The EFSA Journal (2008) 590, 1-76

Glucosinolates as undesirable substances in animal feed[1]

Scientific Panel on Contaminants in the Food Chain

(Question N° EFSA-Q-2003-061)

Adopted on 27 November 2007

PANEL MEMBERS

Jan Alexander, Guðjón Atli Auðunsson, Diane Benford, Andrew Cockburn, Jean-Pierre Cravedi, Eugenia Dogliotti, Alessandro Di Domenico, Maria Luisa Férnandez-Cruz, Peter Fürst, Johanna Fink-Gremmels, Corrado Lodovico Galli, Philippe Grandjean, Jadwiga Gzyl, Gerhard Heinemeyer, Niklas Johansson, Antonio Mutti, Josef Schlatter, Rolaf van Leeuwen, Carlos Van Peteghem and Philippe Verger.

SUMMARY

Glucosinolates (alkyl aldoxime-O-sulphate esters with a β-D-thioglucopyranoside group) occur in important oil- and protein-rich agricultural crops, including among others *Brassica napus* (rapeseed of Canola), *B. campestris* (turnip rape) and *Sinapis alba* (white mustard), all belonging to the plant family of *Brassicaceae*. They are present in all parts of these plants, with the highest concentrations often found in seeds. Several of these *Brassica* species are important feed ingredients and some species are also commonly used in human nutrition such as cauliflower, cabbages, broccoli and Brussels sprouts. Glucosinolates and their breakdown products determine the typical flavour and (bitter) taste of these vegetables.

[1]For citation purposes: Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission on glucosinolates as undesirable substances in animal feed, *The EFSA Journal* (2008) 590, 1-76

The individual glucosinolates vary in structure and the configuration of their side chain. They are hydrophilic and rather stable and remain in the press cake of oilseeds when these are processed and de-oiled. However, glucosinolate producing plants as well as some microorganisms contain specific β-thioglucosidases (denoted as myrosinases). In the intact plant these enzymes are separated from the glucosinolates and sequestered in aqueous vacuoles. Upon plant damage (including chewing during ingestion) the myrosinases are released, and initiate in the presence of water the conversion of glucosinolates into diverse breakdown products, including isothiocyanates, oxazolidinethiones (5-vinyl-2-oxazolidinethione and 5-vinyl-1,3 oxazolidine-2-thione), thiocyanates, nitriles, epithionitriles and other indol-3-ylmethyl derivatives. The biological effects of plant glucosinolates in mammalian species are predominantly related to these glucosinolate-derived compounds. They interfere with iodine uptake (thiocyanate ion) and the synthesis of thyroid hormones triiodothyronine (T_3) and plasma thyroxine (T_4) (5-vinyloxazolidine-2 thione), leading eventually to hypothyroidism and enlargement of the thyroid gland (goitre). As a consequence of these changes in thyroid function, clinical signs of toxicity described in farm animals include growth retardation, reduction in performance (milk and egg production), impaired reproductive activity, and impairment of liver and kidney functions, the latter are likely being associated with the formation of nitriles. Data on the toxicity of individual glucosinolates for food-producing animal species are very limited, and in most cases only the total glucosinolate content in a given feed material, measured indirectly though the quantification of hydrolysable glucose, is available. Only for rapeseed meal or press cakes comprehensive feeding trials in farm animals have been conducted, resulting in the recommendation to restrict the total glucosinolate content to 1 – 1.5 mmol per kg feed for monogastric animals, and to even lower concentrations in feeds for young animals.

In recognition of potential adverse effects exerted at high concentrations of glucosinolates, selection of plant varieties with low glucosinolate content (in addition to a low content of erucic acid in the oil) commenced more than three decades ago, resulting in the use of varieties, particularly rapeseeds, with a low glucosinolate content[2]. The common practices of selecting low-glucosinolate plant varieties as forage plants and processing crops with a potential high glucosinolate concentration prior to use, together with experience-based recommendations for maximal inclusion rates into animal diets given in textbooks, have proven to be effective measures to avoid intoxications and production losses in farm animals, and the undesirable fishy taint in animal-derived products. However, it is recommended that the available advanced analytical techniques should be applied to quantify the major glucosinolates in these forage plants

[2] Rapeseed with less than 1 % erucic acid in the oil is denoted single low varieties. When the glucosinolate level in addition were reduced to less than 20 mmol/kg seed dry matter they were denoted as double low varieties and Canola when the glucosinolate content was below 30 mmol/kg defatted meal.

with the aim to more accurately define animal exposure. This applies particularly to new or re-emerging oilseed crops, such as *Camelina sativa*, that may contain long-side chain glucosinolates, which are not detected in other common *Brassica* species.

Following exposure of farm animals to forages and concentrates containing glucosinolates, a carry over of glucosinolates and their associated breakdown products into edible tissues, milk and eggs has been described, but the rate of carry-over is very low. The measurable residues in dairy milk corresponding to approximately 0.1 % of the given glucosinolate dose, the residues in muscle tissues and organs were even lower. In certain breeds of laying hens, excretion of glucosinolate-derived compounds may convey an undesirable fishy taint to the eggs. However, all measured concentrations in animal-derived products are much lower than those found in vegetables for human consumption, and are unlikely to induce adverse health effects in consumers.

KEYWORDS: Glucosinolates, *Brassicaceae*, feed, isothiocyanates, glucosinolate-derived compounds, 5-vinyloxazolidine-2-thione, *Camelina sativa*, carry-over, animal health, human health.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE REQUESTOR

1. General background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed[3] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[4].

The main modifications can be summarised as follows

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction of the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination ("early warning system") and to take measures to reduce or eliminate the contamination ("pro-active approach").

In particular the introduction of the principle of non-dilution is an important and far- reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public and animal health protection. The deletion of the possibility of dilution is a powerful means to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied by the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)[5].

[3] OJ L140, 30.5.2002, p. 10
[4] OJ L 115, 4.5.1999, p. 32
[5] Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003[6] provides a comprehensive overview of the possible risks for animal and public health as the consequence of the presence of undesirable substances in animal feed.

It was nevertheless acknowledged by SCAN itself and by the Standing Committee on the Food Chain and Animal Health that for several undesirable substances additional detailed risk assessments are necessary to enable a complete review of the provisions in the Annex.

2. Specific background

Glucosinolates are a well-defined group of plant produced allelochemicals exclusively found in dicotyledoneae, where they are limited in occurrence to the plant order *Capparales* and only some few of other higher plants. It is a group of compounds that call for attention owing to the agricultural and horticultural important crops belonging to the family *Brassicaceae*, and for subtropical and tropical areas also for plants belonging to other families of the order *Capparales* as defined in a broad sense. At present, more than 140 different glucosinolates have been identified, and the interest devoted to this group of natural products is caused by the appreciable biological effects of both the intact glucosinolates and especially the complex group of glucosinolate transformation products produced in non-enzymatic and enzymatic reactions. Depending on the concentration and structural types of these compounds, their biological effects can be toxic, anti-nutritional or beneficial to health.

Volatile mustard oils including allyl isothiocyanate and goitrin (5-vinyloxazolidine-2-thione or 5-VOT) are listed as undesirable substances in Annex of Directive 2002/32/EC. In this annex *Camelina sativa* is listed as a botanical impurity.

However, there is a renewed interest in *Camelina* and some other cruciferous species as oilseed crop because of an increasing demand for alternative low-input oilseed crops with the potential for use for food and non-food purposes of the seed oil. SCAN[7] indicated that it is difficult to see why the *Camelina* species was included in the Annex to Directive 2002/32/EC. The possibilities for use of the *Camelina* seed meal after extraction of the oil for animal feeding require a consideration and evaluation of the potential effects, glucosinolates present in *Camelina* may have. It was indicated by SCAN that it is unlikely that either the seeds of this plant or any other plant parts would have adverse effects on livestock when present at the low levels associated with

[6] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)
[7] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 9.2.5. *Camelina sativa*

contamination, but this does not cover potential problems associated with use of *Camelina* as an alternative oilseed crop.

Given the growing interest in *Camelina sativa* as an alternative oilseed crop for the production of oil and the possible use of by-products in animal feed, a deletion of *Camelina sativa* from the list of undesirable substances should be urgently considered after the availability of the scientific opinion.

SCAN concluded[8] that risk assessments could be made for some of the compounds presumed responsible for their toxicity such as isothiocyanates.

The ease of microscopic detection of botanical contaminants is inversely related to the degree of processing, particularly grinding, of feedingstuffs. However because of its flexibility and capacity, microscopy should remain as one of the methods needed for the detection of botanical contaminations. Therefore SCAN recommended that it would be advantageous if the physical detection of the presence of a potentially toxic contaminant could be supported or even better replaced by a quantitative chemical analysis of the specific substance responsible for the toxicity and maximum levels set accordingly based on a risk assessment of the toxic compound.

Thus for mustard seeds (to include the sum of *Brassica juncea*, *Brassica nigra* and *Brassica carinata*) their presence could be considered to be tolerated provided that the limit set for allyl isothiocyanates from whatever source, is not exceeded. SCAN was of the opinion that 5-vinyloxazolidine-2-thione (5-VOT) is not relevant to the control of contamination and should therefore be excluded from the list of undesirable substances.

Isothiocyanates, 5-VOT and various types of other glucosinolate-derived products are toxicants at different concentrations. They are produced from the parent compounds by non-enzymatic and by enzymatic action when biological material is disrupted in the presence of moisture. It is therefore important that methods of analyses are used ensuring reliable determinations of all of the toxic compounds and their concentrations.

[8] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 9.5. Conclusion and point 9.6 Recommendations.

TERMS OF REFERENCE AS PROVIDED BY THE REQUESTOR

The European Commission requests the EFSA to provide a scientific opinion on the presence of glucosinolates in animal feed.

This scientific opinion should

- determine the most appropriate method to ensure full conversion from the glucosinolates to (allyl) isothiocyanates to reflect the maximum possible concentration of (allyl) isothiocyanates
- assess whether 5-vinyloxazolidine-2-thione is relevant to the control of contamination
- determine the toxic exposure levels of glucosinolates/(allyl)isothiocyanates for the different animal species of relevance (difference in sensitivity between animal species) above which
  - signs of toxicity can be observed (impact on animal health)
  - the level of transfer/carry over of glucosinolates/(allyl)isothiocyanates from the feed to the products of animal origin results in unacceptable levels of these undesirable substances or possibly their toxic metabolites in the products of animal origin in view of providing a high level of public health protection.
- determine the importance of the botanical impurities *Brassica juncea* (Indian, Chinese, Sareptian or Brown mustard), *Brassica nigra* (Black mustard) *and Brassica carinata* (Ethiopian mustard), which are currently listed in the Annex to Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed, as sources of the presence of glucosinolates/(allyl)isothiocyanates in animal feed
- determine in particular the importance of the botanical impurity *Camelina sativa*, which is currently listed in the Annex to Directive 2002/32/EC, as potential source of the presence of glucosinolates/(allyl)isothiocyanates in animal feed.
- identify other botanical impurities which could possibly contribute significantly to the presence of glucosinolates/(allyl)isothiocyanates in animal feed.
- identify feed materials which could be considered as sources of contamination by glucosinolates/(allyl)isothiocyanates and the characterisation, insofar as is possible, of the distribution of levels of contamination.
- assess the contribution of the different identified feed materials as sources of contamination by glucosinolates/(allyl)isothiocyanates
  - to the overall exposure of the different relevant animal species to glucosinolates/(allyl)isothiocyanates,

- to the impact on animal health,
- insofar as relevant, to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and carry over rates.

- identify possible gaps in the available data which need to be filled in order to complete the evaluation.

ACKNOWLEDGEMENT

The Scientific Panel on Contaminants in the Food Chain wishes to thank Christer Andersson, Leon Brimer, Bruce Cottrill, Johanna Fink-Gremmels, Jerzy Jaroszewski and Hilmer Soerensen for the preparation of the draft opinion.

ASSESSMENT

1. Introduction

Glucosinolates are secondary metabolites of plant species belonging to the order *Brassicales* (AGPII system, previously denoted Capparales), including among others the family *Brassicaceae*, the so-called mustard and cabbage family (Frohne and Pfander, 2005; Kliebenstein *et al.,* 2005; Tian *et al.*, 2005). Many common vegetables such as broccoli, Brussels sprouts, cabbage and cauliflower, which are traditionally important constituents of the human diet, belong to this plant family. At the same time the genera *Brassica*, *Crambe*, *Sinapis* and *Raphanus* include important oil- and protein-rich agricultural crops used for the production of plant oils, such as rapeseed oils. The press cake is used as animal feed material. The increased demand for plant oils for technical purposes has recently raised interest in *Camelina sativa* (false flax) and its possible use as an alternative oilseed crop and as a source of materials for animal feed (Matthäus and Zubr, 2000).

Glucosinolates represent a diverse class of alkyl aldoxime-O-sulphate esters with a β-D-thioglucopyranoside group attached to the hydroximine carbon in Z-configuration to the sulphate group. They can be synthesised from seven amino acids including alanine, (iso) leucine, tyrosine, tryptophan, valine, phenylalanine, methionine and chain elongated homologues of methionine. According to differences in their structure, more than 120 individual compounds have been characterized, and it is likely that this number will increase when more plant varieties have been

analysed. According to their structure, glucosinolates have been classified as aliphatic, aromatic, ω-methylthioalkyl and heterocyclic (indole-) glucosinolates (Fahey *et al.,* 2001).

Glucosinolates and their miscellaneous breakdown products are generally known as mustard oil glucosides or thioglucosides. They confer the distinctive flavour and bitter taste to among others *Brassica nigra* (black mustard) and *Brassica oleracea* (cauliflower, broccoli, cabbage, Brussels spouts). The intensity flavour and taste depends on the glucosinolate concentration of an individual plant and the degree of hydrolysis releasing an array of breakdown products. In oilseed crops (*Brassica napus* (rapeseed of Canola), *B. campestris* (turnip rape) and *Sinapis alba* (white mustard)) the hydrophilic glucosinolates remain in the seed meal after extraction of the oil and hence are present in the seed meal fraction used in animal diets.

For the producing plants, glucosinolates are part of an innate defence system (for reviews see Rask *et al.,* 2000, Kliebenstein *et al.,* 2005). They convey different signals to herbivorous insects in attracting parasitic wasps and favouring or opposing oviposition by insects. The defence system involves also thioglucosidases commonly known as myrosinases. In the intact plant, glucosinolates and myrosinases are sequestered in different compartments. Upon plant damage (for example by insects, or by chewing during ingestion of plant material), an enzymatic reaction takes place catalysed by myrosinases, and resulting in the formation of isothiocyanates, thiocyanates, oxazolidinethiones (including 5-vinyl-2-oxozolidinethione (VOT) and 5-vinyl-1,3-oxyzolodine-2-thione (5-VOT) as well as nitriles and epithionitriles.

The toxicity of glucosinolates for humans and farm animals has been associated particularly with the formation of thiocyanates, oxazolidinethiones and nitriles. These compounds interfere with iodine uptake (thiocyanates) and the synthesis of the thyroid hormones T_3 and T_4, (oxazolidinethiones) leading eventually to hypothyroidism and enlargement of the thyroid gland (goitre) (for review see Griffiths *et al.*, 1998, Halkier and Gershenzon, 2006). As a consequence of these changes on thyroid function, the metabolism in almost all tissues, including the reproductive organs is affected. Subsequently, a reduction in the fertility of male and female animals is observed. Moreover, various products of glucosinolate hydrolysis cause irritation of the gastro-intestinal mucosa followed by local necroses, and hepatotoxicity (Mawson *et al.*, 1994a,b; Mithen *et al*., 2000; Burel *et al*., 2001; Conaway *et al*., 2002). Subsequently, the major clinical signs of toxicity described in farm animals include growth retardation, reduction in performance (milk and egg production), impaired reproductive activity, and impairment of liver and kidney functions, the latter being attributed to the formed nitriles (Mawson *et al.*,1994a).

Early experimental data had indicated that isothiocyanates at certain dose levels are mutagenic and weakly genotoxic in various assays through the generation of cellular oxidative stress. The evidence of carcinogenicity in experimental animals is equivocal. More recently, interest has focused on the anti-carcinogenic properties of isothiocyanates (as present in *Brassica* vegetables).

These anticarcinogenic effects have been attributed to an inhibition of cytochrome P450 enzymes, and a concomitant induction of phase II detoxifying enzymes, thereby preventing the activation of pro-carcinogens and improving their conjugation and elimination (Holst and Williamson, 2004). These anticarcinogenic effects have been a subject of numerous publications. The relevant mechanistic and epidemiological data have recently been summarised by Hidgon *et al.* (2007).

2. Chemistry of glucosinolates

The structural features common to glucosinolates are the β-D-thioglucopyranoside group attached to C-O as cis or Z- in the N-hydroximine sulphate ester group and the side chain (R-group) attached at C-O (Figure 1). This gives the total functional group known as the glucosinolate group, with semi-systematic names of individual glucosinolates based on the name for the R- and R'-groups used as a prefix to the word -glucosinolate. The glucosinolate structure was originally confirmed by X-ray investigations on synthesised sinigrin (Ettlinger and Lundeen, 1956, 1957; Marsh and Waser, 1970). This structure is found to be a general feature of all glucosinolates investigated by ^{1}H- and ^{13}C-NMR spectroscopy (Olsen and Sørensen, 1981; Sørensen, 1990; Prestera *et al.*, 1996; Karcher and El Rassi, 1999; Matthäus and Luftmann, 2000; Buskov *et al.*, 2000a,b; Bellostas *et al.*, 2007).

Structural differences between individual glucosinolates are due either to substituents on the thioglucoside group (R'- ester groups, Figure 1) or to structurally different side chains (R – groups) (for a review see Fahey *et al.,* 2001, providing the chemical configuration of 120 common glucosinolates). The side chains determine 4 major classes, including allylglucosinolates, benzyl glucosinolates, 2-hydroxy-3-buteny glucosinolates and 4-methylsulfinylgutyl glucosinolates (Halkier and Gershenzon, 2006). In addition, long-side chain glucosinolates have been described to occur in *Camelina sativa* as discussed below. Owing to the strongly acidic sulphate group, glucosinolates occur as salts. This fact, together with the presence of the glucoside group, gives the glucosinolates their hydrophilic properties and thus, polar solvents such as water are needed to dissolve these compounds. Ester groups, as substituents on the thioglucoside part of glucosinolates, were originally described for sinapoyl- and isoferuloyl derivatives (Lincheid *et al.,* 1980; Bjerg and Sørensen, 1987a; Sørensen, 1990). These glucosinolate derivatives are relatively unstable in alkaline solutions, and they are not substrates for the enzymes myrosinases [EC 3.2.1.147] and sulfatases [EC 3.1.6.1]. Chiral centres in glucosinolate side chains are introduced by oxidation/hydroxylation resulting in enantiomers with different biochemical properties (Bjerg *et al.,* 1989; Palmieri *et al.,* 1998).

