

The role and effects of glucosinolates of *Brassica* species – a review

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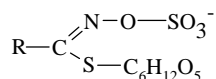
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ABSTRACT

Glucosinolates are the substituted esters of thio amino acids and their synthesis is based on the corresponding amino acids. Methionine and cysteine are the natural donors in the case of the *Brassica* plants and *L*-tryptophane in the indole glucosinolates, respectively. In *Brassica* genus, alkenyl glucosinolates are mostly present and their content and composition differ as far as the development stage and the part of the plant are concerned. The indole glucosinolates are present in a minority level. Their role of sulphur supply is questioned by their very low content between 2% in the beginning of vegetation and 0.1% in its end. Glucosinolates are discussed mostly from the aspect of their anti-nutrition, anti-microbial, anti-fungicidal, and anti-bacterial effects and as being natural bio-fumigants. Their decomposition products have the mentioned properties. The products originate by prepared passive protection by the two-component system. From the aspect of these properties, it is useful to divide them into the following three groups according to the characters of their decomposition products. The first group (I.), whose hydrolysis in the neutral and alkaline environment creates iso-thio-cyanates. These bioactive compounds form the natural protection of the plant with bio-fumigatory effects particularly. Their anti-nutritive effects can be compensated by iodine, contrary to the second group (II.). This group is created by hydroxy-glucosinolates, whose decomposition products – iso-thio-cyanates – are not stable and they cycle while producing substituted 2-oxazolidinethione (goitrine – VTO). These glucosinolates represent a serious problem in feed industry since the VTO has a strong goitrogenic property. The third group (III.) – glucosinolates containing the indole group or the benzene ring (Sinalbin), create thio-cyanates during their hydrolysis. The role of indole glucosinolates has not been completely clarified so far. Their anti-carcinogenic effects are studied and they fulfil the role of an active protection.

Keywords: *Brassica* genus; glucosinolates; biosynthesis; hydrolysis; iso-thio-cyanates; physiological function; anti-nutritive effects; anti-bacterial, anti-microbial and anti-fungicidal properties

From the chemical aspect, glucosinolates are the substituted esters of thio-amino acids with the following chemical structure (Ettlinger 1956):



The glucosinolate biosynthesis is based on the corresponding amino acids as shown in the following schema: amino acid → N-hydroxy-amino acid → aldoxime → thio-hydroxy acid → de-sulpho glucosinolate → glucosinolate (Halkier and Du 1997). During the first phase, the modification of the original amino acid occurs, which differs according to the character of the *R* side chain of the synthesised glucosinolate. The donor of the thio-group participates in this biosynthesis. Methionine or cysteine amino acids are these natural donors. These amino acids are significant amino acids of the *Brassica* plants. By the prolongation of the carbon chain, homo-methionine amino acid originates and 2-amino-6-methyl-thio-hexane and 2-amino-7-methyl-thio-heptane acids are the other products (Magrath et al. 1994). Apart from the alkenyle glucosinolates, the *Brassica* plants contain also the glucosinolates with the indole group, whose biosynthesis starts from *L*-tryptophane (Kutáček et al. 1962), however, contrary to methionine, the chain prolongation does not occur. Glucose is transferred in its activated form by uridine-biphosphate-glucose (UDPG). The sulphate

group is then transferred by phospho-adenosine-phosphosulphate (PAPS). So, the glucosinolates of the *Brassica* plants originate simultaneously in two biosynthetic pathways where the formation of progoitrine and gluconapine participates in the general direction and in the biosynthesis course in the most significant manner (Zukalová et al. 1986).

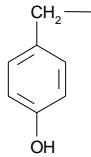
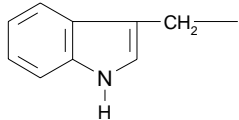
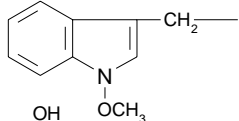
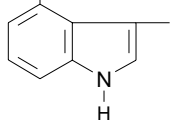
So far, more than 90 glucosinolates (Fenwick et al. 1983) have been determined and further are discovered due to employing more advanced analytical methods exploiting the liquid and gas chromatography, in particular. For the *Brassica* genus, 8 the most significant substances have been identified so far. The sinalbin cyclic glucosinolate is contained in white mustard plant (Table 1).

The *R* group structure is derived from amino acids and it can be of aliphatic, cyclic or hetero-cyclic characters (Table 1).

In *Brassica* genus, alkenyl-glucosinolates are mostly present and their content and the composition differ as far as the development stadium and the specific part of the plant are concerned. The indole-glucosinolates are presented in their minority.

