The Thai Journal of Pharmaceutical Sciences

Volume 28
Issue 1 *2004 (issue 1-2)*

Article 7

1-1-2004

Antimutagenicity of Different Lyophilized Ripe Bananas on Mutagens in Ames Test and Somatic Mutation and Recombination Test(ฤทธิ์ต้านสารก่อกลายพันธุ์ของผงกล้วยต่างชนิด...

Kalyarat Kruawan

Kaew Kangsadalampai

Komchanok Limpichaisopon

นิพนธ์ต้นฉบับ

ฤทธิ์ต้านสารก่อกลายพันธุ์ของผงกล้วยต่างชนิดกันในเอมส์เทสต์และ Somatic Mutation and Recombination Test

กัลยารัตน์ เครือวัลย์, แก้ว กังสดาลอำไพ *, และ กรชนก ลิมปิชัยโสภณ

สถาบันวิจัยโภชนาการ มหาวิทยาลัยมหิดล ศาลายา นครปฐม 73170

* *ผู้เขียนที่สามารถติดต่อได้ โทรศัพท์: (662) 8002380 ต่อ 399, โทรสาร: (662) 4419344, ที่อยู่อิเลคโทรนิค: nukks@mahidol.ac.th*

บทคัดย่อ

การวิจัยนี้ได้ใช้เอมส์เทสต์ในการศึกษาฤทธิ์การยับยั้งของผงแห้งของกล้วยสุกที่ทำจากกล้วย 3 ชนิด คือ กล้วยน้ำว้า กล้วยหอม และกล้วยไข่ ต่อสารก่อกลายพันธุ์ที่เกิดจากปฏิกิริยาระหว่างไนไตรทและอะมิโนไพรีน (การทดสอบรูปแบบที่ 1) หรือสารสกัดเนื้อเข้มข้น (การทดสอบรูปแบบที่ 2) พบว่าในการทดสอบรูปแบบที่ 1 ทุกตัวอย่างมีประสิทธิภาพสูงในการยับยั้งการสร้างสารก่อกลายพันธุ์แบบ frameshift ใน *Salmonella typhimurium* สายพันธุ์ TA 98 ในขณะเดียวกันได้ตรวจพบการยับยั้งการสร้างสารก่อกลายพันธุ์แบบ base pair substitution ระดับปานกลางในสายพันธุ์ TA 100 ส่วนการทดสอบรูปแบบที่ 2 พบว่าตัวอย่างผงกล้วยมีผลต้านการสร้างสารก่อกลายพันธุ์แบบ frameshift ได้เล็กน้อย และไม่สามารถลดการสร้างสารก่อกลายพันธุ์แบบ base pair substitution ได้ การลดการเกิดสารก่อกลายพันธุ์ในการทดสอบทั้งสองแบบนั้น น่าจะมีความเกี่ยวข้องกับการที่ผงกล้วยจับตัวกับสารตั้งต้นในปฏิกิริยา (ไนไตรท, อะมิโนไพรีน หรือสารสกัดเนื้อเข้มข้น) ที่ใช้ในการสร้างสารก่อกลายพันธุ์ด้วยปฏิกิริยาไนโตรเซชั่น ความแตกต่างของโครงสร้างของสารตั้งต้นของรูปแบบการทดสอบที่ต่างกันน่าจะมีผลต่อศักยภาพในการยับยั้งการเกิดสารก่อกลายพันธุ์ของตัวอย่างผงกล้วย นอกจากนี้ผลการต้านการก่อกลายพันธุ์ของผงกล้วยในการยับยั้งการเกิด wing spot ที่กระตุ้นให้เกิดโดยยูรีเทนในแมลงหวี่สายพันธุ์ที่มีระบบเอนไซม์ในร่างกายสูงได้ถูกทำการทดลอง โดยแบ่งการศึกษาออกเป็น 2 แบบ คือ co-administration โดยให้หนอนแมลงหวี่อายุ 3 วัน กินอาหารที่มีทั้งผงกล้วยและยูรีเทน โดยหนอนที่ใช้ได้จากการผสมพันธุ์ระหว่างตัวเมียบริสุทธิ์สายพันธุ์ *ORR;flr*^{3} และ ตัวผู้สายพันธุ์ *mwh/mwh* ส่วนอีกแบบคือ prefeeding ที่ให้พ่อแม่แมลงหวี่ผสมกันในภาชนะบรรจุอาหารที่ผสมผงกล้วยเพื่อให้หนอนแมลงหวี่ได้กินผงกล้วยก่อนที่จะนำหนอนอายุ 3 วันให้ได้รับอาหารมีสารยูรีเทน (prefeeding type 1) หรืออาหารที่มีผงกล้วยและยูรีเทนผสมกัน (prefeeding type 2) จนกระทั่งกลายเป็นตัวเต็มวัยจึงตัดปีกมาส่องดูความผิดปกติของขนบนปีก ผลการทดสอบพบว่าการศึกษาแบบให้กินอาหารที่ผสมผงกล้วยก่อนที่จะได้รับสารยูรีเทน (prefeeding study) สามารถยับยั้งการก่อกลายพันธุ์ได้ดี ทั้งนี้อาจเกิดเนื่องมาจากระบบการเปลี่ยนแปลงยูรีเทนในตัวแมลงหวี่ด้วยสารที่มีอยู่ในผงกล้วย

