Food Chemistry **27** (1988) 141–150

Glucosinolate, Carotene and Cadmium Content of *Brassica oleracea* Grown on Municipal Sewage Sludge

Renee M. Goodrich,[a] Robert S. Parker,[b]
Donald J. Lisk[c] & Gilbert S. Stoewsand[a]

[a] Department of Food Science and Technology, Cornell University,
Geneva, NY 14456, USA
[b] Division of Nutritional Sciences and [c] Toxic Chemical Laboratory,
Cornell University, Ithaca, NY 14853, USA

(Received 21 May 1987; accepted 8 June 1987)

ABSTRACT

Broccoli, brussels sprouts and cabbage grown on municipal sewage sludge-amended soil were analyzed by HPLC for glucosinolates and β-carotene. Cadmium was also determined in these vegetables. A number of quantitative changes in specific glucosinolate levels were observed in broccoli harvested from sludge-amended soil, but the total glucosinolate content in broccoli or brussels sprouts was not changed. Cabbage grown on sludge-amended soil was significantly lower in glucosinolates and β-carotene and significantly higher in cadmium. Although it is unlikely that cadmium caused these reduced levels of glucosinolates and β-carotene, municipal sewage sludge-amended soil appears to change the constituents of specific Brassica *vegetables associated with their quality and healthful attributes.*

INTRODUCTION

The need to find inexpensive ways of disposing of vast amounts of municipal sewage sludge has led the US, as well as other industrialized countries, to explore utilization of some of it as fertilizer for the production of fruits and vegetables (US EPA, 1981). Although certain nutrients are added to the soil, numerous subtle toxicologic effects and biologic changes have been observed in vegetables and other plants (Babish *et al.*, 1984).

Glucosinolates (thioglucosides) are sulfur-containing compounds present

Food Chemistry 0308-8146/88/$03·50

in *Brassica* vegetables (Kjaer, 1976). The glycosyl component is β-D-glucopyranose and all glucosinolates probably have the anti configuration, with respect to the sulfate and R groups, as shown in Fig. 1 (Ettlinger & Lundeen, 1956). The glucosinolates are anions and occur in plants mostly as potassium salts (Tookey *et al.*, 1980). Upon hydrolysis, they are responsible for the characteristic flavors of *Brassica* foods and condiments such as mustard seed and horseradish. At high intake levels certain glucosinolates are associated with toxic effects, especially goiter development (Nishie & Daxenbichler, 1980), but indole glucosinolates have been shown to inhibit chemical carcinogenesis (Stoewsand *et al.*, 1978; Wattenberg & Loub, 1978). β-Carotene has also been described as an anticarcinogenic substance (Peto *et al.*, 1981).

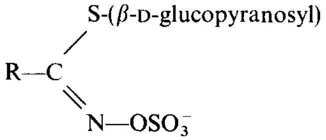

Fig. 1. Constitutional and stereochemical structure of glucosinolates. Trivial names for common glucosinolates, along with the nature of R (parentheses), are as follows; glucocapparin (methyl); glucoiberin (3-methylsulphinylpropyl); progoitrin (2-hydroxybut-3-enyl); gluconapin (3-butenyl); sinigrin (2-propenyl); glucoraphanin (4-methylsulphinylbutyl-); glucobrassicanapin (4-pentyl); gluconasturtiin (2-phenylethyl); glucobrassicin (3-indolylmethyl); neoglucobrassicin (1-methoxy-3-indolylmethyl); no trivial names for 4-hydroxyglucobrassicin or 4-methoxyglucobrassicin.

Since there is evidence that municipal sewage sludge spread on soils changes the level of glucosinolates in *Brassica* vegetables (Miller *et al.*, 1983; Stoewsand *et al.*, 1986), the purpose of this study was to quantitate these putative changes in broccoli, brussels sprouts and cabbage. In addition, a preliminary study indicated that the level of carotenoids was lowered in sludge-grown vegetables. Therefore, β-carotene was measured, as well as cadmium, as this element is also known to accumulate in many sludge-grown crops (Babish *et al.*, 1984).

