

## CHAPTER V

### RESULTS

#### 5.1 Total Phenolic Content, Anthocyanin Content, and Antioxidant Activities of Fermented Pigmented Rice

Total phenolic content, anthocyanin content, and antioxidant activities of six cultivars of pigmented rice, both unfermented and fermented ones, are shown in Table 5.1. It was observed that total phenolic content of fermented rice was significantly higher than that of its corresponding unfermented one. The highest total phenolic content (2086.9 mg gallic acid equivalent/100 g dry weight) belonged to fermented Riceberry (Figure 5.1A). The anthocyanin content of unfermented rice samples of the current study ranged from 0.4 to 111.1 mg cyanidin 3-glucoside/100 g dry weight whereas that of fermented rice samples ranged from 1.5 to 122.2 mg cyanidin 3-glucoside/100 g dry weight. Fermented black glutinous rice exhibited the highest anthocyanin content (approximately 122.2 mg cyanidin 3-glucoside/100 g dry weight) (Figure 5.1B). The DPPH antioxidant activity of unfermented rice had an average of 2.3 to 4.3 mmol Trolox equivalent/100 g dry weight while fermented rice had an average of 3.4 to 5.3 mmol Trolox equivalent/100 g dry weight. The highest DPPH antioxidant activity (5.3 mmol Trolox equivalent/100 g dry weight) was found in fermented black glutinous rice (Figure 5.2A). The FRAP values of unfermented rice ranged from 0.9 to 1.9 mmol Fe(II)/100 g dry weight whereas those of fermented rice ranged from 1.3 to 2.9 mmol Fe(II)/100 g dry weight. It was noted that fermented rice had significantly higher reducing abilities than that of unfermented rice of same variety. The fermented black glutinous rice had the highest FRAP values (2.9 mmol Fe(II)/100 g dry weight) (Figure 5.2B).

## **5.2 Mutagenicity of Unfermented and Fermented Pigmented Rice**

Table 5.2 shows the number of surviving adult flies obtained from the larvae brought up on negative control medium, positive control medium, and each experimental medium containing unfermented or fermented rice substituted for 50, 75 or 100 percent of corn flour. The percentages of surviving adult flies brought up on all experimental media are higher than 50%. The results indicate that all concentrations of each sample used were non-toxic for further study.

Each sample was evaluated for its mutagenicity. The results in Table 5.3 indicate that each sample was not mutagenic because it did not induce the frequencies of mutant spots at every testing concentration to be higher than that of the negative control group. The highest concentration of sample substituted for 100 percent of corn flour that provided higher than 50 percent of surviving flies was determined for its antimutagenicity.

**Table 5.1** Total phenolics, anthocyanins, and antioxidant activities of unfermented and fermented pigmented rice <sup>1,2</sup>.

Type of Rice	Variety	Total phenolics <sup>3</sup>	Anthocyanins <sup>4</sup>	DPPH <sup>5</sup>	FRAP <sup>6</sup>
Unfermented	Sung Yod	490.9±31.6 <sup>B</sup>	0.6±0.1 <sup>B</sup>	3.1±0.4 <sup>A</sup>	1.2±0.2 <sup>B</sup>
	Mon Poo	455.5±23.3 <sup>B</sup>	0.4±0.1 <sup>B</sup>	3.2±0.5 <sup>A</sup>	1.4±0.1 <sup>B</sup>
	Hom Mali Daeng	431.6±27.5 <sup>B</sup>	1.5±0.1 <sup>A</sup>	3.2±0.5 <sup>A</sup>	1.3±0.2 <sup>B</sup>
	Hom Nil	741.8±19.6 <sup>B</sup>	15.1±0.4 <sup>B</sup>	2.3±0.3 <sup>B</sup>	0.9±0.1 <sup>B</sup>
	Riceberry	1575.6±18.4 <sup>B</sup>	24.9±0.1 <sup>B</sup>	4.1±0.3 <sup>B</sup>	1.6±0.2 <sup>B</sup>
	Black glutinous rice	1109.0±39.6 <sup>B</sup>	111.1±1.6 <sup>B</sup>	4.3±0.3 <sup>B</sup>	1.9±0.2 <sup>B</sup>
Fermented	Sung Yod	1204.9±61.9 <sup>baA</sup>	1.8±0.2 <sup>daA</sup>	3.7±0.4 <sup>caA</sup>	1.7±0.1 <sup>cdA</sup>
	Mon Poo	1117.3±112.7 <sup>baA</sup>	1.5±0.2 <sup>daA</sup>	4.0±0.3 <sup>bcA</sup>	1.8±0.1 <sup>caA</sup>
	Hom Mali Daeng	1026.4±91.6 <sup>3baA</sup>	1.7±0.1 <sup>daA</sup>	4.2±0.2 <sup>bcA</sup>	1.8±0.1 <sup>bcA</sup>
	Hom Nil	1222.7±68.6 <sup>baA</sup>	17.6±0.6 <sup>caA</sup>	3.4±0.3 <sup>caA</sup>	1.3±0.2 <sup>daA</sup>
	Riceberry	2086.9±9.7 <sup>aA</sup>	34.2±1.6 <sup>baA</sup>	5.0±0.5 <sup>abA</sup>	2.2±0.2 <sup>baA</sup>
	Black glutinous rice	2064.2±40.2 <sup>aA</sup>	122.2±2.6 <sup>aA</sup>	5.3±0.4 <sup>aA</sup>	2.9±0.1 <sup>aA</sup>

<sup>1</sup>Values are presented as means±SD (n = 3 samples).

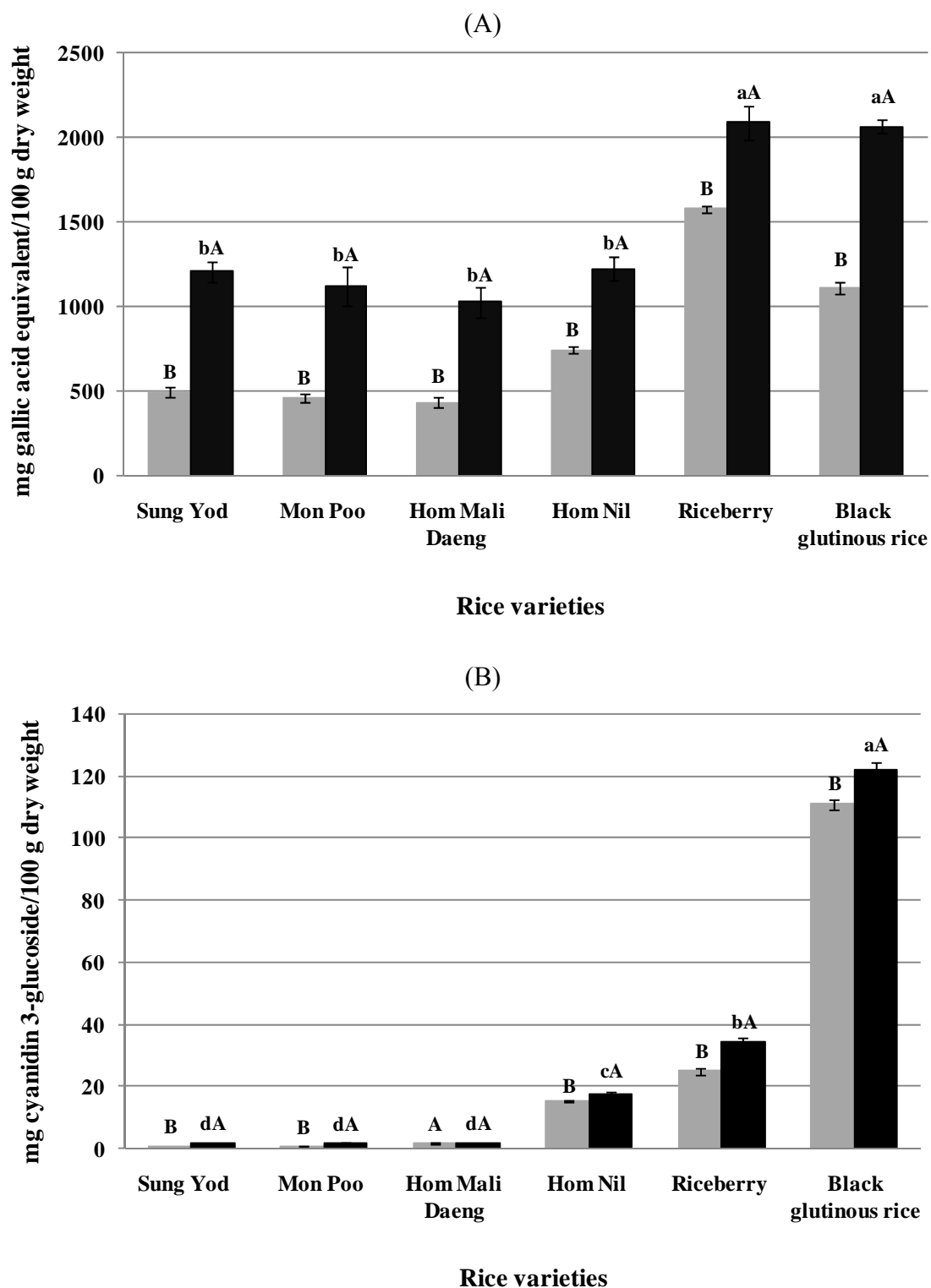
<sup>2</sup>Data with different upper case letters (A,B) indicate significant differences (p < 0.05) between unfermented and fermented rice of the same variety, while those with different lower case letters (a,b) indicate significant differences (p < 0.05) among different varieties of fermented rice.

<sup>3</sup>Express as mg gallic acid equivalent/100 g dry weight.