A distinct group of glucosinolates have recently been described, containing long aliphatic side chains. For example, *Camelina sativa* seeds contain significant amounts of (R)-9-methylsulfinylnonylglucosinolate and (R)-10-methylsulfinyldecylglucosinolate.

Upon plant damage or ingestion, glucosinolates are hydrolysed by myrosinases (thioglucoside glycohydrolase or β-thioglucosidases) and converted into a number of breakdown products. The first step is the hydrolysis of d-glucose catalysed by the plant's myrosinases (EC 3.2.1.147), resulting in an unstable thiohydroximate-O-sulfonate (Figure 1). At neutral pH, the sulphate is released, and by Lossen rearrangement the isothiocyanate (R-N=C=S) is formed. 2-hydroxy-glucosinolates form unstable isothiocyanates, which cyclize to oxazolidine-2-thiones (Figure 2). At an acidic pH or in the presence of Fe^{2+} ions, the formation of nitriles is favoured, whereas epithiospecific protein (ESP) promotes the formation of epithionitriles. The hydrolysis of indole glucosinolates is different, and the formed isothiocyanates are converted to further metabolites including indole-methanols, ascorbic acid conjugates and oligometric mixtures (not shown) (for more details see Rask *et al.*, 2000, Halkier and Gershenzon, 2006).

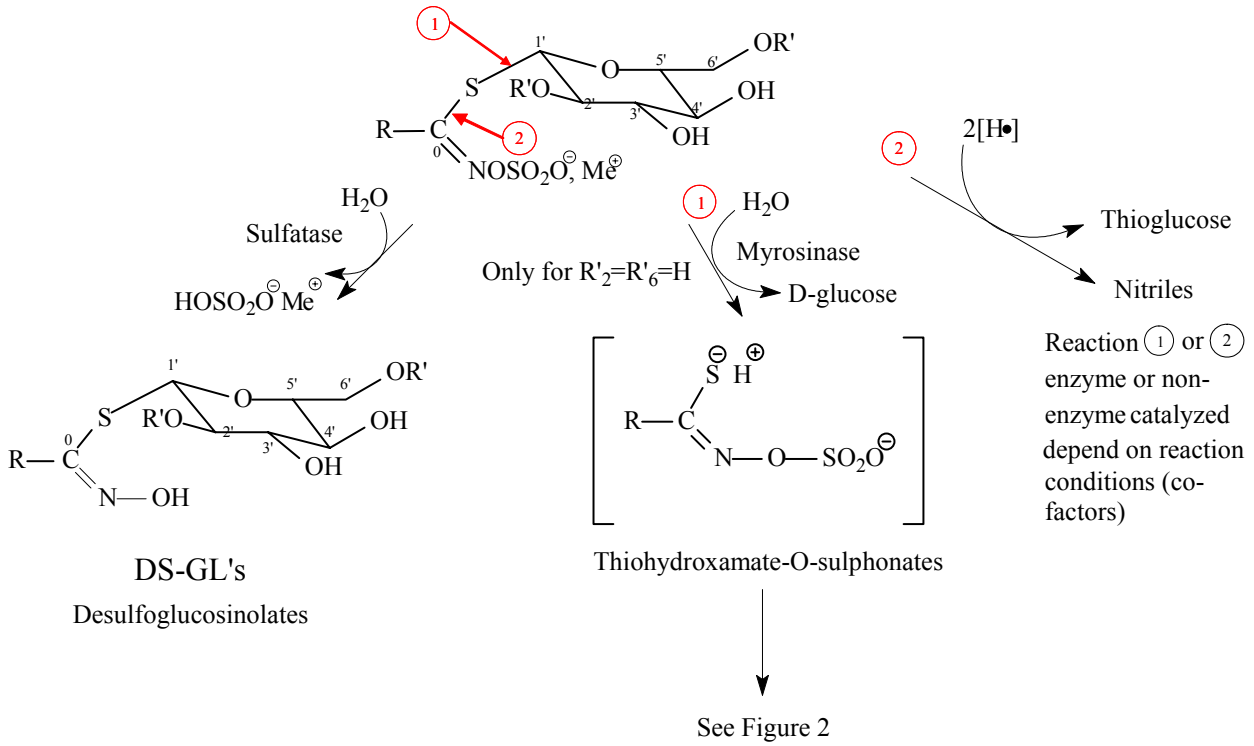


Figure 1. Glucosinolates with R'_2=R'_6=H are transformed into desulphoglucosinolates by catalysis with sulphatase and into thiohydroxamates by myrosinase catalysed hydrolysis (reaction 1). Reaction 2 resulting in nitriles can occur both as an enzyme catalysed and a non-enzymatic process catalysed by e.g. ferro ion, and in all cases with use of two redox equivalents.

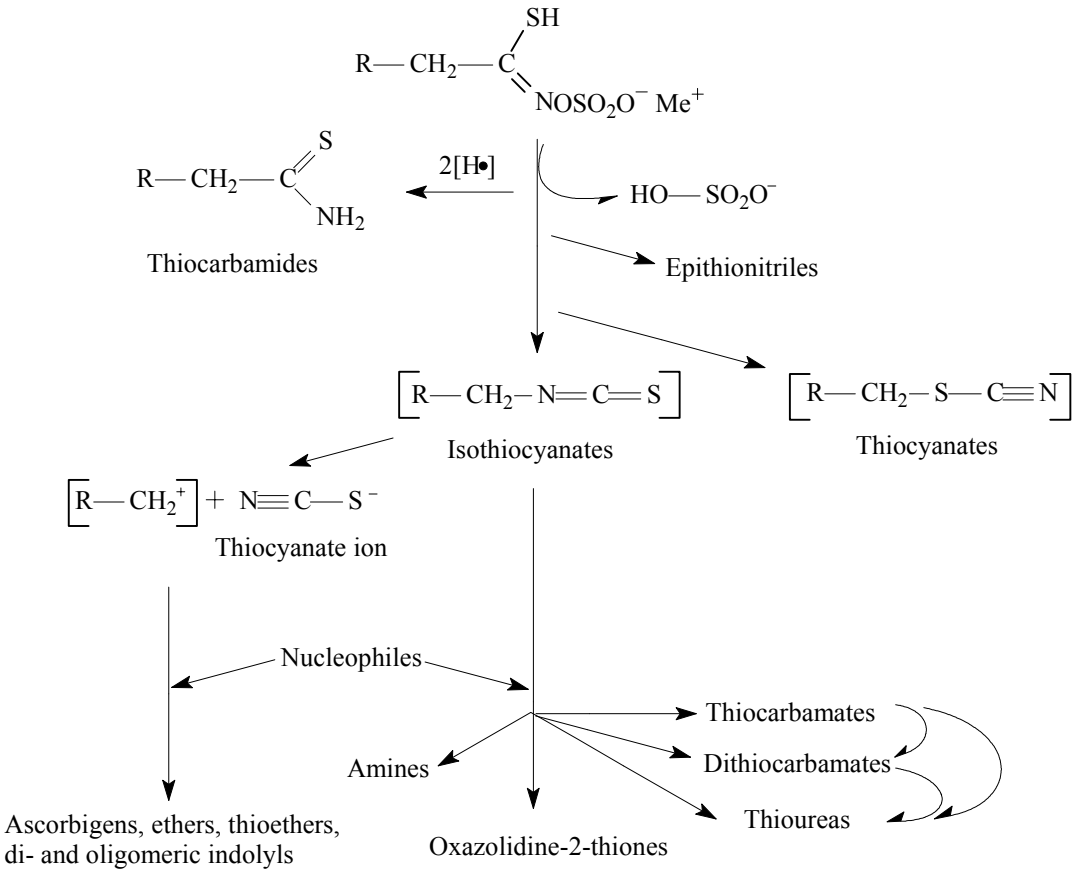


Figure 2. Transformation of reactive thiohydroxamate-O-sulphonates and isothiocyanates into various bioactive products. Thiocarbamide formation requires two redox equivalents, contrary to formation of thiocyanates, epithionitriles and isothiocyanate derived products. Oxazolidine-2-thiones are formed in fast reactions when the parent glucosinolates have a hydroxyl group at C-2.

The ratio between these reaction products varies in individual plants depending on the patterns of glucosinolates originally present, as well as environmental factors such as pH, and the presence of ferrous ions. In addition, the rate of expression of individual plants of an epithiospecific protein (ESP), which is associated with myrosinases, determines the metabolic conversion of glucosinolates. In the presence of ESP predominantly epithionitriles (see Figure 1) will be produced, whereas isothiocyanate results from hydrolysis of glucosinolates in the in the absence of ESP (Halkier and Gershenzon, 2006).

3. Methods of analysis

Different analytical procedures have been developed to quantify the total concentrations, or the amount of individual glucosinolates in plant materials. The most conventional and rapid method to estimate the concentration of total glucosinolates (all compounds with a β-D-thioglucopyranoside group) is an enzymatic assay in which glucosinolates are hydrolysed by commercially available myrosinase. The glucose generated during this process is then converted into gluconate-6-phosphate in the presence of hexokinase and glucose-6-phosphate dehydrogenase. This reaction leads to the formation of nicotinamide adenine dinucleotide phosphate (NADPH) (from $NADP^+$) which can be determined spectrophotometrically at 340 nm (Gardrat and Prevot, 1987) or at 520 nm by using additional redox coupling (Sørensen *et al.,* 1999). The total glucosinolate content is then expressed usually as μmol glucose released per g product and measured indirectly from the NADPH content.

Various modifications of gas chromatographic (GC) methods have also been used for analysis of glucosinolates. Previously gas chromatography analysis of trimethylsilyl derivatives of de-sulfoglucosinolates was considered as a rapid method of analysis (Olsen and Sørensen, 1980; Thies, 1980).

At present, high performance liquid chromatography (HPLC) is the most widely used method and using a HPLC method for analysis of de-sulphoglucosinolates (enzymatically desulfated) is recommended by the International Organisation for Standardization (ISO), the European Committee for Standardization (CEN) and the European Commission as the official method[9] for rapeseed-specific glucosinolates. Reference material containing these de-sulphoglucosinolates is available at the IRMM[10] (Institute for Reference Materials and Measurements). The methods comprising a desulfation step, however, do not allow the analysis of those glucosinolates which have substituents on the thioglucose part, or which have acidic groups in the side chain (R-group) (Sørensen, 1990). Hence several other analytical methods focus on the determination of intact glucosinolates either by HPLC (Helboe *et al.,* 1980; Bjerg and Sørensen, 1987a,b) or by capillary electrophoresis (Michaelsen *et al.,* 1992; Bjergegaard *et al.,* 1994; Buskov *et al.*, 1997; Sørensen *et al.,* 1999; Karcher and El Rassi, 1999). In addition, X-ray fluorescence has become common for fast analysis (Wathelet *et al.,* 2006).

For confirmatory analyses of individual glucosinolates, HPLC separation (either by isocratic paired ion or gradient HPLC) can be combined with electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS) (Ciska and Pathak, 2004; Tian *et al*., 2005). Recently, an improved hydrophilic interaction liquid chromatography (HILIC) method has been developed,

[9] OJ,L170, 03.07.1990, 27-34.
[10] see http://www.irmm.jrc.be/rmcatalogue/searchResultrmcatalogue.do

which can be used for the separation of glucosinolates across a wide spectrum of polarity (Wade *et al.*, 2007). This method, as well as the previously described high-speed counter-current chromatography (Fahey *et al.*, 2003) can be also used to isolate larger quantitative of biologically active glucosinolates.

4. Statutory limits for glucosinolates in feed materials and results on occurrence reported by Member States

The Council Directive 2002/32/EC prescribes that all products intended for animal feed must be of sound, genuine, and of merchantable quality and should pose neither a risk to animal health and productivity nor to human health or the environment. Annex 1 to Council Directive 2002/32/EC lists a number of compounds that are considered undesirable in animal feeds and prescribes maximum limits in different feed commodities, see Table 1.

Table 1: Undesirable feed components containing glucosinolates as listed currently in Annex 1 of Directive 2002/32/EC.

Undesirable substances (or plant materials)	**Product intended for animal feed**	**Maximum content in mg/kg relative to a feedingstuff with a moisture content of 12 %**
Volatile mustard oil	Feed materials with the exception of: - rapeseed cakes	100* 4000*
	Complete feedingstuffs with the exception of: - complete feedingstuffs for cattle, sheep and goats (except young animals) - complete feedingstuffs for pigs (except piglets) and poultry	150* 1000* 500*
Camelina - *Camelina sativa* (L.) Crantz	All feedingstuffs	Seeds and fruit of the plant as well as their processed derivates may only be present in feedingstuffs in trace amounts, which are not quantifiable
Indian mustard - *Brassica juncea* (L.) Czern. and Coss. ssp. *intergrifolia* (West.) Thell.		
Sareptian mustard – *Brassica juncea* (L.) Czern. and Coss. ssp. *juncea*		
Chinese mustard - *Brassica juncea* (L.) Czern. and Coss. ssp. *juncea* var. *lutea* Batalin		
Black mustard – *Brassica nigra* (L.) Koch		
Ethiopian mustard – *Brassica carinata* A. Braun		

* expressed as allyl isothiocyanate

Only a limited amount of data from monitoring programs regarding the occurrence of glucosinolates in crops harvested in EU Member States is available, and the results are given below. Denmark reported the analyses of total glucosinolates in 104 samples of rapeseed and feedingstuffs performed between 1998 and 2004. The limit of detection (LOD) was 1 mmol/kg. Estonia analysed 75 samples of total glucosinolates in rape cake between May 2004 and March 2005. The LOD was 1.5 mmol/kg. Belgium analysed 16 samples between 2000 and 2002 in different complete feeds for content of isothiocyanate. The LOD was 100 mg/kg.

Table 2. Results from glucosinolate measurements as provided by Member States

Country	**Type**	**Number of samples**	**Total glucosinolate (**mmol/kg**), mean (min-max)**
Denmark	Rape seeds, (whole, expeller or extracted)	59	11.2 (<LOD* - 21.6)
	Complementary feed for cattle	42	3.4 (<LOD - 15.3)
	Complete feed	2	both <LOD
Estonia	Rape cake	75	22.35 (7.1 – 80.6)

Country	**Type**	**Number of samples**	**Isothiocyanate (mg/kg), mean (min-max) or result**
Belgium	Complete feed for poultry	9	172 (<LOD – 235)
	Complete feed for laying hens	2	223, 235
	Complete feed for rabbits	2	223, 235
	Complete feed for dogs	3	< LOD

* Concentration values reported as below the LOD were considered equal to LOD/2.

5. Occurrence in feed materials

As mentioned above, members of the family *Brassicaceae* are the most important sources of glucosinolates (Frohne and Pfander, 2005; Kliebenstein *et al*., 2005; Tian *et al.,* 2005). The concentration of glucosinolates varies significantly, however, between different plant species, and is influenced by climate, soil, agricultural technology (use of fertilizers) and other environmental factors. In recognition of the adverse effects, selection of plant varieties with a low glucosinolate content commenced already more than 25 years ago, resulting in the use of varieties, particularly rape seeds, with a much lower content of glucosinolates, which were denoted initially as null (low in the content of erucic acid in the oil) or double null varieties (also low in glucosinolates),

or low and double low varieties, and more recently addressed as Canola[11]. These double-low varieties have replaced conventional rapeseed varieties in Europe and North America, but not in other countries with (partly) tropical climates, including India and China.

In addition, with the aim to increase palatability and to reduce the toxicity of glucosinolates several technical processes have been developed that successfully reduce the concentration of glucosinolates in oil seed products.

5.1. Content in different plants

The glucosinolate content within a given plant species can vary considerably. For example, in the 113 varieties of turnip greens (*Brassica rapa*), the glucosinolate content ranged from 7.5 to 74 mmol/kg dry weight of vegetable (Padilla *et al*., 2007). Comparable detailed investigations are often lacking for other crops, which are only used for oil production and/or in animal feeds.

5.1.1. Oilseed rape

Although *B. napus* and *B. rapa* have been grown for many years as a source of oil, high levels of glucosinolates in the meal remaining after oil extraction made it unsuitable for use as livestock feed. In 1967, seeds from plants of the Polish variety *B. rapa var. Bronowski* were found to be low in glucosinolates, and subsequent breeding programmes resulted in varieties of rapeseed which have combined low levels of both glucosinolates and erucic acid (known as double low varieties, or Canola) (Eskin *et al*., 1996). In Canada and the USA, the standard for glucosinolate content in dried Canola meal is set at a maximum of 30 mmol/kg dry matter. In the EU, compensatory payments to the producers of rapeseed are restricted to those sowing certified seed of double low varieties, which is defined in the regulation as seeds with a maximum glucosinolate concentration of 25 mmol/kg (at a moisture content of 9 %)[12]. Typical values in UK rapeseed meals are in the range of 10 - 14 mmol/kg fresh weight; this contrasts with levels of 90 - 186 mmol glucosinolates/kg commonly observed in the meal from the original varieties (Lardey and Kerley, 1999). The major glucosinolates in rapeseed meals are progoitrin and epigoitrin

[11] Rapeseed with less than 1 % erucic acid in the oil is denoted single low varieties. When the glucosinolate level in addition were reduced to less than 20 μmol/g seed dry matter they were denoted as double low varieties and Canola when the glucosinolate content was below 30 μmol/g defatted meal.

[12] Regulation (EC) No 2316/1999 of 22 October 1999 laying down detailed rules for the application of Council Regulation (EC) No 1251/1999 establishing a support system for producers of certain arable crops. OJ L 280, 30. 10. 1999, p. 43-65.

followed by gluconapin and glucobrassicanapin. The individual composition of rapeseed meals varies considerably depending on the geographic region and the agricultural practice applied (for details see Tripathi and Mishra, 2007).

