Study on the Enhancement of Antioxidant Properties of Rice Bran Using Mixed-Bacteria Solid-State Fermentation

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Abstract: Rice bran is usually mixed into animal feeds or discarded directly because of its harsh taste and undesirable flavor. Its bioavailability and exploitation are limited. In order to enhance the antioxidant properties of rice bran, the solid-state fermentation of rice bran with mixed bacteria was adopted in addition to the optimization of the fermentation technology. The bioactive constituents of water-soluble extracts and the in vivo antioxidant activity were compared before and after fermentation. The results revealed that the DPPH free radical scavenging rate of the water-soluble rice bran extracts under optimized fermentation technology conditions reached 86.38%, which was a 54.06% increase compared to that of raw materials. The mixed-bacteria solid-state fermentation increased the levels of bioactive constituents, including the polyphenols, flavonoid, protein, uronic acid, mannose, catechinic acid, caffeic acid, and ferulic acid contents. In a zebrafish model, the water-soluble fermented rice bran extracts (FRBE) displayed superior protective effects against 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH)-induced reactive oxygen species (ROS) production, lipid peroxidation, and cell death, and FRBE significantly improved SOD and CAT activity against the induced AAPH. Taken together, mixed-bacteria solid-state fermentation could change the bioactive constituents and enhance the antioxidant activity of water-soluble extracts from rice bran.

Keywords: rice bran; solid-state fermentation; bioactive constituents; antioxidant activity; zebrafish

1. Introduction

China is the largest global producer and consumer of rice, resulting in the production of at least 10 million tons of rice bran every year [1]. Rice bran is a waste product of the rice milling process that is usually mixed into animal feeds or discarded directly because of its harsh taste and undesirable flavor caused by lipid oxidation [2]. Its bioavailability and exploitation are limited. However, rice bran is rich in dietary fibers, phyto-antioxidant compounds, proteins, lipids, and vitamins [1,3] and can be used as an important functional food ingredient [4]. Solid-state fermentation, as a biotechnological process, is used to enhance the nutritional qualities, bioactive ability, and sensorial properties of foods [5–7]. It is well known that the fermentation of rice bran produces biologically active metabolites such as phenolic acid, proteins, uronic acid, and ferulic acid [8,9]. These bioactive constituents are key compounds in antioxidant activities. The majority of previous research regarding the antioxidant activity of fermented rice bran has adopted single strains such as *Rhizopus oryzae*, *Aspergillus*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Bacillus* as starter cultures to improve the antioxidant properties [3,10–13]. However, there are only few studies available on the effect of mixed-bacteria solid-state fermentation on the antioxidant properties of rice bran. Dang et al. (2017) [14] found that employing two fungi (*Aspergillus oryzae* and *Rhizopus oryzae*) in mixed solid-state rice bran extract cultures resulted in higher antioxidant activities than when single cultures were used. Schmidt et al.
evaluated the extracts obtained by the solid-state fermentation of rice bran with a reduced DPPH free radical ability. Compared to the native bran, the fermented rice bran extracts showed an inhibition potential for DPPH. In this study, rice bran was subjected to solid-state fermentation using mixed bacteria (S. cerevisiae, B. subtilis, and L. plantarum). In order to enhance the antioxidant properties of the rice bran, the fermentation technology for rice bran was optimized by a single-factor experimental design using the DPPH free radical scavenging rate as an index. Then, the bioactive constituents and antioxidant activities of rice bran extracts in zebrafish were studied before and after fermentation to illustrate the enhancement of the antioxidant properties of rice bran using mixed-bacteria solid-state fermentation, with the aim of improving the comprehensive utilization of rice bran.

