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Full Length Research Paper

Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo

S.C. KOBAWILA, D. LOUEMBE*, S KELEKE, J. HOUNHOUIGAN, C. GAMBA

Faculté des Sciences, BP 69, Brazzaville-Congo / BP 1286, Pointe-Noire, Congo.

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Cassava roots and leaves constitute energy-rich and protein-rich foods, respectively, for the populations in Central Africa, where they are consumed as staple foods. But cassava roots and leaves contain some cyanide in the form of cyanogenic glucosides, notably the linamarine, which can constitute a poison for the consumers when roots or leaves are processed improperly. Cassava roots and leaves processing in Congo, as in most central African countries, involve fermentation. The fermentation of the cassava roots is a lactic fermentation (pH 3.8) with *Lactobacillus* as dominant microflora whereas that of the cassava leaves is an alkaline fermentation (pH 8.5) where *Bacillus* constitute the main microflora. The hydrolysis of cyanogenic glucosides takes place as well in acid medium during the cassava tubers fermentation as in basic medium with the cassava leaves fermentation. The cyanide content decreases during the fermentation of cassava roots and leaves by more than 70% through the activities of the bacterial produced linamarase, allowing the hydrolysis of cyanogenic glucosides. Certain lactic bacteria present in the environment of fermentation are resistant to the strong cyanide concentrations of between 200 and 800 ppm.

Key words: Fermentation, cassava roots, cassava leaves, lactic acid bacteria, bacillus, cyanogenic glucosides.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) leaves are wide and palmated and include 5 in 7 lobes. They are carried by a long and thin petiole. Bunch of mature roots from 30 to 120 cm long and from 4 to 15 cm in diameters appear in the ground. The root is composed of two parts: the external part constitutes the skin and the internal part the pulp.

Cassava roots are quantitatively the third most important food in the tropics, after rice and corn. It is an important source of calories because it covers 60% of the daily calorific needs of the populations in tropical Africa and in Central America Nartey (1978). In central Africa, notably in Congo, cassava roots are mainly consumed in the form of cassava bread, locally named chikwangue. Besides, the cassava leaves are used for human consumption because of their high nutritional value. Cassava leaves are rich in proteins (17-34% dry weight basis), in minerals and vitamins. Cassava leaves proteins is rich in most of the essential amino acids except methionine and phenylalanine (Eggum, 1970; Ravindran et al., 1988; Gomez et al., 1985; Rogers et al., 1963; Ross et al., 1969). But cassava roots and leaves are also rich in cyanide in the form of cyanogenic glucosides, linamarine and lotaustraline (Montgomery 1980; Dunstan et al., 1996) in a ratio of 93:7 (Butler et al., 1965). Cassava is classified according to the cyanhydric acid content into 3 categories:

- Very toxic variety with more than 100 mg HCN/kg of pulp.
- Moderately toxic variety with 50-100 mg HCN/kg of pulp.
- Not toxic variety with less than 50 mg HCN/kg of pulp.

The hydrolysis of cyanogenic glucosides by the endogenous or microbial linamarase enzyme releases the cyanhydric acid, which is toxic. Cyanogenic

*Corresponding author. E-mail: d.louembe@laposte. net, Tel: 00 242 662 05 72 / 00 242 668 69 31, Fax: 00 242 94 39 81.

glucosides are thus responsible for the toxicity of unfermented roots and leaves of cassava (Howlett et al., 1990; Mlingi et al., 1991). Unhydrolysed linamarine, remaining in cassava roots and leaves after fermentation, can constitute a health problem for the consumers (Gomez et al., 1985; Cooke, 1978; Ikediobi et al., 1980; Nartley, 1968). Indeed, the chronic exposure to cyanide due to the consumption of nondetoxified cassava products is associated to a certain number of diseases inferred by the cyanide, including goitre, dwarfism and the tropical ataxic neuropathy. It is particularly a problem in the regions where cassava is the major source of calories (Balagopalan et al., 1988; Tewe, 1984; Umoh et al., 1985; Oke, 1980). Cyanhidric acid is lethal at a consumption dose of 0.5 to 3.5 mg per kilogram body weight.

