

Impact of Different Levels of a Traditional Starter on the Fermentation of Cassava Dough for *Attiéké* Production

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ABSTRACT

This study aimed to assess the biochemical and microbiological changes during fermentation of cassava doughs initiated by a traditional starter at different inoculation rates. The cassava doughs inoculated with different levels of starters were fermented at 35°C for 18 h. The most important changes in biochemical (decrease of pH, increase in titrable acidity, sugar consumption and gas released) and microbiological properties were observed in the 10 and 15% inoculated dough. Citric (188.96 mg/100 g), malic (99.7 mg/100 g) and oxalic (31.8 mg/100 g) acids were the main organic acids in the unfermented dough. Their concentrations decreased during fermentation to low or undetected levels. At the end of fermentation, lactic and acetic acids became the main acids in the dough (respectively 482.35 mg/100 g and 236.66 mg/100 g for the 8% inoculated dough). This study allows to better understanding of cassava dough fermentation for *attiéké* production and also indicates optimal starter rate and duration for controlled fermentation.

Keywords: *attiéké* fermentation, cassava dough, fermentation rates, traditional starter culture

INTRODUCTION

Cassava, the enlarged root of *Manihot esculenta* Crantz, is a staple food for over 500 million people in the developing world (Cock 1985) of which 200 million live in Sub-Saharan Africa (Madeley 1993). It has important agronomic advantages, but it has also two important deficiencies. Firstly, the bitter varieties contain the toxic cyanogenic glucosides linamarin and (to a lesser extent) lotaustralin and secondly, it is very poor in protein, containing only about 1% (Sanni *et al.* 2002). Therefore, it is traditionally processed into a wide variety of products with different local names (*fufu*, *agbelima*, *chikwangue*, *farhina*) (Longe 1980; Hahn 1989; Amoa-Awua 1996). The most popular processing method, however, especially for varieties high in cyanogenic glucosides, is fermentation. In Ivory Coast, the most popular food derived from fermented cassava is *attiéké*.

Attiéké was originally prepared and consumed exclusively in a restricted ethnocultural setting (Adjoukrou, Ebrié, Alladjan, Avikam, Ahizi) living in the Laguna area in the south of the country. In recent years, the product has become popular beyond the boundaries of this area. However, despite of this popularity, *attiéké* production remains traditional and is mainly done by women in small-family or semi-artisanal scale.

Cassava fermentation during *attiéké* production requires the use of a traditional starter whose preparation method varies according to ethnic groups. This starter, usually prepared by cooking and fermenting whole-peeled cassava roots for 72 h, constitutes the main source of microorganisms active in the dough fermentation. It is mixed with the peeled roots during grinding in no exactly defined proportions, at the convenience of the producer. For this reason, the quality of the final product may not always be predictable or controllable. This is a major problem typically associated with traditional fermented foods in Africa (Kimaryo *et al.* 2000). Their preparation generally relies on chance inoculation and the result is often a product of inconsistent quality, poor hygiene, poor nutritional value and short shelf-life.

Many studies on some physical and biochemical changes during cassava processing in Ivory Coast have been performed (Aboua 1995, 1996), and only recent ones (Assanvo *et al.* 2006; Coulin *et al.* 2006) dealt with the microbial composition of the starter. But none of these included any kinetic measurement of biochemical and microbial changes during fermentation (substrate consumption, synthesis of fermentation products, microbial growth), and such measurements are required to demonstrate the role of microorganisms involved and to provide an overall understanding of the process.

Therefore, this study was carried out to assess the impact of incorporating different levels of traditional starter on biochemical and microbiological changes during the fermentation of cassava for *attiéké* production.

MATERIALS AND METHODS

Materials

Traditional starter: Samples of ready-to-use traditional starter were obtained at a small-scale (women's enterprise) *attiéké* production in Abidjan, Ivory Coast.

Cassava roots: 12 months-old freshly harvested cassava roots of the bitter variety were obtained from a farm of the University of Abobo-Adjamé (Abidjan).

Fermentation of cassava dough

About 4 kg of freshly harvested cassava roots of the bitter variety were peeled, washed and grated with a traditional grater. The cassava mash obtained was divided into 5 parts of 500 g each. Four of the 5 parts were inoculated with the following traditional starter rates 5, 8, 10, 15% (w/w) and the fifth part without any inoculum was used as control. The five doughs obtained were then incubated at 35°C for fermentation. Fermentation was monitored over time for 18 h and samples of fermenting dough were aseptically taken for different analyses at the beginning, and after 6, 12 and 18 h of fermentation.

