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DETERMINATION OF CYANOGENIC COMPOUNDS IN CASSAVA DURING HEATED AIR DRYING*

CONTRÔLE DE LA TENEUR EN COMPOSÉS CYANOGÉNIQUES DU MANIOC AU COURS DU SÉCHAGE À L'AIR CHAUD*

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SUMMARY

Cassava is a staple food for approximately 800 million people in tropical countries. This root comprises mainly starch, but also contains a high concentration of cyanogenic glycosides which may give rise to hydrocyanic acid (NARTEY, 1981). Traditional cassava-based foods prepared in the home are devoid of hydrocyanic acid. However, the drying method used for preparation of animal feed does not eliminate cyanogenic glycosides. The purpose of this study was to investigate the relation between thin-bed drying (crossed-flow) and cassava detoxification.

A spectrophotometric method has been developed to measure the kinetics of elimination of these cyanogenic compounds. More than 70% of total and bound cyanide was eliminated using a drying temperature of 60°C, an air speed of 1.5 m/s and 25% relative humidity.

Key-words: *drying, spectrophotometric analytical assay, cyanogenic compounds, cassava.*

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RÉSUMÉ

Le manioc constitue dans les pays tropicaux un aliment de base pour environ 800 millions de personnes. Cette racine, constituée principalement d'amidon, contient cependant une forte teneur en glucosides cyanogéniques susceptibles de donner de l'acide cyanhydrique (NARTEY, 1981). Les préparations culinaires traditionnelles permettent de détoxifier en grande partie le manioc. Cependant, l'utilisation de la technique de séchage pour la fabrication d'aliment du bétail n'élimine pas les composés cyanogéniques.

Ce travail a pour but d'étudier le couplage séchage air chaud-détoxification du manioc. Pour cela, une méthode spectrophotométrique de dosage des composés cyanogéniques a été mise au point et utilisée pour mesurer la cinétique de disparition de ces composés cyanogéniques. Dans les conditions suivantes de séchage : température 60°C, vitesse 1,5 m/s et 25 % d'humidité relative, plus de 70 % des cyanures totaux et liés sont éliminés.

Mots clés : *séchage, dosage spectrophotométrique, composés cyanogéniques, manioc.*

1 - INTRODUCTION

Cassava is grown in tropical countries between latitudes 30° N and 30° S where it constitutes a staple food for about 800 million people (PHILIPS, 1982). However, cassava is not only used for human consumption. This starch-rich root is also used as animal feed in many countries. The E.E.C., for instance, imported 25,4 million tonnes of dry cassava chips between 1986 and 1989 (I.T.P., 1989).

The main defect of cassava is that it contains cyanogenic glycosides (linamarin and lotaustralin) liable to produce hydrocyanic acid (NARTEY, 1981), which causes goitre, cretinism and anorexic ataxia in the consuming populations (SILVESTRE and ARRAUDEAU, 1983). In addition, cassava is rapidly altered and the root is only stable for two days at room temperature or for one week when stored at 4°C.

Cassava present in animal feed constitutes an inexpensive source of starch, and stabilisation and detoxification treatments should not, therefore, augment the cost price. The treatment should simultaneously stabilise and detoxify the cassava. Traditional methods of transformation of cassava consist of a series of treatments (including, peeling, grating, fermentation, boiling, frying, sun drying and grinding) which render it palatable, digestible and non-toxic (HAHN, 1982).

Sun drying is the most common method nowadays. This technique does not require a sophisticated infrastructure and the production costs are low. Moreover, sun drying eliminates a large proportion of the hydrocyanic acid (GOMEZ *et al.*, 1984*a*; COOKE and MADUAGWU, 1978; BOURDOUX *et al.*, 1982). However, drying is slow (two days) (GOMEZ *et al.*, 1984*b*) and depends on the climatic conditions. In addition, since there is no control of the treatment conditions, product quality is inconsistent (SALGADO, 1988).

