CHAPTER 4

Chemical Hazards and Their Control: Endogenous Compounds

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INTRODUCTION

Raw materials of vegetable origin may contain natural toxic or antinutritional compounds, endogenous constituents that are synthesized by the plant itself. *Antinutritional* means a deleterious effect due to the hindrance of uptake or use of other components in the diet. Examples of antinutritional compounds include tannins, which among others bind to proteins, making them unacceptable as substrates for proteases; proteinase inhibitors, which inhibit proteinases such as trypsin and chymotrypsin; phytate, which binds a number of minerals, making them unavailable for uptake; and thiaminase, which degrades vitamin B_1. An effect may be due to the parent compound or to metabolites that are formed in the gut or in the organism after absorption.

Although a few toxic and antinutritional compounds found in plants are proteins, most are low molecular weight compounds. Examples of proteins are ricin,[53] which is found in the seeds of *Ricinus communis* L. (castor bean); lectins,[213] found especially within the legumes; and the proteinase inhibitors, which are also common in legumes.[62] The smaller molecules with deleterious effects belong to the groups of compounds that are normally classified as secondary metabolites. The number of secondary constituents isolated from plants, fungi, and animals is high. Luckner worked with 26 biosynthetic groups divided into 107 subgroups, many of which contained more than 1,000 structures.[155] The majority of these compounds was found in plants. The alkaloids, for example, have had the attention of phytochemists for more than 150 years. In 1950, approximately 2,000 alkaloids were recognized; by 1970, the number had increased to approximately 4,000; 20 years later, approximately 10,000 were known.[217] It is necessary, then, to focus on the most important endogenous plant toxins as seen from a food and feed point of view.

The most prominent constituents known to restrict the nutritional value of food or fodder include certain nonprotein amino acids, alkaloids and glycosides, together with the tannins. However, knowledge concerning the influence of fermentation on these agents is very limited except for certain of the glycosides. Because a number of very important commodities of food and fodder worldwide do contain toxic glycosides,[46] the occurrence and effects of toxic glycosides, and their fate during food fermentations, will be presented in this chapter.

TOXIC AND ANTINUTRITIONAL GLYCOSIDES IN FOOD AND FEED

Glycosides consist of one or more genins (aglycones) to which one or more mono- or oligosaccharides are linked. The glycosidic linkage(s) may differ (i.e., one differentiates between O-, S-, and C-glycosides) (Figure 4–1). If the sugar part is a glucose moiety, it is called a *glucoside*. A number of different glycosides and oligosaccharides causing physiological effects (toxins) or reduced uptake or use of nutrients after ingestion are known in the plant kingdom. So

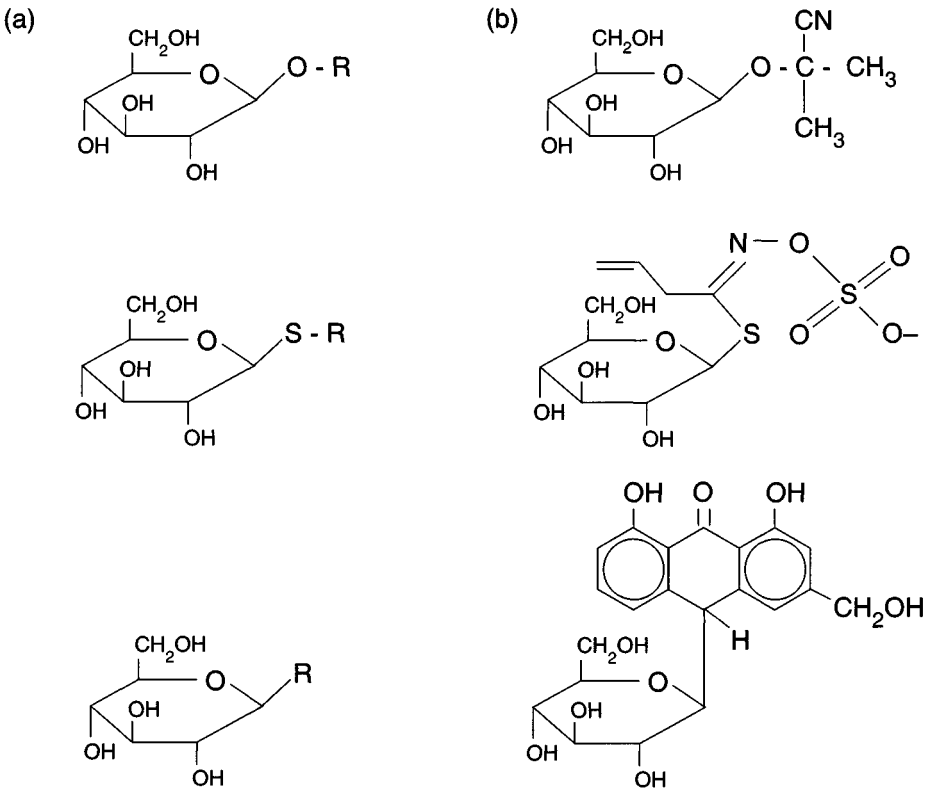

Figure 4–1 (a) The general structure of O-, S-, and C-glucosides as representatives of the broader groups of O-, S-, and C-glycosides, respectively. At top, an O-glucoside; middle, an S-glucoside; bottom, a C-glucoside. (b) Examples of naturally occurring O-, S-, and C-glucosides. Top (Linamarin—a cyanogenic glucoside found in cassava), middle (Sinigrin—a glucosinolate), and bottom (Barbaloin—an anthrone C-glucoside from *Aloe* spp.; laxative). *Note:* R = aglycone (= genin).

are a number of bitter-tasting glycosides that reduce the palatability of the plant (Table 4–1). A few of these glycosides have been shown to be protective to the plant;[129,205] however, most have only been recognized as toxic to domestic animals or humans. The broad range of compounds listed in Table 4–1 illustrates the diversity of chemical structures found even within the restricted field of toxic and antinutritional glycosides and oligosaccharides. Because this diversity also means a broad range of different mechanisms of action, the most important compounds are described in the following paragraphs.

