Antimutagenicity of Some Thai Dishes on Urethane Induced Somatic Mutation and Recombination in *Drosophila melanogaster*

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ABSTRACT

This study examined the mutagenicity of Thai dishes, namely Thai main dishes (Tom Yam Kung, Kaeng Liang, Kaeng Som Pak Ruam, NamPrik Kapi, Nam Prik Makam, and Yam Tua Pu) and Thai one dish meals (Khaow Yam Pak Tai, Khanomjeen Nam-ngiew and Khaow Man Som Tam). The antimutagenicity of the samples on urethane (URE) induced somatic mutation and recombination in *Drosophila melanogaster* was also determined. Eighty trans-heterozygous *Drosophila melanogaster* larvae, aged three-days old, obtained from virgin *ORR*; flr^3 virgin female and *mwh* male were transferred to a test tube containing each Thai dish mix with regular medium (mutagenicity study) or regular medium containing 36 mM URE (antimutagenicity study) until they became adult flies. The ratios (w/w) of Thai dish and a mixture of regular medium or regular medium containing URE were 1:1, 1:2 and 1:4. The occurrences of mutant spots on the round wing of surviving flies were analyzed. It was found that all Thai dishes were not mutagenic. The antimutagenicity of three kinds of Thai dishes at ratios of 1:1 and 1:2 were 61-94 percent inhibition and at a ratio of 1:4 were about 45 – 83 percent inhibition. The antimutagenic mechanisms were not clearly elucidated in this study but rather suggested the effects of many antimutagens in the components of each dish. The findings from the present experiment seems to justify the claim that Thai dished are good for health, aside from its superb sensory attributes as produced by mixtures of different ingredients.

Keywords: Mutagenicity, antimutagenicity, Thai dishes, urethane, SMART

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Introduction

Many traditional cuisines, such as Mediterranean cookery, Japanese food preparation, and Thai diet are interesting because of their health benefits. Thai traditional diet is characterized with high amount of vegetables, fruits, herbs and spices.[1] Thai people consume in large quantity mixtures of various kinds of spices and prepared as curry pastes. Several studies reported that components of diet could be a major factor in modulating the risk of cancer, for instance, Thai edible plants have been reported for their antimutagenic or anticarcinogenic potency, *in vitro* and *in vivo*.[2-5] However, most studies used the extract or the unprocessed plants rather than the dishes of complex mixtures of many ingredients that may interact with each other. Only curry pastes which are mixtures of dried chilies, shallots, garlic and other ingredients depending on types of curry paste have been evaluated for possible antigenotoxicity.[6] They are used as the main ingredient in Thai curry dishes but no *in-vivo* study to evaluate the antimutagenic effect of Thai dishes was undertaken. Therefore, the *Drosophila* somatic mutation and recombination test (SMART) system has been employed in the present study to assess the effect of various Thai dishes in modulating the genotoxicity of urethane which is a promutagen metabolically activated by the cytochrome P-450 enzyme system.[7]

Materials and Methods

Chemicals and Samples: Urethane (URE) was purchased from Sigma Chemical (St. Louis, Mo, USA). Food Chemistry Division (Institute of Nutrition Mahidol University) provided the dishes for this experiment. Ingredients of each Thai dish are shown in Table 1. Each sample was homogenized as paste and kept refrigerated until used. Other chemicals were of laboratory grade.

Experimental Design Virgin females of Oregon wing flare strain (*ORR/ORR*; *flr*^{3}*/TM3, Ser)* were mated with males of multiple wing hair strain *(mwh/mwh)* on regular medium to produce *trans*-heterozygous larvae of improved high bioactivation cross (IHB). Both strains were obtained from the Institute of Toxicology (Swiss Federal Institute of Technology, and the University of Zurich) and maintained on the regular medium modified from the formula of Roberts[8] which had propionic acid (0.01 ml) as a preservative.

Appropriate amount of each Thai dish was added to regular medium at the ratio of 1:1, 1:2, or 1:4 w/w and it was homogenized; the final percentage of sample in each experimental media was 50%, 33% or 25%, respectively. Equal amount of each mixed medium was transferred into a 15 ml test tube. Each medium was used as an *experimental medium* for mutagenicity testing of each dish. URE (36 mM) was substituted for deionized water in the regular medium and was used as a *positive control medium*. An *experimental medium containing URE* was prepared by adding each Thai dish into the positive control medium at the same ratio described above and homogenized. Equal amount of each mixed medium was transferred into a 15 ml test tube. This medium was used for antimutagenicity study. The mutagenicity of each sample (in the experimental medium) was assayed as described by Graf *et al*.[9] and the antimutagenicity of each sample was assayed using the experimental medium containing URE. The larvae were maintained on medium at 25$\pm$1°C until pupation. The surviving adult flies bearing the marker trans-heterozygous (*mwh+/+flr*^{3}) indicated with round wings were collected. Subsequently, the wings were removed, mounted and scored under a compound microscope for recording of the wing spot.

