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Genotoxic effects of crude juices from *Brassica* vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells

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Abstract

Crude juices of eight *Brassica* vegetables as well as juices and extracts of spices and phytopharmaceutical preparations from cruciferous vegetables were tested for induction of point mutations in *Salmonella* TA98 and TA100, repairable DNA damage in *E.coli* K-12 cells and clastogenic effects in mammalian cells. In bacterial assays, all juices caused genotoxic effects in the absence of metabolic activation, the ranking order being: Brussels sprouts > white cabbage > cauliflower > green cabbage > kohlrabi > broccoli > turnip > black radish. In experiments with mammalian cells, six juices induced structural chromosome aberrations. Brussels sprouts, white and green cabbage caused the strongest effects (800 μl of juice induced a 5-fold increase over the background). In sister chromatid exchange assays, positive results were measured as well, but the effects were less pronounced. With all juices the genotoxic effects seen in mammalian cells were paralleled by a pronounced decrease in cell viability. Column fractionation experiments showed that 70–80% of the total genotoxic

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activity of the juices is found in the fraction which contains isothiocyanates and other breakdown products of glucosinolates, whereas phenolics and flavonoids contributed to a lesser extent to the overall effects. On the basis of these findings, and considering the negative results obtained with non-cruciferous vegetables (tomato, carrot and green pepper), it seems likely that the genotoxic effects of the juices are due to specific constituents of cruciferous plants such as glucosinolates and/or their breakdown products, in particular, isothiocyanates, which we found previously to be potent genotoxins in bacterial and mammalian cells. Finally, spices (mustards and horse radish paste) and phytopharmaceutical preparations were tested in bacterial assays. Mustards and horse radish caused very weak effects while most of the pharmaceutical preparations gave negative results, except cabbage tablets, which caused a strong and dose dependent induction of his^+ revertants in *Salmonella* TA100. The present findings clearly indicate that cruciferous vegetables contain DNA damaging constituents. These observations are in contrast to earlier findings, which emphasized the antimutagenic effects of vegetable juices and also raise the question whether greatly increased consumption of *Brassica* vegetables or their concentrated constituents as a means for cancer prevention is indeed recommendable.

Keywords: *Brassica* vegetables; Isothiocyanates; Mutagenicity; Clastogenicity

1. Introduction

In the last decade results of numerous carcinogenicity studies have been published which show that breakdown products of glucosinolates such as isothiocyanates (ITCs) and indoles, which are contained in *Brassica* vegetables, inhibit the carcinogenic effects of specific environmental genotoxins such as nitrosamines and polycyclic aromatic hydrocarbons (PAHs) [1–4]. In all these studies, relatively large doses of model carcinogens were administered after or concomitant with the putative anticarcinogen [3]. Subsequent biochemical investigations demonstrated that the anticarcinogenic effects of ITCs against nitrosamines are due to inhibition of phase I enzymes, which are responsible for their bioactivation [4], and induction of phase II enzymes such as glutathione *S*-transferases, which play a key role in the detoxification of PAHs [5]. Furthermore, a few epidemiological studies indicated that dietary intake of cruciferous vegetables is inversely correlated with the incidence of specific cancers especially in the colon and breast [6,7]. All these findings have led to the assumption that increased intake of cruciferous vegetables may reduce cancer risks in humans. Subsequently, the production of phytopharmaceutical drugs from cruciferous was started for the purpose of cancer prophylaxis [8].

We have recently tried to establish an in vivo model (animal mediated assay with bacterial indicators), which enables the detection of antimutagenic effects of dietary constituents [9–11]. In these studies, we found protective effects of phenethyl isothiocyanate against nitrosamines and heterocyclic amines [9–11], but we also observed that some ITCs act as genotoxins by themselves [12]. These unexpected findings are in compliance with reports from other experiments, which demonstrated that some ITCs cause point mutations [13], repairable DNA damage in bacterial assays [14], and clastogenic effects in mammalian cells in vitro [15–17].

The aim of the present study was to investigate the potential genotoxic effects of crude juices of ITC containing *Brassica* vegetables in assays with bacterial and mammalian cells. As bacterial indicators, we used *Salmonella typhimurium* strains TA98 and TA100. In addition, differential DNA repair assays were performed with a pair of *E. coli* strains which differ vastly in their repair capacity (343/765, *uvr*$^+$/*rec*$^+$; 343/753, *uvr*B/*rec*B). The juices were also tested in experiments with Chinese hamster ovary (CHO) cells and SV_{40}-transformed Indian Muntjac (SVM) cells for induction of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs). In parallel, the histidine contents of the juices were measured with HPLC to exclude false positive results in Ames tests and the total ITC contents of the juices was determined photometrically to find out if the total ITC contents correlate with the mutagenic potential of the juices. Since it is well documented that flavonoids and phenolics contained in plants can cause genotoxic effects [18], the native juices were separated into ITC and flavonoid containing fractions and tested in bacterial and mammalian cells. In order to obtain information about the impact of food processing and digestion on the mutagenic potential of the juices, we also studied the effects of heating and acidification of the juices. Simultaneously, commercially available phytopharmaceutical drugs and spices prepared from cruciferous vegetables were tested for genotoxic effects.

2. Materials and methods

2.1. Chemicals

Benzyl isothiocyanate (BITC), allyl isothiocyanate (AITC), indole-3-carbinol, β-indolylacetonitrile, 1,6-dinitropyrene, sodium azide, 1,3-benzodithiole-2-thione and benz(a)pyrene were from Sigma (St. Louis, USA). Antibiotics, bromodeoxyuridine (BrdU) and colcemid were from Gibco (UK). Methyl isothiocyanate (MITC) was bought from Aldrich (Milwaukee, WI, USA)

2.2. Media

Bacterial media were from Difco (Detroit, MI, USA). Minimal glucose plates, phosphate buffered saline (PBS) and liver S9 mix were composed exactly as described by Maron and Ames [19]. Neutral red agar medium (NR-S) and peptone streptomycin boullion (PEP-S) were prepared as described by Mohn [20]. Ham's F-10 and Minimal Essential Medium for mammalian cell cultivation and trypsin were from Gibco (UK).

