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Effect of solid-state fermentation on amino acid profile and phytochemicals of red rice bran

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ABSTRACT: We aimed to evaluate the effects of solid-state fermentation (SSF) on red rice bran, specifically for a new rice variety (RD69,Tubtim Chumphae) in Thailand. Fermentation of red rice bran with *Aspergillus oryzae* was continued for 6 days at 30 °C, and LC MS/MS was used to determine the free amino acid composition, antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power, presence of phenolic and flavonoid compounds, and cytotoxicity in HeLa cells. The results showed that SSF significantly increased ($p < 0.05$) the total amino acid content by 59.00% after 5 days of fermentation. Fermented red rice bran (FRRB) contained a significant increase ($p < 0.05$) in antioxidant activity and compounds, which reached their maximum levels by fermentation for 5 days. FRRB also exhibited increased cytotoxicity in HeLa cells compared with unfermented rice bran. The increases of cytotoxicity and antioxidant activities were due to enzymatic hydrolysis and fermentation, in correspondence with the increase in phenolic and flavonoid compounds, suggesting that enzymatic hydrolysis improves the nutritional and phytochemical properties. Notably, the duration of fermentation is a key to optimizing the biological activity of the fermented product.

KEYWORDS: rice bran, amino acid composition, phytochemical, solid-state fermentation

INTRODUCTION

Rice bran is one of the byproducts of rice processing, and it is a source of nutrients and phytochemical compounds. Rice bran is rich in polysaccharides, proteins, micronutrients, and phenolic compounds [1]. Rice bran extracts are also rich in phenolic compounds, and pigmented rice (black and red) exhibits functional properties due to its antioxidant and bioactive compounds (including anticancer) and hypoglycemic activities [2, 3]. Additionally, pigmented rice is a source of phenolic and flavonoid compounds that have important biological functions [4]. Phenolic compounds have shown biological activity against inflammation and activation of the immune system against cancer cells [5]. A new Thai rice variety, known as RD69 (Tubtim Chumphae), contains high antioxidant, phenolic, and flavonoid contents. The name "Tubtim Chumphae" refers to the red pericarp, comparable to the red of a ruby. Tubtim Chumphae rice bran extract protects against oxidative stress and endothelial dysfunction in nitric oxide-deficient hypertension rats [6]. However, the amino acid profile of Tubtim Chumphae has not been well investigated.

Fermentation is a chemical process in food that leads to changes in nutritional and biochemical qualities activated by microbial enzymes [1]. This process increases the concentration of vitamins, essential amino acids, proteins, antinutrients, and flavors; and it also enhances the aroma in food [7]. Changes in the biological components of fermented foods can also enhance the antioxidant activity of phenolic compounds [8]. Several studies have investigated the health advantages of fermented foods for improving treatment of diseases; for example, type 2 diabetes, impaired glucose metabolism, obesity, hyperlipidemia, hypertension, and cancer [9, 10]. Fermentation of cereals has benefits such as the synthesis of bioactive compounds, that act as antimicrobial, antioxidant, and anticancer agents, and the development of functional foods [11].

Solid-state fermentation (SSF) is a fermentation technique that improves the process's efficiency and the functions of the final products [12]. Moreover, this process has the potential to enhance antioxidant activity and phenolic compounds in foods [13]. SSF has been used on solid substrates such as wheat, rice bran, and oats. This technique involves fungi and other microorganisms that require less moisture content [12]. Several studies have revealed that SSF of rice bran has enhanced its anti diabetes, antioxidant, and anti cancer activities [1, 14]. *Aspergillus oryzae* is a well-known fungus that has great potential to produce bioactive compounds by SSF. Interestingly, wheat grain fermented with *A. oryzae* increased the total phenolic content by approximately 22-fold. Nevertheless, some

research has demonstrated that fermentation could decrease the phenolic content [15].

