

CHAPTER IV

RESULTS

Instant noodle is one of the most popular fast food in Thailand because it is cheap and convenient for rushing lifestyle. In Japan, instant noodle is the best innovation in 2000. The processing of instant noodle described briefly below is necessary in order to understand the outcome of the present finding. Generally, raw material such as wheat flour, egg, salt, water, base solution are mixed well. The mixture is sent to a continuous dough shelter and it was slit to be noodle. The noodle is sent to a steam cooker for ripening for 2 min; then, it is cut into piece before being sprayed with mostly chicken extract. The noodle is fried in a continuous fryer at 160 °C for 2 min. The fried noodle is cooled and packed in a plastic bag with a small package of seasoning.

All works done in this experiment were performed to observe the potential mutagenicity expressed as numbers of histidine (His)⁺ revertants per plate of specific strains of *Salmonella typhimurium* of Ames test after the water extracts of noodles, seasonings and noodles cooked with seasonings were nitrosated in acid solution pH 3. Samples were drawn from the three major brands of popular flavour instant noodles, namely *Minced Pork flavour* one, *Pa-lo Duck flavour* one and *Sour Shrimp flavour* one. Tables 1 and 2 show the relationship between amount of the extracts of samples both the nitrite treated samples or the untreated ones and histidine revertants. Table 3 shows the specific mutagenic activity (number of revertants/g) and total mutagenicity (number of revertants/package) on both tester strains in order to reveal a possible hazard of one who consume such product in the presence of nitrite during gastric digestion.

4.1 Mutagenicity of Extracts from Samples

All extracts from samples, namely noodle, seasoning and noodle cooked with seasoning did not show any mutagenicity on *Salmonella typhimurium* strains TA 98 and 100. Testing was done in the absence of metabolic activation in order to imitate the situation that needed for the expression of such mutagens occurred from nitrite treatment. When each extract was interacted with excess amount of sodium nitrite in an acid solution pH 3-3.5, mutagenic activity on both tester strains was expressed according to the criteria of Ames test (Tables 1 and 2). Overall results suggested that the extracts from all samples contained some compounds readily to interact with nitrite to produce direct mutagen causing frameshift and base-pair substitution mutation on the tester strains. The mutagenicity expressed in a dose-response manner on both strains.

Extracts from Noodle: MIs of most extracts from nitrite treated noodles were higher on *S. typhimurium* TA 98, a frameshift mutation detector, than those on *S. typhimurium* TA 100, a base-pair change mutation detector (Tables 1 and 2). It was speculated that the nitrite treated of extract from noodle number 5 of Pa-lo duck flavour exhibited the lowest number of revertants per plate as also indicated that its MI showed no difference in the two tester strains. When test was performed on the nitrite treated extract from noodle number 4 of Pa-lo duck flavour, the highest number of revertants per plate and the highest MI were obtained on strain TA 98. Among the extracts from noodle of Minced pork flavour, the nitrite treated one of sample 3 exhibited the strongest mutagenicity on strain TA 100.

Extracts from Seasoning: Most nitrite treated extracts from samples expressed their mutagenicity with a dose response relationship (Tables 1 and 2). The exception was found on the nitrite treated extract from sample 4 of Pa-lo duck flavour

and from sample number 9 of sour shrimp flavour that the mutagenicity on *S. typhimurium* TA 100 decreased when the amount of sample tested increased. It was suggested that at the highest amount of extract incorporated on plate (11.74 mg/plate and 9.82 mg/plate, respectively) of seasoning preparations posted their toxic effect on the tester strain.

Mutagenic potential revealed from the study on seasoning suggested that the nitrite treated extract from sample number 1 of Minced pork flavour exhibited the strongest mutagenicity, indicated as MI, of all sample at 11.55 mg of the extract on *S. typhimurium* TA 98 while the same phenomenon on *S. typhimurium* TA 100 was shown when the study was performed on 5.87 mg of the extract from sample number 4 of Pa-lo duck flavoure after nitrite treatment.

Lower MIs were obtained from the study on nitrite treated extracts from sample number 7 of Sour Shrimp flavour and sample number 5 of Pa-lo duck flavour on *S. typhimurium* TA 98. The weakest mutagenicity on *S. typhimurium* TA 100 was belonged to that of sample number 7 of Sour Shrimp flavour.

