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A study of the role of tissue disruption in the removal of cyanogens during cassava root processing

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Sun-drying as a means to reduce the cyanogen content of cassava roots is commonly known to be inefficient. This paper reports on modifications to the processing procedure used for sun-drying and their effectiveness at removing potentially toxic cyanogenic glucosides. Commonly used processing methods were compared. Crushing cassava root pieces prior to drying was found to significantly improve the efficiency of cyanogen removal by, on average, 22% during laboratory experiments and 12% during field trials. The crushing procedure was optimized and a low cost prototype crusher developed. A reduction in the processing time resulted from crushing the root disks prior to drying. The processing method involving crushing was ranked second in terms of efficiency of cyanogen removal in a comparative study of sun-dry processing methods that are commonly used in East Africa. Pounding cassava to small pieces in a traditional pestle and mortar prior to drying was the most efficient, providing 90% removal of cyanogens. Pounding and crushing cassava prior to sun-drying were significantly better than all other root preparation pre-treatments evaluated. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In cassava (*Manihot esculenta* Crantz), cyanogenic glucosides are synthesized in the leaves and stored in the roots (Mkpong *et al.*, 1990). This class of compounds is present in a number of plant groups. The cyanogenic compounds are thought to provide a nitrogen store and their potential toxicity provides a deterrent against predators and disease (Kakes, 1990). This provides the plants with resistance that enables them to thrive under hostile conditions. The presence of potentially toxic compounds in food for human consumption is not uncommon. Societies that regularly consume such crops have developed processing methods that reduce the levels of toxic compounds to provide an edible product.

Cassava is an important staple for resource poor farmers in Sub-Saharan Africa. Various methods are currently used to process cassava roots into storable products (Natural Resources Institute, 1992). Methods of processing may be simple, such as the sun-drying of peeled cassava whole roots, a product known as makopa in Tanzania; to more involved methods that require grating, fermentation and roasting steps, giving a product called gari in West Africa. The efficiency of processing methods in the removal of cyanogens varies, and the critical steps in processing that are important in removing the cyanogenic compounds have been investigated for a number of methods (Mlingi *et al.*, 1995; Essers *et al.*, 1995*a*,*b*; Vasconcelos *et al.*, 1990; Westby and Choo, 1995).

The critical stages fundamental to the removal of cyanogens during processing include tissue disruption and drying. Tissue disruption can be brought about by: physiological deterioration mediated by endogenous enzymes; exogenous microbial enzymes during lactic or fungal fermentation; or by physical shearing by means of pounding, grating and chopping. A breakdown of the root cell integrity allows the enzyme linamarase to catalyse the hydrolysis of the cyanogenic glucosides to glucose and the corresponding cyanohydrins. The cyanohydrins then decompose spontaneously or through the action of the enzyme α-hydroxynitrile lyase (Conn, 1969; Mkpong *et al.*, 1990; Fomunyam *et al.*, 1985). Removal of the cyanohydrins at pH > 5 and their subsequent breakdown product hydrogen cyanide can be brought about by thorough drying (Mlingi *et al.*, 1995; Essers *et al.*, 1995*b*).

Processing by sun-drying fresh cassava roots has been investigated by Mlingi *et al.* (1995) in response to an outbreak of acute intoxication in communities of Southern Tanzania. The health problems were attributed to

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the consumption of insufficiently processed cassava during a period of severe food shortage (Mlingi *et al.*, 1992). In this region, cassava is a main staple and has an important role in food security.

Health disorders such as acute intoxication, chronic aggravation of goitre or konzo (Rosling, 1987; Tylleskär *et al.*, 1992) occur in regions where food insecurity forces processors to deviate from their normal cassava processing practices. In Southern Tanzania, bitter cassava varieties with correspondingly high levels of cyanogenic glucosides, are processed into storable products by the simple sun-drying of whole roots or chips. The product, termed makopa, can be stored for up to one year. In a survey of 31 households in a district of Southern Tanzania, cyanogenic glucoside levels were highly variable with an average 145 ± 26 mg HCN equiv./kg dry weight and a range of 67–757 mg HCN equiv./kg dry weight (Mlingi *et al.*, 1995). During the period of severe food shortage, processors deviated from normal practices and processed chinyanya, a rapid processing method taking only one day. Although this method was shown to reduce cyanogens to low levels in trials undertaken by Mlingi *et al.* (1995), the level of the cyanohydrins remained high, 23 ± 14 mg HCN equiv./kg dry weight, due to inadequate drying of the flour. The cyanohydrins are considered to be of greater toxic potential than cyanogenic glucosides (Carlsson *et al.*, in press). Consumers of chinyanya were exposed to these high levels of cyanogens in their diet over a period of time and this resulted in acute intoxication (Mlingi *et al.*, 1992).

