

## Ultraviolet Radiation Reduces Microbial Contaminants while Increasing Antioxidant Activities in Black Jasmine Rice Pericarp Beverage

Kanokwan Tandee<sup>1\*</sup>, Suthaya Phimphilai<sup>1</sup>, Somkiat Jaturonglumert<sup>1</sup>,  
Aphiwat Teerawutgulrag<sup>2</sup>, Jatuphon Varith<sup>1</sup>, Surat Nuglor<sup>1</sup>, and Kajorndaj Phimphilai<sup>3</sup>

Received: 12 June 2015; Accepted: 13 July 2015

### Abstract

Black jasmine rice (*Oryza sativa* var. Hom Nil) is a great source of antioxidants mainly present in its pericarp, and the beverage extracted from this part is considered a functional food. However, production of beverage normally involves a heat treatment for safety purpose that possibly destroys some antioxidants, Ultraviolet (UV) radiation is thus proposed as an alternative process to reduce microbial contaminants and its effect on antioxidant activities is also determined. UV treatment through 6×6 W lamps at the lowest flow rate of 0.4 L/min could decrease ( $p>0.05$ ) the total plate count as well as yeast and mold count in the beverage for 0.39 and 0.75 log CFU/mL, respectively. Antioxidant activities, analyzed by ABTS and FRAP assays, in the black jasmine rice pericarp beverage, were significantly ( $p<0.05$ ) increased after UV treatment at a flow rate of 0.4, 0.7, or 1.0 L/min. Results suggest the multiple times of UV radiation should be used to effectively inhibit the microbial contaminants while antioxidants should be re-evaluated to ensure their stability. Black jasmine rice pericarp beverage, which is disinfected by UV treatment to meet a microbiological standard, could be a functional product without any health risk.

**Keywords:** ultraviolet radiation, rice pericarp beverage, microbial contaminant, antioxidant activity

### Introduction

Black rice contains higher contents of protein, total essential amino acids, vitamin B1, and minerals when compared to common rice. Its pericarp is also abundant in anthocyanins, e.g. cyanidin 3-glucoside and peonidin 3-glucoside, as well as antioxidants, e.g.  $\gamma$ -tocotrienol,  $\alpha$ -tocopherol,  $\beta$ -,  $\gamma$ -,  $\delta$ -tocopherols and  $\alpha$ -,  $\delta$ -tocotrienols<sup>1,2</sup>. Functional beverage produced from black jasmine rice, which is high in these compounds, could promote consumer health by inhibiting inflammation, allergy, cancer, and insulin resistance<sup>3-6</sup>. While several methods were used to extract natural compounds from plant materials<sup>7</sup>, optimal conditions of ultraviolet (UV) radiation and ultrasonication (US) were suggested for production of red rice extract drink, resulting in optimal antioxidant

activities and significantly lower microbial counts<sup>8</sup>. Microorganisms remaining in this non-thermally processed product might be, nevertheless, over a limit of microbiological standard that is less than 100 CFU/mL of *Bacillus cereus*, 100 CFU/mL of *Clostridium perfringens*, 100 CFU/mL of yeast and mold, 2.2 MPN/100mL of coliform bacteria, as well as an absence of *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*<sup>9,10</sup>. Low-acid canned foods were thus commonly heat-treated for safety purposes but possibly lost their nutritional value as well. UV radiation that has a germicidal activity by creating a thymine dimer in DNA<sup>11</sup> is, therefore, proposed as a non-thermal treatment after extraction of functional ingredients from black jasmine rice pericarp.

<sup>1</sup> Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, 50290 Thailand

<sup>2</sup> Faculty of Science, Chiang Mai University, Chiang Mai, 50200 Thailand

<sup>3</sup> Faculty of Engineering, Chiang Mai University, Chiang Mai, 50200 Thailand

\* Email: ktandee@gmail.com

Previous studies indicated that UV effectively reduced the microbial contaminants in grape juice, wine, coconut beverage, orange juice, apple juice, guava-and-pineapple juice, mango nectar, strawberry nectar, and tropical juice<sup>12-15</sup>. The objective of this study was to evaluate the effectiveness of UV radiation in disinfecting the black jasmine rice pericarp beverage. Additionally, the stability of antioxidants present in this beverage was ascertained for the side effect of UV treatment.

## Materials and Methods

### 1. Raw Material

Organic black jasmine rice (*Oryza sativa* var. Hom Nil) was purchased from AGRI CMU SHOP, Chiang Mai University after harvest in Phrao, Chiang Mai.