MATERIALS AND METHODS

Experimental plots and plantings

The sludge was obtained from the Ley Creek Sewage Treatment Plant in Syracuse, NY. Wastewater entering this plant is treated to yield an anaerobically digested, waste-activated sludge. No chemicals are added during the treatment. Four years prior to results reported in this study, sludge was applied to 0·04 hectares of a Hudson, silt-loam soil, pH 5·7, in Ithaca, NY, at the rate of 224 metric tons per hectare. The sludge was spread evenly over the plots, and then was tilled with a rotary cultivator. The sludge (pH 6·7) had a fertilizer equivalent of 1·67–1·12–0·13 (N–P–K) and contained 68% ash. A similar plot of the same soil type was prepared and amended at the same N–P–K rate with cow manure to serve as a control. The final pH of the sludge-amended and control soils was 6·2 and 6·0, respectively.

In the spring, 'Early One' broccoli (*Brassica oleracea*), 'Jade Cross E' (hybrid) brussels sprouts (*Brassica oleracea*; gemmifera group) and 'Green Winter' cabbage (*Brassica oleracea* var. capitata) were planted as transplants in the sludge-amended and control plots surrounded by black plastic strips, 30 in wide. Mature vegetables were harvested in the fall for analysis. Before freeze-drying, the broccoli, brussels sprouts and cabbage had a moisture content of approximately 89%, 85% and 94%, respectively.

Glucosinolate analysis

The method used for preparation of aqueous vegetable extracts containing the glucosinolate fraction was that of Miller & Stoewsand (1983). Glucosinolates were analyzed using high-pressure liquid chromatography (HPLC) following the on-column desulphation method of Truscott *et al.* (1983). Briefly, the extracts were applied to DEAE-Sephadex A-25 columns. After washing with water, the glucosinolates were converted to corresponding desulpho-derivatives by addition of aryl sulphatase solution. After overnight incubation, the desulphoglucosinolates were eluted with water. The eluate was filtered prior to analysis by HPLC.

Two Rainin Rabbit-HP pumps were coupled to a Tracor 970A variable wavelength detector. A wavelength setting of 227·5 nm was used to monitor all separations. The chromatographic unit was operated at a constant solvent flow of 1 ml/min and the column (4·6 × 22 mm, RP-18 Brownlee 5μ) was held at 35°C. The solvent program consisted of 100% water for 10 min, a gradient of 0–12% acetonitrile over the next 30 min and 12% acetonitrile for an additional 25 min. A known volume of sample was injected and peak areas and retention times were measured using a Hewlett–Packard 21 MX E-Series computer system. Response factors were compared to purified glucosinolates. Where actual standards were unavailable, peaks were identified by comparison to literature values for glucosinolate retention times (Minchinton *et al.*, 1982). The values for total glucosinolates were obtained by summation of the amount of individual glucosinolates in each vegetable sample.

Carotenoid analysis

Freeze-dried, powdered vegetables were homogenized with 30 volumes of a hexane–acetone–absolute ethanol–toluene solvent (10:7:6:7) containing 0·01% butylated hydroxytoluene and 0·01% butylated hydroxyanisole as antioxidants. The suspension was then transferred to 50-ml screw-cap glass tubes. After addition of 0·5 ml H_2O and replacement of the headspace with nitrogen, the tubes were shaken mechanically with periodic sonication for 2 h in the dark and allowed to stand overnight in a refrigerator. The extracts were then filtered, the residue extracted a second time, and the filtrates combined and dried under a stream of nitrogen. Chlorophylls were removed by saponification of the extracts with 5% KOH in methanol. After adding water, the carotenoids were partitioned into hexane and ethyl acetate. The extracts were then dried under nitrogen and redissolved in acetonitrile–methanol (1:1) for HPLC analysis. The carotenoids were analyzed using a 25-cm Beckman C-18 ODS analytical column, a Waters (Millipore Corp., Milford, MA) M45 HPLC equipped with a HP 1040A diode-array spectrophotometric detector and a mobile phase of methanol–acetonitrile–chloroform–water (60:25:15:0·4) at 1·6 ml/min. Peaks classified as carotenoids were based on their UV–visible spectra. β-Carotene was further identified by comparing the retention time with that of a pure standard. Peak areas were converted to mass using an external quantitative β-carotene standard. Under the conditions used, the retention time of β-carotene was approximately 20 min.

Cadmium analysis

After wet-ashing the dried vegetables with nitric, perchloric and sulfuric acids (Pinta, 1973), the analysis of cadmium was accomplished by conventional stripping voltametry using a Princeton Applied Research Corp. Model 174 polarographic analyzer (Gajan & Larry, 1972).

