ANTIOXIDANT ACTIVITY AND ANTIMUTAGENICITY OF HOM NIL RICE AND BLACK GLUTINOUS RICE

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ABSTRACT: Raw, cooked and fermented Hom-Nil rice and black glutinous rice (*Oryza sativa*) extracts were investigated for their antioxidant activity and antimutagenicity. Each rice extract exhibited high antioxidant activity. The antioxidant activity and total phenolic contents of black glutinous rice extracts were higher than that of Hom Nil rice extracts. In addition, antioxidant activity and total phenolic content of fermented rice extract was higher than those of both raw rice extract and the cooked rice extract respectively. These rice extracts were not mutagenic in the Ames test using *Salmonella typhimurium* strains TA98 and TA100 without metabolic activation at pH 3.0-3.4. After nitrite treatment, rice extracts exhibited their mutagenicity on both tester strains. Their antimutagenicity against nitrite-treated 1-aminopyrene was evaluated. Each rice extract possessed antimutagenic activity. The antimutagenicity of black glutinous rice extracts was higher than that of Hom Nil rice extracts. Fermentation might be a good process to increase the antimutagenicity. The protective effects of these rice extracts might be due to the presence of antimutagenic components that were supposed to be flavonoids which might scavenge of the toxic compounds or/and inhibit bacterial enzyme. Thus, the results from this study make such rice potentially useful in dietary antioxidant and chemoprevention.

Keywords: Hom Nil rice, black glutinous rice, mutagenicity, antioxidant, Ames test, nitrite

INTRODUCTION: The incidence of the proximal stomach cancer has increased markedly over the past 20 years. While the responsible agent remains unidentified, it is likely that environmental factors, such as the diet, play a role in the rising incidence of this cancer. For many years, there has been interest in nitrite as a potential pre-carcinogen for gastric cancer. Acidification of nitrite in the stomach produces nitrosative species, which can form potentially carcinogenic *N*-nitroso compounds[1)]. Phytochemicals which are bioactive nonnutrient compounds found in plant foods (e.g. fruits, vegetables, grains) possess biologic effects associated with reduced risk of various diseases such as cancer. The polyphenols are some of the most studied compounds and can be further divided into flavonoids including flavonols, flavones, catechins, flavanones, and anthocyanins[2)]. Anthocyanins has many biological properties such as prevention of inflammation[3)], reduction of DNA cleavage[4)], antimutagenicity in bacterial model[5)], and gastric protective effects[6)]. One of the good sources of anthocyanins is black rice[7)]. The two kinds of black rice (*Oryza sativa*) that are mostly consumed in Thailand are Hom Nil rice and black glutinous rice. This study was aimed to investigate the antioxidant activity and antimutagenicity of Hom Nil rice and black glutinous rice extracts against mutagenicity of nitrite-treated 1-aminopyren.

MATERIALS AND METHODS:

Chemicals

1-Aminopyrene (1-AP) was obtained from Aldrich, St. Louis, USA. E. Merck (Darmstadt, Germany) supplied methanol and Bacto agar. Oxoid nutrient broth No.2 was purchased from Oxoid Ltd. (Basingstoke, Hants, England). Sodium nitrite was purchased from BDH Chemicals Ltd. (Poole, England). Other chemicals were of laboratory grade.

Sample preparations

Hom-Nil rice and black glutinous rice were purchased from a supermarket in Bangkok. Raw rice was prepared by only washing it with tap water while cooked rice was prepared by using an automatic electric rice cooker with distilled water (water 2 liter: rice 1 kg). Fermented rice was

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prepared by mixed 1 piece of traditional starter for 1 kg of raw rice with cooked rice and left in room temperature for 48 h. Each rice sample was dried at 40 °C in hot air oven until dried and then it was grounded in an electrical blender to be fine powder before the extraction. The powder (1000 g) was soaked for 1 day in 1.5 liter of acid alcohol (0.1 N acetic acid in 70% ethanol) and repeated this step for 3 times. All of the filtrates were evaporated and it was dried in freeze dryer. The rice extract was protected from light and store below 5°C until used.

