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Cyanogenic Glucoside Content of Manioc

III. Fate of Bound Cyanide on Processing and Cooking

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Abstract : On preparation of manioc starch, bound cyanide is converted to free cyanide and removed with the wash water. Whereas, aging of moist manioc chips leads to a decrease in total cyanide content, aging of whole tubers results in increased total cyanide content of the flours prepared by these processes. The cyanogenic glucosides of manioc flour are partly lost when bread and 'roti' are prepared. However, 'roti' contains free cyanide. Preparation of 'pittu' from manioc flour and fried manioc chips from fresh manioc gave products with negligible free cyanide but nearly the full amount of bound cyanide. The above results are discussed in terms of the linamarin-linamarase reaction.

1. Introduction

Manioc contains cyanogenic glucosides and also the enzyme (linamarase) which causes its hydrolysis. In the plant material the enzyme and glucosides are localised such that they do not interact unless the cells are damaged. When this occurs, reactions take place which result in the liberation of cyanide.[2]

Traditionally manioc has been consumed in this country in the form of the boiled product (the cyanogenic glucoside content of boiled manioc has been reported previously[4]). However, the manioc tuber has poor storage characteristics and in addition to quick microbial spoilage, it has also been reported that the cyanide content of the tuber increases on keeping.[5] In this study we present results that are consistent with the above report. As a result, it is clear that if manioc is to be used extensively, it must be processed to a form with better keeping qualities.

The two main forms of processed manioc are starch and flour. Starch is the product of a wet extraction process of manioc and contains very little cyanide,[4] while flour prepared by a dry-milling process contains considerable quantities of cyanogenic glucosides when no special detoxification process is used.[3] Manioc flour would be used in preference to manioc starch as a substitute for wheat flour in several bakery products and other traditional types of food such as ' roti ', ' pittu ', etc. because of its lower cost. Although methods of detoxifying flour were available,[3] the flour used for cooking purposes usually contains sizeable amounts of cyanogenic glucosides.

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In this study, the effect of cooking processes such as frying, roasting, baking and steaming on the cyanogenic glucosides of the manioc flour used has been investigated. The fate of bound cyanide when starch is prepared and also when manioc is processed after aging of the moist chips has been studied.

All assays have been done after enzymic hydrolysis of cyanogenic glucosides using excess linamarase to ensure complete hydrolysis.[4]

2. Experimental

2.1. Sampling

2.1.1. *Tubers for aging experiments* (*for section* 3.3)

Manioc (50 to 60 lbs) of the same variety was harvested with supervision and divided at random into 10 batches each containing 5 to 6 lbs. Each batch contained about 10 tubers. Two batches were processed for each experimental point.

2.1.2. *Sampling of chips* (*for section* 3.2)

Method (1) : Manioc was sampled by the method of quartering described earlier[4] which were uniformly chipped to give 2 samples of similar cyanide content.

Method (2) : Manioc (about 20 lbs) was chipped and sampled into batches as described.[3]

2.2. Preparation of manioc flour

The manioc chips (spread out as much as possible to give quick drying) were dried in a forced draft oven at 55° C. For aging experiments, each experimental point was done in duplicate. The dried chips (of each sample) were powdered separately and the total cyanide content determined. Flours for the preparation of ' pittu ', ' roti ' and bread were assayed and used after sieving (600μ).

2.3. Preparation of manioc starch

Manioc starch was prepared by homogenisation of the peeled, washed manioc tuber (approx. 600 g) with 1 litre of water in a Waring blendor. The starch milk was sampled for total cyanide determination and separated from the pulp by sieving to give the 1st pulp wash. The pulp was washed twice with similar volumes of water and sieved each time to give the 2nd and 3rd pulp washes. The remaining material which does not pass through the sieve (unbroken cellular material) is termed the pulp waste. After the specified time, the wash water of each pulp wash was separated from the starch cake to give the 1st starch wash waters. Each starch cake was resuspended in water and left to settle to give the three 2nd starch washes. Free and bound cyanide were determined on each of the starch washes, the pulp waste and starch (on the former directly and on the other two after drying in a forced-draft oven for 24 h).

