



## CHAPTER IV

### RESULTS

In the present study, the Ames assay was used to determine the mutagenic activity of vegetable extract, 1-AP and beef extract. Furthermore, 1-AP or beef extract is an indirect mutagen requiring metabolic activation. When it was treated with nitrite in acid condition pH 3.0-3.5 (37°C, 4 hours), it gave rise to positive Ames mutagenicity.

#### 4.1 Positive Controls

##### *Nitrite Treated Beef Extract*

The study of mutagenicity of nitrite treated or no treated beef extract in the absence of metabolic activation on *S. typhimurium* TA98 and TA100 (Table 4.1) showed that the higher the amount of the nitrite treated beef extract, the higher the mutagenicity index (MI) obtained from both TA98 and TA100. As a result, the 3.2-mg/plate concentration of nitrite treated beef extract was chosen as a standard for later experiments.

Table 4.1 Mutagenicity of the beef extract treated with and without nitrite in acid condition pH 3.0-3.5 without metabolic activation on *Salmonella typhimurium* TA98 and TA100.

Beef extract	Amount weight of beef residue (mg/plate)	Number of His <sup>+</sup> <sup>a</sup> (revertants/plate)		Mutagenicity Index (MI) <sup>b</sup>	
		TA98	TA100	TA98	TA100
With nitrite	0 <sup>c</sup>	13±4	102±5	1.00	1.00
	0.4	26±3	184±24	2.00	1.80
	0.8	60±5	214±21	4.62	2.10
	1.6	80±9	313±21	6.15	3.07
	3.2	109±26	578±88	8.38	5.67
Without nitrite	0 <sup>c</sup>	13±4	102±5	1.00	1.00
	0.4	12±7	104±15	0.92	1.02
	0.8	17±5	115±23	1.31	1.13
	1.6	23±8	125±20	1.77	1.23
	3.2	25±5	137±24	1.92	1.34

20 µl (TA98) or 40 µl (TA100) of 1-aminopyrene (0.0375 mg/ml) interacted with excess amount of sodium nitrite induced 578 revertants/plate or 1243 Revertants/plate respectively

<sup>a</sup> Data are expressed as means ± SD of triplicate plates from two independent experiments (N=6).

<sup>b</sup> Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the beef extract divided by that of spontaneous revertants.

<sup>c</sup> Spontaneous revertants.

## 4.2 Mutagenicity of Chloroform Extracts from the Vegetables

The mutagenicity of chloroform extracts from vegetables was evaluated on *S. typhimurium* TA98 (Table 4.2) and TA100 (Table 4.3). The result shows that when nitrite was added, almost all of the vegetable extracts showed mutagenicity on both strains except Pak Chee Lao, its showed mutagenic activity only on *S. typhimurium* TA100. Whereas no mutagenic activity was observed when there was no presence of nitrite, except in Pak Ka Yang at the concentration of 0.190 mg/plate on *S. typhimurium* TA98.

### 4.3 Mutagenicity of Ethanol Extracts of the Vegetables

The mutagenicity of ethanol extracts from vegetables was evaluated on *S. typhimurium* TA98 (Table 4.4) and TA100 (Table 4.5). The result shows that when nitrite was added, the mutagenicity index increased in direct proportion to the concentration of all vegetable extracts.

Table 4.2 Mutagenicity of the chloroform extracts of the vegetables in acid solution pH 3.0-3.5 on *S. typhimurium* TA98 (frameshift mutation) without metabolic activation.

