

CYANOGENIC GLYCOSIDES - THEIR ROLE AND POTENTIAL IN PLANT FOOD RESOURCES

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Review



ABSTRACT

Plants have metabolites and mechanisms that provide them with the basic building blocks for germination, growth, and reproduction processes, while providing them with protection and supporting their adaptation to environmental stresses. Due to their multifunctional significance, cyanogenic glycosides (CNG) indicate the importance of their role in the plant organism. Their diversity and biochemical origin are worth noting. Paradoxically, several nutritionally important food resources of plant origin are characterized by the presence of cyanogenic glycosides in various tissues. Processing approaches of plant food resources ensure a reduction in the content of these ingredients to an acceptable safe level. Different bacteria-based biotechnological processes are applied to minimize the content of CNG in food products. For the usability and identification of the added value of plant food resources, it is important to know the functions and importance of antinutritional components of metabolism with a consequent impact on nutrition and health. In the review we provided a comprehensive view of the importance and potential of CNG in plants with a focus on food resources, where the model object was presented by linseed.

Keywords: cyanogenic glycosides, plant food resources, linseed, *Linum usitatissimum* L.

INTRODUCTION

Natural phytotoxins called cyanogenic glycosides are produced by over 3000 plant species within 130 different families many of which are consumed by humans (Nielsen *et al.*, 2016). These water-soluble secondary metabolites have a major role in the defense mechanism, regulation of the cell signaling and growth (Mosayyebi *et al.*, 2020). In addition, the endogenous turnover of cyanogenic glycosides without the release of a hydrogen cyanide (HCN) may offer plants an important source of reduced nitrogen and glucose at specific developmental stages (Pičmanová *et al.*, 2015). In the edible plants were found at least 25 known cyanogenic glycosides (Bolarinwa *et al.*, 2016). The first studies of their biosynthesis were initiated in 1967 with linen flax seedlings (Sørensen, Neilson, and Møller, 2018). The biosynthetic pathway is catalyzed by multifunctional cytochrome P450 enzymes (CYP79 and CYP71) in combination with the P450 redox partner NADPH oxidoreductase and UDP-glucosyltransferase. These enzymes are organized into a dynamic enzyme complex termed a metabolon to ensure a rapid metabolism (Gleadow and Møller, 2014). Cyanogenic glycosides are considered as anti-nutrients what means that a breeding process is set up for optimizing the composition of their beneficial nutrients and minimizing cyanogenic glycosides in the main product (Hartanti and Cahyani, 2020). Therefore, these two sides of cyanogenic glycosides lead the breeding activities to find balance between defensive responses and food safety (Sun *et al.*, 2017).

CHARACTERISTICS OF CYANOGENIC GLYCOSIDES

Cyanogenic glycosides are a large group of the herbal secondary metabolites composed of non-sugar aglycone of α -hydroxynitrile-type and sugar moiety (glycone) linked via a glycosidic bond (Mosayyebi *et al.*, 2020). Cyanogenic glycosides are located in the vacuoles separated from their hydrolytic enzymes (β -glucosidases) which are in a cytosol (Duodu and Apea-Bah, 2017). In case of a cell disruption, glycosides are rapidly broken up to sugar and a cyanohydrin which is immediately degenerated to the hydrogen cyanide (HCN) and a remaining aldehyde or a ketone (Nyirenda, 2020). The general structure of cyanogenic glycosides is presented in the figure 1 where the aglycone part is labeled as R1 and four possible positions of substituents attached to the glucose moiety as R2 – R5 (Yulvianti and Zidorn, 2021).

The cytochrome P450 (CYP) monooxygenases are probably the most catalytic biocatalysts widely founded in different life forms including multicellular eukaryotes (animals and plants), unicellular eukaryotes (fungi and protists), and prokaryotes (bacteria and archaea). They constitute a huge and intricate gene superfamily of heme-thiolate proteins that play an important role in the metabolism of various substrates (Jiu *et al.*, 2020). Eukaryotic P450s are type-II membrane-anchored enzymes that serve as molecular platforms for anchoring metabolons to the endoplasmic reticulum. This dynamic multitasking organelle is connected to several compartments of the cell and is involved in many processes such as protein synthesis, signaling, and primary and secondary metabolism (Griffing *et al.*, 2017). Its membrane is highly crowded with proteins which can be inadvertently competing and in hazardous crosstalk for common substrates with each other within the overlapping P450-mediated metabolic pathways (Guigas and Weiss, 2016). To avoid complications the enzymes are formed into the metabolon what is a highly specialized and dynamic complex of sequential biosynthetic enzymes responding to environmental conditions (Bassard, Møller and Laursen, 2017). In the figure 2 is a model of the metabolon responsible for linamarin synthesis in flax which includes the two membrane-anchored cytochrome P450 enzymes (CYP79D1 and CYP71E7), the membrane-anchored cytochrome P450 reductase and the soluble UDP-glucosyl transferase UGT85K (<https://www.genome.jp/kegg>, Kyoto Encyclopedia of Genes and Genomes). The first enzyme is a member of the CYP79 family and catalyzes the conversion of an amino acid to the corresponding aldoxime. CYP71 family member subsequently converts the aldoxime to a cyanohydrin which is afterwards stabilized by glucosylation to form the final cyanogenic glycosides in a reaction catalyzed by a UGT from the UGT85 family (Hansen *et al.*, 2018). Nowadays, the cytochrome P450 aggregates more than 18687 protein-coding genes which are helpfully organized into families and subfamilies in view of the percentage of an amino acid similarity (Alzahrani and Rajendran, 2020).

