CHAPTER IV

RESULTS

The aim of this study was to determine whether 4 kinds of mushrooms namely, *Agaricus bisporus*, *Lentinus edodes*, *Pleurotus ostreatus* and *Pleurotus abalones* under 3 forms (fresh, blanched, and fermented) could modulate the *in vivo* induction of mutation and mitotic recombination in somatic cells by urethane. The experiment was conducted to evaluate the effect of urethane and all mushroom extracts on survival of flies. Simultaneous administration of urethane along with each mushroom sample to 3-day-old larvae was firstly performed to investigate the antimutagenicity against urethane. Secondly, pre-feeding of each sample before administration of urethane was done in order to observe the effect of mushroom extracts on biotransformation of urethane in early stage of larvae. All experiments were performed twice in order to see the reproducibility of the modulating effect of each sample. Distilled water was used as a negative control and produced the spontaneous mutation of each experiment. The results of wing analysis are given on the three categories of small single spots (1 or 2 cells), large single spots (3 or more cells), and twin spots as well as total spots. In addition, the mutation index and a numbers of surviving flies are examined.

4.1 Preliminary Study: Survival of Adult Flies and Mutagenicity of Mushroom Extracts

4.1.1 Survival of Adult Flies

4.1.1.1 Fed on mutagenic testing sample mediums

Table 5 shows a number of surviving flies derived from larvae fed on the positive control (standard medium containing urethane), negative control (standard medium), and mutagenic testing sample medium (mushroom extract medium). Two-milliliter of each sample; namely, fresh button mushroom (FBM), blanched button mushroom (BBM), fermented button mushroom (PBM), fresh shiitake mushroom (FSM), blanched shiitake mushroom (BSM), fermented shiitake mushroom (PSM), fresh oyster mushroom (FOM), blanched oyster mushroom (BOM), fermented oyster mushroom (POM), fresh abalone mushroom (FAM), blanched abalone mushroom (BAM) and fermented abalone mushroom (PAM) was substituted for water in the standard medium as described in the previous chapter.

Percentages of surviving flies fed on negative control was within the range of 76 - 95 (trial 1), and 76 - 92 (trial2) and surviving flies fed on positive control was within the range 69- 80 (trial 1) and 69- 89 (trial 2), respectively; while that obtained from larvae fed on the button mushroom medium was 70- 90, shiitake mushroom medium was 84- 95, oyster mushroom medium was 74- 98, and abalone mushroom medium was 88- 96.

Table 5 Survival rates of flies fed on the mutagenic testing mushroom extracts

Type of medium		% of survival	
Period 1[a]	Period 2[b]	Trial 1	Trial 2
Button mushroom			
Standard	Standard (negative control)	80	76
Standard	Positive control	72	69
Standard	Mutagenic testing samples		
	- Fresh	76	74
	- Blanched	90	84
	- Fermented	77	70
Shiitake mushroom			
Standard	Standard (negative control)	95	92
Standard	Positive control	75	80
Standard	Mutagenic testing samples		
	- Fresh	88	89
	- Blanched	89	95
	- Fermented	84	86
Oyster mushroom			
Standard	Standard (negative control)	82	90
Standard	Positive control	80	89
Standard	Mutagenic testing samples		
	- Fresh	95	94
	- Blanched	98	92
	- Fermented	74	95
Abalone mushroom			
Standard	Standard (negative control)	76	87
Standard	Positive control	69	75
Standard	Mutagenic testing samples		
	- Fresh	96	94
	- Blanched	88	93
	- Fermented	89	90

a = Mating parent flies fed on standard medium until 3 day- old larvae.

b= 3- day old larvae were collected and transferred to another media.

4.1.1.2 Survival of adult flies in simultaneous feeding studies

Table 6 shows a number of surviving flies derived from larvae fed on positive control (standard medium containing urethane), standard medium (negative control medium), sample medium (only 1 ml of distilled water was substituted with the mushroom extract) and experimental medium (mushroom extract medium containing urethane).

In button mushroom extracts: Percentages of surviving flies fed on the negative control medium were 80 (trial 1) and 91 (trial 2), and survival rate of flies fed on positive control medium were 73% (trial 1) and 78% (trial 2); while that obtained from larvae fed on the fresh sample medium were 82% (trial 1) and 94% (trial2), blanched sample medium were 76% (trial 1) and 78% (trial 2), fermented sample medium were 72% (trial 1)and 87% (trial 2). And the larvae fed on the fresh experimental medium were 78% (trial 1) and 79% (trial 2), blanched experimental medium were 82% (trial 1) and 89% (trial 2), and fermented experimental medium were 73% (trial 1) and 83% (trial 2).

In shiitake mushroom extracts: Percentages of surviving flies fed on the negative control medium were 82 (trial 1) and 89 (trial 2), and survival rate of flies fed on positive control medium were 73% (trial 1) and 83% (trial 2); while that obtained from larvae fed on the fresh sample medium were 68% (trial 1) and 63% (trial2), blanched sample medium were 62% (trial 1) and 76% (trial 2), fermented sample medium were 60% (trial 1)and 63% (trial 2). And the larvae fed on the fresh experimental medium were 60% (trial 1) and 70% (trial 2), blanched experimental medium were 62% (trial 1) and 70% (trial 2), and fermented experimental medium were 55% (trial 1) and 60% (trial 2).

In oyster mushroom extracts: Percentages of surviving flies fed on the negative control medium were 79 (trial 1) and 81 (trial 2), and survival rate of flies fed on positive control medium were 76% (trial 1) and 78% (trial 2); while that obtained from larvae fed on the fresh sample medium were 70% (trial 1) and 75% (trial2), blanched sample medium were 71% (trial 1) and 78% (trial 2), fermented sample medium were 68% (trial 1)and 73% (trial 2). And the larvae fed on the fresh experimental medium were 71% (trial 1) and 73% (trial 2), blanched experimental

medium were 66% (trial 1) and 68% (trial 2), and fermented experimental medium were 63% (trial 1) and 67% (trial 2).

