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Factors affecting cyanide content in cassava (*Manihot esculenta* Crantz)

[Faktor yang mempengaruhi kandungan sianida dalam ubi kayu (*Manihot esculenta* Crantz)]

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Key words: cassava, cyanide content, detoxification methods, *Manihot esculenta*

Abstrak
Rasa bimbang tentang keracunan akibat memakan ubi dan pucuk ubi kayu mendorong kajian dijalankan untuk menentukan faktor yang menyebabkan kandungan sianida yang tinggi serta cara penyahtoksikan yang boleh diikuti. Usia pokok atau peringkat pertumbuhan tidak mempengaruhi kandungan sianida dalam ubi atau pucuk. Kawalan genetik mungkin lebih ketara kerana varieti dapat dikenal pasti berdasarkan kandungan sianida yang tinggi atau rendah dalam pucuk atau ubi. Walau bagaimanapun, kandungan sianida yang rendah dalam isi ubi tidak menjamin pucuk dan daunnya selamat dimakan. Kemarau didapati cenderung meningkatkan kandungan sianida dalam ubi varieti yang boleh dimakan. Penyediaan ulam secara tradisional dengan mencelur pucuk ubi dapat mengurangkan sebahagian sahaja sianida yang ada. Pengubahsuaian cara ini (dengan mencincang dan merendam pucuk sebelum mencelur) dapat mengurangkan kandungan sianida lagi.

Abstract
Concern over poisoning as a result of eating cassava roots or shoots has led to these studies to determine factors which lead to high cyanide content in these tissues and how detoxification can be carried out. Plant age or stage of development does not seem to have a clear influence on root or shoot cyanide content. Genetic control on cyanide content seems significant as varieties may be identified according to low or high cyanide contents in their shoots or roots. A low cyanide content in the root flesh is no guarantee of 'safe' cyanide levels in the shoots or leaves. Drought tends to increase cyanide content in edible cassava varieties. The traditional method of preparing *ulam* from cassava shoots by blanching is effective in removing only part of the cyanide present. Modifications to the method (chopping and soaking the shoots before blanching) can reduce the cyanide level further.

Introduction

In common with a few other crop species, cassava (*Manihot esculenta* Crantz) produces cyanide in a bound form (cyanogenic glucoside) which is found in all its plant tissues (Nambisan 1992). Free cyanide or HCN, a highly volatile and soluble substance, is released when cell walls are broken down by injury (due to pest damage or mechanical means), thereby liberating the enzyme linamarase which acts on the bound cyanide.

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In the early days of taxonomy, cassava was arbitrarily divided into two species, *M. utilissima* and *M. dulcis*, which were supposed to categorize the high (bitter) and low (sweet) cyanide varieties respectively. However, this classification is spurious, since cyanide content in the roots varies on a continuous scale among varieties, with no real distinct demarcation between 'bitter' and 'sweet' varieties. Moreover, bitterness in taste is not always an indication of cyanide level (Dufour 1994). Other bitter chemical factors present in the root are involved (Bokanga 1994).

Indeed, there have been instances when a so-called 'sweet' variety may produce roots with high cyanide content. In 1989, there was a case publicized in the local newspapers about a family in Felda Titi, Jelebu, Negeri Sembilan, who suffered the effects of cyanide poisoning after eating fried slices of the edible variety Medan. A 2-year-old child died after eating the fried cassava for both tea and dinner.

What then is a 'safe' level of cyanide for cassava to be consumed? Bolhuis (1954) suggested a rough guideline, based on an adult weighing 50 kg:

Cyanide content	Safety level
<50 µg/g*	Innocuous
>50 to <100 µg/g	Moderately poisonous to poisonous
>100 µg/g	Very poisonous

*content in fresh sample of root flesh

The operative phrase here is 'an adult weighing 50 kg'. What this implies is that a child who weighs much lighter will not be able to tolerate the cyanide levels considered 'safe' for an adult.

