

CHAPTER V

RESULTS

5.1 Total Phenolic Content and Antioxidant Activities of the Samples

The total phenolic contents of the extracts from raw eggplants were different from those of the corresponding processed ones (Table 5.1 and transformed to Figure 5.1). The total phenolic contents (mg gallic acid equivalent/g dry weight) of the extract from raw Ma Khuea Pro were 12.70 (trial 1) and 7.00 (trial 2), from raw Ma Khuea Lueng were 6.57 (trial 1) and 11.93 (trial 2) and from raw Ma Khuea Muang Glom were 1.92 (trial 1) and 1.45 (trial 2). Steaming increased the total phenolic contents of Ma Khuea Pro to 37.50 (trial 1) and 31.17 (trial 2), of Ma Khuea Lueng to 24.23 (trial 1) and 28.87 (trial 2) and of Ma Khuea Muang Glom to 19.70 (trial 1) and 22.21 (trial 2). Concerning on frying process, the total phenolic contents (mg gallic acid equivalent/g dry weight) of Ma Khuea Pro and Ma Khuea Muang Glom increased, compared to their corresponding raw ones, to 28.73 and 19.30; respectively; while the total phenolic content of Ma Khuea Lueng maintained with 6.83 in trial 1. In trial 2, the total phenolic contents increased to 16.67 (Ma Khuea Pro), 13.33 (Ma Khuea Lueng) and 20.45 (Ma Khuea Muang Glom) compared with raw ones.

The antioxidant activities of the methanolic extracts from raw, steamed and fried eggplant samples determined by DPPH assay and FRAP assay are shown in Table 5.1 and transformed to Figure 5.2 and Figure 5.3; respectively. The reduction of DPPH by antioxidants in the samples was expressed as mM Trolox/g dry weight. Heat treatment increased the scavenging activity of eggplants on DPPH radicals. The DPPH scavenging activity of extracts from raw Ma Khuea Pro was 0.02 (both trial 1 and 2), from Ma Khuea Lueng was 0.05 (both trials 1 and 2) and from Ma Khuea Muang Glom were 0.03 (trial 1) and 0.04 (trial 2); after being steamed, the DPPH scavenging activity increased to 0.10, 0.10 and 0.11; respectively in both trial 1 and 2. Frying increased the DPPH scavenging activity of Ma Khuea Pro (0.08 in trial 1 and 0.09 in

trial 2) and of Ma Khuea Muang Glom (0.11 both trial 1 and 2) but maintained the DPPH scavenging activity of Ma Khuea Lueng with 0.04 in trial 1 and 0.05 in trial 2.

The FRAP (The Ferric Reducing Antioxidant Power) values ($\mu\text{M Fe(II)}/\text{g}$ dry weight) of the extract from raw samples were 29.20 and 24.87 (Ma Khuea Pro in trials 1 and 2), 50.80 and 56.27 (Ma Khuea Lueng in trials 1 and 2) and 45.70 and 38.16 (Ma Khuea Muang Glom in trials 1 and 2); the values increased after the samples were steamed to 153.57 and 161.60 (Ma Khuea Pro in trials 1 and 2), 143.47 and 143.58 (Ma Khuea Lueng in trials 1 and 2) and 251.13 and 228.61 (Ma Khuea Muang Glom in trials 1 and 2), respectively. In addition, the FRAP values of Ma Khuea Pro, Ma Khuea Lueng and Ma Khuea Muang Glom in trial 1 after being fried increased to 95.97, 82.23 and 228.60 $\mu\text{M Fe(II)}/\text{g}$ dry weight, respectively. In trial 2, frying increased the FRAP value to 119.57 (Ma Khuea Pro), 66.38 (Ma Khuea Lueng) and 213.58 (Ma Khuea Muang Glom) compared with their corresponding raw ones.

Table 5.1 Effect of different heat treatments on total phenolic content and antioxidant activities of 0.5 g eggplant extracts. Data are presented as means of composite (n = 3) samples.

Extract	Assigned treatment	Total phenolic content		DPPH assay		FRAP** value	
		(mg gallic acid equivalent/g dry weight)		TEAC*		(μM Fe(II)/g dry weight)	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Ma Khuea Pro	raw	12.70	7.00	0.02	0.02	29.20	24.87
	steaming	37.50	31.17	0.10	0.10	153.57	161.60
	frying	28.73	16.67	0.08	0.09	95.97	119.57
Ma Khuea Lueng	raw	6.57	11.93	0.05	0.05	50.80	56.27
	steaming	24.23	28.87	0.10	0.10	143.47	143.58
	frying	6.83	13.33	0.04	0.05	82.23	66.38
Ma Khuea Muang Glom	raw	1.92	1.45	0.03	0.04	45.70	38.16
	steaming	19.70	22.21	0.11	0.11	251.13	228.61
	frying	19.30	20.45	0.11	0.11	228.60	213.58

* Trolox equivalent antioxidant capacity expressed as mM Trolox/g dry weight.