Cyanogenic Glycosides

Cyanogenesis (cyano—Greek [kyanos = blue] and genesis—Greek [creation]) means the formation of cyanide/hydrogen cyanide, or HCN. Organisms that possess the ability to release cyanide may be termed cyanogenic or cyanophoric (phoros—Greek [bearing]). If the cyanide is formed from the breakdown of another compound, this compound is called a cyanogenic (cyanogenetic) compound, or simply, a *cyanogen*. Cyanogens include cyanogenic glycosides, cyanogenic lipids, cyanohydrins, and

Table 4–1 Toxic, Antinutritional, and Bitter-Tasting Glycosides and Oligosaccharides

Compound group or compound	*Examples*	*Toxicity, taste, etc.*
	O-Glycosides, Sugar Esters, and Oligosaccharides In sources of food and feed	
Cyanogenic glycosides	Linamarin in *Manihot esculenta,* Euphorbiaceae, in general widespread in the plant kingdom (Tracheophyta and Spermatophyta)[52,129,182]	Acute and chronic toxicity due to release of HCN; neurotoxicity of intact glycosides discussed; bitter taste
Glycoalkaloids	Chachonin and solanin in *Solanum tuberosum,* Solanaceae (Angiospermae)[140,206]	Corrosive to the gastrointestinal tract; upon absorption, acutely toxic due to several mechanisms; bitter taste
Glycosides of organic nitriles	Simmondsin in *Simmondsia californica* (Jojoba), Buxaceae (Angiospermae)[2,277]	Causes chronic toxicity of unknown mechanism
Glycosides and sugar esters of aliphatic nitrocompounds	Miserotoxin in *Astragalus* spp., Fabaceae (= Leguminosae; Angiospermae)[160,204]	Acutely toxic to ruminants; inhibit the TCA-cycle of the cells
Methylazoxymethanol (MAM) glycosides	Cycasin in *Cycas* spp., Cycadaceae (Gymnospermae)[22,154]	Carcinogenic
Naringin	In *Citrus* spp., espec. *C. paradisi* (grapefruit), Rutaceae (Angiospermae)[227]	Bitter taste
Oligosaccharides	In seeds of several legume spp., Fabaceae (= Leguminosae; Angiospermae)[94,215]	Flatulence-producing
Platyphylloside	In *Betula pendula*, Betulaceae (Angiospermae)[262]	Antinutritional (deterrrent) to several animal species
Polyphenols	2-hydroxyarctiin in *Carthamus tinctorius* (Safflower), Asteraceae (= Compositae; Angiospermae)[98,203]	Cathartic (laxative); bitter taste
Ptaquiloside	In *Pteridium aquilinum*, Polypodiaceae (Tracheophyta)[249,250]	Acutely toxic and carcinogenic
Saponins	Triterpene or steroid saponins in *Quinoa* spp., *Borassus flabellifer, Glycyrrhizae glabra* and *Balanites* spp. (Angiospermae)[74,95,125]	Some atoxic, other mildly to strongly toxic; several are bitter tasting
Vicine and convicine	In *Vicia faba* (faba bean), Fabaceae (= Leguminosae; Angiospermae)[276]	Acutely toxic to glucose-6-phosphate dehydrogenase-deficient individuals
	In medicinal and toxic plants	
Carboxyatractyloside (CAT) and related compounds	CAT in *Atractylis gummifera*, Asteraceae (= Compositae; Angiospermae)[49,187]	Acutely toxic; inhibit mitochondrial oxidative phosphorylation

continues

Table 4–1 continued

Compound group or compound	*Examples*	*Toxicity, taste, etc.*
Cardeno- and bufodienolides	"Digitalis" glycosides in *Digitalis spp.* (cardiac glycosides), Scrophulariaceae (Angiospermae)[161,225]	Acutely toxic to the heart
Cucurbitacins	Cucurbitacin L in *Citrullus colocynthis,* Cucurbitaceae (Angiospermae), some cucurbitacins also present in food plants (ref. text below)[103,126]	Intensely bitter substances, some of which are acutely toxic
Glycosides of Vitamin D_3	Glycosides of $1\alpha,25\text{-}(OH)_2D_3$ in *Solanum glaucophyllum,* Solanaceae (Angiospermae)[279]	Chronic toxicity (vitamin D intoxication—calcinoses)
Ranunculin	In *Ranunculus* and *Caratocephalus* spp., Ranunculaceae (Angiospermae)[180,195]	Acutely toxic; irritant to mucous membranes; Upon absorption, it affects several organs such as the heart, the lungs, etc.
	C-Glycosides (Some Also Occurring as O-Glycosides) In medicinal plants	
Anthraquinone, anthrone, and dianthrone glycosides	Sennosides in *Cassia angustifolia,* Fabaceae (= Leguminosae; Angiospermae)[152,280]	Laxative effect; some compounds are drastica
	S (Thio)–Glycosides In food and feed resources	
Glucosinolates	In many species within the families of Capparales (Angiospermae)[23]	Chronic toxicity due to release of thiocyanate and other compounds; sharp (burning) taste

cyanogenic epoxides.[31,182] Cyanogenesis has been detected in prokaryotes, fungi, plants, and animals. Cyanogens have been isolated from a great number of organisms; the glycosides, however, have been isolated only from plants and insects.[182]

The release of cyanide from a cyanogen implies the degradation of the compound, a reaction that may be either spontaneous or enzyme catalyzed.[52,182] Cyanogenic lipids and cyanogenic glycosides are broken down to cyanohydrins (hydroxynitriles); these are cyanogens in themselves (Figure 4–2). The cyanogenesis starts on crushing of the tissue, the cyanogens, and the degradative enzymes being compartmentalized either at the subcellular level or at tissue level in the intact plant.[211]

The glycosides are the most common cyanogens, and comprise more than 60 structures.[146,241] They were recognized early as substances that are poisonous to animals.[86] Cyanogens are of some systematic importance at the level of higher plant taxa,[182,265] and within certain families and genera.[182,194,242] The ingestion of cyanide and cyanogenic compounds may lead to acute[172] as well as chronic intoxications, the latter including the central nervous system (CNS) syndrome, konzo.[269,270]

