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Are Whole Extracts and Purified Glucosinolates from Cruciferous Vegetables Antioxidants?

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Fruits and vegetables contain several classes of compounds that can potentially contribute to antioxidant activity, including vitamins, simple and complex phenolics, sulphur-containing compounds and glucosinolates. The glucosinolates are found in high concentration in many cruciferous vegetables, and it is well established that their breakdown products induce endogenous antioxidant defences such as quinone reductase and glutathione S-transferase in cells and *in vivo*. Despite the anticarcinogenic effect of these compounds in animal models, the direct antioxidant properties of this class of compounds have not been systematically studied. We therefore examined the free radical-scavenging properties of representative extracts and of purified glucosinolates from cruciferous vegetables, by measuring their effect on ascorbate- or NADPH/iron-induced peroxidation of human liver microsomes, ascorbate/iron-induced peroxidation on phospholipid liposomes, iron chelation and hydroxyl radical scavenging using the deoxyribose assay, total antioxidant potential using ABTS (2,2'-azinobis(3-ethyl-benzothiazoline-6-sulphonate)) and the bleomycin assay. Most of the extracts from cruciferous vegetables exhibited some antioxidant properties, although extracts from cooked Brussels sprouts increased the rate of microsomal lipid peroxidation. The effects in these assays were dependent upon processing and species of crucifer, and the glucosinolate content appeared to play a minor role in these effects, since purified glucosinolates exhibited only weak antioxidant properties. The total antioxidant activities of extracts from cooked and autolysed Brussels sprouts were identical within experimental error. This is probably due to the content of phenolics which is unaltered by autolysis, despite the differences between these samples in other assays especially NADPH-iron-induced lipid peroxidation of human liver microsomes. The results demonstrate that glucosinolates are unlikely to account for the direct antioxidant effects of extracts from cruciferous vegetables.

Key words: cruciferous vegetables, glucosinolate, pro-oxidant, antioxidant, lipid peroxidation, DNA damage

Abbreviations: QR, Quinone reductase; ABTS, 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulphonate); TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances

INTRODUCTION

There is substantial epidemiological evidence to show that a diet high in fruit and vegetables

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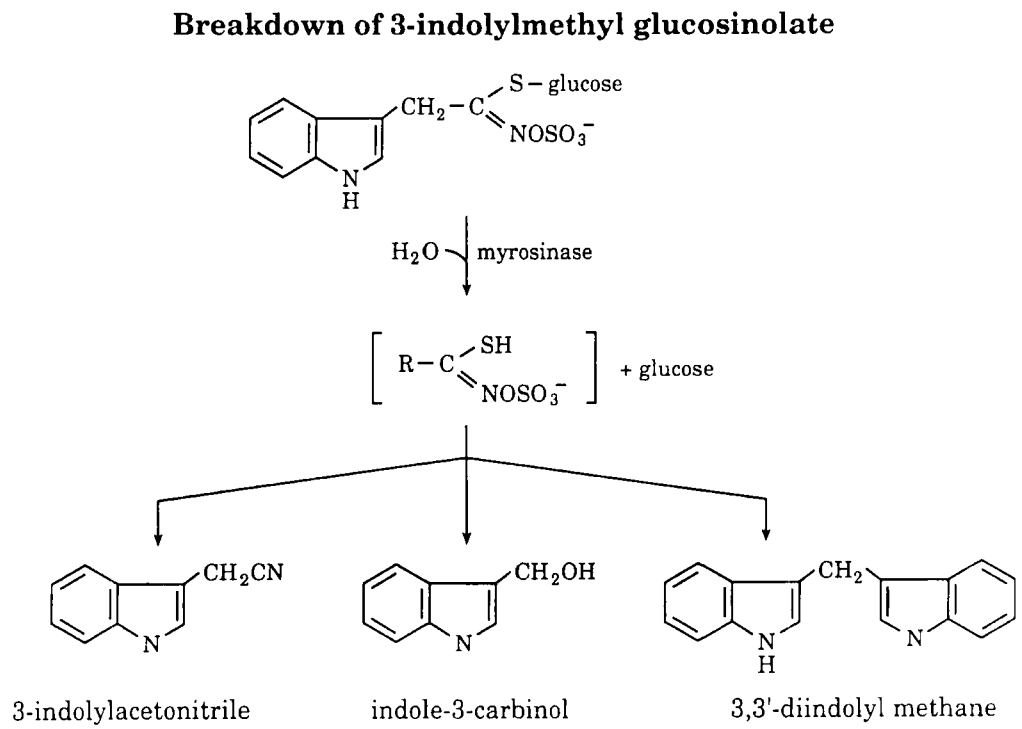
protects against chronic diseases in humans.[1,2] The World Health Organisation recommend the consumption of at least 5 portions of fruit and vegetables per day, including at least one crucifer. The actions of compounds from fruit and vegetables are multifactorial[3]:

