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DISCUSSION PAPER ON CYANOGENIC GLYCOSIDES

Prepared by the Electronic Working Group members (Australia, New Zealand, Netherlands, Brazil, European Community, Indonesia, Denmark, Ghana, Thailand, Tonga, Vanuatu, FAO, WHO and Nigeria).

Scope

1. This discussion paper seeks to provide an overview of the available data on cyanogenic glycosides. The paper focuses on provision of general information on cyanogenic glycosides, levels that have been reported in foods, public health and safety issues both chronic and acute, terminology, methods of analysis and the regulatory status of cyanogenic glycosides in food. The specific intent of the discussion paper is to gather and consider what data are currently available, in order that JECFA could re-consider these data and advise on public health implications in regard to cyanogenic glycosides in food. Were JECFA to re-evaluate cyanogenic glycosides, CCCF would be in a position to consider appropriate risk management options in regard to cyanogenic glycosides in food.

Background

2. The Codex Alimentarius Commission (CAC) concurred with the recommendation of the 59th Session of the Executive Committee[1] to adopt the Proposed Draft Standard for Bitter Cassava, as elaborated by the Committee on Fresh Fruits and Vegetables (CCFFV)[2], at Step 5 and that, as a separate issue, the Committee on Contaminants in Foods (CCCF) should consider the safety levels of hydrogen cyanide (HCN) proposed in the Standard, with a view to a re-evaluation of cyanogenic glycosides (CG) by JECFA.

3. The CAC (4 July 2008) sent the draft Standard for Bitter Cassava back to CCFFV for further work (step 6) on the labelling and processing of bitter cassava due to the recognised safety concerns if cassava is consumed without adequate processing, with a view to referral to the Codex Committee on Food Labelling (CCFL) for re-consideration.

4. The proposed levels for HCN are indicated in footnote 2 of the Draft Standard for Bitter Cassava and are as follows *in italic*:

[Bitter varieties of cassava are those that contain more than 50 mg/kg but less than 200 mg/kg HCN (fresh weight basis). In any case, cassava must be peeled and fully cooked before being consumed.]

[1] Alinorm 07/30/3 para.30
[2] Alinorm 07/30/35 paras 73-82 and Appendix VI

5. The CCCF at its Second Session held in April 2008 considered the need for a re-evaluation of cyanogenic glycosides by JECFA.

6. Pivotal to considerations on the safety of bitter and sweet cassava is whether the current preparation instructions are adequate to ensure safe consumption of these foods. It is unclear what level of processing is required for different initial levels of cyanogenic glycosides in bitter cassava. For example, it is not clear to what extent, following peeling and cooking, additional preparation techniques are necessary to adequately reduce the risk for cassava with 50 mg/kg HCN (fresh weight basis) compared with 200 mg/kg HCN (fresh weight basis).

7. The CCCF noted that potential excessive dietary exposure to cyanogenic glycosides, mainly from cassava but also from other products, was assessed at the 39th Meeting of JECFA (1992) and that, due to lack of quantitative toxicological and epidemiological information at that time, JECFA could not conclude on a safe level of dietary exposure for this naturally occurring toxicant. However, JECFA (WHO 1993) had also concluded that a level of up to 10 mg/kg HCN in the Standard for Edible Cassava Flour (CODEX STAN 176-1989) was not associated with acute toxicity. A review of the available data by the European Food Safety Authority in 2004 reached a similar conclusion.

8. There are a number of FAO publications addressing good agricultural and manufacturing practices for the growing and processing of cassava, including other ongoing work in this field, to assist countries with the cultivation, processing and handling of this product[3]. This information should be taken into account if a Code of Practice or an ML is considered necessary for cyanogenic glycosides in the future.

9. The CCCF agreed that an electronic working group (EWG), led by Australia, prepare a Discussion Paper which should include an overview of available data on cyanogenic glycosides with a view to possible re-evaluation by JECFA.

10. This discussion paper focuses on three key aspects that need addressing before CCCF could consider risk management options in regard to HCN and cyanogenic glycosides in food:

- the potential health consequences of HCN and substances in foods that may result in dietary exposure to HCN (i.e. cyanogenic glycosides and cyanohydrins). In particular the acute and chronic dietary risk for consumers;
- the current inconsistent regulatory limits and terminology for cyanogenic glycosides and cyanohydrins in food, including in existing and proposed Codex standards; and
- the need for JECFA to review limits for cyanogenic glycosides and cyanohydrins following consideration of all currently available data.

Introduction

General information on cyanogenic glycosides

11. Cyanogenic glycosides occur in at least 2000 plant species of which many are used in food. The major cyanogenic glycosides foods used for human consumption are summarised below:

Cyanogenic glycoside	Common name	Latin name
Linamarin	Cassava Lima beans	*Manihot esculenta* *Manihot carthaginensis* *Phaseolus lunatus*

[3] Alinorm 08/31/41
http://www.fao.org/docrep/006/y0169e/y0169e00.htm
http://www.fao.org/docrep/009/a0154e/a0154e00.htm
http://www.fao.org/docrep/009/y1177e/y1177e00.htm
ftp://ftp.fao.org/docrep/fao/007/y5271e/y5271e00.pdf
ftp://ftp.fao.org/docrep/fao/007/y2413e/y2413e00.pdf
http://www.fao.org/docrep/007/y5287e/y5287e00.htm
ftp://ftp.fao.org/docrep/fao/007/y5548e/y5548e00.pdf
http://www.fao.org/docrep/009/x4007e/x4007e00.htm

Dhurrin	Sorghum	*Sorghum album* *Sorghum bicolor*
Amygdalin	Almonds	*Prunus amygdalus*
Lotaustralan	Cassava Lima beans	*Manihot carthaginensis* *Phaseolus lunatus*
Prunasin	Stone fruits	*Prunus species (P. avium, P.padus, P. persica, P. macrophyl*
Taxiphyllin	Bamboo shoots	*Bambusa vulgaris*
Linustatin	Flax seed Cassava	*Linum usitatissimum* *Information needed?*
Neolinustatin	Flax seed	*Linum usitatissimum*
Sambunigrin	Elderberries	*Sambucus nigra*

Cassava is also known by the other common names: manioc, manihot, and yucca. Cassava originates in Latin America and was later introduced into Asia and Africa (FSANZ 2004).