The toxic compounds originate by the hydrolytic decomposition of the aliphatic glucosinolates (Table 1). This hydrolysis runs due to the action of myrosinase enzyme and it is located separately in the idioblast cells, while glucosinolates are located in the parenchymatous

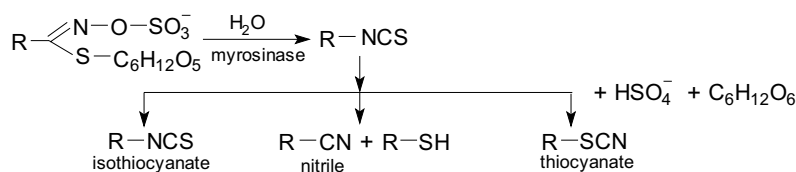
Table 1. Systematic and trivial names of main glucosinolates of *Brassica* species

Systematic name	Trivial name	R
I. aliphatic		
2-propenyl (allyl)-GSL	sinigrin	$\text{CH}_2=\text{CH}-\text{CH}_2$
3-butenyl-GSL	gluconapin	$\text{CH}_2=\text{CH}-(\text{CH}_2)_2$
4-pentenyl-GSL	glucobrassicinapin	$\text{CH}_2=\text{CH}-(\text{CH}_2)_3$
II. hydroxy-aliphatic		
2-hydroxy-3-butenyl-GSL	progoitrin	$\text{CH}_2=\text{CH}-\text{CHOH}-\text{CH}_2$
2-hydroxy-4-pentenyl-GSL	napoleiferin	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{CHOH}-\text{CH}_2$
III. cyclic		
4-hydroxybenzyl-GSL	sinalbin	
hetero-cyclic (indolyl)		
3-indolylmethyl-GSL	glucobrassicin	
1-methoxy-3-indolylmethyl-GSL	neoglucobrassicin	
4-hydroxy-3-indolylmethyl-GSL	4-hydroxyglucobrassicin	

I. iso-thio-cyanates originate by hydrolysis, II. oxazolidine-2-thions originate by hydrolysis, III. thio-cyanates originate by hydrolysis
 GSL = glucosinolates

tissues (Guignard 1980a, b). Damage of these tissues, both by grinding, digestion, mechanical injury and damage by insect, their connection (merging) and self-hydrolysis occur.

No primary physiological role is attributed to glucosinolates. However, as the secondary metabolites and in their intact form they have a series of effects and the role of being the supplier of sulphur is attributed to them.



In progoitrin (*R* is $\text{CH}_2=\text{CH}-\text{CHOH}-\text{CH}_3$), the hydrolytically created 2-hydroxy-3-butenyl-iso-thio-cyanate cycles while producing 5-vinyl-2-oxazolidinethione (VTO) (alkyl-2-oxazolidinethione for napoleiferine).



Glucosinolates as sulphur supply

Rape plant is a demanding crop as far as the sulphur nutrition is concerned, for example, Mc Grath and Zhao (1996) state the need of 16 kg of sulphur for ensuring the production of 1 metric ton of rape plant seeds. Sulphates are the main uptake form of sulphur for the plants, for which the necessity of active uptake is assumed for get-

ting over the negative electro-chemical gradient beyond the cell plasmalemma. SO_4^{2-} metabolising of the organic compounds, similarly as for NO_3^- , requires their reduction (Mengel and Kirkby 1982). Sulphur is a building element of the essential amino acids (methionine, cysteine), which are essential parts of quality (full scope) proteins. The synthesis of the amino acids can be considered as primary metabolites. Glucosinolates, as the secondary metabolites, are the minority component of the sulphur compounds. The seed is the primary distribution place of glucosinolates. Their minimum content was found in the vegetative parts of the rape plant. With advancing vegetation, the shift of their higher content was recorded in the following vegetation parts of rape plant: roots, stems, and flowers, compared to the leaves.

Glucosinolates create less than 5% of total sulphur in the start of vegetation in the individual plant parts and their content decreases in the course of growth. The low content of glucosinolates in the rape plant vegetation parts questions their supply function (Zhao et al. 1993a, b, Fieldsend and Milford 1994), while the content of the total sulphur and sulphates in the leaves during maturing in the wrapping layers of the siliques of rape plants, reflects the nutritional status of rape plant in the best way.

Sulphates represent the substantial component of the content of the total sulphur in the vegetative parts of rape plants. It is still a question, if it is the exhibition only of the low effectiveness of their exploitability for the synthesis of the primary and secondary metabolites or the mineral form of sulphur – the sulphates in rape plant fulfil further mission.

