Effect on Urethane Induced Mutagenicity in *Drosophila melanogaster* of Different Germinated Unpolished Rice and the Thai Desserts Made from Them

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Abstract

Four kinds of rice (Oryza sativa L.), namely polished Jasmine rice, germinated unpolished Jasmine rice and germinated unpolished red Jasmine rice, germinated unpolished Homnil rice were proved to have antioxidant activity and phenolic compounds. Each type of rice was blended and used to make rice crispy, dried rice ball and steamed banana cake. The desserts were evaluated for their modulating effect on urethane induced wing spots in *Drosophila melanogaster*. In the evaluation, three-day-old trans-heterozygous larvae (*mwh flr*$^{+}$*/mwh TM3*) were transferred to the *Drosophila* medium that each dessert was substituted for corn flour (used for the mutagenicity evaluation of each sample) and the *Drosophila* medium that each dessert and 20 mM urethane were substituted for corn flour and water (used for antimutagenicity evaluation of each sample). The wings of the surviving flies were analyzed for the occurrence of mutant spots. Most of the samples were non-mutagenic. Although the *Drosophila* medium prepared from each germinated unpolished rice meal increased the mutagenicity of urethane (possibly be due to the high content of polyunsaturated fatty acids that might lead to the generation of free radicals in test organism) almost all of the desserts made from the three type of geminated unpolished rice reduced the mutagenicity of urethane at various degrees. It was suspected that some components, besides the rice meal in the deserts, might be the modulators of urethane biotransformation.

Key words: Antimutagenicity, Antioxidant, Germinated unpolished rice, Thai desserts

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ผลต่อฤทธิ์ก่อกลายพันธุ์ของยูรีเทนในแมลงหวี่ของข้าวกล้องงอกบางชนิดและขนมไทยที่ทำจากข้าวดังกล่าว

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บทคัดย่อ

ข้าว 4 ชนิด คือ ข้าวขาวหอมมะลิ ข้าวหอมมะลิกล้องงอก ข้าวหอมมะลิแดงกล้องงอกและข้าวหอมนิลกล้องงอกที่ได้รับการวิเคราะห์ว่ามีฤทธิ์ต้านอนุมูลอิสระและมีสารประกอบฟีนอลิคนั้น ถูกนำมาทำขนม 3 ชนิด คือ ข้าวพอง ข้าวตูและขนมกล้วย เพื่อศึกษาฤทธิ์ก่อกลายพันธุ์และฤทธิ์ต้านการก่อกลายพันธุ์ในแมลงหวี่ (*Drosophila melanogaster*) ด้วยวิธี somatic mutation and recombination test การทดลองทำโดยนำหนอนแมลงหวี่ trans-heterozygous อายุ 3 วันที่ได้รับจากการผสมพันธ์ระหว่างแมลงหวี่ตัวเมียสายพันธุ์ *ORR flare hair* กับแมลงหวี่ตัวผู้สายพันธุ์ *mwh/mwh* ไปเลี้ยงในอาหารปกติที่แป้งข้าวโพด ถูกแทนที่ด้วยตัวอย่างข้าวหรือขนม เพื่อศึกษาฤทธิ์ก่อกลายพันธุ์ และในอาหารปกติที่แป้งข้าวโพด ถูกแทนที่ด้วยตัวอย่างข้าวหรือขนม และน้ำถูกแทนที่ด้วยสารละลาย 20 mM urethane เพื่อศึกษาฤทธิ์ต้านการก่อกลายพันธุ์ของตัวอย่างอาหารดังกล่าว ปีกของแมลงหวี่ที่รอดชีวิตถูกนำมาวิเคราะห์ความผิดปกติของขนและพบว่าทุกตัวอย่างไม่ก่อให้เกิดการกลายพันธุ์ สำหรับการศึกษาฤทธิ์ต้านการก่อกลายพันธุ์พบว่า ข้าวกล้องงอกทุกชนิดเพิ่มฤทธิ์ก่อกลายพันธุ์ของยูรีเทนมากขึ้น อาจเนื่องจากข้าวกล้องนั้นมีปริมาณของกรดไขมันไม่อิ่มตัวสูงจึงทำให้มีอนุมูลอิสระเกิดขึ้น ส่วนขนมไทยเกือบทุกชนิดสามารถลดฤทธิ์การก่อกลายพันธุ์ของยูรีเทนได้ เนื่องจากในขนมอาจมีสารที่ไปปรับเปลี่ยนกระบวนการเปลี่ยนแปลงยูรีเทนที่ออกฤทธิ์ก่อกลายพันธุ์