13. While there are approximately 1200 species of bamboo, only a few have been used as human food in Asia and Australia (personal communication from Australian Commercial Bamboo Corporation Ltd. In 2004). Those currently used in Australia that Food Standards Australia New Zealand (FSANZ) is aware of include:

- *Dendrocalamus asper;*
- *Dendrocalamus latiflorus;*
- *Bambusa oldhamii; and*
- *Phyllostachys pubescens.*

Dendrocalamus apser is the most important species in for shoot production in Thailand while *Dendrocalamus latiflorus* and *Bambusa oldhamii* are the most important in Taiwan. The different bamboo species also have different levels of cyanide (FSANZ 2004).

http://www.foodstandards.gocv.au/_srcfiles/Cyanogenic%20glycosides.doc

14. Cyanogenic glycosides (CG) may be defined chemically as glycosides of the α-hydroxynitriles and are secondary metabolites produced by plants. They are amino acid-derived plant constituents. The biosynthetic precursors of the CGs are different L-amino acids. These amino acids are hydroxylated, then the *N*-hydroxylamino acids are converted to aldoximes which are in turn converted into nitriles and hydroxylated to α-hydroxynitriles and then glycosylated to CGs (Vetter, 2000; in FSANZ, 2004). All known CGs are β-linked, mostly with D-glucose. There are at least 2650 species of plants that produce CGs and usually a corresponding hydrolytic enzyme (beta-glycosidase). The enzyme and CG are brought together when the cell structure of the plant is disrupted with subsequent breakdown to saccharide and a cyanohydrin. The cyanohydrin can then rapidly decompose to HCN and an aldehyde or a ketone (Hosel, 1981; Moller and Seigler, 1999; in FSANZ, 2004).

15. Linamarin and to a lesser degree, lotaustralin are the major cyanogenic glycosides in cassava. Under appropriate conditions, linamarin is converted to acetone cyanohydrin and glucose, and the acetone cyanohydrin decomposes to form acetone and hydrocyanic acid. Under appropriate conditions, lotaustralin is converted to butanone cyanohydrin and glucose, and the butanone cyanohydrin decomposes to form butanone and hydrocyanic acid.

16. The major cyanogenic glycosides found in the edible parts of plants and their structures are summarized in Table 1 (**Attachment 1**; Cheeke, 1989; WHO, 2003; EFSA, 2004). The sources of some flavouring substances contain cyanogenic glycosides, including amydgdalin, sambunifrin and prunasin (EFSA, 2004).

17. The release of HCN from cyanogenic glycosides can occur as a result of enzymatic hydrolysis by β-3-glucosidases following maceration of the plant tissue, or by the gut microflora (WHO, 1993; EFSA 2004).

Throughout this discussion paper the terms 'total hydrogen cyanide (HCN)' and 'total hydrocyanic acid' will be used to describe the hydrogen cyanide from cyanogenic glycosides, cyanohydrins and 'free' hydrogen cyanide.

Regulation of levels of cyanogenic glycosides in food

18. The Codex Alimentarius Commission has developed and published standards for Sweet Cassava, Edible Cassava Flour and Gari (a product obtained from processing cassava tubers) (also spelt as 'garri').

The key aspects of these standards are:

- sweet cassava is defined as a raw product containing less than 50 mg/kg of 'hydrogen cyanide';
- edible cassava flour is defined as a product suitable for direct human consumption and the level of 'total hydrocyanic acid' in the flour must not exceed 10 mg/kg; and
- for gari, another product for direct human consumption, the 'total hydrocyanic acid' must not exceed 2 mg/kg as 'free' hydrocyanic acid.

Australia and New Zealand

19. The Australia New Zealand Food Standards Code (the Code) includes a prohibition on the sale of cassava other than 'sweet cassava' (Standard 1.4.4 – Prohibited and Restricted Plants and Fungi). Consistent with the existing Codex standard, sweet cassava' is defined in the Code (Standard 1.1.2 – Supplementary Definitions for Foods) as 'those varieties of cassava roots grown from *Manihot esculenta Crantz* of the *Euphoribiacae* family that contain less than 50 mg per kg of HCN (fresh weight basis)'. The Code includes a requirement for raw sweet cassava to be labelled or accompanied by a statement indicating that sweet cassava should be peeled and fully cooked before being consumed (Standard 1.2.6 – Directions for Use and Storage). Standard 1.2.6 also includes a requirement for bamboo shoots to be fully cooked before being consumed. Documentation describing the development of these food regulatory measures for cassava and bamboo shoots is available via the following link: http://www.foodstandards.gov.au/standardsdevelopment/proposals/proposalp257preparationofcassava21august2002/index.cfm

20. The Code also includes the following levels for hydrocyanic acid in the following foods to which flavourings have been added: 25 mg/kg in confectionery; 5 mg/kg in stone fruit juices; 50 mg/kg in marzipan; 1 mg/kg per 1% alcohol in alcoholic beverages.

European Union

21. In the EU, Annex II of Directive 88/388 on flavourings sets the following maximum permitted levels of HCN in foodstuffs and beverages to which flavourings or other food ingredients with flavouring properties have been added: 1 mg/kg in foodstuffs, 1 mg/kg in beverages, with the exception of 50 mg/kg in nougat, marzipan or its substitutes or similar products, 1 mg per percent of alcohol in alcoholic beverages and 5 mg/kg in canned stone fruit (EEC 1998).

22. In the UK the ML of cyanide/hydrocyanic acid is regulated under the terms of *The Flavourings in Food Regulations 1992*. Otherwise the cyanide/hydrocyanic acid content of food is not specifically regulated except under the terms of the Food Safety Act 1990 which make it an offence to sell or possess for sale food which is injurious to health.

Public health and safety

23. The toxicity of a cyanogenic plant depends primarily on the potential that its consumption will produce a concentration of HCN that is toxic to exposed humans. If the cyanogenic plant is inadequately detoxified during processing or preparation of the food, the CG in the food can be toxic. If the cyanogenic plant is consumed directly, the beta-glycosidase can be released and is then active until the low pH of the stomach deactivates the enzyme, releasing at least some of the HCN from the CG. It is possible that part of the enzyme fraction can be reactivated in the alkaline environment of the gut releasing more HCN from the CG (WHO, 1993). In regard to cassava, previous reports have suggested that ingestion of poorly processed cassava roots is associated with the incidence of ataxic

neuropathy (konzo) in African countries. In addition, the negligible protein and essential amino acid content (particularly sulphur-containing amino acids) in cassava is also known to impair cyanide detoxification mechanisms in the body (Teles, 2002).

24. There are limited studies undertaken on the toxicity of cyanogenic glycosides since the evaluation by JECFA at its 39th meeting in 1993. An overview of the key toxicological studies on HCN and cyanogenic glycosides is detailed in **Attachment 2**. The primary toxicological endpoint of concern for acute HCN exposure is inhibition of mitochondrial oxidation via HCN shutting down the electron transport chain of the inner membrane of the mitochondria (Cheeke, 1989). The cyanide ion inhibits enzymes associated with cellular oxidation and causes death through energy deprivation. The symptoms, which occur within a few minutes, may include headache, nausea, vomiting, giddiness, palpitations, hyperpnoea then dyspnoea, bradycardia, unconsciousness and violent convulsions, followed by death (EFSA 2004).

25. The chronic uptake of hydrocyanic acid, in sub-acutely toxic doses, may be involved in the pathogenesis of certain conditions including disturbance of thyroid function and neuropathies. Human cassava-eating populations show ophthalmological and neurological symptoms which are associated with exposure to hydrocyanic acid, though it is likely that other nutritional or metabolic deficiencies affecting the cyanide detoxification mechanism are also involved (e.g. sulphate and zinc deficiencies). Several epidemiological studies in cassava-eating populations, which established an association between cyanide exposure and spastic paraparesis, amblyobia ataxia or tropical ataxia neuropathy (TAN) and possibly goitre have also been considered. However, the data are highly confounded by other nutritional and environmental factors. Suitable long-term toxicity studies in animals fed a diet containing hydrocyanic acid or linamarin are lacking (JECFA, 1993; Abuye et al, 1998; Teles 2002).

