Journal of Agri-Food and Applied Sciences
Available online at jaas.blue-ap.org
©2016 JAAS Journal. Vol. 4 (3), pp. 53-59, 31 October, 2016
E-ISSN: 2311-6730

ASSESSING THE MICROBIOLOGICAL AND CHEMICAL CHARACTERISTICS DURING TRADITIONAL CASSAVA STARTER "*EBRIÉ*" PRODUCTION

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Received: 2 October, 2016 *Accepted: 11 October, 2016* *Published: 31 October, 2016*

ABSTRACT

The aim of this study was to characterize the different biochemical and microbiological changes and the effect of the cassava variety during the fermentation of traditional cassava starter. Two cassava varieties, (IAC) and (Bonoua) were investigated at different fermentation times. The load of microorganisms (lactic acid bacteria, coliforms, yeast and mould, aerobic mesophilic, *Bacillus*) and biochemical parameters (pH, titratable acidity, total and reducing sugar) were assessed. During fermentation, progressive acidification was observed in the pulp of both cassava varieties (0.02-0.12%). The sugar consumption was observed during cassava fermentation (2.4-1.1 mg/g). Rapid growth of coliforms (total and thermotolerant) was obtained between 0 and 48 hours followed by a decrease after 48 hours. The fermentative microflora (lactic acid bacteria, yeast and mould, *Bacillus*) growth was recorded up to 72 hours before a drop. Contrary to the cassava variety, fermentation time has a significant influence on the evolution of biochemical and microbiological parameters.

Keywords: *Fermentation time, Cassava variety, Artisanal starter.*
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INTRODUCTION

Cassava is an essential resource in the African diet. Its roots, rich in starch are an excellent source of calories for people in developing countries in general, and Côte d'Ivoire in particular. It was estimated that the crop provides about 40% of all the calories consumed in Africa (Umeh and *al.,* 2007). It helps to reduce caloric deficits in food rations.

Manihot esculenta Crantz, species used in Côte d'Ivoire, is a plant of the *Euphorbiaceae* family, rich in starch and low in protein. Several varieties exist, but it is possible to group them into two groups: the sweet variety and the bitter one. Cassava ranks second after yam among food crops with a production of 1 582000 tonnes in 1992 (Assanvo and *al.,* 2006). If the sweet variety is mainly used in the preparation of "Foutou", "Bèdècouman", "Akpessi", the bitter variety is traditionally processed into a wide variety of foods with different local names such as "*Attiéké*", "*Placali* "," *Attoukpou* ", " *Konkondé* "(Kakou, 2000).

Fermentation plays an important role in the preparation of dishes including the second group of aforementioned cassava-based foods. It is a step that involves microbial activity. Thus, the artisanal starter of cassava constitutes the main source of

microorganisms used like inoculum of these different food products (Assanvo and *al.,* 2002). In Côte d'Ivoire, there are several methods for the production of the starter from cassava and according to specific method used the inoculums take various names such as "*Ebrié*", "*Adjoukrou*", "*Alladjan*" and "*Aboure*". These different types of inoculums are usually obtained after 72 hours of fermentation. The fermentation process that induces a succession of microorganisms (Umeh and Odibo, 2014) appears spontaneously through the development of the epiphytic microflora (Giraud and *al.,* 1995).

In Côte d'Ivoire, studies relative to the evolution of microorganisms during the production of the starter from cassava and the influence of cassava variety on the microflora and biochemical, were undertaken. These studies had involved "Alladjan" starter (Abodjo and *al.,* 2010) and "Adjoukrou" starter (Tetchi and *al.,* 2012). The objective of this study was within the same framework. Indeed, it was to follow the evolution of microorganisms during the preparation of "Ebrié" starter and the impact of cassava varieties (bitter variety, sweet variety) on this microflora and biochemical parameters.

MATERIALS AND METHODS

Materials

Fresh cassava roots of eight to nine months-old were collected from a farm in Akeïkoi village, near of Abobo in Côte d'Ivoire. The cassava varieties selected comprised a local variety with low cyanide content, namely Bonoua and a bitter variety with high cyanide content, IAC (Improved African Cassava).

