



CHAPTER IV

RESULTS

4.1 Antioxidant Assays

The ethanolic extracts from Hom Nil rice and black glutinous rice (raw, cooked and fermented) were assessed for their antioxidant activities (determined using DPPH scavenging capacity assay and ferric reducing antioxidant power assay) and phenolic contents (determined using Folin-Ciocalteu reagent) since the antioxidant capacities were influenced by many factors, which cannot be fully described by a single method. The antioxidant activity and the total phenolic contents of raw Hom Nil rice extract (RH), cooked Hom Nil rice extract (CH), fermented Hom Nil rice extract (FH), raw black glutinous rice extract (RB), cooked black glutinous rice extract (CB) and fermented black glutinous rice extract (FB) are shown in Figure 16 and Table C-1 (Appendix C).

The reduction of DPPH by antioxidant in the rice extracts expressed as mg of Trolox equivalent antioxidant capacities (TEAC) was between 0.117 and 1.245 mg TEAC/g dry weight of rice extract. The percentage of radical scavenging activity of each sample which calculated from TEAC was between 24.26 and 98.41%. The FRAP (Ferric Reducing Antioxidant Power) value of each sample was between 360.87 and 6797.89 mg Fe(II)/g dry weight of rice extract. The amount of total phenolic content of each rice extract was in the range from 38.56 to 458.37 mg GAE/g dry weight of rice extract.

Using 8 mg/ml of each crude extract in all assays, the rankings of all samples concerning their antioxidant activity and amount of total phenolic content were $RB > CB > RH > CH > FB > FH$; raw black glutinous rice had the highest antioxidant activity and total phenolic content.

Percentage yield of each rice extract calculated from raw material dry weight basis was $RH = 0.8\%$, $CH = 0.6\%$, $FH = 27.3\%$, $RB = 1.7\%$, $CB = 0.6\%$, and $FB = 12.8\%$. Thus, calculating based on their raw material dry weight basis; it was found that fermented rice had higher both antioxidant activity and phenolic contents than raw or cooked rice.

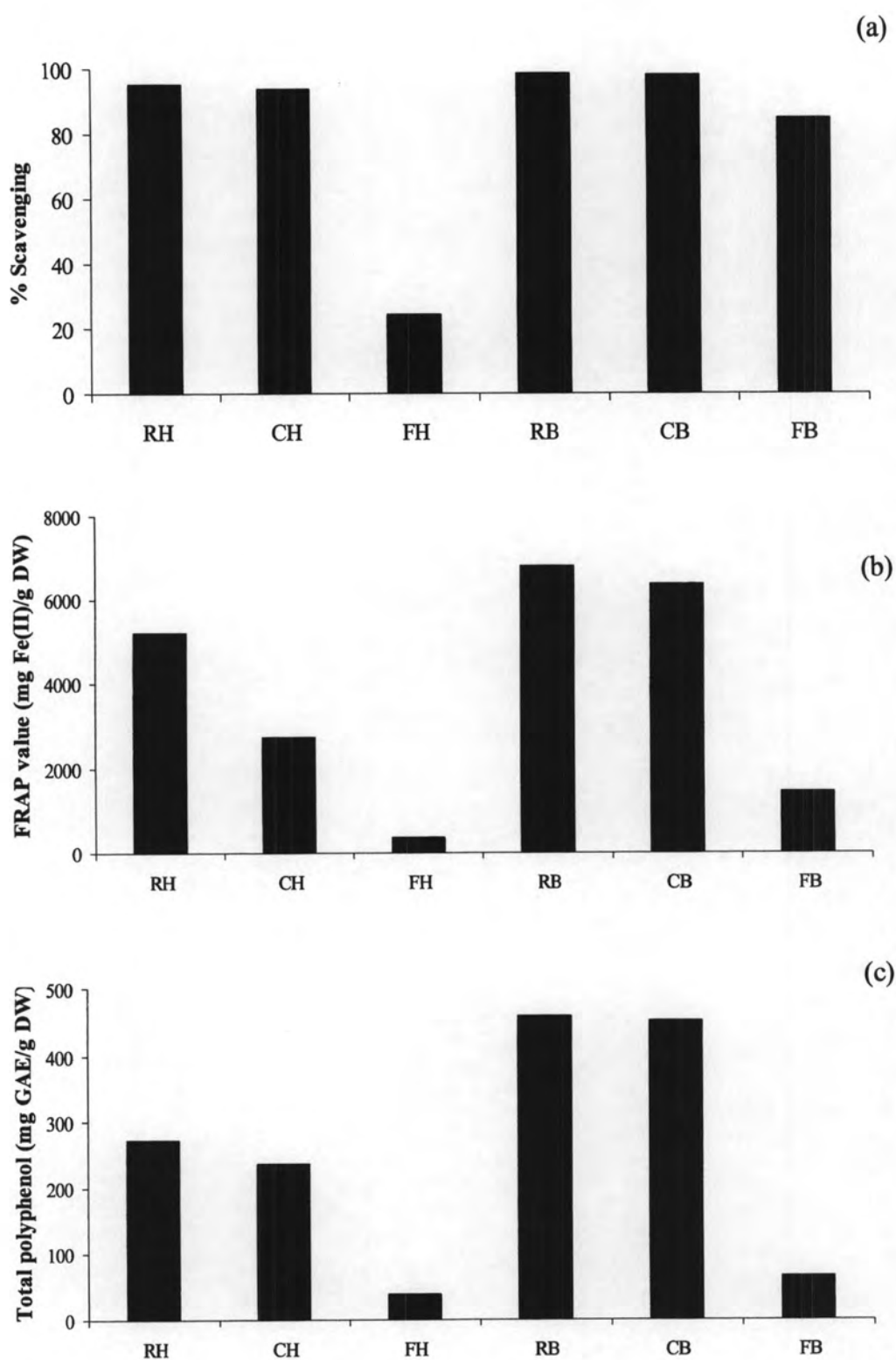


Figure 16 Antioxidant activity determined by DPPH scavenging assay (a), Frap assay (b) and determination of total phenolic content (c) of the rice extracts; raw Hom Nil rice extract (RH), cooked Hom Nil rice extract (CH), fermented Hom Nil rice extract (FH), raw black glutinous rice extract (RB), cooked black glutinous rice extract (CB) and fermented black glutinous rice extract (FB).

4.2 Mutagenicity of Chicken Extract

The chicken extract was investigated for its mutagenicity without and with sodium nitrite treatment in acid condition (pH 3.0-3.5). It was found that only being interacted with excess sodium nitrite in acid solution, it induced both *S. typhimurium* strains TA98 and TA100 revertants to be higher than 2 times of spontaneous control with a dose response manner as shown in Table C-2 (Appendix C). Therefore, 7.2 mg/plate of chicken extract with sodium nitrite treatment was chosen to be the positive mutagen in this study.

4.3 Mutagenicity of Rice Extracts

Rice extracts prepared from raw, cooked and fermented Hom Nil rice and black glutinous rice were investigated for their mutagenicity on *S. typhimurium* and the results were shown in Figures 17 for TA98 and Figures 18 for TA100, and also in Tables C-3 and C-4 (Appendix C). All of the rice extracts (0.8, 1.2 and 1.6 mg/plate) were not mutagenic towards both *S. typhimurium* strains. After being treated with sodium nitrite, all extracts expressed their mutagenicity index to be higher than 2 on both tester strains in a dose response manner. Exceptions were found at low dose (0.8 mg/plate) of FB and all doses of FH for both tester strains that the extracts were not turned to be mutagenic. The results indicated that black glutinous rice and Hom Nil rice extracts had direct mutagenicity only after being treated with sodium nitrite.

