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Use of starter cultures of lactic acid bacteria, yeasts, bacilli and moulds in the fermentation of cassava dough for *attieke* (an ivoirian fermented food) preparation

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ABSTRACT: Starter cultures of lactic acid bacteria (*Lactobacillus plantarum* and *Leuconostoc mesenteroides*), Bacilli (*Bacillus amyloliquefaciens* and *Bacillus subtilis*), yeasts (*Candida tropicalis*) and moulds (*Rhizopus oryzae*), isolated from traditional cassava ferments used for the prepration of the three main attieke types (*Adjoukrou, Alladjan* and *Ebrie*) were tested singly or in combination in cassava fermentation for their contribution to acid production, protein enrichment, detoxification and softening. All tested species and traditional cassava ferments showed an ability to ferment cassava dough by generating significant changes of at least one of the tested parameters. But mixed starter cultures were more efficient than the single cultures developed and their corresponding cassava traditional inocula. The three mixed starter cultures set displayed good acidification (until 7 times more), improved protein content of cassava dough and better softening and rapid and intense elimination of cyanogenic glucosides (more than 50% in only 6 h). Thus, it appears that the controlled mixed starter cultures were showed to have the most interesting technology profiles for any application at small or large scale.

KEYWORDS: Acidifying power, cassava dough fermentation, detoxication, starter cultures, softening.

1 INTRODUCTION

In Cote d'Ivoire, the most popular processing method of cassava (*Manihot esculenta* Crantz), however, especially for varieties high in cyanogenic glucosides, is fermentation. And, the most popular food derived from fermented cassava is *attieke*, a steamed granular cassava meal ready-to-eat, couscous-like product, with slightly sour taste and whitish colour [1]. Cassava fermentation during *attieke* production requires the use of a traditional starter whose preparation method varies according to ethnic groups [2]. 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. Fermentation relies on the autochthonous microbial populations to start the process. Spontaneous fermentations like back slopping typically result from the competitive activities of a variety of autochthonous and contaminating microorganisms. Those best adapted to the conditions during the fermentation process will eventually dominate. However, initiation of a spontaneous or back slopping process takes a relatively long time, with a high risk for failure. Failure of fermentation processes can result in spoilage and/or the survival of pathogens, thereby creating unexpected health risks in food products [3], [4]. Thus, from both a hygiene and safety point of view, the use of starter cultures is recommended, as it would lead to a rapid acidification of the product and thus inhibit the growth of spoilage and pathogenic bacteria [4], and to a product with consistent quality.

Indeed, according [5] and [6], the use of starter cultures would be an appropriate approach for the control and optimisation of the fermentation process in order to alleviate the problems of variations in organoleptic quality and microbiological stability observed in African indigenous fermented foods, and thus would contribute to reduce processing time [7]. The development of starter cultures is one of the prerequisites for the establishment of small-scale industrial production of fermented foods in Africa [5]. The primary consideration before introducing starter cultures for traditional

small-scale fermentations should be whether these would significantly contribute to an improvement of processing conditions and product quality with respect to: rapid or accelerated acidification, an improved and more predictable fermentation process, desirable sensory attributes, improved safety and reduction of hygienic risks [8]. Therefore, a thorough understanding of the fermentation process is required. The knowledge gained with controlled starter culture may also benefit those operating at a very small scale and practising backslopping.

No starter cultures are commercially available yet for the small-scale processing of traditional African foods [8], and particularly for attieke, despite the notoriety e that the product has won in the last decade throughout the West African region and some european countries. Moreover, many studies have been achieved with principal objectives to contribute to the improvement of the product quality through the control of the fermentation process [9], [10], [11]. Most of these studies reported that lactic acid bacteria, yeasts, bacilli and moulds are the predominant microorganisms found in *attieke* fermentation. Furthermore, in our recent studies, the potentialities of each one of these microorganisms in vitro has been well established and those functionally most effective for cassava fermentation during *attieke* preparation were identified [12], [13], [14]. Therefore, this study aimed to select different species of LAB, yeasts, bacilli and moulds as appropriate starters cultures for the 3 main *attieke* types of Côte d'Ivoire, with respect to their contribution to acidification, detoxication, protein enrichment and dough texture during cassava fermentation in *attieke* preparation.

2 MATERIALS AND METHODS

2.1 CASSAVA ROOTS AND STRAINS USED

2.1.1 CASSAVA ROOTS

Mature cassava roots of IAC variety were obtained from a farm of University Nangui Abrogoua at Abidjan (Ex-University of Abobo-Adjame).