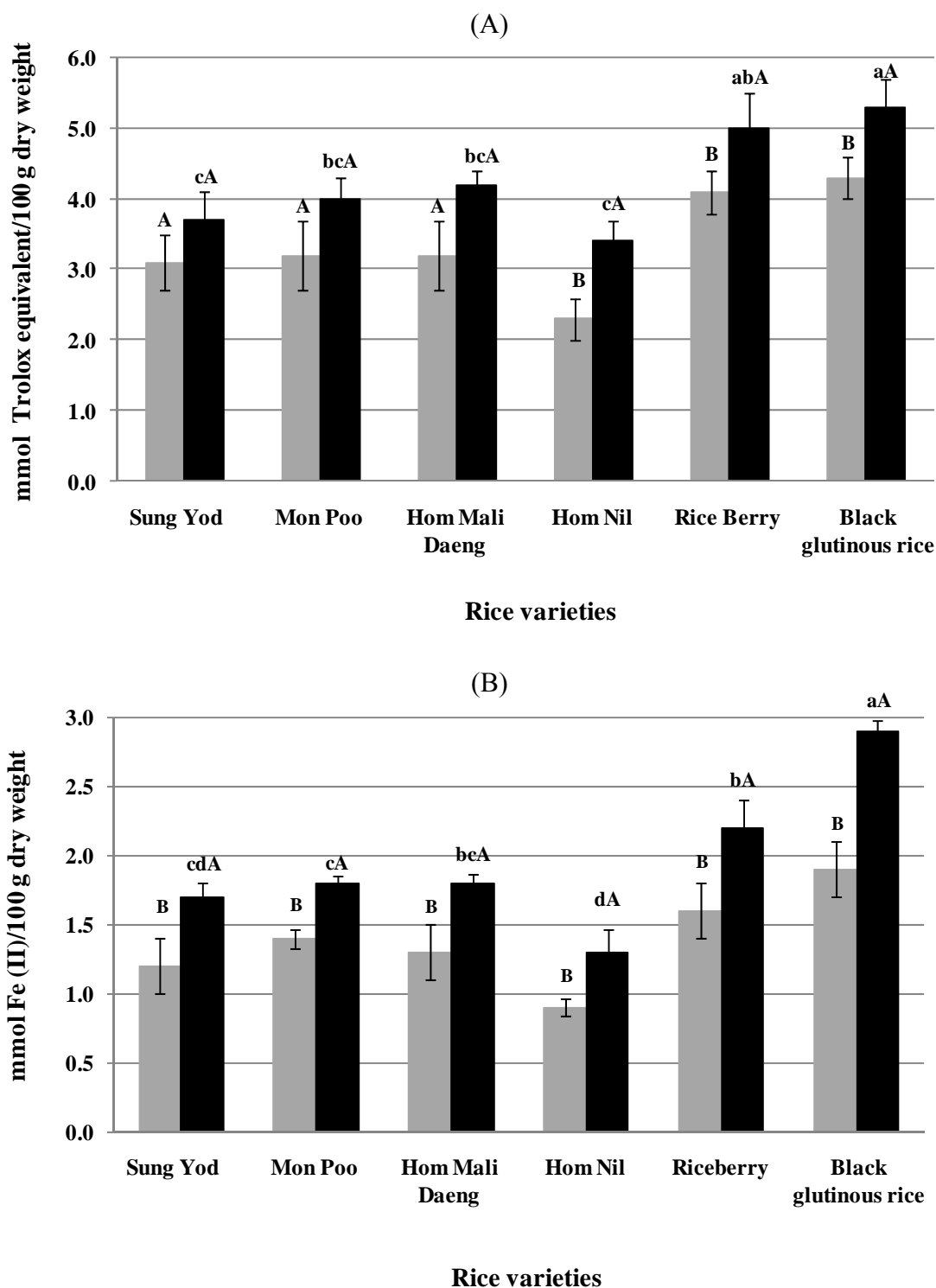
<sup>4</sup>Express as mg cyanidin 3-glucoside/100 g dry weight.

<sup>5</sup>Express as mmol Trolox equivalent/100 g dry weight.

<sup>6</sup>Express as mmol Fe(II)/100g dry weight.



**Figure 5.1** Total phenolic (A) and anthocyanin (B) contents of unfermented  and fermented  pigmented rice. Data with different upper case letters (A,B) indicate significant differences ( $p < 0.05$ ) between unfermented and fermented rice of the same variety, while those with different lower case letters (a,b) indicate significant differences ( $p < 0.05$ ) among different varieties of fermented rice.



**Figure 5. 2** DPPH antioxidant activity (A) and ferric reducing antioxidant power (B) of unfermented  and fermented  pigmented rice. Data with different upper case letters (A,B) indicate significant differences ( $p < 0.05$ ) between unfermented and fermented rice of the same variety, while those with different lower case letters (a,b) indicate significant differences ( $p < 0.05$ ) among different varieties of fermented rice.

**Table 5.2** The percentages of surviving adult flies brought up on control and experimental media\*.

Treatment	Variety of rice	% Substitution for corn flour	No. of surviving flies	
			Trial 1	Trial 2
Distilled water	-	-	97	95
20 mM urethane	-	-	85	88
Unfermented rice	Sung Yod	50	94	89
	Sung Yod	75	86	91
	Sung Yod	100	90	94
	Mon Poo	50	92	93
	Mon Poo	75	95	97
	Mon Poo	100	95	86
	Hom Mali Daeng	50	91	90
	Hom Mali Daeng	75	94	83
	Hom Mali Daeng	100	96	88
	Hom Nil	50	85	92
	Hom Nil	75	92	86
	Hom Nil	100	91	97
	Riceberry	50	87	90
	Riceberry	75	83	94
	Riceberry	100	89	86
	Black glutinous rice	50	96	92
	Black glutinous rice	75	92	87
	Black glutinous rice	100	97	91

**Table 5.2** The percentages of surviving adult flies brought up on control and experimental media\* (cont.).

Treatment	Variety of rice	% Substitution for corn flour	No. of surviving flies	
			Trial 1	Trial 2
Distilled water	-	-	78	93
20 mM urethane	-	-	89	85
Fermented rice	Sung Yod	50	91	81
	Sung Yod	75	84	75
	Sung Yod	100	94	99
	Mon Poo	50	73	92
	Mon Poo	75	92	84
	Mon Poo	100	71	94
	Hom Mali Daeng	50	87	96
	Hom Mali Daeng	75	82	87
	Hom Mali Daeng	100	96	89
	Hom Nil	50	92	73
	Hom Nil	75	78	79
	Hom Nil	100	83	91
	Riceberry	50	74	84
	Riceberry	75	90	72
	Riceberry	100	74	93
	Black glutinous rice	50	81	79
	Black glutinous rice	75	94	73
	Black glutinous rice	100	82	93

\*Each sample was substituted for 25, 50, 75 or 100 % corn flour in the standard medium in order to obtain the experimental media.

**Table 5.3** Wing spot test data obtained with unfermented or fermented pigmented rice.

Trial	Treatment	Variety of rice	% Substitution for corn flour	Spots per wing (No. of spots from 40 wings)*			
				Small single m=2	Large single m=5	Twin single m=5	Total m=2
1	Distilled water	-	-	0.250(10)	0.050(2)	0.025(1)	0.325(13)
	20 mM urethane	-	-	15.250(610)+	5.025(201)+	1.250(50)+	21.525(861)+
	Unfermented rice	Sung Yod	50	0.375(15)i	0.150(6)i	0	0.525(21)i
		Sung Yod	75	0.325(13)i	0.050(2)i	0	0.375(15)i
		Sung Yod	100	0.300(12)i	0.200(8)i	0.075(3)i	0.575(23)i
		Mon Poo	50	0.250(10)i	0.050(2)i	0.025(1)i	0.325(13)i
		Mon Poo	75	0.250(10)i	0.175(7)i	0.075(3)i	0.500(20)i
		Mon Poo	100	0.325(13)i	0.100(4)i	0.050(2)i	0.475(19)i
		Hom Mali Daeng	50	0.350(14)i	0.050(2)i	0.050(2)i	0.175((7)-
		Hom Mali Daeng	75	0.200(8)-	0.100(4)i	0	0.300(12)-
		Hom Mali Daeng	100	0.425(17)i	0	0	0.100(4)i
		Hom Nil	50	0.275(11)i	0	0	0.275(11)-
		Hom Nil	75	0.450(18)i	0.025(1)i	0.025(1)i	0.500(20)i
		Hom Nil	100	0.400(16)i	0.050(2)i	0	0.450(18)i



**Table 5.3** Wing spot test data obtained with unfermented or fermented pigmented rice (cont.).

Trial	Treatment	Variety of rice	% Substitution for corn flour	Spots per wing (No. of spots from 40 wings)*			
				Small single m=2	Large single m=5	Twin single m=5	Total m=2
		Riceberry	50	0.375(15)i	0.050(2)i	0	0.425(17)i
		Riceberry	75	0.300(12)i	0	0	0.300(12)i
		Riceberry	100	0.350(14)i	0	0	0.350(14)i
		Black glutinous rice	50	0.425(17)i	0.025(1)i	10.025(1)i	0.475(19)i
		Black glutinous rice	75	0.325(13)i	0	10.025(1)i	0.350(14)i
		Black glutinous rice	100	0.375(15)i	0.050(2)i	0	0.425(17)i
	Fermented rice	Sung Yod	50	0.275(11)i	0.050(2)i	0	0.325(13)i
		Sung Yod	75	0.300(12)i	0	0	0.300(12)i
		Sung Yod	100	0.225(9)i	0.025(1)i	0.025(1)i	0.275(11)i
		Mon Poo	50	0.250(10)i	0	0	0.250(10)i
		Mon Poo	75	0.325(13)i	0.025(1)i	0.025(1)i	0.375(15)i
		Mon Poo	100	0.400(16)i	0.025(1)i	0.025(1)i	0.450(18)i

