New Zealand Food Safety
Haumaru Kai Aotearoa

Evaluation of food safety risks associated with foods containing cyanogenic glycosides

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Scientific Interpretative Summary

This Scientific Interpretative Summary is prepared by New Zealand Food Safety (NZFS, a business unit of Ministry for Primary Industries (MPI)) risk assessors to provide context to the following report for MPI risk managers and external readers.

FW22009: Evaluation of food safety risks associated with foods containing cyanogenic glycosides

Plants contain substances which can pose potential risk to consumers, one of these being cyanogenic glycosides (CG). CG are converted to hydrogen cyanide (HCN) by enzymatic action on plant tissue or the action of gut microflora in animals and humans. The consumption of foods that contain CG can result in acute or chronic cyanide poisoning. The amount of CGs in food is usually reported as the level of releasable HCN.
In 2020, three New Zealanders required hospital treatment after consuming raw apricot kernels. MPI has also seen an increase in consumer and importer queries on importing these products. Whilst they are not permitted for sale, there is no regulatory intervention to prevent the import of such products.

To ensure we have an adequate understanding of the nature and level of risk presented by CG, MPI contracted the Institute of Environmental Science and Research (ESR) to undertake a review of the latest information on CG in plant-based foods.

With regard to the types of foods containing CG and HCN, the literature did not identify any high-HCN foods likely to be consumed in New Zealand, other than those previously examined. High levels of HCN have been detected in kernels of most species of stone fruit and pome fruit seeds namely apples, however, there is no evidence to suggest that stone fruit kernels or pome fruit seeds are consumed as food items in New Zealand. A study that looked at levels of HCN in taro leaves concluded that commercially available taro leaves in New Zealand did not contain detectable HCN, albeit in contrast to African studies.

A considerable difference is reported between the HCN content of sweet and bitter almonds. Previous analysis on almonds available at retail in New Zealand suggests they are from sweet cultivars which have been shown to have a substantially lower HCN concentration than bitter cultivars. The presence of almonds from bitter cultivars in the New Zealand food supply would be a cause for concern.

Various methods of processing are reported to reduce but not completely remove cyanogenic potential from CG-containing foods. It was identified that the reductions in cyanogenic potential reported are due to the interaction of CGs and β-glucosidase enzymes, and the subsequent release of volatile HCN. Additionally, these processes are enhanced with moderate heat.

Incidents of acute cyanide poisoning following consumption of plant material are mainly reported following consumption of stone fruit kernel, inadequately prepared (usually bitter) cassava, or bitter almonds. Despite the reasonably high concentrations of CGs in linseed, there have been no reports of intoxication following linseed consumption. It is likely that this is due to the structure of the CGs present in linseed and their likely greater resistance to microbial hydrolysis in the human gut.

A limited range of risk management measures have been applied to control the risks associated with cyanogenic substances in the food supply via prohibition of certain plant materials or through applying maximum limits (MLs) for HCN in certain foods.
This report provides a useful resource to aid understanding of and provide information to support risk management decisions relating to cyanogenic glycosides in plant-based foods.

Science for Communities

EVALUATION OF FOOD SAFETY RISKS ASSOCIATED WITH FOODS CONTAINING CYANOGENIC GLYCOSIDES

May 2022

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PREPARED FOR:	**Ministry for Primary Industries, project code CFS2101/406663, Evaluation of food safety risks associated with foods containing cyanogenic glycosides**
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More than 2000 plant species are believed to contain cyanogens (compounds with the potential to form hydrocyanic acid (HCN)). Cyanogenic glycosides (CG) account for at least 90% of the total cyanide-producing potential in most plant species.

Enzymes capable of releasing cyanide from the glycoside (β-glucosidase) are present in plant material, physically isolated from the glycosides. During tissue disruption, the glycosides and the enzyme are able to react. Additionally, some gut microflora contain β-glucosidase enzymes and are able to hydrolyse cyanogenic glycosides during their passage through the gut.

All animal species are susceptible to cyanide poisoning. Cyanide poisoning primarily occurs through inhibition of the mitochondrial enzyme cytochrome c oxidase. This results in disruption of the cellular energy production processes, as the cells of an organism are unable to create adenosine triphosphate (ATP), resulting in histotoxic hypoxia.

The current study was commissioned to establish a risk profile of cyanogenic glycosides in plant-based foods, that will be used by MPI's Imported Food and Food Science and Risk Assessment teams, to assist in addressing risk management questions regarding the levels of hydrocyanic acid in imported foods and whether they present a significant food safety risk to New Zealand consumers.

Results from studies with potential relevance to the New Zealand food supply did not identify any high-HCN foods likely to be consumed in New Zealand, other than those previously examined. While high levels of HCN have been detected in the kernels of most species of stone fruit, these items are generally not considered to be food items in New Zealand. Similarly, high concentrations of HCN have been reported in pome fruit seeds, particularly apples. There is no evidence to suggest that these seeds are an intentionally consumed food item in New Zealand.

Taro leaves have previously been analysed for HCN in New Zealand and were found to not contain detectable HCN. Studies carried out in Africa suggest that the taro root can contain moderate concentrations of HCN (17-21 mg/kg), with one study suggesting markedly higher concentrations.

Study reports indicate a substantial difference between the HCN content of sweet and bitter almonds. The limited analyses previously carried out on almonds available at retail in New Zealand suggests these are probably from sweet cultivars. The presence of almonds from bitter cultivars in the New Zealand food supply would be a cause for concern.

While the impact of a number of food processing steps in the cyanogenic potential of various foods has been examined, it is likely that in most cases the reductions in cyanogenic potential reported are due to the interaction of CGs and β-glucosidase enzymes and the subsequent release of volatile HCN. These processes will be enhanced under conditions of moderate heat.

Most of the studies summarised in the current report indicate that food processing can decrease, but not completely remove the cyanogenic potential of CG-containing foods.

The cyanogenic potential of foods can be determined indirectly by converting CGs to HCN or the CGs can be determined directly, usually by liquid chromatography (direct methods). The results of indirect methods align directly with current regulatory limits, while direct or indirect chromatographic methods offer greater sensitivity (lower LODs) and are useful for determining hydrocyanic acid levels less than 10 mg/kg.

Incidents of acute cyanide poisoning following consumption of plant material are mainly reported following consumption of stone fruit kernel, inadequately prepared (usually bitter) cassava, or bitter almonds. Despite the reasonably high concentrations of CGs in linseed, there have been no reports of intoxication following linseed consumption. It is likely that this is due to the structure of the CGs present in linseed and their likely greater resistance to microbial hydrolysis in the human gut.

It appears that, as long as cases of cyanide intoxication are treated within a few hours of consumption of the cyanogenic food, complete recovery is likely. However, fatalities have occurred.

The occurrence of chronic disease, such as konzo, appear to be restricted to developing countries and situations where chronic consumption of a cyanogenic staple food (e.g. cassava) occurs in the presence of multiple nutrient deficiencies.

A limited range of risk management measures have been applied to control the risks associated with cyanogenic substances in the food supply. Maximum limits have been derived for HCN by Codex (cassava flour and gari), the European Union (apricot kernels and specified flavouring substances) and Australia/New Zealand (RTE cassava chips and specified flavouring substances).

1. INTRODUCTION

More than 2000 plant species are believed to contain cyanogens (compounds with the potential to form hydrocyanic acid (HCN)) (Davis, 1991). Cyanogenic glycosides (CG) account for at least 90% of the total cyanide-producing potential in most plant species.

Enzymes capable of releasing cyanide from the glycoside (β-glucosidase) are present in plant material, physically isolated from the glycosides. During tissue disruption, the glycosides and the enzyme are able to react. Additionally, some gut microflora contain β-glucosidase enzymes and are able to hydrolyse cyanogenic glycosides during their passage through the gut (EFSA, 2007).

All animal species are susceptible to cyanide poisoning (EFSA, 2007). Cyanide poisoning primarily occurs through inhibition of the mitochondrial enzyme cytochrome c oxidase. This results in disruption of the cellular energy production processes, as the cells of an organism are unable to create adenosine triphosphate (ATP), resulting in histotoxic hypoxia.

1.1 PREVIOUS NEW ZEALAND STUDIES

During 2010-2013, the New Zealand Food Safety Authority (NZFSA, now incorporated into the Ministry for Primary Industries) commissioned a number of studies related to the cyanogenic potential of foods:

- A survey of food available in New Zealand (n = 100) for which there was evidence of cyanogenic potential (Cressey and Saunders, 2010). Foods analysed included cassava and cassava products, bamboo shoots, almonds and almond products, apple products, linseed/flaxseed and linseed/flaxseed products, stone fruit products, beans and seeds, and miscellaneous products. All food samples were analysed by an acid hydrolysis-colourimetric method (Haque and Bradbury, 2002).
- A survey of the cyanogenic potential of ready-to-eat cassava chips (n = 100) available in New Zealand (Cressey and Saunders, 2011).
- A survey of retail apple juice samples from Australia and New Zealand (n = 98) and in-process samples (n = 10) from a New Zealand juice manufacturer. Due to the low concentration of HCN in apple juice, some samples were analysed by a more sensitive chromatographic method (Saunders and Cressey, 2012).
- A survey of apricot kernels (n = 28) and products containing apricot kernels, infant apple puree, linseed-containing bread, and cassava and bamboo shoots, analysed before and after cooking (Saunders and Cressey, 2013).
- A literature review of factors influencing the metabolism of cyanogenic glycosides and the production of hydrocyanic acid *in vivo* (Cressey, 2013).

The results from the first four of these studies were published, along with an associated risk assessment, by Food Standards Australia New Zealand (FSANZ) (FSANZ, 2014).

1.2 SCOPE OF THE CURRENT STUDY

The current study was commissioned to establish a risk profile of cyanogenic glycosides in plant-based foods, that will be used by MPI's Imported Food and Food Science and Risk Assessment teams, to assist in addressing risk management questions regarding the levels of hydrocyanic acid in imported foods and whether they present a significant food safety risk to New Zealand consumers.

The outcomes will be used by MPI to inform whether a survey protocol should be developed for the collection and testing of samples, which may inform a change to food standards in the long term.

Specific aspects to be addressed by the risk profile include:

- the types of foods that possibly contain CG and HCN (including raw and processed foods) and concentrations reported.
- the effects of different processing treatments, such as drying, cooking, roasting, blanching, grinding, steaming, on the levels of HCN.
- the available analytical methods for HCN and HCN potential (CGs) in food.
- the adverse health effects, including any reports of hospitalisation and death.
- the likely acute and chronic exposure of NZ consumers to HCN in food products (e.g. consumption data and assumptions), including commentary on new information on metabolism of different CGs.
- regulatory limits set by Australia, Codex and other countries for HCN in food.

In this document the following conventions will be used:

- CG/CGs will refer to the intact cyanogenic glycosides, such as linamarin and amygdalin.
- HCN will refer to the actual or theoretical amount of total hydrocyanic acid that may be released due to the presence of CGs in a plant material or food item.

2. ADVERSE HEALTH EFFECTS

2.1 HUMAN TOXICITY

Potential toxicity of cyanogenic glycosides arises from enzymatic degradation to produce hydrogen cyanide, resulting in acute cyanide poisoning. Clinical symptoms of acute cyanide poisoning include rapid respiration, drop in blood pressure, rapid pulse, headache, dizziness, vomiting, diarrhoea, mental confusion, stupor, blue discolouration of the skin due to lack of oxygen, twitching, and convulsions (FSANZ, 2004; Speijers, 1993).

The human acute lethal oral dose of HCN is reported to be 0.5-3.5 mg/kg bw (EFSA, 2016). The threshold for human toxicity has been report to be a blood cyanide concentration of 0.5 mg/L, with levels of 2.5-3 mg/L being potentially lethal (EFSA, 2016).

Several diseases are associated with chronic dietary intake of cyanogenic glycosides, although there is some debate over the causal relationships due to confounding nutritional factors (Davis, 1991; FSANZ, 2004; Speijers, 1993). For example, malnourished individuals appear to be more susceptible to the effects of cyanogenic glycosides. Populations with a reliance on cyanogenic foods, such as in African regions where cassava is a staple, have developed food preparation procedures that largely detoxify the food. However, the cyanogenic glycoside content of available food and adherence to detoxifying procedures are reported to be variable. Chronic diseases associated with dietary intake of cyanogenic glycosides include:

- Konzo is a motor neuron disease characterised by irreversible weakness in the legs. In severe cases, patients are not able to walk, and speech and arms may be affected. Konzo particularly affects children and women of childbearing age in East Africa in times of food shortage and is associated with a high and sustained intake of cassava (*Manihot esculenta*) in combination with a low intake of protein (Davis, 1991; FSANZ, 2004). More recent studies have suggested that impaired neurocognition and poor neurodevelopmental trajectories may be associated with konzo (Kashala-Abotnes *et al.*, 2019).
- Tropical ataxic neuropathy (TAN) describes several neurological symptoms affecting the mouth, eyesight, hearing, or gait of mostly older males and females. TAN is attributed to cyanide exposure from the chronic consumption of foods derived from cassava (FSANZ, 2004).
- Goitre and cretinism due to iodine deficiency can be exacerbated by chronic consumption of insufficiently processed cassava. Cyanogenic glycosides from cassava are detoxified to thiocyanate that competes with iodine in the thyroid, effectively increasing the dietary requirement for iodine (FSANZ, 2004).

2.2 CASE REPORTS

While the following case reports are not the only reports in the scientific literature, most reports published since 2010 are included. These summaries should be considered as representative of causative foods, symptoms exhibited, and treatments administered.

2.2.1 Apricot kernels

A previously-healthy 27-month-old boy was admitted to the emergency department (ED) after presenting to the hospital with episodes of fainting after having consumed numerous apricot kernels (Kaya *et al.*, 2012). The patient was tachycardic (128 beats/minute) and hypothermic (35°C). The patient was treated with intravenous hydroxycobalamine and activated charcoal. Later sodium bicarbonate was administered to correct metabolic

acidosis. Blood cyanide was not determined for technical reasons. The patient recovered and was discharged on the second day of hospitalisation.

A 28-month-old girl presented to ED with sudden-onset unconsciousness and seizures (Sahin, 2011). Her parents reported that she had eaten approximately 10 apricot kernels. Dicobalt edetate (a cyanide antidote) was given 10 hours after presentation. Whole blood cyanide was 3 mg/L at approximately 20 hours after presentation. The patient remained on supportive care in the intensive care unit (ICU), but died on the 22nd day of hospitalisation.

A previously-healthy 4-year-old boy (16 kg) presented to an emergency department with symptoms of nausea, vomiting, and sudden onset of unconsciousness (Ekinci *et al.*, 2019). The parents reported that he had consumed 15-20 apricot kernels one hour before admission. The patient was comatose, unresponsive to painful stimuli, with deep and irregular respiration. Blood cyanide could not be determined due to lack of laboratory capability, but based on the history of apricot kernel consumption cyanide poisoning was diagnosed. The patient was treated by gastric lavage, activated charcoal ingestion and hydroxocobalamin (vitamin B12) by intravenous infusion (twice). The patient recovered fully and was discharged after three days.

A 35-year-old mentally ill woman presented at ED after consuming 20-30 apricot kernels (10-15 g) (Cigolini *et al.*, 2011). Admission was approximately 30 minutes after consumption, at which time the patient appeared asymptomatic. Gastric lavage was administered. Approximately 70 minutes post-consumption, the patient experienced headache, nausea and dyspnoea and was found to have metabolic acidosis. The patient was treated with amyl nitrite and sodium thiosulphate and then hydroxycobalamin. The patient was transferred from ED after 24 hours, with normal clinical signs.

A previously healthy 41-year-old woman consumed approximately 30 apricot kernels (estimated total weight of 15 g) (Suchard *et al.*, 1998). Within 20 minutes she reported symptoms of weakness and numbness to a friend. The friend called emergency services who found the case unresponsive. Following admission to ED an emergency physician recognised cyanide poisoning and administered amyl nitrate, sodium nitrite and sodium thiosulphate as an antidote. Following an immediate improvement in the patient's condition, activated charcoal was given. The patient was discharged, in a stable condition, on the second hospital day.

A 58-year-old man, receiving palliative chemotherapy for metastatic colon cancer was found to have abnormal liver chemistry (Seghers *et al.*, 2013). The patient admitted to consumption of approximately 70 apricot kernels per day for the last 45 days, as an alternative cancer treatment. Testing did not show lactic acidosis. Elevated blood thiocyanate concentrations were found. The report authors speculated that the level of apricot kernel intake should have generated near-lethal levels of cyanide, but symptoms of cyanide poisoning were not apparent.

Thirteen patients were admitted to a paediatric intensive care unit in Kayseri, Turkey, between 2005 and 2009 with cyanide intoxication associated with consumption of apricot kernels (Akyildiz *et al.*, 2010). The majority of patients were female (9 of 13). The mean onset time for symptoms was 60 minutes (range 20 minutes to 3 hours). Although poisoning was mild in some patients, intoxication required mechanical ventilation in four patients. Metabolic acidosis was observed in nine patients, which was treated with sodium bicarbonate. All patients recovered and were discharged after a mean interval of 3.1 days (range 2-6 days).

2.2.2 Almonds

A 5-year-old boy with no significant medical history was brought to a paediatric ED by his mother after developing symptoms including dizziness, confusion, somnolence and vomiting (Mouaffak *et al.*, 2013). The patient developed tonic-clonic seizures and became comatose.

It was determined that, 5 hours earlier, the patient had consumed about 10 bitter almonds. Testing revealed metabolic acidosis. The child was transferred to ICU, where he was monitored and supported on oxygen. The patient's breathing and neurological functions recovered after 3 hours.

A 58-year-old woman was admitted to ICU with sudden headaches, dizziness, vomiting and generalised seizures (Sanchez-Verlaan *et al.*, 2011). Severe lactic acidosis was determined and the patient was treated with fluids, norepinephrine, insulin, and sodium bicarbonate. Blood cyanide was found to be 2.77 mg/L, prompting immediate infusion with hydroxocobalamin, resulting in complete recovery and discharge after three days. Her husband recalled that she had consumed about 50 bitter almonds approximately 2 hours before admission.