Cassava's artisanal starter production

Tubers of each variety were washed in clean water, peeled, chopped into pieces of roughly 10-15 cm and of approximately 500g then boiled. For each variety, three batches of 5 samples of a cube were made up. A sample of each variety was immediately filed into a sterile stomacher then placed in a cooler to be transported to the laboratory for analysis corresponding to time 0 hour. The four other samples were filed 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 analysis.

Measurement of pH and determination of titratable acidity

The pH of the roots of cassava in fermentation was determined using a pH-meter (p107, CONSORT, bioblock Scientific, France). Titratable acidity was determined using the standard method described. 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 0.1N 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 and *al.,* 2000).

$$\%\, d'acidité = \frac{N_b \times V_b \times 0{,}09}{W_b} \times 100$$

V_b = volume of the base used;
0.009 acid milliequivalent factor for lactic acid;
N_b= normality of base used
Nb=normality of the base used;
W_b= sample weight

Determination of total sugars

A chemical conversion of total sugars into 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% leads acetate solution and 10 mL of chlorhydric acid 12N were succesively 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 method of Miller (1959).

Determination of total reducing sugars

Total reducing sugars were determined on a dry matter base according to the dinitrosalicylic acid (DNSA) method described by Miller (1959) and use by Kimaryo and al. (2000).

Enumeration of microoganisms

Preparation of stock solution inoculation of agar plates cultivation and quantification of microorganisms were carried out according to Coulin and al. (2006). For all determinations, 10 g of the samples were homogenised in a stomacher with 90 mL of sterile peptoned buffered wter (Difco, Becton Dickinson, Sparks, MD, USA). Tenfold serial dilution was prepared and spread-plated for microorganisms count.

Total aerobic mesophilic counts were determined on plate count agar (PCA Oxoid LTD, Basingstore, Hamsphire, England) after incubation at 30°C for 48 h. Enumeration of lactic acid bacteria was carried out using De Man Rogosa and Sharpe (MRS, Merck 10660, Merck, Darmstadt, Germany). Enterococci were counted by cultivation on Bile Esculin Azide agar (BEA, ISO 7899/1) at 37°C for 24-48 hours. The enumeration of coliforms was obtained by cultivation for 24 hours on crystal-violet neutral re bile lactose agar (VRBL, AFNOR NF ISO) at 30°C for the total coliforms and 44°C for the thermotolerant coliforms. Yeasts and moulds were enumerated on plates of Sabouraud chloramphenicol agar (Fluka, Biochemica 89579, Sigma-Aldrich Chemie Gmbh, India) incubated at 30°C for 4 days. *Bacillus* were enumerated according to Buttiaux and al. (1974) method.

Statistical Analysis

The results obtained from the fermented cassava were subjected to analysis of variance (ANOVA) and the Turkey multiple range test to separate the means at the significance defined at $P< 0.05$ using R version 3.2.3 software with Rcmdr package.

RESULTS AND DISCUSSION

RESULTS

During fermentation process, there was significant decrease in pH while titratable acidity increased significantly for both cassava varieties (fig 1). At time 0 hour sweet cassava had pH 6.1 and bitter variety presented pH 6 and showed 0.025% and 0.02% respectively for titratable acidity. At the end of fermentation period, bitter variety had lower pH 4.3 and titratable acidity about 0.12% than sweet variety which showed pH 4.6 with 0.09% of titratable acidity. Titratable acidity values were higher in the bitter variety, statistical analysis did not show any significant difference ($p>0.05$) between two cassava varieties used.

