



## ACTIVITY OF SELECTED MIXED STARTER CULTURES FOR SPECIFIC PRODUCTION OF THREE MAIN TYPES OF ATTIEKE IN CÔTE D'IVOIRE

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**Abstract:** *In this study, mixed potential starter cultures have been set up to control the fermentation of cassava dough for the production of each type of attieke in Côte d'Ivoire. The results showed that potential starter culture from Ebrie was the most acid with pH and total titratable acidity values respectively of 4.68 and 3.22±0.2 % after 24 hours fermentation. Also several organic acids were detected in the potential starter cultures, lactic and acetic acids being predominant. Lactic acid content reached 670±19.4 mg/100g in inoculum from Adjoukrou group, 580±9 mg/100g in and 778±13 mg/100g in those from Ebrie group. For acetic acid, its highest rate (347±14 mg/100g) was obtained in Alladjan inoculum and the lowest (195.2 ±14 mg/100g) in Ebrie's one after 24 hour fermentation. Fumaric, citric, tartaric and propionic acids have not been detected. Volatile substances (acetaldehyde, ethanol) were produced throughout the fermentation by the potential starter cultures formed. The microbial analysis showed that highest loads were represented by lactic acid bacteria with values of 7.2 ± 0.8.10<sup>7</sup> CFU / g; 2.9 ± 0.7.10<sup>7</sup> CFU / g and 7.6 ± 1.10<sup>6</sup> CFU / g respectively in the doughs inoculated with the selected starters Adjoukrou, Alladjan and Ebrie. The production of attieke specific to each ethnic group with potential starter cultures showed that these attieke were of acceptable quality. The importance of mixed potential starter cultures formed could be necessary to standardize at small and industrial scale the process of production each type of attieke in Côte d'Ivoire.*

**Keywords:** *starter cultures, organic acids, standardization, attieke*

### 1. Introduction

In Côte d'Ivoire, Cassava roots are processed into about ten dishes of which the most known are attoukpou, gari, placali and attieke [1]. The major fermented plant food of the country is Attieke. It is a steamed granular cassava (*Manihot esculenta* Crantz) meal ready-to-eat, couscous-like product, with slightly sour taste and whitish colour [2]. In the past, this food was prepared and consumed exclusively by the Adjoukrou, Ebrie, Alladjan, Avikam and Ahizi peoples of Côte d'Ivoire. The processing of cassava into attieke requires several difficult steps.

Generally, roots are peeled, cut into pieces and then washed three times with fresh water. Before milling, 5% - 10% (w/w) of inoculum, 10% (v/w) water and about 0.1% (v/w) of palm oil are added and the pieces are ground into a fine paste and left to ferment for about 12 to 15 hours at room temperature (30°C - 37°C). After fermentation, the dough is continuously pressed and then sieved and granulated. The resulting grains are dried in the sun for a few minutes to half an hour. Then, the fibres and dirt are removed by sprinkling after drying. Finally, the grains are steamed for about 20 to 25 minutes. [3]. The attieke obtained is packed in plastic

bags, hermetically sealed for sale on local and international markets. But, the production of high quality attieke is often associated with specific locations and specific ethnic groups in Côte d'Ivoire, thus generating several types of attieke found on various markets with specific characteristics and differently appreciated by consumers. The ethnic groups Adjoukrou, Ebrie and Alladjan are recognized as the best producers and consumers of attieke in Côte d'Ivoire. Besides these groups, their products are also consumed on a large scale in urban areas, particularly in Abidjan. Yet, the fermentation of cassava during the production of attieke requires the use of a traditional ferment. The method of preparing this ferment varies according to ethnic groups [4]. However, this ferment is the main source of microorganisms active in the fermentation of dough. Fermentation relies on the autochthonous microbial populations to start the process. Spontaneous fermentations typically result from the competitive activities of a variety of autochthonous and contaminating microorganisms. Thus, 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 [5, 6]. Thus, in a framework to develop starter culture for controlled fermentation and production of regional specific attieke, with greater consistency in quality and safety, the use of starter cultures is recommended, as it would lead to control and optimisation of the fermentation process in order to alleviate the problems of variations in

