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Antioxidant and antityrosinase activities in germinated brown rice of indigenous Thai cultivars

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Abstract The antioxidant and antityrosinase activities were examined in germinated brown rice of three indigenous Thai cultivars, namely Riceberry (purple), KDML 105 R-PSL-2 (red) and KDML 105 (white), which are commonly grown in Buriram province of Thailand. Germination was induced by steeping brown rice of each cultivar in distilled water (water: grain ratio = 2:1) at ambient temperature for 6 h. After low temperature induction at 8 – 10 ℃ for 24 h, the rice kernels were allowed to germinate in a double-layered cotton cloth in the dark at ambient temperature for 0, 24 and 48 h, in which the antioxidant and antityrosinase activities of germinated brown rice were evaluated. The results revealed that germinated brown rice of all studied cultivars appeared to display higher levels of antioxidant and antityrosinase activities than ungerminated brown rice. The results showed 24-48 h germination regimes were most effective in maximizing the antioxidant and antityrosinase activities in germinated brown rice. Under such conditions, the maximum quantities of total phenolics, total flavonoids and antityrosinase were detected in Riceberry (25.06 mg GAE/g DW), KDML 105 R-PSL-2 (95.67 mg QE/g DW) and Riceberry (31.33%), respectively. Again, the highest DPPH value of 81.52% was detected in Riceberry and the highest ABTS value of 67.92% in KDML 105 R-PSL-2. Additionally, a strong correlation between antioxidant, antityrosinase and germination time was detected. The information obtained from this study should provide a foundation for future research aiming at the development of functional foods and/or cosmetics.

Keywords: ABTS, DPPH, flavonoid, *Oryza sativa*, phenolic

Introduction

Melanin in human skin, which is synthesized as a key defense mechanism against ultraviolet (UV) radiation from the sun, plays a central role in skin color and pigmentation. When exposed to UV radiation, melanogenesis is stimulated via the enzyme tyrosinase, which may lead to undesirable pigmentation (Jin *et al.*, 2015; Lan *et al.*, 2013). In addition to exogenous chemicals and endogenous metabolic processes in the human body or in food, UV radiation

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can trigger the production of reactive oxygen species (ROS), which can result in cell death and tissue damage, protein degradation in extracellular matrix, and elastin and collagen breakdown, leading to skin aging and cancer (Lin *et al.*, 2015a; Tiraravesit *et al.*, 2015). Accordingly, inhibition of tyrosinase along with ROS scavenging is a potentially valuable strategy for improving skin color and skin health. In particular, the search for bioactive compounds with high antioxidant and antityrosinase activities from natural sources is among the hottest themes in medicinal and cosmetic fields (Di Petrillo *et al.*, 2016; Pintus *et al.*, 2015).

Rice (*Oryza sativa* L.) is one of the important food crops feeding over 50% of the world's population, particularly in Asia (Thuengtung *et al.*, 2018). Recently, due to its high nutritional quality and biological activity, brown rice has been categorized as one of the potent functional foods (Ding *et al.*, 2018). However, brown rice is not extensively consumed since it is poorly cooked and has hard texture owing to high fiber contents in the bran (Kaur *et al.*, 2017). Meanwhile, germinated brown rice is increasingly gaining attention, especially in Asian countries, owing to its improved eating quality and potential health-promoting functions (Cho and Lim, 2016; Patil and Khan, 2011; Wu *et al.*, 2013). Germination of brown rice causes significant improvements in the levels of nutrients and bioactive compounds, including γ-aminobutyric acid (GABA), phenolic acids, tocotrienols, ferulic acid, γ-oryzanol, potassium, magnesium, zinc and dietary fiber (Chompoopong *et al.*, 2016; Lin *et al.*, 2015b). In comparison to white or polished rice, these germinated components were 10-fold higher for GABA and nearly 4-fold higher for dietary fibers, vitamin E, niacin and lysine (Cho and Lim, 2016).