Some background on the Felda Titi case might provide pointers on why the variety Medan became poisonous. The crop was harvested at close to 2 years, whereas Medan is usually harvested after 10–12 months. The crop was grown in a backyard plot on a patch of sandy soil. Thus, the higher than normal levels of cyanide in the roots could be due to age, or to water stress (because of the sandy soil). It has been suggested that drought is a factor which enhances cyanide content in the roots (de Bruijn 1973; CIAT 1989; Ekanayake 1994). Other factors suspected of having an influence include soil fertility, crop age and flooding (Ekanayake 1994). Also, another family who ate the same source of cassava but boiled suffered no ill effects, suggesting that poisoning is related to the method of cooking. Ameny (1990) reported that steamed roots contained cyanide in the moderately poisonous range, whereas boiled roots were in the innocuous range.

Another pertinent point is that the cyanide level of the root is no indication of the cyanide levels of the shoots and leaves. 'Sweet' or edible varieties will still have leaves with dangerous levels of cyanide. Leaves have been determined to be the site of cyanogenesis (Koch et al. 1992), and as a rule contain much higher levels of cyanide than the roots, often in excess of 400 µg/g fresh sample. In most of Southeast Asia, there is a tradition of eating the shoots of cassava as a vegetable *(ulam)*, but this can be dangerous if no proper precautions are taken during preparation of the dish. However, in the absence of cyanide, cassava shoots are rich nutritionally in protein as well as in vitamins A and C (Lancaster and Brooks 1983).

It is, therefore, the objective of these series of studies to identify the predisposing factors in order that they may be avoided and so eliminate further instances of cyanide poisoning through eating cassava.

Materials and methods

The most popular edible variety, Medan, was used in these trials – by itself or in conjunction with other cassava varieties. Woody stem cuttings of 20 cm length were used for planting. Cyanide content was estimated by the modified sodium picrate paper method which measures the intensity of the colour change by a spectrophotometer (Tan and Noor Auni 1981). While this method provides a mere estimation of the free cyanide in fresh plant samples, and is not as precise as the enzymatic and silver nitrate chemical assays, it is nevertheless useful enough for comparisons of relative cyanide contents.

A sample of plant including roots was collected from the backyard plot of the home in Felda Titi, Jelebu, Negeri Sembilan, where the cassava poisoning was reported. The plant was positively identified by its morphological characteristics to be the variety Medan. When the flesh from a root sample was tested, it was found to be in the very poisonous range (190 µg/g). Ordinarily, Medan root flesh contains cyanide <50 µg/g.

Effect of crop age on root cyanide content

Planting materials from the Medan plant sample collected from Felda Titi were planted and harvested at 3-month intervals, starting from 12 months after planting and thereafter till 24 months. Root samples collected at each harvest were tested for cyanide content in both the root rind and flesh.

In a parallel study, planting materials of Medan from the MARDI cassava germplasm collection were planted in a block, and harvested at monthly intervals, starting at 8 months after planting, through to 24 months after planting. Cyanide contents in the root rind and flesh were similarly assayed.

Effect of drought on root and shoot cyanide contents

Medan was planted together with Black Twig (high cyanide variety) and Perintis (moderate cyanide variety) in soil contained in half oil drums (one plant to each half drum). Holes had been bored in the bottom of the drums for drainage, and the drums placed on zinc sheets to prevent the cassava roots from growing through the holes into the soil in search of water. These drums were watered daily to field capacity. Water was withdrawn (drought conditions) at specific stages and for specific durations by fastening black plastic sheets around base of the stem of the cassava plant and covering the whole surface of the soil in the drums.

There were two phases to this trial. The first fixed the duration of drought at 3–4 weeks, and varied the stage of crop growth when drought was imposed (at 4, 6 and 8 months). The second phase fixed the crop stage when drought was imposed (based on the results of the first phase) and varied the duration of drought (1, 2 and 3 weeks).

In both studies, a control was included in which no drought was imposed throughout the 9-month trial period. The experimental design used in both instances was completely randomized design with four replications.

Effect of harvest stage on shoot cyanide content

While it has been established that cyanide levels in leaves decrease with leaf age (Joachim and Pandittesekere 1944; de Bruijn 1971; Gondwe 1974; Williams 1979), there is little published information on whether cyanide content in the leaves and shoots varies when collected at different stages of cassava growth. If fluctuations or distinct changes exist, they might be important for identifying the best and safest time to harvest cassava shoots for *ulam*.