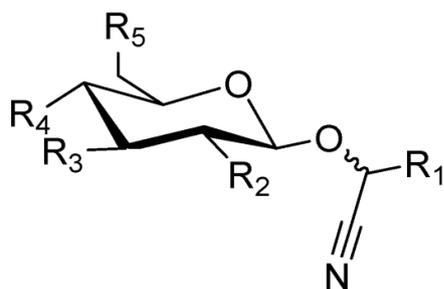


Figure 1 General structure of cyanogenic glycoside (Yulvianti and Zidorn, 2021).

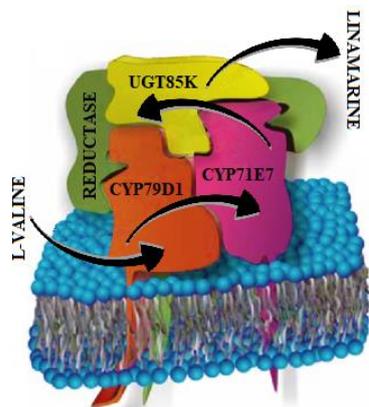


Figure 2 Metabolon responsible for linamarin synthesis from (Yulvianti and Zidorn, 2021). L-valine in flax (Bak et al., 2006, modified by Harenčár).

Synthesis of cyanogenic glycosides

Cyanogenic glycosides in plant are derivatives of five amino acids (L-valine, L-isoleucine, L-leucine, L-phenylalanine, and L-tyrosine) and the non-proteinogenic amino acid cyclopentenyl glycine (Nyirenda, 2020) (Table 1). The most represented cyanogenic glycosides in flax (*Linum usitatissimum* L.) are linamarin and lotaustralin with their diglucosidic forms linustatin and neolinustatin (Zuk et al., 2020). The biosynthetic pathways of these two respectively four secondary metabolites (Figure 3) are quite similar and initiated by N-hydroxylations of L-valine (linamarin) or L-isoleucine in case of lotaustralin. This reaction can be done with use of the same valine or isoleucine N-monoxygenases e.g., CYP79D1, CYP79D2, CYP79D3, CYP79D4. The next step includes three different activities which are isomerization of the substrate (E) isomer ((E)-2-methylpropanal-oxime or (1E,2S)-2-methylbutanal oxime) to the (Z) isomer, dehydration, and C-hydroxylation by the same (E)-2-methylbutanal oxime monooxygenase (CYP71E7). The acetone cyanohydrin β -glucosyltransferase from family UGT85K provides the last step of the cyanogenic glycoside biosynthesis as an attachment of the glucose coming from the UDP- α -D-glucose. The diglucosidic forms linustatin and neolinustatin are created by an addition the second glucose with other UDP-glucosyltransferase (MetaCyc, 2021).

CYANOGENIC GLYCOSIDES IN PLANT FOOD PRODUCTS

The cyanide poisoning in humans is a serious health issue especially in the tropical regions where cassava or bamboo shoots are a primary nutritional staple (Panter, 2018). Cyanide inhibits a utilization of an oxygen and increases the anaerobic metabolism what lead to an excess of lactic acid and metabolic acidosis and finally to cell death through energy deprivation (Dusemund et al., 2017). The human body can tolerate a low level of HCN and converts it into the thiocyanate which is excreted in a urine (Grant, 2016). First clinical signs becoming evident within 30 minutes afterwards an ingestion of a toxic dose (Dalefield, 2017). Sublethal doses could lead to headache, hyperventilation, vomiting, weakness, abdominal cramps, and partial circulatory and respiratory systems failure (Castada et al., 2020). The toxic threshold value for cyanide in blood is considered to be between 0.5 (~20 μ M) and 1.0 mg.L⁻¹ (~40 μ M) and the lethal threshold value ranges between 2.5 (~100 μ M) and 3.0 mg.L⁻¹ (~120 μ M) (Schrenk et al., 2019). The acute lethal oral dose of cyanide is established in humans between 500 and 3500 μ g.kg⁻¹ of body weight. Daily consumption of 20 μ g cyanide.kg⁻¹ may cause chronic and 80 μ g CN.kg⁻¹ acute signs (Lindinger, 2019). Whereas these molecules are chemically quite stable in acidic and alkaline conditions the intermediate cyanohydrins (α -hydroxynitriles) are stable only in acidic media and spontaneously dissociate into the CN at neutral and alkaline pH (Schrenk et al., 2019).