26. Overall, the database for the toxicity of hydrocyanic acid and cyanogenic glycosides is incomplete and limited particularly with respect to chronic intake although there may be sufficient data to establish an acute reference dose (ARfD).

Terminology

27. The cyanogenic glycoside content of foods is often reported as mg/kg of HCN in the food. This reflects the 'total hydrocyanic acid' content of the food which is often determined by measuring the HCN evolved following enzyme or acid hydrolysis of the cyanogenic glycoside and related cyanohydrins. Some texts refer to 'total hydrocyanic acid' as the 'cyanogenic potential' of a food or as 'bound' or also as 'free and combined' hydrocyanic acid.

28. The term 'HCN' is the term recommended by the International Union of Pure and Applied Chemists for hydrogen cyanide. The Codex Standards for 'sweet' and 'bitter' cassava refer to levels of 'hydrogen cyanide' on a raw food or 'fresh weight' basis. However, other Codex Standards refer to 'total hydrocyanic acid' (Edible Cassava Flour) and 'total hydrocyanic acid' 'determined as free hydrocyanic acid' (Gari). The Codex General requirements for Natural Flavourings refer to 'total hydrocyanic acid (free and combined)'. Therefore, even in Codex standards, there does not appear to be a term that is used consistently to describe the total HCN including hydrogen cyanide from all the cyanogenic glycosides.

29. Australia has recently considered terminology as part of the work undertaken in relation to total HCN in ready-to-eat cassava chips[4].

http://www.foodstandards.gov.au/_srcfiles/P1002%20Hydrocyanic%20acid%20in%20cassava%20chips%20AppR%20FINAL.pdf#search=%22p1002%22

The following definition was considered appropriate for 'hydrocyanic acid, total' for ready-to-eat cassava chips to assist in facilitating compliance monitoring of HCN:

> **Hydrocyanic acid, total** means any hydrocyanic acid including hydrocyanic acid evolved from linamarin, lotaustralin, acetone cyanohydrin and butanone cyanohydrin during or following

[4] ready-to-eat cassava chips' are those foods which contain cassava and are represented as snack foods suitable for consumption in the same state in which they are sold, i.e. with no further preparation and ready for immediate consumption. These foods are often represented as 'chips', 'crisps', 'crackers', 'vege crackers' or with other snack food terms. This term does not include processed cassava foods which would not be considered snack foods such as desserts e.g. tapioca pudding.

either enzyme hydrolysis or acid hydrolysis, expressed as milligrams of hydrocyanic acid per kilogram of ready-to-eat cassava chips.

Analytical methods for detection of cyanogenic glycosides

30. Maximum levels for total HCN in certain foods are prescribed in specific regulations in some countries. Some of these regulations have been in place for many years.

31. There are published methods that could be effectively used for measuring total HCN in foods (EFSA 2007). Current methods used to detect HCN in place consist of the following:

- Colorimetry (Essers et al. 1993);
- Gas chromatography (Murphy et al., 2006; Shibata et al., 2004);
- Picrate paper kits (Yeoh and Sun, 2001);
- Biosensors (Mak et al., 2005; Keusgen et al., 2004); and
- Fluoremetry (Sumuyoshi et al., 1995; Sano et al., 1992)

32. Although the acid hydrolysis method is applicable to all plants, it has been suggested that it is much more difficult to use and less accurate compared to the picrate method which may allow better determination of the total HCN content of any plant and food (Haque and Bradbury 2002). A new method is being developed by CEN/TC327 committee Animal Feeding stuffs, which will replace the method described in EC regulation 71/250/EEC.

Levels of cyanogenic glycosides in foods

33. A range of levels of total HCN in edible plants and in food ingredients with flavouring properties have been reported in the literature (WHO, 1993; EFSA, 2004; **Attachment 3**) and these levels can vary depending on the processing methods employed. Caution should be exercised in interpreting results for total HCN in foods as it has been reported that some analytical methods may not always ensure complete hydrolysis of the CG and cyanohydrins.

Cassava

34. Cassava grows well in a tropical climate and is eaten in Africa, Pacific Island Countries, South America and regions of Asia including Indonesia (Knudsen et al 2005). Cassava is consumed in a number of forms: flour, root slices, grated root (baked, steamed or pan fried), steamed whole root and tapioca pearls made as a pudding (Knudsen et al 2005). Processed cassava is one of the major staple foods consumed by the population of the Democratic Republic of Congo (DRC) and may provide more than 60% of the daily energy requirements (Ngudi et al 2002).

35. There are a number of varieties of cassava, each of which has varying total HCN levels according to the altitude, geographical location and seasonal and production conditions (Oluwole et al 2007). In drought conditions there is an increased total HCN content due to water stress (Cardoso et al 2005). Values from 15-400 mg/kg fresh weight of total HCN in cassava roots have been reported in the literature (FSANZ 2004), although there are reports of even higher levels[5] (Oluwole et al, 2007; Cardoso et al 2005) depending on location of the crops. Sweet varieties of cassava (low total hydrocyanic acid content) will typically contain approximately 15-50 mg/kg total HCN on a raw food or 'fresh weight' basis. Sweet varieties of cassava can be processed adequately by peeling and cooking (e.g. roasting, baking or boiling), whereas bitter varieties of cassava (high total HCN content) require more extensive processing, involving techniques such as heap fermentation which take several days (FSANZ 2004). Samples of processed cassava roots (referred to as 'cossettes') from a range of markets in the DRC were found to have total HCN levels <10 mg/kg (Ngudi et al, 2002). Bitter varieties have been reported as not normally being commercially traded (Knudsen et al 2005); however, due to cassava now being a critical staple crop in a number of countries, it is likely that there is increased trade in varieties of bitter cassava.

36. During the processing of manioc flour, the cyanide content was assessed (Chiste et al 2005). In the peeled roots, grated roots, pressed mass and flour the total cyanide content were 154.40, 167.68,

[5] http://online.anu.edu.au/BoZo/CCDN/one.html

66.59, 5.19 mg HCN/kg, respectively. One reason for the cyanide content decreasing from the roots to the flour was due to the heating process employed during the processing phase.

37. Total cyanide ranged from 55.58 to 157.17 mg HCN/kg in "tucupi" (a liquid product from a fermentation of bitter cassava pressed mass). Sixty percent (60%) of the samples contained more than 100mg/kg of HCN, however this product is subject to a high temperature before consumption by humans (Chiste et al, 2007).

