

# Changes in Antioxidant Activity and Antioxidative Compounds of Brown Rice after Pre-germination

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**Abstract** Effect of pre-germination on antioxidant activity and quantity of some antioxidative compounds of a set of different types of brown rice was investigated. Quantification of these antioxidative compounds by GC-MS and LC-ESI-MS revealed that the contents of tocopherols, tocotrienols, and  $\gamma$ -oryzanols, as well as some simple phenolic and heterocyclic compounds increased in all rice samples in the same range after the pre-germination process. Differences in variety, growing location, and amylose content of the rice samples had little effect on these chemical alterations. The findings in this work indicate that the pre-germination process is an effective economical procedure to improve nutritional benefits of rice grain as being a functional food product.

**Keywords:** pre-germinated brown rice, antioxidant activity, tocopherols, tocotrienols,  $\gamma$ -oryzanols, phenolics

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## 1. Introduction

Rice is one of the most important crops in the world. It is consumed as a staple food by more than half of the world's population and approximately 95% of its production is in Asia. Besides being a main energy source, rice also provides a number of trace minerals in addition to several of the B vitamins, including niacin and thiamine [1]. Brown rice is especially important for health proposes because its bran components of pericarp, seed coat, nucellus, and aleurone layer contain a number of functional components, such as vitamin E and  $\gamma$ -oryzanols [2]. A number of studies have shown that tocopherol functions as the most effective lipid-soluble antioxidant that prevents the propagation of free radical reaction, thereby protecting tissues from free radical-mediated degenerative disease and aging [3]. Moreover, tocopherols provide immune protection [4], antiproliferative [5] and anticlotting effects [6], and reduce LDL oxidation, platelet adhesives, and thrombosis [7,8].  $\gamma$ -Oryzanols, the unique rice bran's component, is an effective agent for decreasing plasma cholesterol, lowering serum cholesterol [9], and decreasing platelet aggregation [10]. It has also been used to treat hyperlipidemia [11] and disorders of menopause [12], and to increase muscle mass [13]. In addition to these functional components, there are phytochemicals responsible for pigments in the pericarp and testa of the colored rice kernel. There is a great diversification of colored or pigmented rice, especially in northern Thailand where black sticky rice is mostly consumed in sweetened form. Recently, some newly developed Thai non-

glutinous black rice varieties have been introduced and have gained increasing popularity due to the prominent therapeutic functions of the components contained in their brans. These functional compounds are reportedly involved in the prevention of various diseases associated with oxidative stress, such as cancer [14]. Because of these health benefits, brown rice is now replacing white rice as a staple food.

Attempts to get the most benefit out of eating rice have resulted in the introduction of pre-germinated brown rice (PBR). In recent decades, germination of rice seeds prior to cooking has proven to provide higher nutritional value compared with the non-PBR. Germination of brown rice can be done by soaking whole kernels of brown rice in water until embryos begins to bud. During the process of germination, the chemical composition of the germinated seeds changes drastically. Pre-germinated brown rice has been reported to contain abundant dietary fiber, vitamins, and minerals [2] and it becomes superior in terms of digestion and absorption. It also provides more sweetness, excellent taste, better texture, and is easier to cook. The change in the nutritional value is partly due to essential biochemical activity that forms compounds and energy, and increase activity of some hydrolytic enzymes [15,16,17]. Although some biological activities related to the added nutritional value of pre-germinated brown rice had been reported [18] and the increase in some functional components, such as  $\gamma$ -aminobutyric acid (GABA), some free amino acids, dietary fibres, ferulic acids,  $\gamma$ -oryzanols, and prolylendopeptidase inhibitor, had been evident, comparative studies on quantities of these biologically active phytochemicals are quite limited. In addition, the diversification of rice due to differences in genetic

characters implies that the chemical alteration of those kinds of rice seeds after the pre-germination process might be different. Accordingly, further study to clarify the changes in quantity of some biologically active compounds, especially those related to antioxidant activity of rice before and after pre-germination, including tocopherols, tocotrienols,  $\gamma$ -oryzanols, some simple phenolics, and heterocyclic molecules has been done and is reported here. Quantification of these compounds was done using gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography with photodiode array detector (HPLC-DAD), and liquid chromatography-electrospray ionisation mass spectrometry (LC-ESI-MS). Data obtained from this study should be useful for developing various kinds of pre-germinated brown rice products with high nutritional benefits.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Plant Materials and Germination of Rice seeds