2.2.3 Cassava

A 79-year old female presented to ED with symptoms including nausea, vomiting, dizziness, and generalized weakness (Patel *et al.*, 2019). The patient's daughter reported that the patient had consumed cassava leaves that had only been steamed before consumption. The patient had commented that the leaves were bitter. Cyanide poisoning was diagnosed and the patient was treated with sodium nitrite and sodium thiosulphate. The patient was discharged after three days, having made a full neurological recovery.

2.2.4 Cherry pits

Cases of cherry pit ingestion reported to a state-wide poison centre in the USA during 2003-2018 were reviewed (Menendez *et al.*, 2019). A total of 698 exposures were identified, of which most (511, 73.2%) related to consumption of cherry pits only, while the balance related to ingestion of cherry pits while eating cherries. In 13.3% of exposure (93) ingestion of pits was associated with consumption of a smoothie, shake or juice. About half of the cases (308, 52%) involved consumption of a single pit, however, the mean and maximum number of pits ingested were 4.4 and 50 pits, respectively. Cases were predominantly in the 0-5 years age group (359, 51.4%). Most ingestions (681, 97.6%) were unintentional. No adverse effects were reported for the majority of ingestions, with only two cases (0.3%) reporting moderate effects. The most commonly reported adverse effects were abdominal pain (12, 1.7%), vomiting (11, 1.6%), and nausea (7, 1.0%).

2.3 OUTBREAKS

2.3.1 Cyanide poisoning

An outbreak of suspected food poising occurred in the Kasese District of Uganda during September 2017 (Alitubeera *et al.*, 2019). The outbreak was identified amongst attendees at a funeral and was traced to consumption of a meal of cassava flour prepared with hot water. The investigation identified 98 cases with two deaths (2% case fatality). The median case age was 10 years, with a range from 11 months to 75 years. Main symptoms included vomiting (95% of cases), diarrhoea (87%), malaise (60%), dizziness (48%), tachypnea (27%), syncope (16%), and tachycardia (10%). Cases were found to have consumed cassava purchased from a single wholesaler (wholesaler A). During the outbreak period, wholesaler A was the main supplier to retailers in the affected area. Samples from the implicated batch of cassava flour contained an average of 88 mg/kg hydrocyanic acid.

A family outbreak of acute HCN intoxication occurred in northeast Brazil in 1990 (Teles, 2002). The family had stolen cassava roots from a neighbour's plantation, believing them to be of a 'sweet' variety. The roots were of a bitter variety and were inadequately cooked due to a lack of water and cooking fuel. Of the eight family members who consumed the cassava, four children died and the adults were hospitalised.

An outbreak of foodborne illness was reported in Santa Cruz, Davao del Sur in the Philippines (Penas *et al.*, 2018). A retrospective cohort study was initiated. Fourteen cases were identified, with two fatalities (2 and 4 years old). All cases had consumed cassava, except for one child who had spat out the cassava meal. Implicated cassava was tested and found to contain 69 mg/kg 'cyanide' (analytical method not reported) and was classified as bitter. The relative risk of illness in those consuming cassava was 208 (95% CI 20-2170). The cassava had been grown with the intention of using it as animal feed and included sweet and bitter varieties. The cassava was harvested without permission and there was some evidence of insufficient food preparation.

2.3.2 Konzo

Konzo is a paralytic disease caused by the combined effects of poor nutrition and routine consumption of highly cyanogenic foods such as cassava. In 1996, an outbreak of cases of illness in the Kahemba zone, in the south-west of the Democratic Republic of Congo, was initially diagnosed as poliomyelitis (Bonmarin *et al.*, 2002). However, the diagnosis was changed to Konzo, following review. A total of 237 cases were identified (prevalence 2.4 per 1000 population). Group interviews did not identify any deficiencies and changes in the way cassava was prepared in the affected areas. The most heavily-affected areas were also very poor and there was evidence that due to poverty, cassava was not mixed with maize for consumption in these areas. This practice increases the sulphur amino acid content of the diet, which contributes to cyanide detoxification.

An investigation in the South-Kivu region, in the east of the Democratic Republic of Congo identified 41 cases of konzo (Chabwine *et al.*, 2011). The cases started to appear around 1996. Cases had a mean age of 16 years, with a mean age at disease onset of 12 years. Preparation of cassava in this region involves peeling, heap fermentation for at least 4 days and sun-drying. The region has been badly affected by war and the resultant disruption of the food supply. Cases reported increased consumption of bitter cassava and abbreviation of the cassava preparation processing, including consumption of unprocessed cassava. Cassava roots were provided from cases' households and were found to contain HCN in the range 5-300 mg/kg, with 5 of 21 samples having HCN contents greater than 50 mg/kg. There was also a high prevalence of malnutrition amongst cases.

2.4 RISK FACTORS

2.4.1 Konzo

While the exact biological mechanisms causing konzo have not been established, there is evidence to suggest that the gut microbiome may be a contributing factor, as microbial hydrolysis of CGs is an important route of cyanogenesis (Bramble *et al.*, 2021). A study was carried out of 180 individuals in the Democratic Republic of the Congo, from villages within a high konzo prevalence zone and a low konzo prevalence zone (Bramble *et al.*, 2021). From each area cohorts of konzo-affected and unaffected individuals were recruited. There was some evidence that children in the konzo-affected region were enriched in bacterial species with β-glucosidase activity, enabling hydrolysis of linamarin from cassava. It also appeared that children in the low konzo region had greater abundance of genes for cyanide detoxifying enzymes.

Konzo was first described in Popakabaka, Democratic Republic of Congo, about 70 years ago (Diasolua Ngudi *et al.*, 2011). Prevalence of the disease in this area is about 1.4%. An investigation in this area found that konzo was significantly associated with the female gender, unmarried status, illiteracy, farming as a main occupation, and consumption of cassava from own farm land. The main food consumed in the region was luku, a cassava flour stiff porridge, which was consumed at least once during the day in 99.2% of households. Median consumption of cassava flour was 304 g/person/day (mean 315

g/person/day). Meals of luku were commonly accompanied by consumption of saka-saka (pounded cassava leaves), cowpeas, sesame, mbondi (*Salacia pynaertii*), mushrooms or mfumbwa (*Gnetum africanum*). The foods in the diet were of poor protein quality, particularly with respect to their content of sulphur-containing amino acids.

2.5 CYANOGENIC POTENTIAL OF DIFFERENT FOODS

Intoxication and disease due to consumption of CG-containing foods has mainly been reported for apricot kernels and cassava.

Abraham *et al*. (2016) used a crossover study design to determine the bioavailability of cyanide from different food sources in 12 healthy, adult volunteers. Volunteers consumed persipan paste (prepared from bitter apricot kernels, HCN content 68 mg/kg), linseed (HCN content 220 mg/kg), apricot kernels (HCN content 3250 mg/kg) or fresh cassava roots (76-150 mg/kg). HCN equivalent content was determined by enzyme hydrolysis followed by titration. Each meal was designed to deliver 6.8 mg of HCN equivalents. Bioavailability of cyanide from the foods was assessed in terms of C_{max}, the peak blood concentration of cyanide (µM). Mean values of C_{max} for the volunteer was in the order cassava (17.0 µM) > apricot kernels (15.5 µM) > linseed (6.4 µM) > persipan (200 g, 3.4 µM) > persipan (100 g, 1.4 µM). A single volunteer also consumed a dose of potassium cyanide (KCN) equivalent to 6.8 mg of HCN.[1] C_{max} following ingestion of the potassium cyanide was 20.1 µM; only slightly higher than C_{max} following ingestion of cassava or bitter apricot kernels, suggesting near-complete release of cyanide from these sources. It was suggested that the lower C_{max} following persipan ingestion was due to the lack of endogenous β-glucosidase in this product, as the product is heated to above 100°C at the end of processing, and slow intestinal passage due to the products high caloric content.

Tran *et al*. (2020) developed a physiologically-based pharmacokinetic (PBPK) model for hydrogen cyanide in humans following oral administration of KCN or four foods (persipan, linseed, apricot kernels or cassava). The resultant model was calibrated using data from published human studies (Abraham *et al.*, 2016; Schulz *et al.*, 1983). The PBPK model was used to determine the maximum amounts of KCN or food that could be ingested without fatal consequences. The amounts of KCN, persipan, linseed, apricot kernels and cassava were 0.18, 8700, 862, 23 and 698 g, respectively. These amounts appear plausible as cases have been admitted to ED after consuming an estimated 10-15 g of apricot kernels.

Cressey (2013) reviewed available information on the metabolism of CGs and concluded that:

"Two processes appear to contribute to the production of cyanide from cyanogenic glycosides; the proportion of the glycoside dose that reaches the large intestine, where most of the bacterial hydrolysis occurs, and the rate of hydrolysis of cyanogenic glycosides to cyanohydrin and cyanide.

It appears that some cyanogenic glycosides are actively absorbed in the jejunum by utilising the epithelial sodium-dependent monosaccharide transporter (SGLT1). A large proportion of ingested prunasin (30-100%) is absorbed intact, while almost no amygdalin is absorbed intact. Linamarin appears to be intermediate between the two, with human studies showing an average of approximately 20% of the ingested dose excreted intact in urine. It is unclear why prunasin is absorbed to a greater extent than linamarin, as both contain monosaccharide moieties, but it must be assumed that the other attached groups (phenyl

[1] The toxicity of HCN is due to the action of the cyanide ion, CN^-. In solution KCN dissociated to the potassium ion (K^+) and the cyanide ion.

and hydrogen for prunasin, and methyl and methyl for linamarin) are able to influence the interaction of the glycoside with the SGLT1.

Two factors appear to influence the rate of cyanide production from cyanogenic glycosides due to bacterial β-glucosidase activity:

- The sugar moiety in the molecule. Cyanogenic glycosides with a gentiobiose sugar, amygdalin, linustatin, and neolinustatin, undergo a two stage hydrolysis, with gentiobiose initially being hydrolysed to glucose to form prunasin, linamarin and lotaustralin respectively.
- The stability of the intermediate cyanohydrin following hydrolysis by bacterial β-glucosidase. For example, hydrolysis of prunasin results in much more rapid cyanide production than hydrolysis of linamarin.

Approximately half of the ingested dose of cyanogenic glycoside is unaccounted for in mass balance studies in animals and humans. Two possible mechanisms have been suggested for the fate of this cyanogenic material:

- Hepatic metabolism to form an unknown metabolite that is subsequently catabolised;
- Conversion of hydrogen cyanide to β-cyanoalanine in a fast reaction and then to asparagine."

These findings are consistent with the experimental evidence of Abraham *et al.* (2016) and provide a theoretical basis for the lower toxicity of linseed, compared to cassava or apricot kernels.

2.6 HEALTH-BASED GUIDANCE VALUES

Both acute reference doses (ARfD) and tolerable daily intakes (TDI) have been established for cyanide and/or CGs from food.

2.6.1 Food Standards Australia New Zealand (FSANZ)

FSANZ established an ARfD of 80 µg/kg bw HCN, based on a no observed adverse effect level (NOAEL) of 70 mg/kg bw for linamarin in a hamster acute exposure study, and application of an uncertainty factor of 100 (FSANZ, 2014). The resultant ARfD of 0.7 mg/kg bw, expressed as linamarin was converted to the HCN equivalent.

2.6.2 Joint FAO/WHO Expert Committee on Food Additives (JECFA)

For the derivation of an ARfD, JECFA derived a lower 95th percentile confidence limit for a benchmark dose equivalent to a 10% increase in response ($BMDL_{10}$) of 85 mg/kg bw of linamarin for an increase in skeletal defects in hamster foetuses following acute maternal exposure (JECFA, 2012). JECFA applied an uncertainty factor of 100 to establish an ARfD of 0.9 mg/kg bw for linamarin, equivalent to 0.09 mg/kg bw for cyanide (90 µg/kg bw).

JECFA also established a provisional maximum tolerable daily intake (PMTDI) based on data from a 13-week rat study, in which sodium cyanide was administered in drinking-water. A BMDL equivalent to a one standard deviation increase in response ($BMDL_{1SD}$) of 1.9 mg/kg bw per day was derived, based on effects on the male reproductive organs. JECFA established a PMTDI of 20 µg/kg bw per day by applying an uncertainty factor of 100.

2.6.3 European Food Safety Authority (EFSA)

EFSA derived a lower ARfD, based on human data (EFSA, 2016). EFSA used a blood concentration of cyanide of 20 µM, reported to be the threshold for toxicity, to extrapolate to an external dose. Using data from the study of Abraham *et al.* (2016), EFSA calculated a mean dose for women of 0.105 mg/kg bw that resulted in a blood cyanide concentration of approximately 20 µM. This was assumed to be a NOAEL. EFSA concluded that a default

factor of 3.16 was not required and that a factor of 1.5 was sufficient to cover any additional variability in toxicokinetics. EFSA also concluded that a toxicodynamic subfactor should be applied, and in the absence of cyanide-specific data on individual sensitivity, adopted the default subfactor of 3.16. The NOAEL was divided by the uncertainty factors of 1.5 and 3.16 to give a (rounded) ARfD of 20 μg/kg bw.

EFSA reconfirmed this ARfD in 2019 and concluded that there was insufficient information to establish a chronic health-based guidance value (EFSA, 2019).

2.6.4 UK Committee on Toxicity (COT)

COT reviewed risks from consumption of apricot kernels in 2006 and derived a 'nominal' ARfD based on the lower end of the lethal dose range in humans (0.5 mg/kg bw) and application of a 100-fold uncertainty factor (COT, 2006). The resultant ARfD of 5 µg/kg bw is somewhat lower than acute exposure guidance values established by other authorities.

2.7 SUMMARY

Incidents of acute cyanide poisoning following consumption of plant material are mainly reported following consumption of stone fruit kernels, inadequately prepared (usually bitter) cassava, or bitter almonds. Despite the reasonably high concentrations of CGs in linseed, there have been no reported intoxications following linseed consumption. It is likely that this is due to the structure of the CGs present in linseed, and their likely greater resistance to microbial hydrolysis in the human gut.

It appears that, as long as cases of cyanide intoxication are treated within a few hours of consumption of the cyanogenic food, complete recovery is likely. However, fatalities have occurred.

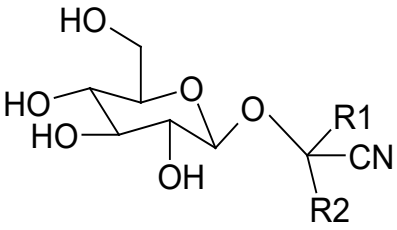
The occurrence of chronic disease, such as konzo, appear to be restricted to developing countries and situations where monotonous consumption of a cyanogenic staple food (e.g. cassava) occurs in the presence of multiple nutrient deficiencies.

3. CYANOGENS IN FOODS

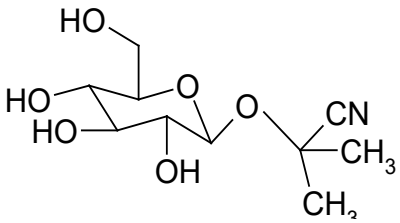
A number of plants and associated plant-based foods naturally contain cyanogenic glycosides (CGs). There are approximately 25 known CGs. The major CGs found in the edible parts of plants are amygdalin (almonds, stone fruit, pome fruit), dhurrin (sorghum), linamarin (cassava, lima beans, linseed/flaxseed, spinach), linustatin (cassava, linseed/flaxseed), lotaustralin (cassava, lima beans), prunasin (stone fruit, pome fruit, pip fruit), and taxiphyllin (bamboo shoots) (Codex Committee on Contaminants in Foods, 2008; Haque and Bradbury, 2002).

The basic structure and two exemplar cyanogenic glycosides are shown in Figure 1.

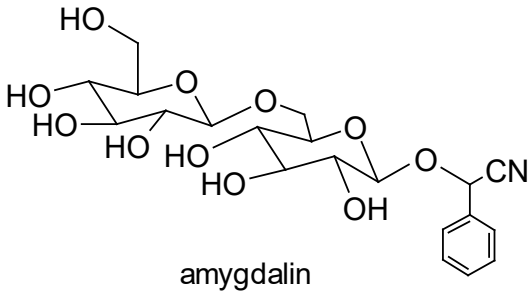
Figure 1: **Cyanogenic glycoside general structure and examples**



Cyanogenic glycoside basic structure



linamarin



amygdalin

R1 may be a methyl group or a phenyl or *p*-hydroxyphenyl group. R2 is most commonly hydrogen, but may also be a methyl or ethyl group. The sugar moiety may be either glucose (monosaccharide) or gentiobiose (disaccharide). Table 1 gives the structure details of the major CGs found in human foods.

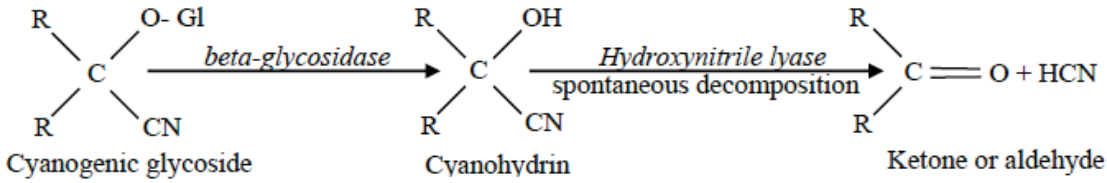
Table 1: Cyanogenic glycosides occurring in human foods

Name	R1	R2	Sugar	Foods
Amygdalin	Phenyl	H	Gentiobiose (R)	Stone fruit, pome fruit
Dhurrin	*p*-hydroxyphenyl	H	Glucose (S)	Sorghum
Linamarin	Methyl	Methyl	Glucose	Cassava, lima beans, linseed
Linustatin	Methyl	Methyl	Gentiobiose	Linseed
Lotaustralin	Methyl	Ethyl	Glucose (R)	Cassava, lima beans, linseed
Neolinustatin	Methyl	Ethyl	Gentiobiose (R)	Linseed
Prunasin	Phenyl	H	Glucose (R)	Ferns
Sambunigrin	Phenyl	H	Glucose (S)	Elderberries
Taxiphyllin	*p*-hydroxyphenyl	H	Glucose (R)	Bamboo shoots

It is immediately apparent that there is a high degree of structural relatedness within this group of compounds. Dhurrin and taxiphyllin are stereoisomers (epimers), as are prunasin and sambunigrin. Removal of a single sugar converts amygdalin to prunasin, linustatin to linamarin, and neolinustatin to lotaustralin.

