

Microbiological and Biochemical Characterization of Cassava Retting, a Traditional Lactic Acid Fermentation for Foo-Foo (Cassava Flour) Production

ALAIN BRAUMAN,* SIMON KÉLÉKÉ, MAURICE MALONGA,
EDOUARD MIAMBI, AND FRÉDÉRIC AMPE†

Laboratoire de Microbiologie et de Biotechnologie, Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), Centre ORSTOM, BP 181, Brazzaville, Congo

Received 5 January 1996/Accepted 6 June 1996

The overall kinetics of retting, a spontaneous fermentation of cassava roots performed in central Africa, was investigated in terms of microbial-population evolution and biochemical and physicochemical parameters. During the traditional process, endogenous cyanogens were almost totally degraded, plant cell walls were lysed by the simultaneous action of pectin methylesterase and pectate lyase, and organic acids (C_2 to C_4) were produced. Most microorganisms identified were found to be facultative anaerobes which used the sugars (sucrose, glucose, and fructose) present in the roots as carbon sources. After 24 h of retting, the fermentation reached an equilibrium that was reproducible in all the spontaneous fermentations studied. Lactic acid bacteria were largely predominant (over 99% of the total flora after 48 h) and governed the fermentation. The epiphytic flora was first replaced by *Lactococcus lactis*, then by *Leuconostoc mesenteroides*, and finally, at the end of the process, by *Lactobacillus plantarum*. These organisms produced ethanol and high concentrations of lactate, which strongly acidified the retting juice. In addition, the rapid decrease in partial oxygen pressure rendered the process anaerobic. Strict anaerobes, such as *Clostridium* spp., developed and produced the volatile fatty acids (mainly butyrate) responsible, together with lactate, for the typical flavor of retted cassava. Yeasts (mostly *Candida* spp.) did not seem to play a significant role in the process, but their increasing numbers in the last stage of the process might influence the flavor and the preservation of the end products.

Natural fermentation of plant material is widely used in underdeveloped countries to transform and preserve vegetables because of its low technology and energy requirements and the unique organoleptic properties of the final product (12). In the case of cassava root, a tuber crop cultivated in most of the tropical world, the fermentation prevents the roots from rapid spoilage after harvest. Cassava roots are more perishable than other tuber crops, such as yam and sweet potato (41). The fermentation of cassava roots, called retting, allows the reduction of potentially toxic endogenous cyanogens, which are present in variable concentrations (300 to 500 ppm) (14), and improves their palatability for further processing. Retting of cassava entails steeping roots in water for 3 to 4 days. During the consequent fermentation, roots are softened (34), the endogenous cyanogenic glycosides (linamarin and lotaustralin) are degraded (3), and characteristic flavors develop (1, 36). In the West and in Asia, retting is used for the treatment of certain *Malvaceae*, such as linen flax (7), and other plants, such as cucumber (39). In central Africa, the retted roots are mainly processed into foo-foo (cassava flour) or chickwangué (cassava bread or stick) (47). These products provide almost 50% of the caloric intake of the population (47). Despite the economic importance of these processed products, most of the published work on cassava retting has focused on the detoxification of the cyanogenic glycosides during fermentation (2, 3, 14, 27) or the

influence of bacterial inoculation on foo-foo flavor and root softening (1, 34, 36). In previous studies on the microbiological aspects of this fermentation for production of foo-foo (34) or related products (9, 37), aerobic or air-tolerant microorganisms were counted or isolated but strict anaerobes were not considered. Measurement of some physical and biochemical changes has also been performed (33, 35, 37, 38), but none of these studies included any kinetic measurement of biochemical changes during retting (e.g., enzyme activities, substrate consumption, synthesis of fermentation products), and such measurements are required to demonstrate the role of the microorganisms isolated and to give an overall understanding of the process.

Therefore, to provide a basis for understanding the microbiology of this fermentation, which is needed to improve the quality of this important staple food, we monitored simultaneously (i) the physicochemical environment, (ii) the composition of the microbial community, (iii) the substrates and products, and (iv) the overall activities of the depolymerizing enzymes involved in cassava retting.

MATERIALS AND METHODS

Origin of the plant material. Cassava roots (*Manihot esculenta* var. MMS6) were harvested near Brazzaville, Congo, 15 months after planting.

Retting procedures. Approximately 100 kg of roots was washed, peeled, and placed in a 200-liter barrel filled with rainwater, which was left at ambient temperature. This retting procedure was repeated four times, always yielding similar results; the data presented are from a single representative retting.