คำสำคัญ: การต้านฤทธิ์ก่อกลายพันธุ์ แอนติออกซิแดนท์ ข้าวกล้องงอก ขนมไทย

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Introduction

Flour made from pigmented rice should be considered as a major ingredient of some Thai snacks since it contains high amount of anthocyanins[1] that are known to have some beneficial functions for consumer such as a anticarcinogenicity[2,3] and antioxidant activity[4,5]. In addition, the flour made form germinated unpolished rice should also be of interest by health concerning consumer since it contains a much higher concentration of essential amino acids,[6] such as lysine, isoleucine, and methionine, than conventional unpolished rice, and over 13 times the amount of γ-aminobutyric acid (GABA)[7]. Apart from changing the level of nutrients, the biochemical activities, which occur during germination, can also generate bioactive components and some of these possess antioxidants, such as ascorbic acid, tocopherols, tocotrienols and phenolic compounds, thus resulting in an increase of antioxidant activity[8]. The preparation of some Thai snacks, namely rice crispy (Khao-pong), dried rice ball (Khao-tu) and streamed banana cake (Khanom-kluay) containing health promoting phytochemicals obtained from germinated pigmented rice was of interest since consuming of all three unattractive traditional Thai snacks has been decreased. Hopefully, they might also have other health benefit, namely antimutagenicity which was the aim of this investigation.

Materials and Methods

Chemicals Urethane was purchased from Sigma Chemical (St. Louis, MO, USA). 2, 4, 6-tripyridly-s-triazine (TPTZ), ferric chloride hexahydrate and ferrous sulfate heptahydrate were furnished from Sigma Chemical (St. Louis, MO, USA). Diethylether and sodium acetate trihydrate were purchased from Merck (Darmstadt, Germany). Propionic acid, 2, 2-diphenyl-1-picrylhydrazl (DPPH), gallic acid and Folin-Ciocalteu reagent were supplied from Fluka Chemika (Buchs, Switzerland). Trolox was brought from Aldrich Chemical (Milwaudee, WI, Germany). All other chemicals and reagents were of analytical grade.

Sample Preparations Four kinds of rice (Oryza sativa L.), namely polished Jasmine rice (used for making each traditional dessert), germinated unpolished Jasmine rice and germinated unpolished red Jasmine rice were purchased from BIG C supercenter (Nakhon Pathom) while germinated unpolished Homnil rice was purchased from Tesco Lotus (Lak-See, Bangkok Metropolitan). Rice was ground to be meal and passed through a 100-mesh sieve before using as the ingredient of streamed banana cake

(Khanom-kluay),[9] dried rice ball (Khao-tu)[10] and rice crispy (Khao-pong)[11]. Each finished product was lyophilized, ground to be powder and stored at room temperature in a desiccator for further studies.

Antioxidant Activity of Rice Meal A portion of each rice meal (2.5 g) was mixed with 80% methanol (50 ml) in a 250-ml Erlenmeyer flask. It was stirred for 2 h at room temperature; then, the extract was filtered through Whatman filter paper No.1 and collected into a glass bottle. The antioxidant capacity measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay for free radical scavenging activity of each methanolic extract was performed as suggested by Fukumoto and Mazza[12] with slight modification[13]. Ferric reducing antioxidant power (FRAP) of methanolic extract was measured according to the procedure described by Griffin and Bhagooli[14] with slight modification[13]. The total phenolic content of methanolic extract from each sample was determined according to the method described by Amarowicz *et al.*[15] with slight modification[13].