กุญแจคำ

กล้วย, ฤทธิ์ต้านการก่อกลายพันธุ์, ปฏิกิริยาไนโตรเซชั่น, อะมิโนไพรีน, สารสกัดเนื้อเข้มข้น, ยูรีเทน

Original Article

Antimutagenicity of Different Lyophilized Ripe Bananas on Mutagens in Ames Test and Somatic Mutation and Recombination Test

Kalyarat Kruawan, Kaew Kangsadalampai *, and Kornchanok Limpichaisopon

Institute of Nutrition, Mahidol University at Salaya, Nakhonpathom 73170, Thailand
* *Corresponding author. Tel: (662) 8002380 ext. 399, Fax: (662) 4419344, E-mail address: nukks@mahidol.ac.th*

Abstract

The Ames test was employed to indicate the inhibitory effects of lyophilized powders prepared from three varieties of ripe bananas, namely, Klouy Nam Wa (Pisang Awak or *Musa* ABB group) Klouy Hom (Gros Michael or *Musa* AAA group), and Klouy Khai (Pisang Mas or *Musa* AA group) on mutagen formation occurring during the reaction between nitrite and aminopyrene (model 1) or commercial beef concentrate (model 2). The results showed that all samples strongly inhibited the formation of frameshift type mutagens during the reaction of model 1 in a dose-related manner while moderate inhibition was observed on formation of base pair substitution type mutagen using *Salmonella typhimurium* strains TA 98 and TA 100 as indicators, respectively. They also slightly reduced the formation of frameshift type mutagen of model 2 while there was no significant effect on base pair substitution type of the same model. Binding of each banana powder to each precursor of direct mutagen, namely nitrite, aminopyrene or beef concentrate during nitrosation was proposed. The difference between structures of mutagen precursors of different models might affect the potency to inhibit the formation of mutagen by the samples. In addition, the antimutagenic effects of the banana powders against the occurrence of wing spots induced by urethane of the improved high bioactivation cross of *Drosophila melanogaster* were determined. Co-administration study was performed by transferring three-day old trans-heterozygous larvae, obtained by mating the virgin *ORR;flr*^{3} females and *mwh/mwh* males, to the medium containing banana powder and urethane until they became adult flies. Prefeeding studies were also performed by mating the parent flies on the medium containing each sample to obtain 3-day-old larvae that were consequently raised on medium containing urethane (type I study) or medium containing urethane and sample (type II study) until they became adult flies. The results showed that the samples were effectively antimutagenic against urethane induced wing spots and percent inhibition was higher in the prefeeding study. The possible explanation may be related to *in vivo* modification of biotransformation of urethane by components of banana.

Key words

Banana, Antimutagenicity, Nitrosation, Aminopyrene, Beef concentrate, Urethane

Introduction

Dietary habits are regarded as possible causative factors in the development of a considerable proportion of human cancer although these observations have not been adequately explained (1). Foods contain mutagens and/or carcinogens, some of which occur naturally, and others that could be introduced during food preparation. Many mutagens and carcinogens have been detected in processed food. Fortunately, many studies indicated that antimutagens and/or anticarcinogens are found in all categories of foods which fruits and vegetables are the main sources. It was suggested that regular consumption of anticarcinogens and antimutagens in diet may be the most effective way of human cancer prevention (2). In Thailand, both unprocessed bananas and their processed products are generally consumed. Ripe bananas are not only cheap but also claimed with its health benefits. Several epidemiological studies on bananas demonstrated that they could decrease the risk of colorectal cancer. A case control study in Uruguay suggested that banana consumption was associated with strong reduction in risk of colorectal cancer (3). Moreover, pharmacological studies showed that bananas had antifungal (4) and antibacterial effects (5) and could prevent peptic ulcer (6). It is also very interesting to explore other benefits of ripe bananas, a cheap fruit commonly eaten by Thai people. Therefore, it is of relevance to investigate the possible antimuta-genicity of ripe bananas through *in vitro* and *in vivo* induction of mutations, and thus, promote the consumption of this fruit.

Materials and Methods

Sample preparation

Three kinds of ripe bananas namely, Klouy Nam Wa (Pisang Awak or *Musa* ABB group), Klouy Hom (Gros Michael or *Musa* AAA group), and Klouy Khai (Pisang Mas or *Musa* AA group) were bought from local markets in Bangkok. During preparation, they were washed thoroughly with water, and peeled. Edible portion was cut and homogenized in a blender; then, it was lyophilized and ground to fine powder.

