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Glucosinolate Content of *Brassica* Vegetables: Analysis of Twenty-Four Cultivars of Calabrese (green sprouting broccoli, *Brassica oleracea* L. var. *botrytis* subvar. *cymosa* Lam.)

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ABSTRACT

The contents of glucosinolates in twenty-four cultivars of calabrese, green sprouting broccoli, have been determined using glucose release and high performance liquid chromatographic methods. The levels of total glucosinolates (range, 42·1–94·5 mg 100 g^{-1} fresh weight; mean, 62·3 mg 100 g^{-1} fresh weight) were comparable with those reported previously for cauliflower, but lower than those found in cabbage and, especially, Brussels sprouts. Analysis of the individual components revealed the main species to be 4-methylsulphinylbutyl glucosinolate (glucoraphanin) and, particularly, glucobrassicin and neoglucobrassicin, containing 3-indolylmethyl and 1-methoxy-3-indolylmethyl side chains, respectively. The individual glucosinolate contents of calabrese and cauliflower are compared and differences are considered in the context of biosynthetic pathways. The consequences of these findings for the flavour and biological properties of calabrese are briefly discussed.

INTRODUCTION

Varying levels of glucosinolates (also known by their historical name, mustard oil glycosides) are found in all members of the Cruciferae (Fenwick *et al.*, 1983*a*), including those used extensively as human foods and animal feedingstuffs. The significance of the presence of these compounds is that they may be broken down by an enzyme, thioglucoside glucohydrolase (EC

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3.2.3.1—commonly called myrosinase), to yield a range of volatile and non-volatile products. The former comprise mainly isothiocyanates and nitriles which contribute to the flavour and aroma of, for example, brassica vegetables and condiments. It is the non-volatile products which have, in recent years, generated most interest, since these include oxazolidine-2-thiones (being goitrogenic, enzyme-inhibitory and possessing adverse sensory properties), hydroxynitriles (causing enlargement of liver, kidney and spleen) and a variety of indoles (which, in contrast, show significant anticarcinogenic and enzyme-inducing effects) (Fenwick *et al.*, 1983*a*).

It is against this background, and because of the significant amounts of brassica vegetables consumed in the UK (Sones *et al.*, 1984*a*), that studies have been conducted into the glucosinolate content and composition of Brussels sprouts (Heaney & Fenwick, 1980), cauliflower (Sones *et al.*, 1984*b*), cabbage, swede and turnip (Sones *et al.*, 1984*c*). Over the same period other workers at the Northern Regional Research Centre Laboratory of the USDA have carried out parallel studies on cabbage (VanEtten *et al.*, 1976), Chinese cabbage (Daxenbichler *et al.*, 1979), turnips and rutabagas (Carlson *et al.*, 1982) and, most recently, radish (Carlson *et al.*, 1985).

Consumption of green vegetables in the UK currently stands at 307 g per person per week, the great majority of which are brassicas. It is expected that this figure will increase in future years given the current dietary recommendations and reported nutritional benefits of leafy vegetables. It is likely, however, that within this expected increase there will be changes in the relative consumption of individual brassicas. Crisp (1976) has pointed out that as living standards increase there is a decrease in consumption of cabbage, collards and kales and an increase in such milder flavoured (and more expensive) cauliflowers, calabrese and Brussels sprouts.

To satisfy the market requirements and ensure constancy of supply, the cultivation of cauliflowers in the UK (331 000 t in 1985, value £71·0 million; harvested in December–May) has been supplemented by the growing of sprouting and Cape broccoli (harvested March–May) and calabrese (harvested June–November). Whilst initially grown for commercial freezing purposes, calabrese is being increasingly supplied to the fresh market. UK production in 1984 was 6000 t with a value of £5·0 million.

It is probable that consumer demand for calabrese, both fresh and frozen, will continue to increase and researches are in progress to develop newer cultivars to optimise agronomic practices and introduce better procedures for packaging, processing and transportation. In view of the interest in the crop, we have examined the glucosinolate contents of a number of cultivars, obtained from the 1985 National Trial by courtesy of staff at the Agricultural Development and Advisory Service (ADAS). The results of this study are reported here.

MATERIALS AND METHODS

Materials and sample preparation

Samples of individual calabrese cultivars were harvested at the Kirton Experimental Horticulture Station, Lincolnshire, and transported to the Institute of Food Research the same day. Upon arrival each sample was stored and frozen at $-40°$. Each sample was part of the 1985 National Trial and had been grown according to prevailing agronomic practices and was not subjected to any experimental treatment.

