TOXICANTS *of* PLANT ORIGIN

Volume II Glycosides

Peter R. Cheeke

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Toxicants of Plant Origin

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Editor

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FOREWORD

Natural toxicants in plants or "nature's pesticides" are increasingly recognized as significant items of the diet of humans and domestic animals. These diverse compounds function as chemical defenses of plants to deter herbivory. Selection of forage and crop plants for resistance against insects and other pests often increases their content of chemical defenses, thus inadvertently adversely affection their nutritional value. With an increasing emphasis on alternatives to agricultural chemicals in control of pests in crop production, natural plant toxicants will assume even more significance. It is appreciated that animal products such as meat, milk, and eggs may also be a means of exposure of humans to plant toxins. Thus knowledge of the metabolic fate of plant toxins in livestock is very important, both in terms of efficient animal production and the safety of animal products in the human diet.

These four volumes are intended to provide a comprehensive, up-to-date treatment of major plant toxins. The authors are authorities in their respective disciplines, and have provided fresh viewpoints and an integration of existing knowledge. These state-of-the-art treatments also draw attention to major areas where additional research is needed.

THE EDITOR

Peter R. Cheeke, Ph.D., is Professor of Comparative Nutrition, Department of Animal Science, Oregon State University, Corvallis. He received his B.S. and M.S. degrees in animal science at the University of British Columbia, Vancouver, in 1963 and 1965, respectively, and his Ph.D. in animal nutrition at Oregon State University in 1969. His research program has reflected a broad interest in livestock production and the components of feeds which adversely affect animal performance, including pyrrolizidine and quinolizidine alkaloids, saponins, protease inhibitors, and glucosinolates. His achievements in this area have resulted in extensive participation in international symposia and conferences. His research has resulted in over 100 technical publications and several books. In addition to work on plant toxins, he has pioneered research on the domestic rabbit as a new livestock species for use as a meat source in developing countries and has extensively studied the relationships between secondary compounds in plants and feeding and digestive strategies in the rabbit. He is recognized internationally for his work in rabbit nutrition, and has received several awards including the Mignini International Award for rabbit research and the outstanding paper award of the American Association of Laboratory Animal Science.

Dr. Cheeke is a member of several scientific organizations including the American Institute of Nutrition and the American Society of Animal Science. His current research emphasizes pyrrolizidine and quinolizidine alkaloid metabolism and the use of the rabbit as an herbivore model for the study of metabolism of plant toxicants.

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Chapter 1

GLUCOSINOLATES

G. R. Fenwick, R. K. Heaney, and R. Mawson

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I. INTRODUCTION

The history of glucosinolate research is now well into its second century. Three main phases may be seen within this period. The first, stretching from 1831, when the first crystalline glucosinolate, sinalbin, was isolated from the seed of white mustard, until about 1950, represented a period of sporadic research, centered on the detailed structural studies of Gadamer at the end of the last century.[1] The second period, from 1950 until 1970, was characterized by a tremendous increase in knowledge about the chemistry of glucosinolates, centered primarily on the research groups of Schulz and Kjaer.[2] This period also saw the correction of the glucosinolate structure proposed by Gadamer and its ultimate confirmation by synthesis and X-ray studies. The final period, from 1970 onward has seen the emphasis on glucosinolate research shift from chemical to biological, prompted initially by the emergence of rapeseed as an oilseed of commerce and the ready availability of the defatted meal

as a protein source of potentially great value for farm animals.[3] Current research is also fueled by the legitimate concerns over the effects of glucosinolates and their products in the human diet.[4] It is, thus, appropriate to consider both aspects of glucosinolates in this chapter.

In common with other natural toxicants, the effects of glucosinolates in animals are readily documented; in comparison those in man are much less easy to identify, being the consequences of long-term, low-level exposure (although, as will be seen, in this context "low" may be something of a misnomer). In addition, there has been considerable interest shown in the anticarcinogenic and enzyme-inducing properties of the products of certain glucosinolates which may be present in the human diet.[5] Thus, although it is impossible yet to provide any measure of risk associated with glucosinolate intake in man, it is apparent that this will reflect an overall balance of deleterious and beneficial properties.

In recent years a number of authoritative reviews of glucosinolates in foods and feedstuffs have been published, notably by research groups from the U.S. Department of Agriculture[6,7] and, in the U.K., the Agriculture and Food Research Council.[1,8] Many of the areas covered in these reviews, notably biosynthesis, enzymic breakdown, and relationship to flavor, have not advanced greatly in the intervening period, and, so in general, earlier references will be cited, the topics being dealt with here only in sufficient depth as to facilitate understanding of the remainder of the chapter. In other areas, notably those of human intake and the relationships of glucosinolates and their products to physiological disturbances in animals and man, the opportunity has been taken to critically assess recent findings.

II. DISTRIBUTION OF GLUCOSINOLATES

It is generally held that glucosinolates are limited to certain families of dicotyledonous angiosperms, predominantly within the order Capparales, *sensu* Cronquist or Taktajan, embracing the Capparaceae, Cruciferae, Moringaceae, Resedaceae,and Tovariaceae.[9] In the limited context of cultivated human foods and animal feedstuffs, it is members of the family Cruciferae which are most important, including oilseeds and forage crops, condiments, relishes, and vegetables[10] (Table 1). At the time of writing no authenticated member of the Cruciferae has been found to be devoid of gucosinolates or, when examined, the associated enzyme, myrosinase (see Section IV.A), and their presence has been suggested to be an important chemotaxonomic criterion for classification within this family.

Recently, claims for the presence of glucosinolates in such botanically diverse species as onion,[11] cocoa,[12] and mushroom[13] have been made, largely on the basis of the presence of trace amounts of known glucosinolate degradation products. Detailed reexaminations of the presence of glucosinolates in onion[14] and cocoa[15] have been conducted, and no evidence for their occurrence has been obtained. At this moment, then, there is little reason to modify the above boundaries of glucosinolate occurrence.