Extracts from Noodle Cooked with Seasoning: MIs of nitrite treated extracts from noodle cooked with seasoning showed their more preference on *S. typhimurium* TA 98 than that on TA 100 (Table 1 and 2). MIs of the nitrite treated extracts from noodle cooked with seasoning showed unexpected higher value than that of the extracts from noodle only or from that of seasoning only of the same sample brand. Exception was noted on sample 7 of sour shrimp flavour which posted the highest mutagenicity index (MI) after nitrite treatment when compared between the data of noodle only and those of seasoning only of the same sample brand on both strains. In addition, the expected values of noodles cooked with seasonings obtained by summing the mutagenicity of individual components (nitrite treated extracts from

noodles and nitrite treated extract from seasonings) were higher than that of the actual values of noodles cooked with seasonings (Table 3).

Significance of Specific Mutagenic Activity: Specific mutagenic activity of sample treated with nitrite expressed as number of revertants per gram and total mutagenic activity expressed as number of revertants per package (Table 3) were presented as expected values and actual values. The expected values of noodles cooked with seasonings were obtained by summing the mutagenicity of individual components from nitrite treated extracts from noodles and nitrite treated extract from seasonings while actual values were obtained from actual experiment. Specific mutagenic activity of nitrite treated samples, namely the extracts from noodle cooked with seasoning, that from noodle only and that from seasoning only (Table 3) revealed that such revertants per package induced by each category of the same brand name were not straight forward as a common summation. The results demonstrated an unexplained effect occurred during cooking of the instant noodle. It was found that actual values of noodle cooked with seasoning of minced pork flavour number 1 showed the lowest value of revertants per gram and revertants per package on both strains. In addition, it was found that number of revertants per gram of seasonings of all samples showed the highest among noodle and noodle cooked with seasoning on both strains. Moreover, seasoning of pa-lo duck flavour number 6 showed the highest number of revertants per package on both strains.

Table 1. Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 98 (frameshift mutation) without metabolic activation

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index (MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Minced pork flavour					
Noodle 1.	0*	15 _± 1	15 _± 1	1.00	1.00
	0.66	19 _± 4	28 _± 4	1.27	1.87
	3.34	18 _± 6	65_±44	1.20	4.33
	6.69	22 _± 5	183_±128	1.53	12.20
	13.39	17 _± 2	209_±142	1.13	13.93
Noodle 2.	0	16 _± 1	16 _± 1	1.00	1.00
	0.78	15 _± 3	14 _± 2	0.94	0.88
	3.90	16 _± 4	39_±15	1.00	2.44
	7.81	13 _± 2	158_±76	0.81	9.88
	15.63	15 _± 2	216_±64	0.94	13.50
Noodle 3.	0	15 _± 1	15 _± 1	1.00	1.00
	0.60	20 _± 7	23 _± 5	1.40	1.53
	3.04	22 _± 5	48_±10	1.47	3.20
	6.09	18 _± 2	109_±19	1.20	7.72
	12.19	17 _± 3	169_±29	1.13	11.27
Seasoning 1.	0	15 _± 1	15 _± 1	1.00	1.00
	0.59	17 _± 7	24 _± 6	1.13	1.60
	2.98	16 _± 3	82_±19	1.13	5.47
	5.97	17 _± 5	116_±34	1.13	7.73
	11.55	21 _± 6	240_±19	1.40	16.00

Table 1. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 98 (frameshift mutation) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index (MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Seasoning 2.	0	28±11	28±11	1.00	1.00
	0.56	24±17	31±13	0.86	1.11
	2.80	29±11	54±11	1.04	1.93
	5.61	34±15	111±18	1.21	3.96
	11.23	28±16	166±75	1.00	5.93
Seasoning 3.	0	28±11	28±11	1.00	1.00
	0.54	28±17	40±12	1.00	1.43
	2.70	30±16	52±16	1.07	1.86
	5.40	35±22	118±17	1.25	4.21
	10.81	28±17	158±24	1.00	5.64
Noodle cooked with Seasoning 1.	0	15±2	15±2	1.00	1.00
	0.58	14±2	20±5	0.93	1.40
	2.91	16±3	44±11	1.07	3.00
	5.82	16±7	74±28	1.07	5.00
	11.65	20±4	133±25	1.40	8.93
Noodle cooked with Seasoning 2.	0	15±1	15±1	1.00	1.00
	0.59	23±8	24±5	1.53	1.60
	2.96	17±5	62±9	1.13	4.13
	5.92	14±3	142±17	0.93	9.47
	11.85	18±5	199±18	1.20	13.27