Vegetable extracts	Amount <sup>a</sup> (mg/plate)	Number of His <sup>+</sup> <sup>b</sup> (revertants/plate)		Mutagenicity Index (MI) <sup>c</sup>	
		w/o nitrite	w/nitrite	w/o nitrite	w/nitrite
Pak Chee Lao	Spontaneous <sup>d</sup>	20±9	20±9	1.00	1.00
	0.050	22±6	28±7	1.10	1.40
	0.100	20±5	28±7	1.00	1.40
	0.201	24±3	39±8	1.20	1.95
Pak Ka Yang	Spontaneous <sup>d</sup>	46±5	46±5	1.00	1.00
	0.048	66±9	123±6	1.43	2.67
	0.095	91±3	135±6	1.98	2.93
	0.190	125±9	159±7	2.72	3.46
Pak Pai	Spontaneous <sup>d</sup>	14±3	14±3	1.00	1.00
	0.049	12±4	32±5	0.86	2.29
	0.099	13±3	51±10	0.93	3.64
	0.197	10±4	48±8	0.71	3.43
Pak Gud	Spontaneous <sup>d</sup>	15±5	15±5	1.00	1.00
	0.048	19±3	39±5	1.27	2.60
	0.097	13±4	53±10	0.87	3.53
	0.194	17±4	46±6	1.13	3.07
Pak Krad Hua Wan	Spontaneous <sup>d</sup>	28±3	28±3	1.00	1.00
	0.051	29±4	67±5	1.04	2.39
	0.102	27±3	107±8	0.96	3.82
	0.204	36±6	114±7	1.29	4.07

20 µl of 1-aminopyrene (0.0375 mg/ml) interacted with excess amount of sodium nitrite induced 624 revertants/plate.

<sup>a</sup> Amount per plate of vegetable extract calculated on the residue.

<sup>b</sup> Data are expressed as means ± SD of triplicate plates from two experiments.

<sup>c</sup> Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the chloroform extract of vegetable divided by that of spontaneous revertants.

<sup>d</sup> Spontaneous mutation (DMSO).

Table 4.3 Mutagenicity of the chloroform extracts of the vegetables in acid solution pH 3.0-3.5 on *S. typhimurium* TA100 (base-pair substitution) without metabolic activation.

Vegetable extracts	Amount <sup>a</sup> (mg/plate)	Number of His <sup>+</sup> <sup>b</sup> (revertants/plate)		Mutagenicity Index (MI) <sup>c</sup>	
		w/o nitrite	w/nitrite	w/o nitrite	w/nitrite
Pak Chee Lao	Spontaneous <sup>d</sup>	117±22	117±22	1.00	1.00
	0.050	129±20	191±16	1.10	1.63
	0.100	135±22	349±17	1.15	2.98
	0.201	142±16	645±21	1.21	5.51
Pak Ka Yang	Spontaneous <sup>d</sup>	119±24	119±24	1.00	1.00
	0.048	117±21	459±49	0.98	3.85
	0.095	123±15	767±67	1.03	6.45
	0.190	143±20	778±31	1.20	6.54
Pak Pai	Spontaneous <sup>d</sup>	114±4	114±4	1.00	1.00
	0.049	103±14	332±36	0.90	2.91
	0.099	105±13	443±30	0.92	3.89
	0.197	85±8	465±42	0.75	4.08
Pak Gud	Spontaneous <sup>d</sup>	105±19	105±19	1.00	1.00
	0.048	124±8	265±13	1.18	2.52
	0.097	86±8	385±9	0.82	3.67
	0.194	109±6	382±8	1.04	3.64
Pak Krad Hua Wan	Spontaneous <sup>d</sup>	129±8	129±8	1.00	1.00
	0.051	128±6	295±20	0.99	2.29
	0.102	122±16	453±42	0.95	3.51
	0.204	104±9	576±27	0.81	4.47

40 µl of 1-aminopyrene (0.0375 mg/ml) interacted with excess amount of sodium nitrite induced 1,215 revertants/plate.

<sup>a</sup> Amount per plate of vegetable extract calculated on the residue.

<sup>b</sup> Data are expressed as means ± SD of triplicate plates from two experiments.

<sup>c</sup> Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the chloroform extract of vegetable divided by that of spontaneous revertants.

<sup>d</sup> Spontaneous mutation (DMSO).