Chemicals

1-Aminopyrene was obtained from Aldrich Chemical Co. (St. Louis, MO, USA). Beef concentrate was acquired commercially from local food store. E. Merck (Darmstadt, Germany) supplied histidine monohydrochloride, dimethyl-sulfoxide (DMSO) and Bacto agar. Oxoid nutrient broth No.2 was supplied by Oxoid Ltd. (Basingstoke, Hants, England). Sodium nitrite was purchased from BDH Chemicals Ltd. (Poole, England). Urethane was purchased from Sigma Chemical (St. Louis, MO, USA). Other chemicals were of laboratory grade.

Ames test

Tester strains

Salmonella typhimurium tester strains used in this study were histidine-dependent strains (His-) TA 98 (indicator of frameshift mutation) and TA 100 (indicator of base pair substitution mutation) provided by Dr. Wannee Kusamran (National Cancer Institute, Ministry of Public Health). Tester strains were checked routinely to confirm genetic features using the procedure described by Maron and Ames (7). Overnight cultures of bacteria inoculated from frozen stock culture (-80°C) in Oxoid nutrient broth No. 2 at 37°C were used for antimutagenicity test.

Mutagenicity of each sample

Direct mutagenicity of the reaction mixture was assayed according to the pre-incubation (at 37°C for 20 min) method of Yahagi *et al.* (8), using *Salmonella typhimurium* in the absence of S-9 mix. Each banana powder (2, 8 or 24 mg) was mixed with appropriate volume of 0.2 N hydrochloric acid (sufficient to acidify the reaction mixture to pH 3.0-3.5) and 250 µl of 2 M sodium nitrite in a test tube. The reaction tube of each model was shaken at 37°C for 4 h; then, it was placed in an ice bath to stop the reaction. In order to decompose the residual nitrite, 250 µl of 2 M or 4 M ammonium sulfamate was added to the reaction mixture; then it was allowed to stand for 10 min in an ice bath. The reaction mixture (100 µl) was mixed with 500 µl

0.5 M sodium phosphate-potassium chloride buffer (pH 7.4), 100 µl of overnight culture of the tester strain and incubated at 37°C in a shaking water bath for 20 min. After incubation, 2 ml molten agar (45°C) containing 5 mM L-histidine and 5 mM D-biotin was added to the tube. The mixture was mixed well and quickly poured onto a minimal agar plate. All plates were inverted and incubated at 37°C for 48 h. Colonies of histidine independence (His^+) were counted manually. Background lawn of each plate was examined for sign of toxic effect using a light microscope. Each experiment was done twice with triplicate plate and the results were reported as means and standard deviations of six plates. The sample expressed its mutagenicity higher than 2 times of spontaneous revertants with a dose-response relationship was evaluated as mutagenic.

Effect of bananas during nitrosation

The inhibitory effect of lyophilized ripe bananas on mutagen formation occurred during the reaction of nitrite and either aminopyrene (model 1) or beef concentrate (model 2) was investigated. Each banana powder (2, 8 or 24 mg) was mixed with 10 µl (for testing on *Salmonella typhimurium* TA 98) or 40 µl (for testing on *Salmonella typhimurium* TA 100) of 1-aminopyrene (0.0375 mg/ml) in the model 1 or 1 ml of the beef concentrate solution (0.5 g total solid/ml) in the model 2. The appropriate volume of 0.2 N hydrochloric acid sufficient to acidify the reaction mixture to pH 3.0-3.5 and 250 µl of 2 M (in the model 1) or 4 M (in the model 2) sodium nitrite were added into the test tube. Incubation and mutagenicity determinations were done as described by Kangsadalampai *et al.* (9). Each experiment was done twice with triplicate plates and the results were reported as means and standard deviations of six plates.

Percent of inhibition is calculated as follows:

$$\text{Percent inhibition} = (A-B)/(A-C) \times 100$$

Where A is the number of histidine revertants induced by the reaction mixture of each model, B is the number of histidine revertants induced by the reaction mixture in the presence of sample, C is the number of histidine spontaneous revertants. The antimutagenic activity is considered strong, moderate or weak when the inhibitory effect is higher than 60%, 40-60% or 20-40%, respectively. When the inhibition is less than 20% (10), no inhibitory effect is pronounced.

Somatic mutation and recombination test (SMART)

Drosophila stocks and media

Two *Drosophila melanogaster* strains were used. Virgin females of *ORR;flr^3/TM3* were crossed with males of mwh/mwh. Both strains were obtained from Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich. They were maintained on negative control medium composed (per tube) of corn flour 0.25 g, sugar 0.2 g, baker's yeast 0.1 g, agar 0.028 g and distilled water 2 ml (11). The addition of urethane solution to make the final concentration of 20 mM instead of distilled water made the medium called a positive control medium. The culture of the flies as well as of the treated larvae in all experiments were maintained at a constant temperature (25 ± 1°C).

Each lyophilized ripe banana (0.25 g) was substituted for corn flour. It was mixed with other ingredients and served as experimental medium, namely, Klouy Nam Wa medium, Klouy Hom medium, and Klouy Khai medium for mutagenesis assay. Urethane solution was substituted for distilled water to obtain experimental medium containing 20 mM urethane for antimutagenicity assay.

