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PRELIMINARY REPORT

On-station trials to investigate options to improve the efficiency of cyanogen removal by rapid cassava processing methods

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Abbreviations

ARI	Agricultural Research Institute
HCN	Hydrogen cyanide
TFNC	Tanzania Food and Nutrition Centre

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Executive summary

(i) Cassava is a secondary staple in the Mtwara Region of Southern Tanzania, however during periods of food shortage it becomes a primary staple having an important role as a food security crop. Sales of cassava roots and products provide income generation. It is the second most important cash crop after cashew nuts.

(ii) Sun-drying is the principle processing method used to process cassava in Mtwara Region. Cassava roots are dried, whole or split, for one to four weeks, depending on the weather. The dried product, *makopa*, can either be pounded into a flour for immediate use, stored for up to a year, or sold in the market. During periods of food shortage, a 1-2 day process involving the pounding of peeled roots, followed by repeated sun-drying and pounding to provide a flour was adopted. The consumption of this product was reported to have resulted in exposure to cyanide during a period of severe prolonged food shortage early 1990s (Mlingi *et al.*, 1992).

(iii) Bound cyanide is present in fresh cassava roots as cyanogenic glucosides, linamarin and lotaustralin. Effective cassava processing methods disintegrate the root tissue completely, thereby releasing an endogenous enzyme, linamarase, that hydrolyses the cyanogenic glucosides to release their corresponding cyanohydrins. These decompose to liberate volatile hydrogen cyanide. To investigate the efficiency of current rapid processing methods including simple options to improve the removal of cyanogens, on-station trials were undertaken at Agricultural Research Institute (ARI) Naliendele in September 1996. The influence on cyanogen removal of: grating versus pounding; incubating or fermenting the root mash; and sun-drying versus roasting was investigated.

(iv) This report provides a preliminary summary of the results of the on-station rapid processing of cassava trials. Further statistical analysis of the results is underway and will contribute towards a further publication on this work.

(v) Several factors were observed to influence the removal of cyanogens including: tissue disruption and particle size; temperature of drying; and the optimisation of linamarase activity through the introduction of a holding period. The processing treatments that provided the optimum conditions for the removal of cyanogens were: (i) grated fermented roasted, (ii) grated incubated roasted and (iii) grated incubated sun-dried. These three treatments provided products with maximum levels of cyanogens of less than 50 mg CNeq/kg dry weight. These methods were considered efficient, bearing in mind that the fresh root from bitter varieties contained on average 562 ± 300 mg CNeq/kg dry weight. The remaining methods gave some products where the maxima was greater than 50 mg CNeq/kg dry weight.

(vi) In the Mtwara region during annual food shortage periods when the stock of *makopa* is depleted, poorly resourced households resort to processing *chinyanya,* a rapid, 1-2 day processing method. *Chinyanya* processing was represented in the trials as a control. In terms of efficiency of cyanogen removal, this treatment ranked last, with an average total cyanogen level 101.9±62.1 mg CNeq/kg dry weight. Simple but significant improvements of this processing method in terms of food safety could be made by the incorporation of a holding period (80% overall improvement of total

cyanogen levels), grating (41%) or roasting (17%) when compared to the standard *chinyanya* processing method.

(vii) Further assessment of the technologies will require evaluation by women processors as part of this participatory research programme. It is recommended that the three optimum treatments, (i) grated fermented roasted, (ii) grated incubated roasted and (iii) grated incubated sun-dried, are included in the evaluation. These products consistently provided a "safe" flour from two cassava varieties that are claimed by farmers to be highly toxic. These methods will also provide products with a range of sensory qualities that will influence their acceptability to consumers.

Introduction

1. Cassava is a secondary staple in the Mtwara Region of Southern Tanzania, however during periods of food shortage cassava becomes a primary staples having an important role as a food security crop. The advantages of cassava over other staple crops is mainly due to its drought tolerance and disease resistance. Sales of cassava roots and products provide income. In recent years, Mtwara has had some success in supplying international markets with dried cassava piece (*makopa*). In the Mtwara Region it is the second most important cash crop after cashew nuts (ARI Naliendele, 1993).

2. Cassava roots are prepared for consumption in different ways in the Mtwara Region (Bainbridge *et al.*, 1997). The main way of preparing cassava is to add the flour, prepared from *makopa,* to boiling water to make a stiff porridge called *ugali* which is consumed with a vegetable relish. Other means of consumption involve the combination of fresh cooked root with a variety of seasonal vegetables.

3. Sun-drying is the principal processing method used to process cassava in Mtwara Region. Cassava roots are dried, whole or split, for one to four weeks, depending on the weather. The dried product, *makopa*, can either be pounded into a flour, stored for up to a year, or sold in the market. During the period of food shortage a 1-2 day process involving the pounding of peeled roots, followed by repeated sun-drying and pounding was adopted. The end product, *chinyanya*, was thus obtained for use the same evening. The consumption of this product was reported to have resulted in exposure to cyanide during a period of food shortage in the early 1990s (Mlingi *et al.*, 1992).

4. An investigative study by Mlingi *et al.* (1995) of cyanogen levels in local cassava varieties and processed products showed that the bitter varieties had a cyanogenic potential of upto 1500 mg HCN equiv./kg dry weight. In a household survey of processed *makopa*, levels of cyanogenic glucosides were 145 ± 26 mg HCN equiv./kg dry weight (n=31), while for *chinyanya*, levels of 95 ± 60 mg HCN equiv./kg dry weight (n=11) were obtained. Although recent work by Carlsson *et al.* (in press) suggests that cyanogenic glucosides, such as linamarin, are only partially degraded in the human gut, the levels observed in this survey were far in excess of what may be considered safe. However, during normal years, consumption of *makopa* does not present a health problem. Interviews with householders indicated that while maize was plentiful, *makopa* was stored for some months, this would be likely to result in a further reduction of the cyanogen levels during storage.

