Chapter 2

Glucosinolates, isothiocyanates and indoles

Although isothiocyanate and indole compounds derived from cruciferous vegetables appear to be structurally unrelated, they are both derived from glucosinolates. These sulfur-containing glycosides are found in the order Capparales, which includes the large family Brassicaceae. The glucosinolate molecule comprises a common glucone moiety and a variable aglucone side-chain, derived from one of seven amino acids. Isothiocyanates are formed by the degradation of glucosinolates that have either aliphatic or aromatic side-chains (Figures 4 & 5), derived (mainly) from methionine and phenylalanine, respectively, whereas indoles are derived from glucosinolates with indolyl side-chains derived from tryptophan (Figures 6 & 7) (Rosa *et al.*, 1997). Cruciferous vegetables also contain other phytochemicals that have been associated with potential health benefits, including phenolics, vitamins and other sulfur-containing compounds.

Isothiocyanates are familiar to many of us as they are largely responsible for the characteristic hot, pungent flavours of salad vegetables such as radish, cress, mustard leaves and watercress, and contribute to the flavour of cooked cruciferous vegetables. Indoles do not contribute to the flavour of crucifers. Many of the biological and chemico-physical properties of these compounds (e.g. volatility and lipophilicity) are determined by the chemical structure of the isothiocyanate side-chain (Figure 5; Appendix 1), which, in turn, is determined by the structure of the parent glucosinolate molecule. The bioactivity of these compounds to humans is thus influenced by a combination of the chemical structure of the isothiocyanates and their concentration.

Major dietary sources of specific isothiocyanates and indoles

The concentrations and forms of glucosinolates in commonly eaten cruciferous vegetables are summarized in Table 6. Several surveys of variations in glucosinolate content between *Brassica* cultivars have been reported, for example for *B. rapa* (Carlson *et al.*, 1987a; Hill *et al.*, 1987) and *B. oleracea* (Carlson *et al.*, 1987b; Kushad *et al.*, 1999), and these are also summarized in Table 6. While over 90 different isothiocyanate (ITC) glucosinolates have been described, only about six occur frequently in the diet (Figure 5; Table 6). Sulforaphane (4-methylsulfinylbutyl-ITC) is obtained predominantly from broccoli but may also be obtained from rocket (*Eruca sativa*) and some cultivars of cabbage and Brussels sprouts. 2-Propenyl ('allyl')-,