2. Materials and Methods

2.1. Materials and Reagents

Rice bran and wheat bran were obtained from Baotou Beichen Feed and Technology Inc. Sodium humate was purchased from Tianjin Guangfu Fine Chemical Research Institute. Nutrient broth and malt extract medium were purchased from Guangdong Huankai Microbial Technology Co., Ltd. (Guangzhou, China). Cellulase, pectinase, and glucanase were purchased from Jinan Nuoneng Biological Engineering Co., Ltd. Standard monosaccharides (St. Louis, MO, USA) (mannose, ribose, rhamnose, glucose, galactose, xylose, arabinose, fucose, galacturonic acid, and glucuronic acid), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), acridine orange (AO), and 2,2′-azobis (2-methylpropionamide) dihydrochloride (AAPH) were purchased from Sigma (St. Louis, MO, USA). 1-phenyl-3-methyl-5-pyrazolone (PMP) and butylated hydroxyanisole (BHA) were purchased from Sinopharm Chemical Reagent Co. (Beijing, China). All other chemicals and reagents were analytical grade. S. cerevisiae (CGMCC 2.119), B. subtilis (CGMCC 1.892), and Lactobacillus plantarum (CGMCC 1.12934) were preserved at the Inner Mongolia Herbivorous Livestock Feed Engineering Technology Research Center (Inner Mongolia Agricultural University).

2.2. Experimental Method

2.2.1. Fermentation of Rice Bran and Optimization of Fermentation Technology

The mixed bacteria were prepared by mixing S. cerevisiae, B. subtilis, and L. plantarum at a ratio of 1:1:1 with a final concentration of $1 \times 10^8$ CFU/mL. S. cerevisiae, B. subtilis, and L. plantarum were cultured in malt extract medium, nutrient broth, and MRS medium, respectively. The main fermentation substrates containing rice bran (80%) and wheat bran (20%) were inoculated with 10% mixed bacteria.

In order to determine the optimal fermentation technology, a single-factor test was adopted to investigate how different conditions influence the DPPH free radical scavenging rate of water-soluble fermented rice bran extracts. The fermentation conditions included fermentation time (0, 48, 72, 96, 120, and 144 h), moisture content (40%, 45%, 50%, 55%, and 60%), sodium humate content (0%, 0.5%, 1%, 2%, and 4%), and different enzymes (cellulase, glucanase, and pectinase) and enzyme dosages (0, 500, 1000, 1500, and 2000 U/g). One factor was changed while the others were held constant in each experiment. The water in the inoculum was taken into account in the initial moisture content. After fermentation, the fermented product was collected and allowed to dry ($45^\circ$C, 24 h) for further extraction and determination of the DPPH free radical scavenging rate. Water-soluble extracts in the rice bran were extracted using the hot water extraction method. The hot water extraction conditions were adopted according to previous methods (Dong et al., 2021) [16]. The extracting conditions included an extraction time of 60 min, an extraction temperature of $85^\circ$C, and a material–water ratio of 1:20 (g/mL).

2.2.2. DPPH Free Radical Scavenging Capacity

The DPPH free radical scavenging capacity was examined using the method reported by Chen et al. [17]. BHA was used as the positive control. A total of 2.0 mL of the sample was mixed with 2 mL of a freshly prepared solution of DPPH (0.2 mmol/L, in 95% ethanol)
at different concentrations (0.5, 1.0, 2.0, and 4.0 mg/mL). The mixture was incubated at room temperature for 30 min in the dark, and the absorbance was measured at 517 nm. All the determinations were carried out in triplicate. The DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity(\%)} = \left(1 - \frac{A_s - A_b}{A_d}\right) \times 100\%$$ (1)

where $A_d$ is the absorbance of the DPPH solution without the sample; $A_s$ is the absorbance of the mixture of the sample with the DPPH solutions; and $A_b$ is the absorbance of the sample solution without DPPH.

2.2.3. Determination of Bioactive Constituents of Water-Soluble Extracts

The total polysaccharide contents of the water-soluble fermented rice bran extracts (FRBE) and water-soluble rice bran extracts (RBE) were determined by the phenol sulfuric acid method, for which glucose was used as a standard [17]. The flavonoid contents were estimated by the colorimetric method [18]. The protein contents were determined by the BCA (bicinchoninic acid) method (Pierce™ BCA Protein Assay Kit), which was adjusted to the microplate assay procedure. The mannose, ribose, rhamnose, glucose, galactose, xylose, arabinose, fucose, and uronic acid contents were determined by HPLC with PMP precolumn derivatization [17]. The polyphenol contents were measured using the Folin–Ciocalteu method, and gallic acid was used as the standard. An appropriate concentration of the sample solution (0.5 mL) was mixed with 10% Folin–Ciocalteu reagent (2.5 mL) and shaken. After 5 min, 2.0 mL of the 7.5% Na$_2$CO$_3$ solution was added to the mixture. The reaction mixture was kept in the dark for 60 min, after which the absorbance was measured at 756 nm. All the determinations were carried out in triplicate.