2.3. Preparation of vegetable juices

Fresh vegetables were bought from local markets, chopped into pieces and juices prepared with a juice maker machine (ELIN T323); subsequently, the juices were centrifuged (9000 $\times$ *g*, 10 min), the supernatants decanted, filtersterilized (0.22 μm) and used immediately in the assays.

In total, eight cruciferous vegetables were tested, namely, Brussels sprouts (*Brassica oleracea* var. *gemmifera*), white cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), green cabbage (*Brassica oleracea* var. *sabauda*), kohlrabi (*Brassica oleracea* var. *gonylodes*), broccoli (*Brassica oleracea* var. *italica*), turnip (*Brassica oleracea* var. *napobrassica*) and black radish (*Brassica oleracea* var. *niger*). The volume of juices recovered from different vegetables of similar weight varied substantially. The amount of vegetable for Brussels sprouts, white cabbage, cauliflower, green cabbage, kohlrabi, broccoli, turnip and black radish required to prepare 1 ml of the corresponding filtersterilized juice was 32.0, 8.8, 22.2, 99.2, 37.3, 14.9, 2.7, and 4.0 g, respectively. In addition, three non-cruciferous vegetables were assayed, namely tomatoes (*Lycopersicon esculentum*), green peppers (*Capsicum annuum*) and carrots (*Daucus carota* ssp. *sativa*).

2.4. Sample preparation from phytopharmaceuticals and and spices

Raddish and sauerkraut juices were from Biotta (Austria), white cabbage and Broccoli tablets were from Bischof (Germany) and Gall Pharma (Austria), respectively. Watercress juice was from Drapel (Austria). Krenn and English special mustards were from Mautner Markhof and Ramsa, respectively (Austria). Prior to the assays, tablets were pulverized, dissolved in phosphate buffered saline solution (PBS), centrifuged and the supernatants filtersterilized. The juices were tested as such except that they were filtersterilized. Paste preparations were dissolved in PBS.

2.5. Indicator cells

Salmonella strain TA98 and TA100 were from B. Ames (Berkley, USA); the *E. coli* strains used in the differential DNA repair assay (*E. coli* 343/753, *uvr*B/*rec*A, *lac*$^+$ and *E. coli* 343/765, *uvr*$^+$/*rec*$^+$, *lac*$^-$) were a gift from G.R Mohn (Bilthoven, Netherlands). CHO cells were a gift from A. Natarajan (University of Leiden, Netherlands). SVM cells were from R.T. Johnson (University of Cambridge, UK).

2.6. Mutagenicity assays

Salmonella/microsome assays were carried out according to the plate incorporation test procedure [19]. Briefly, 0.1 ml of stationary phase overnight cultures of strains TA98 and TA100 were plated onto selective media plates with 100–400 μl of filtersterilized juices in the presence or absence of rat liver S9 mix [19] and 2 ml of top agar. The number of His$^+$ revertants was counted after 48 h of incubation at 37°C.

Differential DNA repair tests with *E. coli* strains were performed as liquid holding assays according to the protocol of Mohn [20] and Knasmüller [21]. Briefly, overnight cultures of the two strains (343/753, *uvr*B/*rec*A, *lac*$^+$ and 343/765, *uvr*$^+$/*rec*$^+$, *lac*$^-$) were mixed 10:1 (final titer of each strain ca. $1-2 \times 10^8$ cells/ml) and 0.1 ml aliquots of the bacterial mix incubated with 100–400 μl of the juices. Rat liver S9 mix or PBS were added to give a final volume of 1 ml. In experiments

with ITCs, the test compounds were dissolved in dimethylsulfoxide and 0.1 ml of these solutions incubated with 0.1 ml of bacterial mix and 0.8 ml PBS. The incubation tubes were rotated at 37°C for 60 min. Subsequently, the mixtures were diluted 10^{-5} and 0.1 ml aliquots thereof plated on neutral red agar plates and incubated for two days at 37°C. The individual strain survival was determined by counting. On the basis of the viability of the two strains, the differential survival rates were calculated [11,21]. 100% survival indicates lack of a repairable DNA-damage; survival $< 100\%$ indicates reduction of the viability of the repair deficient strain relative to the repair proficient wild type due to genotoxic effects.

2.7. Cytogenetic assays with CHO and SVM cells

CHO cells (10^5) were seeded on petri dishes (94/16 mm) in 10 ml Ham's F-10 medium supplemented with 10% foetal calf serum and antibiotics (100 IU/ml penicillin and 125 μg/ml dihydrostreptomycin sulphate) and grown in a humidified incubator (5% CO_2) at 37°C. The same number of SVM cells was seeded in culture flasks (25 cm^2) in 3 ml Eagle's Minimal Essential Medium supplemented with 2 mM glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin and 10% foetal calf serum. Cultivation conditions were the same as for CHO cells.

After 24 h, cells in logarithmic growth phase were exposed to the juices (40–100 μl/ml medium) for 1 h (pH and osmolality values of the medium were corrected). Subsequently, the media were removed and replaced with fresh supplies. After further incubation for a period of 18 and 24 h for CHO and SVM cells, respectively, with the last 1 h in the presence of colcemid (0.1 μg/ml), chromosome preparations were made, stained with crystal violet and scored for induction of CAs. For each experimental point, 100 cells were evaluated. For SCE assays, the cells were grown and treated as described above, but after treatment with the different juices the cells were grown in the presence of BrdU for 20 (CHO) or 48 h (SVM). Chromosomes were prepared and stained according to the method of Alves and Jonasson [22]. For each experimental point, 25 cells were evaluated. The mitotic index (MI) was determined by counting at least 4000 cells and recording the number of cells in metaphase for each treatment. Each assay was repeated at least twice.