(a) Some antioxidants inhibit formation of carcinogens from precursors, including prevention of the formation of mutagens by colonic flora[3]. The ability of whole extracts of cruciferous vegetables to act as prooxidants or antioxidants has been poorly studied. Some cruciferous vegetables contain quercetin and kaempferol[4,5], which can exert some antioxidant properties *in vitro*[6]. Indole-3-carbinol is an autolytic breakdown product of indolyl glucosinolates and is found in many crucifers especially broccoli, Brussels sprouts[7] and Savoy cabbage[8]. Indole-3-carbinol inhibited CCl_4-induced lipid peroxidation of rat liver microsomes, but a relatively high concentration (50% inhibition at 35–40 μM) was required[9,10].

(b) Blocking agents prevent carcinogens from reaching or reacting with critical sites, especially by induction of phase II enzymes, such as glutathione S-transferase and quinone reductase. There is a growing number of compounds identified in cruciferous vegetables which possess this activity, predominantly derived from autolytic breakdown products of glucosinolates (Scheme 1). Although most glucosinolates themselves are not able to induce phase II enzymes,[11] breakdown products such as sulphoraphane[12] are potent inducers.

(c) Suppressing agents inhibit carcinogenesis



SCHEME 1 Structure of a glucosinolate (3-indolylmethyl glucosinolate) and of some of its typical breakdown products as catalysed by myrosinase.

when administered subsequent to a carcinogen. Compounds from crucifers such as indole-3-carbinol have this activity[13].

Experimental studies on animals and some studies on humans have demonstrated a broad range of effects on feeding cruciferous vegetables or compounds purified from them, especially induction of both cytochrome P-450 and phase II enzymes.[14–20] However, these effects require breakdown of constituents either by endogenous plant enzymes[21] or by microflora in the gut.[22] Most of these studies have not examined the antioxidant properties of extracts from crucifers or from constituent compounds, although it has been shown that feeding mice a diet of cabbage (20% w/w of the total diet) led to a reduction in hepatic TBA-reactive material[23] and also that indole-3-carbinol is a weak inhibitor of lipid peroxidation.[9,10]

We have examined the antioxidant properties of extracts from cruciferous vegetables, of isolated glucosinolates and of glucosinolate breakdown products using ascorbate and NADPH/iron peroxidation of human liver microsomes, ascorbate/iron-induced peroxidation of phospholipid liposomes, iron chelation and hydroxyl radical scavenging using deoxyribose, total antioxidant potential using ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate)) and the bleomycin-induced DNA damage assay. The results show that crucifer extracts can be pro- or antioxidant depending on processing of the vegetable and on the variety. These effects do not appear to be mediated by glucosinolates or their breakdown products, which have only weak antioxidant properties.

MATERIALS AND METHODS

Materials

Authenticated cultivars of cruciferous vegetables were supplied from the National Institute of Agricultural Botany. Sample used were: Brussels sprouts (Roger), *Brassica oleraceae* L. var. *gemmifera*; Broccoli, *Brassica oleraceae* L. var. *botrytis* subvar. *cymosa* ('Purple' broccoli is the description for winter grown broccoli, and calabrese usually refers to spring grown); Cabbage (autoro (red) and white), *Brassica oleraceae* L. var. *capitata*; Cauliflower (Diana), *Brassica oleraceae* L. var. *botrytis* subvar. *cauliflora*. Portions of healthy human liver were kindly supplied by the Transplant Unit of Addenbrookes Hospital, Cambridge, UK and were stored at –40°C. Biochemicals and other reagents were of the highest quality available from Sigma Chemical Company, Poole, UK, or from BDH, Poole, UK. Trolox was from Hoffman La Roche Switzerland.

