Mutagenicity and Antimutagenicity in the Somatic Mutation and Recombination Test Using *Drosophila melanogaster* of Battered and Fried Thai Dishes

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Abstract

Seven battered and fried Thai dishes (BFTDs) were evaluated for their mutagenicity and antimutagenicity in the somatic mutation and recombination test using *Drosophila melanogaster*. Three-day old trans-heterozygous larvae, obtained by mating the virgin *ORR; flr*^{3} females and *mwh/mwh* males, were transferred to the medium containing each BFTD (for mutagenicity study) and the medium containing each BFTD and urethane (for antimutagenicity in the co-administration study) until they became adult flies. The antimutagenicity studies were also performed by mating the parental flies on the medium containing each sample to obtain the three-day-old larvae that were consequently raised on the medium containing only urethane (the pre-feeding study type 1) or medium containing urethane and each sample (the pre-feeding study type 2 study) until they became adult flies. The wings of the surviving flies were analyzed for the occurrence of mutant spots. The result showed that none of the samples was mutagenic; however, each sample decreased the mutagenicity of urethane indicating the presence antimutagens of herbs and spices. It was noted that the inhibitory effect of most samples in the co-administration study was nearly the same as of the pre-feeding study type 1 and the inhibitory effect of most samples was the best in the pre-feeding study type 2. It was suggested, by the results according to the experimental design, that the components of most samples should not trap urethane but induced the detoxifying system that was stable throughout the whole period of study.

Keywords: Antimutagenicity, Battered and fried Thai dishes, Urethane, SMART

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ฤทธิ์การก่อกลายพันธุ์และฤทธิ์ต้านการก่อกลายพันธุ์ของยูรีเทนในแมลงหวี่ของข้าวแกงทอด

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บทคัดย่อ

ข้าวแกงทอด 7 ชนิด ได้รับการศึกษาถึงศักยภาพในฤทธิ์ก่อกลายพันธุ์และฤทธิ์ต้านการก่อกลายพันธุ์โดยใช้แมลงหวี่ (*Drosophila melanogaster*) ตามวิธี Somatic Mutation and Recombination Test ในการศึกษาฤทธิ์ก่อกลายพันธุ์ (Mutagenicity) นั้นได้นำหนอนแมลงหวี่ trans-heterozygous อายุ 3วัน (จากการผสมพันธุ์ตัวผู้ *mwh* และตัวเมีย *ORR/flr*^{3}) ไปเลี้ยงในอาหารมีตัวอย่างข้าวแกงทอดผสมอยู่ ส่วนการศึกษาฤทธิ์ต้านการก่อกลายพันธุ์(Antimutagenicity) นั้นได้นำหนอนแมลงหวี่ trans-heterozygous ไป เลี้ยงในอาหารที่มีตัวอย่างผสมและมี 20 mM ยูรีเทน (เรียกว่า Co-administration study) และ ทำการศึกษาหนอนแมลงหวี่ที่ได้รับอาหารที่ทดสอบตั้งแต่แรกเกิด (เรียกว่า pre-feeding study) โดยเลี้ยงไข่ในอาหารที่ผสมกับตัวอย่างจนกลายเป็นหนอนอายุ 3 วัน จากนั้นนำหนอนแมลงหวี่มาเลี้ยงในอาหารที่มี 20 mM ยูรีเทน (เรียกว่า pre-feeding study type 1) หรือ อาหารที่มีตัวอย่างและ 20 mM ยูรีเทน (เรียกว่า pre-feeding study type 2) จนกระทั่งหนอนกลายเป็นแมลงหวี่ตัวเต็มวัย จึงนำไปตัดปีกเพื่อวิเคราะห์หาความผิดปกติของขน ซึ่งพบว่าข้าวแกงทอดแต่ละชนิดไม่ก่อกลายพันธุ์ในแมลงหวี่ แต่สามารถลดฤทธิ์การก่อกลายพันธุ์ของยูรีเทนได้ จึงตั้งสมมุติฐานว่า เนื่องจากสมุนไพรและเครื่องเทศต่างๆที่เป็นส่วนประกอบในข้าวแกงทอดมีฤทธิ์การต้านการก่อกลายพันธุ์ อีกทั้งในการศึกษาแบบ co-administration study พบว่าข้าวแกงทอดโดยส่วนใหญ่ มีศักยภาพในการต้านการก่อกลายพันธุ์ของยูรีเทนคล้ายคลึงกับผลจากการศึกษาแบบ pre-feeding study type 1 โดยที่การศึกษาแบบ pre-feeding study type 2 มีศักยภาพในการต้านการก่อกลายพันธุ์ของยูรีเทนได้สูงสุด จึงสรุปว่าองค์ประกอบส่วนใหญ่ของข้าวแกงทอดไม่น่าจับยูรีเทน แต่คงไปกระตุ้นระบบกำจัดสารพิษให้สูงขึ้นตลอดระยะเวลาของการศึกษา

