Effect of Preparation and Temperature Treatments on Antimutagenicity against Urethane in *Drosophila melanogaster* and Antioxidant Activity of Three *Allium* Members

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ABSTRACT

We investigated whether the effects of preparations (pounding or chopping) and heat treatments (100°C or 200°C) modified the antimutagenicity of garlic, shallot and onion against urethane induced somatic mutation and recombination in *Drosophila melanogaster*. Three-day old trans-heterozygous larvae (*mwh flr*$^{+}$*/mwh TM3*) were transferred to an experimental medium (containing a treated sample) that had 20 mM urethane. The wings of surviving flies were analyzed for occurrence of mutant spots. The results showed that all treated samples still had both antimutagenicity and antioxidant activity (determined using DPPH scavenging capacity and ferric reducing antioxidant power) and phenolic compounds (determined using Folin-Ciocalteu reagent). Treating garlic with 100°C and 200°C before preparations slightly reduced its antimutagenicity. It was proposed that heat treatment slightly destroyed alliinase; thus, the formation of allicins and other organosulfur compounds (commonly turn to be alkylsulfides or allicin derivatives which are the inducers of phase 2 detoxification system) from alliin was reduced. On the other hand, pounding and chopping before applying heat treatments reduced the antimutagenicity of shallot and onion while heat treatment had lower effect if the samples still be a bulb or cut into large piece. It was proposed that the formation of sulfur containing compounds derived from isoalliin by alliinase during pounding and chopping were very labile to atmosphere during the 10 min standing at room temperature. Thus the effect of preparation and heat treatment unequally influenced on the antimutagenicity and the antioxidant activity including total phenolic compounds of three *Allium* members.

Keywords: Antimutagenicity, SMART, antioxidant activity, allium, processing, heat

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Introduction

Experimental and epidemiological studies provided evidence in support of association between garlic intake and reduced cancer risk[1] including reduction of esophageal, mammary, skin, pulmonary, forestomach, colon, and lung tumors [2-4]. These anticarcinogenic effects, as other biological properties of garlic and onion, are attributed to specific organosulfur compounds present in very high levels in these vegetables. The protective effects of these naturally occurring substances against cancer have been supported by many experimental studies with animal models [5,6]. One mechanism may be the modification of carcinogen metabolism via the modulation of drug-metabolizing enzymes. The organosulfur compounds were shown to alter cytochromes P450 (CYP) and phase II detoxication enzymes in different tissues in rats and mice [7-9]. The organosulfur compounds are efficient inhibitors of CYP2E1 in the rat liver [10,11]. In addition, organosulfur compounds have been reported to increase the activity of the phase II detoxification enzymes in a variety of rat tissues[12,13]. These organosulfur compounds are produced through enzymatic hydrolysis of non-volatile sulfur storage compounds, termed *S*-alk(en)yl-L-cysteine sulfoxides. These compounds were only generated during tissue damage and preparation. However, most studies used the extract or the synthesized chemical rather than the cooked *Alliums* plants simulating home preparation. Therefore, the aim of this study was to investigate the effect of preparations and heat treatments on the antimutagenicity against urethane induced somatic mutation and recombination in *Drosophila melanogaster* and their antioxidant activity of three *Allium* namely, garlic, shallot and onion.

Materials and Methods

Chemicals and Sample preparation: Urethane (URE) was purchased from Sigma Chemical (St. Louis, Mo, USA). Other chemicals were of laboratory grade. Garlic, shallot and onion obtained from a local market at Salaya district were peeled, washed with tap water and drained. The samples were divided into seven groups (Table 1). The first group was studied as raw. Samples in the second and third ones were pounded in a mortar, left at room temperature for 10 min and heated at 100°C or 200°C. In the fourth and fifth ones, we chopped each sample into small pieces (0.5 x 0.5 mm), left at room temperature for 10 min and heated at 100°C or 200°C. In the sixth and seventh groups, no preparation was applied to garlic and shallot whereas onion bulb was cut into 4 large pieces (in order to reduce its size to fit the stainless tube), left at room temperature for 10 min and heated at 100°C or 200°C. The temperatures of 100°C and 200°C represent the temperatures of common boiling and stir-fry in oil, respectively. We put each sample in a clean stainless steel tube and placed it in a water bath (100°C) or oil bath (200°C) for 5 min; then, we placed it in an ice bath and adjusted moisture to its original content.

