Different Antimutagenicity against Urethane between Conventionally and Organically Grown Cruciferous Vegetables (*Brassica* spp.)

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ABSTRACT

We lyophilized conventionally and organically grown *Brassica* vegetables (white cabbage, red cabbage, Chinese kale, Chinese mustard and cauliflower) and determined for their antimutagenicity against urethane in *Drosophila melanogaster.* We transferred three-day old trans-heterozygous (*mwh flr+/mwh TM3*) larvae from regular medium to experimental medium that had 20 mM urethane as the co-administration study. In the pre-feeding studies, we mated the parental flies on the experimental medium to obtain three-day old larvae that were subsequently raised on the regular medium containing urethane as the type 1 study or the experimental medium containing urethane as the type 2 study. The mutant spots of the wings from the surviving flies were analyzed. In the co-administration study, the antimutagenicity of conventional Chinese kale, Chinese mustard and cauliflower was higher than that of the organic ones while organic white cabbage had higher antimutagenicity than that of conventional one. In the pre-feeding studies, most samples (except organic cauliflower) exhibited their antimutagenicity. The antimutagenicity of the samples might be due to induction the phase 2 detoxifying enzyme system of *Drosophila* by isothiocyanates commonly found in *Brassica* vegetables. Since most organically grown vegetables are vulnerable to insect infestation that initialize the hydrolysis of their glucosinolates to be unstable isothiocyanates; therefore, the antimutagenicity of organic Chinese kale, Chinese mustard and cauliflower was lower than that of conventional ones. We also found no difference among red cabbages. Surprisingly, the antioxidant activity (DPPH scavenging capacity and ferric reducing antioxidant power) and amount of phenolic compounds (determined using the Folin-Ciocalteu reagent) of all organic vegetables were higher than that of the conventional ones.

Keywords: Conventional vegetables, antimutagenicity, antioxidant activity

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Introduction

Brassica vegetables have been reported as good sources of antimutagens. Several epidemiological studies showed that they were associated with reduction of cancer[1]. Further studies of experimental animals also demonstrated that feeding some of these vegetables could inhibit the development of some chemically induced carcinogenesis[2,3] and resulted in the induction of phase 2 detoxifying enzymes such as glutathione-S-transferase. It has been reported that natural compounds in these vegetables were effective in protection against chemical carcinogenesis by modulating carcinogen metabolism.[4] Therefore, they are now being favorite vegetable for Thai people. However, farmers in some area of Thailand heavily use chemicals and fertilizers to increase the yield in conventional cultivation. There is no information that different types of growing, namely conventional and organic ones have any effect on the contents of natural compounds in *Brassica* vegetables. Therefore, it is of interest to investigate the difference between various conventional and organically grown *Brassica* vegetables on modulation of genotoxicity of urethane in somatic mutation and recombination test using *Drosophila melanogaster* and on the content of phenolic compound and antioxidant activity.

Materials and Methods

Chemicals Urethane (URE) was purchased from Sigma Chemical (St. Louis, MO, USA). 2, 4, 6-tripyridyl-s-triazine (TPTZ), Ferric chloride hexahydrate, and Ferrous sulfate heptahydrate were purchased from Sigma Chemical (St. Louis, Mo, USA). 2, 2- diphenyl-1-picrylhydrazl (DPPH), Gallic acid and Folin-Ciocalteu reagent were purchased from Fluka Chemika (Buchs, Switzerland). Trolox was purchased from Aldrich Chemical (Milwaukee, WI, Germany). Other chemicals were of laboratory grade.

Samples Commercial conventional *Brassica* vegetables were purchased from three local markets in Bangkok. Organically grown vegetables were purchased from supermarkets and came from several producers such as Doi Kham, Wung Nam Khiew etc. They were certified by Organic Agriculture Certification of Thailand (ACT) or Agriculture Ministry of Agriculture and Cooperatives. Sample from different producers was mixed equally, lyophilized and homogenized to be powder and kept in a refrigerator.

Experimental Design 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 modified from the formula of Roberts[5] which had propionic acid (0.01 ml) as a preservative.

