

# Inhibition of Oxidative DNA Damage *In Vitro* by Extracts of Brussels Sprouts

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Cruciferous vegetables have cancer preventive effects which may be due to reduction of oxidative DNA damage. We investigated the effect of an aqueous extract of cooked Brussels sprouts on formation of 7-hydro-8-oxo-2'-deoxyguanosine (8-oxodG) in calf thymus DNA *in vitro*. Damage was induced by a Fenton reaction, UVC (254 nm), UVA (365 nm), sunlamp light, and methylene blue with visible light.

The extract inhibited 8-oxodG formation in all systems except visible light with methylene blue. The IC<sub>50</sub> values were 6-20 µg/ml corresponding to the extract of 5-20 g of Brussels sprouts distributed in a volume of 50 L. The protective effect in the Fenton reaction was unaffected by addition of EDTA. After HPLC separation fractions were identified with similar DNA protective effects. Sinigrin, a glucosinolate abundant in Brussels sprouts, co-eluted with the most effective fraction and had DNA protective effects. In comparison with other antioxidants the patterns of effect of the extract in the five damage systems were more similar to that of sodium azide than to those of dimethylsulfoxide and vitamin C.

Constituents of Brussels sprouts can protect DNA by direct scavenging, e.g. hydroxyl radical and other oxidants, without prooxidant effects at concentrations potentially achievable by modest intake of the vegetable.

**Keywords:** Brussels sprouts, glucosinolate, ROS scavenger, 8-oxodG, DNA damage, Fenton reaction, chemoprevention, antioxidant

**Abbreviations:** 8-oxodG, 7-hydro-8-oxo-2'-deoxyguanosine; dG, deoxyguanosine; DMSO, dimethylsulfoxide; IC<sub>50</sub>, concentration giving 50% inhibition; ITC, isothiocyanate; NaN<sub>3</sub>, sodium azide; ROS, reactive oxygen species; TLC, thin-layer chromatography; UV, ultraviolet light

## INTRODUCTION

Cruciferous vegetables, e.g. broccoli, cauliflower, kale, and Brussels sprouts, have significant cancer preventive effects, as shown in epidemiological and animal carcinogenesis studies.<sup>[1-3]</sup> A specific cancer preventive mechanism of these vegetables could be related to a reduction of oxidative DNA damage, in particular guanine oxidation, which is the most abundant and mutagenic oxidative

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modification.<sup>[4]</sup> Oxidative DNA damage is frequently determined in terms of the level of the oxidatively modified nucleoside 8-oxodG, which has been widely used as a biomarker of oxidative DNA damage.<sup>[4,5]</sup> Indeed, in humans a diet rich in Brussels sprouts reduced the urinary excretion of 8-oxodG.<sup>[6]</sup> Homogenates and an aqueous extract of this vegetable had similar effects in rats, and also reduced the target organ levels of 8-oxodG after administration of the liver carcinogen, 2-nitropropane.<sup>[7]</sup>

The apparent protective effects of cruciferous vegetables against cancer are thought to be partly due to their high content of glucosinolates, which distinguish them from other vegetables.<sup>[8]</sup> Many of the breakdown products of these glucosinolates have potent effects on various phases I and II enzymes which could relate to the chemopreventive effects.<sup>[9]</sup> However, the constituents of these vegetables could also include significant amounts of antioxidants with direct scavenging effects. Such DNA protective effects have previously been found *in vivo* and *in vitro* by other plant products, in particular teas and extracts thereof.<sup>[10,11]</sup>

*In vitro* oxidative DNA damage can be induced by various radical generating systems. Hydroxyl radicals can be generated in a Fenton reaction system composed of iron and hydrogen peroxide.<sup>[12]</sup> 8-OxodG is formed at high rate in DNA by visible light and a photosensitizer, such as methylene blue, which has usually been ascribed to generation of singlet oxygen.<sup>[13,14]</sup> UV light, including UVA to UVC, can also induce the formation of 8-oxodG in DNA by mechanisms which may involve singlet oxygen.<sup>[12,15-17]</sup>

In the present study the inhibition of 8-oxodG formation in calf thymus DNA by an aqueous extract of microwave cooked Brussels sprouts was investigated in *in vitro* systems involving Fenton reactions, UVA, UVC, sunlamp light and visible light with methylene blue. In addition, the extract was separated into distinct fractions by preparative HPLC. These fractions and sinigrin, an abundant glucosinolate in Brussels sprouts, as well as some known ROS scavengers, i.e. DMSO,

vitamin C and sodium azide (NaN<sub>3</sub>), were also tested for their abilities to protect against DNA damage.