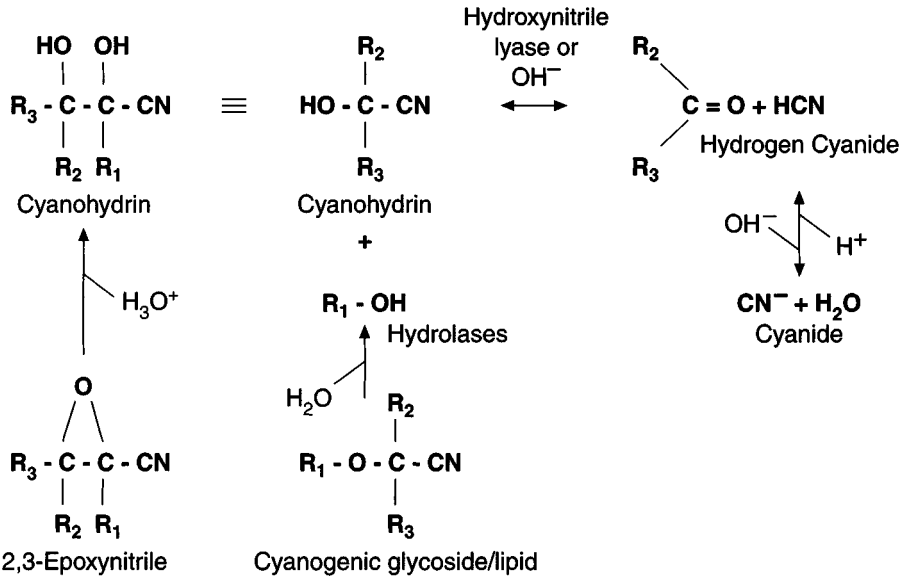

Figure 4–2 The interrelationship between cyanogenic compounds and cyanide/hydrogen cyanide. In a cyanogenic glycoside, R_1 is a saccharide moiety; in a cyanogenic lipid, an acyl moity. Hydrolases: glycosidase(s)—Refer also to Figure 4–3—or lipase. Note that the cyanohydrin formed upon hydrolysis of one of the three types of cyanogens (epoxynitriles, glycosides, or lipids) is a cyanogen itself.

Glycoalkaloids

Steroidal alkaloids and alkaloid glycosides occur throughout the genus *Solanum* (Solanaceae). The common potato (*S. tuberosum)* contains in its edible tuber the two compounds α-chaconin and α-solanin.[140] The total content may vary from 10 mg/kg to 390 mg/kg, with a mean of 73 mg/kg.[96] In other species of *Solanum* and closely related genera, different glycosides and free genins may dominate.[92,206] Gastrointestinal absorption of steroidal alkaloid glycosides varies between animal species. Some hydrolysis of the glycosidic bond and further metabolism seem to occur in different animal species, as judged from analyses comparing the serum level of α-chaconin and/or α-solanin to that of total alkaloids at different times after ingestion.[140] The toxicity of the potato glycosides to humans includes gastrointestinal upset with diarrhea, vomiting, and abdominal pain. In severe cases, neurological symptoms, some of which are clearly a result of the acetylcholinesterase inhibitor activity of these glycosides,[216] are seen.[140] Both α-chaconin and α-solanin, together with their aglycones, are teratogenic in one or more animal models.[96] However, Kuiper-Goodman & Nawrot[140] did not find the suggested association of the consumption of blighted potatoes during pregnancy with increasing incidences of spina bifida substantiated.

Methylazoxymethanol Glycosides

Glycosides of methylazoxymethanol (MAM) have been found only in cycads (*Macrozamia* and *Cycas* spp). The concentration is high in seeds; smaller quantities are found in stems and leaves.[154] Extensive losses of sheep have occurred in Australia due to consumption of *Macrozamia* and *Cycas* spp.[113] The first isolation of a MAM glycoside, macrozamin (the β-primeverosid of MAM), was from seeds of *M. spiralis* Miq., an Australian cycad. Today, other MAM glycosides are known, among these cycasin (the β-D-glucopyranosid of MAM), which

was shown to be characteristic of, and exclusive to, all the genera of cycads.[154] The relative concentrations of cycasin to macrozamin in ripe seeds differ within the cycad genera.[177] The glycosides release MAM on the hydrolysis, which is catalyzed by β-glycosidases. MAM is a mutagenic and carcinogenic alkylating agent.[163,178]

Oligosaccharides

Flatulence is a common phenomenon that is associated with the ingestion of legumes, among others, and caused by the microbial fermentation of low molecular weight sugars. Many of these sugars are α-galactosides because humans do not have α-galactosidase in their digestive tract.[104] The legume oligosaccharides, raffinose, stachyose, and verbascose,[215] are of particular interest. Soybeans contain (by weight) approximately 1% of raffinose and 2.5% of stachyose;[215] the winged bean contains 1–2% of raffinose, 2–4% of stachyose, and 0.2–1% of verbascose.[94]

Ptaquiloside

Bracken fern(s) (*Pteridium* spp.) found throughout the world causes cancer of the urinary bladder in ruminants and is the only higher plant shown to cause cancer naturally in animals.[249] Enzootic hematuria, the clinical name given to the urinary bladder neoplasia of ruminants, tends to occur persistently in localized bracken-infested regions. The major carcinogen of bracken is the mutagenic and clastogenic norsesquiterpene glucoside, ptaquiloside, which in laboratory animals has been shown to be carcinogenic *per os*.[111,190,249] Bracken has further been associated with carcinoma of the upper digestive tract of cattle, where it is believed to transform the bovine papilloma (type 4) to a malign tumor. After hydrolysis of the glucoside, the genin is partly converted under alkaline conditions to a dienone, which can then undergo further reactions to form adducts with DNA bases. A preliminary investigation of the alkylation patterns produced has been presented.[250] Bracken fern is acutely toxic to several farm animals such as horses, cattle, and sheep, the syndromes being different for the different animal species.[82] The administration of pure ptaquiloside to a calf resulted in the same symptoms as known for the bracken intoxications of this species, thus demonstrating that the causative principle of cattle bracken poisoning is ptaquiloside.[112]

