A study on cyanide levels in Cassava and some of its products in some South Pacific Island Countries

Table of Contents **Page Number**

1.0 Summary

Cassava (also known as tapioca or manioc) is an important root crop in many Pacific Islands countries both for food security and as a cash crop.

Cassava can produce reasonable yields on relatively infertile soil and statistics show that it is the crop with highest mean yield in the Pacific (Pacific Economic Survey). Cassava has a flexible harvest period and has traditionally been used as a reserve in case of natural disasters such as cyclones and droughts.

Post harvest activities include milling and drying are not complicated or capital intensive thus can be conducted on farm or village level. Cassava can be processed into a range of products that can be used by numerous industries. In the Pacific, cassava is normally boiled as a household staple food and as livestock feed.

It is increasingly being exported to countries like Australia and New Zealand where a significant number of people of Pacific Islands origin reside.

Cassava contains naturally occurring, but potentially toxic compounds called cyanogenic glycosides, which release hydrogen cyanide (HCN) as a result of enzymatic hydrolysis following maceration of the plant tissue.

Cassava and its products have drawn attention by international food safety regulators on a number of occasions. From a Pacific perspective, two major events are worth recalling in context:

In July 2003, the Codex Alimentarius Commission adopts Standard for “Sweet” Cassava.

In January 2008, test conducted by the New South Wales Food Authority found cassava chips manufactured in Australia from cassava pellets imported from Indonesia to having high cyanide content (10mg/kg to 145mg/kg). This led to a warning not to buy or consume the Piranha brand of cassava cracker snack foods and a voluntary product recall by the manufacturer.

In response to these findings, Food Standards Australia and New Zealand (FSANZ) proposed the inclusion in the FSANZ Code of a maximum level of 10mg/kg for hydrocyanic acid in ready-to-eat cassava chips. This food regulatory measure was aimed at the reduction dietary exposure to hydrocyanic acid from ready-to-eat cassava chips.

Also in 2005, after similar concerns were raised by Australia and New Zealand about possible health effects of cassava, a proposed a ban on imports of raw cassava containing greater than 50mg/kg of hydrogen cyanide was mooted. Through the Codex Process, a policy of labeling such cassava as “bitter” has been proposed.

This could pose a threat to Pacific industries based on cassava processing as levels in raw cultivars may vary widely with maximum levels of up 200mg/kg. Existing studies suggests only about 50% of cyanide is removed when cassava is boiled.

Hence a study was initiated under the FAO Regional Program for Food Security to explore ways to minimize the cyanide content in cassava and its products and thus judge the feasibility of meeting this FSANZ requirement. This would be achieved by determining through desk study and laboratory analysis the effect on cyanide based on

- Agronomic factors such as cultivar, stress, soil type, fertilization
- Processing techniques such as cooking, soaking, fermenting, drying
- Harvest/post harvest practices eg age at harvest, housing, storage time and temperature

Cassava samples from three countries namely Fiji, Tonga and Vanuatu were analyzed. The cyanide content of raw cassava for these countries had a range of 13– 151 mg/kg of raw cassava. All Fijian cassava cultivars available (21) at the Koronivia Research Station germplasm farm were analyzed. Cyanide levels for these varied from 13 mg/kg to a high of 97 mg/kg of raw cassava. A repeat analysis of some varieties after a period of about 11 months from replanted crops gave similar values to those obtained earlier. A total of 48 samples from Tonga were analyzed with values ranging from 18 mg/kg to 151 mg/kg raw sample. Ten samples from Vanuatu had cyanide levels of 26 mg/kg to 78 mg/kg raw sample. A flour sample from the same country had cyanide content of 57 mg/kg while cassava chips had 60 mg/kg of hydrogen cyanide. A cassava flour sample from Palau had 56 mg/kg.

Effects of storage and processing of raw cassava were studied to determine their effects on cassava cyanide content. A single cassava variety (commonly sold locally and also exported) from a farm in Fiji was collected. Samples were kept at ambient temperature and analyzed raw and cooked at two days intervals. It was noted that cyanide levels dropped by about 30% after four days of storage. Boiling resulted in a 50% loss of cyanide content, 80% loss when grated and boiled, and about 50% when grated and cooked in earth oven. Studies of the effects of boiling in 2010 after two tropical cyclones showed lower boiling losses than those done in 2009. A repeat boiling process gave lower cyanide losses at around 42%. It was noted that the cooked samples were not as soft even after prolonged boiling. This suggests that cassava texture changed at maturity after cyclone winds affected the plants a few months prior to harvesting and consequently cyanide removal is less. Traditionally, such cassava is called “kadrala” (candle) in Fijian as it stays hard even after cooking. “Bila”, a cassava product made by soaking cassava for a few days and then wrapping in leaves and boiling resulted in a 99.9% loss in cyanide. This seems to suggest that prolonged soaking and fermentation can effectively detoxify cassava of cyanide. Ten samples of various cassava chips produced in Fiji by a single manufacturer and bought from Fijian super markets were analyzed giving values of around 20mg HCN/kg of cassava chips. A Fijian cassava chips manufacturer provided raw cassava, raw cassava chips blanched and un-blanched and finished products. Results showed that cyanide content decreased by 45 % (22 ±2 mg/kg) for un-blanched chips to over 50% (18±1 mg/kg) for blanched ones thus blanching cassava chips before frying helps in cyanide removal.

In an attempt to study the effects of agronomic conditions such as drought, a single variety of cassava from drier parts of Fiji's Viti Levu were studied. Results seems to show that for the same variety, the cyanide content of cassava from the drier side of Viti Levu was more than that of the wetter side. This is consistent with studies in Africa where seasonal variations has been linked to variations in cassava cyanide levels with higher concentrations recorded in drought conditions. Anecdotal information from Tonga also suggests that during droughts, cassava becomes "bitter" suggesting high cyanide content. Cassava samples from Tonga analyzed at that time on average also showed higher levels than those found in this study, perhaps reflecting the porous nature of the soil and thus greater water stress.

