ฤทธิ์ต้านการก่อกลายพันธุ์ของมะเขือม่วงไทยที่ผ่านกระบวนการปรุงอาหารจากการเหนี่ยวนำให้เกิดการก่อกลายพันธุ์ในแมลงหวี่ด้วยสารยูรีเทน

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บทคัดย่อ

มะเขือเป็นแหล่งที่ดีของสารต้านอนุมูลอิสระ และกระตุ้นการกำจัดสารพิษพวกอิเล็คโตรไฟล์ คณะผู้วิจัยศึกษาผลของมะเขือม่วงกลมดิบ และที่ผ่านการปรุงอาหารต่อการต้านการก่อกลายพันธุ์ของสารก่อกลายพันธุ์มาตรฐานยูรีเทนทดสอบในแมลงหวี่ โดยมะเขือม่วงกลมดิบถูกตัดเป็นชิ้นเล็กและแบ่งออกเป็นสองส่วน ส่วนแรกถูกแช่แข็งทำให้เป็นผงและเก็บไว้เป็นตัวอย่างกลุ่มควบคุม ส่วนที่สองถูกนึ่ง จากนั้นนำมาแช่แข็งและทำให้เป็นผง โดยครึ่งหนึ่งถูกเก็บเป็นกลุ่มตัวอย่างนึ่ง และอีกครึ่งที่เหลือถูกทอดจัดเป็นกลุ่มตัวอย่างทอด จากการศึกษาพบว่ากลุ่มตัวอย่างดิบและกลุ่มที่ได้รับการปรุงอาหารมีฤทธิ์ต้านอนุมูลอิสระและปริมาณสารฟีนอลนิก กลุ่มตัวอย่างที่ผ่านการนึ่งทั้งที่ทอดและไม่ทอดเพิ่มฤทธิ์ต้านอนุมูลอิสระและปริมาณสารฟีนอลิกเมื่อเปรียบเทียบกับกลุมตัวอย่างดิบ จากนั้นศึกษาฤทธิ์ต้านการก่อกลายพันธุ์ในแต่ละความเข้มข้นซึ่งตัวอย่างมะเขือถูกผสมพร้อมกับอาหารมาตรฐานและน้ำกลั่นหรือสารยูรีเทน โดยหนอนแมลงหวี่ชนิดพันธุ์ทางถูกเลี้ยงในหลอดทดลองที่มีอาหารดังกล่าวข้างต้น เมื่อแมลงหวี่โตเต็มวัยปีกของตัวที่รอดตายถูกนำมาวิเคราะห์ ผลการศึกษาพบว่าไม่มีกลุ่มตัวอย่างใดทำให้เกิดการกลายพันธุ์ ทั้งยังแสดงฤทธิ์ต้านการก่อกลายพันธุ์ โดยแต่ละกลุ่มตัวอย่างที่ถูกทดสอบพร้อมกับสารยูรีเทนนั้นสามารถลดจำนวนของจุดที่ก่อกลายพันธุ์ทั้งหมดต่อปีก เป็นไปได้ว่าสารออกฤทธิ์สำคัญของมะเขือม่วงกลมเช่นกลุ่มของโพลีฟีนอล อาจเป็นตัวยับยั้งการออกฤทธิ์ทางชีวภาพของยูรีเทน นอกจากนี้ผลของกระบวนการปรุงอาหารอาจเพิ่มความสามารถในการออกฤทธิ์ต้านการก่อกลายพันธุ์ของมะเขือม่วงกลมได้ขึ้นกับชนิดหรือวิธีการปรุงอาหาร

คำสำคัญ: มะเขือม่วงกลม ฤทธิ์ต้านการก่อกลายพันธุ์ ฤทธิ์ต้านอนุมูลอิสระ แมลงหวี่

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Antimutagenic Effect of Cooking Treatments of Thai Purple Eggplant (*Solanum melongena* L.) Fruit on Urethane-Induced Somatic Mutation and Recombination in *Drosophila melanogaster*