2.4. Preparation and sampling of cooked products

Manioc bread (30% manioc flour and 70% wheat flour) was prepared by incubating the mixture (containing yeast and other requirements) for 3 h and 5 h and baking at 220°C for 25 min. A wheat flour bread control was used as a blank. The bread crust ($\sim$ 1 cm) was separated from the rest of the loaf and both types of sample were cut into pieces about 1 cm cube. Random samples containing about 10—15 g dry weight were withdrawn for cyanide and moisture determination.

Manioc 'roti' was prepared by mixing manioc flour and scraped coconut in the ratio of 2 : 1. A small amount of water was added to give the correct consistency and the mixture made into a mass of approximately $\frac{1}{2}$ cm thick and 15 cm diameter and left to stand (for the given time) before roasting. Roasting was done on an aluminium plate heated by a direct flame, a little coconut oil being smeared to prevent sticking. After the roasting process, the roti was cut up into pieces of about 1 cm square and random samples were withdrawn for analysis. Parallel wheat flour samples gave no blank values on assaying for total cyanide.

Manioc pittu was prepared using a 1 : 1 mixture of scraped coconut and manioc flour. Water was added to the mixture until discrete pellets formed and the mixture was immediately introduced into a steamer of about 7 cm diameter and 20 cm long and the pittu steamed for 15 min over boiling water. The pittu was then crushed and sampled for analysis.

Fried manioc was prepared by frying chips approximately 2 mm thick in coconut oil until slightly brown in colour (moisture level of 1—2%). The fried chips were powdered for the determination of free and bound cyanide, moisture and linamarase activity.

2.5. Determination of free and bound cyanide

This was done by the methods described.[4] However, for studies on cooked manioc products, about 300 units of linamarase were used on samples of about 15 g dry weight.

2.6. Linamarase activity

In this study we have attempted to gauge the relative linamarase activity of samples of flour by estimating the amount of cyanide released at fixed intervals after incubating 10 g dry weight of flour with 100 ml of water. This is not the absolute linamarase activity as the substrate linamarin (in the material) is not saturating. There also appeared to be a time-lag for this reaction possibly for the absorption of water by the flour. We would prefer to call this set of data an 'enzyme activity index' and it is felt that this index is more significant than absolute linamarase activity in this instance.

3. Results

3.1. Fate of cyanide in the preparation of starch

Results showed that more than 80% of the glucoside was converted to cyanide and removed in the wash water (Table 1). Results of other experiments showed that complete conversion of the glucoside to cyanide may occur as early as 4 h after disintegration of the cells (depending on the material used). It was also found that both the pulp waste and the starch, after drying in a forced-draft oven, contained only small quantities of total cyanide.

TABLE 1. Fate of cyanide during processing of manioc to starch.

		Cyanide *mg/kg Tuber (fresh wt)*	
Pulp wash	Starch wash	Free	Bound
1st	1st	117	<3
2nd	1st	13	<3
3rd	1st	<3	<3
1st	2nd	4	—
2nd	2nd	<3	—
3rd	2nd	<3	—

The original manioc sample contained 163 mg CN^-/kg fresh weight. The pulp was washed 3 times (1st, 2nd and 3rd pulp washes) and sieved and left to settle for 24 h. The supernatant (1st starch wash) was tested for free and bound cyanide and the starch cake resuspended in water and left to settle for a further 24 h for the 2nd starch wash.

— not determined.

3.2. Effect of aging of moist chips

These experiments were done to check the commonly believed hypothesis that moist chips on aging followed by drying gave reduced total cyanide levels in the flour prepared from it. The experiments were done with two methods of sampling. Both methods resulted in the duplicate samples of flours giving total cyanide levels that tallied to within 15% error. Table 2 shows the results obtained using sampling method 1. Each batch originated from different manioc samples and a strict comparison on the effect of aging cannot be made from one batch to another due to differences in enzyme levels from batch to batch. The results show that there is a progressive loss of cyanide with aging. A similar conclusion was reached with method 2 of sampling (Table 3) where all points were obtained from the same batch of manioc. It must be noted, however, that although 16 to 24 h aging results in the loss of a large part of the cyanide, the chips frequently showed sliminess and odour associated with bacteriological spoilage.

TABLE 2. Effect of aging moist manioc chips.