Sample preparation for bacterial enumeration. Sampling was performed every 12 h for the first 2 days and then daily until retting was completed. Six randomly selected root sections were cut into 0.5-cm-diameter cubes and mixed under sterile conditions. A 60-g sample was diluted in 540 ml of sterile peptonized water reduced by boiling and addition of cysteine-HCl (0.1% [wt/vol]); this corresponded to a 10^{-1} dilution. The solution was then mixed in a blender (Turmix ME 88; SOFRACA, Bioblock, Strasbourg, France) and serially diluted in sterile peptonized water for aerobic counts or, for anaerobic counts, in an-

* Corresponding author. Present address: Laboratoire d'Ecophysiologie des Invertébrés, Université Paris XII, 93110 Créteil, France. Phone: (33) 1 45 17 05 07. Fax: (33) 1 45 17 15 05. Electronic mail address: brauman@orstom.fr.

† Present address: INSA, Département de Génie Biochimique et Alimentaire, Complexe Scientifique de Rangueil, 31077 Toulouse cédex, France.



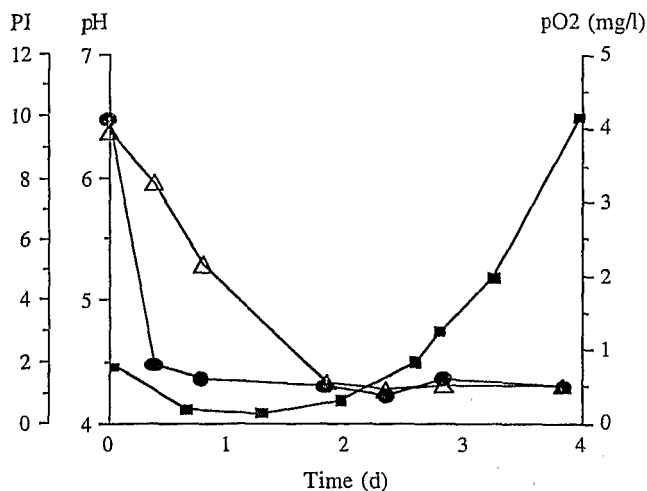


FIG. 1. Evolution of some physicochemical parameters in cassava roots during retting. Symbols: ●, pO₂ (mg/liter); Δ, pH; ■, penetrability index (PI).

aerobic Hungate tubes containing 9 ml of sterile water reduced with 0.1 ml of Na₂S · 9H₂O (5%) and gassed with CO₂-N₂ (20:80) as previously described (23).

Bacterial enumeration. Two types of enumeration were performed. The first method, bacterial most-probable-number determination, involves bacterial growth in liquid culture for the enumeration of glucose- or lactose-fermenting bacteria (GFB and LFB, respectively). Successive dilutions were inoculated (three tubes per dilution) using strict anaerobic techniques (21). Most-probable-number values were obtained by using McCrady's tables (28). The second method involves plate counts on solid medium. A 0.1-ml sample of an appropriate dilution was inoculated in triplicate on solid medium. All the plates were incubated at 30°C, and the numbers of CFU were determined after 48 and 72 h of incubation.

Media and characterization of the isolates. GFB and LFB were enumerated in liquid medium containing (per liter) 0.5 g of trypticase, 0.5 g of yeast extract, 0.5 g of cysteine-HCl, 0.1 g of sodium acetate, 0.005 g of resazurin, 20 ml of Widdel mineral solution (48), 1 ml of trace-element solution (48), and either 2 g of glucose (for GFB) or 5 g of lactate (for LFB). The anaerobic Hungate technique (21) modified by the use of syringes (26) was employed throughout this study. After being boiled the medium was cooled under a continuous flow of O₂-free N₂, adjusted to pH 7.2, dispensed in 9-ml aliquots into Hungate tubes, and then reduced with 0.1 ml of Na₂S · 9H₂O (5%). The tubes were gassed with CO₂-N₂ (20:80) and sterilized (35 min at 110°C).

Lactic acid bacteria (LAB) were enumerated on MRS agar medium (13) supplemented with 0.1% aniline blue. Plate enumeration was preferred to the liquid-culture method because it facilitated the isolation and further characterization of the strains involved in the process. A 0.1-ml sample of each appropriate dilution was placed in a covered petri dish with agar medium and kept at 45°C. Subcultures were further purified by repeated plating. Strains were identified using the criteria previously described (18).

For yeast enumeration, 0.1 ml of each appropriate dilution was spread (in triplicate) on potato dextrose agar (40 g/liter), pH 3.5, containing 50 mg of chloramphenicol per liter. Subcultures were further purified by repeated plating on potato dextrose agar. Isolates were characterized to the genus level according to the criteria of Lodder (24) and Barnett et al. (4). API tests (API 5030 strips; Biomérieux, Marcy l'Etoile, France) were used for the determination of carbohydrate sources.