III. CHEMICAL ASPECTS OF GLUCOSINOLATES

A. Structure

The common skeletal structure for glucosinolates which is currently accepted is shown in Figure 1 (I). Based upon the work of Ettlinger and Lundeen,[16] the structure has been confirmed by X-ray studies[17] and direct synthesis.[18] The significant features of the structure are a sulfonated oxime grouping, which has been shown to be *anti* with respect to the side chain, R, and *syn* with respect to a thioglycosidic moiety. In almost all cases the sugar is (or, rather has been assumed to be) D-glucose, although a glucosinolate isolated from radish seed was found to possess a 6-sinapoyl-β-D-glucose group.[19] Danish workers have suggested that "bound" glucosinolates, containing sinapic, malic, and caffeic acids esterified to the sugar moiety, also occur naturally, although no evidence has yet been published to support these claims.[20]

Table 1
ECONOMICALLY IMPORTANT GLUCOSINOLATE-CONTAINING PLANTS

White mustard	*Sinapis alba*
Brown mustard	*Brassica juncea*
Abyssinian mustard	*B. carinata*
Horseradish	*Armoracia lapathifolia*
Wasabi	*Wasabi japonica*
Radish	*Raphanus sativus*
Cress	*Lepidium sativum*
Indian cress	*Tropaeolum majus*
Water cress	*Nasturtium officinalis*
	B. oleracea L.
Kohlrabi	var. *gongylodes*
Cabbage (red, white)	var. *capitata*
Cabbage (savoy)	var. *sabauda*
Brussels sprouts	var. *gemmifera*
Cauliflower	var. *botrytis* subvar. *cauliflora*
Sprouting broccoli, calabrese	var. *botrytis* subvar. *cymosa*
Kale	var. *acephela*
Pe-tsai	*B. pekinensis*
Pak-choi	*B. chinensis* var. *chinensis*
Turnip	*B. campestris* L. spp. *rapifera*
Turnip rape	*B. campestris* L. spp. *oleifera*
Swede, rutabaga	*B. napus* L. var. *napobrassica*
Rapeseed	*B. napus* L. var. *napus*

The glucosinolate side chain may comprise aliphatic (saturated and unsaturated), aromatic, or heteroaromatic groupings. Common substituents include hydroxyl groups (which may occasionally be glycosylated), terminal methylthio groups (and oxidized analogues), esters, and ketones.[1] As will be seen, the side chain determines the chemical nature of the products of enzyme hydrolysis and, thereby, their biological effects and potencies. A number of important glucosinolates in plants consumed by animals and man are listed in Table 2, together with the trivial nomenclature which, although discouraged, is still used.

B. Separation and Isolation of Pure Glucosinolates

The vast majority of the 100 different glucosinolates which are now known have not been isolated in the pure state. In recent years there has been a need for pure glucosinolates, initially as chromatographic standards and, thereafter, for assessing their antinutritional and toxicological properties. While methods for the synthesis of glucosinolates have been described, in general, isolation rather than synthesis has been employed. In some cases (for example, benzyl and 4-hydroxybenzyl glucosinolates in *Lepidium sativum* and *Sinapis alba* seed, respectively), botanical sources contain essentially only a single glucosinolate, but generally, complex mixtures occur, so that their separation and isolation/purification is a challenge.

In most cases, seed meal (defatted, ground seed) is the preferred source, but whether this or other plant material is to be used, extreme care must be taken to ensure that glucosinolates are not decomposed (enzymically or chemically) during extraction and isolation. Commonly, plant material is extracted with boiling aqueous alcohol, the glucosinolates being separated as a class by anionotropic alumina column chromatography. Separation of individual glucosinolates has been achieved by passage through Sephadex® G-10 or A-25,[21] but recently Peterka and Fenwick have found flash chromatography on reverse-phase bonded octadecyl silane to be effective.[22] Separation and isolation may also be possible using preparative

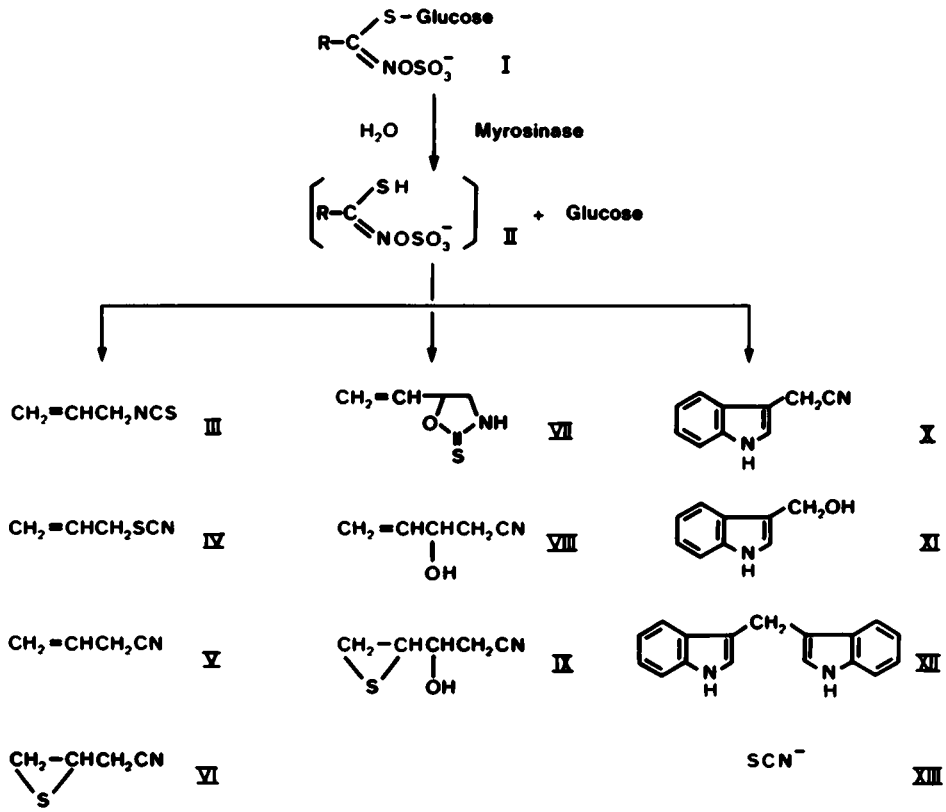

FIGURE 1. Enzymatic breakdown of glucosinolates. The products, which are characteristic of the three classes of glucosinolates referred to in the text, include prop-2-enyl isothiocyanate (III), prop-2-enyl thiocyanate (IV), 1-cyanoprop-2-ene (V), 1-cyano-2,3-epithiopropane (VI), 5-vinyloxazolidine-2-thione (VII), 1-cyano-2-hydroxybut-3-ene (VIII), 1-cyano-2-hydroxy-3,4-epithiobutane (IX), 3-indolylacetonitrile (X), indole-3-carbinol (XI), 3,3'-diindolylmethane (XII), and thiocyanate ion (XIII). (From Watson, D. H., *Natural Toxicants in Food, Progress and Prospects*, Ellis Horwood, Chichester, 1987, 78. With permission.)

reverse-phase high-performance liquid chromatography (HPLC) and volatile buffers, but details are not yet available.[23] It is considered that complete structural elucidation should now include both the side chain, R (Figure 1, I), and the sugar moiety. To assist in the direct structural elucidation, or confirmation of glucosinolate identity, detailed rationalization of mass spectrometry (MS) (EI, CI, and FAB) fragmentation processes[24] and nuclear magnetic resonance (NMR) spectra[25] have been published.