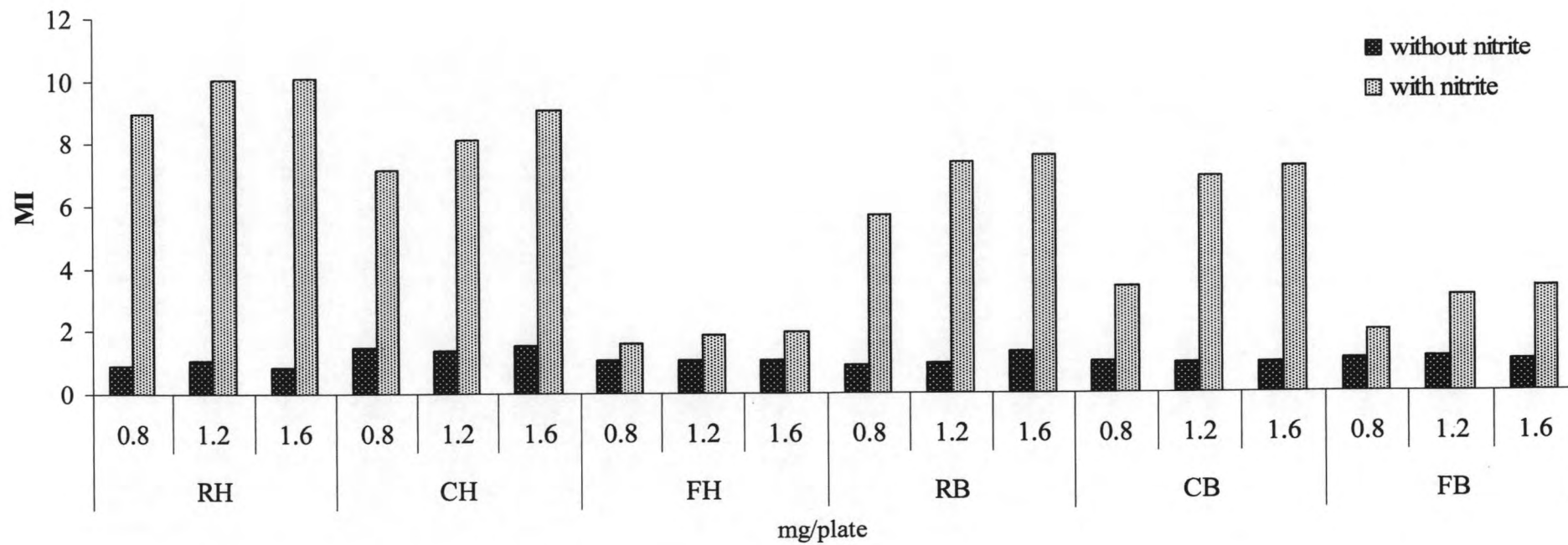


Figure 17 Mutagenicity index of the rice extracts without and with sodium nitrite treatment in acid condition (pH 3.0-3.5) on *S. typhimurium* TA98

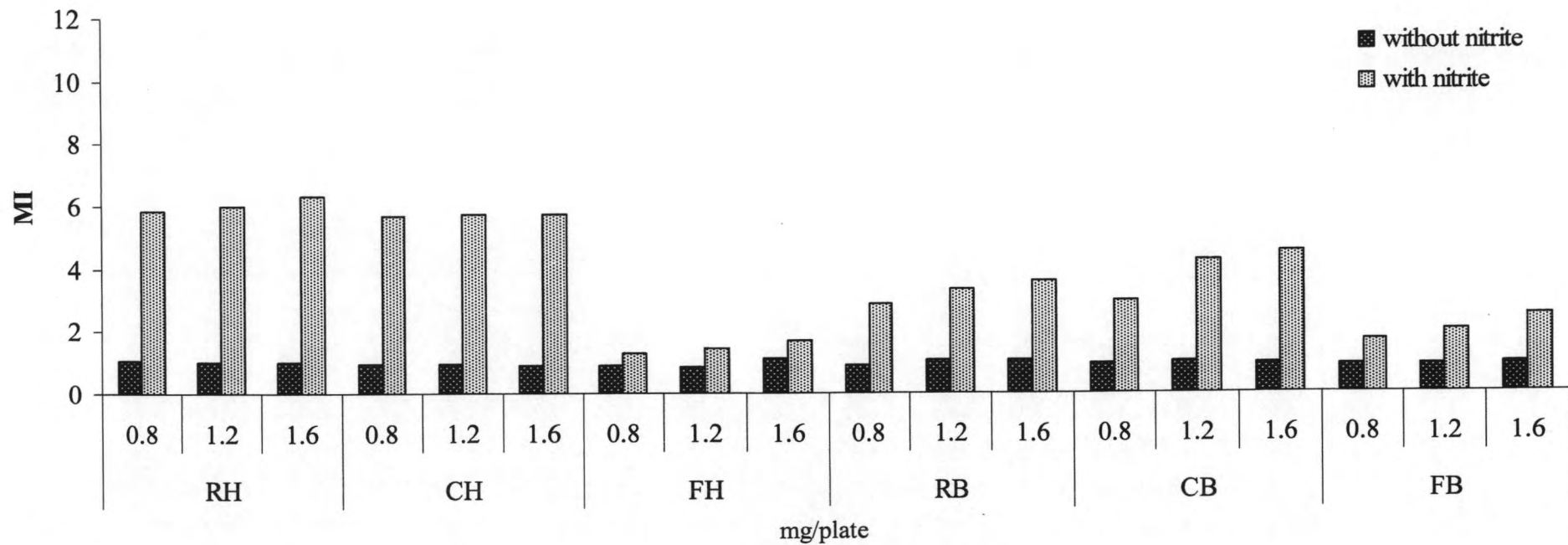


Figure 18 Mutagenicity index of the rice extracts without and with sodium nitrite treatment in acid condition (pH 3.0-3.5) on *S. typhimurium* TA100

4.4 Antimutagenicity of Rice Extracts on Mutagen Formed During Nitrite Treated Chicken Extract

This study revealed the effects of Hom Nil rice and black glutinous rice extracts on the mutagenicity of direct mutagen occurred from the reaction between chicken extract and sodium nitrite on *S. typhimurium* TA98 and TA100 and the results was shown in Figure 19 and 20, and also in Table C-5 (Appendix C). The results showed that the mutagenicity, on *S. typhimurium* TA98 and TA100, of sodium nitrite treated chicken extract was weakly to moderately inhibited by nearly all rice extracts. The enhancing effect on mutagenicity of sodium nitrite treated chicken extract was found in CH toward *S. typhimurium* TA98 and RH toward *S. typhimurium* TA100; however, the enhancing effect was negligible. The inhibitory effect of black glutinous rice extracts seemed to be higher than that of Hom Nil rice extracts. Fermentation seemed to be a good process to increase the antimutagenicity against nitrite treated chicken extract.