Six varieties of cassava with varying levels of cyanide in their leaves (as determined from a preliminary screening of the germplasm collection) were planted in the field at MARDI Serdang, and harvested periodically for shoots. The varieties as well as their leaf cyanide and crude protein contents are as shown in *Table 1*.

A randomized complete block design with four replicates was adopted. Twenty-five plants of each variety were grown at the normal 1.0 m x 1.0 m spacing, and normal agronomic practices for cassava (Chan et al. 1983) followed.

For *ulam*, the usual practice is to harvest the shoot including the first three to

Table 1. Cyanide and crude protein contents of six cassava varieties used in the study

Variety	Cyanide content (μg/g)	Crude protein content (%)
Merah Jambu	25	25
Kuning 2	40–150	23
Black Twig	210–440	27
Medan	220–350	25
Lemak	310–920	24
Keriting	680	34

five newly opened leaves. The first harvest commenced at 15 weeks after planting, and harvests were repeated at 14-day intervals thereafter until 55 weeks. At each harvest, the shoots of one plant were collected from each plot.

The shoots were prepared in the traditional way (for making *ulam*) by blanching the whole shoots in boiling water in less than 10 s. The cooled samples were then tested for cyanide content. Duplicate samples of each harvested shoot were assessed.

At the end of 12 months, the plants were harvested for their roots. Samples of the roots were used to determine the cyanide contents of the flesh and rind in each clone. Again, duplicate samples were used.

Effect of preparation method on shoot cyanide content

Shoots of the same six varieties were collected as above, and prepared in one of the following ways, before testing for cyanide.

- Blanching whole shoots in boiling water.
- Chopping up shoots (about 2 mm wide) before blanching.
- Blanching and then chopping the shoots.

A control was included where the chopped raw shoots were tested for cyanide. A completely randomized design was used.

For the blanched leaves, excess water was squeezed out before taking the usual 2-g sample for assaying cyanide. To simulate the acid conditions of the stomach, a solution of 0.5% 1N hydrochloric acid was used to release any bound form of cyanide still remaining in the blanched leaves. Twenty drops of acid were added before proceeding with the rest of the procedure for determining cyanide content. Duplicate assessments were made from each harvested sample.

The dry matter content of all the samples were determined by oven-drying (at 70 °C) the balance of the shoots not used in the cyanide assays.

Modifying preparation methods for further reducing cyanide content in shoots

Blanching in boiling water may deactivate the enzyme linamarase which releases cyanide from cyanogenic glucoside in the cells. Hence, not much of the cyanide is lost by blanching either as vapour or through dissolution into the cooking water. Subsequently when eaten, the material comes into contact with the acid contents of the stomach (simulated by the addition of hydrochloric acid) which are able to release the bound cyanide.

The idea behind this study was to allow more time for the cyanide to be released and escape harmlessly into the air (as vapour) or dissolved in water. The preparation methods in the above study were modified accordingly.

- Shoots chopped, soaked in water at room temperature for 10 min before blanching.
- Whole shoots blanched, chopped and soaked in water at room temperature.
- Whole shoots blanched and soaked in water at room temperature.

The control (chopped raw shoots) was also included. Shoots of the variety Medan were used to test each preparation method.

Results and discussion

Cyanide content in cassava roots in relation to growth stage

Cyanide contents in the roots of variety Medan tested up to 24 months' growth did not indicate any trend towards increasing with age *(Figure 1* and *Figure 2)*. The results also prove that the Medan sample from Felda Titi was not a 'mutant' form of the normal Medan variety. Thus, it would seem that under normal conditions of cultivation, root cyanide in Medan does not increase with a delay in harvest.