C. Chemical Stability

The effects of chemical treatments on the breakdown of a variety of glucosinolates are indicated in Table 3. In general, nitriles are produced although, as will be seen, glucosinolates possessing a β-hydroxy group yield atypical products such as thionamides and oxazolidinones. Oxazolidine-2-thiones, the important products of enzymic breakdown (see later), have not been identified. The effect of ferrous ion, illustrated in Table 3, is not produced by other ions, such as ferric, cobaltous, nickel, or stannous.[29] The result of conventional and microwave heating on prop-2-enyl glucosinolate in aqueous solution or model systems (soya flour) have been studied,[31] although the products of breakdown were not identified. Decomposition in the soya model system was a function of both the initial moisture level and the period of microwave heating. MacLeod[32] has attributed the adverse flavor of cooked cabbage and other brassicas to chemical, rather than enzymic, degradation products of glucosinolates, especially nitriles.

Table 2
TRIVIAL NOMENCLATURE AND STRUCTURES OF MAIN GLUCOSINOLATES OCCURRING IN EDIBLE PLANTS

Prop-2-enyl	Sinigrin
But-3-enyl	Gluconapin
Pent-4-enyl	Glucobrassicanapin
2-Hydroxybut-3-enyl	Progoitrin, *epi*-Progoitrin
2-Hydroxypent-4-enyl	Gluconapoleiferin
3-Methylthiopropyl	Glucoiberverin
4-Methylthiobutyl	Glucoerucin
5-Methylthiopentyl	Glucoberteroin
3-Methylsulfinylpropyl	Glucoiberin
4-Methylsulfinylbutyl	Glucoraphanin
4-Methylsulfinylbut-3-enyl	Glucoraphenin
3-Methylsulfonylpropyl	Glucocheirolin
4-Methylsulfonylbutyl	Glucoerysolin
Benzyl	Glucotropaeolin
2-Phenylethyl	Gluconasturtiin
4-Hydroxybenzyl	Glucosinalbin
3-Indolylmethyl	Glucobrassicin
1-Methoxy-3-indolylmethyl	Neoglucobrassicin
4-Methoxy-3-indolylmethyl	—
1-Hydroxy-3-indolylmethyl	—

Table 3
PRODUCTS OF CHEMICAL BREAKDOWN OF GLUCOSINOLATES

Glucosinolate	Conditions	Product(s)	Ref.
Prop-2-enyl	H_2O, 100°, 5 h	1-Cyanoprop-2-ene	26
	119°, 2 h	1-Cyanoprop-2-ene	26
	175°	1-Cyanoprop-2-ene	27
	Fe^{2+}, 100°, 15 min	1-Cyanoprop-2-ene; but-3-ene thioamide	28
Benzyl	200°	Benzyl nitrile; benzyl isothiocyanate	27
	119°, 2 h	Benzyl nitrile	26
	Fe^{2+}, H_2O	Benzyl nitrile	29
2-Phenylethyl	150°	2-Phenylethyl isothiocyanate; 1-cyano-2-phenylethane	27
(*R*) and (*S*)-2-hydroxybut-3-enyl	200°	1-Cyano-2-hydroxybut-3-ene	27
	H_2O, 100°, 5 h	1-Cyano-2-hydroxybut-3-ene	26
	119°, 2 h	1-Cyano-2-hydroxybut-3-ene	26
	Fe^{2+}	1-Cyano-2-hydroxybut-3-ene; 3-hydroxypent-4-enethioamide	29
	borate, pH 8—12	5-Vinyloxazolidinone	30
2-Hydroxy-2-phenylethyl	Fe^{2+}	1-Cyano-2-phenyl-2-hydroxy butane; 3-hydroxy-3-phenyl propanethionamide	29

IV. BIOCHEMICAL ASPECTS OF GLUCOSINOLATES

A. Glucosinolate-Degrading Enzymes

Disruption of plant tissue results in a loss of glucosinolates due to hydrolysis by the enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) present in all glucosinolate-containing plants. Although found in specialized "myrosin" cells, myrosinase activity appears not to be restricted to those cells.[33] Myrosin cells are differentiated early in leaf development and are, in effect, diluted with leaf expansion. Myrosinase occurs in a number of isoenzymic forms, but the number and activity of these isoenzymes seems not to be correlated with particular glucosinolates.[34] Myrosinase shows activity over a wide pH range[35] and is stable at temperatures up to 60°C, with an optimum activity at around 50°C. The reaction with the glucosinolate substrate releases a stoichiometric amount of glucose, and the resulting aglucone (II) undergoes a spontaneous Lossen rearrangement with elimination of bisulfate (Figure 1). The nature of the breakdown products is affected by a number of cofactors which are discussed below. Autolysis or hydrolysis due to endogenous enzyme can occur in a somewhat random manner, but the reaction, which is the basis for a number of quantitative analytical methods, can be controlled by adding myrosinase from an exogenous source (usually *S. alba*) to an inactivated extract. Glucosinolates are also susceptible to hydrolysis by sulfatase (notably that from the edible snail *Helix pomatia*), releasing sulfate and yielding the corresponding desulfoglucosinolate. This reaction is commonly used as a highly specific cleanup step in the analysis of glucosinolates.[24]