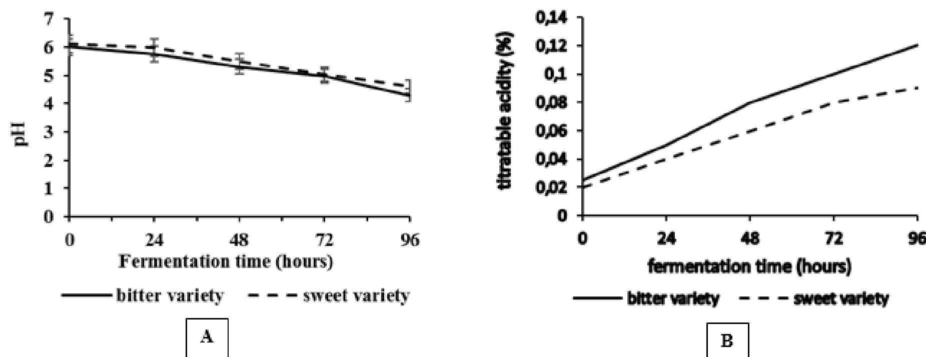

Figure 1. Changes in pH (A) and titratable acidity (B) during cassava lactic acid fermentation

Total sugars content, initially 2.4 mg/g and 2.7 respectively for sweet variety and bitter variety, decreased with the increase of the duration of fermentation for all variety reaching 1.1 (bitter variety) and 1.2 mg/g (sweet variety) at 96 hours (**fig 2 A**). During the first 48 h of fermentation, the lowering of reducing sugars content was observed from 1.1 to 0.9 mg/g and 1.4 to 1.2 mg/g in the fermented pieces of respectively bitter variety and sweet variety. After 48 h, there was a significant decrease in the concentration of reducing sugars for all varieties.

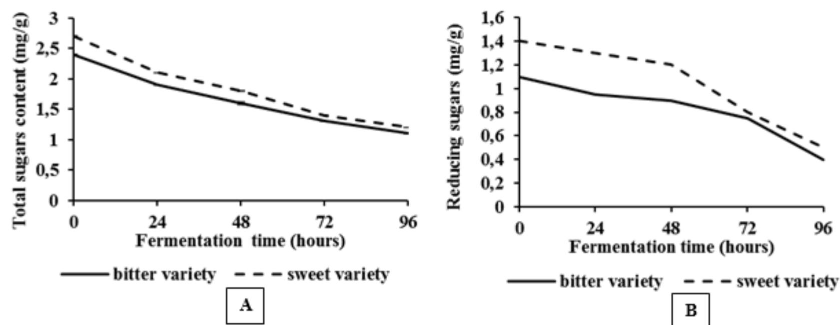

Figure 2. Changes in total sugars (A) and reducing sugars (B) during cassava lactic acid fermentation

The counts of total aerobic mesophilic increased with time, reaching highest values after 48 hours for bitter variety (7.78 log_{10} cfu/g) and sweet variety (7.6 log_{10} cfu/g) before decreasing slightly respectively to 7.5 log_{10} cfu/g and 7.45 log_{10} cfu/g at the end of fermentation (Fig 3). The coliforms had identical evolution like total aerobic mesophilic (Fig 4). During the first 48 h of fermentation there was significant increase of coliforms counts for both cassava variety before decreasing significantly.

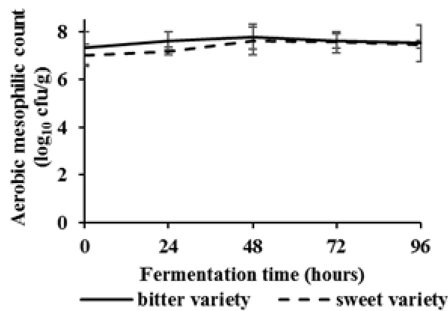

Figure 3. Evolution of total aerobic mesophilic during "*Ebrié*" process for the production of stater culture