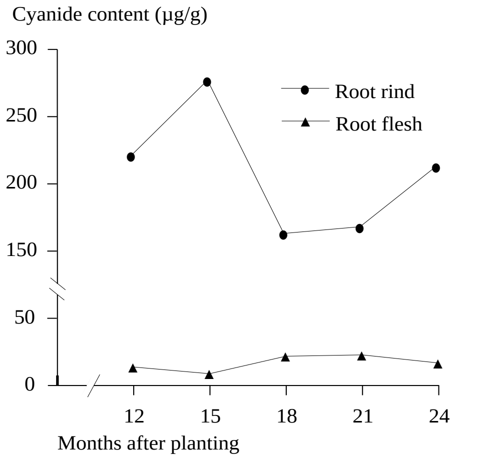


Figure 1. Cyanide contents in the root rind and flesh of cassava variety Medan (from Felda Titi) from 12 to 24 months after planting

Effects of drought on cyanide content in cassava roots

The first part of the study showed no significant differences for cyanide contents in shoots when drought was imposed at different stages of crop growth *(Table 2)*. Nevertheless, there was some indication that withdrawing water at 8 months of crop growth increased cyanide content, especially in the root (both rind and flesh) when compared with drought at 6 months.

Based on these results, the second part of the study was carried out, with different durations of drought imposed at 8 months. In this case, cyanide content in the shoots was significantly affected by duration of drought. In Medan (and to some extent, Perintis), the highest cyanide content was detected when stress was imposed for the duration of 1 week *(Table 3)*. This difference was not found in Black Twig.

Although no significant duration differences were detected for cyanide in the root flesh, the value was highest in Medan and Perintis (both edible varieties) when water was withheld for 3 weeks, whereas in Black Twig (inedible variety), the highest cyanide was detected when the drought lasted 2 weeks *(Table 3)*. For Perintis, normally with flesh cyanide levels lower than Black Twig, the cyanide content was found to be higher (84 µg/g) than that of Black Twig (55–69 µg/g) when water stress lasted 3 weeks.

The results of the above studies, however, were neither very conclusive nor convincing as whether drought in fact

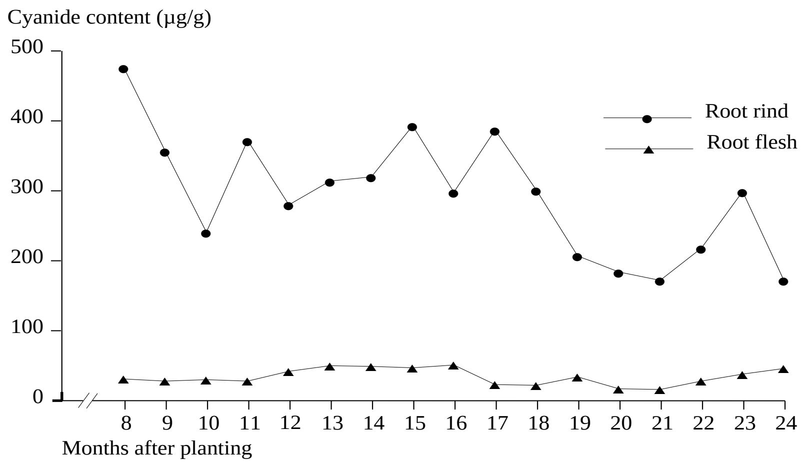


Figure 2. Cyanide contents in the root rind and flesh of cassava variety Medan (from MARDI germplasm) from 8 to 24 months after planting

Table 2. Cyanide contents in the shoot, root rind and flesh of three cassava varieties with drought imposed at different crop growth stages

Variety	Crop stage (months)	Cyanide content (µg/g)		
		Shoot	Rind	Flesh
Medan		180b*	467a	67b
Perintis		537a	288b	146a
Black Twig		289b	251b	125ab
	4	340a**	321ab	107ab
	6	332a	282b	93b
	8	365a	399a	144a
	Control	304a	339ab	106ab
Medan	4	196a	473a	49a
	6	109a	428a	58a
	8	231a	446a	108a
	Control	184a	519a	55a
Perintis	4	575ab	307ab	140a
	6	607a	200b	118a
	8	527ab	373a	186a
	Control	439b	271ab	140a
Black Twig	4	249a	184b	131a
	6	280a	216b	104a
	8	337a	378a	139a
	Control	289a	225ab	124a
C.V. (%)		33.6	32.8	41.8

*Mean over crop growth stages **Mean over varieties

Values in the same grouping within the same column with the same letter are not significantly different from one another according to the LSD test at $p = 0.05$

increases the cyanide content in cassava. There were some indicative trends, but then the magnitude of increase was not substantial, and indeed in the second study, the cyanide levels in the root flesh of Medan were far below what was recorded in the sample taken from Felda Titi. In the first study, cyanide content in the root flesh of Medan conformed with what was normal for this variety in the control where no water stress whatsoever was imposed (only 55 µg/g), but rose to 108 µg/g when drought was imposed at 8 months after planting.