Table 4.4 Mutagenicity of the ethanol extracts of the vegetables in acid solution pH 3.0-3.5 on *S. typhimurium* TA98 (frameshift mutation) without metabolic activation.

Vegetable extracts	Amount <sup>a</sup> (mg/plate)	Number of His <sup>+</sup> <sup>b</sup> (revertants/plate)		Mutagenicity Index (MI) <sup>c</sup>	
		w/o nitrite	w/nitrite	w/o nitrite	w/nitrite
Pak Chee Lao	Spontaneous <sup>d</sup>	27±8	27±8	1.00	1.00
	0.051	39±7	45±11	1.44	1.67
	0.103	33±5	73±4	1.22	2.70
	0.206	32±7	75±4	1.19	2.78
Pak Ka Yang	Spontaneous <sup>d</sup>	18±3	18±3	1.00	1.00
	0.049	20±2	118±24	1.11	6.56
	0.097	19±2	122±27	1.06	6.78
	0.195	16±2	161±8	0.89	8.94
Pak Pai	Spontaneous <sup>d</sup>	16±2	16±2	1.00	1.00
	0.049	17±2	72±9	1.06	4.50
	0.098	19±2	95±10	1.19	5.94
	0.196	19±3	151±9	1.19	9.44
Pak Gud	Spontaneous <sup>d</sup>	15±2	15±2	1.00	1.00
	0.055	15±4	27±3	1.00	1.80
	0.110	15±2	42±10	1.00	2.80
	0.220	17±3	59±3	1.13	3.93
Pak Krad Hua Wan	Spontaneous <sup>d</sup>	22±3	22±3	1.00	1.00
	0.050	18±2	43±5	0.82	1.95
	0.100	19±3	53±4	0.86	2.41
	0.199	25±5	93±9	1.14	4.23

20 µl of 1-aminopyrene (0.0375 mg/ml) interacted with excess amount of sodium nitrite induced 657 revertants/plate.

<sup>a</sup> Amount per plate of vegetable extract calculated on the residue.

<sup>b</sup> Data are expressed as means ± SD of triplicate plates from two experiments.

<sup>c</sup> Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the chloroform extract of vegetable divided by that of spontaneous revertants.

<sup>d</sup> Spontaneous mutation (DMSO).

Table 4.5 Mutagenicity of the ethanol extracts of the vegetables in acid solution pH 3.0-3.5 on *S. typhimurium* TA100 (base-pair substitution) without metabolic activation.

Vegetable extracts	Amount <sup>a</sup> (mg/plate)	Number of His <sup>+</sup> <sup>b</sup> (revertants/plate)		Mutagenicity Index (MI) <sup>c</sup>	
		w/o nitrite	w/nitrite	w/o nitrite	w/nitrite
Pak Chee Lao	Spontaneous <sup>d</sup>	105±11	105±11	1.00	1.00
	0.051	108±11	227±22	1.03	2.16
	0.103	136±3	279±11	1.30	2.66
	0.206	126±5	454±19	1.20	4.32
Pak Ka Yang	Spontaneous <sup>d</sup>	105±5	105±5	1.00	1.00
	0.049	118±5	278±16	1.12	2.64
	0.097	115±21	309±17	1.10	2.94
	0.195	115±5	473±16	1.10	4.50
Pak Pai	Spontaneous <sup>d</sup>	119±10	119±10	1.00	1.00
	0.049	113±6	276±11	0.95	2.32
	0.098	97±8	275±20	0.82	2.31
	0.196	106±8	422±20	0.89	3.55
Pak Gud	Spontaneous <sup>d</sup>	104±6	104±6	1.00	1.00
	0.055	96±6	207±11	0.92	1.99
	0.110	101±5	256±10	0.97	2.46
	0.220	102±3	412±9	0.98	3.96
Pak Krad Hua Wan	Spontaneous <sup>d</sup>	102±4	102±4	1.00	1.00
	0.050	126±6	201±18	1.24	1.97
	0.100	121±6	309±4	1.19	3.03
	0.199	120±9	514±18	1.18	5.04

40 µl of 1-aminopyrene (0.0375 mg/ml) interacted with excess amount of sodium nitrite induced 1,194 revertants/plate.