Preparation of extracts

Extracts from cruciferous vegetables were made as previously described.[21] Briefly, this involved rapid freezing in liquid nitrogen, followed by freeze drying and grinding the material to a fine powder. 'Raw' extracts were made by adding 70% methanol (30 ml to 0.5 g powder) and boiling for 15 min. Methanol was completely removed by rotary evaporation, and the sample volume adjusted with water. Extracts were centrifuged for 5 min at 5000 g, and then sterile filtered before use. The process of chopping, pureeing or chewing involves some autolysis (ie hydrolysis of some of the plant compounds by endogenous enzymes). To mimic this autolytic process, some samples, after drying and grinding, were moistened with water and incubated for 1 h at room temperature, after which methanol was added to 70% v/v. Additionally, some vegetables were cooked by boiling for 15 min, and then freeze dried and extracted as described above for 'raw' vegetables. The resultant extracts contained no methanol, and some characteristics are shown in Table 1. The conditions of extraction denature endogenous plant enzymes and hence none are present in the final extract. The protocol described is optimised for extraction of glucosinolates, flavonoids, phenolics and other compounds that are soluble in

TABLE 1 Characterisation of extract from cruciferous vegetables.

Source of extract	variety or type	preparation	content of ascorbate[1]	induction (%) of QR[2]	pH of extract
Brussels sprouts	Roger	cooked	nd[3]	14	5.9
Brussels sprouts	Roger	autolysed	nd	63	6.0
Broccoli	purple	raw	nd	41	6.0
Red cabbage	Autoro	autolysed	nd	24	6.0
Cabbage	white	autolysed	nd	51	6.0
Cauliflower	Diana	autolysed	nd	<10	6.0

[1]Estimated using ^{1}H-NMR (limit of detection about 5 μM).
[2]Induction of quinone reductase (QR) measured in hepa1c1c7 cells, with compound to be tested at 1.6 mg extract per ml culture medium, for 24 h[21]. Results are expressed as a percentage change in QR activity relative to a control with no extract.
[3]nd, not detected.

70% methanol from the vegetables, with only minimal extraction of lipid-soluble vitamins and carotenes.

Preparation of glucosinolates

Glucosinolates (Table 2 and Scheme 1) were isolated and purified from plant sources.[8,24] Myrosinase (thioglucose glucohydrolase EC 3.2.3.1) was prepared[25] and purified as described previously,[26] and glucosinolates were treated with this enzyme at pH 3.1 and 6.6.[11] HPLC analysis[8] revealed that all of the glucosinolates were completely hydrolysed by this procedure.

Preparation of microsomes and liposomes

Microsomes were freshly prepared from human liver (stored at –70°C) by the method of Lambert,[27] using a buffer of 20 mM BisTris propane pH 7.0/0.25 M sucrose/0.15 M KCl. Microsomes were washed with 150 mM Tris/Cl pH 8.0 to remove extraneous cytosolic proteins. Assay of lactate dehydrogenase and glutamate dehydrogenase activity showed that the vesicles were free from cytosolic and mitochondrial contamination respectively, and the amount of acid phosphatase activity showed that lysosomal contamination was minimal.[27,28]

TABLE 2 Properties and distribution of glucosinolates used in this study.

common name	side chain	good sources[1]:	Induction (%) of QR[2] treated at pH 3.1	myrosinase pH 6.6	myrosinase pH 3.1
Sinalbin	p-hydroxyl benzyl	white mustard	24	<10	<10
Gluconapin	but-3-enyl	Brussels sprouts	<10	<10	<10
Glucoiberin	3-methyl sulphinyl-propyl	broccoli , Brussels sprouts, cabbage	<10	76[3]	46

[1]Data from references 7, 8, 41 and 57.
[2]Induction of quinone reductase (QR) measured in hepa1c1c7 cells, with compounds at 15 μM or [3]1.8 μM for 24 h (reference 11). Results are expressed as a percentage change in QR activity to a control with no extract.