Determination of pH and total titrable acidity

Thirty g of fermenting cassava dough sample were blended with 70 ml of sterile distilled water and filtered through a Whatman filter paper No. 1. The pH of 30 ml of the filtered solution was determined using a pH meter (P107, CONSORT, Bioblock Scientific, France). Total Titrable Acidity was determined using the standard method described by Amoa-Awua *et al.* (1996) on 10 ml of filtered solution. The total titrable acidity was calculated as a percentage of lactic acid using the relationship:

$$\text{Total titrable acidity (\%)} = \frac{V_b \times N_b \times 0.09 \times 100}{W_s}$$

where, V_b = volume of the base used; 0.09 acid milliequivalent factor for lactic acid; N_b = normality of the base used; W_s = sample weight.

The determinations were done in triplicates and the mean value recorded.

Determination of total and reducing sugars

Water-soluble carbohydrates were determined by the phenol sulphuric acid method according to Dubois *et al.* (1956) and the values were expressed in g/100 g of fresh dough, while the reducing sugars were quantified as described by Bernfeld (1955) and expressed in mg/100 g of fresh matter.

Gas released from fermenting dough

The volume of gas produced during fermentation was measured on 300 g of cassava dough using an experimental device described by Pol (1996).

Analysis of organic acids and ethanol

Ten grams of cassava fermenting doughs were ground in a mortar with 15 ml of 0.1 N H_2SO_4 . The obtained mixtures were centrifuged for 10 min at $12000 \times g$ and then the supernatants were filtered through a Millipore membrane (0.20 μm pore size) (Sartorius 16534-100, Barcelona, Spain). Organic acids in the supernatants were assayed by High-Pressure Liquid Chromatography (LC-6A, Shimadzu corporation, Japan) using an ion exclusion ORH-801 column (300 mm \times 6.5 mm) (Interchrom, France). Running conditions were: mobile phase, H_2SO_4 40 mmol L^{-1} ; flow rate, 0.8 ml min^{-1} ; wave length, 210 nm; room temperature (25°C). The separated components were detected with an UV spectrophotometric detector (SPD-6A, Shimadzu Corporation, Japan).

Ethanol was assayed by a gas chromatograph (GC-14A, Shimadzu Corporation, Japan). The instrument was equipped with Porapak Q 100/120 column (1.80 m \times 5 \times 3). Two microlitres extracts were injected using the following temperature programme: 2 min at 60°C, increased to 150°C at 10°C min^{-1} and held constant at 240°C for 30 min. Detector was at 250°C and helium used as carrier gas at 2 kg/cm². All results were the means of three determinations.

Enumeration of microorganisms

Preparation of stock solutions, inoculation of agar plates, cultivation and quantification of microorganisms were carried out according to Coulin *et al.* (2006). For all determinations, 10 g of the samples were homogenized in a stomacher with 90 ml of sterile peptone buffered water (AES Laboratoire, COMBOURG France). Ten-fold serial dilutions of stomacher fluid were prepared and spread-plated for determination of microorganism counts. Enumeration of lactic acid bacteria was carried out using plates of De Man, Rogosa and Sharp agar (MRS, Merck 10660, Merck, Darmstadt, Germany) which were incubated in an anaerobic jar at 30°C for 3 days. Yeasts were enumerated on plates of Sabouraud chloramphenicol agar (Fluka, Bochemica 89579, Sigma-Aldrich Chemie GmbH, Inda) incubated at 30°C for 4 days. Aerobic mesophiles were enumerated on plates of Plate Count Agar (PCA Oxoid Ltd., Basingstore, Hampshire, UK) and incubated at 30°C for 2 days.

Statistical analysis

Experimental results of the three fermentation repetitions for each starter rate were subjected to analysis of variance (ANOVA) and differences between means were assessed by Duncan's new multiple range test at the significance defined at $P \leq 0.05$, using Statistica software (Statistica, 99th Edn).