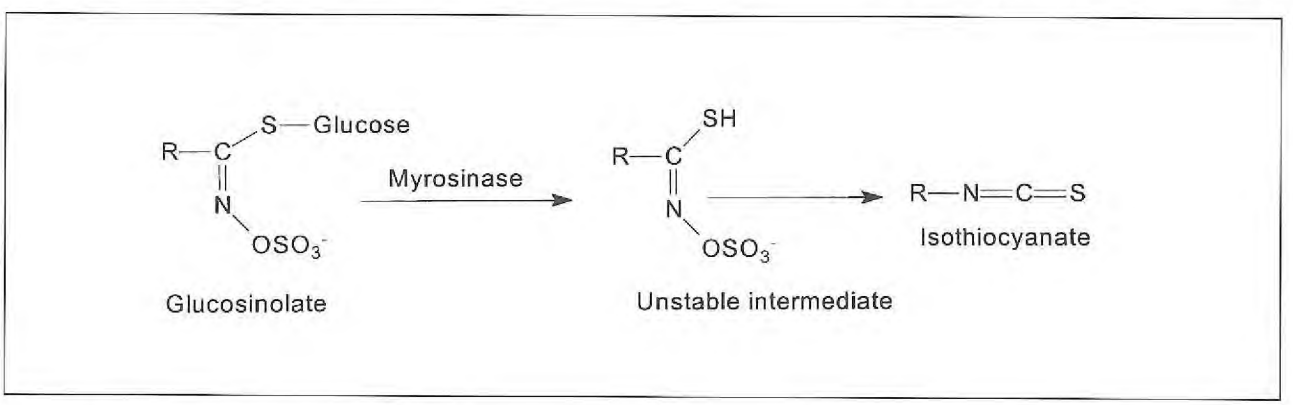

Figure 4 General scheme of the hydrolysis of glucosinolates to isothiocyanates

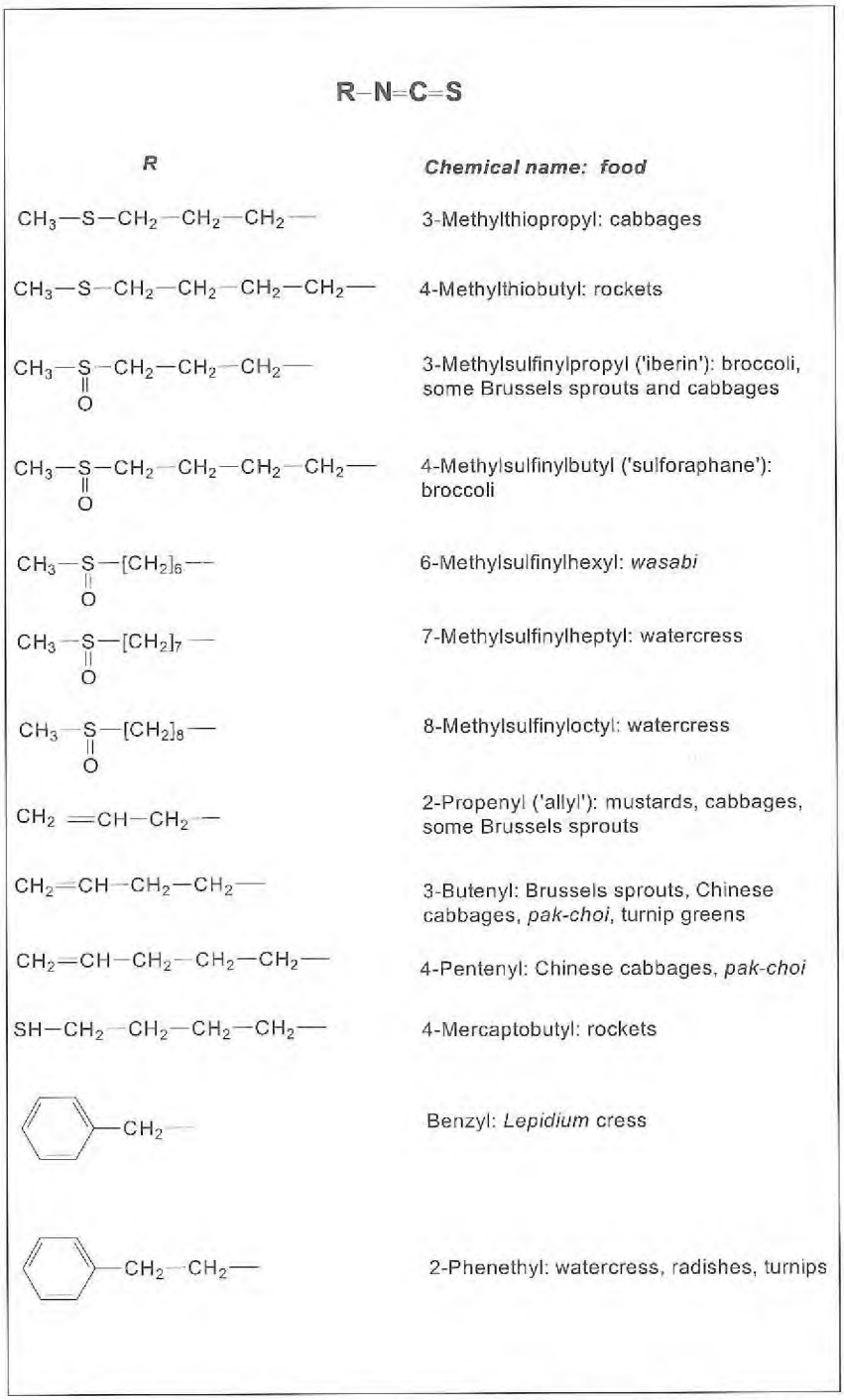

Figure 5 Structures of isothiocyanates and side-chain structures (R) found in commonly eaten cruciferous vegetables

3-butenyl- and 3-methylsulfinylpropyl-ITC ('iberin') are derived from consumption of certain cultivars of *B. oleracea*. Propenyl-ITC can also be obtained from leafy mustard vegetables. 3-Butenyl- and 4-pentenyl-ITC are obtained from leafy *B. rapa* crops and particularly Chinese cabbage. Phenyl-ethyl-ITC is obtained from watercress and to a lesser extent from root crops such as turnips and rutabaga. 6-Methylsulfinylhexyl-ITC is derived from the Japanese vegetable *wasabi* (*Wasabia japonica*). Benzyl-ITC is relatively rare in the diet, as it is obtained from *Lepidium* (cress) species. In addition to cruciferous vegetables, isothiocyanates may also be derived from mustard condiments, which are eaten mainly in western countries. Hydroxy-benzyl-ITC is found in 'English' mustard and is obtained from the seeds of white mustard, *Sinapis alba*. Allyl- (2-propenyl-) and 3-butenyl-ITCs are found in French and American mustards and are derived from the seeds of brown (or Indian) mustard, *B. juncea*. Allyl-ITC is also derived from the grated roots of horseradish (*Armoracia rusticana*), which is used as a condiment in western Europe and North America. Cruciferous vegetables may also be processed by pickling or fermenting, as in sauerkraut in western Europe and in some forms of *kimchi* in the Korean peninsula. Consumption of these products is an additional source of isothiocyanates in the diet.

Six different indolyl glucosinolates have been identified, but only four have been found in cruciferous crops (Figure 7), and, of these, only two, indolylmethyl ('glucobrassicin') and 1-methoxy-3-indolylmethyl ('neoglucobrassicin'), are found frequently. The types of indole glucosinolates vary from species to species (Table 6), and there is wide variation in the concentrations of specific indole glucosinolates. In broccoli, the concentration of

Table 6. Main glucosinolate side-chains of isothiocyanates occurring in cruciferous vegetables

Cruciferous vegetable	Glucosinolate side-chain	Content (μmol/100 g fresh weight)
Broccoli (*B. oleracea* var. *italica*)[a]	3-Methylsulfinylpropyl	0–330
	4-Methylsulfinylbutyl	29–190
	Indole-3-methyl	42–100
	1-Methoxyindole-3-methyl	2–18
Cabbage (*B. oleracea* var. *capitata*)[b]	2-Propenyl	4–160
	3-Methylsulfinylpropyl	5–280
	Indolylmethyl	9–200
Brussels sprouts (*B. oleracea* var. *gemmifera*)[c]	2-Propenyl	4–390
	3-Methylsulfinylpropyl	0–150
	3-Butenyl	0–220
	4-Methylsulfinylbutyl	0–23
	2-Hydroxy-3-butenyl	1–300
	Indole-3-methyl	45–470
	1-Methoxyindole-3-methyl	2–34
Cauliflower (*B. oleracea* var. *botrytis*)	2-Propenyl	1–160
	3-Methylsulfinylpropyl	0–330
	4-Methylsulfinylbutyl	2–190
	Indole-3-methyl	14–160
	1-Methoxyindole-3-methyl	1–32
Kale (*B. oleracea* var. *acephala*)	2-Propenyl	62–200
	3-Methylsulfinylpropyl	0–50
	3-Butenyl	6–38
	2-Hydroxy-3-butenyl	17–130
	Indole-3-methyl	67–160
Rape (*B. rapa*, including Chinese cabbage and turnip tops)[d]	3-Butenyl	38–290
	4-Pentenyl	20–150

All the crops can also contain low concentrations of other glucosinolates (Carlson *et al.*, 1987a,b; Hill *et al.*, 1987; Rosa *et al.*, 1997; Kushad *et al.*, 1999). The concentrations must be interpreted with care, as different methods of analyses were used in the different studies, and the concentrations are affected by a wide range of environmental factors. In addition, generalizations cannot be made about the glucosinolate content of cabbages and Brussels sprouts as there are large genetic differences between cultivars. The values quoted indicate the greatest range of values in the reviews referred to above.

[a] All broccoli cultivars produce predominantly 4-methylsulfinylbutyl glucosinolate, the precursor of sulforaphane.

[b] Some cabbage varieties produce the elongated glucosinolates, 3-butenyl and 4-methylsulfinylbutyl; some also produce their precursors, 3-methylthiopropyl and 4-methylthiobutyl.

