical Biochemistry*, 239:70-76.
15. Pinsirodom, P. and Changnoi, W. (2001). Comparison of total polyphenol content and antioxidant potential of extracts obtained from seeds of different citrus fruits cultivated in Thailand. *Food Chemistry*, 34:300-307.
16. Reddy, N.R., Pierson, M.D., Sathe, S.K., Salunkhe, D.K. (1989). Phytates in Cereals and Legumes. CRC Press, FL.
17. Raweewat, L. (2009). Effect of quick-cooking rice processes on antioxidant properties, polyphenol and phytate content of color brown rice, [M.Sc. thesis]. Maejo University, Chiang Mai, Thailand, p.122.
18. Yeh, A.I. and Chiang, P.Y. (2002). Effect of soaking on wet-milling of rice. *Journal of Cereal Science*, 35:85-94.
19. Fernandez-Orozco, R., Frias, J., Zielinski, H., Piskula, M.K., Kozłowska, H. and Vidal V.C. (2008). Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiate* cv. *emerald*, *Glycine max* cv. *jutro* and *Glycine max* cv. *merit*. *Food Chemistry*, 111:622-630.
20. Tian, S., Nakamura, K. and Kayahara, H. (2004). Analysis of phenolic compounds in white rice, brown rice and germinated brown rice. *Journal of Agricultural and Food Chemistry*, 52:4808-4813.
21. Suwannanon, U. and Jaimyangyeun, S. (2008). Effect of temperature, soaking and germinating on radical scavenging activity of germinated brown rice. *Journal of Agricultural Science*, 39:429-432.

22. Jianfen, L., Han, B.Z., Nout, M.J.R. and Hamer, R.J. (2009). Effect of soaking and phytase treatment on phytic acid, calcium, iron and zinc in rice fractions. ***Food Chemistry***, 115:789-794.