RESULTS AND DISCUSSION

Microbial growth

The high counts of microorganisms found in cassava doughs during fermentation indicated that this fermenting medium is well-adapted for microbial activities. Growth of total aerobic mesophiles, lactic acid bacteria and yeasts in fermenting dough inoculated with different traditional starter rates are illustrated in Figs. 1-3. Their initial counts at the beginning of the fermentation were very different ($P < 0.05$) and increased proportionally with the increase of the added starter rate. Total aerobic mesophiles corresponded approximately to the sum of lactic acid bacteria and yeasts. Their counts were 2.1×10^6 , 3.7×10^8 , 6.7×10^8 , 7.1×10^8 and 7.3×10^8 cfu/g, respectively in the control and the

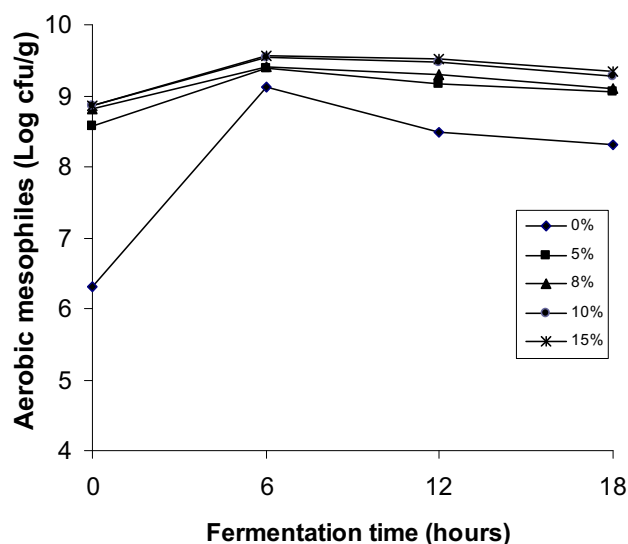


Fig. 1 Evolution of the aerobic mesophiles load during fermentation at 35°C of cassava dough inoculated with different traditional starter rates.

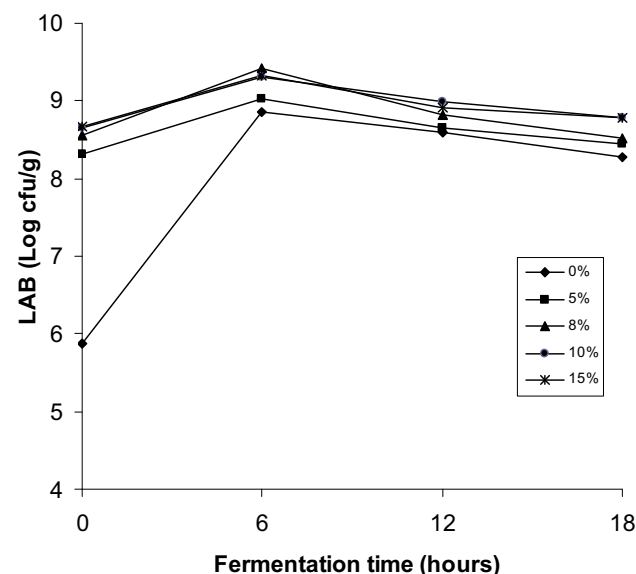


Fig. 2 Evolution of lactic acid bacteria load during fermentation at 35°C of cassava dough inoculated with different traditional starter rates.

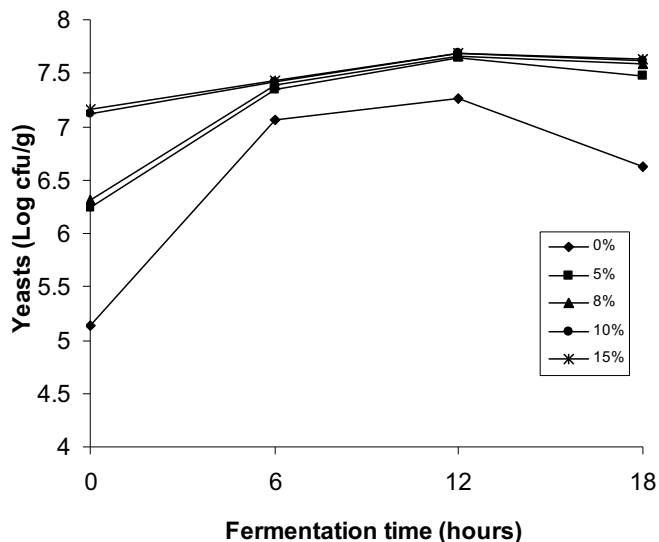


Fig. 3 Evolution of yeasts load during fermentation at 35°C of cassava dough inoculated with different traditional starter rates.