Overall the levels of hydrogen cyanide found in raw cassava in countries being studied were between 10 and 160mg/kg raw cassava. Cassava is normally consumed after being processed and boiling is a major processing method. Based on Codex limit of 50mg hydrocyanic acid/kg raw product, a number of varieties tested for these countries will be exceeding this limit.

It is recommended that blanching be used in cassava chips manufacturing so that the proposed (and Codex) level of 10mg/kg hydrocyanic acid can be achieved. The cassava flour tested had levels above the recommended 10mg/kg.

Table 1 : Summary of Cyanide Contents for Parameters Tested

Type of Cassava	Total Number of samples	Cyanide content Range (mg/kg)	Average Cyanide content (mg/kg)
Raw - Fiji	80	33– 92	39
- Tonga	48	19 – 130	61
- Vanuatu	10	26 -78	47
Stored	40	15 – 42	31
Processed - boiling	40	7 – 22	12
- Soaking	10	-	<0.1
- others	40	6 – 35	16
Chips	14	18 – 32	23
Flour	2	56 – 57	56

The method described by Cooke (1976) was verified and adopted as the analytical procedure to determine cyanide levels in cassava and cassava products in this project. For the first time linamarase extracted from cassava leaves petiole and purified was used.

Both the method and the isolated linamarase were validated using commercial linamarin. A cyanide recovery of 98-105% was obtained. In addition the recommended A_{620} for 1µg HCN to be 0.741 was achieved.

2.0 Aim of Study

Overall Objective

To sustain and enhance the food security and livelihood of Pacific Island farmers and processors by assisting them to successfully produce/trade/export their produce in compliance with food safety standards for cassava and cassava products

The purpose of the study is to conduct a scientific assessment to determine ways along the food chain/commodity pathway to minimize the hydro cyanic acid content in cassava and its products and thus provide methods of meeting the impending FSANZ and Codex requirement. This would be achieved by determining, through desk study and analysis, the effect on cyanide content based on:

- Agronomic factors (e.g. cultivars, stress, soil type, fertilization)
- Processing techniques (e.g. cooking, soaking, fermenting, drying, extrusion)
- Harvest/post harvest practices (e.g. age at harvest, housing, storage time and temperature) Cyanide content in various processed cassava to note how much cyanide has been lost.

The aim of this study is to assess

2.1 Cyanide content in different cultivars and how different agronomic conditions also affect cyanide content

2.2 Cyanide content in various processed cassava to determine how different processing techniques can help reduce cyanide content

2.3 To provide information to relevant stakeholders on cyanide contents in cassava cultivars and cassava products.

3.0 Literature Review

3.1. Cassava : the plant, distribution and a food source

Cassava, now widely grown in the Pacific is a domesticated plant derived from one or more species of the Genus Manihot in the Euphorbiaceae family (Allem, 2002). Cultivated cassava is usually *Manihot esculanta Crantz.* Since its domestication thousands of years ago in the Amazon region, cassava is now spread around the world and is widely cultivated for consumption in the tropics and sub-tropic. Thousands of cultivars are in existence most adapted to local conditions. Cassava is a very rustic crop that grows well under marginal conditions where other crops could not survive and does not require a large amount of agricultural input (e.g., water, fertilizer and pesticides). Most cassava varieties are drought tolerant, resistant to most diseases and are naturally tolerant to acidic soils making cassava a fundamental food security component in marginal agricultural land.

Cassava is grown primarily for its starchy tuberous roots, which are important staple for more than 800 million people, mostly in sub-Sahara but also in other parts of Africa, Asia and South America (Burns et.al. 2010.). It has also become a very important root crop in the Pacific Island where not only it has become a dietary staple but also extensively traded as a raw product or in processed form like chips and flour. A variety of other products specific to different Pacific Islands are available.

3.2 Cassava and Cyanide

Manihot belongs to the same sub-family as rubber (*Hevea brasiliensis*) and like rubber contains both cyanogenic glucosides and latex (Jorgensen et al., 2005).

A food safety problem with cassava is that cassava roots contain considerable quantities of cyanide which occurs in the form of cyanogenic glycosides, primarily linamarin and a small amount of lotaustralin (Uyoh et al., 2007). These cyanogenic glycosides break down to release toxic hydrogen cyanide gas during digestion (Poulton, 1988). The consumption of cassava can therefore be harmful to human health. Despite the presence of these naturally occurring toxins, millions of people all over the world have been safely consuming cassava for hundreds of years. The on-going challenge is to ensure that the presence of these cyanogenic glycosides are minimized through proper understanding and possibly control of factors that affect cyanogenic glycoside content of cassava. Roots and leaves contain the highest amount of linamarin (Cereda and Mattos, 1996).

3.3 Factors Affecting Cyanide Content of Cassava

3.3.1 Cultivar.

Thousands of cassava cultivars have been developed that are adapted to local conditions and differ in their ability to tolerate pest and diseases, yield, nutritional and cooking qualities of food products. Cassava is propagated clonally from stem cuttings so there is minimal variation between individuals of one cultivar when grown under the same environmental conditions. All cassava cultivars contain cyanogenic glucosides however a wide variation in the concentration of cyanogens exists among different cultivars. This can range from 1 to 2,000 mg/kg (Cardoso et al., 2005, CIAT 2007). Cultivars with <100mg/kg hydrogen cyanide are called sweet while those >100mg/kg are called bitter (Wheatley et al., 1993). A study in Fiji by Aalbersberg and Limalevu (1991) on 17 different cultivars grown in the same environmental conditions agreed with literature observations. The 17 different cultivars had cyanide levels of 14 – 121 mg/kg.

3.3.2 Climatic Conditions

Cassava, a perennial shrub thrives in tropical and sub-tropical conditions. In general, the crop requires a warm humid climate. Temperature is important, as all growth stops at about 10ºC. Typically, the crop is grown in areas that are frost free the year round. The highest root production can be expected in the tropical lowlands, below 150 m altitude, where temperatures average 25-27°C, but some varieties grow at altitudes of up to 1500 m.