Cyanide content in cassava shoots in relation to growth stage

Over the whole period of harvesting shoots, i.e. from 15 to 55 weeks after planting, at no time did the cyanide content of all the test varieties drop below 100 µg/g fresh weight *(Figure 3A)*. Cooke and de la Cruz (1983), working on a single variety, showed that there was not much variation in leaf cyanide level over 18 months. The cyanide level in the shoots of the variety Keriting was the highest among the six varieties over most of the period of study. This variety which has characteristically crinkled leaves *(Plate 1)* is sometimes found on sale in local weekly farmer markets.

Although some fluctuations were observed, particularly in the more poisonous varieties, they appear to be somewhat inversely related to the amount of rainfall: cyanide levels being lower in the rainy months and higher in the drier months *(Figure 3B)*. A similar relationship was reported by Simwambana et al. (1992).

What is pertinent is that it would appear that there is no 'safe' stage during

Table 3. Cyanide contents in the shoot, root rind and flesh of three cassava varieties with drought imposed for different durations

Variety	Duration (weeks)	Cyanide content (µg/g)		
		Shoot	Rind	Flesh
Medan		213b*	180a	16b
Perintis		252a	149ab	53a
Black Twig		169c	137b	62a
	1	243a**	139ab	36a
	2	208ab	111b	36a
	3	176b	184a	58a
	Control	219ab	187a	45a
Medan	1	256a	168ab	13a
	2	202ab	101b	5a
	3	163b	242a	34a
	Control	231ab	210a	13a
Perintis	1	292a	112ab	34b
	2	254a	96b	34b
	3	221a	180ab	84a
	Control	242a	209a	57ab
Black Twig	1	180a	137a	62a
	2	167a	137a	69a
	3	146a	131a	55a
	Control	185a	142a	64a
C.V. (%)		25.9	48.1	61.4

*Mean over durations **Mean over varieties

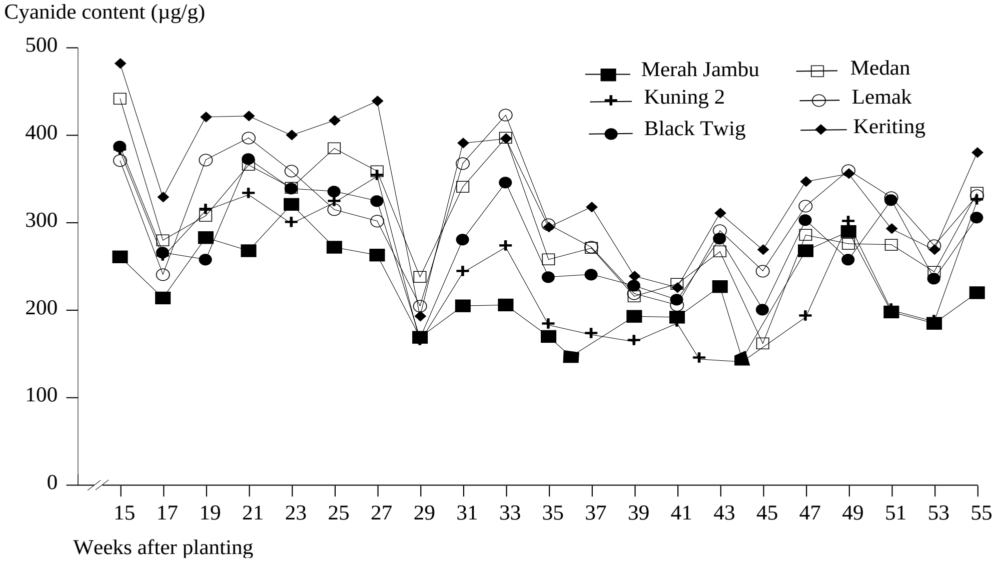
Values in the same grouping within the same column with the same letter are not significantly different from one another according to the LSD test at $p = 0.05$

cassava growth for harvesting shoots to be eaten as *ulam*.