For further considerations concerning the physiological effects of glucosinolates according to the character of the decomposition products (Table 1), their division into three groups is useful:

The compound first group (I.) (Table 1), in which iso-thio-cyanates creation by their hydrolysis in the neutral and alkaline environment is the most frequent. These bio-active compounds have the following effects:

- 1. The anti-nutritive effects.** They bind iodine selectively and they prevent thyroidal gland from the iodine intake. This effect can be compensated by the addition of iodine salts into food or feeding mixtures.
- 2. The anti-microbial, anti-fungicidal, anti-bacterial and thyroidal properties, which create the natural protection of the plant itself.** Glucosinolates are listed amongst the natural pesticides, which are produced by the higher plants for increasing their resistance against the unfavourable effects of the predators, competitors and parasites since they exhibit the toxic or repellent effects and, therefore, they have important position in the protective mechanism of rape plant against pests and diseases (Mithen 1992, Wallsgrove et al. 1999). Two significant classes of the natural pesticides, among them even glucosinolates can be listed, are created by phyto-alexines and phyto-anticipines. The basic difference between the two listed classes is

based on the mechanism of their creation: Phyto-alexines originate as the result of an external influence induced by the modified metabolic activity of the plant *de novo* (they represent the active protective mechanism). Phyto-anticipines originate from already created precursors, which are produced by a healthy plant from the very beginning of its growth start and that serve only as the passive protection against possible pests. Glucosinolates are the typical example of such precursors. The two-component system of glucosinolates – myrosinase represents an in advance prepared protective system, which is activated only due to an attack and subsequent damage of the plant tissue, after which the enzymatic hydrolysis of glucosinolates occurs with the creation of bio-active iso-thio-cyanates. These volatile compounds – iso-thio-cyanates have a wide spectrum of their anti-microbial effects and they act as repellents against certain species of insect (Giamoustaris and Mithen 1995).

- 3. The function of glucosinolates as bio-fumigants.** It is based on the same hydrolytic principle as the natural protection of the plant with the difference that the ploughed in biomass of green manure leaves the bioactive iso-thio-cyanates in soil, which have the significant bio-fumigatory effects (Kirkegaard et al. 1999) for the subsequent cultivation of vegetables, in particular. The same effect have even the post-harvest residues of rape plants, which, due to iso-thio-cyanates contained in them, have the significant bio-fumigatory effects for the subsequent crops and, therefore, the rape plant is the unique remedial pre-crop for cereals. Iso-thio-cyanates are the significant substance of the synthetic bio-fumigants, where, apart from the aliphatic iso-thio-cyanates (Sarwar et al. 1998), are also aromatic forms that exhibit higher toxicity.

The second group (II.) are hydroxy-glucosinolates (Table 1). This group is, as far as its composition is concerned, much smaller, however, from the anti-nutritional effect, it is the most significant. The decomposition products of hydroxy-glucosinolates-iso-thio-cyanates are not stable and they cycle while creating the substituted 2-oxazolidinethione (goitrine – VTO).

The anti-nutritive effects

These glucosinolates represent a serious problem in feed industry since they decrease the exploitability of the rape plant meals and scrapes during feeding of farm animals. The decomposition product – goitrine – is strongly goitrogenic substance. It inhibits the synthesis of the thyroxine and tri-iodine-thyronine thyroidal hormones. Its negative effect cannot be corrected by the increased intake of iodine (Virtanen 1963, Thompson 1983) and, therefore, it is the limiting factor – due to its nutritional properties – in the exploitation of the rape plant meals and scrapes in feeding mixtures. Their high content in the rape plant seeds led to minimising of these substances

Table 2. The contents of the individual glucosinolates of *Brassica* genus ($\mu\text{mol/g}$ extr. meal)

Variety	Glucosinolates (GSL)				
	sinigrin	gluconapine	glucobrassicinapine	progoitrine	ΣGSL
Lirajet ³	–	4.13	0.47	12.18	16.78
Pronto ⁴	–	4.38	0.47	9.60	14.45
Lirajet GMO ¹	–	1.95	0.14	5.85	7.94
Ms8-Rf ₃ ²	–	2.08	0.25	6.08	8.41
Jet Neuf ⁵	–	32.48	2.70	90.43	130.61
<i>Brassica juncea</i>	122.41	14.11	0.94	3.98	141.44
Rex turnip rape	–	36.97	11.76	6.32	55.05

¹ transgenic rape plant 00 – Roundup Ready

² transgenic rape plant 00 – Basta resistant (Liberty Link)

³ lineal rape plant 00

⁴ hybrid rape plant 00

⁵ lineal rape plant 0

Data from analytical laboratory of Department of Crop Production, Czech University of Agriculture in Prague

using the genetic and breeding means. The current assortment of the 00-cultivated rape plants has the glucosinolate content decreased to the tenth of the original content of the content of the high erucic and zero erucic acid rape plant so called 0-rape seed varieties (Table 2). Progoitrine is contained in the rape plant seeds at the level of about 71% of the total glucosinolates and, therefore, it is the most risky aspect from the feed industry point of view. Contrary to this, in turnip rape and *Brassica juncea*, the protective and bio-fumigatory effects should prevail due to the prevailing composition of the glucosinolate of the Ist class.