Resistance to cyanide was determined in the following manner. Filter-sterilized potassium cyanide was added to MRS broth at the following concentrations: 0, 100, 200, 300, 500, 650, and 1,000 ppm. After cyanide addition, the media were adjusted to pH 7.5 and then inoculated with the following strains: *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum*. After 48 h at 30°C, bacterial growth was quantified spectrophotometrically at 600 nm.

Physicochemical parameters. (i) **pH and pO₂ of retting juice.** A 50-ml sample of retting juice was collected for estimation of pH (measured with a CG 838 pH meter from SCHOTT Geräte, Mainz, Germany) and oxygen partial pressure (pO₂) (measured with an OXI 91 from WTW, Weilheim, Germany).

(ii) **Penetrability index.** The penetrability index was used as an indicator of root softening during retting. A penetrometer (model 10-SUR; PNR, Berlin) was used on six randomly cut sections according to a published procedure (1).

(iii) **Cyanide content.** Total and free cyanide was assayed by enzymatic methods according to a published procedure (19).

(iv) **Sugars and metabolites.** The concentrations of sugars, volatile fatty acids, lactate, and ethanol in the roots were assayed by high-pressure liquid chromatography (Thermo Separation Products, San Jose, Calif.). Columns (Bio-Rad Laboratories, Richmond, Calif.) were as follows: (i) a fast carbohydrate column

with a 0.6-ml/min flow of ultrapure water (pH 6) at 70°C, for monosugar analysis; (ii) an Aminex HPX42A column with a 0.3-ml/min flow of ultrapure water (pH 6) at 70°C, for analysis of polysaccharides with degrees of polymerization of 2 to 10; and (iii) an Aminex HPX87H column with a 0.8-ml/min flow of H₂SO₄ (6 mM) at 60°C, for analysis of organic and volatile fatty acids. A refractometer was used for detection (RefractoMonitor IV; Thermo Separation Products).

Enzyme assays. (i) **Enzyme crude extracts.** An 80-ml sample of 0.1 M citrate buffer, pH 6.5, was added to 40 g of cassava pulp, which was then homogenized in a Waring blender, kept overnight at 4°C, and then centrifuged at 12,000 × g for 30 min. The supernatant was lyophilized and then resuspended in 0.1 volume of citrate buffer.

(ii) **Linamarase.** Linamarase activity was measured by using the chromogenic substrate *p*-nitrophenol-β-D-glucopyranoside (20 mM) in 0.1 M sodium phosphate assay buffer, pH 6.8, for 1 h at 25°C. The reaction was stopped by addition of an equal volume of 0.2 M sodium borate (pH 9.8), and *p*-nitrophenol was quantified spectrophotometrically at 400 nm (20). One unit of activity was defined as the amount releasing 1 μmol of *p*-nitrophenol per min.

(iii) **Cellulase and xylanase.** The activities of cellulase and xylanase were assayed by incubation of 100 μl of retting juice with 100 μl of substrate and 50 μl of MacIlvaine buffer at pH 5.8 and 4.4 (25). Reducing sugars were determined by the Somogyi-Nelson procedure (32, 42). The substrates were microcrystalline cellulose (100 mg/ml) and xylane (18 mg/ml). One unit of activity was defined as the amount releasing 1 μmol of glucose or xylose equivalent per min.

(iv) **Pectin methylesterase.** Pectin methylesterase was assayed by titration as previously described (2). One unit of activity was defined as the amount neutralizing 1 μmol of COO⁻ per min.

(v) **Polygalacturonate lyase.** This enzyme was assayed by the change of absorbance at 235 nm according to the procedure of Starr et al. (43). One unit of activity was defined as the amount forming 1 μmol of one unsaturated bond (between C-4 and C-5 in the galacturonide) per min.

(vi) **Polygalacturonase.** This enzyme was assayed by viscosimetry as previously described (2). One unit of activity was defined as the amount releasing 1 μmol of hexose per min.

(vii) **Protein content.** Protein content was estimated by a published method (5), with serum albumin as a standard.

RESULTS

Physicochemical environment. From the first hours of retting, a lowering of pH and a rapid drop in pO₂ occurred in the retting juice (Fig. 1). After the second day of fermentation, both parameters stabilized (to around pH 4.5 and 0.05 mg of oxygen per liter, respectively). After the second day of retting, the plant cells altered considerably, inducing the softening of the roots as evidenced by the increase in the penetrability index (Fig. 1). The cell walls were progressively lysed, releasing starch grains into the retting juice, which gave the retting medium a characteristic white color. Despite the destruction of the plant cells, loss of dry matter during the process was low (<20% of total dry matter) (data not shown).