Traditional technologies have been developed in Central Africa to eliminate cyanhydric acid in cassava roots and leaves, such that they are suitable for human consumption. "*Bikedi*" is a fermented cassava root food obtained by retting of cassava in Congo (Dunican, 1990; Lancaster et al., 1982; Ongusua et al., 1983), while "*ntoba mbodi*" is a vegetable obtained by semi-solid fermentation of cassava leaves. The sensory characteristics (colour, texture, smell, taste) of the final products depend on the type of cassava roots and leaves, on fermentation conditions and on the microorganisms involved. Improvement of the traditional processes and quality control require a better understanding of the traditional fermentation process to obtain "*bikedi*" and "*ntoba mbodi*". This study was carried out to assess the chemical and microbiological changes during natural lactic fermentation of cassava roots and alkaline fermentation of cassava leaves to produce *bikedi* and *ntoba mbodi*.

MATERIALS AND METHODS

Retted cassava roots

18-month-old freshly harvested cassava roots (*Manihot esculenta* Var. Ngansa) were obtained from Agri-Congo (Brazzaville). The roots were processed in a traditional production unit in Brazzaville according illustrated in Figure 1. In the first process, roots are peeled and cleaned in the water. They are then immersed in the water for fermentation for 3 days. After fermentation, the softened cassava product, *bikedi*, is removed from the water. In the second method, the whole roots of cassava (unpeeled) are immersed in the water for fermentation for 3 days. After fermentation and peeling, the softened cassava product, *bikedi*, is removed from the water.

Roots are retted for 6 days in tanks containing 20 L of water at ambient temperature (28 to 32°C). The samplings were conducted during the retting for the determination of pH values and cyanide content.

Fermented cassava leaves

Two weeks to 3 months old cassava leaves were harvested in the cassava plantations of Brazzaville's and of Pointe-Noire's neighborhood. After harvesting, the cassava leaves are exposed to the sun, at ambient temperature, for 2 to 3 h. Stalks and petioles were removed and the leaves cut in fragments. Cut leaves are then cleaned in water, then drained and finally packed in the clean leaves of papaw (*Papaya carica*) at the rate of 150 grams per package for fermentation for 2 to 4 days after which the product, *ntoba mbodi,* is obtained.

Determination of the pH values and cyanide content

Sixty grams of cassava roots sampled during fermentation are pounded in Waring blendor, then homogenized into 100 ml of sterile distilled water. Broyat is filtered on Whatman GF/A (9 cms in diameter) and the pH of the filtrate measured using a Consort P307 pH-meter. pH of the retting water was measured on 10 ml sample, using the same method.

Twenty grams of cassava leaves sampled during fermentation are pounded in Waring blendor, then suspended in 50 ml of sterile distilled water. The pH value is measured with a Jenco model 6071 pH-meter according to the procedure described by Fleming et al. (1983).

Linamarin, cyanohydrins and free cyanides content in fermenting material are determined according to the method of Cooke (1978) modified by Giraud et al. (1993). Linamarase activity was determined according to the method described by Okafor et al. (1990).

Isolation of linamarase-producing lactic acid bacteria

Ten fragments of cassava roots in the course of retting are taken at random and cut in small fragments. 60 g of these small fragments are pounded and homogenized in 540 ml of water by means of Waring blender. Decimal dilutions are prepared from this suspension and Petri dishes containing culture media is sowed with 0.1 ml of these dilutions. For cassava leaves, 10 g of the fermentation sample was crushed in the Waring blender and suspended in 90 ml of sterile peptone water. Dilutions are prepared from this suspension. The following media and the culture conditions were used:

1. PCA (Plate Count Agar) for total mesophile microflora; culture at 30°C for 24 to 72 h.
2. MRS at pH 5.5 for lactic acid bacteria; seeding in layer and incubation at 30°C for 24 to 72 h.
2% malt with rose bengal and 1% for yeasts culture at 37°C for48 h.
3. PDA (Potatoes Dextrose Agar) acidified to pH 3.5 with 10% tartric acid and addition of 0.5% choramphenicol for selection of yeasts and molds; seeding in surface and incubation at 30°C for 3 to 5 days.
4. BP (Baird Parker) at pH 7.2 for selection of staphylococci; seeding in surface and incubation at 30°C for 24 to 72 h.
Agar lactose in desoxycholate pH 7.3 for pathogenic enterobacteria; seeding in double layer and incubation at 30°C for 24 to 72 h.
5. EMB (gelose eosine of methyl alcohol) for enterobacteria; culture anaerobically at 30°C for 24 to 48 h.
6. TSN (Trypticase Sulfite Néomycine) pH 7.2 for clostridium; seeding in surface and incubation anaerobically at 30°C for 24 to 72 h.
7. NAA (Nutrient Agar soluble Amidon, sigma), pH 6.7 – 6.8 for amylolytic bacteria, seeding in surface by means of glass balls and incubation at 30°C for 48 to 72 h.
8. JP2, pH 6.7-6.8 for amylolytic bacteria; seeding in surface and incubation at 28°C for 48 - 72 h, all blue colonies without catalasic activity, presenting a beach of starch hydrolysis after exposure to the vapors of iodine are counted.