Each lyophilized conventional or organic vegetable was mixed with dry ingredients of regular medium at the ratio of 1:1, 1:2, or 1:4 w/w in a 10 x 150 mm test tube in order to obtain the final total solid of 0.58 g. Two ml of deionized water was added; the mixture was heated in a boiling water bath until it became sticky. The final percentage of sample in each *experimental medium* was 11.24, 5.62 or 2.81%, respectively. These media were used for mutagenicity evaluation. URE (20 mM) was substituted for deionized water in the regular medium and was used as a *positive control medium*. Antimutagenicity studies were performed using the medium (containing the highest amount of sample that gave more than 50% survival) that distilled water was substituted by 2 ml URE.

The mutagenicity of each sample (in the experimental medium) was assayed as described by Graf *et al*.[6] The larvae were maintained on medium at 25+1^{o}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 and recorded number of the wing spots. Induction frequencies of wing spots of conventional or organic vegetable treated groups were compared with that of the deionized water 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[7]. 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" was the number of total spots per wing induced by URE, "b" was the number of total spots per wing induced with URE administered with each vegetable. It was proposed that percent of inhibition between 0–20%, 20–40%, 40–60% and higher than 60% would indicate negligible, weak, moderate and strong antimutagenicity, respectively.

Each sample (0.5 g) was stirred twice with 80% methanol (50 ml) at room temperature for 2 h. The solution was filtered through cotton mesh and Whatman filter paper No. 1. DPPH assay for free radical scavenging activity of each methanolic extract was performed as suggested by Fukumoto and Mazza.[8] Ferric reducing antioxidant power (FRAP) was measured according to the procedure described by Griffin and Bhagooli.[9] The total phenolic content of methanolic extract from each sample was determined according to the method described by Amarowicz *et al*.[10]

Results

The surviving of adult flies fed on most samples, except that of the studies on high concentration of conventional Chinese mustard and Chinese kale, were more than fifty percents. None of the samples was mutagenic since they did not significantly induce the frequencies of mutant spots to be higher than that of the negative control ($p<0.05$) (data not shown). Co-administrating (Table 1) of urethane with each sample indicated that most conventional vegetables had greater antimutagenicity than organic ones. The percent inhibition on mutagenicity of URE with conventional Chinese mustard, Chinese kale and cauliflower were 65.93%, 50.30% and 33.03%, respectively while that of organic Chinese mustard, Chinese kale and cauliflower were 34.07%, 37.68%, 5.17%, respectively. On the other hand, organic white cabbage expressed its higher antimutagenicity (36.02 % inhibition) than that of conventional one (20.99% inhibition). The antimutagenicity between conventional red cabbages (26.89%) and organic one (23.75%) was nearly the same. In the pre-feeding studies (Table 2) most samples, except organic cauliflower, exhibited their antimutagenicity. Conventional Chinese mustard and Chinese kale revealed better inhibition of urethane-induced mutant spots (54.07% and 49.16%, respectively) in type 2 experiment than that in the type 1 study (21.20% and 21.56%, respectively). On the other hand, Organic Chinese kale in type 1 study had better inhibition (35.72%) than that of the type 2 study (16.33%).

The antioxidant activity of each conventional and each organic vegetables is shown in Table 3. The reduction of DPPH by antioxidants in the samples expressed as the percentage of radical scavenging activity was between 66.37 to 16.10%. In addition, the FRAP values (µM ferrous tripyridyltriazine) was between 225.88 to 1020.61 µM. The total phenolic contents of each sample varied between 53.51 to 195.74 mg gallic acid equivalent per liter. The antioxidant activity and amount of phenolic compounds of all organic vegetables were higher than that of the conventional ones. Organic red cabbage expressed the highest activities and phenolic compounds as conventional white cabbage had the lowest value.

Discussion

Safety of conventional and organic vegetables: Most vegetables were safe in terms of mutagenicity as seen from the results of SMART (data not shown). The adult flies fed on high concentration of conventional Chinese mustard and Chinese kale had lower survival rate than fifty percents. It was proposed that chemical pesticide residue generally found on conventional Chinese mustard and Chinese kale might be lethal to *Drosophila* larvae. Alternatively, some natural pesticides (isothiocyanates) in each sample might retard the growth of larvae or even killed them. Lichtenstein *et al*.[11] found that root extracts of Brussels sprouts were very toxic to *Drosophila melanogaster* and *Musca domestica* (common housefly). They suggested that the toxicity was in most cases strongly correlated with phenylethyl isothiocyanate content. Therefore, the amount of Chinese mustard or Chinese kale in the experimental medium was reduced to 5.62% (medium concentration) while that of other samples was 11.24% (high concentration).

