Detection of cyanide and comparison of two methods for its detoxification in cassava (*Manihot esculenta* Crantz)

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Recibido: 29 de octubre del 2000. Aceptado: 29 de octubre del 2001.

Palabras clave: *Manihot esculenta* Crantz, cianuro, yodo, detoxificación, cromatografía de gases.
Key words: *Manihot esculenta* Crantz, cyanide, iodine, detoxification, gas chromatography.

RESUMEN. La yuca (*Manihot esculenta* Crantz) es un producto agrícola extensamente difundido en la mayoría de los países tropicales. Sin embargo, su consumo pudiera agravar los trastornos provocados por la deficiencia de yodo, entre los que se destacan el bocio, retardo mental y cretinismo, debido a la presencia de significativas cantidades de compuestos cianogénicos en las raíces y hojas de diferentes variedades de esta planta. Por ello, se propone una metodología para la determinación cuali y cuantitativa de cianuro (HCN) en la yuca utilizando la cromatografía de gases de espacio de cabeza (HS-GC). Se determinó el contenido de HCN en una muestra de yuca sin detoxificar (cáscara: 40 ppm y masa: 2 838 ppm, Factor de respuesta (FR) de 4, 86 y tiempo de análisis 90 min), utilizando un cromatógrafo de gases con columnas empacadas y detector de ionización por llama de hidrógeno. Esta fue la técnica empleada para comparar la influencia de la biofermentación seguida de secado al sol (cáscara: 0 ppm y masa: 0,4 ppm) y del secado directo al sol (cáscara: 8 ppm y masa: 2 037 ppm), en la concentración final de cianuro de las muestras, para lo cual, se analizaron las cáscaras y la masa comestible después de procesadas. Se logró disminuir el contenido de HCN aplicando los métodos de detoxificación mencionados y se observó que la cáscara tiene mayor cantidad de este compuesto que la parte comestible aún después de procesadas. Se concluyó que la biofermentación es el método más eficaz de detoxificación de la yuca, pues garantiza productos con muy poco HCN residual.

ABSTRACT. *Cassava* (*Manihot esculenta* Crantz) is an agricultural product widely diffused in most of the tropical countries. However, its consumption could aggravate a variety of health problems caused by insufficient iodine in the diet, such as bocium, mental handicap and cretinism, due to the presence of significant quantities of cyanogenic compound in the roots and leaves of different varieties of this plant. That is why a methodology for the qualitative and quantitative determination of cyanide (HCN) in the cassava, using the Head Space-Gas Chromatography technique, is proposed. The HCN content was determined in a *cassava* sample without a detoxification method (shell: 40 ppm and mass: 2 838 ppm, Response Factor (RF): 4,86 and time of analysis 90 min), using a gas chromatograph with packed columns and flame ionization detector. This was the technique used to compare the influence of two traditional processing methods for the removing of cyanogenic glycosides: The biofermentation followed by sun-drying (shell: 0 ppm and mass: 0,4 ppm) and of the direct sun- drying (shell: 8 ppm and mass: 2 037 ppm), in the final concentration of HCN of the samples, by analyzing the shells and the eatable mass after having processed. It was possible to reduce the HCN content applying both detoxification methods, being observed that the shell still contained more HCN than the eatable part after having been processed. It was concluded that the biofermentation is the most effective method for cassava detoxification because it guarantees products with very little residual HCN.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an agricultural product widely diffused in the most part of the tropical countries in Africa, Latin America and Asia,[1] where the advantages reported by its easy harvest, its high productivity in terms of calories by unit of cultivable area for a unit of time, its development, even in poor soils and under adverse climatic conditions and its resistance to pest and rodents, make possible to place it in one of the most important cultures for feeding, with rice, wheat and corn.[2]

However, its consumption could cause noxious effects for human health, considering the presence of significant amounts of cyanogenic compounds in roots and leaves of different varieties of this plant.[3] Those elements can increase the disorders provoked by iodine deficiency, remarkably bocium, mental handicap and cretinism, depending on many factors, such as: the genetic variety of Cassava, the frequency of its consumption, the effectiveness of the method for processing the roots for the consumption and the concentration of cyanogenic glucosides in the fresh roots.[4,5]