The role of cassava in food security is increasing with expanding populations, depletion of soil fertility and failure of traditional crops. Maintaining the quality of cassava products may be achieved in the long term by the identification and introduction of low cyanogenic varieties: however, this will take many years for national breeding programmes to achieve. The short-term solution requires the development or identification of improved processing methods that are culturally and technically acceptable to the cassava consumers in marginal areas. The reduction in the processing time is a key criterion during periods of food shortage.

This paper reports upon a comparative study of current and modifications of processing by sun-drying that are used or could be used by cassava consumers in Africa and more specifically in Southern Tanzania. Specific attention was given to crushing the roots prior to sun-drying since tissue disruption is known to be critical to cyanogen reduction.

METHOD

Cassava varieties used

Laboratory trials were undertaken using cassava variety MCol1684, a high cyanogenic variety, supplied by the Centro Internacional de Agricultura Tropical, Cali, Colombia. In field trials, local varieties provided by Ukiriguru Agricultural Research Institute, Tanzania, were used. These included: the bitter varieties, Liongo and Eala; and the bitter/sweet variety, Mamasheri. All varieties were planted 12 months in advance of harvesting.

Crushing cassava roots

In the laboratory, the crushing of cassava root pieces was first investigated using an electrically operated Macklow-Smith Compressor. This allowed the surface of the crushing plates to be varied and the degree of compression to be adjusted. A 1 mm mesh was used as the optimal plate surface for facilitating the disintegration of the roots in the experiments using the Macklow-Smith Compressor. Using the information gained from the mechanical crusher in terms of force required to crush a transverse root disk and the optimal surface texture, a manual crusher was developed. This was comprised of a wooden block that was split into an upper and a lower portion. The internal surfaces of the wooden block were serrated, the direction of the grooves of the upper and lower portion being perpendicular to each other. A spacer was inserted between the upper and lower portions to regulate the aperture between the block to 2 cm and ensure an even force across the surface of the root disks. The crushing block was placed into a lever press, a 2 m long arm was used to apply a downwards pressure. The availability and low cost of materials was an important consideration in the design of the apparatus.

Investigation of the influence of the degree of crushing of fresh root tissue on the removal of cyanogens

Four kilos of peeled cassava roots (MCol1684) were randomly divided into three replicate samples. Each replicate was treated using a standard procedure as follows. Each root was transversely sectioned into lengths of 4 and 1 cm consecutively along their length. The 4 cm disks were put aside for processing, and the 1 cm disks for each batch were chopped into 1 cm cubes. The cubes were mixed and sampled for dry weight and cyanogen determination. This is referred to as the fresh root (FR) sample.

The remaining 4 cm disks of each replicate were again sub-divided into four batches. From each replicate, the batches were treated as follows: (i) disks were oven-dried; (ii) disks of 4 cm height were crushed to 3 cm height and oven-dried; (iii) disks were crushed to 2 cm height and oven-dried; and (iv) disks were crushed to 1 cm height and oven-dried. All disks were crushed in the press (Macklow-Smith Compressor) to a 3, 2 and 1 cm aperture, respectively. Only one batch was processed to 1 cm height due to the limitations of the compressor. The crushed disks were placed on drying racks in a fan-assisted oven for 60 h at 50°C. The preparation

of the roots for drying and sampling for analysis was completed within 2 h.

After drying disks from a given batch were combined and pounded in a pestle and mortar to a flour. After mixing the flour, a sample was taken for cyanogen and dry weight determinations.