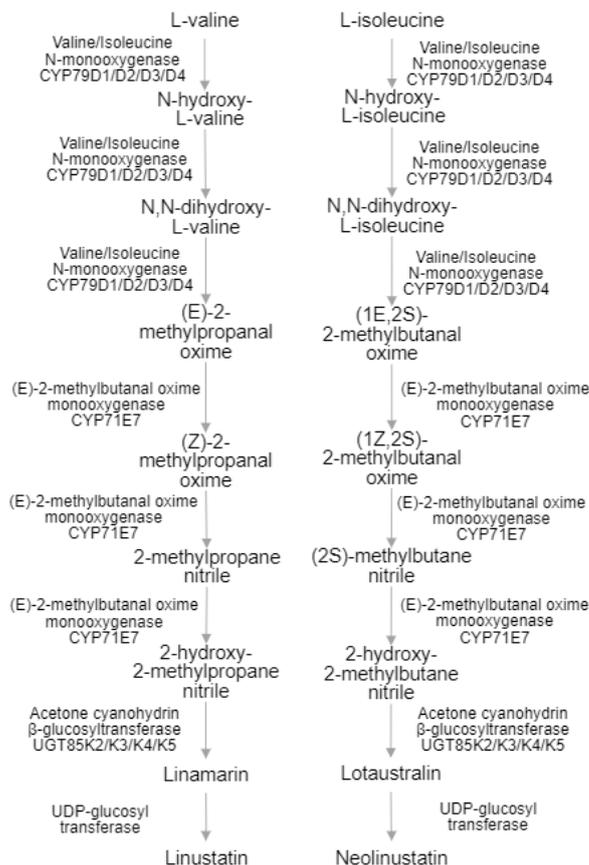


Figure 3 Biosynthetic pathway of cyanogenic glycosides, linustatin and neolinustatin in flax (elaborated by Harenčár).

The molecular bonds are hydrolyzed and catalyzed by a two-step enzymatic reaction utilizing β -glucosidases and hydroxy nitrile lyases. Both are synthesized by the same plant but stored in a different location. While the cyanogenic glycosides are stored in the vacuoles and mainly in leaf tissues, β -glucosidases are in the apoplasmic space commonly attached to the cell walls, in the cytoplasm, vesicles or chloroplast and the hydroxy nitrile enzymes are accumulated in the cytoplasm (Vetter, 2017). The mammalian organisms have developed several metabolic pathways for the detoxification of the cyanide. About 70% of cyanide dose is metabolized in the presence of a sulfur donor thiosulfate and a sulfur transferase rhodanese into the thiocyanate which does not block an electron transport in the mitochondrial respiratory chain. High doses of thiocyanate may affect a thyroid function and goiter especially in an iodine deficiency (Eisenbrand and Gelbke, 2016). Cyanide can react also in another detoxification pathway with L-cystine through intermediate β -thiocyanoalanine to 2-amino-2-thiazoline-4-carboxylic acid. The further detoxification pathway is the reaction of cyanide with an endogenous α -ketoglutarate to form α -ketoglutarate cyanohydrin or reaction with cysteine disulfide groups in serum albumin into a hydroxocobalamin (vitamin B12b) or methaemoglobin. The complete hydrolysis of 1 g linamarin generates 109 mg of HCN and all mentioned metabolites are excreted in the urine (Schrenk et al., 2019). The content of the cyanogenic glycosides in oil varieties of flaxseed planted in Czech Republic achieved the level from 1.9 to 5.5 g.kg⁻¹. The value of linamarin was in range of 0.05 to 0.2 g.kg⁻¹, linustatin 1.2 to 3.5 g.kg⁻¹ and neolinustatin of 0.6 to 2.2 g.kg⁻¹ (Bjelková et al., 2017). Russo and Reggiani (2014) measured the total content of cyanogenic glycosides in flaxseed flour from 0.74 to 1.60 g.kg⁻¹ CN⁻ where oil varieties reached a higher value than fiber. Content of linamarin was ranged of 0.02 to 0.18 g.kg⁻¹, linustatin 0.28 to 0.95 g.kg⁻¹ and neolinustatin from 0.06 to 0.85 g.kg⁻¹. In the research of (Waszkowiak et al., 2015) the total content of HCN in hexane defatted flaxseed was approximately 0.3 g.kg⁻¹. The content of linamarin was ~0.03 g.kg⁻¹, lotaustralin ~0.04 g.kg⁻¹, linustatin ~2.9 g.kg⁻¹ and neolinustatin ~1.3 g.kg⁻¹. Consuming of 30 grams flaxseed with a cyanogenic precursor content of 0.2 g.kg⁻¹ seed will result in an average peak blood cyanide concentration of 5 μ mole.L⁻¹ (Abraham, Buhrke and Lampen, 2016). It is important to emphasize that one of the important factors influencing the content of CNG in flaxseed is genotype (Bjelková et al., 2017). Currently, the cyanogenic glycosides are still sold as an anticarcinogenic medicine (e.g., vitamin B17 - laetrile) in many countries whereas in the USA (where was this theory originated) it has been proven to be dangerous and ineffective in cancer treatment and all products have been withdrawn (Süli, Sobeková and Bujdošová, 2017).

Table 1 The overview of CNG by biochemical origin based KEGG database (<https://www.genome.jp/kegg/>, elaborated by Ražná)