** Ferric reducing antioxidant power.

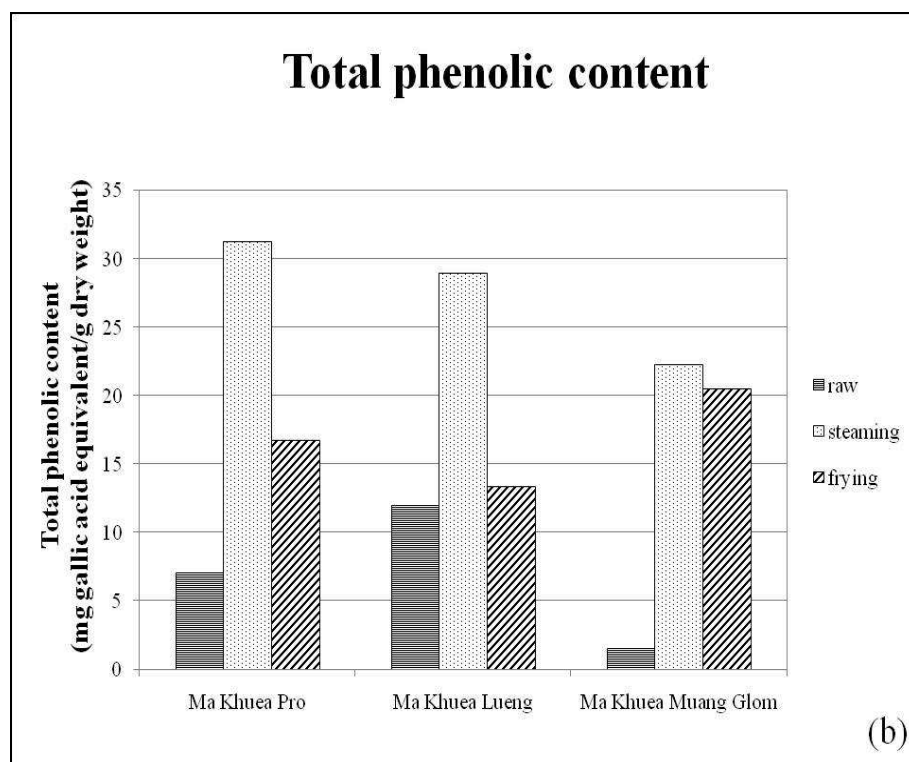
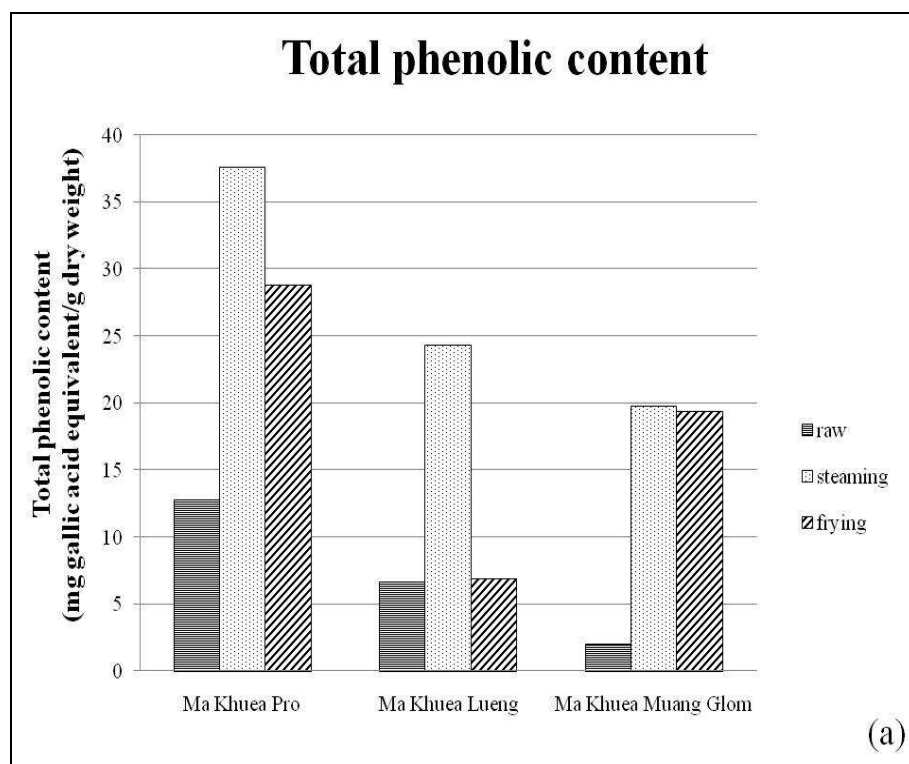


Figure 5.1 Total phenolic content of methanolic extracts of 0.5 g eggplant Trial 1 (a) and Trial 2 (b).

Data are expressed as means.