Somatic Mutation and Recombination Test The antimutagenicity of each freshly prepared sample or germinated rice meal was determined twice. The test was performed as described by Graf *et al.*[16]. Three-day-old, trans-heterozygous larvae (*mwh flr*$^{+}$*/mwh TM3*) were transferred to the standard (*Drosophila*) medium[17] that each dessert or germinated rice meal was substituted for corn flour (used for the mutagenicity evaluation of each sample) and the *Drosophila* medium that each dessert or germinated rice meal was substituted for corn flour and 20 mM urethane was substituted for water (used for antimutagenicity evaluation of each sample) as suggested by Laohavechvanich[18]. The standard medium was used as the negative control and that containing 20 mM was used as the positive control. The wings of the surviving flies were analyzed for the occurrence of mutant spots. The wing spots data were evaluated using the statistical procedure as described by Frei and Wurgler[19].The estimation of spot frequencies and confidence limits of the estimated mutation frequency were performed with significance level of $\alpha = \beta = 0.05$. The antimutagenicity of each sample was determined from the percentage of inhibition calculated as following:

$$\text{Percentage of inhibition} = ((a-b)/a)\ 100$$

When a is the frequency of spots induced by urethane alone and b is the frequency of spots induced by urethane in the presence of sample. It is proposed that percentages of inhibition between 0-20 represent a negligible effect while expression

of percent inhibitions between 20-40, 40-60 and more than 60 are the evidences of weak, moderate and strong antimutagenicity, respectively as suggested by Abraham[20].

Results

The antioxidant activity of all types of rice meal is shown in Figure 1. The reduction of DPPH by antioxidants in the samples expressed as the percentage of radical scavenging activity was between 18.08 to 90.31%. In addition, the FRAP values (μM ferrous tripydyltriazine) was between 72.55 to 1025.24 μM. The total phenolic contents of each sample were between 17.5 to 158.14 mg gallic acid equivalents per liter. It revealed that rice meal used in this study contained antioxidants and certain amount of phenolic compounds.

All samples were determined for its mutagenicity and antimutagenicity. None of the sample was mutagenic (data not shown). The traditional steam banana cake and dried rice ball made from polished Jasmine rice exhibited their moderate antimutagenicity (percentages of inhibition were between 40-60) while that of traditional rice crispy made from polished Jasmine rice was weak inhibitor. It was noted that there was no clear difference between the results obtained between the traditional desserts and their corresponding substituted ones in terms of antimutagenicity (Figure 2).

Discussion

It revealed that rice and germinated unpolished rice exhibited antioxidant activity and certain amount of phenolic compounds; therefore, the desserts made from them should have these properties. Germinated seed gives other benefit to consumer since López-Amorós[21] found that after germination, beans and peas had a significant increase in antioxidant activity. This ensured the healthy benefit of consumers in consuming the modified Thai desserts of this study. In addition, pigmented rice namely, germinated unpolished red rice, germinated unpolished Homnil rice consist of anthocyanins[22] and the major anthocyanins extracted from black rice express its potential to scavenge free radicals, including both oxygen- and nitrogen-derived radicals[23]. Compounds which possess antioxidant activity can inhibit mutation and cancer because they can scavenge free radicals or induce antioxidant enzymes[24]. It was documented that *N*-hydroxyurethane, a urethane metabolite,[25,26] was hydrolyzed by esterase to generate hydroxylamine and exerted its mutagenic effect in multiple organs via generating $O_2^{\cdot -}$ and $NO^{\cdot}$ to cause oxidation and depurination of DNA[27]. Methanol extract

from each germinated unpolished rice was a good source of natural antioxidants; the amount was higher than that of polished Jasmine rice. Thus, in this study, antioxidant activity from germinated unpolished rice might reduce $O_2^{\cdot}$- and/or $NO^{\cdot}$ in urethane metabolism.

Polished Jasmine rice meal or most germinated unpolished rice meal administered with urethane to *Drosophila* larvae surprisingly increased the mutagenicity expressed as induced wing spots. Kantong[28] reported that total urethane induced spots per wing of larvae increased when they were fed on a medium containing cooked rice, namely polished rice, unpolished rice, sticky unpolished rice or Mun-pu rice. The polyunsaturated fatty acid composition of dietary fat should be associated with increased oxidative stress in tissues[29]. Hence, it is hypothesized that higher content of polyunsaturated fatty acids in unpolished rice might lead to the generation of free radicals in fruit fly. Further investigation on the effect of polyunsaturated fatty acids on free radical generation in fruit fly should be done.

The present investigation showed that Thai desserts could inhibit the mutagenicity of urethane. It was possibly due to the ingredients of each dessert or some compounds occurring during dessert processing; it should not be the effect of germinated rice flour substitution. For instance, streamed banana cake made of polished Jasmine rice meal and germinated unpolished rice meal were both effectively antimutagenic against urethane with slight difference. This indicated that the antimutagenicity of this dessert might be due to other ingredients e.g. banana. Kruawan *et al.*[30] elucidated the inhibitory effect of the powders prepared from three varieties of ripe bananas on mutagenicity of urethane in *Drosophila melanogaster*.