## MATERIALS AND METHODS

### Chemicals

Sinigrin (allyl-glucosinolate), calf thymus DNA, 8-oxodG, 2'-deoxyguanosine (dG), sand/celite, nuclease P1 and reagents were purchased from Sigma Co. (St. Louis, MO, USA); alkaline phosphatase was purchased from Boehringer Mannheim Co. (Indianapolis, IN, USA); ascorbic acid, DMSO, NaN<sub>3</sub>, and different isothiocyanates, i.e. allyl-, phenyl-,  $\beta$ -phenylethyl-, phenethyl-, hexyl-, propyl-, butyl-, and benzyl-ITC, were from Aldrich Co. (Milwaukee, WI, USA).

### Preparation of Brussels Sprouts Extract

Fresh Brussels sprouts (*Brassica oleracea L. var. gemmifera*) were obtained from an organic grower in Odsherred, DK and stored at  $-80^{\circ}\text{C}$ . An aqueous extract was prepared as previously described.<sup>[7]</sup> One kg Brussels sprouts was ground in a model MP-80 home-mixer (Braun Multipress, Germany) with 100 ml distilled water, and the juice and residues were cooked together in a microwave oven for 3 min. The cooked materials were squeezed through gauze and the residues were repeatedly washed with distilled water until the extract was colourless. The combined solution (cooked juice and water extract from the residues) was frozen overnight at  $-80^{\circ}\text{C}$  and lyophilized. The dry extract powder was kept in a closed bottle at  $4^{\circ}\text{C}$ . The powder was re-dissolved in distilled water and centrifuged at 4000g for 5 min before experiments. A small residue was not soluble in water or usual solvents and was discarded. An extract of green bean (*Phaseolus vulgaris L.*) and endive (*Cichorium intybus L.*) from a local market was prepared similarly.

### Chromatographic Analysis of the Brussels Sprouts Extract

The aqueous solution of the extract powder of Brussels sprouts was analysed by thin-layer chromatography (TLC) and HPLC. TLC was performed on a TLC aluminium sheet with silica gel 60 F<sub>254</sub> (Merck, Darmstadt, Germany) and developed with isopropanol-ethyl acetate-water (4:4:1). The spots on the plate were visualized under an UV lamp (254 nm) and compared with those of standard compounds, i.e. sinigrin, gluconapin, allyl-ITC and phenyl-ITC.

For HPLC the extract powder solution (9 mg/ml) was decolorized on a column of sand/celite in a Pasteur pipette (glass) and then centrifuged through a Whatman filter (30 kDa, Polysulphone, Maidstone, England) at 5000g for 30 min. The supernatant (100 µl) was loaded onto a D-7000 HPLC system (Merck, Hitachi Ltd., Japan) with a C<sub>18</sub> Nucleosil preparative column (250 × 16 mm I.D., particle size of 5 µm, Kanuer, Berlin, Germany). The column was eluted with a methanol (10%, v/v) acetate buffer (0.2 M, pH 7.0) mobile phase at a flow rate of 1.5 ml/min and fractions were collected by a model 202 fraction collector (Gilson, Middleton, WI, USA) monitored with a UV detector at 226 nm (the UV absorbance maximum of glucosinolates). The fractions corresponding to the seven major peaks of the chromatogram were pooled and lyophilized. The fractions were stored at 4°C until use.

The possible presence of catalase in the Brussels sprouts extract was assayed by incubation with hydrogen peroxide, which was quantitated by phenol red and horse-radish peroxidase.<sup>[18]</sup> The concentration of ascorbate in fresh extract powder solutions was assayed by HPLC with electrochemical detection.<sup>[19]</sup>

### DNA Exposure

The samples were composed of calf thymus DNA (300 µg) in 1.8 ml phosphate buffer (10 mM, pH 7.4) and 200 µl of test solution which consisted of

a series of concentrations of either the aqueous solution of extract powder of Brussels sprouts, or sinigrin, or aliquots of each of the seven fractions obtained by preparative HPLC. Additional experiments were performed with the alternative radical scavengers, i.e. DMSO and NaN<sub>3</sub>, and eight different isothiocyanates, which had to be dissolved in DMSO, Tween-80 or ethanol. All experiments were performed at least three times.

For the Fenton system the reaction mixture included 5 or 25 µM FeCl<sub>2</sub> with 5 µM H<sub>2</sub>O<sub>2</sub>, and the reaction was performed at 37°C for 30 min by gentle shaking in the dark. In additional experiments freshly prepared EDTA (final concentration of 100 µM) was added just before iron.

