VII^th Symposium of the International Society for Tropical Root Crops,
Gosier (Guadeloupe), 1-6 July 1985, Ed. INRA, Paris, 1988.

NUTRITIONAL ASPECTS OF CASSAVA STORAGE AND PROCESSING

Aspects Nutritionnels du Stockage et du Traitement du Manioc

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SUMMARY

Recent cassava work at TDRI has focussed on the cyanide and phenolic components. Cassava contains cyanogenic glucosides which on tissue damage are hydrolysed via cyanohydrins to HCN. Medical studies have stressed the importance of the long term effects of dietary cyanide. An enzymatic assay developed at TDRI can permit measurement of total cyanide, non-glucosidic (free) cyanide and HCN. Cyanide losses during simple processing of cassava pieces (drying, boiling, soaking) are limited by the hydrolysis of the cyanogenic glucosides. A key factor in cyanide removal from desintegrated tissues is the conversion of non-volatile cyanohydrins to HCN. The interaction of these factors is discussed with respect to traditional processing. The rapid post harvest physiological deterioration of roots appears to be essentially due to wound responses. These include increased activity of phenylalanine ammonia lyase, peroxidase and polyphenol oxidase; formation of phenols such as leucoanthocyanidins, catechins, scopoletin and condensed tannins; and the formation of wound periderm. The influence of storage humidity on these responses is described. The possible anti-nutritional effects of condensed tannins is discussed.

RESUME

Les travaux récents du TRDI (Institut Tropical de Recherche et de Développement) ont porté sur les composés cyannurés et phénoliques. Le manioc contient des glucosides cyanogéniques qui, quand les tissus sont endommagés, sont hydrolysés en cyanhydrines puis en HCN. Des études médicales ont souligné l'importance des effets à long terme des régimes contenant des composés cyanurés.

Une étude enzymatique entreprise au TRDI peut permettre

de mesurer le cyanure total, le cyanure non glucosidique (libre) et le HCN. Les pertes en cyanure pendant le traitement de morceaux de manioc (séchage, ébullition, trempage) sont limitées par l'hydrolyse des glucosides cyanogéniques. Un facteur-clé pour extraire le cyanure de tissus désintégrés est la conversion des cyanhydrines non volatiles en HCN. L'intéraction de ces facteurs est discutée par rapport au traitement traditionnel. La rapide détérioration physiologique après récolte des racines semble être essentiellement la réponse à des blessures. Celles-ci comprennent une activité accrue de phénylalanine ammonia lyase, peroxidase et de polyphenol oxidase; la formation de phénols tels que les leucoanthocyanidines, les catechines, la scopoletine et des tannins condensés; et la formation de périderme de blessure. L'influence de l'humidité de stockage sur ces réponses est discutée. Les effets anti-nutritionnels possibles des tannins condensés sont discutés.

2. INTRODUCTION - Cyanide Aspects

World production of cassava is about 120 million tonnes per year, and this has been estimated to provide a major source of calories for about 500 million people (Cock, 1985). Cassava contains the cyanogenic glucosides linamarin and lotaustralin which on tissue damage are hydrolysed to the corresponding cyanohydrins and hence to hydrogen cyanide, by the endogenous enzyme linamarase (Conn. 1969). Cassava is one of the few human food crops in which the content of cyanide can cause nutritional problems (Coursey, 1973; Cooke and Coursey, 1981). Traditonal cassava processing is unlikely to remove all the cyanide (Cooke and Maduagwu, 1978; Oke, 1983), the presence of which is responsible for the chronic toxicity associated with the continued ingestion of cassava products (Ermans et al, 1980). Recent medical studies (Delange and Ahluwalia, 1983) have stressed the need for screening of cassava to locate lower cyanide lines, and for extended studies of the effects of cassava processing on residual cyanide contents.

