

Antimutagenicity of Black Glutinous Rice and Hom Nil Rice

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ABSTRACT

*Black glutinous rice (*Oryza sativa* var. *glutinosa*) and Hom Nil rice (*Oryza sativa*) are colored rice varieties with antioxidant properties, popular among health conscious consumers. We studied the antimutagenicity of raw, cooked and fermented samples against two direct mutagens – nitrite-treated 1-aminopyrene and nitrite treated chicken essence – in an Ames assay using *Salmonella typhimurium* strains TA 98 and TA 100. The extract of each sample with acid alcohol reduced the mutagenicity of both mutagens. In addition, the antimutagenicity of the extracts from the samples made of black glutinous rice was higher than that of the extracts from samples made of Hom Nil rice. The protective effects of these rice varieties might be due to the presence of some phytochemicals, including anthocyanins, which are the main antioxidant. The selected rice varieties might be appropriate for p*

Keywords: Ames test, antimutagenicity, black glutinous rice, Hom Nil rice, nitrite

INTRODUCTION

The colored rice varieties most consumed in Thailand are black glutinous rice (*Oryza sativa* var. *glutinosa*) and Hom Nil rice (*Oryza sativa*). Black rice is a good source of anthocyanins, namely cyanidin-3-O-glucoside and peonidin-3-O-glucoside, which are localized in the pericarp and aleurone layers of the seeds (Abdel-Aal et al., 2006; Zhang et al., 2006). Sriseadka et al. (2012) identified eleven flavonoids and their glycosides in the extracts from the bran of seven Thai black rice varieties. With potent antioxidant capacity, anthocyanins exhibit various bioactivities, including preserving vision and preventing Alzheimer's disease (Shih et al., 2010; Miyake et al., 2012). Since the incidence of cancer has increased markedly over the past 20 years and become the first cause of death among Thai (National Statistical Office, 2013), this study was aimed to investigate the antimutagenicity of black glutinous rice and Hom Nil rice against mutagenicity of nitrite-treated 1-aminopyrene and nitrite-treated chicken essence using the Ames test.

MATERIALS AND METHODS

Chemicals and precursors of direct mutagens

E. Merck (Darmstadt, Germany) supplied Bacto agar and methanol. Oxoid nutrient broth No.2 was obtained from Oxoid Ltd. (Basingstoke, Hants, England). Sodium nitrite was purchased from BDH Chemicals Ltd. (Poole, England). Commercial chicken essence obtained from a local convenience store was evaporated in a vacuum rotary evaporator (Rotavapor® R II, Buchi (Thailand) Ltd., Bangkok, Thailand) at 45°C until a sticky mass, dried in a freeze dryer (Epsilon 1-4 LSCplus, Martin Christ, Osterode, Germany), dissolved in distilled water to the final concentration of 0.486 g/ml and autoclaved before use as a precursor of a direct mutagen. 1-Aminopyrene (1-AP), used as another precursor of a direct mutagen, was purchased from Aldrich, St. Louis, USA. Other chemicals were of laboratory grade.

Sample preparation

Hom-Nil rice and black glutinous rice were purchased from a supermarket in Bangkok. Raw rice was washed with tap water. One portion of rice (1 kg) was cooked in an automatic electric rice cooker with distilled water (2000 ml). Fermented rice was prepared by mixing 1 kg of cooked rice with an appropriate amount of a traditional fermentation starter; then, the mixture was left at room temperature for 48 h. Each fermented rice sample was dried in a hot air oven (40°C); then, it was ground in an electrical blender, soaked in 1.5 liter of acid alcohol (0.1 N acetic acid in 70% ethanol) per 1000 g rice powder for 1 day and filtered. This step was repeated twice and the whole filtrate was pooled and evaporated using a rotary evaporator (Rotavapor® R II, Buchi (Thailand) Ltd., Bangkok, Thailand) and finally dried in a freeze dryer (Epsilon 1-4 LSCplus, Martin Christ, Osterode, Germany). All samples were protected from light and stored below 5°C until use. Each extract (0.216 g) was dissolved in 1 ml of distilled water and filtered through a sterilized 0.20 µm-membrane (Minisart® syringe filter, Sartorius Thailand Co. Ltd., Bangkok, Thailand) filter set.