2.1.2 STRAINS USED

strains used in this study were isolated and pre-selected from three different types of ready to use cassava traditional inocula, with regard to ethnical groups (*Ebrie*, *Adjoukrou* and *Alladjan*) which produce them, and taken in small-scale *attieke* production in the three main processing zones (Abidjan, Dabou and Jacqueville) in Côte d'Ivoire (Table 1). These strains were previously characterized and tested in vitro for their technological properties, interesting for fermentation [11], [12], [13], [14].

Table 1. Starters cultures tested in this study. Strains have been preselected with regards to their origin and their enzymatic activities in our previous studies

Origin of starter culture	Codes	Species
Adjoukrou	LABX2	*Leuconostoc mesenteroides ssp mesenteroides*
	BX5	*Bacillus amyloliquefaciens*
	LVX14	*Candida tropicalis*
	MX4	*Rhizopus oryzae*
	SMX	Mixed culture of LABX2- BX5- LVX14- MX4
Alladjan	LABY9	*Lactobacillus plantarum*
	BY4	*Bacillus amyloliquefaciens*
	LVY3	*Candida tropicalis*
	MY2	*Aspergillus oryzae*
	SMY	Mixed culture of LABY9- BY4- LVY3- MY2
Ebrie	LABZ46	*Lactobacillus plantarum*
	BZ15	*Bacillus subtilis*
	LVZ18	*Candida tropicalis*
	MZ4	*Rhizopus oryzae*
	SMZ	Mixed culture of LABZ46 -BZ15 -LVZ18- MZ4

2.2 PREPARATION, INOCULATION AND FERMENTATION OF CASSAVA DOUGH

2.2.1 PREPARATION OF STARTER CULTURES

The cultures of LAB, Bacilli., yeasts and moulds isolated from traditional cassava inocula as described above were used. The cultures had been stored at -80°C in sterile cryotubes containing appropriate broth medium with 20% (v/v) glycerol until needed. LAB were cultivated by streaking on MRS agar (Conda, Spain) and incubated anaerobically at 30°C for 24 h. Bacilli were cultivated by streaking on Plate Count Agar containing 2% of soluble starch and incubated at 30°C for 24 h. Yeasts and moulds were cultivated by streaking on Sabouraud chloramphenicol agar and incubated at 30°C for 72 h. After incubation, a colony was picked from each pure culture plate, grown successively in appropriate broth before centrifugation at 7500 g for 15 min. The pellet was washed in peptone physiological salt solution, centrifuged again, redistributed in peptone physiological salt solution and the diluted to yield an optical density equivalent between 5 and 7 Mac Farland (McF), equivalent to 10^6 cells/mL.

2.2.2 PREPARATION OF CASSAVA

Cassava roots were firstly washed in a mixture of 1% mercuric chloride in 70% alcohol, peeled with flame sterilized knives and grated on an autoclave-sterilized hand grater. The sterile resulting cassava dough was then divided into 7 parts of 250 g each.

2.2.3 FERMENTATION

In controlled fermentations, four (4) of the 7 parts of 250 g cassava dough were inoculated each with 100 mL of LAB, Bacilli, yeasts or moulds suspension. The fifth part was inoculated with 100 mL of a mix of the different single suspensions while the sixth part was inoculated with 10% (w/w) of traditional cassava inoculum as practiced on *attieke* production sites. The seventh part, without any inoculum was used as control. Inoculated samples were thoroughly homogenated and incubated at 35 °C and fermentations were monitored over time for 24 h. Sample of fermenting doughs were aseptically taken for different analyses at the beginning, and after 6, 12, 18 and 24 h. For microbial softening activities, tests were done with sterile cassava pulp cut into rings of 4 cm long and 10 cm of diameter and inoculated as described above.

2.3 BIOCHEMICAL ANALYZES

2.3.1 DETERMINATION OF PH AND TOTAL TITRABLE ACIDITY

Thirty (30) 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 was determined on 30 mL of the filtered solution using a pH meter (P 107, CONSORT, Bioblock Scientitific). Total titratable acidity (TTA) was determined by titrating 30 mL of the filtered solution against 0.1 M NaOH using phenolphthalein as indicator. TTA was calculated as percentage of lactic acid as described by [15].