Phospholipid liposomes were prepared as previously described.[29]

Ascorbate/Fe(III)-induced peroxidation

Microsomes or liposomes (0–0.2 mg liver microsomal protein or 0–0.6 mg phosphatidylcholine) were suspended in 150 mM KCl containing 0.2 mM $FeCl_3$ and various amounts of test material added. Peroxidation was started with ascorbate (final concentration 0.05 mM), with a final volume of 0.4 ml. Samples were incubated at 37°C for 40 min, and reactions terminated by the addition of 0.8 ml of 20% (w/v) trichloracetic acid/0.4 % thiobarbituric acid/0.25 M HCl and 0.01 ml of butylated hydroxytoluene (5 % w/v) in ethanol. The production of TBARS was measured after incubation at 80°C for 15 min.[30] Extracts and compounds alone (without microsomes or phospholipid) showed no significant reaction in the thiobarbituric acid assay.

NADPH/iron-induced peroxidation

Liver microsomes (0–0.4 mg protein) were suspended in BisTris propane buffer (20 mM, pH 7.0) containing: 150 mM KCl; 0.8 mM ADP; 0.2 mM $FeCl_3$ (freshly prepared in water) and test samples. Peroxidation was started with NADPH (final concentration 0.4 mM) in a total volume of 0.4 ml. Samples were incubated and processed as described above.

All peroxidation experiments were carried out over a range of concentrations of microsomes or lipids. Employing just one concentration to assess antioxidant properties can lead to misleading results, since the peroxidation becomes saturated at high concentrations of microsomes.[29,31–33] The data are therefore presented as the amount (μg) of dry weight that gave rise to 50 % inhibition of peroxidation at 0.3 and 0.5 mg/ml for microsomes and phosphatidylcholine liposomes respectively (see discussion in Plumb *et al.*[29]).

Deoxyribose damage

Hydroxyl radical scavenging and iron chelation assays were performed using deoxyribose as described[29,34].

Reduction of Fe(III) by ascorbate

The ability of test substances to affect the reduction of Fe(III) by ascorbate was assessed as described by Plumb *et al.*[29]

Total antioxidant activity

Total antioxidant activity was measured by the ABTS (2,2′-azinobis(3-ethyl-benzothiazoline-6-sulphonate)) method,[35] which is based on the relative ability of antioxidants to scavenge the radical cation of ABTS ($ABTS^{\cdot+}$), a green chromophore generated from interaction between activated metmyoglobin, hydrogen peroxide and ABTS. The extent of the quenching of $ABTS^{\cdot+}$ absorption at 734 nm by antioxidants in the medium under investigation is compared to standard amounts of the synthetic antioxidant. Trolox gives a measure of the total antioxidant activity.

Bleomycin assay

The ability of the test substances to reduce iron *in vitro* was performed as described previously,[36] in the absence of ascorbate. Positive controls were performed containing ascorbate (0.2 mM) or propyl gallate (100 mM).

Determination of protein concentration

Microsomal protein concentrations were determined by the bicinchoninic acid method[37] using bovine serum albumin as standard.

RESULTS

Effects of extracts on lipid peroxidation

The effects of extracts from cruciferous vegetables on enzymic lipid peroxidation of human liver microsomes is shown in Figure 1a. There is a pronounced promotion of NADPH/iron-induced peroxidation by extracts from cooked Brussels sprouts, and this effect is much greater than when