It was indicated that dried rice ball, rice crispy either made of polished Jasmine rice or germinated unpolished rice was effectively antimutagenic against urethane. The antimutagenicity of dried rice ball and rice crispy made of polished Jasmine rice was possibly due to Maillard reaction products (MRPs) occurring during dessert processing. Further research on *analysis* of the MRPs in each sample should be done since Yen *et al.*[31] reported that sugars-tryptophan and xylose-amino acids, strongly inhibited the mutagenicity of 2-amino-3-methylimidazo (4,5-f) quinoline (IQ), 3-amino-1,4-dimethyl-5H-pyridol-(4,3-b) indole (Trp-P-1) and 2-amino-6-methyldipyrido(1,2-a:3',2'-d) imidazole (Glu-P-1) in Ames test.

In conclusion, the antimutagenicity of each Thai dessert made from different

germinated unpolished rice was not clearly different from that of its corresponding traditional one that was made from polished Jasmine rice. However, the consumers who consume Thai desserts made as suggested by the results of this study should at least receive the benefit of other antioxidant occurring during germination.

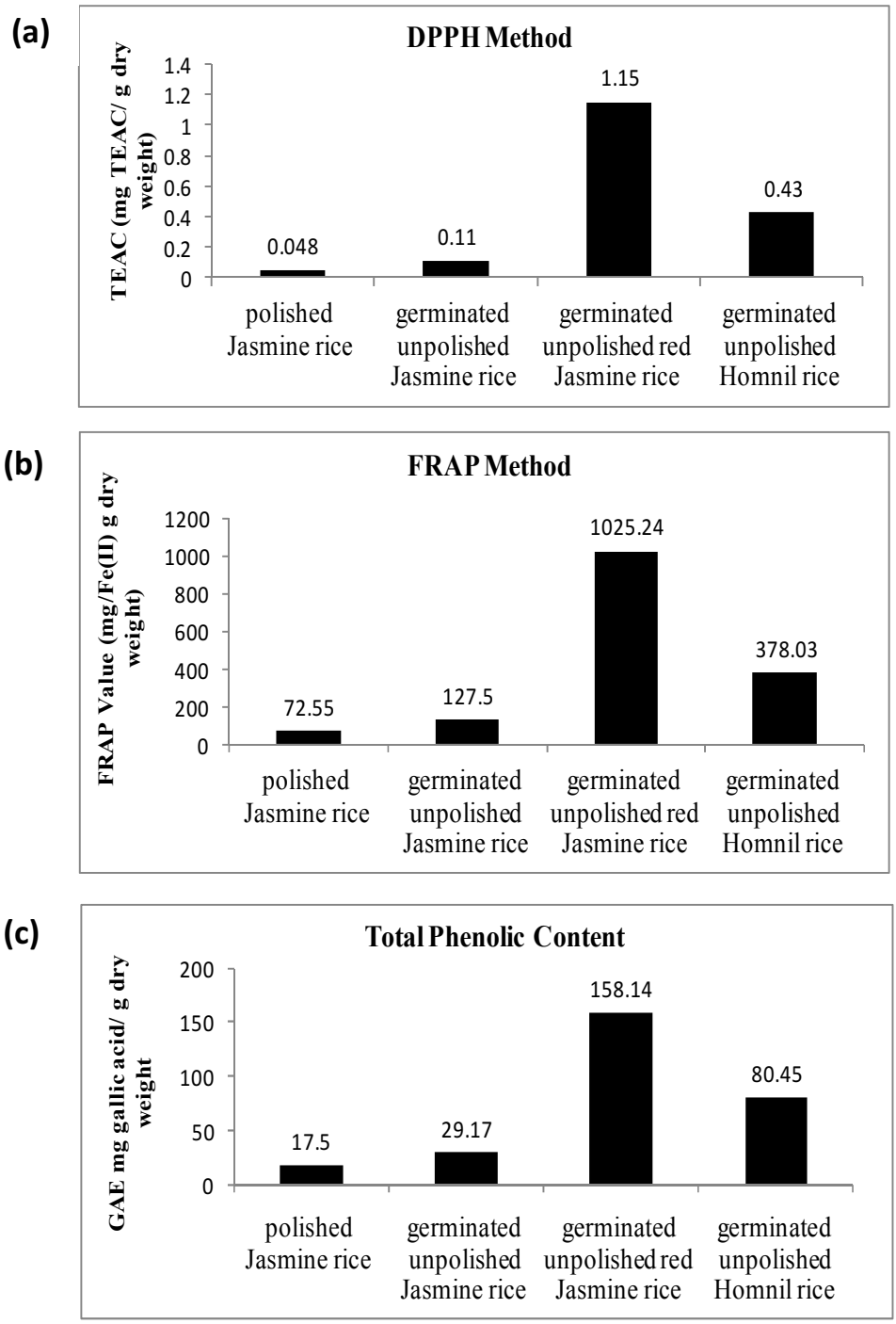


Figure 1 Antioxidant activity in DPPH assay (a), antioxidant activity in FRAP assay (b) and total phenolic content (c) of methanolic extracts of polished Jasmine rice, germinated unpolished rice.