2.3.2 DETERMINATION OF PROTEINS CONTENT

Crude protein content was determined from dosage of total nitrogen by Kjeldahl method acocording [16]. One gram of sample was mineralized at 400 ºC for 2 h in concentrated sulfuric acid (20 mL) in the presence of a catalyst (1 g Selenite of sodium + 1 g copper sulfate + 20 g of potassium sulphate). Ten milliliters of mineral solution added to 10 mL sodium hydroxide solution (40%) were distilled and the distillate collected in 20 mL of boric acid was titrated with sulfuric acid (0.1N) in the presence of a mixed coloured indicator (methyl red and bromocresol green). With this method, all nitrogen compounds were assayed. The conversion of nitrogen to proteins was carried out by a conversion factor of total nitrogen in proteins which was 100/16 = 6.25.

2.3.3 DETERMINATION OF HYDROCYANIC ACID CONTENT

The hydrocyanic acid content of fermenting dough samples was determined following by the method of alkalinity titration [17]. Twenty seven (27) g of samples were soaked in a mixture of water (200 mL) and orthophosphoric acid (10 mL) for 18 h. The mixture was then distilled by internment in water vapor and the distillate was collected in a solution of NaOH (5%). After diluting 100 ml of the distillate to 2/5, the solution was titrated with silver nitrate solution (0.02N) in the presence

of 8 ml potassium iodide. Cyanogenic ions in aqueous solution complexed silver ions. AgNO3 molecule reacted with two molecules of HCN, representing 54 g of HCN for an AgNO3 solution of concentration 1N. For a solution of 1 mL of concentration 0.02N AgNO3 will require (54 g × 0.02 / 1) 1.08 g of HCN.

2.3.4 SOFTENING MEASUREMENT

The penetrometry index was used as an indicator of cassava softening during fermentation. A portable penetrometer (model10-SUR;PNR, Berlin) was used on five randomly cut sections according to a published procedure [18] with five repetitions for each root section. Softening of cassava was measured on an interval of 12 h of fermentation during 108 h.

2.4 EXPRESSION AND STATISTICAL ANALYSIS OF DATA

Excepted for softening activity, data were expressed as a change in a particular activity during time. For example, the acidification activity measured as pH changes was calculated as ΔpH ($\Delta pH = pH_{\text{at time}} - pH_{\text{zero time}}$) between zero time and 12 h and between 12 h and 24 h. The data obtained were subjected to analysis of variance (SAS/Stat, 1996) and mean differences determined by Duncan's multiple range test ($P < 0.05$). Performance of each starter culture tested was illustrated by principal component analysis (PCA). The results of the PCA were represented by one plot, combining tested parameters and starter cultures.

3 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 ACIDIFICATION RATE OF CASSAVA DOUGH

The acidification rate of cassava dough by the various starter cultures tested in this study was presented in Table 2. This acidification rate, expressed as change in pH and total titratable acidity was the most important the first 12 h of fermentation for both doughs inoculated with mixed stater cultures and cassava traditional inocula, and also for some single cultures of LAB; and this whatever the origin of isolates. Indeed, mixte cultures SMX, SMY and SMZ rapidly decreased the pH of doughs after 12 h by 2.27, 2.16 and 2.7 units respectively, with an equivalent of TTA of 1.7, 1.54 and 2.4%. Traditional inocula showed similar pH decrease, but with lower acid production. Among single starter cultures, only LABZ46 from Ebrie origin showed ability to decrease pH similar to the mixed cultures (Table 2).

3.1.2 PROTEIN ENRICHMENT

As observed for acidification rates, the enrichment of cassava dough in proteins during fermentation significantly varied from one starter culture to the other (Table 2). SMX showed the better protein enrichment capacity with a value of 7.15 mg/100 g only after 12 h of fermentation, followed by SMZ (5.75 mg/100 g) and the traditional inocula FTX, FTY and FTZ with respectively 5.7, 5.31 and 5.09 mg of proteins produced by 100 g of dough. In the second part of fermentation, protein enrichment was the highest for the different moulds single cultures MX4 (6.82 mg/100 g), MY2 (4.2 mg /100 g) and MZ4 (4.36 mg/100g).

3.1.3 DETOXICATION OF CASSAVA DOUGH

The initial HCN content in cassava dough was 155 mg/100 g. Detoxication of this dough was observed during fermentation, with a highest activity during the first 12 h. whatever the origin, controlled mixed cultures (SMX, SMY and SMZ) had the highest detoxication powers, by reducing cyanide by more than 82%. Traditional inocula and some single cultures, particularly those of LAB (LABX2, LABY9 and LABZ46) showed similar detoxication activities to those of controlled mixed cultures, but at a lesser extent. The other single cultures detoxication powers were significantly low (Table 2).