2.8. Determination of histidine and isothiocyanate contents

Histidine release was determined by HPLC analysis according to the OPA method [23,24]. Quantitative determination of ITC contents was carried out according to Zhang et al. [25] with the standard addition method using BITC.

2.9. Column fractionation of phenolics and flavonoids

Fractionation of flavonoid content of the crude vegetable juices was performed following the method of Grisebach and Barz [26]. Briefly, M-N-polyamide-SC-6-

powder (Machery and Nagel, corn size 0.05–0.16 mm) was suspended in methanol/water (1:1). The suspension was filled in a glass column (200 × 10 mm, I.D., enlarged at the top to 25 mm) and pressed with a glass rod until 100 mm height was reached. The column was washed with 50 ml double distilled water and 15 ml of the juices were applied to the polyamide column. After the solution had percolated, the column was washed with 20 ml distilled water and ITCs and other compounds were eluted. Afterwards phenolic acids and flavonoids were eluted with methanol.

3. Results

3.1. Bacterial tests

Table 1 depicts the results of mutagenicity tests with crude juices from cruciferous vegetables and non-cruciferous vegetables, as well as extracts from spices and phytopharmaceutical preparations of cruciferous vegetables origin, in *Salmonella* strains TA98 and TA100 without metabolic activation. All juices caused a dose dependent increase in the number of revertants, the base substitution indicator strain being consistently more sensitive than the frameshift strain. The highest mutant number was induced by Brussels sprouts followed by white cabbage and green cabbage. Among the pharmaceutical preparations, pronounced mutagenicity was observed only with cabbage tablets while mustards and horse radish caused weak effects (an increase by ≤ 1.5 fold over the background level at the highest concentration). The number of His^+ revertants induced by any of the juices from non cruciferous vegetables (tomato, green pepper and carrot) did not exceed the spontaneous background level. The table also gives the respective histidine and ITC contents of genotoxic test substances. These values vary over a broad range, 6.7–60 and 0.2–8.9 μg/ml for histidine and ITCs, respectively. In differential DNA repair assays with *E. coli*, only juices of cruciferous vegetables were tested and pronounced effects were detected with all of them. The ranking order of genotoxic activity seen in these assays was similar to that observed in the Ames test. The effect of addition of S9 mix on the mutagenic effects of the juices in strain TA100 is depicted in Fig. 1. It can be seen that the mutagenic effects were markedly reduced in the presence of exogenous metabolic activation. Similar effects were seen in *Salmonella* strain TA98 and *E. coli* assays as well and genotoxic effects were reduced by about 20–30% (data not shown).

Fig. 2 depicts the results obtained with the different ITCs and other breakdown products of glucosinolates, namely, indole-3-carbinol and β-indolylacetonitrile, in differential DNA-repair assays. It can be seen that all three ITCs caused pronounced induction of repairable DNA damage whereas only a marginal effect was seen with β-indolylacetonitrile and no effect was caused by indole-3-carbinol under identical experimental conditions.

Table 1
Induction of His^+ revertants in *Salmonella* typhimurium strains TA98 and TA100 by crude vegetable juices[a]

Test substance	Histidine content, (μg/ml)	ITC content, (μg/ml)	Mutagenic response[b]							
			TA98				TA100			
			100 μl	200 μl	300 μl	400 μl	100 μl	200 μl	300 μl	400 μl
1. Vegetable juices										
White cabbage	8.0	0.2	56 ± 7	68 ± 4	98 ± 12	185 ± 8	267 ± 11	397 ± 21	458 ± 15	576 ± 20
Turnip	8.2	6.3	33 ± 4	48 ± 9	46 ± 6	59 ± 9	149 ± 11	187 ± 9	238 ± 16	244 ± 14
Broccoli	60.0	3.8	50 ± 4	53 ± 6	79 ± 10	91 ± 7	167 ± 16	224 ± 9	278 ± 11	307 ± 15
Kohlrabi	20.0	1.6	38 ± 6	48 ± 7	69 ± 3	101 ± 7	190 ± 14	245 ± 6	301 ± 19	322 ± 10
Cauliflower	56.0	1.2	48 ± 7	57 ± 3	111 ± 8	138 ± 9	184 ± 11	268 ± 6	317 ± 9	396 ± 16
Brussels sprouts	75.0	1.9	77 ± 4	118 ± 6	232 ± 8	266 ± 7	193 ± 11	317 ± 16	522 ± 12	587 ± 19
Green cabbage	17.0	0.5	64 ± 4	71 ± 9	117 ± 6	150 ± 71	149 ± 13	196 ± 8	266 ± 11	351 ± 18
Radish	6.7	8.9	33 ± 4	47 ± 4	71 ± 10	110 ± 6	123 ± 11	144 ± 9	170 ± 5	202 ± 13
Tomato	nd	nd	39 ± 6	38 ± 2	49 ± 5	47 ± 4	133 ± 8	159 ± 7	143 ± 14	167 ± 10
Green pepper	nd	nd	44 ± 8	40 ± 5	49 ± 7	58 ± 8	119 ± 13	138 ± 4	130 ± 6	154 ± 12
Carrot	nd	nd	30 ± 3	34 ± 7	51 ± 9	49 ± 3	134 ± 7	149 ± 11	153 ± 7	155 ± 14
2. Phytopharmaceutical Preparations and spices										
Sauerkraut	nd	nd	36 ± 6	43 ± 8	49 ± 3	40 ± 6	141 ± 17	153 ± 10	189 ± 6	170 ± 13
White cabbage	7.6	0.4	31 ± 3	54 ± 4	53 ± 8	58 ± 10	144 ± 23	173 ± 19	318 ± 21	368 ± 52
Broccoli	nd	nd	33 ± 4	37 ± 7	38 ± 4	46 ± 3	130 ± 7	141 ± 19	133 ± 23	169 ± 10
Watercress	nd	nd	29 ± 2	34 ± 5	40 ± 9	34 ± 9	142 ± 7	139 ± 5	157 ± 11	174 ± 17
Krenn	nd	nd	38 ± 5	40 ± 3	49 ± 2	58 ± 8	158 ± 9	187 ± 9	256 ± 20	272 ± 17
English mustard	nd	nd	36 ± 9	32 ± 7	43 ± 8	40 ± 6	139 ± 13	192 ± 10	238 ± 16	256 ± 14
Radish	nd	nd	39 ± 3	51 ± 4	49 ± 3	52 ± 7	128 ± 6	142 ± 8	194 ± 16	264 ± 15

