

CHAPTER IV

RESULTS

4.1 Mutagenicity of Beef Concentrate

Beef concentrate was investigated for its mutagenicity both before and after nitrite treatment in acid condition pH (3-3.5). It was found that only after interacting with excess nitrite in acid solution beef concentrate induced both *S. typhimurium* strains TA98 and TA100 revertants to be higher than 2 times of spontaneous control with a dose response manner (Tables 3 and 4). Exceptions were found at dose of 4.98 and 9.96 mg/plate of beef concentrate on *S. typhimurium* TA100. It was noticed that the increase of revertants per plate of beef concentrate depended on doses only toward *S. typhimurium* TA100.

Table 3 Mutagenicity of beef concentrate after nitrite treatment towards *Salmonella typhimurium* TA98 without metabolic activation

Original wet weight of beef (mg/plate)	No. of revertants/plate ^a		MI ^c	
	without nitrite	with nitrite ^b	without nitrite	with nitrite
0 ^c	13±3	-	1.00	-
4.98	24±13	29±3^d	1.85	2.23
9.96	18±7	29±12	1.38	2.23
19.92	17±6	30±8	1.31	2.31
39.84	24±9	41±8	1.85	3.15

^aData are expressed as means ± standard deviations of six plates from two different experiments.

^bThe reaction with nitrite was done in a mild acid solution containing 0.25 ml 2 M sodium nitrite (final concentration was 500 mM) .

^cMutagenicity index is calculated by the average value of number of histidine revertants/plate of sample divided by that of spontaneous.

^dBold numbers indicate positive metabolic response.

^eSpontaneous revertants

Table 4 Mutagenicity of beef concentrate after nitrite treatment towards *Salmonella typhimurium* TA100 without metabolic activation

Original wet weight of beef (mg/plate)	No. of revertants/plate ^a		MI ^c	
	without nitrite	with nitrite ^b	without nitrite	with nitrite
0 ^e	143±14	-	1.00	-
4.98	155±8	208±29	1.08	1.45
9.96	176±18	239±19	1.23	1.67
19.92	218±34	297±17^d	1.52	2.08
39.84	252±25	465±42	1.76	3.25

^aData are expressed as means ± standard deviations of six plates from two different experiments.

^bThe reaction with nitrite was done in a mild acid solution containing 0.25 ml 2 M sodium nitrite (final concentration was 500 mM) .

^cMutagenicity index is calculated by the average value of number of histidine revertants/plate of sample divided by that of spontaneous.

^d Bold numbers indicate positive metabolic response.

^e Spontaneous revertants

4.2 Mutagenicity of Extracts from Some Edible Mushrooms

Various mushroom extracts prepared from button, shiitake, abalone and oyster mushroom were investigated for their mutagenicity both with and without nitrite treatment in acid condition (pH 3.0-3.5).

Tables 5 and 6 show the mutagenicity of the extracts from these edible mushrooms after nitrite treatment towards *S. typhimurium* strains TA98 and TA100 respectively. It was indicated that no extract was mutagenic to both strains of *S. typhimurium*. After being treated with nitrite, mushroom extracts expressed their mutagenicity with revertants to be higher than 2 times of spontaneous control on both tester strains when high amounts of mushroom extracts were used.

Table 5 Mutagenicity of extracts from some edible mushrooms after nitrite treatment towards *Salmonella typhimurium* TA98 without metabolic activation

Mushrooms	Amount of mushroom extracts (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Button	0 ^e	24 \pm 12	-	1.00	-
	10	28 \pm 22	28 \pm 14	1.17	1.17
	50	29 \pm 19	47 \pm 25	1.21	1.96
	100	25 \pm 17	50\pm34^d	1.04	2.08
	200	26 \pm 24	66\pm15	1.08	2.75
Shiitake	0 ^e	12 \pm 3	-	1.00	-
	10	12 \pm 6	12 \pm 2	1.00	1.00
	50	13 \pm 3	19 \pm 4	1.08	1.58
	100	6 \pm 2	23 \pm 4	0.50	1.92
	200	10 \pm 4	57\pm24	0.83	4.75
Oyster	0 ^e	35 \pm 5	-	1.00	-
	10	43 \pm 17	50 \pm 8	1.23	1.43
	50	48 \pm 7	63 \pm 32	1.37	1.80
	100	39 \pm 6	91\pm6	1.11	2.60
	200	43 \pm 8	108\pm23	1.23	3.09
Abalone	0 ^e	16 \pm 5	-	1.00	-
	10	14 \pm 5	14 \pm 6	0.88	0.88
	50	13 \pm 5	29 \pm 15	0.81	1.81
	100	14 \pm 3	42\pm18	0.88	2.63
	200	11 \pm 3	68\pm19	0.69	4.25

^aData are expressed as means \pm standard deviations of six plates from two different experiments.

^bThe reaction with nitrite was done in mild acid solution containing 0.25 μ l 2 M sodium nitrite (final concentration was 500 mM).

^cMutagenicity index is calculated by the average value of number of histidine revertants/plate of sample divided by that of spontaneous.

^dBold numbers indicate positive metabolic response.

^eSpontaneous revertants.