A major reason for slow progress in these two areas was the tediousness, lack of accuracy and reproducibility of standard assay methods for total cyanide in cassava. This situation was improved by the development of an enzyme assay (Cooke, 1978) which achieves a rapid and quantitative hydrolysis of the cyanogenic glucosides, and obviates the need for steam distillation or aspiration. Minor variations in the assay procedure permit measurement of total cyanide, non-glucosidic (free) cyanide and HCN (Cooke and De La Cruz, 1982(b). This is an important factor because these different forms of cyanide respond differently to cassava processing and have different toxicities. Application of this assay method has been the subject of collaborative research between TDRI and CIAT (Cooke, 1979; Gomez et al, 1980; Gomez and Valdivieso, 1984), and between TRDI and IITA (Cooke et al, Cooke and Maduagwu, 1978; IITA, 1982). Results of these studies, and applications of this assay at other research centres are discussed in the next section.

Recently, slight modifications to the enzyme assay have been introduced by some research centres. At IITA (Rao and Hahn, 1984) the final stage of quantitation of free cyanide using pyridine pyrazolone (Cooke, 1979) has been automated using a Technicon Auto Analyser. This offers an acceleration of the enzyme assay stage, which may, however, be limited by the sample preparation stage. Ikediobi et al (1980) suggested the use of picrate to replace the pyridine pyrazolone stage of the enzyme assay. The advantage is the avoidance of use of pyridine. However, the latter method (Cooke, 1979) is at least ten times more sensitive, and more importantly the picrate reaction is very unspecific (Zitnak, 1973) leading to interference by many low molecular weight substances common in plant extracts. Workers at the Central Tuber Crops Research Institute, India (Nambisan and Sundaresan, 1984) have suggested replacing the preparation of cassava extracts in phosphoric acid (Cooke, 1979) with extraction in warm 80 per cent ethanol. This necessitates subsequent evaporation of the ethanol, and only permits assay of the cyanogenic glucosides present rather than all the three types of cyanide described above. This is an important limitation because the free cyanide (especially cyanohydrin) often represents a major component of the total cyanide present in processed cassava (eg Maduagwu and Fafunso, 1980).

3. Effects of Processing on the Cyanide Content of Cassava: Processing of Cassava Pieces

Cassava roots are traditionally processed by a wide range of methods to reduce their toxicity, improve their palatability and convert the perishable fresh roots into stable products. These methods comprise combinations of drying, soaking, boiling and fermentation of the roots. All of these processes decrease the total cyanide content, but the data reported in many earlier studies employed doubtful traditional assay methods, and with limited regard for sampling problems (Cooke and Coursey, 1981).

Preliminary studies in collaboration with IITA (Cooke and Maduagwu, 1978) of the processing of cassava pieces indicated that the residual cyanide concentrations are greater than earlier studies had suggested, and that bound cyanide (cyanogenic glucosides) represents the major component.

Drying chips (mean dimensions 40 mm x 8.2 mm x 6.8 mm) of peeled cassava roots in a forced-air drier showed that about 25% - 30% of the bound cyanide was removed at 47°C and 60°C, whereas faster drying at 80°C or 100°C resulted in only a 10% - 15% decrease in bound cyanide. The corresponding losses of free cyanide were 80% - 85% at the lower temperatures, and over 95% at the higher temperatures (Cooke and Maduagwu, 1978). The non-glucosidic

fraction of the total cyanide present in both fresh roots and chipped peeled roots is usually 10% or less (Cooke, 1978). Consequently the decrease in total cyanide content through air-drying is small. The slower drying rates achieved by sun-drying produce greater losses of bound cyanide consistent with the inverse relationship between drying rate and cyanide loss observed in the air-drying experiments (Cooke and Maduagwu, 1978). Studies at CIAT (Gomez et al, 1984; Gomez and Valdivieso, 1984) have indicated similar behaviour with whole root chips of differing root ages. The cyanide elimination found in these studies is greater than those found with the peeled root chips, probably because the former demonstrate a faster hydrolysis of bound cyanide due to the higher linamarase concentration in the peel (Cooke and Coursey, 1981).