Statistics

The analysis of variance for all data was accomplished using Duncan's multiple range test of chemical means (Steel & Torrie, 1960). Significant differences are at $P \leq 0{\cdot}05$.

RESULTS

The chromatogram of HPLC-purified desulphoglucosinolates in the extract of freeze-dried, soil-grown brussels sprouts is shown in Fig. 2. The mean

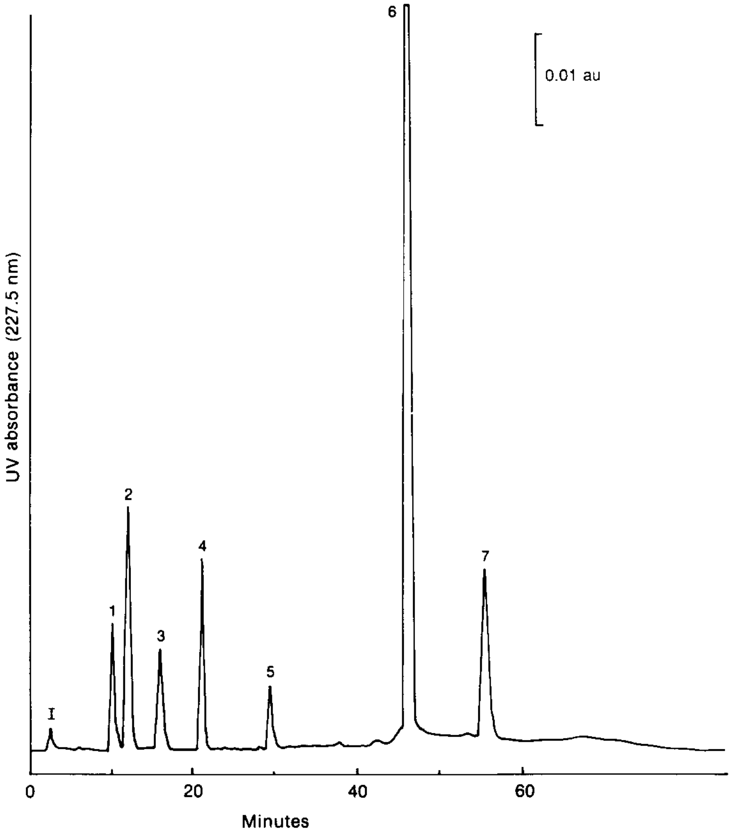

Fig. 2. Chromatogram of HPLC-purified desulphoglucosinolates derived from soil-grown brussels sprouts. Numbered peaks correspond to the following compounds: (1) glucoiberin; (2) progoitrin; (3) sinigrin; (4) glucoraphanin; (5) 4-hydroxyglucobrassicin; (6) glucobrassicin; (7) 4-methoxyglucobrassicin. Peak I is a solvent peak. Sensitivity: 0·08 arbitrary absorption units (au) at full scale.

total glucosinolate, as well as individual non-indole and indole glucosinolate, in each of the three *Brassica* vegetables is shown in Table 1 as mg/g dry basis. The essential features of the significant differences shown in Table 1 are as follows. Mean glucosinolate values sharing any superscript letter are not significantly ($P \leq 0{\cdot}05$) different. Using the comparison of glucoiberin content as an example, the level in control broccoli ($1{\cdot}27 \pm 0{\cdot}01^{ab}$ mg/g) is not statistically different from sludge-grown cabbage ($0{\cdot}73 \pm 0{\cdot}10^{bc}$ mg/g). The largest amount of a specific glucosinolate was indole glucobrassicin in brussels sprouts. Neoglucobrassicin was observed only in broccoli. Cabbage contained the lowest total glucosinolate.

Table 2 shows the mean β-carotene values of the *Brassica* vegetables.

TABLE 1

Effect of Sludge on Glucosinolate Levels (mg/g dry basis) as the Mean of Duplicate Freeze-dried Samples from Control (C) and Sludge-grown (S) *Brassica* Vegetables