2.2.4. Phenolic Acid Composition

The quantitative analyses of the phenolic compounds of the FRBE and RBE were performed using a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) consisting of a VWD-3100 variable wavelength detector and a C-18 column (4.6 mm × 250 mm, 5 µm). Briefly, the sample (0.5 g) was ground with liquid nitrogen and dissolved in 8 mL of 80% ethanol with ultrasonic extraction at 100 Hz for 30 min. The extract was centrifuged at 10,000 × g for 10 min (4 °C). The supernatant was dried in a rotary evaporator at low pressure until no alcohol remained, and the residue was dissolved in 20 mL of ultrapure water with the pH adjusted to 7.0 with 1 mol/L NaOH. Then, 20 mL of ethyl acetate reagent was added to the solution, shaken for 10 min, and transferred to a separating funnel for separation. The aqueous phase was extracted with 20 mL of ethyl acetate once, and the combined ester phase was denoted as a neutral phenol. Then, the aqueous phase was adjusted to 2.0 with a 6 mol/L hydrochloric acid solution and extracted with 20 mL of ethyl acetate twice. The combined ester phase was denoted as an acidic phenol. Finally, the neutral phenol and acidic phenol were combined and concentrated to dry at 35 °C. The residue was dissolved in 60% methanol and transferred to a 10 mL brown volumetric flask and filtered through a 0.22 µm membrane. The resulting solution was analyzed using a Diamonsil C18 liquid chromatogram column (4.6 × 250 mm, 5 µm) and a variable wavelength detector (VWD). Elution was performed with a mixture of a phosphate buffer solution (pH 7.0) and acetonitrile in a ratio of 82:18 (v/v) at a flow rate of 1.0 mL/min. The standard phenolic acids used for the analyses were detected at different wavelengths: catechuic acid at 280 nm and caffeic acid and ferulic acid at 320 nm.

2.2.5. Antioxidant Activity in Zebrafish

Collection of Zebrafish Embryos

On the previous day, nine fish (three females and six males) were kept in a hatchery with the following conditions: 28 ± 0.5 °C and a 10/14 h dark/light cycle. In the morning
(at a set light), the nine breeding fishes interbred for 1 h; the embryos were obtained from natural spawning; and the collection of embryos was completed in Petri dishes.

Exposure of Embryos to AAPH

For the screening of zebrafish embryos that were fertilized and developed to 7–9 h post fertilization (hpf), the embryos were seeded in 24-well plates at different concentration of the sample (0.1, 0.2, 0.3, and 0.4 mg/mL) with 10 embryos per well. After 1 h, the embryos were treated with a final concentration of 20 mM AAPH up to 24 hpf. Some of the embryos were rinsed using fresh water for the determination of antioxidant enzyme activity (CAT and SOD). The other embryos were washed with fresh embryo medium and incubated up to 72 hpf. The zebrafish larvae that hatched from the embryos were used for further intracellular reactive oxygen species (ROS) production, cell death, and lipid peroxidation measurements. The embryos cultured without any treatment served as a control. The AAPH group had the addition of only AAPH. Four experimental groups had the addition of AAPH as well as 0.1, 0.2, 0.3, and 0.4 mg/mL sample, respectively.

