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Cyanogenic Glucoside Content of Manioc
II. Detoxification of Manioc Chips and Flour

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Abstract : *Manihot esculenta* Crantz contains cyanogenic glucosides which remain unhydrolysed even after processing to manioc flour unless special precautions are taken. This paper presents a method by which the total cyanide content of manioc chips and flour can be reduced to approimately 5% of the original value. All assays of bound cyanide and total cyanide were done after the enzymic release of cyanide from linamarin.

1. Introduction

Manioc, *Manihot esculenta* Crantz, contains cyanogenic glucosides (linamarin and lotaustralin) which on interaction with the enzyme linamarase liberate hydrogen cyanide. Most methods of detoxification[2] are based either on destroying linamarase (boiling) or by allowing enzyme and substrate to interact (crushing, grinding, etc.) and driving off the HCN formed. The former method has the disadvantage that considerable quantities of cyanogenic glucosides may remain unhydrolysed;[4] the toxicity (direct or indirect) of these glucosides on ingestion is difficult to evaluate.[3] The second method is enzyme dependent and as the quantity of enzyme in the edible part of the tuber varies considerably from variety to variety,[1] on occasion long incubation periods have to be given in order to reduce the total cyanide content to relatively low values. In addition, handling and redrying crushed or ground material is a rather troublesome operation.

Recently, Rajaguru[5] outlined a process by which manioc chips can be detoxified. His process as described involved drying chips at 100 to 120°C, soaking the dried chips in water (18 to 24 h) and redrying at the same temperatures. This resulted in a product with very low total cyanide content, but it had an undesirable appearance caused by the harsh conditions of processing.

This paper describes a modification of the Rajaguru[5] method, data being presented on the detoxification of manioc chips by the use of different conditions of drying and varying periods of soaking. As a result of this study, it is possible to predict the minimum conditions required for processing manioc chips such that the total cyanide

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content of the chips is reduced to the relatively low levels of 5 to 10 p.p.m. In addition, the study has given some insight into the mechanism of detoxification of this "dry-soak-dry" technique. Measurements of total cyanide and bound cyanide were done after *enzymic* release of cyanide;[4] this analytical technique confers an added advantage over previous studies concerned with the detoxification of manioc.

2. Experimental

2.1. Preparation of material

The edible part of raw manioc (20 to 40 kg) was chipped into slices approximately 3 mm thick. These were dried (unless otherwise specified) in a forced-draft oven at different temperatures.

Manioc flour was prepared by grinding the dried chips (6 to 14% moisture) in an edge runner mill.

2.2. Sampling

The chips were sampled at random and the adequacy of sampling tested by determination of the cyanide content of a series of samples followed by the determination of the standard deviation of the sampling procedure. Sample size was in the order of 100 to 250 g (dried chips) or 200 to 500 g (wet chips); the total number of chips was 50 to 125.

2.3. Detoxification procedure and assays

Chips dried as described in text were soaked in 10 times their weight of water in an open vessel for 2 to 24 h; duplicate experiments were carried out for each soaking time. The soak water (200 ml) was examined for free cyanide and bound cyanide.[4] The total cyanide[4] of the homogenised wet chip (samples of 15 to 20 g dry weight) was also determined. The soaked chips were then dried under various conditions (see 3 Results) for 24 h, ground into flour and the total residual cyanide determined (15 g samples). This method of assay is not reliable for values below 8 p.p.m.

3. Results

3.1. Sampling

Batches of dried chips sampled by the procedure outlined gave the results shown in Table 1, which show that the sampling procedure adopted was adequate.

TABLE 1. Sampling of dried manioc chips.

Batch No.	Cyanide content (p.p.m. dry wt)
1	209 ± 11
2	64 ± 4
3	138 ± 7
4	101 ± 6
5	135 ± 13

See Section 2.2 for details.