Table 1 Treatments of samples of this study

Group	Sample	Preparation	Heat treatment	Assigned treatment
1	Garlic, shallot and onion	No	No	raw
2	Garlic, shallot and onion	Pounding	100	pound/T100
3	Garlic, shallot and onion	pounding	200	pound/T200
4	Garlic, shallot and onion	chopping	100	chop/T100
5	Garlic, shallot and onion	chopping	200	chop/T200
6	Garlic and shallot	No	100	T100
	Onion	Cut into 4 big pieces	200	T200
7	Garlic and shallot	No	100	T100
	Onion	Cut into 4 big pieces	200	T200

Experimental Design Each sample (10 g) was stirred 2 times with 80% methanol (25 ml) at room temperature for 1 h. The solution was filtered through cotton mesh and Whatman filter paper No. 1. Each extract was assayed for its antioxidant activity and total phenolic content. The DPPH assay was estimated

using the procedure described by Fukumoto and Mazza [14] with some modifications. The extract was allowed to react with the stable radical (DPPH) in order to evaluate the free radical scavenging activity. After incubation at 37°C for 30 min, the absorbance of the solution was read in a microplate reader. The activity was monitored by the decrease of the absorbance at 520 nm. The ferric reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 600 nm was performed after 8 min using a microplate reader [15]. The total phenolic content of each extract was determined colorimetrically with the Folin–Ciocalteu reagent, according to the method described by Amarowicz *et al.*[16] and modified the procedures of measurement by using a microplate reader. The plate was mixed well and the absorbance of blue colored mixtures was recorded at 750 nm with microplate reader after 30 min incubation.

Virgin females of Oregon wing flare strain (*ORR/ORR*; *flr*^{3}*/TM3, Ser)* were mated with males of multiple wing hair strain *(mwh/mwh)* on regular medium to produce *trans*-heterozygous larvae of improved high bioactivation cross (IHB). Both strains were obtained from the Institute of Toxicology (Swiss Federal Institute of Technology, and the University of Zurich) and maintained on the regular medium as suggested by Roberts[17] that had propionic acid (0.01 ml) as a preservative. The experiments were firstly conducted to obtain the range of suitable concentrations of each treated *Alliums* sample that provided more than 50% survival of adult flies and also explored whether each treated sample was mutagenic in SMART. The ratios of sample to regular medium in the preparation of experimental media used in this investigation were 1:25 w/w and 1:40 w/w for treated garlic and 1:1 w/w and 1:2 w/w for treated shallot and treated onion. Each experimental medium was homogenized using Ultra-Turrax ® T25 basic (IKA Labortechnik, Malaysia) in a beaker placed in an ice bath. The medium (4.0 g) was transferred into a 15-ml test tube for mutagenicity assay. Regular medium was used as a negative control and the regular medium containing urethane (1335 ppm) was used as a positive control. An experimental medium containing URE was used for antimutagenicity study.

The mutagenicity of each sample (in the experimental medium) was assayed as described by Graf *et al*. [18] and the antimutagenicity of each sample was assayed using the experimental medium containing URE. The larvae were maintained on medium at 25±1°C until pupation. The surviving adult flies bearing the marker trans-heterozygous (*mwh+/+flr*^{3}) indicated with round wings were collected. Subsequently, the wings were removed, mounted and scored under a compound microscope for recording of the wing spot. Induction frequencies of wing spots of *Allium* sample treated groups were compared with that of the negative control group. The estimation of spot frequencies and confidence limits of the estimated mutation frequency were performed with significant level of $\alpha = \beta = 0.05$. A multiple-decision procedure was used to decide whether a sample was positive, weak positive, inconclusive or negative mutagen as described by Frei and Wurgler [19].