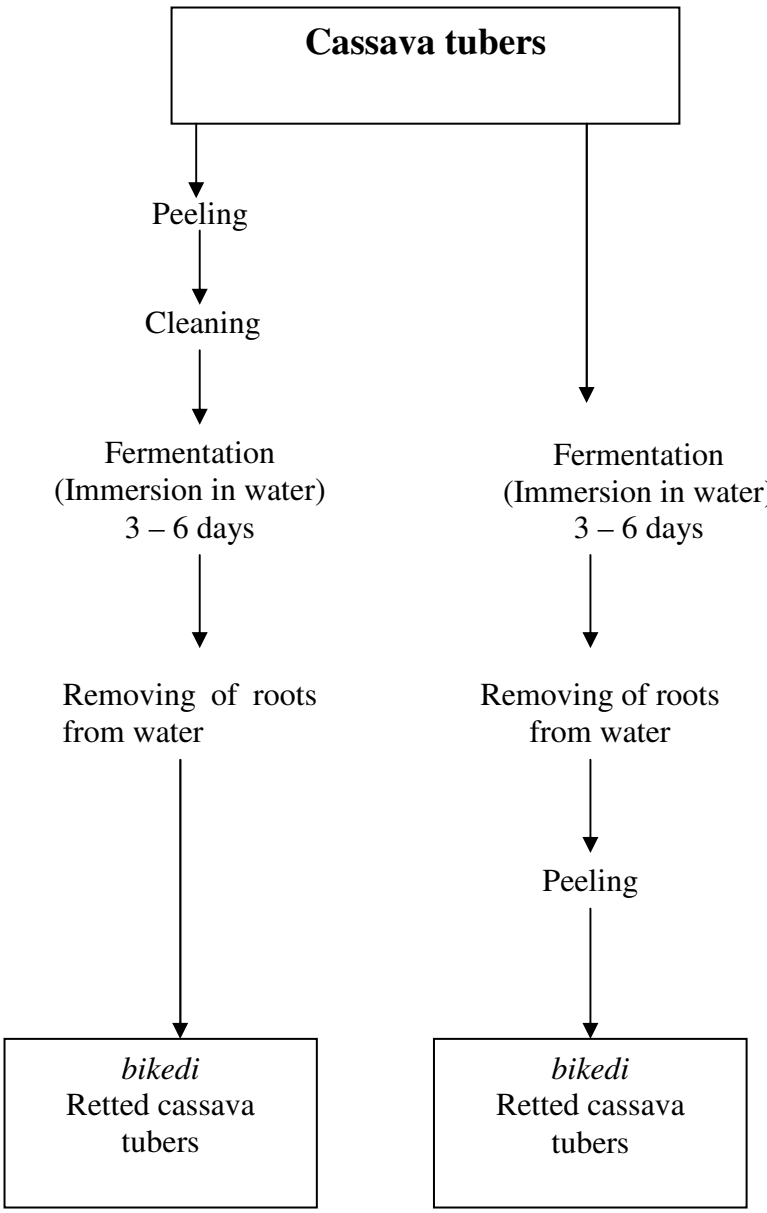

Figure 1. Production of retted roots, *bikedi*.

9. PFP in petri dishes for pectinolytic bacteria; incubation aerobically at 37°C for 4 to 5 days; hydrolysis of the pectin is detected by the presence of depression around colonies.
10. Glucose Yeast Agar (G.Y.A) for zymomonas; culture in double layers at 25°C for 48 h.
11. Terzaghi medium (M17) for lactic streptococci; culture in double layer at 30°C for 48 to 72 h.

Petri dishes are sowed according to method of Miles and Mistra described by Collins et al. (1979). After culture, colonies taken at random various fermentation times are purified and subjected to various physiological and biochemical tests according to the method described by Harrigan et al. (1976). The identification was made according to Buchanan and al. (1974).

RESULTS

pH values and titratable acidity

A fast decrease of the pH was observed both in the retting water and in the roots from 7.2 to 3.8 after 96 h, but the reduction of the pH is faster in the water than in the roots (Figure 3). It can be noticed that cassava root retting is made in acid medium.

In the fermented leaves, the pH, approximately 6.5 at the beginning of the fermentation, increases strongly

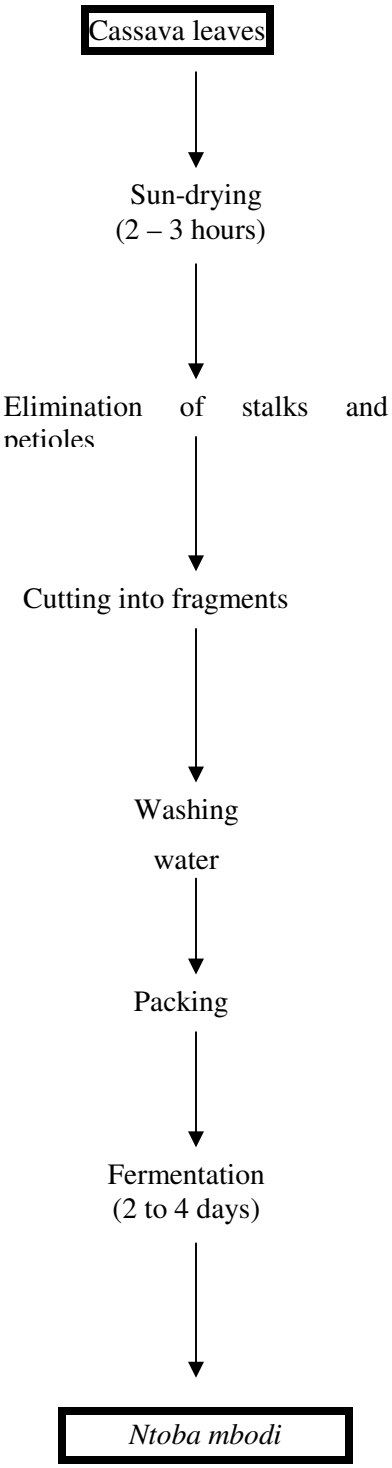

Figure 2. Production of *ntoba mbodi*.