Saponins

A great number of food and feed plants contain saponins. Saponins may belong either to the group of pentacyclic triterpenoid saponins or to the steroidal saponins. The latter include in a broad sense the steroidal alkaloid glycosides that are found, for example, in potatoes. Although certain saponins such as the medicinally used quillaja saponin have been known for centuries to damage mucous membranes,[125] most saponins are considered quite unproblematic when they are administered orally. Saponin fractions from certain *Yucca* spp. have even been used as a feed additive to promote growth of, for example, turkeys.[71] However, concerns have been raised recently that saponins in food or feed may promote oral sensitisation to allergens through their membranolytic action in the gastrointestinal tract, resulting in enhanced uptake of the allergens.[128] This concern is based on the fact that saponins have been shown to act as oral adjuvants.[59,132,158] Food plants that may contain considerable amounts of saponins include the seeds of *Quinoa* spp., fruits of *Borassus flabellifer* (palmyrah) and *Balanites* spp., and roots and stolons of *Glycyrrhizae glabra* (licorice) (Table 4–1). The palmyrah fruits are fermented to wine (palm wine) in Sri Lanka, whereas experimental solid-state tempeh fermentations have been described for quinoa. However, no information is available concerning the fate of the saponins in any of these products.

Vicine and Convicine

Vicia faba (faba/fava bean), *V. harbonensis,* and *V. sativa* contain the two glycosides, vicine and convicine,[219] which after hydrolysis in the intestine and uptake of the genins (divicine and isouramil) cause hemolytic anemia (favism) in

glucose-6-phosphate dehydrogenase-deficient individuals.[166,284] Together with condensed tannins, these two glycosides limit the use of the proteinaceous raw faba beans as feed for monogastric animals.[167,276] Vicine and convicine have not been detected in significant concentrations in other plant species.

Cucurbitacins

Cucurbitacins were first characterized as the bitter compounds of cucumbers, marrows, and squashes (Cucurbitaceae). The cucurbitacins as a group are thought to be among the most bitter substances known to man. Cucurbitacin B can be detected in dilutions as low as 1 ppb, and the glycosides of cucurbitacin E at 10 ppb.[175] Cucurbitacins make up a group of oxygenated tetracyclic triterpenes, some of which occur as glycosides.[103] Some cucurbitacins are not only bitter, but also toxic. Thus, the lethal dose for 10% of a test group of mice (LD_{10} orally mice) of cucurbitacin B is approximately 5 mg/kg b.w.[103] This is quite strong toxicity, as seen from the fact that it is equal to the lowest dose used in the International Organization for Economic Cooperation and Development Guidelines test for acute oral toxicity (Fixed Dose procedure, guideline no. 420).

Glucosinolates

In 1990, more than 100 glucosinolates were already known.[252] They occur in Capparales, Salvadorales, Violales, Euphorbiales, and Tropaeolales within Violiflorae sensu Dahlgren.[57,58] Reasons for interest in glucosinolates or glucosinolate-containing plants are the various antinutritional and toxic effects, the flavors, and the positive physiological effects associated with these constituents and their byproducts.[23] Rape (*Brassica napus, B. campestris,* and *B. juncea)* is among the most important crop containing glucosinolates. Seeds of these species contain approximately 400 g of oil and approximately 250 g of protein per kg. However, the use of rapeseed meals as a protein source in livestock rations and human diets is limited because of compounds associated with the protein fractions. These include phytic acid, phenolic compounds, and glucosinolates. Rapeseed that is bred to contain less than 2% erucic acid in its oil and less than 30 μg/g of aliphatic glucosinolates is termed "double low" or "canola." All pure glucosinolates tested in animal diets have caused antinutritional or toxic effects even when they were in concentrations relevant to levels based on double-low rapeseed as the protein source.[243]

RISKS ASSOCIATED WITH THE OCCURRENCE OF TOXIC GLYCOSIDES IN DIFFERENT COMMODITIES

Regarding toxins in food, the compounds that call for discussions in further detail are the cyanogenic glycosides, but also the MAM glycosides, ptaquiloside, the saponins, the favism agents (vicine and convicine), and the glucosinolates.

Cyanogenic Glycosides

Although discussions concerning a toxicity of intact cyanogenic glycosides may be found, the literature at present concludes that known intoxication syndromes, whether acute or chronic, are mainly due to HCN that is formed from the compounds.[235,236]

A plant containing cyanogenic glycosides may or may not contain enzymes that catalyze their breakdown (i.e., hydrolases [β-glycosidases] and cyanohydrin lyases). These are stored separately from the glycosides.[211] When a tissue containing both cyanogenic glycosides and these enzymes is crushed, enzyme(s) and substrate(s) are brought together and hydrolysis and further lysis (i.e., cyanogenesis) starts. Thus, the intake of raw or processed cyanogenic material normally will mean an intake of a mixture of the genuine glycoside(s) and accompanying hydrolysis products. Tissues that only contain the glycosides (and not the enzymes) will only give rise to exposure to the genuine glycoside(s).

Although cyanogenic glycosides may undergo acid hydrolysis,[30,83] the conditions in the

stomach of a nonruminant, together with the very short residence time, will let the main fraction pass to the intestine. In the intestine, the glycosides will be absorbed, as shown for linamarin in a number of animal species and in humans,[19,37,110,208] and for prunasin and amygdalin in different animal species,[44,220,221,235] or it will be hydrolyzed by microorganisms.[28,44,210] In humans, Carlson *et al.*[43] very recently found that approximately 25% of linamarin ingested in a stiff porridge prepared from cassava flour was absorbed and excreted unchanged in the urine, whereas a little less than 50% was converted to cyanohydrin or cyanide and absorbed as such. The rest could not be accounted for. Most or all of the absorbed glycosides will be excreted in the urine, as shown for both linamarin and amygdalin in animals and humans.[7,37,110,173] HCN as well as cyanohydrins will give rise to cyanide exposure through absorption and nonenzymatic lyses of the cyanohydrins.

Whereas the absorbed glycosides will be excreted unchanged in the urine, the HCN will be totally or partly metabolized, the main metabolite being the goitrogenic compound, thiocyanate. The rate of this conversion will depend on the nutritional status of the individual. Current knowledge concerning the known biomarkers for cyanide exposure (acute and long term) and their use in clinical and experimental toxicology was reviewed by Rosling.[231] The detoxification processes (metabolization) and methods for the estimation of the sulphane sulphur pools available for this were reviewed by Westley.[281] Acute human intoxications have been described as a result of the intake of cassava products and almonds, whereas sorghum and cyanogenic acacia leaves and pods have caused veterinary intoxications.

