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

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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 ensured 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 pericarpis also abundant in anthocyanins, e.g. cyanidin 3-glucoside and peonidin 3-glucoside,as well as antioxidants, e.g. γ-tocotrienol, α-tocopherol, β-, γ-, δ-tocopherols and α-, δ-tocotrienols[1,2]. 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[3-6].While several methods were used to extract natural compounds from plant materials[7], 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[8]. 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/mLof yeast and mold, 2.2 MPN/100mL of coliform bacteria, as well asan absence of *Salmonella, Staphylococcus aureus, Listeria monocytogenes,* and *Escherichia coli*[9,10]. 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[11] is, therefore, proposed as a non-thermal treatment after extraction of functional ingredients from black jasmine rice pericarp.

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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[12-15].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[8]. 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 kH_z, 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 differentconditions 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^{-4} dilution. Bacterial number was determined using a pour plate technique[16]. 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[16]. 0.1 mL of each dilution was spread on patato 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 countin a log scale per 1 mL of rice drink (log CFU/mL).

4. Antioxidant activities

4.1 $ABTS^{\bullet+}$ radical cation decolorization assay

The assay protocol was previously described by Re and colleagues[17]. ABTS radical cation ($ABTS^{\bullet+}$) was prepared by mixing 7 mM ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); (Sigma-Aldrich, Germany)] with 2.45 mM $K_2S_2O_8$ (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 $ABTS^{\bullet+}$ assay, 20 µL of sample was mixed with 80 µL of water and 2 µL of $ABTS^{\bullet+}$ working solution for 3 min and an absorbance at 734 was read using Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid; Sigma-Aldrich) as a standard. Each sample was analyzed in duplicate. Concentration of anti-oxidants 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[18]. FRAP working reagent was freshly prepared before use by mixing 25

mL of 300 mM acetate buffer [3.1 g $C_2H_3NaO_2 \times 3H_2O$ (Fisher Scientific, UK) and 16 mL of $C_2H_4O_2$ (Merck) per 1 L of buffer], 2.5 mL of 10 mMTPTZ solution [2,4,6-tripyridyl-*s*-triazine (Sigma-Aldrich) in 40 mMHCl (Merck)], and 2.5 mL of 20 mM $FeCl_3 \times 6H_2O$ (Merck). To perform the FRAP assay, 20 µL of sample was mixed with 60 µL of water and 600 µL of FRAP working reagent for 4 min and an absorbance at 593 was read using $FeSO_4$ (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 $FeSO_4$.

4.3 Total polyphenol content (TPC) assay

The assay protocol was previously described by Pinsirodom and Changnoi[19]. 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% Na_2CO_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 -0.75log CFU/mL, which was lower than previous studies[12,15,20]. 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.

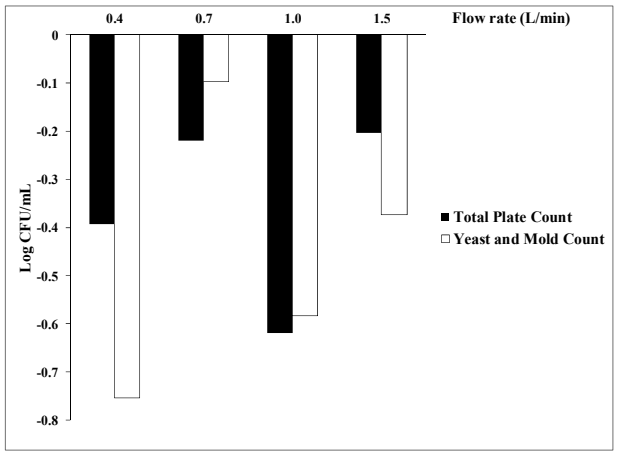

Figure 1 Reduction of microbial contaminants in the black jasmine rice pericarp beverage after UV treatment at 4 different conditions

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 conditionwere 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 forthe 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 $ABTS^{\bullet+}$, FRAP, and TPC assays, inthe 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[21-22]. It is possibly due to the different variety of rice used.

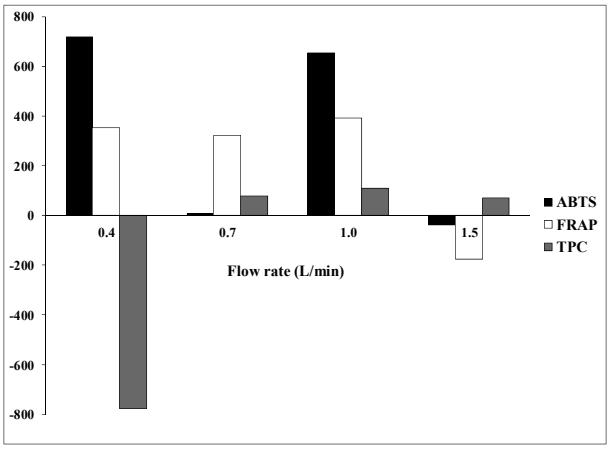

Figure 2 Changes in antioxidant activities, which were analyzed by $ABTS^{\bullet+}$, FRAP, and TPC assays, inthe black jasmine rice pericarp beverage after UV treatment at 4 different conditions

$ABTS^{\bullet+}$and FRAP assays, which are based on a single electron transfer as mechanism of free radical quenching reaction[23], 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[23].

Antioxidant activities, which were measured by $ABTS^{\bullet+}$assay, were found in a range of -38.21 to 719.80 mg Troloxequivalent/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 $FeSO_4$/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[24-25].

Antioxidants as determined by TPC assay were found in a range of -776.31 to 109.17 mg gallic acidequivalent/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 $ABTS^{\bullet+}$and FRAPS 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.

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