Similarly, both soaking peeled root chips in water at 30°C or cooking in boiling water demonstrated that the free cyanide could be removed relatively easily. The bound cyanide decreases at a much slower rate: 55% of the bound cyanide had been removed after 25 minutes in boiling water (at which time these small chips were thoroughly cooked), whereas à negligible decrease occurred on rapid stirring in cold water after 4 hours (Cooke and Maduagwu, 1978). The bound cyanide only begins to decrease after the onset of fermentation, as indicated by a drop in pH and the disruption of the cassava tissue.

Recent studies in collaboration with the National Biological Institute, Indonesia (Cooke and Basuki, 1985) of the Indonesian fermented cassava food'tapé ketela' indicated that the cyanide contents are comparable to those found in commercial samples of fresh peeled "sweet" roots. The tapé process involves a yeast/mould fermentation of boiled, peeled roots. The small reduction in total cyanide content relative to the fresh cassava emphasises again the importance of the hydrolysis of the cyanogenic glucosides as a limiting step in total cyanide loss for products based on cassava pieces. Many traditional cassava preparation methods are based on cassava pieces and the need to investigate the chronic toxicity implications of the residual bound cyanide is emphasised by these studies. Other traditional cassava preparation methods are based on disintegrated or homogenized tissues in which the bound cyanide is more rapidly converted to non-glucosidic cyanide. The differences in rates of cyanide loss in this type of product are described below.

4. Effects of Processing on the Cyanide Content of Cassava :

Processing of Disitegrated or Homogenized Tissues

Cassava processing based on disintegrated tissues would be expected to produce much reduced residual levels of cyanide, because of the much greater contact between linamarase and the cyanogenic glucosides which occurs after tissue damage. An extreme example of this is the traditional extraction of cassava starch. This process consists of wetmilling the washed roots, washing the starch from this milled pulp on vibrating trays or in mixing tanks, sedimenting the starch and sun-drying the product. A collaborative research project between the Food Technology Research Centre, CITA, Costa Rica, and TDRI analysed the reduction in cyanide at each stage of this process (Arguedas and Cooke, 1982). The cassava used is usually harvested when the plant is between 8 and 20 months of age, but the root cyanide concentration is similar in this age range (Cooke and De La Cruz, 982(a)).

More than 80 per cent of the cyanogenic glucoside content is rapidly hydrolysed to free cyanide following tissue disintegration during milling. The highest proportion of the cassava cyanide appears in the wash water (40% - 70%); the freshly sedimenting starch containing about 8% - 14% of the cyanide present in the raw material. This is further reduced by the sedimentation and sun-drying to less than 1 per cent in the starch product. The key step in obtaining these very low residual cyanide concentrations is the initial tissue disintegration in the presence of excess water which permits the rapid hydrolysis of the glucosides. The resulting free cyanide is much easier to remove than bound cyanide (as described in 3.) and the extended mixing in water; soaking with associated fermentation, and slow sun-drying constitute an efficient process for removing this residual cyanide.

This example is extreme in that it is an example of cassava extraction rather than cassava processing. Most cassava processing does not achieve this rapid hydrolysis of the bound cyanide because either the degree of tissue disintegration is considerably less than that encountered in starch extraction, and/or because the reduced amount of water present does not facilitate linamarase action or free cyanide elution as effectively as that described above. Many of the traditional African cassava based foods (Coursey, 1973) are based on soaking of disintegrated cassava tissues with associated fermentation and subsequent cooking and/or drying. Recent results obtained by IITA and other workers using the enzyme assay for cassava cyanide (Maduagwu, 1979; Maduagwu and Fafunso, 1980; IITA, 1982) have indicated that these type of products show an 80% - 95% reduction in total

cyanide content relative to the fresh peeled cassava. Studies of the gari fermentation process (Maduagwu, 1983) suggested that cassava linamarase, rather than enzymes produced by the fermentation microorganisms, is primarily responsible for the glucoside hydrolysis.