Release of hydrogen cyanide (hydrocyanic acid or HCN) from CGs can occur through enzymatic hydrolysis by endogenous β-glucosidase, following maceration or wounding of the plant, or by gut microflora, following ingestion of the plant material (Codex Committee on Contaminants in Foods, 2008). The general pathway is shown in Figure 2.

Figure 2: Principal pathway of cyanide production from cyanogenic glycosides



Reproduced from EFSA, 2007

In linseed/flaxseed this process involves two distinct β-glucosidase enzymes (Shahidi and Wanasundara, 1997). Following breakdown of the plant matrix, linustatinase is able to hydrolyse the gentiobiose moiety of linustatin and neolinustatin to glucose, producing linamarin and lotaustralin, respectively. A linamarase β-glucosidase then hydrolyses these glycosides to the corresponding cyanohydrin.

3.1 PLANT SOURCES OF CYANOGENIC GLYCOSIDES

In the following sections, information is presented on the CG content of various foods and commodities. In some cases the results relate to the conversion of CGs to HCN and subsequent determination of HCN by colourimetric or chromatographic methods. In other studies the CGs are determined directly by chromatographic methods. For these studies, concentrations have also been converted to 'HCN equivalents'; the theoretical amount of HCN that could be released from the amount of CG present.

There is some evidence to suggest that for some foods, the theoretical HCN equivalents may not be achieved by hydrolysis of the CGs. Zhong *et al*. (2020) analysed eight CGs in cassava, linseed, bamboo shoots, sorghum, and apricot kernels by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) and compared results to those from an acid hydrolysis-colourimetric method (details not given). While there was reasonable agreement between the two approaches for cassava, with the colourimetric results equating to 40-90% of the theoretical results, for linseed, measurements of HCN release were all less than 30% of the theoretical results, and for apricot kernels, HCN release was less than 50% of the theoretical amount. Due to methodological issues, comparisons were not possible for bamboo shoots and sorghum.

3.1.1 Cassava and cassava products

Cassava (*Manihot esculenta*) cultivars have been biofortified with provitamin A to assist in combatting vitamin A deficiency (Boakye Peprah *et al.*, 2020). Ten of these 'yellow' cassava cultivars were compared to traditional white cultivars for a number of factors, including their cyanogenic potential. Cultivars were grown across three locations in Ghana. While total HCN content of cassava samples varied, there was little evidence of an effect due to location or cultivar. All total HCN concentrations were in the range 9.9 to 47.8 mg/kg.

Twenty cassava cultivars (10 'local' and 10 improved) were grown in the Mbankomo regions of Cameroon (Njankouo Ndam *et al.*, 2019). Following harvesting of the roots, the cortex (skin) and parenchyma (flesh) were separated and analysed for HCN by a colourimetric picrate paper method. HCN concentrations were higher in the cortex, with concentration ranges of 98-155 and 120-210 mg/kg for improved and local varieties, respectively. The corresponding concentrations in the parenchyma were 61-120 and 79-180 mg/kg for improved and local varieties, respectively. HCN content did not correlate to any morphological characteristics of the cassava cultivars.

Cassava root and cassava products available in Singapore were sampled and analysed (O'Brien *et al.*, 2021) for HCN by the method of Essers *et al.* (1993). This is a colourimetric method, using isonicotinate and 1,3-dimethyl barbiturate as the colour reagent. Fresh cassava roots (n = 66) had a mean (range) HCN concentration of 59 (26-120) mg/kg on a fresh weight basis. The HCN content of root pieces (n = 20) was markedly lower with a mean (range) of 39 (21-80) mg/kg fresh weight. Dried cassava chips (n = 5), for home frying had HCN concentration similar to the fresh roots (mean 62 mg/kg, range 47-89 mg/kg) on a fresh weight basis, but on a dry weight basis the concentrations were lower than in fresh roots or root pieces.[2] HCN concentrations in cassava flour and ready-to-eat (RTE) cassava chips were generally less than 20 mg/kg fresh weight, with concentrations on a dry weight basis not being markedly different, due to the low moisture content of these products.

Surveys of HCN in processed cassava chips available in Australia and New Zealand have detected concerning levels in some products (Cressey and Saunders, 2011; Miles *et al.*, 2011). FSANZ implemented a maximum limit of 10 mg/kg HCN. Following implementation of the standard, the New South Wales Food Authority (NSW Food Authority) carried out a survey, collecting samples of 325 products and analysing them by a colourimetric picrate paper method (NSW Food Authority, 2013). The majority (71%) of products contained HCN concentrations below the maximum limit of 10 mg/kg. However, 15 samples (4.6%) contained HCN concentrations greater than 100 mg/kg, with the highest reported concentrations (up to 268 mg/kg) in products from India.

[2] All results were reported on a fresh weight basis and a dry weight basis. However, the moisture content of 'fresh'products varied from 2.0% in RTE chips to 65.3% in fresh root.

Six samples of cassava were analysed by UPLC-MS/MS for linamarin and lotaustralin (Zhong *et al.*, 2020). Linamarin concentrations were in the range 190-920 mg/kg, with lotaustralin concentrations in the range 25-97 mg/kg. Concentrations of the two CGs were correlated. Theoretical HCN contents were in the range 23-111 mg/kg HCN equivalents, while measured HCN contents (acid hydrolysis-colourimetric) were in the range 10-76 mg/kg HCN equivalents.

Cassava leaves are also used as a food. HCN concentrations in the leaves are generally higher than in the root and a concentration of 530 mg/kg was determined by the picrate paper method in cassava leaves from plants greenhouse grown in Germany (Latif *et al.*, 2019).

3.1.2 Taro

Taro (*Colocasia esculenta*, as known as cocoyam) is a common food in the Pacific, with both the root and leaves used as food. These food products are now commonly available in New Zealand.

Taro grown in Nigeria was reported to contain 740 mg/kg HCN, determined by alkaline titration (Igbabul *et al.*, 2014). However, this figure is substantially higher than reported in two other Nigerian studies, which reported concentrations of 21 mg/kg HCN by alkaline titration (Abdulrashid and Agwunobi, 2009) and 17 mg/kg HCN by the picrate paper method (Olajide *et al.*, 2011).

3.1.3 Gadung

Gadung (*Dioscorea hispida*), also known as the Indian three-leaved yam, is a tuberous plant used as a dietary staple in parts of Indonesia (Kumoro *et al.*, 2011). The HCN content of the fresh tubers, determined by alkaline titration, was 84 mg/kg HCN.

3.1.4 Sorghum

The domesticated species of sorghum (*Sorghum bicolor*) was compared to seven wild species under normal and drought situations (Cowan *et al.*, 2020). The cyanogenic potential, determined by acid hydrolysis and the colourimetric König reaction, was determined in leaf, sheath, and root tissue. Analyses were carried out on freeze-dried material, and while not explicitly stated, it is likely that analytical results are on a dry weight basis. Under normal conditions the leaf HCN concentration of *S. bicolor* was 520 mg/kg. Water deficit resulted in an approximately doubling of the HCN content of leaves. The wild sorghum species had markedly lower leaf HCN concentrations (maximum 2 mg/kg) and concentrations were not significant affected by water deficit. The wild sorghum species had similar sheath HCN contents (80-400 mg/kg) to *S. bicolor* (230 mg/kg). While water deficit increased the sheath HCN content of *S. bicolor* to 1030 mg/kg, the sheath HCN contents of wild species were either unchanged or decreased under water deficit conditions. The full range of HCN responses (increase, decrease, no change) was seen in root HCN under water deficit.

HCN-producing potential of leaves, sheaths, and roots was determined from 3 to 35 days post-germination in plants of *S. bicolor* and two wild species (Cowan *et al.*, 2021). HCN content of plant materials generally decreased with increasing plant maturity.

Three samples of sorghum were analysed by UPLC-MS/MS for dhurrin (Zhong *et al.*, 2020). Concentrations were in the range 45-550 mg/kg, equating to theoretical HCN contents in the range 4-47 mg/kg HCN equivalents.

3.1.5 Beer and brewing ingredients

Concentrations and changes in concentrations of mycotoxins and the CGs linamarin and lotaustralin were followed through the steps in brewing of two beer types, with analyses performed by a multi-analyte LC-MS/MS method (Mastanjević *et al.*, 2018). CG concentrations were highest in the malt, with linamarin concentrations of 19.1 and 19.8

mg/kg (2.1 and 2.2 mg/kg HCN equivalents) and lotaustralin concentrations of 21.9 and 21.5 mg/kg (2.3 and 2.2 mg/kg HCN equivalents). CG concentrations decreased through the brewing process (see section 3) with final concentration in the brewed beer of 0.36 and 0.64 mg/kg (0.04 and 0.07 mg/kg HCN equivalents) for linamarin and 1.66 and 4.53 mg/kg (0.17 and 0.47 mg/kg HCN equivalents) for lotaustralin. This is the first report of CGs in beer, however, concentrations in the finished beer are very low.

3.1.6 Linseed/flaxseed

In response to a call for data on HCN in foods by the European Food Safety Authority (EFSA), data were received on linseed (n = 58) (EFSA, 2019). The mean concentration was 192 mg/kg HCN.

Linseed (*Linum usitatissimum*) samples (n = 4) were analysed for HCN by ion chromatography, following acid hydrolysis and distillation (Cho *et al.*, 2013). The mean concentration (range) was 300 (262-345) mg/kg. This study reported results in terms of the cyanide ion (CN^-), rather than HCN and reported results on a dry weight basis. Based on a moisture content of 7%[3], the mean concentration would be 289 mg/kg HCN. These concentrations were somewhat higher than the mean concentration found in linseed analysed in New Zealand (127 mg/kg HCN) (Cressey and Saunders, 2010).

A novel quantitative nuclear magnetic resonance (qNMR) technique was used to determine the concentrations of linustatin and neolinustatin in linseed cultivars (Roulard et al., 2017). Linustatin concentrations were in the range 910 to 2670 mg/kg dry weight, 780–2720 mg/kg for neolinustatin, and 1980–5130 mg/kg for total CGs (sum of linustatin and neolinustatin). HCN potential contents range from 130 to 330 mg/kg seed. Based on a moisture content for linseed of 7%, this range equates to 120-310 mg/kg HCN on a fresh weight basis.

A HPLC method was used to determine linustatin, neolinustatin, and linamarin in 21 linseed cultivars (Russo and Reggiani, 2014). Results were expressed as cyanide ion equivalents. Three groups of linseed cultivars were included, cultivars used for oil production, fibre production, and intermediate-type cultivars. Oil-type cultivars tended to have higher CG content than fibre-type cultivars, although there was overlap. Overall, the range of CG contents were in the range 740-1600 mg/kg CN equivalents (770-1660 mg/kg HCN equivalents).

A Canadian study determined linustatin and neolinustatin in five commercial linseed cultivars and six advanced breeding lines using a novel gas chromatography-flame ionisation detector (GC-FID) method, following silylation of seed extracts (Shahwar *et al.*, 2019). The linustatin contents of the linseed cultivars were in the range 2000-5700 mg/kg and neolinustatin contents were in the range 900-3900 mg/kg. Analytical results for individual cultivars were presented graphically, but approximate HCN equivalent could be calculated and fell within a fairly narrow range (290-370 mg/kg HCN equivalents). Linustatin was the dominant CG in all samples. Concentrations of the two CGs appeared to be negatively correlated with cultivars with the highest linustatin concentrations having the lowest neolinustatin concentrations.

A novel extraction method was developed for the simultaneous analysis of linseed CGs and lignans by UHPLC with electrospray ionisation high-resolution mass spectrometry (UHPLC-ESI HRMS) (Zhao *et al.*, 2019). The method was then used to determine the CG content of two linseed types. The CG contents of the two linseeds were near identical with approximately 1400 mg/kg of linustatin and 1500 mg/kg of neolinustatin (total of 190 mg/kg HCN equivalents).

[3] https://fdc.nal.usda.gov/fdc-app.html#/food-details/169414/nutrients Accessed 20 December 2021

Four samples of linseed were analysed by UPLC-MS/MS for linustatin, neolinustatin, linamarin, and lotaustralin (Zhong *et al.*, 2020). Linustatin (220-2830 mg/kg) and neolinustatin (300-3650 mg/kg) were the dominant CGs, with considerably lower concentrations of linamarin (19-27 mg/kg) and lotaustralin (3-12 mg/kg). In contrast to the study of Shahwar *et al.* (2019), concentrations of linustatin and neolinustatin were correlated. Theoretical HCN contents were in the range 36-420 mg/kg HCN equivalents, while measured HCN contents (acid hydrolysis-colourimetric) were in the range 1-79 mg/kg HCN equivalents. The directly determined HCN concentrations appear low for linseed and it is unfortunate that more details of the method were not provided. Experience at ESR suggests that CGs in linseed are more resistant to hydrolysis than other CGs and specific method variations are required (Cressey and Saunders, 2010).

3.1.7 Legumes

While Lima beans have been reported to contain high levels of cyanide (Codex Committee on Contaminants in Foods, 2008), a Korean study detected only trace amount of cyanide in 16 assorted bean samples, including red bean (*Phaseolus angularis*), Lima bean (*P. limensis*), mung bean (*P. radiatus*), and kidney bean (*P. vulgaris*) (Cho *et al.*, 2013).

3.1.8 Elderberry

Elderberry (*Sambucus nigra*) is not grown commercially in New Zealand, but the tree is widespread, and its fruit are harvested for personal use or for production of natural health products.

Samples of American elderberry were analysed for total HCN, by the picrate paper method (Bradbury *et al.*, 1999), and for individual CGs by ultra-high-performance liquid chromatography with mass spectrometric detection (UHPLC-MS/MS) (Appenteng *et al.*, 2021). Total HCN was not detected in a commercial elderberry juice sample. Samples (juice, skin, stem, and seeds) were obtained from two elderberry cultivars (Ozone and Ozark), with low concentrations of total CGs determined (2.6-9.2 mg/kg). The highest concentrations were detected in stem, followed by skin. Total CG concentrations in juice were about 3 mg/kg. Further analysis of pooled samples of five elderberry cultivars showed a similar pattern, with highest concentrations in the stem (37 mg/kg) and green berries (26 mg/kg), and lowest concentrations (<10 mg/kg) in juice and ripe berries. Analysis of individual CGs in all tissue types detected (in order of decreasing concentration) amygdalin, prunasin, dhurrin and linamarin. However, it should be noted that the method used was unable to distinguish between the diastereomers prunasin and sambunigrin.

Elderberries grown in Slovenia were analysed for CGs by a HPLC-diode array detection (DAD) method (Senica *et al.*, 2016b). Only sambunigrin was detected at a concentration of 18.8 mg/kg, equivalent to 1.7 mg/kg HCN equivalents. Four products were prepared from the elderberries; juice, liqueur, tea, and spread. The sambunigrin contents of these four products (based on manufacture from equal amounts of fruit) were 10.6, 0.8, 3.8, and 0.8 mg/kg, respectively. The authors of this study ascribed the lower levels of sambunigrin in liqueur and spread to thermal degradation.

Sambunigrin, prunasin, and amygdalin isomer were determined in berries of three elderberry (*Sambucus*) species; black elderberry (*S. nigra*), dwarf elderberry (*S. ebulus*), and red elderberry (*S. racemosa*), by LC-MS/MS (Senica *et al.*, 2019a). CG concentrations were highest in black elderberry and lowest in red elderberry. Patterns of CGs varied, with prunasin the dominant CG in black elderberry, amygdalin isomer in dwarf elderberry and sambunigrin in red elderberries. Expressed as HCN equivalents, the CG contents of the three species were 4.9, 1.8 and 0.3 mg/kg HCN equivalents for black, dwarf and red elderberry, respectively. There was also considerable variability in patterns of CG content between different plant parts. For black elderberry, the highest CG concentrations were observed in the leaves, while for the other two species the highest concentrations were found in the flowers.

3.1.9 Apples and other pome fruit

Dried seeds from 15 apple cultivars were analysed for amygdalin by HPLC (Bolarinwa *et al.*, 2015). The amygdalin content was in the range 950 to 3910 mg/kg, equating to 60 to 200 mg/kg of HCN. Apple juices prepared from the flesh of apples did not contain detectable amygdalin, but juice prepared from apple cores contained up to 430 mg/L of amygdalin (38 mg/L HCN equivalents). Juice prepared from the whole fruit contained intermediate concentrations of amygdalin, with a maximum of 90 mg/L (8 mg/L HCN equivalents). Amygdalin was detected in all commercially-available pressed/squeezed apple juices (n = 10), with amygdalin concentrations in the range 10-39 mg/L (0.9-3.4 mg/L HCN equivalents). Considerably lower amygdalin concentrations were detected in commercially-available long-life apple juices (n = 17), with amygdalin detected in 9 of 17 samples at concentrations in the range 1 to 7 mg/L (0.09-0.6 mg/L HCN equivalents).

Seeds from apples and pears were analysed for amygdalin by HPLC (Bolarinwa *et al.*, 2014). The concentrations in a single composite of each were 3000 and 1300 mg/kg, respectively for apples and pears, or 180 and 77 mg/kg HCN equivalents. Only very low concentrations of amygdalin were detected in apple juices, puree, and cider (maximum concentration 90 mg/kg or 5 mg/kg HCN equivalents).

A Korean study determined the cyanide content of apple seeds, apple skin, and edible apple flesh by ion chromatography, following acid hydrolysis and distillation (Cho *et al.*, 2013). Mean concentrations were 124 and 0.4 mg/kg for apple seed and skin, respectively. Only trace amounts of cyanide were detected in the edible flesh. This study reported results in terms of the cyanide ion (CN^-). The mean concentrations in seeds and skin would equate to 129 and 0.4 mg/kg HCN, respectively.