38. "Multimistura" is composed of 5% cassava leaves, bran of wheat and rice, corn and wheat flours and other ingredients. This food is used as a complement to other food for children with malnutrition. The total cyanide content in cassava leaves powder was 85 mg/kg. This concentration is obtained when the leaves are broken and kneaded before the drying process (Helbig et al, 2008). According to Brazilian legislation, the maximum level of HCN permitted in "Multimistura" is 4mg/kg. (Brazil, 2000)

Bamboo

39. Bamboo shoots may contain as much as 1000 mg/kg total HCN, which is generally higher than the amounts reported in cassava tubers, however, the total HCN content is reported to decrease substantially following harvesting. The bamboo shoots sold commercially as food can be processed adequately by boiling before consumption (FSANZ 2004).

Linen flax

40. Typical levels of total HCN mg/kg in linen flax (flax, linseed (*Linum usitatissimum* L.) may not be greater than 500 mg/kg as previously suggested (refer to **Attachment 3**). A German study on 48 samples of 11 varieties reported a level between 217-541 mg/kg (Schilcher & Wilkens-Sauter 1986). Haque & Bradbury (2002) has found levels varying from 140-370 mg/kg in samples from Australia, New Zealand and Canada. Oomah et al (1992) have reported on HCN levels in seeds from Canada varying from 266-363 mg/kg and Wanasundara et al. (1999) have reported on a level of 373 mg HCN/kg seed in a single sample of seeds from Canada.

41. The major glycosides reported in linseed are not linamarin but the diglycosides linustatin and neolinustatin (Niedzwiedz-Siegien 1998, Schilcher & Wilkens-Sauter 1986**).** However, Omah et al. (1992) reported small amounts of linamarin in 8/10 samples of linseed and Schilcher & Wilkens-Sauter (1986) found traces of linamarin in some samples.

Processing of foods to reduce total HCN

42. Proper processing of cyanogenic glycoside-containing foods will reduce the risk to consumers. In regard to cassava, the level of total HCN depends on the variety of cassava tuber, the growing conditions and the methods of processing. The relative level of each cyanogenic component in turn depends on the cyanogenic reaction pathway at the different stages of process, as illustrated in Figure 1. Cyanogenesis is initiated when the plant tissue is damaged. If any of the processing steps do not take place or are interrupted, the final cassava may contain unacceptably high levels of total HCN. For more detail refer to **Attachment 4**.

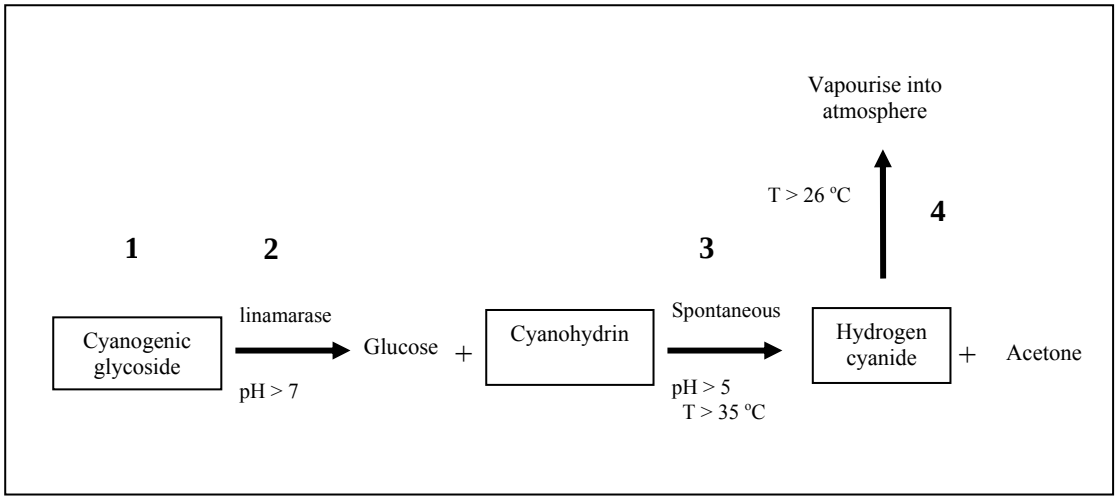


Figure 1: Cyanogenesis reaction pathway and steps in cassava processing. 1: nature of tuber; 2: grating/rasping, soaking, fermentation; 3: sun/oven drying; 4: sun drying, hot manufacturing process (steaming, frying).

43. Processing methods generally adopted for cassava include peeling, soaking, fermentation, boiling or cooking, drying, and pounding/millings (Padmaja 1995). However, the different varieties of cassava have been found to have different cyanogen elimination profiles during the cooking of cassava roots. There also appears to be differences in heat stability of β-glucosidase activity of cassava roots which protects the enzyme from total deactivation during cooking (Ravi and Padmaja 1997). 'Cossettes' (processed cassava roots) one of the most popular cassava product in the Democratic Republic of Congo are processed by soaking or immersing fresh bitter cassava roots (whole or peeked) in a stream or stationary water for at least 3 days to allow them to ferment until they become soft. The fermented roots are then taken out, peeled and sundried on racks, roofs of houses which can take from 2-5 days (Hahn; cited in Ngudi et al 2002).

44. It has been suggested that sun drying and heap fermentation were inadequate to reduce HCN levels in cassava in the Nampula Province of Mozambique to the WHO level of 10 ppm (Cardoso et al 2005). Reductions in HCN levels in cassava have been achieved by thoroughly mixing cassava flour with water and allowing the cassava to stand in an open vessel for 5h before cooking, although this method is dependent on there being an adequate amount of linamarase in order for breakdown of linamarin to occur (Bradbury 2006).

45. The drying process seems to reduce the level of cyanide in addition to affecting the enzyme activity. Drying in the sun was more effective at decreasing the level of cyanide when compared to oven drying to 60º C (82 to 94% versus 68 to 76%, respectively). It was observed that most of the cyanide present in the foliage drying in the sun was composed of free cyanide (62 to 77%), while the foliage dried at 60 ° C; there was only 24 to 36% of free cyanide (Gómez and Valdivieso 1985). Another study evaluated the effect of three drying temperatures (45ºC, 60°C and 75°C) on the level of cyanide from leaves of five varieties of cassava. It was noted that the lowest levels were found in dried leaves to 60^{oC}, ranging from 7.7 to 15 mg/100 g of dry matter (Padmaja, 1989).

CONCLUSIONS

46. There is a wide range of reported levels of cyanogenic glycosides and their derivatives in edible plants and foods derived from these plants. The toxicity of cyanogenic glycosides and their derivatives is dependent on release of HCN from plant tissue or the action of gut microflora in animals or humans. JECFA concluded that a level of up to 10 mg/kg HCN in the Standard for Edible Cassava Flour (CODEX STAN 176-1989) was not associated with acute toxicity (WHO, 1993). A review of

the available data by European Food Safety Authority (EFSA Journal) in 2004 reached a similar conclusion.

