Effect of UV C irradiation on secondary metabolites profile and antioxidant activity of germinated brown rice

S Aisyah*, A Nurjanah, H S H Munawaroh and Zackiyah

Department of Chemistry Education, Universitas Pendidikan Indonesia, Bandung, Indonesia

*Corresponding author’s e-mail: siti.aisyah@upi.edu

Abstract. This study aims to investigate the effect of UV C irradiation on the secondary metabolites profile and the antioxidant activity of brown rice during two days germination. The change on the metabolite profile was identified using UPLC-ESI-QTOF, while antioxidant activity was performed using DPPH assay. The UPLC-ESI-QTOF chromatogram showed that three new peaks were found only in the germinated brown rice (GBR) that was irradiated by UV C. Based on the mass spectroscopy data, one of those three compounds was tentatively identified as glycosylated flavonoid, while the rest remained undetermined. The antioxidant activity of brown rice increased after two days germination, whilst there were no differences between antioxidant activities of germinated brown rice with UV C irradiation and that of without UV C irradiation.

1. Introduction
As many Asian countries, rice is considered as the main carbohydrate source in Indonesia, compared to other carbohydrate sources such as cassava, potato, and corn. Statistical data showed that the consumption of rice in Indonesia per capita in one week during 2007 to 2014 was up to 1.7 Kg [1], of which white rice is the most consumed rice type due to the superior eating texture. Unfortunately, previous research has shown that high consumption of white rice has been linked to the higher risk of chronic diseases such as type 2 diabetes. Substitution of one-third serving a day of the white rice by the whole grain including brown rice (BR), has been suggested to decrease the risk [2].

Brown rice contains bran layers and embryo, existing of nutritional and biofunctional compounds including dietary fibres, γ-oryzanol, vitamins, and minerals [3]. Previous studies have shown that germination process on brown rice might improve not only the eating texture when cooked but also increase the nutritional and biofunctional compounds. Moreover, some healthy associated compound such as gamma-aminobutyric acid (GABA) have been found in germinated brown rice [4].

On the other hand, the presence of induced metabolites might be caused by the disturbance from the surrounding environment including UV irradiation [5]. Several studies have shown that UV C irradiation increase the content of bioactive compounds and antioxidants in fruits and vegetables. It has been shown that the UV C irradiation enhanced the phenolic content and antioxidant activity of dried lemon pomace powder [6]. UV light was also found to induce flavonoid and phenylamide compounds in rice leaves [7, 8]. This suggests that treatment with UV C has the potential to increase the content of bioactive compounds. During the process of rice production, ultraviolet (UV) rays originating from the sun are
naturally used during the planting period of rice to drying rice process. However, the UV rays used are only UV A rays and some UV B rays, while UV C rays from the sun are retained by the ozone layer [9].

In this study, we investigated the effect of UV C irradiation on the secondary metabolite profile and the antioxidant activity of germinated rice. The results of this study are expected to provide information on new methods to produce rice with higher secondary metabolite content as well as higher antioxidant activity.

2. Methods

2.1. Materials
Unpolished brown rice (pandan wangi var.) were purchased commercially from PT. Kampung Kearifan Indonesia (Jakarta, Indonesia). Sodium hypochlorite was purchased from local market. UHPLC grade acetonitrile (ACN), water, and acetic acid were purchased from Merck (Darmstadt, Germany). Analysis grade methanol was purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from R&M chemicals (Fisher Scientific, Canada).

2.2. Rice germination
The germination process was performed in a lab-scale sprouting machine that was reported previously [10]. Briefly, brown rice were subjected to three stages as follows: (i) first stage was surface-sterilizing using 0.07% sodium chloride for 30 minutes then rinsed with several time with aquadest; (ii) second stage was soaking for 24 hours at room temperature; (iii) third stage was germination at 26°C with 99% relative humidity for two days in the dark. For the UV C irradiated rice, the germination was performed for 2 days with UV C irradiation for 30 minutes/day during germination period. The germinated brown rice with and without UV C irradiation were then dried for 6 hours in an oven at 40°C, until its moisture content less then 10%. The dried rice were milled using high energy milling HEM-E3D. The rice powder were then sieved, using a mesh size of 70 mesh and then stored at -4°C before used.

2.3. Rice Extraction
The dried powder of the rice were extracted based on Ti et al. method with modifications [11]. The extraction was performed for 30 minutes in water bath-ultrasonicator (W-211, Japan). The dried rice powder (0.2 gram) was extracted using 25 ml of 80% methanol (v/v). The mixture was centrifuged at 2500 rpm for 10 minutes and then filtered. The residue was re-extracted two times with the same procedure. The filtrate was collected and then concentrated using a rotatory vacuum. The concentrated extract was dissolved with 80% (v/v) methanol until it reached the final volume of 10 ml for further analysis.