Antimutagenicity assay

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 antimuta-genicity assay. The mutagenicity of each sample was also determined as described by Graf *et al.* (12). Antimutagenesis of each sample was evaluated in a co-administration study and two types of prefeeding studies.

Co-administration study was employed by letting the virgin *ORR;flr^3* females and *mwh/mwh*

males mate on the negative control medium. Six days after mating, the 3-day-old larvae in equal batches, were transferred to an experimental medium (containing 20 mM urethane), the positive control medium and the negative control medium.

During the prefeeding studies, the tester flies mated on the experimental medium until the larvae were 3 days old. The larvae were transferred in equal batches to the positive control medium (prefeeding type I study) or the experimental medium containing 20 mM urethane (prefeeding type II study) at 25 ± 1°C and brought up on the media to be adult flies. Concurrent running of a negative control group and a positive control group were also performed.

After metamorphosis, the surviving adult flies were collected from the tubes on days 10-12 after egg laying and stored in 70% ethanol. Only the flies bearing the marker trans-heterozygous (*mwh*+/+*flr*^{3}) as indicated with round wings were chosen as suggested by Graf and Schaik (13). Subsequently, the wings were removed, mounted and scored under a compound microscope for recording of the wing spot as described by Graf *et al.* (12). Different types of spots, namely single spots showing either the multiple wing hairs (*mwh*) or the flare (*flr*^{3}) phenotype, and twin spots showing adjacent *mwh* and *flr*^{3} areas were recorded separately. The wing spots data were evaluated as described by Frei and Würgler (14). 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.

Results and Discussion

As a result of interaction with excess nitrite in acid solution, all samples were mutagenic toward both tester strains at high doses (Table 1). The results indicated that ripe bananas contained some convertible compounds that could interact with nitrite to produce direct mutagens. This health hazard may occur if the consumers eat any ripe bananas with food containing nitrite salt as a preservative. Many researchers (15-17) suggested that direct mutagens formed from nitrite and mutagen precursors in the acid solution of the stomach could be one of the etiologies of human stomach cancer.

We have two models that produce two direct mutagens for the Ames test. Aminopyrene and beef concentrate separately nitrosated in acid condition (simulated to the gastric digestion pH of 3.0-3.5, 37°C, 4 h) can induce positive revertants of *Salmonella typhimurium* strains TA 98 and TA 100. The products of the models do not require metabolic activation before expressing their mutagenicity; thus, they are suitable for the determination of the counteracting activity of the bananas on the mutagen that may cause mutation to the stomach cells. The product(s) formed during the reaction of the beef extract and sodium nitrite produced several heterocyclic amine compounds, and they were direct-acting mutagenic towards both strains TA 98 and TA 100 (18-20). The mutagens produced from such model, namely, the nitro derivatives of imidazoquinoline, and of imidazoquinoxaline (IQ-type heterocyclic amines) showed almost the same mutagenicity either in the absence or presence of metabolic activation.

The results showed that all samples in the present investigation strongly inhibited the formation of mutagens during the reaction of nitrite and aminopyrene (model 1) in a dose-related manner on strain TA 98 while they moderately inhibited the mutagenicity when strain TA 100 was used (Table 2). Since β-carotene is present in bananas, β-carotene may be one of the agents that are responsible for the antimutagenic effect against aminopyrene during nitrosation. From ASEAN Food Composition Tables 2000 (21), the level of β-carotene in Klouy Nam Wa, Klouy Hom and Klouy Khai are approximately 87, 77 and 190 μg/100 g dried weight, respectively. However, various carotenoids that possibly co-exist with β-carotene in banana may also have antimutagenic activity. One of them was α-carotene that showed higher potency than β-carotene in suppressing experimental carcinogenesis (22). Ong *et al.* (23) stated that β-carotene acted against the mutagenicity of the extracts of coal dust, diesel emission particles,

Table 1. Mutagenicity of nitrite-treated lyophilized ripe bananas on *Salmonella typhimurium* strains TA 98 and TA 100

Bananas	Amount (mg/plate)	Number of His^+ revertants[b]/plate	
		TA 98	TA 100
Klouy Nam Wa	0[a]	23 ± 5	105 ± 19
	2	25 ± 7	133 ± 12
	8	33 ± 5	**257 ± 11**
	24	**60 ± 6**	**332 ± 20**
Klouy Hom	0[a]	16 ± 2	119 ± 12
	2	27 ± 4	**259 ± 44**
	8	**60 ± 5**	**525 ± 25**
	24	**65 ± 10**	**509 ± 29**
Klouy Khai	0[a]	15 ± 2	86 ± 6
	2	**36 ± 3**	125 ± 7
	8	**71 ± 5**	**247 ± 36**
	24	**103 ± 7**	**396 ± 60**

[a] negative control (distilled water)
[b] mean value of triplicate plates from two independent experiments and its standard deviation.

airborne particles, fried beef, and tobacco snuff that contained some nitroarenes such as 2-nitrofluorene, 1-nitropyrene and 1,8-dinitropyrene on *S. typhimurium* strain TA 98.