	Broccoli		*Brussels sprouts*		*Cabbage*	
	C	*S*	*C*	*S*	*C*	*S*
Total glucosinolates	11·59 ± 0·12^{b}	12·13 ± 0·86^{b}	29·70 ± 2·14^{a}	32·31 ± 1·14^{a}	7·11 ± 0·62^{c}	1·59 ± 0·46^{d}
Non-indole GS						
Glucocapparin	0·0^{b}	0·60 ± 0·25^{a}	0·0^{b}	0·0^{b}	0·0^{b}	0·0^{b}
Glucoiberin	1·27 ± 0·01^{ab}	0·0^{c}	1·52 ± 0·33^{ab}	1·14 ± 0·40^{ab}	1·88 ± 0·22^{a}	0·73 ± 0·10^{bc}
Progoitrin	0·06 ± 0·00^{c}	2·17 ± 0·95^{a}	2·94 ± 0·25^{a}	1·60 ± 0·31^{ab}	0·45 ± 0·06^{bc}	0·04 ± 0·01^{c}
Gluconapin	0·0^{b}	0·05 ± 0·01^{a}	0·0^{b}	0·0^{b}	0·0^{b}	0·0^{b}
Sinigrin	0·05 ± 0·00^{d}	0·0^{d}	1·58 ± 0·20^{b}	1·13 ± 0·21^{bc}	2·62 ± 0·35^{a}	0·52 ± 0·12^{cd}
Glucoraphanin	4·45 ± 0·07^{a}	0·0^{c}	2·37 ± 0·43^{b}	1·40 ± 0·54^{b}	0·27 ± 0·03^{c}	0·08 ± 0·02^{c}
Glucobrassicanapin	0·11 ± 0·00^{b}	5·77 ± 1·75^{a}	0·0^{b}	0·0^{b}	0·0^{b}	0·0^{b}
Gluconasturtiin	0·0^{b}	0·0^{b}	0·0^{b}	0·14 ± 0·07^{a}	0·02 ± 0·02^{b}	0·0^{b}
Indole GS						
Glucobrassicin	4·96 ± 0·07^{c}	0·45 ± 0·15^{d}	17·81 ± 0·70^{b}	23·07 ± 2·74^{a}	1·44 ± 0·19^{cd}	0·20 ± 0·20^{d}
4-Hydroxyglucobrassicin	0·0^{d}	0·0^{d}	0·62 ± 0·04^{a}	0·36 ± 0·05^{b}	0·20 ± 0·03^{c}	0·02 ± 0·02^{d}
4-Methoxyglycobrassicin	0·0^{c}	0·0^{c}	2·86 ± 0·21^{b}	3·47 ± 0·16^{a}	0·14 ± 0·10^{c}	0·0^{c}
Neoglucobrassicin	0·70 ± 0·04^{b}	3·09 ± 0·16^{a}	0·0^{c}	0·0^{c}	0·0^{c}	0·0^{c}

Different letter superscripts indicate significant ($P \leq 0·05$) differences in all vegetables for each glucosinolate.

TABLE 2

β-Carotene (μg/g dry basis) of the Mean of Duplicate Freeze-dried Samples from Control (C) and Sludge-grown (S) *Brassica* Vegetables

Vegetable	*β-Carotene (μg/g)*	
	Control soil	*Sludge-amended soil*
Broccoli	395 ± 1	402 ± 49
Brussels sprouts	36 ± 4	64 ± 11
Cabbage	4·1 ± 0·1	0·25 ± 0·05

All means are significantly ($P \leq 0{\cdot}05$) different except the control and sludge-grown broccoli.

TABLE 3

Cadmium Level (μg/g) of Duplicate Freeze-dried Samples of *Brassica* Vegetables Grown on Control (C) or Sludge-amended (S) Soil

Vegetable	*Cadmium content*	
	Control soil	*Sludge-amended soil*
Broccoli	0·05 ± 0·01	0·78 ± 0·05
Brussels sprouts	0·06 ± 0·01	0·51 ± 0·02
Cabbage	0·35 ± 0·01	1·47 ± 0·02

All vegetables except the control broccoli and brussels sprouts have significantly ($P \leq 0{\cdot}05$) different mean values.

Cabbage showed the lowest level of β-carotene, with yet a significantly lower value for cabbage grown on sludge.

Cadmium levels (Table 3) in cabbage were significantly higher than in broccoli or brussels sprouts. The sludge-amended soil-grown vegetables showed significantly increased levels of cadmium, which were especially high in cabbage.

DISCUSSION

The four non-indole glucosinolates of brussels sprouts are eluted before 25 min, as seen in Fig. 2. Similar elution times for the desulphoglucosinolates have been reported (Minchinton *et al.*, 1982). Peaks 5 and 7 (4-hydroxyglucobrassicin and 4-methoxyglucobrassicin, respectively) were first identified in *Brassica* vegetables and seeds by Truscott *et al.* (1982*a*,*b*) using the same reversed-phase HPLC method.

