International Food Research Journal 17: 563-573 (2010)

Biochemical and microbial changes during traditional spontaneous lactic acid fermentation process using two varieties of cassava for production of a "Alladjan" starter

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Abstract: Cassava roots are used as human food in many processed forms and products. Fermentation is the initial step needed in the development of various flavour precursors in the cassava and generally conducted as traditional, indigenous processes leading sometimes to the undesirable quality of final products depending probably on the variety (bitter or sweet) of cassava. Both sweet and bitter varieties were purchased from Abidjan and then used for preparation of an Ivorian traditional starter. Lactic acid fermentation was carried out to investigate which variety between sweet and bitter ones is the best raw material for the best quality of starter. During fermentation, progressive acidification was observed in the pulp of both of sweet and bitter cassava varieties. At the beginning of fermentation process total reducing sugars content in bitter cassava pulp is higher than in sweet cassava. Total reducing sugars content decreased and reached the relatively same value of 0.7% in both sweet and bitter cassava. After 48 hours of fermentation, counts of most of micro organisms increased and reached 8 -10 $\log_{10}$ CFU/g and the lowest increase was recorded in the population of Enterococci. Among anaerobic fermenting bacteria, lactic acid bacteria were predominant. Among coliforms, thermotolerant counts population decreased significantly and reached 6 $\log_{10}$ CFU/g in the final stage. Yeast and moulds counts decreased slightly to 6.86 - 7.321 $\log_{10}$ CFU/g at the end of fermentation. The extensive scrutiny of microbial population revealed that micro flora consisted of Aerobic mesophilic counts, coliforms, lactic acid bacteria, enterococci, yeast and moulds in both of varieties

Keywords: Cassava fermentation, starter culture, biochemical, microbial changes

Introduction

Natural fermentation of plant material is one of most food technologies processing used in tropical countries to transform and preserve vegetables because of its low cost and energy requirements and the specific organoleptic properties of the product resulted (Daeschel *et al*., 1987). In the case of, the fermentation prevents the manioc (*Manihot esculenta* Crantz), a tuber crop cultivated in underdeveloped world from rapid spoilage after harvest (Brauman *et al*., 1996). Cassava tubers are more perishable than other tuber crops, such as yam and sweet potato. As a 1–2 m high shrub which, due to its high yields of starchy roots on marginal land, cassava is a major staple crop for over 500 million people in the developing world (Cock, 1982). Cassava ranks fourth on the list of main food crops in developing regions after rice, wheat and maize (Mingli *et al*., 1992). Cassava is a major staple crop for over 500 million people in the developing world (Cock, 1982). It is cultivated mainly by resource-limited small farmers for its starchy roots, and widely consumed in warm tropical areas (Niba *et al*., 2001). Cassava roots are used as human food either fresh when low in cyanogens; it's the case of sweet variety; or in many processed forms and products, mostly starch, flour, and for animal feed; it's the case of bitter variety. Over 90% of the cassava processed in Africa is used for human nutrition as fermented products (Mensah, 1997). Several varieties of cassava exist but it is possible to gather them in two groups: the sweet ones and the bitter ones (Assanvo *et al*., 2002). Indeed; if in Côte d'Ivoire sweet cassava may be consumed directly mainly by preparation of the dishes such as "Foutou", "Bèdècouman", "Akpessi" and braised roots, but bitter ones are traditionally processed into a wide variety of foods with different local names such as "*attiéké"*, "Placali", "Attoukpou" and "Konkondé" (Amoa-Awua, 1996, Assanvo *et al*., 2002). The quality of cassava product is largely dependent on the technologies processing. Processing technologies

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consist of a combination of procedures such as peeling, boiling, steaming, pounding, slicing, grating, roasting, soaking, pressing and fermentation (Hahn, 1989). The fermentation is controlled by several microorganisms, ones of whom have positive effects such us product preservation, flavour development, cyanide reduction and changes in functional properties (Akindahunsi *et al*., 1999). Unfortunately other micro organisms such as high load of coliforms contribute to the degradation of the quality of final products due their potential pathology. In order to produce these fermented food products, cassava roots are peeled, cut in pieces, washed and grated. The starter is prepared by storing boiled cassava roots for generally three days in an unwashed jute bag previously used for inoculums preparation (Coulin *et al*., 2006). In Côte d'Ivoire, there are several methods for the preparation of the starter from cassava and according to specific method used the inoculums take various names such as "*Adjoukrou*", "*Alladjan*". Both varieties of cassava (bitter and sweet) could be used for the preparation of starter culture. 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 (Maduagwu, 1983; Ayernor, 1985; Ampe and Brauman, 1995), on the influence of bacterial inoculation on the physico-chemical characteristics (Nzigamasabo and Hui, 2005), on the characterisation of biochemical properties of lactic acid bacteria isolated (Kostinek *et al*., 2007) or on the microbial changes (Tsav-wua *et al*., 2004 ; Coulin *et al*., 2006) during retting of cassava. Only a few studies dealt with the changes of biochemical and microbial qualities depending cassava variety of the starter obtained from fermented cassava (Kimaryo *et al*., 2000).