Table 1 Antimutagenicity of each sample on URE (20 mM) in *Drosophila melanogaster* in the co-administration study

Sample	Vegetables	No. of wing	Spots per wing (Number of Spots), statistical diagnoses*				% Inhibition (rate**)
			Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
Negative control		40	0.20 (8)	0.05 (2)	0.05 (2)	0.30 (12)	
Positive control (urethane)		38	11.13 (423)+	6.03 (229) +	0.97 (37) +	18.13 (689)+	
white cabbage	conventional	40	8.93(357) +	4.18(167) +	1.23(49) +	14.33(573) +	20.99(w)
	organic	40	6.75(270) +	3.90(156) +	0.95(38) +	11.60(464) +	36.02(w)
red cabbage	conventional	39	7.36(287) +	4.13(161) +	1.77(69) +	13.26(517) +	26.89(w)
	organic	40	7.25(290) +	4.90(196) +	1.68(67) +	13.83(553) +	23.75(w)
Negative control		40	0.15(6)	0.05(2)	0	0.20(8)	
Positive control (urethane)		40	6.60(264) +	5.13(205) +	0.75(30) +	12.48(499) +	
Chinese mustard	conventional	20	2.60(52) +	1.60(32) +	0.05(1)i	4.25(85) +	65.93(s)
	organic	40	4.45(178) +	3.13(125) +	0.65(26) +	8.23(329) +	34.07(w)
Chinese kale	conventional	40	2.53(101)+	3.28(131)+	0.40(16)+	6.20(248)+	50.30(m)
	organic	40	4.08(163)+	3.20(128)+	0.50(20)+	7.78(311)+	37.68(w)
Negative control		40	0.40(16)	0.05(2)	0.00	0.45(18)	
Positive control (urethane)		40	7.48(299)+	4.90(196)+	1.18(47)+	13.55(542)+	
cauliflower	conventional	40	4.50(180)+	3.75(150)+	0.83(33)+	9.08(363)+	33.03(w)
	organic	40	8.55(342)+	3.10(124)+	1.20(48)+	12.85(514)+	5.17(n)

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

Antimutagenicity of conventional and organic vegetables: Overall results of the present investigation showed that most conventional and organic *Brassica* vegetable could reduce the mutagenicity of URE. The antimutagenicity against URE of these vegetables might belong to the fact that URE is metabolically activated by cytochrome P-450 (CYP-450) enzyme system to vinyl epoxide[12,13] that is further detoxified with glutathione-S-transferase (GST) conjugation,[14] therefore, any substance that is an inducer of GST or an inhibitor of CYP-450 system is antimutagen. Chemoprevention by cruciferous vegetables is associated with a significant increase in activities of the phase 2 detoxification enzymes, namely GST and NADPH: quinine reductase resulting in less initiation of chemical-induced carcinogenesis.[15] Indole-3-carbinol was also found to be an inducer of enzymes involved in the detoxification of xenobiotics.[16,17] The anticarcinogenic action of isothiocyanates, normal constituents of *Brassica* species, against nitrosamines was proposed to be due to inhibition of bioactivation of the nitrosamines, the CYP2E1-dependent activity.[18] An inhibitor of CYP2E1 namely, phenylethyl isothiocyanates (PEITC) effectively blocked the bioactivation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in rat and also induce a number of phase 2 enzymes.[19]

Table 2 Antimutagenicity of each sample on URE (20 mM) in *Drosophila melanogaster* in the pre-feeding type 1 and 2 studies.