In abalone mushroom extracts: Percentages of surviving flies fed on the negative control medium were 78 (trial 1) and 82 (trial 2), and survival rate of flies fed on positive control medium were 74% (trial 1) and 77% (trial 2); while that obtained from larvae fed on the fresh sample medium were 73% (trial 1) and 78% (trial2), blanched sample medium were 76% (trial 1) and 68% (trial 2), fermented sample medium were 77% (trial 1) and 83% (trial 2). And the larvae fed on the fresh experimental medium were 68% (trial 1) and 74% (trial 2), blanched experimental medium were 66% (trial 1) and 69% (trial 2), and fermented experimental medium were 73% (trial 1) and 69% (trial 2).

All samples surviving rate of flies more than 50%, it suggested that all samples were not toxic to larvae and flies. Sample medium seemed to show survival rate higher than that group treated with corresponding medium containing urethane.

Table 6 Survival rate of adult flies in simultaneous study obtained from 3- day old larvae (*mwh+/+flr*^{3}) introduced to either the sample mediums or the experimental mediums

Type of medium		% of survival	
Period 1[a]	Period 2[b]	Trial 1	Trial 2
Button mushroom			
Standard	Standard (negative control)	80	91
Standard	Positive control	73	78
Standard	Samples		
	- Fresh	82	94
	- Blanched	76	78
	- Fermented	72	87
Standard	Experimental		
	- Fresh	78	79
	- Blanched	82	89
	- Fermented	73	83
Shiitake mushroom			
Standard	Standard (negative control)	82	89
Standard	Positive control	73	83
Standard	Samples		
	- Fresh	68	63
	- Blanched	62	76
	- Fermented	60	63
Standard	Experimental		
	- Fresh	60	70
	- Blanched	62	70
	- Fermented	55	60

Table 6 (Continued)

Type of medium		% of survival	
Period 1	Period 2	Trial 1	Trial 2
Oyster mushroom			
Standard	Standard (negative control)	79	81
Standard	Positive control	76	78
Standard	Samples		
	- Fresh	70	75
	- Blanched	71	78
	- Fermented	68	73
Standard	Experimental		
	- Fresh	71	73
	- Blanched	66	68
	- Fermented	63	67
Abalone mushroom			
Standard	Standard (negative control)	78	82
Standard	Positive control	74	77
Standard	Samples		
	- Fresh	73	78
	- Blanched	76	68
	- Fermented	77	82
Standard	Experimental		
	- Fresh	68	74
	- Blanched	66	69
	- Fermented	73	69

a = Mating parent flies fed on standard medium until 3 day- old larvae.

b= 3- day old larvae were collected and transferred to another media.

4.1.1.3 Survival of adult flies in pre- feeding studies

Table 7 shows the survival rate of adult flies of the pre- feeding study. Parent mating flies were fed on the sample medium, after that 3- day- old larvae switching to either standard medium (sample control type I experiment) or sample medium (sample control type II experiment) or standard medium containing urethane (type I experiment) or experimental medium (type II experiment) until the larvae became to be adult flies. Because the extraction from 100 g/ml of shiitake mushroom was toxic to the parent flies; therefore the concentration was reduced to 5 g/ml and was used for the experiment. The survival rates of adult flies that obtained from pre- feeding experiment are shown in Table 7.

In button mushroom extracts: Percentages of surviving flies fed on the negative control medium were 91 (trial 1) and 94 (trial 2), and survival rates of flies fed on positive control medium were 95% (trial 1) and 97% (trial 2); while that obtained from larvae fed on the type I fresh experimental medium were 91% (trial 1) and 93% (trial2), type I blanched experimental medium were 85% (trial 1) and 90% (trial 2), type I fermented experimental medium were 69% (trial 1)and 71% (trial 2). And surviving rates of the larvae fed on type II fresh experimental medium were 82% in both trials, type II blanched experimental medium were 88% (trial 1) and 90% (trial 2), and type II fermented experimental medium were 732 (trial 1) and 74% (trial 2).

In shiitake mushroom extracts: Percentages of surviving flies fed on the negative control medium were 80 (trial 1) and 82 (trial 2), and survival rates of flies fed on positive control medium were 75% (trial 1) and 72% (trial 2); while that obtained from larvae fed on the type I fresh experimental medium were 73% (trial 1) and 77% (trial2), type I blanched experimental medium were 60% (trial 1) and 55% (trial 2), type I fermented experimental medium were 66% (trial 1)and 58% (trial 2). And surviving rates of the larvae fed on type II fresh experimental medium were 59% (trial 1) and 61% (trial 2), type II blanched experimental medium were 68% (trial 1) and 71% (trial 2), and type II fermented experimental medium were 62% (trial 1) and 58% (trial 2).

In oyster mushroom extracts: Percentages of surviving flies fed on the negative control medium were 75 (trial 1) and 87 (trial 2), and survival rates of flies fed on positive control medium were 69% (trial 1) and 75% (trial 2); while that obtained from larvae fed on the type I fresh experimental medium were 60% (trial 1) and 68% (trial2), type I blanched experimental medium were 61% (trial 1) and 69% (trial 2), type I fermented experimental medium were 62% (trial 1)and 71% (trial 2). And surviving rates of the larvae fed on type II fresh experimental medium were 65% (trial 1) and 77% (trial 2), type II blanched experimental medium were 72% (trial 1) and 81% (trial 2), and type II fermented experimental medium were 71% (trial 1) and 81% (trial 2).

In abalone mushroom extracts: Percentages of surviving flies fed on the negative control medium were 83 (trial 1) and 92 (trial 2), and survival rates of flies fed on positive control medium were 76% (trial 1) and 84% (trial 2); while that obtained from larvae fed on the type I fresh experimental medium were 71% (trial 1) and 80% (trial2), type I blanched experimental medium were 69% (trial 1) and 80% (trial 2), type I fermented experimental medium were 68% (trial 1)and 73% (trial 2). And surviving rates of the larvae fed on type II fresh experimental medium were 71% (trial 1) and 77% (trial 2), type II blanched experimental medium were 70% (trial 1) and 73% (trial 2), and type II fermented experimental medium were 67% (trial 1) and 75% (trial 2).