The eight rice samples, *Oryza sativa* L., used in this study were rice varieties from the Bureau of Rice Research and Development, Rice Department of Thailand. They were two groups, one white and one pigmented, each group included both glutinous and non-glutinous samples. Also, the white non-glutinous group was divided into low and high amylose rice. The varieties of each of the two rice groups were low amylose white non-glutinous rice, Patumthani 1 (PT 1) and white non-glutinous jasmine rice, KhaoDawk Mali 105 (KDML 105), high amylose white non-glutinous rice, Chainat 1 (CN 1) and Rice Department 31 (RD 31), white glutinous rice, Rice Department 6 (RD 6), pigmented non-glutinous rice, SangyodMuangPattalung (SY), and pigmented glutinous rice, Niaw Dam (ND). Each of these rice varieties was grown in different locations in Thailand. The rice samples of the same variety but from a different growing locations are indicated by a numeral suffix to their names.

Germination of rice seeds was done by soaking the seeds in water at room temperature for four hours. The seeds were then washed and incubated in a closed container for six hours. After this, the seeds were again washed and then subjected to further incubation in a closed container for 14 hours. If the resulting germinated part was not 0.25–1.0 mm long, the seeds were subjected to incubation again until germination was confirmed. The germinated seeds were dried at 50°C, and the moisture content was controlled between 8 and 12%. These pre-germinated rice samples were kept at -20 °C until analyzed.

#### 2.1.2. Reagents and Chemicals

Tocopherols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -isoforms),  $\gamma$ -oryzanols, and 1,1-diphenyl-2-picryl hydrazyl (DPPH) were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Hexane, isopropanol, methanol, absolute ethanol, 1,4-dioxane, acetonitrile, dichloromethane, acetic acid were purchased from Merck (Darmstadt, Germany). AR grade of standard compounds 2-furanmethanol, 2,3-

dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 5-hydroxymethyl-2-furancarboxaldehyde, 2-methoxy-4-vinylphenol, 2-methoxy-phenol, and 1,2-benzendiol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

### 2.2. Antioxidant Activity Against 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radicals

Rice samples, each 20–40 g, were extracted with 150 mL of methanol by continuously shaking for 30 min. The extraction was done in three replicates and the resulting extracts were combined and concentrated using a rotary evaporator (model R-124, Buchi, Switzerland). Determination of the radical-scavenging activities of these rice extracts was performed following the method reported previously [19]. A 3.0-mL portion each of non-PBR and PBR extracts at concentrations of 40 and 20 mg/mL, respectively, was added into a glass tube containing 1.0 mL of 0.3 mM methanolic DPPH. The mixture was vigorously shaken and left to stand for 20 min in a dark space. The absorbance at 517 nm of this reaction solution was measured by a Genesys R-20 spectrophotometer (Rochester, Madison, WI, USA). The control solution was prepared by mixing all reagents except the rice sample extracts. The radical-scavenging effect was derived using the following equation:

$$\text{scavenging capacity (\%)} = 100 - \left[ \frac{\left( \begin{array}{l} \text{Ab of sample} \\ - \text{Ab of blank} \end{array} \right)}{\times 100 / \text{Ab of control}} \right]$$

Scavenging activity of the rice extracts was also estimated from the percentage of the DPPH reduction by calculating the IC<sub>50</sub> values (concentration in mg/mL that caused 50% inhibition of DPPH radicals) using a non-linear regression analysis.

### 2.3. Analysis of Simple Antioxidants Using GC-MS

Thirty grams of each rice sample was extracted with 80 mL of methanol for 30 min with continuous shaking. The extracts were filtered and concentrated under reduced pressure on a rotary evaporator and the residue was adjusted with methanol to make a final volume of 1.0 mL. GC-MS analysis was performed on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). A capillary AT-5MS column (5% phenylmethylpolysiloxane) with a dimension of 30 m  $\times$  0.25 mm I.D. and a 0.25  $\mu$ m film thickness (Deerfield, IL, USA) was used. The injector temperature was set at 230 °C. The flow rate of helium carrier gas was 1.0 mL/min. The oven temperature was started at 60 °C and was followed by a 3 °C/min temperature ramp up to 280 °C. The temperature of GC-MS transfer line was set at 280 °C. An Agilent 5973A mass spectrometer (Agilent Technologies, Atlanta, GA, USA), which utilized electron impact ionization, was used as a chromatographic detector. The ion source temperature was at 280 °C. A scanned mass range of m/z 29–600 was used and the detector voltage was set at 1150 V. Identification of the detected components was mainly done by matching their mass spectra with the reference spectra in the NIST 11 Mass

Spectral Library. In addition, published Kováts indices and retention time of the standards were used to aid structural confirmation for some interesting compounds.