Oxidative-Stress-Induced Intracellular ROS Production, Cell Death, and Lipid Peroxidation Measurements and Image Analysis

Intracellular ROS production, cell death, and lipid peroxidation in zebrafish embryos were detected using an oxidation-sensitive fluorescent probe dye (DCFH-DA), AO, and diphenyl-l-pyrenylphosphine (DPPP), respectively (Chen et al., 2020) [18]. The zebrafish larvae were transferred into the wells of the 24-well plates and treated with DCFH-DA solution (20 µg/mL), AO solution (25 µg/mL), and DPPP solution (25 µg/mL). Then, the plates were incubated in the dark at 28.5 ± 1 °C for 1 h. They were rinsed using clean water at 28 + 0.5 °C before observation and photographed under the microscope with a CoolSNAP-Pro color digital camera (Olympus, Tokyo, Japan). The fluorescence intensity of individual larvae was quantified using the Image J program.

Antioxidant Enzyme Activity Measurements

Catalase (CAD) and superoxide dismutase (SOD) were determined according to commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.3. Statistical Analysis

All the experiments were conducted in triplicate. The UNIVARIATE procedure of SAS was used to test the normality of the data before any further analyses were carried out. Data were subjected to ANOVA using the MIXED procedure of SAS (SAS 9.2, SAS Institute Inc., Cary, NC, USA). Means ± standard errors (S.E.) were reported, and means were separated by LSD multiple comparisons. Differences were accepted as statistically significance at $p < 0.05$.

3. Results and Discussion

3.1. Single-Factor Experimental Results of Fermentation Technology

The effect of fermentation time on the DPPH free radical scavenging rate is presented in Figure 1a. The DPPH free radical scavenging rate of the rice bran decreased slightly at 48 h after fermentation, then increased gradually with the increasing fermentation time, and stabilized at 72 h after fermentation. The DPPH free radical scavenging rate at 72, 96, 120, and 144 h after fermentation was significantly higher than at 0 h and 48 h after fermentation ($p < 0.05$), and the DPPH free radical scavenging rate at 72, 96, 120, and 144 h after fermentation had no significant difference. Thus, the optimal fermentation time was 72 h. The highest DPPH free radical scavenging rate (74.77%) was observed at a 50% moisture content, and no difference was found between a 50% or 60% moisture content. (Figure 1b). We chose 50% as the best moisture content for rice bran fermentation considering the costs. With increasing sodium humate content, the DPPH free radical scavenging rate first increased and then decreased (Figure 1c). We chose the addition of
1.0% sodium humate with a DPPH free radical scavenging rate of 81.22%. Figure 1d shows the effect of different enzymes on the DPPH free radical scavenging rate. When 500 U/g glucanase was added to the rice bran, the DPPH free radical scavenging rate of the rice bran was significantly higher than that of the other groups ($p < 0.05$). The effect of the amount of glucanase on the DPPH free radical scavenging rate of the rice bran was further studied (Figure 1e). The DPPH free radical scavenging rate of the fermented rice bran with different amounts glucanase was significantly higher than that without glucanase ($p < 0.05$). There was no significant difference between the 500 U/g and 1000 U/g glucanase groups. Considering the cost, we suggest adding 500 U/g glucanase in rice bran fermentation.

![Figure 1](image-url)

**Figure 1.** Effect of different fermentation technologies on the DPPH free radical scavenging rate of rice bran. Columns not sharing a common letter showed significant differences ($p < 0.05$). (a) Fermentation time, h; (b) moisture content, %; (c) sodium humate content, %; (d) different enzymes; (e) the amount of glucanase, U/g.
Solid-state fermentation (SSF) requires only a small amount of water, close to that found in the natural environment of the microorganisms. This allows SSF to exert a positive economic and environmental impact when used in place of conventional submerged fermentation. The choice of suitable fermentation technology is an important factor that strengthens the functional properties of grain byproducts. Based on our previous tests, fermentation time, moisture content, sodium humate, and different enzymes and enzyme dosages were identified as crucial factors affecting the bioactive constituents and antioxidant activities of the fermented substrates. The results of this experiment showed that with a moisture content of 50%, a fermentation time of 72 h, and the addition of 1.0% sodium humate and 500 U/g glucanase, the DPPH free radical scavenging rate of the rice bran increased to 86.38%, which was a 54.06% increase compared to that of the raw materials (56.07%).