### 2. Preparation of Black Jasmine Rice Pericarp Beverage

Beverage was extracted from black jasmine rice pericarp as previously described<sup>8</sup>. Briefly, 2kg of rice was washed twice in a tap water and then spread to a thin layer before radiated with 4×15 W UV lamps (Sylvania, Japan) for 30 min. After that, 8L of commercial drinking water (Singha, Thailand) was added into an ultrasonicator model code W-113SANPA (Micromechatronics, USA) filled with the radiated rice. US condition was set at 28 kHz, 50°C for 60 min and the resulting extract was treated in a customized UV disinfection system equipped with 6×6 W lamps at 4 different conditions as described in (Table 1).

**Table 1** Conditions of UV treatment used in this study.

Flow rate (L/min)	Contact time (min)
0.4	4.50
0.7	2.57
1.0	1.80
1.5	1.20

### 3. Microbiological Analyses

1 mL of sample was serially diluted in 9 mL of 0.1% peptone water to the 10<sup>-4</sup> dilution. Bacterial number was determined using a pour plate technique<sup>16</sup>.

1 mL of each dilution was inoculated on Petri dish before approximately 15 mL of 45°C plate count agar (Himedia) was poured and mixed thoroughly. All dilution was plated in duplicate. Plates were incubated at 35°C for 48 h before colonies were enumerated.

Numbers of yeast and mold were evaluated using a spread plate technique<sup>16</sup>. 0.1 mL of each dilution was spread on potato dextrose agar (Himedia), which pH adjusted to 3.5 using 10% tartaric acid (Ajax Finechem, Australia), and all dilution was plated in duplicate. Plates were incubated at 25°C for 5 days before colonies were enumerated.

Dilution that showed 30-300 colonies of bacteria per plate or 10-150 colonies of yeast and mold per plate was selected for calculating a microbial count in a log scale per 1 mL of rice drink (log CFU/mL).

### 4. Antioxidant activities

#### 4.1 ABTS<sup>•+</sup> radical cation decolorization assay

The assay protocol was previously described by Re and colleagues<sup>17</sup>. ABTS radical cation (ABTS<sup>•+</sup>) was prepared by mixing 7 mM ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); (Sigma-Aldrich, Germany)] with 2.45 mM K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (Sigma-Aldrich) at an equal volume and allowing the mixture to stand in the dark at room temperature for 12-16 h. This stock solution could be stable for 2 days and used to prepare the working solution by diluting with water (Merck, Germany) to an absorbance of 0.7-0.9 at 734 nm. To perform the ABTS<sup>•+</sup> assay, 20 µL of sample was mixed with 80 µL of water and 2 µL of ABTS<sup>•+</sup> working solution for 3 min and an absorbance at 734 was read using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich) as a standard. Each sample was analyzed in duplicate. Concentration of antioxidants was obtained by fitting a change in absorbance of samples to a standard curve of Trolox.

#### 4.2 Ferric reducing ability power (FRAP) assay

The assay was slightly modified from a protocol developed by Benzie and Strain<sup>18</sup>. FRAP working reagent was freshly prepared before use by mixing 25

mL of 300 mM acetate buffer [ $3.1 \text{ g C}_2\text{H}_3\text{NaO}_2 \times 3\text{H}_2\text{O}$  (Fisher Scientific, UK) and  $16 \text{ mL of C}_2\text{H}_4\text{O}_2$  (Merck) per 1 L of buffer], 2.5 mL of 10 mMTPTZ solution [2,4,6-tripyridyl-s-triazine (Sigma-Aldrich) in 40 mM HCl (Merck)], and 2.5 mL of 20 mM  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  (Merck). To perform the FRAP assay, 20  $\mu\text{L}$  of sample was mixed with 60  $\mu\text{L}$  of water and 600  $\mu\text{L}$  of FRAP working reagent for 4 min and an absorbance at 593 was read using  $\text{FeSO}_4$  (Merck) as a standard. Each sample was analyzed in duplicate. Concentration of antioxidants was obtained by fitting a change in absorbance of samples to a standard curve of  $\text{FeSO}_4$ .

#### 4.3 Total polyphenol content (TPC) assay

The assay protocol was previously described by Pinsirodom and Changnoi<sup>19</sup>. 0.5 mL of sample was mixed with 9.5 mL of water and 0.5 mL of Folin-Ciocalteu's reagent (Merck) for 5 min and 2 mL of 10%  $\text{Na}_2\text{CO}_3$  (Ajax Finechem) was then added. The solution was allowed to stand at room temperature for 10 min before an absorbance at 730 was read using gallic acid (Sigma-Aldrich) as a standard. Each sample was analyzed in duplicate. Concentration of antioxidants was obtained by fitting a change in absorbance of samples to a standard curve of gallic acid.