Table 7 Survival rates of adult flies obtained from 100 larvae (*mwh+/+flr*^{3}) pre-fed on the sample medium for 3 days and followed by either the positive medium (type I experiment) or the experimentaln media containing 20 mM URE (type II experiment).

Type of medium		% of survival	
Period 1[a]	Period 2[b]	Trial 1	Trial 2
Button mushroom			
Standard	Standard (negative control)	91	94
Standard	Positive control	95	97
Type I experiment			
Samples			
- Fresh	Standard	93	97
- Blanched	Standard	82	86
- Fermented	Standard	69	72
Samples			
- Fresh	Positive control	91	93
- Blanched	Positive control	85	90
- Fermented	Positive control	69	71
Type II experiment			
Samples	Samples		
- Fresh	- Fresh	79	84
- Blanched	- Blanched	79	83
- Fermented	- Fermented	73	72
Samples	Experimental		
- Fresh	- Fresh	82	82
- Blanched	- Blanched	88	90
- Fermented	- Fermented	72	74
Shiitake mushroom[c]			
Standard	Standard (negative control)	80	82
Standard	Positive control	75	72
Type I experiment			
Samples			
- Fresh	Standard	72	74
- Blanched	Standard	73	80
- Fermented	Standard	68	71

Table 7 (Continued)

Type of medium		% of survival	
Period 1[a]	Period 2[b]	Trial 1	Trial 2
Type I experiment			
Samples			
- Fresh	Positive control	73	77
- Blanched	Positive control	60	55
- Fermented	Positive control	66	58
Type II experiment			
Samples	Samples		
- Fresh	- Fresh	78	76
- Blanched	- Blanched	76	79
- Fermented	- Fermented	70	73
Samples	Experimental		
- Fresh	- Fresh	59	61
- Blanched	- Blanched	68	71
- Fermented	- Fermented	62	58
Oyster mushroom			
Standard	Standard (negative control)	75	87
Standard	Positive control	69	75
Type I experiment			
Samples			
- Fresh	Standard	66	72
- Blanched	Standard	84	94
- Fermented	Standard	88	92
Samples			
- Fresh	Positive control	60	68
- Blanched	Positive control	61	69
- Fermented	Positive control	62	71
Type II experiment			
Samples	Samples		
- Fresh	- Fresh	65	77
- Blanched	- Blanched	72	81
- Fermented	- Fermented	71	81

Table 7 (Continued)

Type of medium		% of survival	
Period 1[a]	Period 2[b]	Trial 1	Trial 2
Abalone mushroom			
Standard	Standard (negative control)	83	92
Standard	Positive control	76	84
Type I experiment			
Samples			
- Fresh	Standard	78	84
- Blanched	Standard	72	81
- Fermented	Standard	76	83
Samples			
- Fresh	Positive control	71	80
- Blanched	Positive control	69	80
- Fermented	Positive control	68	73
Type II experiment			
Samples	Samples		
- Fresh	- Fresh	75	86
- Blanched	- Blanched	80	82
- Fermented	- Fermented	74	79
Samples	Experimental		
- Fresh	- Fresh	71	77
- Blanched	- Blanched	70	73
- Fermented	- Fermented	67	75

a = Mating parent flies fed on any media until 3 day- old larvae.

b= 3- day old larvae were collected and transferred to another media.

c= Shiitake mushroom extracts in 5% w/v substituted for water and mixed with other components.

4.1.2 Mutagenicity of mushroom extracts

The data shows in Table 8 indicated that all the samples did not express their genotoxic activity since a numbers of spots per wing were not statistically different from that of control and the survival of adult flies fed on each mutagenic sample medium was higher than 50%.

Table 8 Mutagenicity of the mushroom extracts reported as wing spot induction on *Drosophila melanogaster* derived from 100 trans-heterozygous *mwh+/+flr*^{3} larvae of the improved high bioactivation cross fed on mutagenic sample medium.

Type of medium		Spot per wing[a] (No. of spots from 40 wings)							
		Trial 1				Trial 2			
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0
Button mushroom									
Standard	Standard (negative control)	0.25(10)	0	0	0.25(10)	0.20(8)	0	0.03(1)	0.23(9)
Standard	Positive control	7.88(315)+	1.75(70)+	0.40(16)+	10.03(401)+	7.45(298)+	2.15(86)+	0.65(26)+	10.25(410)+
Standard	Mutagenic samples								
	- Fresh	0.25(10)	0.10(4)-	0-	0.35(14)-	0.18(7)-	0-	0-	0.18(7)-
	- Blanched	0.10(4)-	0.03(1)-	0-	0.13(5)-	0.13(5)-	0-	0-	0.13(5)-
	- Fermented	0.13(5)-	0.03(1)-	0-	0.15(6)-	0.13(5)-	0-	0-	0.13(5)-
Shiitake mushroom									
Standard	Standard (negative control)	0.10(4)	0.05(2)	0.03(1)	0.18(7)	0	0.13(5)	0	0.13(5)
Standard	Positive control	6.63(265)+	3.55(142)+	1.23(59)+	11.40(456)+	4.15(166)+	3.15(126)+	0.90(36)+	8.20(328)+

Table 8 (Continued)