Oil seed rape meal is used as a feed for many classes of livestock. The amounts used in rations vary according to the nutrient requirements of the livestock and the cost of rapeseed meal relative to other feeds. The following guidelines have been suggested as maximum inclusion rates for different species (Ewing, 1998).

Table 3. Concentrate inclusion rate (%) of oilseed rape meal by species (from Ewing, 1998)

Ruminants	**% inclusion**	**Pigs**	**% inclusion**	**Poultry**	**% inclusion**
Calf	5	Creep	0	Chick	0
Dairy	25	Weaner	0	Broiler	2.5
Beef	25	Grower	2.5	Breeder	0
Lamb	5	Finisher	5	Layer	2.5
Ewe	20	Sow	2.5		

These are only very general guidelines, and the limits may have been set on the basis of factors such as the protein quality, digestibility as well other adverse effects affecting the health of livestock. In order to provide for a degree of caution, Roth-Maier (1999) recommended a maximum of 10 % Canola meal in the diet for non-ruminants. Research has shown, however, that higher inclusion rates may be fed without a health risk for animals. In practice, however, the high fibre content of Canola meal is now recognised as the primary obstacle to its use in poultry and, to a lesser extent pigs, which require higher dietary protein and energy levels. For ruminants, low-glucosinolate rapeseed meal can be used as the sole protein supplement without any apparent adverse effects on the health of the animals (Allison *et al.*, 2001). Again, the quality of the protein in rapeseed meal, its price relative to other protein supplements, and the intended level of production are major factors limiting the use of rapeseed meal in ruminant diets.

Most of the rapeseed meal used in livestock feeds is processed using pre-press solvent extraction (for details see section 5.2.). However, increasing amounts of full-fat rapeseed, after heat treatment and particle size reduction, are being used, especially in broiler and pig weaner diets,

where it may be included up to a level of 10 % (Roth-Maier, 1999). One problem associated with the use of full fat Canola seed is that during feeding or processing (grinding) glucosinolates come into contact with myrosinase, enabling the formation of toxic products with antithyroid activity. Treatment with moisture and heat partially reduces this effect (Schöne *et al.,* 1994), but heat treatment alone appears to be insufficient to entirely eliminate antithyroid activity because glucosinolates remain intact even though myrosinase may be inactivated.

5.1.2. Forage Brassica crops

Forage Brassicas are cool-weather crops that are well adapted for growing in Northern Europe. They are grown as root and forage crops principally for feeding to ruminant livestock, and may be divided into 5 main types:

1. Forage rape (*Brassica napus*) producing progoitrin, glucobrassin and neoglucobrassin.
2. Kale (*Brassica oleracea*) known to produce sinigrin, glucobrassin and progoitrin
3. Turnips (*Brassica rova* known to contain progoitrin, gluconasturtiin and R-2-hydroxy-4-pentylglucosinolates) and
4. Leafy turnips or forage *Brassica* hybrids (e.g. with *Brassica campestris* and others)
5. Swedes (beetroot; *Brassica napus ssp. rapifera)*

For the first three, the leaves and stems represent the main part of the crop that is fed, and livestock usually graze them *in situ*. For turnips and swedes, both the roots (bulbs) and leaves are fed. The development of varieties with partially exposed roots makes them more suitable to grazing animals. Alternatively, the roots may be harvested and stored prior to feeding. Although *Brassica oleracea* is usually fed fresh it may be ensiled, and it has been shown that the ensiling process markedly reduces glucosinolate concentrations in kale.

Forage *Brassica* are fast growing and have high yields of biomass, and their high digestibility makes them useful supplementary forages for ruminant livestock. They have been used extensively in Europe as livestock forage, especially by sheep, for at least 600 years, and were widely grown in Europe for much of the last century. More recently the area sown to these crops has declined as a result of improvements in grassland management and developments of alternative forage crops (e.g. forage maize) (Milne, 1990).

In common with other *Brassica* crops, glucosinolates may be present and therefore restrict their use. However, glucosinolates levels can vary between individual plant varieties, and are influenced by factors such as climatic conditions, soil type and fertiliser application (Milne,

1990). Gustine and Jung (1985) reported that phosphorus fertilisation and high levels of nitrogen increased the glucosinolate content of a variety of forage brassicas, but high nitrogen application at lower phosphorus levels did not affect glucosinolate production. Plant growth stage also affects glucosinolate content (Clossais-Besnard and Larher, 1991). The concentrations of glucosinolates are generally higher in turnip roots than in the tops or the leaves. As glucosinolates have an important role in the defence system of plants against micro-organisms and insects (as mentioned above), insect attacks of roots have been shown to result in changes in both the concentration and the relative proportions of glucosinolates in the root tissue (Birch *et al.,* 1990). As a result of these diverse factors that influence the actual formation of glucosinolates in an individual plant, it is difficult to predict the risks to livestock associated with feeding forage *Brassica* crops. Total estimates of glucosinolates in *Brassica* crops are of limited predictive value, since different glucosinolates have different thresholds of toxicity.

In addition to causing metabolic disturbances (goitrogenic effects) in livestock, glucosinolates have been implicated in fish-like taints in animal products. Mawson *et al*., (1995) concluded that although glucosinolates and their breakdown products might pass into products such as meat, milk or eggs in small amounts, this was generally unlikely to result in a noticeable deterioration in their taste. Mawson *et al*. (1995) estimated that when feeding low glucosinolate rapeseed meal as the sole protein supplement to dairy cows, the level of rapeseed glucosinolate breakdown products in milk would be unlikely to exceed 0.1 μmol/L oxazolidinethione, 10 μmol/L unsaturated nitriles and 100 μmol/L thiocyanate. At these levels there was no evidence to indicate any negative effects of glucosinolates breakdown products on the sensory properties of milk. Similarly, with the inclusion of 20 % low-glucosinolate rapeseed meal in diets, no impairment on visual scores or sensory evaluation was found in pig or broiler carcasses (Mawson *et al*., 1995). However, glucosinolate taint of eggs is more common, particularly as a fishy odour in the eggs of brown-shelled-egg layers. As a result only very low levels of rapeseed meals can be fed to layers without the risk of fishy taint in eggs (Mawson *et al.*, 1995), and in practice many feed compounders (producers of mixed feeds) do not include any rapeseed meal at all (see also Chapter 10).

Recently genetically modified myristic acid-rich rapeseed, in which an acyl-thioesterase gene is inserted, has been developed. In these varieties, the myristic and palmitic acid content is increased from 0.1 – 11.4 % and from 3.6 – 20 %, respectively, at the expense of oleic acid, which is significantly reduced. At the same time, the glucosinolate content increased from approximately 12 to 19 mmol/kg in the genetically modified plant. This increase was accompanied by a lower tolerance (changes in thyroid iodine metabolism) of pigs, which were given feed containing 15 % of the genetically modified rapeseed (Böhme *et al.*, 2007).

5.1.3. *Camelina sativa*

As mentioned above, there is a renewed interest in *Camelina sativa* as a oilseed crop because of an increasing demand for alternative low-input oilseed crops with the potential for use for food and non-food purposes of the seed oil. In *Camelina sativa,* the total content of glucosinolates has been reported to range between 14.5 - 23.4 mmol/kg (Matthäus and Zubr, 2000) and 21 - 34 mmol/kg (Lange *et al.*, 1995). This content is higher (up to 2 times) than the concentrations in double-null rapeseeds, but much lower than that in mustard and crambe. In contrast to other *Brassica* crops, the major seed glucosinolates have long aliphatic side chains, such as (R)-10-methylsulfinyl-decylglucosinolate (glucocamelinin), which constitutes the major component (62 - 72 %) (Schuster and Friedt, 1998). Other glucosinolates of quantitative importance are (R)-11-methyl-sulfinylnonyl-glucosinolate, (R)-9-methylsulfinylnonylglucosinolate, and sinapine and sinalbin. For sinapine, however, the content present in *Camelina sativa* was much lower than that in mustard and rapeseed, ranging from 1.7 - 5.4 mg/g (5 - 17 mmol/kg) compared to 7 - 13 mg/g (22 - 41.8 mmol/kg) in rapeseed (Matthäus and Zubr, 2000). In comparative investigations on 10 genotypes of *Camelina sativa* the total glucosinolate content varied between 13.2 - 36.2 mmol/kg with a mean of 24 mmol/kg (Schuster and Friedt, 1998).

5.1.4. Botanical impurities

Botanical impurities including *Brassica juncea* (brown mustard), *Brassica nigra* (black mustard) and *Brassica carinata* (Ethiopian mustard) have been found in various feed materials. According to older literature references (summarised by Van Etten *et al.,* 1969), these plants produce predominantly the glucosinolates sinigrin and sinalbin. Sinigrin is also found in kale and other *Brassica* species, and contributes to the bitter taste of these plants. Data on the toxicity of sinigrin and sinalbin are scarce. In *vitro* experiments show that the parent sinigrin is less toxic than the related isothiocyanates (allyl-isothiocyanate and phenyl-isothiocyanate) (Musk *et al.,* 1995). In addition, sinigrin – like other glucosinolates – was found to protect rats in model experiments against chemically induced cancer (Tanaka *et al.,* 1992). No data are available on the toxicity of sinalbin and sinigrin in farm animal species.

5.2. Methods to reduce the glucosinolate content in feed materials.

In consideration of the adverse health effects of glucosinolates in animal feeds, a number of detoxification methods have been tested and implemented into feed processing (for review see Tripathi and Mishra, 2007). The first measure to prevent adverse health effects was the broad

application of alkaline-extruded rapeseed meal, as it is known that glucosinolates and their degradation products are unstable under alkaline conditions (Barret *et al.,* 1998).

Other techniques used and also reviewed in detail by Tripathi and Mishra (2007) are:

Heat treatment

Heat treatment decreases the glucosinolate content of rapeseed meal. Wet heating or pressure cooking has been shown to be more effective compared to dry heating and depending on pre-conditioning of the meal, heating time and temperature, reduction ranged from 630 - 950 μmol/mmol (63 – 95 %) depending on the commodity. Such heat-treated rapeseed meal has been fed to cows as the sole protein supplement. Milk yield, protein output, and dietary nitrogen utilization were improved in dairy cows, and the calves born from these cows did show an improved growth performance. However, high temperatures (>110ºC, 30 min) impair protein quality and this seems to improve the rumen stability of the applied proteins. In non- ruminants, restricting such heating to a temperature of 100ºC (30 min) would reduce total glucosinolate content by 50 % without affecting protein quality. 4-hydroxy-glucobrassicin has been shown to be cleaved more readily than aliphatic glucosinolates i.e. progoitrin and glucanapin. Pelleting of feed, which involves heating, failed to reduce the toxicity of Crambe meal (Walling *et al.,* 2002).

Water treatment

Oil-free Brassica meals have been soaked with water in a ratio of 1:5 (w/v) for 0 - 12 hours and linear glucosinolate hydrolysis has been shown to vary from 360 to 900 μmol/mmol (36 – 90%) with faster hydrolysis for gluconapin compared to sinigrin and pentenyl glucosinolates. Water treated *Brassica juncea* meal (water:meal = 5:1 for 8 hours) fed as the sole protein supplement to growing lambs, improved the utilization of nutrients and the growth rate (as compared to animals given untreated meal). This method is also effective in removing glucosinolates from rapeseed meals and *Moringra oleifera* kernels. However, the high loss of dry matter in the water used for extraction limits the wide use of this method in practice.

Treatment with water and metal solutions

Copper sulphate treatment (3.1 g/L) of 1 kg of rapeseed meal (dried at 60°C) was found to be the only effective treatment with metals to reduce the glucosinolate level by 900 μmol/mmol (90 %) including the inactivation of isothiocyanates and oxazolidinethione. In addition, when copper sulphate treated rapeseed meal (80 or 160 g $CuSO_4$ per kg feed) was included in the rapeseed meal containing diet of broilers and pigs, weight gain, thyroid function, iodine status, serum Zn content and alkaline phosphatase activity were improved as compared to the group fed rapeseed alone. The mechanisms involved in the reduction of toxicity are not fully understood, but it has been speculated that copper could shift the glucosinolate degradation or enhance the production of volatile metabolites (isothiocyanates and/or nitriles) or amines (allylamine or thiourea).

Solid state fermentation

Fermentation of sterilized rapeseed meal (121°C, 15 min) has been performed using *Rhizopus oligosporus* and *Aspergillus* spp. under aerobic solid state fermentation for 10 days at 25°C (meal:water ratio 1:3) and resulted in the inactivation of myrosinase and the reduction of total glucosinolates by 431 µmol/mmol and thiooxalidone by 340 mg/g. At 30°C and 60 - 96 hours of fermentation, glucosinolates were completely degraded. Such reduction during fermentation has been speculated to be due to the utilization of glucose and sulphur moieties of the compounds by microbial enzymes. Autolysis of glucosinolates under these moisture and temperature conditions has also been observed particularly for volatile metabolites.

Microwaving, micronisation and extrusion

This technique has been shown to be effective for rapeseed meal and glucosinolate decomposition ranging from 70 to 254 µmol/mmol, using microwave irradiation at a frequency of 2450 MHz for 2.5 minutes (moisture 13 g/kg, 24 hours at 4ºC). Decomposition increased with moisture content and length of exposure to microwave heating. Feeding trials with mice and growing pigs showed an improved feed intake, digestibility and nutritive value (feed conversion) although growth depression and goitrogenic effects were still observed in mice.

Taken together, these results show that the investigated methods can reduce total glucosinolate content and improve dry matter intake and the biological value of the protein fraction in feed materials.

6. Estimating the intake by farm livestock

The intake of glucosinolates by livestock and poultry is determined by the amount of glucosinolate-containing feeds consumed, and the glucosinolate content in those feeds. Glucosinolates are found in all parts of the plant and up to fifteen different glucosinolates have been found in the same plant, but only one or two are generally present in significant quantities in a given plant species (Cooper and Johnson, 1984). In general, levels are highest in the seeds. Glucosinolate concentrations differ also according to the age of the plant, plant health and nutrition, and climatic conditions as mentioned above.

Glucosinolates are also very unpalatable, causing animals to reduce their feed intake and resulting in lower growth rates (Bell and Shires, 1982; Bourdon and Aumaitre, 1990).

For non-ruminant livestock, exposure to glucosinolates comes mainly from oilseed rape meal. Under current EU legislation, producers have to keep the maximum level of total glucosinolates in seeds of oilseed rape below 25 mmol/kg to receive support (see chapter 5.1.1). Seeds typically

have average oil contents of 45%, with up to 40% extracted by solvent extraction. Since glucosinolates are retained in the oil-free meals, this maximum permissible level corresponds to a concentration in meal of approximately 42 mmol/kg, although as discussed above (see section 5.1.1. and Table 2) most samples have lower concentrations. Individual dietary exposure depends on the inclusion rates in the animals' diets, which vary between species. Tolerance studies in animals (see section 7.3.) have therefore been based on various inclusion rates and various products (high and low glucosinolate content), but it is often difficult to relate to this maximum exposure levels, because in many studies the actual glucosinolate content was not measured or reported.

In the diet of ruminants, glucosinolates may also be present in certain forage *Brassica* crops. Therefore, for ruminants glucosinolates may be present in both the concentrate portion of the diet (as rapeseed meal) as well as in the forages. Forages may represent a significant proportion of the total diet, although ruminant diets should not contain more than 75 percent *Brassica* forage because the fibre content of *Brassica* crops is too low for maintenance of proper rumen activity (Anonymous, 1993). As discussed above, glucosinolate concentrations in forage brassicas can be very variable, and treatment of the plant material (e.g. wilting, ensiling) and the conditions under which hydrolysis occurs, influence the degree of toxicity (Cooper and Johnson, 1984).

7. Toxicity of glucosinolates

7.1. Mechanisms of toxicity of glucosinolates

The toxicity of glucosinolate is generally attributed to the isothiocyanates, thiocyanates, oxazolidinethiones and nitriles, originating from enzymatic cleavage of glucosinolates by myrosinase (Burel *et al*., 2001). Thiocyanate and oxazolidinethione anions are known to compete with iodine in two ways, by inhibiting its uptake through competition with the sodium-iodide symporter (the common term for combined transporters), and binding of iodine to tyrosine residues of thyroglobulin at high concentrations (Burel *et al*., 2001; De Groef *et al*., 2006). Hence one of the common symptoms of glucosinolate exposure is the impairment of the thyroid function, resulting in a hypertrophy of this endocrine gland (goitre). Clinical signs secondary to hypothyroidism following exposure to toxic concentrations include impairment of growth, reduced feed conversion and impairment of growth, fertility and reproduction (Mawson *et al*., 1994a,b; Mithen *et al*., 2000; Burel *et al*., 2001; Conaway *et al*., 2002).

In addition, irritation of the gastro-intestinal mucosa followed by local necroses, hepatotoxicity and nephrotoxicity have been observed, commonly attributed to the presence of nitriles. Feed

aversion seems to be associated with the bitter taste of sinigrin and progoitrin, which is converted into goitrin for example by heat treatment (van Doorn *et al.,* 1998).

In contrast to the various reports on ITC toxicity, the toxic potential of the stable long side chain glucosinolates and their metabolites remains largely unknown. A typical example of a plant species that seems to contain significant amounts of these long side chain glucosinolates is *Camelina sativa* (Matthäus and Zubr, 2000) as mentioned above. Seeds of this plant contain glucosinolates that have long aliphatic side chains, such as the (R)-9-methylsulfinylnonylglucosinolate and (R)-11-methylsulfinyldecylglucosinolate. The toxicity of such compounds as well as their transformation products has not been investigated as yet as they do not occur in the commonly investigated *Brassica* crops. However, a recent publication (Ryhänen *et al.,* 2007) has investigated the effect of a *Camelina sativa* expeller cake on the growth performance and the results are presented in the next chapter.