Table 2. Biochemical parameters (expressed as a variation between 0 / 12h and 12 / 24 h of fermentation) analyzed during fermentation of cassava doughs inoculated with various controlled starter cultures and traditional cassava ferments

	Biochemical parameters							
	ΔpH		ΔTA (%)		ΔProtein (mg/100g)		ΔHCN (mg/100g)	
Inocula	Δ (0h/12h)	Δ (12h/24h)	Δ (0h /12 h)	Δ (12h/24h)	Δ (0h/12h)	Δ (12h/24h)	Δ (0h/12h)	Δ (12h/24h)
Control	0,04±0,001	0,15±0,002	0,0005±0,00	0,0004±0,00	0,05±0,001	0,1±0,0005	2,5±0,02	3±0,4
LABX2	1,34±0,01	0,96±0,01	0,02±0,006	0,027±0,009	0,89±0,07	0,81±0,07	90±7,01	24,6±3,7
BX5	0,94±0,05	0,72±0,07	0,064±0,01	0,065±0,006	0,7±0,2	0,95±0,2	82,56±6	12,4±7,01
MX4	0,5±0,01	0,56±0,001	0,01±0,009	0,045±0,002	2,34±0,84	6,82±0,84	55±4,9	20±2,8
LVX14	0,69±0,02	0,09±0,06	0,063±0,08	0,033±0,006	1,3±0,7	2±0,09	68±9,7	10±1,9
SMX	2,27±0,53	0,45±0,01	1,7±0,01	0,29±0,001	7,15±0,21	2,49±1	104,9±7,13	28,9±7,7
FTX	2,06±0,05	0,58±0,03	0,84±0,05	0,56±0,005	5,7±0,81	0,4±0,1	95,7±11,01	30,3±8,7
LABY9	1,27±0,09	1,07±0,1	0,24±0,01	0,36±0,01	3,91±0,83	0,7±0,2	106,41±9,9	9,6±4,9
BY4	0,78±0,1	0,22±0,01	0,16±0,05	0,045±0,00	0,3±0,01	1±0,005	56±13,7	33±7,2
MY2	0,52±0,03	0,48±0,07	0,025±0,002	0,2±0,005	2±0,88	4,2±1,5	23±4	15±8,1
LVY3	0,67±0,09	0,4±0,01	0,029±0,01	0,01±0,00	3,91±0,5	0,55±0,005	32±0,01	19±1,11
SMY	2,16±0,01	0,25±0,06	1,54±0,2	0,3±0,001	3,61±1,01	2,85±0,91	120,75±8,9	7,15±1,09
FTY	2,3±0,06	0,07±0,03	0,79±0,05	0,64±0,07	5,31±0,64	0,26±0,08	114±778	10,5±0,01
LABZ46	2,72±0,1	0,07±0,02	0,46±0,07	0,42±0,002	0,25±0,007	0,49±0,02	90±0,9	20±5,6
BZ15	1,07±0,01	0,32±0,01	0,08±0,001	0,075±0,011	0,55±0,06	0,55±0,47	55±3,77	14±4,9
MZ4	0,7±0,005	0,6±0,09	0,015±0,01	0,034±0,001	0,94±0,5	4,36±1,14	11±0,31	34±6,08
LVZ18	0,81±0,007	0,42±0,08	0,06±0,007	0,023±0,00	0,99±0,007	2,96±0,11	40±5,8	20±8,8
SMZ	2,7±0,01	0,44±0,01	2,4±0,2	0,42±0,006	5,75±1,06	1,11±0,005	113,8±10,3	6,2±1,01
FTZ	2,07±0,009	0,62±0,04	0,86±0,03	1,08±0,03	5,09±0,9	0,2±0,003	107,21±9,2	22,8±0,98

FTX, FTY and FTZ = cassava traditional inocula respectively from *Adjoukrou*, *Alladjan* and Ebrie origins

3.1.4 SOFTENING OF CASSAVA

The penetrometry index of cassava pulp at the beginning of fermentation was 16.5±0.7 Kg/cm^2. Cassava pulp were fairly affected by the different inocula during the first 60 hours of fermentation, particularly by strains of traditional inocula and controlled mixed cultures which penetrometry index ranged from 16.5±0.7 Kg/cm^2 at 13±0.5 Kg/cm^2. However, exception was made by single cultures BZ15 and MX4 which penetrometry index a value of 11±1.4 Kg/cm^2 differed significantly ($P < 0.05$) than the other strains for the same fermentation period (Fig. 1). But after this period, softening became more intense particularly with mixed culture which reached values ranging between 1.75±0.3 and 7±0.3 Kg/cm^2. Only single culture MX4 reached value (2.5±0.7 Kg/cm^2) near to this of the starter culture (SMX) with the best penetrometry index value. Softening activities from the other starter cultures, particularly the yeasts remained very low with values between 16.5±0.7 and 13.5±0.5 Kg/cm^2 at the end of fermentation (Fig.1).