Type of medium		Spot per wing[a] (No. of spots from 40 wings)							
		Trial 1				Trial 2			
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0
Standard	Mutagenic samples								
	- Fresh	0.05(2)-	0-	0-	0.05(2)-	0.08(3)-	0.03(1)-	0-	0.10(4)-
	- Blanched	0.10(4)-	0.05(2)-	0.03(1)-	0.18(7)	0-	0.03(1)-	0-	0.03(1)-
	- Fermented	0.05(2)-	0-	0-	0.05(2)-	0-	0.05(2)-	0.03(1)-	0.08(3)-
Oyster mushroom									
Standard	Standard (negative control)	0	0.13(5)	0	0.13(5)	0.25(10)	0.08(3)	0.03(1)	0.35(14)
Standard	Positive control	4.15(166)+	3.15(126)+	0.90(36)+	8.20(328)+	5.0(200)+	3.58(143)+	1.08(43)+	9.65(386)+
Standard	Mutagenic samples								
	- Fresh	0.10(4)-	0-	0-	0.10(4)-	0.08(3)-	0.05(2)-	0.03(1)-	0.15(6)-
	- Blanched	0.08(3)-	0.05(2)-	0-	0.13(5)-	0.13(5)-	0.05(2)-	0-	0.18(7)-
	- Fermented	0.05(2)-	0-	0-	0.05(2)-	0.18(7)-	0.05(2)-	0-	0.23(9)-

Table 8 (Continued)

Type of medium		Spot per wing[a] (No. of spots from 40 wings)							
		Trial 1				Trial 2			
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0
Abalone mushroom									
Standard	Standard (negative control)	0.2(8)	0	0.03(1)	0.23(9)	0.25(10)	0	0	0.25(10)
Standard	Positive control	7.45(298)+	2.15(86)+	0.65(26)+	10.25(410)+	11.0(440)+	1.75(70)+	0.4(16)+	13.15(321)+
Standard	Mutagenic samples								
	- Fresh	0.13(5)-	0-	0-	0.13(5)-	0-	0.05(2)-	0.03(1)-	0.08(3)-
	- Blanched	0.05(2)-	0.03(1)-	0-	0.08(3)-	0.1(4)-	0.03(1)-	0.03(1)-	0.15(6)-
	- Fermented	0.05(2)-	0.05(2)-	0-	0.1(4)-	0.08(3)-	0.03(1)-	0-	0.1(4)-

a = Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with distilled water: + = positive, - = negative, I = inconclusive; m = multiplication factor. Probability levels: $\alpha = \beta = 0.05$. One- sided statistical tests.

b = Mating parent flies fed on any media until 3 day- old larvae.

c = 3- day old larvae were collected and transferred to another media.

4.2 Actual Study: Mutagenic Modulation of Mushroom extracts

4.2.1 Simultaneous feeding study

Table 9 shows the frequencies of different categories of wing spots induced by 4 kinds of mushrooms extracts. It is shows that the fresh (FBM) and blanched (BBM) button extracts increased the frequency of mutant spots ($p<0.05$). The mutation indices (MIs) of urethane are modified by FBM and BBM to be 1.28 (Trial 1), 1.16 (Trial 2) and 1.21 (Trial 1), 1.61 (Trial 2). The trend reduction of wing spots induced by urethane was observed when the extract from fermented button mushroom (PBM) to be 0.82 and 0.92 for trial 1 and trial 2, respectively. The mutation indices of urethane are modified by blanched shiitake mushroom (BSM) to be 0.92 and 1.12 in trial 1 and trial 2, respectively but the frequencies of wing spots induced by urethane were decreased in fresh shiitake mushroom extract (FSM); 0.7 (Trial 1) and 0.55 (Trial 2), and fermented shiitake mushroom extract (PSM) showed strong antimutagenicity; 0.15 (Trial 1) and 0.17 (Trial 2). The MIs of urethane obtained from a number of wing spots of flies derived from larvae fed from fresh oyster mushroom (FOM) are 1.24 (trial 1) and 1.08 (trial 2), blanched oyster mushroom (BOM) are 0.87 (trial 1) and 0.75 (trial 2), and fermented oyster mushroom (POM) are 0.72 and 0.87, for trial 1 and trial 2, respectively. Working on fresh abalone mushroom (FAM), blanched abalone mushroom (BAM) and fermented abalone mushroom (PAM), the frequencies of wing spots induced by urethane slightly increased. The mutation indices of FAM, BAM, and PAM are 0.92 and 1.21, 1.01 and 1.22, and 1.06 and 0.91, for trial 1 and trial 2, respectively. Thus, the results indicated that FSM, PSM, BOM, and POM might have some constituents that could inhibit the genotoxicity of urethane and PSM has a stronger inhibitory effect. Mutagenic potentiator found in the extracts from FBM, BBM, FOM, FAM, and BAM. Expressly, BBM showed a potent mutagenicity potentiator.

Table 9 Wing spot induction on *Drosophila melanogaster* derived from 100 trans-heterozygous *mwh+/+flr*^{3} larvae of the improved high bioactivation cross fed on experimental media in simultaneous feeding study.

Type of medium		Spots per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Button mushroom											
Standard	Standard (negative control)	0.01(4)	0	0	0.01(4)		0.08(3)	0	0	0.08(3)	
Standard	Positive control	3.93(157)+	3.43(137)+	0.55(22)+	7.90(316)+	1.00	4.18(167)+	2.13(85)+	0.38(15)+	6.68(267)+	1.00
Standard	Samples										
	- Fresh	0.05(2)-	0.08(3)-	0-	0.13(5)-		0.08(3)	0.05(2)	0-	0.13(5)	
	- Blanched	0.08(3)-	0-	0-	0.08(3)		0.18(7)	0.03(1)-	0-	0.20(8)	
	- Fermented	0.18(7)-	0-	0-	0.18(7)-		0-	0-	0-	0-	
Standard	Experimental										
	- Fresh	6.58(263)+	2.73(109+	0.80(32)+	10.10(404)+	1.28	4.05(162)+	2.88(115)+	0.83(33)+	7.75(310)+	1.16
	- Blanched	5.90(236)+	2.98(119)+	0.65(26)+	9.53(381)+	1.21	5.53(221)+	3.98(159)+	1.23(49)+	10.73(429)+	1.61
	- Fermented	4.18(167)+	1.88(75)+	0.43(17)+	6.48(259)+	0.82	3.43(137)+	2.13(85)+	0.65(26)+	6.20(248)+	0.92

Table 9 (Continued)