**Table 5.3** Wing spot test data obtained with unfermented or fermented pigmented rice (cont.).

Trial	Treatment	Variety of rice	% Substitution for corn flour	Spots per wing (No. of spots from 40 wings)*			
				Small single m=2	Large single m=5	Twin single m=5	Total m=2
		Hom Mali Daeng	50	0.600(24)i	0	0	0.600(24)i
		Hom Mali Daeng	75	0.475(19)i	0.025(1)i	0.025(1)i	0.525(21)i
		Hom Mali Daeng	100	0.425(17)i	0	0	0.425(17)i
		Hom Nil	50	0.525(21)i	0.025(1)i	0	0.550(22)i
		Hom Nil	75	0.350(14)i	0	0.025(1)i	0.375(15)i
		Hom Nil	100	0.400(16)i	0.025(1)i	0	0.425(17)i
		Riceberry	50	0.425(17)i	0.025(1)i	0.025(1)i	0.475(19)i
		Riceberry	75	0.425(17)i	0.050(2)i	0	0.475(19)i
		Riceberry	100	0.475(19)i	0.025(1)i	0	0.500(20)i
		Black glutinous rice	50	0.575(23)i	0	0	0.575(23)i
		Black glutinous rice	75	0.300(12)i	0.025(1)i	0.025(1)i	0.350(14)i
		Black glutinous rice	100	0.425(17)i	0	0	0.425(17)i

**Table 5.3** Wing spot test data obtained with unfermented or fermented pigmented rice (cont.).

Trial	Treatment	Variety of rice	% Substitution for corn flour	Spots per wing (No. of spots from 40 wings)*			
				Small single m=2	Large single m=5	Twin single m=5	Total m=2
2	Distilled Water	-	-	0.400(16)	0	0	0.400(16)
	20 mM Urethane	-	-	14.475(579)+	5.454(218)+	2.800(112)+	22.725(909)+
	Unfermented rice	Sung Yod	50	0.375(15)-	0	0.025(1)i	0.400(16)-
		Sung Yod	75	0.450(18)i	0	0	0.450(18)i
		Sung Yod	100	0.500(20)i	0.025(1)i	0	0.525(21)i
		Mon Poo	50	0.425(17)i	0.025(1)i	0.025(1)i	0.475(19)i
		Mon Poo	75	0.575(23)i	0	0	0.575(23)i
		Mon Poo	100	0.550(22)i	0	0	0.550(22)i
		Hom Mali Daeng	50	0.475(19)i	0.025(1)i	0.025(1)i	0.525(21)i
		Hom Mali Daeng	75	0.425(17)i	0.025(1)i	0	0.450(18)i
		Hom Mali Daeng	100	0.650(26)i	0	0	0.650(26)i
		Hom Nil	50	0.400(16)i	0.025(1)i	0.025(1)i	0.450(18)i
		Hom Nil	75	0.600(24)i	0	0	0.600(24)i
		Hom Nil	100	0.500(20)i	0	0	0.500(20)i

**Table 5.3** Wing spot test data obtained with unfermented or fermented pigmented rice (cont.).

Trial	Treatment	Variety of rice	% Substitution for corn flour	Spots per wing (No. of spots from 40 wings)*			
				Small single m=2	Large single m=5	Twin single m=5	Total m=2
		Riceberry	50	0.425(17)i	0.050(2)i	0.025(1)i	0.500(20)i
		Riceberry	75	0.475(19)i	0.025(1)i	0	0.500(20)i
		Riceberry	100	0.400(16)i	0.050(2)i	0.025(1)i	0.475(19)i
		Black glutinous rice	50	0.425(17)i	0.025(1)i	0.025(1)i	0.475(19)i
		Black glutinous rice	75	0.550(22)i	0.050(2)i	0	0.600(24)i
		Black glutinous rice	100	0.400(16)i	0.025(1)i	0	0.425(17)i
	Fermented rice	Sung Yod	50	0.425(17)i	0.025(1)i	0	0.450(18)i
		Sung Yod	75	0.525(21)i	0	0	0.525(21)i
		Sung Yod	100	0.350(14)i	0.050(2)i	0	0.400(16)i
		Mon Poo	50	0.400(16)i	0.025(1)i	0	0.425(17)i
		Mon Poo	75	0.475(19)i	0	0	0.475(19)i
		Mon Poo	100	0.500(20)i	0	0	0.500(20)i

**Table 5.3** Wing spot test data obtained with unfermented or fermented pigmented rice (cont.).

Trial	Treatment	Variety of rice	% Substitution for corn flour	Spots per wing (No. of spots from 40 wings)*			
				Small single m=2	Large single m=5	Twin single m=5	Total m=2
		Hom Mali Daeng	50	0.425(17)i	0	0	0.425(17)i
		Hom Mali Daeng	75	0.375(15)i	0.025(1)i	0	0.400(16)i
		Hom Mali Daeng	100	0.450(18)i	0.075(3)i	0	0.525(21)i
		Hom Nil	50	0.475(19)i	0.025(1)i	0	0.500(20)i
		Hom Nil	75	0.575(23)i	0.050(2)i	0	0.625(25)i
		Hom Nil	100	0.400(16)i	0.025(1)i	0.025(1)i	0.450(18)i
		Riceberry	50	0.450(18)i	0	0	0.450(18)i
		Riceberry	75	0.525(21)i	0	0	0.525(21)i
		Riceberry	100	0.400(16)i	0.050(2)i	0	0.450(18)i
		Black glutinous rice	50	0.350(14)i	0.025(1)i	0.050(2)i	0.425(17)i
		Black glutinous rice	75	0.450(18)i	0	0	0.450(18)i
		Black glutinous rice	100	0.450(18)i	0	0.025(1)i	0.475(19)i

\*Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with negative control: + = positive; - = negative; i = inconclusive; Probability level:  $\alpha = \beta = 0.05$ . One-sided statistical tests.

### **5.3 Antimutagenicity of Six Cultivars of Pigmented Rice**

Fermented rice gave greater inhibitory effect than that of unfermented rice of the same variety in both trials. Table 5.4 (also was transformed to be Figure 5.3) shows that unfermented rice namely, Sung Yod, Mon Poo, Hom Mali Daeng, and Hom Nil varieties exhibited negligible antimutagenicity ( $< 20\%$  inhibition) while Riceberry and black glutinous rice had weak antimutagenicity (20-40% inhibition) in both trials 1 and 2. Being fermented, the antimutagenicity of each rice increased e.g. Mon Poo, Hom Nil, and Hom Mali Daeng turned to be weakly antimutagenic (20-40% inhibition) while Sung Yod, Riceberry and black glutinous rice turned to be moderately antimutagenic (40-60% inhibition) in both trials 1 and 2.

**Table 5.4** Antimutagenicity against urethane in *Drosophila melanogaster* of unfermented and fermented pigmented rice.

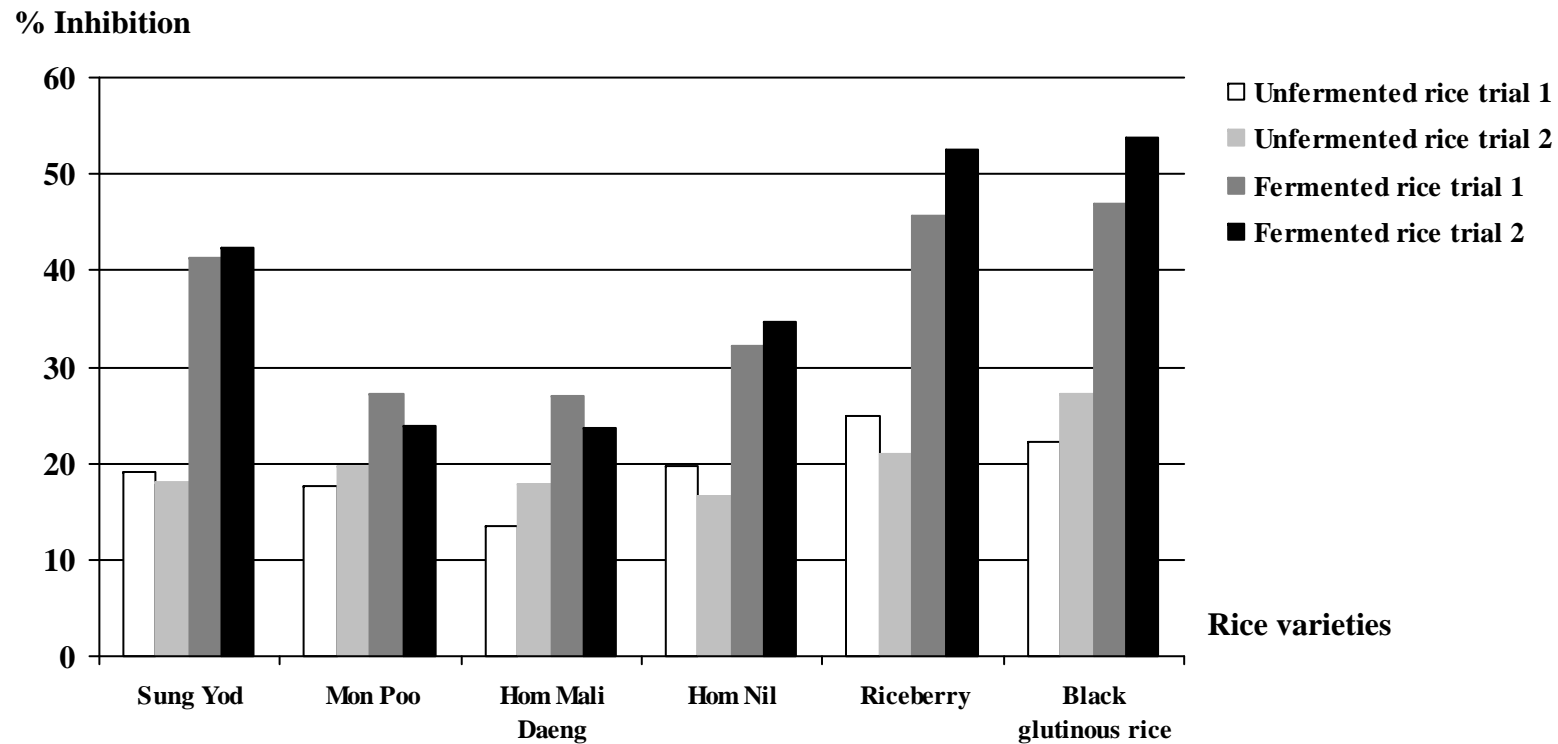
Trial	Treatment	Variety of rice	Spots per wing (No. of spot from 40 wings) <sup>1</sup>				% inhibition <sup>2</sup>
			Small single m=2	Large single m=5	Twin single m=5	Total m=2	
1	Distilled water	-	0.500(20)	0.050(2)	0.025(1)	0.575(23)	-
	20 mM urethane	-	14.725(589)+	5.050(202)+	2.350(94)+	22.125(885)+	-
	Unfermented rice <sup>3</sup>	Sung Yod	12.300(492)+	3.700(148)+	1.900(76)+	17.900(716)+	19.10
		Mon Poo	12.250(490)+	3.725(149)+	2.250(90)+	18.225(729)+	17.63
		Hom Mali Daeng	13.075(523)+	4.075(163)+	2.00(80)+	19.150(766)+	13.45
		Hom Nil	12.525(501)+	3.175(127)+	2.050(82)+	17.750(710)+	19.77
		Riceberry	12.500(500)+	2.525(101)+	1.600(64)+	16.625(665)+	24.86
		Black glutinous rice	12.900(516)+	2.800(112)+	1.525(61)+	17.225(689)+	22.15
	Fermented rice <sup>3</sup>	Sung Yod	9.650(386)+	2.225(89)+	1.100(44)+	12.975(519)+	41.36
		Mon Poo	12.025(481)+	3.00(120)+	1.100(44)+	16.125(645)+	27.12
		Hom Mali Daeng	10.850(434)+	3.350(134)+	1.950(78)+	16.150(646)	27.01
		Hom Nil	11.100(444)+	2.250(90)+	1.650(66)+	15.00(600)+	32.20
		Riceberry	8.550(342)+	2.725(109)+	0.725(29)+	12.00(480)+	45.76
		Black glutinous rice	8.225(329)+	2.375(95)+	1.150(46)+	11.750(470)+	46.89

