JNSF 1 - 2

*Journal of the National Science Council of Sri Lanka*, 1 (1973), 83--96

# Manioc : Selected Topics

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(Accepted for publication : October 4, 1973)

## 1. *Introduction*

The Manioc (cassava, mandioca, yuca) plant originating in tropical America, is widely cultivated in Africa, Asia and Latin America. There are over 200 cultivars of the plant and these have been classified on external morphology[81], cyanogenic glucoside content and other criteria. Workers tend to classify the varieties of the plant in different ways : *Manihot utilissima* Pohl and *Manihot esculenta* Crantz are considered to be one species by some groups[54] while others[31] class these two as different species.

Most of the data on manioc in this review has been taken from work published under these two names. They have been treated as synonyms including all varieties yielding an edible tuber.

The notion[86] that "sweet" varieties of manioc that contain *no* cyanogenic compounds are available, has not been recognised in any of the extensive studies on this aspect[40,65]. It is common to refer to "*sweet*", "*average toxic*" and "*bitter*" varieties of manioc ; these terms generally refer to those varieties in which the edible portion contains less than 50, 50-- 100 and more than 100 mg of cyanide per kg flesh respectively. However, this classification is basically incorrect, as the cyanide content of a single cultivar varies when it is grown under different environmental conditions[54]. Further, Sinha and Nair[89] have shown that the bitterness of tubers cannot be attributed to the concentration of cyanogenic glucosides alone.

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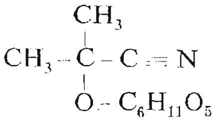
## 2. *Cyanogenic Glucosides of Manioc*

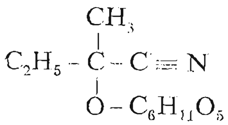
2.1. **Chemistry:** There are two cyanogenic glucosides of manioc: *Linamarin*[71] (A) and smaller amounts of *Lotaustralin*[11] (B).

(A)



$$CH_3-\overset{CH_3}{\underset{O-C_6H_{11}O_5}{C}}-C\equiv N$$

(B)



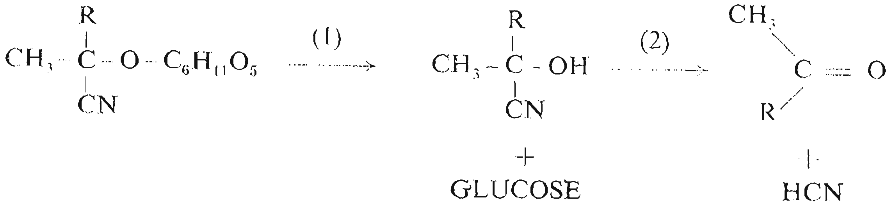
$$C_2H_5-\overset{CH_3}{\underset{O-C_6H_{11}O_5}{C}}-C\equiv N$$

In nature, linamarin was found to be the β anomer[18]. The amino acids valine and isoleucine were found to be the precursors for the aglycone moieties of the glucosides (A) and (B) respectively[70]. Methods are available for the isolation and purification of linamarin[18,99]. The method by Wood[99] has been tested by one of the authors and it was found that the glucosides can be isolated efficiently by this method (~0.3% the wet weight of rind for local types).

The glucosides which are soluble in water, are stable to boiling but are decomposed at 150°C[17] *and* by the enzyme linamarase (linase) which acts on both linamarin and lotaustralin[70] to produce glucose, hydrogen cyanide and the corresponding ketone. Conn[22] has described the action of hydrolysing the glucoside to HCN as a two step process :

(*a*) The conversion of the glucoside to the corresponding cyanohydrin by a specific β glucosidase (linamarase)

(*b*) Hydrolysis of the cyanohydrin by a hydroxynitrilelyase to give HCN and the corresponding ketone (acetone or propanone).



$$CH_3-\overset{R}{\underset{CN}{C}}-O-C_6H_{11}O_5 \xrightarrow{(1)} CH_3-\overset{R}{\underset{CN}{C}}-OH + \text{GLUCOSE} \xrightarrow{(2)} \overset{CH_3}{\underset{R}{C}}=O + HCN$$

$R = CH_3,\ C_2H_5$

Linamarase is reported to be deactivated at 72°C.