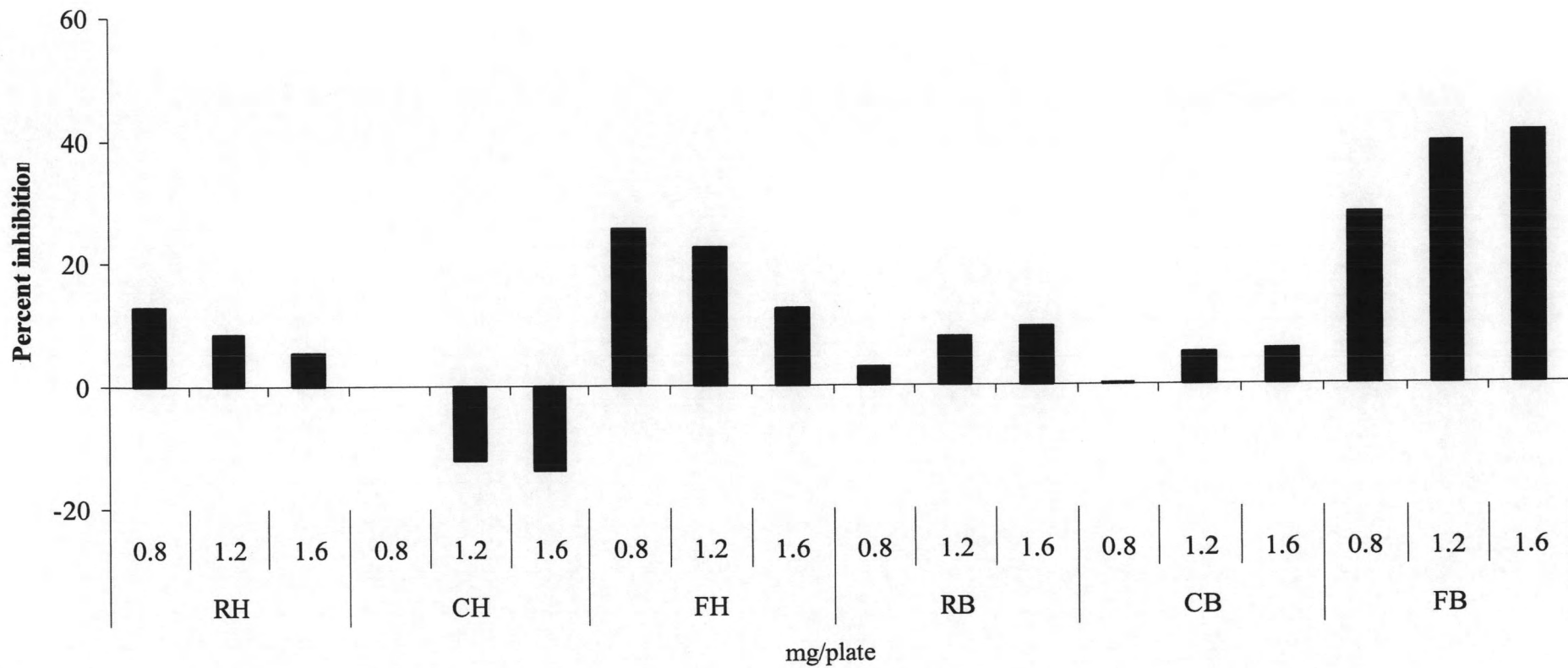


Figure 19 Effect of the rice extracts on the mutagenicity of mutagen formed during sodium nitrite treated chicken extract on *S. typhimurium* TA98

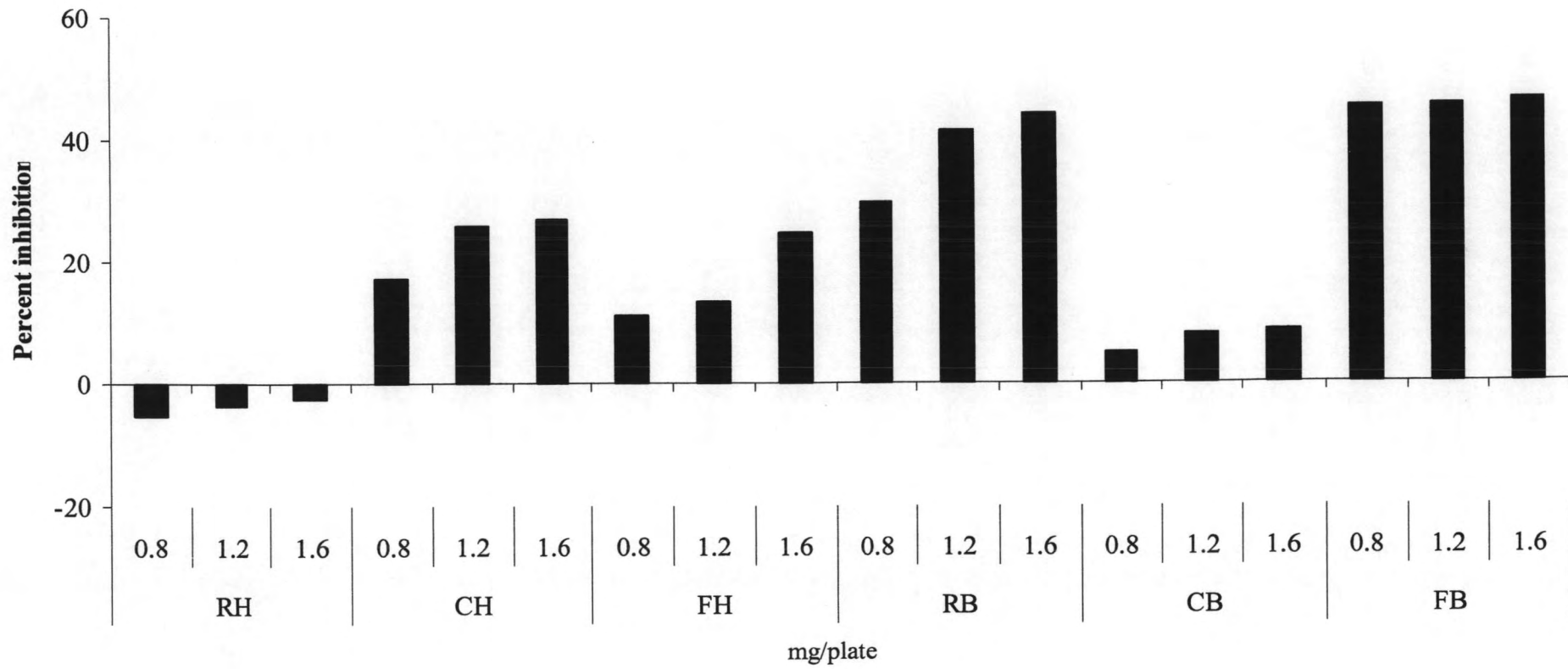


Figure 20 Effect of the rice extracts on the mutagenicity of mutagen formed during sodium nitrite treated chicken extract on *S. typhimurium* TA100

4.5 Antimutagenicity of Rice Extracts on Mutagen Formed During Nitrite Treated 1-Aminopyrene

Figures 21, 22, and Table C-6 (Appendix C) revealed the effects of Hom Nil rice and black glutinous rice extracts on the mutagenicity on *S. typhimurium* TA98 and TA100 of sodium nitrite treated 1-aminopyrene. The results showed that the mutagenicity on *S. typhimurium* TA98 and TA100 of sodium nitrite treated 1-aminopyrene were inhibited by all of the rice extracts with a dose response manner. The moderate to strong inhibiting effects of the rice extracts were obviously found on *S. typhimurium* TA98, while there were only negligible, weak or moderate inhibiting effects on *S. typhimurium* TA 100. However, FH has less inhibiting activity than that of others.