Table 6 Mutagenicity of extracts from some edible mushrooms after nitrite treatment towards *Salmonella typhimurium* TA100 without metabolic activation

Mushrooms	Amount of mushroom extracts (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Button	0 ^e	127 \pm 13	-	1.00	-
	10	106 \pm 14	214 \pm 81	0.83	1.69
	50	96 \pm 20	413\pm132^d	0.76	3.25
	100	118 \pm 17	536\pm183	0.93	4.22
	200	133 \pm 20	597\pm190	1.05	4.70
Shiitake	0 ^e	132 \pm 10	-	1.00	-
	10	140 \pm 17	140 \pm 10	1.06	1.06
	50	129 \pm 14	213 \pm 12	0.98	1.61
	100	126 \pm 18	251 \pm 41	0.95	1.90
	200	169 \pm 64	418\pm30	1.28	3.16
Oyster	0 ^e	122 \pm 14	-	1.00	-
	10	124 \pm 18	151 \pm 15	1.01	1.23
	50	126 \pm 16	308\pm18	1.03	2.52
	100	108 \pm 15	486\pm22	0.89	3.98
	200	128 \pm 31	524\pm44	1.05	4.30
Abalone	0 ^e	119 \pm 23	-	1.00	-
	10	203 \pm 90	313 \pm180	1.71	2.63
	50	234 \pm 106	350 \pm183	1.97	2.94
	100	233 \pm 86	403\pm211	1.96	3.39
	200	229 \pm 102	437\pm124	1.92	3.67

^aData are expressed as means \pm standard deviations of six plates from two different experiments.

^bThe reaction with nitrite was done in a mild acid solution containing 0.25 ml 2 M sodium nitrite (final concentration was 500 mM) .

^cMutagenicity index is calculated by the average value of number of histidine revertants/plate of sample divided by that of spontaneous.

Bold numbers indicate positive metabolic response.

^eSpontaneous revertants.

4.3 Mutagenicity of Concentrate from Beef Boiled with Various Amounts of Some Edible Mushrooms

In order to study the effect of selected edible mushrooms on the formation of the mutagen precursor that could interact with nitrite, beef (100 g) and each mushroom in different amounts (5, 10 or 20 g) were boiled with 200 ml distilled water for 6 hours.

Tables 7 and 8 show the mutagenicity of concentrates from beef boiled with edible mushroom namely, button, shiitake, abalone or oyster on *Salmonella typhimurium* strains TA98 and TA100 respectively. The results show that almost all nitrite treated concentrates from beef boiled with each mushroom expressed the revertant colonies to be higher than 2 times of spontaneous control with dose response manner on both *S. typhimurium* strains TA98 and TA100. Exception was detected on the concentrate from beef boiled with 5 g of shiitake mushroom which showed no mutagenicity on *S. typhimurium* TA98. None of the untreated concentrates was positive mutagenic on both tester strains.

The effects of selected edible mushrooms on the formation of mutagen precursor that could interact with nitrite were evaluated by slopes from graphs between mutagenicity index and amounts of concentrate from beef boiled with edible mushrooms (Figures 9-12 and Table 9). It was proposed that the slopes should decrease when the amount of mushroom increased if such mushroom extract reduced the formation of mutagen precursor. However the results show that all selected edible mushroom extracts did not affect on the formation of mutagen precursor after nitrite treatment.

Table 7 Mutagenicity of concentrates from beef boiled with various amounts (g) of some edible mushrooms treated with nitrite towards *Salmonella typhimurium* TA98 without metabolic activation

Sample	Amount of Samples (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Button Mushroom					
5 g	0 ^c	14 \pm 5	-	1.00	-
	10	15 \pm 3	16 \pm 6	1.07	1.38
	20	19 \pm 7	28 \pm 2	1.36	1.86
	40	22 \pm 9	45\pm12^d	1.57	2.95
	80	17 \pm 9	77\pm39	1.21	3.43
10 g	0	13 \pm 3	-	1.00	-
	10	14 \pm 5	18 \pm 10	1.08	1.38
	20	18 \pm 5	37\pm10	1.38	2.85
	40	18 \pm 4	40\pm19	1.38	3.08
	80	17 \pm 5	84\pm29	1.31	6.46
20 g	0	14 \pm 5	-	1.00	-
	10	11 \pm 6	28\pm4	0.79	2.00
	20	15 \pm 5	32\pm7	1.07	2.29
	40	19 \pm 7	81\pm9	1.36	5.79
	80	22 \pm 9	96\pm5	1.57	6.86

Table 7 Mutagenicity of concentrates from beef boiled with various amounts (g) of some edible mushrooms treated with nitrite towards *Salmonella typhimurium* TA98 without metabolic activation (continued)

Sample	Amount of Samples (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Shiitake mushroom					
5 g	0 ^e	13 \pm 3	-	1.00	-
	10	12 \pm 5	13 \pm 5	0.92	1.00
	20	16 \pm 4	18 \pm 3	1.23	1.38
	40	17 \pm 3	19 \pm 5	1.31	1.46
	80	17 \pm 5	19 \pm 7	1.31	1.46
10 g	0	13 \pm 3	-	1.00	-
	10	14 \pm 4	22 \pm 3	1.08	1.69
	20	13 \pm 4	24 \pm 5	1.00	1.85
	40	17 \pm 3	29\pm8^d	1.31	2.23
	80	14 \pm 5	46\pm16	1.08	3.54
20 g	0	13 \pm 3	-	1.00	-
	10	12 \pm 3	15 \pm 4	0.92	1.15
	20	12 \pm 3	17 \pm 5	0.92	1.31
	40	13 \pm 4	22 \pm 7	1.00	1.69
	80	13 \pm 5	34\pm4	1.00	2.62