[c] Surprisingly, the glucosinolate content of Brussels sprouts varies: some cultivars produce high concentrations of 2-propenyl, while others produce high concentrations of 3-butenyl and 2-hydroxy-3-butenyl. Recently developed cultivars either have low concentrations of these glucosinolates or moderate concentrations of 3-methylsulfinylpropyl or 4-methylsulfinylbutyl.

[d] Cultivars tend to have both 3-butenyl and 4-pentenyl (and hydroxylated forms) or just 3-butenyl

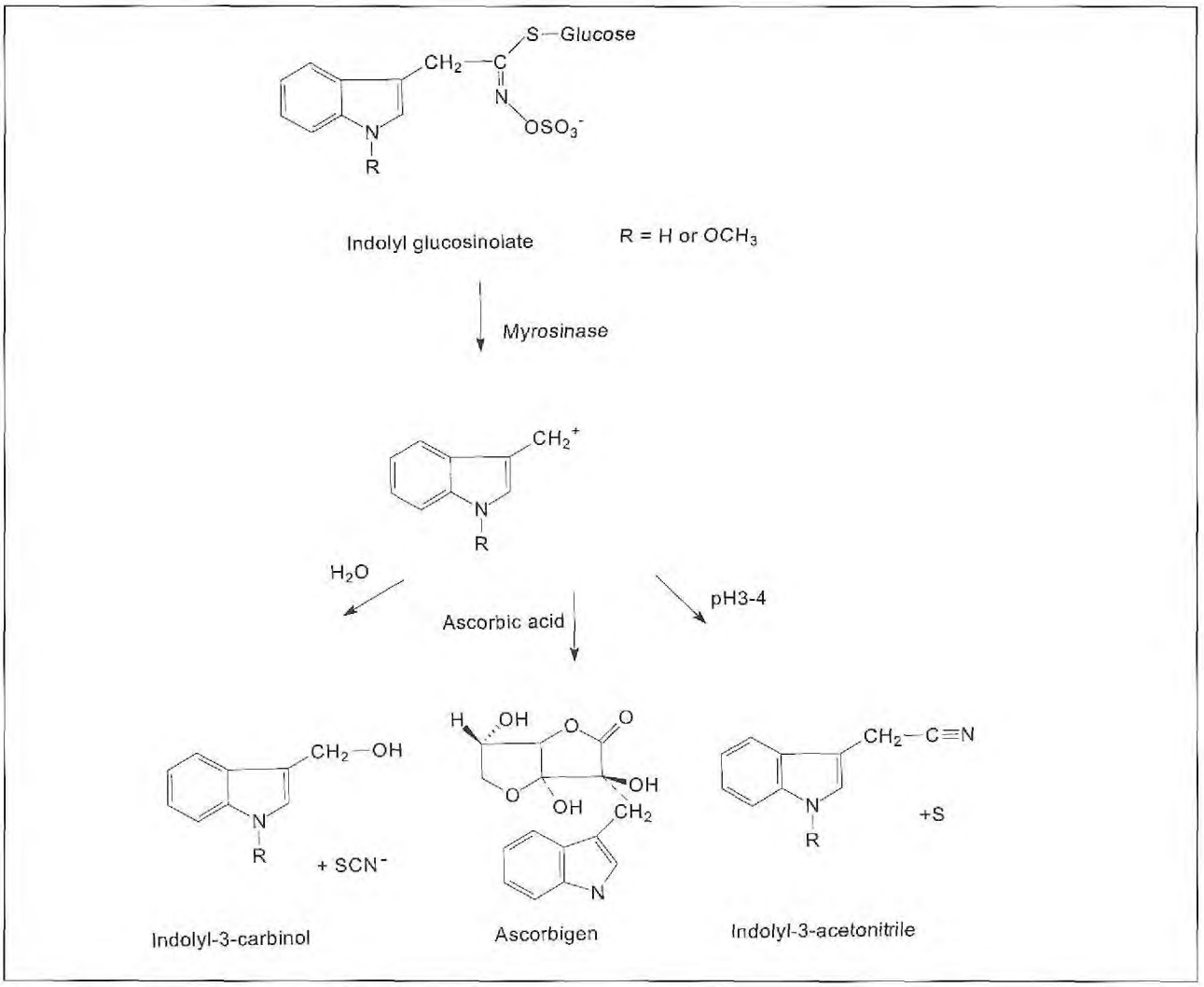

Figure 6 Degradation pathways of indole glucosinolates

neoglucobrassicin was found in some studies to be even higher than that of glucobrassicin (Vang *et al.*, 2001; Vallejo *et al.*, 2003a). In Brussels sprouts, neoglucobrassicin occurs at a much lower concentration than glucobrassicin (Kushad *et al.*, 1999; Ciska *et al.*, 2000), while 4-hydroxy-3-indolylmethyl glucosinolate and glucobrassicin are found at similar concentrations in cauliflower (Kushad *et al.*, 1999). In white cabbage, the main indole glucosinolate is glucobrassicin, and 4-methoxy-3-indolylmethyl glucosinolate is present at half the concentration (Ciska *et al.*, 2000).

Four factors interact to determine the exposure of the gastrointestinal tract to isothiocyanates and indoles:

- the biochemical genetics of the biosynthesis of glucosinolates and isothiocyanates within the crop plant, which determines the chemical structure of the glucosinolate side-chain and partially determines the overall amount;
- abiotic and biotic environmental factors, which can influence the overall amount of isothiocyanates produced by the plant;
- post-harvest storage, processing and cooking; and
- the thioglucosidase activity of the intestinal microbial flora.