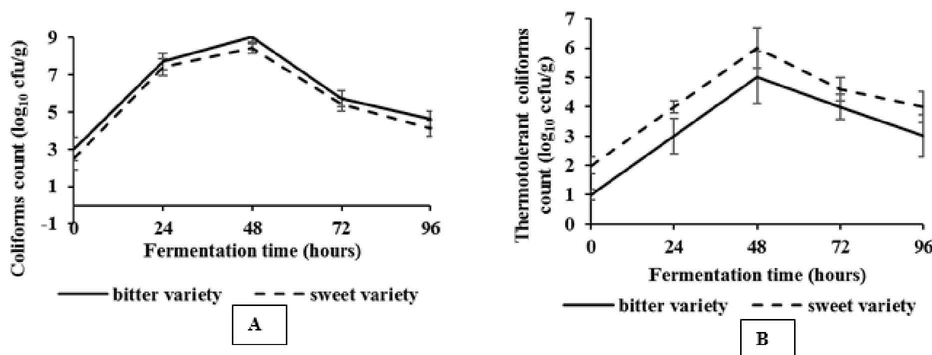

Figure 4. Evolution of total coliforms (A) and thermotolerant (B) during "*Ebrié*" process for the production of stater culture

At time 0 h, we have isolated 1.22 log_{10} cfu/g (bitter variety) and 1 log_{10} cfu/g (sweet variety) for yeast and mould (Fig 4 A). These numbers increased slightly for the first 48 hours and significantly between 48 hours and 72 hours reaching highest values for bitter (6.11 log_{10} cfu/g) and the sweet (3.6 log_{10} cfu/g) varieties. After 72 hours, yeast and mould decreased slightly to 6 log_{10} cfu/g and 3 log_{10} cfu/g respectively for bitter and sweet varieties at the end of the fermentation processing.

Like yeast and mould, the lactic acid bacteria number increased during fermentation for both varieties and reached the highest loads, 8.87 log_{10} cfu/g and 7.47 log_{10} cfu/g respectively for bitter and sweet varieties only after 72 hours and decreased until the end of fermentation for the sweet variety (7.07 log_{10} cfu/g) whereas the bitter variety (8.89 log_{10} cfu/g) (Fig 4 B) slightly increased.

Initially, loads of *Bacillus* were 2.2 log_{10} cfu/g and 2 log_{10} cfu/g repectively for bitter and sweet varieties (Fig 5). These loads increased significantly for the first 48 hours and slightly between 48 hours and 72 hours reaching highest values, 8.34 log_{10} cfu/g (bitter variety) and 8.96 log_{10} cfu/g (sweet variety) within 72 hours. After 72 hours the loads decreased slightly to 8.07 log_{10} cfu/g and 8.83 log_{10} cfu/g respectively for bitter and sweet varieties at the end of the fermentation process.

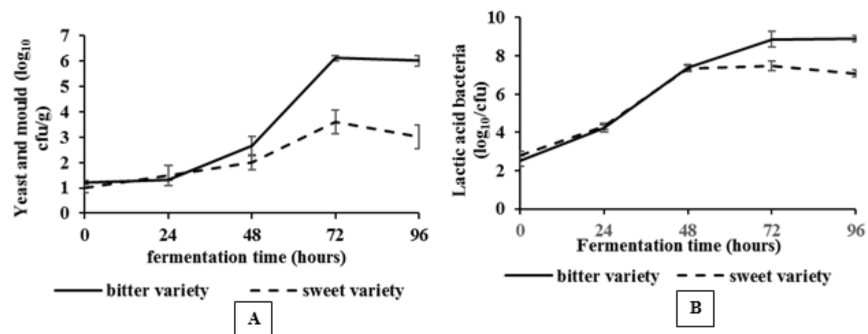

Figure 4. Evolution of yeast and mould (A) and Lactic acid bacteria (B) during "*Ebrié*" process for the production of stater culture

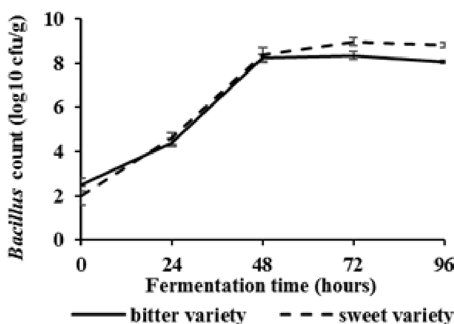

Figure 5. Evolution of *Bacillus* during "*Ebrié*" process for the production of stater culture