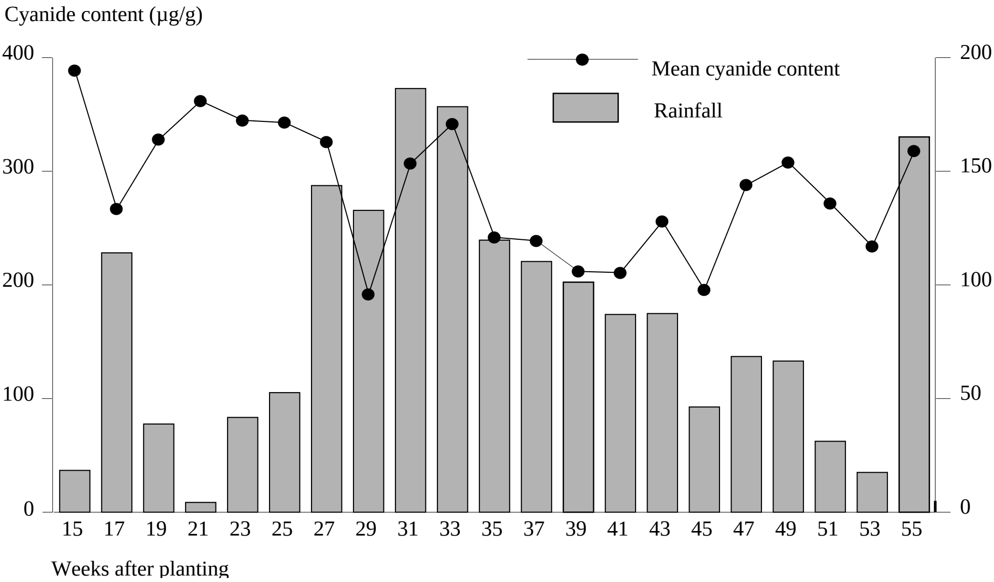
The cyanide contents of the flesh and rind provide the guideline for defining an edible cassava variety. The rind of all varieties tend to be high in cyanide, whereas in the edible varieties, the cyanide in the flesh is much lower, usually less than 50 µg/g. This means that Kuning 2, Medan and Merah Jambu, despite their high rind cyanide contents, can be considered edible because of low contents in the flesh *(Table 4)*. By contrast, non-edible varieties have high cyanide (>50 µg/g) in both rind and root flesh. Black Twig is a typical example. It is most widely cultivated in Malaysia as an industrial variety, used for starch extraction.

Plate 1. Variety Keriting has characteristically crinkled leaves

As mentioned earlier, the edible varieties do not necessarily yield shoots which are safe to eat as well. The cyanide



A. Cyanide content in the shoots of six cassava varieties at different stages of crop growth (15–55 weeks)



B. Relationship of mean cyanide content in the shoots of six cassava varieties at different crop growth stages and rainfall

Figure 3. Cyanide content of cassava shoots at different crop growth stages

contents in the shoots of Kuning 2, Medan and Merah Jambu are all higher than 100 µg/g. In fact, Medan ranked among the highest in shoot cyanide content (in excess of 200 µg/g for most of the period of sampling).

Cyanide content in cassava shoots in relation to preparation methods

On a fresh-weight basis, the shoots of Keriting contained significantly higher cyanide than the other five varieties. Preparation methods produced significant

Table 4. Cyanide contents in the shoots, root rind and flesh of six cassava varieties (fresh-weight basis)

Variety	Cyanide content (μg/g)		
	Shoot[1]	Root rind[2]	Root flesh[2]
Merah Jambu	224a	245a	42c
Kuning 2	240a	237a	20c
Black Twig	282b	211ab	77b
Medan	299bc	249a	28c
Lemak	342d	201ab	72b
Keriting	309c	158b	112a

[1]Mean cyanide content over 21 harvests (15–55 weeks)
[2]Cyanide content at final harvest (12 months)
Values within a column with the same letter are not significantly different from one another according to the new DMRT at p = 0.05