Various methods of *assay* for the cyanogenic glucosides have been used The assay generally has three phases :

(i) liberation of HCN from the glucoside

(ii) isolation of the cyanide *and*

(iii) quantitation of the cyanide.

Autolysis of the glucoside following maceration, acid hydrolysis, addition of extra enzyme or a combination of these methods yields HCN which is then distilled and estimated after reaction with alkaline picrate, silver nitrate, benzidine derivatives, ferrous sulphate or with other reagents that form coloured compounds with the cyanide.

It appears that most of the early controversy on HCN content of manioc and manioc products, was due to incomplete liberation of HCN, by the methods of assay used.

Most of the early assays [24, 53, 71], depended on either autolysis with endogenous linamarase or on acid hydrolysis with varying strengths of sulphuric acid. Autolysis is not satisfactory as endogenous enzyme levels are low and it therefore requires long incubation times, during which competing reactions appear to lower the total potential yield of the cyanide[80]. Acid hydrolysis has been a popular method inspite of the studies of Wood[99] who warned that amides and ammonia are formed under these conditions, which lowers the amount of HCN released, drastically. In order to test this theory, pure linamarin was hydrolysed with varying normalities of sulphuric acid[48]. The results (*Table I*) showed that :

(*a*) Reduced yields are obtained with acid hydrolysis

(*b*) The normality of acid used affects yield markedly.

A similar conclusion has been reached with studies using plant tissue[80].

Table I. Acid hydrolysis of Linamarin

| Treatment | HCN released (%) | Apparent glucoside remaining* (%) |
|---|---|---|
| Enzyme hydrolysis | 83 | |
| Acid Hydrolysis† | | |
| 0.1 N acid | 02 | 81 |
| 0.2 N | 02 | |
| 1 N | 08 | 41 |
| 2 N | 28 | 37 |
| 4.5 N | 47 | 05 |
| 7 N | 55 | 02 |
| 9 N | 53 | 02 |
| 12 N | 51 | 02 |
| 14 N | 34 | 00 |
| 18 N | 08 | 00 |

†Linamarin with the appropriate strength of acid was steam distilled for 1.5 hours.

*The glucoside remaining after acid hydrolysis was determined by adjusting pH to 5·0 followed by hydrolysis by the enzyme. The results are corrected for residual free cyanide from acid treatment (approximately 2%).

Wood[99], de Bruijn[25] and Zitnak[102] have recommended the addition of extra enzyme for assays. This concept has been shown to be correct by recent studies by our group[80], at the CISIR.

The isolation of the cyanide has also produced problems. Air aspiration, a popular method of isolation of HCN formed, has been found wanting[80]. The pH of the medium is important, as very acid pH results in increased binding of HCN[80, 102].

Zitnak[102] has recently outlined the problem arising out of poor sampling techniques which can give misleading results. This problem together with the incorrect assumption that acid treatment results in complete hydrolysis reduces the value of results obtained in many early studies, on cyanogenic glucoside content of manioc.

2.2 **Factors affecting glucoside concentration:** The concentration of cyanogenic glucosides in manioc depends on the variety, stage of development and environment[25]. All parts of the plant contain these compounds. The concentration varies markedly from tissue to tissue, the tubers and the leaves containing the largest amount of glucoside[65]. However, there are variations in glucoside content within the same tissue *e.g.* the young leaves have more toxic factors than the old leaves. The proximal end of the root has more glucoside than the distal end[25].

Soil conditions affect glucoside content markedly *e.g.* while increased nitrogen and drought increased glucoside content, increased potassium and farmyard manure decreased its level. Glucoside content was also found to increase at the onset of rains[25].

Several other factors seemed to affect glucoside concentration : (*a*) shading of plants increased the level in the leaves and reduced the level in the roots ; (*b*) planting of cuttings vertically resulted in less glucoside in the tubers than if the cuttings were planted horizontally ; (*c*) ringing of the stem and leaf elimination resulted in at least a temporary decrease in the toxicity of tubers[25]. Such data are interesting and perhaps with more work the information may be applied practically.