คำสำคัญ: ฤทธิ์ต้านการก่อกลายพันธุ์ ข้าวแกงทอด ยูรีเทน วิธี SMART

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Introduction

Thai traditional diet is characterized with high amount of vegetables, fruits, herbs and spices[1]. Several studies reported that components of diet could be a major factor in modulating the risk of cancer, for instance, Thai dishes have been reported for their antimutagenic potency, *in vitro* and *in vivo*[2-5]. Battered and fried Thai dish (BFTD) is a new form of Thai fast food currently favorite for ones who have less duration of lunch hour or as a snack for young adult. Hageman *et al*.[6] performed a frying experiment carried out under laboratory conditions and they indicated that mutagenic polar substances were formed during repeated and prolonged use of deep-frying fat. Since BFTD is prepared by deep-fat frying process, it was interested to determine whether BFTD was mutagenic and could modify the mutagenicity of urethane in somatic mutation and recombination test (SMART) using *Drosophila melanogaster*.

Materials and Methods

Chemicals and Samples: Urethane (URE) was purchased from Sigma Chemical (St. Louis, Mo, USA). Glycerol was bought from Farmitalia Carlo Erla (Milan, Italy). Other chemicals were of laboratory grade. The battered and fried Thai dishes (BFTDs) of rice fried with sausage and ham, rice fried with crab meat, rice with pork fried with garlic and pepper, rice with shrimp paste and fried Indo-Pacific Mackerel, rice fried with holy basil and pork, rice with spicy shrimp soup, and rice fried with salted fish were purchased from two suppliers, namely "Friday Market" (Mahidol University at Salaya, Nakhorn Pathom Province) and "Wanglaung market" (Bangkoknoi, Bangkok Metropolitan).

Sample Preparation: The samples from different sources were separately studied as the study 1 and the study 2. Each sample obtained as a frozen precooked product was fried in palm oil at 150 °C for 2 min. Excessive oil was drained on paper towels. The finished product was dried in a freeze dryer for approximate 3 days depending on moisture content of each sample. The freeze-dried sample was blended in an electrical blender to be powder and kept frozen until used.

***Drosophila* medium**: The standard medium was the formula developed by Roberts[7]. Sugar (0.20 g), agar (0.028 g), yeast (0.10 g) and corn flour (0.25 g) were blended and boiled in a 15-ml test tube containing 2 ml of water until the mixture became glutinous. It was then left for 10 min and covered with a cotton plug. Appropriate amount of each BFTDs was added to standard medium at the ratio of 1:1, 1:3, or 1:7 w/w and the mixture

was homogenized; the final percentage of sample in each experimental medium was 50%, 25% or 12.5%, respectively.

Somatic Mutation and Recombination Test: Before running each antimutagenicity assay, survival rate of *Drosophila* fed on each experimental medium was noted. Only the amount of sample in the medium that gave higher than 50 percent of surviving flies was selected for further mutagenicity and antimutagenicity assays. The mutagenicity of each sample was determined as described by Graf *et al*.[8]. Antimutagenicity of each sample was evaluated in a co-administration study and two types of pre-feeding studies described earlier[9]. The wing spots data were evaluated as described by Frei and Würgler[10].

Percent inhibition of each sample is calculated as follows:

Percent of modification = ((A-B)/A) x 100

Where A is the number of total spots per wing of positive urethane control group, B is the number of total spots per wing of each experimental group. It was proposed that percent of inhibition between 0–20%, 20–40%, 40–60% and higher than 60% were classified as negligible, weak, moderate and strong antimutagenicity, respectively.

Results

The number of surviving adult flies brought up on all experimental media was higher than 50%; thus, it indicated that none was too toxic for further testing. The mutagenicity of each BFTD was evaluated from the data of total spots per wing obtained from surviving flies. None of the samples was mutagenic since they did not significantly induce the frequencies of mutant spots to be higher than that of the negative control ($p<0.05$) (data not shown).