Table 1. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 98 (frameshift mutation) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index (MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Noodle cooked	0	28±11	28±11	1.00	1.00
with Seasoning 3.	0.56	25±14	30±16	0.83	1.07
	2.82	30±13	58±19	1.00	2.07
	5.65	30±17	110±9	1.00	3.93
	11.31	33±9	162±24	1.10	5.78
Pa-lo duck flavour					
Noodle 4.	0	17±3	17±3	1.00	1.00
	0.64	19±5	28±4	1.12	1.65
	3.20	18±5	42±7	1.06	2.47
	6.40	17±2	137±7	1.00	8.06
	12.80	18±3	249±34	1.06	14.65
Noodle 5.	0	20±2	20±2	1.00	1.00
	0.75	15±2	16±5	0.75	0.80
	3.75	16±17	21±4	0.80	1.05
	7.50	15±3	32±2	0.75	1.60
	15.00	18±2	50±9	0.90	2.50
Noodle 6.	0	17±3	17±3	1.00	1.00
	0.62	18±2	22±2	1.12	1.29
	3.13	18±4	41±19	1.06	2.41
	6.27	19±4	113±21	1.12	6.65
	12.54	16±3	192±31	1.00	11.35

Table 1. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 98 (frameshift mutation) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index (MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Seasoning 4.	0	14 _± 5	14 _± 5	1.00	1.00
	0.58	16 _± 5	26 _± 3	1.14	1.86
	2.93	16 _± 5	51_±6	1.14	3.64
	5.87	15 _± 2	105_±13	1.07	7.50
	11.74	19 _± 4	118_±27	1.36	8.43
Seasoning 5.	0	20 _± 12	20 _± 2	1.00	1.00
	0.50	16 _± 5	22 _± 5	0.85	1.15
	2.54	16 _± 5	37_±5	0.80	1.85
	5.08	18 _± 6	80_±8	0.90	4.00
	10.16	19 _± 3	114_±19	0.95	5.70
Seasoning 6.	0	18 _± 3	18 _± 3	1.00	1.00
	0.46	14 _± 2	34 _± 13	0.78	1.89
	2.30	19 _± 3	51_±12	1.06	2.83
	4.60	17 _± 2	120_±22	0.94	6.67
	9.21	19 _± 5	182_±82	1.06	10.11
Noodle cooked with Seasoning 4.	0	17 _± 3	17 _± 3	1.00	1.00
	0.47	18 _± 4	19 _± 5	1.06	1.12
	2.35	15 _± 2	39_±6	0.88	2.29
	4.70	18 _± 5	105_±5	1.12	6.18
	9.40	18 _± 3	143_±14	1.06	8.41

Table 1. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 98 (frameshift mutation) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Noodle cooked	0	20±2	20±2	1.00	1.00
with Seasoning 5.	0.49	15±4	21±4	0.75	1.05
	2.49	16±6	34±11	0.80	1.70
	4.98	18±4	82±9	0.90	4.10
	9.97	16±3	124±12	0.80	6.20
Noodle cooked	0	18±3	18±3	1.00	1.00
with Seasoning 6.	0.54	13±6	26±3	0.72	1.44
	2.71	17±2	48±9	0.94	2.67
	5.42	14±2	129±16	0.78	7.17
	10.84	16±5	140±30	0.89	7.78
Sour shrimp flavour					
Noodle 7.	0	17±3	17±3	1.00	1.00
	0.77	19±2	23±6	1.12	1.35
	3.88	19±1	34±5	1.12	2.00
	7.76	22±3	102±10	1.29	6.00
	15.53	20±3	148±19	1.18	8.71
Noodle 8.	0	19±3	19±3	1.00	1.00
	0.73	20±2	20±6	1.05	1.11
	3.66	19±3	30±10	1.00	1.58
	7.32	17±2	84±5	0.89	4.42
	14.65	15±1	133±14	0.79	7.00