Apple seeds from Golden Delicious apples grown in four countries (Azerbaijan, Russia, Serbia and Slovenia) and cold stored for up to eight months were analysed for amygdalin, neoamygdalin, and prunasin by LC-MS/MS (Senica *et al.*, 2019b). Concentrations of neoamygdalin were at least an order of magnitude less than the other two CGs. At harvest, there was some geographical variation in the CG content of the apple seeds, with the highest concentrations in seeds from Azerbaijan (amygdalin 850 mg/kg, prunasin 1320 mg/kg, HCN equivalents 170 mg/kg) and the lowest in seeds from Slovenia (amygdalin 520 mg/kg, prunasin 260 mg/kg, HCN equivalents 55 mg/kg). Changes in CG content of seeds during cold storage of apples was variable, however overall the CG content increased, particularly in seeds from apples from Azerbaijan. After eight months of cold storage, seeds from apples from Azerbaijan contained 1470 mg/kg of amygdalin (73% increase) and 2010 mg/kg of prunasin (52% increase), equating to 270 mg/kg HCN equivalents. The authors noted that CG content tended to increase with biotic stress and that the region of Azerbaijan where the apples were grown experienced less precipitation than the other sites and the soil had a higher salt content due to proximity to the Caspian Sea.

Seeds from seven apple cultivars grown in Slovenia were analysed for amygdalin, neoamygdalin, and prunasin by LC-MS/MS (Senica *et al.*, 2017). Amygdalin and prunasin concentrations were in the range 1190-1630 mg/kg and 610-1410 mg/kg, respectively. Neoamygdalin concentrations were 1-2 orders of magnitude lower. Expressed as HCN equivalents, CG concentrations were in the range 130-230 mg/kg. In addition, seeds from two pear cultivars were analysed, with only amydgalin detected, at low concentrations (46-47 mg/kg, 2.7-2.8 mg/kg HCN equivalents).

3.1.10 Miscellaneous fruit

In a study carried out in the Democratic Republic of the Congo, fruits eaten by local people and primates were tested for the presence of HCN (Bondjengo *et al.*, 2017). The presence of HCN was determined qualitatively by a spot test (Feigl and Anger, 1966). HCN was detected in three of 75 fruit samples, although no limit of detection was reported for the method. The fruit species containing HCN were *Camptostylus mannii, Dasylepsis seretii,* and *Oncoba*

welwitschia. Common names for these species were not identified and it is highly unlikely that they are currently available in New Zealand.

Fruit juice samples available in Nigeria (*n* = 35), including apple (*n* = 5), berry (*n* = 6), orange (*n* = 6), mango (*n* = 2), peach (*n* = 4), pineapple (*n* = 2), and mixed (*n* = 10) were analysed for a range of natural toxins, including amygdalin and prunasin (Ayeni *et al.*, 2020). Amygdalin and prunasin were detected in 14 of 35 (40%) and 15 of 35 (43%) of samples, respectively, with detection mainly in apple and peach juices. The highest detected concentrations were 359 µg/L (amygdalin, mixed juice) and 1004 µg/L (prunasin, mixed juice). The HCN equivalents of the maximum concentrations are 0.02 mg/L and 0.09 mg/L HCN for amygdalin and prunasin, respectively.

Loquat (*Eriobotrya japonica*) seeds were analysed for amygdalin and prunasin by LC-MS/MS and for HCN by enzymatic hydrolysis, steam distillation, and colourimetric determination (Tanaka *et al.*, 2020). Mean concentrations of amygdalin and prunasin were 5900 and 760 mg/kg, respectively. This equates to 420 mg/kg HCN equivalents and the determined HCN concentration was very close to this (410 mg/kg HCN). Free cyanide (44 mg/kg) was also detected in the seeds. It is unknown whether loquat seeds are eaten.

3.1.11 Macadamia nuts

Flowers, leaves, nuts, and husks of macadamia (*Macadamia integrifolia*) were obtained from Guangxi Province, China (Castada *et al.*, 2020). The CG, dhurrin, was analysed by high-performance liquid chromatography with diode array detection (HPLC-DAD) and HCN was analysed an alkaline picrate colourimetric method. Dhurrin concentrations were highest in macadamia flowers (37 mg/g) and leaves (8.5 mg/g), and substantially lower in nuts (0.04 mg/g or 40 mg/kg) and husks (0.05 mg/g or 50 mg/kg). A similar trend was seen for total HCN, with concentrations of 42, 17, 0.7 and 0.9 mg/kg HCN in flowers, leaves, nuts, and husks, respectively.

3.1.12 Almonds and almond products

Almonds are the edible seeds of *Prunus dulcis* otherwise known as *Prunus amygdalus.* Almond cultivars include sweet and bitter types, with the bitter types containing higher levels of CGs.

In response to a call for data on HCN in foods by the European Food Safety Authority (EFSA), data were received on sweet almonds (*n* = 35) and bitter almonds (*n* = 3) (EFSA, 2019). The mean concentrations were 4.5 and 1440 mg/kg HCN for sweet and bitter almonds, respectively. Data were also provided on almond-containing products; macaroons and amaretti (*n* = 204), and marzipan (*n* = 130). Mean HCN concentrations for these food types were 12.5 and 8.4 mg/kg HCN, respectively, with 95th percentile concentrations of 26 and 30 mg/kg, respectively.

A Tunisian study examined the HCN potential of three sweet and three bitter almond samples (Chaouali *et al.*, 2013). Total HCN was determined by a titrimetric method. Mean (range) HCN content of sweet and bitter almonds were 25 (16-32) mg/kg and 1060 (920-1220) mg/kg, respectively. The HCN contents of five brands of almond syrup were considerably lower; in the range 1-3 mg/kg.

Wild strains of almond (*Prunus amygdalus*) are used as a source of traditional medicines in Iran (Amjadian *et al.*, 2020). Four species of wild almonds from the Zagros mountains were sampled and the seeds analysed for amygdalin, prunasin, and linamarin. As expected, the predominant CG was amygdalin, with concentrations in the range 176-3076 mg/100 g dry weight (1800-31,000 mg/kg dry weight, almonds have a low moisture content (<5%) and concentrations on a fresh weight basis will not differ greatly). Prunasin concentrations were in the range 83-386 mg/100 g (830-3900 mg/kg dry weight). Linamarin is not usually associated with almonds, but was detected in samples of *Prunus orientalis* at concentrations

in the range 25-90 mg/100g (250-900 mg/kg dry weight). The HCN equivalents of the maximum detected concentrations are 1800, 360, and 100 mg/kg HCN for amygdalin, prunasin and limamarin, respectively. These results are higher, but within the same order of magnitude as reported by Chaouali *et al.* (2013) for bitter almonds.

A range of processed almond products (toasted almonds, almond milk, almond cocoa dessert, almond flour, and marzipan) were analysed for amygdalin by HPLC (Bolarinwa *et al.*, 2014). The highest concentration (120 mg/kg or 7 mg/kg HCN equivalents) was detected in toasted almonds.

3.1.13 Apricot kernels and apricot kernel products

A Tunisian study determined the HCN content of five samples of apricot kernels by a titrimetric method (Chaouali *et al.*, 2013). Concentrations were in the range 540 to 1190 mg/kg, with a mean concentration of 850 mg/kg. It was not reported whether the kernel had the skin on or if the skin had been removed.

A Korean study determined the cyanide content of apricot kernels with (n = 10) and without skin (n = 13) by ion chromatography, following acid hydrolysis and distillation (Cho *et al.*, 2013). The mean concentrations (ranges) were 1680 (6-2260) mg/kg and 22.9 (ND-153) mg/kg, respectively. This study reported results in terms of the cyanide ion (CN^-), rather than HCN and reported results on a dry weight basis. New Zealand apricot kernels have been reported to have a moisture content of 4.7% (Beyer and Melton, 1990). The mean cyanide concentrations from this study would equate to 1660 and 22.7 mg/kg HCN on a fresh weight basis.

As part of a wider study, the amygdalin content of kernels from Spanish apricot kernels, bought in the UK, was determined by HPLC (Bolarinwa *et al.*, 2014). Analyses appear to have been carried out on a single composite sample. The amygdalin content of apricot kernels was 14,400 mg/kg equating to 850 mg/kg HCN equivalents.

The CG content of apricot kernels from apricots grown in Slovenia was determined by LC-MS/MS (Senica *et al.*, 2016a). Concentrations of amygdalin and prunasin were 1020 and 2160 mg/kg, respectively, equating to a total HCN content of 260 mg/kg HCN equivalents. Kernels were broken and steeped in alcohol to produce a liqueur. CGs were determined in the liqueur on steeping days 8, 16, 24, and 32. There was little change in the CG content during this period with the final CG concentrations of amygdalin and prunasin being 32.7 and 5.0 mg/L, respectively, equating to 2.4 mg/L HCN equivalents. However, no mass balance was carried out to determine the final distribution of the CGs between kernels, liqueur, and loses to the environment.

A Chinese study examined the development of prunasin and amygdalin in apricot kernels from 20 to 90 days after flowering (Deng *et al.*, 2021). The study included seven bitter and three sweet varieties. Neither of the CGs were detected at any stage in the kernels of sweet varieties. In bitter varieties, prunasin developed early and reached peak concentrations about 30-40 days after flowering. However, by 60 days after flowering prunasin concentrations had decreased to negligible levels. Amygdalin concentrations increased sharply from 50 days post flowering but in most bitter cultivars amygdalin concentrations decreased again after 70 days after flowering. At fruit maturity, kernel amygdalin concentrations were in the range 11,000-50,000 mg/kg dry weight. The final amygdalin concentrations equate to 1000 to 4400 mg/kg HCN equivalents on a dry weight basis.

Two samples of apricot kernels were analysed by UPLC-MS/MS for amygdalin and prunasin (Zhong *et al.*, 2020). Amygdalin (370-1850 mg/kg) and prunasin (14-73 mg/kg) concentrations were quite low for apricot kernels (23-115 mg/kg HCN equivalents). Measured HCN contents (acid hydrolysis-colourimetric) were even lower (9-51 mg/kg HCN equivalents).

Kernels from six apricot cultivars grown in Slovenia were analysed for amygdalin, neoamygdalin and prunasin by LC-MS/MS (Senica *et al.*, 2017). Amygdalin and prunasin concentrations were in the range 630-2160 mg/kg and 65-1660 mg/kg, respectively. Neoamygdalin concentrations were 1-2 orders of magnitude lower. Expressed as HCN equivalents, CG concentrations were in the range 43-270 mg/kg. Again, these HCN concentrations appear low for apricot kernels.

3.1.14 Other stone fruit and stone fruit products

The black cherry (*Prunus serotina*) is an invasive plant species in the forests of many European countries. Black cherries were collected from forests in northern Poland and the whole fruit, including stones, were extracted and analysed for the CGs, amygdalin and prunasin, by HPLC-MS (Brozdowski *et al.*, 2021). Amygdalin and prunasin were detected at concentrations of 19.2 and 11.9 mg/kg fresh weight, respectively (1.7 and 1.1 mg/kg HCN equivalents, respectively).

A Korean study determined the cyanide content of peach kernels (n = 14) by ion chromatography, following acid hydrolysis and distillation (Cho *et al.*, 2013). The mean concentration (range) was 880 (440-1040) mg/kg. This study reported results in terms of the cyanide ion (CN^-), rather than HCN and reported results on a dry weight basis. Assuming the moisture content of peach kernels is similar to apricot kernels (4.7%), the mean cyanide concentrations from this study would equate to 870 mg/kg HCN on a fresh weight basis. The same study also determined cyanide in the seeds of other *Prunus* spp., including cherry (*P. aviun*, mean = 520 mg/kg CN), plum (*P. domestica*, mean = 660 mg/kg CN), and Japanese apricot (*P. mume*, mean = 1090 mg/kg CN). In all cases, the skin and flesh of the fruit contained only low concentration (<3 mg/kg CN) of cyanide.

Kernels were recovered from a range of stone fruit, including cherries, nectarines, peaches, and plums, and were analysed for amygdalin by HPLC (Bolarinwa *et al.*, 2014). Concentrations were in the range 120-17,500 mg/kg or 7-1030 mg/kg HCN equivalents. The lowest concentration was in nectarine kernel, while the highest concentrations were in plums. However, the amygdalin content of plum kernels varied by a factor of 40 across different plum types. The same study also examined the amygdalin content of apricot, peach, and plum fruit products, with the highest concentration (60 mg/kg or 3.5 mg/kg HCN equivalents) detected in canned peach slices.

The CG content of cherry kernels from cherries grown in Slovenia was determined by LC-MS/MS (Senica *et al.*, 2016a). Concentrations of amygdalin and prunasin were 730 and 650 mg/kg, respectively, equating to a total HCN content of 100 mg/kg HCN equivalents. Kernels were broken and steeped in alcohol to produce a liqueur. CGs were determined in the liqueur on steeping days 8, 16, 24, and 32. During steeping, the amygdalin content of the liqueur decreased by 35%, while prunasin concentrations decreased by 49%. The final concentrations (32 days) of amygdalin and prunasin were 13.4 and 2.7 mg/L, respectively, equating to 1.0 mg/L HCN equivalents. However, no mass balance was carried out to determine the final distribution of the CGs between kernels, liqueur, and losses to the environment.

Kernels from cultivars of cherries (n = 4), peaches (n = 7), and plums (n = 9) grown in Slovenia[4] were analysed for amygdalin, neoamygdalin, and prunasin by LC-MS/MS (Senica *et al.*, 2017). While there was marked cultivar to cultivar variability, no obvious differences between stone fruit species were apparent. Amygdalin and prunasin concentrations were in the range 100-2880 mg/kg and 170-2340 mg/kg, respectively. Neoamygdalin concentrations

[4] This study also included analysis of apricot kernels. Those results are reported separately under the section on apricot kernels and apricot kernel products

were 1-2 orders of magnitude lower. Expressed as HCN equivalents, CG concentrations were in the range 120-310 mg/kg.

3.1.15 Vegetables

Seeds of cucurbit vegetables (courgette, cucumber, marrow, melon, and squash) were analysed for amygdalin by HPLC (Bolarinwa *et al.*, 2014). Amygdalin concentrations were uniformly low (maximum 210 mg/kg or 12 mg/kg HCN equivalents). Cucurbit seeds are not commonly consumed as a food separate from the whole vegetable.

Samples (n = 60) of two traditional Namibian leafy vegetables, *mutete* (*Hibiscus sabdariffa*) and *omboga* (*Cleome gynandra*), were analysed for a range of fungal and plant secondary metabolites by LC-MS/MS (Misihairabgwi *et al.*, 2019). The analytical screen included the CGs, linamarin and lotaustralin. It should be noted that the multi-analyte method performed relatively poorly for these analytes, with spike recoveries of 35-79%. However, the spiked concentrations were also very low (65 and 26 µg/kg for linamarin and lotaustralin, respectively, or 0.007 and 0.003 mg/kg HCN equivalents, respectively). Both CGs were detected in 65% of *mutete* samples, with maximum concentrations of linamarin and lotaustralin of 0.40 and 0.078 mg/kg, respectively (0.04 and 0.008 mg/kg HCN equivalents, respectively). Linamarin was not detected in any *omboga* samples, while lotaustralin was detected in 60% of samples, with concentrations up to 1.5 mg/kg (0.16 mg/kg HCN equivalents).

3.1.16 Bamboo shoots

Emerging bamboo shoots can contain high concentrations of the CG taxiphyllin (Rawat *et al.*, 2015). However, considerable inter-varietal variation was demonstrated by analysis of fresh shoots from 21 bamboo species, using the colourimetric method of Haque and Bradbury (2002). HCN concentrations were in the range 32-1950 mg/kg. By far the lowest concentrations (32 mg/kg and 36 mg/kg) were detected in *Chimonobuambusa callosa* and *Phyllostachys mannii*, respectively, while *Bambusa* spp. and *Dendrocalamus* spp. all contained more than 400 mg/kg.

Fresh juvenile shoots of 15 bamboo species from the Manipur region of India were analysed for HCN by the spectrophotometric picrate paper method (Devi, 2018). HCN contents of bamboos were in the range 56 to 1350 mg/kg HCN, with the highest concentrations in *Dendrocalamus* spp. and the lowest concentrations in *Chimonobuambusa callosa*. There was a high degree of consistency between the results of this study and those of Rawat *et al.* (2015).

In another study on shoots of bamboo species from the Manipur region, Waikhom *et al*. (2013) demonstrated gradients in HCN content along the bamboo shoot, with the lowest concentrations at the base (200-920 mg/kg HCN) and highest concentrations at the tip (300-2600 mg/kg HCN). This is consistent with a plant defence role for HCN, as the high concentrations in the tip will provide greater protection for the actively growing component of the plant. As with other studies in the same region, the lowest HCN concentrations were seen in *Chimonobuambusa callosa.*

Shoots from four bamboo species (*B. bambos, B. tulda, D. strictus,* and *D.asper*) were collected from locations in central India (Pandey and Ojha, 2014). HCN content was determined by a spectrophotometric method. The HCN content of fresh shoots was in the range 110-180 mg/kg.

Six samples of bamboo shoots were analysed by UPLC-MS/MS for taxiphyllin (Zhong *et al.*, 2020). The bamboo species were not identified. Taxiphyllin was only able to be quantified in one sample, with a taxiphyllin concentration of 460 mg/kg (40 mg/kg HCN equivalents).

3.1.17 Supplements

Four supplements, produced in Hungary, China, Slovakia, and the Czech Republic and reported to contain apricot kernel, peach kernel or amygdalin were analysed for amygdalin by HPLC-DAD (Boháčová *et al.*, 2019). Amygdalin was detected at concentrations of 24, 991, 21 and 0.7 mg/g. The highest concentration was for a supplement containing pure amygdalin. The first sample (24 mg/g) was for a 400 mg capsule containing 160 mg of ground apricot kernel, which would equate to a HCN concentration for the apricot kernel of about 5000 mg/kg. The third result (21 mg/g) was for a 500 mg capsule contain 300 mg of ground apricot kernel, equating to a concentration of HCN in the apricot kernel of approximately 3000 mg/kg. These concentrations are consistent with the HCN content of skin-on apricot kernels (Saunders and Cressey, 2013). The peach kernel product (0.7 mg/g) contained 25.8 mg of peach kernel extract in a 165 mg granule, suggesting a HCN concentration of 390 mg/kg in the peach kernel extract.