3.2. Effect of conditions of drying of fresh chips on total cyanide content

Studies on this aspect showed, as expected, that drying conditions (temperature and rate of drying) have effect on the cyanide content of the dried chip (Table 2). Use of a forced-draft oven induces a faster rate of drying and generally less loss of cyanide when compared with the still oven at the same temperatures.

TABLE 2. Effect of drying conditions on cyanide content of chips.

Batch		1			2			3		
Type of oven		A	B	B	A	B	B	A	A*	A
Temperature		55°	80°	100°	70°	70°	100°	60°	65°	100°
Cyanide content	1	142	254	155	273	116	85	496	504	480
(p.p.m. dry wt)	2	133	267	164	253	127	99	461	482	475

Original total cyanide (p.p.m. dry wt) of fresh chips was 207 ± 25, 270 ± 55 and 426 ± 39 for batches 1, 2 and 3 respectively.

A Forced draft oven
B Still oven
A* Dried for 7 h, left overnight and redried.

Other results show that sun drying of chips gives about the same total cyanide as drying at 55° in a forced draft oven. It is likely that the values obtained for fresh manioc are low, due to losses during homogenisation of the large samples used. The large standard deviations obtained for the fresh material probably arise as a result of difficulties in handling the large samples of homogenised material, a part of which has to be introduced into the distillation flask as quickly as possible.

3.3. Effect of soaking time of the dried chip on total cyanide content

Chips from separate batches of manioc were sun-dried (3 days) or oven dried (24 h) at 55°, 75° and 95°C. Results (Tables 3 — 6) showed that : (1) whatever the drying conditions, both free cyanide and glucoside diffuse out of the cells, (2) as soaking time increases there is a gradual conversion of bound cyanide to free cyanide, (3) increase in soaking time results in lower cyanide content in the redried chip and (4) the higher the temperature of the second drying, the lower the cyanide content of the dried chip.

TABLE 3. Cyanide distribution after soaking in water.

	Cyanide content (p.p.m. relative to original dry wt)					
	Wash water		Wet chip	Dry chip* (total CN)		
Soak time (h)	Free	Bound	(total CN−)	Sun	55°	100°
3	25	50	34			23
6	46	63	27	24		10
9	53	41	11 (?)	14	8	
12	60	24	24	5		
18	71	12	14		10	

Chips used were sun dried. For experimental details see Section 2.3.
*The second drying was performed at the given conditions.

TABLE 4. Cyanide distribution after soaking in water.

Soak time (h)	Cyanide content (p.p.m. relative to original dry wt)			
	Wash water		Wet chip	Dry chip
	Free	Bound	(total CN^-)	(total CN^-) (55°)
2	15	21	21	10
4	39	11	15	14
8	46	3(?)	21	08
12	46	9	15	11
16	41	5	21	06
20	34	3	14	05
24	25	2	11	05

Chips used were dried at 55°C. For experimental details see Section **2.3.**

TABLE 5. Distribution of cyanide after soaking in water.

Soak time (h)	Cyanide content (p.p.m. relative to original dry wt)				
	Wash water		Wet chip	Dry chip	
	Free	Bound	(total CN^-)	(total CN^-) 55°	95°
3	39	44	38	16	
6	62	29	28		9
9	64	17	28		5
13	79	05	28		2
18	75	05	25	6	

Chips used for the experiment were dried at 75°C. For further experimental details see Section **2.3.**

TABLE 6. Distribution of cyanide after soaking in water.

Soak time (h)	Cyanide content (p.p.m. relative to original dry wt)			
	Wash water		Wet chip	Dry chip
	Free	Bound	(total CN^-)	(total CN^-) 60°
3	67	20(?)	21	11
6	78	22	20	9
10	98	10	16	7
14	96	08	19	6
24	88	08	19	

Chips used were dried at 95°C. For further experimental details see Section **2.3.**

The ratio of free cyanide to bound cyanide (a reflection of enzyme activity) appears to depend mainly on the material rather than the drying temperature. In other words, the original level of enzyme in the plant material appears to be more important than the variation in the extent of enzyme denaturation during the different drying conditions used in the preparation of chips. This is easily seen when the data in Table 4 are compared with those in Table 7 where although in both sets of data the chips have been dried at 55°C the bound cyanide in the latter is converted to free cyanide very slowly.