organoleptic quality and microbiological stability observed attieke in Côte d'Ivoire. Many previous studies were carried out before putting the establishment of starter cultures specific to the three main types of attieke in Côte d'Ivoire. These are studies conducted by [7] based on screening microorganisms for their abilities to produce  $\alpha$ -amylase,  $\beta$ -glucosidase, pectinase and their rate acidification. These strains used, have been characterized and identified by morphological, biochemical as well as molecular approaches [8, 9]. The optimization of  $\alpha$ -Amylase production by selection of microorganisms have been realized by [10] in the same way, 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 [11]. From these studies, the controlled mixed starter cultures have been shown to be the most interesting technology profiles for any application at small or large scale. Therefore, this study was carried out to provide sufficient knowledge about the final biochemical and microbiological characteristics of potential starter cultures in order to control and standardize the cassava fermentation process for each types of attieke produced in Côte d'Ivoire.

## **2. Materials and methods**

### *2.1. Cassava roots*

The cassava roots used for this study were of the IAC variety harvested from a field at Nangui Abrogoua University in Abidjan.

### *2.2. Strains used*

Strains used in this study were isolated and pre-selected from three different types of ready to use cassava traditional inoculum, with regard to ethnical groups

(Ebrie, Adjoukrou and Alladjan) which produce them 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 for their technological properties, interesting for fermentation [7-11].

**Table 1**

**Potential starter cultures specific to the three main types of attieke in Côte d'Ivoire.**

Strains have been preselected with regards to their origin and characterized, tested for their technological properties interesting for cassava fermentation in our previous studies

Origin of starter	Potential starter cultures	codes of potential starter cultures
Adjoukou	LABX2- BX5- LVX14- MX4	SS.Ad
Alladjan	LABY9-BY4- LVY3 - MY2	SS.Al
Ebrie	LABZ46-BZ15-LVZ18-MZ4	SS.Eb

SS.Ad: Mixed culture of LABX2 (*Leuconostoc mesenteroides ssp mesenteroides*), BX5 (*Bacillus amyloliquefaciens*), LVX14(*Candida tropicalis*) and MX4(*Rhizopus oryzae*) selected as microbials component of potential starter Adjoukou

SS.Al: Mixed culture of LABY9 (*Lactobacillus plantarum*), BY4 (*Bacillus amyloliquefaciens*), LVY3 (*Candida tropicalis*) and MY2 (*Aspergillus oryzae*) selected as microbials component of potential starter Alladjan

SS.Eb: Mixed culture of LABZ46 (*Lactobacillus plantarum*), BZ15 (*Bacillus subtilis*), LVZ18 (*Candida tropicalis*) and MZ4 (*Rhizopus oryzae*) selected as microbials component of potential starter Ebrie

### 2.3. Preparation of microbials suspensions and potential starter cultures for attieke production

#### 2.3.1. Preparation of the microbial suspension

The strains that constitute each mixed starter culture as described in the table 1, has 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<sup>6</sup> cells/mL. The mix of the different single suspensions (100 mL of LAB suspension, of 100 mL of Bacilli suspension, 100 mL of yeasts suspension and 100 mL of moulds suspension) constitutes the suspension of a potential starter specific to a type of attieke.

#### 2.3.2. Preparation of selected starters for attieke production

The starter selected was obtained by peeling the roots and cooking them for 20 min. After cooling to 30°C, 250 g of

cooked roots were crushed in each stomacher sachet containing 100 mL of inoculum (suspension of a potential starter) at  $10^6$  cells/mL. The cassava dough obtained was fermented for 24 hours at room temperature ( $28^{\circ}\text{C}\pm 2$ ). After 24 hours, the dough obtained was the selected starter (mixed), typical to each traditional ferment. All fermentations were conducted for 24 hours and samples were taken every 6 hours for biochemical and microbiological analysis. After all the analyses, the preparation of attieke from each selected starter was carried out using the artisanal attieke preparation method described by [4] in Côte d'Ivoire.