For the light induced damage the samples were placed in 12.5 ml standard plastic containers (2.6 cm diameter). For exposure to sunlamp light (Höhensonne 1000 Impuls, without infrared bulbs, emitting light from 240 to 580 nm, Philips, Holland) a distance of 35 cm from the solution surface was used and the time was 2 min. The effect was 9.12 kJ/cm<sup>2</sup> at this exposure distance. Samples were also exposed to UVA (Hi-intensity UV lamp, B-100AP filter, CA, USA) 365 nm for 30 min with an effect of 0.3 J/cm<sup>2</sup> or UVC (Universal UV lamp TL-900, Germany) at 254 nm for 3 min, both at an exposure distance of 6 cm. Samples with methylene blue 0.5 or 5 µM on ice were exposed to visible light from a 1000 W halogen tungsten lamp for 2 min at a distance of 20 cm.

### Analysis of 8-oxodG and dG in Calf Thymus DNA

After exposure of the DNA samples 5 M NaCl was added to a final concentration of 1 M followed by 50 µl of HCl (250 mM) and two volumes (5 ml) of ice-cold 96% ethanol. The DNA was allowed to precipitate at -20°C overnight followed by centrifugation at 4000g for 5 min. The DNA precipitate was washed with 70% ethanol, dried in air, and then dissolved in 200 µl sodium acetate buffer (20 mM, pH 4.8). The solution was digested to

nucleoside level at 37°C with 20 µl of Nuclease P1 (5 U/sample) for 30 min, and with 20 µl alkaline phosphatase (1 U/sample) for 60 min. The samples were centrifuged at 5000g for 35 min at 4°C.

An aliquot of the supernatant was injected into a chromatographic column (ODS 250 mm × 4.6 mm I.D., particle size of 5 µm, Beckman, CA, USA). The column was eluted with acetonitrile (5%, v/v) in sodium phosphate buffer pH 5.7 at a flow rate of 1 ml/min. The dG in the effluent was quantified by a UV detector (Merck-Hitachi L-4000; Darmstadt) at 254 nm and the 8-oxodG by an ESA Coulochem II electrochemical detector (Bedford Inc., MA, USA) with a 5011 analytical cell set at 120 and 280 mV at electrodes 1 and 2, respectively. Quantification was performed by calibration with pure 8-oxodG and dG. The chromatographic data were processed by Merck-Hitachi software.

### Statistics

IC<sub>50</sub> was determined by curve fitting to a log function by means of Statistic 5.0. Data were analysed with one-way analysis of variance.

## RESULTS

### Chromatographic Analysis and Preparative Fractionation

The aqueous extract powder of Brussels sprouts was prepared with a yield of approximately 64 g from 1 kg Brussels sprouts. Thin-layer chromatography of the aqueous extract of Brussels sprouts showed spots with *R<sub>F</sub>*-values corresponding to those of glucosinolates and isothiocyanates (Figure 1). The *R<sub>F</sub>*-values of the isothiocyanates, i.e. allyl-ITC, phenyl-ITC and others (not shown) were all very similar but distinct from those of the glucosinolates i.e. sinigrin and gluconapin (Figure 1).

The preparative HPLC of the aqueous extract consistently resulted in a chromatogram with

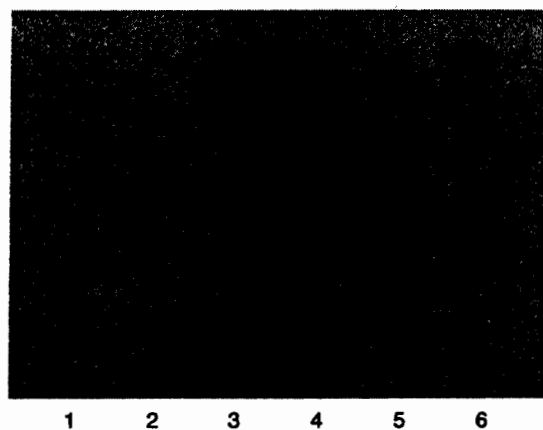


FIGURE 1 TLC of the aqueous extract of Brussels sprouts (5) and the reference compounds: sinigrin (1), gluconapin (2), allyl-ITC (3), phenyl-ITC (4) and a mixture of 1, 2, 3 and 4 (6) were performed on an TLC aluminium sheet with silica gel 60 F<sub>254</sub> and developed with isopropanol-ethyl acetate-water (4:4:1). The spots on the plate were visualized under an UV lamp (254 nm).

seven major peaks when monitored at 226 nm (Figure 2). The corresponding fractions were collected for inhibition experiments and their distinct retention times were confirmed by repeating the HPLC analysis with injection of the individual peak. Pure sinigrin eluted with a retention time identical to that of peak 3 of the chromatogram (Figure 2). Under the conditions used it was not possible to obtain chromatographic peaks after injection of allyl-ITC, phenyl-ITC and other ITC compounds.