Therefore, to provide a basis for understanding the changes in biochemical and the microbial qualities of starter and in order to improve the quality of this important staple food whatever the cassava variety we monitored simultaneously (i) the physico-chemical environment by the changes in the pH, titratable acidity and total reducing sugars (TRS), (ii) the composition and the evolution of the microbial community by the counts of viable micro organisms during the preparation of an Ivorian traditional starter named "*Alladjan*" starter.

Materials and Methods

Collection of cassava tubers and fermentation processing

Fresh cassava tubers comprising both sweet and bitter varieties were purchased at march –april 2007 from Akéikoi, a big village located not far 3 km around of Abidjan, the capital of Côte d'Ivoire a moderate hot rainy region with an average of 28 - 29°C during the harvest season, a low altitude, below 500 m, 70 - 80 mm/month rainfall. Other sweet tubers were harvested from the experimental station of "*Bonoua"* an Ivorian local variety and tubers of bitter variety were collected from station of Improved African Cassava (IAC) variety. Tubers of each variety were washed in clean water, braised, peeled then chopped out of pieces of roughly 10–15 cm and of approximately 500g. For each variety, three batches of 5 samples of a cube were made up. A sample of each variety was immediately filled into a sterile stomacher then placed in a refrigerator in the presence of the ices for being transported to the laboratory for analyses corresponding to time 0 hour. The four other samples were filled individually into a jute bag already used as fermentation vessels for precedent lactic acid fermentation of cassava. The hermetically sealed bags were then stored at ambient temperature and left to ferment spontaneously during four days. A sample of each variety was taken each day during fermentation to carry out analyses.

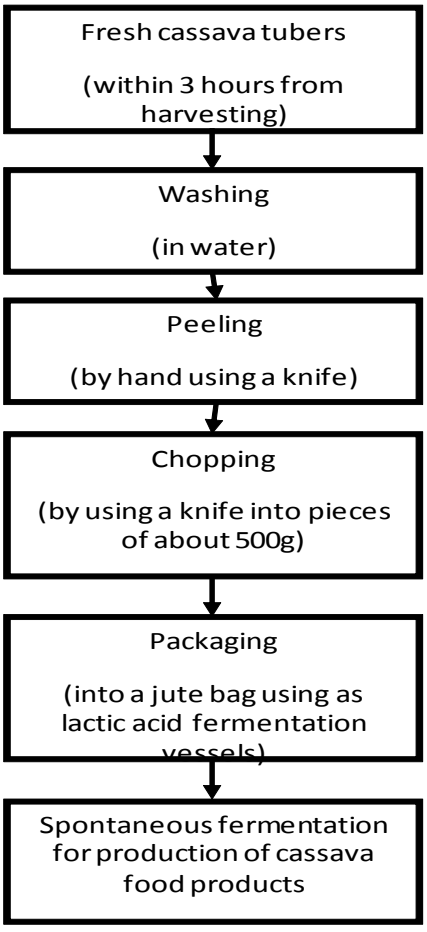

Figure 1. Scheme of cassava pieces natural lactic acid fermentation processing

Measurement of pH and determination of titratable acidity

The pH of the roots of manioc in fermentation was determined using a pH-meter (P.107 Consort) by direct reading. Crushed samples of about 10 g were crushed and homogenized with 90 mL distilled water and the pH of each homogenate was determined according to Anonyme (2001). Titratable acidity was determined using the standard method described by Amoa-Awua et al. (1996). About 10 g of cassava sample were blended with 100 mL of the distilled water and filtered through successively two Whatman filter papers. Titratable acidity of fermenting cassava was determined by titrating a volume of an aliquot of cassava filtrate with 0.1 N NaOH, using 1% phenolphthalein as the indicator in order to determine the amount of acid (as lactic acid) in the sample by using following relationship (Kimaryo et al., 2000):

$$Titrable\ acidity = \frac{V_b \times N_b \times 0.009 \times 100}{W_b}$$

Where,

V_b= volume of the base used;

0.009 acid milliequivalent factor for lactic acid;

N_b=normality of the base used;

W_b=sample weight.