Table 7 Mutagenicity of concentrates from beef boiled with various amounts (g) of some edible mushrooms treated with nitrite towards *Salmonella typhimurium* TA98 without metabolic activation (continued)

Sample	Amount of Samples (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Oyster mushroom					
5 g	0 ^e	9 \pm 2	-	1.00	-
	10	14 \pm 3	15 \pm 4	1.56	1.67
	20	17 \pm 6	18\pm7^d	1.89	2.00
	40	17 \pm 7	21\pm3	1.89	2.33
	80	13 \pm 7	24\pm7	1.44	2.67
10 g	0	16 \pm 5	-	1.00	-
	10	12 \pm 6	19 \pm 6	0.75	1.19
	20	12 \pm 5	18 \pm 7	0.75	1.13
	40	20 \pm 4	25 \pm 9	1.25	1.56
	80	18 \pm 5	47\pm7	1.13	2.94
20 g	0	16 \pm 5	-	1.00	-
	10	18 \pm 8	19 \pm 5	1.13	1.19
	20	23 \pm 11	24 \pm 5	1.44	1.50
	40	21 \pm 7	36\pm14	1.31	2.25
	80	18 \pm 5	44\pm22	1.13	2.75

Table 7 Mutagenicity of concentrates from beef boiled with various amounts (g) of some edible mushrooms treated with nitrite towards *Salmonella typhimurium* TA98 without metabolic activation (continued)

Sample	Amount of Samples (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Abalone mushroom					
5 g	0 ^e	12 \pm 2	-	1.00	-
	10	15 \pm 3	12 \pm 3	1.25	1.00
	20	13 \pm 5	15 \pm 3	1.08	1.25
	40	11 \pm 2	29\pm3^d	0.92	2.42
	80	22 \pm 8	31\pm6	1.83	2.58
10 g	0	12 \pm 2	-	1.00	-
	10	16 \pm 5	21 \pm 6	1.33	1.75
	20	14 \pm 4	25\pm8	2.08	2.08
	40	18 \pm 5	37\pm8	3.08	3.08
	80	21 \pm 2	41\pm10	3.42	3.42
20 g	0	13 \pm 3	-	1.00	-
	10	11 \pm 4	19 \pm 4	0.85	1.46
	20	13 \pm 5	21 \pm 4	1.00	1.62
	40	16 \pm 6	30\pm9	1.23	2.31
	80	18 \pm 8	47\pm8	1.38	3.62

^aData are expressed as means \pm standard deviations of six plates from two different experiments.

^bThe reaction with nitrite was done in mild acid solution containing 0.25 μ l 2 M sodium nitrite (final concentration was 500 mM).

^cMutagenicity index is calculated by the average value of number of histidine revertants/plate of sample divided by that of spontaneous.

^dBold numbers indicate positive metabolic response.

^eSpontaneous revertants.

Table 8 Mutagenicity of concentrates from beef boiled with various amounts (g) of some edible mushrooms treated with nitrite towards *Salmonella typhimurium* TA100 without metabolic activation

Sample	Amount of Samples (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Button Mushroom					
5 g	0 ^e	134 \pm 7	-	1.00	-
	10	138 \pm 38	185 \pm 21	1.03	1.38
	20	193 \pm 40	249 \pm 5	1.44	1.86
	40	193 \pm 49	395\pm52^d	1.44	2.95
	80	200 \pm 72	459\pm85	1.49	3.43
10 g	0	132 \pm 26	-	1.00	-
	10	116 \pm 13	128 \pm 26	0.88	0.97
	20	132 \pm 19	222 \pm 51	1.00	1.68
	40	153 \pm 40	279\pm24	1.16	2.11
	80	188 \pm 16	572\pm84	1.42	4.33
20 g	0	134 \pm 7	-	1.00	-
	10	152 \pm 13	218 \pm 21	1.13	1.63
	20	169 \pm 39	261 \pm 20	1.26	1.95
	40	177 \pm 34	451\pm29	1.32	3.37
	80	256 \pm 53	760\pm57	1.91	5.67

Table 8 Mutagenicity of concentrates from beef boiled with various amounts (g) of some edible mushrooms treated with nitrite towards *Salmonella typhimurium* TA100 without metabolic activation (continued)

Sample	Amount of Samples (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Shiitake mushroom					
5 g	0 ^e	142±14	-	1.00	-
	10	122±18	132±18	0.86	0.92
	20	131±26	156±11	0.92	1.09
	40	154±17	222±38	1.08	1.55
	80	130±70	292±23^d	0.92	2.04
10 g	0	154±12	-	1.00	-
	10	176±27	223±20	1.14	1.45
	20	163±18	229±28	1.06	1.49
	40	185±19	321±23	1.20	2.08
	80	204±21	427±36	1.32	2.77
20 g	0	154±12	-	1.00	-
	10	130±12	183±30	0.84	1.19
	20	140±27	193±14	0.91	1.25
	40	150±20	242±13	0.97	1.57
	80	180±16	325±41	1.17	2.11

Table 8 Mutagenicity of concentrates from beef boiled with various amounts (g) of some edible mushrooms treated with nitrite towards *Salmonella typhimurium* TA100 without metabolic activation (continued)