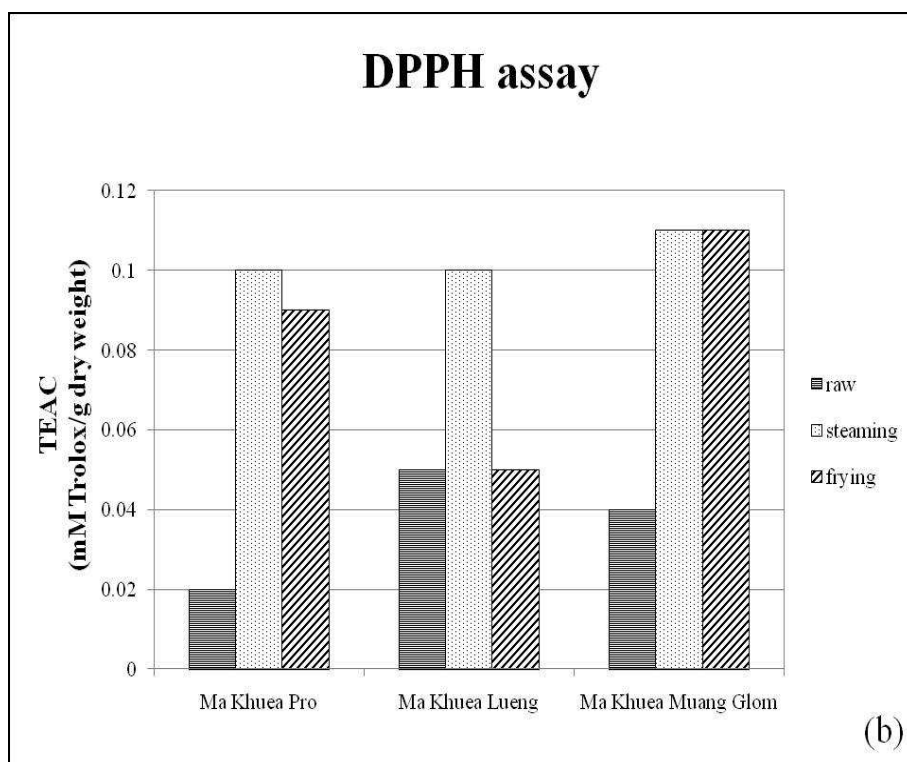
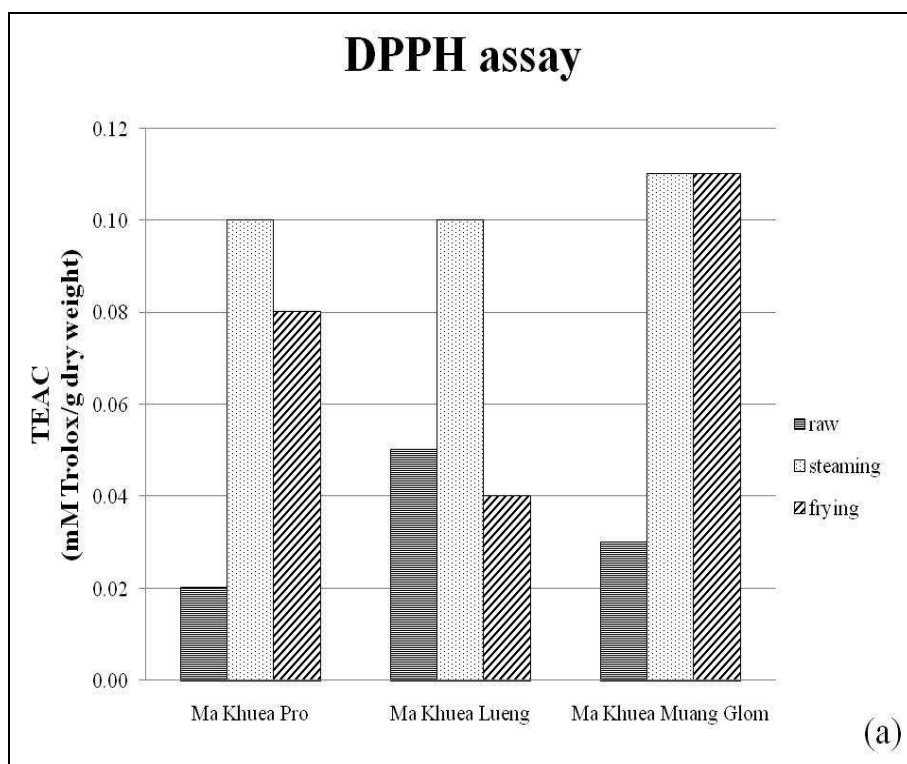


Figure 5.2 Antioxidant activity in DPPH assay of methanolic extracts of 0.5 g eggplant Trial 1 (a) and Trial 2 (b).

Data are expressed as means.