7.2. Mutagenicity, genotoxicity and carcinogenicity

Glucosinolates and some of their metabolites have been shown to be mutagenic and weakly genotoxic (Mawson *et al*., 1994a,b; Mithen *et al*., 2000; Burel *et al*., 2001; Conaway *et al*., 2002). For example, crude juice extracts of a number of *Brassica* vegetables all caused genotoxic effects in the absence of metabolic activation measured as point mutations in *Salmonella* strains, repairable DNA damage in *E. coli* K-12 cells, and clastogenic effects in cultured mammalian cells. In mammalian cells, chromosomal aberrations and sister chromatid exchanges were also described (Kassie *et al.*, 1999). A high oral dose of allyl ITC (25 mg/kg in corn oil given 5 times/week by gavage for 103 weeks in F344 rats resulted in increased incidences of epithelial hyperplasia and transitional-cell papillomas of the urinary bladder in males, whereas in females subcutaneous fibrosarcomas were observed at the same dose (NTP, 1982) A parallel study was conducted in B6C3F1 mice. The reviewers concluded that under the conditions of this bioassay, allyl isothiocyanate was carcinogenic for male F344/N rats, causing transitional-cell papillomas in the urinary bladder. Evidence for associating allyl isothiocyanate with subcutaneous fibrosarcomas in female F344/N rats was equivocal. Allyl isothiocyanate was not carcinogenic for B6C3F1 mice of either sex (NTP, 1982). Phenylethyl isothiocyanate was shown to be an inhibitor of tumour formation at several sites in rats and in lungs of A/J mice in various assays (Chung *et al.,* 1996; Stoner *et al.,* 2002; Adam-Rowell, 1993; Rao *et al.*, 1995). However, investigations with indole-3-carbinol gave conflicting results: when administered before or at the same time as a chemical carcinogen, it was found to inhibit the developments of cancers of the breast, stomach, colon, lung and liver (Guo *et al.*, 1995). In contrast, in other studies in which indole-3-carbinol was administered after the carcinogen (post initiation) an enhancement of

cancer of the liver, thyroid, colon and uterus was observed in rats (Kim *et al.,* 1994, Stoner *et al.*, 2002; Yoshida *et al.*, 2004). The exact mechanisms underlying these controversial results are not entirely elucidated, but the therapeutic use of these compounds has been questioned, despite the increasing epidemiological evidence that higher intake of cruciferous vegetables is associated with a decreased cancer risk in humans (reviewed by Higdon *et al.,* 2007).

7.3. Adverse effects on livestock

Intoxications following the consumption of glucosinolate-containing plants have been described in all major farm animal species. However, many of the reports refer to old case studies in which the actual intake (dose) and the nature of the glucosinolate or products derived therefrom are not given. Moreover, the clinical cases described below are reported in relation to the ingestion of rapeseeds and rapeseed meal prior to the implementation of double-low varieties. Common clinical signs following exposure to intoxicating levels of glucosinolates are:

- alterations of the thyroid metabolism and enlargement of the thyroid gland following the ingestions of isothiocyanates and oxazolidinethione (Spiegel *et al*., 1993)
- irritation and local necroses of the gastro-intestinal mucosa by alkyl isothiocyanates (Martland *et al.,* 1984)
- growth retardation (Kloss *et al.,* 1994; Schöne *et al.*, 1997a)
- liver damage with increased enzyme leakage (Umemura *et al*., 1977; Campbell, 1979; Martland *et al*., 1984).
- impairment of fertility following long-term exposure to glucosinolate containing plants (Taljaard, 1993; Ahlin *et al.,* 1994)
- transient impairment of locomotion, behavioural changes and disorientation ('rape blindness') probably caused by isothiocyanates (Schmid and Schmid, 1992; Rodriguez, 1997)

7.3.1. Adverse effects in pigs

Pigs are among the most sensitive animal species regarding acute adverse effects of glucosinolates. Typical effects attributable to rapeseed meal produced from rape with high glucosinolate levels, were a delayed sexual maturity, impaired conception rates and a decrease in the number of piglets born alive, and weaned. Schöne *et al*. (1997b) reported the effects of

feeding diets containing either 100 g (equivalent to 2 mmol total glucosinolates/kg) or 250 g (equivalent to 10 mmol total glucosinolates/kg diet) of rapeseed meal per kg feed to sows in late gestation and during lactation (Schöne *et al.,* 1997b; Schöne *et al.,* 2001). These diets resulted in a slight decrease in litter weight and hypothyroidism in the piglets, probably due to a reduced iodine excretion with the sows' milk.

Similar symptoms including hypothyroidism, characterised by low levels of thyroid hormones and increase in thyroid gland weight (Spiegel *et al.,* 1993), as well as reduced feed intake and growth retardation (Schöne *et al.,* 1997a) were consistently reported from other studies, but details of exposure were not described. Previous experiments had indicated that diets with a total glucosinolate level below 1 mmol/kg did not induce significant adverse effects, whereas levels exceeding 1.34 mmol/kg resulted in reduced feed intake and growth (Bowland, 1975). Dietary levels between 9 - 10.1 mmol/kg induce iodine deficiency and an increase in the serum level of T_3 and T_4 followed by thyroid hypertrophy (Mawson, 1994a,b). From these experimental data it was concluded that dietary total glucosinolate levels for pigs should remain below 2.1 mmol/kg diet and that when diets containing glucosinolates are used, sufficient iodine (1000 μg/kg diet) should be supplemented (Opalka *et al.,* 2001; Schöne *et al.,* 2001). In turn this implies that for rape varieties with reduced glucosinolate content (double null varieties) inclusion rates of 10 - 12 % rapeseed meal in pig diets are tolerable. However, in the reproductive phase, lower inclusion rates (2.5 %) are recommended.

7.3.2. Adverse effects in poultry

Feeding rapeseed meal containing high glucosinolate levels (the concentration is not reported) in amounts of 200 g/kg diet to laying hens resulted in liver haemorrhage, liver enlargement, reticulolysis (lysis of the endoplasmatic reticulum of hepatocytes) and lymphoproliferation. The high glucosinolate level diet also significantly reduced egg production and plasma urate levels (Martland *et al.,* 1984). At lower concentrations, glucosinolates decreased only feed intake and weight gain (Kloss *et al.,* 1994). In this study, meals containing low glucosinolate levels (concentration not given) were fed to 7-day-old broiler chicks for 14 days with an inclusion rate of 10 % of the diet.

Little effect on growth rates of broiler chicks was reported at total glucosinolate levels of 2 - 4 mmol/kg of diet (equivalent to about 20 % Canola meal in the diet), although a significant reduction in daily weight gain was evident when the inclusion rates in the diet exceeded 10 mmol/kg (Mawson *et al*., 1994a,b).

In turkeys fed rapeseed products for 16 weeks (glucosinolate concentration not given), liver cirrhosis accompanied by hydropericardium was observed. Histological examination revealed an extensive fibrosis, characterized mainly by reticular fibres, and centrolobular degeneration of parenchymal liver cells. At twelve weeks, multiple focal necrotic sites appeared, preceding the ultimate fibrotic degeneration (Umemura *et al.*, 1977). As this study dates back to 1977, it can be assumed that it was conducted with high glucosinolate (wild-type) rapeseed.

In a recent 37-day study, starting with 1-day broiler chickens, the effect of *Camelina sativa* expeller cake (5 and 10 %) on their growth performance and their meat quality was investigated. Overall, at both concentrations *Camelina sativa* cake significantly impaired the growth of the broiler chickens in a linear fashion between day 15 and day 37 as well as their feed intake. However, the relative organ weights of the thyroid glands were not increased, and there were no gross signs of liver toxicity in these animals (Ryhänen *et al.*, 2007).

The effects of *Camelina sativa* cake on the performance and meat quality of broilers (male and females) has been investigated recently in a 38 day experiment with a diet containing 0, 5, 10, 15, 20 or 25 % of the cake (6 x 2 factorial design). All birds received the same starter diet containing 22 % crude protein from day 1 to 9 and the experimental grower diets containing 20 % crude protein and were fed from day 10 to 37. The growth of broilers during the entire experiment decreased with increasing dietary *Camelina sativa* cake with the most remarkable reduction of the growth with diets contained 20 and 25 %.

The negative effect on growth of broilers was more remarkable for males than for females. However, the differences in feed intake between broilers fed 0, 5, 10, 15, and 20 % cake were rather small, except for birds fed 25 % cake for which the feed intake decrease was very clear. With increasing *Camelina sativa* cake content in feed (20 – 25 %), the relative organ weights of the thyroid glands (thyroid weight expressed as percentage of body weight) increased linearly ($p<0.05$). The abdominal fat proportion (% of body weight) decreased linearly with increasing dietary *Camelina sativa* cake ($p<0.001$). However, these effects of *Camelina sativa* cake on the performance results of broilers were marginal and not significant when diets contained 5 and 10 %. The authors concluded that these concentrations could be used in broiler feeds since there were no remarkable effects on performance, but a clear positive effect on the fatty acid composition of breast meat (Venäläinen *et al.*, 2005, unpublished research report provided to EFSA).

Valkonen *et al.* (2004, unpublished research report provided to EFSA) performed a 42-day feed study by adding to the feed of egg-laying hens 0, 5 or 15 % of *Camelina sativa* cake. Overall, the highest egg productivity and optimal fatty acid content was seen in the hen group that had been given the diet containing 5 % *Camelina sativa* cake. However, laying hens given 15 % of

Camelina sativa cake produced more soft eggs than in the other group and their feed intake was clearly lower.

In summary, the studies performed by Valkonen *et al.* and Venäläinen *et al.* demonstrate that the use of *Camelina sativa* in the feed of chickens at low concentrations (5 %) does not exert toxic effects on the animals, but would optimise the fatty acid content in eggs and meat. However, the data presented by Ryhänen *et al.,* 2007 show an impairment of growth and feed intake in young broilers already at an inclusion rate of *Camelina sativa* of 5 %, although no significant effects on the thyroid gland were noticed at this exposure level. Hence it was concluded that even at an inclusion rate of 5 % of *Camelina sativa* expeller cake, adverse effects in chickens might occur.

7.3.3. Adverse effects in ruminants

Glucosinolate poisoning in **cattle** has been reported by several authors (Mason and Lucas, 1983; Taljaard, 1993; Lardy and Kerley, 1994; Morton and Campbell, 1997; Morton and Morton, 1997; Rodriguez, 1997; Katamoto *et al.,* 2001). Clinical symptoms include poor productivity, reduced fertility (prolonged interval from calving to conception) and poor body condition (Taljaard, 1993). However, in general, ruminants are considered to be less sensitive to glucosinolates in their feed, as compared to monogastric species (including pre-ruminant calves), as the rumen flora degrades various glucosinolate breakdown products.

Mason and Lucas described in 1983 a case of an acute intoxication of cattle by *Brassica oleracea* ssp. *acephala*, resulting in acute death within 24 hours of two cows and acute illness in one cow out of thirty exposed animals (the glucosinolate concentrations are not given). The primary pathological lesions were vascular damage and oedema of the rumen mucosa (Mason and Lucas, 1983).

Morton and Campbell reported photosensitization as an important clinical sign in a number of cases of *Brassica* intoxications in cattle (Morton and Campbell, 1997).

Growing cattle tolerated dietary concentrations of glucosinolates up to a level of 10 - 15 mmol/kg and did not show any detrimental effects on growth and feed conversion (Bush *et al.,* 1978). Calves tolerated a level of 7.71 mmol/kg (Mawson *et al.,* 1994b).

Cows showed signs of toxicity and thyroid dysfunction and depressed fertility following a daily intake of 44 mmol/day (equivalent to 31 mmol/kg dry matter) (Ahlin *et al.,* 1994).

In an investigation by Ahlin *et al.* a reduction in fertility and a mild suppressive effect on thyroid function were found after feeding cows a ration containing more than 3 kg of double-low rapeseed per day during three consecutive lactations (Ahlin *et al*., 1994). The authors suggested

that an amount of 3 kg per day could be considered as maximal tolerable inclusion rate of double-low rapeseed in the diet of dairy cows.

A report of glucosinolate poisoning in **lambs** mentions swayback, anaemia and visibly enlarged thyroid glands as major clinical signs (Taljaard, 1993). Lambs feeding on Brassica developed hypothyroidism (Cox-Ganser *et al.,* 1994). Body weight losses were reported in sheep following a glucosinolate intake of 2.5 – 7.6 mmol/day (equivalent to 1.2 - 2.2 mmol/kg feed dry matter) (Mandiki *et al.,* 2002). In lambs, dietary levels of up to 10 mmol/kg affected digestibility only insignificantly without any clinical signs of intoxication, whereas levels above 10 mmol/kg reduce growth (Standford *et al*., 2000). Glucosinolate intakes of 0.24 - 0.69 µmol/day affected thyroid function in lambs prior to weaning, whereas after weaning only much higher levels of 1.6 - 3.9 mmol/day affected thyroid function. This relatively high tolerance of sheep suggests that at least sheep for fattening might tolerate diets with low glucosinolate rapeseed as the sole protein source (Mabon *et al.,* 2000).

Ewes showed an impaired fertility with significantly reduced oestradiol levels following dietary exposure to 1.2 - 1.6 mmol/kg dry matter (Mandiki *et al.*, 2002). In male animals fed high glucosinolate *Brassica juncea* meal (glucosinolate amount not known), reduced testosterone levels and impaired semen quality were reported. These effects were reversible when the diet was supplemented with iodine (Pattanaik *et al.*, 2004).

7.3.4. Adverse effects in horses

In horses, there is only one case report dealing with rapeseed oil intake in association with respiratory disease (Dixon and McGorum, 1990), but this report does not provide information on the clinical signs induced.

7.3.5. Adverse effects in other animal species

Rabbits

A report from 1928 (reviewed by Mawson *et al.,* 1994b) described the effects of feeding high levels of cabbage to rabbits. The animals developed goitre, and the causative agent was later identified as the thiocyanate ion. The goitrogenic effect was also associated with a low iodine status in the diet (Mawson *et al.,* 1994b). High levels of glucosinolates (17.6 - 25.3 mmol/kg diet) caused growth depression and increased mortality. Hence it was recommended to adjust the diet for rabbits to a maximal level of 8 mmol/kg. This suggestion is confirmed by Tripathy *et al.* stating that fattening rabbits tolerated a diet with up to 7.9 mmol/kg (Tripathy *et al.,* 2003).

Minks

The effect of rapeseed oil from double-low-varieties in wet compounded diets (0, 1.5 or 3l %) on plasma thyroxin (T_4), reproductive performance and kit weight gain during lactation was investigated with 3 groups of 20 mink females. Plasma T_4 was significantly lower in lactating female minks compared to non-pregnant females. None of the other parameters were affected by the experimental treatment with rapeseed oil (Tauson and Neil, 1994).

Fish

Adverse effects of glucosinolates have been found in rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) and relate mostly to thyroid dysfunction (Hardy and Sullivan, 1983; Hossain and Jauncey, 1988; Leatherland *et al.*, 1987; Burel *et al*., 2000). In fish, as in other animal species, the dietary amounts of glucosinolates have been expressed as intact glucosinolate and metabolites or metabolites alone, and for this reason, they are highly variable. The typical features for thyroid toxicity in fish are: hypothyroidism associated with lower levels of T_3 and T_4, an increase in the volume of the thyroid gland and the height of epithelial follicle cells (thyroid tissue hyperactivity), and a compensating up-regulation of deiodinase activity. In a study of Burel *et al*. (2000) two rapeseed meals with two levels of glucosinolates (5 and 41 mmol/kg dry matter, respectively) were incorporated into the diet of juvenile trout at levels of 300 and 500g/kg diets. From the thyroid effects observed, the authors concluded that even a diet with 300 g/kg could not be used in trout because of significant adverse effects on thyroid function. Subsequently, in another study by Burel *et al.* (2001) two rapeseed meals that contained glucosinolates at concentrations of 26 and 40 mmol/kg were incorporated at increasing levels (10, 20 and 30 %) in the diet of juvenile rainbow trout. Adverse effects on thyroid function were observed after 14 days already at the lowest exposure level (10 % rape seed meal). Dietary supplementation with T_3 or iodine increased plasma T_3 levels and reduced the adverse effects, whereas thyroid-stimulating hormone (TSH) had no effect on T_3 plasma levels. In a 3rd study, Burel *et al.* (2000) exposed rainbow trout to diets containing 1.4 – 19.3 mmol glucosinolates/kg feed. Even at the lowest concentrations, a significant reduction in growth and histological alterations in the thyroid gland were observed, and hence the authors concluded, that the true no-effect level is even lower than 1.4 mmol. This level is also lower than the tolerance level for pigs (estimated to vary between 2 – 4 mmol/kg glucosinolates), although pigs had been considered as most sensitive animal species regarding goitrogenic effects of glucosinolates.

8. Toxicokinetics, metabolism and tissue distribution

Studies describing the metabolism and toxicokinetics of a number of glucosinolates and their metabolites have been the subject of extensive investigations for rodents and man (Conaway *et*

al., 2002; Gorler *et al.,* 1982; Ionanou *et al.*, 1984; Eklind *et al.,* 1990; Bollard *et al.,* 1997; Kassahun *et al.,* 1997; Conaway *et al.*, 1999; Rouzaud *et al.*, 2003; Anderton *et al.*, 2004). These studies indicate that the metabolism of isothiocyanates produced after myrosinase activity, and particularly the microbial hydrolysis of the glucosinolates in the gastrointestinal tract shows wide qualitative and quantitative interspecies differences. Isothiocyanates are conjugated with glutathione by glutathione-S-transferases, followed by excretion in urine as mercapturic acids (Rouzaud *et al.,* 2003). This pathway was demonstrated in experiments with rats for all investigated allyl, benzyl, butyl, phenetyl isothiocyanates, and sulphoraphane, since these compounds are excreted as the N-acetylcysteine conjugate or mercapturic acid form in the urine at levels of 40 - 60, 10 - 20, 62, 80 - 86 and 67 % of the dose, respectively (Gorler *et al.,* 1982; Ionanou *et al.*, 1984; Eklind *et al.,* 1990; Bollard *et al.,* 1997). In contrast, the alkyl moiety of the synthetic phenetyl isothiocyanates is oxidized in the liver, conjugated to glucuronic acid, and then excreted in the bile (Conaway *et al.,* 1999). Again species differences have been observed between guinea pigs and rabbits, which excrete primarily the mercaptopyruvic acid conjugate of benzyl isothiocyanates. These species also do not readily form mercapturic acids after administration of other types of xenobiotics (Gorler *et al.,* 1982). In contrast, the dog excretes the glycine conjugate, hippuric acid, possibly because of hydroxylation of the benzylic moiety with subsequent oxidation and conjugation of benzoic acid (Gorler *et al.,* 1982; Conaway *et al.*, 2002). Data from studies in mice demonstrated the excretion of the mercapturic acids for some compounds such as the allyl and phenyl isothiocyanates but these only constituted 6 – 10 % and 9 – 15 % of the dose, respectively, and most of the excreted metabolites correspond to cysteine conjugates of thiocyanate (90 % for allyl isothiocyanates) and the mercaptopyruvic acid conjugate of phenyl isothiocyanates (26 - 32 %) (Eklind *et al.,* 1990; Bollard *et al.,* 1997). A recent study on the pharmacokinetics of the isothiocyanates indole-3-carbinol as a hydrolysis product of glucobrassicin showed rapid absorption, distribution, and elimination from the plasma and the tissues, falling below the limit of detection within 1 hour. Six-fold higher concentrations in the liver as compared to the plasma were found. The metabolic route for this compound involves acid condensation to carboxylic acid and a minor oxidative metabolite carboxylaldehyde metabolite (Anderton *et al.,* 2004). Various studies on the metabolism and excretion of glucosinolate-containing cruciferous vegetable were also conducted in humans, given the evidence of a potential anti-carcinogenic effect. In humans urinary dithiocarbamate levels were correlated with the glucosinolate/isothiocyanates profiles in vegetables and condiments. Taken together, these studies show significant interspecies differences in the metabolism and toxicokinetics of glucosinolates. Moreover, these studies refer to the impact of the intestinal flora contributing to the hydrolysis of glucosinolates. Detailed data on the metabolism of glucosinolates in livestock are lacking, but species differences can be expected to occur as well.