The Brussels sprouts extract contained no detectable amounts of catalase activity or ascorbate. The UV absorbance spectra and extinctions (per µg) were similar in extracts of Brussels sprouts and beans and endives.

### Inhibition of 8-oxodG Formation

All the experimental systems, i.e. UVA, UVC, sunlamp, a Fenton reaction and methylene blue with visible light, gave rise to the generation of 8-oxodG in calf thymus DNA (Figure 3). The 8-oxodG yield was low with UVA, possibly due to a low light fluence from the lamp. The yield was

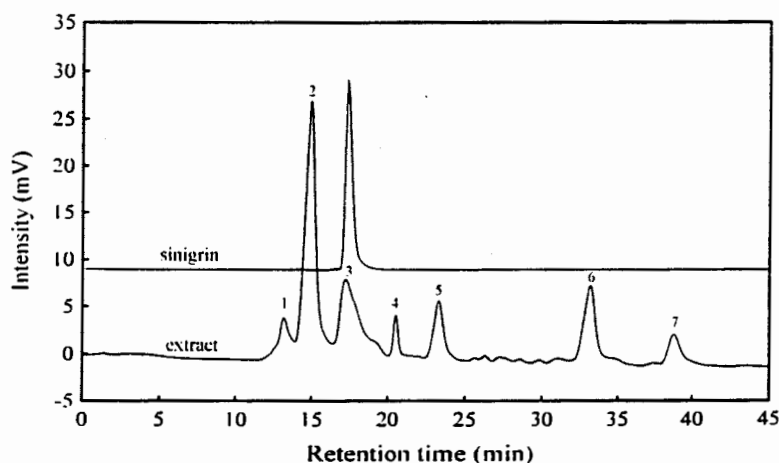


FIGURE 2 Chromatograms of an aqueous extract of Brussels sprouts and sinigrin by high-performance liquid chromatography (HPLC) analysis.

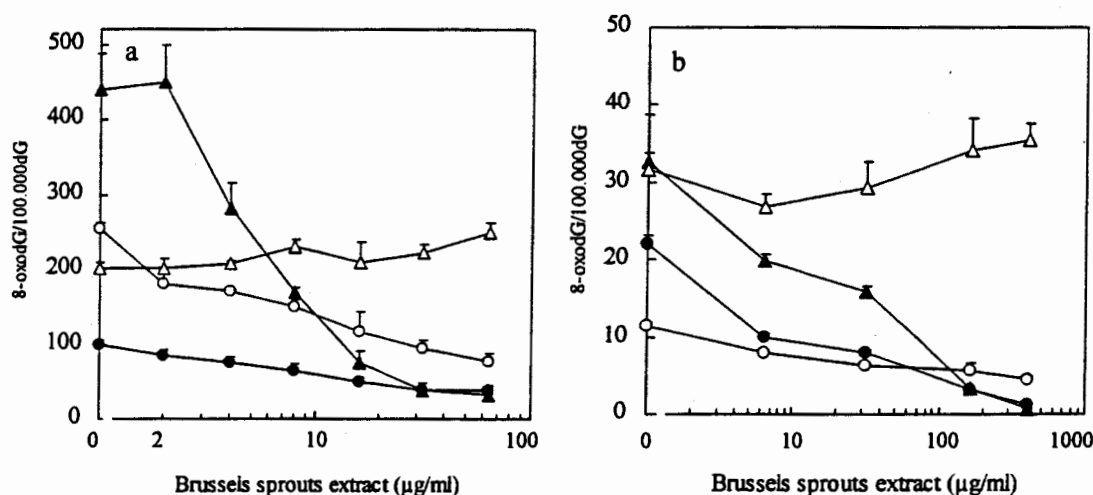


FIGURE 3 The relationship between the concentrations of a Brussels sprouts extract and the effect on formation of 8-oxodG in calf thymus DNA (a): by a Fenton reaction ( $5\ \mu\text{M}$  or  $25\ \mu\text{M}$   $\text{FeCl}_2$  with  $5\ \mu\text{M}$   $\text{H}_2\text{O}_2$  ( $\bullet$ ,  $\circ$ )), sunlamp light ( $\blacktriangle$ ), methylene blue ( $5\ \mu\text{M}$ ) with visible light ( $\triangle$ ); (b): without exposure ( $\circ$ ) and by UVA ( $365\ \text{nm}$  ( $\blacktriangle$ )), UVC ( $254\ \text{nm}$  ( $\bullet$ )), and methylene blue ( $0.5\ \mu\text{M}$ ) with visible light ( $\triangle$ ). Data are mean with SD of 3–6 experiments.