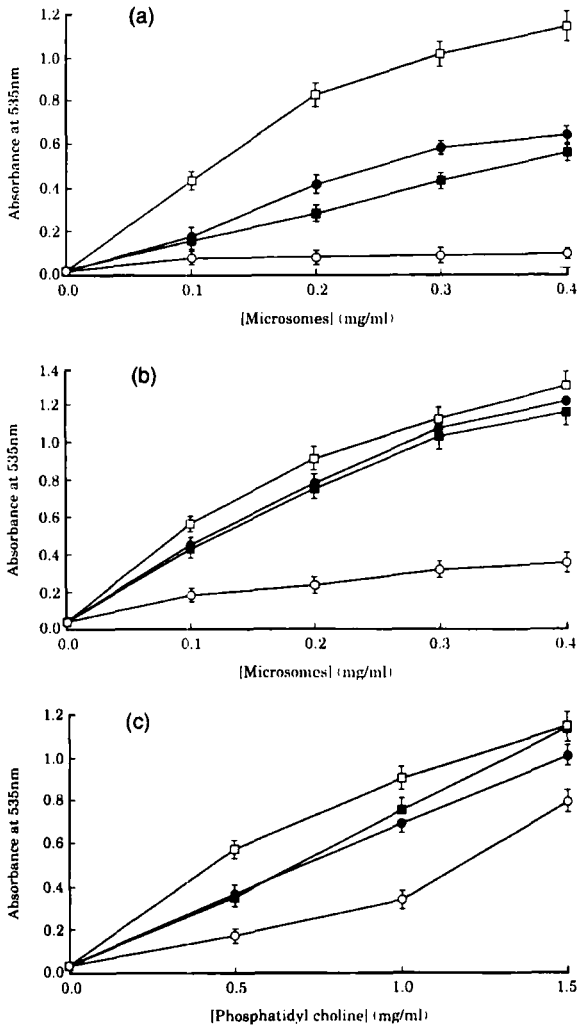


FIGURE 1 Effect of extracts from cruciferous vegetables on peroxidation. Extracts (330 μg dry weight/ml reaction mixture) from cooked Brussels sprouts (□), autolysed Brussels sprouts (●) and from raw Broccoli (○) were added to the assay mixture. Peroxidation was measured using the TBARS method at A_{535} as described in Materials and Methods, and compared to controls with no extract (□). In the absence of microsomes, no TBARS were observed. The error bars indicate standard deviation of 4 replicates. (a) Enzymic peroxidation of human liver microsomes was initiated by NADPH/iron and contained ADP (0.8 mM). Non-enzymic peroxidation of (b) human liver microsomes or (c) phosphatidyl choline liposomes was initiated by ascorbate/iron.

the extract is prepared from autolysed material. Extract from raw broccoli, however, was an effective antioxidant. The pro-oxidant effect of extract from cooked Brussels sprouts was also observed in non-enzymic peroxidation of human liver

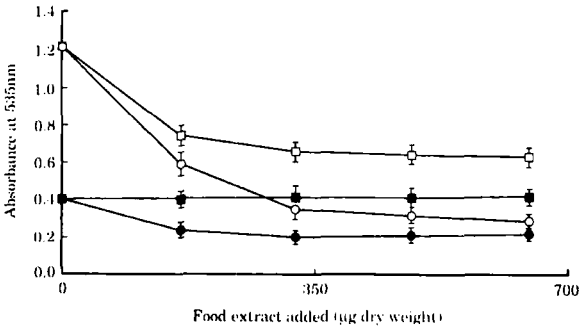


FIGURE 2 Scavenging of hydroxyl radicals and iron binding of extracts from cruciferous vegetables as determined using the deoxyribose assay. Extracts from cooked Brussels sprouts (squares) or from autolysed white cabbage (circles) were added to the deoxyribose assay (reaction volume of 0.4 ml) in the presence (open symbols) and absence (shaded symbols) of EDTA (0.1 mM).

microsomes (Figure 1b) and of phospholipid liposomes (Figure 1c), although the effect was much less pronounced. Extracts from several cruciferous vegetables were tested in this way and the results are summarised in Table 3. Clearly, although some extracts from Brussels sprouts were pro-oxidant, the remaining samples all showed some antioxidant activity. Control experiments showed that the extracts did not themselves generate a chromogen in the assay, or interfere with chromogen development.

Hydroxyl radical scavenging, iron reduction and iron chelation by extracts

We also examined the effect of extracts from cooked Brussels sprouts, when compared to the autolysed vegetable, on hydroxyl radicals. Figure 2 shows data on the effects of extracts from cooked Brussels sprouts and autolysed white cabbage on the deoxyribose assay. This assay, in the presence of EDTA, measures the ability of the extract to scavenge hydroxyl radicals.[34,38] As can be seen from the data, extracts from white cabbage were much better hydroxyl radical scavengers than extracts from cooked Brussels sprouts. The data for all of the extracts are summarized in Table 4.