within the first 12 h and becomes basic (pH 8.1) after 24 h of fermentation (Figure 4) to reach pH 8.5 at the end of fermentation. Fermentation of cassava leaves so takes place in basic environment. The titratable acidity

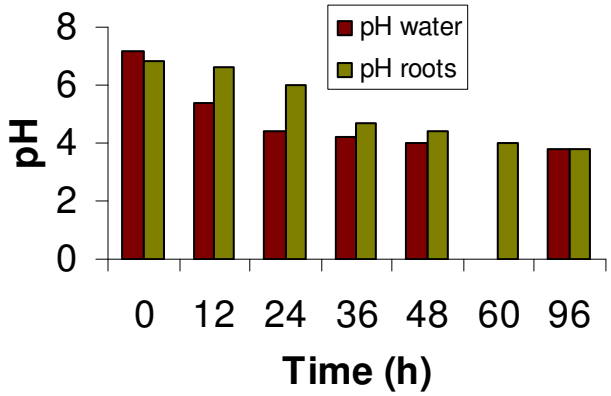

Figure 3. Change of pH during the cassava roots retting.

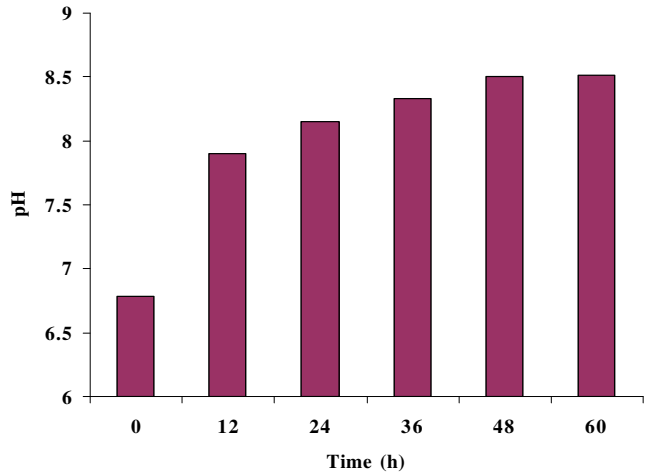

Figure 4. Evolution of pH during the cassava leaves fermentation.

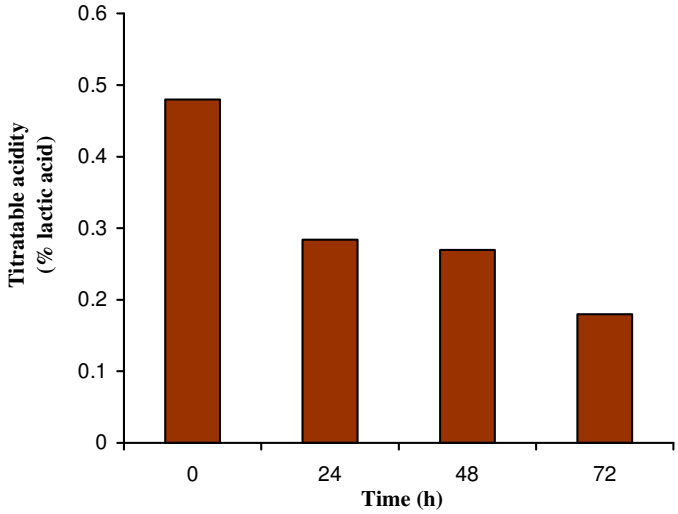

Figure 5. Evolution of the titratable acidity during the cassava leaves fermentation.

decreases during the fermentation of cassava leaves (Figure 5), which is an indication that alkaline compounds are produced during fermentation.