Cyanogenic compounds. Cyanide compounds responsible for the toxicity of cassava roots were assayed throughout the process. Linamarin, the main cyanogenic glycoside, was highly degraded (95%) after 4 days of retting. An appreciable decrease in cyanogen content was found after 2 days of fermentation, which could be attributed to the pH decrease within the roots. The residual cyanogen in the last stage of retting (50 ppm) was almost entirely in the form of cyanohydrin or free cyanide.

Microflora. The composition of the microflora was estimated throughout the process (Table 1). Because of the anoxic

TABLE 1. Evolution of microflora during cassava retting

Microflora constituent	log ₁₀ cells per g (dry wt) at time (h):						
	0	12	24	36	48	60	72
GFB	4.4	ND ^a	8.7	ND	12	12	12
LAB	4.2	7.0	7.7	8.8	9	12	12
LFB	4.2	ND	3.5	ND	3.5	ND	2.9
Yeasts	<1	ND	0.5	ND	2.3	ND	3.1

^a ND, not determined.

TABLE 2. Sugar and metabolite contents of cassava roots during retting

Sugar or metabolite	Content (g/100 g [dry wt]) at time (h):						
	0	12	24	36	48	60	72
Sugars degraded							
Maltotriose	0.02	0.02	ND ^a	0.02	0.04	0.05	ND
Maltose	0.02	0.11	ND	0.03	0.11	0.06	0.008
Sucrose	4.21	3.31	3.49	0.45	0.33	0.32	ND
Glucose	1.26	1.21	1.52	1.35	1.22	0.77	0.63
Fructose	0.96	0.92	1.2	0.95	0.59	0.39	0.51
Products							
Lactate	ND	0.2	0.23	1.28	2.03	1.36	0.93
Acetate	0.08	0.10	0.25	0.47	0.47	0.26	0.23
Propionate	ND	ND	0.02	ND	0.11	0.06	0.03
Butyrate	ND	ND	ND	0.13	0.15	0.23	0.38
Ethanol	ND	0.05	0.13	0.16	0.18	0.16	0.23

^a ND, not detected.

conditions (Fig. 1), particular attention was given to the facultative and strict anaerobes. Results show that the microflora consisted of facultatively anaerobic fermenting bacteria (GFB) numbering between 10^9 and 10^{12} cells per g (dry weight). Among them, LAB were predominant. They had reached 10^7 cells per g (dry weight) after 12 hours of fermentation and $>10^{12}$ cells per g (dry weight) at the end of the process; LAB then accounted for more than 99% of the total microflora. Similar results were found when the enumeration was performed with the retting juice instead of the roots.

One hundred and four LAB strains were isolated and further characterized. An evolutionary trend in the composition of the LAB population during retting was found. This was reproducible in the four rettings studied. In the roots, LAB represented the main epiphytic flora (67% of total fermenting bacteria). Several species were identified and their relative importance (percentage of total LAB) estimated; they were *Lactobacillus coprophilus* (53.3%), *Lactobacillus delbrueckii* (13.3%), *Lactobacillus fermentum* (6.7%), *Leuconostoc mesenteroides* (20%), and *Lactococcus lactis* (6.7%). In the first step of the fermentation, this flora was supplanted by *Lactococcus lactis* (65% at 24 h) and then by the heterofermenting LAB *Leuconostoc mesenteroides*, which accounted for 59 and 71% of total LAB after 48 and 72 h, respectively. In the final stage, we observed a significant increase in the homofermenting population (mainly *Lactobacillus plantarum*), which could represent up to 100% of total LAB after 8 days of fermentation. The effect of free cyanide on LAB growth was investigated with the three most representative strains of the process: *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum*. The three strains were resistant to free cyanide at growth medium concentrations of up to 100 ppm. *Lactococcus lactis* was found to be the most resistant (still growing with 650 ppm of cyanide), followed by *Lactobacillus plantarum* (maximum resistance, 400 to 500 ppm) and *Leuconostoc mesenteroides* (maximum resistance, 100 to 200 ppm).

Strict anaerobes were enumerated on lactate (LFB) as the numerous LAB prevented the use of other substrates, such as sugars, for enumeration. The production of relatively high butyrate concentrations together with the presence of sporulated rods in the counting tubes strongly suggested the presence of *Clostridium* spp. The isolation of strains with phenotypes close to that of *Clostridium butyricum* has confirmed these observations (22).