It has also been suggested that growth regulators like NAA, IAA *etc.* may be used to produce tubers with reduced glucoside[46, 88].

2.3 **Elimination of cyanogenic compounds:** Both enzyme and glucoside are found throughout the plant (the enzyme is present in largest quantities in the leaf where it can be found in quantities over 100 fold that of the tubers). However, in fresh tissues the enzyme and substrate are not in contact (intracellular localisation?). Crushing and soaking in water facilitates contact between the two and hydrogen cyanide is liberated[53]. Most methods of detoxification involve this principle[71]; but it is critical to give the enzyme sufficient time to act[67].

Boiling has been used to eliminate toxic factors. This has been successful in the case of leaves where enzyme concentration is high and diffusion barriers are small[25]. Boiling tissues with low concentration of enzyme while causing elimination of most of the cyanide, probably results in the quick elimination of enzymic activity (which accounts for the remaining glucoside). According to de Bruijn[25], 90% of the glucoside was still present after boiling. However, earlier studies[23, 78] did not report any glucoside after boiling (poor analysis?). Several intermediate values have been reported. The amount of glucoside could be predicted to vary widely depending on:

(i) the extent tubers are sliced
(ii) the amount of washing
(iii) enzyme activity
(iv) time taken for the temperature to rise to 70° C.
(v) initial concentration of glucoside, etc.

Adriaens[2] suggested that glucose can detoxify HCN formed but this has been disclaimed by de Bruijn[25].

2.4. **Ingestion of toxic factors:** Toxic factors of manioc include free cyanide, cyanohydrins and glucoside. The toxicity of cyanide is well known. The lethal dose of prussic acid varies from person to person. The average lethal dose for an adult is reported to be between 50 and 60 mg[54] but the amount also appears to be affected by the type of other foods eaten with it[72]. The lethal effect of cyanide is caused by its reaction with the iron in hemoglobin (and possibly cytochromes) and copper in cytochrome oxidase. However, very little free cyanide (less than 5 p.p.m.) is consumed after the normal cooking procedures in this country for manioc : *peel, cut, wash, boil in open vessel, throw out water and reboil.* On the other hand, considerable amounts of glucoside could be ingested especially if the initial glucoside content is high. It might be expected that quantities of glucoside consumed might, in some cases, exceed the toxic level if it were free cyanide. Although the glucoside itself may not be toxic it has to be considered as a potential source of cyanide.

It is possible that the reason why deaths recorded due to manioc consumption are rare, is due to a detoxification mechanism suggested by Clark[19]. His hypothesis is that the sulphoamino acids of proteins react with the cyanogenic glucoside and prevent the liberation of HCN. The hypothesis has been given more credence by his later studies[20], which showed that high cassava diets short in methionine resulted in symptoms of HCN poisoning and by those of Osuntokun *et al*[73] and Montgomery[66] who showed that consumption of manioc as a staple food (2 to 3 meals a day) was associated with a rise of the blood thiocyanate level. However, there seems to be no evidence that it is the glucoside and not free cyanide that is detoxified.

More recently Montgomery[66] outlined the *pathways of metabolism* of inorganic cyanide. At least three pathways appear to exist : (*a*) the conversion of cyanide to thiocyanate by thiosulphate catalysed by the enzyme rhodanase, (*b*) the conversion of cyanide to thiocyanate by reaction with 3 - mercaptopyruvate catalysed by another sulphotransferase. Cysteine and cystine are the precursors of 3 - mercaptopyruvate, (*c*) uptake of cyanide by vitamin B - 12.

The concept that lethal quantities of cyanide could be ingested as glucoside without toxification is significant when one considers the effect said to be caused by some vegetable juices on manioc consumers. It has been commonly reported that if uncooked vegetable foods are taken with manioc, the consumer is subject to violent discomfort that may even result in death[54]. In this country, ginger is popularly believed to cause this effect. It is also believed that some vegetable juices contain an enzyme that will decompose linamarin. It seems equally possible that these vegetable juices may be interfering with a detoxification mechanism.