Determination of total sugars content (TS)

A chemical conversion of total sugars in to reducing sugars is already before the evaluation of total sugars. Ten grams of fermenting cassava aliquot to be analysed for the total sugars were blended with 50 mL of distilled water and the jar rinsed twice with 50 mL of distilled water. One millilitre of 10% lead acetate solution and 10 mL of chlorhydric acid 12N were successively added to 40 mL of cassava solution. The obtained mixing is boiled at temperature of 76°C during 20 minutes. After cooling at ambient temperature, three drops of phenolphthalein were added and the acidic solution is neutralised by addition NaOH solution 6N. Final solution is completed to 100 mL by addition of distilled water and constitutes a reducing sugar solution. Total reducing sugars were determined as previously described by the method of Miller (1959).

Determination of total reducing sugars content (TRS).

Total reducing sugars were determined on a dry matter base, according to the dinitrosalicilic acid (DNSA) method described by Miller (1959) and used by Kimaryo et al. (2000). Ten grams of the fermenting cassava aliquot to be analysed for the total reducing sugars were blended with 50 mL of distilled water and the jar rinsed twice, first with 50 mL of distilled water and then with 20 mL of the same and the total contents transferred into one flask. Then 2 mL of the blend were centrifuged in an Eppendorf tube for 5 min. The supernatant was diluted five times and 1 mL of DNSA working solution were mixed with 2 mL of the diluted sample in a test tube and incubated at 100°C for 5 min in a water bath, then cooled down to room temperature. The absorbance of the colour developed was read at 540 nm and amounts of reducing sugars were determined by using a fructose standard curve prepared using concentrations ranged from 0 to 400 µg.mL-1 fructose in distilled water.

Microbiological analyses

For all determinations, about 10 g of freshly peeled and washed tubers of cassava and cubes filled into jute bags undergoing fermentation taken at regular intervals of 24 h until the end of the fermentation during 4 days were individually homogenized in a stomacher with 90 mL of sterile diluents containing 0.85% NaCl and 0.1% peptone (Difco, Becton Dickinson, Sparks, MD, USA) according to Coulin et al. (2006). Tenfold serial dilutions of stomacher fluid, ranging from 10-2 to 10-8, were prepared. From each dilution, 0.1 mL of the lowest dilution (10-1) and other dilutions was surface plated for the determination of microbial counts in each sample of the respective agar media, followed by incubation at 30°C as described by Kimaryo et al. (2000). Total aerobic mesophilic counts were determined on plate count agar (PCA) (Difco) incubated at 30°C for 48 h. Enumeration of lactic acid bacteria was carried out using De Man, Rogosa and Sharpe (MRS) (Merck) medium after incubation at 30°C for 48h (Reuter, 1985). Enterococci were detected on citrate azide tween carbonate (Merck) medium and the plates incubated at 30°C for up to 4 days. Typical representative colonies were picked from plates at the higher dilutions, purified and their presumptive identity verified by microscopy (Kimaryo et al., 2000). Bacteria were enumerated on plates bearing 10 to 200 colonies. Yeasts and moulds were enumerated on plates of Sabouraud chloramphenicol agar incubated at 30°C for 4 days. The results (N) were expressed in CFU/g using the following formula (Afnor, 2002):

$$N = \frac{\sum C}{V(n_1 + 0{,}1n_2)d}$$

Where:

$\sum C$ is the sum of colonies count on all retained successive dilution Petri dishes;

V is the volume of inoculums spread-plated onto

culture medium;
n_1 is the number of Petri dishes retained to the first dilution;
n_2 is the number of Petri dishes retained to the second dilution;
d is the dilution rate of the first retained dilution.