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Abstract

Eggplant fruit is a good source of antioxidant and stimulate the detoxification of electrophiles. Here, we investigated the effects of raw and cooking processes of Thai round purple eggplant fruit (*Solanum melongena* L. 'Ma Khuea Muang Glom') against urethane-induced mutagenesis in *Drosophila melanogaster*. Raw sample was cut into small-thin pieces and divided into two portions; the first one was lyophilized and kept as an untreated control. The second portion was steamed and lyophilized; the half of it was kept as the steamed sample and the other one was fried in palm oil, served as the fried sample. We found that both untreated and treated samples had antioxidant activities and contained total phenolic contents. Steaming with/without frying resulted in increasing of antioxidant activity and total phenolic contents compared with those of the raw lyophilized one. For mutagenic approach, an experimental *Drosophila* medium containing mixture of standard medium powder and lyophilized sample (1:0.25, 1:0.5, or 1:1 w/w) and 2 ml of distilled water or 20 mM urethane were prepared for mutagenic or antimutagenic evaluations of the samples, respectively. Trans-heterozygous larvae (mwh flr+/mwh TM3) were transferred to the appropriate medium. The wings of surviving flies were analyzed for occurrence of mutant spots. None of the samples was mutagenic. Eggplant expressed its antimutagenicity in this experiment; along with urethane treatment, the samples could reduce the number of total induced spots per wing. Bioactive components of eggplant, possibly polyphenols, might inhibit bioactivation of urethane. It was noted that cooking treatments could increase antimutagenesis of the samples depending on types of treatment.

Keywords: Round purple eggplant, Antimutagenicity, *Drosophila melanogaster*

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Introduction

Vegetables, such as eggplant, pepper and tomato, of the *Solanaceae* family contain high level of phenolic contents. Eggplants are especially a rich source of phenolic phytochemicals which show high free radical scavenging-linked antioxidant activity[1]. Matsubara and colleague (2005)[2] found that eggplant extracts suppressed the development of blood vessels required for tumor growth and metastasis. Moreover, the solamargine and solasonine extracts from *Solanum melongena* L. inhibited the growth of cancer cells, including human colon cancer cell line (HT 29) and human liver cancer cell line (Hep G2)[3]. Since Thai people consume both raw and processed eggplants, however; there was no study on the antimutagenicity of processed Thai eggplant against any standard mutagens. Therefore, this study was aimed to investigate the antimutagenicity of raw, steamed or fried *S. melongena* L. against urethane induced somatic mutation and recombination in *Drosophila melanogaster*. The antioxidant activity and total phenolic contents of the samples were also determined.

Materials and Methods

Chemicals and reagents

Urethane was purchased from Sigma Chemical (St. Louis, Missouri, USA). 2, 4, 6-tripyridyl-s-triazine (TPTZ), Ferric chloride hexahydrate, and Ferrous sulfate heptahydrate were purchased from Sigma Chemical (St. Louis, Missouri, USA). 2, 2-diphenyl-1-picrylhydrazl (DPPH), Gallic acid and Folin-Ciocalteu reagent were purchased from Fluka Chemika (Buchs, Switzerland). Trolox was purchased from Aldrich Chemical (Milwaukee, WI, Germany). Other chemicals were of the laboratory grade.

Sample Preparation

Thai round purple eggplant (*S. melongena* L. 'Ma Khuea Muang Glom') was purchased from three retail stores of Salaya community market, Nakhonpathom province and pooled to be a composite sample. Raw sample was cut into small-thin pieces and divided into two portions; the first one was lyophilized and kept as an untreated control sample. The second portion was steamed for 2 min and lyophilized. Then, the half of it was kept as the steamed sample. In order to reduce the uptake of oil by fried sample, as suggested by Debnath *et al*. (2003)[4], the last portion was cut into small pieces (2 mm thickness)and were steamed for 2 min. Subsequently, they were lyophilized and deep-fried at 120-140˚C for 10 sec in palm oil. It was labeled as fried sample. The processes assigned to the preparation of fried sample also inhibited polyphenol

oxidases[5] that could be activated after the vegetables were cut and exposed to oxygen. These enzymes can oxidize polyphenolic compounds which is the cause of their radical-scavenging activities loss[6]. The samples were kept in vacuum bags and stored in desiccators at room temperature for further studies.