Table 1. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 98 (frameshift mutation) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Noodle 9.	0	19 \pm 3	19 \pm 3	1.00	1.00
	0.58	22 \pm 1	16 \pm 2	1.16	0.89
	2.92	16 \pm 4	33 \pm 6	0.84	1.74
	5.84	18 \pm 1	102\pm30	0.95	5.37
	11.69	19 \pm 5	149\pm25	1.00	7.84
Seasoning 7.	0	22 \pm 4	22 \pm 4	1.00	1.00
	0.58	14 \pm 4	35 \pm 13	0.64	1.59
	2.90	24 \pm 6	46\pm11	1.09	2.09
	5.81	16 \pm 1	84\pm10	0.77	3.82
	11.63	18 \pm 6	126\pm20	0.86	5.73
Seasoning 8.	0	19 \pm 3	19 \pm 3	1.00	1.00
	0.48	17 \pm 4	28 \pm 2	0.89	1.47
	2.44	18 \pm 6	66\pm13	0.95	3.47
	4.89	20 \pm 2	145\pm27	1.05	7.63
	9.78	13 \pm 2	243\pm45	0.68	12.79
Seasoning 9.	0	20 \pm 2	20 \pm 2	1.00	1.00
	0.49	20 \pm 4	30 \pm 10	1.00	1.50
	2.45	16 \pm 6	80\pm25	0.80	4.00
	4.91	16 \pm 2	185\pm19	0.80	9.25
	9.82	21 \pm 6	230\pm9	1.05	11.50

Table 1. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 98 (frameshift mutation) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Noodle cooked	0	22±4	22±4	1.00	1.00
with Seasoning 7.	0.55	18±3	34±9	0.82	1.59
	2.79	21±10	54±23	0.95	2.45
	5.58	17±5	183±14	0.77	8.32
	11.17	23±3	288±20	1.05	13.09
Noodle cooked	0	19±3	19±3	1.00	1.00
with Seasoning 8.	0.53	15±2	26±7	0.79	1.42
	2.69	16±3	66±13	0.84	3.47
	5.38	14±3	160±27	0.74	8.42
	10.77	18±2	232±28	0.95	12.26
Noodle cooked	0	20±2	20±2	1.00	1.00
with Seasoning 9.	0.47	20±2	35±5	1.00	1.75
	2.37	14±2	73±29	0.70	3.65
	4.74	20±4	126±49	1.00	6.30
	9.48	12±5	200±59	1.10	10.00

* spontaneous revertants

^a The reaction with nitrite was done in a mild acid solution containing 250 µl of 0.5 M sodium nitrite and each selected dose of sample.

^b Data are expressed as means and standard deviation of four plates from two experiments.

^c Mutagenicity index is calculated from the average value of number of histidine revertants/plate of sample mixture divided by that of spontaneous.

Bold figures indicate positive mutagenic response.

Table 2. Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 100 (base-pair substitution) without metabolic activation

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Minced pork flavour					
Noodle 1.	0*	96±7	96±7	1.00	1.00
	0.66	86±5	90±5	0.91	0.94
	3.34	92±10	159±26	0.96	1.66
	6.69	88±16	285±84	0.92	2.97
	13.39	88±6	436±120	0.92	4.54
Noodle 2.	0	95±12	95±12	1.00	1.00
	0.78	85±6	116±11	0.89	1.23
	3.90	97±13	216±22	1.02	2.27
	7.81	101±10	439±53	1.06	4.62
	15.63	95±18	598±42	1.00	6.29
Noodle 3.	0	96±7	96±7	1.00	1.00
	0.60	84±8	97±18	0.89	1.01
	3.04	96±20	200±17	1.01	2.08
	6.09	92±10	406±47	0.96	4.23
	12.19	97±11	751±97	1.01	7.82
Seasoning 1.	0	96±7	96±7	1.00	1.00
	0.59	99±13	146±9	1.03	1.53
	2.98	90±14	425±134	0.94	4.43
	5.97	100±13	600±135	1.05	6.25
	11.55	95±9	895±199	0.99	9.32