#### 4. Statistical analyses

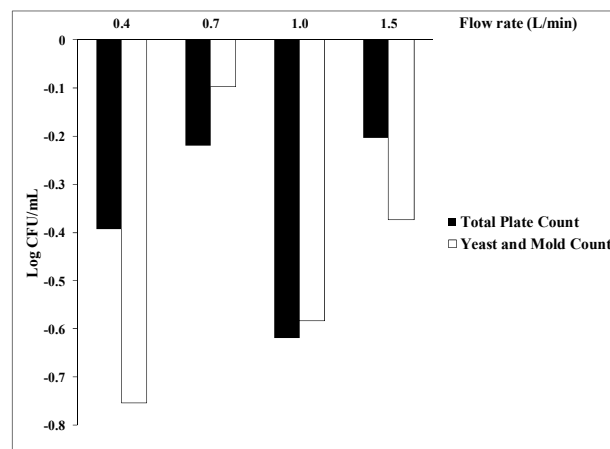
To determine the statistical differences among UV conditions, Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD) tests were used at 95% confidence (SPSS version 11.5.0; IBM, USA).

## Results and Discussions

### 1. UV Treatment Decreases Microbial Counts in the Black Jasmine Rice Pericarp Beverage.

Reductions of microbial contaminants in the black jasmine rice pericarp beverage after UV treatment at 4 different conditions are shown in (Figure 1) Values were in a range of -0.10 to -0.75 log CFU/mL, which was lower than previous studies<sup>12,15,20</sup>. This is possibly due to the lower dosage used in this study since a germicidal effect of UV is dose and time dependent. A lower flow rate generally resulted in a greater microbial reduction although abnormality was observed at a flow rate of 0.7

L/min after a different batch of black jasmine rice was used.

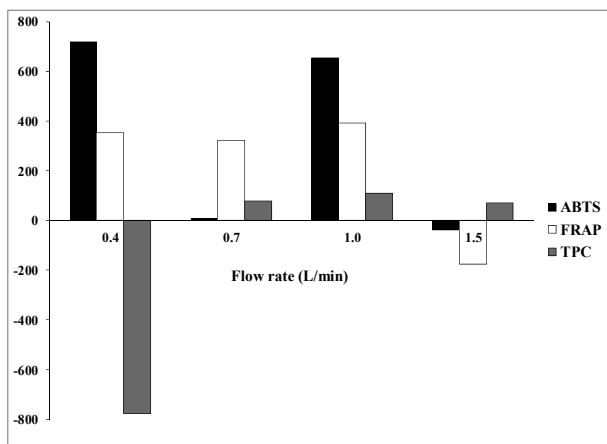


**Figure 1** Reduction of microbial contaminants in the black jasmine rice pericarp beverage after UV treatment at 4 different conditions

Mesophilic bacteria after UV treatment was lowered in a range of -0.20 to -0.62 log CFU/mL and UV treatment at a flow rate of 0.4 L/min showed the maximum reduction, although this condition did not result in a significant number ( $p > 0.05$ ) when compared to a control beverage. Meanwhile, decrease in yeast and mold after UV treatment was found in a range of -0.10 to -0.75 log CFU/mL. Yeast and mold present in the black jasmine rice pericarp beverage were also insignificantly ( $p > 0.05$ ) decreased after UV treatment at a flow rate of 0.4 L/min. Since microbial contaminants remained in the beverage treated at this condition were 4.51 log CFU/mL for mesophilic bacteria and 2.06 log CFU/mL for yeast and mold, multiple times of UV radiation are recommended for the effective disinfection of the black jasmine rice pericarp beverage.

### 2. UV Treatment Increases Antioxidant activities in the Black Jasmine Rice Pericarp Beverage.

Changes in antioxidant activities, which were analyzed by ABTS<sup>+</sup>, FRAP, and TPC assays, in the black jasmine rice pericarp beverage after UV treatment at 4 different conditions were shown in (Figure 2) Values were in a range of -776.31 to 719.80, which was higher than previous studies<sup>21-22</sup>. It is possibly due to the different variety of rice used.