dough inoculated with 5, 8, 10 and 15% of starter. These counts increased differently with time, reaching highest values after 12 h for 10% (3.0×10^9 cfu/g) and 15% (3.4×10^9 cfu/g) before decreasing slightly to 1.9×10^9 and 2.2×10^9 cfu/g at the end of the fermentation (**Fig. 1**). The 5 and 8% inoculated doughs had statistically the same aerobic mesophile loads during the fermentation. Examination of their colonies, cell morphologies and count of other microorganisms indicated that substantial part of aerobic mesophiles were similar to lactic acid bacteria enumerated in the fermenting doughs. Indeed, lactic acid bacteria are often micro-aerophilic and able to grow on PCA (Sefa-Dedeh *et al.* 2004). During the dough fermentations, they became dominant and contributed the most to the acidification of the product. The initial load was different and increased with the level of inoculation. This load was very low (7.4×10^5 cfu/g) in the control and comprised between 2.1×10^8 cfu/g and 4.8×10^8 cfu/g for the other doughs. Their number increased during fermentation for all doughs and reached the highest loads only after 6 hours before decreasing until the end of fermentation. No significant difference was observed in lactic acid bacteria evolution in the 10 and 15%

inoculated doughs (**Fig. 2**). Yeast load was very low (1.3×10^5 cfu/g) in the control and comprised between 1.8×10^6 cfu/g and 1.5×10^7 cfu/g for the other doughs just after inoculation. During fermentation, an increase was observed for all doughs, reaching highest values for the 10 (4.9×10^7 cfu/g) and 15% (5.0×10^7 cfu/g) inoculated doughs after 12 hours (**Fig. 3**). Our results are generally consistent with reports on other cassava fermentations (Amoa-Awua *et al.* 1996; Assanvo *et al.* 2006; Coulin *et al.* 2006). The different starter rates used in this study evidenced the differences in microbial loads observed just after inoculation and during fermentation. The use of such starters not only results in a decrease in time for the fermentation of *attiéké* compared with other fermented cassava products such as gari or chiwangue, prepared without inocula (Regez *et al.* 1987; Coulin *et al.* 2006), but also in rapid softening of cassava dough by the activity of microbial enzymes. That clearly explained the results obtained for the control. The similar microbial evolution observed in doughs inoculated respectively with 10 and 15% of starter indicated that 10% was the limit rate of inoculation and above this level, the fermentation could lead to an undesirable product quality. Toka (1998) estimated that beyond 10% of inoculum, the resulting fermented dough was not suitable for *attiéké*, but could be processed into *placali*, a long time fermented cassava product of the Ivory Coast.

In other respect, the dynamics of growth, survival and biochemical activity of microorganisms in foods are the result of stress reaction in response to the changing of the physical and chemical conditions into the food micro-environment (e.g. the gradient of pH, oxygen, water activity salt and temperature) and the ability to colonise the food matrix and grow with spatial heterogeneity (Giraffa 2004). The rapid increase in microorganism loads the first 12 hours of fermentation was due to the abundance of nutrient useful for their growth and also to absence of inhibitory substances. After this period, growth in the fermenting medium became hard because of the decrease of nutrients, competition between microorganisms, high acidity, explaining the cell mortalities. The microbial changes in fermenting doughs are concomitant with important physico-chemical changes which vary according to inoculation rates.

Changes in pH and total titrable acidity

pH is a critical factor in developing flavour and aroma of foods (Montet *et al.* 2006; Panda *et al.* 2007). **Table 1** gives

Table 1 Changes in pH, total titrable acidity, total sugars and reducing sugars during the fermentation at 35°C of cassava doughs inoculated with various traditional starter rates.