The purpose of this study was to perform SSF with *A. oryzae* using the new red rice bran (Tubtim Chumphae) variety to evaluate the potential improvement in its nutritional value and biological properties and to determine the duration of fermentation required for such improvement. Total content of free amino acids and phenolic compounds, antioxidant activity, and cytotoxic effects of ethanol extracts of fermented red rice bran (FRRB) were examined. Our data revealed that fungal fermentation might be useful in enhancing the nutritional value and biological activity of rice bran, and FRRB could be a potential therapeutic method for the prevention or the treatment of cancer.

MATERIALS AND METHODS

Materials

Individual standards of amino acids, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine, Folin-Ciocalteu reagent, gallic acid, and catechin were purchased from Sigma-Aldrich (MO, USA). Dimethyl sulfoxide was purchased from Labscan (Thailand). All the laboratory chemicals and reagents employed in the study were analytical grade. Red rice (Tubtim Chumphae) was cultivated by the Chumphae Rice Research Center, Rice Department, Thailand. The fungal strain *A. oryzae* was cultured by the Department of Microbiology, Faculty of Science, Khonkaen University, Thailand. HeLa cells, a human cervical cancer cell line, were purchased from the American Type Culture Collection (ATCC; USA).

Fermentation

The fermentation procedure was initiated by soaking red rice bran (100 g solids) in 200 ml of distilled water overnight at room temperature. After removing the water, the soaked red rice bran was sterilized by autoclaving (121 °C, 15 min). Then, the cooled red rice bran was inoculated with 0.1% *A. oryzae* spore powder, and the mixture was incubated at 30 °C for 6 days.

Total amino acid analysis

For sample preparation, a 100 mg of FRRB was finely ground to powder under liquid nitrogen. A volume of 0.5 ml of 0.05 M aqueous HCl-ethanol (1:1, v/v) was mixed and vortexed for 5 min with a vortex mixer (Labmart, LM-3000, Malaysia). The obtained mixture was centrifuged, using a centrifuge (Hettich GmbH, Germany), at 4 °C 12,100×*g* for 15 min. The clear supernatant was analyzed by LC MS/MS, using an LC MS-8030 triple-quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) operated in the ESI mode and a Shimadzu LC-20AC series HPLC system (Shimadzu, Kyoto, Japan).

The content of amino acids was determined using a method previously reported by Chumroenphat et al [16].

The HPLC analysis was operated under the following conditions: flow rate, 0.2 ml/min; temperature of the column oven, 38 °C; and autosampler, at 4 °C. The mobile phases were prepared by (A) combining water with 0.1% (v/v) formic acid, and (B) the solution was diluted with the methanol (1:1). The autosampler needle was purged with methanol before and after aspiration of the sample.

The MS/MS mode was operated under the following conditions: Multiple Reaction Monitoring (MRM) mode was used; capillary voltage was set at 4.5 kV (positive mode, ESI (+)); cone voltage was set at 1.72 kV; and ion source temperature was set at 400 °C. The amino acids were identified by their m/z values and by comparison to the retention time of standards. All other settings were analyte-specific and were auto-optimized by flow injection of 2 µl of solution in methanol containing 1 ppm of one analyte. The results of the auto-optimizations were summarized in Table S1.

Extraction for determination of biological activity

The extraction method used for both fermented and unfermented samples was carried out according to Shin et al [17]. In the first step of the procedure, the samples were briefly defatted by dispersing them in *n*-hexane (1:10, w/v) twice at room temperature prior to alcohol extraction. The samples were extracted in 50% aqueous ethanol (1:20, w/v) by stirring at room temperature for 12 h. The extracted filtrate was evaporated using a rotary evaporator (Heidolph Laborota 4000 efficient, Germany) under reduced pressure at 40 °C. The extracts were dried in a freeze-drier (Model Coolsafe 55, Scanvac, Lynge, Denmark) and stored at −20 °C until analysis.

Determination of antioxidant activity

The antioxidant activities of RRB and FRRB samples were examined using the following antioxidant assays.

DPPH radical scavenging activity assay

The presence of radical scavenging activity was evaluated according to the DPPH assay described by Brand-Williams et al [18]. The assay based on the reduction of DPPH radicals was evaluated by measuring the absorbance at 517 nm with a GENESYS 10 S UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Trolox was used as a positive control. The assay was performed in triplicate. The radical scavenging activity was calculated as a percentage using the following equation:

$$\% \text{ scavenging activity} = 100 \times [1 - (A_c/A_d)]$$

where A_c is the absorbance of the sample extract added to the DPPH solution, and A_d is the absorbance of the control or DPPH solution only.