TABLE 3 Effects of cruciferous extracts on lipid peroxidation.

Source	Treatment	NADPH/Fe peroxidation Human liver microsomes	Ascorbate/Fe peroxidation Human liver microsomes	Phospholipid liposomes
Brussels sprouts	Autolysis	500 (pro)[1]	>700	>700
Brussels sprouts	Cooked	170 (pro)	>700 (pro)	>700 (pro)
Broccoli	Raw	260	>700	430
Red cabbage	Autolysis	400	>700	400
White cabbage	Autolysis	>700	>700	>700
Cauliflower	Autolysis	>700	>700	>700

[1]Results are expressed as the dry weight (μg) required to give 50% inhibition or 50% promotion (pro) at a microsome concentration of 0.3 mg protein/ml. (see Materials and Methods). All data are obtained from a range of at least 5 concentrations of extract at 4 concentrations of microsomal protein (0.1 to 0.4 mg/ml). In our system, butylated hydroxytoluene inhibited NADPH/Fe peroxidation and ascorbate/Fe peroxidation with I_{50} (50% inhibition) of 0.5 and 2.5 μM respectively. For ethoxyquin, I_{50} was 0.07 and 40 μM respectively.

In the absence of EDTA, iron is present in the assay as Fe^{3+} and not Fe^{3+}-EDTA. This 'free' form of iron binds weakly to deoxyribose. In this case, hydroxyl radical scavengers inhibit only poorly, but compounds which bind iron more tightly than deoxyribose are effective inhibitors. From Figure 2 and Table 4, it can be seen that the extract from white cabbage contains the most metal binding compounds. The effect of autolysis and cooking on Brussels sprouts is very pronounced in this experiment: extracts from autolysed Brussels sprouts exhibited strong metal binding capacity, whereas extracts from the cooked vegetable were very poor. Certain compounds such as flavonoids are known to bind metals,[4] but the effect of cooking on this activity is not known.

The extracts were assessed for their ability to influence ascorbate-mediated reduction of Fe^{3+} (Table 4). All exerted only a very minor effect on this reaction.

Total antioxidant activity of extracts

The ABTS method[35] was used to evaluate the total antioxidant potential of some of the extracts (Table 5). Extracts from broccoli and from red cabbage were much more effective in this system than extracts from Brussels sprouts. This is

TABLE 4 Effect of extracts on hydroxyl radical scavenging, iron chelation and iron reduction.

Source	Treatment	Deoxyribose assay A_{535}(+ EDTA)	A_{535}(− EDTA)	Iron reduction[2] (% of control)
Control	water	1.80[1] ± 0.12	0.34 ± 0.03	100
Brussels sprouts	Cooked	0.90 ± 0.08	0.33 ± 0.02	90
Brussels sprouts	Autolysed	0.61 ± 0.05	0.18 ± 0.01	102
Broccoli	Raw	0.83 ± 0.06	0.28 ± 0.02	118
Red cabbage	Autolysed	0.55 ± 0.04	0.27 ± 0.02	120
White cabbage	Autolysed	0.39 ± 0.04	0.16 ± 0.01	118
Cauliflower	Autolysed	0.84 ± 0.05	0.21 ± 0.01	98
Positive control	Hypotaurine (50 mM)	0.35 ± 0.03	0.12 ± 0.01	—

[1] All figures are given as the effect of 660 μg dry weight in each assay, as described in Materials and Methods, and each value is the mean and S.E.M. of 4 determinations.
[2] Control A_{562} value was 0.90 ± 0.01. The assay was performed in the presence of ascorbate (50 μM) as described in reference 29.

TABLE 5 Total antioxidant activity of extracts from cruciferous vegetables.