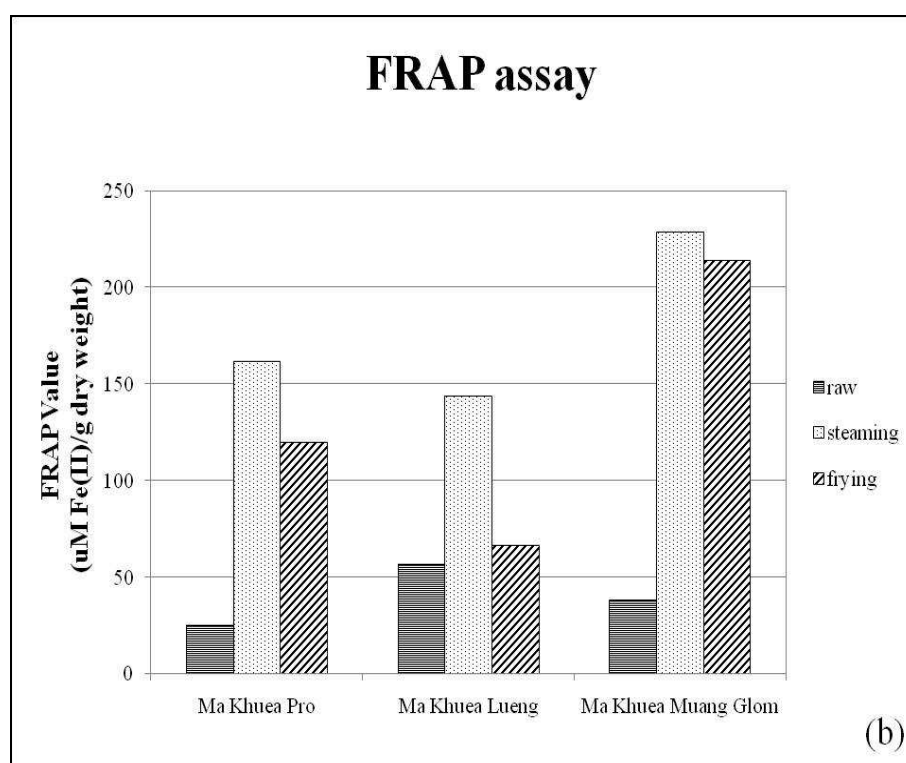
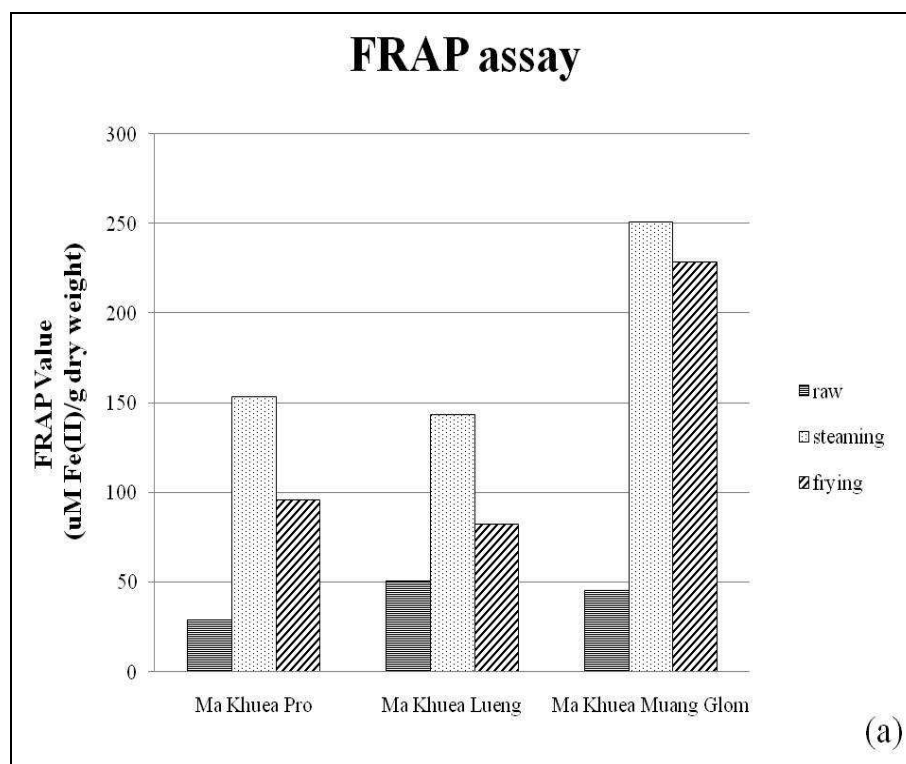


Figure 5.3 Antioxidant activity in FRAP assay of methanolic extracts of 0.5 g eggplant Trial 1 (a) and Trial 2 (b).

Data are expressed as means.

5.2 Mutagenicity of the Samples

Each sample was mixed with all dry ingredients of the standard medium to have the final 12.5, 25 and 50 percentages of samples in the mixtures. Toxicity of each sample was determined and shown in Table 5.2. The percentages of surviving adult flies fed on experimental medium containing 12.5, 25 and 50% Ma Khuea Pro are between 90 and 99%, 85 and 95% and 69 and 89%, respectively; whereas those fed on negative and positive control medium are 99 and 73%, respectively. In addition, the percentages of surviving flies fed on experimental medium containing 12.5, 25 and 50% Ma Khuea Lueng are in the range of 92 to 100%, 90 to 96% and 88 to 90%, respectively and those fed on negative and positive control medium are 93 and 64%, respectively. The percentages of surviving flies fed on experimental medium containing 12.5 and 25% Ma Khuea Muang Glom are 100 % while those obtained from flies fed on medium containing 50% Ma Khuea Muang Glom are between 86 and 99%. Therefore, the experimental medium containing 50% sample supplementation was selected to be tested for its mutagenicity.

Table 5.3 shows the mutagenicity (expressed as range of total induced spots per wing) of Ma Khuea Pro (0.4 to 0.875), Ma Khuea Lueng (0.4 to 0.65) and Ma Khuea Muang Glom (0.3 to 0.5); while that of the negative control is 0.525 to 0.575 and of the positive control is 12.9 to 16.031. It indicates that none of eggplant was mutagenic.

Table 5.2 The percentage of surviving adult flies fed on each experimental medium containing eggplant.