Type of medium		Spot per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Shiitake mushroom											
Standard	Standard (negative control)	0.10(3)	0	0	**0.10(3)**		0.08(3)	0	0	**0.08(3)**	
Standard	Positive control	3.93(157)+	3.43(137)+	0.55(22)+	**7.90(316)+**	1.00	4.16(154)+	2.14(79)+	0.38(14)+	**6.28(247)+**	1.00
Standard	Samples										
	- Fresh	0.08(3)-	0-	0-	**0.08(3)-**		0.08(3)-	0.08(3)-	0-	**0.15(6)**	
	- Blanched	0.13(5)	0-	0-	**0.13(5)**		0.03(1)-	0.03(1)-	0-	**0.05(2)-**	
	- Fermented	0.05(2)-	0-	0-	**0.05(2)-**		0.08(3)	0.03(1)-	0-	**0.10(4)**	
Standard	Experimental										
	- Fresh	2.80(112)+	2.20(88)+	0.50(20)+	**5.50(220)+**	0.70	2.45(98)+	1.05(42)+	0.18(7)+	**3.68(147)+**	0.55
	- Blanched	4.08(163)+	2.80(112)+	0.38(15)+	**7.25(290)+**	0.92	4.50(180)+	2.38(95)+	0.60(24)+	**7.48(299)+**	1.12
	- Fermented	0.73(29)+	0.45(17)+	0.03(1)-	**1.18(47)+**	0.15	0.85(34)+	0.18(7)+	0.08(3)+	**1.10(44)+**	0.17

Table 9 (Continued)

Type of medium		Spot per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Oyster mushroom											
Standard	Standard (negative control)	0.08(3)	0.03(1)	0	0.10(4)		0.10(4)	0	0.03(1)	0.13(5)	
Standard	Positive control	2.75(110)+	2.68(107)+	0.78(31)+	6.20(248)+	1.00	5.48(219)+	1.75(70)+	0.80(32)+	8.03(321)+	1.00
Standard	Samples										
	- Fresh	0.08(3)-	0.05(2)-	0.03(1)-	0.15(6)-		0.23(9)+	0.03(1)-	0-	0.25(10)+	
	- Blanched	0.03(1)-	0-	0-	0.03(1)-		0.08(3)-	0.05(2)-	0-	0.13(5)	
	- Fermented	0-	0-	0.03(1)-	0.03(1)-		0.15(6)-	0-	0.03(1)-	0.18(7)-	
Standard	Experimental										
	- Fresh	4.20(168)+	2.73(109)+	0.75(30)+	7.68(307)+	1.24	4.80(192)+	2.6(104)+	1.28(51)+	8.68(347)+	1.08
	- Blanched	2.68(107)+	2.23(89)+	0.50(20)+	5.40(216)+	0.87	3.18(127)+	1.78(71)+	1.05(42)+	6.00(240)+	0.75
	- Fermented	2.63(105)+	1.75(70)+	0.08(3)+	4.45(178)+	0.72	4.35(174)+	1.73(69)+	0.88(35)+	6.95(278)+	0.87

Table 9 (Continued)

Type of medium		Spot per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Abalone mushroom											
Standard	Standard (negative control)	0.08(3)	0.03(1)	0	**0.10(4)**		0.10(4)	0	0	**0.10(4)**	
Standard	Positive control	2.75(110)+	2.68(107)+	0.78(31)+	**6.20(248)+**	1.00	2.42(92)+	0.71(27)+	0.39(15)+	**3.53(134)+**	1.00
Standard	Samples										
	- Fresh	0.05(2)-	0.08(3)-	0.03(1)-	**0.15(6)-**		0.15(6)	0.03(1)-	0-	0.18(7)	
	- Blanched	0.10(4)	0-	0-	**0.10(4)-**		0-	0-	0-	0-	
	- Fermented	0.08(3)-	0.05(2)-	0.03(1)-	**0.15(6)-**		0.13(5)-	0-	0-	**0.13(5)-**	
Standard	Experimental										
	- Fresh	2.35(94)+	2.98(119)+	0.35(14)+	**5.68(227)+**	0.92	2.05(82)+	1.63(65)+	0.60(24)+	**4.28(171)+**	1.21
	- Blanched	2.85(114)+	2.95(118)+	0.45(18)+	**6.25(250)+**	1.01	2.65(106)+	1.03(52)+	0.35(14)+	**4.30(172)+**	1.22
	- Fermented	3.65(146)+	2.38(95)+	0.58(23)+	**6.60(264)+**	1.06	2.28(91)+	0.68(27)+	0.25(10)+	**3.20(128)+**	0.91

a. = Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with distilled water: + = positive, - = negative, i = inconclusive; m = multiplication factor. Probability levels: $\alpha = \beta = 0.05$. One-sided statistical tests.

b = Mating parent flies fed on any media until 3 day- old larvae.

c = 3- day old larvae were collected and transferred to another media.

4.2.2 Pre-feeding Study

Effect of each mushroom extract

Fresh button mushroom (FBM) and blanched button mushroom (BBM) reduced the number of total wing spots induced by urethane in type I experiment but FBM induced the mutageincity of urethane in type II experiment. However, PBM reduced total wing spots in both type I and type II experiments. For type I experiment the mutation indices (MIs) of urethane were modified by FBM and BBM to be 0.86 and 0.86, respectively. For type II experiments were MIs of urethane are modified by FBM to be 1.54 and 1.53 in trial 1 and trial 2, respectively. MIs of urethane were modified by PBM to be 0.77 and 0.80 for type I and type II experiments, respectively. It was also found the same trend in the second trial (Table 10).

Fresh shiitake mushroom (FSM) in type I and type II experiments, blanched shiitake mushroom (BSM) in type I experiment, the wing spots were not different from that of urethane control group. But BSM, by contrast, reduced the total wing spots induced by urethane in type II experiment. MIs of urethane were modified by that medium to be 0.82 and 0.67 for trial 1 and trial 2, respectively. Fermented shiitake mushroom (PSM) decreased the total wing spots induced by urethane in type II experiments. MIs of urethane were modified by this medium to be 0.78 and 0.85 for type II experiment in trai1 and trial 2, respectively. But, in type I experiment, the wing spots were not much different from that of urethane control group (Table 11).