Frozen samples were ground at $-20°$ and a representative sample (100–150 g) sealed in a plastic container and stored at $-40°$. This was necessary to prevent any myrosinase-induced glucosinolate breakdown. The ground material was kept cold in dry ice, a portion (20 g) weighed into a pre-cooled thermos flask and then extracted with boiling, aqueous methanol as described previously (Heaney & Fenwick, 1980). The combined extracts were evaporated *in vacuo* to approximately 25 ml and the volume adjusted to 50 ml with distilled water. Following thorough shaking the samples were frozen at $-20°$ until required for analysis.

Analysis of glucosinolates

Total glucosinolates were measured using the microcolumn glucose release method (Heaney & Fenwick, 1981). Individual glucosinolates were measured, following enzymatic desulphation, by high performance liquid chromatography (Spinks *et al.*, 1984). Benzyl glucosinolate (glucotropaeolin) was used as an internal standard. Response factors of 0·8 and 0·51 were calculated for 2-hydroxybut-3-enyl and 3-indolylmethyl glucosinolates (progoitrin and glucobrassicin, respectively). Other indole glucosinolates were assigned a response factor of 0·5. An arbitrary response factor of 1·0 was assigned to remaining glucosinolates since available reference compounds were of insufficient purity. Individual glucosinolates were identified by comparison of their retention times with those of reference compounds, confirmation being achieved by LC–MS.

RESULTS

The individual glucosinolates identified in the 24 cultivars of calabrese are shown in Table 1 with the levels presented in Table 2. The total glucosinolate contents ranged from 42·1 mg $100\,g^{-1}$ fresh weight (AVX7901 31/8) to 94·5 mg $100\,g^{-1}$ fresh weight (Corvet), with a mean of 62·3 mg $100\,g^{-1}$ fresh weight.

TABLE 1
Glucosinolates identified in Calabrese

Code	*Trivial Name*	*Glucosinolate side chain*
I	Glucoiberin	3-methylsulphinylpropyl
II	Progoitrin	2-hydroxybut-3-enyl
III	Sinigrin	Prop-2-enyl
IV	Glucoraphanin	4-methylsulphinylbutyl
V	Gluconapoleiferin	2-hydroxypent-4-enyl
VI	4-Hydroxyglucobrassicin	4-hydroxy-3-indolylmethyl
VII	Glucobrassicin	3-indolylmethyl
VIII	4-Methoxyglucobrassicin	4-methoxy-3-indolylmethyl
IX	Neoglucobrassicin	1-methoxy-3-indolylmethyl

Examination of the individual glucosinolate content revealed three glucosinolates which, together, generally contributed >90% of the total figure. These were the 3-indolylmethyl, 1-methoxy-3-indolylmethyl and 4-methylsulphinylbutyl species, glucobrassicin, neoglucobrassicin and glucoraphanin, respectively. The indole glucosinolates were confirmed by comparison with standards and by LC–MS, and 4-methylsulphinylbutyl glucosinolate was identified from LC–MS alone; the desulphoglucosinolate possessed a molecular ion of $m/z358$, subsequent fragmentation being consistent with the expected structure (Eagles *et al.*, 1980). Smaller amounts of 2-hydroxypent-4-enyl glucosinolate (gluconapoleiferin) and 4-methoxyglucobrassicin were also found in all cultivars. Twenty-one cultivars also contained 2-hydroxybut-3-enyl glucosinolate (progoitrin) and thirteen, 3-methylsulphinylpropyl glucosinolate (glucoiberin). There were sporadic instances of the presence of prop-2-enyl glucosinolate (sinigrin, 3 cultivars $<1\,mg\ 100\,g^{-1}$ fresh weight), and 4-hydroxyglucobrassicin (3 cultivars $<1\,mg\,100\,g^{-1}$ fresh weight).

Of the three major glucosinolates, the content of the 4-methyl sulphinylbutyl compound ranged between $2{\cdot}4\,mg\,100\,g^{-1}$ fresh weight (AVX 7901 31/8) and $27{\cdot}0\,mg\,100\,g^{-1}$ fresh weight (Citation). The mean figure for this glucosinolate was $15{\cdot}1\,mg\,100\,g^{-1}$ fresh weight. Inspection of Table 2 reveals that the cultivar possessing the lowest level was AVX 7901 (two samples harvested one week apart).