Results

Changes in pH and titratable acidity of cassava varieties during fermentation

The results of chemical properties of retting of pieces of selected both cassava varieties at different fermentation times are shown in Figures 2a and 2b. Progressive acidification during lactic acid fermentation was observed. At time 0 hour sweet cassava variety pieces had pH 6.6 and bitter variety presented pH 5.9 and both of varieties showed approximately the same titratable acidity near 0.025%. During fermentation processing, there is significant decrease in pH while titratable and acidity increased significantly for both cassava varieties. Fermented pieces characteristics did not differ significantly ($P < 0.05$) in acidity properties in both of cassava varieties even if the pH of bitter variety remained lower than that of sweet variety throughout fermentation. At the end of the fermentation period, pieces of bitter variety roots had lower pH (3.9) and titratable acidity near about 0.08% than sweet variety pieces which showed pH 4.5 with 0.12% of titratable acidity.

Variation of total sugars (TS) content of cassava roots during fermentation

Figure 3 illustrates the changes of total sugars content in both cassava varieties studied during the fermentation. At time 0 hour sweet cassava tubers contained about 2.2 while bitter tubers showed around 1.3 mg per g of cassava pulp. During the first 24 h of fermentation, total sugar content of sweet cassava decreases significantly and reaches 1.43 mg per g of pulp whereas there is a slight decrease in bitter variety which presents 1.16 mg per g of pulp. After 24 h of fermentation, sweet tubers show faster decrease of total sugars content than bitter tubers and at the end of the fermentation period both of varieties show the same content of total sugars of 0.75-0.8 mg per g of pulp.

Changes in total reducing sugars (TRS) content of cassava roots during fermentation

The Figure 4 shows the changes in the content of TRS of both studied cassava varieties throughout the lactic acid fermentation. During the first 24 h of fermentation, a lowering TRS content was observed from 1.23±0.04 to 0.91±0.08 mg/g in the fermented pieces of bitter cassava but the concentration of TRS remained constant ranged between 1.3±0.02 and 1.4±0.04 mg/g in pieces of sweet roots. Between 24 and 72 h of fermentation, there was a significant decrease in the concentration of TRS of pieces of sweet cassava; 50% was degraded and in the same period a slight decrease of TRS content was revealed in pieces of bitter cassava with the degradation of only 20% of TRS. At 72 h of fermentation, the same concentration of TRS was found in fermented pieces of cassava whatever the variety. During the fourth day of fermentation (from 72 to 96 h) only a little TRS was degraded in fermented pieces of bitter cassava where the concentration of TRS remained constant around 0.7-0.75 mg/g and in sweet cassava's roots the concentration of TRS decreased from 0.73±0 to 0.57±0.02 mg/g.

Microflora

The composition of micro flora was determined throughout the process of fermentation of both varieties of cassava (Table 1). Particular attention was given on main group of aerobic, facultative and anaerobic micro organisms. The extensive scrutiny showed that in the first day of the fermentation, the micro flora consisted of Aerobic mesophilic counts, coliforms, lactic acid bacteria (LAB), enterococci, yeast and moulds numbering between 3 and 4 $\log_{10}$ CFU/g (dry weight) in roots of both studied varieties of cassava. The micro flora was supplanted by Aerobic mesophilic which reached maximal value about 10 $\log_{10}$ CFU/g. Among anaerobic fermenting bacteria, LAB were predominant during the fermentation processing whatever the variety of cassava. After 48 h of fermentation, counts of most of micro organisms increased and reached 8 $\log_{10}$ CFU/g but the minimal increase was recorded in the population of enterococci which had reached 7 $\log_{10}$ CFU/g. Among coliforms counts, thermotolerant counts population decreased significantly in the final stage and reached 6 $\log_{10}$ CFU /g whatever the variety of cassava. Yeast and moulds appeared before the beginning of the fermentation of the cassava roots whatever the variety. And at time 0 h, we have isolated 5 $\log_{10}$ CFU/g, their number increasing and duplicating to a maximum of 8.70 $\log_{10}$ CFU/g after 48 h of the process. Yeast and moulds counts decreased slightly to 6.86 - 7.32 $\log_{10}$ CFU/g at the end of the fermentation processing in both of cassava varieties roots.