Recent work by our group[51] have shown that very small amounts of cyanide can be released from linamarin by ginger extracts; the effect is only $10^{-4}$ to $10^{-5}$ times that from an equal weight of manioc and is likely to be due to the effect of non-specific $\beta$ glucosidases. Hence it is difficult to explain the popularly believed effects on this basis. However, it is not possible to rule out adverse effects being caused by eating manioc and ginger together because the mechanism of the effect may not be as what it was hitherto assumed.

It has also been suggested that gut flora can cause the conversion of glucoside to cyanide.[5] However work in this laboratory[51] has shown that coliforms (7 types tested) cannot do this ; work with other bacteria is planned.

Another problem is the popular belief of toxification after the consumption of old and damaged tubers. Although these tubers will contain more free cyanide, the latter will be driven off to a large extent by boiling. These tubers generally have bluish or greyish areas said to be due to the formation of metal cyanides. Some metal cyanides are insoluble and would not be eliminated by standard cooking procedures but would be converted to free cyanide in the stomach. More scientific data are needed to answer three questions in this area :

(i) how relatively toxic are these old tubers ?

(ii) are metal cyanides the only possible cause for the presumed increase in toxicity ?

(iii) do cyanohydrins play any role in this connection ?

All these indicate that provided the tubers are fresh, and normal precautions (of eliminating cyanide) are followed, there would be little, if any, danger of loss of life. Worthy of more consideration is the possibility that repeated sub-lethal doses of cyanide (or glucoside) might cause cumulative poisoning.

Sub-lethal doses of cyanide (and/or glucoside) are reported to cause high blood thiocyanate levels which are associated with neurological diseases[73,74]. No doubt some of the diseases attributed to manioc have been due to nutritional imbalance caused by regular consumption of manioc without an adequate supplement of proteins and vitamins. However, Nigerian Ataxis Neuropathy was not relieved by the administration of vitamins, the lack of which was supposed to cause the disease. Although evidence is not complete it is highly suggestive. Extensive manioc consumption (staple food, 2—3 meals a day over a period of years) is also associated with:

(i) Goitre[25,33,34] probably due to higher levels of thiocyanate which is reported to be goitrogenic

(ii) Lebers Optic Atrophy[98] which is reported to be due to hereditary defect in cyanide detoxification *and*

(iii) K Diabetes[76].

It might be pertinent to note that most methods of preparation of manioc products still result in residual glucoside.[53] Also, several studies have shown the presence of glucoside and/or cyanide in manioc chips and flour, in some cases the quantity being high[80,85] while other studies did not detect toxic factors[8].

The conclusion reached is that several aspects pertaining to the toxic factors in manioc are still unknown. The situation demands extensive research of an interdisciplinary nature.

## 3. *Products from Manioc*[96]

Two common forms of manioc carbohydrate are available (i) *Starch* and (ii) *Flour*. Starch is the pure carbohydrate while flour, (the ground product of chips) in addition to starch, contains protein, cellulose and other constituents of the tuber cells.

Two types of *starch* can be prepared from manioc : (i) edible starch and (ii) industrial starch. The essential difference between the two are that edible starch needs : (*a*) better raw material and hygiene ; (*b*) equipment made of brass, bronze, aluminium or stainless steel[9] or other equipment which would not form insoluble products with HCN (such as iron or mild steel). Some questions may be raised on the use of some of the above materials suggested by Biema & Shipman[9] ; (*c*) precautions to be taken during the settling and drying process to prevent microbial growth ; (*d*) water which has been treated (e.g. lime-alum) and is bacteriologically satisfactory.

Studies in several laboratories, including the CISIR[49], have shown that a clean manioc starch is not difficult to prepare. However, the big problem is the efficient disintegration of cells ; although manioc contains 28—35% starch, generally only two thirds of this is available due to incomplete disintegration of cells.

Manioc consists mainly of carbohydrate which is highly digestible[12,41]. However, its protein content is very low[61] (around 1%) but the quality of proteins is good. Due to its low *protein* content, manioc consumption in the absence of adequate protein is not advisable. Studies have shown, however, that if an additional protein source is provided, manioc could be used extensively as a staple food (assuming of course, that cyanide has been eliminated). Protein sources that can be used include groundnut[28,68,69] gram flour and fish flour[87], chick pea and coconut soya flour[94]. Yeast protein and skim milk have also been used as a protein supplement.