Batch No.	Time of aging (h)	Total cyanide mg/kg (dry wt) Initial (no aging)	Final
1	4	233	246
2	4	134	114
3	8	288	134
4	8	349	154
5	16	334	176
6	16	267	86
7	24	350	74
8	24	157	42

Manioc was sampled by method 1, chipped, dried at 55°C for 24 h in a forced draft oven and powdered after which total cyanide was determined.

TABLE 3. Effect of aging moist manioc chips.

Time of aging (h)	Total cyanide mg/kg (dry wt) A	B
0	179	153
4	148	151
8	118	134
16	75	73
24	33	32

Manioc was sampled as in method 2, chipped, dried at 55°C for 24 h and powdered. Duplicate samples (A and B) were tested for total cyanide after aging (room temperature).

3.3. Aging of manioc tubers

This set of experiments was done in order to determine if the total cyanide content of manioc flour was affected by the aging of the tubers. Results obtained showed that duplicate preparations of flour showed deviations on some occasions. It was felt that this was due to inadequate sampling of tubers (the cyanide content of tubers varies very widely and therefore a representative sample of tubers can be obtained by using a very large number of tubers per sample). However, the results obtained (Table 4) show a definite trend of increased cyanide content, the magnitude of which tallied with the findings of Rajaguru.[5]

TABLE 4. Effect of aging manioc tubers.

Batch	Time of aging (h)	Total cyanide mg/kg (dry wt) A	B
1	1	102	106
	6	147	104
	11	106	129
	24	136	151
	31	137	157
2	1	70	92
	24	134	159
	48	150	121
	72	152	216
	96	187	198

Duplicate batches of manioc tubers (A and B) sampled as in methods (Section **2.1.1**) were chipped, dried at 55° C for 24 h, powdered and total cyanide determined after aging for the above periods.

TABLE 5. Cyanogenic glucoside content of manioc 'roti'.

Batch	Incubation time (min)	Cyanide in 'roti' mg/kg manioc flour (dry wt) Free	Bound
A	10	24	44
	20	16	35
	30	24	14
B	0	15	63
	30	13	48
	60	21	36
C	0	29	29
	30	38	08
	60	25	06
	120	32	08

Original flour for Batches A, B and C were 138, 106 and 106 mg CN^-/kg (dry weight) respectively. 'Roti' was roasted after incubating the flour—coconut mixture for the above times.

3.4. Cyanogenic glucoside content of manioc 'roti'

Preparation of 'roti' (roasting) using samples of manioc flour of known total cyanide content showed (Table 5) that there was: (1) a loss in bound cyanide and (2) an increase in free cyanide. The loss of bound cyanide increased with (i) time of incubation before roasting and (ii) linamarase activity of flour (Table 6). The prepared roti (after roasting) contained no linamarase activity.

TABLE 6. Enzyme activity index of manioc flours.

Batch	Time of incubation (min)	Cyanide liberated mg/kg (dry wt)
A	0	14
	15	15
	30	49
	60	73
B	0	18
	15	31
	30	49
	60	56
C	0	08
	15	38
	30	71
	60	92

Batches A, B and C were the same as those given in Table 5. Cyanide liberated by linamarase present in the flour was determined after incubation for the above times.

3.5. Cyanogenic glucoside content of manioc bread

A similar loss of total cyanide was observed on baking. The loss in the crust was more than that in the inner portion of the bread (Table 7). This reduction is possibly due to the decomposition of the glucoside (which undergoes thermal decomposition at $\sim$ 150°C) ; the temperature of the surface of the bread being higher than the inner portion by more than 100°C.

3.6. Cyanogenic glucoside content of manioc 'pittu'

'Pittu' (which is prepared by a process of steaming) was found to lose very little total cyanide (Table 8). The preparation was steamed immediately after mixing. Pittu samples had, when present, relatively small amounts of free cyanide and no linamarase activity.

TABLE 7. Cyanogenic glucoside content of manioc bread.

Time of fermentation (h)	Part of bread analysed	Total cyanide mg/kg manioc flour (dry wt)
3	Crust	47
		53
5	Crust	40
		30
3	Inner portion	97
		103
5	Inner portion	104
		106

Manioc flour sample used contained 196 mg total CN^-/kg (dry weight). Wheat flour blanks were equivalent to 5 and 7 mg CN^-/kg for the crust and inner portion of the bread respectively.

TABLE 8. Cyanogenic glucoside content of manioc 'pittu'.