3.2. Analysis of Bioactive Constituents of Water-Soluble Extracts

As shown in Table 1, the water-soluble polysaccharide content of the FRBE and RBE were 450 ± 2 mg g\(^{-1}\) and 552 ± 3 mg g\(^{-1}\), respectively. The FRBE had a significantly lower polysaccharide content than the RBE (\(p < 0.05\)), whereas the protein, polyphenol, flavonoid, and uronic acid contents of the FRBE were significantly higher than those of the RBE (\(p < 0.05\)). The ribose, rhamnose, glucose, galactose, xylose, arabinose, and fucose contents of the FRBE were significantly lower than those of the RBE. However, the mannose content of the FRBE was remarkably higher than that of the RBE (\(p < 0.05\)). It is possible that the bacteria partly utilized the rice bran polysaccharides and lead to protein accumulation in the process of fermentation. The changes in the contents of mannose, ribose, rhamnose, glucose, galactose, xylose, arabinose, fucose, and uronic acid may be due to the fermentation. Studies have reported that fermentation could decompose the cell wall structure of cereal bran, leading to an increase in digestible protein and total phenolic content [2,19]. The increase in the polyphenol and flavonoid contents in the FRBE may be attributed to the degradation of the rice bran cell wall during fermentation. These results indicate that the fermentation changed the content of the active substances in the water-soluble rice bran extracts.

Table 1. Bioactive constituent contents in water-soluble rice bran extracts.

| Soluble Bioactive Constituents | RBE          | FRBE          |
|-------------------------------|--------------|---------------|
| Polysaccharide content (mg g\(^{-1}\) dry matter) | 552 ± 3 \(^a\) | 450 ± 2 \(^b\) |
| Polyphenol content (mg g\(^{-1}\) dry matter) | 16 ± 0.4 \(^b\) | 32 ± 0.1 \(^a\) |
| Flavonoid content (mg g\(^{-1}\) dry matter) | 6 ± 0.0 \(^b\) | 9 ± 0.0 \(^a\) |
| Protein content (mg g\(^{-1}\) dry matter) | 54 ± 0.5 \(^b\) | 76 ± 0.4 \(^a\) |
| Uronic acid (mg kg\(^{-1}\) dry matter) | 1 ± 0.1 \(^b\) | 16 ± 0.2 \(^a\) |
| Mannose (mg kg\(^{-1}\) dry matter) | 7617 ± 2 \(^b\) | 9321 ± 3 \(^a\) |
| Ribose (mg kg\(^{-1}\) dry matter) | 1590 ± 2 \(^a\) | 91 ± 3 \(^b\) |
| Rhamnose (mg kg\(^{-1}\) dry matter) | 597 ± 2 \(^a\) | 107 ± 3 \(^b\) |
| Glucose (mg kg\(^{-1}\) dry matter) | 145,909 ± 13 \(^a\) | 13,241 ± 7 \(^b\) |
| Galactose (mg kg\(^{-1}\) dry matter) | 11,450 ± 5 \(^a\) | 1091 ± 7 \(^b\) |
| Xylose (mg kg\(^{-1}\) dry matter) | 23,721 ± 13 \(^a\) | 1259 ± 11 \(^b\) |
| Arabinose (mg kg\(^{-1}\) dry matter) | 24,258 ± 4 \(^a\) | 1336 ± 3 \(^b\) |
| Fucose (mg kg\(^{-1}\) dry matter) | 324 ± 1 \(^a\) | 36 ± 1 \(^b\) |

There were significant differences between the data of groups of the same category with different superscript letters (\(p < 0.05\)).

3.3. Analysis of Water-Soluble Phenolic Acid Composition

Table 2 shows that the water-soluble phenolic acids in the rice bran mainly consisted of ferulic acid, catechin, and caffeic acid, and the ferulic acid content was the highest. Compared with the RBE, the ferulic acid, catechin, and caffeic acid contents of the FRBE increased considerably (\(p < 0.05\)). Schmidt et al. (2014) [15] also found that the ferulic acid content of water-soluble rice bran extracts showed significant improvement, up to 765 mg/g. This is consistent with the results of our experiment. Phenolic acids belong to
the polyphenols. The increase in the content of water-soluble phenolic acids in FRBE may be attributed to the degradation of the rice bran cell wall during fermentation.