Induction frequencies of wing spots of Thai dishes treated groups were compared with that of the deionized water negative control group. The estimation of spot frequencies and confidence limits of the estimated mutation frequency were performed with significant level of $\alpha = \beta = 0.05$. A multiple-decision procedure was used to decide whether a sample was positive, weak positive, inconclusive or negative mutagen as described by Frei and Wurgler.[10]

Antimutagenicity was estimated using percentage of inhibition of total spots per wing calculated as follows: percentage of inhibition = (a-b)/a x 100. Where “a” is the number of total spots per wing induced by

URE, "b" is the number of total spots per wing induced with URE administered with each Thai dish. 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

Table 1 presents the common ingredients and amount of each recipe. All Thai dishes, namely Thai main dishes (Table 2) and Thai one-dish meals (Table 3) reduced the number of URE-induced wing spots when each dish, along with URE, was administered to the three-days-old larvae. Most dishes added to the positive control medium at the ratio 1:1 and 1:2 showed strong antimutagenicity against the genotoxicity of URE (61-94% inhibition). Only the ratio 1:4 of some sample to the positive control medium revealed moderate antimutagenicity. Similar trends were obtained in both first and second trials. Only Kanomjeen Nam-ngiew (Table 3) showed weak to strong antimutagenicity effect against URE depending on the amount of the dish in the medium. This revealed that percentage of inhibition is dependent on the amount of each Thai dish added to the fly medium.

Discussion

Safety of Thai Dishes: Traditional Thai dishes are safe in terms of mutagenicity as resulted from *Drosophila melanogaster* tests. The average size and survival rates of adult flies obtained from larvae fed on medium containing each Thai dish with 1:1 ratio did not show any difference compared with the control group (fed on regular medium). Only the larvae fed on the highest amount (1:1 ratio) of either Nam Prik Makam or Nam Prik Kapi had smaller size and lower number of surviving adult flies. These dishes that contain table salt might retard the growth of larvae or even killed the larvae. Analysis performed by the Division of Food Chemistry, Institute of Nutrition, showed that both Nam Prik Kapi and the Nam Prik Makam contained 22 mg sodium per 100 g. Kangsadalampai and Sommani[11] found that size of *Drosophila* larvae fed on salty fermented soybean products namely; soy paste (26 mg sodium chloride per g) and sufu which was preserved bean curd (37 mg sodium chloride per g) were smaller than that of the negative control group and also had lower survival rates. However, this should not pose any problem to consumer since both dishes have strong flavor (i.e. hot, salty, sweet and sour) because only small amount is consumed with large amount of fresh, steamed or boiled vegetables or deep-fried mackerels.

The high unsaturated fatty acids content of vegetable oil used in Nam Prik Makam could contribute to high level of free radicals that may cause toxicity on *Drosophila melanogaster*. This organism generally lacks superoxide dismutase,[12,13] thus, some have no resistance to its toxic content. To prevent this effect of experimental medium to fly development, the amount of sample incorporated in the regular medium was reduced to 1:2 and 1:4.

Antimutagenicity of Thai dishes: URE is metabolically activated by cytochrome P-450 enzyme system (7). Vinyl epoxide, the reactive intermediate of URE metabolism, is the carcinogenic active metabolite.[14] Kemper[15] reported the carcinogenic metabolites of URE are detoxified with glutathione-S-transferase (GST) conjugation. Substantial information indicated that the mutagenicity of URE decreased in the presence of antimutagens or anticarcinogens in many food and beverages. Overall results of the present investigation showed that most Thai dishes could reduce the mutagenicity of URE. This protective outcome could be the result of more than one mechanisms and the antimutagenicity could be the total effect of all ingredients in each Thai dish.

