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Microbiological and physico-chemical dynamics during the fermentation of the millet-based (*Pennisetum glaucum*) *Ablo* and the sorghum-based (*Sorghum bicolor*) *Ablo* in the Republic of Benin

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Abstract

Ablo is wet bread, slightly salty and sweet, steamed and sold in the form of pellets. The study has for objective to follow microbiological and physicochemical changes during the fermentation of the millet's dough and sorghum's dough for the production of two new types of Ablo. The methodology adopted consisted in performing production's essay followed by analyses in the laboratory. The dominant micro flora of the fermentation of millet-based Ablo and sorghum-based Ablo was constituted of lactic bacteria and yeasts and moulds. The evolution of lactic bacteria was inversely proportional to the decrease of the pH and the material dry during fermentation.

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Introduction

Fermentation is a desirable process of biochemical changes caused by microorganisms and their enzymes on products of first transformation. Fermentation is one of the oldest and most economical methods of production and preservation of food (Steinkraus *et al*., 1983; Cooke *et al*., 1987; Chavan and Kadan, 1989; Padonou, 2010). It is a technology on which depend millions of people in the countries of the third world for the conservation of their food to make them available to average consumers (Kalui *et al*., 2010). For several decades, fermented products have a great importance in the diet of the African (Odunfa), 1985. In Africa, these fermented food products are particularly used as products of weaning for small children (Kalui *et al*., 2008, Tchekessi *et al*., 2013). There are two main types of fermentations that often take place at the same time in spontaneous processes from hydrocarbon substances: lactic fermentation and alcoholic fermentation. Lactic fermentation is a method of transformation very widespread in the tropics. It allows to lower cost to give added value to agricultural produce, improve food safety and the preservation of foods resulting from (Cooke *et al*., 1987; Nout and Motarjemi, 1997). It's happen by the acidification of medium reactional, resulting from the transformation of carbohydrates into lactic acid primarily. We'll talk about homolactic fermentation when the process results in the exclusive formation of lactic acid and the lactic bacteria that can lead to this result are called homofermentative.

When at the end of the process you get a mixture of lactic acid and ethanol with a release of gas we're talking about heterolactic fermentation result of the activity of heterofermentative lactic bacteria (Padonou, 2010). Sometimes the fermentation results in the formation of a mixture of lactic acid and acetic acid: this is the way bifidum characteristic fermentation of *Bifidobacterium bifidum* (Orla-jensen, 1924), a bacterium known for its probiotic role for the human organism. All these reactions are accompanied by the production of energy in the form of ATP.

This study focused on Ablo which is a moist bread shaped ball, very consumed in Benin, especially in large cities (Ahoyo *et al*., 2013; Dansou, 2013; Houssou *et al*., 2014; Aboudou *et al*., 2014; Houssou *et al*., 2015). This dough fermented made the object of a summary description of its preparation by Nago (1998), of the assessment of the traditional processes of its preparation by Ahokpè (2005), of the artisanal technical system of its preparation by Aholou-yeyi (2007), of the assessment of the microbial flora during its fermentation by Banon (2012) and of the bibliographic synthesis of its synthesis by Bokossa *et al*. (2016). The presented information is gotten following experiences of consistent production of analyses in the laboratory.

The study permitted us to master the physico-chemical and microbiological faculty of the two cereals used to produce *Ablo*. The study aims to follow microbiological and physicochemical changes during the fermentation of the dough of millet and the dough of sorghum intended for the production of two new types of Ablo.

Materials and methods

The productions were made in the research unit in safety health food (URSSA) of the laboratory of Microbiology and of the food Technologies (LAMITA) in the Faculty of science and technology (FAST) of the University of Abomey-Calavi (UAC).

Materials

Plant material

Sorghum (*Sorghum bicolor*) of red color designated in local language fon by "abokun" and the small millet (*Pennisetum glaucum*) greenish color called "likun" in fon were used. Wheat flour also served as plant material. These cereals were purchased at the Dantokpa Cotonou's international market.