B. Products of Enzyme Hydrolysis

Myrosinase-induced hydrolysis of glucosinolates yields a wide variety of products, the exact nature of which is determined by a number of factors including pH, the presence of certain cofactors, and, most importantly, the structure of the parent glucosinolate.[1,6] Most glucosinolates can be conveniently divided into three groups according to the products of their hydrolysis (Figure 1). By far the largest group are those glucosinolates generally having either an alkyl or alkenyl side chain which on hydrolysis with myrosinase at pH 5 to 7 yields glucose and, following a loss of bisulfate, primarily isothiocyanates (III). Glucosinolates possessing a β-hydroxyl substituent form unstable hydroxyisothiocyanates which spontaneously cyclize to oxazolidinethiones (such as VII). The third group having an indole nucleus are hydrolyzed via an unstable isothiocyanate to the 3-carbinol (XI) and thiocyanate ion (XIII); the former can react further to yield diindolylmethane (XII). In more acid conditions of hydrolysis (pH 3), all three groups produce increasing amounts of nitriles (V, VIII, X). Significant amounts of such products are also produced during autolysis of cruciferous plant tissue, even when the pH would be expected to favor isothiocyanate formation. Recently, Uda et al.[36,37] have offered explanations for this effect. While ferrous ion markedly depressed myrosinase-induced isothiocyanate formation under acid conditions, it had little effect at pH 7.5. The authors suggested that the ferrous ion was acting on the aglycone rather than on the parent glucosinolate, since rate of release of glucose was unaffected by ion concentration. Further support was forthcoming from the finding that, in combination, ferrous ion and thiols inhibited isothiocyanate formation at neutral pH. Experiments using silver sinigrate as an aglucone model led the authors to propose a mechanism whereby ferrous ion and thiols together facilitate desulfuration of the aglucone (yielding nitriles) at the expense of the isothiocyanate-producing Lossen rearrangement. The involvement of thiols may also, at least in part, explain the reduction in nitrile production which is evident after heating and which has been attributed to destruction of a thermolabile "nitrile-forming factor."[6]

Ferrous ion is also a necessary cofactor, together with an inactive epithiospecifier protein[6,7,38] for the myrosinase-induced addition of sulfur across a terminal double bond (e.g., the formation of the episulfides VI or IX). Epithiospecifier protein does not occur in all cru-

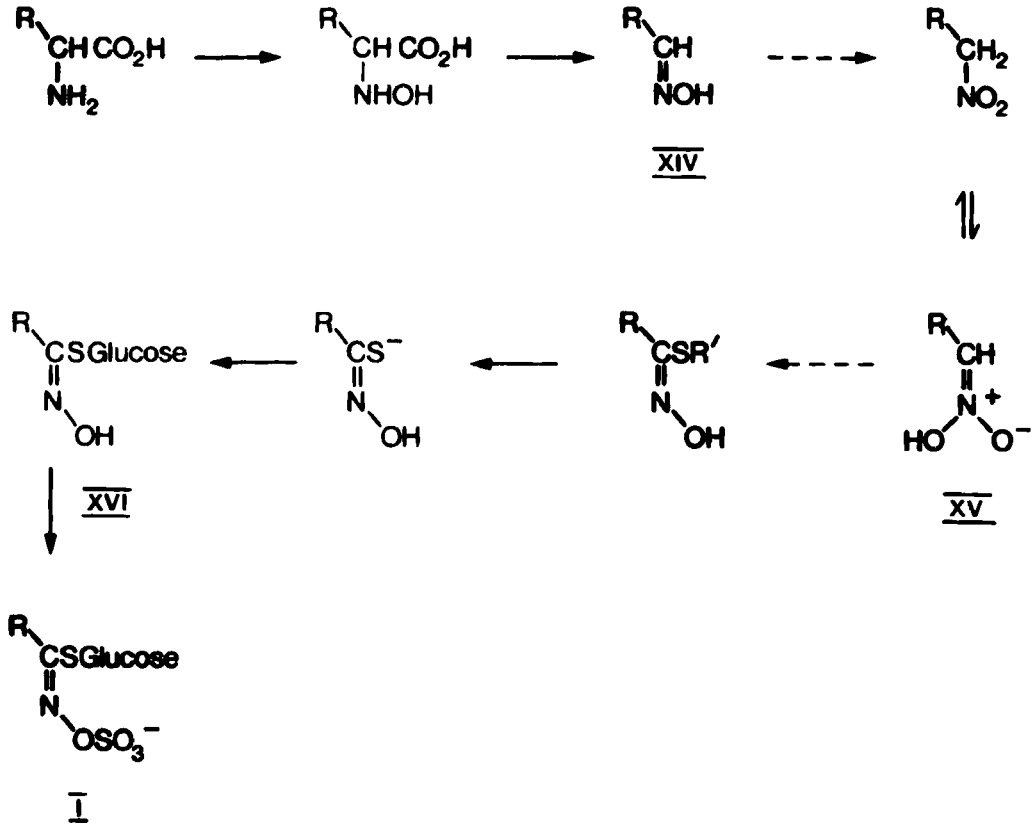

FIGURE 2. The biosynthesis of glucosinolates. (From Bell, E. A. and Charlwood, B. V., Eds., *Encyclopaedia of Plant Physiology*, Vol. 8, Springer-Verlag, Heidelberg, 1980, 503. With permission.)

ciferous plants, and recently MacLeod and Rossiter have speculated that it is absent from those species which do not contain glucosinolates possessing terminally-unsaturated side chains.[39] The presence of only a trace of such a glucosinolate, however, appears to be necessary for the presence of this cofactor.

Various mechanisms have been offered to explain the formation of episulfides.[38-41] Kinetic evidence suggests that epithiospecifier protein acts as a noncompetitive inhibitor of myrosinase[38] while the sulfur atom is transferred to the terminal site by a substantially intramolecular process.[41] In marked contrast, the factors responsible for the unusual formation of organic thiocyanates (IV), rather than isothiocyanates, in certain species are as yet little understood.