3.1.18 Medicinal plants

A Korean study determined the cyanide content of a range of medicinal plants by ion chromatography, following acid hydrolysis and distillation (Cho *et al.*, 2013). Apart from the materials summarised elsewhere in this section (flaxseed, apricot kernels, peach kernels), the highest cyanide concentrations were reported for purslane (*Portaluca oleracea*; mean = 1150 mg/kg) and seeds of *Prunus nakii* (mean = 870 mg/kg). This study reported results in terms of the cyanide ion (CN^-), rather than HCN and reported results on a dry weight basis.

An Indian study examined the HCN content of medicinal plants by aqueous incubation, followed by colourimetric determination with picrate (Gulhane and Jadhao, 2019). The plants were *Bombax ceiba* (cotton tree), *Leea macrophylla*, *Argyreia nervosa* (Hawaiian baby woodrose), *Begonia crenata*, *Clerodendrum infortunatum* (bhat), *Dioscorea bulbifera* (air potato, potato yam), *Dioscorea pentaphylla* (fiveleaf yam), and *Abrus precatorius* (jequirity bean). HCN contents of plant tissues were in the range 51-137 mg/kg. It is unknown whether any of these plant materials are available in New Zealand.

3.1.19 Summary

Results from studies with potential relevance to the New Zealand food supply are summarised in Table 2. Overall, the results in Table 2 do not identify any high-HCN foods likely to be consumed in New Zealand, other than those previously examined, as detailed in section 1.1 of this report (FSANZ, 2014). While high levels of HCN have been detected in the kernels of most species of stone fruit, these items are generally not considered to be food items in New Zealand. Similarly, high concentrations of HCN have been reported in pome fruit seeds, particularly apples. There is no evidence to suggest that these seeds are an intentionally consumed food item in New Zealand.

Taro leaves have previously been analysed for HCN in New Zealand and were found to not contain detectable HCN (Cressey and Saunders, 2010). Studies carried out in Africa suggest that the taro root can contain moderate concentrations of HCN (17-21 mg/kg), with one study suggesting markedly higher concentrations (Igbabul *et al.*, 2014).

The information in Table 2 indicates a substantial difference between the HCN contents of sweet and bitter almonds. The limited analyses previously carried out on almonds available at retail in New Zealand suggest these are probably from sweet cultivars (Cressey and Saunders, 2010). The presence of almonds from bitter cultivars in the New Zealand food supply would be a cause for concern.

Table 2: Summary of studies on the cyanogenic glycoside content of plant materials and foods

Country	Product description	Analytical method	Number of samples	CG content as CG Mean (range), mg/kg fresh weight	CG content as HCN Mean (range), mg/kg fresh weight	Reference
Cassava						
Ghana	Peeled and washed roots	Alkaline titration	30		32.3 (9.9-47.8)	(Boakye Peprah *et al.*, 2020)
Cameroon	Peeled and washed roots	Spec (picrate)	-Improved (10) Cortex Parenchyma -Local (10) Cortex Parenchyma		 (98-155) (61-120) (120-210) (79-180)	(Njankouo Ndam *et al.*, 2019)
Singapore	Retail products	Spec (isonicotinate/ barbiturate)	Fresh roots (66) Root pieces (20) Flour (8) Dried chips (5) RTE chips (1)		59 (26-120) 39 (21-80) 12 (2-20) 62 (47-89) 17	(O'Brien *et al.*, 2021)
Australia	RTE cassava chips)	Spec (picrate)	325		(<10-268)	(NSW Food Authority, 2013)
China	Peeled roots	LC-MS/MS Spec (isonicotinate/pyrazolone)	6	LIN 530 (190-920) LOT 55 (25-97)	49 (10-76)	(Zhong *et al.*, 2020)
Germany	Cassava leaves	Spec (picrate)	1		530	(Latif *et al.*, 2019)
Taro						
Nigeria	Root	Alkaline titration	1		740	(Igbabul *et al.*, 2014)
Nigeria	Root	Alkaline titration	1		21	(Abdulrashid and Agwunobi, 2009)
Nigeria	Root	Spec (picrate)	1		17	(Olajide *et al.*, 2011)
Gadung						
Indonesia	Tuber	Alkaline titration	1		84	(Kumoro *et al.*, 2011)
Sorghum						
China	Seed	LC-MS/MS	3	DHU 272 (45-548)	23 (4-47)[a]	(Zhong *et al.*, 2020)
Beer						
Croatia	Brewed beer	LC-MS/MS	2	LIN 1.7 and 4.5 LOT 0.36 and 0.64	0.21 and 0.54[a]	(Mastanjević *et al.*, 2018)
Linseed/flaxseed						
Europe	Linseed	Various	58		192	(EFSA, 2019)
Republic of Korea	Linseed	IC	4		289 (252-333)[b]	(Cho *et al.*, 2013)
France	Linseed	NMR	42	LIU (910-2670) NEO (1980-5130)[c]	(120-310)[a,b]	(Roulard *et al.*, 2017)
Italy	Linseed	HPLC	21		(770-1660)	(Russo and Reggiani, 2014)
Canada	Linseed	GC-FID	11	LIU (2000-5700) NEO (900-3900	(290-370)[a]	(Shahwar *et al.*, 2019)

Country	Product description	Analytical method	Number of samples	CG content as CG Mean (range), mg/kg fresh weight	CG content as HCN Mean (range), mg/kg fresh weight	Reference
China	Linseed	UPLC-HRMS	2	LIU 1400 NEO 1500	190	(Zhao *et al.*, 2019)
China	Linseed	LC-MS/MS Spec (isonicotinate/pyrazolone)	4	LIU 1840 (220-2650) NEO 2260 (300-3650)	270 (36-420)[a]	(Zhong *et al.*, 2020)
Elderberry and elderberry products						
Slovenia	Elderberry, whole Elderberry juice Elderberry liqueur Elderberry tea Elderberry spread	HPLC-DAD	1 1 1 1 1	SAM 18.8 SAM 10.6 SAM 0.8 SAM 3.8 SAM 0.8	1.7 1.0 0.1 0.3 0.1	(Senica *et al.*, 2016b)
Slovenia	Berries of: Black elderberry (*Sambucus nigra*) Dwarf elderberry (*S. ebulus*) Red elderberry (*S. racemose*)	LC-MS/MS	 1 1 1	 SAM 22.5 PRU 27.5 AMY 4.9 SAM 0.6 PRU 6.8 AMY 19.0 SAM 1.5 PRU 0.9 AMY 0.7	 4.9 1.8 0.3	(Senica *et al.*, 2019a)
Pome fruit and pome fruit products						
United Kingdom	Dried seed Commercial apple juices - Pressed/squeezed - Long-life	HPLC-DAD	15 10 17	AMY 2200 (950-3900) AMY 28 (10-39) AMY <10 (ND-7)	130 (60-200) 2 (<1-2) All <1	(Bolarinwa *et al.*, 2015)
United Kingdom	Apple seeds Apple juice Apple and beetroot juice Apple juice UHT Apple puree Pear seeds	HPLC-DAD	1 1 1 1 1 1	AMY 3000 AMY 90 AMY 20 AMY 4 AMY 20 AMY 1300	180 5 1.2 0.2 1.2 77	(Bolarinwa *et al.*, 2014)
Republic of Korea	Apple seeds Apple skin Apple flesh	IC	6 6 6		129 (108-184) 0.4 (Trace-1.3) Trace	(Cho *et al.*, 2013)
Azerbaijan Russia Serbia Slovenia	Apple seeds	LC-MS/MS	1 1 1 1	AMY 850 PRU 1320 AMY 650 PRU 1090 AMY 800 PRU 900 AMY 520 PRU 260	170 140 130 55	(Senica *et al.*, 2019b)
Slovenia	Apple seeds	LC-MS/MS	7	AMY 1400 (1190-1630) PRU 1040 (610-1410)	180(130-230)	(Senica *et al.*, 2017)

Country	Product description	Analytical method	Number of samples	CG content as CG Mean (range), mg/kg fresh weight	CG content as HCN Mean (range), mg/kg fresh weight	Reference
	Pear seeds		2	AMY 47 (46-47)	2.8 (2.7-2.8)	
Apricot kernel and apricot kernel products						
Tunisia	Apricot kernels	Alkaline titration	5		850 (540-1190)	(Chaouali *et al.*, 2013)
Republic of Korea	Apricot kernels - With skin - Without skin	IC	 10 13		 1660 (6-2230)[d] 22.7 (ND-151)	(Cho *et al.*, 2013)
United Kingdom	Spanish apricot kernels	HPLC-DAD	1 (composite)	AMY 14,400	850	(Bolarinwa *et al.*, 2014)
Slovenia	Apricot kernels Apricot liqueur	LC-MS/MS	1	AMY 1020 PRU 2160 AMY 32.7 PRU 5.0	260 2.4	(Senica *et al.*, 2016a)
China	Bitter apricot kernels	HPLC-UV	7	AMY 39,000 (11,000-50,000)	2300 (1000-4400)[c]	(Deng *et al.*, 2021)
China	Apricot kernels	LC-MS/MS Spec (isonicotinate/pyrazolone)	2	AMY 370 and 1850 PRU 14 and 73	23 and 115[a] 9 and 51	(Zhong *et al.*, 2020)
Slovenia	Apricot kernels	LC-MS/MS	6	AMY 1600 (630-2160) PRU 950 (63-1650)	180 (43-270)	(Senica *et al.*, 2017)
Other stone fruit and stone fruit products						
Poland	Black cherry (*Prunus serotina*), whole fruit (including stones)	HPLC-MS	10	AMY 19 PRU 12	2.8	(Brozdowski *et al.*, 2021)
Republic of Korea	Peach kernels Peach skin Peach flesh Cherry kernels Cherry skin Cherry flesh Plum kernel Plum skin Plum flesh Apricot skin Apricot flesh Nectarine skin Nectarine flesh	IC	14 11 11 1 1 1 5 8 8 2 2 4 4		870 (440-1030)[c] Trace Trace 540[c] Trace Trace 680 (520-1070)[c] 2.8 (ND-14)[c] Trace 0.5 (ND-1.0)[c] Trace Trace Trace	(Cho *et al.*, 2013)
United Kingdom	Seeds/kernels: Cherry Nectarine Peach Plum Sliced, canned fruit: Apricot Peach Prune	HPLC-DAD	 2 1 1 5 1 1 1	 AMY 2700 and 3900 AMY 120 AMY 6800 AMY 6300 (440-17,500) AMY 50 AMY 60 AMY 30	 160 and 230 7 400 370 (26-1030) 3 3.5 2	(Bolarinwa *et al.*, 2014)
Slovenia	Cherry kernel	LC-MS/MS	1	AMY 730 PRU 650	100	(Senica *et al.*, 2016a)

Country	Product description	Analytical method	Number of samples	CG content as CG Mean (range), mg/kg fresh weight	CG content as HCN Mean (range), mg/kg fresh weight	Reference
	Cherry liqueur		1	AMY 13 PRU 3	1	
Slovenia	Seeds/kernels: Cherry Peach Plum	LC-MS/MS	 4 7 9	 AMY 2040 (1300-2880) PRU 920 (430-1370) AMY 1300 (290-2200) PRU 1600 (900-2040) AMY 1700 (170-2300) PRU 1700 (170-2400)	 200 (120-300) 220 (100-300) 260 (130-310)	(Senica *et al.*, 2017)
Bamboo shoots						
India	Bamboo shoots, various species	Spec (picrate)	21		960 (30-1950)	(Rawat *et al.*, 2015)
India	Bamboo shoots, various species	Spec (picrate)	15		820 (56-1350)	(Devi, 2018)
India	Bamboo shoots, various species - Base - Middle - Tip	Spec (picrate)	15		 590 (200-920) 1120 (210-2240) 1590 (300-2600)	(Waikhom *et al.*, 2013)
India	Bamboo shoots, four species	Spec (not stated)	4		150 (110-180)	(Pandey and Ojha, 2014)
China	Bamboo shoots	UPLC-MS/MS	6	TAX 460	40	(Zhong *et al.*, 2020)
Tree nuts and tree nut products						
China	Macadamia nuts	HPLC-DAD Spec (picrate)	1	DHU 40	0.7	(Castada *et al.*, 2020)
Europe	Almonds - Sweet - Bitter Macaroons and amaretti Marzipan	Various	 35 3 204 130		 4.5 1440 12.5 8.4	(EFSA, 2019)
Tunisia	Almonds - Sweet - Bitter Almond syrup	Alkaline titration	 3 3 5		 25 (16-32) 1060 (920-1220) (1-3)	(Chaouali *et al.*, 2013)
United Kingdom	Toasted almonds Almond milk Almond cocoa dessert Almond flour Marzipan	HPLC-DAD	1 1 1 1 1	AMY 120 AMY 50 AMY 40 AMY 30 AMY 20	7 3 2 2 1	(Bolarinwa *et al.*, 2014)

Spec: spectrophotometric, LC-MS/MS: liquid chromatography-tandem mass spectrometry, IC: ion chromatography, following acid hydrolysis and distillation, NMR: nuclear magnetic resonance, HPLC: high-performance liquid chromatography, GC-FID: gas chromatography-flame ionisation detection, UPLC-HRMS: ultra-high-pressure liquid chromatography-high resolution mass spectrometry, HPLC-DAD: HPLC-diode array detection, HPLC-UV: HPLC with UV spectrophotometric detection, UPLC-MS/MS: Ultra high pressure chromatography- tandem mass spectrometry

AMY: amygdalin, DHU: dhurrin, LIN: linamarin, LIU: linustatin, LOT: lotaustralin, NEO: neolinustatin, PRU: prunasin, SAM: sambunigrin, TAX: taxiphyllin

[a] Theoretical, calculated from concentration of cyanogenic glycosides

[b] Converted to a fresh weight basis using a moisture content of 7%

[c] Dry weight basis

[d] Converted to a fresh weight basis using a moisture content of 4.7%

4. EFFECT OF PROCESSING

During the course of food processing there is potential for cyanogenic glycosides to be degraded to HCN by endogenous β-glucosidase enzymes. Depending on the processing, the volatile HCN formed may be lost to the environment.

4.1 FREEZING

4.1.1 Apple juice

Freezing of apple juice did not have a significant impact on the amygdalin content (Bolarinwa *et al.*, 2015).

4.2 DRYING

4.2.1 Cassava

The flesh (parenchyma) of 20 cassava cultivars (10 local and 10 improved) was sliced into chips and sun-dried for five days (Njankouo Ndam *et al.*, 2019). The HCN content of the chips reduced by approximately 47-48%.

4.2.2 Bamboo shoots

Shoots of four bamboo species (*Bambusa nutans*, *Dendrocalamus hamiltonii, D. latifloris,* and *D. sikkimensis*) grown in the Manipur region of India, with CG levels determined by the picrate paper method in the range 1020-1340 mg/kg HCN fresh weight, were dried in an oven at 60°C for 24 hours (Devi, 2018). Post-drying the CG concentrations were in the range 42-77 mg/kg HCN (-92 to -97%).

4.3 PULPING/GRINDING/MASCERATION

As both CGs and the enzymes that hydrolyse them are usually present in different parts of the plant tissue, breaking down the plant material and leaving for a period of time may substantially reduce the HCN potential.

4.3.1 Cassava leaves

Cassava leaves were processed to produce a pulp and were left at 25°C for 48 hours (Latif *et al.*, 2019). The initial HCN concentration of 530 mg/kg decreased to less than 100 mg/kg after 3 hours and continued to decrease more slowly for the remainder of the study period. After 48 hours, the final concentration of HCN was approximately 20 mg/kg (96% reduction).

4.4 SOAKING

Soaking of cyanogenic plant material can transfer CG and HCN to the soak water and when combined with ultrasound may provide an opportunity for β-glucosidase enzymes to be mobilised and hydrolyse CGs present.

4.4.1 Bamboo shoots

Soaking of fresh shoots from the bamboo species *Dendrocalamus hamiltonii* and *D. giganteus* in water for 12 hours resulted in 50 and 64% reductions in the HCN content, respectively (Rawat *et al.*, 2015). Increasing the soaking time to 24 hours increased the degree of HCN reduction to 85 and 82%, respectively. The HCN content of the soaking water was not determined and it is uncertain whether the reductions were due to destruction of CGs or their transfer to the water.

Shoots of four bamboo species (*Bambusa nutans*, *Dendrocalamus hamiltonii, D. latifloris,* and *D. sikkimensis*) grown in the Manipur region of India, with CG levels determined by the picrate paper method in the range 1020-1340 mg/kg HCN fresh weight, were soaked in water for 12 hours (Devi, 2018). Post-soaking the CG concentrations were in the range 330-410 mg/kg HCN (-67 to -71%). Further samples of the bamboos were preserved in brine (5% salt) for 1, 3, 6, or 12 months. CG concentrations decreased substantially in the first month, to concentrations in the range 40-60 mg/kg HCN (-96% for all species). A more gradual decrease in CG content occurred over the subsequent 11 months, with final concentrations in the range 4-24 mg/kg HCN (-98 to >-99%).

4.4.2 Apricot kernels

Apricot kernels can be 'debittered' by soaking in water or acidic solutions (Zhang *et al.*, 2019). Vacuum, microwave, or ultrasound can be applied to facilitate this process. Zhang *et al.* (2019) examined the impact of different debittering procedures on the residual amygdalin content of the kernels. After removal of the skin, kernels contained 64,700 mg/kg of amygdalin (3800 mg/kg HCN equivalents). A sub-sample of the kernels was boiled for 10 minutes to inactivate β-glucosidase. Debittering by soaking in water at 55°C for 2 hours, with β-glucosidase inactivated, decreased the amygdalin content to 24,900 mg/kg (1470 mg/kg HCN equivalents). Using ultrasound (300 W and 59 kHz) resulted in improved debittering, with amygdalin reduced to 9,400 mg/kg (550 mg/kg HCN equivalents). Application of ultrasound-assisted debittering to kernels with β-glucosidase not denatured, resulted in even greater decreases, to 2,800 mg/kg of amygdalin (95.7% reduction, 165 mg/kg HCN equivalents).