Biological material

The instant yeast (*Saccharomyces cerevisiae*) of trademark PASHA made in Turkey by DOSU MAYA MAYACILIK A.S. Company certified ISO 9001: 2008 has been used. It was purchased at the Dantokpa Cotonou's international market. It is used as a leaven in the manufacturing technology of the Ablo (Ahokpe, 2005; Aholou-Yeyi, 2007; Bokossa *et al*., 2013).

Laboratory equipment

The analysis material consists of classical material used for microbiological and Physico-chemical handling.

Other materials

The material used for the different manufacturing was constituted of ingredients (sugar, salt) and standard production equipment of the Ablo such as basins, plastic buckets, pots, a spatula, a whip, grinding wheels, a sieve and a fireplace. The water of the national society of waters in Benin (SONEB) was also used.

Methods

Experimental method

The production tests were conducted according to the original method described by Aholou-Yeyi (2007) modified. The difference in this technology was the use of other types of cereals such as millet and sorghum and the reduction of the fermentation time. Each test was repeated three times in the laboratory. Fermentation lasted six hours. We realized tacking every two hours starting from the time zero (t_0). Different microbiological and physicochemical analyses were conducted on these samples.

Methods of Analysis

Microbiological analyses

Microbiological analyses consisted of the enumeration of total flora, yeasts and moulds and lactic bacteria depending on the duration of fermentation by crops on media synthetic. The counts were taken by counting the colonies (Guiraud and Galzy, 1980). Microbiological analyses were conducted in three repetitions on each sample of the products.

Preparation of suspensions mothers and decimal dilutions

Before seeding, it was imperative to sterilize glassware and prepare the suspension-mother and the successive decimal dilutions thereof. Here, all operations were conducted in the field of the flame of the Bunsen burner or under the hood. According to the AFNOR (1999), test socket for the preparation of suspensions-mothers was 10g for each sample. To get the suspension-mother, these 10g of sample were collected aseptically in a previously sterilized Erlenmeyer. Then, 90ml of water solution sterile peptone (EPS) prepared following the indications of the manufacturer have been added. The mixture was homogenized with the brand vortex Homogenizer. One milliliter of this suspension-mother has been taken and added to the first tube of a series of test tubes to screw containing 9ml of the solution of dilution and homogenization has been carried out. The same procedure was repeated by taking 1ml of the resulting suspension this time, and by adding it to the tube in the series and so on until the last decimal dilution desired.

Enumeration of total flora

From each prepared decimal dilution, 1 ml was taken and introduced into a sterile Petri dish box. 10 to 15ml of the previously melted plate Count Agar (PCA Oxoid CM 0325) solid medium was added, and then all was perfectly homogenized. After complete solidification, plates were incubated at 30°C for 48 to 72 hours.

Enumeration of yeasts and moulds

The medium that was used for their research was the word Dextrose Agar (Oxoid CM 0041) containing chloramphenicol (0.05 g/L). Previously prepared and sterilized, 10 to 15ml of Sabouraud have been sunk in sterile boxes of Petri dish and left to solidify. From each prepared dilution of decimal, 0.1ml was collected and spread on the surface of the agar using a spreader rake. The counting of colonies was made after incubating plates at 25°C for 3 to 5 days.

Enumeration of lactic bacteria

Lactic bacteria count was based on a culture in depth of 1ml of each prepared dilution of decimal. So, 1ml of each prepared dilution was placed in sterile Petri dish boxes. Then, 10 to 15ml of medium of Man Rogosa Sharpe Agar (MRS Agar CM 0361) have been added. After solidification, a second layer was conducted. Petri boxes were incubated at 30°C for 48 h.

Expression of the results

The results were obtained from the count of net settlements. The counts were taken by dials. The results were expressed in CFU per gram of product according to the method described by Bokossa in 2007.