9. Carry-over and tissue residues

A number of carry-over studies addressing the transfer of glucosinolates from feed to animal products have been carried out and are reviewed in Mawson *et al.* (1995). Most of these studies were performed in the 1970s and 1980s and current reports on the transfer of glucosinolates or isothiocyanates into milk, meat, or eggs using state of the art analytical techniques are not available. Furthermore, no carry-over study is available for the long side-chain glucosinolates present in *Camelina sativa* press cakes.

In dairy cows, the carry-over of glucosinolates from rapeseed meal containing 20.5 and 36.2 mmol/kg, respectively, was studied. Although these glucosinolate levels induced alterations of the thyroid function in the exposed animals, no measurable amounts of intact glucosinolates were detected in milk. Similarly the glucosinolate aglucones, isothiocyanates or vinyl oxazolidinethione, were not transferred into milk. Small amounts of unsaturated nitrile (1-cyano-2-hydroxy-3-butene, 38 µmol/L) were measurable in the milk of animals fed the high glucosinolate diet, but not in the milk of the low-glucosinolate group. In addition, inorganic thiocyanate (28.6 and 34.9 µmol/L respectively) was excreted in the milk (Papas *et al.,* 1979). Other early studies have also measured unsaturated nitrile (5.9 - 22.4 µmol/L and thiocyanate (32 - 182.8 µmol/L after feeding rapeseed meal to cows with varying amounts of glucosinolates (3.1 - 76.1 µmol/L) depending on the rapeseed cultivars selected (summarized by Mawson *et al.,* 1995). Older studies had also indicated that goitrin (S-5-vinyl-oxazolidine-2-thione) may occur in cow‘s milk when animals were fed on rapeseed extract cakes containing 6 g of progoitrin/kg over a period of 7 days (Virtanen *et al.,* 1963). After milking the cows twice a day, the goitrin content of the heated milk samples was determined by a HPLC-method within 2 hours. When rape cake was given with an inclusion level of 0.39, 1.9 and 3.9 % of the diet, goitrin values were 37, 163 and 707 µg/L milk respectively. Overall these values represent a 0.1 % transfer of the original progoitrin content in the feed in to the milk. Goitrin was below the detection limit of 7 µg/L 12 hours after the last rape feeding (Bachmann *et al.,* 1985). No evidence of negative effects on the carcass quality (flavour) was provided in any of these studies.

In conclusion, when applying low glucosinolate rapeseed meal as the sole high protein component of concentrate mixture for cows, the level of rapeseed glucosinolate breakdown products did not exceed 0.1 µmol/L oxazolidinethione, 10 µmol/L unsaturated nitriles and 100 µmol/L thiocyanate. At these levels, no evidence for a negative influence of glucosinolate breakdown products on the sensory properties of milk could be found (Mawson *et al.,* 1995).

In **pigs or broilers** even at an inclusion level of 17 - 20 % of rapeseed meal into the diet, no impairment on visual scores or sensory (fishy taint) evaluation was found in carcasses. However, progoitrin has been shown to affect the taint in eggs, particularly in birds with genetically conditioned susceptibility (brown layers) and fishy taint was observed even at 0.3 µmol progoitrin per gram in the feed, corresponding to approximately 0.5 mmol/kg total glucosinolates. Assuming a threefold higher threshold for white layers (1 µM progoitrin in 1 g diet) the adverse fishy taint can be expected when 10 % rapeseed are included in the diet, and therefore only very low glucosinolate rapeseed meals can be fed to layers without the risk of fishy taint in eggs (Mawson *et al.,* 1995). None of the residue studies provided evidence for residue deposition of glucosinolate breakdown products in meat or organs (liver, kidney).

10. Human dietary exposure

Consumption of cruciferous vegetables results in human dietary exposure to glucosinolates of several milligrams per day. Adequate data for the total glucosinolate content in food were available from eighteen published studies providing 140 estimates for forty-two food items. The highest glucosinolate values were for garden cress (*Lepidium sativum*) (3.89 mg/g), while the lowest values were for Pe-tsai Chinese cabbage (*Brassica rapa*) (0.20 mg/g). Considerable variability was observed in the values reported for the same vegetable by different studies, with a median difference between the minimum and maximum values of 5.8-fold. Data for the average glucosinolate loss during cooking were limited but were approximately 36 % (McNaughton and Marks, 2003). The consumption of *Brassica* vegetables containing glucosinolates, as a source of isothiocyanates, is promoted due to their potential anticarcinogenic properties.

Comparing this human exposure to glucosinolates originating from vegetable consumption (which vary between 0.8 to 20 mmol/kg dry weight) (Kushad *et al*., 1999) with the potential exposure from milk (containing the highest level of thiocyanate of 0.18 mmol/L and goitrin of 0.7 mmol/L), it is evident that animal-derived products would contribute little to human dietary exposure, even if accidentally high inclusion rates of rapeseed meal are used in the animal's diet.

CONCLUSIONS

- Glucosinolates comprise a large group of structurally diverse compounds that are produced by many *Brassicaceae*, particularly by the genera *Brassica*, *Sinapis* and *Raphanus*. Outside Europe other families and genera that contain glucosinolates are important local feed materials, but these are not commercially produced and hence do not enter the European

market. Glucosinolates occur in all parts of the plant and act as innate defence system against insects, fungi and bacteria. The highest concentrations are generally found in the seeds.

- The most commonly used *Brassica* forages are forage rape (*Brassica napes*), Leafy turnips (*Brassica campestris*), kale (*Brassica oleracea*), turnips (*Brassica rova*) and Swedes (*Brassica napus ssp rapifera*.) In addition, press cakes of oilseed plants, particularly rapeseeds (including Canola) are regularly used as protein- and fat-rich feed material. The glucosinolate content and composition of these crops vary according to the geographic region of origin.

- In the intact plant cell, glucosinolates are stable as they are separated from the major degrading enzymes (myrosinase isoenzymes). During oil pressing, glucosinolates remain intact, and are present in the remaining press cakes/de-oiled meals, which are used in animal feeds. In cases of plant damage during harvest, storage and processing but also during chewing by animals, enzymatic and non-enzymatic reactions yield various transformation products, including isothiocyanates, thiocyanates, nitriles, epithionitriles and oxazolidinethiones.

- Isothiocyanates, thiocyanate ions and oxazolidinethiones, including goitrin (5-vinyloxazolidine-2-thione) as a prominent example, inhibit iodine uptake and the biosynthesis of the thyroid hormones T_3 and T_4, leading to hypothyroidism and subsequent enlargement of the thyroid gland. The changes in thyroid function account for many of the clinical signs associated with dietary exposure of animals to glucosinolates such as growth retardation and impaired fertility. Some of the glucosinolate breakdown products, particularly the nitriles, may cause mucosal irritation in the gastro-intestinal tract and transient impairment of liver and kidney functions.

- Adverse effects in animals have been generally correlated to the amount of total glucosinolates (generally measured indirectly by enzymatically released glucose) in the diet, despite the fact that individual glucosinolates vary in their toxicity. If the amount of total glucosinolates is measured, impurities caused by the presence of *Brassica juncea* , *Brassica nigra* and *Brassica carinata* seeds, currently allocated to the group of undesirable botanical impurities, would be detected as well.

- In contrast to the current provisions in the legislation of undesirable substances in animal feed, the measurement of 5-VOT does not provide addition information on the toxicity of feed materials, as this compound is volatile and not formed as cleavage product by all glucosinolates.

- Data on the toxicity of individual glucosinolates for food-producing animal species are very limited. The common practice of selecting low-glucosinolate plant varieties as forage plants and of processing crops with a potential high glucosinolate concentration prior to use, together with experience-based recommendations for maximal inclusion rates into animal diets given in textbooks, have proven to be effective measures to avoid intoxications and production losses in farm animals, and the undesirable fishy taint in animal-derived products. Among the techniques used in practice to reduce the amount of glucosinolates in press cakes from oil seeds, soaking with copper sulphate solutions and water extraction were found to be the most effective procedures to remove intact glucosinolates as well as their degradation products.
- Data on carry-over of glucosinolates into milk, meat and eggs are limited. Carry-over into milk is estimated to remain below 0.1 % of the total concentration of glucosinolates in the diet. In comparison with the dietary intake of glucosinolates originating from *Brassica* vegetables (including broccoli, cabbage, cauliflowers and Brussels sprouts) the contribution of glucosinolates and their breakdown products in animal-derived products to human exposure is very low.
- *Camelina sativa* is an emerging oil seed crop. Hence the seeds and products of *Camelina sativa* cannot be regarded as botanical impurity. When *Camelina sativa* expeller cake was fed to chickens it has been found to be toxic at very low inclusion rates (5 %). Whether or not this can be attributed to the presence of glucosinolates or to other constituents in *Camelina sativa* is presently unknown.
- In *Camelina sativa* the main seed glucosinolates have long aliphatic side chains, e.g. (R)-9-methylsulfinylnonylglucosinolate and (R)-10-methylsulfinyl-decylglucosinolat. The toxicity of these compounds and the nature of their breakdown products are at present unknown. Therefore, the CONTAM Panel concluded that no inclusion level in animal diet can be defined at present.

RECOMMENDATIONS

- Sensitive and reliable methods have been developed to measure the content of a large number of individual glucosinolates in plant materials. These methods should be validated and applied for the analysis of feed materials.
- Analytical methods for long side-chain glucosinolates should be developed, as these occur in emerging low-input oil seeds such as *Camelina sativa* and probably in other glucosinolate-producing plants.

- The potential adverse effects of *Camelina sativa* should be investigated, in particular the stability, oral bioavailability and toxicity of long side chain glucosinolates.
- To calculate inclusion rates of glucosinolate-containing feed materials into the diet of individual animal species, analyses of the total glucosinolate can be recommended, particularly for rapid screening of rapeseed products.

REFERENCES

Adam-Rodwell, G., Morse, M.A. and Stoner, G.D. 1993. The Effects of Phenethyl Isothiocyanate on Benzo[a]pyrene-Induced Tumors and DNA Adducts in A/J Mouse Lung. Cancer Lett 71: 35-42.

Ahlin, K.A., Emanuelson, M. and Wiktorson, H. 1994. Rapeseed products from double-low cultivars as feed for dairy cows: effects of long term feeding on thyroid function, fertility and animal health. Acta Veterinaria Scandinavia 35: 37-53.

Allison, R.D., Moss, A.R. and Blake, J.S. 2001. The effect of feeding rations containing heat treated rapeseed meal, lupins and beans to lactating dairy cows on milk yield and quality. Proceedings of the British Society of Animal Science: 4.

Anderton, M.J., Manson, M.M., Verschoyle, R.D., Gescher, A., Lamb, J.H., Farmer, P.B., Steward, W.P. and Williams, M.L. 2004. Pharmacokinetics and tissue disposition of indole-3-carbinol and its acid condensation products after oral administration to mice. Clin Cancer Res 10(15): 5233-41.

Anonymous, 1993. Strategies for extending the grazing season. Agronomy Facts 41. Penn State University. http://www.forages.psu.edu/agfacts/agfact41.pdf

Bachman, R., Theus, R., Luthy, J. and Schlatter, C. 1985. [The occurrence of goitrogenic substances in milk. 1. Release of goitrin in the milk of cows fed on rapeseed extract cakes]. Article in German. Z Lebensmittel-Unters –Forsch 181: 375-78.

Barrett, J.E., Klopfenstein, C.F. and Leipold, H.W. 1998. Alkaline heating of canola and rapeseed meals reduces toxicity for chicks. Plant Foods Hum Nutr 152: 9-15.

Bell, J.M. and Shires, A. 1982. Comparison and digestibility by pigs of hull fractions from rapeseed cultivars with yellow or brown seed coats *Brassica campestris, Brassica napus.* Canadian Journal of Animal Science 62: 557-565.

Bellostas, N., Kachlicki, P., Sørensen, J.C. and Sørensen, H. 2007. Glucosinolate profiling of seeds and sprouts of B. oleracea varieties used for food. Scientia Horticulturae 114: 234-242.

Birch, A.N.E., Griffiths, D.W. and MacFarlane Smith, W.H. 1990. Changes in forage and oilseed rape (Brassica napus) root glucosinolates in response to attack by turnip root fly (Delia floralis). Journal of the Science of Food and Agriculture 51: 309–320.

Bjerg, B. and Sørensen, H. 1987a. Quantitative analysis of glucosinolates in oilseed rape based on HPLC of desulfoglucosinolates and HPLC of intact glucosinolates. In Wathelet J.-P. (Ed), World Crops: Production, Utilization, Description. Glucosinolates in Rapeseeds: Analytical Aspects, Martinus Nijhoff Publishers, Kluwer Academic Publishers Group. Dordrecht/Boston/Lancaster. 13, 125-150.

Bjerg, B. and Sørensen, H. 1987b. Isolation of intact glucosinolates by column chromatography and determination of their purity. In Wathelet, J.-P. (Ed), World Crops: Production, Utilization, Description. Glucosinolates in Rapeseeds: Analytical Aspects, Martinus Nijhoff Publishers, Kluwer Academic Publishers Group. Dordrecht/Boston/Lancaster 13: 59-75.

Bjerg, B., Eggum, B.O., Jacobsen, I., Otte, J. and Sørensen, H. 1989. Antinutritional and toxic effects in rats of individual glucosinolates (+/- myrosinase) added to a standard diet (2). Journal of Animal Physiology and Animal Nutrition 61: 227-244.

Bjergegaard, C., Li.P. W., Michaelsen, S., Møller, P., Otte, J. and Sørensen, H. 1994. Glucosinolates and their transformation products- compounds with a broad biological activity. Bioactive Substances in Food of Plant Origin 1: 1-15.

Böhme, H., Rudloff, E., Schöne, F., Schumann, W., Hüther, L., Flachowsky, G. 2007. Nutritional assessment of genetically modified rapeseed synthesizing high amounts of mid-chain fatyy acids including production responses of growing-finishing pigs. Arch. Anim. Nutr. 61: 308-316.

Bollard, M., Stribbling, S., Mitchell, S. and Caldwell, J. 1997. The disposition of allyl isothiocyanate in the rat and mouse. Food Chem Toxicol 35: 933-943.

Bourdon, D. and Aumaitre, A. 1990. Low-glucosinolate rapeseeds and rapeseed meals: effect of technological treatments on chemical composition, digestible energy content and feeding value for growing pigs. Animal Feed Science and Technology 30: 175-191.

Bowland, J.P. 1975. Evaluation of low glucosinolate-low erucic acid rapeseed meal as the protein supplement for young growing pigs, including effects on blood serum constituents. Can J Anim Sci 55: 409–416.

Burel, C., Boujard, T., Escaffre, A.M., Kaushik, S.J., Boeuf, G., Mol, K.A., Van der, G.S., and Kuhn, E.R. 2000. Dietary low-glucosinolate rapeseed meal affects thyroid status and nutrient utilization in rainbow trout (Oncorhynchus mykiss). Br J Nutr 83: 653-664.

Burel, C., Boujard, T., Kaushik, S.J., Boeuf,G., Mol, K.A., Van der, G.S., Darras, V.M., Kuhn, E.R., Pradet-Balade, B., Querat, B., Quinsac, A., Krouti, M. and Ribaillier, D. 2001. Effects of rapeseed meal-glucosinolates on thyroid metabolism and feed utilization in rainbow trout. Gen Comp Endocrinol 124: 343-358.

Bush, R.S., Ncholson, J.W.G., MacIntyre, T.M. and McQueen, R.E., 1978. A comparison of Candle and Tower rapeseed meal in lamb, sheep and beef steer rations. Can J Anim Sci 58: 369–376.

Buskow, S. Sørensen, H, Sørensen, J.C. and Sørensen, S. 1997. Quantitative extraction of oil from plant materials with supercritical carbon dioxide. Pol.J Food Nutr Sci 6/47(3): 115-124.

Buskov, S., Hasselstrøm, J., Olsen, C.E., Sørensen, H., Sørensen, J.C. and Sørensen, S. 2000a. Supercritical fluid chromatography as method of analysis for determination of 4-hydroxybenzylglucosinolate degradation products. Journal of Biochemical and Biophysical Methods 43: 157-174.

Buskov, S., Olsen, C.E., Sørensen, H. and Sørensen, S. 2000b. Supercritical fluid chromatography as basis for identification and quantitative determination of indol-3-ylmethyloligomers and ascorbigens. Journal of Biochemical and Biophysical Methods 43: 175-195.

Campbell, L.D. 1979. Incidence of liver haemorrhage among white leghorn strains fed on diets containing different types of rapeseed meals. British Poultry Science, 20, 239-246.

Chung, F.L., Kelloff, G., Steele, V., Pittman, B., Zang, E., Jiao, D., Rigotty, J., Choi, C.I. and Rivenson, A. 1996. Chemopreventive efficacy of arylalkyl isothiocyanates and N-acetylcysteine for lung tumorigenesis in Fischer rats. Cancer Res 56: 772-778.

Ciska, E. and Pathak, D.R. 2004. Glucosinolate derivatives in stored fermented cabbage. Journal of Agricultural and Food Chemistry 52: 1938-1943.

Clossais-Besnard, N. and Larher, F. 1991. Physiological role of glucosinolates in Brassica napus. Concentration and distribution pattern of glucosinolates among plant organs during a complete life cycle. Journal of the Science of Food and Agriculture 56: 25–38.