Yeasts appeared only after 48 h, their numbers increasing to

a maximum of 10^3 to 10^5 CFU per g (dry weight) at the end of the process. All the strains found belonged to the genus *Candida*.

Substrates and products. Sucrose was the main and preferred growth substrate for all of the microorganisms. Of the initial sucrose content (4.21 g/100 g [dry weight]), 90% was degraded during the first 36 h (Table 2). In the same time period, the glucose and fructose concentrations remained fairly constant. These monosaccharides were then slowly metabolized. The presence of other oligosaccharides was also investigated. Only a little maltose and maltotriose were detected, and their concentrations did not vary significantly during the fermentation. In parallel with sucrose degradation, the accumulation of fermentation products in the medium was observed (Table 2). High amounts of lactate (2.03 g/100 g [dry weight]) and, to a lesser extent, acetate (0.47 g/100 g [dry weight]) were produced during the first 2 days of fermentation. In the same period of time, ethanol was also produced (0.18 g/100 g [dry weight] after 2 days). After 36 h, butyrate was detected in the roots. During the third day of fermentation, the concentrations of lactate and acetate decreased to 0.93 and 0.23 g/100 g (dry weight), respectively, whereas the ethanol concentration remained constant and the butyrate concentration increased gradually to reach 0.38 g/100 g (dry weight) at the end of the fermentation.

Enzymatic activities. Depolymerizing and detoxifying enzymes were assayed throughout the fermentation (Table 3). No cellulase or xylanase activity was found. High pectin methyl-esterase activity was found from the onset to the end of the process (1,400 to 2,200 U/100 g [dry weight]). Significant polygalacturonate lyase activity was found after 24 h and until the end of the fermentation (24 and 45 U/100 g [dry weight], respectively). Endopolygalacturonase activity was also detected, but the levels found were low and not reproducible. Linamarase activity was high (50 U/100 g [dry weight]) during the first 2 days of fermentation and slowly decreased until the end of the process (8.5 U/100g).

DISCUSSION

The results presented here provide the first overall kinetic study of cassava spontaneous fermentation. Cassava fermentation was shown to be a complex microbial process in which a small amount of LAB rapidly replaced the epiphytic microflora and governed the retting of cassava roots. This dominance within the fermentation process could be explained by several factors. (i) As facultative anaerobes, LAB could develop from the onset of retting, when oxygen was still present in the medium, and thanks to their high growth rates on the fermentable sugars present (sucrose, glucose, and fructose), they could overcome the other flora. (ii) LAB produced high amounts of lactic acid, leading to a rapid drop in pH to around 4.5 as previously found during the preparation of foo-foo (34, 35) or lafun, a similar product (37); the environment then became selective against less acid-tolerant microorganisms, as occurs during sauerkraut fermentation (40). (iii) LAB strains isolated

TABLE 3. Enzyme activities in cassava roots during retting

Enzyme	Activity ($\mu\text{mol}/\text{min} \cdot 100 \text{ g}$ [dry wt]) at time (h):				
	0	24	48	72	96
Pectin methyl-esterase	1,414	1,616	1,869	2,070	2,171
Polygalacturonate lyase	ND ^a	24	54	45	45
Linamarase	50	55	31.5	22	8.5

^a ND, not detected.

were well adapted to this toxic environment, as they were all resistant to free cyanide at high concentrations (100 ppm) which inhibited other aerobic organisms. (iv) In addition, strains of *Lactococcus lactis* isolated during the fermentations described here were found to produce bacteriotoxins (data not shown), another way to limit the development of other microorganisms.

As far as LAB are concerned, a three-step microbial succession trend was observed: the epiphytic homofermenting microflora was rapidly supplanted by *Lactococcus lactis* and then by the heterofermenting *Leuconostoc mesenteroides*, which governed the process. Finally, *Lactobacillus plantarum* became the dominant flora in the last hours. The same pattern was observed in earlier studies of foo-foo (34) and lafun production (37). However, the production of ethanol and acetate, together with the dominance of *Leuconostoc mesenteroides* within the fermentation, makes the heterofermenting part of the process more important than previously thought (34, 36). This pattern of population succession was observed in other vegetable fermentations, like those of cucumber (39) and sauerkraut (12, 40). The dominance of heterofermenting LAB (*Leuconostoc mesenteroides*) over homofermenting lactobacilli in cassava roots is a general feature of plant materials (12). The rapid growth of *Lactococcus lactis* in the early stage of retting could be due to its high resistance to cyanide together with its linamarase activity. Its comparatively low, growth-limiting internal pH and its ability to maintain a pH gradient at high organic acid concentrations contribute to the ability of *Lactobacillus plantarum* to terminate these plant fermentations (29). Moreover the lower growth rate of this species on cassava as compared with those of the other LAB (17) may explain why this species was not present in the earlier stage of the process.