also low for UVC due to a short exposure period. Prolonged exposure, however, caused extensive fragmentation of the DNA rendering isolation impossible.

The aqueous solution of extract powder of Brussels sprouts reduced the formation of 8-oxodG in a concentration dependent manner in all systems except that with methylene blue and visible light (Figure 3) with maximal inhibition

of 60–100% (Table I). However, there were substantial differences in the potency. Thus, the rank order of the  $\text{IC}_{50}$  values of the extract was  $\text{UVC} = \text{sunlamp light} < \text{Fenton system} < \text{UVA}$  (Table II). When EDTA ( $100\ \mu\text{M}$ ) was included in the Fenton system, the extract of Brussels sprouts inhibited the formation of 8-oxodG to the same extent as in the absence of EDTA (Figure 4). Sinigrin was less potent than the extract of

TABLE I Effect of a Brussels sprouts extract in terms of inhibition in % of 8-oxodG formation in calf thymus DNA by reactive oxygen species generating systems

Brussels sprouts extract ( $\mu\text{g/ml}$ )	Sunlamp light	$\text{FeCl}_2$ 5 $\mu\text{M}$ + $\text{H}_2\text{O}_2$ 5 $\mu\text{M}$	$\text{FeCl}_2$ 25 $\mu\text{M}$ + $\text{H}_2\text{O}_2$ 5 $\mu\text{M}$	UVA 365 nm	UVC 254 nm
4	38 $\pm$ 18.6	21 $\pm$ 7.8	33 $\pm$ 3.7	—	—
6.4	—	—	—	39 $\pm$ 10.0	55 $\pm$ 8.4
8	62 $\pm$ 6.0	34 $\pm$ 8.4	41 $\pm$ 10.0	—	—
16	83 $\pm$ 5.1	50 $\pm$ 6.6	48 $\pm$ 2.7	—	—
32	91 $\pm$ 0.4	61 $\pm$ 8.6	63 $\pm$ 10.9	52 $\pm$ 4.9	64 $\pm$ 7.4
64	93 $\pm$ 1.8	63 $\pm$ 7.0	70 $\pm$ 11.8	—	—
160	—	—	—	90 $\pm$ 1.0	86 $\pm$ 17.6
400	—	—	—	98 $\pm$ 0.4	95 $\pm$ 7.2

Data are from at least three experiments with each assay performed in triplicate; — = not determined.

TABLE II  $\text{IC}_{50}$  values for inhibition of 8-oxodG formation in calf thymus DNA by UVA, UVC, sunlamp light, Fenton reaction and visible light with methylene blue ( $\mu\text{g/ml}$  and mM)

Exposure systems	Brussels sprouts extract $\mu\text{g/ml}$	Sinigrin $\mu\text{g/ml}$ (mM)	$\text{NaN}_3$ $\mu\text{g/ml}$ (mM)	DMSO $\mu\text{g/ml}$ (mM)	Vitamin C $\mu\text{g/ml}$ (mM)
UVA	20.0	n.i.	26.0 (0.4)	prom	n.i.
UVC	6.0	n.i.	390 (6.0)	n.i.	n.i.
Sunlamp light	6.0	10.0 (0.025)	—	11.2 (0.14)	< 17.6 (0.1)
$\text{FeCl}_2$ 5 $\mu\text{M}$ + $\text{H}_2\text{O}_2$ 5 $\mu\text{M}$	17.5	—	—	—	—
$\text{FeCl}_2$ 25 $\mu\text{M}$ + $\text{H}_2\text{O}_2$ 5 $\mu\text{M}$	14.5	40.0 (0.10)	65.0 (1.0)	1.3 (0.02)	prom
Methylene blue 0.5 $\mu\text{M}^a$	n.i.	n.i.	n.i.	n.i.	—
Methylene blue 5 $\mu\text{M}^a$	n.i.	n.i.	n.i.	n.i.	352 (2.0)

— = not determined; n.i. = no inhibition; prom = promotion; <sup>a</sup> = + visible light.