Investigation of changes in cyanogen levels during drying of crushed root tissue

Roots (3.5 kg, peeled) of variety MCol1684 were divided into three replicates. The fresh roots were prepared and sampled as described above. From each replicate the 4 cm root disks were divided into two batches. The first batch was placed on a drying rack. The second batch was crushed as described above, to a height of 2 cm. Samples were taken for analysis at time 0, 48, 95 and 165 h for each treatment.

Cyanogen reduction in crushed and non-crushed root disks (using the prototype crusher)

Two kilos of peeled cassava roots (MCol1684) were randomly divided into 10 replicate samples. The roots were prepared and a fresh root sample taken as described above.

In each replicate, the 4 cm root disks were randomly divided into two batches, crushed root (CR) and standards (STD) root disks. The CR root disks were crushed using the prototype, wooden crusher. Each root disk was placed one at a time into the manual crushing device and a lever action was used to crush cassava to a 2 cm height. The crushed disks were then placed alongside the standard disks on the drying rack and dried in an air-assisted oven at 50°C for 60 h. Dried disks for each batch were combined, pounded and sampled for dry weight and cyanogen analyses.

Comparison of residual cyanogen levels in product of traditional and experimental processing methods

Field trials were undertaken at Ukiriguru Agricultural Research Institute and analyses of samples were carried out by the Tanzania Food and Nutrition Centre and of the Natural Resources Institute.

For each of the three varieties available, a quantity of roots was harvested and immediately divided into five replicate samples by random selection. Each variety was then randomly divided again into six batches. To each batch a particular treatment was applied.

After peeling: batch (1) was taken for analysis of cyanogens and dry matter (FR); (2) was chopped into finger size chips (FS); (3) remained as whole roots (WR); (4) was cut into 4 cm length disks (SD); (5) was cut into 4 cm disks and crushed (CD) using the prototype crusher; while (6) was pounded in a pestle and mortar (CY). All processing treatments and sampling of fresh roots were completed within 2 h from the time of harvesting. The processing of the three varieties was started on consecutive days.

The cassava treatments were dried on mesh trays. Due to the variability of the weather conditions, sun-drying was undertaken in a ventilated glass house. The cassava pieces were turned or mixed on a daily basis. The termination of drying was decided upon by processors who were experienced in processing cassava. The criteria they used included whiteness in colour and snapping sound when broken. Once dried, the batches were pounded in a pestle and mortar, and a sample was taken for extraction of cyanogens, dry weight determination and storage. The following drying periods were required: CY for 2 days; FS for 3 days; CD for 5–6 days; SD for 6–7 days; and WR for 7–9 days.

Cyanogen determination

All dried cassava products were pounded into a flour using a pestle and mortar prior to sampling for subsequent analysis. Extraction and analysis of cyanogens from fresh root and dried flour samples was undertaken as described by O'Brien *et al.* (1992). Moisture was determined by a gravimetric procedure using drying to a constant weight. Sample extracts for cyanogen determination were maintained at 6°C and analysed within 6 weeks. Flour samples were maintained at freezer temperatures of −15°C.

Statistical analysis

Treatment and variety effects were assessed through analysis of variance. Comparisons among treatments that were of particular interest were also made, and differences assessed for significance using a *t*-test.

RESULTS

Investigation of the influence of the degree of crushing of fresh root tissue on the removal of cyanogens

Cassava root disks were crushed by vertical action in controlled laboratory experiments using a Macklow-Smith Compressor. Using a compressor, it was determined that the use of textured surface provided by pieces of wire mesh placed on the crushing plates, resulted in optimal tissue disruption whereby root disks remained intact (in one piece). Root disks that were successfully crushed were softened due to a high degree of tissue disruption resulting from a large number of longitudinal fissures throughout the tissue. The textured surface of the crushing plates prevented the movement of the root disks thus avoiding a shearing action. When shearing occurred the root disk split into a number of pieces that remained firm.