TABLE 7. Nature of cyanide in wash water using chips dried at 55°C.

	Cyanide content of wash water (p.p.m. relative to original dry wt)	
Soak time (h)	Free	Bound
6	32	149
12	104	133
18	169	70
24	152	83

For further experimental details see Section **2.3.**

4. Discussion

The level of cyanide in dried chips (of the same batch) depends on the extent of interaction between enzyme and substrate. Increase in drying temperature will affect this interaction by : (1) increasing disorganisation of the intracellular structure of cells and hence promoting contact between enzyme and substrate, (2) increasing rate of reaction over the range of temperature where the enzyme is not deactivated and (3) increasing rate of drying of tissue and therefore reducing interaction. It appears from this study that rate of drying is the vital factor. Quick drying in a forced draft oven results in very little loss of cyanide. Results using the closed oven (slower drying) are more difficult to interpret. These results are interesting and deserve further investigation.

These studies have also cleary shown that the "dry-soak-dry" technique can be used to reduce the total cyanide in chips to only 5 to 10 p.p.m. (about 5% the original levels).

The mechanism appears to operate as follows :—

(i) Loss of moisture from the chips (initial drying) causes an increase in permeability of the cell membrane.

(ii) Soaking in water (3 to 6 h) results in the loss of diffusible material (redrying of soaked chips have shown a 7 to 10% loss in dry weight) including free cyanide and cyanogenic glucosides; the loss of total cyanide is therefore largely enzyme independent.

(iii) Continued soaking in water does not significantly alter the residual total cyanide in the chip but has been shown to result in a conversion of bound cyanide to free cyanide in the wash water (a similar situation probably exists in the state of cyanide within the chip). This conversion is probably brought about by released linamarase. This happens even in the chips dried at 95°C showing that although isolated linamarase in solution is deactivated within 3 min at this temperature it is much more stable within the tissue.

(iv) Redrying of the chip brings about further loss of free cyanide. Raising the drying temperature lowers the total cyanide content still further. This is probably due to facilitation of the decomposition of acetone cyanohydrin.

On the basis of these studies we predict that sun drying, soaking in water for 9 to 12 h and sun drying again would reduce the total cyanide content of chips to about 10 p.p.m. A short oven drying at 100°C is likely to halve this value. The advantages of such a process would be : (1) it is largely enzyme independent and variation in internal levels of linamarase will not appreciably affect the final result, (2) the quality of the flour produced is satisfactory and (3) the process is cheap and can be carried out with minimum machinery at the site of production.

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References

1. COURSEY, D. G. (1973) *In Chronic cassava toxicity : proceedings of an interdisciplinary workshop, London, England,* 29-30 *January* 1973, pp. 27-36. Ottawa : International Development Research Centre. (IDRC-010e).
2. DE BRUIJN, G. H. (1973) *In Chronic cassava toxicity : proceedings of an interdiciplinary workshop, London, England,* 29-30 *January* 1973, pp. 43-48. Ottawa : International Development Research Centre. (IDRC-010e).
3. JANSZ, E. R., JEYARAJ, E. E., PIERIS, N. & ABEYRATNE, D. J. (1974) *J. Natn. Sci. Coun. Sri Lanka* **2**(1) : 57-66.
4. PIERIS, N., JANSZ, E. R. & KANDAGE, R. (1974) *J. Natn. Sci. Coun. Sri Lanka* **2**(1) : 67-76.
5. RAJAGURU, A. S. B. (1973) *Proc. Ceylon Ass. Advmt Sci.* **29**(1) : 70-71.