The plant produces best when rainfall is fairly abundant, but it can be grown where annual rainfall is as low as 500 mm or where it is as high as 5,000 mm. The plant can stand prolonged periods of drought in which most other food crops would perish. This makes it valuable in regions where annual rainfall is low or where seasonal distribution is irregular. In tropical climates the dry season has about the same effect on Cassava as low temperature has on deciduous perennials in other parts of the world. The period of dormancy lasts two to three months and growth resumes when the rains begin again. Cassava is drought resistant and grows well in poor soil (Java Cassava, 2007).

The problem however is that cyanide content of cassava tends to increase during periods of droughts and or prolonged dry weather due to water stress on the plant (Bokanga et al., 1994). For example in Mozambique, about 55% of the sweet fresh roots were extremely toxic and the remainder moderately so during drought like conditions. Similar observations were recorded in The Democratic Republic of Congo (Gitebo et al. 2009), and various citations in Africa (Cardoso et al., 2005). Splittstoesser and Tunya (1992) reported that cassava grown in wet areas contain relatively lower amount of cyanide than those grown in drier areas.

3.3.3 Fertilizer

There is a general consensus that crop yields do increase with application of fertilizer, there is debate however on the relationship between addition of fertilizer and cyanide content of cassava.

Studies in the Philippines (Rolinda et.al, 2008) concluded that application of fertilizer does not significantly affect cyanide content. It further suggested that the amount of nutrient in the soil does not considerably contribute to the cyanogenic character of the cultivar. In Ethiopia, Endris (1977) suggested that the cyanogenic content of cassava roots were significantly reduced by potassium application.

3.3.4 Harvesting

Harvesting of cassava can be done throughout the year when the roots reach maturity. Maturity differs from one variety to another, but for food, the tubers can be harvested at almost any age below 12 months (FAO, 1977) and can remain the soil for up to three years after maturity (Lebot, 2009).

Harvesting is still generally a manual operation. Before harvest, the plants are "topped" (stalks are cut off 40-60 cm above ground) leaving an adequate length of stalk as a handle for pulling. In light soils the roots are slowly drawn from the soil simply by pulling the stems or with the help of a kind of crowbar and the tubers are cut off the stock. In heavier soils, digging up the roots before the plant is pulled out may be required (Java Cassava, 2007). Once the plants have been topped, uprooting must not be delayed, as sprouting causes a drastic fall in the starch content of the tubers. While the effect of harvesting method on cyanide is not clear, injuring the roots increases rate of post harvest deterioration.

3.3.5 Age of Cassava at Harvesting

A study by Hidayat et al. (2002) on ninety nine variety of cassava showed that there is a significant correlation between cyanide potential of roots and leaves. The cyanide content was higher in younger leaves compared to older ones, suggesting that cyanide potential of roots drops as plant ages. This seems to agree with investigations by Chotineeranati, et al (2006). Cooke and Elba,(1982) reported that the root parenchymal tissue and root cortex were not significantly different between 6 and 14 months; both tissues displayed peak concentrations at 6 and 14 months.

3.3.6 Post Harvest Practices

Post-harvest deterioration (PHD) is the most important cause of loss in cassava production and this is mainly as a result of microbial invasion of the tuber (Okigbo et al 2009). PHD can render cassava unpalatable and un-marketable within 24-72 h (Rielly et al. 2004). Cassava must also be processed before being eaten.

The Amerindians, who first cultivated cassava, over the years, have devised numerous processing techniques not only to increase palatability and extend shelf life, but also to decrease its cyanogenic potential. Today, a great diversity of processing methods are found in the various parts of the world where cassava is consumed (Lancaster et al.,1982). These methods consist of different combinations of peeling, chopping, grating, soaking, drying frying, boiling and fermenting. In Africa where cassava flour is a major product, wetting (Bradbury 2006; Cumbana et al. 2007) is an effective method of cyanide removal.

In the Pacific where cassava is a dietary staple, boiling freshly harvested cassava roots is the norm. Cooke and Maduagwa (1999) reported a 55% reduction of bound cyanide by cooking of cassava. Similar figures (50-60%) were reported by Aalbersberg and Limalevu (1991) and 25-75% by Nambisan and Sundarsan (1985). The figures by Nambisan and Sundersan were dependent on cooking time and chips size, with smaller chips size recording highest cyanide losses.

Methods which use grating and crushing are very effective in removing cyanide because of the intimate contact in the finely-divided wet parenchyma between linamarin and the hydrolyzing enzyme linamarase, which promotes rapid breakdown of linamarin to hydrogen cyanide gas that escapes into the air (Cardoso et al., 2005). This in combination with wetting, fermentation and drying can reduce cyanide contents up to 99%.

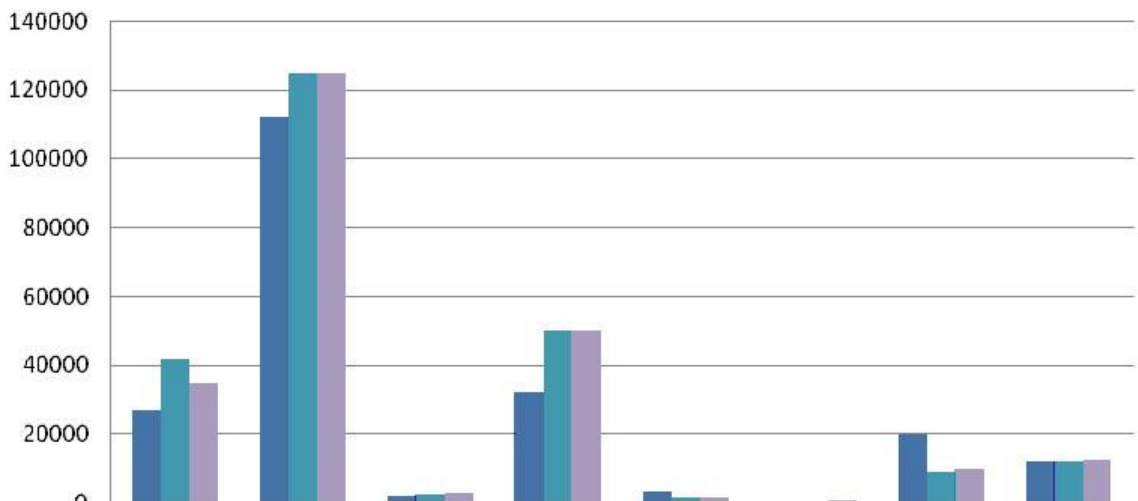
Cassava is generally harvested fresh and boiled in preparations where roots are eaten. Storage at ambient temperature may take up to three days. Storage of cassava roots at ambient temperatures for up to four days can result in cyanide loss of up to 7% (Idowu and Akindele, 1994).