Antimutagenicity was estimated using percentage of inhibition of total spots per wing calculated as follows: percentage of inhibition = (a-b)/a x 100. Where “a” is the number of total spots per wing induced by URE, “b” is the number of total spots per wing induced with URE administered with each sample. It was proposed that percent of inhibition between 0–20%, 20–40%, 40–60% and higher than 60% were rated as negligible, weak, moderate and strong antimutagenicity, respectively.

Result and Discussion

Antioxidant activity and amount of total phenolic compounds They are shown in Table 2. The reduction of DPPH by antioxidants in the samples is expressed as Trolox equivalent antioxidant capacity (mg TEAC/g wet weight) of garlic, shallot and onion are between 0.10 to 0.31, 0.19 to 0.31 and 0.07 to 0.25, respectively. The FRAP values (mg Fe (II)/g wet weight) of garlic, shallot and onion are between 103.93 to 277.84, 254.76 to 396.35 and 130.87 to 329.39, respectively. Overall results suggest that *Allium* sample that were pounded and heated at 200°C had the highest antioxidant activity. Total phenolic contents (expressed as mg gallic acid/g wet weight) of garlic, shallot and onion are in the range from 85.01 to 192.66, 91.89 to 130.67 and 29.41 to 95.89, respectively. The chopping and heating at 200°C applied to onion and shallot make them

had the highest amount of phenolic compounds while heating at 200°C without pretreatment resulted the all samples had the lowest value.

Table 2 Antioxidant activity and total phenolic content of methanolic extracts of samples.

Sample	Assigned treatment	DPPH assay		FRAP** value	Total phenolic content (GAE***)
		TEAC*	% Scavenging		
Garlic	Raw	0.18	22.41	142.69	171.16
	Pound T100	0.15	18.35	137.04	147.87
	Pound T200	0.31	38.35	277.84	154.14
	Chop T100	0.10	11.81	110.17	105.31
	Chop T200	0.12	14.29	118.74	115.47
	T100	0.16	19.05	139.38	192.66
	T200	0.10	12.40	103.93	85.01
Shallot	Raw	0.23	28.54	296.89	115.49
	Pound T100	0.21	26.27	314.07	112.03
	Pound T200	0.31	38.92	396.35	130.67
	Chop T100	0.20	25.10	290.38	94.37
	Chop T200	0.24	29.82	315.88	94.37
	T100	0.24	30.05	310.09	127.70
	T200	0.19	23.44	254.76	91.89
Onion	Raw	0.19	29.44	275.38	86.69
	Pound T100	0.12	18.08	203.23	69.97
	Pound T200	0.25	38.18	329.39	95.89
	Chop T100	0.11	15.51	169.05	74.15
	Chop T200	0.14	21.31	211.46	65.16
	T100	0.12	17.98	218.21	74.98
	T200	0.07	9.55	130.87	29.41

* TEAC = Trolox equivalent antioxidant capacity (mg TEAC/g wet weight).

** FRAP values = The FRAP values of the extracts were determined using the standard curve (mg Fe(II)/g wet weight).

*** GAE = gallic acid equivalent of the extracts were determined using the standard curve (mg gallic acid/g wet weight).

Preparation and heat treatment applied to *Allium* samples increased their antioxidant activity. After garlic is cut, chopped or crushed, alliin is immediately transformed to alkyl thiosulfinates by allinase and converted spontaneously and quickly to alkyl sulfides[20]. The garlic derivatives, diallyl sulfide, diallyl disulfide and allylmethyl sulfide possess antioxidant activities[21]. It was found that blanching (100°C for 90s) and frying (without fat in a pan at 100°C during 10 min.) and then microwaving (65-70°C) of garlic (6 min) and onions (5 min) did not decrease the amounts of their bioactive compounds and the level of antioxidant activities[22]. The brown color occurring during heat treatment of garlic sample in this investigation was due to non-enzymatic browning mechanism [23]. It is the amino-carbonyl (Maillard) reaction products of amino acids and sugars. Ide *et al.*[24] revealed that fructosyl arginine, a Maillard reaction product of aged garlic extract contributed to the antioxidant activity of the garlic preparation.