#### *2.4. Biochemical and Microbial analyses*

##### *2.4.1. Biochemical analysis*

Forty grams (40 g) of starter cultures samples selected were ground in 300 ml of distilled water in a porcelain mortar and then centrifuged at 4000 tours/min for 30 min. The pH was determined on 50 ml of the supernatant using a pH-meter (P107 Consort). Total titratable acidity (TTA) was determined by titrating 30 ml of supernatant used for pH determination against 0.1 M NaOH using phenolphthalein as indicator. TTA was calculated as percentage of lactic acid. Water-soluble carbohydrates were determined by the phenol sulphuric acid method according to [12] and the values were expressed in g/100 g of fresh matter, while the reducing sugars were quantified as described by [13] and expressed in mg/100 g of fresh matter. Organic acids of samples were before extracted and then analyzed by high performance liquid chromatography using an ion exclusion ORH-801 column (300 mm $\times$ 6.5 mm) (Interchrom, France) as achieved by [14]. Running conditions were: mobile phase, H<sub>2</sub>SO<sub>4</sub> 40 mmol.L<sup>-1</sup>; flow rate, 0.8

ml.min<sup>-1</sup>; wave length, 210 nm; room temperature (25 °C). The separated components were detected by an UV spectrophotometric detector (SPD-6A, Shimadzu Corporation, Japan). Volatile compounds were determined by gas chromatography (Shimadzu CG-14A, Shimadzu Corporation, Japan) following the method used by [15].

##### *2.4.2. Microbial analysis*

Microbial analyses were carried out to determine the microbiological loads of inoculum samples. Preparation of stock solutions, inoculation of agar plates, cultivation and quantification of microorganisms were carried out according to [16]. For all determinations, 10 g of samples were homogenized in a stomacher with 90 ml of sterile peptoned buffered water (AES Laboratoire, COMBOURG France). Tenfold serial dilutions of stomacher fluid were prepared and spread plated to determine microorganism counts. Yeasts and moulds were enumerated on plates of Sabouraud chloramphenicol agar (Fluka, Bochemica 89579, Sigma-Aldrich Chemie GmbH, Inda) incubated at 30 °C for 4 days. Bacili species were enumerated on plates Mossel agar (AES Laboratoire, COMBOURG France) after incubation at 30°C for 2 days. Enumeration of LAB was carried out using plates of de Man, Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany) which were incubated under anaerobic conditions (Anaerocult A, Merck) at 37° C for 72 h.

#### *2.5. Statistical analysis*

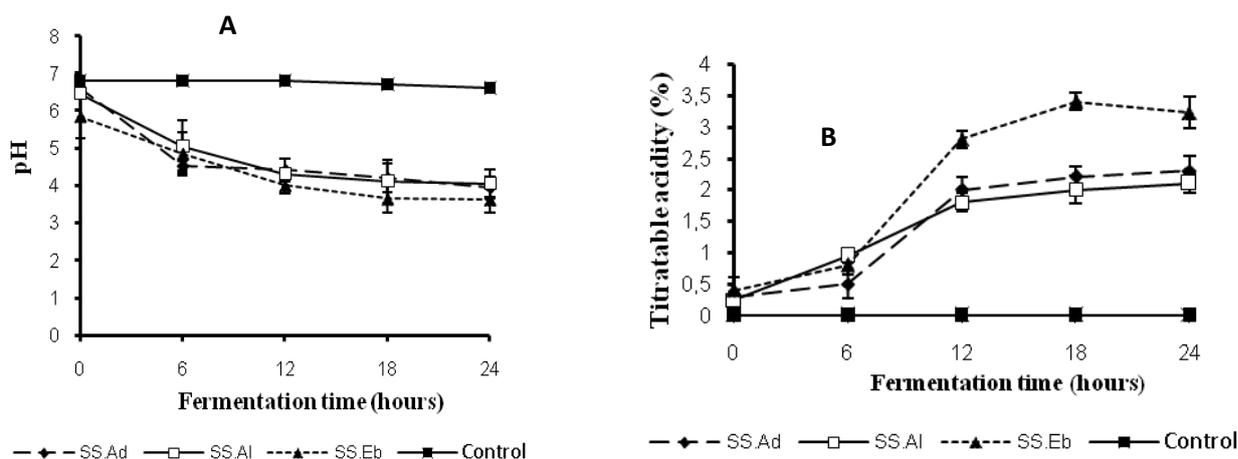
One-way analyses of variance based on DUNCAN multiple tests with significant level  $\alpha=0.05$  were performed in order to compare biochemical and microbial characteristics samples and also

to determine significant differences between potential starter cultures types. The software used for the statistical evaluation was XLSTAT (Addinosoft Inc.).