Biochemical origin of CNG	Type of CNG	Source
CNG derived from phenylalanine	Amygdalin	<i>Prunus dulcis</i> (Mill.) D. A. Webb, <i>P. persica</i> (L.) Batsch, <i>P. armeniaca</i> L., <i>P. avium</i> (L.) L., <i>Malus domestica</i> Borkh.
	Anthemis glycoside A	<i>Anthemis altissima</i> L.
	Anthemis glycoside B	<i>Anthemis altissima</i> L.
	Lucumin	<i>Clerodendrum grayi</i> Munir
	Prunasin	<i>Prunus</i> sp. L., <i>Olinia</i> sp. Thunb., <i>Senegalia greggii</i> A.Gray
	(R) – Vicianin	<i>Vicia angustifolia</i> L.
	Zierin	<i>Sambucus nigra</i> L., <i>Apis cerana</i> , <i>Xeranthemum inapertum</i> (L.) Mill., <i>X. cylindraceum</i> (L.) Mill.
CNG derived from tyrosine	Dhurrin	<i>Sorbus bicolor</i> L.
	p-Glycosyloxymandelonitrile	<i>Manihot esculenta</i> Crantz, <i>Bambusa</i> sp. Schreb.
	Proteacin	<i>Lomatia</i> sp. R.Br.
	Taxiphyllin	<i>Bambusa</i> sp. Schreb.
	Triglochinin	<i>Araceae</i> Juss.
CNG derived from valine or isoleucine	Linamarin	<i>Phaseolus lunatus</i> L.
	Linustatin	<i>Linum usitatissimum</i> L.
	Lotaustralin	<i>Manihot esculenta</i> Crantz
	Neolinustatin	
	Sarmentosin	<i>Abraxas glossulariata</i> L., <i>Sedum stenopetalum</i> Pursh., <i>Rhodiola</i> sp. L., <i>Piper sarmentosum</i> Roxb.
CNG derived from leucine	Cardiospermin	<i>Cardiospermum halicacabum</i> L.
	Heterodendrin	<i>Acacia</i> sp. DC., <i>Rhodiola</i> sp. L., <i>Vachellia</i> sp. <i>Wight & Arn.</i>
	Proacaciberin	<i>Acacia sieberiana</i> DC.
	Proacacipetalin	<i>Acacia sieberiana</i> DC.
CNG derived from nonprotein amino acid	Deidaclin	<i>Passiflora</i> sp. L., <i>Turnera</i> sp. L., <i>Adenia globosa</i> Engl., <i>Kiggelaria africana</i> L.
	Gynocardin	<i>Baileyxylon lanceolatum</i> C.T.White, <i>Achariaceae</i> Harms
CNG derived from other origin	Acalyphin	<i>Acalypha indica</i> L.
Others	Cycasin	<i>Cycas revoluta</i> Dumort, <i>Zamia pumila</i> L.
	Ranunculiln	<i>Ranunculaceae</i> Juss.

Legend: CNG – cyanogenic glycosides

Processing approaches for CNG reduction content in plant food resources

The risk of poisoning within many other plants consumed by humans is negligible due to very easy removing the toxic HCN from food by grinding, drying in air, soaking in water or other thermal treatment that in combination with effective human detoxifying mechanisms contributes to the denaturation of enzymes (Kudlak, Wiczerzak and Namieśnik, 2017).

Hydrogen cyanide is released from CNG-containing foods during food digestion or digestion, which hydrolyzes these substances. The aim of individual processes of processing plant food resources is to reduce the content of hydrogen cyanide (HCN) to an acceptable safe level. Such processes are fermentation, boiling, cooking, drying (oven drying, freeze drying), grinding, soaking, peeling, roasting (Bolarinwa et al., 2016; Chongtham et al., 2021). The list of plant species containing CNG mainly in seeds/stones includes apples, apricots, cherries, peaches, plums, quinces, almonds, cassava, bamboo shoots, flaxseed, lima beans, chickpeas, cashews, but also food marzipan ingredients, alcoholic beverages from stone fruit (Bolarinwa et al., 2016) (Table 2). Fermentation has proven to be the most suitable method of processing bamboo shoots in terms of reducing CNG content (Chongtham et al., 2021).

Development of extraction methodology for cyanogenic glycosides is focused on identification and optimization of methods of disturbing the integrity of the tissue (e.g., seeds), extraction solvent composition, extraction time, repeat extraction and other factors (Barthet and Bacala, 2010). After extraction of cyanogenic glycosides from flaxseed, where the extraction efficiency was almost 88% for linustatin and neolinustatin, the ground flaxseed was stable up to 7 day at room temperature, refrigeration or freezing, and frozen for at least 2 weeks. Extracts of CNG were stable for 1 week at room temperature and at least 2 weeks under 4°C or frozen.

There are approaches based on bacteria-based degradation of cyanogenic glycosides in plant food resources. Menom et al. (2015) identified lactic acid bacteria capable of amygdalin degradation. Amygdalin fermentation by strains from *Lactobacillus plantarum* group represent a potential approach to biotransformation of amygdalin for food improvement and safety. Seven strains of *L. plantarum* showed great variation in the ability to degrade amygdalin, linamarin, linustatin and neolinustatin. This was also the case of another tested strains of *Candida tropicalis* (Lei et al., 1999). Also, *Bacillus* species were screening for their ability to degrade linamarin in cassava and amygdalin (Abban et al., 2013). As study showed, some *Bacillus* spp. isolates (*B. subtilis*, *B. licheniformis* and *B. sonorensis*) can hydrolyze CNG during cassava fermentation at pH 4.5-5.0. Research results suggest that, co-culture of the most active strains regarding *beta*-glucosidase activity, seem to degrade linamarin faster than the monoculture (Lei et al., 1999).

Antimicrobial activity of CNG

Amygdalin from crushed apple seeds has shown the ability to inhibit the growth of two Gram-positive species (*Staphylococcus aureus* and *Streptococcus pyogenes*) and two Gram-negative species (*Escherichia coli* and *Pseudomonas aeruginosa*) pathogenic bacteria. The zone of activity inhibition was highlighted with increasing concentration of undiluted extract (Mhawesh et al., 2018).

The hot apricot seed extract significantly inhibited the growth of the tested bacterial strains, of which *Staphylococcus aureus* showed the highest sensitivity. The highest antibacterial substance was shown by the aqueous extract of apricot seeds (Abtahi et al., 2008).