Preparation of standard direct mutagens

The direct mutagens were prepared as suggested by Kangsadalampai et al. (1995). Briefly, chicken essence solution (200 µl) or 1-AP solution (0.075 mg/ml; 10 µl for testing on TA98 or 20 µl for testing on TA100) 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 sodium nitrite (2 M) and 550 µl hydrochloric acid (0.2 N) 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 ammonium sulfamate (2 M) was added. The reaction mixture was allowed to stand for 10 min in an ice bath before being subjected to the antimutagenicity assay.

Antimutagenicity test

Salmonella typhimurium tester strains TA98 and TA100 were obtained from Dr. Wannee Kusamran of the National Cancer Institute (Bangkok) and manipulated as suggested by Maron and Ames (1983). The antimutagenicity against both direct-acting mutagens by each sample was determined as suggested by Kangsadalampai and Suharitamrong (1996). Briefly, 100 µl of each culture of tester strain add to the test tube containing 500 µl of phosphate buffer (pH 7.4), 100 µl of different concentrations of each sample and 100 µl of a direct mutagen (described above) were incubated at 37°C for 20 min in a shaking water bath. Then, top agar (2 ml) containing 5 mM L-histidine and 5 mM D-biotin was added to the mixture. The tube was mixed well and quickly poured onto a minimal 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 were counted. The antimutagenicity was calculated as percentage of inhibition as follows:

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

where A is the number of histidine revertants induced by a direct mutagen (nitrite treated chicken essence or nitrite treated 1-AP), B is the number of histidine revertants induced by corresponding mutagen in the presence of rice extract and C is the number of spontaneous revertants (negative control). The antimutagenicity levels were categorized as follows: >60% strong, 60-41% moderate, 40-21% weak and 20-0% negligible, as suggested by Calomme et al. (1996).

RESULTS

All of the extracts from the samples, namely raw black glutinous rice extract (RB), cooked black glutinous rice extract (CB), fermented black glutinous rice extract (FB), raw Hom Nil rice extract (RH), cooked Hom Nil rice extract (CH) and fermented Hom Nil rice extract (FH), possessed a dose-inhibition relationship towards the mutagenicity of nitrite treated chicken essence and nitrite treated 1-AP (Figure 1). On *S. typhimurium* TA98, most extracts strongly inhibited (>60% inhibition) the mutagenicity of nitrite treated 1-AP, while most rice extracts negligibly inhibited (≤20% inhibition) the mutagenicity of nitrite treated chicken essence. Fermented Hom Nil rice extract was the only sample that had a greater inhibitory effect on nitrite treated chicken essence than on nitrite treated 1-AP. The antimutagenicity against nitrite treated 1-AP on *S. typhimurium* TA100 was less than that against TA98. However, the inhibitory effect of most samples on the mutagenicity of nitrite treated chicken essence on *S. typhimurium* TA100 was higher than that against TA98. The extract from the sample made of black glutinous rice (raw, cooked or fermented) possessed higher inhibitory effect (weak-moderate) on *S. typhimurium* TA100 than that (negligible-weak) of the extract from the sample made of Hom Nil rice (raw, cooked or fermented). In addition, the extract from cooked rice had a lower inhibitory effect than that of the extract from raw rice; the effect increased if rice was fermented.