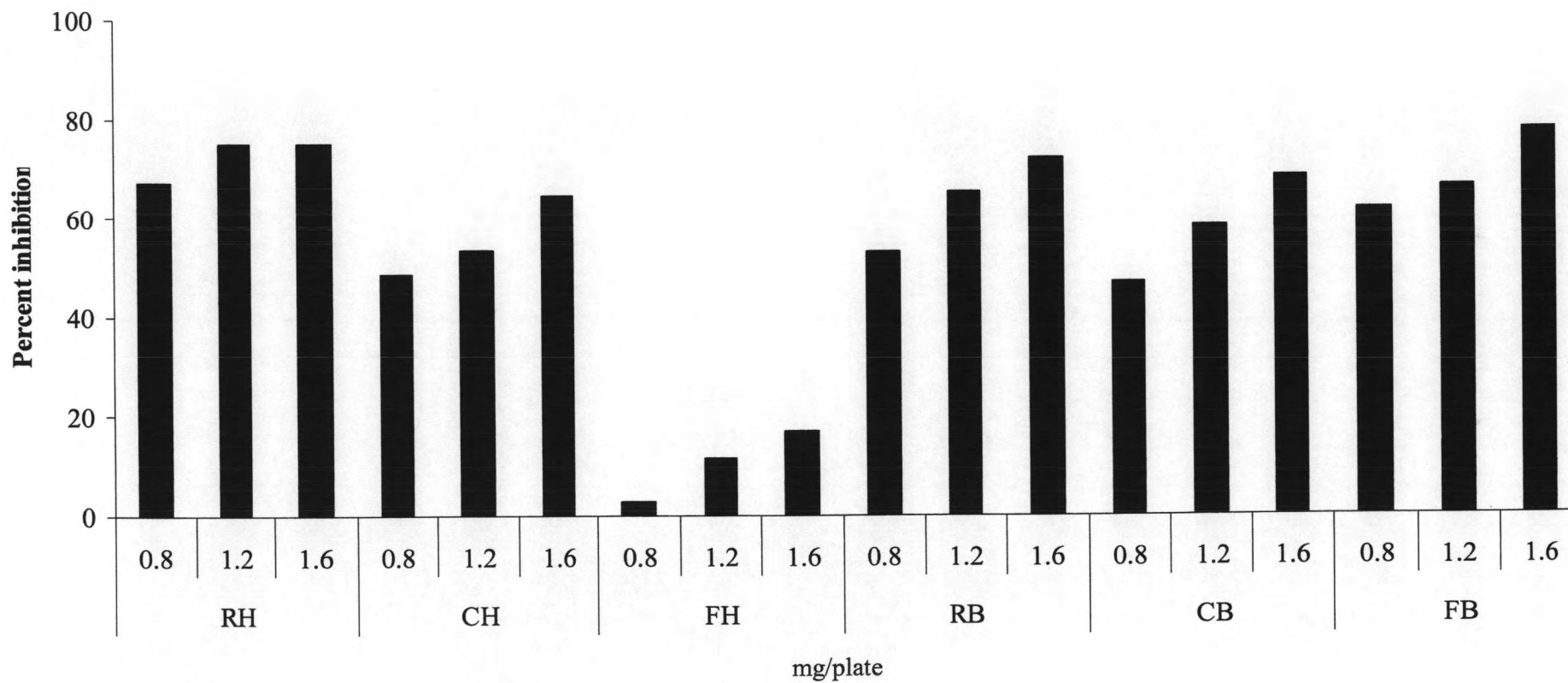


Figure 21 Effect of the rice extracts on the mutagenicity of mutagen formed during sodium nitrite treated 1-aminopyrene on *S. typhimurium* TA98

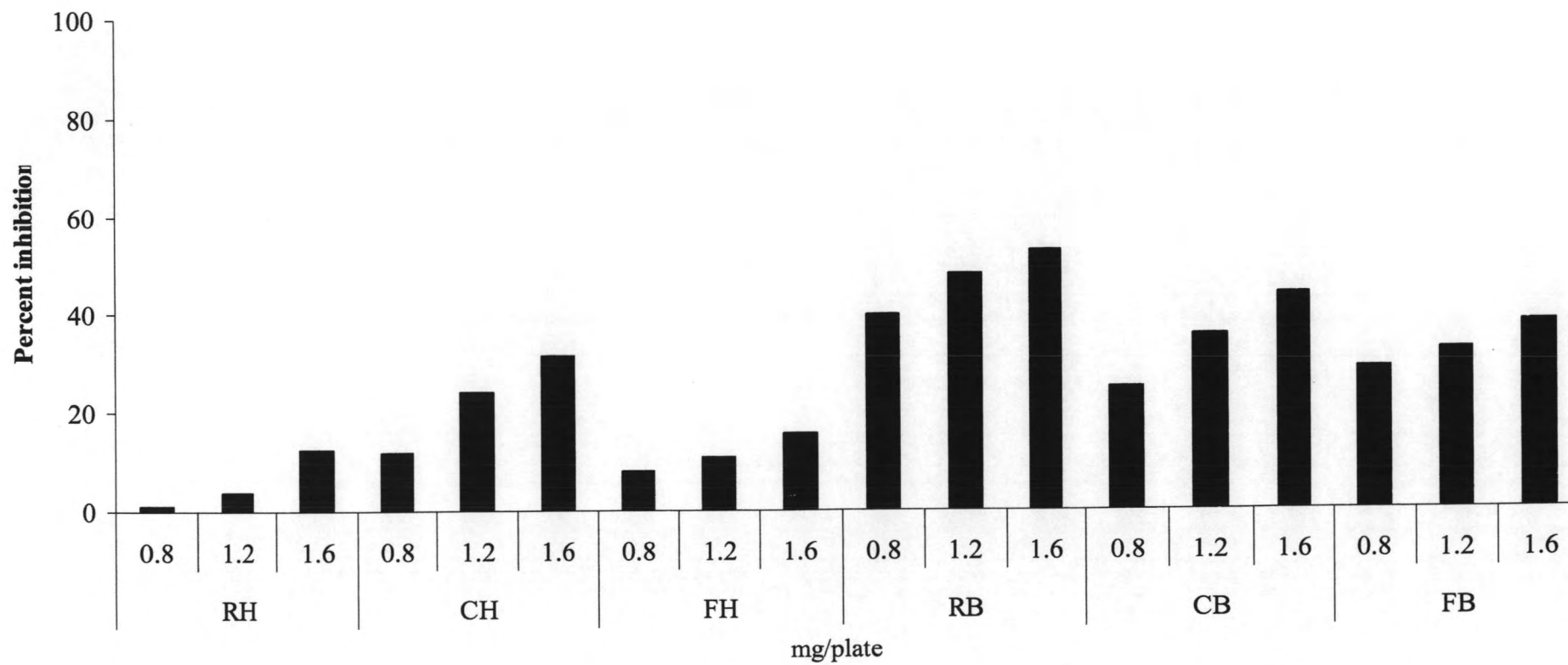


Figure 22 Effect of the rice extracts on the mutagenicity of mutagen formed during sodium nitrite treated 1-aminopyrene on *S. typhimurium* TA100

4.6 Anti-Mutagen Forming Effect of Rice Extracts during Treatment Chicken Extract with Sodium Nitrite

The effects of Hom Nil rice and black glutinous rice extracts on the formation of direct mutagen occurred during the reaction between chicken extract and sodium nitrite was studied. The mutagenicity of the reaction mixtures on *S. typhimurium* TA98 and TA100 were shown in Figures 23 and 24 respectively, and also in Table C-7 (Appendix C). The presence of rice extract, except each of the fermented one, during sodium nitrite treatment of chicken extract increased the revertant colonies of *S. typhimurium* both strains TA98 and TA100.