Table 1 Ingredients of each Thai dish

Dish	English name	Ingredients of each Thai dish	
		Sub-group	Food item and amount per recipe (g)
Kaeng Liang	Thai style vegetable soup	main	peeled pumpkin (121), hairy basil leaves (73), ivy gourd leaves (81), sponge gourd (109), mushrooms-straw (150), bottle gourd (100), soup stock (1188),
		chili paste	pepper (3.5), peeled shallot, sliced (89), shrimp paste (20), ground dried shrimp (44)
		seasoning	fish sauce (17)
Kaeng Som Pak Ruam	Sour and spicy curry	main	snake head fish (215), meat snake-head fish (110), water (1069), long beans (210), young water melon (240), cabbage (230), sesbania flowers (150)
Nam Prik Kapi*	Dried shrimp paste dip	main	grilled shrimp paste (43), hot chili (4.6), peeled garlic (18.9), ground dried shrimp (3.5), pea aubergine (36), ripe ma-euk, sliced (20), old round aubergine seed (4), red hot chili (1)
		seasoning	fish sauce (28), lime juice (50), palm sugar (47)
Nam Prik Makam*	Tamarind dip	main	peeled young tamarind (95), hot chili (2.8), chopped peeled garlic (24), grilled shrimp paste (28), soybean oil (23), ground dried shrimp (12)
Tom Yam Kung	Sour and spicy prawn soup	main	giant fresh water prawn (441), mushroom-straw (420), young galangal (15.4), kaffir lime leaves (1.5), lemon grass (29), hot chili (4.7), coriander leaves (7), soup (832)
		seasoning	fish sauce (73.6), lime juice (73.8)
Khanom-jeen Nam-ngiew	spicy rice noodles	Main	Thai noodle or Khanom Jeen (400), small cubes pork blood (138), pork chop (75), small tomato (139.4), dried red cotton flowers (2.3), pork cartilage stock (600), chopped pork cartilage (262), water (865)
		chili paste	dried chili (11.5), shrimp paste (3.7), sliced shallots (44.4), sliced peeled garlic (10), sliced galangal (2.8), sliced coriander rhizomes (2.9), dried fermented soybean (22.5), vegetable oil (16.8), fish sauce (62)
		side dish (vegetables)	mung bean sprouts (200), sliced fermented chinese cabbage (80), spring onion, sliced (12), sliced coriander leaves, (4), lime juice (26.8), fried garlic (7.4), fried-dried chili (5.8)
Khaow Man Som Tam	Oily Rice With Spicy Papaya Salad	Khaow-Man	rice (250), coconut milk (356), grated coconut (300), water (238), sugar (16), salt (4), pandatus leaves (2 leaves)
		Som Tam	raw papaya (227), peeled garlic (5), dried chili (3), pepper (0.1), tamarind extract (31.5), fish sauce (46.5), palm sugar (62.8), lime juice (29.5), ground dried shrimp (10), hot chili (2.2), small lime peels (8), vegetables lettuce (75), coral leaves (20)
Khaow Yam Pak Tai	Rice salad	main	cooked rice (780), fried sun-dried rice (167), ground dried shrimp (73), roasted grated coconut (96), lime juice (96), ground chili (8.3),pounded budu (250), salty budu (125), palm sugar (190), pounded lemon grass (30), pounded galangal (18), kaffir lime leaves (3.7), pounded shallot (57.7), water (505)
		side dish (vegetable/fruit)	long bean (249), mung bean sprout (395), sliced cucumber (181), fine sliced kaffir lime leaves (11), fine sliced lemon grass (120), fine sliced wild betel leaves (27), pomelo, edible portion (454)