	Starter rates	Fermentation time			
		0 h	6 h	12 h	18 h
pH	Control	6.54 ± 0.06 a	5.81 ± 0.28 a	4.81 ± 0.20 a	4.66 ± 0.01 a
	5%	6.29 ± 0.06 b	4.83 ± 0.07 b	4.53 ± 0.35 a	4.38 ± 0.18 ab
	8%	6.18 ± 0.0 bc	4.72 ± 0.13 b	4.51 ± 0.35 a	4.36 ± 0.20 ab
	10%	6.14 ± 0.09 bc	4.69 ± 0.11 b	4.50 ± 0.34 a	4.25 ± 0.01 b
	15%	5.98 ± 0.12 c	4.65 ± 0.14 b	4.50 ± 0.33 a	4.36 ± 0.18 ab
Total titrable acidity (%)	Control	0.09 ± 0.01 a	0.21 ± 0.08 a	0.44 ± 0.10 a	0.41 ± 0.14 a
	5%	0.11 ± 0.01 ab	0.52 ± 0.04 b	0.69 ± 0.05 b	0.69 ± 0.06 b
	8%	0.13 ± 0.03 abc	0.54 ± 0.06 b	0.71 ± 0.01 b	0.72 ± 0.01 b
	10%	0.15 ± 0.02 bc	0.59 ± 0.08 b	0.72 ± 0.01 b	0.75 ± 0.01 b
	15%	0.16 ± 0.03 c	0.60 ± 0.16 b	0.74 ± 0.12 b	0.76 ± 0.12 b
Total sugars [†]	Control	2.19 ± 0.23 a	1.18 ± 0.41 a	0.73 ± 0.28 a	0.50 ± 0.17 a
	5%	2.19 ± 0.23 a	0.81 ± 0.38 a	0.57 ± 0.26 a	0.40 ± 0.08 a
	8%	2.19 ± 0.23 a	0.74 ± 0.32 a	0.52 ± 0.24 a	0.36 ± 0.11 a
	10%	2.19 ± 0.23 a	0.49 ± 0.17 a	0.44 ± 0.22 a	0.34 ± 0.13 a
	15%	2.19 ± 0.23 a	0.58 ± 0.29 a	0.47 ± 0.28 a	0.35 ± 0.18 a
Reducing sugars*	Control	1.51 ± 0.20 a	1.33 ± 0.09 a	1.52 ± 0.50 a	0.78 ± 0.02 a
	5%	1.51 ± 0.20 a	2.82 ± 1.60 a	3.49 ± 3.10 a	2.27 ± 1.57 a
	8%	1.51 ± 0.20 a	4.17 ± 3.08 a	3.01 ± 2.55 a	2.16 ± 2.51 a
	10%	1.51 ± 0.20 a	3.86 ± 1.50 a	1.58 ± 0.55 a	1.09 ± 0.00 a
	15%	1.51 ± 0.20 a	2.96 ± 0.88 a	2.03 ± 0.29 a	2.05 ± 0.56 a

Values are means of three determinations; In a column, means values followed by different superscript are statistically different ($P \leq 0.05$) (Duncan's multiple range test).

*: mg/100 g of fresh matter; †: g/100 g of fresh matter

the change in pH and total titrable acidity during dough fermentations. The rate and extent of pH decline is indicative of the fermentation intensity. In our study, the pH of the unfermented dough was 6.54 with a total titrable acidity value of 0.09%. The initial parameters were significantly modified after adding the traditional starter. This is due to the fact that the starter itself was acidic with a pH value of 4.86 ± 0.1 . During the fermentation, the pH gradually decreased to reach 4.66, 4.38, 4.36, 4.25, and 4.38 after 18 hours respectively in the control and the 5, 8, 10 and 15% inoculated doughs. Contrarily to the pH, the total titrable acidity increased to reach 0.41, 0.69, 0.72, 0.75 and 0.76% respectively in the control and in the other doughs. Similar results were found by Coulin *et al.* (2006) and Djè *et al.* (2008). The decrease of pH and the rapid increase of the total titrable acidity during the cassava dough fermentation were probably due to the accumulation of organic acids mainly lactic and acetic acids produced during the fermentation (Giraud *et al.* 1998; Kobawila *et al.* 2005; Coulin *et al.* 2006; Panda *et al.* 2007). A combined culture of yeasts and Lactobacilli has also been reported to bring about more significant decrease in pH and simultaneous increase in acidity in fermented millet (Khetarpaul and Chauhan 1990; Mugula *et al.* 2003). Statistical analyses showed significant differences ($P < 0.05$) between the control and the other doughs due to its low load of microorganisms. This demonstrated the necessity of the use of the starter for a rapid acidification in a short time. Among the inoculation rates, 10% seemed to be the most appropriate for an optimal acidification.

Total and reducing sugars

Total sugars content, initially at 2.19 ± 0.23 g/100 g of fresh matter decreased proportionally with the increase of the duration of fermentation for all doughs. This decrease was more accentuated after only 6 hours of fermentation in the doughs inoculated with 10% (0.49 ± 0.17) and 15% (0.58 ± 0.29) than the others (Table 1). But, the differences observed were not statistically significant ($P > 0.05$). The reducing sugars content increased after 6 hours of fermentation before decreasing until the end of the fermentation. It was clear that, the initial elevation in sugar levels during natural fermentation was due to starch degradation by amylases and might have an effect of increasing the microbial counts during fermentation, causing an elevation in acidity and a subsequent drop in pH, while subsequent decrease in sugar content could be due to utilization by fermenting microflora as a carbon source. Similar results were found by several groups (Mbugua *et al.* 1983; Odunfa and Ade-

yele 1987; Umeta and Faulks 1988; Khetarpaul and Chauhan 1990, 1991). Moreover, according to Spier *et al.* (2006), 30°C combined with 90% moisture lead to highest α -amylase production for cassava starch hydrolysis and fermentable sugar production for further acidification. However, all the fermentable sugars generated after starch hydrolysis were not converted to organic acids. A substantial portion was probably utilized by microorganisms present in the fermentation medium for their normal metabolism.