The third group (III.) are glucosinolates containing the indole group or the benzene ring (sinalbin). Iso-thiocyanates are formed by their hydrolysis. Currently, they appear in larger amounts in the seeds of the low-glucosinolate rape plants.

The anti-nutritional properties

It is not completely clear if they are the cause of the nutritional or toxic problems. Currently, their anti-carcinogenic properties and their function in the metabolism of the growth hormones are studied. Food is considered to be the most significant factor playing its role in the cancer incidence (Doll and Peto 1981) due to the fact that the human beings intake an enormous amount of the substances in food. These substances significantly speed up the cancerogenesis process. Therefore, a significant attention is paid to the natural substances contained in the food of plant origin (so called phyto-chemicals) since the great number of epidemiological studies documents the positive effects of increased consumption of fruits and vegetables (Block et al. 1992, Steinmetz and Potter 1996). Particularly, the results related to the *Brassica* vegetables are outstanding in the prevention of the large intestine and rectum cancer, breast cancer and cancer of stomach and lungs (Verhoeven et al. 1996). The anti-carcinogenic ef-

fects of the *Brassica* vegetables are attributed even to some decomposition products of glucosinolates – indoles and iso-thiocyanates. Currently, the metabolism of carcinogens and the capacity of the biologically active substances to intervene in it are studied.

The natural protection of plant

These glucosinolates in the *Brassica* plants participate in the active protection and they fulfil the role of phytoalexines (Takasugi et al. 1986), since Griffiths et al. (1994) have proved the fact that the increased biosynthesis of glucosinolates is triggered after the attacking by some species of pests. These are indole-glucosinolates, which are capable to release auxin during the effect of the extraordinary external factors (frost, diseases) on the plant. Therefore, the indole metabolism of the *Brassica* plants represents not the main way but one of further pathways of the auxin formation during its critical need. The study of the indole glucosinolates and their metabolism requires – due to their low light and thermal stability, and even due to quick changes during a day, a completely independent study and it will be the subject of the independent and very demanding work.

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ABSTRAKT

Úloha a účinky glukosinolatů druhů *Brassica* – studie

Glukosinoláty jsou substituované estery thioaminokyselin a jejich biosyntéza vychází z příslušných aminokyselin. Přírodním donorem v případě brukvovitých je aminokyselina methionin nebo cystein a u indolových glukosinolatů *L*-tryptofan. U rodu *Brassica* jsou to především alkenylglukosinoláty a jejich obsah a složení se liší vývojovým stadiem a částí rostliny, v níž se vyskytují. Indolové glukosinoláty jsou v minoritním zastoupení. Úloha zásobárny síry je zpochybněna jejich velmi nízkým obsahem, pohybujícím se mezi 2 % na počátku vegetace a 0.1 % na jejím konci. Glukosinoláty jsou především diskutovány z pohledu svých účinků antinutričních, antimikrobiálních, antifungicidních, antibakteriálních a jako přirozené biofumiganty. Tyto vlastnosti mají jejich rozkladné produkty, vzniklé připravenou pasivní obranou dvousložkovým systémem glukosinolatů – myrosinasa. Z hlediska těchto účinků je užitečné podle charakteru rozkladných produktů jejich rozdělení do tří skupin. Do první skupiny (I.) patří glukosinoláty, které hydrolyzou v neutrálním a alkalickém prostředí tvoří

isothiokyanáty, přičemž tyto bioaktivní sloučeniny vytvářejí především přirozenou ochranu vlastní rostliny s biofumigačními účinky. Jejich antinutriční účinky jsou kompenzovatelné jódem na rozdíl od druhé skupiny (II.), kterou tvoří hydroxyglukosinoláty, jejichž rozkladné produkty isothiokyanáty nejsou stabilní a cyklizují za vzniku substituovaného 2-oxazolidinethionu (goitrinu – VTO). Tyto glukosinoláty představují závažný problém v krmivářství vzhledem k tomu, že VTO je silně strumigenní. Třetí skupinou (III.) jsou glukosinoláty obsahující indolovou skupinu nebo benzenové jádro (sinalbin), jejich hydrolýzou vznikají thiokyanáty. Úloha indolových glukosinolátů není zatím zcela jasná. Je známo, že plní funkci aktivní obrany, a studují se jejich antikarcinogenní vlastnosti.

Klíčová slova: rod *Brassica*; glukosinoláty; biosyntéza; hydrolýza; isothiokyanáty; fyziologická funkce; antinutriční účinky; antibakteriální, antimikrobiální a antifungicidní vlastnosti

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