The compressor allowed variations in the degree of crushing to be investigated. It was noted that the degree

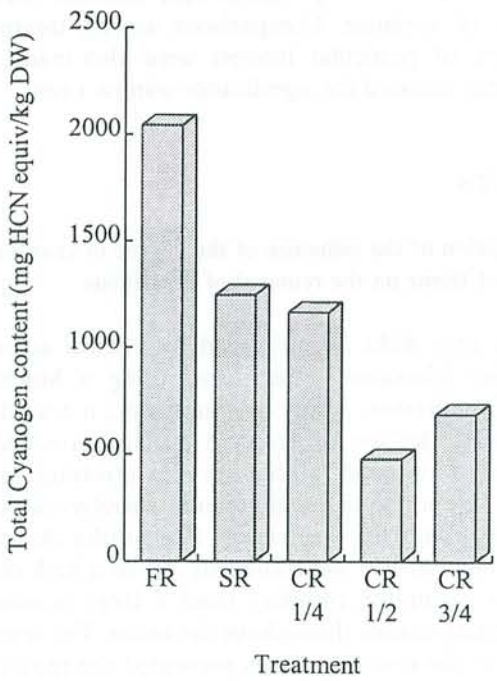
of visible tissue disruption was directly proportional to the level of crushing. Crushing the root disks from 4 cm to 3 and 2 cm height resulted in a mean of 5% and 38% improvement compared to standard disks in the removal of cyanogens, respectively (refer to Fig. 1). An increase in the level of crushing from 4 cm to 1 cm height resulted in a greater degree of tissue disruption but there was no further improvement in cyanogen removal (results of one replicate only, hence significance of the data could not be determined).

Investigation of changes in cyanogen levels during drying of crushed root tissue

Investigation of cyanogen removal during drying was made, whereby levels present in standard disks and those crushed from 4 cm to 2 cm height were monitored over a 165 h period (refer to Fig. 2). Cyanogen levels in both standard and crushed root disks decreased rapidly for the first part of the drying period and then levelled off as the moisture content was reduced. Crushing the disks had an influence on the rate of drying; moisture contents were 12% and 22% after 48 h for crushed and standard disks, respectively. The cyanogen levels of the crushed roots were observed to level out approximately 50 h prior to the standard, non-crushed disks.

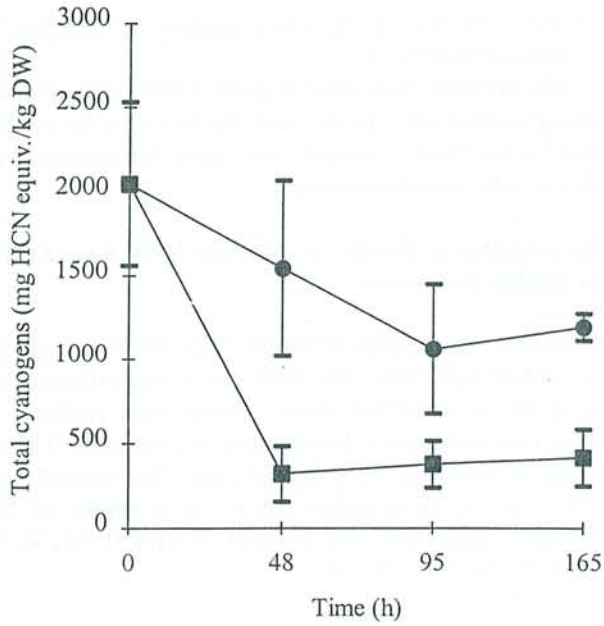
Cyanogen reduction in crushed and non-crushed root disks (using the prototype crusher)

Using the prototype lever crusher to process, a highly significant difference ($p \leq 0.001$ for 10 replicates) in the efficiency of cyanogen removal from root disks as compared to standard non-crushed disks was observed. An improvement in the cyanogen levels from a mean 1040 mg HCN equiv./kg dry weight (50% reduction) to 470 mg HCN equiv./kg dry weight (77% reduction) was observed (Table 1). Thus, a 22% improvement in levels of cyanogens was brought about by crushing the root disks prior to drying. Using the information gained a sampling regime was determined for the field trials. For the 10 replicates taken the least significant difference (LSD), using the t value at 0.025 significance level with the degrees of freedom equal to 10, was 12%. Therefore, for five replicates the LSD would be 21%. The mean values in the trial gave a mean difference of 50%, hence, in subsequent trials five replicates were used that would enable the detection of differences greater than 21%.