The expected and observed mutagenicity indices (MI) of the product of sodium nitrite treated chicken extract in the presence of each rice extract on *S. typhimurium* TA98 and TA100 were compared and shown in Tables 9 and 10 respectively. It was found that the observed MI was rather lower than that of the expected MI on both *S. typhimurium* TA98 and TA100. Only the observed MI of sodium nitrite treated chicken extract in the presence of CB (0.8 mg/plate) was higher than the expected MI on *S. typhimurium* TA98; however, it was judged as negligible effect with -3.36 % modification.

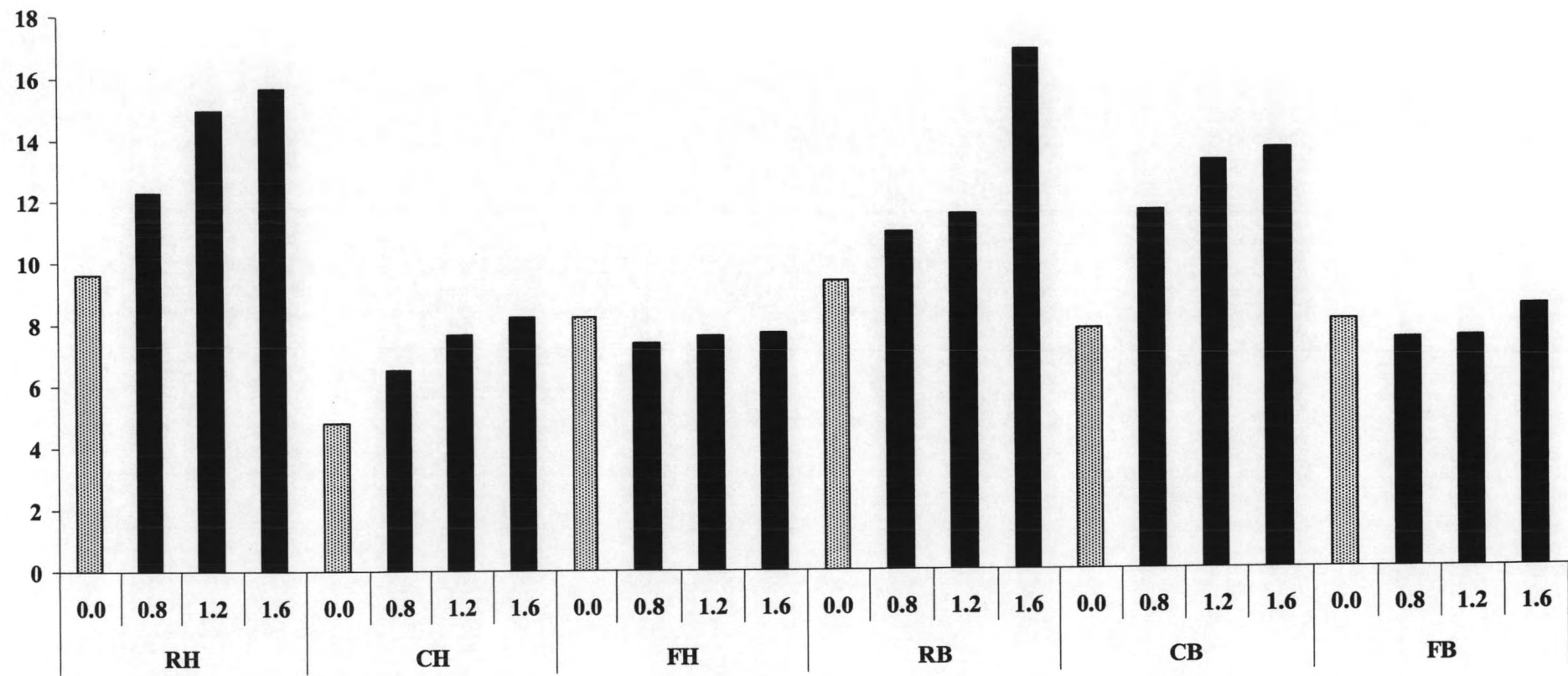


Figure 23 Mutagenicity index of chicken extract treated sodium nitrite in the presence of each rice extract on *S. typhimurium* TA98

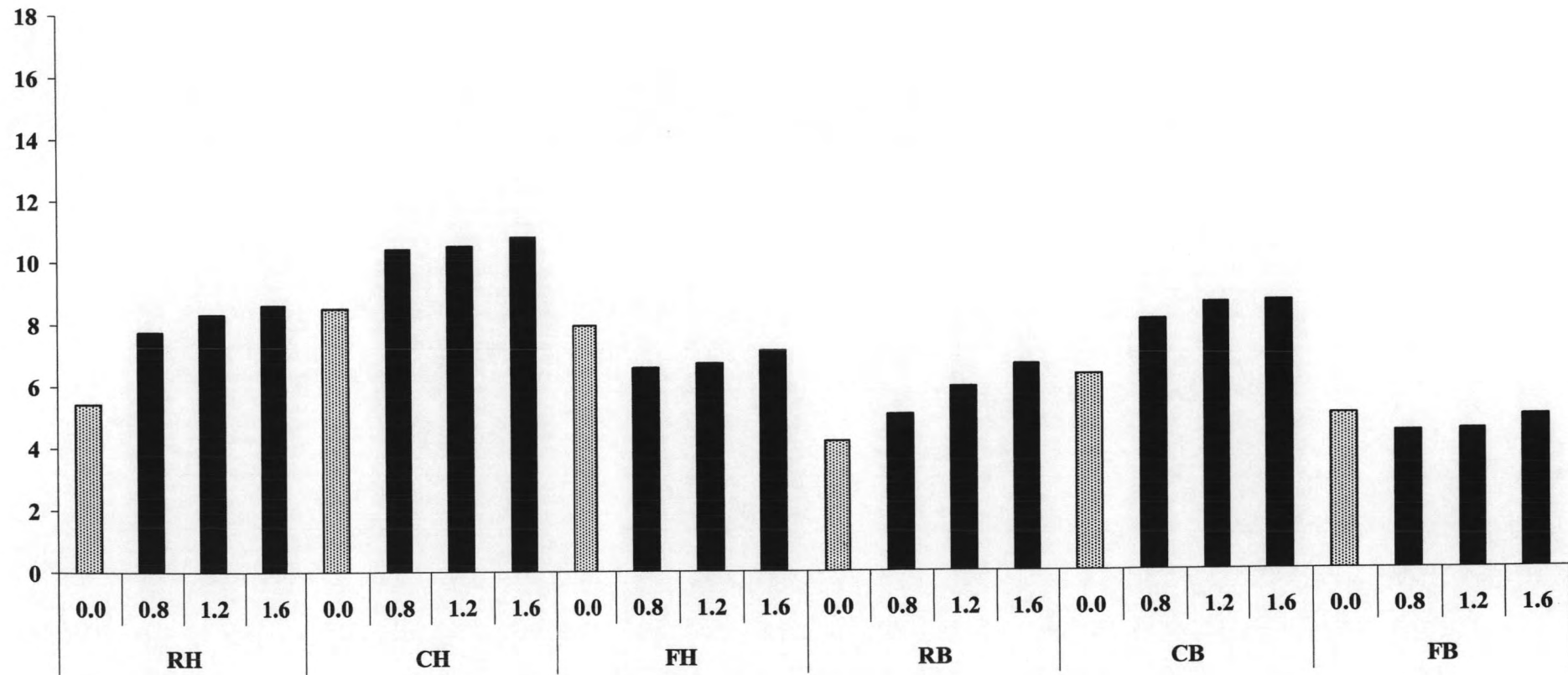


Figure 24 Mutagenicity index of chicken extract treated sodium nitrite in the presence of each rice extract on *S. typhimurium* TA100