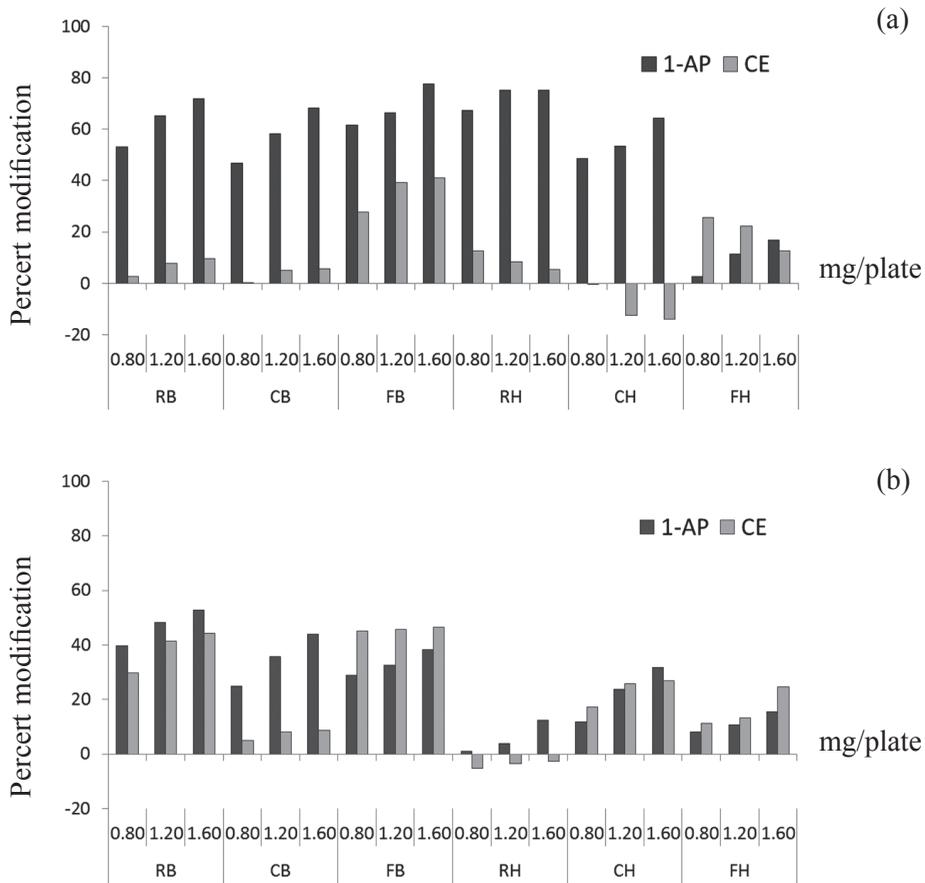


Figure 1. Effect of the extracts from different samples made of each rice varieties on the mutagenicity of sodium nitrite treated 1-AP or sodium nitrite treated chicken essence on *S. typhimurium* TA98 (a) and TA100 (b) without metabolic activation; raw Hom Nil rice extract (RH), cooked Hom Nil rice 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

The result that rice extracts inhibited the mutagenicity of both direct mutagens towards both *S. typhimurium* tester strains indicated that they reduced both frame-shift mutation (TA98) and base-pair substitution mutation (TA100). Anthocyanins, the flavonoids of rice extract, might act as antimutagenic compounds (Edenharder and Tang, 1997) against both mutagens, possibly via the inhibition of the activation of intermediate nitro-compounds by bacterial nitroreductase (Kuo et al., 1992) and/or O-acetyltransferase (Edenharder and Tang, 1997).

The inhibitory effect of the extract from samples made of black glutinous rice on *S. typhimurium* TA100 was higher than that of the extract from samples

made of Hom Nil rice, suggesting differences between the two varieties in terms of amount and/or types of active compounds. Moreover, Đorđević et al. (2009) suggested that the method of sample preparation should be of concern since fermentation enhanced the levels of antioxidant activity and improved the bioactive potential of rice. Lee and Chou (2006) reported that fermentation increased the content of aglycone, the bioactive isoflavone. Moreover, Vipassanatham et al. (2012), who cooked or cooked/fermented both glutinous rice and Hom Nil rice, found that each sample expressed its antimutagenicity against the indirect mutagen urethane in a somatic mutation and recombination test using *Drosophila melanogaster*. Therefore, the result obtained from this study suggested that the consumption of (cooked or fermented) Hom Nil rice or (cooked or fermented) black glutinous rice as a dessert after a meal might provide protection against the mutagenicity of compounds obtained from the interaction between nitrite ion (e.g. of fermented meat products) and some convertible compounds of food during the acidic conditions of stomach digestion.

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