Table 2. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 100 (base-pair substitution) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Seasoning 2.	0	90 \pm 7	90 \pm 7	1.00	1.00
	0.56	89 \pm 9	140 \pm 16	0.99	1.56
	2.80	82 \pm 19	299\pm24	0.92	3.32
	5.61	88 \pm 7	581\pm41	0.98	6.46
	11.23	95 \pm 19	840\pm112	1.06	9.33
Seasoning 3.	0	90 \pm 7	90 \pm 7	1.00	1.00
	0.54	88 \pm 15	132 \pm 19	0.99	1.47
	2.70	99 \pm 20	294\pm46	1.10	3.27
	5.40	88 \pm 8	563\pm14	0.99	6.26
	10.81	82 \pm 12	856\pm26	0.91	9.51
Noodle cooked with Seasoning 1.	0	100 \pm 8	100 \pm 8	1.00	1.00
	0.58	94 \pm 11	124 \pm 8	0.94	1.24
	2.91	96 \pm 9	226\pm6	0.96	2.26
	5.82	97 \pm 16	398\pm100	0.97	3.98
	11.65	99 \pm 17	593\pm47	0.99	5.93
Noodle cooked with Seasoning 2.	0	96 \pm 7	96 \pm 7	1.00	1.00
	0.59	80 \pm 14	104 \pm 23	0.83	1.08
	2.96	84 \pm 11	293\pm23	0.89	3.05
	5.92	84 \pm 3	617\pm128	0.89	6.43
	11.85	83 \pm 9	829\pm71	0.86	8.64

Table 2. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 100 (base-pair substitution) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Noodle cooked	0	90±7	90±7	1.00	1.00
with Seasoning 3.	0.56	87±12	107±12	0.97	1.19
	2.82	83±15	165±29	0.92	1.83
	5.65	98±6	376±34	1.09	4.18
	11.31	98±15	682±63	1.09	7.58
Pa-lo duck flavour					
Noodle 4.	0	91±6	91±6	1.00	1.00
	0.64	88±9	104±21	0.97	1.14
	3.20	87±14	179±20	0.96	1.97
	6.40	92±9	308±13	1.02	3.38
	12.80	85±8	539±25	0.93	5.92
Noodle 5.	0	86±13	86±13	1.00	1.00
	0.75	80±21	102±14	0.93	1.19
	3.75	88±7	118±6	1.02	1.37
	7.50	98±21	147±16	1.14	1.71
	15.00	97±6	215±23	1.13	2.50
Noodle 6.	0	91±6	91±6	1.00	1.00
	0.62	86±11	106±6	0.95	1.16
	3.13	103±6	214±11	1.13	2.35
	6.27	88±24	428±60	0.97	4.70
	12.54	97±9	616±60	1.07	6.77

Table 2. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 100 (base-pair substitution) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Seasoning 4.	0	91 _± 10	91 _± 10	1.00	1.00
	0.58	78 _± 8	173 _± 25	0.87	1.90
	2.93	82 _± 17	380_±26	0.90	4.18
	5.87	90 _± 2	1,013_±21	0.99	11.13
	11.74	80 _± 13	670_±91	0.88	7.36
Seasoning 5.	0	86 _± 13	86 _± 13	1.00	1.00
	0.50	93 _± 20	130 _± 18	1.08	1.51
	2.54	82 _± 13	224_±14	0.97	2.60
	5.08	88 _± 18	395_±50	1.03	4.60
	10.16	92 _± 14	562_±55	1.07	6.53
Seasoning 6.	0	91 _± 9	91 _± 9	1.00	1.00
	0.46	82 _± 10	172 _± 36	0.89	1.90
	2.30	93 _± 9	302_±43	1.02	3.32
	4.60	91 _± 19	660_±17	1.00	7.25
	9.21	93 _± 9	831_±73	1.02	9.13
Noodle cooked with Seasoning 4.	0	91 _± 6	91 _± 6	1.00	1.00
	0.47	84 _± 8	124 _± 28	0.92	1.36
	2.35	97 _± 12	204_±52	1.07	2.24
	4.70	91 _± 7	505_±22	1.00	5.55
	9.40	95 _± 17	621_±31	1.04	6.82