Our results also show that in cassava fermentations in which sucrose, glucose, and fructose are present simultaneously, sucrose is the preferred substrate. Growth of LAB on mixtures of sucrose and either glucose or fructose is not known, and this is the first evidence that sucrose inhibits glucose and fructose consumption in vivo. Such a preference for a disaccharide was previously observed during growth of *Lactococcus lactis* on lactose-galactose mixtures; lactose was degraded prior to galactose when it was transported via its phosphotransferase system (46). In *Lactococcus lactis* and *Streptococcus mutans*, sucrose is mainly transported through a high-affinity phosphotransferase system (45), but further evidence is needed before conclusions about the mechanisms responsible for substrate preference with mixtures of sucrose and either glucose and fructose can be made.

Another major characteristic of the rettings described here was the very rapid drop in pO_2 . The installation of reducing conditions enabled the anaerobic microflora to develop. These anoxic conditions have not been reported before but clearly explain the disappearance of the epiphytic molds observed by Oyewole and Odunfa (37) and confirm that organisms such as *Geotrichum* spp. cannot play a significant role in retting, contrary to the suggestion of Collard and Levi (9).

The importance of strict anaerobes, which had not been previously investigated, was demonstrated in this study both through the production of typical fermentation products (butyrate and, to a lesser extent, propionate) and the isolation of *Clostridium* spp. (22). Contrary to the other major fermented vegetables, for which the presence of butyric acid-forming clostridia is seen as an indication of spoilage, in cassava retting, these organisms contribute to the flavor of cassava fermented products. They might also play an important role in the destruction of plant cell walls, as strains with pectinolytic activities have been isolated. Previous reports have indicated the

presence of clostridia in the retting of linen flax (*Clostridium felsineum*) and hemp (7) and in the latter stage of the lactic acid fermentation of olives (15) but not in cassava retting. Clostridia such as *C. butyricum* could resist the acidic conditions of retting, as acid-producing clostridial strains are still able to grow at low pH (pH 4.5) in the presence of 5 g of butyrate or acetate per liter (11). Butyrate, propionate, and ethanol seem to be characteristic of retting, since these products were not detected in other cassava fermentations, such as that used for gari preparation (44).

Yeasts only appeared at the end of retting and could play an important role in the case of prolonged storage. However, contrary to previous suggestions (9, 34, 37), they could not play a significant role in the fermentation process.

Retting allowed the elimination of more than 90% of endogenous cyanide compounds in the roots. This elimination mostly occurred after 48 h, when the endogenous cassava linamarase reached its optimum pH (5.5 [10]). LAB linamarase may participate in the degradation (this work), and the bacterial pectinases have also been shown to help the process (2). Interestingly, the removal of linamarin during retting was slower than that observed during the fermentation of cassava for the production of gari, in which it was eliminated in less than 5 hours (16). In the case of the preparation of gari, prior grating favored the contact between the linamarase, located in the plant cell walls, and its substrate (linamarin), located in the cell vacuoles (30). On the other hand, the slower fall in pH compared with that observed during gari production allowed a greater dissociation of the cyanhydrin into free cyanide, a process which is inhibited at pH values below 5.5 (10). Total free cyanide levels measured in the retted root at the end of fermentation (between 10 and 60 ppm) were comparable to those measured in similar fermentations (14, 44). They remained higher than the maximum recommended intake (10 ppm) (6, 8). However, in the production of chickwango and foo-foo, retted roots are subjected to subsequent processes which further decrease their free cyanide content to 6 to 8 ppm (47).

Softening allows further processing of cassava roots. The presence of high pectinase activities together with the absence of cellulase and xylanase indicated that the former are responsible for the softening. However, the presence of pectin methyl-esterase has already been reported in traditional rettings (38) and in an artificial inoculation of cassava with *Corynebacterium* spp. (34). As was the case for linen flax (31), cassava softening can be characterized by the dissociation of cellulose fibers from their pectin cement because of the action of enzymes, such as hydrolases and lyases, on the pectin glycosidic linkages. The fact that softening began on the second day of retting indicates that the bacteria responsible for this phenomenon were acid-tolerant anaerobes. The recent isolation of pectinolytic *Clostridium* spp. (22) supports this hypothesis.

From all these results, spontaneous cassava fermentation can be seen as the combination of a typical natural heterolactic acid fermentation of a plant material, such as cucumber or sauerkraut, and a spontaneous retting (i.e., softening) resembling that of linen flax.