The studies of Prof Ermans and his collaborators in Zaire (Delange and Ahluwalifa, 1983) have indicated that despite the considerable reduction in cyanide achieved by traditional fermentation processes, these foods are associated with chronic cyanide toxicity in populations who depend on a poor diet - especially with respect to sulphur containing amino acids and iodine. Consequently, it is pertinent to consider what other factors may limit the reduction of cyanide contents in disintegrated cassava tissues.

The free cyanide produced by hydrolysis of the cyanogenic glucosides is a mixture of cyanohydrin resulting from linamarase action on the glucoside (Conn. 1969) and HCN resulting from chemical or enzymatic (Conn. private communication) hydrolysis of the cyanohydrin. A study (Cooke and De La Cruz, 1982(b) of disintegrated cassava tissues in water and buffer solutions at different temperatures has indicated that the autolytic conversion of cyanogenic glycosides to free cyanide is rapid at pH's near 6. The subsequent slower conversion of the non-volatile cyanohydrin component to HCN is a key factor in the loss of total cyanide from the tissue homogenates. This is illustrated in Fig. 1 which shows the changes in the ratios of the three types of cyanide in parenchymal homogenates maintained at 37°C at (a) pH 6.3, and at (b) pH 4.8. The homogenates at pH 6.3 lost about 25% of the total cyanide after 4 h, the non-glucosidic cyanide content increased to 55% of the total cyanide content, while the HCN proportion increased to 23%. At pH 4.8 both linamarase activity and cyanohydrin breakdown were depressed (Cooke, 1978) but to differing extents (Fig. 1(b)). Only 20% of the total cyanide was present as free cyanide after 4 h, ie about one-third of that present at pH 6.3, but instead of a proportional total cyanide loss there was a negligible loss. The porportion of HCN after 4 h was 3%, suggesting that the key factor was cyanohydrin breakdown to HCN.

In homogenates maintained at 52°C conversions of bound cyanide to free cyanide and cyanohydrin to HCN were more rapid (Fig. 2), but the total cyanide losses were in agreement with the hypothesis described. Total cyanide losses after 4 h at pH 6 and 5 were 48% and 20% respectively, yet the conversion of total to free cyanide were similar. The relative proportions of HCN were quite different: 70% and 35% emphasing again the importance of cyanohydrin decomposition to HCN. The importance of cyanohydrin decomposition