Table 2. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 100 (base-pair substitution) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Noodle cooked with Seasoning 5.	0	86±13	86±13	1.00	1.00
	0.49	92±18	133±21	1.07	1.55
	2.49	92±14	196±41	1.07	2.28
	4.98	81±10	357±38	0.94	4.15
	9.97	94±15	515±51	1.10	5.99
Noodle cooked with Seasoning 6.	0	91±9	91±9	1.00	1.00
	0.54	82±13	139±18	0.90	1.53
	2.71	91±18	260±38	1.00	2.86
	5.42	90±8	584±39	0.99	6.43
	10.84	107±15	752±44	1.18	8.26
Sour shrimp flavour					
Noodle 7.	0	92±4	92±4	1.00	1.00
	0.77	88±14	91±12	0.95	0.98
	3.88	79±12	148±25	0.85	1.59
	7.76	93±18	231±33	1.00	2.48
	15.53	87±13	441±86	0.94	4.74
Noodle 8.	0	95±10	95±10	1.00	1.00
	0.73	79±8	115±10	0.83	1.21
	3.66	94±13	177±7	0.99	1.86
	7.32	79±6	323±28	0.83	3.40
	14.65	98±11	562±15	1.03	5.92

Table 2. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 100 (base-pair substitution) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Noodle 9.	0	95±10	95±10	1.00	1.00
	0.58	87±10	86±13	0.92	0.91
	2.92	90±10	200±43	0.95	2.11
	5.84	87±17	371±57	0.92	3.91
	11.69	92±15	630±120	0.97	6.63
Seasoning 7.	0	94±12	94±12	1.00	1.00
	0.58	98±8	109±23	1.04	1.16
	2.90	92±15	254±9	0.98	2.70
	5.81	87±9	401±19	0.93	4.27
	11.63	74±11	584±121	0.79	6.21
Seasoning 8.	0	95±10	95±10	1.00	1.00
	0.48	85±9	144±30	0.89	1.52
	2.44	88±21	386±10	0.93	4.07
	4.89	87±15	718±39	0.92	7.57
	9.78	90±19	700±152	0.95	7.38
Seasoning 9.	0	91±11	91±11	1.00	1.00
	0.49	89±12	164±40	0.98	1.80
	2.45	94±21	463±35	1.04	5.09
	4.91	104±11	900±51	1.14	9.89
	9.82	109±26	726±93	1.20	7.99

Table 2. (Continued) Number of histidine revertants and Mutagenicity Index (MI) of extracts from instant noodles after nitrite treatment^a on *S. typhimurium* TA 100 (base-pair substitution) without metabolic activation.

Sample	Amount of Extract of Sample (mg/plate)	No. of revertants/plate ^b		Mutagenicity index(MI) ^c	
		without nitrite	with nitrite	without nitrite	with nitrite
Noodle cooked	0	94±12	94±12	1.00	1.00
with Seasoning 7.	0.55	98±15	125±23	1.04	1.33
	2.79	103±20	281±12	1.10	2.99
	5.58	91±16	515±30	0.97	5.48
	11.17	102±12	800±44	1.09	8.51
Noodle cooked	0	95±10	95±10	1.00	1.00
with Seasoning 8.	0.55	98±15	125±23	1.04	1.33
	2.69	103±20	281±12	1.10	2.99
	5.38	91±16	515±30	0.97	5.48
	10.77	86±14	718±42	0.91	7.56
Noodle cooked	0	91±11	91±11	1.00	1.00
with Seasoning 9.	0.47	87±10	142±12	0.96	1.56
	2.37	96±7	360±89	1.05	3.96
	4.74	90±11	623±146	0.99	6.85
	9.48	100±4	771±46	1.10	8.47

* spontaneous revertants

^a The reaction with nitrite was done in a mild acid solution containing 250 µl of 0.5 M sodium nitrite and each selected dose of sample.

^b Data are expressed as means and standard deviation of four plates from two experiments.

^c Mutagenicity index is calculated from the average value of number of histidine revertants/plate of sample mixture divided by that of spontaneous.