Various other methods like storage in moist sawdust, stored in ground, piling in heap and watered daily, clamping, plastic bags, and finally frozen as the practice for fresh cassava that are exported. In Africa, production of cassava flour is the main storage method.

3.4 Cassava and Trade

Cassava is exported to Australia and New Zealand and is the second major root crop imported into New Zealand behind taro and ahead of yam. Some 1400 tonnes cassava was imported into New Zealand in 1999 the major exporter being Fiji with Tonga capturing about a third of the market. Pacific Islands are also the major cassava exporter to Australia (FSANZ, 2004).

Fig:1 Cassava Production in the Pacific (tonnes)



	FIJ	PNG	SI	T-L	CI	SAM	TON	FSM
1998	27136	112000	2000	32092	3000	250	20000	11800
2002	41432	125000	2500	50000	1600	300	9000	11800
2007	34500	125000	2500	49720	1500	370	9700	12000

Source: FAOSTAT Agriculture Production Statistics

In the ten years between 1995 and 2005, two Pacific Island countries, Fiji and Tonga ranked amongst the top of cassava exporters in the world.

Table 2 : Top Exporters of Cassava 1995- 2005 (US$'000)

	Year			Average annual growth, 00-05 (%)	Percentage of total	
	1995	2000	2005		1995	2005
Thailand	330,703	288,988	428,866	8.22	71.31	68.77
Viet Nam	14,155	25,008	68,741	22.41	3.05	11.02
Costa Rica	18,200	27,301	64,799	18.87	3.92	10.39
Indonesia	65,115	20,435	35,126	11.44	14.04	5.63
Ecuador	54	2,143	4,382	15.38	0.01	0.70
The Netherlands	1,028	15,674	4,171	-23.26	0.22	0.67
Ghana	873	645	3,518	40.41	0.19	0.56
France	158	1,527	1,989	5.42	0.03	0.32
Cameroon	8	187	1,469	51.04	0.00	0.24
Brazil	46	272	1,399	38.80	0.01	0.22
Nicaragua	121	101	984	57.56	0.03	0.16
Fiji	271	450	900	14.86	0.06	0.14
Venezuela	266	-	816		0.06	0.13
Belgium	-	2,315	678	-21.78	0.00	0.11
Philippines	1,205	453	674	8.29	0.26	0.11
Suriname	142	3	565	186.50	0.03	0.09
Malaysia	202	371	448	3.84	0.04	0.07
Spain	13	452	373	-3.76	0.00	0.06
Colombia	22	139	364	21.22	0.00	0.06
Tonga	39	157	350	17.42	0.01	0.06

Source: AusAid/SADC: Trade Information Brief Cassava, (undated) p.39

3.5 Cassava and Health

Despite the presence of these naturally occurring toxins, millions of people all over the world have been safely consuming cassava for hundreds of years. Usually, cassava is well processed before being consumed. Inadequate processing however may result in appreciable amounts of cyanogenic glycosides remaining and this may pose a public health risk (FSANZ 2008.).

Konzo is a condition resulting from the excessive ingestion of cyanide compounds from inadequately prepared cassava and cassava products is characterized by irreversible paralysis of the legs in and other developmental disorders. They occurs mainly amongst children and women of child bearing age in Democratic Republic of Congo, where a reported 100,000 cases exist, Tanzania, Mozambique, Central African Republic, Cameroon and probably other countries (CCDN News, 2007). Tropical Ataxic

Neuropathy (TAN) is another syndrome attributed to dietary cyanide exposure from inadequately prepared cassava. In contrast to Konzo, TAN is a progressive disorder that mainly affects older adults (CCDN News, 2008). It has been shown that different varieties of cassava have varying cyanide content and this quantity is affected by climatic conditions and other factors (Raji et al., 2007, CIAT, 2007). Studies in Africa have linked varying cyanide content in cassava to seasonal changes with higher concentrations of cyanide recorded in drought conditions. Iodine deficiency diseases are exacerbated by the intake of cyanogenic plants such as cassava.

4.0 Methodology

4.1 Sample Collection

In response to the invitation to participate in the research project, samples were received from Tonga, Vanuatu and Fiji. Forty eight samples (not necessarily different cultivars) were received from the Ministry of Agriculture, Tonga while ten samples (of ten different cultivars) were obtained from Vanuatu through the Secretariat of the Pacific Community.

All the twenty one different cultivars available at Fiji's Ministry of Agriculture Kornivia Research Station germ-plasm farm were analyzed. A repeat analysis of six cultivars from the same farm was done after a period of about eleven months was facilitated by the Secretariat of the Pacific Community. A farm in Nasinu (close to Suva) was used to obtain a single variety, identified as Niumea. This sample was used to assess cyanide content at different storage intervals and also the various preparation methods. Another farm was used for a repeat of the above using a single variety identified as "Natoaika". Further, a number of samples of the commonly sold varieties (identified as Nadelei and Niuqini, both have characteristic rough brownish outer skin and pinkish inner skin) were obtained from market vendors. These two varieties represent about 70% of all cassava grown in Fiji

Cassava chips were obtained from supermarkets around Fiji's capital Suva. These cassava chips were manufactured by Flour Mills of Fiji Limited. The same company provided us with three samples of raw cassava to finished product; the samples were for three production batches

4.2 Sample Storage and Preparation

Samples were analyzed fresh as required while others were stored frozen at -18^{0}C whilst awaiting analysis. Samples were thawed, peeled, and middle portion of a whole cassava was diced, (well mixed if more than one cassava stick was submitted) and a portion taken for analysis. Freezing does not affect cyanide content.