Treatment		Percent of surviving flies		
		12.50% sample addition*	25% sample addition	50% sample addition
Ma Khuea Pro	raw	99	95	89
	steaming	90	85	86
	frying	96	89	69
Ma Khuea Lueng	raw	100	94	88
	steaming	93	96	90
	frying	92	90	90
Ma Khuea Muang Glom	raw	100	100	87
	steaming	100	100	99
	frying	100	100	86

Percent of surviving flies of negative (water) control ranges from 76-99

Percent of surviving flies of positive (urethane) control ranges from 64-100

*Each lyophilized sample was mixed well with 0.58 g fly medium containing all components but water in a beaker to obtain an experimental medium with the 12.5, 25 or 50 percent sample addition

Table 5.3 Mutagenicity of each eggplant in adult flies bringing up on each experimental medium.

Treatment		Wings	Spots per wing* (No. of spots from wings)			
			Small single m= 2	Large single m= 5	Twin m= 5	Total m= 2
Negative control		40	0.525 (21)	0	0	0.525 (21)
Positive control/URE		40	7.925 (317)+	4.250 (170)+	0.725 (29)+	12.900 (516)+
Ma Khuea Pro	raw	40	0.325 (13)i	0.050 (2)i	0.025 (1)i	0.400 (16)i
	steaming	40	0.775 (31)i	0.025 (1)i	0.075 (3)i	0.875 (35)i
	frying	40	0.500 (20)-	0.025 (1)i	0	0.525 (21)-
Negative control		40	0.525 (21)	0.050 (2)	0	0.575 (23)
Positive control/URE		40	8.300 (332)+	4.400 (176)+	0.950 (38)+	13.650 (546)+
Ma Khuea Lueng	raw	40	0.475 (19)-	0.075 (3)i	0.100 (4)i	0.650 (26)-
	steaming	40	0.475 (19)-	0	0	0.475 (19)-
	frying	40	0.325 (13)-	0.075 (3)i	0	0.400 (16)-
Negative control		34	0.412 (14)	0.088 (3)	0.029 (1)	0.529 (18)
Positive control/URE		32	12.563 (402)+	2.469 (79)+	1.000 (32)+	16.031 (513)+
Ma Khuea Muang Glom	raw	30	0.267 (8)-	0.033 (1)-	0	0.300 (9)-
	steaming	32	0.375 (12)-	0.031 (1)-	0	0.406 (13)-
	frying	30	0.433 (13)i	0.067 (2)-	0	0.500 (15)-

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würglur (1988) for comparison with negative control:

+ = positive; - = negative; i = inconclusive; Probability level: $\alpha = \beta = 0.05$. One side statistical tests.

5.3 Antimutagenicity of Samples

All samples expressed their antimutagenicity in this experiment (Tables 5.4, 5.5, 5.6 and transformed to Figure 5.4). The administration of each sample along with urethane to 3-day-old larvae reduced number of induced spots per wing. The percentage of inhibition on urethane mutagenicity was calculated to show the relationship between the mutagenicity of urethane in the presence and absence of raw, steamed and fried eggplants.

Figure 5.4 demonstrates that steaming and frying decreased the antimutagenicity of Ma Khuea Pro (Figure 5.4a). Surprisingly, steaming increased the antimutagenicity of Ma Khuea Lueng (Figure 5.4b) and Ma Khuea Muang Glom (Figure 5.4c) compared with that of their corresponding raw ones.

Table 5.4 Antimutagenicity of Ma Khuea Pro against urethane induced wing spots in *Drosophila melanogaster*

Trial	Treatment	Wings	Spots per wing* (No. of spots from wings)				% Inhibition
			Small single m= 2	Large single m= 5	Twin m= 5	Total m= 2	
1	Negative control	32	0.813 (26)	0.031 (1)	0.031 (1)	0.875 (28)	-
	Positive control/URE	34	7.500 (255)+	6.000 (204)+	0.853 (29)+	14.353 (488)+	-
	Ma Khuea Pro raw/URE	32	4.750 (152)+	3.094 (99)+	0.781 (25)+	8.625 (276)+	40 (w)
	steaming /URE	32	5.188 (166)+	3.375 (108)+	0.969 (31)+	9.531 (305)+	34 (w)
	frying/URE	30	5.767 (173)+	4.167 (125)+	0.867 (26)+	10.800 (324)+	25 (w)
2	Negative control	36	0.528 (19)	0.056 (2)	0.056 (2)	0.639 (23)	-
	Positive control/URE	34	10.088 (343)+	6.206 (211)+	1.118 (38)+	17.412 (592)+	-
	Ma Khuea Pro raw/URE	30	6.067 (182)+	2.167 (65)+	0.367 (11)+	8.600 (258)+	51 (m)
	steaming /URE	30	6.767(203)+	1.867 (56)+	0.733 (22)+	9.367 (281)+	46 (m)
	frying/URE	34	8.676 (295)+	2.324 (79)+	0.735 (25)+	11.735 (399)+	33 (w)

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würglur (1988) for comparison with negative control:

+ = positive; - = negative; i = inconclusive; Probability level: $\alpha = \beta = 0.05$. One side statistical tests.

Antimutagenic potential: (w) = weak, (m) = moderate, (s) = strong.