Ferric reducing antioxidant power (FRAP) assay

The reducing power of the sample extracts was determined using the method described by Benzie and Strain [19] with slight modification. FRAP solution consisting of 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3\,H_2O$ and 16 ml $C_2H_2O_2$, pH 3.6) and 10 mM 2,4,6-tripyridyl-S-triazine was mixed with 75 µl of each of the extracts. After 30 min of incubation, the absorbance was measured at 593 nm. The standard curve of Trolox was linear between 20 and 100 µg/ml.

Determination of antioxidant compounds

The antioxidant compounds of the sample extracts included the total phenolic and flavonoid contents.

Total phenolic content (TPC)

The determination of TPC was performed using Folin-Ciocalteu reagent according to the method described by Yawadio et al [20]. The absorbance was measured at 765 nm. Gallic acid was applied as a standard, and the TPC was reported as mg of gallic acid equivalents.

Total flavonoid content (TFC)

The TFC was determined by colorimetric analysis according to the method described by Abu-Bakar et al [21]. The extracts (1 mg/ml) were mixed with 0.5 ml of 2% aluminum chloride in ethanol and incubated for 60 min at room temperature. The absorbance was measured at 420 nm. The TFC was reported as mg of catechin equivalents.

Cytotoxicity assay

To determine the effect of extracts on HeLa cell viability, the sulforhodamine B (SRB) assay was used according to Buranrat et al [22]. The extracts were weighed and dissolved in 10% (v/v) dimethyl sulfoxide. Cells were cultured with each of the extract at various concentrations (0–5000 µg/ml) for 24–72 h, and cell viability was determined by SRB. After incubation, cells were fixed with 10% trichloroacetic acid, stained with 0.4% SRB, washed with 1% acetic acid, and solubilized with 10 mM Tris base. The optical density at 540 nm was measured by spectrophotometry (*Opsys* MR™ Microplate Reader; Dynex Technologies, Chantilly, VA, USA). The percentage cytotoxicity was calculated using the following formula [23]:

$$\% \text{ cytotoxicity} = 100 - ((A_s - A_b)/(A_c - A_b)) \times 100$$

where A_s = Absorbance value of sample, A_b = Absorbance value of blank, and A_c = Absorbance value of control.

Statistical analysis

All statistical analyses were performed using STATA version 13.0 software (Stata Corp, College Station, TX). The data are presented as the mean ± standard deviation (SD) of the measurements of triplicate samples. Statistical significance between groups was analyzed by one-way ANOVA followed by Tukey's post hoc test. The comparison of means was considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Amino acid determination

The type and content of 20 amino acids in FRRB samples were studied using LC MS/MS, and the results were shown in Table 1. The levels of total amino acids at 4, 5, and 6 days of fermentation were significantly increased (ANOVA, Tukey post hoc, $p < 0.05$) compared with unfermented red rice bran. The total amino acid contents reached the maximum on the 5th day. After 4 days of fermentation, the increases in total amino acid contents were relatively minimal, indicating that the microorganism growth occurred mainly within three days. Notably, the essential amino acids methionine, phenylalanine, isoleucine, leucine, and lysine increased greatly in 5 days of fermentation to 2.03, 2.40, 2.14, 2.10, and 2.84 times, respectively. The concentration of total essential amino acids in the fermented substrate also increased by 9.23%, while the total non-essential amino acids decreased by 18.97%.