**Figure 2** Changes in antioxidant activities, which were analyzed by ABTS<sup>+</sup>, FRAP, and TPC assays, in the black jasmine rice pericarp beverage after UV treatment at 4 different conditions

ABTS<sup>+</sup> and FRAP assays, which are based on a single electron transfer as mechanism of free radical quenching reaction<sup>23</sup>, resulted in a similar trend of antioxidant activities. More specifically, the activities were promoted by a lower flow rate of UV treatment but inhibited at a higher flow rate. However, contradiction was observed in a TPC assay where other phenolic compounds, such as flavonoids, lignins, and tannins, were analyzed as well<sup>23</sup>.

Antioxidant activities, which were measured by ABTS<sup>+</sup> assay, were found in a range of -38.21 to 719.80 mg Trolox equivalent/mL. Increase in these activities was significantly ( $p < 0.01$ ) higher at a flow rate of 0.4, 1.0, and 0.7 L/min, respectively. Using FRAP assay, antioxidant activities were found in a range of -175.67 to 391.87 mg FeSO<sub>4</sub>/50mL and significantly ( $p < 0.01$ ) higher at a flow rate of 1.0, 0.4, and 0.7 L/min, respectively. UV treatment might induce enzymes involved in the antioxidant synthesis after exposure to oxidative stress as described in previous studies, resulting in a high level of antioxidant activities<sup>24-25</sup>.

Antioxidants as determined by TPC assay were found in a range of -776.31 to 109.17 mg gallic acid equivalent/50mL. Increase in antioxidants in the black jasmine rice pericarp beverage was significantly ( $p < 0.01$ ) higher at a flow rate of 1.0, 0.7, and 1.5 L/min, respectively. The lower content of total polyphenol at a flow rate of 0.4

L/min is possibly due to the degradation of some compounds, which are not involved in any antioxidant activities, after a long period of UV exposure. Overall, UV treatment at the lowest flow rate of 0.4 L/min is suitable for the black jasmine rice pericarp beverage since microbial contaminants were maximally reduced and antioxidant activities, which were measured by ABTS<sup>+</sup> and FRAP assays, were maximally increased when compared to a control beverage.

## Conclusion

UV radiation could be a promising alternative to heat treatment of black jasmine rice pericarp beverage due to the reduction of microbial contaminants and the increase of antioxidant activities. The lowest flow rate of 0.4 L/min was recommended as a condition of UV disinfection system, although multiple passes are necessary to ensure the microbiological safety of final product.

## Acknowledgement

This research was financially supported by the Agricultural Research Development Agency (Thailand). We greatly appreciate the technical assistance from Miss Sookhathai-Yaibua and Miss Prapatsara Supamongkol, undergraduate students in Food Science and Technology Program at Maejo University.

## References

- [1] Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G., and Berghofer, E. Physicochemical and Antioxidative Properties of Red and Black Rice Varieties from Thailand, China and Sri Lanka, *Food Chem.*, 2011; 124(1): 132-140.
- [2] Yoshida, H., Tomiyama, Y., and Mizushima, Y. Lipid Components, Fatty Acids and Triacylglycerol Molecular Species of Black and Red Rices, *Food Chem.*, 2010; 123(2): 210-215.
- [3] Chen, M., Choi, S., Kozukue, N., Kim, H., and Friedman, M. Growth-Inhibitory Effects of Pigmented Rice Bran Extracts and Three Red Bran Fractions against Human Cancer Cells: Relationships with Composition and Antioxidative Activities, *J. Agr. Food Chem.*, 2012; 60(36): 9151-9161.