Sample	Vegetables	Pre-feeding type	No. of wing	Spots per wing (Number of Spots), statistical diagnoses*				% Inhibition (rate**)
				Small single (m=2)	Large single(m=5)	Twin (m=5)	Total (m=2)	
Negative control			40	0.73(29)	0	0	0.73(29)	
Positive control			34	6.56(223)+	4.41(150)+	0.53(18)+	11.50(391)+	
White cabbage	conventional	1	39	4.85(189)+	2.79 (109)+	0.38(15)+	8.03(313)+	30.21(w)
		2	40	4.90(196)+	2.10(84)+	0.58(23)+	7.58 (303)+	34.13(w)
White cabbage	organic	1	40	4.45(178)+	2.83(113)+	0.53(21)+	7.80(312)+	32.17(w)
		2	40	4.93(197)+	3.00(120)+	0.65(26)+	8.58(343)+	25.43(w)
Negative control			40	0.45(18)	0.08(3)	0	0.53(21)	
Positive control			40	6.68(267)+	3.70(148)+	0.50(20)+	10.88(435)+	
Red cabbage	conventional	1	40	4.70(188)+	2.35(94)+	0.48(19)+	7.53(301)+	30.80(w)
		2	40	4.45(178)+	2.20(88)+	0.45(18)+	7.10(284)+	34.71(w)
Red cabbage	organic	1	40	5.25(210)+	2.35(94)+	0.78(31)+	8.38(335)+	22.99(w)
		2	40	5.08(203)+	3.00(120)+	0.73(29)+	8.80(352)+	19.08(n)
Negative control			40	0.30(12)	0.08(3)	0	0.38(15)	
Positive control			40	6.73(269)+	4.58(183)+	0.68(27)+	11.98(479)+	
Chinese mustard	conventional	1	39	5.28(206)+	3.44(134)+	0.72(28)+	9.44(368)+	21.20(w)
		2	12	5.25(63)+	0.25(3)i	0	5.50(66)+	54.07(m)
Negative control			40	0.45(18)	0.05(2)	0	0.50(20)	
Positive control			38	6.89(262)	3.76(143)	0.82(31)	11.47(436)	
Chinese mustard	organic	1	40	4.60(184)+	3.55(142)+	0.50(20)+	8.65(346)+	24.61(w)
		2	40	4.80(192)+	3.38(135)+	0.68(27)+	8.85(354)+	22.87(w)
Negative control			40	0.45(18)	0.05(2)	0	0.50(20)	
Positive control			38	6.89(262)+	3.76(143)+	0.82(31)+	11.47(436)+	
Chinese kale	conventional	1	40	5.25(210)+	3.15(126)+	0.60(24)+	9.00(360)+	21.56(w)
		2	36	3.33(120)+	2.25(81)+	0.25(9)+	5.83(210)+	49.16(m)
Chinese kale	organic	1	40	4.60(184)+	2.23(89)+	0.55(22)+	7.38(295)+	35.72(w)
		2	40	5.53(221)+	3.23(129)+	0.85(34)+	9.60(384)+	16.33(n)
Negative control			40	0.55(22)	0.05(2)	0.05(2)	0.65(26)	
Positive control			36	9.31(335)+	6.00(216)+	1.36(49)+	16.67(600)+	
Cauliflower	conventional	1	20	5.50(110)+	4.45(89)+	0.75 (15)+	10.70(214)+	35.80(w)
		2	36	6.33(228)+	2.92(105)+	0.67(24)+	9.92(357)+	40.50(m)
Cauliflower	organic	1	40	8.00(320)+	6.33(253)+	1.50(60)+	15.83(633)+	5.05(n)
		2	37	8.22(304)+	6.16(228)+	1.68(62)+	16.05(594)+	3.68(n)

* Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (1988) for comparison with distilled water (negative control); + = positive; - = negative; i = inconclusive; m = multiplication factor. Probability level: (= (= 0.05. One-sided statistical tests. **w = weak antimutagenicity, m = moderate antimutagenicity, s = strong antimutagenicity.

Table 3 Antioxidant activity and total phenolic content of methanolic extracts of each conventional or organic sample.

Sample	Vegetables	% DPPH Scavenging activity*	FRAP values**	Total phenolic content GAE (mg/l)***
White cabbage	conventional	16.095	225.889 ± 17.80	53.519 ±17.46
	organic	22.533	342.278 ±19.37	114.630 ±46.17
Red cabbage	conventional	66.370	801.722 ±42.87	174.259 ±32.85
	organic	73.316	1020.610 ±40.41	195.741 ±30.84
Chinese mustard	conventional	28.378	272.556 ±13.92	89.815 ±14.50
	organic	34.773	565.333 ±19.70	134.630 ±54.82
Chinese kale	conventional	19.017	398.111 ±20.63	110.556 ±18.36
	organic	33.799	482.833 ±5.55	172.407 ±32.51
Cauliflower	conventional	25.498	347.000 ±12.92	69.444 ±10.94
	organic	34.096	473.944 ±35.38	153.148 ±19.06

*150 µM DPPH in 80% methanol had been used for this investigation

** FRAP values = µM ferrous tripyridyltriazine form after the addition of sample; *** GAE = gallic acid equivalent (mg gallic acid/l)