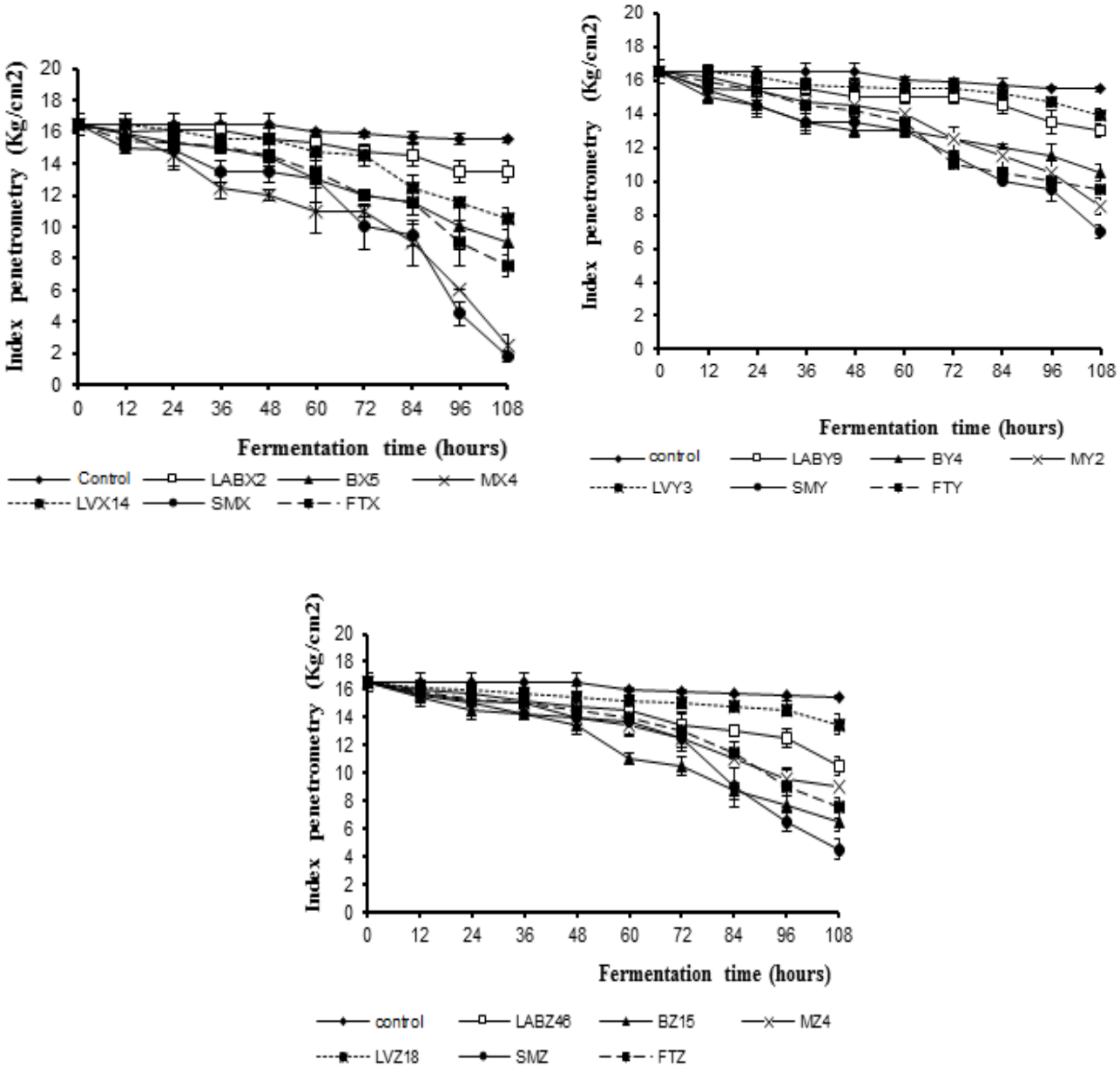

Fig. 1. Softening activity (expressed wy penetrometry Index) during fermentation of cassava inoculated with various controlled starter cultures and traditional cassava ferments

3.1.5 SELECTION OF STARTER CULTURES BY PRINCIPAL COMPONENT ANALYSIS (PCA)

The Principal Component Analysis (PCA), made from results obtained for the variables "acidifying power" "protein enrichment", "detoxification" and "softening" at the different periods of cassava dough fermentation allowed to classify the 15 controlled starter cultures and the 3 traditional inocula on the basis of their technological potential, knowing that in most cases there is no correlation between these activities.