Gas released from the fermentation

Heterofermentative lactic acid fermentation occurs in most of the spontaneously fermented cassava products (Giraud *et al.* 1998). In this kind of fermentation, microorganisms produce lactic acid and other compounds such as acetic acid, ethanol and CO₂ by the phosphocetolase way. The results showed differences ($P < 0.05$) in gas production for each inoculation rate (Fig. 4). Gas released during fermentation could be associated to the dynamics of microbial changes. Degradation activity of fermenting microflora was higher in the 10 and 15% inoculated doughs. Indeed, 15% lead to the highest value (133.5 ml) of gas released only after 3 hours, due to the high microbial load at the beginning of the fer-

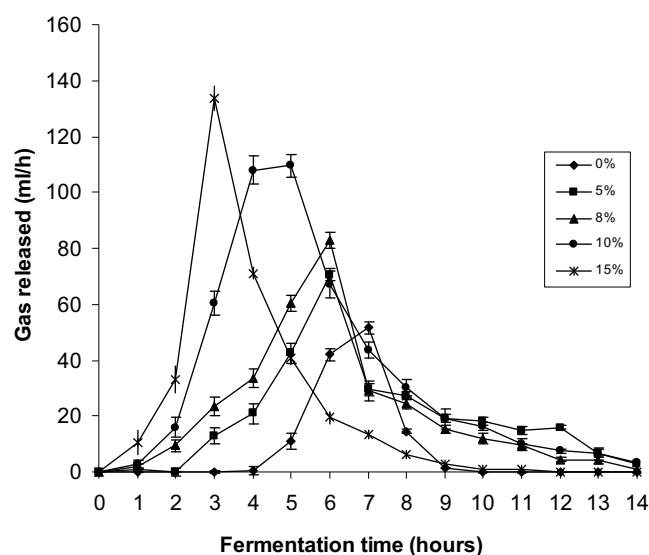


Fig. 4 Gas released during the fermentation of cassava dough inoculated with various level of traditional starter at 35°C.

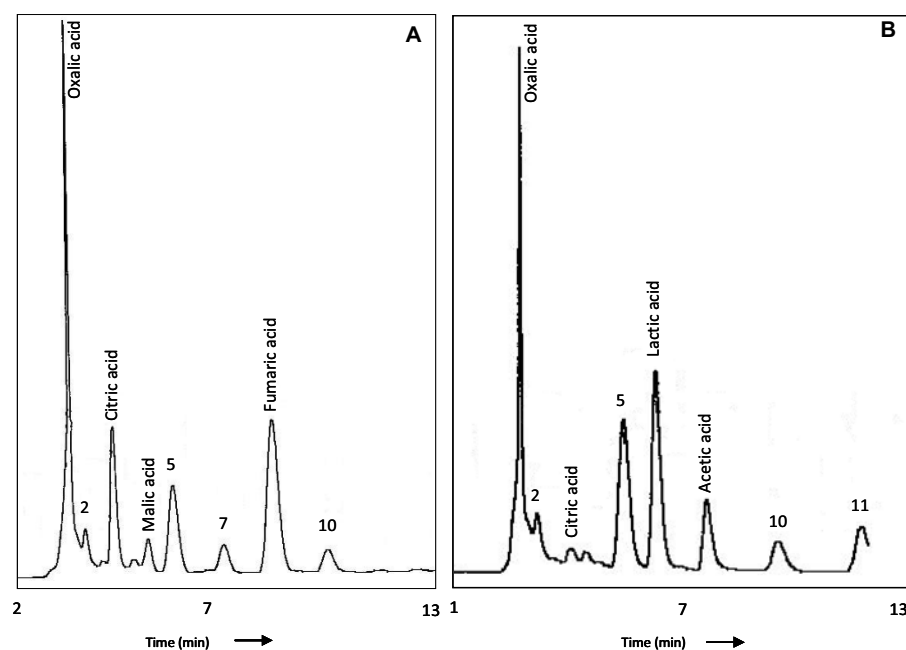


Fig. 5 HPLC profile of organic acids from the 10% inoculated dough at the start of fermentation (A) and after 18 h (B) of fermentation at 35°C.