[a]The spontaneous reversion rate was 49 ± 7 and 131 ± 9 mutants/plate for strains TA98 and TA100, respectively; 1,6 dinitropyrene (1 μg/plate) was used as a positive control in strain TA98 and induced 426 ± 14 mutants/plate. Sodium azide (1.5 μg/plate) was the positive control in strain TA100 and induced 662 ± 23 His^+ revertants/plate.
[b]Values give means ± S.D. of the His^+ revertants of three plates per experimental point.

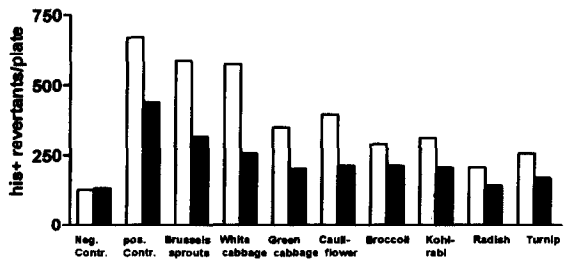

Fig. 1. Influence of addition of rat liver S9 mix on the mutagenic effects of various vegetable juices in *Salmonella* TA100. Symbols: blank bars, His^+ revertants induced in absence of S9 mix; shaded bars, His^+ revertants induced in the presence of S-9 mix. Three plates were evaluated per experimental point.

3.2. Clastogenic effects in mammalian cells

All juices from cruciferous vegetables were tested in CHO and SVM cells for induction of CAs and SCEs (Table 2). With both endpoints, the order of activity of the juices was Brussels sprouts > Chinese cabbage > green cabbage > broccoli ≈ cauliflower ≈ kohlrabi. With rettich and turnip juices, weak effects were observed. The extent of induction of SCEs was in no case more than 2-fold over the background level while the induction of CAs by Brussels sprouts and white and green cabbage was > 5-fold compared to the negative control value. The last column of Table 3 gives the mitotic indices (MI): The rate of cellular division was inversely related to the amount of juices used in the test. At the highest concentration tested, determination of cytogenetic effects was not possible due to severe cytotoxicity.

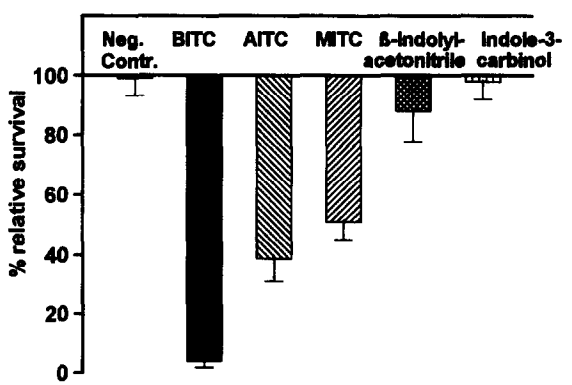

Fig. 2. Comparative genotoxicity of benzyl isothiocyanate (BITC), allyl isothiocyanate (AITC), methyl isothiocyanate (MITC), indole-3-carbinol and β-indolylacetonitrile in differential DNA repair assays with *E. coli*. The indicator bacteria were incubated with 5.0 μg/ml of the respective test substances for 2 h, diluted with PBS and 0.1 ml aliquots were plated onto NR-S plates. After an incubation period of 24 h at 37°C, the relative survival of the two strains was determined by counting.

Table 2
Induction of genotoxic effects in differential DNA repair assays with *E. coli* K-12 cells by crude vegetable juices[a]

Vegetable	Relative survival (%), *E coli* 434/753 (*uvr*B/*rec*A) vs. *E.coli* 343/765 (uvr^+/rec^+)			
	100 μl	200 μl	300 μl	400 μl
White cabbage	76 ± 19	57 ± 12	39 ± 6	24 ± 4
Turnip	89 ± 17	70 ± 10	58 ± 11	34 ± 6
Broccoli	79 ± 13	61 ± 7	47 ± 4	22 ± 2
Kohlrabi	65 ± 13	61 ± 7	41 ± 11	33 ± 6
Cauliflower	51 ± 9	44 ± 4	37 ± 3	14 ± 2
Brussels sprouts	58 ± 7	34 ± 5	30 ± 1	9 ± 5
Green cabbage	78 ± 16	66 ± 10	39 ± 4	32 ± 6
Radish	86 ± 11	68 ± 7	53 ± 9	47 ± 14

[a]Numbers are means ± SD of three plates per experimental point. The differential survival rate in the untreated control was 100% ± 7, in the positive control (2 μg/ml streptozozocin) 12% ± 7.