Citrus lanatus seed extract containing cyanogenic glycosides showed antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Proteus mirabilis* and *Streptococcus pyogenes* (Nwankwo et al. 2014). Phytochemical screening of inner bark of *Atuna racemosa* reveals the presence of several secondary metabolites including cyanogenic glycosides which showed antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa* (Nadayag et al., 2019). Antibacterial, antifungal and antiparasitic activities of extracts of Mexican medicinal plant *Laenecia confusa* of *Asteraceae* family were confirmed. Extracts including cyanogenic and cardiotoxic glycosides demonstrated antibacterial activity against *Staphylococcus aureus* and antiparasitic activity against *Leishmania donovani* and were also found to be cytotoxic to macrophages (Martinez Ruiz et al., 2012).

The oils extracted from seeds of *Brassica nigra* and *Cucurbita pepo* showed antibacterial activities on fresh hospital isolates *Staphylococcus aureus* and *Escherichia coli*. A higher zone of inhibition of 60% on *S. aureus* were produced by oil from *C. pepo* whose phytochemical analyses revealed the content of tannins, flavonoids, saponins, cyanogenic glycosides and cardiac glycosides, while the oil from *B. nigra* does not consists cyanogenic glycosides and showed lower inhibitory effect on *S. aureus*. However, both oil extracts showed similar inhibitory effects on *E. coli* (Obi et al., 2009).

Nwaogu et al. (2008) conducted the antimicrobial screening of leaf and root extracts from *Landolphia owariensis* against clinical isolates of *Staphylococcus* spp., *Proteus* spp. and *Escherichia coli*. The phytochemical screening indicated the presence of cyanogenic glycosides only in root extract. Although, the response of tested strains to extracts was concentration dependent, dehydrogenase activity was inhibited by the root extract at a higher concentration range than the leaf extract.

THE ROLE OF CYANOGENIC GLYCOSIDES IN PLANTS

Plants produce many phytochemicals and metabolites enabling interactions with their biotic and abiotic environment (Thodberg et al., 2020). Release of hydrogen cyanide makes an effective chemical defense against generalist herbivores and pathogens (Hansen et al., 2018). Many authors termed this defense ability as a cyanide bomb (Yeats, 2018). The ability to synthesize cyanogenic glycosides is at least 300 million years old and distributed among 130 families in pteridophytes (ferns), gymnosperms and angiosperms (flowering plants) (Van Ohlen, Herfurth and Wittstock, 2017). Ferns and gymnosperm species contain aromatic cyanogenic glycosides derived from either tyrosine or phenylalanine whereas angiosperms contain aliphatic as well as aromatic cyanogenic glycosides derived from valine, leucine, isoleucine or tyrosine and phenylalanine, respectively. Few plants species such as passiflora contain cyanogenic glycosides derived from the non-protein amino acid cyclopentenyl glycine (Bak et al., 2006). This fact indicates that the cyanogenic glycosides were initially only aromatic and afterwards aliphatic (Mobot, 2021). The functions of cyanogenic glycosides and

hydrogen cyanamide lie in the expression of floral regulative, antioxidant and hypoxia-responsive genes and catalase activity. In the buds influence the level of hydrogen peroxide, calcium, ethylene, proline putrescine and phytohormones, buds opening and seed germination (Nielsen et al., 2016; Ionescu et al., 2017). The other potential physiological functions are elimination of reactive oxygen and osmoprotective function associated with tolerance to abiotic stresses including drought, salt, temperature, and oxidative and nutrient stresses (Hayes et al., 2015). The plants have developed the endogenous turnover pathways enabling utilization of nitrogen as ammonia without any release of hydrogen cyanide and detoxification pathway to avoid hydrogen cyanide intoxication in which β -cyanoalanine synthase catalyzes the conversion of hydrogen cyanide into β -cyanoalanine. In subsequent reaction is hydration of β -cyanoalanine catalyzed by type 4 nitrilase resulting in the production of asparagine or aspartate and ammonia (Del Cueto et al., 2017). The followed figure 4 displays different functions of cyanogenic glycosides in plants. The investigation of the role of CNG in plants has been part of several research projects (Table 2).

Table 2 Overview of some of the research intentions of the importance of CNG in the species consumed (elaborated by Ražná)

Species	Name	Tissue type	Type of CNG	The purpose of the investigation	Reference
<i>Hordeum vulgare</i> L.	Barley	Seeds, roots, seedlings		The role of CNG as nitrogen storage compounds	Forslund and Jonsson, 2006
<i>Linum usitatissimum</i> L.	Flax	Linseed	Linustatin Neolinustatin	CNG validation in agri-food	Zhong et al., 2020
<i>Malus domestica</i> Borkh.	Apple	Apple seeds	Cyanogenic glycosides	Environmental conditions, long term storage effect	Senica et al., 2019
<i>Phaseolus lunatus</i> L.	Lima Bean	Seeds, seedlings leaves	Linamarin	The role in defense or plant nutrition	Cuny et al., 2019
<i>Prunus armeniaca</i> L.	Apricot	Fruit, kernel	Prunasin, Amygdalin	Accumulation pattern and fruit parameters	Deng et al., 2021
<i>Prunus avium</i> (L.) L.	Sweet Cherry	Flower buds	Prunasin, Amygdalin	CNG validation in agri-food	Zhong et al., 2020
<i>Prunus dulcis</i> (Mill.) D. A. Webb	Almond	Flower buds, roots, leaves, kernels	Prunasin, Amygdalin	Controlling flower development and dormancy	Del Cueto et al., 2017
				Controlling flower development, dormancy	Del Cueto et al., 2017
				Intraspecific variability	Dicenta et al., 2002
<i>Sambucus canadensis</i> L.	American Elderberry	Fruit tissues, pressed juice samples, seeds	Total cyanogenic potential	Potential toxicity issues	Appenteng et al., 2021
<i>Sorghum bicolor</i> (L.) Moench	Sorghum	Sorghum rice	Dhurrin	CNG validation in agri-food	Zhong et al., 2020
<i>Vitis vinifera</i> L.	Grapevine	Mature leaves	Prunasin Sambunigrin	Polymorphism and developmental pattern	Franks et al., 2004