ACKNOWLEDGMENTS

The authors acknowledge the scientific assistance of O. Mavoungou and S. Trèche and the technical assistance of G. Eboungabeka.

This work was supported by EEC program STD 2 from DG XII, grant TS2A-0226.

REFERENCES

1. Ampe, F., A. Agossou, S. Trèche, and A. Brauman. 1994. Cassava retting: optimization of a traditional fermentation by experimental research meth-

- odology. *J. Sci. Food Agric.* 65:355-361.
2. Ampe, F., and A. Brauman. 1995. Origin of enzymes involved in detoxification and root softening during cassava retting. *World J. Microbiol. Biotechnol.* 11:178-182.
 3. Ayernor, G. 1985. Effects of the retting of cassava on product yield and cyanide detoxification. *J. Food Technol.* 20:89-96.
 4. Barnett, J. A., R. W. Payne, and D. Yarrow. 1983. *Yeasts: characteristics and identification.* Cambridge University Press, Cambridge.
 5. Bensadoun, A., and D. Weinstein. 1976. Assay of protein in the presence of interfering materials. *Anal. Biochem.* 70:241-250.
 6. Blanshard, A. F. J., M. T. Dahniya, H. N. Poulter, and A. J. Taylor. 1994. Quality of cassava foods in Sierra Leone. *J. Sci. Food Agric.* 64:425-432.
 7. Chesson, A. 1978. The maceration of linen flax under anaerobic conditions. *J. Appl. Bacteriol.* 45:219-230.
 8. CODEX Alimentarius Commission. 1989. Codex regional standards, part C. Food and Agriculture Association, Rome.
 9. Collard, P., and S. Levi. 1959. A two-stage fermentation of cassava. *Nature (London)* 183:620-621.
 10. Cooke, R. D., G. G. Blake, and J. M. Battershill. 1978. Purification of cassava linamarase. *Phytochemistry* 17:381-383.
 11. Crabbenbam, P. M., O. M. Neijssel, and D. W. Tempest. 1985. Metabolite and energetic aspects of the growth of *Clostridium butyricum* on glucose in chemostat culture. *Arch. Microbiol.* 142:375-382.
 12. Daeschel, M. A., R. E. Anderson, and H. P. Fleming. 1987. Microbial ecology of fermenting plant material. *FEMS Microbiol. Rev.* 46:357-367.
 13. de Man, J. C., M. Rogosa, and M. E. Sharpe. 1960. A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* 23:130.
 14. El Tinay, A. H., P. L. Bureng, and E. A. E. Yas. 1984. Hydrocyanic acid levels in fermented cassava. *J. Food Technol.* 19:197-202.
 15. Gilliland, J. R., and R. H. Vaughn. 1946. Characteristics of butyric acid bacteria from olives. *J. Bacteriol.* 46:315-322.
 16. Giraud, E. 1993. Ph.D. thesis. University of Aix-Marseille II, Marseille, France.
 17. Giraud, E. 1995. Personal communication.
 18. Giraud, E., A. Brauman, S. Kéléké, B. Lelong, and M. Raimbault. 1991. Isolation and physiological study of an amylolytic strain of *Lactobacillus plantarum*. *Appl. Microbiol. Biotechnol.* 36:379-383.
 19. Giraud, E., and M. Raimbault. 1992. Degradation of the cassava linamarin by lactic acid bacteria. *Biotechnol. Lett.* 14:593-598.
 20. Hosel, W., and W. Barz. 1975. β -Glucosidases from *Cicer arietinum* L. *Eur. J. Biochem.* 57:607-616.
 21. Hungate, R. E. 1969. A roll tube method for the cultivation of strict anaerobes, p. 117-132. *In* J. R. Norris and D. W. Ribbons (ed.), *Methods in microbiology*, vol. 3B. Academic Press, New York.
 22. Kéléké, S. 1996. Unpublished results.
 23. Labat, M., and J. L. Garcia. 1986. Study on the development of methanogenic microflora during anaerobic digestion of sugar beet pulp. *Appl. Microbiol. Biotechnol.* 25:163-168.
 24. Lodder, J. 1970. *The yeasts: taxonomic study.* Elsevier/North-Holland, Amsterdam.
 25. MacIvaine, T. C. 1921. A buffer solution for colorimetric comparison. *J. Biol. Chem.* 49:183-188.
 26. Macy, J. M., J. E. Snellen, and R. E. Hungate. 1972. Use of syringe methods for anaerobiosis. *Am. J. Clin. Nutr.* 25:1318-1323.