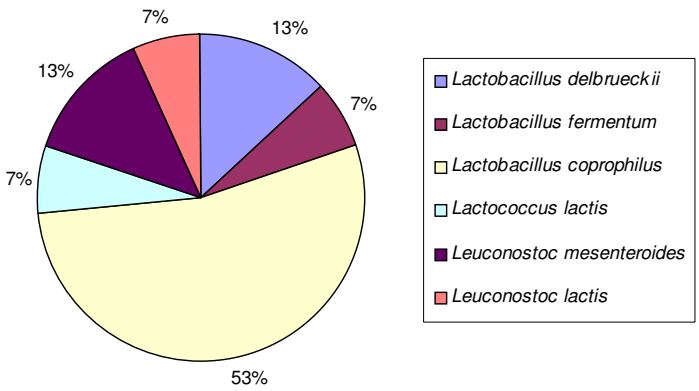

Figure 6. Distribution of lactic bacteria in the fresh cassava roots.

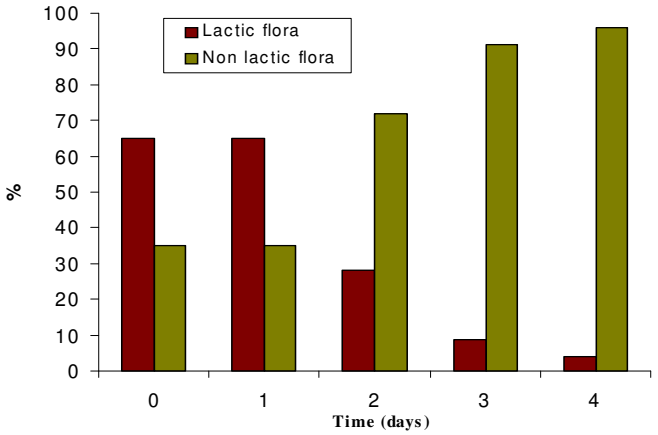

Figure 7. Evolution of lactic and non lactic microflora during the ntoba mbodi production.

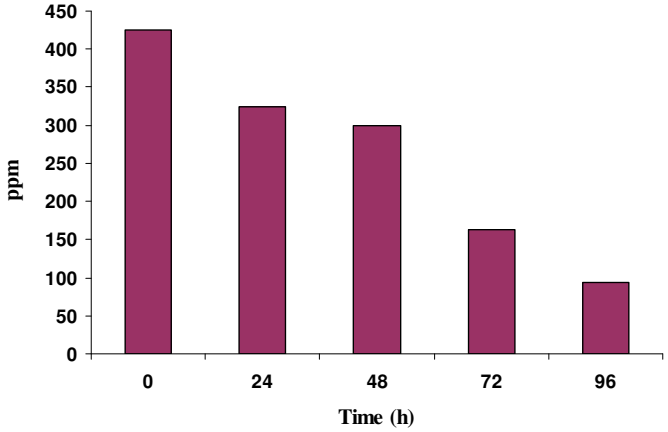

Figure 8. Total cyanide concentration during the *bikedi* production.

Microbial populations in the fermented products

The bacterial population is essentially constituted of lactic bacteria, notably lactobacillus (73.3%) including *Lactobacillus coprophilus* (53.3%), *Lactobacillus fermentum* (6.7%), *Lactobacillus delbrueckii* (13%). The remainder is made up mainly of cocci (26.7%), of which *Lactococcus lactis* (6.7%), *Leuconostoc mesenteroïdes* (13.3%) and *Leuconostoc lactis* (6.7%) are prominent (Figure 6).

Yeast, notably *Saccharomyces cerevisiae* and *Candida*, increases and becomes important at the end of the retting. The molds disappear after 3 days of fermentation.

Lactic microflora in the fermented cassava leaves was mainly constituted of *Lactobacillus plantarum*, *Lactobacillus fermentum, Lactobacillus spp, Lactococcus lactis and Pediococcus cerevisiae.* Their counts falls very strongly, from 65% to 4% of total flora, while non lactic flora including mainly bacillus increases from 34.97% at the beginning of fermentation to 95.92% at the end (Figure 7). The non lactic microflora is constituted mainly of *Bacillus subtilis, Bacillus macerans, Bacillus pumilus, Bacillus spp*. It also includes *Erwinia spp, Staphylococcus sciuri, Staphylococcus xylosus and Micrococcus varians*. These *Bacillus* posess proteolytic activities during the fermentation.