*Generally consumed with a combination of various vegetables

Table 2 Effect of each Thai main dishes on URE-treated *Drosophila melanogaster*

Sample	% of sample in the fly medium	Spots per wing a (Number of spots from 40 wings)				Percent Inhibition	Antimuta-genicity classification
		Small single (m=2.0)	Large single (m=5.0)	Twin (m=5.0)	Total (m=2.0)		
First trial							
Water	-	0.2(8)	0.00(0)	0.00(0)	0.2(8)	-	-
36 mM Urethane	-	13.30(532)+	2.68(107)+	0.30(12)+	16.28(651)+	0	-
Tom Yam Kung	50	2.85(114)+	0.98(39)+	0.22(9)+	4.05(162)+	76	strong
	33	2.95(118)+	0.60(24)+	0.10(4)+	3.65(146)+	78	strong
	25	5.70(228)+	1.15(46)+	0.10(4)+	6.95(278)+	59	moderate
Kaeng Liang	50	1.80(72)+	0.05(2)+	0.00(0)	1.85(74)+	87	strong
	33	3.18(127)+	0.40(16)+	0.10(2)+	3.63(145)+	74	strong
	25	5.13(205)+	0.85(34)+	0.20(8)+	6.18(247)+	56	moderate
Kaeng Som Pak Ruam	50	3.40(136)+	0.97(39)+	0.08(3)+	4.45(178)+	68	strong
	33	3.32(133)+	1.45(58)+	0.17(7)+	4.95(198)+	65	strong
	25	4.78(191)+	2.45(98)+	0.32(13)+	7.55(302)+	46	moderate
Nam Prik Kapi	50	3.58(143)+	0.25(10)+	0.15(6)+	3.98(159)+	78	strong
	33	3.60(144)+	0.55(22)+	0.23(9)+	4.38(175)+	76	strong
	25	6.35(254)+	1.25(50)+	0.22(9)+	7.82(313)+	57	moderate
Nam Prik Makam	50	2.56(41)+	0.38(6)+	0.00(0)	2.94(47)+	83	strong
	33	6.40(256)+	2.00(80)+	0.30(13)+	8.70(348)+	49	moderate
	25	1.65(66)+	0.48(19)+	0.07(3)+	2.20(88)+	86	strong
Yam Tua Pu	50	3.85(154)+	0.85(34)+	0.15(6)+	4.85(194)+	71	strong
	33	3.70(148)+	0.70(28)+	0.40(16)+	4.80(192)+	71	strong
	25	4.40(176)+	1.52(61)+	0.40(16)+	6.32(253)+	62	Strong
Second trial							
Water	-	0.18(7)	0.00(0)	0.00(0)	0.18(7)	-	-
36 mM Urethane	-	12.77(511)+	4.00(160)+	0.28(11)+	17.05(682)+	0	-
Tom Yam Kung	50	1.88(75)+	0.32(13)+	0.15(6)+	2.35(94)+	86	strong
	33	3.78(151)+	1.25(50)+	0.25(10)+	5.28(211)+	69	strong
	25	4.85(194)+	1.35(54)+	0.15(6)+	6.35(254)+	63	Strong
Kaeng Liang	50	1.33(53)+	0.02(1)+	0.00(0)	1.35(54)+	91	strong
	33	1.82(73)+	0.43(17)+	0.05(2)+	2.30(92)+	85	strong
	25	3.00(120)+	0.92(37)+	0.08(3)+	4.00(160)+	73	Strong
Kaeng Som Pak Ruam	50	3.10(124)+	0.90(36)+	0.28(11)+	4.28(171)+	71	strong
	33	3.83(153)+	1.40(56)+	0.32(13)+	5.55(222)+	63	strong
	25	4.88(195)+	2.52(101)+	0.28(11)+	7.68(307)+	49	moderate
Nam Prik Kapi	50	3.48(139)+	0.60(24)+	0.10(4)+	4.18(167)+	76	strong
	33	4.15(166)+	0.95(38)+	0.20(8)+	5.30(212)+	70	strong
	25	6.52(261)+	0.92(37)+	0.28(11)+	7.72(309)+	56	moderate
Nam Prik Makam	50	4.15(83)+	0.45(9)+	0.30(6)+	4.90(98)+	71	strong
	33	4.18(167)+	1.60(64)+	0.22(9)+	6.00(240)+	65	strong
	25	4.92(197)+	0.93(37)+	0.35(14)+	6.20(248)+	63	strong
Yam Tua Pu	50	4.78(191)+	0.90(36)+	0.27(11)+	5.95(238)+	65	strong
	33	4.12(165)+	0.75(30)+	0.48(19)+	5.35(214)+	69	strong
	25	4.88(195)+	1.90(76)+	0.27(11)+	7.05(282)+	59	moderate

[a] Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with deionized water : + = positive, - = negative; m = multiplication factor. Probability levels: $\alpha = \beta = 0.05$. Using one-sided statistical tests.

The modulation detoxifying system could be a mechanism to inhibit the mutagenicity of URE. Citrus plants used in Thai dishes, namely, lemon grass, kaffir lime leaves and lime juice contain some bitter compounds e.g., limonene, naringenin, naringin, diosmin, tangeretin and rutin. Many citrus flavonoids (phenolic compounds) have been reported for their antimutagenicity against many mutagens by modulating the detoxifying enzymes of the host.[16,17] In this study, garlic and shallot, the most common herbal

Table 3. Effect of each Thai one-dish meal on URE-treated *Drosophila melanogaster*

