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Short Communication

Effect of germination on total phenolic content and antioxidant properties of 'Hang' rice

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Abstract

'Hang' rice is the rice product from the folk wisdom of traditional northeast region of Thailand. The process of making consists of soaking, steaming, drying and dehusking without polishing. Nowadays, the process includes rice germination which enhance GABA compound (γ-aminobutyric acid). This research studied the effect of germination on total phenolic content and antioxidant properties of 'Hang' rice. It was prepared with three varieties including black waxy rice, red non-waxy rice (red Jasmine) and white non-waxy rice (KDML105) that determined the amount of total phenolic content, total flavonoid content and antioxidant properties by FRAP and DPPH assays. Result showed the total phenolic content of germinated black waxy rice and Jasmine KDML105 were higher than non-germinated rice, while germinated red jasmine rice were lower than non-germinated rice. The germinated jasmine KDML105 showed the highest total phenolic content (437.16 mgGAE/g). While, the total flavonoid content showed the highest value (92.66±0.98 mgCE/100g) in black waxy rice. For the antioxidant properties, germinated 'Hang' rice showed antioxidant activity higher than non-germinated rice by FRAP and DPPH assay of all three varieties. This study suggests that germination have the effect on the antioxidant properties.

Introduction

'Hang' rice product originated northeast of Thailand. The traditional 'Hang' rice process consists of soaking, steaming, drying and dehusking without polishing. Currently, the process includes rice germination. 'Hang' rice is a high nutritional value rice product. The steaming process can reduce broken grain and inactivate enzymes, but it decreases GABA content due to heat loss.

Germination is the one process to produce 'Hang' rice that increasing the bioactive compounds and nutritional quality. Many researchers reported that the germination of rice increases the content of γ-aminobutyric acid (GABA) and antioxidants such as phenolic compounds, γ-oryzanol and vitamin E (Moongngarm and Saetung, 2010; Kim *et al*., 2012). However, never studies have been reported the antioxidant activity in 'Hang' rice. Therefore, the objective of the research to investigated the total phenolic content and antioxidant properties of germinated and non-germinated 'Hang' rice.

Materials and Methods

Chemicals

Folin-Ciocalteu reagent was purchased from LOBA CHEMIE PVT, LTD (Mumbai, India). Gallic acid, trolox, tripyridyltrizine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemical Co., (St. Louis, Mo, USA).

Preparation of rice extract

Rice powders of three varieties were obtained by milling rice grain in a local grinding mill and passed through a 500 µm sieve screen. The powder samples were extracted with 80% methanol (1:10 w/v) at room temperature for 24 h. After filtration by filter paper (Whatman no.4), the filtrates were evaporated to remove the solvent by using rotary vacuum evaporator. The residues were resuspended in water and then freeze-dried. The dried extract powders were obtained and stored at -20°C before use.

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu reagent, according to method of

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Lai *et al.* (2009). The 0.1 ml of the extract solution (1 mg/ml distilled water) was transferred into a test tube containing 2 ml of 2% Na_2CO_3 and then mixed thoroughly with 0.1 ml of 50% Folin-Ciocalteu reagent. The mixture was mixed using vortex mixer and then allowed to stand for 30 min in the dark. The absorbance of rice extracts was measured in spectrophotometer at 750 nm. The blank consisted of a solution only with the Folin-Ciocalteu reagent (without the extract). Total phenolic content was calculated from a calibration curve obtained with gallic acid.

Determination of total flavonoid content

The total flavonoid content was determined using the colorimetic method (Vichanpong *et al*., 2010). The 1 ml of the extract solution (1 mg/ml distilled water) was transferred into a volumetric flask containing 5 ml of distilled water. Then 0.1 of 5% sodium nitrite was added, after 5 min, 0.3 ml of 10% aluminium chloride was added and diluted to volume with distilled water. The total flavonoid content was calculated from a calibration curve obtained with catechin.

Evaluation of DPPH radical-scavenging

The free radical-scavenging activity of the rice extracts was evaluated using the stable radical DPPH, according to the method of Lai *et al*. (2009). Briefly, 0.1 ml of the extract solution (1 mg/ml distilled water) was transferred into a test tube and then mixed thoroughly with 3.9 ml of methanol and 1.0 ml of DPPH solution (1.0 mM in methanol). After incubation for 30 min, the absorbance of the mixtures at 517 nm was determined using spectrophotometer. The percentage of DPPH radical scavenging activity of each rice extracts was calculated as:

$$\text{DPPH radical scavenging activity (\%)} = [1 – (A_{517\text{ nm sample}} / A_{517\text{ nm control}})] \times 100$$

The percentage of DPPH radical-scavenging activity was plotted against the rice extract concentration to determine the amount of extract necessary to decrease DPPH radical concentration by 50% (IC_{50}).

Evaluation of Ferric reducing antioxidant power (FRAP)

The FRAP method was using by Butsat and Siriamornpun (2010). The stock solution included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was warmed at 37^{o}C before use. The extracts (300 µl) were allowed to react with 1.7 ml of FRAP solution. After 60 min, the absorbance was measured in spectrophotometer at 593 nm. The FRAP was calculated from a calibration curve obtained with $FeSO_4$/g.