Another possible explanation of antimutagenicity found in both models was the binding of theprecursors of direct mutagens; namely, nitrite, aminopyrene or compounds in beef concentrate to banana fiber during nitrosation (desmutagenic action). Kangsadalampai *et al.* (24) revealed that plant fiber could trap some nitrite in the reaction mixture of nitrosation. The mutagenicity of frameshift type mutagen of beef concentrate (model 2) decreased slightly while no effect on base pair substitution type of the same model was observed (Table 3). In addition, banana fiber may have less ability to absorb the mutagen precursors found in beef concentrate or the nitrosated products than aminopyrene. This phenomenon may be due to the difference in the structures of the precursors and of mutagenic products generated from different models that responded to the effect of the bananas.

In vivo testing with *Drosophila melanogaster* showed that ripe bananas used in this study contained no mutagen (Table 4). The samples ineffectively acted against the mutagenicity of urethane in the co-administration study (Table 5); however, their antimutagenicity clearly showed in the prefeeding study (Table 6). The inhibitory effect of the sample was nearly equal in both types of prefeeding experiments. This could be that banana may induce the detoxifying enzyme systems. The induction effect lasted longer even though the larvae in type I experiment were not continuously fed on the sample on day 4. Jang *et al.* (25) stated that the ethyl acetate soluble fraction of the methanol extract of *Musa x paradisiaca* cultivar significantly induced the enzyme quinone

Table 2. Effects of lyophilized ripe bananas presented in the reaction between aminopyrene and nitrite on *Salmonella typhimurium* strains TA 98 and TA 100

Bananas	Amount (mg/plate)	Number of His^+ revertants [b]/plate			
		TA 98		TA 100	
		Mean ± SD	Percent inhibition [c]	Mean ± SD	Percent inhibition [c]
Klouy Nam Wa	0[a]	453 ± 54	-	998 ± 110	-
	2	222 ± 20	51	538 ± 78	46
	8	163 ± 42	64	340 ± 57	66
	24	68 ± 11	85	379 ± 20	62
Klouy Hom	0[a]	582 ± 24	-	970 ± 47	-
	2	363 ± 64	38	788 ± 34	19
	8	340 ± 65	57	623 ± 56	36
	24	166 ± 35	72	493 ± 47	49
Klouy Khai	0[a]	677 ± 35	-	1025 ± 33	-
	2	295 ± 10	56	913 ± 95	11
	8	161 ± 20	76	372 ± 68	64
	24	122 ± 6	82	412 ± 50	60

[a] positive control (nitrite treated aminopyrene)
[b] mean value of triplicate plates from two independent experiments and its standard deviation.
[c] Percent inhibition = (A-B)/(A-C) x 100. Where A is the number of histidine revertants induced by the reaction mixture of each model, B is the number of histidine revertants induced by the reaction mixture in the presence of sample, C is the number of histidine spontaneous revertants.

reductase (QR) in cultured Hepa1C1C7 (mouse hepatoma) cells. Phase II drug-metabolizing enzymes such as QR is primarily responsible for the metabolic detoxification of some chemical carcinogens and other harmful oxidants. Therefore, induction of QR is a major mechanism of protection against tumor initiation. In addition, consumption of banana could significantly reduce the number of esophagus cancer in male patient (26). A case-control study conducted in Uruguay by Deneo-Pellegrini *et al.* (3) revealed that the strongest protection of colorectal cancer was observed among banana eaters. Also, Lohsoonthorn and Danvivat (27) found a protective effect provided by banana in a case-control study for colorectal cancer risk factors conducted in Bangkok, Thailand. The data suggested that nitrite-treated meat increased colorectal cancer risk while dietary fiber e.g., in bananas, decreased cancer risk.

While banana is a safe food, it is important to avoid some nitrite containing food items that could initiate the formation of mutagen during stomach digestion. The presence of banana powder in the stomach showed antimutagenicity benefit, as shown in the *in vitro* testing on nitrite treated aminopyrene. In addition, *in vivo* study using *Drosophila melanogaster* showed that consumption of each banana powder for a period of time prior to the consumption of mutagenic compound could

Table 3. Effects of lyophilized ripe bananas presented in the reaction between beef concentrate and nitrite on *Salmonella typhimurium* strains TA 98 and TA 100

Bananas	Amount (mg/plate)	Number of His$^+$ revertants [b]/plate			
		TA 98		TA 100	
		Mean ± SD	Percent inhibition [c]	Mean ± SD	Percent inhibition [c]
Klouy Nam Wa	0[a]	222 ± 18	0	564 ± 40	0
	2	184 ± 6	17	582 ± 30	0
	8	136 ± 29	39	578 ± 33	0
	24	PK	0	PK	0
Klouy Hom	0[a]	183 ± 15	0	739 ± 49	0
	2	167 ± 6	9	657 ± 38	11
	8	147 ± 18	20	663 ± 56	10
	24	127 ± 14	31	627 ± 48	15
Klouy Khai	0[a]	217 ± 13	0	654 ± 44	0
	2	198 ± 15	9	625 ± 85	4
	8	176 ± 23	19	543 ± 84	17
	24	172 ± 22	21	563 ± 78	14