Fresh oyster mushroom (FOM) and blanched oyster mushroom (BOM) increased the total wing spots induced by urethane in type I and type II experiments. On the other hand, fermented oyster mushroom (POM) reduced the total wing spots induced by urethane in both type I and type II experiment. (MIs) of urethane were modified by FOM to be 1.83 and 1.89 for type I and type II experiments, respectively. (MIs) of urethane were modified by BOM to be 1.90 and 1.75 for type I and type II experiments, respectively. And (MIs) of urethane were modified by POM to be 0.65 and 0.59 for type I and type II experiments, respectively. It was also found the same trend in the second trial (Table 12).

Fresh abalone mushroom (FAM) reduced the total wing spots induced by urethane in type I experiment but this sample, the frequencies of mutant spots between

flies obtained from larvae pre- fed with FAM medium in type II experiment and positive control were not much different. MIs of urethane were modified by FAM in type I experiment to be 0.58 and 0.56 for trai1 and trial 2, respectively. Blanched abalone mushroom (BAM) and fermented abalone mushroom (PAM) reduced the total wing spots induced by urethane in both type I and type II experiments. MIs of urethane were modified by BAM to be 0.73 and 0.78 for type I and type II experiment, respectively. And MIs of urethane were modified by PAM to be 0.50 and 0.61 for type I and type II experiment, respectively. It was also found the same trend in the second trial (Table 13).

The results suggest that fresh mushroom extracts namely, button mushroom exhibited a moderate mutagenicity potentiator induced by urethane in pre- feeding type II experiment. Furthermore, fresh and blanched oyster mushroom showed a strong mutagenicity potentiator induced by urethane both in type I and type II experiment. On the other hand, blanched mushrooms sample namely, button mushroom in type I experiment, shiitake mushroom in type II experiment and abalone mushroom in both type I and type II experiments trend to decreased mutagenicity induced by urethane. In addition, all fermented mushrooms decreased the number of total mutant spots. Similar trend was observed when the experiment was repeated, thus mushrooms fermentation process may changed some composition or chemical substance to decreased mutagenicity.

Table 10 Wing spot induction in pre-feeding study of button mushroom extracts on *Drosophila melanogaster* derived from 100 trans-heterozygous *mwh+/+flr*^{3} larvae of the improved high bioactivation cross.

Type of medium		Spots per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Standard	Standard (negative control)	0.08(3)	0.03(1)	0	**0.10(4)**		0.10(4)	0.05(2)	0.03(1)	**0.18(7)**	
Standard	Positive control	3.90(156)+	1.95(78)+	0.75(30)+	**6.60(264)+**	1.00	5.50(220)+	2.53(101)+	1.35(54)+	**9.38(375)+**	1.00
Type I experiment											
Samples[d]											
- Fresh	Standard	0.03(1)-	0.05(2)-	0	0.08(3)-	0.012	0.08(3)-	0.10(4)-	0-	0.18(7)-	0.019
- Blanched	Standard	0.08(3)-	0.03(1)-	0-	0.10(4)	0.015	0.10(4)-	0.05(2)-	0-	0.15(6)-	0.016
- Fermented	Standard	0.08(3)-	0.05(2)-	0-	**0.13(5)-**	0.014	0.08(3)-	0.05(2)-	0-	0.13(5)-	0.014
Samples[d]											
- Fresh	Positive control	3.75(150)+	1.38(55)+	0.55(22)+	**5.68(227)+**	0.86	3.75(150)+	2.73(109)+	1.90(76)+	**8.38(335)+**	0.89
- Blanched	Positive control	3.28(131)+	1.78(71)+	0.65(26)+	**5.70(228)+**	0.86	4.75(190)+	2.60(104)+	0.90(36)+	**8.25(330)+**	0.88
- Fermented	Positive control	2.50(100)+	1.85(74)+	0.93(37)+	**5.28(211)+**	0.80	3.63(145)+	2.70(108)+	1.85(74)+	**8.18(327)+**	0.87

Table 10 (Continued)

Type of medium		Spots per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Type II experiment											
Samples [d]	Samples[d]										
- Fresh	- Fresh	0.03(1)-	0.08(3)-	0-	**0.10(4)-**	0.015	0.08(3)-	0.13(5)-	0-	**0.20(8)-**	0.021
- Blanched	- Blanched	0.08(3)-	0-	0-	**0.08(3)-**	0.012	0.10(4)-	0.18(7)-	0-	**0.28(11)-**	0.030
- Fermented	- Fermented	0.13(5)	0-	0-	**0.13(5)-**	0.020	0.05(2)-	0-	0-	0.05(2)-	0
Samples [d]	Experimental[e]										
- Fresh	- Fresh	7.48(299)+	1.83(73)+	0.85(34)+	**10.15(406)+**	1.54	10.28(411)+	2.73(109)+	1.30(52)+	**14.30(572)+**	1.53
- Blanched	- Blanched	4.90(196)+	1.25(50)+	0.35(14)+	**6.50(260)+**	0.98	6.55(262)+	2.85(114)+	1.05(42)+	**10.45(418)+**	1.11
- Fermented	- Fermented	3.28(131)+	1.40(56)+	0.43(56)+	**5.10(204)+**	0.77	5.30(212)+	1.70(68)+	0.50(20)+	**7.50(300)+**	0.80

a = Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with distilled water: + = positive, - = negative, I = inconclusive; m = multiplication factor. Probability levels: $\alpha = \beta = 0.05$. One-sided statistical tests.

M.I. = Mutation index was calculated from the number of induced total spots per wing subtracted by the value of the control group.

b = Mating parent flies fed on any media until 3 day- old larvae.

c = 3- day old larvae were collected and transferred to another media.

d = The medium prepared by substituted 1 ml distilled water with 1 ml of the mushroom extract.

e = The medium prepared by mixing the remaining ingredients (except distilled water) with 1 ml of the sample extract and 1ml urethane (40mM).