Statistical analysis

All experiments were carried out in triplicate and the results were expressed as mean values + standard deviation (SD). These means were compared using the Duncan Multiple Range Test.

Results and Discussion

Total phenolic content

In this study, the total phenolic content of germinated and non-germinated 'Hang' rice investigated, ranging from 159 to 437.16 mgGAE/g (Table 1). Germinated 'Hang' rice was significantly ($p<0.05$) higher total phenolic content in varieties black waxy rice and white non-waxy rice (416.16 - 437.16 mgGAE/g) than in varieties red non-waxy rice (159.00 mgGAE/g). However, non-germinated 'Hang' rice in red non–waxy rice showed higher (355.16 mgGAE/g) than germinated rice (159.00 mgGAE/g). These results were similar to those reported by Cáceres *et al.* (2014). Germination improved GABA, total phenolic content and antioxidant activity in Ecuadorian brown rice sprouts. However, many factors had the effect of total phenolic content such as extraction methods, varieties, growing conditions and germination time (Ti *et al*., 2014).

During hydrothermal treatment of Hang rice production, the phenolic compounds were affected by heat and the steep water might partially remove the soluble phenolic compounds of the grain (Scaglioni *et al.,* 2014). Pascual *et al*. (2013) reported high losses of α-tocopherol and γ--oryzanol after parboiling in brown rice.

Total flavonoid content

The evaluation of total flavonoid content of germinated and non-germinated 'Hang' rice was presented in Table 2. It ranged from 3.66 to 92.66 mgCE/100g. Germinated 'Hang' rice was significantly ($p<0.05$) higher total flavonoid content in varieties black waxy rice (92.66 mgCE/100g) than non-germinated rice (38.11 mgCE/100g). This results was opposite in varieties red non–waxy rice. The germination did not effect to increasing of total flavonoid content in brown rice. It could explain that bound forms of flavonoids are stable transformation (Ti *et al*., 2014).

Table 1. Total phenolic content of non-geminated and germinated 'Hang' rice extract (mgGAE/g)

Varieties	Processing	
	Germinated	Non-germinated
Black waxy rice	416.16a ± 6.33	275.83b ± 16.36
Red non-waxy rice (red Jasmine)	159.00b ± 7.96	355.16a ± 3.39
White non-waxy rice (KDML105)	437.16a ± 6.51	322.00b ± 5.86

In each row, different letters mean significant differences ($p<0.05$).

Table 2. Total flavonoid content of non-geminated and germinated 'Hang' rice extract (mgCE/100g)

Varieties	Processing	
	Germinated	Non-germinated
Black waxy rice	92.66a ± 0.98	38.11b ± 2.04
Red non-waxy rice (red Jasmine)	25.77b ± 4.07	52.88a ± 1.75
White non-waxy rice (KDML105)	3.66a ± 1.18	7.55a ± 1.66

In each row, different letters mean significant differences ($p<0.05$).

Table 3. DPPH radical-scavenging activity of non-geminated and germinated 'Hang' rice extract (% scavenging effect)

Varieties	Processing	
	Germinated	Non-germinated
Black waxy rice	63.26a ± 0.09	37.93b ± 1.56
Red non-waxy rice (red Jasmine)	89.23a ± 0.99	84.73a ± 0.42
White non-waxy rice (KDML105)	73.60a ± 1.63	69.10b ± 2.57

In each row, different letters mean significant differences ($p<0.05$).

Antioxidant activity of 'Hang' rice extracts

In this study, the antioxidant activities were evaluated by DPPH and FRAP methods (Table 3 and 4). Results showed that all of varieties germinated 'Hang' rice had higher antioxidant activity than non-germinated rice excepted red non-waxy rice in DPPH method. During germination, enzyme synthesis could enhance the intrinsic phytochemical compounds and antioxidant activity. It was also in agreement with previous reports that germinated brown rice showed a higher the flavonoid contents than non-germinated brown rice (Ti *et al.*, 2014). However, the significant differences in antioxidant activities of 'Hang' rice obtained from different varieties that had effects to quantities of phenolics, flavonoids, tocopherols, tocotrienols and γ-oryzanol compounds (Chotimarkorn *et al*., 2008).

Table 4. Ferric reducing antioxidant power (FRAP) of non-geminated and germinated 'Hang' rice extract (μmol $FeSO_4$/g)

Varieties	Processing	
	Germinated	Non-germinated
Black waxy rice	352.33a ± 18.85	279.66b ± 27.81
Red non-waxy rice (red Jasmine)	372.33a ± 18.56	232.66b ± 25.30
White non-waxy rice (KDML105)	252.66a ± 41.15	99.00b ± 32.66

In each row, different letters mean significant differences ($p<0.05$).

Conclusion

Germination had the effect to phenolic content, flavonoid content and antioxidant activity that occurred from the change in chemical compounds and bioactive compounds in 'Hang' rice during germination. However, increasing or decreasing of its depended on varieties which related to seasons, growing, harvesting, storage management and process of 'Hang' rice.

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