Table 9 Antagonistic interaction^a between each rice extract and chicken extract during nitrite treatment on *S. typhimurium* TA98

Rice extract		Mutagenicity Index (MI) ^c				% Modification ^f
Name	Amount ^b (mg/plate)	A sodium nitrite treated chicken extract ^d	B sodium nitrite treated rice extract ^e	Summation of A and B (Expected)	Chicken extract treated with sodium nitrite in the presence of rice extract (Observed)	
Hom Nil rice						
Raw	0.8	9.60	8.98	18.58	12.27	33.98
	1.2	9.60	10.01	19.61	14.93	23.85
	1.6	9.60	10.10	19.70	15.67	20.47
Cooked	0.8	4.83	7.17	12.00	6.53	45.62
	1.2	4.83	8.13	12.96	7.67	40.86
	1.6	4.83	9.07	13.90	8.22	40.86
Fermented	0.8	8.24	1.61	9.85	7.38	25.05
	1.2	8.24	1.85	1.09	7.62	24.47
	1.6	8.24	1.96	10.20	7.71	24.36

Table 9 Antagonistic interaction^a between each rice extract and chicken extract during nitrite treatment on *S. typhimurium* TA98 (continued)

Rice extract		Mutagenicity Index (MI) ^c				% Modification ^f
Name	Amount ^b (mg/plate)	A sodium nitrite treated chicken extract ^d	B sodium nitrite treated rice extract ^e	Summation of A and B (Expected)	Chicken extract treated with sodium nitrite in the presence of rice extract (Observed)	
Black glutinous rice						
Raw	0.8	9.40	5.67	15.07	10.93	27.45
	1.2	9.40	7.38	16.78	11.53	31.27
	1.6	9.40	7.58	16.98	16.87	0.67
Cooked	0.8	7.83	3.40	11.23	11.61	-3.36
	1.2	7.83	6.88	14.71	13.22	10.13
	1.6	4.83	9.07	13.90	8.22	9.64
Fermented	0.8	8.06	1.97	10.03	7.44	25.75
	1.2	8.06	3.10	11.16	7.50	32.77
	1.6	8.06	3.33	11.39	8.50	25.34

^a Difference between the MI of sodium nitrite treated rice extract plus MI of sodium nitrite treated chicken extract and MI of chicken extract treated with sodium nitrite in the presence of rice extract on *S. typhimurium* TA98. ^b Amount per plate of rice extracts. ^c Mutagenicity Index is calculated from the average value of a number of histidine revertants/plate of the rice extracts divided by that of spontaneous revertants. ^d Mutagenicity index of sodium nitrite treated chicken extract in each experiment from Table 15. ^e Mutagenicity index of sodium nitrite treated rice extract from Table 11. ^f Percent modification = $\frac{\text{Expected MI} - \text{Observed MI}}{\text{Expected MI}} \times 100$

Expected MI

Table 10 Antagonistic interaction^a between each rice extract and chicken extract during nitrite treatment on *S. typhimurium* TA100

Rice extract		Mutagenicity Index (MI) ^c				% Modification ^f
Name	Amount ^b (mg/plate)	A sodium nitrite treated chicken extract ^d	B sodium nitrite treated rice extract ^e	Summation of A and B (Expected)	Chicken extract treated with sodium nitrite in the presence of rice extract (Observed)	
Hom Nil rice						
Raw	0.8	5.44	5.86	11.30	7.75	31.40
	1.2	5.44	6.00	11.44	8.33	27.17
	1.6	5.44	6.33	11.77	8.64	26.55
Cooked	0.8	8.53	5.70	14.23	10.44	26.65
	1.2	8.53	5.73	14.26	10.52	26.23
	1.6	8.53	5.76	14.29	10.80	24.41
Fermented	0.8	7.96	1.25	9.21	6.56	28.71
	1.2	7.96	1.44	9.40	6.73	28.35
	1.6	7.96	1.68	9.64	7.14	25.87

Table 10 Antagonistic interaction^a between each rice extract and chicken extract during nitrite treatment on *S. typhimurium* TA100 (continued)

Rice extract		Mutagenicity Index (MI) ^c				% Modification ^f
Name	Amount ^b (mg/plate)	A sodium nitrite treated chicken extract ^d	B sodium nitrite treated rice extract ^e	Summation of A and B (Expected)	Chicken extract treated with sodium nitrite in the presence of rice extract (Observed)	
Black glutinous rice						
Raw	0.8	4.17	2.89	7.06	5.05	28.48
	1.2	4.17	3.37	7.54	5.93	21.37
	1.6	4.17	3.63	7.80	6.65	14.77
Cooked	0.8	6.33	2.99	9.32	8.08	13.32
	1.2	6.33	4.27	10.60	8.60	18.86
	1.6	6.33	4.49	10.82	8.65	20.09
Fermented	0.8	5.02	1.70	6.72	4.44	33.90
	1.2	5.02	2.00	7.02	4.46	36.42
	1.6	5.02	2.48	7.50	4.91	34.58

^a Difference between the MI of sodium nitrite treated rice extract plus MI of sodium nitrite treated chicken extract and MI of chicken extract treated with sodium nitrite in the presence of rice extract on *S. typhimurium* TA100. ^b Amount per plate of rice extracts. ^c Mutagenicity Index is calculated from the average value of a number of histidine revertants/plate of the rice extracts divided by that of spontaneous revertants. ^d Mutagenicity index of sodium nitrite treated chicken extract in each experiment from Table 16. ^e Mutagenicity index of sodium nitrite treated rice extract from Table 12. ^f Percent modification = $\frac{\text{Expected MI} - \text{Observed MI}}{\text{Expected MI}} \times 100$

Expected MI

4.7 Anti-Mutagen Forming Effect of Rice Extracts during Treatment

1-Aminopyrene with Sodium Nitrite

The effects of Hom Nil rice and black glutinous rice extracts on the formation of direct mutagen occurred during the reaction between 1-aminopyrene and sodium nitrite on *S. typhimurium* TA98 and TA100 were shown in Figures 25 and 26 respectively, and also in Table C-8 (Appendix C). After sodium nitrite treatment, 1-aminopyrene in the presence of each rice extract was positive mutagenic with a dose-response manner on both *S. typhimurium* strains. The presence of nearly all rice extracts during nitrite treatment of 1-aminopyrene increased number of revertant colonies of *S. typhimurium* TA98 and TA100. Exception was found in all doses of CH on both tester strains, high dose (1.6 mg/plate) of RH on TA98 and all doses of CB on TA100. High dose (1.6 mg/plate) of RH and all doses of CH inhibited the number of *S. typhimurium* TA98 revertants induced by sodium nitrite treated 1-aminopyrene, while all doses of CH and CB decreased the mutation of *S. typhimurium* TA100. The results indicated that the extracts from cooked rice might reduced the formation of mutagen occurred during the reaction between 1-aminopyrene and sodium nitrite.