## 2.4. HPLC-DAD and HPLC-MS Analysis of Tocopherols, Tocotrienols, and $\gamma$ -oryzanols

Ten grams of each rice sample was added to 100 mL of ethanol and sonicated for 30 min. Each extract was filtered and evaporated to dryness. These dry residues were adjusted to make a volume of 2.0 mL with hexane or ethanol for analysis of tocopherols, tocotrienols, or  $\gamma$ -oryzanols. The HPLC system consisted of an Agilent HPLC 1100 connected to a DAD detector (Model G1315 A, Agilent Technologies, Palo Alto, CA, USA). Chromatographic separations of tocopherols and tocotrienols were done on a VertiSep™ UPS Silica (5 $\mu$ m, 4.6  $\times$  250 mm) column. Chromatographic separation of  $\gamma$ -oryzanols was done on a Vertical-C18 (5  $\mu$ m, 4.6 $\times$ 250 mm) column. The mobile phases used were a mixture of hexane/1,4-dioxane (100:6, v/v) and a mixture of methanol/acetonitrile/dichloromethane/acetic acid (40:30:27:3, v/v/v/v) for analysis of tocopherols and tocotrienols, and  $\gamma$ -oryzanols, respectively. The flow rate of both systems was 1.0 mL/min. The UV detections were at 295 nm for tocopherols and tocotrienols and at 330 nm for  $\gamma$ -oryzanols. Identification of these vitamin E analogues was done using the Agilent HPLC 1100 combined with a single quadrupole mass spectrometer (Model G 1946 A; Agilent Technologies) via an orthogonal electrospray ionization (ESI) interface. Nitrogen gas was used as drying gas and was used in the collision induced dissociation (CID) process. The electrospray ionization operating parameters were: ionisation mode, positive; nebuliser pressure, 32 psi; drying gas flow rate, 12 L min<sup>-1</sup>; drying gas temperature, 350°C; capillary voltage, 4000 V; and fragmentor voltage, 130V. The quadrupole temperature was 100°C, and the electron multiplier voltage was 2670V.

## 3. Results and Discussion

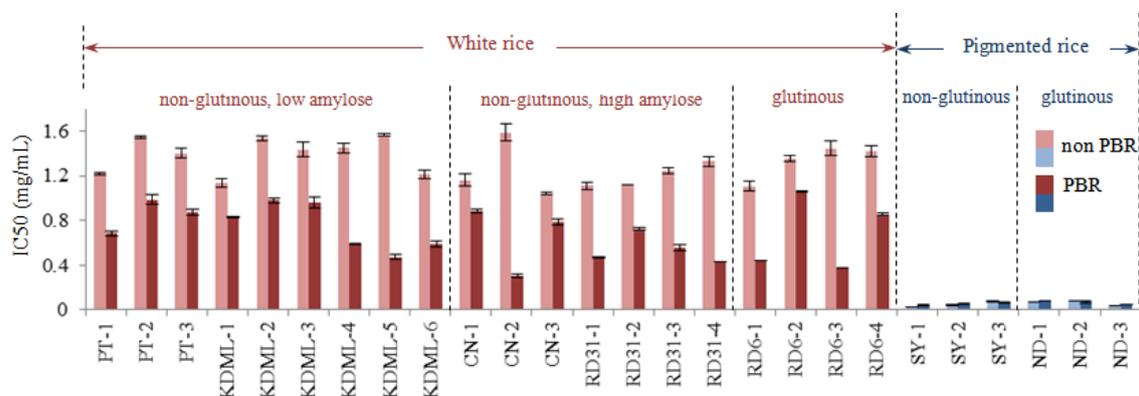
The process of germination is known to enhance the bio-availability of many nutrients, such GABA, vitamins,

niacin, dietary fiber, magnesium, potassium, and zinc, in cereal seed. It also causes a decrease in some anti-nutrients, such as phytic acid [20]. Likewise, the biochemical activities occurring during germination can generate some phytochemicals that play an important role in biological activities involving human health [18].

In this study, changes in antioxidant activity between PBR and non-PBR were observed, along with changes in content of some bioactive compounds, such as tocopherols, tocotrienols,  $\gamma$ -oryzanols, simple phenolics, and heterocyclic antioxidants. Some of these changes were found to be dependent on the type, variety, and amylose content of the rice samples as well as on their growing locations.