Source	Treatment	mean ± standard deviation of 3 measurements
Brussels sprouts	Autolysed	0.75 ± 0.04
Brussels sprouts	Cooked	0.79 ± 0.04
Broccoli	Raw	3.84 ± 0.08
Red cabbage	Autolysed	4.21 ± 0.17

The values shown are relative to the trolox C standard (value = 1) as described previously.[35]

compatible with the observation[4,5] that the quercetin and kaempferol content of Brussels sprouts is lower than that of broccoli or red cabbage. Furthermore, no significant difference in activity between extracts from cooked and autolysed sprouts was observed.

Effects of isolated glucosinolates on lipid peroxidation

It is clear from the preceding experiments that both the species and the method of processing strongly influence the behaviour of the extracts in inhibiting or promoting lipid peroxidation, scavenging hydroxyl radicals and chelating iron. One major group of bioactive component of cruciferous vegetables is the glucosinolates and their breakdown products[39,40] (Scheme 1). We therefore examined the effect of glucosinolates and their breakdown products (see Table 2) upon lipid peroxidation.

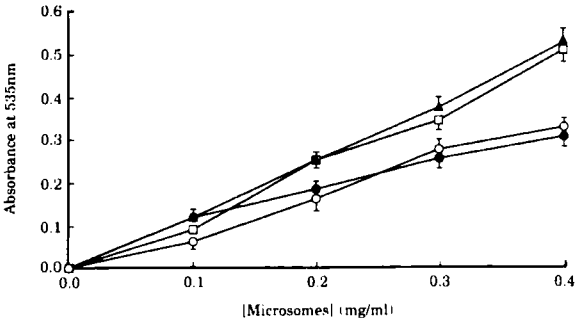


FIGURE 3 Effect of gluconapin and its breakdown products on enzymic peroxidation of human liver microsomes. Gluconapin (original concentration of 0.15 mg/ml) treated with myrosinase as pH 6.6 (●) or 3.1 (○), or untreated gluconapin (0.15 mg/ml) (□), was added to human liver microsomes. Peroxidation, initiated by NADPH/iron, was measured by the TBARS method at A_{535}. The control (▲) is in the absence of added gluconapin.

TABLE 6 Effects of glucosinolates on lipid peroxidation and DNA damage.

Glucosinolate	Treatment	Human liver microsomes NADPH/Fe peroxidation	Ascorbate/Fe peroxidation	Bleomycin assay
		A_{535}	A_{535}	A_{535}
Control	water	0.50 ± 0.04	1.1 ± 0.06	<0.05
Sinalbin[1]	none	0.38 ± 0.03	1.1 ± 0.07	<0.05
Glucoiberin	none	0.40 ± 0.05	1.2 ± 0.08	<0.05
Gluconapin	none	0.52 ± 0.05	1.1 ± 0.06	<0.05
Gluconapin	myrosinase pH 6.6[2]	0.30 ± 0.02	1.1 ± 0.05	ND
Gluconapin	myrosinase pH 3.1[2]	0.32 ± 0.03	1.1 ± 0.05	ND
Gluconapin	boiled for 15 min	0.50 ± 0.04	ND[3]	<0.05
Gluconapin	Boiled with myrosinase pH 6.6[2]	0.50 ± 0.03	ND	<0.05
Ascorbate	0.2 mM	ND	ND	1.5 ± 0.11
Positive control	Propyl gallate (100 mM)	ND	ND	0.24 ± 0.02

[1]Concentration of all glucosinolates was 0.15 mg/ml. The results are the mean of 4 determinations.
[2]Compared to control containing myrosinase alone.
[3]Not determined

Untreated gluconapin has no effect on NADPH/Fe-catalysed peroxidation of microsomes (Figure 3). After myrosinase-catalysed breakdown at either acid or neutral pH, the resulting mixture of compounds showed a significant inhibition of peroxidation. Treating the glucosinolate at 100°C for 15 min in the presence or absence of myrosinase gave the same result as the untreated glucosinolate, which proves that active myrosinase and not heat is required to increase the antioxidant capacity of gluconapin. Sinalbin and glucoiberin both showed a slight inhibition of peroxidation when untreated (Table 5), but this was not altered after myrosinase-treatment at either pH. None of the glucosinolates affected ascorbate/Fe-catalysed peroxidation of microsomes, which suggests that the glucosinolates are not affecting the iron availability for the peroxidation reaction, and myrosinase itself showed no effect on peroxidation.