The indole glucosinolates ranged from $11{\cdot}6\,mg\ 100\,g^{-1}$ fresh weight to $35{\cdot}7\,mg\,100\,g^{-1}$ fresh weight in the case of the 3-indolylmethyl compound, and from $9{\cdot}3\,mg\,100\,g^{-1}$ fresh weight to $49{\cdot}1\,mg\,100\,g^{-1}$ fresh weight for the 1-methoxy analogue. In all cases the combined indole glucosinolates contributed well over 50% of the total glucosinolate content and, in some cases (for example, AVX 7901, Green Duke, Green Thistle), this figure rises to well above 80%.

TABLE 2
Glucosinolate Content of Calabrese mg/100 g Fresh Weight

	I	II	III	IV	V	VI	VII	VIII	IX	*Total by HPLC*	*Total by glucose release*	*Total sum HPLC as % of G. Rel*
* OCTAL CAN 67		2·6		12·6	1·5		21·3	1·2	28·3	67·5	66·0	102·3
† ASMER PRIMAR (AP70)		2·8		10·1	1·3	0·6	13·9	2·4	9·3	40·4	44·6	90·6
† SGI		2·1		10·1	1·3		20·1	1·7	25·3	60·6	56·2	107·6
** AVX 7901 23/8	6·9			3·3	1·9	0·4	17·0	2·9	27·5	59·9	53·8	111·3
** AVX 7901 31/8	1·2	0·6		2·4	1·4		15·1	1·1	28·3	50·1	42·1	119·0
** MERCEDES		0·6		14·1	1·6		13·4	2·7	45·4	77·8	63·3	122·9
* LASER	1·0	0·6		25·0	1·9		21·3	1·1	29·1	80·0	76·3	104·8
** AVX 7361 (CAZ 54)		0·5		17·7	1·3		12·5	1·6	24·3	57·9	55·8	103·8
† SG 706		3·8		8·7	2·3		25·0	2·4	13·5	55·7	54·4	102·3
† GREEN DUKE (CAH 61)		0·6		11·8	1·8		12·4	1·9	47·9	76·4	55·6	137·4
† GREEN CHARGER (CAW 62)		0·7		15·2	1·3		18·5	2·5	17·2	55·4	51·7	107·1
** FUTURA	3·5	0·5		7·8	2·2		7·8	2·2	35·2	59·2	43·8	135·1
** GREEN THISTLE	0·6	0·9		11·8	1·7		11·6	3·8	49·1	79·5	57·9	137·3
* NEPTUNE	4·8	3·9	0·6	19·6	2·8	0·4	18·7	4·0	32·2	87·0	79·1	110·0
† EMPEROR		0·5		13·6	1·9		13·8	1·2	12·3	43·3	42·5	101·9
† GREEN VALIENT	3·0	3·1	0·4	14·4	2·6		18·7	2·2	9·3	53·7	51·5	104·3
* SEPTAL		4·9		17·3	2·0		18·2	2·2	27·9	72·5	61·8	117·3
** CITATION		0·6		27·0	2·0		18·8	2·9	21·1	72·4	75·9	95·4
* CRUISER		8·2		24·4	1·8		15·0	3·7	34·0	88·0	88·1	99·9
** ORION	1·8			11·9	1·9		15·5	1·9	41·2	74·2	55·6	133·5
** AURIGA	0·9	0·6		21·2	1·9		30·2	3·6	21·9	80·3	81·3	98·8
BI 85189	6·1			14·7	1·7		24·6	2·7	13·0	62·8	54·0	116·3
* SKIFF	0·6	3·6		19·7	1·9		28·7	3·0	24·1	82·2	67·9	121·0
† IDOL	7·1			18·9	1·0		35·7	1·9	19·0	83·6	84·1	99·4
* CORVET	3·4	9·2	0·9	23·5	3·2		25·3	3·8	27·3	98·5	94·5	104·2
Mean	3·1	2·4	0·6	15·1	1·8	0·5	18·9	2·4	26·5	68·6	62·3	
Minimum	0	0	0	2·4	1·0	0	11·6	1·2	9·3	40·4	42·1	
Maximum	7·1	8·2	0·9	27·0	3·2	0·6	35·7	4·0	49·1	98·5	94·5	

* Netherlands variety. ** North American variety. † Far East variety.