Furthermore, evaluation of the efficiency of treatment requires a reliable assay method. Numerous methods of varying specificity and sensitivity have been developed, using the extraction of cyanogenic compounds (NAMBISAN and SUNDARESAN, 1984; IKEDIOBI *et al.*, 1980), hydrolysis of cyanogenic glycosides (BRUNS *et al.*, 1970; COOKE, 1978; BRIMER *et al.*, 1983), and assay of cyanide ions (SILVESTRE and ARRAUDEAU, 1983; ASMUS and GARSHAGEN, 1953*a*; DALGAARD and BRIMER, 1984; IZOMKUM-ETIOBHIO *et al.*, 1987). Recently, COOKE (1978) and NAMBISAN and SUNDARESAN (1984) have developed efficient assays, although the latter does not allow determination of all the cyanogenic compounds in cassava (free cyanides, hydrocyanic acid, cyanhydrins and cyanogenic glycosides) (MONROY, 1988). We were therefore obliged to develop a special assay method.

This article describes a protocol for analysis of the different cyanogenic compounds in cassava and the effect of drying at 60°C on cassava chips.

2 - MATERIALS AND METHODS

2.1 Pilot drier

The pilot drier was designed to perform heated air drying under well-defined conditions and to measure the drying rate. It comprised:
- a unit for controlling the temperature, moisture content and speed of the air;
- a measurement cell; the product to be dried is placed on a supported grill. During measurement, the air flow can be diverted to allow determination of the mass. The duration of this diversion of the air is programmable.

Probes were used to monitor various characteristics of the air and the cassava (MONROY, 1988).

Since the characteristics of the drying air were present, recordings were made of the time-dependent changes in the mass of a thin layer of cassava chips (290 g and 1 cm thick, i.e. a load of 11.8 kg/m^2) and in the surface temperature. The measurements were recorded by a data logger.

2.2 Drying tests

The following protocol was used to determine the detoxification kinetics:

Selection of the desired drying conditions

The adjustment and stabilisation of the drying conditions were performed in the same way as for the determination of drying kinetics.

Sample preparation

Wax was first removed from the surface of the cassava roots, which were then cut into chips (3 x 3 x 50-100 mm^3). The chips were mixed to produce a

uniform sample and 290 g were placed on the drying grill. The time lapse between the cutting of the roots and the start of drying was about five minutes.

Drying

Determination of the kinetics of elimination of cyanides was performed by taking cassava samples during drying and assaying the residual cyanide content. The drying air characteristics were: temperature 60°C, speed 1.5 m/s, relative humidity 25%.

Sampling

Samples were taken by deviating the air flow and removing the drying grill. They were weighed immediately and ground with 0.1 M phosphoric acid (1/3, m/v) to inactivate endogenous linamarase in the cassava. The samples were stored in stoppered test-tubes before assay.

2.3 Cassava

Two varieties of cassava were examined in this study:
- The assay was developed using the Costa Rica variety waxed at the production site and then stored in a cold room at 2°C during use.
- The second variety used (M COL 1684) was supplied by CIAT-Colombia (International Centre of Tropical Agriculture). It was treated on site with a fungicidal solution and arrived in the laboratory five days after harvesting. Upon arrival it was immediately waxed and stored in a cold room at 2°C. This variety was used to determine the kinetics of detoxification and drying.

2.4 Dry matter

The dry matter content of the chips was determined after 24-hour drying in a forced air circulation oven set at 105°C.

2.5 Reagents

All reagents used were of analytical quality (Prolabo). The chloramine T solution and the barbituric acid-pyridine reagent were prepared as described by NAMBISAN and SUNDARESAN (1984).

2.6 Determination of cyanogenic compounds *(fig. 1)*

The bound forms were first hydrolysed by linamarase prepared from cassava peel according to the protocol of NAMBISAN and SUNDARESAN (1984) and stored at 2°C protected from light. They were then transformed into glucose and cyanhydrins, which gave hydrocyanic acid and a ketone when hydrolysed in an alkaline medium.

The rate of alkaline hydrolysis increases with pH (FOMUNYAM *et al.*, 1985). The general reaction of the transformation of cyanogenic glycosides was:

Each cassava sample taken during drying was divided into three parts for measurement of HCN concentrations:

1) in the initial phosphoric extract (free cyanides);
2) in the extract treated with NaOH (free cyanides and cyanhydrins);
3) in the extract treated with linamarase and alkalised (total cyanides).