Sample	Amount of Samples (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Oyster mushroom					
5 g	0 ^e	128 \pm 7	-	1.00	-
	10	133 \pm 12	154 \pm 15	1.04	1.20
	20	130 \pm 40	170 \pm 16	1.02	1.33
	40	158 \pm 23	236 \pm 25	1.23	1.84
	80	212 \pm 59	356\pm37^d	1.66	2.78
10 g	0	119 \pm 11	-	1.00	-
	10	132 \pm 14	170 \pm 11	1.11	1.43
	20	154 \pm 16	197 \pm 12	1.29	1.66
	40	173 \pm 20	260\pm22	1.45	2.18
	80	223 \pm 34	375\pm84	1.87	3.15
20 g	0	119 \pm 11	-	1.00	-
	10	122 \pm 56	180 \pm 32	1.03	1.51
	20	195 \pm 68	222 \pm 48	1.64	1.87
	40	200 \pm 56	297\pm38	1.68	2.50
	80	206 \pm 22	206\pm128	1.73	3.50

Table 8 Mutagenicity of concentrates from beef boiled with various amounts (g) of some edible mushrooms treated with nitrite towards *Salmonella typhimurium* TA100 without metabolic activation (continued)

Sample	Amount of Samples (μ l)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Abalone mushroom					
5 g	0 ^e	136 \pm 27	-	1.00	-
	10	142 \pm 23	157 \pm 36	1.04	1.15
	20	139 \pm 31	178 \pm 53	1.02	1.31
	40	163 \pm 38	271 \pm 87	1.20	1.99
	80	207 \pm 23	436\pm117^d	1.52	3.21
10 g	0	136 \pm 27	-	1.00	-
	10	144 \pm 23	202 \pm 19	1.06	1.49
	20	176 \pm 23	259 \pm 52	1.29	1.90
	40	213 \pm 18	309\pm24	1.57	2.27
	80	216 \pm 59	471\pm67	1.59	3.46
20 g	0	132 \pm 26	-	1.00	-
	10	116 \pm 32	138 \pm 14	0.88	1.05
	20	123 \pm 12	168 \pm 8	0.93	1.27
	40	117 \pm 15	249 \pm 20	0.89	1.89
	80	159 \pm 11	387\pm46	1.20	2.93

^aData are expressed as means \pm standard deviations of six plates from two different experiments.

^bThe reaction with nitrite was done in mild acid solution containing 0.25 μ l 2 M sodium nitrite (final concentration was 500 mM).

^cMutagenicity index is calculated by the average value of number of histidine revertants/plate of sample divided by that of spontaneous.

^dBold numbers indicate positive metabolic response.

^eSpontaneous revertants.