The relatively large amount of glucosinolates in brussels sprouts observed in this study (Table 1) was also reported by Sones *et al.* (1984), but those investigators also showed gluconapin and neoglucobrassicin as major glucosinolates in brussels sprouts and cabbage. That finding was not evident in this study. In comparing glucosinolates in soil-grown vegetables (controls) to the sludge-grown vegetables, many differences are evident (Table 1). Although control and sludge-grown broccoli had similar levels of total glucosinolates, sludge-grown broccoli was devoid of the two glucosinolates containing a sulphinyl group (glucoiberin and glucoraphanin), while the control broccoli samples contained appreciable amounts of those compounds. Glucobrassicanapin and neoglucobrassicin, present only in broccoli, were significantly increased in the sludge-grown vegetables. Brussels sprouts harvested from control soil or sludge treatments also contained similar levels of total glucosinolate. Differences in levels of a specific glucosinolate were not as prevalent in brussels sprouts grown on soil or sludge as observed with broccoli. Gluconasturtiin was the only specific glucosinolate not present in control brussels sprouts that was present (0·14 mg/g) in the sludge-grown vegetables. As previously reported (Miller *et al.*, 1983; Stoewsand *et al.*, 1986), sludge-grown cabbage had significantly lower total glucosinolate levels, but the glucosinolate profile was similar to the cabbage control.

Cabbage was also significantly lower in β-carotene (Table 2) than broccoli and brussels sprouts with further lowered values in sludge-grown cabbage. These results may be partially explained by the fact that the cabbage was harvested, freeze-dried and stored in a refrigerator one year prior to the harvesting of the other two vegetables. Dehydrated foods are susceptible to the loss of provitamin A (carotene) during storage (MacKinney *et al.*, 1958) because of oxidation (Labuza *et al.*, 1970). In addition, broccoli is reported to be higher in provitamin A than brussels sprouts or cabbage, with cabbage containing the relatively lowest levels of carotenoids (USDA, 1984). The level of β-carotene was similar in the soil-grown or sludge-amended soil-grown broccoli, while a comparatively higher β-carotene level was observed in the brussels sprouts grown on sludge-amended soil (Table 2).

Cadmium was increased in all of the sludge-grown vegetables, but the cadmium content of sludge-grown cabbage was significantly higher than the broccoli or brussels sprouts (Table 3). However, it is unlikely that cadmium at this concentration caused the reduction of glucosinolates or β-carotene. In a preliminary study with spinach grown on similar sludge-amended soil, we observed no reduced β-carotene levels, yet the spinach contained 11·5 μg/g of cadmium. This leafy vegetable is known to accumulate cadmium and other heavy metals (Bingham, 1979).

In conclusion, sludge-amended soils produce significant changes in specific glucosinolate levels in broccoli and brussels sprouts, and significant reduction of glucosinolate and β-carotene with increased cadmium levels in cabbage. These changes, although accomplished by unknown mechanisms, indicate modification of vegetable quality as well as their health attributes (i.e. modification of β-carotene and indole glucosinolate content) when *Brassica* vegetables are grown on municipal sewage sludge-amended soils.

ACKNOWLEDGEMENTS

The authors are grateful to Dr R. J. W. Truscott for kindly supplying the indole glucosinolate standards and Hoffman-LaRoche Inc. for the β-carotene standard. We also appreciate the assistance of J. L. Anderson, C. A. Bache and W. H. Gutenmann on all phases of this study.

REFERENCES

Babish, J. G., Stoewsand, G. S., Scarlett Kranz, J. M., Boyd, J. N., Ahrens, V. D. & Lisk, D. J. (1984). Toxicologic studies associated with the agricultural use of municipal sewage sludge and health effects among sewage treatment plant workers. *Reg. Toxicol. Pharmacol.*, **4**, 305–21.

Bingham, F. T. (1979). Bioavailability of Cd to food crops in relation to heavy metal content of sludge-amended soil. *Environ. Health Perspect.*, **28**, 39–43.

Ettlinger, M. G. & Lundeen, A. J. (1956). The structures of sinigrin and sinalbin, an enzymatic arrangement. *J. Amer. Chem. Soc.*, **78**, 4172–3.