 27. Maduagwu, E. N. 1983. Differential effects on the cyanogenic glycoside content of fermenting cassava root pulp by β -glucosidase and microbial activities. *Toxicol. Lett.* 15:335-339.
 28. McCrady, M. H. 1918. Tables for rapid interpretation of fermentation tube results. *Can. Public Health J.* 9:201.
 29. McDonald, L. C., H. P. Fleming, and H. M. Hassan. 1990. Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. *Appl. Environ. Microbiol.* 56:2120-2124.
 30. Mkpogon, O. E., H. Yan, G. Chism, and R. T. Sayre. 1990. Purification, characterization and localisation of linamarase in cassava. *Plant Physiol.* 93:176-181.
 31. Morvan, O., A. Jauneau, C. Morvan, M. Demarty, and C. Ripoll. 1985. Degradation of pectic substances in green flax fibre by *Ervinia carotovora*. *Ann. Appl. Biol.* 112:107-116.
 32. Nelson, N. 1944. Photometric adaptation of Somogyi method for determination of glucose. *J. Biol. Chem.* 153:375-380.
 33. Ogunsa, O. A. 1980. Changes in some chemical constituents during the fermentation of cassava roots (*Manihot esculenta*, Crantz). *Food Chem.* 5:249-255.
 34. Okafor, N., B. Ijioma, and C. Oyolu. 1984. Studies on the microbiology of cassava retting for foo-foo production. *J. Appl. Bacteriol.* 56:1-13.
 35. Oteng-Gyank, G. K., and C. C. Anuonye. 1987. Biochemical studies on the fermentation of cassava (*Manihot utilissima* pohl). *Acta Biotechnol.* 7:289-292.
 36. Oyewole, O. B. 1990. Optimization of cassava fermentation for fufu production: effects of single starter cultures. *J. Appl. Bacteriol.* 68:49-54.
 37. Oyewole, O. B., and S. A. Odunfa. 1988. Microbiological studies on cassava fermentation for "lafun" production. *Food Microbiol.* 5:125-133.
 38. Oyewole, O. B., and S. A. Odunfa. 1992. Extracellular enzyme activities during cassava fermentation for 'fufu' production. *World J. Microbiol. Biotechnol.* 8:71-72.
 39. Pederson, C. S., and M. N. Albury. 1961. The effect of pure culture inoculation on fermentation of cucumber. *Food Technol.* 15:351-354.
 40. Pederson, C. S., and M. N. Albury. 1969. *The sauerkraut fermentation.* N. Y. State Agricultural Experiment Station bulletin no. 824. Cornell University, Geneva, N.Y.
 41. Poulter, D. 1995. Foreword, p. 9-13. *In* E. Agbor, A. Brauman, D. Griffon, and S. Trèche (ed.), *Cassava food processing.* Orstom Edition, Paris.
 42. Somogyi, M. 1945. Determination of blood sugar. *J. Biol. Chem.* 160:61-68.
 43. Starr, M. P., A. K. Chatterjee, P. B. Starr, and G. E. Buchanan. 1977. Enzymatic degradation of polygalacturonic acid by *Yersinia* and *Klebsiella* species in relation to clinical laboratory procedures. *J. Clin. Microbiol.* 6:379-386.
 44. Steinkraus, K. H. 1983. Acid fermented cereal gruels, p. 189-198. *In* K. H. Steinkraus (ed.), *Handbook of indigenous fermented foods.* Microbiology series, vol. 9. Marcel Dekker, Inc., New York.
 45. Thompson, J. 1987. Sugar transport in the lactic acid bacteria, p. 13-38. *In* J. Reizer and A. Peterkofsky (ed.), *Sugar transport and metabolism in Gram-positive bacteria.* Ellis Horwood Ltd., Chichester, England.
 46. Thompson, J., K. W. Turner, and T. D. Thomas. 1978. Catabolite inhibition and sequential metabolism of sugars by *Streptococcus lactis*. *J. Bacteriol.* 133:1163-1174.
 47. Trèche, S., and J. Massamba. 1995. Les modes de transformation traditionnels du manioc au Congo, p. 133-150. *In* E. Agbor, A. Brauman, D. Griffon, and S. Trèche (ed.), *Cassava food processing.* Orstom Edition, Paris.
 48. Widdel, F., and N. Pfennig. 1984. Dissimilatory sulfate or sulfur-reducing bacteria, p. 663-679. *In* N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, 8th ed. Williams & Wilkins Co., Baltimore.

AUGUST 1996

Volume 62 Number 8

Applied and Environmental Microbiology

Published
monthly by
the American
Society for
Microbiology



PM 287
Phyto ^{Bot}

ISSN: 0099-2240