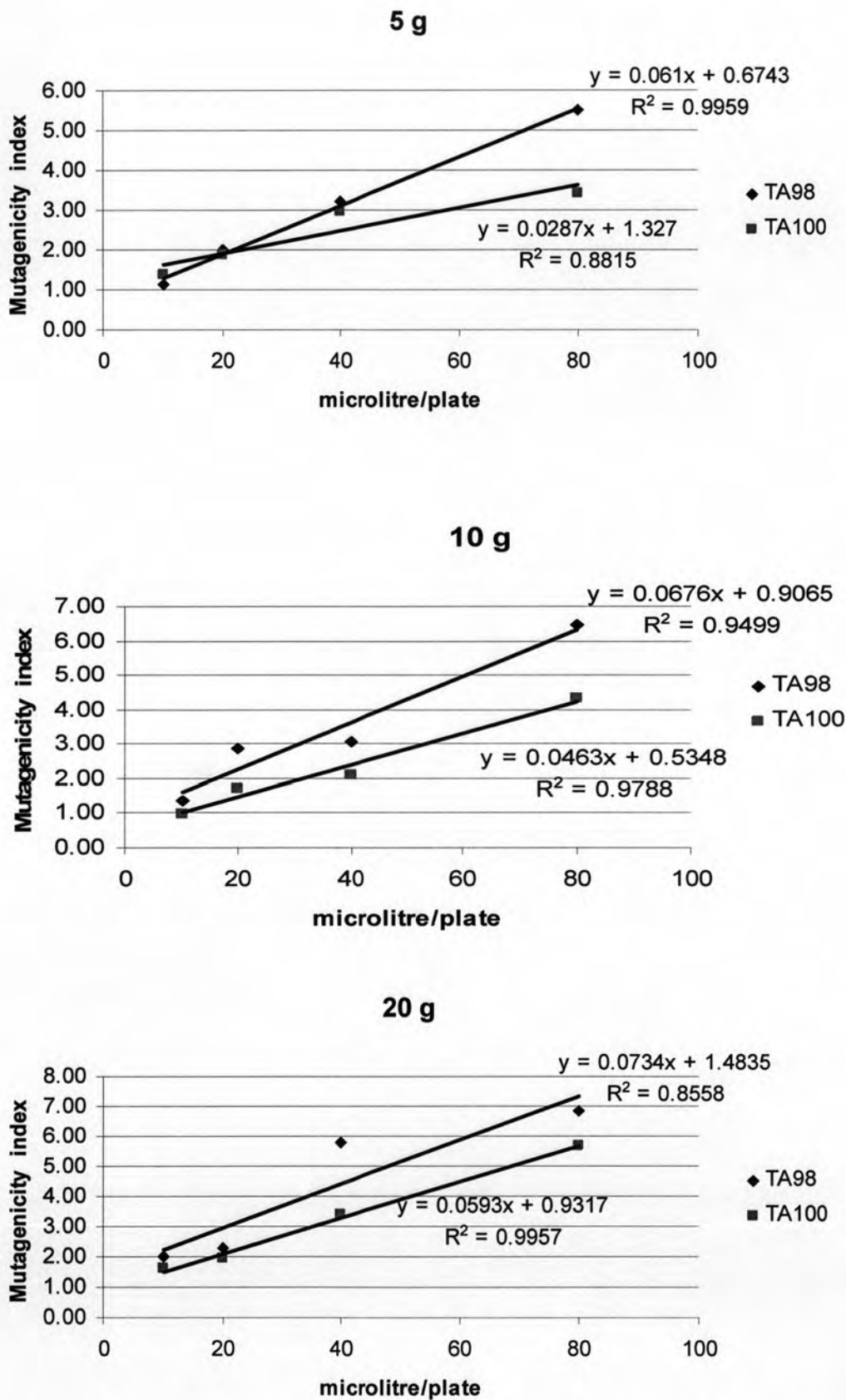


Figure 9 Mutagenicity index of concentrates from beef boiled with button mushroom in different amounts (5, 10 or 20 g)

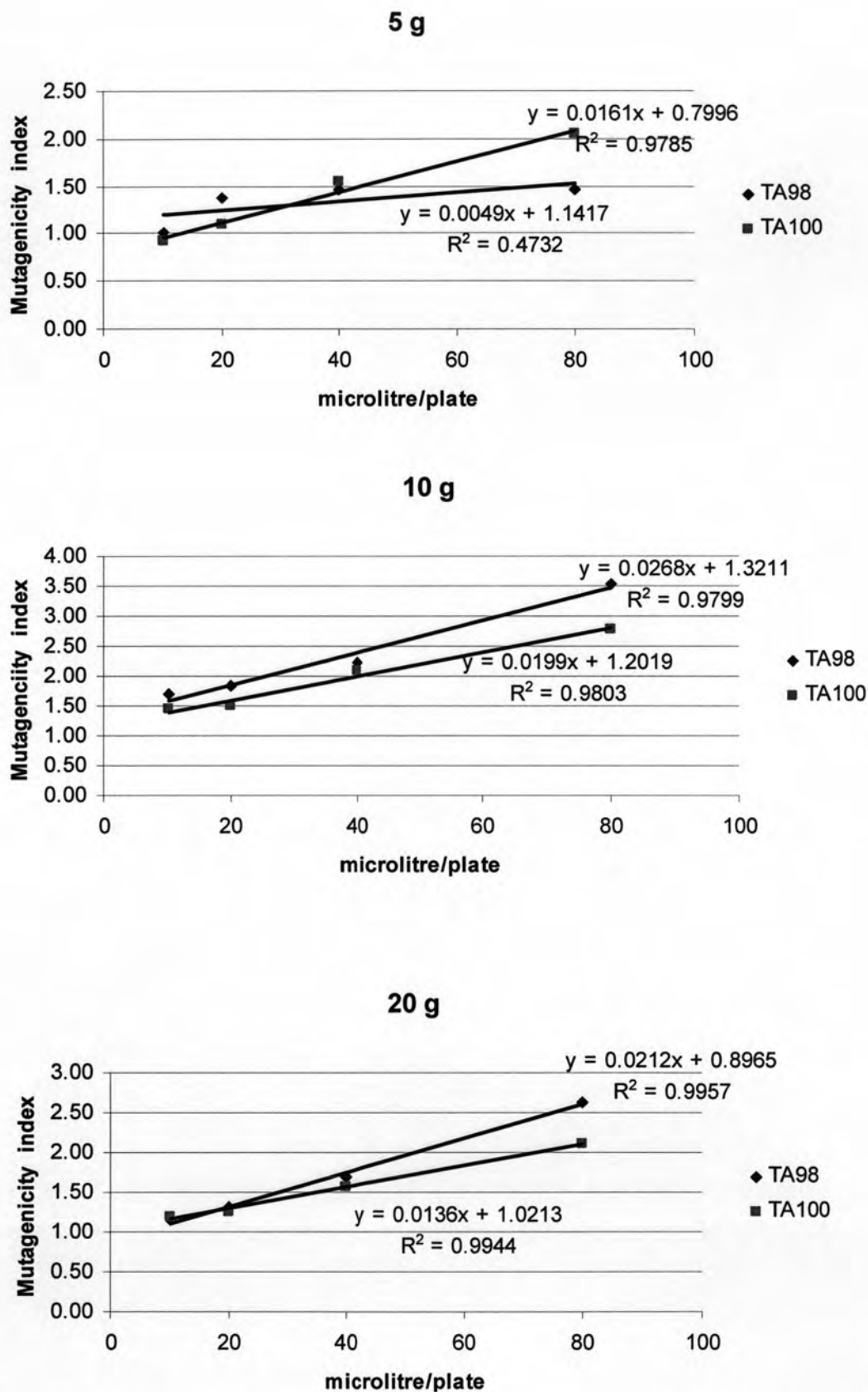


Figure 10 Mutagenicity index of concentrates from beef boiled with shiitake mushroom in different amounts (5, 10 or 20 g)