The bound cyanides comprised the cyanogenic glycosides and the cyanhydrins, the free cyanides (hydrocyanic acid), and the total cyanides the sum of free and bound cyanides.

In this way it was possible during drying to determine the level of each cyanogenic compound.

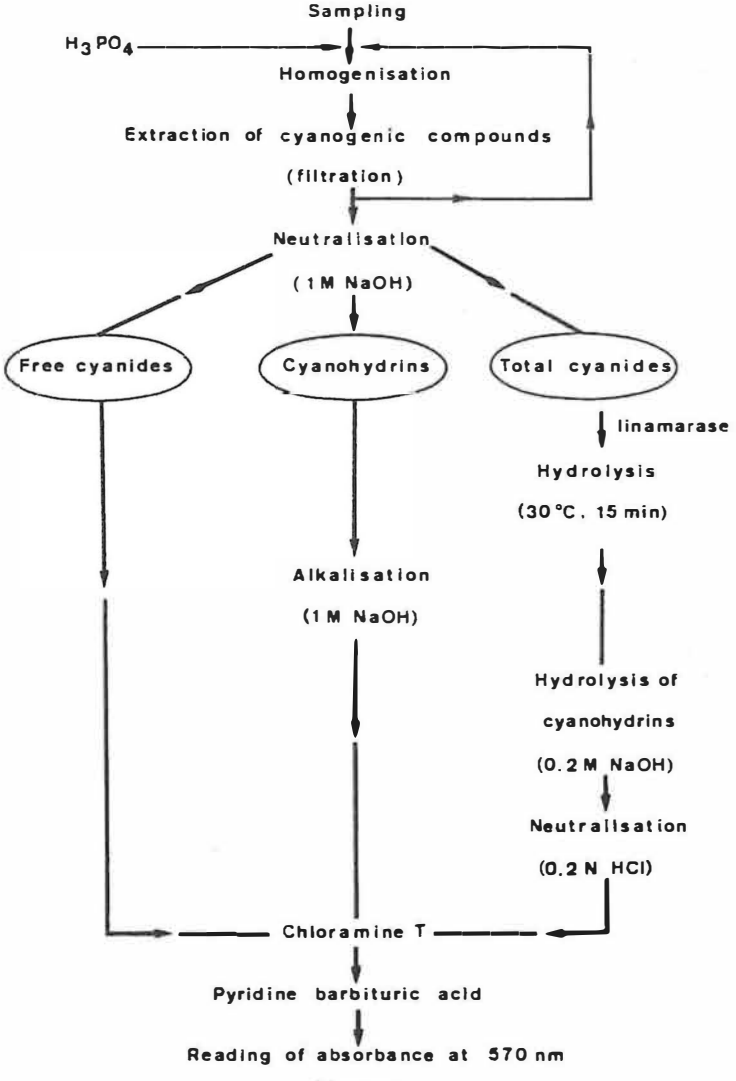


Figure 1
Diagrammatic representation of the assay of cyanogenic compounds

2.7 Determination of cyanide ion concentration by visible spectrophotometry

The assay of hydrocyanic acid used for the three samples was based on the reactions proposed by ASMUS and GARSHAGEN (1953b). Cyanide ions react with chloramine T to form a chlorocyanic compound, which in turn reacts with barbituric acid-pyridine (BAP) to give violet-coloured bis-methine barbituric acid.

Dilutions performed 4 minutes after addition of BAP ensured that the highest concentration of cyanide ions did not exceed 4 mg/l, which corresponded to an optical density of 0.8. The absorbance at 570 nm was read using a Varian Techtron 635 spectrophotometer (wavelength range 190-900 nm, resolution ±0.5 nm). The assays were performed in triplicate.

Calibration was carried out under the same conditions using KCN solutions of various concentrations *(table 1)*.