**Table 5.4** Antimutagenicity against urethane in *Drosophila melanogaster* of unfermented and fermented pigmented rice (cont.).

Trial	Treatment	Variety of rice	Spots per wing (No. of spot from 40 wings) <sup>1</sup>				% inhibition <sup>2</sup>
			Small single m=2	Large single m=5	Twin single m=5	Total m=2	
2	Distilled water	-	0.575(23)	10.025(1)	-	0.600(24)	-
	20 mM urethane	-	12.550(502)+	4.600(184)+	2.225(89)+	19.375(775)+	-
	Unfermented rice <sup>3</sup>	Sung Yod	11.750(470)+	2.600(104)+	1.525(61)+	15.875(635)+	18.06
		Mon Poo	13.275(531)+	1.550(62)+	0.725(29)+	15.550(622)+	19.74
		Hom Mali Daeng	11.375(455)+	3.200(128)+	1.350(54)+	15.592(637)+	17.81
		Hom Nil	12.125(485)+	2.825(113)+	12.00(48)+	16.150(646)+	16.65
		Riceberry	10.150(406)+	3.750(150)+	1.400(56)+	15.300(612)+	21.03
		Black glutinous rice	9.550(382)+	2.925(117)+	1.650(66)+	14.125(565)+	27.10
	Fermented rice <sup>3</sup>	Sung Yod	8.075(323)+	1.950(78)+	1.125(45)+	11.150(446)+	42.45
		Mon Poo	9.525(381)+	3.675(147)+	1.550(62)+	14.750(590)+	23.87
		Hom Mali Daeng	9.050(362)+	3.425(137)+	2.300(92)+	14.775(591)+	23.74
		Hom Nil	9.657(387)+	1.850(74)+	1.150(46)+	12.675(507)+	34.58
		Riceberry	6.875(275)+	1.175(47)+	1.150(46)+	9.200(368)+	52.52
		Black glutinous rice	5.550(222)+	2.300(92)+	1.100(44)+	8.950(358)+	53.80

<sup>1</sup>Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with negative control: + = positive; - = negative; i = inconclusive; Probability level:  $\alpha = \beta = 0.05$ . One-sided statistical tests. <sup>2</sup>Percentage of inhibition between 0-20, 20-40, 40-60 and more than 60 are the evidences of negligible, weak, moderate, and strong antimutagenicity, respectively. <sup>3</sup>Each dried fermented rice was substituted for 100 % corn flour in the standard medium.





**Figure 5.3** Antimutagenicity effect of unfermented and fermented pigmented rice against urethane in SMART. It is proposed that percentage of inhibition between 0–20, 20–40, 40–60 and higher than 60 indicates negligible, weak, moderate and strong antimutagenicity, respectively (Abraham, 1994).

## 5.4 The Optimal Fermented Rice for Using in the Formulation of a New Functional Food Product

### 5.4.1 Ranking and Scoring of the Dried Fermented Rice

All types of dried fermented rice were ranked from the highest score of 6 to the lowest score of 1 according to the results of total phenolic content, anthocyanin content, antioxidant activities or antimutagenicity. All the scoring values of each sample were summed. The fermented black glutinous rice that has the highest summed score was determined for its nutritive values and used in the formulation of a new functional food product namely, cereal bar.

**Table 5.5** Rank and score of total phenolic content, anthocyanin content, antioxidant activities, and antimutagenicity of each dried fermented rice.

Fermented rice	Score <sup>1</sup>					Sum score
	Total phenolics	Anthocyanins	DPPH	FRAP	% inhibition	
Sung Yod	3	2	2	2	4	13
Mon Poo	1	1	3	3	2	10
Hom Mali Daeng	2	3	4	4	1	14
Hom Nil	4	4	1	1	3	13
Riceberry	6	5	5	5	5	26
Black glutinous rice	5	6	6	6	6	29

<sup>1</sup>To rank the samples, from the highest score of 6 to the lowest score of 1, according to the result obtained from tables 5.1 and 5.4.

### 5.4.2 Nutritive Values of Selected Pigmented Rice

Fermented black glutinous rice has the highest summed score concerning total phenolic content, anthocyanin content, antioxidant activities, and antimutagenicity. Therefore, it was selected to be analyzed for its nutritive values as reported in Table 5.6. The nutritive values of unfermented cooked rice were also

determined for comparison. It was noted that cooked rice contained higher amount (about 2%) of carbohydrate than that of fermented rice.

**Table 5.6** Nutritive values of cooked and fermented black glutinous rice

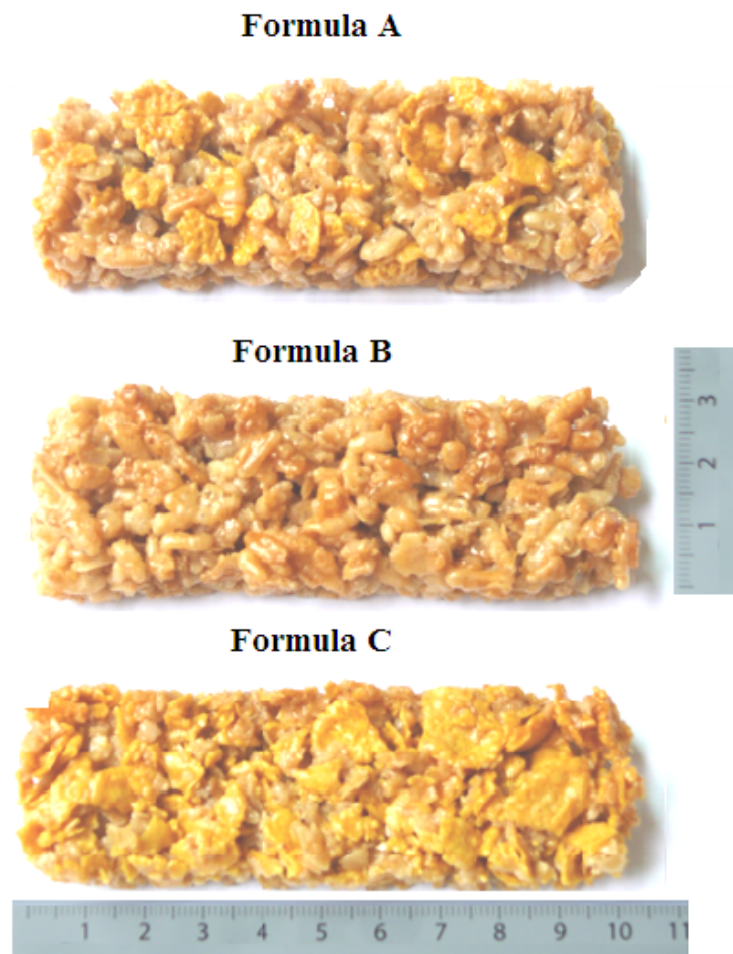
Composition	Amount <sup>1</sup> (g per 100 g dry weight)	
	Fermented rice	Cooked rice
Moisture (g)	4.1	3.8
Ash (g)	1.6	1.4
Protein (g)	11.6	10.4
Fat (g)	4.5	3.8
Carbohydrate (g)	82.3	84.5
Energy (calorie)	416	412

<sup>1</sup> Results are mean of duplicate analyses.

## 5.5 Cereal Bar Containing Dried Fermented Rice

### 5.5.1 Preparation of Control Cereal Bar

The control cereal bar was prepared as suggested by Ryland *et al.* (2010) with minor modification. Three formulas (A, B and C) of the control cereal bar were evaluated in a sensory screening test in order to determine their acceptability. Table 5.7 shows that the mean values of general appearance, color, overall acceptability, odor, taste, and texture scores were not significantly different. Therefore, formula C was selected as the control formula because it had the highest (7.0) overall acceptability score. The appearance of control cereal bars is shown in Figure 5.4.