3.3. Fractionation experiments

In order to obtain information about the components of the vegetable juice which might be responsible for the genotoxic effects, the juices were subjected to column fractionation. The results of representative experiments performed with flavonoid and ITC containing fractions of the juices in bacterial and CHO cells are depicted in Fig. 3. It can be seen that the mutagenicity of the ITC containing fraction accounts for 70–80% of the total activity while only 20–30% of the activity could be attributed to the flavonoid fraction.

3.4. Impact of heating and acidification

Fig. 4 shows the results of experiments in which the effects of heating and acidification on the mutagenic effects of three selected vegetable juices were measured in *Salmonella* TA100. Heating the juices at 100°C for 5 min caused an increase in the mutagenic activity (significant enhancement with white cabbage and broccoli juices and a moderate increase with Brussels sprouts). In contrast to heating, acidification of the juices (pH 2.0) reduced mutagenic activities.

4. Discussion

The results of the present experiments indicate that *Brassica* vegetables contain compounds which cause point mutations and repairable DNA-damage in bacteria and induction of CAs and SCEs in mammalian cells. Since genotoxicity was observed without metabolic activation, the effects can be attributed to direct acting

Table 3
Induction of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) with vegetable juices[a]

Vegetable	Dose (μl/ml) medium	Chromatid aberrations (%)	Chromosomal aberrations (%)	Total no. of cells with aberrations (%)	SCEs/cell	Mitotic index (%)
Brussels sprouts	80	18.6 ± 2.0	11.0 ± 2.0	27.6 ± 1.5	14.96 ± 1.1	0.8 ± 0.06
	60	10.3 ± 2.5	5.3 ± 1.5	11.6 ± 2.0	11.3 ± 2.0	1.7 ± 0.08
	40	6.0 ± 2.0	3.3 ± 0.5	9.0 ± 2.6	–	2.4 ± 0.02
White cabbage	80	15.3 ± 2.0	7.3 ± 1.5	19.6 ± 1.5	14.44 ± 0.9	1.0 ± 0.04
	60	8.0 ± 2.0	4.3 ± 0.5	10.5 ± 0.7	9.8 ± 0.52	2.1 ± 0.1
	40	4.6 ± 0.6	3.6 ± 1.2	6.3 ± 0.57	–	3.0 ± 0.1
Green cabbage	80	11 ± 1.5	4.0 ± 1.0	13.6 ± 1.5	10.02 ± 2.2	1.3 ± 0.06
	60	8.3 ± 0.6	3.0 ± 0.0	9.6 ± 1.5	8.91 ± 1.84	4.3 ± 0.6
	40	4.3 ± 1.5	1.0 ± 0.0	4.3 ± 0.57	–	5.1 ± 1.2
Broccoli	80	7.3 ± 1.5	4.3 ± 1.5	10.3 ± 0.57	8.64 ± 1.33	2.8 ± 0.08
	60	6.3 ± 1.2	3.0 ± 1.0	7.6 ± 0.57	7.42 ± 197	6.4 ± 1.4
	40	3.6 ± 1.2	1.3 ± 0.6	3.0 ± 0.0	–	7.0 ± 0.8
Cauliflower	80	8.0 ± 0.0	3.6 ± 1.2	8.6 ± 1.5	10.76 ± 1.08	1.9 ± 0.08
	60	6.3 ± 1.5	2.0 ± 1.0	6.6 ± 1.5	6.42 ± 0.82	4.8 ± 1.4
	40	4.6 ± 2.0	1.0 ± 0.0	4.0 ± 0.0	–	5.1 ± 1.8
Kohlrabi	80	8.0 ± 1.0	2.6 ± 0.6	9.3 ± 2.0	8.21 ± 1.75	5.6 ± 1.2
	60	5.0 ± 0.0	1.0 ± 0.0	6.0 ± 0.0	7.77 ± 2.01	6.0 ± 2.2
	40	3.3 ± 0.6	–	3.0 ± 1.0	–	7.6 ± 1.8
Positive control		14.3 ± 1.5	7 ± 2	19.3 ± 2.0	16.46 ± 4.8	81.8 ± 0.8
Negative control		2.0 ± 1.0	0.7 ± 0.6	2.0 ± 0.0	7.33 ± 0.57	7.6 ± 0.6

[a] 10^5 cells were seeded on petri dishes, cultivated for one cell cycle and exposed for 60 min to various concentrations of freshly prepared, filter sterilized juices and treated further as described in Materials and methods. BITC (1.2 μg/ml) was used as a positive control.