C. Biosynthesis

Many studies, conducted over a decade ago, have led to the view that most glucosinolates are derived from amino acids, with most lying on a common biosynthetic pathway, indole glucosinolates being apparent exceptions. A comprehensive review has been presented by Underhill and Wetter,[42] and more recent advances have been discussed by Kjaer[43] and Underhill.[44]

The currently accepted pathway (illustrated in Figure 2) involves *N*-hydroxylation and oxidative decarboxylation to yield an aldoxime (XIV) which is a common intermediate for the biosynthesis of glucosinolates and cyanogenetic glycosides. It is only recently, however, that in *Carica papaya* an example has been found of the co-occurrence of these classes of secondary metabolites. It has been suggested that for glucosinolate biosynthesis this aldoxime

undergoes oxidation to a nitro compound, the *aci*-tautomer of which (XV) may be envisaged as the site for introduction of the thioglycoside-*S*. In studies reported by Underhill and Wetter,[42] cysteine-*S* was most readily incorporated to produce a thiohydroxamic acid. UDP-Glucose-mediated *S*-glucosylation yields the penultimate desulfoglucosinolate (XVI), the final product being obtained following reaction with PAPS, 3'-phosphoadenosine-5'-phosphosulfate. While some glucosinolates, for example, those possessing methyl, isopropyl, or 4-hydroxybenzyl side chains, are clearly derived from corresponding protein amino acids (alanine, valine, and tyrosine), others require elaboration, as can be seen from Table 2, most commonly homologation, elimination or addition, and hydroxylation. There is ample evidence that such processes can occur at various points along the biosynthetic pathway. In a few cases both modified amino acids and glucosinolates containing corresponding side chains have been identified.[42] A large family of glucosinolates contain homologous series possessing a terminal methylthio grouping (or oxidized analogues, methylsulfinyl, methylsulfonyl). These are considered to be derived from methionine by acetate-type chain elongation. Terminal elimination of the elements of methylthiol affords another series of glucosinolates possessing a terminal ω-double bond. In view of their current importance in rapeseed breeding, it is surprising that more research has not been carried out on the biosynthesis of indole glucosinolates, their interconversion, and, most importantly, the point where their biosynthesis deviates from that of most other glucosinolates.

Not all of the common amino acids have side chains which are found in corresponding glucosinolates, but, as Kjaer has pointed out,[43] in view of the well-defined and ordered processes involved, the future identification of these "missing" compounds should occasion little surprise.

D. Metabolism

Metabolic studies on glucosinolates are relatively few. The breakdown of 2-hydroxybut-3-enyl glucosinolate to (in part) 5-vinyloxazolidine-2-thione has been reported in both man and the rat.[45] However, in retrospect both the purity of the chemicals and the analytical methods employed may be questioned. Recently various glucosinolates have been shown to be broken down when incubated with human fecal extract, although the products have not as yet been identified.[46] Little or no breakdown occurred under conditions (e.g., pH, digestive enzymes) simulating those of the stomach. The breakdown of 2-hydroxybut-3-enyl glucosinolate to 5-vinyloxazolidine-2-thione has been suggested to occur in rats[47] and poultry[48] on the basis of the known antithyroid and enzyme-inhibiting properties of the latter, but Smith and Campbell[49] observed greater amounts of nitriles in the crops of fowl fed rapeseed meal. Various studies have indicated that intact glucosinolates in the rat are probably broken down, but the nature of the products is generally unknown. Because of the nature of the resulting physiological effects the assumption is often made that these products are similar, if not identical to, those obtained following treatment with plant myrosinase.

Recently Macholz and co-workers[50] have found traces (<1%) of benzyl glucosinolate in urine and feces following the feeding of this glucosinolate to rats. Benzyl nitrile and benzyl isothiocyanate were found in the feces, the former alone in the thyroid, but no quantitative information was given in the preliminary description of this work. When 2-hydroxybut-3-enyl glucosinolate was fed to germ-free rats, larger amounts were excreted than was the case for conventional animals. Products "other than 5-vinyloxazolidine-2-thione" were observed, one of which may have been the 4-hydroxy metabolite reported earlier.[1] Independently, Nugon-Baudon and Szylit[51] have found that rapeseed meal is of equivalent nutritional value to soya meal only when fed to germ-free rats or chickens. Rat commensal microflora was responsible for the goitrogenic effect, hypertrophy of liver and kidneys, and growth retardation. In the fowl the commensal microflora showed a dramatic goitrogenic influence but had only a marginal effect on growth. When germ-free rats or chickens were

inoculated with the total fecal flora of the alien species, the goitrogenic effect was maintained, but the depressive influence of the rat microflora on growth was lost when implanted into chickens.

V. ANALYSIS

The analysis of glucosinolates has been a fruitful area of research for many years. Indeed, methods for the analysis of glucosinolates and their products have been described for over a century, and there have been few major analytical techniques which have not been applied to these compounds. There are many methods currently used for glucosinolate analysis, having been developed in response to the specific, and sometimes mutually exclusive, requirements of industry, researcher, and legislator. The development of methods for the analysis of glucosinolates and their products has been discussed by McGregor et al.[24] In this section it is only relevant to review those methods currently widely used, and, in this context, it is appropriate to consider the analysis of total and individual glucosinolates separately.

A. Total Glucosinolates

Measurement of the total glucosinolate content is particularly useful for plant breeders and legislators, when concerns about the nutritional value (and, hence, complement of individual glucosinolates) are not paramount. Most methods are based upon the measurement of a product obtained following the controlled enzymic hydrolysis with myrosinase. Usually glucose is measured, spectrophotometrically or by a chromatographic procedure. Interfering substances may be removed by ion exchange[52] or microcolumn methods.[53] The measurement of enzymically released sulfate has been proposed but has been little used. Recently, Møller et al.[54] have adapted a method, based upon the observed color reaction between glucosinolates and tetrachloro(IV)palladate, and have automated this for the screening of spring-grown rapeseed. Specific concerns over the availability of a reliable, rapid method suitable for the crushing industry have been addressed recently by Thies[55] and Smith and Dacombe.[56] There would be obvious advantages if a nondestructive method of analysis could be developed, and a number of groups have explored the potential of NIR, so far with little success.[57] A recent paper[58] has described the use of X-ray fluorescence, the time of analysis being less than 1 min. The cost of the equipment may be the major factor mitigating against the wider, industrial use of this method.

The specific requirements of the plant breeder — speed, inexpensiveness, simplicity, and the ability to analyze as little as half a cotyledon — have been addressed by glucose-release methods, where the level of glucose (and, hence, glucosinolates) is revealed by change of color on a test paper or stick.