For the samples that were analyzed at intervals, the samples were analyzed at Day 0, (day of harvest) Day 2, Day 4 and Day 6 of storage at ambient temperature. The samples at these intervals were separated into two portions where one portion was analysed raw while the other was boiled and analyzed.

Two traditional Fijian products "Bila" and "Vakalavalava" were prepared as follows:

The "vakalavalava" was prepared by grating the cassava and wrapping it in breadfruit leaves. Two parcels were boiled while the two others were cooked in a traditional earth oven.

Fig 2 : Vakalavalava

"Bila" was prepared (from cassava at Day 0) by peeling and dicing a few sticks of cassava to about 1 – 2 cm^3 and then soaked in water for three days. After the three days, the cassava was mashed and squeezed dried to certain moisture content. Sugar and finely grated coconut were added and thoroughly mixed and made in to dough like mix. A "loaf" measuring 2cm in diameter and about 30cm long is wrapped in "vasili" leaf (*Cordyline terminalis*), fasten and boiled. The "bila" samples used in this project was prepared by a lady proficient in making bila. A composite sample was analyzed.

Fig 3 : Bila

For cassava chips, composite 45g packs were grounded, weighed in soxhlet thimbles and defatted with diethyl ether for about 9 hours. Fat content was determined to allow for final measurement to be corrected for fat. The defatted sample was analyzed for cyanide.

4.3 Analytical Method

Following the method by Cooke (1978) cassava roots were washed, peeled and diced. The diced samples were thoroughly mixed and 60 grams was extracted with about 150 mls of 0.1M orthophosphoric acid. Sample was filtered and volume of extract measured and recorded. 0.1 mL of sample, 0.1ml of linimarase and 0.4 mL of pH buffer 7 are incubated in shaking water bath at 30ºC for 15 minutes. At the end of 15 minutes period, 0.6 mL of 0.2M NaOH is added to stop the reaction of linimarase. Color was developed by adding 0.2 ml of Chloramin T reagent followed by pyridine/pyrazolone reagent. The sample was left to stand for 90 minutes for color to develop. All samples were analyzed in triplicates. A series of standards ranging from 0-1.65 μg of HCN were prepared and color developed as above. The absorbance of both standards and sample were spectrophotometrically read at 620 nm.

The cyanide content of sample was determined from the standard calibration curve. Amount of cyanide in 100g sample was computed using the formula:

$$\text{mg/kg cyanide} = \frac{\mu\text{g/mL of cyanide x final volume (mLs) x 10}}{\text{Sample wt}}$$

Where: μg/mL CN is obtained from calibration curve
Final volume - volume of sample measured from filtered extract
Sample weight - weight of sample extracted

4.4 Method Verification

Since linimarase extracted from cassava leaves petiole was being used in our laboratory for the first time, this was verified to demonstrate that it is workable and at the same time validating the method.

The use of linimarase derived from cassava leaves is being trialed for the first time and will be adopted as part of the methodology if it is proved to be workable. Benefits derived from this include saving in costs for purchasing and preserving commercially prepared enzyme and acquisition of skills in adapting appropriate science. Given that the method by Cooke is the method of choice, the usage of in-house linimarase will be ongoing.

Using commercially available linimarin (A.G. Scientific Inc., San Diego, CA), a solution having an equivalent amount of 1.22μg/mL of HCN was prepared. Varying amounts of linimarin was incubated using extracted linimarase (various volumes of 1.22 μg/ml stock) and cyanide released was measured. Recovery was determined by the ratio of recovered cyanide to theoretical level in the linimarin.

Fig 4 : Extraction of Linimarase from Cassava Leaf Petiole

4.5 Reporting of Results

The mean and standard deviation (in parenthesis) of the replicates were reported in mg cyanide/kg sample. The chips results were reported as original chips after amount of fat removed was factored back into the cyanide value obtained (cyanide was determined on defatted sample).

5.0 Results

5.1 Method Verification

Linimarin was prepared to produce an equivalent amount of 1.22 μg/mL of HCN. Various volumes were incubated with results as follows.

Table 3 : Percentage Recovery of Linimarin using 0.1 mL Linimarase

Volume of 1.22 μg/ml Linamarine Incubated	Theoretical Amount of HCN Present (μg)	Actual Amount Determined (μg)	Recovery (%)
0.1 mL	0.122	0.117	96
0.2 mL	0.244	0.240	98
0.4 mL	0.488	0.503	103
0.5 mL	0.61	0.634	104
1.0 mL	1.22	1.259	105

A recovery range of 95-105% was obtained. This indicated that the method was working well and samples can be analyzed.

The method is simple and reliable and was easy to start up given that a spectrophotometer and incubators already exist in our laboratory. An added advantage is the ability to extract linimarase and successfully use it, removing the need to buy the expensive commercial item. The method can comfortably analyze five samples (in triplicate) per day.

5.2 Cyanide Levels of Fresh Samples

5.2.1 Fiji Samples

Table 4 (in Annex) gives a summary of the cyanide content of different Fijian cultivars from this work and that by Aalbersberg and Limalevu (1990).

It is noted that most the values obtained in this work were less than those obtained by Aalbersberg and Limalevu (1990). A possible reason would be that samples were obtained after a period of extended dry weather in the previous study while current research was carried out after a period of extended wet weather. The New Guinea and Coci varieties obtained very similar values in both the studies.

Also noteworthy is that about 81% of cultivars tested had cyanide levels of less than 50mg/100g fresh. Nadelei, a variety that is commonly grown in Fiji had 35 mg/kg cyanide content that is classified as sweet and should be grown by farmers. Another variety known by some farmers as Natoaika is grown by farmers in the South Eastern part of Fiji for export also falls in the "sweet cassava" category.This means that they can traded as sweet cassavas per Codex Standards for cassava (Codex Standard 283-2003; Codex Standard 300-2010). These two varieties are worth considering for commercial cultivation in view of export market requirements.