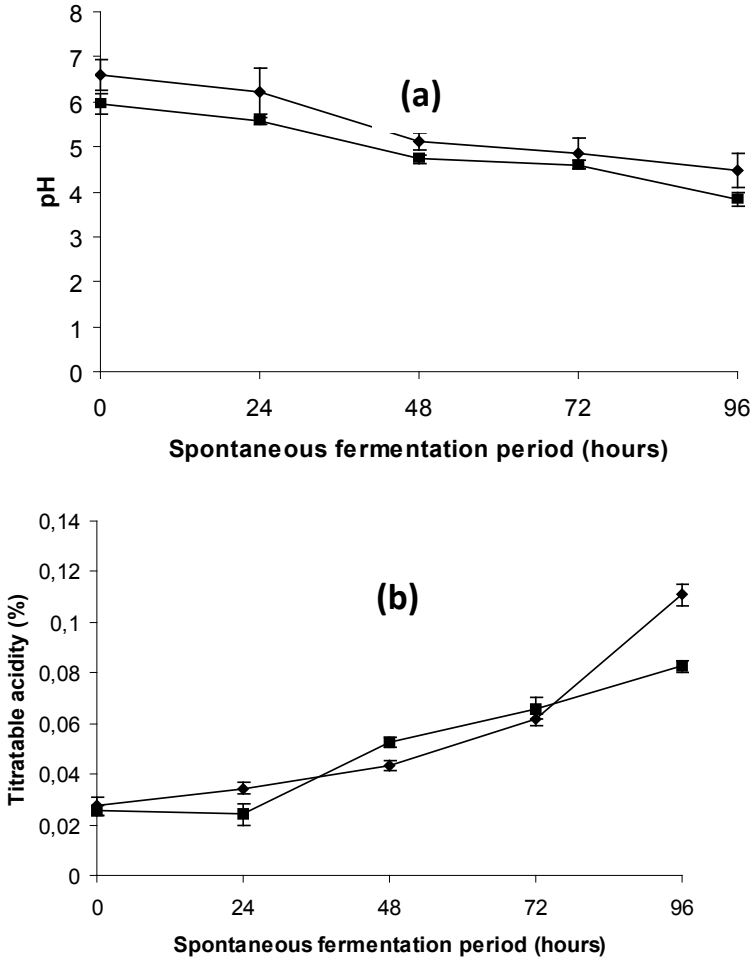

Figure 2. Changes in pH (a) and titratable acidity (b) during cassava lactic acid fermentation. Symbols: ♦, sweet variety roots, ■, bitter variety roots. Error bars represent

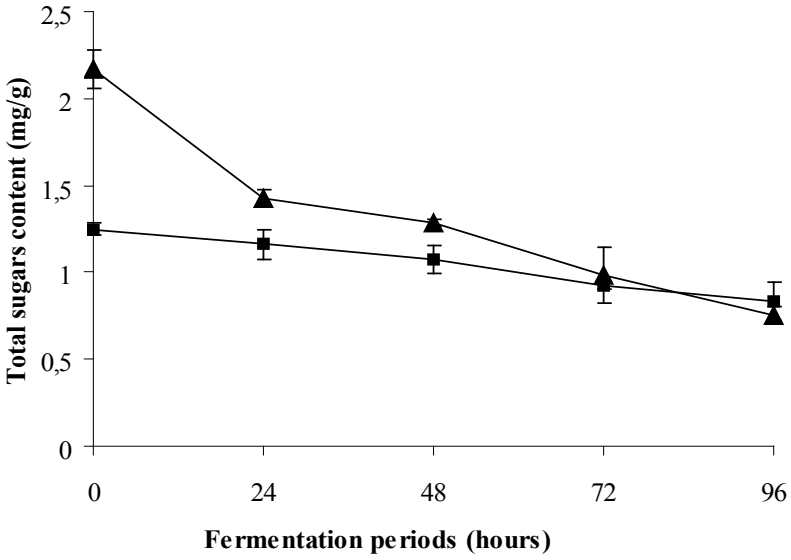

Figure 3. Change in total sugars during cassava lactic acid fermentation.
Symbols: ♦, sweet variety roots, ■, bitter variety roots. Error bars represent standard deviations.