### 3. Results and Discussion

The present study on potential starter cultures specific to the three main types of attieke (Adjoukrou, Alladjan and Ebrie) was performed firstly to establish a scientific database of characteristics of these potential starter cultures, and secondly to produce attieke with the potential culture starters set up to ensure the quality of each type of attieke in Côte

d'Ivoire. During various fermentation tests made, there was a significant acidification resulting in a rapid reduction in the pH during the first twelve (12) hours, an increase in the titratable acidity rate and a significant production of organic acids, particularly lactic and acetic acids. All these inocula were acid with different degrees of acidity. Potential starter culture from Ebrie is the most acid with pH and total titratable acidity values respectively of 4.68 and 3.22±0.2 %. These values were respectively 4.26 and 2.3±0.2 % for potential starter culture from Adjoukrou group and of 4.49 and 2.09±0.1 for Alladjan type after 24 hours fermentation (Fig. 1).



**Fig.1. pH (A) and Titratable acidity (B) of cassava doughs inoculated with the three types of starters selected during fermentation. SS.Ad: selected starter Adjoukrou, SS.Al: starter selected Alladjan, SS.Eb: selected starter Ebrie**

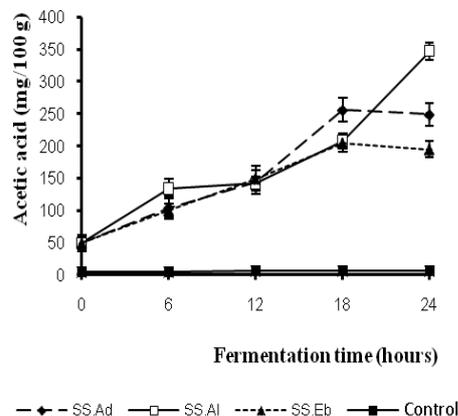
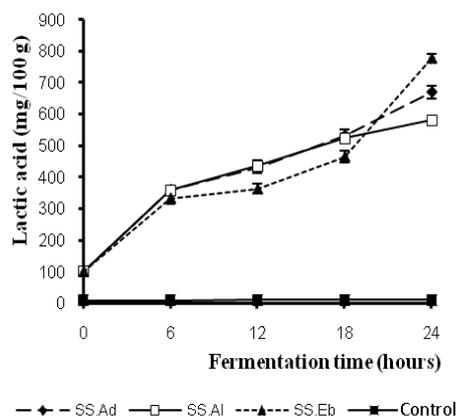
Also, as it could be seen in the figure 2, in the potential starter cultures, lactic and acetic acids were predominant. Lactic acid content reached 670±19.4 mg/100g in

inocula from Adjoukrou group, 580±9 mg/100g in and 778±13 mg/100g in those from Ebrie group (Fig. 2A). For acetic acid, its highest rate (347±14 mg/100g)

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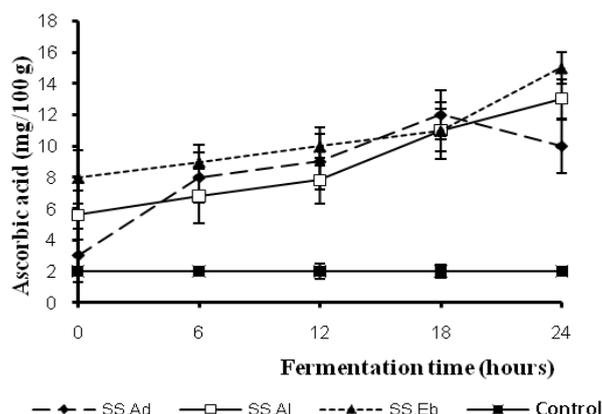
was obtained in Alladjan inocula and the lowest ( $195.2 \pm 14$  mg/100g) in Ebrie's one after 24 hours fermentation (Fig.2B).