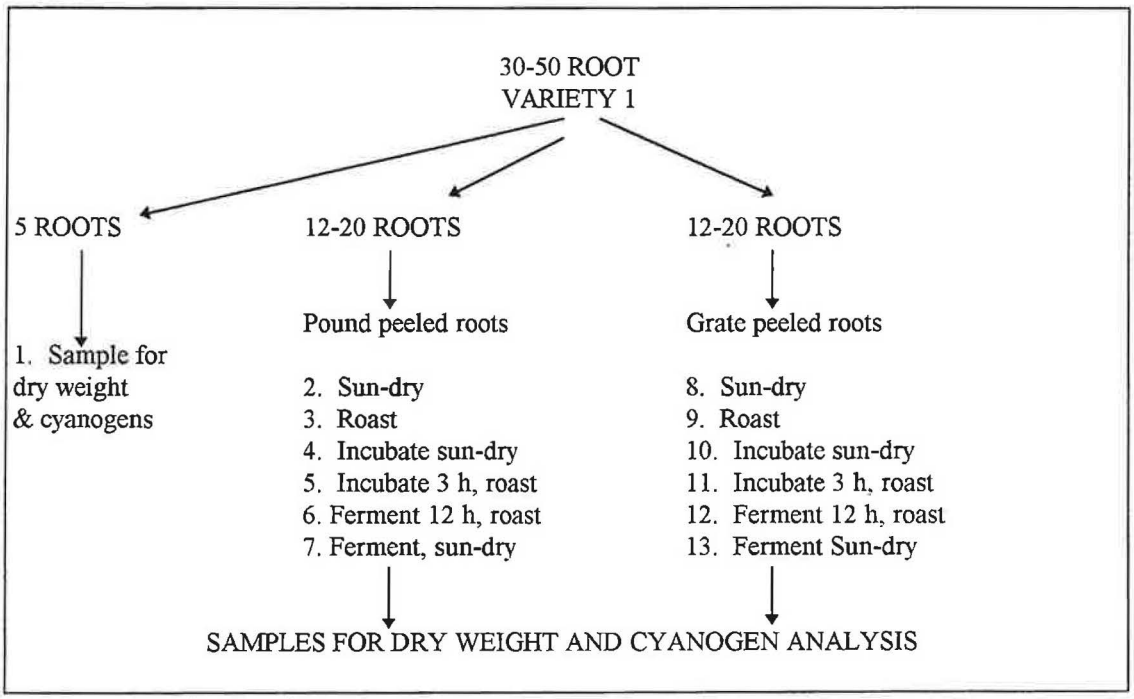
5. Cyanide in fresh cassava roots is present in a bound form, the cyanogenic glucosides. Effective cassava processing methods disintegrate the root tissue completely, thereby releasing an endogenous enzyme, linamarase that hydrolyses the cyanogenic glucosides to their corresponding cyanohydrins which decompose to liberate volatile hydrogen cyanide. Processing methods that involve a high level of root tissue disruption can reduce the cyanogens in cassava to negligible levels rendering the end product safe for consumption. The pounding step in *chinyanya* processing achieves this in part, however, improvement to the degree of disruption and release of hydrolytic enzymes could be made by use of grating as an alternative.

Similarly, improvement may be achieved by introducing a holding or incubation step after root disintegration, allowing the hydrolytic enzymes longer time period to act on their substrates (van der Grift *et al.*, 1996).

6. To investigate the efficiency of current rapid processing methods including simple steps to improve the removal of cyanogens, on-station trials were undertaken at Agricultural Research Institute (ARI) Naliendele in September 1996. Processing steps under investigation were either suggested during a participatory rural appraisal study with village women processors (Bainbridge *et al.*, 1997) or have been reported in recent literature (van der Grift *et al.*, 1996). The influence on the cyanogen levels of: grating versus pounding; incubating or fermenting the cassava root mash; and sun-drying versus roasting was investigated.

Investigation of rapid processing methods

7. On-station trials were undertaken at ARI Naliendele. Two cassava varieties that were considered very bitter by local farmers were harvested from local farms immediately prior to use. These varieties were called Emmanuel and Limbanga. The influence on the cyanogen levels of key steps in the processing methods including: grating versus pounding; incubating or fermenting; and sun-drying versus roasting was investigated. Fresh roots were analysed to obtain the initial cyanogenic potential of the starting material and 12 processing treatments were applied as illustrated in Figure 1.



Number 1-12 refer to various treatment.

Figure 1: Processing treatment and sampling flow diagram.

8. Each treatment was undertaken using the two varieties and five replicates for each variety ie a total of 10 replicates for each treatment. For example, for replicate 1 approximately 30-50 roots on one variety were harvested early in the morning, on the same day these roots were sampled, peeled, washed and processed using the 12 treatments (detailed in Figure 1). On subsequent days the remaining replicates were processed (refer to Appendix 1 for the procedure used). Samples were taken for immediate extraction of cyanogens and dry weight analysis, the remaining flour was frozen for storage. The analysis of cyanogens and pH were undertaken at the Tanzania Food and Nutrition Centre (TFNC).

Results and discussion

9. This report provides a preliminary summary of the results of the on-station rapid processing of cassava trials. Further statistical analysis of the results is underway and will contribute towards a further publication on this work.

10. The 12 treatments to which the cassava roots were subjected had a varied degree of influence on the removal of cyanogens from the final product. Table 1 indicates the treatments and the average residual cyanogens levels ranked in terms of efficiency of removal of total cyanogens. Fresh root material had an average total cyanogens level of 562.4 mg CNeq/kg dry weight. By processing using the various treatments the level was reduced to an average of 12.6-101.9 mg CNeq/kg dry weight of total cyanogens in the final products. This corresponds to a 82-98 % reduction in the total cyanogen levels. The level of residual cyanogens is highly dependent on the processing treatment and the unit steps involved.

Table 1: Cyanogen levels obtained in the fresh roots and after treatment to various processing methods, ranked in order of efficiency of cyanogen removal.