Physicochemical analysis

They consisted in the determination of the dry matter, water content, pH, titratable acidity according to the duration of fermentation. The physicochemical analyses were performed in three repetitions on each sample.

Rate of dry matter and water content

The dry matter content was determined according to the method AACC44-15A (AACC, 1984). The water content has been deducted by the above formula:

Dry water (%) = 1 - matter content (%)

pH and titratable acidity

The pH and titratable acidity were determined according to the modified method of Nout *et al.* (1989).

Statistical analyses of the data

The collected data were analyzed using SPSS 16 and MINITAB 14 software. MINITAB 14 software was used to verify the conditions of application of the statistical tests. These were made with the software SPSS 16 which has to do the analyses of variance (ANOVA) and Tukey test for the comparison of averages. The chosen significance level was 5% $p < 0.05$.

Results

The determination of pH, titratable acidity and dry matter and enumeration of microorganisms have allowed to appreciate their evolution during the fermentation of the dough.

Physicochemical analysis showed that the pH decreased during the fermentation. At the same time, the titratable acidity increased following a similar rhythm to that of the pH decrease. Thus, the pH was 5.40±0.11 to 3.27±0.12 in the dough of millet and 5.80±0.23 to 3.50±0.18 in the dough of sorghum in six hours of fermentation while the titratable acidity varied from 0.67±0.11 to 2.97±0.66 in the dough of millet and 0.59±0.06 to 2.8±0.23 in the dough of sorghum for the same period (Fig. 1 and Fig. 2).

Dry matter decreased gradually in favor of water content during the fermentation. Indeed, the dry matter varied from 39.20±1.06 to 34.66%±0.87 in the dough of millet and 40.60±2.10 to 33.12%±1.02 in the dough of sorghum in six hours of fermentation. By contrast, the water content was 60.80%±1.06 to 65.34±0.87% in the dough of millet and 59.40%±2.10 to 66.88%±1.02 in the dough of sorghum for the same duration of fermentation (Fig. 3 and Fig. 4).

The total flora load has increased during the first two hours of fermentation, then beyond this period, it has experienced a substantial reduction. it spent 7.16±0.20 to 7.44±0.12 Log_{10} cfu/g in the dough of mil and 6.26±0.36 to 6.61±0.18 Log_{10} cfu/g in the dough of sorghum after two hours of fermentation and 6.38±0.11 Log_{10} cfu/g in the dough of millet and 5.95±0.47 Log_{10} cfu/g in sorghum dough after six hours of fermentation (Fig. 5 and Fig. 6).

The number of yeasts and mould and lactic bacteria varied significantly during the fermentation of dough (Fig.7 and Fig.8). Indeed, yeasts and molds in number at the beginning of the fermentation had a slow increase up to two hours of fermentation. After this time, this increase had become important then declined gradually after four hours of fermentation (Fig.7 and Fig.8) process. Lactic bacteria had exponential growth during the fermentation (Fig. 7 and Fig. 8).

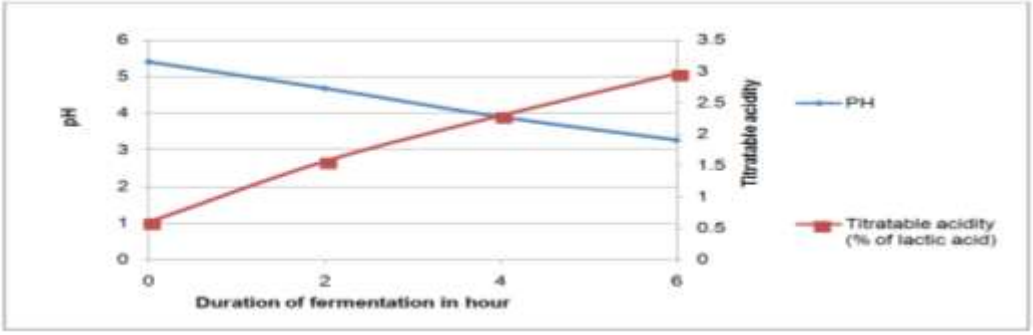

Fig. 1. Evolution of pH and titratable acidity of the dough of millet in function of the duration of fermentation