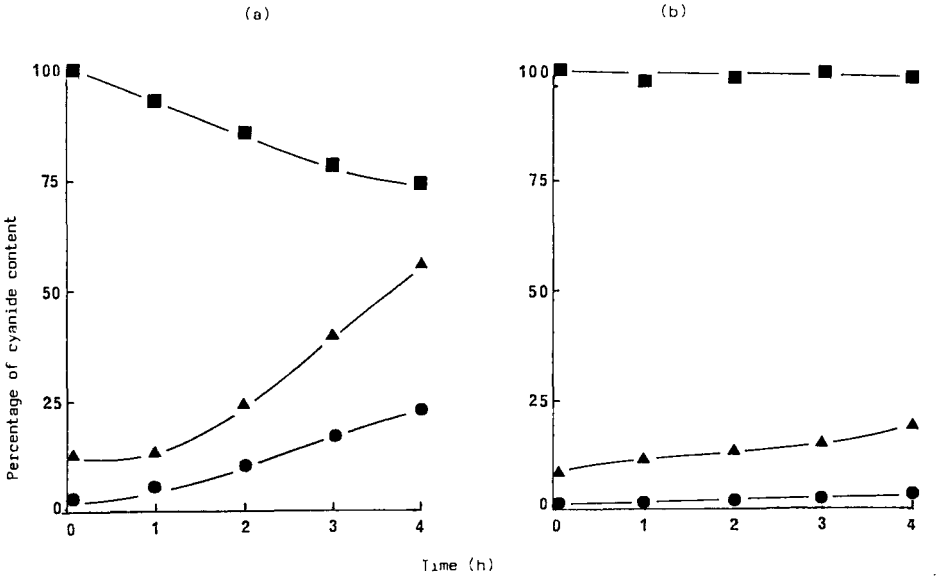

Figure 1

Cyanide stability in root parenchymal tissue homogenates at 37°C: (a) homogenised in water (pH 6.3); (b) homogenised in acetate (0.1 M) buffer pH 4.8. The total cyanide concentration ■ at each interval is expressed as the percentage of the initial total cyanide concentration in the homogenate. The non-glucosidic (free) cyanide concentration (▲) and the hydrogen cyanide (●) concentrations are expressed as percentages of the total cyanide concentrations at each interval. The homogenate pH's did not vary more than 0.1 pH unit during the 4 h period.

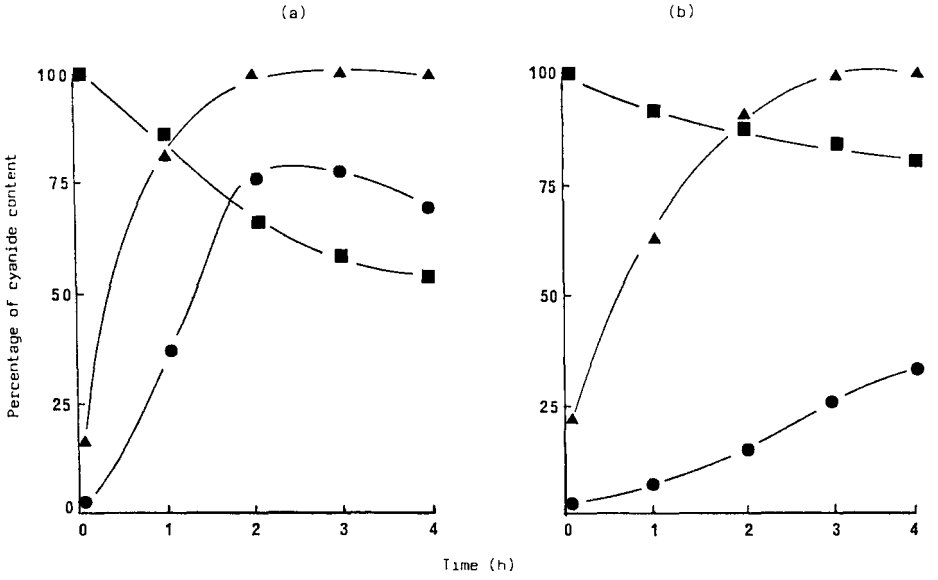

Figure 2

Cyanide stability in root parenchymal tissue homogenates at 52°C: (a) homogenised in water (pH 6.2); (b) homogenised in acetate (0.1 M) buffer pH 5.0. Symbols are as described in Figure 1. The homogenate pH's did not vary more than 0.1 pH unit during the 4 h period.

to cyanide loss is consistent with the similar rates of total cyanide loss from parenchymal, cortex, leaf and whole root homogenates, despite their different linamarase activities (Cooke and De La Cruz, 1982(b)).

The rate of cyanohydrin decomposition to HCN is very pH dependent (Cooke, 19878). Traditional processing methods that involve extensively disintegrated tissues usually undergo lactic acid fermentation (Okafor, 1977) with a consequent decrease in pH to less than 4.0. This hinders total cyanide loss by retarding the rate of cyanohydrin decomposition. This perhaps explains why such products retain considerable residual cyanide.

In conclusion, the key factor determining the rate of cyanide loss during cassava processing based on cassava pieces is likely to be he hydrolysis of the cyanogenic glucosides. In processes based on disintegrated cassava tissues, the conversion of the cyanohydrins to HCN is likely to become limiting. The relative importance of these two steps depends of the proportion of damaged tissue (degree of tissue disintegration) and moisture content ie the linamarase activity on the cyanogenic glucosides; and on the pH of the mixture. The interaction between these two factors is the subject of further research.