Conaway, C.C., Jiao, D., Kohri, T., Liebes, L. and Chung, F.L. 1999. Disposition and pharmacokinetics of phenethyl isothiocyanate and 6-phenylhexyl isothiocyanate in F344 rats. Drug Metab Dispos 27: 13-20.

Conaway, C.C., Yang, Y.M. and Chung, F.L. 2002. Isothiocyanates as cancer chemopreventive agents: their biological activities and metabolism in rodents and humans. Curr.Drug Metab 3: 233-255.

Cooper, M.R. and Johnson, A.W. 1984. Poisonous Plants in Britain and their effects on Animals and Man. Ministry of Agriculture, Fisheries and Food Reference Book 161. Her majesty's Stationary Office, London.

Cox-Ganser, J.M., Jung, G.A., Pushkin, R.T. and Reid, R.L. 1994. Evaluation of Brassicas in grazing systems for sheeep: II. Blood composition and nutrient status. Journal of Animal Science 72: 1832-1841.

De Groef, B., Decallonne, B.R., Van der, G.S., Darras, V.M. and Bouillon, R. 2006. Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. Eur J Endocrinol 155: 17-25.

Dixon, P.M. and McGorum, B. 1990. Oilseed rape and equine respiratory disease. Vet Rec 126(23): 585.

Eklind, K.I., Morse, M.A. and Chung, F.L. 1990. Distribution and metabolism of the natural anticarcinogen phenethyl isothiocyanate in A/J mice. Carcinogenesis 11(11): 2033-6.

Eskin, N.A.M., McDonald, B.E., Przybylski, R., Malcolmson, L.J., Scarth, R., Mag, T., Ward, K. and Aldoph, D. 1996. Canola Oil. In: Bailey's Industrial Oil and Fat Products. Vol. 2:

Edible Oil and Fat Products: Oils and Oil Seeds. 5th Edition. Y.H. Hui, ed. John Wiley and Sons, Inc. New York.

Ettlinger, M.G. and Lundeen, A.J. 1956. The structure of sinigrin and sinalbin; An enzymatic rearrangement. Journal of American Chemistry Society 78: 4172-4173.

Ettlinger, M.G. and Lundeen, A.J. 1957. First synthesis of a mustard oil glucoside: the enzymatic Lossen rearrangement. Journal of the American Chemistry Society 79: 1764-1765.

Ettlinger, M. and Kjær, A. 1968. Sulfur compounds in plants. Recent advances in phytochemistry. Mabry, T. J., Alston, R.E. and Runeckles, V. C. (Eds.) North Holland Publishing Co., Amsterdam. Vol 1: pp. 59-144.

Ewing, W.N. 1998. The Feeds Directory: Commodity Products Guide. Context Publications, Ashby de la Zouch, UK, pp. 118.

Fahey, J.W., Zalcman, A.T. and Talalay, P. 2001. The chemical diversity and distribution of gluocosinolates and isothiocyanates among plants. Phytochemistry 56(1): 5-51.

Fahey, J.W., Wadem K.L., Stephenson, K.K. and Chou, F.E. 2003. Separation and purification of glucosinolates from crude plant homogenates by counter-current chromatography. J Chromatography 996: 85-93.

Frohne, D. and Pfander, H.J. 2005. Poisonous Plants. A handbook for Doctors, Pharmacists, Toxicologists, Biologists and Veterinarians. London, Manson Publishing Ltd.

Gardrat, C. and Prevot, A. 1987. Quantitative determination of glucosinolates in rapeseeds and rapeseed meals by an enzymic method. Rev Fr Crops Gras 34: 457–461.

Gorler, K., Krumbiegel, G., Mennicke, W.H. and Siehl, H.U. 1982. The metabolism of benzyl isothiocyanate and its cysteine conjugate in guinea-pigs and rabbits. Xenobiotica 12: 535-542.

Griffiths, D.W., Birch, A.N.A. and Hillmann, J.R., 1998. Antinutritional compounds in the Brassicaceae – Analysis, biosynthesis, chemistry and dietary effects. J. Hort. Sci. Biotech. 73: 1-18.

Guo, D., Schut, H.A., Davis, C.D., Snyderwine, E.G., Bailey, G.S. and Dashwood, R.H. 1995. Protection by chlorophyllin and indole-3 carbinol against 2 amino-1-methyl-6-phenylimidazo[4,5]pyridine (PhIP)-induced DNA adducts and colonic aberrant crypts in F344 rats. Carcinognesis 16: 2931-2937.

Gustine, D. and Jung, G. 1985. Influence of some management parameters on glucosinolate levels in Brassica forage. Agronomy Journal 77: 593–597.

Halkier, B.A. and Gershenzon, J. 2006. Biology and biochemistry of glucosinolates. Annual Review of Plant Biology 57 (1): 303-333.

Hardy, R.W. and Sullivan, C.V. 1983. Canola meal in rainbow trout (Salmo gairdneri) production diets. Can J Fish Aquat Sci 40: 281-286.

Helboe, P., Olsen, O. and Sørensen, H. 1980. Separation of glucosinolates by high-performance liquid chromatography. Journal of Chromatography 197: 199-205.

Higdon, J.V., Delage, B., Williams, D.E. and Dashwood, R.H. 2007. Cruciferous vegetables and human cancer risk: epidemiological evidence and mechanistic basis. Pharmacological Research 55: 224-236.

Holst, B. and Williamson, G. 2004. A critical review of the bioavailability of glucosinolates and related compounds. Nat Prod Rep 21: 425-447.

Hossain, M.A. and Jauncey, K. 1988. Toxic effects of glucosinolate (allyl isothiocyanate) (synthetic and from mustard oilcake) on growth and food utilization in common carp. Indian J Fish 35: 186-196.

Ioannou, Y.M., Burka L.T. and Matthews, H.B. 1984. Allyl isothiocyanate: comparative disposition in rats and mice. Toxicol Appl Pharmacol 75: 173–181.

Karcher, A. and El Rassi, Z. 1999. Capillary electrophoresis of glucosinolates and their degradation products. Electrophoresis 20: 3181-3189.

Kassahun, K., Davis, M., Hu, P., Martin, B. and Baillie, T. 1997. Biotransformation of the naturally occurring isothiocyanate sulforaphane in the rat: identification of phase I metabolites and glutathione conjugates. Chem Res Toxicol 10: 1228-1233.

Kassie, F., Pool-Zobel, B., Parzefall, W. and Knasmuller, S. 1999. Genotoxic effects of benzyl isothiocyanate, a natural chemopreventive agent. Mutagenesis 14: 595-604.

Katamoto, H., Nishiguchi, S., Harada, K., Ueyama, I., Fujita, T. and Watanabe, O. 2001. Suspected oriental mustard (Brassica juncea) intoxication in cattle. Veterinary Records 149(7): 215-216.

Kim D.J., Lee, K.K., Han, B.S., Ahn, B., Bae, J.H. and Jang, J.J. 1994. Biphasic modifying effect if indole-3-carbinol on diethylnitrosamine-induced preneoplastic glutathione S-transferase placenta form-positive liver cell foci in Sprague Dawley rats. Jpn J Cancer Res. 85: 578-583.

Kliebenstein, D. J., Kroymann, J. and Mitchell-Olds, T. 2005. The glucosinolate-myrosinase system in an ecological and evolutionary context. Curr Opin Plant Biol 8(3): 264-71.

Kloss, P., Jeffrey, E., Wallig, M., Tumbleson, M., Parsons, C., Johnson, L. and Reuber, M. 1994. Efficacy of feeding glucosinolate-extracted crambe meal to broiler chicks. Poult Sci 73(10): 1542-51.

Kushad, M.M., Brown, A.F., Kurilich, A.C., Juvik, J.A., Klein, B.P., Wallig, M.A. and Jeffery, E.H. 1999. Variation of glucosinolates in vegetable crops of Brassica oleracea. J Agric Food Chem 47: 1541-1548.

Lange, R., Schumann, W., Petrzika, M., Busch, H. and Marquard, R. 1995. Glucosinolates in linseed dodder. Fat Sci Technol 97: 146-152.

Lardy, G.P. and Kerley, M. S. 1994. Effect of increasing the dietary level of rapeseed meal on intake by growing beef steers. Journal of Animal Science 72: 1936-1942.

Leatherland, J.F., Hilton, J.W. and Slinger, S.J. 1987. Effects of thyroid hormone supplementation of canola meal-based diets on growth, and interrenal and thyroid gland physiology of rainbow trout (Salmo gairdneri). Fish Physiol Biochem 3: 73-82.

Lincheid, M., Wendisch, D. and Strack, D. 1980. The structures of sinapic acid esters and their metabolism in cotelydons of Raphanus sativus. Zeitschrift für Naturforschung 35 C: 907-914.

Mabon, N., Mandiki, S.N.M., Derycke, G., Bister, J.-L., Wathelet, J.P., Paquay, R. and Marlier, M. 2000. Chemical changes and influence of rapeseed antinutritional factor on lamb physiology and performance. Part 3. Antinutritional factor in plasma and organ. Anim Feed Sci Technol 85: 111–120.

Mandiki, S.N.M., Derycke, G., Bister, J.L Mabon, N., Wathelet, J.P., Marlier, M. and Paquay, R., 2002. Chemical changes and influence of rapeseed antinutritional factors on gestating and lactating ewes. Part1: Animal performance and plasma hormones and glucose. Anim Feed Sci Technol 98: 25-35.

Marsh, R.E. and Waser, J. 1970. Refinement of the crystal structure of sinigrin. Acta Crystallographica Section B 20: 1030-1037.

Martland, M.F., Butler, E.J. and Fenwick, G.R. 1984. Rapeseed induced liver haemorrhage reticulolysis and biochemical changes in laying hens: the effects of feeding high and low glucosinolate meals. Research in Veterinary Science 63: 298-309.

Mason, R.W. and Lucas, P. 1983. Acute poisoning in cattle after eating old non-viable seed of chou moellier (Brassica oleracea convar. Acephala). Australian Veterinary Journal 60: 272-273.

Matthäus, B. and Luftmann, H. 2000. Glucosinolates in members of the family Brassicaceae: Separation and identification by LC/ESI-MS-MS. Journal of Agricultural and Food Chemistry 48: 2234-2239.

Matthäus, B. and Zubr, J. 2000. Variability of specific components in Camelina sativa oilseed cakes. Industrial crops and products 12: 9-18.

Mawson, R., Heaney, R.K., Zdunczyk, Z., and Kozlowska, H. 1994a. Rapeseed meal-glucosinolates and their antinutritional effects. Part 3. Animal growth and performance. Nahrung 38, 167-177.

Mawson, R., Heaney, R.K., Zdunczyk, Z., and Kozlowska, H. 1994b. Rapeseed meal-glucosinolates and their antinutritional effects. Part 4. Goitrogenicity and internal organs abnormalities in animals. Nahrung 38: 178-191.

Mawson, R., Heaney, R.K., Zdunczyk, Z. and Kozowska, H. 1995. Rapeseed meal-glucosinolates and their antinutritional effects. Part 6. Taint in end-products. Nahrung 39: 21-31.

McNaughton, S.A. and Marks, G.C. 2003. Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. Br J Nutr 90: 687-697.

Michaelsen, S., Møller, P. and Sørensen, H. 1992. Factors influencing the separation and quantitation of intact glucosinolates and desulfoglucosinolates by micellar electrokinetic capillary chromatography. Journal of Chromatography 608: 363-374.

Milne, J.A. 1990. Brassica leaf and root crops: A review of research findings in relation to animal production. In: Milk and Meat from Forage (BGS Occasional Symposium No. 24), Pollott G.E. (Ed.), British Grassland Society, Reading, UK, pp191-201.

Mithen, R.F., Dekker, M., Verkerk, R., Rabot, S. and Johnson, I.T. 2000. The nutritional significance, biosynthesis and bioavailability of glucosinolates in human food. Journal of the Science of Food and Agriculture 80: 967-984.

Morton, J.M. and Campbell, P.H. 1997. Disease signs reported in south-eastern Australian dairy cattle while grazing Brassica species. Australian Veterinary Journal 75: 109-113.

Morton, J.M. and Morton P.C. 1997. Polioencephalomalacia in cattle in New Zealand fed chou moellier (*Brassica oleacrea*). New Zealand Veterinary Journal 45(1): 37-39.

Musk, S.R.R., Smith, T.K. and Johnson, I.T. 1995. On the cytotoxicity and genotoxicity of allyl and phenethyl isothiocyanates and their parent glucosinolates sinigrin and gluco-nasturtiin. Mutation Research 348: 19-23.

NTP (National Toxicology Program), 1982 NTP carcinogenesis bioassay of allyl isothiocyanate (CAS No. 57-06-7) in F344N rats and B6C3F1 mice (gavage study). Technical Report Series No. 234.

http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr234.pdf

Olsen, O. and Sørensen, H. 1980. Glucosinolates and amines in Reseda media. Phytochemistry 19: 1783–1787.

Olsen, O. and Sørensen, H. 1981. Recent advances in the analysis of glucosinolates. Journal of American Oil and Chemistry Society 58: 857-865.

Opalka, M., Dusza, L., Koziorowski, M., Staszkiewicz, J., Lipinski, K. and Tywonczuk, J. 2001. Effect of long-term feeding with graded levels of low glucosinolate rapeseed meal on endocrine status of gilts and their piglets. Livestock Production Science 69 (3): 233-243(11).

Padilla, G., Cartea, M.E., Velasco, P., de, H.A. and Ordas, A. 2007. Variation of glucosinolates in vegetable crops of Brassica rapa. Phytochemistry 68: 536-545.

Palmieri, S., Rollin, P., Sørensen, H. and Sørensen, S. 1998. Enzyme technology for a potential glucosinolate utilization in Agro-Industry. Agro Food Industry High Tech 9(1): 24-27.

Papas, A., Ingalls, J.R. and Campbell, L.D. 1979. Studies on the effects of rapeseed meal on thyroid status of cattle, glucosinolate and iodine content of milk and other parameters. J Nutr 109: 1129-1139.

Pattanaik, A.K., Khan, S.A., Mohanty D.N. and Vashaney, V.P. 2004. Nutritional performance, clinical chemistry and semen characteristics of goats fed mustard (Brassica juncea) cake based supplements with or without iodine. Small Rumin Res 54: 51-59.

Prestera, T., Fahey, J.W., Holtzclaw, W.D., Abeygunawardana, C., Kachinski, J.L. and Talalay, P. 1996. Comprehensive chromatographic and spectroscopic methods for the separation and identification of intact glucosinolates. Analytical Biochemistry 239: 168-179.

Rao, C.V., Rivenson, A., Simi, B., Hamid, R., Kelloff, G.J., Steele, V. and Reddy, B.S. 1995. Enhancement of experimental colon carcinogenesis by dietary 6-phenylhexyl isothiocyanate. Cancer Res 55: 4311-4318.

Rask, L., Andreasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B. and Meijer, J. 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol Biol 42: 93-113.

Rodriguez, R.A., Maciel, M.G. and de Ochoteco, M.B. 1997. Blindness in Holando Argentine calves due to ingestion of turnip (*Brassica campestris*). Veterinario Argentina 14: 601-605.

Roth-Maier, D.A. 1999. Investigations on feeding full-fat canola seed and canola meal to poultry. Proceedings 10th International Rapeseed Congress, Canberra, Australia, 1999.

Rouzaud, G., Rabot, S., Ratcliffe, B. and Duncan, A.J. 2003. Influence of plant and bacterial myrosinase activity on the metabolic fate of glucosinolates in gnotobiotic rats. Br J Nutr 90: 395-404.

Ryhänen, E.-L., Perttilä, S., Tupasela, T., Valaja, J., Eriksson, C. and Larkka, K. 2007. Effect of Camelina sativa expeller cake on performance and meat quality of broilers. Journal of the Science of Food and Agriculture 87 (8): 1489-1494.

Schmid, A. and Schmid, H. 1992. Rapeseed poisoning of wild herbivores. Tierarzl Prax 20: 321-325.

Schöne, F., Kirchheim, U. and Schumann, W. 1994. Glucosinolate degradation by rapeseed myrosinase and effect on rapeseed acceptability by growing pigs. Animal Feed Science and Technology 48: 229-235.

Schöne, F., Rudolph, B., Kirchheim, U. and Knapp, G. 1997a. Counteracting the negative effects of rapeseed and rapeseed press cake in pig diets. British Journal of Nutrition 78: 947-939.

Schöne, F., Leiterer, M., Jahreis, G. and Rudolph, B. 1997b. Effect of rapeseed feedstuff with different glucosinolate conaten and iodine administration on gestating and lactating sow. Zentralblatt für Veterinärmedizin Reihe A 44: 325-339.

Schöne, F., Leiterer, M., Hartung, H., Jahreis, G. and Tischendorf, F. 2001. Rapeseed glucosinolates and iodine in sows affect the milk iodinie concentration and the iodine status of piglets. British Journal of Nutrition 85: 659-670.

Schuster, A. and Friedt, W. 1998. Glucosinolate content and composition as parameters of quality of Camelina seed. Ind Crops Prod 7: 297-302.

Sørensen, H. 1990. Glucosinolates: Structure-Properties-Function. In: Shahidi, F. (Ed), Rapeseed/Canola: Production, chemistry, nutrition and processing technology. Van Nostrand Reinhold publisher, Chapter 9: 149-172.

Sørensen, H., Sørensen, S., Bjergegaard, C. and Michaelsen, S. 1999. Chromatography and capillary electrophoresis in food analysis. Royal Society of Chemistry, UK, ISBN 85404-561-9, 470 pp.

Spiegel, C., Bestetti, G.E., Rossi, G.L. and Blum, J.W. 1993. Normal circulating triiodothyronine concentration are maintained despite severe hypothyroidism in growing pigs fed rapeseed presscake meal. Journal of Nutrition 123: 1554-1561.

Stanford, K., Wallins, G.L., Smart,W.G. and McAllister, A.A. 2000. Effect of feeding canola screenings on apparent digestibility, growth performance and carcass characteristics of feedlot lambs. Can J Anim Sci 80: 355–365.

Stoner, G., Casto, B., Ralston, S., Roebuck B., Pereira C. and Bailey G. 2002. Development of a multi-organ rat model for evaluating chemopreventive agents: efficacy of indole-3-carbinol. Carcinogenesis 23: 265-272.