mentation. But, this value quickly declined and became almost nil at the 9th hour of fermentation. The volume of gas released (108 ml) at the 4th hours in the 10% inoculated dough was constant for 2 h because of an abundance of sugar, before progressively decreasing until the end of fermentation. The other rates lead to maximal volumes of 51, 71 and 83 ml after 7, 6 and 6 h, respectively for the control and the 5 and 8% inoculated dough, due to their relative small microbial loads. The differences observed in the release of gas could also be explained by the fact that starch hydrolysis and fermentation of simple sugars were naturally most intensive at highest rates of inoculation due the load

and the faster microflora development (Gotcheva *et al.* 2001). In all cases, due to the decrease of heterofermentative bacteria and alcoholic yeasts, the decrease of nutrients, important changes in the fermenting medium, competition between microorganisms for rare carbon source, the amount of gas released became almost nil after 12 hours of fermentation.

Changes in organic acids and ethanol

The changes in the concentration of organic acids during cassava dough fermentation are shown in Figs. 5A, 5B.

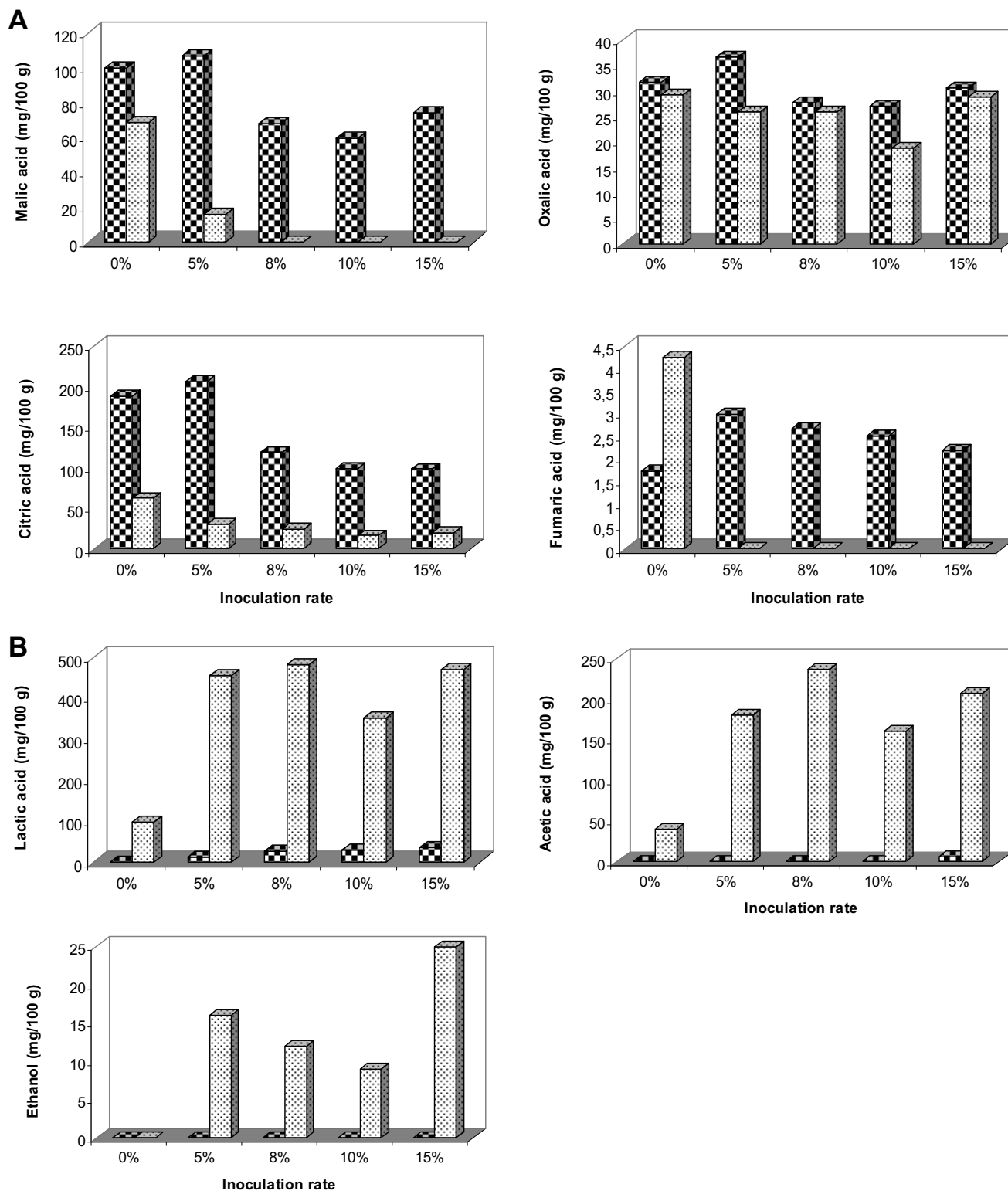


Fig. 6 (A) Initial concentrations of main organic acids of the various inoculated dough at the start (■) and after 18 h of fermentation (▣) at 35°C. (B) Content of main metabolites produced during the fermentation at 35°C for 18 h of cassava doughs inoculated with different traditional starter rates: ■ start of fermentation, ▣ after 18 h of fermentation.

Many organic acids were detected in cassava doughs at the beginning of the fermentation (Fig. 6).

The unfermented dough contained important amounts of citric acid (188.96 mg/100 g of fresh matter), malic acid (99.71 mg/100 g) and oxalic acid (31.85 mg/100 g). It contained low amounts of fumaric acid (1.73 mg/100 g). Lactic, acetic, propionic, tartaric and ascorbic acids were not detected in the dough before inoculation. The low amounts of lactic and acetic acids in the inoculated doughs were probably brought by the traditional starter which contained substantial amounts of these organic acids. We have been unable to find data in the literature with which to compare these values.

After 18 hours of fermentation, important changes occurred in organic acid concentrations. Many of them completely decreased or disappeared and new organic acids were synthesized. Indeed, citric acid, the major organic acid before fermentation was significantly ($P < 0.05$) reduced of 66.95, 85.54, 79.02, 83.58 and 78.99% within 18 hours respectively in the control and the 5, 8, 10, and 15% inoculated doughs. Malic acid was also reduced within 18 hours to undetectable levels in 8, 10 and 15% inoculated doughs, but 15.84 mg/100 g and 68.61 mg/100 g remained respectively in the control and in the 5% inoculated dough (significant difference at 0.05). Fumaric acid was also reduced to undetectable levels excepted in the blank were the amount increased significantly from 1.73 to 4.25 mg/100 g of fresh matter. And oxalic acid fairly decreased of 6.07% for the 15% inoculated dough and 30.68% for the 10% within 18 hours. The decrease to low or undetectable levels of these organic acids was probably due to their metabolization by microorganisms during fermentation. Kennes *et al.