		Cyanide, mg/kg manioc flour (dry wt)	
Batch	Original flour	Free cyanide in 'pittu'	Bound cyanide 'pittu'
B	106	N.D.	101
C	106	N.D.	89
D	178	09	124

'Pittu' was prepared without an incubation period. The product did not have linamarase activity.

N.D. Not detected.

3.7. Cyanogenic glucoside content of fried manioc chips

Manioc chips (50 to 60% moisture) on frying also did not lose significant amounts of total cyanide when the final moisture content was 1 to 2%. However, on overfrying some loss of cyanide was observed probably due to the thermal decomposition of the glucoside (Table 9). In this case too, very small amounts of free cyanide were present in the product. Linamarase activity was absent in the fried chips.

TABLE 9. Cyanogenic glucoside content of fried manioc chips.

	Total original cyanide mg/kg (dry wt)	Free cyanide mg/kg (dry wt)	Bound cyanide mg/kg (dry wt)
1.	105	03	103
2.	107	03	71*
3.	454	12	407

Sampling was done by method 1. Fried chips were tested for free and bound cyanide. Fried chips did not contain linamarase activity.

*Chips were charred.

4. Discussion

The fate of bound cyanide in manioc and its products depends mainly on one factor, *viz.* the linamarin-linamarase reaction. The reaction is dependent on : (1) enzyme substrate contact, (2) enzyme activity of material and (3) rate and extent of deactivation of enzyme (reversible deactivation by drying of material or irreversible deactivation by denaturation heating in liquid medium). In specialized instances, as in the soaking of the dried chip, permeability of the tissue to the glucoside is also significant.[3] The absence of free cyanide in products depends mainly on how much the system is heated, not only for removing free HCN but also for the thermal decomposition of the cyanohydrins.

Results of experiments described in this paper and those described previously[3] show that fast drying prevents enzyme activity. This results in no significant hydrolysis of the glucoside and therefore a product (dried chip or flour) with the full complement of bound cyanide as well as an enzyme system that can be reactivated by moistening. It is this type of flour which is generally used for cooking purposes.

Preparation of flour by the method of keeping in the shade for 24 h causes the loss of 60 to 80% of the bound cyanide (increased enzyme substrate contact due to autolysis of the tissue) but levels of total cyanide are greater than that observed by the process described earlier;[3] in addition flour obtained by this method may not be bacteriologically suitable for human consumption.

Use of flours containing linamarase (that can be activated) in addition to bound cyanide is not desirable for cooking purposes unless either the enzyme is deactivated before or during cooking and any free cyanide is driven off by the cooking process.

This appears to be the case with manioc ' pittu ' and with fried manioc chips which contain high levels of bound cyanide (quick deactivation of linamarase) but no significant amounts of free cyanide (any free cyanide being driven off by cooking). Although the relative absence of free cyanide is encouraging it is felt that it would be far more desirable if the bound cyanide is also eliminated before the flour is used.[1]

The situation with manioc 'roti' is more serious as the final roasting does not remove the free cyanide formed. The experiments on 'roti' clearly illustrate that the factor involved is the linamarin-linamarase reaction. The enzyme activity index of the flour used was C > A > B. This tallied with residual bound cyanide in the 'roti' which was in the order of B > A > C. The experiments also clearly illustrate the effect of incubation time on the system. Free cyanide levels in the 'roti' do not have any fixed trend. This is understandable as the level of free cyanide is dependent on the amount of heat the material is subjected to, which, in these experiments, cannot be effectively controlled.

The experiment with manioc bread, in addition, shows the effect of temperature on total cyanide content. The outer crust of the bread is subject to a very high temperature which no doubt causes the lower total cyanide content of the crust in comparison to that of the inner part of the bread. Whether this is due to thermal decomposition of the glucoside or of the cyanohydrin or both is still under investigation.

The results concerning the aging of tubers is consistent with the general idea of old manioc being more toxic than fresh manioc ; the total cyanide levels of the flour show a general trend of increased cyanide content with aging. Inadequate sampling reduces the values of these results, but the trend closely resembles that reported by Rajaguru[5] recently. Increased cyanide in the unpeeled tuber could be caused by either a synthesis of glucoside in the edible part of the tuber or a migration of glucoside from the rind (which is rich in glucoside) to the edible part of the tuber.

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