The percent inhibition on urethane mutagenicity was calculated to show the relationship between the mutagenicity of urethane in the presence and absence of each BFTD in *Drosophila* medium. The data given in Figure 1 demonstrate that the percent inhibition of each sample is within the range of 25.79 % (BFTD of rice fried with sausage and ham in the study 2) to 54.42% (BFTD of rice with pork fried with garlic and pepper in the study 1). Most BFTDs decreased number of wing sports induced by urethane in this investigation.

The data shown in Figure 2A and B reveal the antimutagenic effect of all BFTDs in the pre-feeding study. Percent inhibitory of each sample in the pre-feeding study type 1 was lower than that of the pre-feeding study type 2. In addition, most samples in the study

2 of pre-feeding were moderate and strong antimutagenic (range 40-60 and 60-100% inhibition).

Discussion

The results confirmed that such samples are safe for most consumers. None of samples was mutagenic since they did not significantly induce the frequencies of mutant spots to be higher than that of the negative control medium. The average size and survival rates of adult flies obtained from larvae fed on medium containing each BFTD did not show any difference compared with those of the control group (fed on standard medium).

The antimutagenicity of BFTDs might be due to their ingredients that can modulate detoxifying enzymatic system. URE is metabolically activated by cytochrome P-450 enzyme system[11] to be vinyl epoxide (the reactive intermediate of URE metabolism) which is the carcinogenic active metabolite [12]. Kemper reported that the carcinogenic metabolites of URE were detoxified with glutathione-S-transferase (GST) conjugation. Substantial information indicated that the mutagenicity of URE decreased in the presence of antimutagens in many food and beverages[13]. In order to clarify such hypothesis, the pre-feeding study types 1 and 2 were performed.

Contacting with the sample only on the early stage (day 0 to day 3) of larval period supposed to induce or inhibit the activity of enzyme in biotransformation system in the pre-feeding study type 1. While continuous feeding of sample to the larvae in the pre-feeding study type 2 allowed them to contact the possible modulators of enzymes in each sample for the whole period of larvae stage. In case that the reduction of urethane induced spots per wing in the co-administration is nearly the same as of the result of pre-feeding type 2 study and the inhibition of both studies is stronger than that of the pre-feeding type 1, it seems that samples can scavenge urethane and/or induced the enzymatic system to detoxify urethane.

Comparing the results between co-administration study and that of pre-feeding type 1, it suggested that the components of most samples should not trap urethane since the inhibitory effect on urethane mutagenicity of each sample in both studies mentioned above were nearly at the same magnitudes. Since the experimental design of the pre-feeding study type 1 did not allow the larvae to contact with urethane in the presence of each sample; therefore, the inhibitory effect should rather belong to the induction of detoxifying enzymes. This also confirmed by the higher inhibitory effect of most samples

determined from the result of the pre-feeding study type 2 that allowed the larvae contacted with the samples from day 0 after hatching until they were collected for wing analysis; thus, the detoxifying enzymes might be stimulated and stable throughout the whole period of study.

Over all result showed that most BFTDs could reduce the mutagenicity of urethane. The protection may be concerned with the effect of some herbs and spices. BFTDs had garlic as a common ingredient. Vegetables, herbs and spices consumption in India and others Asian nations are claimed to exhibit potential anticarcinogenicity and other health benefits[14]. It was shown that some organosulfur compounds in garlic could increase the expression of glutathione-S-transferase (GST) in red blood cells of rat[15]. Guyonnet *et al.* [16] showed that these CYP modifications can lead to an enhancement or a decrease in the mutagenicity of carcinogens in the Ames test. Abraham[17] also suggested that interaction among the naturally occurring compounds present in each herb might be of significance concerning its antimutagenicity. Small amount of antimutagenic substances such as organic sulfur containing compound, β-carotene, flavonoids presented in each BFTD may not exert its significant protective effects by itself; however, the significant in vivo antigenotoxic effect may be the bio-interaction of most compounds to produce the additive or synergistic effect. Many citrus flavonoids (phenolic compounds) have been reported for their antimutagenicity against many mutagens by modulating the detoxifying enzymes of the host[18-19]. Chili pepper is a common spice in Thai cuisine and also in some BFTDs of this study. Laohavechvanich *et al.* [20] revealed that hot chili peppers prepared as raw paste (chili ground in water), pickled in vinegar or stir-fried in palm oil were antimutagenic against urethane induced *Drosophila melanogaster* wing hairs. Holy basil which is the main flavor in BFTD of rice fried with holy basil and pork may be the main protecting factor against mutagenicity of URE. The effect of alcoholic extract of holy basil leaves reported by Banerjee *et al.*[21] in elevating the levels of cytochrome P450, cytochrome b5, aryl hydrocarbon hydroxylase in liver and enhanced glutathione-*S*-transferase (GST) while reduced glutathione level in the liver, lung and stomach of mice might explain why the mutagenicity of urethane reduced in this study.