**Figure 5.4** Appearance of control cereal bars.

**Table 5.7** Sensory acceptability scores of cereal bars (control formula)<sup>1,2,3</sup>.

Formula	Before testing		After testing			
	General appearance	Color	Overall acceptability	Odor	Taste	Texture
A	6.9±1.4 <sup>a</sup>	7.1±1.3 <sup>a</sup>	6.9±1.5 <sup>a</sup>	6.7±1.5 <sup>a</sup>	6.6±1.4 <sup>a</sup>	6.5±1.8 <sup>a</sup>
B	6.7±1.5 <sup>a</sup>	6.6±1.5 <sup>a</sup>	6.7±1.7 <sup>a</sup>	6.5±1.4 <sup>a</sup>	6.5±1.6 <sup>a</sup>	6.2±2.2 <sup>a</sup>
C	7.0±1.3 <sup>a</sup>	6.8±1.5 <sup>a</sup>	7.0±1.4 <sup>a</sup>	6.7±1.5 <sup>a</sup>	6.8±1.5 <sup>a</sup>	6.7±1.5 <sup>a</sup>

<sup>1</sup>Values are presented as means±SD from randomized complete block design (n = 30).

<sup>2</sup>Data with different lower case letters indicate significant differences (p < 0.05) among formulas within the same characteristic.

<sup>3</sup>Nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

### **5.5.2 Cereal Bar Containing Fermented Black Glutinous Rice**

The fermented black glutinous rice was substituted for corn flakes and crispy rice of the control cereal bar (Table 4.1). Other ingredients were kept constant in odor to focus mainly on the effect of dried fermented rice on the quality of cereal bar. The appearance of cereal bars containing dried fermented black glutinous rice is shown in Figure 5.5.

#### **5.5.2.1 Sensory Screening Test on New Formulated Cereal Bar**

The cereal bar containing dried fermented black glutinous rice was evaluated using the sensory screening test to obtain the level of dried fermented black glutinous rice that could be introduced into the new product. Table 5.8 shows the sensory acceptability scores from a screening test of the cereal bars containing dried fermented black glutinous rice. It is indicated that the addition of dried fermented black glutinous rice affected on general appearance, color, overall acceptability, sweet, odor, and texture scores. The acceptability scores namely, general appearance, overall acceptability, and odor decreased as the percentage of dried fermented black glutinous rice increased. The mean scores of color, sweetness, and texture indicated that formula D was appropriate. Therefore, the formulation D was selected for in-house consumer test and storage test since it had the highest overall acceptability score (7.0 overall acceptability score). It was called “new cereal bar”

#### **5.5.2.2 In-House Consumer Test**

An in-house consumer test was performed to compare the acceptability between control cereal bar (formula C) and new cereal bar (formula D). Sensory acceptability scores shown in Table 5.9 indicates that the score of general appearance, color, overall acceptability, taste, and texture scores were not significantly different. However, the score of odor of formula D was significantly lower than that of the control cereal bar (formula C). It is noted that addition of dried fermented black glutinous rice had effect on odor score.

**Formula D**



**Formula E**



**Formula F**



**Figure 5.5** Appearance of cereal bars containing dried fermented black glutinous rice.

**Table 5.8** Sensory acceptability scores from a screening test of the cereal bars containing dried fermented black glutinous rice<sup>1,2</sup>.

Formula	Before testing		After testing			
	General appearance <sup>3</sup>	Color <sup>4</sup>	Overall acceptability <sup>3</sup>	Sweet <sup>4</sup>	Odor <sup>3</sup>	Texture <sup>4</sup>
D	7.1±1.3 <sup>a</sup>	3.2±0.5	7.1±1.3 <sup>a</sup>	3.2±0.7	6.5±1.3 <sup>a</sup>	3.1±0.5
E	6.4±1.5 <sup>a</sup>	3.4±0.7 <sup>*</sup>	6.0±1.8 <sup>b</sup>	3.3±0.6 <sup>*</sup>	6.2±1.6 <sup>a</sup>	3.5±0.9 <sup>*</sup>
F	5.4±1.6 <sup>b</sup>	4.0±1.0 <sup>*</sup>	5.9±1.7 <sup>b</sup>	3.4±0.9 <sup>*</sup>	6.1±1.4 <sup>a</sup>	2.4±0.9 <sup>*</sup>

<sup>1</sup>Values are presented as means±SD from randomized complete block design (n = 30).

<sup>2</sup>Data with different lower case letters indicate significant differences (p < 0.05) among formulas within the same characteristic.

<sup>3</sup>Nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

<sup>4</sup>Five-point just-about-right scale (1 = much too little, 3 = just-about- right, 5 = much too much)

<sup>\*</sup>Significant difference (p < 0.05) from the score of 3 (just-about-right).



**Table 5.9** Sensory acceptability scores between control cereal bar and new cereal bar <sup>1,2,3</sup>.

Formula <sup>4</sup>	Before testing		After testing			
	General appearance	Color	Overall acceptability	Odor	Taste	Texture
C (Control)	6.2±1.7 <sup>a</sup>	6.2±1.8 <sup>a</sup>	6.1±1.7 <sup>a</sup>	6.2±1.7 <sup>a</sup>	6.3±1.8 <sup>a</sup>	5.9±2.1 <sup>a</sup>
D	6.3±1.8 <sup>a</sup>	6.3±1.8 <sup>a</sup>	6.1±1.8 <sup>a</sup>	5.6±1.8 <sup>b</sup>	6.0±2.0 <sup>a</sup>	6.1±1.8 <sup>a</sup>

<sup>1</sup>Values are presented as means±SD from randomized complete block design (n = 50).

<sup>2</sup>Data with different lower case letters indicate significant differences (p < 0.05) between formulas within the same characteristic.

<sup>3</sup>Nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

<sup>4</sup>Formulation corresponded to the alphabet shown in Tables 4.1 and 4.3.

## **5.6 Characteristics of Cereal Bar Containing Dried Fermented black glutinous Rice**

### **5.6.1 Physical Properties**

The physical properties including color, water activity, and texture of the cereal bar containing dried fermented rice (formulas D, E and F) and those of the control cereal bar (formula C) were determined. The results are shown in Table 5.10. It reported that the color values ( $L^*$ ,  $a^*$  and  $b^*$ ) decreased with an increasing level of dried fermented black glutinous rice. The water activity values of all cereal bars containing dried fermented black glutinous rice and the control cereal bar were significantly different. It was noted that water activity values increased corresponding with an increase in the amount of dried fermented black glutinous rice. Dried fermented black glutinous rice was found to be an important factor that influenced of hardness. The higher the dried fermented black glutinous rice content, the lower the hardness. There was no significant difference in fracturability force between the control cereal bar and the cereal bar containing dried fermented black glutinous rice.

**Table 5.10** Physical properties of the control cereal bar and cereal bars containing different percentage of dried fermented rice<sup>1,2</sup>.

Physical property	Formula <sup>3</sup>			
	C	D	E	F
Color value <sup>4</sup>				
L*	65.05±0.60 <sup>a</sup>	42.75±0.59 <sup>b</sup>	37.68±0.19 <sup>c</sup>	27.47±0.45 <sup>d</sup>
a*	+4.97±0.09 <sup>a</sup>	+4.89±0.27 <sup>ab</sup>	+4.45±0.10 <sup>bc</sup>	+4.11±0.12 <sup>c</sup>
b*	+32.89±0.73 <sup>a</sup>	+14.91±0.23 <sup>b</sup>	+9.27±0.51 <sup>c</sup>	+2.40±0.41 <sup>d</sup>
Water activity	0.49±0.01 <sup>a</sup>	0.51±0.01 <sup>b</sup>	0.54±0.01 <sup>c</sup>	0.59±0.01 <sup>d</sup>
Texture				
Hardness force (g)	1138±4 <sup>a</sup>	647±1 <sup>b</sup>	301±4 <sup>c</sup>	223±2 <sup>d</sup>
Fracturability distance (mm) <sup>5</sup>	4.0±0.2 <sup>a</sup>	3.9±0.4 <sup>a</sup>	3.0±0.1 <sup>a</sup>	3.6±0.1 <sup>a</sup>

<sup>1</sup>Values are presented as means±SD (n = 15 samples).

<sup>2</sup>Data with different lower case letters indicate significant differences (p < 0.05) among formulas within the same property.

<sup>3</sup>Formula C: control cereal bar, Formulas D, E and F: containing dried fermented black glutinous rice 14.75%, 22.13% and 29.51% w/w, respectively.

<sup>4</sup>L\*= lightness (0: black; 100: light), a\*= -a: greenness; +a: redness, b\*= -b: blueness; +b: yellowness.

<sup>5</sup>The shorter distance (mm) indicates the greater fracturability.

### 5.6.2 Total Phenolic content and Anthocyanin Content, and Antioxidant Activities

It was observed that the new cereal bar (formula D) had significantly higher total phenolic content, anthocyanin content, and antioxidant activities (DPPH antioxidant activity and FRAP values) than those of the control cereal bar (formula C) approximately 4, 2, 2, and 4 folds, respectively (Table 5.11).

**Table 5.11** Total phenolic content, anthocyanin content, and antioxidant activities of the control cereal bar and new cereal bar<sup>1,2</sup>.

Property	Cereal bars formula <sup>3</sup>	
	C	D
Total phenolics <sup>4</sup>	92.3±5.1 <sup>b</sup>	371.5±14.8 <sup>a</sup>
Anthocyanins <sup>5</sup>	1.9±0.1 <sup>b</sup>	3.5±0.1 <sup>a</sup>
DPPH <sup>6</sup>	0.3±0.1 <sup>b</sup>	0.7±0.1 <sup>a</sup>
FRAP <sup>7</sup>	0.2±0.1 <sup>b</sup>	0.8±0.1 <sup>a</sup>

<sup>1</sup>Values are presented as means±SD (n = 3 samples).

<sup>2</sup>Data with different lower case letters indicate significant differences (p < 0.05) between formulas within the same property

<sup>3</sup>Formula C: control cereal bar, Formula D: new cereal bar

<sup>4</sup>Express as mg gallic acid equivalent/100 g dry weight.

<sup>5</sup>Express as mg cyanidin 3-glucoside/100 g dry weight.

<sup>6</sup>Express as mmol Trolox equivalent/100 g dry weight.

<sup>7</sup>Express as mmol Fe(II)/100 g dry weight.