### 3.1. Antioxidant Activity against DPPH Radicals

The DPPH radical scavenging assay that was employed for evaluating the anti-oxidative properties of PBR and non-PBR brown rice samples revealed a dramatic difference between IC<sub>50</sub> values of the white and pigmented groups of rice samples. Pigmented rice samples possessed quite strong antioxidant activities, 0.04-0.08 mg/mL for PBR and 0.02-0.08 mg/mL for non-PBR, compared with those of white rice, 0.30-1.06 mg/mL for PBR and 1.04-1.59 mg/mL for non-PBR. All white rice extracts had increases in their radical scavenging activities after pre-germination, with changes in IC<sub>50</sub> of some white rice samples up to more than 200% (Figure 1). Whereas no significant changes in DPPH radical scavenging activity occurred between pigmented rice samples with and without a pre-germination process. The much higher antioxidant activities of pigmented rice samples could be attributed to the presence of pigment compounds bearing an antioxidative property, such as flavonoids, especially anthocyanins, and carotenoids, which were reported previously [21,22]. Apart from possessing a strong radical scavenging property, anthocyanins found in rice and other food plants were reported to have other biological effects, including anti-mutagenic and anti-carcinogenic activity, reduction of atherosclerotic plaque formation, inhibition of aldose reductase, and amelioration of pathogenesis of hyperlipidemia and diabetes [23,24,25].



**Figure 1.** Antioxidant activities of the extracts from PBR and non-PBR measured by DPPH assay. PT 1: PathumThani 1, KDML 105: KhaoDawk Mali 105, CN: Chai Nat 1, RD 31: Rice Department 31, SY: SangyodMuangPattalung, RD 6: Rice Department 6, ND: Niew Dum

### 3.2. Tocopherol, Tocotrienol and $\gamma$ -oryzanol Contents

The contents of tocopherols, tocotrienols, and  $\gamma$ -oryzanols increased after pre-germination. These increases were likely dependent on the rice variety rather than on

bran color. Separation of all tocopherol and tocotrienol analogues was accomplished within 25 minutes by the use of normal-phase HPLC. The quantitative results shown in Figures 2, 3, and 4 reveal that PBR has greater contents of tocopherols, tocotrienols, and  $\gamma$ -oryzanols. The contents of  $\alpha$ -tocopherol (Figure 2A) of both non-PBR and PBR follow the same trend as those of tocotrienol analogues, as well as  $\gamma$ -oryzanols (Figure 4), but the contents pattern was completely different from that of  $\gamma$ -tocopherol (Figure 2B). The content of  $\gamma$ -tocopherol is quite constant for each rice variety regardless of its growing location. The white non-glutinous high amylose rice CN had the highest content of  $\gamma$ -tocopherol and also had a moderate content of  $\alpha$ -tocopherol, both of which make it a white rice variety rich in total tocopherols. The pigmented glutinous rice ND is second to white non-glutinous high amylose rice CN in total tocopherols. This ND rice also contains a high amount of  $\gamma$ -oryzanols, the same as does white glutinous rice RD 6.

The total tocopherol content ranged from 0.019-0.061  $\mu\text{g/g}$  in non-PBR and from 0.021-0.078  $\mu\text{g/g}$  in PBR. The  $\gamma$ -oryzanols content of non-PBR was 6.50-26.4  $\mu\text{g/g}$  and was 7.90-27.0  $\mu\text{g/g}$  in PBR. The highest tocopherol contents were from pigmented glutinous rice ND and were 0.061 and 0.078  $\mu\text{g/g}$  for non-PBR and PBR, respectively. The second highest tocopherol contents were from high amylose white rice CN and were 0.052 and 0.062  $\mu\text{g/g}$  for non-PBR and PBR, respectively. The pigmented glutinous rice ND had the highest amounts of  $\gamma$ -oryzanols, these

being 26.5  $\mu\text{g/g}$  in non-PBR and 27.0  $\mu\text{g/g}$  in PBR. These  $\gamma$ -oryzanols amounts were followed by those in the white sticky rice RD 6, which contained 20.9 and 27.2  $\mu\text{g/g}$  in non-PBR and PBR, respectively. No significant alteration was observed in each derivative of  $\gamma$ -oryzanols.