A plethora of studies have been implemented to assess the antioxidant properties in germinated brown rice, with only few studies focused on determining the tyrosinase inhibitory effect. Thus, investigation of the antityrosinase activity of germinated brown rice is of great importance. Previous studies have unravelled that during germination changes in the biochemical components and bioactive substances of germinated rice are influenced by several factors, such as grain moisture contents, cultivars, and germination time and temperature (Kaur *et al.*, 2017; Lin *et al.*, 2015b). Accordingly, optimization of the germination conditions for the highest contents of antioxidants and antityrosinase in germinated brown rice is of particular interest.

In this study, germination conditions for brown rice were studied to determine the optimal conditions for the maximal production of antioxidants and antityrosinase that could be utilized for skin-protective and cosmetic purposes. In this attempt, the crude extracts of germinated brown rice of three

indigenous Thai cultivars germinated under different conditions were compared for their antioxidant and antityrosinase activities and were benchmarked against those of ungerminated brown rice.

Materials and methods

Chemicals and reagents

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH), Folin–Ciocalteu's reagent and 6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid (trolox) were obtained from Fluka (Buchs, Switzerland). Ascorbic acid, tyrosinase, Kojic acid and 3,4-Dihydroxyl-L-phenylalanine (L-DOPA) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). All analytical grade solvents including methanol and dimethyl sulfoxide (DMSO) were obtained from Fluka (Buchs, Switzerland).

Rice samples and germination process

The brown rice of three indigenous Thai cultivars, namely Riceberry (purple), KDML 105 R-PSL-2 (red) and KDML 105 (white), was obtained from local agricultural cooperative markets in Buriram province of Thailand. First, the rice grains (500 g) of each cultivar were thoroughly cleaned and defective grains were excluded. Then, the brown rice was steeped in distilled water (water: grain ratio = 2:1), at ambient temperature (25 ℃) for 6 h. Following 24-h cold induction at 10 ℃, the steeped rice grains were left to germinate in the dark in a double-layered cotton cloth at ambient temperature for 0, 24 and 48 h. The germinated brown rice was collected and dried at 60 ℃ for 6 h, ground to a fine powder and sieved through a 2-mm mesh before storage in zip-lock plastic bags at –20 ℃.

Extraction of the germinated brown rice was carried out using maceration method by dissolving 1 g of the germinated brown rice powder in 10 mL of methanol and the mixture was left in the dark for 24 h. After filtration through a 0.45-μm filter membrane, the filtrate was centrifuged at 10,000 rpm for 10 min and analyzed for the antioxidant and antityrosinase activities.

Determination of total phenolic and total flavonoid contents

Total phenolic content was quantified by Folin-Ciocalteu colorimetric method (Pang *et al*., 2018) with minor modifications. In brief, a 10-μL volume of the extract (100 mg/mL) was reacted with 100 μL of 10% Folin-Ciocalteu's

reagent and 90 µL of 7.5% Na_2CO_3. After vigorous shaking and 30-min settlement at ambient temperature, the mixture was measured at 765 nm. Gallic acid was used as the standard and total phenolic content was expressed in mg gallic acid equivalent per g dry weight (mg GAE/g DW). Total flavonoid content was assessed by the aluminum chloride colorimetric method (Do et al., 2014) with trivial modifications. In brief, a 10-µL volume of the extract (100 mg/mL) was added to 20 µL of 5% $NaNO_3$ and the mixture was allowed to settle at ambient temperature for 6 min. Subsequently, the mixture was reacted with 20 µL of 10% $AlCl_3$ solution and left to stand for 6 min. Then, a 150-µL amount of 1 M NaOH was added and the mixture was allowed to settle for 10 min. The resultant mixture was measured at 510 nm. Quercetin was employed as the standard and total flavonoid content was expressed in mg quercetin equivalent per g dry weight (mg QE/g DW).