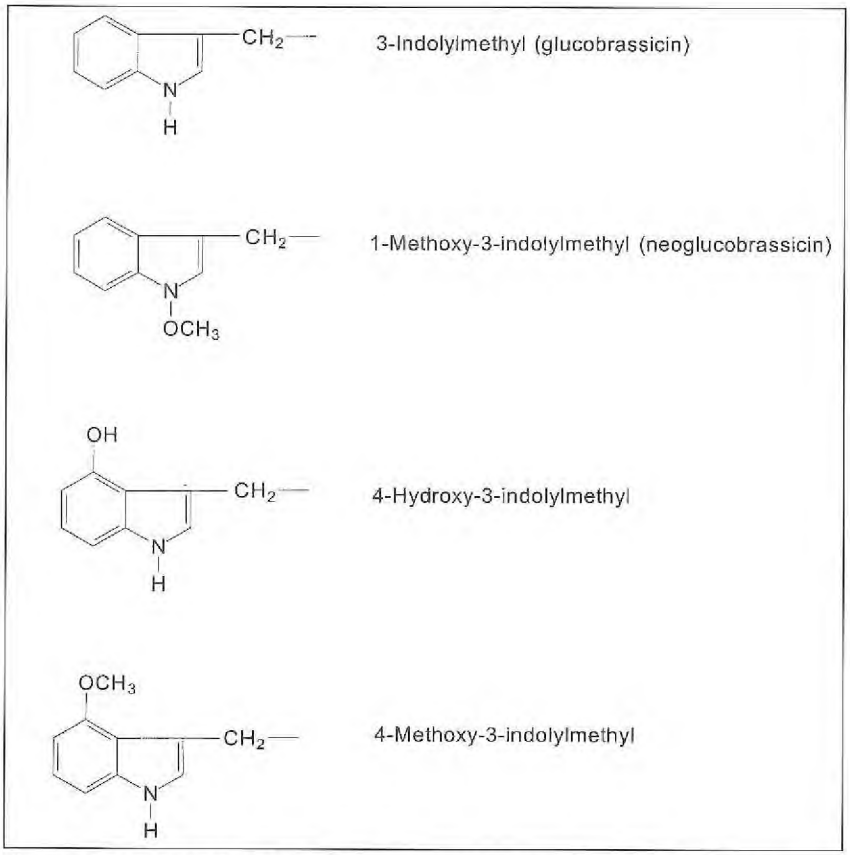

Figure 7 Structures of the four indolyl glucosinolates found in cruciferous crops

Biochemical genetics of glucosinolate biosynthesis

Side-chain structures

Glucosinolates with more than 115 side-chain structures derived from one of eight amino acids (alanine, valine, leucine, isoleucine, phenylalanine, methionine, tyrosine and tryptophan) have been described. These glucosinolates all form isothiocyanates upon hydrolysis, except for those derived from tryptophane, which release indoles. The glucosinolate contents of wild and cultivated plants have been surveyed by Daxenbichler *et al.* (1991) and Fahey *et al.* (2001).

Biosynthesis of these glucosinolates and their resultant isothiocyanates or indole derivatives can be separated into four parts: amino acid chain elongation, core glucosinolate synthesis, chain modification and production of isothiocyanates and indoles (Figure 8). The side-chain structure of glucosinolates in any one genotype is under strict genetic control, whereas the overall level is determined by interactions between genotype and environment. Only an overview is provided here; for further details, see Halkier and Du (1997) and Mithen (2001).

Amino acid elongation

While some glucosinolates are synthesized from chain-elongated forms of valine and phenylalanine, about 50% of all known glucosinolates, only from the Brassicaceae and the Capparaceae, are synthesized from elongated forms of methionine. Methionine can be elongated by the addition of one to nine methyl groups, but most taxa within the Brassicaceae have only a restricted chain length, which can be divided into three classes: 'short chains', from the addition of one, two, three or (rarely) four methyl groups to methionine, such as found in *Brassica* crops; 'long chains', from the addition of five or six methyl groups; and 'very long chains' from the addition of seven, eight or nine methyl groups.

Biochemical studies in which [^{14}C]acetate and ^{14}C-labelled amino acids were administered, with subsequent analysis of the labelled glucosinolates, suggest that the amino acid elongation is similar to that which occurs during synthesis of leucine from 2-keto-3-methylbutanoic acid and acetyl coenzyme A (Strassman & Ceci, 1963; Graser *et al.*, 2000). The amino acid is transaminated to produce an α-keto acid, followed by condensation with acetyl coenzyme A, isomerization involving a shift in the hydroxyl group, and oxidative decarboxylation to result in an elongated keto acid which is transaminated to form the elongated amino acid. It is likely that the elongated keto acid can undergo further condensation with acetyl coenzyme A to result in multiple chain elongations.

Molecular genetics studies in *Arabidopsis thaliana* resulted in identification of a small gene family, designated *MAM* (*methylthioalkylmalate*), which catalyse condensation of the keto acids with acetyl coenzyme A (Kroymann *et al.*, 2001). Differences in expression and allelic variants of the *MAM* genes appear to explain the variety of chain lengths observed, although the details remain to be clarified (Kroymann *et al.*, 2003). Thus, differences in the chain length of