DISCUSSION

When compared with other brassica vegetables previously examined, the total glucosinolate contents of the calabrese cultivars examined here are nearer to those of swede/turnip (Sones *et al.*, 1984*c*) and cauliflower (Sones *et al.*, 1984*b*), rather than cabbage (Sones *et al.*, 1984*c*) or Brussels sprouts (Heaney & Fenwick, 1980). The botanical relationship between cauliflower and calabrese, and the similar physiological states of their edible portions, has prompted a comparison of their individual glucosinolate contents.

According to Sones *et al.* (1984*b*), the major glucosinolates in cauliflower were those containing prop-2-enyl-, 3-methylsulphinyl propyl-, 3-indolylmethyl- and 1-methoxy-3-indolylmethyl side chains. In the present study the latter two compounds, together with 4-methylsulphinylbutyl glucosinolate, predominate. In the case of cauliflower, 3-indolylmethyl glucosinolate (range 7·2–78·9 mg $100\,g^{-1}$ fresh weight) was present in much larger amounts than its 1-methoxy analogue (range 0·6–16·5 mg $100\,g^{-1}$ fresh weight). In calabrese the reverse is true, with more 1-methoxy-3-indolyl methyl than 3-indolylmethyl glucosinolate. This clearly suggests that the metabolic transformation of the indole ring to the 1-methoxylated analogue is favoured in calabrese.

Studies have shown that glucosinolates are derived from amino acids and that most lie on a common biosynthetic pathway (Underhill & Wetter, 1973; Underhill, 1980). The final stages of biosynthesis comprise the introduction of the S-glucose moiety and sulphation. Prior to these stages, the glucosinolate side chain is elaborated. Prop-2-enyl glucosinolate, common in brassicas, is formed by chain elongation and CH_3SH elimination reactions from methionine. A number of other aliphatic glucosinolates may be considered as biosynthetically related to this compound, via further elaboration of the side chain (Fig. 1). This may involve chain elongation to, initially, but-3-enyl glucosinolate, addition of CH_3SH, yielding 3-methylthiopropyl glucosinolate or, after initial elongation, the homologous 4-methylthiobutyl species, β-hydroxylation, to form the ubiquitous 2-hydroxybut-3-enyl glucosinolate. Once formed, ω-methylthio species may be oxidised to the corresponding ω-methylsulphinyl and, less commonly, the ω-methylsulphonyl species.

In cauliflower prop-2-enyl glucosinolate, 2-hydroxybut-3-enyl glucosinolate and 3-methylsulphinylpropyl glucosinolate predominate reflecting the presence of independent biosynthetic pathways involving (i) chain elongation and hydroxylation and (ii) terminal addition and oxidation. This is supported by the presence of, with a few notable exceptions, low (5 mg $100\,g^{-1}$ fresh weight) levels of the 'intermediate' but-3-enyl and 3-methylthiopropyl glucosinolates.