2.4. Determination of Secondary Metabolites Profiles with UPLC-ESI-QTOF
The rice extracts were analyzed using the same method as reported previously [10]. UPLC-ESI-QTOF Xevo ToF-1 was used in positive mode. The column used was C-18 ACQUITY UPLC® BEH Shield RP18 (1.7 μm VanGuard™ Pre-Column 3/Pk (2.1x5 mm). The solvents used were water (solvent A) and acetonitrile (solvent B). The ratio of solvent A:B was 30:70, with a flow rate of 0.200 ml/min. The sample volume was 7.5 μL. The mode of operation for mass spectroscopy was ESI (+); capillary voltage: 3.0 KV; cone voltage: 60 V; low collision energy (CE): 6.0 V; acquisition range: 100-1000 Da.

2.5. Determination of Antioxidant Activity
The method used for antioxidant activity in this research was DPPH method, according to Lee et al. (2003) with some modifications [12]. 5 mL of DPPH (10.7 ppm) was added to 1 ml of sample and then incubated for 10 minutes in the dark. Absorbance was measured using UV-Vis Spectrophotometry at 517 nm wavelength. Percentage of antioxidant activity was calculated using the following formula (1).
Antioxidant Activity = \frac{\text{Abs. DPPH control} - \text{Abs. DPPH of test}}{\text{Abs. DPPH control}} \times 100\% \quad (1)

\text{Abs. DPPH control: absorbance of DPPH prior to reacting with the sample}
\text{Abs. DPPH of test: absorbance of DPPH after reacting with the sample}

3. Results and Discussion

3.1. Germination process of brown rice with and without UV C irradiation

In this study, brown rice were subjected to germination for 2 days, of which non treated brown rice (BR) were used as a control. The germination was performed in two conditions: (i) germination in the dark for 2 days (GBR) and germination with UV C irradiation (GBR-UV) for 30 minutes/day during the 2 days germination period. It can be seen that the morphology of GBR-UV were shorter and drier than that of GBR (Figure 1).

![Figure 1](image)

Figure 1. Ungerminated (BR), germinated without UV (GBR), and germinated with UV C of brown rice (GBR-UV)

3.2. Effect of UV C irradiation on secondary metabolite profile of germinated brown rice

The chromatogram of brown rice extracts showed that the germination process, with and without UV C irradiation, altered their secondary metabolite profile. In general, there were twenty peaks, eluted at retention time ($t_R$) of 0.95 to 13.53 min (Figure 2) with different peak intensities. The first eight peaks had higher intensity than the other peaks. The sum and intensities of the peaks varied depended on the treatments (Figure 2). Eleven compounds were found in BR, while twelve compounds were found in GBR and GBR-UV (Figure 2). Although the compound number was not so divergent, some compounds were found only in BR extract, while some compounds were found only in GBR extract (Table 1). For instance, compounds 6 and 10 were found in the BR extract only, and cannot be spotted in the GBR. Interestingly, peaks 8, 9, 12, 15 and 19 were only can be found in the GBR (Figure 1). On the other hands, there were peaks that were found only in GBR-UV. Germinated brown rice with UV C irradiation induced three peaks that were absent in both non treated and germinated in dark, namely peak 11, 18, and 20 at the retention time of 5.0.0, 13.02, and 13.53 minutes, respectively. It appears that some compounds were induced during germination (with or without UV C irradiation), while some of them were loss.

The identification of compounds in BR, GBR and GBR-UV was performed using comparison of mass spectra data of the compounds, including the retention time ($t_R$) and mass per charge (m/z) of the parent and fragment ion, found in the extracts with mass spectra data of published literatures (Table 1). However, due to the limited published literature data, most of the peaks cannot be identified (unknown compounds). Some flavonoids glycosylated and phenolic acids, including hydrocaffeic and caffeic acid, might be tentatively determined based on the fragment ions. In addition, one of the induced peaks by UV C irradiation was tentatively identified as flavonoids glycosylated, while the two of them remained undetermined. In contrast to secondary metabolites in rice leaves produced when induced by UV irradiation, in a study conducted by Park et al., it was reported that the compounds induced when the
rice leaves were irradiated by UV C were phenyl amide compounds and only sakuranetin of the group aglycone flavonoids [8].

Figure 2. RP-UHPLC−MS profile of 80% (v/v) methanol extracts of ungerminated (BR), germinated without UV (GBR), and germinated with UV (GBR-UV) of brown rice. Peak numbers refer to compounds in Table 1.
Table 1. Compounds Tentatively Identified by RP-UHPLC-MS in the extracts of ungerminated brown rice (BR), germinated brown rice without UV (GBR), and germinated brown rice with UV (GBR-UV)