<sup>a</sup> Amount per plate of vegetable extract calculated on the residue.

<sup>b</sup> Data are expressed as means ± SD of triplicate plates from two experiments.

<sup>c</sup> Mutagenicity Index (MI) is calculated from the average value of a number of histidine revertants/plate of the chloroform extract of vegetable divided by that of spontaneous revertants.

<sup>d</sup> Spontaneous mutation (DMSO).

#### 4.4 Antimutagenicity of the Vegetable Extracts.

Table 4.6 reveals the effects of chloroform from vegetables on the mutagenic activity of nitrite treated 1-AP and nitrite treated beef extract on *S. typhimurium* TA98 and TA100 in the absence of metabolic activation. The results show that the mutagenicity of nitrite treated 1-AP was inhibited by all of the chloroform extracts from vegetables on *S. typhimurium* TA98 and TA100. On the nitrite treated beef extract model, chloroform extracts of Pak Ka Yang and Pak Pai show antimutagenic activity. However, the chloroform extracts from Pak Chee Lao, Pak Gud and Pak Krad Hua Wan show enhanced mutagenicity on nitrite treated beef extract.

Table 4.7 reveals the effect of ethanol extracts from vegetables on the mutagenic activity of nitrite treated 1-AP and nitrite treated beef extract on *S. typhimurium* TA98 and TA100 in the absence of metabolic activation. The results show that the ethanol extracts of Pak Ka Yang and Pak Pai reduced mutagenicity of nitrite treated beef extract on *S. typhimurium* TA100. Accordingly, ethanol extracts from all vegetables showed their enhancing mutagenic effect of nitrite treated beef extract on *S. typhimurium* TA98.



Table 4.6 Effect of the chloroform extracts of the vegetables on the mutagenicity of nitrite treated 1-aminopyrene and nitrite treated beef extract on *S. typhimurium* strains TA98 and TA100.

Extract of vegetables	Amount <sup>a</sup> (mg/plate)	1-aminopyrene				beef extract			
		TA98		TA100		TA98		TA100	
		Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>
Pak Chee Lao	Spontaneous <sup>d</sup>	23 ± 2		104 ± 21		34 ± 12		105 ± 17	
	0 <sup>e</sup>	667 ± 21		1392 ± 42		101 ± 6		586 ± 18	
	0.63	659 ± 34	+ 1.24	1203 ± 82	+ 14.67	95 ± 4	+ 8.96	593 ± 45	- 1.46
	1.26	550 ± 46	+ 18.17	642 ± 48	+ 58.23	158 ± 9	- 85.07	613 ± 34	- 5.61
	2.52	307 ± 88	+ 55.90	931 ± 85	+ 35.79	125 ± 16	- 35.82	604 ± 46	- 3.74
Pak Ka Yang	Spontaneous <sup>d</sup>	25 ± 3		110 ± 6		21 ± 4		107 ± 9	
	0 <sup>e</sup>	651 ± 95		1339 ± 56		108 ± 4		508 ± 15	
	0.59	636 ± 98	+ 2.40	1076 ± 82	+ 21.40	121 ± 5	-14.94	454 ± 6	+ 13.47
	1.18	548 ± 91	+ 16.45	1019 ± 100	+ 26.04	95 ± 4	+ 14.94	437 ± 19	+ 17.71
	2.36	216 ± 43	+ 69.49	602 ± 15	+ 59.57	102 ± 7	+ 7.00	408 ± 16	+ 24.94
Pak Pai	Spontaneous <sup>d</sup>	16 ± 2		114 ± 5		25 ± 5		103 ± 8	
	0 <sup>e</sup>	623 ± 58		1572 ± 195		103 ± 5		559 ± 42	
	0.62	506 ± 66	+ 19.28	570 ± 66	+ 68.72	57 ± 2	+ 58.97	435 ± 9	+ 27.19
	1.24	176 ± 68	+ 73.64	440 ± 45	+ 77.64	61 ± 4	+ 53.85	365 ± 10	+ 42.54
	2.48	54 ± 50	+ 93.74	299 ± 44	+ 87.31	51 ± 3	+ 66.67	356 ± 8	+ 44.52