Indeed, this acidification is attributed to the LAB that colonizes the selected starters set up.



A

C



**Fig. 2. Production of organic acids by the selected starters in the various cassava doughs in fermentation.** Lactic acid (A); Acetic acid (B); Ascorbic acid (C). SS.Ad: selected starter Adjoukrou, SS.Al: starter selected Alladjan, SS.Eb: selected starter Ebrie

These results were in agreement with those of [17, 18]. These authors attribute to lactic bacteria the main role of rapid acidification of food products. Indeed, the high activity of producing lactic acidity or titratable acidity is related to the diversity of microorganisms with important enzymatic activities ( $\alpha$ -amylase,  $\beta$ -glucosidase and

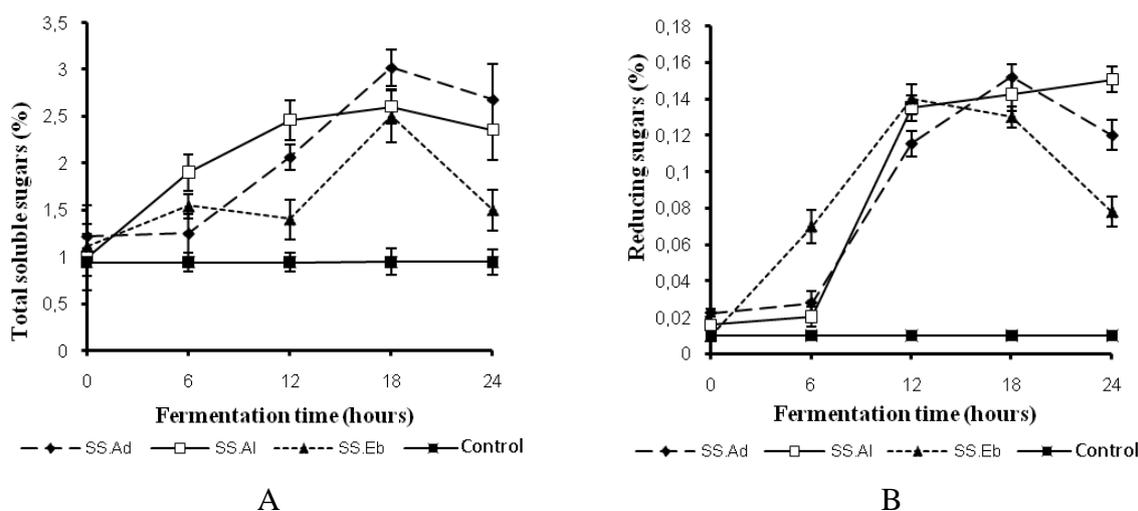
pectinase). It could be suggested a co-metabolism between yeasts and other microorganisms especially lactic acid bacteria, whereby bacteria produce acid in the medium for yeast growth then these yeasts provide vitamins and other growth factors for bacteria. Thus, it could be said that starters set up have a microflora

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capable for the production of attieke. In addition, [14] stated that the high content of lactic acid and acetic acid in the fermented dough would clearly result in a safe action against pathogens. Here, the presence of pathogens has not been sought. However, also high content of organic acids, especially acetic acid, could have an adverse effect on the sensory properties of the finished product [4].

Acidification was more intense in the dough inoculated with these starters selected than that established by [14] during his study on the performance of traditional ferments Adjokrou, Allandjan and Ebrie. The acidification contributes to the improvement of the taste, the texture and the life time of the final product, it could be stated that the organoleptic

qualities of the finished product in each selected starter will be improved more than those in each traditional ferment. These results are consistent with those [19, 20]. Acidification is an essential parameter for the organoleptic characteristics of fermented foods. Although organic acids, mainly lactic and acetic acids are the main metabolites of cassava fermentation, it is also obvious that their synthesis by microorganisms is possible thanks to the degradation of cassava constituents (starch, soluble sugars, etc.). This degradation of soluble sugars in the doughs inoculated by selected starters was total after 18 hours of fermentation except for the selected starter Ebrie which degrades the reducing sugars by  $14 \pm 0.008\%$  after 12 hours of fermentation (Fig. 3).