The  $\text{IC}_{50}$  values were calculated by curve fitting to a log function of data from three independent sets of experiments.

Brussels sprouts as an inhibitor of the formation of 8-oxodG by sunlamp light and the Fenton reaction but the rank order of the  $\text{IC}_{50}$  values for these two systems were similar (Table II). Sinigrin at concentrations up to 750  $\mu\text{M}$  did not affect 8-oxodG formation by UVA, UVC or methylene blue with visible light.

Different patterns were observed when some other scavengers, i.e. DMSO, Vitamin C and  $\text{NaN}_3$  were tested (Table II). Sodium azide was an inhibitor of 8-oxodG formation in the UVA, UVC and Fenton systems. DMSO was the most potent scavenger with respect to the Fenton system, whereas it promoted the effect of UVA with a doubling of the 8-oxodG formation at 200  $\mu\text{M}$ . Vitamin C had no effect against UVA and UVC and was a strong prooxidant in the Fenton system with a doubling of the 8-oxodG formation at 56  $\mu\text{M}$ .

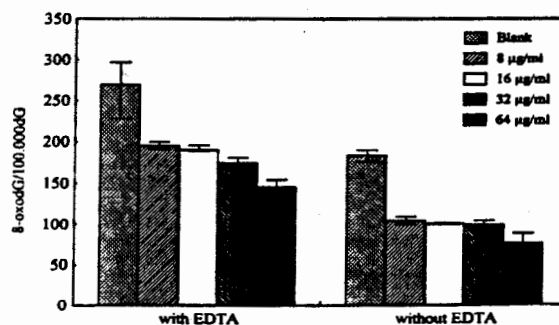


FIGURE 4 The effect of a Brussels sprouts extract on formation of 8-oxodG in calf thymus DNA by a Fenton reaction (25  $\mu\text{M}$   $\text{FeCl}_2$  with 5  $\mu\text{M}$   $\text{H}_2\text{O}_2$ ) with or without EDTA (100  $\mu\text{M}$ ). Data are mean with SD of 3 experiments.

The seven fractions from the HPLC separation of the Brussels sprouts extract were compared with respect to their abilities to inhibit 8-oxodG formation induced by the Fenton reaction,

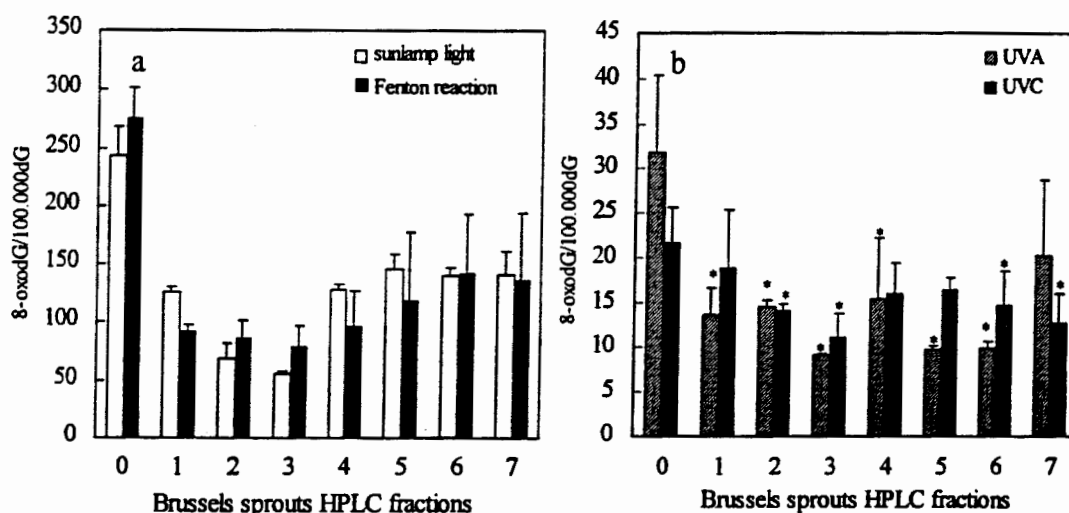


FIGURE 5 Effects of HPLC fractions of a Brussels sprouts extract on formation of 8-oxodG in calf thymus DNA by sunlamp light, a Fenton reaction ( $25 \mu\text{M FeCl}_2$  with  $5 \mu\text{M H}_2\text{O}_2$ ), UVA (365 nm) and UVC (254 nm). Values are mean with SD from four (a) and three (b) experiments. (a) The effects of peak 1–7 were all significantly different from the control sample which was no addition of HPLC fraction ( $P < 0.01$ ); (b) \* denotes significantly different from the control sample ( $P < 0.05$ ).