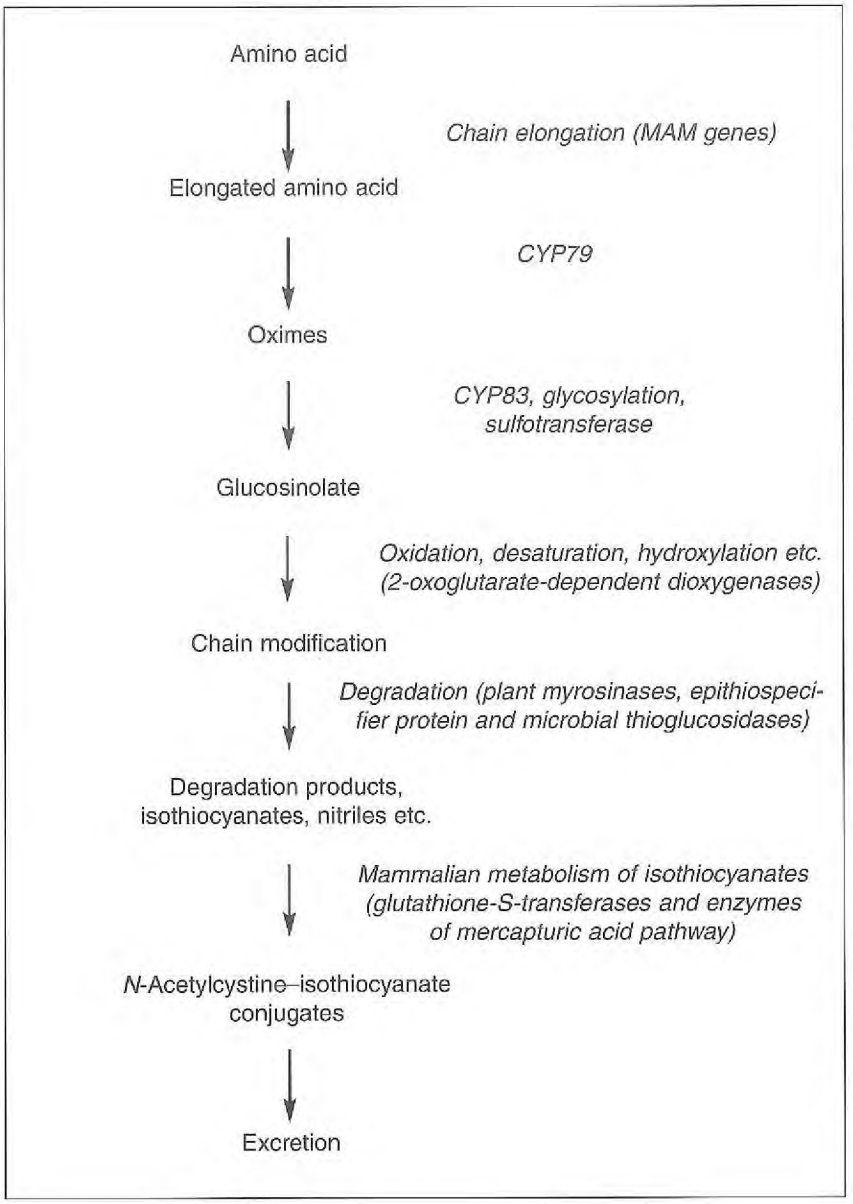

Figure 8 Biosynthesis and metabolism of glucosinolates and isothiocyanates
Indole glucosinolate degradation products are not metabolized via the mercapturic acid pathway

methionine-derived glucosinolates in cruciferous crops is probably due to allelic differences in *MAM* genes that result in different specificities of their enzymic products for elongated keto acids.

Core glucosinolate biosynthesis

The first step in glucosinolate biosynthesis is conversion of the amino acid to an oxime. Recent studies, largely with *A. thaliana*, suggest that all amino acid–oxime conversions are catalysed by cytochrome P450 (CYP) monooxygenases of the CYP79 family. The best characterized system is conversion of tyrosine and phenylalanine to their corresponding oximes (Du *et al.*, 1995; Bak *et al.*, 1999) as precursors of benzyl and hydroxybenzyl glucosinolates. In *Arabidopsis*, oximes from chain-elongated methionine homologues are formed by the action of the *CYP79F1* and *CYP79F2* genes. The first of these can catalyse many homologues of chain-elongated forms of methionine, whereas the latter can catalyse only long-chain methionine homologues.

In *Arabidopsis*, conversion of the oxime to the thiohydroximate is probably catalysed by another cytochome P450, CYP83B1 (Hansen *et al.*, 2001). As thiohydroximates would be toxic to plant tissue, they are effectively detoxified by glycosylation by a soluble UDPG:thiohydroximate S-glucosyltransferase to produce a desulfoglucosinolate (Reed *et al.*, 1993; Grootwassink *et al.*, 1994; Guo & Poulton, 1994). This is sulfated by a soluble 3′-phosphoadenosine 5′-phosphosulfate: desulfoglucosinolate sulfotransferase (Jain *et al.*, 1990), to produce the glucosinolate.

Chain modification

The chain structure of glucosinolates derived from any amino acid can undergo modification. As for other aspects of glucosinolates, most attention has been paid to methionine-derived compounds.

After the biosynthesis of methylthioalkyl glucosinolates from methionine, the side-chain can undergo various modifications. Initial oxidation results in methylsulfinylalkyl, which accumulates in broccoli. Additional oxidation results in methylsulfonylalkyl glucosinolates, which accumulate in certain non-cultivated cruciferous vegetables. Removal of the methylsulfinyl group and desaturation result in alkenyl glucosinolates. Alternatively, hydroxylation

results in 3-hydroxypropyl or 4-hydroxybutyl glucosinolate (Mithen *et al.*, 1995; Giamoustaris & Mithen, 1996; Hall *et al.*, 2001). There is considerable scope for variation in this part of the pathway. For example, 4-methylsulfinylbutenyl glucosinolate, found exclusively in *Raphanus*, probably results from desaturation of the corresponding methylsulfinylbutyl glucosinolate but without associated methylsulfinyltransferase activity. The alkenyl glucosinolates 3-butenyl and 4-pentenyl can undergo β-hydroxylation, with important consequences for the nature of the hydrolytic products. While the details of the biochemical processes and the genes that determine these modifications are not fully understood, studies with *Arabidopsis* suggest that many of the processes are due to the activity of 2-oxogluturate-dependent dioxygenases (Hall *et al.*, 2001). As for side-chain elongation, genetic control of the side-chain structure is very strict. Thus, a specific genotype always produces the same chain structure, regardless of the environment in which it is grown.

Indole glucosinolates derived from tryptophan are also modified by addition of hydroxyl or methoxy groups (Figure 7), but the biochemical mechanisms and genetic basis of these modifications are unknown. Although modification of the side-chains of isothiocyanates, producing glucosinolates, is under strict genetic control, environmental factors can affect modification of indole side-chains.

Genetic basis of glucosinolate accumulation

While several genes that determine side-chain structure have now been cloned, the genetic control of total glucosinolate accumulation is still far from understood. Most studies have been conducted on spring or winter rape (*B. napus*), in which breeders have sought to reduce the content of 2-hydroxy-3-butenyl glucosinolate ('progoitrin') owing to the goitrogenic activity of its major hydrolysis product, 5-vinyloxazolidine-2-thione (goitrin), when incorporated into animal feed. Thus, glucosinolate concentrations have been reduced from greater than 80 μmol/g to less that 5 μmol/g in spring rape and to less than 15 μmol/g in winter rape. This was shown to be a complex genetic trait determined by alleles at four or five quantitative trait loci (Toroser *et al.*, 1995; Howell *et al.*, 2003). Studies in broccoli have focused on increasing the concentration of methylsulfinylalkyl glucosinolates. Surveys of glucosinolates in existing broccoli cultivars showed that the concentration of 4-methylsulfinylbutyl glucosinolates varied from 0.8 mmol/g dry weight to 21.7 mmol/g (Kushad *et al.*, 1999). In an effort to obtain higher concentrations, Faulkner *et al.* (1998) used *B. villosa* and other wild members of the nine *B. oleracea* species complex. Hybrids between these accessions and broccoli contained concentrations in excess of 80 μmol/g dry weight. After several backcrosses, isothiocyanate-enriched broccoli was developed from these initial hybrids (Mithen *et al.*, 2003). Some of the quantitative trait loci important in enhancing the glucosinolate concentration of broccoli are in positions in the genome similar to those that determine reduction of glucosinolate levels in rape. Identification of the genes involved in these quantitative trait loci might facilitate a genetic approach to enhancing glucosinolates in horticultural crops.