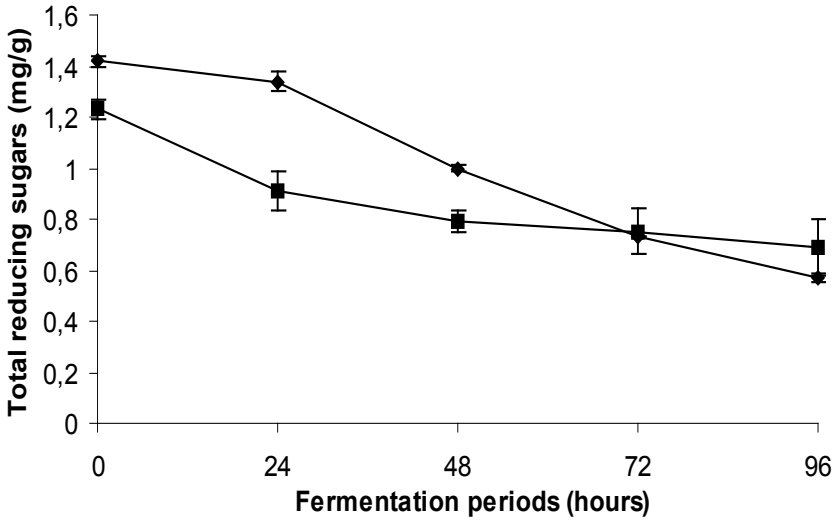

Figure 4. Change in total reducing sugars during cassava lactic acid fermentation.
Symbols: ♦, sweet variety roots, ■, bitter variety roots. Error bars represent standard deviation

Table 1. Evolution of microflora during "alladjan" process for the production of "starter" culture

Microflora constituent		Log_{10} CFU/g (dried wt) at fermentation time (h)				
		0	24	48	72	96
Aerobic mesophilic count	Sweet variety roots	5.51	9.20	10.22	9.87	8.96
	Bitter variety roots	5.53	9.20	9.98	9.73	8.61
Coliforms count	Sweet variety roots	3.77	7.90	8.78	7.51	7.44
	Bitter variety roots	3.60	7.01	8.51	7.55	7.24
Thermotolerant coliforms count	Sweet variety roots	3.61	7.74	8.38	7.22	6.42
	Bitter variety roots	3.35	7.76	8.17	6.90	5.96
Lactic acid bacteria (LAB) count	Sweet variety roots	4.51	7.81	8.53	8.56	7.88
	Bitter variety roots	4.36	7.33	8.22	8.06	7.82
Enterococci count	Sweet variety roots	4.88	7.32	7.41	7.52	7.53
	Bitter variety roots	5.26	7.10	7.10	7.45	7.24
Yeast and moulds	Sweet variety roots	4.72	7.71	8.71	7.80	7.33
	Bitter variety roots	4.84	6.79	8.49	7.33	6.86

Discussion

During fermentation processing, there were significant decrease in pH and increase in the titratable acidity. Other reports showed that fermentation lowers the pH (Dziedzoave et al., 2000; Kostinek et al., 2007). Analyses reveal that at the beginning of the fermentation, bitter cassava tubers had lower pH value than sweet cassava tubers. Lower pH in bitter tubers could be probably due to the presence of higher cianhydric acid (HCN) concentration in bitter cassava than in sweet cassava. Indeed, according to most studies, low pHs are always associated with high acidities but titratable acidity is a better indicator of acidity than pH (Nazaruddin et al., 2006, Hii et al., 2009). So despite of lower pH measured in bitter tubers than sweet tubers, both varieties have the same titrabale acidity. In others respects HCN could not be titrated together titratable acidity and the values of the titratable acidity found in both cassava varieties could be due to the sensitivity of the titration method. After all, most studies showed plainly that bitter cassava is more acidic than sweet cassava. The drop of pH and the increase of titratable acidity in the pulp of cassava could be attributed to the accumulation of some organic acids such as lactic and acetic acids (Guiraud et al., 1998; Almeida et al., 2007) throughout the cassava fermentation which is generally spontaneous lactic

acid fermentation. Cassava fermentation is associated with the fermentative activities of bacteria and yeasts (Moorthy and Mathew, 1998). According to Brauman et al. (1996) sucrose was the main and preferred growth substrate for all of the micro organisms. During such fermentation, monosaccharides (glucose and fructose) coming from the break down of sucrose were then slowly metabolized into organic acids by facultative anaerobic micro organisms such as lactic acid bacteria. LAB produced high amounts of lactic acid as previously demonstrated by Coulin et al. (2006) who concluded that most of acids produced during this fermentation are lactic acid and the quantities of acetic acid being generally three times less. That leads to a rapid drop in pH to around 4.5 as previously found during the preparation of cassava fermented products such as foo-foo, "attiéké" and lafun (Okafor et al., 1984, Oyewole and Odunfa, 1988; Oyewole, 1990; Coulin et al., 2006).