The alterations in amino acid profiles may vary depending on the fungal metabolism [24]. Inoculating with *A. oryzae* for 5 days of fermentation increased the total amino acid content of FRRB by 1.69 times, which was significantly higher than that of the unfermented rice bran. The increase in total amino acids may be due to small-molecule peptides formed by the amino acids from decomposed FRRB protein, a result of extracellular proteases produced by *A. oryzae*. Notably, amino acids are a rich source of nitrogen for living organisms [25, 26]. The increased amino acid content could positively affect the bioactivity of FRRB because it might contribute to antioxidant and metal-chelating activities [27]. Furthermore, enzymatic hydrolysis could influence structure, texture, and bioactive properties of proteins [28]. However, the total amino acids on the 1st and 2nd day of fermentation decreased from day 0, possibly because some amino acids were metabolized after two days of fermentation. According to Shin et al [17], most of the microorganisms grew after the initial two days of fermentation, and the hydrolytic enzymes were active in the late stage of fermentation.

Antioxidant activity by DPPH and FRAP assays

The antioxidant activity of the ethanol extracts from FRRB samples was determined. The outcome of DPPH radical scavenging was presented as the inhibition

Table 1 Amino acid content of 20 amino acids in FRRB samples.

Amino acid	Amino acid content at different fermentation durations (μg/g)						
	0 day	1 day	2 days	3 days	4 days	5 days	6 days
Aspartic acid	13.31 ± 1.43^{c}	7.68 ± 0.38^{e}	9.21 ± 1.06^{d}	8.88 ± 0.14^{d}	15.13 ± 0.26^{b}	16.30 ± 0.32^{a}	15.02 ± 0.11^{b}
Glutamic acid	66.39 ± 0.58^{b}	46.38 ± 1.18^{e}	61.31 ± 1.10^{c}	52.22 ± 1.11^{d}	67.67 ± 0.74^{b}	79.46 ± 2.43^{a}	69.16 ± 1.44^{b}
Serine	6.75 ± 0.31^{d}	2.80 ± 0.18^{f}	4.48 ± 0.04^{e}	7.47 ± 0.48^{c}	9.53 ± 0.44^{b}	11.61 ± 0.44^{a}	11.61 ± 0.44^{a}
Asparagine	8.43 ± 0.77^{a}	1.04 ± 0.09^{f}	1.77 ± 0.14^{e}	4.08 ± 0.09^{d}	7.82 ± 0.09^{b}	7.25 ± 0.32^{c}	7.50 ± 0.12^{c}
Threonine*	11.19 ± 0.84^{b}	2.02 ± 1.27^{f}	5.95 ± 0.01^{e}	8.32 ± 0.25^{d}	11.37 ± 0.25^{b}	13.23 ± 0.23^{a}	10.14 ± 0.31^{c}
Glutamine	11.83 ± 0.87^{d}	3.61 ± 0.13^{f}	8.59 ± 0.21^{e}	18.85 ± 0.80^{c}	30.89 ± 1.51^{b}	35.42 ± 1.64^{a}	31.21 ± 0.94^{b}
Tyrosine	164.46 ± 4.50^{c}	98.34 ± 1.24^{d}	131.79 ± 4.60^{e}	152.54 ± 1.99^{d}	218.20 ± 2.91^{b}	251.71 ± 2.45^{a}	221.10 ± 3.10^{b}
Glycine	ND	ND	ND	ND	ND	ND	ND
Proline	23.91 ± 0.24^{a}	18.71 ± 0.08^{b}	13.05 ± 1.31^{d}	11.14 ± 0.09^{e}	6.38 ± 0.36^{f}	15.95 ± 1.17^{c}	12.52 ± 0.91^{d}
Alanine	20.77 ± 0.18^{c}	23.23 ± 0.48^{b}	11.59 ± 0.50^{d}	8.75 ± 0.37^{e}	8.73 ± 0.27^{e}	28.15 ± 0.68^{a}	24.46 ± 0.61^{b}
Methionine*	289.96 ± 13.01^{d}	137.27 ± 2.17^{f}	188.04 ± 4.75^{e}	284.24 ± 3.00^{d}	466.08 ± 3.77^{c}	589.75 ± 6.80^{a}	491.25 ± 5.01^{b}
Valine*	38.92 ± 2.26^{d}	18.59 ± 0.27^{g}	29.27 ± 0.87^{f}	32.30 ± 0.21^{e}	45.77 ± 0.26^{c}	56.04 ± 1.19^{a}	49.12 ± 0.13^{b}
Phenylalanine*	87.53 ± 1.98^{f}	48.86 ± 0.91^{g}	93.11 ± 2.27^{e}	116.72 ± 2.60^{d}	144.37 ± 0.61^{c}	209.31 ± 2.76^{a}	156.15 ± 3.26^{b}
Isoleucine*	68.92 ± 1.39^{e}	32.74 ± 0.20^{g}	55.76 ± 0.13^{f}	80.32 ± 1.31^{d}	104.33 ± 1.23^{c}	148.83 ± 2.49^{a}	114.65 ± 3.01^{b}
Leucine*	63.80 ± 1.91^{e}	30.97 ± 0.72^{g}	50.65 ± 1.82^{f}	73.72 ± 0.43^{d}	94.09 ± 1.84^{c}	132.26 ± 1.34^{a}	114.01 ± 1.19^{b}
Tryptophan*	79.83 ± 3.20^{a}	56.26 ± 0.47^{c}	34.75 ± 1.34^{e}	36.92 ± 1.01^{e}	65.66 ± 0.69^{b}	56.68 ± 0.81^{c}	53.41 ± 0.32^{d}
Cysteine	ND	ND	ND	ND	ND	ND	ND
Histidine*	8.72 ± 0.48^{c}	4.22 ± 0.75^{e}	2.69 ± 0.37^{f}	6.53 ± 0.30^{d}	13.24 ± 0.27^{a}	11.51 ± 0.31^{b}	9.32 ± 0.25^{c}
Lysine*	13.12 ± 0.23^{e}	3.42 ± 0.17^{g}	6.13 ± 1.65^{f}	19.24 ± 0.36^{d}	30.46 ± 1.52^{c}	37.29 ± 1.88^{a}	34.12 ± 0.67^{b}
Arginine	87.95 ± 0.47^{d}	62.04 ± 0.69^{e}	64.16 ± 1.45^{e}	79.06 ± 1.08^{f}	95.10 ± 1.20^{c}	105.12 ± 1.11^{a}	98.81 ± 1.09^{b}
Total	1065.79 ± 34.65^{d}	598.18 ± 11.38^{g}	772.30 ± 23.62^{f}	1001.30 ± 15.62^{e}	1434.82 ± 18.22^{c}	1805.87 ± 28.37^{a}	1523.56 ± 22.90^{b}