Determination on antioxidant activities and total phenolic content

Each sample (0.5 g) was stirred with 80% methanol (50 ml) at room temperature for 2 h. The solution was filtered through Whatman filter paper No. 1 and collected in a glass bottle. Each methanolic extract was assayed for 2, 2- diphenyl-1-picrylhydrazl (DPPH) free radical scavenging activity[7], ferric reducing antioxidant power (FRAP)[8], the total phenolic content[8] with modification as suggested by Kruawan and Kangsadalampai (2006)[9].

Somatic Mutation and Recombination Test (SMART)

Each lyophilized sample (0.15 g, 0.29 g or 0.58 g) was added and mixed well with 0.58 g of standard medium[10] containing all ingredients but water (at the ratio of 1:0.25, 1:0.5, or 1:1 w/w) in a beaker. Then 0.58 g of the mixture was transferred to a test tube and 2 ml deionized water was added. Each mixture was boiled in a water bath until it became sticky. This experimental medium was used for mutagenic determination and also as a sample control in antimutagenic determination. The standard medium was used as a negative control while the standard medium containing 20 mM urethane was used as a positive control.

The mutagenicity of each sample (in the experimental medium) was assayed as described by Graf *et al*. (1984)[10]. 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 standard medium to produce trans-heterozygous larvae of improved high bioactivation (IHB) cross; both strains were kindly provided by Professor Graf U. (University of Zurich, Switzerland). One hundred larvae were transferred to each experimental medium and maintained at 25±1ºC until pupation. The surviving adult flies bearing the marker trans-heterozygous (*mwh flr*$^+$*/mwh TM3*) indicated with round wings were collected. Subsequently, 20 wings were removed, mounted on a glass slide. Number of spots per wing was scored under a compound microscope. Induction frequencies of wing spots of eggplant treated groups were compared with that of the deionized water negative control group. The resulting wing spots were classified as indicated in Figure 1.

The highest concentration of sample providing higher than 50% survival was selected to determine for its antimutagenicity. In the determination, 2 ml 20 mM urethane was substituted for deionized water in the experimental medium; then the experiment was performed as did in the mutagenic determination. The antimutagenicity of each sample was determined from the percentage of inhibition calculated as following:

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 percentage of inhibition between 0-20 represented a negligible effect while expression of percent inhibition between 20-40, 40-60 and more than 60 were the evidences of weak, moderate and strong antimutagenicity, respectively[9].