Total cyanide concentration during fermentation

The cyanide content in cassava roots falls progressively during fermentation, from 414 to 93 ppm (77.53% reduction; Figure 8). The fermentation is thus a detoxication process.

The cyanide content varies very little during the first 24 h of fermentation, but decreases drastically from 1158 to 339.6 mg/kg after 48 h of fermentation (Figure 9), which corresponds to 70.67% reduction. The fermentation is also a detoxication process as in cassava roots fermentation.

After fermentation, there is another 22 to 30% of cyanhydric acid content in retted cassava roots and fermented cassava leaves. The change in cyanhydric acid content during cooking is depicted in Figure 10. The cyanhydric acid rate becomes zero after one minute of cooking of fermented cassava leaves.

Many strains of isolated lactic bacteria possess linamarase activity including *Lactococcus lactis, Leuconostoc mesenteroides, Lactobacillus plantarum* and one strain of *Lactobacillus sp.* (Table 1). Elimination of cyanogenic glucosides is thus ensured, at least partially, by the action of the bacterial enzymes.

Some of the lactic acid bacteria such as *Lactobacillus coprophilus, Lactobacillus delbrueckii, Lactobacillus fermentum, Leuconostoc mesenteroides, Lactobacillus plantarum* and *Lactococcus lactis* have the capacity to

Table 1. Bacterial strains producing β-glucosidase activity.

Strains	Number of Strains studied	Number of β-glucosidase producing strains	β-glucosidase activity (10^{-4} µM/ml/mn)
Lactococcus lactis	45	26	6.28
Leuconostoc mesenteroides	22	19	25.18
Lactobacillus plantarum	5	2	3.08
Lactobacillus sp.	2	1	1.22

Table 2. Sensitivity of the lactic acid bacteria strains to cyanide.

Tested microorganisms	Tolerated maximal concentration (ppm)
Lactobacillus coprophilus	200
Lactobacillus delbrueckii	200
Lactobacillus fermentum	400
Leuconostoc mesenteroides	500
Lactobacillus plantarum	600
Lactococcus lactis	800

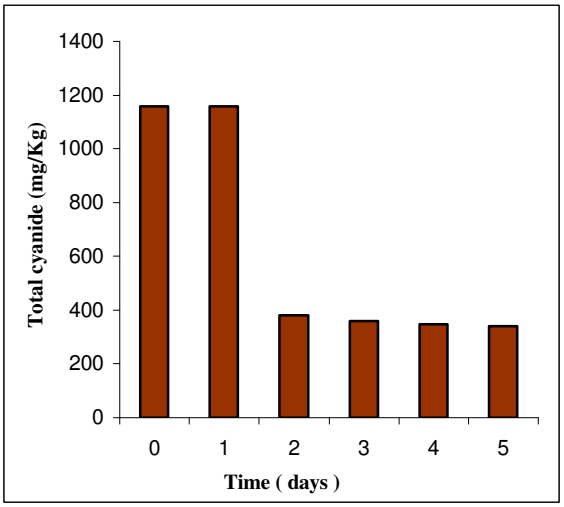

Figure 9. Total cyanide concentration during the cassava leaves fermentation.