Bold figures indicate positive mutagenic response

Table 3 Specific mutagenic activity of instant noodles expressed as revertants/gram and Total mutagenic activity expressed as revertants/package with correlation from linear regression on *Salmonella typhimurium* TA 98 (frameshift mutation) and TA 100 (base-pair substitution)

Sample	No. of revertants/g ^a		No. of revertants/package ^b		r (correlation) ^c	
	TA 98	TA 100	TA 98	TA 100	p<0.05	
			TA 98	TA 100	TA98	TA 100
Minced pork Flavour 1						
Noodle	1,210	2,095	66,913	115,853	0.715	0.920
Seasoning	18,197	67,074	92,622	341,406	0.978	0.937
Noodle cooked with Seasoning						
Expect*	2,576	7,320	159,535	457,259	-	-
Actual**	959	4,108	60,321	258,393	0.951	0.972
Minced pork Flavour 2						
Noodle	1,342	3,322	89,511	221,577	0.887	0.975
Seasoning	12,448	66,949	54,522	293,236	0.867	0.970
Noodle Cooked with Seasoning						
Expect*	2,026	7,242	144,033	514,813	-	-
Actual**	1,394	5,695	96,743	395,233	0.975	0.963
Minced pork Flavour 3						
Noodle	1,200	5,067	60,600	255,883	0.968	0.983
Seasoning	9,965	57,980	47,832	278,304	0.938	0.991
Noodle Cooked with Seasoning						
Expect*	1,959	9,659	108,432	534,187	-	-
Actual**	1,280	5,441	70,016	297,622	0.954	0.984

Table 3 (Continued) Specific mutagenic activity of instant noodles expressed as revertants/gram and Total mutagenic activity expressed as revertants/package with correlation from linear regression on *Salmonella typhimurium* TA 98 (frameshift mutation) and TA 100 (base-pair substitution).

Sample	No. of Revertants/g ^a		No. of Revertants/package ^b		r (correlation) ^c	
	TA 98	TA 100	TA 98	TA 100	p<0.05	
					TA98	TA 100
Pa-lo duck flavour 4						
Noodle	2,340	4,520	125,938	243,266	0.979	0.994
Seasoning	8,667	56,250	64,309	417,375	0.914	0.754
Noodle Cooked with Seasoning						
Expect*	3,106	10,787	190,247	660,641	-	-
Actual**	1,297	5,467	69,324	292,211	0.971	0.958
Pa-lo duck flavour 5						
Noodle	406	1,558	20,969	80,470	0.931	0.949
Seasoning	6,999	33,879	67,890	328,626	0.966	0.977
Noodle Cooked with Seasoning						
Expect*	1,447	6,667	88,859	409,096	-	-
Actual**	1,187	4,712	74,282	294,876	0.973	0.975
Pa-lo duck flavour 6						
Noodle	1,188	3,657	62,607	192,723	0.958	0.978
Seasoning	15,125	69,732	125,537	578,775	0.884	0.963
Noodle Cooked with Seasoning						
Expect*	3,083	12,647	188,144	771,498	-	-
Actual**	1,108	5,725	64,928	335,485	0.909	0.969

Table 3 (Continued) Specific mutagenic activity of instant noodles expressed as revertants/gram and Total mutagenic activity expressed as revertants/package with correlation from linear regression on *Salmonella typhimurium* TA 98 (frameshift mutation) and TA 100 (base-pair substitution).

Sample	No. of Revertants/g ^a		No. of Revertants/package ^b		r (correlation) ^c	
	TA 98	TA 100	TA 98	TA 100	p<0.05	
					TA98	TA 100
Sour shrimp flavour 7						
Noodle	1,558	3,983	71,979	184,014	0.968	0.958
Seasoning	7,876	37,432	53,556	254,537	0.956	0.962
Noodle Cooked with Seasoning						
Expect*	2,368	8,273	125,535	438,551	-	-
Actual**	2,343	6,157	123,007	323,242	0.986	0.992
Sour shrimp flavour 8						
Noodle	1,168	4,652	51,625	205,618	0.972	0.994
Seasoning	14,479	42,573	117,279	344,841	0.971	0.882
Noodle Cooked with Seasoning						
Expect*	3,229	10,524	168,904	550,459	-	-
Actual**	2,012	5,982	111,666	332,001	0.971	0.966
Sour shrimp flavour 9						
Noodle	1,106	4,420	58,418	233,464	0.943	0.966
Seasoning	11,842	39,159	119,604	395,505	0.956	0.823
Noodle Cooked with Seasoning						
Expect*	2,828	9,995	178,022	628,969	-	-
Actual**	1,732	6,984	102,326	412,614	0.909	0.935

a Calculated from linear regression. Result expressed per gram

b Calculated from linear regression. Result expressed per package

c p< 0.05, n=24

*Expect revertants are calculated from summation of revertants from noodle and from seasoning

**Actual revertants are received from actual experiment.