Pretreatment and heat treatment effect on antimutagenicity The data given in Table 3 show the percentage of inhibition (antimutagenicity) and rate of each sample. Treated garlic reduced the mutagenicity

Table 3 Wing spot induced by 20 mM urethane (URE) co-administrated with each sample in *Drosophila melanogaster*

Sample	Trial	Assigned treatment	Mutagen	Sample: regular medium (w/w)	No. of wings	Spots per wing (Number of Spots), statistical diagnoses[a]				% Inhibition (rate[**])
						Small single	Large single	Twin	Total	
Garlic	1	-	-	-	40	0.15(6)	0	0	0.15(6)	-
		-	URE	-	40	6.53(261)+	3.00(120)+	0.33(13)+	9.85(394)+	-
		Raw	URE	1:25	28	2.25(63)+	0.64(18)+	0.32(9)+	3.21(90)+	77(s)
				1:40	36	3.22(116)+	1.08(39)+	0.44(16)+	4.75(171)+	57(m)
		Pound T100	URE	1:25	32	2.75(88)+	1.06(34)+	0.13(4)+	3.94(126)+	68(s)
				1:40	40	1.58(63)+	0.98(39)+	0.30(12)+	2.85(114)+	71(s)
		Pound T200	URE	1:25	40	0.93(37)+	0.80(32)+	0.15(6)+	1.88(75)+	81(s)
				1:40	28	2.18(61)+	0.93(26)+	0.29(8)+	3.39(95)+	76(s)
		Chop T100	URE	1:25	40	1.50(60)+	0.85(34)+	0.15(6)+	2.50(100)+	75(s)
				1:40	40	1.78(71)+	1.15(46)+	0.28(11)+	3.20(128)+	68(s)
		Chop T200	URE	1:25	40	1.63(65)+	1.20(48)+	0.20(8)+	3.03(121)+	69(s)
				1:40	40	2.23(89)+	1.68(67)+	0.28(11)+	4.18(167)+	58(m)
		T100	URE	1:25	40	3.88(155)+	1.28(51)+	0.45(18)+	5.60(244)+	43(m)
				1:40	40	4.70(188)+	1.33(53)+	0.43(17)+	6.45(258)+	35(w)
		T200	URE	1:25	40	2.53(101)+	1.23(49)+	0.28(11)+	4.03(161)+	59(m)
				1:40	40	2.53(101)+	1.43(57)+	0.35(14)+	4.30(172)+	56(m)
	2	-	-	-	40	0.18(7)	0	0.03(1)	0.20(8)	
		-	URE	-	40	5.25(210)+	2.78(111)+	0.48(19)+	8.50(340)+	
		Raw	URE	1:25	40	2.83(113)+	0.93(37)+	0.33(13)+	4.08(163)+	52(m)
				1:40	40	3.68(147)+	1.20(48)+	0.38(15)+	5.25(210)+	38(w)
		Pound T100	URE	1:25	28	2.00(56)+	0.68(19)+	0.25(7)+	2.93(82)+	76(s)
				1:40	40	1.95(78)+	0.95(38)+	0.23(9)+	3.13(125)+	63(s)
		Pound T200	URE	1:25	36	1.28(46)+	0.89(32)+	0.25(9)+	2.42(87)+	74(s)
				1:40	40	1.90(76)+	1.00(40)+	0.25(10)+	3.15(126)+	63(s)
		Chop T100	URE	1:25	40	1.40(56)+	1.10(44)+	0.33(13)+	2.83(113)+	67(s)
				1:40	40	2.15(86)+	1.35(54)++	0.30(12)+	3.80(152)+	55(m)
		Chop T200	URE	1:25	28	1.71(48)+	1.25(35)+	0.21(6)+	3.18(89)+	74(s)
				1:40	40	0.18(7)i	1.38(55)+	0.33(13)+	1.88(75)+	78(s)
		T100	URE	1:25	40	3.05(122)+	0.88(35)+	0.30(12)+	4.23(169)+	50(m)
				1:40	40	2.98(19)+	1.08(43)+	0.35(14)+	4.40(176)+	48(m)
		T200	URE	1:25	36	1.61(58)+	0.89(32)+	0.36(13)+	2.86(103)+	70(s)
				1:40	36	1.86(67)+	1.36(49)+	0.33(12)+	3.56(128)+	62(s)