Acute Intoxication

Acute intoxications in humans caused by the intake of insufficiently processed cassava meals have been reported from nearly all parts of the cassava consuming area, although it must be emphasized that the published reports are very scarce in relation to the extensive use of cassava as human food.[5,76,78,172] The symptoms of acute intoxication include vomiting, nausea, headache, dizziness, difficulty with vision, and collapse.[172]

Chronic Intoxication Syndromes

Evidence has accumulated that cyanide exposure from the diet is a causative factor in konzo,[115,269] and may aggravate iodine deficiency disorders.[60] The influence, if any, on the development of special types of diabetes remains a matter of discussion.[3,263] Symptoms and diagnosis of konzo have been described by Rosling & Tylleskär.[232]

Based on the knowledge available concerning the toxicity of cyanide and cyanogenic glycosides, the Joint World Health Organization (WHO)/Food and Agricultural Organization (FAO) Expert Commitee on Food Additives and Contaminants (JECFA) tried to estimate a safe level for the intake of cyanogenic glycosides by humans. The committee concluded that "because of lack of quantitative toxicological and epidemiological information, a safe level of intake of cyanogenic glycosides could not be estimated." However, the committee also concluded that "a level of up to 10 mg HCN/kg of product is not associated with acute toxicity."[253(p332)] Thus, no authority has yet felt confident to set scientifically based safe levels for the intake of one or more of the known cyanogenic glycosides (or their products of degradation), that is, levels that take the risk(s) for the development of chronic intoxications into consideration. In acknowledgement of this, the "International Workshop on Cassava Safety," held in Ibadan, Nigeria in 1994, concentrated on making recommendations concerning steps to be taken in research; in breeding programs; and in information to extension workers in the agricultural, food, and nutrition sectors.[10]

Long before humans knew the identity of cyanide, they did know that bitter cassava is a good starch crop, but that it must be detoxified before consumption.[67,69] Today, we know that this is because of its content of the cyanogenic glucosides, linamarin and lotaustralin. Overviews of the occurrence of cyanogenic glycosides in plants used for human or animal consumption are provided in Conn[51] and Jones.[129] Some of the

important species of plants have been subjected to selection/breeding for a low total cyanogenic potential (TCP). Examples of the constituents and the TCP of economically important crops are provided in the following sections, together with some remarks concerning their importance as food or feed commodities.

Phaseolus lunatus (Seeds) and Other Beans. Seeds from several species of legumes are used for human consumption, many of which contain toxic and antinutritional substances. Thus, seeds from, for example, *P. lunatus, P. aureus, Cajanus cajan, Canavalia gladiata,* and *Vigna unguiculata* have been examined due to concerns about the possibility for cyanide intoxications.[63,192] All of these species are known to be cyanogenic in one or more tissues.[242] *P. lunatus* contains linamarin as its main cyanogenic constituent; the cyanogens have not been identified in the other species.[242] Only *P. lunatus* has been subjected to investigations concerning the variation in the cyanogenic potential.[21] However, several of the other species certainly may contain toxic amounts of cyanogens in the seeds.[192]

Prunus Species (Seeds). Peach, plum, cherry, apricot, and almond (family Amygdalaceae sensu Dahlgren) are all drupes (stone fruits) of great importance to man. Cyanogenic glycosides typical for Amygdalaceae are phenylalanine derived.[182] Thus, the ripe seeds of *P. persica* (peach), *P. domestica* (plum), *P. avium/cerasus* (cherry), *P. dulcis (P. amygdalus)* (almond), and *P. armeniaca* (apricot) all contain amygdalin as the major cyanogenic constituent. The total cyanogenic potential per gram dry weight of whole fruit rises during the early development, and the relative composition of cyanogens changes from 100% prunasin in the beginning to nearly 100% of amygdalin in the ripe seed.[90,170,189] Amygdalin and different *Prunus* seeds have, in spite of their ineffectivity, been commercially promoted for years as medicines to treat different cancers.[108]

- *Almond*—This tree is very widely cultivated around the Mediterranean. The naming of the species and its varieties/cultivars has changed through time.[99] The tree comes in two varieties, var. *dulcis* and var. *amara*, of which var. *amara* contains high concentrations of amygdalin in its ripe seeds (also denoted "bitter almonds").[39,50,99] The seeds are used in confectionary and bakery.[99] They contain approximately 50% of lipids, the oil being used in cosmetics and dermatology.[39,99] Bitter almonds (but also, e.g., apricot seeds) are also used to produce an essential (volatile) oil called "oil of almonds." This competes with synthetic benzaldehyde as a source of flavor.[39] Only few references exist concerning the content of cyanogenic glycosides in almonds.[50,90] Conn[50] found bitter almond seeds to release 290 mg of HCN/100 g of seed. According to Sturm,[260] commercial sweet almonds from California in general contain less bitter seeds (approximately 1%) than the 2–3% that is normally found in the Mediterranean ones.
- *Apricot*—Apricots have considerable economic importance for several countries such as Italy, the production of which was approximately 200,000 tons in 1988.[259] Different products are marketed from apricots, including fresh, dried, and canned fruits; nectar; jam; and distilled liqueur.[176] The number of varieties and hybrids of apricots are numerous.[174] Thus, Audergon *et al.*[14] tested more than 400 varieties as part of a physicochemical characterization program. Several marketed products of apricots require destoning,[55] leaving the stones as a byproduct from which oil can be extracted. The use of the seed/presscake is, however, restricted by the toxicity.[266] Depending on the variety and type of apricot, the apricot stone is relatively small, representing 6–8% of the fruit weight, even if it can sometimes reach 10%.[176] To the best knowledge of the present author, no investigations have been published concerning the variation in content of amygdalin in seeds of different cultivars. However, as part of investigations concerning the microbial degradation of cyanogens in such

seeds, Tuncel *et al.* [266,267] analyzed two batches of bitter and one of sweet Turkish apricot seeds, obtained on the commercial market. The bitter ones were found to contain approximately 52 and 92 μmol/g d.w., respectively; the sweet ones contained approximately 2.5 μmol/g.