Antioxidant Assay

8 mg/ml in 80% methanol of each rice extract was used in antioxidant assays. Each rice extract was run simultaneously. All data were presented as means of at least triplicate experiments. 2, 2′-Diphenyl-1-Picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays were used to determine the antioxidant activity of the rice extracts. The procedure of DPPH assay was described by Fukumoto and Mazza[8] with slight modifications. The absorbance of the solution was read in microplate reader (Sunrise, Tecan Co., Austria) using a 520 nm filter. The antioxidant activity of the rice extracts was determined using the standard curve expressed as mg of Trolox Equivalent Antioxidant Capacity (TEAC)/g dry weight of rice extract. The radical scavenging activity was also calculated as a percentage of DPPH scavenging activity using the equation: % scavenging activity = $100 \times (1\text{-}A_E/A_D)$, where A_E is the absorbance of the solution when the rice extract is added, and A_D is the absorbance of the DPPH solution with nothing added. FRAP assay was used by its ability to reduce the Fe^{3+}/ferricyanide complex by forming ferrous products. Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 600 nm as described by Griffin and Bhagooli[9]. An aqueous solution of Fe^{2+} ($FeSO_4.7H_2O$) was used to making standard curve. The FRAP values of the extracts expressed as mg of ferrous iron (Fe (II))/g dry weight of rice extract. Total phenolic content of each rice extract was determined using Folin-Ciocalteu reagent[10-12]. The absorbance of the solution was read in microplate reader using a 750 nm filter. The amount of total polyphenols was calculated as a Gallic Acid Equivalent (GAE) from the calibration curve of gallic acid standard solutions; expressed as mg gallic acid equivalent/g dry weight of rice extract.

Nitrite treatment

An aliquot of rice extract (50, 75, 100 µl) was measured into a sterile test tube and the volume was adjusted to 200 µl with sterile distilled water. It was then mixed with 250 µl 2 M sodium nitrite and 550 µl 0.2 N hydrochloric acid to acidify the reaction mixture to pH 3-3.4 and incubated at 37°C in a shaking water bath for 4 h. The reaction was stopped by placing the tube in an ice bath for 1 min and 250 µl of 2 M ammonium sulfamate was added. The reaction mixture was allowed to stand for 10 min in an ice bath before being subjected to the mutagenicity assay.

Ames mutagenicity assay

The plate incorporation procedure of Maron and Ames[13] was used for mutagenicity and antimutagenicity testing on *Salmonella typhimurium* TA98 and TA100 without metabolic activation, with the inclusion of a pre-incubation step[14], and with some minor modifications. Positive controls using 1-AP (0.075 mg/ml) 10 µl (tested on TA98) or 20 µl (tested on TA100) treated with nitrite in acid solution and negative controls were included in each assay. The untreated or nitrite-treated sample as described (100 µl) was mixed with 500 µl of 0.5 M phosphate buffer (pH7.4), 100 µl of each tester strain for a pre-incubation step and incubated at 37°C in a shaking water bath. After incubation, 2 ml of top agar containing 0.5 mM L-histidine and 0.5m M D-biotin (45°C) was added, mixed well and poured onto aminimal glucose agar plate. The plate was rotated to achieve uniform colony distribution and incubated at 37°C in darkness for 48 h. Number of His^+ revertant colonies was counted. The mutagenicity of each sample was pronounced when number of histidine revertants per plate was higher than twice of spontaneous revertants with a concentration-response relationship[11].

Antimutagenicity assay

Each rice extract (50, 75, 100 µl) was added to a test tube containing the 4-hour incubated

mixture of sodium nitrite treated chicken extract (1250 µl) and adjusted the volume into 1350 µl with sterile distilled water before pre-incubation process. Then the mixture was determined for its mutagenicity by Ames test. The antimutagenic activity was calculated as percentage of modification as follow:

$$\% \text{ Inhibition} = (A-B)/(A-C) \times 100$$

Where A is the number of histidine revertants induced by nitrite treated standard mutagen (1-AP), B is the number of histidine revertants induced by mutagen in the present of rice extract and C is the number of spontaneous revertants (negative control). The inhibition of mutagenicity may be divided into four classes as follows: more than 60%: strong; 60-41%: moderate; 40-21%: weak; 20-0%: negligible[15].