[a] positive control (nitrite treated beef concentrate)
[b] mean value of triplicate plates from two independent experiments and its standard deviation.
PK = partial killing effect
[c] Percent inhibition = (A-B)/(A-C) x 100. Where A is the number of histidine revertants induced by the reaction mixture of each model, B is the number of histidine revertants induced by the reaction mixture in the presence of sample, C is the number of histidine spontaneous revertants.

Table 4. Mutagenicity of lyophilized ripe bananas (0.25 g/tube) reported as wing spot induction on *Drosophila melanogaster* derived from trans-heterozygous *mwh+/+flr*^{3} larvae of the improved high bioactivation cross fed medium containing each sample

Sample	Spots per wing (No. of spots from 40 wings) [a]			
	Small Single Spots m = 2	Large Single Spots m = 5	Twin Spots m = 5	Total Spots m = 2
Water	0.10 (4)-	0.05 (2)-	0-	0.15 (6)-
Urethane	9.03 (361)+	3.05 (122)+	1.30 (52)+	13.38 (535)+
Klouy Nam Wa	0.05 (2)-	0.03 (1)-	0.03 (1)-	0.10 (4)-
Klouy Hom	0.05 (2)-	0.03 (1)-	0.03 (1)-	0.13 (5)-
Klouy Khai	0.05 (2)-	0.10 (4)-	0.03 (1)-	0.18 (7)-

[a] Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (14) for comparison with deionized water; +, Positive; -, Negative; m, Multiplication factor. Probability levels: $\alpha = \beta = 0.05$. One-sided statistical tests

Table 5. Antimutagenicity of lyophilized ripe bananas (0.25 g/tube) on urethane (20 mM) in the co-administration study expressed as reduction of mutant wing spots in *Drosophila melanogaster* derived from 3-day-old trans-heterozygous *mwh+/+flr*^{3} larvae

Sample	Spots per wing (Number of spots from 40 wings) [a]				Percent inhibition [b]
	Small single Spot m=2	Large single Spot m=5	Twin spot m=5	Total Spot m=2	
Trial I					
Water	0.10 (4)-	0 (0)-	0.08 (3)-	0.18 (7)-	-
Urethane	8.28 (331)+	4.10(164)+	1.98 (79)+	14.35 (574)+	-
Klouy Nam Wa	7.48 (299)+	3.23 (129)+	1.70 (68)+	12.40 (496)+	14
Klouy Hom	6.85 (274)+	3.00 (120)+	1.85 (74)+	11.70 (468)+	18
Klouy Khai	8.00 (320)+	3.30 (132)+	1.20 (48)+	12.50 (500)+	13
Trial II					
Water	0.05 (2)-	0.03 (1)-	0.05 (2)-	0.13 (5)-	-
Urethane	8.93 (357)+	1.63(65)+	1.50 (60)+	12.05 (482)+	-
Klouy Nam Wa	7.18 (287)+	2.25 (90)+	1.58 (63)+	11.00 (440)+	9
Klouy Hom	6.63 (265)+	1.98 (79)+	2.08 (83)+	10.68 (427)+	11
Klouy Khai	7.68 (307)+	2.58 (103)+	1.20 (48)+	11.45 (458)+	5

[a] Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (14) for comparison with deionized water; +, Positive; -, Negative; m, Multiplication factor. Probability levels: $\alpha = \beta = 0.05$.
[b] 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

Table 6. Antimutagenicity of lyophilized ripe bananas against urethane-induced mutant wing spots in the prefeeding studies on *Drosophila melanogaster* derived from trans–heterozygous (*mwh+/+flr*^{3}) larvae

Sample	Spots per wing (No. of spots from 40 wings) [a]				Percent inhibition [c]
	Small single m=2	Large single m=5	Twin m=5	Total m=2	
Klouy Nam Wa (Trial I)					
Water	0.10 (4)	0.05 (2)	0	0.15 (6)	-
Urethane	6.90 (276)+	1.75 (70)+	0.85 (34)+	9.50 (380)+	-
Prefeeding Type I [b]	4.35 (174)+	0.95 (38)+	0.55 (22)+	5.85 (234)+	38
Prefeeding Type II [c]	3.50 (140)+	1.35 (54)+	0.60 (24)+	5.45 (218)+	43
Klouy Nam Wa (Trial II)					
Water	0.05 (2)	0.10 (4)	0.03 (1)	0.18 (7)	-
Urethane	1.20 (302)+	1.20 (48)+	1.10 (44)+	9.85 (394)+	-
Prefeeding Type I	4.50 (180)+	0.55 (22)+	0.35 (14)+	5.40 (216)+	45
Prefeeding Type II	3.70 (148)+	0.65 (26)+	0.90 (36)+	5.25 (210)+	47