The result that most conventional vegetables had greater antimutagenicity than organic ones might be due to the fact that most organically grown vegetables were vulnerable to insect infestation that initialize the hydrolysis of their indole glucosinolates (glucobrassicins) found in the family *Brassicaceae*[20] to be unstable indole-3-carbinol. Indole-3-carbinol is reported to upregulate the gene expression of the phase 1 enzyme and the phase 2 enzymes GST and oxidoreductases in prostate and breast cancer cells.[21,22] However, indole-3-carbinol is unstable in aqueous solutions.[23] Therefore, the antimutagenicity of organic Chinese kale, Chinese mustard and cauliflower was lower than that of conventional ones. In addition, Velasco *et al*.[24] evaluated the changes in the total and individual glucosinolate concentrations of kale (*B. oleracea acephala*) after insect attack in four locations in northwestern Spain. They found that total and individual glucosinolate concentrations related to insect attack. Leaves damaged by lepidopterous insect contained lower total glucosinolate content (25.8 μmol g/dw) than undamaged leaves (41 μmol g/dw) and the amounts of sinigrin, glucoiberin, and glucobrassicin were also lowest in insect-damaged leaves. Because chewing insects causes tissue disruption thereby bringing glucosinolates into contact with myrosinase and resulting in the production of a variety of toxic degradation products including isothiocyanates (natural pesticide in plant against insects), hence glucosinolates in plant tissue is reduced.[25]

The result that organic white cabbage had higher antimutagenicity than that of conventional one was an exception. It might be because management practices (e.g. fertilizer application) have its specific influences on the contents of glucosinolates. Dick-Hennes *et al*.[26] found that a reduction in mineral nitrogen application caused an increase in the non-protein sulphur content in kohlrabi, cabbage and radish. It also indirectly affected the increased availability of methionine as a precursor of alkyl glucosinolate. Krumbein *et al*.[27] found that an increased level of mineral nitrogen fertilizer in a field experiment with broccoli decreased the content of the alkyl glucosinolates namely glucoraphanin and glucoiberin. Therefore, the explanation why conventional white cabbage had less antimutagenicity might be due to heavy application of mineral N fertilizer that affected the synthesis of alkyl glucosinolate group which are the inducers of detoxifying system.

The results from pre-feeding study on both types of vegetables showed that the larvae fed conventional grown vegetables especially Chinese kale and Chinese mustard had greater antimutagenicity against urethane induced wing spots. This suggests that these two vegetable could synthesis any compounds that were capable of stimulating the detoxification system of the fly. On the other hand, the finding those larvae fed on organic vegetables for longer time decrease the detoxification against urethane.

Antioxidant activity and total phenolic content: Epidemiological data strongly suggest that vegetables having antioxidant activities have strong protective effects against major degenerative diseases including cancer and cardiovascular diseases.[28] The information obtained from this study revealed that *Brassica* vegetables both organically and conventional grown vegetables contained antioxidant activity.

It was interesting that all organic vegetables contained higher antioxidants and amount of phenolic compounds. The application of pesticides and fertilizers has been previously reported to modulate the biosynthesis of phenolics in plants and the use of them has been found to decrease in phenolic compound content in apple fruits.[29,30] Conventional agricultural practices utilize levels of synthetic pesticides that can result in a disruption of the natural defense production of phenolic metabolites in the plant (may reduce the need for natural plant defense).[31] Since no synthetic pesticide is used in organic vegetable production; therefore, such produce has more susceptibility to the action of herbaceous insects. From this reason, the plant synthesizes higher amounts of phenolic compounds as a mean to defend itself.[32,33] Most studies on the mechanism conferring plant resistance report that an increase in both the phenolic compounds and the activity of phenoloxidase (and oxidative enzymes in general) is associated with an improvement of the resistance against phytopathogens and herbivorous animals.[34,35] Fertilization is an important aspect to consider when comparing organic and conventional agriculture. Toor *et al*.[36] revealed in their study that the mean total phenolic content of tomatoes grown using chicken manure and grass-clover mulch was higher than the tomatoes grown with mineral nutrient solutions. The presence of easily-accessible nitrogen in conventional soil and making nitrogen more available for the plants may decrease the production of phenolic antioxidants as plants devote a larger proportion of resources to growth rather than the biosynthesis of polyphenols (plant secondary metabolites).[37,38] Organic fertilization typically does not provide nitrogen in easily-accessible form resulting on restricted plant growth due to slow nutrient availability in the organic treatments. This may be responsible for an increased production of phytochemical content and phenolic metabolites.[39] Haukioja *et al.*[40] showed that a negative correlation between growth and the synthesis of secondary carbon containing metabolites (C-compounds). When nitrogen is readily available, plants will primarily make compounds with high nitrogen content (e.g., proteins for growth) but when nitrogen is limited availability, metabolism changes more towards carbon-containing compounds such as starch, cellulose, and non-N containing secondary metabolites such as phenolics and terpenoids. The relative differences in the release of nutrients from various fertilizers could lead to different carbon/nitrogen ratios in plants and this in turn could lead to a difference in the production of secondary metabolites.[33] Therefore, the increase in polyphenol content observed in organic vegetable may support the hypothesis of enrichment in plant defense mechanisms against stresses such as pest attack or infestations and the hypothesis of slow release of nutrients by application of organic fertilizer through an increase in endogenous polyphenols.