- *Peach*—Much of the same that has been said for apricot can be said for peach. Seeds from *P. persica* Batsch (peach) also contain amygdalin as their major cyanogenic constituent.[193] Kupchella & Syty[141] analyzed the total cyanogenic potential of the seeds from an undefined cultivar and found it to correspond to a content of amygdalin of approximately 2.45% w/w.

Linum usitatissimum (Seeds = Linseed/Flaxseed). Flax is grown for two main purposes, fibers and seeds. Different cultivars are used for the two products. Whole seeds are used as a laxative due to the swelling seed coat polysaccharides.[200] Both full-fat flaxseed flour and defatted meal from the oil extraction are on the commercial market, the latter in two qualities, with 30% and 40% protein, respectively.[198] Flax is one of the major industrial oilseeds traded in world markets. Global production for crop year 1994–1995 was 2.44 million metric tons, with Canada contributing a major share. Flaxseed oil is used for a multitude of purposes, the oil being priced up to four times that of the whole seed.[198] The extraction cake (linseed meal) is traditionally used for fodder purposes. Recently, research into the refinement of flax products has accelarated. Thus, two patents have been issued for the use of flaxseed polysaccharide (gum) for cosmetic and medical preparations,[13,196] and an optimization of protein extraction from defatted flaxseed meal has been presented.[199]

Until 1980, linamarin was thought to be the main cyanogen in linseed. However, looking for the factor(s) in linseed meal responsible for its protective effect against selenium toxicity, Smith *et al.*[251] isolated two new cyanogenic glycosides (linustatin and neolinustatin). A later TLC-based investigation concerning the concentrations of different cyanogenic glycosides in a linseed sample gave the following μmol/g: linustatin+neolinustatin 4.6, linamarin 0.46, and lotaustralin 0.36,[32] pointing to linustatin and neolinustatin as the major cyanogenic constituents. This was further confirmed by a high performance liquid chromatography (HPLC) analysis of 48 samples, which on the other hand only found traces of linamarin and lotaustralin.[240] However, a recent investigation showed quite some variation between 10 cultivars. Two contained no linamarin, whereas in the cultivar Vimy, 7.8% of the weight of the total cyanogenic glycosides were linamarin.[201] This is close to the findings of Brimer *et al.*[32] for an unspecified sample. Frehner *et al.*[90] analyzed both the cyanogenic potential and the relative cyanogen composition during fruit development—one cultivar. As in *Prunus* seeds, the monoglucosides predominated at anthesis, shifting toward diglycosides during maturation. Rosling[230] found the cyanogenic potential of a nonspecified number of commercially sold linseed in Sweden to range from 4 mmol/kg to 12 mmol/kg (112–336 mg kg^{-1} HCN). The acute lethal dose is less than 2 mmol in 24 hours in sick and malnourished patients.[270]

Manihot esculenta (Roots and Leaves). The genus *Manihot* (Euphorbiaceae) incorporates more than 200 species, all originating in tropical America, from where several have been spread to other continents. Thus, *M. esculenta* Crantz (cassava) is today grown as a major source of starch in tropical Africa, India, Indochina, Indonesia, and Polynesia.[184] As early as 1605, Clusius reported that cassava could be toxic to man. The two cyanogenic glucosides, linamarin and lotaustralin, are responsible for this.[70,183] The cyanogenic potential (CNp) of several cassava germplasm collections has been investigated. Thus, Aalbersberg & Limalevu[1] analyzed 28 cultivars grown in Fiji, and found a variation from approximately 15 mg to 120 mg HCN equivalents/kg f.w. Dufour[67,69] looked at 14 cultivars of the Tukanoan Indians in northwest Amazonia and found very high levels (310–561 mg HCN eq./kg f.w.) in *Kii* (toxic cultivars) and 171 mg HCN eq./kg f.w. in the only *Makasera*

(nontoxic/safe cultivar) grown. The Tukanoan Indians clearly expressed that they preferred toxic varieties as the main staple (70% of calorie intake) component of their diet. In this connection, it should be noted that the so-called "safe" (*Makasera*) cultivar had a higher CNp than the 100 ppm (f.w.) that was proposed as the upper limit by Koch[67,69] based on acute toxicity. Finally, Bokanga[24] examined 1,768 different cassava collections and found that the content of the central pith of the root varied from approximately 1 mg to more than 530 mg HCN equivalent/ kg d.w. The peel surrounding the pith has a much greater content, as have the leaves.[24] No acyanogenic cassava was found. While discussing the cyanogenic potential in this precise way, it should be born in mind that variations of up to 100% may be recorded between roots of the same plant.[24] It has also been shown that age, agricultural practices,[73] and environment may have a strong influence on its cyanogenic potential.[24,26]

The leaves of *M. esculenta* also serve as food and feed.[25] The cyanogenic potential of leaves from the same plant is less variable than that of the roots,[24] and is usually 5 to 20 times higher on a fresh weight basis.[25] The high content in the leaves normally does not present a problem for their use in food, given the methods generally used in their preparation.[25] In contrast, the roots of many cultivars, if not properly processed, have actually caused both acute and chronic intoxications worldwide. However, it should be emphasized again that the cassava root (even highly cyanogenic types) is a very valuable and irreplaceable crop. To ensure its safe use in every community under all conditions, the effectiveness of the different processing techniques (under rural as well as industrialized conditions) needs to be verified and the knowledge spread.[10]