Sample	Percent of sample in the fly medium	Spots per wing [a] (Number of spots from 40 wings)				Percent inhibition	Antimutagenicity classification
		Small single m=2.0	Large single m=5.0	Twin m=5.0	Total m=2.0		
First trial							
Water	-	0.13(5)	0.02(1)	0.00(0)	0.15(6)	-	-
Urethane	-	10.92(437)+	2.88(115)+	0.27(11)+	14.07(563)+	0	-
Khaow Yam Pak Tai	50	0.90(36)+	0.08(3)+	0.00(0)	0.98(39)+	93	strong
	33	1.68(67)+	0.18(7)+	0.02(1)+	1.88(75)+	87	strong
	25	2.40(96)+	0.35(14)+	0.10(4)+	2.85(114)+	80	strong
Kanomjeen Nam-ngiew	50	3.02(121)+	1.08(43)+	0.25(10)+	4.35(174)+	74	strong
	33	5.72(229)+	1.70(68)+	0.33(13)+	7.75(310)+	54	moderate
	25	4.20(168)+	1.9(76)+	0.15(6)+	6.25(250)+	63	strong
Khaow Man Som Tam	50	1.65(66)+	0.40(16)+	0.12(5)+	2.17(87)+	87	strong
	33	3.80(152)+	1.48(59)+	0.20(8)+	5.48(219)+	66	strong
	25	3.75(150)+	1.85(74)+	0.15(6)+	5.75(230)+	65	strong
Second trial							
Water	-	0.18(7)	0.00(0)	0.00(0)	0.18(7)	-	-
Urethane	-	11.72(469)+	4.78(191)+	0.28(11)+	16.78(671)+	0	-
Khaow Yam Pak Tai	50	0.95(38)+	0.05(2)+	0.00(0)	1.00(40)+	94	strong
	33	1.78(71)+	0.02(1)+	0.05(2)+	1.85(74)+	89	strong
	25	2.55(102)+	0.35(14)+	0.00(0)+	2.90(116)+	83	strong
Kanomjeen Nam-ngiew	50	2.52(101)+	0.68(27)+	0.12(5)+	3.32(133)+	81	strong
	33	4.00(160)+	1.75(70)+	0.20(8)+	5.95(238)+	65	strong
	25	7.03(281)+	3.40(136)+	0.25(10)+	10.68(427)+	38	weak
Khaow Man Som Tam	50	3.37(135)+	1.00(40)+	0.25(10)+	4.62(185)+	70	strong
	33	4.30(172)+	1.48(59)+	0.20(8)+	5.98(239)+	61	strong
	25	4.07(163)+	1.30(52)+	0.28(11)+	5.65(226)+	63	strong

[a] Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with deionized water : + = positive, - = negative; m = multiplication factor. Probability levels: $\alpha = \beta = 0.05$. Using one-sided statistical tests.

ingredients in Thai dishes were used. Many organosulfur compounds such as diallyl sulfide (DAS) and diallyl disulfides (DADS) could increase the expression of glutathione-*S*-transferase (GST) in red blood cells of rats.[18] These compounds modulated levels of cytochrome P450 isozymes and increased activity of epoxide hydrolase and glutathione-*S*-transferase[19,20] and reduced the genotoxicity of aflatoxin B_1 and *N*-nitrosodimethylamine (NDMA) in rat.[21] Curry pastes commonly consumed in Thailand contain garlic and shallot as major ingredients, showed antimutagenicity against URE in *Drosophila melanogaster.*[6]

Many carotenoids found in ivy gourd, pumpkin, pepper and hairy basil showed their antimutagenic activities in many studies.[22,23] Carotenoids are known antioxidants both *in vitro*[24] and *in vivo*[15], therefore, they can counteract some mutagens that require metabolic activation through cytochrome P-450 system[25,26] to oxidise them to ultimate mutagens. Further studies to explain the antimutagenic mechanism of carotenoids are still necessary. Moreover, some components that may be present in Thai dishes such as organosulfur compounds and flavonoids inhibited DNA-adduct by scavenging the reactive species of the mutagen.[27,28]

Since the study was conducted as the co-administration of URE with each dish, desmutagenic activity of some components may interfere with the availability of URE in young larvae. Many vegetables, herbs and spices contain dietary fiber and chlorophyll. Antimutagenicity was observed when some dietary

fiber such as lignin and suberin adsorb the mutagens[29-31] and chlorophyll formed complex with mutagens.[32] *In vitro* or *in vivo* studies on free radical scavenging activities of dietary fiber such as pectin on colon mucosa of rats were reported. Alkali-lignin inhibited both enzymatic and non-enzymatic lipid peroxidation on cell culture. Lignin and ferulic acid in wheat bran acted as a nitrite scavenger on cell culture.[33-35] However, there has been no information on the scavenging activity of these compounds on URE; thus, further investigations would be relevant.

It seems to justify the claim that Thai dishes are good for health, aside from its superb sensory attributes as produced by mixtures of different ingredients. The protective effects of each dish may be due to the presence of antimutagenic ingredients. However, this study investigated only the result of co-administration of various Thai dishes with URE. The level of protection may be clearer when the experiments are extended to be pre-feeding study.

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