References

1. Zhang Y, Talalay P. Anticarcinogenic activity of organic isothiocyanates: chemistry and mechanisms. *Cancer Res* 1998;28:323-33.
2. Stoewasand GS, Babish JB, Wimbery HC. Inhibition of hepatic toxicities from polybrominated biphenyls and aflatoxin B1 in rats fed cauliflower. *Environ Patho Toxicol* 1978;2:399-406.
3. Boyd JN, Babish JG, Stoewsand GS. Modification by beet and cabbage diets of aflatoxin B1-induced rat plasma α-foetorotein elevation, hepatic tomorigenesis and mutagencity of urine. *Food Chem Toxicol* 1982;20:47-52.
4. Bradfield CA, Chang Y, Bjeldanes LF. Effects of commonly consumed vegetables on hepatic xenobiotic metabolizing enzymes in the mouse. *Food Chem Toxicol* 1985;23:899–904.
5. Roberts DB. Basic Drosophila care and techniques. In: Roberts DB, editor. Drosophila: a practical approach. Oxford: IRL Press; 1986. p. 1-38.
6. Graf U, van Schaik N. Improved high bioactivation cross for the wing somatic mutation and recombination test in Drosophila melanogaster. *Mutat Res* 1992;271:59-67.
7. Frei H, Würgler FE. Statistical methods to decide whether mutagenicity test data from Drosophila assays indicate a positive, negative, or inconclusive result. *Mutat Res* 1988; 203: 297-308.
8. Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J Agric Food Chem* 2000;48:3597-604.
9. Griffin SP, Bhagooli R. Measuring antioxidant potential in corals using the FRAP assay. *J Exp Marine Biol Ecol* 2004;302:201-11.
10. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, *et al.* Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem* 2004;84: 551-62.
11. Lichtenstein EP, Morgan DG, Mueller CH. Naturally Occurring Insecticides in Cruciferous Crops. *J Agric Food Chem* 1964;12:158-61.
12. Schlatter J, Luitz WK. The carcinogenic potential of ethyl carbamate (urethane): risk assessment at human dietary exposure levels. *Food Chem Toxicol* 1990;28:205-11.
13. Dahl GA, Miller JA, Miller EC. Vinyl carbamate as a promutagen and a more carcinogenic analog of ethyl carbamate. *Cancer Res* 1978;38:3793-804.