Comparison between the expected and observed mutagenicity indices (MI) of the sodium nitrite treated 1-aminopyrene in the presence of each rice extract on *S. typhimurium* TA98 and TA100 were compared and shown in Tables 11 and 12 respectively. It was found that nearly all observed MIs of *S. typhimurium* TA98 induced by the sodium nitrite treated 1-aminopyrene in the presence of each rice extract were higher than the expected MIs; thus, the samples could enhance the mutation of the tester strain. On TA 100, the observed MI of the sodium nitrite treated 1-aminopyrene in the presence of each rice extract were less than the expected MI; this results showed weak to moderate inhibitory effects. Exception was found at all doses of FH on *S. typhimurium* TA100 that showed weak to strong mutagenicity enhancing effects while FB had negligible effect. These results suggested that only CH had the inhibiting effect against sodium nitrite treated 1-aminopyrene.

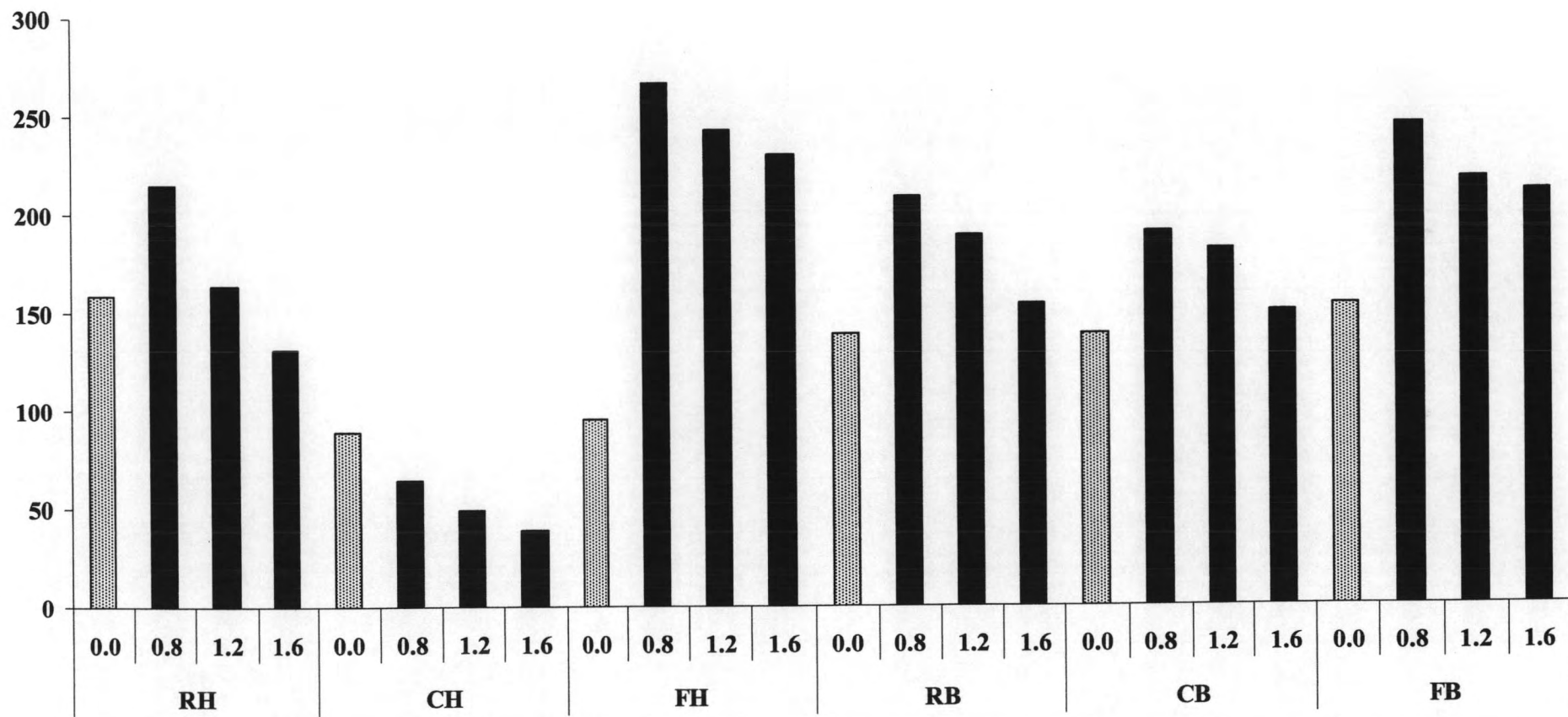


Figure 25 Mutagenicity index of 1-aminopyrene treated sodium nitrite in the presence of each rice extract on *S. typhimurium* TA98