The current international standard method[59] for glucosinolate content of rapeseed has a number of limitations. Two procedures are required: measuring isothiocyanates (by gas chromatography [GC]) and oxazolidine-2-thiones (spectrophotometrically). The "total" figure arrived at by summing these contributions underestimates the real total because indole glucosinolates (which may, however, be estimated independently via the thiocyanate ion formed upon myrosinase hydrolysis[24]) are not included. Moreover, this underestimate is more serious in low (improved) glucosinolate seed. A matter of considerable additional confusion to the layman is that isothiocyanate levels are specified in terms of prop-2-enyl (allyl) isothiocyanate. This compound is absent in rapeseed, and legislation exists in the U.K. and elsewhere prohibiting the use as animal feedstuffs of seed meals containing (precursors of) this substance. It seems likely that this method, which does give some indication about the individual glucosinolate composition, will shortly be replaced by a chromatographic method allowing separation and analysis of individual glucosinolates.

B. Individual Glucosinolates

Individual glucosinolates may be separated and quantified by GC or HPLC. Methods based upon the separation of desulfoglucosinolates, produced enzymatically, using temperature-programmed GC conditions are now in official use in Canada and Europe.[60] While certain indole glucosinolates can be separated and quantified by this method, problems with 4-hydroxy-3-indolylmethyl glucosinolate, which only recently has been found to be present in rapeseed, have meant that attention has been turned to HPLC. Methods based upon both intact glucosinolates and desulfoglucosinolates have been described,[24] with the latter having received more support.

Glucosinolate breakdown products may be determined by a variety of techniques including HPLC (oxazolidine-2-thiones, indoles), GC (nitriles, isothiocyanates), and spectrophotometry (thiocyanate ion).[24] While these methods have proved effective for analysis of plant material, there have been few applications to biological samples, although oxazolidine-2-thiones have been monitored in milk by HPLC[61] and in plasma and urine by capillary GC.[62]

VI. LEVELS OF GLUCOSINOLATES IN FOODS AND FEEDSTUFFS

While glucosinolates have been studied in a wide variety of plants, quantitative studies have been confined mainly to cruciferous plants having economic importance as human foods and animal feedstuffs. Brassicas are widely consumed in the human diet, fresh, cooked, or othrwise processed and constitute the major source of glucosinolates.[1,4] While 10 to 20 individual glucosinolates are usually present in brassica species, only a few predominate. There is a large variation in the absolute amounts of glucosinolates both in individual cultivars of the same species and between species (Table 4). Factors which affect total glucosinolate contents are discussed in the following section.

In general, it has been found that the same glucosinolates usually occur in a particular subspecies irrespective of genetic origin. There is, however, a marked variation between cultivars in the absolute amounts of glucosinolates. This is reflected in the wide ranges in the levels of individual glucosinolates in the species listed in Table 5. It should be emphasized that this table lists major glucosinolates, and for a consideration of the total complement the individual references should be examined. Investigations conducted on North American cultivars employed methods based upon analysis of hydrolysis products so that distinction between the individual indole glucosinolates was impossible (all contributing to the figure obtained by thiocyanate ion release).

The relative distribution of glucosinolates has been shown in the case of brussels sprouts to be characteristic of a particular variety affording a possible chemotaxonomic tool. Such cultivar specificity is not, however, exhibited by other important species such as oilseed rape which poses particular problems in defining distinctness for establishing breeders' rights.

A. Foods

There have been many studies of the nature and amount of glucosinolates in crops commonly consumed by man, and an indication of the average levels and range of these compounds in some of the more important crops is given in Table 5. It should, however, be noted that, in general, these studies used representative samples of material as marketed, and breeding lines may be significantly different. Although only a minor component of the Western diet, radishes are an important feature of the Japanese diet[62a] and are the only vegetable in which 4-methylsulfinylbut-3-enyl glucosinolate occurs.[72] Cruciferous seeds contain high amounts of glucosinolates, and, although only a small proportion of the Western diet, mustard (*S. alba* and *Brassica juncea*) contains large amounts of 4-hydroxybenzyl and but-3-enyl glucosinolates, respectively, which in the form of their enzymic breakdown products are responsible for the desirable pungency and flavor of these condiments.

Table 4
TOTAL GLUCOSINOLATE CONTENTS μg/g OF AGRICULTURALLY IMPORTANT PLANTS[a]

Species	Glucosinolate content		Ref.
	Range	Mean	
Cabbage			
White	260—1,060	530	63
	420—1,560		64
Red	410—1,090	760	63
Savoy	470—1,240	770	63
	1,210—2,960		64
Chinese cabbage	170—1,360	540	65
Brussels sprouts	600—3,900	2,000	66
	1,070—2,760	1,770[b]	67
Cauliflower	610—1,140		68
	100—180	161[b]	67
Calabrese	420—950	620	69
Swede (peeled)	200—1,090	550	70
	1,130—2,310		71
Turnip (peeled)	210—600	420	70
	970—2,270		71
Radish			
Oil	920—1,120	1,010	72
European	340—570	450	72
European-American			
Red	420—1,170	680	72
White	570—1,190	770	72
Black	1,240		72
Japanese daikon	660—2,530	1,390	72
Korean	704—1,650	1,090	72
Horseradish	33,200—35,400		73
Mustard			
White	22,000—52,000		74
Brown	<44,000		74
Black	18,000—45,000		74
	33,000—60,000		75
Rapeseed			
High glucosinolate	>42,000	(equivalent 100 μmol/g defatted meal)	
Intermediate glucosinolate	25,000	(60 μmol/g)	
Low glucosinolate	13,000	(30 μmol/g)	
Canola	20,000[c]		
Spring (Danish)	5,000—10,000	(10—20 μmol/g)	

[a] Additional comprehensive data on the glucosinolate content of U.S.-grown broccoli, brussels sprouts, cauliflower, collards, kale, mustard greens, kohlrabi, and turnips has recently been published.[76,77]
[b] Calculated from data on cooked vegetables.
[c] Specification 13,000 μg/g (30 μmol/g defatted meal) excluding indole glucosinolates.