47. Due to lack of quantitative toxicological and epidemiological information at that time, JECFA in 1993 could not establish a safe level of intake for cyanogenic glycosides. The database for the toxicity of hydrocyanic acid and cyanogenic glycosides is incomplete and limited, particularly with respect to chronic intake. Reviews by other regulatory bodies (UK Committee on Toxicity and EFSA) have suggested that there is no suitable long-term study in animals or humans treated with either HCN or cyanogenic glycosides to establish a PTDI. Therefore, it would be appropriate that further quantitative data be sought on chronic exposure in animals or humans with either HCN or individual cyanogenic glycosides with a view to establishing a PTDI. Further information is also needed on toxicokinetics of cyanogenic glycosides. Given the potential acute public health implications of cyanogenic glycosides and their metabolites, it would be appropriate to compile and review the available data on the toxicity of cyanogenic glycosides and update the 1993 JECFA toxicological evaluation.

48. The toxicity associated with cyanogenic glycosides and their derivatives in food can be reduced by appropriate preparation of the plant material prior to consumption. However, it is unclear what measures would be sufficient to reduce the potential for toxicity in humans following consumption of cyanogenic glycosides, particularly foods that may contain high levels e.g. bitter cassava, bitter almonds and apricot kernels. For this reason, it would be appropriate to compile the available preparation and processing measures and consider the need for a Code of Practice for the production and processing of foods containing cyanogenic glycosides, including for specific foods.

49. In order to allow a thorough consideration of the public health implications associated with total HCN in foods, data on total HCN in foods should be compiled along with the analytical methods used to generate these data. To complement these data, information on the regulatory limits that apply in specific countries or regions would also enable the data on total HCN in foods to be interpreted.

50. Due to the lack of a universally acceptable term for total HCN, it is necessary to consider whether there is a need for an appropriate descriptor for total HCN in food, including the cyanogenic glycosides and cyanohydrins. This would include consideration of the basis for the descriptor and the form of the food to which the descriptor should apply e.g. ‘as is’ or ‘dry weight basis’, ‘raw food basis’ or ‘fresh weight basis’. This would involve considering the different terms that are used for reporting cyanogenic glycosides, cyanohydrins and hydrogen cyanide in foods and determining if a universally acceptable term can be developed. This should then prompt a review of the existing terms used in relevant Codex standards to address the current inconsistencies in these standards.

51. To facilitate consistent monitoring of total HCN in food, information on the methods used to monitor total HCN in food should be compiled and considered. This could form part of the considerations in any future Code of Practice or maximum level for cyanogenic glycosides in food.

RECOMMENDATIONS

52. As a result of the request from the CCCF to consider the available data on cyanogenic glycosides, and the recent issues in regard to ready-to-eat cassava chips in Australia, the EWG recommends the following:

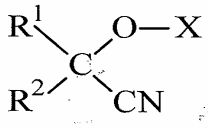
- JECFA is requested to re-consider the data available on cyanogenic glycosides and advise on the public health implications of cyanogenic glycosides and their derivatives in food. In particular, whether there are sufficient data to establish an appropriate health standard, such as an acute reference dose or tolerable daily limit, for cyanogenic glycosides or their derivatives present in food.
- JECFA to consider whether or not the current level of up to 10mg/kg HCN in the Standard for Edible Cassava Flour is still an appropriate level which is not associated with acute toxicity, and whether this level would be applicable to any other HCN-containing food.
- JECFA to consider what levels of these cyanogenic glycosides and their derivatives may be appropriate in food, including levels that are appropriate to minimise any risks to public health from the consumption of foods containing cyanogenic glycosides and their derivatives.
- JECFA to consider what an appropriate descriptor for total HCN in food could be.

- Taking into account any assessment by JECFA, that CCCF consider developing a Code of Practice for producing, processing and marketing of foods which may contain cyanogenic glycosides or their derivatives. In consultation with CCFL, this would also include whether further information requirements are necessary for these foods to ensure adequate processing of cyanogenic glycoside-containing foods by consumers before consumption.
- Following receipt of any risk assessment advice from JECFA, CCCF and CCMAS should review the current relevant Codex Standards to ensure these standards are consistent in relation to any limit for cyanogenic glycosides and their derivatives in food

Attachment 1

HCN

Table 1. General structure of cyanogenic glycosides



Name	Formula Mol. mass CAS-number	R^1	R^2	X	Configuration	Occurrence*
Amygdalin	$C_{20}H_{27}NO_{11}$ 457.4334 29883-15-6	Phenyl	H	Gentiobiose	R	Almonds, Peach, Apricot, Prune, Cherry, Apple & Quince kernels
Linamarin	$C_{10}H_{17}NO_6$ 247.2474 554-35-8	Methyl	Methyl	Glucose	-	Cassava, Lima bean, (Flax seed)
Prunasin	$C_{14}H_{17}NO_6$ 295.29 99-18-3	Phenyl	H	Glucose	R	Ferns, e.g. Bracken fern, Rowanberries
Linustatin	$C_{16}H_{27}NO_{11}$ 409.39 72229-40-4	Methyl	Methyl	Gentiobiose	-	Flax seed, Cassava
Lotaustralin	$C_{11}H_{19}NO_6$ 261.272 534-67-8	Methyl	Ethyl	Glucose	R	Lima bean, (Cassava), (Flax seed)
Neolinustatin	$C_{17}H_{29}NO_{11}$ 423.42 7229-42-6	Methyl	Ethyl	Gentiobiose	R	Flax seed
Sambunigrin	$C_{14}H_{17}NO_6$ 295.29 138-53-4	Phenyl	H	Glucose	S	Elderberries
Taxiphyllin	$C_{14}H_{17}NO_7$ 311.29 21401-21-8	p-Hydroxy-phenyl	H	Glucose	R	Bamboo shoot
Dhurrin	$C_{14}H_{17}NO_7$ 311.29 499-20-7	p-Hydroxy-phenyl	H	Glucose	S	Durra, (Sorghum)

* minor sources are indicated between parenthesis

On hydrolysis, one gram of the respective cyanogenic glycosides can liberate the following quantities of HCN: amygdalin, 59.1 mg HCN (equivalent to 56.9 mg CN^-), linamarin 109.3 mg HCN (equivalent to 105.2 mg CN^-) and prunasin 91.5 mg HCN (equivalent to 88.1 mg CN^-).

Attachment 2

TOXICOLOGY

Australia recently reviewed the toxicology of HCN and specific cyanogenic glycosides in light of high levels of HCN detected in Australian ready-to-eat cassava chips. http://www.foodstandards.gov.au/_srcfiles/P1002%20Hydrocyanic%20acid%20in%20cassava%20chips%20AppR%20FINAL.pdf#search=%22cassava%20chips%22

After oral administration HCN is readily absorbed and rapidly distributed in the body and is known to combine with iron in both methaemoglobin and haemoglobin present in erythrocytes (WHO, 1993; USEPA, 1993; EFSA, 2004). Following oral administration, a proportion of ingested linamarin is absorbed and excreted unchanged in the urine (Barrett *et al.*, 1977; Hernandez *et al.*, 1995; Carlsson *et al.*, 1999).The remainder is enzymatically converted to hydrocyanic acid by micro-organisms in the gastrointestinal tract (Frakes *et al.,* 1986a; Carlsson *et al.*, 1999). The hydrocyanic acid absorbed from the gut is metabolically converted to the less toxic thiocyanate (Carlsson *et al.*, 1999). Other detoxification pathways include combination with vitamin B12 or some sulphur-containing amino acids (Askar, 1983; Ludwig *et al*., 1975; Freeman, 1988). Acute toxicity results when the rate of absorption of hydrocyanic acid is such that the metabolic detoxification capacity of the body is exceeded.