* (1991) reported the ability of *Lactobacillus plantarum* to ferment citrate in the presence of yeast under acid conditions. Other studies with some lactic acid bacteria strains showed that organic acids (principally malic and citric acids) in wine are completely consumed before the sugars are totally exhausted (Saguir and Manca de Nadra 1996; Rozès *et al.* 2003).

On the other hand, lactic and acetic acids were produced in the largest amounts reaching respectively about 482.35 mg/100 g and 236.66 mg/100 g of fresh matter after 18 hours in the 8% inoculated dough, rather closely followed by doughs with 15, 5 and 10% of inoculum. Many studies reported the accumulation of organic acids mainly lactic and acetic acids produced during lactic acid fermentation (Giraud *et al.* 1998; Kobawila *et al.* 2005; Coulin *et al.* 2006; Panda *et al.* 2007). Low amount of ethanol was also detected in all inoculated doughs with highest value (25 mg/100 g of fresh matter) in the 15% inoculated dough. Its presence was also mentioned in other cassava fermentation (Brauman *et al.* 1995). The low amounts of organic acids and ethanol found in the 10% inoculated dough evidenced the intense microbial activity occurring in this dough. Indeed, due to the rapid disappearing of sugar, in the medium, organic acids could have been utilized by microorganisms for their own metabolism or for the production of substances involved in organoleptic properties of *attiéké*. Propionic and butyric acid have been reported in other cassava fermentations (Brauman *et al.* 1995). Their absence in our study could be explained by the rapid decrease of pH, inhibiting the microorganisms at the base of the production of such volatile acids.

Organic acids detected at the end of the fermentation are those which play important roles in organoleptic properties of *attiéké*. But their high amounts could have negative consequences on odour and taste, important properties of organoleptic quality of *attiéké*.

CONCLUSION

The results of the present work indicate that important changes occurred during the fermentation of cassava dough, initiated by a traditional starter. This study evidenced the need to use such starter to shorten the fermentation time to up to 12 hours and the most important changes were ob-

tained in the 10% inoculated doughs. No difference in biochemical and microbiological changes was observed above this level of inoculation. Standardization of condition for *attiéké* production using controlled fermentation should be considered in future research.

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