The potential toxicity of highly demanded foods, makes necessary the use of fast and reliable analytical methods for detecting toxic substances, as well as the qualitative and quantitative determination of

cyanogens (linamarin, lotaustraline, cyanohydrins and free cyanide) present in Cassava. For that purpose, different techniques are applied, as the Head space-gas chromatography, characterized by its speed and accuracy for obtaining the results.[6-11]

On the other hand, the main purpose of the Cassava processing for its consumption is precisely to remove the content of the cyanogenic glucosides or of the products of its enzymatic degradation,[12] for that reason, many traditional methods changing according to the cultural preferences of each region have been developed, among them, the biofermentation is relevant by its effectiveness, performed by immersion in water, after sun drying.[13]

However, because of certain circumstances, like wars or natural catastrophes, that have induced determinate population sectors to obviate the more appropriate detoxification methods of the Cassava, by appealing to other faster and sometimes less effective methods, as is the case of sun drying without previous fermentation.[14] This has provoked consequent chronic poisonings caused by the presence of residual cyanogenic glucosides in the products resulting from those alternative methods to process Cassava.

For that reason, the present paper had as aim, the preparation of a gas chromatography technique to be used in determining cyanide (HCN) in Cassava and comparing two detoxification methods of this tuber, by using the proposed chromatography technique.

MATERIALS AND METHODS

Method for determining cyanide in Cassava

Materials

The plant material is triturated in a mortar after freezing with liquid Nitrogen, to reach the smallest particle possible.

Methodology

An amount of 500 mg of powdered sample are placed to react in a 12 mL glass flask, with 0.39 mL distilled water, 0.1 mL sodium sulphate and 10 μL acetonitrile. The flask is hermetically closed and exposed at 70 °C in water-bath for 70 min . After this period, 1 mL gas is extracted to be injected in the chromatograph under the following operation conditions:

Equipment

PYE UNICAM Gas chromatograph GCD model; detector, flame ionization (FID):300 °C, injector: 250 °C furnace: 150 °C refilled pyrex column with porapak Q of 80-100 mesh. Porter gas flow (nitrogen) 25 mL/min

A standard solution with 0.38 mL distilled water, 0.1 mL phosphoric acid, 0.1 g sodium sulphate, 10 µL acetonitrile and 10 µL potassium cyanide (10 mg/mL) dissolved in sodium hydroxide 1 mol/L, this solution is subject to the same conditions as the samples. The cyanide concentration is determined through the Internal Standard Method, for what the acetonitrile is used.

Comparison of two methods for Cassava detoxification

A comparative analysis between two methods commonly used for the elaboration of Cassava in Angola. To do that, the CENSA 6329 genetic variety of Cassava, harvested in San José de las Lajas at the beginning of February and collected the first days of October 2000.

Biofermentation and sun-drying (Angolan traditional Method)

Cassava is immersed in drinking water for 72 or 96 h according the temperature (25-35 °C), the period (relative humidity) and the medium (type of microbiota).

In this conditions, Cassava is softened, gases are removed through the water surface and a strong odor of butyric acid and other organic acids that indicate the end of fermentation. The roots are removed from the water, the peel is removed and Cassava is put for sun drying, so the odor disappears. Finally, the pieces are carried to the mill to obtain the flour, a grayish-white powder similar to the wheat flour.

Sun-drying without previous biofermentation (Alternative method)

Cassava is peeled, cut in small pieces and put under the sun. Once they are dried, the pieces are carried to the mill (or crumbled in mortars) for obtaining the powder or fuba.

Using four combinations between the two processing methods described above and two parts of the tubercle performed the comparative study: the bark and the mass. Three samples were analyzed for each combination through the determination of the content of free cyanide. As control, peel and mass of this variety were used without any previous detoxification treatment.

RESULTS

The average response factor of the studied replicas was 4. 86 with a time of analysis of 90 min (Fig. 1. The control sample (Fig. 2) showed an HCN content significantly different ($p < 0, 05$), with 40 ppm in the bark and 2 838 ppm in the mass. The biofermentation followed by sun-drying (Fig. 3) reduced the HCN content in the bark to 0 ppm and in the mass to 0.4 ppm, while the direct sun-drying without previous fermentation (Fig. 4) caused a decrease of this value in the bark to 8 ppm and in the mass to 2 037 ppm.