4.4.3 Cassava leaves

Pulped cassava leaves were mixed with water (control) or 0.4% sodium bicarbonate and incubated at 55°C for 6 hours (Latif *et al.*, 2019). Only minor differences in the decrease in HCN were observed, with a 90% reduction in HCN in water and a 93% reduction in sodium bicarbonate. However, it appears that CG hydrolysis is initially more rapid in sodium bicarbonate solution. The pH of the cassava leaf-water mixture was not reported and it is not possible to surmise how different the pHs of the test solutions may have been.

In a further experiment as part of the same study, pulped cassava leaves were dispersed in water and incubated at 55°C for 0.25 hours, either in a static water bath or with ultrasonification (35 kHz, 500 W) (Latif *et al.*, 2019). Both ultrasound-treated and non-treated samples showed the same level of HCN reduction (84%).

4.4.4 Linseed

Soaking of defatted linseed flour in water (temperature not stated) resulted in decreases in the HCN content from an initial concentration of 319 mg/kg, determined by alkaline titration, to 245 (-28%), 193 (-39%), 182 (-43%), and 165 (-48%) mg/kg after 12, 24, 36, and 48 hours, respectively (Safdar *et al.*, 2020). In a further experiment, the linseed flour was added to water and ultrasound (300 W power, 20 kHz frequency) was applied. This substantially increased the rate of HCN removal with concentrations of 211 (-34%), 178 (-44%), 73 (-77%), and 25 (-92%) mg/kg achieved after sonication for 5, 10, 15, and 20 minutes, respectively.

4.4.5 Taro

Taro root was soaked in water at room temperature for 72 hours, then drained and sun-dried (Olajide *et al.*, 2011). The HCN content, determined by the spectrophotometric picrate paper method, reduced from 17.1 to 7.5 mg/kg (-56%).

4.4.6 Gadung

Gadung, with an initial CG content (alkaline titration) of 84 mg/kg, was soaked in static water for 24, 48, or 72 hours (Kumoro *et al.*, 2011). At the end of these periods only very modest

decreases in the CG content were observed, with CG contents of 82, 82, and 81 mg/kg after 24, 48, and 72 hours, respectively. It is uncertain why the decreases were so minimal, but it may be due to low concentrations of β-glucosidase enzymes in the tuber. However, greater reductions were observed when the tubers were placed in flowing water, with an approximate 50% reduction seen after 2.5 hours in water flowing at 5 x 10^{-5} m^3/s.

4.5 HEAT TREATMENT

4.5.1 Apple juice

Holding apple juice at room temperature for 120 minutes decreased the amygdalin content by 11-19% (Bolarinwa *et al.*, 2015). Reductions increased with holding time, with a 10 minute hold of Egremont Russet apple juice achieving a 2% reduction, while a 120 minute hold achieved a 13% reduction. Surprisingly, pasteurisation at 75°C did not appear to have any impact on the activity of the endogenous β-glucosidase.

4.5.2 Bamboo shoots

Boiling of fresh shoots from the bamboo species *Dendrocalamus hamiltonii* and *D. giganteus* in water for 10 minutes resulted in 68 and 77% reductions in the HCN content (Rawat *et al.*, 2015). Boiling for 20 minutes resulted in further reductions to 86 and 87% total reductions, respectively. HCN content was determined by the colourimetric method of Haque and Bradbury (2002). The cooking water was not analysed and it is uncertain whether the CGs were destroyed or transferred to the cooking water.

Shoots of four bamboo species (*Bambusa nutans*, *Dendrocalamus hamiltonii, D. latifloris,* and *D. sikkimensis*) grown in the Manipur region of India, with CG levels determined by the picrate paper method in the range 1020-1340 mg/kg HCN fresh weight, were boiled in water for 20 minutes (Devi, 2018). Post-boiling the CG concentrations were in the range 170-240 mg/kg HCN (-81 to -83%).

Shoots from four bamboo species (*B. bambos, B. tulda, D. strictus,* and *D.asper*) were collected from locations in central India (Pandey and Ojha, 2014) and boiled in water, 1% NaCl, 5% NaCl, or 10% NaCl for 10, 15, 20, or 25 minutes. CG content was determined by a spectrophotometric method. For all species, the CG content of the shoots decreased with boiling time, but without a consistent effect due to the salt content of the boiling water. After 25 minutes of boiling the CG content of shoots was 20 mg/kg or less in all cases.

4.5.3 Cassava leaves

Cassava leaves were pulped and left to stand at temperatures of 25, 35, 55, 75, or 85°C for 36 hours (Latif *et al.*, 2019). Residual HCN concentrations were in the range 3-5% of the initial leaf concentration, with no significant difference between temperatures. Samples were then dispersed in water and heated at 25 or 55°C for 6 hours. The higher temperature was chosen as it is the temperature optimum for the linamarase enzyme. Incubation at 55°C reduced the HCN content to 50 mg/kg (91% reduction), compared to 78 mg/kg (85% reduction) for the sample incubated at 25°C.

4.5.4 Linseed

Roasting of linseed using infra-red radiation (1150 W/m^2) was reported to reduce HCN content by up to 59% (Tuncel *et al.*, 2017). HCN was determined titrimetrically. The HCN content of the raw linseed was 218 mg/kg. Depending on the wavelength of the radiation and the roasting time, the HCN content was reduced to 88-144 mg/kg (-34 to -59%).

Oven heating ground linseed (130°C) for 10 or 20 minutes reduced the HCN concentration from 377 mg/kg to 316 (-16%) and 290 (-23%), respectively (Feng *et al.*, 2003). Autoclaving (120°C, pressure 16.5 kg/cm^2) had a greater effect, reducing the HCN concentration to 265

mg/kg (-30%). Microwave heating (750 W, maximum output for 4 minutes) was even more effective, reducing the HCN content to 63.5 mg/kg (-88%).

Ground and defatted linseed was dispersed on glass plates and subjected to microwave heating for 3, 6, 8, or 12 minutes at 450 W output power (Safdar *et al.*, 2020). The initial HCN content, determined by alkaline titration, of 319 mg/kg reduced to 231 (-28%), 169 (-47%), 121 (-62%), and 93 (-71%) mg/kg at 3, 6, 8, and 12 minutes, respectively. Boiling of the linseed flour in water resulted in decreases to 94 (-71%), 77 (-76%), 58 (-82%), and 18 (-94%) mg/kg after boiling for 15, 20, 25, and 30 minutes, respectively. Autoclaving (not stated, but presumably of the dry flour) at 120°C resulted in decreases to 200 (-37%), 169 (-47%), 139 (-63%), and 119 (-63%) mg/kg after autoclaving for 5, 10, 15, and 20 minutes, respectively. The authors of this study suggested that the temperature-mediated reductions in HCN content were due to stimulation of the activity of the native β-glucosidase enzyme. However, this seems unlikely as the temperatures involved in these studies would have denatured the enzyme. The greater reduction during boiling may have been the result of off-gassing of HCN formed following milling and solubilisation of CGs or HCN into the boiling water.

4.5.5 Taro

Sliced, unpeeled taro root was boiled in water for 15 minutes, then drained and sun-dried for 12 days (Olajide *et al.*, 2011). The HCN content, determined by the spectrophotometric picrate paper method, reduced from 17.1 to 7.3 mg/kg (-57%).

The HCN content of raw taro (sliced and sun dried) was compared to taro that was sliced, boiled for 30 minutes and then sun-dried (Abdulrashid and Agwunobi, 2009). HCN was determined by titration. The HCN content reduction from 21 to 9.2 mg/kg (-56%).

4.5.6 Gadung

Gadung tubers that had previously been immersed in running water to remove about half of the CG content were steamed for up to 2 hours (Kumoro *et al.*, 2011). The CG content, determined by alkaline titration, decreased from 46 mg/kg HCN before steaming to 30 mg/kg HCN after 2 hours of steaming (-35%).

4.6 PELLETING

Ground plant material can be formed into pellets through application of pressure and increased temperature.

4.6.1 Linseed

Ground raw linseed with an initial HCN content of 377 mg/kg was pelleted using a laboratory pellet mill (Feng *et al.*, 2003). A single pelleting process reduced the HCN content to 327 mg/kg, a 13% reduction. The pelleted linseed had a temperature of 31.5°C. Repeating the pelleting process three or six times produced further decreases in HCN concentrations, with reduction approximately correlated with the number of pelleting cycles; 29% reduction for three cycles and 55% reduction for six cycles. The temperature of the pelleted meal increased with each cycle, to 33.9°C after three cycles and 38.0°C after six cycles.

4.7 EXTRUSION

4.7.1 Linseed

The impact of extrusion parameters (initial moisture content, temperature) on the CG content of a linseed co-extrudate was examined (Čolović *et al.*, 2015). The CG content of the initial material, as determined by alkaline titration was 126 mg/kg HCN. While CG content generally decreased with increasing extrusion temperature, the impact of initial moisture

content did not appear to follow any pattern. The lowest final CG content (25 mg/kg) was achieved with an initial moisture content of 11% and an extrusion temperature of 70°C.

4.8 BREWING

4.8.1 Beer

Linamarin and lotaustralin were determined in malt, mash, wort, boiled wort, inoculated wort, and beer (Mastanjević *et al.*, 2018). While the concentrations were reasonably low in the malt (4.4 mg/kg HCN equivalents for the sum of linamarin and lotaustralin), concentrations decreased markedly during the mashing process, by approximately 96% for linamarin and 83% for lotaustralin. Mashing involves mixing of malt with water. The reduction in CG concentrations suggests that residual β-glucosidase activity was present in the malt, allowing hydrolysis of CGs to HCN. Reductions in CG concentrations through the remaining brewing steps were modest.

Sorghum beer is commonly produced in parts of Africa. During malting (wetting and germination of the grain) concentrations of the CG dhurrin were shown to increase markedly from 35 and 44 mg/kg in unmalted brown and white sorghum, determined by HPLC, to 320 and 220 mg/kg (28 and 19 mg/kg HCN equivalents) in the same sorghum samples (Tokpohozin *et al.*, 2018). It is likely that even greater amounts of dhurrin are produced but hydrolysed by native β-glucosidases, as the malted sorghums were also found to contain 330 and 470 mg/kg of *p*-hydroxybenzaldehyde, the immediate hydrolysis product of dhurrin. Kilning (drying) of the malted sorghum resulted in a small decrease in dhurrin content and an almost complete removal of *p*-hydroxybenzaldehyde. The next step in the brewing process is mashing, where malt is mixed with water, allowing the starch-degrading enzymes produced during malting to convert starch to sugars (saccharification). The mashes of brown and white sorghum malts contained 4.4 and 11.0 mg/L of dhurrin, respectively. Heating the mash to 40°C for 30 minutes reduced these concentrations to 1.4 and 5.2 mg/L of dhurrin, respectively, presumably due to the action of native β-glucosidase (dhurrinase). Acidification of the mash with lactic acid bacteria (*Lactobacillus plantarum* or *L. paracasei*) strains known to be good sources of β-glucosidase further reduced dhurrin to undetectable levels in brown sorghum mash and 2.3 mg/L in white sorghum mash (0.2 mg/L HCN equivalents).

4.9 ENZYMATIC TREATMENT

4.9.1 Cassava leaves

Pulped cassava leaves were dispersed in 0.1 M sodium citrate, containing 0.32% of a plant cell wall-degrading enzyme (Multifect®, mainly cellulase) and incubated at 55°C for 4 hours (Latif *et al.*, 2019). Cassava leaf pulp dispersed in citrate buffer, without enzyme, was used as a control. There were relatively minor differences in the level of HCN reduction between the enzyme-treated and non-enzyme-treated samples. In the enzyme-treated sample, HCN decreased from 559 mg/kg to 100 mg/kg (82% reduction), while in the citrate buffer control HCN was reduced to 118 mg/kg (79% reduction).

4.10 MICROBIAL TREATMENT

4.10.1 Cassava

Bacteria able to utilise linamarin were isolated from the peel of cassava root (Murugan *et al.*, 2012). A strain of *Bacillus subtilis* was identified with particularly high linamarase activity. Cassava flour with a HCN content of 210 mg/kg (picrate paper method) was treated either by soaking overnight in water or with an enzyme extract from *B. subtilis*. The final HCN concentrations were 35 and 8 mg/kg, respectively.

4.11 FERMENTATION

4.11.1 Cassava

Decreases in HCN were studied during the production of local foods from cassava root in Cameroon (Njankouo Ndam *et al*., 2019). Gari is produced by peeling, grating, dry fermentation, drying, and roasting of cassava. This process resulted in an approximate 80% reduction in HCN concentrations. Fufu is produced by peeling and immersing in water for five days (wet fermentation). The root is then crushed and sun dried. Processing resulted in a 89-94% reduction in the HCN content and prepared fufu contained HCN concentrations less than 10 mg/kg. Although the processes used in the production of gari and fufu are referred to as fermentation, it does not appear to be a true fermentation process. Rather, it appears that the processes are intended to allow the natural β-glucosidase enzymes in the cassava to hydrolyse the CGs.

Changes in the HCN content of cassava flour were followed during ethanol fermentation (Qin *et al*., 2021). HCN was determined following distillation by two colourimetric methods and by ion chromatography. HCN concentrations were determined after steaming, saccharification, and yeast fermentation. The cassava flour contained 43 mg/kg HCN equivalents. The concentration of HCN was reduced by 73% after steaming, with minor further reductions after saccharification and fermentation, giving a total reduction of 82%. It appears likely that enzymatic hydrolysis during the early stages of steaming would be the major reduction process.

4.11.2 Taro

Taro root was grated, packed into an air-tight bag, pressed and allowed to stand for 72 hours, then sun-dried for 4 days (Olajide *et al.*, 2011). The standing period allowed fermentation to occur. The HCN content, determined by the spectrophotometric picrate paper method, reduced from 17.1 to 7.2 mg/kg (-58%).

Taro root was sliced and naturally fermented in water at 30°C for 24, 48, or 72 hours (Igbabul *et al.*, 2014). All samples were dried at 65°C in an air draught oven. HCN was determined in all samples by titration. The HCN content reduced from 740 mg/kg in the unfermented control to 500 mg/kg after 24 hours, 100 mg/kg after 48 hours, and 10 mg/kg after 72 hours.

4.11.3 Bamboo shoots

Shoots of four bamboo species (*Bambusa nutans*, *Dendrocalamus hamiltonii, D. latifloris,* and *D. sikkimensis*) grown in the Manipur region of India, with CG levels determined by the picrate paper method in the range 1020-1340 mg/kg HCN fresh weight, were fermented in air-tight polythene bags at room temperature for 1, 3, 6, or 12 months (Devi, 2018). As with brine storage, the greatest reduction in CG content occurred during the first month with concentrations reducing to 65-110 mg/kg HCN (-92 to -94%). The decrease in CG content was more gradual during the following 11 months, with final CG concentrations in the range 23-55 mg/kg HCN (-96 to 98%).

4.12 SOLVENT EXTRACTION

4.12.1 Linseed

Defatted linseed flour was added to methanol/ammonia/water of differing ratios, stirred (500 rpm) for 15 minutes, followed by recovery of the flour by vacuum filtration and freeze-drying (Safdar *et al.*, 2020). HCN reduction was related to the methanol proportion of the extraction solvent, with the initial concentration (319 mg/kg, determined by alkaline titration) reduced to 278 (-13%), 187 (-41%), 88 (-73%), and 56 (-82%) mg/kg for methanol proportions of 75, 80,

85, and 90%, respectively. Insufficient solvent compositions were examined to determine if the proportion of ammonia and/or water had an impact on HCN reduction.

4.13 SUMMARY

While the impact of a number of food processing steps in the cyanogenic potential of various foods has been examined, it is likely that in most cases the reductions in cyanogenic potential reported are due to the interaction of CGs and β-glucosidase enzymes and the subsequent release of volatile HCN. These processes will be enhanced under conditions of moderate heat.

Most of the studies summarised above indicate that food processing can decrease, but not completely remove the cyanogenic potential of CG-containing foods.

5. ANALYTICAL METHODS

The analysis of foodstuffs for total hydrocyanic acid generally takes one of two forms, either cyanide is released as volatile hydrogen cyanide by the action of enzymes or dilute acids and subsequently quantified (indirect), or the concentration of intact CGs are themselves determined (direct).

5.1 INDIRECT ANALYSIS OF CYANOGENIC GLYCOSIDES AS TOTAL HCN

Indirect analysis of foodstuffs for total cyanide aims to release HCN from CGs with the HCN subsequently detected and quantified by various methods including colourimetric, spectrometric and chromatographic.

5.1.1 Sample preparation and extraction

One of the most important aspects of the total hydrocyanic acid determination in foodstuffs containing CGs is the preparation of samples for analysis (Cho *et al.*, 2013). It is related to the fact that cyanide ions are not stable and occur in various forms. The presence of matrix interference must also be considered in the preservation and preparation procedures (Jaszczak *et al.*, 2017).

For optimal yields of cyanide from CG-containing foodstuffs, samples should be finely ground. Yields from fresh foodstuffs have been shown to depend, in part on temperature with yields of cyanide highest if the material is ground in liquid nitrogen, lower in dry ice, and lowest if ground in buffer at room temperature. Some researchers have ground samples in acidic or basic media to inhibit β-glucosidases, adjusting the pH to a value at which the enzyme is active just before the sample is sealed in the reaction flask (Brinker and Seigler, 1989).

5.1.2 Analysis of released HCN

Interferences

Interferents present in the sample or in solvents, reagents and on glassware can interfere with sample analysis. While a distillation step can eliminate or reduce many interferences, chlorine and sulphide are potentially problematic.

Oxidising agents such as chlorine, e.g. bleach, decompose most cyanides in solution and can result in decreased apparent cyanide concentration. Sulphide (S^{2-}) (as well as sulphites SO_3^{2-} and native sulphur) also reacts with cyanide to form thiocyanate also reducing apparent cyanide concentration in the sample.