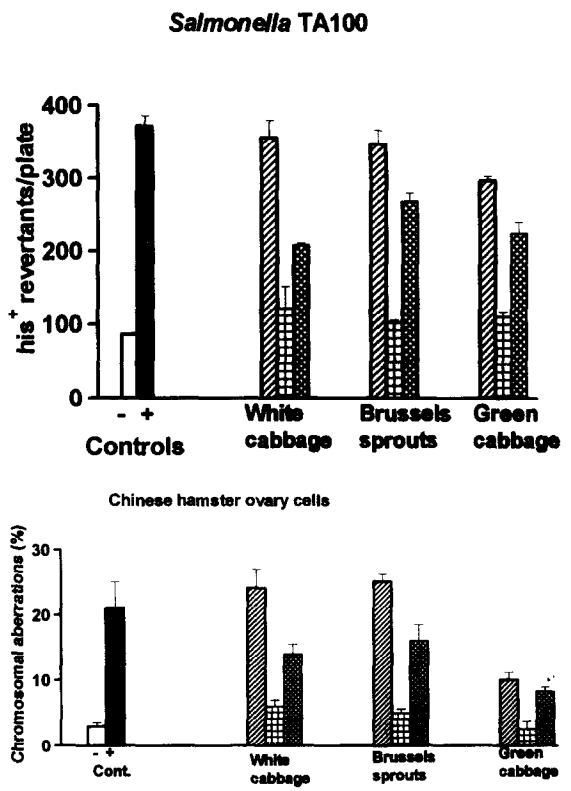

Fig. 3. (a) and (b) Genotoxicity of crude juices and their fractions in *Salmonella* TA100 (a) and CHO cells (b). Vegetable juice (15 ml) was applied to a polyamide column and washed with 20 ml of distilled water. Subsequently, the column was further eluted with methanol to extract phenolic acids and flavonoids. Methanol was evaporated and the residue diluted in distilled water to give a volume equal to that of the isothiocyanate fraction. Both fractions were filtersterilized and tested in parallel with the native juice as described in the material and methods section. Symbols: hatched pattern, whole juice; squares pattern, flavonoid/phenol enriched fractions; criss-cross pattern, residual fractions containing glucosinolates and their metabolites. Positive controls for experiments with *Salmonella* and CHO cells were Na-azide (1.5 μg/plate) and BiTC (12 μg/ml) respectively.

genotoxins. The results of bacterial assays give evidence that these compounds induce base substitutions rather than frameshift mutations. In assays with mammalian cells, CAs were induced by the vegetable juices with higher efficiency in comparison to SCEs.

Since genotoxicity was restricted only to juices of cruciferous vegetables, it is likely that the effects are due to the presence of glucosinolates, which are specific constituents occurring predominantly in this plant family [27,28]. These compounds are hydrolyzed enzymatically to various biologically active metabolites such as ITCs, nitriles, thiocyanates and goitrogens [27]. As described above, we and other groups found that certain ITCs cause genotoxic effects in bacterial and mammalian cells [12,15–17]. The results of the mutagenicity experiments in which flavonoid and

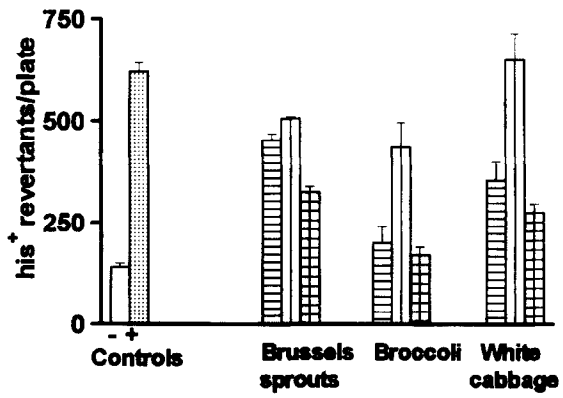

Fig. 4. Effect of heating and acidification of crude juices on the mutagenic activity in *Salmonella* TA100. Crude juices were heated shortly (5 min, 100°C) or acidified (pH 2.0) and tested in plate incorporation assays. Symbols: horizontal lines, untreated juice; vertical lines, heated juice, squares pattern-acidified juice. Na-azide (1.5 μg/plate) was used as a positive control.

phenol enriched fractions caused only a minor part of the total genotoxicity of the crude juices is in agreement with our presumption that glucosinolates and/or their breakdown products are primarily responsible for the genotoxicity of the native juices. Moreover, the assumption that ITCs were, at least partly, responsible for the DNA damaging effects of *Brassica* vegetables is supported by the fact that both pure ITCs and native juices were direct acting genotoxins, caused predominantly base substitution in bacteria, and unlike many other genotoxins, induced a high frequency of CAs but only a low number of SCEs which is a typical feature of radiomimetic compounds [17]. Moreover, other breakdown products of glucosinolates such as indole-3-carbinol and β-indolylacetonitrile induced no or only marginal effects in differential DNA-repair assays. The latter compound was also found to be devoid of mutagenic activity in *Salmonella*/microsome assays with TA100 and TA98 both in the presence and absence of metabolic activation [29].

The results of our analytical measurements also showed that the ITC concentrations in the juices are sufficiently high to be attributed to the genotoxic effects of the juices. However, the degree of genotoxicity of the various juices did not correlate with their total ITC contents. This might be due to the fact that different *Brassica* vegetables contain a variety of ITCs, which differ substantially in their DNA-damaging effects: for example, benzyl-ITC is a much stronger genotoxin than phenethyl-ITC and allyl-ITC in bacterial DNA-repair assays [14] and the latter compound was devoid of any clastogenic effects in SVM cells [16]. About 22 ITCs have been identified from edible plants [27]; however, genotoxicity data are available only for very few of them. Therefore, it is likely that the presence of specific, highly DNA-reactive ITCs account for the genotoxicity of the juices rather than the total ITC contents.

Acidification of the juices resulted in a reduction of mutagenic effects and this can be taken as an indication that the DNA damaging properties of constituents of *Brassica* vegetables are reduced during the digestion process in the stomach. In this context, it is notable that hydrolysis of glucosinolates under acidic conditions favours the preferential formation of nitriles rather than ITCs [30]. Heating of the native juices, however, increased the mutagenic potential. This latter finding is in agreement with earlier studies which reported an increased enzymatic and non-enzymatic hydrolysis of glucosinolates to isothiocyanates at high temperatures [30].

Also, phytopharmaceutical preparations contain bioactive compounds which induce dose dependent genotoxic effects similar to those seen with crude extracts denoting the possible health risks associated with high consumption of these plant drugs for the sake of cancer prophylaxis. For example, a daily intake of 900 mg of Brassica tablets is recommended by the manufacturer, but we have found that 0.2 ml aqueous extract prepared from 2.1 mg of the pulverized tablet induces about 100 His^+ revertants in *Salmonella* TA100.