DISCUSSION

The Cassava capacity to produce HCN is owed to the presence of significant amounts of two cyanogenic glucosides linamarin and lotaustraline, as well as, of products by their enzymatic hydrolysis (cyanohydrins and cyanhydric acid) in the roots and leaves of different varieties of this plant.[15] Figure 5 shows the degradation process of linamarin, that occurs in the cell vacuoles when entering in contact with the endogen enzyme, linamarase.

The most part of the analytical methods used in determining HCN in Cassava are composed of three steps:

(i) Removal of the cyanogenic compounds of the plant material.

(ii) Hydrolysis of the cyanogenic glucosides to release cyanohydrins and then cyanide.

(iii) Finally, CHN determination.[16]

The removal of the cyanogenic compounds from the plant material is usually performed by using diluted acid, because the linamarase enzyme is inactivated at a very low pH.[17] The hydrolysis of the cyano-

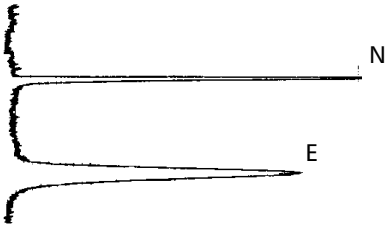

***Fig. 1**. Chomatogram of the standard solution. E Internal standard (acetonitrile). N HCN.*

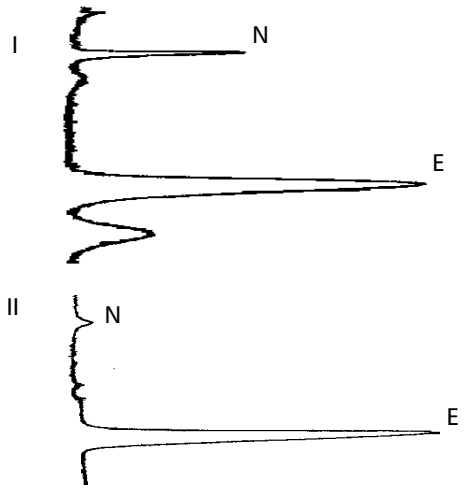

***Fig.2**. Chromatograms of the control sample. I Bark. II. Mass. E Internal standard (acetonitrile). N HCN.*

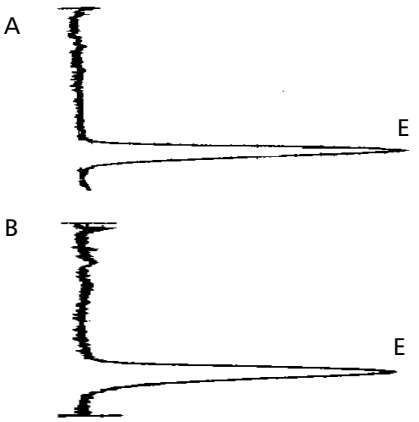

***Fig. 3**. Chromatograms of a sample subjected to biofermentation and sun-drying. A Bark. B Mass. E Internal standard (acetonitrile).*

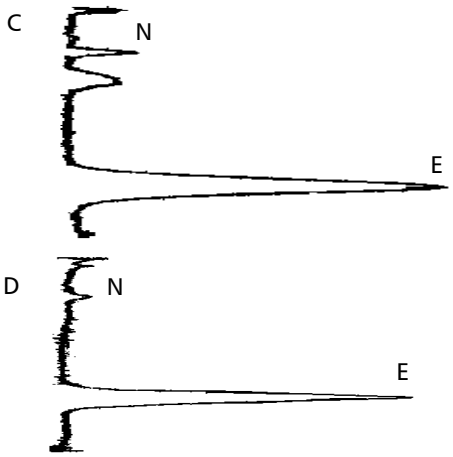

***Fig. 4**. Chromatograms of a sample subjected to sun-drying. C Bark. D Mass. E Internal standard. N HCN.*

genic glucosides can occur by autolysis, performed by some of the linamarase inherent of the plant,[18] enzymatic hydrolysis, with addition of exogenous linamarase[19] or acid hydrolysis, in H_2SO_4 2 mol/L at 100 °C .[20] Finally, after alkalinizing the medium, the HCN determination is performed by using several methods including colorimetry, electrochemistry, HPLC or gas chromatography.