A repeat analysis of six varieties from the Koronivia Research Station, except for one variety, gave similar results to those obtained some twelve months earlier (Table 5). The only significant difference was the Merelesita cultivar. The 9 mg/kg obtained this time was closer to that previously obtained (14 mg/kg) by Aalbersberg and Limalevu (1990). The variation in values is indicative of the various factor affecting cyanide content of cassava as well as the uneven distribution of cyanide within a given cassava root.

Table 5 : Results of Repeated Analyses and Earlier Analysis of Some Cultivars from Koronivia Research Station

Cultivar	Total Cyanide (mg/kg) - 2010	Total Cyanide (mg/100g) - 2009
Beqa	30(1)	26(2)
Nadelei	40(1)	35(3)
Sokobale	25(1)	29(0)
Merelesita	9(0.5)	39(1)
Vula tolu	27(2)	23(0)
Tavioka Falawa	15(1)	15(0.9)

Standard Deviation in parenthesis

5.2.2 Tonga

Table 6 (in Annex) gives the cyanide content of the Tonga samples with values between 18 – 151 mg/kg obtained. These were somewhat lower than those that were analyzed at the IAS laboratory in 2004 with values ranging from 41 – 218 mg/kg of cassava. This could have been due to soil rehabilitation processes undertaken by the Ministry of Agriculture, Tonga in an attempt to reduce cyanide levels in cassava (pers. comm.). The values obtained would mean that only 20% of the samples tested were below 50mg/kg and therefore would fall under the Code definition of sweet cassava.

A possible reason for the higher cyanide levels in Tonga is the soil type. With a more loose texture (pers. comm.), water retention is low, exposing cassava to water stress situation, a contributing factor to cyanide elevation.

5.2.3 Vanuatu

The Vanuatu samples (Table 7, in Annex) had cyanide contents of between 26 – 78mg/kg sample. It was note that 60% of all cultivars tested hand total cyanide content of less than 50mg/kg. Further, it was observed Vanuatu cultivars had a lower maximum cyanide level compared to those of Fiji and Tonga. While proper comparisons are difficult to make due to unknown common identity of cultivars, soil and climatic conditions in Vanuatu may contribute to lower cyanide levels in the cultivars analyzed.

5.3 Cyanide Levels of Cassava Stored at Ambient Temperature

Table 8 : Cyanide Content of Fiji Cassava Stored at Ambient Temperature (Standard deviation in parenthesis)

Days of Storage	Averaged Total Cyanide (mg/kg)	Average % Decrease
0	38 (2)	-
2	36 (2)	5%
4	30 (1)	21%
6	23 (1)	39%

A total of forty analyses covering the four periods were carried out. A general trend observed was the decrease in cyanide levels over the six days of storage at ambient temperature. An overall average decrease of 39% was recorded after 6 days of storage. Normally cassava is eaten within 2-3 days of harvest. It was noted that the cassava started to turn brown after day 2 and in some cases rotten at day 6. It was further observed that cassava that got damaged post harvest had an accelerated rate of post harvest deterioration (PHD).

At garden levels, it may be recommended that cassava be uprooted gently so as avoid detaching (thus injuring) the root from the stump, and this be carefully cut off to avoid damaging cassava parenchyma tissue thus delaying PHD.

5.4 Cyanide Content of Boiled Cassava after Different Storage Intervals

Table 9 : Averaged Cyanide Content of Cassava Boiled after Different Storage Intervals (Standard deviation in parenthesis)

Days of Storage	Total Cyanide (mg/kg)
0	11 (3)
2	7 (0)
4	12 (0)
6	10 (1)

Again another 40 analyses covering the four periods were carried out. It is interesting to note that while total cyanide in cassava decreased with increased storage time, the amount of cyanide remaining after boiling the stored samples did not change much. This suggested that a fraction of cyanide only can be removed by storage and or boiling while a certain amount cannot be removed by these processes. While the percentage cyanide loss after boiling was about 52%, cassava analyzed after the Fiji Cyclones averaged at around 42%. It was further observed that cassava roots that were affected by a cyclone that hit Fiji months earlier did not soften to the desired texture when boiled and seems to retained more cyanide over the 6 day- period compared to pre-cyclone results.

It was note that the texture of the cassava was not soft even after prolonged boiling. This suggests that varieties that do not soften well on boiling will lose less cyanide when boiled and may not be a desirable variety.

5.5 Effect of Various Processing Methods on Cyanide Content

Table 10 : Cyanide Content of Cassava prepared under Various Processing Methods (Standard deviation in parenthesis)

Cooking Process/Product	Total Number of samples	Cyanide content Range (mg/kg)	Average Cyanide content (mg/kg)	Average % Cyanide lost
Whole cassava – earth oven	10	19 – 35	27	37
Grated cassava – earth oven (Vakalavalava)	10	10 – 16	14	63
Grated cassava – boiled (vakalavalava)	10	6 – 8	7	80
Soaking/Cooking- (Bila)	10	<0.1	<0.1	99.9
Cassava Chips	10	14 – 21	18	?
Cassava Chips- Raw, un-blanched	1	-	32	15
Cassava Chips – Raw Blanched	1	-	25	36
Cassava Chips – un-blanched	1	-	22	45
Cassava Chips- blanched	1	-	18	53
Flour	2	56 – 57	56	?

? = no data available since original raw cassava to make product was not tested.

5.5.1 Boiling

Fresh cassava is generally boiled in Pacific Islands where it is eaten as a staple. It is peeled and cut into pieces of about 40cm. Those with a larger girth are generally split into two to aid cooking. It was found that about 50% of cyanide was removed. Also observed was that when cassava does not soften enough when boiled (occurs when plants are disturbed during cyclones or plant tops being trimmed and plant re-grows), around 40% of cyanide was removed. This is most likely due to reduced interaction with cooking water thus reducing removal of cyanide by hydrolysis.

When cassava was and grated boiled, cyanide loss of around 80% was recorded. The high percentage losses were expected since grating allow endogenous linimarase to come in contact with linimarin releasing HCN. Boiling the grated sample further improved cyanide removal by hydrolysis and leaching.