The first two principal component explained 76.94% of the variation in the data, with the first component (F1) accounting for more than 55% of the variation and the second component (PC2) accounting for 21.04% of the variation. The contribution of the combined data to the principal component is illustrated in Fig. 2. When inocula were plotted on the space created by the two dimensions, they were distinctly divided along both axes, described by the variables "acidity produced","protein

enrichment", "detoxification" and "softening". The proximity of the vector extremity, representing a variable with the circle of correlation gave information on the quality of representation of this variable, showing that these variables (interest activities) were well represented in the factorial plan (not showed). However, we clearly observed the low correlation between these variables due to the angle formed between vectors of each variable globally considered regardless of fermentation time. Contrarily, data obtained per fermentation time for each variable appeared to be strongly correlated between them, regarding their low angle (near 0 °). This seemed rational, since it was the same metabolic activity of interest analyzed along fermentation.

The distribution of strains in the factorial plan according to axes F1 and F2 allowed to distinguish then into different groups, especially the axis F2, separating the single starter cultures to the mixed ones (controlled and traditional inocula). Indeed, mixed starter cultures SMY, SMZ, and traditional inocula (FTX, FTY and FTZ) formed a distinct group in the positive direction of axis F1. They generally possessed higher acidification, detoxifying and protein enrichment levels. Within this group, the proximity of traditional inocula from each other showed that they had almost similar activities, but equally distinct, or even lower than those of controlled mixed starters, relating to the position deviation between both types of inocula. Another group was formed, opposing the first one in the positive part of axis F2, composed by mixed starter SMX and single starter MX4 which demonstrated significant softening activity and best protein enrichment after 24 h of culture. Next to each of these two groups, different types of single starter cultures were positioned. On the one hand, LAB single cultures which are closer to the group of inocula with high acidifying and detoxification activities, probably possessed intermediary activities; and on the other hand, Bacilli and moulds single cultures showed intermediary activities as mainly concern the softening activity. Therefore, PCA method offers an enough powerful discriminatory analysis to sort or elect the most effective starter.