Table 4.6 Effect of the chloroform extracts of the vegetables on the mutagenicity of nitrite treated 1-aminopyrene and nitrite treated beef extract on *S. typhimurium* strains TA98 and TA100 (continued).

Extract of vegetables	Amount <sup>a</sup> (mg/plate)	1-aminopyrene				beef extract			
		TA98		TA100		TA98		TA100	
		Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>
	Spontaneous <sup>d</sup>	21 ± 4		108 ± 24		38 ± 3		111 ± 6	
	0 <sup>e</sup>	673 ± 85		1250 ± 54		109 ± 7		582 ± 27	
Pak Gud	0.61	770 ± 46	- 14.88	1264 ± 75	+ 1.23	130 ± 3	- 29.58	604 ± 25	- 4.67
	1.22	639 ± 76	+ 5.21	862 ± 60	+ 33.98	120 ± 4	- 15.49	629 ± 30	- 9.98
	2.44	276 ± 47	+ 60.89	700 ± 65	+ 44.00	122 ± 15	- 18.31	585 ± 14	- 0.64
	Spontaneous <sup>d</sup>	30 ± 9		107 ± 47		20 ± 5		106 ± 6	
	0 <sup>e</sup>	579 ± 43		1319 ± 48		107 ± 7		580 ± 27	
Pak Krad	0.64	378 ± 59	+ 36.61	863 ± 67	+ 37.13	162 ± 4	- 63.22	521 ± 22	+ 12.45
Hua Wan	1.28	504 ± 73	+ 13.66	944 ± 77	+ 30.94	128 ± 8	- 24.14	529 ± 24	+ 8.65
	2.56	241 ± 34	+ 61.57	461 ± 50	+ 70.79	138 ± 10	- 35.63	532 ± 36	+ 10.13

<sup>a</sup> Amount per plate of vegetable extract calculated on the residue.

<sup>b</sup> Data are expressed as means ± SD of triplicate plates from two experiments.

<sup>c</sup> + or - indicates that the extract decreased or increased the mutagenicity of the model, respectively.

<sup>d</sup> Spontaneous revertants

<sup>e</sup> No extract was added to the standard mutagen, namely nitrite treated 1-aminopyrene (0.12 µg/plate for *Salmonella typhimurium* TA98 and 0.24 µg/plate for *Salmonella typhimurium* TA100)

Table 4.7 Effect of the ethanol extracts of the vegetables on the mutagenicity of nitrite treated 1-aminopyrene and nitrite treated beef extract on *S. typhimurium* strains TA98 and TA100.