Determination of antioxidant activities

In order to evaluate the antioxidant capacity of the extracts, DPPH and ABTS free-radical scavenging assays were employed. DPPH free-radical scavenging method was conducted as per the method of Promden et al. (2014) with trivial modifications. Briefly, a 10-µL volume of the extract (100 mg/mL) was mixed with 190 µL of 100 µM DPPH methanolic solution. Following 30-min incubation at 37 ℃ in the dark, the mixture was measured at 515 nm. Ascorbic acid served as the positive controls and percent DPPH scavenging was calculated as follows: DPPH scavenging (%) = $(1 - A_s/A_c) \times 100$, where A_c and A_s denote the absorbance of the control and sample, respectively. ABTS free-radical scavenging method was implemented according to Shen et al. (2017) with some modifications. In brief, a 10-µL amount of the extract (100 mg/mL) was mixed with 190 µL of ABTS free-radical solution. After 6-min incubation at 37 ℃ in the dark, the mixture was measured at 734 nm. Trolox was utilized as the positive controls and percent ABTS scavenging was calculated as follows: ABTS scavenging (%) = $(1 - A_s/A_c) \times 100$, where A_c and A_s are the absorbance of the control and sample, respectively.

Tyrosinase inhibitory assay

The tyrosinase inhibitory activity of the extracts was determined using the dopachrome method described by (Likhitwitayawuid and Sritularak, 2001) with some modifications. The extracts, tyrosinase enzyme and L-DOPA solutions were dissolved in 50% v/v dimethyl sulfoxide (DMSO) and 0.1 M phosphate buffer solution (pH 6.8). The *o*-diphenolase activity of mushroom tyrosinase

was determined in 96-well plates, in which 10 µL of the extract (100 mg/mL) were mixed with 70 µL of 0.1 M phosphate buffer (pH 6.8) and 10 µL of mushroom tyrosinase (100 U/mL). After a 10-min pre-incubation process at 37 ℃, the mixture was added 10 µL of 2.5 mM L-DOPA and incubated at 37 ℃ for another 40 min to allow the conversion of L-DOPA to dopachrome, in which a change in color from colorless to orange occurred. Then, the mixture was measured at 475 nm. Kojic acid served as the positive control, the reaction mixture in the absence of the enzyme acted as blank and 50% DMSO instead of the extract served as the negative control. The inhibitory activity of the extract was expressed as % inhibition and was calculated as follows: % inhibition = $(1 - A_s/A_c) \times 100$, where A_c and A_s represent the absorbance of the control and sample, respectively.

Statistical analysis

All treatments and determinations were implemented in triplicate and the data are expressed as the mean ± one standard deviation. One-way analysis of variance followed by Duncan's multiple range tests were employed for analyzing the variance ($p < 0.05$) of the data.

Results

Total phenolic and total flavonoid contents

As presented in Table 1, it was observed that significant differences in the total phenolic and total flavonoid contents were detected between ungerminated and germinated rice. A 48-h germination process resulted in the highest total phenolic content in Riceberry (25.06 mg GAE/g DW) and KDML 105 (16.04 mg GAE/g DW). Meanwhile, the highest total phenolic content of 24.92 mg GAE/g DW was observed for KDML 105 R-PSL-2 subjected to a 24-h germination process. As with the total flavonoid contents, germinating brown rice of KDML 150 R-PSL-2 and KDML 105 for 24 h yielded the highest total flavonoid content of 95.67 and 43.00 mg QE/g DW, respectively. Meanwhile, a 48-h germination regime was most suitable for the maximum total flavonoid content of 88.89 mg QE/g DW in Riceberry.

Table 1. Effect of germination time on the total phenolic and total flavonoid contents of germinated brown rice of Riceberry, KDML 105 R-PSL-2 and KDML 105

Total phenolic /total flavonoid contents	Germination time (h)	Rice cultivars		
		Riceberry	KDML 105 R-PSL-2	KDML 105
Total phenolic content (mg GAE/g DW)	0	9.74±0.70c	16.09±1.09b	9.18±0.55b
	24	19.51±1.62b	24.92±2.61a	8.80±0.50b
	48	25.06±1.94a	20.37±2.47b	16.04±0.93a
Total flavonoid content (mg QE/g DW)	0	41.11±3.37c	27.00±1.46c	30.78±3.10b
	24	65.67±6.08b	95.67±2.40a	43.00±1.45a
	48	88.89±3.56a	57.56±2.83b	41.33±5.57a

Results are expressed as mean ± standard deviation.
Mean values followed different letters in the same column are significantly different at $p < 0.05$.