Table 3 Wing spot induced by 20 mM urethane (URE) co-administrated with each sample in *Drosophila melanogaster*(continued)

Sample	Trial	Assigned treatment	Mutagen	Sample: regular medium (w/w)	No. of wings	Spots per wing (Number of Spots), statistical diagnoses*				% Inhibition (rate**)
						Small single	Large single	Twin	Total	
Shallot	1	-	-	-	40	1.08(43)+	0.85(34)	0.23(9)	2.15(86)+	
		-	URE	-	40	5.13(205)+	3.13(125)+	0.60(24)+	8.85(354)+	
		Raw	URE	1:1	40	3.95(158)+	0.68(27)-	0.18(7)i	4.80(192)+	46(m)
				1:2	40	5.83(233)+	1.05(42)+	0.18(7)i	7.05(282)+	20(w)
		Pound T100	URE	1:1	40	4.40(176)+	2.25(90)+	0.45(18)+	7.10(284)+	20(w)
				1:2	40	4.05(162)+	1.63(65)+	0.45(18)+	6.13(245)+	31(w)
		Pound T200	URE	1:1	40	5.18(207)+	0.68(27)+	0.25(10)+	6.10(244)+	31(w)
				1:2	40	3.48(139)+	1.53(61)+	0.45(18)+	5.45(218)+	38(w)
		Chop T100	URE	1:1	40	7.08(283)+	1.25(50)+	0.43(17)+	8.75(350)+	1(n)
				1:2	40	6.98(279)+	1.83(73)+	0.80(32)+	9.60(384)+	-8
		Chop T200	URE	1:1	40	6.28(251)+	1.10(44)+	0.48(19)+	7.85(314)+	11(n)
				1:2	40	5.50(220)+	1.63(65)+	0.55(22)+	7.68(307)+	13(n)
		T100	URE	1:1	40	3.25(130)+	1.58(63)+	0.40(16)+	5.23(209)+	41(m)
				1:2	40	4.70(188)+	0.70(28)-	0.20(8)i	5.60(224)+	37(w)
		T200	URE	1:1	40	2.95(118)+	0.35(14)-	0.05(2)-	3.35(134)+	62(s)
				1:2	40	2.48(99)+	1.58(63)+	0.30(12)+	4.35(174)+	51(m)
	2	-	-	-	40	0.15(6)	0.05(2)	0.03(1)	0.23(9)	
		-	URE	-	40	7.48(299)+	3.28(131)+	0.43(17)+	11.18(447)+	
		Raw	URE	1:1	40	5.83(233)+	0.70(28)+	0.30(12)+	6.83(273)+	39(w)
				1:2	40	8.38(335)+	1.13(45)+	0.30(12)+	9.80(392)+	12(n)
		Pound T100	URE	1:1	38	8.34(317)+	1.79(68)+	0.47(18)+	10.61(403)+	10(n)
				1:2	40	9.05(362)+	1.45(58)+	0.93(37)+	11.43(457)+	-2
		Pound T200	URE	1:1	40	9.20(368)+	1.23(49)+	0.50(20)+	10.93(437)+	2(n)
				1:2	36	6.69(241)+	2.19(79)+	0.94(34)+	9.83(354)+	21(w)
		Chop T100	URE	1:1	40	9.40(376)+	1.55(62)+	0.80(32)+	11.75(470)+	-5
				1:2	40	6.35(254)+	2.15(86)+	1.20(48)+	9.70(388)+	13(n)
		Chop T200	URE	1:1	40	8.28(331)+	2.48(99)+	1.03(41)+	11.78(471)+	-5
				1:2	38	7.24(275)+	3.24(123)+	1.11(42)+	11.58(440)+	2(n)
		T100	URE	1:1	34	2.88(98)+	2.09(71)+	0.53(18)+	5.50(187)+	58(m)
				1:2	40	7.63(305)+	1.30(52)+	0.68(27)+	9.60(384)+	14(n)
		T200	URE	1:1	40	5.05(202)+	0.70(28)+	0.35(14)+	6.10(244)+	45(m)
				1:2	30	4.50(135)+	1.53(46)+	0.70(21)+	6.73(202)+	55(m)