**Fig. 3. Utilization of sugars during fermentation of cassava doughs inoculated with the three types of starters selected.** Total sugars (A); reducing sugars (B). SS.Ad: starter selected Adjokrou, SS.Al: starter selected Allandjan, SS.Eb: starter selected Ebrie

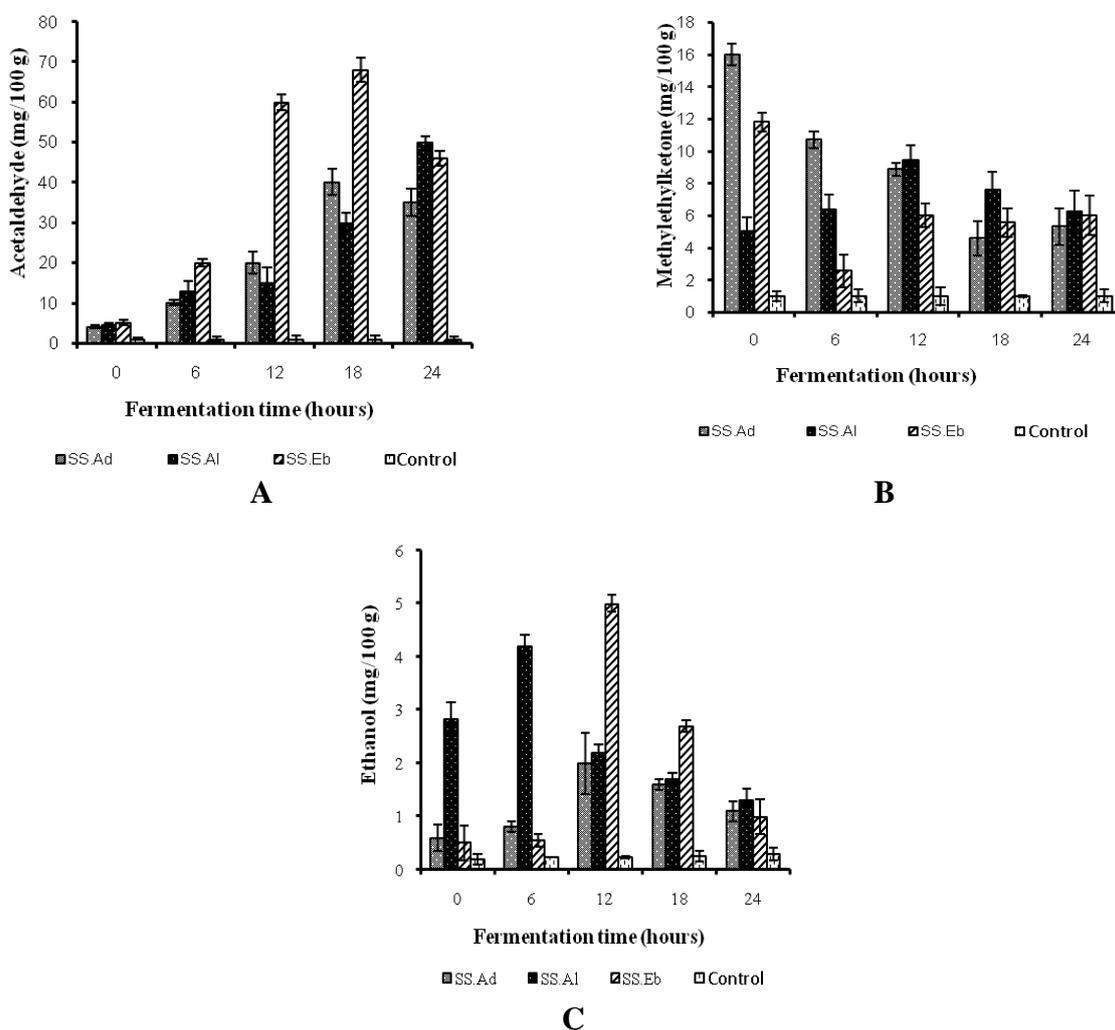
In fact, the evolution of sugar content is the result of two phenomena: the consumption of sugars by microorganisms and the degradation of complex sugars into simple sugars. According to [21] the degradation of complex sugars into fermentable sugars leads to an increase in sugars in the culture

medium. For [22], the variation of sugars in the medium is due to the amylolytic activity of fermentative microorganisms. The metabolic activity of these fermentative microorganisms would also lead in addition to the production of organic acids, to the production of