Table 5. Effect of preparation methods on cyanide content in the shoots of six cassava varieties (fresh-weight basis)

Variety	Cyanide content of shoots (μg/g)				
	P1	P2	P3	P4	Variety mean
Merah Jambu	269	135	274	116	198b
Kuning 2	287	148	248	153	209b
Black Twig	332	166	201	163	216b
Medan	302	206	216	171	224b
Lemak	246	138	246	138	192b
Keriting	419	183	349	182	283a
Preparation method	309a	163c	256b	154c	220

P1 = control (raw chopped shoots)
P2 = shoots chopped and blanched
P3 = shoots blanched then chopped
P4 = whole shoots blanched (traditional method)
Means with the same letter are not significantly different from one another according to the new DMRT at p = 0.05

reductions in cyanide content compared with the control, but there was no significant interaction between variety and preparation method.

The traditional method of preparing cassava shoots for *ulam* was generally effective in removing part (about 50%) of the cyanide in the shoots *(Table 5)*. However, the balance of the cyanide left in the shoots is still considered poisonous (154 μg/g). This does not pose much of a problem for an adult because of the small amount of shoots eaten in relation to his body weight. Besides, cassava shoots are not eaten daily as *ulam*. Nevertheless, these levels are dangerous for small children, especially if they eat as many shoots as an adult.

On a dry-weight basis, there were no statistically detectable differences among the six varieties, the four methods of preparation nor the interaction between clone and preparation method for cyanide level in the shoots.

Cyanide content in cassava shoots with modifications to preparation method

Modifying the preparation method by including an additional step of soaking in water (at room temperature) was successful in reducing the cyanide level further in the shoots of Medan. It has been possible to further reduce the cyanide content in the blanched shoots to a level of 110 µg/g when these were minced and soaked in water after blanching *(Table 6)*. This may be the result of allowing time for the free cyanide released by the enzyme linamarase from the cells (ruptured by mincing the leaves) to dissolve in the water. Immediate blanching in boiling water after mincing probably deactivated the enzyme so that the cyanide remained in the bound form within the cells. It is possible that the cyanide is subsequently released in the stomach when it comes into contact with the hydrochloric acid present there, and this is supported by the results of the earlier study.

It may be concluded that although cassava shoots may represent an important source of protein, especially for the rural population, when eaten as *ulam*, there is in fact no 'safe' period for harvesting these shoots as far as cyanide content is concerned. Also, cassava varieties which are well-known as having edible roots (i.e. <50 µg/g in the root flesh) are not guaranteed to have low cyanide in their shoots and leaves. Shoots (and leaves) invariably contain more cyanide than the root flesh on a fresh-weight basis.

While the traditional method of preparing cassava shoots for *ulam* (i.e. blanching the whole shoot) is effective in reducing cyanide content by half, the remaining cyanide is still present at potentially lethal levels. By introducing the two extra steps of chopping the shoots after blanching and soaking them in water for about 10 min before serving will help bring down the cyanide content further to a level around 100 µg/g. Chopping or crushing the leaves prior to boiling have been recommended in place of boiling whole leaves (Charavandapavan 1944; de Bruijn 1971; Williams 1979). Also, work by Padmaja et al. (1994) shows that cyanide in whole leaves can also be reduced substantially by placing them in cold water which is then slowly brought to boil.

Connoisseurs of cassava shoots as *ulam* are well advised to give attention to preparation methods which reduce the cyanide content. Young children should be discouraged from eating cassava shoots, or at least should only be allowed to eat small amounts, as the remaining cyanide in the blanched shoots can still be toxic when body weight is low.

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Table 6. Effect of modified preparation methods on cyanide content in the shoots of cassava variety Medan

Modified preparation method	Cyanide content (µg/g)	
	Fresh basis	Dry basis
Chopped and soaked before blanching	138b	636ab
Blanched, chopped and soaked	110b	434b
Blanched and soaked	184ab	874a
Raw, chopped (control)	246a	904a

Values within a column with the same letter are not significantly different from one another according to the new DMRT at p = 0.05

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