Sorghum Species (Leaves and Seeds). Seedlings of *S. bicolor* (Poaceae) and other *Sorghum* species synthesize the cyanogenic glucoside dhurrin that is localized in the ariel shoots of the plant.[101] Thus, three-day-old etiolated seedlings of *S. vulgare* (i.e., the name for any cultivated grain sorghum) was found to contain up to 15 μmoles/g.[4] The content in mature leaves is much lower. The concentration depends on species, subspecies, and race/cultivar, and is also influenced by ecological factors.[65] Although most intoxications are seen in cattle browsing a newly sprouted field, forage may not be totally safe.[65,282] Grain sorghums constitute an important part of human nutrition in several semi-arid areas of the world.[61,88] Generally, the grains are considered completely safe for human consumption,[88,136] although the digestibility and biological value are not always high as a result of the occurrence of quite high concentrations of phytate and polyphenolics in several cultivated types.[88,120] Especially in Sudan, sorghum is irreplaceable, being the traditional stable food.[64] Although sorghum seeds in general are safe, germinated seeds are not. In certain African countries, germinated sorghum seeds are used traditionally for the production of malt,[88] which in turn is used for the brewing of alcoholic beverages[64] and for the production of the baked products called *Hulu-mur*.[64] According to FAO,[88] the traditional methods of preparation of these products remove the dhurrin effectively; however, it is stressed that the existence of these products must not be seen as an indication of sprouted sorghums being safe—they are not.[88]

MAM Glycosides

A metabolic fate and mechanism of toxicity, including the same alkylating end product as with dimethylnitrosamine, has been proposed for the MAM that is released from the MAM glycosides.[209] Thus, cycasin has been shown to be toxic to a number of animals, causing hepatic lesions and demyelination with axonal swelling in the spinal cord.[22,246] Cow's milk may be a vector of transmission of plant toxins. Thus, Mickelsen *et al.*[168] showed that MAM can pass into the milk of lactating rats, causing tumors in the offspring. The seeds of several *Cycas* spp. are traditionally eaten in Australia[22] and on certain islands.[150,254] A special neurological syndrome occurring on the island of Guam, and termed *Guam ALS-PDC,* has been hypothesized to be due to the intake of seeds of *C. circinalis*.[150,254] In 1987, Spencer *et al.*[254] pro-

posed that the causative factor of this syndrome was the neuroexcitotoxic amino acid β-N-methylamino-L-alanine (BMAA). However, a number of subsequent investigations doubted this, as reviewed by Stone[258] in an article on the gradual disappearance of this disease. Thus, it may never be known whether the MAM glycosides could have a role in this disease, though it remains a possibililty given the spinal cord lesions reported in goats as a result of chronic intake of cycasin.[246]

Ptaquiloside

The carcinogenicity of ptaquiloside demonstrated in feeding experiments with rats, mice, hamsters, guinea pigs, and cattle, among others, is alarming because the young shoots of bracken fern are highly regarded as a tasty dish in Japan.[111] Hence, this intake of bracken has been linked to high incidences of stomach cancer in Japan,[111] and in Costa Rica among people who have been exposed to milk that was produced in bracken-infested grasslands.[6] The theory has been supported by the finding of a high tumor incidence in rats and mice that were fed milk from cows that had been fed with dietary complements of bracken, and by the subsequent demonstration of ptaquiloside in bovine milk.[6]

Saponins

Food and feed containing saponins include soybean, guar, quinoa, balanites fruits, and others. Besides the membranolytic action of many saponins, certain of these compounds exert special effects due to the structure of their aglycone.[286] Such effects include (1) lowering of blood cholesterol;[144] (2) reversible sodium retention and potassium loss leading to hypertension, water retention, and electrolyte imbalance (e.g., glycyrrhizinic acid found in licorice root, the roots and stolons from *Glycyrrhiza glabra,* and for products to which licorice root extract, or glycyrrhizinic acid, has been added);[100,133,239,256] and (3) crystal formation in the liver and biliary system, which may inhibit the excretion of phylloerythrin (from chlorophyll degradation), causing a subsequent photosensitation as seen in "Geeldikkop" (a *Tribulus terristris* intoxication).[131] A number of saponins are bitter. The occurrence of bitter saponins in palmyrah (*Borassus flabellifer* L) fruit pulp thus reduces the use of juices based on this fruit.[74] Likewise, seeds of *Chenopodium* spp. used for human consumption (*C. quinoa* [quinoa], *C. pallidicaule* [canihua], and *C. berlandieri* ssp. *nuttaliae* [Safford] Wilson and Heiser [huauzontle]) contain bitter saponins,[95,107,226] most of which are concentrated in the outer layers of the grain.[40,41,223]

Vicine and Convicine

Favism is characterized by anemia, jaundice, and hemoglobinuria, and may develop in subjects with glucose-6-phosphate dehydrogenase (G6PD) deficiency as a consequence of faba bean intake. Favism has also been reported in breast-fed infants whose mothers had eaten faba beans, and in newborn infants.[54] More than 300 variants of G6PD are known.[273] In addition, an association between the genotype of ACP_1 (human red cell acid phosphatase) and favism has been shown, and a possible biochemical mechanism has been proposed.[27] Most cases of G6PD deficiency described in the past were from Italy and other countries around the Mediterranean, that is, patients with the common Mediterranean B-form of G6PD, rather than the common African A (-) form.[273] However, recent investigations have shown that subjects with variants that result in a relatively mild G6PD deficiency may also develop favism.[93,181] Preventive measures and treatments have been described elsewhere.[102,162,188]

Glucosinolates

The most prominent toxic manifestation of glucosinolates in humans is the occurrence of goiter.[214] In animal experiments, this and other effects were generally more pronounced when myrosinases were included in the diet.[252] The effects seen were related to differences in the side chains and to chirality.[252] The fact that there are several mechanisms behind the toxic and antinutritional effects has also been very re-

cently stressed by the results of the most detailed studies on the degradation products of various glucosinolates.[23] These authors presented an overview of the different degradation products formed from glucosinolates, which also include, for example, oligomers. From the degradation of glucobrassicin (an indole glucosinolate), indolyl-3-methanol is formed in considerable amounts, but it disappears very quickly, giving rise to, among others, appreciable amounts of thiocyanate ion. No organic isothiocyanates and thiocyanates are formed. In contrast, the degradation of various aliphatic glucosinolates results in the formation of nitriles as well as isothiocyanates and thiocyanates.[23] Toxic effects of glucosinolates in *B. oleracea* have been reviewed by Stoewsand[257] and those of crambe (*Crambe abyssinica*) meal fed to broiler chicks by Kloss *et al.*[137] The mechanism behind the observed decrease in cancer risk for people on diets with a high content of cruciferous vegetables has been investigated by Wallig *et al.*[274]

VARIATION IN TOXIN CONCENTRATION AMONG VARIETIES AND CULTIVARS: THE INFLUENCE OF TRADITIONAL DOMESTICATION AND MODERN BREEDING