The primary toxicological endpoint of concern for HCN is inhibition of mitochondrial oxidation, which, if the level of exposure to HCN exceeds the capacity of normal physiological detoxification mechanisms, may rapidly lead to death (Gettler & Baine, 1938; WHO, 1993; NTP, 1993; EFSA, 2004). Clinical manifestations of acute cyanide poisoning, especially non-lethal doses, are often non-specific and mainly reflect those of oxygen deprivation of the heart and brain. Typically these effects include headaches, dizziness, stomach pain, or mental confusion (Montgomery, 1969; Gosselin *et al*., 1976). As these symptoms closely resemble that of over indulgence or mild GIT disturbance, and the dose response curve is steep, individuals exposed to high levels of HCN may not recognise warning symptoms before consuming a lethal dose. This is likely to be particularly true for young children (Geller *et al*, 2006). Death in humans has been reported from HCN doses as low as 0.58 mg/kg bw.

HCN is a normal component of mammalian physiology, and efficient mechanisms for its detoxification are present. Cyanide clearance is very rapid and its half life is short, 14 minutes in rats. For acute toxicity the maximum systemic exposure (C_{max}) is the primary determinant of toxicity rather than the average exposure over a period of time (AUC). An individual consuming a near lethal dose over a few minutes for example would have normal background levels of blood cyanide within approximately 2 hours (around 6 half lives). Thus, the appropriate toxicological reference value must reflect acute rather than cumulative toxicity, and dietary exposure must reflect intakes at a single sitting rather than averages over longer periods.

The acute toxicity of hydrocyanic acid in mouse, rat, rabbit and dog are quite similar with the median oral lethal doses (50% death) estimated to be 3-4 mg cyanide/kg bw in rats and rabbits. In dogs the median lethal dose was 2 mg cyanide/kg bw whereas in mice it was 6 mg cyanide/kg bw with potassium cyanide (Conn, 1979). Based on analyses of cyanide contents in tissues and in gastrointestinal tract contents from fatal poisoning cases (and comparative kinetics with dogs), Gettler & Baine (1938) estimated that death in cases of suicide occurred after absorption of an average of 1.4 mg hydrocyanic acid/kg bw; the lowest fatal absorbed dose was 0.58 mg hydrocyanic acid/kg bw. However, the oral lethal dose of hydrocyanic acid in the four cases of suicide reported by Gettler & Baine which were calculated from the total amount of hydrocyanic acid absorbed in the body at the time of death, and from the amount of hydrocyanic acid found in the digestive tract, differed considerably (calculated as mg hydrocyanic acid): 1450 (62.5 kg bw), 556.5 (74.5 kg bw), 296.7 (50.7 kg bw), and 29.8 (51 kg bw). This corresponds to doses varying from 0.58 mg/kg bw to 23 mg/kg bw.

Indications of teratogenicity in offspring from hamsters treated with 120 or

140 mg/kg bw linamarin (equivalent to 13.1 and 15.3 mg hydrocyanic acid/kg bw, respectively) on day 8 of gestation were only observed at maternally toxic doses (Frakes *et al*, 1986b).

Experimental data on chronic toxicity and carcinogenicity are not available. Overall, the mutagenicity tests conducted with hydrocyanic acid and cyanides at gene and/or chromosome level did not reveal a genotoxic potential (De Flora, 1981, cited in EPA, 1993; Kushi *et al*., 1983, cited in EPA, 1993; Leuschner *et al*, 1989). The US EPA considers that it is not classifiable as a carcinogen (US EPA, 1993).

Cyanide poisoning by ingestion of foods containing a cyanogenic glycoside such as cassava seems to occur very rarely in regions where they do form major components of the diet, but it is reported more frequently in children in tropical countries, where such foods are more important parts of the diet. Numerous cases of acute cyanide poisoning after ingestion of cassava have been reported in children in tropical countries (Dawood, 1969; Cheok, 1978; Akintonwa, 1992; Arrifin, 1992; Espinoza *et al.*, 1992; Ruangkanchanasetr *et al.*, 1999).

Children seem to be more susceptible than adults to poisoning by ingestion of cyanogenic foods such as cassava and often developing more severe toxicity than adults concurrently ingesting cassava. The apparently greater vulnerability of children to poisoning by cyanogenic foods is likely to be due to their lower body mass.

Long-term consumption of cassava containing high levels of cyanogenic glycosides, usually when constituting the principal source of calories, and associated with malnutrition and protein and vitamin deficiencies, has been associated with neurological diseases involving tropical ataxic neuropathy and endemic spastic paraparesis. In areas with low iodine intake, development of hypothyroidism and goitre, sometimes accompanied by the neurological diseases, has also been linked to cassava (JECFA, 1993; Abuye et al, 1998). While daily cyanide exposure has been estimated to be 15–50 mg/day in endemic areas, owing to the limitations of data on exposure, which is likely to be quite variable, and the potential impact of confounders, such as general malnutrition, low protein content of the diet, and iodine status, the available data do not provide meaningful information on a dose–response for cyanide.

Acute toxicity

For acute toxicity the concept of an acceptable daily intake (ADI) or Tolerable Daily Intake (TDI) may not be appropriate. For acute toxicity the appropriate reference value is the acute reference dose (ARfD), the maximum amount that, confidently, can be safely consumed in a single meal or a single day.

In order to establish a health standard to determine a safe dose for bitter apricot kernel consumption the UK Committee on Toxicity reported that the database for the toxicity of cyanide and cyanogenic glycosides in humans was incomplete. It acknowledged that the reported acute lethal oral dose for cyanide in humans was in the range 0.5 to 3.5 mg/kg bw. They applied a 100-fold safety factor (10 to account for inter-individual variability and 10 to extrapolate from an effect level to a no effect level, taking into account the steep dose-response relationship) to the lowest lethal dose (0.5 mg/kg bw). This indicated that a dose of 0.005 mg/kg bw would be unlikely to cause acute effects, ie. a 'nominal' acute reference dose (ARfD).

The estimated lethal hydrocyanic acid dose (0.5 mg/kg bw) used to establish this 'nominal' acute reference dose is based on only one individual who ingested an unspecified cyanide salt preparation to commit suicide (Gettler & Baine, 1938). Nevertheless, there is little doubt that this health standard would be protective but it may be overly conservative because it takes no account of the different toxicokinetics for amygdalin (the cyanogenic glycoside present in apricot kernels) that involves bacterial enzymatic conversion to hydrocyanic acid once ingested. In contrast ingested hydrocyanic acid is rapidly absorbed unchanged from the GI tract.