The proposed technique did not include hydrolysis or alcalinization of the medium, so it only allowed the determination of HCN present after removal of the cyanogenic compounds with phosphoric acid 0.1 mol/L and not those of the others. This could be a disadvantage of the method, since even though the linamarin toxicity after absorption is probably very low, it is unknown if linamarin can be hydrolyzed in certain body tissues or by microorganisms sometimes present in intestinal flora, while cyanohydrins are spontaneously hydrolyzed at pH similar to those of the gut and so, they can become as toxic as HCN.

However, this methodology can be used for the preliminary follow-up and the comparison of the effectiveness of methods for processing Cassava for consumption, through the determination of the residual HCN content.

The dehydration degree reached after sun drying depends on the initial humidity intrinsic in Cassava roots, the particle size, the material, the environmental temperature and humidity, the solar radiations and the wind, among other factors.[21]

It is not recommended as a unique detoxification process for the Cassava varieties with a high content of cyanogenic compounds, where the decrease of the size of the pieces to shorten the drying time, results in less hydrolysis of linamarin and therefore, in a higher amount of residual cyanide,[22] while a slow drying can cause the appearance of fungi and it is not known if this process favors micotoxines.[23] Moreover, the linamarin concentration during the sun-drying process exponentially decreases, since they stabilize when an intrinsic humidity level of 15 % is reached. This can explain the fact that the sun-dried roots without a previous fermentation have shown bigger HCN residual when lately analyzed in this paper.

On the other hand, during the period of immersion in water, the microorganisms present in the roots ferment the soluble carbohydrates to cause the formation of lactic acid, mainly, and some acetic acid, that is why the medium pH decreases from 6.0 to 3.8 approximately.[16] These fermenting microorganisms cause a softening of the pulp, what is accompanied by an increase of the hydrolysis of the cyanogenic glucosides by the endogenous linamarase and the microbial ß-glucosidases. The latter sun-drying process completely removes the cyanohydrins content.[24] This explains the total reduction of

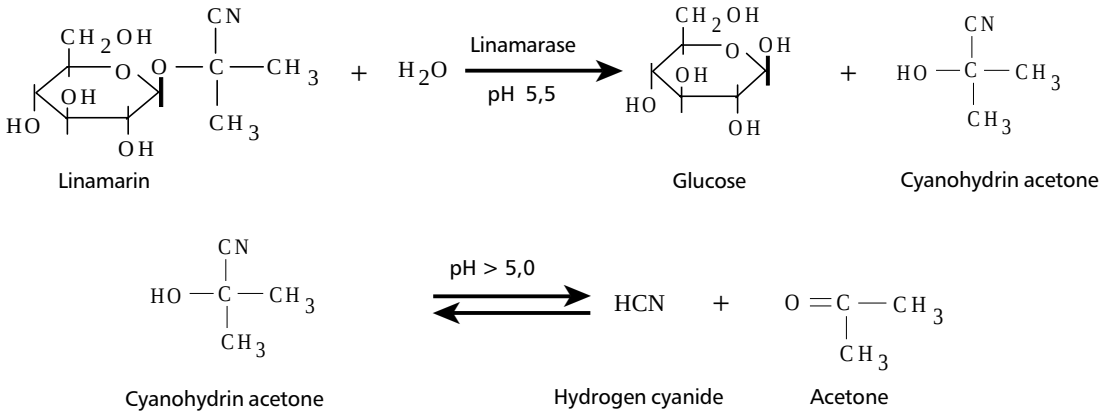

***Fig. 5**. Degradation process of linamarin.*

the HCN levels in the roots subjected to sun drying with previous fermentation in water observed in the present paper.

In general, with both detoxification method of Cassava, the authors obtained a considerable reduction of the content of residual HCN, both in the bark and the mass, when compared to control. Nevertheless, with the traditional Angolan biofermentation and sun-drying method we obtained, for the studied genetic variety of Cassava, a reduction of residual cyanide to limits lower than the detectable one by the used chromatographic method, that is why it is recommended to use it for processing Cassava for consumption.

Moreover, the authors recommend to go on working in order to reach the determination, not only of free cyanide, but also of the other cyanogenic compounds present in the different varieties of Cassava. This method, with the appropriate modifications, could be applied for every type of plant samples.

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