B. Feedstuffs

Brassicas find widespread use in animal feedstuffs, both as forage crops, such as fodder rape, kale, cabbage, swedes, and turnips, and as oilseed crops such as rape or mustard which, after removal of the oil, provide a meal rich in protein. In general the glucosinolates of forage crops reflect those of vegetable crops grown for human consumption, and, until recently, problems of thyroid disorder in animals grazing such crops were addressed by

Table 5
CONTENT OF MAJOR INDIVIDUAL GLUCOSINOLATES (μg/g) IN AGRICULTURALLY IMPORTANT CROPS[a]

	A	B	C	D	E	F	G	H	I	J
White cabbage		35—590				46—270		0—144		
		74—414				23—890				
Red cabbage		6—102	16—53			22—143		150—390		
Savoy cabbage		0—160				70—420				
		125—646	19—64			334—1290				
Brussels sprouts		11—1560	30—500							
Cauliflower		10—627			0—295	6—419				
Calabrese						0—71		151—270		
Swede	0—240						37—380			4—330
Turnip	0—180		4—501	4—250			9—137			24—220
Radish, oil									594—768	
European									178—343	
European-American									292—1,070	
Japanese									498—2,230	
Korean									494—1,193	
Chinese cabbage			0—250	13—275						
Horseradish		27,000—29,000								
Mustard										
White										
Brown		320—8,000	33,000—69,000	0—320						
Black		18,000—45,000								
Rapeseed										
High			8,800—9,600	2,200—2,700						
Low			500—700	200—300						

	K	L	M	N	O	P	Q	R	S
White cabbage						51—510[b]			
						8—83			

Table 5 (continued)
CONTENT OF MAJOR INDIVIDUAL GLUCOSINOLATES (μg/g) IN AGRICULTURALLY IMPORTANT CROPS[a]

	K	L	M	N	O	P	Q	R	S
Red cabbage						155—330[b]			
Savoy cabbage						300—526[b]			
						24—126			
Brussels sprouts						40—990			
Cauliflower						0—101			
Calabrese		24—82				189—357	265—491		
Swede	0—110	220—670			5—470		70—570[b]		
Turnip		0—450	0—190		28—553	60—560[b]			
Radish, oil									
European									
European-American									
Japanese									
Korean									
Chinese cabbage	4—257	3—194			22—320			97—547[b]	
Horseradish					4,200—7,200				
Mustard									
White				22,000—52,000					
Brown									
Black									
Rapeseed									
(Danish Spring)									
High		26,000—28,000	1,300—1,600			<100			1,600—1,900
Low		1,200—1,700	<100			<200			1,200—1,600

[a] Data from References 63 to 75. Key to column heads as follows: A — 1-Methylpropyl glucosinolate; B — Prop-2-enyl glucosinolate; C — But-3-enyl glucosinolate; D — Pent-4-enyl glucosinolate; E — 3-Methylthiopropyl glucosinolate; F — 3-Methylsulfinylpropyl glucosinolate; G — 4-Methylthiobutyl glucosinolate; H — 4-Methylsulfinylbutyl glucosinolate; I — 4-Methylsulfinylbut-3-enyl glucosinolate; J — 5-Methylthiopentyl glucosinolate; K — 5-Methylsulfinylpentyl glucosinolate; L — 2-Hydroxybut-3-enyl glucosinolate; M — 2-Hydroxypent-4-enyl glucosinolate; N — 4-Hydroxybenzyl glucosinolate; O — 2-Phenylethyl glucosinolate; P — 3-Indolylmethyl glucosinolate; Q — 1-Methoxy-3-indolylmethyl glucosinolate; R — 4-Methoxy-3-indolylmethyl glucosinolate; S — 4-Hydroxy-3-indolylmethyl glucosinolate.

[b] Indole glucosinolates assayed as a group.

Table 6
MEAN DAILY INTAKES OF INTACT GLUCOSINOLATES

(mg/person/day)

	U.K.[83]	Canada[84]	U.S.[a]	Holland[67]
Cabbage	19.4 (14.0)[b]	3.4	8.5[c]	nd
Brussels sprout	17.2 (9.4)	1.5	0.6[d]	(6.5)
Cauliflower	6.4 (4.4)	1.2	2.7[e]	(1.2)
Broccoli		1.4	3.3[f]	nd
Swede/turnip	3.1 (1.6)	5.8		nd
Total	46.1 (29.4)	13.5	15.1	(7.7)

[a] Calculated.
[b] Figures in brackets refer to calculations based upon cooked vegetable data.
[c] Including 1.0 mg from canned sauerkraut.
[d] Including 0.5 mg from frozen brussels sprouts.
[e] Including 0.6 mg from frozen cauliflower.
[f] Including 1.4 mg from frozen broccoli.

assaying for thiocyanate ion content. Recent studies have shown, however, that rape and radicole, unlike kale, contain significant amounts of 2-hydroxybut-3-enyl glucosinolate, the precursor of the potent goitrogen 5-vinyloxazolidine-2-thione, and it was concluded that its level should be closely monitored in future breeding programs.[78,79] Glucosinolate levels in cruciferous seeds are generally much higher than in the corresponding vegetative material, and the high content of glucosinolates in rapeseed is one of the main factors limiting its use as a protein supplement in animal feeds,[3] as is described in Section VIII. However, intensive breeding programs have produced much improved rapeseed varieties having much lower levels of glucosinolates.[75] The glucosinolate content of high and low rapeseed cultivars is shown in Table 5.

C. Food Chain

Glucosinolates, or more accurately their breakdown products, can pass indirectly into the human diet in foods derived from animals feeding on cruciferous material. Little is known about the metabolic fate of glucosinolates in the digestive tract, but it has been suggested that in Finland endemic goiter may be related to goitrogens in milk.[80] VanEtten et al.[81] have conducted a detailed study into the possible occurrence of glucosinolate hydrolysis products in the organs and meat of cattle fed rations containing crambe seed meals, but no such products could be detected. Low levels of 1-cyano-2-hydroxybut-3-ene (1 to 3 mg l^{-1}) have been detected[82] in milk following the feeding of rapeseed meal to dairy cattle; given the toxicological properties of this compound further studies using more modern analytical techniques would appear justified. Recently, 5-vinyloxazolidine-2-thione levels of up to 1 mg l^{-1} have been found in milk following the feeding of rapeseed meal to cattle,[61] and under certain circumstances the levels may be even higher.

D. Glucosinolate Intake

Information on glucosinolate intake is shown in Table 6. The most comprehensive study has been conducted in the U.K. where a mean daily intake of 46 mg has been calculated.[83] This is certainly an underestimate since the calculation takes no account of processed or home-grown vegetables. Seasonal dietary trends and, in particular, the consumption of large amounts of fresh glucosinolate-rich brussels sprouts result in a much higher-mean glucosi-

nolate intake in the winter months (58.8 mg vs. 32.9 mg in the summer). When this information, together with social and agronomic factors, is taken into account, it is calculated that 5% of the U.K. population (95th percentile) consume >300 mg daily. It is pertinent to contemplate whether consumers and legislators would be so sanguine about the daily consumption of 300, 30, or even 3 mg of a synthetic chemical ("additive") for which equally little biological information was available.