Treatment	Cyanogen level (mg CNeq/kg dry weight)				Dry weight (%)
	Total cyanogens	Linamarin	Cyano-hydrin	Cyanide	
Fresh root	562.4±299.7	548.3±224.3	9.7±7.5	4.4±1.9	64.8±3.2
Pounded sun-dry	101.8±60.4	95.9±61.2	4.2±3.7	1.7±0.5	7.1±1.7
Pounded roasted	82.5±64.9	77.8±64.4	2.1±1.2	2.6±0.4	5.0±2.0
Pounded, fermented sun-dried	78.9±28.4	29.8±34.3	44.5±18.9	4.5±1.2	8.3±1.0
Grated roasted	76.1±75.0	73.0±74.7	1.3±1.0	1.8±0.6	7.2±1.2
Grated sun-dry	68.5±81.8	64.1±80.8	2.7±2.9	1.7±0.6	6.9±1.2
Grated fermented sun-dried	41.7±15.7	5.3±4.9	33.4±16.0	3.0±1.1	7.6±1.2
Pounded, incubated sun-dry	39.6±27.1	27.2±27.1	9.7±4.4	2.4±1.0	8.1±2.4
Pounded, fermented roasted	33.3±16.2	11.1±9.3	17.2±8.8	4.9±1.5	7.3±1.8
Pounded, incubated roasted	29.0±15.5	24.0±15.1	2.3±1.1	2.7±0.8	5.3±0.9
Grated incubated sun-dry	18.0±10.8	8.7±10.2	7.3±7.7	2.0±0.6	7.5±1.8
Grated, incubated roasted	12.5±8.2	3.9±7.4	6.7±11.6	1.8±0.5	7.1±1.2
Grated fermented roasted	12.6±4.9	1.4±1.4	7.8±3.7	3.4±0.9	6.0±2.0

Standard deviation is given as σ_{n-1}.

Cassava root disintegration

11. Root disintegration is a critical step in bringing about the enzymic breakdown of the cyanogenic glucosides. Vasconcelos *et al.* (1990) have shown that the majority of cyanogenic glucosides present were hydrolysed after grating cassava roots. In this study, two methods of root disintegration were used, pounding which is the commonly used unit step in the Mtwara Region and grating, which is widely used in West Africa to produce high quality cassava products.

Table 2: Cyanogen levels for treatments that contrast in root disintegration method

Treatment	Cyanogenic glucoside levels (mg CNeq/kg dry weight)	
	Pounded	Grated
Sun-dry	95.9±61.2	64.1±80.8
Roasted	77.8±64.4	73.0±74.7
Incubated sun-dry	27.2±27.1	8.7±10.2
Incubated roasted	24.0±15.1	3.9±7.4
Fermented sun-dried	29.8±34.3	5.3±4.9
Fermented roasted	11.1±9.3	1.4±1.4

Standard deviation is given as σ_{n-1}.

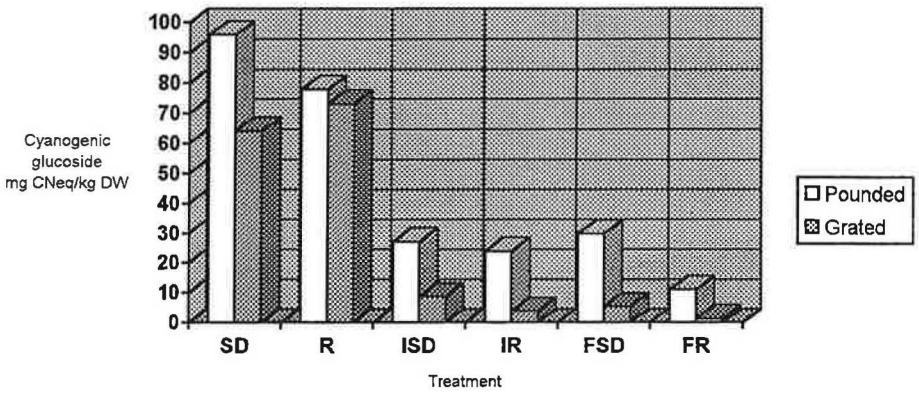


Figure 2: Cyanogen levels for treatments that compare root disintegration methods

Key: SD - sun-dried; R - roasted; ISD - incubated sun-dried; IR - incubated roasted; FSD - fermented sun-dried; FR - fermented roasted.

12. The data given in Table 2 and Figure 2 indicate that when grating is compared to pounding the former is more efficient in facilitating the degradation of cyanogenic glucosides. For each pair of treatments, the grated samples remained with lower residual levels of the glucosidic cyanogens. This was attributed to the more extensive degree of tissue disruption resulting from grating as in contrast to pounding. The level of tissue and hence cellular disruption dictate to what degree the cellular compartmentalisation is breached and the linamarase able to come into contact with its substrates the cyanogenic glucosides. Hydrolysis releases the non-glucosidic cyanohydrins which are unstable at pH >5 and the final product in the pathway is the

cyanide ion. The latter is volatile as hydrogen cyanide at temperature above 25°C and is thus removed from the system through drying.

Drying method

13. The removal of both glucosidic and non-glucosidic cyanogens is influenced by the method and rate of drying. Table 3 the residual levels for paired treatments allows a comparison of the drying methods to be made, ie sun-dry and roasting.

Table 3: Cyanogen levels for treatments that contrast in drying method

Treatment	Cyanogen levels (mg CNeq/kg dry weight)			
	Sun-dried		Roasted	
	Cyanogenic glucoside	Non-glucosidic	Cyanogenic glucoside	Non-glucosidic
Pounded	95.9±61.2	5.9±4.1	77.8±64.4	4.7±1.2
Grated	64.1±80.8	4.4±3.2	73.0±74.7	3.1±1.3
Pounded, incubated	27.2±27.1	12.3±5.2	24.0±15.1	4.9±0.9
Grated incubated	8.7±10.2	9.3±7.9	3.9±7.4	8.5±11.5
Pounded, fermented	29.8±34.3	49.1±19.4	11.1±9.3	22.1±9.7
Grated fermented	5.3±4.9	36.4±16.5	1.4±1.4	11.2±4.2
Treatment	Moisture content (%)			
Pounded	7.1±1.7		5.0±2.0	
Grated	6.9±1.2		7.2±1.2	
Pounded, incubated	8.1±2.4		5.3±0.9	
Grated incubated	7.5±1.8		7.1±1.2	
Pounded, fermented	8.3±1.0		7.3±1.8	
Grated fermented	7.6±1.2		6.0±2.0	

Standard deviation is given as σ_{n-1}.