### 5.6.3 Antimutagenicity of Cereal Bar

Table 5.12 shows the number of surviving adult flies obtained from the larvae brought up on negative control medium, positive control medium, control cereal bar, and new cereal bar. The percentages of surviving adult flies are higher than 50%. The results indicated that all samples were non-toxic for mutagenicity testing. The results of Table 5.13 indicate that all samples were not mutagenic since they did not induce the frequencies of mutant spots, at any testing concentrations, to be higher than that of the negative control group. The highest substitution for 100% corn flour in the fly medium of the sample providing more than 50% survival of flies was determined for its antimutagenicity.

**Table 5.12** The percentage of surviving adult flies fed on different media<sup>1</sup>.

Treatment	% Substitution for corn flour in the standard medium	Number of surviving adult flies	
		Trial 1	Trial 2
Distilled water	-	97	88
20 mM urethane	-	82	91
Control cereal bar	50	95	79
	75	87	93
	100	93	96
New cereal bar	50	89	84
	75	91	89
	100	95	95

<sup>1</sup>Each sample was substituted for 50, 75 or 100 % corn flour in the standard medium in order to obtain the experimental media.

**Table 5.13** Mutagenicity of the control cereal bar and new cereal bar.

Trial	Treatment	%Substitution for corn flour in the standard medium	Spots per wing (No. of spot from 40 wings) statistic diagnoses <sup>1</sup>			
			Small single M =2	Large single m=5	Twin single m=5	Total m=2
1	Distilled water	-	0.300(12)	0.750(3)	0	0.375(15)
	20 mM urethane	-	10.575(423)+	6.900(276)+	7.375(295)+	24.850(994)+
	Control cereal bar	50%	0.275(11)-	0.100(4)i	0	0.400(16)i
		75%	0.400(16)i	0.025(1)-	0.025(1)i	0.450(18)i
		100%	0.325(13)i	0	0.050(2)i	0.375(15)-
	New cereal bar	50%	0.425(17)i	0.025(1)-	0	0.450(18)i
		75%	0.400(16)i	0.025(1)-	0.025(1)i	0.450(18)i
		100%	0.400(16)i	0.050(2)-	0	0.450(18)i

**Table 5.13** Mutagenicity of the control cereal bar and new cereal bar (cont).

Trial	Treatment	Substitution for corn flour in the standard medium	Spots per wing (No. of spot from 40 wings) statistic diagnoses <sup>1</sup>			
			Small single m=2	Large single m=5	Twin single m=5	Total m=2
2	Distilled water	-	0.125(5)	0.025(1)	0	0.150(6)
	20 mM urethane	-	15.525(621)+	5.075(203)+	4.100(164)+	24.700(988)+
	Control cereal bar	50%	0.150(6)i	0.025(1)i	0	0.175(7)i
		75%	0.175(7)i	0	0.025(1)i	0.200(8)i
		100%	0.200(8)i	0	0	0.200(8)i
	New cereal bar	50%	0.100(4)i	0.025(1)i	0.025(1)i	0.150(6)i
		75%	0.750(3)i	0	0	0.750(3)-
		100%	0.225(9)i	0	0	0.225(9)i

<sup>1</sup>Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with negative control: + = positive; - = negative; i = inconclusive; Probability level:  $\alpha = \beta = 0.05$ . One-sided statistical tests.

#### **5.6.4 Antimutagenicity of the New Cereal Bar**

The new cereal bar had greater inhibitory effect than that of the control cereal bar in both trials. Table 5.14 shows that the control cereal bar exhibited negligible antimutagenicity (< 20% inhibition) while the new cereal bar had weak antimutagenicity (20-40% inhibition) in both trials 1 and 2.

#### **5.6.5 Nutritive values**

The control cereal bar and new cereal bar were analyzed moisture, ash, protein, fat, carbohydrate, energy, soluble and in soluble dietary fiber. The data in Table 5.15 showed that the new cereal bar contained higher amount (about 60%) of total dietary fiber than that of the control cereal bar.



**Table 5.14** Antimutagenicity of the control cereal bar and new cereal bar.

Trial	Treatment	Spots per wing (No. of spots from 40 wings) statistic diagnoses <sup>1</sup>				%Inhibition <sup>2</sup>
		Small single m=2	Large single m=5	Twin single m=5	Total m=2	
1	Distilled water	0.300(12)	0.025(1)	0.025(1)	0.350(14)	-
	20 mM Urethane	14.475(579)+	6.200(248)+	3.150(126)+	23.825(953)+	-
	Control cereal bar <sup>3</sup>	16.900(676)+	3.875(155)+	2.850(114)+	23.625(945)+	0.84
	New cereal ba <sup>3</sup>	11.400(546)+	3.025(121)+	1.600(64)	16.025(641)+	32.74
2	Distilled water	0.250(10)	0.500(2)	0.750(3)	0.375(15)	-
	20 mM Urethane	16.575(663)+	5.150(206)+	1.925(77)+	23.650(946)+	-
	Control cereal bar <sup>3</sup>	16.250(650)+	4.325(173)+	4.425(117)+	23.500(940)+	0.63
	New cereal bar <sup>3</sup>	11.275(451)+	2.675(107)+	1.475(59)+	15.425(617)+	34.36

<sup>1</sup>Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with negative control: + = positive; - = negative; i = inconclusive; Probability level:  $\alpha = \beta = 0.05$ . One-sided statistical tests.

<sup>2</sup>Percentage of inhibition between 0-20, 20-40, 40-60 and more than 60 are the evidences of negligible, weak, moderate, and strong antimutagenicity, respectively.

<sup>3</sup>Substituted for 100% corn flour in the standard medium.

**Table 5.15** Nutritive values of the control cereal bar and new cereal bar (g/serving)<sup>1,2</sup>.

Composition	Cereal bar formula	
	Control	New cereal bar
Moisture	3.1	3.1
Ash	0.6	0.5
Protein	2.6	2.9
Fat	5.1	5.3
Carbohydrate	28.5	28.2
Total dietary fiber	0.8	1.4
Soluble dietary fiber	0.04	0.6
Insoluble dietary fiber	0.8	0.8
Energy (calorie)	171	172

<sup>1</sup>Results are means of duplicate analyses.<sup>2</sup>40g/serving size according to regulation number 182 of the Thai FDA.

## 5.7 Effect of Storage on Various Properties of the New Cereal Bar

The new cereal bars were kept in vacuum-sealed laminated aluminum foil bags at ambient temperature (approximately 28°C). The sensory acceptability of the new cereal bars stored during 0-90 days (Table 5.16) indicated that general appearance, color, overall acceptability, odor, taste, and texture scores were not significantly different.

During storage for 90 days, the color, water activity, and texture of the new cereal bar were determined. The results are shown in Table 5.17. It reported that the color values (L\*, a\* and b\*) and water activity were not significantly different. The hardness of the product increased as the period of storage was extended while the fracturability decreased along the storage. The total phenolic content, anthocyanin content, and antioxidant activities (DPPH antioxidant activity and FRAP values) did not change during storage for 90 days. The results are reported in Table 5.18 and Figures 5.6 and 5.7.

**Table 5.16** Sensory acceptability scores of the new cereal bar during storage for 90 days<sup>1,2,3</sup>.

Storage time (days)	Before testing		After testing			
	General appearance	Color	Overall acceptability	Odor	Taste	Texture
0	7.1±1.3 <sup>a</sup>	7.1±1.3 <sup>a</sup>	6.9±1.4 <sup>a</sup>	6.6±1.8 <sup>a</sup>	6.7±1.6 <sup>a</sup>	6.9±1.5 <sup>a</sup>
45	6.8±1.2 <sup>a</sup>	6.8±1.4 <sup>a</sup>	6.8±1.7 <sup>a</sup>	6.6±1.5 <sup>a</sup>	6.7±1.3 <sup>a</sup>	6.6±1.3 <sup>a</sup>
90	6.2±1.6 <sup>a</sup>	6.6±1.7 <sup>a</sup>	6.6±1.4 <sup>a</sup>	6.3±1.5 <sup>a</sup>	6.3±1.9 <sup>a</sup>	6.2±1.6 <sup>a</sup>

<sup>1</sup>Values are presented as means±SD from randomized complete block design (n = 30).

<sup>2</sup>Data with different lower case letters indicate significant differences (p < 0.05) among storage times within the same characteristic.

<sup>3</sup>Nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

**Table 5.17** Physical properties of the new cereal bar during storage for 90 days<sup>1,2</sup>.

Physical property	Storage time (days)		
	0	45	90
Color value <sup>3</sup>			
L*	44.29±0.46 <sup>a</sup>	44.46±1.23 <sup>a</sup>	45.68±1.82 <sup>a</sup>
a*	+4.95±0.21 <sup>a</sup>	+5.04±0.18 <sup>a</sup>	+5.23±0.06 <sup>a</sup>
b*	+15.53±0.93 <sup>a</sup>	+16.95±0.32 <sup>a</sup>	+17.27±1.57 <sup>a</sup>
Water activity	0.51±0.01 <sup>a</sup>	0.51±0.01 <sup>a</sup>	0.52±0.01 <sup>a</sup>
Texture			
Hardness force (g)	1235±3 <sup>c</sup>	2922±2 <sup>b</sup>	3303±3 <sup>a</sup>
Fracturability distance (mm) <sup>4</sup>	3.6±0.4 <sup>a</sup>	2.7±0.2 <sup>b</sup>	2.4±0.1 <sup>b</sup>

<sup>1</sup>Values are presented as means±SD from randomized complete block design (n = 30).

<sup>2</sup>Data with different lower case letters indicate significant differences (p < 0.05) among storage times within the same property.

<sup>3</sup>L\*= lightness (0: black; 100: light), a\*= -a: greenness; +a: redness, b\*= -b: blueness; +b: yellowness.

<sup>4</sup>The shorter distance (mm) indicates the greater fracture.

**Table 5.18** Total phenolic content, anthocyanin content, and antioxidant activities of the new cereal bar during storage for 90 days<sup>1,2</sup>.