Glucosinolate hydrolysis and formation of isothiocyanates

When tissue is disrupted, an endogenous plant thioglucosidase ('myrosinase', see below) causes cleavage of the thio–glucose bond to give rise to unstable thiohydroximate *O*-sulfonate (Figure 4). This aglycone spontaneously rearranges to several products. Most frequently, it undergoes a Lossen rearrangement to produce an isothiocyanate. If the glucosinolate side-chain contains a double bond, and in the presence of an epithiospecifier protein (see below), the isothiocyanate may rearrange to produce an epithionitrile (Figure 9a). If the glucosinolate lacks a double bond, the sulfur may be lost and a nitrile formed. (Figure 9b). A few glucosinolates have been shown to produce thiocyanates, although the mechanism by which this occurs is unknown. Aglucones from glucosinolates which contain β-hydroxylated side-chains, such as progoitrin found in the seeds of oilseed rape and in the edible parts of Chinese cabbage and Brussels sprouts, spontaneously cyclize to form the corresponding oxazolidine-2-thiones (Figure 9c).

Glucosinolate hydrolysis and formation of indoles

The formation of indoles from indole glucosinolates has been reviewed by Vang and Dragsted (1996). After tissue disruption, myrosinase cleaves the thioglucose bond, like other glucosinolates, but the resulting isothiocyanate is unstable and degrades to the corresponding alcohol (Figure 5). The alcohol can condense to 3,3′-diindoylmethane. As with methionine-derived glucosinolates, indolyl-3-acetonitrile can be formed instead of an unstable isothiocyanate. The factors that determine the degradation pathway are largely unknown. Moreover, in the acidic conditions of the stomach, indole-3-carbinol (and related products from other indolyl glucosinolates) can undergo several condensation reactions, to produce at least 15 different oligomeric products (Anderton *et al.*, 2003). Indole-3-carbinol can also react with ascorbic acid to form ascorbigen (Piironen & Virtanen, 1962; Hrncirik *et al.*, 2001). As for isothiocyanate-producing glucosinolates, if cooking

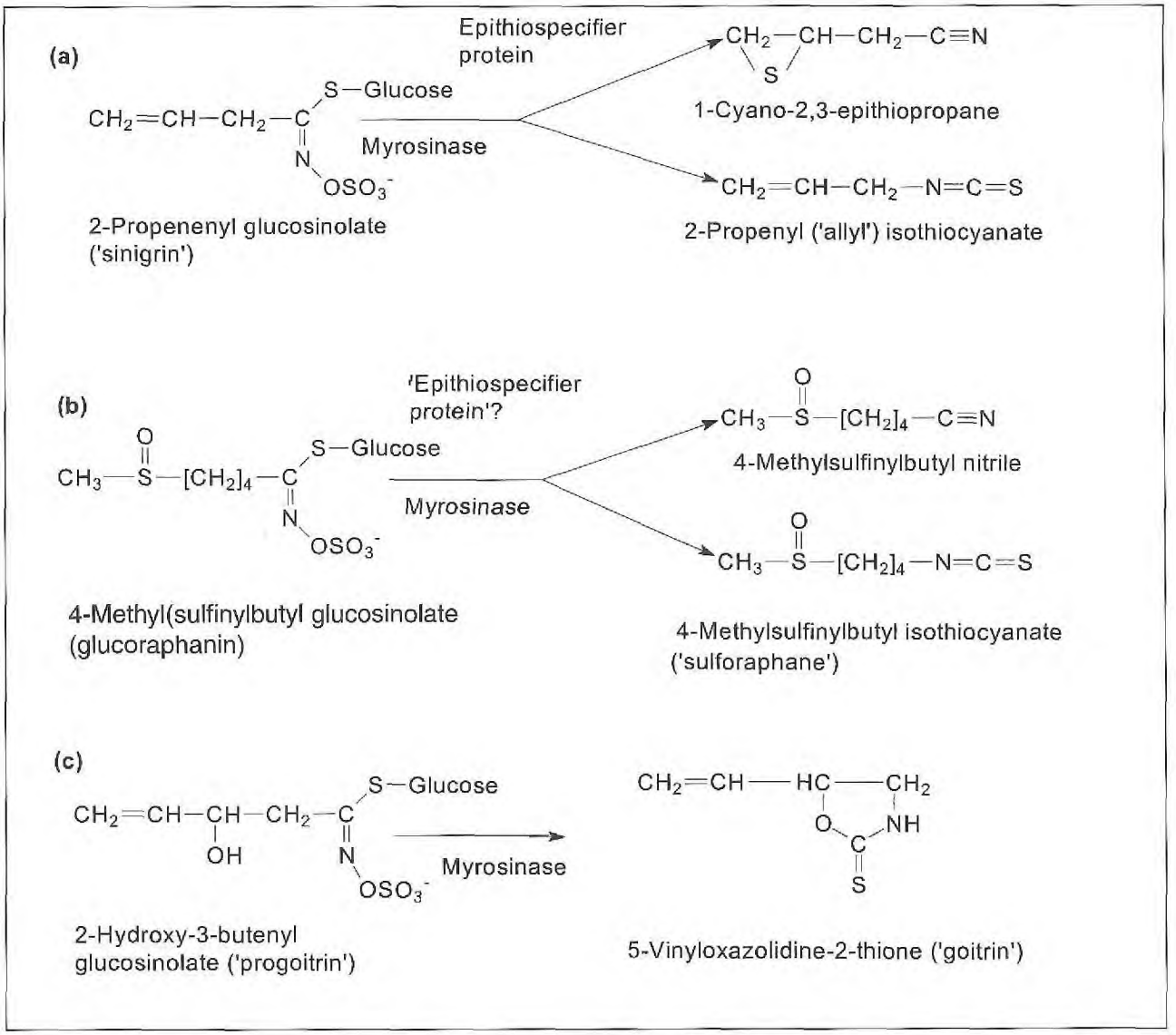

Figure 9 (a) Production of 2-propenyl- or 1-cyano-2,3-epithiopropane by hydrolysis of 2-propenyl glucosinolates, which are a result of expression of the epithiospecifier protein. **(b)** Production of 4-methylsulfinylbutyl isothiocyanate or nitrile by hydrolysis of the corresponding glucosinolate, as a result of expression of epithiospecifier-like protein. **(c)** Production of 5-vinyloxazolidine-2-thione (goitrin) from 2-hydroxy-3-butenyl glucosinolate

denatures myrosinase activitiy, the intact indole glucosinolate can be degraded in the colon, in which the contrasting pH may result in production of different degradation products and their absorption into the bloodstream.