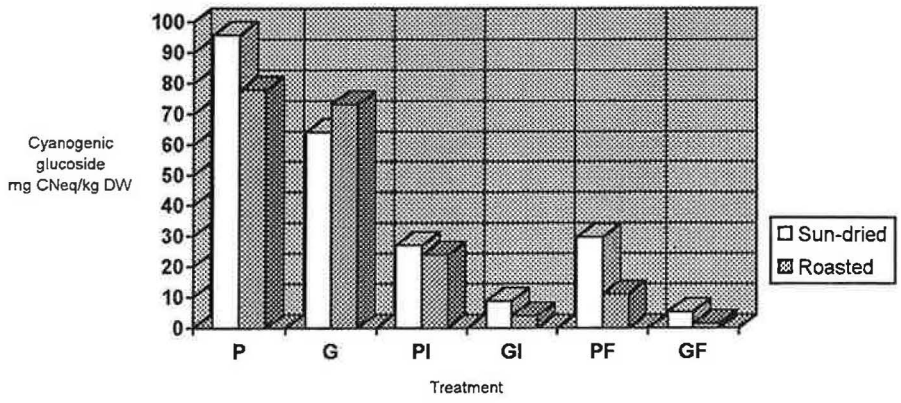


Figure 3: Cyanogen levels for treatments that compare drying methods

Key: P - pounded; G - grated; PI - pounded incubated; GI - grated incubated; PF - pounded fermented; GF - grated fermented.

14. Figure 3 illustrates the comparison of drying method on the residual level of cyanogenic glucosides. In the majority of cases it can be observed that treatments that included roasting remained, to a variable extent, with lower levels of cyanogenic glucosides than those that were sun-dried. This observation was attributed to the activity of the linamarase which is influenced by temperature and moisture content.

15. Work by Yeoh (1989) has shown that linamarase in the root cortex has a temperature optimum of 55°C. At this temperature it is reasonably stable, incurring a 10 % activity loss after 30 min at 60°C. The increase in local temperature of the material during the roasting would provide heat to increase the rate of reaction until such time that the enzyme was denatured. The data indicates that a greater difference in residual levels was observed for the pounded treatments as compared to the grated. This may be due to the thermo-dynamics of heating the material, the larger pieces taking longer to heat to the core, thus delaying the denaturation of linamarase as compared to the more rapid heating of the smaller grated particles. This would explain the observation that for grated material higher glucosidic levels were observed due to the rapid denaturation of the linamarase enzyme.

15. At moisture contents of below 12 % the activity of linamarase is impeded. The rate of drying can influence the period of time when the linamarase is active. However, the extended activity of the enzyme through slow drying rates and the increased release of enzyme thorough disintegration of the tissue are dynamic. In the majority of paired treatment it was observed that the influence of greater tissue disruption exceeded the benefit of a slower rate of drying.

16. The level of non-glucosidic cyanogens was low for all treatments except those that involved fermentation. It is known that the intermediate cyanohydrins are stabilised by acidic pH <5 (Formunyam *et al.*, 1985). In treatments where fermentation was used, the pH was on average 5.0, ranging from 4.3 to 6.7. The levels of non-glucosidic cyanogens are particularly high, 49.1±19.4 and 36.4±16.5 mg CNeq/kg dry weight, in the fermented and sun-dried treatments. These high levels were attributed to the higher moisture levels in the products of sun-dried products. In all paired comparisons non-glucosidic levels were higher in sun-dried treatments. On roasting, the non-glucosidic levels in the fermented products were more acceptable at 22.0±9.6 and 11.3±4.3 mg CNeq/kg dry weight for pounded and grated treatments respectively. The higher temperatures reached during roasting were more efficient at driving off the cyanohydrins that volatilise at 83°C (Formunyam *et al.*, 1985). The consumption of non-glucosidic compounds are generally considered to result in greater exposure to cyanide than results from the consumption of cyanogenic glucosides, hence, these levels are unacceptable in terms of food safety.

Holding period

17. Previous research by van der Grift *et al.* (1996) has indicated that providing a period of time for the linamarase enzyme to work prior to drying allows a greater degree of cyanogen removal. In the trials as reported here, two holding period were investigated, 3 hr, as recommended by van der Grift's work and 12 hr. The latter holding period would allow fermentation of the mash to occur thus providing the final

product with the lactic flavour that is preferred by some communities in the Mtwara Region. The results obtained are given in Table 4 and illustrated in Figure 4.

Table 4: The influence of incubating and fermenting the disintegrated cassava mash on the residual glucosidic and non-glucosidic cyanogen levels.

Treatment	Cyanogenic glucoside levels (mg CNeq/kg dry weight)			
	Pounded		Grated	
	Sun-dried	Roasted	Sun-dried	Roasted
No holding period	95.9±61.2	77.8±64.4	64.1±80.8	73.0±74.7
Incubated for 3 hr	27.2±27.1	24.0±15.1	8.7±10.2	3.9±7.4
Fermented for ~12 hr	29.8±34.3	11.1±9.3	5.3±4.9	1.4±1.4
	Non-glucoside levels (mg CNeq/kg dry weight)			
No holding period	5.9±4.1	4.7±1.2	4.4±3.2	3.1±1.3
Incubated for 3 hr	12.3±5.2	4.9±0.9	9.3±7.9	8.5±11.5
Fermented for ~12 hr	49.1±19.4	22.1±9.7	36.4±16.5	11.2±4.2

Standard deviation is given as σ_{n-1}.