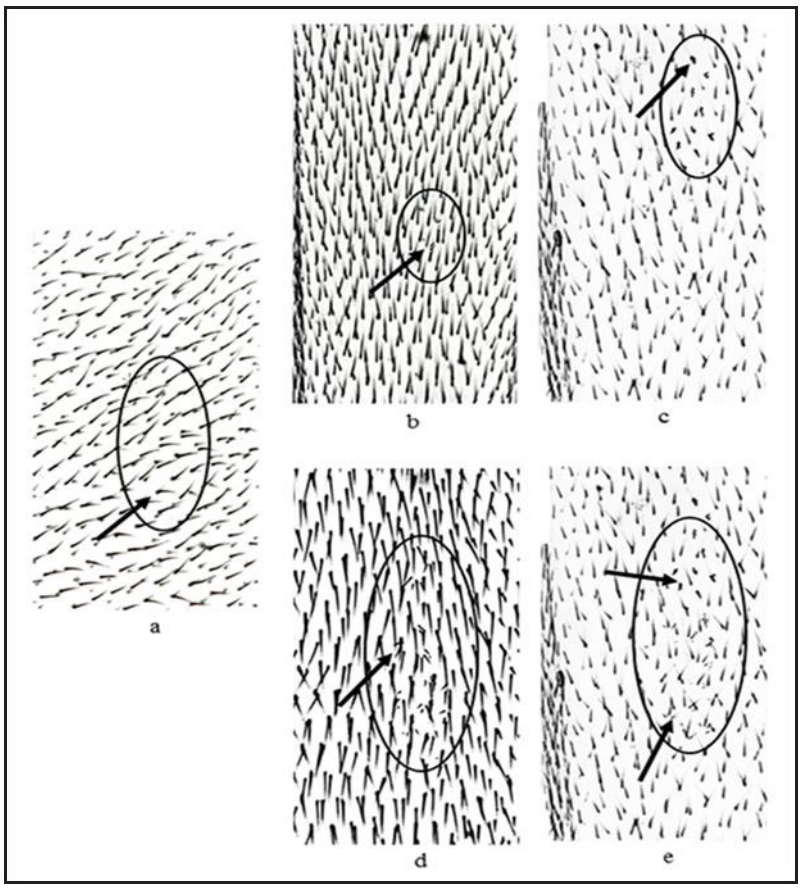

Figure 1. The different types of wing hair mutation (By courtesy of Assoc. Prof. Kaew Kangsadalampai); a) no mutation spot (one single spot on wing), b) small single spots of mwh on wing, c) large single spots of flare on wing, d) large single spots of mwh on wing, e) twin spots (mwh and flare) on wing.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD) of three experiments and the antioxidant results were processed using one way analysis of variance ($p < 0.05$) using SPSS. For SMART data, the estimation of spot frequencies and confidence limits of the estimated mutation frequency were performed with significant level of $\alpha = \beta = 0.05$, using one-sided statistical tests. A multiple-decision procedure was used to decide whether a sample was positive, weak positive, inconclusive or negative mutagenicity as described by Frei and Würgler (1988)[11].

Results and Discussion

Antioxidant activity and total phenolic contents of different cooking treatments of eggplant

The antioxidant activity and total phenolic contents of the methanolic extracts from raw, steamed and fried *S. melongena* L. samples are shown in Table 1. The radical scavenging by the extract from each sample was expressed as μM Trolox equivalent (TE)/g dry weight (DW). Cooking treatments increased the scavenging activity on DPPH radicals of eggplant. The DPPH scavenging activity of extracts from raw eggplant were 40.87; after being steamed or fried, the DPPH scavenging activity approximately increased to 110. In FRAP assay, the reducing power values of the extract from raw eggplant were 45.70; the values increased after the sample were steamed to 251.13 μM Fe (II)/g DW. In addition, the FRAP values of the eggplant extract after being fried increased to 228.60 μM Fe(II)/g DW compared with that of its corresponding raw one. The total phenolic contents of the extract from raw sample were 392.28 mg gallic acid equivalent (GAE)/100g DW. Steaming increased the total phenolic contents of eggplant extract to 1970.11 mg GAE/100g DW. Concerning on frying process, the total phenolic contents of this eggplant extract increased to 1930.26 mg GAE/100g DW compared with that of the raw one (Table 1).

Table 1. Antioxidant activity and total phenolic content of Thai round purple eggplant with different cooking treatments

Treatment	DPPH (μmole TE/g DW)	FRAP (μM Fe(II)/g DW)	Total phenolic content (mg GAE/100g DW)
Raw	40.87 ± 0.33	45.70 ± 3.12	392.28 ± 0.74
Steaming	110.01 ± 0.54*	251.13 ± 0.87*	1970.11 ± 2.13*
Frying	110.74 ± 0.31*	228.60 ± 0.21*	1930.26 ± 0.58*

Data are presented as mean ± SD (n = 3). *$p < 0.05$ versus raw treatment in each assay.