14. Kemper RA, Myers SR, Hurst, HE. Detoxification of vinyl carbamate epoxide by glutathione: Evidence for participation of glutathione-S-transferases in metabolism of ethyl carbamate. *Toxicol Appl Pharm* 1995;135:110-18.
15. Wattenberg LW, Loub WD, Lam LK, *et al.* Dietary constituents altering the responses to chemical carcinogens. *Fed Proc* 1976;35:1327-31.
16. Sparnins VI, Venegas PL, Wattenberg LW. Glutathione-S-transferase activity: enhancement by compounds inhibiting chemical carcinogenesis and by dietary constituents. *J Natl Cancer Inst* 1982;68:493-5.
17. Cha YN, Thompson DC, Heine HS, *et al.* Differential effects of indole, indole-3-carbinol and benzofuran on several microsomal and cytosolic enzyme activities in mouse liver. *Korean J Pharmacol* 1985;21:1-11.
18. Chung FL. Chemoprevention of lung carcinogenesis by aromatic isothiocyanates. In: Wattenberg LW et al, editor. Cancer Chemoprevention. Boca Raton (FL): CRC Press; 1992. p. 227-45.
19. Guo Z, Smith TJ, Wang E, *et al.* Effects of phenethyl isothiocyanates, a carcinogenesis inhibitor, on xenobiotic-metabolizing enzymes and nitrosamine metabolism in rats. *Carcinogenesis* 1992;13:2205-10.
20. McDanell R, McLean AEM, Hanley AB, *et al.* Chemical and biological properties of indole glucosinolates (glucobrassicins). *Food Chem Toxicol* 1988;26:59-70.
21. Li Y, Li X, Sarkar FH. Gene expression profiles of I3C and DIM-treated PC3 human prostate cancer cells determined by cDNA microarray analysis. *Nutr Cancer* 2003;33:1011–9.
22. Ociepa-Zawal M, Rubis B, Lacinski M, *et al.* The effect of indole-3-carbinol on the expression of CYP1A1, CYP1B1 and AhR genes and proliferation of MCF-7 cells. *Acta Biochim Pol* 2007;54:113–7.
23. Leete R. 3-Hydroxymethylindoles. *J Amer Chem Sot* 1959;81:6023-7.
24. Velasco P, Cartea ME, Lez CG, *et al.* Factors Affecting the Glucosinolate Content of kale (Brassica oleracea acephala Group). *J Agric Food Chem* 2007;55:955-62.
25. Wittstock U, Halkier BA. Glucosinolate research in the Arabidopsisera. *Plant Sci* 2002;7:263–70.
26. Dick-Hennes E, Büning-Pfaue H. In: Schreier and Winterhalter, editor. Progress in flavour precursor studies. USA: Allured Publishing Corporation; 1992. p. 185-8.
27. Krumbein A, Schonhof I, Rühlmann J, *et al.* Influence of sulphur and nitrogen supply on flavour and health-affecting compounds in Brassicaceae. In: Horst WJ et al, editor. Plant Nutr. Netherlands: Kluwer Press; 2001. p. 294-5.
28. Block G, Patterson B, Subar, A. Fruits vegetables and cancer prevention. *Nutr Cancer* 1992;18:1–29.
29. Lea AGH, Beech FW. The phenolic of ciders: effect of cultured conditions. *J Sci Food Agric* 1978;29:493-6.
30. Nicolas JJ, Richard-Forget FC, Goupy PM, *et al.* Enzymatic browning reactions in apple and apple products. *Food Sci Nutr* 1994;34:109-57.
31. Macheix JJ, Fleuriet A, Billot J. Changes and metabolism of phenolics compounds in Fruits. In: Macheix JJ et al, editor. Fruit Phenolics. 1st ed. Boca Raton (FL): CRC Press; 1990. p. 149-221.
32. Soleas GJ, Diamandis EP, Goldberg DM. Resveratrol: a molecule whose time has come? And gone?. *Clin Biochem* 1997;30:91–113.
33. Brandt K, Mølgaard JP. Does it enhance or reduce the nutritional value of plant foods?. *J Sci Food* Agric 2001;81: 924–31.
34. Lattanzio V, De Cicco V, Venere D, *et al.* Antifungal activity of phenolics against fungi commonly encountered during storage. *Italian J Food Sci* 1994;6:23-30.
35. Ohazurike NC, Arinze AE. Changes in phenol oxidase and peroxidase levels in cocoyam tubers of different postharvest ages infected by Sclerotium rolfsii sacc. *Nahrung* 1996;40:25-7.
36. Toor RK, Savage GP, Heeb A. Influence of different types of fertilizers on the major antioxidant components of tomatoes. *Food Comp Anal* 2006;19:20–7.

37. Sander JF, Heitefuss R. Susceptibility to Erysiphe graminis f. sp. tritici and phenolic acid content of wheat as influenced by different levels of nitrogen fertilization. *J Phytopathol* 1998;146:495-507.

38. Stout MJ, Brovont RA, Dufffey SS. Effect of nitrogen availability on expression of constitutive and inducible chemical defenses in tomato. *J Chem Ecol* 1998;24:945-63.

39. Brandt K, Mølgaard JP. Food quality and organic agriculture. In: Kristiansen P et al, editor. Advances in Organic Agriculture. Australia: CSIRO Publishing; 2006. p. 305-27.

40. Haukioja E, Ossipov V, Koricheva J, *et al.* Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization?. *J Chemoecology* 1998;8:133-9.