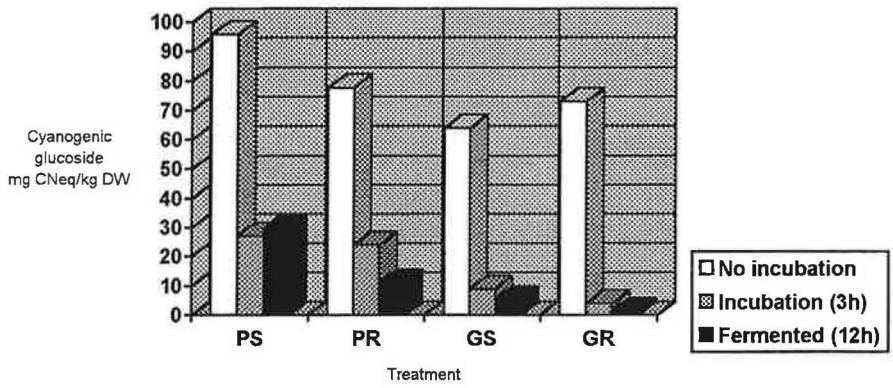


Figure 4: Cyanogen levels for treatments that compare holding periods

Key: PS - pounded sun-dried; PR - pounded roasted; GS - grated sun-dried; GR - grated roasted.

18. The introduction of a 3 hr holding period resulted in a substantial improvement in the removal of glucosidic cyanogens of: 72 and 69 % for pounded, sun-dried and roasted material respectively; and 86 and 94 % for grated, sun-dried and roasted material. In the majority of cases this was improved by a further of 5 % on average for increasing the holding period from 3 to 12 hr. This strongly supports van der Grift's research findings. Incorporation of a 3 hr holding period is a highly effective means of improving rapid processing methods.

19. For the non-glucosidic cyanogen levels (refer to Table 4) an increase directly proportional to the length of the holding period was observed, hence, high levels for the products of 12 hr fermentation were observed. As stated earlier, this could be attributed to the decrease in pH that was observed due to lactic fermentation causing the stabilisation of the cyanohydrins. Without the use of roasting where high

temperature promotes the volatilisation of cyanohydrins, the fermentation and sun-drying option should be used cautiously.

Conclusion

20. Several factors were observed to influence the removal of cyanogens during processing including: tissue disruption and particle size; temperature during drying; and prolonging the activity of linamarase through the introduction of a holding period. The processing methods that provided the optimum conditions for the removal of cyanogens were: (i) grated fermented roasted, (ii) grated incubated roasted and (iii) grated incubated sun-dried. Using these three methods, products were prepared from highly cyanogenic roots (562 ± 300 mg CNeq/kg dry weight) that had cyanogen levels with ranges of less than 50 mg CNeq/kg dry weight. The remaining methods gave some products where the cyanogen level maxima were greater than 50 mg CNeq/kg dry weight.

21. In the Mtwara region during food shortage periods when the stock of *makopa* is depleted, women processors resort to processing *chinyanya,* using a rapid processing method (Bainbridge *et al.*, 1997). The treatment that best represented the *chinyanya* method was the pounded and sun-dried treatment. In terms of efficiency of cyanogen removal, this treatment ranked last with an average total cyanogen levels 102 ± 62 mg CNeq/kg dry weight. Simple but significant improvements of this product in terms of food safety could be effected by the incorporation of a holding period (80% overall improvement in cyanogen levels), grating (41%) or roasting (17%). Incorporation of a 3 hr holding period to the current rapid processing method would not require the introduction of a new technology but may require two days to allow thorough drying. The alternative options, including grating and roasting, would require participatory evaluation at household and village level as both require additional resource inputs. The rapid method of processing tested can provide high quality flours in a short period of time that could provide food safety and improved market opportunities for cassava flour.

22. Further evaluation of the technologies will require evaluation by women processors as part of participatory research programme, this will be addressed by the next phase of this project. It is recommended that the three options (i) grated fermented roasted, (ii) grated incubated roasted and (iii) grated incubated sun-dried be included in the evaluation. These products consistently provided a "safe" flour from two cassava varieties that are claimed by farmers to be highly toxic. These methods will also provide a range of sensory qualities that will influence their acceptability. In addition the market acceptability of the "improved flour" and potential for income generation will be assessed.

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Appendix 1 - Procedure for processing trials

Root sampling

The cassava roots were placed in a row according to variety, Emmanuel and Limbanga. Roots were discarded if they were diseased or badly damaged. To sample, the roots were counted and every fifth root was selected for pounding, grating or analysis to give three piles.

Sample code

The following sample code was used:

FR1-2:	where FR is fresh root, 1 is rep 1 and 2 is variety 2.
PS1-2:	where P - pounded, S - sun-dry, 1 - batch number and 2 is variety.
PR1-2:	where P is pounded, R - roast, 1 - batch number and 2 is variety.
PIS1-2	where P is pounded, I is incubate, S is sun-dry
PIR1-2	where P is pounded, I is incubate, R is roast
PFR1-2	where P is pounded, F - fermented and R - roast, 1 is rep number and 2 is variety

GS, GR, GIS, GIR and GFR were codes used for treatments that included grating.

Root preparation for analysis

One pile of roots was washed, peeled and left whole until all are ready for the chopping. A sample for dry weight and cyanogen determinations was randomly selected and prepared for analysis as follows: a longitudinal quarter of each root was taken and chopped into 1 cm x 1 cm pieces; the pieces were placed in a bucket and randomised; duplicate 10 g portions were taken for dry weight analysis and one 50 g portion for extraction of cyanogens; extraction for cyanogens was undertaken immediately using the O'Brien *et al.* (1991) method. Cyanogen extracts were stored in the refrigerator and samples of flour were placed in two polythene bags and frozen.

Processing

For the remaining roots one pile was pounded and one grated simultaneously. For pounding, the roots were cut into small pieces and pounded in a traditional pestle and mortar until the roots were disintegrated. Roots were grated using a manually operated grater. The grated and pounded mash was first collected in a separate sacks and dewatered by twisting the sack to pressurise the water out. The mash was divided into equal portions for further steps in the processing method.