So whatever the variety of cassava changes in acidities are the same during lactic acid fermentation. However, after 96 h of fermentation, the pH value of the bitter variety remains weak while titratable acidity is not very significant compared to the titratable acidity of sweet cassava roots. These results confirm that titratable acidity is a better indicator of acidity than pH and are similar to those obtained by Obilie and Okomas (2004). Lower pH in the fermented pulp of bitter cassava could be explained by the presence of high amount of cyanhydric acid accumulated through the degradation of the cyanogenic glycosides such as amygdalin, linamarin, and linseed cyanogens by lactic acid bacteria (Lei et al., 1999). At the pH about 5, the cyanohydrins formed disintegrate to form HCN (Fomunyam et al., 1985) which would be evaporated latter from the fermented cassava pulp. Indeed, fermentation allowed the elimination of more than 90% of endogenous cyanide compounds in the cassava roots after 48 h.

The accumulation of HCN in the bitter pulp did not contribute much to the increase of titratable acidity because of its volatility. After 72 h of fermentation, acidity is almost identical in the pulps of both of cassava varieties and the statistical analysis did not reveal any significant difference. These results could be linked to the high content of TRS in sweet cassava pieces which would be converted in to high concentrations of lactic and acetic acids.

Higher content of total sugar in sweet cassava tubers than bitter tubers is reasonable but the faster decrease of total sugars during fermentation of sweet cassava variety could be explained by the faster growth of fermentative micro flora such as LAB than during fermentation of bitter cassava variety. Indeed, recently Panda et al. (2008) have clearly demonstrated that some LAB as Lactobacillus plantarum could produce α-Amylase and then produce some reducing sugars from hydrolyse of starch. The same content of TS reached in both cassava varieties at the end of the fermentation could be explained by the reduction of fermentative micro flora by the production of antibiotic agent as Nisine (Valat et al., 2003) which could inhibit the growth of others bacteria and finally lead to the necrosis of biomass particularly in sweet cassava pulp.

The decrease of the content of total reducing sugars contributed to the production of lactic acids during the lactic acid fermentation. Even if Panda et al. (2008) demonstrated that Lactobacillus plantarum could produce α-Amylase and reducing sugars from hydrolyse starch, the decrease of reducing sugars concentration could be explained by the activities of total fermentative micro flora which metabolized and converted them in to energy for their growth and in organic acids. Probably accumulation of reducing sugars resulted from hydrolyse of starch by some LAB was less than utilization of them for the growth of total biomass. These results are similar to those obtained by Kouassi (2006) which observed in addition the drastic reduction of total sugars content a fall of reducing sugars concentration after 12 h of fermentation of cassava. Although the initial content of reducing sugars was more significant in the sweet variety compared to the bitter variety we noted the same contents of TRS at the end of the fermentation processing whatever the cassava variety. We could conclude from these observations that reducing sugars were significantly degraded faster in the roots of sweet cassava than in the bitter variety. In parallel with sugars degradation, the accumulation of fermented metabolites such as lactic and acetic acids was observed. The availability of high content of TRS as substrate for the growth of micro organisms, the presence of aerobic conditions and the using of jute bags already as fermentation vessels favour the strong development of diverse groups of micro organisms during cassava fermentation.

Whatever the variety of cassava, the changes in the content of micro organisms throughout cassava lactic acid fermentation was similar but we observed that micro flora was slightly and more abundant in roots of sweet cassava than in bitter cassava. In each cassava variety, the total aerobic mesophile counts corresponded approximately to the sum of LAB, coliforms, Enterrococci, and yeast and moulds as recorded by Coulin et al. (2006) during "attiéké" fermentation. These counts appear to be high but seem closed to the finding of Coulin et al. (2006).

The high bacterial load recorded in this study may be attributed to the poor microbial quality of jute bags ever used for precedent cassava lactic acid fermentation. This high bacterial content included the normal LAB involved in the lactic acid fermentation as well as others undesirable bacterial such as coliforms. The high coliform counts were probably induced by the contamination of exposure the jute bags to environmental atmosphere in the course of fermentation processing as well as by the faecal contamination. Coliforms are generally very thermal sensitive so their high number during the preparation of starter culture could be favoured by the absence of thermal step in the course of fermentation processing. However, it has been reported that the presence of coliforms in fermented cassava could contribute a health hazard to the consumer and could also act as potential spoilage agent of fermented cassava dishes and foods (Okpokiri et al., 1985; EEC, 2005). So improvements in production hygiene and in selection of raw materials are required for "Alladjan" starter. In Côte d'Ivoire, there are several methods of preparation of the starter culture from cassava and according to each method used the inoculums take various names such as "Adjoukrou", "Alladjan". Both varieties of cassava (bitter and sweet) could be used for the preparation of starter culture. These products provide almost 50% of the caloric intake of the most Ivorian population. 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 (Maduagwu, 1983; Ayernor, 1985; Ampe and Brauman, 1995). Some studies like this present report may be conducted in order to valorize most local food crops and improved the production hygiene and the quality of traditional final products.