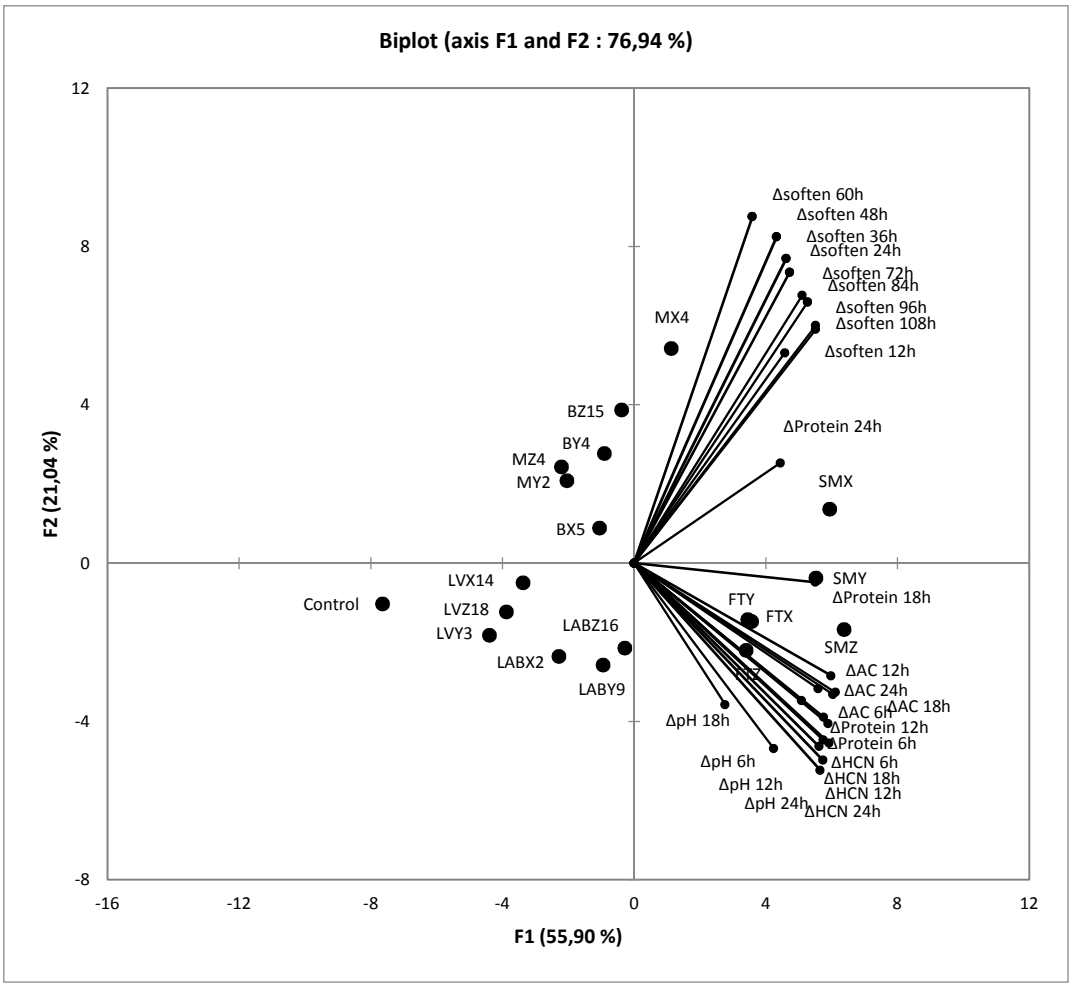

Fig. 2. Principal Component Analysis of technological profiles of various controlled starter cultures and traditional cassava ferments from the three main attieke production ethnic groups, tested during fermentation of cassava.

3.2 DISCUSSION

Our previous studies allowed us to preselect LAB, Bacilli, yeasts and moulds strains according to their acidifying power, their potential to excrete α amylases, pectinases and β-glucosidase, interesting properties for the bioconversion of cassava, in synthetic media (in vitro tests). The present study assessed the capacities of the 15 preselected strains to create significant changes in a complex medium which is cassava dough.

Strains of *Bacillus* species (*Bacillus amyloliquefaciens* BX5, *Bacillus amyloliquefaciens* BY4 et *Bacillus subtilis* BZ15) et *Lactobacillus plantarum* LABY9, preselected for their cassava broth acidifying abilities, were not yet able to display significative acidifying power in real medium of cassava dough when inoculated in single culture. Only *Leuconostoc mesenteroides* spp *mesenteroides* LABX2 et *Lactobacillus plantarum* LABZ46 showed important acidifying power when cultivated in single cultures on cassava dough. But, after only 12 h of fermentation, the acidity produced by mixed culture from *Adjoukrou* origine (SMX) was 3.7 time higher than those of *Leuconostoc mesenteroides* spp *mesenteroides* LABX2 displaying significant acid production and 1.3 time this of the traditional inocula from the same origin. In the same way, for starter cultures from *Alladjan* origin, which for most of them did showed interesting acid production in cassava dough, controlled mixed starter culture showed an acid production 7 times higher particulary against its corresponding best acidifier *Lactobacillus plantarum* LABY9. Rapid acidification during dough fermentation as performed by controlled mixed starter cultures is a technological characteristic that is of paramount importance as reported by [19]; notably, in the preservation and microbial safety of fermented foods, thus promoting the microbial stability of the final products of fermentation. Our results could not be explained based on an additive effect of activities from microorganisms constituting the mixed starter cultures, because selected Bacilli, yeasts and moulds strains showed very fair acidifying power in cassava dough when inoculated as single cultures. It would thus be a potentiating effect. It means that, in a mixed starter culture, the acidifying power of lactic acid bacteria strain (*Leuconostoc mesenteroides* spp *mesenteroides/dextranicum* ou *Lactobacillus plantarum)* was increased by the activity of the other microorganisms and / or vice versa. Lactic acid bacteria (LAB) and other microorganisms have been reported to be present in most of the African indigenous fermented foods [5], [20], [21], [22], [23], [24], [25], [26]. Stable co-metabolism between LAB and yeasts is common in many foods, enabling the utilization of substances that are otherwise non fermentable (for example starch) and thus increasing the microbial adaptability to complex food ecosystems [27], [28], [29]. Thus, it has been suggested that the proliferation of yeasts in foods is favoured by the acidic environment created by LAB while the growth of bacteria is stimulated by the presence of yeasts, which may provide growth factors, such as, vitamins and soluble nitrogen compounds [20]. Moreover, This potentiation may depend on lactic acid bacteria proportion. In fact, in the traditional inocula, where variations of acidity were lower compared to those in mixed starter cultures, lactic acid bacteria proportions were estimated at 23.7% of cocci and 18.7% of bacilli according to Djeni [30]. However mixed cultures in this study consisted each either of 25% of *Leuconostoc mesenteroides* spp *mesenteroides* or 25% of *L. plantarum*. Also, α-amylase excreted by preselected *Candida tropicalis* and moulds strains may indirectly contribute to the potentiation of acidifying power of lactic bacteria. Indeed, α amylases excreted would provide necessary amounts of reducing sugars for LAB strains through a direct metabolism convert glucose into lactic acid [31]. Conversely metabolites from LAB (*Leuconostoc mesenteroïdes* or *L. plantarum*) may contribute to improving the acidifying power of other mixed cultures component strains.