Key: FR - fresh root; SR - standard non-crushed root 4 cm in height disk; CR 1/4 - 4 cm crushed to 3 cm, CR 1/2 - 4 cm to 2 cm, CR 3/4 - 4 cm to 1 cm.

Fig. 1. Optimization of cyanogen removal by variation in the degree of crushing.



Key: ● Standard disk; ■ crushed disk

Fig. 2. Cyanogen removal from standard and crushed root disks against time, with error bars representing plus or minus one standard deviation.

Table 1. Levels of cyanogens in standard and crushed root disks of cassava obtained when using the prototype crusher

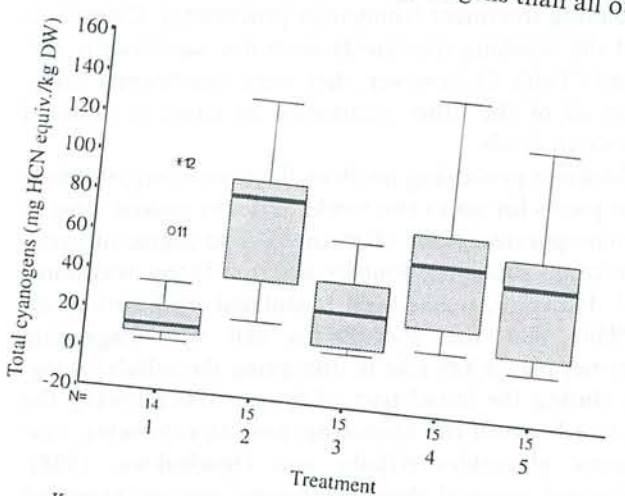
Treatment	Total cyanogen content (mg HCN equiv./kg dry weight)[a]	Total per cent reduction (%)
Fresh root	1942 ± 281	
Standard root disk	1040 ± 270	50 ± 12
Crushed root disk	470 ± 175	77 ± 9

[a]Mean (with standard deviation σ_{n-1}), number of observations was 10.
Mean of difference between standard and crushed = 570 mg HCN equiv./kg DW.
Standard error of difference = 102.
t value = 5.6.
95% Confidence interval is that 570 ± 230 mg HCN equiv./kg DW difference with 9 degrees of freedom.

Comparison of residual cyanogen levels in product of traditional and experimental processing methods

The degree of cyanogen reduction achieved by various means of traditional and improved processing methods was investigated by means of field trials carried out in Tanzania. Local, high cyanogenic varieties, Liongo and Eala, had initial mean total cyanogen levels of 122 and 309 mg HCN equiv./kg dry weight, respectively. The bitter/sweet variety, Mamasheri, had initial cyanogen levels of 293 mg HCN equiv./kg dry weight (Fig. 3). Overall the processing treatment reduced the cyanogen levels in the range of 71–90%.

Ranking in order of efficiency (Fig. 3), chinyanya (pounding prior to sun-drying) processing was found to be most efficient with a 90% reduction in cyanogen levels. This was closely followed by crushed disks using the prototype crusher with an 88% reduction. There was no significant difference detected between these two methods in terms of cyanogen reduction (Table 2). Chinyanya and crushed disk processing methods were significantly better at removing cyanogens than all other processing methods, to at least the 5% significance level. There was a strong significant difference detected at the 1% level between the crushed and standard root disks, corresponding to a 12% improvement. Of the remaining three methods, makopa (whole roots sun-dried) processing ranked third with a 82% reduction in levels, followed by standard uncrushed disks and finger-sized makopa at 76 and 71% reduction, respectively.



Key: 1 - Pounded (chinyanya); 2 - finger-sized pieced (finger-sized makopa); 3 - crushed disk; 4 - standard disk; and 5 - whole root (makopa).
* Value more than 3 box-lengths from 75th percentile.
0 Value more than 1.5 box-lengths from 75th percentile.

Fig. 3. Box plot of residual cyanogen levels in products of various sun-drying processing methods.