Legend: CNG - cyanogenic glycosides

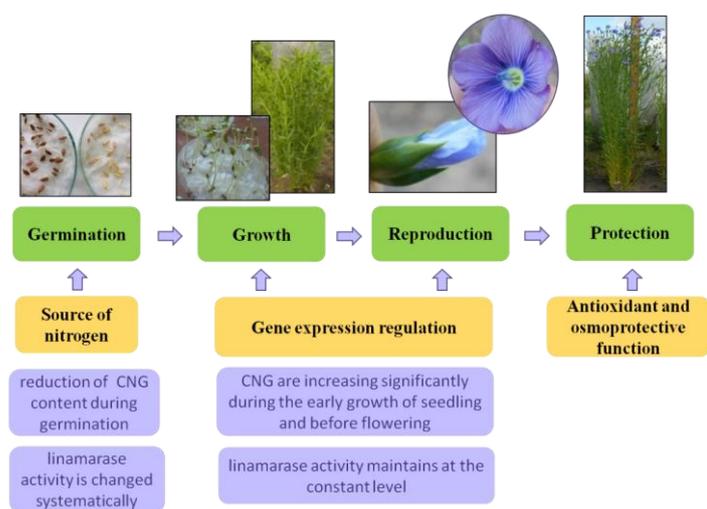


Figure 4. A summary of CNG functions in plants (photo by Ražná and Hlavačková)

Representation of CNG in individual parts of the flax plant

Flax is one of the most frequently used sources of food products and nutritional supplements and is also a crop to which intensive attention is paid to the overall importance of cyanogenic glycosides. The potential concentration of HCN in various parts of flax may differ and depends on a location and year of production whereas the most important factor is a cultivar (Oomah, Mazza, and Kenaschuk, 2002). Large amount of cyanogenic glycosides accumulates in the seed, young

seedlings, leaves, growing shoot and flowers (Siegien, 2007). Plant seed germination causes *de novo* synthesis of bioactive phytochemicals such as ascorbic acid, phenylic acids, flavonoids, free and poly amino acids. On the other hand, in flaxseed was observed a reduced content of oil, cyanogenic glycosides and trypsin inhibitor during 8 days of germination (Wang et al., 2016). In another research, on the 5th day the content of linustatin, neolinustatin and lotaustralin significantly decreased however the content of linamarin was higher (Li et al., 2019). In other 5-days oil seedlings, depending on a higher intensity and longer exposure time of a white light with the highest temperature 30°C the cyanogenic glycosides content was enhanced. An enzyme activity of linamarase was the highest at 20°C, especially in light-grown seedlings and the lowest in extreme 15 and 30°C temperature. Water stress reduced the cyanogenic glucoside level and linamarase activity by more than twice (Niedzwiedz-Siegien and Gierasimiuk, 2001). The cyanogenic diglucosides linustatin and neolinustatin are the main in developing embryos and mature seed. After germination are rapidly depleted and the monoglucosides linamarin and lotaustralin were also found in leaves, flowers and developing embryos. Roots and stems contained relatively low concentrations of cyanogenic glycosides (Muir and Westcott, 2003). High levels of linamarin and lotaustralin were found in leaves throughout the vegetation period, but the highest amounts were in flowers (Niedzwiedz-Siegien, 1998). The total content of cyanoglycosides increases significantly during the early growth of seedlings and before flowering (Adamczuk, Siegien, and Ciereszko, 2015). The flaxseed content of HCN in period from anthesis (0 days after flowering) to maturity (40 DAF) was on the decrease from 2-3 mmol HCN to 0.4-1.3 mmol.dm⁻³ (Figure 4). A broad maximum of HCN per flax fruit appeared between 7 and 18 DAF and was characterized by a high variability among the samples. A ratio between monoglucosides and diglucosides was shifted from 100% monoglucosides at anthesis (0-18 DAF) to 100% diglucosides at maturity (25-40 DAF). The monoglucosides linamarin and lotaustralin produced in parental tissues of the flax plant would be translocated into the growing seeds as the corresponding diglucosides linustatin and neolinustatin which persist in the seed until the seed will be germinated (Frehner, Scalet and Conn, 1990). The presence of forms of

CNG in the various development stages and plant parts of flax is shown in Figure 5.

An integrated approach to the identification of the formation and deposition of cyanogenic glycosides in the seed tissues of flaxseed has made it possible to localize these components in the germ layer (ovaries), in the endosperm and in the embryo. In contrast to the lignans that are part of the seed coat (Dalisy et al., 2015).