Table 6. (continued)

Sample	Spots per wing (No. of spots from 40 wings) [a]				Percent inhibition [c]
	Small single m=2	Large single m=5	Twin m=5	Total m=2	
Klouy Hom (Trial I)					
Water	0.05 (2)	0.05 (2)	0.03 (1)	0.13 (5)	-
Urethane	6.40 (256)+	2.15 (86)+	0.70 (28)+	9.25 (370)+	-
Prefeeding Type I	2.85 (114)+	0.65 (26)+	0.45 (18)+	3.95 (158)+	57
Prefeeding Type II	2.30 (92)+	0.60 (24)+	0.70 (28)+	3.60 (144)+	61
Klouy Hom (Trial II)					
Water	0.05 (2)	0	0.08 (3)	0.13 (5)	-
Urethane	6.65 (266)+	2.35 (94)+	1.05 (42)+	10.05 (402)+	-
Prefeeding Type I	2.70 (108)+	0.55 (28)+	0.95 (38)+	4.20 (168)+	58
Prefeeding Type II	2.50 (100)+	0.90 (36)+	0.65 (26)+	4.05 (162)+	60
Klouy Khai (Trial I)					
Water	0.10 (4)	0.05 (2)	0	0.15 (6)	-
Urethane	7.35 (294)+	2.15 (86)+	1.70 (68)+	11.20 (448)+	-
Prefeeding Type I	4.90 (196)+	1.10 (44)+	0.25 (10)+	6.25 (250)+	44
Prefeeding Type II	4.10 (164)+	0.60 (24)+	0.45 (18)+	5.15 (206)+	54
Klouy Khai (Trial II)					
Water	0.10 (4)	0.05 (2)	0	0.15 (6)	-
Urethane	6.60 (264)+	1.90 (76)+	1.00 (40)+	9.5 (380)+	-
Prefeeding Type I	4.60 (184)+	0.35 (14)+	0.90 (36)+	5.85 (234)+	39
Prefeeding Type II	3.90 (156)+	0.75 (30)+	0.65 (26)+	5.30 (212)+	44

[a] Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (14) for comparison with deionized water; +, Positive; -, Negative; m, Multiplication factor. Probability levels: $\alpha = \beta = 0.05$. One-sided statistical tests.

[b] Prefeeding Type I or [c] Prefeeding Type II = 0.25 mg/ml of banana was fed to newborn larvae for 3 days followed with standard medium containing urethane or banana medium containing urethane, respectively.

[c] 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

decrease the formation of the mutant cells. In conclusion, ripe banana is a good natural food for health-conscious consumer in order to prevent an adverse effect of mutagens in daily meals.

References

1. R. Doll and P. Peto. The causes of cancer: Quantitative estimates of avoidable risks of cancer in the US today. *J. Natl. Cancer* **66**: 1191-1308 (1981).
2. L. R. Ferguson. Antimutagens as cancer chemopreventive agents in diet. *Mutat. Res.* **49**: 725-737 (1994).
3. H. Deneo-Pellegrini, E. De Stefani, and A. Ronco. Vegetables, fruits, and risk of

colorectal cancer: A case-control study from Uruguay. *Nutr. Cancer* **25**: 297-304 (1996).