Taljaard, T.L. 1993. Cabbage poisoining in ruminants. Journal of South African Veterinary Associaction 64: 96-100.

Tanaka, T., Kojima, T., Morishita, Y. and Mori, H. 1992. Inhibitory effects of the natural products indole-3-carbinol and sinigrin during initiation and promotion phases of 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. Jpn J Cancer Res 83: 835-42.

Tauson, A.H. and Neil, M. 1994. Plasma thyroxine concentration in non-pregnant and lactating mink, and effect of dietary rapeseed oil in the reproduction period. Arch Tierernährung 46(1): 103-9.

Tian, Q., Roselot, R.A. and Schwartz, S.J. 2005. Quantitative determination of intact glucosinolates in broccoli, broccoli sprouts, Brussels sprouts, and cauliflower by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. Analytical Biochemistry 343: 93-99.

Thies, W. 1980. Analysis of glucosinolates via "on-column" desulfation. In: J.K. Daun et al. (ed.) Proc Symp Analytical Chemistry of Rapeseed and its Products Winnipeg, pp. 66-71.

Tripathi, M.K., Mishra, A.S., Misra, A.K., Prasad, R., Modal, D. and Jakhmola, R.C. 2003. Effect of graded levels of high glucosinolate mustard (Brassica juncea) meal inclusion on nutrient utilization, growth performance, organ weight and carcass composition of growing rabbits. World Rabbit Sci 11: 211-266.

Tripathi, M.K. and Mishra, A.S. 2007. Glucosinolates in animal nutrition: A review. Animal Feed Science and Technology 132: 1–27.

Umemura, T., Yamashiro, S., Bhatnagar, M.K., Moody, D.L. and Slinger, S.J. 1977. Liver fibrosis of the turkey on rapeseed products. Research in Veterinary Science 23: 139-145.

Van Doorn, H.E., van Holst, G.J., van Der Kruk, G.C., Raaijmakers-Ruijs, N.C. and Postma, E. 1998. Quantitative Determination of the Glucosinolates Sinigrin and Progoitrin by Specific Antibody ELISA Assays in Brussels Sprouts. J Agric Food Chem 46(2): 793-800.

Van Etten, C.H., Gagne, W.E., Robbins, D.J., Booth, A.N., Daxenbichler, M.E. and Wolf, I.A. 1969. Biological evaluation of Crambe seed meals and derived products by rat feeding. Ceral Chem 42: 507-514.

Virtanen, A.I., Kreula, M. and Kiesvaara, M. 1963. Investigations on the alleged goitrogenic properties of milk. Z Ernährungswiss 3: 23-37.

Wade, K.L., Garrard, I.J. and Fahey, J.D. 2007. Improved hydrophilic interaction chromatographic method for the identification and quantification of glucosinolates. J Chromatography A 1154: 469-472.

Wallig, M.A., Belyea, R.L. and Trumbleson, M.E., 2002. Effect of pelleting on glucosinolates content in Crambe meal. Anim. Feed Sci Technol 99: 205-214.

Wathelet, J.-P., Marlier, M., Severing, M., Boenke, A. and Wagstaffe, P.J. 2006. Measurement of glucosinolates in rapeseeds. Natural Toxins 3: 299-304.

Yoshida, M., Katashima, S., Ando, J., Tanaka, T., Uematsu, F., Nakae, D. and Maekawa, A. 2004. Dietary indole-3-carbinol promotes endometrial adenocarcinoma development in rats initiated with N-ethyl-N'-nitro-N-nitrosoguanidine, with induction of cytochrome P450s in the liver and consequent modulation of estrogen metabolism. Carcinogenesis 25: 2257-64.

DOCUMENTATION PROVIDED TO EFSA

Valkonen, E., Valaja, J., Venäläinen, E., Tupasela, T. and Hiidenhovi, J. 2004. False flax cake in the feed of egg-laying hens. TEST 71, THE 10313504. 04.10.2004. Research report made available to EFSA.

Venäläinen, E., Valaja, J., Valkonen, E., Hiidenhovi, J. and Tupasela, T. 2005. Effects of false flax (Camelina sativa) cake on performance and meat quality of broilers. 20 p. Raisio Oyj 31.10.2005. Research report by MTT AgriFood Research Finland made available to EFSA.

Submission of occurrence data

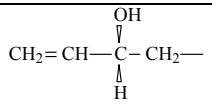
Belgium. The Federal Agency for the Safety of the Food Chain.

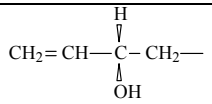
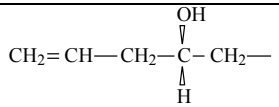
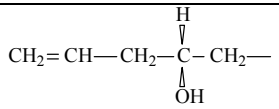
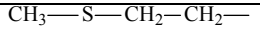
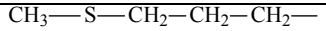
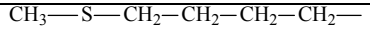
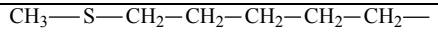
Denmark. Ministry of Food, Agriculture and Fisheries. The Danish Plant Directorate.

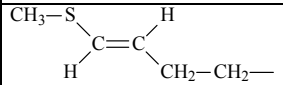
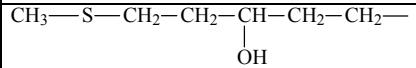
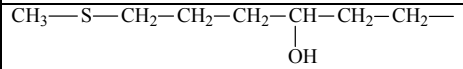
Estonia. Ministry of Agriculture, Estonian Control Centre of Plant Production.

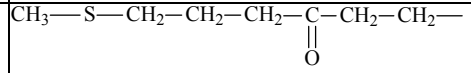
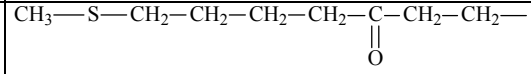
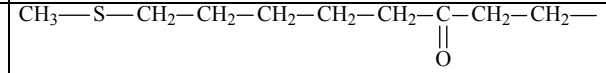
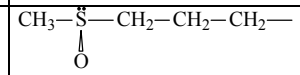
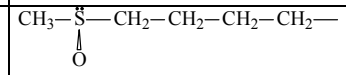
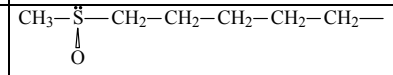
ANNEX. STRUCTURES OF GLUCOSINOLATES

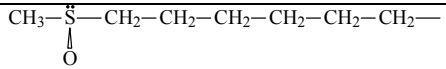
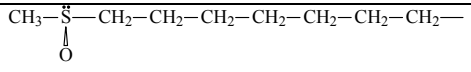
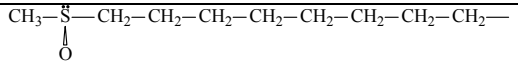
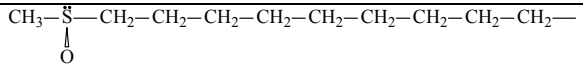
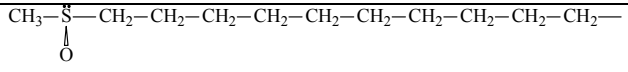
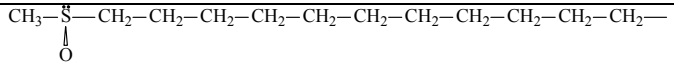
Table A1. Structures and numbers of glucosinolates together with semi systematic names (Ettlinger and Kjær, 1968; Olsen and Sørensen, 1981; Sørensen, 1990) and trivial names which indicate plant source.

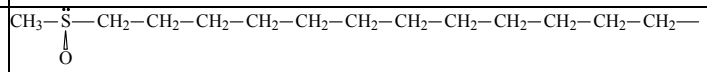
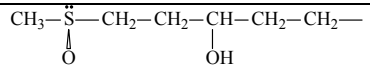
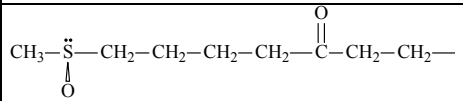
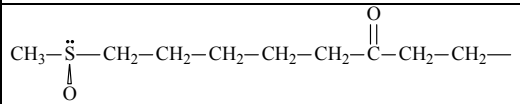
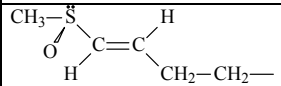
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
0.1	$CH_2=CH—CH_2—$	Prop-2-enylglucosinolate	sinigrin
0.2	$CH_2=CH—CH_2—CH_2—$	But-3-enylglucosinolate	gluconapin
0.3	$CH_2=CH—CH_2—CH_2—CH_2—$	Pent-4-enylglucosinolate	glucobrassicanapin
0.4	$CH_2=CH—CH_2—CH_2—CH_2—CH_2—$	Hex-5-enylglucosinolate	glucowasabiamin
0.5	$CH_2=CH—CH_2—CH_2—CH_2—CH_2—CH_2—$	Hept-6-enylglucosinolate	glucowasajaponicain
1.1		(2R)-2-Hydroxybut-3-enylglucosinolate	progoitrin

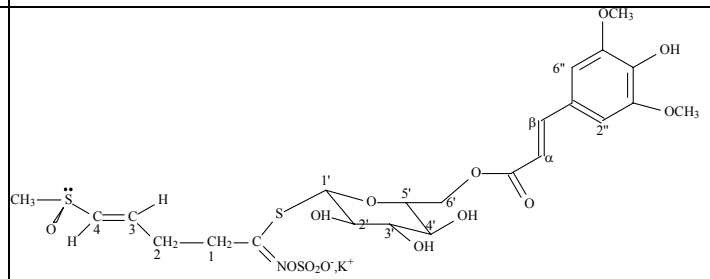
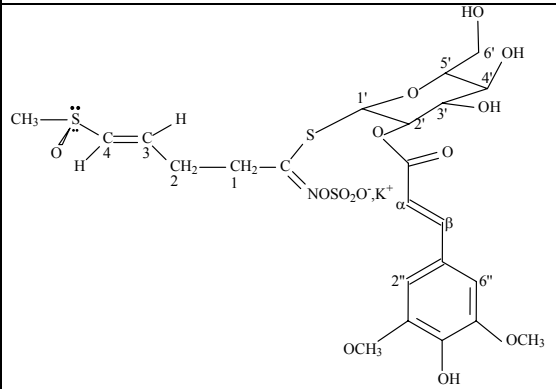
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
1.2		(2S)-2-Hydroxybut-3-enylglucosinolate	epiprogoitrin
1.3		(2S)-2-Hydroxypent-4-enylglucosinolate	napoleiferin
1.4		(2R)-2-Hydroxypent-4-enylglucosinolate	epinapoleiferin
2.1		2-Methylthioethylglucosinolate	glucoamoracialapathifolin
2.2		3-Methylthiopropylglucosinolate	glucoibervirin
2.3		4-Methylthiobutylglucosinolate	glucoerucin
2.4		5-Methylthiopentylglucosinolate	glucoberteroin

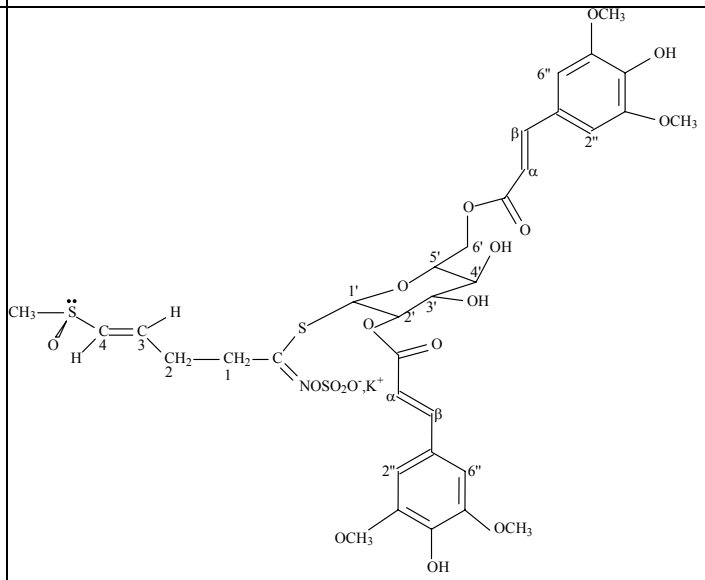
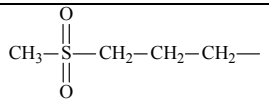
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
2.5	$CH_3—S—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—$	6-Methylthiohexylglucosinolate	glucoarabidopthalianin
2.6	$CH_3—S—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—$	7-Methylthioheptylglucosinolate	glucoarabishirsutain
2.7	$CH_3—S—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—$	8-Methylthiooctylglucosinolate	glucoarabishirsuin
2.8	$CH_3—S—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—$	9-Methylthiononylglucosinolate	glucoarabispurpureain
2.9	$CH_3—S—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—$	10-Methylthiodecylglucosinolate	
2.10		4-Methylthiobut-3-enylglucosinolate	glucoraphasatin
2.11		3-Hydroxy-5-methylthiopentylglucosinolate	
2.12		3-Hydroxy-6-methylthiohexylglucosinolate	

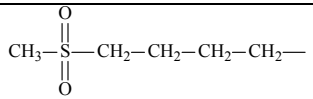
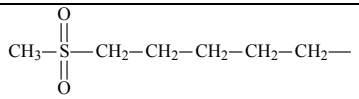
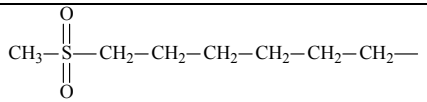
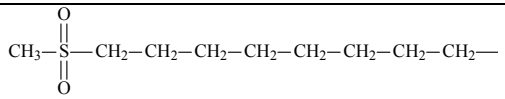
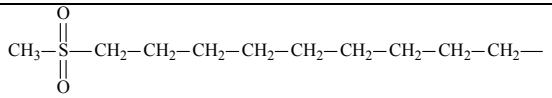
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
2.13		3-Oxo-6-methylthiohexylglucosinolate	
2.14		3-Oxo-7-methylthioheptylglucosinolate	
2.15		3-Oxo-8-methylthiooctylglucosinolate	
3.1		(R)-3-Methylsulfinylpropylglucosinolate	glucoiberin
3.2		(R)-4-Methylsulfinylbutylglucosinolate	glucoraphanin
3.3		(R)-5-Methylsulfinylpentylglucosinolate	glucoalyssin

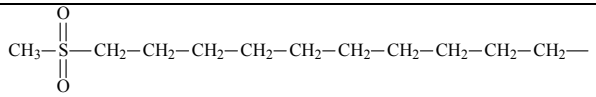
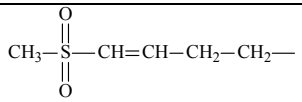
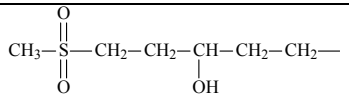
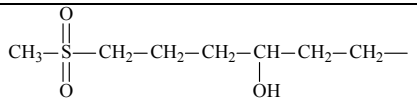
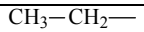
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
3.4		(R)-6-Methylsulfinylhexylglucosinolate	glucohesperalin
3.5		(R)-7-Methylsulfinylheptylglucosinolate	glucoarabidopsithalianin
3.6		(R)-8-Methylsulfinyloctylglucosinolate	glucohirsutin
3.7		(R)-9-Methylsulfinylnonylglucosinolate	glucoarabin
3.8		(R)-10-Methylsulfinyldecylglucosinolate	glucocamelein
3.9		(R)-11-Methylsulfinylundecylglucosinolate	gluconesliapaniculatin

Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
3.10		(R)-12-Methylsulfinyldodecylglucosinolate	glucocamelinain
3.11		(R)-3-Hydroxy-5-methylsulfinylpentylglucosinolate	glucoerysimumhieracifolinin
3.12		(R)-3-Oxo-7-methylsulfinylheptylglucosinolate	
3.13		(R)-3-Oxo-8-methylsulfinyloctylglucosinolate	glucoarabishirsutain (skal ændres)
3.14		(R)-4Methylsulfinylbut-3-enylglucosinolate	glucoraphenin

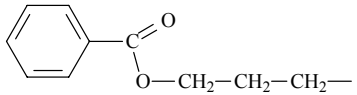
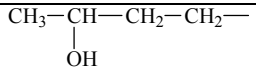
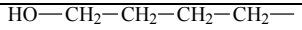
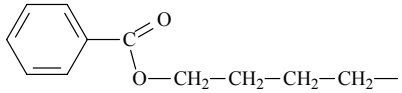
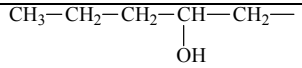
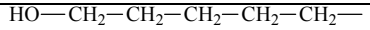
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
3.15		6'-Sinapoyl-(R)-4methylsulfinylbut-3-enylglucosinolate	6'-Sinapoylglucoraphenin
3.16		2'-Sinapoyl-(R)-4methylsulfinylbut-3-enylglucosinolate	2'-Sinapoylglucoraphenin

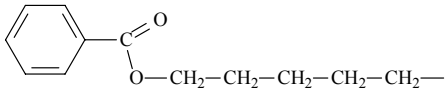
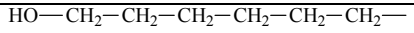
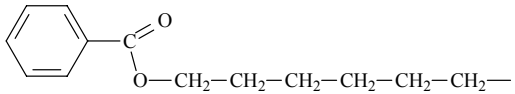
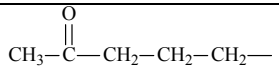
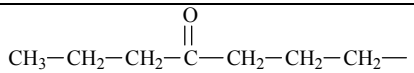
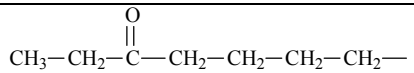
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
3.17		2,6'-Disinapoyl-(R)-4methylsulfinylbut-3-enylglucosinolate	2',6'-Disinapoylglucoraphenin
4.1		3-Methylsulfonylpropylglucosinolate	glucocheirolin

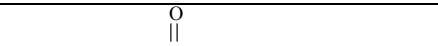
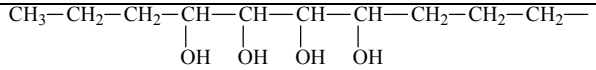
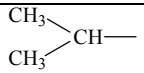
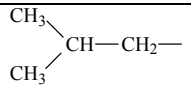
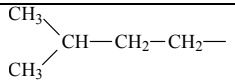
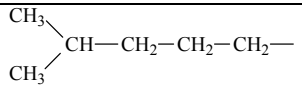
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
4.2		4-Methylsulfonylbutylglucosinolate	glucoerysolin
4.3		5-Methylsulfonylpentylglucosinolate	glucoerysihieracifoliumin
4.4		6-Methylsulfonylhexylglucosinolate	
4.5		7-Methylsulfonylheptylglucosinolate	
4.6		8-Methylsulfonyloctylglucosinolate	