Myrosinases

Bones and Rossiter (1996) and Rask *et al.* (2000) have reviewed the data on myrosinases, and only a brief summary is provided here. Many myrosinase isozymes have been detected in glucosinolate-containing plants, and myrosinase (i.e. thioglucosidase) activity has also been detected in insects, fungi and bacteria. In plants, the expression of isozymes varies both between species and within the organs

of the same individual (Lenman *et al.*, 1993). In general, isozymes with activity towards the glucosinolate chain structure appear to have little substrate specificity, although a myrosinase highly specific to epiprogoitrin was described in *Crambe* (Bernardi *et al.*, 2003). Molecular studies with *A. thaliana, Brassica* and *Sinapis* have shown that myrosinases comprise a gene family (Xue *et al.*, 1992) with three subclasses, denoted MA, MB and MC. Members of each of these subfamilies occur in *A. thaliana* (Xue *et al.*, 1995). As would be expected, many more copies occur in the *B. napus* genome, because of genome replication. All myrosinases are glycosylated, and the extent of glycosylation varies between subclasses. It is likely that the subdivision of myrosinases will be revised as new data on sequences become available. MB and MC myrosinases are linked with myrosinase-binding proteins and myrosinase-associated proteins, respectively (Lenman *et al.*, 1990). The roles of these proteins are not understood, but they seem to have no significance for the generation of glucosinolate degradation products after consumption of cruciferous vegetables.

Epithiospecifier protein

The epithiospecifier protein was first described by Tookey (1973) and was purified from *B. napus* (Bernardi *et al.*, 2000; Foo *et al.*, 2000). This protein appears to have no inherent enzymatic activity; it does not interact with glucosinolates but only with the unstable thiohydroximate *O*-sulfonate after myrosinase activity. Foo *et al.* (2000) suggested that the mode of action of epithiospecifier protein is similar to that of a cytochrome P450, such as in iron-dependent epoxidation reactions. While this protein has been considered only in the context of the generation of epithionitriles, it might also be involved in the production of nitriles from glucosinolates, such as those with methylsulfinylalkyl side-chains found in broccoli and watercress. In this case, the sulfur from the glucone is lost, as it cannot be re-incorporated into the degradation product since it lacks a terminal double bond.

The ratio of isothiocyanates:nitriles varies according to genotype (Matusheski *et al.*, 2003; Mithen *et al.*, 2003), and this may be related to expression of the epithiospecifier protein. Glucosinolates in ecotypes of *A. thaliana* vary with respect to the relative ratio of isothiocyanate to epithionitrile or to nitrile they form after tissue disruption, due partly to allelic variation at a quantitative trait locus associated with gene coding for epithiospecifier protein (Lambrix *et al.*, 2001). Likewise, while in standard cultivars of broccoli hydrolysis results in about a 20:80% ratio of isothiocyanates:nitriles, isothiocyanate enriched broccoli produces about 95% isothiocyanates after hydrolysis (Mithen *et al.*, 2003).

Factors that affect glucosinolate concentrations

Abiotic and biotic environmental factors

In general terms, while the ratio of individual glucosinolates within a particular class (e.g. those derived from methionine) is relatively constant and is unaffected by environmental factors, the total concentration is affected by several such factors. Our understanding of these is relatively poor, and it is likely that there are often significant genotype–environment interactions. Soil fertility is probably a major factor. Zhao *et al.* (1994) reported that the sulfur and nitrogen supply affected the glucosinolate content of rapeseed and described minor alterations in the ratios of individual methionine-derived glucosinolates and larger alterations in the ratio of indolyl:methionine-derived glucosinolates. In contrast, sulfur fertilization alone had either a small effect or no effect at all on the glucosinolate content of broccoli (Vallejo *et al.*, 2003a,b). It is likely that any effect of sulfur depends strongly on the nitrogen supply. Soil fertilization (combined N and S) was reported to have a significant effect on glucosinolates in broccoli (Mithen *et al.*, 2003). This study also showed that certain genotypes have a greater response than others. Nitrogen fertilizer in a hydroponic system enhanced the concentration of glucosinolates in *pak-choi* (Shattuck & Wang, 1994). Water stress induced glucosinolates in rapeseed and rape (Bouchereau *et al.*, 1996; Jensen *et al.*, 1996) and is likely to have a similar effect in horticultural cruciferous vegetables. The effect of temperature has not been studied in detail, and, like other environmental factors, affects general plant growth parameters. Pereira *et al.* (2002) reported that temperature affected the glucosinolate concentrations in seedlings ('sprouts') of broccoli, those grown at higher temperatures having more glucosinolates than those grown at lower temperatures.

Not only abiotic factors but also insects and pathogens induce glucosinolate accumulation, although the effects are mainly on indolyl glucosinolates as opposed to methionine-derived (isothiocyanate-producing) glucosinolates (Birch *et al.*, 1992; Shattuck & Wang, 1994). Jasmonic acid, a signalling molecule involved in several plant–insect or –pathogen interactions, also induced glucosinolates (Bodnaryk, 1994).

Post-harvest storage, processing and cooking

Few studies have been conducted on the effects of post-harvest events on the concentration of glucosinolates in horticultural cruciferous vegetables.

Vallejo *et al.* (2003c) reported a loss of 70–80% of the total glucosinolate content after a period of simulated cold storage during transport and storage before sale. Storage of cut cabbage reduced methionine-derived glucosinolates, as expected, but enhanced the concentration of indolyl glucosinolates (Verkerk *et al.*, 2001). Storage of broccoli under various conditions also enhanced indole glucosinolates (Hansen *et al.*, 1995).