Table 1
Concentrations (mg/100 ml) of KCN used in standard solutions

mg KCN/100 ml	ppm CN^-
2	8
3	12
4	16
5	20
6	24
7	28
8	32
9	36
10	40

2.8 Extraction methodology

A modified version of the method of NAMBISAN and SUNDARESAN (1984) was used in which 0.1 M orthophosphoric acid (COOKE, 1978) was used in place of hot 80% ethanol (65-70°C) for the extraction of cyanogenic glycosides from cassava. The extractive capacity of orthophosphoric acid is the same as that of ethanol, and it also inactivates endogenous linamarase. Furthermore, evaporation of ethanol results in loss of free hydrocyanic acid.

2.9 Production and stability of coloured compound

The development of colour during the reaction was monitored in order to ensure that the absorbance measurements were performed at the optimal moment. Figure 2 shows that the absorbance reached its maximal value 3.5 minutes after addition of BAP and remained stable for a further 15 minutes *(fig. 2)*. These data are in agreement with those reported by ASMUS and GARSCHAGEN (1953*a*) and NAMBISAN and SUNDARESAN (1984).

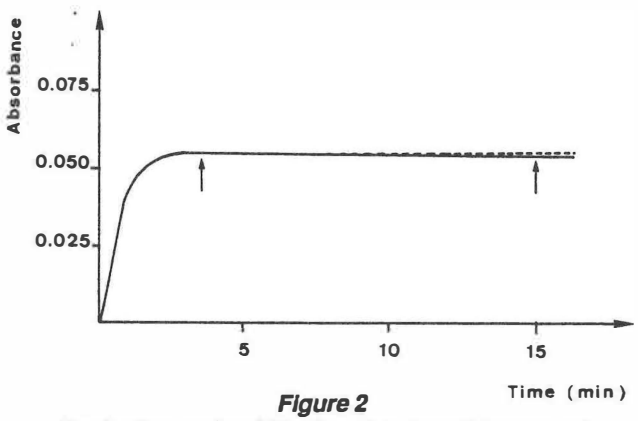


Figure 2
Production and stabilisation of coloured compound

2.10 Enzymatic activity of linamarase extracts

Enzymatic activity was determined using the method of IKEDIOBI *et al.* (1980) using ß-phenyl-4-nitro-D-glucopyranoside as substrate. The unit of linamarase activity was defined as the quantity of enzyme producing an absorbance change of 0.001 at 400 nm during one minute of reaction under the assay conditions. 1 ml of linamarase extract contained about 20 enzymatic units.

3 - RESULTS AND DISCUSSION

3.1 Composition of cassava

The initial proportions of cyanogenic compounds in the cassava were as follows:

68% cyanogenic glycosides
< 1% cyanhydrins
31% hydrocyanic acid

3.2 Kinetics of drying of cassava

Preliminary studies of the drying kinetics were performed in order to determine:

- the drying time for the cassava chips;
- the dry matter content of the cassava at the time of sampling.

The cassava was dried at 60°C for 40 min *(fig. 3)*, instead of 6 h in a heated air drier (cocurrent) (COOKE and MADUAGWU, 1978) or 24 h in an oven (GOMEZ *et al.*, 1984*b*), i.e. for a time comparable to that used by SALGADO (1988) with a heated air drier (cross-current).