The increase of acidity during fermentation could contribute probably to inhibition acts of LAB which developed abundantly against other undesirable micro organisms resulted in a decrease growth of coliforms as shown by their low number at the end of the fermentation. The lowering of their counts can be also explained by the fact that they do not play an important role for the acidification during fermentation. They probably produce relatively high levels of cell-wall degrading enzymes which contributed to textural changes in the cassava pulp (Mante et al., 2003; Obilie et al., 2003) or also antibiotic agent as Nisine (Valat et al., 2003).

Numbered yeast and moulds in roots of cassava before starting fermentation were probably sourced from the jute bags used for an anterior process of cassava fermentation. Initial high count of yeasts and moulds would be in conformity with the idea that such moulds may be indigenous to cassava roots but certain strains of them can be potentially implicated with mycotoxins food poisoning (Tsav-wua et al., 2004). Abundance of substrates such as sugars, acidic pH environment and probably temperature conditions attained by the roots were certainly favourable for their growth. Although yeast and moulds are known to have an important role in food processing particularly in fermentation, the excessive growth moulds may be undesirable. Indeed as described by Moorthy and Mathew (1998) cassava fermentation is associated with the fermentative activities of bacteria and yeasts. But the decrease of their population is probably due to the depletion of substrates and the bacterial competition although their counts remained relatively constant at the end of the fermentation as observed Coulin et al. (2006) during "attiéké" fermentation. LAB remained relatively high throughout the fermentation indicating high microbial stability while coliforms, yeast and moulds decrease after 48 h of lactic acid fermentation into both cassava varieties. A rapid increase in LAB during the preparation of starter culture is generally consistent with reports on other cassava fermentations (Obilie et al., 2004; Coulin et al., 2006). Although the depletion of substrates such as TRS, the numbers of LAB remained relatively constant like favoured by the acidification of pulp and resistant to the competition of others bacterial micro organisms, yeast and moulds. Enterococci counts remained relatively constant throughout the fermentation. They seemed not sensitive to the acidification of pulp and benefited from the metabolites sourced from the growth of others micro organisms such as LAB. Their counts indicated that they can be considered as parasite and they did not play a determinant role in the acidification of pulp during cassava lactic acid fermentation.

This study carries out the similar changes in biochemical properties and in microbial qualities to those previously described by most authors (Tsav-wua et al., 2004; Nzigamasabo and Hui, 2005; Coulin et al., 2006; Kostinek et al., 2007) in different environmental conditions for the production of numerous and different traditional foods but the results from this report have the merit to be obtained in the same condition and time from two varieties of cassava tubers.

Conclusion

The results of this study emphasize the independence of the preparation of starter culture on cassava lactic acid fermentation. They showed

that whatever the variety of cassava roots used for the production of starter culture, the biochemical and microbial properties are approximately the same during the same period of fermentation and under the same conditions. In addition, this study revealed the progressive acidification of the pulp and the growth of diverse groups of micro organisms in starter culture which will determine the quality of cassava fermentation processing. The lactic acid fermentation of cassava is mainly bacterial. Among fermentative bacterial, LAB are predominant even if yeast and moulds can be developed. However, high coliforms content in fermented cassava could contribute a health hazard to the consumer and could also act as potential spoilage agent of fermented cassava dishes and foods. So such starter with high level of coliforms could not be used as starter. The process could be improved by reduction of the coliforms content. The understanding of the preparation of a starter culture can be used for further development aimed to prepare a defined starter (defined micro flora) in order to more control the lactic acid fermentation processing so as to improve the quality of tropical traditional fermented cassava dishes and foods.

Acknowledgements

Authors gratefully acknowledge selected cassava growers of Akéïkoi, a big village located not far 3 km around of Abidjan for their hearty welcome, their open collaboration and for providing all required cassava varieties and quantities of roots samples.

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