Table 3 Wing spot induced by 20 mM urethane (URE) co-administrated with each sample in *Drosophila melanogaster* (continued)

Sample	Trial	Assigned treatment	Mutagen	Sample: regular medium (w/w)	No. of wings	Spots per wing (Number of Spots), statistical diagnoses*				% Inhibition (rate**)
						Small single	Large single	Twin	Total	
Onion	1	-	-	-	40	1.08(43)	0.85(34)	0.23(9)	2.15(86)+	
		-	URE	-	40	5.13(205)+	3.13(125)+	0.60(24)+	8.85(354)+	
		Raw	URE	1:1	40	5.63(225)+	0.50(20)-	0.15(6)i	6.28(251)+	29(w)
				1:2	40	5.18(207)+	0.90(36)-	0.30(12)i	6.38(255)+	28(w)
		Pound T100	URE	1:1	40	8.28(331)+	0.68(27)-	0.25(10)i	9.20(368)+	-4
				1:2	40	8.68(347)+	1.38(55)+	0.60(24)+	10.65(426)+	-20
		Pound T200	URE	1:1	40	6.03(241)+	1.48(59)+	0.70(28)+	8.20(328)+	7(n)
				1:2	40	4.73(189)+	2.00(80)+	0.73(29)+	7.45(298)+	16(n)
		Chop T100	URE	1:1	40	7.43(297)+	0.70(28)-	0.28(11)i	8.40(336)+	5(n)
				1:2	40	7.35(294)+	1.20(48)+	0.43(17)+	8.98(359)+	-1
		Chop T200	URE	1:1	40	7.23(289)+	1.38(55)+	0.70(28)+	9.30(372)+	-5
				1:2	40	4.90(196)+	1.00(40)+	0.60(24)+	6.50(260)+	27(w)
		T100	URE	1:1	40	6.55(262)+	0.95(38)+	0.43(17)+	7.93(317)+	10(n)
				1:2	40	3.25(130)+	1.35(54)+	0.43(17)+	5.03(201)+	43(m)
		T200	URE	1:1	30	4.20(126)+	3.13(94)+	1.20(36)+	8.53(256)+	28(w)
				1:2	36	2.89(104)+	1.64(59)+	0.47(17)+	5.00(180)+	49(m)
	2	-	-	-	40	0.15(6)	0	0	0.15(6)	
		-	URE	-	40	5.33(213)+	2.73(109)+	0.48(19)+	8.53(341)+	
		Raw	URE	1:1	40	7.55(302)+	0.75(30)+	0.30(12)+	8.60(344)+	-1
				1:2	40	7.20(288)+	1.38(55)+	0.30(12)+	8.88(355)+	-4
		Pound T100	URE	1:1	40	5.58(223)+	1.45(58)+	0.50(20)+	7.53(301)+	12(n)
				1:2	40	5.55(222)+	1.65(66)+	0.55(22)+	7.75(310)+	9(n)
		Pound T200	URE	1:1	40	6.10(244)+	1.58(63)+	0.63(25)+	8.30(332)+	3(n)
				1:2	40	7.00(280)+	2.98(119)+	1.10(44)+	11.08(443)+	-30
		Chop T100	URE	1:1	40	7.35(294)+	0.90(36)+	0.40(16)+	8.65(346)+	-1
				1:2	40	6.23(249)+	1.38(55)+	0.53(21)+	8.13(325)+	5(n)
		Chop T200	URE	1:1	40	9.10(364)+	1.58(63)+	0.70(28)+	11.38(455)+	-33
				1:2	40	7.23(289)+	1.65(66)+	0.85(34)+	9.73(389)+	-14
		T100	URE	1:1	40	7.95(318)+	0.90(36)+	0.43(17)+	9.28(371)+	-9
				1:2	40	5.85(234)+	1.45(58)+	0.60(24)+	7.90(316)+	7(n)
		T200	URE	1:1	40	4.30(172)+	1.80(72)+	0.53(21)+	6.63(265)+	22(w)
				1:2	38	4.13(157)+	1.66(63)+	0.47(18)+	6.26(238)+	30(m)