Table 11 Wing spot induction in pre-feeding study of shiitake mushroom extracts on *Drosophila melanogaster* derived from 100 trans-heterozygous *mwh+/+flr*^{3} larvae of the improved high bioactivation cross.

Type of medium		Spots per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Standard	Standard	0.10(4)	0.05(2)	0	0.15(6)		0.18(7)	0.03(1)	0	0.20(8)	
Standard	Positive control	5.638 (225)+	1.95 (78)+	0.98 (39)+	8.55 (342)+	1.00	7.10(284)+	2.45(98)+	1.28(51)+	10.83(433)+	1.00
Type I experiment											
Sample [d]											
- Fresh	Standard	0.05(2)-	0.05(2)-	0-	0.10(4)-	0.015	0.10(4)-	0.08(3)-	0-	0.18(7)-	0.016
- Blanched	Standard	0.05(2)-	0-	0.03(1)-	0.08(3)-	0.012	0.10(4)-	0.13(5)-	0-	0.23(9)-	0.021
- Fermented	Standard	0.08(3)-	0-	0.05(2)-	0.13(5)-	0.019	0.08(3)-	0.05(2)-	0-	0.13(5)-	0.012
Sample[d]											
- Fresh	Positive control	5.45(218)+	2.60(104)+	1.15(46)+	9.20(368)+	1.08	8.46(330)+	2.39(92)+	0.90(35)+	11.72(457)+	1.06
- Blanched	Positive control	6.95(278)+	2.08(83)+	0.85(34)+	9.88(395)+	1.16	7.05(282)+	2.32(93)+	1.05(42)+	10.43(417)+	0.96
- Fermented	Positive control	4.98(199)+	2.23(89)+	1.40(56)+	8.60(344)+	1.01	5.48(219)+	2.58(103)+	1.33(53)+	9.38(375)+	0.87

Table 11 (Continued)

Type of medium		Spots per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Type II experiment											
Sample [d]	Sample [d]										
- Fresh	- Fresh	0.08(3)-	0.10(4)-	0-	0.18(7)-	0.026	0.13(5)-	0.05(2)-	0-	0.18(7)-	0.016
- Blanched	- Blanched	0-	0.08(3)-	0-	0.08(3)-	0.012	0.05(2)-	0.05(2)-	0.08(3)-	0.18(7)-	0.016
- Fermented	- Fermented	0-	0.05(2)-	0.03(1)-	0.08(3)-	0.012	0-	0.13(5)-	0-	0.13(5)-	0.012
Sample [d]	Experimental [e]										
- Fresh	- Fresh	5.28(211)+	2.23(89)+	0.70(28)+	8.20(328)+	0.96	6.35(254)+	2.00(80)+	0.98(39)+	9.33(373)+	0.86
- Blanched	- Blanched	4.83(193)+	1.35(54)+	0.80(32)+	6.98(279)+	0.82	5.23(209)+	1.35(54)+	0.68(27)+	7.25(290)+	0.67
- Fermented	- Fermented	3.83(153)+	1.88(75)+	0.70(28)+	6.40(256)+	0.78	5.60(224)+	2.43(97)+	1.13(45)+	9.15(366)+	0.85

a = Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with distilled water: + = positive, - = negative, I = inconclusive; m = multiplication factor. Probability levels: $\alpha = \beta = 0.05$. One-sided statistical tests.

M.I. = Mutation index was calculated from the number of induced total spots per wing subtracted by the value of the control group.

b = Mating parent flies fed on any media until 3 day- old larvae.

c = 3- day old larvae were collected and transferred to another media.

d = The medium was substituted mushroom extract (5% w/v) and was mix with another components.

e = The medium prepared by mixing the remaining ingredients (except distilled water) with 1 ml of the sample extract and 1ml urethane (40mM).

Table 12 Wing spot induction in pre-feeding study of oyster mushroom extracts on *Drosophila melanogaster* derived from 100 trans-heterozygous *mwh+/+flr*^{3} larvae of the improved high bioactivation cross.

Type of medium		Spots per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Standard	Standard	0.03(1)	0.03(1)	0.05(2)	0.10(4)		0.15(6)	0.03(1)	0.03(1)	0.20(8)	
Standard	Positive control	3.28(131)+	1.75(70)+	0.75(30)+	5.78(231)+	1.00	3.11(118)+	1.66(63)+	1.08(41)+	5.84(222)+	1.00
Type I experiment											
Sample [d]											
- Fresh	Standard	0.05(2)-	0.05(2)-	0-	0.10(4)-	0.017	0.10(4)-	0.08(3)-	0-	0.18(7)-	0.031
- Blanched	Standard	0.05(2)-	0-	0.03(1)-	0.08(3)-	0.013	0.10(4)-	0.13(5)-	0-	0.23(9)-	0.039
- Fermented	Standard	0.08(3)-	0.03(1)-	0-	0.10(4)-	0.017	0.08(3)-	0.05(2)-	0-	0.13(5)-	0.022
Sampled											
- Fresh	Positive control	6.20(248)+	3.20(128)+	1.15(46)+	10.55(422)+	1.83	5.30(212)+	2.55(102)+	1.50(60)+	9.35(374)+	1.60
- Blanched	Positive control	6.75(270)+	2.80(112)+	1.43(57)+	10.98(439)+	1.90	5.60(224)+	4.20(168)+	1.75(70)+	11.50(462)+	1.98
- Fermented	Positive control	2.43(97)+	1.00(40)+	0.30(12)+	3.73(149)+	0.65	2.55(102)+	1.33(53)+	0.68(27)+	4.55(182)+	0.78

Table 12 (Continued)