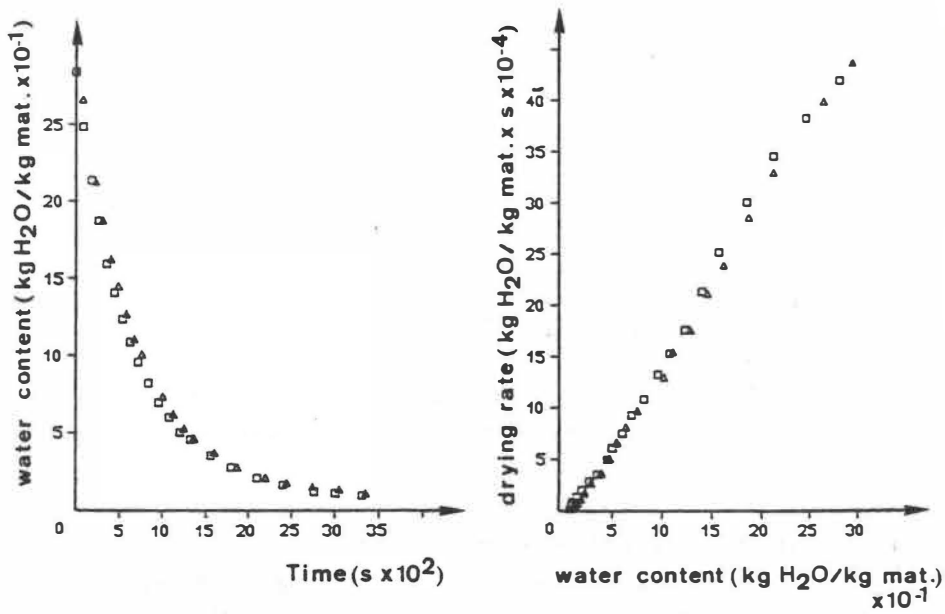


Figure 3
Drying kinetics of cassava variety MCOL 1684

Two tests performed under the same conditions in order to evaluate the repeatability of the measurements gave identical kinetics *(fig. 3)*.

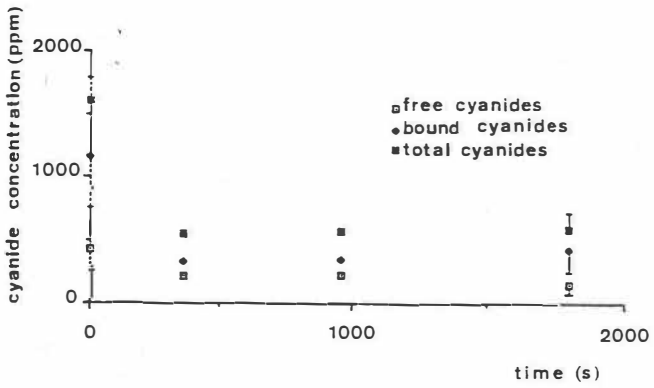
3.3 Kinetics of elimination of cyanides from cassava during drying

Two drying tests were carried out. A series of samples were taken during monitoring of cyanogenic compounds, principally bound cyanides (linamarin and lotaustralin), which are the compounds most difficult to eliminate. Samples were taken every 10 minutes, the time required for sampling and inactivation of the enzyme.

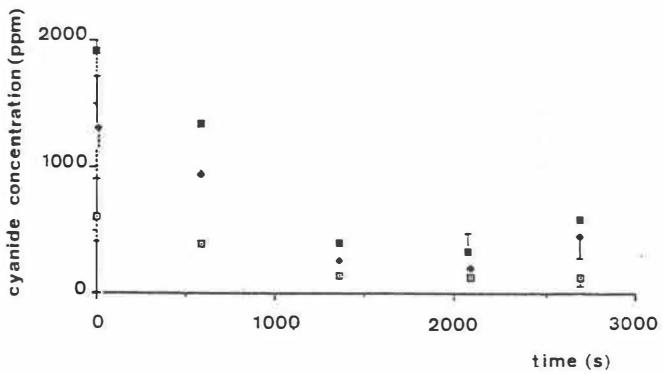
The two assays were performed with samples taken from the same batch of cassava chips under the same drying conditions (60°C, 1.5 m/s, 25% relative humidity). While the first batch was drying, the second was kept in a plastic bag in the cold room at 0°C. After the first test (i.e. after 40 minutes), this sample was removed and dried immediately.

The following observations were made during these test:

1) Figures 4a and 4b show that the effect of drying on elimination of the cyanogenic compounds was only seen during the first 15 minutes of drying. The total cyanide content stabilised between 500 and 600 ppm, corresponding to a loss of about 70% compared with the initial content. Likewise, 70% of the bound cyanides were eliminated.



(a)



(b)

Figure 4
Cyanide elimination kinetics during drying

2) The effect of drying on the cyanogenic compounds must be interpreted in light of the uncertainty of the measurements. The half-intervals at 95% confidence for zero time and for the end of drying are indicated in figures 4a and 4b.