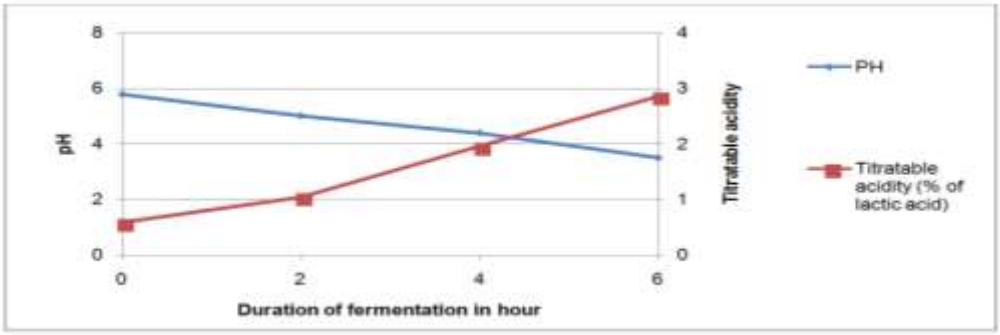

Fig. 2. Evolution of pH and titratable acidity of sorghum's dough in function of duration of fermentation dough

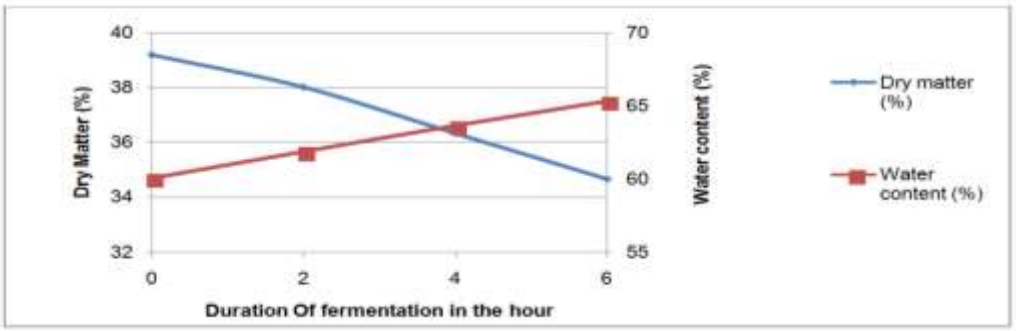

Fig. 3. Evolution of the dry matter and the water content of the dough of millet in function of the duration of fermentation

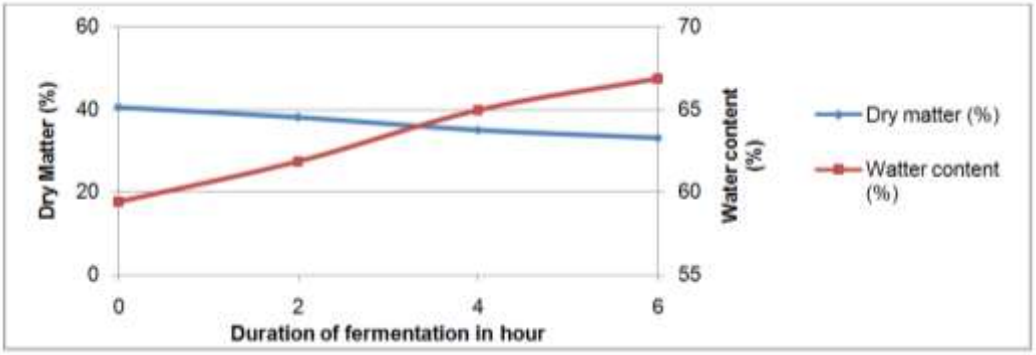

Fig. 4. Evolution of dry matter and the water content of the dough of sorghum in function of the duration of fermentation