Table 2. Probability level of differences for processing cassava by varied means compared to the crushing method

Processing method	Significance level of comparison between crushing and other treatments
'R	—
'Y	< 0.5
'R	< 0.1
5	< 0.0001
)	< 0.01

y: Fresh root (FR); Chinyanya (CY); Makopa (WR); Fin--sized makopa (FS); Standard disk (SD); Crushed disk)).

DISCUSSION

Investigations of cassava processing using traditional methods have been studied since the identification of human health problems resulting from exposure to cassava cyanogens (Rosling, 1987). Outbreaks reported occurred mainly in East Africa, where the principal processing methods involve sun-drying. The aim of this study was to investigate the potential for making simple improvements to the traditional processing of cassava using sun-drying.

It has been shown in previous research that tissue disruption is a critical step in optimizing the removal of potentially toxic cyanogens from cassava (Jones *et al.*, 1994; Vasconcelos *et al.*, 1990). Breakdown of the root cellular structure allows endogenous linamarase, situated on the cell wall, to come in contact with its substrate cyanogenic glucosides, which are situated in the cytoplasm (Mkpong *et al.*, 1990; White *et al.*, 1994).

Previous work by Jones *et al.* (1994) compared different process variables on the cyanogen content of cassava. Pressing prior to drying was one of the least effective treatments when compared to mechanical mincing or rasping; however, the degree of tissue disruption resulting from the pressing treatment was minimal. Mlingi *et al.* (1995), during studies of traditional processing methods used in Tanzania, found that chinyanya, a product processed by pounding fresh roots into small pieces and sun-drying, had far lower residual cyanogen levels than the commonly used product of sun-dried whole roots, known as makopa.

A study of novel means for disrupting the tissue structure of fresh cassava roots was made. The influence of crushing the roots was investigated through laboratory trials and looked promising. This pre-drying treatment was then compared to other established methods through on-station trials in Tanzania. The pre-drying treatments of fresh peeled roots under investigation were: crushing, a novel step that was promising during laboratory based trials; pounding, as in chinyanya processing; and chipping, as for finger-sized makopa. These were compared to two standard treatments: no treatment of whole roots, as for makopa processing; and non-crushed disks.

The sampling strategy adopted during this experimental work was designed to take into account the high degree of heterogeneity of cyanogen levels in cassava roots. Using the least significant difference determined

from the laboratory experiments, the least number of replicates possible to detect a difference in the comparative field study was determined. Although full cyanogen analyses were undertaken, giving values for linamarin, cyanohydrins and free cyanide, the results for total cyanogens alone are reported for fresh and processed flour samples. In all samples, the major cyanogens present were cyanogenic glucosides. The non-glucosidic levels were negligible in the fresh root samples analysed because the cyanogenic glucosides remain intact in freshly harvested roots (White *et al.*, 1994). In flour samples, the lack of lactic fermentation, which is known to stabilize the cyanohydrins and the low moisture content after drying, both contributed to negligible non-glucosidic levels being present in the products (Banea *et al.*, 1992; Mlingi *et al.*, 1995).

The most suitable means to crush cassava roots was to cut them into transverse sections along the length of the root. The root disks were crushed by placing them in a press and applying a downwards force across the cut surface. Using the Macklow-Smith Compressor to crush root disks, it was observed during the laboratory trials that the degree of tissue disruption was directly proportional to the level of crushing force.

The crushing action led to a distortion of the cassava root disks and a softening of the tissue due to longitudinal fissures along the root length. This effectively increased the surface area to volume ratio, allowing a greater area from which moisture could evaporate. In addition, fluid was expelled during crushing. The combined influence of the increased surface area to volume ratio and lowered moisture content due to crushing resulted in an increase in the drying rate. Incorporating a crushing step into the processing procedure resulted in a highly significant improvement of 38% in the removal of cyanogen (Fig. 1).