Extract of vegetables	Amount <sup>a</sup> (mg/plate)	1-aminopyrene				beef extract			
		TA98		TA100		TA98		TA100	
		Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>
Pak Chee Lao	Spontaneous <sup>d</sup>	23 ± 2		104 ± 21		34 ± 12		105 ± 17	
	0 <sup>e</sup>	667 ± 21		1392 ± 42		101 ± 6		586 ± 18	
	0.64	508 ± 50	+ 24.69	917 ± 145	+ 36.88	99 ± 9	+ 2.99	578 ± 48	+ 1.66
	1.28	658 ± 42	+ 1.40	865 ± 47	+ 40.92	98 ± 7	+ 4.48	506 ± 69	+ 16.63
	2.56	688 ± 139	- 3.26	728 ± 75	+ 51.55	128 ± 6	- 40.30	601 ± 59	- 3.12
Pak Ka Yang	Spontaneous <sup>d</sup>	25 ± 3		110 ± 6		21 ± 4		107 ± 9	
	0 <sup>e</sup>	651 ± 95		1339 ± 56		108 ± 4		508 ± 15	
	0.61	653 ± 58	- 0.32	938 ± 91	+ 32.63	104 ± 4	+ 4.60	409 ± 7	+ 24.69
	1.22	622 ± 58	+ 4.63	633 ± 62	+ 57.45	127 ± 6	- 21.84	424 ± 4	+ 20.95
	2.44	114 ± 5	+ 4.95	658 ± 58	+ 55.41	103 ± 8	+ 5.75	463 ± 15	+ 11.22
Pak Pai	Spontaneous <sup>d</sup>	16 ± 2		114 ± 5		25 ± 5		103 ± 8	
	0 <sup>e</sup>	623 ± 58		1572 ± 195		103 ± 5		559 ± 42	
	0.61	584 ± 43	+ 6.43	996 ± 60	+ 39.51	212 ± 30	- 139.74	411 ± 12	+ 32.49
	1.22	603 ± 76	+ 3.29	1238 ± 49	+ 22.91	176 ± 19	- 93.59	384 ± 7	+ 38.38
	2.44	597 ± 29	+ 4.28	691 ± 68	+ 60.43	130 ± 16	-34.62	416 ± 15	+ 31.36

Table 4.7 Effect of the ethanol extracts of the vegetables on the mutagenicity of nitrite treated 1-aminopyrene and nitrite treated beef extract on *S. typhimurium* strains TA98 and TA100 (continued).

Extract of vegetables	Amount <sup>a</sup> (mg/plate)	1-aminopyrene				beef extract			
		TA98		TA100		TA98		TA100	
		Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>	Number of His <sup>+</sup> revertants/plate <sup>b</sup>	%Modification <sup>c</sup>
	Spontaneous <sup>d</sup>	21 ± 4		108 ± 24		38 ± 3		111 ± 6	
	0 <sup>e</sup>	673 ± 85		1250 ± 54		109 ± 7		582 ± 27	
Pak Gud	0.69	648 ± 59	+ 3.83	1250 ± 173	0.00	132 ± 39	- 32.39	616 ± 8	- 7.22
	1.38	669 ± 87	+ 0.61	1412 ± 168	- 14.19	125 ± 25	- 22.54	597 ± 14	- 3.18
	2.76	652 ± 43	+ 3.22	1070 ± 126	+ 15.76	120 ± 11	- 15.49	591 ± 12	- 1.91
	Spontaneous <sup>d</sup>	30 ± 9		107 ± 47		20 ± 5		106 ± 6	
	0 <sup>e</sup>	579 ± 43		1319 ± 48		107 ± 7		580 ± 27	
Pak Krad	0.62	589 ± 92	- 1.82	726 ± 105	+ 48.93	127 ± 9	- 22.99	569 ± 57	+ 2.32
Hua Wan	1.24	614 ± 119	- 6.38	706 ± 65	+ 50.58	133 ± 16	- 29.89	545 ± 16	+ 7.38
	2.48	620 ± 106	- 7.47	557 ± 105	+ 62.87	114 ± 11	- 8.05	541 ± 19	+ 8.23

<sup>a</sup> Amount per plate of vegetable extract calculated on the residue.

<sup>b</sup> Data are expressed as means ± SD of triplicate plates from two experiments.

<sup>c</sup> + or - indicates that the extract decreased or increased the mutagenicity of the model, respectively.

<sup>d</sup> Spontaneous revertants

<sup>e</sup> No extract was added to the standard mutagen, namely nitrite treated 1-aminopyrene (0.12 µg/plate for *Salmonella typhimurium* TA98 and 0.24 µg/plate for *Salmonella typhimurium* TA100)