* Essential amino acid.
ND: Not detected. Values are shown as mean ± SD ($n = 3$ measurements). The means with different letters in each row are significantly different ($p < 0.05$).

Table 2 Antioxidant activity and compounds of FRRB samples at different fermentation durations.

Fermentation duration (day)	Antioxidant activity		Antioxidant compounds	
	DPPH (% scavenging activity)	FRAP (mmol $FeSO_4$/100 g dry weight)	TPC (mg/100 g)	TFC (mg/100 g)
0	56.12 ± 0.77^{f}	232 ± 32.01^{e}	7.82 ± 0.32^{g}	10.24 ± 1.11^{f}
1	61.99 ± 2.02^{e}	445 ± 47.81^{d}	16.48 ± 1.81^{f}	19.56 ± 5.54^{e}
2	66.94 ± 0.84^{d}	654 ± 90.74^{c}	20.44 ± 0.92^{e}	20.89 ± 0.64^{e}
3	68.60 ± 1.12^{d}	693 ± 24.91^{c}	33.39 ± 3.44^{d}	25.65 ± 2.23^{d}
4	73.03 ± 2.11^{c}	782 ± 56.36^{b}	40.89 ± 0.45^{b}	36.12 ± 0.96^{a}
5	88.27 ± 1.31^{a}	891 ± 45.15^{a}	43.07 ± 2.89^{a}	33.12 ± 1.43^{b}
6	79.61 ± 1.07^{b}	753 ± 13.89^{b}	38.17 ± 1.88^{c}	30.89 ± 2.11^{c}

Values are shown as mean ± SD of triplicate measurements. The means with different letters in each column are significantly different ($p < 0.05$).