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

of urethane. Heating without pretreatment of garlic showed lesser antimutagenicity compared with other ones which had pretreatments. On the other hand, pretreatment to shallot and onion resulted in less antimutagenicity. Feeding higher concentration (3.85%) of treated sample gave greater antimutagenicity than feeding the lower one (2.44%). In addition, the antimutagenicity of all treated samples slightly decreased when they were stored for 1 month. This indicated that preparations and heat treatments influenced on the antimutagenicity of each *Allium* members in this study.

Reduction of genotoxicity of a mutagen by natural compounds in garlic and onion through the inhibition of mutagen activation is not surprised while pretreatment and temperature could modulate the detoxification systems of *Drosophila melanogaster* has been revealed in this study. The chemoprotective effects of diallyl sulfide, a flavor component of garlic, were attributed to its inhibitory effects on CYP2E1-mediated bioactivation of certain carcinogenic chemicals [25]. The bioactivation of acrylamide was diminished by applying the CYP2E1 inhibitor diallyl sulfide [26]. Diallyl disulfide was the most effective organosulfur compounds since it induced all Phase II enzyme activities studied. The induction of extrahepatic detoxication enzymes may contribute to the protection provided by organosulfur compounds against carcinogenesis in several organs[7]. Organosulfur compounds were generated only during tissue damage and preparation [27].

Heat treatment to unprepared garlic made it had lesser antimutagenicity since alliinase seemed to be inactivated before it could transform inactive precursor of sulfur containing compounds. Contrastingly, pounding or chopping the sample before heat treatment could demonstrate garlic's antimutagenicity. Song and Milner[28] revealed that crushing and leaving garlic for 10 min before microwave heating for 60 s prevented the total loss of allyl sulfur compounds. However, it was shown that heat treatment of chopped garlic might allow allicin and sulfides generated on the surfaces might disappear and only a small amount of alliin remained in the pieces [29].

Pretreatment of shallot and onion by pounding or chopping before they were heated resulted lesser antimutagenicity compared with that of the raw one. It is nearly the same as of garlic that when the cells of onion and shallot are disrupted the enzyme alliinase is released to hydrolyse the S-alk(en)yl cysteine sulphoxides to produce pyruvate, ammonia and many volatile sulfur compounds associated with flavor and odor[30]. The most important compound found in sliced onions after 1 min emission of volatiles is propanethial *S*-oxide which disappears after 30 min [31]. This shows that naturally formed components of onion and shallot are not stable even at room temperature.

The antimutagenicity of treated samples slightly decreased after 1-month storage. This might be due to the fact that some antimutagens were not stable. Ichikawa *et al*. [32] studied the changes in organosulfur compounds in garlic cloves during storage at different temperatures. They demonstrated that isoalliin produced enzymatically from γ-L-glutamyl-S-(trans-1-propenyl)-L-cysteine was chemically converted to cycloalliin which was not trans-formed into thiosulfinates; therefore, it reduced the inducer of phase 2 detoxifying system.

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