Sun-drying

To sun-dry, the mash was spread out on a raised platform made from palm matting. The material was agitated 3-4 times during the drying period in order to facilitate drying and check for completion. If drying was not complete by late afternoon, the material was collected in a basket and drying resumed early the following day. Timing of all activities was recorded. Once considered dry by women experienced in processing cassava, the material was collected, randomised and sampled for immediate extraction of cyanogens and determination of dry weight analysis and pH value.

Roasting

The grated and pounded mash was placed in the sun for half an hour. For the grated treatment the mash was passed through a sieve to remove fibres and separate the particles. The pounded and roasted material was placed in a hot frying pan and agitating it until a dry. Care was taken to avoid over cooking and the formation of gelatinised lumps. The dried material was collected in a bucket, randomised, a sample extracted for cyanogen extraction and dry weight analysis the remainder was stored.

Incubated sun-dry/roast

The pounded or grated material was allowed to stand in the shade for three hours. The material was then put out to dry or was roasted as described above

Fermentation

The pounded or grated material was placed in a sack, tied with string and placed in the shade with a heavy weight placed on top to further dewater the material during fermentation. The mash was allowed to stand for approximately 12 hr, after this time the material was randomised and split, one portion was taken to be roasted the other to be sun-dried as described above.

Appendix 2: Processing trials data - cyanogen levels, moisture content and pH.

Sample		mg CNeq/kg DW				Moisture	
	Total	Lin	Cyan	CN	I/F		pH
FR1-L	599.7	570.3	21.5	7.8	29.4	63.84	
FR2-L	649.8	637.4	6.3	6.1	12.4	63.13	
FR3-L	433.7	425.7	3.8	4.2	8.0	63.73	
FR4-L	67.6	65.0	1.4	1.2	2.6	61.07	
FR5-L	497.0	488.5	5.6	3.0	8.6	65.47	
FR1-E	913.3	898.8	9.4	5.2	14.6	64.43	
FR2-E	538.2	521.5	10.6	6.1	16.7	65.48	
FR3-E	817.5	789.7	24.0	3.7	27.7	72.98	
FR4-E	478.7	471.4	4.6	2.7	7.3	65.63	
FR5-E	628.5	614.7	9.4	4.4	13.8	62.65	
PR1-L	29.6	23.9	2.7	3.0	5.7	4.50	6.3
PR2-L	184.9	180.6	1.7	2.6	4.3	6.78	6.9
PR3-L	117.7	111.9	3.7	2.1	5.8	3.63	6.2
PR4-L	18.0	15.4	0.3	2.3	2.6	3.65	6.1
PR5-L	12.9	9.3	1.4	2.1	3.6	5.32	6.5
PR1-E	160.1	155.6	1.8	2.8	4.6	4.14	6.5
PR2-E	17.1	13.3	0.4	3.4	3.8	2.99	6.2
PR3-E	89.4	84.0	2.8	2.7	5.4	9.96	6.2
PR4-E	55.1	50.3	2.1	2.7	4.8	4.66	6.1
PR5-E	139.9	133.4	3.9	2.6	6.5	4.81	6.4
GR1-L	42.3	36.4	3.0	3.0	5.9	6.63	6.4
GR2-L	194.2	190.7	0.9	2.6	3.5	8.36	7.0
GR3-L	147.4	142.9	2.8	1.7	4.5	8.49	6.3
GR4-L	10.4	8.6	0.4	1.4	1.8	9.11	6.4
GR5-L	6.5	4.0	0.9	1.6	2.4	6.44	6.7
GR1-E	91.3	88.7	0.9	1.8	2.6	5.14	6.7
GR2-E	4.7	2.9	- 0.1	1.9	1.8	6.99	6.5
GR3-E	21.3	18.7	1.5	1.1	2.6	6.46	6.4
GR4-E	54.7	51.7	1.1	1.9	3.0	7.64	6.4
GR5-E	187.9	185.3	1.5	1.1	2.6	6.31	6.4
PS1-L	88.7	75.1	10.8	2.8	13.6	7.13	6.3
PS2-L	205.1	199.8	3.3	1.9	5.2	7.72	6.6
PS3-L	98.1	93.7	3.0	1.3	4.3	4.98	6.2
PS4-L	50.2	47.4	1.8	1.1	2.9	6.79	6.7
PS5-L	1.9	- 3.1	3.4	1.6	5.0	7.43	6.3
PS1-E	185.6	182.5	1.6	1.5	3.1	4.29	6.4
PS2-E	83.4	80.0	1.4	2.0	3.4	6.61	6.2
PS3-E	70.4	57.1	11.3	2.0	13.3	9.50	5.6
PS4-E	101.2	97.9	1.4	1.9	3.3	9.50	5.6
PS5-E	133.7	128.6	3.9	1.3	5.1	6.95	6.2
GS1-L	11.3	3.1	6.1	2.1	8.2	7.13	6.6
GS2-L	249.1	239.0	7.5	2.6	10.1	7.90	6.8
GS3-L	21.7	12.9	6.7	2.1	8.8	4.65	6.3
GS4-L	11.6	9.0	1.3	1.3	2.5	6.98	6.3
GS5-L	15.7	12.9	1.2	1.6	2.8	7.13	6.7
GS1-E	62.9	61.3	- 0.3	2.0	1.7	4.79	7.0
GS2-E	28.1	26.4	- 0.1	1.8	1.7	7.62	6.7
GS3-E	63.9	61.6	1.4	1.0	2.3	7.47	6.4
GS4-E	36.7	33.7	1.1	2.0	3.1	7.48	6.4
GS5-E	184.1	181.3	2.0	0.8	2.8	7.48	6.4
PIS1-L	82.6	61.3	16.3	4.9	21.3	7.15	5.8
PIS3-L	21.2	5.8	13.3	2.1	15.5	6.14	5.7
PIS4-L	18.9	13.7	3.9	1.3	5.2	6.48	6.2
PIS5-L	12.0	2.4	7.7	1.9	9.6	7.84	5.8
PIS1-E	82.7	77.5	3.8	1.5	5.2	5.76	6.3
PIS2-E	23.0	11.3	9.1	2.6	11.7	7.91	5.4
PIS3-E	42.9	28.4	12.1	2.4	14.5	12.40	5.3
PIS5-E	27.3	9.4	15.5	2.4	17.9	7.45	5.0
PIS2-L	64.1	54.1	7.7	2.3	10.0	7.43	6.5