percentage, and the results were shown in Table 2. The FRRB samples showed a significant increase (ANOVA, Tukey post hoc, $p < 0.05$) in the inhibition percentages compared with the unfermented rice bran (day 0). The inhibition highest percentage (88.27%) was found on day 5, followed by day 6 and day 4 ($p < 0.05$). In addition, the inhibitory concentration at 50% (IC_{50}) of the FRRB sample on day 5 was 41.00 μg/ml at five different concentrations (20–100 μg/ml). The antioxidant activities of the FRAP assay were expressed as the concentrations of antioxidants with a ferric reducing ability equivalent to that of 1 mM $FeSO_4$. The sample extract of FRRB on day 5 showed the maximum activity (Table 2), and the extract from day 0 showed the minimum activity; whereas the IC_{50} of FRAP capacity was 53.19 μg/ml. Fermented rice bran is rich in polyphenolic compounds and several bioactive compounds. The products of the fermentation process have many pharmacological effects, including antioxidant and anticancer activities [29–31]. In this study, the bioactive compounds (TPC and TFC) and the biological activity (antioxidant and cytotoxicity to cancer cells) of FRRB on days 1–6 were investigated. Several studies have shown that SSF is a useful method for producing secondary metabolites that can be applied to the production of drugs, food supplements, and fortified functional food products [32–34]. Among these processes, fungal strains are widely used for the improvement of phenolic compounds in natural substrates [35]. Our results showed that all red rice bran had antioxidant activity, but FRRB possessed higher activity than unfermented red rice bran, especially at the 5th day of fermentation. The inhibition percentage of the FRRB (98.41%), determined by the DPPH assay,

was higher than that of the unfermented rice bran (78.32%); and the FRAP assay showed a similar trend. Our results were consistent with Purewal et al [36], who showed that SSF with *A. oryzae* increased antioxidant properties in fermented millet. In addition, they explained that microbial transformation during SSF induced various biochemical changes in millet koji, and these specific changes resulted in an increase in the antioxidant properties of the extracts. Postemsky et al [33] also observed that black rice bran subjected to SSF with *A. awamori* showed increased antioxidant activity, with the highest antioxidant activity on fermentation day 3. Moreover, they explained that SSF was an effective process to increase the phenolic content and antioxidant activity. Therefore, our results revealed that FRRB increased antioxidant activity compared with unfermented red rice bran.

Antioxidant compounds

All results of the analysis of antioxidant compounds, including TPC and TFC, were shown in Table 2. Less TPC was observed on day 0 than on all other fermentation durations, with the highest content on fermentation day 5. There were significant differences among the different durations of fermentation (ANOVA, Tukey post hoc, $p < 0.05$). The TPC of the FRRB extract ranged from 7.82 to 38.17 mg gallic acid equivalents/g dry weight. The TFC was analyzed as a proxy for bioactive compounds in the extracts, and the highest TFC of the FRRB extract was observed on fermentation day 4. Significant differences were found among the different days of fermentation ($p < 0.05$).

Many studies have hypothesized that microbial enzymes may hydrolyze cells and result in the release of TPC during fermentation [36–38]. Corresponding with our results, the TPC and the TFC of FRRB were higher than those of unfermented red rice bran, and the duration of fermentation significantly ($p < 0.05$) affected both the TPC and the TFC. According to previous studies, red-brown rice extract fermented for 4 days at 30 °C had more polyphenol compounds than unfermented red-brown rice extract [17], which was similar to our results of higher antioxidant activity and concentrations on fermentation day 5. This improvement was explained by the fact that the TPC in rice was increased by microbial enzymes during fermentation, resulting in enhanced antioxidant activity. Moreover, TPC in fermented rice was increased 5 times, while TFC was increased 2.5 times, by the fermentation process [38]. In contrast, Olukomaiya et al [15] showed that SSF of canola meal with *A. sojae* at 30 °C for 7 days reduced the TPC. They assumed that the decrease in TPC may be due to the buildup of free radicals during fungal metabolism. The free radicals, then, interacted with antioxidants and degraded upon exposure to factors such as higher temperatures, higher oxygen concentrations, and moisture.