Gajan, R. J. & Larry, D. (1972). Determination of lead in fish by atomic absorption spectrophotometry and by polarography: I. Development of the methods. *J. Assoc. Off. Anal. Chem.*, **55**, 727–32.

Kjaer, A. (1976). Glucosinolates in the *Cruciferae*. In: *The biology and chemistry of the* Cruciferae (J. G. Vaughn, A. J. MacLeod & B. G. M. Jones (Eds)), Academic Press, London.

Labuza, T. P., Tannenbaum, S. R. & Karel, M. (1970). Water content and stability of low moisture and intermediate-moisture foods. *Food Tech.*, **24**, 543–50.

MacKinney, G., Luckton, A. & Greenbaum, L. (1958). Carotene stability in stored dehydrated carrots. *Food Tech.*, **12**, 164–6.

Miller, K. W. & Stoewsand, G. S. (1983). Dietary brussels sprouts and glucosinolate influence on rat hepatic polysubstrate monooxygenases. *J. Plant Foods*, **5**, 67–74.

Miller, K. W., Boyd, J. N., Babish, J. G., Lisk, D. J. & Stoewsand, G. S. (1983). Alteration of glucosinolate content, pattern and mutagenicity of cabbage (*Brassica oleracea*) grown on municipal sewage sludge-amended soil. *J. Food Safety*, **5**, 131–43.

Minchinton, I., Sang, J., Burke, D. & Truscott, R. J. W. (1982). Separation of desulphoglucosinolates by reversed-phase high-performance liquid chromatography. *J. Chromatography*, **247**, 1141–8.

Nishie, K. & Daxenbichler, M. E. (1980). Toxicology of glucosinolates, related compounds (nitriles, R-goitrin, isothiocyanates), and vitamin U found in *Cruciferae. Food Chem. Toxicol.*, **18**, 159–72.

Peto, R., Doll, R., Buckley, J. D. & Sporn, M. B. (1981). Can dietary beta-carotene materially reduce human cancer rates? *Nature*, **290**, 201–8.

Pinta, M. (1973). *Detection and determination of trace elements*, Ann Arbor Science, Ann Arbor, Michigan, 453–4.

Sones, K., Heaney, R. K. & Fenwick, G. R. (1984). An estimate of the mean daily intake of glucosinolates from cruciferous vegetables in the UK. *J. Sci. Food Agric.*, **35**, 712–20.

Steel, R. G. D. & Torrie, J. H. (1960). *Principles and procedures of statistics*, McGraw-Hill, New York.

Stoewsand, G. S., Anderson, J. L. & Lisk, D. J. (1986). Changes in liver glutathione S-transferase activities in coturnix quail fed municipal sludge-grown cabbage with reduced levels of glucosinolates. *Proc. Soc. Exptl Biol. Med.*, **182**, 95–9.

Stoewsand, G. S., Babish, J. G. & Wimberly, H. C. (1978). Inhibition of hepatic toxicities from polybrominated biphenyls and aflatoxin B_1 in rats fed cauliflower. *J. Environ. Path. Toxicol.*, **2**, 399–406.

Tookey, H. L., Van Etten, C. H. & Daxenbichler, M. E. (1980). Glucosinolates. In: *Toxic constituents of plant foods* (2nd edn) (I. E. Liener (Ed.)), Academic Press, New York.

Truscott, R. J. W., Burke, D. G. & Minchinton, I. R. (1982*a*). The characteristics of a novel hydroxyindole glucosinolate. *Biochem. Biophys. Res. Comm.*, **107**, 1258–64.

Truscott, R. J. W., Minchinton, I. R., Burke, D. G. & Sang, J. P. (1982*b*). A novel methoxyindole glucosinolate. *Biochem. Biophys. Res. Comm.*, **107**, 1368–75.

Truscott, R., Minchinton, I. & Sang, J. (1983). The isolation and purification of indole glucosinolates from *Brassica* species. *J. Sci. Food Agric.*, **34**, 247–54.

US Department of Agriculture (USDA) (1984). *Composition of foods: Vegetables and vegetable products.* USDA Handbook 8-11. US Government Printing Office, Washington, DC.

US Environmental Protection Agency (US EPA) (1981). *Land application of municipal sewage sludge for the production of fruits and vegetables.* A statement of federal policy and guidance. Joint Policy Statement SW-905.

Wattenberg, L. W. & Loub, W. D. (1978). Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. *Cancer Res.*, **38**, 1410–13.