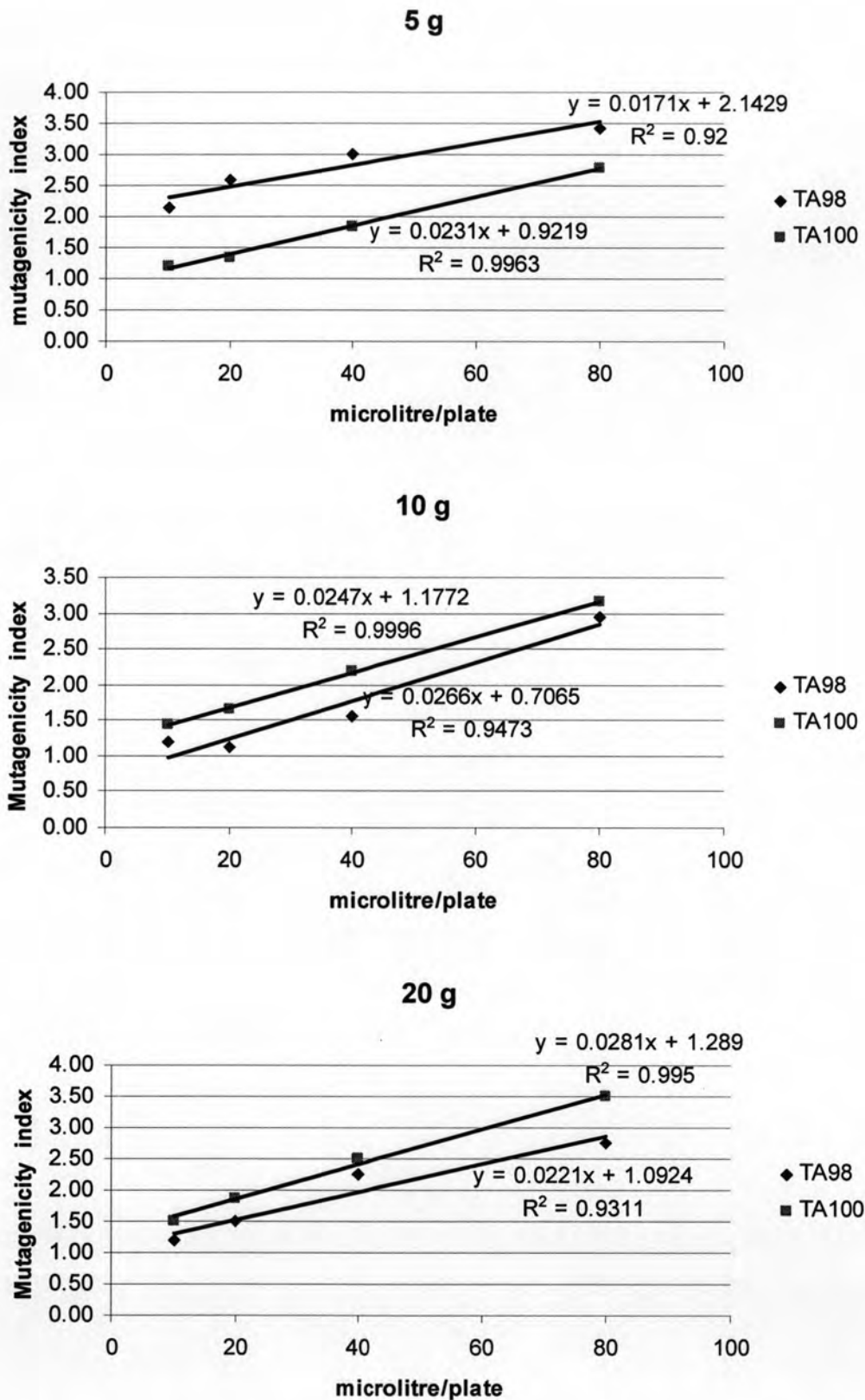


Figure 11 Mutagenicity index of concentrates from beef boiled with oyster mushroom in different amounts (5, 10, or 20 g)