Several toxic glycosides (including various saponins and cyanogenic glycosides, etc.) are known to be bitter tasting in addition to toxic. Hence, the term "bitter," as opposed to "sweet," has been used traditionally to designate naturally occurring or selected groups within a plant species that contain high amounts of the toxic (and bitter) substance. Depending on the view of the botanical author, the groups in question may be divided on the level of variety, form, or cultivar. Examples of plant species for which the division bitter/sweet has been used are *P. dulcis* and other *Prunus* spp. (containing amygdalin), as well as *M. esculenta* (cassava, containing linamarin), and in quinoa.[139] In most such cases, a correlation between the toxicity (content of glycoside) and the degree of bitterness of the plant part has been established. However, it is only seldom that a proper investigation concerning the degree to which this correlation holds has been performed. Thus, a positive correlation, but with exceptions, was found in a number of smaller studies on cassava roots.[247] Hence, King & Bradbury[135] took up the challenge of investigating in more detail the bitter-tasting substances in cassava parenchyma and cortex. Linamarin was found to be the sole contributer to bitterness present in the parenchyma; a new structure (isopropyl-β-D-apiofuranosyl-(1–6)-β-D-glucopyranoside) contributing in the cortex of some cultivars. This is in agreement with a very recent study from Malawi,[234] which compared the content of cyanogenic glucosides in the cortex of 492 cassava roots with their taste as estimated by a taste panel. The correlation had an $r^2 = 0.96$ when looking at the cultivar level.

It is well documented, at least for a number of cyanogenic plant species, that the concentration of both the glycosides and the enzymes degrading them can show a discrete variation (polymorphism) as well as a continous one. The polymorphism is genetically based, whereas the continous variations observed may be both genetically and environmentally influenced.[24,26,116–119,130,159,186] The genetic polymorphism (discrete variation, chemical races) with respect to the occurrence of both cyanogenic constituents and hydrolytic enzymes makes it difficult to define what is meant by a "cyanogenic species." Furthermore, it should be noted that the cyanohydrin lyase, which cleaves the cyanohydrins formed after the hydrolysis of the glycoside(s), may be expressed in certain organs and not in others. Thus, White *et al.*[283] recently showed that this enzyme, although present in the leaves, is not expressed in the roots of cassava. This observation explains why very high intermediate concentrations of cyanohydrins are formed during the processing of cassava roots. The environmental influences mentioned above may furthermore mean that certain plants will be found positive at some times of the year and negative at others.

Increased use of more highly cyanogenic cultivars of cassava among small farmers has been reported from several places. Thus, Dufour reported on a clear preference for Kii (toxic variet-

ies) for most purposes by the Tukanoan Indians,[67,69] whereas Aalbersberg & Limalevu[1] stated that planting of the toxic (bitter) cultivars increased in New Guinea. Also, Onabolu *et al.*[197] found that the three most commonly grown cultivars in Ososa (a semi-urban farming community approximately 80 km east of Lagos, Nigeria), where cassava has been the main staple for decades, were all stated to be poisonous and to need processing. However, this was not regarded as a disadvantage. Farmers' reasons for preferentially growing cassava cultivars providing bitter roots were studied in Malawi.[48] In many traditional agricultural communities, the farmers (often the women) judge the "safeness" of the roots by chewing a small piece. According to Dufour,[67,69] the Tukanoan Indians appear to be able to distinguish accurately less from more poisonous cultivars by the taste. Very recent studies from Malawi prove such a procedure to exist, and to be very effective (Chiwona-Karltun, personal communication, October 2000).

Several of the species within the family Cucurbitaceae, which are used as human food, naturally contain cucurbitacins in amounts that are unacceptable to the market. However, intense domestication and breeding have resulted in cultivars low in bitter compounds.[126,127] Breeding programs for curcurbits are constantly aware of the bitterness.[84]

Great variations (0–13000 μg/g) may also be found in the content of ptaquiloside in bracken fern as a result of both ecological and genetic variation, a tendency for higher contents being reported when originating in relatively colder climates.[249] In addition, *P. esculentum* contains the cyanogenic glucoside, prunasin, the concentration of which similarly has been related to climatic conditions.[153]

Also, quinoa cultivars vary concerning the quantitative content of saponins, and the tradition has, as for other crops, been working with so-called sweet and bitter varieties.[139]

For *V. faba,* it should be mentioned that, although Duc *et al.*[66] gave the first report of a gene that codes for nearly a zero content of vicine and convicine, present-day cultivars contain approximately 7 and 2.5 mg g^{-1} respectively.[276]

REMOVAL OF TOXINS THROUGH PROCESSING

Traditional Household versus Modern Industrial Processing

When discussing the removal of toxic and antinutritional constituents, one must distinguish between traditional household processing and industrial processing. The two procedures may use different starting materials and will have different means of analyzing these and different methods available for processing. The priorities may indeed be very different when choosing between slow versus fast processing methods, and between processes that require low input of water and/or energy as compared to methods requiring a high input. The general trend of a greater number of traditional food fermentation processes being industrialized has been discussed recently by Rombouts & Nout.[228]

A number of industrial processing methods or laboratory methods meant for industrial development were investigated quite early on for soybean (e.g., removal of oligosaccharides and proteinase inhibitors)[47,56,91,109,224,245,248,261,264] and for cruciferous plants (glucosinolates).[20,72,85,151,255] Later, linseed (flax, cyanogenic glycosides),[149,156,157,275] jojoba meal (organic nitrile glycosides),[2] citrus juices (limonoids and naringin),[105,145,212,229,244] cotton seeds (gossypol),[179] and quinoa seeds (saponins)[95,226] were focused on. For commodities such as cassava roots and leaves, lima beans, cycas seeds, bracken leaves, and yam tubers (alkaloids as well as terpenes), most methods described and investigated scientifically are actually household processing methods. A look at the processing of cassava roots will help illustrate the important characteristics for each of the two sectors.

Household Processing (Cassava Roots)

Both in South America and in sub-Saharan Africa, sweet and bitter roots are generally regarded as two well-known different crops, and most traditional methods of processing that have been studied have proven very effective in re-

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