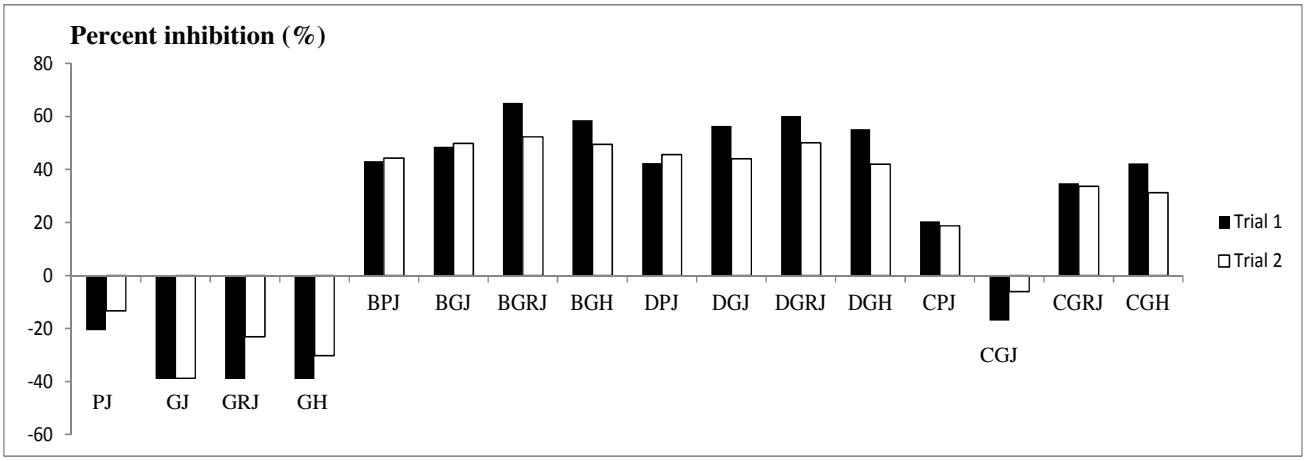


Figure 2 Percent inhibition of each Thai dessert on urethane (20 mM) induced somatic mutation and recombination in *Drosophila melanogaster* derived from trans-heterozygous (*mwh+/+flr*^{3}) larvae in trials 1and trial 2.

(PJ-Polished Jasmine rice, GJ-germinated unpolished Jasmine rice, GRJ-germinated unpolished red Jasmine rice, GH-germinated unpolished Homnil rice, BPJ-steam banana cake made from polished Jasmine rice, BGJ-steam banana cake made from germinated unpolished Jasmine rice, BGRJ-steam banana cake made from germinated unpolished red Jasmine rice, BGH-steam banana cake made from germinated unpolished Homnil rice, DPJ-Dried rice ball made from polished Jasmine rice, DGJ-Dried rice ball made from germinated unpolished Jasmine rice, DGRJ-Dried rice ball made from germinated unpolished red Jasmine rice, DGH-Dried rice ball made from germinated unpolished Homnil rice, CPJ-Rice crispy made from polished Jasmine rice, CGJ-Rice crispy made from germinated unpolished Jasmine rice, CGRJ-Rice crispy made from germinated unpolished red Jasmine rice, CGH-Rice crispy made from germinated unpolished Homnil rice).

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