Effects of extracts from Brassicas and of glucosinolates in the bleomycin assay

We tested extracts from both cooked and autolysed sprouts, and purified glucosinolates treated with myrosinase, in the bleomycin assay. In the absence of ascorbate (Table 6), none of the extracts, and none of the treated or untreated glucosinolates, were able to reduce iron, which is consistent with data in Table 4.

DISCUSSION

Previous studies have shown that Brussels sprouts, cabbage and broccoli exhibit antitumour effects when administered to rodents.[41,42] These vegetables exert multiple biological effects. Brussels sprouts and cabbage in the diet increased the level of glutathione S-transferase in rat liver[43–45] and mouse liver.[23] Cruciferous vegetable consumption may also lead to a modest increase in cytochrome P450 in humans,[46] some cell lines[47] and in animals.[43] The induction of enzyme activity is usually ascribed to the glucosinolates, which are present at high concentration in cruciferous vegetables. Intact glucosinolates are absorbed in the upper gastrointestinal tract of rodents,[48] and do not induce cytochrome P-450s.[49] On the other hand, breakdown products from glucosinolates such as isothiocyanates, generated by the action of myrosinase[50] or by gut microflora,[51] are potent inducers of glutathione S-transferase,[52,53] can induce or inhibit cytochrome P450[54] and are absorbed in the distal gastrointestinal tract.[48] A significant component of the anticancer effects of cruciferous vegetables appears due to the induction of phase II enzymes, which increases the efficiency of both detoxification and endogenous defences against free radicals. This mechanism may act together with the direct free radical scavenging components of the vegetables. These components include flavonoids and vitamins C and E, and possibly the glucosinolates themselves. Very few studies have looked at the direct antioxidant and free-radical scavenging effects of extracts from cruciferous vegetables and none have examined glucosinolates.

Our results show that some of the extracts of cruciferous vegetables exhibit antioxidant properties. This is expected owing to the presence of flavonoids and other antioxidants expected to be present in these extracts. Extract from broccoli is almost as effective at inhibiting NADPH/Fe-catalysed lipid peroxidation as extract from apple or tarragon.[29] We have determined if one of the major bioactive components of crucifers, the glucosinolates, contribute to this antioxidant activity. It is already known that broccoli is high in indolyl-glucosinolates[39] which break down to give a range of compounds including indole-3-carbinol, a weak antioxidant against microsomal peroxidation (50 % inhibition at 30–40 μM[10,55]). In isolation, the parent glucosinolates exhibited only weak antioxidant properties compared to the effects of whole extracts from cruciferous vegetables. Treatment with the endogenous plant enzyme myrosinase only exerted a small effect on these properties, this being dependent on the nature of the side chain of the parent glucosinolate.

It therefore follows that most of the direct antioxidant action of the crucifers is not due to the glucosinolate content. The total antioxidant activity of the extracts probably involves the hydroxylated phenol and polyphenol content of these vegetables.[6] For example, broccoli and red cabbage are higher in quercetin and kaempferol than Brussels sprouts, and, in addition, red cabbage is rich in anthocyanins that give its red colour.[4,5]

The compound(s) responsible for the prooxidant effect of cooked Brussels sprouts is not known, and our results indicate that it is unlikely to be glucosinolates or their breakdown products, although it could be reaction products of glucosinolates with other compounds. This prooxidant effect, however, was not related to iron reduction. It may be significant that extracts from cooked Brussels sprouts were poorest at chelating iron and at hydroxyl radical scavenging and this may be related to the effect on lipid peroxidation. It is known that the content of intact glucosinolates is much higher in cooked crucifers than in autolysed,[19] and that ascorbigens (reaction products between vitamin C and glucosinolate breakdown products) are increased in cooked crucifers,[56] but the compound(s) responsible for the effects observed here remain to be isolated.

Conclusions

Extracts from some Brassica vegetables show significant antioxidant properties, but these properties are not due to glucosinolates. Therefore, the bioactive properties of the glucosinolates are due to modulation of antioxidant defences and not to free radical scavenging properties of these compounds.

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