After pre-germination, all rice samples had increased levels of vitamin E (tocopherols) and  $\gamma$ -oryzanols, which are the main antioxidants in rice bran [17]. Both vitamin E and  $\gamma$ -oryzanols have been reported as significant antioxidants, thereby protecting cells from the oxidative damage of plasma, very low-density lipoprotein, cellular proteins and DNA and from membrane degeneration [26,27]. On the basis of the results of this study, high amylose white rice (CN) and pigmented sticky rice (ND) have the highest contents of total tocopherols and  $\gamma$ -oryzanols and, therefore, have the potential to develop pre-germinated brown rice products.

In this study, although there was an increasing tendency of both tocopherols and  $\gamma$ -oryzanols after pre-germination, there was a weak correlation ( $R^2 = 0.06$ ) in the concentrations of tocopherols and  $\gamma$ -oryzanols in many kinds of rice. A correlation plot confirmed that biosyntheses of tocopherols and  $\gamma$ -oryzanols are clearly separated and are not controlled by the same chromosomal genes shown previously [28]. Therefore, the germination process would activate overall metabolisms of compounds and energy essential for the formation of seeding than would some biosynthesis pathways of specific compound.

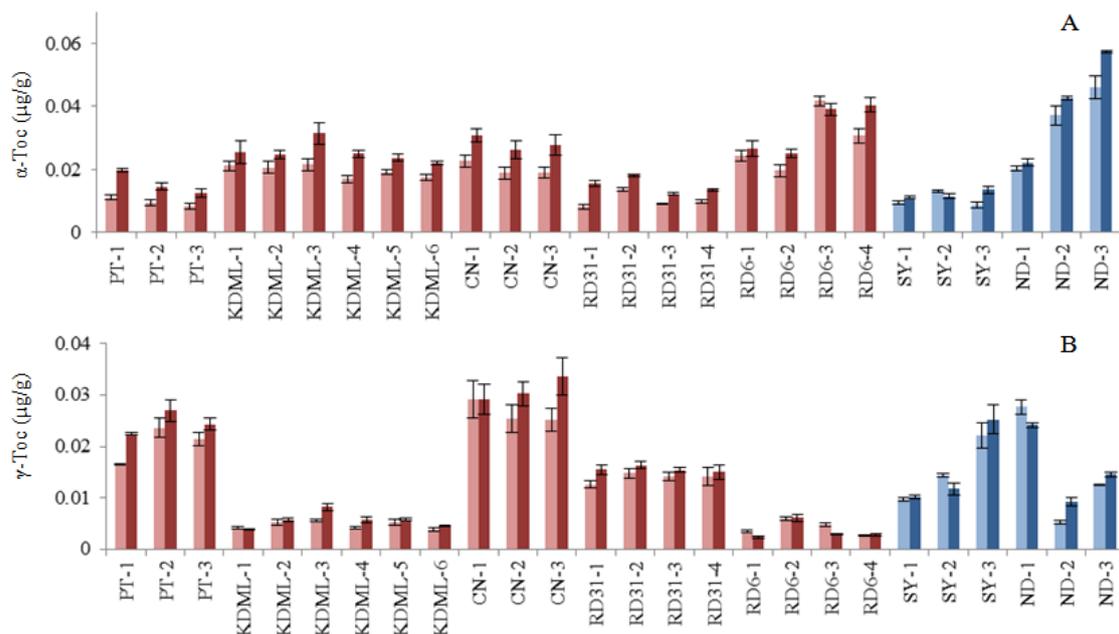


Figure 2. Contents of  $\alpha$ -tocopherol (A) and  $\gamma$ -tocopherol (B) in PBR and non-PBR samples