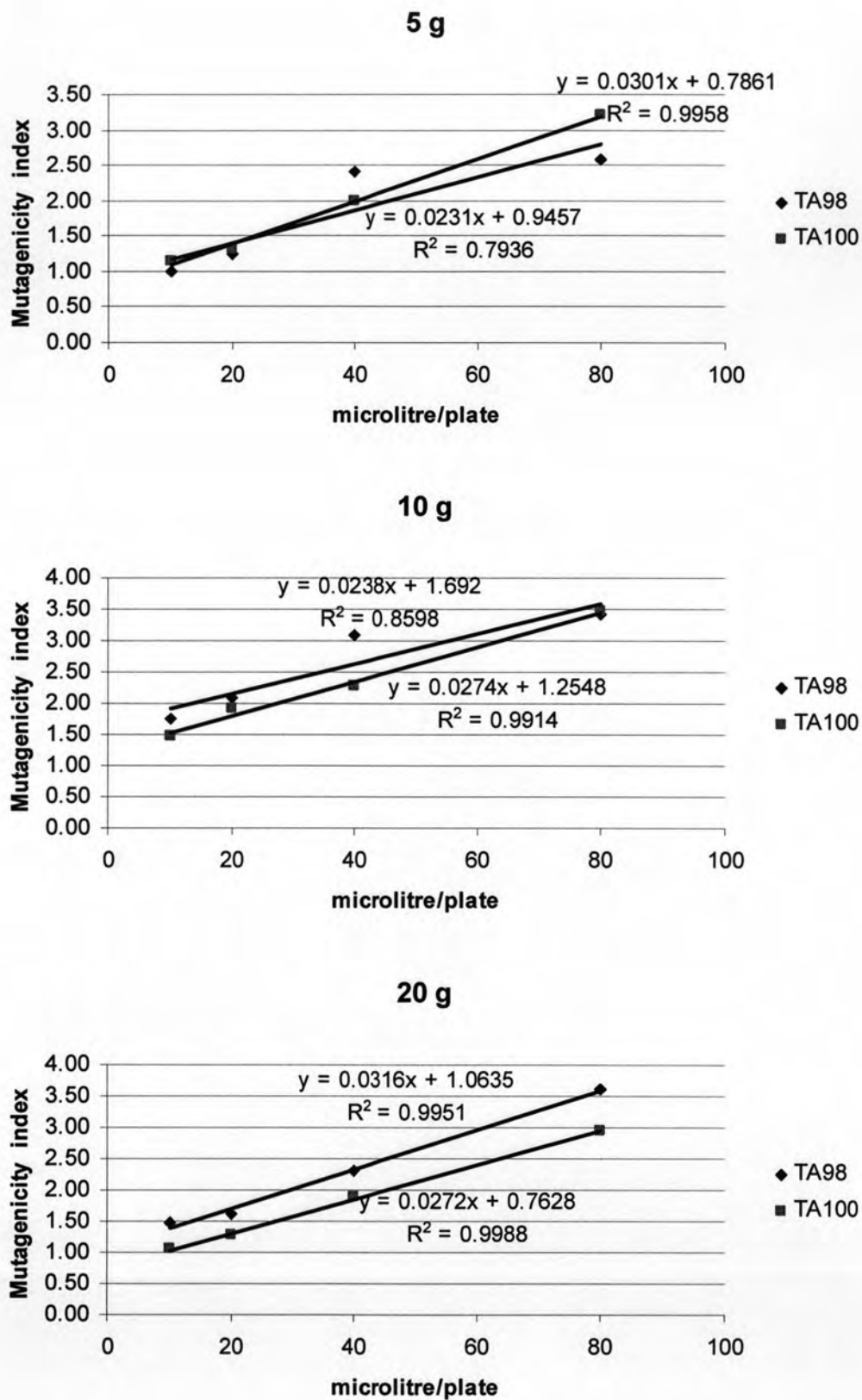


Figure 12 Mutagenicity index of concentrates from beef boiled with abalone mushroom in different amounts (5, 10 or 20 g)

Table 9 Slopes from the graphs of the mutagenicity of concentrates from beef boiled with various amounts (g) of some edible mushrooms treated with nitrite towards *Salmonella typhimurium* TA98 and TA100 without metabolic activation

Mushrooms	Amounts of original dried mushrooms (g)	Slopes of Graphs	
		TA98	TA100
Button	5 g	0.0610	0.0287
	10 g	0.0676	0.0463
	20 g	0.0734	0.0593
Shiitake	5 g	0.0049	0.0161
	10 g	0.0268	0.0199
	20 g	0.0212	0.0136
Oyster	5 g	0.0171	0.0231
	10 g	0.0266	0.0247
	20 g	0.0221	0.0281
Abalone	5 g	0.0231	0.0301
	10 g	0.0238	0.0274
	20 g	0.0316	0.0372

4.4 Effect of Extracts from Selected Edible Mushrooms on the Mutagenicity of Beef Concentrate

To examine the effect of different concentrations of selected edible mushroom extracts on the formation of direct mutagen occurred during the reaction between beef concentrate and nitrite. Each mushroom extract namely button, shiitake, abalone and oyster was mixed with beef concentrate and treated with nitrite in the acid condition (pH 3.0-3.5).

Tables 10 and 11 show the mutagenicity of the mixtures obtained from different concentrations of each mushroom extract and beef concentrate (80 μ l/plate) on *S. typhimurium* TA 98 and TA 100 respectively. All untreated samples exhibited no detectable mutagenicity on both tester strains. After nitrite treatment, almost all of nitrite treated mixtures were positive mutagenic with dose response manner on these tester strains without metabolic activation. However, the extract from abalone mushroom showed the decrease of mutagenicity on both tester strains when the highest amount (100 μ l) of the extract was used.

Comparison between the expected (calculated) and actual (observed) mutagenicity indices (MIs) of the nitrite treated mixture of beef concentrate (80 μ l) and mushroom extract on *S. typhimurium* TA 98 and TA 100 are shown in Tables 12 and 13 respectively. It was found that almost all observed MIs of the mixtures of beef concentrate and mushroom extracts were less than the expected MIs that obtained from summation of each individual MI on both *S. typhimurium* TA 98 and TA100, then percent modifications of these mixtures were positive. Different results were found on button mushroom extracts at dose of 50 and 100 μ l/plate and oyster mushroom extract at dose of 100 μ l/plate towards *S. typhimurium* TA 98 and percent modifications of these mushroom mixtures were negative.

Table 10 Effect of extracts from some edible mushrooms on mutagenicity of beef concentrate (80µl) towards *Salmonella typhimurium* TA98 without metabolic activation

Mushrooms	Amount of mushroom extracts (µl)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Button	0 ^e	9±2	-	1.00	-
	10	16±4	35±20^d	1.78	3.89
	50	15±7	79±20	1.67	8.78
	100	18±3	104±35	2.00	11.56
Shiitake	0 ^e	16±2	-	1.00	-
	10	16±10	22±11	1.00	1.38
	50	15±7	33±11	0.94	2.06
	100	23±13	55±15	1.44	3.44
Oyster	0 ^e	16±2	-	1.00	-
	10	20±7	17±15	1.25	1.06
	50	10±10	58±36	0.63	3.63
	100	18±2	97±40	1.13	6.06
Abalone	0 ^e	16±2	-	1.00	-
	10	14±5	19±13	0.88	1.19
	50	10±5	34±14	0.63	2.13
	100	9±6	24±12	0.56	1.50

^aData are expressed as means ± standard deviations of six plates from two different experiments.