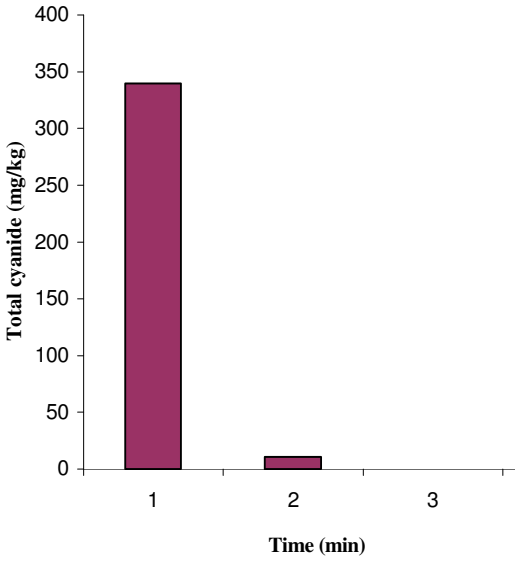

Figure 10. Total cyanide concentration during the cooking of cassava fermented leaves.

resist to strong concentrations of free cyanide, from 200 to 800 ppm (Table 2). These lactic bacteria have a selective advantage with regard to the others in the fermentation medium.

DISCUSSION

In this study, the cyanogenic glucosides content in the roots and freshly harvested leaves of cassava are 414 and 1158 ppm, respectively (Figures 8 and 9). In literature the contents vary according to the studied varieties and it is between 137 and 1515 ppm (Gomez et al., 1984; O'Brien et al., 1992; Agbor Egbe et al., 1995).

After the traditional fermentation of roots and leaves of cassava, the cyanogenic glucosides content is reduced significantly by 70 to 75% (Louembe et al., 1997; Kobawila et al., 2003). Our results confirm those obtained by Agbor-Egbe et al. (1995) confirming fermentation is then a very effective process for elimination of endogenous cyanic compounds from cassava roots.

The inhibitive effect of the cyanide on the lactic acid

bacteria is weak because these bacteria tolerate high concentrations of cyanide (800 ppm; Louembe et al., 1997), while the growth of the other bacteria, such as *E. coli*, is totally inhibited by a cyanide concentration of 2 to 3 ppm (Knowles, 1976). Giraud (1993) reports that the growth of lactic bacteria strains is inhibited by concentrations of cyanide close to 1000 ppm.

This resistance property is responsible for the dominance of lactic acid bacteria in natural microflora of cassava retting. It shows that these microorganisms are adapted well to the contents of cyanide present in cassava-retting roots. Vasconcelos et al. (1990) observed that the degradation of cyanogenic compounds during the fermentation of cassava, leads to the accumulation of free cyanide, which can reach 200 ppm in the fermenting medium. The linamarase produced by the cassava lactic acid bacteria, notably *Leuconostoc mesenteroides* and *Lactococcus lactis*, and the endogenous linamarase contribute to the process of detoxification. Besides, hydrolysis of cyanogenic glucosides (Louembe et al., 1997; Kobawila et al., 2003; Vasconcelos et al., 1990; Okafor et al., 1986) takes place in acid environment (pH 3.8) during lactic fermentation of cassava roots as well as in basic environment (pH 8.5) during alkaline fermentation of the cassava leaves.

The decrease of pH during the fermentation of cassava roots results from the production of organic acids by lactic acid bacteria, which constitute the dominant microflora (Malonga et al., 1993; Malonga et al., 1996). The alkaline pH during the fermentation of cassava leaves could be due to amines produced by *Bacillus* (Louembe et al., 2003). Certain strains of *Bacillus*, notably *Bacillus pumilus*, have the capacity to use cyanhydric acid for their nutrition (Knowles, 1976; Skowronski et al., 1969). They can thus contribute to the reduction of the cyanide content in the medium of fermentation.

The alkaline pH would facilitate reduction of the cyanogenic glucosides content because cyanohydrin acetone, produced by the hydrolysis of linamarin, is cleaved spontaneously when pH is above 5.0 or by the action of hydroxynitril lyase to give acetone and cyanhydric acid, (Nartley, 1968; Conn, 1969; Cooke et al., 1978; Formunyam et al., 1985). Also, cyanhydric acid boiling point is 25.7°C (Gomez et al., 1985; Cooke, 1978; Ikediobi et al., 1980).

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