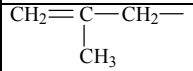
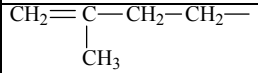
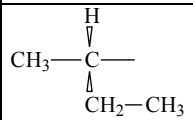
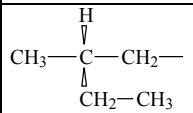
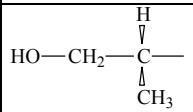
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
4.7		9-Methylsulfonylnonylglucosinolate	
4.8		4-Methylsulfonylbut-3-enylglucosinolate	
4.9		5-Methylsulfonyl-3-hydroxypentylglucosinolate	
4.10		6-Methylsulfonyl-3-hydroxyhexylglucosinolate	
5.1		Methylglucosinolate	glucocapparin
5.2		Ethylglucosinolate	glucoslepidiin

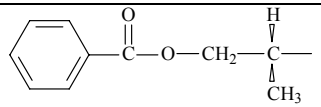
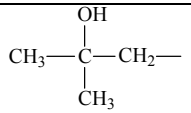
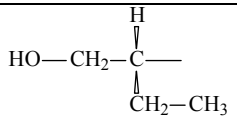
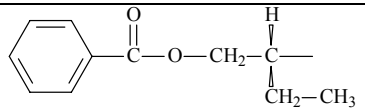
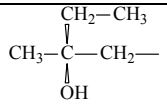
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
5.3	$CH_3-CH_2-CH_2-$	Propylglucosinolate	
5.4	$CH_3-CH_2-CH_2-CH_2-$	Butylglucosinolate	glucocapparisflexuosain
5.5	$CH_3-CH_2-CH_2-CH_2-CH_2-$	Pentylglucosinolate	glucokohlrabiin
5.6	$CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-$	Hexylglucosinolate	glucoraphasativusain
5.7	$CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-$	Heptylglucosinolate	
5.8	$CH_3-CH_2-CH_2-CH=CH-$	Pent-1-enylglucosinolate	
6.1	$HO-CH_2-CH_2-$	2-Hydroxyethylglucosinolate	glucocapparimasiakain
6.2	$CH_3-\underset{\displaystyle OH}{\underset{\vert}{CH}}-CH_2-$	2-Hydroxypropylglucosinolate	glucoarabidopsithalin
6.3	$HO-CH_2-CH_2-CH_2-$	3-Hydroxypropylglucosinolate	glucoerysimumhieracifolium

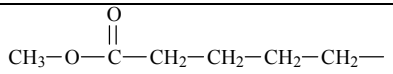
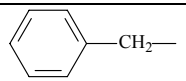
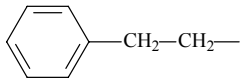
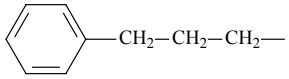
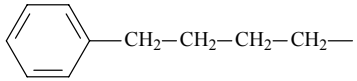
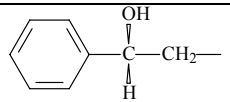
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
6.4		3-Benzoyloxypropylglucosinolate	glucomalcolmiin
6.5		3-Hydroxybutylglucosinolate	glucocappariflexin
6.6		4-Hydroxybutylglucosinolate	glucoarabidopsithalianain
6.7		4-Benzoyloxybutylglucosinolate	
6.8		2-Hydroxypentylglucosinolate	glucoarmoracialapathin
6.9		5-hydroxypentylglucosinolate	glucoarabidopsin

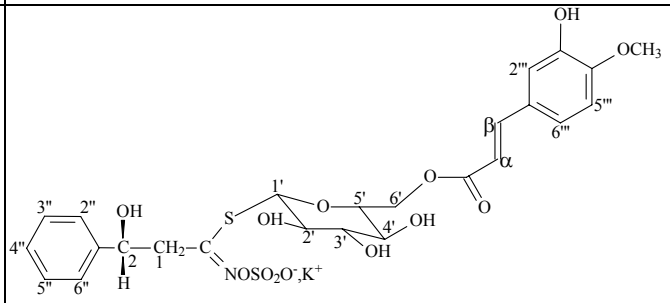
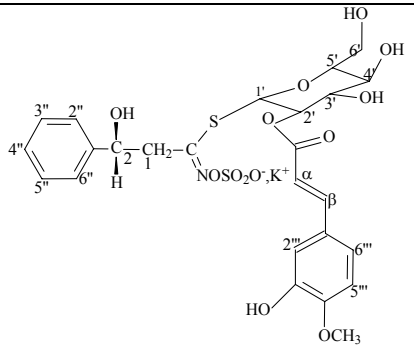
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
6.10		5-Hydroxybenzoyloxypentylglucosinolate	glucoarabidopsithain
6.11		6.Hydroxyhexylglucosinolate	
6.12		6-Hydroxybenzoyloxyhexylglucosinolate	
6.13		3-Oxopentylglucosinolate	
6.14		5-Oxoheptylglucosinolate	glucocapparisangulatain
6.15		6-Oxoheptylglucosinolate	gluconorcappasalin

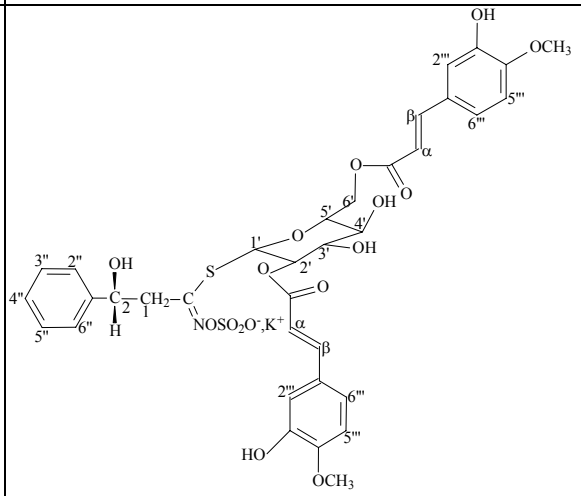
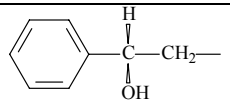
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
6.16		5-Oxooctylglucosinolate	glucocappasalin
6.17		4,5,6,7-Tetrahydroxydecylglucosinolate	glucocapparisgrandisin
7.1		Isopropylglucosinolate	glcuputranjivin
7.2		Isobutylglucosinolate	glucoconringianin
7.3		3-Methylbutylglucosinolate	glucoarmoracialapathin ?ændres
7.4		4-Methylpentylglucosinolate	glucoraphanusativasin

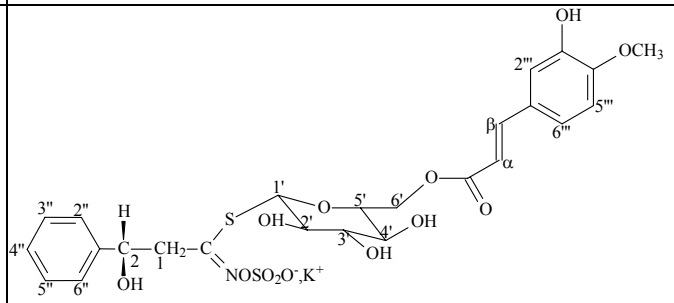
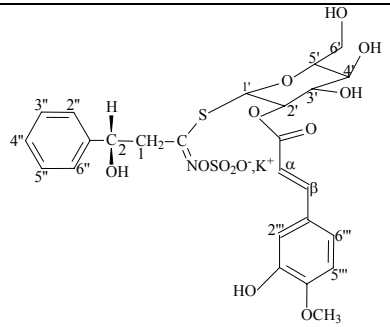
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
7.5		2-Methylprop-2-enylglucosinolate	
7.6		3-Methylbut-3-enylglucosinolate	glucocapparilinearisin
7.7		(1S)-Methylpropylglucosinolate	glucocochlearin
7.8		(2S)-2-Methylbutylglucosinolate	glucojiaputin
8.1		(1R)-2-Hydroxy-1-methylethylglucosinolate	glucosisymbrin

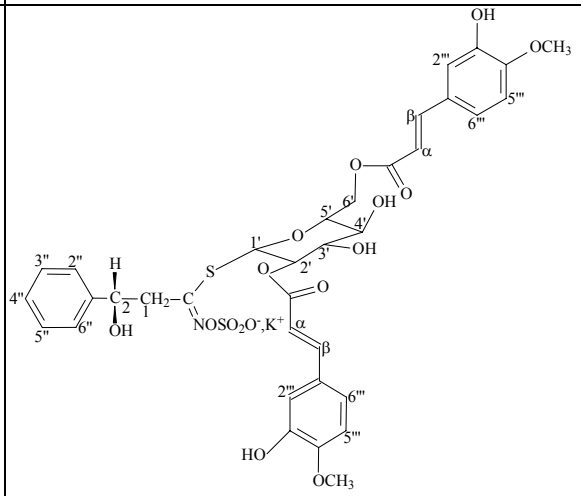
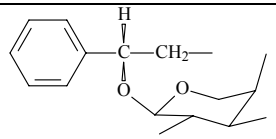
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
8.2		(1R)-2-Benzoyloxy-1-methylethylglucosinolate	glucobenzosisymbrin
8.3		2-Hydroxy-2-methylpropylglucosinolate	glucoresedalbain
8.4		(1R)-2Hydroxy-1-ethylethylglucosinolate	glucosisaustriacin
8.5		(1R)-Benzoyloxy-1-ethylethylglucosinolate	glucoaustriacuin
8.6		(2R)-2-Hydroxy-2-methylbutylglucosinolate	glucocleomin

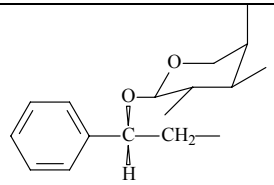
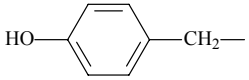
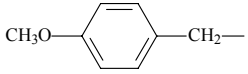
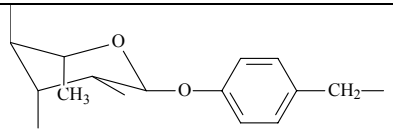
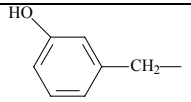
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
9.1		4-Methoxy-4-oxobutylglucosinolate	glucoerypestrin
10.1		Benzylglucosinolate	glucotropaeolin
10.2		Phenethylglucosinolate	gluconasturtiin
10.3		3-Phenylpropylglucosinolate	glucoarmoracialapicin
10.4		4-Phenylbutylglucosinolate	glucoarmoracialafolicin
11.1		(2R)-2-Hydroxy-2-phenylethylglucosinolate	glucosibarin

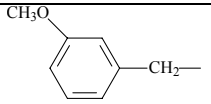
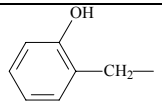
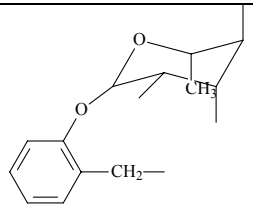
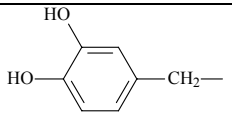
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
11.2		6'-Isoferuloyl-(2R)-2-Hydroxy-2-phenylethylglucosinolate	6'-isoferuloylglucosibarin
11.3		2'-Isoferuloyl-(2R)-2-Hydroxy-2-phenylethylglucosinolate	2'-isoferuloylglucosibarin

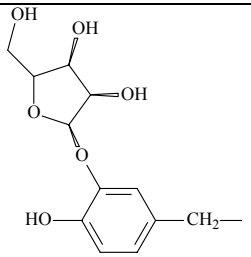
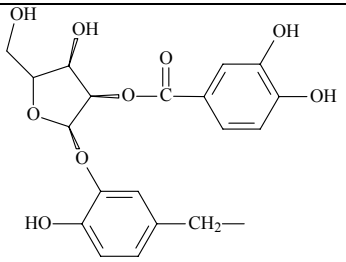
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
11.4		2',6'-Diisoferuloyl-(2R)-2-Hydroxy-2-phenylethylglucosinolate	2',6'-isoferuloylglucosibarin
11.5		(2S)-2-Hydroxy-2-phenylethylglucosinolate	glucobarbarin

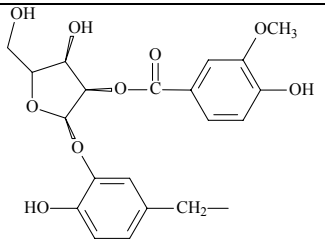
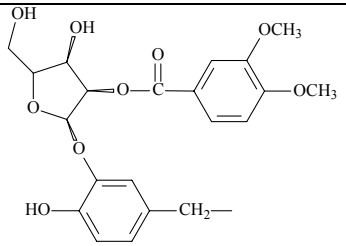
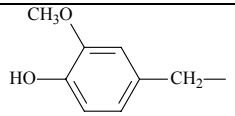
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
11.6		6'-Isoferuloyl-(2S)-2-Hydroxy-2-phenylethylglucosinolate	6'-isoferuloylglucobarbarin
11.7		2'-Isoferuloyl-(2S)-2-Hydroxy-2-phenylethylglucosinolate	2'-isoferuloylglucobarbarin

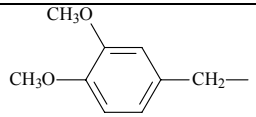
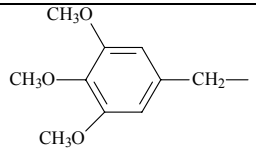
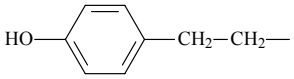
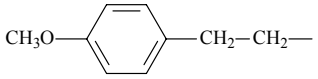
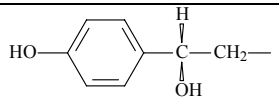
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
11.8		2',6'-Diisoferuloyl-(2S)-2-Hydroxy-2-phenylethylglucosinolate	2',6'-diisoferuloylglucobarbarin
11.9		(2S)-2-α-L-Arabinopyranosyloxy-2-phenylethylglucosinolate	glucosesamoidin

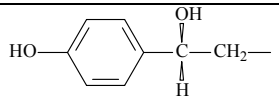
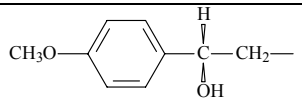
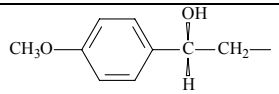
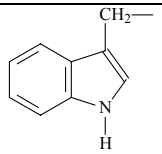
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
11.10		(2R)-2-α-L-Arabinopyranosyloxy-2-phenylethylglucosinolate	glucocanescenein
12.1		4-Hydroxybenzylglucosinolate	sinalbin
12.2		4-Methoxybenzylglucosinolate	glucoaubrietin
12.3		4-α-L-Rhamnopyranolsyloxybenzyl-glucosinolate	glucomoringain
12.4		3-Hydroxybenzylglucosinolate	glucolepigramin

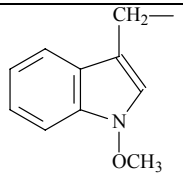
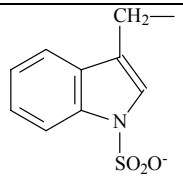
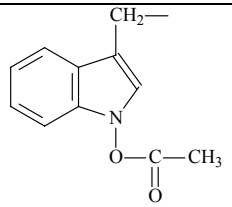
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
12.5		3-Methoxybenzylglucosinolate	glucolimnanthin
12.6		2-Hydroxybenzylglucosinolate	glucoreluteolain
12.7		2-Rhamnopyranosyloxybenzyl-glucosinolate	glucoreodoratain
12.8		3,4-Dihydroxybenzylglucosinolate	glucohesmatrolin

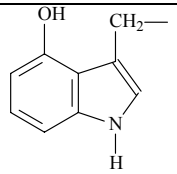
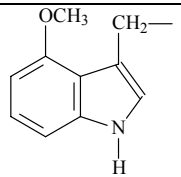
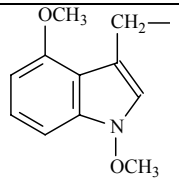
Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
12.9		4-Hydroxy-3-apiofuranosyloxybenzylglucosinolate	glucohesmatrolalin
12.10		4-Hydroxy-3-[2'-(3,4-dihydroxybenzoyl)apiofuranosyloxy]benzylglucosinolate	

Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
12.11		4-Hydroxy-3-[2'-(4-hydroxy-3-methoxybenzoyl)apiofuranosyloxy]benzylglucosinolate	
12.12		4-Hydroxy-3-[2'-(3,4-dimethoxybenzoyl)apiofuranosyloxy]benzyl glucosinolate	
12.13		4-Hydroxy-3-methoxybenzylglucosinolate	glucoelongatin

Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
12.14		3,4-dimethoxybenzylglucosinolate	glucolongifoliain
12.15		3,4,5-trimethoxybenzylglucosinolate	glucolepidiumhyssopifolium
12.16		2-(4-Hydroxyphenyl)ethylglucosinolate	
12.17		2-(4-Methoxyphenyl)ethylglucosinolate	
12.18		(2S)-2-Hydroxy-2-(4-hydroxyphenyl)ethylglucosinolate	glucoarabihirsuin

Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
12.19		(2R)-2-Hydroxy-2-(4-hydroxyphenyl)ethylglucosinolate	glucoarabihirin
12.20		(2S)-2-Hydroxy-2-(4-methoxyphenyl)ethylglucosinolate	
12.21		(2R)-2-Hydroxy-2-(4-methoxyphenyl)ethylglucosinolate	
13.1		Indol-3-ylmethylglucosinolate	glucobrassicin

Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
13.2		N-Methoxyindol-3-ylmethylglucosinolate	neoglucobrassicin
13.3		N-Sulfoindol-3-ylmethylglucosinolate	sulfoglucobrassicin
13.4		N-Acetylindol-3-ylmethylglucosinolate	N-acetylglucobrassicin

Number	Structure of side chain (or complete glucosinolate)	Semisystematic name	Trivial name
13.5		4-Hydroxyindol-3-ylmethylglucosinolate	4-hydroxyglucobrassicin
13.6		4-Methoxyindol-3-ylmethylglucosinolate	4-methoxyglucobrassicin
13.7		1,4-Dimethoxyindol-3-ylmethylglucosinolate	