In a toxicokinetic study by Frakes *et al.,* (1986a) 4/20 hamsters orally dosed with

0.44 mmol/kg bw amygdalin (201 mg/kg bw) died. The blood cyanide concentrations following amygdalin treatment reached their highest level (130 nmol/mL) 1 h after dosing and remained elevated until 3 h after treatment. This cyanide concentration in blood is similar to that achieved following oral administration of 0.44 mmol/kg bw linamarin (108 mg/kg bw), namely 116 µmol/L and also corresponds closely with concentrations in blood known to be lethal in humans ie, 100 – 115 µmol/L (Geller *et al.*, 2006).

In a developmental study a single dose of linamarin administered by gavage to hamsters on day 8 of gestation identified a possible NOAEL of 70 mg/kg bw (Frakes *et al.*, 1985). This study investigated the teratogenic potential after a single dose of 70, 100, 120 or 140 mg/kg bw linamarin on day 8 of gestation. Although no deaths were observed at the next higher tested dose of 100 mg/kg bw in the teratogencity study a follow-up toxicokinetic study by the same investigators using a larger number of non-pregnant hamsters revealed that deaths and clinical signs occurred at 108 mg/kg bw (Frakes *et al.*, 1986a). This information casts doubt on whether 70 mg/kg bw may be a 'true' NOAEL because a larger number of hamsters per group may reveal a significant incidence of clinical signs.

Using a NOAEL of 70 mg/kg bw and applying of 100-fold safety factor gives an acute reference dose (ARfD) of 70/100 = 0.7 mg linamarin/kg bw. A 100-fold safety factor could be applied to account for intra-species variability in sensitivity and an inter-species extrapolation. The linamarin ARfD equates to an ARfD for HCN of 0.08 mg/kg bw. As the lowest reported fatal absorbed dose for HCN is 0.58 mg/kg bw, the ARfD for hydrocyanic acid provides a margin of exposure of 7 which, given the steep dose response curve for HCN toxicity is considered to be appropriate.

Additional support for use of the hamster as a relevant surrogate for human risk assessment comes from the observation that, in adult humans, the blood cyanide level which is regarded as 'toxic' and causing clinical signs following acute exposure is generally considered to be ≥ 1 mg/L (39 µmol/L), whereas a 'fatal' concentration is generally considered to exceed 2.6 to 3 mg/L (100 – 115 µmol/L) (Geller *et al.*, 2006). These concentrations which are considered lethal in humans show remarkably good agreement with the levels which caused death in hamsters after an oral dose of 0.44 mmol linamarin/kg bw. The cyanide concentration in plasma following linamarin treatment reached a maximum of 116 µmol/L.

The ARfD of 0.7 mg linamarin/kg bw is also supported by the absence of any adverse effects in volunteers following the dietary ingestion of linamarin in a cassava-based porridge at doses ranging between 1 and 2.5 mg linamarin/kg bw (Carlsson *et al.*, 1999). This metabolic fate study in humans may not be suitable to establish an ARfD because of the inadequate range of clinical parameters measured and reported. Moreover the use of the highest tested dose in this volunteer study would result in a lower ARfD once a 10-fold safety factor for intra-species variability has been applied. Clinical signs and symptoms of acute cyanide toxicity in humans are subtle and studies designed to monitor effects need protocols which include the monitoring of headaches, dizziness, stomach pain, or mental confusion. The Carlsson *et al.*, study (1999) does not indicate if such monitoring took place.

Provisional Tolerable Daily Intake

Table 4 shows PTDI levels which have been established by several regulatory agencies. The PTDI values range from 0.02 mg/kg bw/day to 0.108 mg/kg bw/day.

Table 4: Health Standards (PTDI) established by other regulatory agencies

Organisation*	**Year**	**NOAEL study**	**NOAEL (mg HCN/kg bw/day)**	**PTDI (mg HCN/kg bw/day)**
JMPR	1965	Two-year rat study; (Howard & Hanzal,1955)	5	0.05
US EPA	1993	Two-year rat study; (Howard & Hanzal,1955)	10.8	0.108
JECFA	1993	-	-	No suitable data available to establish PTDI
EFSA	2004			
UK COT	2006			
CoE	2000	Several epidemiological studies	0.19	0.02
CoE	2005	Three-month rat study; (NTP, 1993)	4.5	0.023
ATSDR	2006	Three-month rat study; (NTP, 1993)	5	0.05
WHO	2004	Six-month study in pigs. (Jackson, 1988)	1.2	0.012
WHO	2007	Three-month rat study;	4.5	0.045

		(NTP, 1993)		

* JMPR, Joint FAO/WHO Meeting on Pesticide Residues; US EPA, US Environmental Protection Agency; JECFA, Joint FAO/WHO Expert Committee on Food Additives; EFSA, European Food Safety Authority; UK COT, UK Committee on Toxicity; CoE, Committee of Experts on Flavourings of the Council of Europe; ATSDR, Agency for Toxic Substances and Disease Registry; WHO, World Health Organization.

JECFA, EFSA and the UK Committee on Toxicity concluded that there was no suitable quantitative long-term toxicity studies in animals treated with either HCN or cyanogenic glycosides and so were unable to establish a PTDI.

Attachment 3

Sources and typical levels of total HCN in edible plants and in food ingredients with flavouring properties (EFSA, 2004; WHO 1993)

Plant	Parts with HCN	Typical levels of total HCN mg/kg	Type of glycoside
Cassava	Root (sweet) Root (bitter)	10-20 15-1120	Linamarin
Lima bean	Seed	100 to 3000 (depending on variety of seed)	Linamarin
Garden bean	Seed	20	Linamarin
Bitter almond	Seed, kernel In almond oil	300 to 3400 800 to 4000	Amygdalin
Apricot	Seed, kernel	120-4000	Prunasin
Peach	Seed, kernel	470	Prunasin
Pea	Seed	20	No information
Soya bean	Shell	1.240	No information
Linen flax	Seed	>500	Linamarin

Food product	Typical levels of total HCN in food
Ground almonds (powder)	1.4 mg/kg
Marzipan and other similar products made from apricot kernels	15-50 mg/kg
Marzipan novelties	<0.8 mg/kg
Almond paste	3 mg/kg
Cherry juice Plum juice Apricot juice Peach juice	0.5 to 12 mg/L 0.33 to 1 mg/L >0.1 to 7.8 mg/L 2.3 to 5.9 mg/L
Stone fruit preserves	0.18 mg/kg
Canned stone fruit	Up to 4 mg/kg
Kirsch (61% alcohol; distilled from cherries)	<10 mg/L
Calvados (40% alcohol, distilled from apples)	<0.5 mg/L
Stone fruit brandies	<3mg/L
Almonds and/or marzipan-containing confectionary and baked goods	Up to 40 mg/kg
Chocolate enrobed marzipan	1.3 mg/kg

Indonesia

Data on the total HCN level on raw cassava and cassava products in Indonesia are:

Item	Range of Total HCN Level	Test Method
Raw cassava	23.65 – 50.65 ppm	Picrate Spectrophotometer (J.Sci Food Agric. 1997)
Cassava products (Cassava flour products and Cassava chips)	0.42 – 16.24 ppm	Picrate Spectrophotometer (J.Sci Food Agric. 1997)

Brazil

In the Table below levels of cyanogenic glycosides in young and old cassava leaves, raw and roasted manioc flour, fermented and no fermented cassava flour are reported (Santana et al, 2008). These levels were determined by the picrate and acid hydrolysis methods (Haque and Bradbury; 2002).