Table 5.5 Antimutagenicity of Ma Khuea Lueng against urethane induced wing spots in *Drosophila melanogaster*

Trial	Treatment	Wings	Spots per wing* (No. of spots from wings)				% Inhibition
			Small single m= 2	Large single m= 5	Twin m= 5	Total m= 2	
1	Negative control	32	0.813 (26)	0.031 (1)	0.031 (1)	0.875 (28)	-
	Positive control/URE	34	7.500 (255)+	6.000 (204)+	0.853 (29)+	14.353 (488)+	-
	Ma Khuea Lueng raw/URE	30	5.833 (175)+	3.633 (109)+	0.967 (29)+	10.433 (313)+	27 (w)
	steaming /URE	30	5.133 (154)+	3.867 (116)+	0.533 (16)+	9.533 (286)+	34 (w)
	frying/URE	30	6.367 (191)+	3.467 (104)+	0.533 (16)+	10.367 (311)+	28 (w)
2	Negative control	36	0.528 (19)	0.056 (2)	0.056 (2)	0.639 (23)	-
	Positive control/URE	34	10.088 (343)+	6.206 (211)+	1.118 (38)+	17.412 (592)+	-
	Ma Khuea Lueng raw/URE	32	9.531 (305)+	3.563 (114)+	0.750 (24)+	13.844 (443)+	20 (w)
	steaming /URE	34	7.500 (255)+	3.588 (122)+	0.853 (29)+	11.941 (406)+	31 (w)
	frying/URE	34	8.235 (280)+	3.088 (105)+	0.912 (31)+	12.235 (416)+	30 (w)

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würglur (1988) for comparison with negative control:

+ = positive; - = negative; i = inconclusive; Probability level: $\alpha = \beta = 0.05$. One side statistical tests.

Antimutagenic potential: (w) = weak, (m) = moderate, (s) = strong.

Table 5.6 Antimutagenicity of Ma Khuea Muang Glom against urethane induced wing spots in *Drosophila melanogaster*

Trial	Treatment	Wings	Spots per wing* (No. of spots from wings)				% Inhibition
			Small single m= 2	Large single m= 5	Twin m= 5	Total m= 2	
1	Negative control	40	0.625 (25)	0.050 (2)	0	0.675 (27)	-
	Positive control/URE	40	10.050 (402)+	1.975 (79)+	0.800 (32)+	12.825 (513)+	-
	Ma Khuea Muang Glom raw/URE	50	6.300 (315)+	2.880 (144)+	0.800 (40)+	9.980 (499)+	22 (w)
	steaming /URE	38	3.947 (150)+	2.132 (81)+	0.816 (31)+	6.895 (262)+	46 (m)
	frying/URE	54	6.667 (360)+	2.278 (123)+	0.537 (29)+	9.481 (512)+	26 (w)
2	Negative control	40	0.425 (17)	0.050 (2)	0.050 (2)	0.525 (21)	-
	Positive control/URE	40	10.325 (413)+	3.350 (134)+	0.625 (25)+	14.300 (572)+	-
	Ma Khuea Muang Glom raw/URE	40	5.775 (231)+	2.975 (119)+	0.800 (32)+	9.550 (382)+	33 (w)
	steaming /URE	40	4.650 (186)+	2.625 (105)+	1.200 (48)+	8.475 (339)+	41 (m)
	frying/URE	34	5.618 (191)+	3.294 (112)+	0.588 (20)+	9.500 (323)+	34 (w)

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würglur (1988) for comparison with negative control:

+ = positive; - = negative; i = inconclusive; Probability level: $\alpha = \beta = 0.05$. One side statistical tests.

Antimutagenic potential: (w) = weak, (m) = moderate, (s) = strong.