Moreover, cassava roots are low in proteins (0.54%) [32]. Roots used in this study have a protein content of 2.05 mg/100g (0.02%), poorer than those used by the mentioned authors in their studies. Protein contents in fermented cassava doughs after 24 h by controlled mixed starters cultures and mould single cultures were relatively low (0.097% to 0.11%), but were about 4 times higher than the initial rate. The results showed that the improvement of protein levels by mixed starter *Adjoukrou*, was mainly due to the activity of *Rhizopus oryzae* MX4. In fact, [32] reported that the presence of proteins during fermentation depends on a good enzymatic activity of the selected strains. However, in doughs inoculated with *Alladjan* or *Ebrie* controlled mixed cultures, protein contents seemed to be related to an additive effect of *Candida tropicalis* and *Rhizopus Oryzae* MX4 or *Mucor* sp. MY1 activities; that is to say the amount of protein produced by each pure culture of the controlled mixed starter, reflected the amount of protein observed in the doughs inoculated with the mixed starters.

A reduction in the cyanogenic potential of cassava occurred during every unit operation in the processing of cassava into *akyeke* (cassava fermented food from Ghana), and this resulted in near detoxification of the product. The greatest loss in the cyanogenic potential occurred during grating and fermentation [33]. In our study, the reduction in hydrogen cyanide (HCN) content of cassava dough by mixed starter cultures was essentially the affair of lactic acid bacteria strains. Indeed, no significative difference was observed in the HCN content of doughs inoculated with controlled mixte starters, cassava traditional inocula and single cultures of LAB during the fermentation. This demonstrates that LAB are a vital link in β-glucosidase production, thus responsible for the elimination of cyanogenic glycosides [34]. The same could be said regarding

the detoxication activities that it did not occur additive or potentiating effect in doughs inoculated with mixed starter cultures. The detoxication activity of a mixed starter was essentially that of the lactic acid bacterium species present in the culture medium. This detoxication was achieved by half with controlled mixed cultures and their corresponding cassava traditional inocula after only 6 h of fermentation, while single LAB monocultures performed the same result after 12 hours. [33] reported four species of LAB (*L. brevis, L. mesenteroides, L. salivarius and L. fermentum*) to be capable of breaking down cyanogenic glucosides and contributed to the detoxification of cassava during the 5 days of *akyeke* fermentation by 56.1% and 62.1% of the total cyanide content, respectively in field and laboratory samples.

Moreover, no softening was obtained in sterile fermentation. According to [35], pectinolytic enzymes of microbial origin are clearly indispensible for the softening to be completed in cassava rettings. Depolymerizing enzymes were not detected in fresh roots, but significant activities were detected after 17 h of fermentation, when the microbial populations were fully established [36]. The results of the present work showed the involvement of moulds and *Bacillus* species in the textural modification of cassava tissue during fermentation. And this is in agreement with the findings of [37] and [38]. If no study reports the contribution of moulds in cassava fermentation, *Bacillus species*, particularly *B. subtilis* was the dominant species identified by [38] as being responsible for textural breakdown of cassava tissue during *agbelima* cassava dough fermentation. [37]. also reported *Bacillus* spp. as one of the isolates from heap, fermenting cassava roots which was able to cause a softening of cassava tissue. Therefore, the important softening activities observed in cassava doughs inoculated with controlled mixed cultures would indoubtely be the potentiation of moulds and *Bacillus* strains activities.

4 CONCLUSION

The bioconversion of cassava dough through the study of acid production, detoxication, protein enrichment and softening clearly indicates that mixed starter cultures from *Adjoukrou Alladjan* and *Ebrie* origins established for controlled fermentation during the preparation of the three main *attieke* types in Côte d'Ivoire were more efficient than the single culture developed and their corresponding cassava traditional inocula. These three mixed starter cultures displayed good acidification, improved protein content of cassava dough and better softening and elimination of cyanogenic glucosides. Thus, it appears that the controlled mixed starter cultures SMX, SMY and SMZ were showed to have the most interesting technology profiles for any application at small or large scale.

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