The increase of total phenolic contents and antioxidant activity after steaming or frying suggested that heat treatments performed in the present study released some phytochemicals from the insoluble portion of eggplants. Thermal treatment significantly increased both phenolic contents and antioxidant activity of sweet corn[12]. Frying samples maintained the total phenolic content and antioxidant activity (except that assayed by FRAP method) of *S. melongena* L. It is possible to hypothesize that anthocyanins might be responsible for increasing or maintaining the antioxidant activities and total phenolic content of *S. melongena* L. Anthocyanins isolated from *S. melongena* L. could both inhibited hydroxyl radical generation and scavenged superoxide[13,14]. However, Harbourne and colleague (2008)[15] indicated that temperature could have a deleterious effect on anthocyanins. Some evidence reported that blanching, boiling and steaming resulted in losses of 59%, 41% and 29%, respectively, in anthocyanin contents of red cabbage[16]. However, chlorogenic and caffeic acids which are the major phenolic compounds in eggplant[17] might be responsible for the maintenance of antioxidant activity and phenolic content of the samples since *S. melongena* L. contained 154 mg/100g of chlorogenic acid and 12.8 mg/100g of caffeic acid[18]. In addition, Scalzo *et al*. (2010)[18] suggested that grilling *S. melongena* L. for 4-5 min or boiling for 10 min increased the amounts of phenolic compounds namely, chlorogenic and caffeic acids. Similar results were also reported by Re *et al*. (2002)[19] who found that heat processing of tomato increased chlorogenic and caffeic acids. As the matter of fact, heat treatment was reported to increase the chemical extractability of phytochemical compounds because it released most phytochemicals from chromotoplasts leading to an increment of concentration[20]. Additionally, it has been exhibited that heating at 98˚C

for 10 min increased the anthocyanin content of cherries, peaches and plums[21].

Antimutagenicity of different cooking treatments of eggplant in SMART

Round purple eggplant (*S. melongena* L.) was processed using two cooking treatments and raw treatment. All samples were determined for its mutagenicity and antimutagenicity. First, each sample treatment was assessed the toxicity on adult flies which obtained from 100 trans-heterozygous (*mwh* $^{+/+}$*flr*^{3}) larvae of IHB cross to experimental medium. Table 2 show that none of the sample was toxic with different concentration. The survivals of adult flies fed on both raw and heat-processed eggplants provided more than fifty percents and the flies had normal size. Only the larvae fed on the highest dose of frying treatment had lower number of surviving flies than urethane and negative control. Since this sample was deep-fried in oil that contained fat, resulting in retarded the growth of larvae. Accordingly, previous study found that high-fat diet caused reduction on survival of flies[22]. However, the surviving flies of the frying treatment had more than fifty percent cutoff. This indicated that no sample was toxic for the tester organism. Thus, the highest concentration (1:1 w/w) of each treatment was selected for mutagenic and antimutagenic evaluations in further analysis.

Table 2. The percentage of survival rate of adult flies fed on each experimental medium containing Thai round purple eggplant with different cooking treatments

Treatment	Percentage of surviving flies (%)		
	Concentration of sample*		
	1:0.25 (w/w)	1:0.5 (w/w)	1:1 (w/w)
Negative control	100	95	100
20 mM Urethane	80	92	94
Raw	100	90	87
Steaming	95	100	99
Frying	80	92	86

* The concentration ratio of each lyophilized sample (0.15 g, 0.29 g or 0.58 g) was added and mixed well with 0.58 g of standard medium containing all ingredients but deionized water (at the ratio of 1:0.25, 1:0.5, or 1:1 w/w) to obtain an experimental medium in a test tube.

In mutagenic testing, the result of this study has revealed that both raw and cooking processes of eggplants were not mutagenic on the somatic cells of *Drosophila* tester (Table 3). Next, antimutagenesis properties of all samples were determined. The administration of each treatment along with urethane to the larvae decreased the number of induced spots per wing, while urethane alone significantly increased the number of spots per wing as shown in Table 4. It was noted that steamed sample had higher antimutagenicity than that of its corresponding raw one. Eggplants have been consumed by Thai people without any toxic indication. Therefore, this experiment confirmed the safety of eggplants for general consumption.