RESULTS:

Antioxidant activity

All of the rice extracts, namely raw Hom Nil rice extract (RH), cooked Hom Nil rice extract (CH), fermented Hom Nil rice extract (FH), raw black glutinous rice extract (RB), cooked black glutinous rice extract (CB) and fermented black glutinous rice extract (FB), possessed high antioxidant activity and high phenolic contents (Table 1). The antioxidant activity and total phenolic contents of black glutinous rice extracts was higher than those of Hom Nil rice extracts. However, calculating based on their raw material dry weight basis; it was found that fermented rice had higher both antioxidant activity and phenolic contents than raw or cooked rice (data not shown).

Mutagenicity

All samples were not mutagenic on *S. typhimurium* strains TA98 and TA100, but exhibited their mutagenicity on both tester strains after sodium nitrite treatment with a concentration-response relationship (data not shown).

Antimutagenicity

The dose-inhibition relationship of rice extracts toward the mutagenicity of nitrite treated 1-AP is shown in Figure 1. The result showed that rice extracts could inhibit the mutagenicity of nitrite treated 1-AP on both *S. typhimurium* tester strains at the dose of 0.8 mg/plate or higher. At the dose of 1.6 mg/plate of rice extract; UH, CH, UB, CB and FB strongly inhibited (>60% inhibition) the mutagenicity of nitrite treated 1-AP on *S. typhimurium* TA98; CH and FB showed weak antimutagenic activity (>20% inhibition), UB and CB showed moderate antimutagenic activity (>40% inhibition) on *S. typhimurium* TA100. On both tester strains, black glutinous rice extract showed higher antimutagenic activity than Hom Nil rice extracts where as FH showed the lowest antimutagenic activity.

Table 1 Antioxidant activity, total phenolic contents and extraction yield of rice extracts

Rice sample	DPPH assay		FRAP[b] value	Total polyphenol content (GAE)[c]	%Yield[d] (dry weight)
	TEAC[a]	% Scavenging			
Hom Nil					
Raw	1.189	95.03	5214.44	272.07	0.8
Cooked	1.173	93.99	2744.36	235.42	0.6
Fermented	0.117	24.26	360.87	38.56	27.3
Black glutinous					
Raw	1.245	98.41	6797.89	458.37	1.7
Cooked	1.236	98.19	6352.16	452.75	0.6
Fermented	1.032	84.71	1461.40	65.21	12.75

All values are the means of three measurements.
[a]TEAC (Trolox equivalent antioxidant capacity) in mg/g dry weight of rice extract.
[b]FRAP assay = The FRAP value of the extract was expressed as mg of ferrous iron (Fe (II))/g dry weight of rice extract.
[c]GAE (The gallic acid equivalent) in mg/g dry weight of rice extract.
[d]%Yield calculated from dry weight of raw material.