In conclusion, the present study assessed the safety of battered and fried Thai dishes. All BFTDs were mutagen free. They were also sources of antimutagens in the somatic mutation and recombination test

(SMART). They also expressed their antimutagenicity against urethane in co-administration and pre-feeding studies of SMART. Each BFTD contained many natural phytochemicals. The data suggest that BFTD should be promoted for ones who omit either meal due to rushing life style and as a good snack for health concerning consumers.

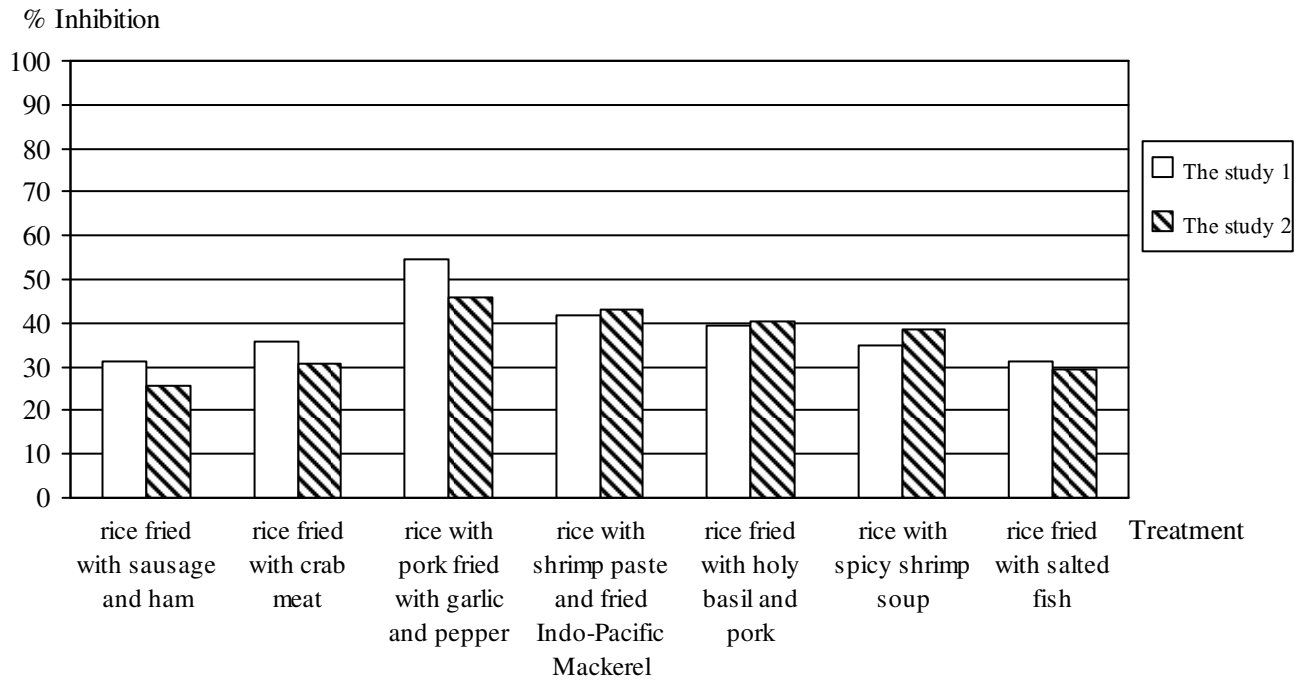
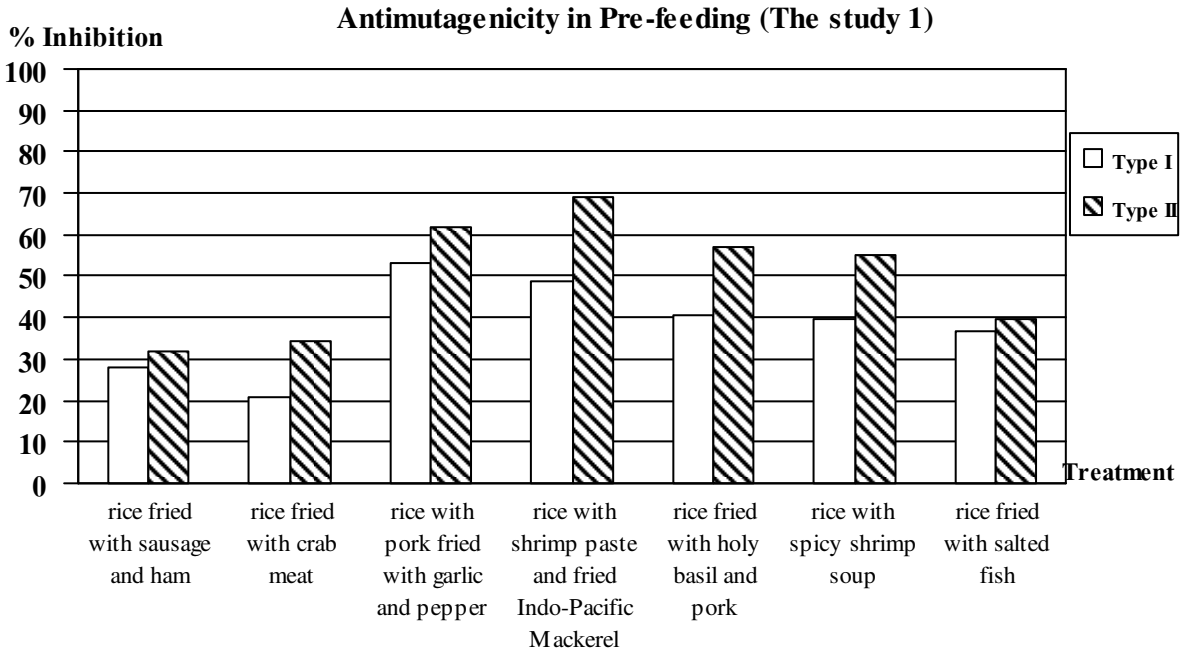