Processed manioc can be used as a source of calories in the manufacture of *food products* such as (i) bread[13,38], roti[53], stringhoppers[42] macaroni[92] and chappati[91]. Drawbacks to its extensive use in bread are, (*a*) the lack of protein which affects the textural quality of the products and (*b*) possible toxic factors. Considerable work has been done to improve textural quality and nutritional value by blending with other flours and by addition of sources of protein[64,27,57,58,59].

Several minor products have been produced for consumption from manioc, such as gari and farinha (microbiologically fermented products) and tapioca ("sago")[47].

Manioc starch has been used as a starting material for the production of *glucose*[10,30,77,84].

Glucose can be produced by acid hydrolysis or enzymically with the starch hydrolysing enzyme glucoamylase. This enzyme has been produced using local raw materials by our group[50] and it is capable of giving a 90% yield (on theoretical), of glucose.

Manioc has been used as a raw material for production by a *fermentation process* of beer[1], ethanol[6], vegetable cheeses[14,21], yeast[29], glycerol[63] and citric acid[75]. Manioc starch also finds a place as a minor component in several food products.

Manioc has been used extensively in animal and poultry *feed*[3,44,62]. Manioc starch has enormous potential for industrial use. The use of manioc starch in the textile[36], paper[4] and adhesive industries is well known. Manioc glue (NaOH treatment) has had considerable use in wood furniture manufacture[35] before the success of synthetic adhesives.

Manioc starch can be used as a raw material for the manufacture of *modified starches*; cationic starch[15]; starch esters[16], dialdehyde starch[95], oxidised starch[55,83,101], $AlCl_3$ treated starch[56]; starch ethers[60]; cross-linked starch[100] etc. The wide applicability of modified starches in industry is well recognised[97]. Manioc starch has properties which differ from that of other starches and it is probable that highly specialised modified starches may be prepared from it.

The use of manioc starch in the *adhesive* industry deserves special mention. This source of starch was considered indispensable in the production of remoistening gums[45]. Alkaline oxidized manioc starch[52] and enzyme modified manioc starch[37] have also been extensively used as adhesives.

Manioc has also been used as source of raw material for the production of acetone and butanol[43], cellulose[63], particle board from stalks[30] and HCN for coagulating rubber latex[90]; manioc leaves have been used as a protein source[32,79,82].

## 4. *Manioc and Pollution*

The manioc starch industry is a significant source of water pollution in Thailand[93] and Brazil[7]. In Thailand, heavy pollution gives a strong offensive odour and the fauna and flora of waterways into which the effluent is discharged are greatly affected[93]. Pollution can be observed even in the small scale operations in Sri Lanka.

The main waste products in a manioc starch industry are (i) pulp wastes and (ii) wash water. The pulp waste contains large amounts of starch and is quickly susceptible to microbial attack. The wash water has a biological oxygen demand of over 4,000 mg/l and contains about 1% total solids[93].

Further, the water will have a cyanide content (free and bound) probably well over the allowed level for surface disposal.

Although waste treatment is desirable it is often restricted by financial resources. The ideal solution would be the utilization of wastes, (which could offset costs of treatment or possibly even result in a profit). Such studies have been made in Thailand[93]. In this country manioc pulp waste has been made use of as a cattle feed after drying and milling. (*Private communication*). Laboratory trials by our research group[49] have shown that manioc pulp waste can be used as a source of carbohydrate for yeast production. A mixture of acid hydrolysed pulp with added wash water and $(NH_4)_2SO_4$ can produce a 15% yield (on manioc pulp) of yeast cell material. The wash water itself supports yeast growth.

## *Acknowledgements*

The authors thank : the Director CISIR for facilities provided, Mr. E. E. Jeya Raj Head, Industrial Microbiology Section CISIR for encouragement during work on the review, Miss L. Wimalasekera and others on the CISIR Library Staff for collecting the literature references on the topic, Mrs. N. M. Pieris, Mr. D. J. Abeyratne and the other Staff of the Industrial Microbiology and Analytical Chemistry Sections of the CISIR for assistance in the laboratory work and finally Mrs. I. Bandara for Secretarial assistance.

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