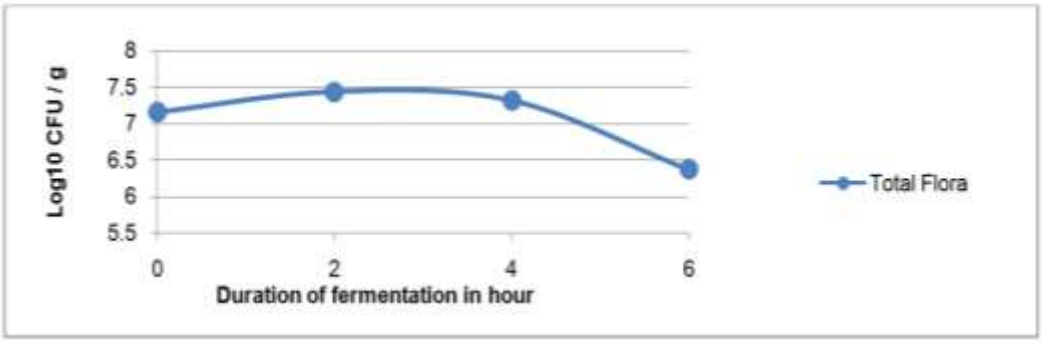

Fig. 5. Evolution of the number of the total flora present in dough of millet in function of the duration of fermentation

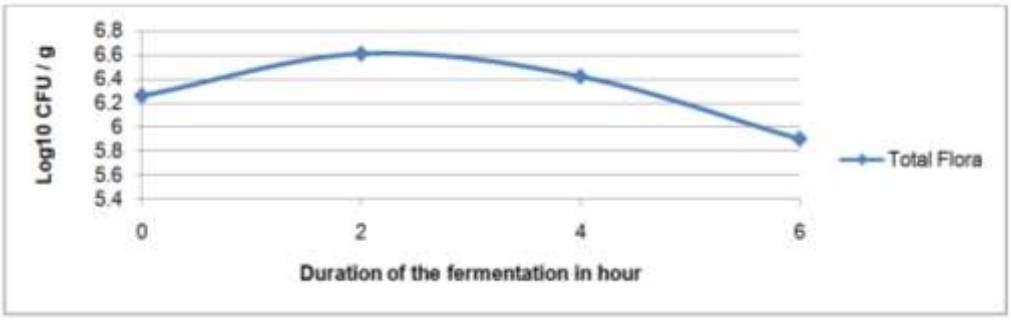

Fig. 6. Evolution of the number of the total flora present in sorghum dough in function of the duration of fermentation

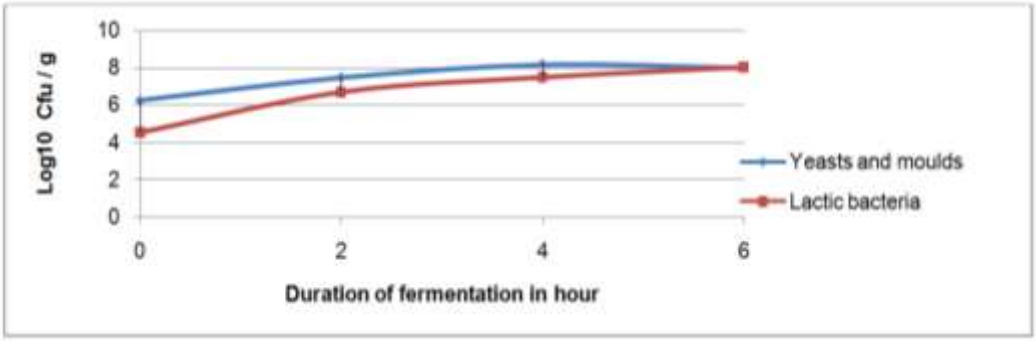

Fig. 7. Evolution of the number of lactic bacteria and yeasts and moulds in the dough of millet function of the duration of fermentation