The level in "bila" was expected since the preparation of "bila" involved days of soaking the cassava allowing cyanide to leach in the process. A combination grating, leaching and fermentation is taking place here and literature has cited that the combination of these process is capable of removing most cyanide from cassava..

5.5.2 Earth Oven

Cassava was both earth oven cooked whole and grated and wrapped in breadfruit leaves.
There was a loss of about 37% cyanide loss whole cassava while grated one lost about 63%. It was noted that earth oven cooked whole cassava lost less cyanide than boiled ones. A possible reason would be that boiled cassava has water interacting will cassava tissue hydrolyzing the linimarin, while the earth oven cooked one allowed for the release of volatile cyanide. The grated one lost about 60% cyanide. Here the combination of temperature and breaking up of cassava tissue enabling the action of linimarase resulted in more cyanide loss.

6.0 Discussions and Conclusion

The Pacific Islands is the major source of raw cassava being exported to Australia and New Zealand. Results from these studies would enable the Pacific Islands to be aware of cultivars that needed to be grown and exported to these two countries. Cultivars that contained less than 50mg/kg cyanide would be the most desirable. Not only will these cultivars facilitate continuous exportation of cassava to Australia and New Zealand, it will also have the potential to reduce health risks associated with cyanide ingestion in-country when eaten.

From data gathered, it can be concluded that a majority of the cultivars grown, consumed and exported in Vanuatu and Fiji are relative safe. This however may not be the case for Tonga where only 20% of the samples analyzed had cyanide contents of <50mg/kg of sample. These figures are debatable since the samples were experimental and may not necessarily reflect cultivars that are widely grown and exported in Tonga.

The “ready to eat” cassava products flour and chips may need to undergo more efficient processing methods to ensure that they contain no more than 10mg cyanide/kg of sample.

7.0 Recommendations

7.1 A concerted effort by organizations such as the South Pacific Community to document cassava varieties in Island states and provide them with a common English identity so that high cyanide varieties are documented. This will help facilitate cassava export at regional level by identifying and exporting sweet varieties only.

7.2 Cassava exporters to submit samples of export batches for cyanide determination. This will enable them to monitor cyanide content in-country to avoid exporting of high cyanide varieties.

7.3 Cassava chips manufacturer to continuously provide their products for analyses to ensure their processes were able to achieve the 10mg/kg cyanide proposed for such products.

ANNEX

Table 4 : Comparison of Total Cyanide in Fijian Cultivars previously Analyzed (Standard deviation in parenthesis)

Cultivar	Total Cyanide (mg/kg) This Study	Total Cyanide (mg/kg) Aalbersberg (1998)
Tavioka Falawa	13 (1)	
Yabia Damu	15 (3)	101
Yabia Vula	16 (1)	93
Vulatolu II	17 (0)	21
Aikavitu	17 (1)	42
Vulatolu	23 (0)	70
Modre	24 (1)	*
Lomaivuna	24 (3)	*
Beqa	26 (2)	121
Coci (selection)	26 (2)	55
Niumea	27 (2)	38
Sokobale	29 (0)	36
Vulatolu Dalip Singh	34 (0)	*
Bele Silika	35 (2)	*
Nadelei	35 (3)	*
Merelesita	39 (1)	14
Vulatolu Yasawa	49 (1)	*
Coci	55 (3)	55
H97	61 (1)	*
New Guinea	82 (3)	80
Navolau	92 (1)	101

***** Cultivars were not previously analyzed

Table 6 : Cyanide Content of Tongan Cassava
(Standard deviation in parenthesis)

Code	Total Cyanide (mg/kg)
01 - FM	59 (4)
02 - FM	83 (4)
03 - FM	130 (2)
04 - FM	81 (5)
05 - FM	76 (3)
06 - FM	48 (6)
07 - FM	15 (4)
08 - FM	135 (1)
01 - FW	54 (2)
02 - FW	51 (1)
03 - FW	44 (1)
04 - FW	47 (2)
05 - FW	63 (4)
06 - FW	28 (3)
07 - FW	47 (3)
08 - FW	18 (1)
01 - TM	37 (2)
02 - TM	137 (3)
03 - TM	56 (1)
04 - TM	64 (1)
05 - TM	84 (8)
06 - TM	120 (2)
07 - TM	53 (1)
08 - TM	69 (1)
01 - TW	44 (3)
02 - TW	91 (7)
03 - TW	76 (4)
04 - TW	74 (1)
05 - TW	79 (2)
06 - TW	101 (2)
07 - TW	72 (4)
08 - TW	48 (6)
01 – PW	19 (1)
02 – PW	28 (3)
03 – PW	22 (2)
04 – PW	37 (3)
05 – PW	48 (2)
06 – PW	31 (2)
07 – PW	28 (3)
08 – PW	61 (5)
01 – PM	52 (1)

02 – PM	53 (6)
03 – PM	36 (2)
04 – PM	35 (3)
05 - PM	33 (1)
06 - PM	37 (4)
07 - PM	54 (1)
08 - PM	45 (4)

Table 7 : Cyanide Content of Vanuatu Cassava
(Standard deviation in parenthesis)

Cultivar	**Total Cyanide (mg/kg)**
Onesua 1	40 (3)
Onesua 2	57 (4)
Onesua 8	64 (1)
Epule	77 (4)
Rice	38 (2)
Ena	34 (2)
Onesua Curry	78 (1)
Tagabe 1	26 (2)
Tagabe 6	26 (3)
Tagabe 14	33 (1)

Table 8 : Cyanide Content of Boiled Cassava after Different Storage Intervals
(Standard deviation in parenthesis)

Days of Storage	**Total Cyanide (mg/*kg*)**
0	11 (3)
2	7 (0)
4	12 (0)
6	10 (1)

References

Aalbersberg, W.G.L., Limalevu, L. 1991. Cyanide content in fresh and processed Fijian cassava (*Manihot esculenta*) cultivars. *Trop. Sci*. 31, 249-256.

Bokanga, M., Essers, S., Pouler, N., Rosling, H., Tewe, O., Asiedu, R., Brader, L. 2004. International workshop on cassava safety. *Acta Horticulturae*. 375, 1-17.