Levels of in cassava leaves and products

Products	**Levels of cyanogenic glycosides (ppm)**			
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**
Young Cassava Leaves (less than 1year)	261.60	235.54	220.41	239.18
Old Cassava Leaves (more than 1year)	356.44	337.95	325.04	339.81
Raw manioc flour Brand 1	0.95	0.24	5.66	2.28
Raw manioc flour Brand 2	14.45	1.43	0.80	5.56
Roasted manioc flour Brand 1	3.17	0.20	26.14	9.83
Roasted manioc flour Brand 2	4.47	2.14	3.33	3.31
No fermented cassava flour Brand 1	1.90	1.90	2.50	2.10
No fermented cassava flour Brand 2	6.10	0.12	1.50	2.57
Fermented cassava flour Brand 1	0.20	0.36	2.06	0.87
Fermented cassava flour Brand 2	0.40	0.08	4.16	1.54

Attachment 4

Processing of cassava to reduce total HCN content

Typical production of cassava flour or starch, especially the large-scale commercial factories, has ensured that processing steps and parameters are effective in eliminating total HCN from cassava. Cassava starch, also known as tapioca starch is one of the most commonly used starches in food manufacturing and functions as a thickener, emulsifier or confectionery ingredient. Levels of total HCN in some modified starches could be as low as 0.01 mg/kg.

Cassava tubers, once harvested, are usually fermented or dried to inhibit deteriorative physiological changes and microbial growth. General processing steps, as labelled in Figure 1, are discussed in relation to the four stages in the cyanogenesis pathway, examining the parameters and techniques that are most effective in eliminating total HCN and maintaining product quality of the final products. When cassava products are found to have unacceptably high levels of total HCN, failures in one of those steps is the most likely cause.

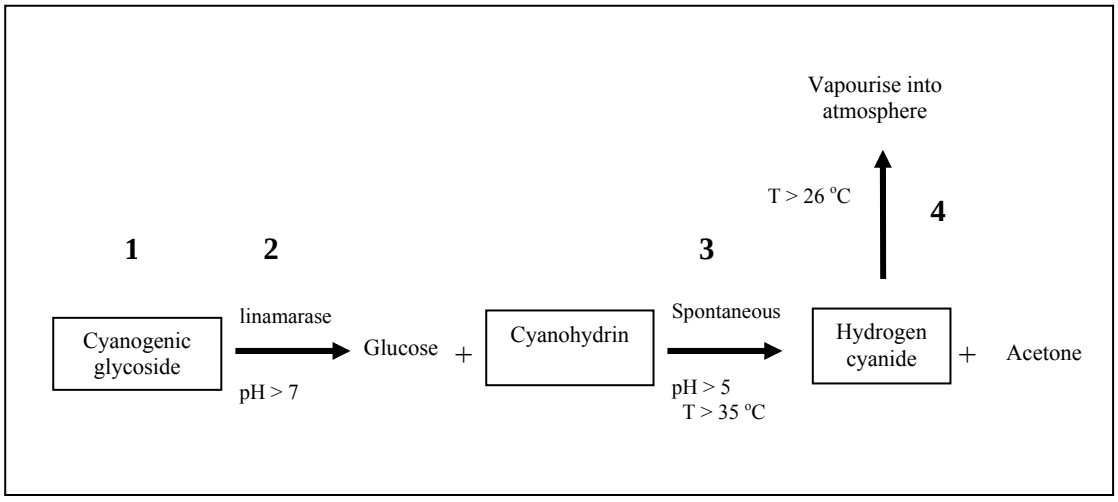


Figure 1: Cyanogenesis reaction pathway and steps in cassava processing. 1: nature of tuber; 2: grating/rasping, soaking, fermentation; 3: sun/oven drying; 4: sun drying, hot manufacturing process (steaming, frying).

Step 1: Nature of the cassava tuber. Tubers with high levels of cyanogenic glycosides are difficult to reduce to an acceptable level through the typical cassava product processes. Bitter cassava tubers have much higher cyanogenic glycosides than sweet cassava varieties and within the sweet varieties, there is a large range of cyanogenic glycoside levels existing in the tubers. Droughts have been shown to stress cassava plants to produce and accumulate high levels of cyanogenic glycosides.

Step 2: Grating, soaking and fermenting. The release of enzymes (e.g. linamarase) from the crushed cell walls and the appropriate conditions for the enzymes to react with the cyanogenic glycosides is critical. If this processing step is shortened or modified, for instance in order to prevent microbial growth or browning of the raw cassava, high levels of cyanogenic glycosides may remain in the products.

Therefore the size of the grated or sliced cassava, the time allowed for the fermentation or soaking to take place and the temperature and pH of the product will each determine how much of the cyanogenic glycoside is reduced. If high heat is used immediately after slicing or grating, for example, in frying of sliced cassava chips or drying in hot ovens, the enzyme would be inactivated and the cooked cassava products would contain high levels of cyanogenic glycosides. If low pH preservatives such as acetic acid and sodium metabisulphites are to be used at this stage, it is possible they would affect the conversion of cyanogenic glycosides.

Step 3: Sun/oven drying. The action of enzymes is continued here, as well as the spontaneous breakdown of cyanohydrins to hydrogen cyanide, at pH over 5 and temperature over 35°C. The final product of this cyanogenesis pathway is the volatile hydrogen cyanide, which vapourises at 26°C. Therefore, if the cassava grits/mash/slices are small and spread thinly in the drying step, the hydrogen cyanide can escape more easily to the atmosphere. The use of hot ovens to hasten the drying process, or when sun-drying is not available, may denature the enzyme or trap the enzyme in the dried cassava matrix and prevent the conversion of the cyanogenic glycosides to volatile hydrogen cyanide. Therefore the cassava products that have been dried too quickly would have the cyanogenic, cyanohydrin and cyanide components trapped in the cassava matrix.

Step 4: Final food product manufacturing process. If hydrogen cyanide is trapped in the dried cassava products (starch, flour or raw chips), further processing of these products may allow the hydrogen cyanide to escape (if the process temperature is higher than 26°C). If the cyanide is still in the cyanogenic glycoside form, a steaming process at a temperature less than 100°C allows the enzymes (e.g. linamarase) to be reactivated and to hydrolyse the glycosides, freeing hydrogen cyanide. However, if the cassava product (slices or chips) is subjected to high heat in frying, the cyanogenic glycoside will remain in the product. http://www.foodstandards.gov.au/_srcfiles/P1002%20Hydrocyanic%20acid%20in%20cassava%20chips%20AppR%20FINAL.pdf#search=%22cassava%20chips%22

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