Property	Storing time (days)		
	0	45	90
Total phenolic content <sup>3</sup>	370.30±5.48 <sup>a</sup>	356.77±9.46 <sup>a</sup>	353.37±9.30 <sup>a</sup>
Anthocyanin content <sup>4</sup>	3.40±0.10 <sup>a</sup>	3.13±0.15 <sup>a</sup>	3.07±0.15 <sup>a</sup>
DPPH <sup>5</sup>	0.66±0.05 <sup>a</sup>	0.58±0.06 <sup>a</sup>	0.57±0.04 <sup>a</sup>
FRAP <sup>6</sup>	0.82±0.04 <sup>a</sup>	0.81±0.03 <sup>a</sup>	0.80±0.02 <sup>a</sup>

<sup>1</sup>Values are presented as means±SD (n = 3).

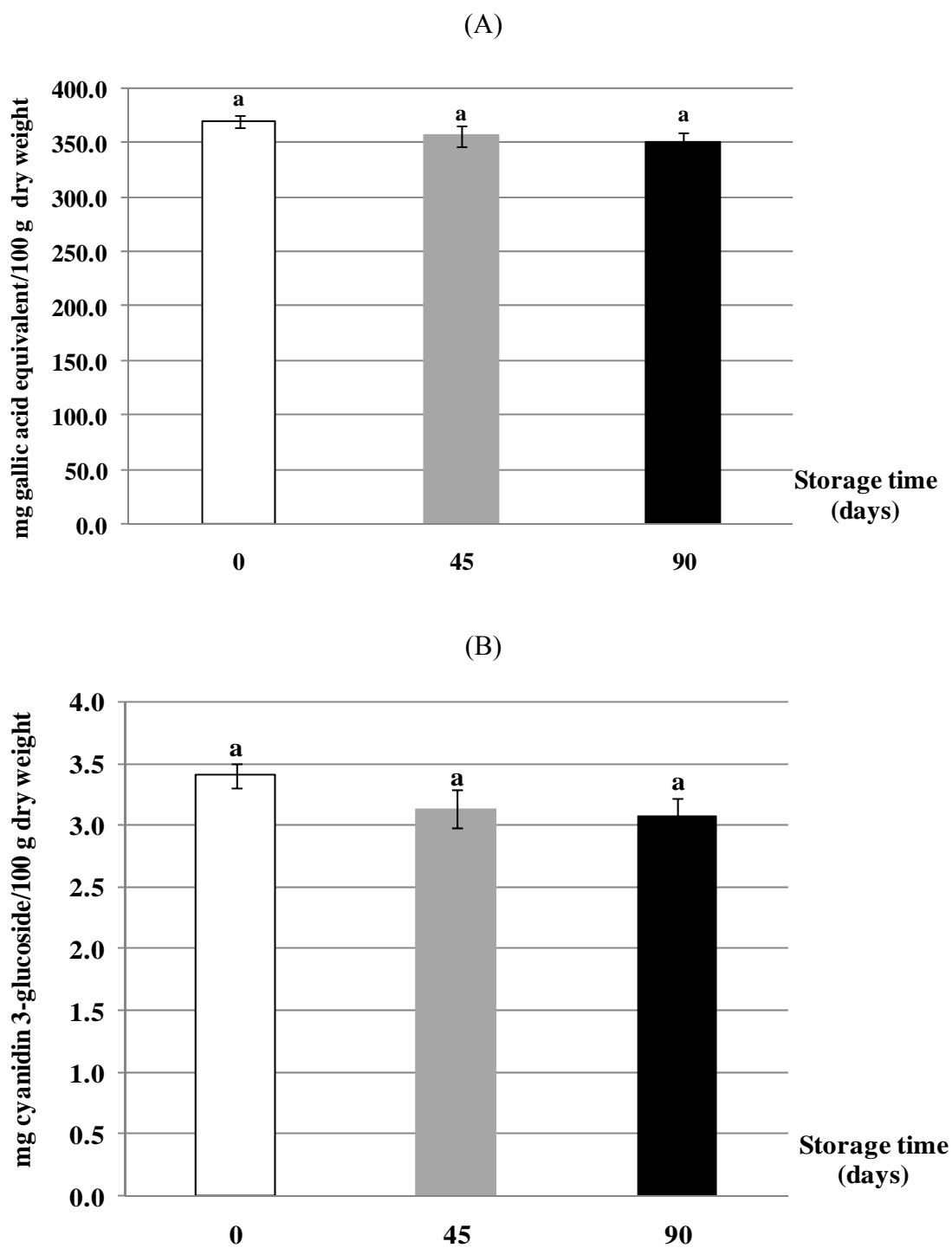
<sup>2</sup>Data with different lower case letters indicate significant differences (p < 0.05) among storage times within the same property.

<sup>3</sup>Express as mg gallic acid equivalent/100 g dry weight.

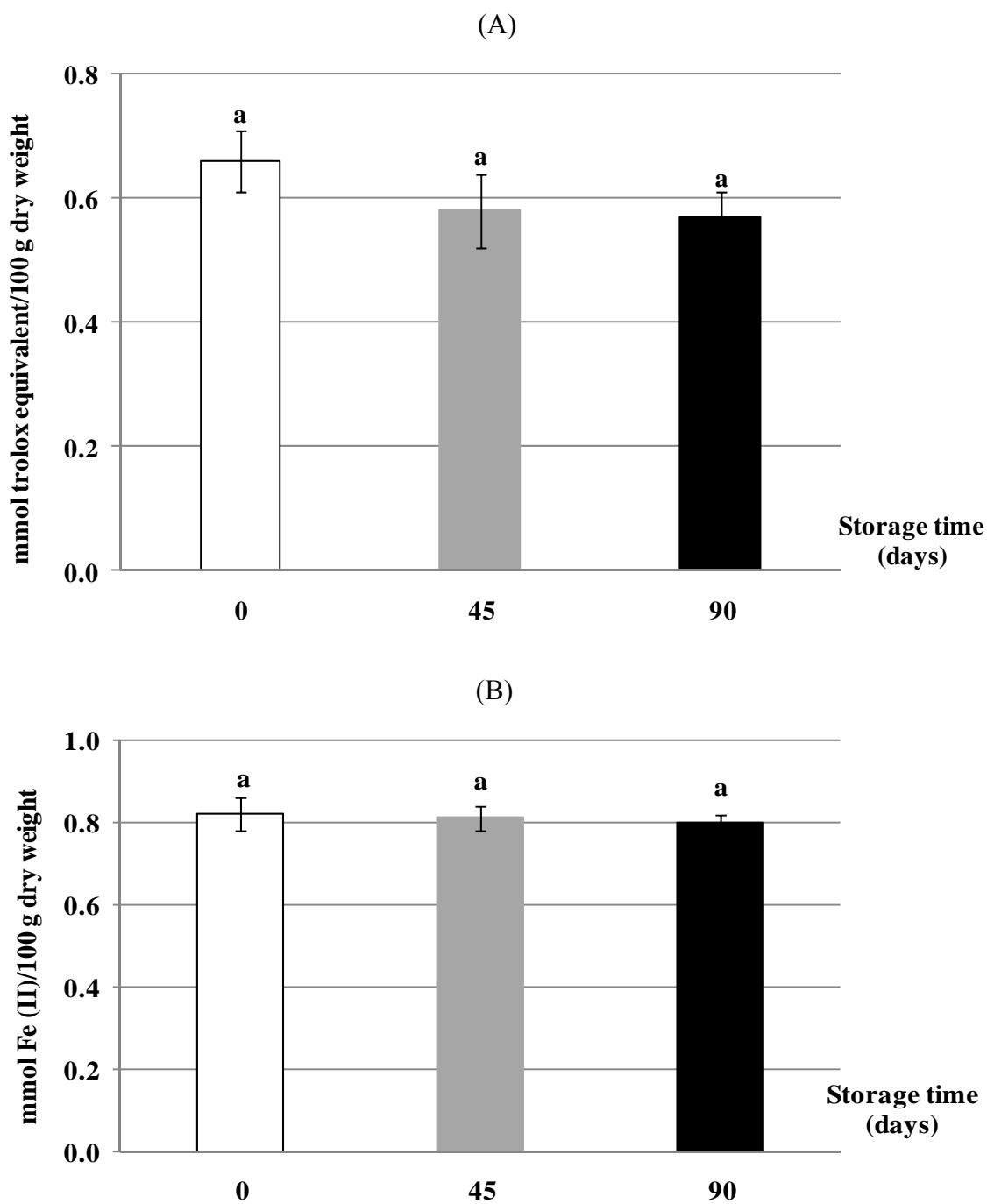
<sup>4</sup>Express as mg cyanidin 3-glucoside/100 g dry weight.

<sup>5</sup>Express as mmol Trolox equivalent/100 g dry weight.

<sup>6</sup>Express as mmol Fe(II)/100 g dry weight.



**Figure 5.6** Total phenolic (A) and anthocyanin (B) contents of new cereal bar during storage for 90 days. Data with different upper case letters indicate significant differences ( $p < 0.05$ ) ( $p < 0.05$ ) among storage times within the same property.



**Figure 5.7** DPPH antioxidant activity (A) and ferric reducing antioxidant power (B) of new cereal bar during storage for 90 days. Data with different upper case letters indicate significant differences ( $p < 0.05$ ) among storage times within the same property.

Table 5.19 shows the number of surviving adult flies obtained from the larvae brought up on negative control medium, positive control medium, and each experimental medium containing the new cereal bar stored for 0, 45 or 90 days. The percentages of surviving adult flies brought up on all media were higher than 50% which indicated that all sample were non-toxic for further mutagenicity study. The results of Table 5.20 indicate that the new cereal bar stored for 0, 45 and 90 days were not mutagenic since they did not induce the frequencies of mutant spots at any testing concentrations to be higher than that of the negative control group. The highest substitution (100%) for corn flour provided higher than 50% survival of flies was determined for its antimutagenicity.

The new cereal bar had weak antimutagenicity (20-40% inhibition) in both trials 1 and 2. The antimutagenic effect slightly decreased with respect to the storage time. The results shown in Table 5.21 (also transformed to be Figure 5.8).