Ascorbic acid will also react with cyanide to decrease apparent concentrations while formaldehyde can also decrease cyanide concentrations but in the presence of ammonia or nitrogen will increase cyanide concentrations. High results may be obtained for samples that contain nitrite (NO_2^-) and/or nitrate (NO_3^-) in excess of 10 mg/kg. During distillation, these compounds will form nitrous acid, which can react with some organic compounds present to produce oximes. These compounds, once formed, can decompose under test conditions to form HCN. Pre-treating samples with sulphamic acid (H_3NSO_3) prior to distillation will eliminate this problem (Jaszczak *et al.*, 2017).

Hydrolysis, extraction and distillation

The volatile nature of HCN imposes some analytical restrictions concerning extraction and hydrolysis of CGs. While hydrolysis of CGs to form HCN can be achieved by acid hydrolysis

or enzymatic degradation, the volatility of HCN means, generally, to ensure all released HCN is retained for analysis, food samples must be incubated in sealed containers (EFSA, 2019). Also, while commercially available β-glucosidase will hydrolyse some CGs, others will be hydrolysed slowly or not at all. To avoid this issue some analysts have employed crude acetone extracts of β-glucosidase sourced specifically from the plant being studied. Amygdalin is the most common CG against which β-glucosidase is very effective (Brinker and Seigler, 1989).

Once the sample has been prepared, extraction, if necessary, is carried out using NaOH or H_3PO_4. Sodium hydroxide has the advantage of binding volatile forms of cyanide in solution if the pH >11. The resultant extract can then be treated with acids or enzymes to produce HCN. Steam distillation of HCN from treated samples into NaOH solution is also employed as a sample clean-up step, having the advantage of eliminating many interfering compounds from a complicated food matrix for liquid chromatographic or gas chromatographic analysis.

Spectrophotometric determination

The most common analytical techniques used for detection and determination of cyanide in properly prepared food samples are spectrometric and chromatographic (Jaszczak *et al.*, 2017).

Cyanide reacts with many compounds to produced coloured products allowing for both qualitative and quantitative visual and spectrometric determination. Two of the easiest and most reliable methods employ either Feigl-Anger or sodium picrate paper (Brinker and Seigler, 1989). Feigl-Anger paper is made using copper ethylacetoacetate and 4,4'-methylenebis(N,N-dimethylaniline). In the presence of HCN a blue coloured complex is formed, its intensity being indicative of the amount of HCN present (Reddy *et al.*, 2016). Sodium picrate paper can be prepared in aqueous solution (unlike Feigl-Anger paper that may not work in a humid environment) using picric acid and sodium carbonate, and forms a red coloured compound when exposed to HCN (Brinker and Seigler, 1989). The reaction of chloramine-T/pyridine-barbituric acid colourimetric methods for measuring cyanide are also a well-established method (Ma and Dasgupta, 2010). The preceding list of colourimetric reagents is by no means exhaustive and does not include fluorometric reagents.

A summary of the method employed by Burns *et al.* (2012) to investigate the cyanide content of cassava products in Australia illustrates how sample treatment and colourimetric reagents can be combined to quantitate HCN in foodstuffs. A suitably prepared sample (100 mg) was added to a small plastic bottle. Enzyme[5] and a suitable buffer were added to liberate HCN from CGs. A plastic support allowed for the suspension of a picrate paper over the sample and the bottle was closed and left at 30°C overnight. The picrate papers were removed, and 5 ml of water used to elute the colour from the paper. The absorbance of the solution was measured at 510 nm. The authors were able to quantify HCN down to 1 mg/kg of sample (Burns *et al.*, 2012).

Hydrogen cyanide can also be liberated from CGs by heating in acid, which is cheaper than β-glucosidase and can hydrolyse a wide range of CGs. Due to the volatility of HCN, such procedures, unless conducted in a sealed glass ampoule or similar, will result in loss of HCN even in well-sealed vials. Such loss can be adjusted for by hydrolysing multiple aliquots of the same sample and determining the HCN content of each as time progresses. Hydrogen

[5] The source and identity of the enzyme was not stated by the authors of this study. The enzyme is presumably β-glucosidase

cyanide concentrations can then be graphed against time and concentrations extrapolated backward to time = 0 to give a total hydrocyanic acid concentration in the sample (Saunders, 2012).

Most colourimetric methods can be subject to interference. The picric acid reagent gives false positives with protoanemonins found in Ranunculaceae (buttercup) species. Breakdown products of glucosinolates found in members of the Brassicaceae (cabbage family) and other families, frequently give false positives (Brinker and Seigler, 1989).

Titrimetric determination

Hydrogen cyanide generated by hydrolysis of CGs in foodstuffs is also amenable to titrimetric quantitation. The Association of Official Analytical Chemists (AOAC) method 49.11.02 Hydrocyanic acid in beans, for example, first macerates 10-20 g of sample in water for 2 hours allowing for endogenous β-glucosidase to generate HCN from CGs, which is then steam-distilled into a known volume of acidified 0.02M silver nitrate ($AgNO_3$) solution. Any cyanide present reacts with silver to form silver cyanide (AgCN) an insoluble white precipitant.

Following filtration to remove the solid precipitant, any remaining $AgNO_3$ is quantitated by titration with 0.02M potassium thiocyanate (KSCN) using a ferric alum indicator and the amount of HCN reacted with $AgNO_3$ determined by difference with 1 ml 0.02M $AgNO_3$ = 0.54 mg HCN.

There are multiple variations to this titrimetric approach including the AOAC alkaline titration method. Similar to that described above, the HCN is steam-distilled into a caustic solution containing a known amount of potassium iodide (KI) that is subsequently titrated with $AgNO_3$, the end point being the appearance of turbidity with 1 ml of 0.02M AgNO3 = 1.08 mg HCN (AOAC International, 2005).

Other colourimetric reagents as described in the section above 'Spectrometric' can be adapted for the quantitation of HCN by titrimetric means.

Chromatographic determination

Hydrogen cyanide produced from foodstuffs for analysis as described above can be determined chromatographically, either as free CN^- in solution or a derivative of CN with a suitable chromophore or fluorophore for liquid chromatography or gas chromatography.

<u>Ion chromatography</u>

The CN^- ion lacks a suitable chromophore for UV-VIS or fluorometric analysis. Detection of free CN^- has therefore frequently employed electrochemical detection techniques. Koch (1983) describes several electrochemical procedures for the analysis of free CN^- using pulse polarography, coulometry, amperometry and ion-selective electrodes. While free CN^- in matrices such as bottled water can be directly determined using ion chromatography, the presence of sulphate and chloride significantly lowers free cyanide recovery, while carbonate increases apparent CN^- concentration (Cengiz *et al.*, 2015).

The method developed by Cho *et al*. (2013) is typical for the analysis of total HCN in foodstuffs. Edible plant material containing CGs was extracted into 0.1M Phosphoric acid (H_3PO_4) and CGs hydrolysed to form HCN by addition of sulphuric acid (H_2SO_4) and heat. The resultant solution was micro-distilled, and CN^- collected in NaOH solution. Two hundred microlitres of distillate was injected onto a IonPac AS7 ion column with a mobile phase consisting of 0.5M Sodium acetate/0.1M NaOH/0.5%(v/v) ethylenediamine. The electrode

cell was equipped with a Ag/AgCl working electrode. A limit of detection of 0.005 mg/L CN^- was achievable.

Gas chromatography

Gas chromatography (GC) is one of the most common methods for analysis of HCN. Common detectors used for analysis of HCN are flame ionisation detection (FID), electron capture detector (ECD), nitrogen-phosphorus detector (NPD) and mass spectrometric (MS) detector. Since HCN is volatile, for detection in biological matrices (and presumably suitably prepared foodstuffs) derivatisation is not always essential (excepting for ECD detectors). In GC analysis, the NPD permits sensitive detection of nitrogen-containing compounds, but the detector is prone to instability and is less sensitive than other types of detector such as ECD and MS. Therefore, the most common pre-analysis step in GC analysis of HCN is sampling from the sample headspace (Logue and Hinkens, 2008).

The use of Solid Phase Micro Extraction (SPME) fibres has also been employed to concentrate HCN from samples for subsequent GC-MS analysis. Zain *et al.* (2017) used carboxen/polydimethylsiloxane (CAR/PDMS) 75 µm thickness and an Agilent GS-GASPRO 30 m length, 0.32 mm ID, 0.25 µm film thickness column with helium as a carrier gas to determine HCN in liquid samples derived from the air. Labelled potassium cyanide ($K^{13}C^{15}N$) was used as an internal standard.

Derivatisation of HCN to form products of larger molecular weight can improve specificity and sensitivity of GC-MS analyses. Osak et al (2021) noted that the low molecular mass of HCN does limit detector performance and many columns poorly retain HCN alone and may require a cryogenic trap to ensure adequate peak resolution. These researchers employed pentafluorobenzylation to create a suitable derivative for GC-MS analysis.

Liquid chromatography

Liquid chromatography has seldom been reported for CN^- determination, except for a few methods involving fluorometric detection after complexation of the analyte by the pyridine/barbiturate reaction (König reaction) or naphthalene-2,3-dicarboxylic acid (NDA) in the presence of taurine (Tracqui *et al.*, 2002). Post-column derivatisation with o-phthalaldehyde has also been reported, however o-phthalaldehyde derivatives are substantially less stable than those of NDA (Sumiyoshi *et al.*, 1995).

Derivatising with NDA has the advantage of being done in aqueous conditions to produce a powerfully fluorescent product that can be analysed using HPLC-FL or LC-MS (Beek and de Jong, 2011; Tracqui *et al.*, 2002). Beek and de Jong (2011) first extracted animal feed samples (compound feeds and feed ingredients) into H_3PO_4. The extract was treated with β-glucosidase to release HCN from CGs and the solution steam-distilled into dilute NaOH after incubating overnight at 37°C to immobilise CN^- in the aqueous phase. Following derivatisation with NDA, the product was analysed by HPLC-FL using a C18 RP-column and a methanol-phosphate buffer mobile phase with fluorescence detection at λ_{ex} = 418 nm and λ_{em} = 460 nm. The limit of quantitation (LOQ) for total HCN in feed was < 1 mg/kg.

5.2 DIRECT ANALYSIS OF CYANOGENIC GLYCOSIDES

5.2.1 Extraction for liquid and gas chromatographic analysis.

Since the direct methods determine individual CGs, these methods are not prone to many of the interference issues surrounding indirect analysis of CGs as HCN. Loss or formation of HCN by interfering compounds is less of a concern, though care must still be taken not to lose cyanide due to the action of endogenous β-glucosidases on CGs. In many instances a

simple extraction into a water-alcohol solution (with possible subsequent clean-up before injection onto the LC) suffices for extraction of CGs for subsequent LC-MS analysis.

As previously noted, there are at least 60 different CGs. The most common CG is amygdalin, that can be found in seeds, pips and kernel of fruit such as apples, peaches, almonds, cherries, plums, and apricots (Jaszczak *et al.*, 2017). However, other important foodstuffs contain different CGs, e.g. cassava contains linamarin and lotaustralin, bamboo contains taxiphyllin, peach and nectarine both contain prunasin. Direct analyses do not convert these individual CGs into HCN, so the analysis must be matrix-specific, considering the specific CGs known to be present. Alternatively, analyses may determine a range of key CGs at once (Saunders, 2012).

Whether the ultimate analytical destination for individual CGs is HPLC-UV or LC-MS, extraction methods appear relatively similar being based upon aqueous solvent extraction, noting however, that matrices containing high levels of lipids such as flax seed, may require defatting by extraction with hexane or similar solvent prior to aqueous-alcohol extraction of CGs (Bacala and Barthet, 2007; Shahwar *et al.*, 2019; Zhao *et al.*, 2019).

A simple HPLC procedure is described in Ngamriabsakul and Kommen (2011) who analysed Thai Pra (*Elateriospermum tapos*) seeds for the presence of amygdalin and prunasin by HPLC-UV. A sample (0.2 g) was extracted with 10 ml of methanol for 12 hours at room temperature, 0.1 g polyvinylpolypyrrolidone being added to remove coloured pigments that might interfere with UV detection.

Using a similar extraction method, Del Cueto *et al.* (2017) were able to detect amygdalin and prunasin in samples of almond and cherry flower buds by homogenising samples in liquid nitrogen and extracting into an 85% methanol solution by boiling for 5 mins and using CG linamarin as an internal standard with subsequent LC-MS analysis.

While testing for amygdalin, dhurrin, prunasin, and linamarin in elderberry, Appenteng *et al.* (2021) determined recoveries of these analytes from various aqueous ethanol and methanol combinations. Highest recoveries were obtained with 75% methanol extraction overnight with shaking at room temperature and 30 mins sonication at 30°C. The samples were further purified by solid phase extraction on a 500 mg C18 Sep-Pak cartridge, washed with 0.1% formic acid (FA) and CGs eluted with various aqueous methanol mixtures, with 30-40% methanol being found to be the best solvent strength for elution of all CGs. Samples were then evaporated under nitrogen, made up in FA solution for subsequent LC-MS analysis.

Zhong *et al.* (2020) employed a similar extraction method to test for eight CGs including: linamarin, linustatin, lotaustralin, neolinustatin, taxiphyllin, dhurrin, amygdalin, and prunasin. One gram of sample ground to flour in liquid nitrogen, was extracted twice into 3 ml of 80% methanol and sonicated for 15 mins. Combined extracts were eluted onto a 200 mg/6 ml hydrophilic-lipophilic SPE cartridge and CGs eluted with 3 ml 30:70, v/v methanol/acetonitrile. The solution was evaporated under nitrogen gas and redissolved in 1 ml of 10% methanol prior to LC-MS/MS analysis.

5.2.2 Chromatography

Most CGs can be readily resolved by liquid chromatography with RP C18 columns with mixtures of water and solvents, methanol, or acetonitrile used as mobile phases (Madrera and Valles, 2021; Ngamriabsakul and Kommen, 2011). These studies used UV-VIS absorption at 218 nm and 214 nm with the latter employing diode array detection (DAD) to detect amygdalin and claimed quantitation limits of approximately 0.08 mg/L and 2.2 mg/kg

respectively. Identification of CGs was determined by retention time (RT) (Ngamriabsakul and Kommen, 2011) or RT and UV-VIS absorption profile (Madrera and Valles, 2021). An extensive list of references for HPLC methods is given in Liu and Wang (2019) with most researchers using wavelengths < 220 nm for detection of amygdalin.

Lacking ideal chromophores, low UV wavelengths are necessary for detection of CGs using LC with UV-VIS detection. Unfortunately, this lends itself to interference as many compounds absorb strongly in this region of the UV-VIS spectrum.

LC-MS/MS analyses appear very similar i.e., RP C18 columns with acetonitrile and water mobile phases mildly acidified with formic acid or acetic acid and using gradient elution often employed. Far greater specificity and sensitivity is achievable with MS compared with HPLC-DAD detection of CGs. The Na^+-adducts ($[M + Na]^+$) under positive ionisation appeared to be the main or unique ions observed for CG analytes. The influence of matrix on analytical signals needs to be ascertained when using LC-MS/MS. This is achieved primarily by determining the difference in response of the same concentration of analyte in matrix-matched and solvent-matched solutions. Issues with ion suppression were partially addressed by diluting the injected sample or including an internal standard (I.S.), e.g., another CG not found in the matrix under investigation.

Zhong *et al.* (2020) were able to analyse for eight key CGs in various matrices including cassava, linseed, bamboo, sorghum, apricot, almond, and lima beans by LC-MS/MS with limits of detection (LOD) ranging from 1 to 25 µg/kg and LOQs ranging from 5 to 100 µg/kg for the listed CGs.

GC analysis of CGs requires derivatisation with most researchers using silanisation with trimethylsilylchlorosilane (TMCS) or similar reagents followed by GC-MS or GC-FID analysis. Shahwar *et al* (2019) employed a DB-17 capillary column (50% phenyl-methylpolysiloxane), 30 m x 0.32 mm x 0.25 mm film thickness and a temperature programme from 190°C to 280°C at 50°C/min using hydrogen as a carrier gas. Linamarin, linustatin, and neolinustatin were detected and quantitated using this method. Such procedures do require a dry sample for derivatisation although a very similar aqueous-solvent extraction method was initially employed prior to derivatisation (Bacala and Barthet, 2007; Barthet and Bacala, 2010).

5.2.3 Other methods for detection and quantitation of CGs

Capillary Electrophoresis (CE)

Capillary electrophoresis is a family of electrokinetic separation methods performed in submillimetre diameter capillaries. Analytes can be separated according to ionic mobility and can be concentrated or "focused" by means of gradients in pH and conductivity. Various means of analyte detection can be used including UV-VIS and fluorescence, CE can also be coupled with MS.

CE methods have been used to quantitate amygdalin in apple core and cherry seed extracts (Feng *et al.*, 2019; Liu and Wang, 2019). The electrolyte solution used was 20 mmol/L borax solution containing 15% methanol. Measurements were carried out at 18 kV voltage and experimental temperature at 30°C. UV detection wavelength was 210 nm with an injection time of 10 seconds. The lowest standard on their standard curve had a concentration of 0.041 mg/L amygdalin (samples were extracted into water and diluted by approximately 25X prior to analysis which equates to an LOQ <1 mg/kg amygdalin in the original sample).

Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance (NMR) is a good tool for qualitative and quantitative analyses of natural products and complex mixtures. Roulard *et al.* (2017) developed an NMR based analysis using ^{1}H and ^{13}C assignments for linustatin, neolinustatin, amygdalin, and related compounds. Flaxseed samples were extracted into a 3:1 methanol/water mixture, extracts were dried under vacuum, and dissolved in 1.5 ml methanol/D_2O 3:1 (v/v). After ultra-centrifugation, 750 µL of supernatant was transferred to the NMR tube. Amygdalin was added as in internal standard before extraction at a concentration of 0.5 mg/ml. Depending on the specific CG, LODs ranged from 7 to 230 mg/kg with LOQs ranging from 10 to 385 mg/kg of seed sample.