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precursors of flavoring compounds such as amino acids that could be either deaminated or decarboxylated to form aldehydes. And these aldehydes would be oxidized or reduced to alcohols [23, 24]. Some food flavor compounds have also been detected in the different fermenting doughs. This was ethanol, acetaldehyde and methylethylketone (MEK) (Fig. 4). During fermentation, large quantities of acetaldehyde were produced by isolates of the selected starter Ebrie with contents of

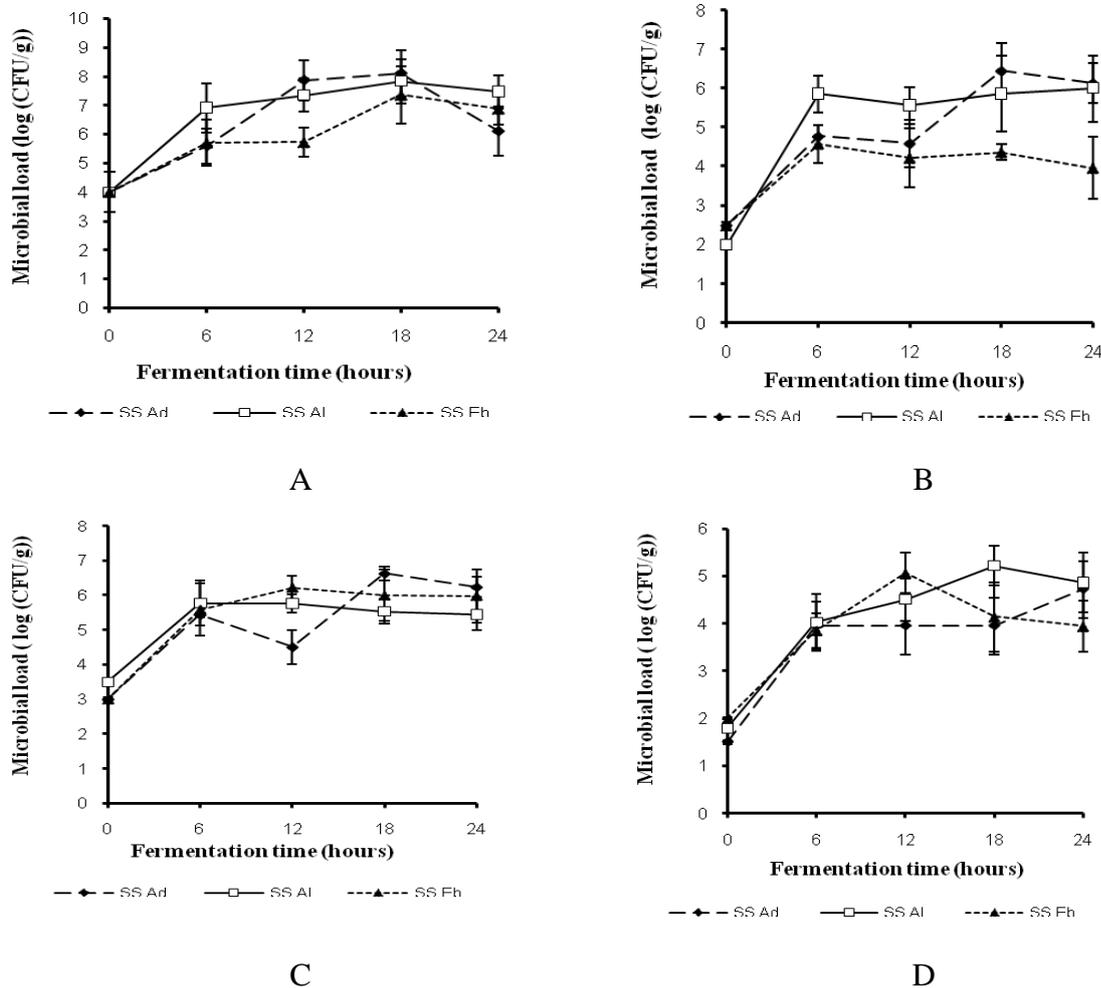
60±2 mg/100g and 68±3 mg/100g after 12 and 18 hours of fermentation, (Fig. 4A). In contrast to acetaldehyde, the quantity of methylethylketone observed in fermented dough was irregular with a high concentration of 9.4±0.94 mg/100g produced by Alladjan starter isolates after 12 hours of fermentation (Fig. 4B). On the other hand, a large content of ethanol (5±0.15 mg/100g) was produced by the Ebrie starter isolates after 12 hours of fermentation (Fig. 4C).