sunlamp light, UVA and UVC (Figure 5). For this experiment each fraction was added to the exposure vials at concentrations corresponding to the yield from  $90 \mu\text{g/ml}$  of the extract powder. The effects of sunlamp light and Fenton reaction were significantly inhibited by all the seven fractions, although with significantly different effects ( $P < 0.01$ ). The maximum inhibition (75%) was achieved with fraction 3 (Figure 5(a)). The UVA-induced 8-oxodG levels were reduced by 50–70% by fractions 1–6 ( $P < 0.05$ ), with fraction 3 being the most efficient (Figure 5(b)). With UVC only fractions 2, 3, 6 and 7 demonstrated significant, but less pronounced inhibitory effects (30–50%,  $P < 0.05$ ), again with fraction 3 as the most effective (Figure 5(b)).

Extracts of green beans and endives prepared similarly to the Brussels sprouts extract had no effect on 8-oxodG formation in the Fenton and sunlamp systems at concentrations up to  $64 \mu\text{g/ml}$  (data not shown). With the different isothiocyanates the experiments were complicated by the solvents used, i.e. DMSO, Tween-80 and ethanol, which had potent inhibiting effects of their own. At the necessary concentrations of

solvents the formation of 8-oxodG was reduced by at least 60% in the Fenton and sunlight systems. The presence of isothiocyanates did not further reduce the formation of 8-oxodG in DNA (data not shown).

## DISCUSSION

In the present study an extract of cooked Brussels sprouts inhibited oxidation of DNA by a number of systems generating reactive oxygen species (ROS) *in vitro*. Although such an effect was not seen in an extract of beans and endives, test of other vegetables will be required to show that it is specific to Brussels sprouts and possibly other cruciferous vegetables. A chromatographically separated fraction appeared to be particularly effective whereas the active compounds have yet to be identified. It appears evident that a number of different constituents contribute to the protection from oxidative DNA damage, including glucosinolates.

Previous studies in humans and rats have shown that cooked Brussels sprouts can reduce

the spontaneous rate of oxidative DNA damage as assessed by the urinary excretion of 8-oxodG, whereas non-cruciferous vegetables had no effect.<sup>[6,7,20]</sup> An aqueous extract similar to the present also decreased the enhanced levels of 8-oxodG in urine and the target organs, liver, kidney and bone marrow, induced by treatment of rats with 2-nitropropane.<sup>[7]</sup> Although isolated indolyl-glucosinolates and their myrosinase generated breakdown products had no effects in rats<sup>[7]</sup> other glucosinolates breakdown products could have protective effects *in vivo* through enhancement of phase II defence enzymes, such as glutathione peroxidase, glutathione transferases and NAD(P)H: quinone reductase. Indeed, Brussels sprouts and isothiocyanates with alkyl, aryl and/or sulphanyl side chains have such effects.<sup>[9,21,22]</sup> Nevertheless, Brussels sprouts have a low content of precursors to sulphoraphane, a particularly potent member of the isothiocyanates and inducer of the antioxidant responsive element effects of phase II detoxification enzymes and other protectants.<sup>[21,23]</sup> Moreover, unpublished data from our laboratory show only a rather modest effect of the Brussels sprouts extract on the relevant phase II enzymes in rat liver. A number of other antioxidant vegetable products, such as teas and their extracts, have been shown to protect from 8-oxodG formation in DNA both by 2-nitropropane and other carcinogens *in vivo* in rats and from oxygen radical generating systems *in vitro*.<sup>[10,24]</sup> Thus, a direct scavenging effect of some constituents could be partly responsible for the protective effects *in vivo* as supported by the present data.