As described above, myrosinase-mediated glucosinolate degradation results in initial formation of an unstable intermediate and then in the formation of an isothiocyanate or a nitrile (Figure 9a and b), the latter due to the presence of an epithionitrile specifier or an epithiospecifier nitrile-like protein. When raw *Brassica* vegetables are macerated, about 80% of the methionine-derived glucosinolates can be converted to nitriles, as opposed to isothiocyanates, the precise ratio of isothiocyanates:nitriles depending on genotype (Mithen *et al.*, 2003). Both myrosinase and ethiospecific protein can be degraded by cooking. Mild cooking, for example steaming broccoli florets for less than 3 min, denatured the epithionitrile specifier protein while leaving at least some of the endogenous myrosinase intact, enhancing the isothiocyanate content. Further cooking, e.g. heating broccoli for 10 or 20 min at 50 °C, subsequently denatured myrosinase, preventing any immediate isothiocyanate production. Thus, the degree of cooking can have significant effects on the delivery of isothiocyanate to the gastrointestinal tract: consumption of raw vegetables may result in exposure to a significant amount of nitriles; mild cooking may result in consumption of large amounts of isothiocyanates, with topical exposure of the upper gastrointestinal tract to biologically significant concentrations; while more extensive cooking prevents isothiocyanate formation, and intact glucosinolates will be consumed which may be degraded to isothiocyanates or other compounds by the intestinal microflora, as discussed below.

Glucobrassicin is chemically and thermally stable, as no degradation was observed after 2 h in aqueous media with pH values ranging from 2 to 11. Moreover, glucobrassicin was weakly degraded by heat treatment (10% after 1 h) (Chevolleau *et al.*, 1997).

Intestinal microbial flora

Cooking glucosinolate-containing vegetables for more than 2 min inactivates myrosinase, as described above. Several studies have shown that isothiocyanate metabolites can be detected in blood and urine after cooked glucosinolate-containing foods are eaten, although to a lesser extent than when raw glucosinolate-containing foods are consumed (Getahun & Chung, 1999; Conaway *et al.*, 2000). A likely source of isothiocyanates is microbial degradation of glucosinolates by the intestinal microflora. Thus, isothiocyanates can be generated by incubation of cooked (i.e. myrosinase denatured) watercress with human fæces under anerobic conditions (Getahun & Chung, 1999). Rouzaud *et al.* (2003) compared urinary isothiocyanate metabolites in gnotobiotic rats harbouring whole human faecal flora and in germ-free rats and found, as expected, that after feeding with glucosino-late-containing food the greatest excretion of isothiocyanates was observed when the plant myrosinase was intact. When the plant myrosinase was denatured by heat treatment, the amount of isothiocyanate produced was estimated to be reduced by > 85% in the gnotobiotic rats. Some isothiocyanates also appeared to be produced in the germ-free rats, and some evidence was obtained that the presence of human faecal flora actually reduced the amount of isothiocyanate available for absorption.

Indole glucosinolates are likely to be degraded in the gastrointestinal tract. When they were fed to rats, similar biological effects were seen as when degradation products were fed, suggesting microbial degradation of intact glucosinolates (Bonnesen *et al.*, 1999).

Estimates of dietary intake of isothiocyanates and indoles

Estimates of dietary intake of isothiocyanates and indoles are clearly needed. The existing databases on consumption of crucifers (section 1) and glucosinolate content (Table 6) might be considered adequate to provide a basis for such estimates, but the wide variation in glucosinolate content due to the factors described above and the difficulty in estimating the conversion of glucosinolates to isothiocyanates and indoles during consumption reduce the confidence with which such estimates can be made.

Measurement of isothiocyanates in vegetables before consumption

Jiao *et al.* (1998) in Singapore and Shapiro *et al.* (1998) in the USA reported the isothiocyanate concentrations found in vegetables. In each case, the vegetables were analysed by condensation with 1,2-benzenedithiol (see section 3), which results in quantification of total isothiocyanates. Endogenous plant myrosinase was denatured by cooking before treatment with exogenous myrosinase (Table 7). Thus, the estimate of isothiocyanate production is probably higher than that which occurs within the gastrointestinal tract, on the assumption that myrosinase activity is a limiting factor.

Intake of indole glucosinolates

Estimates of the average daily intake of indole glucosinolates are based on

Table 7. Isothiocyanate (ITC) content of cruciferous vegetables in Singapore and the USA

Vegetable	Mean content (µmol/100 g fresh weight (range))	
	Singapore (Jiao *et al.*, 1998)	USA (Shapiro *et al.*, 1998)
Broccoli (*B. oleracea* var. *italica*)	38.6 (10.1–62.0)	6.7
Cabbage (*B. oleracea* var. *capitata*)	27.5 (11.9–62.7)	4.4
Cauliflower (*B. oleracea* var. *botrytis*)	11.6 (2.7–24.0)	
Kale (*B. oleracea* var. *acephala*)		18.2
Kai lan (*B. oleracea* var. *alboglabra*)	15.4 (3.1–35.9)	
Turnip (*B. rapa* var. *rapa*)		1.4
Bok choi (*B. rapa* var. *chinensis*)	4.9 (2.0–7.5)	
Choi sum (*B. rapa* var. *parachinensis*)	11.1 (3.5–23.4)	
Watercress (*Nasturtium officinale*)	81.3 (17.1–144.6)	
Kai choi (*B. juncea* var. *rugosa*)	71.2 (25.6–138.4)	

the intake of specific cruciferous vegetables and their content of the various indole glucosinolates. The estimates are only approximate, as the concentrations of indole glucosinolates vary considerably depending on growing conditions, the cultivar, storage and preparation conditions, as considerable amounts of glucosinolates can be lost during storage and processing.

Sones *et al.* (1984) estimated the per-capita intake of indole glucosinolates in the United Kingdom to be 19.4 mg/day for glucobrassicin and 3.1 mg/day for neoglucobrassicin. Vang and Dragsted (1996) estimated the per capita intake in Denmark to be 5 mg/day for glucobrassicin and 0.5 mg/day for neoglucobrassicin, and the respective values in Finland to be 2.5 mg/day and 0.3 mg/day. Broadbent and Broadbent (1998a) in the USA estimated the per capita intake of glucobrassicin to be 8.1 mg/day. The total intake of indole glucosinolates, including the two major ones and both fresh and cooked cruciferous vegetables, was about 22.5 mg/day per capita. The more recent estimates indicate wide variations between countries, which are due to the use of different methods for estimating intake of cruciferous vegetables and indole glucosinolates.