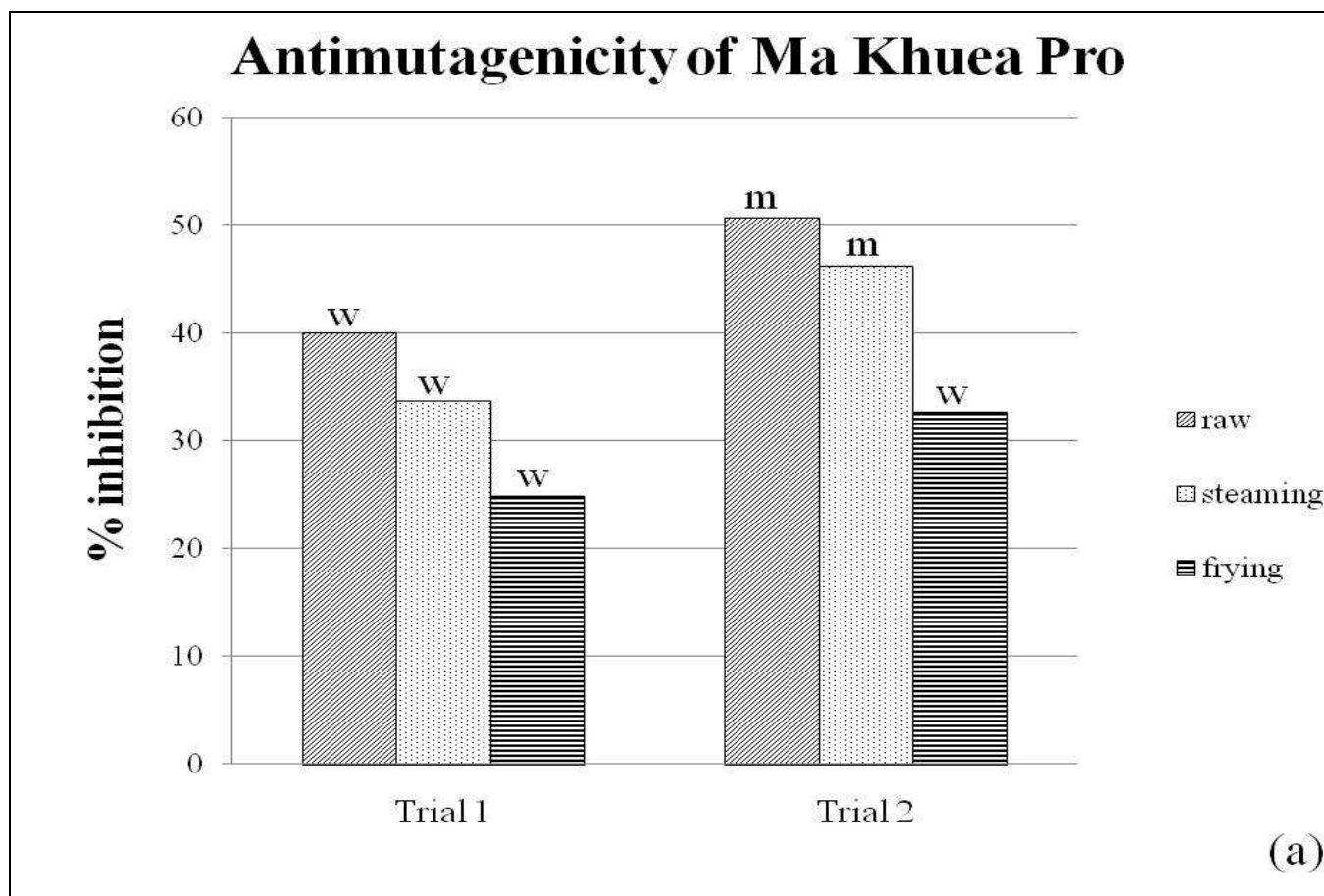


Figure 5.4(a) Percent inhibition of Ma Khuea Pro on urethane (20 mM) induced somatic mutation and recombination in *Drosophila melanogaster* derived from trans-heterozygous ($mwh/+flr^3$) larvae. Antimutagenicity potential: (w) = weak, (m) = moderate, (s) = strong)