Table 2. The phenolic acid composition of water-soluble rice bran extracts.

| Compound                        | Content (mg kg\(^{-1}\) dry matter) | RBE       | FRBE      |
|---------------------------------|--------------------------------------|-----------|-----------|
| Catechinic acid                 | 4 ± 0.3\(^{b}\)                      | 10 ± 0.3\(^{a}\) |
| Caffeic acid                    | 1 ± 0.1\(^{b}\)                      | 6 ± 0.3\(^{a}\) |
| Ferulic acid                    | 641 ± 1\(^{b}\)                      | 721 ± 1\(^{a}\) |

There were significant differences between the data of groups of the same category with different superscript letters (\(p < 0.05\)).

3.4. Antioxidant Activity in Zebrafish

3.4.1. Reactive Oxygen Species Production

Zebrafish have become a preferred model animal in many studies, such as those concerning toxicology and pharmacological research [20]. They have been widely used to study antioxidant stress due to their advantages of fast growth and reproduction, fecundity, low costs, and transparent embryos [20,21]. After the fermentation discussed above, we found that fermentation exhibited more prominent effects on the DPPH free radical scavenging rate and bioactive constituents of water-soluble extracts of rice bran. It is well known that AAPH can cause oxidative damage to cells. In this study, we evaluated the protective effects of RBE and FRBE against AAPH-induced oxidative stress in a zebrafish model. The level of ROS production in cells was detected by a fluorescent probe and DCFH-DA; the cell death rate was tested with acridine orange, which was caused by the AAPH treatment, and the lipid peroxidation rate in cells was detected by a fluorescent probe and DPPP [22,23].

The effects of RBE and FRBE on ROS production in AAPH-treated zebrafish are shown in Figure 2a,b. Compared with the control group, the ROS of the AAPH group increased dramatically (174.78%), indicating that the model was established successfully. The ROS production induced by AAPH was significantly reduced by the RBE and FRBE in a concentration-dependent manner at different concentrations (0.2 mg/mL, 0.3 mg/mL, and 0.4 mg/mL). The level of ROS production in the zebrafish exposed to RBE and FRBE at 0.4 mg/mL was significantly reduced to 89.46% and 80.65%, respectively. Furthermore, at the concentration of 0.1 mg/mL, the ROS production of the zebrafish treated with FRBE was significantly lower than in those treated with RBE and AAPH. These results indicate a reduction in ROS generation after RBE and FRBE treatment, and the protective effects of FRBE were greater than those of RBE.

3.4.2. Lipid Peroxidation of Zebrafish Embryos

In fact, LPO is one of the main harmful variations induced by oxygen free radicals, which can generate free radicals, damage the structure and function of biological cells, and interfere with the synthesis of nucleic acids and proteins [24]. Thus, the LPO level is another essential measure of oxidative stress.

Figure 3a,b show different groups of LPO rates visually. The LPO rate was increased by 150% after being treated with AAPH. The FRBE treatments at all the concentrations (0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, and 0.4 mg/mL) significantly decreased the LPO rates to 102.99%, 89.09%, 89.48%, and 102.57%, respectively. In contrast, at the concentrations of 0.2 mg/mL and 0.3 mg/mL, the levels of LPO rate in zebrafish exposed to the RBE were significantly reduced to 109.03% and 108.29%, respectively. The LPO levels of the zebrafish exposed to FRBE at all concentrations were significantly lower than in those exposed to RBE. These results suggest that RBE and FRBE can inhibit lipid peroxidation under a certain concentration and that the protective effects of FRBE were stronger than those of RBE.
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DPPP [22,23].