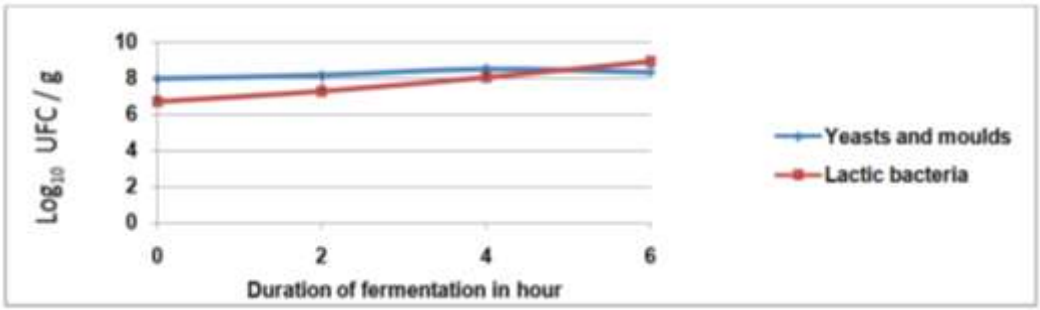

Fig. 8. Evolution of the number of lactic bacteria and yeasts and moulds in the dough of sorghum in function of the duration of fermentation

Discussion

This study shows that the pH decreases during the fermentation for the benefit of the titratable acidity that increases following a rhythm similar to that of the pH decrease. These results are in agreement with those of Hounhouigan, 1994; Michodjehoun, 2000; Mugula *et al.,* 2002; Kanninkpo, 2011 and Bokossa-Yaou *et al.*, 2013 on other fermented products who said that the gradual decline of the pH and the increase of titratable acidity that are observed during fermentation are characteristic of flours of cereals in fermentation. These observations are similar to those of Odunfa and Adeleye (1985), which explained the decline of pH seen during the fermentation of the 'kankey" (fermented dough of corn-based Ghanaian origin) by the increase in the number of lactic bacteria. Thus, the bacteria metabolize the fermentable sugars to produce many metabolites including lactic acid leading to an increase in titratable acidity and a drop in pH during the fermentation. This explains the decrease in pH during the fermentation process.

The dry matter is gradually reduced in favor of moisture during the fermentation. The same comments were made by Jansen, 1990; Hounhouigan *andal.*, 1993 and by Vieira-dalode, 2008 who reported that this reduction is due to absorption of the material then a production of water by lactic bacteria and yeasts in the metabolic reactions.

The total flora load increases during the first two hours of fermentation then knows beyond this period a significant reduction. The same observations were made by Banon, 2012; Tchekessi, 2012 and Bokossa *et al.*, 2013 on other fermented cereals products that have attested that the fall of the load in total flora is due to the decline in the pH during the fermentation.

The number of yeasts and moulds and lactic bacteria vary significantly during the fermentation of dough. These results are consistent with those obtained by Luis *et al.*, 2003; Vieira-dalode, 2008; Odunfa, 1985; Hounhouigan *et al.*, 1993a; Hounhouigan *et al.*, 1993b and Larry, 1995 which have shown that the association of yeasts and lactic bacteria found in fermentations spontaneous basis of corn and a lot of other cereals.

According to Steinkraus, 1996 this would be the result of a symbiotic association between yeasts and lactic bacteria. This author has shown that lactic bacteria create an acidic environment for the proliferation of yeasts that produce in turn to vitamins and other compounds of bacteria growth.

The same dynamics of fermentation has been reported by Luis *et al.* 1996 who observed a significant increase in the burden of lactic bacteria and yeast during the 15 hours of the fermentation's process of potopoto, a Congolese product fermented corn sprouted.

Conclusion

The results of this work show that the dominant microflora of the fermentation of sorghum-based Ablo and millet-based Ablo consists of lactic bacteria and yeast and mold. Then, the evolution of lactic bacteria is inversely proportional to the decrease in pH and dry matter during the fermentation.

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