- [4] Fan, J., Choi, K., and Han, G. Inhibitory Effects of Water Extracts of Fermented Rice Bran on Allergic Response, *Food Sci. Biotechnol.*, 2010; 6: 1573-1578.
- [5] Guo, H., Ling, W., Wang, Q., Liu, C., Hu, Y., and Xia, M. Cyanidin 3-Glucoside Protects 3T3-L1 Adipocytes against H<sub>2</sub>O<sub>2</sub> - or TNF- $\alpha$ -Induced Insulin Resistance by Inhibiting c-Jun NH2-Terminal Kinase Activation, *Biochem. Pharmacol.*, 2008; 6: 1393-1401.
- [6] Min, S., Ryu, S., Kim, D. Anti-Inflammatory Effects of Black Rice, Cyanidin-3-O- $\beta$ -d-Glycoside, and Its Metabolites, Cyanidin and Protocatechuic Acid, *Int. Immunopharmacol.*, 2010; 10(8): 959-966.
- [7] Oroian, M. and Escriche, I. Antioxidants: Characterization, Natural Sources, Extraction and Analysis, *Food Res.* 2015; 74: 10-36.
- [8] Tandee, K., Phimphilai, S., Jaturonglumlert, S., Teerawutgulrag, A., Varith, J., Nuglor, S., and Phimphilai, K. Effect of Ultraviolet Radiation and Ultrasonication on Quality of Red Rice Extract Drink, *RMUTI J., Special issue*, 2015; 217-222.
- [9] Ministry of Public Health. Article No. 356: Beverages in Closed Containers. Royal Thai Government Gazette, 2013; 87: 93-97.
- [10] Ministry of Public Health. Article No. 364: Food Standard on Pathogens. Royal Thai Government Gazette, 2013; 148(13): 42.
- [11] Ortoneda, M., Cullen, J.D, Phipps, D.A., and Al-Shamma'a, A.I. Use of UV Light for Environmentally Friendly Microbial Control, Online, 2010.
- [12] Fredericks, I.N., du Toit, M., and Krügel, M., Efficacy of Ultraviolet Radiation as an Alternative Technology to Inactivate Microorganisms in Grape Juices and Wines, *Food Microbiol.*, 2011; 28(3): 510-517.
- [13] Gabriel, A.A., Aguila, M.L.C., and Tupe, K.A.M. Application of Ultraviolet-C Radiation to Inactivate Acid-and-Desiccation Stressed *Salmonella Enterica* in Young and Mature Coconut Liquid Endosperm Mix Beverage, *Food Control*, 2011; 51: 425-432.
- [14] HakguderTaze, B., Unluturk, S., Buzrul, S., Alpas, H. The Impact of UV-C Irradiation on Spoilage Microorganisms and Colour of Orange Juice, *J. Food Sci. Tech.*, 2015; 52(2): 1000-1007.
- [15] Keyser, M., Müller, I.A., Cilliers, F.P., Nel, W., and Gouws, P.A. Ultraviolet Radiation as a Non-Thermal Treatment for the Inactivation of Microorganisms in Fruit Juice, *Innov. Food Sci. Emerg. Technol.*, 2008; 9(3): 348-354.
- [16] Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International, Washington D.C., 2005.
- [17] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. Antioxidation Activity Applying an Improved ABTS Radical Cation Decolorization Assay, *Free Radic. Biol. Med.*, 1999; 9(10): 1231-1237.
- [18] Benzie, I.F.F. and Strain, J.J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": the FRAP Assay, *Anal. Biochem.*, 1996; 239(1): 70-76.
- [19] Pinsirodom, P. and Changnoi, W. Comparison of Total Polyphenol Content and Antioxidant Potential of Extracts Obtained from Seeds of Different Citrus Fruits Cultivated in Thailand, *Food* 2002; 32(4): 300-307.
- [20] Tran, M. and Farid, M. Ultraviolet Treatment of Orange Juice, *Innov. Food Sci. Emerg. Technol.*, 2004; 5(4): 495-502.
- [21] Aguilar-Garcia, C., Gavino, G., Baragaño-Mosqueda, M., Hevia, P., and Gavino, V.C. Correlation of Tocopherol, Tocotrienol,  $\gamma$ -Oryzanol and Total Polyphenol Content in Rice Bran with Different Antioxidant Capacity Assays, *Food Chem.*, 2007; 102(4): 1228-1232.
- [22] Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G., and Berghofer, E. Physicochemical and Antioxidative Properties of Red and Black Rice Varieties from Thailand, China and Sri Lanka, *Food Chem.*, Vol.124, No.1, 1 January 2011, 132-140, 2011.
- [23] Wootton-Beard, P.C., Moran, A., Ryan, L., Stability of the Total Antioxidant Capacity and Total Polyphenol Content of 23 Commercially Available Vegetable Juices before and after *in vitro* Digestion Measured by FRAP, DPPH, ABTS and Folin-Ciocalteu Methods, *Food Res. Inter.*, 2011; 44(1): 217-224.

- [24] Erkan, M., Wang, S., and Wang, C. Effect of UV Treatment on Antioxidant Capacity, Antioxidant Enzyme Activity and Decay in Strawberry Fruit, *Postharvest Biol. Technol.*, 2008; 48(2): 163-171.
- [25] González-Aguilar, G.A., Zavaleta-Gatica, R., and Tiznado-Hernández, M.E. Improving Postharvest Quality of Mango 'Haden' by UV-C Treatment, *Postharvest Biol. Technol.*, 2007; 45(1): 108-116.