In 1978, Mullin and Sahasrabudhe[84] reported a mean daily intake of 7.9 mg glucosinolates on the basis of Canadian dietary data. The analytical techniques available at that time mean that this is an underestimate. Using data obtained by various workers on North American- and (where the former is unavailable)* U.K.-grown vegetables, the present authors have calculated a mean daily intake of 13.5 mg glucosinolates. When the same procedure is applied to American dietary tables, the mean daily intake is calculated to be 15.1 mg. Recently Gramberg and colleagues[67] have reported mean daily glucosinolate intakes from brussels sprouts and cauliflower in the Netherlands to be 6.5 and 1.2 mg, respectively. These values are calculated on the basis of cooked vegetables, and, given earlier findings, the combined intake (based upon fresh produce) would likely be rather higher, perhaps 12 to 14 mg.

From these comparisons it may be seen that the U.K. has the highest intake of glucosinolates, thus justifying the continuing programs of research on dietary glucosinolates in that country. However, populations in certain Oriental countries consume much higher amounts of crucifers and so, at least potentially, are exposed to much higher levels of glucosinolates. In Japan,[85] for example, the average daily consumption (1975 figures) of radish, cabbage, and Chinese cabbage is 75.5 g and of fermented root and leaf vegetables (some of which will be crucifers) 37.2 g. In contrast, the total daily crucifer intake in the U.K., from which the figures shown in Table 6 were obtained is 45.2 g. Information on the glucosinolate content of Japanese vegetables is, unfortunately, limited, but recently Carlson et al.[72] have examined various types of radish, including Japanese daikon (Table 4). The mean daily glucosinolate intake in Japan *due to daikon alone* may thus be calculated as 46 mg. Differences in food preparation practices may reduce the actual intake of glucosinolates significantly (although a proportion of this "loss" may be revealed as an increase in the intake of glucosinolate breakdown products).[1] Thus, boiling has been shown to reduce glucosinolate levels in brassica vegetables such that the U.K. mean daily intake, calculated on a cooked-vegetable basis, is reduced to 29.4 mg.[83] Intact glucosinolates are readily broken down under fermentation conditions.[86]

VII. FACTORS AFFECTING GLUCOSINOLATE LEVELS

Although it has been shown that the relative amount of glucosinolates in a particular subspecies is comparatively stable, absolute levels are subject to a wide variety of influences. These have been reviewed in detail by Fenwick et al.[1] and consequently will be discussed only briefly here.

A. Genetic and Botanical

Glucosinolate levels in cruciferous seeds have been manipulated to advantage by plant breeders. Mustard quality has been enhanced by increasing the levels of glucosinolates, whereas breeding from selected lines of oilseed rape has reduced the glucosinolate levels in the seed to around 10% of those in high-glucosinolate varieties. In rapeseed, only alkenyl glucosinolates are reduced, and indole glucosinolates, which remain unaffected, assume greater significance.[87] In cruciferous vegetables, the flavor is due to certain glucosinolates,

See comment at foot of Table 4.

and manipulation of the relative amounts in order to reduce levels of undesirable precursors such as 2-hydroxybut-3-enyl glucosinolate may be a more desirable goal. In this context the finding that F_1 hybrid brussels sprouts have a glucosinolate content close to the average of both parent lines may be significant.[88] Differences have been noted between vegetables grown in North America and the U.K. Both individual and total glucosinolates differ, suggesting both genetic and agronomic factors are involved.[64]

Absolute and relative amounts of glucosinolates are determined by the part of the plant examined, with leaves, stems, and roots showing wide differences.[1] Apical leaves of brussels sprout plants have been found to contain up to fourfold more 3-indolylmethyl glucosinolate than mature leaves, and the glucosinolate content of fully expanded leaves decreases rapidly with the onset of senescence. VanEtten et al.[89] have examined the variation of individual glucosinolates within the pith, leaves, and cambial cortex of red, white, and savoy cabbage. Glucosinolates occurred in relatively low abundance in the older, outer leaves; within the cabbage head proper, the highest concentration was found in the cambial cortex, being approximately double that in the pith and leaves. When seeds of rape were germinated, isothiocyanates were decreased, and nitriles increased in comparison to the behavior of the autolyzed seed.[90] This is consistent with changes in the microenvironment (pH, ion concentration) during germination. Highest glucosinolate concentrations have been found in seeds from the top siliques of the lowest branch of *B. campestris*,[91] and, overall, higher levels were found in siliques from upper, rather than basal, portions of the branch. Such variability has obvious implications for the screening of plant-breeding programs.

B. Agronomic

The use of sulfur-based fertilizers has been shown to increase glucosinolate levels, and it has been suggested that nitrate application results in a lowering of values. Stress factors such as drought and close plant spacing are also known to increase levels.[1] The effect of sowing time on glucosinolate content of harvested rapeseed has recently been emphasized.[92] Harvesting techniques have been suggested to be responsible for the observed instability of glucosinolates in some low-glucosinolate cultivars of oilseed rape,[93] with harvested seed sometimes containing twice the glucosinolate content of sown seed. This problem may have important implications in the context of any legislation governing the levels of glucosinolates in internationally traded seed.

C. Processing

Although some cruciferous crops for human consumption are eaten fresh, most are processed in some way. Such processes include cutting or chopping, cooking and blanching, freezing, pickling, and fermenting. Any process which causes disruption of cellular integrity results in some hydrolysis occurring with a loss of glucosinolates. Although blanching and cooking inactivate the myrosinase, some loss of glucosinolates occurs through leaching into the cooking water, and it has been shown that boiling of brassica vegetables in the normal manner reduces glucosinolates by up to 50%.[83] Enzymic reactions continue during storage; thus, a loss of glucosinolates in sauerkraut is accompanied by the formation of nitriles.[94] The dehydration of *Wasabia japonica* by air drying or freeze drying has been found by Kojima et al.[95] to be associated with only minor losses of glucosinolates.

The processing of rapeseed includes steps which inactivate the myrosinase, but further processing of the rapeseed meal to reduce glucosinolate content (a goal which does not necessarily equate with detoxification) has been superceded to some extent by the success of plant breeding. However, processing remains an option to achieve greater utilization of this valuable protein source.[96]