5. Cassava Phenolics and the Physiological Deterioration of the Roots

The root tubers of cassava are much more perishable than are the other major root crops. This has been attributed to the fact that unlike the storage organs of other crops they exhibit no dormancy, have no function in propagation and possess no bud primordia from which regrowth can occur (Coursey and Booth, 1977; Passam and Noon, 1977). A collaborative programme between TDRI and CIAT studied different low-cost storage techniques for cassava roots (Booth, 1976). This programme permitted a study of the post harvest deterioration of cassava, which occurs in two separate phases: (i) physiological of primary deterioration often begins within one day of harvest and is characterised by blue or brown discolouration of the vascular bundles of the root tubers ("vascular streaking"); (ii) microbial or secondary deterioration usually occurs later than (i) and involves a wide spectrum of fungi and bacteria, causing a variety of wet and dry rots (Booth, 1976; Rickard and Coursey, 1981).

The TDRI/CIAT programme evaluated a number of methods which would maintain an increased storage humidity, thus "curing" the roots, which eliminates physiological

deterioration (Booth, 1977; Cock, 1985). Similar effects can be achieved by storage in polyethylene bags or cling or shrink films (Thompson and Arango, 1977). These authors also demonstrated the control of spoilage using antimicrobial treatments. CIAT have recently extended this work (CC Wheatley, personal communication) combining fungicide treatment with storage in polythene bags. Pruning the plants several weeks before harvest prevents physiological deterioration (Wheatley et al, 1983), but this causes a reduction in root eating quality and lengthens the cooking time (Cock, 1985).

Recent studies of the physiological deterioration of roots at TDRI indicate that this is due to wound responses comparable to those observed in other plant storage organs. The initial response involves occlusion of the xylem vessels and production of phenolic compounds in the storage parenchyma; carbohydrates, lipids and lignin-like materials were shown to be the major components of the occlusions (Rickard, 1983; Rickard, 1985). Related responses include: increased activity of phenylalanine ammonia lyase, an enzyme associated with phenol biosynthesis; increased activity of peroxidase and polyphenol oxidase; and the consequent formation of phenols/polyphenols including leucoanthocyanidins, catechins, scopoletin and condensed tannins, and often the slower formation of a wound periderm. In cassava, the responses did not remain localised at wound surfaces in roots when held at low storage humidity but spread through the roots causing a discolouration of the vascular tissue and storage parenchyma. Roots stored at high humidity showed a more typical wound response with localised production of phenols and periderm formation (Rickard, 1985).

6. Phenols and Cassava Quality

The studies of the phenolic interactions involved in the physiological deterioration of cassava roots, described in the previous section, led to an appraisal of methods of condensed tannin (proanthocyanidins, ie polymers of flavan-3-ols and flavan 3-4 diols) assay in cassava. Various studies have shown that tannins act as growth depressing factors which decrease protein digestibility (Swain, 1979). A modification of Hagerman and Butler's (1978) protein precipitation method was found to be the most sensitive assay method. The protein precipitation studies gave similar results (Rickard, unpublished results) with dried and chipped commercial cassava samples as with high tannin sorghum. The values were 2 - 10 times higher than those obtained using freeze-dried fresh cassava, suggesting that the post harvest deterioration changes in phenolics (Rickard, 1985) are having an impact under commercial conditions of chip and pellet production.

Early research on cassava roots as a feed for pigs and poultry indicated lower growth rates and feed conversion efficiencies than animals on cereal-based diets, even if the diets were supplemented with proteins (Cock, 1985). More recently (Walker, 1983), methionine supplementation has been found to be important; the usual explanation being its rôle in the detoxification of the cyanide present. However, the phenolics present may also have a nutritional rôle; this is the subject of further research at TDRI since the phenolic spectrum present in cassava products will depend on the post harvest storage and processing procedures employed.

The nature of the phenolics present is likely to have an effect on the flavour and acceptability of cassava based foods. The flavouring constituents of cassava, gari and farinha are the subject of a related research programme at TDRI (Dougan et al, 1983).

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