Type of medium		Spots per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Type II experiment											
Sample[d]	Sample [d]										
- Fresh	- Fresh	0.08(3)-	0.03(1)-	0-	**0.10(4)-**	0.017	0.08(3)-	0.05(2)-	0.03(1)-	**0.15(6)-**	0.026
- Blanched	- Blanched	0.08(3)-	0.05(2)-	0-	**0.13(5)-**	0.022	0.13(5)-	0.05(2)-	0-	**0.18(7)-**	0.031
- Fermented	- Fermented	0.05(2)-	0-	0-	**0.05(2)-**	0.008	0.18(7)-	0.03(1)-	0-	**0.20(8)-**	0.034
Sample [d]	Experimental[e]										
- Fresh	- Fresh	6.40(256)+	2.78(111)+	1.73(69)+	**10.90(436)+**	1.89	5.18(207)+	2.53(101)+	1.68(67)+	**9.38(375)+**	1.60
- Blanched	- Blanched	5.88(235)+	2.70(108)+	1.55(62)+	**10.13(405)+**	1.75	5.53(221)+	3.40(136)+	2.08(83)+	**11.00(440)+**	1.88
- Fermented	- Fermented	1.80(72)+	1.20(48)+	0.40(16)+	**3.40(136)+**	0.59	2.18(87)+	1.18(47)+	0.30(12)+	**3.65(146)+**	0.62

a = Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with distilled water: + = positive, - = negative, I = inconclusive; m = multiplication factor. Probability levels: $\alpha = \beta = 0.05$. One-sided statistical tests.

M.I. = Mutation index was calculated from the number of induced total spots per wing subtracted by the value of the control group.

b = Mating parent flies fed on any media until 3 day- old larvae.

c = 3- day old larvae were collected and transferred to another media.

d = The medium prepared by substituted 1 ml distilled water with 1 ml of the mushroom extract.

e = The medium prepared by mixing the remaining ingredients (except distilled water) with 1 ml of the sample extract and 1ml urethane (40mM).

Table 13 Wing spot induction in pre-feeding study of abalone mushroom extracts on *Drosophila melanogaster* derived from 100 trans-heterozygous *mwh+/+flr*^{3} larvae of the improved high bioactivation cross.

Type of medium		Spots per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Standard	Standard	0.15(6)	0.03(1)	0	**0.18(7)**		0.20(8)	0.03(1)	0.03(1)	**0.25(10)**	
Standard	Positive control	6.50(234)+	2.39(86)+	1.03(37)+	**9.92(357)+**	1.00	7.23(289)+	1.68(67)+	1.13(45)+	10.03(401)+	1.00
Type I experiment											
Sample [d]											
- Fresh	Standard	0.05(2)-	0.05(2)-	0-	**0.10(4)-**	0.01	0.10(4)-	0.08(3)-	0-	**0.18(7)-**	0.018
- Blanched	Standard	0.05(2)-	0-	0.03(1)-	**0.08(3)-**	0.008	0.10(4)-	0.13(5)-	0-	**0.23(9)-**	0.023
- Fermented	Standard	0.08(3)-	0-	0.05(2)-	**0.13(5)-**	0.013	0.08(3)-	0.05(2)-	0-	**0.13(5)-**	0.013
Sample [d]											
- Fresh	Positive control	4.58(183)+	0.93(37)+	0.25(10)+	**5.75(230)+**	0.58	3.88(155)+	0.93(37)+	0.78(31)+	5.58(223)+	0.56
- Blanched	Positive control	5.38(215)+	1.23(49)+	0.68(27)+	**7.28(291)+**	0.73	6.33(253)+	0.78(31)+	0.53(21)+	7.63(305)+	0.76
- Fermented	Positive control	3.20(128)+	1.15(46)+	0.63(25)+	**4.98(199)+**	0.50	5.28(211)+	1.03(41)+	0.70(28)+	7.00(280)+	0.70

Table 13 (Continued)

Type of medium		Spots per wing [a] (No. of spots from 40 wings)									
		Trial 1					Trial 2				
Period 1[b]	Period 2[c]	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.	Small single spots m=2.0	Large single spots m=5.0	Twin spots m=5.0	Total spots m=2.0	M.I.
Type II experiment											
Sample [d]	Sample [d]										
- Fresh	- Fresh	0.13(5)-	0-	0-	**0.13(5)-**	0.01	0-	0.05(2)-	0.03(1)-	**0.08(3)-**	0.008
- Blanched	- Blanched	0.05(2)-	0.03(1)-	0-	**0.08(3)-**	0.008	0.10(4)-	0.03(1)-	0.03(1)-	**0.13(5)-**	0.013
- Fermented	- Fermented	0.05(2)-	0.05(2)-	0-	**0.10(4)-**	0.010	0.08(3)-	0.03(1)-	0-	0.10(4)-	0.010
Sample [d]	Experimental [e]										
- Fresh	- Fresh	7.05(282)+	3.18(127)+	1.30(52)+	**11.53(461)+**	1.16	6.67(268)+	1.98(79)+	1.23(49)+	**9.90(396)+**	0.99
- Blanched	- Blanched	6.00(240)+	1.10(44)+	0.63(25)+	**7.73(309)+**	0.78	6.75(270)+	1.38(55)+	0.60(24)+	**8.73(349)+**	0.87
- Fermented	- Fermented	4.60(184)+	1.00(40)+	0.48(19)+	**6.08(243)+**	0.61	5.75(230)+	1.20(48)+	0.40(16)+	**7.35(294)+**	0.73

a = Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with distilled water: + = positive, - = negative, I = inconclusive; m = multiplication factor. Probability levels: $\alpha = \beta$ =0.05. One-sided statistical tests.

M.I. = Mutation index was calculated from the number of induced total spots per wing subtracted by the value of the control group.

b = Mating parent flies fed on any media until 3 day- old larvae.

c = 3- day old larvae were collected and transferred to another media.

d = The medium prepared by substituted 1 ml distilled water with 1 ml of the mushroom extract.

e = The medium prepared by mixing the remaining ingredients (except distilled water) with 1 ml of the sample extract and 1ml urethane (40mM).