Figure 1 Antimutagenicity of each BFTD on urethane (20mM) induced somatic mutation and recombination in *Drosophila melanogaster* derived from trans-heterozygous (*mwh+/+flr*^{3}) larvae in the co-administration.

(A)



(B)

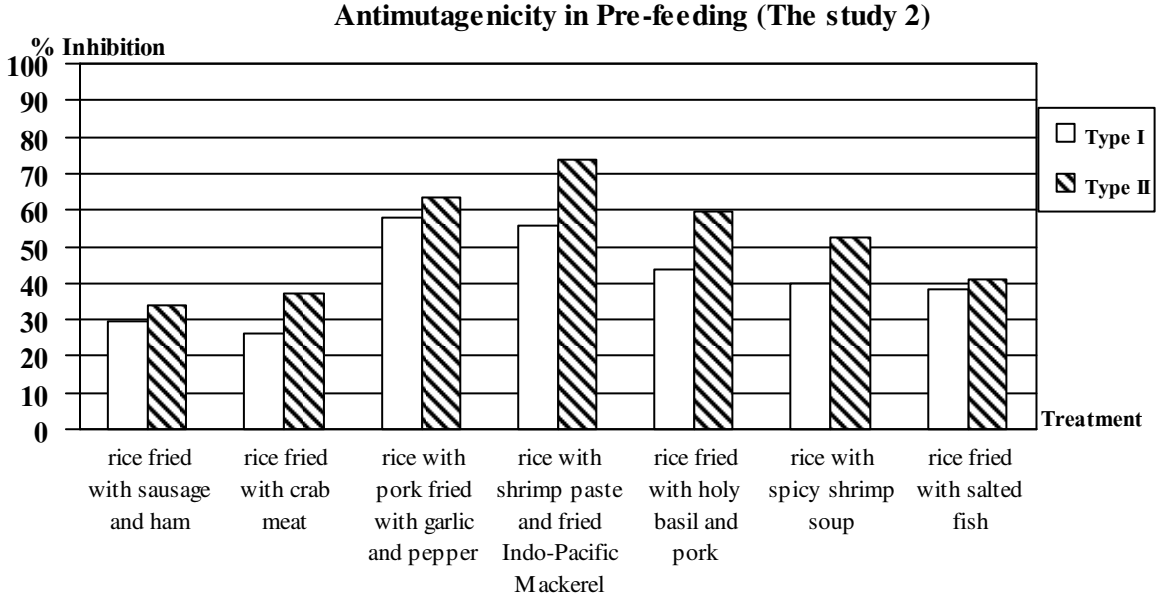


Figure 2 Antimutagenicity of each BFTD on urethane (20mM) induced somatic mutation and recombination in *Drosophila melanogaster* derived from trans-heterozygous (*mwh+/+flr*^{3}) larvae in the pre-feeding studies.

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