The present investigation showed that *S. melongena* L. could inhibit the mutagenicity of urethane. Lee *et al*. (2004)[3] found that solamargine and solasonine extracts from *S. melongena* L. attenuated the growth of human colon cancer cell line (HT 29) and human liver cancer cell line (Hep G2). Steaming increased the antimutagenicity of *S. melongena* L. compared with that of the raw one. However, frying decreased the antimutagenicity of steamed *S. melongena* L. to be nearly the same as that of its corresponding raw one. It might be possible to hypothesize that the components of *S. melongena* L. eggplant might induce the activity of glutathione-*S*-transferase in the phase 2 of xenobiotic metabolism which increases the detoxification of vinyl carbamate epoxide (active metabolite of urethane). Nisha and colleague (2009)[23] indicated that *S. melongena* L. had 0.53 mg/100 g of anthocyanin which had the ability to induce phase 2 detoxifying enzymes in cultured cells[24]. Treatment of rat liver clone 9 cells with 50 μM anthocyanins[25] and non-cancerous breast cells with 10-20 μg/ml anthocyanins[26] enhanced their antioxidant capacity by activating glutathione-related enzymes (glutathione reductase, glutathione peroxidase) and glutathione-*S*-transferase as well as the activity of NAD(P)H: quinone reductase. *S. melongena* L. was determined to contain 154 mg/100g of chlorogenic acid and 12.8 mg/100g caffeic acid[18]. It was found that caffeic acid isolated from *Syzygium cumini* L. (Jamun or black plum) increased glutathione and increased glutathione-S-transferase[27]. Thus, it is warrant that anthocyanin and caffeic acid isolated from *S. melongena* L. should be evaluated for their inducibility on the activities of glutathione-S-transferase.

Table 3. Mutagenicity of the different cooking treatments of Thai round purple eggplant in *Drosophila melanogaster*

Treatment	Spots per wing* (Number of spots from 40 wings)			
	Small single (m = 2)	Large single (m = 5)	Twin (m = 5)	Total (m = 2)
Negative control	0.412(14)	0.088(3)	0.029(1)	0.529(18)
20 mM Urethane	12.563(402)+	2.469(79)+	1.000(32)+	16.031(513)+
Raw	0.267(8)-	0.033(1)-	0	0.300(9)-
Steaming	0.375(12)-	0.031(1)-	0	0.406(13)-
Frying	0.433(13)i	0.067(2)-	0	0.500(15)-

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler[11] for comparison with deionized water: + = positive, - = negative, i = inconclusive,
m = multiplication factor. Probability levels: $\alpha = \beta = 0.05$, using one-sided statistical tests.

Table 4. Antimutagenicity of the different cooking treatments of Thai round purple eggplant against urethane-induced somatic mutation and recombination wing spots in *Drosophila melanogaster*

Treatment	Spots per wing* (Number of spots from 40 wings)				% Inhibition (rate)#
	Small single (m = 2)	Large single (m = 5)	Twin (m = 5)	Total (m = 2)	
Negative control	0.425(17)	0.050(2)	0.050(2)	0.525(21)	-
20 mM Urethane	10.325(413)+	3.350(134)+	0.625(25)+	14.300(572)+	-
Raw/urethane	5.775(231)+	2.975(119)+	0.800(32)+	9.550(382)+	33 (w)
Steaming/urethane	3.947(150)+	2.132(81)+	0.816(31)+	6.895(262)+	46 (m)
Frying/urethane	5.618(191)+	3.294(112)+	0.588(20)+	9.500(323)+	34 (w)

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler[11] for comparison with deionized water: + = positive, m = multiplication factor. Probability levels: $\alpha = \beta = 0.05$, using one-sided statistical tests.

Antimutagenic classification: (w) = weak antimutagenicity, (m) = moderate antimutagenicity

Conclusion

The present study exhibits that Thai round purple eggplant (Ma Khuea Muang Glom) with different cooking treatment protect against urethane-induced mutagenesis in somatic mutation and recombination wing spots in *D. melanogaster*. It might be postulated that components of this eggplant were antimutagens that warrant research. For the cooking treatment such as fried eggplant, which indicate its opportunity to be developed as health-benefit snack containing both antioxidant and antimutagenicity.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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