4. L. Ranasinghe, B. Jayawardena, and K. Abeywickrama. Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L. M. Perry against crown rot and anthracnose pathogens isolated from banana. *Lett. Appl. Microbiol.* **35**: 208-211 (2002).
5. H. Ono, S. Tesaki, S. Tanabe, and M. Watanabe. 6-Methylsulfinylhexyl isothiocyanate and its homologues as food-originated compounds with antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. *Biosci. Biotechnol. Biochem.* **62**: 363-365 (1998).
6. P. Pannangpetch, A. Vuttivirojana, C. Kularbkaew, S. Tesana, B. Kongyingyoes, and V. Kukongviriyapan. The antiulcerative effect of Thai *Musa* species in rats. *Phytother. Res.* **15**: 407-410 (2001).
7. D. M. Maron and B. N. Ames. Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* **113**: 173-215 (1983).
8. T. Yahagi, M. Nagao, Y. Seino, T. Matsushita, T. Sugimura, and M. Okada. Mutagenicity of N-nitrosamines on *Salmonella*. *Mutat. Res.* **48**: 121-130 (1977).
9. K. Kangsadalampai, C. Butryee, and K. Manoonphol. Direct mutagenicity of the polycyclic aromatic hydrocarbon-containing fraction of smoked and charcoal-broiled foods treated with nitrite in acid solution. *Food Chem. Toxicol.* **35**: 213-218 (1996).
10. M. Calomme, L. Pieters, A. Vlietinck, and D. V. Berghe. Inhibition of bacterial mutagenesis by *Citrus* flavonoids. *Planta Med.* **62**: 222-226 (1996).
11. D. B. Roberts. Basic *Drosophila* care and techniques. In D. B. Roberts (ed.), Drosophila: *A Practical Approach*, IRL Press, Oxford, 1986, pp. 1-38.
12. U. Graf, F. E. Würgler, A. J. Katz, H. Frei, H. Juon, C. B. Hall, and P. G Kale. Somatic mutation and recombination test in *Drosophila melanogaster*. *Environ. Mutagen.* **6**: 153-188 (1984).
13. U. Graf and N. van Schaik. Improved high bioactivation cross for the wing somatic mutation and recombination test in *Drosophila melanogaster*. *Mutat. Res.* **271**: 59-67 (1992).
14. H. Frei and F. E. Würgler. Statistical methods to decide whether mutagenicity test data from *Drosophila* assays indicate a positive, negative, or inconclusive result. *Mutat. Res.* **203**: 297-308 (1988).
15. M. Higashimoto, Y. Akada, M. Sato, T. Kinouchi, T. Kuwahara, and Y. Ohnishi. Inhibitory effects of tea extracts on the mutagenicity of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid on treatment with nitrite in the presence of ethanol. *Food Chem. Toxicol.* **38**: 7-13 (2000).
16. A. Laires, J. Gaspar, H. Borba, M. Proenca, M. Monteiro, and J. Rueff. Genotoxicity of nitrosated red wine and of the nitrosatable phenolic compounds present in wine: Tyramine, quercetin and malvidine-3-glucoside. *Food Chem. Toxicol.* **31**: 989-994 (1993).
17. K. Wakabayashi, M. Nagao, T. H. Chung, M. Yin, I Karai, M. Ochiai, T. Tahira, and T. Sugimura. Appearance of direct-acting mutagenicity of various food stuffs produced in Japan and Southeast Asia in nitrite treatment. *Mutat. Res.* **158**: 119-24 (1984).
18. M. Tsuda, C. Negishi, R. Makino, S. Sato, Z. Yamaizumi, T. Hirayama, and T. Sugimura. Use of nitrite and hypochlorite treatments in determination of the contributions of IQ type and non-IQ type heterocyclic amines to the mutagenicities in crude pyrolyzed materials. *Mutat. Res.* **147**: 335-341 (1985).
19. R. Munzner. Modifying action of vegetable juice on the mutagenicity of beef extract and nitrosated beef extract. *Food Chem. Toxicol.* **24**: 847-849 (1986).
20. T. Sugimura, K. Wakabashi, M. Nagao, and H. Esumi. *Food Nutrition and Chemical Toxicity. A New Class of Carcinogens: Heterocyclic Amines in Cooked Food*, Smith-Gordon, London, 1993, pp. 1561-1577.
21. P. Puwastien, B. Barlingame, M. Raroengwichit, and P. Sungpuag. *Asean Food*

Composition Table 2000, ASEAN Network of food Data System, Institute of Nutrition, Mahidol University, Bangkok, 2000.

22. H. Nishino, H. Tokuda, M. Murakoshi, Y. Satomi, M. Masuda, M. Onozuka, S. Yamaguchi, J. Takayasu, J. Tsuruta, M. Okuda, F. Khachik, T. Narisawa, N. Takasuka, and M. Yano. Cancer prevention by natural carotenoids. *Biofactors* **13**: 89-94 (2000).
23. T. Ong, W. Z. Whong, J. D. Stewart, and H. E. Brockman. Comparative antimutagenicity of 5 compounds against 5 mutagenic complex mixtures in *Salmonella typhimurium* strain TA 98. *Mutat. Res.* **222**: 19-25 (1989).
24. K. Kangsadalampai, C. Butryee, and P. Lasoha-vechvanich. Antimutagen formation and nitrite scavenging by unprocessed or processed fruit and vegetable fibers. In H. Ohigashi, T. Osawa, J. Terao, S. Watanabe, and T. Yoshikawa (eds.), *Food Factors for Cancer Prevention*, Springer-Verlag, Tokyo, 1997, pp. 166-169.
25. D. S. Jang, E. J. Park, M. E. Hawthorne, J. S. Vigo, J. G. Graham, F. Cabieses, B. D. Santarsiero, A. D. Mesecar, H. H. Fong, R. G. Mehta, J. M. Pezzuto, and A. D. Kinghorn. Constituents of *Musa x paradisiaca* cultivar with the potential to induce the phase II enzyme, quinone reductase. *J. Agric. Food Chem.* **50**: 6330-6334 (2002).
26. U. W. De Jong, N. Breslow, J. G. Hong, M. Sridharan, and K Shanmugaratnam. Aetiological factors in oesophageal cancer in Singapore Chinese. *Int. J. Cancer* **13**: 291-303 (1974).
27. P. Lohsoonthorn and D. Danvivat. Colorectal cancer risk factors: A case-control study in Bangkok. *Asia Pac. J. Public Health* **8**: 118-122 (1995).