5.3 SUMMARY

The cyanogenic potential of foods can be determined by converting CGs to HCN, followed by determination of the cyanide ion by spectrophotometric, titrimetric or chromatographic techniques (indirect methods). Alternatively, the CGs can be determined directly, usually by liquid chromatography (direct methods). The results of indirect methods align directly with current regulatory limits, in determining hydrocyanic acid, and methods based on spectrophotometry and titration can be applied by basically equipped laboratories. Direct or indirect chromatographic methods offer greater sensitivity (lower LODs) and are useful for determining hydrocyanic acid levels less than 10 mg/kg.

6. EXPOSURE ASSESSMENT

Human health concerns associated with exposure to CGs generally relate to acute exposure, from a single meal or from a single day of food consumption. While JECFA have established a chronic health-based guidance value (JECFA, 2012), other authorities have concluded that the evidence base is insufficient to derive a chronic value.

Two main approaches have been taken to estimate acute dietary exposure to dietary CGs:

- EFSA carried out a probabilistic acute dietary exposure assessment (EFSA, 2019). This involves use of food consumption data for a single survey day or for surveys that examined multiple days, considering each day separately. For each survey day, acute dietary exposure was estimated by multiplying the total consumption amount for each relevant food category by an occurrence level randomly drawn among individual results available for that food category. Respective intakes of the foods consumed that day were summed and finally divided by the individual's body weight.
- JECFA carried out deterministic acute dietary exposure assessments (JECFA, 2012), using a high percentile of the food consumption distribution for relevant foods. The percentile used was the 97.5th if sufficient data were available, but for some less commonly consumed foods the 95th or 90th percentile was used. The maximum CG/HCN concentration from the available data set was used. Variations on this approach have been used. For example, in the FSANZ assessment of hydrocyanic acid in ready-to-eat cassava chips used a nominal 200 g consumption level to assess dietary exposure for a 20 kg child (FSANZ, 2008).

While there are New Zealand-specific data available to conduct probabilistic acute dietary exposure assessments, using data from the national nutrition surveys (Ministry of Health, 2003; University of Otago and Ministry of Health, 2011), the following sections will summarise New Zealand data that may be used for deterministic acute dietary exposure estimates. Food consumption information is summarised for foods discussed in section 2 of this report.

6.1 CASSAVA AND CASSAVA PRODUCTS

Cassava is not a commonly consumed food in New Zealand. Food balance sheets (FBS) for New Zealand, available through the Food and Agriculture Organization of the United Nations (FAO)[6] for the year 2019 gave a per capita availability of cassava and products for New Zealander of 0.26 kg/person/year (0.7 g/person/day).

The microdata for the 2008/09 Adult Nutrition Survey (adults 15+ years) (University of Otago and Ministry of Health, 2011) include 28 records of consumption of cassava (boiled or baked). These servings were consumed by 25 consumers, 0.5% of the survey cohort. The mean serving size was 336 g, with a 95th percentile of 704 g and a maximum of 1017 g. The microdata from the 2002 National Children's Nutrition Survey (children 5-14 years) (Ministry of Health, 2003) included 29 records of cassava consumption, relating to 25 survey respondents (0.7% of the survey cohort). The mean, 95th percentile, and maximum serving sizes were 138, 497 and 1080 g, respectively.

Cassava is also consumed in the form of ready-to-eat chips/crisps. Across the two nutrition surveys, eight servings were reported of 'crisps, tapioca' or 'crisps, vegetable, nfs' (not further specified), with a mean of 47 g and a maximum of 150 g. If it is assumed that

[6] https://www.fao.org/faostat/en/#data/FBS Accessed 6 April 2022

consumption of different types of crisp (potato, cereal, vegetable) will be similar, considerably more data are available. The Adult Nutrition Survey reports 357 records related to consumption of crisps, with a mean, 97.5th percentile and maximum of 50, 150, and 250 g, respectively. The Children's Nutrition Survey reports 1498 records related to consumption of crisps, with a mean, 97.5th percentile, and maximum of 28, 100, and 240 g, respectively.

6.2 TARO

Taro root, leaves, and stalk can all be consumed, however, CG information was only found for taro (cocoyam) root.

The Adult Nutrition Survey reports 111 records related to consumption of taro root, with a mean, 97.5th percentile and maximum of 262, 803, and 1410 g, respectively. The Children's Nutrition Survey reports 100 records related to consumption of crisps, with a mean, 97.5th percentile and maximum of 134, 503, and 570 g, respectively.

6.3 GADUNG

It is uncertain whether gadung is consumed in New Zealand. Given that gadung is consumed as a staple root crop, if consumed in New Zealand consumption levels would be expected to be similar to cassava root.

The FAO/WHO Chronic individual food consumption database – summary statistics (CIFOCOss)[7] does not include information on gadung consumption.

6.4 SORGHUM

There is no evidence of consumption of sorghum in New Zealand, either from FBS data or national nutrition surveys. However, this may be due to sorghum being consumed as an ingredient of processed foods.

The FAO/WHO CIFOCOss contains information on consumption of sorghum flour and grain. However, sorghum consumption was not reported for any westernised countries, with data only reported for Burkina Faso, China, Mozambique, and the Republic of Korea.

6.5 BEER

The Adult Nutrition Survey reports 682 records related to consumption of beer, with a mean, 97.5th percentile, and maximum of 822, 4040 and 16200 g, respectively. It should be noted that only a single study to date has reported the HCN content of beer and this was very low (see section 3.1.5).

6.6 LINSEED/FLAXSEED

There are 11 records of linseed consumption in the Adult Nutrition Survey and none in the Children's Nutrition Survey. The mean and maximum consumption amounts from these data are 8.6 and 28 g, respectively. There are too few data to determine robust percentiles.

The most common food source of linseed is as a component of mixed grain breads, usually in association with soy.

The Adult Nutrition Survey reports 138 records related to consumption of linseed-containing bread, with a mean, 97.5th percentile, and maximum of 75, 158, and 219 g, respectively. The Children's Nutrition Survey reports 25 records related to consumption of linseed-containing

[7] https://apps.who.int/foscollab/Download/DownloadConso Accessed 6 April 2022

bread, with a mean, 95th percentile, and maximum of 65, 130 and 172 g, respectively. Linseed-containing breads typically contain 7-8% linseed and a 75 g portion of linseed-containing bread would contain 5-6 g of linseed.

6.7 ELDERBERRY

The available resources do not contain any information on consumption of elderberries or elderberry-containing products.

The FAO/WHO CIFOCOss database contains a small number of records on elderberry consumption in Austria. Overall these data only represent four consumers and due to these small consumer numbers, they have not been summarised here.

It is possible that data on a similar small fruit, such as currants (black or red) could be used as a surrogate for elderberries. However, the Adult Nutrition Survey contains a single record related to consumption of redcurrants, none related to blackcurrants, and five related to currants, that appear to refer to dried fruit.

6.8 POME FRUIT AND POME FRUIT PRODUCTS

Most of the information on the CG content of pome fruit relate to the pome fruit seeds. There is no evidence of pome fruit seeds as an intentionally consumed food in New Zealand or internationally. There is little evidence of CGs in the flesh of pome fruit.

CGs are occasionally reported in pome fruit juices and have been detected in apple juices available in New Zealand (Saunders and Cressey, 2012).

The Adult Nutrition Survey reports 86 records related to consumption of apple juice or apple-based juice, with a mean, 97.5th percentile, and maximum of 313, 1020, and 2080 g, respectively. The Children's Nutrition Survey reports 25 records related to consumption of apple juice or apple-based juice, with a mean, 95th percentile, and maximum of 248, 343, and 1320 g, respectively.

6.9 ALMONDS AND ALMOND PRODUCTS

The Adult Nutrition Survey reports 65 records related to consumption of almonds, with a mean, 97.5th percentile, and maximum of 22, 86, and 206 g, respectively. The Children's Nutrition Survey reports five records related to consumption of almonds, with a mean and maximum of 11 and 24 g, respectively.

There is a single record of consumption of almond meal by an adult and no records of marzipan consumption for either cohort. It is probable that these foods have been used as ingredients in other food items.

There is no mention of almond milk in either data set. However, it is likely that this food will be more apparent in any future surveys. This is also likely to be true for almond meal, as this product is used in some gluten-free formulations.

6.10 APRICOT KERNELS AND APRICOT KERNEL PRODUCTS

No New Zealand-specific information was found on consumption of apricot kernels or apricot kernel products.

The FAO/WHO CIFOCOss database contains a single record of a single consumer of apricot kernels, for a Bulgarian male consuming 25 g.

FSANZ carried out dietary exposure assessment for HCN from apricot kernels based on two scenarios (4 or 32 kernels/day), rather than any food consumption data (FSANZ, 2014).

EFSA also noted the absence of food consumption data for apricots kernels and based their assessment on scenarios of 10 or 60 kernels/day, to represent the general population and cancer patients, respectively.

6.11 OTHER STONE FRUIT AND STONE FRUIT PRODUCTS

The literature information on CGs in other stone fruits related to the CG content of their kernels. There is no evidence that these are intentionally consumed in New Zealand. No additional information is available from the FAO/WHO CIFOCOss database.

For dietary exposure assessment to CGs in these products, a scenario-based approach, such as those used for apricot kernels may be appropriate.

6.12 BAMBOO SHOOTS

Somewhat surprisingly, the Adult Nutrition Survey contains no reports of consumption of bamboo shoots, while the Children's Nutrition Survey contains two records of consumption of 20 g of 'bamboo' (presumably bamboo shoots).

The FAO/WHO CIFOCOss database contains a number of entries for consumption of bamboo shoots, although for most non-Asian countries the numbers of consumers are very small. Consolidated (both genders, all age groups) data for the United States provides summary statistics for 510 consumers, with consumer mean and 97.5th percentiles of 5.8 and 18.7 g/day, respectively. Consolidated data for the Finnish population (35 consumers) gives a similar consumer mean consumption level of 5.2 g.

6.13 CHRONIC DIETARY EXPOSURE

Ideally, estimation of chronic dietary exposure considers the sum of all the long-term contributions to daily dietary exposure. The foods most commonly associated with cyanide intoxication following food consumption are not commonly consumed foods in New Zealand. Consequently, estimation of chronic dietary HCN exposure using population mean food consumption levels is unlikely to identify dietary situations of concern. Use of 24-hour dietary recall (24HDR) information from the national nutrition surveys has the potential to identify individual consumers with dietary habits that place them at greater risk of cyanide intoxication. However, food consumption information from this source will not include instances of consumption of items such as apricot kernel.

It is also worth noting that adverse health effects from chronic consumption of cyanogenic foods have not been reported in developed countries and have mainly been reported in regions of Africa, where a monotonous diet of cassava is found in association with a wide range of nutrient deficiencies.

7. REGULATIONS

Risks associated with cyanogenic plant materials and their products can be controlled by prohibitions on certain plant materials for use in the food supply or by applying maximum limits (MLs) for cyanogens in particular foods.

7.1 AUSTRALIA/NEW ZEALAND

The Australia New Zealand Food Standards Code includes both of the risk management measures mentioned above for control of cyanogens in the food supply. Specifically:

- *Standard 1.4.4 Prohibited and restricted plants and fungi* and the associated *Schedules 23* and *24* specify:
    - Cassava (*Manihot esculenta*), other than sweet cassava, is a prohibited plant. Sweet cassava is defined in *Standard 1.1.2* as "those varieties of cassava roots grown from *Manihot esculenta Crantz* of the *Euphoribiacae* family that contain less than 50 mg/kg of hydrogen cyanide (fresh weight basis)".
    - An exception for raw apricot kernels (*Standard 1.4.4-5*), such that they "may be used as an ingredient in a food for sale if the kernels have been or will be subject to processing or a treatment that renders them safe for human consumption". This exemption is related to *Standard 1.1.1 – 10(5)(f)* that prohibits retail sale of raw apricot kernels.
- *Standard 1.1.1 Contaminants and natural toxicants* and the associated *Schedule 19* specify MLs for hydrocyanic acid (HCN) for:
    - Confectionery 25 mg/kg
    - Stone fruit juices 5 mg/kg
    - Marzipan 50 mg/kg
    - Ready-to-eat cassava chips 10 mg/kg
    - Alcoholic beverages 1 mg per 1% alcohol content
- *Standard 1.2.6 – 2(c)* specifies labelling requirements for raw bamboo shoots and raw sweet cassava concerning preparation before consumption:
    - raw bamboo shoots—a statement indicating that bamboo shoots should be fully cooked before being consumed,
    - raw sweet cassava—a statement indicating that sweet cassava should be peeled and fully cooked before being consumed.

7.2 CODEX

Codex has also developed a range of risk management instruments for the control of HCN in the food supply. These include:

- *General Standard for Contaminants and Toxins in Food and Feed (CXS 193-1995)* contains MLs for cassava flour (10 mg/kg total hydrocyanic acid) and gari (2 mg/kg free hydrocyanic acid) (Codex Alimentarius Commission, 2016). Gari is the finished product obtained by artisanal or industrial processing of cassava tubers. The processing consists of peeling, washing and grating of the tubers, followed by

fermentation, pressing, fragmentation, granulation, drying if necessary, sifting, and suitable heat treatment (Codex Alimentarius Commission, 1995).

- *Code of practice for the reduction of hydrocyanic acid (HCN) in cassava and cassava products* (Codex, 2013). The code outlines procedure for the production of gari, fufu, dried cassava chips, and other cassava products.

7.3 EUROPEAN UNION

As a component of Commission Regulation (EC) No. 1881/2006 (EC, 2006), the European Union has developed an ML for HCN in apricot kernels, specifically for unprocessed, whole, ground, milled, cracked, chopped apricot kernels placed on the market for the final consumer. The ML is 20 mg/kg hydrocyanic acid, including hydrocyanic acid bound in cyanogenic glycosides.

The European Commission is currently considering additional MLs for linseed and derived products, almonds, and cassava, and cassava products.[8]

Similar to FSANZ Standard 1.1.1, Regulation (EC) No 1334/2008 governs the use of flavourings and food ingredients with flavouring properties in foods (EC, 2008b). The regulation also provides maximum levels of certain substances naturally present in flavourings and food ingredients with flavouring properties. A maximum level for HCN of 50 mg/kg has been established for nougat, marzipan or its substitutes, or similar products, of 5 mg/kg in canned stone fruits and of 35 mg/kg in alcoholic beverages.

Regulation (EC) No 110/2008 governs the definition, description, presentation, labelling, and protection of geographical indications of spirit drinks and establishes a maximum content of HCN of 7 g/hL of 100% volume alcohol (70 mg/L) in stone-fruit marc spirits and stone-fruit spirits (EC, 2008a).

7.4 CANADA

Division 15 of the Canadian Food and Drug Regulations[9] covering adulteration of food contains a list of contaminants and other adulterating substances in foods. The list specifies total extractable cyanide to not exceed 20 ppm (20 mg/kg) in apricot kernels for human consumption.

7.5 USA

No regulations relevant to CGs were found for the USA.

[8] https://ec.europa.eu/food/system/files/2021-09/reg-com_toxic_20210621_sum.pdf Accessed 15 March 2022
[9] https://laws-lois.justice.gc.ca/eng/regulations/C.R.C.%2C_c._870/page-1.html Accessed 11 April 2022

8. CONCLUSIONS

A wide variety of plants contains CGs. CGs can break down to produce HCN either through the action of enzymes present in the plant material or due to hydrolysis by microbial enzymes in the human gut.

Results from studies with potential relevance to the New Zealand food supply did not identify any high-HCN foods likely to be consumed in New Zealand, other than those previously examined (see section 1.1) (FSANZ, 2014). While high levels of HCN have been detected in the kernels of most species of stone fruit, these items are generally not considered to be food items in New Zealand. Similarly, high concentrations of HCN have been reported in pome fruit seeds, particularly apples. There is no evidence to suggest that these seeds are an intentionally consumed food item in New Zealand.

Taro leaves have previously been analysed for HCN in New Zealand and were found to not contain detectable HCN (Cressey and Saunders, 2010). Studies carried out in Africa suggest that the taro root can contain moderate concentrations of HCN (17-21 mg/kg), with one study suggesting markedly higher concentrations (Igbabul *et al.*, 2014).

Study reports indicate a substantial difference between the HCN contents of sweet and bitter almonds. The limited analyses previously carried out on almonds available at retail in New Zealand suggests these are probably from sweet cultivars (Cressey and Saunders, 2010). The presence of almonds from bitter cultivars in the New Zealand food supply would be a cause for concern.

While the impact of a number of food processing steps on the cyanogenic potential of various foods has been examined, it is likely that in most cases the reductions in cyanogenic potential reported are due to the interaction of CGs and β-glucosidase enzymes and the subsequent release of volatile HCN. These processes will be enhanced under conditions of moderate heat.

Most of the studies summarised in the current report indicate that food processing can decrease, but not completely remove, the cyanogenic potential of CG-containing foods.

The cyanogenic potential of foods can be determined indirectly by converting CGs to HCN or the CGs can be determined directly, usually by liquid chromatography (direct methods). The results of indirect methods align directly with current regulatory limits, while direct or indirect chromatographic methods offer greater sensitivity (lower LODs) and are useful for determining hydrocyanic acid levels less than 10 mg/kg.

Incidents of acute cyanide poisoning following consumption of plant material are mainly reported following consumption of stone fruit kernel, inadequately prepared (usually bitter) cassava, or bitter almonds. Despite the reasonably high concentrations of CGs in linseed, there have been no reported cases of intoxication following linseed consumption. It is likely that this is due to the structure of the CGs present in linseed and their likely greater resistance to microbial hydrolysis in the human gut.

It appears that, as long as cases of cyanide intoxication are treated within a few hours of consumption of the cyanogenic food, complete recovery is likely. However, fatalities have occurred.

The occurrence of chronic disease, such as konzo, appear to be restricted to developing countries and situations where monotonous consumption of a cyanogenic staple food (e.g. cassava) occurs in the presence of multiple nutrient deficiencies.

A limited range of risk management measures have been applied to control the risks associated with cyanogenic substances in the food supply. Maximum limits have been derived for HCN by Codex (cassava flour and gari), the European Union (apricot kernels and specified flavouring substances) and Australia/New Zealand (RTE cassava chips and specified flavouring substances).

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