**Table 5.19** The percentage of survival adult flies fed on control and experimental medium<sup>1</sup>.

Storage (days)	Treatment	%Substitution for corn flour in the standard medium	Number of surviving flies	
			Trial 1	Trial 2
0	Distilled water	-	86	89
	20 mM urethane	-	76	85
	New cereal bar	50	79	96
		75	98	91
		100	96	85
45	Distilled water	-	97	83
	20 mM urethane	-	70	77
	New cereal bar	50	71	92
		75	93	88
		100	69	62
90	Distilled water	-	92	77
	20 mM urethane	-	76	67
	New cereal bar	50	97	86
		75	88	97
		100	91	85

<sup>1</sup>Each sample was substituted for 25, 50, 75 and 100 % corn flour in the standard medium in order to obtain the experimental media.

**Table 5.20** Mutagenicity of the new cereal bar stored for 0, 45 or 90 days.

Trial	Storage (days)	Treatment	%Substitution for corn flour in the standard medium	Spots per wing (No. of spots from 40 wings) statistic diagnoses <sup>1</sup>			
				Small single m=2	Large single m=5	Twin single m=5	Total m=2
1	0	Distilled water	-	0.100(4)	0	0	0.100(4)
		20 mM urethane	-	11.450(458)+	2.700(108)+	2.375(95)+	16.525(661)+
		New cereal bar	50	0.225(9)i	0	0	0.225(9)i
			75	0.125(5)i	0	0.050(2)i	0.175(7)i
			100	0.200(8)i	0	0	0.200(8)i
	45	Distilled water	-	0.200(8)	0.025(1)	0	0.225(9)
		20 mM urethane	-	7.975(319)+	7.350(294)+	2.625(105)+	17.950(718)+
		New cereal bar	50	0.225(9)i	0.050(2)i	0	0.275(11)i
			75	0.250(10)i	0	0	0.250(10)i
			100	0.175(7)i	0.025(1)i	0	0.200(8)i

**Table 5.20** Mutagenicity of the new cereal bar stored for 0, 45 or 90 days (cont.).

Trial	Storage (days)	Treatment	%Substitution for corn flour in the standard medium	Spots per wing (No. of spots from 40 wings) statistic diagnoses <sup>1</sup>			
				Small single m=2	Large single m=5	Twin single m=5	Total m=2
2	90	Distilled water	-	0.050(2)	0.025(1)	0.025(1)	0.075(3)
		20 mM urethane	-	12.950(518)+	2.875(115)+	1.725(69)+	17.550(702)+
		New cereal bar	50	0.175(7)i	0	0	0.175(7)i
			75	0.100(4)i	0.025(1)i	0	0.125(5)i
			100	0.075(3)i	0.050(2)i	0	0.125(5)i
	0	Distilled water	-	0.275(11)	0.025(1)	0	0.300(12)
		20 mM urethane	-	9.875(395)+	3.975(159)+	4.550(182)+	18.400(736)+
		New cereal bar	50	0.200(8)-	0	0.050(2)i	0.250(10)-
			75	0.225(9)-	0	0	0.225(9)-
			100	0.325(13)i	0.025(1)i	0	0.350(14)i

**Table 5.20** Mutagenicity of the new cereal bar stored for 0, 45 or 90 days (cont.).

Trial	Storage (days)	Treatment	%Substitution for corn flour in the standard medium	Spots per wing (No. of spots from 40 wings) statistic diagnoses <sup>1</sup>			
				Small single m=2	Large single m=5	Twin single m=5	Total m=2
	45	Distilled water	-	0.325(13)	0.025(1)	0	0.350(14)
		20 mM urethane	-	12.975(519)+	3.575(143)	2.075(83)+	18.625(745)+
		New cereal bar	50	0.475(19)i	0	0	0.475(19)i
			75	0.375(15)i	0.025(1)i	0.025(1)i	0.425(17)i
			100	0.300(12)i	0.025(1)i	0	0.325(13)i
	90	Distilled water	-	0.250(10)	0.025(1)	0	0.275(11)
		20 mM urethane	-	11.700(468)+	4.825(193)+	1.575(63)+	18.100(724)+
		New cereal bar	50	0.450(18)i	0.025(1)i	0	0.475(19)i
			75	0.300(12)i	0	0	0.300(12)i
			100	0.400(16)i	0	0.025(1)i	0.425(17)i

<sup>1</sup>Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with negative control: + = positive; - = negative; i = inconclusive; Probability level:  $\alpha = \beta = 0.05$ . One-sided statistical tests.

**Table 5.21** Antimutagenicity of the new cereal bar stored for 0, 45 and 90 days.

Trial	Storage (days)	Treatment	Spots per wing (No. of spots from 40 wings) statistic diagnoses <sup>1</sup>				% Inhibition <sup>2</sup>
			Small single	Large single	Twin single	Total	
			m=2	m=5	m=5	m=2	
1	0	Distilled water	0.175(7)	0.050(2)	0	0.225(9)	-
		20 mM urethane	14.200(568)+	6.000(240)+	3.150(126)+	23.350(934)+	-
		New cereal bar <sup>3</sup>	11.025(441)+	2.775(111)+	1.600(64)	15.400(616)+	34.05
	45	Distilled water	0.225(9)	0	0.025(1)	0.250(10)	-
		20 mM urethane	19.000(760)+	7.450(298)+	3.125(125)+	29.575(1183)+	-
		New cereal bar <sup>3</sup>	15.700(628)+	3.625(145)+	3.675(147)+	23.000(920)+	22.23
	90	Distilled water	0.400(16)	0	0	0.400(16)	-
		20 mM urethane	17.275(691)+	5.975(239)+	2.450(98)	25.700(1028)+	-
		New cereal bar <sup>3</sup>	12.700(508)+	5.125(205)+	2.675(107)+	20.500(820)+	20.23

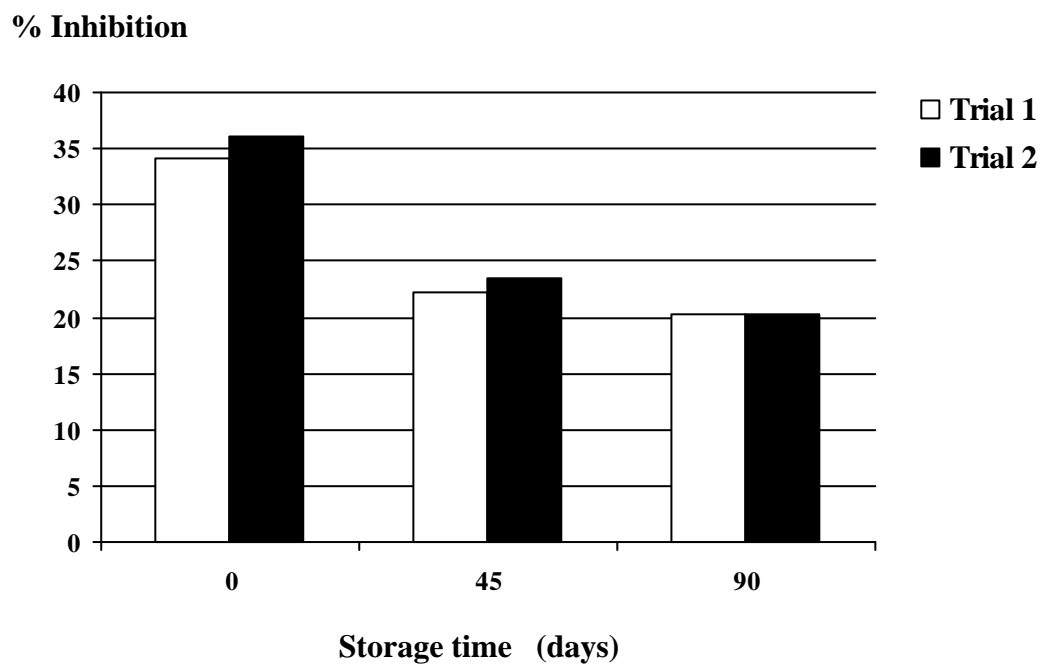
**Table 5.21** Antimutagenicity of the new cereal bar stored for 0, 45 and 90 days (cont.).

Trial	Storage (days)	Treatment	Spots per wing (No. of spot from 40 wings) statistic diagnoses <sup>1</sup>				% Inhibition <sup>2</sup>
			Small single m=2	Large single m=5	Twin single m=5	Total m=2	
2	0	Distilled water	0.45(18)	0.025(1)	0.025(1)	0.500(20)	-
		20 mM urethane	13.100(524)+	5.375(215)+	2.00(80)+	20.475(819)+	-
		New cereal bar <sup>3</sup>	10.775(431)+	1.100(44)+	1.225(49)+	13.00(524)+	36.02
	45	Distilled water	0.175(7)	0.075(3)	0	0.250(10)	-
		20 mM urethane	18.425(737)+	5.975(239)+	1.525(61)+	25.925(1037)+	-
		New cereal bar <sup>3</sup>	10.825(433)+	4.05(162)+	4.815(195)+	19.750(790)+	23.38
	90	Distilled water	0.35(14)	0	0	0.35(14)	-
		20 mM urethane	16.500(660)+	7.300(292)+	3.550(142)+	27.350(1094)+	-
		New cereal bar <sup>3</sup>	13.575(543)+	6.425(257)+	1.800(72)+	21.800(872)+	20.29

<sup>1</sup>Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Wurgler (1988) for comparison with negative control: + = positive; - = negative; i = inconclusive; Probability level:  $\alpha = \beta = 0.05$ . One-sided statistical tests.

<sup>2</sup>Percentage of inhibition between 20, 20-40, 40-60 and more than 60 are the evidences of negligible, weak, moderate, and strong antimutagenicity, respectively.

<sup>3</sup>Each cereal bar was substituted for 100 % corn flour in the standard medium.



**Figure 5.8.** Antimutagenicity effect of the new cereal bar stored for 0, 45 and 90 days against urethane in SMART. It is proposed that percentage of inhibition between 0–20, 20–40, 40–60 and higher than 60 indicates negligible, weak, moderate, and strong antimutagenicity, respectively (Abraham, 1994).