^bThe reaction with nitrite was done in a mild acid solution containing 0.25 ml 2 M sodium nitrite (final concentration was 500 mM) .

^cMutagenicity index is calculated by the average value of number of histidine revertants/plate of sample divided by that of spontaneous.

^dBold numbers indicate positive metabolic response.

^eSpontaneous revertants

Table 11 Effect of extracts from some edible mushrooms on mutagenicity of beef concentrate (80µl) towards *Salmonella typhimurium* TA100 without metabolic activation

Mushrooms	Amount of mushroom extracts (µl)	No. of revertants/plate ^a		MI ^c	
		without nitrite	with nitrite ^b	without nitrite	with nitrite
Button	0 ^e	128±7	-	1.00	-
	10	143±79	502±106^d	1.12	3.92
	50	221±17	692±246	1.73	5.41
	100	243±73	766±202	1.90	5.98
Shiitake	0 ^e	154±26	-	1.00	-
	10	240±63	315±140	1.56	2.05
	50	270±128	484±128	1.75	3.14
	100	284±30	644±105	1.84	4.18
Oyster	0 ^e	154±26	-	1.00	-
	10	289±38	449±85	1.88	2.92
	50	297±110	491±222	1.93	3.19
	100	241±114	787±295	1.56	5.11
Abalone	0 ^e	154±26	-	1.00	-
	10	190±108	529±78	1.23	3.44
	50	137±51	573±93	0.89	3.72
	100	183±88	451±185	1.19	2.93

^aData are expressed as means ± standard deviations of six plates from two different experiments.

^bThe reaction with nitrite was done in a mild acid solution containing 0.25 ml 2 M sodium nitrite (final concentration was 500 mM) .

^cMutagenicity index is calculated by the average value of number of histidine revertants/plate of sample divided by that of spontaneous.

^dBold numbers indicate positive metabolic response.

^eSpontaneous revertants

Table 12 Comparison between the calculated mutagenicity index obtained by summing the mutagenicity index of individual components^a and observed values of nitrite treated mixture of beef concentrate (80µl) and mushroom extract on *Salmonella typhimurium* TA98 without metabolic activation

Mushrooms	MI ^b				Percent modification ^e
	A	B	Summation	Actual	
	nitrite treated beef concentrate ^c	nitrite treated mushroom extract ^d	of A and B (Expected)		
Button	3.15	1.17	4.32	3.89	9.95
	3.15	1.96	5.11	8.78	-71.82
	3.15	2.08	5.23	11.56	-121.03
Shiitake	3.15	1.00	4.15	1.38	66.75
	3.15	1.58	4.73	2.06	56.45
	3.15	1.92	5.07	3.44	32.28
Oyster	3.15	1.43	4.58	1.06	76.86
	3.15	1.80	4.95	3.63	26.67
	3.15	2.60	5.75	6.06	-5.39
Abalone	3.15	0.88	4.03	1.19	70.47
	3.15	1.81	4.96	2.13	57.06
	3.15	2.63	5.78	1.50	74.05

^a Nitrite treated extract from edible mushrooms and nitrite treated beef concentrate.

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

^c Mutagenicity index of nitrite treated beef concentrate (80 µl) from Table 8.

^d Mutagenicity index of nitrite treated mushroom extracts (10, 50 or 100 µl) from Table 6.

^e Percent modification = $\frac{\text{Expected MI} - \text{Actual MI}}{\text{Actual MI}} \times 100$

Table 13 Comparison between the calculated mutagenicity index obtained by summing the mutagenicity index of individual components^a and observed values of nitrite treated mixture of beef concentrate (80µl) and mushroom extract on *Salmonella typhimurium* TA100 without metabolic activation

Mushrooms	MI ^b				Percent modification ^e
	A	B	Summation	Actual	
	nitrite treated beef concentrate ^c	nitrite treated mushroom extract ^d	of A and B (Expected)		
Button	3.25	1.68	4.94	3.92	20.65
	3.25	3.24	6.50	5.41	16.77
	3.25	4.21	7.47	5.98	19.95
Shiitake	3.25	1.05	4.31	2.05	52.44
	3.25	1.61	4.86	3.14	35.39
	3.25	1.90	5.15	4.18	18.83
Oyster	3.25	1.23	4.48	2.92	34.82
	3.25	2.51	5.77	3.19	44.71
	3.25	3.97	7.23	5.11	29.32
Abalone	3.25	2.64	5.88	3.44	41.50
	3.25	2.95	6.19	3.72	39.90
	3.25	3.40	6.64	2.93	55.87

^a Nitrite treated extract from edible mushrooms and nitrite treated beef concentrate.

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

^c Mutagenicity index of nitrite treated beef concentrate (80 µl) from Table 9.

^d Mutagenicity index of nitrite treated mushroom extracts (10, 50 or 100 µl) from Table 7.

^e Percent modification = $\frac{\text{Expected MI} - \text{Actual MI}}{\text{Actual MI}} \times 100$