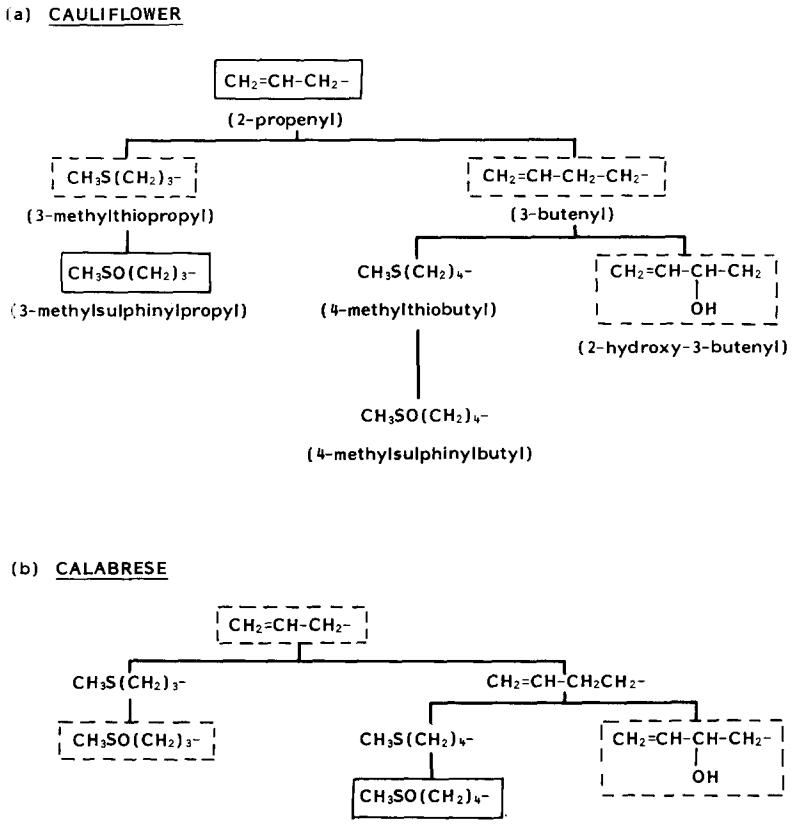

Fig. 1. Biosynthetic relationship of side chains in major aliphatic glucosinolates in cauliflower and calabrese. Major glucosinolates present in cauliflower and calabrese are contained in a solid box, minor glucosinolates in a broken box and absent glucosinolates are not boxed.

In contrast, in the present study, both prop-2-enyl and but-3-enyl glucosinolates were absent or, in a few cases, present at less than 1 mg $100\,g^{-1}$ fresh weight. Low levels (< 3 mg $100\,g^{-1}$ fresh weight) of 2-hydroxy-but-3-enyl glucosinolate were found in all calabrese cultivars, indicative of the presence of the β-hydroxylating enzyme system, but it was clear that the main biosynthetic pathway after initial chain elongation was that involving sequential terminal addition of CH_3SH and oxidation (yielding 4-methylsulphinylbutyl glucosinolate; 2·4–27·0 mg $100\,g^{-1}$ fresh weight). In contrast, terminal addition to the original prop-2-enyl glucosinolate precursor yielding 3-methylthiopropyl glucosinolate was, however, followed by much reduced S-oxidation, as evidenced by the low level of 3-methylsulphinylpropyl glucosinolate (0·7 mg $100\,g^{-1}$ fresh weight), suggesting that the S-oxidation process has very specific structural requirements.

Such subtle differences between cauliflower and calabrese emphasise, yet again, that considerable areas remain to be understood in our knowledge of glucosinolate biosynthesis and interconversion.

Whilst the overall contents of total glucosinolates, as estimated by glucose release (42·1–94·5 mg $100\,g^{-1}$ fresh weight; mean 62·3 mg $100\,g^{-1}$ fresh weight) and HPLC summation (40·4–98·5 mg $100\,g^{-1}$ fresh weight; mean 68·6 mg $100\,g^{-1}$ fresh weight), are in reasonable agreement, there is obvious disparity in a small number of cases. Thus (Table 2), if the HPLC figures are expressed as a percentage of those obtained from glucose release measurements, cultivars Mercedes (123%), Green Duke (137%), Tuliva (135%), Green Thistle (137%), Orion (134%) and Skiff (121%) all appear to be anomalous. Both HPLC and glucose release analyses have been repeated on these samples, without any significant differences emerging. Whilst the full reason for the differences between the two methods remains to be elucidated, five of these cultivars contain very high levels of 1-methoxy-3-indolylmethyl glucosinolate (>35 mg $100\,g^{-1}$ fresh weight), which, according to HPLC, contribute $\sim 70\%$ to the total content. Thus the determination of the correct response factors becomes a matter of the utmost importance and it seems probable that the figure of 0·50 assigned is an error. At present, solutions are being sought to this problem via the use of post column reaction (McGregor, 1985) and isolation and purification of appropriate standards (Hanley *et al.*, 1983; 1984).

Typical brassica flavour is due in considerable degree to glucosinolate-derived volatile isothiocyanates and nitriles (MacLeod, 1976). Of the glucosinolates found in calabrese only those possessing prop-2-enyl, 3-methylsulphinylpropyl and 4-methylsulphinylbutyl chains would be expected to produce such products. Thus, compared with cabbage, Chinese cabbage and Brussels sprouts, calabrese would be expected to be considerably less strongly flavoured and to be less pungent. In addition, bitterness, a flavour defect in certain Brussels sprouts (Fenwick *et al.*, 1983b), has been linked to high levels of prop-2-enyl and/or 2-hydroxybut-3-enyl glucosinolates and thus, on the basis of Table 2, would seem to be unlikely to be associated with calabrese.

Rather more importantly, recent researches in a number of laboratories (see McDannell *et al.*, 1987) have demonstrated that anticarcinogenic and enzyme-inducing effects of brassica vegetables are due primarily to indole glucosinolate breakdown products. Calabrese would seem to be comparable to cauliflower and swede as a source of indole glucosinolates, being especially rich in 1-methoxy-3-indolylmethyl glucosinolate, the biological properties of which, along with those of its hydrolysis products, are presently under investigation.

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