It is generally assumed that a causal relationship exists between the genotoxic potential of chemicals and their carcinogenicity. The present observations show that cruciferous vegetables contain constituents which are mutagenic in bacteria and mammalian cells. Most of the currently known food carcinogens cause genotoxic effects in short term assays in vitro and several of these compounds (e.g. heterocyclic aromatic amines) have been detected initially in in vitro bacterial tests. Ames et al. [31,32] has stressed that plants contain numerous genotoxic and carcinogenic 'natural pesticides' which may pose higher risks than synthetic chemicals for human health. Many of these plant constituents occur in small quantities in spices or in plants used for the preparation of beverages and it is also possible that cruciferous plants which are consumed in large quantities by humans and animals are a major source of exposure to natural genotoxins. Although it is generally accepted that in vitro assays are useful tools for the detection of environmental mutagens, further experimental work will be necessary to clarify the question whether the compounds responsible for the effects of the cruciferous vegetables are active under in vivo conditions and if these findings are of human relevance. Most epidemiological studies currently available have shown protective effects of cruciferous vegetables against specific forms of cancer, in particular against tumors of the lower bowel. On the other hand, it has also been demonstrated that some natural and synthetic anticarcinogens may cause cancer by themselves, especially when administered at increased dose levels [33]. A typical example is indole-3-carbinol, a breakdown product of glucosinolates, which reduces tumor induction by polycyclic aromatic hydrocarbons [34] and also acts as a tumor promotor by binding to the aromatic hydrocarbon receptor [35].

We have recently studied the genotoxic effects of various ITCs under in vivo conditions and found positive effects in particular in the gastrointestinal tract, albeit at relatively high doses [12]. In animal carcinogenicity studies, allyl-ITC was found to induce transitional cell papillomas and epithelial hyperplasia in male Fisher rats [36] while experimental feeding of phenethyl-ITC alone or in combination with a tobacco specific nitrosamine resulted in a slight (statistically not

significant) increase in pancreatic tumors (1). Moreover, dehydrated cabbage fed to dimethylhydrazine treated mice caused a higher incidence and multiplicity of colon tumors in females while males were little affected by cabbage apart from a lower incidence of adenocarcinomas [37].

Whilst epidemiological data indicate that consumption of cruciferous vegetables at current levels is beneficial in regard to cancer reduction in humans, the present findings as well as earlier studies raise the question whether increased consumption of *Brassica* vegetables should be recommended before detailed investigations clarify the balance of potential adverse and beneficial effects and their mechanisms.

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References

[1] M.A. Morse, C. Wang, G.D. Stoner, S. Mandal, P.B. Conran, S.G. Amin, S.S. Hecht and F.L. Chung, Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA adduct formation and tumorigenicity in the lung of F344 rats by dietary phenethyl-isothiocyanatec, Cancer Res., 49 (1989) 549–553.

[2] L.W. Wattenberg, Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine or benz(a)pyrene on pulmonary and forestomach neoplasia in A/J mice, Carcinogenesis, 8 (1987) 1971–1973.

[3] Y. Zhang and P. Talalay, Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanism, Cancer Res., 54 (Suppl.) (1994) 1976s–1981s.

[4] F.L. Chung, Chemoprevention of lung carcinogenesis by aromatic isothiocyanates, In: L.W. Wattenberg, M. Lipkin, C.W. Boone and G.J. Kelloff (Eds.), Cancer Chemoprevention, CRC press, Boca Raton, 1992, pp 227–245.

[5] P. Talalay, Mechanisms of induction of enzymes that protect against chemical carcinogenesis, Advan. Enzyme Regul., 28 (1989) 237–250.

[6] G. Block, B. Patterson and A. Subar, Fruit, vegetables and cancer prevention: A review of the epidemiological evidence, Nutr. Cancer, 18 (1992) 1–29.

[7] K.A. Steinmetz and J.D. Potter, Vegetables, fruit and cancer. I. Epidemiology. Cancer Causes and Control, 2 (1991) 325–357.

[8] H. Hu and S. Kotha, The role of nutrition in mitigating environmental insults: policy and ethical issues, Environ. Health Perspect., 103 (Suppl.) (1995) 185–190.

[9] S. Knasmüller, W.W. Huber, H. Kienzl and R. Schulte-Hermann, Inhibition of repairable DNA damage in *E. coli* K-12 cells recovered from various organs of nitrosamine treated mice by vitamin A, phenethyl isothiocyanate, oleic acid and triolein, Carcinogenesis, 13 (1992) 1643–1650.

[10] S. Knasmüller, W. Parzefall, H. Huber, H. Keinzl and R. Schulte-Hermann, Genotoxic effects of nitrosamines and cooked food mutagens in various organs of mice and their modification by dietary factors, In: K.W. Waldron, L. Johnson and G.R. Fenwick (Eds.), Food and Cancer Prevention: Chemical and Biological Aspects of Anticarcinogens and Antimutagens, Royal Society of Chemistry, Cambridge, 1993, pp. 47–53.

[11] S. Knasmüller, M.D. Friesen, J.A. Holme, J. Alexander, R. Sanyal, F. Kassie and H. Bartsch, Effects of phenethyl isothiocyanate on the metabolism and genotoxicity of dimethylnitrosamine and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP), Mutat. Res., 350 (1996) 93–102.

[12] F. Kassie, S.R.R. Musk, W. Parzefall, I.T. Johnson, G. Sontag, R. Schulte-Hermann and S. Knasmüller, Antigenotoxic and genotoxic effects of naturally occurring isothiocyanates In: H. Kozlowska, J. Fornal and Z. Zdunczyk (Eds.), Proc. Int. Euro Food Tox IV; Bioactive Substances in Food of Plant Origin, Vol. 2, Polska Akademia Nauk Olsztyn, 1994, pp. 501–507.

[13] T. Yamaguchi, Mutagenicity of isothiocyanates, thiocyanates and thioureas on *Salmonella typhimurium*, J. Agric. Biol. Chem., 44 (1980) 3017–3018.