| Peak no. | Tentative compounds                        | Retention time (min) | m/z (parent ion) | Distribution peaks in extracts |
|---------|--------------------------------------------|----------------------|-----------------|-------------------------------|
| 1       | Unknown                                    | 0.95                 | 178.0679        | ✓                             |
| 2       | Unknown                                    | 1.14                 | 526.7372        | ✓                             |
| 3       | Hydrocaffeic acid derivative 1             | 1.82                 | 467.9528        | ✓                             |
| 4       | 6'-O-feruloylsucrose                       | 2.04                 | 519.9201        | ✓                             |
| 5       | Flavonoid glycosilated 1                   | 2.35                 | 495.9638        | ✓                             |
| 6       | Hidrocaffeic acid                          | 2.48                 | 183.9990        | ✓                             |
| 7       | Hydrocaffeic acid derivative 2             | 3.06                 | 515.9164        | ✓                             |
| 8       | Unknown                                    | 3.26                 | 416.8736        | ✓                             |
| 9       | Caffeic Acid                               | 4.17                 | 181.9169        | ✓                             |
| 10      | Unknown                                    | 5.05                 | 377.0094        | ✓                             |
| 11      | Flavonoid glycosilated 1                   | 5.07                 | 658.9622        | ✓                             |
| 12      | Flavonoid glycosilated 2                   | 6.34                 | 495.1987        | ✓                             |
| 13      | Unknown                                    | 6.83                 | 395.9943        | ✓                             |
| 14      | Unknown                                    | 8.74                 | 602.9640        | ✓                             |
| 15      | Unknown                                    | 8.82                 | 550.2068        | ✓                             |
| 16      | Unknown                                    | 10.51                | 522.1942        | ✓                             |
| 17      | Unknown                                    | 12.05                | 424.0126        | ✓                             |
| 18      | Unknown                                    | 13.07                | 596.0142        | ✓                             |
| 19      | Unknown                                    | 13.07                | 522.2078        | ✓                             |
| 20      | Unknown                                    | 13.53                | 648.0056        | ✓                             |

3.3. Effect of UV C irradiation on antioxidant activity of germinated brown rice

The antioxidant activity of BR changed after germination with or without UV C irradiation. The antioxidant activity of BR rose slightly after germination in the dark, from 11.2% to 22.5%. However, the antioxidant activity of germination with UV C irradiation had no different compared to germination without UV C irradiation. This higher antioxidant activity of GBR was consistent with the results of the antioxidant analysis of germinated rice by Ti et al. using the method of Ferric Reducing Antioxidant Power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) analysis, that germination process increase the antioxidant activity of brown rice [11].

![Figure 3](image-url)  
**Figure 3.** Antioxidant activity (% AA) of ungerminated (BR), germinated without UV (GBR), and germinated with UV (GBR-UV) of brown rice
4. Conclusion
Germination with or without UV C irradiation altered the profile of secondary metabolites and antioxidant activities of brown rice. The UV C irradiation induced three peaks in brown rice, of which one of them was tentatively identified as flavonoid glycosylated while two of the peaks remained undetermined. The antioxidant activities of red rice that were germinated with or without UV C irradiation were higher than that of brown rice.

5. References
[1] BPS 2015 Bada Pusat Statistik Konsumsi Rata-rata per Kapita Seminggu Beberapa Macam Bahan Makanan Penting 2007-2014
[2] Sun Q, Spiegelman D, van Dam R M, Holmes M D, Malik V S, Willett W C and Hu F B 2010 White rice, brown rice, and risk of type 2 diabetes in US men and women Arch. Intern. Med. 170 961-9
[3] Moongngarm A, Daomukda N and Khumpika S 2012 Chemical Compositions, Phytochemicals, and Antioxidant Capacity of Rice Bran, Rice Bran Layer, and Rice Germ APCBEE Procedia 2 73-9
[4] Cho D-H and Lim S-T 2016 Germinated brown rice and its bio-functional compounds Food Chem. 196 259-71
[5] Boue S M, Cleveland T E, Carter-Wientjes C, Shih B Y, Bhatnagar D, McLachlan J M and Burow M E 2009 Phytoalexin-enriched functional foods J. Agric. Food Chem. 57 2614-22
[6] Papoutsis K, Vuong Q V, Pristijono P, Golding J B, Bowyer M C, Scarlett C J and Stathopoulos C E 2016 Enhancing the Total Phenolic Content and Antioxidants of Lemon Pomace Aqueous Extracts by Applying UV-C Irradiation to the Dried Powder Foods 5
[7] Kodama O, Miyakawa J, Akatsuka T and Kiyosawa S 1992 Sakuranetin, a Flavanone Phytoalexin from ultraviolet-irradiated rice leaves vol 31
[8] Park H L, Yoo Y, Hahn T-R, Hee Bhoo S, Wang G-L and Cho M-H 2014 Antimicrobial Activity of UV-Induced Phenylamides from Rice Leaves vol 19
[9] Diffey B L 2002 Sources and measurement of ultraviolet radiation Methods 28 4-13
[10] Nurnaista Y, Aisyah S and Munawaroh H 2018 Secondary metabolites profiles and antioxidant activities of germinated brown and red rice. In: Journal of Physics: Conference Series: IOP Publishing p 012204
[11] Ti H, Zhang R, Zhang M, Li Q, Wei Z, Zhang Y, Tang X, Deng Y, Liu L and Ma Y 2014 Dynamic changes in the free and bound phenolic compounds and antioxidant activity of brown rice at different germination stages Food Chem. 161 337-44
[12] Lee S C, Kim J H, Jeong S M, Kim D R, Ha J U, Nam K C and Ahn D U 2003 Effect of far-infrared radiation on the antioxidant activity of rice hulls J. Agric. Food Chem. 51 4400-3