Monitoring the cyanogen levels of standard and crushed disks during drying, revealed that the levels in both, rapidly decreased during the initial 95 h (9% moisture content) and 50 h (12% moisture content), respectively, and then levelled out. Levelling of the rate of cyanogen removal during drying was also observed by Mlingi *et al.* (1995) at 12% moisture content and by Essers *et al.* (1995*b*) at 13–18%. It has been hypothesized that at these low moisture contents, the linamarase activity and that of other cell wall degrading enzymes is substantially decreased (Essers *et al.*, 1995*a*; Iwatsuki *et al.*, 1984; Okolie and Oguchukwu, 1988). The removal of cyanogens therefore does not only depend on the degree of tissue disruption, but is also influenced by the rate of drying. Crushing the root tissue resulted in an increased efficiency of cyanogen removal compared to standard root batches even though the time allowed for the linamarase to be active was approximately 45 h less than that for non-crushed disks.

In practical terms, the influence of the degree of cellular disruption and drying rate on the efficiency of cyanogen removal can be illustrated by observations made during the comparative study of processing methods. Of the methods studied, the ranking in descending order of efficiency was as follows: chinyanya; crushed disk; makopa; standard uncrushed disk; and finger-sized makopa.

Chinyanya was the most efficient method for removing cyanogens (90% reduction). It is a method whereby the tissue is highly disintegrated by the pounding action, resulting in a rapid breakdown of the cyanogenic glucosides. This was supported by the work of Vasconcelos *et al.* (1990) and Jones *et al.* (1994), who observed complete removal of cyanogenic glucosides after a high degree of tissue disruption brought about by grating and mincing. The small pieces also dried rapidly in up to 2 days. This was due to the greatly increased surface area to volume ratio of the pieces when compared to, for example, makopa processing.

The crushing method ranked second and was of similar efficiency to chinyanya processing with an 88% reduction in cyanogen levels. Crushing the roots involved a lower degree of tissue disruption but has a longer period of high moisture content compared to the pounding treatment (chinyanya processing). Chinyanya and the crushing treatments were not significantly different (Table 2), however, they were significantly better than all of the other treatments in terms of residual cyanogen levels.

Makopa processing involves the sun-drying of whole root pieces for up to two weeks and was ranked third in the comparative study of methods. The degree of tissue disruption brought about by external forces was minimal. However, it has been postulated by Essers *et al.* (1995*b*) that the endogenous cell wall degrading enzymes play a key role in disrupting the cellular integrity during the initial part of drying thus allowing the contact between the linamarase and its substrates, cyanogenic glucosides (Okolie and Oguchukwu, 1988). Cyanogen removal therefore occurs over an extended time period and 82% of the initial level was removed during these trials.

The standard, non-crushed disk method of processing was less efficient in cyanogen removal (76%) than the makopa method due to the increase in the surface area to volume ratio resulting in an increase in the drying rate and consequently reduction in the available time for the action of the hydrolytic enzymes. The finger-sized makopa method ranked last in efficiency (71% reduction). The larger surface area to volume ratio of the chips combined with minimal tissue disruption resulted in high levels of residual cyanogens. Jones *et al.* (1994) observed that compared to the manual slicing of cassava roots mechanical slicing of chips of similar dimensions resulted in an increase in the levels of cyanogens removed. It was postulated that this was due to the reduced level of tissue disruption resulting from the precise incisions of a sharp knife as compared to the rough blades of a mechanical chipper.

CONCLUSION

Crushing cassava roots prior to sun-drying significantly improves the removal of cyanogen over those methods that involve drying of root pieces of similar dimensions. However, it provides no significant benefit in terms of cyanogen removal as compared with methods currently in use in East Africa that involve a high level of root disintegration. A key advantage of the crushing over the pounding method, termed chinyanya, is that the former produces a product that can be stored in the same way as traditional dried pieces (makopa).

The level of cyanogen removal is directly related to the balance between the degree of tissue disruption and rate of drying. This was illustrated by the comparative study of processing methods currently used. Inhibition of the enzyme systems at low moisture content can result in the cyanogenic glucosides being trapped in the dried matrix. Sun-drying of cassava is generally considered to be the least efficient of the various categories of processing commonly practised in Africa (Mlingi *et al.*, 1995). Processing steps such as crushing and pounding may be incorporated prior to sun-drying increase the efficiency of cyanogen removal. However, sun-drying alone as a processing method for highly cyanogenic cassava varieties remains inadequate if levels are to be reduced to the safe limits of 10 mg HCN equiv./kg dry weight recommended by the FAO/WHO (1991).

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