A previous *in vitro* study has reported relatively modest antioxidant properties or even some pro-oxidant effects of Brussels sprouts extracts prepared by methanol extraction of freeze-dried and ground material that was cooked or autolyzed.<sup>[25]</sup> Similarly, some isolated glucosinolates from cruciferous vegetables, sinalbin, glucoiberin and gluconapin, and their breakdown products had almost no antioxidant effects.<sup>[25]</sup> However, none of the assays in that study included DNA

oxidation. In a deoxyribose based assay an extract from autolyzed Brussels sprouts showed iron binding capacity and modest hydroxyl radical scavenging property.<sup>[25]</sup> In contrast, the DNA protective effect of the present Brussels sprouts extract in the Fenton system was independent of the presence of EDTA indicating that it is related to hydroxyl radical scavenging rather than iron chelation. Thus, differences in extract preparation and assay targets may explain the apparent differences between the present data and the study by Plumb *et al.*<sup>[25]</sup>

Brussels sprouts contain 0.4% (w/w) glucosinolates,<sup>[26]</sup> mainly sinigrin, glucobrassicin, gluconapin and progoitrin, of which the first two are predominant.<sup>[27]</sup> In agreement with this the TLC analysis of the present Brussels sprouts extract showed spots corresponding to sinigrin, gluconapin and their breakdown products, such as allyl-ITC, phenyl-ITC etc. Although, all the seven fractions from the HPLC separation of the Brussels sprouts extract reduced the levels of 8-oxodG significantly in the Fenton and sunlamp systems and most of them had effects with respect to UVA and UVC, fraction 3 was particularly effective in all systems. Unfortunately, there was not sufficient material to study detailed concentration-effect relationships. Sinigrin coeluted with fraction 3 in HPLC analysis. However, on a weight basis it was less potent and could only contribute to a minor extent to the effect of the extract in Fenton reaction and sunlamp light. Eight isolated isothiocyanates, one group of the breakdown products of glucosinolates, did not seem to have DNA protective effects, although definitive conclusions cannot be drawn because of the solubility problems in the present study. Brussels sprouts are not particularly rich in the antioxidants, kaempferol and quercetin known from e.g. broccoli.<sup>[28]</sup> The lack of effect of an extract of green beans and endives in the present study and of a similar homogenate in rats *in vivo* indicate that the active compounds are not abundant in all vegetables.<sup>[7]</sup> Accordingly, the autolytic process starting with myrosinase catalysed



cleavage of the glucosidic bond of the glucosinolates in Brussels sprouts generating a number of reactive products, the subsequent cooking and/or reactions with hydroxyphenols and polyphenols could be required for the protective effects found in this study and in rats *in vivo*.<sup>[7]</sup> Indeed, extracts of fermented black tea were more potent in scavenging hydrogen peroxide and quenching UV induced 8-oxodG formation in DNA than the corresponding extracts of green tea rich in the known catechin antioxidants.<sup>[10]</sup>

The formation of 8-oxodG in DNA by UV light *in vitro* has been suggested to be due to singlet oxygen based on the enhancement by deuterium oxide and the quenching effect of sodium azide.<sup>[17,29]</sup> Methylene blue and visible light induced 8-oxodG in DNA as in many other studies.<sup>[13]</sup> However, there was no effect of sodium azide in the present study, which could question the involvement of singlet oxygen. Nevertheless, singlet oxygen generated by methylene blue and visible light has been shown to oxidise 8-oxodG further at a high rate, which is reduced by sodium azide.<sup>[14]</sup> Accordingly, scavenger effects may be difficult to assess in such systems although different concentrations of methylene blue yielding highly different 8-oxodG levels gave similar results concerning the scavengers. Moreover, in the present and other studies sodium azide was also an efficient scavenger of hydroxyl radical generated in the Fenton reaction.<sup>[30]</sup> The Brussels sprouts extract was in general similar to sodium azide with quenching of the effects of both UV and Fenton reaction on DNA. In agreement with other studies DMSO enhanced the effect of UVA<sup>[30]</sup> whereas vitamin C was the only compound able to quench the effect of methylene blue and visible light and a potent prooxidant in the Fenton reaction as previously shown.<sup>[12]</sup> Thus, the pattern of effects of the Brussels sprouts extract appears similar to that of extracts of teas, in particular black tea.<sup>[10]</sup>

Brussels sprouts are important vegetables for consumption. The yield of extract powder from Brussels sprouts was about 64 g/kg. Thus, the

present IC<sub>50</sub> values of 6–20 µg/ml correspond to the intake of 5–20 g Brussels sprouts assuming complete bioavailability of the active constituents and a volume of distribution of 50 L. Even a modest intake of Brussels sprouts may contribute to protection against oxidative DNA damage and thus mutagenesis and carcinogenesis.

In conclusion, the present study has shown that constituents of cooked Brussels sprouts have potent inhibitory effects on oxidation of DNA *in vitro* possibly through scavenging hydroxyl radical and other oxidants. Although the active compounds have yet to be identified, even a modest intake of Brussels sprouts could suffice in DNA protection if the bioavailability is not a problem.

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