3) Storage of the second sample in the cold room modified the proportion of cyanogenic compounds: the decrease in total cyanides is explained by the drop in free cyanide levels. This prevented us from comparing the two batches from the same sample (see figures 4a and 4b).

COOKE and MADUAGWU (1978) eliminated about 25 to 30% of bound cyanides from peeled cassava chips (40 x 8.2 x 6.8 mm^3) after 6-hour drying at 45 and 60°C in a parallel air flow drier. We eliminated more than twice this amount of bound cyanides using a shorter (8-fold) drying time. This improved elimination is due to the absence of peeling (linamarase is predominantly present in the peel) and to the size of the chips. Hydrolysis of cyanogenic glycosides is initiated when tissues are ruptured and the enzyme and substrate come into contact. Hydrolysis increases as the chip size decreases, and NAMBISAN

and SUNDARESAN (1985) were therefore able to eliminate between 96.8 and 98.5% of cyanide from cassava by grinding followed by sun drying. In contrast, GOMEZ *et al.* (1984*b*) only destroyed 41% of bound cyanide by drying whole cassava roots in an oven at 60°C, but eliminated 85% of the free cyanide. The drying used in the present study was, however, less efficient than sun drying in the elimination of bound cyanide (GOMEZ *et al.*, 1984*a*; GOMEZ and VALDIVIESO, 1984; MADUAGWU and ADEWALE, 1981).

The surface temperature (*) of the cassava was monitored during drying in order to determine why the elimination of cyanides occurred mainly during the first 15 minutes of drying. The water activity (Aw) at the surface of the cassava was deduced from the surface temperature (ABLAN, 1985). The time at which the surface temperature reached 60°C (i.e. a water activity at the surface of less than 0.3, figure 5) coincided with cessation in elimination of bound cyanide.

The temperature and water activity are two complementary parameters reflecting the drying process. Maximal enzymatic activity occurs at a temperature of between 30 and 32°C and the enzyme is inactivated between 80 and 85°C. At 60°C, the enzymatic activity should be very low. After 15 minutes of drying, the overall water content was low and there was not enough water at the surface (Aw = 0.3) to allow contact between the enzyme and substrate. Hence, as the drying temperature gradually increased, the elimination of glycosides decreased.

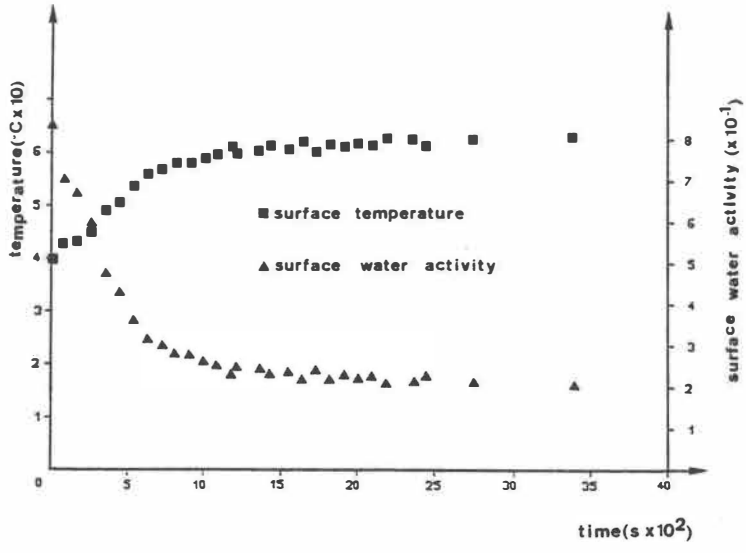


Figure 5
Changes in surface water activity and temperature during drying

(*) Given the size of the chips, the surface temperature is taken as the temperature of the chip.

4 - CONCLUSIONS

We have developed an analytical protocol for differential assay of cyanogenic compounds in cassava. In terms of the drying rate and the elimination of total cyanides from cassava chips, we have found that at the same temperature drying by heated air is more efficient than drying with a parallel air flow and by oven drying. Nonetheless, to complete this work it would be desirable to determine the effects of the drying conditions (temperature, speed and relative humidity of air) on the cyanide content of cassava. This study is currently under way.

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