Seedling derived and regenerated *in vitro* shoots from hypocotyl showed about twice higher accumulation of linamarin and lotaustralin in light conditions. The low content of these compounds in roots was unchanged regardless of light conditions. Tiny quantity was also detected in callus tissue, but only under light conditions (Siegjeń, Adamczuk and Wróblewska, 2013). The content of cyanogenic compound is positively correlated with amount of protein in the seed and the level of nitrogen fertilization. The lower nitrogen supply can keep the content of cyanogenic glycosides at the required level (Klein et al., 2017).

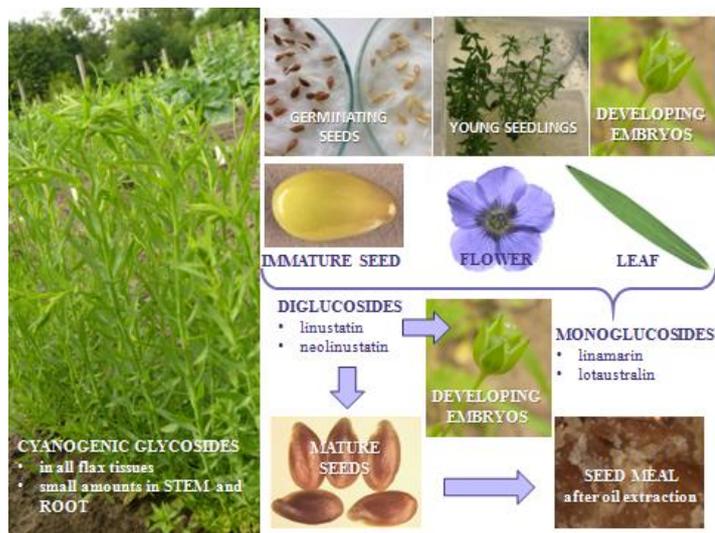


Figure 5 The presence of forms of CNG in the various development stages and plant parts of flax (photo by Nůžková).

CNG as part of plant defense mechanism

Secondary metabolites in plant species fulfill specific functions, such as attracting insects to transfer pollen, animals to eat the fruits and subsequent pollen transfer; and they can act as natural pesticides. They can protect the plants from pathogenic microorganisms and herbivores. These defending substances are permanently the part of the plants (Heldt and Heldt, 2005). Very effective defense system is formed in plants because the presence of two different toxic substances. When the cell is injured by feeding animals, the glycosidase encounters cyanogenic glycosides. The remaining substance of glucose residue hydrolysis is cyanhydrin. It is very unstable, and it is degraded spontaneously to prussic acid and an aldehyde. The detoxification of the aldehydes, which are usually very toxic, is even more difficult for feeding animals than that of prussic acid. The prussic acid (HCN) is also very toxic, and it inhibits cytochrome oxidase, which is final step of the respiratory chain. The plants are protecting themselves by the fact that the prussic acid is in the bound form as cyanogenic glycosides because they have a mitochondrial respiratory chain also. Decomposition to the prussic acid and aldehydes is accelerating by enzyme a hydroxynitrile lyase (Heldt and Heldt, 2005; Ballhorn, 2011).

Cyanogenic glycosides and their corresponding degrading enzymes (β -glucosidases; hydroxynitrile lyases) are part of a preformed defense system. Thus, they can be regarded as phytoanticipins (Pičmanová et al., 2015). Some insects are strongly associated with their cyanogenic host plants. They sequester the cyanogenic glycosides from these plants as well as carry out de novo biosynthesis of these compounds. Contrary to phytoalexins that are synthesized de novo after the plant is exposed to microbial attack, i.e., being produced in response of elicitors or stressors, the phytoanticipins are not formed in the tissue or released from preexisting plant constituents. These substances are plant antibiotics presented in tissue prior to infection, serving as the basis of pest tolerance (Oros and Kállai, 2019).

Cyanide production in leaves has role to herbivore defense, but ecological function of cyanogenic precursor in seeds is still not entirely clear. One option may be that during seed germination these compounds are transferred to the growing seedling for their defense, and in addition cyanogenic glycosides can store nitrogen that is required for seedling grow, or they can act as germination inhibitors (Ballhorn, 2011).

It is believed that by hydrolysis of CNGs during seed germination, they are thought to be used as a nitrogen source for amino acid synthesis and, at the same time, the released hydrogen cyanide (HCN) performs a protective function of germs against pests (Krech and Fieldes, 2003). The process of cyanogenesis leads to serious problems for feeding animals while cyanogenic glycosides as defensive secondary metabolites play important roles in plant development and response to adverse environment (Pičmanová et al., 2015).

In the genus *Phaseolus*, the only species in which the content of cyanogenic glycosides has been recorded is lima bean (*Phaseolus lunatus* L.). The cyanogenic glycosides contained in the seeds of this species are related to the defense mechanisms of the seedlings against the leaf pest (*Spodoptera littoralis*), but do not have a negative effect on germination and further growth. The results confirmed that seeds with a higher CNG content provide better protection of the primary leaves of seedlings and point to their defensive rather than nutritional role in the seeds. The authors observed a demonstrable positive correlation between CNG content, defined by the level of linamarin ($\text{mg}\cdot\text{g}^{-1}$), in seeds and CNG content in cotyledons of seedlings. However, no significant correlation was observed between CNG content in seeds and in primary and secondary leaves. The level of linamarin in germinating seeds increased by 100% compared to seed and by 230% when comparing the level of linamarin between germinating seeds and young seedlings (Cuny et al., 2019).