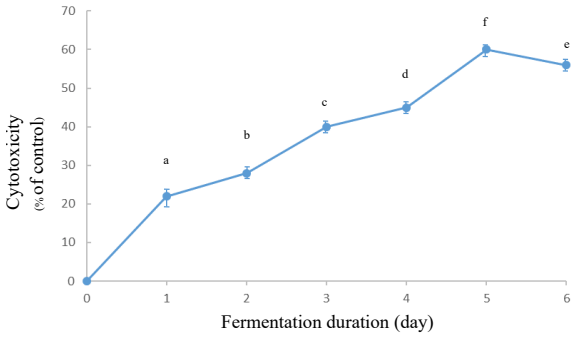

Fig. 1 Cytotoxicity of FRRB sample extracts on HeLa cell after different durations of fermentation introduced at a concentration of 5000 μg/ml and incubated for 72 h. Values are expressed as the percentage of cytotoxicity ($n = 3$). The results are presented as the percentage of the untreated control groups (0 μg/ml). The means with different letters on the graph lines indicate that the values are significantly different (Tukey's post hoc test, $p < 0.05$) from each other.

Cell cytotoxicity

The cytotoxicity of FRRB on HeLa cancer cells was determined by the SRB assay. The FRRB extracts exhibited cytotoxic effects against the HeLa cancer cells, and the highest levels of cytotoxicity was found on fermentation day 5 (Fig. 1). The changes of cytotoxicity during fermentation were related to the change in TPC, suggesting that phenolic acids were the main bioactive compounds in FRRB extracts responsible for cytotoxicity in HeLa cells. The SSF with *Aspergillus* sp. was also enhanced by fungal enzyme, hydrolyzing insoluble matrices to phenolic compound products. Hence, the enhancemed phenolic compounds increased the antioxidant activity and cytotoxicity activity [17].

CONCLUSION

This study demonstrated that amino acid profile, antioxidant activity, antioxidant compound content, and cytotoxic effect of red rice bran (Tubtim Chumphae) could be increased by SSF. In addition, the duration of fermentation was essential for enhancing its nutritional value and biological activity. The biological activity of FRRB indicated that it contained elevated levels of bioactive compounds with potential application in the development of therapeutic strategies for prevention or treatment of cancer.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2022.131.

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Appendix A. Supplementary data

Table S1 MRM conditions for amino acid on LC/MS/MS.

No.	Amino acid	Precursor ion $[M+H]^+$ (m/z)	Product ion (m/z)	Q1 Pre Bias (V)	Collision energy (V)	Q3 Pre Bias (V)
1	Aspartic acid	134.00	74.00	−15.00	−15.00	−16.00
2	Glutamic acid	148.00	84.00	−17.00	−18.00	−18.00
3	Serine	148.00	60.00	−13.00	−12.00	−10.00
4	Asparagine	133.00	74.00	−13.00	−17.00	−16.00
5	Threonine	120.00	74.00	−15.00	−74.00	−20.00
6	Glutamine	147.00	84.00	−25.00	−18.00	−19.00
7	Tyrosine	182.00	136.00	−21.00	−13.00	−16.00
8	Glycine	76.00	30.00	−13.00	−12.00	−12.00
9	Proline	116.00	70.00	−12.00	−17.00	−16.00
10	Alanine	90.00	44.00	−10.00	−13.00	−18.00
11	Methionine	150.05	104.00	−20.00	−18.00	−29.00
12	Valine	118.00	72.00	−18.00	−13.00	−14.00
13	Phenylalanine	166.00	120.00	−14.00	−14.00	−18.00
14	Isoleucine	132.00	86.00	−14.00	−10.00	−18.00
15	Leucine	132.00	86.00	−26.00	−27.00	−29.00
16	Tryptophan	205.00	146.00	−12.00	−12.00	−14.00
17	Cysteine	122.00	59.00	−12.00	−24.00	−28.00
18	Histidine	156.05	110.05	−12.00	−13.00	−23.00
19	Lysine	147.00	84.00	−13.00	−13.00	−15.00
20	Arginine	175.00	70.00	−17.00	−17.00	−18.00