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Sample	mg CNeq/kg DW					Moisture	
	Total	Lin	Cyan	CN	I/F		pH
GIS1-L	10.7	3.9	4.5	2.3	6.8	6.79	5.9
GIS3-L	33.1	1.9	28.4	2.7	31.2	5.65	5.5
GIS4-L	7.0	1.6	3.9	1.5	5.4	6.97	6.5
GIS5-L	8.4	0.6	6.0	1.8	7.8	6.78	6.0
GIS1-E	26.4	22.8	1.7	1.9	3.6	5.81	6.7
GIS2-E	9.5	2.4	5.1	1.9	7.1	7.95	5.9
GIS3-E	23.8	16.7	6.0	1.0	7.1	10.65	5.8
GIS5-E	18.8	7.2	10.1	1.5	11.6	7.31	6.2
GIS2-L	34.4	28.4	3.9	2.1	6.0	6.92	6.8
GIS4-E	7.7	0.9	3.5	3.2	6.7	10.65	5.8
PIR2-L	42.4	36.5	3.3	2.5	5.9	6.55	6.8
PIR4-E	24.8	19.1	2.9	2.8	5.7	4.98	6.1
PIR1-L	33.5	28.1	3.1	2.3	5.4	5.33	6.2
PIR3-L	28.3	23.0	2.4	2.9	5.4	4.32	6.2
PIR4-L	14.2	10.7	1.8	1.7	3.5	5.28	6.2
PIR5-L	7.1	1.7	1.1	4.3	5.4	7.28	6.0
PIR1-E	53.7	48.8	2.2	2.8	4.9	4.30	6.3
PIR2-E	10.5	7.3	- 0.2	3.4	3.2	4.80	6.2
PIR3-E	46.2	41.1	3.5	1.6	5.1	4.98	6.1
PIR5-E	29.2	24.3	2.7	2.2	5.0	5.15	6.1
GIR2-L	23.2	16.0	4.2	3.0	7.2	8.07	6.8
GIR4-E	11.1	6.3	3.2	1.6	4.8	5.97	6.2
GIR1-L	7.1	3.7	1.4	2.1	3.4	9.28	6.3
GIR3-L	15.0	8.5	4.8	1.6	6.5	5.64	6.2
GIR4-L	3.4	0.6	1.2	1.6	2.7	7.97	6.4
GIR5-L	3.7	0.3	1.7	1.6	3.3	7.45	6.0
GIR1-E	13.4	9.4	1.7	2.3	4.0	6.64	6.5
GIR2-E	9.4	0.9	7.1	1.5	8.6	5.80	6.3
GIR3-E	9.5	5.7	2.5	1.4	3.9	5.97	6.2
GIR5-E	28.8	- 11.9	39.3	1.4	40.7	7.84	6.2
PFR1-L	15.2	8.8	3.3	3.1	6.3	8.92	6.3
PFR2-L	24.8	7.5	10.8	6.4	17.2	7.13	5.8
PFR3-L	30.5	8.7	15.7	6.1	21.9	5.98	5.2
PFR4-L	13.0	3.0	7.2	2.8	10.0	4.97	4.8
PFR5-L	24.8	3.6	16.5	4.7	21.2	5.98	5.0
PFR1-E	27.9	5.4	17.4	5.2	22.6	4.79	4.5
PFR2-E	38.7	12.6	22.5	3.7	26.2	8.32	4.9
PFR3-E	63.2	35.2	23.9	4.2	28.0	9.73	6.7
PFR4-E	39.6	12.5	21.1	6.0	27.2	9.74	4.7
PFR5-E	55.1	14.1	33.9	7.1	40.9	7.09	4.4
GFR1-L	7.0	1.7	2.4	2.9	5.3	9.16	6.4
GFR2-L	20.3	2.3	13.5	4.4	17.9	8.49	5.5
GFR3-L	12.9	- 0.0	9.2	3.7	12.9	6.65	5.0
GFR5-L	10.2	- 1.0	6.7	4.4	11.1	4.48	4.8
GFR1-E	17.0	3.3	9.8	3.9	13.7	3.79	4.6
GFR3-E	12.4	1.9	8.6	1.9	10.5	4.47	4.5
GFR4-E	5.8	0.2	2.8	2.8	5.6	4.49	4.5
GFR5-E	14.9	2.3	9.7	2.8	12.5	6.80	4.3
GFR4-L	-	-	-	-	-	4.97	4.8
GFR2-E	-	-	-	-	-	8.44	4.7
PFS1-L	140.6	118.4	18.5	3.7	22.2	9.50	6.0
PFS2-L	57.8	10.3	40.5	7.0	47.5	7.64	5.9
PFS3-L	70.6	17.6	48.0	5.0	53.0	6.64	5.1
PFS4-L	38.6	18.4	16.6	3.6	20.2	8.43	4.8
PFS5-L	57.5	2.6	49.9	5.1	55.0	7.99	5.0
PFS1-E	70.7	10.7	56.1	3.8	60.0	10.23	4.5
PFS2-E	73.2	34.7	35.1	3.4	38.5	8.80	4.9
PFS3-E	98.7	53.2	41.9	3.5	45.4	7.94	4.6
PFS4-E	83.7	20.6	58.8	4.3	63.1	7.94	4.6