During germination, the content of neolinustatin was higher in the seeds and the content of lotaustralin was higher recorded in the cotyledons of the seedlings. However, the distribution of CNG did not support the assumption that the content of these components in the hypocotyl is responsible for the protection of seedlings against soil pests. In flax as well as other cyanogenic plants, HCN, as a product of CNG hydrolysis, is expected to play a much more important role in regulating seed germination and prolonged growth of germline hypocotyl than as a potential nitrogen source for amino acid synthesis by mediating ethylene production to help overcome dormancy of seeds and stimulates growth. The activity profile of linamarin-cleaving linamarase and lotaustralin points to its role during the early stages of germination and during cotyledon expansion. In young flax seedlings, linamarase activity is stable (Fieldes and Gerhardt, 2001).

The role of CNG in environmental adaptation of plants

Plants that produce hydrogen cyanide (HCN) are an important part of the human diet. All plants produce small amounts HCN as a by-product of ethylene biosynthesis. However, some plant species can release large amounts of hydrogen cyanide from endogenous secondary metabolites - cyanogenic glycosides (Gleadow and Møller, 2014). Genetic conditionality and heredity of cyanogenesis are governed by Mendelian patterns of heredity, but its quantitative expression is very plastic depending on the tissue or growth phase. Elevated concentrations of hydrogen cyanide have been reported in connection with adverse environmental conditions, such as lack of light, low temperatures and drought, as well as lack of soil moisture (Gleadow and Møller, 2014). Accumulation of high concentrations of dhurrin in sorghum plants was confirmed during drought stress (O'Donnell et al., 2013).

It has been shown that environmental stress result in decrease in oil content in flax. However, significant changes in the content of cyanogenic glycoside in flaxseed under different environmental stress were confirmed together with significant varietal differences in CNG content (Daun and Przybylski, 2000).

The polymorphism of cyanogenic glycosides is subject to specific environmental conditions and plant ontogenesis (Figure 6). CNGs improve phenotypic plasticity by increasing the ability of plants to adapt to changing environmental conditions. Given the high proportion of foods derived from cyanogenic crops, it will be important to predict the response of the genome of these species to environmental changes in the context of food safety (Gleadow and Møller, 2014).

The mandelonitrile which is part of cyanogenic glycoside biosynthesis was tested in peach plant response to abiotic and biotic stress factors (salt and virus infection) (Bernal-Vicente et al., 2018). The study showed reduced impact of both stresses on plant development of mandelonitrile treated seedlings.

Cyanide potential of sorghum depends on genotype, environmental factors, and crop management (Emendack et al., 2018). Different (40) sorghum lines were tested on dhurrin content in leaves under water deficit stress. The lines (5) with very low dhurrin content were identified across developmental stages a water deficit stress. In general, younger plants (30 days old) content less cyanogenic glycoside - dhurrin than older ones. Water stress applied shortly just before flowering manifested itself in a reduction of dhurrin in leaves, while the same stress applied in post-flowering phase caused an increase of dhurrin.

The study of diurnal regulation of cyanogenic glycosides in cassava showed that linamarin content increased during the dark period and on other side the light period caused rapid decrease in linamarin content suggesting its additional role in anti-oxidative stress as a ROS scavenger (Schmidt et al., 2018). *In silico* analyses of promoter sequences of genes involved in CNG biosynthesis revealed a presence of light, abiotic stress and development-related transcription factor binding motifs. Similarly, the presence of cyanogenic glycoside - dhurrin in sorghum supports its role in reducing oxidative stress (O'Donnell et al., 2013).

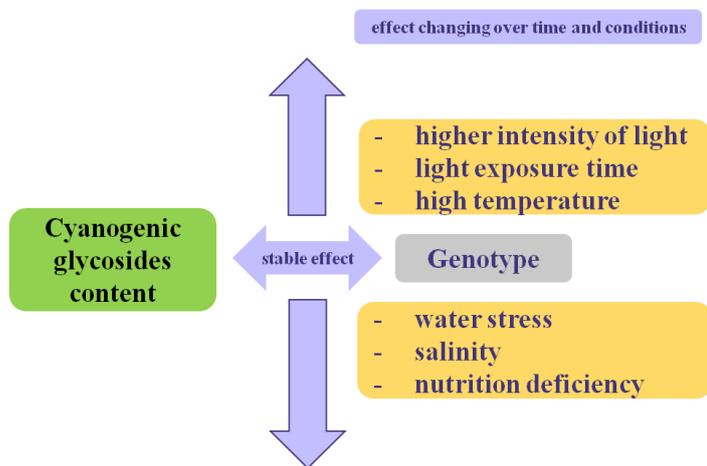


Figure 6 Factors affecting the metabolism activity of cyanogenic glycosides

CONCLUSION

The growing trend of searching for alternative food sources is conditioned on the one hand by the increasing interest of people in the quality of nutrition, but also by the growing incidence of nutrition-related diseases. The presence of secondary metabolites in plants is justified and it is therefore important to perceive their importance in the context of the functioning of the whole organism. From this point of view, it is necessary to find a new potential of secondary metabolites for nutrition and food use. Plant cyanogenic glycosides represent naturally occurring phytochemicals, secondary metabolites produced by plant biochemical machinery as part of various defense, nutritional and adaptive processes. Cyanogenic glycosides can be beneficial in many applications (a) to agriculture for development of bio-based agents in plant protection, (b) to plant adaptation potential against environmental stress and (c) for health and hygiene requirements (antimicrobial properties). The identification of added value of plant food resources is closely connected with the knowledge of the overall metabolism of plants and the role of individual components in the harmonious context of the plant organism.

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