[14] F. Kassie, G. Sonntag, S.R.R. Musk, B. Pool-Zobel and S. Knasmüller, Genotoxische Effekte von Isothiocyanaten und isothiocyanathältigen Gemüsesäften, In: 15. Jahrestagung der Gesellschaft für Umweltmutationsforschung, Tierversuche in der Mutations- und Krebsforschung: Notwendigkeit und in vitro Alternativen, Gmunden, 1995, pp. 52.

[15] S.R.R. Musk and I.T. Johnson, In vitro genetic toxicity testing of isothiocyanates in the human diet, Mutagenesis, 7 (1992) 383.

[16] S.R.R. Musk and I.T. Johnson, The clastogenic effects of isothiocyanates, Mutat. Res., 300 (1993) 111–117.

[17] S.R.R. Musk, S.B. Astley, S.M. Edwards, P. Stephenson, R.B Hubert and I.T. Johnson, Cytotoxic and clastogenic effects of benzyl isothiocyanate towards cultured mammalian cells, Food and Chem. Toxicol., 33 (1995) 31–37.

[18] H.F. Stich, The beneficial and hazardous effects of simple phenolic compounds, Mutat. Res., 259 (1991) 307–324.

[19] D.M. Maron, and B.N. Ames, Revised methods for the Salmonella mutagenicity test, in: B.J. Kilbey, M.S Legator, W. Nichols and C. Ramel (Eds.), Handbook of Mutagenicity Test Procedures, Elsevier, Amsterdam, 1984, pp. 93–141.

[20] G.R. Mohn, The DNA-repair host mediated assay as a rapid and sensitive in vivo procedure for the determination of genotoxic factors present in various organs of mice. Some preliminary results with mitomycin C, Arch. Toxicol., 55 (1984) 268–271.

[21] S. Knasmüller, A. Wottawa and W. Fielder, Distribution and retention of bacterial cells in various organs of rodents, In: A. Leonard and M. Kirsch-Volders (Eds.), Proc. XVIth Ann. EEMS Meet, Brussels (1986) pp: 165–168.

[22] P. Alves and J. Jonasson, New staining method for the detection of sister chromatid exchanges in BrdU-labelled chromosomes, J. Cell Sci., 32 (1987) 185–195.

[23] B.N. Jones, Amino acid analysis and enzymatic sequence determination of peptides by an improved *o*-phthaldialdehyde precolumn labelling procedure, J. Liq. Chromatogr., (1981) 565–586.

[24] W. Beinert, Amino acid analysis by automatic precolumn derivatization with OPA, Merck Anal. Inf. No., 0013 (1991).

[25] Y. Zhang, C. Cho, G.H. Posner and P. Talalay, Quantitation of isothiocyanates by cyclocondensation with 1,2 benzenedithiol, In: K. W. Waldron, L. Johnson and G.R. Fenwick (Eds.), Food and Cancer Prevention, Chemical and Biological Aspects of Anticarcinogens and Antimutagens, Royal Society of Chemistry, Cambridge, 1993, pp. 421–425.

[26] H. Grisebach and W. Barz, Biochemie der Flavonoide. Naturwiss., 56 (1969) 538–544.

[27] G.R. Fenwick, R.K. Heaney and W.J. Mullin, Glucosinolates and their breakdown products in food and food plants. CRC Crit. Rev. Food Sci. Nutr., 18 (1983) 123–201.

[28] C.H. Van Etten, M.E. Daxenbichler, H.L. Tookey, W.F. Kwolek, P.H. Williams and O.C. Yoder, Glucosinolates: Potential toxicants in cabbage cultivars. J. Amer. Soc. Hort. Sci., 105 (1980) 710–714.

[29] K. Wakabayashi, M. Nagao, M. Ochiai, T. Tahira, Z. Yamaizumi and T. Sugimura, A mutagen precursor in Chinese cabbage, indole-3-acetonitrile, which becomes mutagenic on nitrite treatment, Mutat. Res., 143 (1985) 17–21.

[30] R. Lange, Glucosinolates in der Ernährung—pro und contra einer Naturstoffklasse, Teil II: Abbau und Stofwechsel, Ernährungs-Umschau, 39 (1992) 292–296.

[31] B.N. Ames, M. Profet and L.S. Gold, Dietary pesticides (99.9% all natural), Proc. Natl. Acad. Sci., 87 (1990a) 7777–7781.

[32] B.N. Ames, M. Profet and L.S. Gold, Nature's chemicals and synthetic chemicals: comparative toxicology, Proc. Natl. Acad. Sci., 87 (1990b) 7782–7786.

[33] B. Bertram and N. Frank, Inhibition of chemical carcinogenesis, Environ. Carcino. Ecotox. Revs., C11(1) (1993) 1–71.

[34] L.W. Wattenberg and W.D. Loub, Inhibition of polycyclic aromatic hydrocarbon induced neoplasia by naturally occurring indoles, Cancer Res., 38 (1978) 1410–1417.

[35] G.S. Bailey, J.D. Hendricks, D.W. Shelton, J.E. Nixon and N.E. Pawlowski, Enhancement of carcinogenesis by natural anticarcinogen indole-3-carbinol, J. Natl. Cancer Inst., 78 (1987) 931–934.

[36] J.K. Dunnick, J.D. Prejan, J. Haseman, R.B. Thompson, H.D. Giles and E.E. McConnell, Carcinogenesis bioassay of allyl isothiocyanate, Fund. Appl. Toxicol., 2 (1982) 114–120.

[37] N.J. Temple and S.M. El-Khalib, Cabbage and vitamin E: their effect on colon tumor formation in mice, Cancer Lett., 35 (1987) 71–77.