Bradbury, J.H. 2006. Simple wetting method to reduce cyanogens content of cassava flour. *J. Food Comp. Anal*. 19, 388-393.

Burns, A., Gleadow, R., Cliff, J., Zacarias, A., Cavagnaro, T. 2010. Cassava: The drought, war and Famine crop in a changing world. *Sustainability* 2, 3572-3607.

Cardoso, A.P., Mirione, E., Ernesto, M., Massaza, F., Cliff, J., Haque, M.R., Bradbury, J.H. 2005. Processing of cassava roots to remote cyanogens. *J. Food. Comp. Anal.* 18, 451-460.

Cassava Cyanide Disease Network News, 2007

Cassava Cyanide Disease Network News, 2008

Centro InternationL de Agricultura Tropical (CIAT). 2007. Improved Cassava for the developing world. Annual report. pp 39.

Cereda, M. P. Mattos, M. C. Y. 1996. Linamarin, the toxic compound of cassava. J Venom. Anim. Toxins. 2 (1).

Cooke,R.D., 1978. An enzymatic assay for the total cyanide content of cassava (*Manihot esculanta* Crantz). Journal of the Science of Food and Agriculture 29, 345-352.

Cooke, R. D. Elba, M. 982.(title) de la Cru 33 (3), 269-275.

Chotineeranat, S., Suwansichon, T., Chompreeda, P., Piyachomkwan, K., Vichukit, V., Sriroth, K., Haruthaithanasan, V. 2006. Effect of root ages on the quality of low cyanide cassava flour from Kasetsart 50. *Kasetsart J. Nat. Sci.* 40, 694-701.

Cumbana, A., Mirione, E., Cliff, J., Bradbury, J.H. 2007. Reduction of cyanide content of cassava flour in Mozambique by the wetting method. *J. Food Chem*. 101 (3), 894-897.

Endris,S. 1977. Cyanogenic potential of cassava cultivars grown under varying levels of potassium nutrition in Southwestern Ethopia. Ethiopian Institute of Agricultural Research (EIAR), Jimma Center, PO Box 192, Jimma

FAO, 1977. Traditional storage of yams and cassava and its improvement- Cassava. http://www.fao/inpho/content/vlibrary/gtzhtml.

Food Standards Australia and New Zealand, 2004. Final Assessment Report, Proposal P257, Advice on the preparation of cassava and bamboo shoots.

Food Standards Australia and New Zealand , 2008. Assessment Report, Proposal P1002, Hydocyanic Acid in ready to eat cassava chips.

Gitebo D.N., Banea-Mayabu J.P., Matadi R.N., Tylleskar T., Gebre-Medhin M., Rosling H. 2009. Geogarphical and Seasonal Association between Linamarin and Cyanide Exposure from Cassaca and the upeer motor neuron disease Konzo in DRC. In CCDN News, Issue 14.
.

Hidayat A., Zuraida N. and Hararida I. 2002. The Cyanogenic Potential of Roots and Leaves of Ninety Nine CASSAVA cultivars. Indonesian Journal of Agricultural Science: 3(1) 25 - 32

Idowu, M.A., Akindele, S.A. 1994. Effect of storage of cassava roots on the chemical composition and sensory qualities of gari and fufu. *Food Chem*. 51 (4), 421-424.

Jorgensen, K., Bat, S., Busk, p.k., Sorenson, C., Olsen, C.E., Pounti-Kaerlas, J. and Moller, B.L.,2005. Cassava plants with depleted cyanogenic glucoside content in leaves and tubers. Distribution of cyanogenic glucosides, their site of synthesis and transport and blockage of the biosynthesis by RNA interference technology. Plant Physiol., 139, 363-364.

Lancaster, PA., Ingram, J.S., Lim, M.Y., Coursey, D.G. 1982. Traditional cassava based foods: survey of processing techniques. *Econ. Bot*. 36, 12-45.

Lebot, V. 2009. Observations tropical roots and tuber crops: cassava, sweet potato, yams and aroids. CABI, Wallingford, UK, 2009.

Ministry of Health Mozanbique. 1984. Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 2. Nutritional factors and hydrocyanic acid content of cassava products. *Bull. World Health Org*. 62 (3), 485-492.

Nambiscan, B., Sundarsan, S. 1985. Effect of processing on the cyanoglucoside content of cassava. J Sci Food Agric. 36, 1197-1203.

Poulton, J. E., 1988. Localization and Catabolism of Cyanogenic Glycosides. In Cyanide Compunds in Biology; Rvered, D.and Harnett, S., Eds.; John Wiley & Sons: Chichester, UK, pp. 67-71.

Raji, A A., LLadeinde, T. A. O., Dixin, A. G. O. 2007. Agronomic traits and tuber quality attributes of farmer grown cassava in landraces in Nigeria. J Tropical Agric. 45 (1-2), 9-13.

Reilly, K. Gomez-Vasquez, R., Buschmann, H., Tohme, J., Beeching, J.R. 2004. Oxidative stress response during cassava post-harvest physiological deterioration. *Plant Mol. Biol*. 56 (4), 625-641.

Rolinda, L., Talatala, R.L., Ma, T.P. and Loreto I., 2008. Cyanide Content of Cassava Cultivars at Different Fertility Levels and Stages of Maturity. Department of Science and Technology-Region 10. http://region 10.dost.gov.ph/index.php..

Splittstoesser, E. E., and Tunya, G.O.,1992. Crop Physiology of Cassava; In Horticulture Reviews Vol 13;Eds Janick, J., pp102-127.

Uyoh, E. A., Udensi, O., Natui, v. And Urua, I., 2007. Effect of different processing methods on cyanide contentof garri from four cultivars of cassava. J of Food, Agriculture and Environment, 5(3&4) 105-107.
Wheately, C. C., Orrego, J.I., Sanchez, T. and Granados, E., 1993. Quality evaluation of cassava core collection at CIAT. In Roca, A.M. and Thro, A.M., Eds: Proceedings of the First International Scientific Meeting of Cassava Biotechnology Network; CIAT, Cali Columbia, pp379-383.