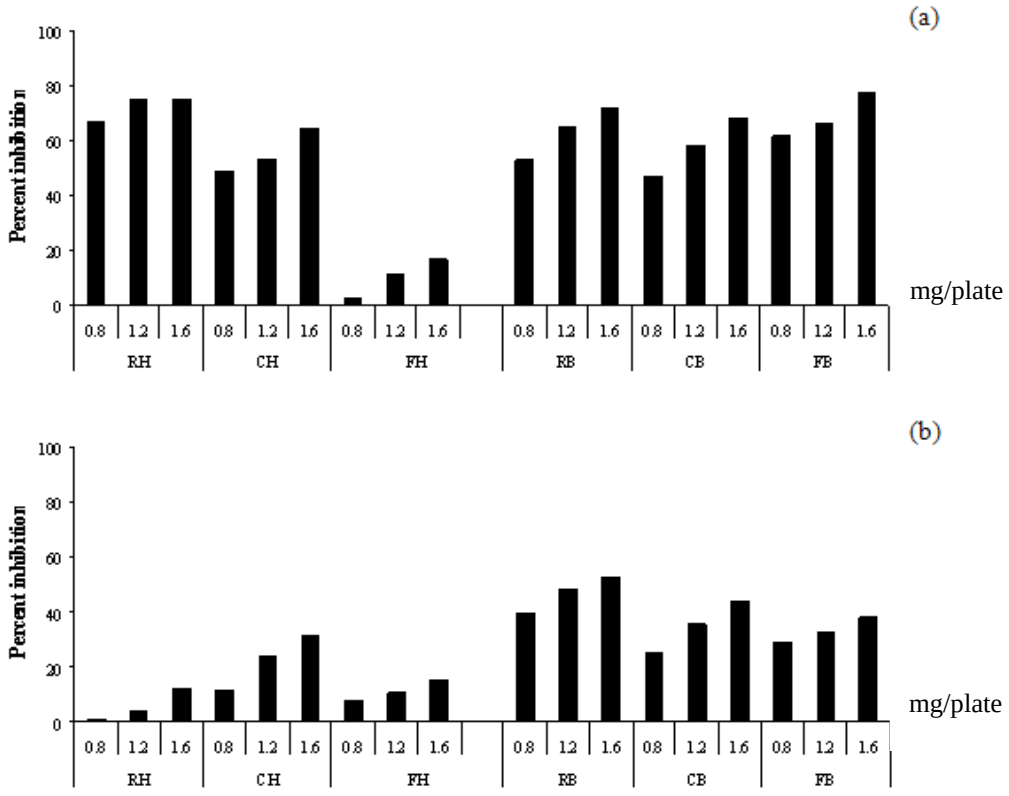

Figure 1 Effect of the rice extracts on the mutagenicity of sodium nitrite treated 1-AP on *S. typhimurium* TA98 (a) and TA100 (b) without metabolic activation at pH 3.0-3.4; raw Hom Nil rice extract (RH), cooked Hom Nil rice extract (CH), fermented Hom Nil rice extract (FH), raw black glutinous rice extract (RB), cooked black glutinous rice extract (CB) and fermented black glutinous rice extract (FB)

DISCUSSION: Hom nil rice and black glutinous rice possessed high antioxidant activity which might be the good sources of antioxidant food for consumers. High level of antioxidant activity and phenolic content in each rice extract might be due to high anthocyanins content present in the rice[16]. Fermented rice had much higher yield than those of raw and cooked rice because the starch was turned to reducing sugar[17,18] that was extracted by acidic alcohol in this study. Therefore, FH and FB had the highest antioxidant activity and the phenolic contents based on dry weight basis of the starting materials. This indicated that fermentation can enhance levels of antioxidant activity and also improve the bioactive potential of the rice[19] which might be due to the increasing of aglycone content, the bioactive isoflavone[20].

Hom-Nil rice and black glutinous rice seem to be safe for consumer because their extracts were not mutagenic towards *S. typhimurium* on TA98 and TA100 at doses tested. However, all samples were mutagenic after being treated with excess sodium nitrite in acid solution. It implied that each rice extract contained certain precursors that could react with nitrite under acidic solution to produce direct mutagenic products causing frame-shift (TA98) and base-pair substitution (TA100). It is not surprised since Hayatsu and Hayatsu[21] reported that boiled rice treated with nitrous acid showed mutagenicity in the absence of metabolic activation but the mutagenicity decreased greatly after treated with S9. The possible precursors contained in the rice extract might be flavonoids[22], indoles, phenolics, and carbolines[23]. These results indicate that consumers

would be in a risk of gastric cancer when consume these two kinds of rice simultaneously with any nitrite containing foods.

Hom Nil rice and black glutinous rice extracts might be the inhibitors of nitrosated products that induced mutations. All of the samples inhibited the mutagenesis of nitrite treated 1-AP on both *S. typhimurium* tester strains may suggest that scavenging of the toxic compounds or/and enzyme inhibition by the rice extracts were the possible mechanism of inhibition. The flavonoids in rice extract, might be the potent antimutagenic compounds against 1-nitropyrene (possibly one of the products occurred during nitrite treatment of 1-aminopyrene)[24] by inhibited the activation of 1-nitropyrene by bacterial nitroreductase[25] or *O*-acetyltransferase[24]. In addition, there were similar trends between the antimutagenicity of black glutinous rice extracts and the antioxidant activity and total phenolic contents assays; it was higher than that of Hom Nil rice extracts.

CONCLUSION: Hom Nil rice and black glutinous rice are safe for consumption in consideration of mutagenicity but consumers should avoid consuming these two kinds of black rice with nitrite containing foods. Furthermore, they are good for human health in terms of their antioxidant activity and their ability to inhibit mutagenicity of direct mutagens.

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