Table 2. Effect of germination time on the antioxidant and antityrosinase activities of germinated brown rice of Riceberry, KDML 105 R-PSL-2 and KDML 105

Antioxidant and antityrosinase activities	Germination time (h)	% Scavenging/inhibition		
		Riceberry	KDML 105 R-PSL-2	KDML 105
DPPH assay	0	49.46±2.41c	37.91±2.47c	27.62±1.22b
	24	65.26±3.82b	78.97±0.65a	30.92±3.10b
	48	81.52±1.99a	71.64±2.14b	36.22±1.73a
ABTS assay	0	24.69±0.51c	31.94±0.61c	13.94±0.53c
	24	58.58±1.72a	67.92±2.30a	21.76±1.94b
	48	48.26±1.80b	61.98±1.35b	43.79±2.66a
L-Dopachrome	0	26.89±3.42a	20.66±1.15a	15.56±1.93b
	24	31.33±1.76a	18.67±2.00a	19.55±1.71a
	48	29.56±2.52a	20.11±1.34a	13.55±2.04b

Results are expressed as mean ± standard deviation.
Mean values followed different letters in the same column are significantly different at $p < 0.05$.

Antioxidant and antityrosinase activities

Germination was found to induce a significant increment in the antioxidant activity of germinated brown rice of all studied cultivars (Table 2). Similar to the total phenolic content, the highest level of antioxidant activity for Riceberry and KDML 105 was observed for a 48-h germination regime, with the % scavenging value of 81.52 and 36.22%, respectively, as indicated by the DPPH assay. The highest level of antioxidant activity for KDML 105 R-PSL-2 was detected when rice brown was left to germinate for 24 h, giving the % scavenging value of 78.97%. According to the ABTS assay, a 24-h germination process was most appropriate for maximizing the antioxidant activity in Riceberry (58.58%) and KDML 105 R-PSL-2 (67.92%). Meanwhile, KDML 105 showed the highest antioxidant activity of 43.79% when the brown rice was allowed to germinate for 48 h.

The tyrosinase inhibitory assay (L-dopachrome) showed that germination only caused a significant increment in the antityrosinase activity in germinated brown rice of KDML 105 subjected to a 24-h germination process, with the % inhibition of 19.55% (Table 2). Significant differences in the antityrosinase activity were not observed for germinated brown rice of Riceberry and KDML 105 R-PSL-2.

Discussion

Results revealed that the total phenolics, total flavonoids, antioxidants and antityrosinase activities of brown rice of all studied cultivars could be enhanced through the germination process, which was well supported by an earlier study (Lin *et al.*, 2015b). The levels of total phenolics, total flavonoids, antioxidants and antityrosinase activities of both ungerminated and germinated brown rice vary across cultivars, indicating that cultivar is one of the important factors contributing to differences in the accumulation of bioactive substances in brown rice during germination. The literature has revealed that water requirement for germination depends on the dormancy period and cultivar (Takahashi, 1984). The steep water triggers cell respiration, cell elongation, secretory activity of the embryo and activation of enzymes (Pyler and Thomas, 2000).

In the present study, germination of brown rice was maintained under aerobic conditions to ensure the high germination rate. Despite the superior ability of rice seeds to germinate and grow at low oxygen conditions in comparison to many other plant species, gaseous concentrations less than 0.3% have been reported to slow down germination, growth and root/shoot ratio (Baker and Hatton, 1987).

Temperature is another factor that affects germination. High germination (90–97%) occurs during the first 48 h at a temperature range of 27–37 ℃. On the other hand, germination rates drop considerably at lower temperatures (Karen and Julia, 2003). It has also been reported that temperatures below 15 ℃ slow down rice germination at the early stage (Cruz and Milach, 2004).

In summary, germinating brown rice resulted in substantial changes in the antioxidant and antityrosinase activities with the significant differences detected between ungerminated and germinated rice. A 24-h germination regime was most suitable for inducing high levels of antioxidants and antityrosinase activities. Temperature and germination time had a profound effect on the contents of antioxidants and antityrosinase activity in germinated brown rice.

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