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Sample	mg CNeq/kg DW					Moisture	
	Total	Lin	Cyan	CN	I/F		pH
PFS5-E	97.2	11.5	80.0	5.7	85.7	8.29	4.3
GFS1-L	20.7	6.5	11.4	2.8	14.2	9.84	6.4
GFS2-L	52.6	12.7	34.3	5.6	39.9	7.61	5.7
GFS3-L	48.4	4.1	40.3	4.0	44.3	5.79	5.1
GFS4-L	26.3	1.3	23.2	1.8	25.0	8.12	4.8
GFS5-L	40.2	1.3	35.8	3.0	38.9	7.45	4.9
GFS1-E	46.7	11.9	31.7	3.1	34.8	6.33	4.6
GFS2-E	24.8	1.7	21.2	1.9	23.1	8.29	4.9
GFS3-E	41.5	10.7	28.9	1.9	30.8	7.12	4.5
GFS4-E	41.7	3.7	35.3	2.7	38.0	7.12	4.5
GFS5-E	74.3	- 1.2	72.0	3.4	75.4	8.61	4.3

Summary table

	Fresh root			
	Average	SD	Min	Max
Total	562.4	229.7	67.6	913.3
Lin	548.3	224.3	65.0	898.8
Cyan	9.7	7.5	1.4	24.0
CN	4.4	1.9	1.2	7.8
I/F	14.1	8.7	2.6	29.4
Mois	64.8	3.2	61.1	73.0
pH			-	-

	Pounded roasted				Pounded incubated roasted				
	Average	SD	Min	Max		Average	SD	Min	Max
Total	82.5	64.9	12.9	184.9	Total	29.0	15.5	7.1	53.7
Lin	77.8	64.4	9.3	180.6	Lin	24.0	15.1	1.7	48.8
Cyan	2.1	1.2	0.3	3.9	Cyan	2.3	1.1	- 0.2	3.5
CN	2.6	0.4	2.1	3.4	CN	2.7	0.8	1.6	4.3
I/F	4.7	1.2	2.6	6.5	I/F	4.9	0.9	3.2	5.9
Mois	5.0	2.0	3.0	10.0	Mois	5.3	0.9	4.3	7.3
pH	6.3	0.2	6.1	6.9	pH	6.2	0.2	6.0	6.8

	Grated roasted				Grated incubated roasted				
	Average	SD	Min	Max		Average	SD	Min	Max
Total	76.1	75.0	4.7	194.2	Total	12.5	8.2	3.4	28.8
Lin	73.0	74.7	2.9	190.7	Lin	3.9	7.4	- 11.9	16.0
Cyan	1.3	1.0	- 0.1	3.0	Cyan	6.7	11.6	1.2	39.3
CN	1.8	0.6	1.1	3.0	CN	1.8	0.5	1.4	3.0
I/F	3.1	1.3	1.8	5.9	I/F	8.5	11.5	2.7	40.7
Mois	7.2	1.2	5.1	9.1	Mois	7.1	1.2	5.6	9.3
pH	6.5	0.2	6.3	7.0	pH	6.3	0.2	6.0	6.8

	Pounded sun-dried				Pounded fermented roasted				
	Average	SD	Min	Max		Average	SD	Min	Max
Total	101.8	60.4	1.9	205.1	Total	33.3	16.2	13.0	63.2
Lin	95.9	61.2	- 3.1	199.8	Lin	11.1	9.3	3.0	35.2
Cyan	4.2	3.7	1.4	11.3	Cyan	17.2	8.8	3.3	33.9
CN	1.7	0.5	1.1	2.8	CN	4.9	1.5	2.8	7.1
I/F	5.9	4.1	2.9	13.6	I/F	22.1	9.7	6.3	40.9
Mois	7.1	1.7	4.3	9.5	Mois	7.3	1.8	4.8	9.7
pH	6.2	0.4	5.6	6.7	pH	5.2	0.8	4.4	6.7

	Grated sun-dried				Grated fermented roasted				
	Average	SD	Min	Max		Average	SD	Min	Max
Total	68.5	81.8	11.3	249.1	Total	12.6	4.9	5.8	20.3
Lin	64.1	80.8	3.1	239.0	Lin	1.4	1.4	- 1.0	3.3
Cyan	2.7	2.9	- 0.3	7.5	Cyan	7.8	3.7	2.4	13.5
CN	1.7	0.6	0.8	2.6	CN	3.4	0.9	1.9	4.4
I/F	4.4	3.2	1.7	10.1	I/F	11.2	4.2	5.3	17.9
Mois	6.9	1.2	4.7	7.9	Mois	6.0	2.0	3.8	9.2
pH	6.5	0.2	6.3	7.0	pH	4.9	0.7	4.3	6.4

	Pounded incubated sun-dried				Pounded fermented sun-dried				
	Average	SD	Min	Max		Average	SD	Min	Max
Total	39.6	27.1	12.0	82.7	Total	78.9	28.4	38.6	140.6
Lin	27.2	27.1	2.4	77.5	Lin	29.8	34.3	2.6	118.4
Cyan	9.9	4.4	3.8	16.3	Cyan	44.5	18.9	16.6	80.0
CN	2.4	1.0	1.3	4.9	CN	4.5	1.2	3.4	7.0
I/F	12.3	5.2	5.2	21.3	I/F	49.1	19.4	20.2	85.7
Mois	8.1	2.4	5.8	12.4	Mois	8.3	1.0	6.6	10.2
pH	5.7	0.5	5.0	6.5	pH	5.0	0.6	4.3	6.0

	Grated incubated sun-dried				Grated fermented sun-dried				
	Average	SD	Min	Max		Average	SD	Min	Max
Total	18.0	10.8	7.0	34.4	Total	41.7	15.7	20.7	74.3
Lin	8.7	10.2	0.6	28.4	Lin	5.3	4.9	- 1.2	12.7
Cyan	7.3	7.7	1.7	28.4	Cyan	33.4	16.0	11.4	72.0
CN	2.0	0.6	1.0	3.2	CN	3.0	1.1	1.8	5.6
I/F	9.3	7.9	3.6	31.2	I/F	36.4	16.5	14.2	75.4
Mois	7.5	1.8	5.7	10.7	Mois	7.6	1.2	5.8	9.8
pH	6.1	0.4	5.5	6.8	pH	5.0	0.6	4.3	6.4