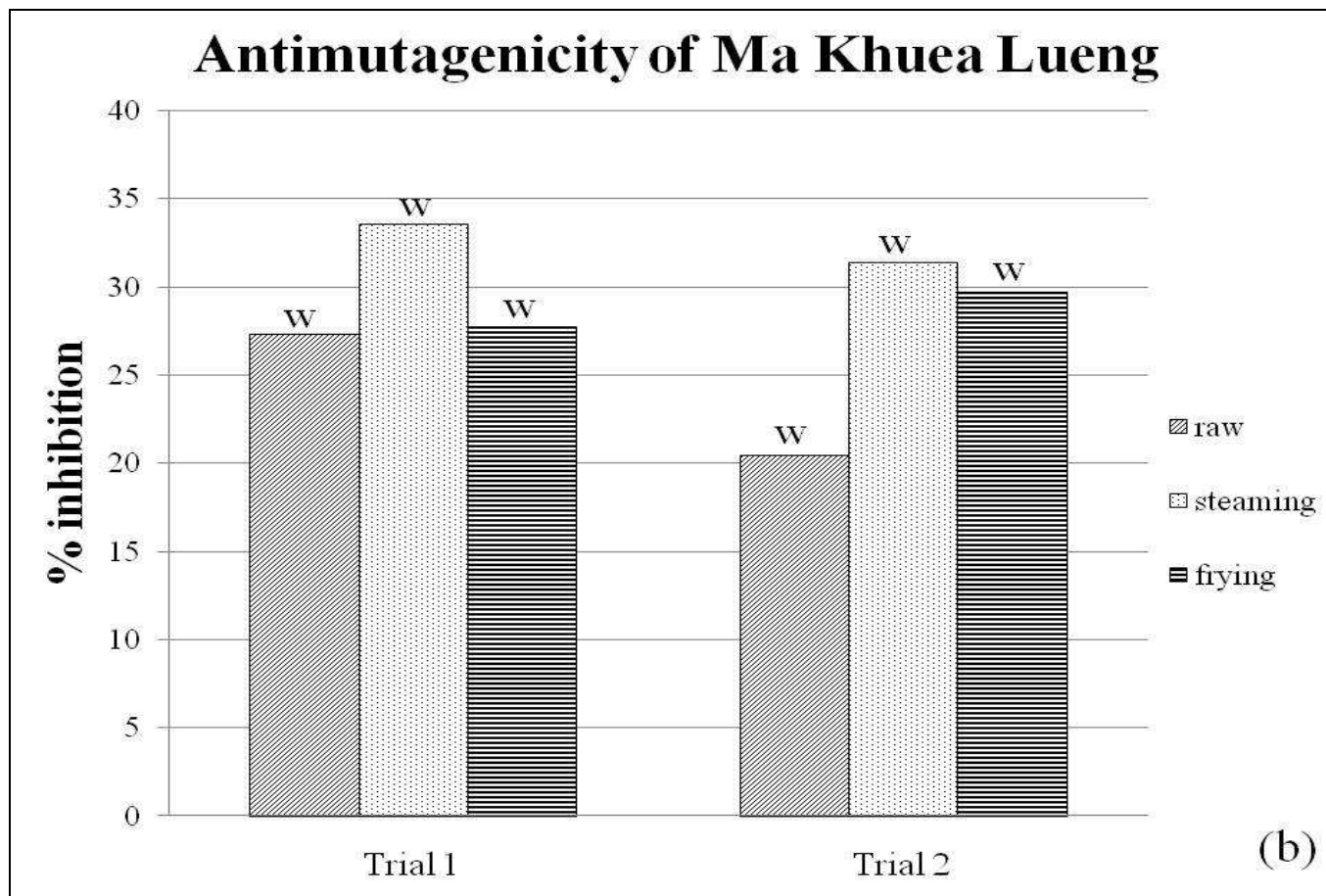


Figure 5.4(b) Percent inhibition of Ma Khuea Lueng on urethane (20 mM) induced somatic mutation and recombination in *Drosophila melanogaster* derived from trans-heterozygous ($mwh/+flr^3$) larvae. Antimutagenicity potential: (w) = weak, (m) = moderate, (s) = strong)

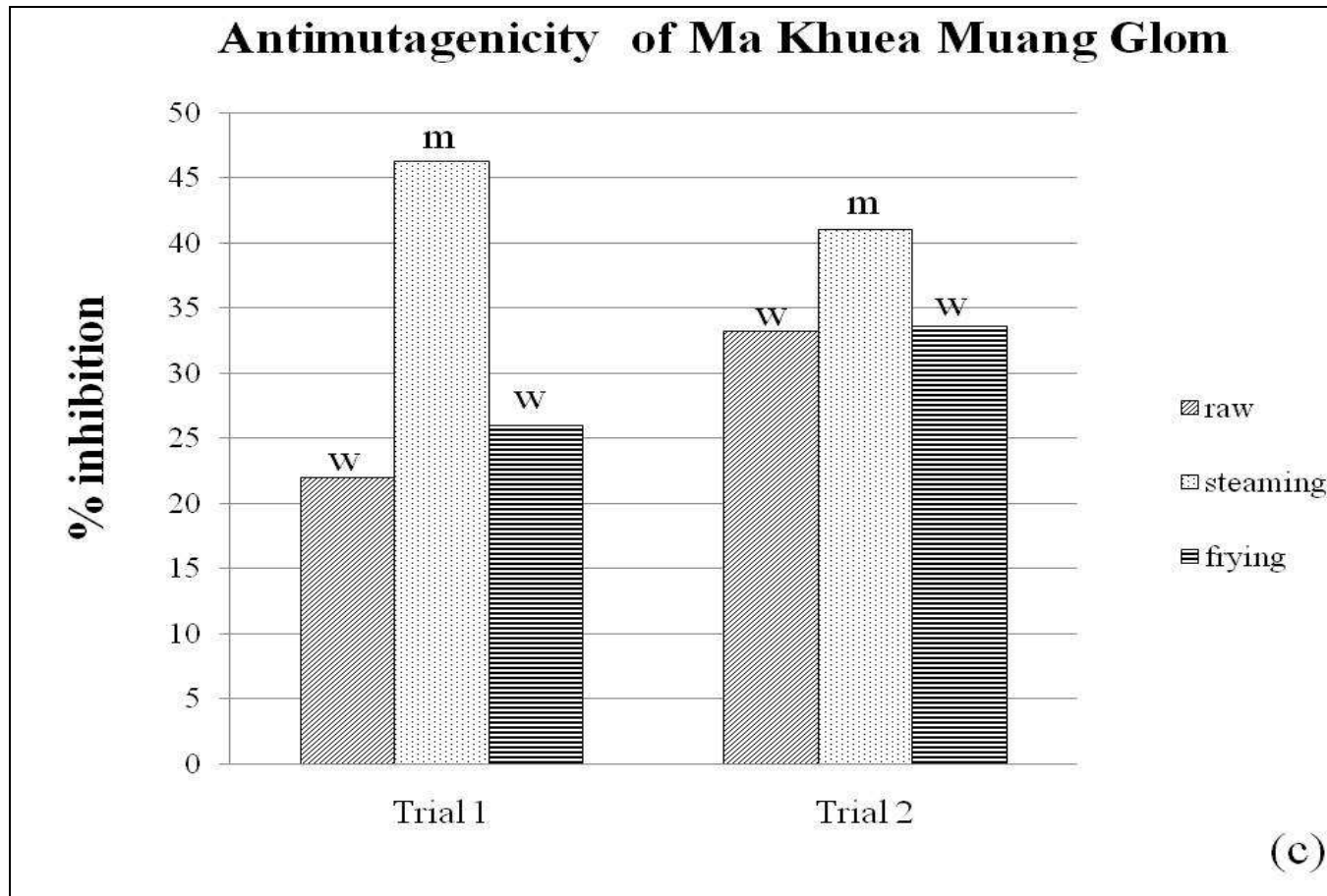


Figure 5.4(c) Percent inhibition of Ma Khuea Muang Glom on urethane (20 mM) induced somatic mutation and recombination in *Drosophila melanogaster* derived from trans-heterozygous ($mwh^{+}/+flr^3$) larvae. Antimutagenicity potential: (w) = weak, (m) = moderate, (s) = strong)