**Fig. 4. Production of volatile compounds by the selected starters in the different fermenting cassava doughs.** Acetaldehyde (A), Methylethylketone (B) and Ethanol (C). SS.Ad: selected starter Adjoukrou, SS.Al: starter selected Alladjan, SS.Eb: selected starter Ebrie

The production of these compounds was irregular during fermentation. Yeast is believed to be the basis of ethanol production [24]. The presence of these volatile compounds depends on several factors such as yeast seeding rate, yeast strain, medium composition, fermentation temperature and aeration [25]. Thus, isolation and enumeration of microorganisms revealed that lactic acid bacteria, Bacilli, yeasts and moulds in varying proportions (Fig. 5). The flora, more abundant was represented by lactic

acid bacteria with loads of  $7.2 \pm 0.8 \cdot 10^7$  CFU/g;  $2.9 \pm 0.7 \cdot 10^7$  CFU/g and  $7.6 \pm 1.1 \cdot 10^6$  CFU/g respectively in the doughs inoculated with the selected starters Adjoukrou, Alladjan and Ebrie after 18 hours of fermentation (Fig. 5A). At the same time, average loads of bacilli were respectively  $1.3 \pm 0.7 \cdot 10^6$  CFU/g,  $9.9 \pm 0.9 \cdot 10^5$  CFU/g and  $9.1 \pm 0.2 \cdot 10^4$  CFU/g (Fig. 5B). Loads of yeasts and moulds were also significantly different ( $p < 0.05$ ) among all these potential starter cultures.



**Fig. 5. Microbial load in cassava doughs inoculated with the three types of starters selected during fermentation.** Lactic Acid Bacteria (A), *Bacillus* (B), Yeast (C) and Mould (D). SS.Ad: selected starter Adjoukrou, SS.Al: starter selected Alladjan, SS.Eb: selected starter Ebrie

This microbial load used during the degradation of soluble sugars remains lower than that obtained by [26] during traditional fermentation. This indicates that the presence and multiplication of these microorganisms with known properties lead to significant changes in each dough controlled by a mixed starter.

Thus, in all the analyses, each mixed ferment was used to prepare a typical attieke for each ethnic group. These attieke in doughs inoculated with the selected starters Adjoukrou, Alladjan and Ebrie were of acceptable quality and appearance similar to traditional attieke. So, the formulation of a mixed starter from a traditional ferment makes it possible to obtain a microflora capable of degradation of cassava starch and the production of organic acids and volatile compounds necessary for the attieke production and flavor.

#### 4. Conclusion

The comparative study of the performance of the three (3) starters of attieke (selected starters Adjoukrou, Alladjan and Ebrie) clearly indicates that the evolution of physico-chemical and microbiological parameters of the dough inoculated with the selected starters Adjoukrou and Alladjan were similar except the ethanol content that was higher in the dough inoculated with the Alladjan starter selected during the first six (6) fermentations. On the other hand, these two (2) doughs differ from the inoculated dough with the selected starter Ebrie by its high titratable acidity rate and its high acetaldehyde content. After having carried out all analyses, each mixed- ferment was used to prepare a typical attieke for each ethnic group. These attieke in doughs inoculated with the selected starters

Adjoukrou, Alladjan and Ebrie were of acceptable quality and appearance similar to traditional attieke.

#### 5. Acknowledgement

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