The free radical inducer AAPH is a kind of hydrophilic small molecular containing
nitric acid, which can cause damaging oxidation reactions. To some degree, oxidative stress
levels can correspond to the cell death rate [25,26]. Cell death has an effect on the main-
tenance of homeostasis, and excessive cell death can destroy the body’s homeostasis [27].
As shown in Figure 4a,b, the AAPH-treated zebrafish displayed significantly increased
cell death rates (150.40%) compared with those in the control zebrafish. The cell death
induced by AAPH was significantly reduced by RBE and FRBE at different concentrations
(0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, and 0.4 mg/mL). Although no significant difference
was detected, the cell death rate of zebrafish exposed to FRBE was lower than in those exposed to RBE (112.11%, 98.71%, 80.35%, and 77.00%). These results suggest that FRBE shows better attenuating effects on
the cell death caused by AAPH in the zebrafish.

Figure 2. Protective effects of RBE and FRBE on AAPH-treated ROS production in zebrafish. (a) ROS levels were measured by image analysis and fluorescence microscopy; (b) ROS levels were measured using Image J software. Experiments were performed in triplicate, and the data are expressed as the means ± SE; Δ p < 0.05 compared to the control group; * p < 0.05 compared to the AAPH-treated group; # p < 0.05 for the comparison between RBE and FRBE.

Figure 3. Protective effect of RBE and FRBE on AAPH-treated lipid peroxidation rate in zebrafish. (a) Lipid peroxidation levels were measured by image analysis and fluorescence microscopy; (b) Lipid peroxidation levels were measured using Image J software. Experiments were performed in triplicate, and the data are expressed as the means ± SE; Δ p < 0.05 compared to the control group; * p < 0.05 compared to the AAPH-treated group; # p < 0.05 for the comparison between RBE and FRBE.

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The first step of the antioxidant system is composed of SOD and CAT. The role of these enzymes is to prevent or reduce the oxidative damage caused by ROS [28]. We measured the SOD and CAT activity of the RBE and FRBE against the AAPH induced in the zebrafish model. As shown in Figure 5a,b, the activity of SOD and CAT of the AAPH-treated zebrafish was more significantly reduced than that of control. The SOD activity induced by AAPH was significantly enhanced by FRBE at the concentration of 0.2 mg/mL. At the concentrations of 0.3 mg/mL and 0.4 mg/mL, the CAT activity induced by AAPH in the FRBE group was significantly higher than that of the AAPH and RBE groups. These results indicate an enhancement of SOD and CAT activity after FRBE treatment.

Figure 4. Protective effect of RBE and FRBE on AAPH-treated cell death in zebrafish. (a) Cell death levels were measured by image analysis and fluorescence microscopy. (b) Cell death levels were measured using Image J software. Experiments were performed in triplicate, and data are expressed as the means ± SE; # p < 0.05 compared to the control group; * p < 0.05 compared to the AAPH-treated group.

3.4.4. Enzyme Activity Assay

Figure 5. Effects of RBE and FRBE on enzyme activity levels in zebrafish exposed to AAPH. (a) Activity of SOD (U mg protein-1); (b) activity of CAT (U mg protein-1). Experiments were performed in triplicate, and the data are expressed as the means ± SE; # p < 0.05 compared to the AAPH-treated group; # p < 0.05 for the comparison between RBE and FRBE.
Mixed-bacteria solid-state fermentation can enhance the protective effects of water-soluble rice bran extracts against AAPH-induced oxidative stress. It was observed that the uronic acid, protein, and phenolic compound contents of cereal bran were closely related to the antioxidant activities \[7,17,29\]. As described previously, the polyphenol, flavonoid, protein, and uronic acid contents were clearly changed after fermentation, which could be the basis for the differences between the RBE and FRBE groups in the measured ROS production, lipid peroxidation, cell death, and SOD and CAT activity.

4. Conclusions

In summary, the DPPH free radical scavenging abilities of rice bran water-soluble extracts and the contents of bioactive constituents were considerably altered by mixed-bacteria solid-state fermentation. Moreover, fermentation improved the protective effects of water-soluble rice bran extracts against AAPH-induced oxidative stress in a zebrafish model. These results provide an experimental basis for the development of novel antioxidant-rich products and suggest that mixed-bacteria solid-state fermentation is applicable to acquiring natural extracts with strengthened antioxidative activity.

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