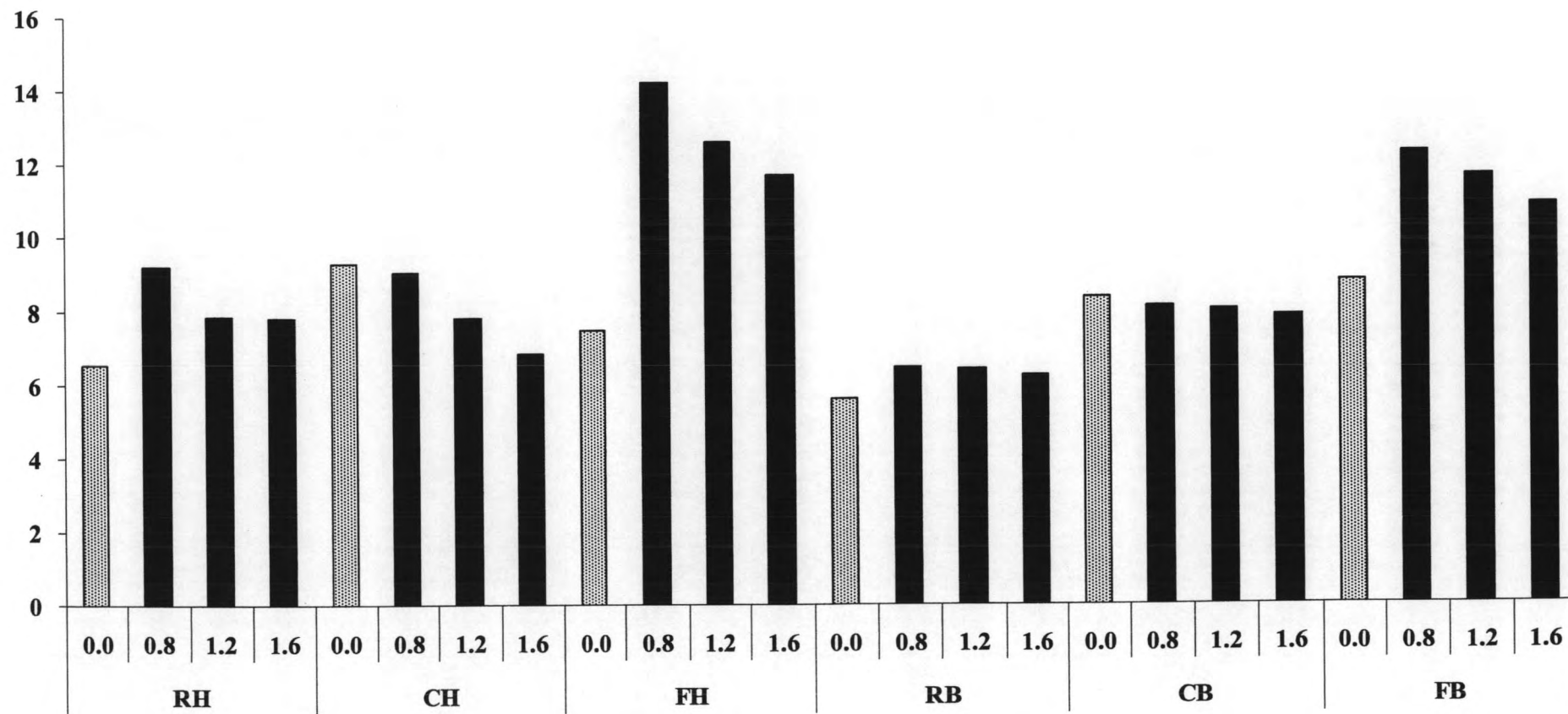


Figure 25 Mutagenicity index of 1-aminopyrene treated sodium nitrite in the presence of each rice extract on *S. typhimurium* TA100

Table 11 Antagonistic interaction^a between each rice extract and 1-aminopyrene during nitrite treatment on *S. typhimurium* TA98

Rice extract		Mutagenicity Index (MI) ^c				% Modification ^f
Name	Amount ^b (mg/plate)	A sodium nitrite treated 1-aminopyrene ^d	B sodium nitrite treated rice extract ^e	Summation of A and B (Expected)	1-aminopyrene treated with sodium nitrite in the presence of rice extract (Observed)	
Hom Nil rice						
Raw	0.8	158.94	8.98	167.92	215.38	-28.26
	1.2	158.94	10.01	168.95	163.50	3.22
	1.6	158.94	10.10	169.04	130.81	22.61
Cooked	0.8	88.70	7.17	95.87	64.57	32.65
	1.2	88.70	8.13	96.83	48.91	49.48
	1.6	88.70	9.07	97.77	38.96	30.16
Fermented	0.8	94.91	1.61	96.52	266.86	-176.49
	1.2	94.91	1.85	96.76	243.14	-151.28
	1.6	94.91	1.96	96.87	230.41	-137.86

Table 11 Antagonistic interaction^a between each rice extract and 1-aminopyrene during nitrite treatment on *S. typhimurium* TA98 (continued)

Rice extract		Mutagenicity Index (MI) ^c				% Modification ^f
Name	Amount ^b (mg/plate)	A sodium nitrite treated 1-aminopyrene ^d	B sodium nitrite treated rice extract ^e	Summation of A and B (Expected)	1-aminopyrene treated with sodium nitrite in the presence of rice extract (Observed)	
Black glutinous rice						
Raw	0.8	139.27	5.67	144.94	208.53	-43.88
	1.2	139.27	7.38	146.65	188.67	-28.65
	1.6	139.27	7.58	146.85	154.33	-5.10
Cooked	0.8	138.89	3.40	142.29	190.11	-33.60
	1.2	138.89	6.88	145.77	181.42	-24.45
	1.6	138.89	7.23	146.12	150.11	-2.72
Fermented	0.8	153.33	1.97	155.30	245.53	-58.10
	1.2	153.33	3.10	156.43	217.47	-39.02
	1.6	153.33	3.33	156.66	211.40	-34.94

^a Difference between the MI of sodium nitrite treated rice extract plus MI of sodium nitrite treated 1-aminopyrene and MI of 1-aminopyrene treated with sodium nitrite in the presence of rice extract on *S. typhimurium* TA98. ^b Amount per plate of rice extracts. ^c Mutagenicity Index is calculated from the average value of a number of histidine revertants/plate of the rice extracts divided by that of spontaneous revertants. ^d Mutagenicity index of sodium nitrite treated 1-aminopyrene in each experiment from Table 19. ^e Mutagenicity index of sodium nitrite treated rice extract from Table 12. ^f Percent modification = $\frac{\text{Expected MI} - \text{Observed MI}}{\text{Expected MI}} \times 100$

Expected MI

Table 12 Antagonistic interaction^a between each rice extract and 1-aminopyrene during nitrite treatment on *S. typhimurium* TA100

Rice extract		Mutagenicity Index (MI) ^c				% Modification ^f
Name	Amount ^b (mg/plate)	A sodium nitrite treated 1-aminopyrene ^d	B sodium nitrite treated rice extract ^e	Summation of A and B (Expected)	1-aminopyrene treated with sodium nitrite in the presence of rice extract (Observed)	
Raw	0.8	6.53	5.86	12.39	9.20	25.77
Hom Nil rice	1.2	6.53	6.00	12.53	7.84	37.45
	1.6	6.53	6.33	12.86	7.79	39.39
Cooked	0.8	9.31	5.70	15.01	9.01	39.77
	Hom Nil rice	1.2	9.31	5.73	15.04	7.80
Hom Nil rice	1.6	9.31	5.76	15.07	6.83	54.71
	Fermented	0.8	7.46	1.25	8.71	14.20
Hom Nil rice	1.2	7.46	1.44	8.90	12.62	-41.78
	1.6	7.46	1.68	9.14	11.70	-27.95

Table 12 Antagonistic interaction^a between each rice extract and 1-aminopyrene during nitrite treatment on *S. typhimurium* TA100 (continued)

Rice extract		Mutagenicity Index (MI) ^c				% Modification ^f
Name	Amount ^b (mg/plate)	A sodium nitrite treated 1-aminopyrene ^d	B sodium nitrite treated rice extract ^e	Summation of A and B (Expected)	1-aminopyrene treated with sodium nitrite in the presence of rice extract (Observed)	
Raw	0.8	5.60	2.89	8.49	6.43	24.27
black glutinous rice	1.2	5.60	3.37	8.97	6.39	28.77
	1.6	5.60	3.63	9.23	6.22	32.63
Cooked	0.8	8.35	2.99	11.34	8.11	28.46
black glutinous rice	1.2	8.35	4.27	12.62	8.01	36.53
	1.6	8.35	4.49	12.84	7.85	38.84
Fermented	0.8	8.77	1.70	10.47	12.33	-17.75
black glutinous rice	1.2	8.77	2.00	10.77	11.96	-8.59
	1.6	8.77	2.48	11.25	10.87	3.38

^a Difference between the MI of sodium nitrite treated rice extract plus MI of sodium nitrite treated 1-aminopyrene and MI of 1-aminopyrene treated with sodium nitrite in the presence of rice extract on *S. typhimurium* TA100. ^b Amount per plate of rice extracts. ^c Mutagenicity Index is calculated from the average value of a number of histidine revertants/plate of the rice extracts divided by that of spontaneous revertants. ^d Mutagenicity index of sodium nitrite treated 1-aminopyrene in each experiment from Table 19. ^e Mutagenicity index of sodium nitrite treated rice extract from Table 12. ^f Percent modification = $\frac{\text{Expected MI} - \text{Observed MI}}{\text{Expected MI}} \times 100$

Expected MI