DISCUSSION

During the fermentation process, there was significant decrease in pH and increase in titratable acidity whatever the variety of cassava roots. Other reports showed that the evolution of pH and titratable acidity during the fermentation process (Umeh and Odibo, 2014, Kostinek and *al.,* 2007). The observed in pH and titratable acidity during the fermentation of cassava roots were probably due to the accumulation of organic acids mainly lactic and acetic acids associated with the fermentative activities of bacteria and yeast (Giraud and *al.,* 1995; Almeida and *al.,* 2007; Coulin and *al.,* 2006). During the fermentation, monosaccharides (glucose, fructose) coming from the breakdown of sucrose was metabolized into organic acids by facultative anaerobic micro organisms such as lactic acid bacteria. The decrease of total sugars during the fermentation was probably linked to the metabolism of sucrose. According to Zhan and al. (2000), the decrease of total sugars was due to the amylolytic activity of the microbiota of the traditional artisanal starter which converted a part of starch in cassava tubers into sugars and consequently into lactic acids during fermentation. Panda and al. (2008) demonstrated that *Lactobacillus plantarum* could produce α-amylase and reduce sugars from hydrolyse starch. The decrease of reducing sugars concentration could be explained by the activities of total fermentative microflora which metabolized and converted them into energy for their growth and into organic acids. The evolution of all biochimical parameters in this study is identical to lactic acid fermentation process for production of a "*Alladjan*" starter.
Whatever the cassava variety, the composition of microorganisms was identical for each microbial group considered. The origin of the microflora in the roots results from several sources: hands, air and bags (Assanvo and *al.*, 2002; Fagbemi and Ijah, 2005). The presence of germ contamination witnesses (coliform), confirm the results of Achi and Akomas (2006). Indeed, the high coliform counts were probably induced by the contamination of the jute bags during the fermentation process. Microbial growth is accelerated between 0 and 48 hours. This could be explained by an apparent lack of competition between microorganisms related to an abundance of substrates. After 48 hours of fermentation, a decline in coliform loads was observed in the different variety of cassava roots. This decrease of coliform loads could be due to substrate depletion and the production of increasingly lactic and acetic acid by the lactic acid and acetic acid bacteria. According to Djoulde (2003), the dissociated acids (lactic and acetic) allow them to enter the bacterial cells where they ionize and build up, causing an internal lowering of the pH and the blocking transport mechanisms.

Lactic acid bacteria, yeast and mold and *Bacillus* have greater loads than coliforms. These microorganisms play a very important role in the fermentation of cassava roots (Assanvo and *al.,* 2006) by the preservation of food, detoxification, production of aroma and enzymes (amylase, cellulase, tannase, and pectinase betaglucosidase) (Schallmey and *al.,* 2004). The exponential growth of these microorganisms extends up to 72 hours whatever the cassava variety. Several studies (Achi and AKomas, 2006;

Padonou and *al.,* 2009; Umeh and Odibo, 2014) have shown the involvement of these microorganisms in the fermentation of cassava tubers. Indeed, lactic acid bacteria can produce lactic acid only (homofermentative bacteria) or lactic acid, acetic acid or ethanol and CO_2 (strict heterofermentative bacteria) (Kostinek and *al.,* 2005; Haydersah, 2010). The genus *Bacillus* is involved in tissue degradation of cassava by the production of enzymes such as polygalacturonase, pectin esterases, cellulases and amylases that it releases (Ouattara and *al.,* 2008, Ehon and *al.,* 2015). Also, Amoa-Awua and al. (1997) have shown the involvement of the molds and yeasts in the cellulase activity during the fermentation of cassava tuber.

CONCLUSION

The results of this study showed that whatever the variety of cassava roots 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. The cassava fermentation for artisanal starter production is widely influenced by the time of fermentation. Rapid growth of coliforms (total and thermotolerant) was obtained between 0 and 48 hours followed by a decrease after 48 hours. The fermentative microflora (lactic acid bacteria, yeasts and molds, *Bacillus*) growth was recorded up to 72 hours before a drop. A decrease of pH, total and reducing sugars and an increasing of acidity were observed during the fermentation. Contrary to the cassava variety, fermentation time was influenced significantly by the evolution of biochemical and microbiological parameters.

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