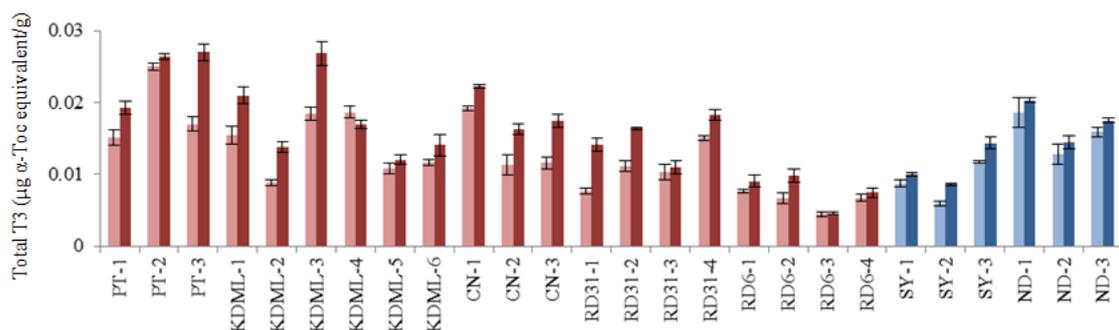


Figure 3. Content of total tocotrienol in PBR and non-PBR samples

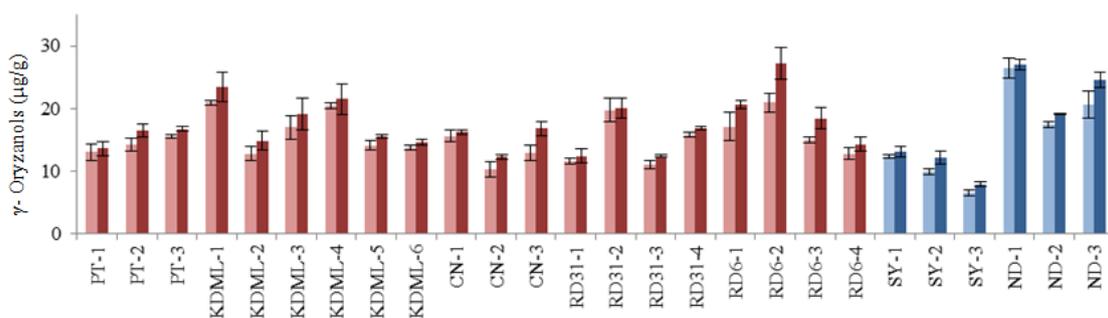


Figure 4. Content of total  $\gamma$ -oryzanol in PBR and non-PBR samples

### 3.3. Relative Contents of Simple Antioxidant and Phenolic Compounds

To clarify some chemical changes of rice samples after the pre-germination process, analysis of simple antioxidants by GC-MS was performed. The result of this was that the relative content of some bioactive constituents in the sets of white and pigmented rice samples had increased. These increased bioactive constituents were those metabolic products of simple carbohydrates: 2-furanmethanol, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), and 5-hydroxymethyl-2-furancarboxaldehyde, and of phenol derivatives: 2-methoxy-4-vinylphenol (4-vinylguaiacol), 2-methoxy-phenol (guaiacol), and 1,2-benzendiol (catechol). These phytochemicals were reported to possess important biological activities and to be especially involved in the reduction of oxidative stress as a major cause of age-related diseases and cancer. Both 2-furanmethanol and 2-methoxyphenols showed high antioxidant activities. Another reported anti-inflammatory agent is 2-methoxyphenol. 5-Hydroxymethyl-2-furancarboxaldehyde had been shown to have anti-platelet activity, while 2-methoxy-4-vinylphenol is a flavoring substance with an antioxidant property [29,30,31]. DDMP, normally a Maillard reaction product of glucose and glycine, has been reported to involve in DNA strand-breaking activity and mutagenicity [32].

The chemical changes during pre-germination occurred in the same manner in both pigmented and white rice samples. In white rice PT and red rice SY, after germination 2-furanmethanol, DDMP and 5-hydroxymethyl-2-furancarboxaldehyde increased greatly but 2-methoxy-4-vinylphenol increased only in the red rice. Black rice ND had an increase of phenolics 2-methoxy-4-vinylphenol, 2-methoxy-phenol, and 1,2-benzendiol. The results showed chemical alternation in both qualitative and quantitative ways that some bioactive compounds occurred only in PBR of some rice varieties. The higher contents of these small bioactive molecules as well as that of the vitamin E and  $\gamma$ -oryzanol groups after the pre-germination process confirmed the merit of consuming PBR for health benefits. The results of this study suggest that PBR presents a good opportunity for the functional food industry. However, a further study should be conducted to find out the most appropriate pre-germination condition that yields the highest quality PBR products.

## 4. Conclusion

The pre-germination effect on antioxidant activity of brown rice occurred mostly in white rice rather than in

pigmented rice. Quantitative analysis of some antioxidants in groups of tocopherols, tocotrienols, and  $\gamma$ -oryzanol, as well as in some simple phenolic and heterocyclic compounds, showed that the contents of these antioxidants increased in all rice samples in the same range after the pre-germination process. Differences in variety, growing location, and amylose content of the rice samples had little effect on these chemical alterations. The findings in this work indicate that the pre-germination process is an effective economical procedure to improve nutritional benefits of rice seeds, even where there are differences in physical appearance and eating quality.

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